Antigen Structure, Processing and Presentation

A Presentation by

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Objectives

- Definition of antigens and epitopes
- Types and sources of antigens
- Antigen processing and presentation
- The roles of Major Histocompatibility Complex (MHC)
- Discuss the role of antigen presentation in generating immunity

Definitions

- **Antigen:**

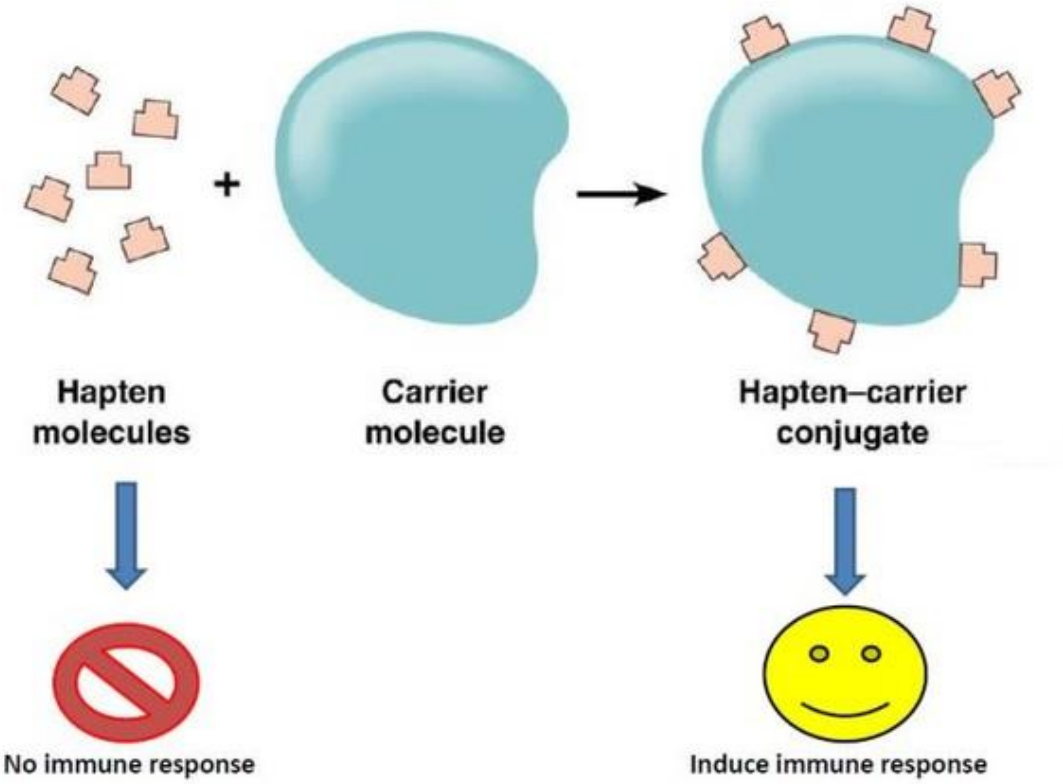
  is any substance that causes your immune system to prompt the generation of antibodies

- **Immunogen**:

  a stimulus that produces a humoral or cell-mediated immune response

- **Haptens**:

  Low molecular weight substances, these substances not immunogenic by itself, if it couples to a larger carrier molecule (albumin, globulins), they become immunogenic



Definitions

- Antigens can be proteins, polysaccharides, conjugates of lipids with proteins (lipoproteins) and glycolipids.
- An antigen may be a foreign substance from the environment such as chemicals, bacteria, viruses, or pollen
- An antigen may also be formed within the body, as with bacterial toxins or tissue cells

Definitions

- **Complete Antigen or Immunogen**
  - Posses antigenic properties denovo, i.e. there are able to generate an immune response by themselves.
  - High molecular weight (more than 10,000)
  - May be proteins or polysaccharides

- **Incomplete Antigen or Hapten**
  - These are the foreign substance, usually non-protein substances
  - Unable to induce an immune response by itself, they require carrier molecule to act as a complete antigen.
    - Serum Protein such as Albumin or Globulin.
  - Low Molecular Weight (Less than 10,000)

Properties that make molecules more effective antigens include:

1. **Foreignness:** foreignness means substances that never contact with lymphocytes in embryo period.
2. **Stable molecules**, ie, molecules that assume and maintain a definite shape.
3. **Larger molecules** with molecular masses between 5000 and 100,000 daltons.
4. **Molecules** that are structurally **complex**, with distinctive shapes and novel subunit combinations
5. **Route of administration**: Parenteral routes are more immunogenic to oral route

Antigenic Determinants-epitopes

- **Epitope** is immunologically active regions of an immunogen (or antigen) that binds to antigen-specific membrane receptors on lymphocytes or to secreted antibodies. It is also called **antigenic determinants.**

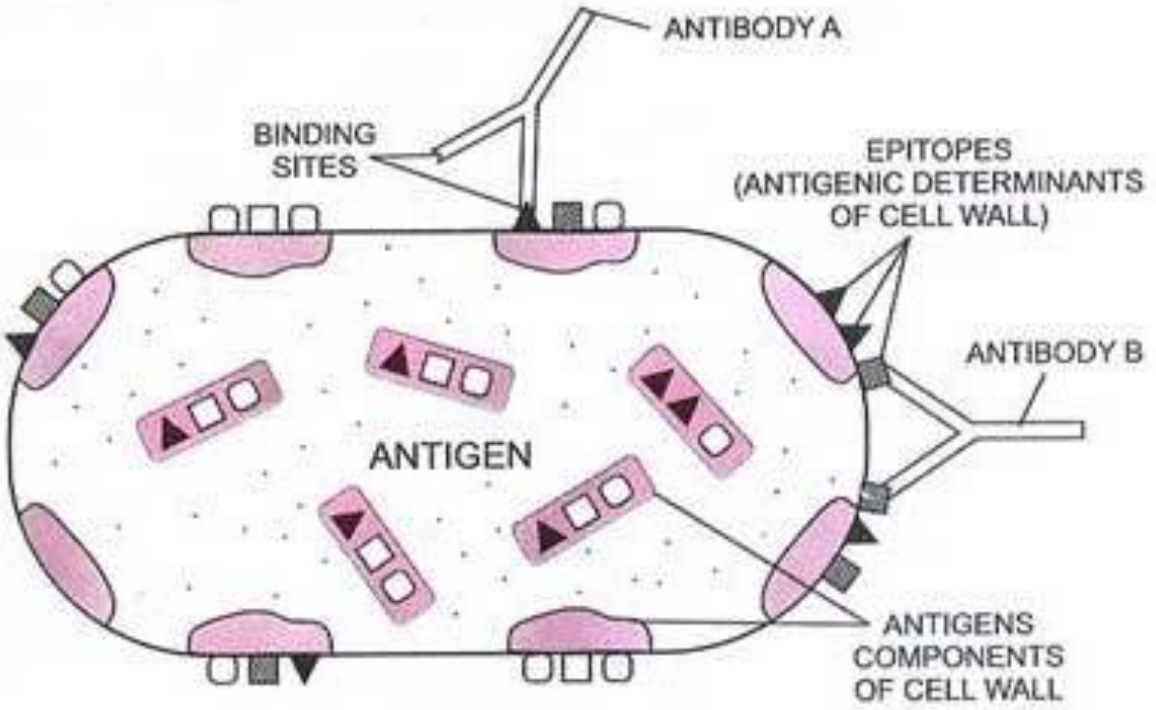


Diagram showing an antigen with epitopes (antigenic determinants). Two attached antibodies are also shown.\

Determinants of Antigenicity

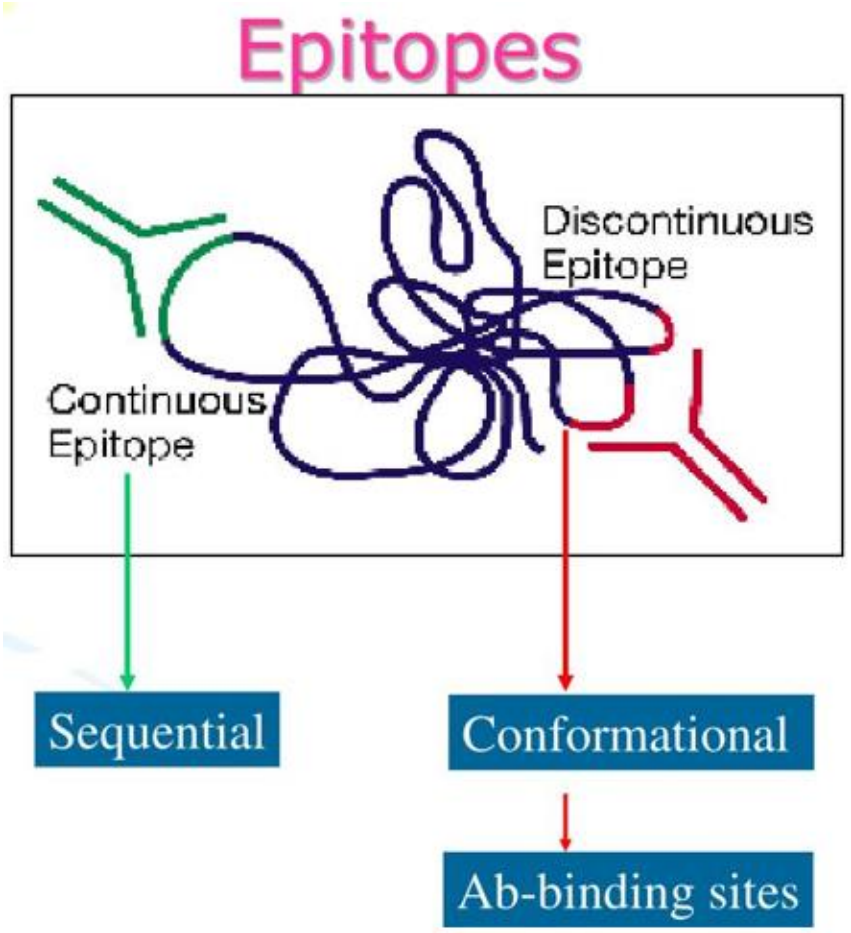
- The whole antigen does not evoke immune response and only a small part of it induces B and T cell response.

- The small area of chemical grouping on the antigen molecule that determines specific immune response and reacts specifically with antibody

Antigenic Determinants-epitopes

- The body recognizes antigens by the three-dimensional shapes or regions called antigenic determinants or epitopes. Sites on or within antigen with which antibodies react
- 2 types of antigenic determinants

1. **Conformational determinants**: amino acid residues that aren't in a sequence but become spatially juxtaposed in the folded protein. They are recognized by B cells or antibody.
2. **Sequential (or linear) determinants**: They are mainly recognized by T cells, but some also can be recognized by B cells.



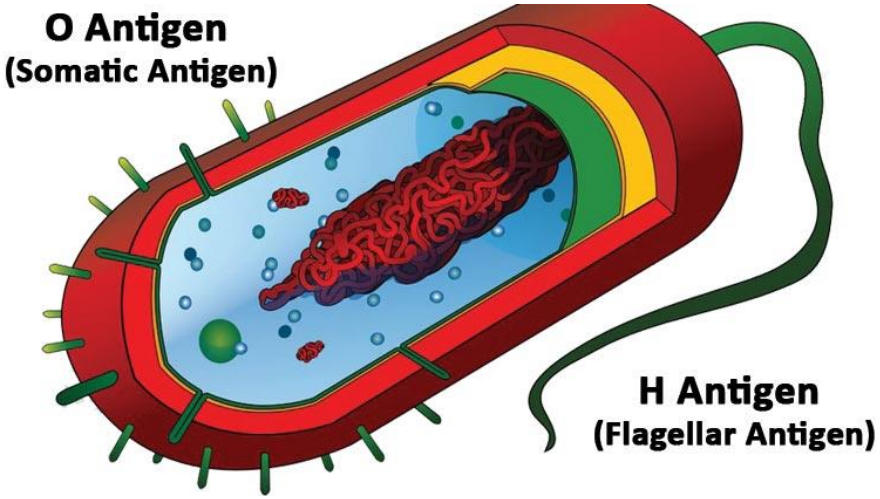
Chemical Nature of Antigens

- **Proteins**
  - The **vast** majority of immunogens
  - Pure proteins or glycoproteins or lipoproteins.
  - Very good immunogens.
- **Polysaccharides**
  - Pure polysaccharides and lipopolysaccharides are good immunogens.
- **Nucleic Acids**
  - Poorly immunogenic.
  - Become immunogenic when single stranded or when complexed with proteins.
- **Lipids**
  - Non-immunogenic, although they may be haptens.

Types of Antigens

- **Exogenous antigens**
- **Endogenous antigens**
- **Autoantigens**

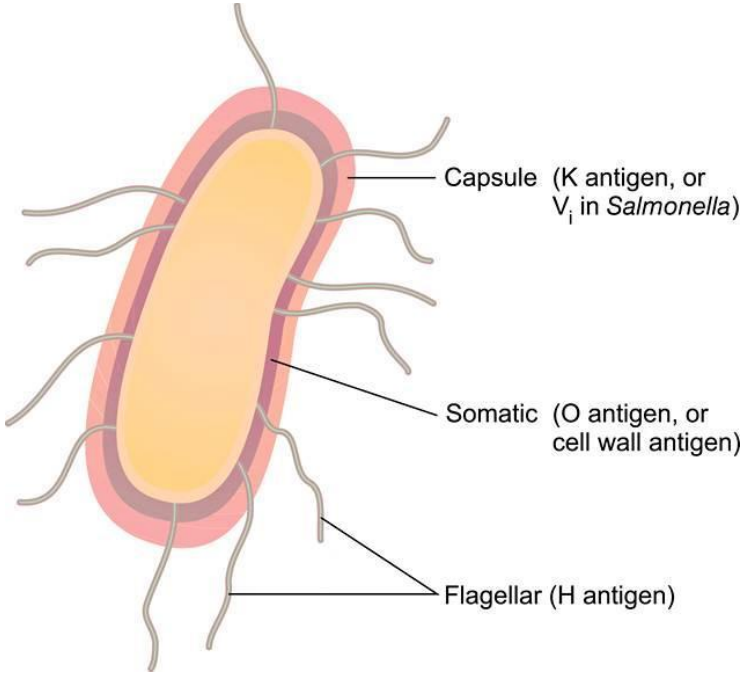
1. Exogenous Antigens



- Exogenous antigens are antigens that have entered the body from the **outside**, for example by **inhalation, ingestion, or injection**

A. Bacterial antigens:

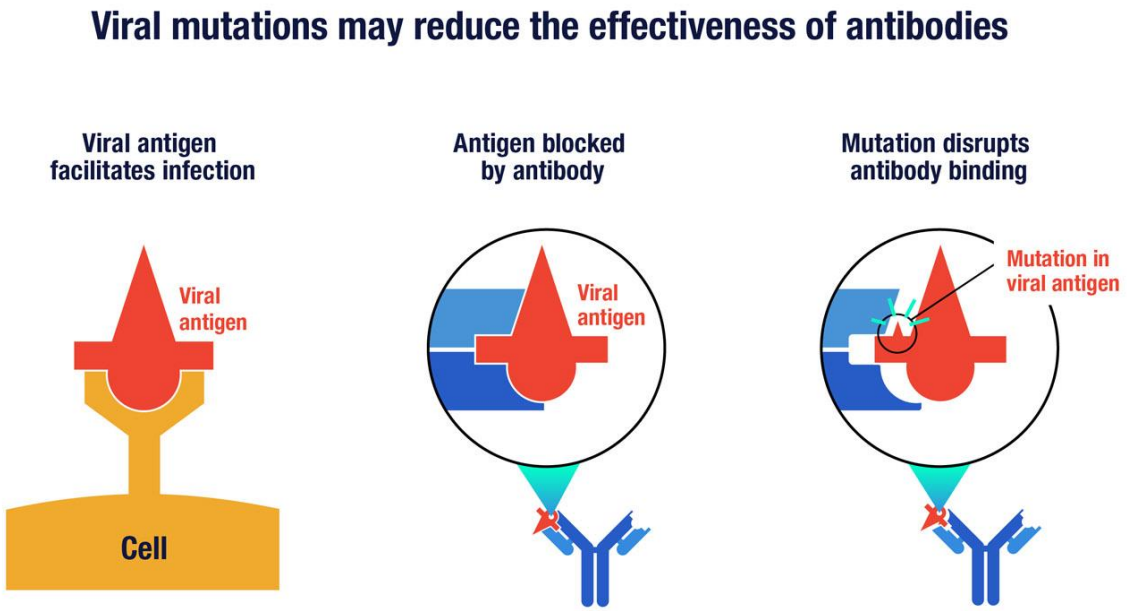
- Antigens related to bacterial cells: Somatic antigen (O), Capsular antigen, Flagellar Ag (H), etc
- Antigen secreted by bacteria: for ex Exotoxins



1. Exogenous Antigens

B. Viral antigens:

- These antigens enters the body or system and start circulating in the body fluids and trapped by the **APCs** (Antigen processing cells such as macrophages, dendritic cells, etc.)
- The uptakes of these exogenous antigens by APCs are mainly mediated by the phagocytosis

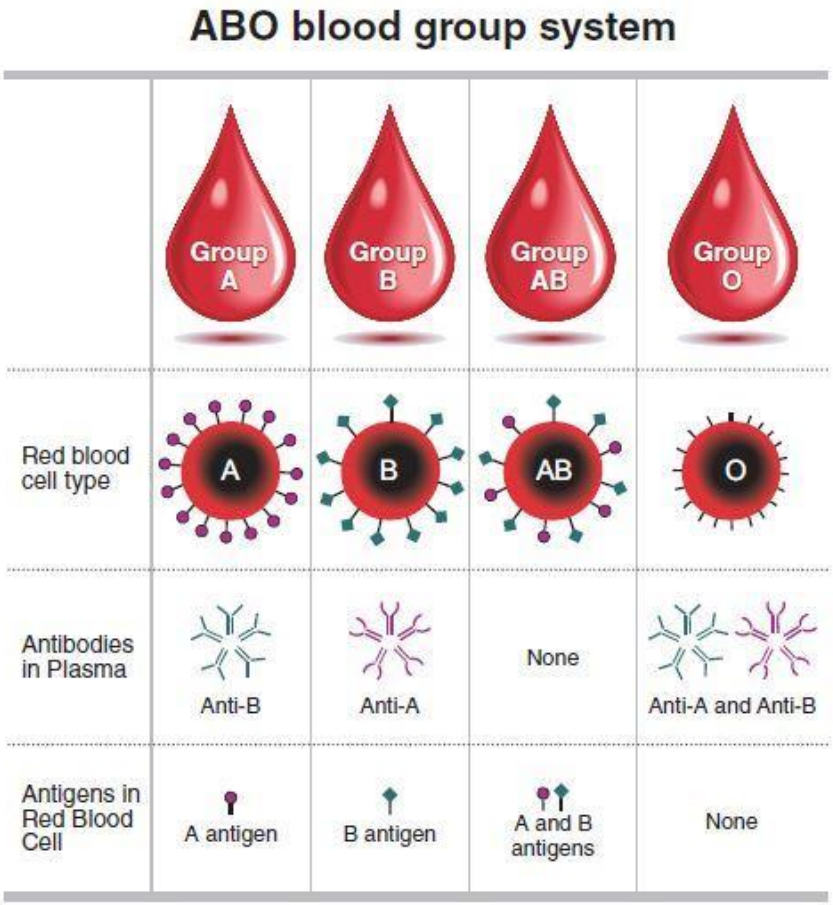


2. Endogenous Antigens

- Endogenous antigens are antigens that have been generated within cells as a result of normal cell metabolism, or because of viral or intracellular bacterial infection
- **Human tissue antigens**:

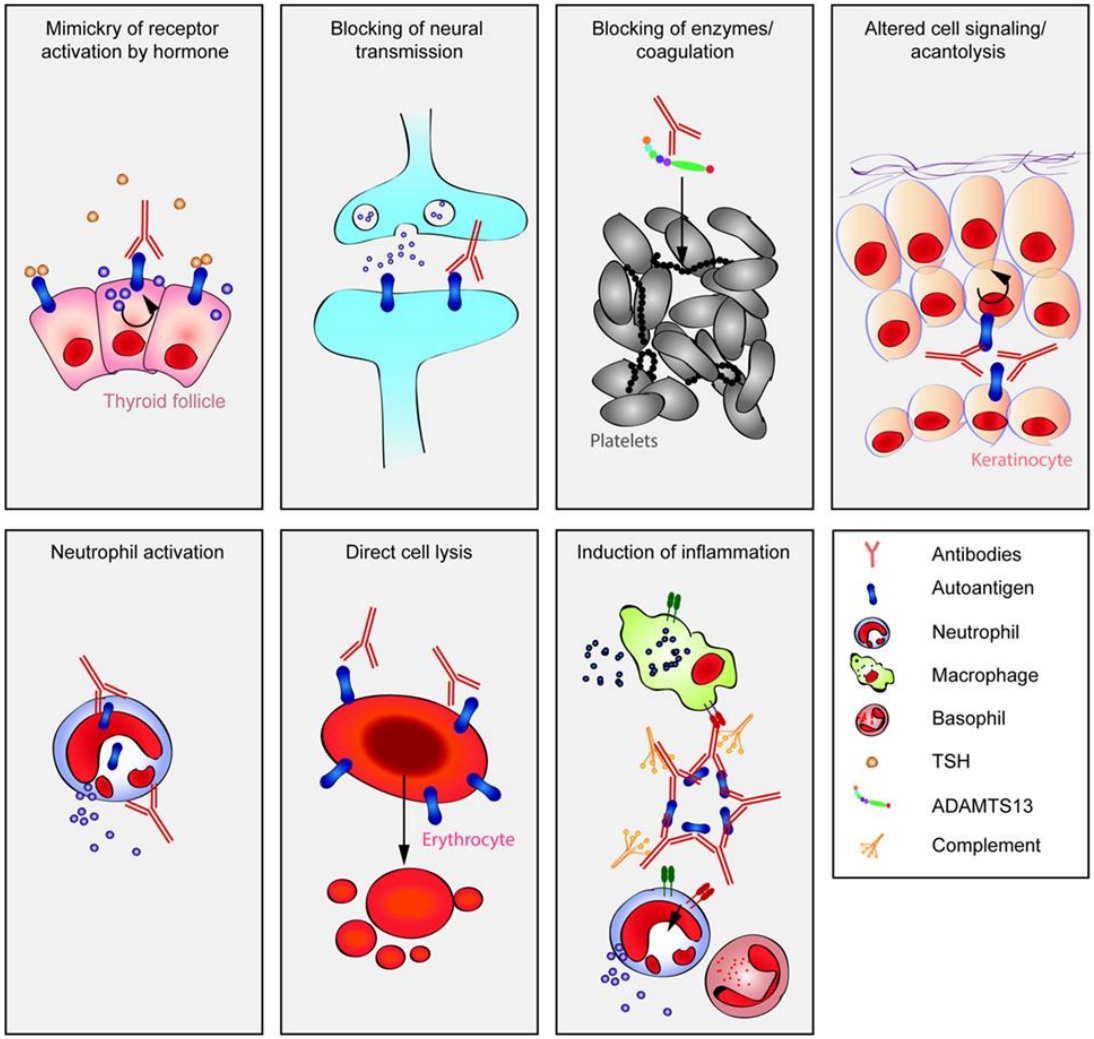
1. **Blood group antigens**: A, B and Rh antigens
2. **Histocompatibility antigens**: Glycoprotein molecules on all nucleotide cells:
   - Major histocompatibility complex antigens (MHC)
   - Human leucocyte antigen (HLA)
3. **Cells infected with viruses**

- Some antigens start out as exogenous antigens, and later become endogenous (for example, intracellular viruses)



3. Autoantigens

- An autoantigen is usually a normal protein or complex of proteins (and sometimes DNA or RNA) that is **recognized** by the immune system of patients suffering from a specific autoimmune disease
- These antigens under normal conditions, not be targeted of the immune system, but due to mainly genetic and environmental factors, the normal immunological tolerance for such an antigen has been lost in these patients



Superantigens (SAgs)

- **SAgs** are a class of antigens that result in excessive activation of the immune system. Specifically it causes:

➢ non-specific activation of multiple clones of T-cells (polyclonal T cell activation) resulting in massive cytokine release.

- They are active at very low concentration causing release of large amounts of cytokines
- The massive T-cell activation and release of large amounts of cytokines cause **systemic toxicity**
- It **does not** lead to acquired immunity i.e no memory
- SAgs are produced by some pathogenic viruses and bacteria most likely as a defense mechanism against the immune system

Superantigens (SAgs)

- **Conventional T-dependent antigen**,
  - only a **small fraction** (1 in 104 -105) of the T cell population is able to recognize the antigen and become activated (monoclonal/oligoclonal response).
- **Superantigens**
  - Polyclonally activate a **large fraction** of the T cells (up to 25%).
  - Examples of superantigens **Bacterial toxins** include:
  - ➢ Staphylococcal enterotoxins (food poisoning),
  - ➢ Staphylococcal aureus toxic shock syndrome toxin (TSST)
  - ➢ Staphylococcal exfoliating toxins (scalded skin syndrome) and
  - ➢ Streptococcal pyrogenic exotoxins (shock).

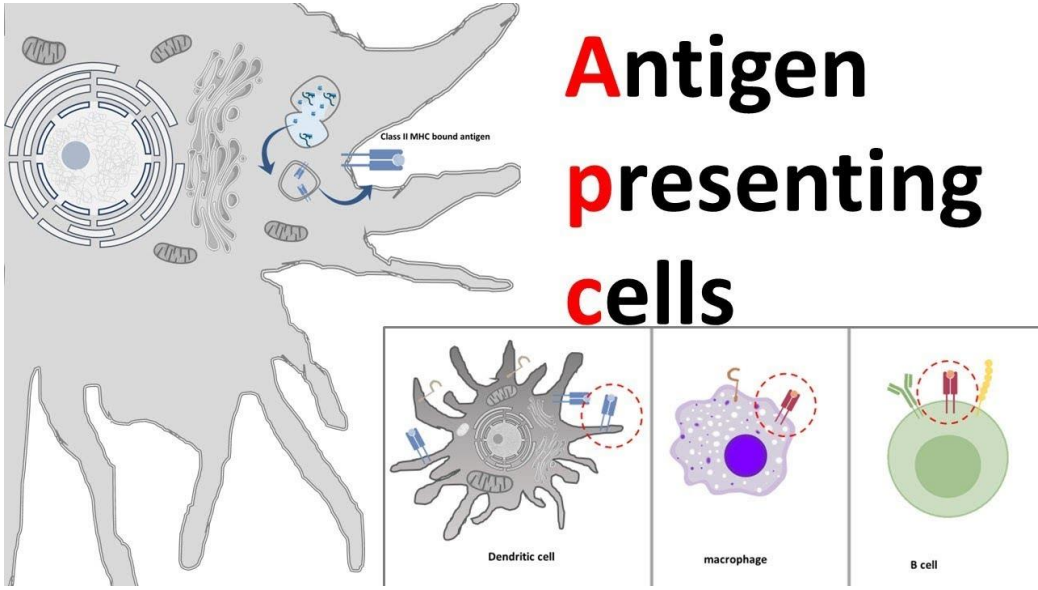
Requirement of T Cells Response

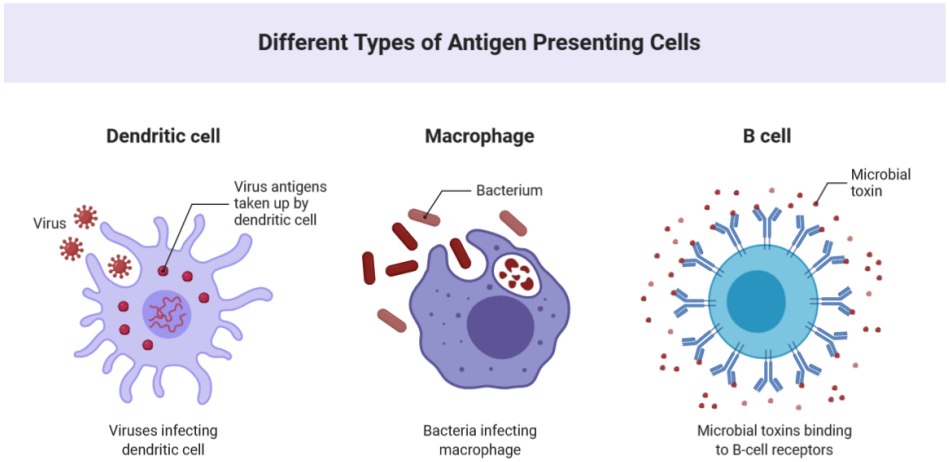
1. **T cells present mainly in lymph and lymphoid organs**, however, microbes usually enter through epithelial cells (like skin, respiratory sys, alimentary track..etc) where T cells number is very low. According, microbial antigens needs to be transported to lymph nodes to enhance chances of encounter with T cells.
2. **T cells can not interact with complex microbial antigen**, antigens need to be captured, processed, and then **presented for T cells** in a specific way in order to allow interactions
3. **T cells** respond only to **protein antigens** and not to other types of **chemical antigens**.

Antigen Presenting Cells (APC)

- A group of immune cells, whose role is to take up, process and present antigenic peptides to T cells.
- **Professional APC**:
  Macrophages, dendritic cells, and B cells, which can express MHC class II molecules
- **Non-professional APC**:

Other cell type capable of expressing MHC class II molecules eg. Endothelial cells, fibroblasts, activated T cell.





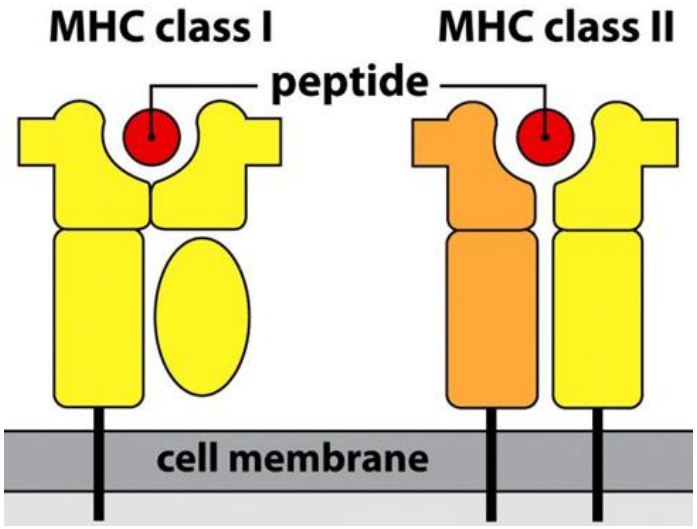
Major Histocompatibility Complex (MHC)

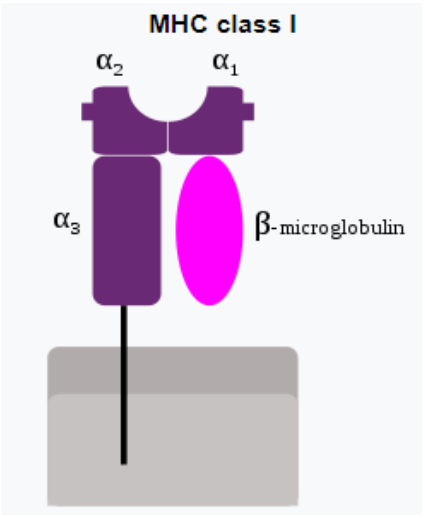
- **MHC molecules:**

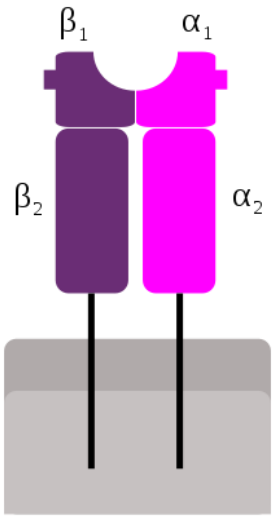
✓ are membrane proteins on APCs that displays peptide antigen for recognition by T cells.

- MHC molecules are the **principle** determinants of **acceptance** or **rejection** of tissue graft
- **Two main classes of MHC**

1. **MHC class I**
2. **MHC class II**



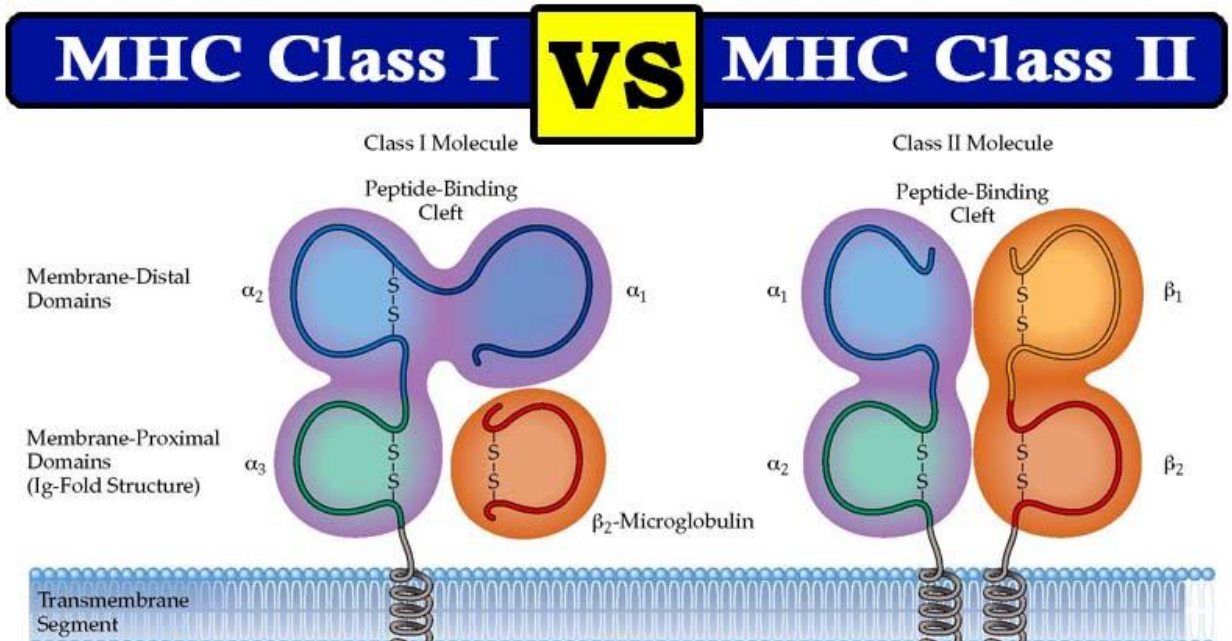




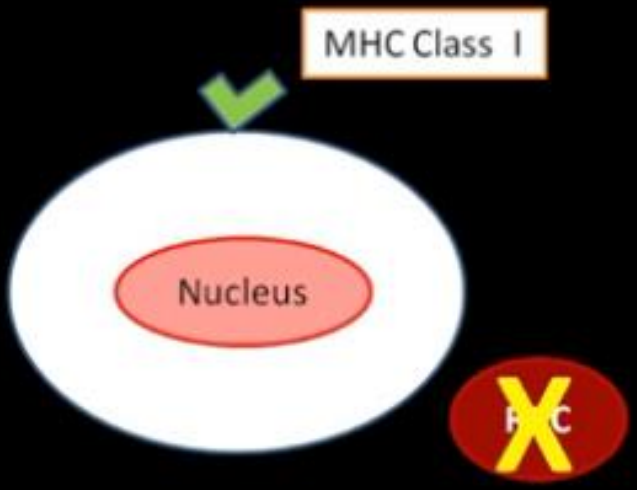
Major Histocompatibility Complex (MHC)

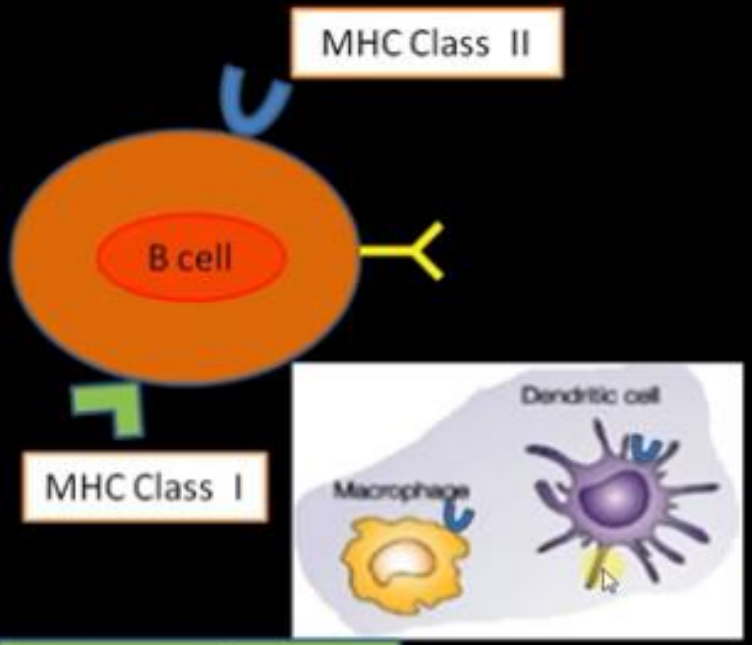
- **MHC class I:**
  - **Function:** Regulation of immune responses to **intracellular** parasites (**endogenous antigens**) such as viruses
  - **All cells** can be infected by viruses, therefore **all** cells **express** MHC class I
  - **Structure**: α1, α2, α3, and β2 macroglobulin
  - Contain a peptide binding cleft that accommodate **8-11** aa
  - Necessary for **CD8+ T** cells activation (cytotoxic T cell)

- **MHC class II:**
  - Regulation of immune responses to **exogenous antigens**, **few cells** are specialized to take up extracellular antigens, and so the distribution of MHC class II **expression** is **restricted** to APCs
  - **Structure**: 2α chains (α1, α2) and 2 β (β1, β2)
  - Contain peptides binding cleft of **10-30** residues
  - Important for binding and activation of **CD4+ T** cells



Difference No: 1



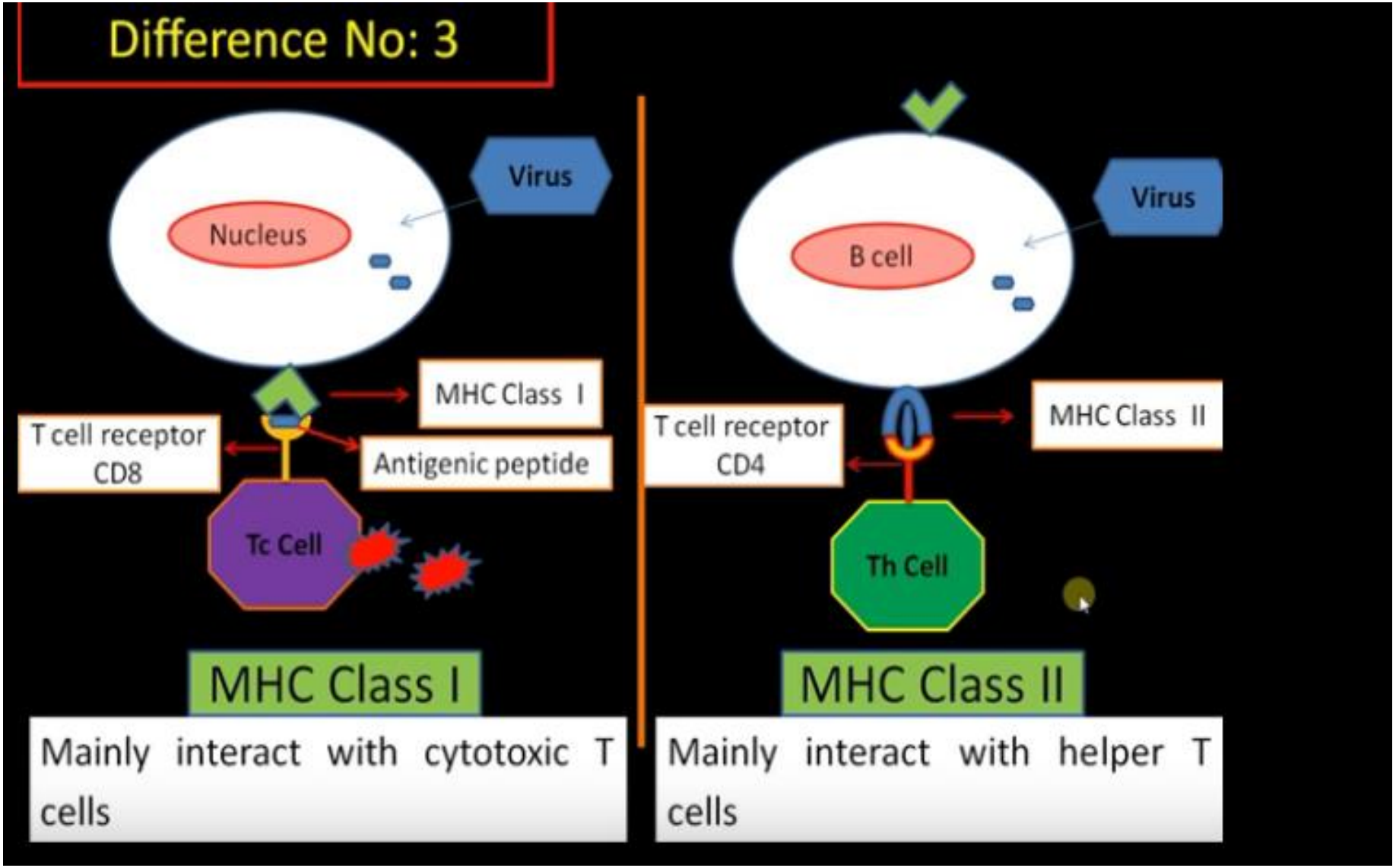


MHC Class I

Present on the surface of all nucleated cells

MHC Class II

Present on the surface of antigen presenting cells such as macrophages, B cells, dendritic cells etc.


Difference No: 3
Virus
Nucleus
MHC Class I
T cell receptor
CD8
Antigenic peptide
Tc Cell
MHC Class I
Mainly interact with cytotoxic T cells
Virus
B cell
MHC Class II
T cell receptor
CD4
Th Cell
MHC Class II
Mainly interact with helper T cells

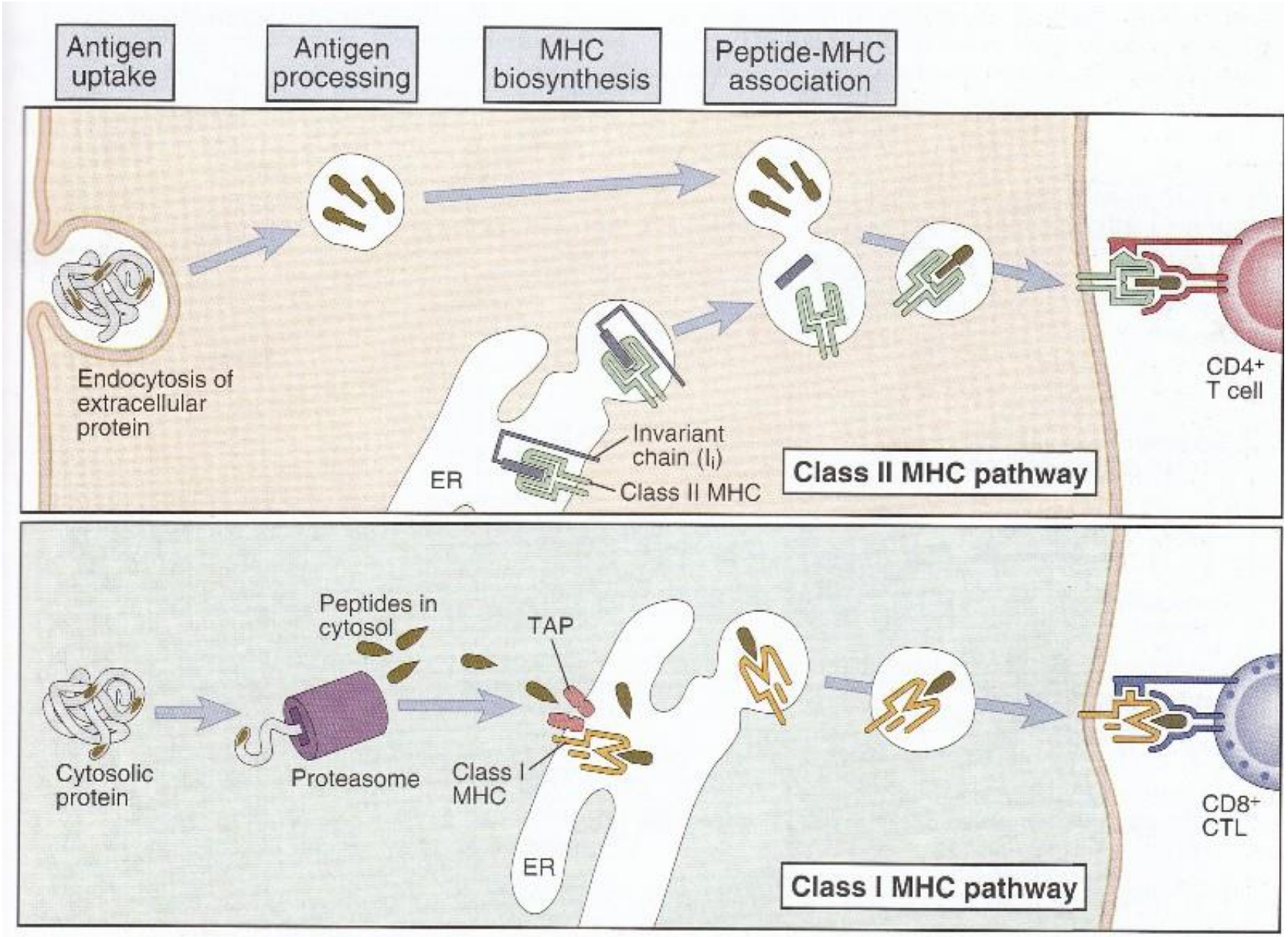
Features of Peptides Binding to MHC Molecules

- MHC molecules present **antigenic peptides** on the surface of cells to be recognized by **specific T-cells.**
- Each MHC molecule displays one peptide at a time
- Peptides are acquired during intracellular assembly
- **Low affinity and broad specificity binding** so many different peptides can bind to the same MHC molecule, and even can bind self peptides
- **Very low off rate**: MHC display bound peptides long enough to be located by T cells
- **Stable expression of MHC molecules** require peptides displaying
- **MHC molecules bind only to peptides** (protein antigen) so T cells can **only** respond to protein antigens

Steps in Antigen Preparation for T cells

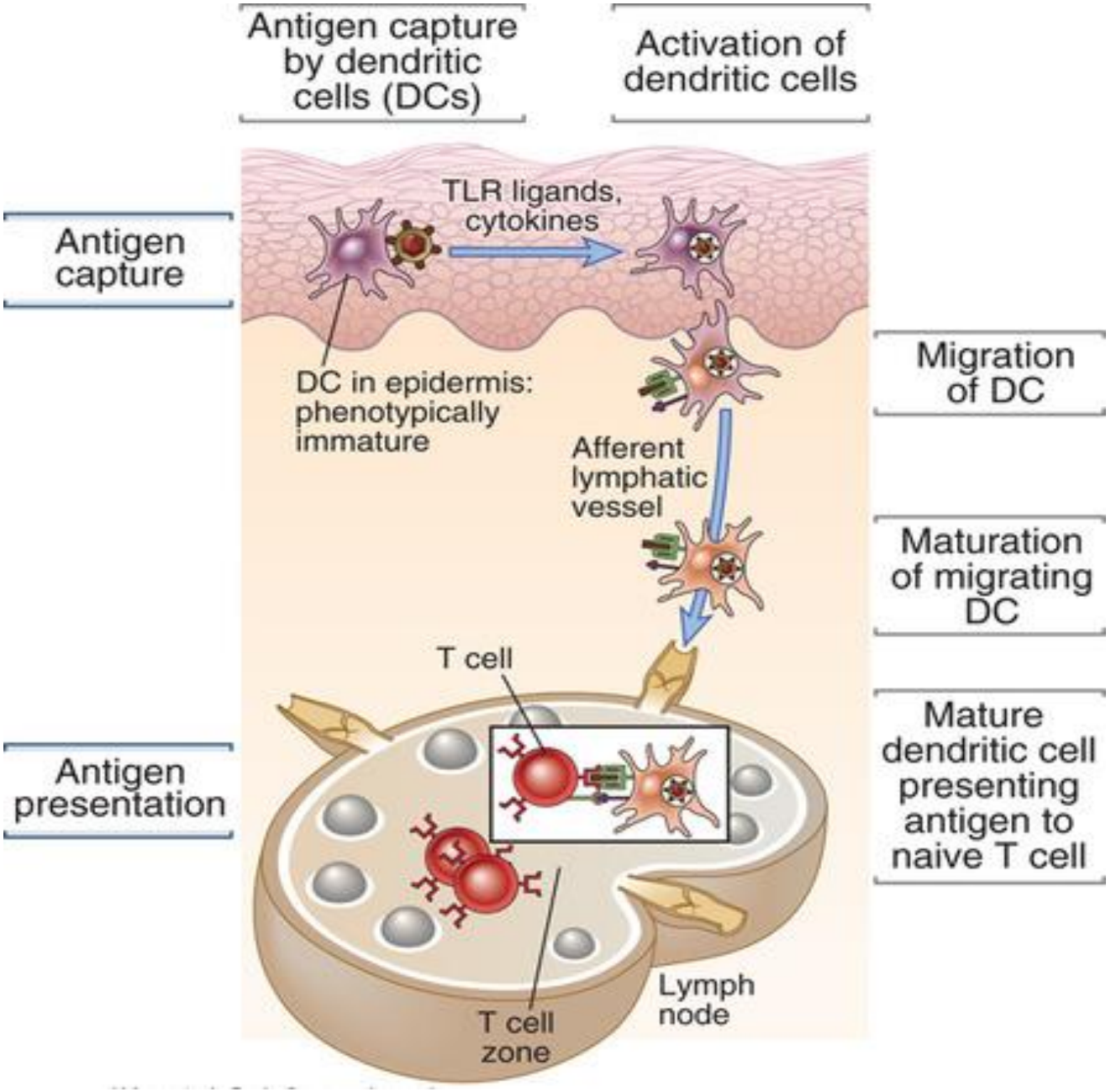
- **Antigens must be prepared in order to be recognized by T cells**

1. **TRANSPORT**: Antigen must be transported to lymph node for proper interaction with T cells
2. **UPTAKE**: Access of native antigens and pathogens to intracellular pathways of degradation
3. **DEGRADATION**: Limited proteolysis of antigens to peptides
4. **ANTIGEN-MHC COMPLEX FORMATION**: Loading of peptides onto MHC molecules
5. **ANTIGEN PRESENTATION**: Transport and expression of peptide-MHC complexes on the surface of cells for recognition by T cells


Antigen uptake
Antigen processing
MHC biosynthesis
Peptide-MHC association
Endocytosis of extracellular protein
ER
Invariant chain (I_i)
Class II MHC
Class II MHC pathway
CD4+ T cell
Peptides in cytosol
TAP
Cytosolic protein
Proteasome
Class I MHC
ER
Class I MHC pathway
CD8+ CTL

1. Antigen Capture and Transport to Lymph Node

- **Immature dendritic cells** (DCs) in the epithelium capture microbial antigens and leave the epithelium
- The DCs **migrate** to draining lymph nodes being attracted by chemokines produced in the nodes
- During their migration the DCs **mature**
- Once at the lymph nodes the DCs start the processing of presenting the antigen to T cells
- DCs at their maturation **express** different membrane proteins, for example **immature** DCs **express** surface receptors essential for **microbial** binding and capture, while **mature** DCs **express MHC** molecules necessary for antigen processing


Antigen capture by dendritic cells (DCs)
Activation of dendritic cells
Antigen capture
TLR ligands, cytokines
DC in epidermis: phenotypically immature
Migration of DC
Afferent lymphatic vessel
Maturation of migrating DC
T cell
Antigen presentation
Mature dendritic cell presenting antigen to naive T cell
Lymph node
T cell zone

2. Uptake of Antigen

- The process of entering the antigens into the cells (mainly APCs) this is important for **exogenous** antigen processing, while **endogenous** antigens are already inside the cells.
- **Uptake by immature DCs**

–**Pinocytosis**: Liquid or small granule

–**Receptor-mediated endocytosis**

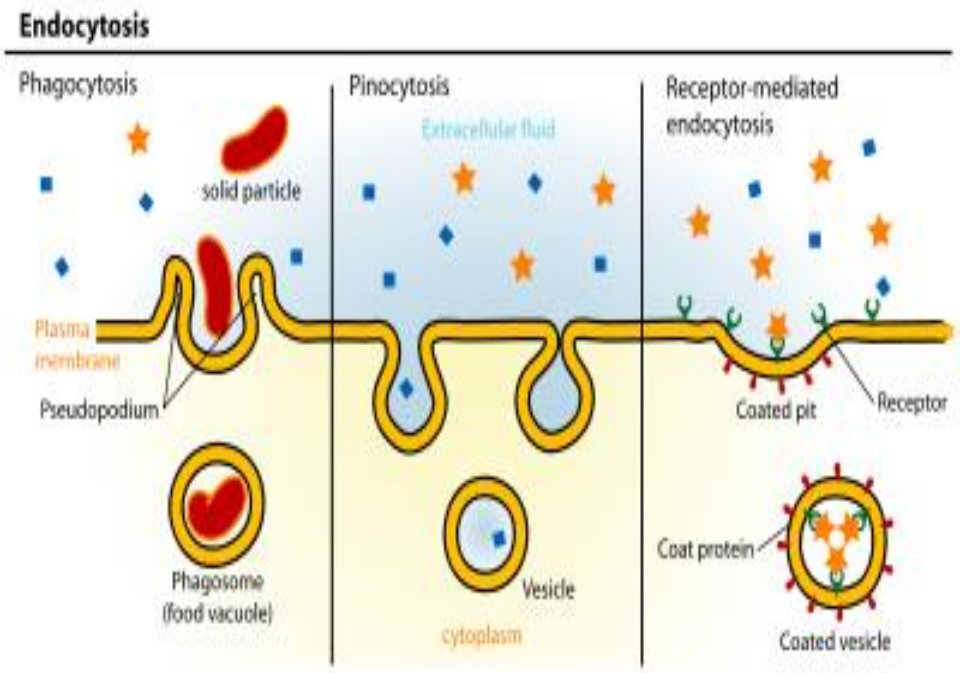
–**Phagocytosis**: Large molecular or microbe

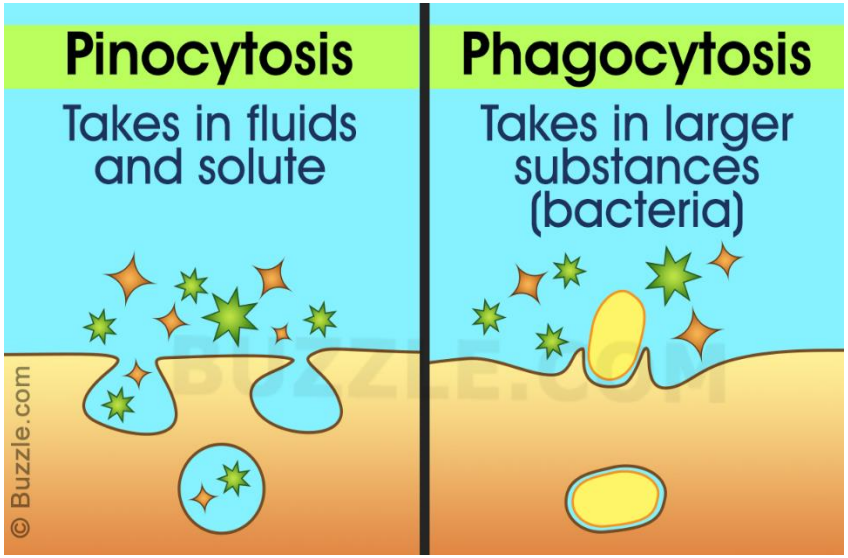
- **Uptake by Macrophage**

–**Phagocytosis**: Large solid or molecular complex

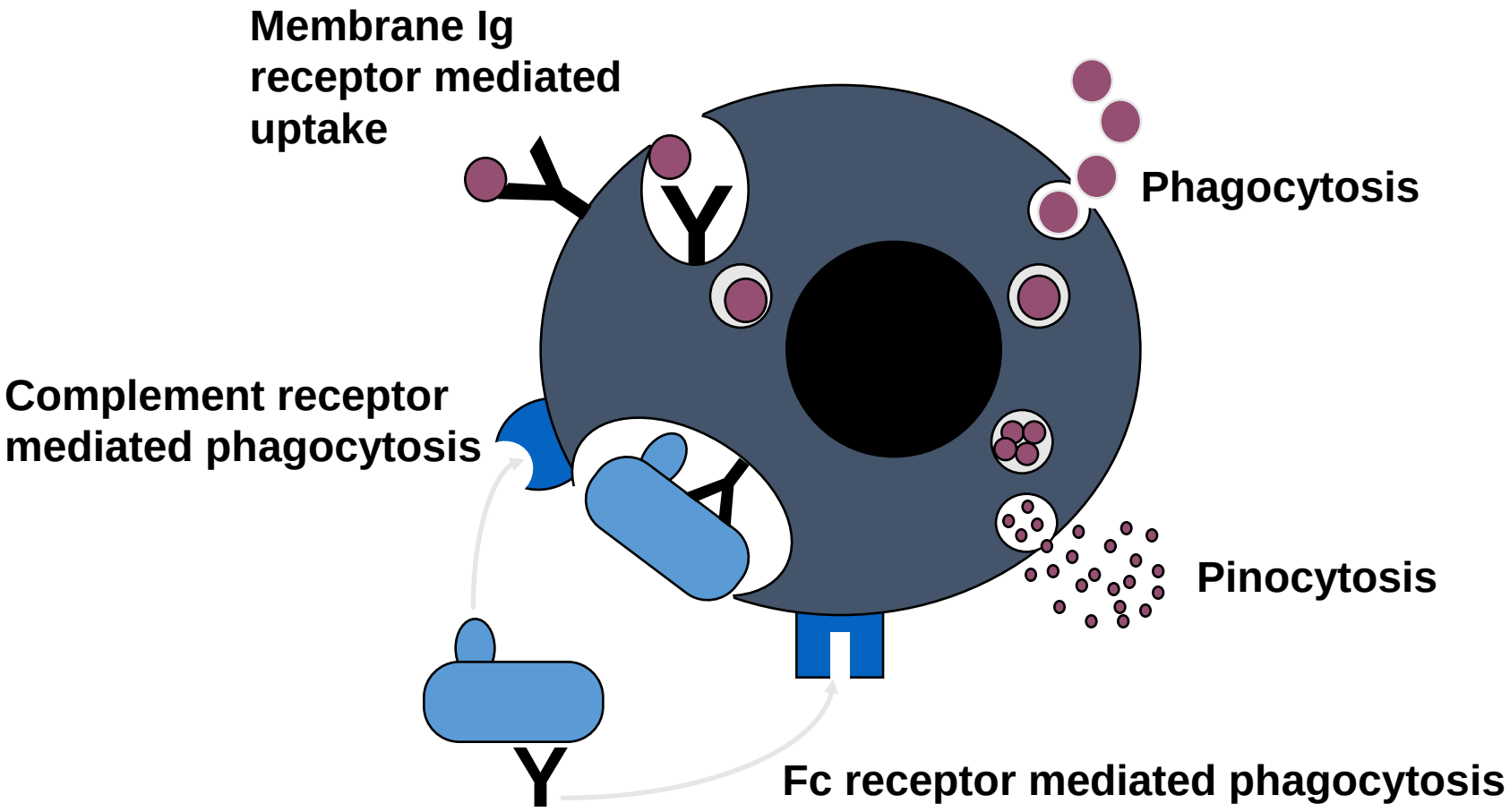
–**Pinocytosis**: Receptor-mediated pinocytosis

–**Endocytosis**: Low levels of particulate or soluble antigens





Uptake of exogenous antigens



Uptake mechanisms direct antigen into intracellular vesicles for exogenous antigen processing

3. Antigen Processing

- Antigens must be processed in order to be recognized by T cells, require 2 main steps
  - Degradation of externally- or internally-derived antigen into short peptide sequences
  - Association of the peptide with MHC molecules

Two antigen-processing pathways

	MHC class I	MHC class II
Major antigen sources	endogenous antigen	exogenous antigen
Processing machinery	proteasome	lysosomal enzymes
Cell type where active	all nucleated cells	professional APCs
Site of antigen-MHC binding	endoplasmic reticulum	lysosome and endosome
MHC utilized	MHC class I	MHC class II
Presents to	CD8+ T cell (Tc)	CD4+ T cells (Th)

4. Antigen Presentation

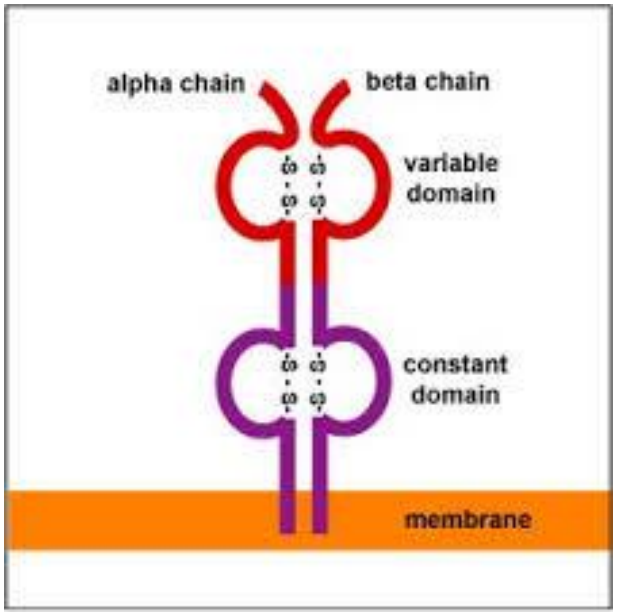
- The activation of T cells via T cell receptors, which specifically recognize antigenic peptide in association with either MHC class I or II molecules on the surface of APC.

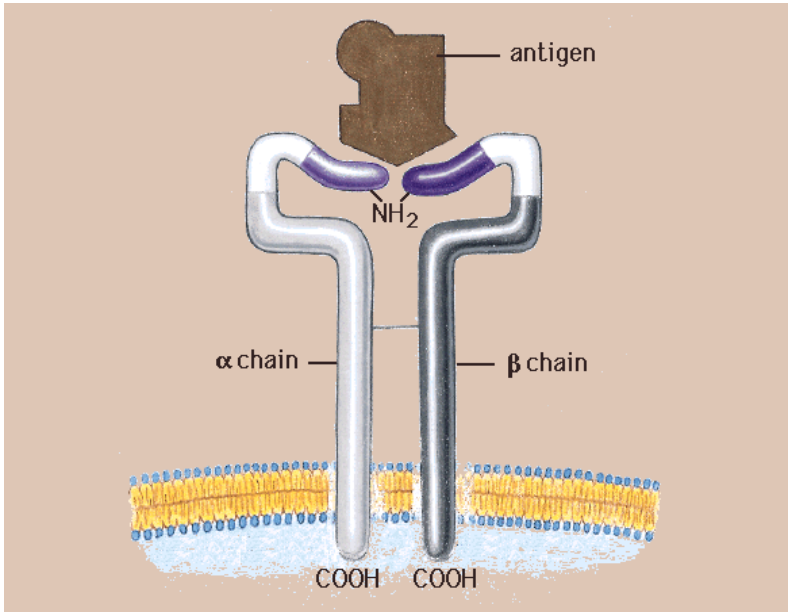
5. Antigen Recognition

- Antigens are recognized by and bind to:

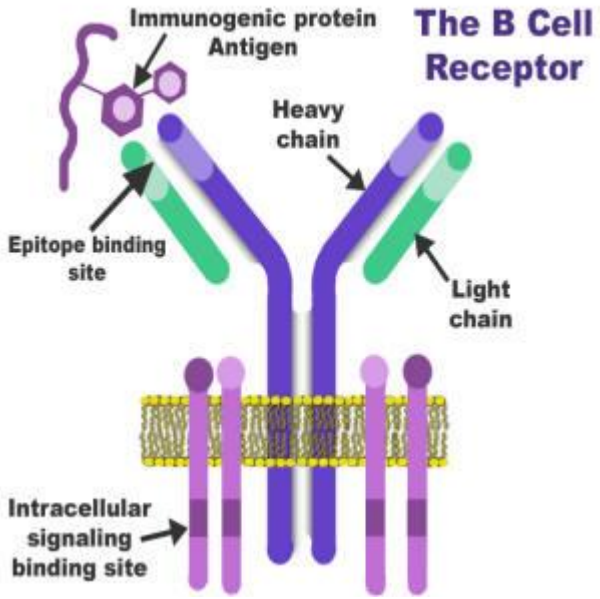
1. **B-cell receptors (BCR)** :
   - These are membrane-bound immunoglobulins (IgM and IgD) on B-cells
   - BCRs can be secreted in plasma as antibodies
2. **T-cell receptors (TCR)**
   - α and β chains anchored to T-cells
   - There is a groove which binds small peptides presented by MHC on surface of APCs

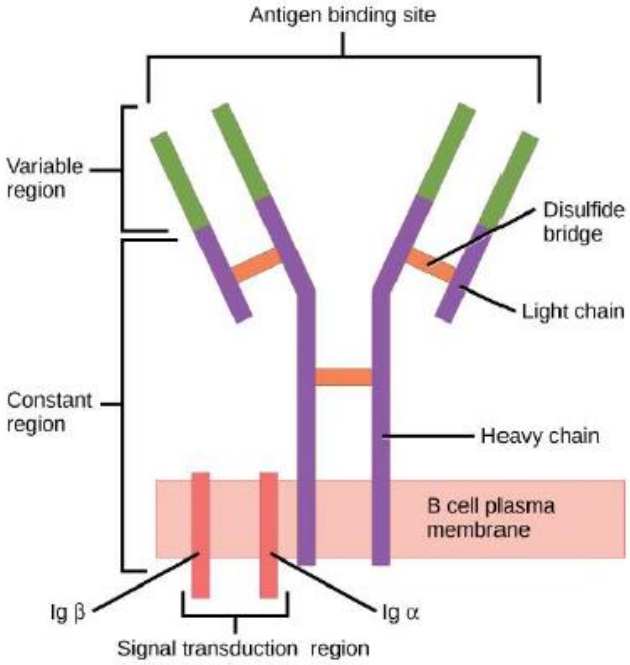
TCR


alpha chain
beta chain
variable domain
constant domain
membrane


antigen
NH2
α chain
β chain
COOH
COOH

BCR


Immunogenic protein Antigen
The B Cell Receptor
Heavy chain
Epitope binding site
Light chain
Intracellular signaling binding site


Antigen binding site
Variable region
Disulfide bridge
Light chain
Constant region
Heavy chain
B cell plasma membrane
Ig β
Ig α
Signal transduction region