

VOL**2****IMMUNOLOGY-II**

BLOCK 3**WORKING OF THE IMMUNE SYSTEM****7**

BLOCK 4**IMMUNE SYSTEM IN HEALTH AND DISEASES****73**

Indira Gandhi
National Open University
School of Sciences

Block

3

WORKING OF THE IMMUNE SYSTEM

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BLOCK 3: WORKING OF THE IMMUNE SYSTEM

Immunology is a rapidly growing field with new discoveries, advances in techniques and previously unappreciated connections coming to light every day. The immune system's job is to help identify and eliminate dangerous germs that enter the body before they can cause disease or damage. There are two types of immunity: innate and adaptive. Innate immunity is the immune system that is present when you are born. Vaccines provide active immunity to disease. Vaccines do not make you sick, but they can trick your body into believing it has disease, so it can fight the disease.

The present volume (Volume 2) comprises 2 blocks namely:

Block 3: Working of the Immune System

Block 4: Immune System in Health and Diseases

Block 3 comprises four units and Block 4 consists of two units in all.

Unit 9 "Working of the Immune System-I" gives an insight of general organization and Inheritance of MHC, structure, function and role of MHC I, and MHC II. It further speaks about antigen processing and presentation: endogenous and exogenous pathways, endogenous or cytosolic pathway of antigen processing and presentation. The unit ends with antigen processing and presentation via exogenous or endocytic pathways".

Unit 10 "Working of the Immune System-II" explains about B cell development which includes antigen dependent and independent phases and regulation of B cell development. Then it discusses about B cell maturation, activation and differentiation. During the activation stage, there is a progressive rise in the affinity (average) of the antibodies generated, which is known as affinity maturation, as well as a change in the antibody isotype produced by the B cells, which is known as class switching.

Unit 11 deals with the T-cell mediated immunity wherein you will learn about the cytotoxic T-cells and Helper-T cells and their activation, maturation, differentiation, and memory cells. The positive selection and negative selection and their role in T cell development are also discussed.

Cytokines are low molecular weight signalling proteins or glycoproteins. They mediate and regulate immunity inflammation and haematopoiesis. WBCs and various other cells secrete cytokines. You will study about components, functions, pathways of complement system in Unit 12. Unit further speaks about complement related diseases and regulation of complement system.

Objectives

After studying this block you will be able to:

- discuss general organization and inheritance of MHC,
- describe structure, function and role of different classes of MHC,
- differentiate between endogenous and exogenous pathways of antigen presentation,
- comprehend proteolytic breakdown of antigen protein into peptides,
- explain B lymphocytes,

- discuss B-cell receptors and their working mechanism,
- explain the development process of B-cells,
- describe regulation of B-cell development,
- define T cell- mediated cellular immunity,
- classify the T cells, and explain the antigen presenting cells and their role in T cell activation,
- differentiate between the cytotoxic T cells and helper T cells,
- describe the T cells positive selection and negative selection,
- discuss the process of T differentiation and maturation,
- explain the memory T cells,
- define cytokines, and enlist the actions, properties and functions of cytokines,
- define complement system and list out its functions and activation pathways,
- give examples of complement related diseases, and
- explain the regulation of the complement system.

UNIT 9

WORKING OF THE IMMUNE SYSTEM-I

Structure

- | | |
|---|--|
| 9.1. Introduction
Objectives | 9.6. Endogenous or Cytosolic pathway of antigen processing and presentation |
| 9.2. General organization and Inheritance of MHC | 9.7. Antigen processing and presentation via Exogenous or Endocytic pathways |
| 9.3. Structure, function and role of MHC I | 9.8. Summary |
| 9.4. Structure, function and role of MHC II | 9.9. Terminal Questions |
| 9.5. Antigen processing and presentation: endogenous and exogenous pathways | 9.10. Answers |

9.1 INTRODUCTION

In this unit, we shall provide some details of the Major Histocompatibility complex (MHC). It's a collection of surface proteins located on the cell membrane of nucleated cells. The term "Histo" refers to tissues, and "compatibility" means cells living together harmoniously. It plays a critical work to identify the antigen between self and non-self body and is also responsible for antigen presentation.

Next, we deal with Antigen-presenting cells, which can process antigens, either exogenously or endogenously. In addition, two types of MHC are working in immunity. First, the MHC II molecules recognize T helper (Th) cells, and second MHC-I molecules identify T cytotoxic (Tc) cells. Antigens found within the cytosol of human cells, such as viral proteins, intracellular bacteria proteins, and tumour antigens, are known as endogenous antigens. Antigens from viruses or cancer cells degrade in the endoplasmic reticulum, where they are converted into peptide fragments that attach to class I MHC molecules. Peptide class I MHC complex is then transported to the cell membrane as a result of this process. However, the specialized antigen presentations cells, including the macrophages, dendritic, and B cells, use the exogenous pathway

to present peptides. These peptides derive from exogenous antigens and are endocytosed and degraded by acid-dependent proteases in endosomes and finally presented on MHC class II molecules, which are recognized by CD4+ T_H cells.

Objectives

After studying this unit, you should be able to:

- ❖ discuss general organization and inheritance of MHC,
- ❖ describe structure, function and role of different classes of MHC,
- ❖ explain the formation of the MHC peptide complex and the location of Antigen processing,
- ❖ differentiate between endogenous and exogenous pathways of antigen presentation,
- ❖ comprehend proteolytic breakdown of antigen protein into peptides, and
- ❖ explain how peptides get from the cytoplasm through endoplasmic reticulum and endocytic vesicles.

9.2 GENERAL ORGANIZATION AND INHERITANCE OF MHC

MHC is also known as the Human Leukocyte Antigen (HLA) complex in humans since this molecule was first studied on leukocytes (Fig. 9.1). In the 1930's Peter Gorer and George D. Snell gave the concept of rejection of foreign tissue to be an immune response to MHC molecules. In 1980, George D. Snell was awarded the Nobel prize in medicine for "discoveries relating to genetically determined features on the cell surface that regulate immunological responses."

MHC plays a significant role in determining whether the transplanted tissue of the donor will be histocompatible or histoincompatible with the recipient's tissue. MHC is a collection of genes present on chromosome 6 and is related to immune system functions. Because distinct alleles exist in different individuals within a population, the MHC genes are highly polymorphic. As a result, the evolution of MHC polymorphism plays an important role in the species' survival. As a result, a population will not succumb to a mutated or a new pathogen; because some individuals can win over the pathogen by developing an adequate immune response (Fig. 9.1).

MHC genes code for antigen-presenting proteins on the cell surface that falls into three categories: MHC-I, MHC-II, and MHC-III molecules. These molecules are with epitopes and mediate intracellular recognition and self/non-self discrimination. As a result, they play a crucial role in both humoral and cell-mediated immune responses. The MHC molecules have a critical role in antigen recognition by appropriate T cells. In addition, they bind and express antigen peptides derived from pathogens on the cell surface for further action. Thus, the immune response is injurious to the pathogen, and infected cells are being killed.

The three different classes of MHC are as follows:

- Class I MHC** molecules are glycoproteins found on all nucleated cells in the body. It shows the processed antigenic peptides to T_C cells (cytotoxic T cells).
- Class II MHC** molecules are also the glycoproteins that are expressed on antigen-presenting cells (APC's - Dendritic cells, Macrophages and B cells). In addition, they present the processed antigenic peptides to T_H cells (helper T cells).
- Class III MHC** molecules are secreted proteins that are highly diverse, both structurally and functionally. They include components of the complement system, tumour necrosis factors, heat shock proteins etc. As they are not membrane proteins, they do not play any role in antigen presentation, although they are critical in immune function.

Heat shock proteins are a group of highly conserved proteins produced by cells in response to various stresses, including heat shock, nutrient deprivation, oxygen radicals, and viral infection. These heat shock proteins are linked to certain autoimmune diseases.

The structure and function of class I and class II MHC molecules are quite similar. Membrane glycoproteins of both types act as highly specialised antigen-presenting molecules in their respective environments. They form stable complexes with antigenic peptides that are displayed on cell surfaces in order for T cells to recognise and recognise the antigens as foreign. Class III MHC molecules, on the other hand, are a category of secreted proteins that have no structural or functional resemblance to class I or class II MHC molecules.

The MHC is extremely polymorphic, and alleles are expressed in a co-dominant manner. In the same cells, both maternal and paternal gene products are expressed. A haplotype is a collection of alleles. Every MHC class I and MHC class II gene has many alleles. Genetic recombination creates new allelic combinations (Fig. 9.1).

MHC genes are a group of genes that code for three different types of MHC molecules and are found in a long stretch of DNA on chromosome 6. Class I MHC is encoded by the A, B, C, E, F, and G genes, while the six D genes encode class II MHC. Many evolutionary biologists have been drawn to MHC because of the enormous levels of allelic variability seen within its genes.

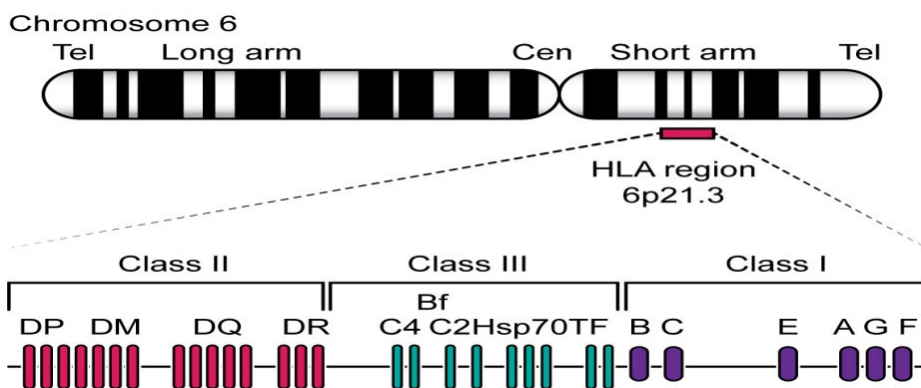


Fig. 9.1: Human chromosome 6:HLA region.

SAQ 1

What is HLA? What is its primary function?

9.3 STRUCTURE, FUNCTION AND ROLE OF MHC I

A long 45 kDa chain (heavy chain) makes up MHC Class I molecules, and it is associated non-covalently with a smaller β 2-microglobulin molecule of 12 kDa (light chain). The α -chain is a polymorphic transmembrane glycoprotein, and polymorphic MHC genes encode it. On the other hand, the β 2-microglobulin molecule is an invariant protein encoded by a highly conserved gene on a separate chromosome. The interaction of chain with β 2-microglobulin is needed for Class I MHC molecule expression on cell membranes. The class I MHC is not expressed on the cell membrane in the absence of the β 2-microglobulin protein. For example, Daudi tumour cells lack class I MHC molecules on the cell surface as they cannot synthesize β 2-microglobulin molecules (Fig. 9.2).

The hydrophobic transmembrane segment and the hydrophilic cytoplasmic tail of the polymorphic transmembrane anchor it in the plasma membrane. The α -chain is divided into three distinct external domains: α 1, α 2, and α 3. A total of 90 amino acids make up each domain (Fig. 9.2). The 25 hydrophobic amino acids in a transmembrane domain are followed by a short stretch of hydrophilic (charged) amino acids. There are 30 amino acids in the cytoplasmic anchor region (Fig. 9.2). There are roughly 90 amino acids in the β 2-microglobulin. It is comparable to the α 3 domain of the α chain in size and organization, but it lacks a transmembrane region. Members of the immunoglobulin super family include both Class I MHC molecules and β 2-microglobulin. The constant area domains of the α 3 domain, β 2-microglobulin, and immunoglobulin were found to be homologous in the research.

Interacting domains are divided into two groups:

- 1) A membrane distal pair consists of domains α 1 and α 2 on either side of the membrane.
- 2) A membrane proximal pair has the α 3 domain and β 2-microglobulin, which are both membrane proximal.

Peptide-binding cleft on the top surface of the class I MHC molecule is formed by the membrane distal domains of the MHC molecule. The antigenic peptide binding cleft is large enough to hold an antigenic peptide of 8 to 10 amino acids (Fig. 10.2). Because it contains a region that interacts with the CD8 membrane receptor on T_C cells, the α 3 domain appears to be quite conserved. The interaction of β 2-microglobulin and a peptide with all three domains of the class I molecule is essential for the class I molecule to achieve its completely folded conformation. Many distinct peptides will be expressed on the cell surface concurrently by MHC class I molecules. Endogenous antigens that come from the cytoplasm are presented by MHC class I glycoproteins.

Thus, MHC class I molecules are responsible for the endogenous antigen processing pathway.

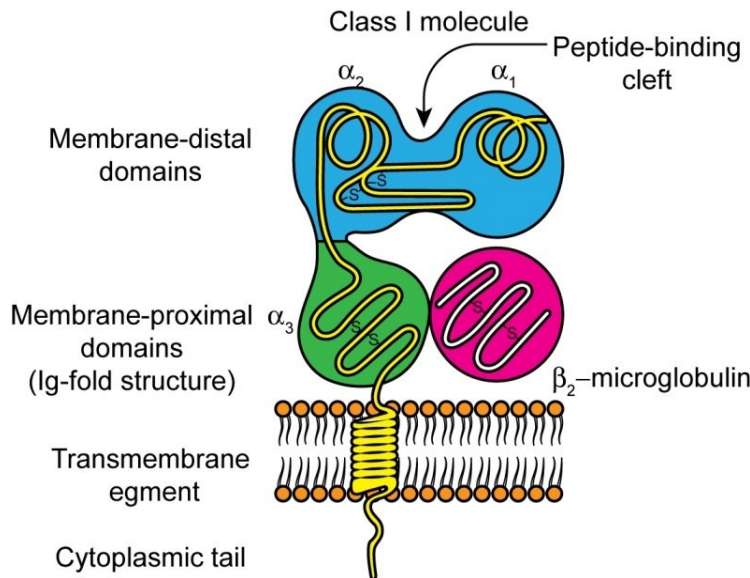


Fig. 9.2: Structure of class I MHC molecule.

SAQ 2

How many interacting domains are present in class I MHC molecule? What are they?

9.4 STRUCTURE, FUNCTION AND ROLE OF MHC II

MHC class II molecules are made up of two polypeptide chains. The α chains are 33 kDa and β 28 kDa, respectively. Non-covalent interactions are related to both the α chain and β chain. Class II and class I MHC molecules are similar, according to X-ray crystallographic results. The class II MHC molecules have a peptide-binding cleft identical to class I MHC molecules (Fig. 10.3). All of the glycoproteins that comprise class II MHC molecules are membrane-bound glycoproteins in a similar manner. Class I MHC molecules have external domains, a transmembrane segment, and a cytoplasmic anchor segment, all of which are present. Each chain of a class II MHC molecule has two outer domains, denoted by the numbers 1 and 2 (Fig. 10.3). Each β chain contains two external domains, β_1 and β_2 domains. The class II MHC molecules are considered as members of the immunoglobulin super family. Studies have revealed homology between the fold structure of α_2 domain and β_2 domain and the immunoglobulin constant region domains.

There are two pairs of domains that interact:

- 1) A membrane distal pair is made up of α_1 and β_1 domains.
- 2) A membrane proximal pair is made up of α_2 domain and β_2 .

The peptide-binding cleft on the top surface of the class II MHC molecule is formed by the membrane distal domains, which are composed of the α_1 and β_1 domains. It is composed of eight antiparallel strands on the bottom and eight antiparallel helices on the sides, which together form the peptide binding

cleft (Fig. 10.3). Class II MHC molecules lack the conserved residues that bind to the terminal residues of short peptides (as shown in class I MHC molecules) and cause an open pocket to form. Class I MHC molecules have these conserved residues. As a result, class I MHC molecules have a socket-like shape, whereas class II MHC molecules have an open-ended groove. There are differences between crystallised class I and class II MHC molecules, according to studies. The class II MHC molecules are a "dimer of dimers" made up of β heterodimers. The dimer is also positioned in such a way that the two peptide-binding clefts face opposing directions. Antigenic peptides bind to class II MHC molecules, then deliver them to CD4 membrane receptors on T_H cells. Exogenous antigens originating extracellularly from foreign entities such as bacteria are presented by MHC class II glycoproteins. MHC class II molecules thus control exogenous antigen processing pathways.

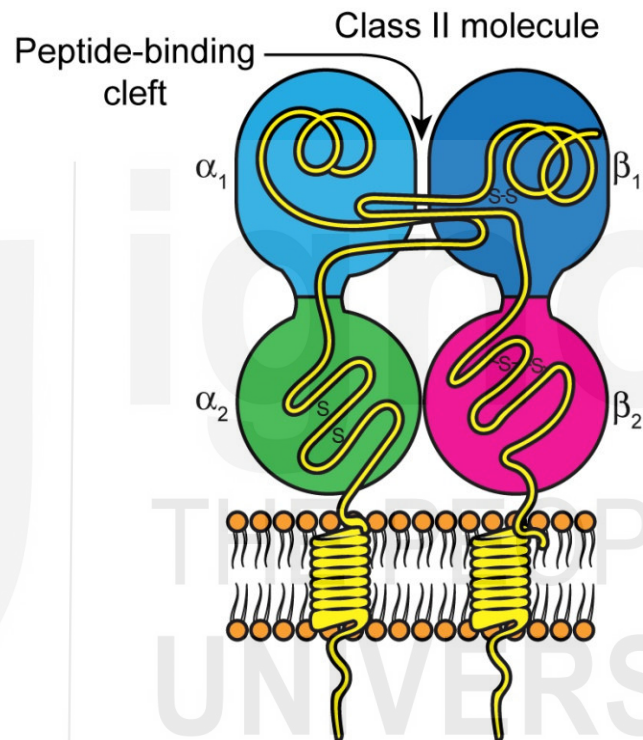


Fig. 9.3: Structure of class II MHC molecule.

SAQ 3

How many interacting domains are present in class II MHC molecule? What are they?

Self MHC restriction: Both class I and class II MHC molecules can only recognize the antigens presented by self MHC molecules. This self MHC restriction occurs in the thymus.

9.5 ANTIGEN PROCESSING AND PRESENTATION: ENDOGENOUS AND EXOGENOUS PATHWAYS

Immune response to antigen processing is a metabolic process in which proteins are broken down into peptides that can be displayed on the cell membrane alongside MHC molecules of class I or class II and recognised by T cells (Fig. 10.5). Immune recognition occurs when cells, such as Antigen-presenting cells (APCs), produce processed antigen on their cell surface and

MHC molecules in a form that T cells can recognise. It is the CD8+ T_C cells that are responsible for the recognition of the antigen presented with the class I MHC molecule. T cells recognize the antigen given in conjunction with the class II MHC molecule on CD4+ T_H cells (Fig. 10.5). It is possible to distinguish two types of antigen processing and presenting pathways, which are distinguished by the types of antigens that need to be processed and presented:

- 1) Antigen processing and presentation via the cytosolic or endogenous pathway.
- 2) Antigen processing and presentation via the endocytic or exogenous pathway.

9.6 ENDOGENOUS OR CYTOSOLIC PATHWAY OF ANTIGEN PROCESSING AND PRESENTATION

In the Endogenous or Cytosolic pathway, the endogenous antigens generated within the cell are processed and presented. For example, tumour cells, intracellular pathogens (*M. tuberculosis*) and virally infected cells (Fig. 9.7). The endogenous antigen is processed and displayed on the cell membrane with a class I MHC molecule, which the CD8+ T_C cell identifies and degrades (Fig. 9.7).

Broadly there are three steps involved in the endogenous pathway.

- 1) Antigen protein degradation into peptides by proteolytic enzymes.
- 2) Peptide's transportation (8 to 16 amino acids) from the cytoplasm to the rough endoplasmic reticulum.
- 3) Assembly of peptides with MHC molecules of class I.

10.6.1 Proteolytic Degradation of Antigen Protein into Peptides

The endogenous antigen proteins are large and cannot bind to class I MHC molecules. They are degraded into short peptides of about 8 to 10 amino acids by the proteasome. The cytosolic proteolytic system in the cell is known as a proteasome. The proteasome's large component (20S) is made-up of 14 subunits (Fig. 10.4). These subunits are arranged in a symmetrical ring structure that resembles a barrel. Protease activity is seen in some of the subunits. Proteasome consists of a narrow channel at each end through which the proteins enter. A small protein called ubiquitin is attached to many proteins which are targeted for proteolysis. The ubiquitin-protein complex is made up of the 20S proteasome and the 19S regulatory components, among other things (Fig. 10.4). This process is ATP dependent. Degradation of ubiquitin-protein complex occurs in the proteasome's central core, and the peptides are released (Fig. 10.4).

Molecular chaperones are protein complexes that play an essential role in protein folding and refolding. The unfolded polypeptides are produced as a by-product of protein synthesis during normal cell growth. However, misfolded proteins occur due to cellular stresses such as heat shock, oxidative stress, & pathological conditions. Therefore, molecular chaperones play a crucial role in protein folding and refolding. Thus, it maintains cell viability.

10.6.2 Peptide Transport from the Cytoplasm to the Rough Endoplasmic Reticulum

The peptides produced by the proteasome in the cytoplasm are transported to the lumen of the rough endoplasmic reticulum by TAP protein (transporter associated with antigen processing). This procedure necessitates ATP hydrolysis. TAP is a two-protein membrane-spanning protein that consists of TAP1 and TAP2 (Fig. 10.6). TAP selects peptides with 8 to 16 amino acids in length, as well as peptides with hydrophobic or basic carboxyl-terminal amino acids, are selected for use as anchor residues in class I MHC molecules (Fig. 10.6). However, the optimal peptide length required for binding to class I MHC molecule is nine. Trimming of peptides occurs with the help of amino peptidase present in the rough endoplasmic reticulum (e.g., ERAP). Therefore, TAP deficiency can lead to autoimmune disease.

10.6.3 Peptide Assembly using a Class I MHC Molecule

The rough endoplasmic reticulum is responsible for the production of the α -chain and 2β -microglobulin components of the class I MHC protein, which are found in the cytoplasm. A need for the formation of class I MHC molecules that can escape the rough endoplasmic reticulum is the binding of peptides into the peptide-binding cleft of the class I MHC molecule. Molecular chaperones are involved in the assembly process. Calnexin is the first molecular chaperone involved in the assembly of class I MHC molecules. Calnexin is a rough endoplasmic reticulum resident membrane protein. It supports the folding of a free class I α -chain by being associated with it. Calnexin is released when the class I MHC molecule binds to 2β -microglobulin, and the class I MHC molecule associates with another chaperone, tapasin (TAP associated protein) and calreticulin. Tapasin enables the acquisition of endogenous antigenic peptides by bringing TAP (transporter) bearing peptides into close proximity with class I MHC molecules. To stabilize the association, ERp57 (an enzyme-like protein) creates a disulphide bond with tapasin and non-covalently interacts with calreticulin. After accumulating antigenic peptides, it facilitates the release of the class I MHC molecule. The class I MHC molecule presents the endogenous antigen to CD8⁺ T_C cells. The CD8⁺ T_C cells (cytotoxic) will recognize the complex and initiate the immune response to lysis the infected cell.

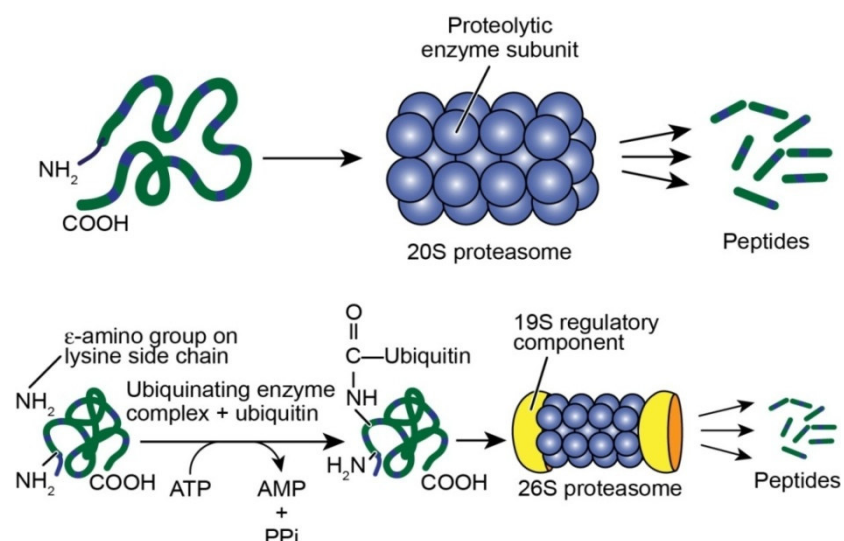


Fig. 9.4: Proteolytic system working on the degradation of antigen protein into peptides.

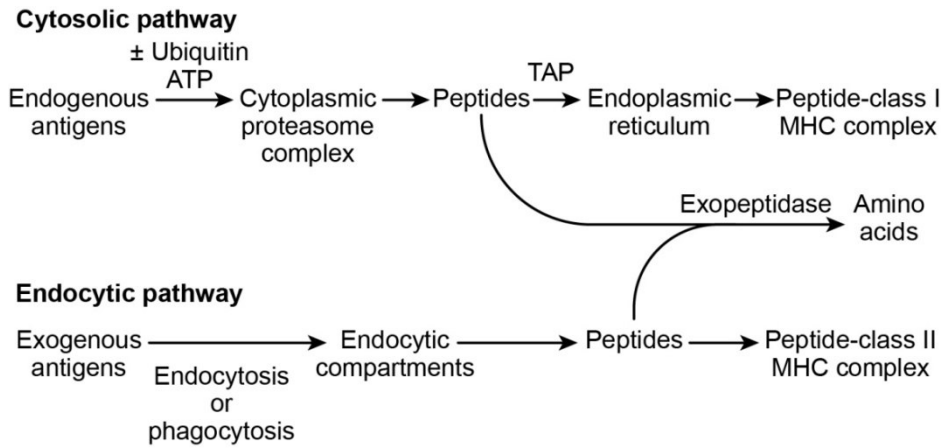


Fig. 9.5: Assembly of peptides in Cytosolic and Endocytic pathway.

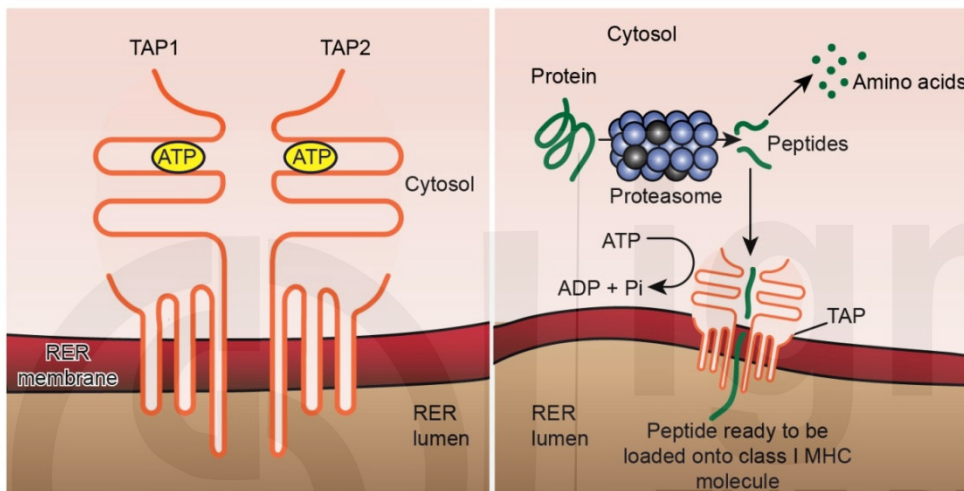


Fig. 9.6: Role of TAP and assembly of peptides with class I MHC molecule.

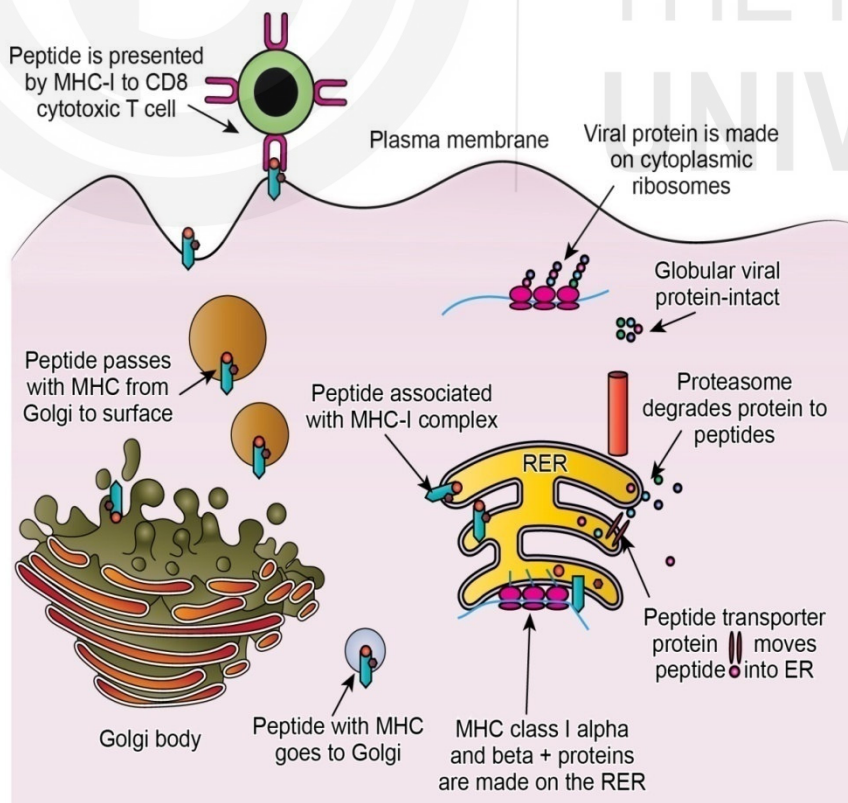


Fig. 9.7: Endogenous or cytosolic pathway of antigen processing and presentation.

SAQ 4

What is the name of the first molecular chaperon in the assembly process?
What is its function?

9.7 ANTIGEN PROCESSING AND PRESENTATION VIA EXOGENOUS OR ENDOCYTIC PATHWAYS

Exogenous antigens produced outside the cell, such as bacteria and intercellular pathogens, are processed and presented in the Exogenous or Endocytic pathway. At first, the antigen is phagocytosed and/or endocytosed by antigen-presenting cells (APC). Both macrophage and dendritic cells internalize the antigen by phagocytosis and endocytosis. Other APCs are either non-phagocytic or poorly phagocytic. For example, the antigen is internalized by B cells receptor-mediated endocytosis as it is non-phagocytic in nature (Table 9.1). Finally, the exogenous antigen is displayed on the cell membrane with a class II MHC molecule, which is recognised and degraded by CD4+T_H cells after it has been displayed (Table 10.1).

Table 9.1: Professional vs. non-professional antigen-presenting cells.

Professional antigen-presenting cells	Non-professional antigen-presenting cells
Dendritic cells Macrophages B cells	Fibroblasts in skin Glial cells in the brain Pancreatic β cells

Let us discuss these terms in this section.

Dendritic cells: These cells are antigen-presenting cells of the mammalian immune system. The primary function of dendritic cells is to capture, process and display it on the T cell surface.

Macrophage: The term macrophage derives from the Greek words "makro," which means "large," and "phagein," which means "to consume." Macrophages are immune system cells that form due to an infection or accumulate dead or dying cells. Macrophages are vast and specialized cells that are capable of recognizing, engulfing, and destroying target cells.

B cells: These cells are the heart of the adaptive humoral immunity, mediating the generation of antigen-specific antibodies against invading infections. B cells develop and originate in bone marrow, the soft fatty substance within bones, and it expresses membrane-bound antibody molecules with specificity to a particular antigen.

Fibroblasts: These cells are responsible for the production of collagen and extracellular matrix components and play a role in the skin's structural maintenance and healing. They are made up of mesoderm and may be found all over the skin.

Glial cells in the brain: The brain and nervous system include non-neuronal cells called glia. Glial cells divide into subtypes, such as astrocytes, oligodendrocytes, and microglia, each with its role. For example, according to new research, microglia, which are also the brain's primary resident immune cells, are thought to offer disease-modifying control of the other significant glial populations, astrocytes and oligodendrocytes.

Pancreatic β cells: Beta cells are found in islets, which are clusters of cells. These cells make insulin, which regulates blood sugar levels. Unfortunately, the immune system erroneously kills beta cells in people with type 1 diabetes. As a result, the pancreas cannot produce insulin without beta cells.

Broadly there are three steps involved in the exogenous pathway:

- 1) In endocytic vesicles, peptides are made from internalized antigens.
- 2) Transportation of class II MHC molecules to Endocytic vesicles.
- 3) Peptide assembly utilizing class II MHC molecules

In endocytic vesicles, peptides are made from internalized antigens.

In the three increasingly acidic compartments of the endocytic processing pathway, the foreign antigen is absorbed and digested into peptides. In the first compartment or early endosomes, the pH is 6 to 6.5 (Fig. 9.8). In the second compartment, or late endosome/endo-lysosome, the pH is 5 to 6. In the third compartment or lysosomes, the pH is 4.5 to 5 (Fig. 9.8). Internalized exogenous antigens travel from one compartment to the next, encountering hydrolytic enzymes and a decreasing pH in each one. The antigen is broken down into 13 to 18 amino acid peptides (Fig. 9.8).

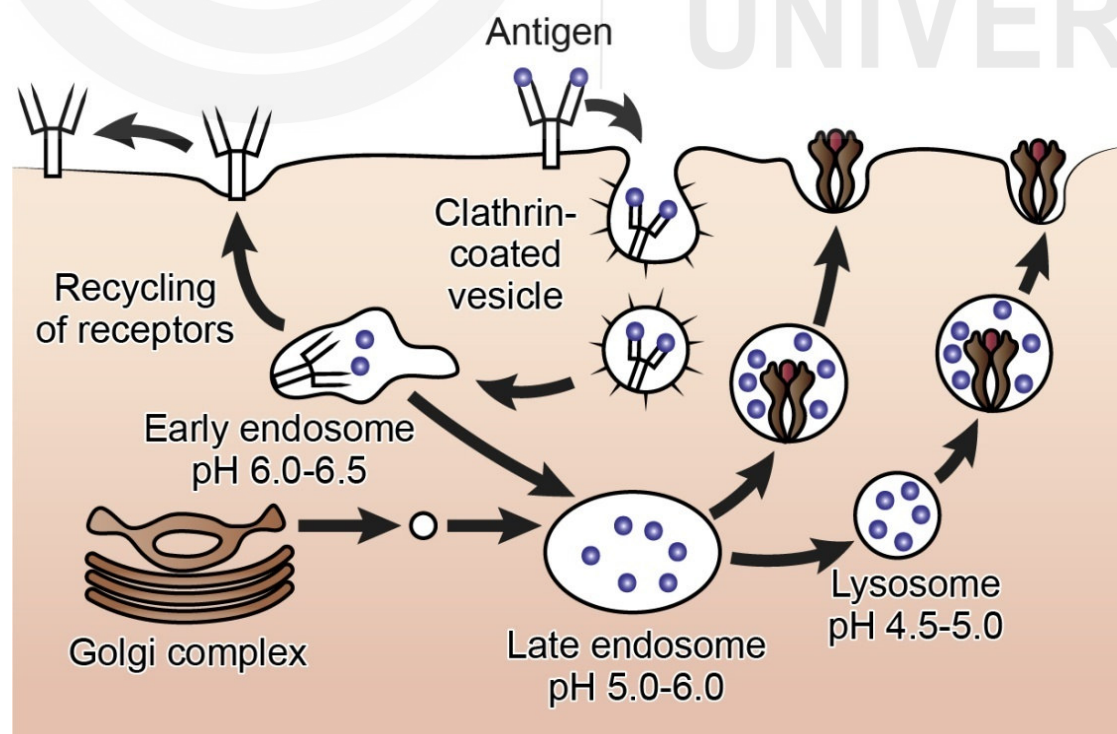


Fig. 9.8: Antibody-mediated endocytosis.

Transportation of class II MHC molecules to Endocytic vesicles

The rough endoplasmic reticulum is where class II MHC molecules are made. The peptide binding cleft of the class II MHC molecule is blocked by a trimer protein called invariant chain (CD74). The invariant chain prevents cellular or endogenous peptides from attaching to the cleft. In addition, the invariant chain's cytoplasmic tail contains a sorting signal that helps class II MHC molecules migrate from the endoplasmic reticulum to endocytic vesicles. (Phagolysosome) (Fig. 9.9).

Assembly of peptides with class II MHC molecules

Proteolytic activity increases in endocytic vesicles, and the invariant chain slowly dissolves, leaving just a little piece known as CLIP (Class II-associated invariant chain peptide) (Fig. 9.9).

CLIP binds to antigenic peptides in the peptide-binding cleft of the class II MHC molecule, preventing them from binding prematurely. After that, a non-classical class II MHC molecule known as HLA-BM removes CLIP from the endosome and substitutes it with an exogenous peptide of 13 to 18 amino acids. The stable class II MHC molecule with antigenic peptide is then presented on the cell surface. T_H cell will recognize the complex and trigger the suitable immune response like secretion of cytokines or chemokines to control the infection (Fig. 9.9).

Cross-presentation is an effective mechanism by which the immune system monitors tissues and phagocytes for the presence of foreign antigen. It is the ability of specific professional APC's (mostly dendritic cells) to take up, process and present extracellular antigens with class I MHC molecules to CD8 TC cells (cytotoxic T cells) instead of complexing with class II MHC molecules. During cross-presentation, the exogenous antigens cross-stimulate the CD8 TC cells via the class I MHC molecules, and the endogenous antigens stimulate the TH cells via class II MHC molecules.

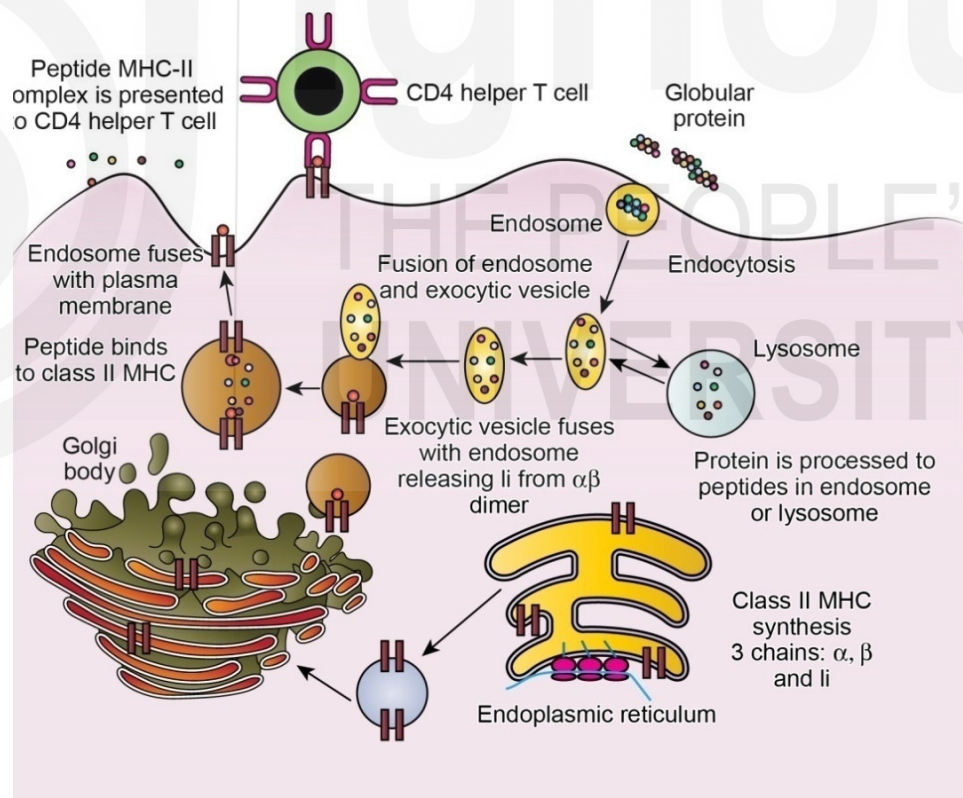


Fig. 9.9: Exogenous or Endocytic pathway of antigen processing and presentation.

9.8 SUMMARY

Let us summarise what you have learnt in this Unit:

- In humans, the MHC is known as the Human Leukocyte Antigen Complex (HLA).

- The MHC genes encode cell-surface antigen-presenting proteins of three classes: MHC-I, MHC-II, and MHC-III.
- Antigen processing enzymes break down proteins into peptides that T cells may recognise by displaying them on the cell membrane using class-I or II MHC molecules.
- The CD8⁺ T_C cells recognize antigen presented along with the class I MHC molecule. The antigen delivered along with the class II MHC molecule is recognized by CD4⁺ T_H cells. Thus, there are two types of antigen processing and presenting pathways: (1) Endogenous or Cytosolic pathway; (2) Exogenous or Endocytic pathway.
- In the Endogenous or Cytosolic pathway, the endogenous antigens generated within the cell are processed and presented. For example, tumour cells, intracellular pathogens (*M. tuberculosis*) and virally infected cells.
- A class I MHC molecule is used to deliver the processed endogenous antigen on the cell membrane, which is recognised and destroyed by CD8⁺ T_C-cells.
- Specialized antigen-presenting cells use the exogenous pathway to present peptides produced from proteins that the cell has endocytosed. MHC class II molecules are used to present the peptides. In endosomes, proteins are endocytosed and destroyed by acid-dependent proteases, which takes roughly an hour.
- The RER synthesises class II MHC molecules. Invariant chain, a trimer protein, blocks the peptide binding cleft of class II MHC molecules (CD74). The invariant chain prevents cellular peptides from attaching to the cleft. The invariant chain's sorting signal increases class II MHC molecule migration from the ER to endocytic vesicles (Phagolysosome).
- On the other hand, the invariant chain is slowly degraded, leaving only a small fragment known as CLIP (Class II-associated invariant chain peptide).
- CLIP hinders antigenic peptide binding. Later, an HLA-BM non-classical class II MHC molecule replaces CLIP with an exogenous peptide. The stable class II MHC molecule with antigenic peptide is then presented on the cell surface. T_H cell will recognize the complex and trigger the suitable immune response like, secretion of cytokines or chemokines to control the infection.

9.9 TERMINAL QUESTIONS

1. Explain, Antigen processing and Antigen presentation?
2. What are the steps involved in the endogenous pathway?
3. What are the steps involved in the exogenous pathway?
4. What are the differences between class I and class II MHC molecules?
5. Discuss the role of invariant chain and CLIP in an exogenous pathway.

9.9 ANSWERS

Self Assessment Questions

1. HLA is Human Leukocyte Antigen. Because the components were first investigated on leukocytes, the MHC is also known as the Human Leukocyte Antigen Complex (HLA) in humans. MHC plays a significant role in determining whether the transplanted tissue of the donor will be histocompatible or histoincompatible with the recipient's tissue.
2. In class I MHC molecule, there are two pairs of interacting domains:
 - i) A membrane distal pair made up of $\alpha 1$ and $\alpha 2$ domains and
 - ii) A membrane proximal pair is made up of $\alpha 3$ domain and $\beta 2$ -microglobulin.
3. In class II MHC molecule, there are two pairs of interacting domains:
 - i) A membrane distal pair made up of $\alpha 1$ and $\beta 1$ domains and
 - ii) A membrane proximal pair is made up of $\alpha 2$ domain and $\beta 2$.
4. Calnexin is the first molecular chaperone engaged in class I MHC molecule building. Calnexin is a rough endoplasmic reticulum resident membrane protein. It supports the folding of a free class I α chain by being associated with it.

Terminal Questions

1. Antigen processing is a metabolic process that breaks down proteins into peptides that T lymphocytes may recognise by displaying them on the cell membrane alongside class-I or class-II MHC molecules. Antigen presentation is the process through which cells, especially antigen-presenting cells (APCs), create processed antigen and MHC molecules in a state that T cells recognise. CD8+ T_C cells recognise the antigen presented with the class I MHC molecule. CD4+ T_H cells recognise the antigen supplied with the class II MHC molecule.
2. Broadly, there are three steps involved in the endogenous pathway:
 - a) Antigen protein is degraded into peptides by proteolytic enzyme.
 - b) Peptides (8 to 16 amino acids) are transported from the cytoplasm to the rough endoplasmic reticulum.
 - c) Assembly of peptides with class I MHC molecule.
3. Broadly, there are three steps involved in the exogenous pathway.
 - In endocytic vesicles, peptides are made from internalised antigens.
 - Endocytic vesicle transport of class II MHC molecules.
 - Peptide assembly using class II MHC molecules.

4. MHC class I and class II molecules differ in the following ways:

MHC Class I molecule	MHC Class II molecule
It can be found on the surface of all nucleated cells. It binds with endogenous antigen. The peptide-binding domain is made up of $\alpha 1$ and $\alpha 2$ chains and can accommodate 8 to 10 amino acid long antigenic peptides. It presents the antigen to CD8 T_C cells.	It is present on the cell surface of APC's. It binds with exogenous antigen. The peptide-binding domain is made up of $\alpha 1$ and $\beta 1$ chains and can accommodate 13 to 18 amino acid long antigenic peptides. It presents the antigen to CD4 T_H cells.

5. The rough endoplasmic reticulum is where class II MHC molecules are made. A trimer protein termed invariant chain blocks the peptide binding cleft of the class II MHC molecule (Li, CD74). The invariant chain prevents the binding of cellular peptides or endogenous peptides to the cleft. The invariant chain's cytoplasmic tail contains a sorting signal that helps class II MHC molecules migrate from the endoplasmic reticulum to endocytic vesicles (Phagolysosome). In endocytic vesicles, proteolytic activity increase, and the invariant chain slowly degrades, leaving just a tiny remnant known as CLIP (Class II-associated invariant chain peptide).

CLIP binds to antigenic peptides in the peptide binding cleft of the class II MHC molecule, preventing them from binding prematurely. CLIP is later removed from the endosome by a non-classical class II MHC molecule termed HLA-BM, which substitutes it with an exogenous peptide of 13 to 18 amino acids. The stable class II MHC molecule with antigenic peptide is then presented on the cell surface. T_H cell will recognize the complex and trigger the suitable immune response like secretion of cytokines or chemokines to control the infection.

WORKING OF IMMUNE SYSTEM-II

Structure

10.1 Introduction	Class Switching
Objectives	Formation of Plasma Cells and Memory B Cells
10.2 B cell receptors	10.7 Humoral Response
10.3 B cell development	Primary Humoral Response
Regulation of B-Cell Development	Secondary Humoral Response
10.4 B cell Maturation	10.8 Terminal Questions
10.5 B cell Activation	10.9 Answers
Cell Independent Activation	
T cell Dependent Activation	
10.6 B cell Differentiation	
Affinity Maturation	

10.1 INTRODUCTION

The development of plasma cells and memory B cells can be divided into three stages: the production of mature, immunocompetent B cells (development), the activation of developed B cells when they communicate with antigen, and the differentiation of activated B cells into plasma cells and memory B cells. In numerous vertebrates, including human and mice, the bone marrow is the production site for B cells. This interaction is a systematic succession of Ig- rearrangement, which advances without antigen. A mature B cell exits the bone marrow with membrane-bound immunoglobulins (mIgM and mIgD) that have a single antigen specificity. These naïve B cells, which have never encountered an antigen, travel through the blood and lymph to the secondary lymphoid organs, most notably the lymph nodes and spleen. If a B cell is activated by an antigen that is specific to an antibody bound to membrane, the cell multiplies (clonal development) and differentiates into antibody-producing plasma and memory B cells.

During the activation stage, there is a progressive rise in the affinity (average) of the antibodies generated, which is known as affinity maturation, as well as a change in the antibody isotype produced by the B cells, which is known as class switching. Antigens are necessary for B cell activation and differentiation. As a result, this is the antigen-dependent stage of B cell development.

Objective

After studying this unit you will be able to:

- ❖ explain B lymphocytes,
- ❖ discuss B-cell receptors and their working mechanism,
- ❖ explain the development process of B-cells,
- ❖ describe regulation of B-cell development,
- ❖ explain maturation and activation of B cells that take place at the germinal centers like affinity maturation, class switching and the formation of plasma cells and memory B cells, and
- ❖ differentiate between primary and secondary humoral responses.

10.2 B-CELL RECEPTORS-BCR (MIG + IG-A/IG-B)

B-cell receptors (BCR) are complexes of transmembrane proteins that are made up of B lymphocyte antigen receptors (mIg) which are membrane bound immunoglobins along with Ig- α /Ig- β . These heterodimers are linked by disulphide bonds. As there is a short cytoplasmic tail present in the mIg, it can recognize the antigens present but cannot transmit the signal to the B-Cells effectively by itself.

Due to this reason mIg forms an association with the Ig- α /Ig- β heterodimer (an accessory molecule) which facilitates the signal transduction process, after the interaction of the mIg with the antigen. This receptor which binds to the antigen Immunoglobulin mIg (membrane bound) heterodimers which are linked by disulphides called as Ig- α /Ig- β . These heterodimers have immunoglobulin fold structures and they have longer cytoplasmic tails as compared to the membrane bound immunoglobins. An mIg molecule is associated with one Ig- α /Ig- β heterodimer (Fig 10.1).

Ig- α is a long cytoplasmic chain and it has around 61 amino acids, whereas the tail of the Ig- β chain consists of around 48 amino acids. Both Ig- α and Ig- β have tails long enough to be capable of interacting with the signaling molecules (intracellular).

B lymphocytes

B lymphocytes develop inside the bone marrow; when they leave it, each possess a remarkable antigen-specific receptors on its membrane (Table 10.1).

B- cell receptor (BCR) is a layer bound immune response molecule. B-cells originate and develop in the bone marrow of many animals, including humans and mice.

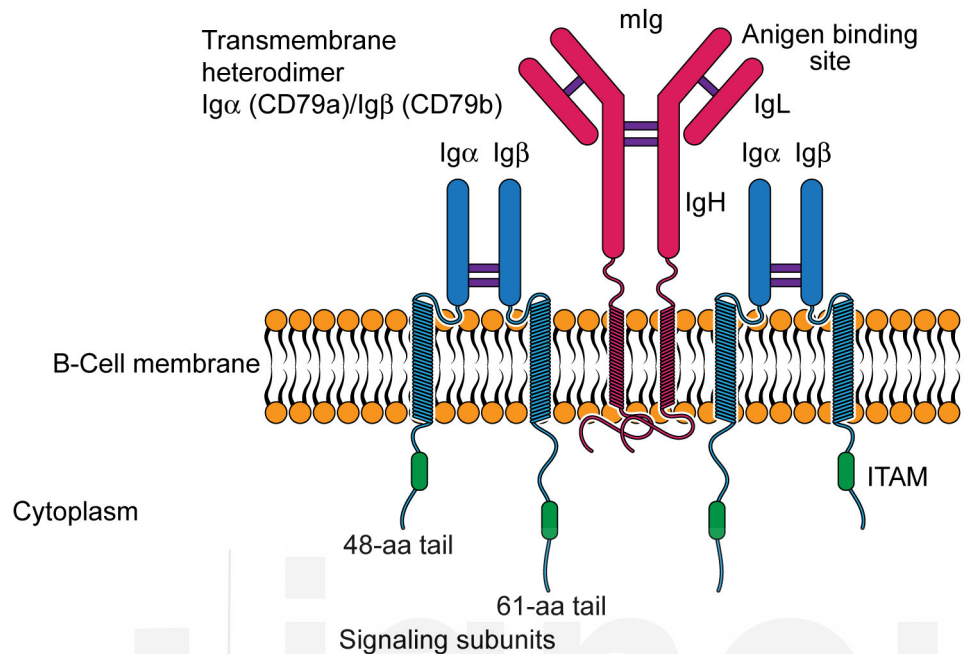


Fig 10.1: Structure of B-cell antigen receptor complex.

They are lymphocytes that are named from the *Bursa of Fabricius*, their site of maturation in birds.

B lymphocyte maturation in mammals like mice and humans 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.

Table 10.1: B-lymphocytes: surface receptor and functions.

Sl. 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 ligand on the surface of helper T cells.

The interaction of naïve 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. Plasma cells rapidly secrete antibodies while the memory cells act on subsequent exposure with the antigen.

Every B cell expresses a B cell receptor (BCR) on its membrane which is a unique antigen binding receptor. When a naïve B cell (one that has not recently experienced antigen) first encounters the antigen that interacts with its membrane bound receptor, becomes activated. It then proliferates into memory B cells and effector B cells called plasma cells (Fig. 10.2).

The humoral branch of our immune system is mediated by antibodies as the effector molecules that are secreted by plasma cells by elimination or binding to antigen and neutralizing it. B cells have around 10^5 molecules of layer bound antibody per cell. All the antibodies on a given B cell have a similar antigenic specificity and can directly target and bind with the antigen.

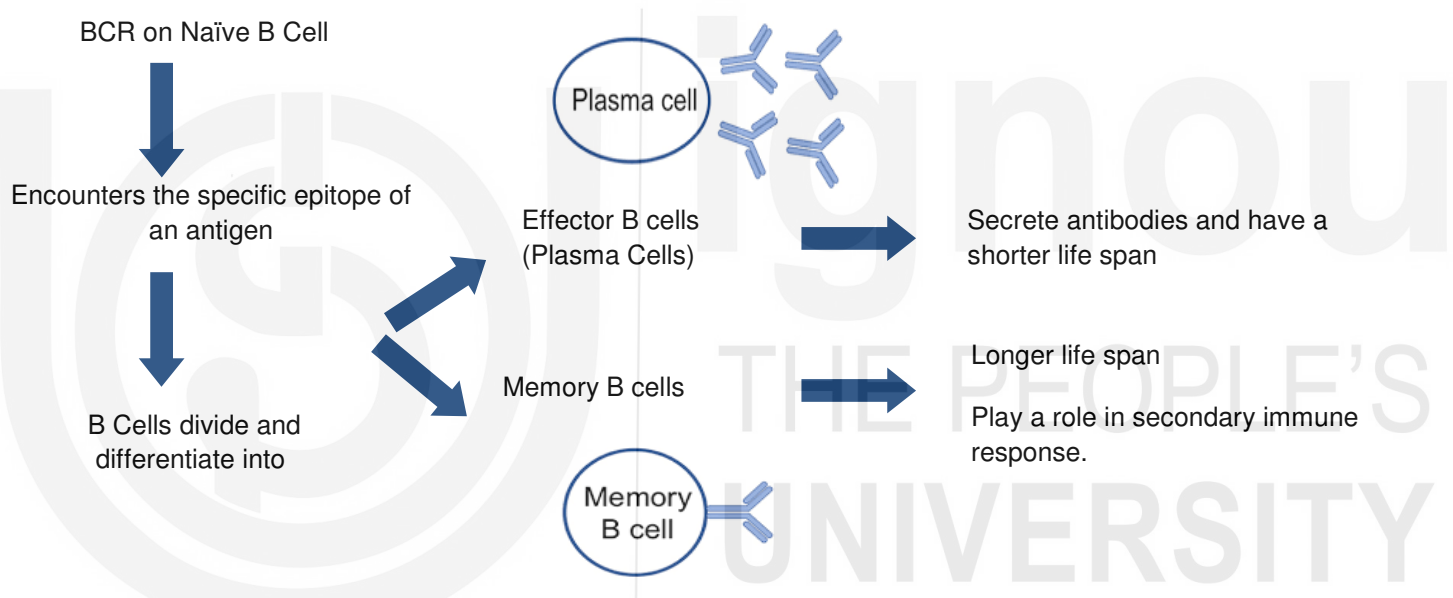


Fig. 10.2: B cells differentiation into plasma cells and memory cells.

10.3 B CELL DEVELOPMENT

The development of the B cells starts when the lymphoid stem cells differentiate into the earliest types of B-lineage cells, known as Progenitor B-cells or B-cell precursors (pro-B cells). These pro-B cells express a transmembrane tyrosine phosphatase protein called CD45R. These pro-B cells are proliferated in the bone marrow, where they fill up the extra vascular spaces which are present between the large sinusoidal areas present in the shaft region of the bone. The stromal cells present in the bone marrow provide the microenvironment needed for the growth and differentiation of the pro-B cells into precursor B cells or pre-B cells.

In the absence of the stromal cells or if these pro-B cells are cultured *in-vivo* after removal from the bone marrow, they will not progress into the maturation and also in the further stages of mature B cells formation.

The stromal cells play two key roles:

- They are responsible for the direct interaction with pro-B cells and pre-B cells.
- They are responsible for the secretion of the various cytokines like IL-7 that play an important role in the process of development.

The process of formation of plasma and memory B cells may be divided into three broad stages.

- Maturation:** Mature and incompetent B cells are generated. During the maturation phase which is antigen independent, the bone marrow generates incompetent B cells which express IgM and IgD.
- Activation:** Mature B cells are activated upon antigen interaction.
- Differentiation:** The formation of activated plasma and memory B cells.

Around 10% of the B cells complete the process of development, reach maturity and exit the bone marrow. The naïve B cells are no longer functional and die within a few days until they are encountered with a soluble protein antigen along with activated T_H cells.

Once the B cells are activated, they start developing within the secondary lymphoid organs. The B cells which have high affinity mlg immunoglobulin molecules are able to differentiate into plasma and memory B cells That can result into the expression of different isotypes due to the process of class switching (Fig. 10.3).

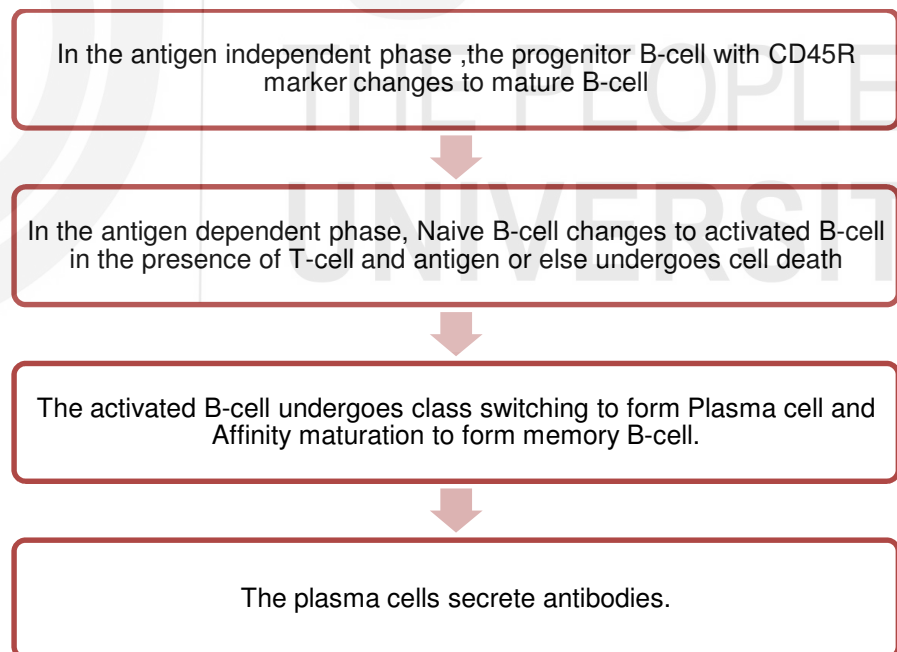


Fig. 10.3: B cell development can be divided into antigen independent phase and antigen dependent phase.

B cell development can be divided into

- Antigen Independent Phase:** The generation and maturation process of the B cells inside the bone marrow is achieved by the rearrangement of the Ig-gene in an orderly manner which takes place in the absence of antigen.

- ii) **Antigen Dependent Phase:** The activation and the differentiation of the B-cells take place in the secondary lymphoid organs like the spleen (Fig. 10.3).

10.3.1 Regulation of B-Cell Development

There are a number of transcription factors which are responsible for the regulation of the expression of various products of genes at different stages of the development of the B-cells. Some of these factors are:

- NF-6B,
- BSAP,
- Ets-1,
- c-Jun,
- Ikaros,
- Oct-2,
- Pu.1,
- EBF,
- BCF,
- E2A.

Like the all other transcription factors in the body, these proteins interact with the enhancer or promotor sequences. This results in either the stimulation or the inhibition of the transcription of the genes associated with that particular factor.

B-cell-specific activator protein (BSAP) is considered to be one of the most important B-cell transcription factors as it functions as the master regulator. This factor is only expressed by the B lineage cells and it has an effect on all the stages of cells that are present during the process of maturation.

There are various evidences that suggest that BSAP also has a role to play in the differentiation events at the final stage, which leads to the production of the two cell types namely- plasma Cells and memory B cells. The memory B cells are the only type of cells from the lineage of B cells that do not express BSAP.

BSAP binds to the promoter or the enhancer region sequences of the various genes that are specific to B cells, which includes examples like Vpre-B genes of the surrogate light chain, the heavy-chain enhancer region, the J-chain gene of polymeric IgM, one of the two enhancers that lie at 3' of the gene in heavy-chain germ-line DNA. BSAP is known to bind to various heavy chain switch sites of the immunoglobulin and to various other genes that are involved in the activation of B-cells.

10.4 B CELL MATURATION

Mature B-cells begin to develop in the embryo and continue to develop throughout life. Before birth, the yolk sac, fetal liver and fetal bone marrow are the major sites for B-cell development. After the birth, bone marrow takes up the role of B-cell maturation when the infant is born.

The lymphoid and myeloid progenitors are formed from the HSC (hematopoietic stem cells) as already discussed in previous units. The myeloid progenitors are responsible for production of myeloid related cells, while the lymphoid progenitors form all the lymphoid cells. B-cell development begins when lymphoid stem cells differentiate into the earliest kinds of B-lineage cells, known as Progenitor B-cells or B-cell precursors (pro-B cells). CD45R is a transmembrane tyrosine phosphatase that is expressed by these pro-B cells (Fig. 10.4).

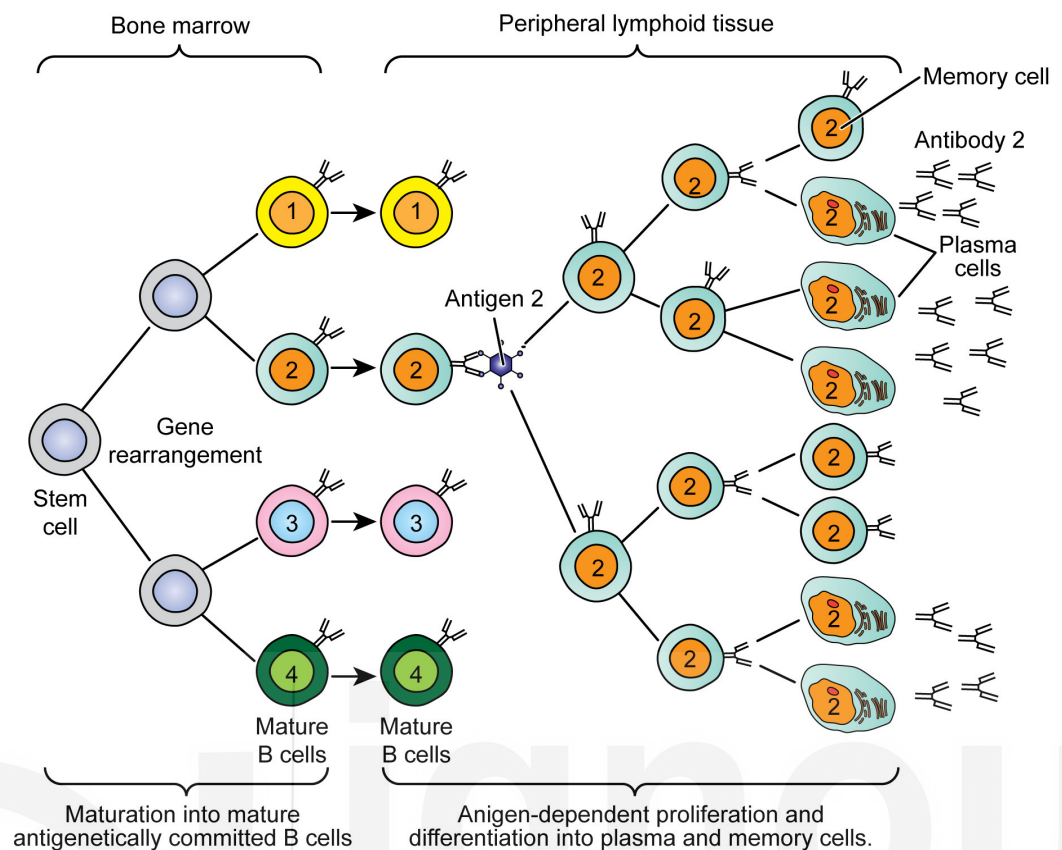


Fig 10.4: B cell maturation.

In the beginning stages of development, a direct contact with the stromal cells of bone marrow is required by the pro-B cells. This is regulated by certain cell-adhesion molecules like the VLA-4 that is present on the pro-B cells and the ligand called as VCAM-1 that is present on the stromal cells.

Following the initial interaction, c-Kit, a receptor on pro-B cells, interacts with stromal stem factor (SCF), which is found on the surface of stromal cells. This causes the tyrosine kinase C-Kit to become activated, causing the pro-B cells to divide and differentiate into pre-B cells, which then begin to express the IL-7 receptor.

The stromal cells release IL-7 which binds to the receptors for IL-7, that initiates the process of conversion of the pro-B cells by maturation into pre-B cells. IL-7 controls the maturation process, where it eventually initiates the process of down-regulating the adhesion molecules on pre-B cells, as the cells that are growing can get detached from the cells of the stroma.

At this stage, pre-B cells no longer require direct contact with stromal cells but continue to require IL-7 for growth and maturation. Pre-B cells proliferate and differentiate eventually to produce immature B cells. The bone-marrow phase of B-cell development culminates in the production of an IgM-bearing immature B cell.

NOTE: The stromal cells play two important roles: they interact directly with pro-B and pre-B cells, and they secrete various cytokines, notably IL-7, that supports the developmental process.

In the current state the B cells are not at their full functional capacity, due to which any antigenic interaction causes anergy and death instead of differentiating and division. The co-expression of both IgG and IgM on membrane is a signal for completed maturation.

The maturation of B cells is dependent on the immunoglobulin DNA rearrangement in stem cells. A DH-to-JH gene rearrangement of the heavy chain is the first step to occur in the stages of pro-B cells, this rearrangement is followed up by the VH-to-DHJH rearrangement. When this rearrangement of the heavy chains is completed, the cells are then called as the pre-B cells.

A rearrangement of the light chain gene is needed for the continuous progression of the formation of immature B cells from the pre-B cells. On the completion of rearrangement of the light chain genes, a specificity for an antigen is determined for the immature B-cells by the heavy-chain VDJ sequence and light-chain VJ sequence of the cell. These immature B cells release the membrane bound IgM (mIgM) onto the surface of the cells.

The co-expression of both the immunoglobins mIgM and mIgD is required in a fully matured B cell, for this progression to take place a change in the processing of the RNA primary transcript of heavy chain, which enables the production of two functional mRNA. One of them encodes for the membrane form of the chain- μ (for mIgM) while the other mRNA encodes for the membrane form of the chain- δ (for mIgD).

NOTE: The stages of development are identified by cell surface markers.

A pattern change in the markers present at the surface shows the progression in the development of mature B cells from progenitor cells.

The following cell surface markers are expressed by the pro-B cells:

- CD45R (a form of tyrosine phosphatase).
- CD19 (B-cell coreceptor).
- CD43 (leukosialin).
- CD24, known as heat stable antigen (HSA).
- C-Kit, present in the cells of stroma and promotes growth.

At the stage of pre-B cells, pre-B cell receptor display is an important feature. After the light chain is rearranged, immunoglobulins which contain both the heavy and the light chains are seen to appear, and the cells, now called as the immature B cells are no longer able to express the CD25 as they do not have the pre-BCR anymore.

When the maturation process takes place in the presence of an antigen, the B cells then produced are committed to a specific antigen i.e., they are only able to express antibodies specific to an antigen.

Mature B cells, also known as naïve B cells, leave the bone marrow and circulate throughout the bloodstream, secondary lymphoid organs, and lymph. When naïve B cells in the lymphoid organs are exposed to an antigen that is specific to them, a process known as **clonal selection**, also known as B cell activation, occurs, in which B cells with membrane bound antibodies specific to a specific type of epitope bind to the antigen containing the specific epitope.

The antigen-activated B cells then undergo clonal expansion (differentiating and proliferating), resulting in a clone of memory B cells and plasma cells. These B cell clones produced share affinity with and specificity for the same antigen.

NOTE: Only 5×10^6 (or around 10%) of the 5×10^7 B cells/day produced in bone marrow of a mouse are capable of being recruited and entering the recirculating B-cell pool, resulting in around 90% of the B cells dying even before they can leave the bone marrow. Negative selection and subsequent elimination (clonal deletion) of immature B lymphocytes that express auto-antibodies for self-antigens in the bone marrow account for a portion of this loss.

SAQ 1

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

- i) Before birth, the yolk sac, fetal liver and fetal bone marrow are the major sites for B-cell development.
- ii) The maturation of B cells is not dependent on the immunoglobulin DNA rearrangement in stem cells.
- iii) Only antigen-activated B cells undergo clonal expression.
- iv) Antigen dependent phase for lymphocytes occurs in secondary lymphoid organs.
- v) Memory B cells release antibodies that circulate in the blood and lymph.

10.5 B CELL ACTIVATION

Mature naïve B cells are able to express the membrane bound immunoglobulin (mIgM and mIgD) of the B-cell receptors (BCRs), both them are of equal specificity of the antigens.

For generation of activation of the B cells, a large number of BCRs are supposed to be brought in close proximity of the surface of B-cells. This BCR clustering results into the activation of multiple Ig- α /Ig- β molecules and leads to the initiation of the signaling process. This cluster formation depends on the antigen's type that is being encountered by the B cells.

10.5.1 T-Cell Independent Activation

Thymus-Independent antigens, or TI antigens, are antigens that can activate B cells even when T_H cells are not present.

The TI antigens may be classified into two categories, each of which has a different mode of activation (Fig 10.5).

- Certain parts of the cell walls of bacteria, like lip polysaccharide, can function as a Type 1 TI antigen also called as TI-1 antigens.
- Antigens that are repetitive molecules like the flagellin in bacteria containing repeating polymeric proteins are the TI-2 antigens or the Type 2 thymus independent antigens.

Activation Signal in TI antigens

- Immune receptors and the Ig are the sites through which B cells are able to bind to the TI-1 antigens. For example B cells bind to the LPS from the Gram-negative bacteria through membrane-bound immunoglobins (mIg) and the toll-like receptor TLR-4, resulting in signalling from both receptors.

C3d complement components crosslink both the mIg and CD21 receptors on B-cells, which are typically bound by T-independent type 2 (TI-2) antigens. The Type 2 antigens can transmit an activation signal by cross linking the 12 and 16 Ig receptors.

The germinal centers develop about 7-10 days after initial exposure to the TI antigens. When the germinal center is in its early stages of formation, the activated B cells go through a period of intensive growth and development. The B cells that are growing are called as centroblasts which are seen to be present in the germinal centers in humans, also in a well-defined dark zone. Centroblasts can be differentiated easily as they are larger in size, have enlarged cytoplasm, diffused chromatin, and the surface immunoglobins are almost near to none.

Centrocytes are eventually formed from the centroblasts and can be defined as small B cells that are non-dividing and are now expressing Ig present on the membrane. The centrocytes are shifted from the areas called as the dark zones into an area that contains follicular dendritic cells which is known as the light zone, where an interaction with the antigens takes place.

10.5.2 T-Cell Dependent Activation

Three signal process takes place in the activation process that is T-cell dependent (Fig. 10.6). Three signals are discussed below:

Signal 1: T-dependent antigen (TD) binds to Ig receptor of B-cells. B cell Ig receptors and antigens that are Thymus dependent, such as TD antigen, bind together.

Signal 2: Co-stimulatory Signal (CD40/CD40L)

Certain antigens are processed and subsequently presented through the Class II MHC molecules to the T helper cells. The T cells then bind to this MHC- antigen complex and the activating signals are delivered to the B cells by the interactions that take place between the CD40 present on the B cells and the CD40L present on the T cells.

Signal 3: Cytokine release (IL-2 and IL-4)

Activating cytokines like the IL-2 along with the IL-4 are then secreted by the T_H cells. These cytokines are then recognized by the surface receptors present on the B cells. These cytokines are responsible for delivering the signals for the process of differentiating, proliferation, and for survival to the B-cells.

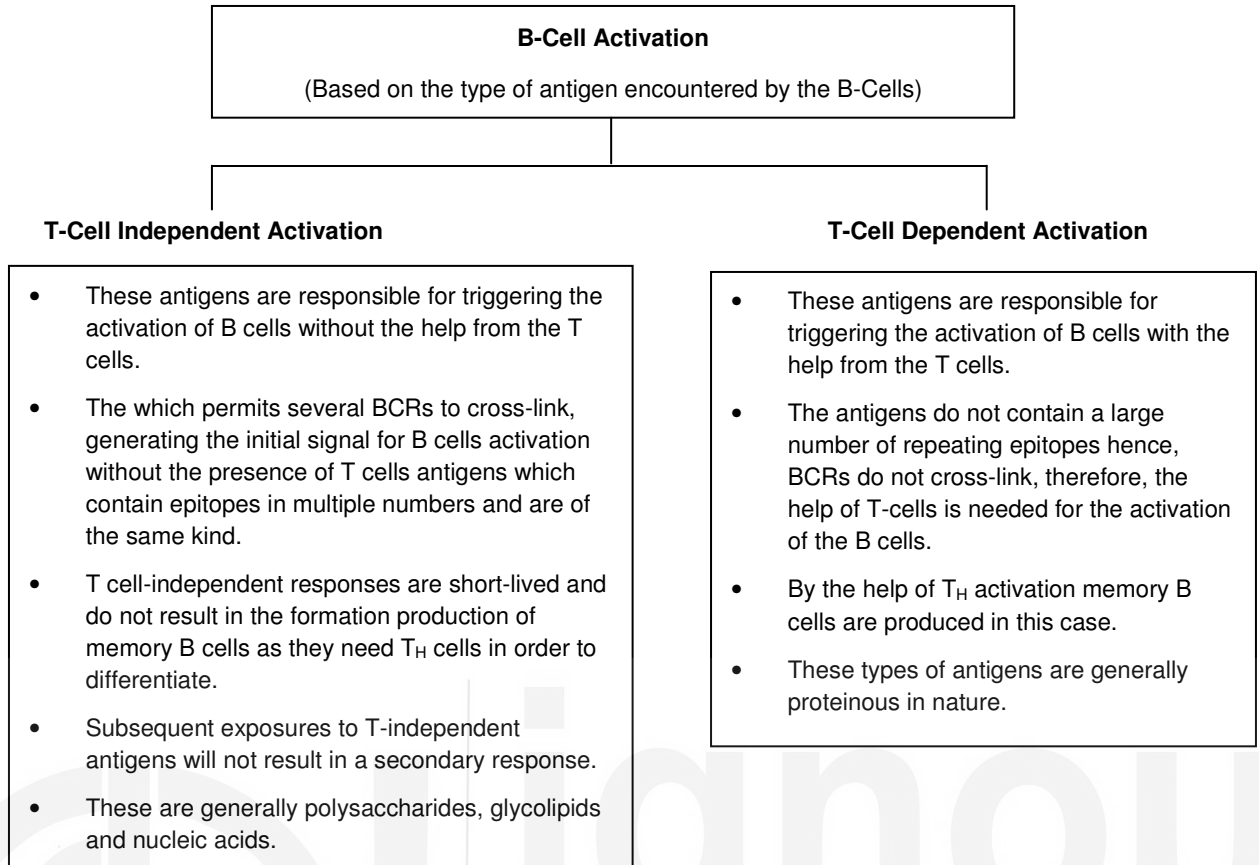


Fig. 10.5: B cell activation can occur by T-Cell independent activation or T-Cell dependent activation.

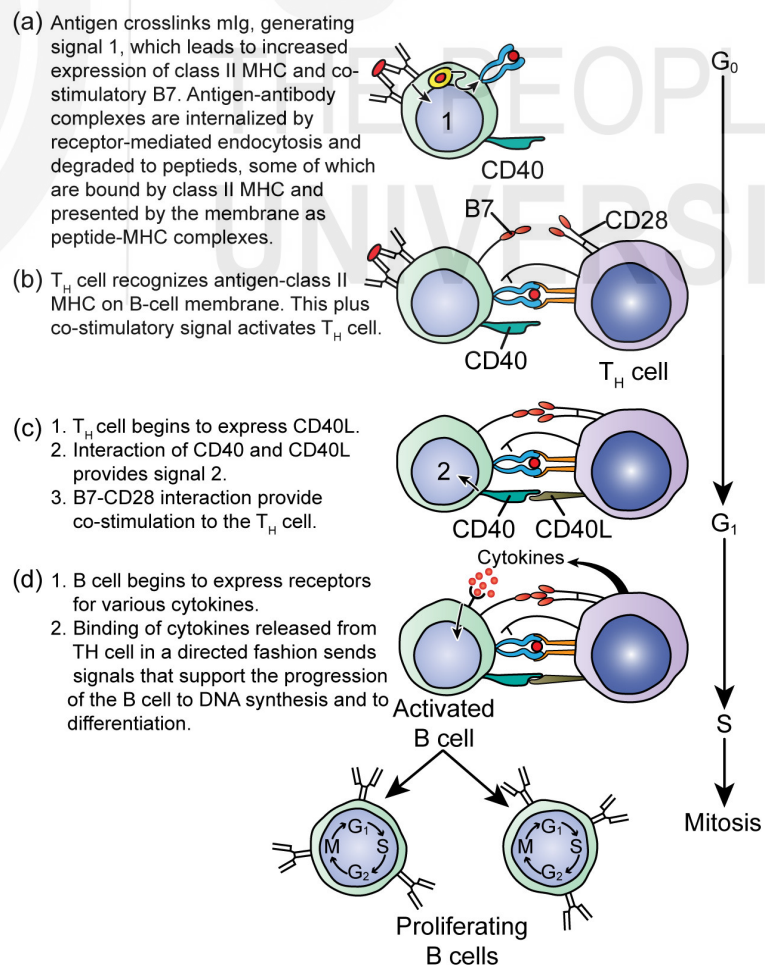


Fig. 10.6: B-cell activation by a T-dependent antigen.

In case of the thymus independent and the thymus dependent antigens the humoral responses vary. A low level of class switching is seen in the response to the TI antigens and is weak. There are no production of the memory B cells along with IgM being the predominant antibody that is being secreted. This shows that the T_H cells play an important role in the production of the memory B cells, the process of affinity maturation and are crucial for the process of class switching.

NOTE: Negative selection of the B cells that are self-Reactive can also take place in the periphery. Certain self-antigens are not able to access the bone marrow, and the B cells that express mlg specific to such antigens are not removed by the process of negative selection and to avoid the auto-immune response due to such self-antigens, self-tolerance processes take place in the peripheral lymphoid tissues to inactivate or devoid them.

10.6 B CELL DIFFERENTIATION

B cells that are stimulated by the antigens start migrating to the germinal centers, resulting in a decrease in Ig expression on the surface and the initiation of rapid cell division and within the dark zones mutations of the rearranged V region genes are seen.

After which, the division process comes to a halt, and the B cells start migrating to a light zone, along with it the surface of Ig expression is increased. These cells are called centrocytes. B cells must interact with T helper cells and follicular dendritic cells in order to survive.

Antigen antibody complexes are bound together by the follicular dendritic cells along their long extensions and B cells compete for antigen binding.

When B cells bind to the antigens and they receive a signal from T_H cells; they start the process of differentiation into either of the two types of cells: plasma cells (secrete antibody) or memory B cells

The process of class switching can also be initiated after the encounter with the T_H cells. One of the major functions from the germinal center is the development of B cells which have higher affinity (Ka_2) from the B cells of lower affinity (Ka_1).

10.6.1 Repeated Selection and Mutation Can Lead to Affinity Maturation

During the process of affinity maturation, the affinity (average) of the antibodies that are being produced in the humoral response is seen to increase by a great amount.

10.6.2 Class Switching

Two important activities are being performed by the antibodies:

- V_H and V_L domains determine binding of the antibodies to specific antigens.

- They participate in various effector functions, the isotypes of the constant domains (heavy chains) determine these functions.

A given V_H domain can be connected to a certain isotype by the use of class switching. The specificity of the antibodies still is able to remain constant while there is a variation in the biological effector activities. A number of cytokines affect the decision of what Ig class is chosen when an IgM-bearing cell undergoes the class switch.

An extensive class switching to the isotypes except IgM is seen in the humoral response generated to the antigens that are dependent on thymus, while the humoral response to the antigens that are independent of thymus is IgM dominant. For the thymus-dependent antigens, interactions between the CD40 that is present on the B cells and the CD40L that is present on the T_h cells is very important for initiating class switching.

NOTE: CD40/CD40L interaction is crucial, as evidenced by X-linked hyper-IgM syndrome (an immunodeficiency disorder in which CD40L expression does not take place).

10.6.3 Formation of Memory B Cells and Plasma Cells

After the selection of the required B cells in the germinal centers which have high-affinity mIg for antigens that are displayed on the follicular dendritic cells, these B cells differentiate into memory B cells and plasma cells.

For the generation of plasma cells, the germinal centers are important, but there are other sites where these types of cells are produced as well.

Immunoglobins that are membrane bound, which can be detectable are lacking in the plasma cells, instead high amounts of secreted antibodies are synthesized (with the rates that are as high as 1000 molecules of Ig per cell per second).

A change in RNA processing is essential for the differentiation of mature B cells into plasma cells, resulting in the creation of the secreted form of the heavy chain rather than the membrane form. The transcription rates of the light and heavy chain genes are much greater in plasma cells than in less-differentiated B cells.

The B cells that are able to survive in the light zone selection in the germinal centers then lead to differentiation in the memory B cells.

10.7 HUMORAL RESPONSE

The intensity and different attributes of the humoral reaction vary greatly depending on whether naïve cells (primary response) or memory lymphocytes (secondary response) are activated. In the two cases, it results in the production of antibodies of various isotypes, each of which has a varied ability to elicit a specific effector capability.

A main consideration in the more-fast beginning and more prominent magnitude of auxiliary reactions is the way that the number of inhabitants in memory B cells explicit for a given antigen is impressively bigger than the number of inhabitants in comparing naive B cells. Besides, memory cells are more effectively enacted than naive B cells.

The increased partiality and numerous isotypes observed in secondary reactions can be attributed to affinity formation and class switching cycles.

The more elevated levels of immune response combined with the higher affinity give a powerful host protection against reinfection. The change in isotype gives antibodies whose effector capacities are especially fit to a given microbe.

The steps of the humoral immune response are as given:

1. **Antibodies Production:** The B cell production occurs in bone marrow. They have B-cell receptors (BCRs) displayed on their surface that are specific for a particular antigen. These mature B-cells that go on to encounter pathogens in lymphoid organs, to where they have migrated.
2. **Activation of B-cells:** The mature B cell now encounters antigen in the lymphoid organs. The antigen binds to the BCR and is internalised by endocytosis. The antigen fragments are presented with MHCII molecules.
3. **B-cell proliferation:** The helper T-cells are activated after they bind to the B-cell and release various cytokines that go on 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 rest state. They are activated on subsequent encounter with the pathogen.
4. **Antigen –antibody reaction:** Now the antigen and antibody react and then the immune complex so formed is cleared from the body.

10.7.1 Primary and Secondary Humoral Response

A lag stage depicts an important antigen response in which naïve B lymphocytes go through clonal determination, clonal development, and differentiation into memory cells or plasma cells. The length of the lag stage varies depending on the antigen. The lag stage is followed by **a logarithmic expansion** in serum antibody, which initially reaches peak, then plateaus for a variable duration before dying (Fig. 10.7). Immunization of mice with an antigen, such as sheep red platelets (SRBCs), usually results in a 3–4 days lag period.

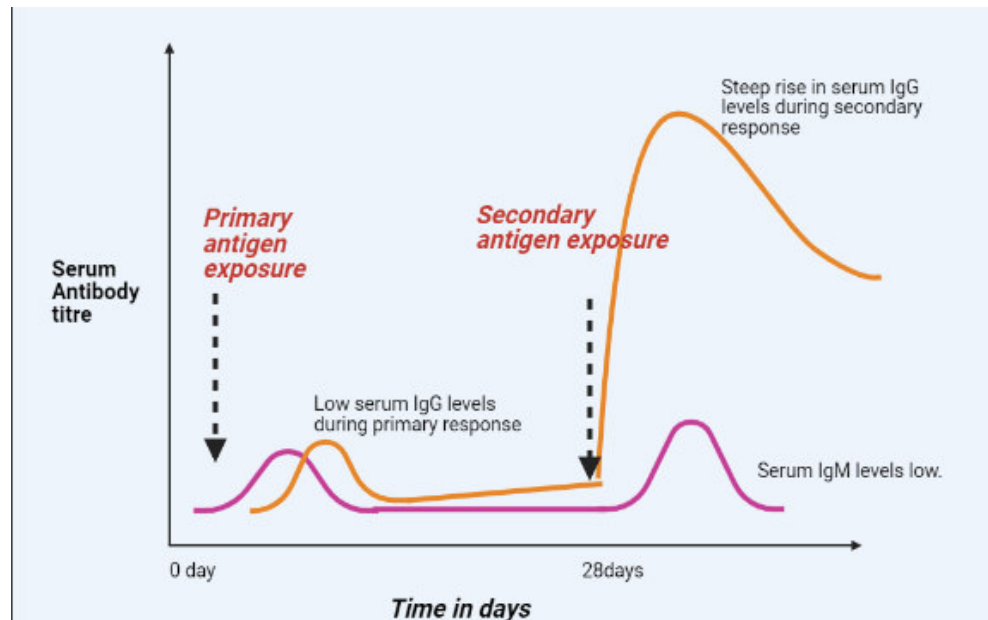


Fig. 10.7: Comparison of serum Ig levels in primary and secondary immune response.

During days 4 and 5, eight or nine progressive cell divisions of activated B cells produce plasma and memory cells. Peak plasma-cell levels are reached by days 4-5; peak serum immunoglobulin levels are reached by days 7-10. IgM is released first during a crucial humoral response, followed by a shift to an increasing amount of IgG. An important reaction can last anywhere from a few days to a few weeks, depending on the antigen's persistence. Memory B cells formed during the main phase of the cell cycle stop dividing and enter the G_0 phase of the cell cycle. These cells have varying life spans, with some surviving for the duration of the individual's life (Table 10.2).

Table 10.2: Differences between the primary and secondary response.

Sl. 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
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.

The ability to foster secondary humoral reaction relies upon the presence of this populace of memory B cells just as memory T cells. Antigen-induced memory cell activation produces an auxiliary antibody that may be distinguished from the essential response in a variety of ways. The secondary response has a shorter lag time, manifests itself to a greater extent, and lasts longer. In the secondary response, immunizers with a greater affinity for the antigen are emitted, and isotypes other than IgM predominate. The fact that the number of residents in memory B cells explicit for a specific antigen is substantially higher than the number of inhabitants in naïve B cells is a vital factor in the quicker commencement and more noticeable magnitude of secondary responses. Furthermore, memory cells are easier to activate than naïve B cells. The strong affinity and various isotypes seen in secondary response are due to cycles of liking development and class exchanging. The greater antibody levels, combined with a much higher affinity, provide a strong host defense against reinfection.

SAQ 2

Fill in the blank:

- i) antigens can stimulate B cells to become activated but require cytokine assistance delivered by helper T cells.
- ii) A longer lag phase is observed in immune response.
- iii) Immunoglobulin heavy-chain gene rearrangement begins in cells.
- iv) The main antibody of secondary responses in the blood is
- v) is the process whereby an activated B cell changes its antibody production from IgM to either IgA, IgG, or IgE.

10.8 SUMMARY

So far, you have learnt that:

- B cells are produced in the bone marrow of many animals, including humans and mice. B cells develop in the bone marrow from multipotent hematopoietic stem cells (HSCs).
- The production of mature, immunocompetent B cells (development), the activation of produced B cells when they interact with antigen, and the differentiation of active B cells into plasma cells and memory B cells are the three steps of plasma cell and memory B cell development.
- B-cell receptors (BCR) are transmembrane protein complexes that consist of membrane bound immunoglobins (mIg) and Ig- γ . In combination with the mIg, these molecules in the heterodimer create the BCR.
- Mature B cells, also known as naïve B cells, exit the bone marrow and circulate throughout the circulation, lymph, and secondary lymphoid organs.

- When naive B cells in the lymphoid organs are exposed to their specific antigen, clonal selection, also known as B cell activation, occurs, in which B cells with membrane bound antibodies specific to a certain kind of epitope attach to the antigen that contains the specific epitope.
- Depending on the molecular class of the antigen, different processes are used to activate B lymphocytes. Protein antigens are classed as T-dependent antigens because they rely on T cells to activate B cells. T-independent antigens, on the other hand, include polysaccharides, lipopolysaccharides, and other nonprotein antigens that can activate B cells without antigen processing and presentation to T cells.
- The body's primary immunological reaction to antigen happens the first time it comes into contact with it.
- Secondary response occurs following a repeat contact with the same antigen that is faster and results in the activation of previously created memory cells. The second reaction differs from the primary response in terms of both quantity and quality.

10.9 TERMINAL QUESTIONS

1. What is BCR? What are its functions? Which 2 Igs make up the BCRs?
2. Briefly explain the cellular processes that occur in the germinal center during B cell development.
3. Write down the significance of class switching.
4. What are the types of B cell activation? On what criteria they are differentiated?
5. Describe the major events of the B-Cell maturation briefly.

10.9 ANSWERS

Self Assessment Questions

1. i) True, ii) False, iii) True, iv) True, v) False.
2. i) T-dependent, ii) Primary, iii) Early pro-B, iv) IgG, v) Class switching.

Terminal Questions

1. Refer to Section 10.2.
2. Refer to Section 10.3.
3. Refer to Section. 10.3
4. Refer to Section 10.5.
5. Refer to Section 10.4.

UNIT 11

WORKING OF THE IMMUNE SYSTEM-III

Structure

11.1 Introduction

Objectives

11.2 T cells: An Overview

T cell Classification

Antigen Presenting Cells

11.3 T Cell Activation

11.4 T Cell Maturation

Positive Selection

Negative Selection

11.5 T Cell Differentiation

Helper T cell Differentiation

Cytotoxic T cells

Memory Cells

11.6 Summary

11.7 Terminal Questions

11.8 Answers

11.1 INTRODUCTION

From the proceeding Units of the Block 1 and 2 of the immunology course, you might have understood the basic concept of immunology, various immunological cells and their role in defense system against harmful pathogenic species. As you know, specialized immune cells play a crucial role to protect our body safe from a wide range of environmental pathogens or infectious diseases. The most important cells of the immune system are the B lymphocyte (B cells) and the T lymphocyte (T cells) which are involved in the Humoral immunity and Cell-mediated immunity, respectively. B and T lymphocytes both work together in a coordinated manner to eliminate pathogenic microbes and viruses from the body.

In Unit 11, you have also studied about the B-cells: their activation, maturation, differentiation (plasma B cells and memory B cells). B lymphocytes deal with the humoral immunity while and T lymphocytes are involved in cell-mediated immunity. However, both B and T lymphocytes originate from the hematopoietic stem cells in the bone marrow. The term "B lymphocytes" refers to the bone marrow or the bursa of Fabricius found in birds where it matures

while T cells mature in the thymus. Hence, the name T lymphocytes. However, many microbes or viruses live inside the host cells, where antibodies cannot reach. Such intracellular microbial infection is killed or neutralized by T cell-mediated immunity.

Therefore, this unit deals with the T-cell mediated immunity wherein you will learn about the cytotoxic T-cells and Helper-T cells and their activation, maturation, differentiation, and memory cells. The positive selection and negative selection and their role in T cell development are also discussed.

Objectives

After studying this unit, you should be able to:

- ❖ define T cell- mediated cellular immunity,
- ❖ classify the T cells,
- ❖ explain the antigen presenting cells and their role in T cell activation,
- ❖ illustrate the structure of CD4 T cells and CD8 T cells,
- ❖ differentiate between the cytotoxic T cells and helper T cells,
- ❖ describe the T cells positive selection and negative selection,
- ❖ discuss the process of T differentiation and maturation, and
- ❖ explain the memory T cells.

11.2 T CELLS: AN OVERVIEW



Prof. Jacques Miller



Dr. Graham Mitchell

Professor Jacques Miller with his Ph.D. student, Dr. Graham Mitchell, discovered the T cells in thymus that are essential for the adaptive immunity. Our body has developed a specialized immune system that protects us from various pathogenic organisms. When a pathogen crosses the first barrier of the innate immune system of the body, the adaptive immunity comes into function. The adaptive immune system works both at the humoral and cellular levels in the human body. B cells and antibodies participate in humoral immunity in the blood and lymph nodes while T cells in cell-mediated immunity.

Recall the structure of the thymus from unit 4, it is the multi-lobed lymphoid organ that is found near the heart. We generally remember cytotoxic T cells as killer cells or helper T cells as helper cells. In fact, T cells originate in the hematopoietic stem cells of the bone marrow and then they migrate to the thymus for their development. T cells mature in the thymus called thymocytes and participate in cell-mediated immunity. T cells are the chief immune cells involved in cell-mediated immunity to prevent infection or destroy intracellular antigens (viral and bacterial infected cells).

The resistance against intracellular infection produced by T lymphocytes is called cell-mediated immunity. T cells are capable to kill the virally infected cells. There are various cells such as T lymphocytes, Antigen presenting cells,

Macrophages, natural killer cells and antibody dependent killer cells (K cells) that work in coordination manner for cell-mediated immunity. The naive T cells are those primary T cells that have not encountered the antigen infected cells. Upon antigen stimulation, naive T cells are activated and express their subsets cells via cell division and expressed specific surface receptors.

T cells attack on those cells which contain foreign antigens as opposed to antibodies. To destroy antigen infected cells, T cells must develop specific receptors to recognize MHC associated foreign antigen peptides processed by antigen-presenting cells (APCs). T cells on their membrane surface have specific various receptors called T cell receptors (TCRs) that recognize specific antigen peptides. TCR consists of two polypeptide chains (α and β) which diverse in nature and binds the epitopes of various antigens processed by antigen-presenting cells (Fig. 11.1).

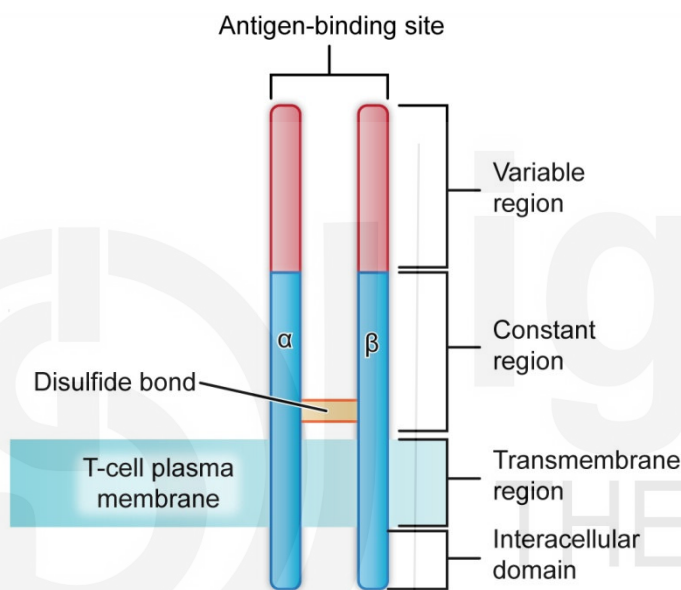


Fig.11.1: Schematic diagram of T cell receptor.

Macrophages are the antigen presenting cells that are involved in detection, phagocytosis and destruction of microbes and other harmful organisms. In addition, they present antigens to T cells and initiate inflammation by releasing cytokines that activate other immune cells.

Natural killer cells are components of innate immune system. It is a cytotoxic cell that can kill the virally infected cells and remove self-tumor cells. Hence it is known as NK cells, K cells, and killer cells.

11.2.1 T cell Classification

All T lymphocytes produce different protein molecules on their surface. These different surface proteins designated as **cluster of differentiation (CD)** and each cell surface protein is assigned a different number which helps in identification of the various types of white blood cells. T cells are divided into two classes: **Cytotoxic T cells** and **Helper T cells** on the basis of surface proteins CD8 or CD4 (Fig.11.2). Both T cells differ in their functions (Table 11.1):

1. **Cytotoxic T cells:** T cells express a CD8⁺ surface marker that recognize class I MHC molecule associated-antigen peptide present on antigen-presenting cells. Therefore, CD8⁺ binding to Class I MHC ensure that cytotoxic T cells only interact with infected target cells. Cytotoxic T cells release proteolytic enzymes to destroy or kill antigen infected cells (viral peptides or bacteria). Hence, they are known as killer T cells.
2. **Helper T cells:** T cells which express CD4⁺ marker Instead of CD8⁺ marker and interacts with class II MHC molecule associated-antigen peptide present on antigen-presenting cells. CD4⁺ binding to the class II

MHC ensures that T_H cells only interact with the APCs and secrete signal molecules i.e., cytokines. Cytokines are co-stimulatory molecules that help in the regulation of other immune cells such as T cells, B cells, dendritic cells, and macrophages. Hence, it is called T helper cells.

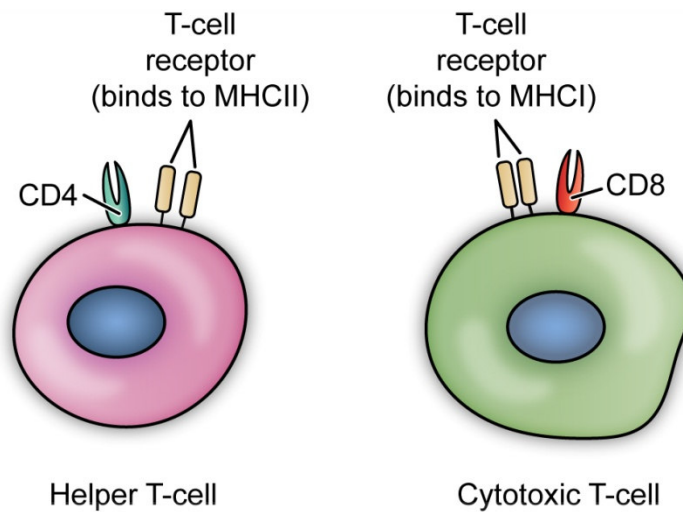


Fig. 11.2 Types of T cells with the specific surface markers.

Table 11.1: Classes of T Cells.

Class	CD Molecules	Activation	Functions
Helper T cells	CD4	Class II MHC on APCs presenting antigens	Regulate the humoral and cellular immunity
			Activate B cells to produce antibodies and cytotoxic, macrophages and NK cells
Cytotoxic T cells	CD8	Class I MHC on APCs or infected nucleated cells	Directly kill pathogens infected cells.

The MHC are the large family of genes encoding surface proteins i.e. Class I MHC and

Class II MHC. These proteins are responsible for intracellular recognition and antigen presentation to T-cell mediated immunity.

11.2.2 Antigen Presenting Cells

Recall the immunological function of the antigen presenting cells and the MHC molecules discussed in unit 10. APCs are a group of immunological cells that display antigen to T cells. APCs are involved in the intracellular digestion of foreign antigens such as (virus, bacteria etc). The dendritic cells, macrophages, and B cells also serve as antigen-presenting cells and are present in high numbers in the lymphoid organs. APCs are necessary for the initial activation of naive T cells. T cells only recognize intracellular antigen peptides bound to MHC molecules. MHC molecules are antigen presenting molecules expressed on all nucleated cells.

The Major Histocompatibility complex (MHC) is a large collection of genes encoding MHC molecules found on all nucleated cells. They are divided into two classes: Class I MHC and Class II MHC both are involved in T-cell

mediated immunity. **Class I MHC molecules** are present on all nucleated cells and present normal self-antigens and non-self pathogens to the effector T cells. On the other hand, **Class II MHC molecules** are only present on the surface of antigen presenting cells (macrophages, dendritic cells), and B cells. They present abnormal or non-self antigen peptides for the initial activation of T cells.

The Class I MHC interacts only with the $CD8^+$ cytotoxic T cells while Class II MHC molecules bind with the $CD4^+$ helper T cells. Fig. 11.3 depicts the steps for antigen processing and MHC-associated antigen presentation by APCs.

1. When a bacterial pathogen interacts with the dendritic cell, it engulfs it within the phagosome via the phagocytosis process.
- 2: The phagosome fuses with the lysosome for hydroxylation of foreign antigen (virus) into the smaller peptides.
- 3: Antigen peptides along with MHC molecules are then displayed for T cell recognition.

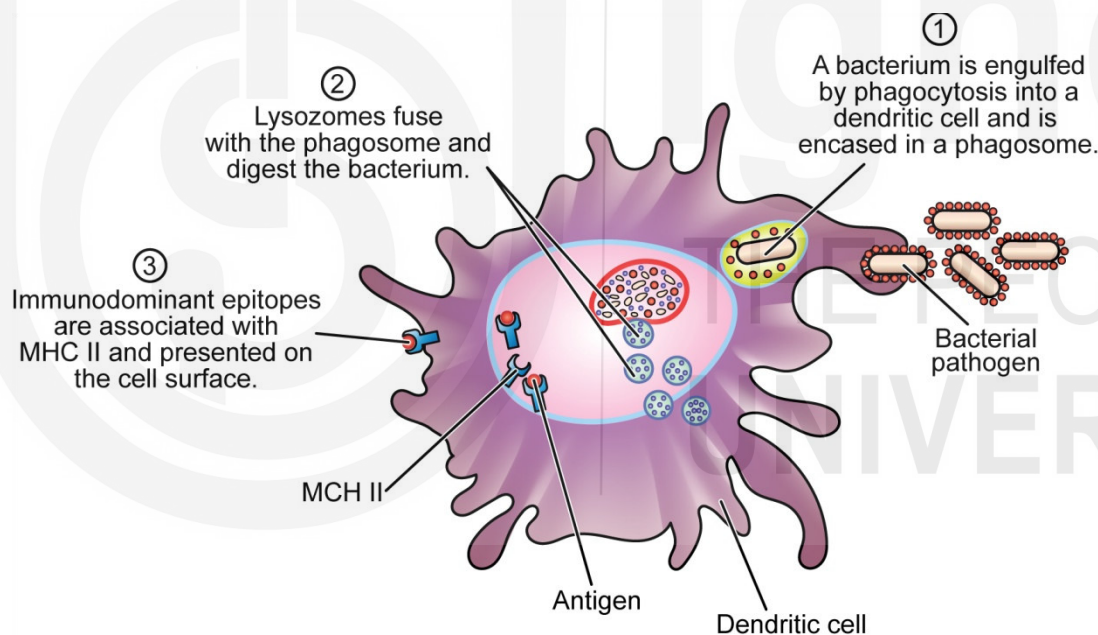


Fig. 11.3: Processing and presentation Antigens via MHC-peptide complex to T cells.

SAQ 1

- a) Choose whether following sentence are true (T) or False (F):
- i) T helper cells possess $CD8^+$ surface markers.
 - ii) Cytotoxic T -cells kills the antigen infected cells.
 - iii) MHC molecule require to present antigen peptide on the surface of APCs.
 - iv) $CD8^+$ marker recognize MHC II complex.

- v) $CD4^+$ marker recognize MHC I complex.
 - vi) All nucleated cells possess MHC I molecule.
 - b) Define the CD and MHC molecules.
 - c) Which type of antigen-presenting molecule is found only on macrophages, dendritic cells, and B cells?
 - d) Which type of antigen-presenting molecule is found on all nucleated cells?
-

11.3 T CELL ACTIVATION

T cell activation is an important step in the cell mediated immunity. To activate, T cells must interact to antigen presenting cells (APCs). Mature naive T cells circulate continuously into secondary lymphoid organs (lymph nodes, spleen etc.) via blood stream to identify antigen infected cells and return back to the blood. T cell only recognise antigen peptides which are properly processed by MHC molecules because T cells do not attend antigens without MHC molecules. Hence, T cells are MHC restricted cells. When mature naive T cell recognize specific antigen peptide: MHC complex on the surface of antigen presenting cells, the activation process is started (Fig. 11.4). There are three steps involved in T cell activation:

Step 1: T cell co-receptors recognise specific antigen peptides presented by MHC molecule on the surface of a dendritic cell.

Step 2: $CD4^+$ T cell receptor binds with the specific antigen as peptide-MHC II complex while $CD8^+$ T cell binds to the peptide-MHC-I complex.

Step 3: Upon proper binding interaction, APCs deliver two distinct signals, one to TCR via peptide-MHC complex, and a second **co-stimulatory** signal (i.e., cytokines) that trigger activation of naive T cells. Activated naive T cells secrete signal molecule i.e., cytokines that trigger activation of the T cells maturation. Activated mature T cells undergo proliferation to express their surface receptor molecules i.e., **cluster of differentiation** (CD). Among the CD molecules, some T cells bear only $CD8^+$ markers which are functionally recognised as cytotoxic T Cells and some T cells have $CD4^+$ surface markers on their surface and such cells are called $CD4^+$ helper T cells.

However, the T cell activation process for cytotoxic and helper T cells is similar for both cells. The key difference only is that $CD4^+$ T cell recognizes foreign peptide via class II MHC molecules and $CD8^+$ T cell recognizes antigen peptide via class I MHC molecules.

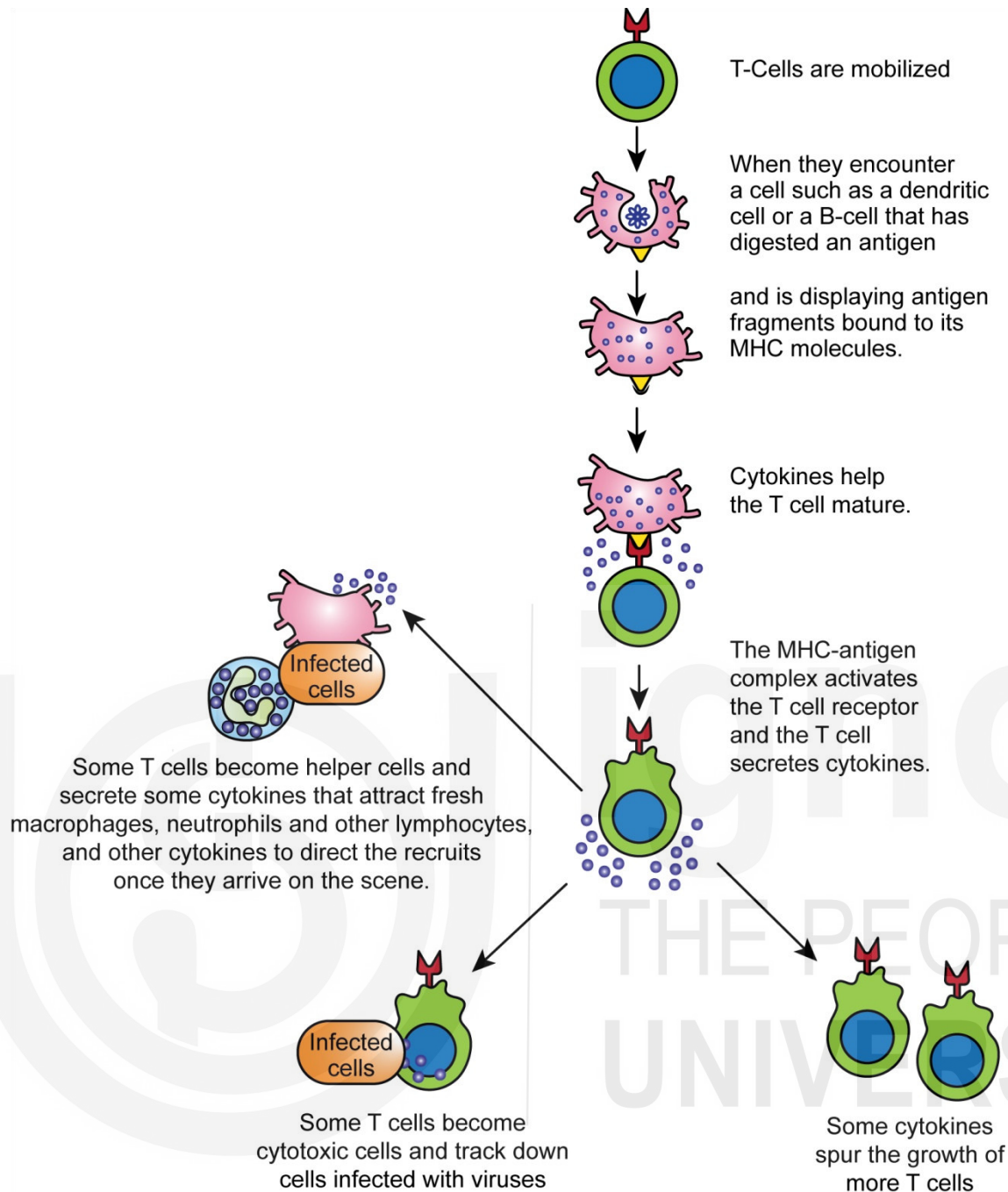


Fig. 11.4: T-cell activation process mediated by intercellular antigens.

SAQ 2

- Antigen presenting cells process and present antigen as
- T cell activation is driven by the
- T cell only recognise antigen peptides that are properly processed by
- Activated mature T cells can undergo differentiation process to produce
- Activated naive T cells secrete

11.4 T CELL MATURATION

Thymus is the main site for T cells maturation. Immature T cells serve as the progenitor cells or naive cells as they have not been presented to the antigen peptide-MHC complex. Thymus executes a mechanism for the selection of effective and non-effective T cells. It provides a suitable microenvironment for T cells development. T cell maturation process within thymus is divided into the positive selection and negative selection which are collectively called thymic selection. Fig. 11.5 shows the overview of positive selection and negative selection.

Let us discuss the positive selection and negative selection.

11.4.1 Positive Selection

Positive selection occurs in the thymic cortical region of thymus where immature T lymphocytes interact with cortical epithelial cells having MHC molecules (MHC Class I and II). In this process, only few T cells are positively selected and the rest of the T cells dies (about 98%) through apoptosis.

During the development, immature T cells express surface receptors along with co-receptors ($CD4^+$ and $CD8^+$) which are referred to as double positive T cells. Only those immature T cells are positively selected, if their receptors (TCRs) recognise properly and interact moderately to the self-peptide: self-MHC-complex presented by thymic cortical epithelial cells. This interaction releases survival signals that prevents T-cells from undergoing cell death. T cells whose receptors do not interact with the cortical epithelial cells, they would not get survival signal and undergo cell death by apoptosis. This process results in MHC restriction.

In positive selection, T cells undergo gene rearrangement and proliferation via mitosis for their maturation process. They are differentiated into distinct types; $CD8^+$ T cells (cytotoxic T cells) or $CD4^+$ T cells (helper T cells). These two distinct mature naive T cells can proliferate and differentiate into their subtypes when they interact again with specific MHC associated antigen peptides. Therefore, positive selection plays an extraordinary role in the T-cells maturation.

11.4.2 Negative Selection

Positively selected T cells further undergo negative selection. The medulla region of the thymus is committed to negative selection. In this process, mature T cells are again presented with the self-peptide-self MHC complex derived from thymic medullary epithelial cells. If TCRs bind tightly to the self peptide-MHC complex, then T-cells receive suicidal response to cell death via apoptosis process. Only those T-cells will survive which possess minimum affinity to the self-peptide-self-MHC complex.

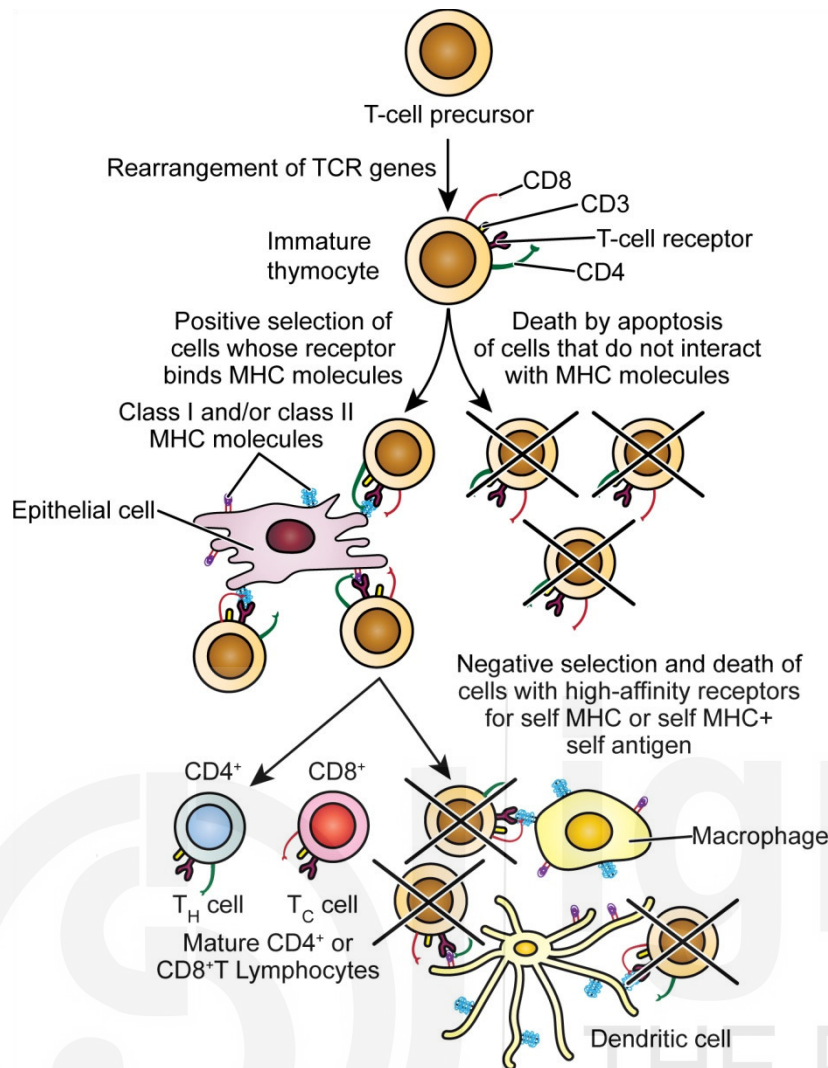


Fig. 11.5: Schematic diagram of T cells positive selection and negative selection.

Credit: Kuby-Immunology

The negative selected T cells are auto-reactive cells (autoimmune cells) which can be harmful to the normal body cells. To overcome this problem, such auto-reactive thymocytes are removed by negative selection. Thus, negative selection is an essential process to protect the normal body cells from the auto-reactive immune cells.

Thus, thymic selection ensures the selection, maturation and expression of distinct class of T cells receptors on their surface. The T cells express different T-cell receptors to recognise self-peptide / non-self-peptide bound to MHC molecules presented by antigen-presenting cells and nucleated cells. The question is yet to be unknown how the T-cell co-receptor distinguishes the self-peptide: self-MHC complex between these opposite affinities during thymocytes development.

SAQ 3

Differentiate between positive selection and negative selection.

11.5 T CELL DIFFERENTIATION

As T cells become properly mature by thymic selection, they migrate from thymus into secondary lymph node, where they interact with antigen presenting cells. Following activation, T cells can differentiate into different subtypes. As you know mature $CD4^+$ TCRs recognise specific antigen peptide-MHC class II complex and $CD8^+$ T CRs recognise specific antigen peptide-MHC class I complex. However, T cell differentiation can take several days or weeks for making their subtypes.

11.5.1 Helper T cell Differentiation

The pathway of T helper cell differentiation is depicted at Fig. 11.6. As mature $CD4^+$ TCRs recognizes specific antigen peptide bound to MHC-II of the APCs, the T helper cell become activate to release cytokines. After being activated, T helper cells undergo clonal selection via proliferation and differentiated into distinct subtypes i.e., T helper 1 cell (T_H1), T helper 2 cells (T_H2) and memory T helper cells etc. (Table: 11.2). There are four distinct T cell subtypes and their functions are summarized in Table 11.2. All of these T helper cell subtypes are characterised by the expression of unique cell surface markers. However, the proliferation and differentiation of distinct T cell lineage depends on complex network of cytokines signalling. These subtypes play diverse roles in ranging from activation of innate immunity to cell mediated immunity (B lymphocytes). Cytokines are a group of small interleukin protein released by activated dendritic cells associated with T cells. They are involved in activation, proliferation, and differentiation of T cells ($CD4^+$ T cells and $CD8^+$ T cells). IL-12 stimulates the activated T helper cells to produce T helper subset i.e., T_H1 , T_H2 , and memory helper T cells and regulatory helper T cell. IL-10 also helps to develop T_H2 cells.

The types of Helper T-cells and their function are summarised in **Table 11.2**

Subtypes of Helper T Cells		
Subtype	Cytokines production	Functions
T_H1 cells	IFN and TNF	Stimulate cytotoxic T cells to produce memory cytotoxic T cells. Host defence protection against virus and bacteria; stimulate NK cells to kill infected cells more effectively
T_H2 cells	IL-4, IL-5 IL-13	Trigger B cell activation and differentiation into plasma cells and memory B cells and direct antibody class switching in B cells
T_H17 cells	IL- 17, IL-21, IL-22	Induce immunity to specific infections such as chronic mucocutaneous infections
Memory helper T cells		“Provide rapid immune response and protection against specific pathogen.

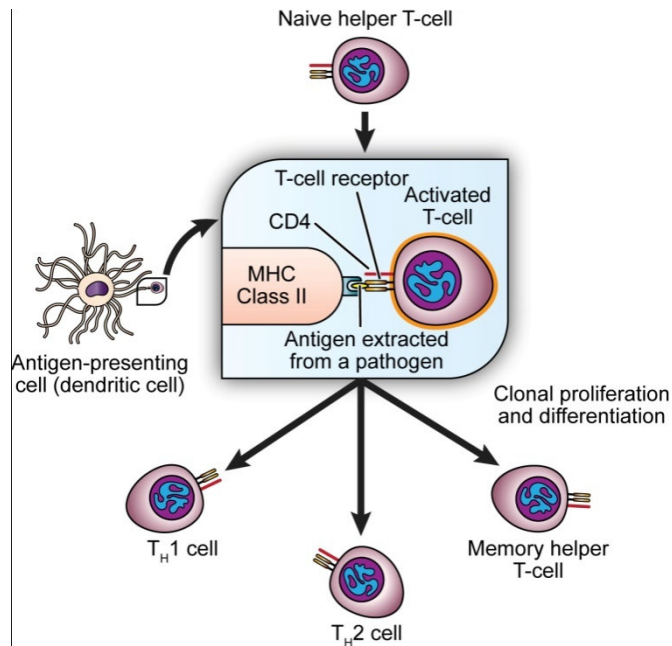


Fig. 11.6: Activation and differentiation of T helper cells into distinct subsets by antigen presenting cells.

11.5.2 Cytotoxic T-cell Differentiation

Like the T helper cell differentiation process, the peptide-MHC I complex and co-stimulatory cytokines initiate the cytotoxic T cells differentiation process. Activated cytotoxic T cells act as effector cytotoxic cells to kill the antigen infected cells (APCs). T_H1 cells secrete cytokines that stimulate the proliferation and differentiation of cytotoxic T cells. Effector cytotoxic T cell releases cytotoxic molecules called perforin and granzymes that destroy the antigen-infected cell (Fig. 11.7).

Effector T cells differ from naive mature T cells as they respond quickly and attack on target cells to destroy intracellular antigens without further differentiation. These effector CD8⁺T cells become memory cells that are ready to respond to subsequent exposures of a specific pathogen. Therefore, a differentiation step is required to make clonal T cells with specific functions that regulate and prevent microbial infection, as well as development of memory cells.

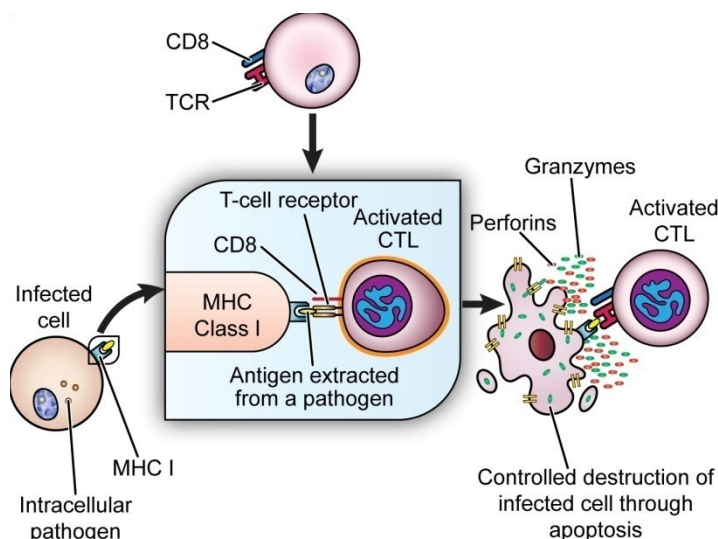


Fig.11.7: Activation of naive cytotoxic T cells by binding interaction of CD8⁺TCR with MHC-I peptide complex on antigen-infected cells.

11.5.3 Memory T Cells

Once effector T cells are fully developed over the period of time, they produce memory T cell via clonal selection of cell division (Fig.11.8). Memory T cells shows immune response when the antigens infect cell second time. They have memory or pre-information for recognizing and kills the specific antigen infected cells. Hence, memory T cells are considered as a secondary immune response.

Memory T cells are long lived immunological cells that produce quick immune response upon re-exposure to the antigens. They are specific for one particular type of antigen. Memory T cells bear either $CD4^+$ or $CD8^+$ marker but not both. These cells act as effector T cells to quickly encounter target cells as they recognise antigen peptide bound MHC molecules on antigen infected cells. These mature cells further proliferate into effective $CD8^+$ T cytotoxic cells and T helper cells that target and kill the antigens (virus).

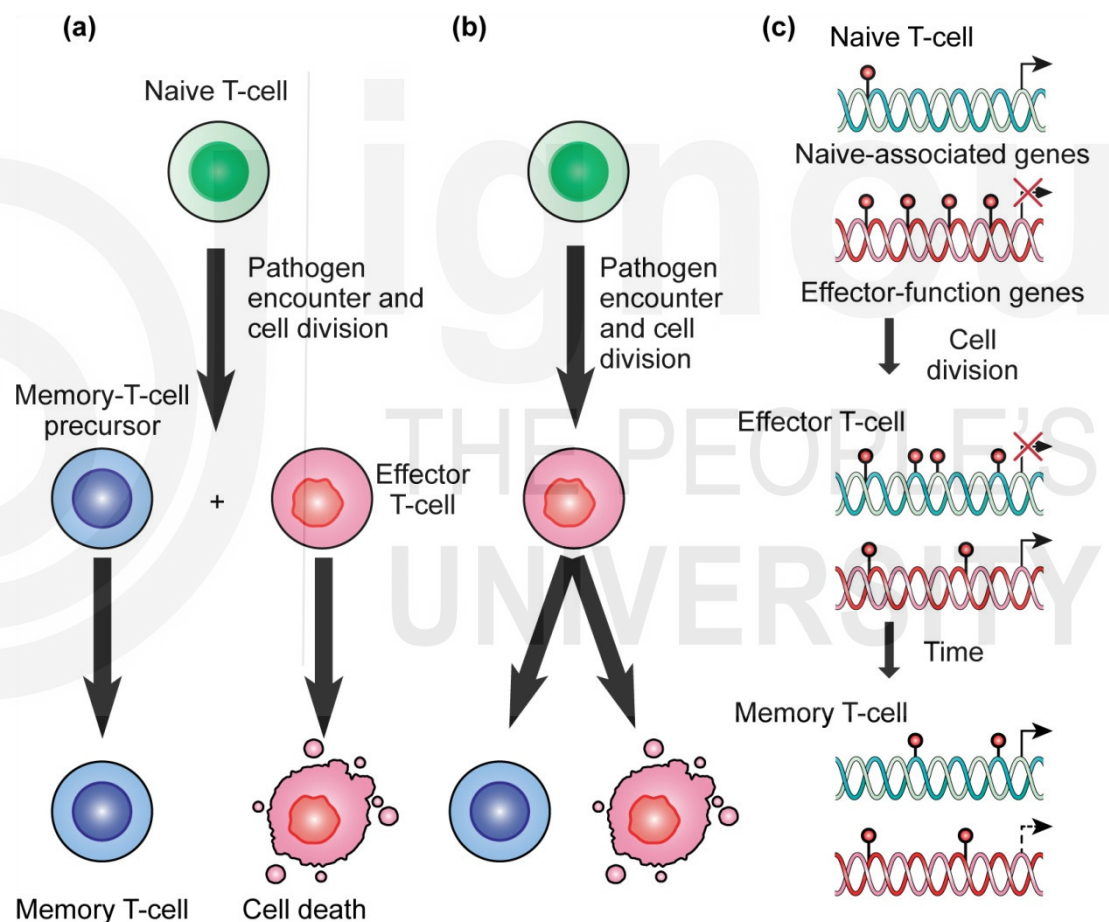


Fig.11.8: Overview of Memory T-cell development.

11.6 SUMMARY

So far, you have learned that:

- Immature or naive T lymphocytes migrate from red bone marrow to the thymus for maturation. These cells are activated by professional antigen presenting cells (Dendritic and macrophages cells). Antigen presenting cells (APCs) present MHC: peptide complex to T cells for their activation that leads to cell mediated immunity.

- T cells activation require three components: T Cell complex-MHC: peptide interaction.
- Each T cells express different version of T-cell receptor that can recognise different sets of MHC-peptide complex expressed on APCs. Each T cell also express co-receptors on their surface such as CD4⁺ or CD8⁺ which facilitates TCR engagement with peptide-MHC complexes on antigen-presenting cells.
- T helper cells carry co-receptor CD4⁺ that recognize class II MHC molecule and T cytotoxic cells bears CD8⁺ co-receptor and recognize class I MHC molecules. These cells initiate cell mediated immunity when their receptors (TCRs) interact with the MHC-peptide complex on the APCs.
- Antigen presenting cells primarily ingest and digest non-self antigen by phagocytosis and process the antigen peptide with the class II MHC for presentation to T cells activation. MHC molecules encodes in large group of genes. All healthy nucleated cells have class I MHC molecules that essential for presentation of normal self-antigens while class II MHC molecules are found only on the surface of antigen presenting cells which play important role in T cells activation.
- Positive selection ensures survival of only those T cells whose TCRs recognize self MHC molecules. Negative selection eliminates those T cell whose TCRs react strongly with self peptide-MHC molecule thereby it inhibits self reactive immune response or auto-immune T cells.
- Activated helper T cells can differentiate into their various subsets such as T_H1, T_H2, T_H17, or **memory T cell subtypes**. Each subset performs different functions to support or stimulate the both adaptive and innate immune system.
- Memory T cells are specific programmed immune cells that respond quickly to secondary antigen exposures.
- Cytotoxic T cells act as effector cells that target and kill antigen infected cells. Killing requires **perforin** and **granzymes** that induce apoptosis to destroy/remove completely infected cells from the body.
- Cytokines are protein molecules that act as signaling molecules for that play an important role in activation, differentiation and regulation of T cells and other immune cells (B Cells).

11.8 TERMINAL QUESTIONS

1. Define what is the cell-mediated immunity.
2. Differentiate between helper T cells and cytotoxic T cells.
3. Explain how antigen presenting cells process and present antigen to T cells.
4. Explain the process of T cell activation.

5. Name the location sites of positive selection and negative selection.
6. Explain the role of MHC molecules play in cell mediated immunity.
7. Explain the thymic selection.
8. What is effector T cells?
9. What are cytokines and how they help in CD4⁺ T cells and CD8⁺ T cells differentiation?

11.7 ANSWER

Self Assessment Questions

1. a) i) F, ii) T, iii) T, iv) T, v) T, vi) T, vii) T.
 (b) The **cluster of differentiation** refers to a group or cluster of different proteins occur on the surface of immunological cells (T cells) and determined by different numbers. It is often abbreviated as CD which are very useful for the identification and characterization of lymphocytes and their different subpopulations. For example, CD4 is a membrane surface glycoprotein found on the immune cells such as T helper cells, monocytes, macrophages, and dendritic cells. T cytotoxic cells have the cluster of determination receptor CD8 and T helper cells CD4 receptor.
 The major histocompatibility complex (**MHC**) refers the set of polymorphic genes (vertebrate DNA) that code for cell surface proteins essential for the T-cell mediated immunity. These genes produce surface proteins are called **MHC molecules**. T cells only recognized only MHC-bound antigen peptide processed by antigen presenting cells.
 (c) MHC-II.
 (d) MHC-I.
2. a) MHC-peptide complex
 b) antigen stimulation.
 c) Antigen presenting cells
 d) T cytotoxic cells and T helper cells
 e) cytokines (lymphokines)
- 3.

Positive Selection	Negative Selection
It occurs in cortical epithelial cells.	It takes place in medulla region of thymus.
Cortical cells produce protective signal upon interaction with the T cell receptors.	It is the process of removal auto reactive T cells.
Only those T cells is positively whose TCRs recognise self MHC -self peptide molecules. If T cell receptor is not capable to interact with self MHC-peptide, they undergo cell death.	Only T cells is eliminated whose TCRs react strongly react with self peptide-MHC molecule.

Terminal Questions

1. Refer to section 11.2.
2. Refer to Table 11.1.
3. Refer to subsection 11.2.2.
4. Refer to section 11.3.
5. Refer to section 11.4.
6. Refer to subsection 11.2.2.
7. Refer to section 11.4.
8. Refer to subsection 11.5.2.
9. Refer to subsection 11.5.1 and 11.5.2.



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WORKING OF THE IMMUNE SYSTEM-IV

Structure

12.1	Introduction	12.6	Functions of Complement
	Objectives	12.7	Complement Related Diseases
12.2	Cytokines and their Action and Properties	12.8	Regulation of Complement System
12.3	Functions of Cytokines	12.9	Summary
12.4	Complement System: Components	12.10	Terminal Questions
12.5	Complement System: Pathways	12.11	Answers

12.1 INTRODUCTION

In this unit, we will study the details of cytokines and complement systems. The cytokines are low-molecular-weight signalling proteins or glycoproteins. They mediate and regulate immunity, inflammation and haematopoiesis. In addition, they act as humoral regulators that modulate individual cells' functions and assist in regulating the development of immune effector cells. In addition, WBCs and various other cells secrete cytokines. Moreover, the recombinant cytokines are pharmaceutical analogues of endogenously produced cytokines to treat multiple infections or immune disorders.

This unit will also study the complement system, which consists of many small proteins found in plasma. It is activated by pathogens directly or indirectly through pathogen-bound antibodies, resulting in a cascade of reactions. There are three pathways to activate a complement: classical, lectin, and alternative and generate active components with various effector functions on the surface of pathogens. For initiation, the classical pathway requires antigen-antibody complexes, whereas the alternative path does not. In addition, when lectin attaches to hexoses containing carbon 3 and 4 - OH groups, such as N-acetyl-d-glucosamine, glucose, fucose, and mannose found on the surfaces of bacteria, yeast, parasites, mycobacteria, and some viruses, the lectin pathway is triggered.

Objectives

After studying this unit, you should be able to:

- ❖ define cytokines,
- ❖ enlist the actions and properties of cytokines,
- ❖ discuss the function of cytokines,
- ❖ explain how Cytokines differ in innate and adaptive immunity,
- ❖ list of recombinant human cytokines and their uses,
- ❖ define complement system and list out its functions,
- ❖ describe various complement activation pathways,
- ❖ explain how does mannose lead to complement activation,
- ❖ give examples of complements related diseases, and
- ❖ explain the regulation of the complement system.

12.2 CYTOKINES AND THEIR ACTION AND FUNDAMENTAL PROPERTIES

Chemokines are a subpopulation of cytokines and represent a class of chemoattractants. Their primary function is to mobilize immune cells from one area to another (Fig.12.1).

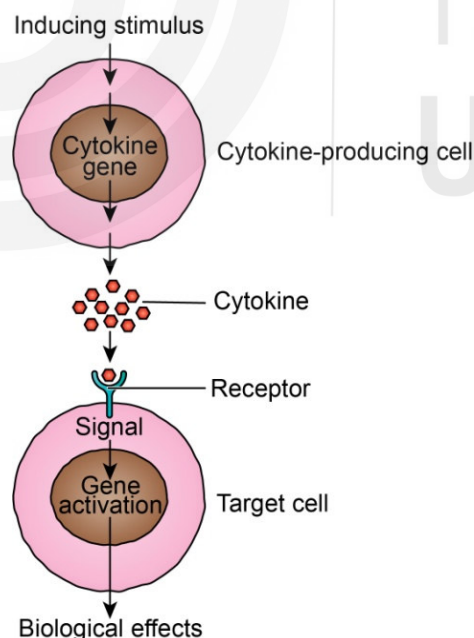


Fig. 12.1: Cytokines as humoral regulators modulate the functions of a target cell.

1. Cytokines and their actions

Cytokines exhibit the following three types of action (Fig.12.2).

- i) **Autocrine action:** Cytokine binds to membrane receptors of the same cell that secreted it.

- ii) **Paracrine action:** A cytokine binds to receptors on a target cell near the producer cell.
- iii) **Endocrine action:** Cytokine travels through circulation and acts on target cells in distant body parts.

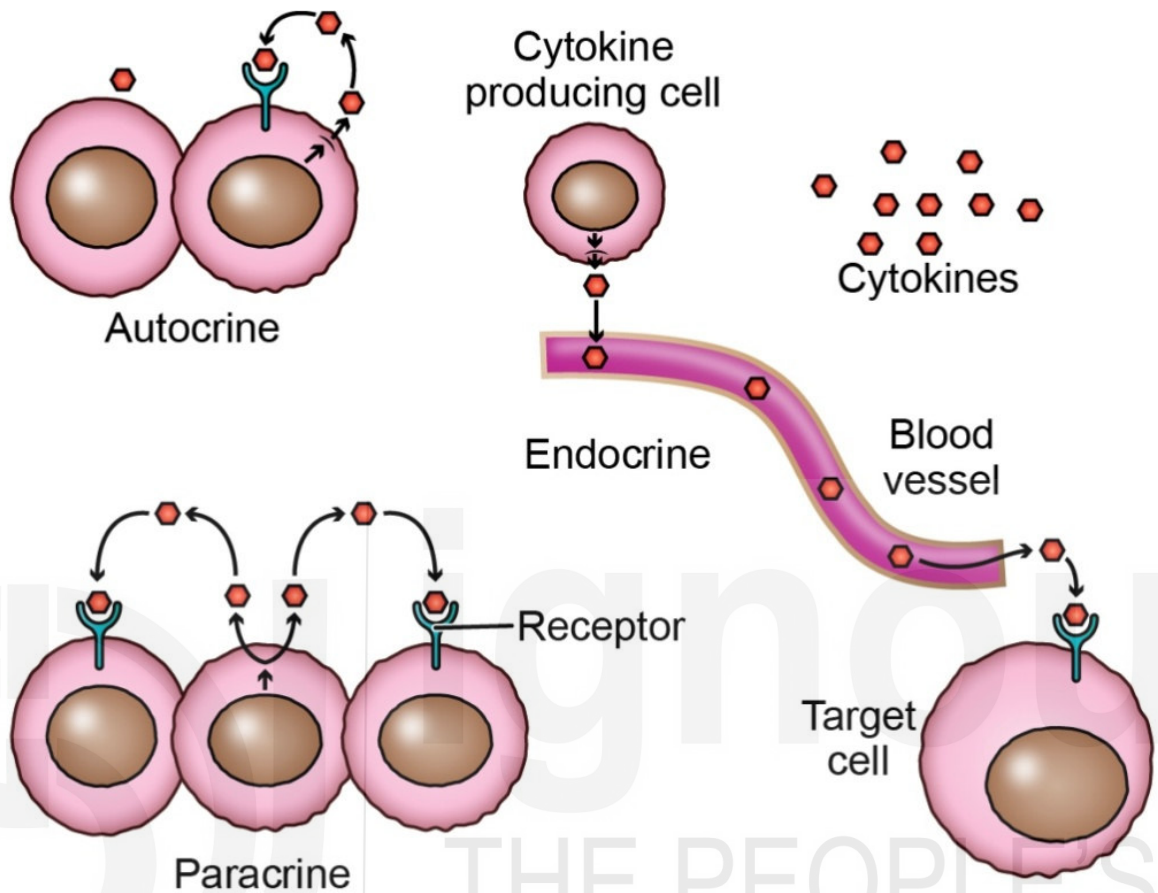


Fig. 12.2: Cytokines exhibiting Autocrine, Paracrine and Endocrine functions.

2. Fundamental properties of cytokines

The properties of cytokines include pleiotropy, redundancy, synergy, antagonism and cascade induction (Fig.12.3). Let us discuss these terms in this section.

- i) **Pleiotropism:** it refers to a cytokine's ability to operate on different cell types and cause various biological effects.
- ii) **Redundancy:** It indicates the property where two or more cytokines have the same functional effects.
- iii) **Synergism:** It refers to the combined effect of multiple cytokines. The combined effect of two cytokines on cellular activity is greater than the additive effects of the individual cytokines.
- iv) **Antagonism:** This property is just opposite to the synergism. In this case, one cytokine inhibits the effects of another cytokine.
- v) **Cascade induction:** It is the multiple-step feed-forward mechanism. The cytokine secreted by one cell type activates a second cell type; the second cell type, in turn, secretes a cytokine that acts on another cell type.

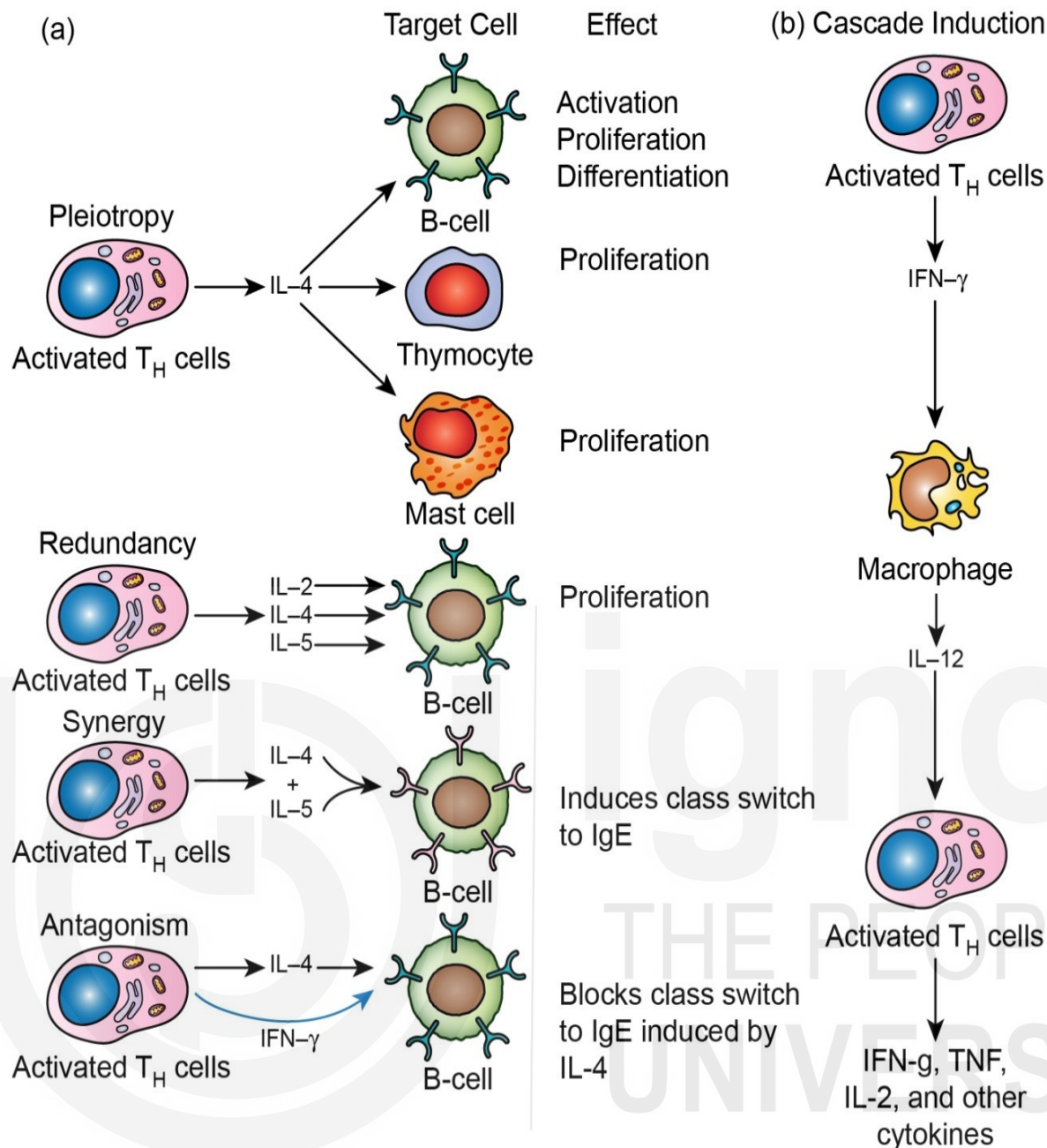


Fig.12.3: Properties of cytokines: Pleiotropy, Redundancy, Synergy, Antagonism and Cascade Induction.

SAQ 1

- What are chemokines? How do they help in immune response?
- Cytokines exhibit the property of Cascade Induction. Explain.

12.3 FUNCTIONS OF CYTOKINES

Based on structural studies cytokines, belong to 6 families. They are the Interleukin family, Hematopoietin family, Interferon family, Tumour necrosis factor family, Chemokines family and Interleukin-17 or Transforming growth factor-beta family. The functions of cytokines belonging to these six groups are described in Table 12.1.

Table 12.1: Functions of Cytokines belonging to six different groups.

S. No.	Cytokines	Functions
1.	Interleukin Family: Members of this family include important proinflammatory mediators between leukocytes. These are produced by T-helper cells and are secreted early in an immune response. IL-1 was the first non-interferon cytokine to be identified. Ligands- IL-1α, IL-1β, IL-1Ra, IL-18, IL-33.	Depending on the Interleukin and cell type, there are a variety of actions. Proliferation and differentiation of cells. Antibody secretion and chemoattractants.
2.	Hematopoietin Family: It is a large family of small cytokine molecules which promote cell proliferation and differentiation. They exhibit functional diversity. As these were structurally characterized first, they are called CLASS I. Ligands- IL-2, IL-3, IL-4, IL-5, IL-6, IL-13, IL-15, IL-21, IL-27.	Promote cell proliferation and differentiation.
3.	Interferon (Class II) Family: Interferons are the first cytokines to be discovered. They are essential modulators of immune responses and have roles in antiviral responses. They are of two types: TYPE I- Macrophages, dendritic cells, and virus-infected cells all release interferons. TYPE II- Activated T and Natural Killer (NK) cells generate various interferons. Ligands- IFN- α, IFN- β, IFN γ -, IL-10, IL-19, IL-20, IL-22, IL-24	Antiviral proteins. Inhibit viral replication and cell proliferation.
4.	Tumor Necrosis Factor Family: Members of this family are involved in immune system development, effector functions, and homeostasis. They may be either soluble or membrane-bound. In addition, they are involved in signal development, activation, or death of specific cells. Ligands- TNF- α, TNF- β, TNF-γ, IL-10, IL-19, IL-20, IL-21, IL-24.	Regulate inflammatory and immune responses like phagocytosis and cytotoxicity.

5.	Chemokines Family: All the members of this family serve as chemoattractants. They stimulate the migration and activation of phagocytes. Ligands- IL-8, RANTES, MIP-1, PF4, MCAF, NAP-2, CCL19, CCL-21	Regulate cell migration, adhesion and activation.
6.	Interleukin 17 Family or Transforming Growth Factor Beta Family: Members of this family are most recently discovered. They promote neutrophil accumulation, activation and are proinflammatory cytokine clusters. Ligands-IL-17(IL17-A), IL17B,C,D and F	Regulate Immune cells.

Cytokines play an important role in both innate immunity and adaptive immunity. Some of the cytokines of innate immunity are listed in Table 12.2, and cytokines of adaptive immunity are listed in Table 12.3.

Table 12.2: Cytokines of Innate Immunity.

S. No.	Cytokines	Principal cell sources	Principal cell targets and biological effects
1.	IL-1(IL-1 α and - β)	Endothelial cells, macrophages, and certain epithelial cells	Endothelial cells in the liver: Activation, Inflammation, Coagulation, and Acute-phase Protein Synthesis
2.	IL-10	Macrophages and T cells	Anti-inflammatory macrophages (APCs) suppress IL-12 cytokine production as well as the expression of co-stimulators and class II MHC molecules
3.	IL-12	Macrophages, B cells, and dendritic cells are all types of immune cells	T _H and NK- Proliferation, IFN- γ synthesis, increased cytolytic activity
4.	TNF	Macrophages, T cells	Tumour cells, Neutrophils, Macrophages Cytotoxicity, induction of cytokine secretion, phagocytosis, Many cells -Apoptosis

Table 12.3: Adaptive Immunity Cytokines.

S. No.	Cytokines	Principal cell sources	Principal cell targets and biological effects
1.	IL-2	T _H 1 cells	T cells and NK cells- Proliferation, activation. B cells- Proliferation, antibody synthesis
2.	IL-4	T _H 2 cells, Mast cells, NK cells	T cells, Mast cells - Proliferation. Macrophages- Inhibition of IFN- γ mediated activation. B cells- Isotype switching to IgE
3.	IL-5	T cells	Eosinophils- Proliferation and differentiation activation. B cells- Proliferation, IgA production
4.	IFN- γ	T cells and NK	Activation of Macrophages Inhibitor of viral replication. Cell proliferation inhibitor. Increased antigen processing and presentation to T cells in various cells due to greater expression of class I and class II MHC molecules.

Recombinant DNA technologies produce several human cytokines. They are being used to treat various infections or immune disorders. Some are listed in Table 12.4.

Table 12.4: List of some Recombinant Human Cytokines and their uses.

S. No.	Recombinant Human Cytokines	Treatment of Disease/Disorder
1.	Recombinant interferon alfa-2a (<i>Roferon-A</i>)	It's a cytokine that's used to treat Kaposi's sarcoma, chronic myelogenous leukaemia, and hairy cell leukaemia, among other diseases.
2.	Peginterferon alfa-2a (<i>Pegasys</i>)	It's a drug that's used to treat hepatitis C. (HCV).

3.	Recombinant interferon-alpha 2b (<i>Intron A</i>)	Hepatitis B, Malignant melanoma, Kaposi's sarcoma, Follicular lymphoma, Hairy cell leukaemia, Warts, and Hepatitis C are all treated with it.
4.	Recombinant interferon-alpha n3 (<i>Alferon N</i>)	It's used to get rid of warts.
5.	GM-CSF (Granulocyte-Macrophage Colony Stimulating Factor)	It's used to help people with lymphoid malignancies rebuild their hematopoiesis following a bone marrow transplant.

SAQ 2

Give one example of recombinant human cytokines and their uses.

12.4 COMPLEMENT SYSTEM: COMPONENTS

The complement system is made up of more than 20 inert proteins that circulate in the blood and tissue fluids. Complements are proteins that participate in the complement system. Complements were found by Jules Bordet (1895) as heat-labile components in the blood that have non-specific antibacterial activity. Soluble proteins and glycoproteins are these supplements. They're mostly made by liver cells. The complement system works as a biological cascade wherein a series of proteins interact highly, each being the catalyst for the next. Thus, the complement system works in both innate and adaptive immunity.

Complement activation is induced by several innate immune components, as well as by an antibody attached to an antigen. The complement cascade boosts phagocytes' and antibodies' ability to remove germs and injured cells, attack the pathogen's cell membrane, and induce inflammation. Components of the Complement system include serum components, membrane regulatory proteins and membrane receptors. Following are the main components of the complement system:

- There are nine central components in the cascade.
- Complements are typically denoted by the capital letter C followed by a number, such as C1, C2, C3, C4, C5, C6, C7, C8, and C9.
- For example, C1 has three sub-units; C1q, C1r and C1s.
- C2-C5 have two components, a and b; the larger subunits are denoted by b, whereas the smaller ones are indicated by a. However, there is an exception wherein C2a is larger than C2b.
- Some components are designated by alphabet, e.g., B and D.
- Multiple activation products such as C3a and C3b.

- Regulators and inhibitors, e.g., Factor H and C4BP.
- Proteases and newly assembled enzymes, e.g., C4b2a and Factor B.
- Effector molecule receptors such as C3aR and C5aR.

12.5 COMPLEMENT SYSTEM: PATHWAYS

The complement system activation occurs via three biochemical pathways (Fig.12.4). They are:

1. Classical pathway: activated by the antigen-antibody reaction.
2. Alternative pathway: activated on microbial cell surfaces.
3. Lectin pathway or Mannose-binding Lectin pathway: activated by plasma lectin binds to mannose residues on microbes.

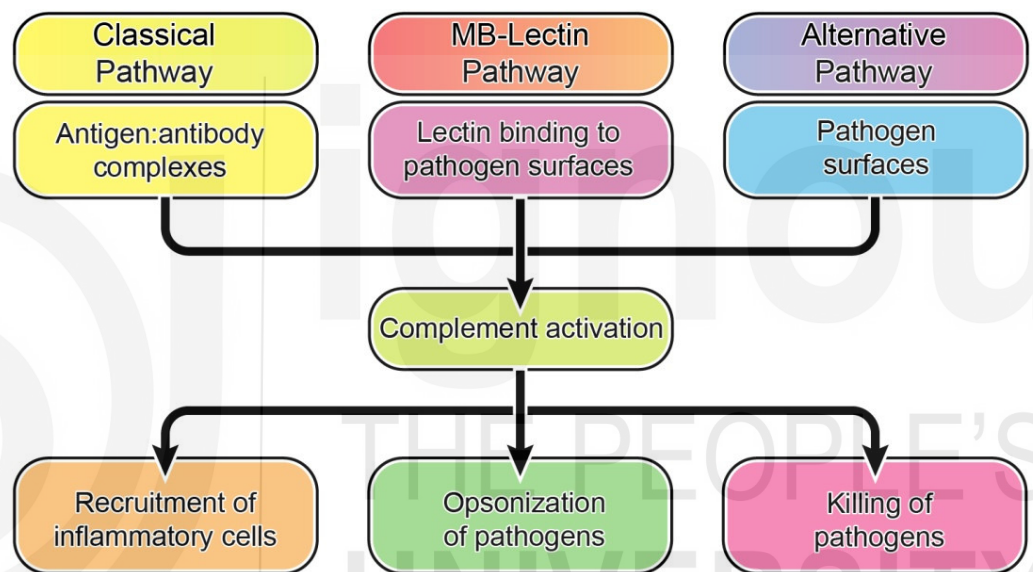


Fig.12.4: Activation of Complement system via three biochemical pathways.

12.5.1 Classical Pathway

Antibody-antigen complexes trigger the classical pathway (Fig.12.5). It involves complement components **C1**, **C2** and **C4**. The antibodies IgM and IgG bind to antigens as they enter into the body. It causes conformational changes in the antibody's Fc region, exposing a binding site for component C1. C1 is a large multimeric protein complex that consists of one C1q, two C1r, and two C1s molecule subunits. C1q interacts with the Fc region of the antigen-bound antibody. The C1r and C1s proteases are involved in the cleavage of C4 and C2. C4 is divided into smaller C4a and larger C4b units when the immune complex links to C1. C4a diffuses away, but activated C4b attaches to the target surface at C1q and attracts C2, which is cleaved into two units, C2a and C2b, in a similar manner (Exception as the larger unit is a and smaller unit is b). The bigger unit, C2a, forms the C4bC2a complex with C4b, while the smaller unit, C2b, diffuses away. C3 is engaged when C4bC2a is active. Because it is responsible for turning C3 into an active form by separating smaller unit C3a from larger unit C3b during the conversion process, the C4bC2a complex is also known as a C3 convertase. C4bC2a is

capable of cleaving a high number of C3 molecules with a single molecule. C3b forms a strong bond with C4bC2a. Some of the C3b molecules do not bind with C4bC2a. Instead, they attach to the microbial cell surfaces or coat immune complexes, act as opsonins, and enhance phagocytosis. The smaller unit C3a activates mast cells, causing the release of histamine. When C3b binds to C3 convertase, it becomes C4bC2aC3b, sometimes known as C5 convertase, because it activates C5.

The C5 convertase enzyme breaks down C5 into smaller C5a and larger C5b pieces. As a result, C5a diffuses away, but C5b is preserved by adhering to C6. C5bC6 then forms a link with C7. The C5bC6C7 complex is subsequently injected into the phospholipid bilayer of the cell membrane, where it interacts with C8. C9 is activated by all of them (C5b678), resulting in the formation of a macromolecular structure known as the membrane attack complex (MAC). This causes pores to form in the bacteria, allowing internal components to flow out and chemicals from the environment to enter. As a result, the cell's osmotic stability is compromised, and lysis ensues as a result of an influx of water and electrolyte loss.

This is particularly effective in Gram-negative bacteria since the development of MAC in the outer membrane of these bacteria is quite straightforward. It is difficult in the case of Gram-positive bacteria because of the presence of a rigid thick layer of peptidoglycan. Smaller complement subunits disperse from the location throughout the pathway, where they bind to particular receptors to cause localised inflammatory reactions.

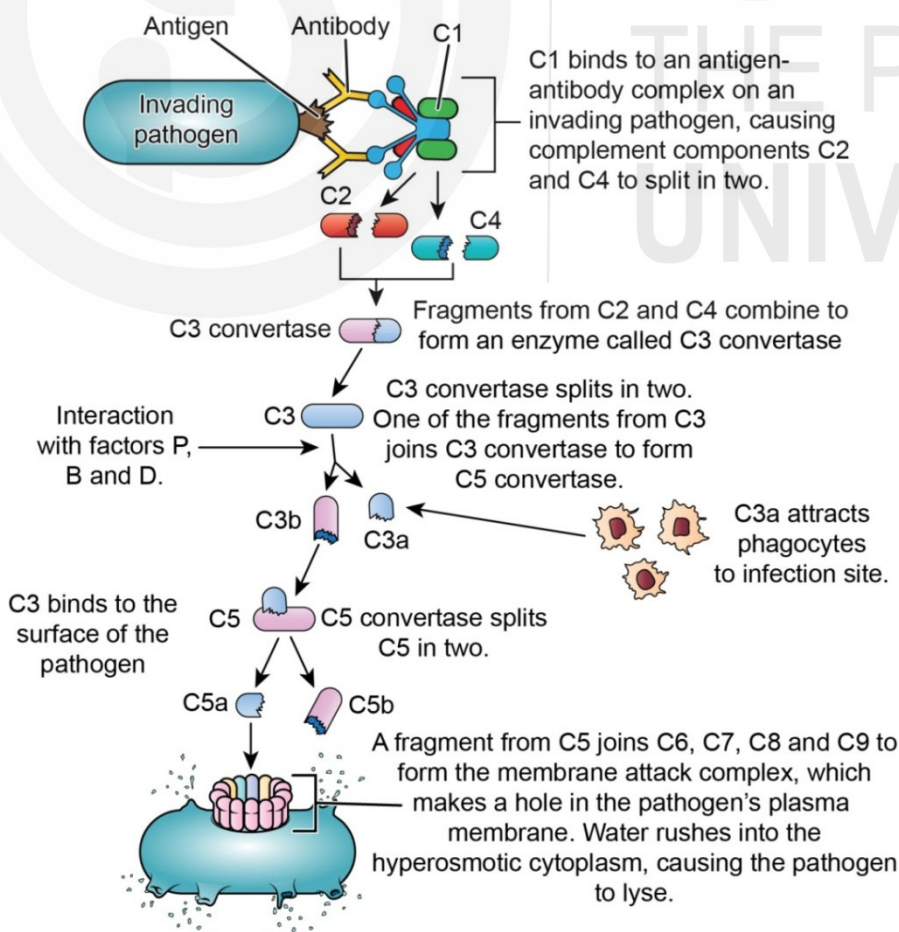


Fig. 12.5: The Classical Pathway.

12.5.2 Alternative Pathway

This pathway is activated by cell surface elements foreign to the host, such as lipopolysaccharide (Fig.12.6), which initiate the process. It does not require an antigen-antibody complex for the initiation of the alternative complement pathway. If a bacterium infiltrates the host body as a result of inflammation, complements travel to the site of infection, where C3 molecules immediately contact antigen and activate.

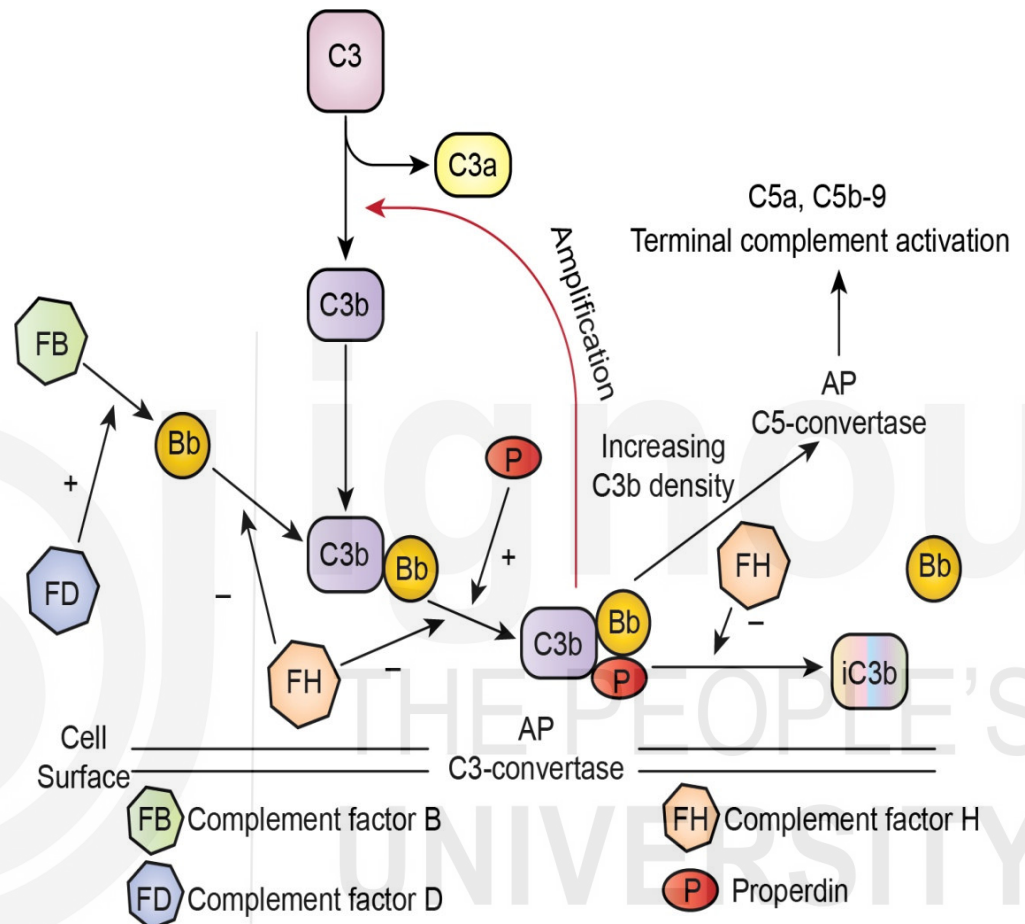


Fig. 12.6: Activation of the Alternative Complement Pathway.

In this pathway (Fig.12.7), serum C3 undergoes slow spontaneous hydrolysis to yield C3a and C3b. The B, D, H, and I factor interact with each other and with C3b to produce a C3 convertase in this pathway.

C3b binds to the surface of foreign cells and subsequently binds to another serum protein known as factor B, which is responsible for the binding. Factor B reveals the location on serum protein D that serves as a substrate for the enzymatically active form of the protein. Then factor D breaks down B into Ba and Bb, resulting in the formation of C3 convertase (C3bBb). According to the conventional pathway, C3 convertase creates C5 convertase, which forms a MAC at the end of the process.

C3bBb can activate more C3; consequently, the pathway is frequently called 'the amplification loop'. Activation of the loop is enhanced in the presence of bacterial and fungal cell walls but is inhibited by chemicals on the surface of normal mammalian cells.

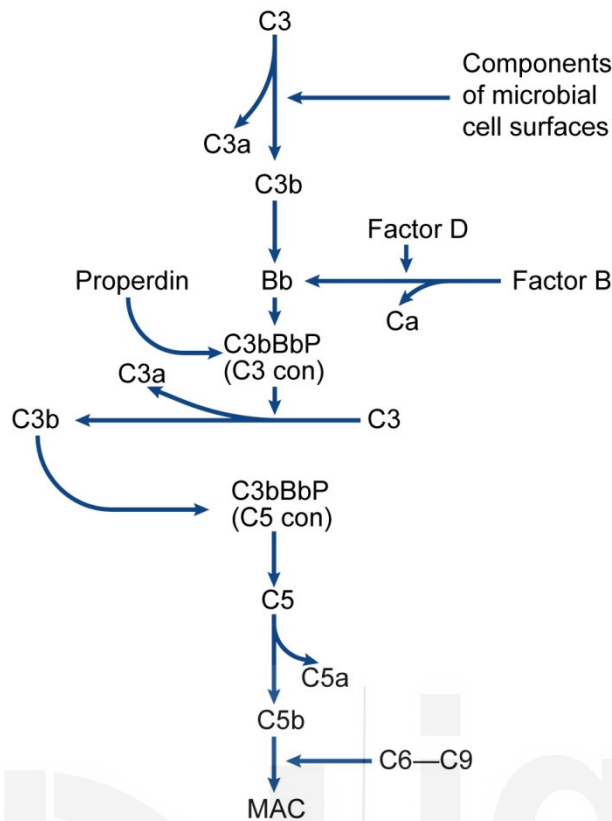


Fig.12.7: The Alternative Pathway.

12.5.3 Lectin Pathway or Mannose-binding Lectin Pathway

Some bacteria such as *Salmonella*, *Listeria*, and *Neisseria* can activate the complement system without having antibodies and endotoxin. Some fungi and viruses, notably the HIV-1 virus, have the ability to activate the MBL pathway. Mannose Binding Lectin (MBL) is an acute-phase protein antibody, and its concentration rises dramatically during inflammation, indicating that the body is responding to the stimulus. It is the mannose residues on glycoproteins or carbohydrates that are present on the surface of microorganisms or the target cell that are recognised and bound by the circulating lectin. The complement system is then activated as a result of this (Fig.12.8).

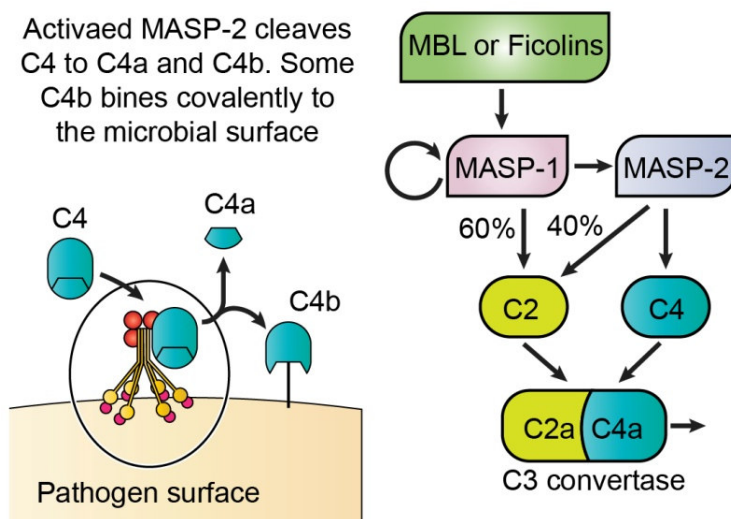


Fig.12.8: Activation of Mannose Binding Lectin Pathway.

MBL pathway resembles the classical pathway of activation of the complement system. Mannose Binding Lectin (MBL) is an acute-phase protein antibody, and its concentration rises dramatically during inflammation, indicating that the body is responding to the stimulus. It is the mannose residues on glycoproteins or carbohydrates that are present on the surface of microorganisms or the target cell that are recognised and bound by the circulating lectin. The complement system is then activated as a result of this (Fig.13.8).

When mannose-binding lectin (MBL) binds to mannose residues on the pathogen surface, the MBL-associated serine proteases MASP-1 and MASP-2 are activated. MASP-1 and MASP-2 cleave C4 and C2 to form C3 convertase C4bC2a, which is analogous to the tetrameric complex formed by C1r and C1s. The procedure now proceeds to make C5 convertase and the MAC, as it did previously.

i) Lytic Pathway

Splitting C5 and attaching C5b to a target kickstarts the lytic pathway. The complements C6, C7, C8, and C9 then join C5b to form the membrane attack complex (MAC). Membrane-attack complex can kill bacteria by lysis when inserted into their outer membrane. Furthermore, if antibodies are bound to the surface of red cells, they can activate the classical and lytic pathways, making them vulnerable to lysis (Fig.12.9).

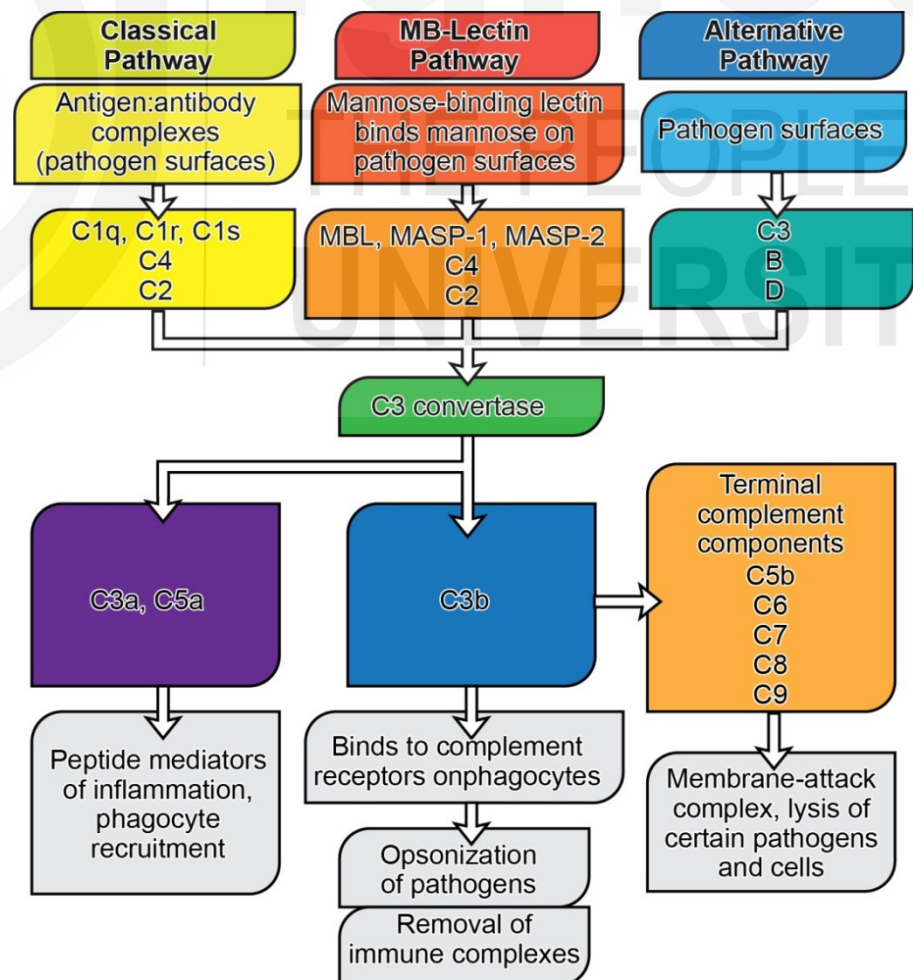


Fig.12.9: Complement Pathways-Classical Pathway, MBL Pathway and Alternative Pathway.

SAQ 3

Which pathways will be followed in the following cases? Give your answer in the space provided.

- i) This process involves many components, including B, D, H, and I, which interact with one another as well as with C3b to produce a C3 convertase enzyme.
- ii) Immune complexes trigger this pathway.
- iii) Antigen-Antibody complexes are not required for inducing this pathway.

ii) Role of C3 in Complement Pathways

The complement system's first step differs in each of the three paths. All of the routes, however, create enzyme complexes, such as C3 convertase, which cleaves C3 into C3a and C3b.

C3 plays an important role in this process by keeping the complement cascade alert. It acts as a point of convergence of all three pathways. It helps in the amplification of the complement response. It helps in better coordination of various immune responses by exerting direct effector functions. C5 convertase is formed when C3b attaches to C3 convertase, and C5 convertase cleaves C5 into C5a and C5b. The activation of other complement system components by C5 convertase, which is produced through the alternative, classical, or lectin pathways, results in the development of the Membrane Attack Complex (MAC), which kills the pathogen in the long term.

12.6 FUNCTIONS OF COMPLEMENT

The primary functions of complements include chemotaxis, opsonization, cell lysis, activation of mast cells, production of antibodies and immune clearance (Fig.12.10).

We will elaborate these terms as follows:

- a) **Chemotaxis:** It is the movement of a cell in response to a chemical stimulus. Complement fragments recruit neutrophils and macrophages to the antigenic region. Complement fragments recruit neutrophils and macrophages to the antigenic region. Receptors for complements, like C5a and C3a, are present on the cell surface of neutrophils and macrophages. They move towards the site of inflammation.
- b) **Opsonization:** It is an immune process that uses opsonins to tag pathogens for elimination by phagocytes. The complement fragment C3b binds to the immune complex or is coated on the pathogen's surface and enhances phagocytic activity. Complements bind to specific receptors on the surface of phagocytic cells, allowing them to engulf the cell.
- c) **Celllysis:** In the presence of C5b6789 components, a membrane attack complex (MAC) is generated, which induces a rupture of the microbiological cell surface and subsequent cell lysis.

- d) **Activation of mast cells:** The proteolytic complement fragments, C5a, C4a, and C3a, bind to mast cells and neutrophils and induce acute inflammation. Because they induce the mast cell degranulation reactions characteristic of anaphylaxis, these peptides are also known as anaphylatoxins. By connecting to certain complement receptors on immune cells, they also induce specific cell activities, inflammation, and the production of immunoregulatory chemicals.
- e) **Production of antibodies:** B cells have a receptor for C3b. Therefore, B cells secrete more antibodies when C3b binds to them. Thus, C3b is an antibody-producing amplifier that helps to destroy invading microorganisms and make the defence mechanism more effective.
- f) **Immune clearance:** The complement system has an anti-inflammatory role because it removes immune complexes from the circulation and stores them in the spleen and liver, thereby reducing inflammation. Furthermore, complement proteins aid in the solubilization of immune complexes and the subsequent clearance of these complexes by phagocytosis.

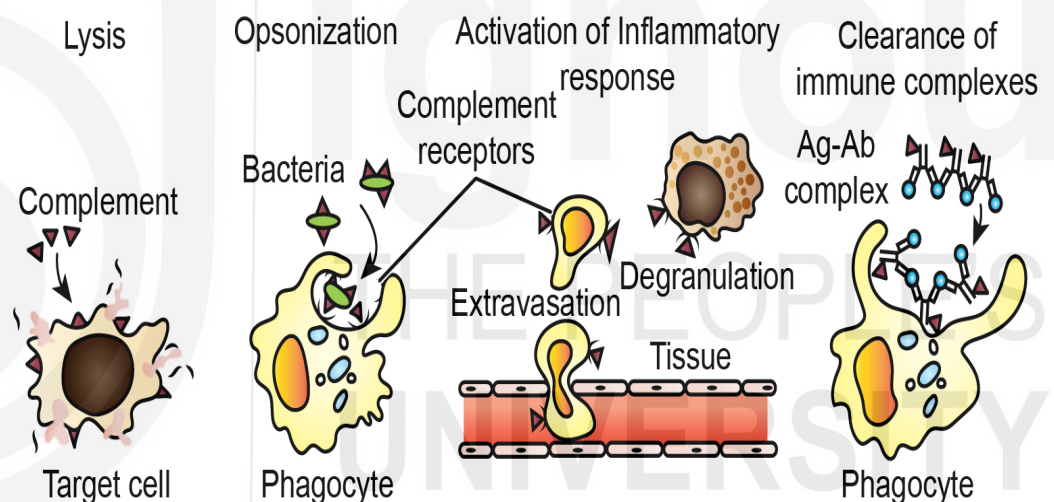


Fig.12.10: Functions of Complements.

12.7 DISEASES RELATED TO COMPLEMENT SYSTEM

Complement-related diseases can be caused by defects in any of the complement cascade's protein or regulatory components.

- C2 and C4 deficiency can lead to systemic lupus erythematosus.
- Pyogenic bacterial infection can be caused by a lack of C3 and factor D.
- C5-C9 deficiency (also known as MAC deficiency) can result in *Neisseria* infections such as gonorrhoea and meningitis.
- Due to a lack of regulatory proteins, complements and sites become too activated, resulting in unwanted inflammation and cell lysis. Such deficiencies result in pyogenic bacterial infection and glomerulonephritis.

- Atypical hemolytic uremic syndrome, age-related macular degeneration, hereditary angioedema, and other diseases can be caused by mutations in the complement regulators factors.
- It is also possible that abnormal stimuli, such as persistent microorganisms, antibodies against self-antigens, or immune complexes accumulated in tissues, will excite the complement system. As a result, even when the system is properly controlled and activated, it has the potential to cause considerable tissue damage.

Let us discuss these terms in this section:

- Systemic Lupus Erythematosus** is a disease in which your immune system attacks your own cells and tissues. It causes inflammation in various parts of the body for some time. It can harm your joints, tendons, kidneys, and skin. It also can affect blood vessels.
- Gonorrhoea and Meningitis:** Gonorrhea is a sexually transmitted bacterial infection that affects both males and females. The urethra, rectum, and throat are the most common sites of gonorrhoea. Gonorrhoea can also infect the cervix in women, while meningitis is a condition in which the fluid and membranes (meninges) that surround your brain and spinal cord become inflamed. Typical signs and symptoms of meningitis include a headache, fever, and stiff neck. A viral infection most commonly causes meningitis, but it can also be caused by bacterial, parasitic, or fungal infections.
- Glomerulonephritis:** Glomerulonephritis is a condition in which the tiny filters in your kidneys become inflamed (glomeruli). Glomeruli remove extra fluid, electrolytes, and waste from the bloodstream and excrete them in the urine. Glomerulonephritis can strike suddenly (acute) or slowly (chronic).
- Hemolytic uremic syndrome** is a condition in which your kidneys' tiny blood vessels become damaged and inflamed. Clots in the vessels can form as a result of this damage. The clots clog the kidneys' filtering system, resulting in kidney failure, which can be life-threatening.
- Age-related macular degeneration:** it is a progressive eye disease that can lead to blindness. In people over the age of 60, it's the leading cause of severe, permanent vision loss. It occurs when the macula, the small central portion of your retina, wears down.
- Hereditary angioedema:** Hereditary angioedema is characterised by recurrent episodes of fluid accumulation outside of blood vessels, which block the normal flow of blood or lymphatic fluid and cause rapid swelling of tissues in the hand, feet, limb, face, intestinal tract, and airway. It's a rare inherited condition.

12.8 REGULATION OF COMPLEMENT SYSTEM

Regulatory mechanisms are required to restrict the complement pathway as it can be highly damaging to host tissues. Complement activation is regulated by

a number of cell membrane and plasma proteins. Different phases in the complement cascade are inhibited by them. The high quantity of sialic acid in the membrane of most mammalian cells causes complement inactivation.

12.9 SUMMARY

Let us summarise what you have learnt in this unit:

- Cytokines are tiny proteins that play critical roles in cell signalling. Cells release them, and they have paracrine, autocrine, and endocrine activities.
- The properties of cytokines include pleiotropy, redundancy, synergy, antagonism, and cascade induction.
- Cytokines are classified into various categories that include chemokines, interferons, and interleukins, lymphokines, tumour necrosis factor, and others.
- A wide spectrum of cells, including immune cells such as macrophages, B lymphocytes, T lymphocytes, and mast cells, release cytokines.
- The complement system comprises more than 20 inactive proteins that circulate in the blood and tissue fluids.
- Complements are proteins that participate in the complement system. The complement system is a biological cascade in which a series of proteins interact in a controlled manner, each acting as a catalyst for the next.
- The complement system activation occurs via three biochemical pathways. They are the classical pathway (triggered by an antigen-antibody interaction), the alternative pathway (triggered on microbial cell surfaces), and the Lectin pathway (also known as the Mannose-binding Lectin pathway in which plasma lectin binds to mannose residues on microbes and activated).
- Regulatory mechanisms are required to restrict the complement pathway as it can be highly damaging to host tissues.
- Complement-related diseases can be caused by any of the complement cascade's protein or regulatory components.

12.10 TERMINAL QUESTIONS

1. What are cytokines?
2. Write your comments on "Cytokines are pleiotropic and redundant".
3. How is the classical complement pathway activated?
4. What triggers alternative complement pathways?
5. Write the salient features of Lectin Pathway.

12.11 ANSWERS

Self Assessment questions

1.
 - a) Chemokines are a subpopulation of cytokines and represent a class of chemo attractants. Their primary function is to mobilize immune cells from one area to another.
 - a) Cascade Induction is the multiple-step feed-forward mechanism. The cytokine secreted by one cell type activates a second cell type; the second cell type, in turn, secretes a cytokine that acts on another cell type.
2. Hepatitis B, malignant melanoma, Kaposi's sarcoma, follicular lymphoma, hairy cell leukaemia, warts, and Hepatitis C are treated with recombinant interferon-alpha 2b (Intron A).
3.
 - a) Alternative Pathway.
 - b) Classical Pathway.
 - c) MBL Pathway.

Terminal Questions

1. Cytokines are soluble proteins with a low molecular weight produced in response to an antigen and act as chemical messengers for controlling the innate and adaptive immune systems.
2. Pleiotropic cytokines are those that act on a variety of cell types rather than just one. Because numerous different cytokines can perform the same job, they are referred to as redundant cytokines.
3. The classical pathway is one of the three complement pathways. Antigen-antibody complexes initiate it, or immune complexes bind with IgG and IgM. In addition, the apoptotic cells, necrotic cells, and acute-phase proteins can also activate this pathway.
4. When the C3b protein directly attaches to a bacterium, the alternative pathway is activated. It results in opsonization and killing of pathogens. Foreign materials and damaged tissues can also initiate it.
5. The lectin complement pathway provides an effective defence against invading pathogens and apoptotic cells in an organism. It is also a proteolytic cascade similar to the classical pathway. In addition, this pathway elicits various effector functions including, phagocytosis, cell lysis, inflammation etc.

SUGGESTED READINGS

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