

# **ANTIGEN, IMMUNOGENICITY AND MAJOR HISTOCOMPATIBILITY COMPLEX**

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# Outlines

- Antigen- explanation and types
- Determinants of immunogenicity
- MHC gene-introduction, polymorphism and inheritance
- Expression and restriction of MHC
- MHC molecules
- MHC-peptide interaction
- Processing and presentation of peptide antigen.

# ANTIGEN

- Any substance that may be specifically bound by **an antibody molecule or T cell receptor**.
- An antigen by itself need not produce an immune response of any kind but it bind specifically with **antibodies and sensitized T-cell** and its ability to bind is referred to as **antigenicity**.

# Contd...

- **Immunogen** ,on the other hand, is a substance which produces an immune response as well as bind to its products i.e. antibodies or sensitized T-cell.
- So an immunogen is an antigen but reverse is not true and its property of producing immune response is called **immunogenicity**.

# HAPTEN

- Small well-defined chemicals which are not immunogenic on their own but will react with preformed antibodies induced by **hapten linked to a “carrier”**, such as **dinitrophenyl (DNP)** or ***m*-aminobenzene sulfonate**.
- Antihapten antibody do not form large complexes with hapten( **monovalent**). However if many hapten combine with carrier, it form large complexes resulting in precipitation and agglutination.

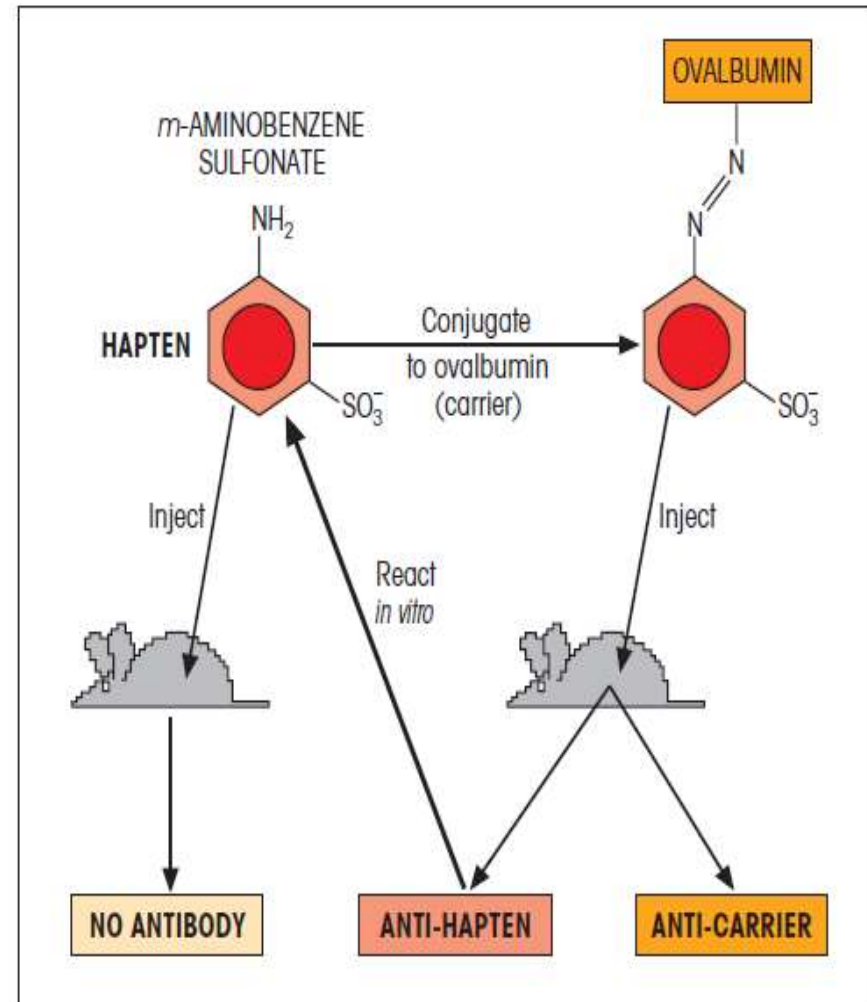


Figure 5.1 A hapten on its own will not induce antibodies. However, it will react *in vitro* with antibodies formed to a conjugate of the hapten with an immunogenic carrier.

# ANTIGENIC DETERMINANT OR EPITOPE

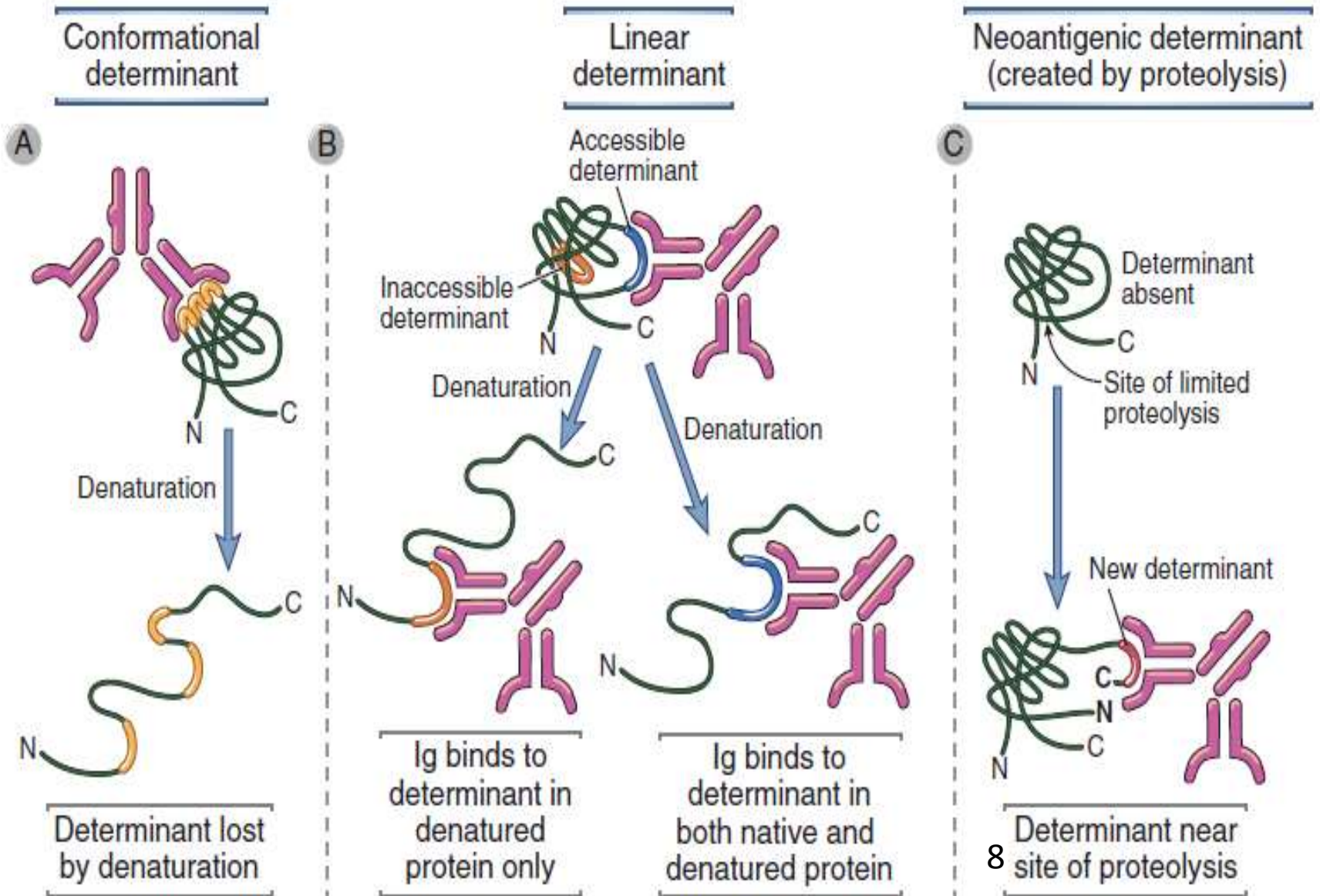
- Part of the antigen which is in contact with the **paratope** or **receptor of lymphocyte**, is designated the **epitope** or **antigenic determinant**.
- Presence of multiple identical determinants in an antigen is referred to as **polyvalency** or **multivalency**.
- B cells and T cells do not recognise the same epitopes on the antigen molecules, but different epitopes.

# Contd...

## ➤ **B-cell epitope:-**

- Recognised in free state without combining with any other molecules.
- **Conformational** , consists of non-adjacent residues placed far away from each other in primary structure. Brought together during three dimensional folding of antigen . Destroyed by denaturation.
- **Linear epitope**, formed by adjacent linear amino acid residues present in accessible region. Not destroyed by denaturation.

# Contd...





# Contd...

## ➤ **T-cell epitope:-**

- Are recognised after combined with MHC molecule.
- Located internally deep and not present on surface, so T-cell epitope undergo antigen processing to make these epitope accessible.
- T-cell epitope recognition involves the formation of a **ternary** complex of MHC, antigen and TCR. In this complex ,the antigen has 2 binding site- one for MHC (**aggretope**) and another for TCR (**epitope**).

# **ANTIGEN TYPES**

# EXOGENOUS AND ENDOGENOUS

- Exogenous antigens, enters into the host from external environment. (eg. Bacterial , viral antigens, some drugs, etc)
- Endogenous antigen, produced within the host.( eg.. Products of genetically mutated cells or virally infected cells or cancerous cell , etc)

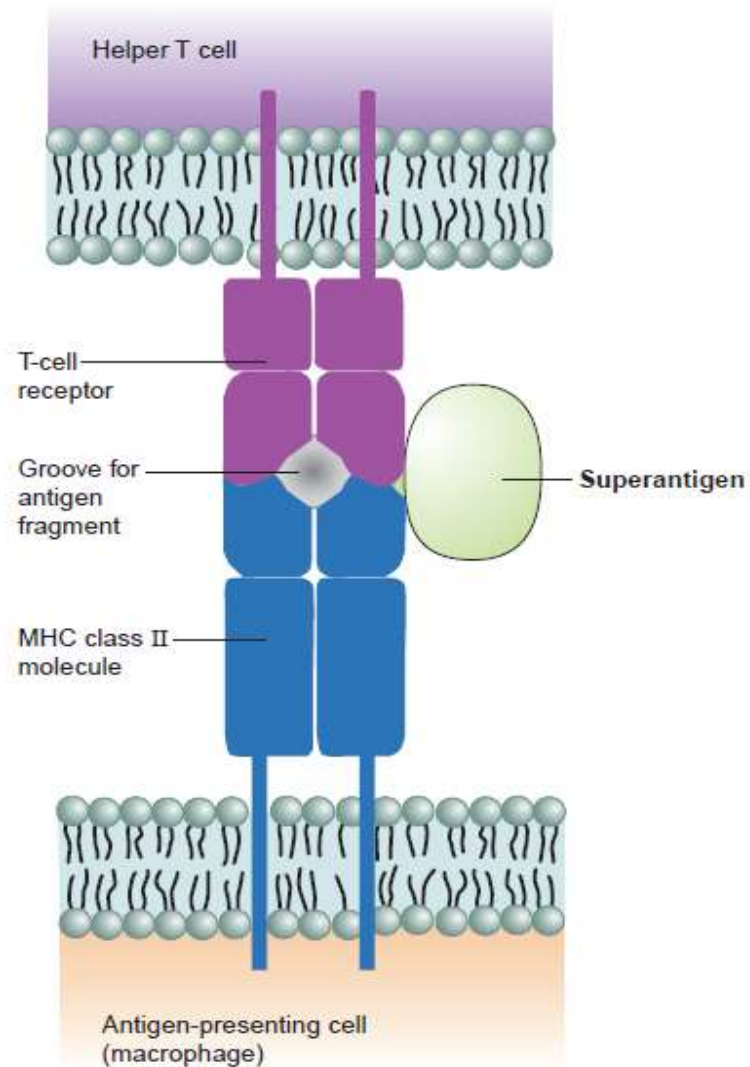
# AUTOANTIGEN,ALLOANTIGEN AND HETEROANTIGEN

- Autoantigen, belonging to the host itself.( eg.. Sperm and corneal protein ).
- Alloantigen, derived from other members of species of host. Important in transplantation and blood transfusion.( eg. Antigen on donor and recipient RBCs are alloantigen to each other)
- Heteroantigen, Antigens from two different species. ( eg..heterophile antigen, human blood group A antigen and pneumococcal polysaccharide type XIV)

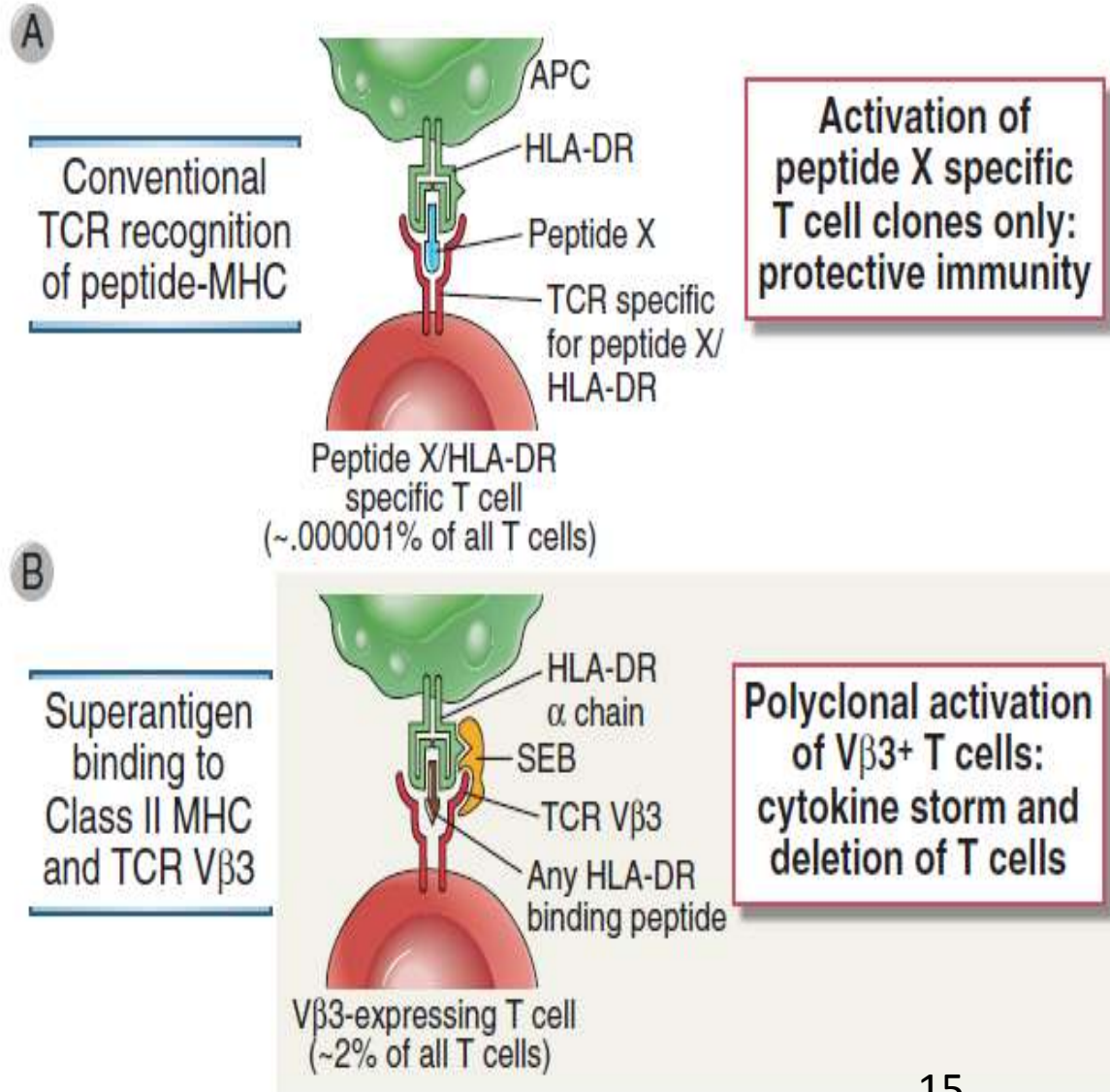
# TD AND TI ANTIGENS

- **T- cell dependent antigens** are those that do not directly stimulate the production of antibody without the help of T cells. Proteins are T-dependent antigens.
- **T-cell independent antigens** are antigens which can directly stimulate the B cells to produce antibody without the requirement for T cell help.  
In general, polysaccharides are T-independent antigens.

# Superantigen



# Superantigen



**FIGURE 15-2 Polyclonal activation of T cells by bacterial superantigens.** A, Conventional microbial T cell antigens, composed of

# IMMUNOGENICITY

- Properties of immunogen,
- Properties of host,
- Route and dose of administration, and
- Adjuvants



# PROPERTIES OF AN IMMUNOGEN

## ➤ Foreignness:-

- Immunogen molecule must be recognised as non-self i.e. foreign by host cell.
- Typically , more distant taxonomically the source of an immunogen from the host, better It is as immunogen. ( eg.. BSA is more immunogenic in chicken than goat).

## ➤ Chemical nature:-

- Protein >polysaccharide>lipids and nucleic acid.
- All protein except gelatin are immunogenic.

# Contd...

## ➤ **Molecular size:-**

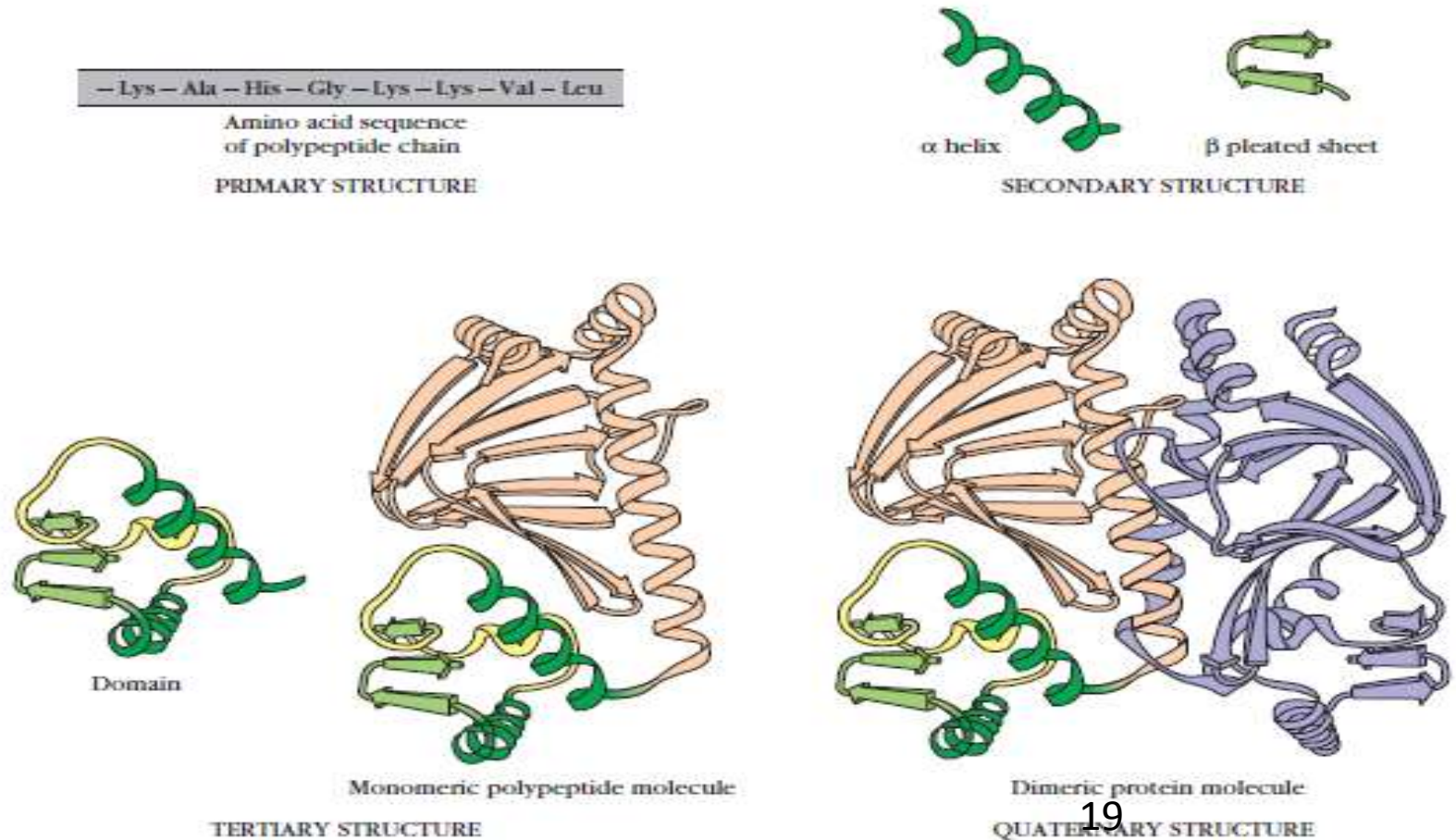
- To become most active immunogen, molecular mass of 10,000 Da is required. Generally, substances with a molecular mass less than 5000–10,000 Da are poor immunogens.

## ➤ **Molecular heterogeneity and structure complexity:-**

- Co-polymers composed of different amino acids or sugars are usually more immunogenic than homopolymers of their constituents.

# Contd...

- Natural proteins, with 20 different AAs and all four levels of protein organization, are highly heterogeneous and complex in structure, that's why they are highly immunogenic.
- Addition of aromatic AAs increase immunological potential.



# Contd...

## ➤ **Susceptibility to antigen processing and presentation:-**

- Antigen is degraded and some selected fragment of it is presented for recognition by T-cell in order to generate an immune response.
- Large, insoluble molecules, being easily phagocytosed, are potent immunogens than small and soluble ones.
- D-amino acids polymer cannot be metabolised in human body and they are not immunogenic.

# HOST CHARACTERS

## ➤ Genetic make up:-

- Genetic basis of MHC, gene encoding BCR and TCR are also involved in immune regulation.

## ➤ Nutritional status:-

- Malnutrition and/or undernutrition also leads to a poor response on immunogenic challenge.

## ➤ Age:-

- Neonates and elderly people show poor immune response as compared to young adults.

# ROUTE AND DOSE OF ADMINISTRATION

- A too little dose fails to elicit an immune response and a too large dose leads to the development of immunological tolerance.
- Eg..  $5 \times 10^{-4}$  mg of purified pneumococcal capsular polysaccharide antigen is sufficient to induce antibody production. But a dose of 0.5 mg does not produce a response.

# Contd...

- Repeated immunogen exposure increase the clonal proliferation of T and B cells to augment the response (booster dose).
- Intravenously administered immunogens go to spleen while suncutaneous route leads the immunogen to regional lymph nodes and hence they are more potential than those administered orally.

# ADJUVANTS

- Substances that potentiate the action of immunogen.
- They are usually mixed with antigen to boost the immune response.
- Also used when antigen is available in small quantity.
- Common examples- alum( aluminium potassium sulfate) and Freund's adjuvants.



# Contd...

## a) **Freunds complete adjuvnts:-**

- Contains antigen in aqueous phase and a suspension of **killed mtb** in the oil phase( water in oil emulsion)

## b) **Freunds incomplete adjuvants:-**

- Same but oil phase contains mannide mono-oleate , rather than mtb.
- *Bordetella pertussis* acts as a good adjuvant for diphtheria and tetanus toxoid in triple vaccine.

# Contd...

- Adjuvant acts in following ways:
  - By prolonging antigen exposure by slowing the release( depot action.)
  - By activating the phagocytes(muramyl dipeptide,IL-11, MHC-II).
  - By activating the TH cells.
  - Granuloma formation.

# MAJOR HISTOCOMPATIBILITY COMPLEX

# Introduction

- Cluster of genes whose products play important role in the development of immune response and discrimination between self and non-self.
- Determine wheather a grafted tissue will be accepted as self (histocompatible) or rejected as non-self (histiincompatible) by the host.

# Contd...

- Formely called **human leucocyte antigen(HLA)**  
(major determinant of rejection) → Jean Dausset
- Also important for acquired immune response  
because  $T_H$  and  $T_c$  donot recognize antigens alone but  
only when they are combined with MHC molecules.

# MHC Genes

- In human beings, are located on short arm of chromosome no. 6 in 4000 kbp long.
- Organized in to three regions: I, II and III.
- Each region contains many gene loci and gene of each region encode separate class of MHC molecules.

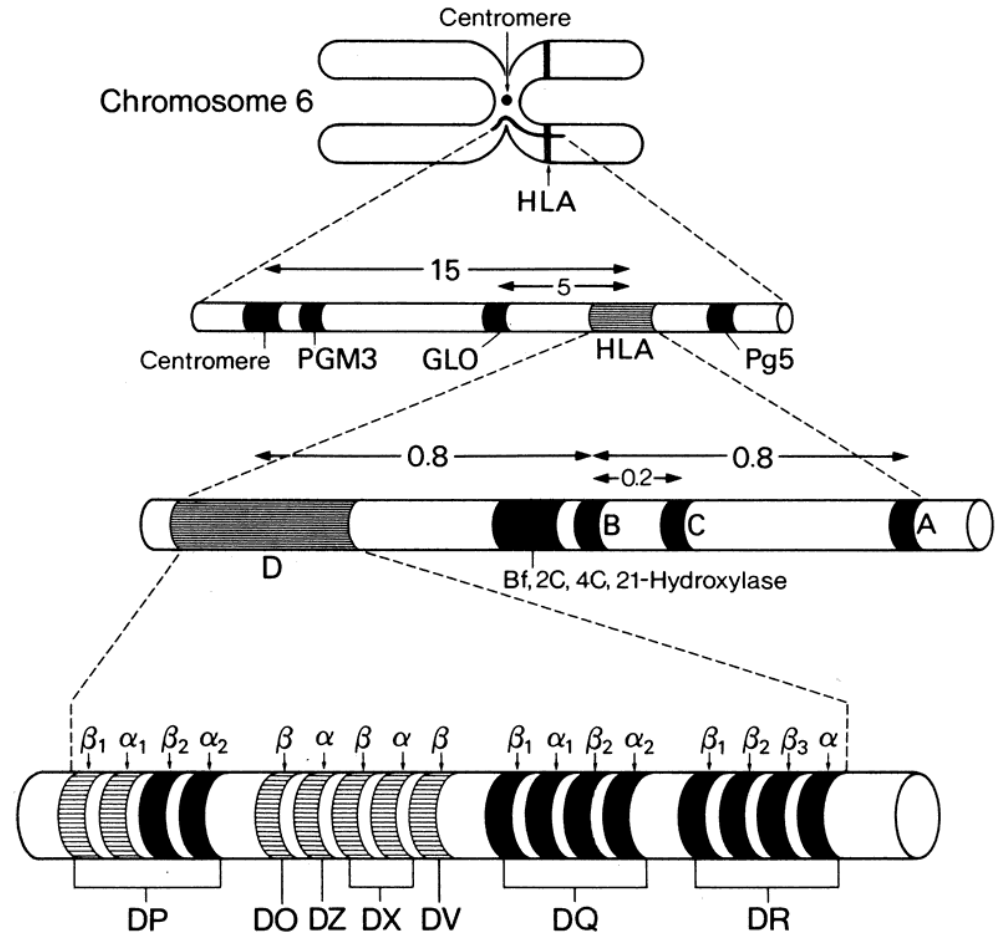
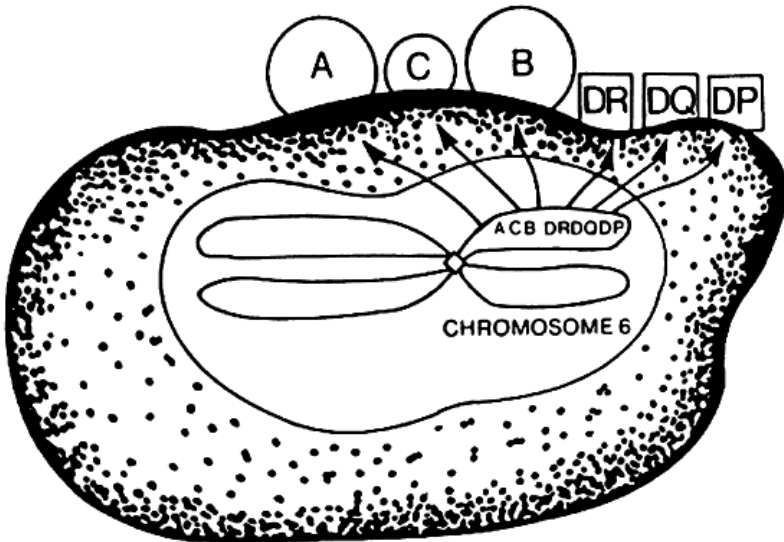
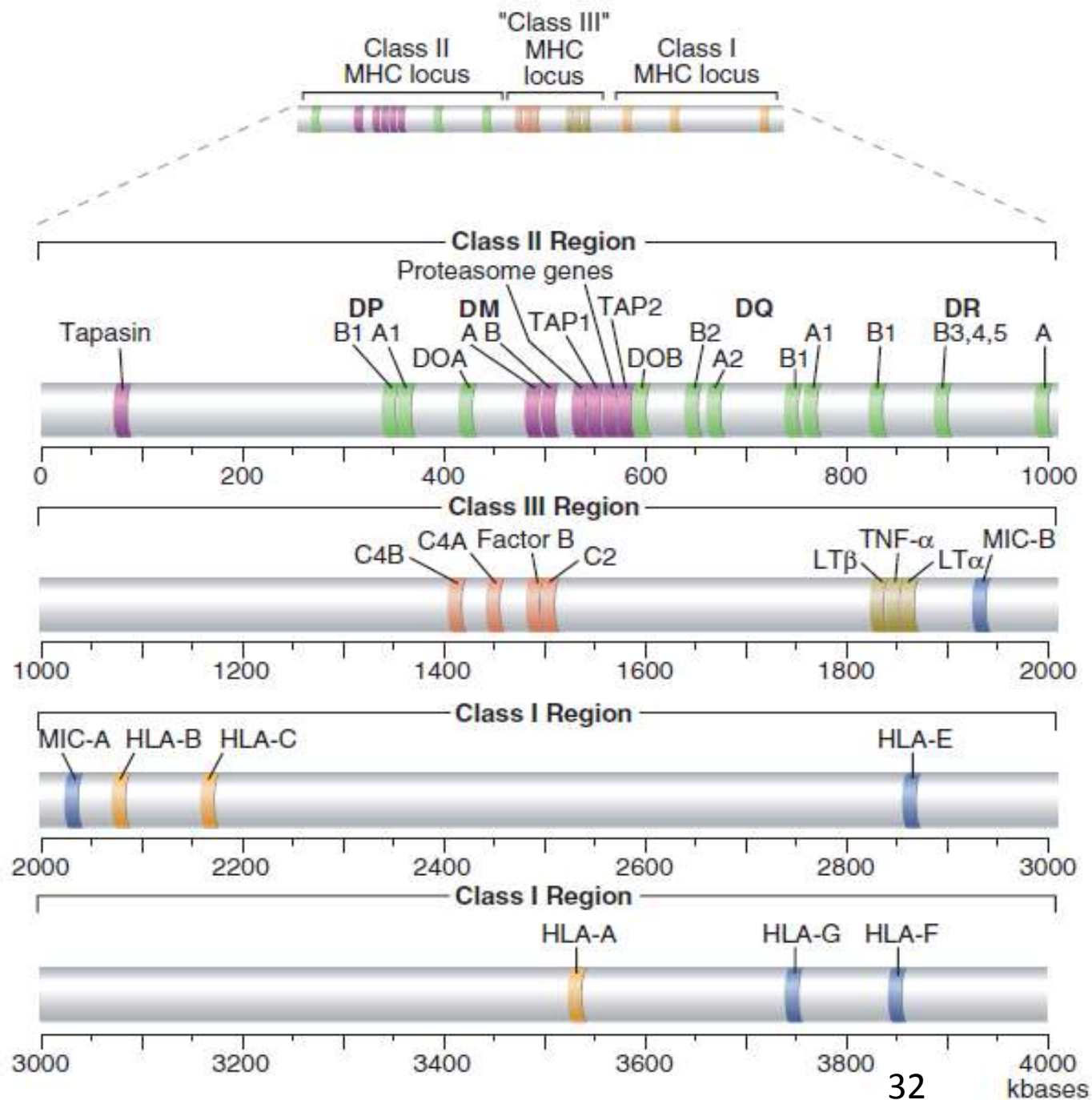


Fig: organization of human MHC complex

# Contd...

- In chromosome no. 6 , region III lies in between region I and II.
- Region I is longest (2000kbp).
- Region II and III are short ( about 1000 kbp).
- Class I MHC genes are organized into three main loci i.e. A , B and C in region I.
- Class II MHC genes are also organized into three main loci i.e. DP, DQ and DR in region II.
- Also many loci in region III.



**FIGURE 6-8 Map of the human MHC.** The genes located within the human MHC locus are illustrated. In addition to the class I and class II



# Polymorphism

- **MHC** genes are highly polymorphic and different individual express different alleles.
- Due to
  - Multiple gene loci,
  - Multiple alleles for different loci, and
  - Codominant expression i.e the two alleles at a gene locus of MHC in the two homogenous chromosomes are expressed together.

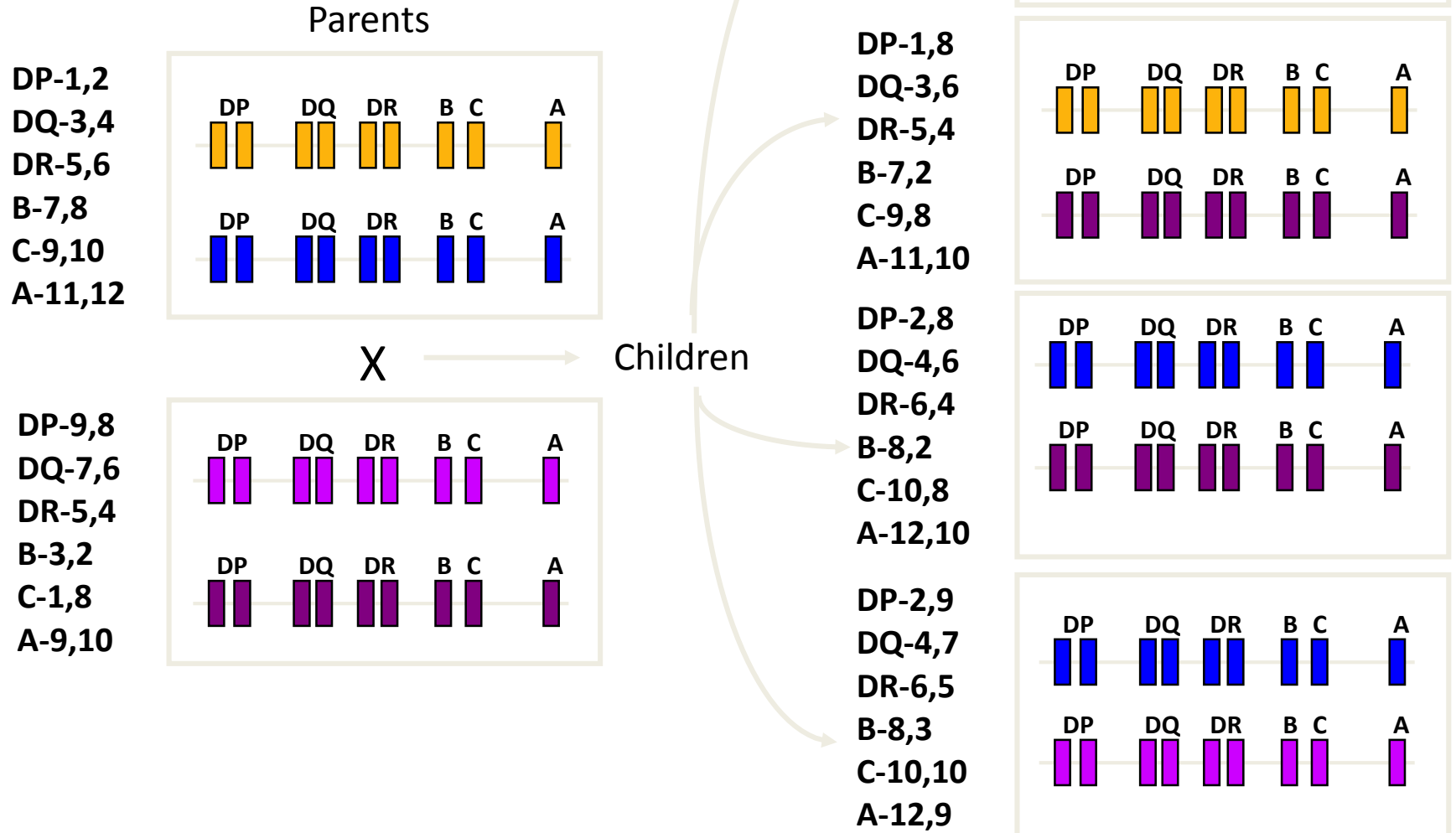
# Class I polymorphism

Locus	Number of alleles (allotypes)
HLA - A	218
HLA - B	439
HLA - C	96
There are also HLA - E, HLA - F and HLA - G	Relatively few alleles

# Class II polymorphism

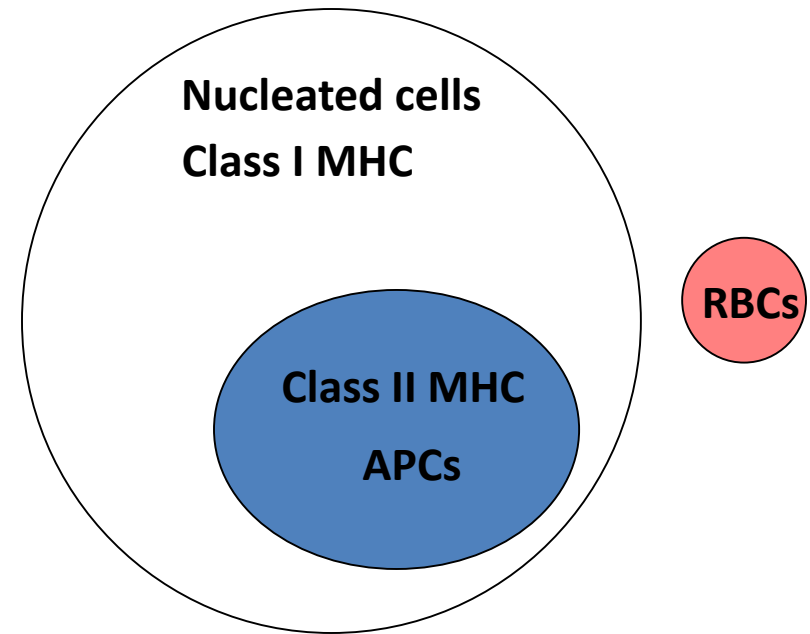
Locus	Number of alleles (allotypes)
HLA - DP <sub>A</sub>	12
HLA - DP <sub>B</sub>	88
HLA - DQ <sub>A</sub>	17
HLA - DQ <sub>B</sub>	42
HLA - DR <sub>A</sub>	2
HLA - DR <sub>B1</sub>	269
HLA - DR <sub>B3</sub>	30
HLA - DR <sub>B4</sub>	7
HLA - DR <sub>B5</sub>	12
There are also HLA - DM and HLA - DO	Relatively few alleles

# Inheritance of MHC haplotypes



# Expression of MHC genes

- **Class I MHC genes** encode glycoproteins expressed on the surface of nearly **all nucleated cells** and the major function of the class I gene products is presentation of peptide antigens to T<sub>c</sub> cells.
- **Class II MHC genes** encode glycoproteins expressed primarily on **antigen-presenting cells (macrophages, dendritic cells, and B cells)**, where they present antigenic peptides to T<sub>H</sub> cells.

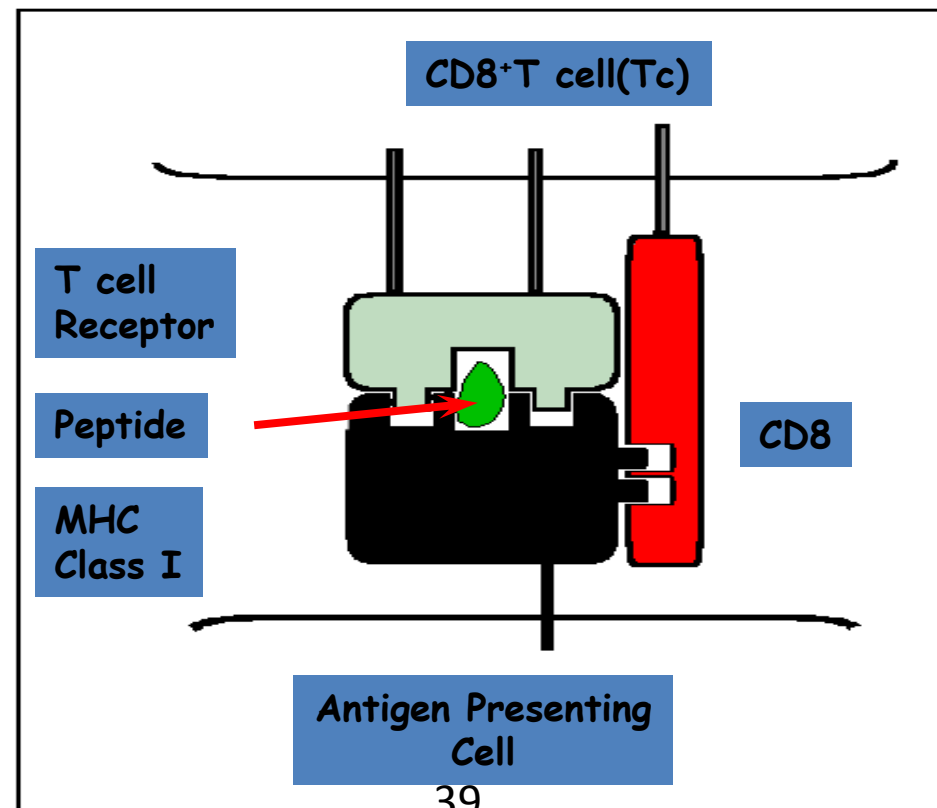
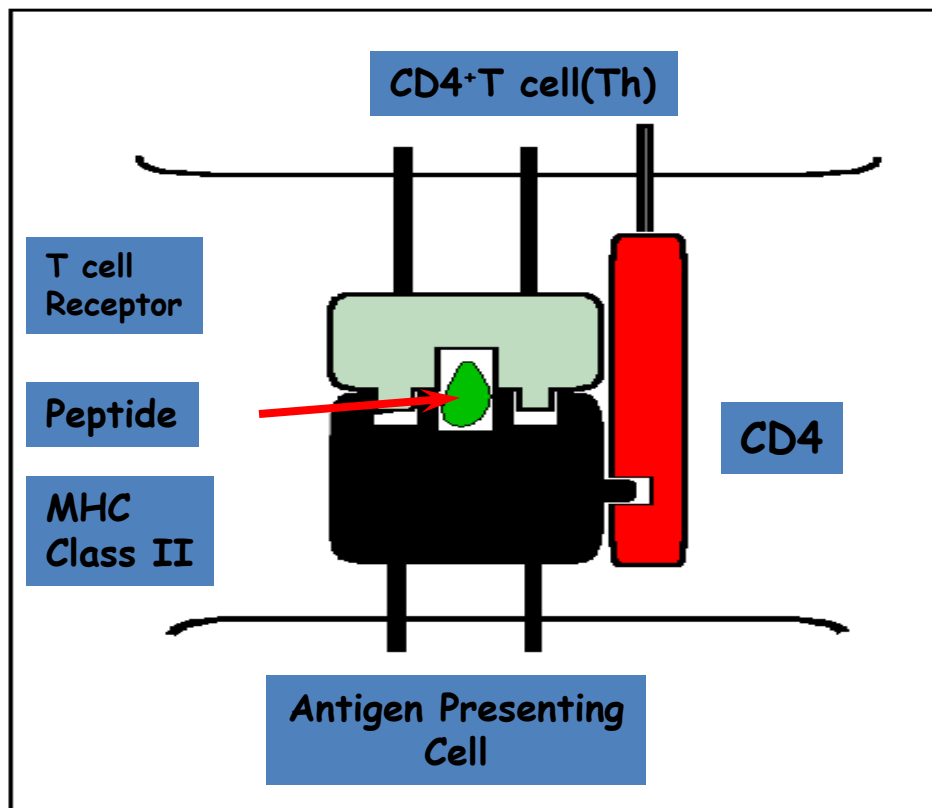


# Contd...

- **Class III MHC genes** encode various secreted proteins having immune functions(eg. complement proteins, TNF, etc)
- Expression of MHC molecule is increased by cytokines.
- Transcription factor is major determinant of MHC molecule synthesis.
  - CIITA (Trans activator), RFX (Trans activator)
- **Some Viruses Decrease MHC Expression**
  - CMV, HBV,etc.

# MHC RESTRICTION

- MHC restriction refers to the fact that T-cell will recognise a peptide antigen only when it is bound to a particular MHC molecule.
- Recognition of antigen by CD8 T-cell is restricted by class I alleles and CD4 helper T-lymphocytes by class II MHC.



# Class I MHC Molecules

- 2 noncovalently linked polypeptide chain, an MHC encoded **44-47 kD  $\alpha$  chain** (heavy) and a non-MHC encoded **12kD  $\beta$ -2 microglobulin** (light).
- $\alpha$  chain is encoded by genes in A,B and C region in HLA complex.
- A chain has 3 parts:-
  - Cytoplasmic domain( $\alpha$ 1,  $\alpha$ 2 and  $\alpha$ 3, each contain about 90 A. As)
  - Transmembrane domain( 25 A.As)
  - Cytoplasmic terminal (30 A.As)

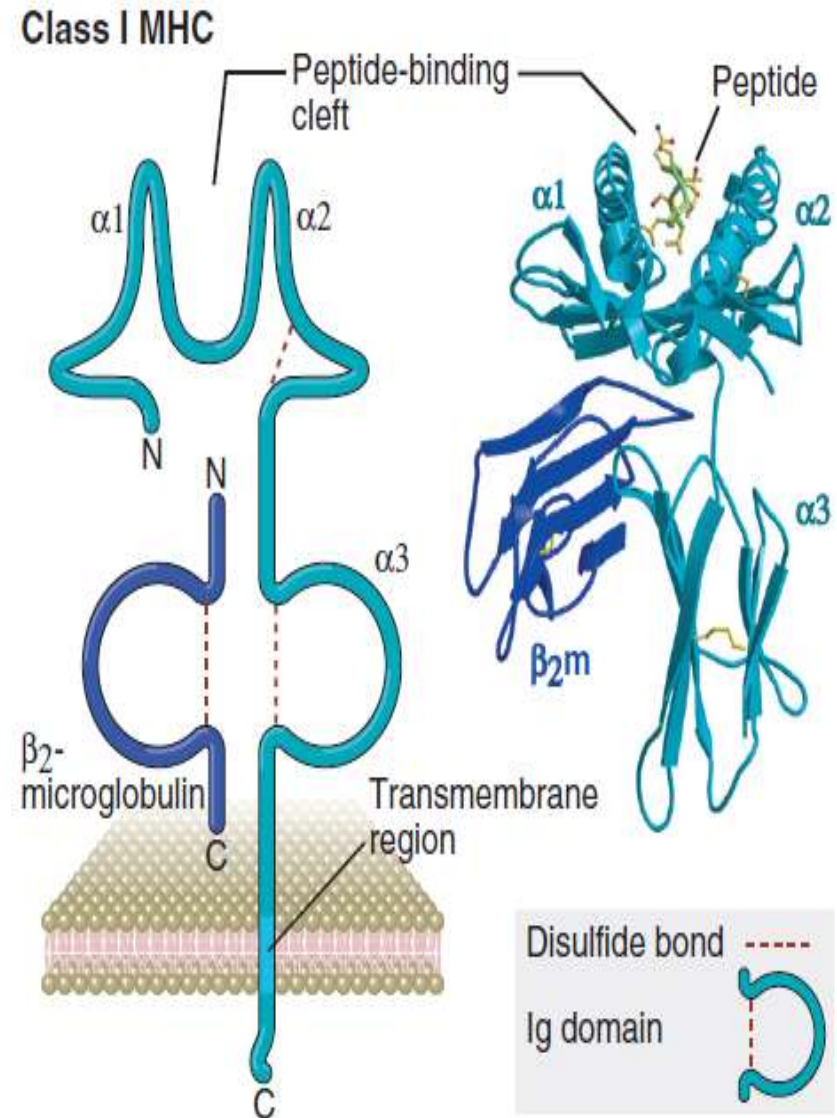


FIGURE 6-10 Structure of a class I MHC molecule. The

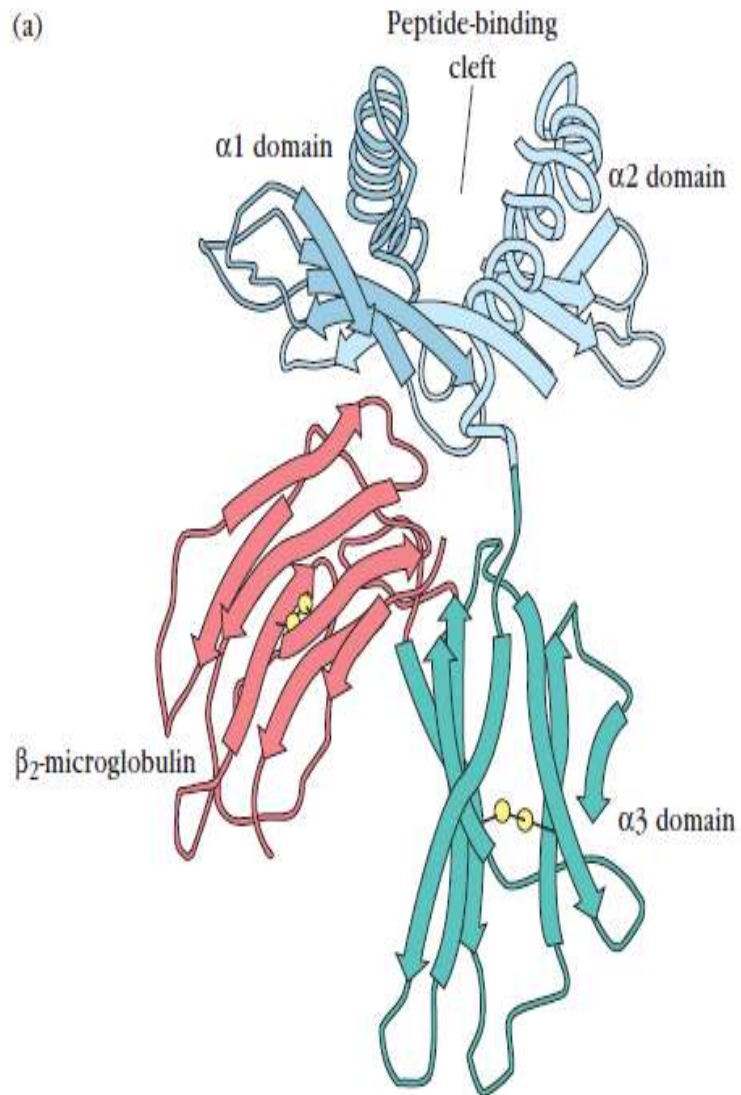


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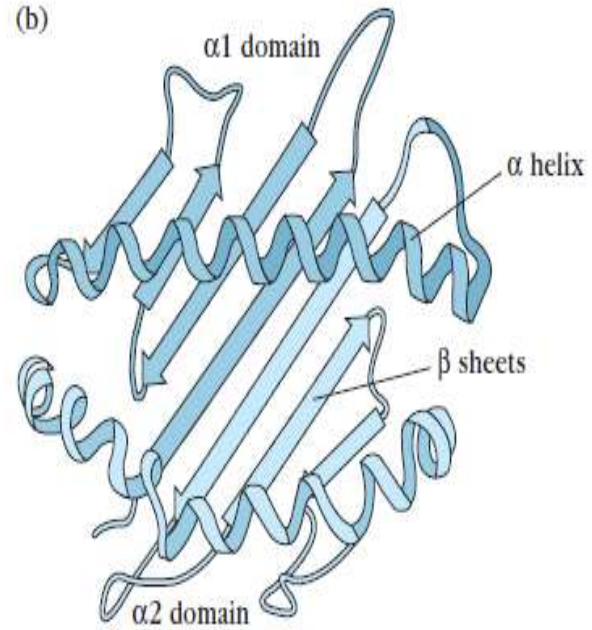
- $\beta$ -2 microglobulin, noncovalently linked to  $\alpha$ 3, is encoded by a gene in chromosome no. 15 and is necessary for expression of class I molecule on cell surface.
- $\alpha$ 1 and  $\alpha$ 2 regions form a deep cleft i.e. antigen binding cleft.
- Dimension of ( $\sim 25 \text{ \AA} \times 10 \text{ \AA} \times 11 \text{ \AA}$ ) to bind peptides of 8 to 11 amino acids.
- End of cleft is closed so that larger peptide cannot be accommodated.
- $\alpha$ 3 region is bind with CD8 T-cell receptor.

# Contd...

(a)



(b)



# Class II MHC Molecules

- Similar to class I molecule.
- 2 non covalently associated chain :- **32-34 kD  $\alpha$  chain ( $\alpha 1$  and  $\alpha 2$ ) and 29-32 kD  $\beta$  chain ( $\beta 1$  and  $\beta 2$ ).**
- Both  $\alpha$  and  $\beta$  chain is encoded by DP, DQ and DR region In HLA complex.
- Each  $\alpha 2$  and  $\beta 2$  domain is followed by transmembrane domain and cytoplasmic domain.
- $\alpha 1$  and  $\beta 1$  domain form antigen binding cleft which is open at both ends and binds peptide antigen of 10-30 A.As.
- $\beta 2$  region is bind with CD8 T-cell receptor.

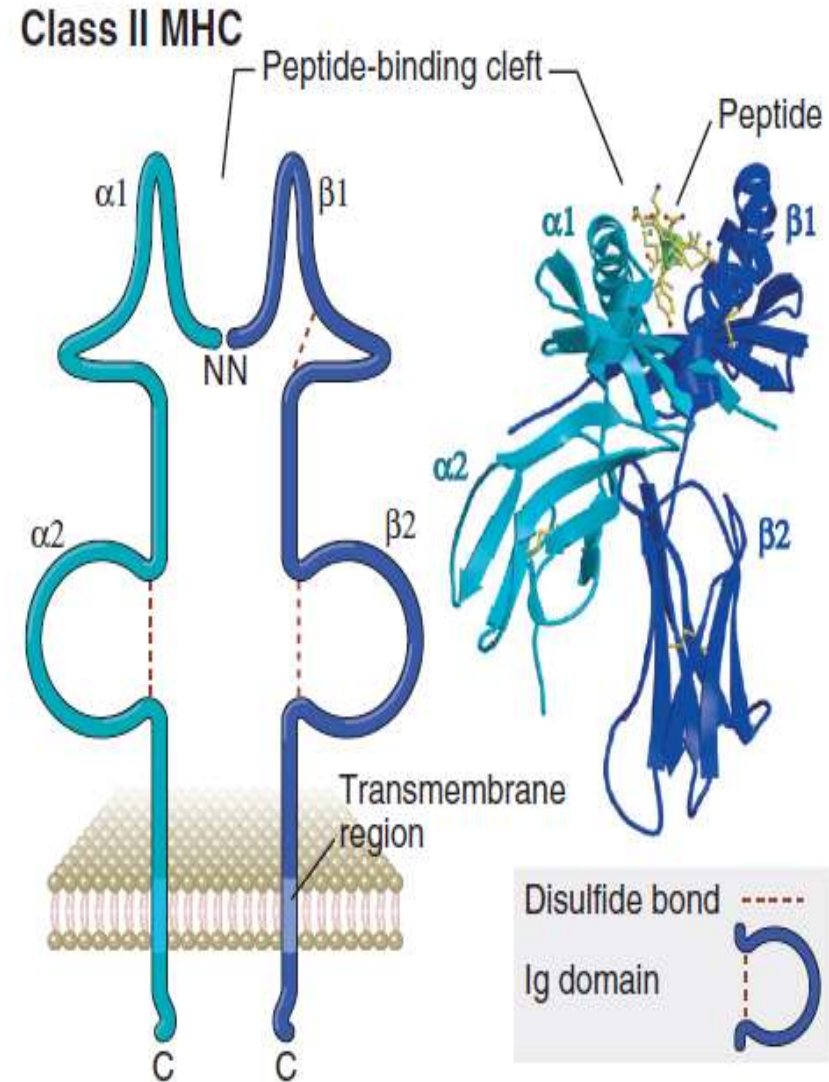
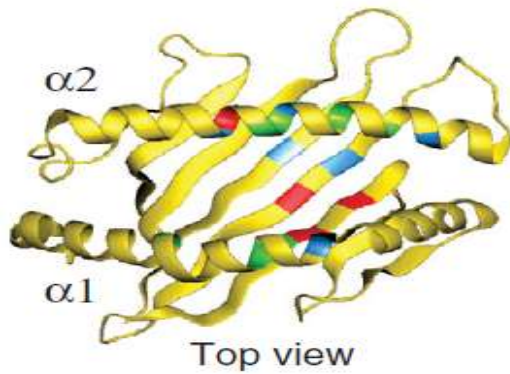


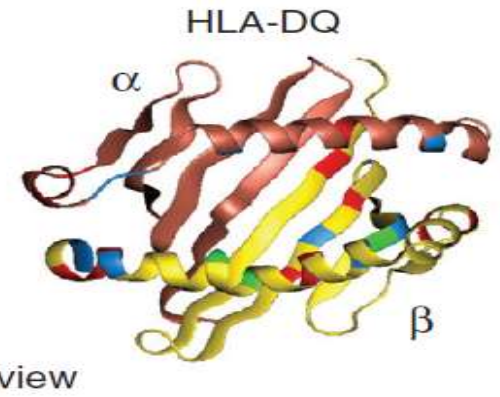
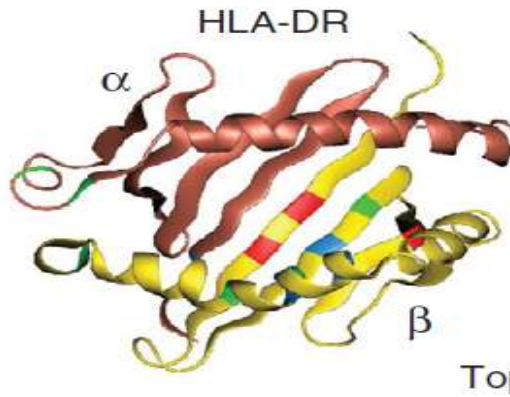
FIGURE 6-12 Structure of a class II MHC molecule. The

# Contd...

**HLA class I**



**HLA class II**



Hypervariable region

# Contd....

**TABLE 6-4 Features of Class I and Class II MHC Molecules**

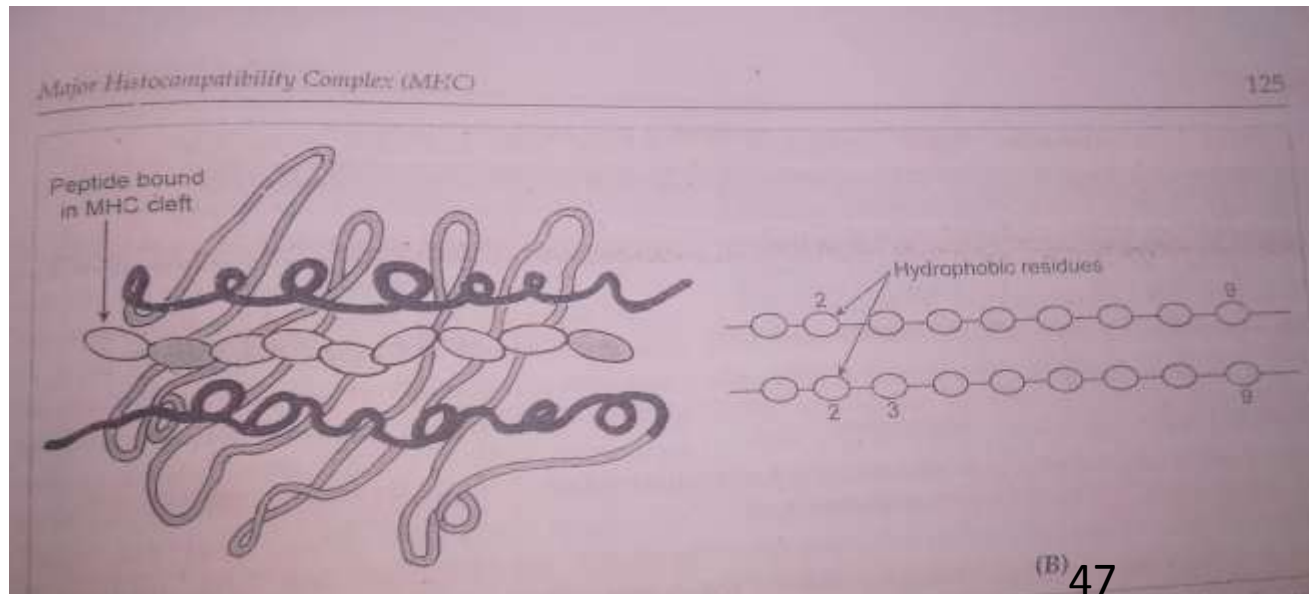
Feature	Class I MHC	Class II MHC
Polypeptide chains	$\alpha$ (44-47 kD) $\beta_2$ - Microglobulin (12 kD)	$\alpha$ and $\beta$
Locations of polymorphic residues	$\alpha$ 1 and $\alpha$ 2 domains	$\alpha$ 1 and $\beta$ 1 domains
Binding site for T cell coreceptor	CD8 binds to $\alpha$ 3 region	CD4 binds to $\beta$ 2 region
Size of peptide-binding cleft	Accommodates peptides of 8-11 residues	Accommodates peptides of 10-30 residues or more
Nomenclature		
Human	HLA-A, HLA-B, HLA-C	HLA-DR, HLA-DQ, HLA-DP
Mouse	H-2K, H-2D, H-2L	I-A, I-E

# Binding of peptide to MHC molecule

- Each class I or class II MHC molecule has a single peptide-binding cleft that binds one peptide at a time, but each MHC molecule can bind many different peptides.
- MHC molecules acquire their peptide cargo during their biosynthesis and assembly inside cells.
- The association of antigenic peptides and MHC molecules is a saturable interaction with a very slow off-rate.
- Very small numbers of peptide-MHC complexes are capable of activating specific T lymphocytes.

# PEPTIDE ANTIGEN AND MHC-I INTERACTION

- Binds endogenous peptide of 8-11 amino acids( nanomers being ideal)
- Hydrophobic amino acids at 2<sup>nd</sup> or 2<sup>nd</sup> and 3<sup>rd</sup> position from N-terminal, called **anchor residues** that insert into cleft.
- In general , any peptide of correct length containing anchor residues can bind to MHC I molecule by hydrogen bonding.



# PEPTIDE ANTIGEN AND MHC-II INTERACTION

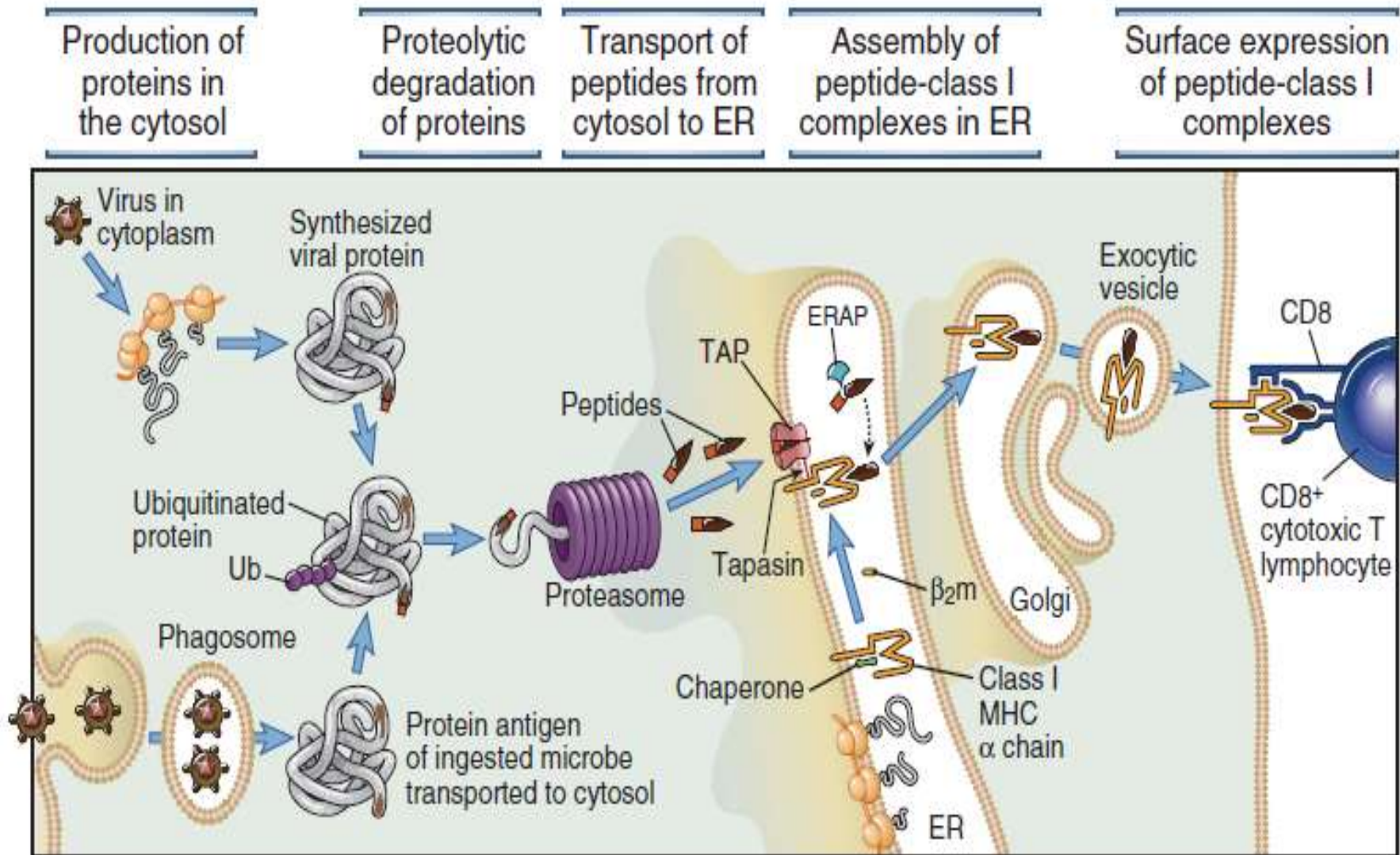
- Binds with exogenous peptide of 10-30 amino acids.
- No anchor residues.
- Central 7-10 amino acids is critical in this peptide binding, usually rich in aromatic and hydrophobic amino acids.
- Hydrogen bond.



# PROCESSING AND PRESENTATION OF PROTEIN ANTIGENS

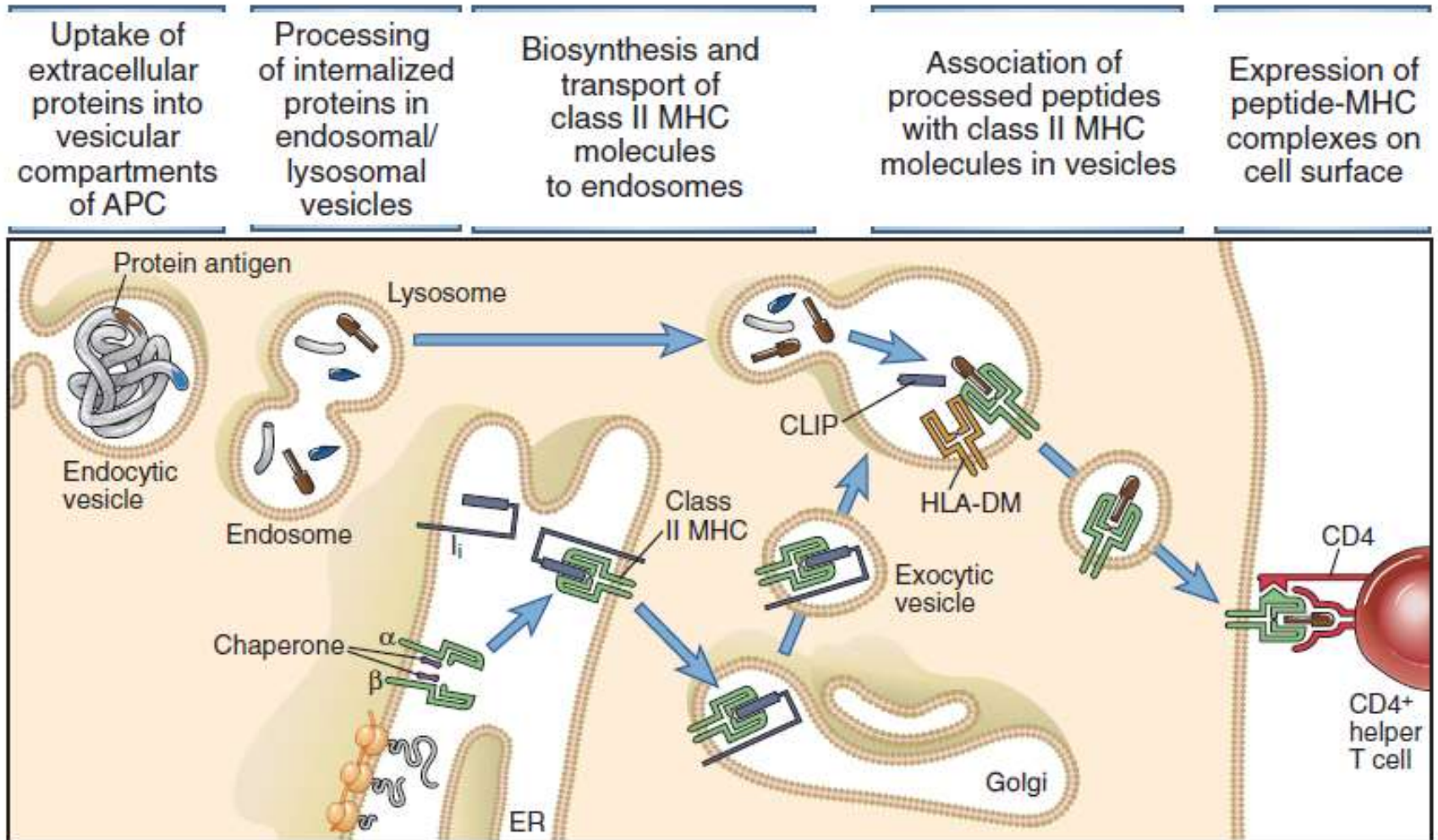
- Antigen processing convert protein antigens present in the cytosol or internalized from the extracellular environment into **peptides** and **load** these peptides onto MHC molecules for display to T lymphocytes.
- Protein antigens that are **present in the cytosol (usually synthesized in the cell)** generate class I-associated peptides that are recognized by CD8+ T cells,
- whereas antigens **internalized from the extracellular environment** into the vesicles of APCs generate peptides that are displayed by class II MHC molecules and recognized by CD4+ T cells.

# Class I MHC Pathway -Cytosolic Proteins



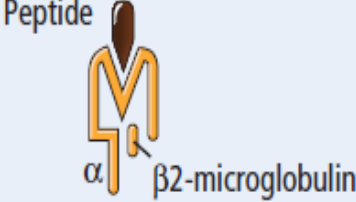
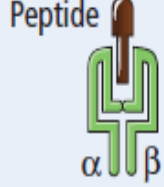
**FIGURE 6-16 The class I MHC pathway of antigen presentation.** The stages in the processing of cytosolic proteins are described in the text. ERAP, endoplasmic reticulum associated peptidase; ER, endoplasmic reticulum;  $\beta_2m$ ,  $\beta_2$ -microglobulin; TAP, transporter associated with antigen processing; Ub, ubiquitin.

# Class II MHC Pathway- Vesicular Proteins



**FIGURE 6-17 The class II MHC pathway of antigen presentation.** The stages in the processing of extracellular antigens are described in the text. CLIP, class II-associated invariant chain peptide; ER, endoplasmic reticulum;  $I_i$ , invariant chain.

# Contd...

TABLE 6-5 Comparative Features of Class I and Class II MHC Pathways of Antigen Processing and Presentation		
Feature	Class I MHC Pathway	Class II MHC Pathway
Composition of stable peptide-MHC complex	Polymorphic $\alpha$ chain, $\beta_2$ -microglobulin, peptide 	Polymorphic $\alpha$ and $\beta$ chains, peptide 
Types of APCs	All nucleated cells	Dendritic cells, mononuclear phagocytes, B lymphocytes; endothelial cells, thymic epithelium
Responsive T cells	CD8 <sup>+</sup> T cells	CD4 <sup>+</sup> T cells
Source of protein antigens	Cytosolic proteins (mostly synthesized in the cell; may enter cytosol from phagosomes)	Endosomal and lysosomal proteins (mostly internalized from extracellular environment)
Enzymes responsible for peptide loading of MHC	Cytosolic proteasome	Endosomal and lysosomal proteases (e.g., cathepsins)
Site of peptide loading of MHC	Endoplasmic reticulum	Specialized vesicular compartment
Molecules involved in transport of peptides and loading of MHC molecules	Chaperones, TAP in ER	Chaperones in ER; invariant chain in ER, Golgi and MIIC/CIIV; DM
APC, antigen-presenting cell; CIIV, class II vesicle; ER, endoplasmic reticulum; MHC, major histocompatibility complex; MIIC, MHC class II compartment; TAP, transporter associated with antigen processing.		

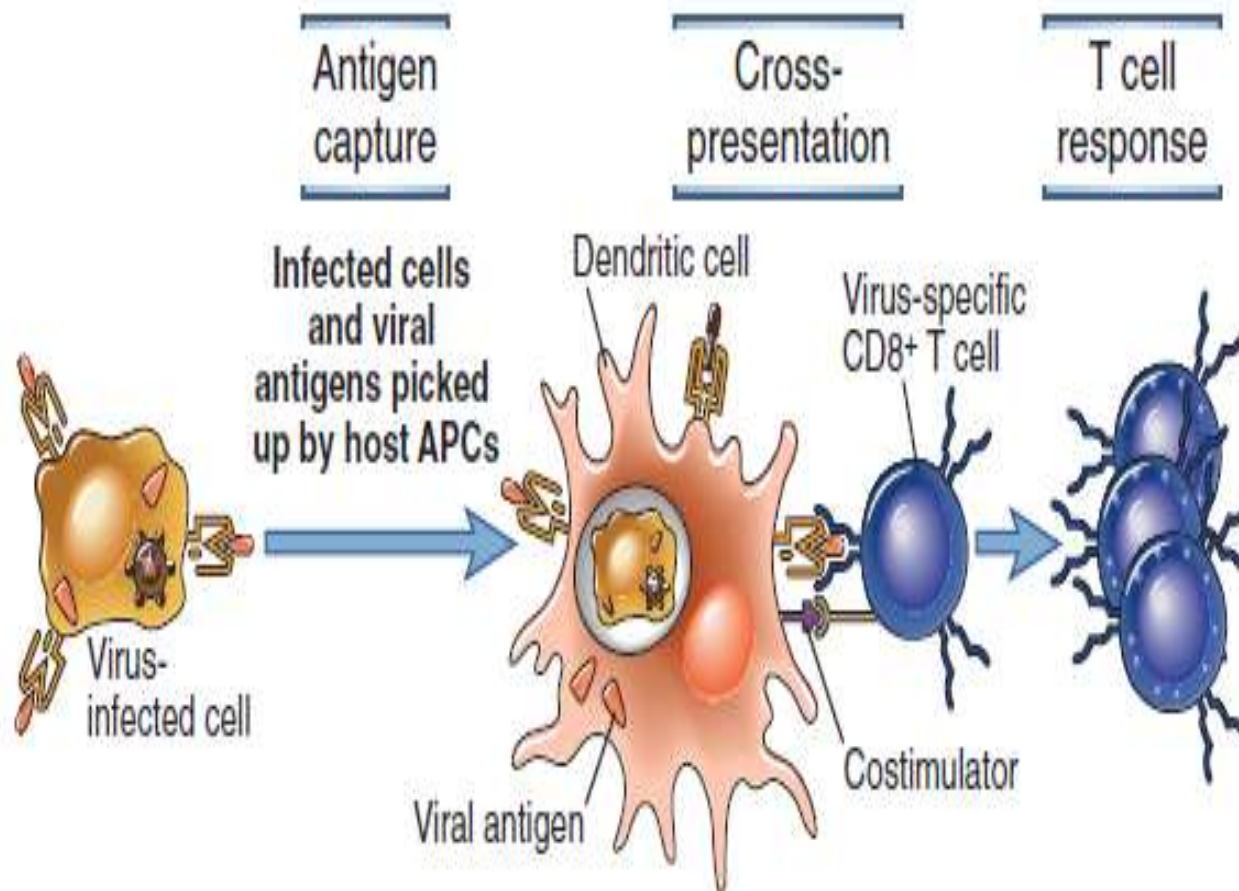


# Contd...

- The fully assembled class I molecule is a **heterotrimer** consisting of an  **$\alpha$  chain**,  **$\beta$ 2-microglobulin**, and a **bound antigenic peptide**, and stable expression of class I molecules on cell surfaces requires the presence of all three components of the heterotrimer.
- The fully assembled class II molecule is a heterotrimer consisting of an  **$\alpha$  chain**, a  **$\beta$  chain**, and a **bound antigenic peptide**, and stable expression of class II molecules on cell surfaces requires the presence of all three components of the heterotrimer.

# CROSS PRESENTATION

- Some dendritic cells have the ability to capture and to ingest virus-infected cells or tumor cells and present the viral or tumor antigens to naive CD8<sup>+</sup> T lymphocytes.



# MHC AND DISEASE SUSCEPTIBILITY

**TABLE 7-4** Some significant associations of HLA alleles with increased risk for various diseases

Disease	Associated HLA allele	Relative risk*
Ankylosing spondylitis	<i>B27</i>	90
Goodpasture's syndrome	<i>DR2</i>	16
Gluten-sensitive enteropathy	<i>DR3</i>	12
Hereditary hemochromatosis	<i>A3</i>	9.3
	<i>B14</i>	2.3
	<i>A3/B14</i>	90
Insulin-dependent diabetes mellitus	<i>DR4/DR3</i>	20
Multiple sclerosis	<i>DR2</i>	5
Myasthenia gravis	<i>DR3</i>	10
Narcolepsy	<i>DR2</i>	130
Reactive arthritis ( <i>Yersinia, Salmonella, Gonococcus</i> )	<i>B27</i>	18
Reiter's syndrome	<i>B27</i>	37
Rheumatoid arthritis	<i>DR4</i>	10
Sjogren's syndrome	<i>Dw3</i>	6
Systemic lupus erythematosus	<i>DR3</i>	5

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