Imperial College London

Antigen processing and presentation of foreign antigens on MHC class II

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We will discuss about...

- Antigens
- Antigen processing: uptake, degradation, complex formation and presentation
- Antigen-presenting cells
- The role of MHC class II in immune response
- The structure of MHC class II
- MHC class II proteins and disease

Learning outcomes

By the end of this lecture you should know that:

- **T and B cells recognise antigen differently**
- **The antigen processing requires metabolic activity**
- **The antigen processing generates antigenic peptides**
- **The antigen processing pathways involves: uptake, degradation, complex formation and presentation**

You should also be able to:

- **Define the role of the class II MHC molecule, invariant chain peptide CLIP and HLA-DM molecule in antigen processing**
- **Describe the structural features of MHC class II molecules**
- **Give examples of immune disease associated with aberrant function of class II MHC molecule.**

Definition of antigen

An **ANTIGEN** is a molecule capable of inducing a specific immune response. The term literally means molecules that **gen**erate **anti**bodies.

It is important to recognize that bacteria or viruses are not themselves antigens but they contain antigens both on their surface and inside them. Such antigens can be isolated and used to safely vaccinate against infection with the whole organism.

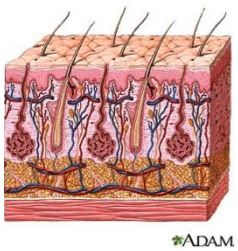
Antigens are not restricted to infectious agents. Tumors often contain modified proteins or proteins not normally expressed which are seen by the immune system as antigens. In autoimmune disease the immune system recognizes normal molecules on the surface of cells as being antigens and these cells are attacked.

Proteins or glycoproteins make the best antigens because they are the best at stimulating antigen-recognition molecules.

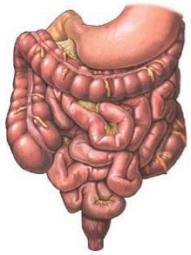
Each part of the antigen that is recognized by either an antibody or a T cell receptor is known as an **epitope**.

[From: '*Immunology for 1st Year Medical Students*' (2009) by Timothy Lee and Andrew Issekutz]

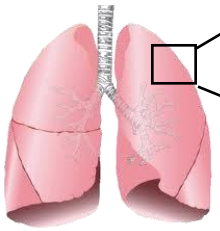
Routes of antigen entry



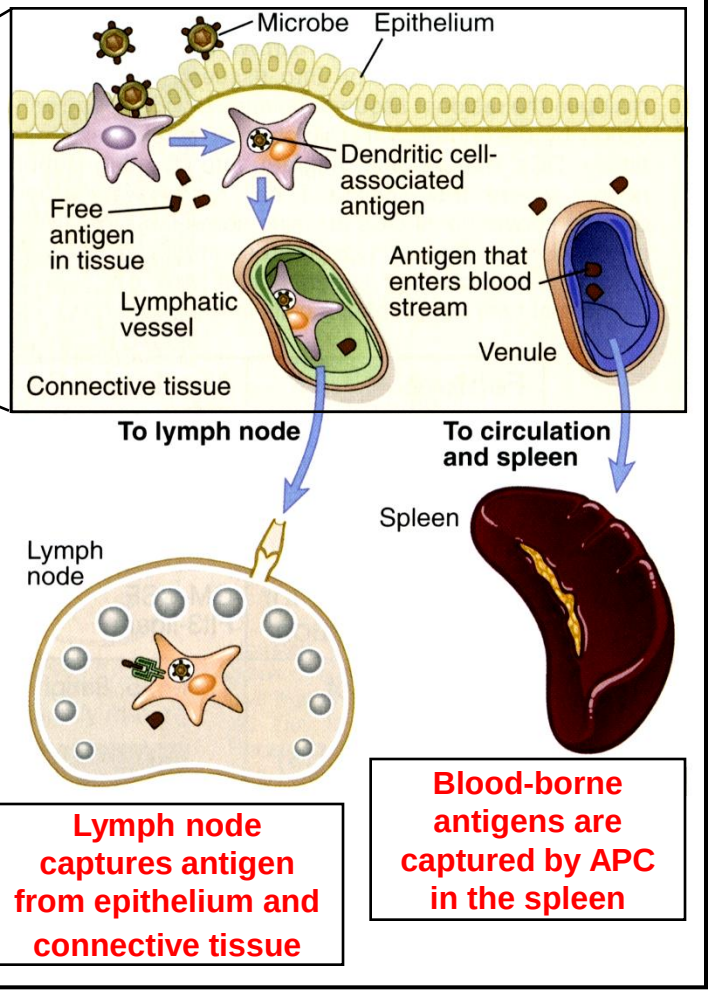
Skin



Gastrointestinal tract

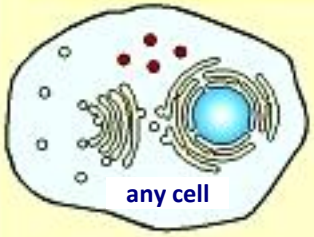
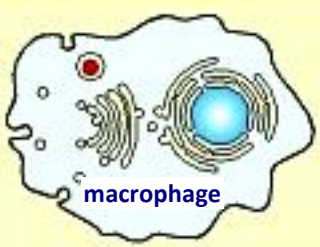
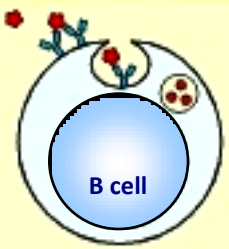


Respiratory tract



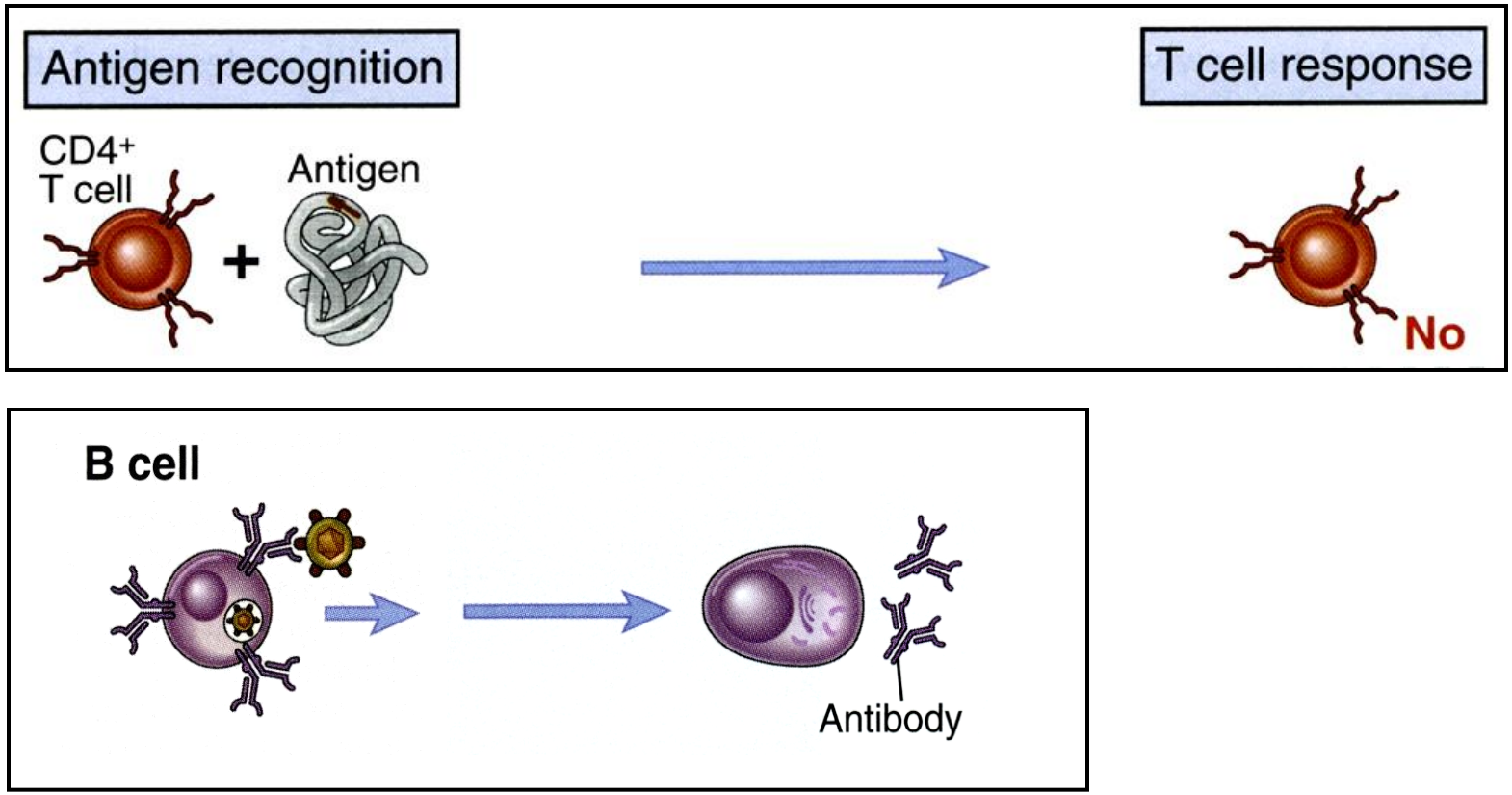
Modified from: *Cellular and Molecular Immunology*; Authors: Abbas AK, Lichtman AH, and Pillai S (Saunders Elsevier).

Pathogens and their products can be found in either the cytoplasm or the vesicular compartments of cells

	Cytosolic pathogens	Intravesicular pathogens	Extracellular pathogens
			
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	Cell death	Activation to kill intravesicular bacteria and parasites	B cells secrete Ig to eliminate extracellular bacteria/toxins

Modified from *Immunobiology;* Authors: Janeway CA, Travers P (Garland Publishing Inc.)

T and B cells recognize antigens differently



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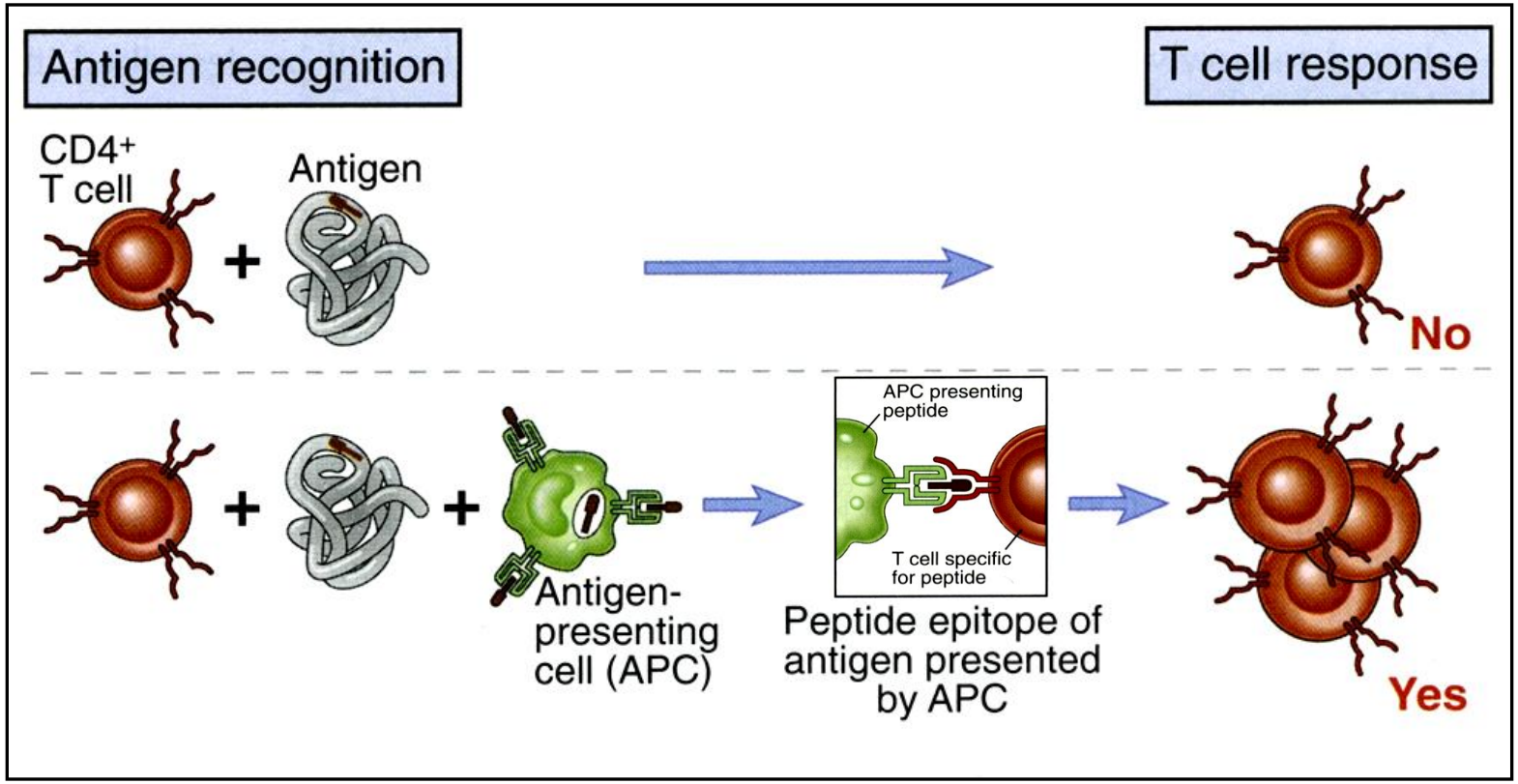
T cell response requires antigen processing and presentation



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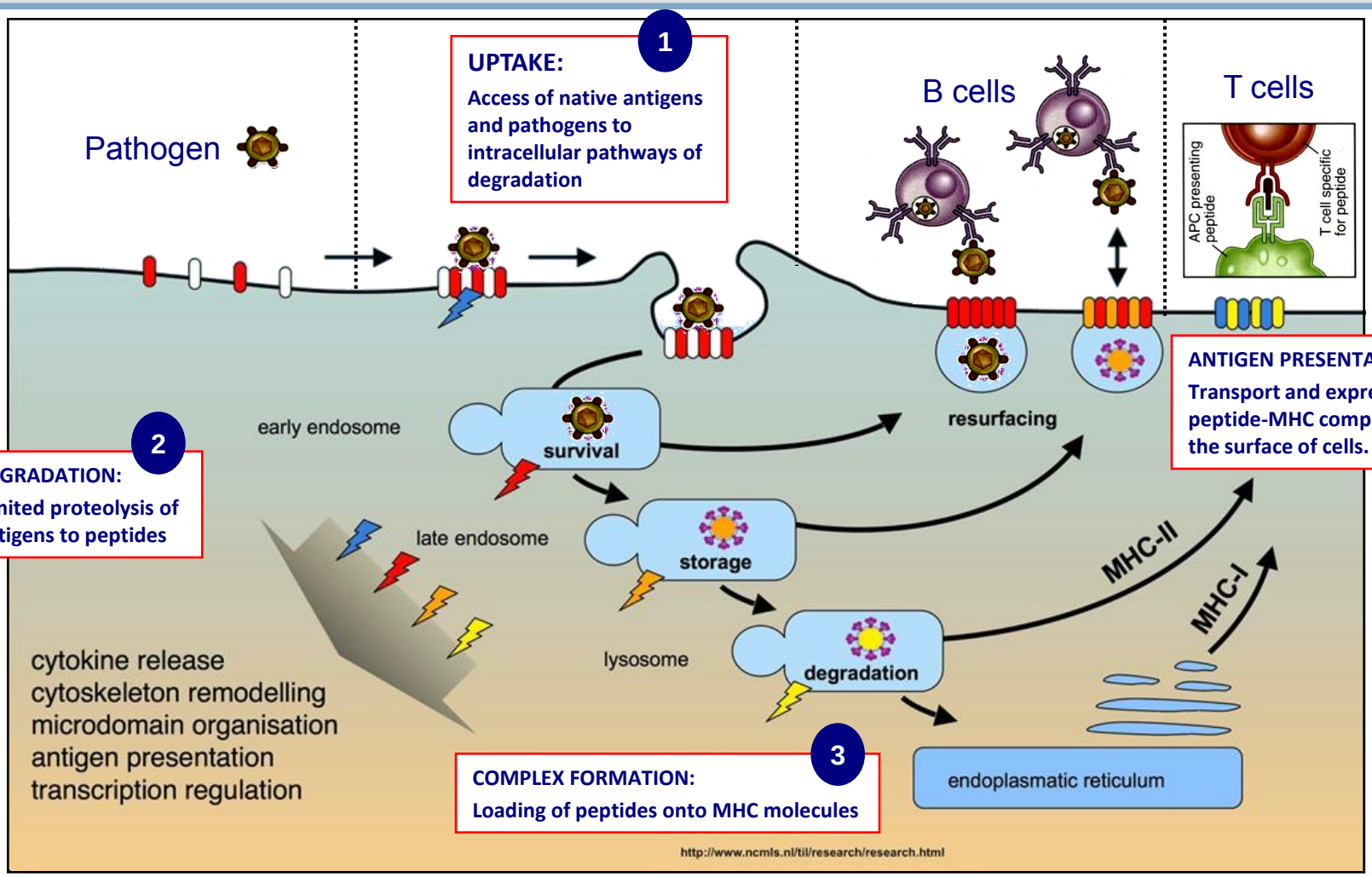
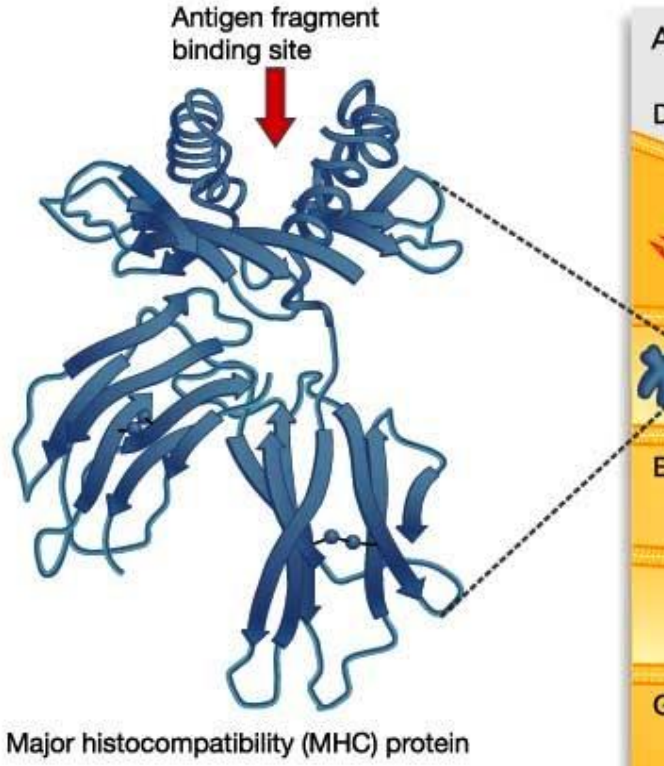
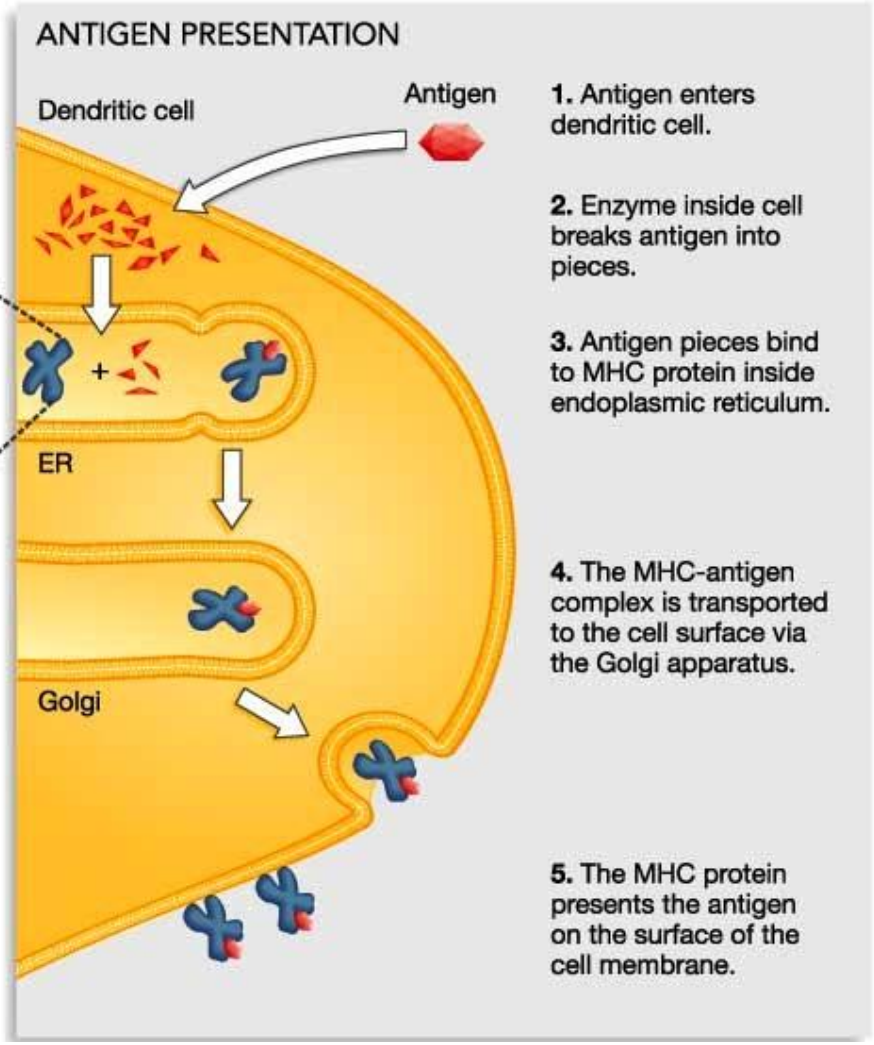
Pathways of antigen processing: overview



The pathways of antigen processing convert protein antigens derived from extracellular space or the cytosol into antigenic peptides, and load these peptides onto MHC molecules for display to T lymphocytes.

Antigen processing and presentation





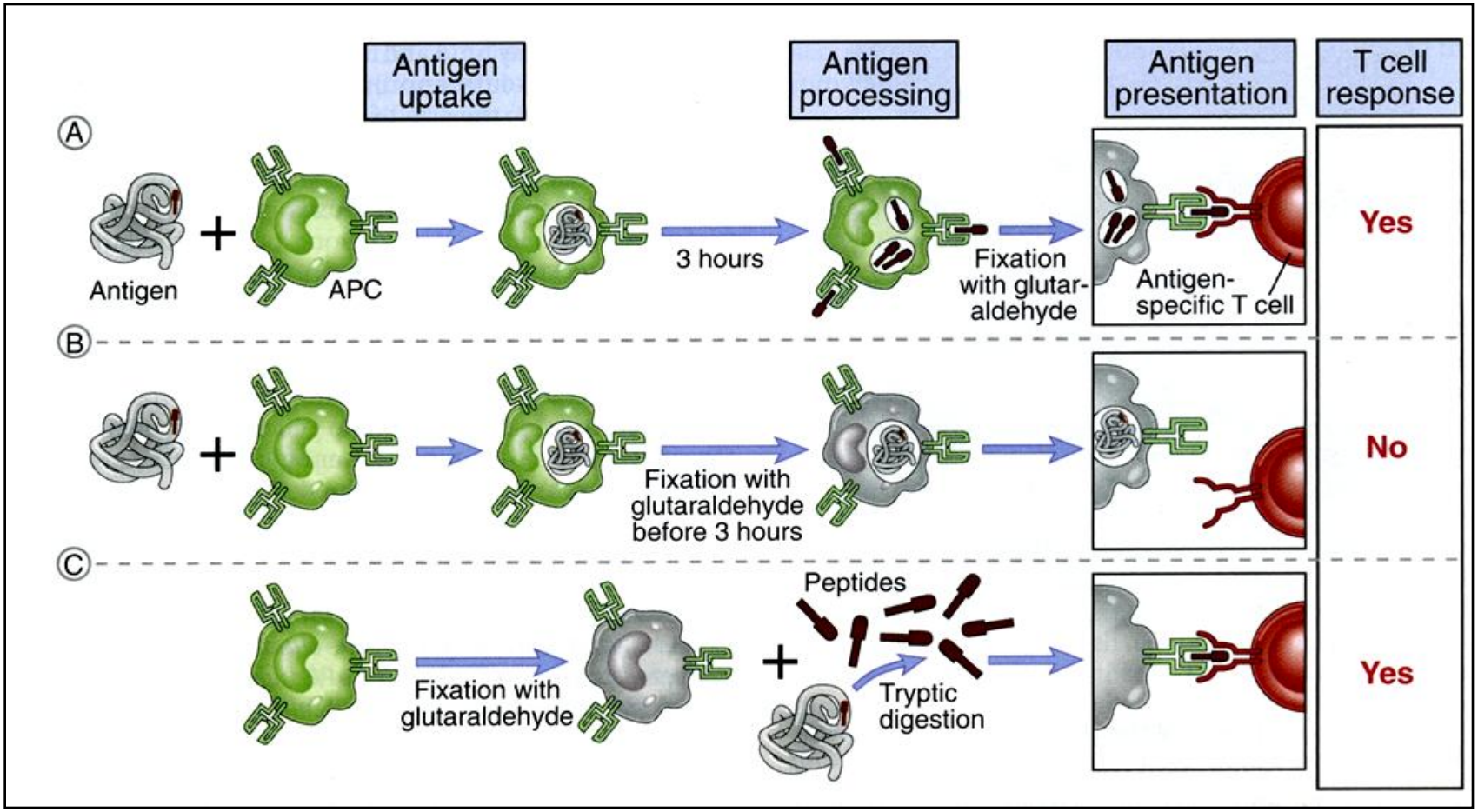
Many different enzymes promote the degradation of protein antigens in the endosomes. The most abundant preoteases are CATHEPSINS, which are thiol and aspartyl proteases with broad substrate specificities

Endosomic digestion: role of Cathepsins

- Cathepsin proteins are a class of globular lysosomal proteases, most of which contain an active-site cysteine residue and a histidine residue.
- The cystein residue acts as a nucleophile; the histidine residue acts as a general base in the hydrolysis of a target peptide bond in the substrate
- The cathepsins have broad specificity albeit some prefer certain amino acids over others in the target sequence.
- Additionally, while most are endopeptidases (cleaving peptide bonds within the target sequence), a few are carboxy or aminopeptidases (cleaving the peptide bond at only the carboxy- or amino-terminal residue).
- Cathepsin S is distinguished from other cysteine proteinases by its limited tissue distribution and better conformational stability at neutral and slightly basic pH.
- While most members of the cathepsin family are expressed in a large variety of tissues and organs, cathepsin S is present predominately in the spleen, lymph nodes, monocytes, macrophages and few other APCs. This unique distribution pattern indicates that cathepsin S is likely to be deeply involved in the immune response.

Villadangos JA, Ploegh HL. (2000). Proteolysis in MHC class II antigen presentation: who's in charge? *Immunity* 12: 233-239.

Metabolically active cells can process antigens



Modified from: *Cellular and Molecular Immunology*; Authors: Abbas AK, Lichtman AH, and Pillai S (Saunders Elsevier)

Note: Antigen presenting cells MUST be viable to process antigen; and DO NOT need to be viable to express MHC molecules and to present antigen.

Antigen-presenting cells (APC)

Cell type	Expression of		Principal function
	Class II MHC	Costimulators	
Dendritic cells	Constitutive; increases with maturation; increased by INF-γ	Constitutive; increases with maturation; inducible by INF-γ, CD40-CD40L interactions	Initiation of T cell responses to protein antigens (priming)
Macrophages	Low or negative; inducibile by INF-γ	Inducible by LPS, INF-γ, CD40-CD40L interactions	Effector phase of cell-mediated immune responses
B lymphocytes	Constitutive; increased by IL-4	Inducible 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	Inducibile by INF-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	Inducibile by INF-γ	Probably none	No known physiologic function

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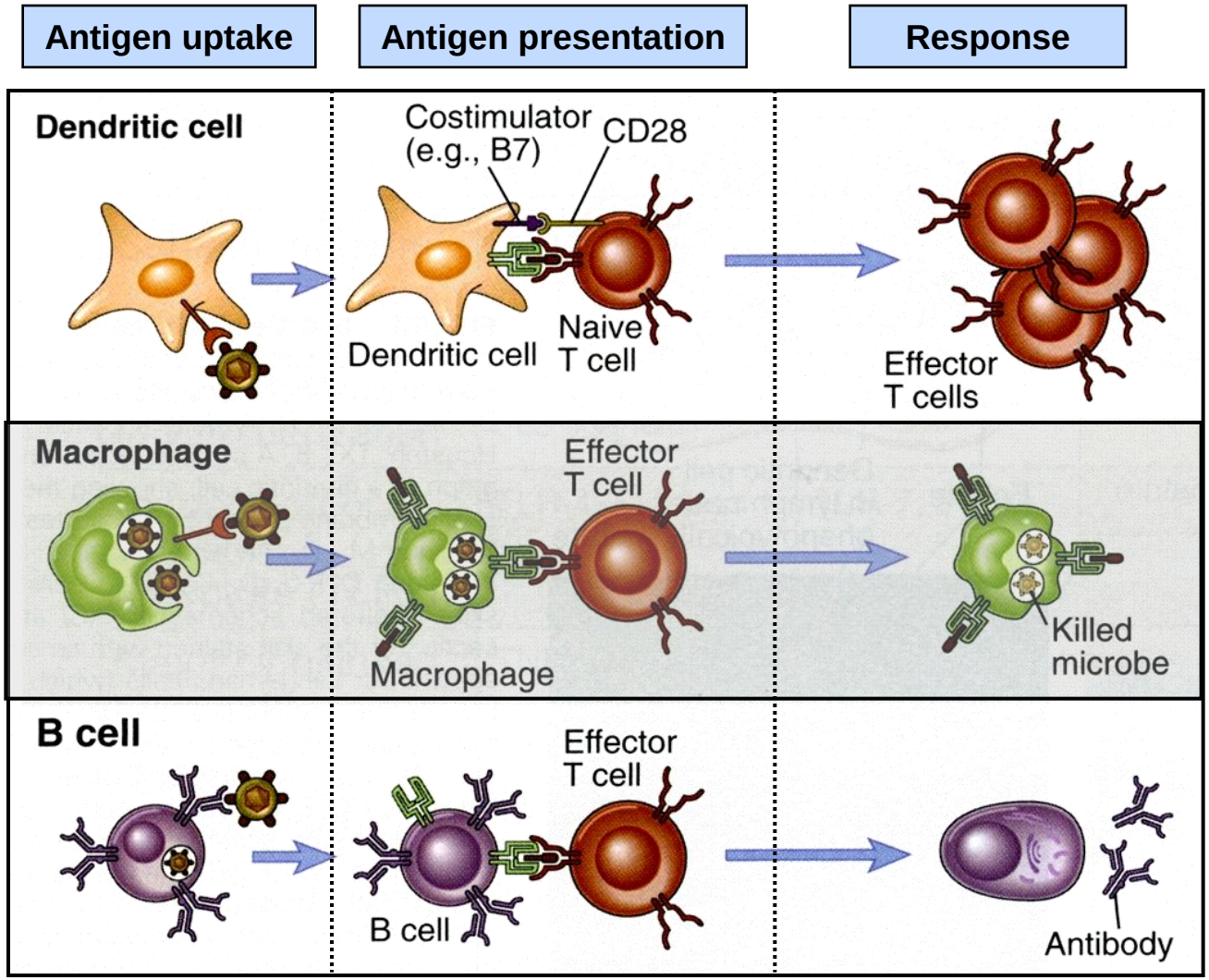
Function of different APC



Naïve T cell activation: clonal expansion and differentiation into effector T cells

Effector T cell activation: activation of macrophages (cell-mediated immunity)

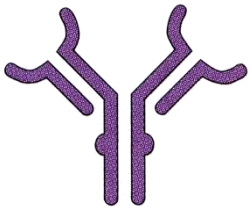
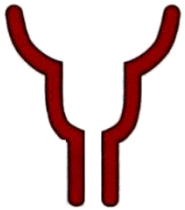
Effector T cell activation: B cell activation and antibody production (humoral immunity)

Modified from: *Cellular and Molecular Immunology*; Authors: Abbas AK, Lichtman AH, and Pillai S (Saunders Elsevier)

Summary : Part 1

- **Antigens are captured, internalized and processed by specialized cells, named APCs (i.e., dendritic cells, macrophages and B lymphocytes).**
- **T cells recognize only processed antigens.**
- **Antigen processing is the conversion of native proteins into peptides, which then associate with dedicated molecules named MHC.**
- **Only metabolic active cells can process antigens.**
- **Antigen presentation does not require metabolically-active cells.**
- **Extra-cellular proteins are internalized into endosomes where they are cleaved by specific enzymes and binds to newly synthesized class II MHC molecules, using specific mechanisms of loading** (discussed later).

Antigen binding molecules

	Immunoglobulin (Ig)	T cell receptor (TCR)	MHC molecules (class I and II)
			
Antigen binding site	Made up of three CDRs in V_H and three CDRs in V_L	Made up of three CDRs in V_α and three CDRs in V_β	Peptide-binding cleft made of α1 and α2 (class I) or α1 and β1 (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

Modified from: *Cellular and Molecular Immunology*; Authors: Abbas AK, Lichtman AH, and Pillai S (Saunders Elsevier)

MHC molecules: class I, class II & class III

Genes of MHC organized in 3 classes:

- **Class I MHC**
  - » Glycoproteins expressed on all nucleated cells
  - » Major function to present processed antigens to CD8+ cells (CTLs)
- **Class II MHC**
  - » Glycoproteins expressed on MΦ, B-cells, DCs
  - » Major function to present processed antigens to CD4+ cells (T cell effectors)
- **Class III MHC**
  - » Products that include other secreted immune protein, such as complement components (e.g., C2, C4, factor B) and some that encode cytokines (e.g., TNF-α) and other inflammatory proteins (Hsp)

MHC gene clusters

- In human, MHC gene clusters are found on chromosome 6
  - Referred to as **HLA** complex
- In mice, MHC gene clusters are found on chromosome 17
  - Referred to as **H-2** complex
- **MHC Class I genes** named in humans: HLA-A, HLA-B and HLA-C (H-2K and H-2D in mice)
- **MHC Class II genes** named in humans: HLA-DR, HLA-DP and HLA-DQ (H-2IA and H-2IE in mice)

MHC gene organization: summary

Human HLA complex

Complex	HLA								
MHC class	II			III		I			
Region	DP	DQ	DR	C4, C2, BF		B	C	A	
Gene products	DP αβ	DQ αβ	DR αβ	C′ proteins	TNF-α TNF-β	HLA-B	HLA-C	HLA-A	

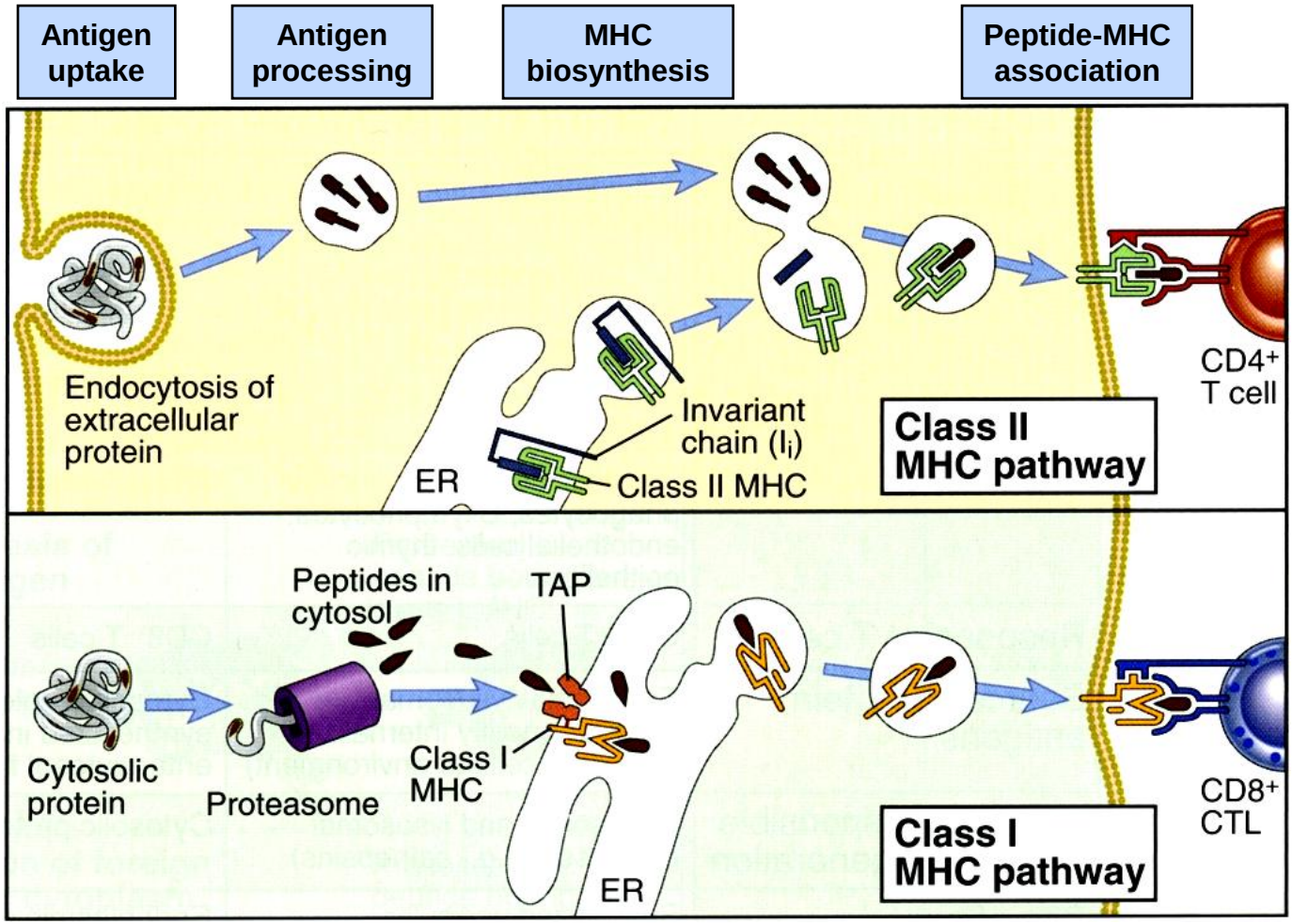
Mouse H-2 complex

Complex	H–2						
MHC class	I	II		III		I	
Region	K	IA	IE	S		D	
Gene products	H–2K	IA αβ	IE αβ	C′ proteins	TNF-α TNF-β	H–2D	H–2L

Role of MHC in antigen presentation

NOTE: Antigens loaded on MHC-II are typically exogenous proteins internalized by the APC or endogenous proteins resident in the endosomal system.

However, antigens presumably excluded from the endosomal system, such as proteins localized in the cytosol or the nucleus, can also be presented by MHC-II molecules



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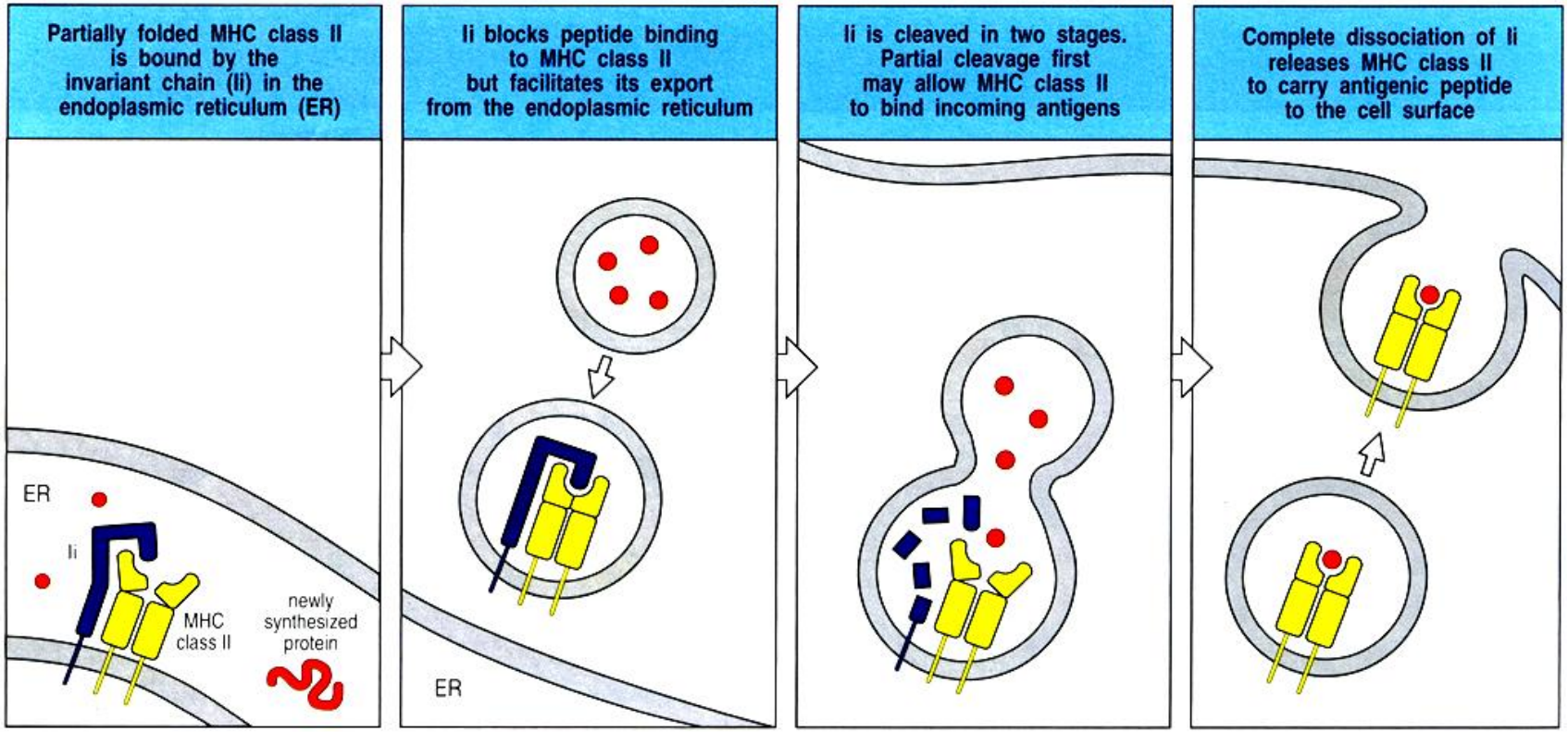
How the peptide loading onto the class II MHC molecule is regulated?

The class II MHC-associated invariant chain (Ii) delays peptide binding

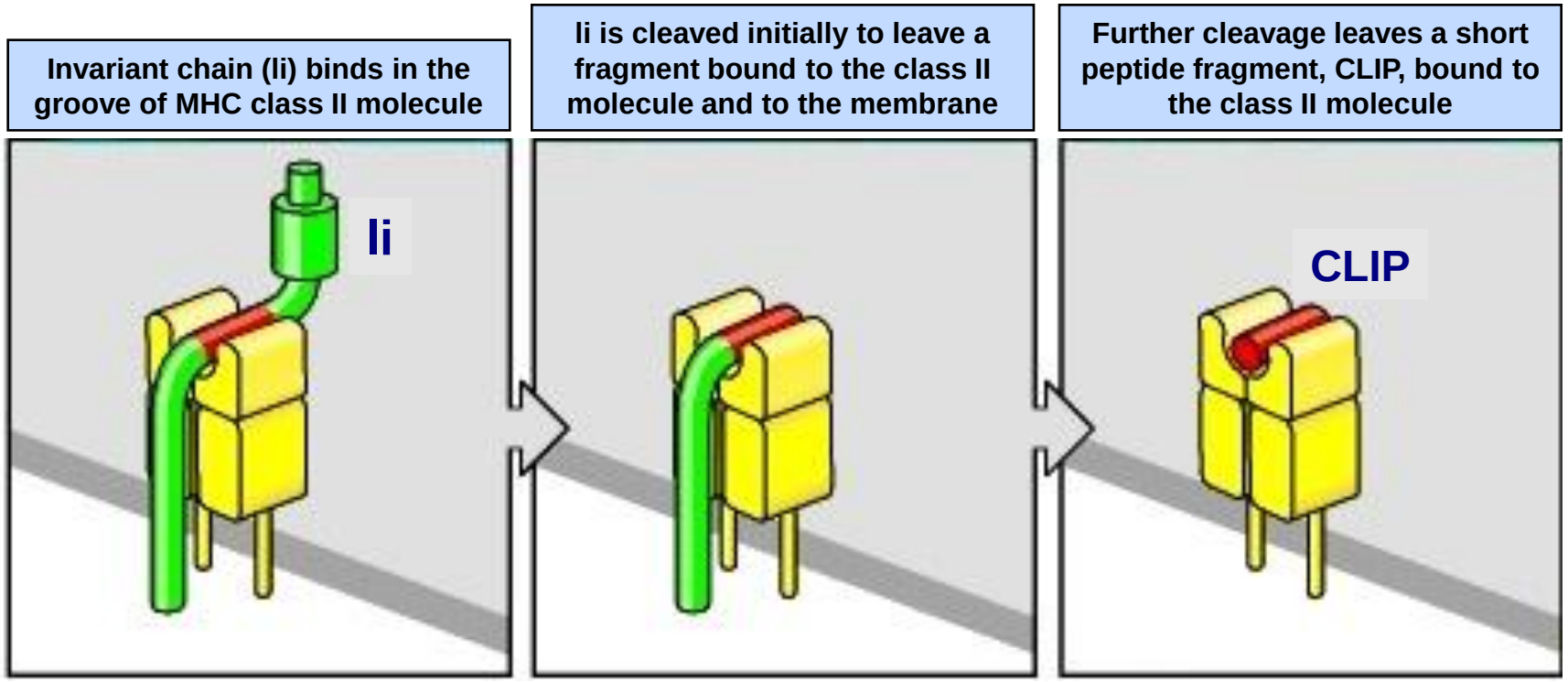


Modified from *Immunobiology;* Authors: Janeway CA, Travers P (Garland Publishing Inc.)

Class II MHC molecules are synthesized in the ER and transported to endosomes with an associated protein called the invariant chain (Ii), which occupies the peptide-binding clefts of MHC II

CLIP blocks the class II MHC binding site

CLass II associated Invariant chain Peptide (CLIP)



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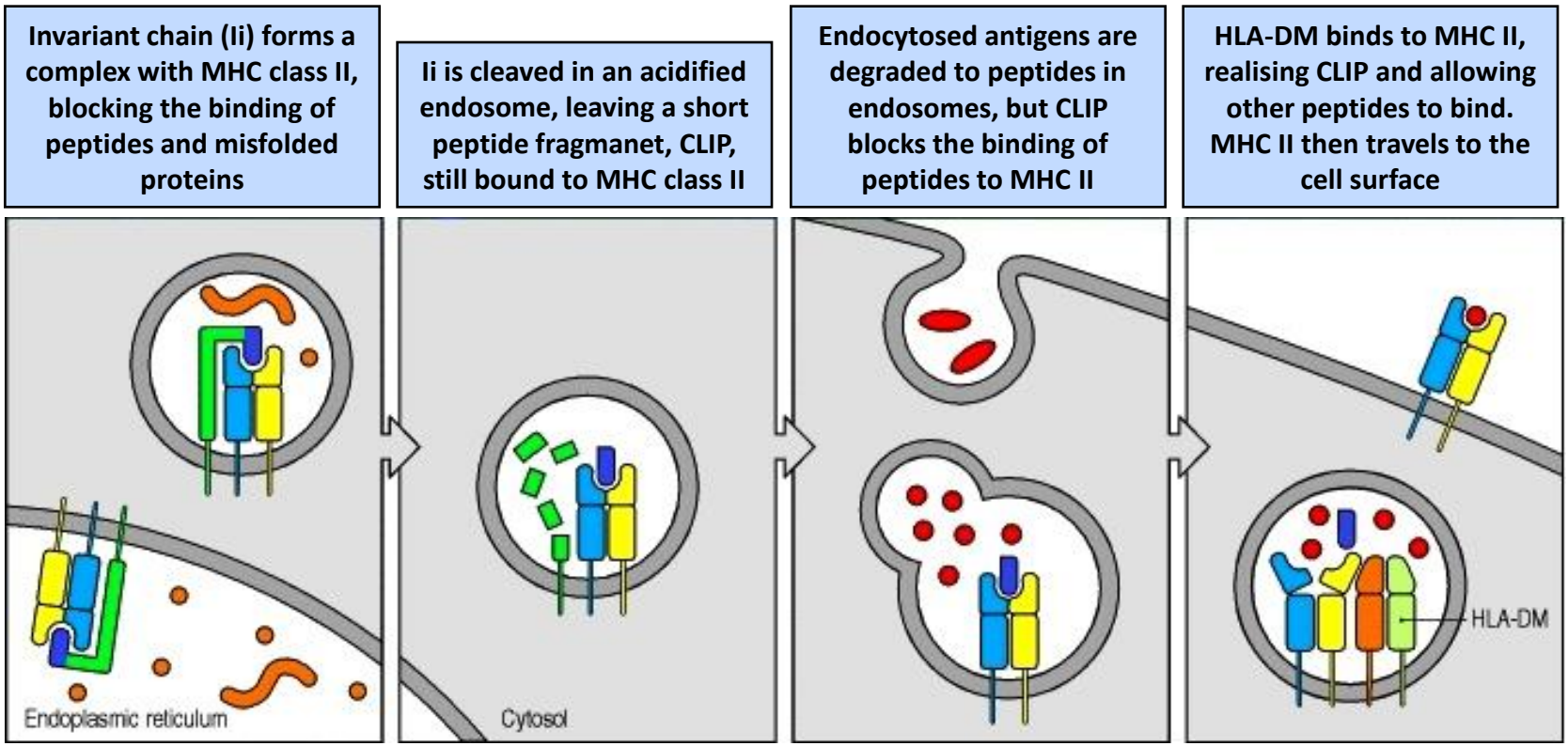
The same proteolytic enzymes, such as cathepsin S, that generate peptides from internalized proteins also act on the invariant chain (Ii), degrading it and leaving only a 24-aa remnant CLIP, which sits in the peptide-binding cleft of MHC-II in the same way of antigenic peptides. Therefore, its removal is obligatory for loading of antigenic peptides.

Chaperone molecule (HLA-DM) allows the binding of peptides to MHC class II molecules



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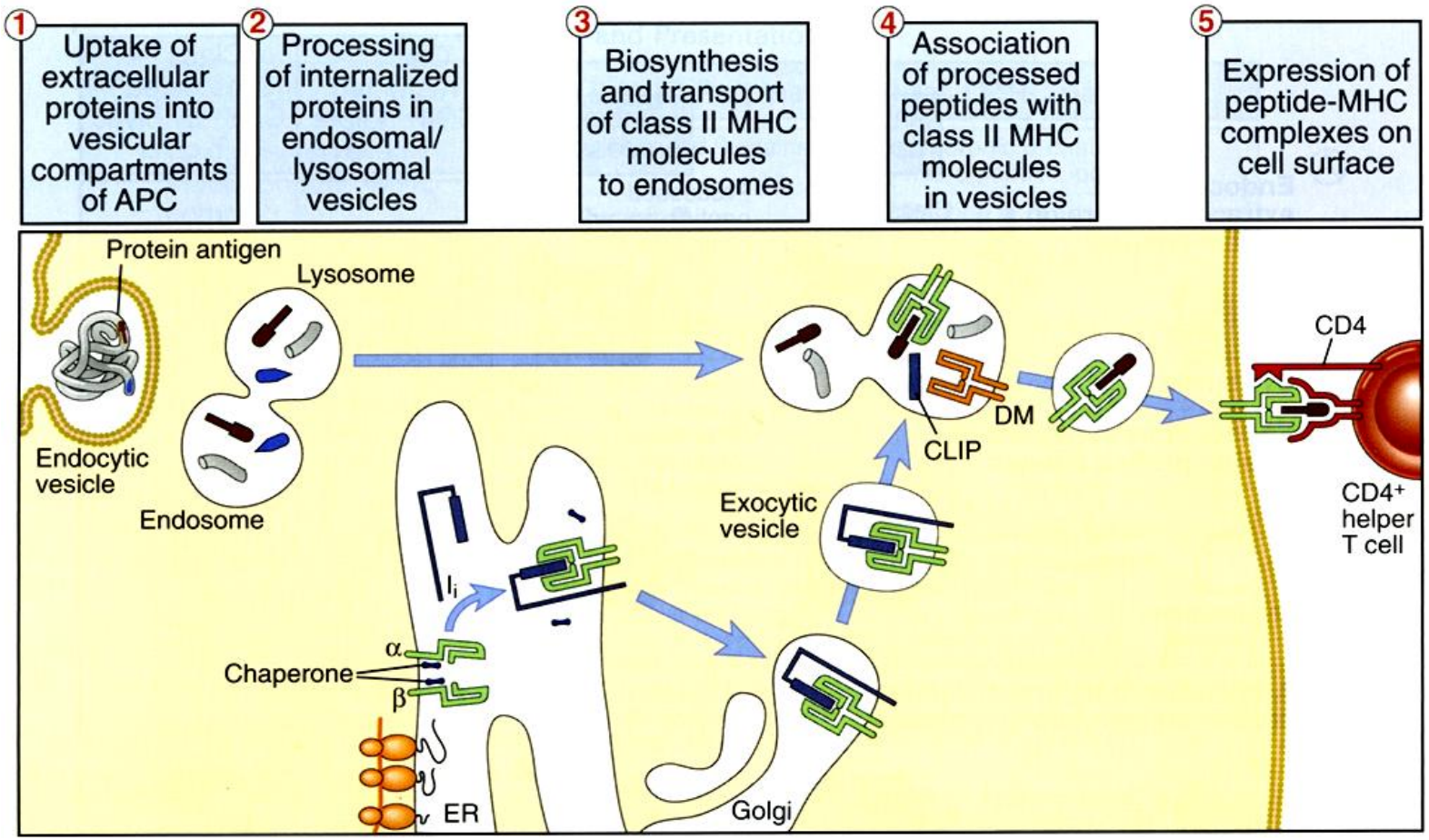
NOTE: **The molecule HLA-DM closely resemble MHC II and act as a molecular chaperone by keeping class II molecules competent for antigenic peptide loading and serves as an editor by favoring presentation of high-stability peptides.**

The class II MHC pathway of antigen presentation: overview



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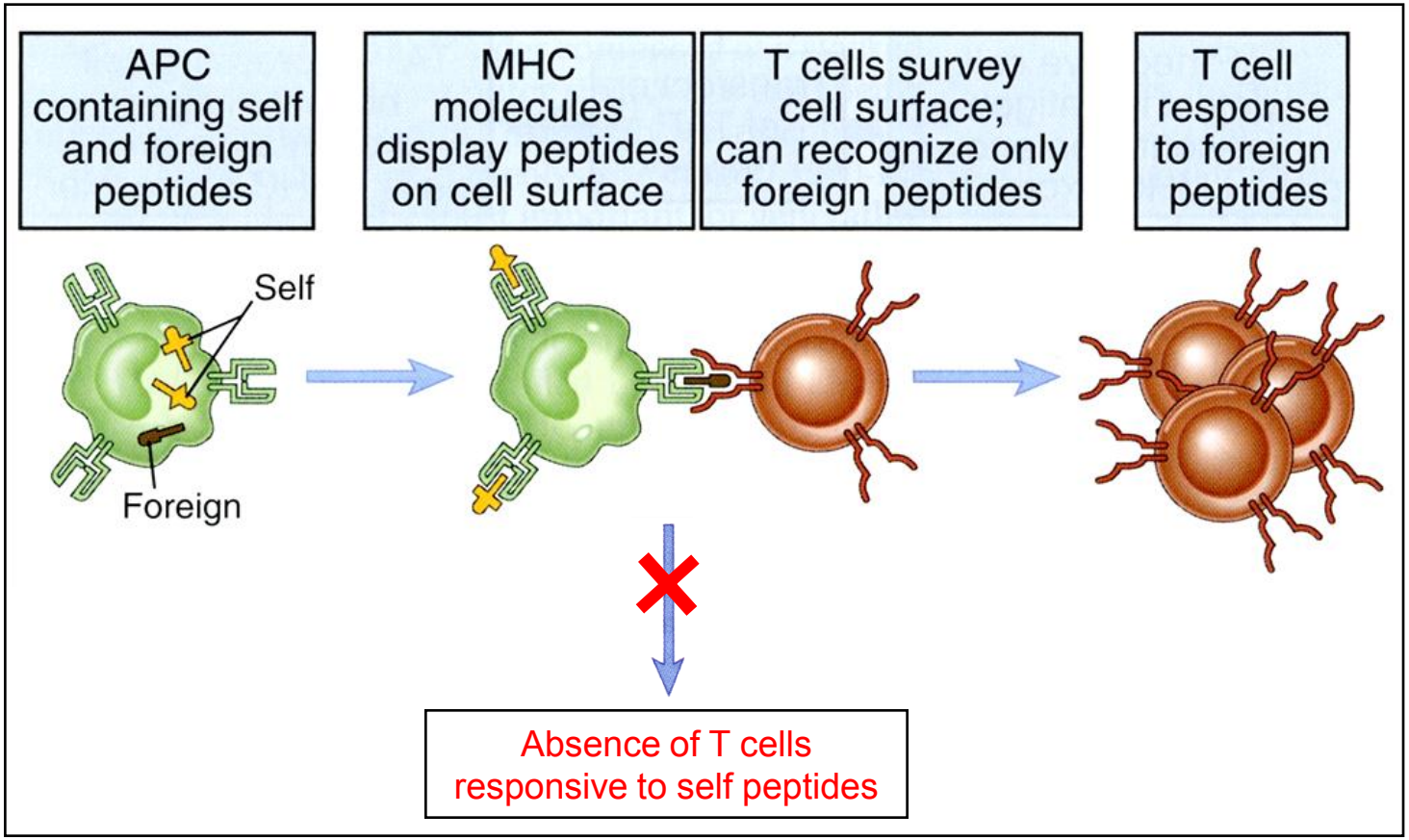
T cell surveillance for foreign antigens



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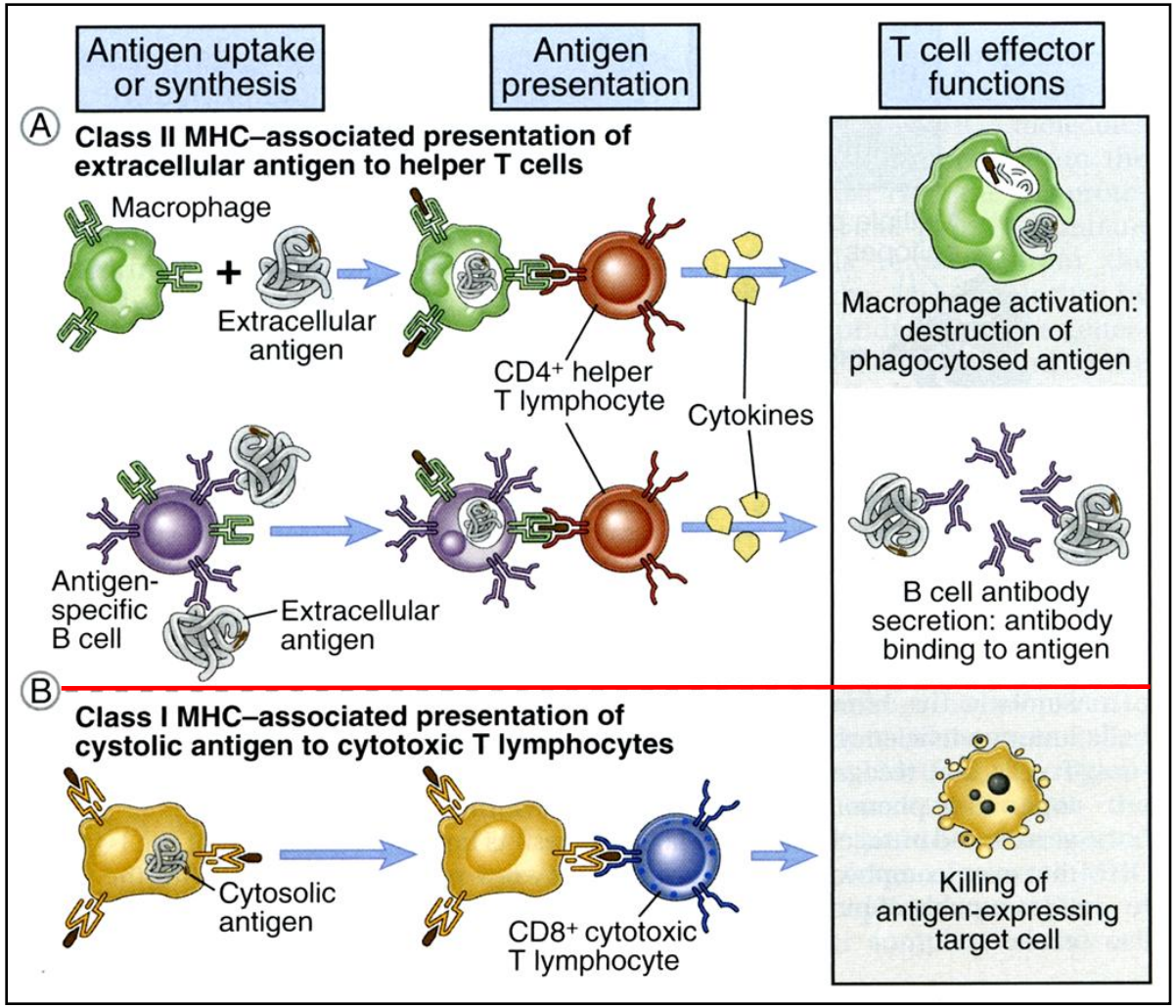
Presentation of extracellular and cytosolic antigens



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Summary : Part 2

- Class II MHC molecules are responsible to display peptides to naïve CD4+ T cells to initiate the primary responses.
- Extra-cellular proteins are internalized into endosomes where they are cleaved by specific enzymes and binds to class II MHC molecules.
- Newly formed MHC II molecules are tightly associated with invariant chain protein Ii in the endoplasmic reticulum.
- Invariant chain Ii stabilises MHC class II by non-covalently binding to the immature MHC class II molecule.
- Changes in pH allows Ii to be proteolytically cleaved into remnant peptides called CLIP, which is then removed when ready to associate with peptides from extra-cellular proteins.

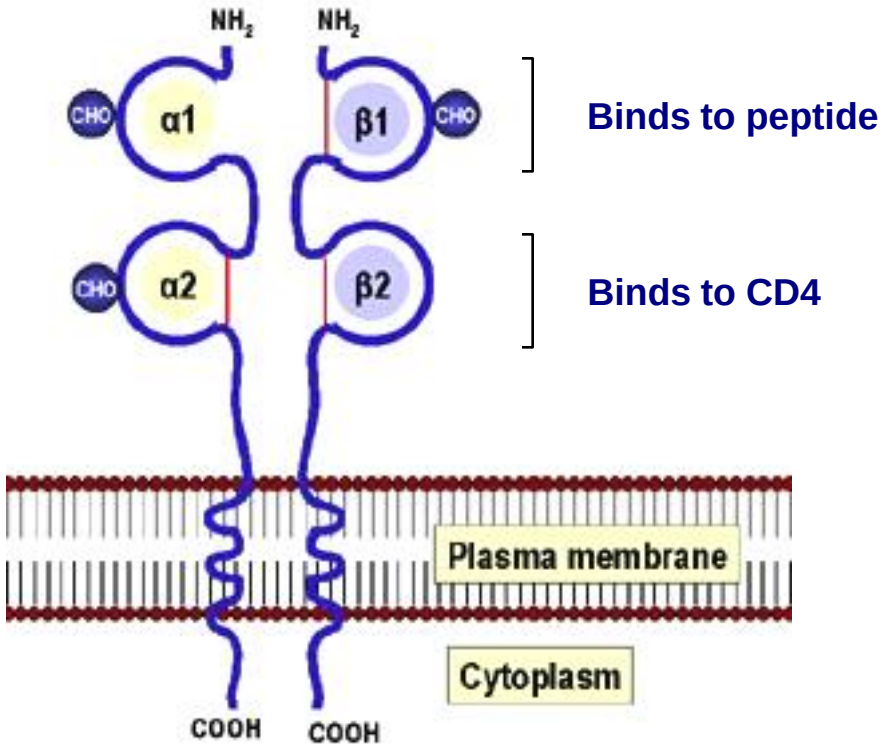
Structure of MHC class II

Two polypeptide chains

- α (34 kDa) and β (29 kDa)

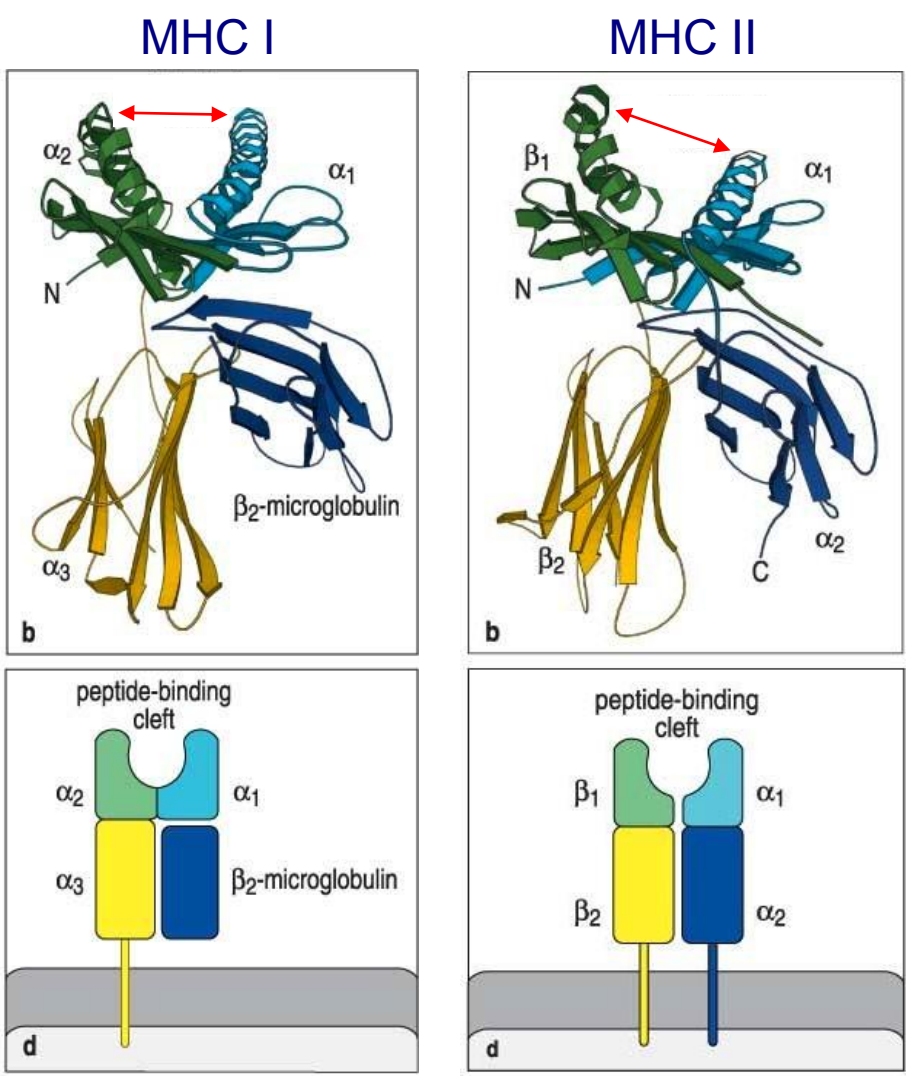
Four regions

- Cytoplasmic region contains sites for phosphorylation and binding to cytoskeleton
- Transmembrane region contains hydrophobic AAs
- Highly conserved α2 and β2 domains binds CD4
- Highly polymorphic peptide binding region formed by ⟨1 and ®1



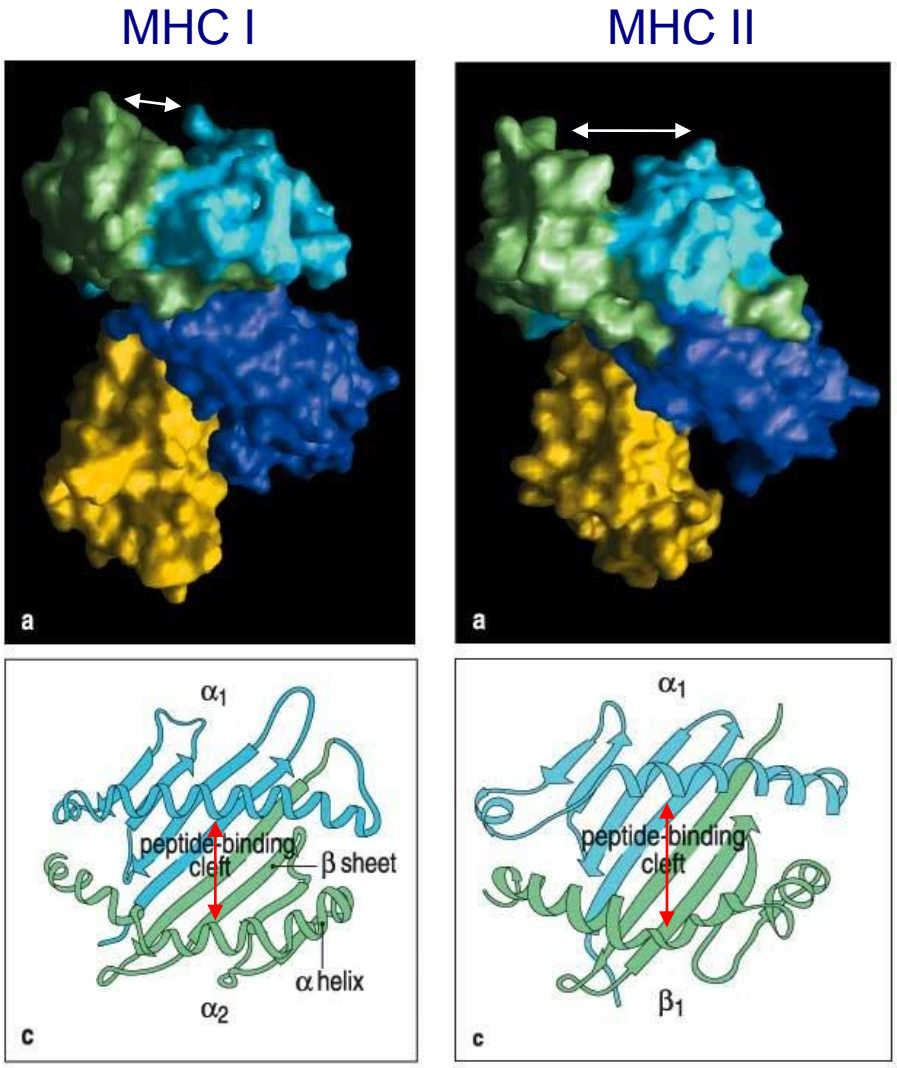
MHC Class I and II molecules: spot the difference … Part 1

MHC I contains 4 domains:
3 formed from the MHC-encoded α chain,
and 1 contributed by
the non-covalently associated β2-microglobulin chain.
Peptide-binding cleft is generated by a1-a2 domains



MHC II consist of a non-covalent complex of two chains, α and β, both of which bind to the membrane. Crystal structure of the MHC class II is very similar to MHC class I, with one main difference: the peptide-binding cleft is more open in MHC II --> peptides are more buried in MHC I, while peptides bound to MHC II are more exposed.

MHC Class I and II molecules: spot the difference … Part 2



The peptide-binding domain consists of a groove, with a floor provided by a β-sheet, and two walls, each formed from an α-helix. This binding groove presents peptides to CD4+ T cells, providing the mechanism by which MHC class II molecules function in the maintenance of self- tolerance and in the induction and regulation of adaptive immune responses against invading pathogens.

Modified from *Immunobiology*; Authors: Janeway CA, Travers P (Garland Publishing Inc.)

HLA and Disease Association

- Certain diseases associated with specific HLA antigens or haplotype.
- Unknown etiologic basis for most of these associations. However, many are autoimmune diseases though to result from variation in immune response from polymorphism at HLA.
- In some cases (ankylosing spondylitis and HLA-B27) there is a strong association: >95% of patients in Norway with ankylosing spondylitis are HLA-B27-positive (although this represents only 5% of patients who are HLA-B27 positive).
- In other cases, disease is due to linkage disequilibrium with HLA (i.e. 21-OHase, hemochromatosis).
- HLA haplotype contributes to disease, along with other genes and environmental causes

Disease-associated MHC class II molecules

Precise regulation of major histocompatibility complex class II (MHC-II) gene expression plays a crucial role in the control of the immune response.

A large number of chronic inflammatory diseases are associated with genes in the MHC class II region.

- **HLA-DR2 is strongly positively correlated with Systemic Lupus Erythematosus (SLE), Narcolepsy and Multiple Sclerosis, and negatively correlated with Type 1 Diabetes Mellitus (IDDM).**
- **HLA-DR3 is correlated strongly with Sjögren's syndrome, myasthenia gravis, SLE, and IDDM.**
- **HLA-DR4 is correlated with the genesis of rheumatoid arthritis, IDDM, and pemphigus vulgaris.**

Note: fewer correlations exist with MHC class I molecules. The most notable and consistent is the association between HLA-B27 and ankylosing spondylitis.

Reference: Janeway CA, Travers P, Walport M, Shlomchik MJ. (2001). Immunobiology. 5th Edition. Garland Publishing, New York.
Reference: Jones EY, Fugger L, Strominger JL, Siebold C. (2006). MHC class II proteins and disease: a structural perspective. Nat Rev Immunol. 6: 271-282.

Conclusions

- T cells recognize antigens only in the form of peptides displayed by the products of self MHC genes on the surface of APCs
- Antigens are captured, internalized and processed by dendritic cells, macrophages and B lymphocytes.
- These pathways of MHC-restricted antigen presentation ensure that most of the body's cells are screened for the possible presence of foreign antigens.
- These pathways also ensure that proteins from extra-cellular pathogens preferentially generate antigenic-peptides bound to class II MHC molecules for recognition by CD4+ helper T cells, which activate effector mechanisms that eliminate extra-cellular antigens.
- On the contrary, cytosolic antigens generate peptides bound to class I MHC molecules for recognition by cytotoxic CD8+ T cells, which function to eliminate cells harboring intracellular pathogens.
- Aberrant MHC class II processing is associated with disease, most commonly autoimmune disease (i.e., SLE, IDDM).

Learning outcomes

At the end of this lecture you should know that:

- **T and B cells recognise antigen differently**
- **The antigen processing requires metabolic activity**
- **The antigen processing generates antigenic peptides**
- **The antigen processing pathways involves: uptake, degradation, complex formation and presentation**

You should also be able to:

- **Define the role of the class II MHC molecule, invariant chain peptide CLIP and HLA-DM molecule in antigen processing**
- **Describe the structural features of MHC class II molecules**
- **Give examples of immune disease associated with aberrant function of class II MHC molecule.**

References

Reviews:

- Vyas JM, Van der Veen AG, Ploegh HL. (2008). **The known unknowns of antigen processing and presentation.** *Nat Rev Immunol.* 8: 607-618.

- Sercarz EE, Maverakis E. (2003). **MHC-guided processing: binding of large antigen fragments.** *Nat Rev Immunol.* 3: 621-629.

- Jones EY, Fugger L, Strominger JL, Siebold C. (2006). **MHC class II proteins and disease: a structural perspective.** *Nat Rev Immunol.* 6: 271-282.

Books:

- **Basic immunology -** Abbas and Lichtman 2006-2007 Elsevier
- **Immunobiology 6th Edition -** Janeway et al., Garland Publishing

....Video time

http://www.youtube.com/user/garlandscience#p/c/7D18C93964A61F67/34/_8JMVq7HF2Y

http://www.youtube.com/user/garlandscience#p/c/7D18C93964A61F67/38/AmPITzBoYWY