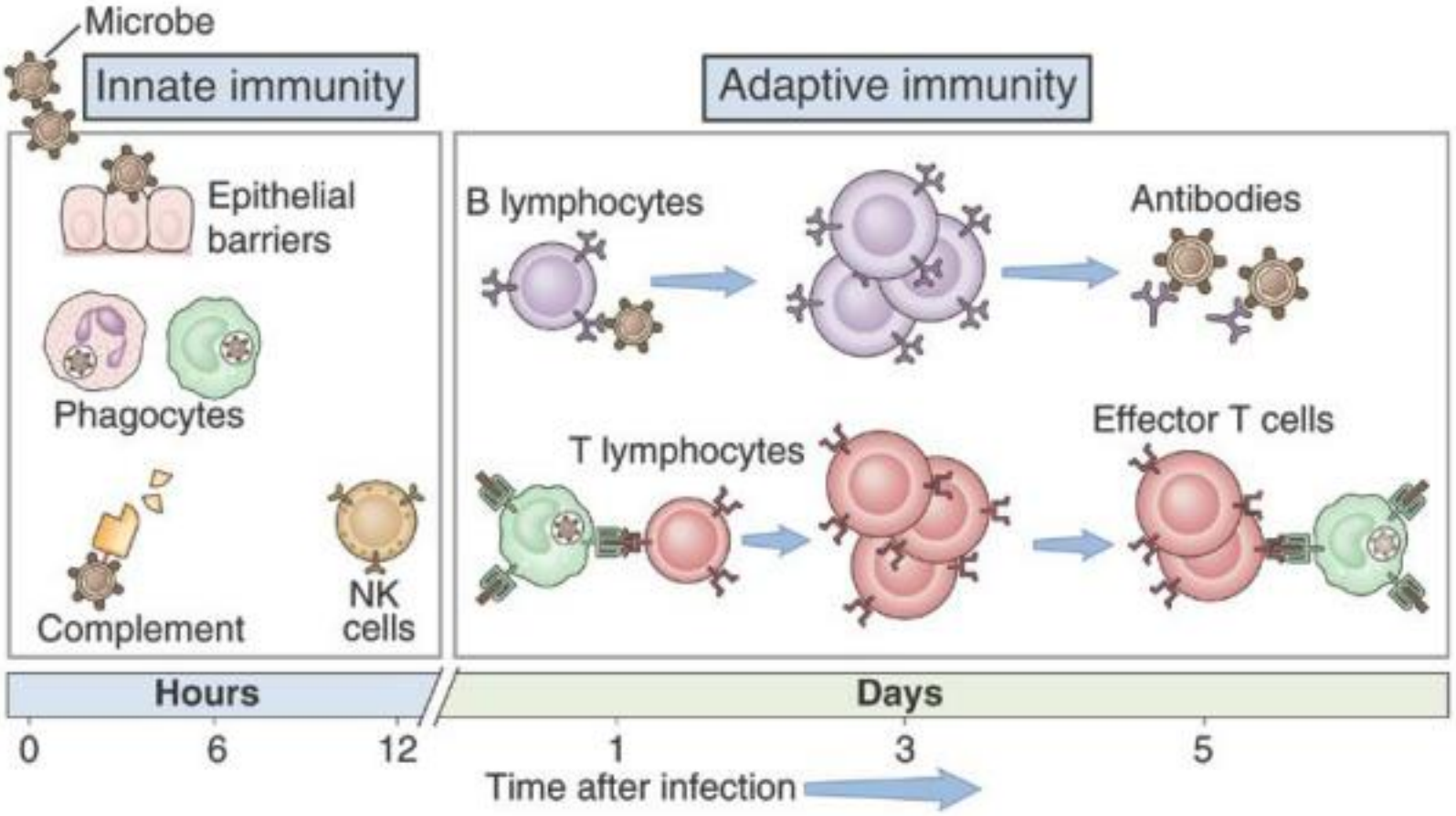
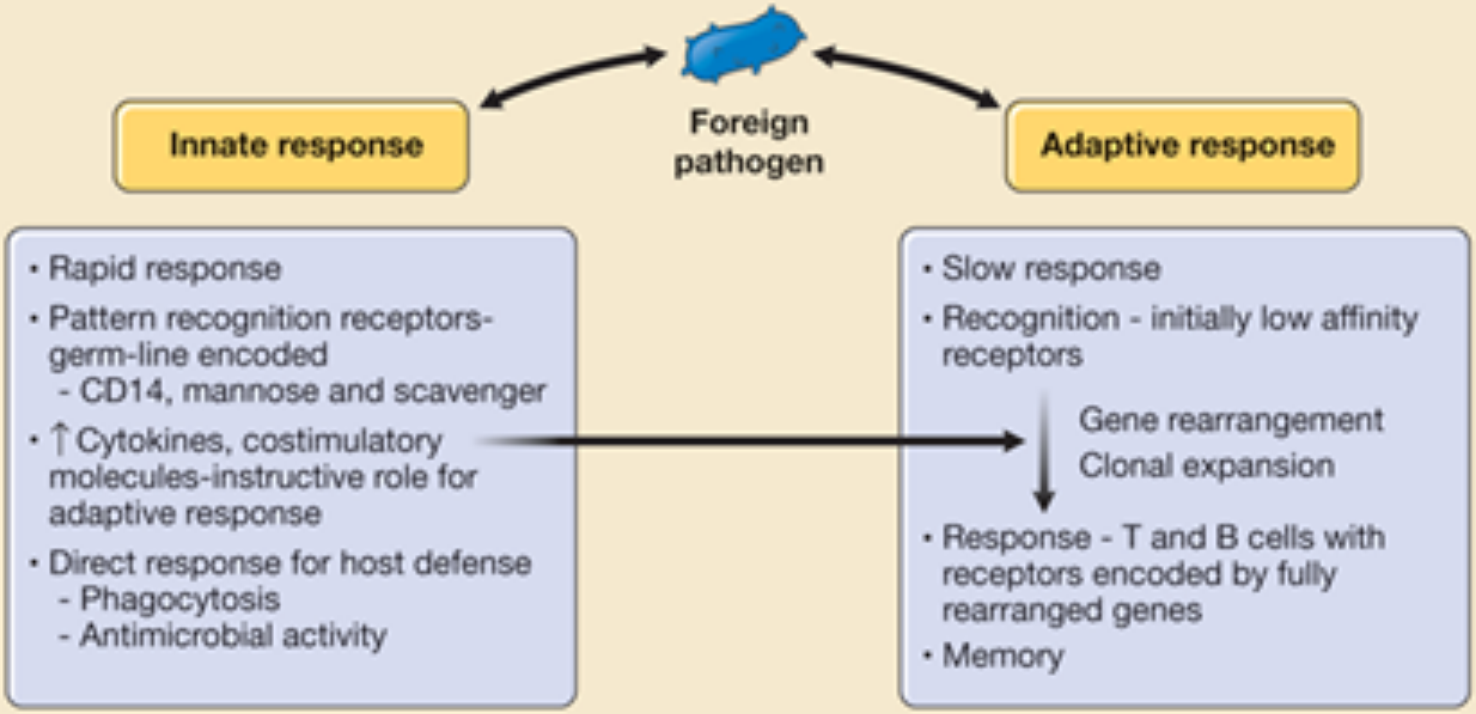
Antigen Processing and Presentation

Assoc. Prof. Dr. Emrah Şefik Abamor

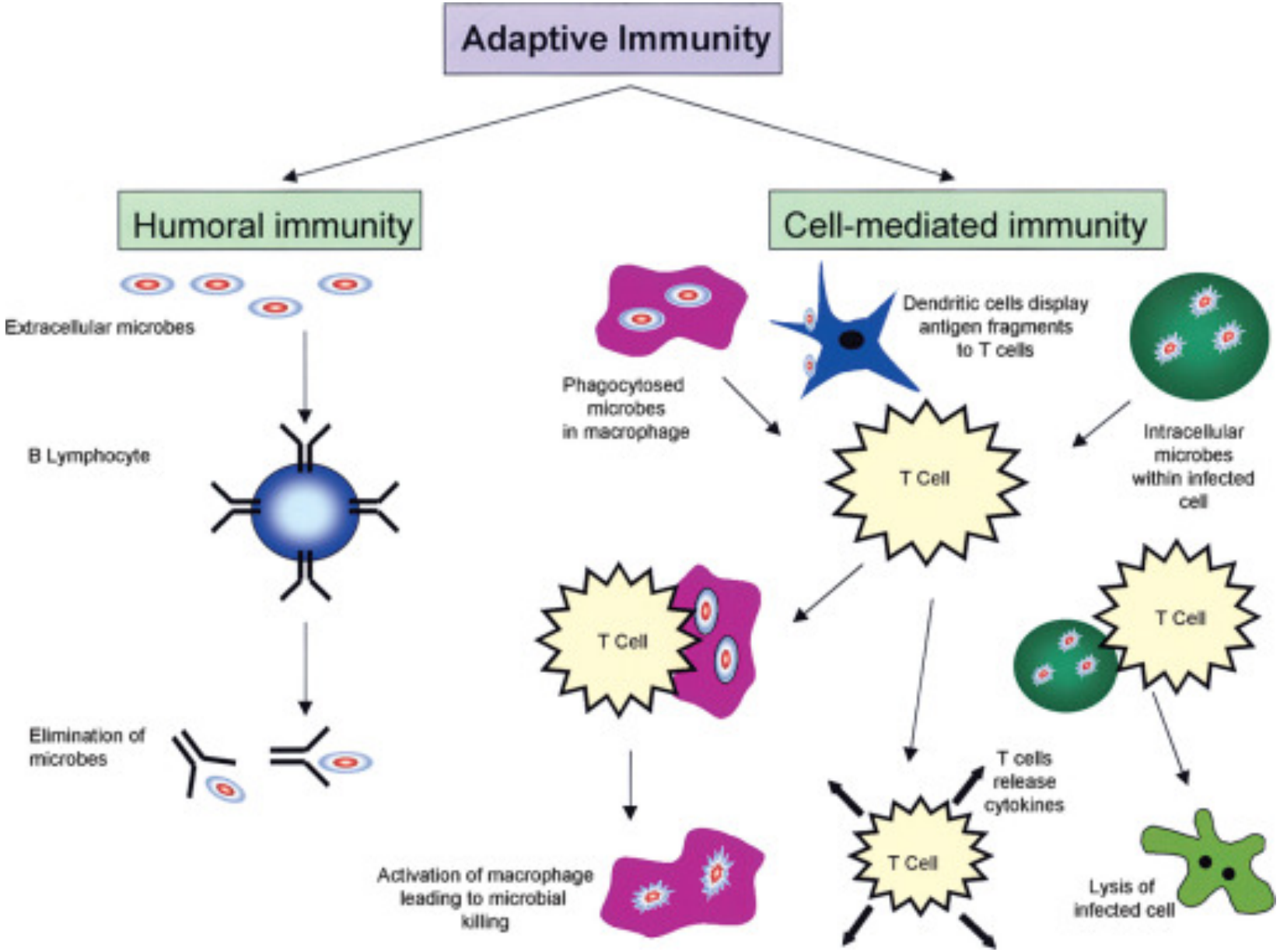
Innate and Adaptive Immunity



The immune response



Source: Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ, Wolff K: *Fitzpatrick's Dermatology in General Medicine, 8th Edition*: www.accessmedicine.com


Adaptive Immunity
Humoral immunity
Cell-mediated immunity
Extracellular microbes
B Lymphocyte
Elimination of microbes
Phagocytosed microbes in macrophage
Dendritic cells display antigen fragments to T cells
Intracellular microbes within infected cell
T Cell
T Cell
T Cell
T Cell
T cells release cytokines
Activation of macrophage leading to microbial killing
Lysis of infected cell

- Both types of acquired immunity are initiated by antigens. Each toxin or organism carries one or more chemicals unique to it.
- **These substances with protein or large polysaccharide structure are called antigens** and acquired immunity is initiated with these building blocks.
- For a substance to be antigenic, **it must possess high molecular weight, 8000 or higher.**

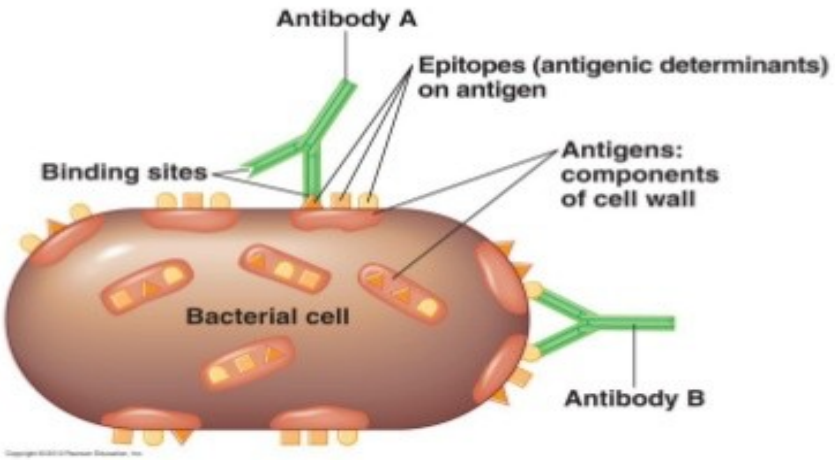
- Antibodies and T-cell receptors interact with only a specific part of an antigenic macromolecule called the **antigenic determinant** or **epitope**.
- Antigenic determinants include **sugars, amino acids and other organic molecules.**
- Surface of a bacterium or virus is a mosaic of proteins, polysaccharides and other macromolecules, each containing different epitopes.

Epitope

- Epitope or antigenic determinant, is the **portion of an antigen that binds to antibody and B cells.**
- **Part of the epitope can also be converted to peptides presented by the major histocompatibility complex and bind to T cells.**

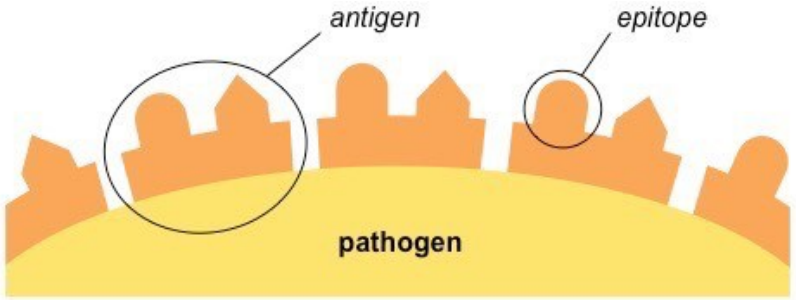
Epitopes or *antigenic determinants*

- **Antigens** are molecules the body recognizes as foreign
- Their binding to defensive cells **can trigger adaptive immune responses**
- Antigens are recognized by the shape of regions called **epitopes**

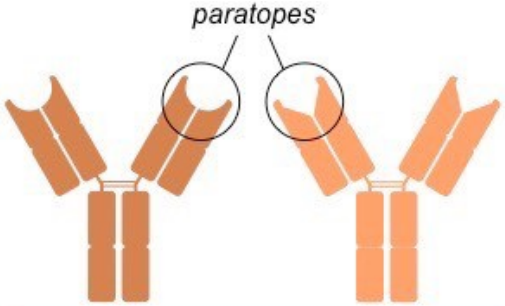


- **Microbial antigens include:**

Components of the cell walls, capsule, flagella or toxins, proteins and glycoproteins of viruses, fungi, or protozoa

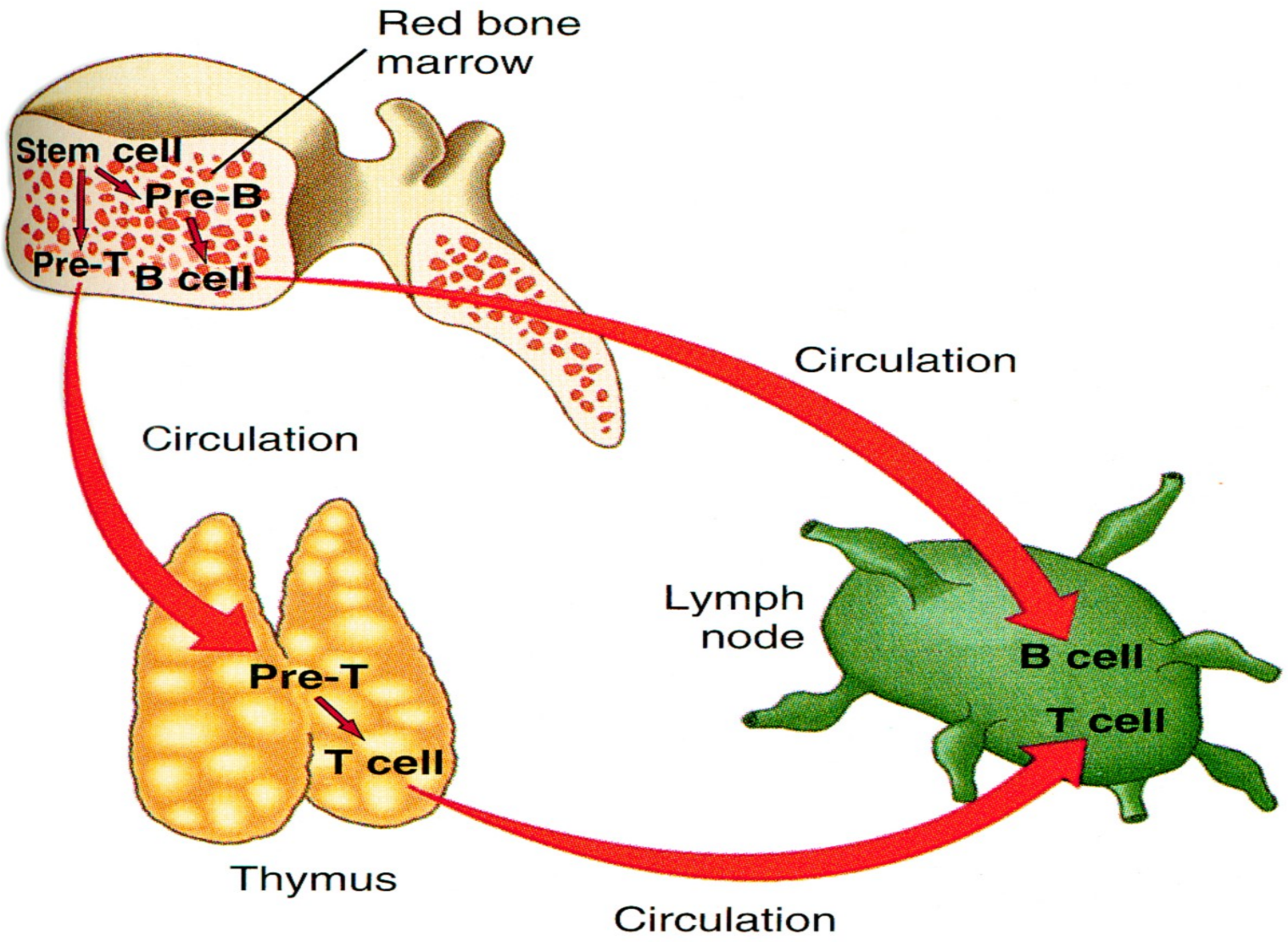


Pathogens possess highly specific antigenic determinants (epitopes)

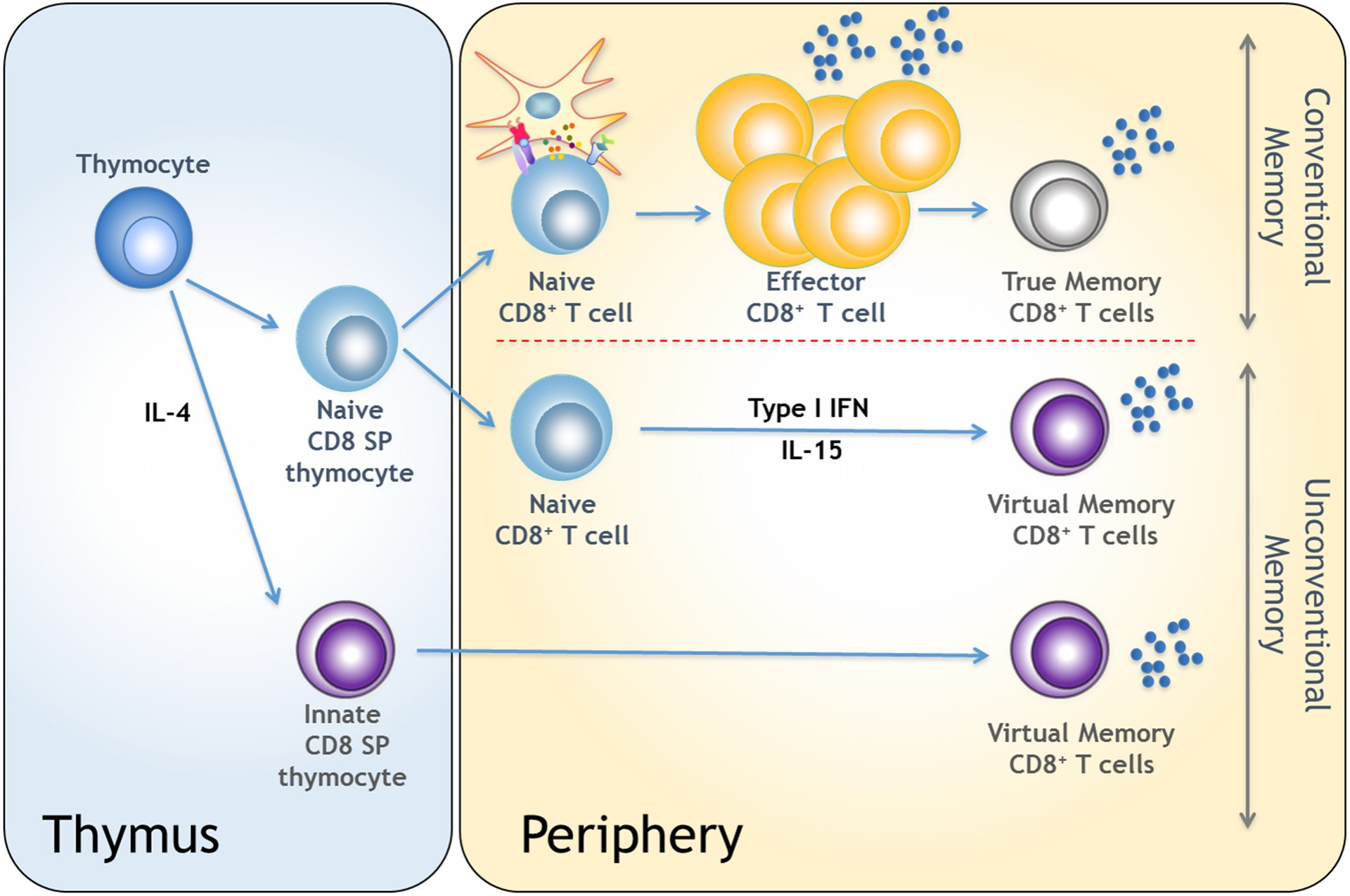


Antibody paratopes are complementary to specific antigenic determinants

Development of T and B cells



- Lymphocytes that will form active T lymphocytes develop in the bone marrow and go to the thymus gland.
- **Here it proliferates by rapidly dividing and diversified in such a way that it can develop a response to a large number of antigens.**
- A large number of different processed **T lymphocytes leave the thymus, dispersing to accommodate in the body lymphoid tissues.**


Thymocyte
IL-4
Naive
CD8 SP
thymocyte
Innate
CD8 SP
thymocyte
Thymus
Naive
CD8+ T cell
Effector
CD8+ T cell
True Memory
CD8+ T cells
Conventional
Memory
Type I IFN
IL-15
Naive
CD8+ T cell
Virtual Memory
CD8+ T cells
Virtual Memory
CD8+ T cells
Unconventional
Memory
Periphery

T CELLS HAVE TWO MAIN SUB-GROUPS

The first group carries CD4 markers (CD4 + T cells) and mainly helps or stimulates the immune response;

The second group carries CD8 markers (CD8 + T cells) and are mainly cytotoxic.

CD4 + T cells recognize specific antigens together with MHC class II molecules, whereas CD8 + T cells recognize antigens together with MHC class I molecules.

Thus, the presence of CD4 or CD8 determines which cell the T cell can interact with.

T CELLS HAVE TWO MAIN SUB-GROUPS

- T cells are divided into CD4 and CD8 subgroups and these subgroups develop by recognizing MHC class II and I molecules and antigen (peptide).
- **CD4 + T cells are subdivided into TH1 and TH2 cells according to their cytokine profile.**

T CELLS HAVE TWO MAIN SUB-GROUPS

- While B cells can recognize the antigen as it is, **T cells respond only when antigens bind to MHC-major histocompatibility complex proteins on the surface of the antigen presenting cell** found in lymphoid tissue.

T CELLS HAVE TWO MAIN SUB-GROUPS

- There are two types of MHC proteins: MHC I and MHC II proteins.
- **MHC I proteins are found on the surface of all body cells except erythrocytes,**
- **MHC II proteins are found only in macrophages, B cells and macrophage-like (dendritic) cells.**

Major Histocompatibility (Tissue Compatibility) Complex (MHC)

MHC genes encode both class I and class II MHC proteins. Class I MHC proteins are found on the surface of all nucleated cells.

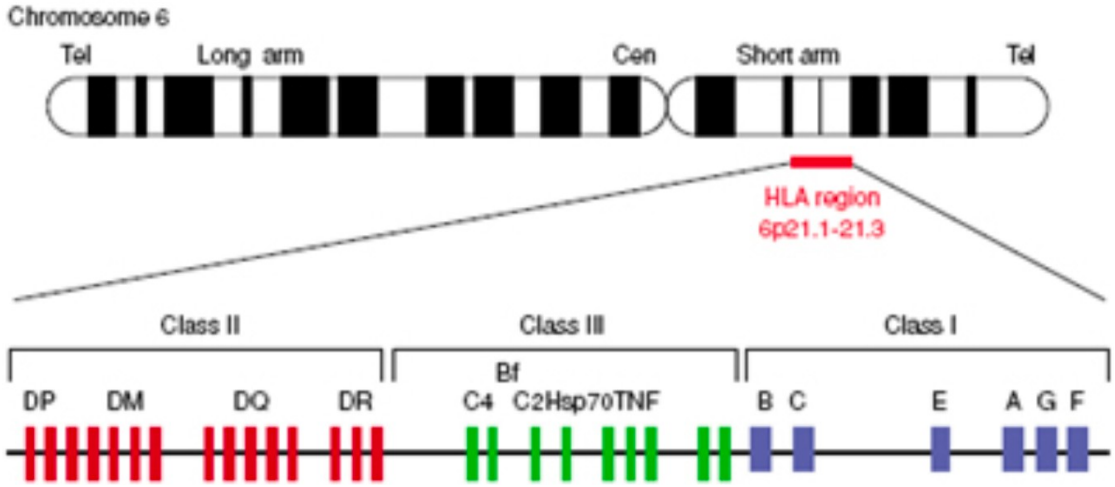
Class II MHC proteins are found only on the surface of B lymphocytes, macrophages and dendritic cells that are fully antigen presenting cells.

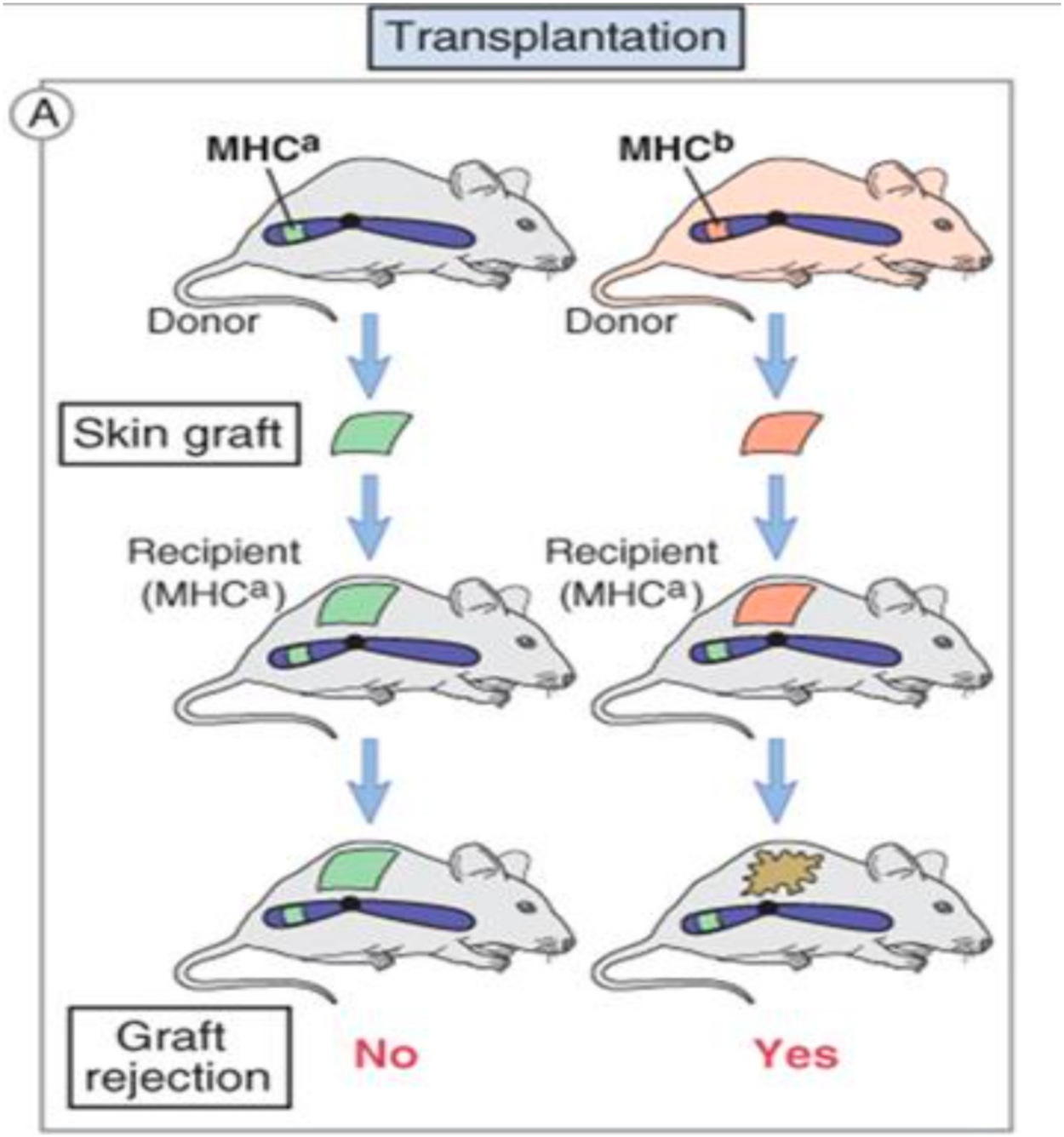
The different cellular distribution of these molecules correlates with the functions of each.

There may be small changes in the amino acid sequence of MHC proteins called polymorphisms. Humans have hundreds of different MHC genes. This polymorphism in MHC genes is the major antigenic barrier to tissue transfer from one individual to another.

Major Histocompatibility (Tissue Compatibility) Complex (MHC)

MHC proteins are encoded by a genetic region found in all vertebrates, called the major histocompatibility complex. MHC proteins are produced by many genes involved in this complex and are often referred to as **human leukocyte antigen (HLA).**



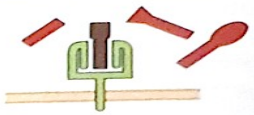
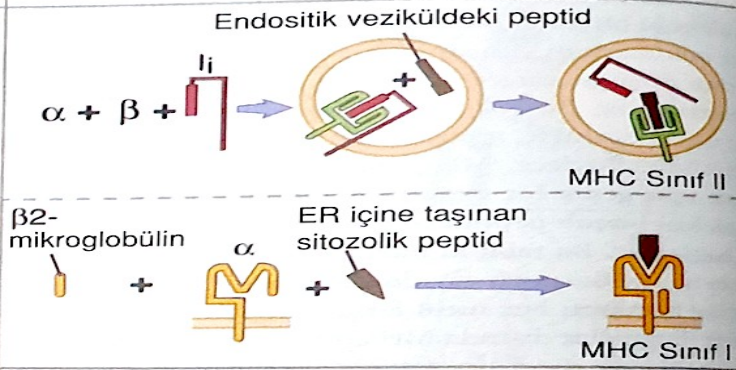
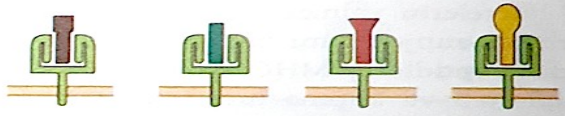
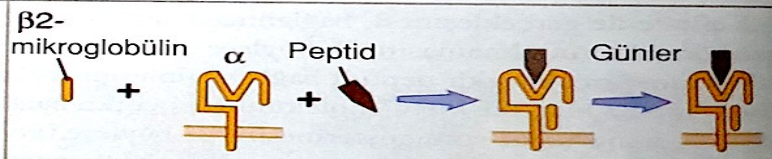
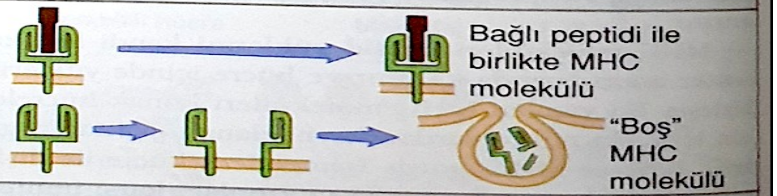
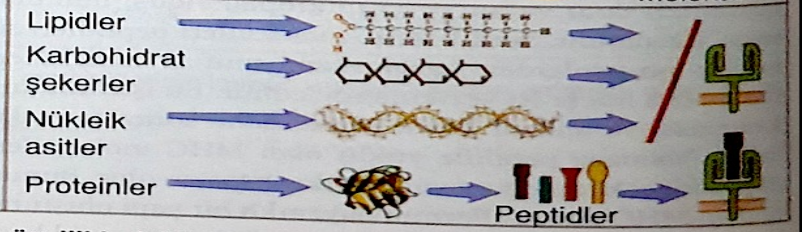

Transplantation
A
MHC^a
MHC^b
Donor
Donor
Skin graft
Recipient
(MHC^a)
Recipient
(MHC^a)
Graft
rejection
No
Yes

Properties and functions of MHC molecules

- Theoretically, it is accepted that there is a distinct T cell clone specific to each peptide of protein antigens found in nature.
- Another important feature of T cells is that in order to recognize an antigen and create a reaction, this antigen must be processed by some cells and presented to them through surface molecules in their membranes.
- **These surface molecules that provide antigen presentation to T cells are MHC molecules.**

Properties and functions of MHC molecules

- MHC molecules are not as specific as T cell receptors.
- Different peptides that are structurally similar can be linked by the same MHC molecule and presented
- T cells are cells with very high antigen specificity.

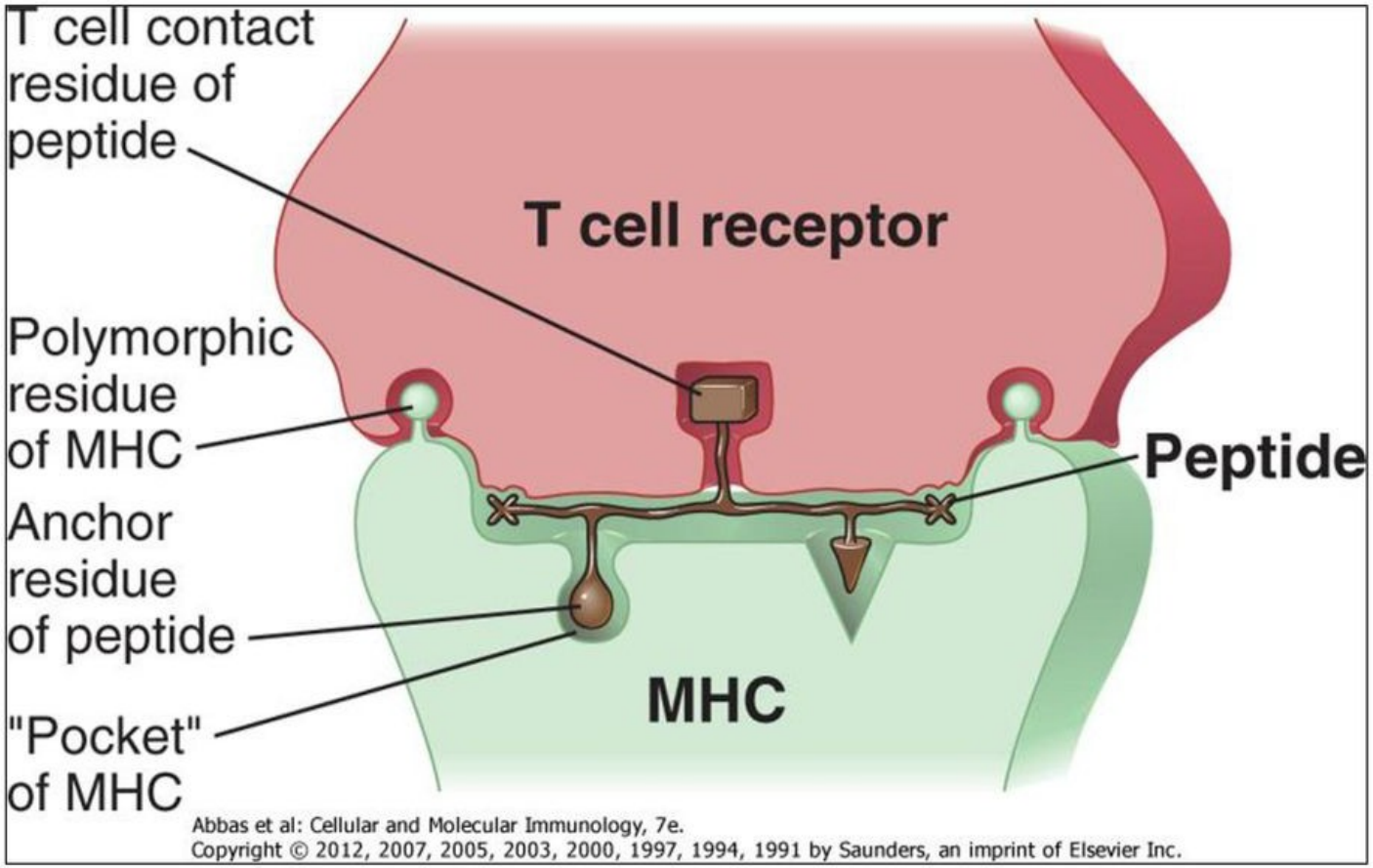
Özellik	Önemi	
Her MHC molekülü bir kerede bir peptidi sunar	Her T hücresi MHC molekülüne bağlı olan tek bir peptide yanıt verir	
Peptidler hücre içi moleküler birleşme/gelişim sürecinde kazanılır	MHC Sınıf I ve Sınıf II molekülleri farklı hücre kompartmanlarından peptidleri sunar	
Düşük afinite, geniş özgüllük	Aynı MHC molekülüne birçok farklı peptid bağlanır	
Çok düşük "kopma" hızı	MHC molekülü bağladığı peptidi T hücresinin bulabileceği kadar uzun süre gösterir	
Stabil ekspresyon için peptid gereklidir	Yalnızca peptid sunan MHC molekülleri T hücrelerince tanınabilecek şekilde eksprese olur	
MHC molekülleri yalnızca peptidleri bağlar	MHC-sınırlı T hücreleri yalnızca protein yapısındaki antijenlere yanıt verir ve diğer kimyasal yapıları tanımaz	

Şekil 3-10 Peptidlerin MHC moleküllerine bağlanmasının özellikleri. MHC moleküllerine peptid bağlanmasının immün yanıt açısından anlamını gösteren bazı önemli özellikleri listelenmiştir.

Properties and functions of MHC molecules

- Another condition necessary for an antigen to generate a T cell response is that the MHC molecule to which the antigen is presented is the organism's own MHC molecule. This is called the **self restriction feature**.
- Accordingly, in order for a T cell to recognize the antigen and form a reaction, the T cell receptor must be physically compatible with the structure of both the antigen and the MHC molecule, as in the key lock model. This can only be achieved in the presence of the unique antigen and self MHC molecule.

Schematic Representation of How a TCR Recognizes the Peptide/MHC complex (pMHC)



Properties and functions of MHC molecules

- Presence of MHC molecules is also important in terms of determining the direction of T cell response.
- During antigen presentation, **MHC I molecules interact with CD8 from T cell coreceptors and, accordingly, CD8 + cytotoxic T cell response occurs.**
- **Similarly, MHC II molecules bind to the CD4 coreceptor, resulting in a CD4 + helper T cell response.**

Presentation to T Helper Cells

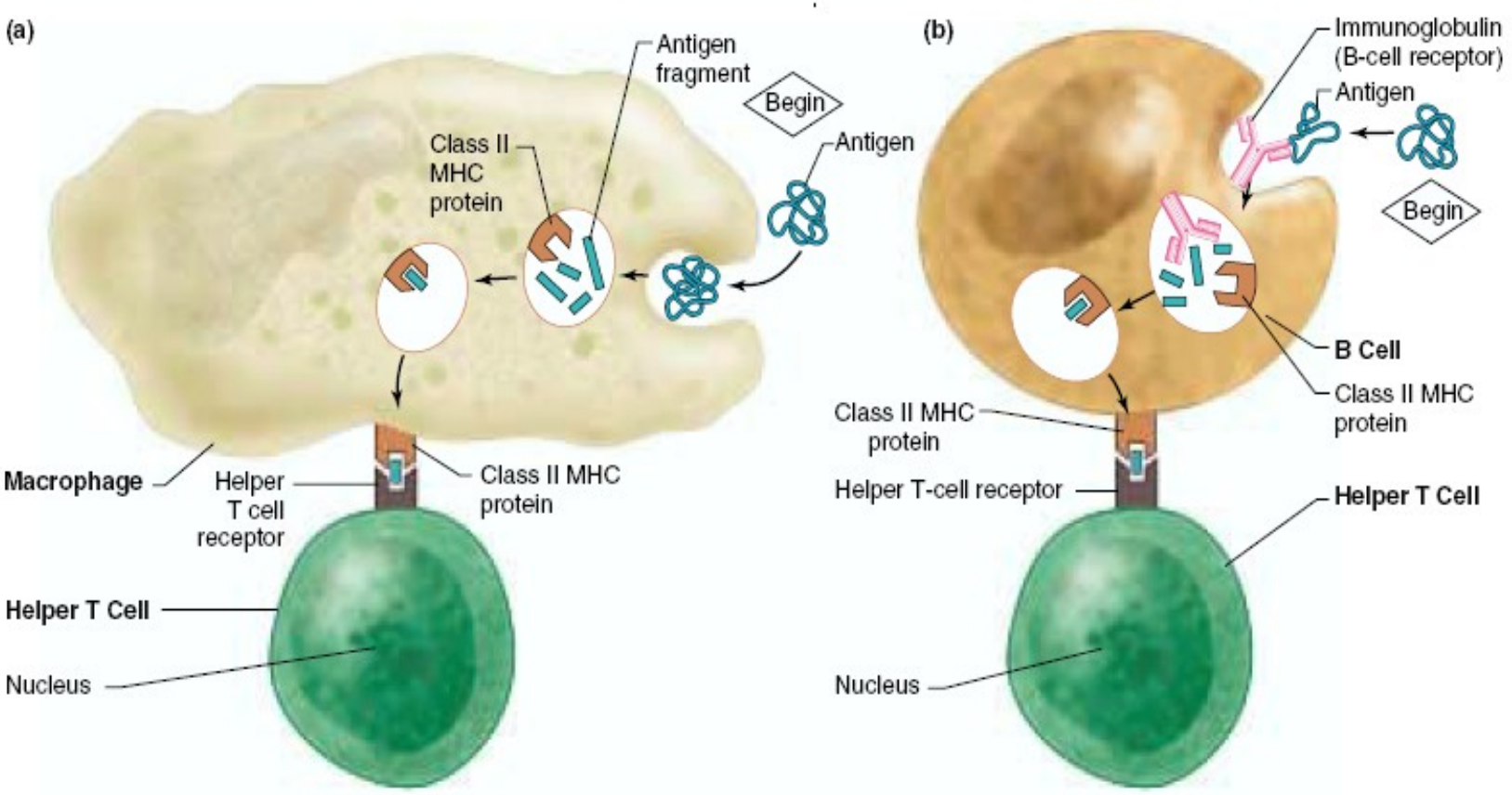


FIGURE 20–10

Sequence of events by which antigen is processed and presented to a helper T cell by (a) a macrophage or (b) a B cell. In both cases, begin the figure with the antigen in the extracellular fluid.

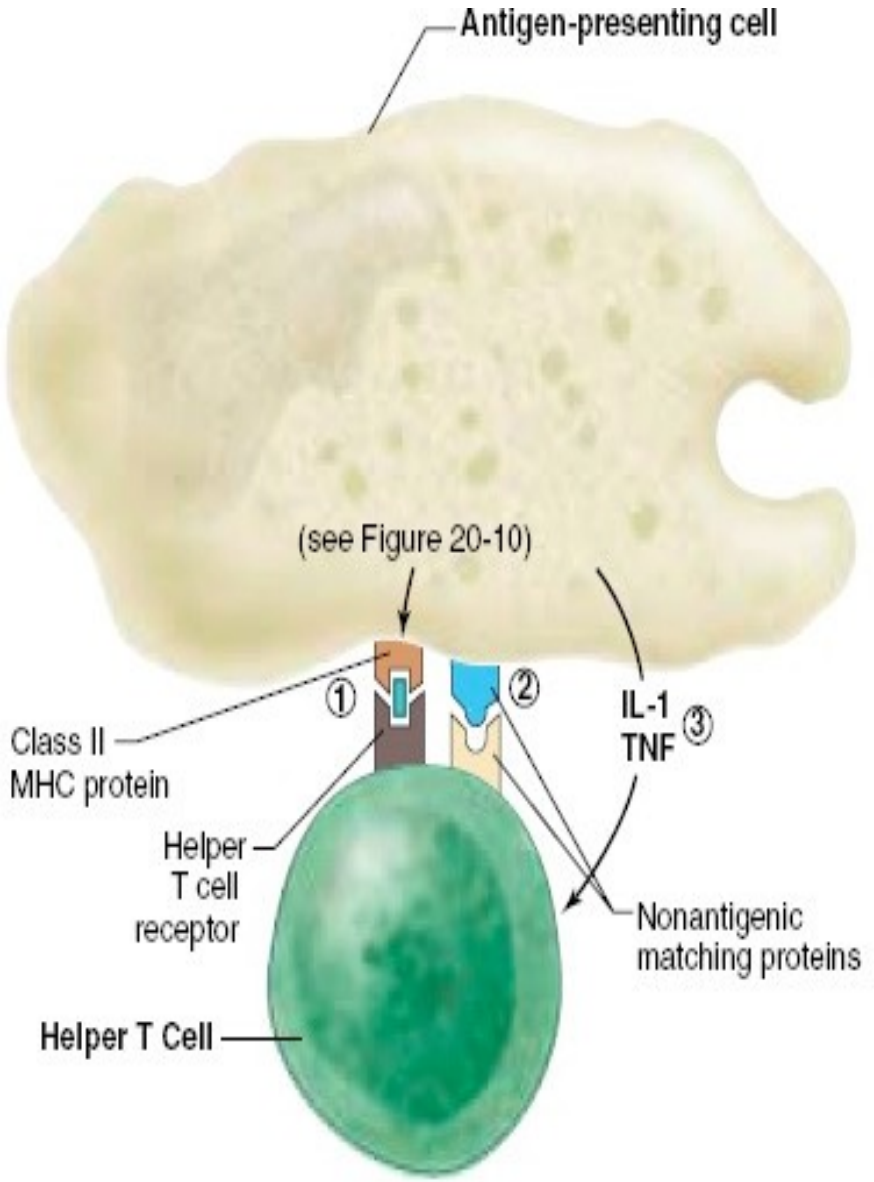
Adapted from Gray, Sette, and Buus.

Three events are required for the activation of helper T cells;

1. **Presentation of antigens**

2. **Binding of non-antigenic proteins in the plasma membranes of APC and helper T cells.**

3. **IL-1 and TNF secretion by APC**



Presentation to Cytotoxic T Cells

- Since MHC I proteins are synthesized in almost every cell, these cells act as antigen-presenting cells for cytotoxic T cells.
- **When a virus enters the host cell, the nucleic acids belonging to the virus cause the production of foreign viral proteins in the host cell.**

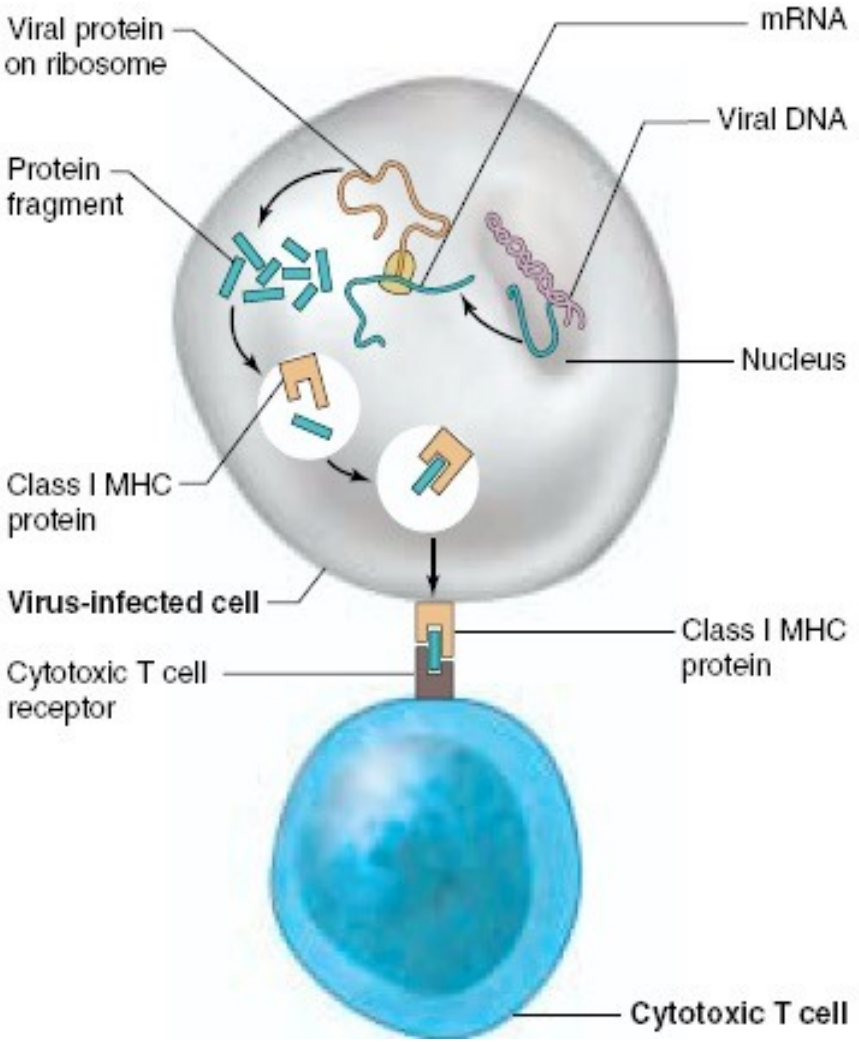
Presentation to Cytotoxic T Cells

- **Viral DNA stimulates infected cell to make viral protein**

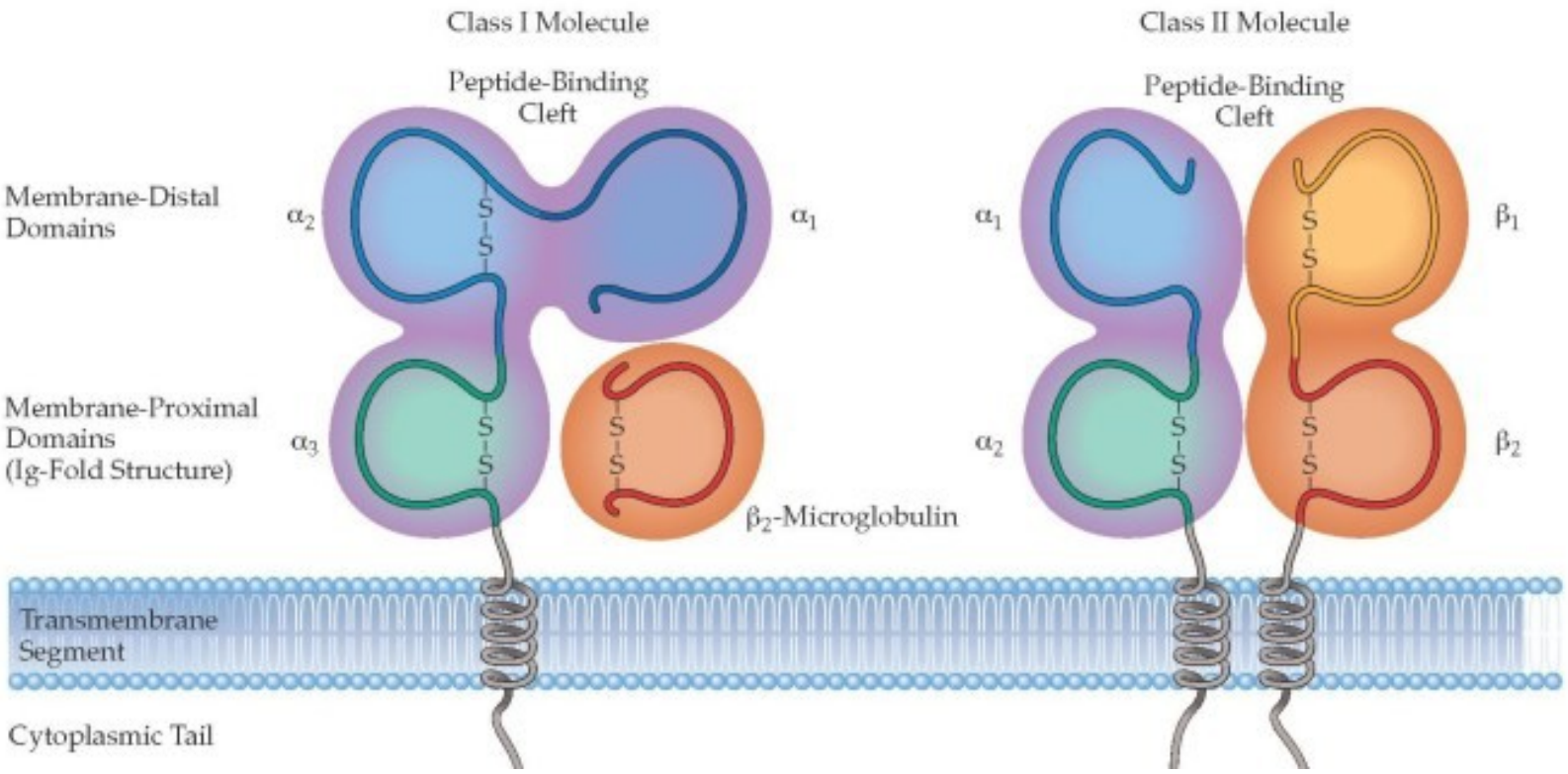
- **Antigenic proteins endogenously produced in virus-infected cells are digested through cytosolic enzymes.**

- **The digestive product of small peptide structures complexes with MHC I proteins in the endoplasmic reticulum.**

- **These complexes then migrate to the plasma membrane.**



Differences between MHC Class I and MHC Class II



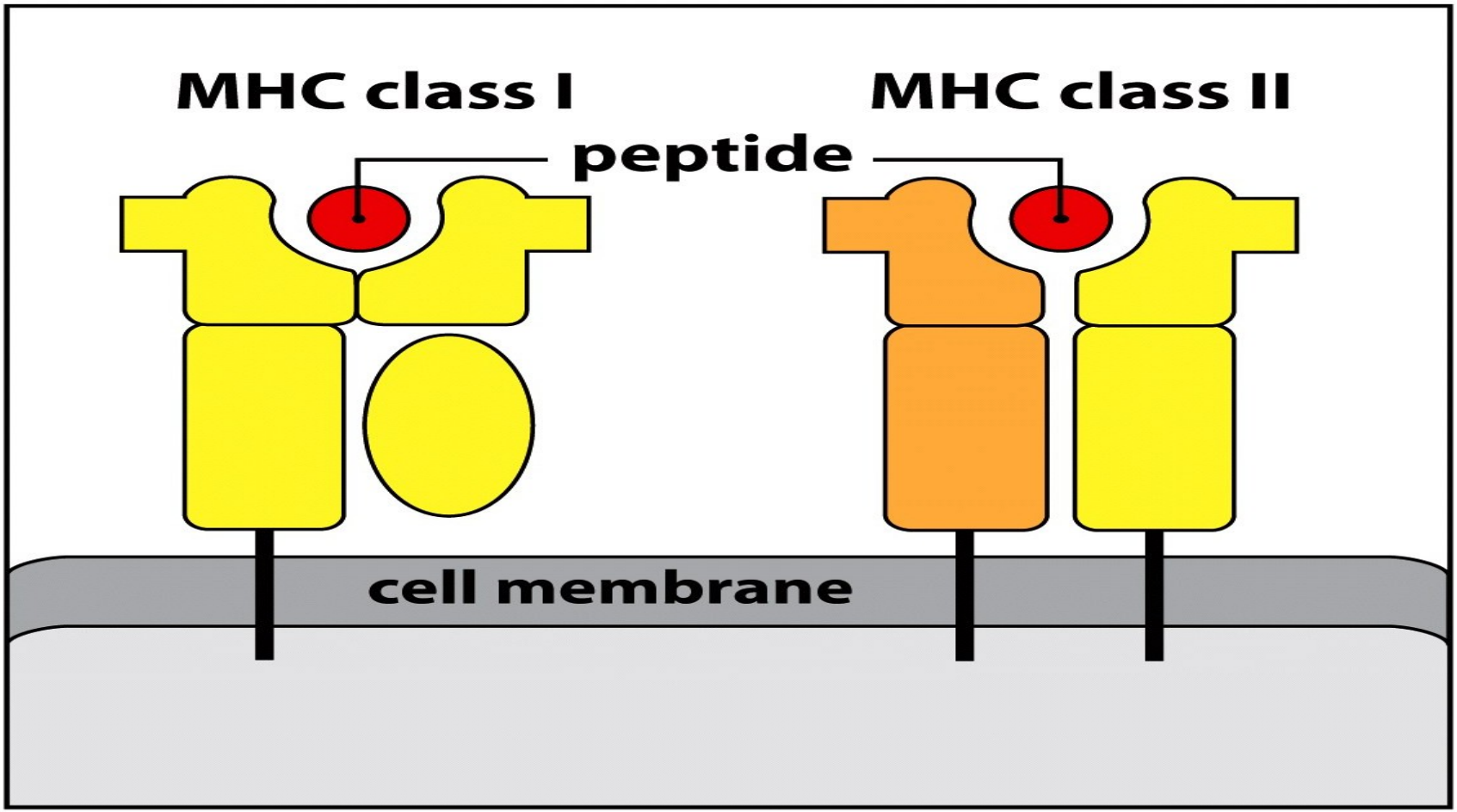


Figure 3.8 The Immune System, 3ed. (© Garland Science 2009)

Antigen Processing and Presentation

- Most of the antigens, especially the protein antigens, must be reduced to a level **that can be detected by the immune system cells in order to create an immune response.**
- This event, called antigen processing, is carried out by certain cells.

Antigen Processing and Presentation

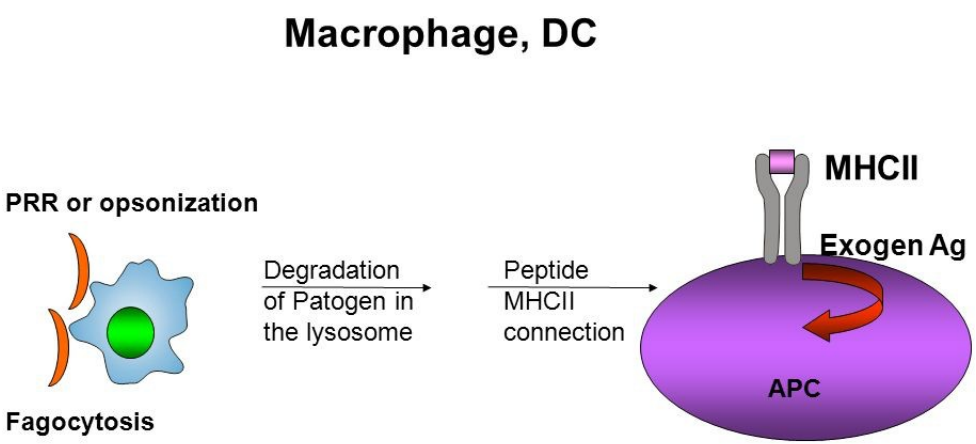
- After processing and separating into small peptide fragments, antigens must be presented to T lymphocytes along with MHC molecules.
- **This process performed by cells that process is called antigen presentation.**
- **Cells that process and present the antigen are commonly called as antigen-presenting cells (APCs).**

Antigen Processing and Presentation

Antigens that need to be processed to stimulate the immune response are divided into two groups as **exogenous** and **endogenous**.

Exogenous antigens

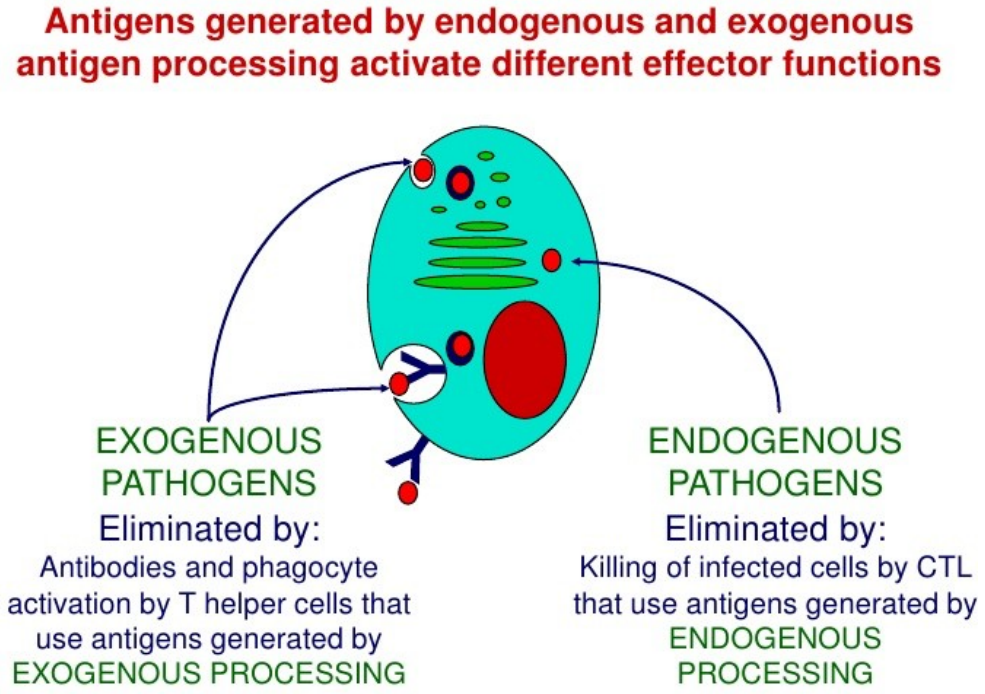
After processing by macrophages, dendritic cells and B lymphocytes, antigens are presented to antigen-sensitive helper T cells along with MHC class II molecules.



Constitutive checking of the extracellular enviroment
(fagocytosis, pinocytosis)

Endogenous antigens

They are presented by any nucleated cells carrying MHC class I molecules, together with these molecules, to antigen-sensitive cytotoxic T cells.



Antigen Presenting Cells

- Macrophages
- Dendritic cells
- B lymphocytes
- Neutrophil, Eosinophil, T cells, B cells NK cells,
- Endothelial cells,
- Fibroblasts, smooth muscle cells

Endogenous Antigen Presentation

- Antigenic protein source:

Proteins living in the host cell

- a- virus, bacteria b- intracellular parasites
- Formation of peptides: **by enzyme complex called proteasome.**

Exogenous antigen presentation

- Antigen binds to APC, is taken into the cell
- antigens accumulate within endosomes
- **Proteolytic destruction occurs in lysosomes and endosomes.**

COMPARATIVE FEATURES OF CLASS II AND CLASS I MHC PATHWAYS OF ANTIGEN PROCESSING AND PRESENTATION

Feature	Class II MHC Pathway	Class I MHC pathway
Composition of stable peptide-MHC complex	Polymorphic α and β chains, peptide 	Polymorphic α chain, β_2-microglobulin, peptide 
Types of APCs	Dendritic cells, mononuclear phagocytes, B lymphocytes; endothelial cells, thymic epithelium	All nucleated cells
Responsive T cells	$CD4^+$ T cells	$CD8^+$ T cells
Source of protein antigens	Endosomal/lysosomal proteins (mostly internalized from extracellular environment)	Cytosolic proteins (mostly synthesized in the cell; may enter cytosol from phagosomes)
Enzymes responsible for peptide generation	Endosomal and lysosomal proteases (e.g., cathepsins)	Cytosolic proteasome
Site of peptide loading of MHC	Specialized vesicular compartment	Endoplasmic reticulum
Molecules involved in transport of peptides and loading of MHC molecules	Chaperones in ER; invariant chain in ER, Golgi and MIIC/CIIV; DM	Chaperones, TAP in ER

Abbreviations: APC, antigen-presenting cell; CIIV, class II vesicle; ER, endoplasmic reticulum; MHC, major histocompatibility complex; MIIC, MHC class II compartment; TAP, transporter associated with antigen processing

Presentation of Superantigens

Some antigens, despite their protein structure, can stimulate helper T lymphocytes without being processed by antigen-processing cells at all.

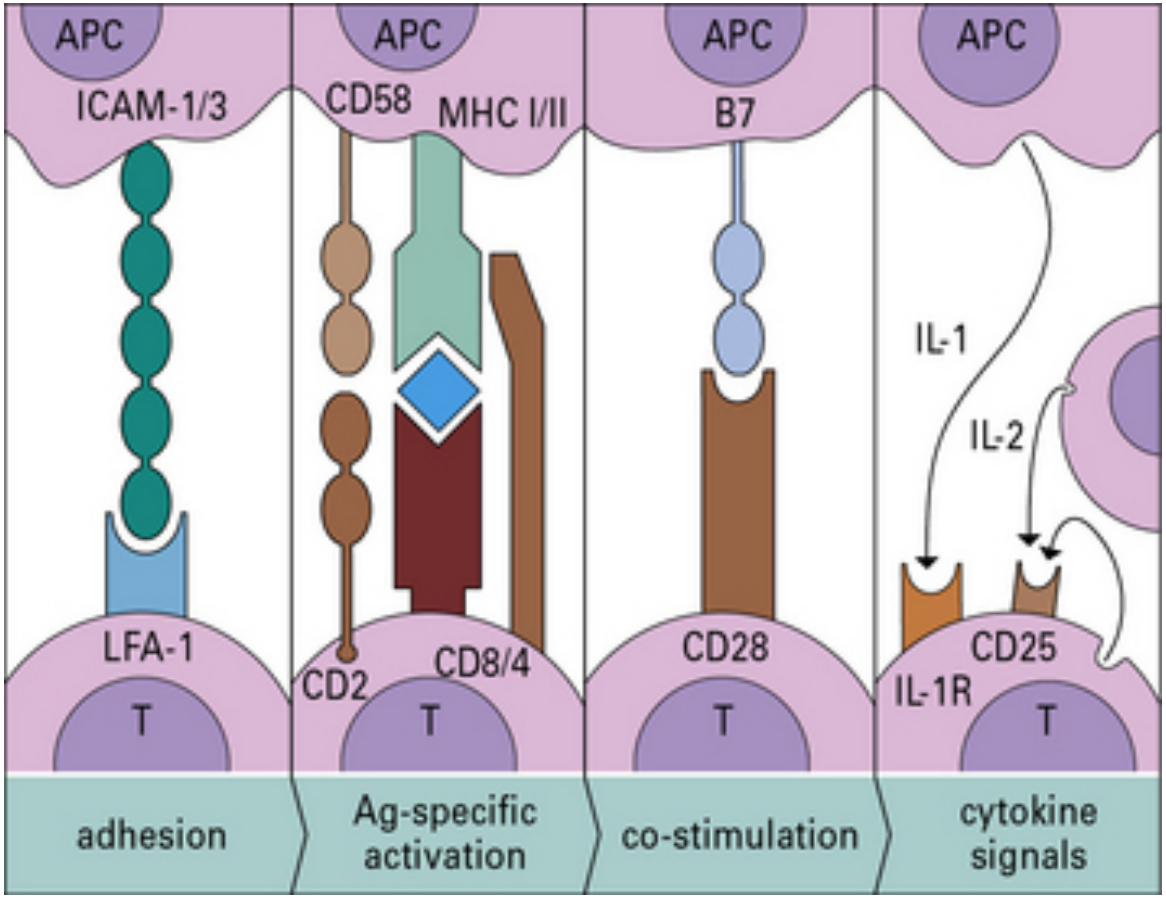
Antigens with this feature are called **superantigens.**

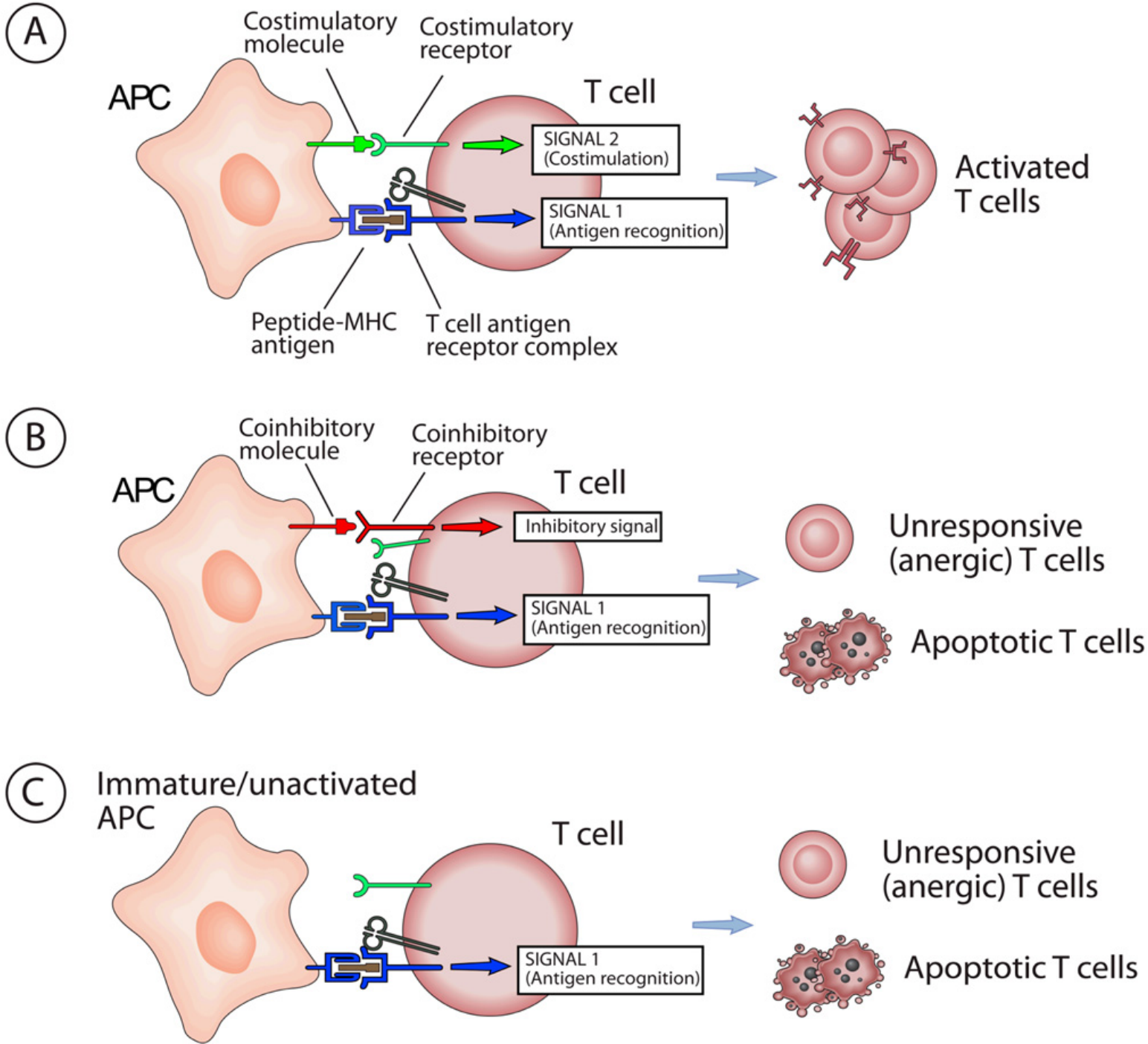
Most of the superantigens are exotoxin molecules belonging to bacteria and these are the strongest antigens.

Presentation of Superantigens

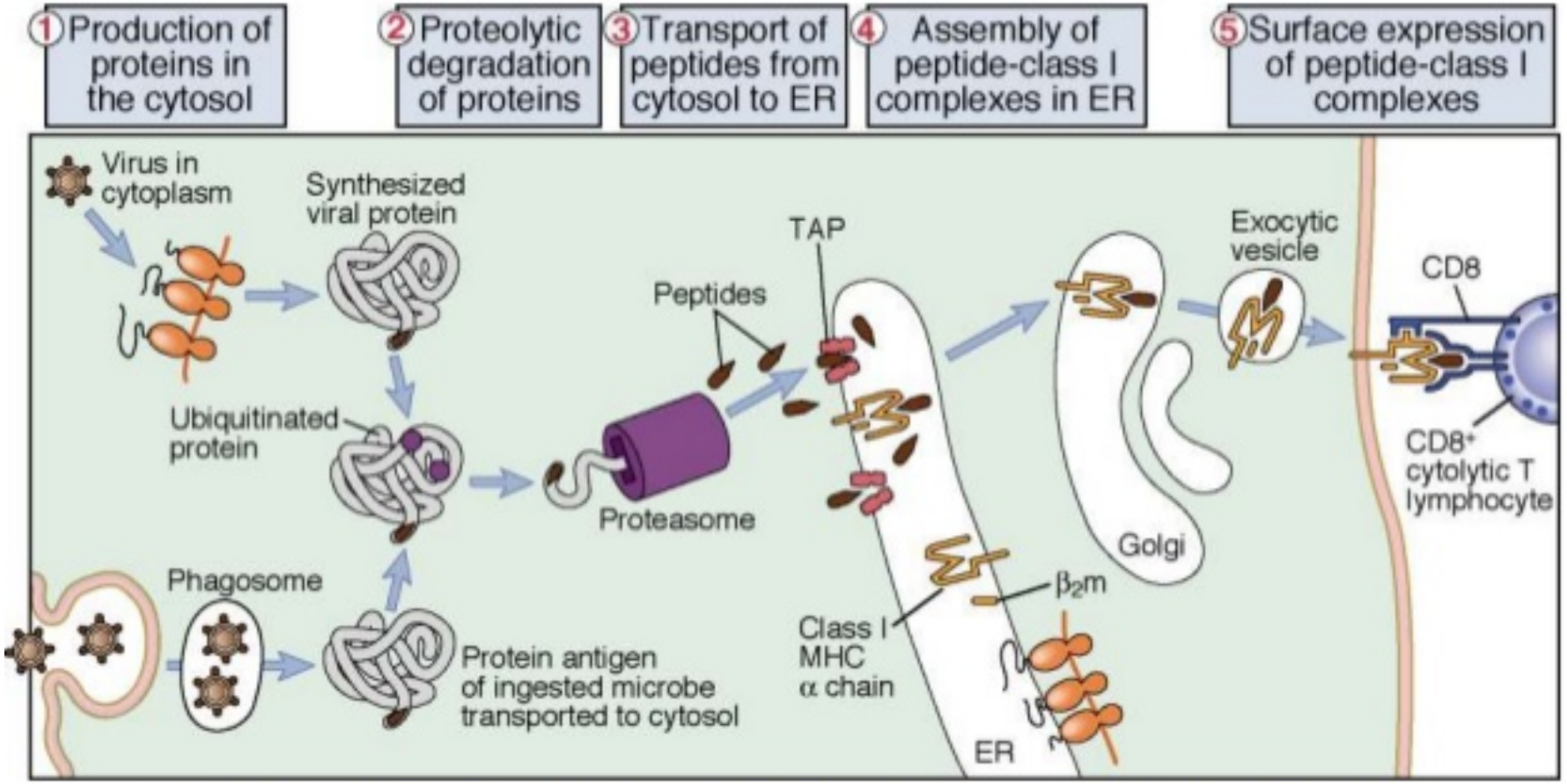
- **Processing of superantigens causes loss of their structure or even their characteristics. They can stimulate different T lymphocytes by binding to class II molecules.**
- Especially bacterial superantigens stimulate helper T lymphocytes and cause excessive cytokine synthesis and systemic toxic effect occurs in the host, specific immunity is suppressed.

Four main steps of antigen presentation




A
Costimulatory molecule
Costimulatory receptor
APC
T cell
SIGNAL 2 (Costimulation)
SIGNAL 1 (Antigen recognition)
Activated T cells
Peptide-MHC antigen
T cell antigen receptor complex
B
Coinhibitory molecule
Coinhibitory receptor
APC
T cell
Inhibitory signal
SIGNAL 1 (Antigen recognition)
Unresponsive (anergic) T cells
Apoptotic T cells
C
Immature/unactivated APC
T cell
SIGNAL 1 (Antigen recognition)
Unresponsive (anergic) T cells
Apoptotic T cells

The class I MHC pathway of processing of endogenous cytosolic protein antigens



Cytoplasmic peptides are actively transported into the ER; class I MHC molecules are available to bind peptides in the ER

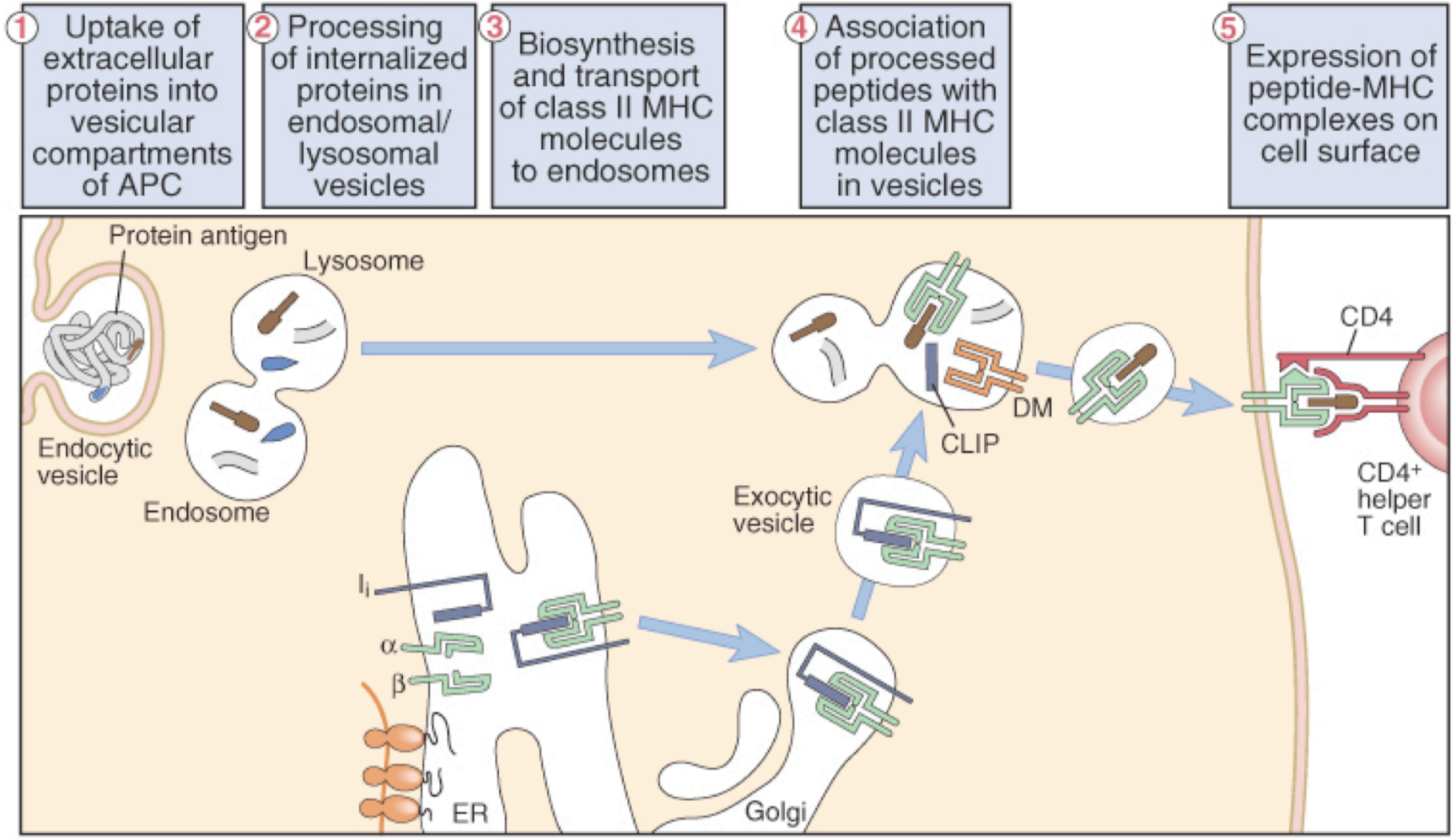


Figure 6-9

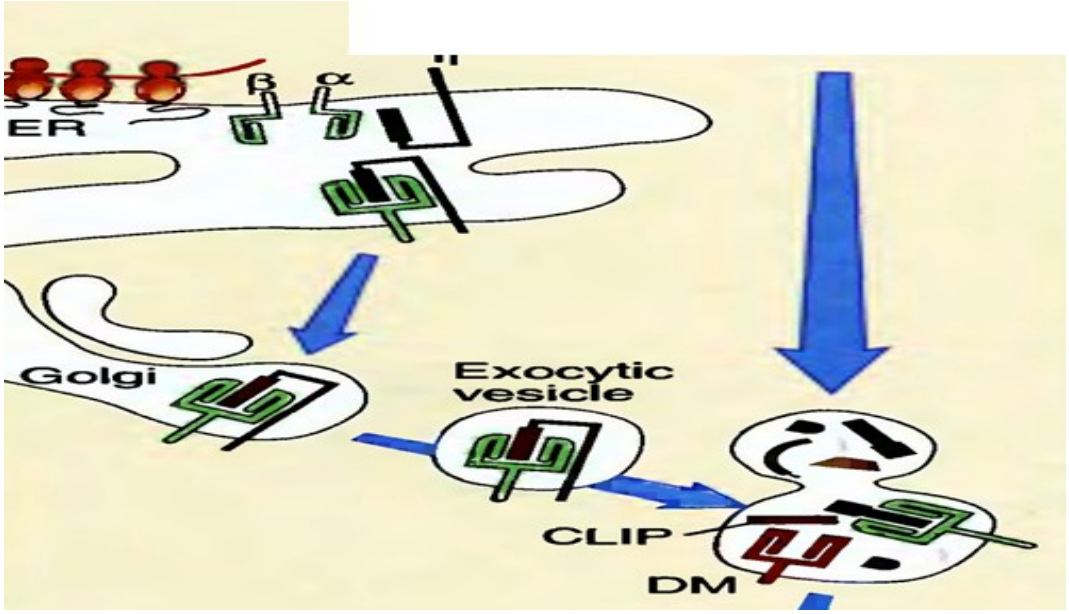
The class II MHC pathway of antigen presentation.

The numbered stages in processing of extracellular antigens correspond to the stages described in the text. APC, antigen-presenting cell; CLIP, class II-associated invariant chain peptide; ER, endoplasmic reticulum; I_i, invariant chain.

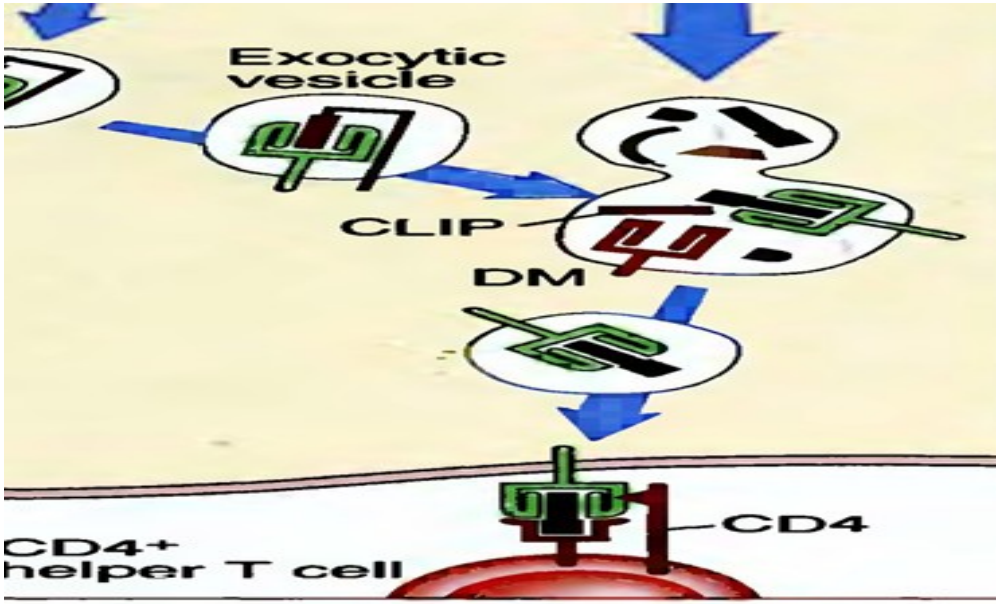
PROCESSING OF ANTIGENS IN VESICULES BY CLASS II MHC

- Microbial proteins taken into APCs arrive in intracellular vesicles called endosomes or phagosomes that can fuse (fusion) with lysosomes.
- Proteins are cut by proteolytic enzymes in these vesicles and converted into peptides in many different lengths and sequences.

- Class II MHC proteins are synthesized in the endoplasmic reticulum of APCs
- Each newly synthesized class II molecule carries with it a class II invariant chain peptide (CLIP), which is called the invariant chain, which is tightly bound to the peptide binding groove of the class II molecules.
- That is, the groove of newly synthesized class II molecules is full.



This class II molecule begins to move to the cell surface by an exocytic vesicle and then combines with an endosomal vesicle containing peptides.
The same endosomal vesicle contains the DM molecule whose task is to remove CLIP.
After the CLIP is removed, the groove of the class II molecule becomes able to accept peptides.



If the class II molecule binds one of the peptides formed from phagocytosed proteins, this complex becomes stable and delivered to the cell surface.

If the class MHC II molecule cannot find a peptide that it can bind, **the empty molecule is unstable and is destroyed by proteases in the endosome.**

Class I MHC Pathway

Injected via Bacterial secretory mechanisms

Phagocytosed

Escape Mechanism

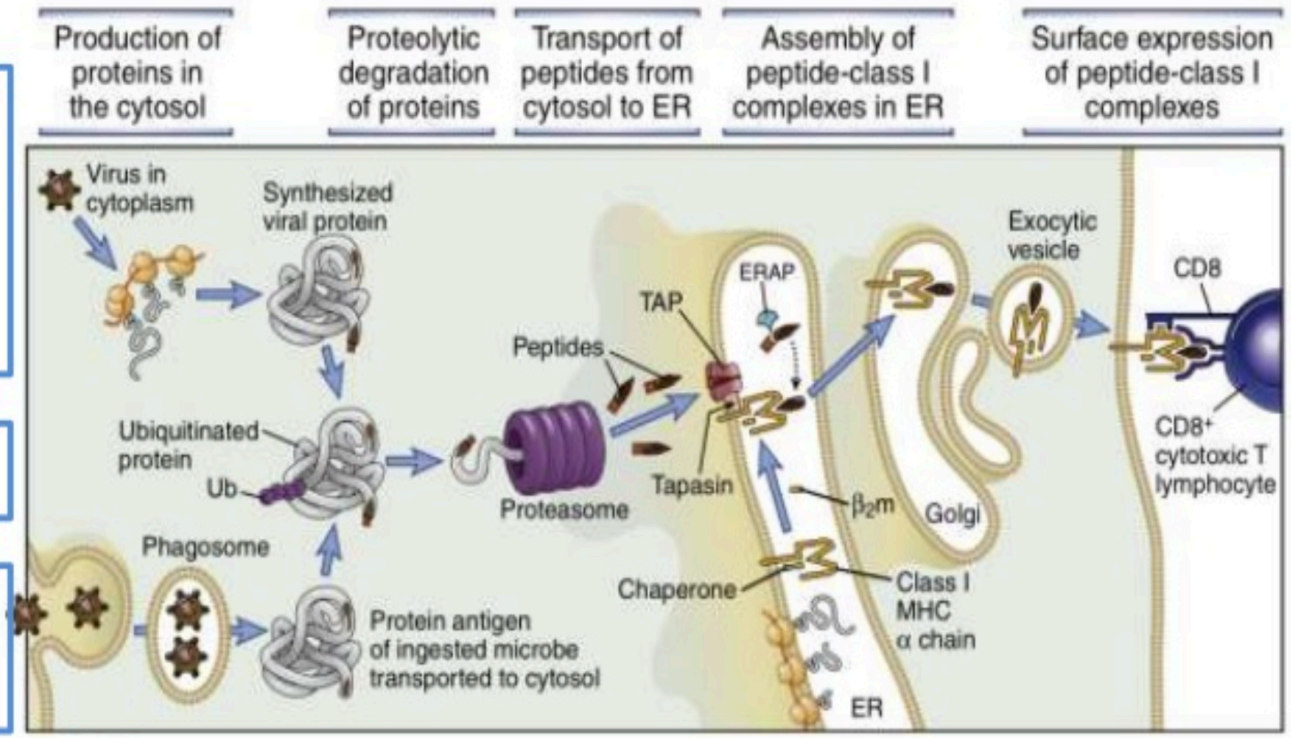


FIGURE 6-16 The class I MHC pathway of antigen presentation. The stages in the processing of cytosolic proteins are described in the text. *ERAP*, endoplasmic reticulum associated peptidase; *ER*, endoplasmic reticulum; β_2m, β_2-microglobulin; *TAP*, transporter associated with antigen processing; *Ub*, ubiquitin.

Class I MHC Pathway

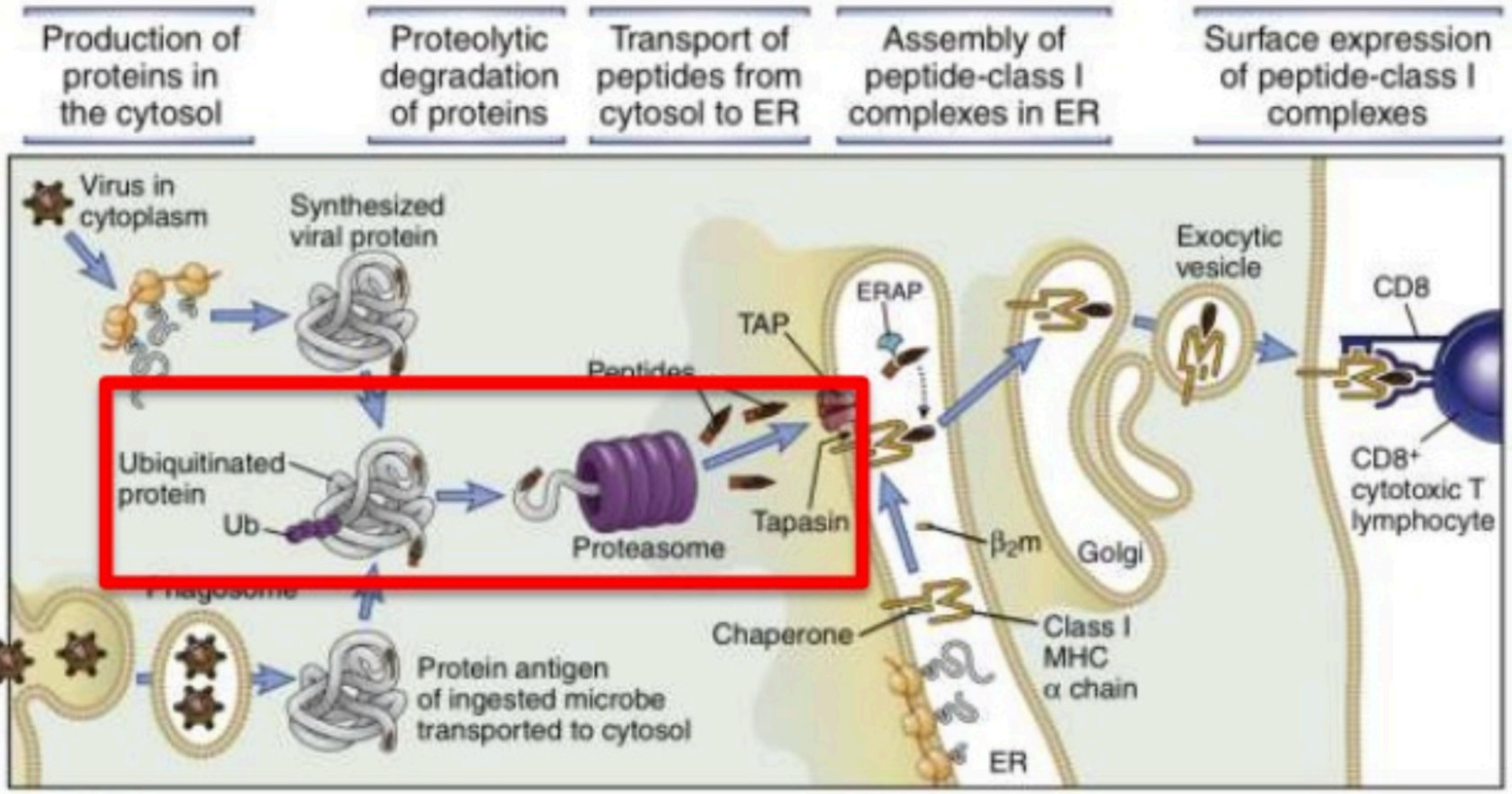
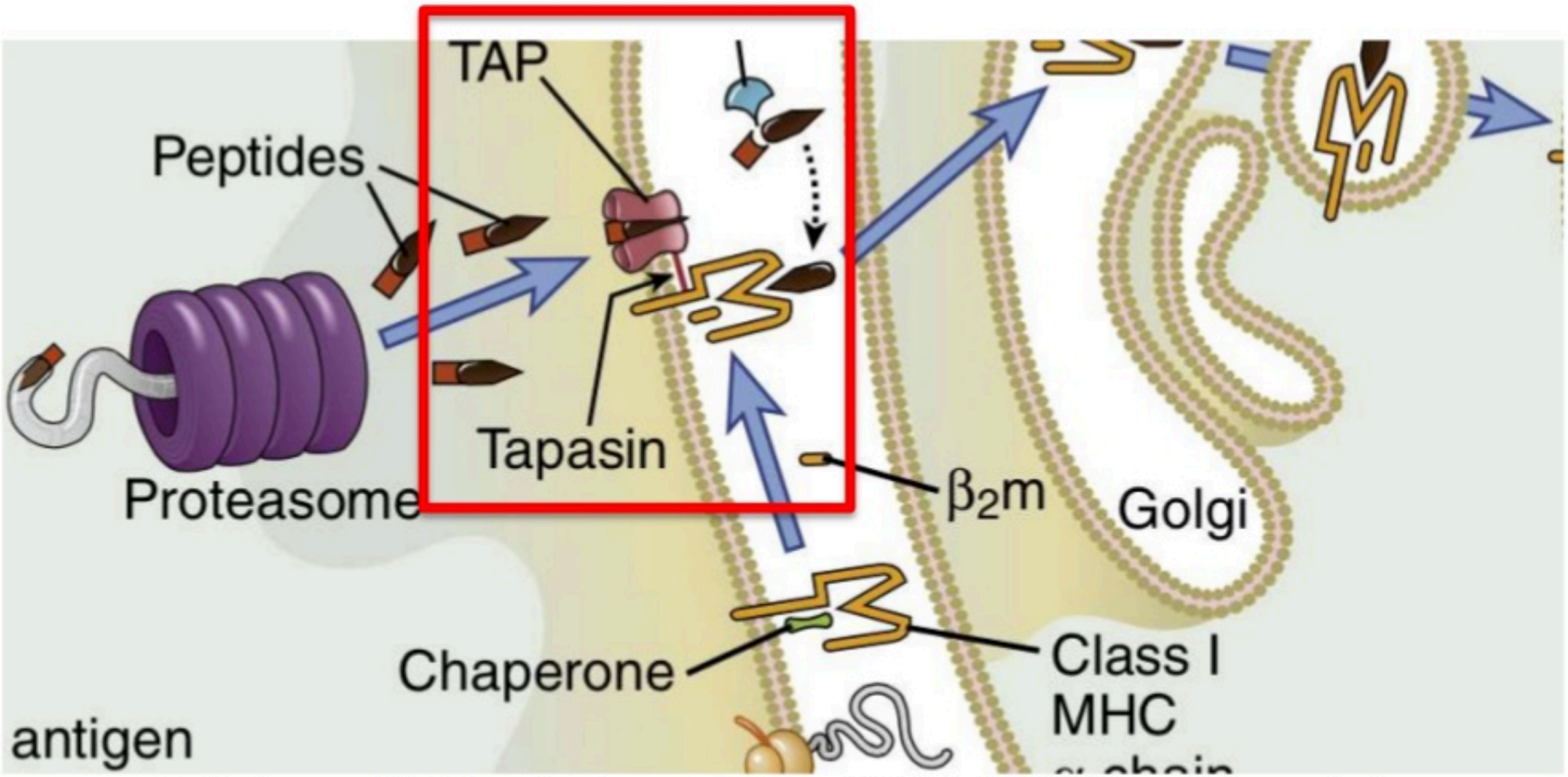


FIGURE 6-16 The class I MHC pathway of antigen presentation. The stages in the processing of cytosolic proteins are described in the text. *ERAP*, endoplasmic reticulum associated peptidase; *ER*, endoplasmic reticulum; β_2m, β_2-microglobulin; *TAP*, transporter associated with antigen processing; *Ub*, ubiquitin.

Class I MHC Pathway

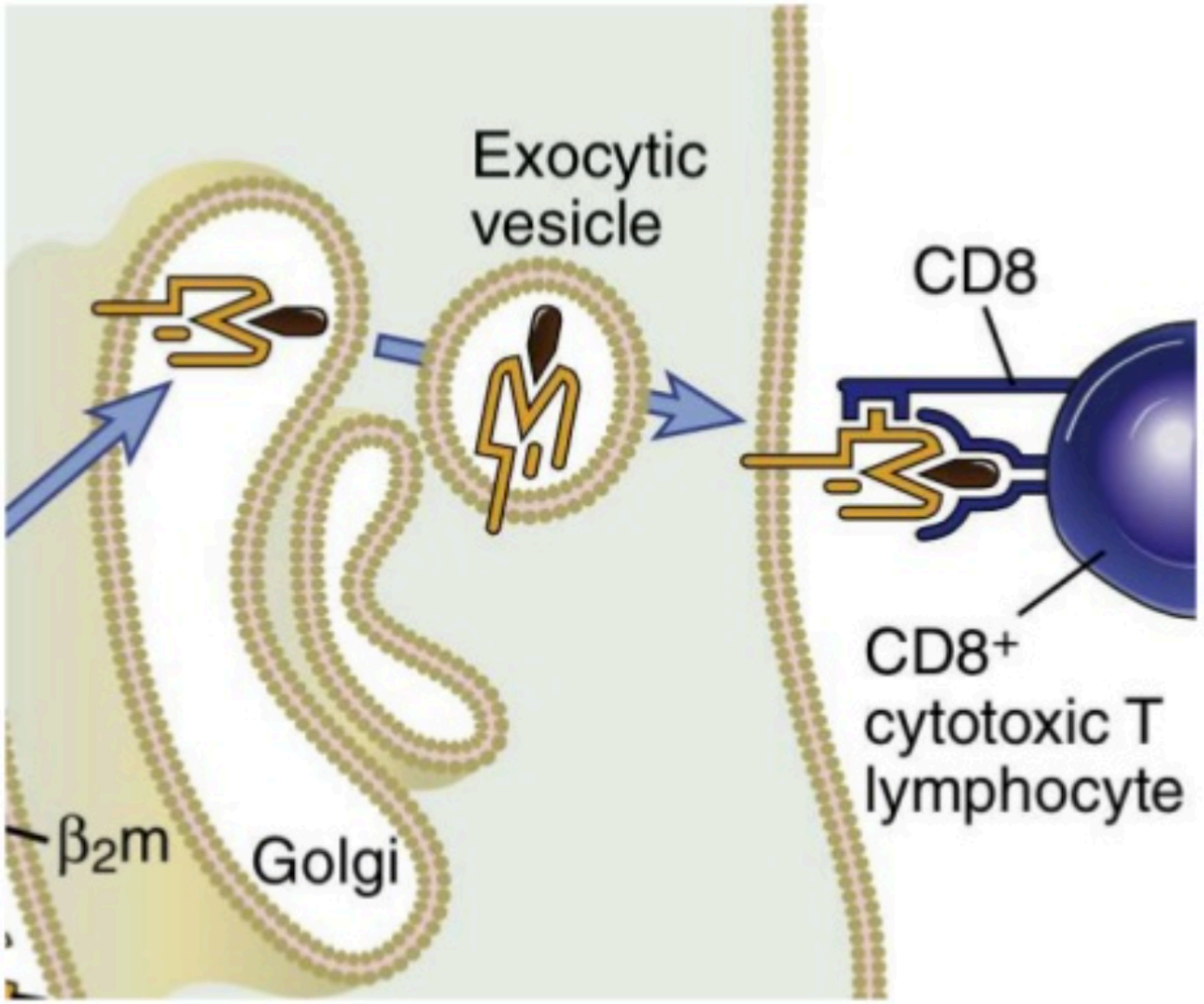


Cellular and Molecular Immunology 8th Ed. (2015) by Abbas et al.

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



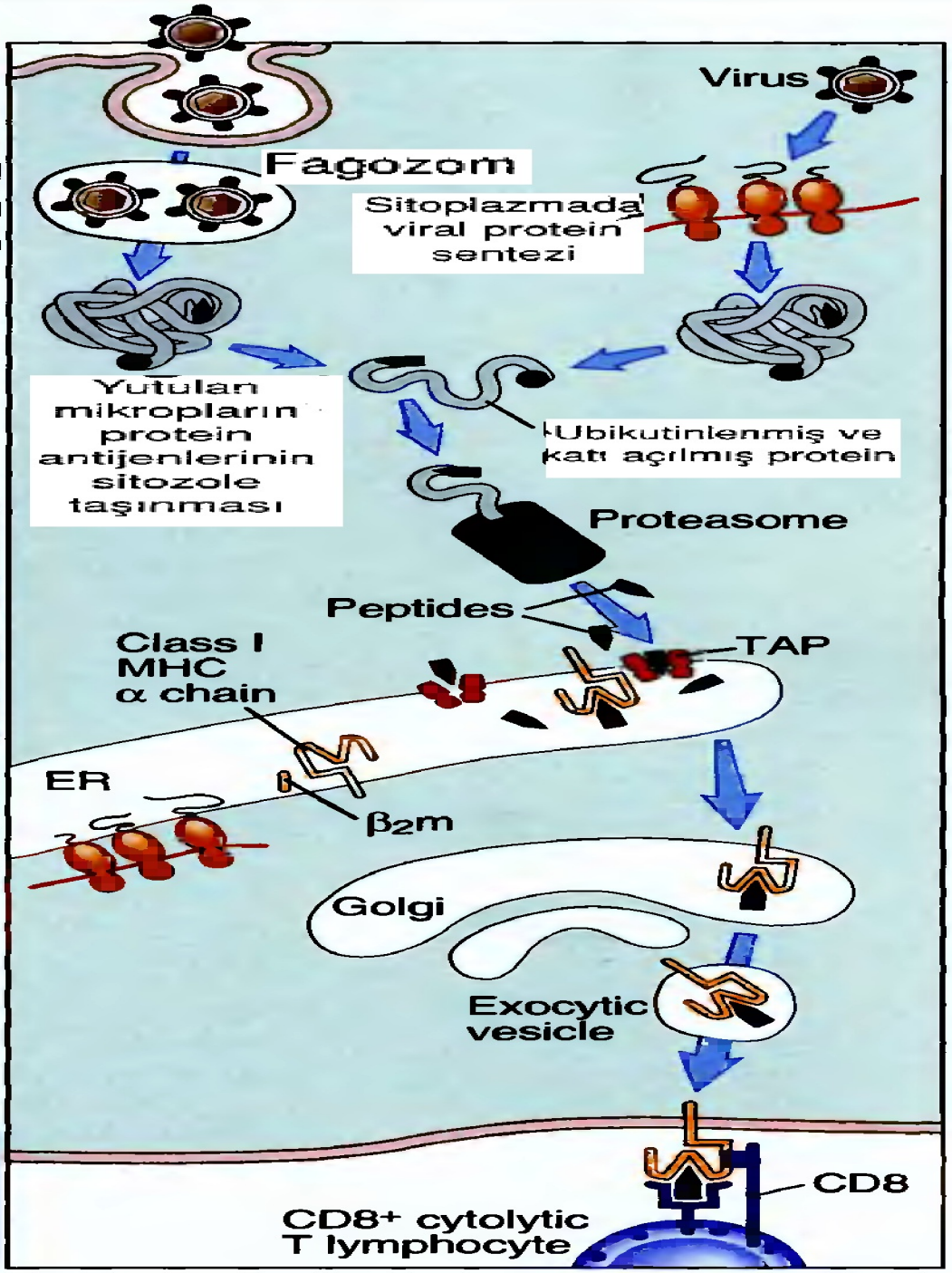
Sitozolde protein üretimi

Sitozolik proteinlerin proteolitik degradasyonu

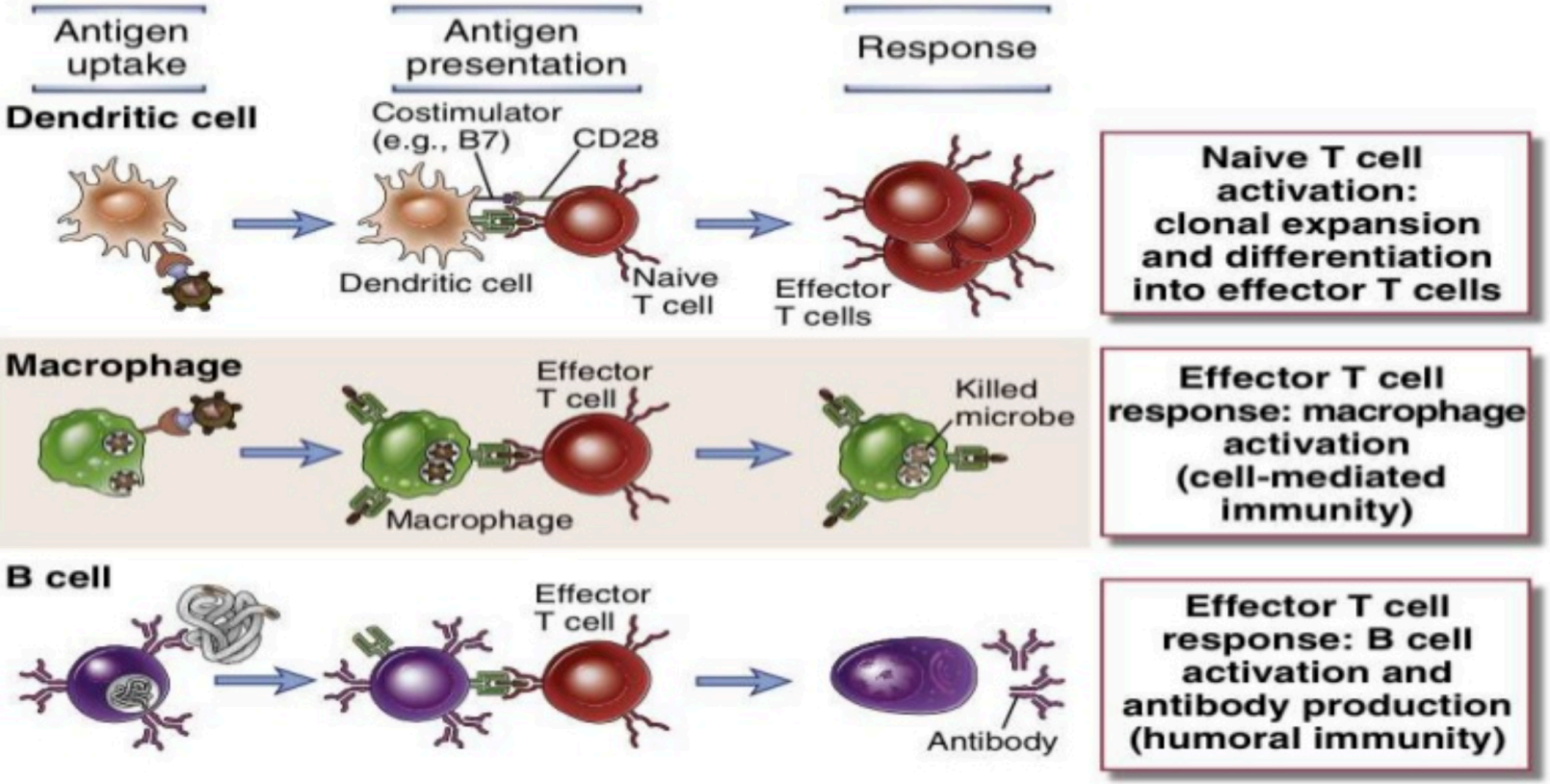
Peptidlerin sitozolden ER'a taşınması

ER de sınıf I-peptid kompleksinin birleşmesi

Sınıf I-peptid kompleksinin yüzey ekspresyonu



ANTIGEN CAPTURE AND THE FUNCTIONS OF ANTIGEN-PRESENTING CELLS



Physiological Importance of Mhc Related Antigen Presentation

MHC molecules can only bind with peptides found inside the cell.

There are antigens phagocytosed or antigens of intracellular pathogens within the cell.

Therefore, T cells can only recognize antigens phagocytosed or intracellular microbes and they are eliminated by T cell-mediated immunity

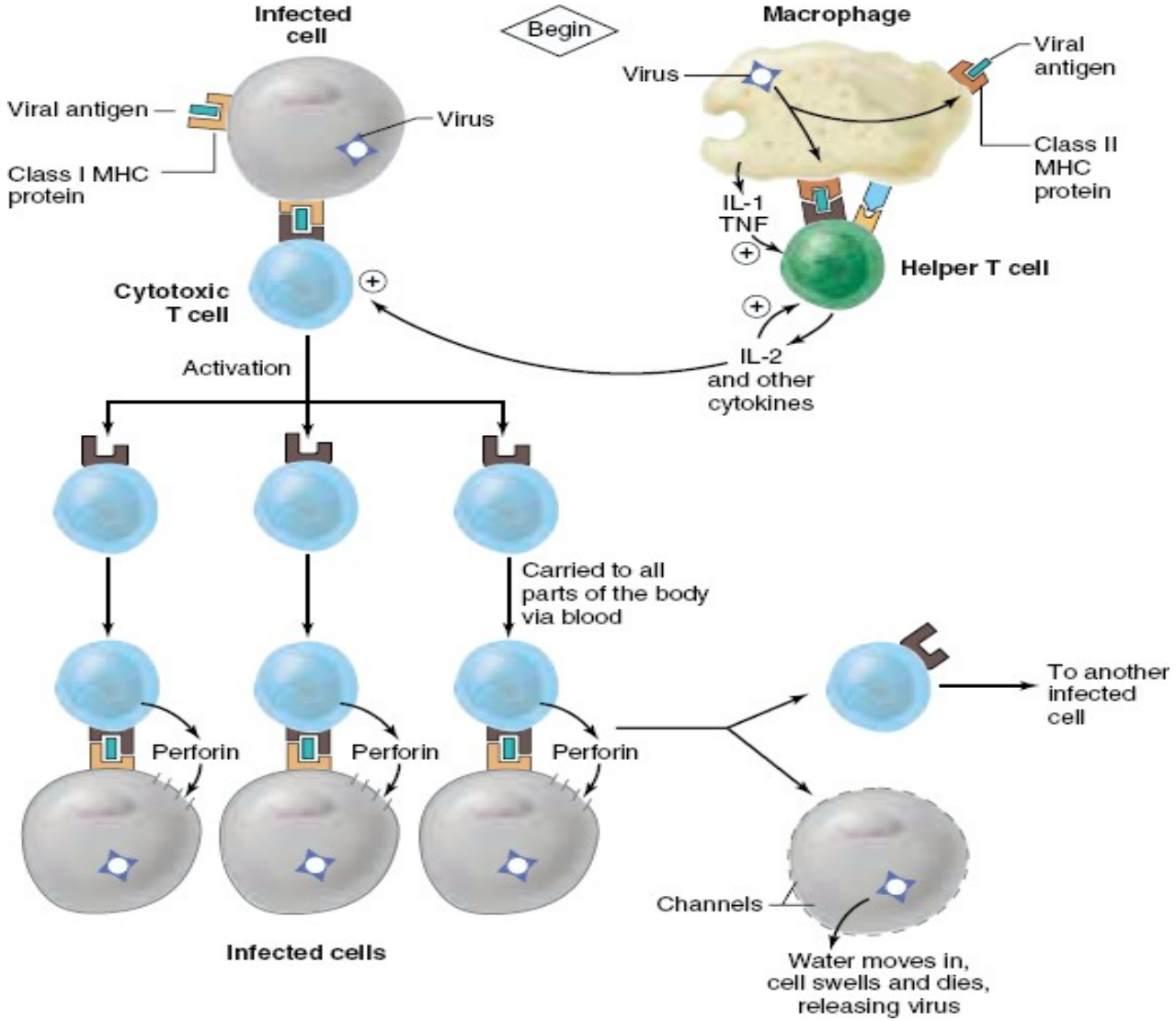
PHYSIOLOGICAL IMPORTANCE OF MHC RELATED ANTIGEN PRESENTATION

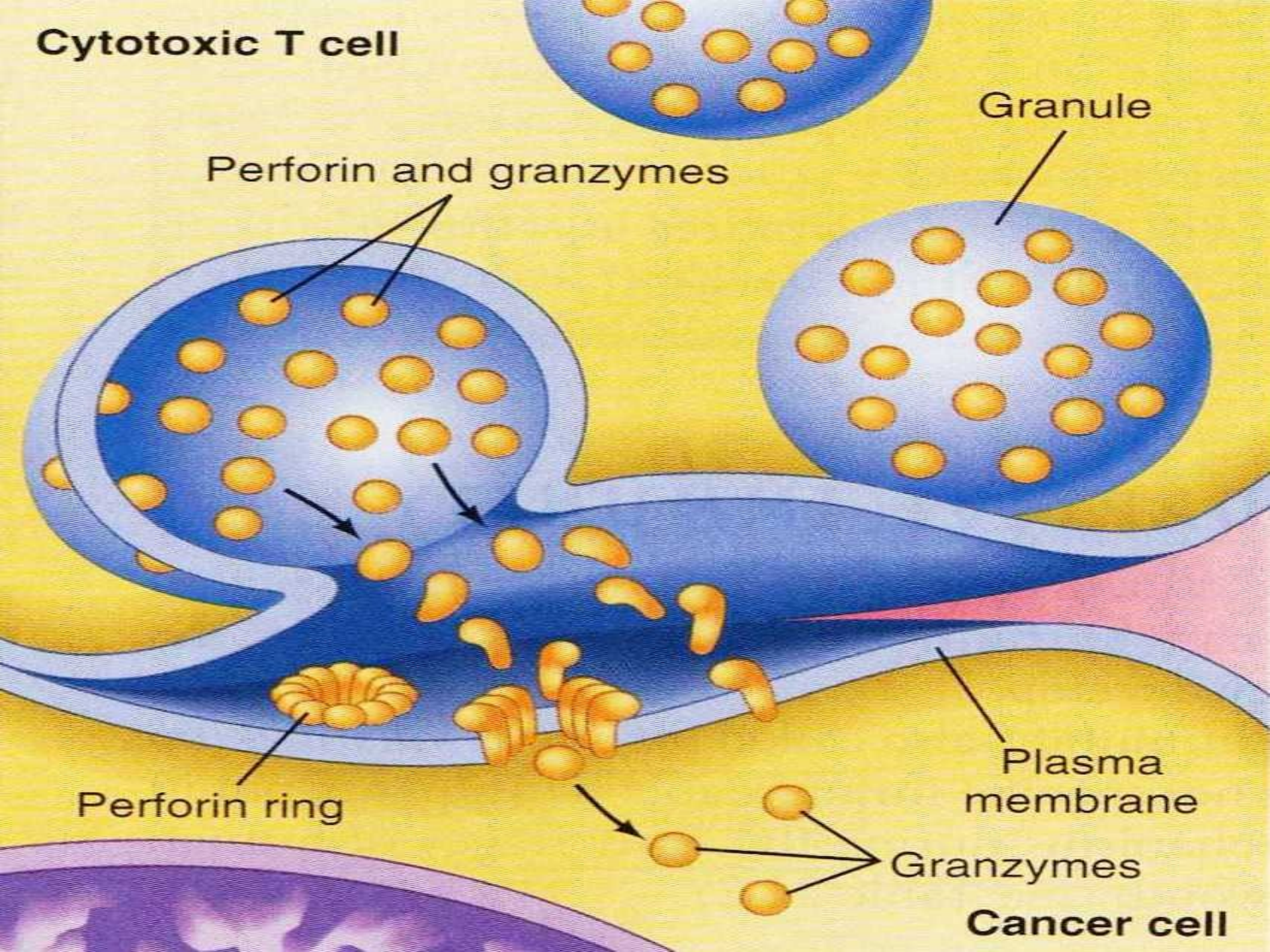
Extracellular microbes are captured by APCs, including B lymphocytes and macrophages, and presented with class II molecules,

MHC II molecules are mainly expressed in these APCs (and dendritic cells).

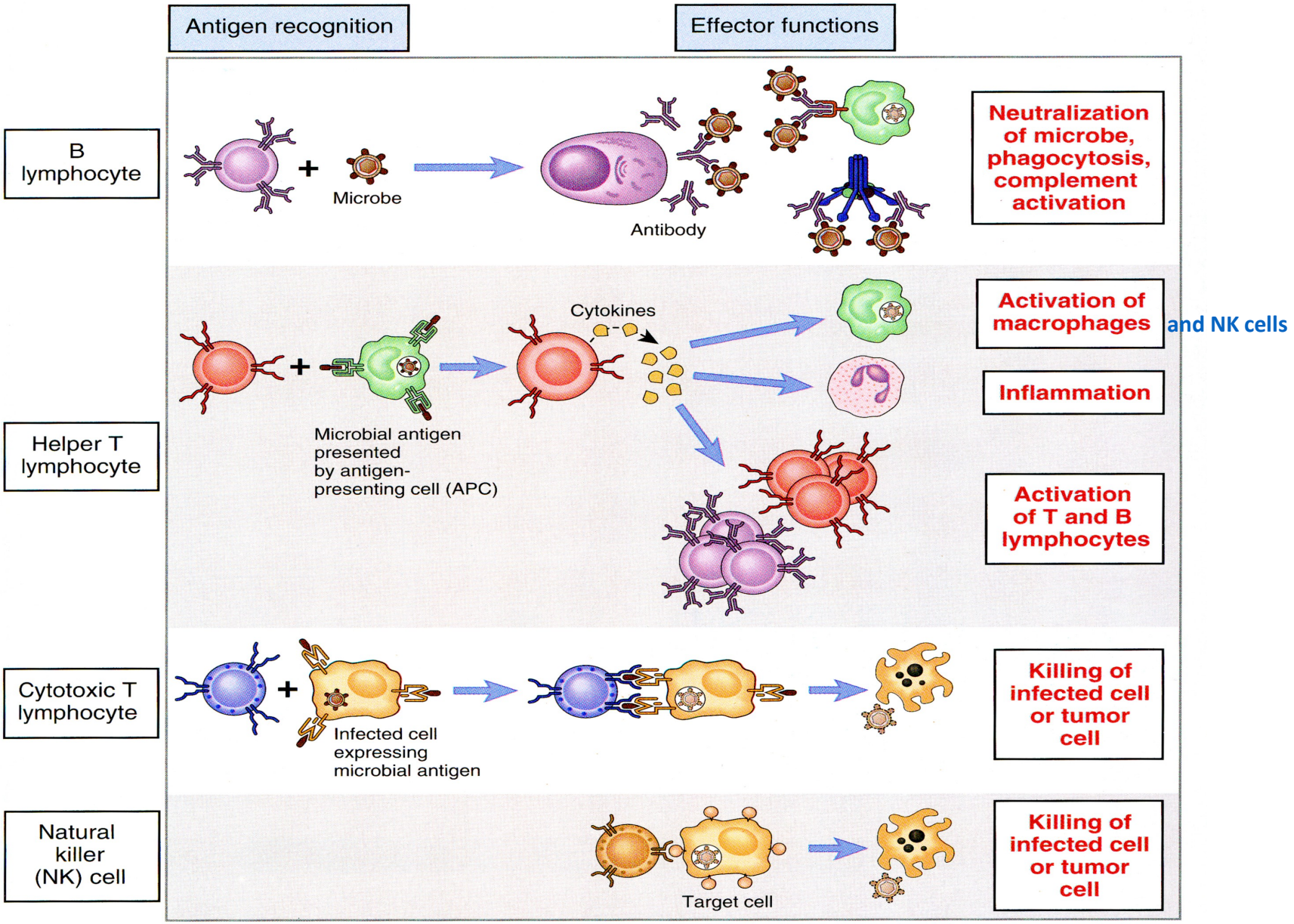
Because of the specificity of CD4 to class II, peptides associated with class II are recognized by CD4 + T cells acting as helper T cells.

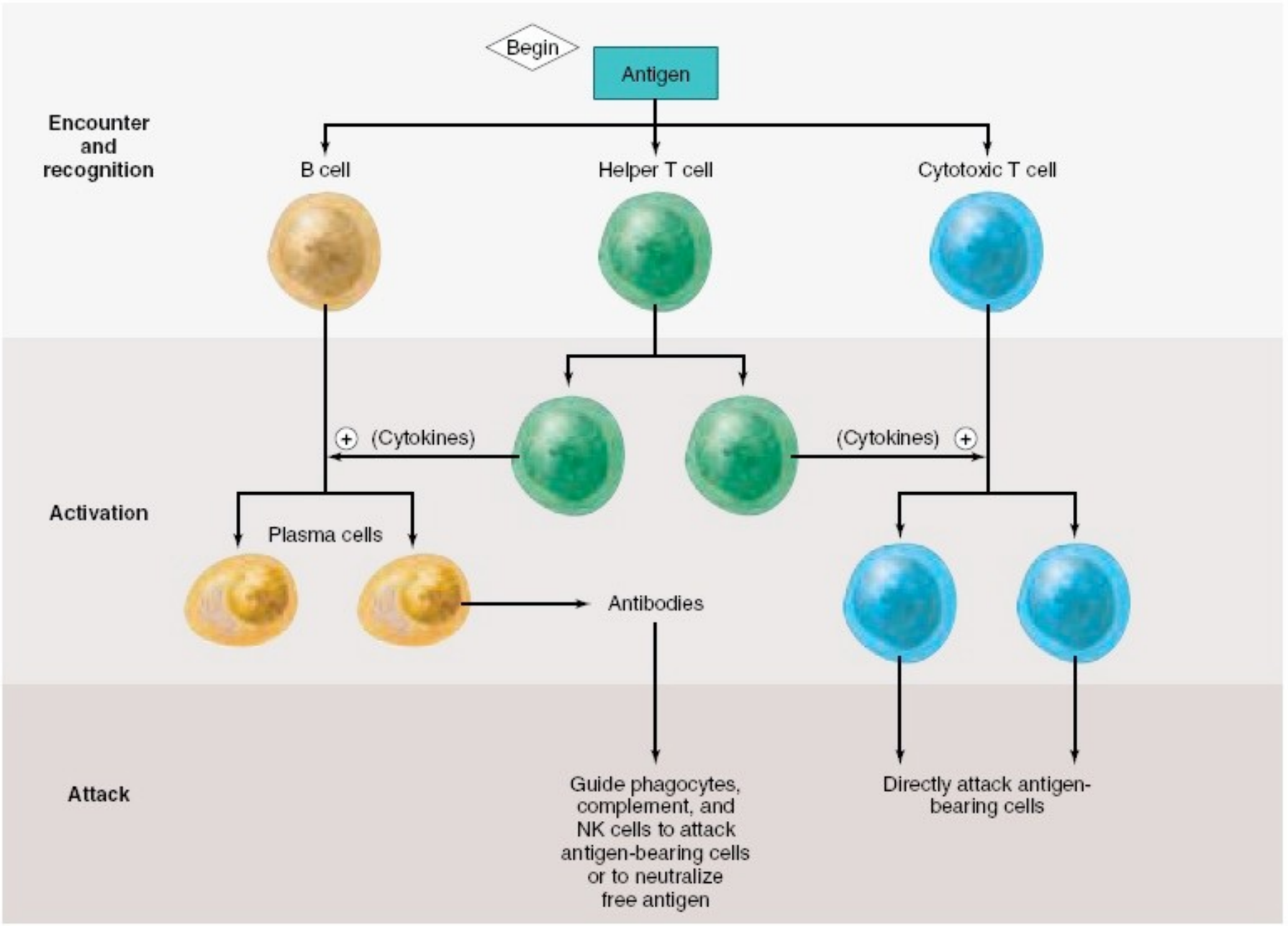
Summary of Events During Killing of Virus-Infected Cells by Cytotoxic T Cells

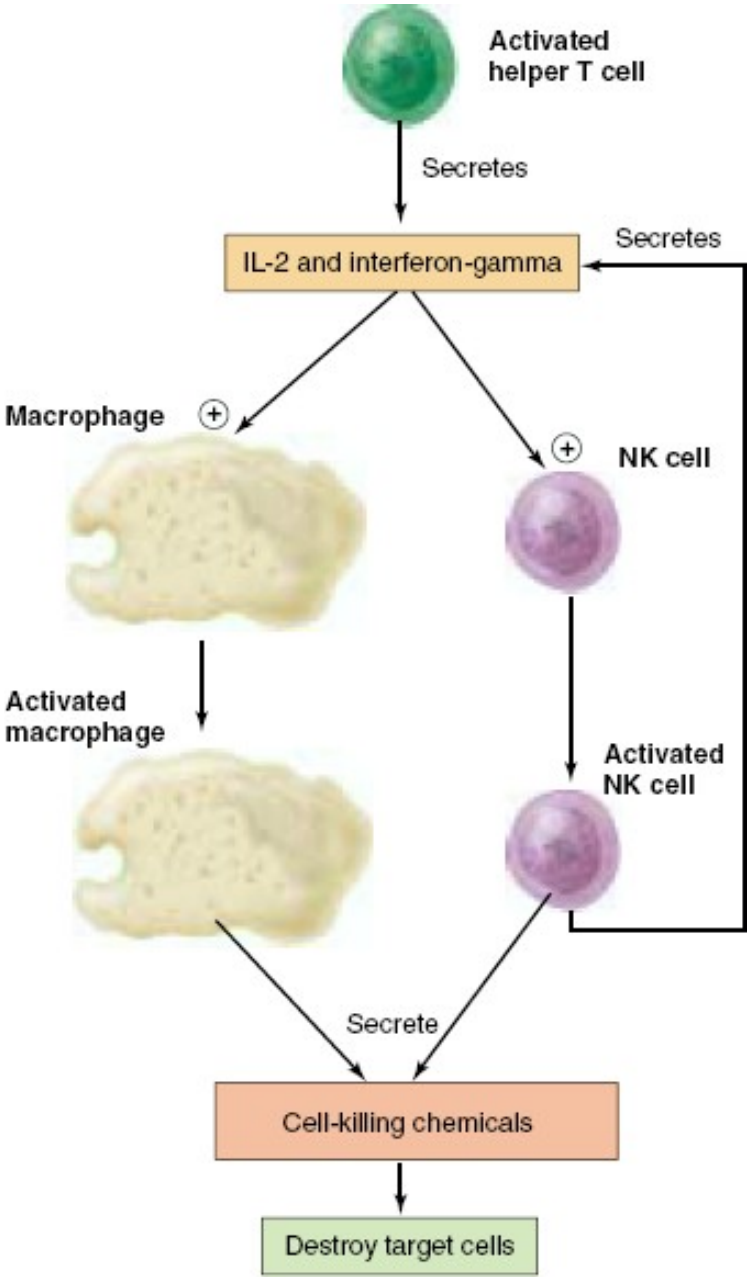



Cytotoxic T cell
Granule
Perforin and granzymes
Perforin ring
Plasma membrane
Granzymes
Cancer cell

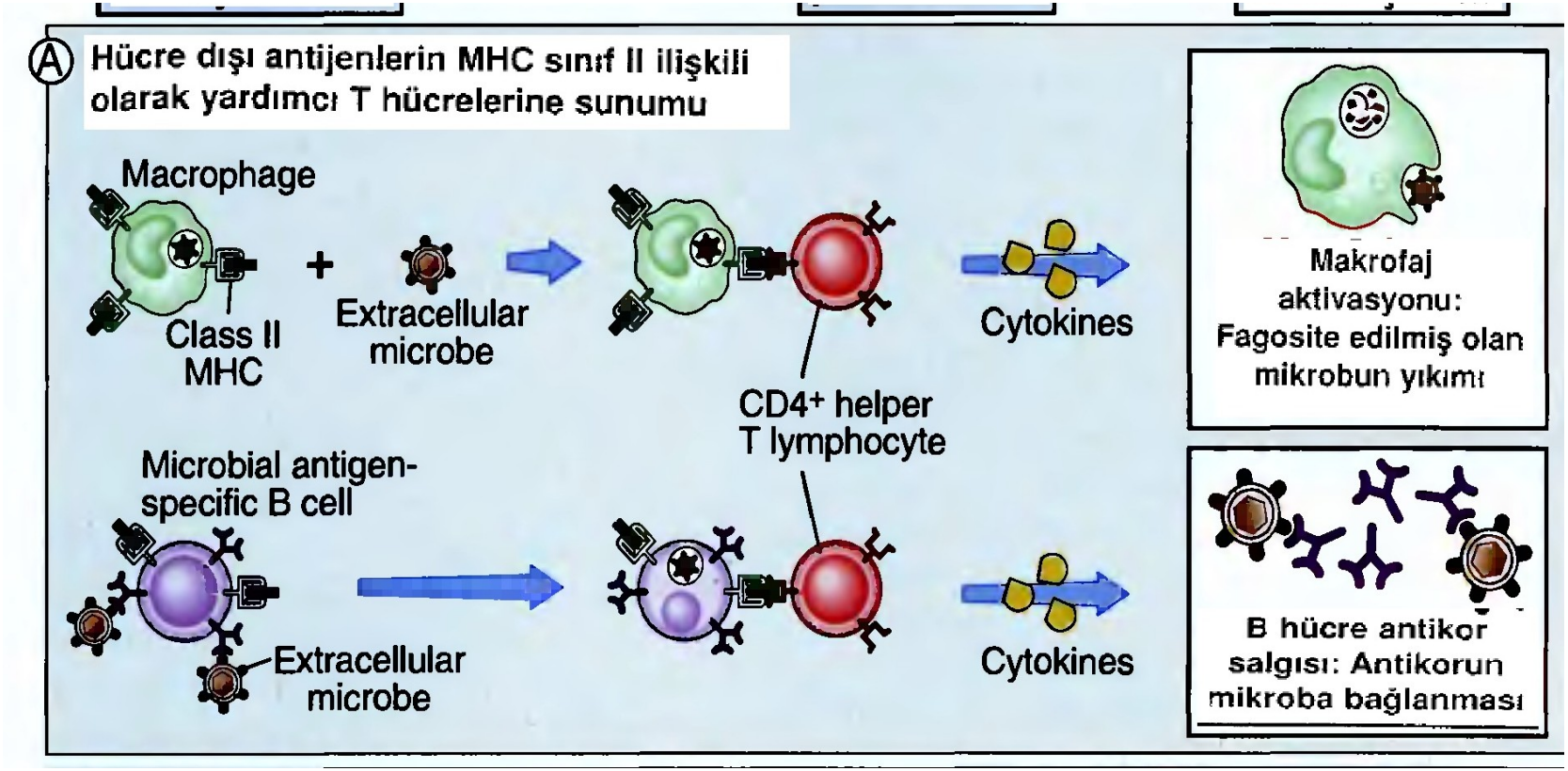
Functions of Lymphocytes




Begin
Antigen
Encounter and recognition
B cell
Helper T cell
Cytotoxic T cell
(+) (Cytokines)
(Cytokines) (+)
Activation
Plasma cells
Antibodies
Attack
Guide phagocytes, complement, and NK cells to attack antigen-bearing cells or to neutralize free antigen
Directly attack antigen-bearing cells

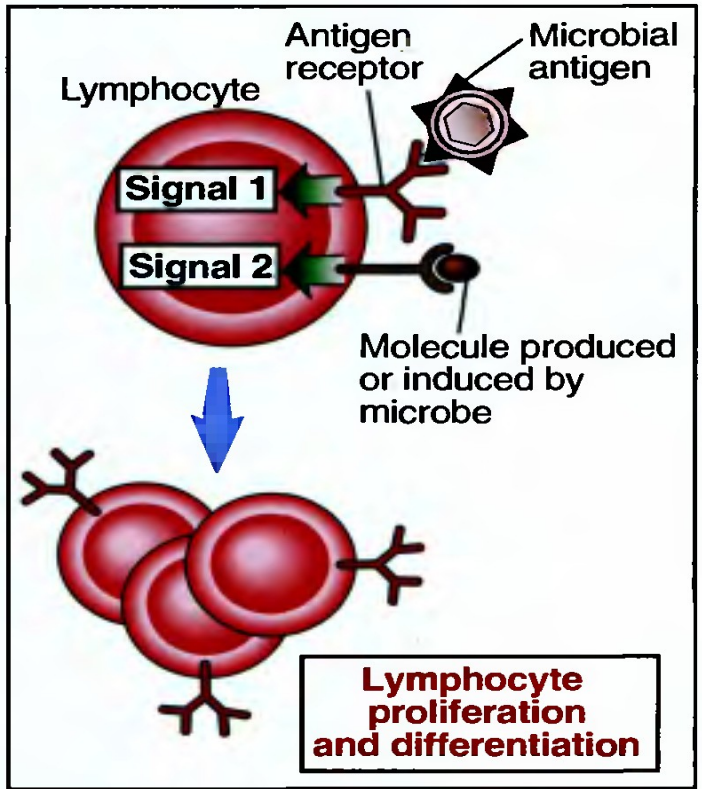

Activated helper T cell
Secretes
IL-2 and interferon-gamma
Secretes
Macrophage
NK cell
Activated macrophage
Activated NK cell
Secrete
Cell-killing chemicals
Destroy target cells

Physiological Importance of MHC Related Antigen Presentation



FUNCTIONS OF APCs OTHER THAN ANTIGEN PRESENTATION

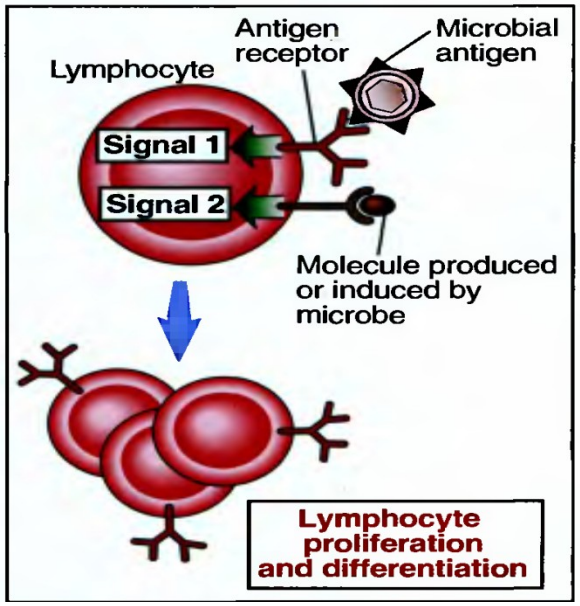
- APCs also provide a "second signal" for T cell activation.
- The antigen itself provides the 1st signal, and microbes or ASHs that meet microbes provide the 2nd signal.



FUNCTIONS OF APCs OTHER THAN ANTIGEN PRESENTATION

The second signal is developed against harmful microbes by the acquired immune response.

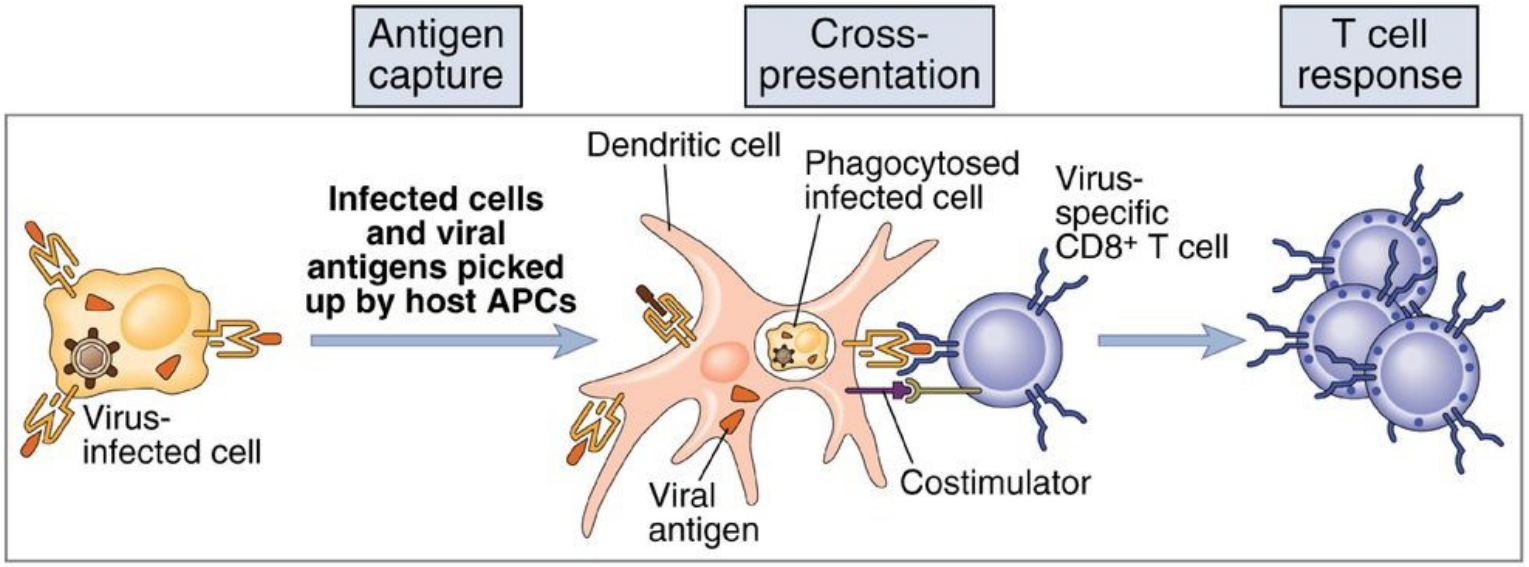
For example, **lipopolysaccharides (LPS), also called endotoxins in bacteria, can create the second signal for lymphocyte activation by activating APCs.**



Cross-Presentation

- Professional antigen presenting cells are dendritic cells, B lymphocytes and macrophages.
- **Dendritic cells can also present exogenous peptides to CD8 + T lymphocytes via MHC class I molecules. This event is called cross-presentation.**

Cross-Presentation of Antigens to CD8+ T cells



Abbas & Lichtman. Cellular and Molecular Immunology, 5th ed. W. B. Saunders 2003

Cross presentation explains how infections of non-professional APCs can lead to the initiation of a CD8 T cell response

CROSS PRESENTATION

- Some dendritic cells have the ability to capture and to ingest virus-infected cells or tumor cells and present the viral or tumor antigens to naive CD8+ T lymphocytes.

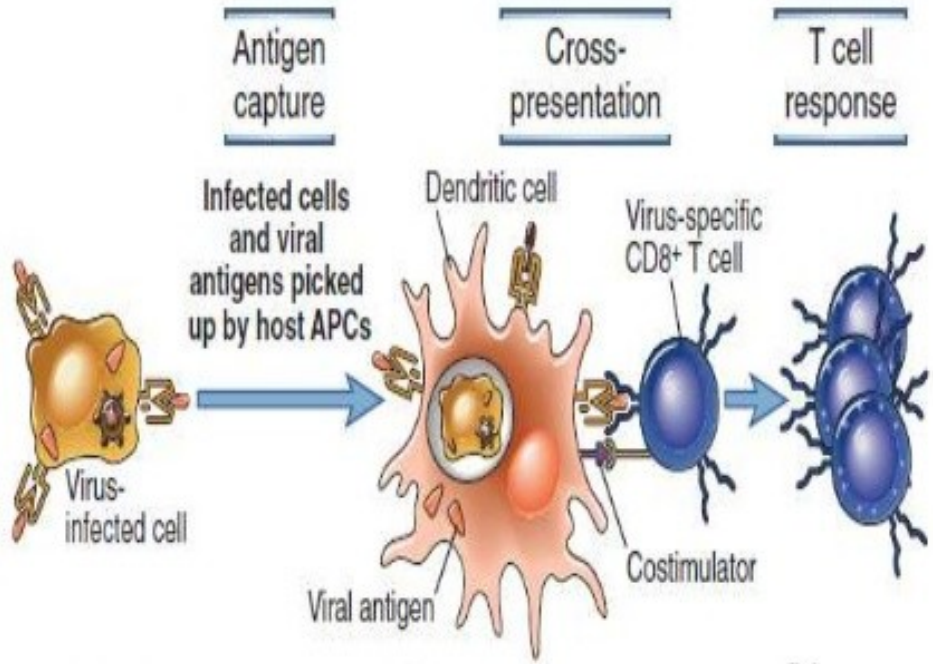
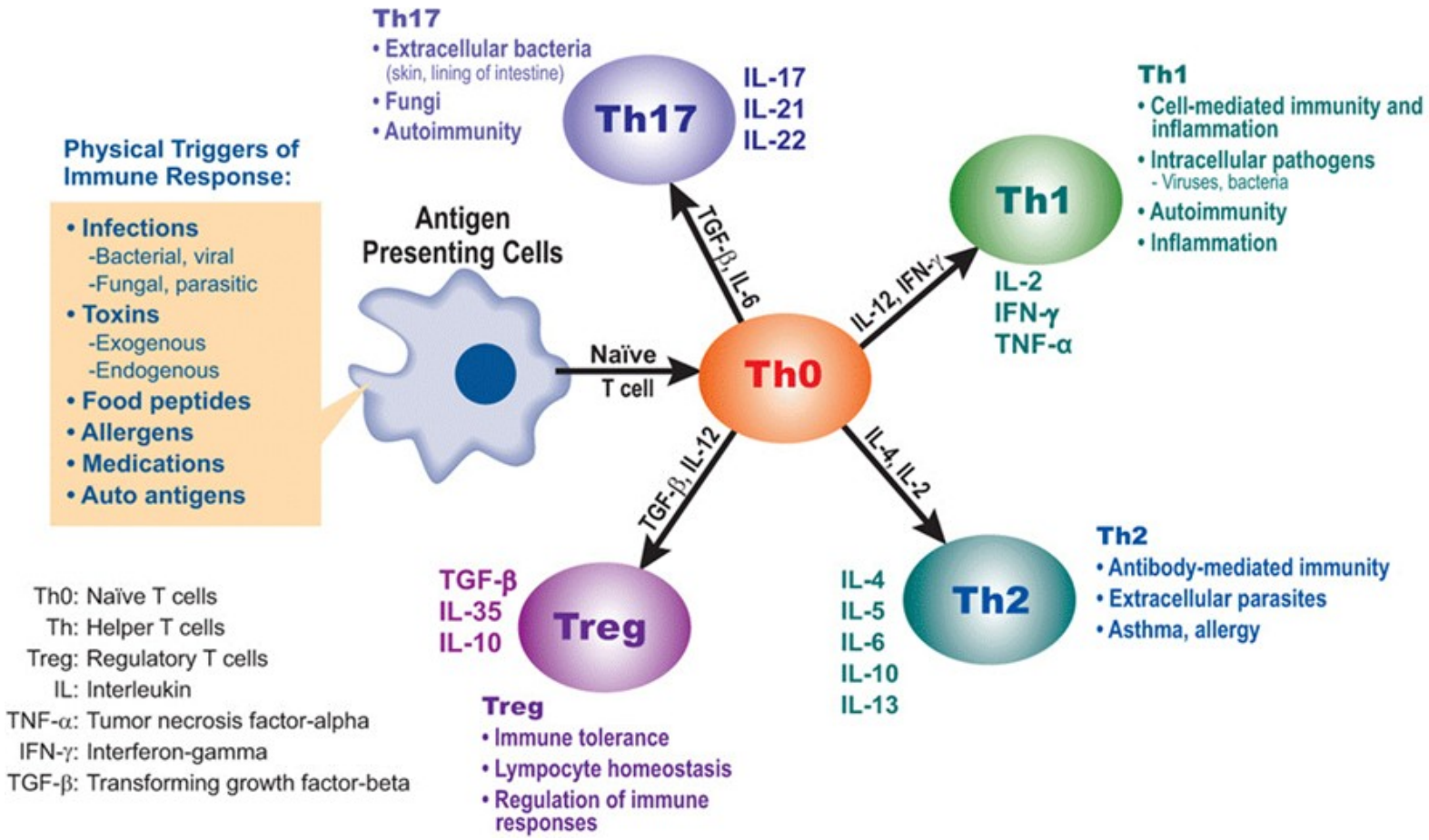


FIGURE 6–20 Cross-presentation of antigens to CD8⁺ T cells. Cells infected with intracellular microbes, such as viruses, are ingested

Th1 cytokines		Th2 cytokines	
IFN-γ	Enhances the microbicidal function of macrophages. Promotes the differentiation of naive helper T cells into Th1 cells. Activates polymorphonuclear leukocytes, cytotoxic T cells, and NK cells.	IL-4	Regulates antibody production, haematopoiesis, and inflammation. Promotes the differentiation of naive helper T cells into Th2 cells. Decreases the production of Th1 cells.
IL-2	Promotes clonal expansion and development of T and B-lymphocytes. Induces expression of adhesion molecules. Enhances the function of NK cells.	IL-10	Inhibits synthesis of Th1 cytokines such as IFN-γ and IL-2. Inhibits antigen-presenting cells.
IL-15	Induces activation and cytotoxicity of NK cells. Activates macrophages. Promotes proliferation and survival of T and B-lymphocytes and NK cells.	IL-8	Promotes neutrophils chemotaxis and degranulation. Promotes tumour angiogenesis.
IL-21	Regulates proliferation and differentiation of T cells, B cells, and NK cells. Potently regulates cellular-mediated immunity and directs cytotoxic T lymphocytes and NK cell effector activity in the clearance of tumours.	IL-13	Inhibits inflammatory cytokine production. Induces immunoglobulin E secretion from B-lymphocytes.


Th17
• Extracellular bacteria
(skin, lining of intestine)
• Fungi
• Autoimmunity
Th17
IL-17
IL-21
IL-22
Th1
• Cell-mediated immunity and inflammation
• Intracellular pathogens
- Viruses, bacteria
• Autoimmunity
• Inflammation
Th1
IL-2
IFN-γ
TNF-α
Physical Triggers of Immune Response:
• Infections
-Bacterial, viral
-Fungal, parasitic
• Toxins
-Exogenous
-Endogenous
• Food peptides
• Allergens
• Medications
• Auto antigens
Antigen Presenting Cells
TGF-β, IL-6
IL-12, IFN-γ
Naïve T cell
Th0
TGF-β, IL-12
IL-4, IL-2
TGF-β
IL-35
IL-10
Treg
Treg
• Immune tolerance
• Lympocyte homeostasis
• Regulation of immune responses
IL-4
IL-5
IL-6
IL-10
IL-13
Th2
Th2
• Antibody-mediated immunity
• Extracellular parasites
• Asthma, allergy
Th0: Naïve T cells
Th: Helper T cells
Treg: Regulatory T cells
IL: Interleukin
TNF-α: Tumor necrosis factor-alpha
IFN-γ: Interferon-gamma
TGF-β: Transforming growth factor-beta