Programme: T. Y. B. Sc.

Subject: Microbiology

Course Code: MIC 108

Course Title: Immunology

Unit: 04 – Major Histocompatibility Complex

Module Name: 4.2 Antigen processing and presentation (Cytosolic and Endocytic pathways)

Module No: 15

Name of the Presenter: Dr. Carolina F. E. Fernandes

Antigen processing and presentation (Cytosolic and Endocytic pathways)

INTRODUCTION

MHC proteins expressed on the cell surface reflect the composition of the proteins inside the cell. A cell that contains no pathogens or foreign antigens will display MHC proteins complexed with self-peptides – those peptides derived from normal catabolism of proteins during cell growth. Cells that have ingested foreign proteins or pathogens produce peptides that also interact with MHC proteins. MHC proteins expressed on the cell surface are complexed with foreign / nonself peptides – function as molecular reference points that permit T cells to identify foreign antigens. Hence it is mandatory that the pathogens should be ingested and degraded into peptides and expressed on the membranes of these cells for recognition by T cells, while B cells can recognize an antigen in its native form directly. The cell types which pick up microbes or microbe-derived antigens for presentation to T cells are called antigen presenting cells (APCs). The process of degradation of microbial proteins to peptides that occurs inside the APCs is called **antigen processing** – is a **metabolic process**.

The peptides are then associated with class I or class II MHC molecules within the APC, and the MHC:peptide complexes are transported to the cell membrane and displayed to the T cell receptors. This process is called **antigen presentation**.

Processing of Antigen is required for recognition by T cells:

T-cell receptor does not recognize free antigens. T-cell receptor recognize only antigen that is bound to MHC molecules. All cells expressing either class I or class II MHC molecules can present peptides to T cells. Class II molecules present processed exogenous antigen to CD4+ TH cells. Thus, antigen recognition by the CD4+ TH cell is class II MHC restricted. Class I molecules present processed endogenous antigen to CD8+ TC cells. Thus, antigen recognition by CD8+ TC cells is class I MHC restricted. Most cells can present antigen with class I MHC while presentation with class II MHC is restricted to APCs

Target cells - are nucleated cells that display peptides (endogenous antigens) associated with class I MHC molecules to CD8+ TC cells – kill target cells. They are cells that have been infected by a virus or some other intracellular microorganisms. They may be also altered self cells such as cancer cells, aging body cells, or allogeneic cells, from a graft.

APCs - Are cells that display peptides associated with class II MHC molecules to CD4+ TH cells.

There are three types of professional APCs – Macrophages, Dendritic cells, B cells.

Characteristics of APCs: Phagocytose the bacteria or bacterial products. Ability to express class II MHC molecules as well as costimulatory membrane molecules such as B7 molecules which provide second signal required for T cell activation. APCs get activated when they internalize the pathogens or products.

Nonprofessional antigen presenting cells – Fibroblasts (skin), glial cells (brain), pancreatic beta cells, thymic epithelial cells, thyroid epithelial cells, vascular endothelial cells – they can be induced to express class II MHC molecules or a stimulatory signal – many function in antigen presentation only for short periods during a sustained inflammatory response.

The immune system uses different pathways to eliminate intracellular and extracellular antigens.

Rule: Exogenous antigens (e.g. bacteria, viruses, toxins - those taken up by endocytosis) are processed in the endocytic pathway and are presented on the membrane of APCs with class II MHC molecules.

Endogenous antigens (e.g. replicating viruses, intracellular pathogens - those generated within the nonphagocytic cells) - are processed by endogenous or cytosolic pathway and are presented on the membrane with class I MHC molecules.

Processing and presentation of endogenous antigens: Cytosolic pathway

Endogenous antigens are synthesized inside a cell; typically they are derived from pathogens – e.g. viruses, bacteria, and parasites - that have infected the cell. MHC I proteins present peptide antigens derived from endogenous protein antigen. Processing of endogenous antigens occurs in the cytoplasm rather than in the acid vesicles. The major mechanisms for generating short peptide fragments is by a cytosolic proteolytic system present in all cells known as the proteasome.

Cytosolic pathway - Peptide antigens generated in the cytosol are translocated by transporter associated with antigen processing (TAP) into the rough endoplasmic reticulum (RER) by a process that requires hydrolysis of ATP. TAP has affinity for peptides containing 8 to 16 amino acids in length. The optimal peptide length for class I MHC binding is around 9 amino acids and this length is achieved by trimming with aminopeptidases present in ER. TAP also favours peptides with hydrophobic or basic carboxyl-terminal amino acids, the preferred anchor residues for class I MHC molecules. **Thus, TAP is optimized to transport peptides that will interact with class I molecules.**

α chain and β2-microglobulin components of class I MHC molecule are synthesized on polysomes along the rough endoplasmic reticulum. Assembly of these components into a stable class I MHC molecular complex that can exit the RER requires the presence of a peptide in the binding groove of the class I molecule. The assembly process involves several steps and includes the participation of molecular chaperones, which facilitates the folding of polypeptides. The first molecular chaperone involved in class I MHC assembly is Calnexin, a resident membrane protein of the endoplasmic reticulum. Calnexin associates with the free class I α and promotes its folding. When β2-microglobulin binds to the α chain, calnexin is released and the class I molecule associates with the chaperone calreticulin and with tapasin.

Once the peptides have entered the ER, they are bound by the MHC I protein, held in place near the TAP site by a group of chaperone proteins such as calnexin, calreticulin, tapasin and ERp57. The MHC I-peptide complex is then released from the chaperone and moves to the cell surface where it integrates into the membrane and can be recognised by TC cells.

Endogenous pathway (class I MHC): 1.Endogenous antigen is degraded by proteasome. 2.Peptide is transported to RER via TAP. 3.Class I MHC α chain binds Calnexin, then β2 microglobulin. Calnexin dissociates. Calreticulin, Tapasin, and ERp57 bind. MHC captures peptide, chaperones dissociate. 4.Class I MHC – Peptide is transported from RER to Golgi complex to plasma membrane.

Processing and presentation of exogenous antigens: Endocytic pathway

APCs can internalize antigen by phagocytosis, endocytosis or both. Once an antigen is internalized, it is degraded into peptides in several acidic compartments of endocytic vesicles. When class II MHC molecules are synthesized within RER, class II α β chains bind an Invariant chain.

Processing and presentation of exogenous antigens: Endocytic pathway

The bound Invariant chain prevents premature binding of peptides to the class II molecule and helps to direct the complex to endocytic compartments containing peptides derived from exogenous antigens. Digestion of the Invariant chain leaves class II-associated invariant chain peptide (CLIP), a small fragment remaining in the binding groove of the class II MHC molecule.

Exogenous antigen is taken up, degraded, routed to endocytic pathway compartments. HLA-DM, a nonclassical MHC class II molecule expressed within endosomal compartments, mediates exchange of antigenic peptides for CLIP. Another non-classical class II MHC HLA-DO may act as a negative regulator of class II antigen processing by binding to HLA-DM and inhibiting its role in the dissociation of CLIP from class II molecules. Peptides assemble with class II MHC molecules by displacing CLIP.

Exogenous pathway (class II MHC)

1. Class II MHC α and β bind Invariant chain, blocking binding of endogenous antigen.
2. MHC complex is routed through Golgi complex to endocytic pathway compartments.
3. Invariant chain is degraded, leaving CLIP fragment.
4. Exogenous antigen is taken up, degraded, routed to endocytic pathway compartments.
5. HLA-DM mediates exchange of CLIP for antigenic peptide.
6. Class II MHC – peptide is transported to plasma membrane.