UNIT 9

WORKING OF THE IMMUNE SYSTEM-I

Structure

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 endosomes and finally presented on MHC class II molecules, which are recognized by CD4+ $\mathbf{T_H}$ 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 torough 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:

a) **Class I MHC** molecules are glycoproteins found on all nucleated cells in the body. It shows the processed antigenic peptides to $\mathbf{T_C}$ cells (cytotoxic T cells).

b) **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 $\mathbf{T_H}$ cells (helper T cells).

c) **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 agroup 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.

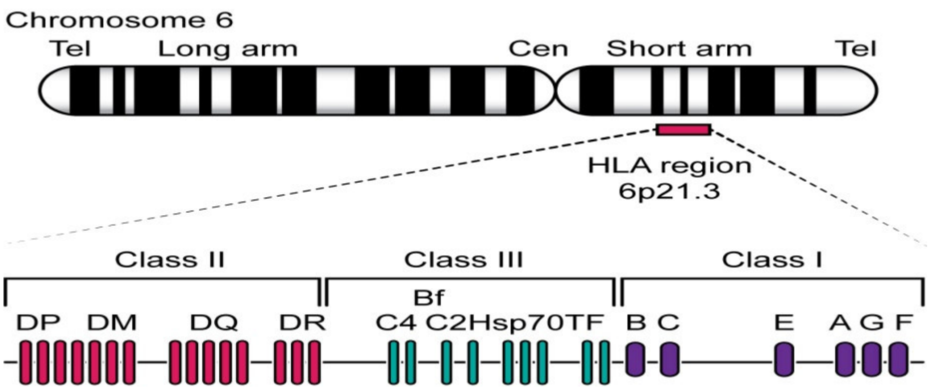

Fig. 9.1: Human chromosome 6:HLA region.

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 associatednon-covalently with a smaller β2-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 β2-microglobulin molecule is an invariant protein encoded by a highly conserved gene on a separate chromosome. The interaction of chain with β2-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 β2-microglobulin protein. For example, Daudi tumour cells lack class I MHC molecules on the cell surface as they cannot synthesize β2-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: α1, α2, and α3. 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 β2–microglobulin. It is comparable to the α3 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 β2–microglobulin. The constant area domains of the α3 domain, β2–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 α1 and α2 on either side of the membrane.

2) A membrane proximal pair has the α3 domain and 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 $\mathbf{T_C}$ cells, the α3 domain appears to be quite conserved. The interaction of 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.

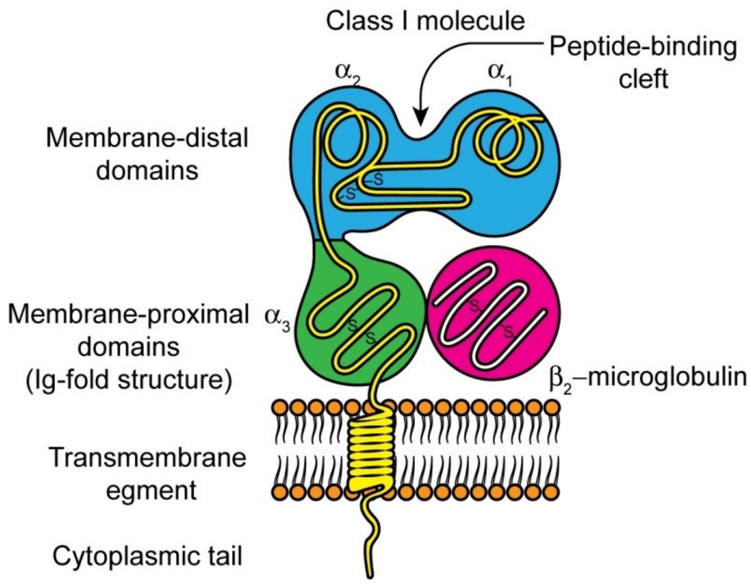

Fig. 9.2: Structure of class I MHC molecule.

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 αchain andβchain. 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, β1 and β2 domains. The class II MHC molecules are considered as members of the immunoglobulin super family. Studies have revealed homology between the fold structure of α2 domain and β2 domain and the immunoglobulin constant region domains.

There are two pairs of domains that interact:

1) A membrane distal pair is made up of α1 and β1 domains.

2) A membrane proximalpair is made up of α2 domain and β2.

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-1domains. 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 ß 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 $\mathbf{T_H}$ 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.

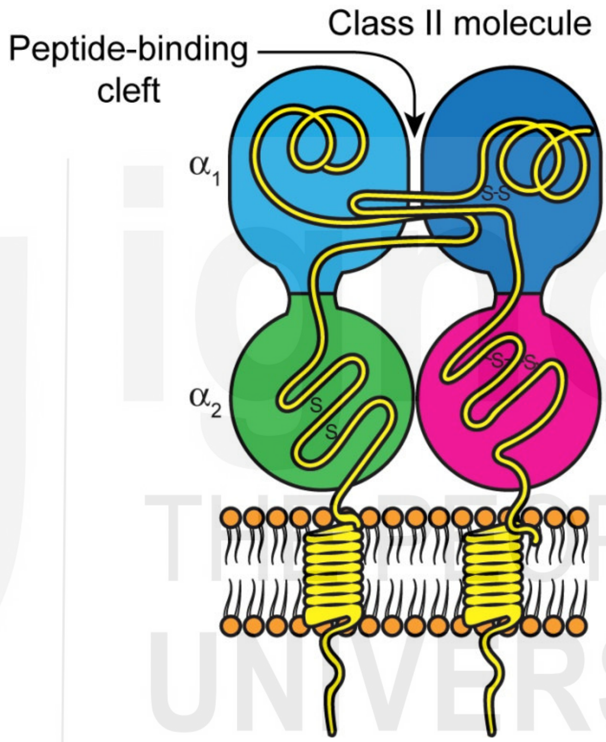

Fig. 9.3: Structure of class II MHC molecule.

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 EXOGENOUSPATHWAYS

Immune response to antigen processing is a metabolic process in whichproteins 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+ $\mathbf{T_C}$ 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+ $\mathbf{T_H}$ 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+ $\mathbf{T_C}$ 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 proteosome. The cytosolic proteolytic system in the cell is known as a proteosome. The proteosome'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. Proteosome 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 proteosome and the 19S regulatory components, among other things (Fig. 10.4). This process is ATP dependent. Degradation of ubiquitin-protein complex occurs in the proteosome'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 proteosome 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 1 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+ $\mathbf{T_C}$ cells. The CD8+ $\mathbf{T_C}$ cells (cytotoxic) will recognize the complex and initiate the immune response to lysis the infected cell.

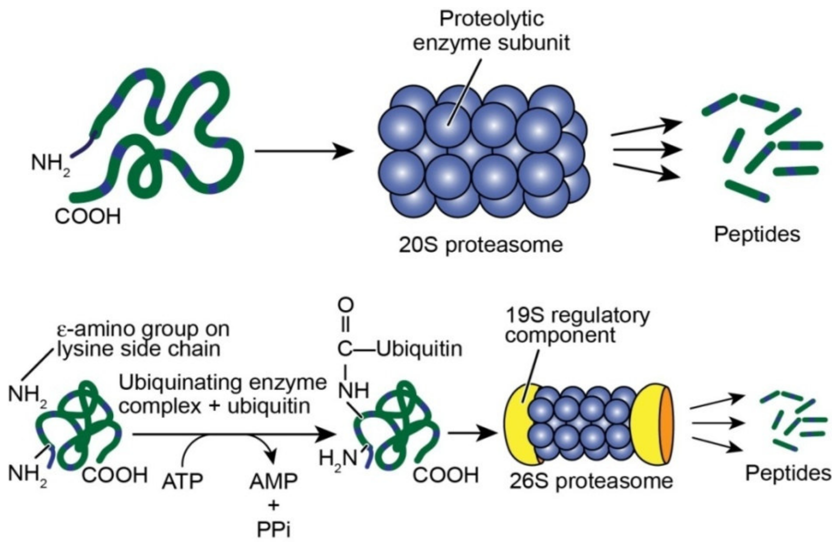

Fig. 9.4: Proteolytic system working on thedegradation of antigen protein into peptides.

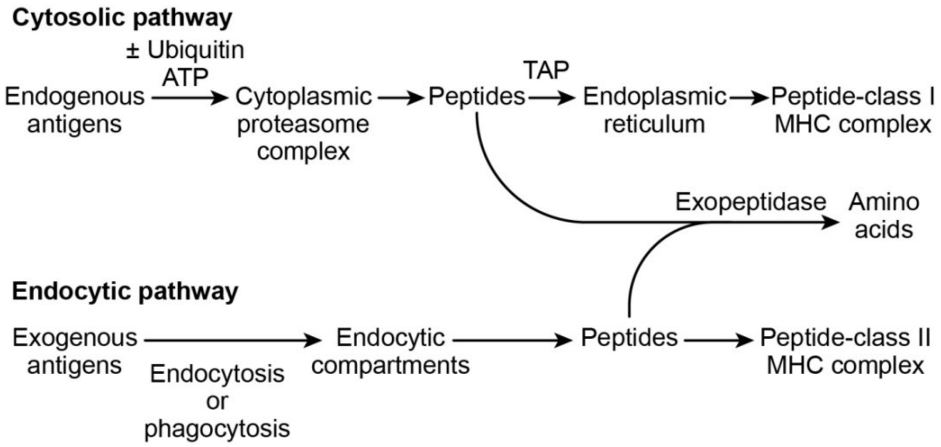

Fig. 9.5: Assembly of peptides in Cytosolic and Endocytic pathway.

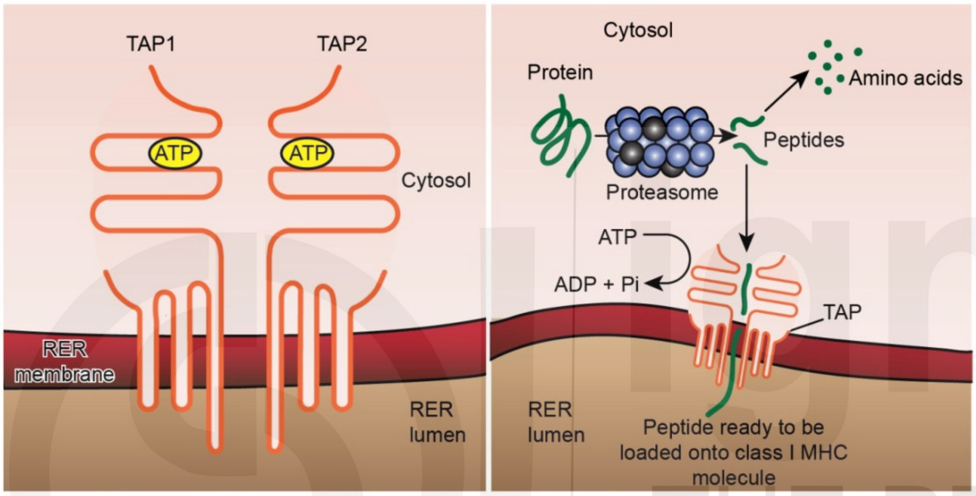

Fig. 9.6: Role of TAP and assembly of peptides with class I MHC molecule.

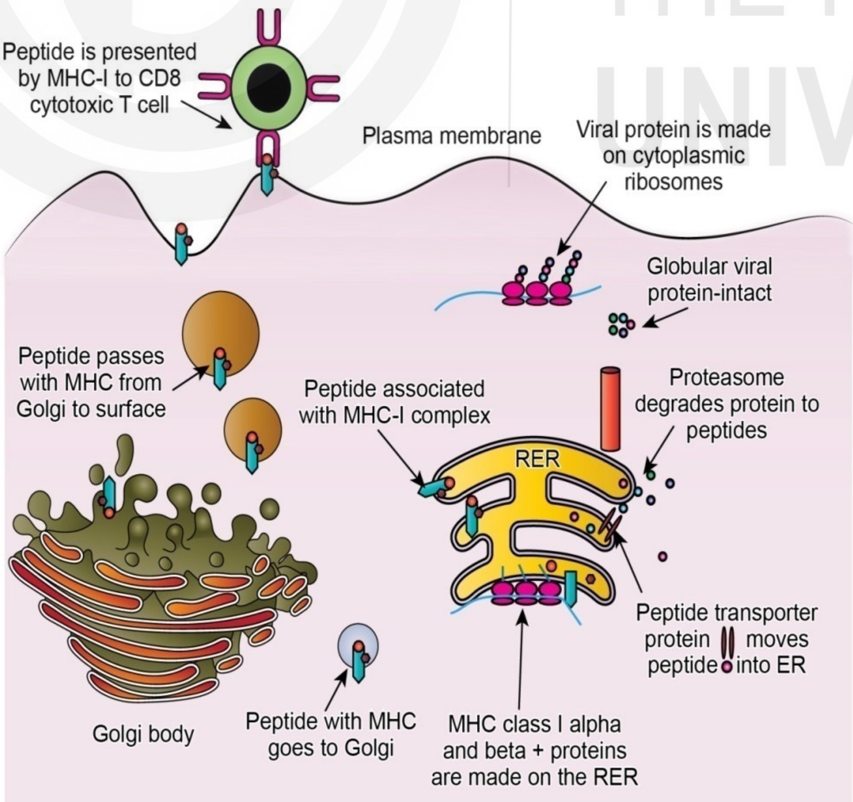

Fig. 9.7: Endogenous or cytosolic pathway of antigen processing and presentation.

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 (Table10.1).

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).

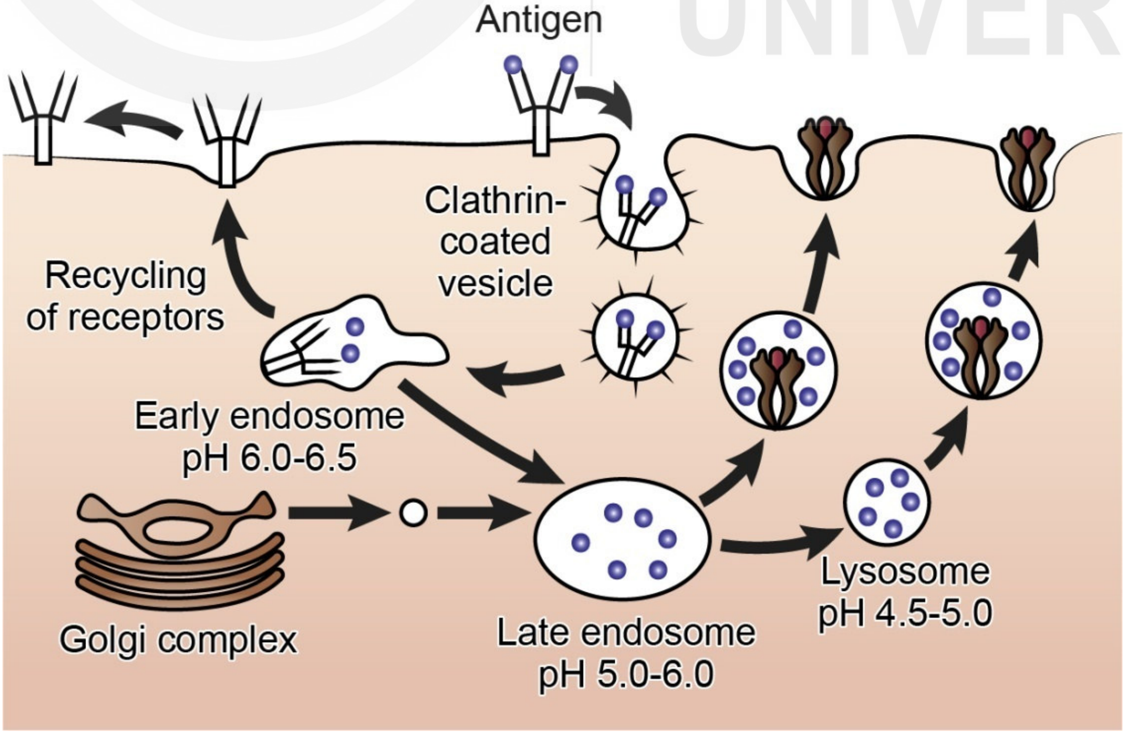

Fig. 9.8: Antibody-mediated endocytosis.

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).

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.

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).

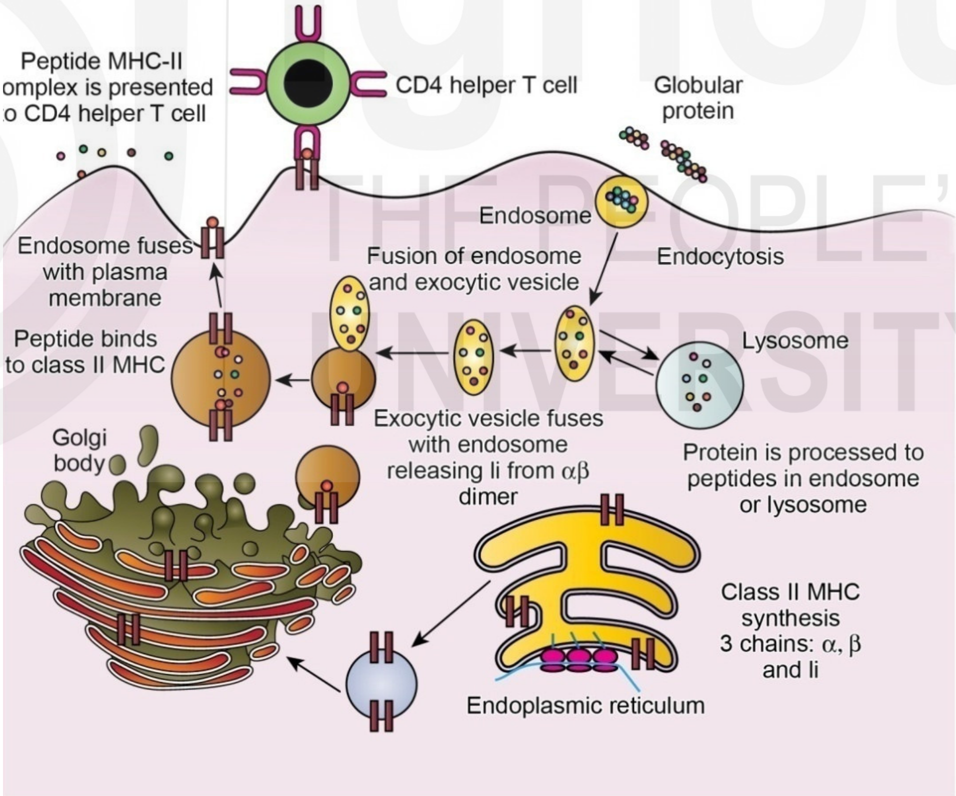

Fig. 9.9: Exogenous or Endocytic pathway of antigen processing and presentation.

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 $CD8^+$ $\mathbf{T_C}$ 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+ $\mathbf{T_H}$ 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+ Tc-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 fragmentknown 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 α1 and α2 domains and

   ii) A membrane proximal pair is made up of α3 domain and β2–microglobulin.

3. In class II MHC molecule, there are two pairs of interacting domains:

   i) A membrane distal pair made up of α1 and β1 domains and

   ii) A membrane proximal pair is made up of α2 domain and β2.

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 1 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+ $\mathbf{T_C}$cells recognise the antigen presented with the class I MHC molecule. CD4+ $\mathbf{T_H}$ 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 α1 and α2 chains and can accommodate 8 to 10 amino acid long antigenic peptides. It presents the antigen to CD8 $\mathbf{T_C}$ cells.	It is present on the cell surface of APC's. It binds with exogenous antigen. The peptide-binding domain is made up of α1 and β1 chains andcan accommodate 13 to 18 amino acid long antigenic peptides. It presents the antigen to CD4 $\mathbf{T_H}$ 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.