

Indira Gandhi
National Open University
School of Sciences

VOL

1

IMMUNOLOGY-I

BLOCK 1

INTRODUCTION TO IMMUNOLOGY

7

BLOCK 2

ANTIGEN-ANTIBODY

109

IMMUNOLOGY

Immunology is the study of the immune system and is a very important branch of the medical and biological sciences. The immune system protects us from infection through various lines of defence. If the immune system is not functioning as it should, it can result in diseases such as autoimmunity, allergy and cancer.

Immunology is the branch of biomedical science that deals with the response of an organism to antigenic challenge and its recognition of what is self and what is not. It deals with the defence mechanisms including all physical, chemical and biological properties of the organism that help it to combat its susceptibility to foreign organism.

The immune system is divided into those which are **static** or **innate** to the organism and those which are responsive, **adaptive** to a potential pathogen.

The innate system is older system in evolutionary terms, that forms the first line of defence. It is non-specific and the resistance is static. (It does not improve with repeated exposure and there is no memory on subsequent exposure). This includes physical defences such as skin and epithelial surfaces, cilia, acidic gastric content, fever etc. Others are biochemical defences such as acute phase reactants and complement, interferon. Cellular components include natural killer cells, RES phagocytes.

The adaptive system is the second line of defence and is activated once the innate system has been overwhelmed. It is specific to the infective agent and can store the information about the invader as the memory to show an enhanced response to subsequent challenge.

The word immunity was derived from the Latin word 'immunis' meaning exempt.

Immunology deals with physical, chemical and physiological characteristics of the components of the immune system *in vitro*, *in situ* and *in vivo*. Immunology has a vast array of uses in several disciplines of science and medical science.

The key primary lymphoid organs of the immune system are thymus and bone marrow and secondary lymphatic tissues such as spleen, tonsils, lymph vessels, lymph nodes, adenoids and skin. The actual components of the immune system are cellular in nature and not associated with any specific organ. They are widely present in circulation throughout the body.

Immunology course is discipline specific elective of fifth semester. It has two volumes i.e. Immunology I and Immunology II. Volume 1 has two blocks i.e. Block I and Block 2 and Volume 2 has two blocks i.e. Block 3 and Block 4. Volume 1 has 8 units and Volume 2 has 7 units.

Block 1 comprises five units. It traces the historical perspective of immunology. Basic concepts of immunology viz. self and non self, Clonal selection theory and major categories of human pathogens and host pathogen interaction are discussed in the introductory unit.

Components, cells and organs of immune system are elaborated in first block. Innate immunity is the inborn immunity that serves as the first line of defence by creating barriers against any pathogens through anatomical, humoral and cellular barriers. Adaptive immunity is specific to antigens and effective in combat against invading microbes. The major population of lymphocytes involved in adaptive immune responses are B and T-lymphocytes. B-cell immune response is also known as **humoral immunity** and T cell immunity is also known as **cellular immunity**.

Immune system of human is a network of organs and tissues connected by blood vessels and lymphatic system. The organs of lymphatic system are **primary** and **secondary** lymphoid organs. Bone marrow and thymus, are primary lymphoid organs. Spleen, tonsils, and lymph nodes are secondary lymphoid organs. All the blood cells including cells of immune system are derived from pHSC stem cells in the bone marrow. The microenvironment in which these cells are found influences their development and on maturation pHSC cells give rise to myeloid and lymphoid progenitors. From myeloid progenitor, four types of cell arise i.e. megakaryocytes, erythroblast, myeloblast and monoblast. The cell types that are principal to immune system are called as lymphocytes that arise from lymphoid progenitor cells.

Block 2 discusses about antigens, antibodies, and their interaction. Antigen are substances that can bind with antibodies or cell receptors which may (immunogen) or may not elicit an immune response (haptens). So all immunogens are antigens but not all antigens are immunogens. Only a small portion of antigen, called as epitope, binds to B and T cells. The mechanism of binding to T and B cells are different. Antigens can be classified as exogenous, endogenous, autoantigens and alloantigens based on origin. Antibodies are a heterogenous group of globulin proteins found in serum collectively called as immunoglobulins (IgG, IgH, IgA, IgE, IgD) that mediate phagocytosis, triggering of inflammation and other important immunological responses. Antibodies can be specific to several epitopes of antigens (polyclonal antibodies) or be specific to only one epitope of the antigen which are called as monoclonal antibodies.

Volume II, Immunology II comprises Blocks 3 and 4 which have seven units in all. It speaks about working of the immune system and role of immune system in fighting diseases. It introduces to structure and function of MHC. Different pathways of antigen presentation (exogenous and endogenous) and processing are also discussed.

T cell activation requires 3 components i.e. T cell complex – MHC: peptide interaction. T helper cells carry co-receptor CD_4^+ that recognise class II MHC molecule and T cytotoxic cells bear CD_8^+ co-receptor and recognise class I MHC molecules. Activated helper T cells can differentiate into various subsets which perform different functions.

Cytokines are diverse groups of non antibody glycoproteins usually smaller than 30kDa that form a part of intercellular signalling molecules secreted by specific cells of immune system. Lymphokines, monokines and interleukins are various types of cytokines. Complement system is an integral part of innate immune system that are formed by number of proteins >20 which are synthesised in liver and circulated in blood and tissue fluids. Complement system induces opsonisation, chemotaxis, inflammation and lysis pathogens.

Pathogens produce diseases in humans by the colonisation of host tissues or by liberating toxins. To protect humans against diseases from microbes, immunisation is used as a means of providing specific protection against pathogens by stimulation of one's immune system. Such specific immunity can be attained either by active or passive immunisation through vaccine.

Objectives

After studying this course you should be able to:

- trace the course of development of the field of immunology,
- explain an overview of the immune system,

- define innate immunity,
- list the various innate immunity barriers,
- discuss the functions of NK cells,
- define complement system and list its functions,
- explain various complement activation pathways,
- gain an insight about the adaptive immune system,
- differentiate between naturally and artificially acquired immunity,
- explain B and T lymphocytes,
- differentiate between humoral and cell-mediated immunity,
- compare and differentiate functions executed by primary and secondary lymphoid organs,
- enlist the biological significance exhibited by lymphoid organs,
- discuss the properties of antigen recognized by the B and T cells,
- list the structure and properties of antigens,
- differentiate between T and B epitopes,
- define adjuvants and discuss their functions,
- explain the immunological variants of antibodies present in mammalian body,
- distinguish between isotype, idiotype and allotype antibodies,
- differentiate between polyclonal and monoclonal antibodies, and
- discuss the principles and applications of important immunological tools, and techniques.

Indira Gandhi
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Block

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BLOCK 1: INTRODUCTION TO IMMUNOLOGY

The immune system is present everywhere in the body. The specialized cells and molecules which comprise the immune system cannot function in isolation. There must be a constant crosstalk and interaction among cells and tissues throughout the body. Immune cells must be able to exchange information or messages with the cells of other major regulatory systems i.e: the brain, the nervous system and endocrine system etc. The most significant response takes place within the tissue. In the past few years immunologists have been able to understand the cellular and molecular features which actually indeed encourage the immune cells to leave the circulation and enter the tissue. In response to a variety of stimuli, the blood and the lymphatic vessels are considered to be the key regulators of immune and inflammatory responses. They provide a dynamic interface between the tissues and the circulating fluids, cells and molecules under certain circumstances.

The immune system is defence system that protects animals against attack by pathogens and cancer. The immune system has two major activities namely recognition and response.

The presents volume (Volume I) comprises 2 blocks namely

Block 1: Introduction to Immunology

Block 2: Antigen–Antibody

Block 1 comprises five units and Block 2 consists of three units in all.

Unit 1 “Overview of the Immune System” gives an insight of historical perspective of immunology. It introduces basic concepts of immunology i.e. self and non self regulation, clonal selection theory and various categories of pathogens. Various types of pathogens, pathogen associated molecular patterns (PAMPs) and host-pathogen interactions are discussed here.

Unit 2 and 3 speak of non specific and specific lines of defence of the body i.e. innate and adaptive immunity respectively. Innate immunity is inborn immunity to certain infection prior to exposure of that disease. Innate immune system serves as the first line of defence by creating anatomical, humoral and cellular barriers.

Unlike innate immunity, adaptive immunity has the ability to recognise specific antigens, develop memory and ability to distinguish between self and non self. Adaptive immunity can be humoral and cell mediated immunity.

Units 4 and 5 explain about cells and organs of immune system. In Unit 4, you will study about primary lymphoid organs (where immune cells develop i.e. bone marrow and thymus) and secondary lymphoid organs (where the immune response is initiated i.e. lymph node, spleen, MALT). Cells of myeloid lineage i.e. granulocytes (neutrophils, eosinophils, basophils, mast cells), Phagocytic cells (monocytes macrophages and dendritic cells) and cells of the lymphoid lineage that include lymphocytes (B and T cells– CD₄ and CD₈) and natural killer cells (NK cells) are discussed in Unit 5.

Block 2 – “Antigen–Antibody” comprises four units. Unit 6 “Antigens” explains about general structure, basic properties of and various types of antigens viz. autoantigens, alloantigens, exogenous and endogenous antigens.

Unit 7 explains about antibodies (Immunoglobulins Igs). Their basic structure and classes (IgG, IgM, IgA, IgE and IgD) are discussed in Unit 7. It speaks about various immunoglobulin variants i.e. isotypes, allotypes and idiotypes. Monoclonal and polyclonal antibodies are discussed and their differences are also explained.

In Unit 8 you will study about mechanisms of antigen-antibody interaction (i.e. precipitation, agglutination, neutralization complement fixation and opsonisation). Basic immunological tools i.e. Western Blot ELISA, flow cytometry and others are also discussed.

Objectives

After studying this block you will be able to:

- trace the course of development of the field of immunology,
- define innate immunity and list the various innate immunity barriers,
- define Toll like Receptors,
- discuss the functions of NK cells,
- define complement system, list its functions and explain various complement activation pathways,
- gain an insight about the adaptive immune system and list the various features of adaptive immunity,
- differentiate between humoral and cell-mediated immunity,
- explain the role of NK cells as a bridge between innate and adaptive immune systems,
- compare and differentiate functions executed by primary and secondary lymphoid organs
- discuss the process of generation, regulation and functions of immune cells,
- explain mechanism of immune responses, and
- discuss the properties of Antigen Recognized by the B and T cells,

UNIT 1

OVERVIEW OF THE IMMUNE SYSTEM

Structure

1.1	Introduction		The Postulates of Clonal Selection Theory
	Objectives		
1.2	Historical Perspective of Immunology	1.5	Introduction to Pathogens
1.3	Introduction to Basic Concepts of Immunology	1.6	Major Categories of Human Pathogens
	The Innate Immune System		Virus
	The Adaptive Immune System		Bacteria
	Humoral Immunity		Protozoa
	Cell Mediated Immune Response		Helminths or Parasitic Worms
	Antigen Processing	1.7	Host-Pathogen Interaction
	Exogenous Antigen Processing		Interaction with the Innate Immune System
	Endogenous Antigen Processing		Mechanisms used by Pathogens to Evade Adaptive Immune Response Mediated Killing
1.4	Self and Non-Self Cells	1.8	Summary
	Tolerance	1.9	Terminal questions
	Autoimmunity	1.10	Answers
	Clonal Selection Theory		

1.1 INTRODUCTION

Immunology is the study of the functioning of immune system. Immune system is a defence system that protects us from invasion by pathogens. Diseases like allergy, autoimmunity and cancer can result if the immune system fails to work properly. The immune system has two components called the **innate** and **adaptive** immune system. Adaptive immune system is further divided into **Humoral** and **Cell Mediated** Immune Systems. Together these systems work to protect the body.

One of the most important characteristics of immune system is the ability to distinguish between self and foreign (cells and molecules). The cells that bind to self-antigens are selectively eliminated during the early developmental stages. **Only activated cells that bind to foreign antigens are clonally selected and propagated.**

This unit also talks about infectious agents called pathogens that attack the body. These pathogens belong to very diverse classes like bacteria, fungi, protozoa, viruses and helminths. The immune system must combat these pathogens and prevent the establishment of infection in the body through various host-pathogen interactions.

The concepts that are being discussed in this unit will be dealt in detail in further Units of this course: You are being introduced to some immunological terminology that will be used throughout this course.

Objectives

After studying this unit, you should be able to:

- ❖ trace the course of development of the field of immunology,
- ❖ explain an overview of the immune system,
- ❖ discuss how the body differentiates between self and foreign molecules,
- ❖ explain the basic causes of autoimmunity,
- ❖ list the postulates of Clonal Selection theory,
- ❖ explain basic terms like pathogenicity and transmission,
- ❖ list the various classes of human pathogens, and
- ❖ give examples of major pathogenic diseases.

1.2 HISTORICAL PERSPECTIVE OF IMMUNOLOGY

- The term 'Immunity' comes from the Latin word 'ímmunis' that means to exempt. Thus immunity refers to a state of protection from disease.
- Thucydides in 430BC while describing a plague in Athens introduced the concept of immunity for the first time.
- Chinese and Turks made the first attempts to provide immunity in the fifteenth century.
- Lady Mary Wortley Montagu observed in 1718 the advantages of variolation of the local population and got her children variolated. In this technique, dried crust from the dried pus of small pox patients has been given to subjects via small cuts or inhalation.
- Edward Jenner, an English physician in 1789 went on to improve this technique. He was surprised to note that the milkmaids who had

previously been infected with cowpox were subsequently found to be immune to smallpox. Jenner concluded that inoculating fluid from cowpox pustules into subjects might protect them from smallpox.

- The next discovery made by Louis Pasteur was a fortunate happening. He had successfully grown the fowl cholera causing bacteria in culture and had then demonstrated that when chickens were injected with that cultured fowl cholera causing bacterium, they developed cholera. He then went on a vacation and could not revive the culture. On returning from the vacation, Pasteur injected the chickens with the old culture and found that the chickens just became ill, but they soon recovered. He then intended to repeat the experiment with a freshly grown culture but due to a limited supply of chickens, he had to inject the same chickens with the fresh culture. To his surprise, the chickens again fell ill and recovered.

Thus, Pasteur put forward the hypothesis that aging of the culture had dampened the virulence of the bacteria. He said that such attenuated pathogen might be administered to individuals to provide protection against the disease. He coined the term 'vaccine' for this attenuated pathogen (Latin: 'vacca' means cow), to honour the work of Edward Jenner in cowpox inoculation to protect against smallpox.

- The first vaccine was administered by Pasteur in 1885 to a young boy named Joseph Meister who had been severely bitten by a rabid dog. Several doses of attenuated rabies viral preparations were given to the boy and thus he was protected from rabies.
- The first insight to the working mechanism of immunity was established by the work of Emil von Behring and Shibasaburo Kitasato in 1890. They showed that immunity against diphtheria could be transferred from immunised patient via serum to non-immunised individual.
- During the next decade, terms like precipitin, antitoxin and agglutinin popped up to indicate the component of serum that performed the roles of clumping bacteria and neutralising toxins. They were all considered to be separate components till Elvin Kabat attributed all these functions to an immunoglobulin molecule. These are now known as antibodies.
- The immunity mediated via these antibodies present in the serum was thus called as humoral immunity.
- Elie Metchnikoff observed that certain cells which he called phagocytes were white blood cells that were able to internalise (phago/endocytose) microbes and other foreign material. He observed that the phagocytic cells were more active in immunized animals and thus he hypothesized that cells were more important for immunity than the serum components. The cells identified as active phagocytic cells were monocytes and neutrophils.
- For a long time the scientists were divided between the humoral and the cell mediated immunity theories. However, it was established later that both work together in the body.

- Interest in cell-mediated immunity was renewed when Merrill Chase successfully transferred immunity against pathogen causing tuberculosis while performing a leukocyte transfer experiment on pigs.
- The lymphocyte was identified in 1950s as an important element of humoral and cell mediated immunity.
- Bruce Glick demonstrated via his experiment on chickens that cell mediated immunity was effected by T-cells and humoral immunity by B-cells.

SAQ 1

Fill in the blanks:

- Variolation was first performed by on her children.
 - observed the phenomenon of phagocytosis for the first time.
-

1.3 INTRODUCTION TO BASIC CONCEPTS OF IMMUNOLOGY

The immune system is a defence system that protects animals against attack by pathogens and cancer. A large number of cells and molecules are produced by the immune system to perform this task.

The immune system has two major activities namely- **recognition and response**.

- The ability to differentiate even single amino acid differences among pathogens and also self from non-self (molecules and cells).
- Once pathogen recognition is complete, an effector response is mounted to neutralize the pathogen or toxin. Subsequent exposure to the same pathogen generates a more intensive memory response.

1.3.1 The Innate Immune system

It is the first line of defence of the body present from the time of birth. The innate immunity components are present before the initiation of infection. It is not specific for a particular pathogen but in general increases the ability of the body to recognise commonly found molecules on the surface of pathogens.

Table 1.1 depicts various barriers and mechanisms of protection.

Table 1.1: Barriers and mechanisms of protection.

Sl. No.	Barriers	Mechanism of protection
1.	Anatomical Barriers	a) The skin has epidermis and dermis that form a covering and prevent pathogen invasion.

		The low pH of secretions by sebaceous and sweat glands is inhibitory to pathogens. b) Mucous membranes form a continuous lining where pathogens are trapped in mucous and are removed. Also, they have to compete with the normal microflora of the body for attachment sites.
2.	Physiological Barriers	a) Temperature-Fever protects against pathogens. The high body temperature of some organisms protects them from diseases. For example, chickens are protected from <i>B. anthracis</i> due to their high body temperature. b) pH- Low pH of gastric secretions inhibits most microbes. c) Antimicrobial substances like lysozyme in tears, saliva.
3.	Phagocytic Barriers	Phagocytic cells engulf, break down and kill the pathogens. Examples are macrophages, neutrophils, monocytes.
4.	Inflammation	There is leakage of fluid from blood vessels due to vasodilation and increase in permeability. There is an influx of cells to the area of pathogen invasion/damage.

1.3.2 The Adaptive Immune System

Foreign pathogens and antigens are selectively recognised and eliminated by the adaptive immune system when the innate immune system fails to defend the body against the pathogen invasion. It is capable of responding only when a stimulus is provided by the innate immune system.

The Adaptive or Acquired immune response takes much longer time than the innate immune response to become activated and respond. It can take days or even weeks but adaptive immunity is more pathogen specific and also has memory. Adaptive immune response is generated post exposure to a pathogenic antigen or after a vaccination. The responses are tailor made to specific antigenic challenges and not present naturally like the innate immune system.

The adaptive immunity can be naturally or artificially acquired:

- Naturally acquired immunity results when the body fights the pathogen and recovers or when preformed antibodies are provided via the placenta and mother's milk.

- Artificially acquired immunity results when killed/attenuated pathogen or toxoid is injected in the body. This process is called Vaccination.

1.3.3 Humoral Immunity

Humoral immunity depends on molecules found in the “*humor*”, that is the body fluids. It is mediated by molecules like antibodies, various complement proteins and antimicrobial peptides. Therefore, it is also as **Antibody Mediated Immunity**.

Humoral immunity involves the production of antibodies and simultaneously occurring events like the activation of helper T-cells and production of cytokines. It also involves the various effector function of antibodies like-

- Neutralization of pathogens and toxins.
- Classical pathway of complement activation.
- Opsonisation (of pathogen) and phagocytosis.
- Eventual pathogen clearing.

1.3.4 Cell mediated Immune Response

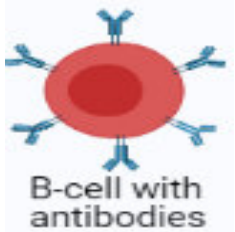
Cell mediated immune response is the method of the body to fight against pathogens like bacteria and viruses. It is also responsible for the killing of cancerous cells and in the graft-rejection process.

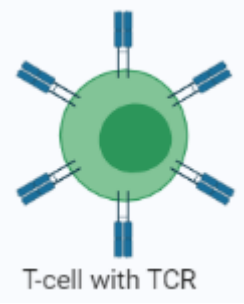
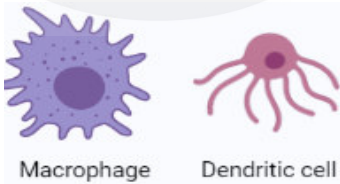
The foreign antigen is recognised by phagocytic cells like macrophages and dendritic cells of the innate immune system. They internalise the pathogen, degrade it and present their antigenic peptides on their surface with MHC Class II molecules. This MHC-antigenic complex is responsible for the activation of adaptive immune system.

CD4⁺ and CD8⁺ cells are formed from T-cell precursors in the thymus. The MHC Class II complex on APCs (Antigen presenting cells) interact with the CD4⁺ T cells while the CD8⁺ T cells interact with the antigen- Class I MHC complex found on infected or cancerous cells. The CD8⁺ T cell transforms into Cytotoxic T lymphocytes (CTL) that are responsible for the destruction of the infected or the cancerous cell.

Table 1.2 describes the major cells involved and their important features.

Table 1.2: Cells and their features in immune response.

Sl. No.	Cell	Major features
1.		- Mature in bone marrow - Express membrane bound antibody molecules with specificity to particular antigen. - When naive B-cell encounters an antigen, it is activated and proliferates to form memory B-

		cells and plasma cells. - Plasma cells secrete antibodies rapidly. - Memory cells get activated on subsequent exposure to the same antigen and produce a much more severe response.
2.	T-cell 	- Formed in bone marrow but matures in thymus. - Express T-cell receptor (TCR) on its surface. - TCR can recognise antigen only when bound to MHC molecules. - Naive T-cell on encountering MHC bound antigen proliferates to produce effector T-cells and memory T-cells. - Helper T-cell (T_H cell) on activation secretes cytokines that further activate cytotoxic T-cells (T_c cells). T_c cells proliferate to form cytotoxic T-lymphocytes (CTL) that kill the pathogen.
3.	Antigen presenting cells 	- Macrophages, dendritic cells and B-lymphocytes. - Express class II MHC molecules on their surface. - They internalise the antigen and degrade it into smaller fragments. The fragment is then expressed with MHC-II molecules on the surface. - They provide co-stimulation signal for T_H cell activation.

1.3.5 Antigen Processing

The T-cell recognises antigen only after it is degraded into smaller peptides that can form complexes with MHC Class I or II molecules. This process that the antigen undergoes is called **Antigen presentation and processing**. The route of antigen entry determines whether the antigen will be presented with Class I or Class II MHC molecules.

Exogenous Antigen Processing

Exogenous antigen is taken up by the cell by phagocytosis or endocytosis and degraded into smaller peptides. This process is performed by antigen presenting cells like macrophages, dendritic cells and B-cells in the Endocytic processing pathway.

The antigenic peptides produced are bound to MHC Class II molecules and transported to the cell surface. Therefore the presentation of exogenous peptides is limited to antigen presenting cells that bear MHC II molecules. Now the CD4 displaying T-cells bind to MHC-II bound antigen. These cells act as helper T-cells.

Endogenous Antigen Processing

Endogenous antigens are produced inside host cells. Common sources of endogenous antigens are cancerous cells and virus infected cells that continuously form viral proteins. They are degraded in the endoplasmic reticulum to smaller fragments. These fragments bind with MHC-I molecules and move to cell surface. Class I MHC-antigen complex is recognised by CD8 bearing T cells. These cells are killed by cytotoxic T-cells.

SAQ 2

State whether the following statements are 'True' or 'False':

- a) T-cells are formed and mature in thymus.
 - b) Endogenous antigens are degraded to smaller fragments in endoplasmic reticulum.
 - c) APCs express class I MHC molecules on their surface.
 - d) Humoral immunity provides protection against cancerous cells.
-

1.4 SELF AND NON-SELF CELLS

It is of critical importance that the immune system should differentiate body's own cells from foreign invaders. This is necessary to avoid attack on self-cells.

The immune system recognises the cells based on antigens present on the surface. The microorganisms have antigens that are absent in the human body and thus recognised as foreign.

The Major Histocompatibility Complex (MHC) is the major self-identification molecule. Each individual has a unique combination of MHC molecules that is recognised as self. When any difference is noted in these molecules in pathogen infected cells, altered cancerous cells or in transplanted tissue, the immune system is activated and attacks these cells.

The B-cells have antibodies attached on their surface that recognise and bind to specific antigens on pathogen surfaces. The T-cell activation occurs via antigen presenting cells (APCs) like macrophages. The pathogen is internalised by APC, broken down and the fragment expressed with MHC molecules present on the surface of APC. The T- cell binds to this presented fragment via specific T-cell receptor (TCR) and this leads to T-cell activation

1.4.1 Tolerance

The ability of T and B cells to ignore or tolerate self-antigens is called as **Immunological tolerance**. Tolerance involves selective elimination of self-reactive cells during development. T-cells that bind to self-antigens are killed in the thymus and likewise B-cells in bone marrow. The tolerance gained by such mechanisms is called **Central tolerance**.

Therefore the immune system is able to develop cells with specificity to antigen only through the process of tolerance and somatic recombination.

1.4.2 Autoimmunity

An **autoimmune disease** occurs when the body attacks its own self-cells tissues and other body constituents. There are several reasons for the development for autoimmunity-

1. The secretion of antibodies by plasma cells requires the activation of B-cells by T-cells. But this need for T-cells can be neglected in certain cases, example; super-antigens.
2. An imbalance between B and T cell functioning. For example; in coeliac disease T-cells recognising gliadin activate B-cells recognising transglutamine.
3. Damage in feedback mechanism mediated by B-cell receptor.
4. Dysregulation of cytokines.
5. Defective apoptosis of dendritic cell may lead to lymphocyte activation and autoimmune conditions, since dendritic cells are antigen presenting cells.
6. Molecular mimicry- the pathogen's antigens closely resemble a molecule in the host. Thus, immune response might be mounted on self-cells in such cases.

SAQ 3

Fill in the blanks:

- a) is an example of autoimmune disease.
- b) The ability of T and B cells to ignore or tolerate self-antigens is called as

1.5 THE CLONAL SELECTION THEORY

The Clonal Selection theory was put forward by *Neils Jerne, F. Macfarlane Burnet* and *David Talmadge*.

Neils Jerne's theory proposed that large amounts of antibodies are present in the serum at all times. In the scenario of invasion by a pathogen, that antibody is selected and produced in large quantities which have specificity for the target antigen.

Burnet expanded Jerne's theory in a paper titled "A modification of Jerne's theory of antibody production using the concept of clonal selection". He later wrote a book called "**The Clonal Selection Theory of Acquired immunity**" and postulated the theory of **Clonal Selection**.

The theory states that each lymphocyte expresses antigen specific membrane receptors that are specific to a particular antigen. The receptor's specificity for a distinct antigen is determined even before the lymphocyte interacts with the antigen. The interaction between the specific receptor and its corresponding antigen causes the activation of B-cells in the secondary lymphoid organs (spleen and bone marrow) where it encounters the antigen (Fig 1.1). The B-cells then proliferate to form a clone of cells with the same antigen specificity as the parent B-cell.

This is the currently accepted model for the immune response to foreign antigens and selection of specific B and T cells to carry out the immune effector functions.

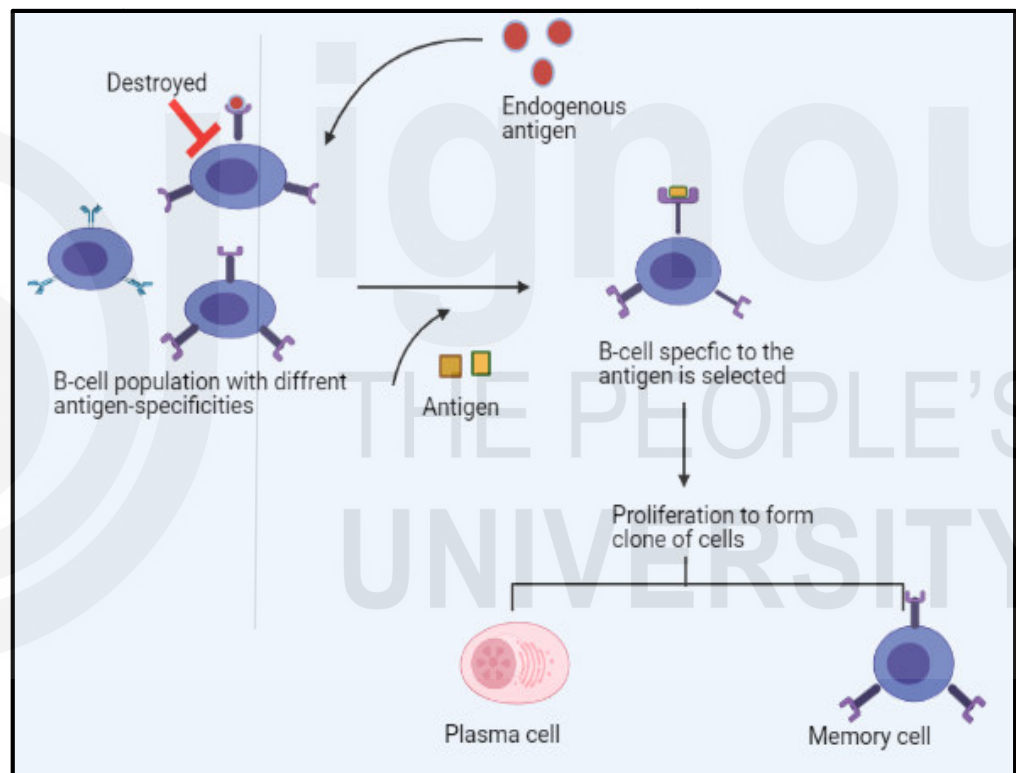


Fig1.1: Clonal selection theory: B-cell specific to antigen is selected and proliferates to form memory and plasma cells.

1.5.1 The Postulates of Clonal Selection Theory

- Each lymphocyte bears a receptor with specificity for a particular antigen.
- The activation of cell occurs only after interaction with that particular antigen.
- A clone of cells is produced with identical antigen specificity as its parent cell.
- Selective destruction of lymphocytes bearing endogenous antigens on their receptors. This prevents the immune system from targeting self-cells.

1.6 INTRODUCTION TO PATHOGENS

The term 'pathogen' comes from the Greek word "*pathos*" meaning suffering. Pathogen is an organism or an infectious agent that can cause disease.

There can be several infectious agents like virus, bacteria, protozoan or fungus. Disease can also be caused by parasitic worms and insect larvae (Table 1.3).

Pathogenicity: It is the potential of a pathogen to cause disease. It is a qualitative term. The pathogenicity of a pathogen depends on several factors like:

- Ability to enter host tissue
- Colonise the host tissue
- Use the host nutrients
- Suppress the host immune system
- Ability to produce toxins

Transmission: The spread of pathogen. It occurs via several routes like faeco-oral route, air, water, through direct and indirect contact, blood, body secretions like milk, saliva and tears.

Table 1.3: Examples of common human pathogens and diseases caused by them.

Sl. No.	Pathogen group	Name of pathogen	Disease Caused
1.	Bacteria 	<i>Klebsiella pneumonia</i> <i>Bacillus anthracis</i> <i>Vibrio cholera</i> <i>Mycobacterium tuberculosis</i>	Pneumonia Anthrax Cholera Tuberculosis
2.	Virus 	<i>Herpes simplex virus</i> <i>Rhinovirus</i> <i>Hepatitis virus</i>	Herpes Common cold Hepatitis
3.	Protozoa 	<i>Plasmodium sp</i> <i>Leishmania donovani</i>	Malaria Leishmaniasis
4.	Helminths 	<i>Ascaris</i> <i>Schistosoma</i>	Ascariasis Schistosomiasis

Microbiology is the study of microorganisms, including microscopic pathogens. The study of parasites and their hosts is called **Parasitology**. The study of the diseases caused by these pathogens is called by **Pathology**.

5.	Fungi 	<i>Candida albicans</i> <i>Aspergillus flavus</i> <i>Dermophytes</i>	Candidiasis Aspergillosis Athlete's foot
----	--	--	--

1.7 MAJOR CATEGORIES OF HUMAN PATHOGENS

A plethora of microorganisms are found in our surroundings and thus the human body is susceptible to attack by many diverse groups of pathogens. A few of them will be discussed here.

1.7.1 Virus

Virus is an infectious agent that is dependent on the host for its metabolic activities and replication. Outside of the host cell, they exist as small inactive particles called as 'virions'.

The virion has:

1. Genetic material in the form of DNA/RNA but never both.
2. A protein coat (capsid) that protects the genetic material.
3. An envelope may also be present- Enveloped viruses.

Interaction of Virus with Innate Immune System

The innate immune system responds to viral infections by producing Interferons- $IFN\alpha$ and $IFN-\beta$ and activation of Natural Killer (NK) cell activation. These Interferons bind to receptors called $IFN\alpha/\beta$ receptor and activate the **JAK-STAT** pathway. This goes on to cause the transcription of many genes whose products have many antiviral roles. For example, RNase L that degrades the RNA of the virus is activated by an enzyme called 2,5- oligo-adenylate-synthetase. This enzyme is a product of one of the activated genes.

Interferon is a protein released by animal cells, usually in response to the entry of a virus, which has the property of inhibiting virus replication.

Virus Neutralisation with Antibodies

Antibodies against specific antigens of the virus prevent viral spread and protect against virus reinfection. Many viruses bind to the cell surface of the host via specific molecules expressed on their surfaces. For example, **Epstein Barr virus** binds to B-cells via type II complement receptors. Antibodies that are targeted to these receptors would inhibit the binding of virus to the host cell surface and thus prevent infection altogether.

Agglutination of the virus can also occur by antibodies and complement proteins. This would promote virus phagocytosis by acting as **opsonins**.

Virus clearance by Cell mediated immunity (Fig 1.3)

The innate immune responses are effective before the establishment of infection, especially if the virus genome has integrated with the host genome. Thus, cell-mediated immune systems are crucial in such scenarios.

The main defence components against viruses are $CD8^+T_C$ cells and $CD4^+T_H$ cells. Various cytokines like TNF, IL-2 and $IFN-\gamma$ are produced by activated T_H1 cells that directly or indirectly protect against the virus.

JAK-STAT Pathway is a chain of interactions between proteins in a cell and is involved in processes, such as immunity, cell division and tumor formation.

- IL-2 helps in bringing precursors of CTL (Cytotoxic T cells) into effector population
- IFN- γ creates an antiviral state
- NK cells are activated by IFN- γ and IL-2 together. NK cells are important in providing protection against the virus in initial stages of the infection till the CTLs are activated.

Epstein Barr Virus (EBV) is the underlying pathogen of infectious mononucleosis, which is a benign, self limiting disease.

Opsonin is an antibody or other substance which binds to foreign microorganisms making them more susceptible to phagocytosis.

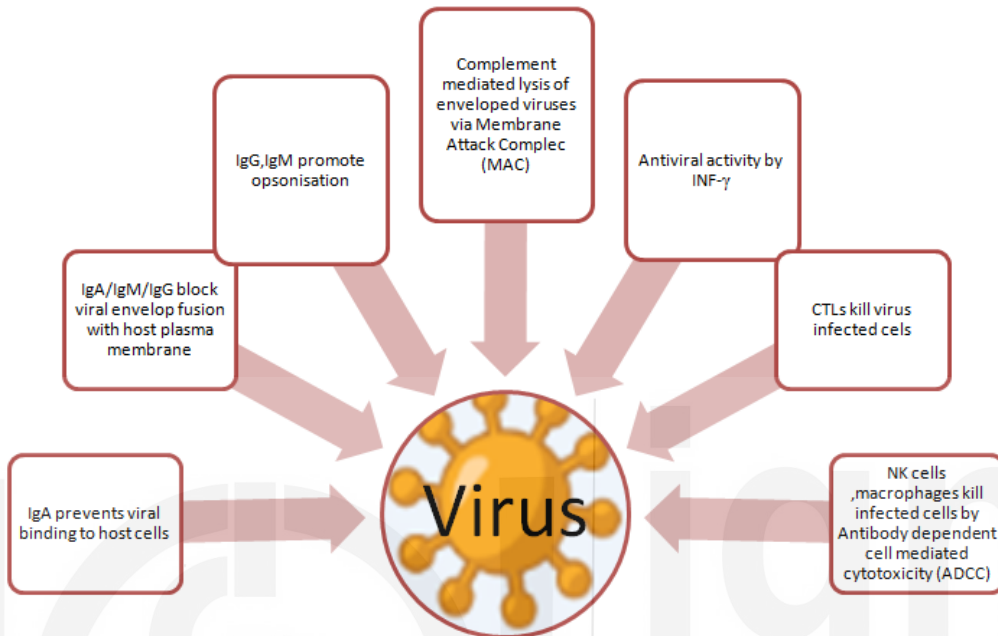


Fig 1.2: The various responses generated to virus

1.7.2 Bacteria

Bacteria can gain entry into the body via cuts, breaks, wounds or through the respiratory, gastrointestinal or genitourinary tracts. The response of immune system depends on both the number of invading bacteria and their virulence-

- Lesser number of bacteria with low virulence are often dealt with tissue macrophages.
- For larger numbers of bacteria with higher virulence, the response is often by the adaptive immune system.

The flowchart (Fig 1.3) depicts the steps in establishment of bacterial infection:

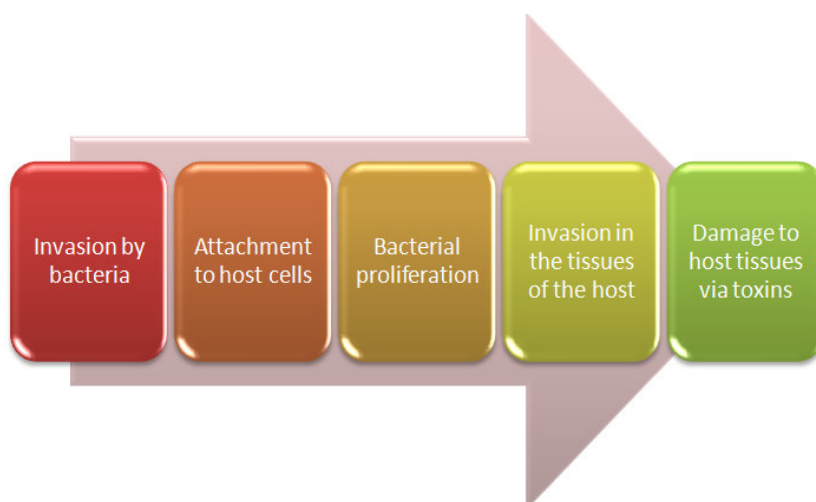


Fig 1.3: The process of establishment of bacterial infection

Intracellular and Extracellular Bacteria

The main response for protection against extracellular bacteria is the humoral immune response. Antibodies are secreted by plasma cells in lymph nodes and in the submucosa of gastrointestinal and respiratory tracts.

Extracellular bacteria may secrete toxins (endotoxins/exotoxins) or effect a local inflammation. The exotoxins are secreted out by the bacteria, like the diphtheria toxin. The endotoxins are usually bacterial cell wall components, like the LPS (lipopolysaccharide).

Protection against intracellular bacteria is provided by activation of NK cells. Cell mediated immune responses are induced in the case of intracellular bacteria, especially delayed type hypersensitivity. Cytokines like interferon- γ secreted by CD4⁺ T cells activate macrophages. The activated macrophages kill the pathogen effectively.

The antibodies can neutralise extracellular bacteria in many ways as shown in Fig 1.4.

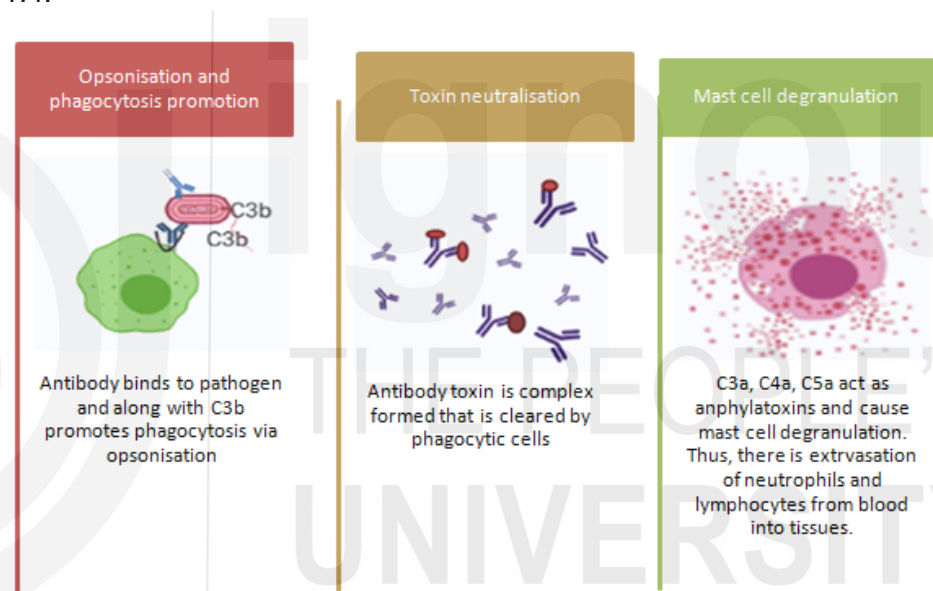


Fig 1.4: The various neutralisation mechanisms of bacteria by antibodies

1.7.3 Protozoa

Protozoa are single celled eukaryotes that are responsible for causing many human diseases like Leishmaniasis, toxoplasmosis, malaria, African sleeping sickness and Chaga's disease.

The location of the protozoa in the body determines the type and effectiveness of immune response developed.

The humoral immunity mostly works against the protozoa in the stages of its lifecycle where they freely move in the blood stream. On the other hand, the cell mediated immunity is most effective in stages where the organism shows intracellular growth.

1.7.4 Helminths or Parasitic Worms

They are large, multicellular worms that are not intracellular pathogens. They reside in the human body but do not normally reproduce there. Although their

larger size makes them more susceptible to the immune attack but often the immune response is poor. Thus once the infection is established, it is often difficult for the immune system to eliminate these parasites.

Helminths cause a variety of diseases in both animals and humans.

1. *Ascaris* – Roundworms present in the small intestine that cause Ascariasis.
2. *Schistosoma*: chronic, debilitating disease called Schistosomiasis is caused by it. The major pathogens in humans are:
 - *S. japonicum*.
 - *S. mansoni*.
 - *S. haematobium*.
3. Pathogens of animals can sometimes also infect humans.
 - *Taenia*- tapeworm that infects cattle, pigs.
 - *Trichinella*- roundworm that is found in pigs.

SAQ 4

Fill in the blanks:

- a) Diphtheria toxin is an
 - b) B-cell activation takes place in the where it encounters the antigen.
 - c) are roundworms present in the small intestine that cause Ascariasis
 - d) The endotoxins are usually bacterial cell wall components, like the, ...
-

1.8 HOST- PATHOGEN INTERACTION

A pathogen must be able to evade the innate and adaptive immune systems if it has to affect the host and cause infection.

1.8.1 Interaction with the Innate immune system

- i) The innate immunity provides a primary effective barrier to the pathogen in the form of skin and mucosal surfaces. This physical barrier is very difficult for the pathogen to invade through the skin and gain entry.
- ii) Additionally, many antimicrobial substances are also secreted by the epithelial cells. For example, the stomach epithelial cells secrete hydrochloric acid which is inhibitory to most microorganisms.
- iii) The alternative complement pathway is activated by many microbial molecules like, the peptidoglycan of bacteria. There is opsonisation of the pathogen by the generation of C3b and thus enhanced phagocytosis is seen.

- iv) Macrophages or endothelial cells produce cytokines (like $\text{TNF-}\alpha$, IL-1) when stimulated by endotoxins. This leads to further activation of macrophages.
- v) The pathogen is phagocytosed by phagocytic cells like macrophages.
- vi) Antiviral response due to the production of interferons.

1.8.2 Mechanisms used by Pathogens to Evade Adaptive Immune Response Mediated Killing

- i). Pathogens shed their antigens or grow inside host cells where they are safe from immune detection. By this mechanism they reduce their antigenic potential.
- ii). Pathogens mimic host cell surface molecules. For example, they may gain membrane molecules with the same amino acids as the host membrane molecules.
- iii). Some pathogens regulate or selectively inhibit the immune responses. This leads to an ineffective immune response against the pathogen.
- iv). Some pathogens bring about variation in their surface molecules continuously. This is achieved by either accumulating mutations gradually or by a rapid change in the surface antigenic molecules.

1.9 SUMMARY

Let us summarise what you have learnt in this Unit:

- The immune system is a defence system that protects animals against attack by pathogens and cancer.
- The body has innate and adaptive immune systems. The innate immune system evolved earlier as it is also present in plants, earthworm and insects. The presence of adaptive immunity is a unique feature of vertebrates.
- The immune system has two major activities namely- **recognition and response**.
- The innate immunity is the first, non-specific line of defence of the body present from the time of birth whose components are present before the initiation of infection.
- Foreign pathogens and antigens are selectively recognised and eliminated by the adaptive immune system.
- The adaptive immune system further has humoral and cell mediated immune systems.
- Self and non-self-recognition are important features of immune system.
- An autoimmune disease occurs when the body attacks its own self-cells tissues and other body constituents.

- The ability of T and B cells to ignore or tolerate self-antigens is called as *Immunological tolerance*.
- Clonal Selection Theory states that each lymphocyte expresses antigen specific membrane receptor that is specific to a particular antigen. It provides a mechanism for generation of diverse antibody specificities.
- The T_H (helper) and T_C (cytotoxic) cells are two subpopulations of T lymphocytes. Helper cells recognise antigen-MHCII complex which regulates immune response by secreting cytokines. Cytotoxic T cells display cytotoxic activity by recognising antigen-MHCI molecules.
- Endogenous antigens are degraded in endoplasmic reticulum and are displayed with MHCI molecules.
- Exogenous antigens are phagocytosed by Antigen presenting cells and they are then broken down to smaller fragments. These fragments are then displayed on surface with MHCII molecules.
- The killing of endogenous pathogens occurs by cell mediated immune system and that of exogenous antigens occurs by humoral system.
- Pathogen is an organism or an infectious agent that can cause disease.
- There are several human pathogens like bacteria, fungi, protozoa, viruses and parasitic worms.

1.10 TERMINAL QUESTIONS

1. Fill in the blanks:
 - a) The Clonal Selection Theory was put forward by....., ..., and
 - b) RNase L that degrades the RNA of the virus is activated by an enzyme called
 - c) Outside of the host cell, viruses exist as small inactive particles called as.....
2. Expand the following terms:
 - a) ADCC
 - b) MAC
3. Write the various steps in the establishment of bacterial infection.
4. State whether the following statements are 'True' or 'False'
 - a) The main response for protection against extracellular bacteria is the adaptive immune response.
 - b) Protection against intracellular bacteria is provided by activation of NK cells
 - c) IFN-γ creates an antiprotozoan state
 - d) Helminths are not intracellular pathogens.

5. Fill in the blanks

- a) virus binds to B-cells via type II complement receptors.
- b) Antibody toxin complex formed is cleared by
- c) A chronic, debilitating disease called Schistosomiasis is caused by.....
- d) An occurs when the body attacks its own self-cells, tissues and other body constituents.

6. Match the following:

Column I	Column II
i) Pneumonia	a) Innate immunity
ii) Measles	b) Exogenous antigen
iii) Endocytic pathway	c) Bacterial disease
iv) Low pH	d) Viral disease

7. Define the following terms:

- a) Tolerance
- b) Autoimmunity
- c) Phagocytic barriers of innate immunity
- d) Inflammation

8. Give an example of each of the following in the space provided-

Bacterial disease that can prevented due to high body temperature	
Protozoan disease	
Autoimmune disorder	
Cytokine	

1.11 ANSWERS

Self Assessment Questions

- 1.
 - a) Lady Mary Wortley Montagu
 - b) Elie Metchnikoff
- 2.
 - a) False- only mature in thymus
 - b) True

- c) False- ClassII MHC
- d) Cell Mediated Immunity
- 3. a) Rheumatoid arthritis
- b) Immunological tolerance.
- 4. a) Exotoxin
- b) Secondary lymphoid organs (spleen and bone marrow)
- c) *Ascaris*
- d) LPS (lipopolysaccharide).

Terminal Questions

- 1. a) *Neils Jerne, F.Macfarlane Burnet and David Talmadge.*
- b) 2,5- oligo-adenylate-synthetase.
- c) Virions
- 2. a) Antibody dependent cell mediated cytotoxicity
- b) Membrane attack complex
- 3. The various steps in the establishment of bacterial infection are-
 - Invasion by bacteria
 - Attachment of bacteria to host cells
 - Bacterial proliferation
 - Invasion of tissues of the host
 - Damage to host tissues via various toxins.
- 4. a) False. It is humoral response
- b) True
- c) False. It creates an antiviral state
- d) True
- 5. a) Epstein Barr virus
- b) Phagocytic cells
- c) *Schistosoma*
- d) Autoimmune disease
- 6. i) c, ii) d, iii) b, iv) a.

7. a) The ability of T and B cells to ignore or tolerate self-antigens is called as Immunological tolerance.
- b) An **autoimmune disease** occurs when the body attacks its own self-cells tissues and other body constituents.
- c) Phagocytic cells engulf, break down and kill the pathogens. Examples are macrophages, neutrophils, monocytes
- d) In inflammation there is leakage of fluid from blood vessels due to vasodilation and increase in permeability. There is an influx of cells to the area of pathogen invasion/damage.
- 8.

Bacterial disease that can be prevented due to high body temperature	Anthrax
Protozoan disease	Malaria by <i>Plasmodium</i>
Autoimmune disorder	Rheumatoid arthritis

UNIT 2

COMPONENTS OF IMMUNE SYSTEM-I

Structure

- | | |
|---|--|
| 2.1. Introduction | 2.4. Complement System |
| Objectives | Functions of the Complement System |
| 2.2. Innate Immunity | The Classical Pathway |
| Anatomical Barriers | The Formation of Membrane Attack Complex |
| Physiological Barriers | The Lectin Pathway |
| Endocytic and Phagocytic Barrier | The Alternative Pathway |
| Inflammatory Barrier | 2.5. Phagocytosis |
| 2.3. Pattern Recognition | 2.6. Inflammation |
| PAMPs- Pathogen Associated Molecular Patterns | Types of Inflammation |
| PRRs- Pattern Recognition Receptors | 2.7. Summary |
| Toll Like Receptors | 2.8. Terminal questions |
| Natural Killer Cells | 2.9. Answers |

2.1 INTRODUCTION

This unit aims to provide you with a picture of the non-specific pathogen evasion mechanisms in our body. The innate immunity which is present from birth is a natural defence system of our body that protects us from a variety of pathogens on a daily basis. Most pathogen groups share similar molecular patterns that can be recognised as foreign and an immediate response can be mounted. As the pathogen enters the body, it is faced with many barriers like the phagocytic and endocytic barriers, wherein the pathogen is engulfed and lysed. The inflammatory response is a cascade of events that is triggered by tissue damage due to a wound or pathogen invasion. This phenomenon serves to remove the damaged cell, prevent further spread of the infection and also promotes the healing process. You shall also come to know about the Complement system that includes inactive proteins and zymogens circulating in the blood, which are activated by several mechanisms on encountering the pathogen.

Objectives

After going through this unit, you shall be able to-

- ❖ define innate immunity
- ❖ list the various innate immunity barriers,
- ❖ describe pattern recognition, Pathogens Associated Molecular Patterns and the receptors involved in this process,
- ❖ define Toll like Receptors,
- ❖ discuss the functions of NK cells,
- ❖ define complement system and list its functions,
- ❖ explain various complement activation pathways,
- ❖ explain the mechanism of phagocytosis using flowchart,
- ❖ explain inflammation and list its types, and
- ❖ describe the mechanism of inflammation.

2.2 INNATE IMMUNITY

It is the first, non-specific line of defense of the body present from the time of birth. The innate immunity components are present before the initiation of infection. **Innate immunity is not specific for a particular pathogen but in general increases the ability of the body to recognise commonly found molecules on the surface of pathogens.** Fig 2.1 depicts the various types of barriers viz. anatomical, inflammatory, physiological and phagocytic which provide immunity to the humans.

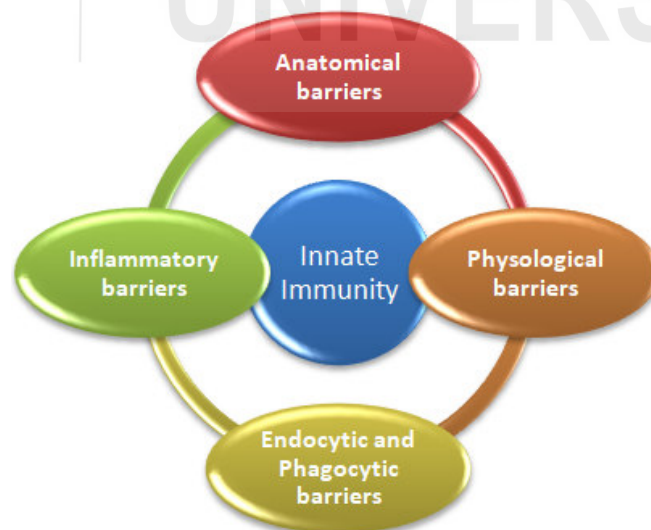


Fig: 2.1: Various barriers that provide immunity.

2.2.1 Anatomical Barriers

It includes skin and mucous membranes of the body. It acts as a primary physical barrier that prevents the entry of microorganisms.

1. **Skin:** The skin of the body is the largest organ and thus serves as a primary barrier. It has two layers- Epidermis and Dermis.

Epidermis is the thin outer layer made of dead cells and has keratin deposition, while the dermis is thicker and has connective tissue, blood vessels and sebaceous and sweat glands. The sebum produced has a low pH around 3-5 and thus effectively prevents the growth of microorganisms.

However, the pathogens may breach the skin via cuts, wounds and other aberrations.

2. **Mucous membranes:** The mucous membranes line the conjunctiva, respiratory, gastro-intestinal and the urogenital tracts of the body. It has a number of defence elements like saliva, tears and mucous secretions that wash off pathogens and also have antimicrobial activity, i.e. kill the pathogens.

Mucous is the viscous fluid secreted by the epithelial cells of the mucous membranes that serves to trap the pathogens.

2.2.2 Physiological Barriers

Include temperature, pH and other soluble and cell associated molecules.

1. **Temperature:** The normal body temperature discourages the growth of most pathogens. For example the high body temperature of chickens provides them immunity for anthrax.
2. **pH:** Microorganisms are very sensitive to pH changes and each microbe has its optimum pH of growth.

The highly acidic pH of the stomach inhibits the growth of most microorganisms. Also the low pH of sebum discourages microbial growth.

3. **Soluble and cell associated molecules:** Various molecules circulate in the body that serve as antimicrobial agents. These molecules and their activity are given in Table 2.1.

Table 2.1: Activity of various antimicrobial agents.

Sr. No.	Molecule	Activity
1.	Lysozyme	Found in tears, saliva and other mucous membrane secretions. It cleaves peptidoglycan of bacterial cell walls and thus eliminates pathogens.
2.	Interferon	Creates an antiviral state in the body.
3.	Complement	Circulating inactive proteins that may be activated by formation of antigen-antibody complexes or by reactions between complement proteins and cell wall components of pathogen.

2.2.3 Phagocytic and Endocytic Barriers

This serves as the next line of defense for those pathogens that have managed to cross the epithelial cell barriers. Endocytosis is the uptake or engulfment of foreign material by the cell, as carried out in *Amoeba*. Phagocytosis is a type of endocytosis carried out by macrophages, neutrophils, dendritic cells and monocytes.

2.2.4 Inflammatory Barriers

It comes into play when tissue damage occurs due to a wound or in events of pathogen invasion. It is a cascade of events. It is one of the earliest responses to infection or irritation. This is stimulated by factors secreted by damaged cells and helps to prevent spread of infection.

Thus we conclude that anatomical and physiological barriers form the **physical barriers** while phagocytic and inflammatory barriers form the **chemical barriers**.

SAQ 1

Fill in the blanks:

- The line the conjunctiva, respiratory, gastro-intestinal and the urogenital tracts of the body.
 - The antimicrobial substance in tear and saliva is
 - Physical barriers include and Barriers.
 - of sebum discourages microbial growth.
 - Lysozyme cleaves of bacterial cell wall.
-

2.3 PATTERN RECOGNITION

It is the ability of the immune system to recognise a class of molecules that are uniquely found in microorganisms and never in multicellular hosts. The innate immune system is capable of recognising such molecules and fight with the invading pathogens based on it.

The molecules capable of pattern recognition can be soluble or cell associated receptors. The complement system involves circulating soluble molecules capable of identifying microbial patterns. In the following subsections, you will study about various types of receptors.

2.3.1 PAMPs- Pathogen Associated Molecular Patterns

They are sugar, protein or nucleic acid motifs present in multiple copies on the surface of bacteria, fungi, viruses and other parasites. They help in the identification of pathogen by the immune system.

2.3.2 PRRs- Pattern Recognition Receptors

These are the host receptors that recognise the Pathogen Associated Molecular Patterns. Some PRRs bind the microbe PAMPs like mannans, lipopolysaccharides and peptidoglycan and induce phagocytosis.

PRRs can be found on plasma membrane or in endolysosomes and in cytosol. This facilitates identification of both intracellular and extracellular pathogens. Example of PRRs are:

1. LLRs- L Type Lectin Receptors

- Mannose Receptor binds to mannans present on bacterial, fungal and parasitic surfaces.
- Dectin-1 binds to β - glucans present on fungal cells.

2. Scavenger Receptors

- SR-A receptor binds to lipopolysaccharides (LPS), lipoteichoic acid (LTA).

Mannans are polysaccharides that are linear polymers of the sugar mannose. Plant mannans have β 1-4 linkages. Mannan may also refer to cell wall polysaccharide found in yeasts.

2.3.3 Toll Like Receptors(TLRs)

The first Toll protein was reported in the fruit fly *Drosophila melanogaster* by Christiane Nüsslein-Volhard and Eric Wieschaus in 1985 and since then 10 TLRs(TLR1–TLR10) have been discovered in humans and 13 (TLR1–TLR13) in mouse.

TLR (Toll-like receptors) are a class of pattern recognition receptors (PRRs) that sense conserved molecular patterns for early recognition of pathogen by initiating innate immune response. They detect a wide variety of patterns associated with pathogens like viruses, bacteria, fungi and parasites as well as damaged tissues.

TLRs are trans membrane proteins (Type I) having three structural domains (Fig 2.2):

1. LRRs -Leucine-rich repeats motif. It helps in the recognition of pathogen.
2. A trans membrane domain.
3. Cytoplasmic Toll/IL-1 receptor (TIR) domain- It initiates signalling by interacting with signal transduction adaptors.

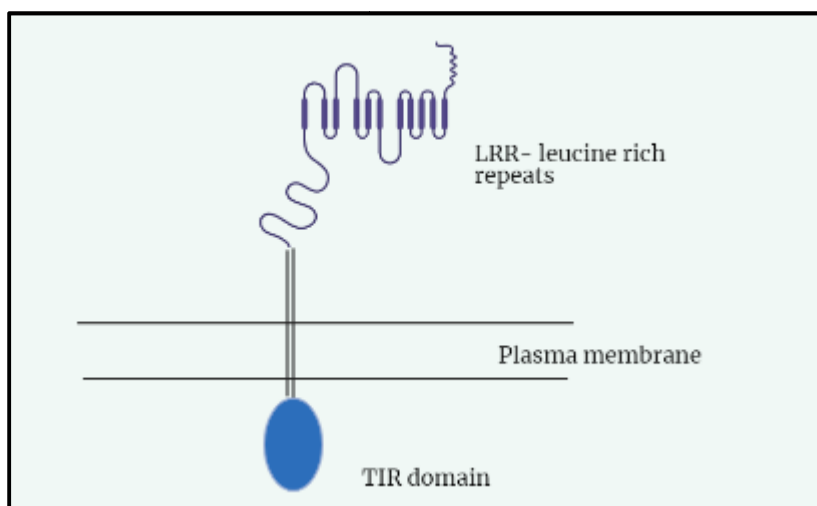


Fig 2.2: The structure of Toll Like Receptors.

Most mammalian species contain the same set of TLR homologs but some exceptions do exist.

They are found widely on leucocyte membranes, on antigen presenting cells like dendritic cells and macrophages, on Natural killer (NK) cells (you will study about NK cells in detail in the following subsection), T cells and B cells (cells of adaptive immunity) and even on normal cells like fibroblasts, epithelial and endothelial cells.

Upon activation, TLRs stimulate the antigen-induced signal transduction pathway by recruiting adaptor proteins in cytosol of the immune cell. Adaptor proteins serve to mediate other protein-protein interactions and then cause the activation of other downstream proteins, like protein kinases (TBK1, IKKi). These downstream proteins further amplify the signal and finally lead to the suppression or upregulation of the genes that carry out the inflammatory responses and other related transcriptional events. Often these events eventually lead to cytokine production and proliferation of cells or enhanced adaptive immunity.

If the TLR identifies a factor on bacteria, it is phagocytosed, digested and the antigen are presented to CD4⁺ T cells. The infected cell may shut down its growth and protein synthesis activity and undergo apoptosis (programmed cell death) if a viral PAMP is identified.

Table 2.2 given below gives the list of a few important TLRs.

Table 2.2: Important Toll Like Receptors (TLRs).

Sr. No.	Toll Like Receptor	Location	Ligand identified
1.	TLR1	Cell surface	Triacylated lipopeptides
2.	TLR2	Cell surface	proteolipids, glycolipids, lipoteichoic acid, zymosan
3.	TLR3	Cell compartment (endosome)	Double stranded RNA
4.	TLR4	Cell surface	Lipopolysaccharide, lipoteichoic acid, heparin sulphate fragments, mannans
5.	TLR5	Cell surface	Bacterial flagellin, profilin

Profilin is an actin binding protein involved in the dynamic turnover and reconstruction of the actin cytoskeleton. It is found in all eucaryotic organisms in most cells.

Virion is an entire virus particle consisting of an outer protein shell (capsid) and inner core of nucleic acid (RNA or DNA)..

2.3.4 Natural Killer cells (NK cells)

NK cells are large granular lymphocytes that are naturally cytotoxic to tumor cells even in the absence of previous immunisation to the tumor. It became clearer later that NK cells are a separate lymphocyte lineage with roles like cytotoxicity and cytokine-producing effector functions. They comprise around 5-10% of the lymphocytes in the body.

They play a crucial role in killing of virus infected cells in the body, graft rejection mechanisms, controlling early signs of cancer and are also found in the placenta during pregnancy.

They contain granules in the cytoplasm that contain lytic enzymes like granzymes and perforins. When such molecules are released in proximity to the infected cell, perforins create pores in the plasma membrane and allow entry of granzymes and other enzymes, thus the cell undergoes apoptosis. Apoptosis is required because lysis of the cell would release the virions and further infect more cells, whereas apoptosis would terminate the virus within the cell itself.

Cytokines like IFN γ and TNF α are also secreted by NK cells. These cytokines act on macrophages and dendritic cells that further enhance the immune response.

NK cells constantly come in contact with many cells and act as surveillance system. Activating and inhibitory receptors are present on the surface of NK cells and a balance of signals from these receptors decides whether or not a cell will be killed by NK cell. Molecules expressed on the surface of infected cells and cancer cells provide positive signals to activating receptors and therefore activate the NK cells. The killing activity of NK cells is checked by signals from inhibitory receptors. Class I MHC molecules are expressed on most normal and healthy cells of the body and these cells are recognised as 'self'. These MHC I molecules are recognised by inhibitory receptors on NK cells that inactivate the NK cells and prevent them from killing any healthy cell. Often infected and cancerous cells lose the MHC I molecules and thus their self-tag. This makes these cells prone and susceptible to killing by NK cells. Perforins and granzymes are released once a cell is marked to be killed by NK cell. This function is important as most immune cells like T-lymphocyte cells are not capable of recognising and eliminating cells with missing MHC class I molecules.

The presence of CD56 and the absence of CD3 (CD56+, CD3-) help in the identification of NK cells.

Functions of NK cells

1. Apoptosis via Cytolytic granules

They contain granules in the cytoplasm that contain lytic enzymes like granzymes and perforins. When such molecules are released in proximity to the infected cell, perforins (cytoplasmic toxic granules) create pores in the plasma membrane and allow entry of granzymes and other enzymes, thus the cell undergoes apoptosis. Granzymes are serine-proteases that are stored as granules that depend on perforins for delivery to target site. They effectively induce apoptosis in cooperation with perforins.

2. Antibody dependent cell-mediated cytotoxicity (ADCC)

It involves the killing of target tumor or infected cell by NK cells with the help of recognition of antitumor/antiviral antibodies produced in response to the antigen on the target cell. The flowchart (Fig 2.3) and (Fig 2.4) depict this process.

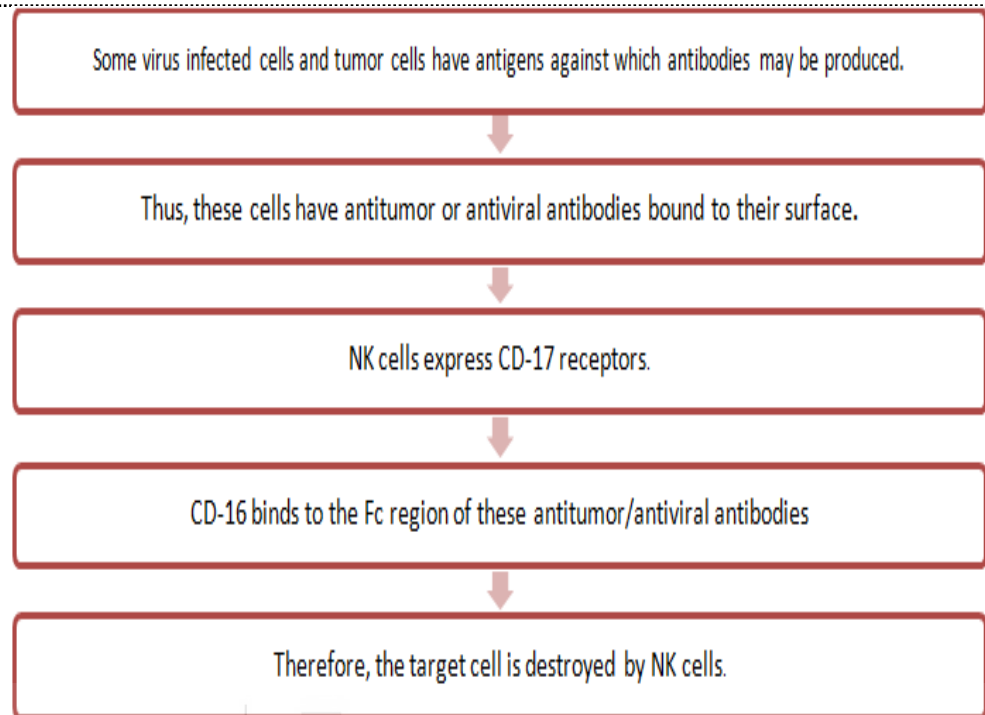


Fig 2.3: Flow chart to depict functions of NK cells.

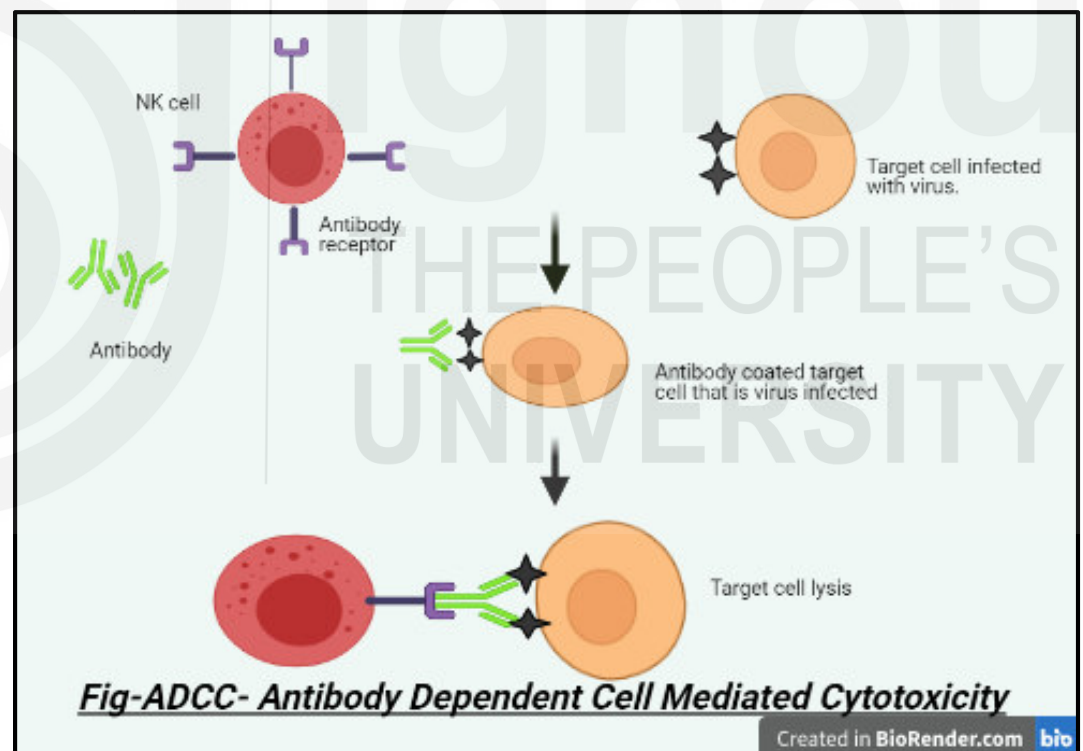


Fig 2.4: Cell killing by ADCC.

3. Surveillance for presence of tumor cells.

NK cells are considered as part of innate immunity since NK cells lack antigen-specific receptors and even target cells without prior exposure to the antigen. This is important as T-cells and other immune cells are unable to recognise cells in the absence of antigens. Once, NK cells recognise tumor cells, cytokines like Tumor Necrosis Factor- α (TNF- α) and Interferon γ (INF- γ) are released to enhance immune response. Before proceeding further, try to attempt the following SAQ.

SAQ 2

- a) State whether the following statements are True or False.
- PAMPs are proteins present in multiple copies on the surface of pathogen.
 - Nucleic acid cannot be recognised by any PRR.
 - TLR is a pattern recognition receptor.
- b) Match the following

Column A	Column B
a) TLR3	i) PRR
b) Scavenger receptor	ii) NK cells
c) Apoptosis via cytolytic granules	iii) Single stranded RNA

2.4 COMPLEMENT SYSTEM

The term “*complement*” was coined by Paul Ehrlich for the activity of blood serum that serves to complete the action of antibodies.

Complement system are group of soluble and cell bound proteins mostly glycoproteins that are synthesized by liver hepatocytes, gastro-intestinal, genitourinary tract epithelial cells, monocytes and macrophages. They circulate in the serum as inactive proenzymes (also called zymogens) and post proteolytic cleavage of inhibitory fragment, the active site is exposed and they change into active molecules. Thus, the complement system or the complement cascade involves a part of immune system that helps antibodies and various immune cells to defend the body against pathogens by promoting inflammation and attacking pathogen's cell membrane.

2.4.1 Functions of complement system

Complement system performs the following functions:

- Causes lysis of cells, pathogens like bacteria and viruses.
- Promotes phagocytosis of various particulate antigens via opsonisation.
- Complement receptors bind to many cells of the immune system. This triggers specific cell functions, causes inflammation and subsequent release of immune- regulatory substances.
- It also helps in the clearance of immune complexes from the body.

There are three established pathways of complement activation namely-

1. The Classical pathway
2. The Alternative pathway
3. The Lectin pathway.

2.4.2 The Classical Pathway

This pathway begins by the formation of a soluble antigen complex with antibody or by the binding of an antibody to an antigen present on a target, for example a bacterium.

It is important to note here that there exists a *C1 macromolecular complex* that is involved in the initiation step (Fig 2.5).

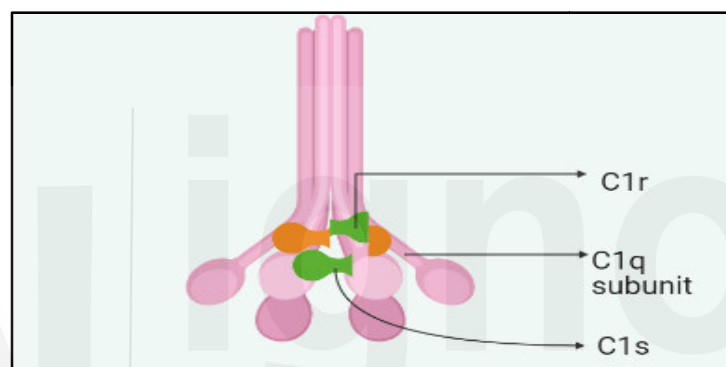


Fig 2.5: The structure of C1 macromolecular complex.

The C1 macromolecular complex has three elements- 6 units of C1q, 2 units of C1r and 2 units of C1s. This C1 complex binds by its globular heads to at least 2 Fc sites on the antibody for stable C1 complex-antibody interaction (Fig. 2.6 and Fig. 2.7).

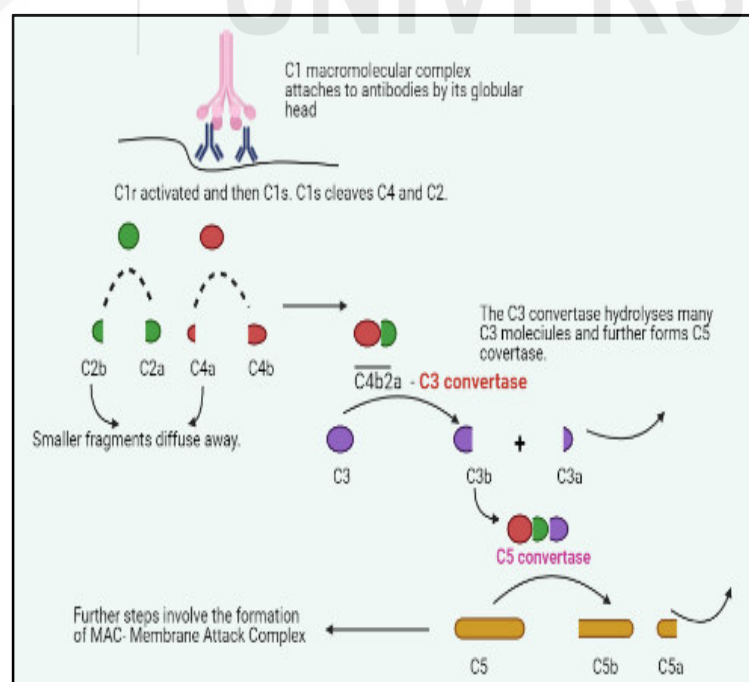


Fig 2.6: The Classical Pathway of Complement Activation.

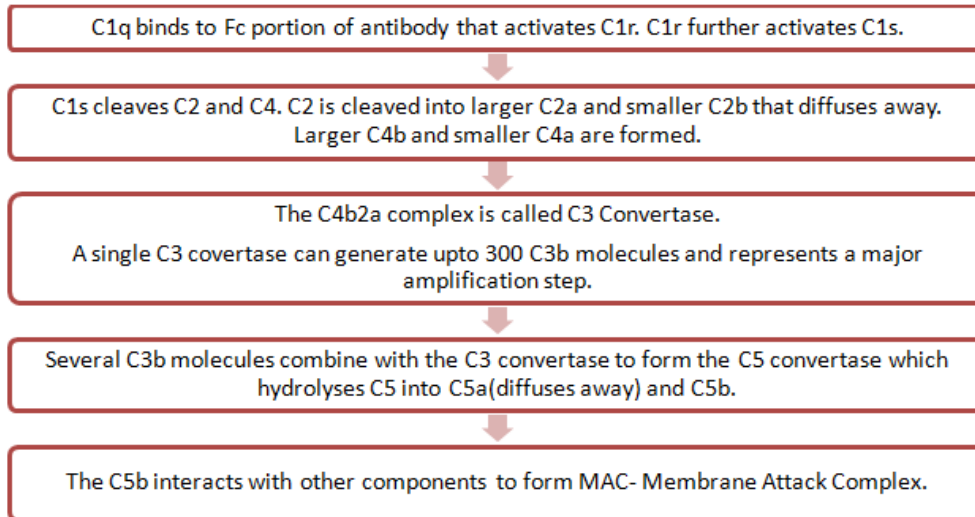


Fig 2.7: Flowchart representing the Classical Pathway.

2.4.3 The Formation of Membrane Attack Complex (MAC)

C5b, C6, C7, C8 and C9 proteins interact to form the MAC. The MAC forms a large pore in the plasma membrane of the target cells that leads to free diffusion of ions.

The C5b formed by cleavage via C5 convertase provides an attachment site for the other components of MAC. C5b is unstable and requires the binding of C6 for stabilisation. Further C5b6 binds to C7 and the complex now has hydrophobic areas exposed that would allow the insertion in the plasma membrane of the target cell.

C5b67 attaches to C8 and the C5b67-C8 complex attached to cells and creates a pore of 10angstrom diameter. But this is just sufficient to lyse red blood cells but not pathogens. Thus, the involvement of C9 is crucial. About 10-17 molecules of perforin like C9 molecules form a complex capable of insertion in pathogen membranes.

Therefore, the completed Membrane Attack Complex consists of C5b67-C8 complex surrounded by a perforin like poly C9 complex. The MAC is capable of killing a variety of cells including Red Blood cells, nucleated cells, bacteria, viruses and parasites.

2.4.4 The Lectin Pathway (Mannan Binding Lectin Pathway)

The Lectin pathway is part of the innate immune system as it does not need the formation of antigen-antibody complex for its activation. Pathogens like *Salmonella*, *Candida albicans*, *Listeria* have mannose residues on the carbohydrates or glycoproteins present on their surface. These mannose residues bind to mannose- binding lectin (MBL) and thus lead to the activation of the Lectin pathway.

Mannose Binding Lectin (MBL) is produced in inflammation as it is an acute phase protein. MASP1 and MASP2 (Mannose associated serine- proteases) bind to MBL and the complex so formed leads to the cleavage of C2 and C4 proteins (Fig 2.8).

The further steps are similar to the Classical pathway of complement activation, which you have studied in subsec. 2.4.2

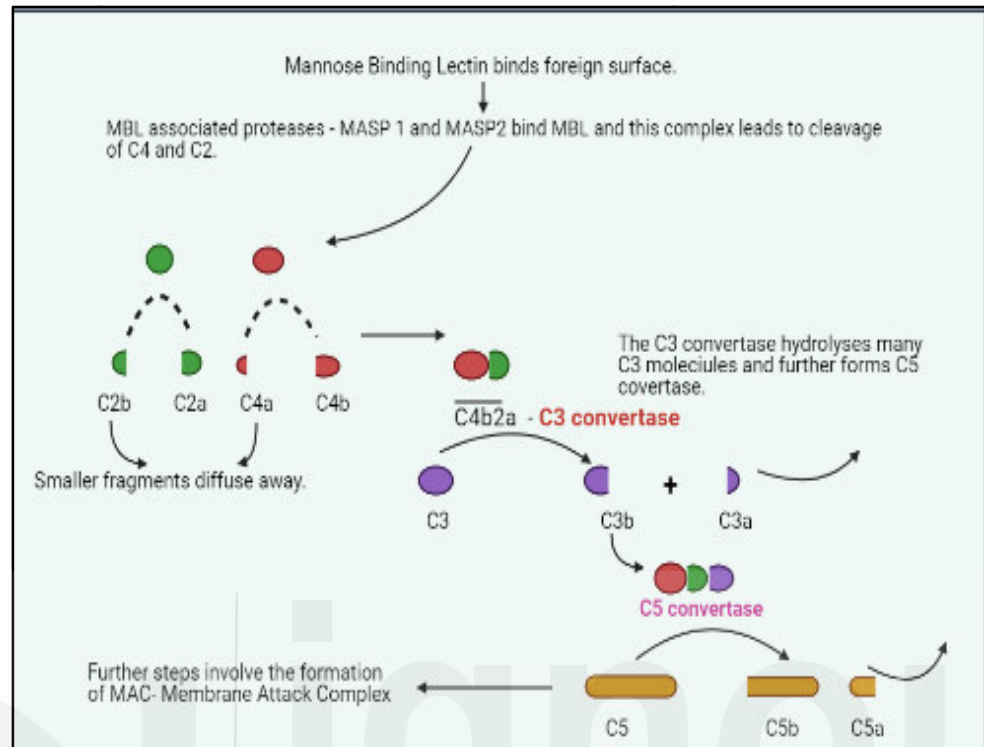


Fig 2.8: The Lectin Pathway.

2.4.5 The Alternative Pathway

The alternative pathway does not need the formation of antigen-antibody complex for its activation. It is initiated by molecules present on pathogen that are foreign to the host. Alternative pathway can be activated by cell wall components from both Gram positive and negative bacteria. Therefore, it is often considered as part of innate immune system. Figs. 2.9 and 2.10 depict the mechanism of Alternative Pathway.

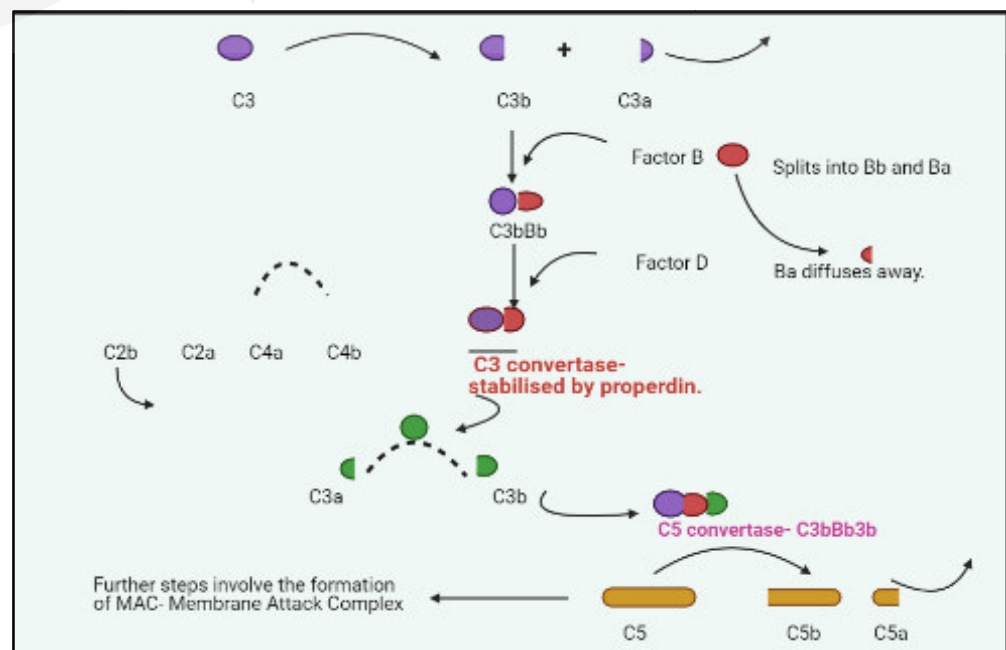


Fig 2.9: The Alternative Pathway.

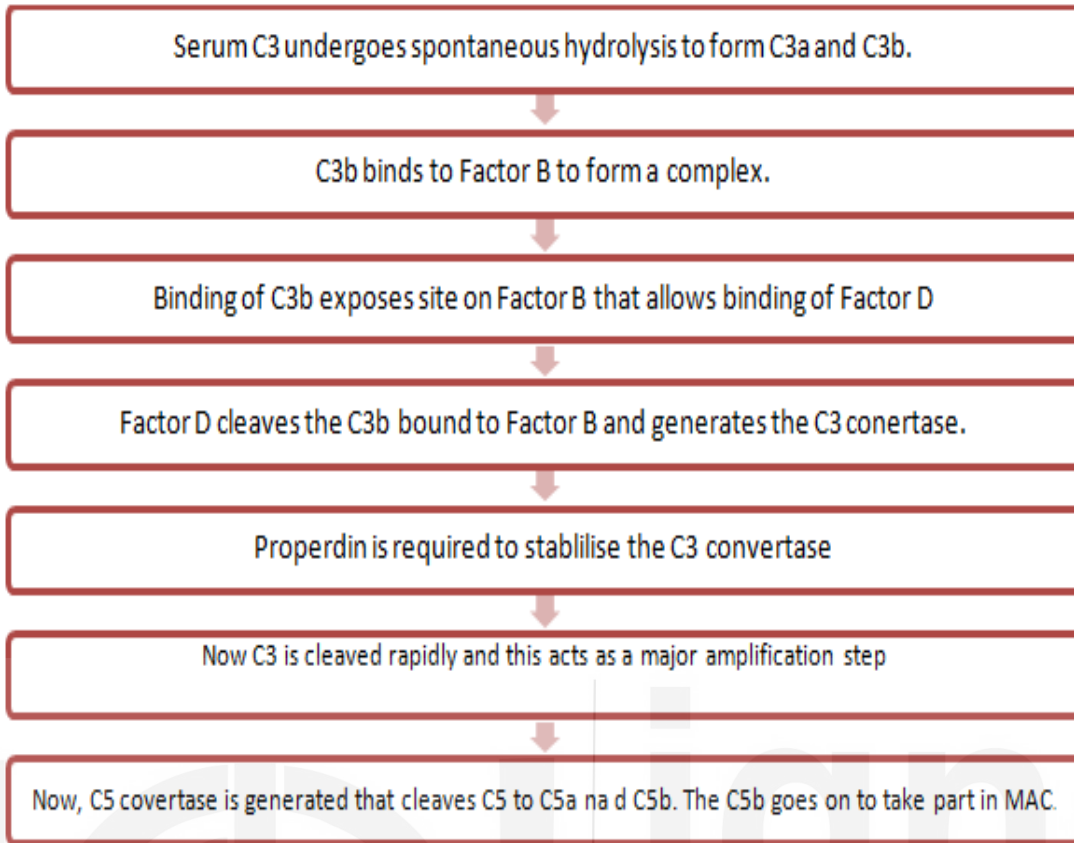


Fig 2.10: Flowchart representing the Alternative Pathway.

SAQ 3

State true or false

- The alternative pathway is a part of the innate immune system as it does not need the formation of antigen-antibody complex for its activation.
- The MAC forms a large pore in the plasma membrane of the target cells that leads to cell division disruption.
- Promotes phagocytosis of various particulate antigens via opsonisation.

SAQ 4

Fill in the blanks

- The term “*complement*” was coined by
- Binding of C3b exposes site on that allows binding of Factor D.
- The C1 macromolecular complex has three elements- 6 units of, 2 units of and 2 units of
- MASP1 and MASP2 (Mannose associated serine- proteases) bind to and the complex so formed leads to the cleavage of C2 and C4 proteins in the Lectin pathway.

2.5 PHAGOCYTOSIS

Cells internalise solid matter like pathogens by a special type of endocytosis called as Phagocytosis. Many cells of the body are capable of performing this task but it is best accomplished by cells called Professional antigen presenting cells that include macrophages, immature dendritic cells and neutrophils. Fig 2.11 and Fig 2.12 depicts the process of phagocytosis.

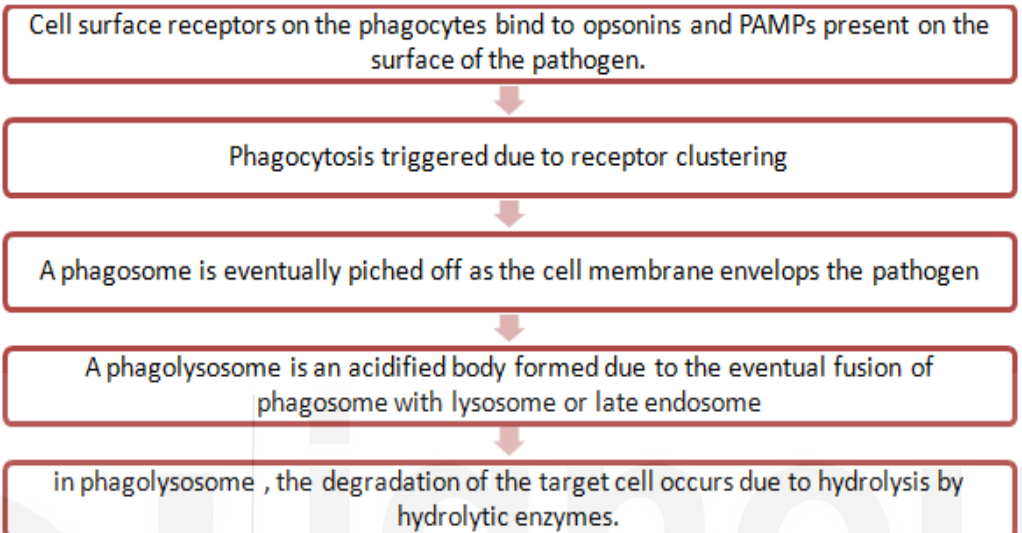


Fig 2.11: Flowchart representing the process of Phagocytosis.

Phagocytosis is an important part of the innate immunity as it does not require antigen specificity and also serves to activate the adaptive immune response. It not only helps to contain and kill pathogen but also in processing antigens for further immune processes.

A number of receptors are involved in the phagocytosis process. This includes the complement receptors and the Fc receptors that serve to help in the phagocytosis of the opsonised pathogen. Other receptors like Toll like Receptors, Lectin and Scavenger receptors are also important in pathogen uptake.

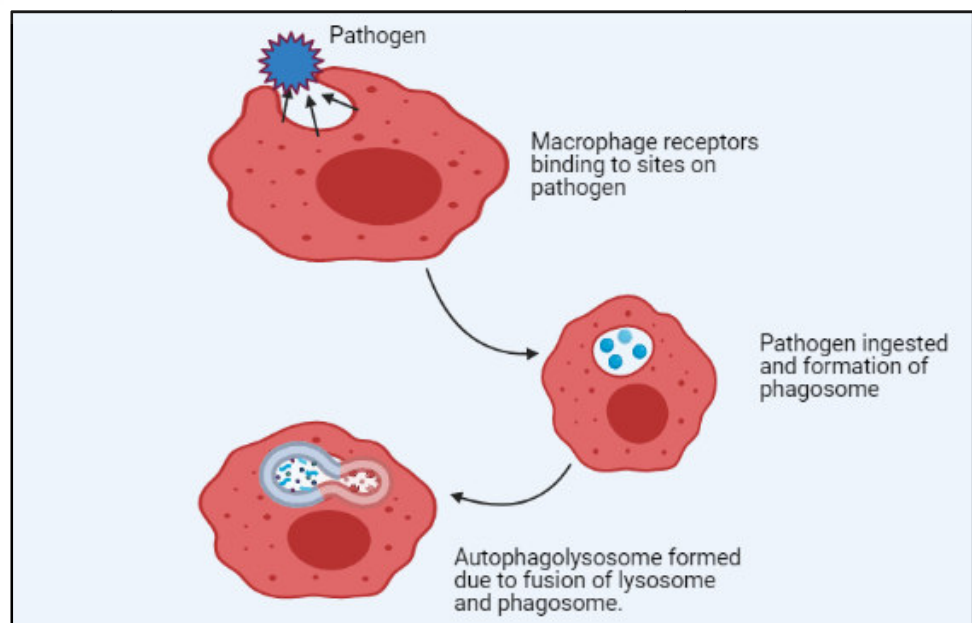


Fig 2.12: Process of Phagocytosis.

2.6 INFLAMMATION

The inflammatory response is a cascade of events that is triggered by damaged tissue due to a wound or pathogen invasion. It is a protective response of the body that involves several elements like immune cells, blood vessels and various molecular mediators. Inflammation is a part of innate immune response as it does not involve pathogen specific mechanisms like adaptive immunity but is a rather generalized response.

The inflammation process takes place to eliminate the cause of infection, remove the damaged tissue and also initiate the healing process of the wound or damage. Important signs of inflammation are given in Fig 2.13

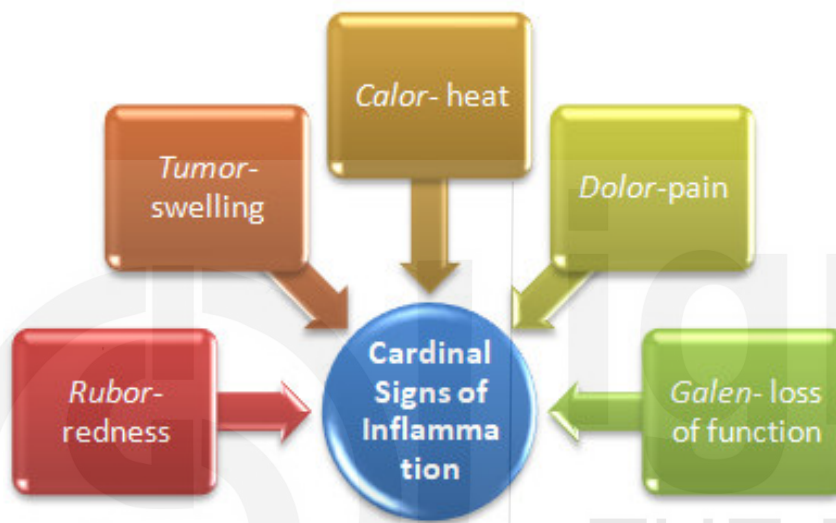


Fig 2.14: The five Cardinal Signs of Inflammation.

2.6.1 Types of Inflammation

There are two types of inflammations.

1. **Acute inflammation** short term (lasts for a few days), triggered immediately after injury of pathogen invasion. Microbial pathogens are recognised by Toll Like receptors (TLRs). Neutrophils and macrophages migrate to the site of inflammation under the influence of cytokines and chemokines.

Acute inflammation is therefore the primary line of defense against pathogen invasion. It is often initiated by resident macrophages, dendritic cells and mast cells that recognise via PRRs, various PAMPs (Pathogen associated molecular patterns) and DAMPs (Damage associated molecular patterns).

Causes of acute inflammation include pathogens, toxins, allergens, burns and frostbites. Sub -acute inflammation lasts longer from 2 to 6 weeks.

2. **Chronic inflammation prolonged, which lasts for months to years.** Macrophages, plasma cells and lymphocytes are the primary cells

involved. Several diseases like allergies, cardiovascular diseases, diabetes and COPD- chronic obstructive pulmonary disease are caused by chronic inflammation.

It is also associated with conditions like hay fever and atherosclerosis. Differences between these two inflammations are provided in Table 2.3.

Table 2.3 Differences between Acute and Chronic inflammation.

Sr. No.	Acute Inflammation	Chronic inflammation
1.	Lasts for a few days. Sub-acute inflammation may last for 2-6 weeks.	Prolonged inflammation- months to years.
2.	Neutrophils are primarily involved	Mainly involves macrophages, lymphocytes and monocytes.
3.	Little or no fibrosis is seen	Marked fibrosis
4.	Immediately occurs post wound/injury	Takes time to develop
5.	Eicosanoids and vasoactive amines are mediators	Involves cytokine growth factors, hydrolytic enzymes and reactive oxygen species
6.	There is eventual healing.	It may lead to fibrosis and tissue destruction.

Now we will discuss about various events that take place during inflammation.

Events in Inflammation process

1. **Vasodilation:** This involves an increase in the diameter and permeability of blood vessels that allows more blood flow at the site of damage or injury. The redness in tissue and increase in temperature at the site of inflammation that begins in the arterioles and eventually leads to engorging of capillaries. The plasma moves out due to the increased permeability of blood vessels that results in larger concentration of cells which enlarge the blood vessels.
2. **Increase in capillary permeability:** This helps in the movement of fluid from enlarged capillaries into the tissue spaces.

There is fluid accumulation that causes oedema.
3. **Influx of phagocytes:** Influx of phagocytes occurs at the inflammation site due to increased permeability of capillaries.

The flowchart illustrates the process of influx of phagocytes (Fig 2.14).

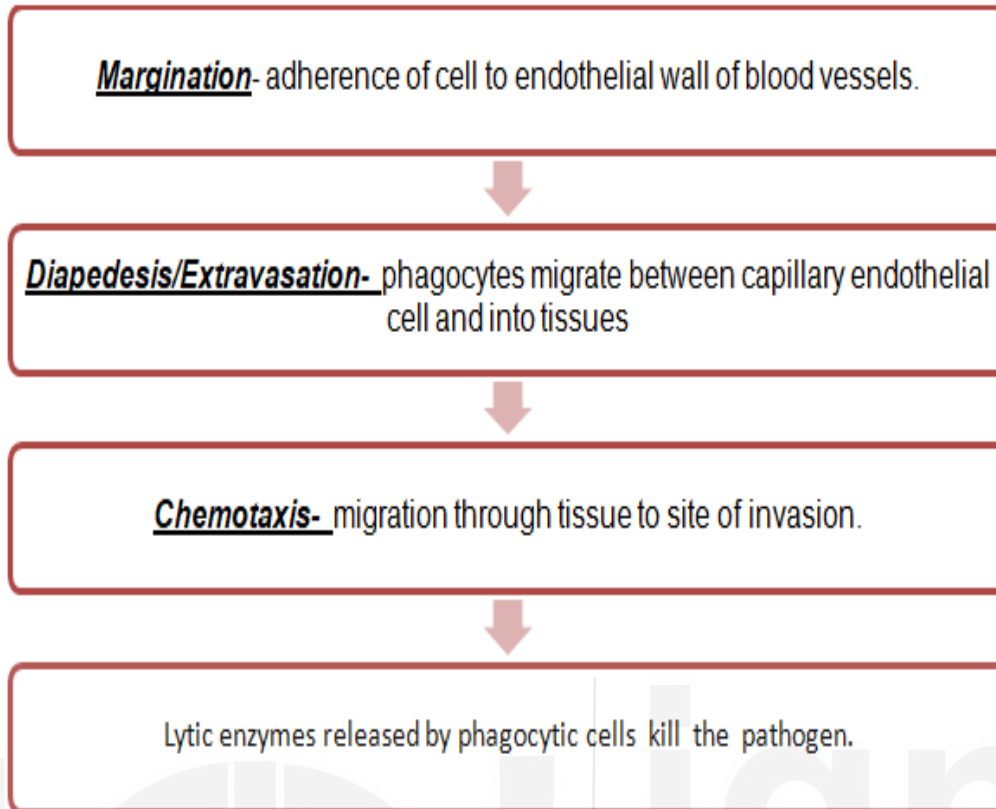


Fig 2.14: Process of Influx of phagocytes.

Chemical Mediators of Inflammation

The chemical mediators of inflammation may be derived from various sources:

- Mediators produced locally by cells at the site of inflammation.
- Mediators sourced from plasma. These are formed in the liver.
- Some mediators are also produced by necrotic cells.

These mediators bind to specific receptors on target cells and bring about their effects. Mediators like lysosomal proteases have direct toxic activity, while some mediators may stimulate the secretion of secondary effector molecules from target cells.

1. Cell Derived Inflammation Mediators

Activated cells (like mast cells) often secrete molecules (like histamine). A variety of molecules may be secreted rapidly by mast cells (Fig 2.15), macrophages and endothelial cells. A few of them have been listed below:

- **Histamine:** It is rapidly secreted by mast cells, basophils and platelets and causes increased vascular permeability and arteriolar dilation. This increased permeability allows the influx of leucocytes that are capable of combatting with the pathogen effectively.

Histamine release occurs when an antigen binds to an IgE antibody present on the surface of mast cells or basophils.

It is responsible for itching and pain of the site of inflammation.

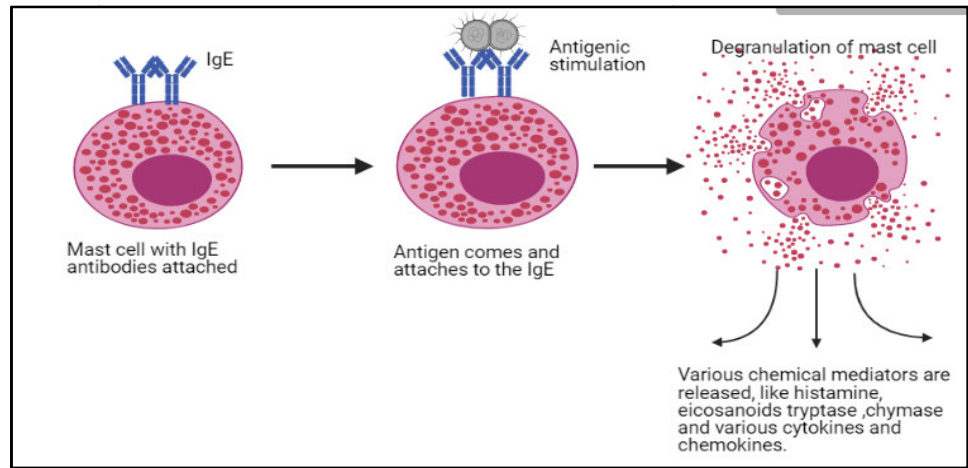


Fig 2.15: Degranulation of Mast cell.

- **Serotonin:** it is a performed mediator with vasoactive roles which is released during platelet aggregation by platelet dense body granules. It plays similar roles than histamine but is less potent.
- **Cytokines:** Activated monocytes and lymphocytes release a variety of polypeptide substances called cytokines. The important cytokines in acute inflammation include Tumor Necrosis Factor (TNF) and Interleukin-1(IL-1) and in chronic inflammation- Interferon- γ (IFN- γ).

The production of TNF and IL-1 is stimulated in response to immune complexes, bacterial endotoxins and products of T lymphocytes.

They are mainly responsible for endothelial activation.

- **Chemokines:** Molecules that serve as chemo-attractants for leucocytes and activate them.

2. Plasma Derived Inflammation Mediators

It includes molecules from three systems, namely- fibrinolytic system, clotting system and the complement. It involves inactive protein molecules that are enzymatically activated by enzymes at the site of inflammation.

- **Hageman Factor:** it is a plasma protein synthesized in the liver that serves as the factor that activates all the three systems.

SAQ 5

Fill in the blanks:

- is main cell involved in acute inflammation.
- The main cells involved in chronic inflammation are, ... and is secreted by mast cells, basophils and platelets and causes increased vascular permeability and arteriolar dilation.

2.7 SUMMARY

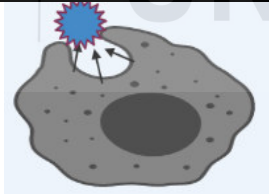
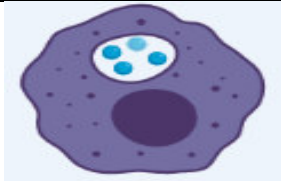
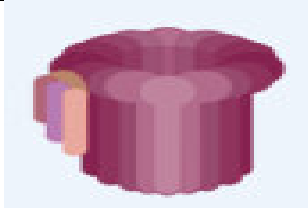
Let us summarise what you have learnt in this unit:

- The innate immunity is the first, non-specific line of defence of the body present from the time of birth whose components are present before the initiation of infection.
- The innate immunity has physical, physiological, phagocytic and inflammation barriers.
- Pattern recognition receptors recognise pathogen associated molecular patterns on pathogens for example- Scavenger receptors.
- Toll like receptors initiate innate immune response by recognising patterns on pathogens.
- NK cells are granular lymphocytes with cytotoxic activity even without prior antigen priming. They have role in killing virus, cancerous cells and in graft rejection.
- Complement system includes circulating serum proteins that are a part of immune system that helps antibodies and other cells to kill the pathogen.
- There are three pathways of complement activation- Classical, Alternative and Lectin pathway. All these pathways are activated differently.
- The Classical pathway of the complement system is activated by the formation of an antigen-antibody complex.
- The Lectin and Alternative pathways are activated by binding of antigenic molecules on pathogen to complement proteins. They do not require the formation of an antigen-antibody complex.
- All the pathways lead eventually to the formation of a Membrane Attack Complex (MAC) that lyses cells by inserting in the plasma membrane of pathogen.
- There are several functions of the complement system like lysis of pathogens, opsonisation, promotion of inflammation and also helps in clearing immune complexes.
- Phagocytosis is a type of endocytosis performed by Antigen Presenting Cells (APCs) through which the pathogen is engulfed.
- Inflammation is a cascade of events triggered due to wound/tissue damage/pathogen invasion. It involves cells, blood vessels and various molecular mediators.
- There are cell derived (e.g.-histamine) and plasma derived mediators (e.g.- Hageman Factor) of inflammation.
- Localised and systemic effects are seen in acute inflammation.

- In allergies, transplants, autoimmune diseases, a chronic inflammatory response is seen.
- Several inflammatory mediators are released due to degranulation of mast cells and activation of macrophages.

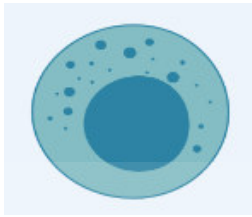
2.8 TERMINAL QUESTIONS

- Fill in the blanks:
 - and are the layers of the skin.
 - The pH of sebum is which is inhibitory to pathogens.
 - protects chickens from anthrax.
 - enzyme is found in tears and saliva and is bacteriolytic.
 - molecules create an antiviral state.
- List the three parts of Toll like Receptors.
- Expand the following terms:
 - PAMPs
 - PRRs
 - DAMPs
 - MBL
- Identify the following diagrams:

Sr. No.	Diagram	Identified as
1.		
2.		
3.		

- List the various steps of inflammation.

6. Explain the mechanism of activation of the Lectin pathway of complement system.
7. Define the following terms:
 - i) Diapedesis
 - ii) Margination
 - iii) Chemotaxis
8. List the various signs of inflammation.
9. Identify the cell given below and write the significance of the granules present.



2.9 ANSWERS

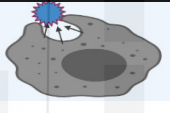
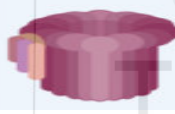
Self Assessment Questions

1.
 - a) Mucosa
 - b) Lysozyme
 - c) Anatomical and physiological
 - d) Low pH
 - e) Peptidoglycan
2.
 - a)
 - i) False – can be nucleic acid, sugars also
 - ii) False, nucleic acid can also be a PAMP
 - iii) True
 - b) a) iii, b) i, c) ii.
3.
 - i) True, ii) False, causes ion loss, ii) True.
4.
 - a) Paul Ehrlich, b) Factor B, c) C1q, C1r, C1s, d) Mannose Binding Lectin.
5.
 - i) Neutrophil
 - ii) Macrophage, lymphocyte and plasma cells
 - iii) Histamine

Terminal Questions

1.
 - a) Dermis and Epidermis, b) 3-5 pH, c) High body temperature, d) Lysozyme, e) Interferons.

2. The three parts of Toll like Receptors are:
 - i) LRRs -leucine-rich repeats motif. It helps in the recognition of pathogen.
 - ii) A trans membrane domain.
 - iii) Cytoplasmic Toll/IL-1 receptor (TIR) domain- It initiates signalling by interacting with signal transduction adaptors.
3.
 - i) Pathogen Associated Molecular Patterns.
 - ii) Pattern Recognition Receptors.
 - iii) Damage Associated Molecular Patterns.
4. Identify the following diagrams:

Sr. No	Diagram	Identified as
1.		Phagocytosis by macrophage
2.		Phagosome
3.		Membrane attack complex

5. The various steps of inflammation are:
 - i) Vasodilation
 - ii) Increase in capillary permeability
6. The Lectin pathway does not need the formation of antigen-antibody complex for its activation. Pathogens like Salmonella, Candida albicans, Listeria have mannose residues on the carbohydrates or glycoproteins present on their surface. These mannose residues bind to mannose-binding lectin (MBL) and thus lead to the activation of the Lectin pathway.
7.
 - i) Margination- adherence of cell to endothelial wall of blood vessels.
 - ii) Diapedesis/Extravasation- Phagocytes migrate between capillary endothelial cell and into tissues.
 - iii) Chemotaxis- Migration through tissue to site of invasion.
8. The various signs of inflammation are:
 - i) Rubor- redness

- ii) Calor- heat
 - iii) Tumor- swelling
 - iv) Dolor-pain
 - v) Galen- loss of function.
9. The cell is a Natural Killer (NK cell). The granules denote perforin and granzymes that help the NK cell to lyse the target cell. When such molecules are released in proximity to the infected cell, perforins create pores in the plasma membrane and allow entry of granzymes and other enzymes, thus the cell undergoes apoptosis.



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COMPONENTS OF IMMUNE SYSTEM-II

Structure

- | | |
|--|--|
| 3.1. Introduction | 3.8. Primary and Secondary Immune Response |
| Objectives | Primary Immune Response |
| 3.2. Adaptive Immunity | The Secondary Immune Response |
| Four Characteristics of Adaptive Immunity Response | 3.9. How do the Innate and Adaptive Immune Systems Work Together? |
| 3.3. B Lymphocytes | Natural Killer Cells-The Bridge between Innate and Adaptive Immune Systems |
| 3.4. T Lymphocytes | 3.10. Acquired Immunity |
| 3.5. Cytokines | Naturally Acquired Immunity |
| Different Features of Cytokines | Artificially Acquired Immunity |
| Nomenclature of Cytokines | 3.11. Summary |
| Functions of some Cytokines | 3.12. Terminal Questions |
| Chemokines | 3.13. Answers |
| 3.6. Humoral Immunity | |
| Antibodies | |
| Steps of Humoral Immunity | |
| 3.7. Cell Mediated Immune Response (CMI) | |

3.1 INTRODUCTION

This unit aims to provide basic concept to the adaptive immune system of the body. The adaptive or acquired immune system comes into action once the pathogen has breached the innate immune system. The innate immune system passes on stimulatory signals to activate the adaptive immunity. We would study about various cells and molecules involved in the adaptive immunity response. B cells on activation produce plasma cells that secrete antibodies and generate memory cells that are dormant cells which are activated on subsequent pathogen exposure. The T cells go on to produce cytotoxic T lymphocytes that are involved in direct killing of the pathogen.

An overview of the humoral and cell mediated immune systems is also provided. The humoral immune response involves various components in the body fluids like antibodies, complements and antimicrobial peptides; while the cell mediated immune system has cell components to neutralize the pathogen. We would also understand the difference between primary and secondary immune responses. Then we would proceed to understand the interrelationship between the innate and adaptive immune systems via NK cells.

Objectives

After studying this unit, you should be able to:

- ❖ gain an insight about the adaptive immune system,
- ❖ differentiate between naturally and artificially acquired immunity,
- ❖ list the various features of adaptive immunity,
- ❖ explain B and T lymphocytes,
- ❖ discuss the role of cytokines and chemokines,
- ❖ give examples of cytokines with their functions,
- ❖ differentiate between humoral and cell-mediated immunity,
- ❖ list the steps of antibody activation,
- ❖ list the features of primary and secondary response, and
- ❖ explain the role of NK cells as a bridge between innate and adaptive immune systems.

3.2 ADAPTIVE IMMUNITY

Foreign pathogens and antigens are selectively recognised and eliminated by the adaptive immune system when the innate immune system fails to defend the body against the pathogen invasion. However, it is capable of responding only when a stimulus is provided by the innate immune system.

The Adaptive or Acquired immune response takes much longer time than the innate immune response to become activated and respond. It can take days or even weeks but adaptive immunity is more pathogen specific and also has memory. Adaptive immune response is generated post exposure to a pathogenic antigen or after a vaccination. The responses are tailor made to challenge specific antigen unlike the innate immune system.

The differences between innate and adaptive immunity are given in Table 3.1.

Table 3.1: Differences between innate and adaptive immunity.

	Lines of defense	Timeline	Cells	Antigen dependency	Example
Innate (Non-specific)	First	Immediate response (0-96 hours)	NK cells, macrophages, neutrophils, dendritic cells, mast cells, basophils, eosinophils.	Independent	Skin, hair, cough, mucous membranes phagocytes, granulocytes.
Adaptive (Specific)	Second	Long term (>96 hours)	T and B lymphocytes	Dependent	Pus, swelling, redness, pain.

3.2.1 Four Characteristics of Adaptive Immune Response

Adaptive immune responses are carried out by white blood cells called lymphocytes. Four major features of adaptive immune response are as follows:

- i) **Antigen specificity:** This allows the immune system to detect even minor differences among antigens. Antibodies can recognize even single amino acid differences among protein molecules.
- ii) **Diversity:** The property of adaptive immune system to generate enormous diversity in the recognition molecules allows it to identify billions of molecules on foreign antigens and pathogens.
- iii) **Immunologic memory:** This attribute of generating memory against specific antigen/ pathogen post primary encounter with the antigen allows providing up to life long immunity to various pathogens due to formation of memory B and T cells after initial exposure.
- iv) **Distinction between self and non-self:** It is critical that the immune system reacts and responds not only to non-self-molecules as response to self-molecules can lead to several fatal conditions like autoimmune disorders.

There are two types of adaptive immunity namely

- a) **Humoral Immune Response** (Antibody Response) mainly involves B cells and antibodies.
- b) **Cell mediated Response** mainly involves T cells.

Before studying Humoral Immune Response and cell mediated response in this unit let us discuss about B and T lymphocytes, cytokines and chemokines in the following section.

3.3 B LYMPHOCYTES

They are lymphocytes that are named so due to their occurrence from the site of maturation in birds called the *Bursa of Fabricius*. In mammals like mice and humans, **B lymphocyte maturation occurs in the bone marrow. They are distinct from other lymphocytes and cells in the respect that they display membrane bound antibody molecules.** These antibody molecules have the same antigen specificity and antigen binding sites.

Several other molecules are found on the B-cell surface (Table 3.2)

Table 3.2: Molecules expressed on B cell surface.

Sr. No	Molecule expressed	Role
1.	B220	Frequently used as marker for precursor B cells and mature B cells.
2.	CR1 and CR2	They are receptors for the complement products to bind.
3.	CD32	Receptor for IgG
4.	MHC Class II molecule	They allow B-cells to act as Antigen Presenting Cells
5.	CD 40	Interacts with the CD40 on helper T cell surface.

Bursa of Fabricius: is a chestnut size sac like lymphoid organ in birds, located dorsal to the rectum, anterior to the sacrum, communicating with the posterior portion of the cloaca. In birds B cells mature here.

The interaction of naive B cells antibodies with antigen, as well as with macrophages and T-cells, causes rapid proliferation of B cells leading to the production of B-cell clones. These cells differentiate into memory B cells and plasma cells (Fig 3.1). **The plasma cells rapidly secrete antibodies while the memory cells act on subsequent exposure with the antigen.**

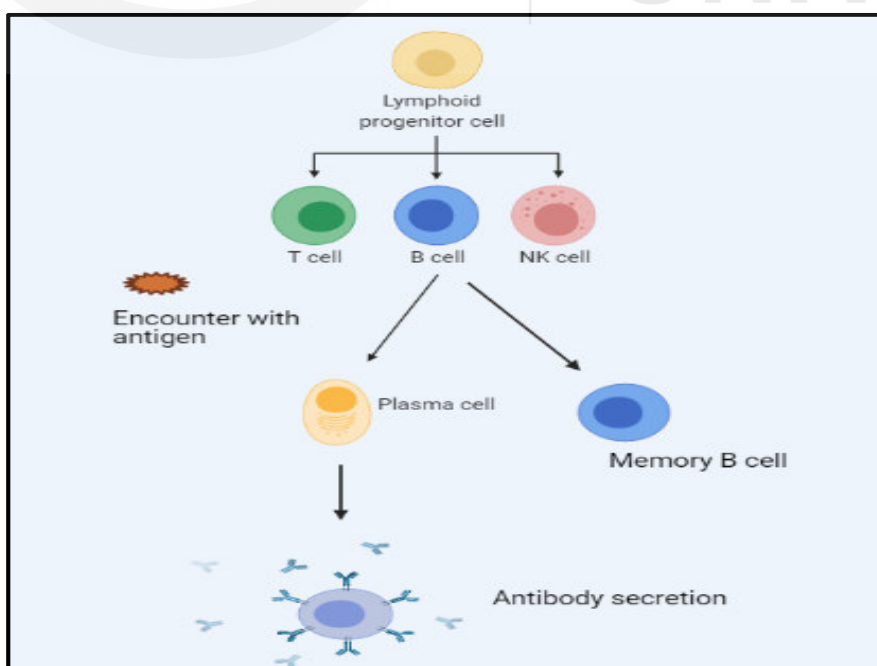


Fig 3.1: Differentiation of B lymphocyte in plasma cells and memory B cell.

3.4 T LYMPHOCYTES

T lymphocytes are cells that mature in the thymus. They have T Cell Receptors (TCRs) that are membrane bound receptors that do not directly recognise the antigen. Most T-cells can recognise antigens only when they are coupled with Major Histocompatibility molecule found on antigen presenting cells like macrophages and dendritic cells and on tumor cells.

Several molecules are displayed on the membrane of T cells.

1. *T-cell Receptor (TCR)*
2. *CD45 and CD28.*

Two distinct subpopulations of T cells can be distinguished based on the expression of CD4 and CD8 molecules:

1. **CD4⁺ cells:** They can recognise antigen bound to class II MHC molecules and usually function as helper T cells (T_H). Activity of T_H cells is depicted in Fig 3.2.
2. **CD8⁺ cells:** They recognise antigen bound to class I MHC molecules and function as cytotoxic T cells (T_C). Fig 3.3 depicts the activity of T_C cells.

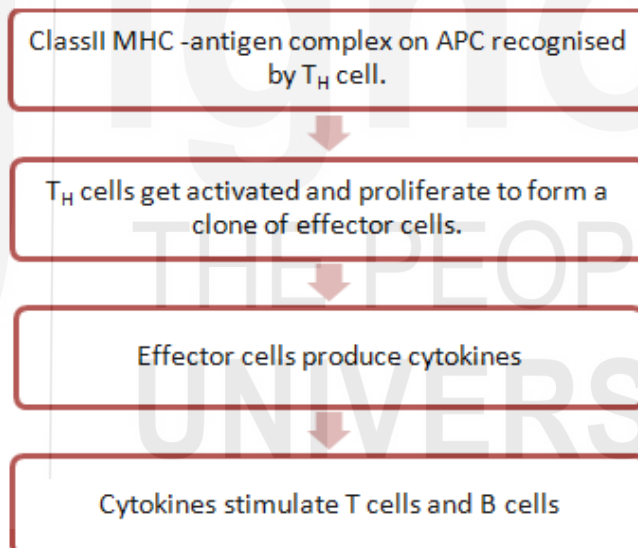


Fig 3.2: Flowchart depicting the activity of helper T-cells (T_H).

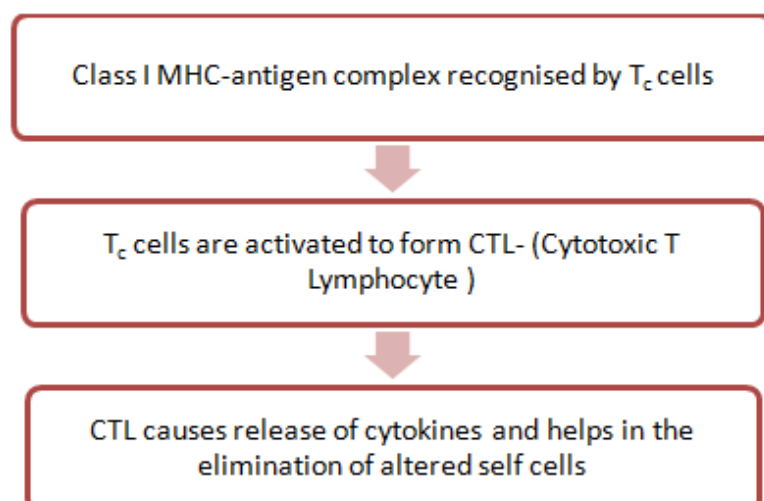


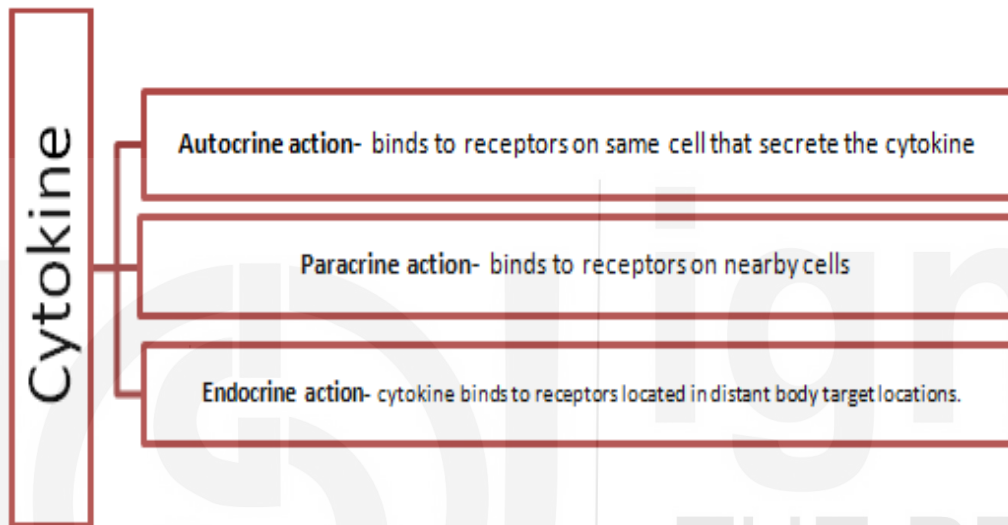
Fig 3.3: Flowchart depicting the activity of cytotoxic T-cells (T_C).

3.5 CYTOKINES

Cytokines are group of proteins secreted by white blood cells and many other cells of the body in response to external stimuli. They are low molecular weight proteins or glycoproteins with regulatory roles.

Cytokines **trigger signal transduction pathways** and **eventually lead to alteration of gene expression** in target cells by **binding to specific receptors located on the target cell**. It is the presence of these receptors that determines whether or not a cell is susceptible of action of a particular cytokine molecule.

The cytokines may have:



Cytokines are involved in the stimulation or inhibiting the activation and proliferation of many cells, regulation of antibody secretion and also the secretion of other cytokines. In this way they are able to regulate the magnitude and duration of the immune response.

The binding of cytokines to the target cells leads to increased expression of cytokine receptors and also causes secretion of more cytokine molecules which go on to affect other target cells. Therefore, even a small amount of cytokines secreted by antigen activated lymphocytes can affect the activity of many immune cells. For example, a plethora of interacting cells like B cells, NK cells, macrophages, T_c cells, granulocytes and HSCs- (Hematopoietic Stem Cells) are activated by the cytokines released by T_H cells.

3.5.1 Different Features of Cytokines

Cytokines exhibit different features as discussed below:

1. **Pleiotropy:** It is the exhibition of different biological effects on different target cells by the same cytokine. For example, IL-4 secreted by activated T_H cells goes on to activate B cell, thymocyte and mast cells.
2. **Redundancy:** It is when the same function is performed by two cytokines. Thus, it becomes difficult to attribute a function to one particular cytokine. For example, IL-2, IL4 and IL-5 secreted by activated T_H cells cause B cell proliferation.

3. **Synergy:** Synergy occurs when the additive function of two cytokines is more than the function of a single cytokine.
4. **Antagonism:** The effect of one cytokine inhibits the function of other cytokine.
5. **Cascade induction:** It is the initiation of a sequence of events where the production of cytokine by a cell activates another cell. Now the activated cell secretes more cytokines which further activate more target cells.

3.5.2 Nomenclature of cytokines

Cytokines are principally named on the basis of the cells that produce them. For example:

- **Lymphokines:** cytokines produced by lymphocytes.
- **Monokines:** cytokines produced by macrophages and monocytes.
- **Interleukins:** cytokines secreted by leukocytes and act on other leukocytes.
- Some cytokines are also called by their common names. For example, tumor necrosis factors.

3.5.3 Functions of some cytokines

Functions performed by some cytokines are as discussed below:

- **TNF- α (tumor necrosis factor- α):** It is synthesized by macrophages and has several functions like: inflammation, formation of acute phase proteins in liver, neutrophil activation and loss of body fat and muscle.
- **Interferon- α :** It is secreted by macrophages. It creates an antiviral state in nucleated cells, activates NK cells and increases the expression of MHC I expression.
- **Interleukin -2:** This is secreted by T-cells and causes B cell proliferation, T cell proliferation and activation of NK cells.
- **Interferon- γ :** This is secreted by CD8⁺ T cells and NK cells. Serves to activate macrophages, stimulates better antigen presentation and increases the overall expression of MHC molecules.

3.5.4 Chemokines

Chemokines are cytokines that act as chemo-attractants. They play an important role in migration of cells between blood and tissues through venules during the process of chemotaxis. (Chemotaxis is the stimulation of cell movement in response to chemical stimuli such as cytokine gradients). Chemokines are also known to influence development of lymphoid organs and T-cell differentiation. They mediate tumour cell metastasis and also function as neuro-modulators in the nervous system.

Chemokine receptors are required for the action of chemokines on the target cells. These receptors belong to G-protein coupled receptors (GPCRs) family and help in initiating intracellular signalling pathways (Fig 3.4).

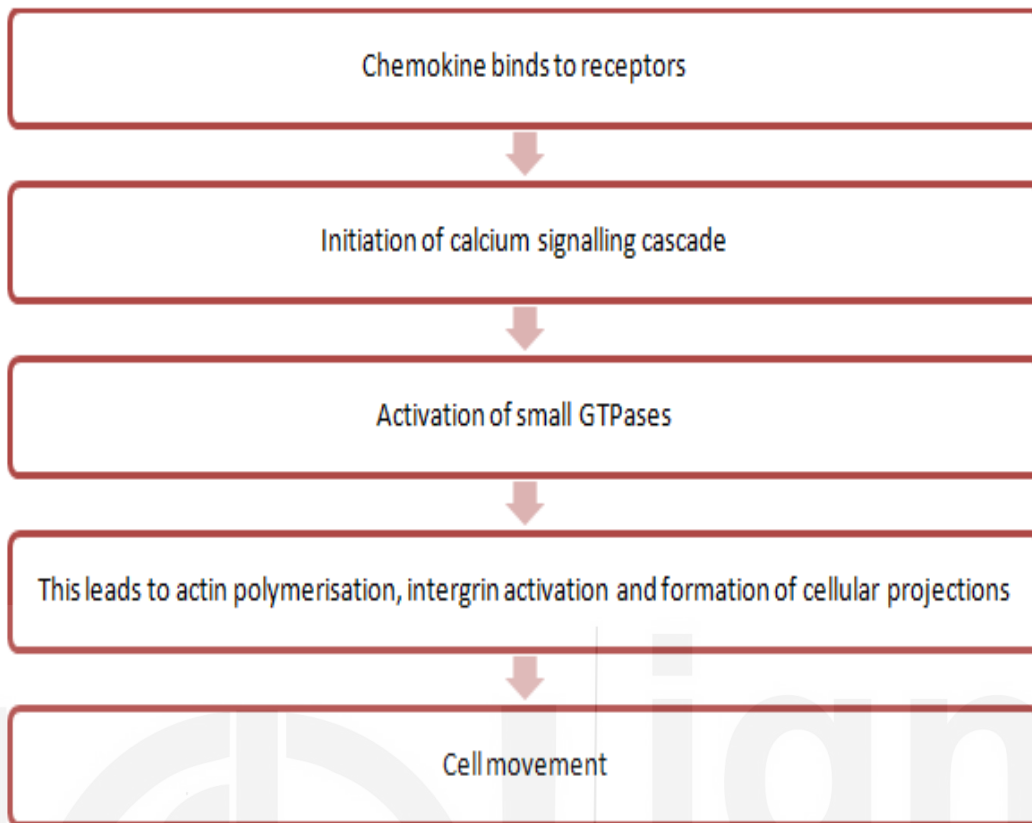


Fig 3.4: Mechanism of action of Chemokines

SAQ 1

State 'True' or 'False':

- B lymphocyte maturation in mammals like mice and humans occurs in the bone marrow.
- Redundancy is not a feature of cytokines.
- TCRs do not directly recognise antigens.

SAQ 2

Match the following items given in Column A with Column B.

Column A	Column B
i) CD45 and CD 28	a) Macrophages
ii) Monokines	b) Receptor for IgG
iii) B-cells	c) Expressed on T-cells
iv) Interferons	d) Bear antibody on surface
v) CD32	e) Antiviral state

3.6 HUMORAL IMMUNITY

Humoral immunity depends on molecules found in the “*humor*”, that is the body fluids. It is mediated by molecules like antibodies, various complement proteins and antimicrobial peptides. Therefore, it is also as **Antibody Mediated Immunity**.

Humoral immunity involves the production of antibodies and simultaneous occurring of events like the activation of helper T-cells and production of cytokines. It is also involved in various effector functions of antibodies like:

- Neutralization of pathogens and toxins.
- Classical pathway of complement activation
- Opsonisation of pathogen and help in phagocytosis.
- Eventual pathogen clearing.

3.6.1 Antibodies

Antibodies are immunoglobulins found in blood, tissue fluids and various secretions of the body. They are globular proteins that are synthesized and produced by plasma cells that are formed by activated B-cells. Five different classes of antibodies are found in the body. They are: **IgG, IgM, IgA, IgE and IgD**. You will study about them in detail in Unit 7 of this course.

Antibodies are used by the adaptive immune system to neutralize various pathogens like viruses and bacteria. The antibodies can bind to specific targets and cause agglutination and precipitation reactions. As a result various immune complexes are formed that can be cleared from the body. Antibodies also promote phagocytosis by various cells like macrophages and activate the complement system.

3.6.2 Steps of Humoral Immunity

Various steps of humoral immunity are discussed below and shown in Fig 3.5.

1. **Production of B-cells:** The production of B cells occurs in bone marrow. They have B-cell receptors (BCRs) displayed on their surfaces which are specific for particular antigens. These mature B-cells that go on to encounter pathogens in lymphoid organs, to where they have migrated.
2. **The activation of B-cells:** The mature B cell encounters antigen in the lymphoid organs. The antigen binds to the BCR which is then internalised by endocytosis. The antigen fragments are then presented with MHC-II molecules.
3. **The proliferation of B-cells:** The helper T-cell get activated on binding to B-cell. The activation of helper T-cells results in release of various cytokines that go on further to activate the B-cells to proliferate rapidly. Soon a clone of B-cells is formed and these cells eventually form memory cells or plasma cells. The plasma cells secrete large amounts of antibodies while the memory cells are in a temporary resting state which gets activated on subsequent encounter with the pathogen.

4. **Antigen –antibody reaction:** The antigen and antibody then react forming an immune complex which is cleared from the body.

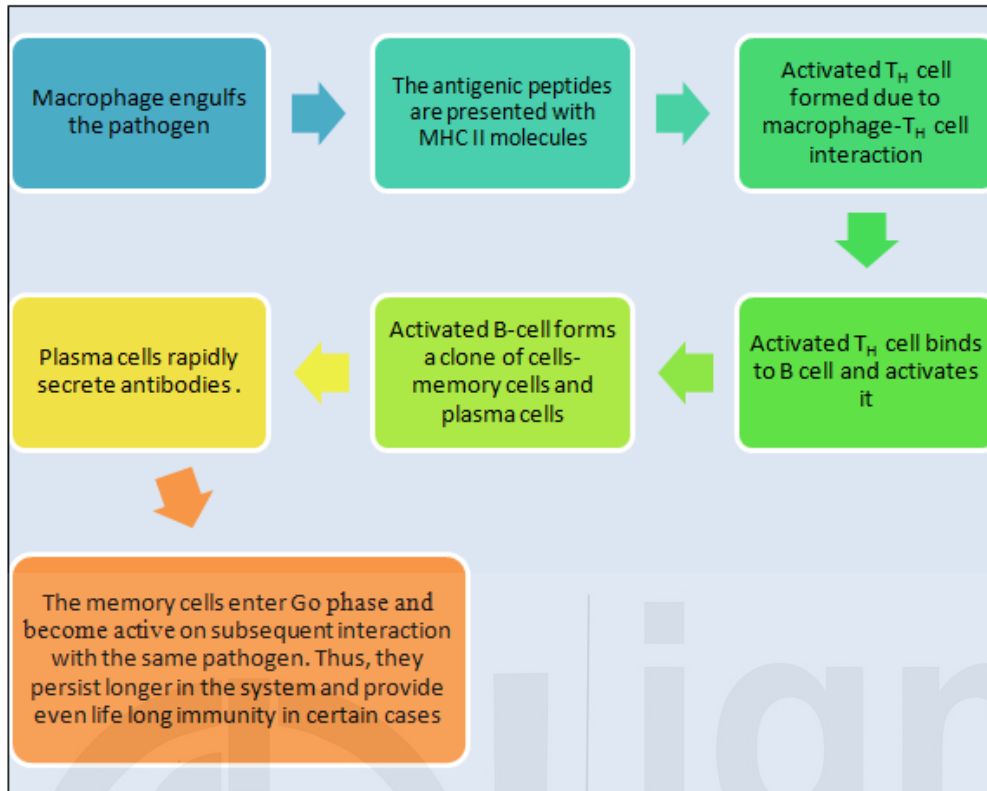


Fig 3.5: The steps of Humoral Immune Response.

3.7 CELL MEDIATED IMMUNE RESPONSE (CMI)

The cell mediated immune response is the method of the body to fight against pathogens like bacteria and viruses. It is also responsible for the killing of cancerous cells and in the graft-rejection process.

The foreign antigen is recognised by macrophages and dendritic cells of the innate immune system. They internalise the pathogen, degrade it and present their antigenic peptides on their surface with class II MHC molecules. This MHC-antigenic complex is responsible for the activation of adaptive immune system.

CD4⁺ and CD8⁺ T cells are formed from T-cell precursors in the thymus. The MHC Class II complex on APCs interact with the CD4⁺ T cells while the CD8⁺ T cells interact with the antigen- Class I MHC complex found on infected or cancerous cells. The CD8⁺ T cells transform into Cytotoxic T lymphocytes (CTL) and are responsible for the destruction of the infected or the cancerous cell. (Fig 3.6)

The CD4⁺ cells prior to the interaction with MHCII- antigen complex present on APCs are called as **naïve cells**. The naïve cell on activation can form memory T cell or other T_H-cell variants-

- Interferons are produced by Type I helper T-cells that cause pathogen digestion with the APCs. They also stimulate CTL and B-cell activity.
- Interleukins that promote B cell activity are produced by Type II helper T-cells.

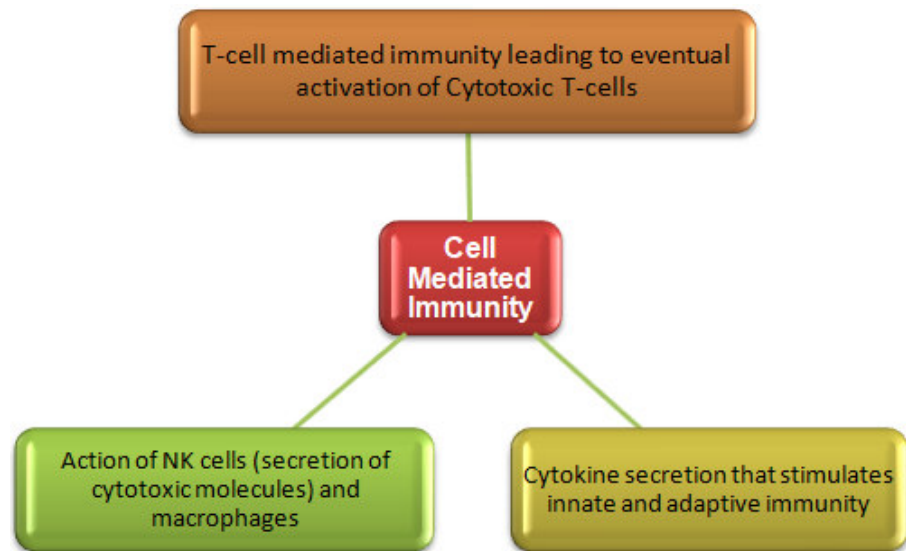


Fig 3.6: Mode of action of Cell-Mediated Immunity.

You have studied about humoral and cell mediated immunity. In Table 3.2 differences between the two types of immunity are listed.

Table 3.2: Differences in Humoral and Cell mediated Immunity.

Sr. No.	Humoral Immunity	Cell-mediated Immunity
1.	The response is mediated by antibodies.	The response is mediated by cells.
2.	Only helper T cells are involved.	Both CD4 ⁺ and CD8 ⁺ T cells are involved.
3.	Mediated by B cells, T cells and macrophages.	Mediated by helper T-cells, cytotoxic T cells, NK cells and macrophages.
4.	Involves B-cell receptors.	Involves T-cell receptors.
5.	Participates in homografts rejections and in GVHD- Graft versus host disease.	The preformed antibodies may lead to early graft rejection.
6.	No role in immune surveillance.	Has role in defense against cancer cells and also in immune surveillance.

SAQ 3

Fill in the blanks with appropriate words:

- CD4⁺ and CD8⁺ T cells are formed from in the thymus.
- The CD8⁺ T cell transforms into that are responsible for the destruction of the infected or the cancerous cell.
- participates in homografts rejections and in GVHD- Graft versus host disease.

3.8 PRIMARY AND SECONDARY IMMUNE RESPONSES

3.8.1 Primary Immune Response

Primary humoral response is generated when primary contact with the exogenous antigen is established. It leads to the production of plasma cells that secrete large amounts of antibodies and memory B cells.

The magnitude and kinetics of primary humoral response depend on several factors like:

- Nature and type of antigen.
- The involvement of adjuvants.
- Route of antigen administration.
- The species of the organism.

The primary response has a long lag period during which several activities occur like; clonal selection of B cells, their clonal expansion and subsequent differentiation into plasma cells or memory cells. An exponential increase in the serum antibody level is seen after the lag phase. A plateau is reached after some time and then the antibody levels gradually decline.

The process of antibody production has four distinct stages as discussed below and shown in Fig 3.7.

1. **Lag phase:** It is the phase when primary contact with the antigen is established. IgM appears and engages the antigenic element. The length of the lag phase is variable. For example- the pneumococcal polysaccharide persists for several hours while the Diphtherial toxin lasts for 2-3 weeks.
2. **Log phase:** Rapid increase in the antibody levels.
3. **Plateau:** An equilibrium state where the antibody production and decay is balanced.
4. **Decline phase:** The antigenic stimulus is removed and the serum antibody level begins to fall.

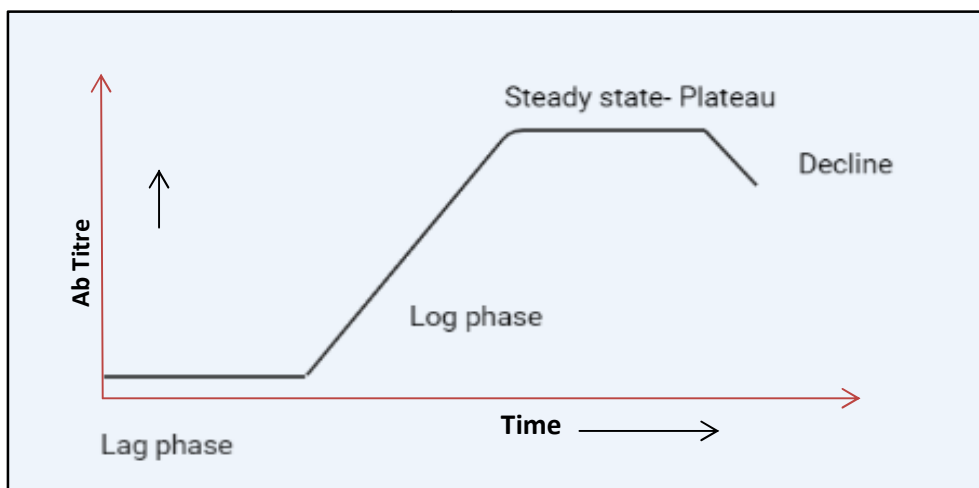


Fig. 3.7: Stages of antibody production.

Initially a rise in the serum IgM levels is seen and later it is replaced by IgG antibody. The IgM has higher avidity, which means that it is capable of engaging more diverse antigens. The IgG on the other hand is more specific to a particular antigen and thus appears in later stages of the immune response. The primary response can last from a few days to a several weeks depending on the antigen and the severity of the infection.

The memory cells that are generated during this process move out of the cell cycle and progress into the G₀ phase, where the cell is less metabolically active. These memory cells last for variable durations in the body and some may last for even the entire lifetime. It is the population of memory T cells and memory B cells that together elicit the secondary immune response on subsequent encounter with the same antigen.

3.8.2 The Secondary Immune Response

The secondary immune response is generated on subsequent exposure to the antigen and its interaction with memory B cells generated during the primary response. In secondary response, the lag phase is shorter but the magnitude is greater and persists longer. There is secretion of antibodies other than IgM that are more specific to the antigen.

Reasons for rapid and higher magnitude response in Secondary Response

1. The population of naive B-cells is much lower than the more antigen specific memory B cells.
2. The memory B cells are more readily activated than naive B-cells.

The production of higher affinity and different antibodies other than IgM is due to the processes of:

1. Affinity maturation
2. Class switching.

Therefore, the antibodies produced are equipped with better effector functions that are customised for a particular pathogen. There is a 100-1000 fold increase in antibody concentration as compared to primary response and the predominant antibody in this case is IgG along with IgA and IgE. In Table 3.3 Primary and Secondary Responses are compared and comparison of Ig levels in serum in both the responses is depicted in Fig. 3.8.

Table 3.3: Differences between Primary and Secondary Immune Responses.

Sr. No.	Primary Response	Secondary Response
1.	Occurs following first exposure to a foreign antigen.	It occurs on encountering the same antigen subsequently.

2.	A longer lag phase is observed that may last from a few days to a few weeks.	The lag phase is very short.
3.	Weaker response.	Effective, rapid and more specific response.
4.	Appears mainly in spleen and lymph nodes.	First seen in bone marrow, then in lymph nodes and spleen.
5.	IgM antibody predominates.	IgG antibody predominates, also small amounts of IgM, IgA, IgE. (Fig 3.8)
6.	The response occurs by naïve B cells and naïve T cells.	Response occurs by memory B and T cells.
7.	Rapid decline in antibody levels is seen and become undetectable after some time.	Antibodies persist for longer time.

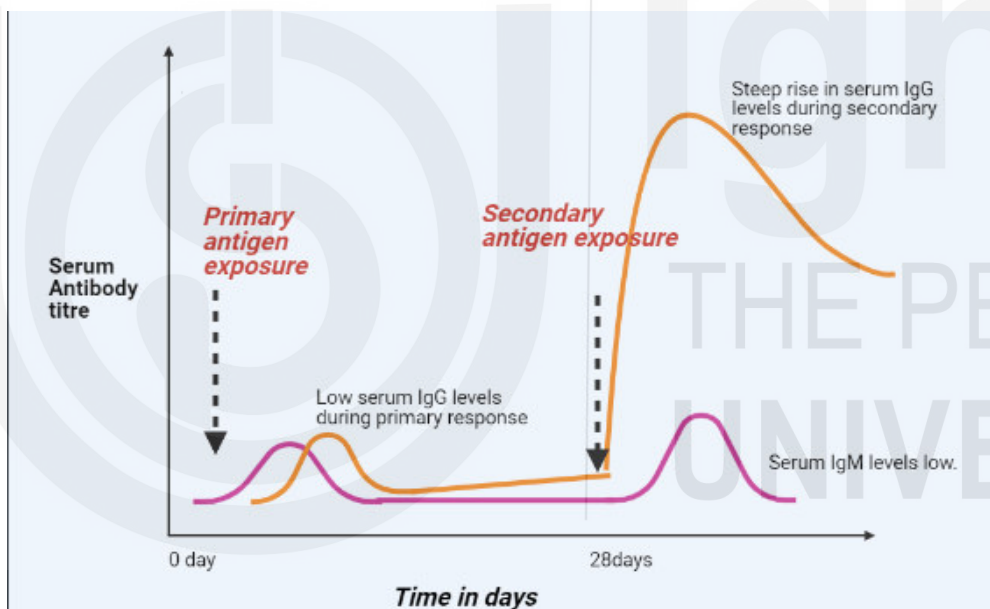


Fig 3.8: Comparison of serum Ig levels in primary and secondary immune response.

3.9 HOW DO THE INNATE AND ADAPTIVE IMMUNE SYSTEMS WORK TOGETHER?

As seen earlier, foreign pathogens and antigens are selectively recognised and eliminated by the adaptive immune system when the innate immune system fails to defend the body against the pathogen invasion. It is capable of responding only when a stimulus is provided by the innate immune system.

An antigen is a non-self or foreign molecule that is recognised by cells of the innate immune system. The information is then passed on to the adaptive immune system. These cells are called antigen-presenting cells (APC) and they recognise, engulf and pass on the activation signal to the adaptive system.

The pathogen/antigen is phagocytosed by APCs and then digested into many smaller fragments. These antigenic fragments are then transferred to the surface of the APC. This fragment will act as a signal for other immune cells activation. Dendritic cells and macrophages act as professional antigen presenting cells.

Once the antigen is phagocytosed, a phagosome is formed which then fuses with a lysosome to form a phagolysosome where the antigen is broken down, and then attached to class II MHC molecules. The class II MHC molecules-antigen peptide complex is then moved to the cell surface and present the antigen peptides. Various immune cells respond when antigen is presented by MHC-II molecules. One of the main cells to respond is Helper T-cells. They release cytokines and cause further activation of cells.

Additionally, the adaptive immunity is fully activated by danger signals displayed on cells. This occurs when the Pattern Recognition Receptors (PRRs) present on APCs like macrophages and dendritic cells recognise general classes of molecules frequently displayed by pathogens but never our own body. These pathogen specific patterns are called Pathogen Associated Molecular Patterns (PAMPs). This triggers events in the cell due to which threat signals are displayed by cells. Also, various cytokines, chemokines and chemotactic lipids are released that bring the adaptive immune system into action. Furthermore, dendritic cells bridges the innate and adaptive immune systems by presenting antigen and communicating the signal of activation to CD4⁺ Helper T lymphocytes.

3.9.1 Natural Killer Cells-The Bridge between Innate and Adaptive Immune Systems

The functional borders between innate and adaptive immune system is blurred due to the sophisticated biological roles played by NK cells.

The immune system has been divided into: Innate immunity and Adaptive immunity. The innate immunity consists of myeloid and lymphoid cells having limited number of germ line-encoded receptors which cause rapid effector functions. The adaptive immune system consists of lymphocytes- the B and T cells that express a wide variety of antigen receptors. The receptors of these cells are produced by site-specific somatic recombination. Naive B and T cells exert their effector functions after undergoing cell division and maturation on encountering the antigen in lymphoid organs.

NK cells are a population of white blood cells that have been classified as lymphoid cells as:

- They originate from the common lymphoid progenitor cells.
- Many lymphoid markers are expressed by them.
- Morphological similarity.

NK cells do not express antigen specific receptors on their surface and are thus considered to be a part of the innate immune system. They are cytolytic cells that can kill tumor cells or virus infected cells even in the absence of any prior immunisation, unlike cytotoxic T-cells.

NK cells are also known to produce many cytokines, chemokines and growth factors. In several pathological and physiological conditions, several cytokines like Interferon γ (IFN- γ), pro-inflammatory cytokines like Tumor necrosis factor- α (TNF- α) and immunosuppressive cytokines like interleukin (IL-10) are also secreted. The NK cells are able to influence T-cell responses through the secretion of interferon γ . There is a direct interaction between naive T cells and NK cells migrating from the site of inflammation in peripheral tissues to secondary lymphoid compartments. Growth factors like G-CSF (granulocyte colony-stimulating factor), IL-3 and as GM-CSF (granulocyte macrophage colony-stimulating factor) are also produced. The chemokine molecules secreted include CCL2, CCL3, CCL4, CCL5 and CCL8. These chemokines play important role in facilitating the presence of NK cells with dendritic cells at the site of inflammation.

The T cell responses are influenced by the killing of target cells by NK cells by probably-

- Decreasing the load of antigen.
- Cross presentation of target cell debris to CD8⁺ cytotoxic T cells.

It is established that NK cells have cytolytic effects against tumor cells or virus infected cells, but they also impact dendritic cells, macrophages and neutrophils due to cytokine production and cytotoxicity. Thus NK cells play an important role in influencing subsequent interaction of B and T cells with antigens. Depending upon the nature of antigen, NK cells can negatively or positively influence the B and T cell of the host immunity through the secretion of IFN- γ and IL-10.

SAQ 4

Do as directed:

- have cytolytic effects against tumor cells or virus infected cells. (Fill the blank)
- Initially a rise in the serum IgM levels is seen in primary immunity and later it is replaced by IgG antibody. (True/False)
- IgM has more affinity than IgG. (True/False)

3.10 ACQUIRED IMMUNITY

There are two types of acquired immunity viz. naturally acquired and artificially acquired immunity (Table 3.4)

Table 3.4: Differences between Naturally Acquired and Artificially Acquired Immunity.

Sr. No	Naturally Acquired Immunity	Artificially Acquired Immunity
1.	<i>Active</i> - This is acquired when the antigen enters the body naturally; the host fights it and recovers naturally.	<i>Active</i> - Killed or attenuated pathogen is introduced in the host. This process is called vaccination.

2.	<i>Passive-</i> It occurs when the antibodies are passed from mother to the foetus through the placenta or when the antibodies are given to child via breast milk.	<i>Passive-</i> Preformed antibodies are given to individual from an immune animal/person.
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3.10.1 Naturally Acquired Immunity

Naturally acquired immunity can be divided as active or passive-

1. **Active:** When a pathogen enters the body, the innate immune system passes on a message for the activation of adaptive immune system via antigen presenting cells. The B and T cells are activated via interaction between cells and the secretion of many cytokines, chemokines and other molecules. Eventually, memory B and T cells are formed and immunologic memory of the antigen/pathogen is retained in the body. Also, receptors specific to the pathogen are acquired by the host. On subsequent exposure to the same pathogen, a much exaggerated secondary response is mounted. This also forms the basis for vaccination.

A wild infection with for example Hepatitis A virus would elicit an immune response. A lifelong protection can be generated post- recovery.

2. **Passive:** New-borns are particularly susceptible to infections as they have no prior exposure to most antigen/pathogens. Thus, the mother provides passive protection to the baby.

The maternal antibodies (MatAb) are transferred from mother to the foetus through the placenta via FcRn receptor found on placental cells. This happens around the end of first trimester. Thus very high antibody levels are found in the baby at the time of birth. These antibodies have almost the same specificity for antigen as the mother. **The only antibody that can cross the placenta is IgG.**

Also, breast milk contains large amounts of secreted antibodies (mainly IgA) that protect the new-born till it becomes capable of synthesizing antibodies itself.

This immunity is however very short lived as no antibodies are synthesized or immunological memory is generated.

3.10.2 Artificially Acquired Immunity

The Artificially acquired immunity can be active or passive.

1. **Active:** This can be acquired by the process of Vaccination. A vaccine contains an antigenic substance that can induce primary immune response without producing any signs or symptoms of the disease. The term “vaccine” was adopted by Louis Pasteur in honour of Edward Jenner who developed the small pox vaccine in 1796.

The vaccination process involves providing immunization by priming the immune system with the antigen deliberately. There are several types of vaccines- their types and example are given in Table 3.5.

Table 3.5: Different types of vaccines and their examples.

Sr. No	Vaccine	Description	Examples
1.	Inactivated vaccines	Composed of potentially pathogenic microorganisms that are no longer capable of causing infection as they have been killed using chemicals/ heat or other treatments.	Cholera Flu Plague
2.	Attenuated vaccines	The potentially pathogenic microorganisms are inactivated/ weakened and are no longer capable of causing infection.	Mumps Rubella virus Yellow fever
3.	Toxoid based vaccines	Used when the disease is caused by pathogenic toxin rather than the pathogen itself.	Tetanus
4.	Subunit/ recombinant vaccine	Immunisation with small pathogenic fragments.	Hepatitis B virus

2. **Passive:** Passive immunisation is critical when the infection is rapidly spreading and there is insufficient time for the body to develop response and protect itself. Artificially acquired passive immunity is due to the intravenous or intramuscular injection of preformed antibodies from human or animal plasma through antiserum therapy and immunoglobulin therapy. This is short lived as no response is generated and antibodies are superficially administered. **It used in the treatment of several types of acute infections and in the treatment of poisoning.**

However, in this method the major drawback is the risk of developing hypersensitivity reactions, especially when gamma globulins are involved from non-human sources.

SAQ 5

Fill in the blanks:

- A vaccine contains an that can induce primary immune response without producing any signs or symptoms of the disease.
- The only antibody that can cross the placenta is
- During primary contact with the antigen, memory of this encounter is generated in our system in the form of
- In vaccine the potentially pathogenic microorganisms are inactivated/weakened and are no longer capable of causing infection.

3.11 SUMMARY

Let us summarise whatever you have learnt in this unit

- Adaptive immune response is generated post exposure to a pathogenic antigen or after a vaccination. The responses are tailor made to specific antigenic challenges and not present naturally like the innate immune system.
- The features of adaptive immunity are diversity, antigen specificity, immunologic memory and self and non-self-differentiation.
- The acquired immunity can be naturally and artificially acquired.
- There are several types of vaccines:-inactivated, attenuated, toxoid and recombinant vaccines.
- B-cells are formed in the bone marrow and on activation form memory and plasma cells.
- A signal transduction pathway is initiated by BCR binding to antigen. This leads to the activation of B-cells.
- T-cells that recognise antigen-MHCII molecule function as helper T-cells.
- T-cells that recognise antigen-MHCI molecule function as cytotoxic T-cells that on activation form cytotoxic T-lymphocytes.
- Cytokines are low molecular weight proteins or glycoproteins with regulatory roles.
- Cytokines trigger signal transduction pathways and eventually lead to alteration of gene expression in target cells by binding to specific receptors located on the target cell.
- Cytokines exhibits several features: redundancy, pleiotropy, synergy and antagonism.
- Humoral immunity is mediated by molecules like antibodies, various complement proteins and antimicrobial peptides. Therefore, it is also known as Antibody Mediated Immunity.
- The cell mediated immune response is the method of the body to fight against pathogens like bacteria and viruses. It is also responsible for the killing of cancerous cells and in the graft-rejection process.
- Primary response is seen on first encounter with the pathogen. It usually has a long lag phase. IgM is the main antibody involved.
- Secondary response is seen on subsequent exposure to the same antigen. It has a much shorter lag phase and is of a greater magnitude.

3.12 TERMINAL QUESTIONS

1. Expand the following terms:

- a) CTL
- b) MHC
- c) T_H cell
- d) APC

2. Match the following:

Attenuated vaccine	Cholera
Toxoid	Mumps
Killed vaccine	Tetanus

3. State as 'True' or 'False'.

- i). Interleukin -10 is a cytokine.
- ii). NK cells need prior antigen exposure to get activated and cause cytolytic activity.
- iii). The memory B-cells are more easily activated than naive B-cells.

4. List the various factors that determine the kinetics of humoral response.

5. Fill in the blanks:

- i). is an example of active artificially acquired immunity.
- ii). is an example of active naturally acquired immunity.
- iii). Passage of antibodies via mother's milk is an example of

6. Why is the IgM that appears early in the infection replaced by IgG antibodies in the later exposure?

7. Label the stages of antibody production given in the figure.



8. Fill in the blanks:
- Activated B cell clones form and cells.
 - B-cells are called so due to their origin in in birds.
 - CD8⁺ T cell activated transform into
 - B-cells mature in lymphoid organ.
9. Provide a name for the following situations.
- IL-4 secreted by activated T_H cells goes on to activate B cell, thymocyte and mast cells.
 - IL-2, IL4 and IL-5 secreted by activated T_H cells all cause B cell proliferation.

3.12 ANSWERS

Self Assessment Questions

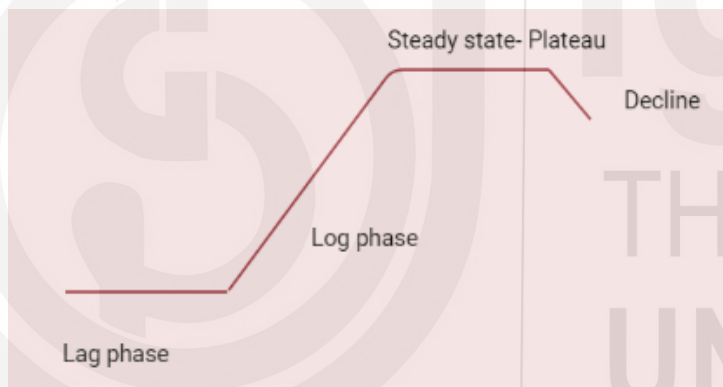
- True,
 - False,
 - True.
- c,
 - a,
 - d,
 - e,
 - b.
- T-cell precursors
 - Cytotoxic T-lymphocyte
 - Humoral immunity
- NK cells,
 - True,
 - False,
 - True.
- Antigenic substance.
 - IgG.
 - Memory B and T cells.
 - Attenuated vaccine.

Terminal Questions

- Cytotoxic T lymphocyte.
 - Major Histocompatibility Complex.
 - Helper T cell.
 - Antigen presenting cell.
- Match the following:

Attenuated vaccine	Mumps
Toxoid	Tetanus
Killed vaccine	Cholera

3. i) True, ii) False, iii) True.
4. Factors that determine the kinetics of humoral response are:
- Neutralization of pathogens and toxins.
 - Classical pathway of complement activation.
 - Opsonisation of pathogen and help in phagocytosis.
 - Eventual pathogen clearing.
5. i) Vaccination.
- ii) Exposure to antigen and natural recovery of the body.
- iii) Passive naturally acquired immunity.
6. Initially a rise in the serum IgM levels is seen and later it is replaced by IgG antibody. IgM has higher avidity, which means that it is capable of engaging more diverse antigens. The IgG on the other hand is more specific to a particular antigen and thus appears in later stages of the immune response.
- 7.



8. i) Memory cells and plasma cells.
- ii) Bursa of Fabricius.
- iii) Cytotoxic T lymphocytes.
- iv) Bone marrow.
9. i) Pleiotropy, ii) Redundancy.

ORGANS OF IMMUNE SYSTEM |

Structure

- | | |
|-------------------------------|------------------------|
| 4.1 Introduction | 4.5 Summary |
| Objectives | 4.6 Terminal Questions |
| 4.2 Lymphoid Organs | 4.7 Answers |
| 4.3 Primary Lymphoid Organs | |
| 4.4 Secondary Lymphoid Organs | |

4.1 INTRODUCTION

In the previous units you have studied about various important components of immune system. You have also learnt about their significant role in providing immunity. Human immune system is made up of many different organs and tissues that are present throughout our body.

In this unit you will be studying about organs of immune system. These organs broadly divided into two types that is primary lymphoid organs and secondary lymphoid organs. However, in your previous courses you might have learnt about these organs and their physiological importance. These organs are located at different parts of your body and also have differences in their activities. Primary lymphoid organs responsible for the development of immune cells whereas secondary lymphoid organs act as site of immune response.

In simple words primary lymphoid organs acts as sites where production of lymphoid cells takes place and secondary lymphoid organs are the battle grounds where antigen and antibody interaction takes place. You would be excited to know the significant role played by bone marrow and thymus in developing the immune cells. While studying this unit you will come across lymphatic nodes and their significance as secondary lymphoid organs in responding and providing immune response against the antigens.

In this context this unit is acting as a connecting link between the various components of immune system and immunogenicity that is exhibited by immune system.

Objectives

After studying this unit you should be able to

- ❖ identify the important organs of immune system,
- ❖ compare and differentiate functions executed by primary and secondary lymphoid organs,
- ❖ explain the involvement of lymphoid organs at various stages of immune response, and
- ❖ enlist the biological significance exhibited by lymphoid organs.

4.2 LYMPHOID ORGANS

In BZYCT-135, you have studied about different organs of our body and their diverse functions. During this you might have noticed that these organs have different structures and functions and are also located throughout the body. The coordination among these organs is very much essential for the maintenance of health. Similarly, there are different lymphoid organs that exist in our body and are performing various functions in the development of immunity or immune responses (Fig 4.1).

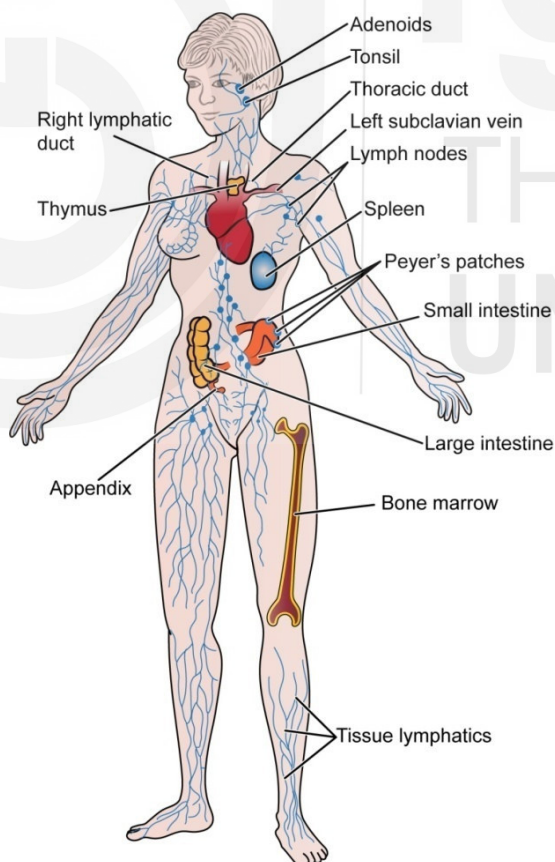


Fig. 4.1: Lymphoid Organs.

These organs can be classified into two major groups that are primary and secondary lymphoid organs. To know more about the role of the lymphatic system in immunity, you are advised to watch the video available at the following link <https://youtu.be/kjLwVqxwaIM>.

4.3 PRIMARY LYMPHOID ORGANS

We all know that B-cells play an important role in the immune system by producing antibodies whereas T-cells have a specific role in performing cell mediated immunity which is significant with respect to controlling viral infections. It is well established that antibodies are clinically essential to fight against covid-19 infection. However specific antibodies levels against severe acute respiratory syndrome corona virus (SARS-CoV-2) dropped below the limit of detection within 2-3 years, whereas the specific memory T-cells have been detected even after 11 years of SARS-CoV-2 infection. More studies are being conducted to understand the protective role of T-cells in controlling SARS-Cov-2 infection (<https://www.nature.com/articles/s41586-020-2550-z>)

Both thymus and bone marrow are considered as central or primary lymphoid organs. These are the sites of lymphocyte maturation. In brief the immature lymphocytes produced during haematopoiesis get mature towards specific antigen inside these primary lymphoid organs. During this maturation process lymphocytes attain the property of '**immunocompetent**' i.e., the ability to show immune response.

You are aware that there are two major types of immune cells like T cells and B cells. *Thymus is the site where T cells arise, however bone marrow is the site for the origin of B cells.* Let us discuss about these two significant primary lymphoid organs in the following sections.

THYMUS

It is located above the heart; it has a flat, bilobed structure (Fig 4.2). These lobes are surrounded by capsule like structure and are separated from each other by a connective tissue strands called as **trabeculae**. Each lobule is divided into two compartments the outer compartment is known as **cortex** which is densely packed with immature T cells that are known as thymocytes. However, the second inner compartment which is known as **medulla** contains few thymocytes.

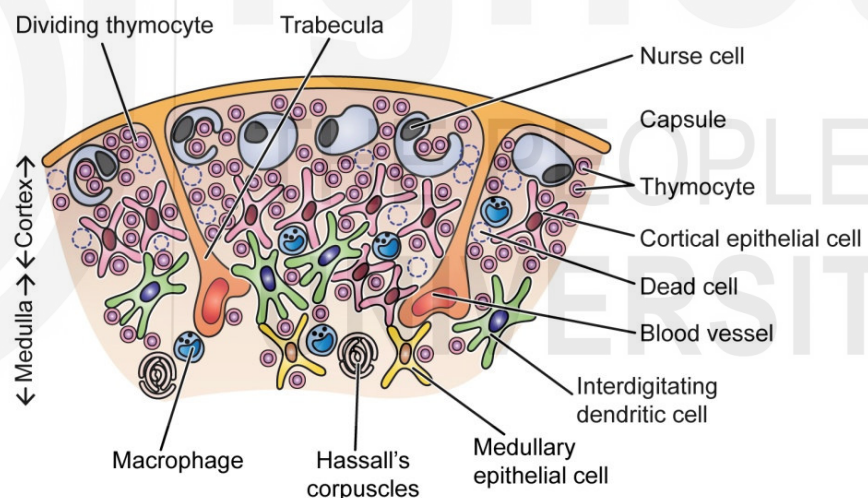


Fig. 4.2: Thymus.

These two compartments of thymus are interconnected by a three-dimensional network composed of epithelial cells, dendritic cells and macrophages. All these three cells are responsible for the development of stromal cell network that plays a significant role in the growth and maturation of thymocytes. Here it is important to know about specific type of epithelial cells that are found in the outer cortex that are known as **nurse cells**. These cells have long membrane extensions and are surrounded by more than 50 thymocytes, altogether they are responsible for formation of large **multicellular complexes**. Thymus also exhibits a unique property of selecting the effective T cells that protect the body from infection. You will know more about this process in Unit 9 where you will be studying about major histocompatibility complexes (**MHC**) and their importance in immune response.

It is important to remember that on the efficiency of thymus or thymic function will be declining with growing age. This decline may be one of the reasons for low immunity during old age in humans. It is interesting to note that the average weight of thymus in infants is around 70 g whereas it is reduced to 3 g in old people.

BONE MARROW

Bone marrow is the soft, spongy, highly vascular tissue found in the medullary cavities of bones (Fig. 4.3 A). In humans it is primarily located in the regions like ribs, vertebrae, sternum, and bones of the pelvis. In adults this site is responsible for production of **red blood cells**, a process that is known as **haematopoiesis**. **Haemopoietic stem cell** is the primary stem cell that are responsible for production all other immune cells. It is interesting to know that this Haemopoietic stem cells are found in foetal liver and bone.

Especially in mammals like humans and rats bone marrow acts as crucial site for the origin and development (**proliferate and differentiate**) of **B cells**. Whereas in case of bird's **bursa Fabricius**, in case of cattle and sheep it is the **foetal spleen** acts as primary lymphoid tissue for maturation proliferation functions of B cells. Even in rabbit gut associated tissue like **appendix** acts as primary lymphoid organ.

Structure of bone marrow

Bone marrow can be divided into two major components or parts the first one is **red** marrow and the second one is **yellow** marrow. Red marrow is majorly found in skeleton bone system and plays a significant role in the formation of blood cells and also to remove the old cells from circulation. This part of the bone consists of haemopoietic stem cells that in turn produce myeloid and lymphoid stem cells. These cells in later stages divided into red blood cells white blood cells and platelets. You will learn more about cells of immune system in the next unit of this course.

Whereas the yellow marrow is predominantly found in soft bone region which is also known as spongy bone. This part of bone marrow is known vascular and consists primarily of fat cells. Though it possesses hemopoietic tissue but it is physiologically inactive.

Microscopic observation of bone marrow depicts hemopoietic tissue islands and adipose cells surrounded by vascular sinuses within a mesh work of trabecular bone (Fig. 4.3 B). Structurally bone marrow can be divided into **vascular and non-vascular regions** that are composed of both cellular and non-cellular components. The non-vascular (peripheral) region is known for **haematopoiesis** which is rich in cells of various lineages and stages of maturity. The cells and tissues that are commonly found in this region include fat cells, collagen fibres, fibroblasts, dendritic cells and bony tissues (trabeculae). Whereas the vascular region (centre) is rich in blood vessels that brings in nutrients and collects the mature blood cells into circulation.

Hence we can conclude that bone marrow is playing a significant role in immune response by producing various lymphoid cells that migrate to secondary lymphoid organs to divide further and also in maturation of B cells. Platelets which play an important role in blood coagulation are also produced in bone marrow.

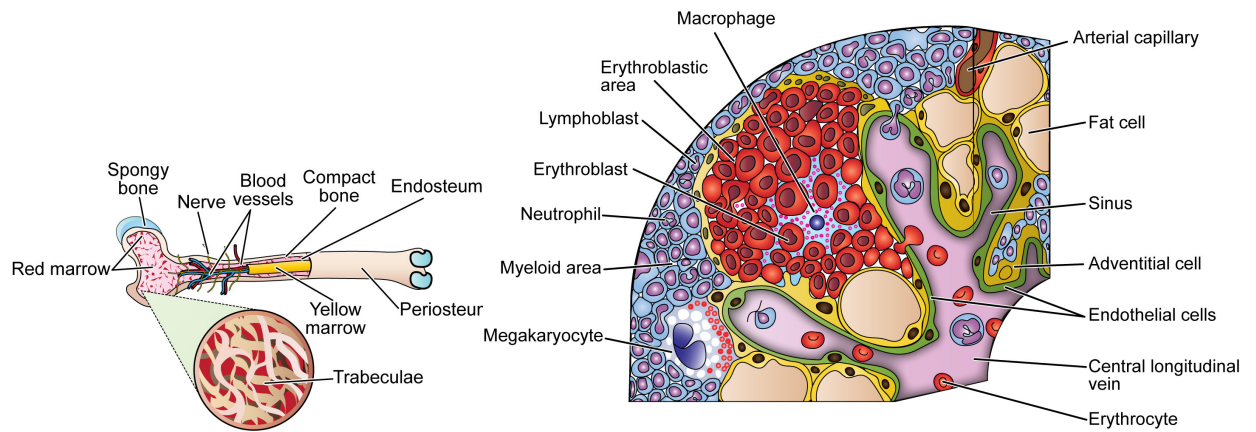


Fig. 4.3: A. Bone marrow B. Microscopic view of Bone Marrow.

SAQ 1

Match the following:

Column A

- i) Thymus
- ii) Bone marrow
- iii) Nurse cells
- iv) Selection effective of T cells
- v) Cortex
- vi) Primary stem cell
- vii) Non-Vascular tissue
- viii) Red bone marrow

Column B

- a) Haematopoietic stem cell
- b) protection from infection
- c) T cell maturation
- d) Densely packed with immature T cells
- e) B cell maturation
- f) formation of multicellular complexes
- g) haematopoiesis
- h) Collection of mature red blood cells

4.4 SECONDARY LYMPHOID ORGANS

This group includes **lymph nodes**, **spleen**, **mucosa associated lymphoid tissues (MALT)** and **gut associated lymphoid tissue (GALT)**. Due to their location in the body, they are known as the peripheral lymphoid organs they mainly involved in trapping the antigen and act as **sites for the interaction** of mature lymphocytes with antigen. Both lymph nodes and the spleen are organised secondary lymphoid organs, as they have specific regions for T cell and B cell activity and also surrounded by fibrous capsule, whereas MALT is an unorganised or less organised. However, MALT is widely distributed in the human body. Let us discuss these secondary lymphoid organs one by one.

i) Lymph nodes

These organs are bean shaped structures composed of reticular network that is filled with lymphocytes, macrophages and dendritic cells (Fig. 4.4). Lymph

nodes are found at the junctions of lymphatic vessels (refer unit 3 of BZYCT-135). Due to their widespread availability, they are the first organs to combat antigens that enter into tissue spaces. The lymphatic fluid that is passing through these nodes brings the antigens and these antigens will be exposed to the phagocytic and dendritic cell network present inside these nodes.

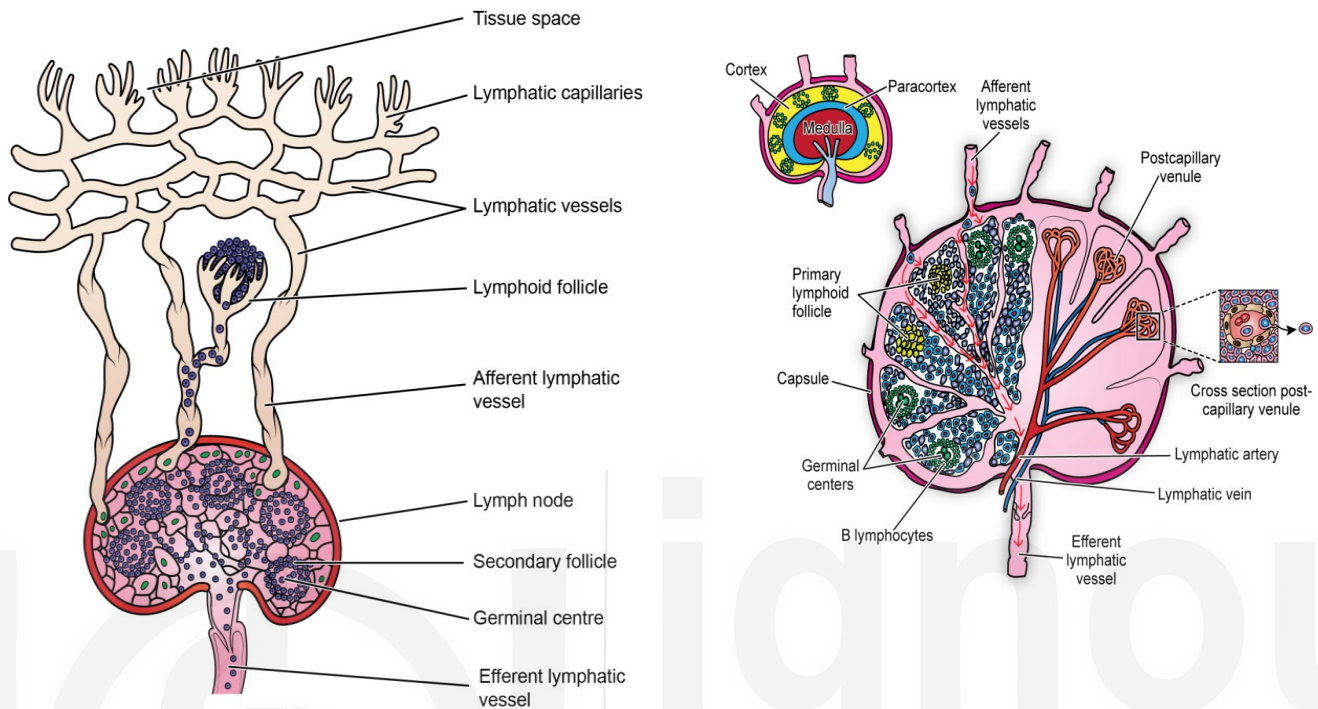


Fig. 4.4: Lymphatic system and Lymph node.

Lymph node consists of three regions the **cortex**, the **paracortex** and the **medulla**. Where cortex contains **B cells** (Lymphocytes), macrophages and follicular dendritic cells in their primary follicles (Fig 4.4). Once after interacting with antigens the primary follicles convert into secondary follicle which is comparatively large in structure and contains a germinal centre.

The para cortex rich in **T lymphocytes** also contains dendritic cells that have migrated from surrounding tissues into the nodes. The last compartment that is medulla that has a smaller number of lymphoid lineage cells and plasma cells that are actively **secreting antibodies**.

If we put the entire mechanism that is taking place inside a lymph node it is like, *"the antigen is trapped, processed and presented to dendritic cells in the paracortex which in turn activates T helper cells. Also, activation of B cells takes place in the presence of T cell rich paracortex"*. These **plasma B cells** are responsible for secreting Ig M and IgG antibodies. Phagocytic cells and dendritic cells are responsible to trap bacteria or any foreign particle that is coming into lymph node. It is interesting to note that this entire process completes in a span of 4 to 7 days. It is also important to know that fluid leaving lymph node through efferent lymphatic vessels is rich in antibodies and concentration of lymphocytes.

ii) Spleen

Spleen located in the left abdominal cavity it is a large ovoid secondary lymphoid organ (Fig. 4.5). Spleen plays an important role in immune response through producing antibodies. We have discussed in the previous

section that lymph nodes filter the antigens present in lymphatic circulation, however, spleen is responsible to **filter the antigens** that are present in blood circulation or blood borne antigens. Here it is important to know that spleen plays a key role in systemic infections. Splenic artery brings in the blood along with antigens, whereas splenic vein takes away the blood that is rich in antibodies. There are two important compartments inside spleen like **red pulp** and **white pulp**. The red pulp consists of sinusoid network, surrounded by numerous macrophages, red blood cells and few lymphocytes. This site is physiologically responsible for the breakdown of defective and dead RBCs, whereas white pulp is rich in T lymphocytes forming a **periarteriolar lymphoid sheath (PALS)**.

The effects of splenectomy (a surgical procedure to remove spleen) on the immune response depends 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 (**bacteraemia**). Ref: Text book of Immunology by Janis Kuby.

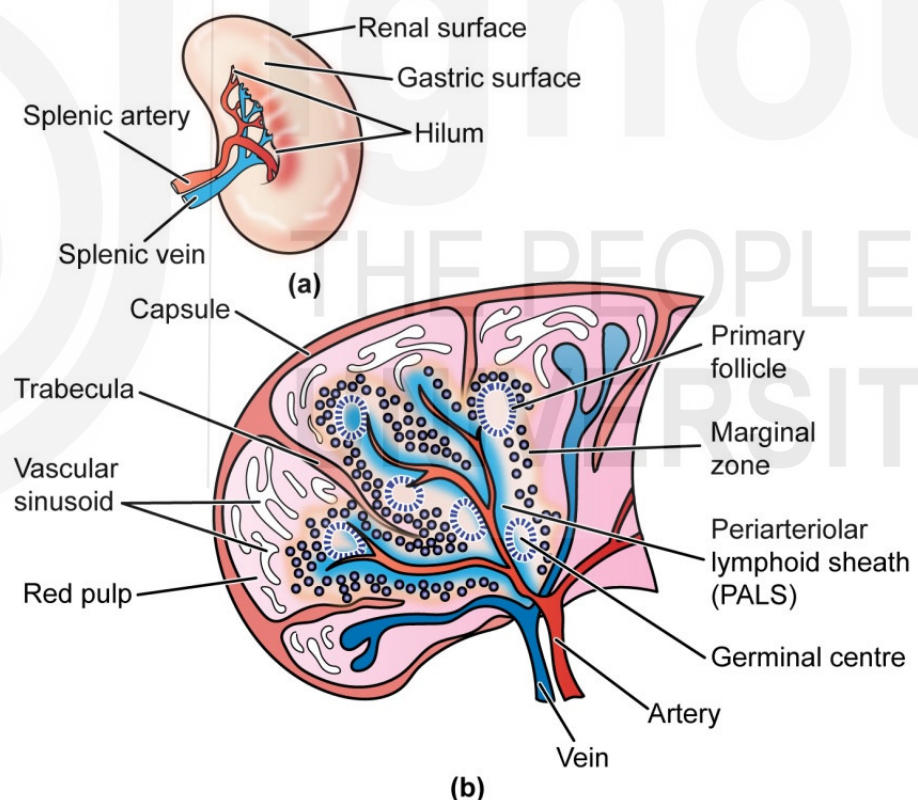


Fig. 4.5: Spleen.

MUCOSAL-ASSOCIATED LYMPHOID TISSUE (MALT)

MALT is a diffuse system of small concentrations of lymphoid tissue found in various submucosal membrane sites of the body. These sites include the gastrointestinal tract, nasopharynx, thyroid, breast, lung, salivary glands, eye, and skin. You would be surprised to know the size of MALT is approximately **400 m²** (nearly the size of a basketball court). MALT lining covers digestive system, respiratory system and urogenital system, that are

major sites of entry for the pathogens. These tissues include loosely arranged lymphocyte clusters around villi and well organised tonsils (Fig. 4.6). MALT can also be classified based on the tissues they are associated with: BALT (Bronchus Associated Lymphoid Tissue), GALT (Gut Associated Lymphoid Tissue), LALT (Larynx Associated Lymphoid Tissue), CALT (Conjunctiva Associated Lymphoid Tissue), NALT (Nasal Associated Lymphoid Tissue), SALT (Skin Associated Lymphoid Tissue), DALT (Duct Associated Lymphoid Tissue) and LDALT (Lacrimal Duct Associated Lymphoid Tissues). Appendix, and Peyer's Patches found in the submucosal layers of the intestinal lining (Fig. 4.7). MALT has the capacity to invade (endocytose) the antigens from the lumen of the respiratory and urogenital tracts. Due to these immune abilities or activities, antibodies are released immediately to fight against the antigens that are entering into our body. It is important to know that, secretions of MALT majorly consist of IgA antibody, hence it is known as **secretory antibody**.

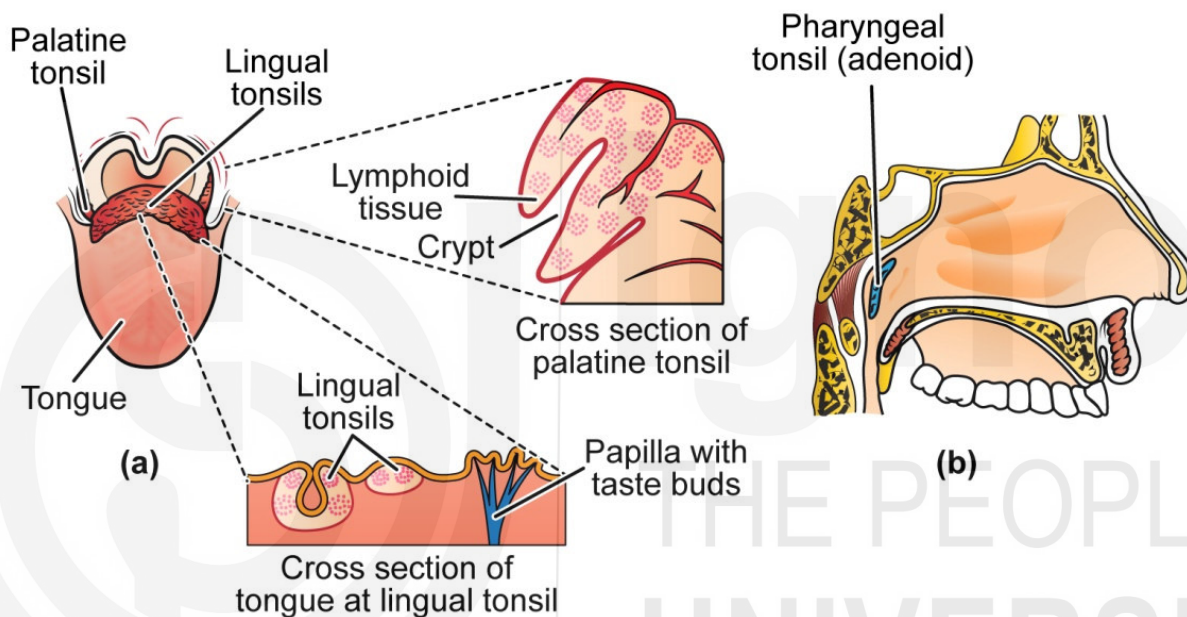


Fig. 4.6: Types of Tonsil.

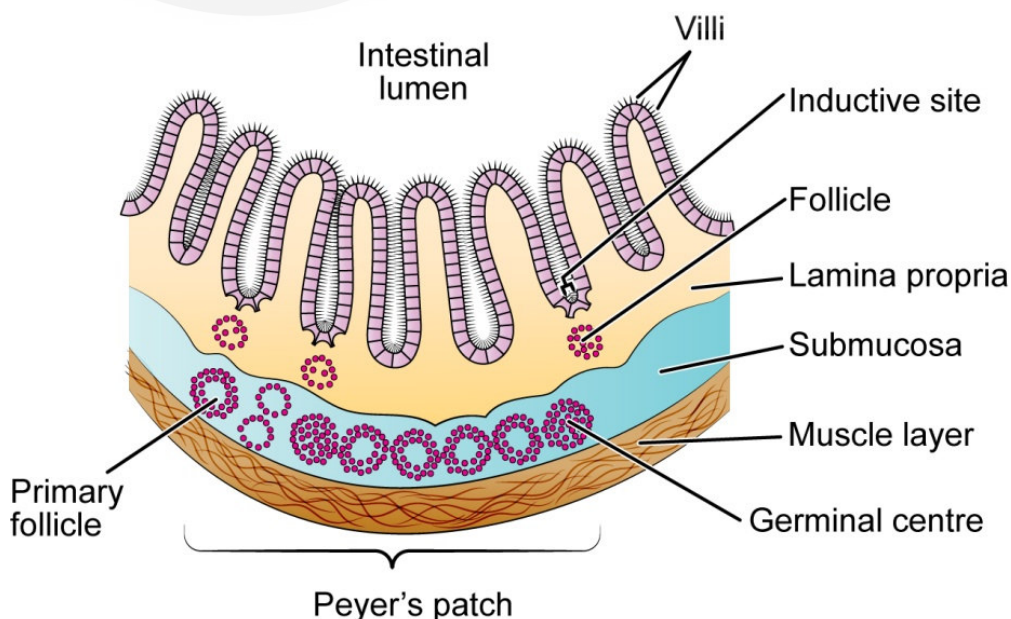


Fig. 4.7: Micro villi of gastrointestinal tract.

SAQ 2

Match the following

Column A	Column B
i) Organised lymphoid organ	a) Plasma B Cells
ii) Lymphoid cortex	b) rich in T cells
iii) Lymphoid paracortex	c) rich in B cells
iv) Secretory antibody	d) Primary lymphoid organs
v) Dendritic cells	e) Trapping bacteria
vi) Red pulp	f) MALT secretions
vii) Spleen	g) Break down of defective RBCs
viii) Ig A	h) Filtering blood borne antigen

Some interesting Facts about our immune system:

Whenever you receive any vaccination or antibiotic through intramuscular injection you might have noticed the development of small swelling/lesions at the site of injection. Have you ever thought what would be the reason for this swelling? This swelling is a by-product of interaction that is taking place between antigen and antibody. In our daily life we came across so many such incidents where our immune response combats with the antigens that are entering into our body. Some of the examples include sneezing when smell something strange and tears against the entry dust mites. These are a kind of defence mechanisms that our body possess as a natural ability.



(Source: pinterest.com)

Have noticed the consequences that took place after meeting any minor injury while playing football or any sport? If not, you will start observing after going through this unit. Let us discuss one small such example, whenever we suffer with small scratches on our elbows and toes (while playing in ground), the next day morning we will notice swelling at the junction of thighs or under arm pits. This swelling is due to the presence of lymph nodes at these regions and their involvement in the battle against the antigens that might have entered through the scratch.

You might have also heard about kids suffering from tonsils whenever they consume something cold or exposed to allergens. Such people with

aggressive immune system are known as hypersensitive. Similar to this adenoid is also one such response developed by our lymphoid organs.



(source:

<https://www.aboutkidshealth.ca/Article?contentid=1220&language=English#article-intro>)

You might be surprised to know that the rise in body temperature during any infectious disease or condition could be due to the immune reactions (production of antibodies) that are taking place in your immune system and the fight exhibited by antibodies against the antigens that entered into your body. Hence, the rise in body temperature indicates that your immune system is responding to the infection that has entered your body. Last but not the least the lesions developed against mosquito bite or bee sting are also part of our immune response.



(source: <https://health.clevelandclinic.org/>)

4.5 SUMMARY

In this Unit you have studied:

- Primary lymphoid organs namely thymus and bone marrow are responsible for the development of immune cells.
- Primary lymphoid organs can be assumed as the centres or points where the production of immune cells occurs.
- Thymus being a small organ in the body is responsible for the maturation of T lymphocytes.
- Bone marrow plays a vital role in the process of haematopoiesis where it produces various lymphoid cells.
- Bone marrow is the site from where B cells are produced. The secondary lymphoid organs like lymph nodes, spleen and mucosa associated lymphoid organs are the sites of immune response.
- The secondary lymphoid organs can be considered as battle grounds where the actual antigen antibody interaction takes place.

4.6 TERMINAL QUESTIONS

1. Write a detailed note on the lymphoid organs that are involved in the maturation of lymphocytes.
2. Describe the structure of bone marrow with a neat diagram.
3. Write a comparative note on the role of Thymus and Bone marrow with respect to immune response.
4. Draw a neat labelled diagram of spleen.
5. Compare and contrast primary and secondary lymphoid organs.

4.7 ANSWERS

Self Assessment Questions

1. i) c, ii) e, iii) f, iv) b, v) d, vi) a, vii) g, viii) h.
2. i) d, ii) c, iii) d, iv) a, v) e, vi) g, vii) h, viii) f.

Terminal Questions

1. Refer to Section 4.3.
2. Refer to Fig. 4.3.
3. Refer to Section 4.3.
4. Refer to Fig. 4.5.
5. Refer to Sections 4.3 and 4.4.

UNIT 5

CELLS OF THE IMMUNE SYSTEM

Structure

- | | |
|---|-----------------------------------|
| 5.1 Introduction | 5.6 Cells of lymphoid lineage |
| Objectives | Lymphocytes (T and B cells) |
| 5.2 Hematopoietic Stem Cells | Natural killer cells |
| 5.3 Hematopoiesis | 5.7 Epitope- Antigen Determinants |
| Genetic Regulation of Haematopoiesis | 5.8 Summary |
| Growth Factors in Haematopoiesis | 5.9 Terminal Questions |
| Nexus between Hematopoiesis and Apoptosis | 5.10 Answers |
| 5.4 Cells of the immune system | |
| 5.5 Cells of Myeloid Lineage | |
| Granulocytes | |
| Phagocytic cells | |

5.1 INTRODUCTION

Immune system is a congregation of cells and organs that functions to provide immunity against microbial infection. Primary and secondary lymphoid organs in human or vertebrate body constitute the immune system. Primary organs provide the conditions necessary for the production and maturation of cells involved in immune response. The secondary organ provides the platform for interaction of antigens and mature lymphocytes. The two organs of immune system are interconnected through network of blood vessels and lymphatic ducts through which cells of immune system circulates to all the tissues and organs of the body. Therefore the cells of immune system are in constant patrol and looking for pathogens. On locating a pathogen/ antigen, they multiply and release alert signals to other cells of immune system. The vital role of recognition of self/ non-self, diversity of forms, specificity for antigens is possessed by lymphocytes. The other leucocytes play the ancillary functions,

such as activating lymphocytes, efficient antigen clearance through phagocytosis, or secreting different immune-effector molecules. The present chapter would concentrate on the development of blood cells, properties of the various cells of immune system, and their interaction with antigens.

Objectives

After studying this unit, you should be able to:

- ❖ discuss the process of generation of immune cells,
- ❖ explain regulation of production of immune cells,
- ❖ describe characteristics and functions of various immune cells,
- ❖ explain mechanism of immune responses and
- ❖ discuss the properties of Antigen Recognized by the B and T cells.

5.2 HEMATOPOEITIC STEM CELLS

Pluripotent cell has two properties: Self renewal and potency. They are capable of giving rise to several different cell types.

Blood cells of our immune system that are formed from stem cells are known as hematopoietic stem cells (HSCs). The term **hematopoiesis** refers to the process of formation of blood cells, their development and differentiation. Hematopoietic stem cells (HSC) are pluripotent cells which have the ability to differentiate in various different blood cells types like granulocytes, erythrocytes, mast cells, monocytes, megakaryocytes and lymphocytes (Fig. 5.1). However, the hematopoietic stem cells have very low population in the bone marrow (less than 1 HSC per 5×10^4 cells). The study of HSC is still a challenge due to its difficulty to growing them in *vitro* conditions.

The HSC remains at a stable level even during adult life due to its ability of self-renewal; however, hematopoietic stem cells exhibit tremendous proliferative potential when there is an increased demand by the body. HSCs develop and mature in the stromal cells meshwork of bone marrow. These stromal cells includes lipocytes, endothelial cells, fibroblasts and macrophages. Stromal cells are non-hematopoietic but nurtures the growth and differentiation of HSCs. They influence stem cell differentiation by providing a cellular matrix and factors that promote growth and differentiation - **hematopoietic-inducing microenvironment (HIM)**.

Hematopoietic stem cells are pluripotent cells which are able to differentiate in various different blood cells types.

Most of these hematopoietic growth factors are soluble agents that enter their target cells through diffusion or through receptors on the surface of stromal cells that are involved in cell-to-cell communication between the reacting cells and the stromal cells. During infection, activated T cells and macrophages induce hematopoiesis by producing hematopoietic growth factors. An average human being is estimated to produce 3.7×10^{11} blood cells every day to maintain steady state levels in the peripheral circulation. During hematopoiesis cell division and differentiation are regulated by apoptosis, the programmed death of cells. Failure in apoptosis may result in leukemia.

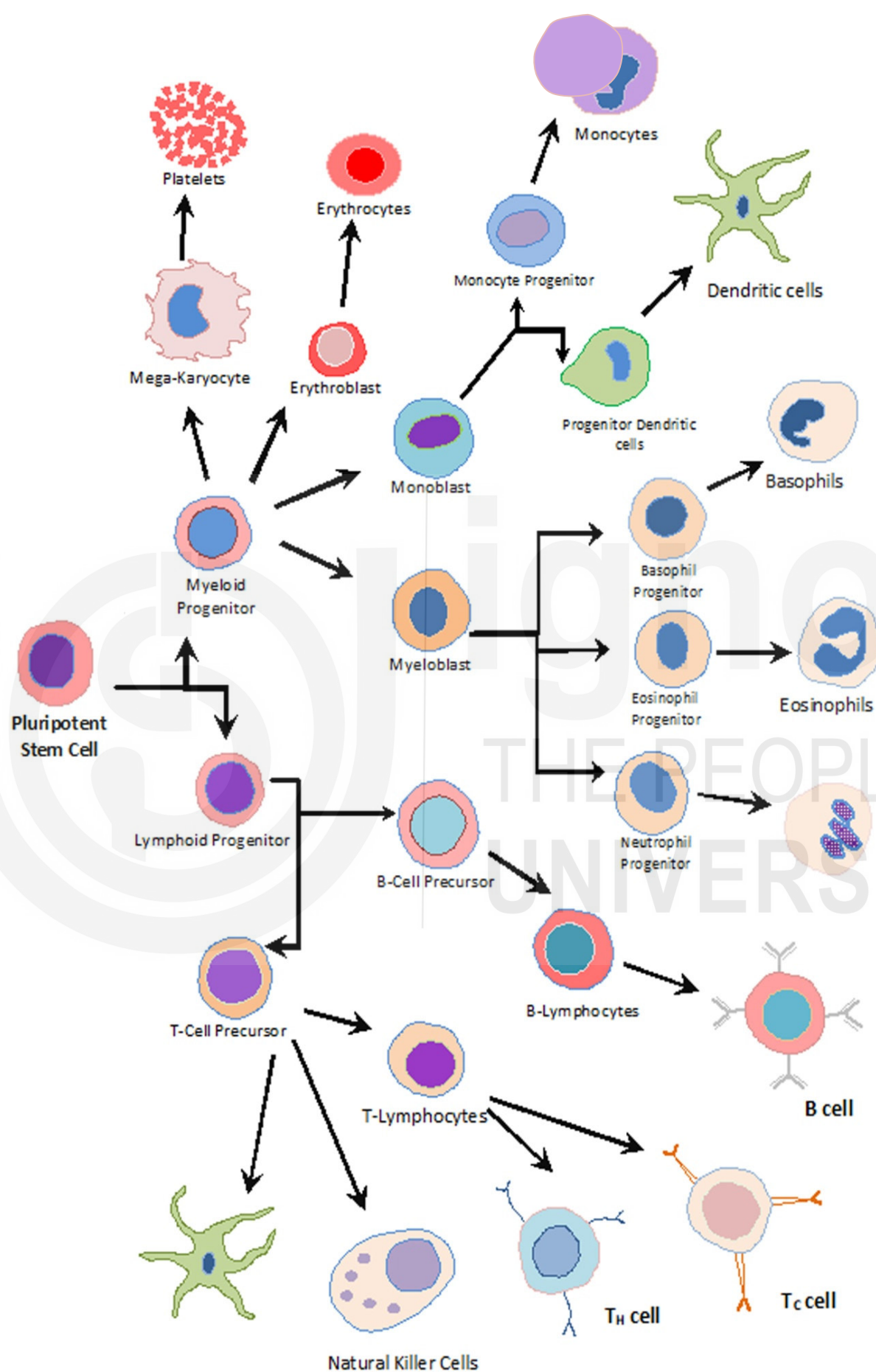


Fig. 5.1: Haematopoiesis.

5.3 HEMATOPOIESIS

In humans, during fetal stage hematopoiesis begins in the first weeks of embryonic development during the yolk sac stage. Then HSC migrates to liver from the yolk sac and subsequently to the spleen. Therefore, during the third to seventh months of gestation these two organs play vital roles in hematopoiesis (Table 5.1).

Table 5.1: Sites of Hematopoiesis in humans

Stage	Sites
Fetus 0–2 months 2–7 months 5–9 months	Yolk sac Liver, Spleen Bone marrow
Infants	Bone marrow
Adults	Vertebrae, ribs, sternum, skull, sacrum and pelvis, proximal ends of femur

Thereafter, bone marrow becomes the primary hematopoietic organ, and hematopoiesis in the liver and spleen ceases. The multipotent hematopoietic stem cells differentiate to give rise to either a **common myeloid or lymphoid progenitor cell**. The differentiation of stem cell is determined by its microenvironment in terms of the nature and amount of growth factors. The ability for self-renewal in HSC is lost once they differentiate into progenitor cells and the fate of their cell lineage gets fixed. A common lymphoid progenitor cells give rise to Lymphoid cells like B-cells, T-cells, NK (Natural Killer) cells and some dendritic cells arise from common lymphoid progenitor. Red blood cells (erythrocytes) and other white blood cells are generated from myeloid stem cells.

5.3.1 Genetic Regulation of Hematopoiesis

The differentiation of pluripotent HSC into different types of cells involves the expression of lineage-specific genes in specific order at specific time period. The product of these genes control stem cell differentiation and act as critical components of regulatory networks. Studies using “**knockout**” mice (replacement or disruption of an existing gene using an artificial piece of DNA) has helped in understanding the genetic regulation of hematopoiesis. When specific gene is knocked out, it failed to generate RBC or specific WBC in the mice. Therefore, it can be inferred that this gene is essential for the development of those cells. Although much remains to be done, number of transcription factors playing important role in hematopoiesis has been identified using this technology. Some of these transcription factors control and regulate several or only single hematopoietic lineages of lymphocyte development. GATA-2, a family member of transcriptional factor which

recognizes a nucleotide motif in target gene that has GATA sequence is one such transcription factor that influences several lineages. A functional GATA-2 gene encoding factor is vital for the development of lymphoid, myeloid and erythroid lineage. Hence, in animals with GATA impaired gene, the progeny gets determinate during the embryonic development period. While transcription factor Ikaros, unlike GATA-2 is crucial for the development of lymphoid lineage cells only. In Ikaros knockout mice, the production of myeloid lineage erythrocytes, granulocytes, and other cells are unaffected and therefore, they survive embryonic development. Nevertheless, they display severely compromised immunity. Usually they die at an early age from infection as they are not able to produce substantial amount of B, T, and NK cells (Table 5.2).

Table 5.2 : Few Transcriptional factors essential for hematopoietic lineages

Factor	Dependent Lineage
GATA-1	Erythroid
GATA-2	Erythroid, myeloid, lymphoid
PU.1	Erythroid (maturation stage), myeloid(later stages), lymphoid
Bmi-1	All hem
Ikaros	Lymphoid
Oct-2	B lymphoid (differentiation of B cells into plasma cells)

5.3.2 Growth Factors in Haematopoiesis

The hematopoietic cells in culture require a variety of growth factors for their survival, proliferation, differentiation and maturation. They are also known as hematopoietic cytokines. These factors are recognized from their ability in promoting formation of colonies in a bone-marrow culture. Colony Stimulating Factor (CSF), a family of acidic glycoprotein is one of the first cytokines to be identified. Another significant hematopoietic cytokine produced in the kidney is erythropoietin (EPO). Erythropoietin is a glycoprotein that plays significant role in inducing production and regulating terminal development of RBCs. Studies have revealed that the capacity of cytokine in promoting growth and differentiation depends on the presence of cytokine receptors in the target cell. Therefore, the fate of progenitor cell is directly related to the type of receptors on the cells surface that are unique to that cytokine. Many different cytokines and their receptors play important roles in inducing cell proliferation, promoting differentiation, maturation, preventing apoptosis and functioning of mature cell. Several other growth factors also function at different stages of haematopoiesis. Some important growth factors are mentioned in the table below (Table 5.3).

Table 5.3: Growth Factors in Hematopoiesis

Acts On	Growth factor type
Stromal cells	IL-1, TNF pluripotent stem cells SCF, Flt-L
Multipotent progenitor cells	IL-3, GM-CSF, IL-6, G-CSF,
Thrombopoietin committed progenitor cells	G-CSF, M-CSF, IL-5, Thrombopoietin

Legend: Flt-L, Flt ligand; G- and GM-CSF, granulocyte and granulocyte-macrophage colony-stimulating factor; IL, interleukin; M-CSF, macrophage colony-stimulating factor; SCF, stem cell factor; TNF, tumour necrosis factor.

Hematopoiesis naturally maintains a steady-state between the development of mature blood cells and loss mainly due to aging. It is estimated that an average human being must produce 3.7×10^{11} WBCs per day to maintain steady-state levels. White blood cells have differential life spans; for instance certain T lymphocytes live from a few days to 20-30 years for neutrophils. However, erythrocyte life span is about 120 days before being phagocytised and digested in the spleen. To maintain the steady state level of different blood cells, hematopoiesis is regulated by complex mechanism. In emergency condition, during haemorrhages or infection, there is an inbuilt adaptability of rapid cell production by ten to twentyfold. Management of steady-state hematopoiesis is carried out in various ways, including: precise quantity and class of cytokines that is released by stromal cells of bone marrow, cytokines produced by activated T cells and macrophages having hematopoietic activity, controlled expression of receptor in stem cells and progenitor cells for hematopoietically active cytokines.

A failure in either of these regulatory frameworks or a combination of them can have significant consequences. Anomalies in the production of hematopoietic cytokines or expression of their receptors may lead to unchecked cell proliferation contributing to the development of leukemia. Finally, in every hematopoietic family the number of cells recruited through cell division and cells destroyed by cell death maintains the cells population. The rate of cell proliferation and differentiation can be affected by one or a combination of regulatory factors. Therefore, the fate of a hematopoietic cell to die is also decided by these factors.

5.3.3 Nexus between Hematopoiesis and Apoptosis

Cells actively bring about their own demise by an induced and ordered process called programmed cell death (PCD) or **apoptosis**. Programmed cell death is a very crucial for steady-state maintenance of different types of cell populations.

A number of molecular steps are involved during apoptosis, such as intracellular protein activation, cell shrinkage, chromatin content condensation, nuclear fragmentation, cleavage in a cell of intra-nucleosomal genetic material, lead to its death. Each white blood cells has a distinct life span after which they die by programmed cell death. For instance, blood circulating in an adult human contains around 5×10^{10} neutrophils which have a life span of just a few

days until the onset of programmed cell death. Therefore, death of the cells along with the continuous production of neutrophils, maintains a steady state of cells number. Leukemia would develop if programmed cell death does not occur. Hence, PCD plays a vital role in keeping a check on proper numbers of hematopoietic progenitor cells. Death of cells can be PCD or from natural process of aging and damage which is termed as necrosis. The former, i.e., Apoptosis is distinctly different from **necrosis** (Fig. 5.2). In necrosis, the damaged cell swells and burst losing its contents which then initiates a harmful inflammatory reaction.

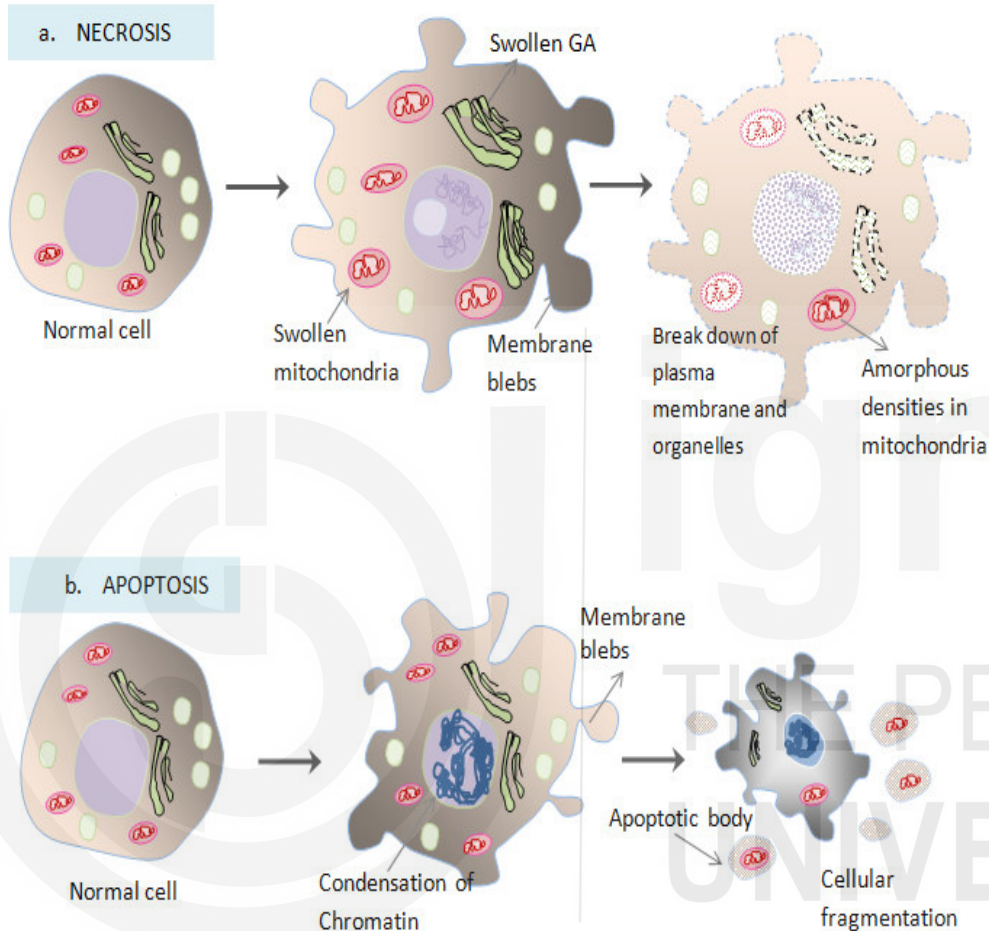


Fig. 5.2: Schematic diagram depicting internal cellular changes occurring during apoptosis (Fig a) and necrosis (Fig. b).

SAQ 1

State whether the following statements are 'True' or 'False':

- i) Hematopoietic stem cells are pluripotent cells.
- ii) Stromal cells affect hematopoietic stem cell differentiation by providing a hematopoietic- inducing microenvironment.
- iii) Necrosis, the cell death-related changes that arise from damage.
- iv) Myeloid stem cells generate progenitor cells of lymphocytes and NK cells.
- v) In humans, during fetal stage hematopoiesis begins in the yolk sac for first weeks of embryonic development.

5.4 CELLS OF IMMUNE SYSTEM

You have already studied in this courses that our immune system can be divided into two types of responses; **innate immunity** and **adaptive immunity/acquired immunity**. The innate immune response is a nonspecific first line of defence. It is a highly complex integration of various components. Cells that are involved in innate immune response comprises of neutrophils, basophils, eosinophils, dendritic cells, mast cells and macrophages. Adaptive immune response is a very specific response as it tailors its attack to a specific antigen previously encountered. Adaptive immune response is mediated by B lymphocytes, T lymphocytes and Natural killer cells. In general, the immune response requires three types of cells, namely lymphocytes, leukocytes (white blood cells) and monocytes (Table 5.4). The diverse collection of immune cells arise from multipotent HSC which differentiates to either a myeloid lineage cells or lymphoid lineage cells.

Table 5.4 : Normal adult blood cell count

Cell Type	Cells/mm ³	Total leukocytes (%)
Red Blood cells	5.0×10^6	
Platelets	2.5×10^5	
Leukocytes	7.3×10^3	
Neutrophil	$3.7-5.1 \times 10^3$	50-70
Lymphocyte	$1.5-3.0 \times 10^3$	20-40
Monocyte	$1-4.4 \times 10^2$	1-6
Eosinophil	$1-2.2 \times 10^2$	1-3
Basophil	$<1.3 \times 10^2$	<1

5.5 CELLS OF MYELOID LINEAGE

Cells from the myeloid lineage are derived from a common myeloid progenitor in the bone marrow during haematopoiesis. Myeloid lineage is a primary component of the innate immune system – the non-specific, first line of defense. Four types of cells arise from common myeloid progenitor cells: megakaryocytes, erythroblast, myeloblast and monoblast. The large multinucleated megakaryocytes cells produce platelets, which are necessary for normal blood clotting. Erythroblast give rise to Red blood cells (RBC). Myeloblast differentiates to form the effectors of the granulocytes including neutrophils, eosinophils and basophils. Monoblast cells mature into monocytes which, in turn, develop into macrophages. White blood cells have primary function of protecting the body from infection. Bone marrow produces white blood cells, and organs including spleen, liver, and kidneys regulate their growth levels. These cells types are broadly characterized into **granulocytes** and **agranulocytes** based on the presence or absence of cytoplasmic granules or sacs.

5.5.1 Granulocytes

Granulocytes are large spherical shaped cells with characteristic lobed nuclei. Their membrane-bound cytoplasmic granules get stained very precisely with Wright's stain. Granulocytes are to a greater or lesser degree, phagocytic cells with much shorter-life span as compared to erythrocytes.

There are three types of granulocytes (Fig. 5.3):

- **Neutrophils:** They are the most abundant white blood cell constituting about 50 – 70% of circulating WBC. They arise from myeloid progenitor cell. The nuclei of neutrophils consist of three to six lobes and due to this nuclear heterogeneity are often referred to as polymorphonuclear leukocytes (PMNs). Since the granules of neutrophils take up both acidic and basic dyes they are termed as neutrophils. Neutrophils are the first cells to arrive at infection site. Neutrophils are drawn to inflammation sites and phagocytic cells that engulf and destroy target cells under the influence of chemotactic factors stimulated by tissue damage (complement protein, clotting proteins and T cell derived cytokines). They release their granules which acts as lysosomes digesting cellular macromolecules and also cause self-destruction.
- **Eosinophils:** Eosinophils constitute 2-4 % of the circulating white blood cells and are around the size of neutrophils. The nucleus of eosinophils is bi-lobbed having a U-shaped appearance under microscope in blood smears. The cytoplasm is granular which stains from brick red to crimson with acidic dyes. Typically, eosinophils are located in the stomach and intestinal connective tissues. They are phagocytic in nature targeting the antigens bound with antibody. This antigen-antibody complex signal the destruction of antigen by eosinophils. Eosinophils are active particularly during parasitic infection like helminths and release a toxin called Major basic protein, which help in removal of the parasite from the body.

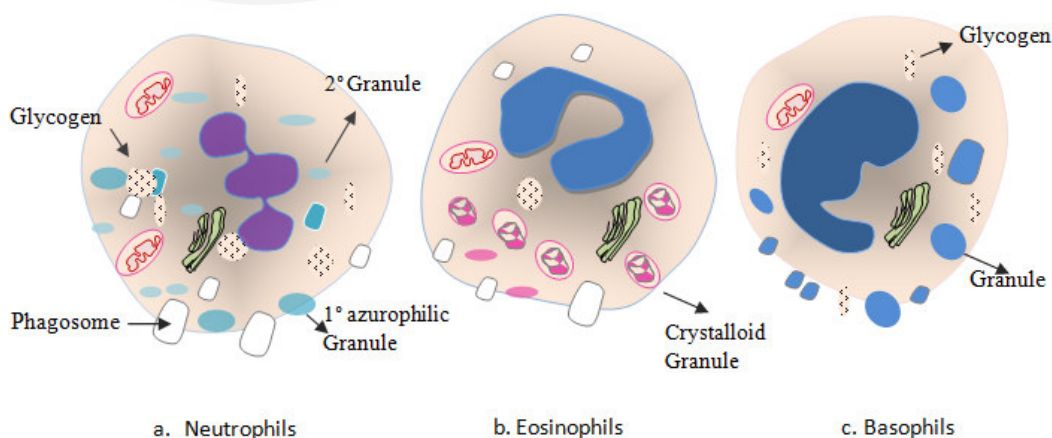


Fig. 5.3: Morphology of granulocytes. a) Neutrophil b) Eosinophil c) Basophil.

- **Basophils:** Basophils are found in the blood in low numbers with an average of just 0.5–1 %. The nucleus is multi-lobed and the cytoplasm contains large, coarse granules containing histamine which have an affinity towards basic dyes. Cross-linking of the IgE causes the basophils

to release histamine. Histamine, an active mediator of blood vessel dilation, causes increased blood flow and capillary permeability to facilitate rapid transfer of white blood cells to infected areas.

- **Mast Cells:** Mast cells are released as undifferentiated cells into the blood during hematopoiesis. They only undergo differentiation after leaving the blood stream and enter into tissues. Mast cells are abundantly found in the connective tissues of different organs, respiratory, genito-urinary, digestive mucosal epithelial tissues and skin. Like basophils, they possess histamine containing cytoplasmic granules in large numbers. These granules also contain important pharmacologically active mediators. Mast cells, along with basophils from the blood play an important role in allergy development.

5.5.2 Agranulocytes

Agranulocytes are the white blood cells that lack visible granules in their cytoplasm and have characteristically larger nucleus. There are two types of agranulocytes: Lymphocytes arising from common lymphoid progenitor cells and monocytes from common myeloid progenitor cells. Monoblast cells of myeloid lineage mature into monocytes which, in turn, develop into macrophages (Fig. 5.4).

Monocytes: Monocytes constitute 3-8 % of white blood cells. They are the greatest in size amongst the white blood cells and have an average diameter of 18 μm . They have pale-blue cytoplasm and a darkly stained purple nucleus that is distinctly U-shaped or kidney-shaped. They differentiate into extremely mobile macrophages or dendritic cells with remarkable appetites as circulating monocytes migrate from blood to tissue.

Macrophages: Macrophages are found scattered in the body, some macrophages take up residence in specific tissues and become fixed macrophages and others remain motile as free/ wandering, macrophages. Free macrophages migrate across the tissues by amoeboid movement. Differentiation of a monocyte into a tissue macrophage involves a number of changes like five to tenfold enlarged cell size; increase in both number and complexity of the intracellular organelles; and attaining increased phagocytic capacity, higher levels of hydrolytic enzymes production, and secretion of a number of soluble factors.

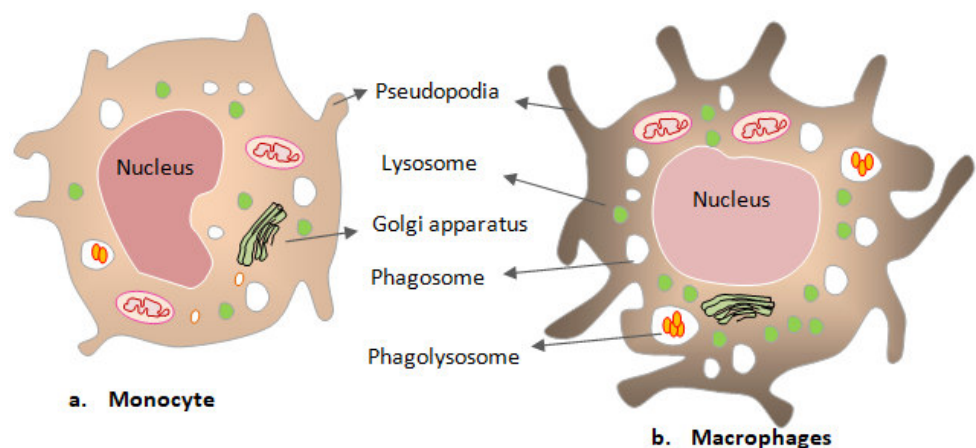


Fig. 5.4: Typical Morphology of a) Monocyte b) Macrophage.

In several tissues, macrophage-like cells perform various roles and are termed accordingly. Macrophages found in the lungs are termed as **alveolar macrophages**, those found in the connective tissues are termed as **histiocytes**, **Kupffer cells in liver**, **mesangial cells**, **osteoclast**, and **microglial cells in kidney, bones, and brain respectively**. During resting state, a number of stimuli activate macrophages. The initial activating stimulus is the phagocytosis of a particulate antigen. The function of macrophage may be further enhanced by cytokines and activators like IFN- γ secreted by activated helper T cells. In contrast to resting macrophages, activated macrophages show greater efficiency in tackling the impending pathogens. These activities include; improved capability in killing phagocytosed microbes, enhanced inflammatory mediators production, and improved capacity for T cell activation. Furthermore, they secrete different cytotoxic proteins that aid killing of a wide variety of pathogens, including intracellular bacteria, virus-infected cells and tumour cells. These activated macrophages also show higher expression of MHC class-II molecules allowing them to behave as antigen-presenting cells more effectively.

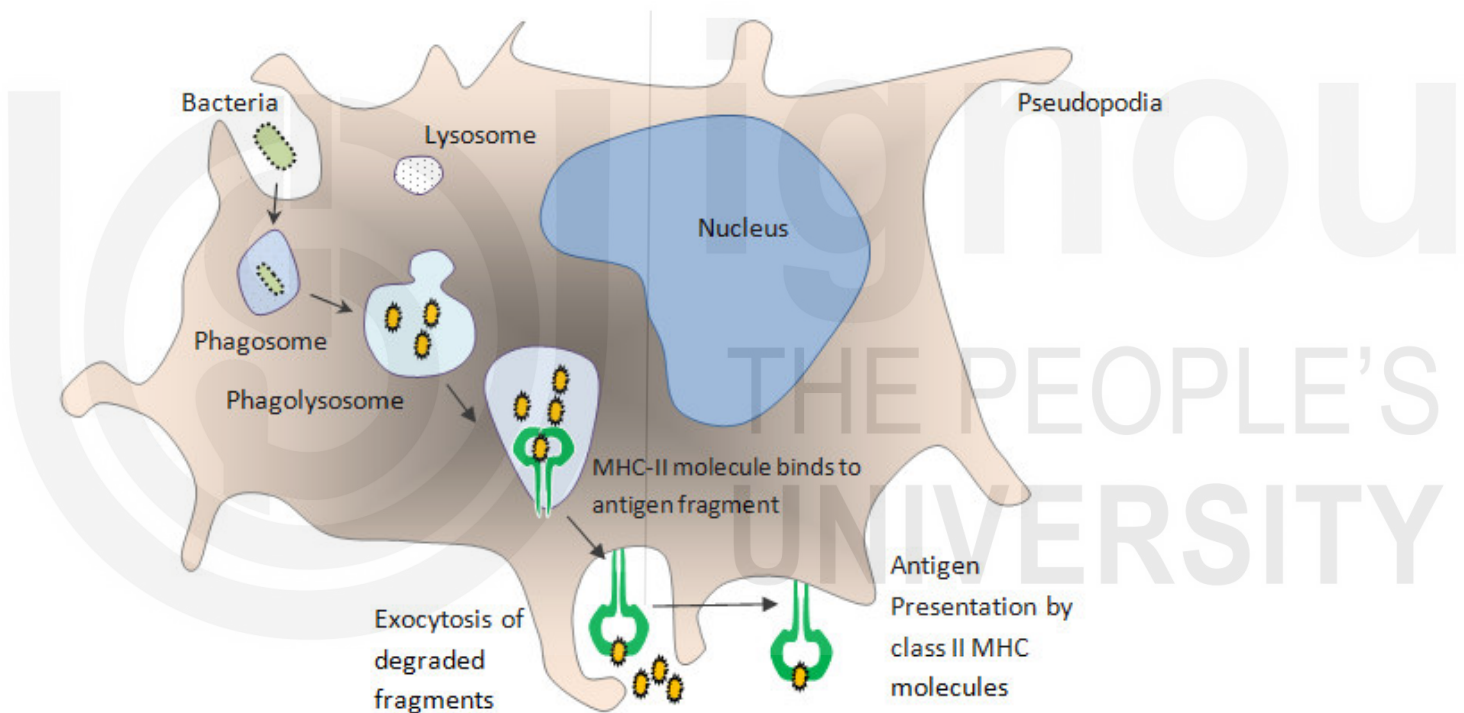


Fig. 5.5: Phagocytosis and exogenous antigen processing by macrophage.

Antigens that are not of intracellular origin – exogenous antigen and antigens whose source of origin is intracellular – endogenous antigen may be ingested and digested by macrophages. During phagocytosis, initially macrophages get attracted and migrate towards various chemical substances produced in an immune response by chemotaxis. In the next step of phagocytosis, the antigen gets adhered to the macrophage cell membrane after which membrane protrusions stretch around the attached material, called pseudopodia. Pseudopodia encircle the attached antigen and fuse with the membrane forming a structure called phagosome. A phagosome moves towards the interior region of the cell and fuses with lysosome. The structure thus formed is called phagolysosome. Lysosome releases hydrolytic enzymes and lysozyme that digest the ingested material which is then eliminated by process

of exocytosis. Macrophages can also deploy alternate mechanism through which antigens could be made more susceptible to phagocytosis. This mechanism is known as Opsonization. In opsonization, an antigen say a bacteria is coated with a appropriate antibody forming an antigen-antibody complex. A second antibody forms a link between the antigen-antibody complex and receptor present on the macrophage membrane. The antigen-antibody complex readily binds to macrophage receptor due to high affinity of the second antibody with the receptor than the complex alone. Hence, this results in enhanced phagocytosis (Fig. 5.6). Therefore such molecules that bind to both the antigen-antibody complex with the macrophage to improves phagocytosis are known as **opsonin**.

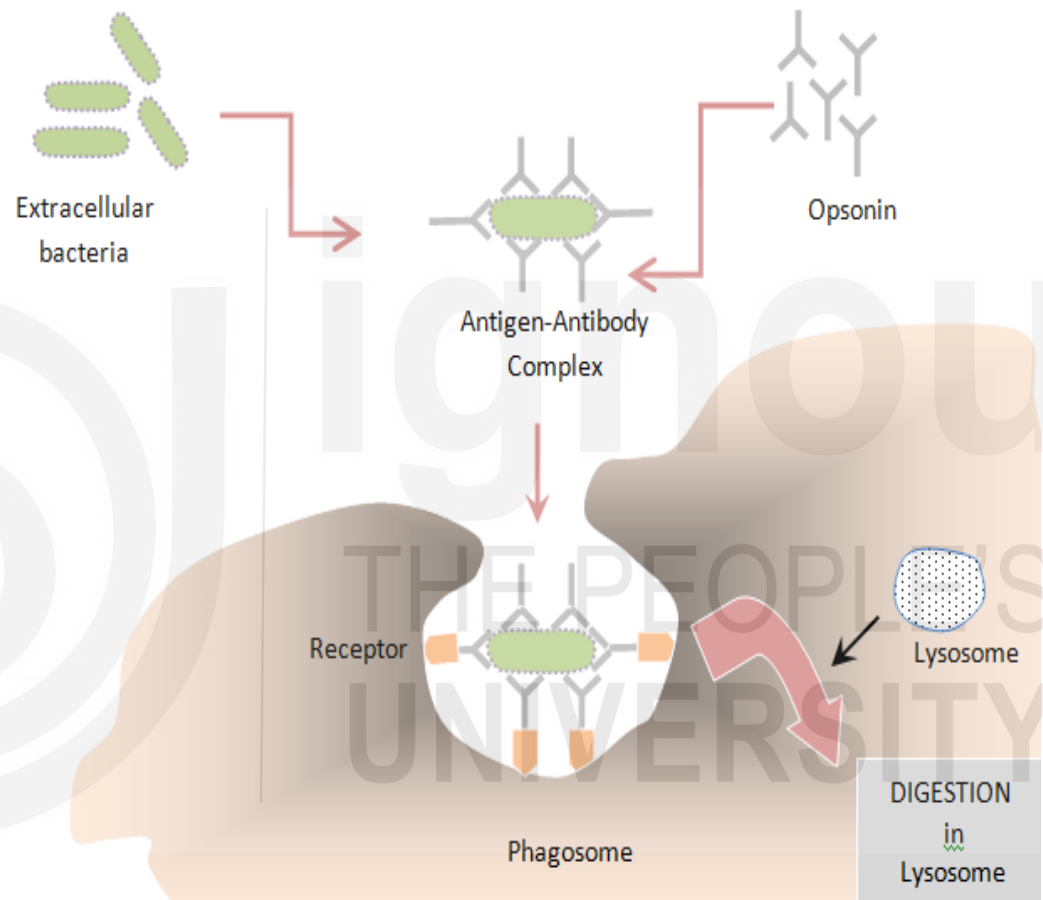


Fig. 5.6: A phagocytic cell recognizes the antibody (opsonin) on the surface of an antigen.

Dendritic cells: Dendritic cells (DC) are located in skin, inner layers of the nose, lungs and gastrointestinal tract. Dendritic cells are identified easily from their characteristic projection of membrane that give the appearance similar to the dendrites of nerve cells. There are four types of dendritic cell (Fig. 5.7). They are: Langerhans cells, intestinal and myeloid dendritic cells arising from myeloid lineage and lymphoid dendritic cells from lymphoid lineage of HSCs at different location. All the four types of dendritic cells constitutively expresses class II MHC molecules and B7 (co-stimulatory molecules) in high levels. Hence, the DCs are much efficient than other APCs which require trigger to function as an antigen presenting cells. The immature DC acquires and processes the antigen through phagocytosis or endocytosis and mature dendritic cells present it to T helper cells.

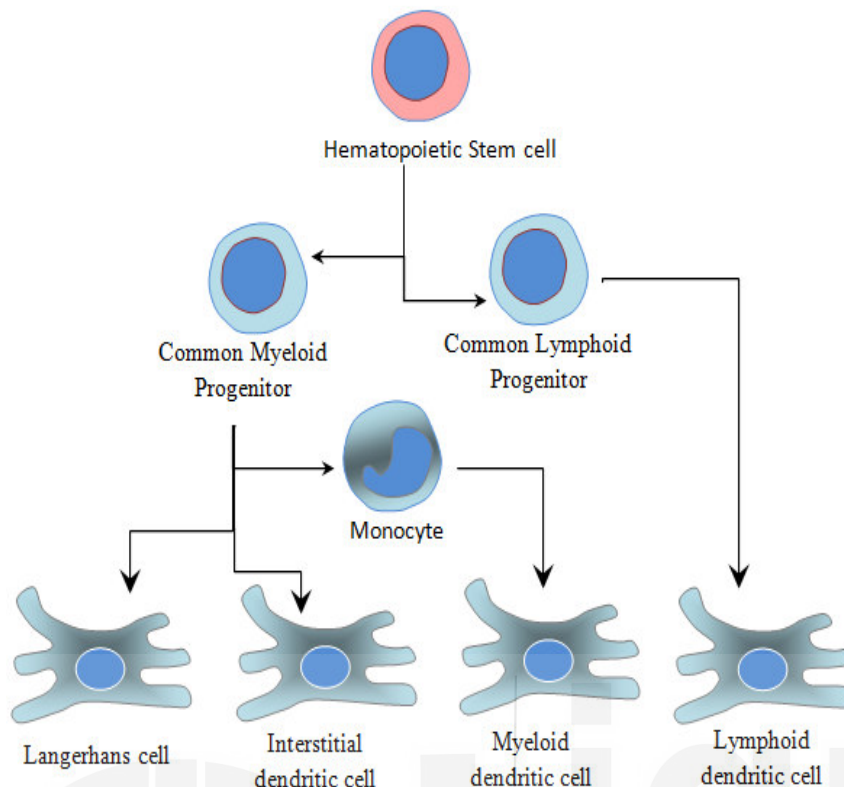


Fig. 5.7: Dendritic cell lineage from HSC.

Following microbial invasion or during inflammation, DCs of common myeloid progenitor (Langerhans and interstitial) migrate and drain into lymph nodes for presenting antigens to Helper T Cells. Some dendritic cells found in the lymph follicle of lymph nodes which are rich in B cells. They are called Follicular dendritic cells due to their exclusive location in the lymph follicles of lymph nodes region. Unlike other dendritic cells, these cells do not participate in antigen presentation since they lack MHC class II molecules on their membrane. On the contrary, they show high level of antibody receptors on their membrane for the binding of antigen-antibody complexes. These immune complexes present in the blood stay in the blood for longer duration and hence leads to an effective immune response.

SAQ 2

Fill in the blanks:

- (i) cell has bi-lobed nucleus that appears U shape in blood smear.
- (ii) Macrophage in the liver is called cell.
- (iii) dendritic cells lack MHC class II in its membrane, hence do not participate in antigen presentation.
- (iv) act as opsonin which improves the process of phagocytosis by macrophages.
- (v) are the first cells to arrive at infection site.

5.6 CELLS OF LYMPHOID LINEAGE

The elemental cells of the immune system responsible for adaptive immunity are the Lymphocytes. They are derived from a common lymphoid progenitor cells. Lymphocyte possesses fundamental immunological trait of specificity, memory, diversity and recognition of self/nonself. Lymphocytes constitute the second most numerous white blood cells which is about 20-40% of the white blood cell population. Morphologically, lymphocyte is characterized by dark purple stained, spherical shaped, large nucleus slightly depressed that occupies most of the cell volume with a pale blue cytoplasm. Lymphocytes are in constant circulation (blood and lymph) through the tissues spaces and lymphoid organs thereby integrating the immune system to a high level. Lymphocytes can be divided into B cells, T cells and natural killer (NK) cells based on their function and cell-membrane components. Unlike B and T cells which possess surface marker, NK cells are large granular lymphocytes without surface markers. Resting lymphocytes are usually small-sized naïve lymphocytes with densely packed chromatin, poorly developed endoplasmic reticulum and Golgi apparatus and a few mitochondria. Once, the antigen is encountered by naïve lymphocytes, these resting lymphocytes are triggered to proliferate. Hence, the cell cycle arrest is withdrawn and these cells enter the cell cycle. During this process, lymphocytes enlarge thrice its size and also organellar complexity is enhanced and these are named as **lymphoblasts** (Fig. 5.8).

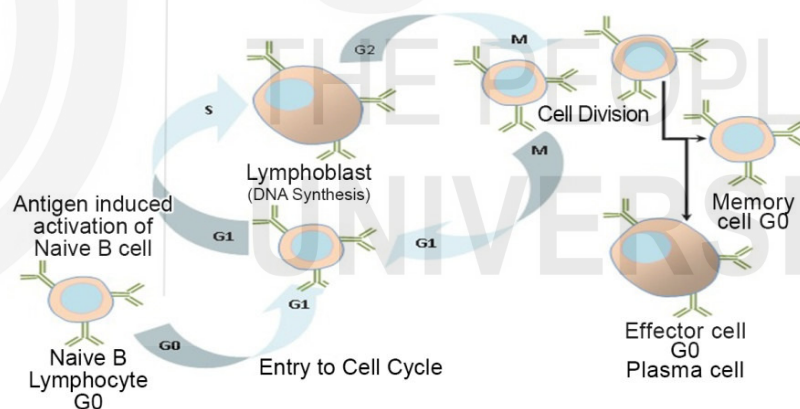


Fig. 5.8: Fate of antigen-activated small lymphocytes.

5.6.1 B lymphocytes

B cells were first discovered in the lymphoid tissue called 'Bursa of Fabricius' which is a tissue associated with gut in birds. In humans and mice B cell maturation takes place in bone marrow. After maturation, B cells migrate to secondary immune organs. B cells constitutes about 10-15% of circulating lymphocytes. B cells on encountering with an antigen, bind with the antigen through their membrane receptors called B cell receptors (BCR). This interaction stimulates rapid division of B cells and differentiation into Plasma and memory cells (Fig. 5.9). Numerous identical copies of antibodies specific to the antigen are produced and released into the bloodstream by these plasma cells to target the circulating antigens. For the same antigen, memory B cells develop immunological memory to impart enhanced reactivity to the

same antigen on the second encounter. In order to kill the antigen, the B cell immune reaction requires the synthesis of antibodies in the blood and lymph, hence, it is also often referred to as **humoral immune response** (humor, or body fluid). Memory B cells have a long life span, therefore, provide life-long immunity against many pathogens. Production of antigen specific antibodies and antigen presentation to T helper cells for their activation are the two most significant functions played by B cells in providing the protection to the body.

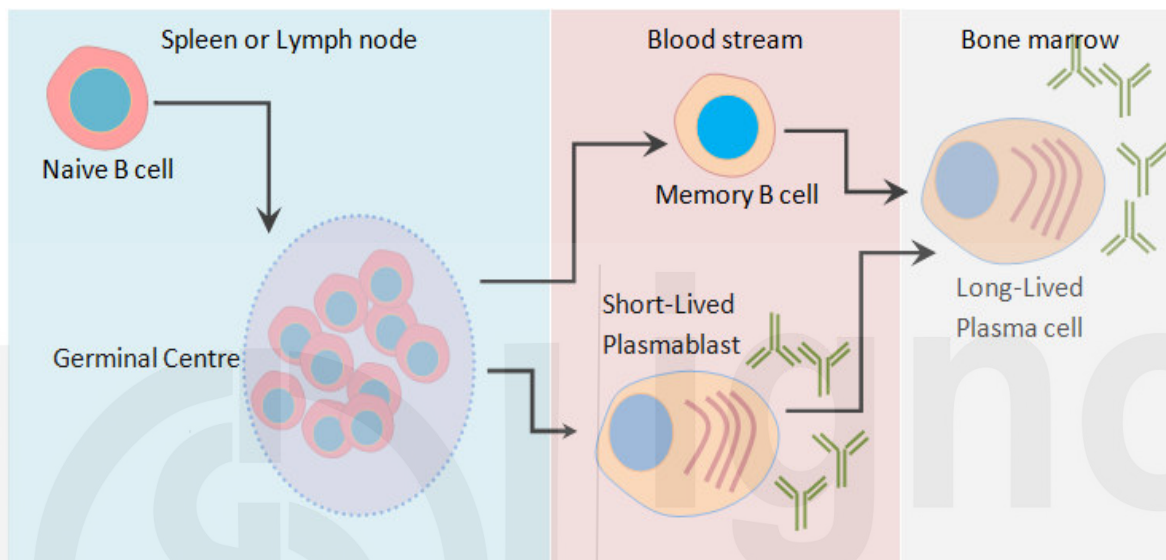


Fig. 5.9: Development of memory B cells and plasma cells.

Mature B cells display different molecules on their membrane. They are;

- i) CD45 is form B220: Marker protein present in mature and immature of B cells. It is, however, not expressed specifically by B-lineage cells, unlike antibodies.
- ii) Class II MHC: Facilitate the B cell to serve in presenting antigens.
- iii) CR1 and CR2: Complement receptors for binding of complements and its products.
- iv) CD32: Receptors for antibody class G (IgG) also designated as FcγRII.
- v) B7-1 and B7-2: Membrane proteins that interact with T_H cell membrane receptors (CD28, CTLA-A).

CD40: Costimulatory protein required for the activation of antigen presentation and crucial role in the survival and production of plasma cells or memory cells.

5.6.2 T Lymphocytes

T lymphocytes or T cells like B cells originate from the bone marrow. They migrate to thymus gland, where they undergo maturation. T lymphocytes response to antigens is responsible for the adaptive immunity or cell-mediated immunity. Unlike humoral immunity, where B cells possess the capability to bind with soluble antigens, T cells cannot bind on its own in cell-mediated

immunity. They require **major histocompatibility complex (MHC)** molecules to present the antigens. MHC molecules are expressed on the cell surface of APCs or cancerous cells or virus infected cells.

T cells can be identified by the presence of two membrane molecules, namely CD4 and CD8 (Fig. 5.10). The CD4 are limited to recognising antigens that are bounded to class II MHC, CD8 is limited to recognising antigen with MHC class I molecules. Additionally, the following membrane molecules are expressed by most mature T cells:

- CD28: a receptor responsible for co-stimulatory function with molecules of B7 family that are present in antigen presenting cells.
- CD45: Tyrosine phosphatase type receptor, present abundantly in T cells as integral membrane protein.

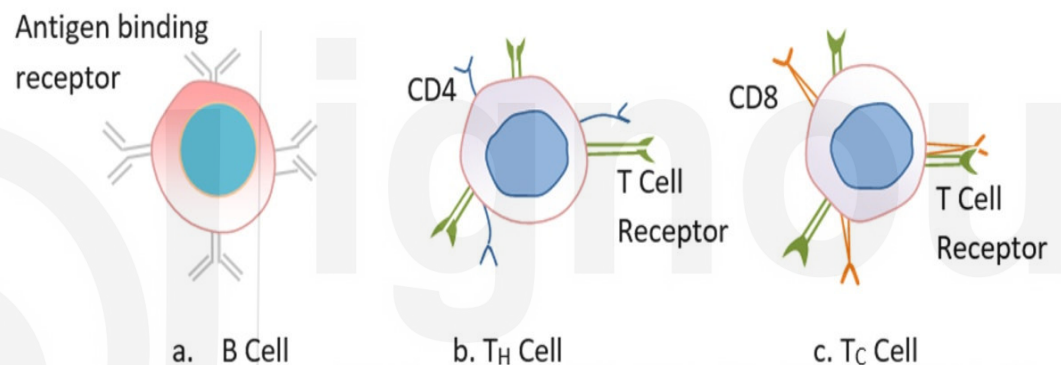


Fig. 5.10: Characteristic membrane molecules on lymphocytes. (a) B cells possess membrane-bound antibody (b) T helper cells (T_H) bear CD4 membrane receptors (c) T Cytotoxic cells (T_C) bear CD8 membrane receptors.

There are mainly three sub-populations (Fig. 5.11):

1. **Helper T cells (T_H):** T cells expressing CD4 molecules are called T_H cells. They are restricted to recognising only those antigens bound to MHC class II molecules.
2. **Cytotoxic T cells (T_C):** T cells expressing CD8 molecules are called T_C cells.
3. **Suppressor T cells (T_S):** T_S are specific T cells involved in suppressing humoral and cell-mediated immune responses.

The activation of T cells therefore, requires specific conditions since T_C and T_H cells are restricted to antigens presented through Class I and II MHC molecules respectively. When T_C cells get activated they result in proliferation and differentiation into effector and memory T cells. Activation of T_H cells produces cytokines (growth factors) that stimulate B cells to proliferate and generate antibodies, trigger macrophages, and T cytotoxic cells to destroy antigen/cells. T_C cells on activation proliferate and differentiate into **cytotoxic T lymphocyte (CTL)**, under the influence of T_H-derived cytokines. The CTL

does not typically secrete many cytokines unlike like the T_C cell, instead they display cytotoxic or cell killing activities. The CTL plays a crucial role in tracking the body's cells and removing any antigen-showing cells, such as virus-infected cells, tumour cells, and tissue graft cells. Those cells that display antigens-MHC class I complex are referred to as **altered self-cells**; these are **CTL targets**.

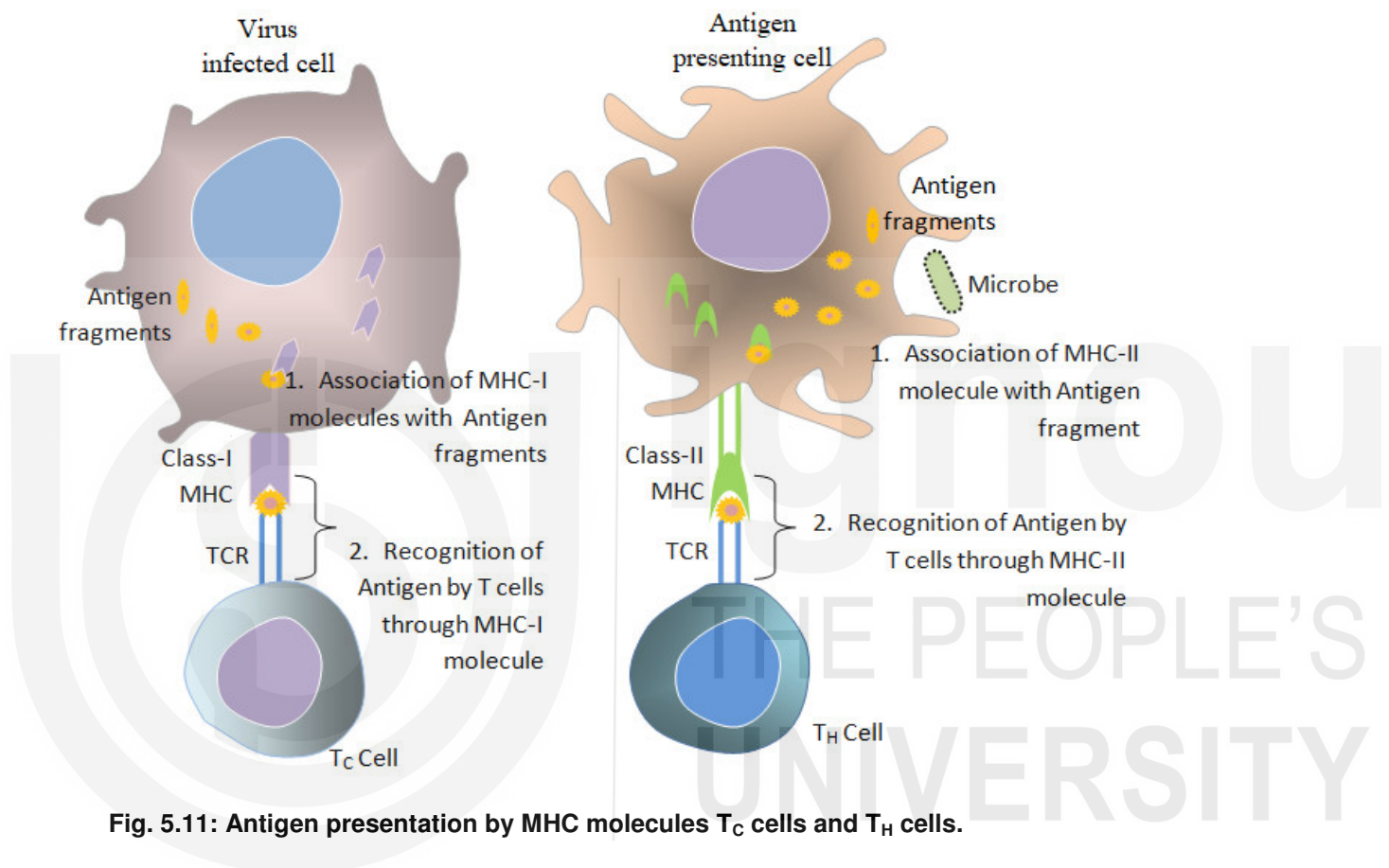


Fig. 5.11: Antigen presentation by MHC molecules T_C cells and T_H cells.

5.6.3 Natural Killer Cells (NK cells)

Natural Killer cells constitute about 5 to 10% of the total lymphocyte population. NK cells do not exhibit specific membrane receptors or molecules like the T and B cells lines. They use different strategies to identify potential target cells. Anomalies like a reduced Class I MHC molecules display, irregular profile of surface antigens that some tumour cells and some virus infected cells exhibit are determined by the receptors of the NK cells. An alternative approach through which NK cells identify and destroy possible target cells is antibody-dependent cell mediated cytotoxicity. In certain tumor cells and virus infected cells, antibodies binds with the antigens that are displayed on the cell surfaces against which antibody mediated immune response can be produced. NK cells express CD16 membrane receptor for antibody (IgG) Fc region and hence, they are able to bind to these antigen coated antibodies and eventually kill these targeted cells (Fig. 5.12).

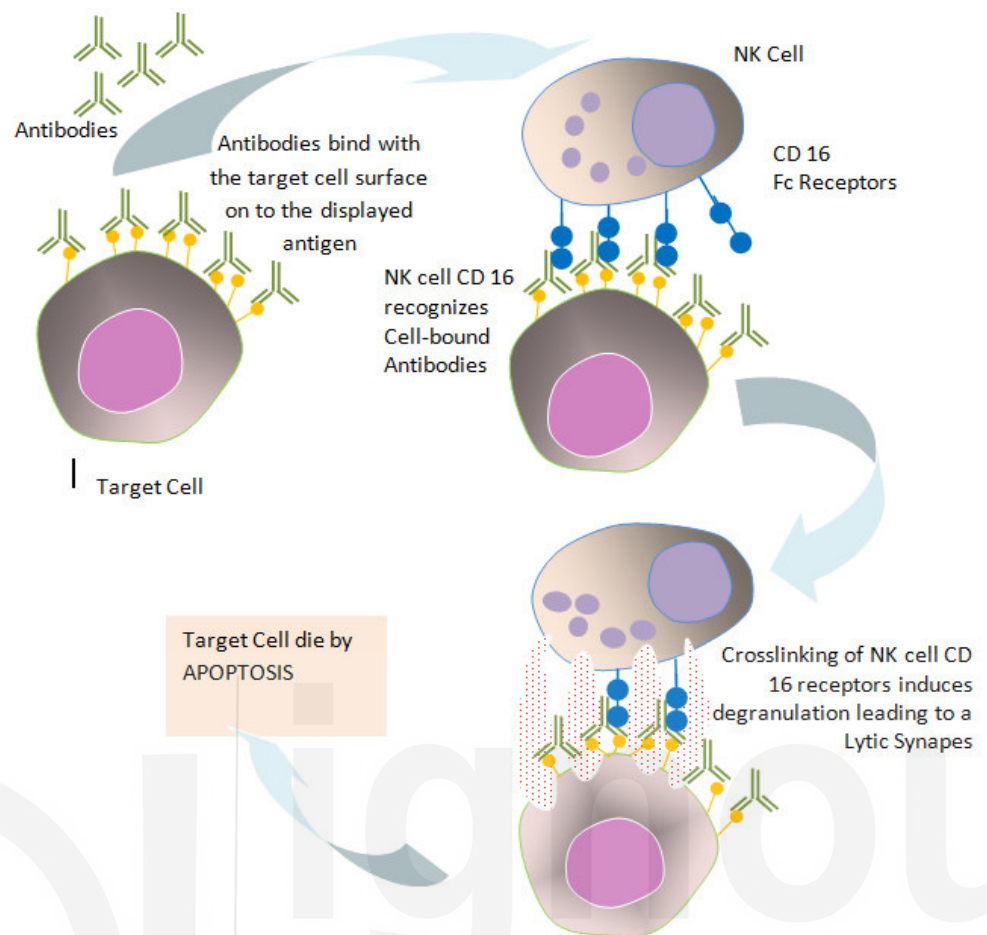


Fig. 5.12: Schematic illustration of Antibody-dependent cell-mediated cytotoxicity (ADCC) mediated by NK cells.

5.7 EPITOPES-ANTIGENIC DETERMINANTS

Immune cells do not interact with the entire antigen but interact with discrete region of an antigen. These immunologically active regions of the antigen are recognised primarily by B and T-lymphocytes, as well as free molecules of antibodies which are known as epitope or antigenic determinant. Studies with small antigens have shown that diverse epitopes present on the same antigenic molecule can be recognised by B and T cells. However, the mode of antigen recognition is distinctive, where a soluble antigen is recognised by antibody in case of B cells. In case of T cells, recognition is made possible through display of antigens peptides on MHC molecules of APCs and altered self-cells. Therefore, the epitopes must be highly accessible and exposed to the surface on the antigen.

5.7.1 Properties of B Cell Epitopes

The ability to function as a B cell epitope depends on the nature of antigen binding site on antibody molecules of B cells. The interaction between B cells and the antigens involves a binary complex comprising of antibody, the membrane receptor and antigen by a weak non-covalent interaction (Fig. 5.13). Hence, having complementary shape is required for the formation of binary complex between the binding site of the antibody and the epitope. This criterion presents certain constraint on the epitope's properties.

The size of the epitope is always smaller than the antibody binding site and the shape of the epitope recognizable by the antibody is determined by the amino acid sequence of the binding site and the chemical environment they generate for any given antigen-antibody reaction. For a proper binding of antigen-antibody, the epitope of B cell should possess on its protein surface a protruding region that is accessible. These regions are usually rich in hydrophilic amino acids. The sizes of B cell epitopes are not constant. B cell epitope proteins are generally large and often associated with the antibody molecule with a larger, flatter, complementary surface. Also, complex proteins comprise numerous overlapping epitopes of B cells, some of which are immuno-dominant. Often, small ligand gets itself attached to antibody's deep pocket region. These ligands include, carbohydrates, haptens, oligonucleotides and peptides.

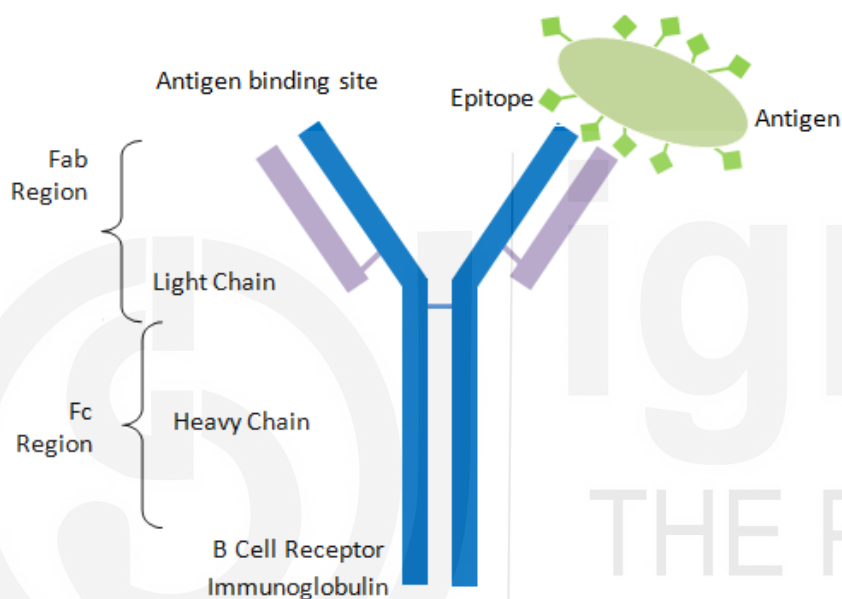


Fig. 5.13: Interaction between B cells and the antigens involves a binary complex.

Table 5.5 B and T cells Epitopes

Features	B Cells	T cells
Interaction with Antigen	Binary complex (Antibody and Antigen)	Ternary complex (TCR, Antigen and MHC molecules)
Binding of soluble antigen	Yes	No
Association of MHC molecules	Not required	Essential to display processed antigen
Chemical nature of antigens	Protein, polysaccharides, lipids	Mostly proteins
Properties of Epitope	Accessible, hydrophilic	Internal linear peptides produced by processing and displayed along with MHC molecules.

5.7.2 Properties of T cell Epitopes

Soluble native antigens are not recognised by T cells directly. However, when the antigens are processed into antigenic peptides and presented on MHC

molecules they can be recognized. The T cell recognized antigenic peptide forms a tri-molecular complex of MHC molecule-antigenic peptide-T cell Receptor (Fig. 5.14). T cell epitopes are mostly internal and are exposed as antigenic peptides after undergoing processing inside APCs or altered self-cells.

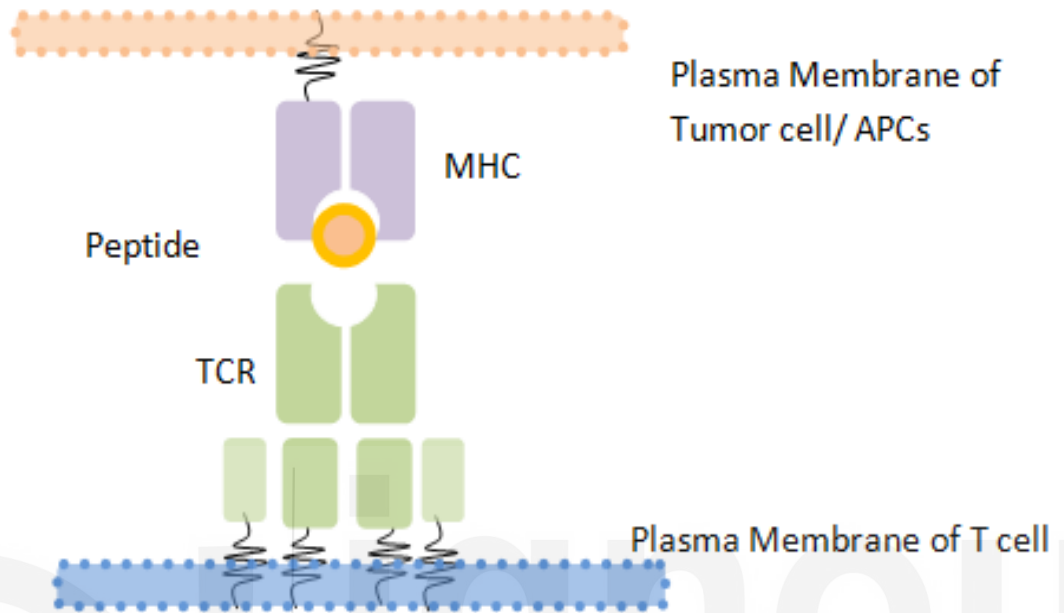


Fig. 5.14: Tri-molecular Complex of TCR-Antigenic peptide-MHC molecule.

5.8 SUMMARY

In this unit you have studied:

- Various types of white blood cells are present in our body and they play an important role in immune response. Lymphocytes are the fundamental immune cells that possess immunological features.
- Blood cells originate from hematopoietic stem cells. Progenitor stem cells arise from the HSC which further differentiate into other specific forms of cells.
- During hematopoiesis, the stem cell differentiation involves the expression of various genes and transcription factors that establish the lineage. Several hematopoietic growth factors (cytokines) cause various blood cells to proliferate and differentiate.
- To maintain a steady-state of blood cells population, hematopoiesis is closely regulated. The programmed cell death balances each of the lineages' cell division and differentiation.
- Macrophages and neutrophils are specialised phagocytotic cells. Opsonization promoted by antibodies coating antigen enhance the phagocytosis.
- Activation of macrophages secrete factors that mediate adaptive immune response. They also present antigens to T_H cells through class II MHC molecules.

- Dendritic cells play an important role in the activation of T_H cells along with macrophages and B cells by processing and presenting antigen bound to class II MHC molecules and by supplying the required co-stimulatory signal. Unlike the other dendritic cells, follicular dendritic cells promote the activation of B cells but play no role in the activation of T cells.
- Lymphocytes are differentiated into three forms namely, B cells, T cells and natural killer (NK) cells based on the presence or absence of specific membrane receptors.
- B and T lymphocytes form the central cells of adaptive immune response. On encountering antigens they undergo proliferation and differentiation into effector and memory cells.

5.9 TERMINAL QUESTIONS

- 1) What is hematopoiesis?
- 2) Differentiate between the B and T cells epitopes.
- 3) Expand the following:
(i) ADCC (ii) CTL (iii) MHC (iv) TCR (v) PMNs

5.10 ANSWERS

Self Assessment Questions

1. True and False
(i) True, (ii) True, (iii) True, (iv) False.
2. Fill in the blanks:
(i) Eosinophils, (ii) Kuffer cells, (iii) Follicular, (iv) Antibodies
(v) Neutrophils.

Terminal questions

- 1) Hematopoiesis refers to the production of all types of blood cells including formation, development, and differentiation of blood cells from hematopoietic stem cells.
- 2) Refer to Table 5.5.
- 3) i) Antibody-dependent cellular cytotoxicity
ii) Cytotoxic T lymphocyte
iii) Major histocompatibility complex
iv) T-cell receptor
v) Polymorphonuclear leukocytes.

SUGGESTED READINGS

1. Judith A Owen; Jenni Punt; Sharon A Stranford; Patricia P Jones; Janis Kuby. Text book of Immunology, New York: W.H. Freeman, ©2013. 7th edition.
2. Online Education Resources.
3. **Kuby** Immunology. W. H. Freeman & Co. Fourth edition, 2000. 670 pages.
4. Immunology by Ivan **Roitt**, Jonathan Brostoff, and David Male. Mosby, London. 6th edition, 2001. 480 pages.
5. Basic Immunology by Abul K. **Abbas** and Andrew H. **Lichtman**, Saunders, 2001. 309 pages.
6. Kuby Immunology 8th edition by Punt, Stranford, Jones and owen. W,H, Freeman McMillan learning., New York.
7. The Cell: A Molecular Approach by Geoffery M Cooper, 8th edition, Oxford University Press.
8. Owen, J. A. Punt, J. Stranford, S. A. Jones, P. P and Kuby, J. (2013). 7thEdition *Kuby Immunology*. New York, USA: W.H. Freeman and Company. ISBN-13: 978-1429219198.
9. Delves, P.J. Martin, S.J. Burton, D.R. and Roitt, I. M. (2017). 13thEdition. *Roitt's Essential Immunology*. New Jersey, USA: Wiley-Blackwell Science. ISBN: 13: 978- 1118415771.

Acknowledgement of Figures

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