MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

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History

- In the mid 1930s, the concept of histocompatibility antigens originated from the work of Peter Gorer, who observed that the rejection of foreign tissue is the result of an immune response to cell-surface molecules.
- During the 1940s, Medawar and his colleagues demonstrated that tissue graft rejection in rabbits was indeed due to an immune response attacking the foreign tissue graft.

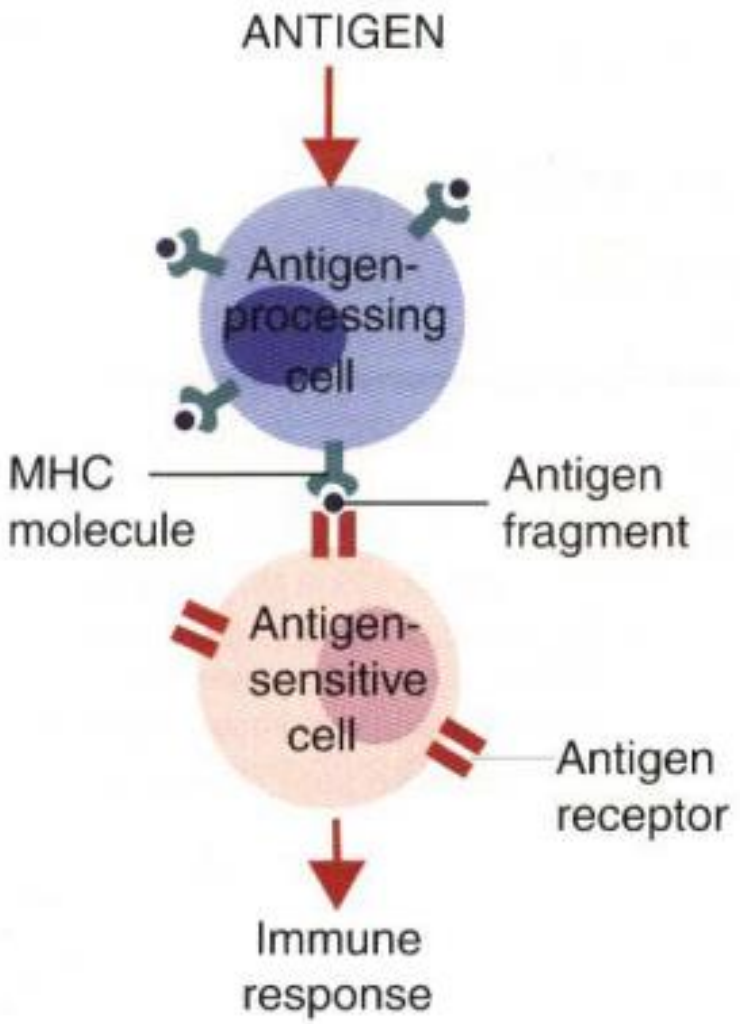
History

- In the 1940s and 1950s by Gorer and George Snell established that antigens encoded by the genes in the group designated II took part in the rejection of transplanted tumors and other tissue.
- Snell called the genes controlling tissue rejection 'histocompatibility (H) genes", In 1980 Snell was awarded the Nobel prize for this work

Histocompatibility Molecules

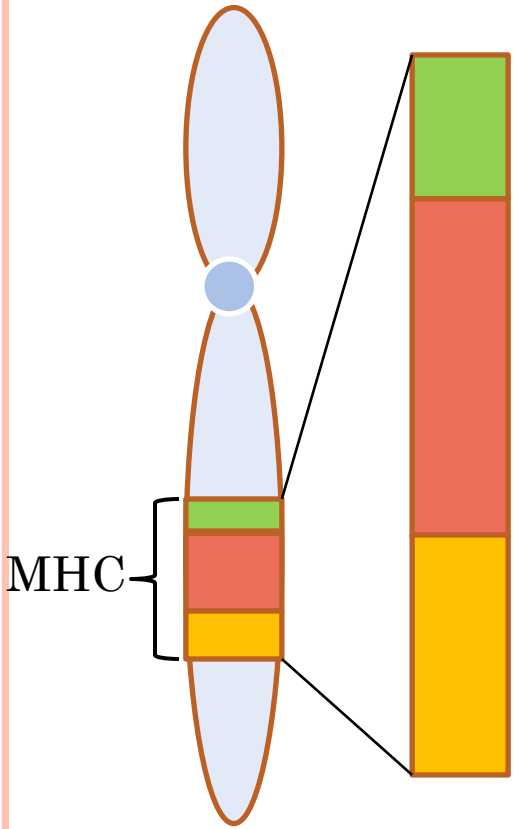
- Antigen presentation:
  - In order to trigger acquired immunity, antigens phagocytosed by cells are degraded.
  - The degraded antigens are presented by these cells on plasma membrane for recognition by T-cells.
- But for this antigen presentation, these antigen fragments are bound to antigen-presenting receptors called **Histocompatibility molecules**.
- Antigens can trigger immune response only when they are bound to these histocompatibility molecules.

- Histocompatibility molecules are glycoproteins encoded by genes belonging to a gene cluster known as **Major Histocompatibility Complex (MHC).**
- Therefore these receptors are also called **MHC Molecules**.
- This gene complex can be very large.
- for example, the human MHC is about 04mb in size, which is about the same size as the total genome of the bacterium *E. coli*



Make up of MHC Gene Cluster

- Three classes of gene loci are found:



Loci I : Code for MHC molecules found on **all nucleated cells**.

subclasses Ia, Ib, Ic and Id.
Ia is highly polymorphic.
Present antigen to cytotoxic T cells.
T cell mediated toxicity.

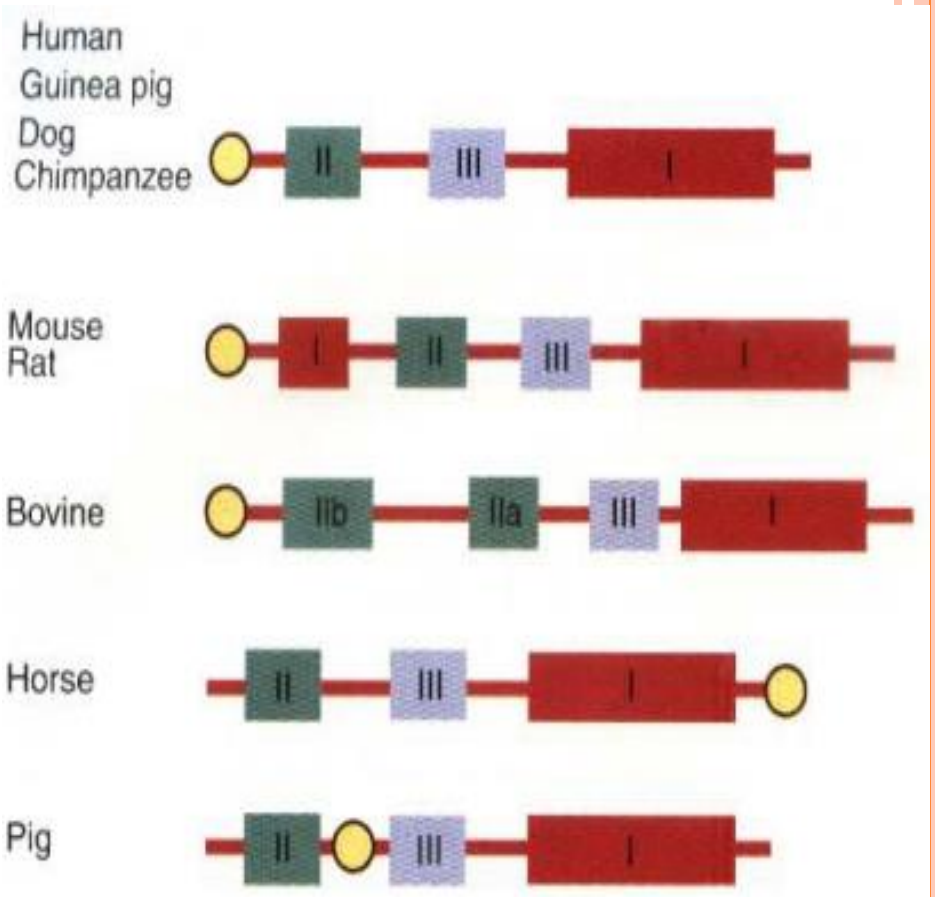
Loci II : Code for MHC found on antigen presenting cells.

Present antigen to helper T cells.
T cell mediated stimulation/help.

Loci III : Code for proteins with diverse functions. Ex. Some complement proteins C2, C4, Factor B, Cytokine TNF-α, some heat shock proteins etc.

- Each MHC contains all three classes of loci, although their name, number and arrangement vary with different species for example
- Human- HLA (6), Dog- DLA (12), Horse- ELA (20), Cattle- BoLA(23), Pig-SLA (7)

- The complete set of alleles found within an animal's MHC is called its MHC Haplotype.



Structure of Class I MHC molecule

- Class I MHC are Glycoproteins expressed on all nucleated cells, for example, in pigs detected on lymphocytes, platelets, granulocyte, hepatocyte, kidney cells and sperms.
- Usually not found on mammalian red cells, gametes, neurons or tropholast cells
- Structure: Made up of two glycoprotein chains
  - One 45 kD α chain, and
  - Second small 12 kD β2 microglobulin (β2 M) chain.
- α chain consists of 5 domains.
- α1 and α2 domains form the antigen binding site.
- β 2 chain stabilize the structure.

- The alpha chain is attached to the cell where as the beta chain is not attached to the cell and is bound non-covalently with the alpha chain.
- There are four discrete domains in a MHC I molecules
  i. External polypeptide binding domain
  ii. Immunoglobulin like domain
  iii. Transmembrane domain
  iv. Cytoplasmic domain
- The external polypeptide-binding domain is made up of 180 amino acids with two homologous segments called as alpha 1 and alpha 2 segments.
- This two segments form a cleft like structure with approximate dimension of (25Å x 10 Å x 11 Å).

- This cleft can take a polypeptide of 9-11 amino acid (epitope) and presents to T cells.
- The immunoglobulin like domain is made up of 90 amino acids and have disulphide bonds bound a loop.
- The amino acids in this region are highly conserved and no variations are found between different MHC I molecules.
- This domain is also named as alpha3 domain and is responsible for binding with CD8 receptor.
- The remaining transmembrane domain and cytoplasmic domain help in anchoring the MHC I molecules to cell surface.

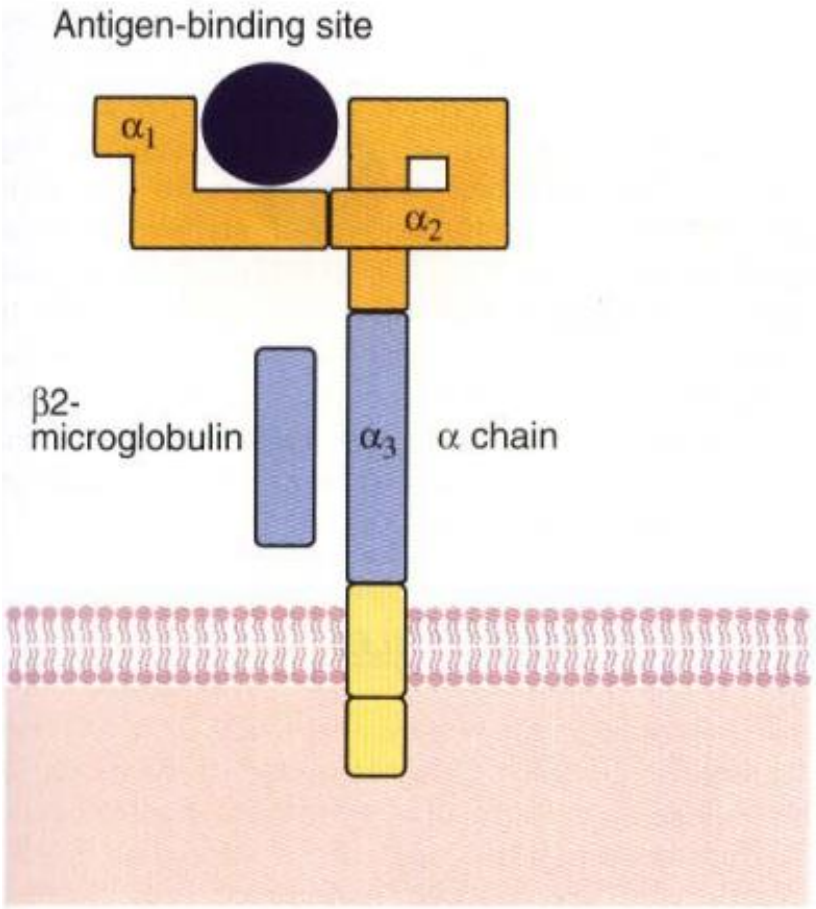


Figure 7-3. Diagram showing the structure of a class Ia MHC molecule on a cell membrane. Its antigen-binding site is formed by the folding of its α_1 and α_2 domains.

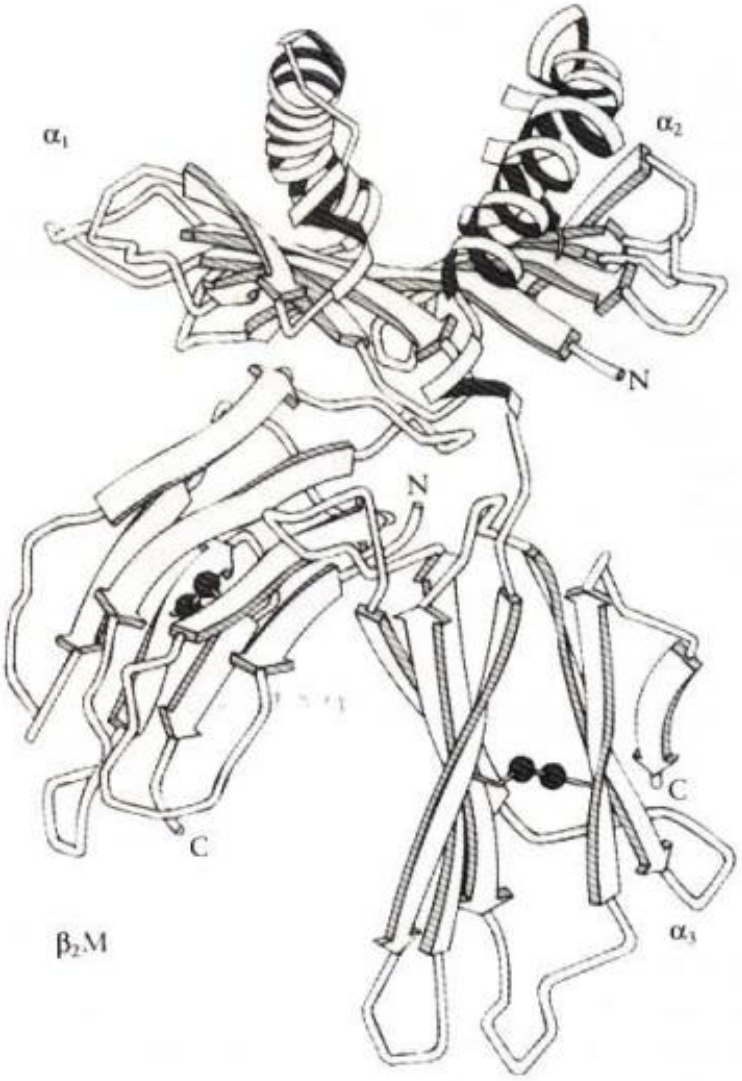


Figure 7-5. Schematic three-dimensional view of the complete structure of HLA-A2 derived by x-ray crystallography. The antigen-binding groove at the top is formed by the α_1 and α_2 domains, whereas the α_3 domain binds to the cell membrane. The β chain (β_2-microglobulin) has no direct role in antigen binding. (From *Nature* 320:506, 1987. Macmillan Magazines Ltd.)

Structure of Class I MHC molecule

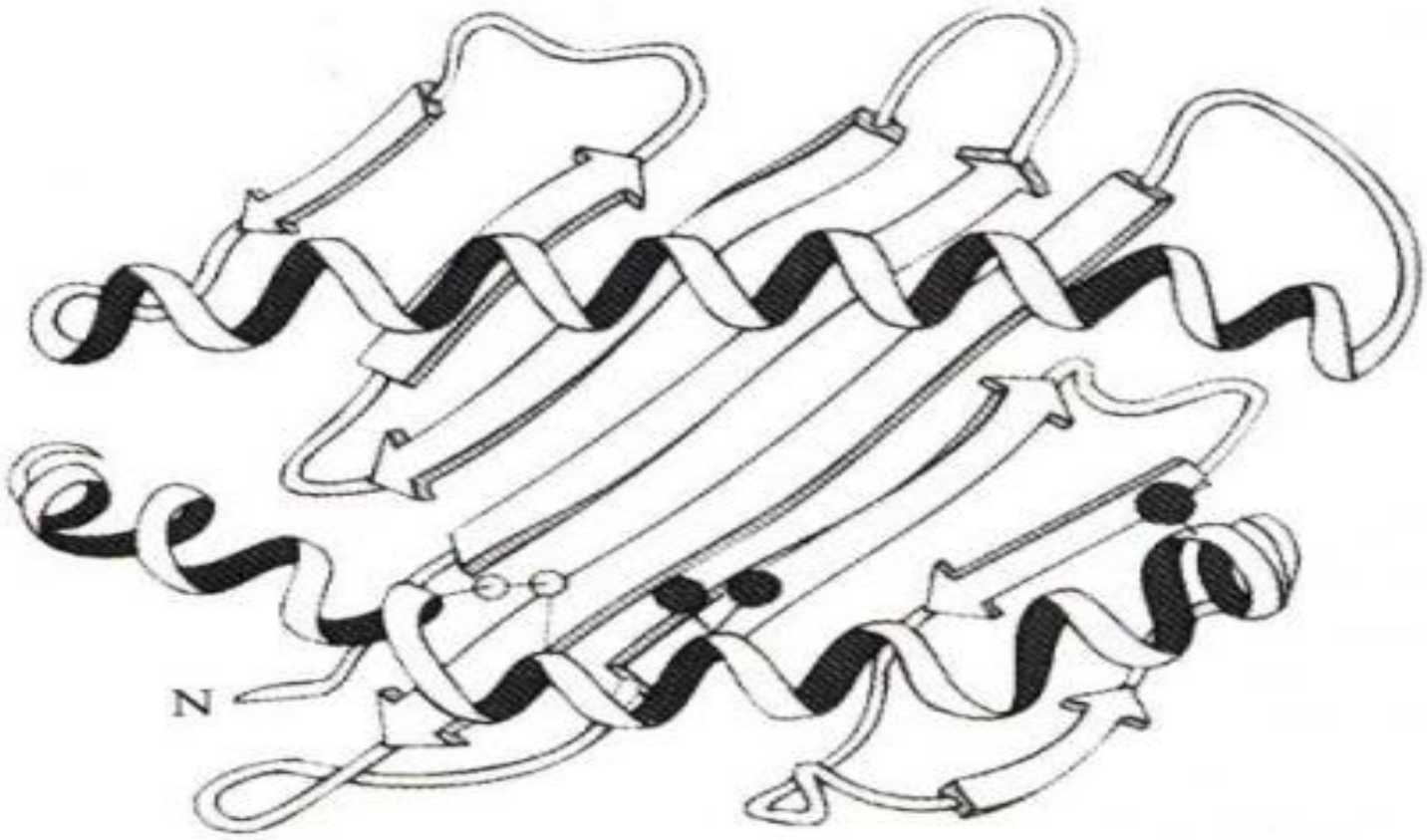
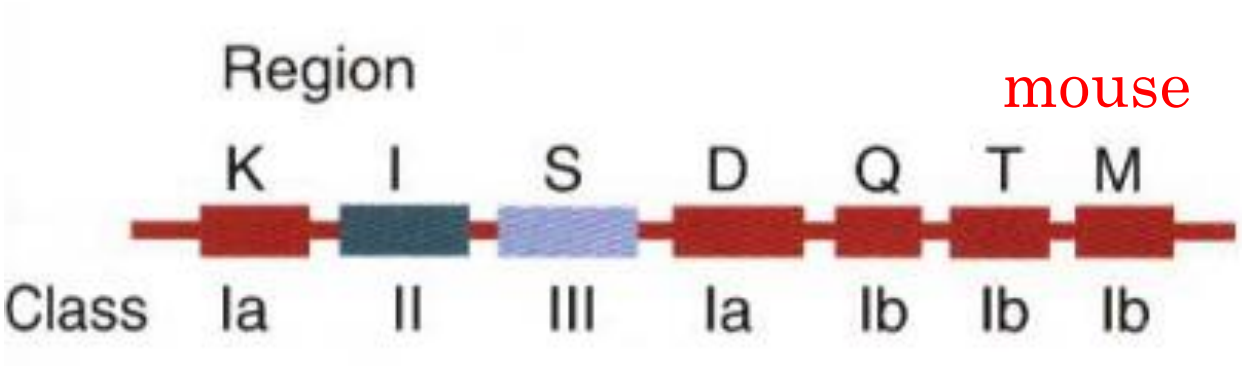


Figure 7-6. A view (from above) of the antigen-binding groove on a MHC class I molecule. The floor of the groove is formed by an extensive β-pleated sheet. The walls of the groove are formed by two parallel α helices. This structure is formed by the folding of the α_1 and α_2 domain of the α chain. (From *Nature* 320:506, 1987. Macmillan Magazines Ltd.)

Gene arrangement

- The total number of class I loci varies greatly between mammals.
- Rats have more than 60, mice 30, human 20, cattle 13 to 15 and pig have 11 but not all these loci as functional and code for cell surface protein.
- For example mice only two or three class Ia gene are expressed.
- In humans the functional loci are called *A, B, C* and in mice they called *K* and *D* (some strains, *L*)

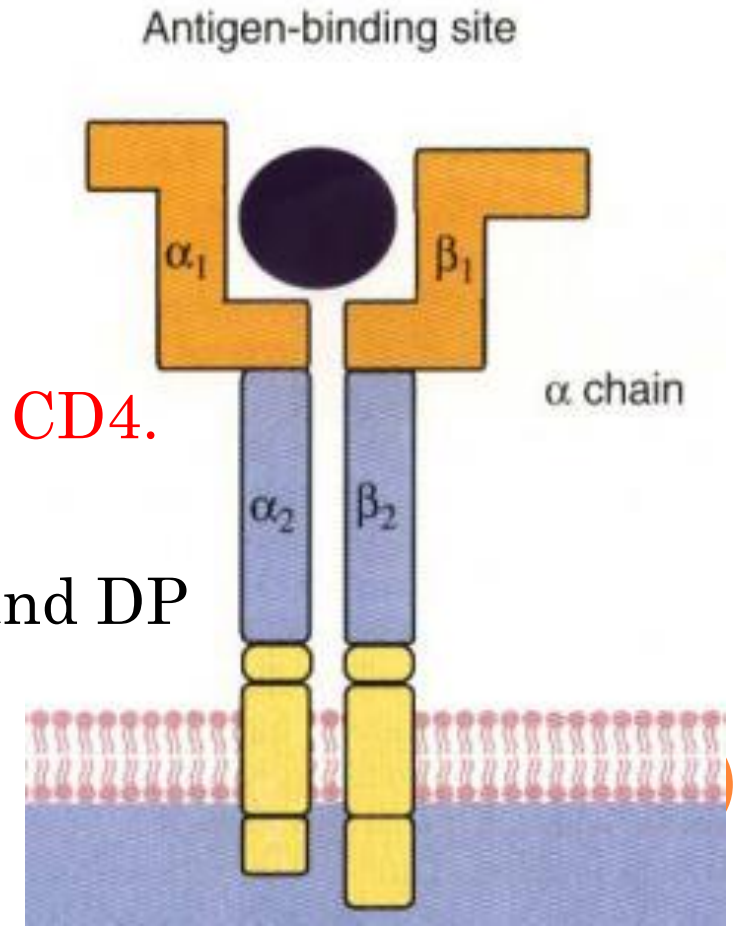


Polymorphism in Class I MHC molecule

- Some of the class Ia gene loci code have a large number of alleles, these allelic differences cause variations in the amino acid sequence of the α1 and α2. This variation is called polymorphism.
- These variable regions are restricted to three to four discrete regions within the α1 and α2 domains while other domains of MHC class Ia molecules are highly conserved and not show evident sequence variation.
- This nucleotide sequence variability in MHC alleles result of
- Point mutation,
- Reciprocal recombination and
- Gene conversion

Structure of Class II MHC molecule

- Class II MHC molecule encode glycoproteins expressed primarily on antigen- presenting cells (macrophages, dendritic cells and B cells) where they present processed antigenic peptides to T_H cells.
- Two protein chains – α and ß.
- α chain = 31 to 34 kDa
- ß chain = 25 to 29 kDa
- Third protein chain = Ii or γ
- The β2 is the binding site for the CD4.
- The α chains are the HLA - DR
- The β chains are the HLA - DQ and DP

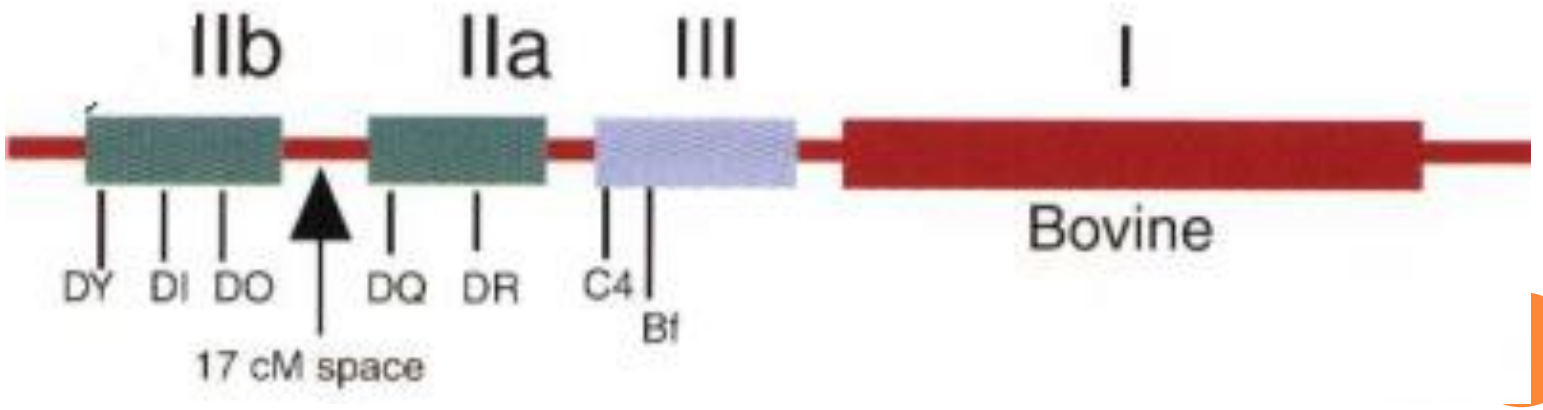


- The alpha chain is slightly larger than the beta chain. As in MHC I molecule the MHC II molecules also contain four discrete domains
  i. External polypeptide binding domain
  ii. Immunoglobulin like domain
  iii. Transmembrane domain
  iv. Cytoplasmic domain
- The external polypeptide binding site of the MHC II molecule is made up 90 amino acids and is formed by interaction of part of alpha and beta chains (alpha 1 and beta 1).
- The immunoglobulin like domain is made up of 90 amino acids and have disulphide bonds bound a loop. This domain is formed by part of alpha and beta chains (alpha 2 and beta 2).

- The amino acids in this region are highly conserved and no variations are found between different MHC II molecules and are responsible for binding with CD4 receptor.
- The remaining transmembrane domain and cytoplasmic domain help in anchoring the MHC II molecules to cell surface.

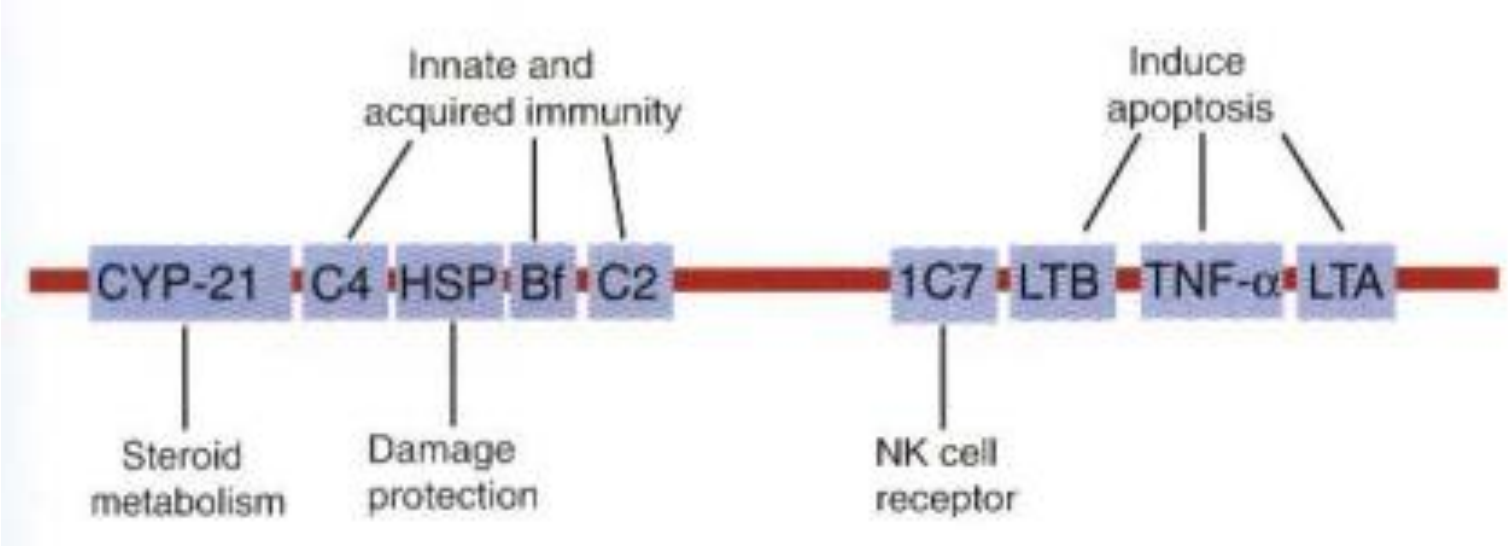
Gene arrangement

- Within the MHC class II region there are six loci arranged in order DP, DOA, DM, DOB, DQ and DR
- Not all loci contain genes for both chain and some contain many pseudogenes. These pseudogenes may used to generate additional class II polymorphisms
- While nonpolymorphic loci such as DM and DO, whose function is to regulate the loading of antigen fragments into the groove



MHC Class III molecules

- They code for proteins with many different functions Such as four genes for complement components: two for C4 and one each for factor B and C2
- Code for the enzyme 21- hydroxylase involved in steroid synthesis, for cytochrome P450, for tumor necrosis factor α, for several lymphotoxins, for some NK cell receptors and for several heat- shock proteins (HSP)



- In mice, MHC is called H2 complex. Present on chromosome 17.

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

- In humans, it is called Human Leukocyte Antigen (HLA) Complex, present on chromosome 6.

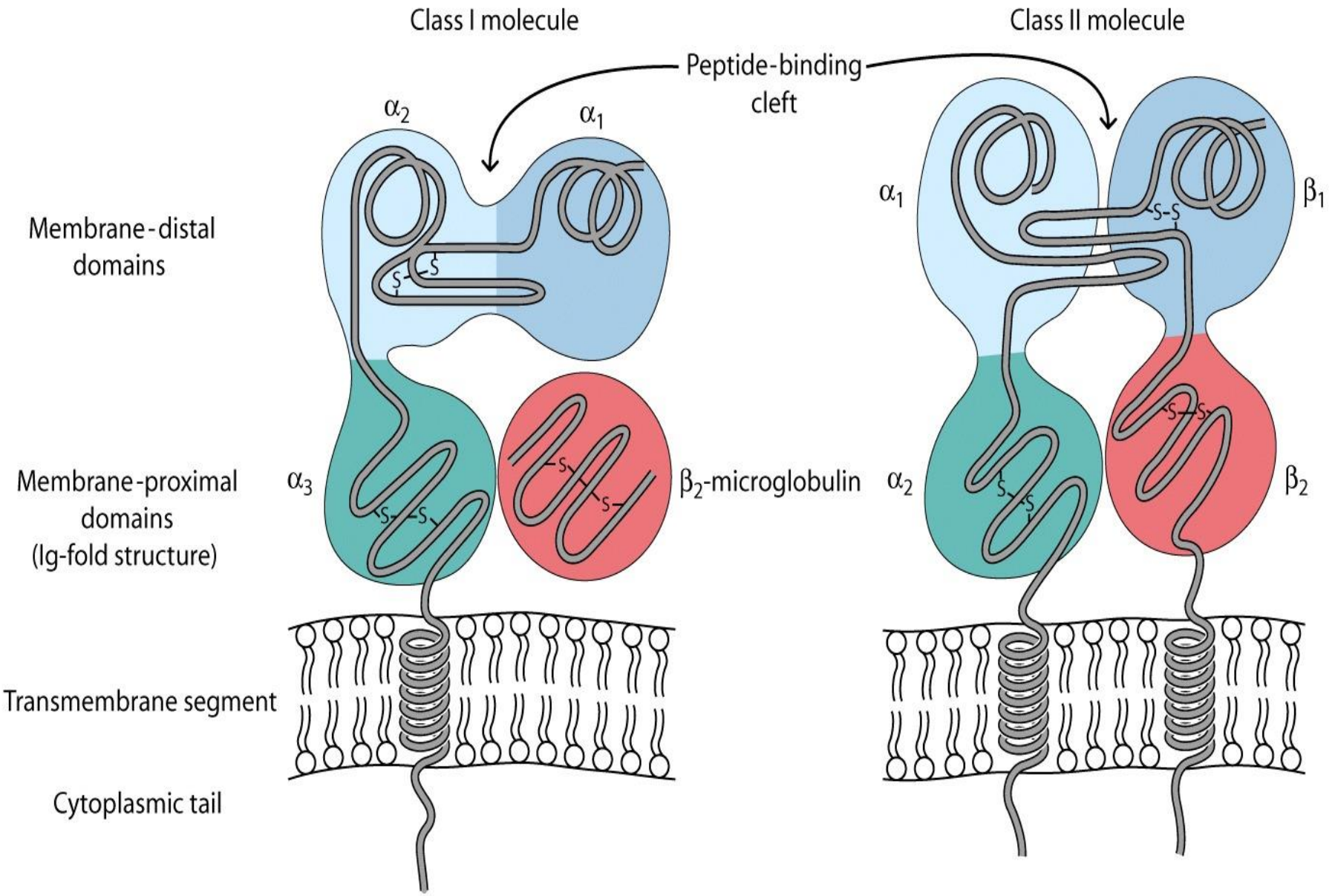
Human HLA complex

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

Comparison of MHC class I & II molecule

Feature	Class I MHC	Class II MHC
Polypeptide chains	α (44-47kD) $ß_2$- Microglobin (12kD)	α (32-34kD) ß (29 -32kD)
Locations of polymorphic residues/ Peptide binding domain	α1 and α2 domains	α1 and ß1 domains
Binding site for T cell corecptor	α3 regions binds CD8	ß2 regions binds CD4
Nature of peptide binding cleft	Closed at one 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 molecules	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
Nomenclature **Human** **Mouse**	 HLA- A, B, C H- K, D, L	 HLA- DR, DQ, DP I- A, E

Comparison of MHC class I & II molecule



Mechanism of MHC molecules–I (Processing of Endogenous Antigens)

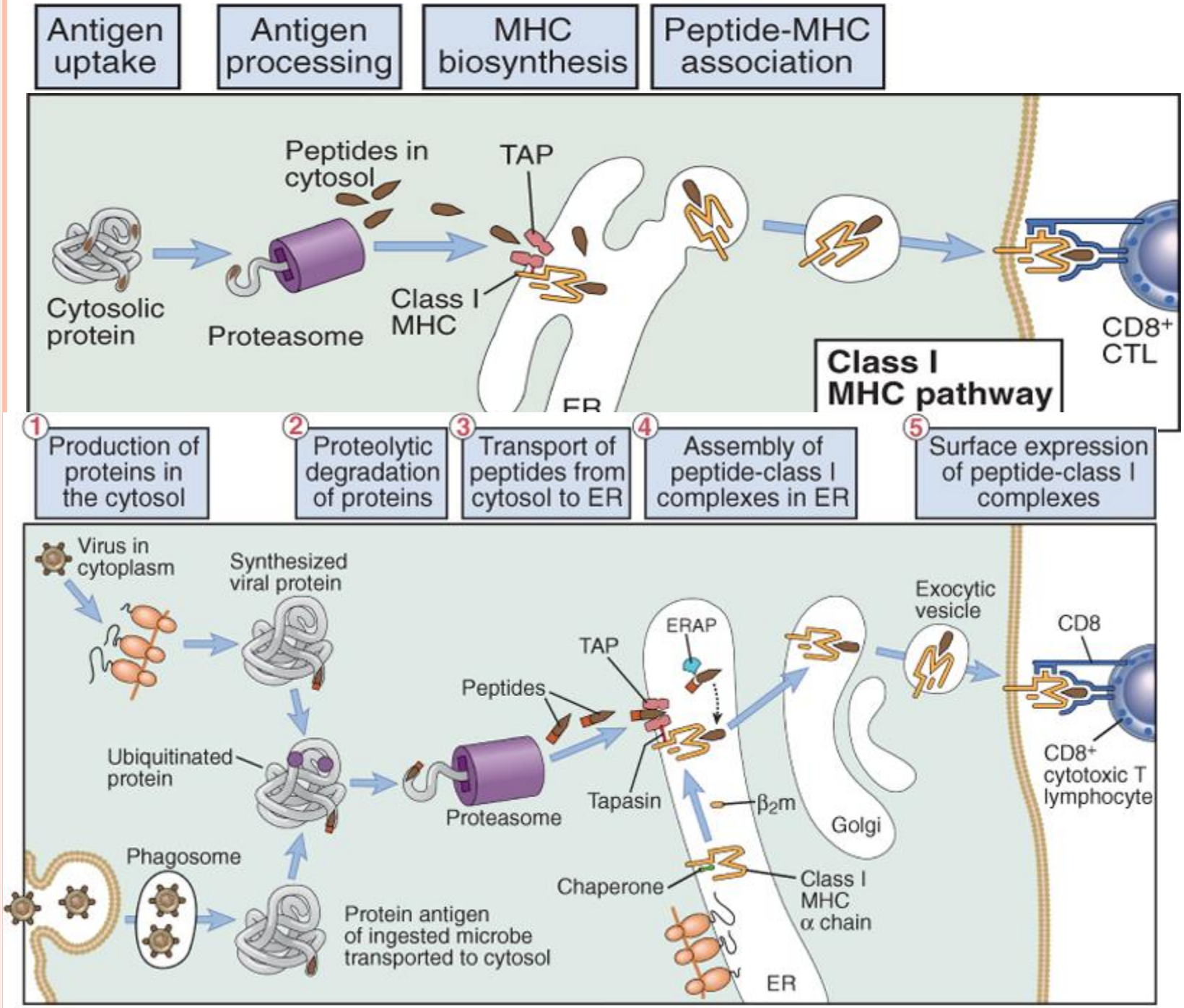
- Endogenous antigens are type of antigens that are generated inside the cells.
- Some of the common examples of endogenous antigens are virus proteins and cancer proteins.
- The antigen processing is taken care by all nucleated cells.
- In other words all nucleated cells act APC for endogenous antigens. The MHC molecules involved in presentation is MHC Class I molecules.

- The peptides (epitopes) that bind to MHC I molecules are proteolytically generated inside the cytoplasm.
- The major mechanism for the generation of peptides from endogenous antigens is proteolysis is special complexes inside the cell called proteosomes.
- These proteosomes are large multiprotein complex with a broad range of proteolytic activity.
- Two different types of proteosomes are found inside the cell. This classification is based on the size.

- One proteosome with 700 kD size appears as cylinder composed of stacked array of four inner and four outer rings with each ring composed of seven distinct subunits. The subunits of inner rings are the catalytic sites for proteolysis.
- The second type of proteosome is of 1500 kD in size and it is composed of 700 kD structure along with additional subunits that regulate proteolytic activity.
- The endogenous antigens that are found inside the cell are taken to this proteosomes by a special polypeptide called ubiquitin.

- Several ubiquitins are found attached to a single endogenous antigen just like string of pearls. This process is called as polyubiquitination.
- The endogenous antigens are broken into small polypeptides of 5-10 amino acids inside the proteosome complexes. This breaking resembles the act of meat grinding in meat grinder.
- The small polypeptides thus formed are modified structurally suitable for fixing into the cleft of MHC I molecule. Now the small peptides are ready for binding with MHC I molecules are for presentation to cytotoxic T cells.

- The two chains of MHC I molecules are synthesised separately at the rough ER and the move to smooth ER where they are assembled into a dimer structure. This structure formation is facilitated and stabilized by calnexin, BiP and TAP proteins.
- Then the MHC I molecules move to proteosomes, bind with small peptides and move through Golgi to the surface of cell by exocytic vesicles. At the surface of the cells they are recognized by cytotoxic cells.



Mechanism of MHC molecules–II (Processing of Exogenous Antigens)

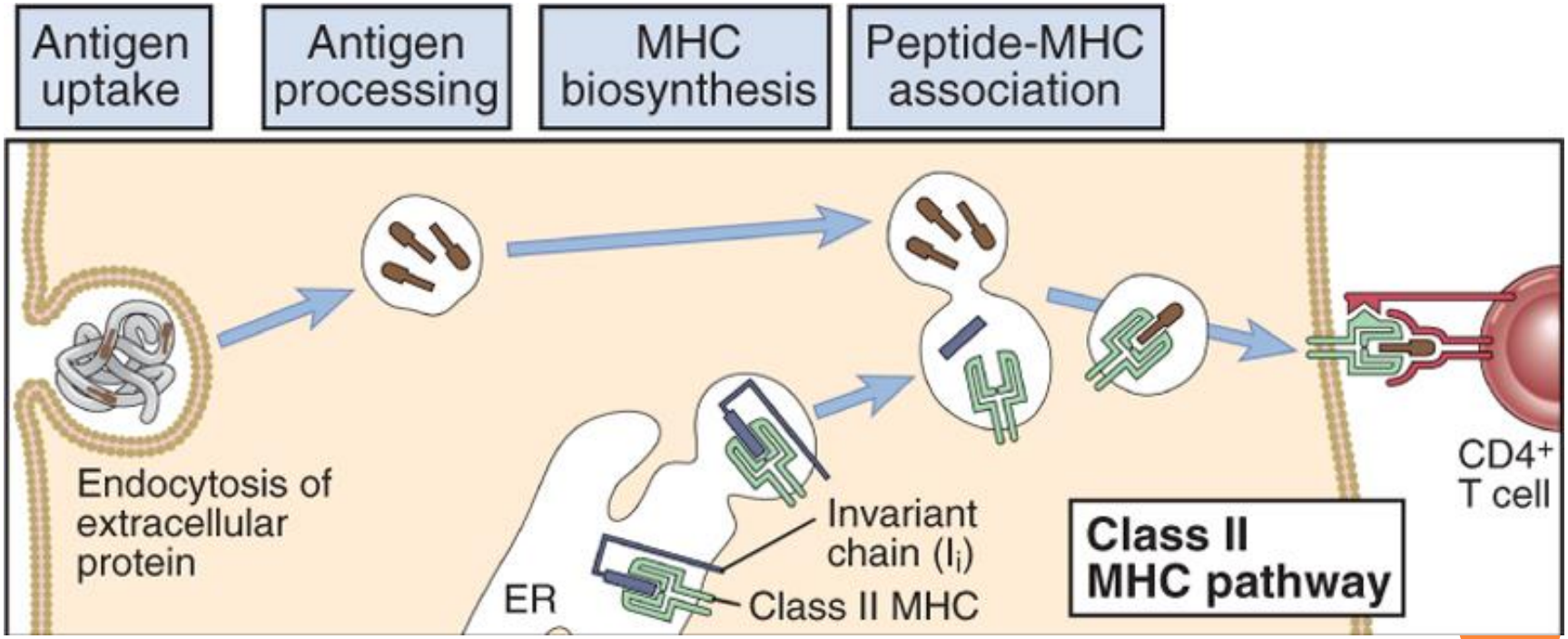
- Internalization of antigen: For antigen processing to take place first the antigens should enter into APC. This usually takes place by phagocytosis, pinocytosis or endocytosis.
- Macrophages on their surface exhibit receptors for Fc portion of antibody. Hence oposonised bacteria can easily be taken into the cell. Besides this macrophages also exhibit receptors that bind with mannose residues of the polysaccharide on bacterial cell wall.
- The internalized antigen becomes localized inside the APC in a membrane bound vesicular structure called endosome.

- The proteolytic processing of proteins takes place either in endosomes or in lysosomes. Both endosomes and lysosomes have an acidic pH that is pre requisite for antigen processing.
- Proteolytic processing requires proteases enzymes and some of the common protease enzymes found in endosomes and lysosomes are cathespin and leupeptin.
- These enzymes break the peptide antigen into small peptides of 10-30 amino acids long that are capable of binding with clefts of MHC II molecules.

- The alpha and beta chains of MHC II molecules are synthesized and associate with each other in endoplasmic reticulum.
- Certain proteins like calnexin that are, help in proper assembly and transport of the MHC molecule.
- Another protein called invariant chain prevents the nascent or any other unfolded peptide in ER from binding to MHC II molecules.
- This invariant chain also directs the MHC II molecule to endosomes or lysosomes where internalised proteins are broken into small peptides. This transport occurs in vesicle like structure. It is called MHC class II compartment or MIIC in macrophages and Class II vesicle or CIIV in B cells.

- Inside the MIIC or CIIC the invariant chain is removed.
- Removal of invariant chain is essential for processed peptides of endogenous antigens to bind with cleft.
- This removal is facilitated by proteases. Initially these enzymes leave a small fragment of invariant chain called Class II associated invariant chain polypeptide (CLIP) in the cleft.
- But this is also removed later. Only after removal of CLIP, the MHC II molecule is ready for binding with peptides of exogenous antigens.
- Each MHC II molecule takes a peptide of 10-30 amino acids length.

- Once, a MHC II molecules bind with peptides they are taken in a vesicle that fuses with plasma membrane and thus the peptides are displayed to T helper cell for binding.



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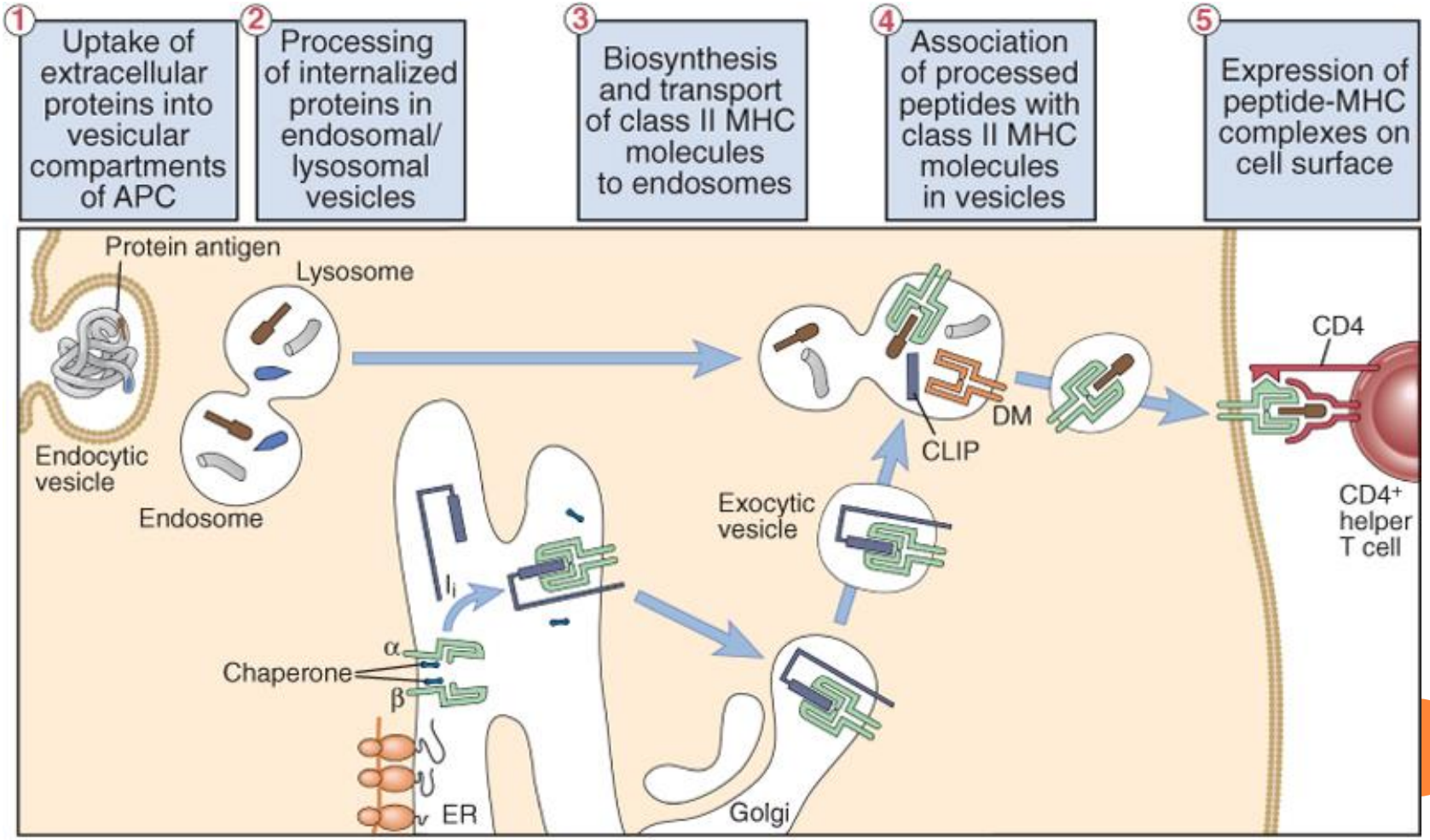


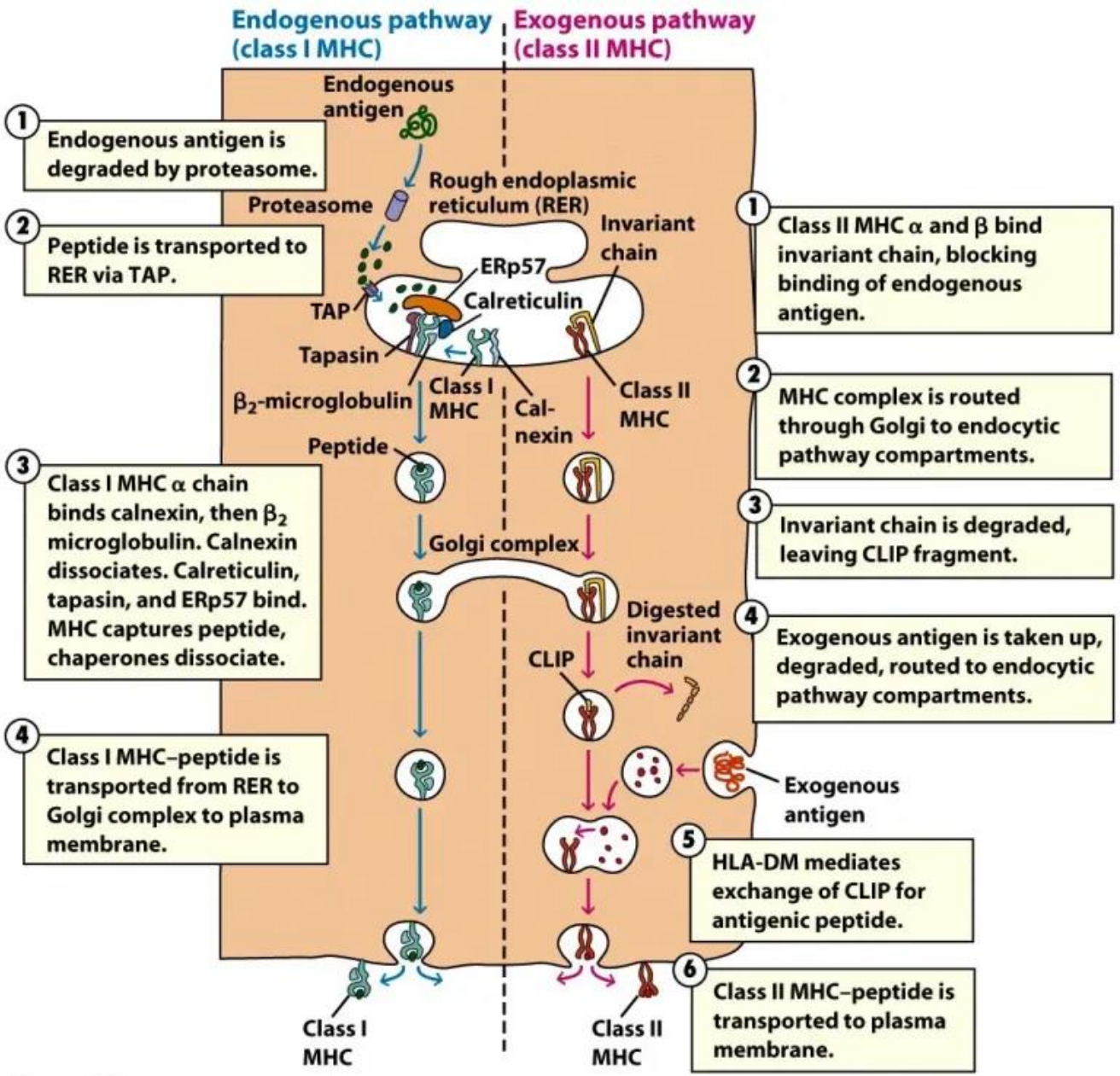


Figure 8-23
Kuby IMMUNOLOGY, Sixth Edition

Function of MHC molecules

- **Specific tissue markers**:- they reflect the specific and unique genetic make-up of an individual thus serve as specific tissue markers, may be identified by in vitro tissue typing.
- **Recognition of foreign antigen:-** MHC molecules can bind specifically to the altered target cells and present the MHC- antigenic protein (peptide) complex to T cells (CD8+/ CD4+) for recognition and destruction. This type of antigen recognition in the context of MHC molecule is referred to as **MHC-restricted antigen recognition**

- Dr. Peter Doherty and his colleague, Rolf zinkernagel were first to show the phenomena of MHC restriction

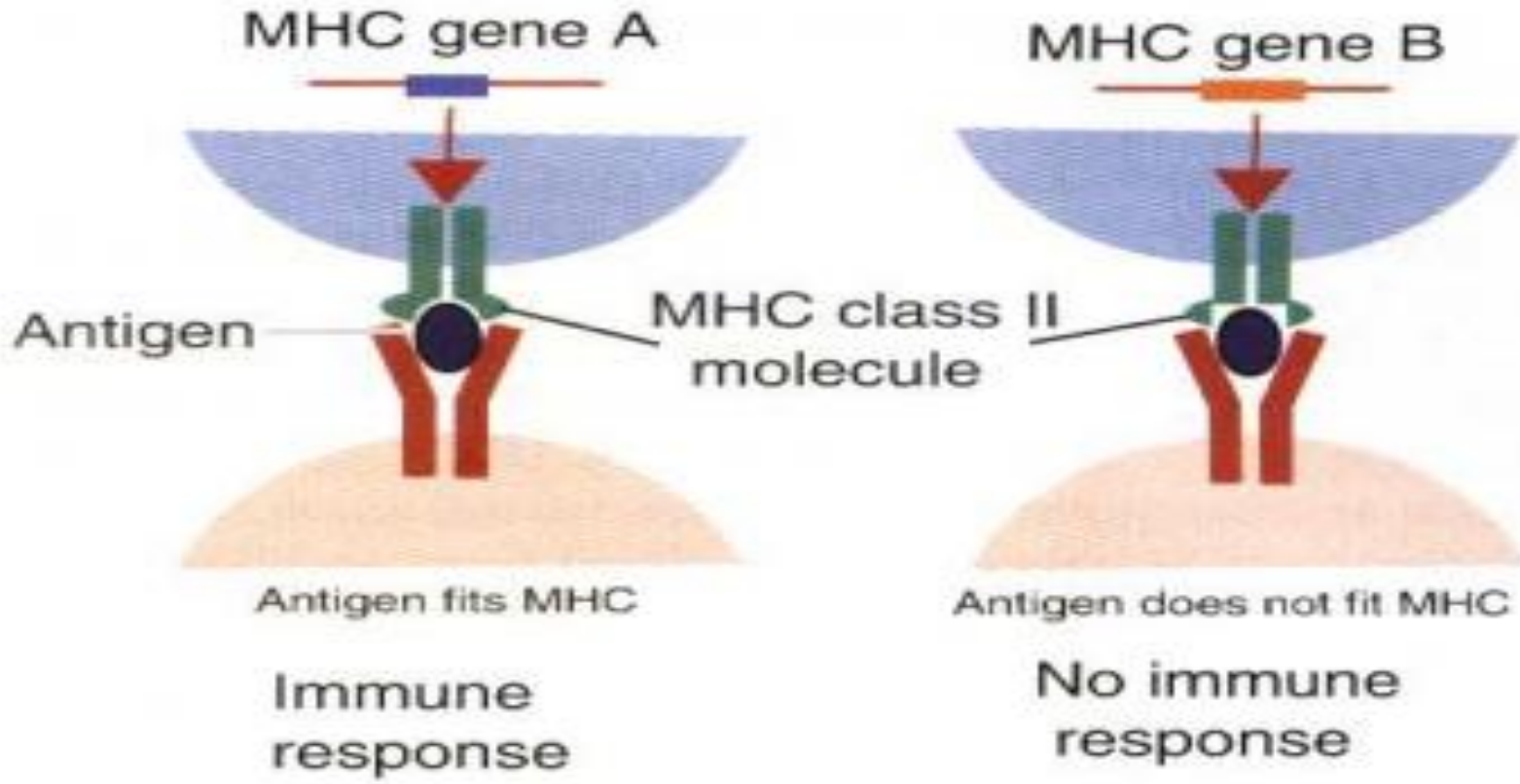


Figure 7-16. MHC molecules regulate the immune response. Only molecules that can bind in the groove of a MHC molecule will trigger an immune response. This is called MHC restriction. Thus the MHC genes that code for these molecules also regulate immune responsiveness.

Function of MHC molecules

MHC and body odors:-

- The class I region of mice, cattle, and pigs contains at least four genes coding for pheromone olfactory receptors.

- As the result, MHC haplotype affects the recognition of individual odors in an allelic –specific fashion and thus influences the mating preferences of mammals

Function of MHC molecules

Graft rejection:-

- MHC molecules act themselves as antigens and can provoke immune response in the recipient—thus transplant rejection.

1) Both TH and TC are activated

- TC cells destroy graft cells by direct contact

TH cells secrete cytokines that attract and activate macrophages, NK cells and polymorphs leading to cellular infiltration and destruction of graft

2)B cells recognize foreign antigens on the graft and produce antibodies which bind to graft cells and

- Activate complement causing cell lysis
- Enhance phagocytosis, i.e. opsonization

3) Immune complex deposition on the vessel walls induce platelets aggregation and microthrombi leading to ischemia and necrosis of graft

Graft Versus Host (GVH) Reaction

* An immunologically competent graft is transplanted into an immunologically suppressed recipient (host)

* The grafted cells survive and react against the host cells i.e instead of reaction of host against the graft, the reverse occurs

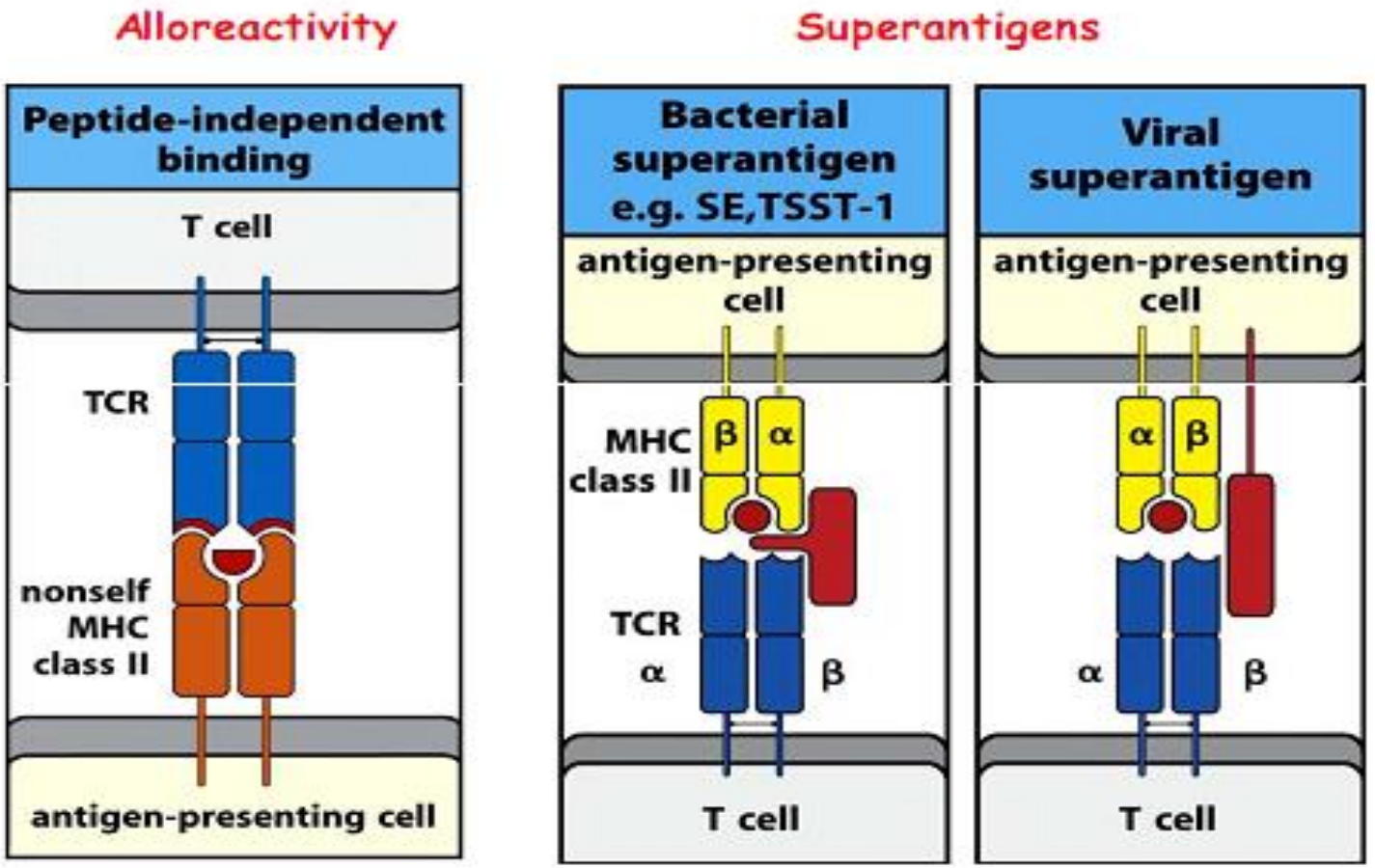
* GVH reaction is characterized by fever, pancytopenia, weight loss, rash , diarrhea, hepatsplenomegaly and death

Diseases associated with MHC molecule

Disease	HLA type
Ankylosing spondylitis	B27
Goodpasture´s syndrome	DR2
Insulin-dependent diabetes mellitus	DQ2
Multiple sclerosis	DR2
Pemphigus vulgaris	DR4
Rheumatoid arthritis	DR4
Systemic lupus erythmatosus	DR3

Alloantigens:- non-self MHC molecules. 1-10% of all T lymphocyte recognizes allogenic MHC- transplant rejection.

Superantigens:- bacterial or viral proteins/lipid/sugars that associate extracellularly with the MHC – II (HLA-DQ) and are recognized by 5- 10% of all T lymphocyte- endotoxic shock.



Thank You

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