Immunology

Antigen Processing and Presentation Pathways

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INTRODUCTION

Antigen recognition by the T cells and B cells require appropriate and processed antigens displayed on the cleft of different MHC molecules. The B cells and T cells recognizes antigens processed by different pathways. The B cells recognizes the soluble form antigens like proteins, nucleic acids, polysaccharides, some lipids and small chemicals (called haptens) while in contrast the T cells recognizes mainly proteins as antigens.

It is important for the T cells to recognize whether antigen is in the cytosol or in the vesicular system. Recognition of the foreign antigen by a T cell requires that the antigen should be degraded to small peptides (process called as antigen processing) and then associated with MHC molecule intracellular and then the peptide – MHC complex is displayed on the cell surface (process known as antigen presentation) where they can be recognized by the T cell receptors on T cells. The pathways leading to the association of peptide fragment to the MHC molecule differs for class I and class II MHC.

Few of the main reasons for antigen processing and presentation are as follows:

- Antigen processing splits the protein into small fragments or subunits of about 8 to 10 amino acids and presents them to T cells. This linear sequence of amino acids help the T cells to identify the sequence better with lesser chances of cross reaction than in case of folded sequences.
- The cell presenting the antigen peptide acts as signal information about the origin of the cell and about the pathway by which the antigen is processed and presented on class I or class II MHC molecule.
- Antigens both extracellular and intracellular are presented by this phenomenon and even if there is no virus, the cell is still processing and presenting its own protein and thus self presentation plays an important role in positive and negative selection process in the thymus.

Each individual human being possesses a characteristic set of histocompatibility antigens. These sets of antigens are acquired through inheritance from individual parents. These antigens play an important role in intracellular recognition and discrimination of cells or graft as “self” or “non-self”. The MHC is a region of multiple loci that plays an important role in tissue or organ transplantation, which is to be accepted as self (histocompatible) or rejected as foreign (histoincompatible) in the recipient body. Histocompatibility antigens on the cells surface thus help in eliminate cells that differ, even slightly from normal one.

This set of histocompatibility antigens is acquired by inheritance from its parents through the activity of the genes that code for these antigens. Few of the histocompatibility antigens are more potent than others in provoking rapid graft rejection, this tight cluster of genes that play a major role in recognition and discrimination between self and non-self is known as **Major Histocompatibility Complex (MHC)**. These genes are inherited along with other antigens, which provoke immune response. MHC genes are organized into regions encoding for three classes of molecules; **Class I MHC, Class II MHC** and **Class III MHC**.

MAJOR HISTOCOMBATIBILITY FACTOR (MHC) and ANTIGEN INTERACTION

Both class I and class II MHC molecules are membrane bound glycoproteins and have been purified and isolated to determine the X-ray crystallography of the extracellular domains.

Class I MHC molecule: It contains a large alpha (α) associated non-covalently with β_2 microglobulin molecule and forms a heterodimer. The α chain of class I MHC is made up of three extracellular domains (α_1, α_2, α_3). The Class I MHC molecules are found in highest concentration on the membrane of B cells, T cells and macrophages. The class I antigen are absent from human red blood cells but they are found on mouse red blood cells.

Class II MHC molecule: It's a glycoproteins consists of two non-covalently linked polypeptide chains, alpha (α) and beta (β) chains. Each chain contains two external domains: α_1 and α_2 domains in alpha chain while β_1 and β_2 domains of beta chain. **The membrane distal domains of both Class I and Class II MHC forms the antigen binding cleft for processed antigens.**

Endogenous antigens, Exogenous antigens and Non-peptide antigens: The **C**lass I MHC molecules binds to processed endogenous antigens that have been processed within the cytoplasm of the cell (like tumor proteins, viral protein, bacterial proteins etc synthesized within the infected cells). The Class II MHC molecules binds to peptides processed from **exogenous antigens**; that are internalized either by phagocytosis or endocytosis followed by processing by the endocytic pathway.

Recent research have shown that the **non-peptide antigens** like lipids and glycolipids molecules produced by the bacteria ***Mycobacterium tuberculosis*** are processed and presented by the members of the CD1 family of non-classical class I molecules.

NECESSITY AND ROLE OF T – CELLS IN PRESENTATION PATHWAYS

Both T helper and T cytotoxic cells contains a membrane bound molecule called as T- cell receptor (TCR) that interact with the antigen peptide combined with Class I or Class II MHC molecule present on target or APC cells. T cells differs from B cell receptor in that the TCR are no as soluble as the B cell receptor are. Further the T cell receptors are associated on the T cell membrane by a signal transducing complex known as CD3 complex.

Experiments by R.M Zinkernagel and P.C Doherty (1974) have demonstrated that antigen recognition by T cells is not only specific for viral antigen but also for a self MHC molecule having same MHC haplotype like that of the T cell and proved the self MHC restriction of the T cell receptor. This phenomenon of self MHC restriction of the T cell receptor distinguishes T cells from B cells.

In the basic structure of TCR; the variable region domains contains three hyper variable regions or complementarity determining regions (CDRs), analogous to those present in immunoglobulins. **These CDRs are the active sites that interact with the antigen epitopes presented on MHC molecule.** CD4 and CD8 co-receptors present in T helper and T cytotoxic cells respectively help in recognition of peptide-MHC complex along with T cell receptor and also play a role in signal transduction pathways **(Figure 1)**. The extracellular domains of CD4 and CD8 bind to the membrane proximal domains of MHC molecules present on the surface APCs and target cells respectively, with processed and presented antigen.

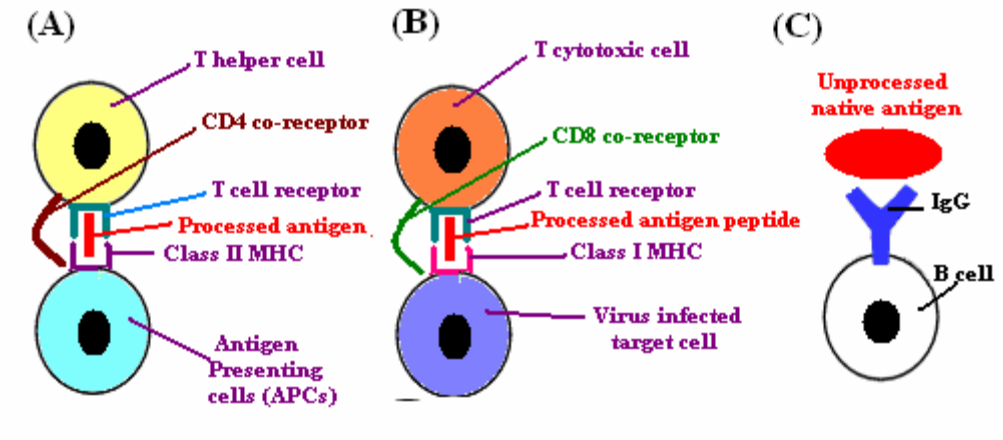


Figure 1. - Schematic diagram showing the interaction of T_H cell, T_C cell and B cell with antigen. (A) The processed antigen peptide produced by exogenous pathway displayed on APCs by Class II MHC interacts with the T cell receptor of TH cell along with CD4 co-receptor that binds to the membrane proximal domains ($\alpha 3$ and β_2 microglobulin) of Class II MHC molecule on APCs. (B) Processed antigen by endogenous pathway displayed by Class I MHC interacts with TCR and CD8 co-receptor of T_C cell. (C) B cell interacts with the native, unprocessed antigen (epitope) directly using the membrane bound immunoglobulin molecule.

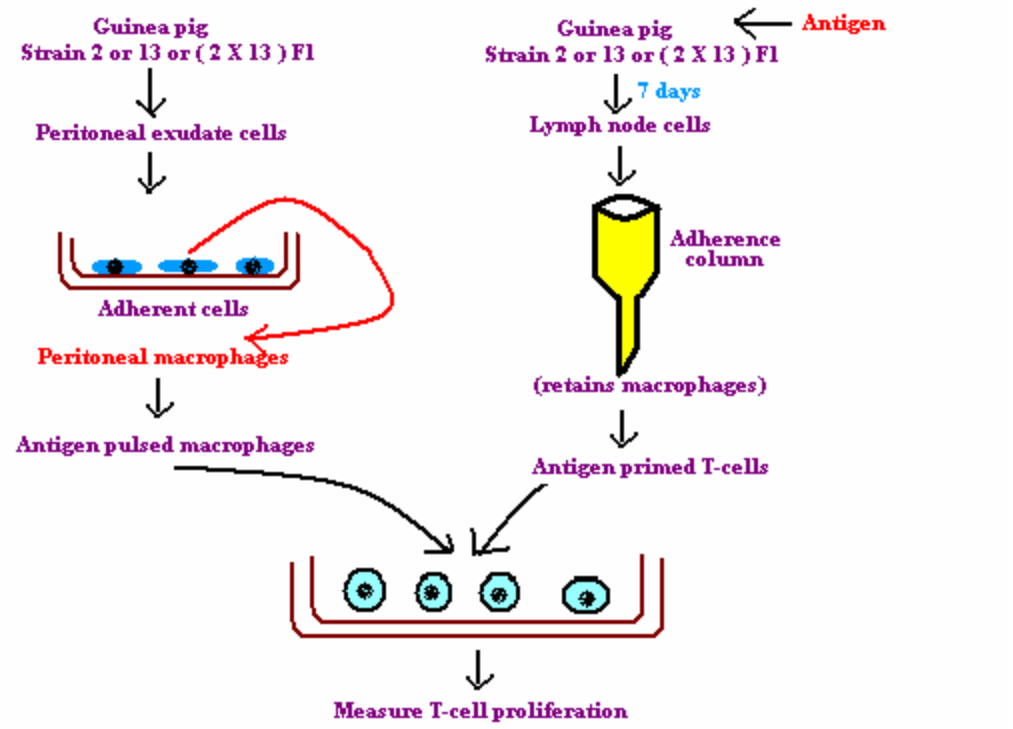
SELF MHC RESTRICTION OF T CELLS

When syngeneic (matching at all MHC loci) mouse T cells, B cells and macrophages are mixed with antigens ***invitro***; antibodies to the antigens are produced showing the immune activation while in case when allogeneic (not matching at MHC loci) mouse immune response cells are used, absence of immune response can be seen even in responder mice (inbred mice having ability to produce IgG antibody on being stimulated with hapten - carrier complex). Thus showing that the ability to recognize antigens are somewhere genetically linked to MHC genes.

T cells are MHC restricted in their ability to recognize antigens. Both $CD4^+$ T helper (T_H) cells and $CD8^+$ T cytotoxic (T_C) cells can recognize antigens only when it is presented with a self MHC molecule (syngeneic MHC) on antigen presenting cells (APCs) or virus infected target cells. This ability of the T cells is known as **self MHC restriction.** This distinguishes the ability of T cells from B cells in recognition of antigen peptide.

Experimental evidences to prove the self MHC restriction of T helper and T cytotoxic cells

A. Rosenthal and E. Shevach, in mid 1970's experimentally demonstrated that antigen-specific proliferation of the T helper cells occurs only when the antigen is presented on macrophages having same MHC haplotype. In their experiment peritoneal exudates cells from guinea pigs of different strains (strain 2, strain 13, strain (2X13) F_1) were initially incubated with an antigen and over a period of time these antigens were internalized by phagocytosis and presented on macrophages. Each of the 'antigen – pulsed' macrophages was subsequently incubated with T helper (T_H) cells from same and different strains **(Figure 2)**. The antigen primed T_H cells showed proliferation only in response to antigen peptide presented by macrophages that shared the same MHC haplotype with the T_H cell.



Antigen Primed T cell	Antigen - pulsed macrophages		
	Strain 2	Strain 13	Strain (2 X 13) F 1
Strain 2	+	–	+
Strain 13	–	+	+
Strain (2 X 13) F 1	+	+	+

Figure 2. – Experimental protocol followed by A. Rosenthal and E. Shevach showing the antigen specific proliferation of T_H cells occurred only in response to antigen presentation by macrophages of same MHC haplotype. Strain 2, Strain 13, and Strain (2X13) F_1 peritoneal macrophages were activated by antigen pulse and were incubated invitro with T_H cells from Strain 2, Strain 13, and Strain (2X13) F_1. Results showed that T_H cells could proliferate only in response to antigen presented by macrophages sharing the same MHC haplotype with T_H cells. **(Rosenthal A.S and Shevach E, 1974, J. Exp. Med. 138: 1194).**

The results indicated that the antigens pulsed macrophages of strain 2 activates T_H cell of strain 2 and strain (2X13) F_1 only while antigen pulsed macrophages of strain 13 activates T_H cells of strain 13 and strain (2X13)F_1. The antigen pulsed macrophages of strain (2X13)F_1 activated T_H cells of all the three strains i.e. strain 2, strain 13, strain (2X13) F_1. These experimental results confirmed that the $CD4^+$ T_H cells are activated by 'antigen pulsed' macrophages only when both $CD4^+$ T_H cells and 'antigen pulsed' macrophages share MHC alleles with one another.

In another experiment P.Doherty and R. Zinkernagel in 1974 demonstrated the self MHC restriction of CD8+ T_C cells. In their protocol lymphocyte choriomeningitis (LCM) virus were immunized into H-2^k mice model, where H-2^k represents the 'k' region of H-2 MHC

haplotype of mice. After few days, spleen of the virus primed H-2^k mice was isolated and was added to target cells of different H-2 haplotypes that were intracellularly labeled with Chromium (Cr^{51}) and these target cells were either infected or not with the LCM virus **(Figure 3).** T_C cell mediated destruction of the target cells were measured by the release of Chromium (Cr^{51}) into the culture medium. They found that the T_C cell lysed only virus infected cell that shared the homologous H-2 haplotype with T cytotoxic (T_C) cells. Thus antigen recognition by CD8+ TC cells was said to be Class I MHC restricted.

Doherty and Zinkernagel in 1996 were awarded with **Nobel Prize** for their major contribution in understanding the cell mediated immunity.

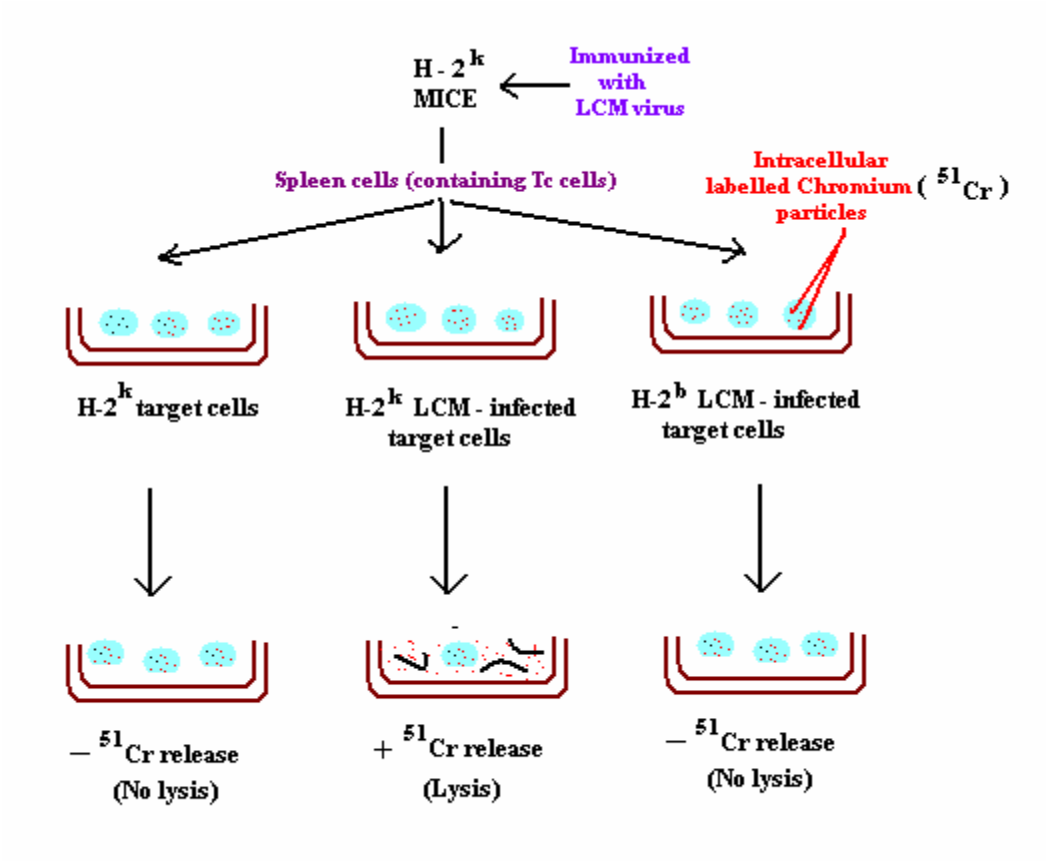


Figure 3. - Experimental procedure followed to prove the self MHC restriction of $CD8^+$ T_C cells by R. Zinkernagel and P. Doherty. In their experiment, mice (H-2^k) were injected with Lymphocytic choriomeningitis (LCM) to induce the production of T cytotoxic cells present in spleen cells. Spleen cells were incubated with LCM infected target cells having different H-2 haplotype and intracellularly labeled with Cr^{51} molecules. The T_C cell mediated target cell killing occurred only in cells having same H-2 haplotype. **(Doherty P.C and Zinkernagel R.M, 1975. J Exp. Med. 141: 502).**

ROLE OF ANTIGEN PRESENTING CELLS (APCs)

Exogenous antigens are processed to small peptides and then presented on cell surface with Class II MHC molecules to $CD4^+$ T_H cells. Only a limited group of cells express Class II MHC along with co-stimulatory activity, these cells are called as Antigen presenting cells (APCs).

Professional antigen presenting cells.

The principal APCs include macrophages, dendritic cells and B cells and are classified as ***professional antigen presenting cells***. The expression of Class II MHC molecules on these

professional APCs are either constitutive or inducible (mainly by IFN-γ in case of macrophages).

Dendritic cells in different locations have different functions and form and are classified by their location. These cells have originated by hematopoietic stem cells through the myeloid lineage and expresses high level of Class II MHC and co-stimulatory signals. Most of the dendritic cells; process and present antigens to T helper cells by the endocytic pathway. Few examples of dendritic cells are Langerhans cells (skin epidermis and mucous membranes), Interstitial dendritic cells (gastrointestinal tract, liver, lung, heart, and kidney), Interdigitating dendritic cells (lymph nodes, spleen, mucosal associated lymphoid tissues (MALT) and thymic medulla), Circulating dendritic cells (found in blood (forms 0.1% of total blood lymphocytes) and lymph).

The follicular dendritic cells (another type of dendritic cells) don't express Class II MHC molecules and doesn't function as antigen presenting cell. But follicular dendritic cells express high level of membrane receptor for antibody and complement and can activate the B cells for immune response.

Macrophage constitutes the mononuclear phagocytic system that also includes monocytes (circulating in blood stream). Macrophages like dendritic cells have different functions in different tissues and are classified by their location like **Osteoclasts** (bones), **Kupffer cells** (hepatic liver cells), **Alveolar macrophages** (lungs), **Histiocytes** (connective tissues), **Mesangial cells** (kidney), **Microglial cells** (brain).

Phagocytosis of foreign antigen acts as stimulus for activation of macrophages and can be further stimulated by interferon gamma (IFN-γ), the most potent activators of macrophages secreted by activated T_H cells.

B lymphocytes (B cells) also act as professional APCs. Mature B cells are different from other lymphocytes in the process of synthesis and display of membrane bound immunoglobulin molecule, which serve as surface receptor. As the Class II MHC molecule is involved in antigen processing and presentation while the co-stimulatory B7-1 and B7-2 molecules are involved in co-stimulatory activity of B cells. The B7-1 and B7-2 are surface molecules that interact with the CD28 and CTLA-4 regulatory molecules on the surface of T helper (T_H) cells, thus activating the immune response more strongly.

Non-professional antigen presenting cells.

Few other cell types are classified as ***non-professional antigen presenting cells,*** and on induction these cells express Class II MHC molecules. Generally these cells function as APCs for short span of time mainly during some inflammatory response. Few of the non-professional APCs are Fibroblasts (Skin), Glial cells (Brain), Pancreatic β cells, Thyroid epithelial cells etc.

Early experimental evidences showing the importance and necessity of Antigen processing and presentation pathways

K. Ziegler and E.R Unanuae observed that antigen processing is necessary for T helper (T_H) cell activation. In first set of experiment when the antigen presenting cells (APCs) were fixed with Para formaldehyde, before exposure to bacterial protein antigens, the T helper (T_H) cell activation by bacterial protein antigen was prevented. In second set, when the APCs fixed 1 to 3 hours after antigen exposure, the APCs were able to activate the T_H cells and in third set, when APCs were fixed before antigen exposure and then incubated with processed antigen peptides (instead of unprocessed antigen protein), they could activate the T_H cell proliferation (**Figure 4**).

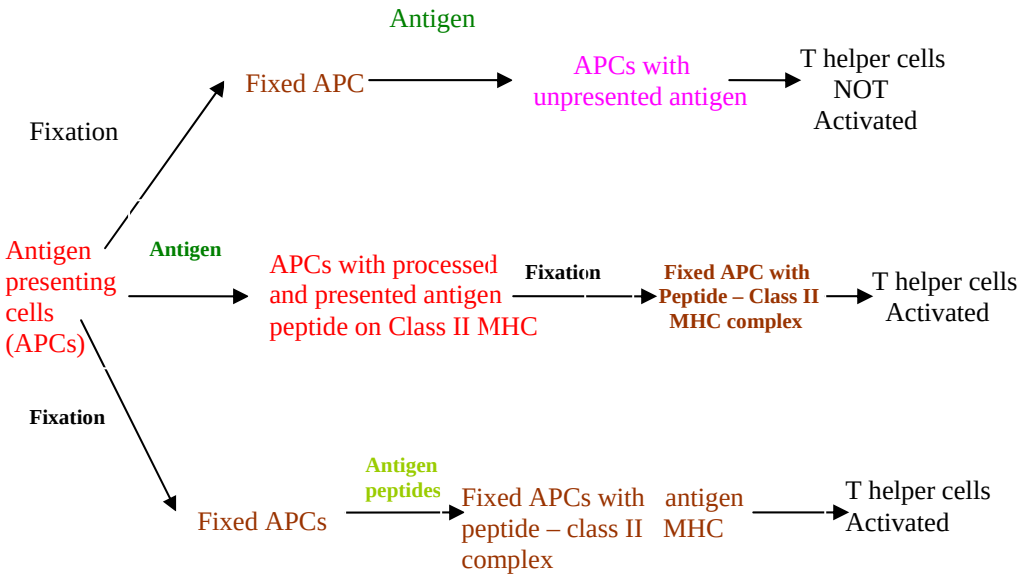


Figure 4.- Overview of the experiment done by K. Ziegler and E.R. Unanuae to demonstrate that antigen processing is necessary for T_H-cell activation.

Thus results demonstrated that the T helper (TH) cell activation occurs only when antigen processed and presented to the T_H cells by antigen presenting cells (APCs). The T_H cell activation was determined by measuring the specific T_H cell response like increase in cytokine secretion.

Cells involved in Antigen presenting pathways

Almost all nucleated cells expresses Class I MHC molecule and they display the processed antigen to $CD8^+$ T cytotoxic cells. These cells are also referred as ***target cells***.

Only a limited group of cells expresses Class II MHC molecule on their surface, that displays the processed peptide antigen to the $CD4^+$ T helper cells and are referred to as ***antigen presenting cells*** (APCs). The professional APCs are macrophages, dendritic cells (Langerhans cells), and B cells. Among these the dendritic cells are the most effective APCs because these cells expresses a high level of Class II MHC molecule along with co-stimulatory activity that further activates the immune response and can also activate the naïve T helper cells.

Presence of two antigen processing and presentation pathways

The T cells must identify the sequence of amino acids inserted on the antigen presenting cleft on the MHC molecule. If the antigen is in the cytosol i.e. endogenous antigen, then the antigen is processed and presented on Class I MHC by a pathway referred as **cytosolic pathway**. The peptide – class I MHC present on the target cell interacts with the T cell receptor (TCR) and CD8 receptor of the T cytotoxic (T_C) cell, thus activating the T_C cells.

If the antigen is in the vesicular system i.e. exogenous antigen, then it proceeds in the Class II route referred as **endocytic pathway**. This route utilizes a $CD4^+$ T helper (T_H) cells that interact with Class II MHC and stimulate the T_H cell activation. Antigen presenting cells (APCs) plays an important role in the endocytic antigen processing and presentation pathway. In professional APCs like B cells, the endocytic pathways initiates by endocytosis that is receptor-mediated endocytosis. The membrane bound IgG antibody acts as receptor, which binds to specific antigen and with the help of these receptors the antigen is engulfed

into the intracellular endosomal system and finally the processed peptides are loaded on Class II MHC molecule and displayed on the surface of the APCs.

Experimental evidence for presence of Cytosolic and Endocytic pathways

L. A. Morrison and T. J. Braciale demonstrated that antigen is processed and presented by two different pathways i.e. Cytosolic and Endocytic pathways; for association with Class I or Class II MHC restricted T cells. Two clones of T_C cells specific for influenza virus were selected for the experiments. One clone was a typical T_C line that recognizes influenza hemagglutinin (HA) – Class I MHC complex while the other typical T_C cell line recognizes influenza hemagglutinin (HA) – Class II MHC complex.

In first set of experiment, a target cell (which expresses both Class I and Class II MHC molecule) were added with infectious influenza virus or with UV-inactivated virus (which is non-infectious and non-replicating but retains antigenic properties). After few hours, the target cells were then incubated subsequently with Class I MHC restricted T_C cells and Class II MHC restricted T_C cells. The results of their experiments demonstrated that Class I MHC restricted T_C responded to target cells treated with infectious influenza virus and not to UV-inactivated virus treated cells whereas the Class II MHC restricted T_C cells responded and activated to target cells with both infectious virus and UV-inactivated treated noninfectious virus.

In the second set of experiments, target cells were incubated with infectious virus along with emetine (inhibitor of viral proteins synthesis) followed by incubation with the Class I and Class II restricted T_C cells. The results showed no lyses of target cells occurred when incubated with Class I restricted T_C cell while lyses of target cells occurred in the aliquot with Class II restricted T_C cells.

In third set of experiments, target cells were incubated with infectious influenza virus along with chloroquine (inhibitor of endocytic processing pathway) followed by incubation with Class I or Class II MHC restricted T_C. The results showed that the Class I restricted T_C cells were stimulated while Class II restricted T_C cells were not stimulated **(Table 1).** These experimental results showed the presence of endocytic processing pathway for exogenous antigens and cytosolic processing pathway for endogenous antigens.

Table 1: Showing the results from the experiment done by L. A. Morrison and T. J. Braciale to demonstrate the presence of two different pathways; cytosolic and Endocytic pathways.

Virus used for the target cells	**Presence of lysis in target cell when treated with Class I restricted T_C cell**	**Presence of lysis in target cell when treated with Class II restricted T_C cell**
Infectious virus	+	+
UV inactivated Virus (non-infectious)	–	+
Infectious virus treated with emetine	–	+
Infectious virus treated with chloroquine	–	–

(+) shows lysis and (-) shows no lysis of target cells

PROCESSING AND PRESENTATION PATHWAYS

Antigen processing and presentation are processes that occur within the virus infected target cells and antigen presentation cells that results in fragmentation and cleavage of protein,

association of the antigen fragment to MHC molecule and finally expression of the peptide – MHC molecules at the cell surface where they can be recognized by the T cell receptor of T helper or T cytotoxic cell **(Figure 5).**

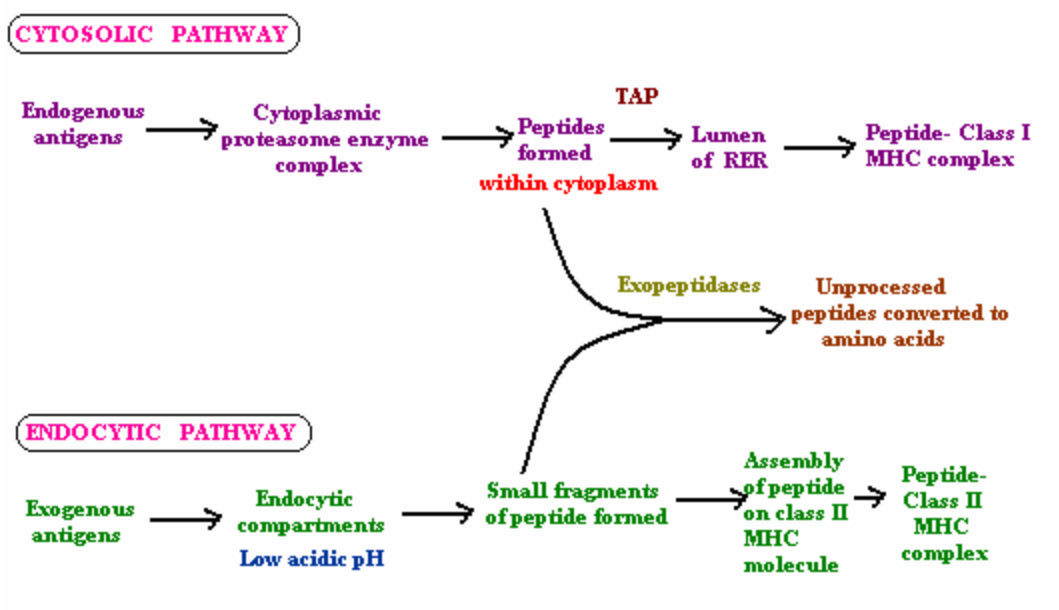


Figure 5 - Overview of cytosolic and endocytic pathways for processing antigen.

Exogenous antigens include the extracellular antigens that are internalized by phagocytosis or endocytosis and are processed by the endocytic pathway. The exogenous pathogens include bacteria, their soluble toxin, extracellular protozoan parasites, viruses and fungi. The exogenous antigen are processed and presented on the membrane of professional antigen presenting cells (APCs) which are recognized by the $CD4^+$ T helper (T_H) cells . APCs like dendritic cells, macrophages and B cells; are cells that have a very active capacity for endocytosis. In the endocytic pathways, the exogenous antigens internalized by phagocytosis or endocytosis are degraded by various hydrolytic enzymes within acidic endocytic compartments.

Class II MHC is routed to endocytic compartments by a number of sequential steps involving its interaction with invariant (Ii) chain followed by CLIP and finally the peptide – class II MHC complex is transferred to the plasma membrane of APCs. This pathway is referred as **Endocytic pathway**.

Endogenous antigens are reproduced within the host cell cytosol. These antigens are mainly the products produced by virus replicating within the infected cell (also known as target cells). As the serum antibody, complement system and phagocytes cannot detect these infected cells due to their size and pathogen being intracellular henceforth cytotoxic T (T_c) cells must be activated to recognize and kill the infected cells. To activate $CD8^+$ T_c cells, the antigens must be presented on class I MHC, which interacts with the CD8 receptor on T_c cells. The pathway by which such endogenous antigens are processed and presented is referred as **Cytosolic pathway.**

Almost all the nucleated cells in the body are equipped with class I MHC. The class I route or cytosolic pathway starts with the proteasome enzyme, which degrades antigen protein into small peptides within the cytoplasm of the target cells. The resulting peptide is transported to the lumen of the rough endoplasmic reticulum (RER) followed by the peptide assembly with

class I MHC molecule. Peptide – class I MHC complex is finally transported from RER through the Golgi complex to the plasma membrane of target cell.

THE CYTOSOLIC PATHWAY FOR ENDOGENOUS ANTIGENS (ENDOGENOUS PATHWAY)

Endogenous means "originating within the organism". The endogenous antigens include the proteins produced by the virus replicating within a target cell. They are degraded to peptides which then associates with Class I MHC molecules and finally is presented on the cell surface of target cell. The detailed description of the cytosolic pathway is as follows:

Peptide Generation by Proteasome

A virus infected cell synthesizes viral proteins on the ribosomes present in the cytoplasm. In order to be presented well, these proteins must be cleaved down into short peptides and then presented on Class I MHC molecule. The cytosolic proteins are degraded by peptides by multifunctional protease complex known as ***proteasome.***

Proteasome complex are very ancient and have been shown to be present in bacterial system also. This enzyme consists of four stacks of seven units. In each of the seven units, the middle stack bears the proteolytic enzyme activity. Functionally proteasome complex is formed of cylindrical arrays of proteolytic enzymes with their active sites facing towards the center of the hollow cylinder. These units' forms a hollow cylinder through which the protein from the cytosol moves in and degradation of the protein complex occurs within the central hollow of the proteasome enzyme complex to form short peptides. The proteasome generally cleaves the peptide bond after 2 or 3 different amino acids in an ATP dependent process. Few inducible proteases are produced which replaces the constitutive proteases in the proteasome complex. Two proteases of these inducible one are encoded from MHC class II region namely LMP2 and LMP7 while a third subunit is coded in response to interferon (which are synthesized in response to virus attack) and not encoded from any MHC regions.

The protein to be cleaved by the proteasome complex is identified by presence of a small protein molecule called as **ubiquitin**; which is attached to the antigenic protein. An ubiquinating enzyme complex brings about the linkage of the ubiquitin group to a lysine amino group near the amino terminal of the antigenic protein **(Figure 6).** Ubiquitin are covalently linked to the proteins to be degraded and this attachment process requires ATP hydrolysis. The Ubiquitin proteasome system is present in number of cellular mechanism like cell cycle, signal transduction and gene expression regulating mechanisms. Cleavage of peptide bonds by proteasome produces peptides, which are now to be transported from the cytosol to the lumen of rough endoplasmic reticulum (RER).

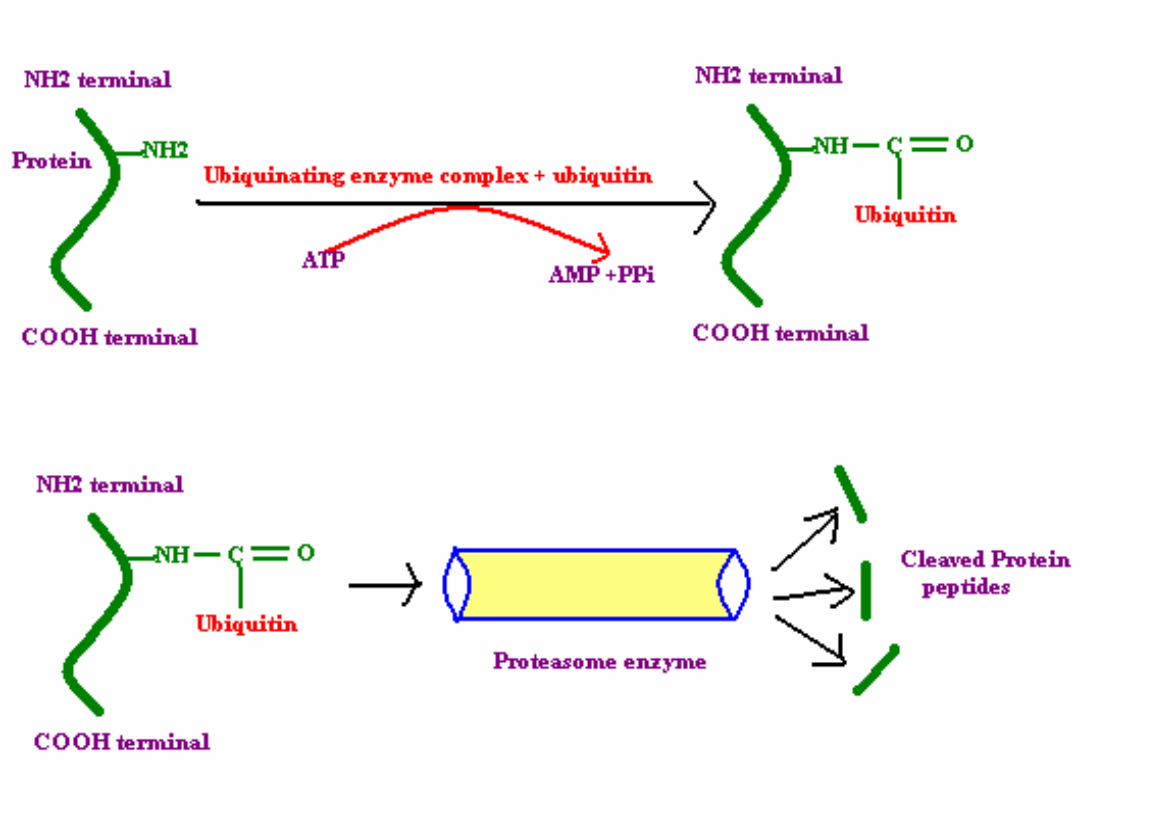


Figure 6. - Process of peptide generation by Proteasome enzyme in the cytosolic pathway. The proteins that are targeted for proteolysis are conjugated with a small protein called as Ubiquitin in a ATP depending process. The ubiquitin attachment is made using Ubiquinating enzyme complex to a lysine amino group near the amino terminal of the protein followed by degradation of the ubiquitin – protein complex in the central hollow channel of the proteasome generating large number of small peptides.

A. Ciechanover, A. Hershko, and Irwin Rose for their outstanding studies done on the importance of proteolytic degradation inside cells and the role of ubiquitin in proteolytic pathways; were awarded with Nobel Prize in Chemistry for the year 2004.

Peptide transport from cytosol to RER through TAP (Transporter protein)

Peptides that were cleaved by the proteasome are now in the cytosol and are to be transferred into the lumen of rough endoplasmic reticulum (RER) for loading on Class I MHC molecule. This peptide transport is done by a transporter protein known as TAP (Transporter Associated with antigen Processing). Tap protein is a membrane spanning heterodimer consisting of two proteins: TAP 1 and TAP 2 **(Figure 7).**

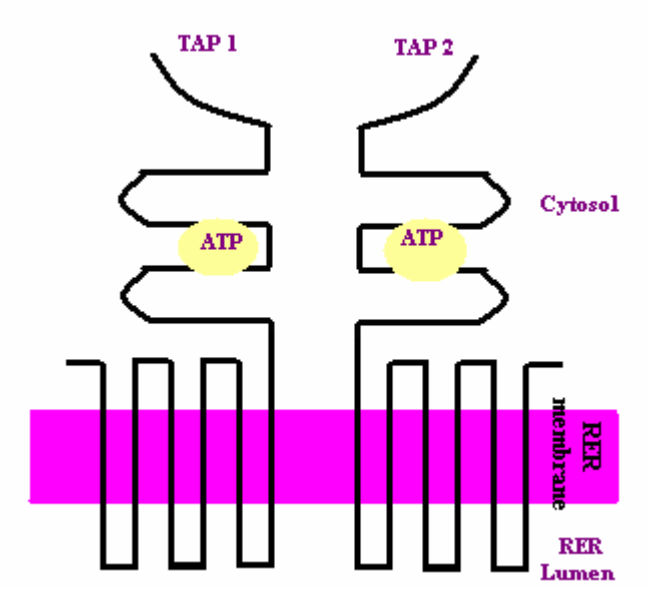


Figure 7.- Schematic diagram of transporter protein TAP (transporter associated with antigen processing) associated with transfer of the proteasome processed peptide into the lumen of RER. It is a membrane spanning heterodimer consisting of two proteins namely TAP1 and TAP2 along with multiple transmembrane segments and each cytosolic domain contains an ATP binding site. Peptide transport is made with hydrolysis of ATP molecule.

Transporter of antigen peptide, TAP 1 and TAP 2 are present in the cytosolic part of RER membrane with ATP binding sites in each of the two transporter proteins while hydrophobic transmembrane domains spanning the RER membrane follows the cytoplasmic domains of TAP1 and TAP2 protein. TAP 1/ TAP 2 heterodimer complex translocates the cytosolic peptides into the lumen of the RER with expenditure and hydrolysis of ATP's.

Assembly of processed peptide with Class I MHC molecule

The alpha (α) chain and β_2 microglobulin domains of Class I MHC are synthesized by the polysomes (large number of ribosomes) present on the rough endoplasmic reticulum (RER). Assembly process that forms stable Class I MHC molecule with antigen peptide involves participation of ***molecular chaperones***, which facilitates proper folding of the polypeptides of class I MHC during assembly process **(Figure 8).**

Newly synthesized partly folded Class I MHC alpha (α) chain is first complexes with a chaperone called as **calnexin**. Calnexin helps the alpha (α) chain of Class I MHC to align in proper shape and fold so that it can bind to β_2 microglobulin properly. When β_2 microglobulin binds to Class I alpha (α) chain, calnexin dissociates and this association of β_2 microglobulin to alpha (α) chain is stabilized by two molecular chaperones: calreticulin and tapasin. Tapasin molecule guides the Class I MHC molecule in contact with the transporter protein TAP in the lumen of RER.

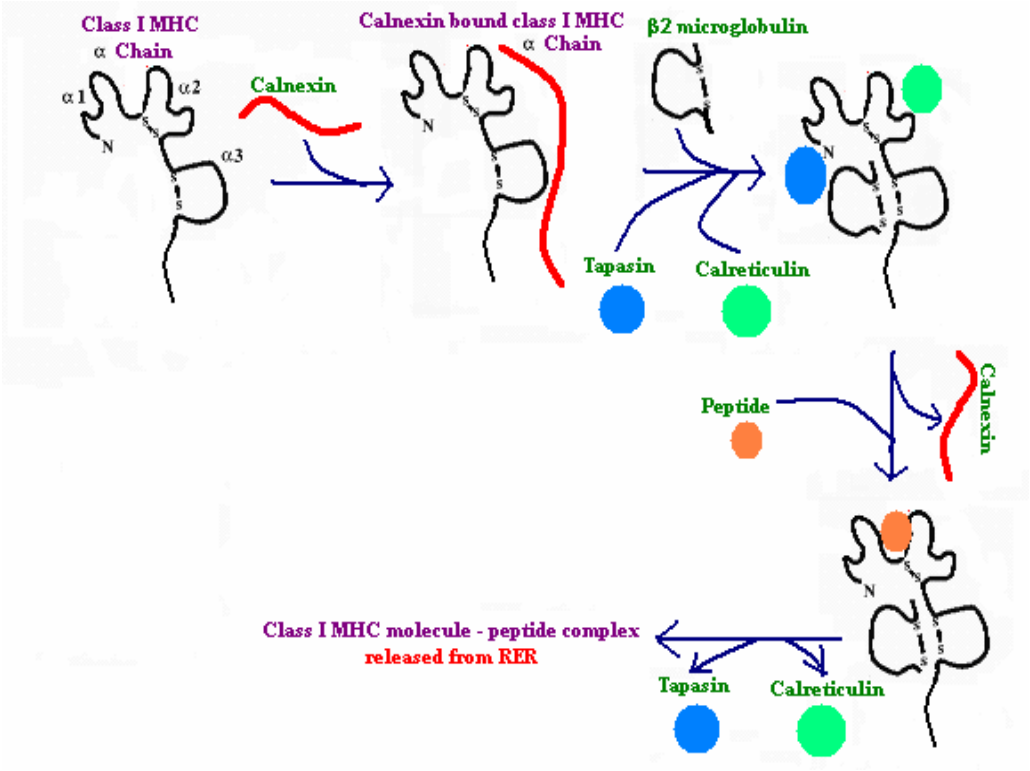


Figure 8. – Assembly of the alpha (a) chain and β_2 microglobulin of the Class I MHC molecule which is further stabilized by peptide binding. The multi-step process is brought about by participation of molecular chaperones like calnexin, tapasin, and calreticulin all of which are dissociated in a sequential manner as shown in the figure. The stabilized peptide – Class I MHC complex is released from RER and proceeds to the cell surface.

When the proper peptide binds properly to the cleft of the MHC Class I molecule, the tapasin and calreticulin gets dissociated from the completely formed peptide – Class I MHC complex. Finally the peptide – Class I MHC complex leaves the RER in micro vesicles through the Golgi passage and reaches the surface of the cell. Once they reach the surface, the $CD8^+$ T_C cell interacts with the T cell receptor and CD8 receptor with the peptide – class I MHC molecule, thus the T_C cell gets activated to destroy the target cell. Unbound peptides are thought to be transported back into the cytoplasm for reprocessing and retransport by another similar pathway for presentation on Class I MHC molecule or degraded by exopeptidase to amino acids. In uninfected target cells, self peptides are processed and presented to Class I MHC molecules and this process is called as **self presentation**, this helps in positive and negative selection in the thymus.

Overview of the Cytosolic pathway

In this pathway, the exogenous antigens are first degraded within the cytoplasm by a proteasome complex. A proteasome complex cleaves peptide bonds between 2 or 3 different amino acids combination by an ATP dependent process. The small peptides are then translocated into the lumen of rough endoplasmic reticulum (RER) by transporter protein TAP (Transporter Associated with antigen Processing). With the use of molecular chaperones like calnexin, tapasin and calreticulin, the β_2 microglobulin domain and processed peptides are attached to the alpha (α) chain of Class I MHC in a sequential manner **(Figure 9).** Finally the peptide – class I MHC are transported from the rough endoplasmic reticulum (RER) through the Golgi complex to the plasma membrane surface.

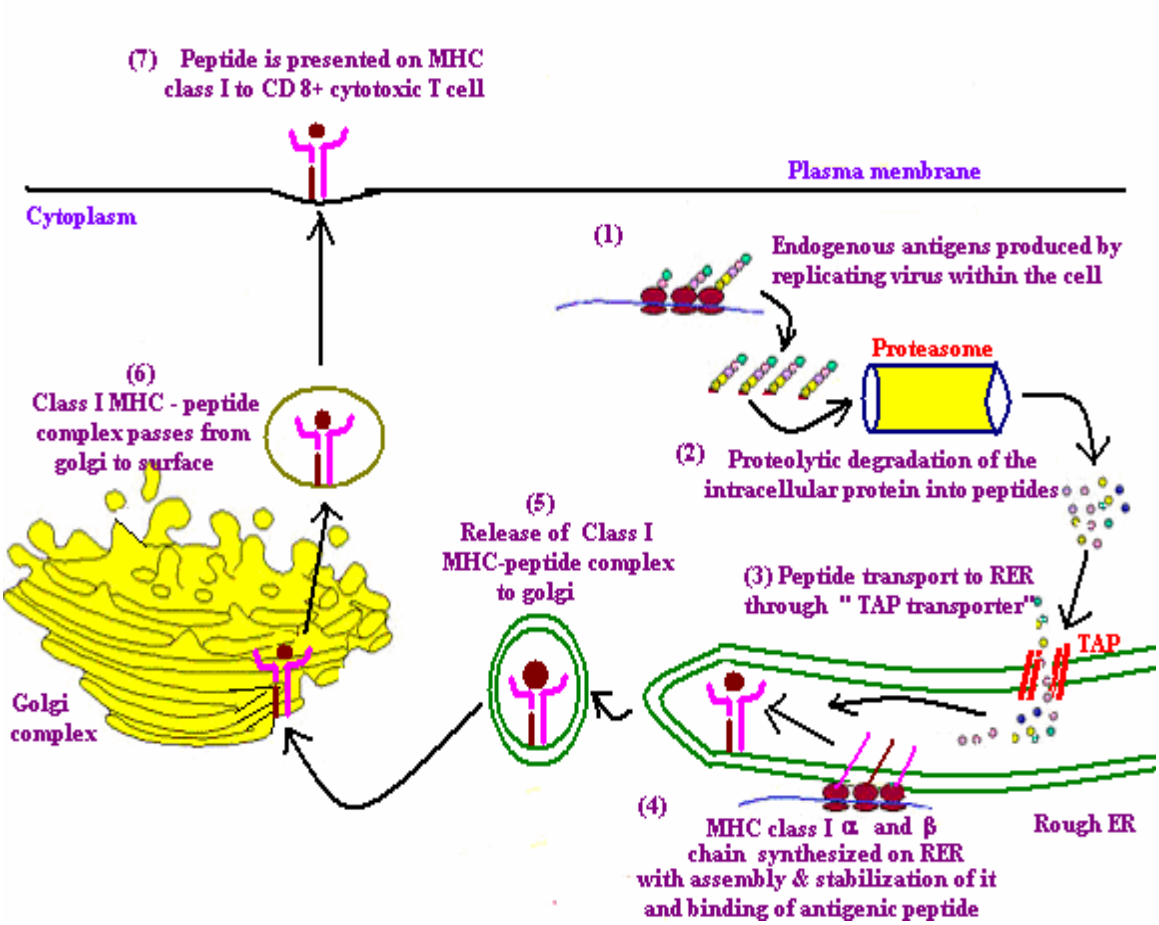


Figure 9. - Overview of the Endogenous pathway or Cytosolic pathway. The endogenous antigens are first degraded by proteasome complex and peptides are then transported across the RER by an ATP dependent transporter protein called TAP. The assembly process leading to formation of stable antigen peptide- class I MHC complex takes place in several steps with the help of molecular chaperones. Finally the Class I MHC – peptide is transported from RER through the Golgi to the plasma membrane.

THE ENDOCYTIC PATHWAY FOR EXOGENOUS ANTIGENS (EXOGENOUS PATHWAY)

Exogenous antigens are processed by the endosomal processing pathway. Antigen presenting cells (APCs) internalize antigens like bacteria, soluble protein antigens, antibody coated viruses by phagocytosis and/or endocytosis. APCs except macrophages are poor phagocytes hence it internalize the protein antigen mainly by endocytosis; which is a receptor mediated endocytosis. Macrophages shows both the phenomenon to engulf the exogenous antigen i.e. phagocytosis as well as endocytosis. While the B cells internalize the antigens by receptor mediated endocytosis and the membrane bound antigen specific IgG molecule acts as the receptor for the process. The internalization is followed by sequential steps and finally the processed peptide is presented on Class II MHC molecule on the surface of APCs; which then activates the $CD4^+$ T_H cells.

Peptide generation in Endocytic vesicles

After internalization, the antigen is transferred to endosomes that become increasingly acidic (lowering of pH) as the unprocessed antigen moves from the plasma membrane farther into the cytoplasm.

The endocytic pathway involves three acidic components with hydrolytic enzymes but varying pH value.

- **Early endosome –**They are the acidic compartments containing hydrolytic enzymes and a pH value of 6.0 to 6.5.
- **Late endosome** – These acidic compartments are also known as endolysosomes having a pH of 5.0 to 6.0.
- **Lysosome** – These compartments are the most acidic one with a pH of 4.5 to 5.0. It contains more than 40 acid dependent hydrolases like nucleases, proteases, phospholipases, lipases, phosphatases and glycosidases.

Internalized exogenous antigen, first moves into early endosome then into late endosome and finally into lysosomes encountering hydrolytic enzymes **(Figure 10)**. Increased acidity activates hydrolytic protease enzymes that cleave the protein antigen into small peptides of 13 to 18 amino acid residues long. The hydrolytic enzymes are functionally most active at low acidic pH, henceforth if the pH of the endosomal compartments is increased by chloroquine or protease enzymes are inhibited by drugs like leupeptin; then the endocytic processing pathway is terminated. As the processed peptide are now passed to be assembled on class II MHC molecule, the receptors that are involved in the receptor mediated endocytosis are recycled back to the plasma membrane and can be again used for the endocytosis process.

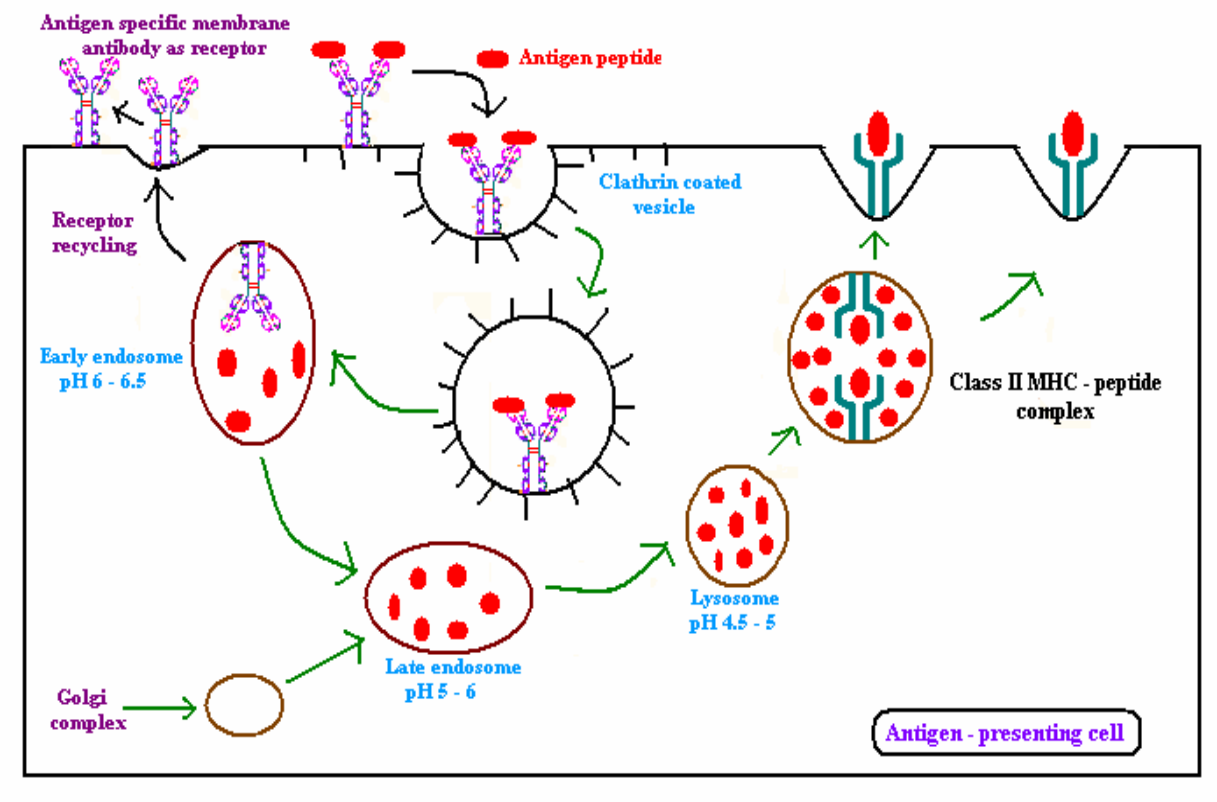


Figure 10. - Peptide generation in the endocytic vesicles. Once an exogenous antigen is internalized, it moves from early to late endosomes and finally to lysosomes with lowering of pH in each of the acidic compartments. The hydrolytic enzymes bring about the degradation of antigen to oligopeptides, which binds to Class II MHC molecule. The cell shown is B cell, which internalize the antigen with the help of membrane bound immunoglobulin molecules which acts as a receptor (receptor mediated endocytosis)

Transport of Class II MHC molecules to endocytic vesicles

Class II MHC alpha (α) and beta (β) chains are synthesized on the polysomes of the rough endoplasmic reticulum (RER) and transported into the RER lumen, where the Class II MHC gets associated and banded by another protein molecule known as **Invariant chain (Ii).**

Invariant chain appears to be involved in proper folding of Class II MHC (α) and beta (β) chains and their exit from the RER **(Figure 11).** Invariant chain also allows the Class II MHC to assemble in the absence of peptide and blocks the association of "self peptide" on to the Class II MHC molecule. Research analysis have shown that the invariant chain contains sorting signals in its cytoplasmic tail which help to direct the fully formed Class II MHC – Invariant chain complex from trans-Golgi network to the endocytic compartments where the invariant chain encounters hydrolytic enzymes.

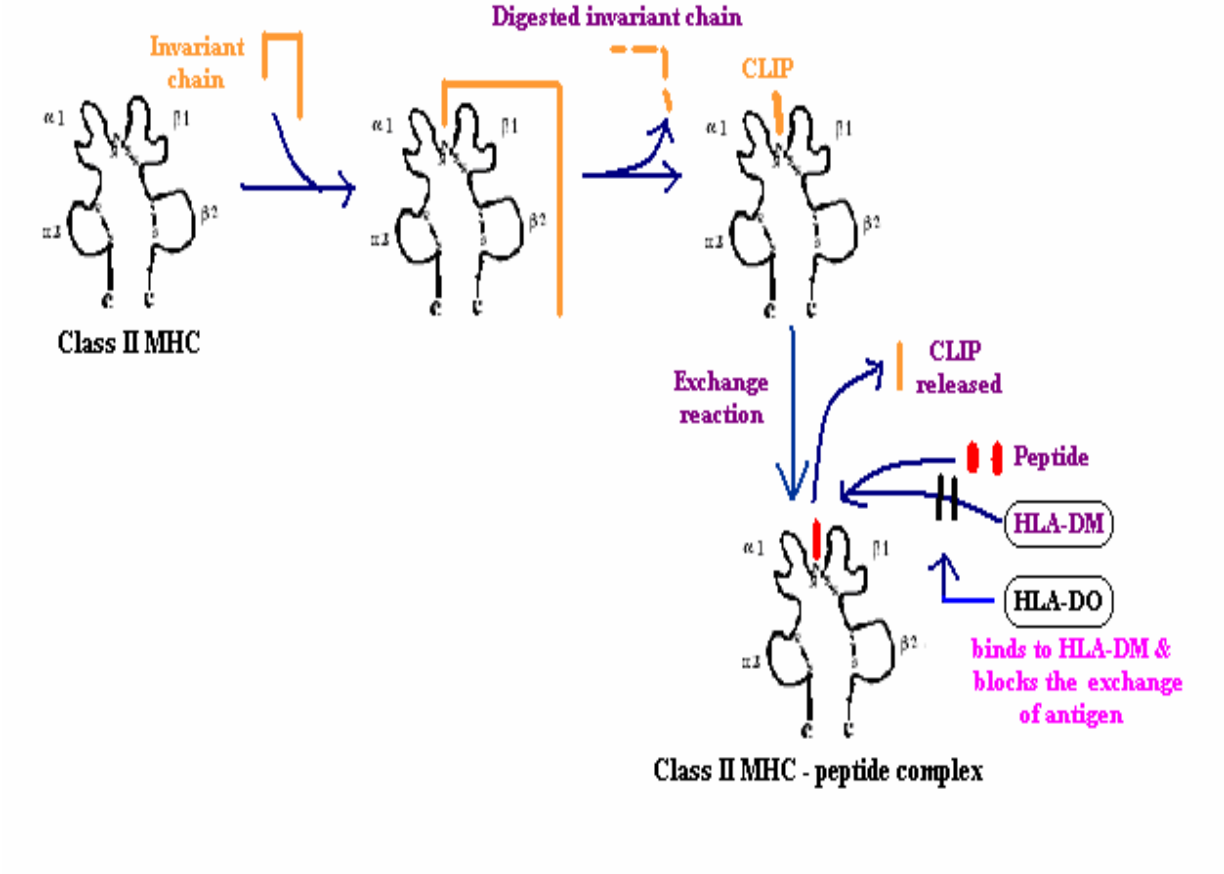


Figure 11. – Assembly of peptides with Class II MHC molecules takes place within the RER. An invariant chain gets bound to the Class II MHC, as it prevents the premature binding of peptides and helps to navigate the complex to endocytic vesicles. In the endocytic compartments the invariant chain is degraded leaving only a small fragment known as CLIP. HLA-DM, a non-classical Class II MHC mediates the exchange of CLIP with an antigen peptide and this exchange reaction can be blocked by a negative regulator called as HLA-DO, which binds to HLA-DM.

The class II MHC – invariant chain complex is transferred through the Golgi complex to a specialized vesicular compartment known as **MIIC** (MHC Class II Compartment) or endosomal compartments; this acidic compartment is equipped with pH dependent proteolytic enzymes where over several hours the Invariant chain starts degrading by hydrolytic enzymes. The Class II MHC – Invariant chain complex moves from RER to Golgi complex and then through specialized vesicles to the first endosomal compartment called as early endosomes. It is then followed by transfer of the complex to late endosome and finally into lysosomes. As the pH decreases progressively in each endosomal compartment, a short

fragment of Invariant chain known as CLIP (for Class II – associated Invariant chain Peptide) remains bound to the antigen binding cleft of Class II MHC and the remaining part of the invariant chain is degraded completely within these highly acidic compartments.

Assembly of Peptide with Class II MHC

The short fragment of invariant chain called as CLIP (for Class II – associated Invariant chain Peptide) occupies the peptide binding cleft, which avoids the premature antigenic peptide binding to the antigen binding site of Class II MHC molecule.

HLA-DM, a non classical Class II MHC alpha (α) and beta (β) heterodimer resembles the classical Class II MHC molecule that felicitates the presentation of processed antigen on the surface of APCs to T helper cells (Figure 12). The HLA-DM molecule is not expressed on the cell surface but plays a major role in loading of antigenic peptide to Class II MHC molecule with removal of the short CLIP fragment.

The exchange reaction of removal of CLIP (for Class II – associated Invariant chain Peptide) with loading of the processed antigen in presence of HLA-DM is inhibited by HLA-DO, which binds to HLA-DM and thereby blocking the HLA-DM role in the exchange reaction for dissociation of CLIP protein from Class II MHC molecule. The non classical Class II MHC molecule HLA-DO acts as a negative regulator to the class II antigen processing and presentation pathway. Finally the processed antigen peptide – Class II MHC complex reaches the plasma membrane of APCs and presents the peptide to $CD4^+$ T_H cells.

Class II MHC – peptide complexes are very stable. The Class II MHC which couldn't bind to any peptide after the dissociation of CLIP protein is highly unstable and is rapidly degraded. When the APCs are not processing any exogenous antigens, then APCs present Class II MHC containing self peptides.

Overview of the Endocytic pathway

Newly synthesized Class II MHC alpha and beta chain heterodimer complex associates with the Invariant chain (Ii) at the peptide binding cleft of the Class II MHC. This binding helps the premature self antigen binding to this heterodimer complex. This Class II MHC – Invariant chain complex is routed through the rough endoplasmic reticulum to the Golgi complex and finally to the endocytic pathway. Within the endosomal compartments, the invariant chain is cleaved and degraded leaving only a small fragment of invariant chain attached to the peptide binding cleft, this short fragment is called as CLIP (for Class II – associated Invariant chain Peptide). In the endocytic compartment, the CLIP protein is replaced by processed antigenic peptides that are formed by sequential and directional degradation process of antigen protein from early endosome to late endosome and finally in lysosome. The exchange reaction takes place in presence of HLA-DM, a non classical Class II MHC molecule. This HLA-DM felicitates the exchange of CLIP with antigen peptide and can be blocked by HLA-DO, another non classical Class II MHC molecule. Finally the Class II MHC – peptide complex moves to the surface of the plasma membrane of APCs.

PROCESSING AND PRESENTATION OF NON PEPTIDE BACTERIAL ANTIGENS

Class I and Class II MHC presentation route are limited to protein peptide processing and presentation pathways only. A little knowledge about the processing pathways for non-peptide molecules is known. It has been shown that for special conditions like presentation of antigenic lipids and glycolipids (derived from pathogenic bacteria like Mycobacterium tuberculosis) are presented on a Class I MHC like molecule called as **CD1 (Figure 12).** This

third pathway for processing and presentation of non peptide antigens derived from bacteria involves this class I like CD1 molecules.

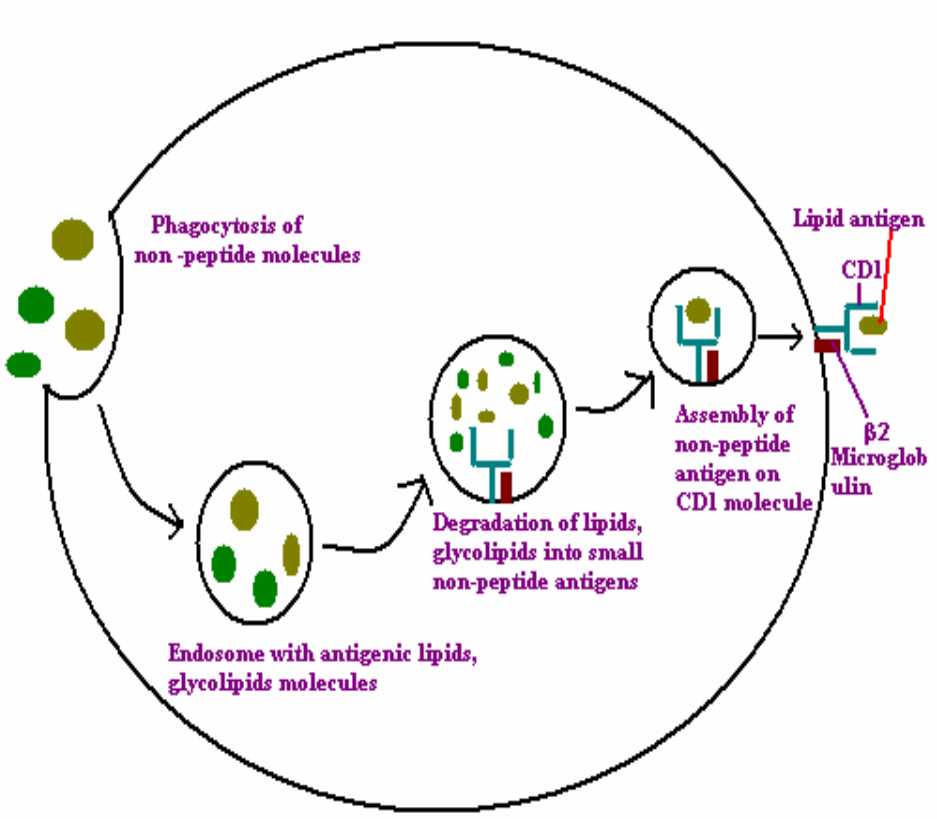


Figure 12. - Presentation of non-peptide bacterial antigens like lipids and glycolipids antigens from ***Mycobacterium*** species. The CD1 molecule associated with β_2 microglobulin presents the lipid or other non-peptide antigens. The CD1- β_2 microglobulin has general structure similar to Class I MHC molecule.

CD1 family of molecules are made up of a CD alpha (α) chain and β_2 microglobulin that forms a complete Class I like CD1. The CD alpha (α) chain resembles and are homologous to the classical Class I alpha (α) chain. CD1 gene products are found on antigen presenting cells (APCs) like macrophages and dendritic cells. The processed lipids, glycolipids, hydrophobic peptides and other non peptide molecules are presented on CD1 that present these molecules to the $CD8^+$ T_C cells, thus activating them. Exact mechanism of non peptide processing and presentation is still not understood completely and research on it is in progress.

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