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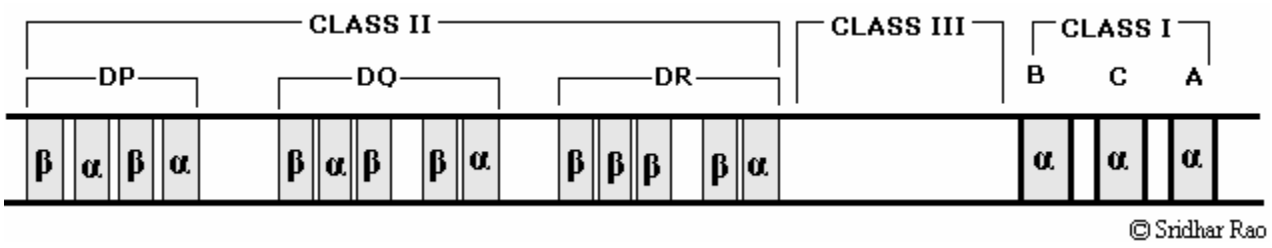
MHC (MAJOR HISTOCOMPATIBILITY COMPLEX)

MHC complex is group of genes on a single chromosome that codes the MHC antigens. Major as well as minor histocompatibility antigens (also called transplantation antigens) mediate rejection of grafts between two genetically different individuals. However, the role played by the major histocompatibility antigens supersedes the minor histocompatibility antigens. HLA (human leukocyte antigens) are the MHC antigens of humans, and called so because they were first detected on leukocytes. H-2 antigens are their equivalent MHC antigens of mouse. A set of MHC alleles present on each chromosome is called an MHC haplotype. Monozygotic human twins have the same histocompatibility molecules on their cells, and they can accept transplants of tissue from each other. Histocompatibility molecules of one individual act as antigens when introduced into a different individual. George Snell, Jean Dausset and Baruj Benacerraf received the Nobel Prize in 1980 for their contributions to the discovery and understanding of the MHC in mice and humans

MHC gene products were identified as responsible for graft rejection. MHC gene products that control immune responses are called the immune response (Ir) genes. Immune response genes influence responses to infections. The essential role of the HLA antigens lies in the induction and regulation of the immune response and defense against microorganisms. The physiologic function of MHC molecules is the presentation of peptide antigen to T lymphocytes. These antigens and their genes can be divided into three major classes: class I, class II and class III.

STRUCTURE:



The MHC complex resides in the short arm of chromosome 6 and overall size of the MHC is approximately 3.5 million base pairs. The complete three-dimensional structure for both class I and class II MHC molecules has been determined by x-ray crystallography. The class I gene complex contains three loci A, B and C, each of which codes of α chain polypeptides. The class II gene complex also contains at least three loci, DP, DQ and DR; each of these loci codes for one α and a variable number of β chain polypeptides. Class III region is not actually a part of the HLA complex, but is located within the HLA region, because its components are either related to the functions of HLA antigens or are under similar control mechanisms to the HLA genes. Class III antigens are associated with proteins in serum and other body fluids (e.g.C4, C2, factor B, TNF, lymphotoxin & heat shock proteins) and have no role in graft rejection.

NOMENCLATURE:
HLA specificities are identified by a letter for locus and a number (A1, B5, etc.), and the haplotypes are identified by individual specificities (e.g., A1, B7, Cw4, DP5, DQ10, DR8). Specificities which are defined by genomic analysis (PCR), are named with a letter for the locus and a four digit number (e.g. A0101, B0701, C0401, etc.)

INHERITANCE:
Histocompatibility genes are inherited as a group (haplotype), one from each parent. Thus, MHC genes are co dominantly expressed in each individual. A heterozygous human inherits one paternal and one maternal haplotype, each containing three Class-I (B, C and A) and three Class II (DP, DQ and DR) loci. Each individual inherits a maximum of two alleles for each locus. The maximum number of class I MHC gene products expressed in an individual is six; that for class II MHC products can exceed six but is also limited. Thus, as each chromosome is found twice (diploid) in each individual, a normal tissue type of an individual will involve 12 HLA antigens. Haplotypes, normally, are inherited intact and hence antigens encoded by different loci are inherited together. However, on occasions, there is crossing over between two parental chromosomes, thereby resulting in new recombinant haplotypes. MHC genes lack

recombinational mechanisms for generating diversity. Many alleles of each locus permit thousands of possible assortments. There are at least 1000 officially recognized HLA alleles. Even single allele may consist of multiple variants resulting in tremendous polymorphism.

EXPRESSION:
Class I antigens are expressed on all nucleated cells (except those of the central nervous system) and platelets. The class II antigens are expressed on antigen presenting cells such as B lymphocytes, dendritic cells, macrophages, monocytes, Langerhans cells, endothelial cells and thymic epithelial cells. On most cell types, IFN-α, IFN-β & IFN-γ, TNF and lymphotoxin increases the level of expression of class I molecules. IFN-γ also induces the expression of MHC II molecules on monocytes, macrophages & vascular endothelial cells. TNF induces expression of Class II molecules on dendritic cells during its maturation. Expression of MHC II on B cells increases on exposure to IL-4.

MHC CLASS I MOLECULE:
Class I MHC molecules contain two separate polypeptide chains, the heavier (44-47 KDa) alpha chain and the lighter (12 KDa) beta chain. The carboxyl end of α chain resides inside the cell while the amino end projects on the surface of cell with a short intervening hydrophobic segment traverses the membrane.

The α chain is coded by the MHC genes and has three globular domains α1, α2 and α3. β2-microglobulin is encoded by a gene on another chromosome. The α3 domain is non-covalently associated with the β2 microglobulin. Both α chain and β2-microglobulin are members of the Ig superfamily. Without the β2 microglobulin, the class I antigen will not be expressed on the cells surface. Individuals with defective β2 microglobulin gene do not express any class I antigen and hence they have a deficiency of cytotoxic T cells.

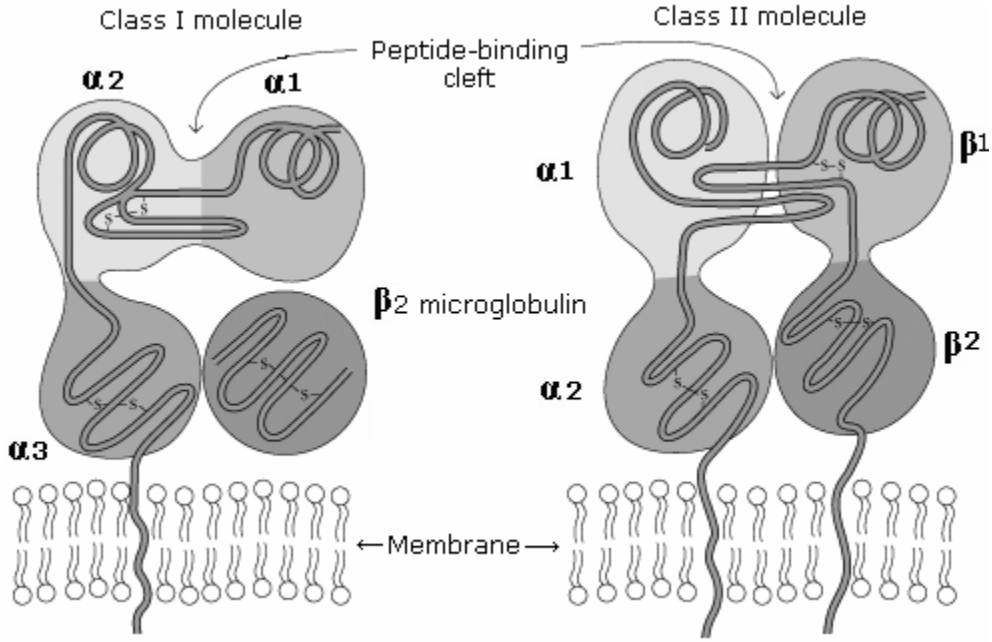
A peptide-binding groove is formed between α1 and α2 helices with beta-pleated sheet as its floor. A peptide of 8-10 amino acids long can be presented in this groove. The alloantigenic sites that carry determinants specific to each individual are found in the α1 and α2 domains. The greatest variability in amino acids (or polymorphism) occurs in the α1 and α2 sequences that line the wall and floor of the groove that binds the peptides. The polymorphism among class I MHC gene products creates variation in the chemical surface of the peptide-binding groove so that various peptide molecules can be accommodated. The specific binding of a peptide molecule in the peptide-binding groove of MHC requires the peptide to have one or more specific amino acid at a fixed position. Such sites are termed anchor sites. The other amino acids can be variable so that each MHC molecule can bind many different peptides.

The α1 and α2 domains also bind T cell receptor (TCR) of $CD8^+$ T lymphocytes. The parts of these domains that are in contact with TCR also show polymorphism. The immunoglobulin-like region of α3 domain is constant (shows no variation) and is non-covalently bound β2 microglobulin. The importance of the highly conserved region of α3 is that CD8 molecules present on $CD8^+$ T lymphocytes binds to this region.

$CD8^+$ T lymphocytes recognizes peptide antigen only when it is presented by the antigen presenting cell in the peptide binding groove of MHC I molecules. Class I molecules present peptide fragments in the cytosol (endogenous antigen, which could be fragments of viral or tumour proteins) to the $CD8^+$ lymphocytes.

MHC CLASS II MOLECULE:
MHC class II molecules comprise two non-identical and non-covalently associated polypeptide chains (α and β). These two chains have amino ends on the surface, a short transmembrane stretch and intracytoplasmic carboxyl ends. Both α chain (34 kDa) and β chain (28 kDa) are MHC-encoded and polymorphic. The domains closest to the membrane in each chain are structurally related to immunoglobulins. With the exception of the α1 domain, all domains are stabilized by disulfide bridges. The β chain is shorter than the α chain and contains the alloantigenic sites. In general α chain of one locus pair best with β chain of the same locus (e.g., DR) and less commonly with β chain of other loci (e.g., DP & DQ). Heterologous pairing (e.g., DQ α from one chromosome and DQ β from another chromosome) as well as polymorphism in β and α chain result in 10-20 number of class II molecules in an individual.

A peptide binding groove is formed in between α1 and β1 domains with a beta pleated floor. As in the case for class I MHC, the greatest polymorphic variability in the amino acids is in those facing the groove. This in turn determines the chemical structure of the groove and influences the specificity and affinity of peptide binding. Peptides associated with class II MHC are 13-25 amino acids long. The ends of peptide binding clefts are open so that peptides of 30 residues or more also can fit. As with class I MHC, anchor sites for one or more amino acids also exist in the groove of the class II MHC molecule. α2 and β2 are largely non-polymorphic. During antigen presentation, CD4 molecule of Helper T lymphocyte binds to β2 domain of the class II MHC molecules.



Because each MHC molecule (I and II) can bind many different peptides, the binding is said to be degenerate. Class I molecules present peptides to and are recognized by CD8+ T cells where as Class II molecules present peptides to and are recognized by CD4+ T cells. This is termed MHC restriction.

ANTIGEN PROCESSING AND PRESENTATION

$CD4^+$ T cells play important role in immune response against foreign protein antigens, help macrophage to destroy phagocytosed microbes and stimulate the proliferation & differentiation of B cells. $CD8^+$ T cells are useful in cytotoxicity against virus infected and tumor cells and take part in regulation of immune response.

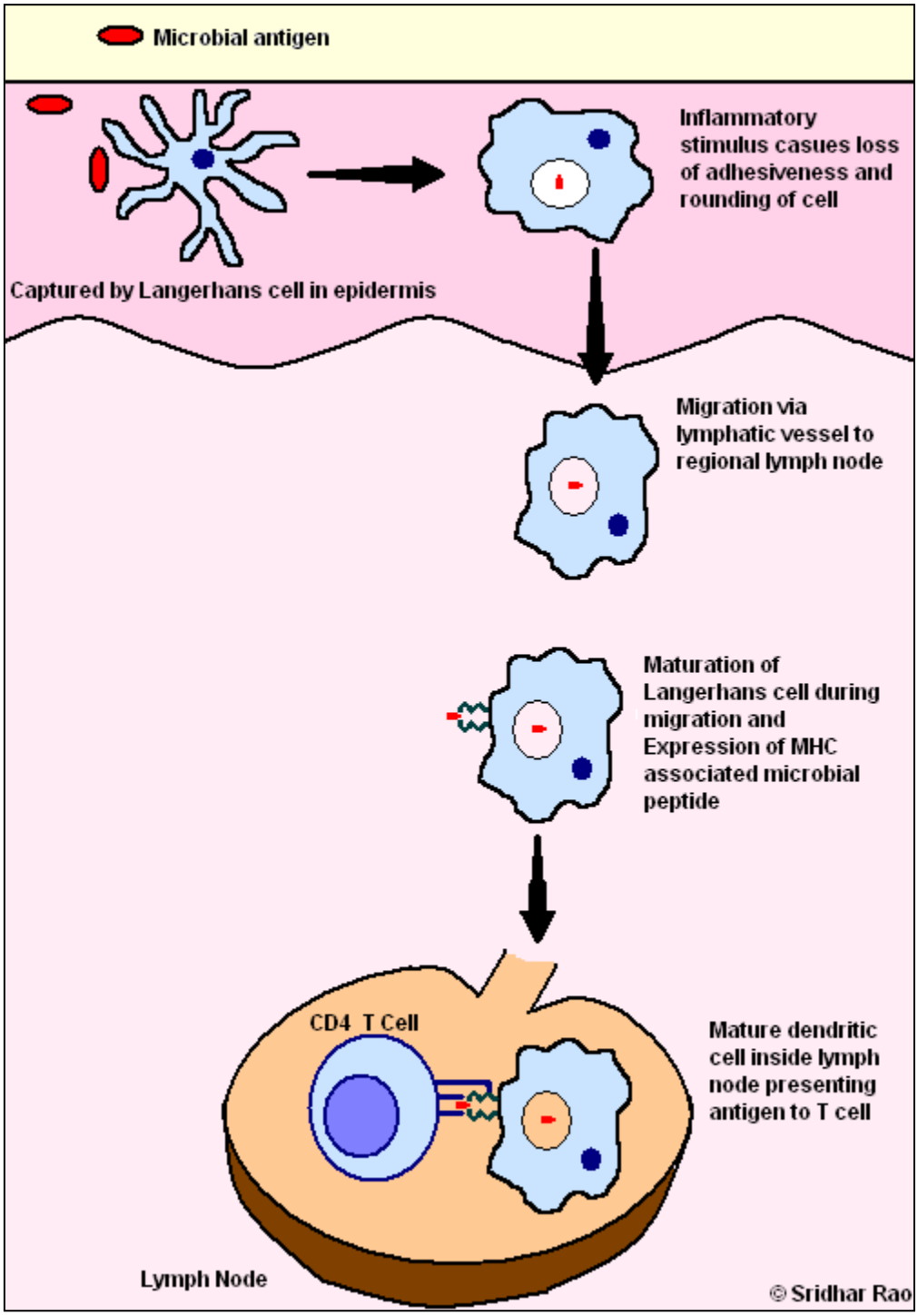
T cells and B cells vary in the manner they recognize antigens. B cells can specifically recognize peptides, proteins, nucleic acids, polysaccharides, lipids & small chemicals where as T cells recognize only peptides. B cells recognize native (secondary & tertiary structure) as well as denatured proteins where are T cells recognize only processed linear peptides. B cells can recognize antigen in free or cell bound form whereas T cells recognize antigen only they are associated with MHC proteins. T cells recognize antigens only when they are presented by self MHC molecules, this is termed self-MHC restriction.

While most cells have the capability of engulfment, only certain cells like monocytes-macrophages, dendritic cells, B lymphocytes are considered professional antigen presenting cells (APC). Nonprofessional APC includes fibroblasts, glial cells, epithelial cells and endothelial cells. For a cell to act as APC, it must have the ability to process phagocytosed antigens and express them along with MHC molecules. Functions of Antigen-Presenting Cells (APC) include antigen collection , antigen concentration , antigen processing, antigen presentation to lymphocytes and provide co-stimulation to T cells. Conversion of phagocytosed native protein to MHC-associated peptide fragments by APCs is called antigen processing.

Microbial antigens from skin, mucosal epithelium and parenchymal organs are drained via lymphatic vessels into draining lymph nodes. Lymph nodes filter the lymph before it reaches the blood. APCs in the spleen capture antigen that reach blood directly from tissue or from lymph that have entered through thoracic duct. Peyer's patches and tonsils also capture any antigen in the mucosa. Foreign antigens are transported to peripheral lymphoid tissues by APCs but some antigens may also be transported to lymph nodes in soluble form. Lymph borne soluble antigen are extracted from fluid by macrophages, dendritic cells and B cells.

Protein administered in soluble form, without adjuvants either fail to induce T cell response or induce a state of unresponsiveness called tolerance. Both the inductor & effector phase of T cell response are triggered by specific antigen recognition. APCs present antigen to naïve T cells as well as to differentiated effector T cells. Since the effect of successful antigen presentation to T cells is seen on APCs, they are both inducers & target of T cell actions.

LANGERHANS CELLS AS APC



Human skin epithelium contains large number of Langerhans cells, which serve to capture foreign antigens and microbes that enter the skin. Numerous projections help Langerhans cell to effectively capture and engulf microbes.

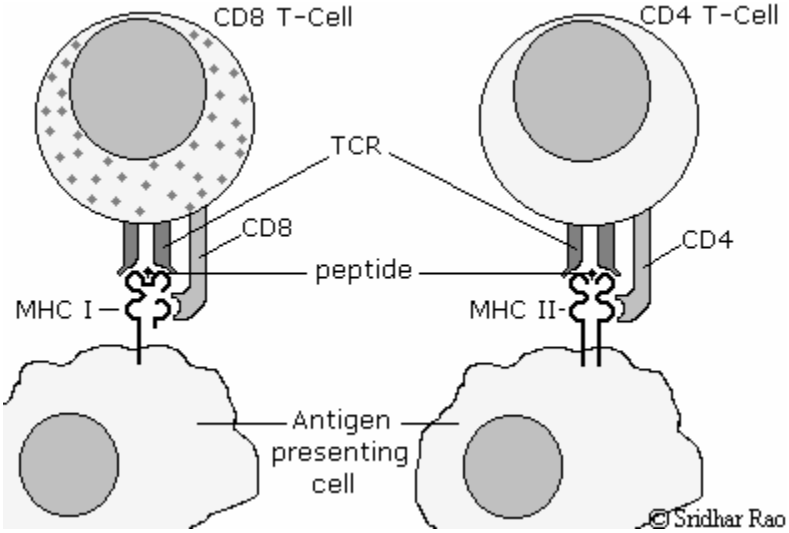
Once phagocytosed, the microbes are taken inside a vacuole called phagosome, which fuses with cytoplasmic lysosome resulting in formation of phagolysosome.

Presence of microbe in the skin epithelium triggers inflammatory response resulting in production of cytokines. Inflammatory cytokines produced during local immune response act on Langerhans cell causing it lose adhesiveness, become rounded and migrate through lymphatic vessels to regional lymph node. These cells express receptors for chemokines that are produced in the lymph node, which direct them to migrate to lymph node.

During migration to lymph node and under influence of cytokines, Langerhans cells undergo maturation. They synthesize MHC II molecules and complex it with the peptides generated from engulfed protein/microbe and present it on their surface. By the time they reside in lymph nodes, these cells become professional APCs and are known as dendritic cells.

Dendritic cells present MHC II-peptide complex to CD4+ T cells.

Antigen presenting cells are typically those cells which can present antigenic peptide associated with MHC class II molecules. All nucleated cells express MHC class I molecules, however only antigen presenting cells express MHC class II molecules. It should be noted that APCs too express class I molecules. MHC II protein expressing cells and MHC I protein expressing cells process antigen differently, but both present MHC protein-peptide complex on their surface. Those peptides presented with Class I proteins are recognized by specific CD8+ T lymphocytes where as those presented with Class II proteins are recognized by CD4+ T cells. How antigens are processed and presented depends on the nature of antigen and route of entry. Most cells of the body can present antigens to lymphocytes through class I MHC proteins, whereas professional APCs present antigen through class II MHC proteins.

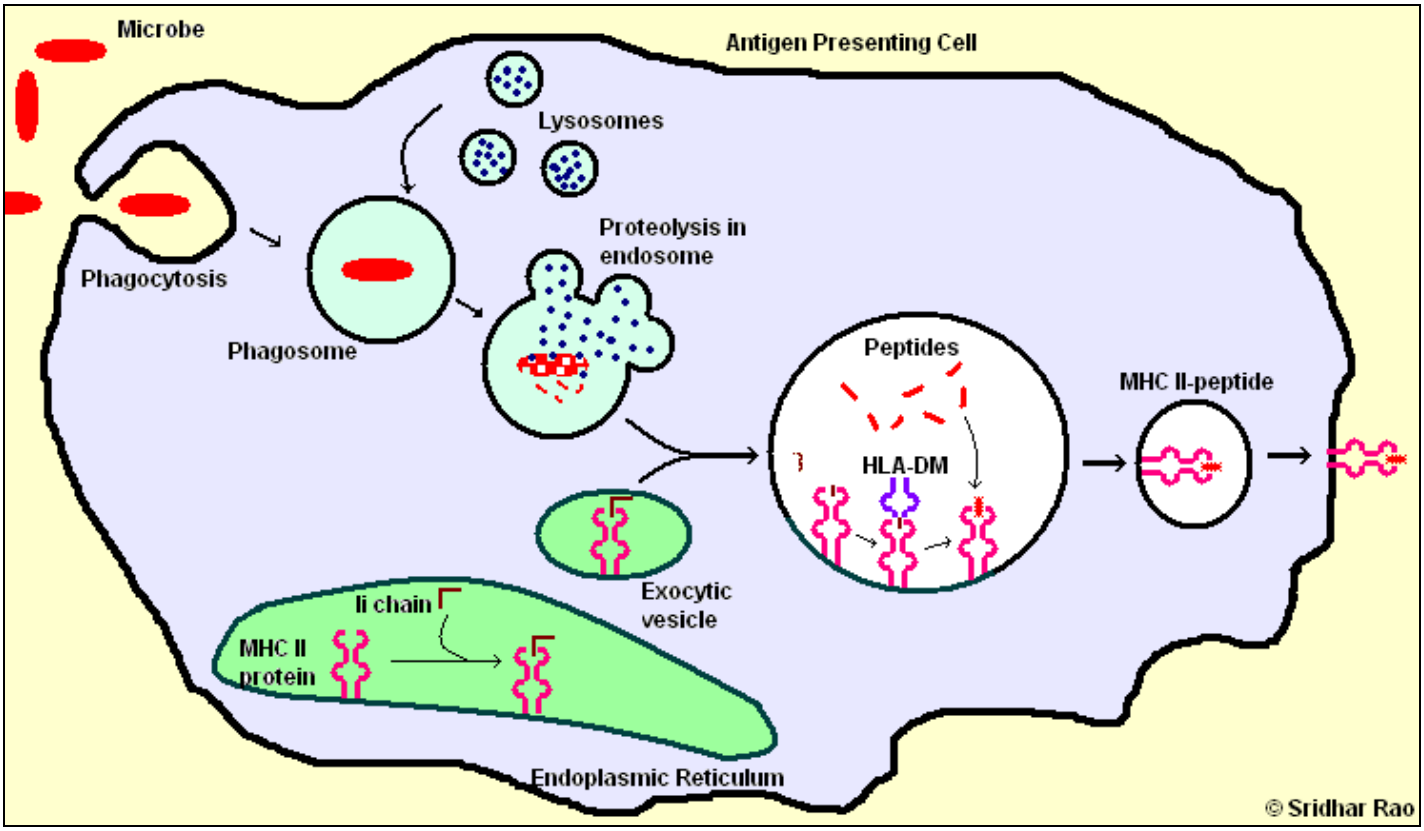


$CD4^+$ Helper T lymphocytes can recognize peptide antigen only when presented along MHC II molecules.

$CD8^+$ Cytotoxic T lymphocytes can recognize peptide antigen only when presented along MHC I molecules.

ANTIGEN PROCESSING AND PRESENTATION BY CLASS II (EXOGENOUS/ENDOCYTIC) PATHWAY

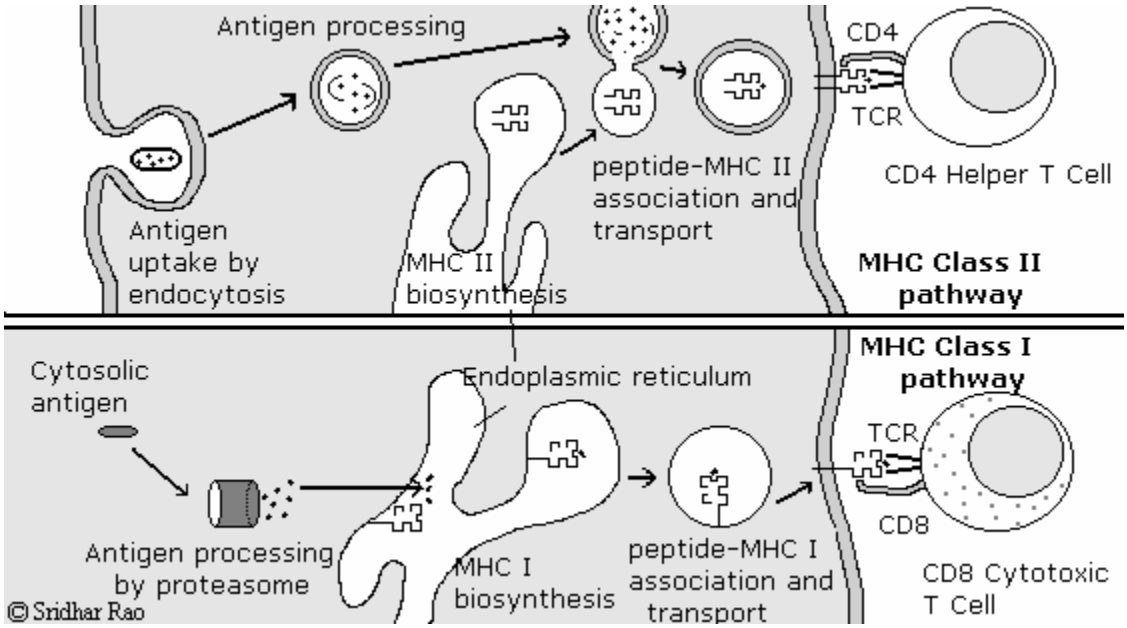
The endocytic or exogenous involves intake of extracellular protein. Macrophages accomplish this by phagocytosis. B cells are not phagocytic and therefore take up antigen by receptor-mediated endocytosis. After internalization, the protein antigens are become localized in intracellular membrane bound vesicles called endosomes, which have acidic pH and are rich in proteolytic enzymes.



These enzymes are proteases (e.g., cathepsins). Internalized proteins are degraded enzymatically in endosome & lysosomes to generate peptides. Although most peptides derived are from internalized extracellular proteins, occasionally cytoplasmic & membrane proteins may also enter. Viral membrane proteins and tumor proteins are processed in this manner. As proteins are being broken down in the endocytic pathway, α and β chain of class II molecules are also synthesized in endoplasmic reticulum.

A protein called Ii (invariant chain) that is also produced in ER, associates itself with newly formed MHC II proteins. It occupies the cleft formed between α1 and β1 domain, preventing binding of any non-specific peptide produced inside ER. Ii protein may promote folding, assembly of MHC II molecules & direct them to peptide containing endosomes. Newly formed MHC class II proteins along with Ii are transported from ER via Golgi in an exocytic vacuole. Endosome containing peptide and exocytic vacuole containing MHC II molecules fuse resulting in formation of MHC II compartment (MIIC). The Ii protein in the cleft is acted upon by proteolytic enzymes in MIIC and all of the invariant chain is degraded except for a small piece left in the peptide-binding cleft called CLIP (class II associated invariant chain peptide). A non-polymorphic HLA-DM serves to extract CLIP from the cleft and facilitates the incorporation of newly generated foreign peptide. The peptide-MHC II complex are delivered to the surface of APC, where they are displayed for recognition by $CD4^+$ T cells. Other contents of MIIC stay inside the vesicle only and only the MHC-peptide complex gets displayed.

COMPARISON OF CLASS I AND CLASS II PRESENTATION PATHWAY



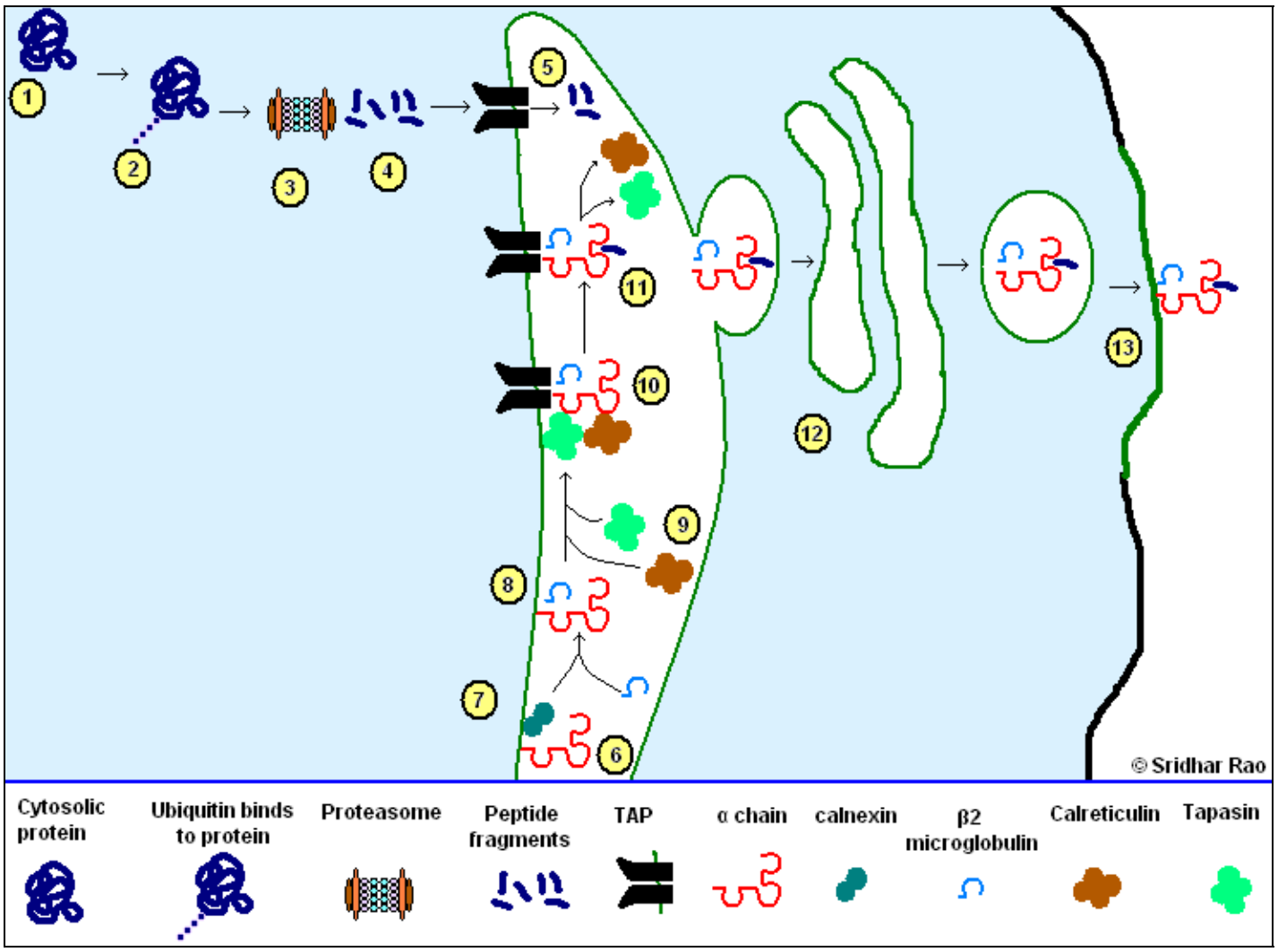
ANTIGEN PROCESSING AND PRESENTATION BY CLASS I (ENDOGENOUS/CYTOSOLIC) PATHWAY

In cells, protein levels are controlled by continuous breakdown and synthesis of proteins. Antigenic peptides that associate with class I molecules are usually derived from virus infection or as a result or normal breakdown of normal cell metabolic products within the cell. Cytosolic proteins include viral antigen, tumor proteins, phagocytosed microbial proteins and normal cellular proteins.

Proteins that are to be broken down by proteolytic degradation bind to a small molecule called ubiquitin in the cytoplasm. Ubiquitin is a small protein that occurs in all eukaryotic cells. Its main function is to mark other proteins for destruction. Several ubiquitin molecules attach to the protein. Binding of this protein is a signal for that protein to be processed into small peptides by the proteosome. The proteosome is a large, cylindrical complex made up of 4 ring subunits that form a central channel. Ubiquitin bound proteins are broken down in the center of the channel into peptides 6-30 amino acids long. Peptides generated in the cytosol are translocated into endoplasmic reticulum (ER) by special transporter protein located in the ER membrane called transporter associated with antigen processing (TAP). TAP is a membrane-spanning molecule made of two proteins TAP1 and TAP2.

MHC class I molecules α chain and β-2 microglobulin are also synthesized inside ER. MHC class I folding requires the help of proteins called molecular chaperones. Chaperones are proteins whose function is to assist other proteins in achieving proper folding. These molecular chaperones help to stabilize the class I molecule until it interacts with β2 microglobulin and peptide. The first of the chaperones to associate with the class I α chain is calnexin. Calnexin promotes proper folding of the class I α chain. When β2 microglobulin binds to the α chain, calnexin is released. Along with β2 microglobulin binding, 2 new chaperones bind, calreticulin and tapasin. Tapasin, brings TAP and class I together to allow the peptide to be loaded into the peptide binding cleft. When the peptide binds, calreticulin and tapasin are released. Peptide transported into ER preferentially bind to Class I molecules only. Once the peptide is bound, the

class I molecule is stable enough to move to the Golgi and then out to the surface of the cell via cellular vesicles. MHC I and peptide complex is then recognized by specific CD8+ T cell.



1: Cytosolic protein, 2: Protein binds to ubiquitin chain, 3: Protein taken to proteasome for proteolysis, 4: Protein broken into small peptides, 5: Peptides transported into ER with aid of TAP, 6: Synthesis of α chain in ER, 7: Calnexin binds to α chain, 8: β2 microglobulin binds to α chain and displaces calnexin, 9: Proteins Calreticulin and Tapasin bind to Class I proteins, 10: Tapasin brings TAP and class I together, 11: Peptide binds to the cleft and proteins Calreticulin and Tapasin leave, 12: MHC I-peptide complex leaves via Golgi in a vacuole, 13, MHC I-peptide complex is expressed on the surface

SIGNIFICANCE OF MHC-PEPTIDE PRESENTATION

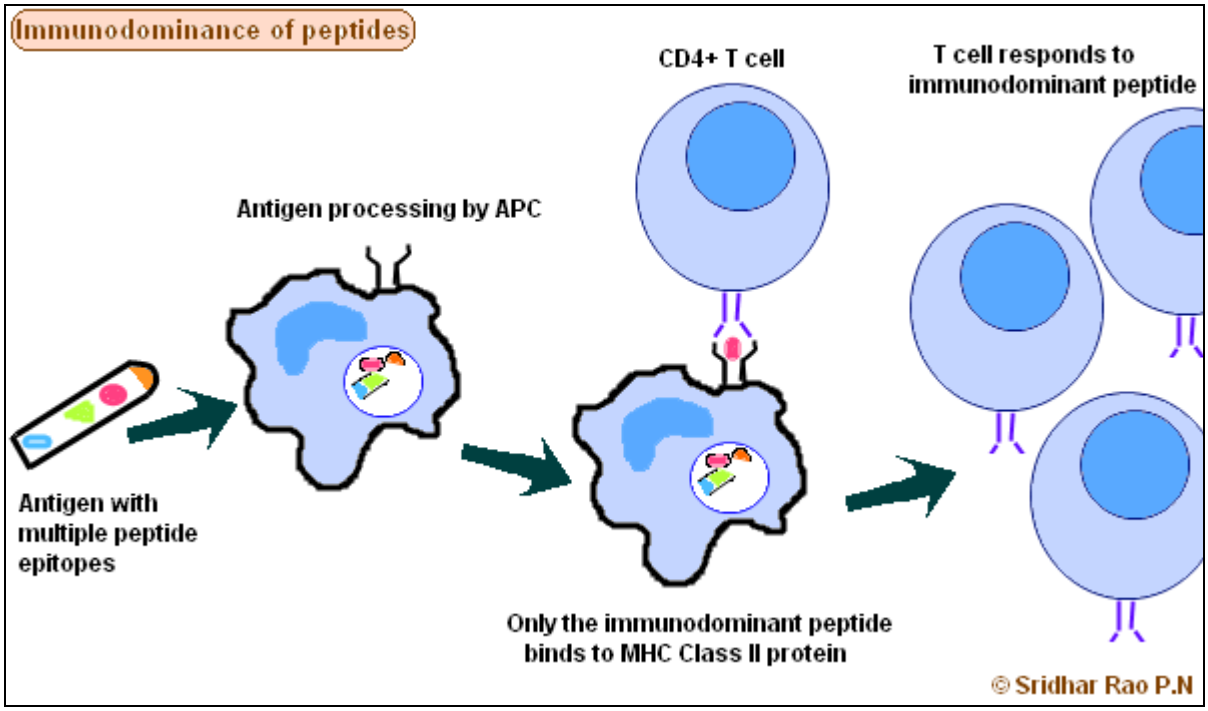
T cells constantly survey the antigens displayed on the surface of presenting cells. Most of the proteins presented are usually self proteins, which are ignored by T cells. Only foreign antigens (microbial or tumor) activate T cells. Often microbes also stimulate expression of co-stimulators on APCs that enhance T cell response, thus favoring response to microbes and not to self antigens.

Presentation of antigens by the pathway they undertake determine which subsets of T cells will respond. Extracellular antigens present antigen to CD4+ T cells, whereas cytosolic antigens are presented to CD8+ T cells. Expression of Class I proteins on most cells ensure that the intracellular pathogens, which are hidden from B cells or antibodies are eliminated by CD8+ T cells.

APCs not only present antigens to T lymphocytes, they are also the targets of T cell effector functions. B cells and macrophage are principal cell types that present antigen to CD4+ T cells. Macrophages that have engulfed microbe but unable to clear it presents microbial peptide antigen with MHC II proteins to antigen-specific CD4+ T cells. The T cell in

turn activates the macrophage and helps to eliminate the microbe. Similarly, B cells present endocytosed antigen with MHC II proteins to CD4+ T cells. The CD4+ T cells then stimulate the B cells finally resulting in production of antibodies against the foreign antigen.

The proteases involved in antigen processing produce a variety of peptides from native proteins, only some of these peptides possess the characteristics that enable them to bind the cleft of MHC molecules. When a protein with multiple antigenic determinants are subjected to antigen processing and presentation, majority of the responding T cells are specific to one or few peptides of the antigen. These are called immunodominant determinants or epitopes.



The immune response (Ir) genes that control the antibody response are the class II MHC structural genes. They influence immune responsiveness because various allelic class II MHC molecules differ in their ability to bind different antigenic peptides and thus stimulate specific T helper cells.