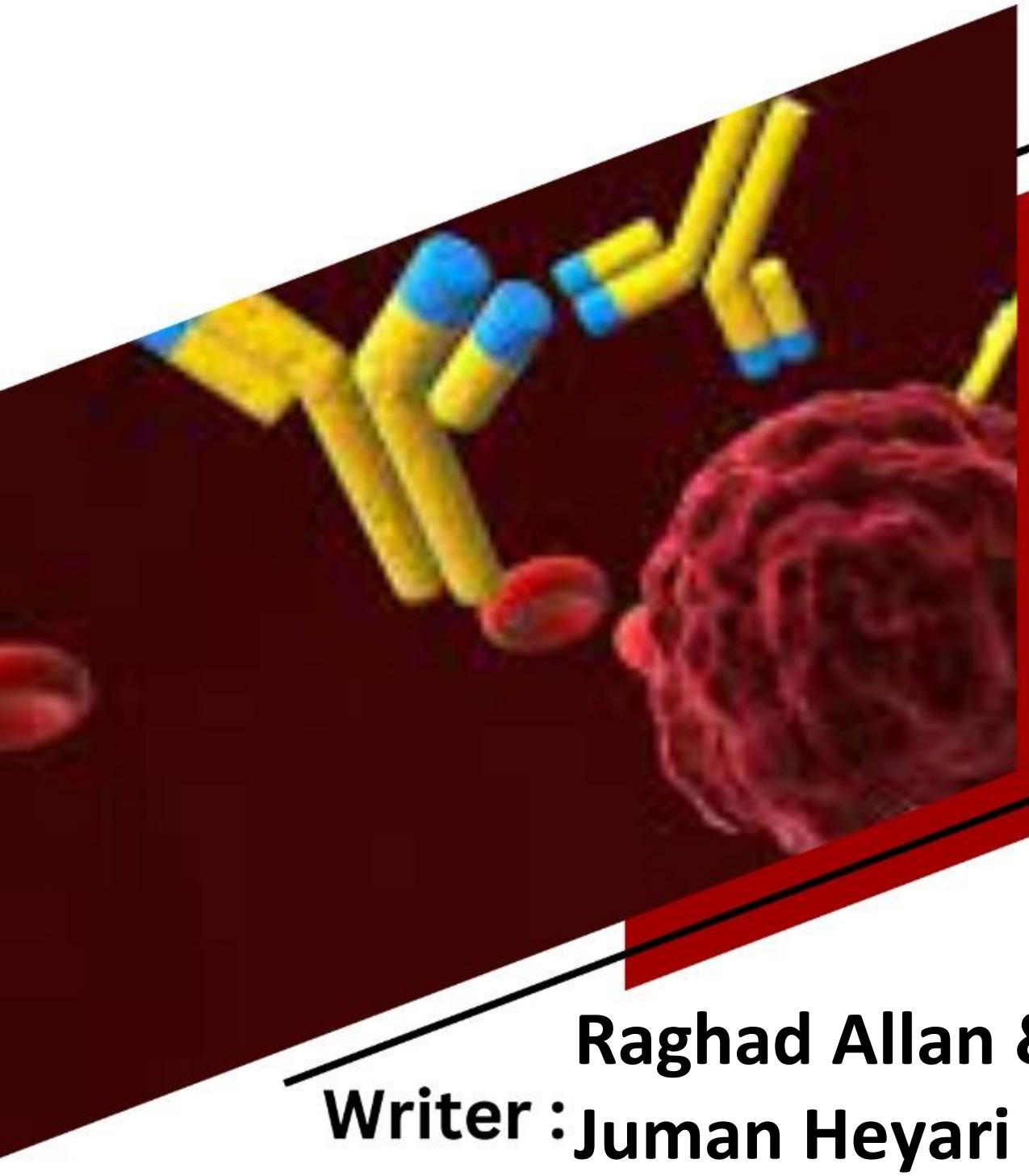
Doctor 021

# IMMUNOLOGY

## Sheet no. 11

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# Antigen capture and presenting to T lymphocytes

❖ This subject links between innate and adaptive immunity, explaining the first step of activation of the **adaptive immune system** specifically **T lymphocytes.**

## FEATURES OF ANTIGENS RECOGNIZED BY T LYMPHOCYTES

**• The antigen receptors of most T lymphocytes, can see only peptide fragments of protein antigens, and only when these peptides are presented by specialized molecules that bind peptides generated inside a host cell and then display them on the cell surface.**

- The **majority** of T lymphocytes ( **Having alpha beta receptors**) recognize only processed proteins (peptide molecules) in contrast to B lymphocytes which can recognize multiple forms of an antigen.
- Those peptides must be loaded on self **MHC molecule** on the surface of cells. **(Antigen presenting cells (APCs) or any nucleated cell)**.
  - **MHC II molecules for APCs and MHC I molecules for other nucleated cell in the body.**

**Remember**:

- MHC (major histocompatibility complex) genes exist on the short arm of $6^{th}$ chromosome, the protein products of those genes are called **HLA (Human leukocyte antigens)** which are ligand for receptors.
- B lymphocytes can recognize the microbe itself, part of the microbe, membrane associated form of the microbe, and soluble (secreted) forms.

**Note:**

- Red blood cells do not express **MHC I** molecules (they are not nucleated cells).
- Even APCs express **MHC I molecules** in addition to the **MHC II molecules.** This is known as **cross linking presentation**, and will be explained soon.

**• Therefore, T cell–mediated immune responses may be generated only against protein antigens that are either produced in or taken up by host cells.**

**• The cells that capture microbial antigens and display them for recognition by T lymphocytes are called antigen-presenting cells (APCs).• The majority of T lymphocytes recognize peptide antigens that are bound to and displayed by major histocompatibility complex (MHC) molecules of antigen-presenting cells.**

- T cells do not recognize polysaccharides, lipids, metals or small chemicals, they only recognize peptides.
- NKT cells and γδ T cells can recognize non-peptide antigens, **(exception of the previous statement)**
- T cells do not recognize soluble nor associated antigens.

- The processing of protein is done by cleaving it into smaller fragments (**peptides**)
- During the processing, the protein will lose its 3d conformation and charge. So that it can fit in the **antigen binding cleft** on the **MHC molecule**.

**• In every individual, different clones of CD4+ and CD8+ T cells can see peptides only when these peptides are displayed by that individual's MHC molecules. MHC restriction**

**• The T cell receptor (TCR) recognizes some amino acid residues of the peptide antigen and simultaneously also recognizes residues of the MHC molecule that is displaying that peptide.**

- T cell receptor recognize polymorphic residues on the MHC molecule as well as on the antigen.

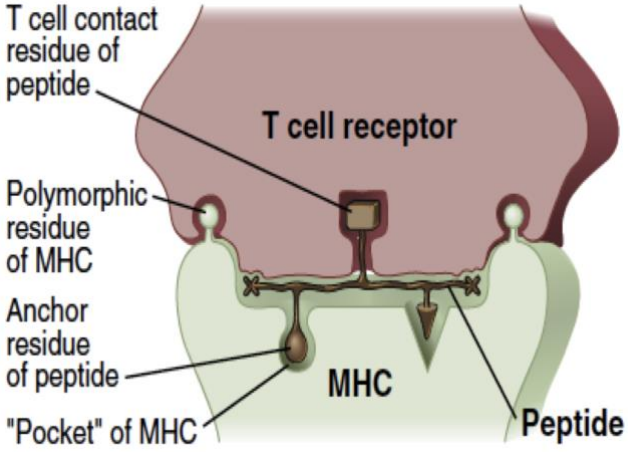


**TABLE 6–1 Features of Antigens Recognized by T Lymphocytes**

| Features of Antigens Recognized by T Cells | Explanation |
|---|---|
| Most T cells recognize peptides and no other molecules. | Only peptides bind to MHC molecules. |
| T cells recognize linear peptides and not conformational determinants of protein antigens. | Linear peptides bind to clefts of MHC molecules, and protein conformation is lost during the generation of these peptides. |
| T cells recognize cell-associated and not soluble antigens. | T cell receptors recognize only MHC-like shapes, and MHC molecules are membrane proteins that display stably bound peptides on cell surfaces. |
| $CD4^+$ and $CD8^+$ T cells preferentially recognize antigens sampled from the extracellular and cytosolic pools, respectively. | Pathways of assembly of MHC molecules ensure that class II molecules display peptides that are derived from extracellular proteins and taken up into vesicles in APCs and that class I molecules present peptides from cytosolic proteins; CD4 and CD8 bind to nonpolymorphic regions of class II and class I MHC molecules, respectively. |

**The rule of Eight:**

- Antigens on **MHC I** are recognized by **CD8+** cells.
- Antigens on **MHC II** are recognized by **CD4+** cells.
- (1*8=8)
- (2*4=8)

# GENERAL PROPERTIES OF APCS FOR CD4+ T lymphocytes.

**• Different cell types function as APCs to activate naïve and previously differentiated effector T cells.**

**• Dendritic cells, macrophages, and B lymphocytes express class II MHC molecules and other molecules involved in stimulating T cells and are therefore capable of activating CD4+ T lymphocytes.**

- Those cells are called **professional phagocytes** or **Antigen presenting cells** since they can express MHC2 molecule, so they can present antigens to CD4+ cells.
- Cd4+ cells which differentiate into types of T helper cells are considered the master regulator to all immune responses ( including other immune cells and humoral immunity).

**• APCs display peptide-MHC complexes for recognition by T cells and also provide additional stimuli to the T cells that are required for the full responses of the T cells.**

# CAPTURE OF PROTEIN ANTIGENS BY ANTIGEN-PRESENTING CELLS

**• Protein antigens of microbes that enter the body are captured mainly by dendritic cells and concentrated in the peripheral lymphoid organs, where immune responses are initiated .**

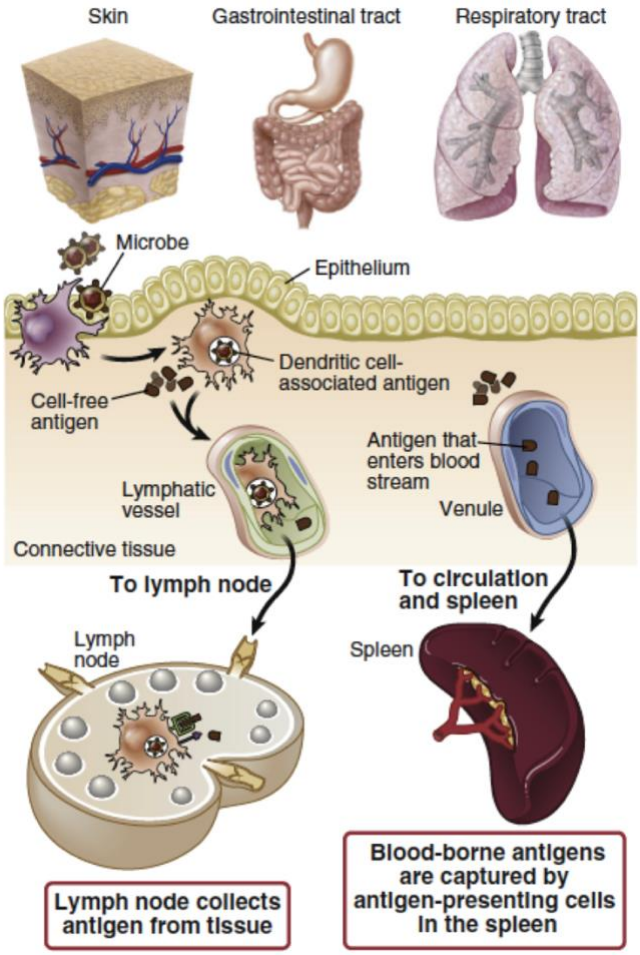
**• Microbes usually enter the body through the skin (by contact), the gastrointestinal tract (by ingestion), and the respiratory tract (by inhalation).**

**• Some insect-borne microbes may be injected into the bloodstream as a result of insect bites, and some infections are acquired through the genitourinary tract.**

**• Microbial antigens can also be produced in any infected tissue**

**Antigens can enter the body from entrances such as**:

- Digestive tract (by the mouth)through digestion.
- Respiratory tract through inhalation.
- Skin through skin abrasion or cuts.
- Genitourinary tract.




- T cells are limited in numbers, meaning they can't patrol all the body. They also can't migrate to microbes, since they do not exist in soluble form.
- So, antigens (if crossed the anatomical barriers) will get engulfed/ captured by APCs **mainly dendritic cells**, and will then migrate into secondary lymphoid tissues (lymph nodes, or spleen in case of blood borne infections).
- There, APCs will have a direct contact with T cell rich zones, meaning they will interact with the T cell receptors, and will eventually induce the activation of those T lymphocytes.

**Note:**

- Anatomical barriers include epithelia and sub-epithelia.
- Dendritic cells are the most efficient antigen presenting cell.

# MORPHOLOGY AND POPULATIONS OF DENDRITIC CELLS

**• There are two major populations of dendritic cells, called classical and plasmacytoid (myeloid), which differ in their locations and responses .**

| Feature | Classical dendritic cells | Plasmacytoid dendritic cells |
|---|---|---|
| Surface markers | CD11c high<br>CD11b high | CD11c low<br>CD11b negative<br>B220 high |
| Major location | Tissues | Blood and tissue |
| Expression of Toll-like receptors | TLRs 4, 5, 8 high | TLRs 7, 9 high |
| Major cytokines produced | TNF, IL-6, IL-12 | Type I interferons |
| Postulated major functions | Induction of T cell responses against most antigens | Antiviral innate immunity and induction of T cell responses against viruses |

- Note that the main function of Classical dendritic cells is the activation of adaptive immunity, while the plasmacytoid ones is the activation of the innate immune system (through the production of Type 1 interferons) as will as the adaptive immune system.

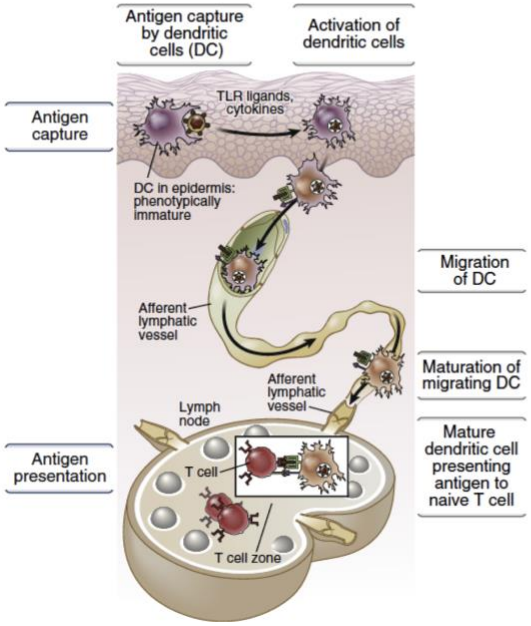
# ANTIGEN CAPTURE AND TRANSPORT BY DENDRITIC CELLS

**• DCs that are resident in epithelia and tissues capture protein antigens and transport the antigens to draining lymph nodes.**

**• The activated DCs (also called mature DCs) lose their adhesiveness for epithelia or tissues and migrate into lymph nodes. The DCs also begin to express a chemokine receptor called CCR7 that is specific for two chemokines, CCL19 and CCL21, that are produced in the T cell zones of lymph nodes.**

- As the dendritic cell **captures** the microbe from its site of entry, it will transform into **mature dendritic cell**, which undergoes changes including the expression of chemokine receptors (mainly CCR7).
- CCL19, CCL21 are chemoattractants, which attract the DC into the lymphoid organ.
- Those changes enable the DC to **present** the antigen to T lymphocyte.

**• DCs can ingest infected cells and present antigens from these cells to CD8+ T lymphocytes, cross presentation, or cross-priming.**




T cell zone

| | Immature dendritic cell | Mature dendritic cell |
|---|---|---|
| Principal function | Antigen capture | Antigen presentation to T cells |
| Expression of Fc receptors, mannose receptors | ++ | – |
| Expression of molecules involved in T cell activation: B7, ICAM-1, IL-12 | – or low | ++ |
| Class II MHC molecules | | |
| Half-life | ~10 hr | >100 hr |
| Number of surface molecules | ~$10^6$ | ~$7 \times 10^6$ |

- The Fc receptors (innate immune receptors), mannose receptors (pattern recognition receptors), become negative or low in mature dendritic cell because its function is only to activate T lymphocytes.
- For the activation of T lymphocytes we need at least 2 signals:
    1. T cell receptor binding to the MHC molecule loaded with a peptide.
    2. **Costimulators** inducing signal transduction and completing the activation of T lymphocytes.
- Note that these **costimulators** are highly expressed in mature dendtitic cells, while negative or low in the immature ones.

- The long half life of the mature dendritic cell gives it enough time to present the antigen to T lymphocyte after arriving to the lymphoid tissue.

# DIFFERENT TYPES OF APC SERVE DISTINCT FUNCTIONS IN T CELL-DEPENDENT IMMUNE RESPONSES

- We have 5 types of antigens presenting cells:

1. Dendritic cells.
2. Macrophages.
3. B cells.
4. Vascular endothelial cells.
5. Thymic epithelial cells.

- Note: the role of the vascular endothelial cells in activation of the adaptive immune system isn't well established, yet the thymic epithelial cells have an established role in the activation of T lymphocyte development in the thymus.

  - **Dendritic cells are the principal inducers of such responses because these cells are located at sites of microbe entry and are the most potent APCs for activating naive T lymphocytes.**
  - **One important type of APC for effector T cells is the macrophage, which is abundant in all tissues. In cell-mediated immune reactions, macrophages phagocytose microbes and display the antigens of these microbes to effector T cells, which activate the macrophages to kill the microbes**
  - **B lymphocytes ingest protein antigens and display them to helper T cells within lymphoid tissues; this process is important for the development of humoral immune responses.**
  - **All nucleated cells (MHCI) can present antigens derived from microbes in the cytoplasm to CD8+ T cells.**

| Cell type | Expression of | | Principal function |
|---|---|---|---|
| | Class II MHC | Costimulators | |
| Dendritic cells | Constitutive; increases with maturation; increased by IFN-γ | Constitutive; increases with maturation; increased by TLR ligands, IFN-γ, and T cells (CD40-CD40L interactions) | Antigen presentation to naive T cells in the initiation of T cell responses to protein antigens (priming) |
| Macrophages | Low or negative; inducible by IFN-γ | Low, inducible by TLR ligands, IFN-γ, and T cells (CD40-CD40L interactions) | Antigen presentation to $CD4^+$ effector T cells in the effector phase of cell-mediated immune responses |
| B lymphocytes | Constitutive; increased by cytokines (e.g., IL-4) | Induced by T cells (CD40-CD40L interactions), antigen receptor cross-linking | Antigen presentation to $CD4^+$ helper T cells in humoral immune responses (T cell–B cell interactions) |

- **This table is important.**

As we can see in the table above:

- Note that the dendritic cell can prime naïve mature T cell, while the Macrophages and the B lymphocytes can activate effector (already activated) T cells.
- Dendritic cells MHC class 2 is constitutive, which means it is always present, if the cell is exposed by a foreign antigen or a stress, it gets upregulated to produce MHC class 2 and this increases with maturation and the presence of INF-γ, Costimulators are constitutive as well, which allows it to activate naïve mature T cells
- While as in macrophages have low or negative MHC class 2 expression which is upregulated by INF-γ, and Costimulators are low as well, yet they are upregulated by inflammation in the microenvironment were CD40 or its ligand bind on T lymphocyte

# PEPTIDE BINDING TO MHC MOLECULES

- As we mentioned before, T cells can only recognize MHC molecules, and on this molecule, there is a binding grove (cleft), where we can find a peptide (a result from protein processing) inside of it.

- **The peptide-binding clefts of MHC molecules bind peptides derived from protein antigens and display these peptides for recognition by T cells.**

- **Each MHC molecule can present only one peptide at a time, because there is only one binding cleft.**
- **MHC molecules bind mainly peptides and not other types of antigens.**
- **MHC molecules acquire their peptide cargo during their biosynthesis, assembly, and transport inside cells. Therefore, MHC molecules display peptides derived from protein antigens that are inside host cells.**
- **In each individual, the MHC molecules can display peptides derived from the individual's own proteins, as well as peptides from foreign (i.e., microbial) proteins.**

# PROCESSING AND PRESENTATION OF PROTEIN ANTIGENS

- **Extracellular proteins that are internalized by specialized APCs (dendritic cells, macrophages, B cells) are processed in late endosomes and lysosomes and displayed by class II MHC molecules.**
- **Whereas proteins in the cytosol of any nucleated cell are processed in proteolytic structures called proteasomes and displayed by class I MHC molecules.**

# PROCESSING OF CYTOSOLIC ANTIGENS FOR DISPLAY BY CLASS I MHC MOLECULES

- Sources of cytosolic proteins:

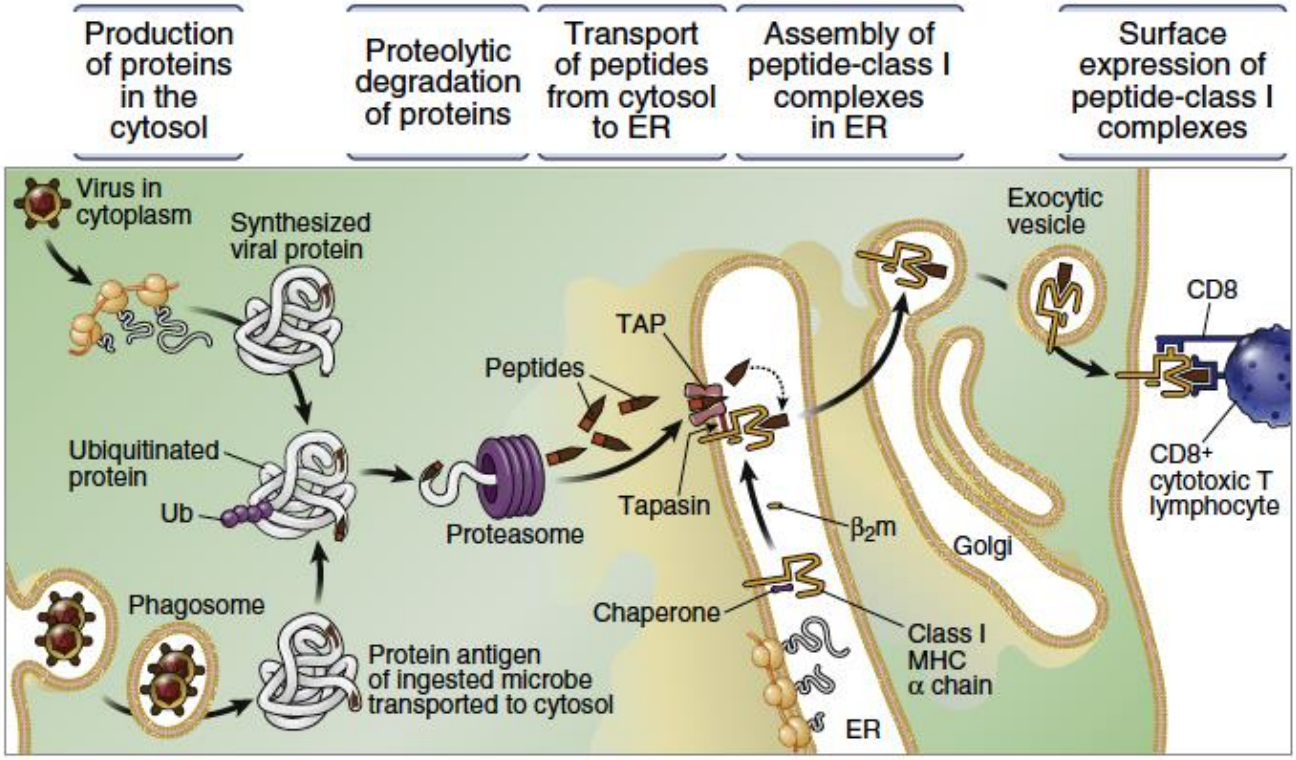
1. Virus
2. Intracellular bacteria
3. Defective protein in tumor cells
4. Entry through endosome and able to egress into the cytosol

- If these proteins enter the cytoplasm, they get tagged with ubiquitin protein , then they enter a **proteasome**, which is a **multienzyme multiprotein complex** (found in the cytoplasm) where protein degradation takes place, at the same time MHC class 1 is being synthesized in the ER (endoplasmic reticulum).

- The loading of MHC happens in the ER, the loaded MHC leaves as an exocytic vesicle to the cell surface to be presented to CD8 cells, and it is called **UPS pathway**.

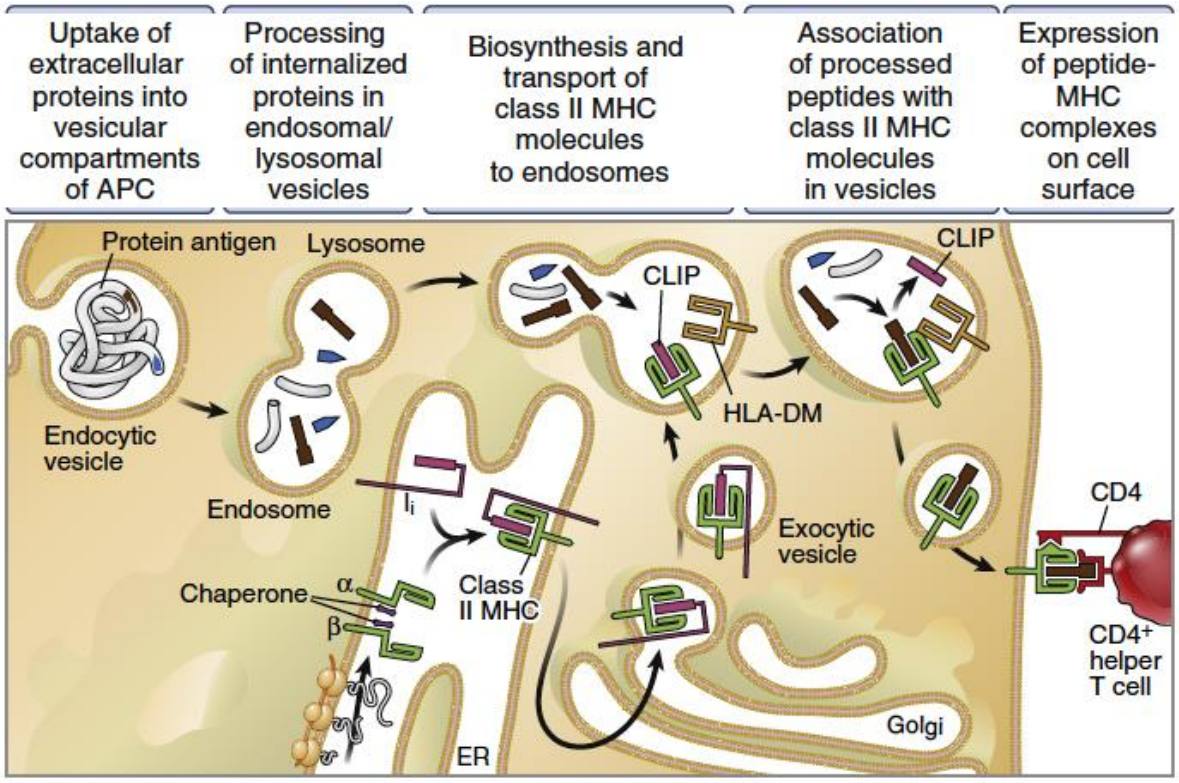
- **Most cytosolic protein antigens are synthesized within cells, and some are phagocytosed and transported into the cytosol.**
- ***Proteolysis of cytosolic proteins.*** **The peptides that bind to class I MHC molecules are derived from cytosolic proteins following digestion by the ubiquitin-proteasome (UPS) pathway.**
- **Peptides generated in the cytosol are translocated by a specialized transporter into the ER, where newly synthesized class I MHC molecules are available to bind the peptides. transporter associated with antigen processing (TAP).**
- ***Binding of peptides to class I MHC molecules.*** **In order to form peptide-MHC complexes, the peptides must be transported into endoplasmic reticulum.**

- Through **UPS**, proteins are degraded into peptides with certain lengths, this way they lose both their shape and charge so that they would fit in the MHC grove.
- when peptides leave the proteasome, there is another protein called **TAP** which is a transporter associated with antigen processing , it is a tunnel that transport the peptides into the ER, where MHC is biosynthesized.
- The empty groove of the MHC will be loaded with peptides by chaperons, to prevent the loading of self-antigens and by which prevents autoimmune responses.
- There is another protein called **Tapasin** found on the membrane of the ER that brings the **TAP** transporter close to the ER, an exchange occur between them, peptide is loaded in MHC class 1 by **Calnexin** and **Calreticulin**, then they leave in vesicles to be presented on the cell surface to be recognized by CD8 cells, there are chaperons (invariant proteins) that go with them, **yet focus to TAP and Tapasin.**




# PROCESSING OF CYTOSOLIC ANTIGENS FOR DISPLAY BY CLASS II MHC MOLECULES

- Most class II–associated peptides are derived from protein antigens that are captured from the extracellular environment and internalized into endosomes by specialized APCs.
- Internalized proteins are degraded enzymatically (cathepsins) in late endosomes and lysosomes to generate peptides that are able to bind to the peptide-binding clefts of class II MHC molecules.
- Class II MHC molecules are synthesized in the ER and transported to endosomes with an associated protein, the invariant chain (Ii), which occupies the peptide-binding clefts of the newly synthesized class II molecules.
- Within the endosomal vesicles, the Ii dissociates from class II MHC molecules by the combined action of proteolytic enzymes and the HLA-DM molecule, and antigenic peptides are then able to bind to the available peptide binding clefts of the class II molecules




- In MHC 2, we talk about the presentation on proteins acquired **extracellularly**, these proteins enter the cell through an endosome - they don't follow UPS pathway because they didn't egress-
- The endosome fuses with a lysosome creating a **phagolysosome**, where pH drops and protein degradation occurs through functioning enzymes (ex: **cathepsin**).
- Protein degradation results in the loss of conformation and charge, which allows peptides to fit in the MHC groove.
- Meanwhile, MHC class 2 is being synthesised in the ER. The presence of chaperones **(invariant chain)** prevents the loading of self-antigens by loading itself the groove until they get to the peptides they want to present
- When the MHC 2 and the peptides meet in the vesicle, the invariant chain then gets trimmed and becomes a **clip** ( class 2 associated protein)
- In the case of MHC 2 the loading of peptides happens in an exocytic vesicle
- A protein called HLA-DM ( human leukocyte antigen) works as a peptide exchanger, through replacing the clip with the antigen peptide.
- The exocytic vesicle with the loaded MHC molecle leaves to the cell surface to be presented for CD4 cells.

| MHC1 | MHC2 |
|---|---|
| Cytosolic proteins | Extracellular proteins |
| Present to CD8 cells | Present to CD4 cells |
| Loading in the ER | Loading in the exocytic vesicle |
| Calnexin and calreticulin chaperones | Invariant chain chaperones |
| UPS pathway | Phagolysosome |

- What we mentioned above is considered the **first signal in activating T cells**

# PRESENTATION OF NONPROTEIN ANTIGENS TO SUBSETS OF T CELLS

- **Several small populations of T cells are able to recognize nonprotein antigens without the involvement of class I or class II MHC molecules, The best defined of these populations are NKT cells and γδ T cells.**
- **NKT cells recognize lipids and glycolipids displayed by the class I–like "non-classical" MHC molecule called CD1.**
- **γδ T cells are a small population of T cells that express antigen receptor proteins that are similar but not identical to those of CD4+ and CD8+ T cells**
- **γδ T cells recognize many different types of antigens, including some proteins and lipids, as well as small phosphorylated molecules and alkyl amines. These antigens are not displayed by MHC molecules, and γδ cells are not MHC restricted.**
- They are the minority
- Normal T cells (α, β) chain composition

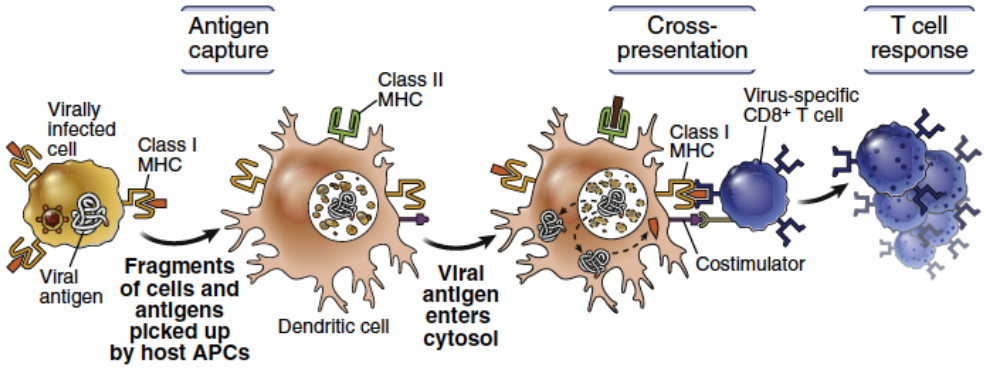
# CROSS-PRESENTATION OF INTERNALIZED ANTIGENS TO CD8⁺ T CELLS

- **Some dendritic cells can present ingested antigens on class I MHC molecules to CD8+ T lymphocytes.**

- **A subset of classical dendritic cells have the ability to ingest infected host cells, dead tumor cells, microbes, and microbial and tumor antigens and transport the ingested antigens into the cytosol, where they are processed by the proteasome. The antigenic peptides that are generated then enter the ER and bind to class I molecules, which display the antigens for recognition by CD8+ T lymphocytes. This process is called cross-presentation (or cross-priming), to indicate that one type of cell, dendritic cells, can present the antigens of other, infected or dying, cells or cell fragments, and prime (or activate) naive T lymphocytes specific for these antigens.**
- **Once the CD8+ T cells have differentiated into CTLs, they kill infected host cells or tumor cells without the need for dendritic cells or signals other than recognition of antigen.**
  - Dendritic cells proven in vivo and vitro to cross present.
  - Primarily, they are professional phagocytes, the original function is to activate CD4 cells, yet they sometimes present MHC 1through UPS system and present the antigen through it to activate CD8 cells (cytotoxic T lymphocytes).
  - Dendretic cells cross present in the existence of:

1. Tumor cells
2. Infected cells that can't upregulate the expression of MHC class1

  - Example on 2 : a virus infected a fibroblast- which is a nucleated cell- this should result in an expression of MHC class1, yet for some reason it didn't, in this case CD8 cells cant recognize and terminate it, to solve this issue, dendritic cells take fragments of the antigen that entered the cell, and present it using MHC class 1 to be recognized by CD8 cells, this cause clonal expansion and they are able to recognize the infected cells and terminate them.




  - Dendritic cells aren't harmed, since their number is limited we need to reserve them.