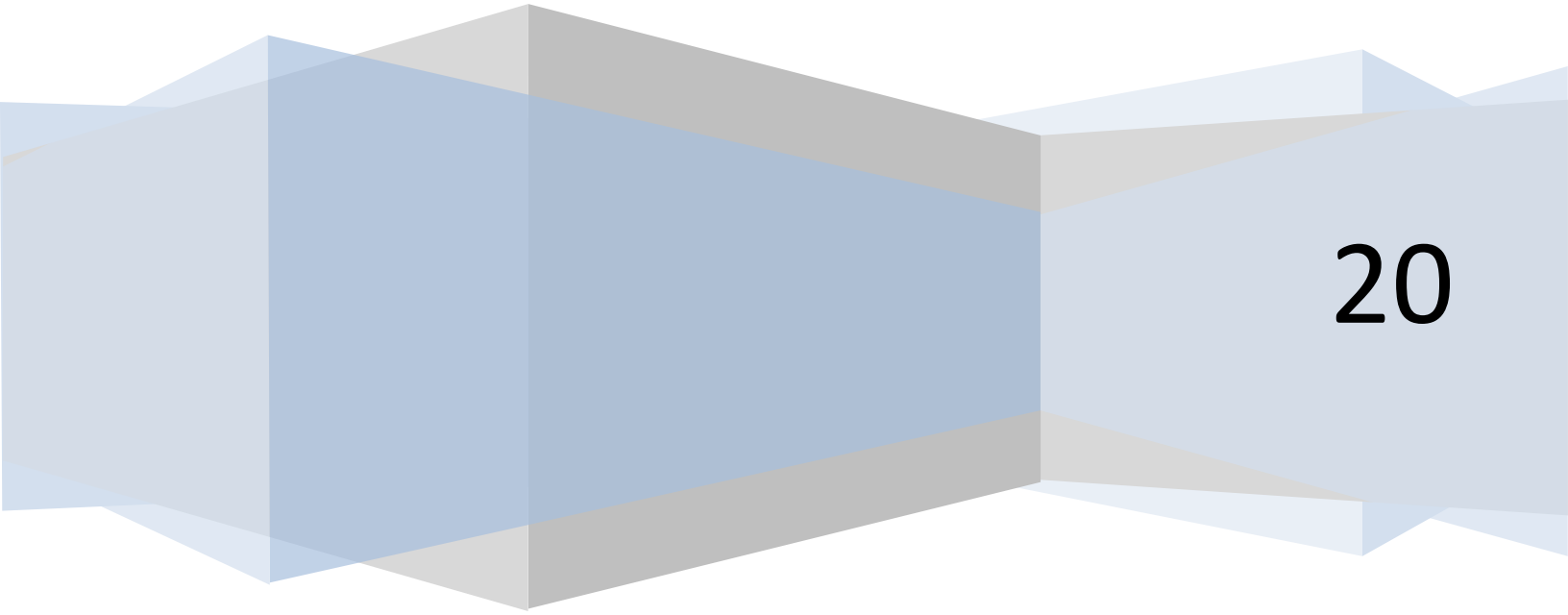
DAV University, Department of Biochemistry

Antigen processing and presentation with MHC

Immunology

Dr. Jasweer Kaur



20

Most Cells Can Present Antigen with Class I MHC; Presentation with Class II MHC Is Restricted to APCs

Since all cells expressing either class I or class II MHC molecules can present peptides to T cells, strictly speaking they all could be designated as antigen-presenting cells. However, by convention, cells that display peptides associated with class I MHC molecules to $CD8^+$ T_C cells are referred to as *target cells;* cells that display peptides associated with class II MHC molecules to $CD4^+$ T_H cells are called **antigen-presenting cells (APCs).** A variety of cells can function as antigen-presenting cells. Three cell types are classified as *professional* antigen-presenting cells: dendritic cells, macrophages, and B lymphocytes. Because nearly all nucleated cells express class I MHC molecules, virtually any nucleated cell is able to function as a target cell presenting endogenous antigens to TC cells. Most often, target cells are cells that have been infected by a virus or some other intracellular microorganism. However, altered self-cells such as cancer cells, aging body cells, or allogeneic cells from a graft can also serve as targets.

Two Processing and Presentation Pathways

The immune system uses two different pathways to eliminate intracellular and extracellular antigens. Endogenous antigens (those generated within the cell) are processed in the *cytosolic pathway* and presented on the membrane with class I MHC molecules; exogenous antigens (those taken up by endocytosis) are processed in the *endocytic pathway* and presented on the membrane with class II MHC molecules (Figure 1).

Endogenous Antigens: The Cytosolic Pathway

The pathway by which endogenous antigens are degraded for presentation with class I MHC molecules utilizes the same pathways involved in the normal turnover of intracellular proteins.

1. Peptides for Presentation Are Generated by Protease Complexes Called Proteasomes

Intracellular proteins are degraded into short peptides by a cytosolic proteolytic system present in all cells. Those proteins targeted for proteolysis often have a small protein, called *ubiquitin,* attached to them . Ubiquitin-protein conjugates can be degraded by a multifunctional protease complex called a **proteasome.** A proteasome can cleave peptide bonds between 2 or 3 different amino acid combinations in an ATP-dependent process.

2. Peptides Are Transported from the Cytosol to the Rough Endoplasmic Reticulum

Peptides generated in the cytosol by the proteasome are translocated by TAP into the RER by a process that requires the hydrolysis of ATP. The transporter protein, designated **TAP** (for **transporter associated with antigen processing**) is a membrane-spanning heterodimer consisting of two proteins: TAP1 and TAP2. In addition to their multiple transmembrane segments, the TAP1 and TAP2 proteins each have a domain projecting into the lumen of the RER, and an ATP-binding domain that projects into the cytosol. Both TAP1 and TAP2 belong to the family of ATP-binding cassette proteins found in the membranes of many cells, including bacteria; these proteins mediate ATP-dependent transport of amino acids, sugars, ions, and peptides. TAP has the highest affinity for peptides containing 8–10 amino acids, which is the optimal peptide length for class I MHC binding. In addition, TAP appears to favor 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 MHC molecules.

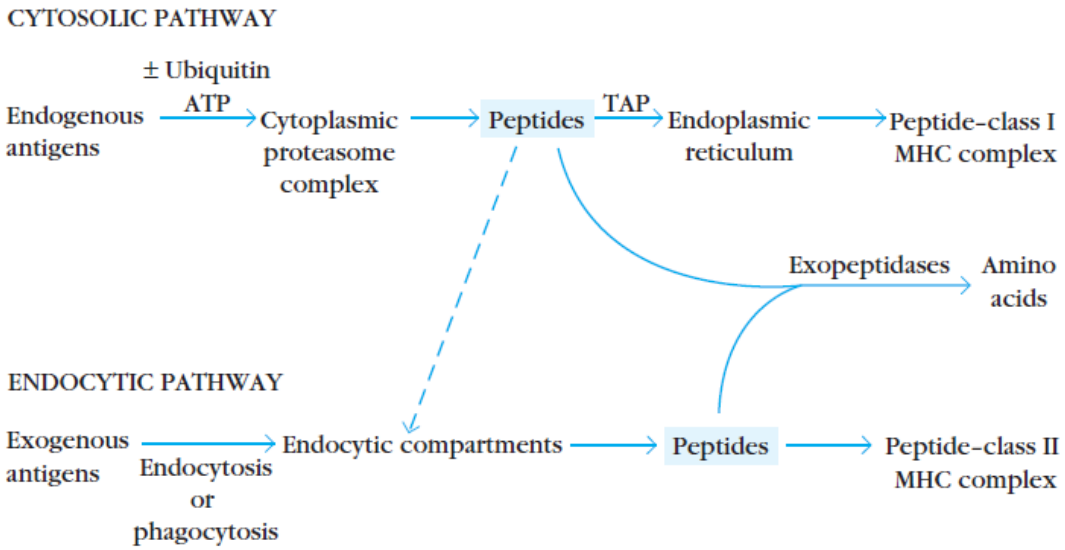


Figure1: Overview of cytosolic and endocytic pathways for processing antigen. The proteasome complex contains enzymes that cleave peptide bonds, converting proteins into peptides. The antigenic peptides from proteasome cleavage and those from endocytic compartments associate with class I or class II MHC molecules, and the peptide-MHC complexes are then transported to the cell membrane. TAP (*t*ransporter of *a*ntigenic *p*eptides) transports the peptides to the endoplasmic reticulum. It should be noted that the ultimate fate of most peptides in the cell is neither of these pathways, but rather to be degraded completely into amino acids.

3. Peptides Assemble with Class I MHC Aided by Chaperone Molecules

Like other proteins, the α chain and β_2-microglobulin components of the 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 facilitate 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 chain and promotes its folding. When β_2-microglobulin binds to the α chain, calnexin is released and the class I molecule associates with the chaperone *calreticulin* and with *tapasin*. Tapasin (TAP-associated protein) brings the TAP transporter into proximity with the class I molecule and allows it to acquire an antigenic peptide. As a consequence of peptide binding, the class I molecule displays increased stability and can dissociate from calreticulin and tapasin, exit from the RER, and proceed to the cell surface via the Golgi. An additional chaperone protein, ERp57, has been observed in association with calnexin and calreticulin complexes.

Exogenous Antigens: The Endocytic Pathway

Antigen-presenting cells can internalize antigen by phagocytosis, endocytosis, or both. Macrophages internalize antigen by both processes, whereas most other APCs are not phagocytic or are poorly phagocytic and therefore internalize exogenous antigen only by endocytosis (either receptor-mediated endocytosis or pinocytosis).

1. Peptides Are Generated from Internalized Molecules in Endocytic Vesicles

Once an antigen is internalized, it is degraded into peptides within compartments of the endocytic processing pathway. antigen takes 1–3 h to transverse the endocytic pathway and appear at the cell surface in the form of peptide–class II MHC complexes. The endocytic pathway appears to involve three increasingly acidic compartments: early endosomes (Ph 6.0–6.5); late endosomes, or endolysosomes (pH 5.0–6.0); and lysosomes (pH 4.5–5.0). Internalized antigen moves from early to late endosomes and finally to lysosomes, encountering hydrolytic enzymes and a lower pH in each. Lysosomes, for example, contain a unique collection of more than 40 acid-dependent hydrolases, including proteases, nucleases, glycosidases, lipases,

phospholipases, and phosphatases. Within the compartments of the endocytic pathway, antigen is degraded into oligopeptides of about 13–18 residues,which bind to class II MHC molecules. It has been suggested that early endosomes from the periphery move inward to become late endosomes and finally lysosomes. Alternatively, small transport vesicles may carry antigens from one compartment to the next. Eventually the endocytic compartments, or portions of them, return to the cell periphery, where they fuse with the plasma membrane. In this way, the surface receptors are recycled.

2. The Invariant Chain Guides Transport of Class II MHC Molecules to Endocytic Vesicles

When class II MHCmolecule are synthesized within the RER, three pairs of class II αβ chains associate with a preassembled trimer of a protein called **invariant chain (Ii, CD74).** This trimeric proteininteracts with the peptide-binding cleft of the class II molecules, preventing any endogenously derived peptides from binding to the cleft while the class II molecule is within the RER (see right side of Figure 2). The invariant chain also appears to be involved in the folding of the class II α and β chains, their exit from the RER, and the subsequent routing of class II molecules to the endocytic processing pathway from the trans-Golgi network. The invariant chain contains sorting signals in its cytoplasmic tail that directs the transport of the class II MHC complex from the trans-Golgi network to the endocytic compartments.

3. Peptides Assemble with Class II MHC Molecules by Displacing CLIP

A short fragment of the invariant chain termed *CLIP* (for *class II–associated invariant chain peptide*) remains bound to the class II molecule after the invariant chain has been cleaved within the endosomal compartment. CLIP physically occupies the peptide-binding groove of the class II MHC molecule, presumably preventing any premature binding of antigenic peptide (see Figure 2). A nonclassical class II MHC molecule called *HLA-DM* is required to catalyze the exchange of CLIP with antigenic peptides. As with class I MHC molecules, peptide binding is required to maintain the structure and stability of class II MHC molecules. Once a peptide has bound, the peptide–class II complex is transported to the plasma membrane,where the neutral PH appears to enable the complex to assume a compact, stable form. Peptide is bound so strongly in this compact form that it is difficult to replace a class II–bound peptide on the membrane with another peptide at physiologic conditions.

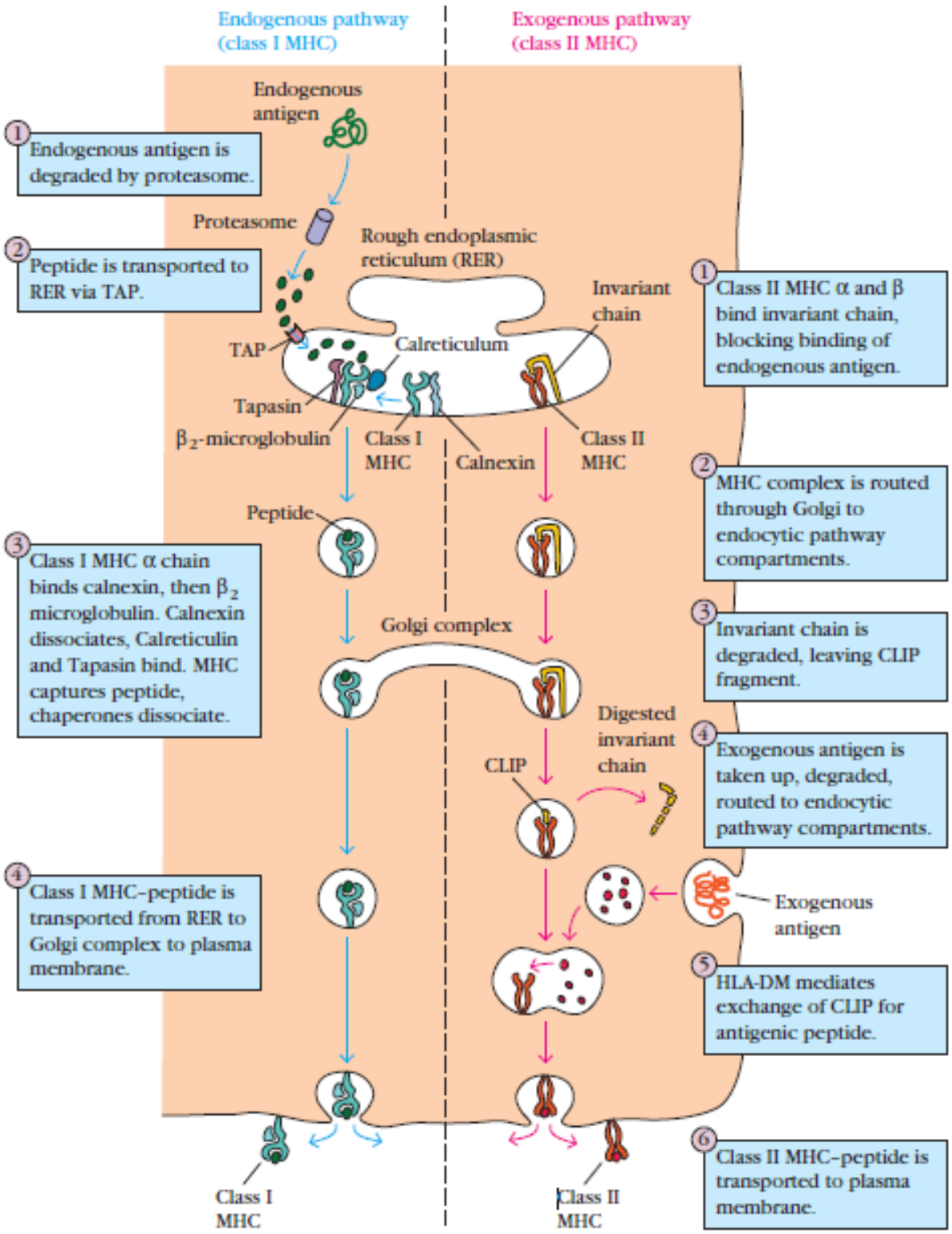


Figure2: Separate antigen-presenting pathways are utilized for endogenous (green) and exogenous (red) antigens. The mode of antigen entry into cells and the site of antigen processing determine whether antigenic peptides associate with class I MHC molecules in the rough endoplasmic reticulum or with class II molecules in endocytic compartments.