

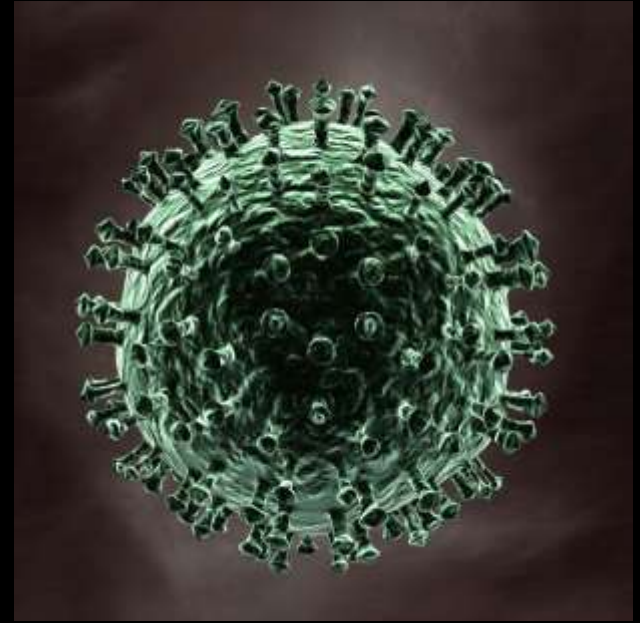


# ANTIGEN PROCESSING and PRESENTATION OF CELLS

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Section of Allergy and Immunology  
University of the Philippines – Philippine General Hospital

# Outline

- I. Properties of Antigens Recognized by T Lymphocytes
- II. Antigen Capture and the Functions of Antigen-Presenting Cells
- III. Processing of Protein Antigens
- IV. Presentation of Non-protein Antigens to Subsets of T Cells



# **PROPERTIES OF ANTIGENS RECOGNIZED BY T LYMPHOCYTES**

# T Lymphocytes

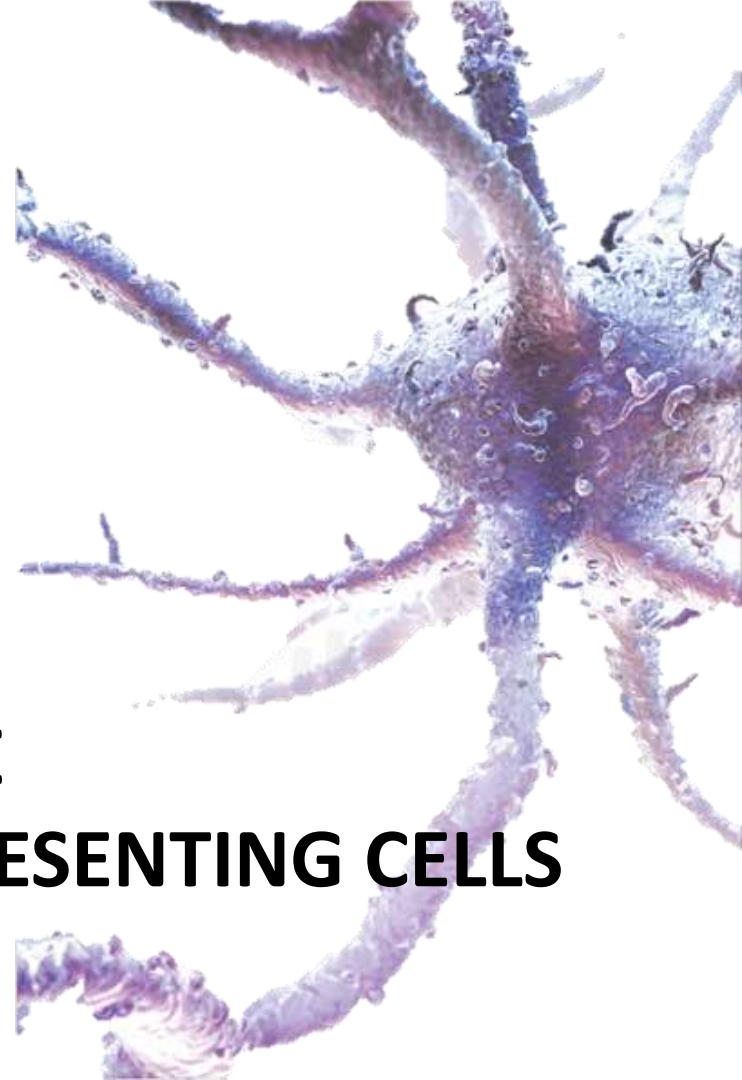
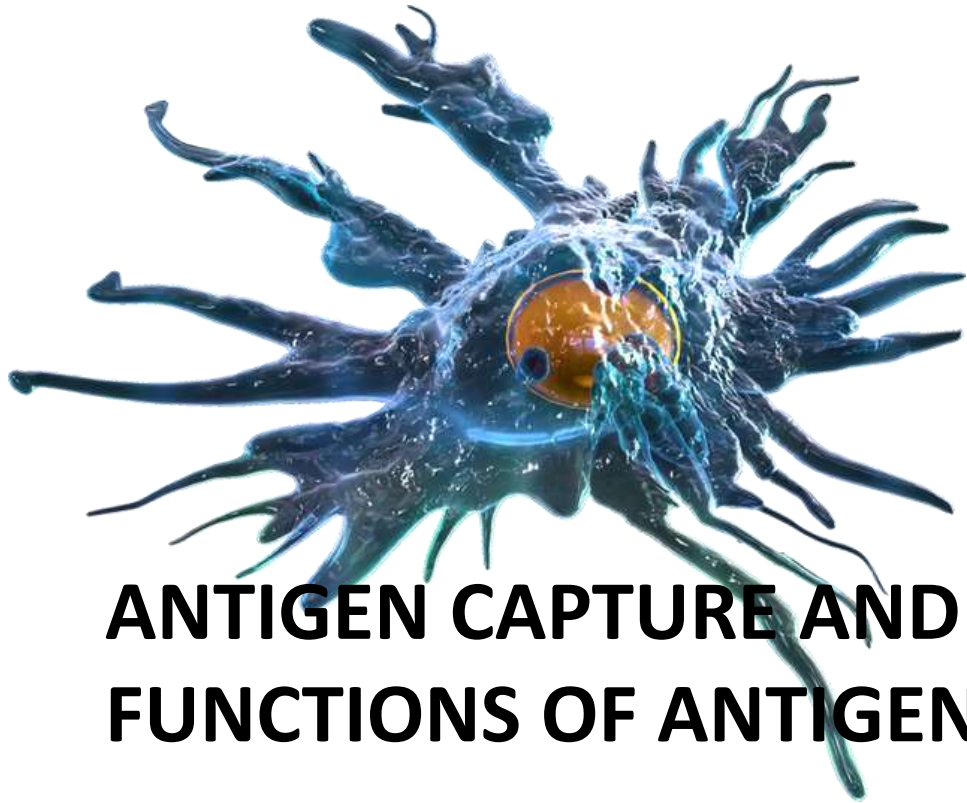
- Principal functions of T lymphocytes
  - a. to ***eradicate*** infections by intracellular microbes
  - b. to ***activate*** other cells, such as macrophages and B lymphocytes.

# T Lymphocytes

- Several challenges to T cells:
  1. **Very few naive T cells** specific for any one antigen
    - APCs
  2. Most T cell functions **require that they interact with other cells.**
    - MHC
  3. Different T cells have to be able to **respond to microbial antigens in different cellular compartments.**

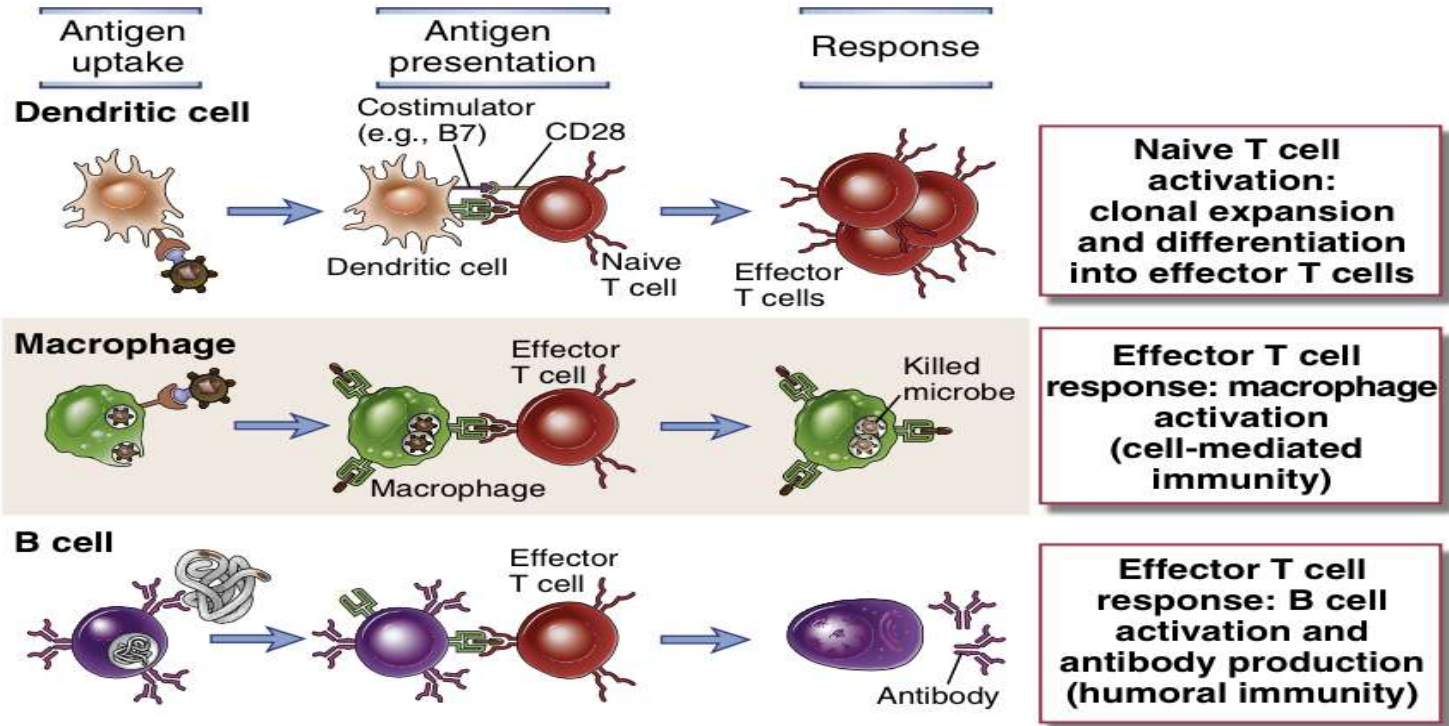
# PROPERTIES OF ANTIGENS RECOGNIZED BY T LYMPHOCYTES

1. Most T Lymphocytes recognize **only short peptides**
  - Induced by foreign protein antigens or small chemical substances
2. Antigen Receptors of CD4+ and CD8+ T cells are **specific for peptide antigens** displayed by MHC molecules
  - TCRs have evolved to be specific for MHC molecules
  - Majority of T cells recognize only peptides



# **ANTIGEN CAPTURE AND THE FUNCTIONS OF ANTIGEN-PRESENTING CELLS**

# ANTIGEN CAPTURE AND THE FUNCTIONS OF ANTIGEN-PRESENTING CELLS





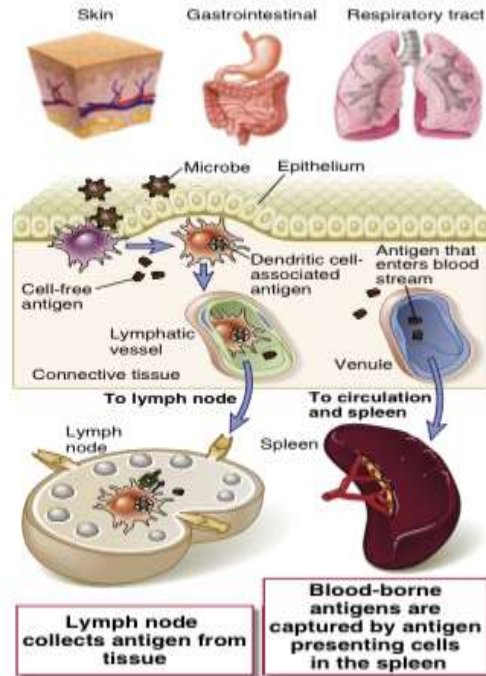
# ANTIGEN CAPTURE AND THE FUNCTIONS OF ANTIGEN-PRESENTING CELLS

- APC function is enhanced by exposure to microbial products
  - Toll-like receptors and other microbial sensors in dendritic cells and macrophages
  - Improved antigen presentation efficiency and APC cytokine production → Increase expression of MHC and costimulators
  - Adjuvants: products of microbes or mimic microbes
    - Enhance expression of costimulators and cytokines
    - Enhance functions of APC's

# ANTIGEN CAPTURE AND THE FUNCTIONS OF ANTIGEN-PRESENTING CELLS

- APCs that present antigen to T cells also receive signals from these Lymphocytes, enhancing their antigen-presenting function
  - Activated CD4<sup>+</sup> express CD40L --- CD40 on dendritic cells and macrophages → IFN- $\gamma$  secretion, activates APC's
    - Leads to increased ability to process and present antigens,
    - Increased expression of costimulators
    - Secretion of cytokines

# Role of Dendritic Cells in Antigen Capture and Display



**FIGURE 6-3** Routes of antigen entry. Microbial antigens commonly

# Role of Dendritic Cells in Antigen Capture and Display

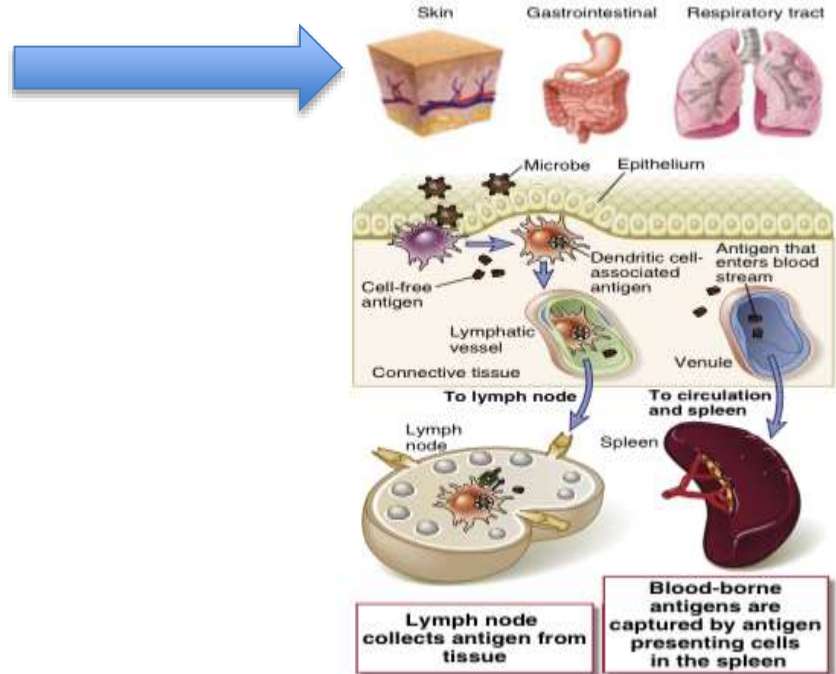


FIGURE 6-3 Routes of antigen entry. Microbial antigens commonly

# Role of Dendritic Cells in Antigen Capture and Display

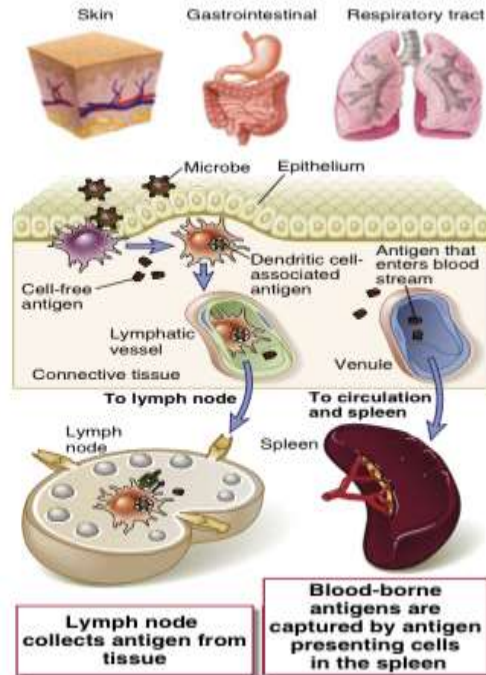


FIGURE 6-3 Routes of antigen entry. Microbial antigens commonly

# 2 Sets of Dendritic Cells

Classical DC	<ul style="list-style-type: none"><li>• Most numerous subset of dendritic cells in the lymphoid organs</li><li>• Mostly derived from myeloid precursors</li></ul>
	<ul style="list-style-type: none"><li>• Constantly sample the environment</li><li>• May also present self antigens for regulation/self-tolerance.</li><li>• Upon encountering microbes/cytokines:<ul style="list-style-type: none"><li>• Upregulate costimulatory molecules</li><li>• Produce inflammatory cytokines</li><li>• Migrate from peripheral tissue to draining lymph node</li></ul></li></ul>

## 2 Sets of Dendritic Cells

### Classical DC

- 2 subsets:
  1. High expression of BDCA-1/CD1c – most potent at driving CD4+ responses
  2. Expression of BDCA-3 – efficient in process of cross-presentation

# 2 Sets of Dendritic Cells

## Plasmacytoid DC

- Resemble plasma cells
- Develop in Bone Marrow from same precursor as Classical DC.
- Found in blood and in small numbers in lymphoid organs
- Poorly phagocytic and do NOT sample environmental antigens
- **Major function:** Secretion of Type I IFN in response to viral infections
- May also differentiate into cells similar to Classical DC and present antigen to Virus-specific T-cells



# Antigen Capture and Transport

Membrane Receptors  
(C-type lectins)

Capture and Endocytose  
microbes or microbial  
products

Process ingested proteins  
into peptides capable of  
binding to MHC

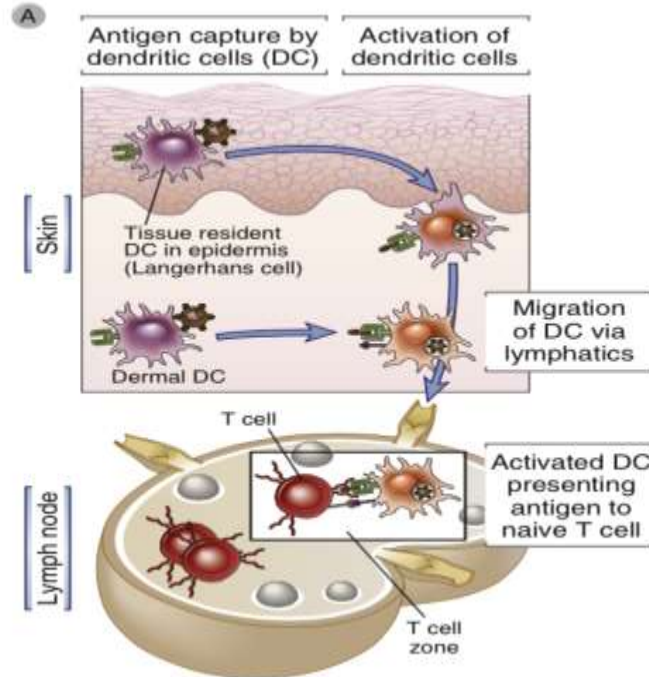


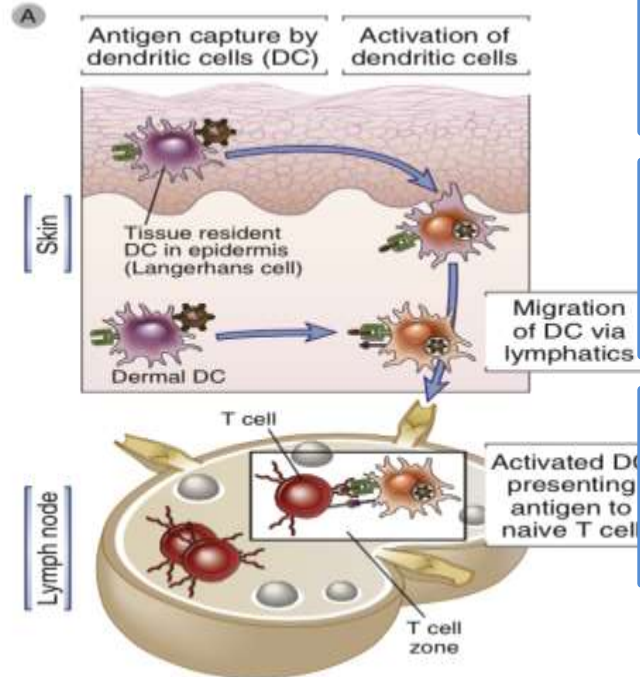
FIGURE 6-5 Role of dendritic cells in antigen capture and presentation

# Antigen Capture and Transport

Membrane Receptors  
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Capture and Endocytose  
microbes or microbial  
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Process ingested proteins  
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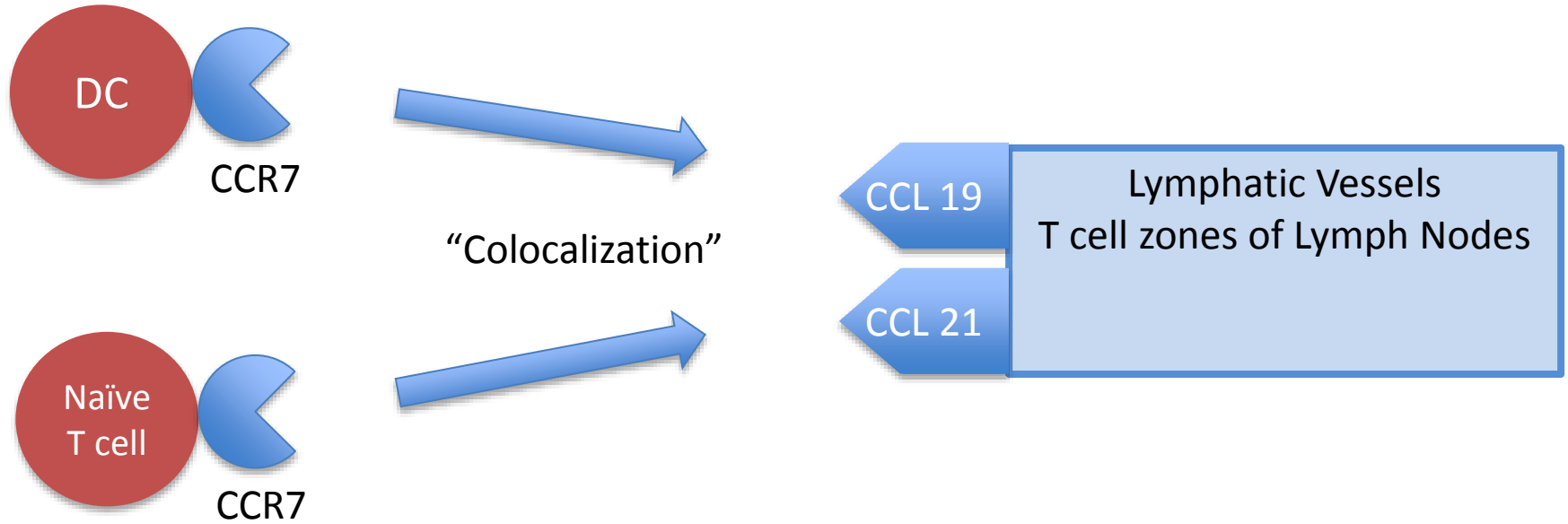
Microbial products  
recognized by TLR

Signals and Cytokines  
activate DC  
(TNF)

Activated DC lose  
adhesiveness and migrate  
to lymph nodes

FIGURE 6-5 Role of dendritic cells in antigen capture and presentation

# Antigen Capture and Transport



# Antigen Capture and Transport



Capture Antigen

Present Antigen to Naïve  
T cells

Activate Lymphocytes

Express high levels of  
MHC

Activated DC develop  
Into potent APCs

B

	<b>Tissue resident dendritic cell</b>	<b>Activated dendritic cell</b>
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 <sup>6</sup>	~7 x 10 <sup>6</sup>

# Antigen Capture and Transport

- Antigens can be transported to lymphoid organs in soluble form
- Afferent Lymphatic Vessel → Subcapsular sinus → FRC conduits → Cortex
- Antigen can be extracted at the conduits, some in the sinuses

# Antigen Capture and Transport

Properties that make DC the most efficient APCs for initiating T cell responses

1. Strategically located at common sites of entry
2. Express receptors that enable capture and response
3. Migrate from epithelia and tissues via lymphatics to T cell zones of LN
4. Mature DC express high levels of peptide-MHC complexes, costimulators, and cytokines

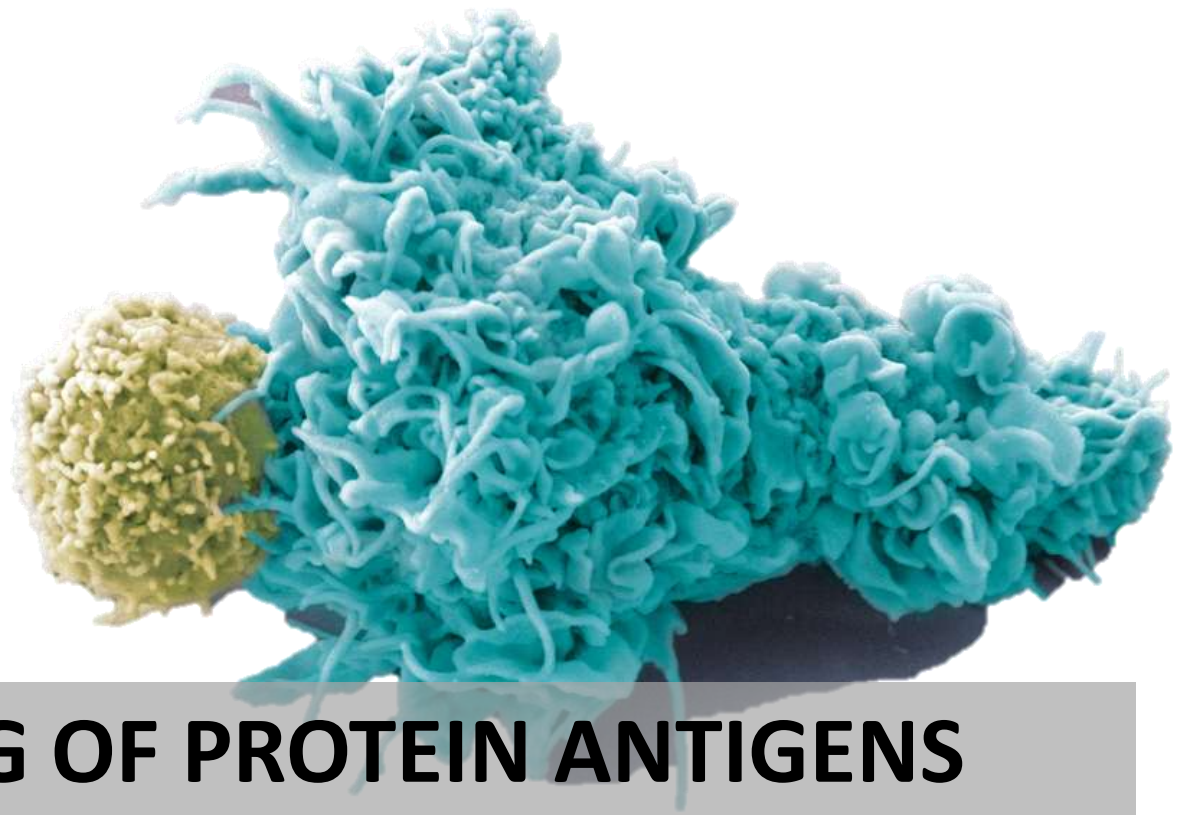
# Antigen Capture and Transport

- Dendritic cells can also ingest infected cells and present antigens to CD8+ T lymphocytes
  - Peptide antigens must be derived from proteins in the cytosol of DC
  - Specialized DC: able to ingest virus-infected cells and deliver viral proteins into their cytosol
  - “Cross-presentation or Cross-priming”



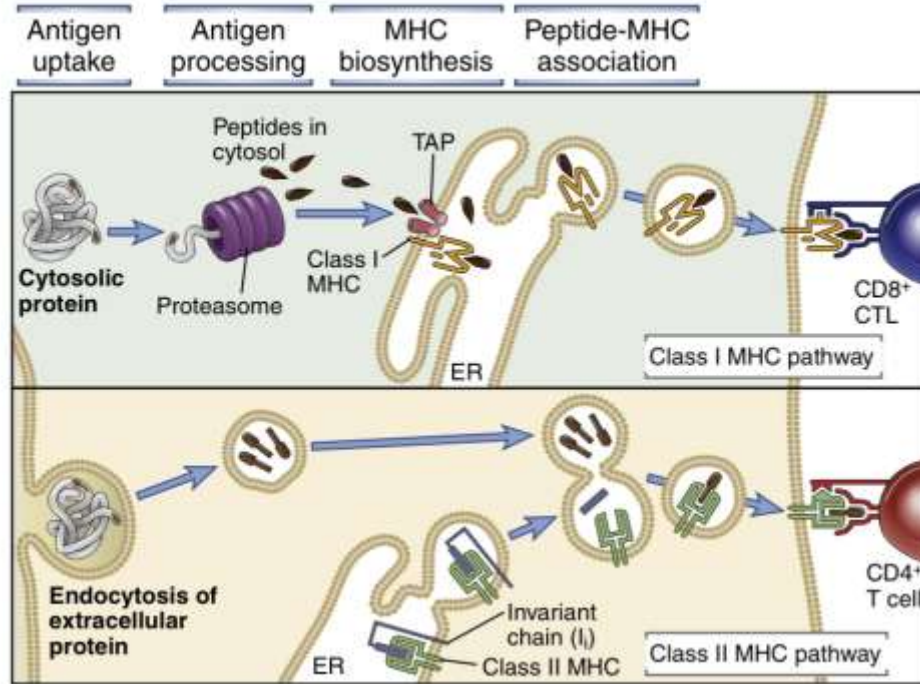
# Function of Other APCs

Cell-mediated Immune Responses	Macrophages present Ag of phagocytosed microbes to effector T cells
Humoral Immune Responses	B lymphocytes internalize protein Ag and present peptides from these proteins to helper T cells.
Nucleated cells	Can present peptides, derived from cytosolic protein antigens → CD8+ CTLs
Other cell types that express MHC class II (endothelial and some epithelial cells)	May present Ag to T cells





# PROCESSING OF PROTEIN ANTIGENS

# PROCESSING OF PROTEIN ANTIGENS

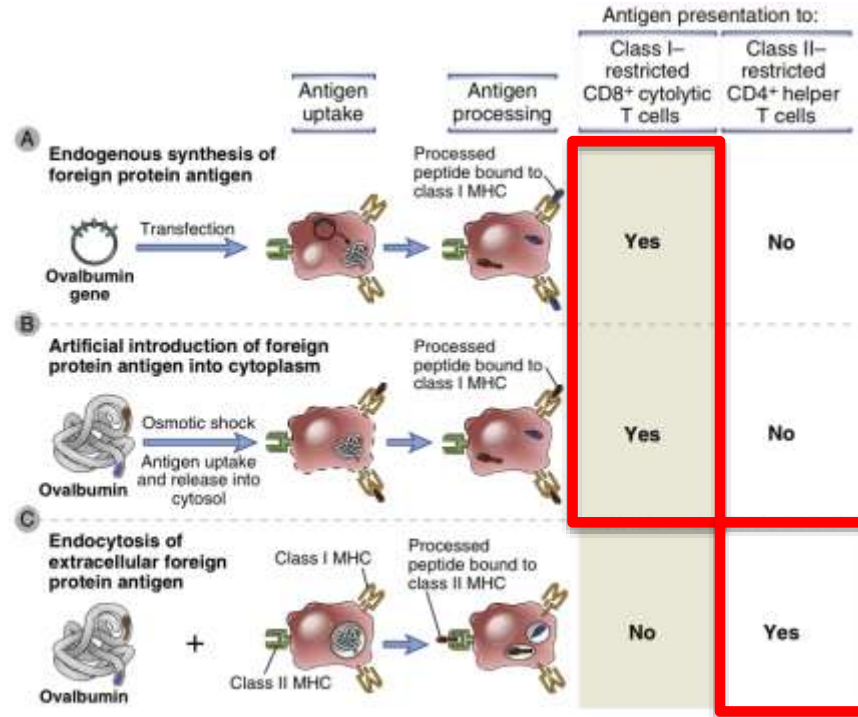


# PROCESSING OF PROTEIN ANTIGENS

**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 Peptide  $\alpha$ $\beta_2$ -microglobulin	Polymorphic $\alpha$ and $\beta$ chains, peptide Peptide  $\alpha$ $\beta$
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	Mainly cytosolic proteins (usually synthesized in the cell; may enter cytosol from phagosomes); also nuclear and membrane proteins	Endosomal and lysosomal proteins (mostly internalized from extracellular environment)
Enzymes responsible for peptide loading of MHC	Proteasomes	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
<i>APC</i> , antigen-presenting cell; <i>CIIV</i> , class II vesicle; <i>ER</i> , endoplasmic reticulum; <i>MHC</i> , major histocompatibility complex; <i>MIIC</i> , MHC class II compartment; <i>TAP</i> , transporter associated with antigen processing.		

# PROCESSING OF PROTEIN ANTIGENS

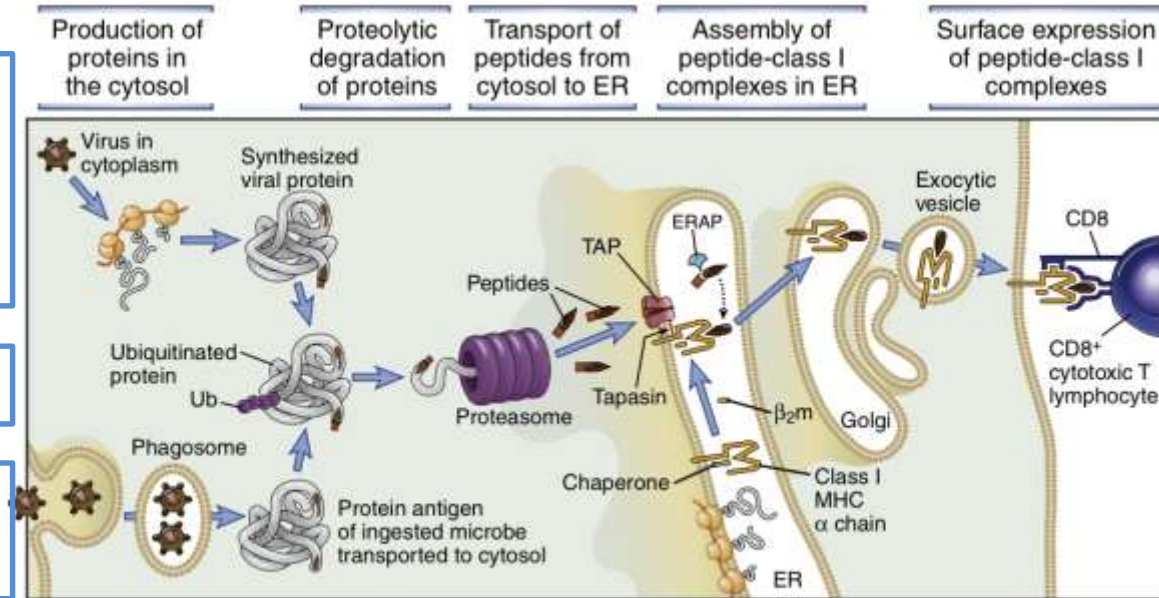


# Class I MHC Pathway

Injected via  
Bacterial  
secretory  
mechanisms

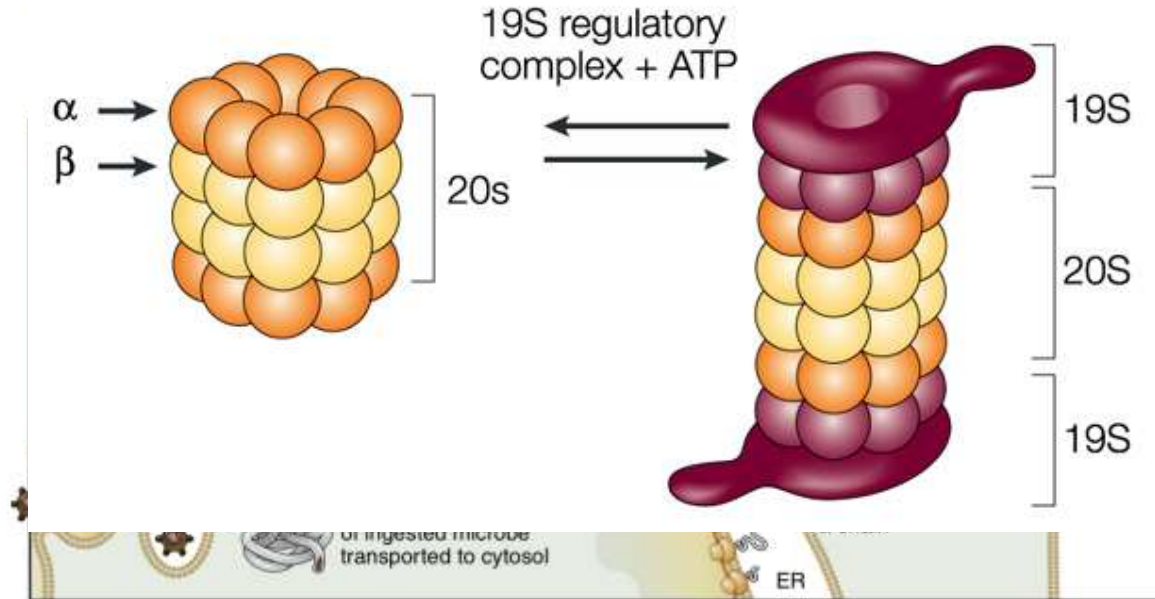
Phagocytosed

Escape  
Mechanism



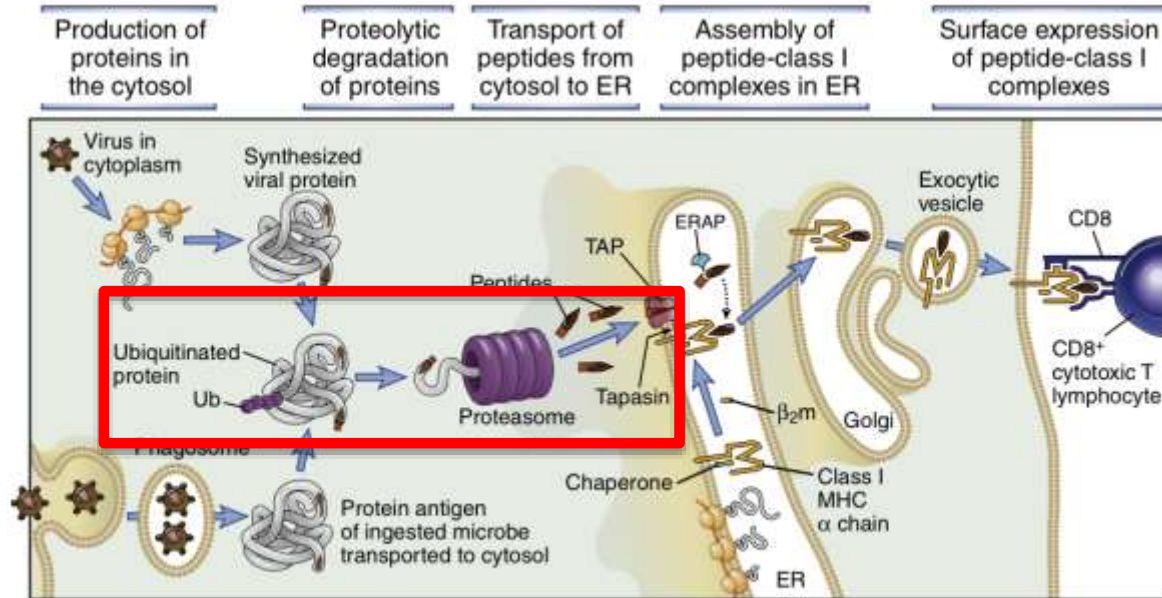
**FIGURE 6-16 The class I MHC pathway of antigen presentation.** The stages in the processing of cytosolic proteins are described in the text. ERAP, endoplasmic reticulum associated peptidase; ER, endoplasmic reticulum; β<sub>2</sub>m, β<sub>2</sub>-microglobulin; TAP, transporter associated with antigen processing; Ub, ubiquitin.

# Class I MHC Pathway



**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.

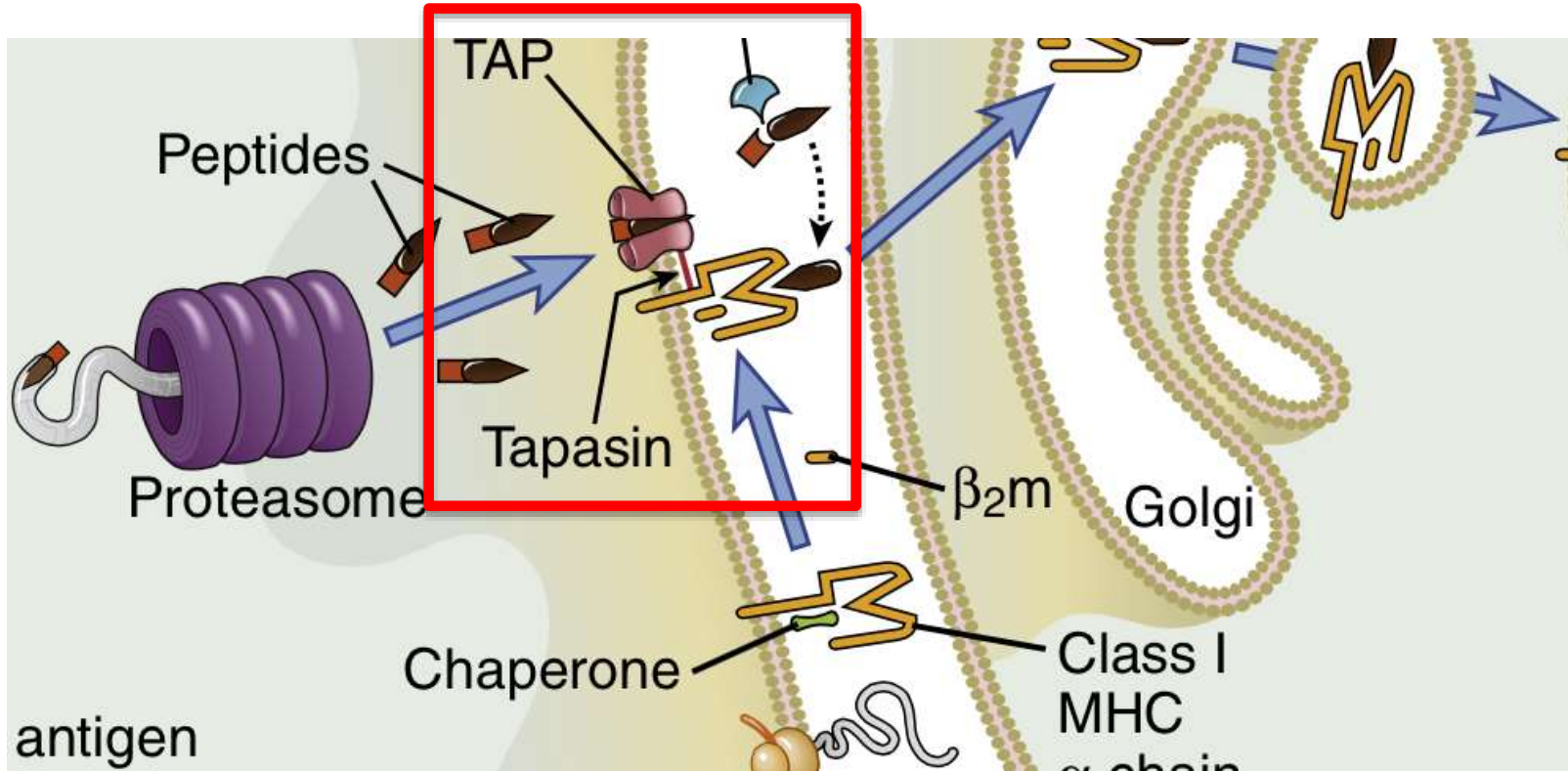
# Class I MHC Pathway



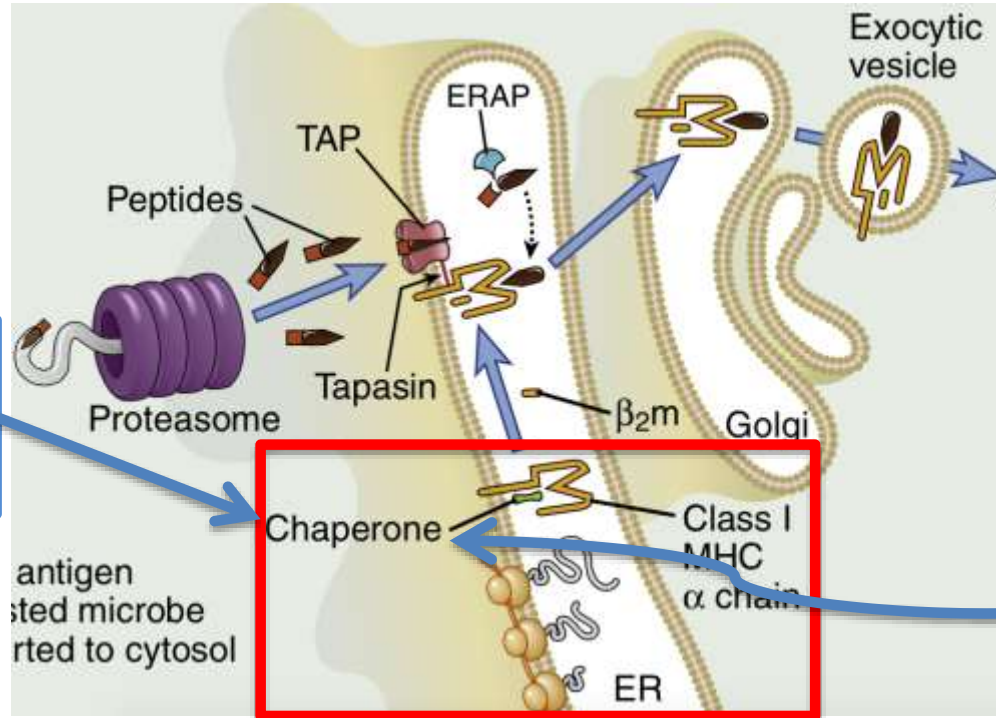
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# Class I MHC Pathway



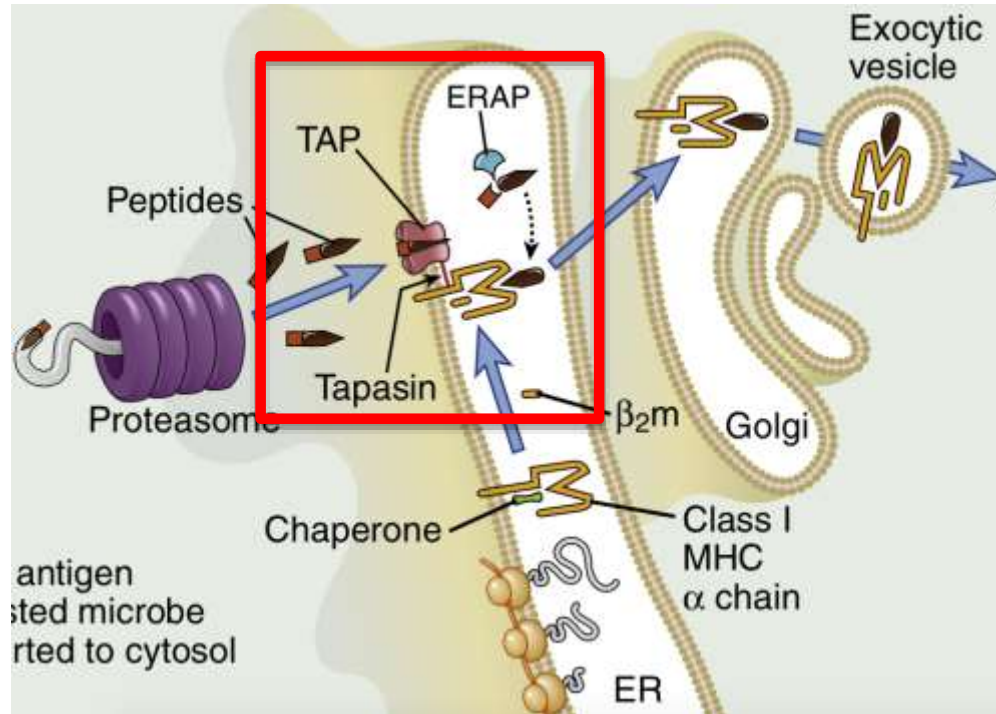
# Class I MHC Pathway



Membrane  
Chaperone:  
Calnexin

Luminal Chaperone:  
Calreticulin

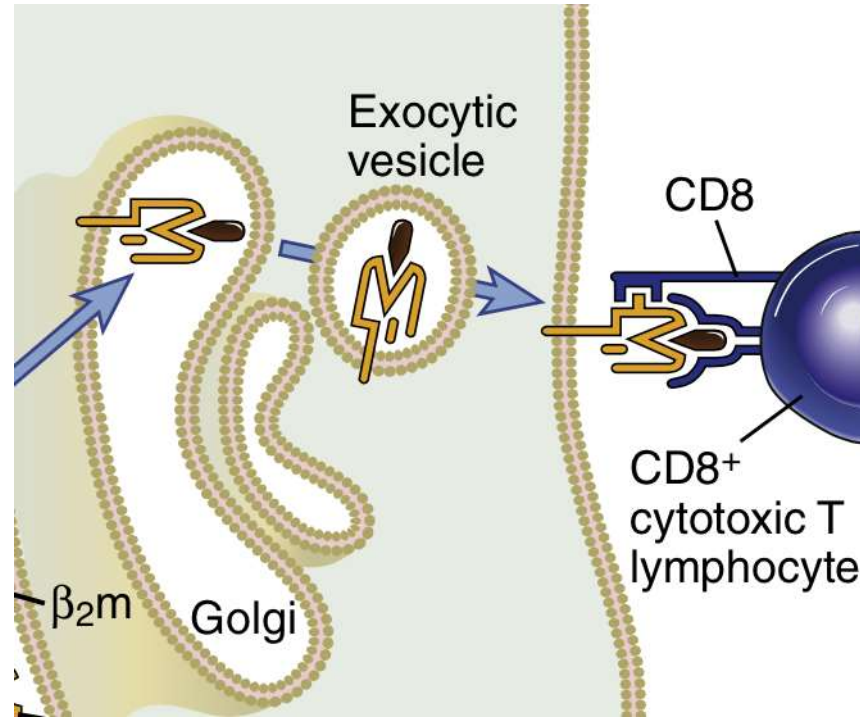
# Class I MHC Pathway



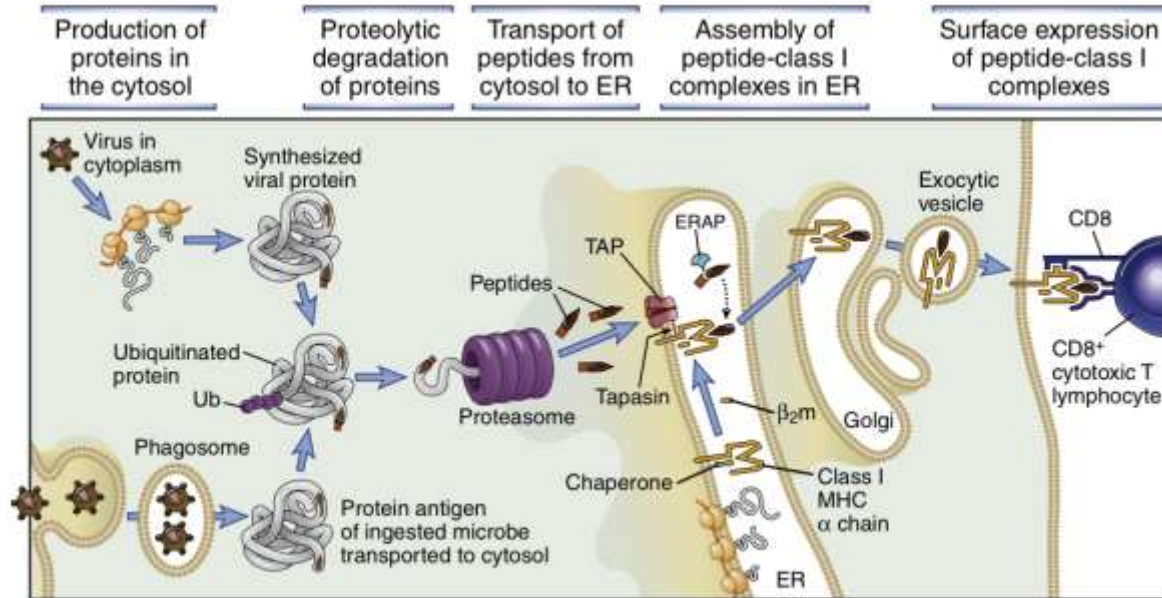
# Class I MHC Pathway

- Peptides transported into ER preferentially bind to Class I MHC but NOT Class II MHC:
  1. Class I attached to TAP complex
  2. Class II molecules are blocked by a protein called the invariant chain

# Class I MHC Pathway

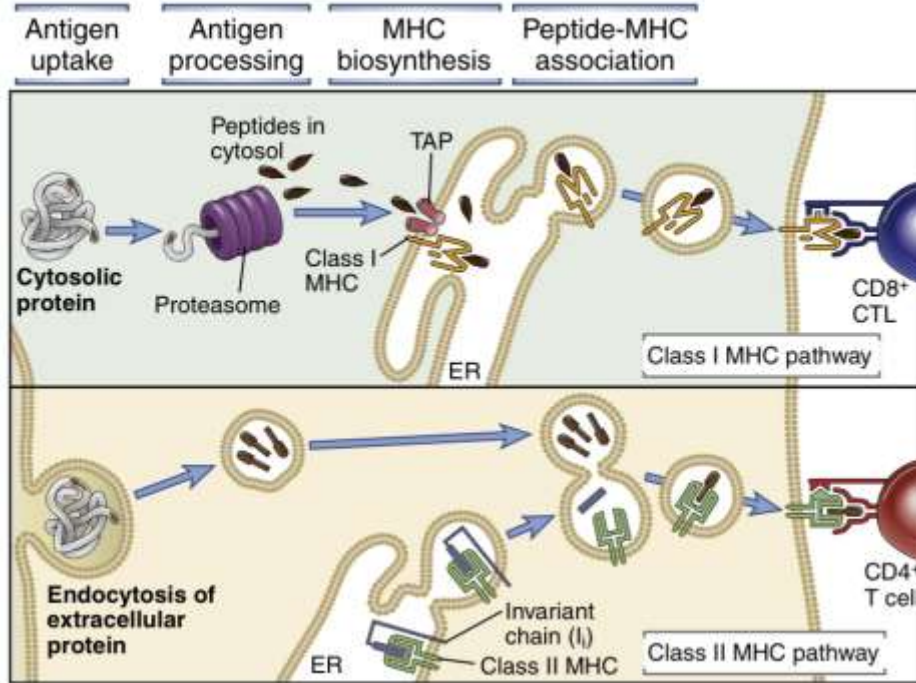


# Class I MHC Pathway



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# MHC PROCESSING

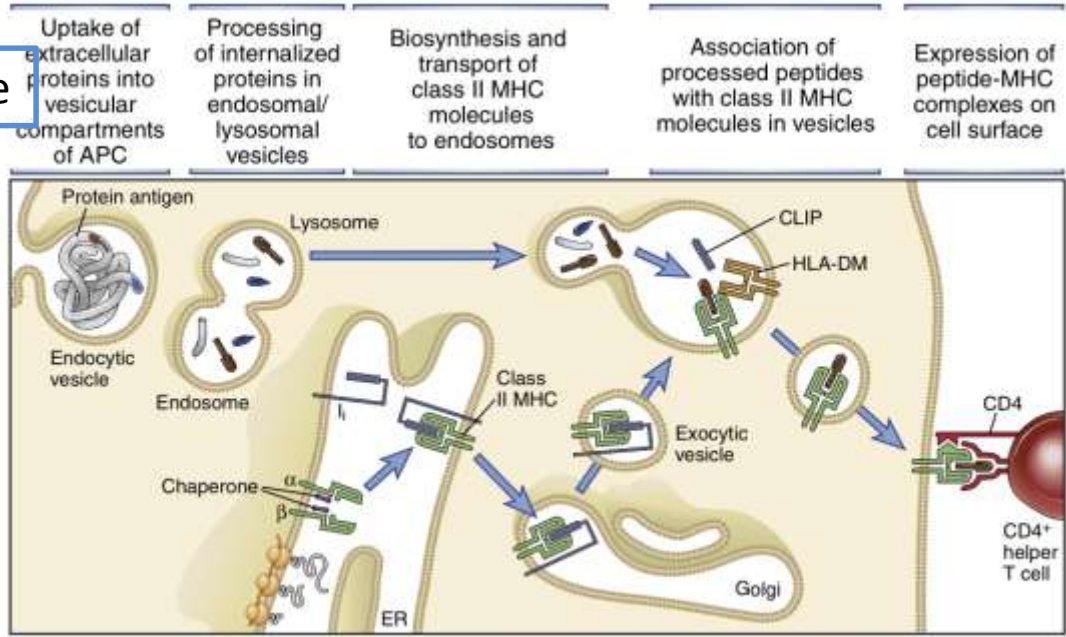


# Class II MHC Pathway

Endosome-Lysosome

Phagolysosomes

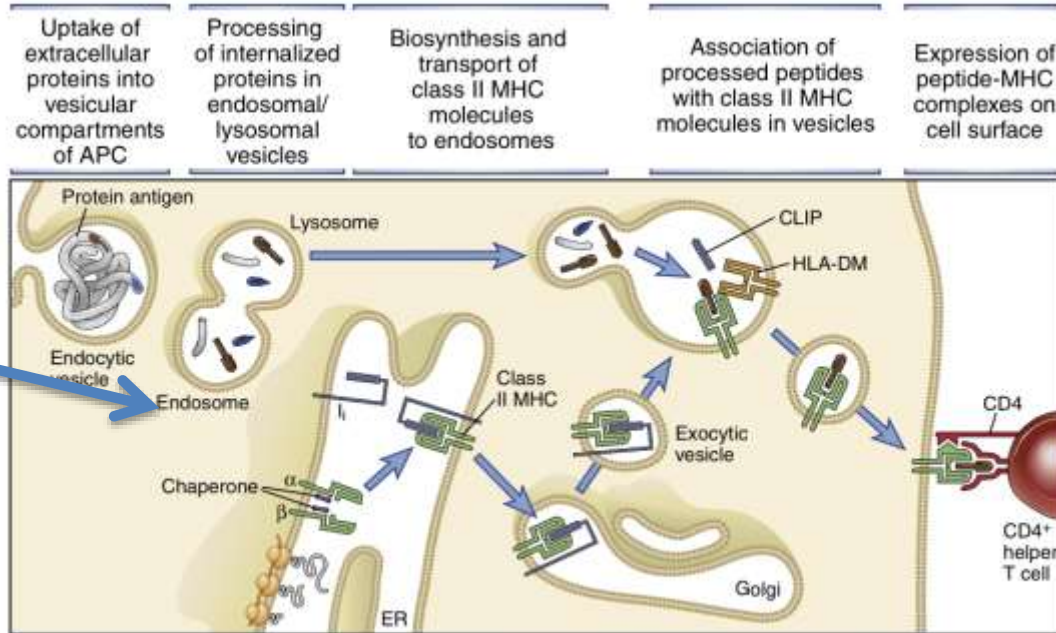
Autophagosomes



**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<sub>i</sub>, invariant chain.



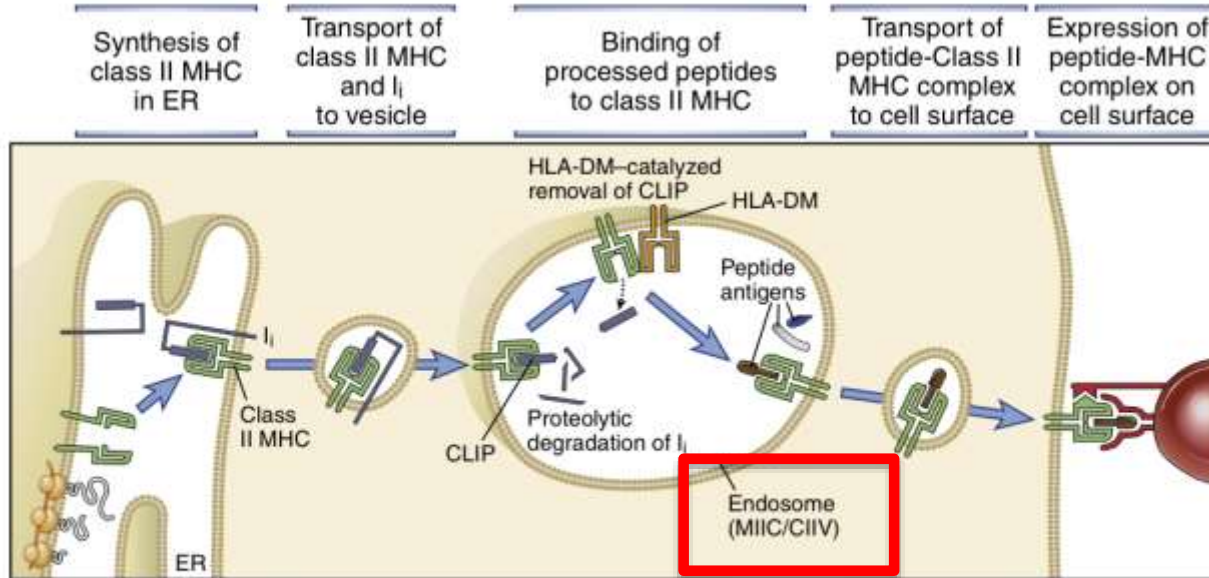
# Class II MHC Pathway



CATHEPSINS

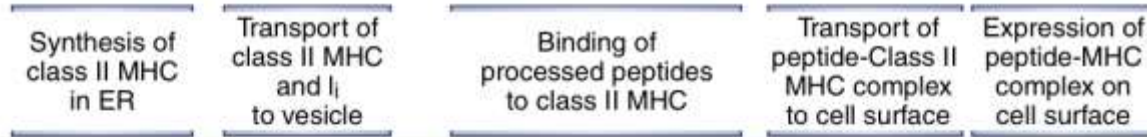
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# Class II MHC Pathway

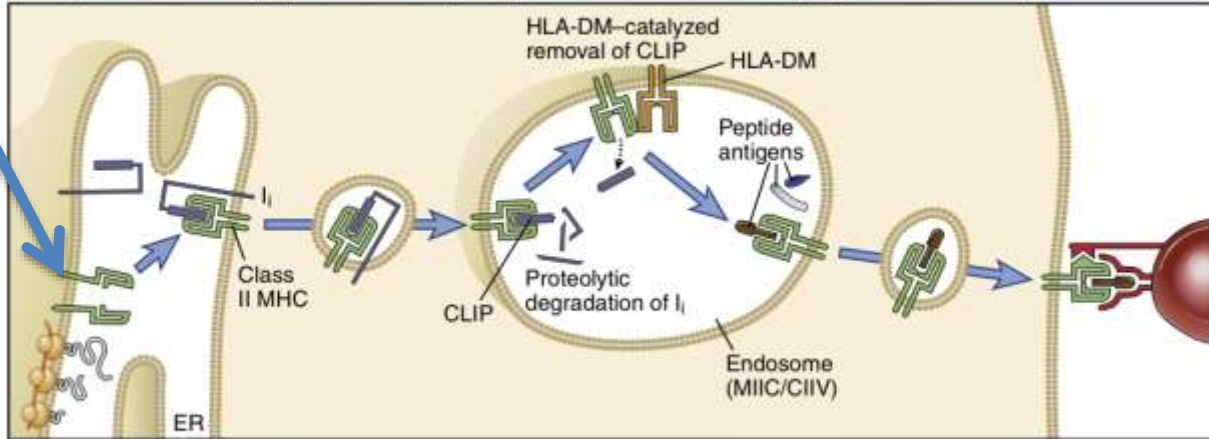


**FIGURE 6-19** The functions of class II MHC-associated invariant chain and HLA-DM. Class II molecules with bound invariant chain, or CLIP, are transported into vesicles, where the I<sub>i</sub> is degraded and the remaining CLIP is removed by the action of DM. Antigenic peptides generated in the vesicles are then able to bind to the class II molecules. Another class II-like protein, called HLA-DO, may regulate the DM-catalyzed removal of CLIP. (not shown). CIIV, class II vesicle.

# Class II MHC Pathway

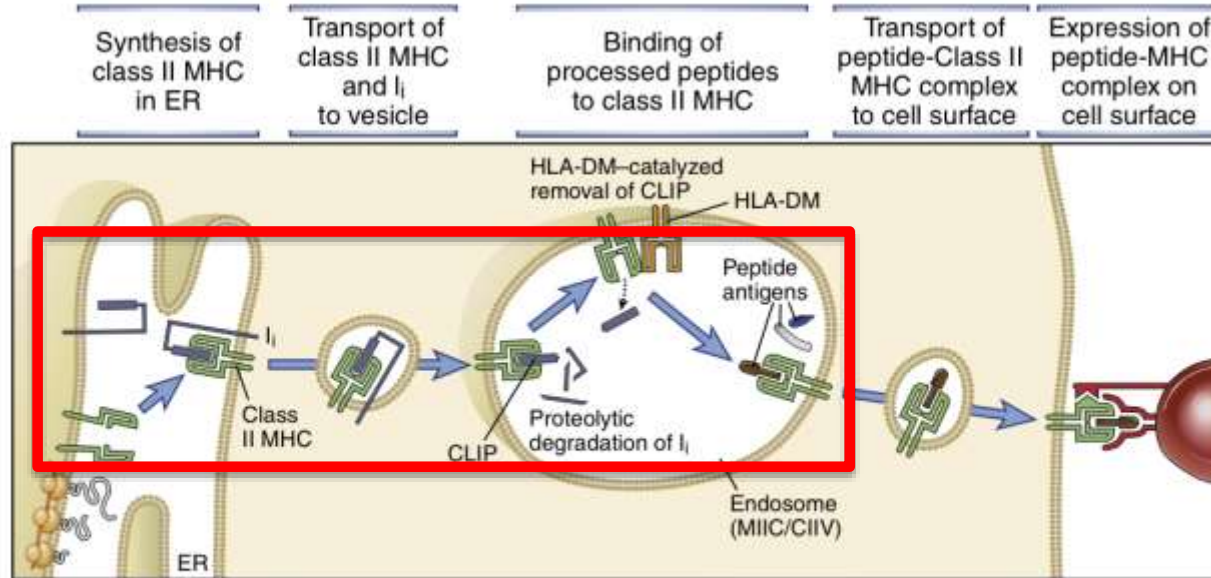


Membrane Chaperone: Calnexin



**FIGURE 6-19** The functions of class II MHC-associated invariant chain and HLA-DM. Class II molecules with bound invariant chain, or CLIP, are transported into vesicles, where the I<sub>i</sub> is degraded and the remaining CLIP is removed by the action of DM. Antigenic peptides generated in the vesicles are then able to bind to the class II molecules. Another class II-like protein, called HLA-DO, may regulate the DM-catalyzed removal of CLIP. (not shown). CIIV, class II vesicle.

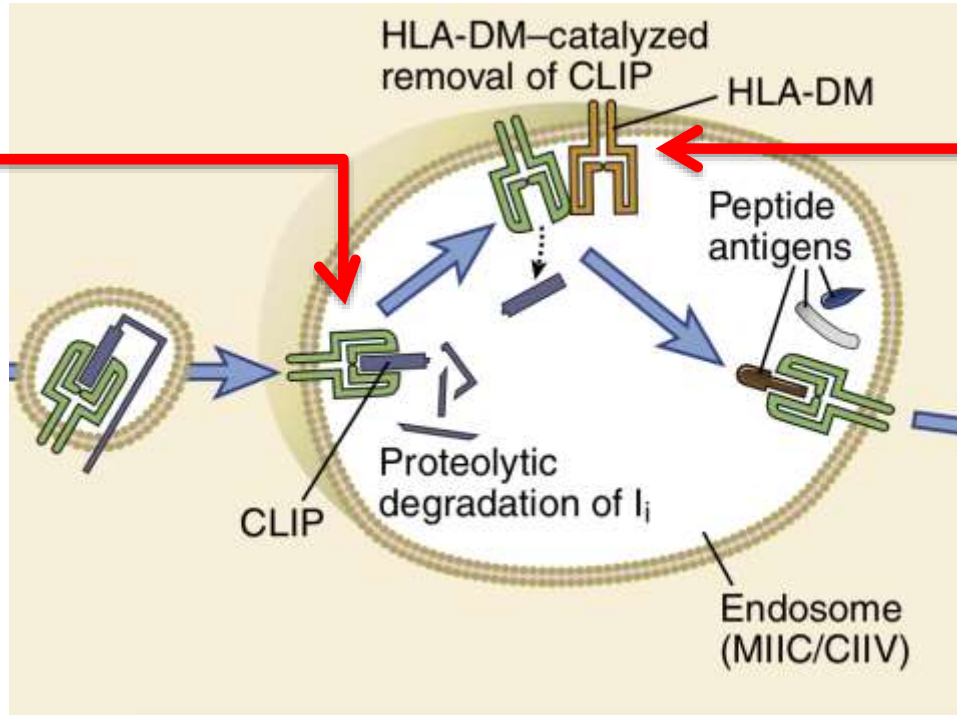
# Class II MHC Pathway



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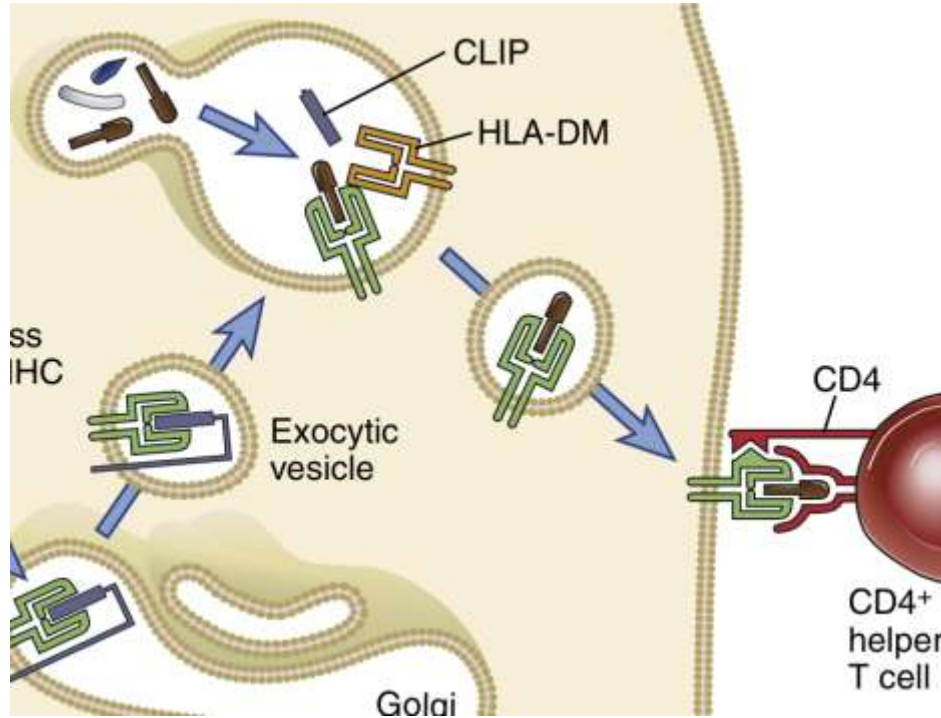
# Class II MHC Pathway

STEP 1:  
Cathepsins degrade  
Invariant Chain  
↓  
CLIP

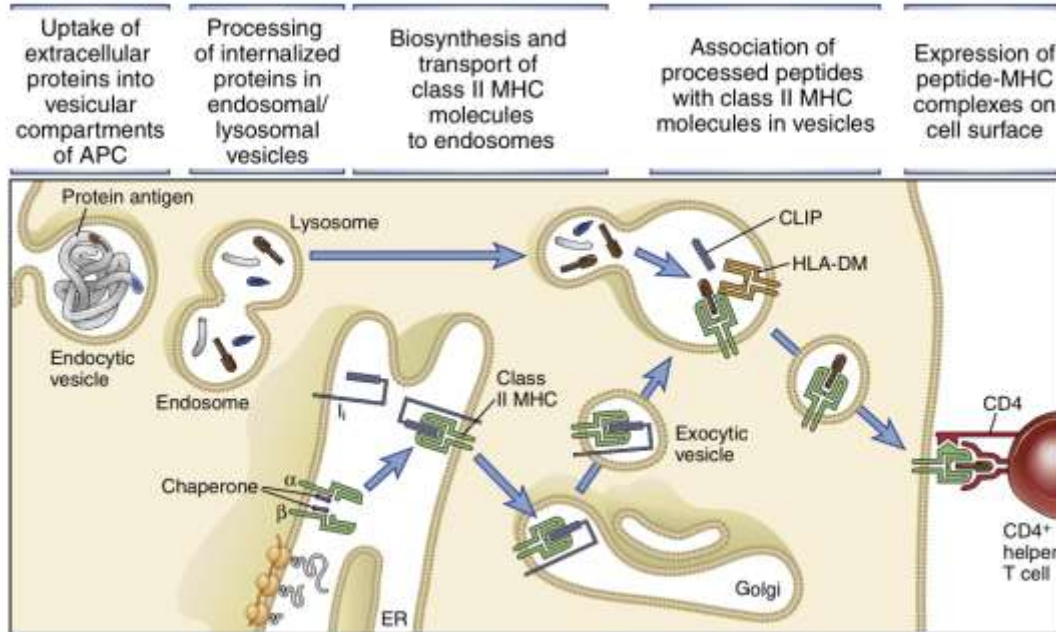


STEP 2:  
HLA-DM removes  
CLIP

# Class II MHC Pathway

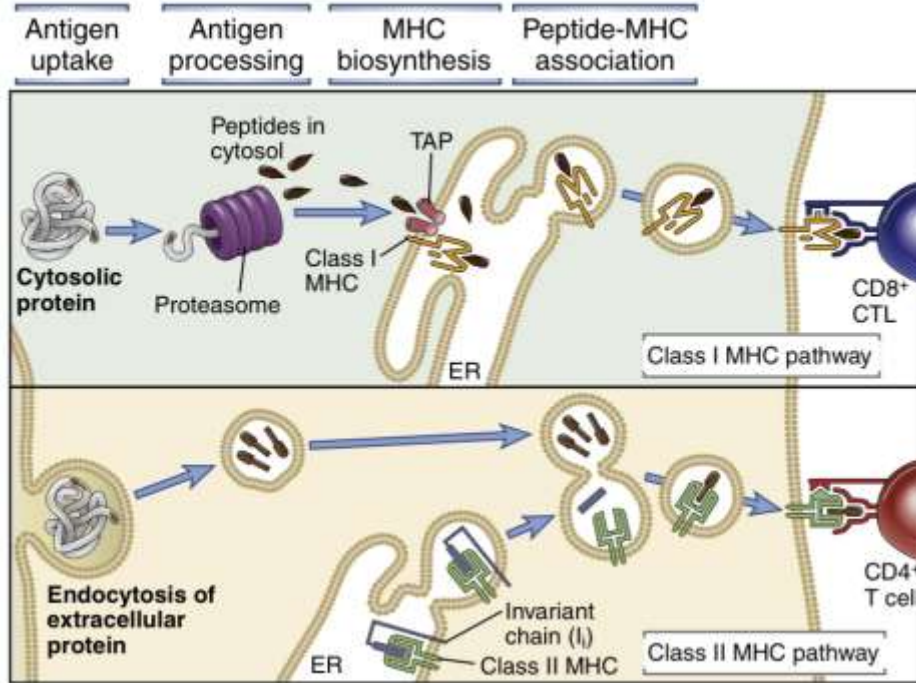


# Class II MHC Pathway



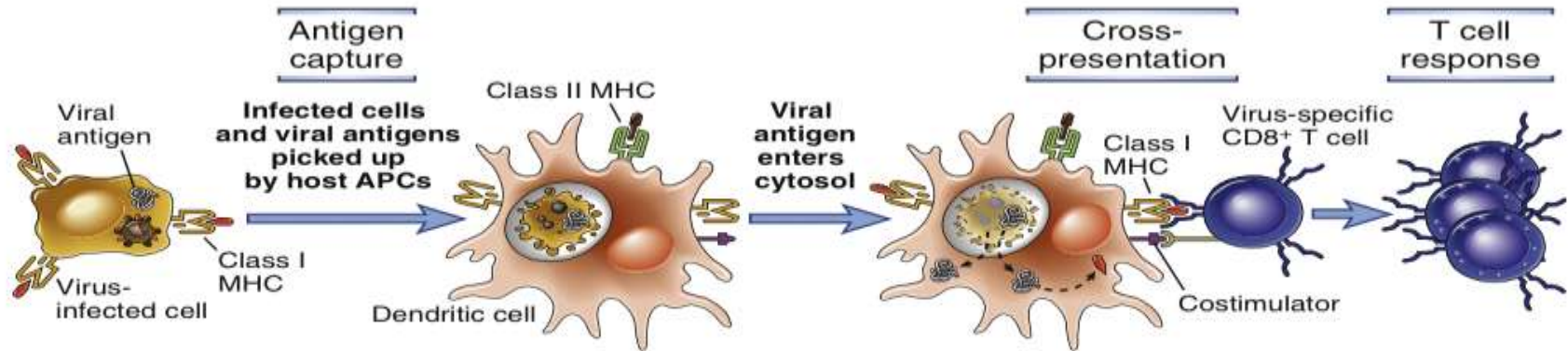
**FIGURE 6-17** The class II MHC pathway of antigen presentation. The stages in the processing of extracellular antigens are described in the text. CLIP, class II-associated invariant chain peptide; ER, endoplasmic reticulum; I<sub>i</sub>, invariant chain.

# MHC PROCESSING



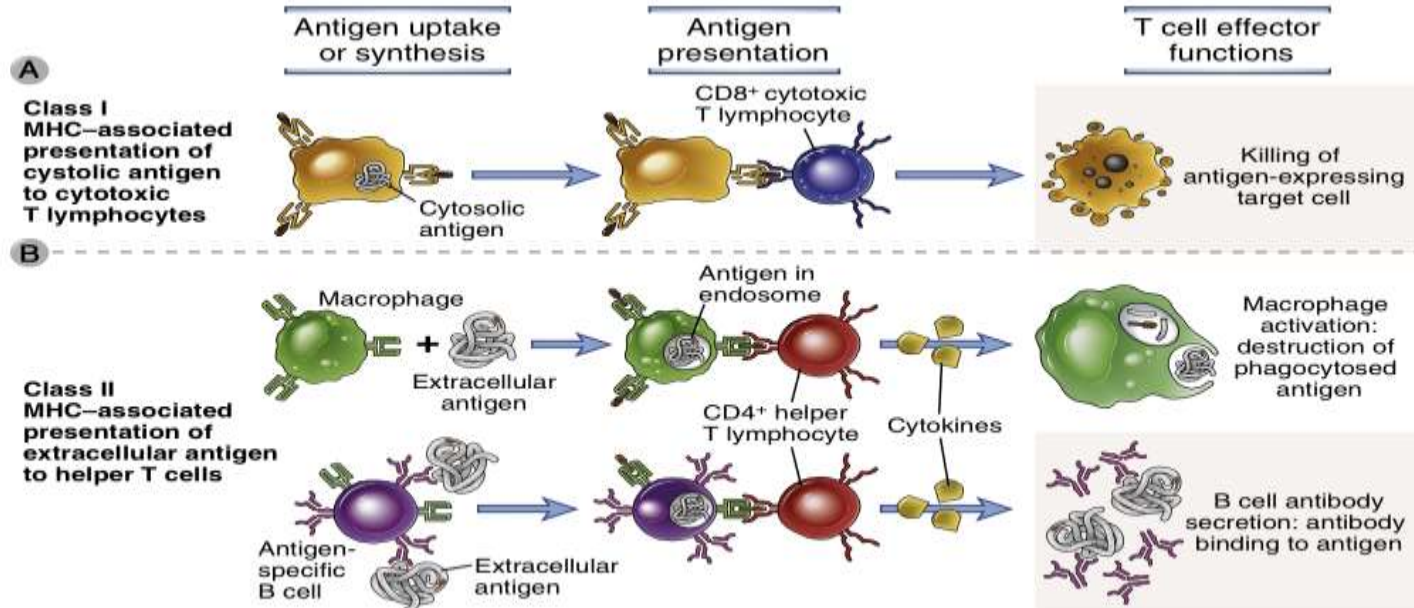


# Cross-Presentation



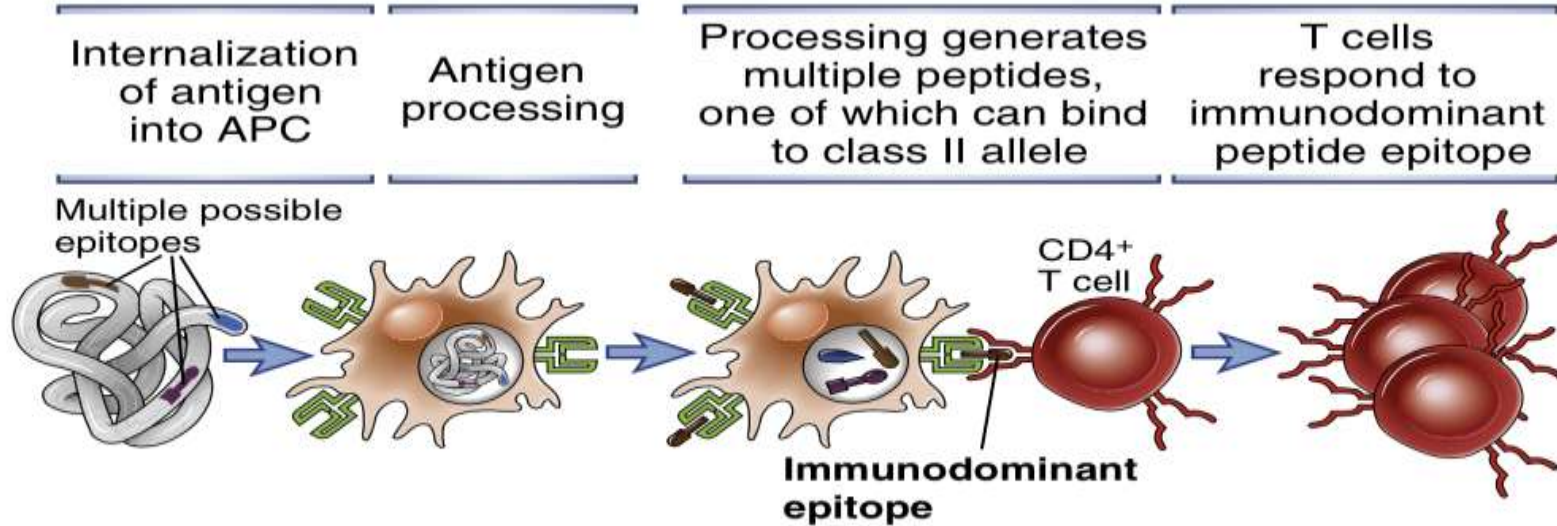
**FIGURE 6-20 Cross-presentation of antigens to CD8<sup>+</sup> T cells.** Cells infected with intracellular microbes, such as viruses, are ingested by dendritic cells, and the antigens of the infectious microbes are transported into the cytosol and processed and presented in association with class I MHC molecules to CD8<sup>+</sup> T cells (see also Fig. 6-16). Thus, dendritic cells are able to present endocytosed vesicular antigens by the class I pathway. Note that the same cross-presenting APCs may display class II MHC-associated antigens from the microbe for recognition by CD4<sup>+</sup> helper T cells.

# Physiologic Significance of MHC-associated Antigen Presentation

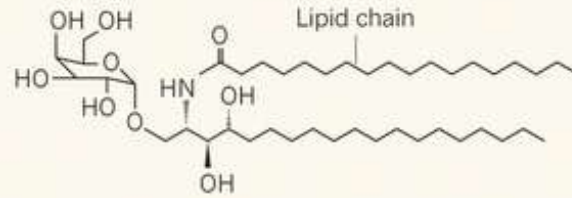
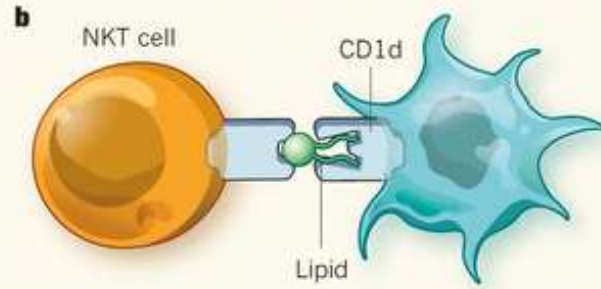


**FIGURE 6-21** Presentation of extracellular and cytosolic antigens to different subsets of T cells. **A**, Cytosolic antigens are presented by nucleated cells to CD8<sup>+</sup> CTLs, which kill (lyse) the antigen-expressing cells. **B**, Extracellular antigens are presented by macrophages or B lymphocytes to CD4<sup>+</sup> helper T lymphocytes, which activate the macrophages or B cells and eliminate the extracellular antigens.

# Physiologic Significance of MHC-associated Antigen Presentation



**FIGURE 6-22 Immunodominance of peptides.** Protein antigens are processed to generate multiple peptides; immunodominant peptides are the ones that bind best to the available class I and class II MHC molecules. The illustration shows an extracellular antigen generating a class II-binding peptide, but this also applies to peptides of cytosolic antigens that are presented by class I MHC molecules.



# PRESENTATION OF NON-PROTEIN ANTIGENS TO SUBSETS OF T CELLS

# Presentation of Non-Protein Antigens

- Small populations of T cells can recognize non-protein antigens without Class I or II MHC: NKT cells and  $\gamma\delta$  T cells.
  - NKT: recognize lipids and glycolipids displayed by CD1
  - $\gamma\delta$  T cells: recognize proteins, lipids, phosphorylated molecules and alkyl amines

# Outline

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# Summary

1. Most T cells recognize antigens only as peptides displayed by the products of self MHC genes on the surface of APCs.
2. MHC is a large genetic region coding for highly polymorphic, co-dominantly expressed class I and class II MHC molecules
3. The expression of MHC gene products is enhanced by inflammatory and immune stimuli, particularly cytokines like IFN- $\gamma$ , which stimulate the transcription of MHC genes.

# Summary

MHC I	MHC II
Composed of an $\alpha$ (or heavy) chain in a non-covalent complex with a $\beta$ 2-microglobulin	Contain two MHC-encoded polymorphic chains, an $\alpha$ chain and a $\beta$ chain.
Recognized by CD8+ T cells	Recognized by CD4+ T cells
Accommodate peptides that are 6 to 16 amino acid residues in length	Allows larger peptides (up to 30 amino acid residues in length or more) to bind
Expressed on all nucleated cells	Expressed mainly on specialized APCs
Cytosolic proteins are proteolytically degraded in the proteasome	Extracellular proteins are internalized into endosomes



**THANK YOU**

# References

- Cellular and Molecular Immunology 8<sup>th</sup> Ed. (2015) by Abbas et al.
  - **Chapter 6:** MHC Molecules and Antigen Presentation to T Lymphocytes

