NATURE OF ANTIGENS AND THE MAJOR HISTOCOMPATIBILITY COMPLEX

Immunogens

- macromolecules capable of triggering an adaptive immune response
  - by inducing the formation of antibodies or sensitized T cells in an immunocompetent host.
- can then specifically react with such antibodies or sensitized T cells.

- **Antigen**
  - refers to a substance that reacts with antibody or sensitized T cells

- What's the difference between Immunogen and Antigen?
  - Immunogen is capable of triggering an immune response.
  - Antigen may not be able to evoke an immune response in the first place.
  - All immunogens are antigens, but not all antigens are immunogens.

Antigens

- Most are proteins or large polysaccharides from a foreign organism.
  - Microbes: Capsules, cell walls, toxins, viral capsids, flagella, etc.
  - Nonmicrobes: Pollen, egg white , red blood cell surface molecules, serum proteins, and surface molecules from transplanted tissue.
- Lipids and nucleic acids are only antigenic when combined with proteins or polysaccharides.
- Molecular weight of 10,000 or higher.
  - Hapten: Small foreign molecule that is not antigenic. Must be coupled to a carrier molecule to be antigenic. Once antibodies are formed they will recognize hapten.

FACTORS INFLUENCING THE IMMUNE RESPONSE

- **(1)Age**
  - older individuals
  - Neonates

  - aging results into decline in immune function. Elders, they often do not respond efficiently to novel or previously encountered antigens.

- **(2)Overall health**
  - Malnourishment
  - Fatigue
  - Stress
  - Affect normal functioning

- **(3)Dose**
  - Generally, the larger the dose of an immunogen one is exposed to, the greater the immune response is.
  - However, very large doses can result in T- and B-cell tolerance, a phenomenon that is not well understood. It is possible that memory cells become overwhelmed and therefore nonresponsive.

FACTORS INFLUENCING THE IMMUNE RESPONSE

- **(4)Route of Inoculation**
  - intravenous (into a vein)
  - intradermal (into the skin)
  - subcutaneous (beneath the skin)
  - oral administration

  - Where the immunogen enters the body determines which cell populations will be involved in the response and how much is needed to trigger a response.

In general, the ability of an immunogen to stimulate a host response depends on the following characteristics:

- **(1) macromolecular size**
  - molecular weight of at least 10,000-- to be recognized by the immune system,
  - molecular weight of over 100,000 daltons-- best immunogens
  - the greater the molecular weight, the more potent the molecule is as an immunogen.

- **(2) chemical composition and molecular complexity**
  - Proteins and polysaccharides--are the best immunogens
  - Proteins
    - are powerful immunogens, because they are made up of a variety of units known as *amino acids.*

- Carbohydrates
  - are somewhat less immunogenic than protein
  - As immunogens, carbohydrates most often occur in the form of glycolipids or glycoproteins.
  - Pure nucleic acids and lipids-- are not immunogenic by themselves, although a response can be generated when they are attached to a suitable carrier molecule

- **(3) foreignness**
  - The immune system is normally able to distinguish between self and nonself, and those substances recognized as nonself are immunogenic.

- **(4) the ability to be processed and presented with MHC molecules**
  - A substance must be subject to antigen processing, which involves enzymatic digestion to create small peptides or pieces that can be complexed to MHC molecules to present to responsive lymphocytes.
  - If a macromolecule can't be degraded and presented with MHC molecules, then it would be a poor immunogen.

Types of antigen:

1. T-independent Antigens
   - Can directly stimulate B cells to produce antibodies
   - Polysaccharides in general
   - Generally more resistant to degradation → persist for longer periods of time → continue to stimulate immune system

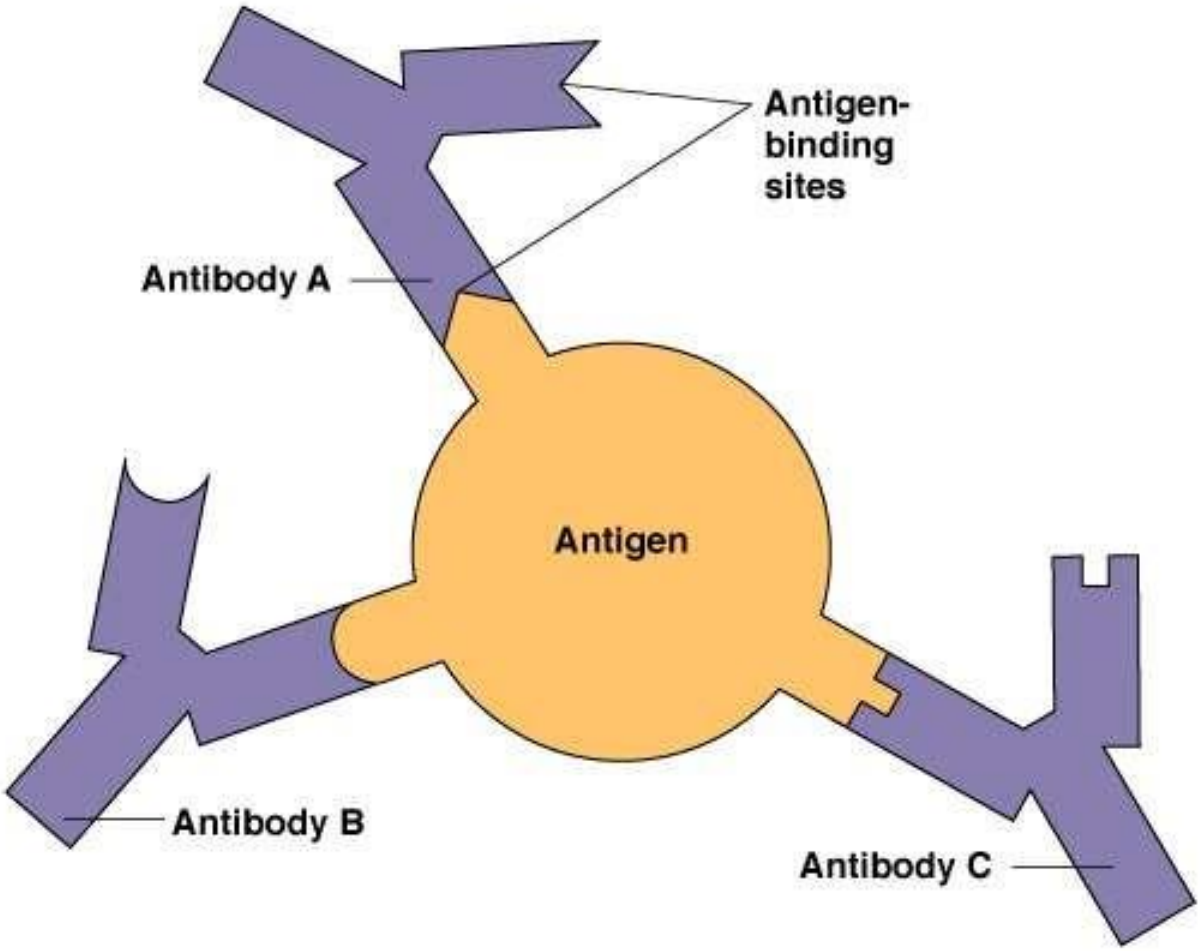
2. T-dependentAntigens
   - **Do not directly stimulate antibody production; need help of T cells**
   - **Usually proteins**

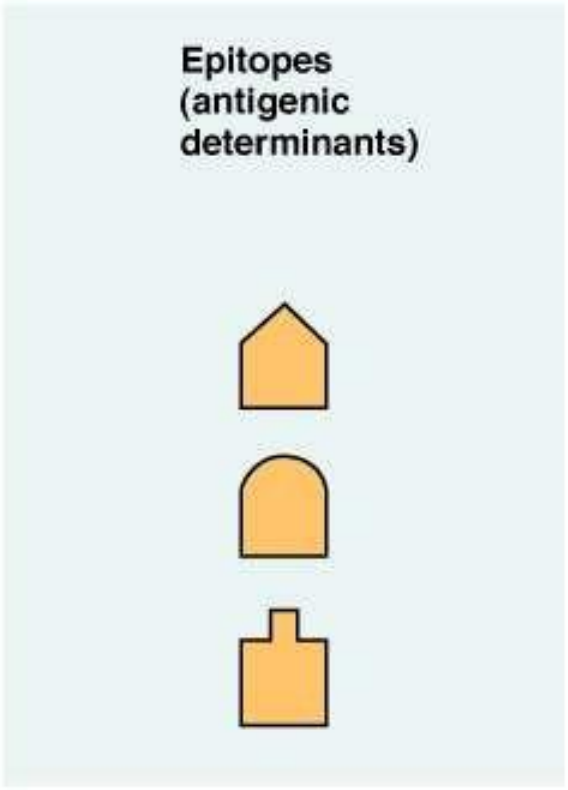
Epitopes

- Small part of an antigen that interacts with an antibody. 10-12 amino acids
- Any given antigen may have several epitopes.
- Each epitope is recognized by a different antibody.

- Large molecules may have numerous epitopes, and each one may be capable of triggering specific antibody production or a T-cell response.
- Epitopes may be repeating copies, or they may have differing specificities. They may also be:
  - sequential or **linear**

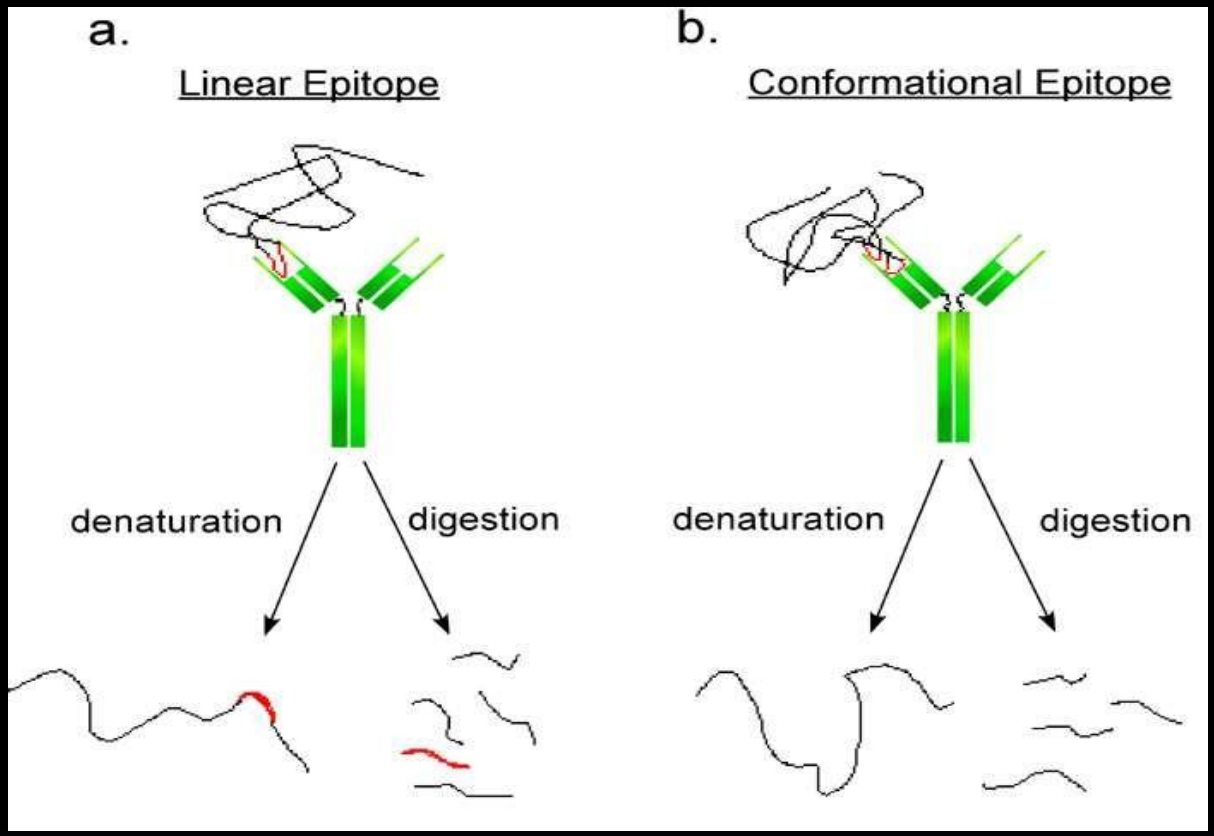
Epitopes: Antigen Regions that Interact with Antibodies





Types:

1. Linear epitope - formed by a specific sequence
2. Conformational epitope - formed by a 3-D structure



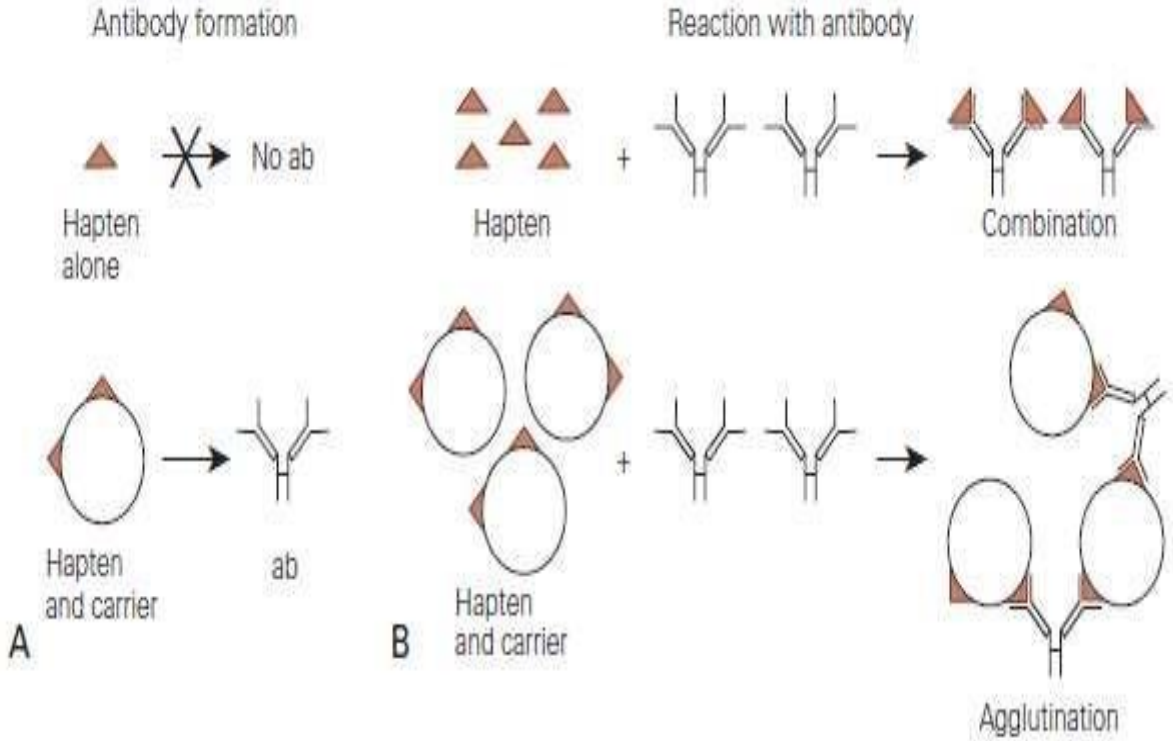
Schematic representation of two antibodies interacting with linear and conformational epitopes.

a.Linear epitopes are ***short and continuous****. After denaturation the linear epitopes* ***may still be able to bind the antibody****.*

b.Conformational epitopes are domains of proteins composed of specific regions of protein chains. After denaturation the discontinuous epitope ***can no longer bind the antibody****.*

HAPTENS

- Molecule that is not immunogenic by itself but can react with specific antibody
- A low MW substance which by itself cannot stimulate an immune response
- Has to be bound to a *carrier molecule (immunogenic molecule)*
- Cannot activate helper T cells → unable to bind to MHC proteins since are not polypeptides
- Univalent → cannot activate B cells by themselves


Antibody formation
Reaction with antibody
No ab
Hapten alone
Hapten
Combination
Hapten and carrier
ab
A
Hapten and carrier
B
Agglutination

RELATIONSHIP OF ANTIGENS TO THE HOST

- Antigens can be placed in broad categories according to their relationship to the host.

- **Autoantigens**
  - are those antigens that belong to the host. These do not evoke an immune response under normalcircumstances.
- **Alloantigens**
  - Are from other members of the host's species, and these are capable of eliciting an immune response.
  - They are important to consider in tissue transplantation and in blood transfusions.
- **Heteroantigens**
  - are from other species, such as other animals, plants, or microorganisms.

ADJUVANTS

- is a substance administered with an immunogen that increases the immune response.
- It acts by producing a local inflammatory response that attracts a large number of immune system cells to the injection site.1

- EXAMPLES:
- (1)Aluminum salts
  - are the only ones approved for clinical use in the United States
  - these are used to complex with the immunogen to increase its size and to prevent a rapid escape from the tissues.
  - It must be injected into the muscle to work.

- The hepatitis B vaccination is an example of using this type of adjuvant.

- (2)Freund’s complete adjuvant
  - consists of mineral oil, emulsifier, and killed mycobacteria (0.5 mg/mL). Antigen is mixed with adjuvant and then injected.
  - It is released slowly from the injection site.
  - Freund’s adjuvant produces granulomas, or large areas of scar tissue, and thus is not used in humans.

(3)Liposomes - defined lipid complexes

(4)Bacterial cell wall components

(5)Polymeric surfactants

- (6)Cholera toxin & E. coli lymphotoxin - potent adjuvants for IgA

Adjuvants are thought to enhance the immune response by:

- prolonging the existence of immunogen in the area
- increasing the effective size of the immunogen
- increasing the number of macrophages involved in antigen processing

MAJOR HISTOCOMPATIBILITY COMPLEX

MAJOR HISTOCOMPATIBILITY COMPLEX

- originally referred to as "*Human Leukocyte Antigens* (HLA)".
  - The French scientist Dausset gave them this name, because they were first defined by discovering an antibody response to circulating white blood cells.

- These antigens are also known as "*Major Histocompatibility Molecules(MHC),* because they determine whether transplanted tissue is HISTOCOMPATIBLE and thus accepted or recognized as foreign and rejected.

- are actually found on **all nucleated cells** in the body
- play a pivotal role in the development of **both humoral and cellular immunity**.
- Their main function is to bring antigen to the cell surface for recognition by T cells
  - T-cell activation will occur only when antigen is combined with MHC molecules.
- Clinically, they are relevant, because they may be involved in transfusion reactions, graft rejection, and autoimmune diseases.

Genes Coding for MHC Molecules (HLA Antigens)

- The MHC system is the most polymorphic system found in humans.
- It is thought that this polymorphism is essential to our survival, because MHC molecules play a pivotal role in triggering the immune response to diverse immunogens.
- Genes coding for the MHC molecules in humans are found on the **short arm of chromosome 6**
- divided into three categories or classes.
  - Class I
  - Class II
  - Class III

- ***GENES:***
  - ***HLA-A*, *HLA-B*, *HLA-C* → code for class I MHC proteins**
  - ***HLA-D (DP, DQ, DR)* →code for class II MHC proteins**
  - Between the class I and class II regions on chromosome 6 is the area of class III genes--which code for complement proteins and cytokines such as tumor necrosis factor.

- Class III proteins are secreted proteins that have an immune function, but they are not expressed on cell surfaces.

- Class I and II gene products are involved in antigen recognition and influence the repertoire of antigens to which T cells can respond.

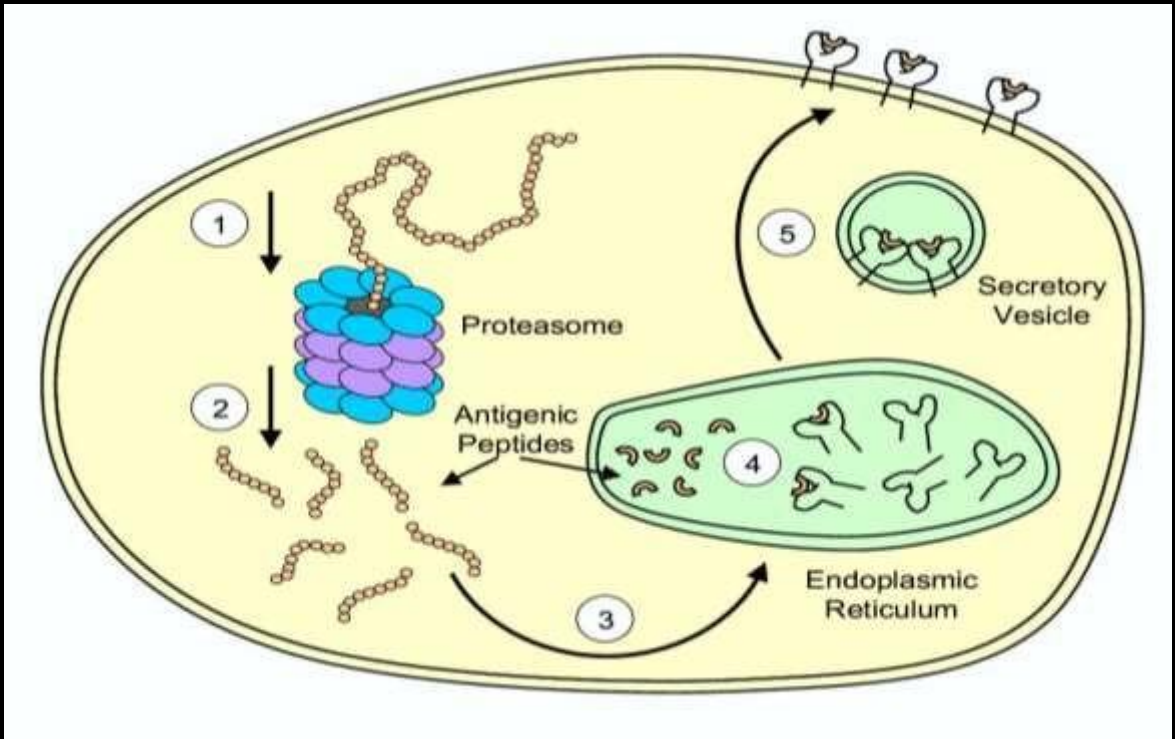
Structure of Class I Molecules

- Found on all nucleated cells and platelets
- Present endogenous peptides
  - They are highest on lymphocytes and low or undetected on liver hepatocytes, neural cells, muscle cells, and sperm.

- Heterodimer --made up of two noncovalently linked polypeptide chains--polymorphic α (heavy) chain non-covalently bound to a β_2-microglobulin (chr. 15)
- Heavy chain composed of:
  1. Hypervariable region – important for recognition of self and non-self
  2. Constant region – CD8+ T cell binding site

- The chain has a molecular weight of 45,000. A lighter chain associated with it, called a β2–microglobulin, has a molecular weight of 12,000 and is encoded by a single gene on chromosome 15 that is not polymorphic.6

- The chain is folded into three domains, 1, 2, and 3, and it is inserted into the cell membrane via a transmembrane segment that ishydrophobic.



Schematic representation depicting processing of antigens presented by class I MHC molecules.

(1) Intracellular proteins are proteolytically degraded within proteasomes →
(2) yields antigenic peptides of 9 – 11 amino acids →
(3) antigenic peptides are transported into the ER →
(4) bind to newly synthesized class I MHC molecules →
(5) Class I MHC-antigenic peptide complexes are exported through the Golgi and to the cell surface, for presentation of antigenic peptide to $CD8^+$ T cells.

Cannon and Pate *Reproductive Biology and Endocrinology* 2003 1:93 doi:10.1186/1477-7827-1-93

- three external domains-- 90 amino acids each
- transmembrane domain--25 hydrophobic amino acids along with a short stretch of about 5 hydrophilic amino acids, and an anchor of 30 amino acids.

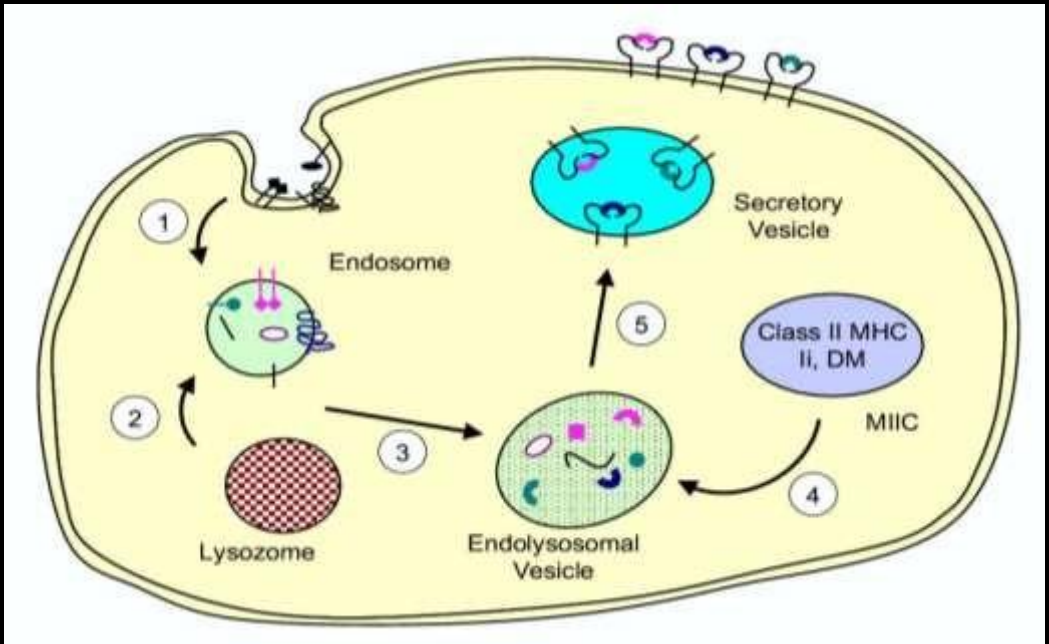
- β2–microglobulin does not penetrate the cell membrane, but it is essential for proper folding of the chain. X-ray crystallographic studies indicate that the 1 and 2 domains each form an alpha helix and that these serve as the walls of a deep groove at the top of the molecule that functions as the peptide-binding site in antigen recognition.

- This binding site is able to hold peptides that are between 8 and 10 amino acids long.
- Most of the polymorphism resides in the 1 and 2 regions, while the 3 and 2 regions are similar to the constant regions found in immunoglobulin molecules.

- The 3 region reacts with CD8 on cytotoxic T cells.
- Another group of molecules called the nonclassical class I antigens are designated E, F, and G. This group of molecules, except for G, are not expressed on cell surfaces and do not function in antigen recognition but may play other roles in the immune response.
- G antigens are expressed on trophoblast cells during the first trimester of pregnancy and are thought to help ensure tolerance for the fetus by protecting placental tissue from the action of NK cells.

Structure of MHC Class II

- *CLASS II MHC MOLECULES*
  - Coded for by *HLA-D (DP,DQ,DR)*
  - Heterodimer → noncovalently associated α chain and β chain
  - Composed of:
    1. Hypervariable region - responsible for polymorphism
    2. Constant region - CD4 T cell binding site
    3. Invariant chain (Ii) - protect the binding site
  - Found on APC's
  - Present exogenous antigens



Schematic representation depicting processing of antigens presented by class II MHC molecules.

(1) Extracellular and integral membrane proteins are internalized into endosomes via endocytosis →

(2) Lysozomes fuse with endosomes.

3) Proteolytic degradation of endocytosed proteins resulting in the generation of antigenic peptides

(4)A specialized subcellualr organelle containing the class II MHC molecules, invariant chain, and DM fuses with the endolysosomal vesicle resulting in proteolytic degradation of invariant chain to CLIP. DM then catalyzes removal of CLIP, and the empty class II MHC molecules then bind antigenic peptides

(5)Class II MHC-antigenic peptide complexes are then exported to the cell surface, for presentation of antigenic peptide to $CD4^+$ T cells.

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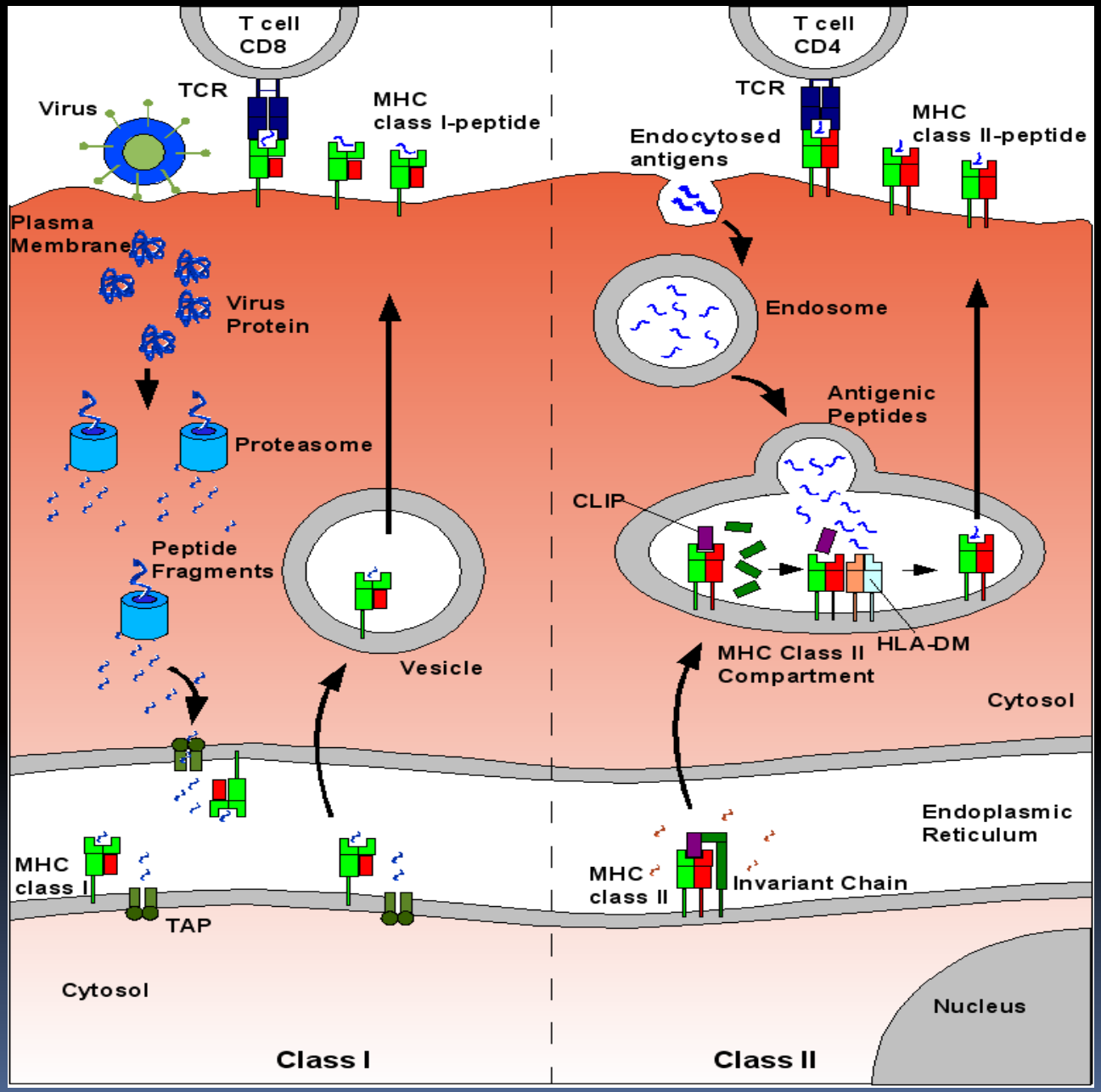
T cell
CD8
TCR
Virus
MHC
class I-peptide
Plasma
Membrane
Virus
Protein
Proteasome
Peptide
Fragments
Vesicle
MHC
class I
TAP
Cytosol
Class I
T cell
CD4
TCR
MHC
class II-peptide
Endocytosed
antigens
Endosome
Antigenic
Peptides
CLIP
HLA-DM
MHC Class II
Compartment
Cytosol
Endoplasmic
Reticulum
MHC
class II
Invariant Chain
Nucleus
Class II

CLASS III MHC MOLECULES

MHC glycoproteins

- Between class I and class II; soluble proteins
- Contain immunologically important genes encoding for:
  1. Cytokines – TNF and lymphotoxin
  2. Complement components – C2 and C4
- Does not have genes that code for histocompatibility antigens

BIOLOGIC IMPORTANCE:

1. Antigen recognition by T cells
   - ✓ CD8 T cells → class I MHC molecules
   - ✓ CD4 T cells → class II MHC molecules
2. Autoimmune diseases occur in people who carry MHC genes (e.g. HLA-B27 in ankylosing spondylitis)
3. Success of organ transplants is determined by compatibility of MHC genes of donor and recipient.

Important Features of Some Human MHC Gene Products

	Class I	Class II
Genetic loci (partial list)	HLA-A, -B, and -C	HLA-DP, -DQ, and -DR
Polypeptide composition	MW 45,000 + β_2M (MW 12,000)	α chain, β chain, and Ii chain
Cell distribution	All nucleated somatic cells	Antigen-presenting cells, activated T cells
Present peptide antigens to	CD8+ T cells	CD4+ T cells
Size of peptide bound	8 - 11 residues	10 - 30 or more residues

Comparison of Class I and Class II MHC Proteins

Feature	Class I MHC	Class II MHC
Present antigen to CD4+ T cells	No	Yes
Present antigen to CD8+ T cells	Yes	No
Found on surface of all nucleated cells	Yes	No
Found on surface of professional APCs	Yes	Yes
Encoded by genes in the HLA locus	Yes	Yes
Expression of genes is codominant	Yes	Yes
Multiple alleles at each gene locus	Yes	Yes
Composed of 2 peptides encoded in HLA locus	No	Yes
Composed of one peptide encoded in the HLA locus & a β2-microglobulin	Yes	No

Structure of Class I I Molecules

- The occurrence of **class II MHC molecules** is much more restricted than that of class I, because they are found primarily on antigen-presenting cells, which include B lymphocytes, monocytes, macrophages, and dendritic cells.
- The major class II molecules—DP, DQ, and DR—consist of two noncovalently bound polypeptide chains that are both encoded by genes in the MHC complex.

- DR is expressed at the highest level, as it accounts for about one-half of all the class II molecules on a particular cell.
- The DR gene is the most highly polymorphic, as 18 different alleles are known at this time.
- Both the chain, with a molecular weight of 33,000, and the chain, with a molecular weight of 27,000, are anchored to the cell membrane.11 Each has two domains, and it is the 1 and the 1 domains that come together to form the peptide-binding site, similar to the one found on class I molecules7,10 (see Fig. 3–5).
- However, both ends of the peptide-binding cleft are open, and this allows for capture of longer peptides than is the case for class I molecules. At least three other class II genes have been described—DM, DN, and DO, the so-called nonclassical class II genes. Products of these genes play a regulatory role in antigen processing.7
- The main role of the class I and class II MHC molecules is to bind peptides within cells and transport them to the plasma membrane, where T cells can recognize them in the phenomemon known as *antigen presentation*.
- T cells can only "see" and respond to antigens when they are combined with MHC molecules. While one individual can express only a small number of MHC molecules, each molecule can present a large number of different antigenic peptides to T cells.
- It is thought that the two main classes of these molecules have evolved to deal with two types of infectious agents:
- those that attack cells from the outside (such as bacteria)
- and those that attack from the inside (viruses and other intracellular pathogens).

- Class I molecules mainly present peptides that have been synthesized within the cell to CD8 (cytotoxic) T cells, while class II molecules present antigen to CD4 (helper) T cells.
- Class II molecules mainly bind exogenous proteins—those taken into the cell from the outsideand degraded.13,14

- Class I molecules are thus the watchdogs of viral, tumor, and certain parasitic antigens that are synthesized within the cell, while class II molecules stimulate CD4 T cells in the case of bacterial infections or the presence of other material that is endocytosed by the cell.13,15
- In either case, for a T-cell response to be triggered, peptides must be available in adequate supply for MHC molecules to bind, they must be able to be bound effectively,

- and they must be recognized by a T-cell receptor.16

- Some viruses, such as herpes simplex and adenovirus, have managed to block the immune response by interfering with one or more processes involved in antigen presentation.
- These viruses are able to maintain a lifelong presence in the host (see Chapter 22 for details).

- The difference in functioning of the two molecules is tied to the mechanisms by which processed antigen is transported to the surface.
- Both types of molecules, however, must be capable of presenting an enormous array of different antigenic peptides to T cells. The chemistry of the MHC antigens controls what sorts of peptides fit in the binding pockets. These two pathways are discussed here.

Role of Class I Molecules

- Both class I and class II molecules are synthesized in the rough endoplasmic reticulum, and for a time they remain anchored in the endoplasmic reticulum membrane.
- Class I molecules, however, actually bind peptides while still in the endoplasmic reticulum.7 In fact, binding helps to stabilize the association of the chain of class I with the B2–microglobulin.16
- However, before binding with antigen occurs, newly synthesized chains freely bind a molecule called *calnexin*. This 88-kd molecule is membrane-bound in the endoplasmic reticulum, and it keeps the chain in a partially folded state while it awaits binding to B2–microglobulin.13,18
- When 2–microglobulin binds, calnexin is released, and three other chaperone molecules—calreticulin, tapasin, and ERp57—are associated with the complex and help to stabilize it for peptide binding17,18
- **(Fig. 3-6).**
- Peptides that associate with the class I molecules are approximately eight to ten amino acids in length and are derived from partial digestion of proteins synthesized in the cytoplasm.

- These intracellular peptides may include viral, tumor, or even bacterial antigens.12 Such peptides may be newly made proteins that fail to fold correctly and hence are defective.

- These are called *defective ribosomal products* (DRiPs).12 Twenty to 70 percent of all proteins synthesized in a cell may fall into this category.8,19 Digestion of these defective or early proteins is carried out by proteases that reside in large cylindrical cytoplasmic complexes called *proteasomes.*
- 13
- Proteasomes are a packet of enzymes that play a major role in antigen presentation.13
- Peptides must be unfolded before entering the cylindrical chamber of the proteosome, and then they are cleaved into the proper size for delivery to class I molecules. Once cleaved, the peptides must then be pumped into the lumen of the endoplasmic reticulum by specialized transporter proteins.7,15
- These two proteins, **transporters associated with antigen processing (TAP1 and TAP2),** are responsible for the adenosine triphosphate–dependent transport, from the cytoplasm to the lumen of the endoplasmic reticulum, of peptides suitable for binding to class I molecules.8,17,18 TAP1 and TAP2 are most efficient at transporting peptides that have 12 amino acids or less.15,17
- Tapasin brings the TAP transporters into close proximity to the newly formed MHC molecules and mediates interaction with them so that peptides can be loaded onto the class I molecules.13,15 Once the chain has bound the peptide, the MHC I-peptide complex is rapidly transported to the cell surface (see Fig. 3–6).6
- Of the thousands of peptides that may be processed in this manner, only a small fraction of them (1 percent or less) actually induce a T-cell response.15 Binding is based on interaction of only two or three amino acid residues with the class I binding groove. Different class I molecules will have slightly different binding affinities, and it is these small differences that determine to which particular antigens one individual will respond.
- It is estimated that a single cell may express about 105 copies of each class I molecule, so many different peptides can be captured and expressed in this manner.10 As few as 10 to 100 identical antigen-MHC I complexes can induce a cytotoxic response.15 In healthy cells, most of these MHC I complexes contain self-peptides that are ignored by the T cells, while in diseased cells, peptides are derived from viral proteins or proteins associated with cancerous states.

- Display of hundreds of class I molecules complexed to antigen allows CD8 T cells to continuously check cell surfaces for the presence of nonself-antigen.
- If it recognizes an antigen as being foreign, the CD8 T cell produces cytokines that cause lysis of the entire cell **(Fig. 3-7).**

Role of Class II Molecules

- Unlike class I molecules, class II molecules must be transported from the endoplasmic reticulum (ER) to an endosomal compartment before they can bind peptides.7
- Dendritic cells are the most potent activators of T cells, and they are excellent at capturing and digesting exogenous antigens such as bacteria. Class II molecules in the endoplasmic reticulum associate with a protein called the **invariant chain (Ii),** which prevents interaction of the binding site with any endogenous peptides in the endoplasmic reticulum.7,13

- The invariant chain is a 31-kd protein that is made in excess so that enough is available to bind with all class II molecules shortly after they are synthesized. Ii may be responsible for helping to bring and chains together in the ER lumen and then moving them out through the Golgi complex to the endocytic vesicles, where digested antigen is found.16
- Because the open structure of class II molecules would permit binding of segments of intact proteins within the ER, Ii serves to protect the binding site.20
- Once bound to the invariant chain, the class II molecule is transported to an endosomal compartment, where it encounters peptides derived from endocytosed, exogenous proteins. Antigen processing may help to unfold molecules and uncover functional sites that are buried deep within the native protein structure.3 The invariant chain is degraded by a protease, leaving just a small fragment called *class II invariant chain peptide* (CLIP) attached to the peptide-binding cleft.14,21 CLIP is then exchanged for exogenous peptides. Selective binding of peptides is favored by the low pH of the endosomal compartment.16 HLA-DM molecules help to mediate the reaction by removing the CLIP fragment. 6,10,21
- Generally, peptides of approximately 13 to 18 amino acid residues can bind, because the groove is open on both ends, unlike class I molecules, which have a closed end.10,14,22,23Within a central core of 13 amino acids, 7 to 10 residues provide the major contact points.10

- Hydrogen bonding takes place along the length of the captured peptide, in contrast to class I molecules, which only bond at the amino and carboxy terminal ends.23,24
- There are also several pockets in the class II proteins that easily accommodate amino acid side chains.
- This gives class II proteins more flexibility in the types of peptides that can be bound.23,24
- Once binding has occurred, the class II protein-peptide complex is stabilized and is transported to the cell surface (see Fig. 3–6). On the cell surface, class II molecules are responsible for forming a trimolecular complex that occurs between antigen, class II molecule, and an appropriate T-cell receptor. If binding occurs with a T-cell receptor on a CD4 T cell, the T helper cell recruits andtriggers a B-cell response, resulting in antibody formation **(Fig. 3-8).**

Clinical Significance of MHC

- Testing for MHC antigens has typically been done, because both class I and class II molecules can induce a response that leads to graft rejection.

- Testing methodology has changed from serological principles to molecular methods, which are much more accurate. The role of the laboratory in transplantation is presented in Chapter 17.

- MHC antigens also appear to play a role in development of autoimmunediseases. The link between MHC antigens and autoimmune diseases is discussed more fully in Chapter 14.
- However, the evidence that both class I and class II moleculesplay a major role in antigen presentation has more far-reaching consequences. They essentially determine thetypes of peptides to which an individual can mount an immune response. Although the MHC molecules typically have a broad binding capacity, small biochemical differences in these proteins are responsible for differences seen in the ability to react to a specific antigen.12 It is possible that nonresponders to a particular vaccine such as hepatitis B do not have the genetic capacity to respond.

- On the other hand, presence of a particular MHC protein may confer additional protection, as the example of HLA B8 and increased resistance to HIV infection shows.8 Therefore, it will be important to know an individual's MHC type for numerous reasons.
- Much of the recent research has focused on the types of peptides that can be bound by particular MHC molecules.
- 23–25 Future developments may include tailoring vaccines to certain groups of such molecules. As more is learned about antigen processing, vaccines containing certain amino acid sequences that serve as immunodominant epitopes can be specifically developed. This might avoid the risk associated with using live organisms. Additionally, if an individual suffers from allergies, knowing a person's MHC type might also help predict the types of allergens to which they may be allergic, because research in this area is attempting to group allergens according to amino acid structure.25 It is likely that knowledge of the MHC molecules will affect many areas of patient care in the future.