Università degli Studi di Padova

Corso di Laurea in
BIOTECNOLOGIE
Piano di studi Farmaceutico

Anno Accademico 2024-2025

Insegnamento di

Immunologia Farmaceutica

La restrizione per MHC
La tipizzazione HLA
Recettori per l'antigene e molecole accessorie dei linfociti T: il TCR α/β e γ/δ. Proteine CD3.
Molecole accessorie e di adesione dei linfociti T

08-04-2025

T-cell recognition of antigen is MHC-restricted

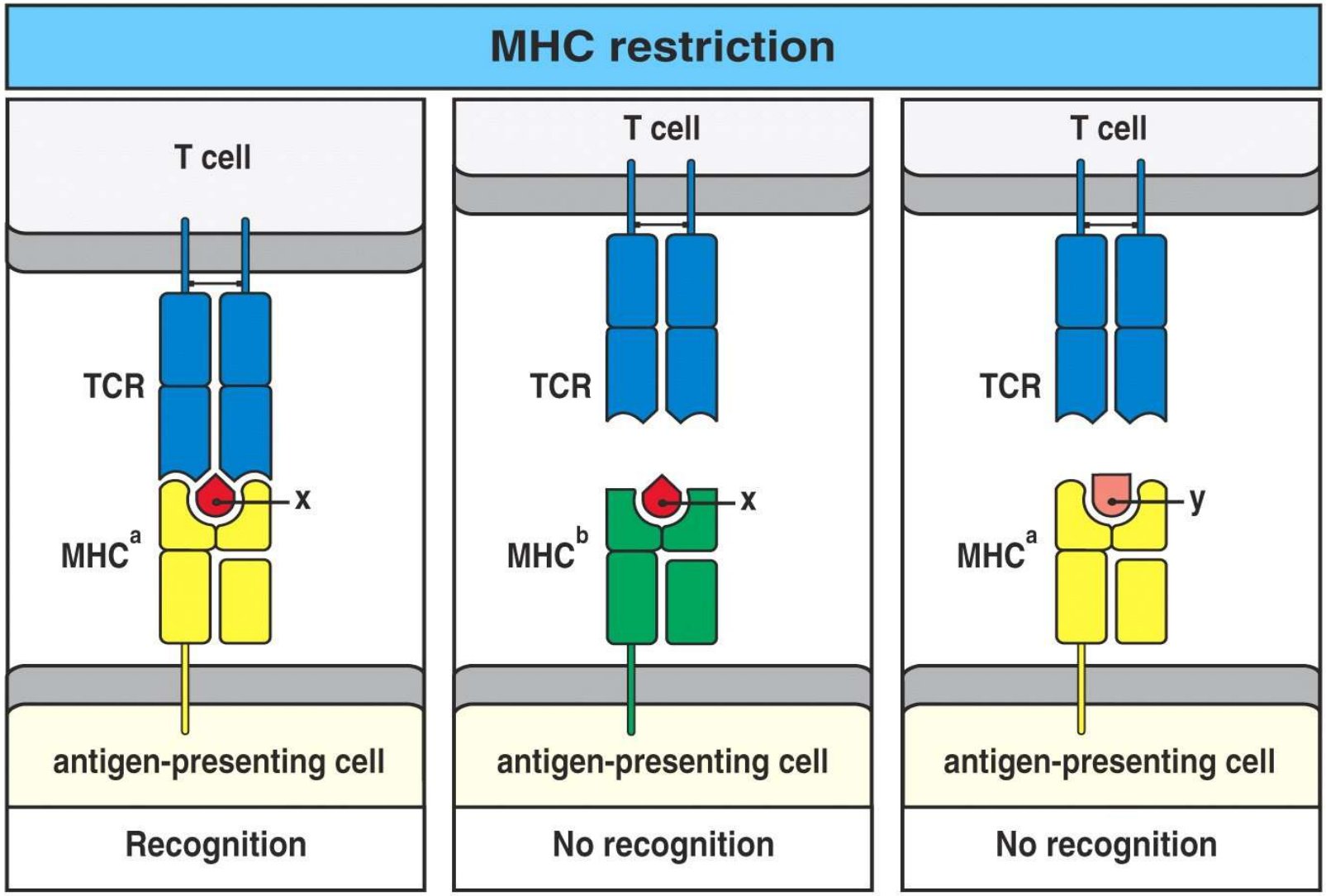
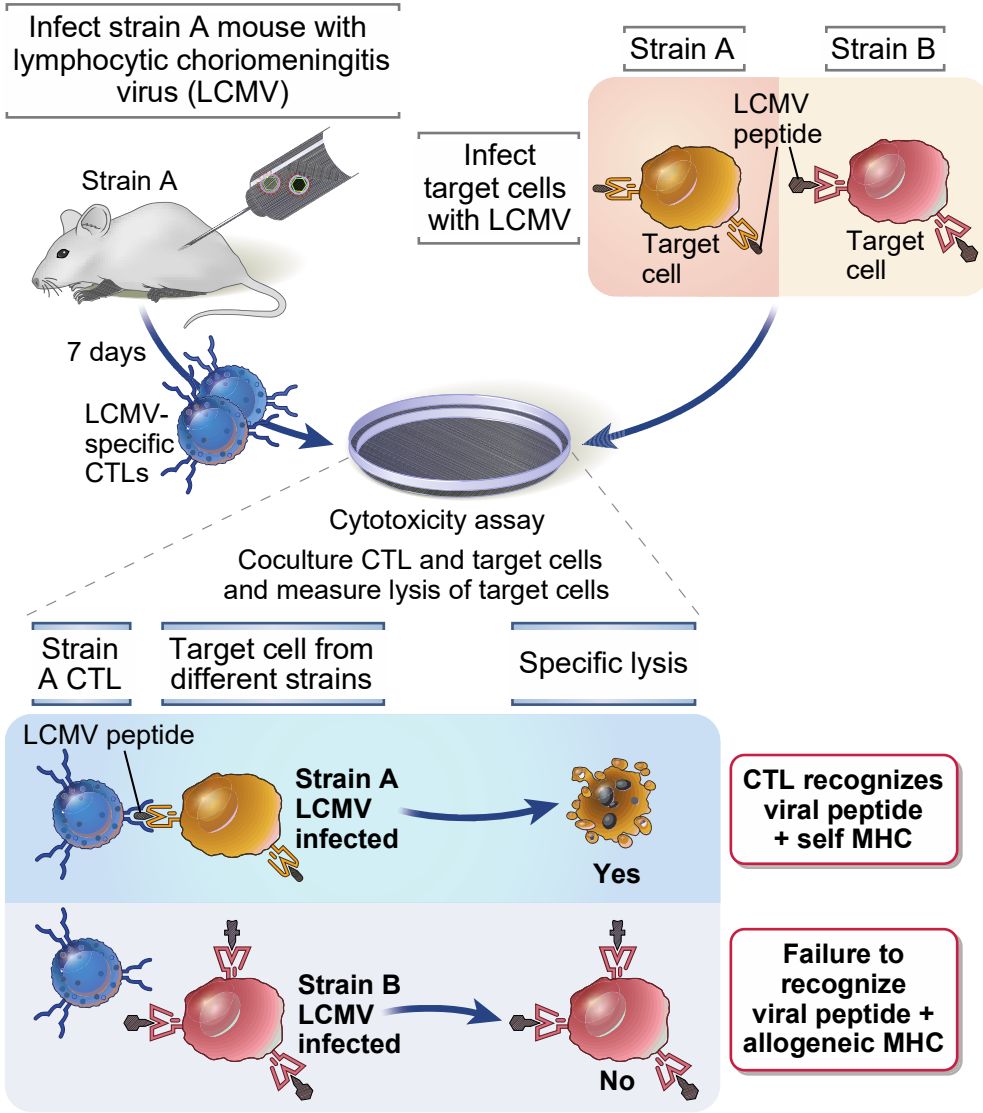


Figure 5-17 Immunobiology, 6/e. (© Garland Science 2005)

Experimental demonstration of MHC restriction



T cells with TCR specific for MHC^a and antigenic peptide x will not recognize either antigenic peptide x presented by MHC^b or Antigenic peptide y presented by MHC^a

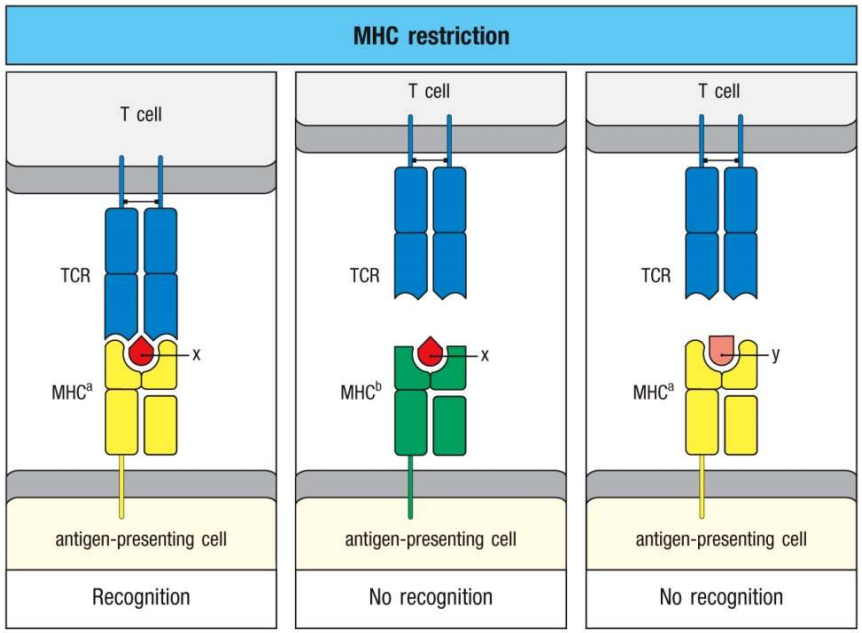


Figure 6.23 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Nobel Prize in Physiology or Medicine 1996

Photo from the Nobel Foundation archive.

Peter C. Doherty

Prize share: 1/2

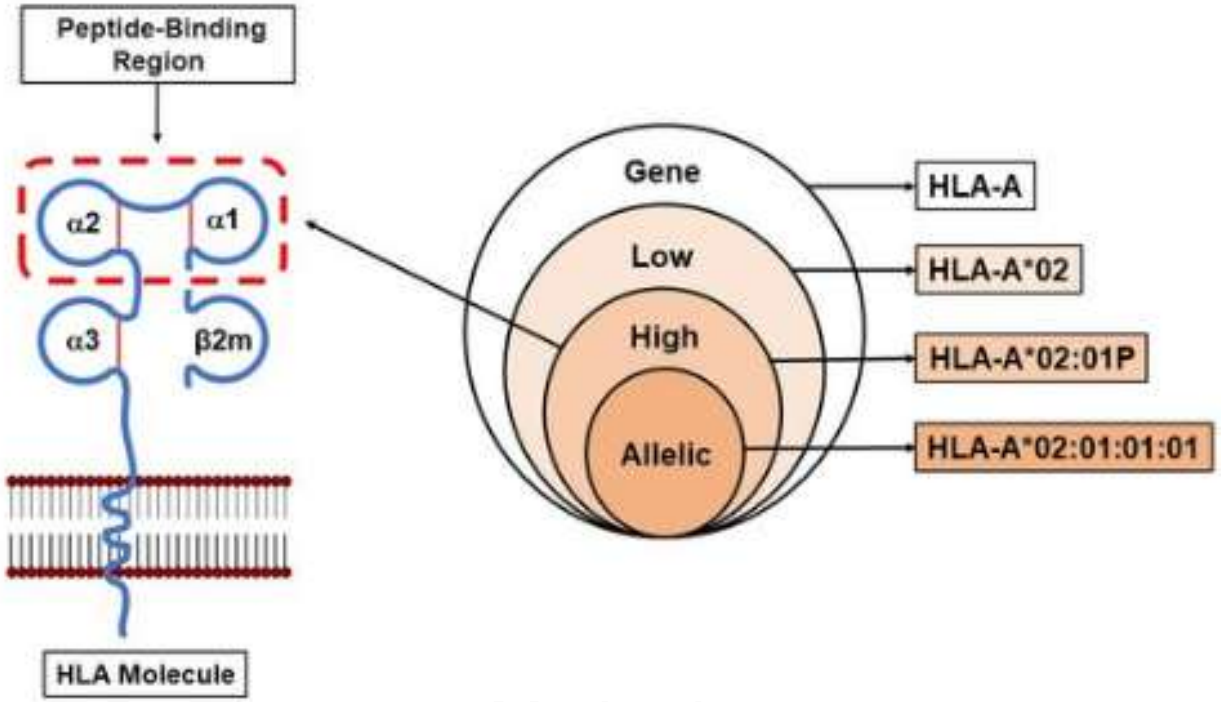
Photo from the Nobel Foundation archive.

Rolf M. Zinkernagel

Prize share: 1/2

The Nobel Prize in Physiology or Medicine 1996 was awarded jointly to Peter C. Doherty and Rolf M. Zinkernagel "for their discoveries concerning the specificity of the cell mediated immune defence"

Tipizzazione HLA



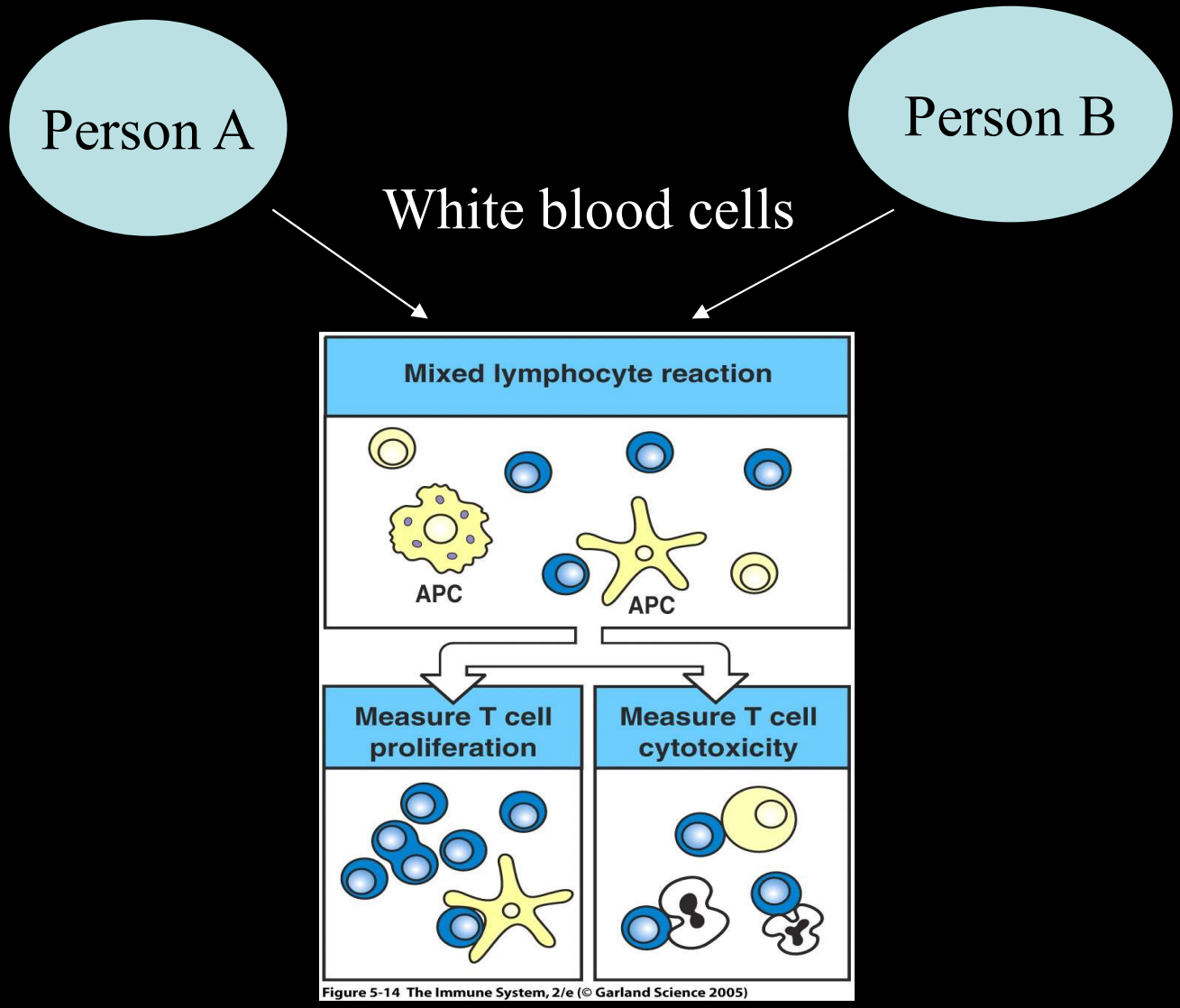
Methods:

1. Serological
2. Molecular
(3. Cellular)

TYPING METHODS

- SEROLOGY used to be the 'gold' standard. Now being superseded by molecular techniques as they become more robust and time efficient
- CELLULAR rarely used now. Orginally used for Class II typing
- MOLECULAR fast becoming the method of choice. Many laboratories test of choice.

Mixed Lymphocyte Reaction (MLR) is used to test for HLA compatibility between individuals



HLA specificities identified by serology versus Molecular methods (February 2007)

Gene	Serology	Molecular
HLA Class I		
HLA-A	28	506
HLA-B	62	851
HLA-C	10	276
HLA Class II		
HLA-DRA1	0	3
HLA-DRB1	25	476
HLA-DRB3	1	44
HLA-DRB4	1	13
HLA-DRB5	1	18
HLA-DQA1	0	34
HLA-DQB1	9	81
HLA-DPA1	0	23
HLA-DPB1	6	126

Steps of Molecular Typing

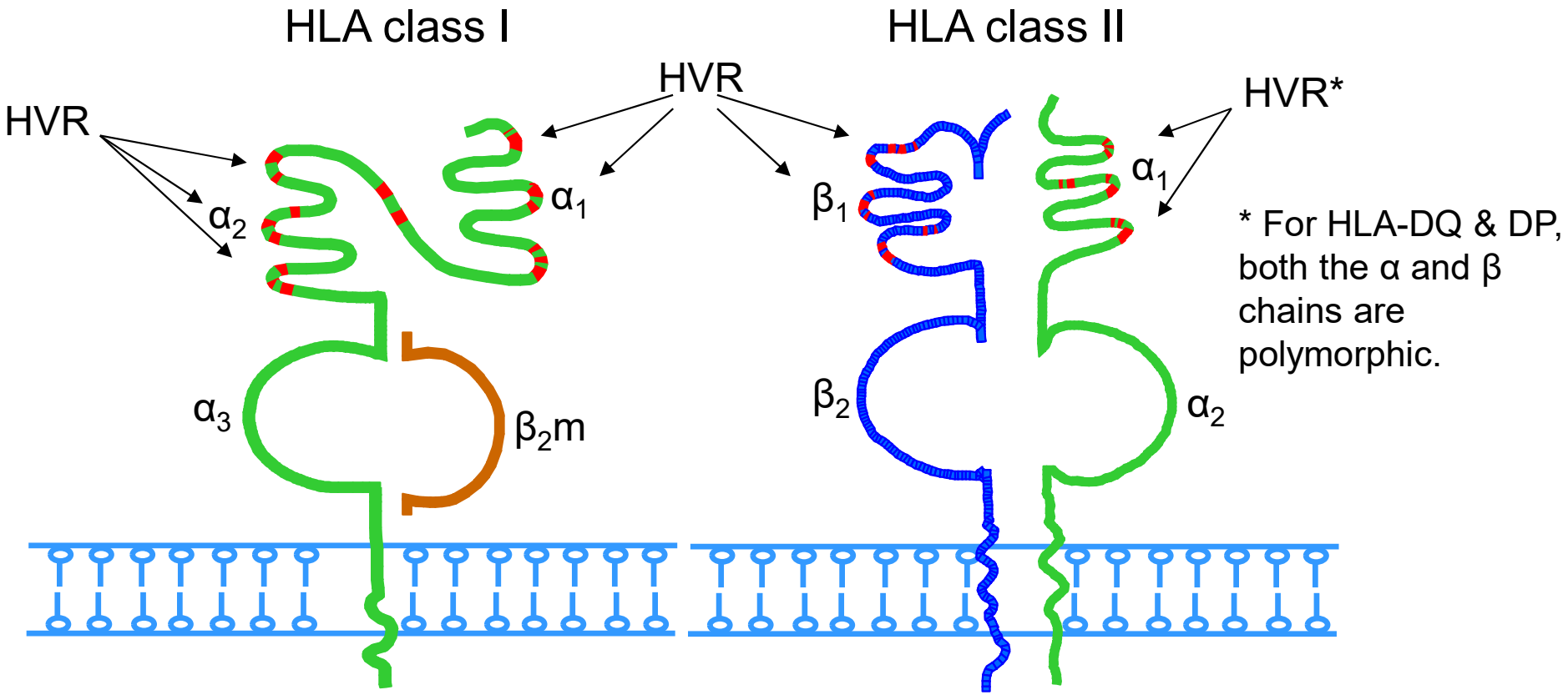
- Extraction of genomic DNA
- Amplification of the genes of interest and
- Detection of sequence polymorphism that define the alleles.

Molecular HLA Typing

DNA based procedures has increased the accuracy of HLA typing and lead to the identification of serologically undetected alleles and of many subtypes of serological specificities.

DNA based methods to type for HLA alleles have therefore focused in the analysis of nucleotide variation occurring in both exon 2 and 3 of class I genes and exon-2 of class II genes.

Target Sequences for HLA Typing



Typing targets polymorphic or hypervariable regions (HVR) that occur largely in the exons encoding the protein domains that form the peptide binding sites:

- For HLA class I, exons 2 and 3 of the heavy chain genes
- For HLA class II, exon 2 of the alpha and beta chains

HLA Typing by Molecular Assays

- Advantages of molecular assays:
  - Increased accuracy and reproducibility
  - No need for viable lymphocytes
  - Option for automation
- Typing resolution:
  - Antigen level: defines groups of HLA alleles that all encode the same serologically defined antigen.
  - Allele level: defines specific HLA alleles.

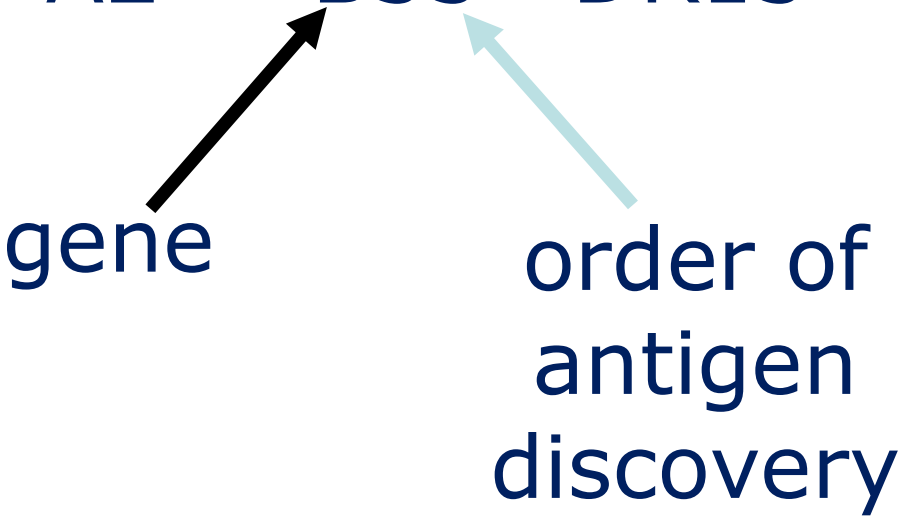
Nomenclature of HLA typing

DNA	Serology
A*0201	A2
A*0202	A2
A*0203	A2
A*0204	A2
A*0205	A2
…	…
A*0273	A2

Examples of serological typing

A10 B15 DR4

A2 B35 DR15



Examples of DNA typing:

HLA Nomenclature

Distinguishes Between Antigen and Allele Level Typing

Antigen Level

Based on Serologic Specificity

HLA- A2
HLA- B7
HLA-Cw8
HLA-DR4
HLA-DR52
HLA-DQ5

Some antigens can be "split" into closely related antigens:
HLA-A9 splits =
HLA-A24
HLA-A23

Allele Level

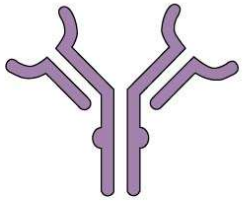
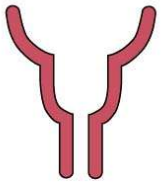
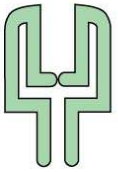
Based on DNA Sequence

DRB1*0401

Locus Allele

Serologic Equivalent
(if known)

Note: Multiple alleles can define the same HLA antigen:
DRB1*0401, *0402, *0405, *0407 = DR4

Feature	Antigen binding molecule		
	Immunoglobulin (Ig) 	T cell receptor (TCR) 	MHC molecules* 
Antigen binding site	Made up of three CDRs in V_H and three CDRs in V_L	Made up of three CDRs in V_a and three CDRs in V_b	Peptide-binding cleft made of a1 and a2 (class I) or a1 and b1 (class II)
Nature of antigen that may be bound	Macromolecules (proteins, lipids, polysaccharides) and small chemicals	Peptide-MHC complexes	Peptides
Nature of antigenic determinants recognized	Linear and conformational determinants of various macromolecules and chemicals	Linear determinants of peptides; only 2 or 3 amino acid residues of a peptide bound to an MHC molecule	Linear determinants of peptides; only some amino acid residues of a peptide
Affinity of antigen binding	K_d 10^{-7} – 10^{-11} M; average affinity of Igs increases during immune response	K_d 10^{-5} – 10^{-7} M	K_d 10^{-6} – 10^{-9} M; extremely stable binding

*The structures and functions of MHC and TCR molecules are discussed in Chapters 5 and 7, respectively.
Abbreviations: CDR, complementarity-determining region; K_d, dissociation constant; MHC, major histocompatibility complex; V_H, variable domain of heavy chain Ig; V_L, variable domain of light chain Ig

Identification of the T-cell Receptor

Much more difficult to identify than the B-cell receptor (Ig)

- Always membrane bound- never secreted

- Specific for antigen AND MHC

-Not an abundant mRNA in T-cells

Consequently the T-cell receptor wasn't isolated and identified until the early 1980's

The T-cell Receptor is Similar to the Fab Fragment of Ig

Member of Ig superfamily

Each chain has two domains: an N-terminal variable region and a C-terminal constant region

Each variable region has 3 hypervariable regions analogous to the CDRs in Ig molecules

Joined by interchain disulfide bond

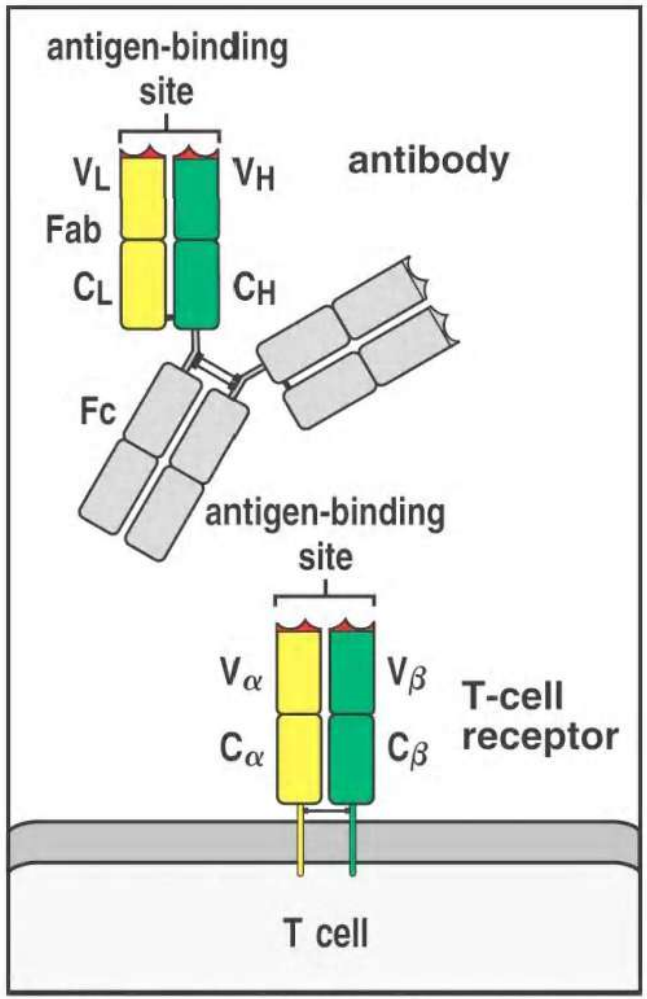
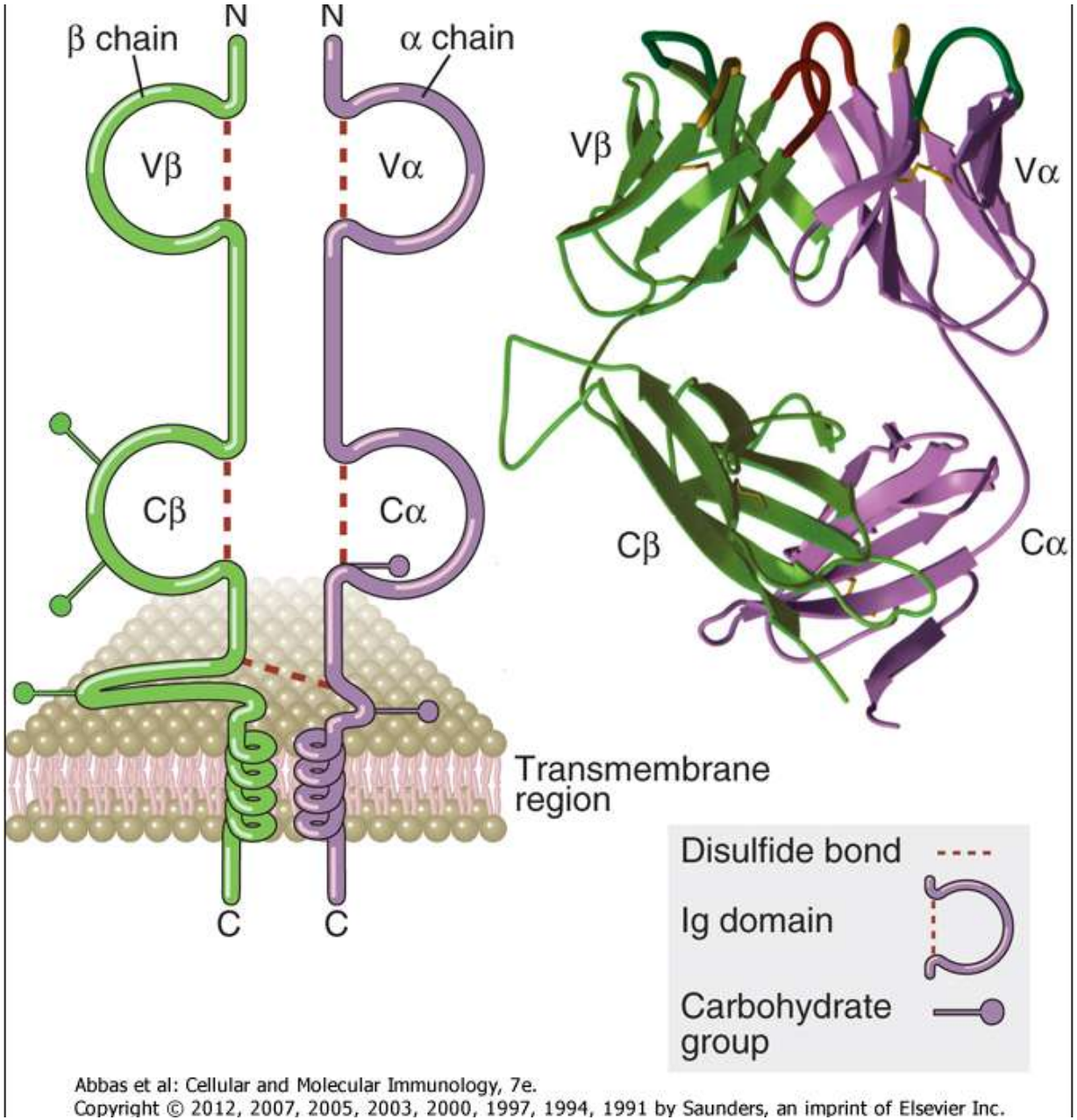


Figure 3-11 Immunobiology, 6/e. (© Garland Science 2005)

Struttura del recettore delle cellule T



Struttura della regione variabile del TCR

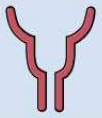
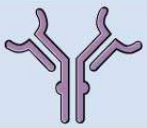
Le regioni V delle catene α e β del TCR contengono nelle loro sequenze brevi tratti in cui si concentra la variabilità tra i diversi TCR: queste regioni *ipervariabili* costituiscono le regioni che determinano la complementarietà (*Complementary Determining Regions*) o CDR.

Ogni regione V sia della catena α che della β contiene 3 CDR.

Le 3 CDR della catena α sono giustapposte alle 3 regioni della catena β a costituire la porzione del TCR che riconosce specificatamente i complessi MHC-peptide.

Il riconoscimento dei complessi peptide/MHC è mediato dalle CDR formate dalle catene α e β del TCR.

Proprietà dei recettori per l'antigene dei linfociti : TCR e Ig

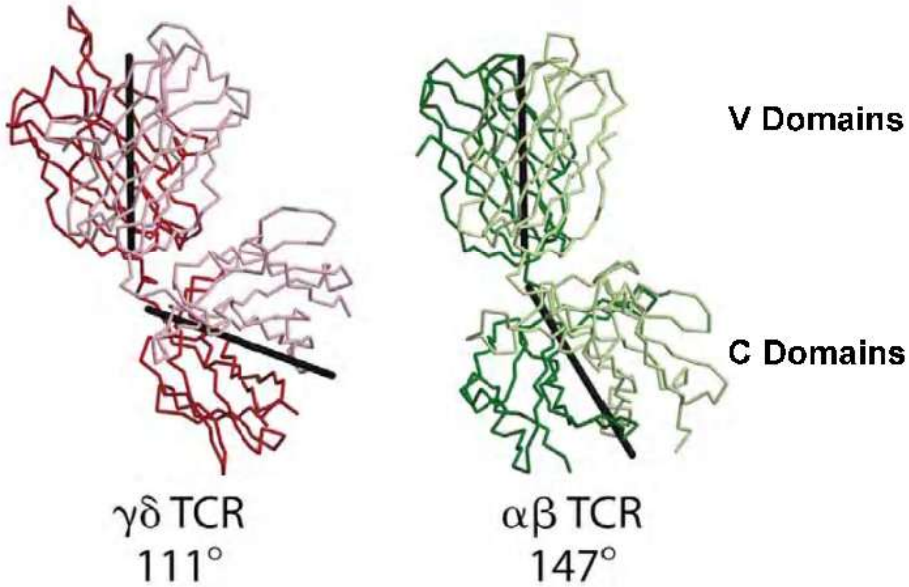
	T cell receptor (TCR) 	Immunoglobulin (Ig) 
Components	a and b chains	Heavy and light chains
Number of Ig domains	One V domain and one C domain in each chain	Heavy chain; one V domain, three or four C domains Light chain; one V domain and one C domain
Number of CDRs	Three in each chain for antigen binding; fourth hypervariable region in b chain (of unknown function)	Three in each chain
Associated signaling molecules	CD3 and z	Iga and Igb
Affinity for antigen (K_d)	10^{-5}–10^{-7} M	10^{-7}–10^{-11} M (secreted Ig)
Changes after cellular activation		
Production of secreted form	No	Yes
Isotype switching	No	Yes
Somatic mutations	No	Yes

Abbreviations: C, constant; CDR, complementarity-determining region; K_d, dissociation constant; V, variable.

γδ T-cell Receptor

Although organization is the same, the structure of the γδ receptor is very different from the αβ receptor

Thought to interact with non-protein antigens that are not processed or associated with classical MHC I or II



Most don't express CD4 or CD8

In humans, γδ T-cells account for only about 5% of all T-cells

TABLE 9-1 Comparison of αβ and γδ T cells

Feature	αβ T cells	γδ T cells
Proportion of $CD3^+$ cells	90–99%	1–10%
TCR V gene germ-line repertoire	Large	Small
CD4/CD8 phenotype		
$CD4^+$	~60%	<1%
$CD8^+$	~30%	~30%
$CD4^+CD8^+$	<1%	<1%
$CD4^-CD8^-$	<1%	~60%
MHC restriction	$CD4^+$: MHC class II	No MHC restriction
	$CD8^+$: MHC class I	
Ligands	Peptide + MHC	Phospholipid antigen

<u>Linfociti T γ/δ</u>

Meno del 5% dei linfociti T negli organi linfoidi periferici

15% dei linfociti presenti tra le cellule epiteliali dell'intestino tenue

40% dei linfociti presenti tra le cellule epiteliali dell'intestino crasso.

I linfociti T sono detti anche *<u>linfociti intra-epiteliali</u>: ruolo importante nella* ***protezione delle superfici mucose.***

The T-cell Receptor Complex

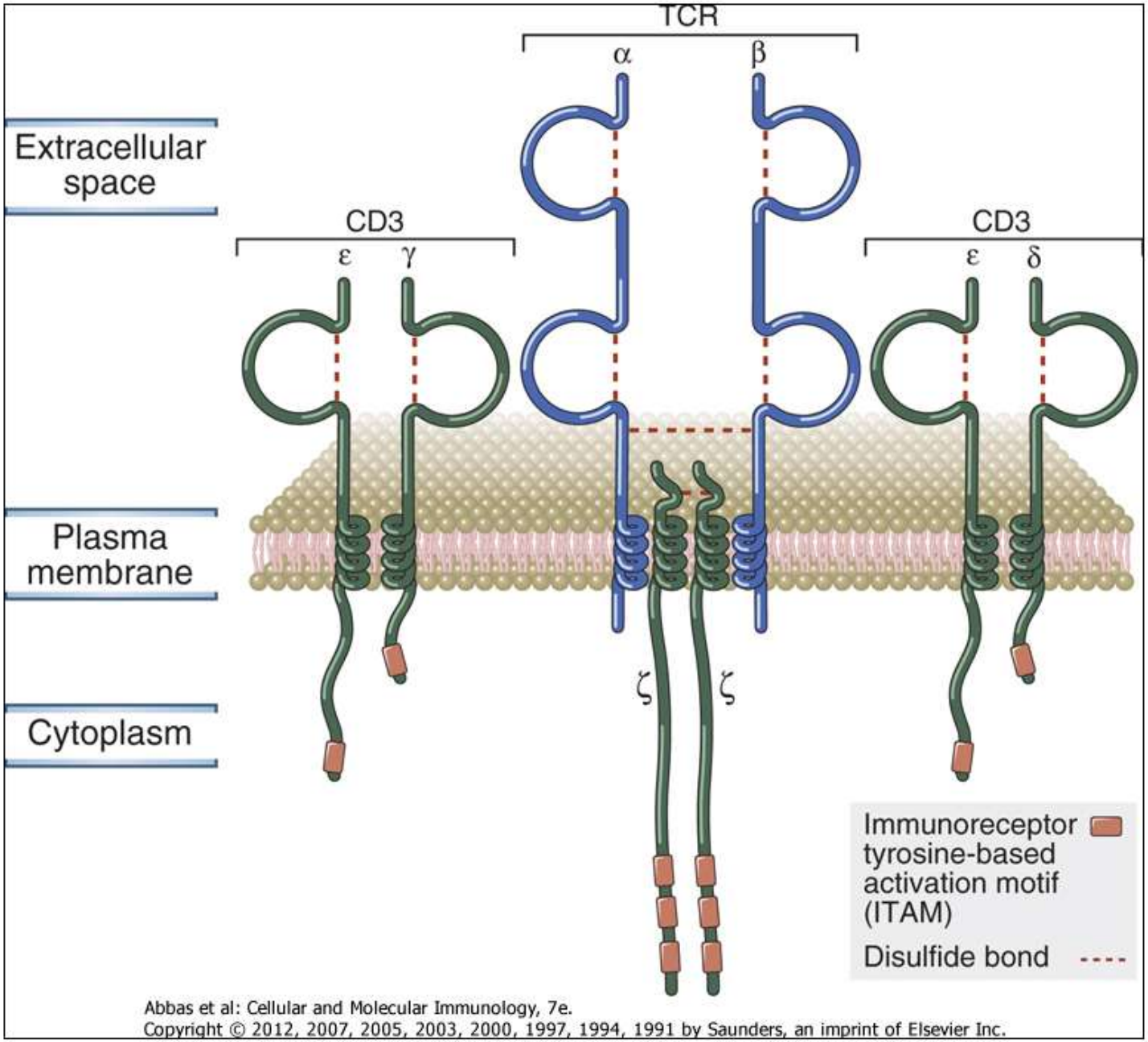
The T-cell receptor is found associated with six other proteins on the surface of cells= CD3 complex

CD3 consists of three dimers

Dimer 1= γ chain and ε chain
Dimer 2= δ chain and ε chain
Dimer 3= two ζ (zeta) chains

CD3 is required for membrane localization of the T-cell receptor and signal transduction following recognition of MHC:antigen complex

Componenti del complesso del TCR



Corecettori e recettori costimolatori dei linfociti T

Oltre al complesso del TCR, altre molecole di membrana contribuiscono in modo importante all'attivazione e alla differenziazione dei linfociti T.

I **corecettori** sono una categoria di proteine di membrana che amplificano il segnale del TCR.

Accessory Membrane Molecules

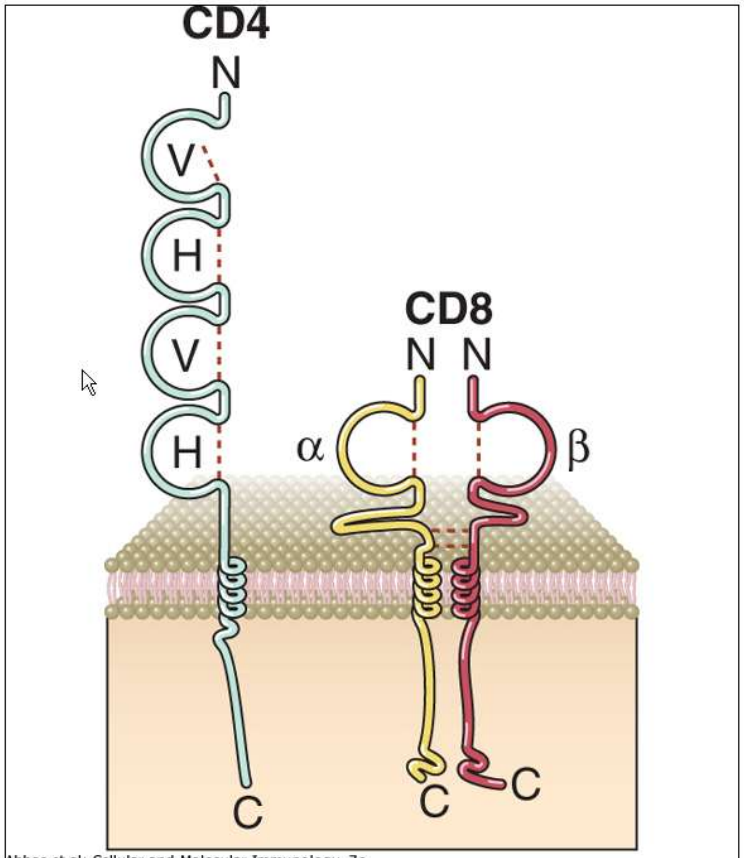
Coreceptors- Involved in MHC:peptide interaction and subsequent signal transduction events

CD4- Monomeric protein, with long cytoplasmic tail

Interacts with $\alpha 2/\beta 2$ domains of MHC II

CD8- Dimer of α and β chains or two α

Interacts with $\alpha 2$ and $\alpha 3$ domains of MHC I, as well as $\beta_2$microglobulin



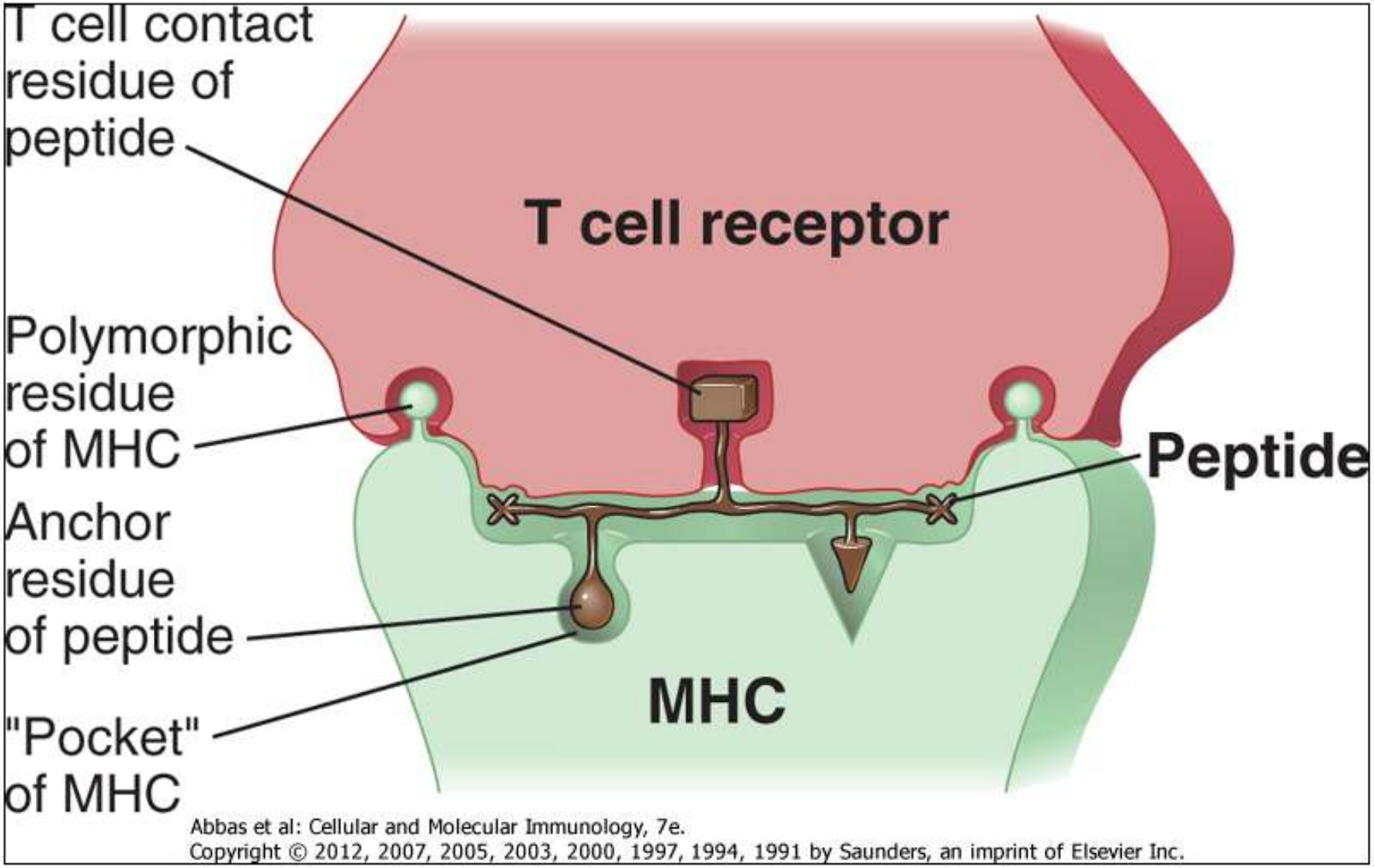
Processazione e presentazione dell'antigene

- Processazione dell'antigene: degradazione di proteine e formazione di peptidi antigenci.
- Presentazione dell'antigene: esposizione del peptide sulla superficie cellulare in associazione a molecole MHC.
- Una volta processati e presentati gli antigeni sono pronti ad interagire con i linfociti T, che riconoscono in modo specifico i complessi peptidi-MHC mediante il TCR.

Caratteristiche degli antigeni riconosciuti dai linfociti T

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 cell-associated and not soluble antigens	MHC molecules are membrane proteins that display stably bound peptides on cell surfaces
$CD4^+$ and $CD8^+$ T cells preferentially recognize antigens sampled from the vesicular 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 class I molecules present peptides from cytosolic proteins; CD4 and CD8 bind to nonpolymorphic regions of class II and class I MHC molecules, respectively

Abbreviations: APC, antigen-presenting cell; MHC, major histocompatibility complex



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- Una volta processati e presentati gli antigeni sono pronti ad interagire con i linfociti T, che riconoscono in modo specifico i complessi peptidi-MHC mediante il TCR.

There are two categories of major intracellular compartments, separated by membranes

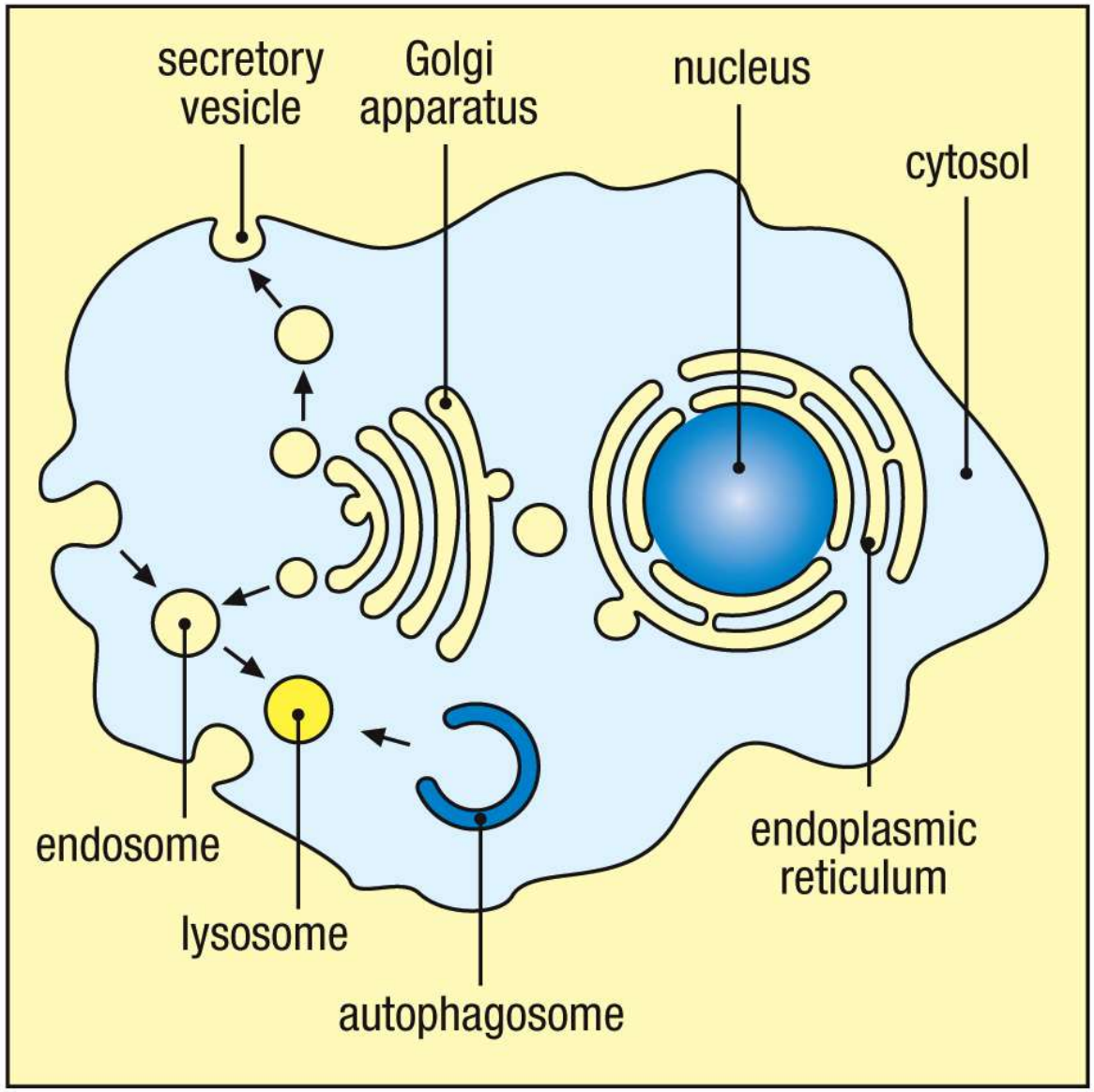


Figure 6.1 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

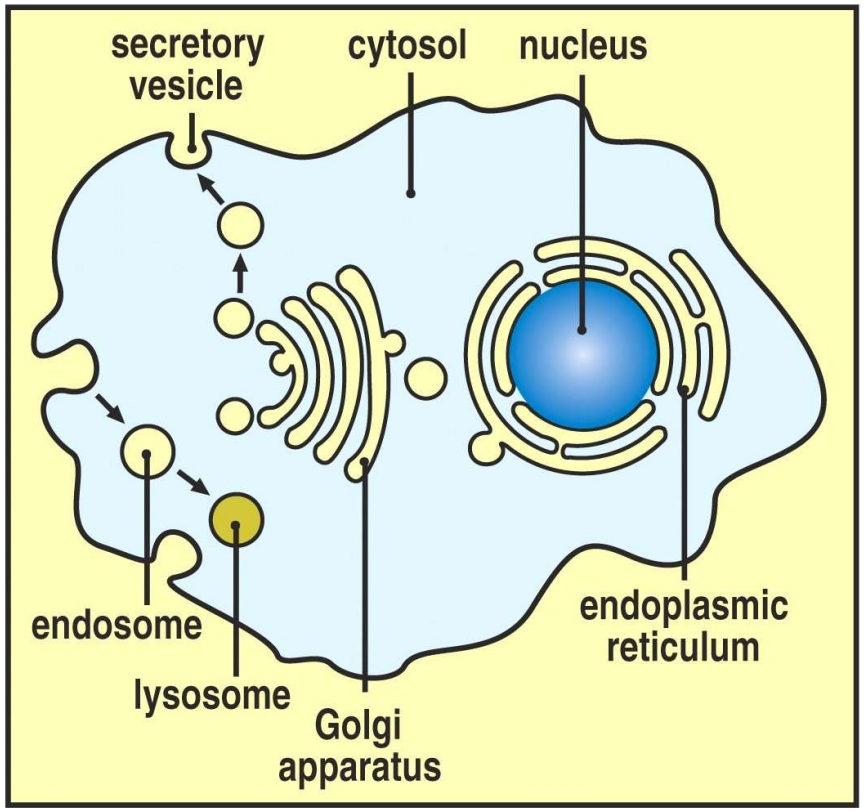


Figure 5-1 Immunobiology, 6/e. (© Garland Science 2005)

Gli antigeni derivano da due fonti:
• possono essere prodotti all'interno della cellula (citosol)
•possono essere acquisiti dalla cellula mediante endocitosi, pinocitosi o fagocitosi.

Ci sono 2 principali compartimenti all'interno delle cellule, separati da membrane.

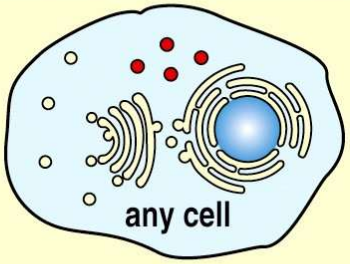
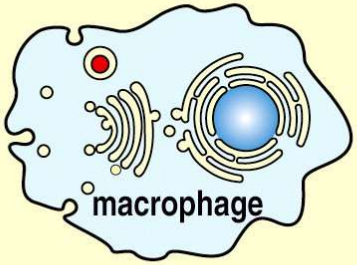
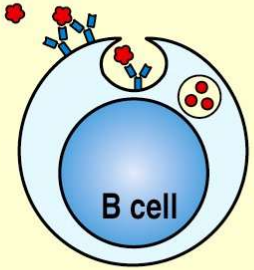
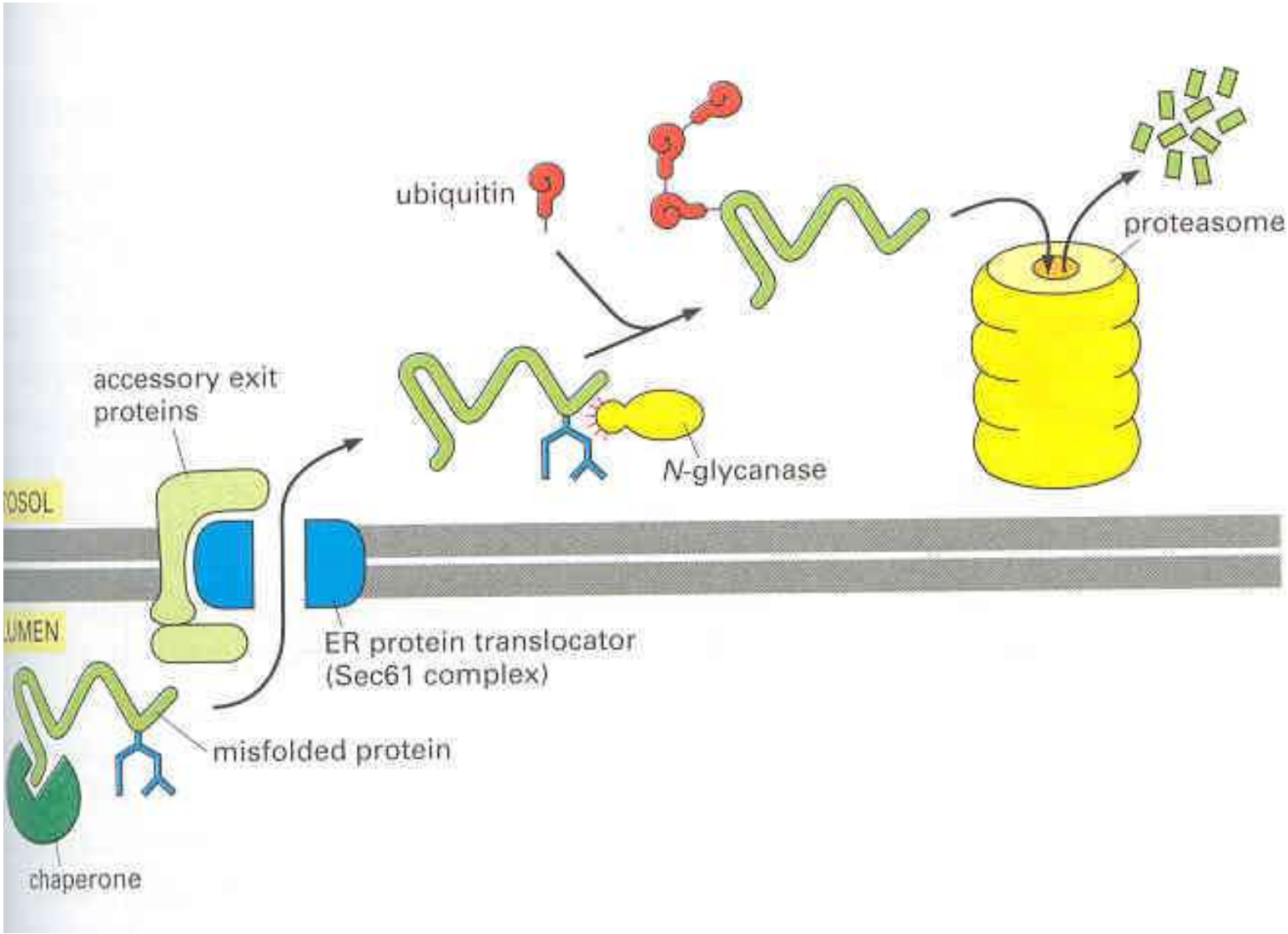
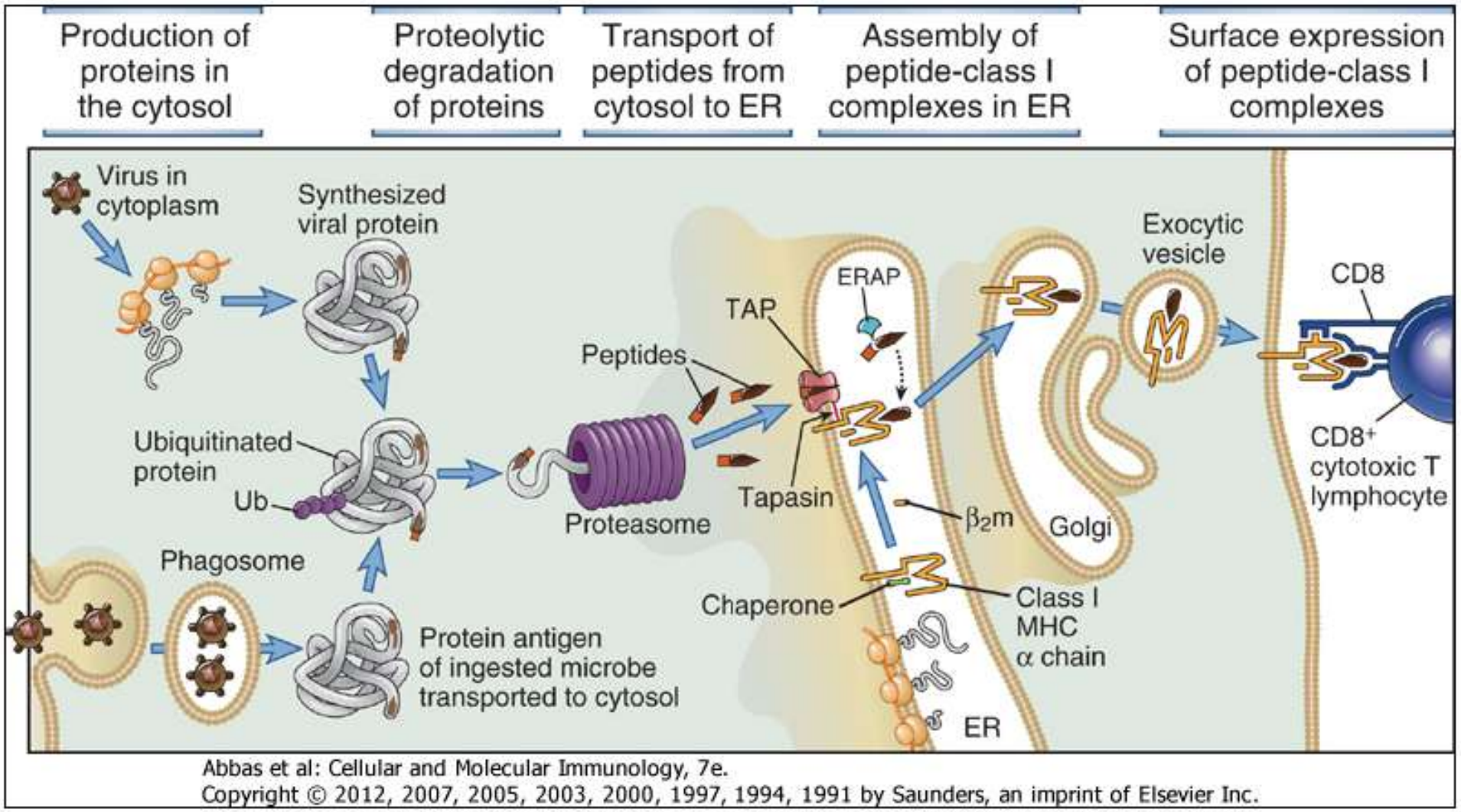
	Cytosolic pathogens	Intravesicular pathogens	Extracellular pathogens and toxins
			
Degraded in	Cytosol	Endocytic vesicles (low pH)	Endocytic vesicles (low pH)
Peptides bind to	MHC class I	MHC class II	MHC class II
Presented to	CD8 T cells	CD4 T cells	CD4 T cells
Effect on presenting cell	Cell death	Activation to kill intravesicular bacteria and parasites	Activation of B cells to secrete Ig to eliminate extracellular bacteria/toxins

Figure 5-2 Immunobiology, 6/e. (© Garland Science 2005)

Protein digestion through ubiquitinilation occurs regularly

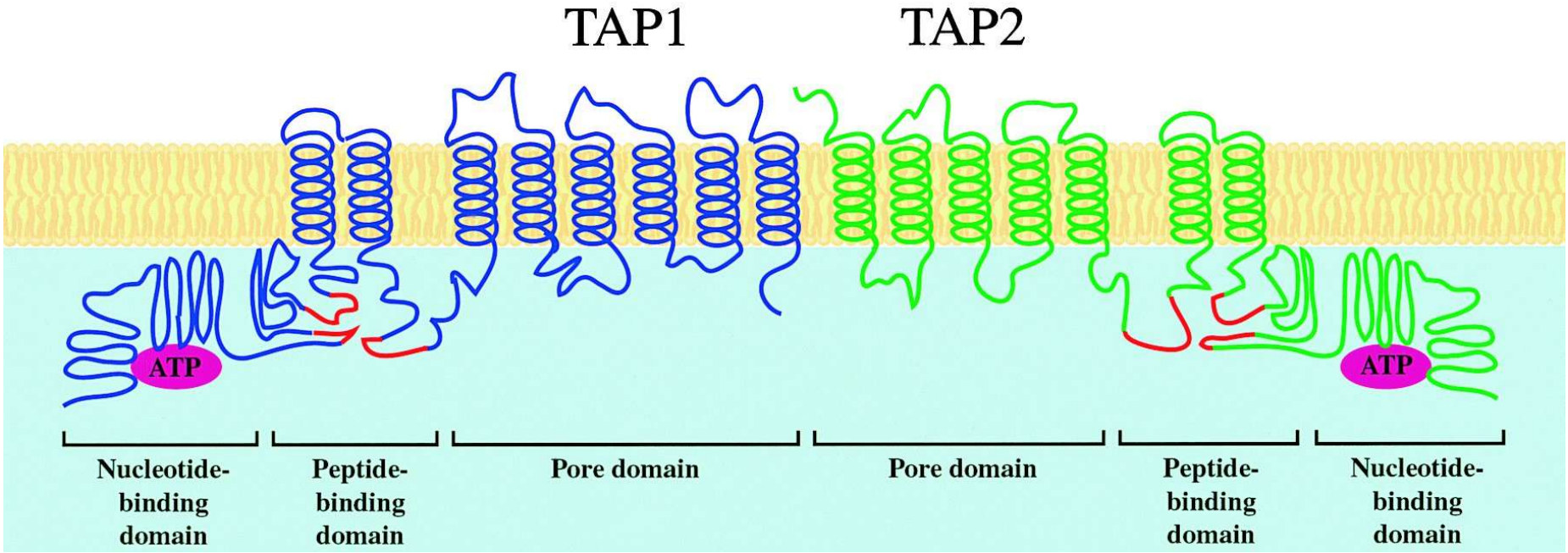


La via di processazione e presentazione dell'antigene di classe I



I TRASPORTATORI DEI PEPTIDI, TAP

(Transporter associated with Antigen Processing) della famiglia dei trasportatori ABC (ATP-Binding Cassette).



- legano e trasportano peptidi di circa 8-13 AA
- peptidi con residui basici o idrofobici al C terminale sono trasportati più efficientemente, e sono anche quelli che legano meglio MHC-I

MHC class I molecules do not leave the endoplasmatic reticulum unless they bind peptides

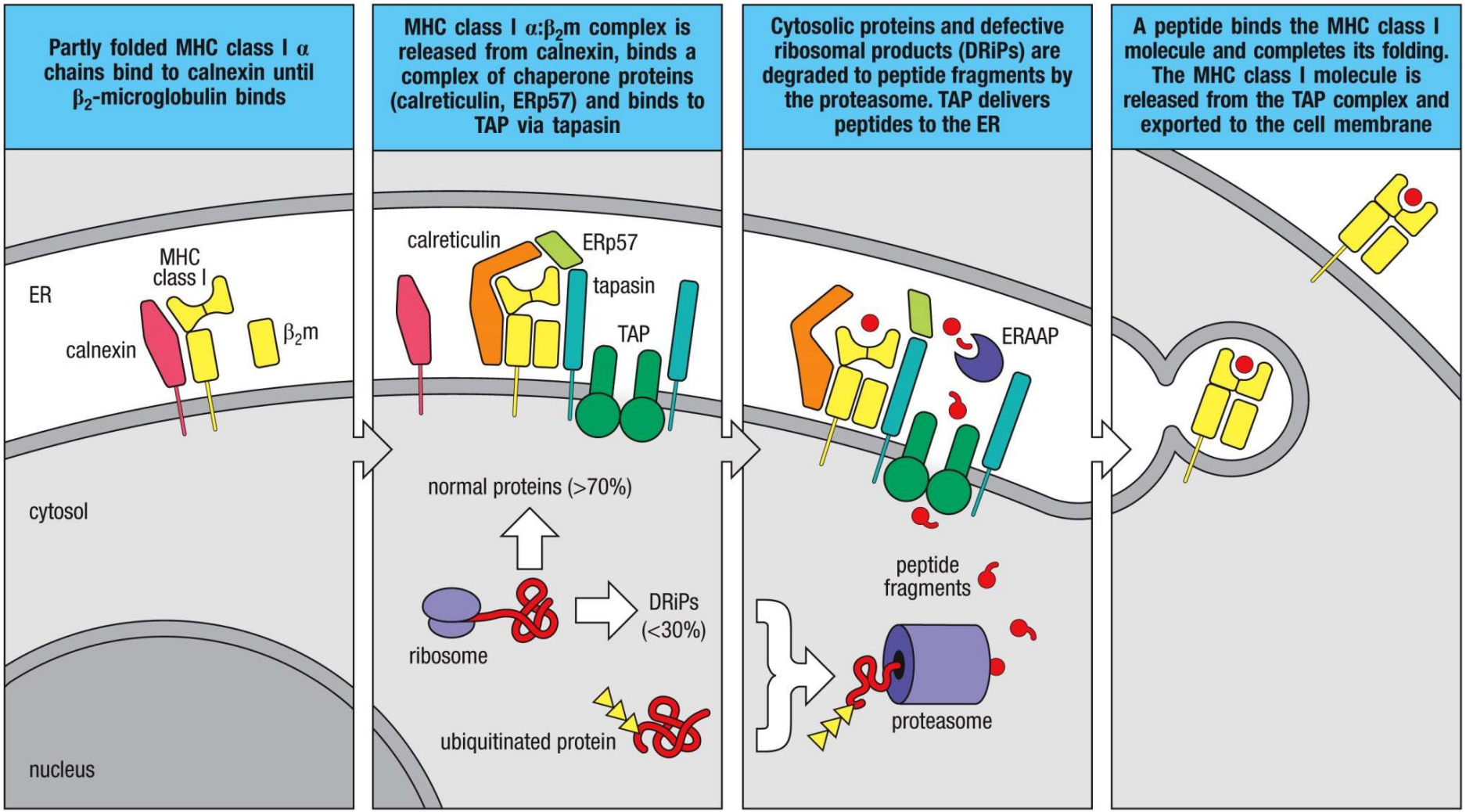
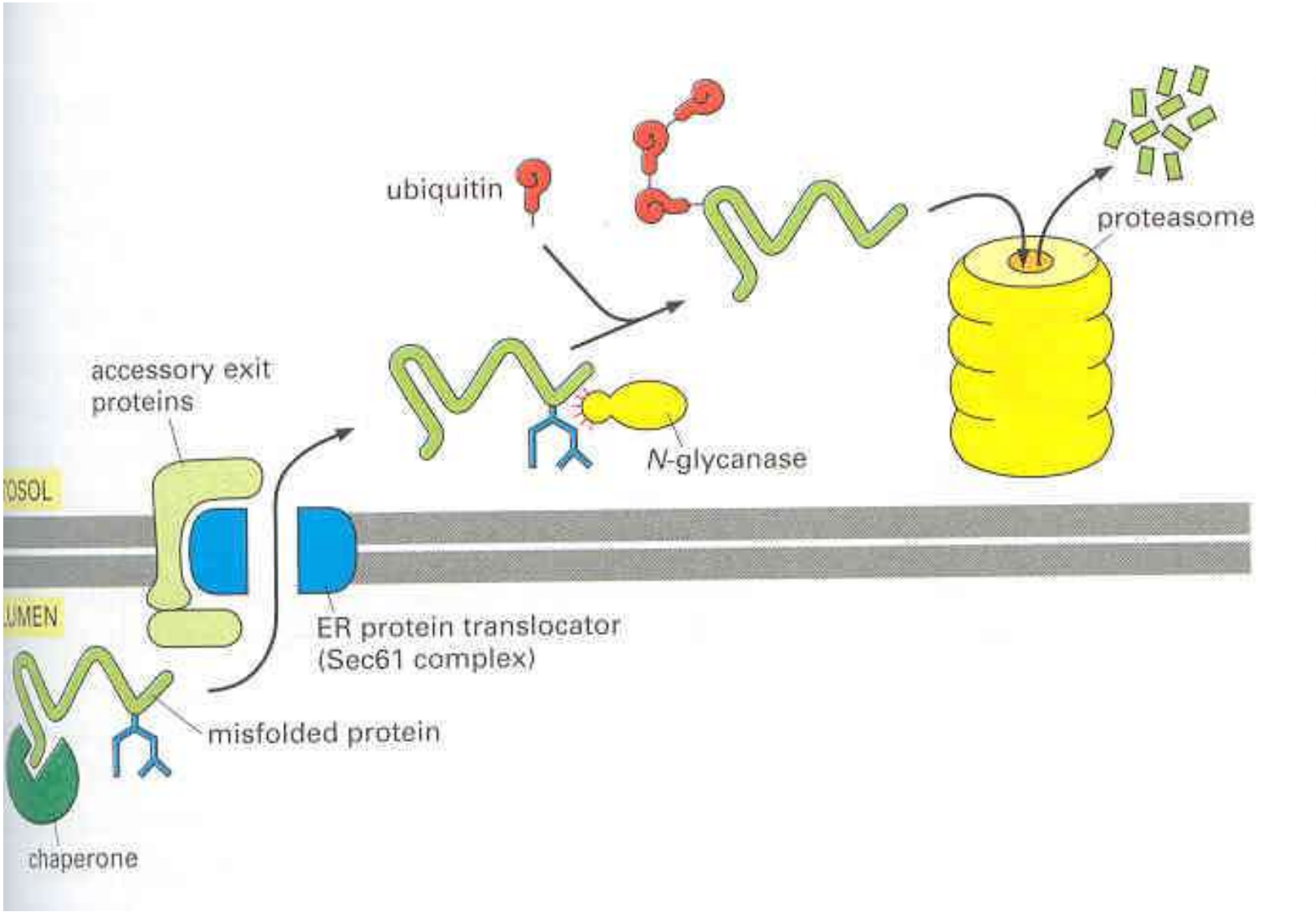


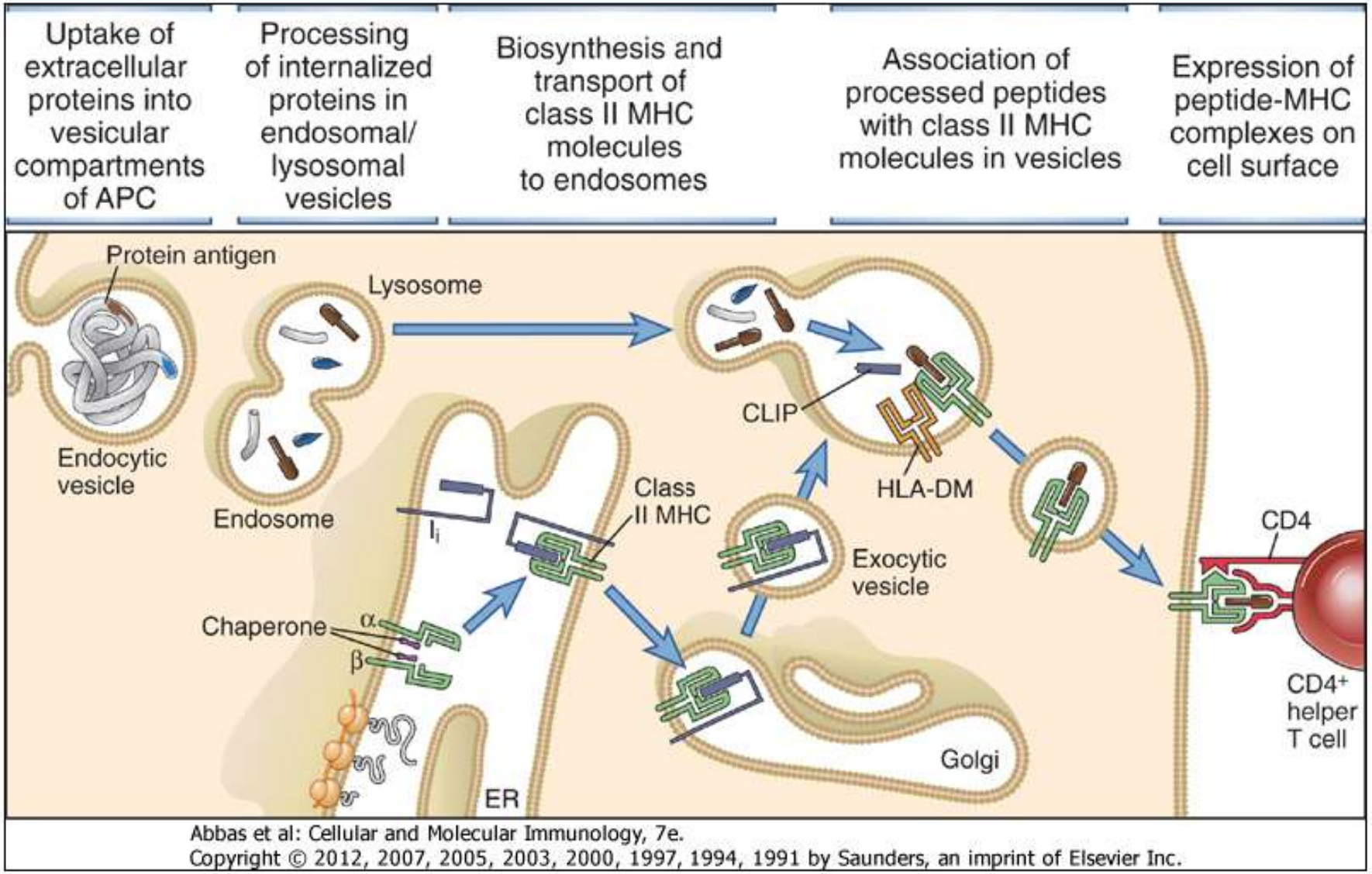
Figure 6.8 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Chaperones: calnexine, calreticuline.

La digestione di proteine ubiquitinate nei proteasomi avviene routinariamente

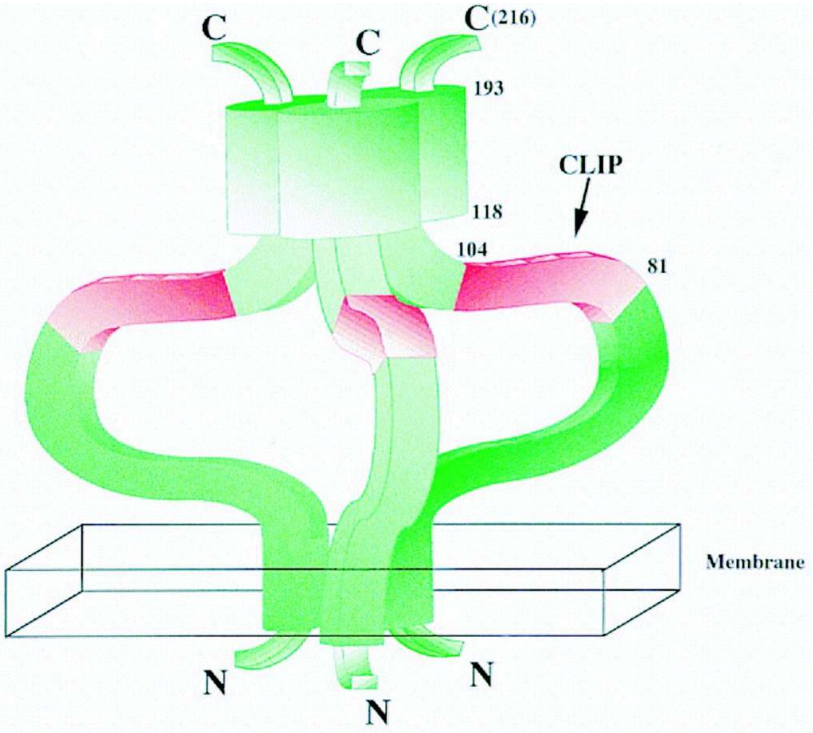


La via di processazione e presentazione dell'antigene di classe II

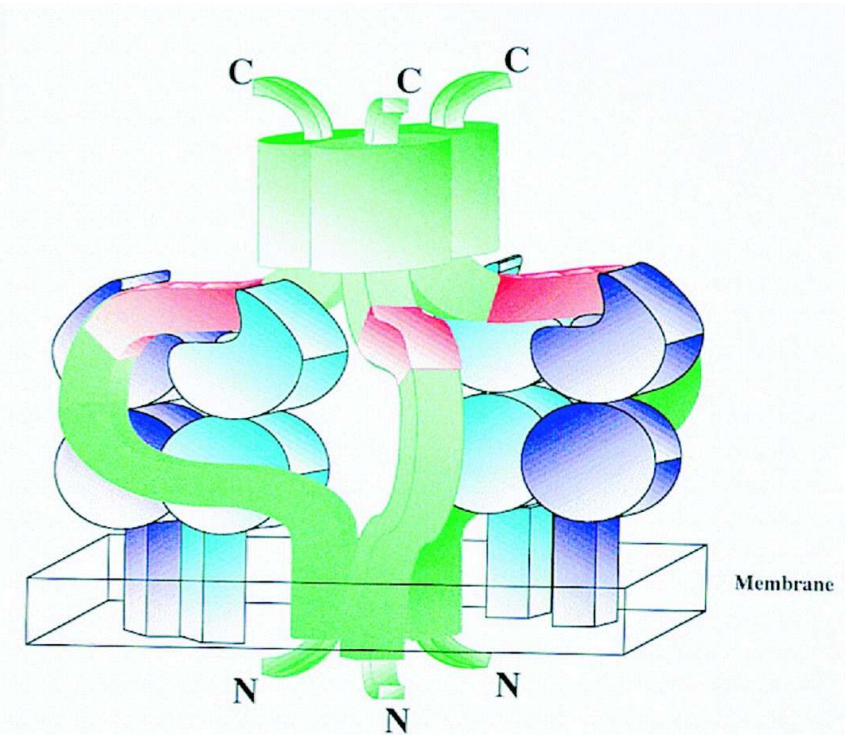


INVARIANT CHAIN (Ii)

trimero Ii



trimero Ii, α E β MHC



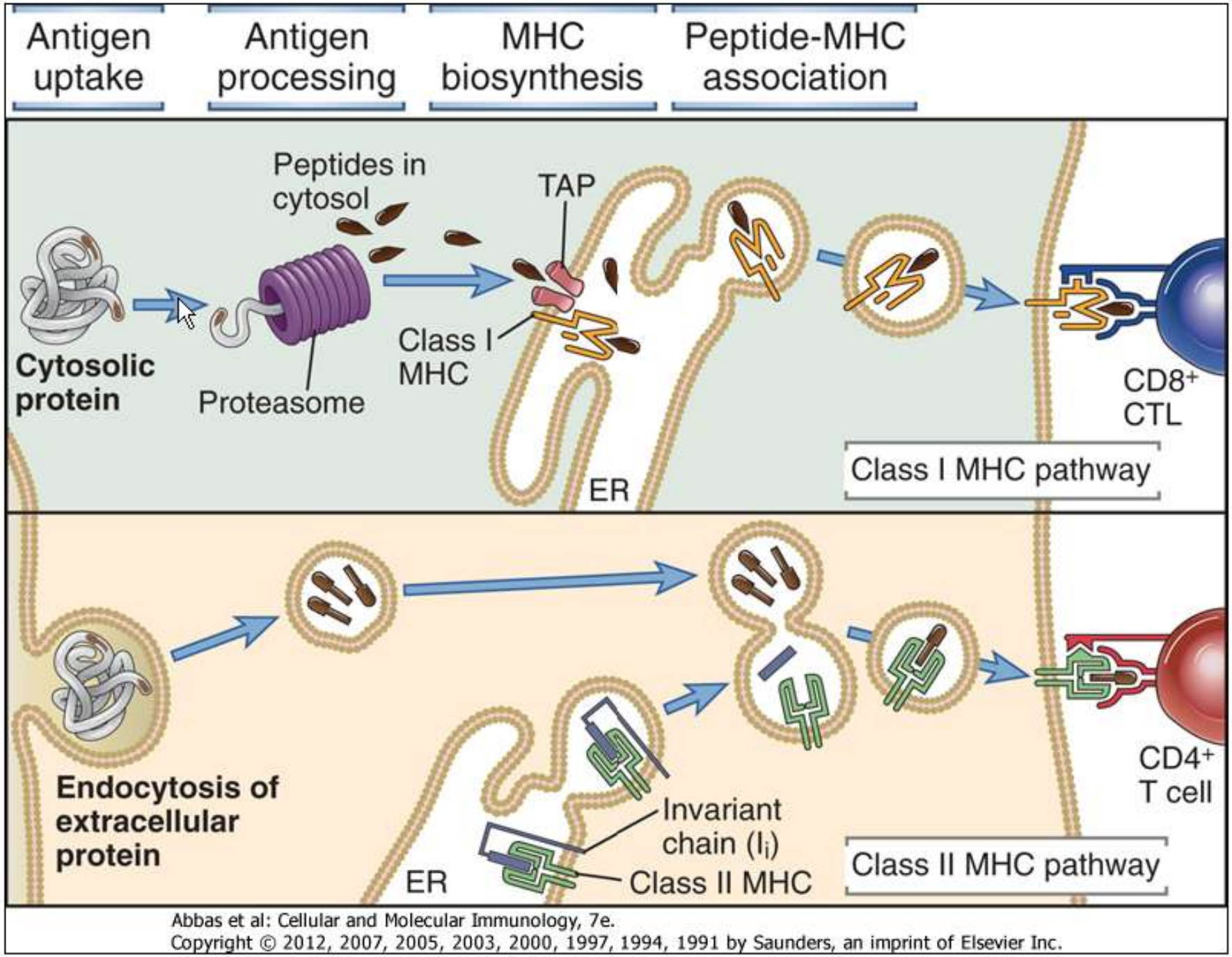
❑Funzioni di Ii:

- ❑ guida il folding delle molecole MHC
- ❑ impedisce che i peptidi antigenici presenti nel RE si possano legare alla molecola MHC di classe II.
- ❑ fornisce un segnale di trasferimento verso il percorso lisosomiale/endosomiale

❑Ii contiene il segmento CLIP (MHC-CLassII associated Invariant chain Peptide), che protegge la tasca del peptide finchè la molecola non arriva alle vescicole endocitiche.

- ❑ Ii viene clivata per produrre CLIP nel sistema endocitico che si inserisce nel sito legante il peptide

❑HLA-DM catalizza il rilascio del frammento CLIP ed il suo rimpiazzo con il peptide antigenico.



https://nerd.wwnorton.com/nerd/179573/r/goto/cfi/204!/4?control=control-toc

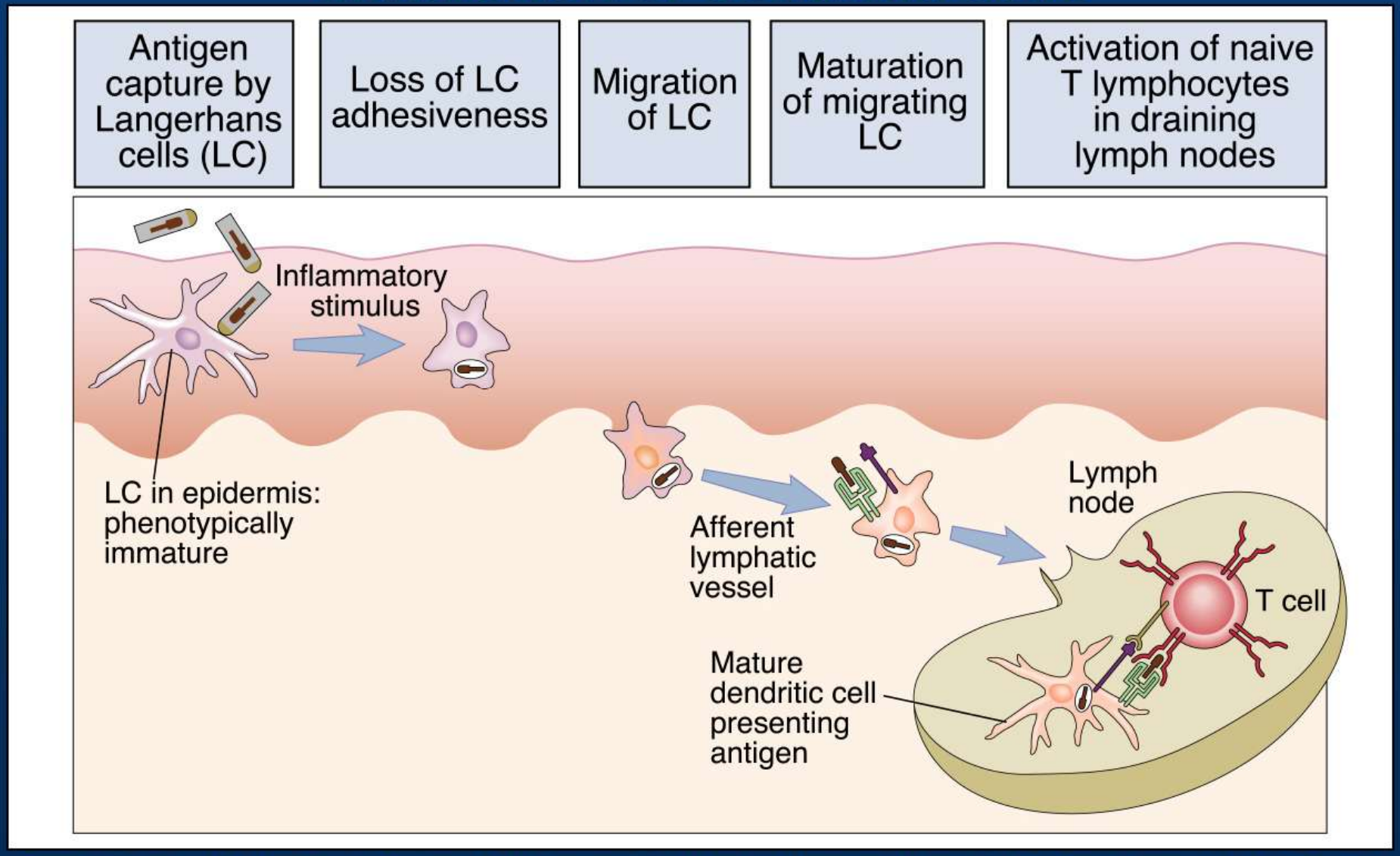
Caratteristiche degli antigeni riconosciuti dai linfociti T

- La maggior parte dei linfociti T riconosce solo PEPTIDI.
- I linfociti T sono specifici per la sequenza aminoacidica del peptide.
- I linfociti T riconoscono e rispondono agli antigeni estranei solo quando i peptidi da essi derivati sono presentati sulla superficie delle APC.
- In ogni individuo i linfociti T riconoscono gli antigeni estranei solo quando i peptidi da essi derivati sono legati a molecole MHC dell'individuo stesso

→ RESTRIZIONE PER MHC SELF!

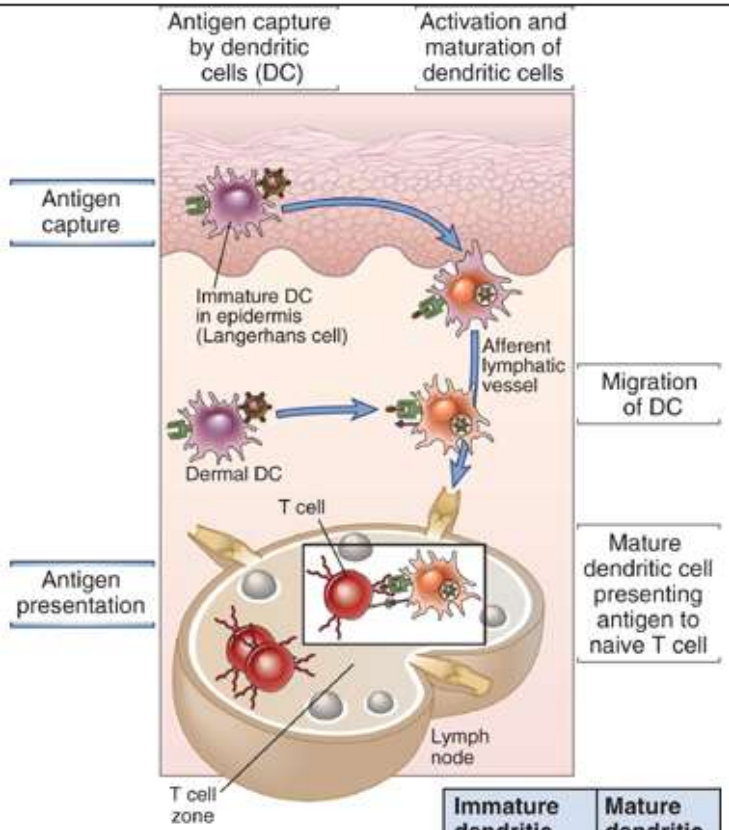
- I linfociti T CD4+ riconoscono peptidi legati a molecole MHC di classe II, mentre i CTL CD8+ riconoscono peptidi legati a molecole MHC di classe I.
- I linfociti T CD4+ ristretti per classe II riconoscono peptidi derivati principalmente da proteine extracellulari internalizzate nelle vescicole endocitiche delle APC, mentre i linfociti T CD8+ ristretti per classe I riconoscono peptidi derivati da proteine citosoliche, solitamente sintetizzate nelle cellule stesse.

Antigen capture, migration, and maturation of dendritic cells



From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 5-4

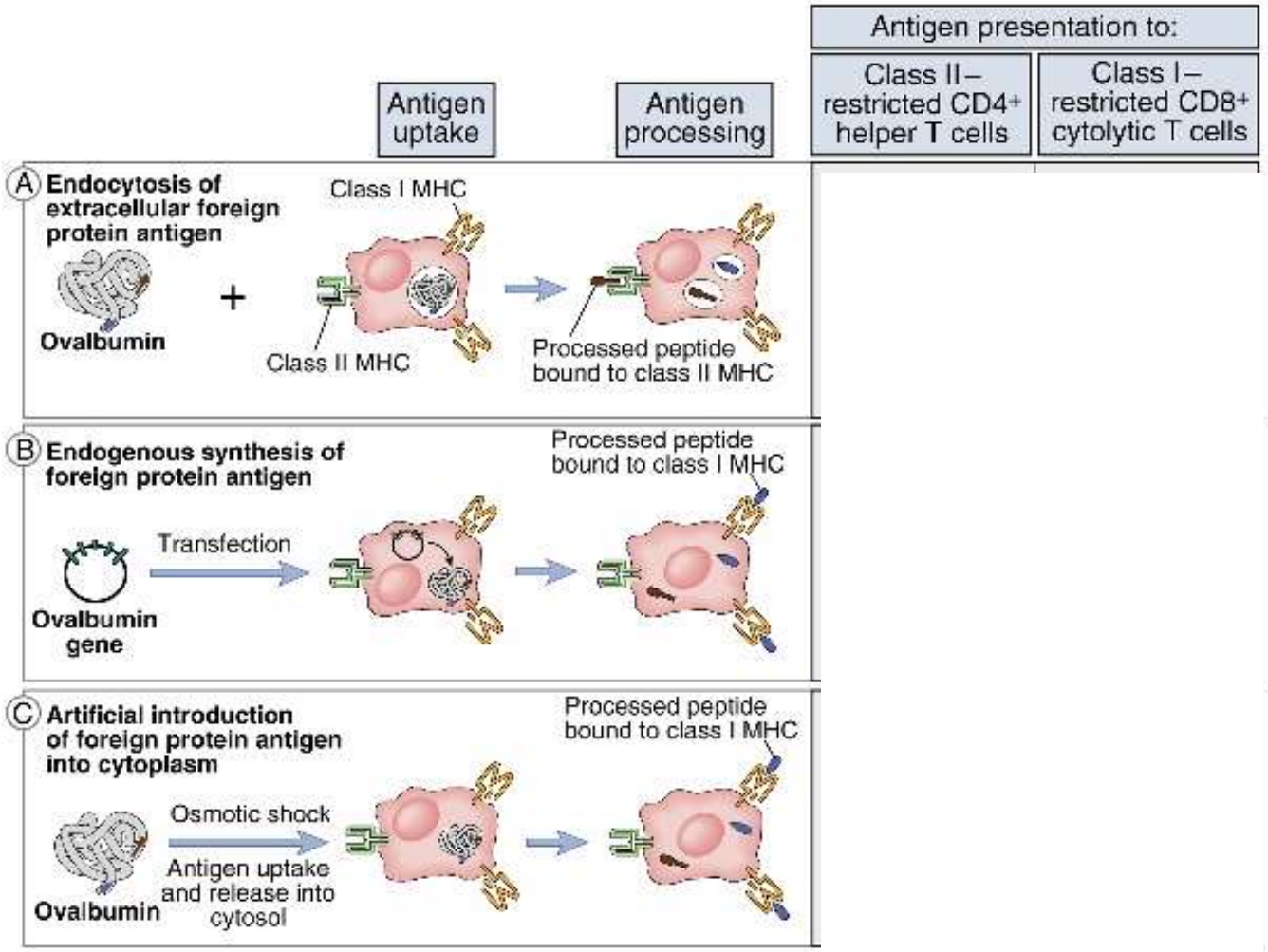
Ruolo delle cellule dendritiche nella cattura e nella presentazione dell'antigene



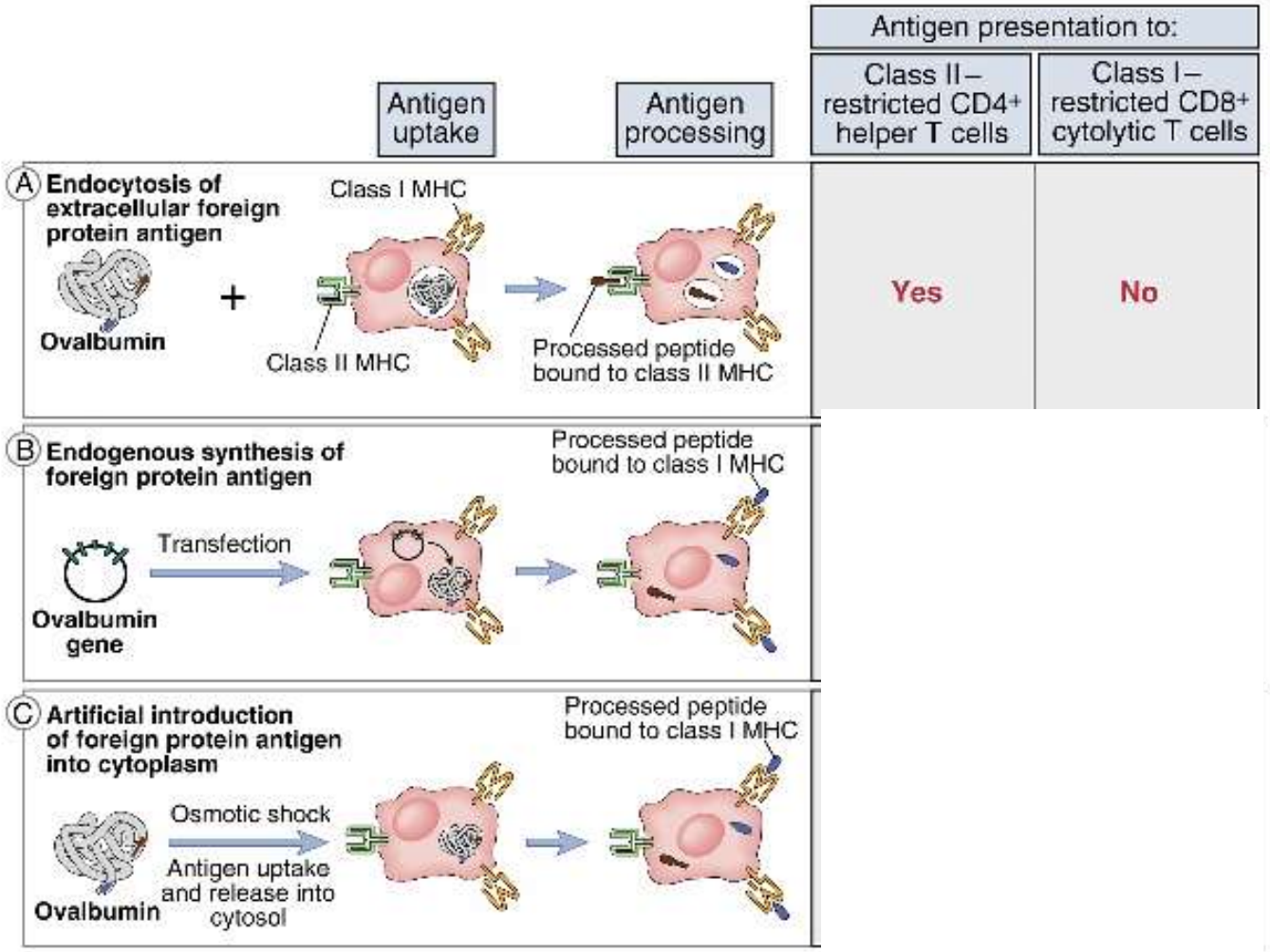
	Immature dendritic cell	Mature dendritic cell
Principal function	Antigen capture	Antigen presentation to T cells
Expression of Fc receptors, mannose receptors	++	–
Expression of molecules involved in T cell activation: B7, ICAM-1, IL-12	– or low	++
Class II MHC molecules		
Half-life	~10 hr	>100 hr
Number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

Table 5–3. Properties and Functions of Antigen-Presenting Cells

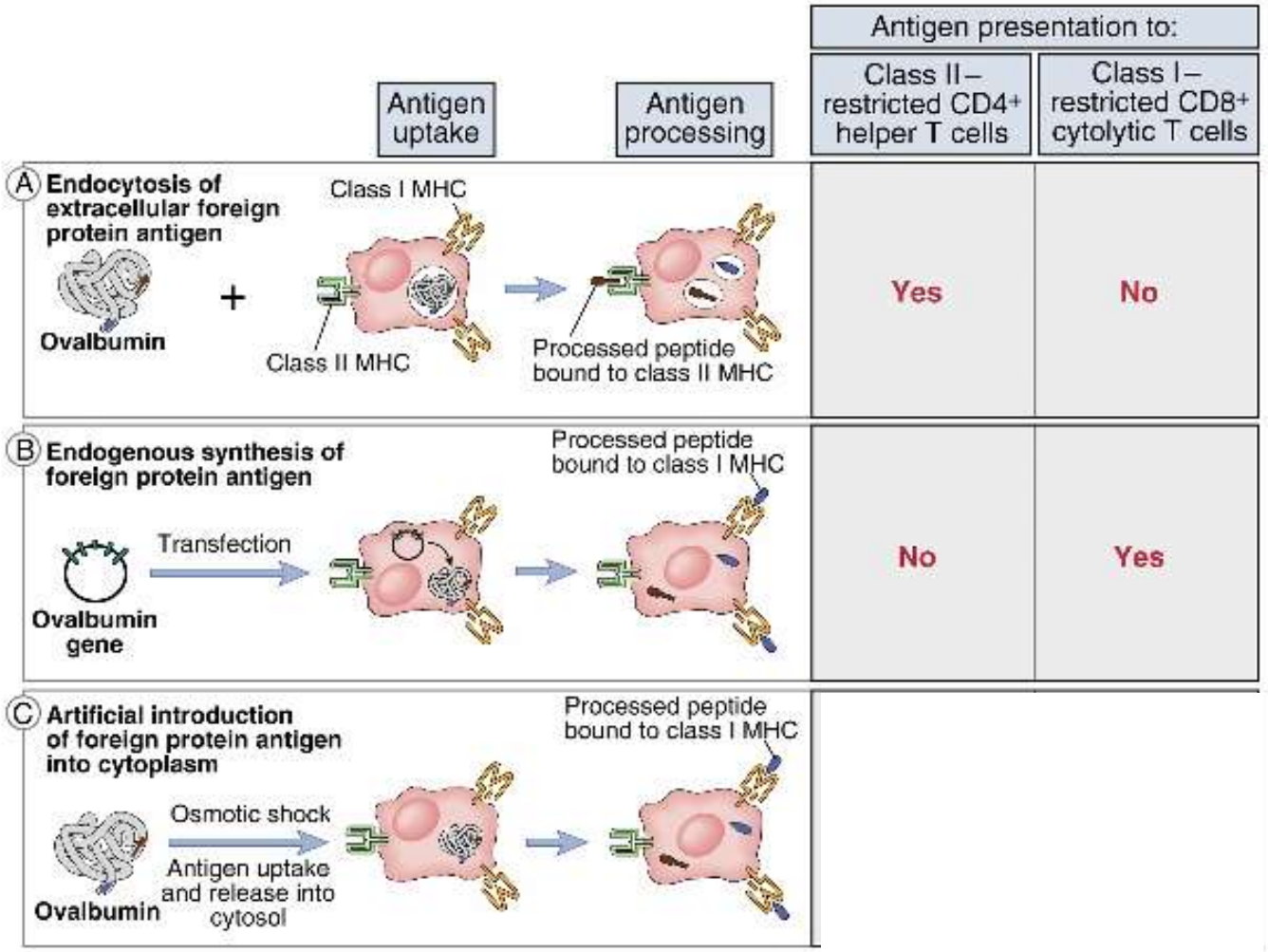
Cell type	Expression of		Principal function
	Class II MHC	Costimulators	
Dendritic cells	Constitutive; increases with maturation; increased by IFN-γ	Constitutive; increases with maturation; inducible by IFN-γ; CD40-CD40L interactions	Initiation of T cell responses to protein antigens (priming)
Macrophages	Low or negative; inducible by IFN-γ	Inducible by LPS, IFN-γ; CD40-CD40L interactions	Effector phase of cell-mediated immune responses
B lymphocytes	Constitutive; increased by IL-4	Induced by T cells (CD40-CD40L interactions), antigen receptor cross-linking	Antigen presentation to $CD4^+$ helper T cells in humoral immune responses (cognate T cell–B cell interactions)
Vascular endothelial cells	Inducible by IFN-γ; constitutive in humans	Constitutive (inducible in mice)	May promote activation of antigen-specific T cells at site of antigen exposure
Various epithelial and mesenchymal cells	Inducible by IFN-γ	Probably none	No known physiologic function



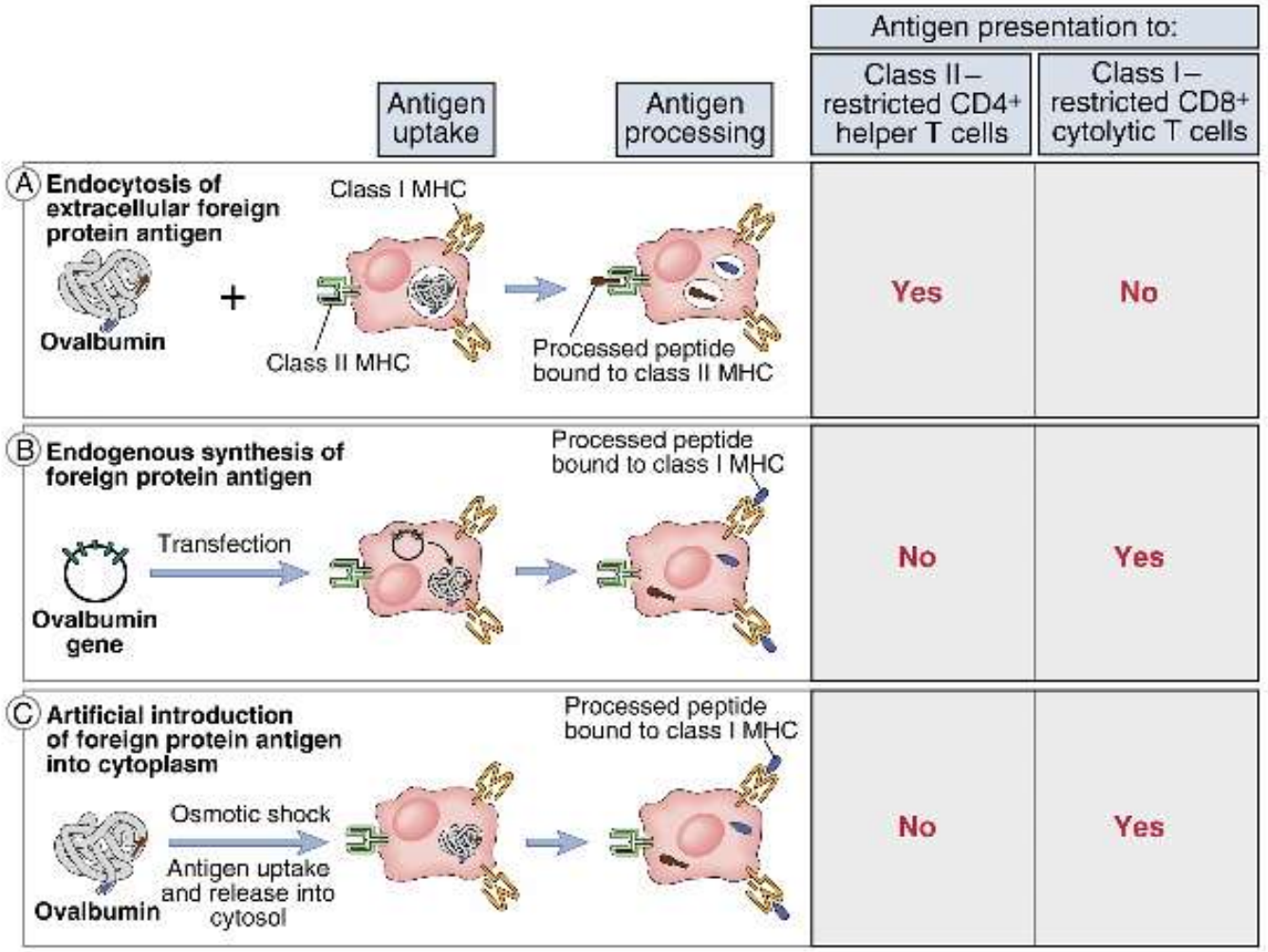
Presentazione di antigeni extracellulari (esogeni) e di citosol (endogeni). When a model protein ovalbumin is added as an extracellular antigen to an antigen-presenting cell that expresses both class I and class II MHC molecules, ovalbumin-derived peptides are presented only in association with class II molecules (A). When ovalbumin is synthesized intracellularly as a result of transfection of its gene (B), or when it is introduced into the cytoplasm through membranes made leaky by osmotic shock (C), ovalbumin-derived peptides are presented in association with class I MHC molecules. The measured response of class II-restricted helper T cells is cytokine secretion, and the measured response of class I-restricted CTLs is killing of the antigen-presenting cells



Presentazione di antigeni extracellulari (esogeni) e di citosol (endogeni). When a model protein ovalbumin is added as an extracellular antigen to an antigen-presenting cell that expresses both class I and class II MHC molecules, ovalbumin-derived peptides are presented only in association with class II molecules (A). When ovalbumin is synthesized intracellularly as a result of transfection of its gene (B), or when it is introduced into the cytoplasm through membranes made leaky by osmotic shock (C), ovalbumin-derived peptides are presented in association with class I MHC molecules. The measured response of class II-restricted helper T cells is cytokine secretion, and the measured response of class I-restricted CTLs is killing of the antigen-presenting cells

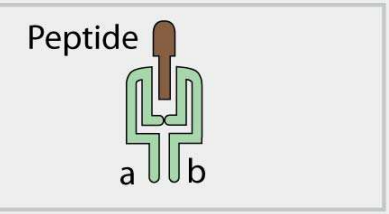
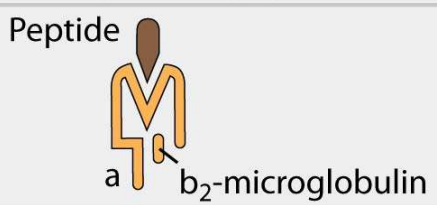


Presentazione di antigeni extracellulari (esogeni) e di citosol (endogeni).



Presentazione di antigeni extracellulari (esogeni) e di citosol (endogeni).

Comparative features of class I and class II MHC pathways of antigen processing and presentation

Feature	Class II MHC Pathway	Class I MHC pathway
Composition of stable peptide-MHC complex	Polymorphic a and b chains, peptide 	Polymorphic a chain, b_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