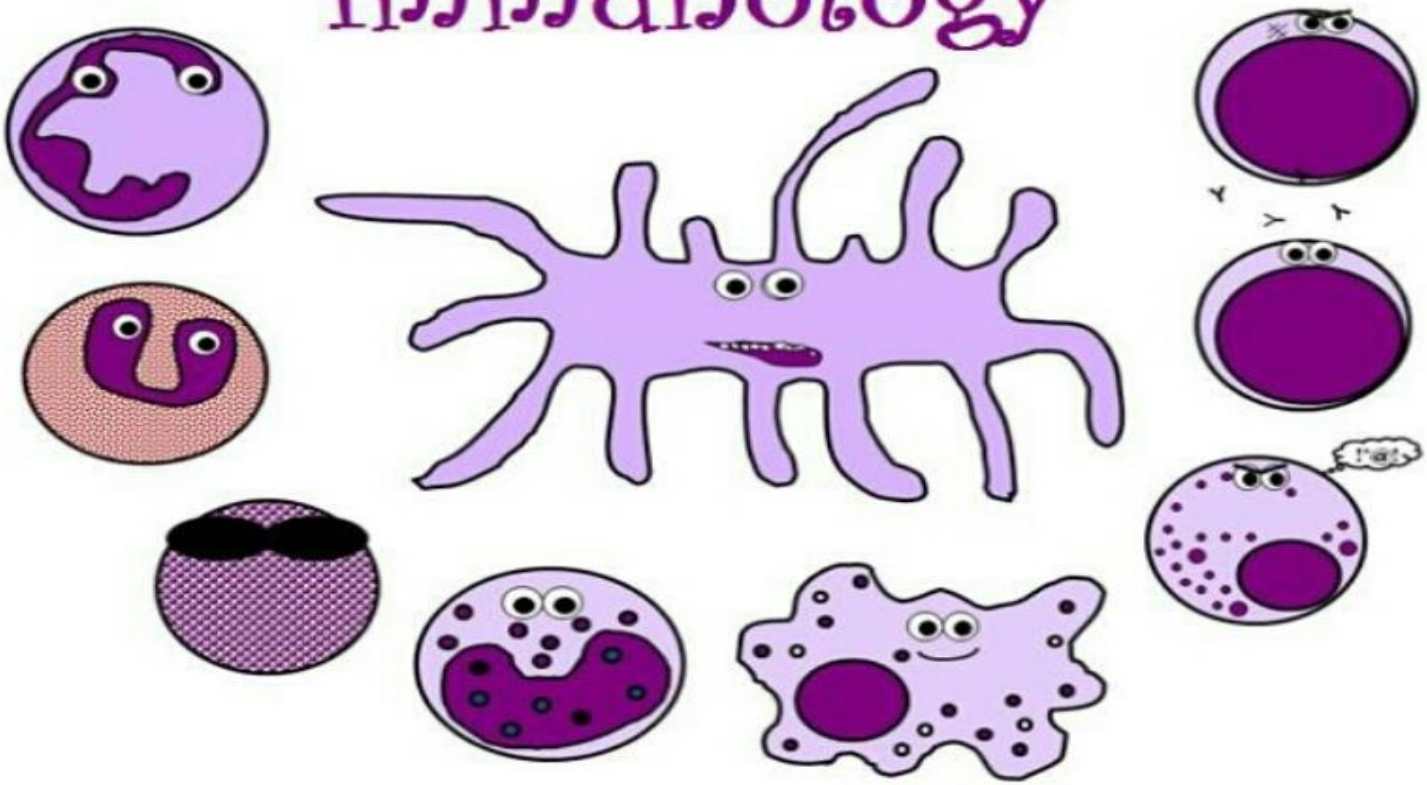
Immunology



Lecture: 5

Subject: Antigen structure, processing and presentation

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Antigen Structure, Processing and Presentation

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General notes

- What is written in **bold** and *italic* it is from doctor explanation during the lecture.
- Coloured statements what doctor emphasized.
- The symbol.  Means it is a new slide not the original

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 i mmunity

Definitions

- Antigen is any substance that causes your immune system to prompts 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 couple to a larger carrier molecule (albumin, globulins), they become immunogenic.
- 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(حبوب اللقاح).
- An antigen may also be formed within the body, as with bacterial toxins or tissue cells.

- ***Antigen does not mean necessarily microorganism***

- ***The main difference between antigen and immunogen is that antigen is associated always with antibody, while immunogen is a general term, that describes any substance that activates immune system.***
- ***for example, cell mediated immunity is carried by T cells that don't produce antibodies, foreign body in this case is immunogen but not necessary antigen***
- ***So we can say that every antigen is immunogen, but not vice versa.***

- Properties that make molecules more effective antigen s include:

1. Foreignness: foreignness means substances that never contact with lymphocytes in embryo period.
2. Stable molecules, ie, molecules that assume and maint ain a definite shape. ***(In order for B-Cells to produce antibodies (AB), it have to study the antigen, producing specific AB, if this antigen changes its shape, it would take more time to give specific AB)***
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. ***(Since simple structures are similar to our self antigens, While complex structures have specific features that stimulate fast specific Anti-bodies formation)***
5. Route of administration: Parenteral routes are more im munogenic to oral route

- ***Bacterial cell walls are famous examples for antigens, such structures are complex, and exposed, therefore highly antigenic.***
- ***Our immune system recognises the most Complicated regions of theses structures,since they are characteristic for this microorganism, this makes theimmune response highly specific***
- ***Sometimes, our immune cells cannot recognise these specific structures (ex: internal domains of the protein), so they are presented by APC.***

Antigenic Determinants-epitopes

- The body recognizes antigens by the three-dimensio nal 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 juxtap osed in the folded protein. They are recognized by B cells or antibody.***(more complex, better as an antigen)***
2. Sequential (or linear) determinants: They are mainly recognized by T cells, but some also can be recognize d by B cells

- ***The Epitope is a (1)small part of the antigen, that's (2)very complex and (3)can trigger the specific immune response.***
- ***Pointes 1, 2 and 3 are the answer of the question: what's the difference between antigen and epitope?***

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, ingest ion, or injection

1. Bacterial antigens:
   - Antigens related to bacterial cells: Somatic antigen (O), Capsular antigen, Flagellar Ag (H), etc
   - Antigen secreted by bacteria: for ex Exotoxins
2. Viral antigens:
   - Protein coat viral antigens. ***(Envolope)***
   - Soluble antigens (soluble nucleoproteins)
   - ***The problem with most viruses, that they are intracellular, so it's difficult to immune system to recognise*** it

2. Endogenous Antigens

- Endogenous antigens are antigens that have been gener ated 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 ***(Viruses may be considered as an e xogenous or endogenous depending on the stage of the life cycle)***

3. Autoantigens

- An autoantigen is usually a normal protein or complex of proteins (and sometimes DNA or RNA, ***ex: SLE***) tha t is recognized by the immune system of patients suffe ring from a specific autoimmune disease
- These antigens under normal conditions, not be target ed of the immune system, but due to mainly genetic an d environmental factors, the normal immunological to lerance for such an antigen has been lost in these patie nts.

Superantigens

- They activate multiple clones of T-lymphocytes
- They are active at very low concentration causing relea se of large amounts of cytokines
- The massive T-cell activation and release of large amo unts of cytokines cause systemic toxicity
- It does not lead to acquired immunity i.e no memory
- Example: Bacterial toxins:
  - Staph. aureus toxic shock syndrome toxin (TSST) and e nterotoxins
  - Strpt. pyogenes pyrogenic toxin A

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, altimetry tract..etc) w here T cells number is very low. According, microbial antigens needs to be transported to lymph nodes to e nhance chances of encounter with T cells
2. T cells can not interact with complex microbial antig en, antigens need to be captured, processed, and the n presented for T cells in a specific way in order to all ow interactions
3. T cells respond only to protein antigens and not to ot her types of chemical antigens

Antigen Presenting Cells

- A group of immune cells, whose role is to take up, proc ess 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 ex pressing MHC class II molecules

  eg. Endothelial cells

  Fibroblasts

  Activated T cell

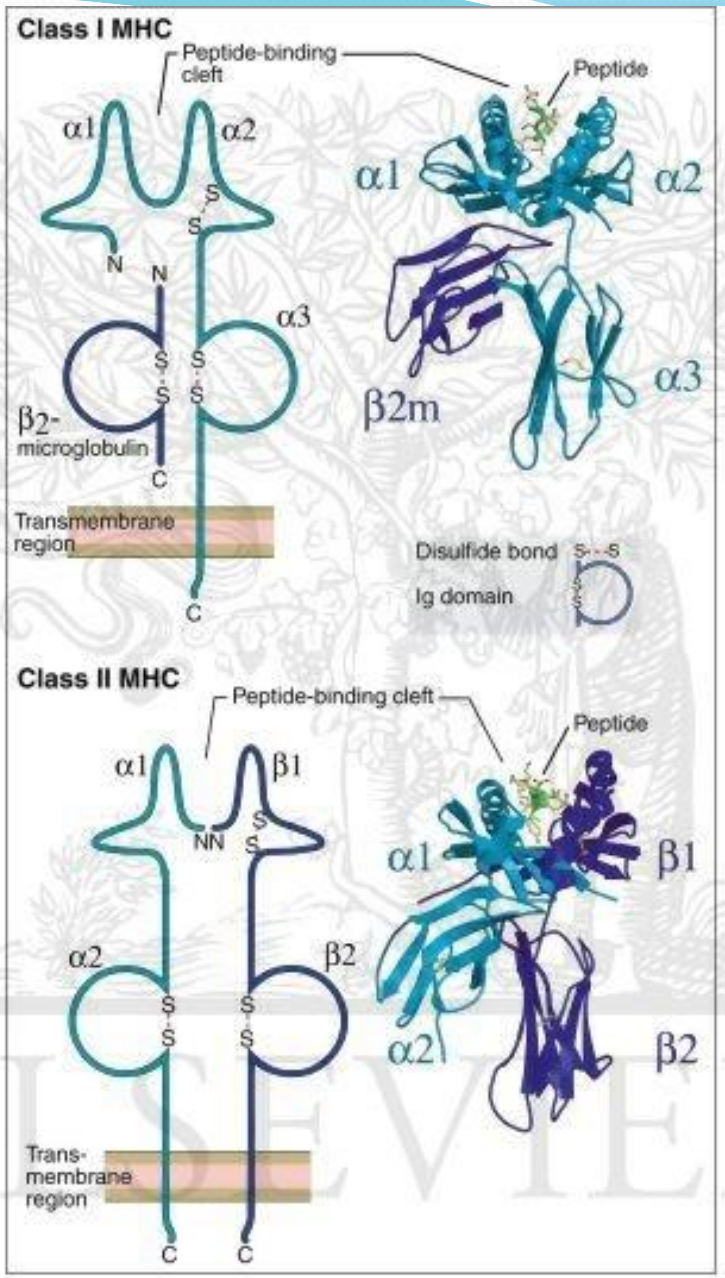
Major Histocompatibility Complex (MHC)

- MHC molecules are membrane proteins on APCs that dis plays peptide antigen for recognition by T cells
- MHC molecules are the principle determinants of accepta nce or rejection of tissue graft. ***(Responsible for self recognition)***
- Two main classes of MHC
- MHC class I:
  - Regulation of immune responses to intracellular parasites (e ndogenous antigens) such as viruses - all cells can be infecte d by viruses, therefore all cells express MHC class I
  - Structure: α1, α2, α3, and β2 microglubuin
  - Contain a peptide binding cleft that accommodate 8-11 aa
  - Necessary for CD8+ T cells activation.

Note:
Helper T cells → CD4+ → Bind MHC II
Cytotoxic T Cells → CD8+ → bind MHC II
Regulator T Cells → CD25+

- 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 rest ricted 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

Notice the peptide binding cleft



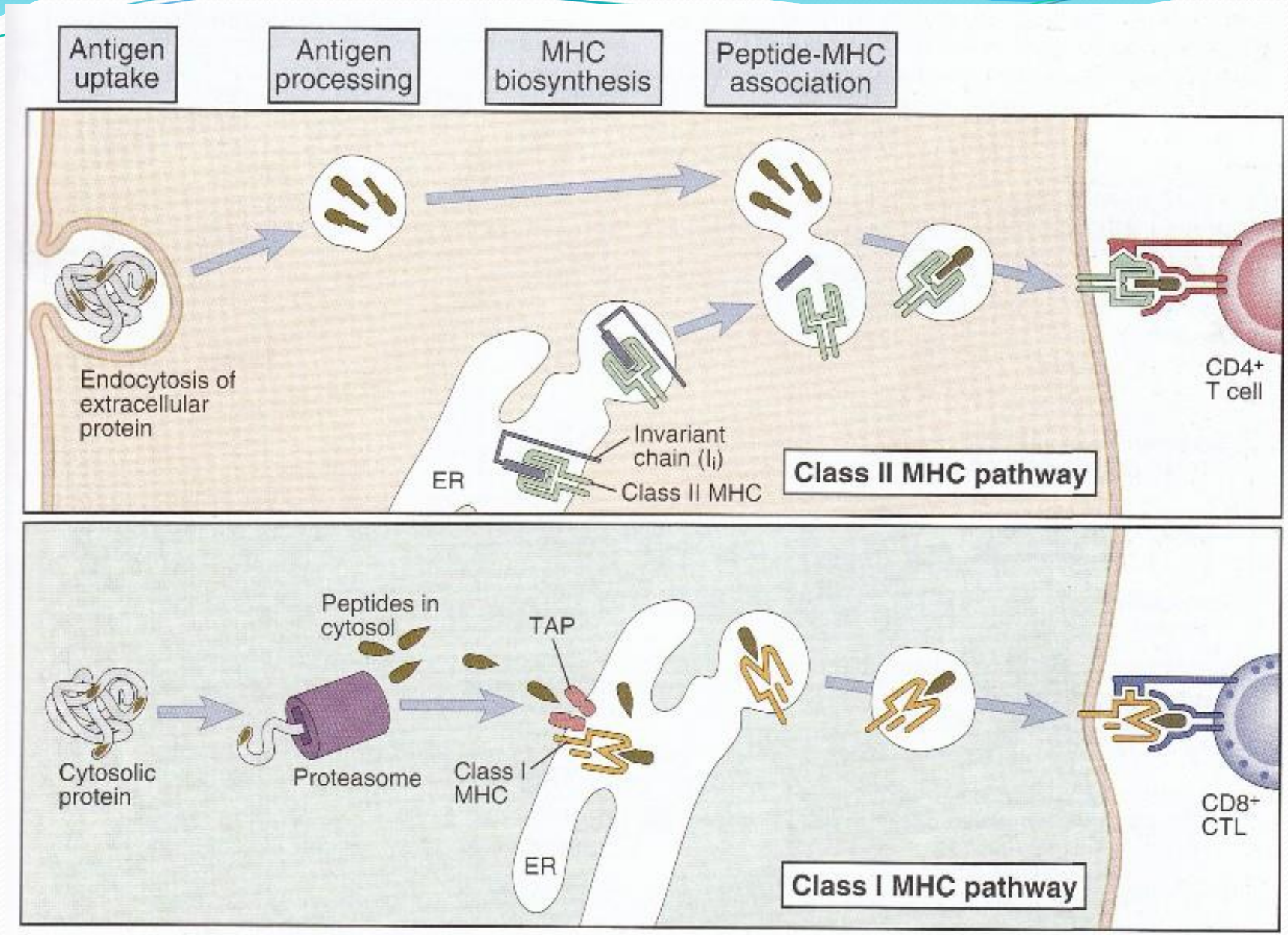
Features of Peptides Binding to MHC Molecules

- Each MHC molecule display 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
6. ***Binding with CD_4 or CD8***

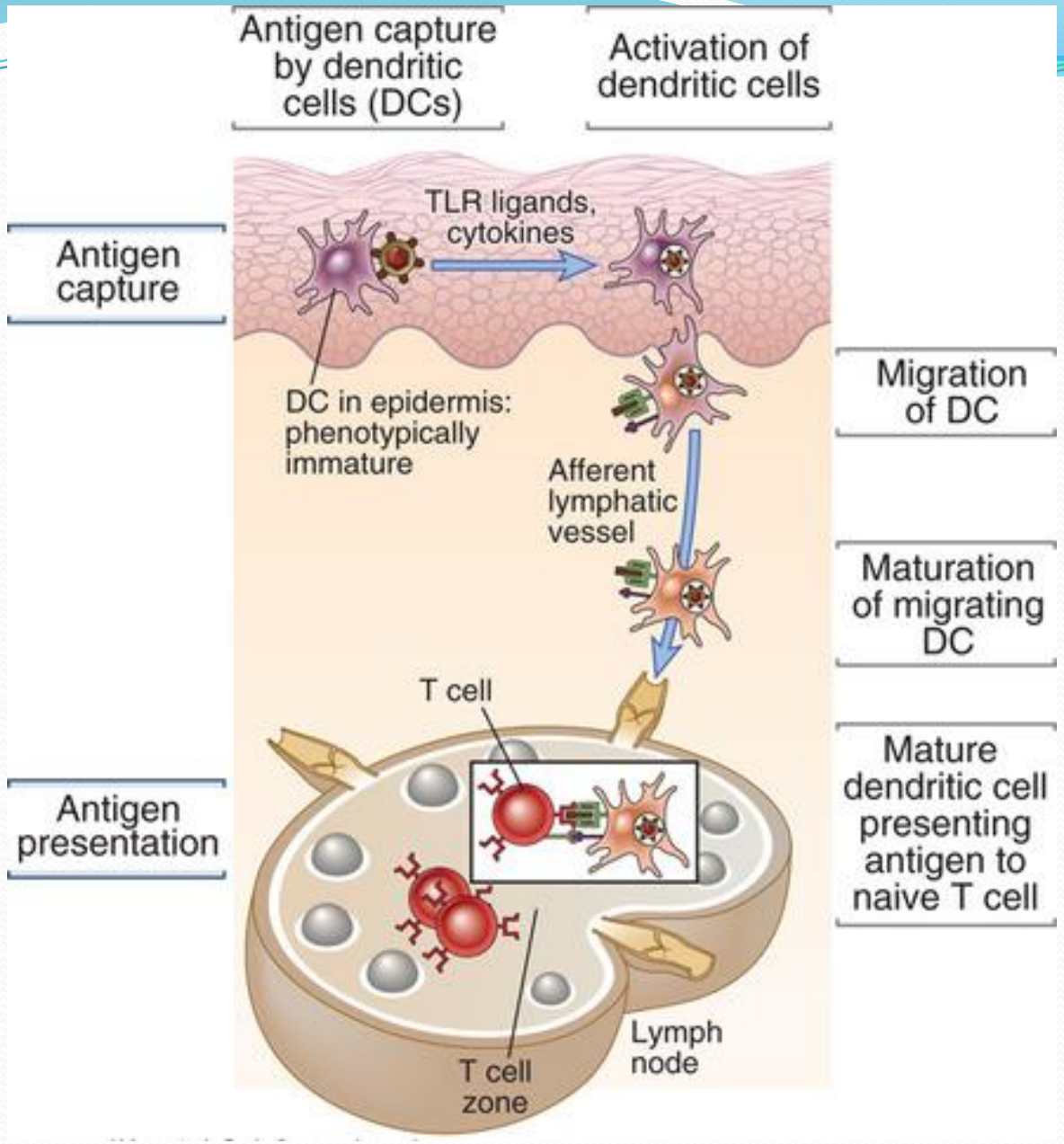

Antigen uptake
Antigen processing
MHC biosynthesis
Peptide-MHC association
Endocytosis of extracellular protein
ER
Invariant chain (I_i)
Class II MHC
Class II MHC pathway
CD4+ T cell
Peptides in cytosol
TAP
Cytosolic protein
Proteasome
Class I MHC
ER
Class I MHC pathway
CD8+ CTL

Conditions of T cell action

- *It deals with protein antigens*
- *The antigen has to be processed by APCs then presented by MHC (MHC I ....CD8+ or T killer / MHC II .... CD4+ ot T helper)*
- *It should be a transport system to bring antigens to lymph nodes because T cells do not go to periphery*

1. Antigen Capture and Transport to Lymph Node

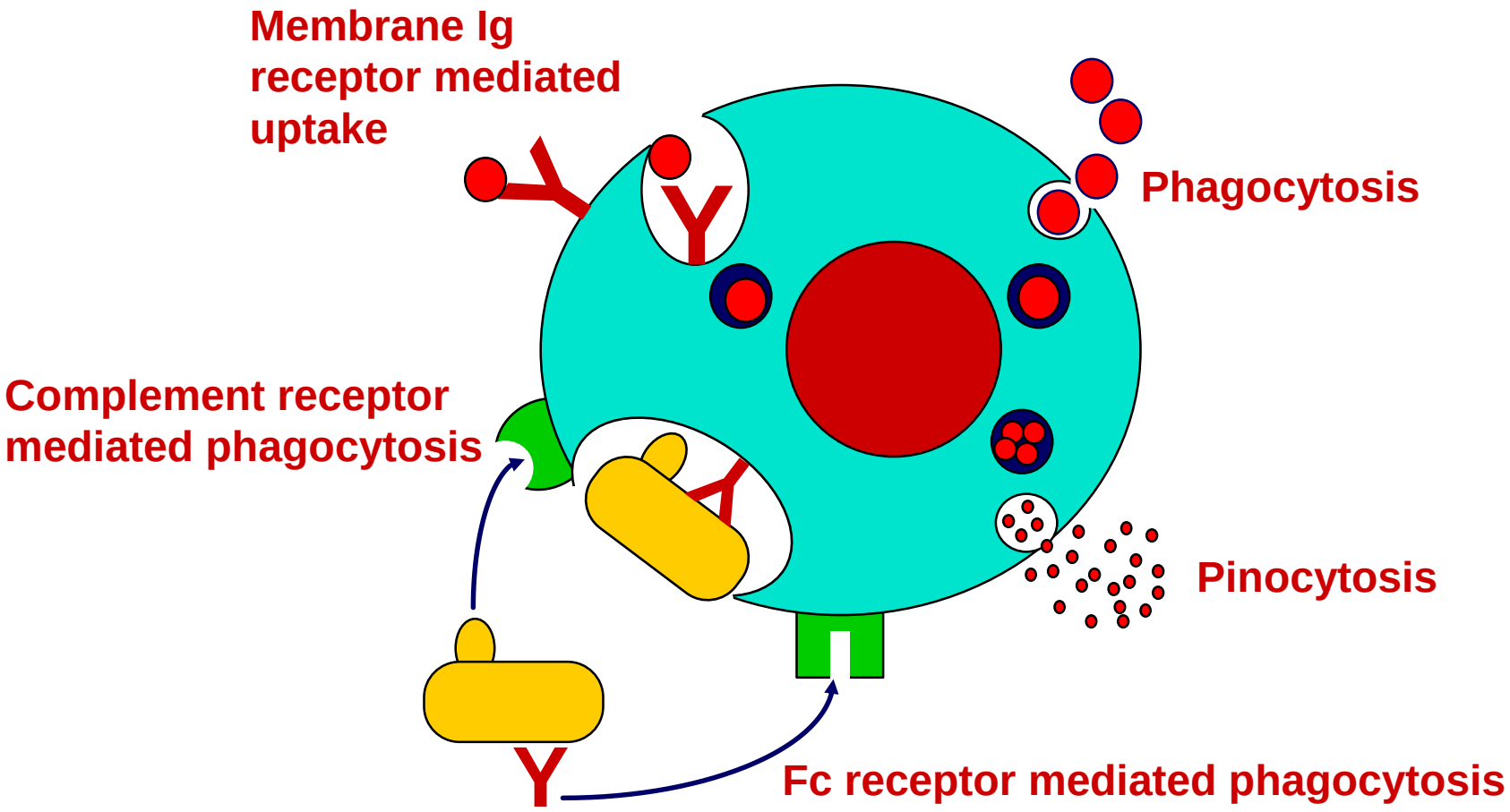
- Immature DCs in the epithelium capture microbial antigens and leave the epithelium
- The DCs migrate to draining lymph nodes being attracted by chemokines produced in the nodes
- During their migration the DCs mature
- Once at the lymph nodes the DCs start the processing of presenting the antigen to 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 (mai nly APCs) this is important for exogenous antigen proc essing, while endogenous antigens are already inside t he 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 antigen s

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

Processing means to degrade the protein (antig en) to small units and identify major units

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.

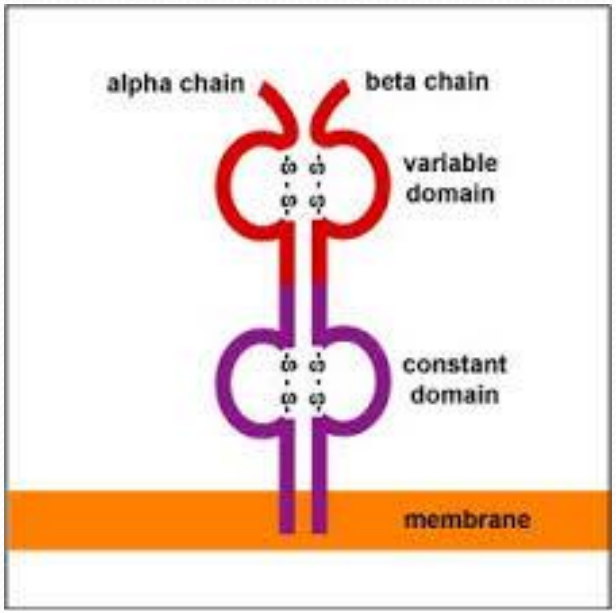
5. Antigen Recognition

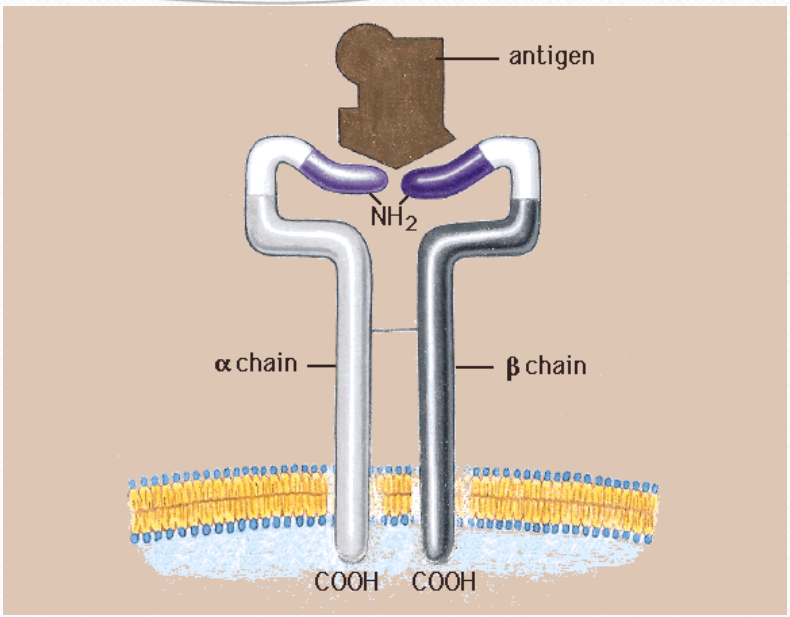
- Antigens are recognized by and bind to:

1. B-cell receptors (BCR) :
   - These are membrane-bound immunoglobulins (IgM an d 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

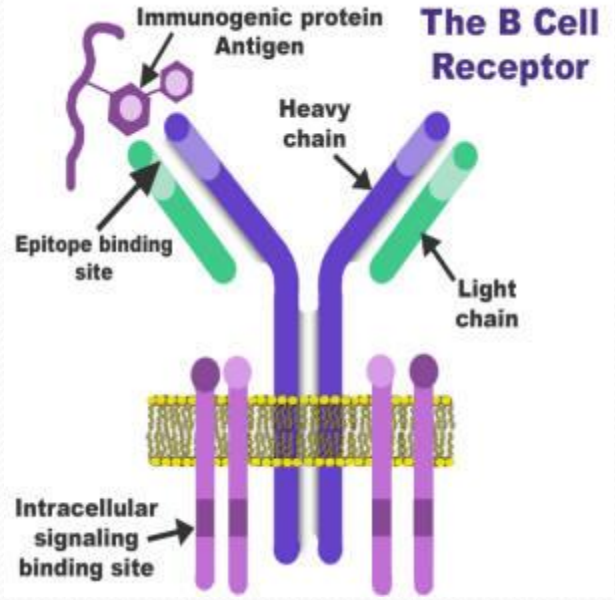
Binding of BCR and TCR to their antigens ca uses activation of B or T cells

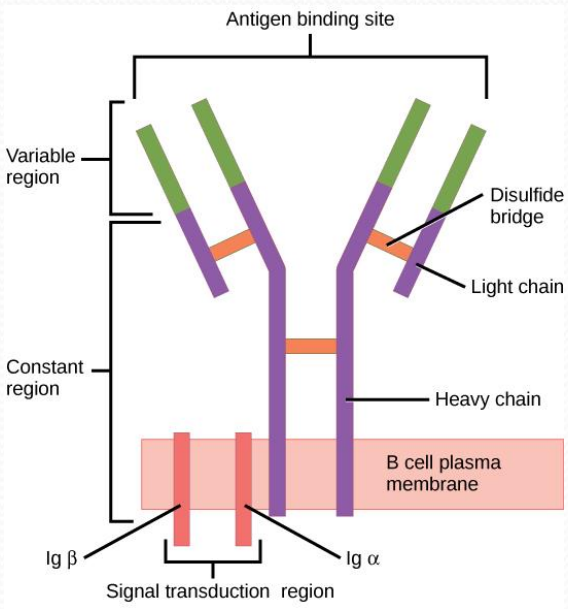
TCR


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