Major histocompatibility complex proteins and antigen processing

Self is distinguished from non self by the display of peptides on cell surface

The immune system must identify and destroy pathogens, but it must also recognize and not destroy the normal proteins and cells of the host organism –the “self”.
Detection of protein antigens in the host is mediated by the MHC
(major histocompatibility complex proteins).

The MHC was studied and so named as a genetic complex that influences the ability of an organism to accept or reject transplanted tissues from an other member of the same species. In mid 1930s, Peter Gorer, used inbred strains of mice to identify blood-group antigens and identified four groups of genes (I-IV).

George Snell and Gorer in 1940s and 1950s established that antigens encoded by group II genes take part in rejection of transplanted tumor. Snell named these genes as “Histocompatibility genes” and current gesignation is Histocompatibility-2 (H-2). Snell was awarded Nobel prize in 1980 for this work while Gorer died.

Every mammalian species possesses a tightly linked cluster of genes, the major histoconpaibility complex (MHC).

The products of MHC play a role in

1. Recognition of self and non self cells

2. It determines whether transplanted tissue is accepted as self or rejected as non self

3. MHC plays a key role in development of both humoral and cell-mediated immune responses. Most T cells recognize antigen only when it is combined with an MHC molecule.

4. It is also responsible for autoimmunity

Mouse H-2 complex

Complex	H-2						
MHC class	I	II		III		I	
Region	K	IA	IE	S		D	
Gene products	H-2K	IA αβ	IE αβ	C′ proteins	TNF-α TNF-β	H-2D	H-2L

Human HLA complex

Complex	HLA							
MHC class	II			III		I		
Region	DP	DQ	DR	C4, C2, BF		B	C	A
Gene products	DP αβ	DQ αβ	DR αβ	C′ proteins	TNF-α TNF-β	HLA-B	HLA-C	HLA-A

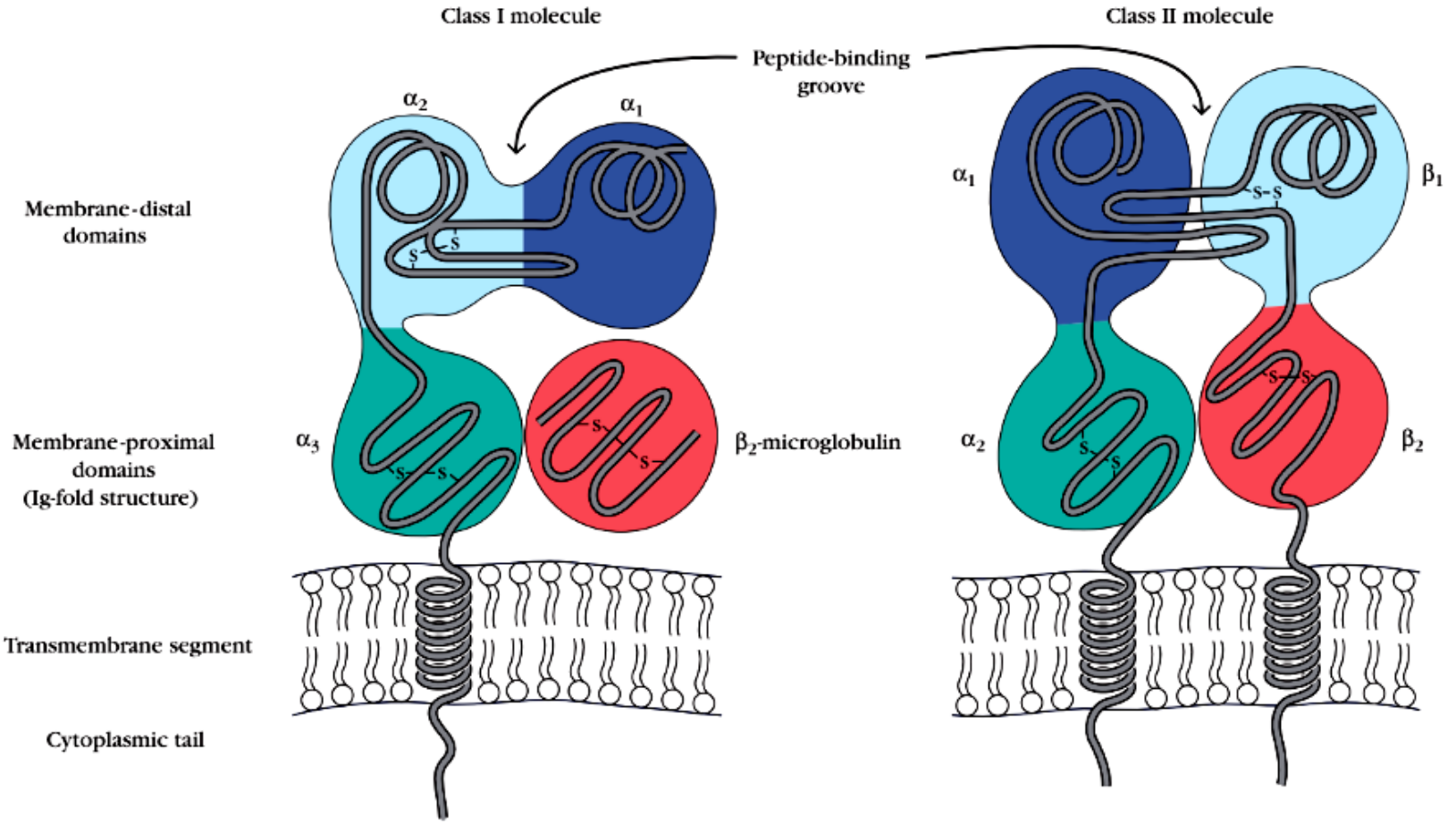
HLA- Human-leucocyte associated antigen

Comparison of the organization of the major histocompatibility complex (MHC) in mice and humans.
The MHC is referred to as the H2 (formerly H-2) complex in mice and as the HLA complex in humans.
In both species, the MHC is organized into a number of regions encoding class I (pink), class II (blue), and class III (green) gene products.
The class I and II gene products shown in this figure are considered to be the classical MHC molecules.
The class III gene products include other immune function–related compounds such as the complement proteins (C') and tumor necrosis factors (TNF and lymphotoxin).

MHC class I genes encode glycoproteins expressed on the surface of nearly all nucleated cells; the major function of the class I gene products is presentation of endogenous (cytosolic) peptide antigens to CD8 T cells.

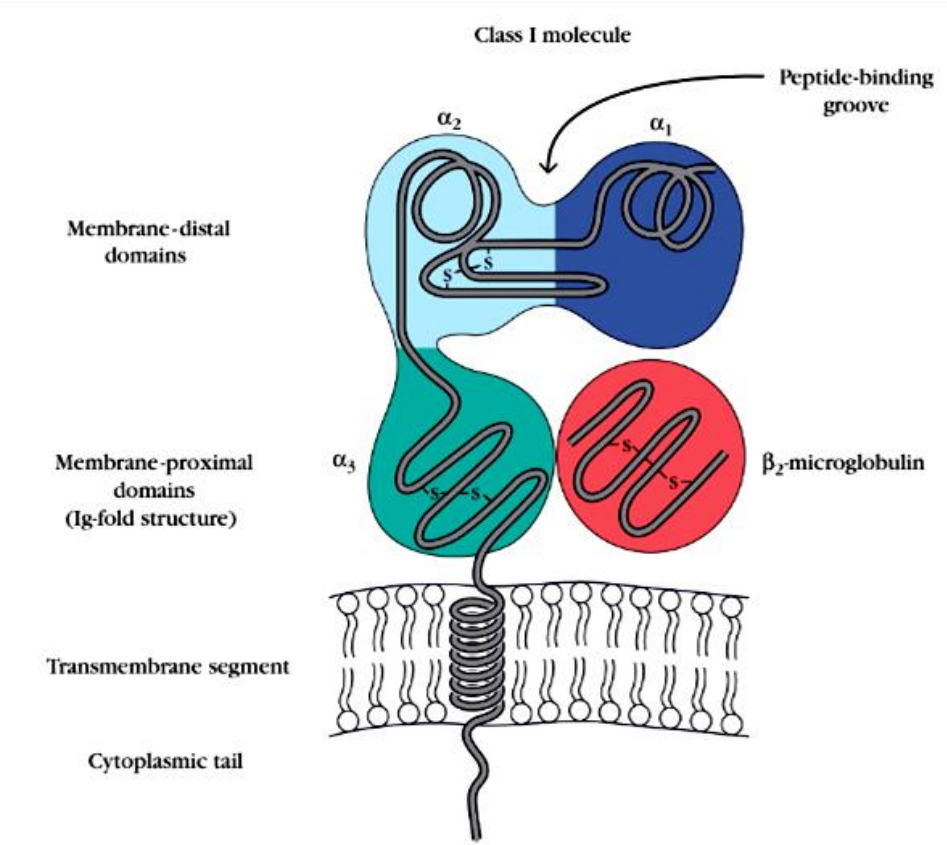
MHC class II genes encode glycoproteins expressed predominantly on APCs (macrophages, dendritic cells, and B cells), where they primarily present exogenous (extracellular) peptide antigens to CD4 T cells.

MHC class III genes encode a diverse set of proteins, some of which have immune functions, but that do not play a direct role in presenting antigen to T cells.



Schematic diagrams of class I and class II MHC molecules showing the external domains, transmembrane segments, and cytoplasmic tails.

The peptide-binding groove is formed by the membrane-distal domains in both class I and class II molecules. The α1 and α2 domains of class I, and the α1 and β1 domains of class II, interact to form the peptide-binding groove. The membrane-proximal domains possess the immunoglobulin domain structure; thus, both MHC class I and II molecules, as well as β microglobulin, are classified as members of the immunoglobulin superfamily.



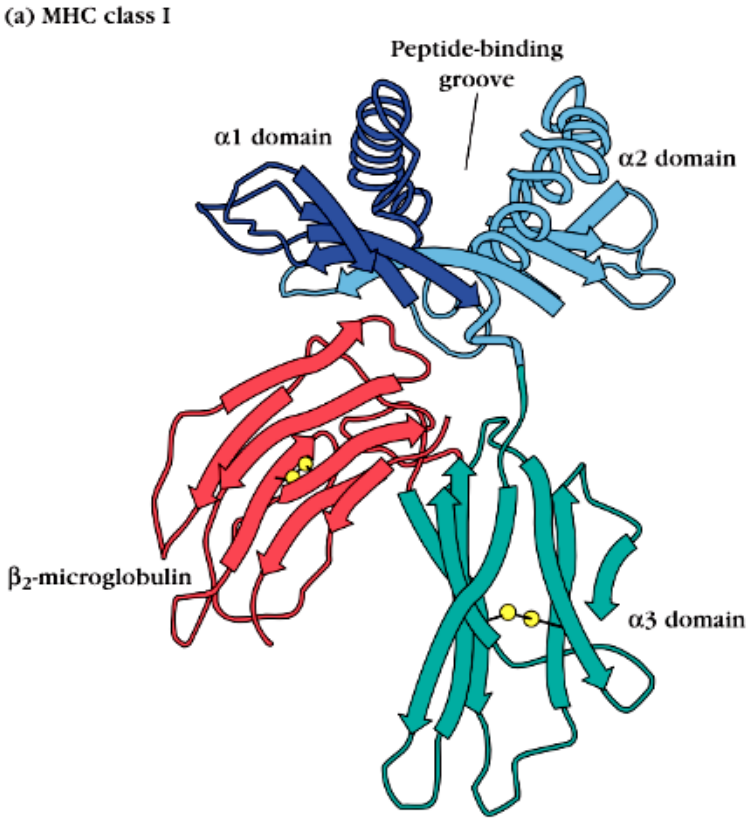
Class I Molecules Consist of One Large Glycoprotein Heavy Chain Plus a Small Protein Light Chain Two polypeptides assemble to form a single MHC class I molecule:

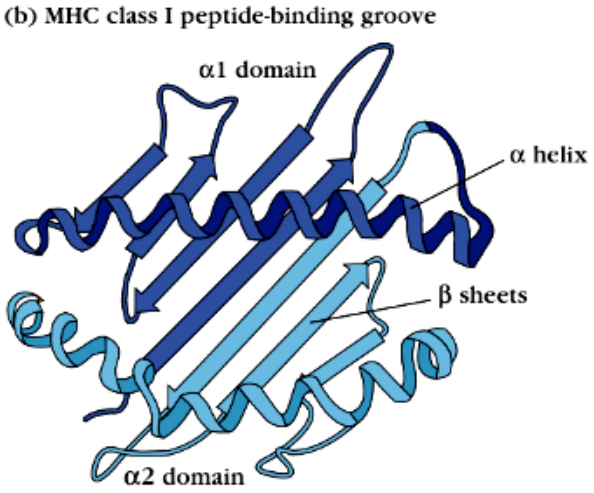
a 45-kilodalton (kDa) α chain and a much smaller 12-kDa β chain, called β -microglobulin.

The α chain is organized into three external domains (α1, α2, and α3), each approximately 90 amino acids long; a transmembrane domain of about 25 hydrophobic amino acids followed by a short stretch of charged (hydrophilic) amino acids; and a cytoplasmic anchor segment of 30 amino acids.

The , β Microglobulin does not contain a transmembrane region and is noncovalently bound to the MHC class I α chain.

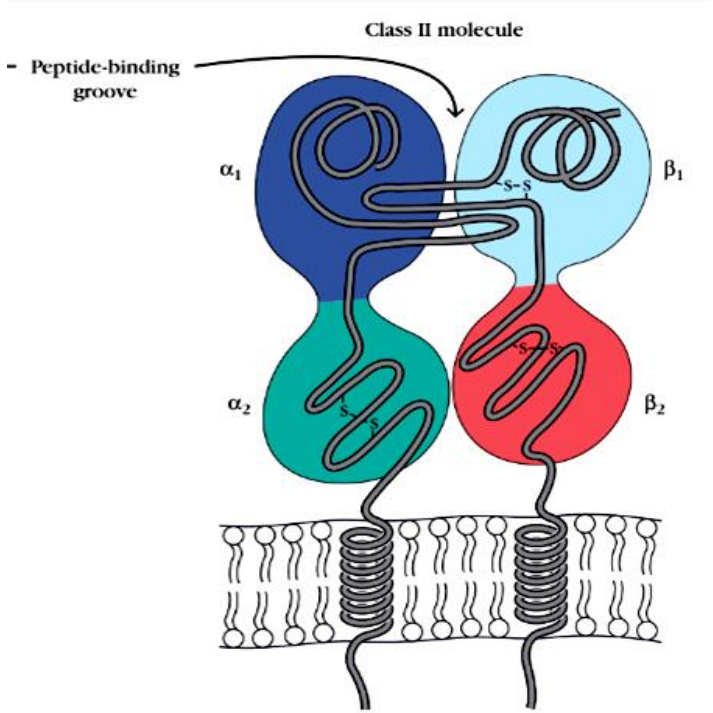
Sequence data reveal strong homology between the α3 domain of MHC class I, β microglobulin, and the constant region domains found in immunoglobulins.





The α1 and α2 domains interact to form a floor of eight antiparallel β strands rimmed by two long α helices . The structure forms a groove, or cleft, with the long α helices on the sides and the β strands as the bottom.
This peptide-binding groove is located on the top surface of the MHC class I molecule, and it is large enough to bind a peptide **of 8 to 10 amino acids**.

During the x-ray crystallographic analysis of class I molecules, small noncovalently associated peptides that had cocrystallized with the protein were found in the groove. The big surprise came when these peptides were later discovered to be fragments of processed self proteins and not the foreign antigens that were expected. MHC molecule/self-peptide complexes are ubiquitous and also play an essential role in regulating tolerance to self-proteins.



Class II Molecules Consist of Two Nonidentical Membrane-Bound Glycoprotein Chains

MHC class II molecules contain two different glycoprotein chains of similar size, a 33-kDa α chain and a 28-kDa β chain, which associate by noncovalent interactions .

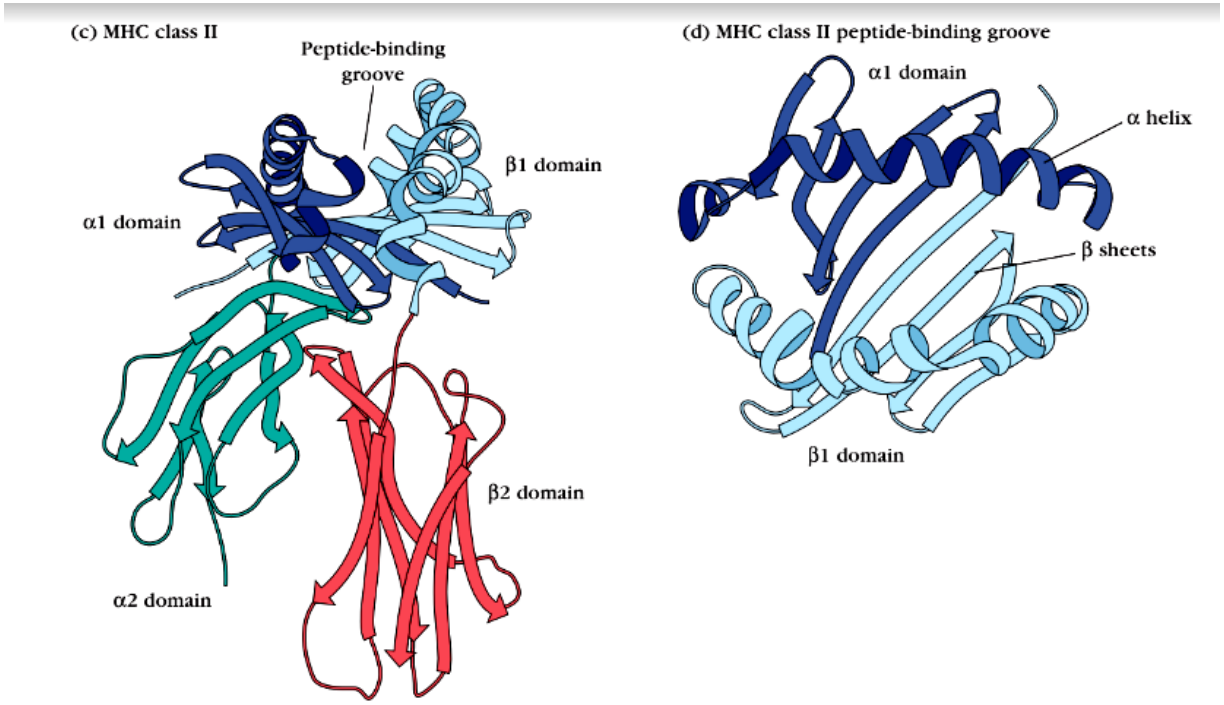
Like the class I α chain, both MHC class II chains are membrane-bound glycoproteins that contain external domains, a transmembrane segment, and a cytoplasmic anchor segment.

Each chain in a class II molecule contains two external domains: α1 and α2 domains in one chain and β1 and β2 domains in the other. The membrane-proximal α2 and β2 domains, like the membrane-proximal α3/β -microglobulin domains of MHC class I molecules, bear sequence similarity to the immunoglobulin fold structure.

For this reason, MHC class II molecules are also classified as belonging to the immunoglobulin super family.

The membrane-distal α1 and β1 domains form the peptide-binding groove for processed antigen.

Although similar to the peptide binding groove of MHC class I, the groove in MHC class II molecules is formed by the association of two separate chains; an important distinction between the class I and class II structures.



The peptide-binding groove of class II molecules, like that found in class I molecules, is composed of a floor of eight antiparallel β strands and two sides of antiparallel α helices.
Class II associated peptides are frequently slightly larger than class I peptides, typically ranging from **13 to 18 amino acids**

Peptide binding by class I and class II MHC molecules

	Class I molecules	Class II molecules
Peptide-binding domain	α1/α2	α1/β1
Nature of peptide-binding cleft	Closed at both ends	Open at both ends
General size of bound peptides	8–10 amino acids	13–18 amino acids
Peptide motifs involved in binding to MHC molecule	Anchor residues at both ends of peptide; generally hydrophobic carboxyl-terminal anchor	Anchor residues distributed along the length of the peptide
Nature of bound peptide	Extended structure in which both ends interact with MHC cleft but middle arches up away from MHC molecule	Extended structure that is held at a constant elevation above the floor of MHC cleft

The major histocompatibility complex is a collection of genes arrayed within a long continuous stretch of DNA on chromosome 6 in humans and on chromosome 17 in mice.

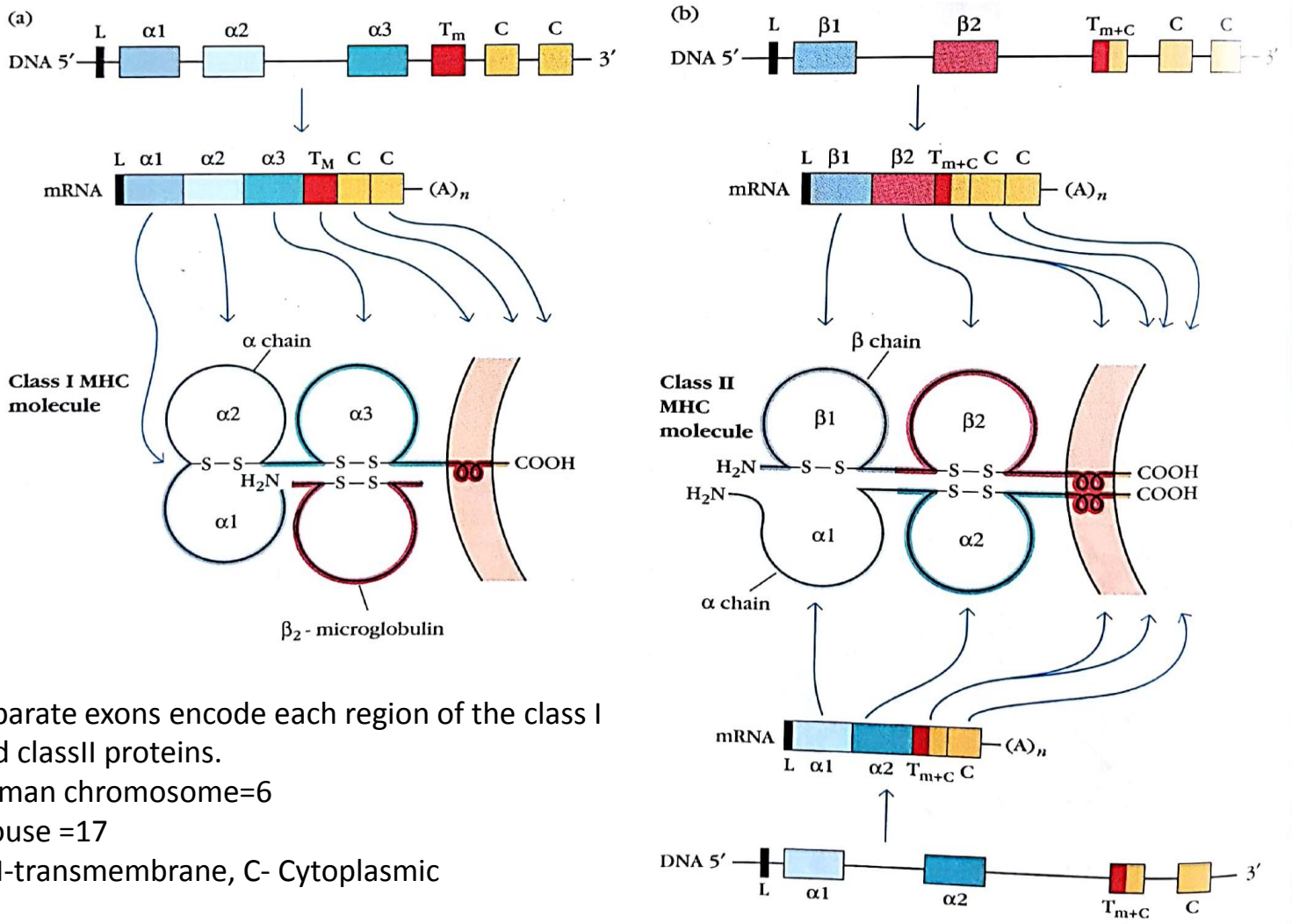
Classical MHC class I (α-chain) and MHC class II (α and β-chain) molecules are encoded within the MHC locus.

The MHC is also referred to as the human leukocyte antigen (HLA) complex in humans and as the H2 complex (previously H-2) in mice.

β -Microglobulin of MHC-I is encoded outside the MHC locus, on a separate chromosome altogether (chromosome 15 in humans and chromosome 2 in mice).

MHC genes are polymorphic (many alleles exist for each gene in the population), polygenic (several different MHC genes for MHC class I and II molecules exist in an individual), and codominantly expressed (both maternal and paternal copies).

Species diversity at the MHC locus imparts an evolutionary survival advantage against mortality from infectious disease.



Separate exons encode each region of the class I and classII proteins.
Human chromosome=6
Mouse =17
TM-transmembrane, C- Cytoplasmic

Schematic diagram of (a) class I and (b) class II MHC genes, mRNA transcripts, and protein molecules.
There is strong correspondence between exons and the domains in the gene products of MHC molecules. Note that the mRNA transcripts are spliced to remove the intron sequences. Each exon, with the exception of the leader (L) exon, encodes a separate domain of an MHC molecule. The leader peptide is removed in a post-translational reaction before the molecule is expressed on the cell surface. The gene encoding 2-microglobulin is located on a different chromosome in both human and mouse.

MHC Polymorphism is primarily limited to the Antigen-binding groove

Although the sequence divergence among alleles of the MHC within a species is very high, this variation is not randomly distributed along the entire polypeptide chain.

Instead, polymorphism in the MHC is clustered in short stretches, largely within the membrane-distal **α1 and α2** domains of class I molecules.

Similar patterns of diversity are observed in the **α1 and β1** domains of class II molecules.

MHC Alleles and Susceptibility to Certain Diseases

Some HLA alleles occur at a much higher frequency in people suffering from certain diseases than in the general population. The diseases associated with particular MHC alleles include autoimmune disorders, certain viral diseases, disorders of the complement system, some neurologic disorders, and several different allergies.

In humans, the association between an HLA allele and a given disease may be quantified by determining the frequency of that allele expressed by individuals afflicted with the disease, and then comparing these data with the frequency of the same allele in the general population.

Such a comparison allows calculation of an individual's **relative risk (RR)**

$$RR = \frac{\text{frequency of disease in the allele}^{+}\text{ group}}{\text{frequency of disease in the allele}^{-}\text{ group}}$$

An RR value of 1 means that the HLA allele is expressed with the same frequency in disease-afflicted and general populations, indicating that this allele confers no increased risk for the disease.

An RR value substantially above 1 indicates an association between the HLA allele and the disease.

For example, individuals with the HLA-B27 allele are 90 times more likely (RR = 90) to develop the autoimmune disease ankylosing spondylitis, an inflammatory disease of vertebral joints characterized by destruction of cartilage, than are individuals who lack this HLA-B allele

Genetic diversity of MHC loci in the human population Data from http://hla.alleles.org, a website maintained by the HLA Informatics Group based at the Anthony Nolan Trust in the United Kingdom, with up-to-date information on the numbers of HLA alleles and proteins. Data as of January 2017

MHC region	HLA locus	Number of allotypes (proteins)
Class I	A	2480
	B	3221
	C	2196
	E	8
	F	4
	G	18
Class II	DMα	4
	DMβ	7
	DOα	3
	DOβ 5	5
	DPα1	22
	DPβ1	591
	DQα1	34
	DQβ1	678
	DRα 2	2
	DRβ1	1440
	DRβ3	106
	DRβ4	42
	DRβ5	39

This enormous polymorphism results in a tremendous diversity of MHC molecules within a species. Even just considering the most polymorphic of the HLA class I genes (A, B, and C), the theoretical number of potential class I haplotypes in the human population is over 40 billion . If the most polymorphic class II loci are considered, the numbers are even more 544 staggering, with over 10 different possible class II haplotypes. Because each HLA haplotype contains both class I and class II genes, theoretically, there could be as many as 10 possible ways to combine HLA class I and II alleles within the human population. Combined with the promiscuity of peptide binding for each of these MHC molecules, this represents an enormous number of different vantage points for TCRs to engage with antigen.

MHC class I expression is found throughout the body

Classical MHC class I molecules are expressed constitutively on almost all nucleated cells of the body. However, the level of expression differs among different cell types, with the highest levels of class I molecules found on the surface of lymphocytes.
On these cells, class I molecules may constitute approximately 1% of the total plasma membrane proteins, or some 5×10 MHC class I molecules per cell.

In contrast, cells such as fibroblasts, muscle cells, liver hepatocytes, and some neural cells express very low to undetectable levels of MHC class I molecules. This low-level expression on liver cells may contribute to the relative success of liver transplants, reducing the likelihood of graft rejection when T cells of the recipient recognize the foreign tissue of the donor.

A few cell types (e.g., subsets of neurons and sperm cells at certain stages of differentiation) appear to lack MHC class I molecules altogether.

However, nucleated cells without MHC class I expression are very rare. Non nucleated cells, such as red blood cells in mammals, do not generally express any MHC molecules, making them poor targets for T cells.

In normal, healthy cells, MHC class I molecules on the surface of the cell will display self peptides resulting from normal turnover of self-proteins inside the cell.
In cells infected with a virus, viral peptides as well as self-peptides will be displayed.

Therefore, a single virus-infected cell can be envisioned as having various class I molecules on its membrane, some displaying a subset of viral peptides derived from the viral proteins being manufactured within.

Because of individual allelic differences in the peptide-binding grooves of the MHC class I molecules, different individuals within a species will have the ability to bind and present different sets of viral peptides (like displaying different pieces of a jigsaw puzzle that represents the whole of that pathogen).

In addition to virally infected cells, altered self-cells such as cancer cells, aging body cells, or cells from an allogeneic graft (tissue from a genetically different individual), also can serve as target cells because of their expression of defective or foreign MHC proteins, and can be lysed by T cells.

The importance of constitutive expression of class I is highlighted by the response of NK cells to somatic cells that lack MHC class I, as can occur during some viral infections.

NK cells can kill a cell that has stopped expressing MHC class I on its surface, presumably because this suggests that the cell is no longer healthy or has been altered by the presence of an intracellular invader.

Expression of MHC Class II molecules is primarily restricted to antigen-presenting cells

MHC class II molecules are found on a much more restricted set of cells than class I, and sometimes only after an inducing event.

As mentioned previously, antigen-presenting cells (APCs) display peptides associated with MHC class II molecules to CD4 T cells, and these cells are primarily specific types of leukocytes. APCs are specialized for their ability to alert the immune system to the presence of an invader and can induce the activation of T-cell responses.

APCs thus play a policing role in the body, with unique authorization to activate an immune response to extracellular infection, as needed.

A variety of cells can function as bona fide APCs. Their distinguishing feature is their ability to express MHC class II molecules and to deliver a costimulatory, or second activating signal, to T cells.

Three cell types are known to have these characteristics and are thus often referred to as **professional antigen-presenting cells (pAPCs):** dendritic cells, macrophages, and B lymphocytes.

These cells differ from one another in their mechanisms of antigen uptake, in whether they constitutively express MHC class II molecules, and in their costimulatory activity, as follows:

Dendritic cells are considered the most powerful and most efficient of the pAPCs. These cells constitutively express high levels of MHC class II molecules and have inherent costimulatory activity, allowing them to quickly activate naïve T cells.

Macrophages must be activated before they express MHC class II molecules or costimulatory membrane molecules such as CD80/86.

B cells constitutively express MHC class II molecules, although at low levels, and possess antigen-specific surface receptors. This makes them particularly efficient at capturing and presenting their cognate antigen, or the specific epitope recognized by their BCR.

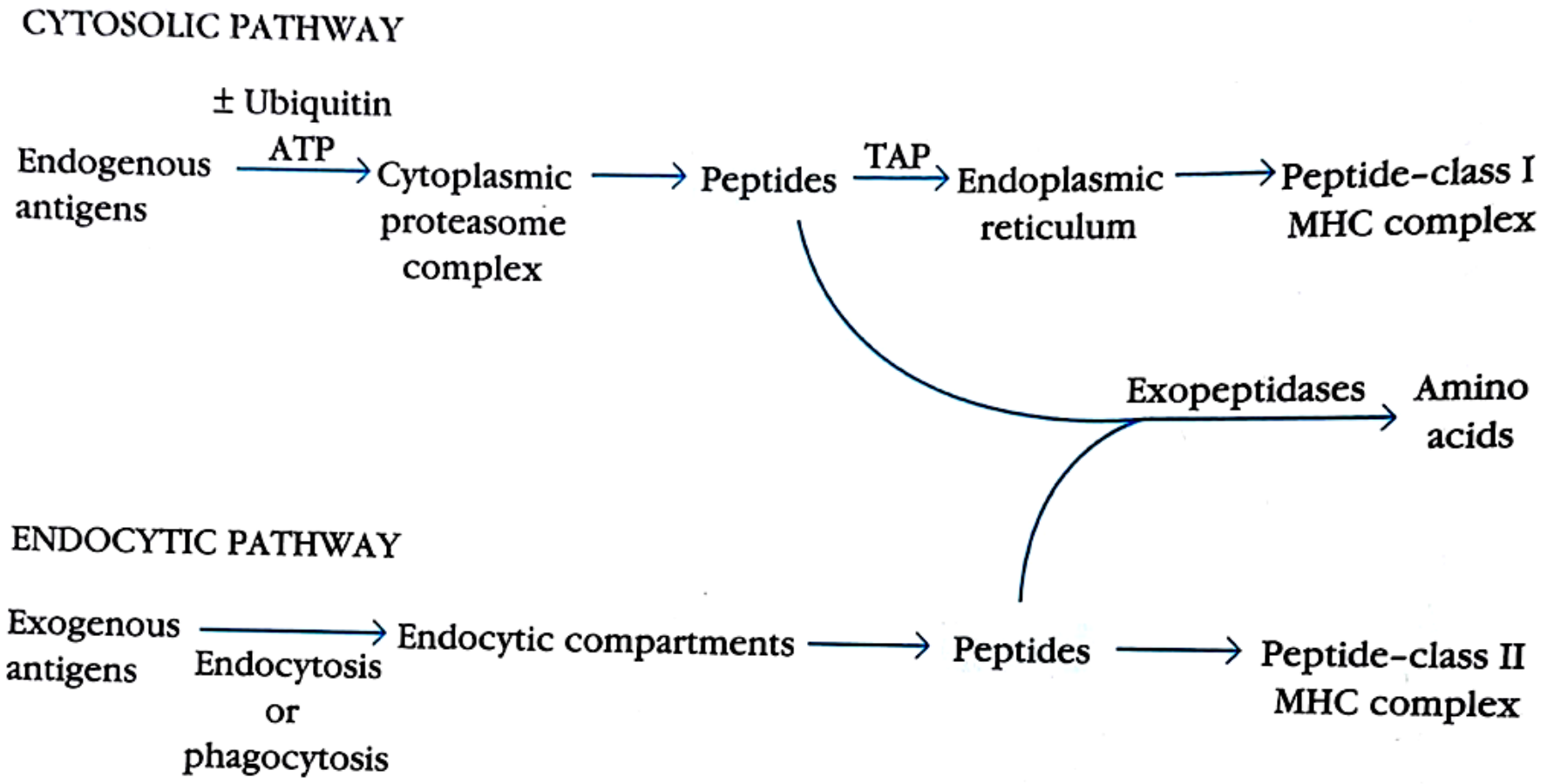
Antigen presenting cells

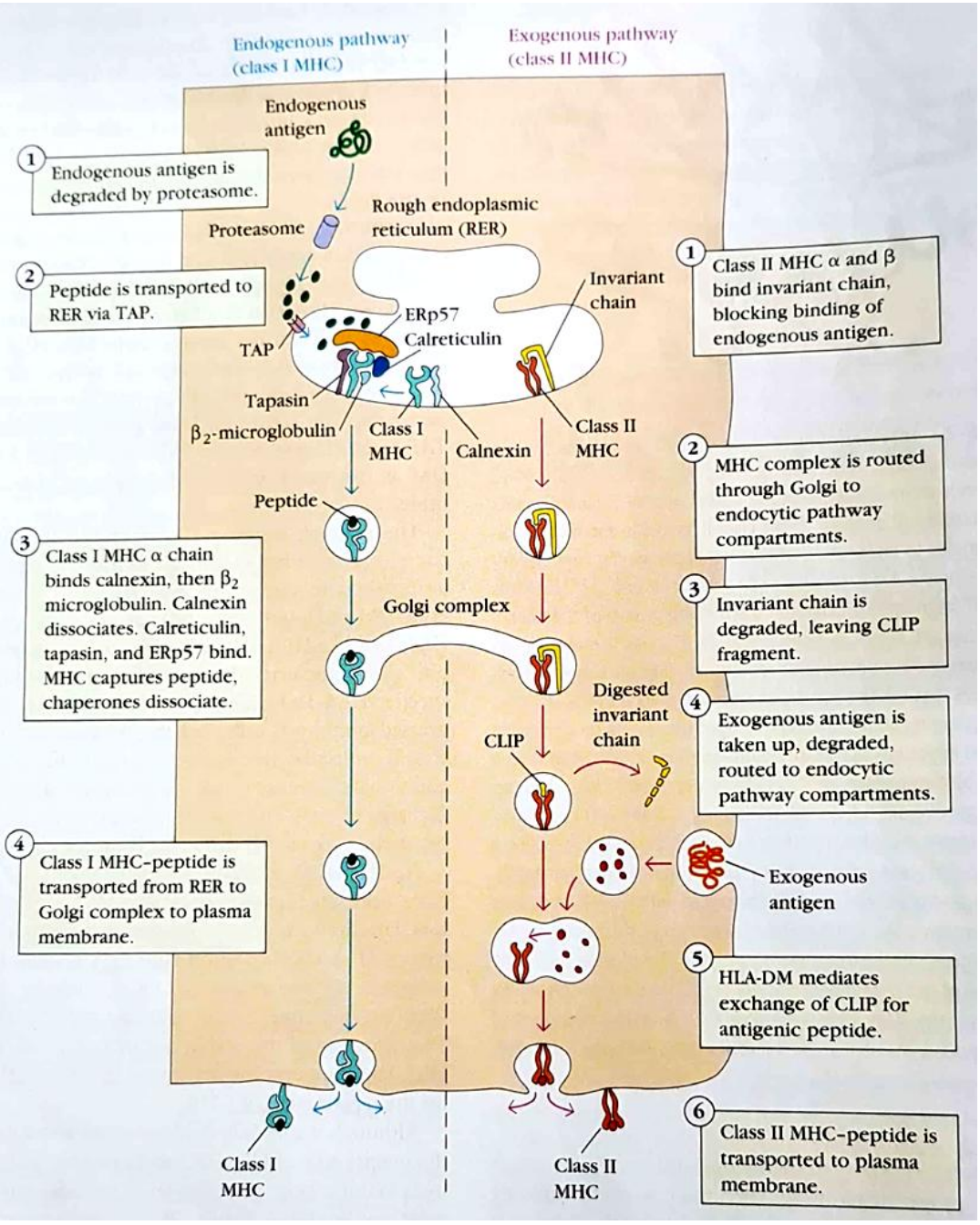
Professional antigen presenting cells	Non professional Antigen presenting cells
Dendritic cells	Fibroblasts (skin)
Macrophages	Glial cells (Brain)
B Cells	Pancreatic beta cells
	Thymic epithelial cells
	Thyroid epithelial cells
	Vascular endothelial cells

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

As a general rule, **endogenous antigens** (those generated within the cell) are processed in the cytosolic or endogenous pathway and presented on the membrane with class I MHC molecules.

Exogenous antigens (those taken up from the extracellular environment by endocytosis) are typically processed in the exogenous pathway and presented on the membrane with class II MHC molecules .


CYTOSOLIC PATHWAY
± Ubiquitin
ATP
Endogenous antigens
Cytoplasmic proteasome complex
Peptides
TAP
Endoplasmic reticulum
Peptide–class I MHC complex
Exopeptidases
Amino acids
ENDOCYTIC PATHWAY
Exogenous antigens
Endocytosis or phagocytosis
Endocytic compartments
Peptides
Peptide–class II MHC complex



Overview of endogenous and exogenous pathways for processing antigen.

In the endogenous pathway (left), antigens in the cytosol are degraded by the proteasome, converting proteins into smaller peptides.

In the exogenous pathway (right), extracellular antigens are engulfed into endocytic compartments, where they are degraded by acidic pH–dependent endosomal and lysosomal enzymes.

The antigenic peptides from proteasome cleavage and those from endocytic compartments associate with MHC class I or II molecules, respectively, and the MHC-peptide complexes are then transported to the cell membrane.

Ultimate fate of most peptides in the cell is neither of these pathways; rather, most are degraded completely into amino acids.

The endogenous pathway of antigen processing and presentation in eukaryotic cells, protein levels are carefully regulated.

Every protein is subject to continuous turnover and is degraded at a rate that is generally expressed in terms of its half-life. Some proteins (e.g., transcription factors, cyclins, and key metabolic enzymes) have very short half-lives.

Denatured, misfolded, or otherwise abnormal proteins also are degraded rapidly. Defective ribosomal products are polypeptides that are synthesized with imperfections and constitute a large part of the products that are rapidly degraded.

The average half-life for cellular proteins is about 2 days, but many are degraded within 10 minutes. The consequence of steady turnover of both normal and defective proteins is a constant deluge of degradation products within a cell. Most will be degraded to their constituent amino acids and recycled, but some persist in the cytosol as peptides.

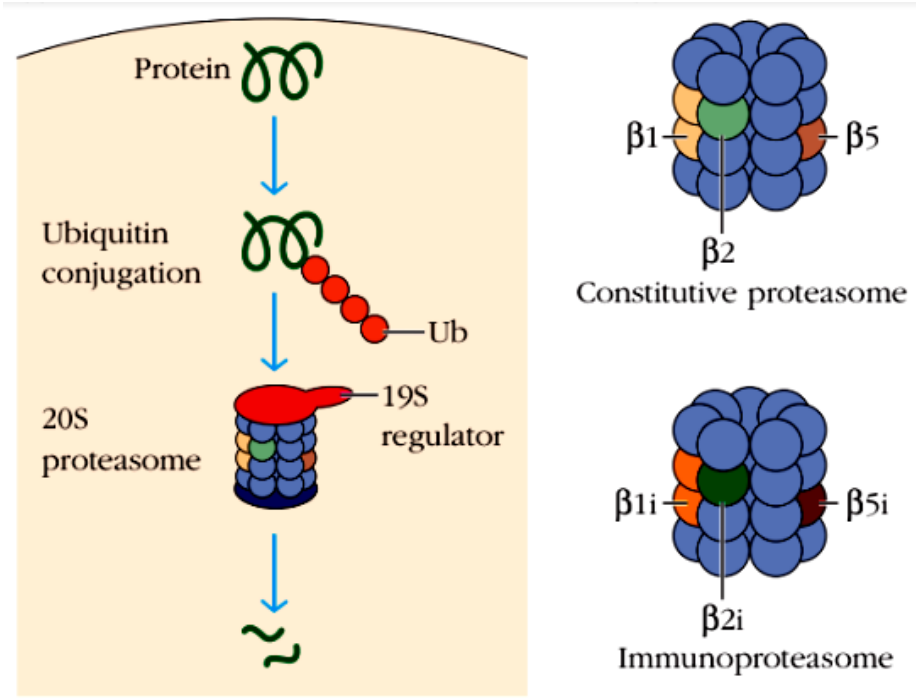
The cell samples these peptides and presents some on the plasma membrane in association with class I MHC molecules, where cells of the immune system can sample these peptides to survey for foreign proteins.

The pathway by which these endogenous peptides are generated for presentation with class I MHC molecules utilizes mechanisms similar to those involved in the normal turnover of intracellular proteins.

Peptides are generated by protease complexes called proteasomes

Intracellular proteins are degraded into short peptides by the proteasome, a cytosolic proteolytic system present in all cells . The large (20S) proteasome is composed of multiple α and β subunits arranged in concentric rings; the α subunits make up the top and bottom rings while the β subunits construct the middle two rings. There are a total of 14 β subunits arrayed in this barrel-like structure of symmetrical rings.

many proteins are targeted for proteolysis when a small protein called ubiquitin is attached to them. These ubiquitin-protein conjugates enter the proteasome complex, consisting of the 20S base and an attached 19S regulatory component, through a narrow channel at the 19S end. The proteasome complex cleaves peptide bonds in an ATP-dependent process. Degradation of ubiquitin-protein complexes is thought to occur within the central hollow core of the proteasome.



Cytosolic proteolytic system for degradation of intracellular proteins. (a) Endogenous proteins may be targeted for degradation by ubiquitin conjugation. These proteins are degraded by the 26S proteasome complex, which includes the 20S constitutive proteasome and a 19S regulator. (b) In activated APCs, several proteins in the constitutive proteasome (β 1, β 2, and β 5) are replaced by proteins encoded by the LMP genes and specific to the immunoproteasome (β 1i, β 2i, and β 5i). This immunoproteasome has increased proteolytic efficiency for creating peptides that can assemble with MHC class I molecules.

The immune system adds a twist to the general pathway of protein degradation to specifically produce small peptides optimized for binding to MHC class I molecules.

In addition to the standard 20S proteasomes resident in all cells, a distinct proteasome of the same size, the immunoproteasome, can be found in APCs and some infected cells.

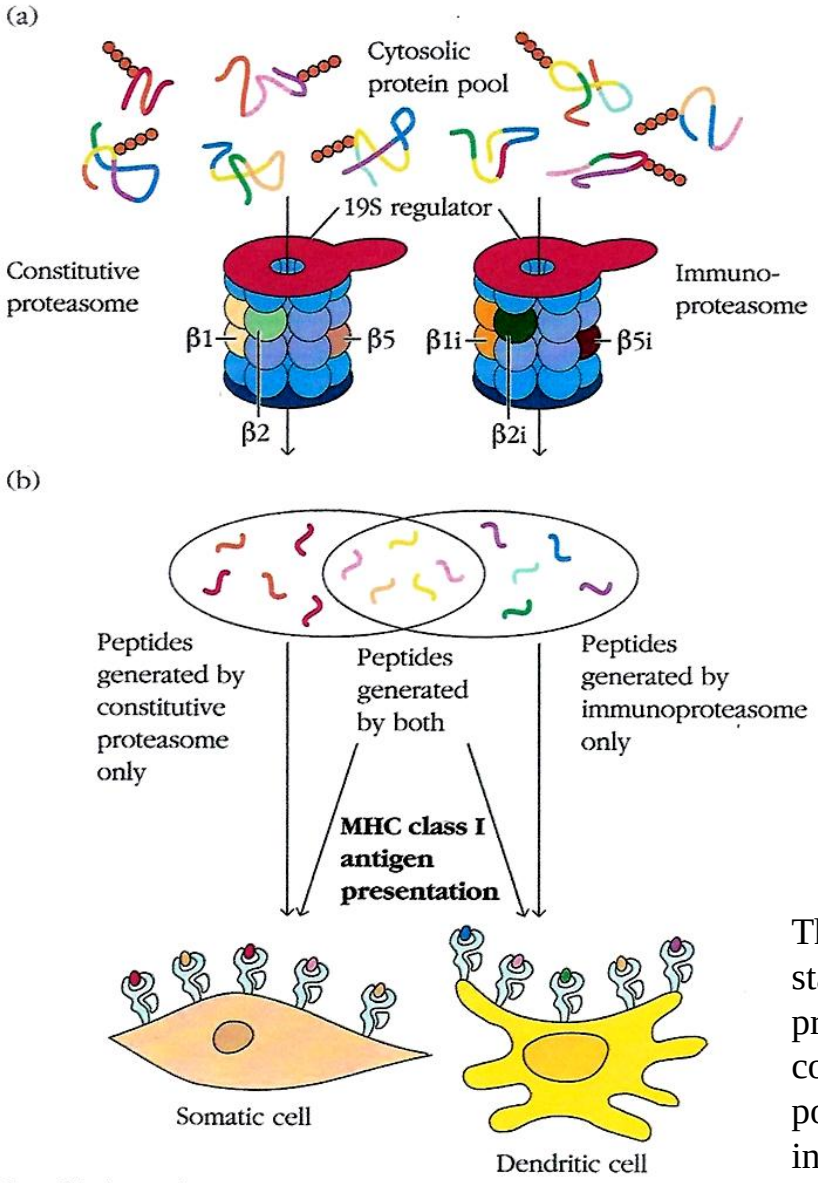
It has the same basic structure as the traditional proteasome with some unique subunit substitutions. In most cells, these new subunits are not constitutively expressed like the other components of the proteasome but are induced by exposure to certain cytokines, such as IFN-γ or TNF. LMP2 and LMP7, genes that are located within the class II region and are responsive to these cytokines, encode replacement catalytic protein subunits that convert standard proteasomes into immunoproteasomes, increasing the efficiency with which cytosolic proteins are cleaved into peptide fragments that specifically bind to MHC class I molecules.

In fact, a subset of the peptides created in the presence of immunoproteosomes is not found in cells lacking these structures.

The half life of an immunoproteasome is shorter than that of a standard proteasome, possibly because the increased level of protein degradation in its presence may have negative consequences beyond the targeting of infected cells.

It is possible that in some cases autoimmunity results from increased processing of self-proteins in cells with high levels of immunoproteasomes.

Proteolytic system for degradation of intracellular proteins.



(a) Endogenous proteins in all cells are targeted for degradation by ubiquitin conjugation. These proteins are degraded by the 26S proteasome complex, which includes the 20S constitutive proteasome and a 19S regulator, generating a pool of peptides for MHC class I loading.

In activated APCs, several proteins in the constitutive proteasome (β1, β2, and β5) are replaced by proteins encoded by the LMP genes (β1i, β2i, and β5i).

(b) This generates an immunoproteasome with increased efficiency for creating unique peptides with a proclivity for assembly with MHC class I molecules

The half life of an immunoproteasome is shorter than that of a standard proteasome, possibly because the increased level of protein degradation in its presence may have negative consequences beyond the targeting of infected cells. It is possible that in some cases autoimmunity results from increased processing of self-proteins in cells with high levels of immunoproteasomes.

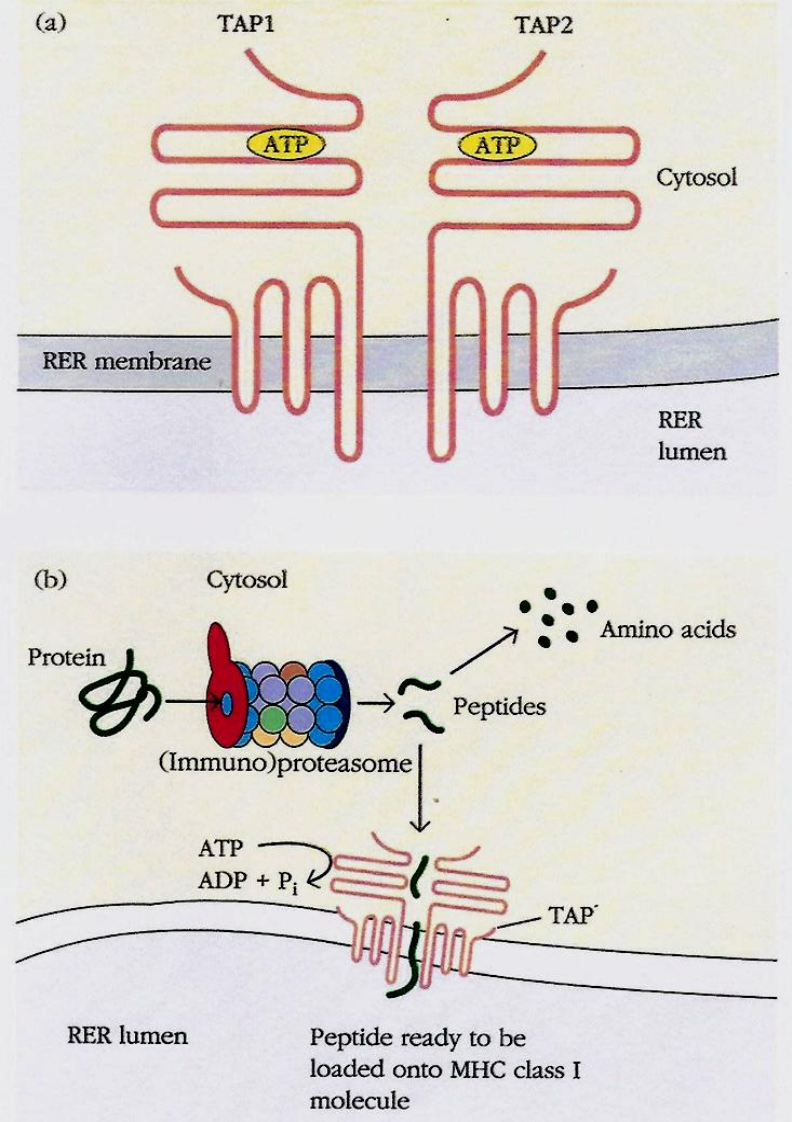
Peptides are transported from the cytosol to the rough endoplasmic reticulum

The transporter protein, designated TAP (transporter associated with antigen processing), is an ER-membrane-spanning heterodimer consisting of two proteins: TAP1 and TAP2. In addition to their 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. This family of proteins mediates ATP-dependent transport of amino acids, sugars, ions, and peptides across membranes.

Peptides generated in the cytosol by the proteasome are translocated by TAP into the RER by a process that requires the hydrolysis of ATP . TAP has affinity for peptides containing **8 to 16 amino acids**. The optimal peptide length for final association with MHC class I is **nine** amino acids.

It was later discovered that longer peptides can also bind but are trimmed by enzymes present in the lumen of the ER, such as ERAP (endoplasmic reticulum aminopeptidase).
In addition, TAP appears to favor peptides with hydrophobic or basic carboxyl-terminal amino acids, the preferred anchor residues for MHC class I molecules .

Thus, TAP is preoptimized to transport peptides that are likely to interact with MHC class I molecules.



TAP(transporter associated with antigen processing), a heterodimer anchored in the membrane of rough endoplasmic membrane (RER). Cytosolic domain contains an ATP binding site and peptide transport depends on hydrolysis of ATP.

In the cytosol, ubiquitinated proteins are directed to a constitutive or immunoproteasome, where they are digested into peptide fragments. These peptides are translocated by TAP into the RER lumen, where, in a process mediated by several other proteins, they will associate with MHC class I molecules.

Chaperones aid peptide assembly with MHC class I molecules

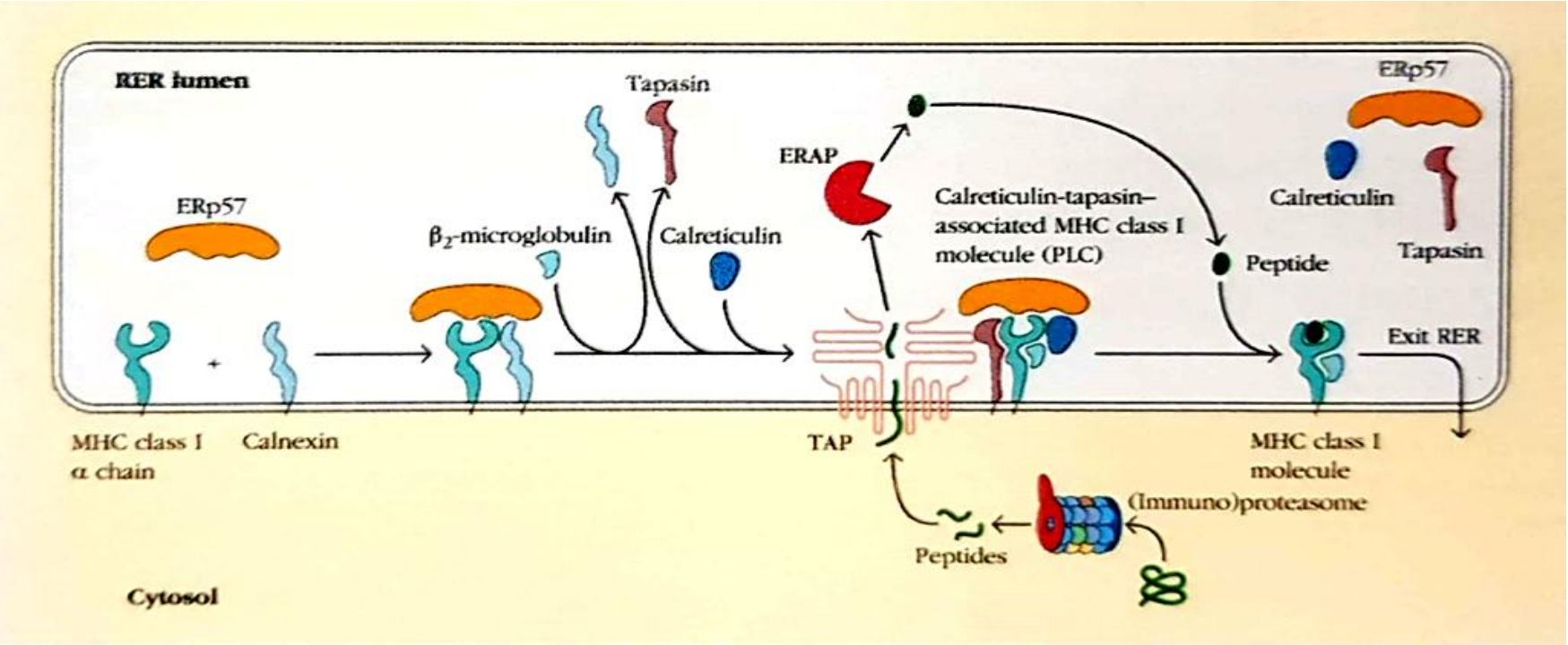
Like other proteins destined for the plasma membrane, the α chain and β -microglobulin components of the MHC class I molecule are synthesized on ribosomes on the RER. Assembly of these components into a stable MHC class I molecular complex that can exit the RER requires the presence of a peptide in the binding groove of the class I molecule. The assembly process involves several steps and includes the participation of **molecular chaperones** that facilitate the folding of polypeptides.

The first molecular chaperone involved in MHC class I assembly is **calnexin**, a resident membrane protein of the ER. ERp57, a protein with enzymatic activity, and calnexin associate with the class I α chain and promote its folding .

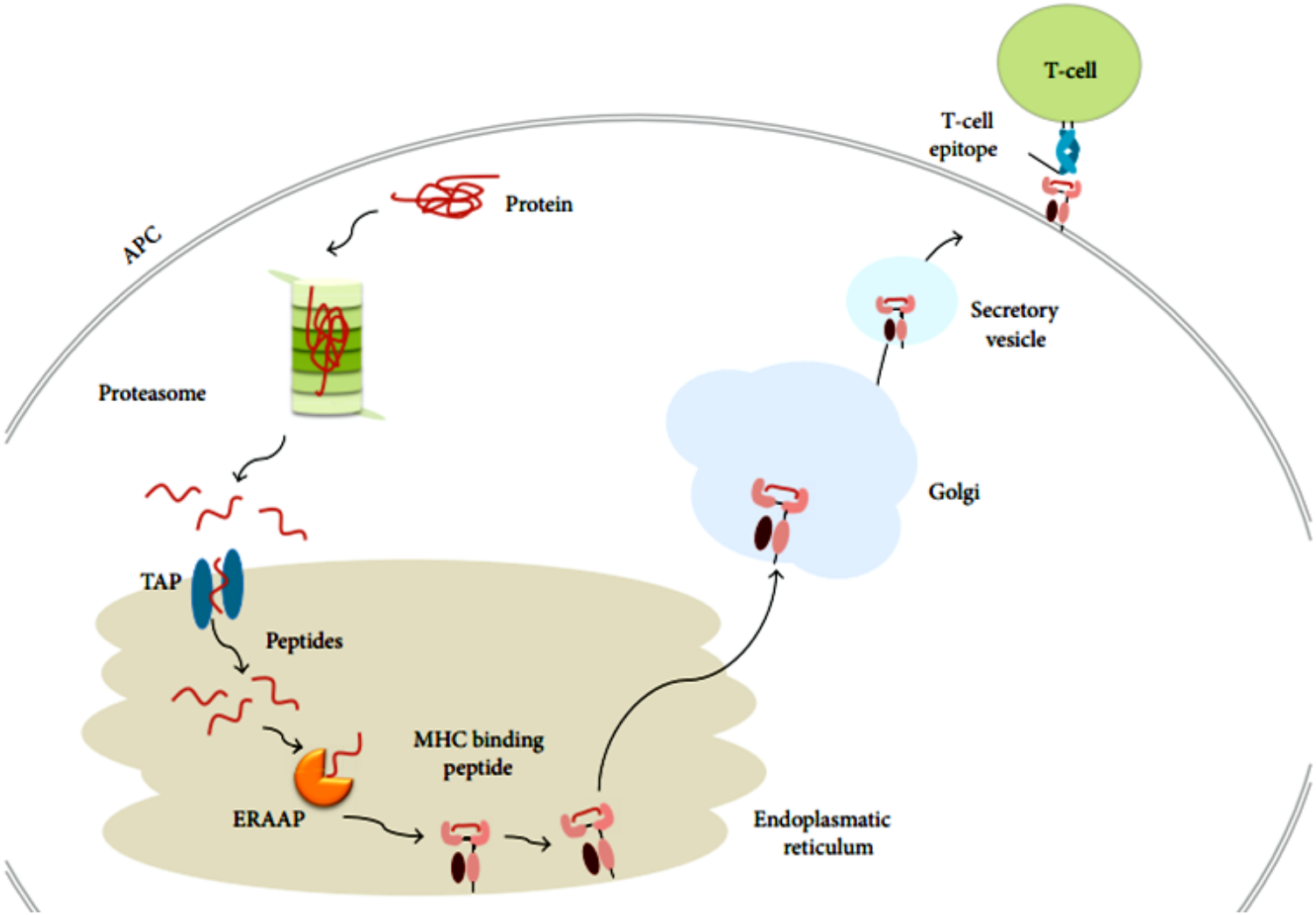
When β -microglobulin binds to the α chain, calnexin is released and the **class I-ERp57 complex** associates with the chaperones **calreticulin** and **tapasin**.
Tapasin (TAP-associated protein) brings the TAP transporter into proximity with the class I molecule and allows it to acquire an antigenic peptide. The TAP protein then promotes peptide capture by the class I molecule before the peptides are exposed to the luminal environment of the RER.

The tapasin-related protein, **TAPBPR** (for TAP-binding protein related), was found to bind MHC class I molecules much like tapasin does. However, TAPBPR appears to be more than just an analogue of tapasin.

The class I molecule can then exit from the RER and proceed to the cell surface via the Golgi complex.

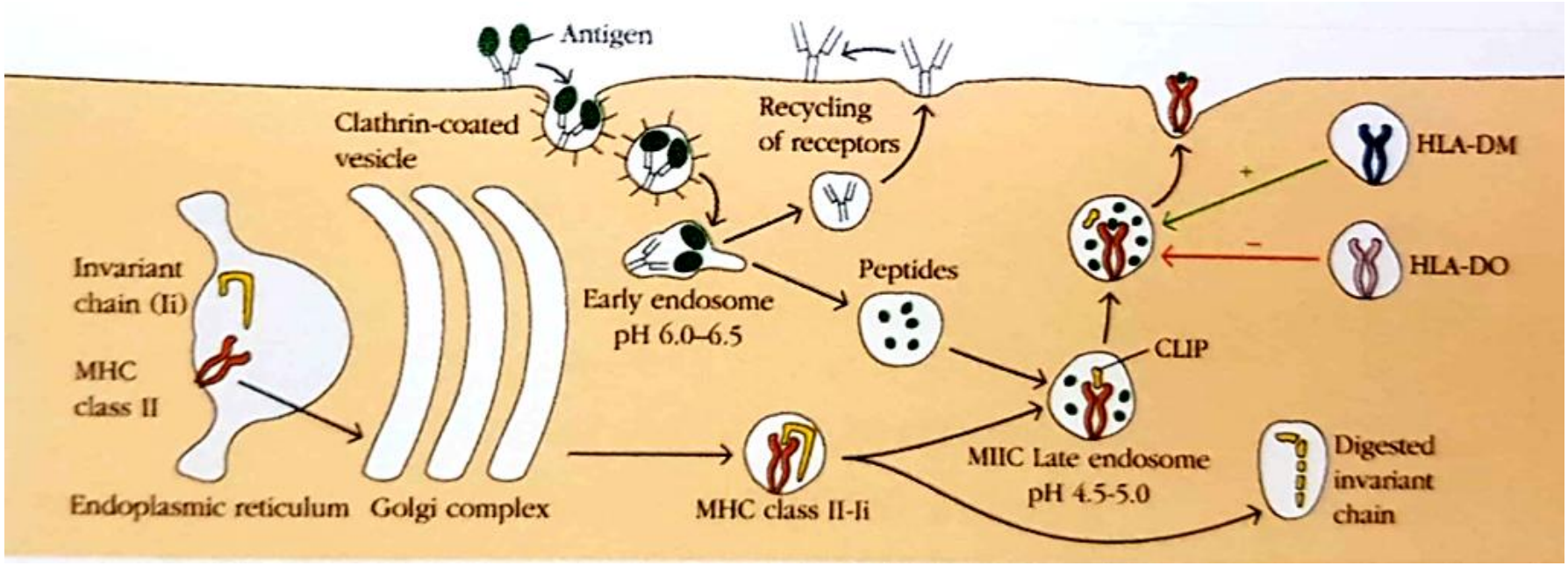


Assembly and stabilization of MHC class I molecules.
Within the rough endoplasmic reticulum (RER) membrane, a newly synthesized class I α chain associates with calnexin (a molecular chaperone) and ERp57 until β -microglobulin binds to the α chain. The binding of β -microglobulin releases calnexin and allows binding to calreticulin and to tapasin, which is associated with the peptide transporter TAP.
This association creates a protein loading complex (PLC) that promotes binding of an antigenic peptide. Antigens in the RER can be further processed via amino peptidases such as ERAP (endoplasmic reticulum amino pepdidase), producing fragments ideally suited for binding to class I. Peptide association stabilizes the class I molecule–peptide complex, allowing it to be transported from the RER to the plasma membrane.



Class I antigen processing. The figure depicts the major steps involved in antigen presentation by MHC I molecules. Proteins are degraded by the proteasome and peptide fragments transported to the endoplasmic reticulum (ER) by TAP where they are loaded onto nascent MHC I molecules. TAP transports peptides ranging from 8 to 16 amino acids. Long peptides cannot bind MHC I molecules but often become suitable for binding after N-terminal trimming by ERAP.

The exogenous pathway of antigen processing and presentation



Generation of antigenic peptides and assembly of MHC class II molecules in the exogenous processing pathway. In the cell shown here, a B cell, exogenous antigen is internalized by receptor-mediated endocytosis (top left), with the membranebound antibody functioning as an antigen-specific receptor. Internalized antigen moves through several acidic compartments ending in specialized MIIC late endosomes, where it is degraded into peptide fragments. Within the rough endoplasmic reticulum (bottom left), a newly synthesized MHC class II molecule binds an invariant chain. The bound invariant chain prevents premature binding of peptides to the class II molecule and helps to direct the complex to endocytic compartments containing processed peptides derived from exogenous antigens. Digestion of the invariant chain leaves CLIP, a small fragment remaining in the binding groove of the MHC class II molecule. HLA-DM, a nonclassical MHC class II molecule present within the MIIC compartment, mediates exchange of antigenic peptides for CLIP. The nonclassical class II molecule 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.

MICC=MHC class II-containing compartment

Exogenous Antigens: The endocytic pathway

Once an antigen is internalized, it is degraded into peptides within compartments of the endocytic processing pathway. internalized antigen takes 1 to 3 hours to traverse the endocytic pathway and appear at the cell surface in the form of class II MHC-peptide complexes.

1. Three increasingly acidic compartments are involved.
   - Early endosome(pH6.0-6.5)
   - Late endosome or endolysosomes (pH 5.0-6.0)
   - Lysosome (pH 4.5-5.0)

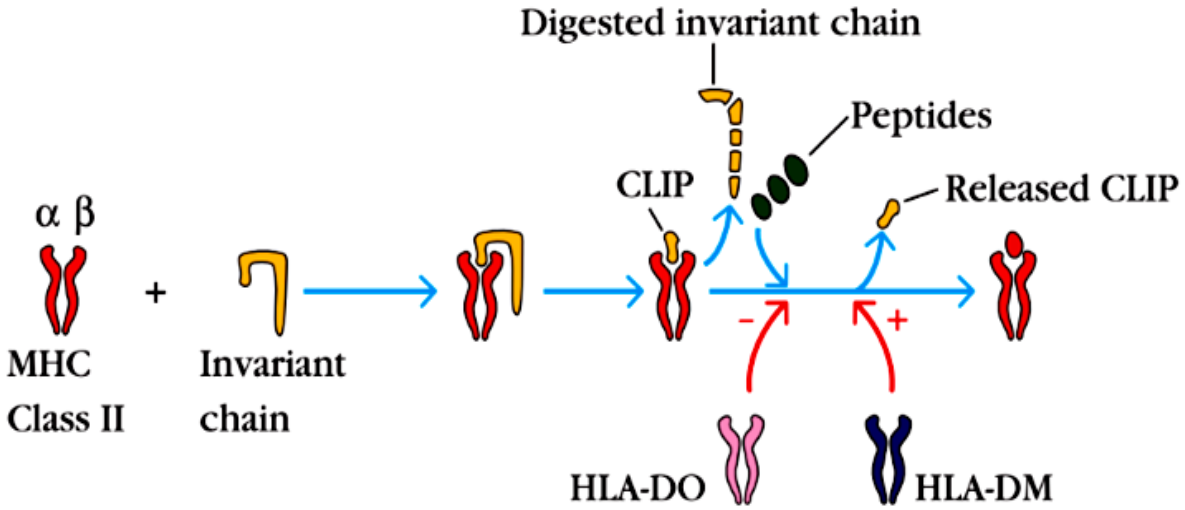
2. Internalized antigen progresses through these compartments, encountering hydrolytic enzymes and a lower pH in each compartment . Antigens are exposed to hydrolytic enzymes of lysosome which contains more than 40 acid-dependent hydrolases (proteases, nucleases, glycosidases, lipases, phospholipases and phosphatases).

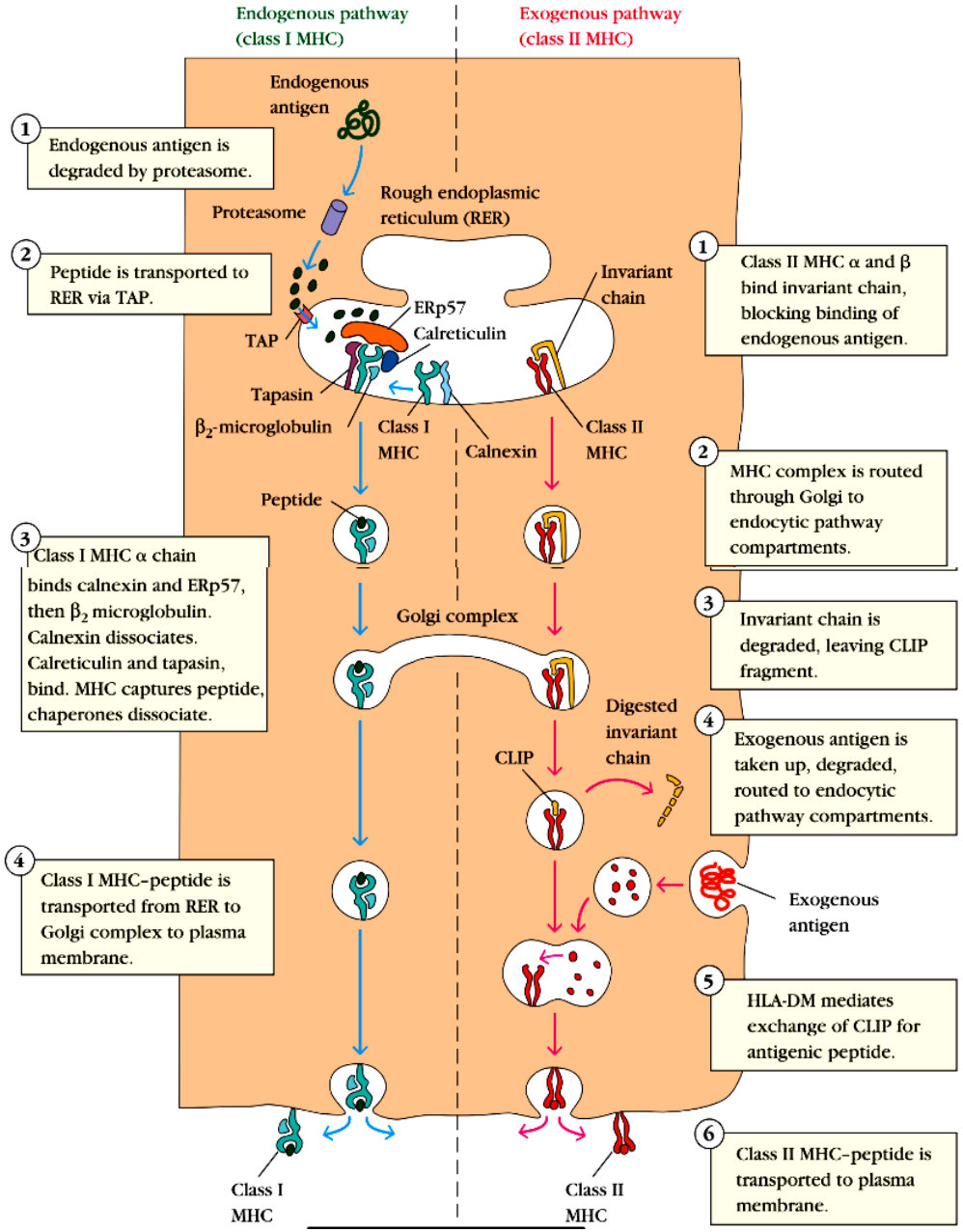
3. Antigen-presenting cells have a unique form of late endosome, the MHC class II-containing compartment (**MIIC**), in which final protein degradation and peptide loading into MHC class II proteins occurs.

4. Within the compartments of the endocytic pathway, antigen is degraded into oligopeptides of about 13 to 18 residues that meet up with and bind to class II MHC molecules in late endosomes.

5. It is suggested that early endosomes from the periphery move inward to become late endosome and finally lysosome. Small transport vesicles carry antigens from one compartment to the next and finally fuse with cell membrane.

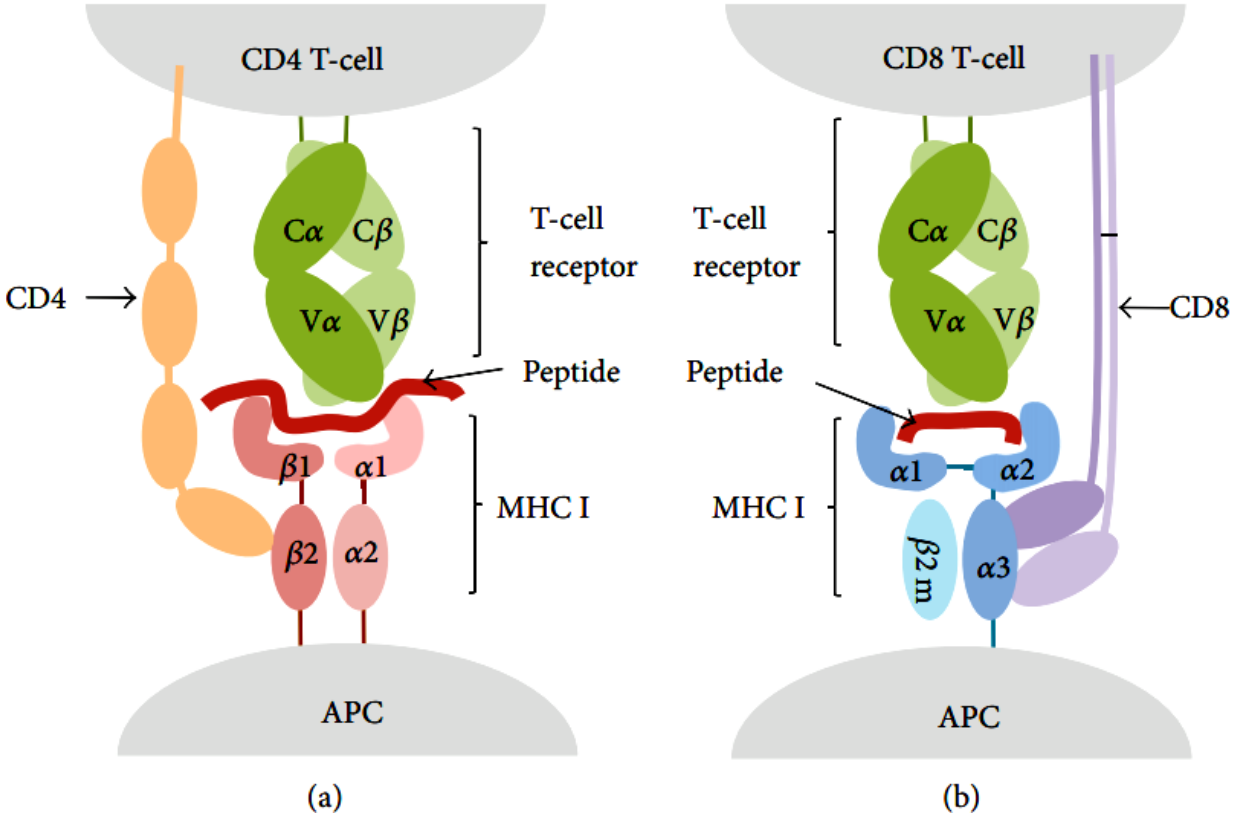
1. Within the rough endoplasmic reticulum, a newly synthesized class II MHC molecule binds an invariant chain. The invarient chain is involved in folding of α and β chains .The bound invariant chain prevents premature binding of peptides to the cleft of class II molecule and helps to direct the complex to endocytic compartments containing peptides derived from exogenous antigens.
2. The invarient chain contains sorting signals in its cytoplasmic tail that directs transport of MHC from trans-Golgi network to endocytic compartments.
3. As proteolytic activity increases in each compartment the varient chain is gradually degraded. Digestion of the invariant chain leaves CLIP (class II associated invarient chain peptide), a small fragment remaining in the binding groove of the class II MHC molecule
4. HLA-DM, a nonclassical MHC class II molecule present within the MIIC (MHC class II-containing compartment compartment,) mediates exchange of antigenic peptides for CLIP. The nonclassical class II molecule 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.



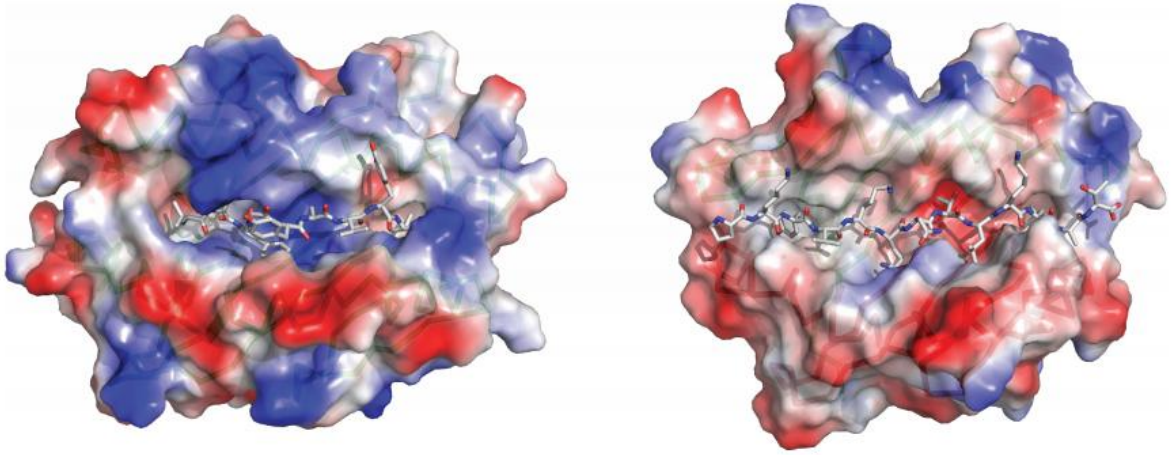


Separate antigen-presenting pathways are utilized for endogenous (green) and exogenous (red) antigens.

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.



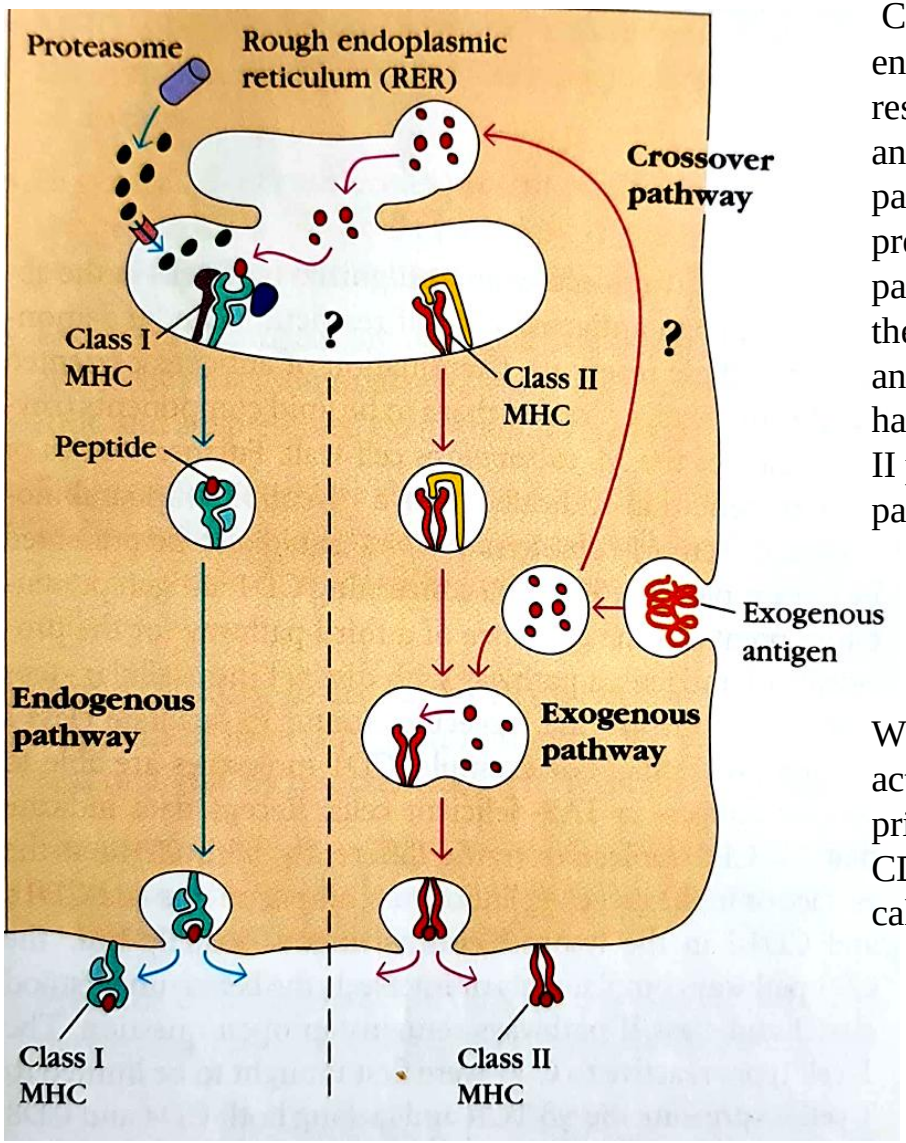
T-cell epitope recognition. T-cell epitopes are peptides derived from antigens and recognized by the T-cell receptor (TCR) when bound to MHC molecules displayed on the cell surface of APCs.
(a) CD4 T-cells express the CD4 coreceptor, which binds to MHC II, and recognize peptides presented by MHC II molecules.
(b) CD8 T-cells express the CD8 coreceptor, which binds to MHC I, and recognize peptides presented by MHC I molecules.



MHC molecule binding groove.
The figure depicts the molecular surface as seen by the TCR of representative MHC I and II molecules.
Binding groove of the MHC I molecule is closed but that of MHC II is open. As a result, MHC I molecules bind short peptides (8–11 amino acids), while MHC II molecules bind longer peptides (9–22 amino acids).

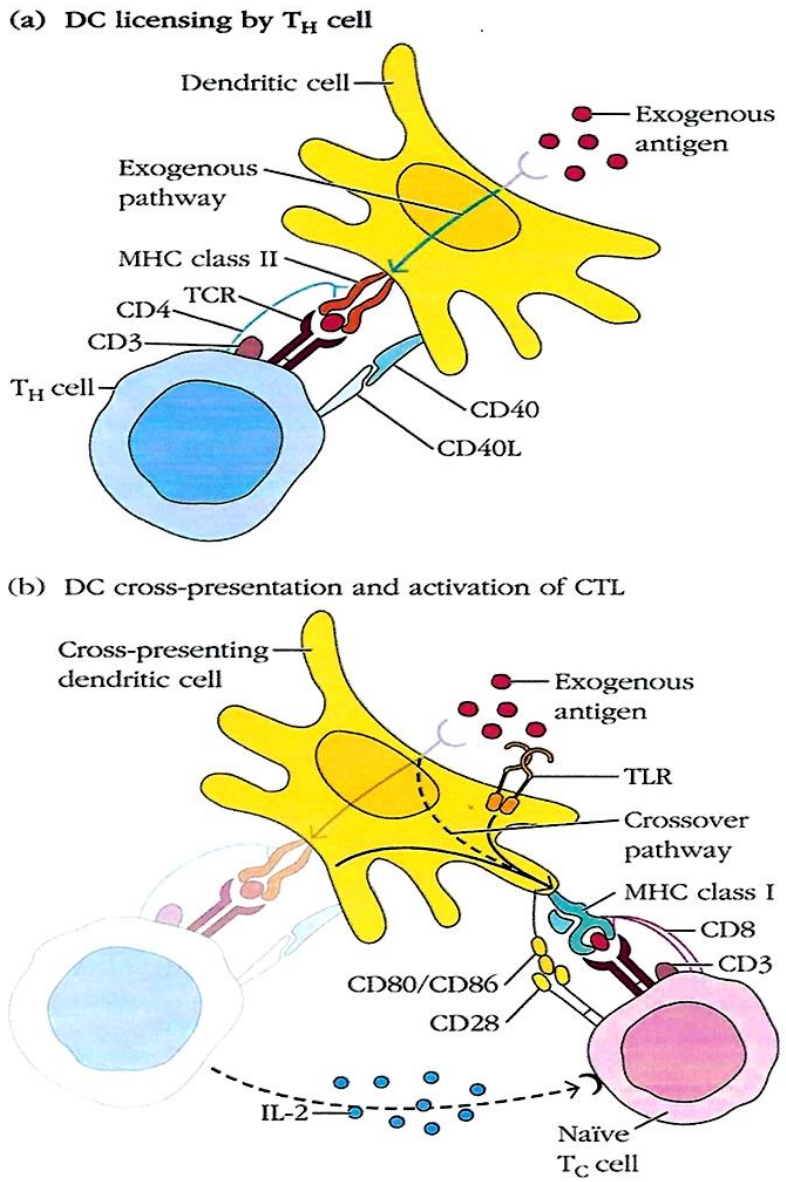
Cross-presentation is the process by which primary antigen presenting cells (APCs), primarily denditic cells (DCs) that have been activated and licensed by antigen-specific T cells, divert exogenously acquired antigen into MHC class I molecules (crossing endogenous and exogenous pathways), allowing activation of CD8 T cells; the responses of these CD8 T cells are responsible for destroying other virally infected target cells that express this same peptide-MHC combination.

In some cases, exogenous antigens internalized by dendritic cells can gain access to the endogenous presentation pathway in a process called cross-presentation, leading to peptide association with MHC class I and engagement with CD8 T cells.



Cross-presentation is blending the exogenous and endogenous pathways in a process that is still being fully resolved. In some instances certain APCs will divert antigen obtained by endocytosis (exogenous antigen) to a pathway that leads to MHC class I loading and peptide presentation to CD8 T cells (like in the endogenous pathway)—in other words, crossing the two pathways. the phenomenon of cross presentation requires that antigens acquired from extracellular sources, normally handled by the exogenous pathway leading to MHC class II presentation, are redirected to a class I peptide loading pathway.

When this form of antigen presentation leads to the activation of a naïve CD8 T cell it is referred to as cross-priming; when it leads to the induction of tolerance in a CD8 T cell, such as when the APCs are not activated, it is called cross-tolerance.



Cross Presentation (DC licensing)

(a) Dendritic cells (DCs) first internalize and process antigen through the exogenous pathway, presenting antigen to CD4 T cells via MHC class II molecules and activating these cells through, among other things, CD40-CD40L engagement.

(b) These activated T cells can then serve as a bridge to help activate CTL responses; they provide local IL-2 to help activate the CTL and they also license the DCs to cross-present internalized antigen in MHC class I, up-regulate costimulatory molecules, and down-regulate their inhibitory counterparts. DC licensing creates an ideal situation for the stimulation of antigen-specific CD8 T-cell responses. When the TLRs (toll like receptors) on these DCs are engaged, this further activates these cells, providing added encouragement for cross-presentation. The dashed arrow indicates antigen directed for cross-presentation.

Presentation of nonpeptide antigens

Some nonprotein antigens are also recognized by T cells. As early as the 1980s, T-cell proliferation was detected in the presence of nonprotein antigens derived from infectious agents. Mycolic acid derived from pathogens such as *Mycobacterium tuberculosis* is one classic example.
These and other small lipid-containing antigens are presented by a small group of structurally similar proteins encoded outside the MHC locus: a group of nonclassical class I molecules, including the CD1 family of proteins and the MHC class I–related protein (MR1).

CD1 and MR1 molecules share structural similarity with classical MHC class I but have more of a functional overlap with MHC class II.

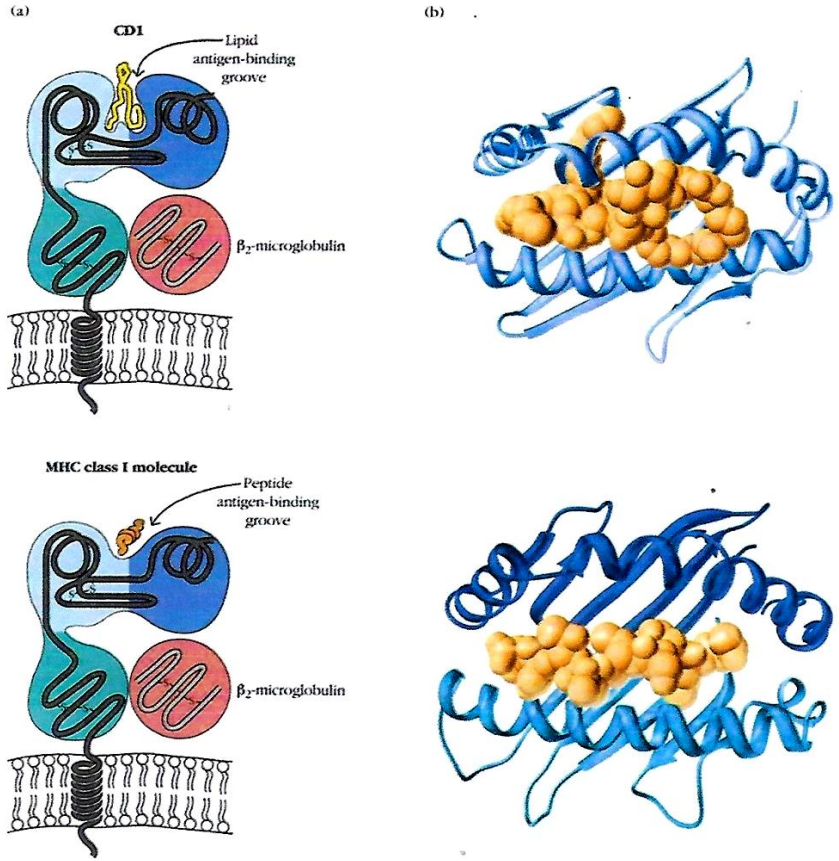
Five human CD1 genes and one MR1 gene have been identified. Most of the proteins encoded by these genes form a transmembrane heavy chain composed of three extracellular α domains, and associate noncovalently with β -microglobulin, much like classical MHC class I.

However, unlike classical MHC molecules, CD1 and MR1 display very limited polymorphism.

In terms of trafficking and expression profile, CD1 and MR1 molecules resemble MHC class II proteins, moving intracellularly to endosomal compartments, where they associate with exogenous antigen.
Like MHC class II molecules, these nonclassical class I proteins are expressed by many immune cell types, including thymocytes, B cells, and DCs, although some members of the family have also been found on hepatocytes and epithelial cells

Non peptide antigens such as lipid and glycolipid derived from Bacteria involves the class I-like CD1 molecules.

The ligands for CD1 and MR1 include several different lipids or lipid-linked molecules, small molecules, and metabolites of vitamin B .
Unlike their peptide counterparts, these moieties fit into deep pockets within the CD1/MR1 binding groove. Crystal structures have demonstrated that CD1 contains a binding groove that is both deeper and narrower than that of classical MHC molecules, and is lined with nonpolar amino acids that can easily accommodate hydrophobic structures. This means that antigen binds to CD1 and MR1 via a deep groove through a narrow opening—like a foot sliding into a shoe.

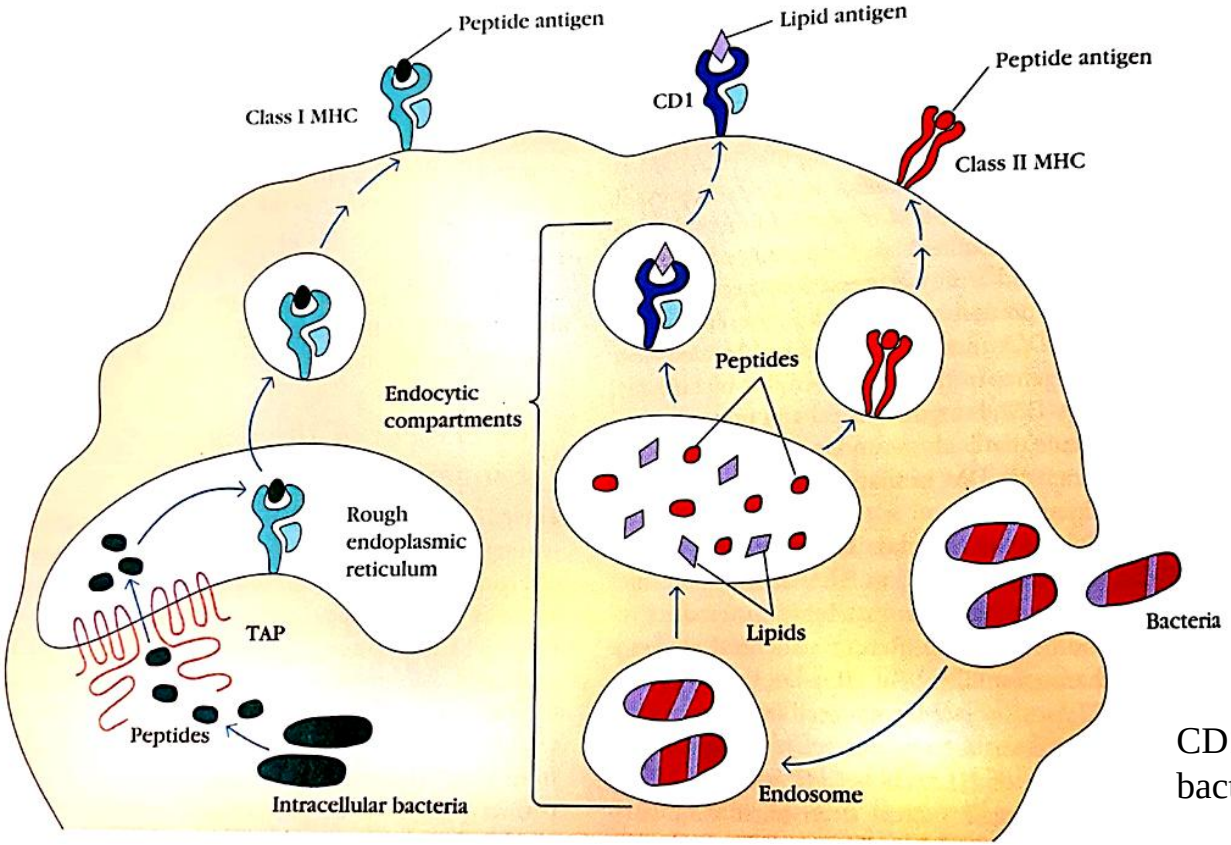


Lipid antigen binding to the CD1 molecule.
(a) Schematic of the deep binding pockets that accommodate lipid antigens in a foot-in-shoe fashion in the members of the CD1 family of nonclassical class I molecules (top), compared with the shallower peptide-binding groove of classical MHC class I (bottom).

(b) Ribbon diagram of the binding groove or pocket of human CD1b complexed with lipid (top) compared with a classical class I molecule binding peptide antigen (bottom).

Non classical MHC molecules like CD1 and MR1 share structural similarity with class I but function more like class II, presenting non protein (lipid and small-molecule) antigens to αβ and γδ T cells. CD1 and MR1 have limited variability, and serve to regulate immune homoeostasis as well as control some infectious agents at mucosal surfaces.

Natural killer (NK) T cells, many skin and mucosal γδ T cells, and T cells responsible for recognizing Mycobacterium tuberculosis recognize CD1 molecules presenting lipid antigens. These and other "invariant" T cells are abundant in the body, especially in mucosal tissues, where they play a long-standing evolutionary role.



CD1 molecule presenting bacterial lipid antigen