

IV.

Antigen Presentation

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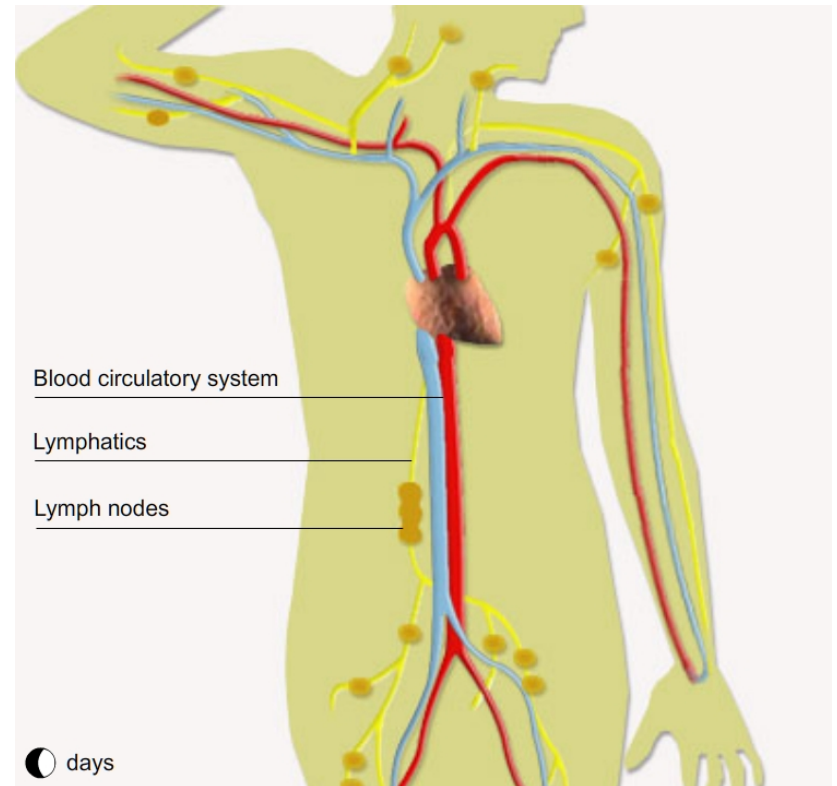
Question

- How do the rare naïve lymphocytes with the adequate receptor enter in contact with an antigen?

The immune system has developed a highly specialized system for capturing and displaying antigens to lymphocytes.

Antigen sampling and presentation: questions

- How are antigens sampled when they enter the body?
- How do B and T lymphocytes recognize antigens?
- How are antigens presented on the cell surface of APCs for recognition by T cells?
- What is the role of MHC molecules as antigen display molecules?



Outline

IV-1 Antigen sampling

IV-2 Dendritic cells

IV-3 Antigen recognition

3.1 Antigen recognition by B cells

3.2 Antigen recognition by T cells

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4.1 The MHC molecule

4.2 The MHC gene complex

4.3 How cells present antigens

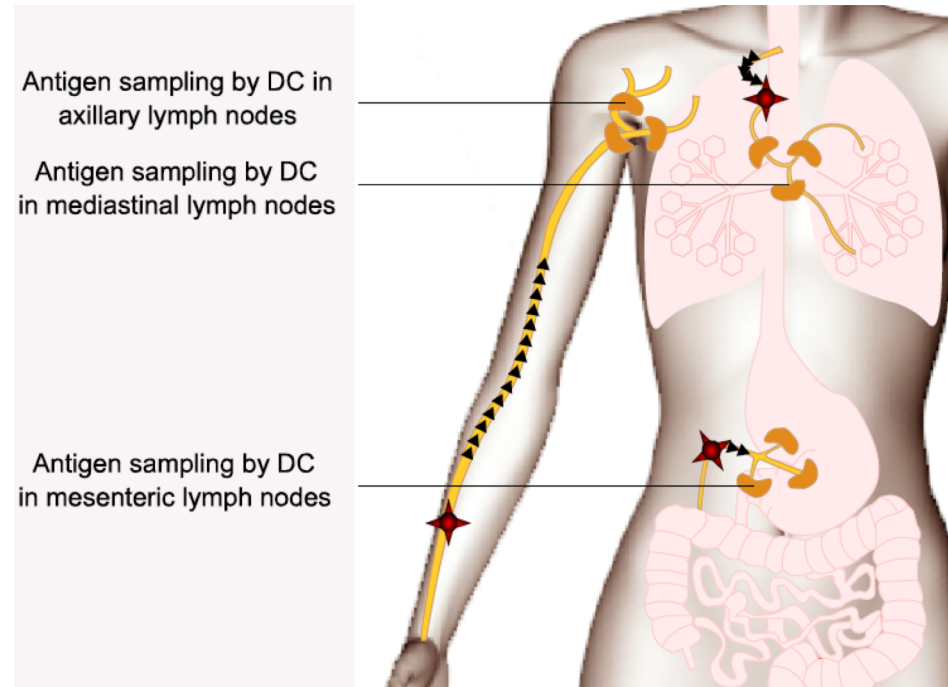
Antigen sampling

Antigen sampling sites

- Since foreign antigens and microorganisms can enter the body at multiple sites, antigen-sampling cells are strategically located at all the possible sites of antigen entry:
 - Skin
 - mucosal surfaces
 - the lymph nodes for antigens that escape sampling in skin or mucosal surfaces and find their way from the tissue spaces to the lymph
 - in the spleen for antigens that gain access to the blood, for instance following bites by insects

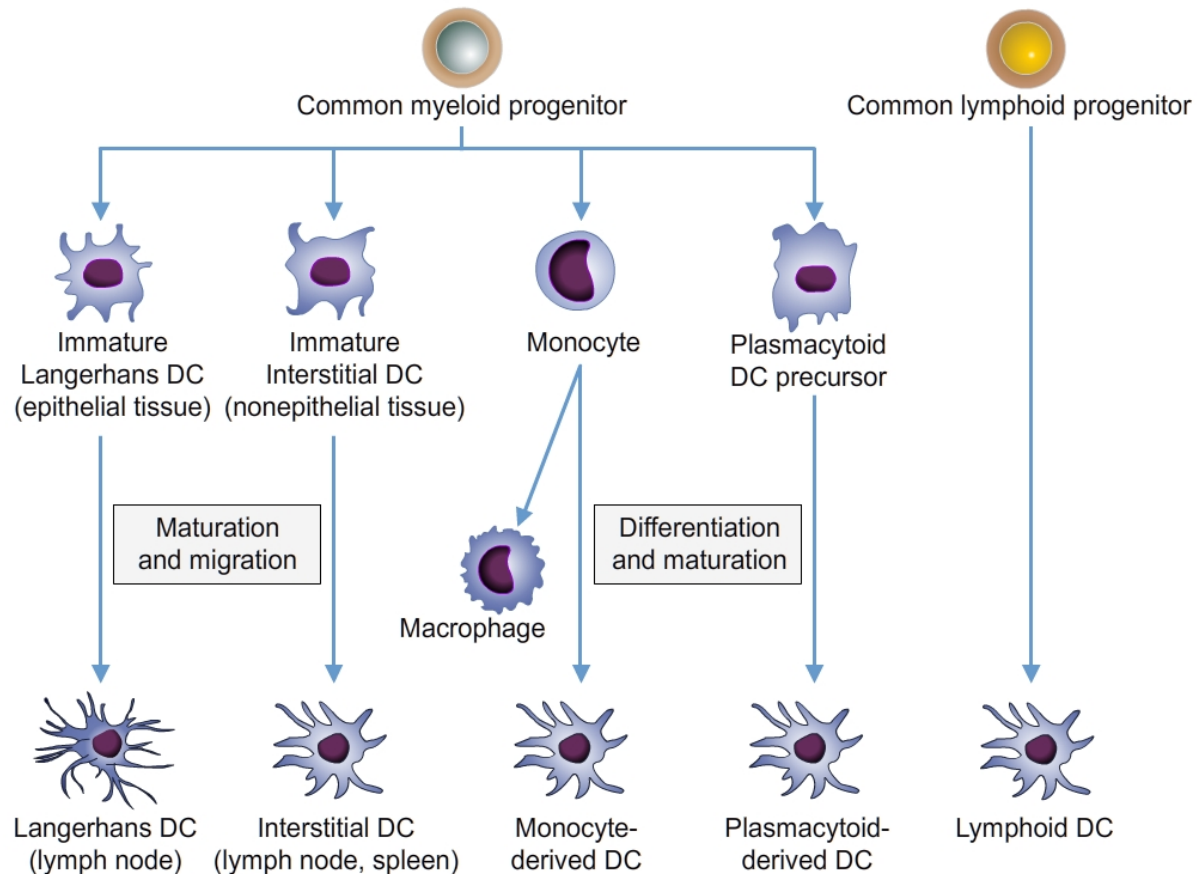
Antigen sampling cells

- the resident antigen-sampling cells.
 - take up antigen and deliver it intact to underlying antigen presenting cells (APCs)
 - Example: epithelial M cells concentrated in MALT
- the migratory hematopoietic antigen-sampling cells (Dendritic cells)
 - take up antigen and transport it to distant lymphoid organs



Dendritic Cells

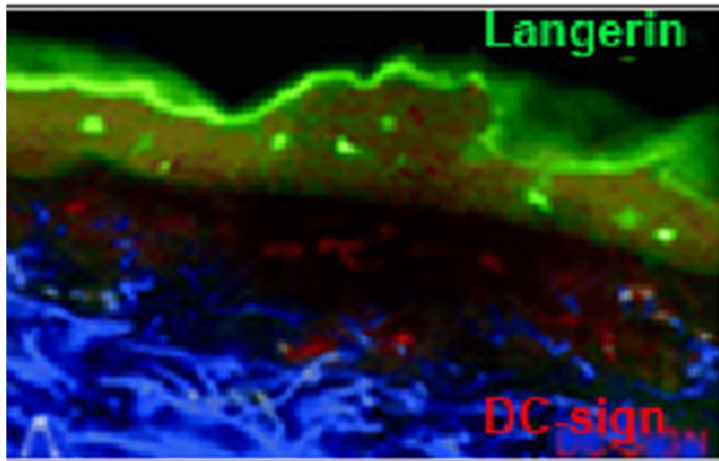
- Heterogeneous population, include plasmacytoid and lymphoid DCs
- Key players in induction of adaptive immunity: integrate danger and antigen signals
- Phagocytic cells which travel from tissues to draining lymph nodes with antigen cargo
- Activate naive T cells in lymph nodes



The skin harbors two populations of antigen-sampling cells

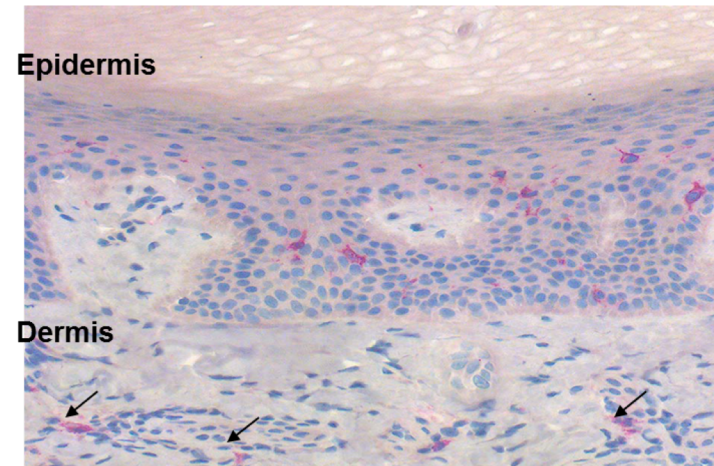
Langerhans cells

- Contain large granules, notably Langerin
- Langerhans cells pick up antigens (including apoptotic skin cells) and migrate to the regional lymph nodes
- reside within the **epidermis**



Dermal dendritic cells

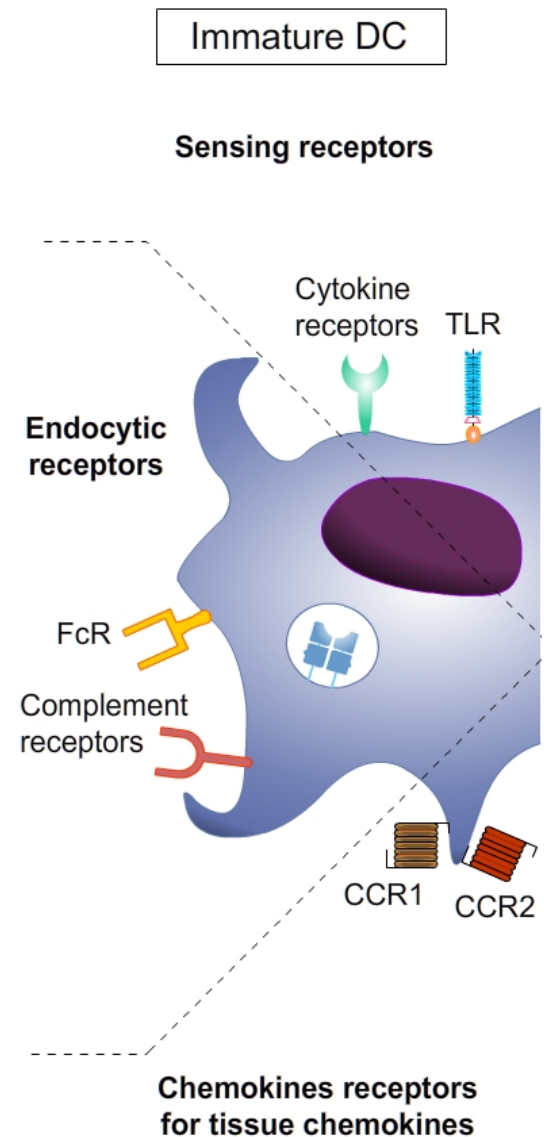
- lack Birbeck granules and Langerin
- reside within the **dermis** (but also derived from the bone marrow)



Immature Dendritic cells

Immature DCs

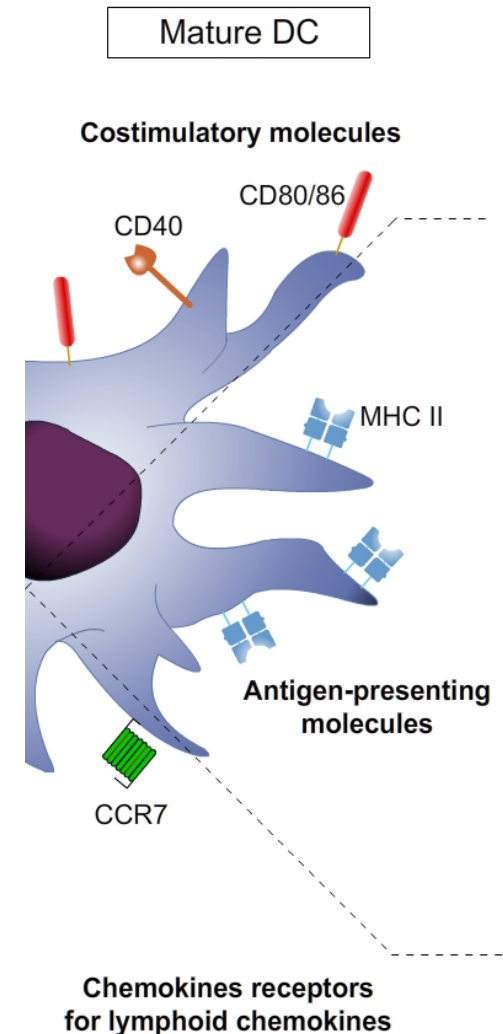
- found in all tissues where antigens may enter the body
- high capacity for antigen uptake but limited capacity for antigen presentation
- express a large number of **receptors**:
 - **chemokine receptors** (CCR1, CCR2, CCR5, CCR6)*
 - **endocytic receptors** by which they can capture antigens
 - non-opsonic receptors (DC-SIGN, mannose receptor) specific for microbial products
 - opsonic receptors (Fc & complement receptors)
 - integrin receptors, which are involved in the uptake of apoptotic bodies
 - **sensing receptors** by which they can integrate information about the antigen
 - TLRs
 - Cytokine receptors



Mature Dendritic cells

Mature DCs

- exclusively found in the T cell areas of secondary lymphoid organs
- high propensity for antigen presentation and T cell activation but reduced ability to capture antigens
- express the following **receptors**:
 - **Chemokine receptor** CCR7, so that they can leave peripheral tissues and migrate to secondary lymphoid organs where ligands for CCR7 are constitutively expressed.
 - **Adhesion molecules** required for interacting with T cells
 - **Cell surface MHC class II** molecules required for antigen presentation.
 - **Co-stimulatory molecules** (CD80, CD86, CD40) required for activation of reactive T cells.
- no longer express endocytic receptors



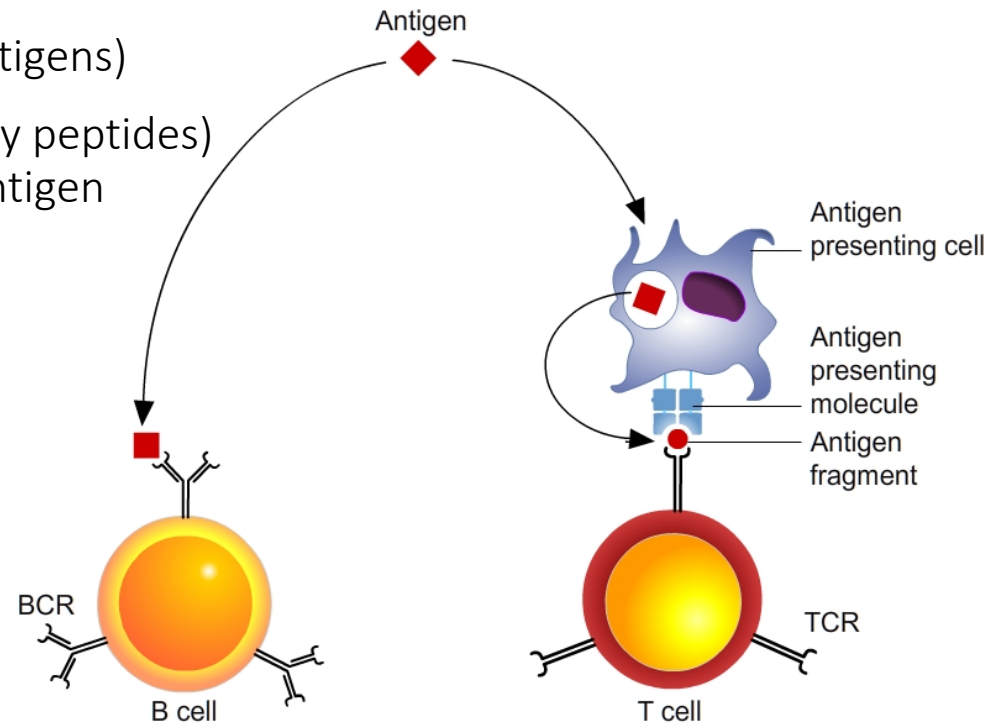
Antigen Recognition

Two main strategies have been developed by the adaptive immune system **to recognize antigen**:

- **Immunoglobulins (Igs)**, expressed by B lymphocytes.
- **T-cell receptors (TCRs)**, expressed by T lymphocytes

Antigen recognition by B cells clearly differs from antigen recognition by T cells:

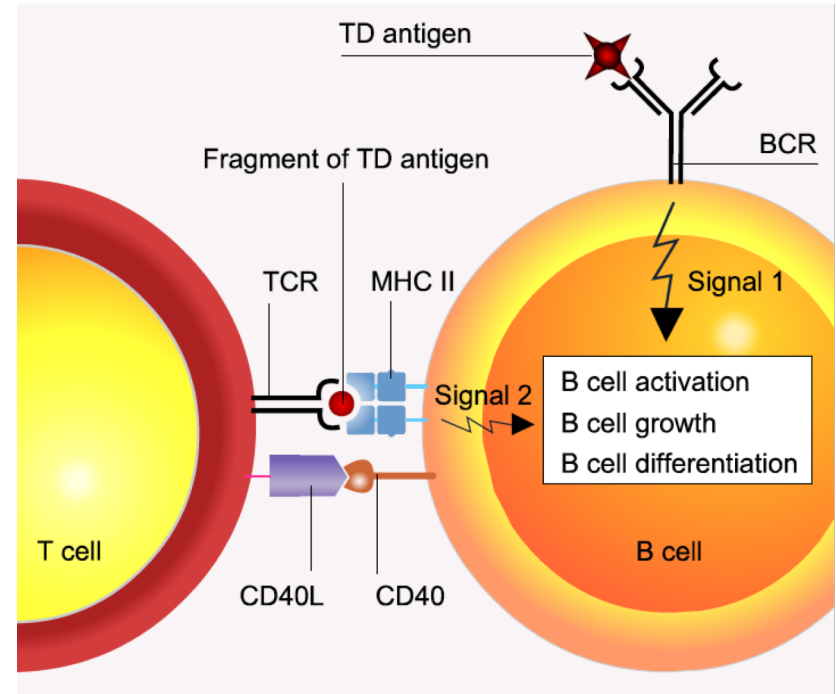
- **B cells** recognize **intact antigens** (native antigens)
- **T cells** recognize **antigen fragments** (usually peptides) exposed on the surfaces of host cells by antigen presenting molecules



Antigen Recognition by B lymphocytes: TD

Thymus dependent (TD)

- TD antigens refer to antigens against which antigen-specific B cells cannot produce antibodies without the cognate help of antigen-specific CD4+ T cells
- Most TD antigens are proteins

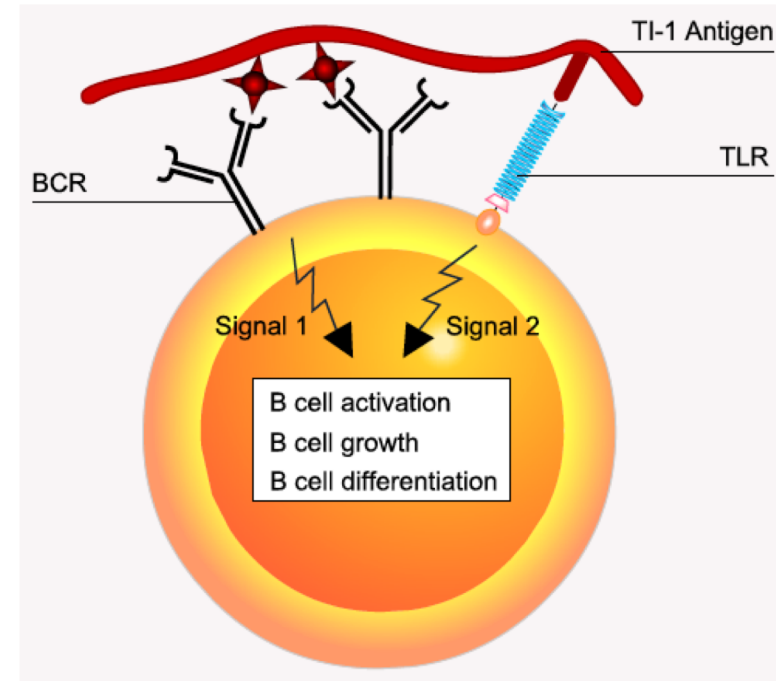


TD-antigens generate only a weak BCR signal. To be activated by TD-antigens, B cells must receive help from activated T helper cells that are specific for the same TD-antigen. Help passes through interaction between CD40L on T cells and CD40 on B cells. For this interaction to occur, the B cell must first display fragments of the TD-antigen on its MHC II molecules so that it can be recognized by the T helper cell.

Antigen Recognition by B lymphocytes: TI-1

Thymus independent type 1 (TI-1) (Non specific)

- TI-1 antigens are polyclonal, non antigen specific activators of B cells that engage co-stimulatory receptors on B cells (e.g. lipopolysaccharide (LPS) from Gram-negative bacterial cell wall which binds to TLR4).
- only generate short-lived IgM responses with no memory
- This likely represents an evolutionarily old arm of host defense against bacteria.

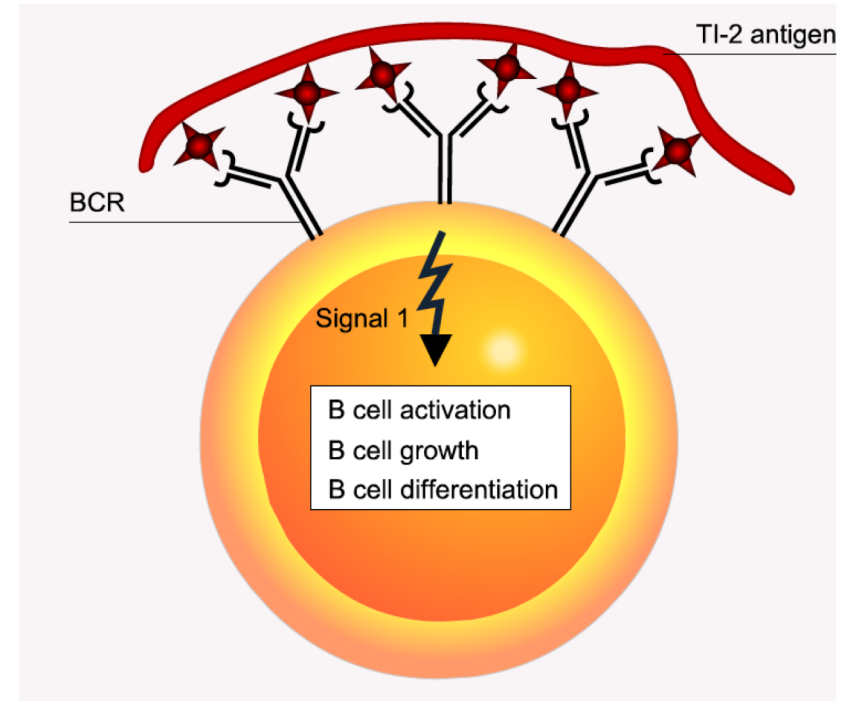


TI-1 antigens cause proliferation and differentiation of B lymphocytes without T cell stimulation and independently of their BCR specificity. TI-1 antigens activate B-cells via Toll like receptors. TI-1 antigens are classified as B-cell mitogens, because they induce numerous cell divisions. In higher concentrations, TI-1 antigens bind to BCR and TLR of various clones of B lymphocytes, which leads to production of polyclonal antibodies. But when the concentration of TI-1 is lower, it can activate only B lymphocytes with specific binding of TI-1 on their BCR, and leads to production of specific antibodies. This part of immune response may be important in some early stages of infection by extracellular pathogens, because it is rapidly activated and does not require T cell help or clonal maturation and expansion.

Antigen Recognition by B lymphocytes: TI-2

Thymus independent type 2 (TI-2) (Non specific)

- TI-2 antigens are molecules of high molecular weight with repetitive epitopes that cause extensive cross-linking of BCRs and stimulate B cell proliferation and differentiation without the cognate help of T lymphocytes.
- Prototype: high molecular weight polysaccharide such as the dextran B512.
- Only generate short-lived IgM responses with no memory



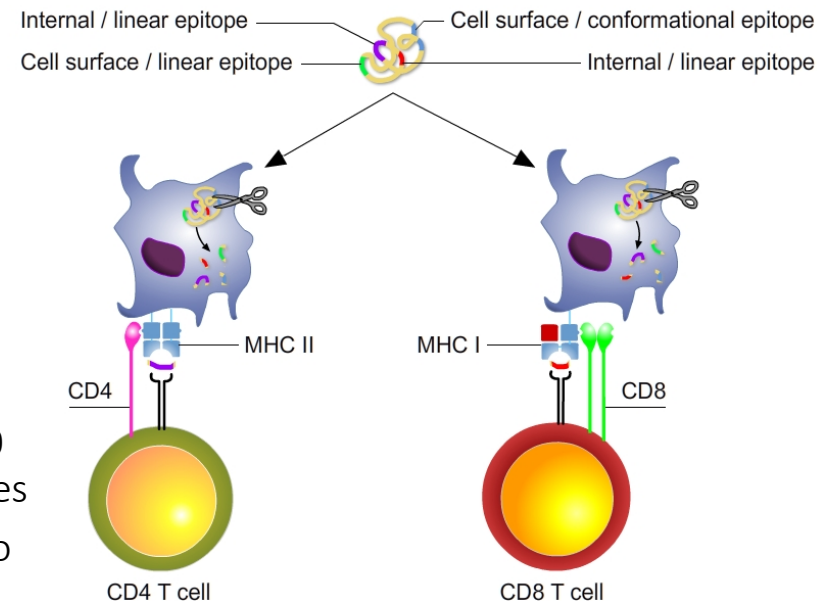
Antigen Recognition by T lymphocytes

TCR-MHC interaction

- T cells detect antigens via T-cell receptors (TCRs) that recognize antigen when presented as short fragments bound to antigen-presenting molecules (MHC) on the surface of antigen-presenting cells (APCs)

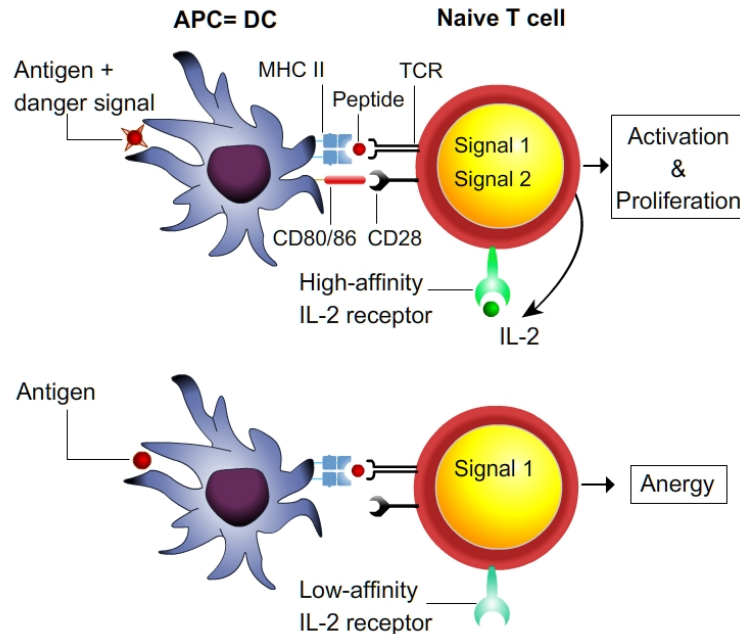
Peptide recognition

- T cells exist as two main populations with distinct antigen recognition strategies:
 - T cells bearing $\alpha\beta$ TCRs recognize peptides of protein antigens in association with MHC molecules
 - CD4+ T helper cells recognize peptides of 10-20 amino acids presented by MHC class II molecules
 - CD8+ T cells recognize peptides of 8 to 10 amino acids presented by MHC class I molecules.
 - T cells bearing $\gamma\delta$ TCRs recognize small phosphorylated molecules and lipidic bacterial antigens bound to evolutionarily conserved and nonpolymorphic class I-like antigen-presenting molecules known as CD1 molecules*



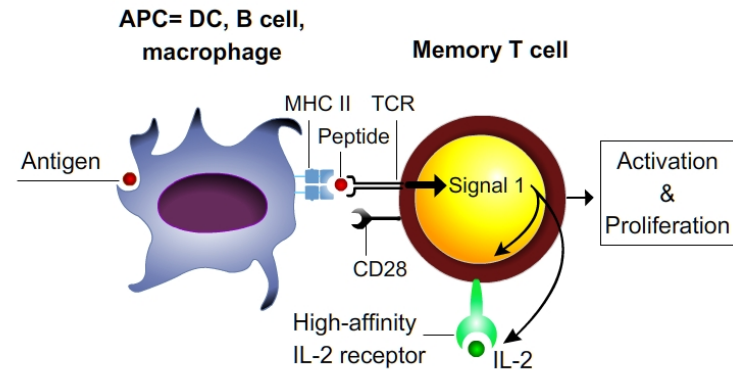
Antigen Recognition by T lymphocytes

Naive T Cells



For a naive T cell to be activated it must integrate two signals: a signal 1 generated by interactions between TCR on the T cell and MHC-peptide complexes on a professional APC and a signal 2 generated by the binding of CD28 on the T cell to the co-stimulatory molecules CD80/86 induced on professional APCs in response to danger signals. Dendritic cells are the only professional APCs able to activate naive T cells *in vivo*.

Memory T Cells



Memory T cells are less dependent on co-stimulation than naive T cells for activation so they can be activated by all professional APCs in the absence of high co-stimulation conditions.

Conclusions | Antigen presentation

- Dendritic cells form a heterogeneous class of cells that initiate T cell responses by capturing and presenting antigens to T cells.
- Antigen recognition by B cells differs significantly from antigen recognition by T cells.
- T cells recognize antigen fragments (usually peptides) exposed on the surface of host cells by antigen presenting molecules (MHC).
- B cells can recognize intact antigens with their BCRs.
- Two modes of B cell activation:
 - Thymus Dependent antigens refer to antigens against which B cells cannot produce antibodies without the cognate help of CD4+ T cells. Most TD antigens are proteins.
 - Thymus Independent antigens refer to antigens against which B cells do not need the help of CD4+ T cells. Most TD antigens are either antigens that also engage co-stimulatory receptors on B cells (TI-1) or are molecules with repetitive epitopes engaging several BCRs (TI-2). TI-2 only generates short-lived IgM responses with no memory.

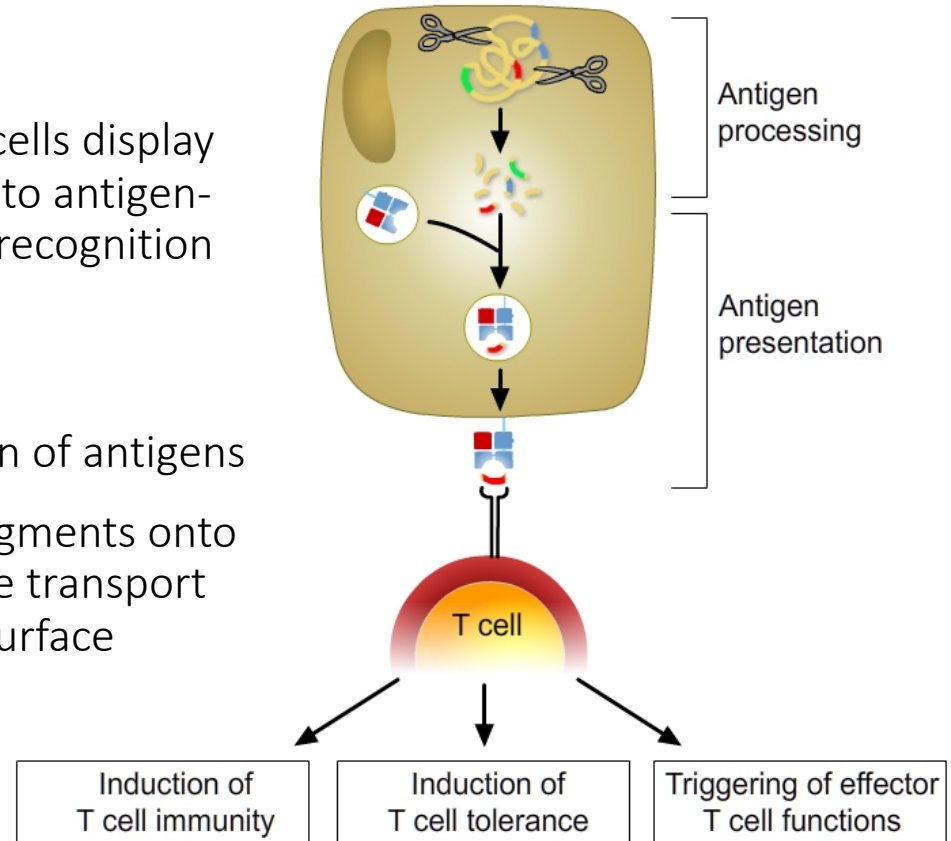
D. Antigen Presentation

What is antigen presentation?

Antigen presentation is the process by which cells display antigen in the form of short fragments bound to antigen-presenting molecules on their cell surface for recognition by T lymphocytes.

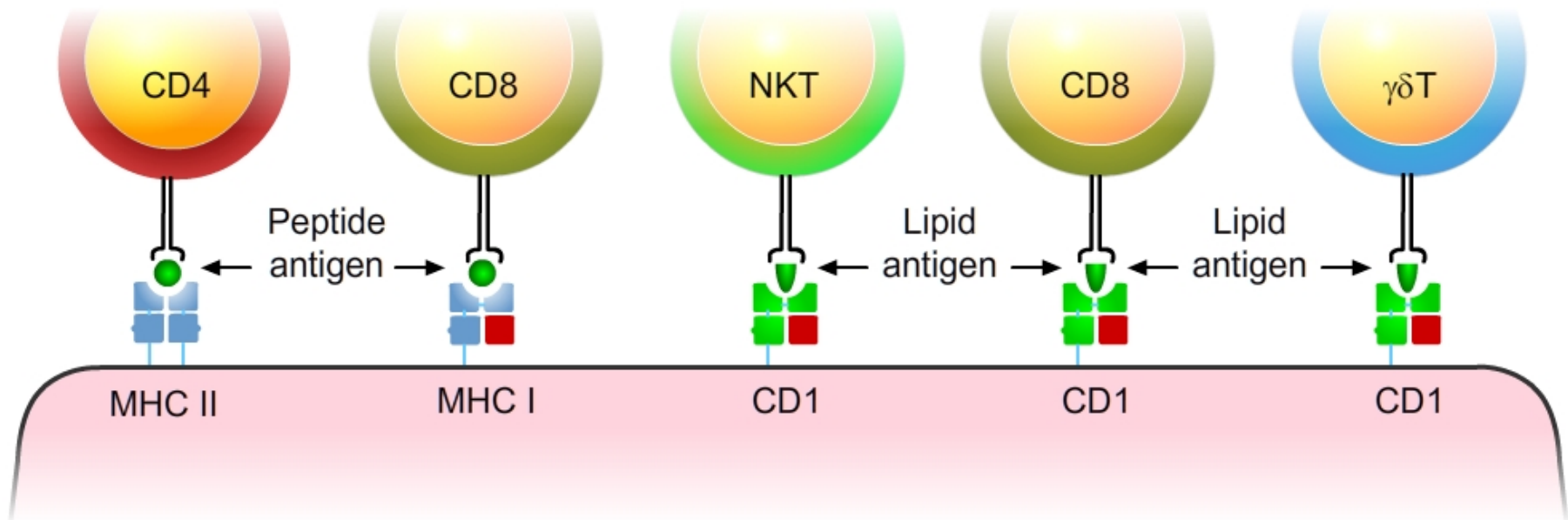
Antigen presentation involves two steps:

- **antigen processing**: intracellular degradation of antigens
- **intracellular loading** of resulting antigen fragments onto intracellular MHC molecules followed by the transport and display of these complexes to the cell surface



Antigen Presentation: MHC molecules

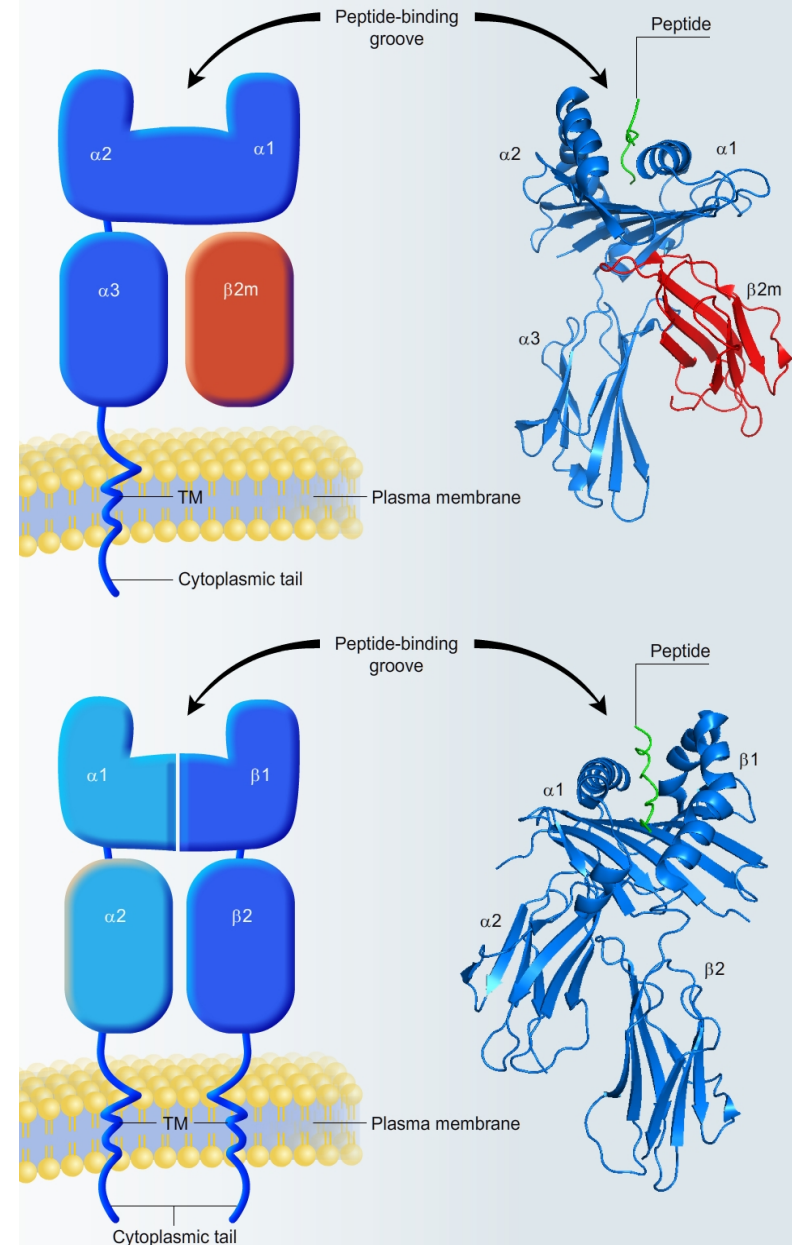
Molecule	Type of antigen	Expressed on	Function
MHC class I	protein	Almost all cells	present peptides to MHC-class I restricted CD8+ T cells
MHC class II	protein	DCs, B cells, macrophages	present peptides to MHC-class II restricted CD4+ T cells
CD1	Lipid and glycolipid	Limited number of cell types	Present lipid antigens to NKT cells, CD8+ T cells and $\gamma\delta$ T cells



Structure of the MHC molecule

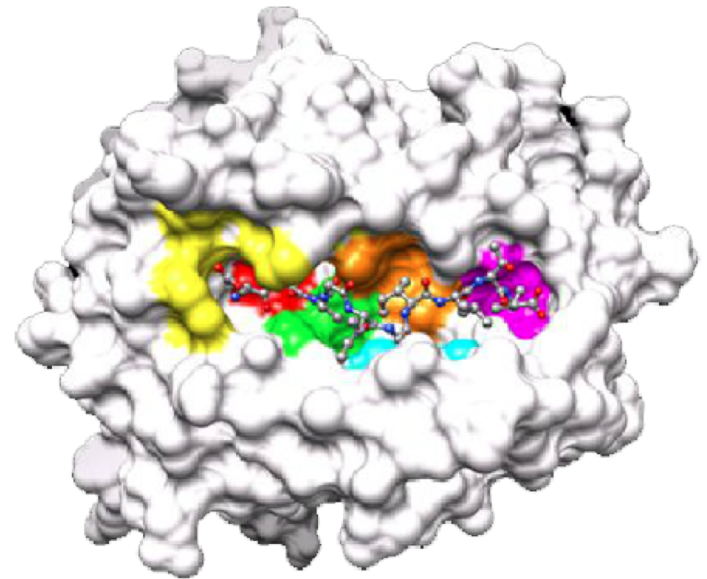
- Both types of MHC molecules contain peptide-binding clefts and invariant portions that bind CD8 or CD4.
- The two outermost domains ($\alpha 1$ and $\alpha 2$ in MHC class I, $\alpha 1$ and $\beta 1$ in MHC class II) are MHC-unique domains.

The crystal structures of class I MHC and class II MHC molecules illustrate the domains of the molecules and the fundamental similarities between them. $\beta 2m$, $\beta 2$ -microglobulin; Ig, immunoglobulin



MHC: The peptide binding domain

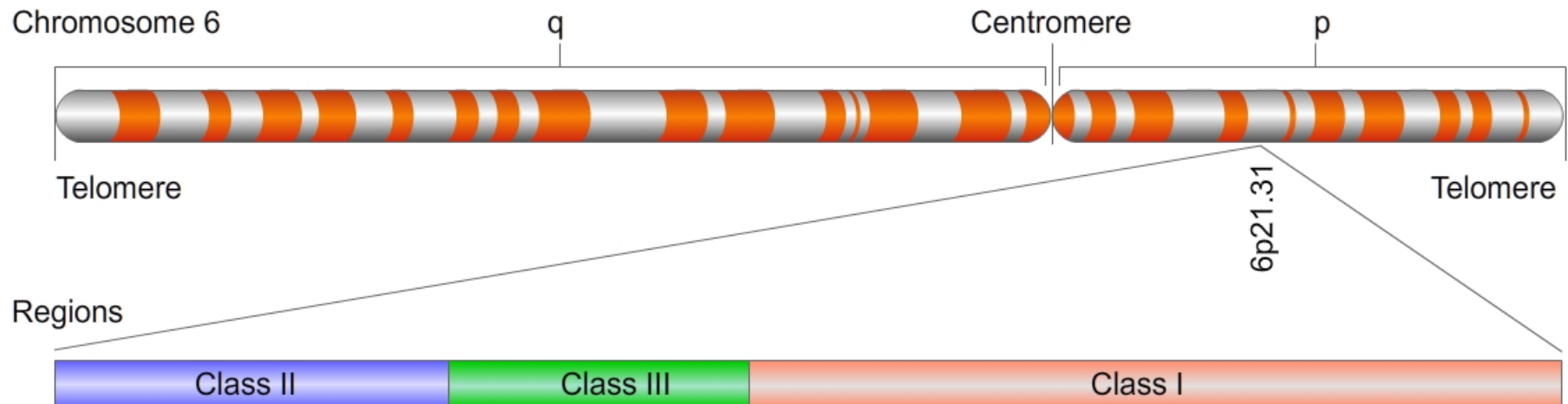
- MHC domains fold together to form a narrow **groove or cleft**.
- This groove forms the **peptide binding site** where peptides are bound and presented to T cells.
- An MHC molecule can accommodate a **limited number** of peptides.



The MHC gene complex

The HLA complex is located on chromosome 6 and covers almost 4 Mb.

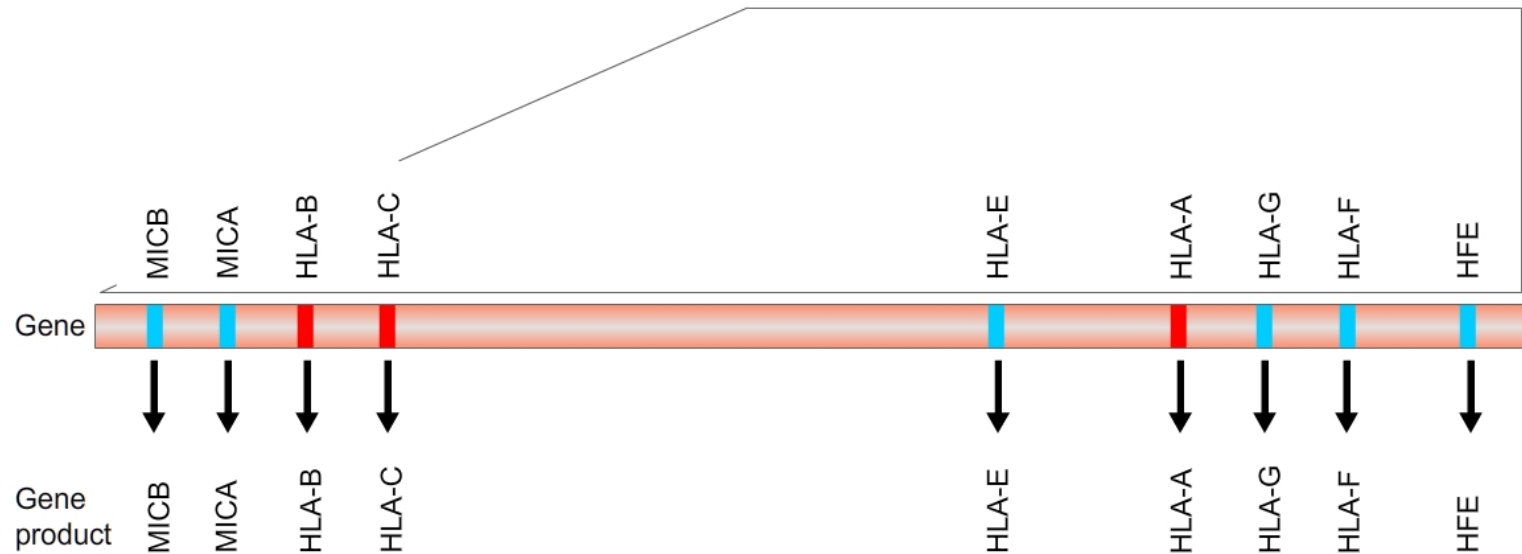
- very **gene-dense** region: contains over 220 identified genes, of which more than 40 encode MHC molecules
- about half are MHC-unrelated genes with **immunological functions**
- many **pseudogenes** or genes with unknown function



The MHC gene complex: HLA Class I

The human class I region spans 2 Mb. It contains:

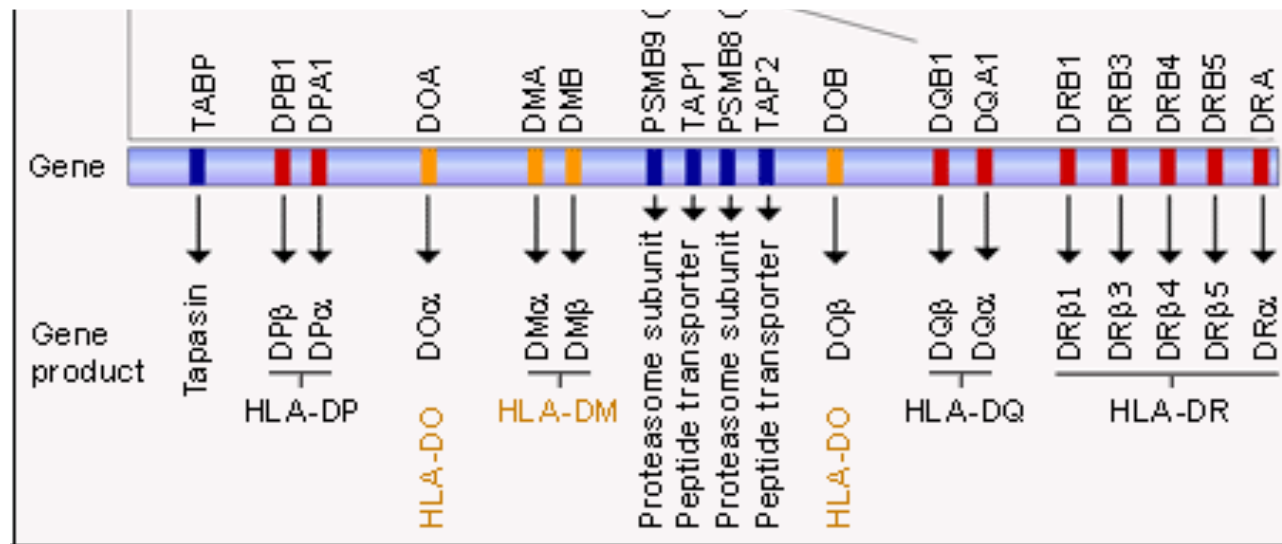
- **HLA-A, B, C** which code for the α chain of “classical” MHC I molecules
- a number of genes coding for the α chain of "non-classical" MHC class I molecules
- pseudogenes and MHC-unrelated genes
- genes with unknown functions



The MHC gene complex: HLA Class II

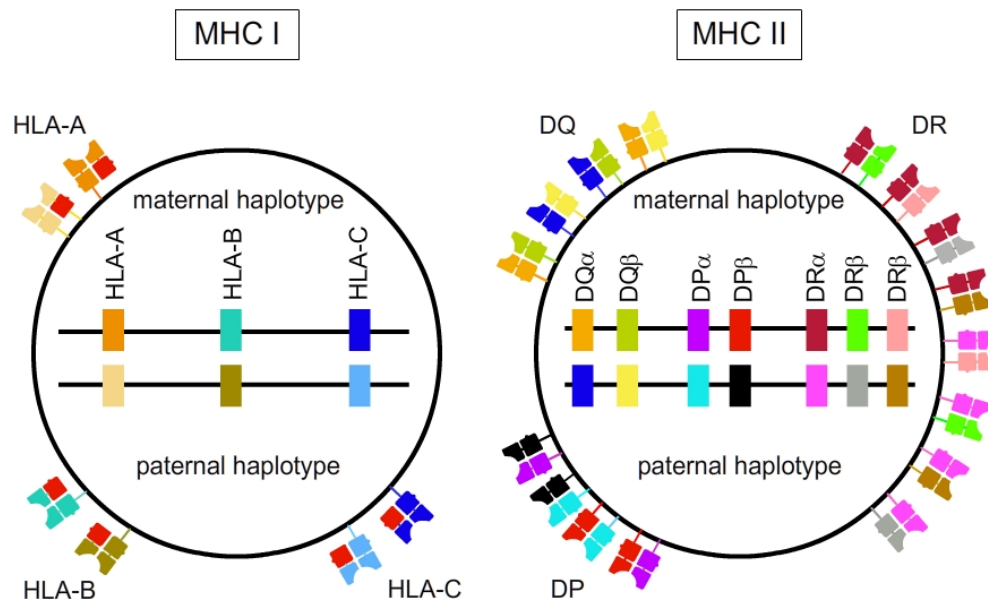
The human class II region spans ~ 750 kb and contains:

- three groups of "classical" class II genes, called HLA-DR, -DP and -DQ
- two pairs of "nonclassical" class II genes, called HLA-DM and HLA-DO
- a series of genes that play a major role in the class I antigen presentation pathway
- pseudogenes



MHC: Co-dominant expression

- MHC expression is **co-dominant**
- **MHC class I**
 - consists of an α chain and β 2-microglobulin
 - Up to **6 different MHC class I** molecules can be expressed on the same cell
- **MHC class II**
 - consists of an α and a β chain
 - α and β chain can pair either in cis (both from the same chromosome) or in trans (one from each chromosome) association
 - Up to **16 different MHC class II** molecules can be expressed on the same cell



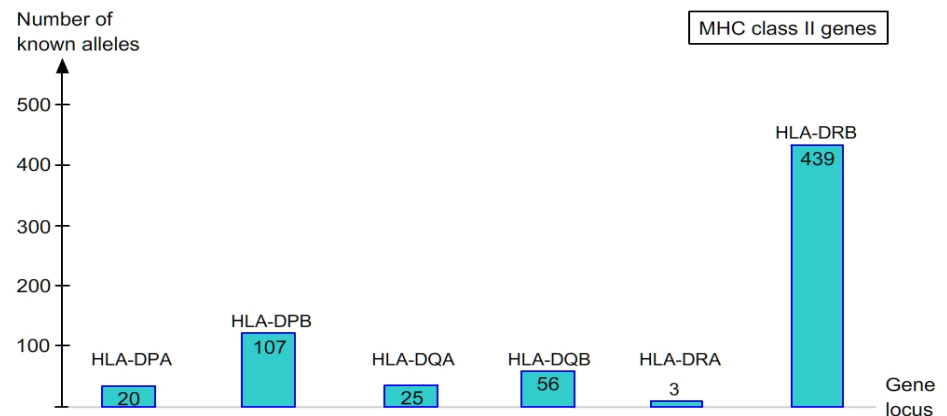
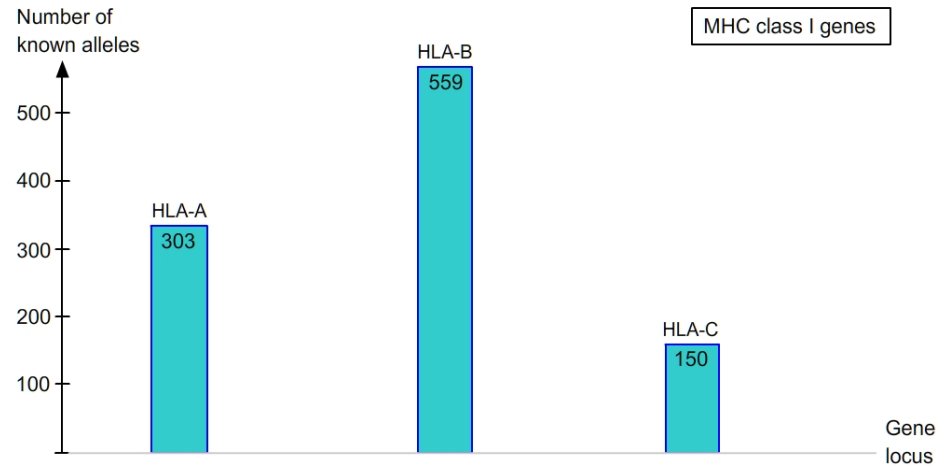
Why so many MHC molecules ?

The causes of this diversity

- MHC expression is co-dominant
- Several genes, and cis/trans pairing for MHC II
- MHC genes are highly polymorphic in human populations

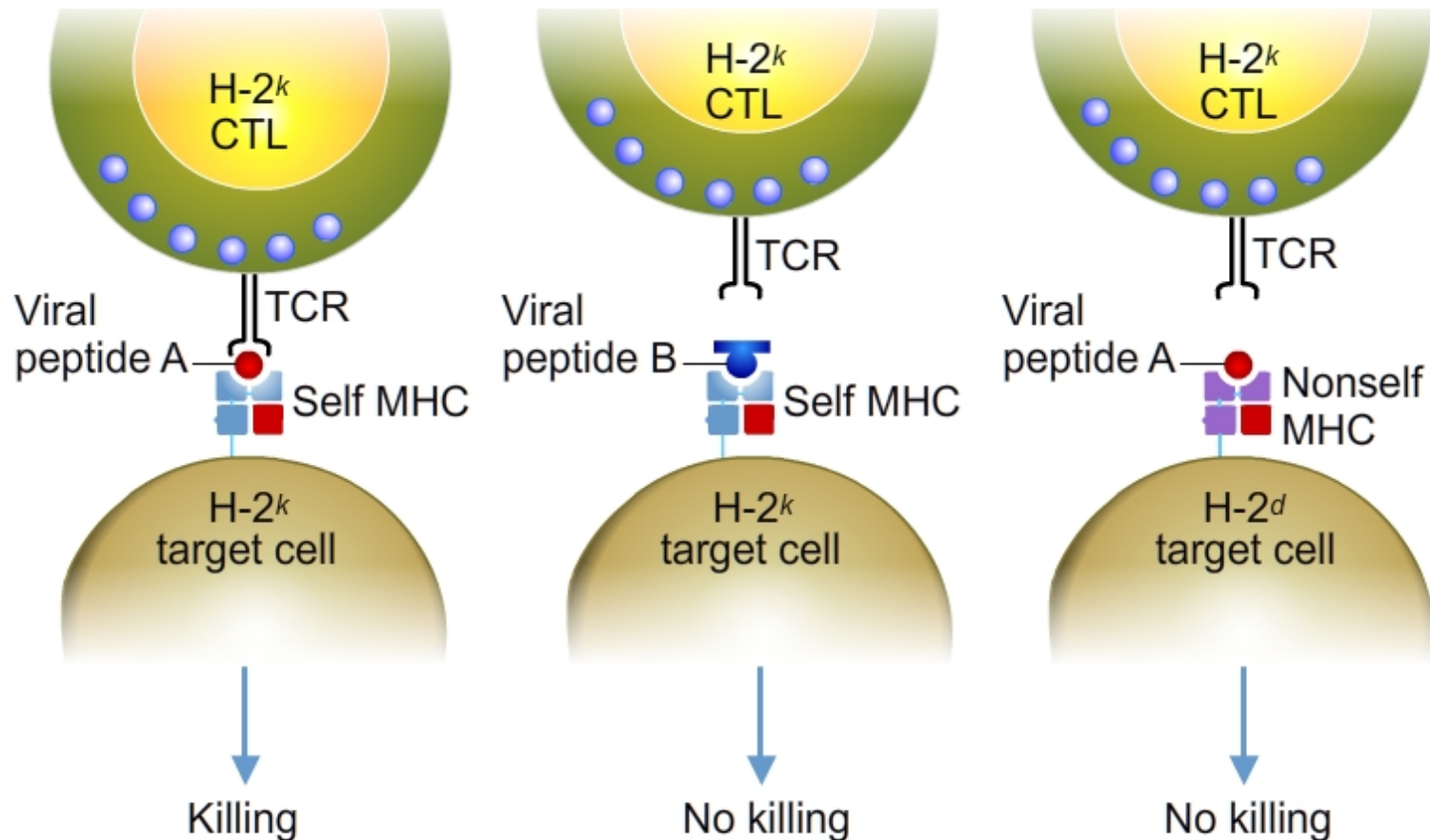
The reasons for this diversity

- Each MHC molecule can present a limited set of peptides → the high number of MHC molecules displayed by individuals increases the number of possible peptides
- Ensures that different individuals can respond differently to different antigens → increases survival at the population level



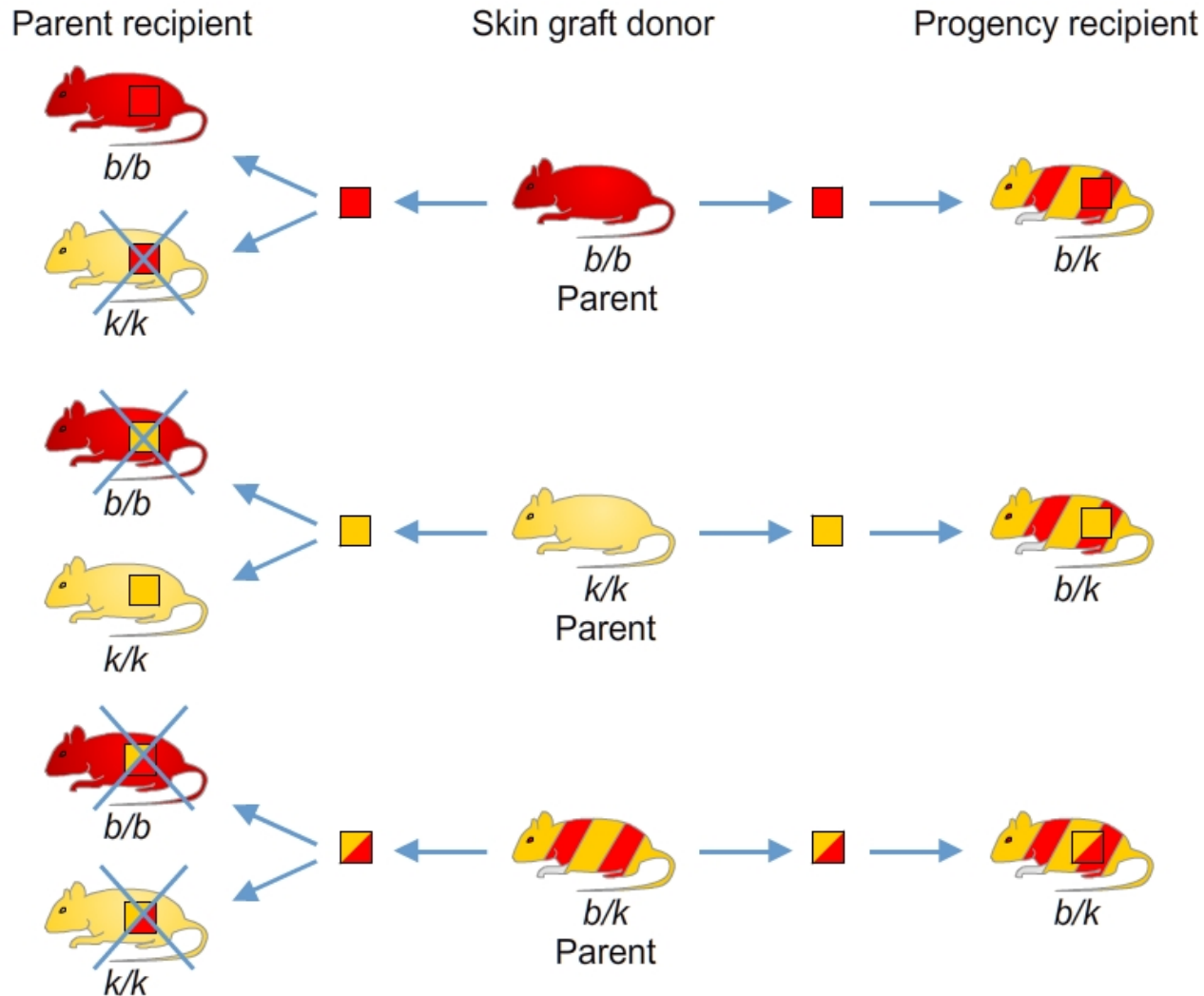
Consequences of MHC diversity (I): self restriction

T cell response are restricted to the self



Consequences of MHC diversity (II): graft rejection

Together with proteins defining the ABO blood group, the diversity of MHC molecules is an obstacle to grafts.



Conclusions | MHC molecules

MHC molecules

MHC are antigen-presenting molecules that present protein antigens to T lymphocytes.

- MHC class I molecules present peptides (from cytosolic proteins) to CD8+ T cells.
- MHC class II molecules present peptides (from endocytosed proteins) to CD4+ T cells.

MHC polymorphism

In any individual, the set of MHC molecules are sufficient to alert T cells to all possible infections.

- MHC polygeny : more than one MHC molecule of each class are expressed in cells
- MHC polymorphism: each MHC molecule exists as multiple variants so in heterozygous individuals, each MHC molecule exists as two variants.
- Binding properties of MHC molecules: each MHC variant is capable of binding a diverse set of peptides.

MHC diversity

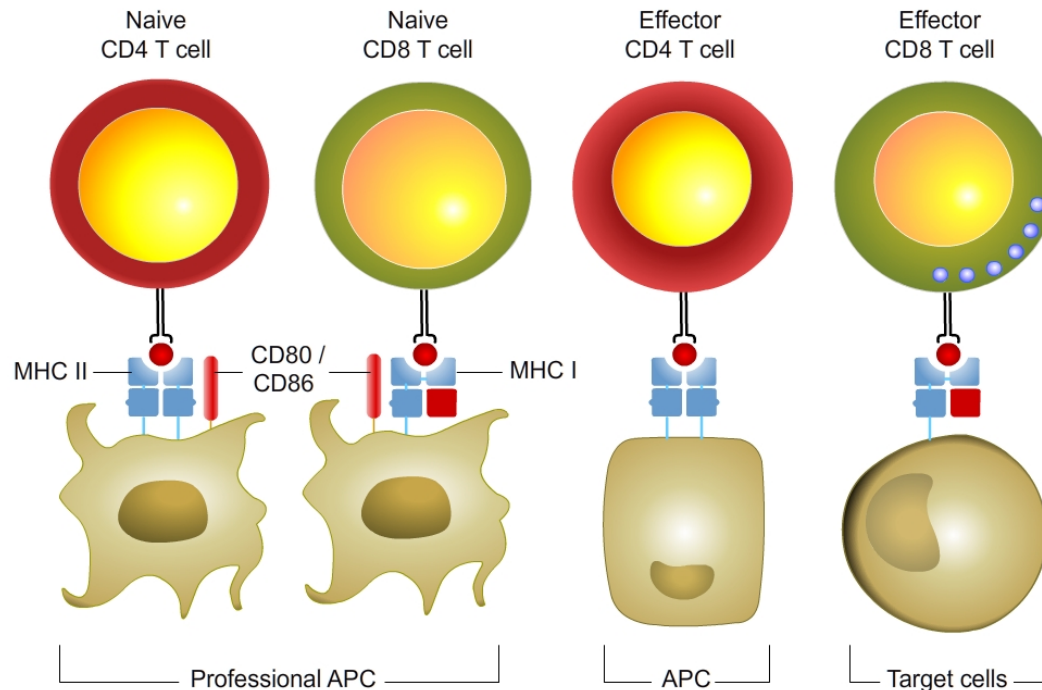
- ensures a high level of peptide presentation for an individual
- explains why two individuals are able to present and to respond to different antigens
- explains the restriction to the self: T cells recognize antigens presented by self MHC.
- provokes graft rejection between individuals

Characteristics of Major Histocompatibility Complex (MHC) molecules

Feature	Significance	
Codominant expression: Both parental alleles of each MHC gene are expressed	Increases number of different MHC molecules that can present peptides to T cells	T cells MHC molecules Parental chromosomes
Polymorphic genes: Many different alleles are present in the population	Ensures that different individuals are able to present and respond to different microbial peptides	
MHC-expressing cell types: Class II: Dendritic cells, macrophages, B cells Class I: All nucleated cells	CD4 ⁺ helper T lymphocytes interact with dendritic cells, macrophages, B lymphocytes CD8 ⁺ CTLs can kill any virus-infected cell	Dendritic cell Macrophage B cell Leukocytes Epithelial cells Mesenchymal cells

Which cells present antigen?

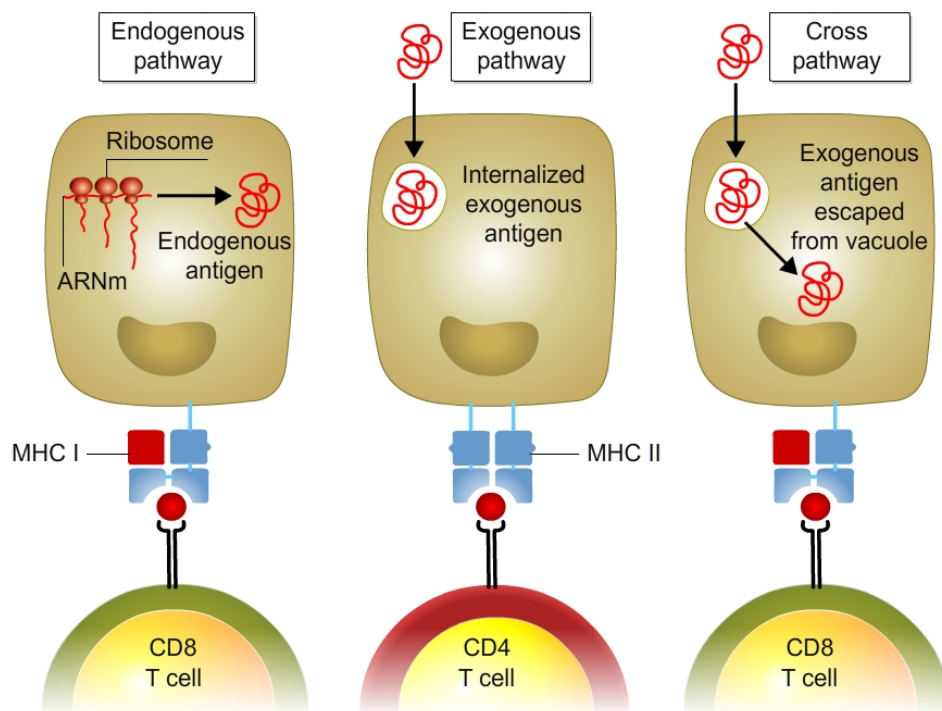
- All cells except red blood cells express MHC class I molecules, can present antigens and can be antigen-presenting cells.
- One restricts however the term **antigen presenting cells (APCs)** to dendritic cells, macrophages and B cells, which deliver a co-stimulatory signal and express MHC II.
- **Dendritic cells (DCs)** are the best professional APCs, since they are the only APCs able to present antigens to naïve T cells and promote their activation.
- **Target cells** refers to cells that present antigens in the context of MHC class I molecules in order to activate cytotoxic effector T cells and eventually be killed by them (e.g. infected or malignant cells)



How do cells present antigen?

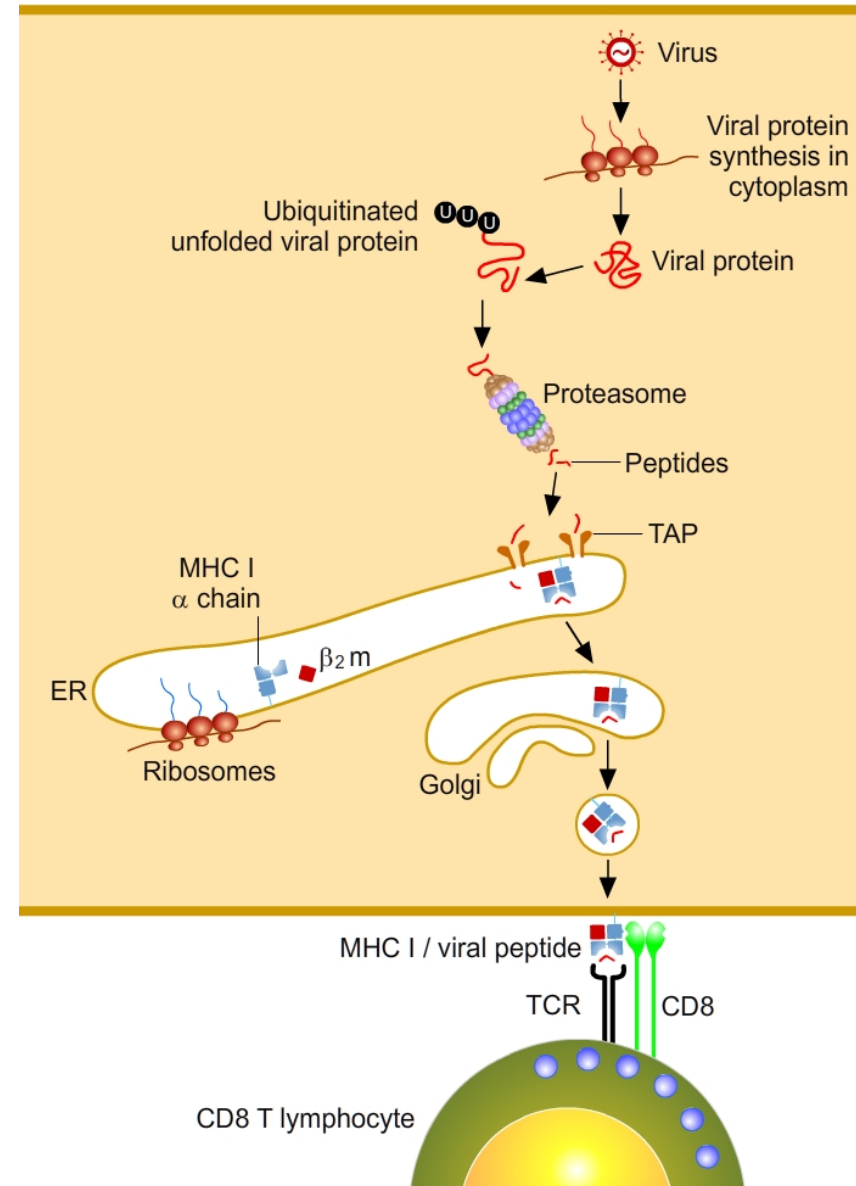
Cells have evolved three pathways for presenting antigen

- The **endogenous pathway** allows cells to present endogenously synthesized proteins on MHC class I molecules for recognition by CD8+ T cells.
- The **exogenous pathway** allows cells to present internalized exogenous antigens on MHC class II molecules for recognition by CD4+ T cells.
- The **cross pathway** allows cells to present exogenous antigens on MHC class I molecules for recognition by CD8+ T cells.



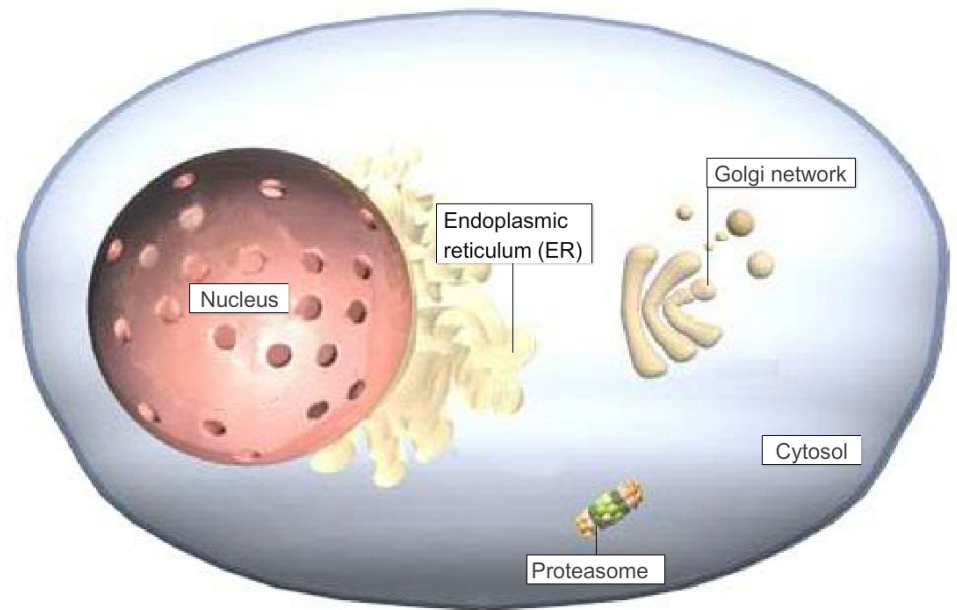
The MHC I presentation pathway: The biosynthetic pathway of MHC class I molecules

- MHC class I biosynthesis starts by the separate translation of the α and β_2m chains in the ER with the support of chaperone proteins.
- Cytoplasmic proteins derived from self or non-self proteins (virus) are unfolded, ubiquitinated, and degraded in proteasomes. The resulting peptides are transported by the TAP transporter into the endoplasmic reticulum (ER), where they bind to newly synthesized class I MHC molecules.
- The peptide-class I MHC complexes are transported to the cell surface and are recognized by CD8⁺ T cells.



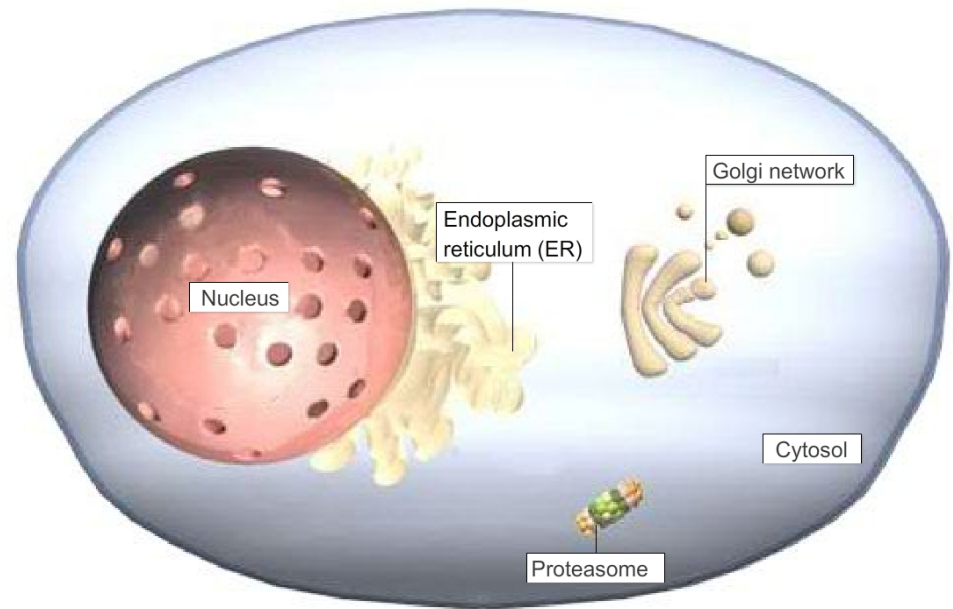
The MHC I presentation pathway: The biosynthetic pathway of MHC class I molecules*

MHC class I biosynthesis starts by the separate translation of the α and β_2m chains in the ER. Newly synthesized class I heavy chains associate with calnexin, a transmembrane ER chaperone protein that protects and stabilizes partly folded heavy chains until they assemble with β_2m . When β_2m binds to the heavy chain, calnexin is replaced by the "peptide-loading complex" including calreticulin, ERp57 and tapasin.



The MHC I presentation pathway: Peptide Degradation and Presentation*

Cytoplasmic self or non self (virus) proteins are unfolded, ubiquitinated, and degraded in proteasomes. The resulting peptides are transported by the TAP transporter into the endoplasmic reticulum (ER), and then bind to newly synthesized class I MHC molecules. The peptide-class I MHC complexes are transported to the cell surface and are recognized by CD8+ T cells.



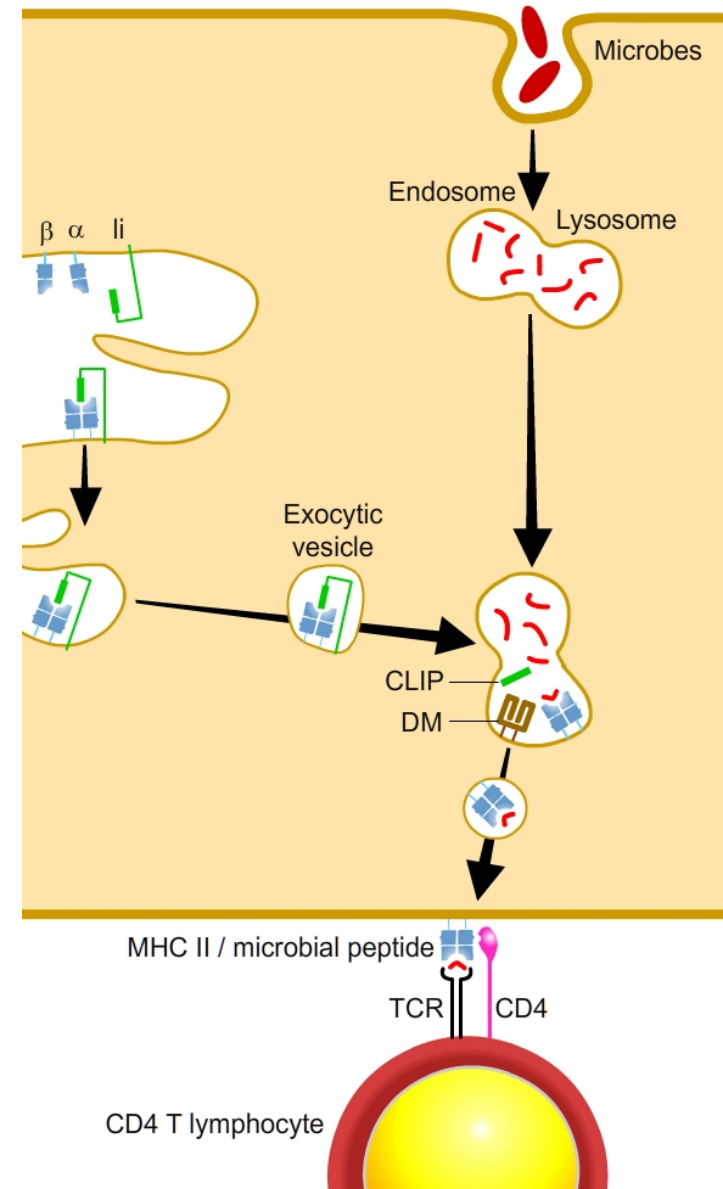
The MHC II presentation pathway

The biosynthetic pathway of MHC class II molecules

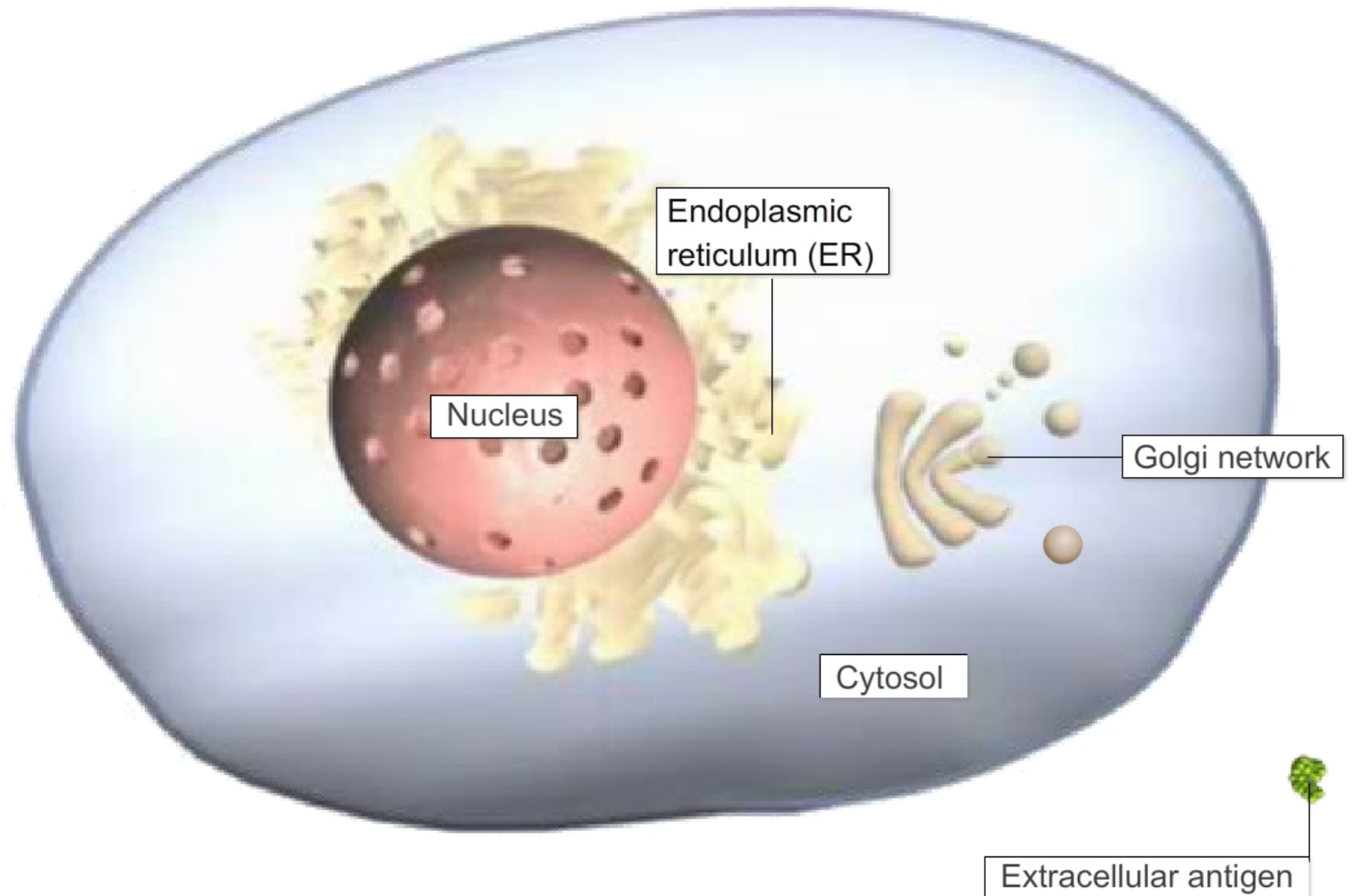
- MHC II molecules are made up of two transmembrane chains (α and β) synthesized and assembled in the ER with a third protein called the invariant chain (Ii). The Ii chain prevents the loading of peptides on class II molecules.
- The $\alpha\beta$ /Ii trimeric complexes are transported from the ER to the Golgi and then sent to the endocytic compartments. Here, Ii is degraded, leaving MHC II molecules free to acquire peptide from endocytosed antigens.

The endocytic protein-processing pathway

- Following internalization (endocytosis), the antigen is enclosed in an endosome that converts to a late endosome, in which the antigen unfolds under low pH. Their fusion with lysosomes creates a highly degradative environment which allows the denaturation and processing of endocytosed antigens into short antigenic peptides.
- In the endosomes, the chaperone HLA-DM promotes the dissociation of the CLIP peptide (a place holder peptide derived from Ii) from MHC class II which allows endosomal peptides to bind.

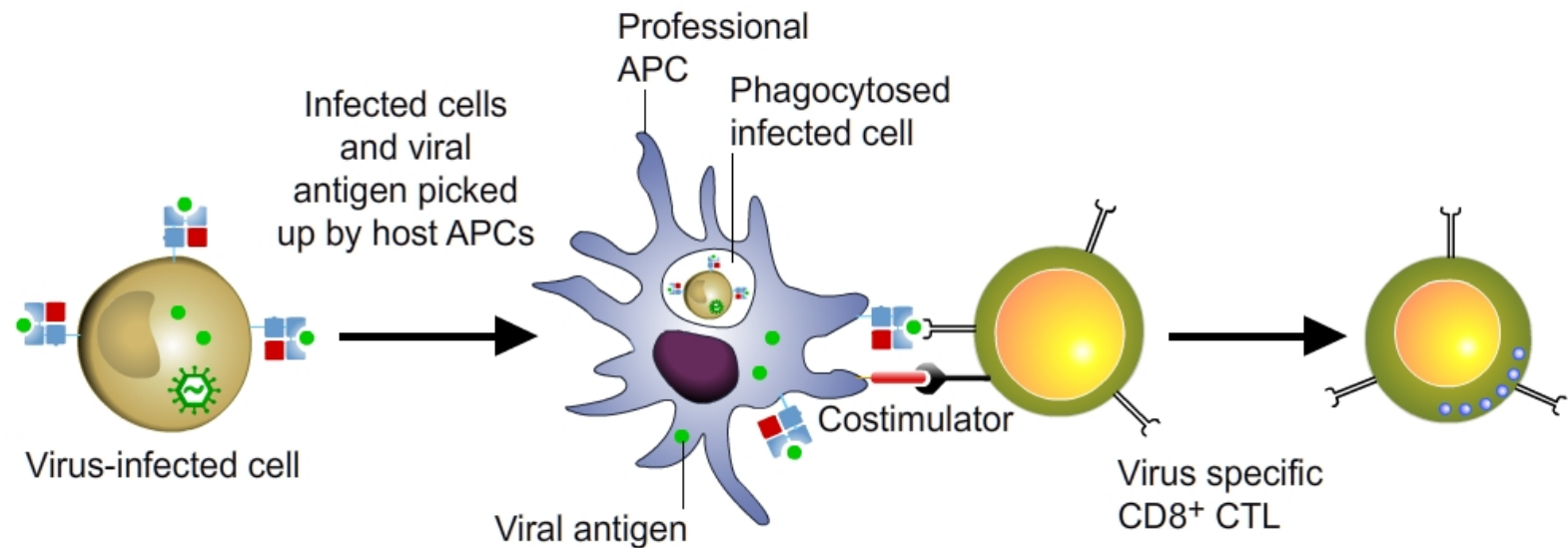


The MHC II presentation pathway*



The cross presentation pathway

- The cross presentation pathway is the process by which DCs present internalized exogenous antigens on MHC class I molecules for recognition by CD8⁺ T cells.
- It allows these cells to generate cytotoxic CD8⁺ T cells against viruses, when the DCs are not the target of the virus and thus do not express viral antigens.



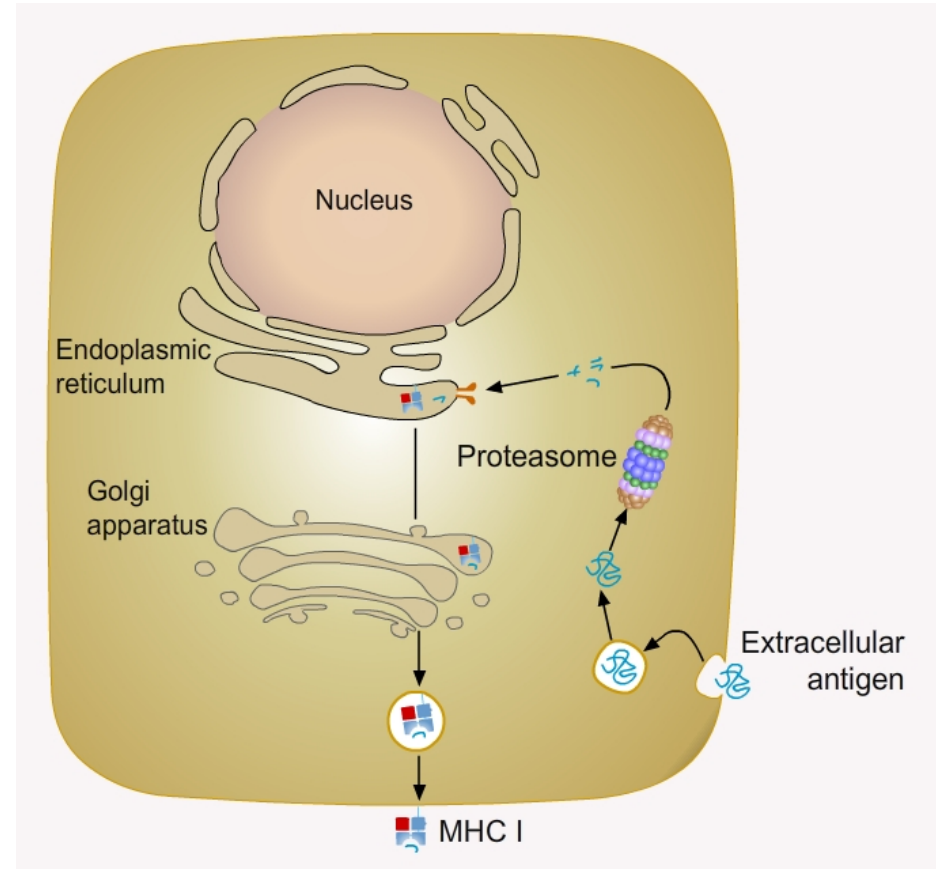
The cross presentation pathway

The cross-presentation pathway requires the encounter between:

- **MHC I molecules** generated in the endoplasmic reticulum (ER)
- **Peptides** derived from endocytosed antigens

This pathway requires exogenous antigens to be re-routed for loading on MHC I molecules, despite their initial sequestration in the endocytic compartments.

The mechanism is not yet fully established.



Conclusions | Antigen presentation

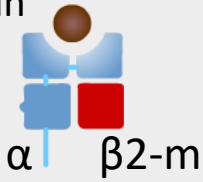
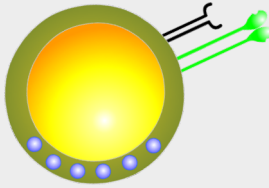
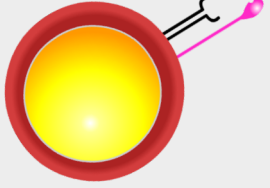
Antigen presentation can take place in different contexts

- In **Dendritic cells**, to initiate T cell responses
- In **B cells**, for B cell/T cell interactions
- In **macrophages**, for macrophage-T cell interactions
- In **any cell**, for the elimination of infected or malignant cells by CTLs
- **Antigen presenting cells (APCs)** (dendritic cells, macrophages and B cells) deliver a co-stimulatory signal and express MHC II.
- **Target cells** refer to cells that present antigens in the context of MHC class I molecules in order to activate cytotoxic effector T cells.

Cells have evolved **three pathways for presenting antigen**

- The **MHC I or endogenous pathway** allows cells to exhibit a representative fraction of their cytosolic content for inspection by MHC I-restricted cytotoxic CD8+ T cells.
 - Cytosolic antigens are degraded by the proteasome. Peptides are transported to the ER by TAP.
 - MHC I molecules acquire peptides in the ER while they are synthesized.
- The **cross-presentation pathway** is specific to DCs. It allows DCs to initiate CD8+ T cell responses against viruses that infect cells other than DCs.
- The **MHC II or exogenous pathway** allows DCs, B cells and macrophages to exhibit a representative fraction of their extracellular environment for inspection by MHC II-restricted helper CD4+ T cells.
 - Endocytosed antigens are degraded along the endocytic pathway
 - MHC II molecules acquire peptides in lysosome/late endosome on their way to the plasma membrane.

Summary: features of antigen processing pathways

Feature	Class I MHC pathway	Class II MHC pathway
Stable peptide-MHC complex	Polymorphic α chain β 2-microglobulin peptide 	Polymorphic α and β chains peptide 
APCs involved	All nucleated cells	DCs, macrophages, B cells, some endothelial cells, thymic epithelial cells
T cells responding	CD8+ T cells (CTLs) 	CD4+ T cells (Th cells) 
Source of protein antigens	Cytosol (synthesized in the cell or delivered from phagosomes)	Endosomes/lysosomes (internalized from exterior milieu)
Enzymes generating peptides	Cytosolic proteasome	Endolysosomal proteases (e.g. cathepsins)
Site of peptide loading on MHC	ER	Vesicular compartment
Transport and loading molecules	TAP	Invariant chain (Ii), DM

APCs, antigen-presenting cells; CTL, cytotoxic T lymphocyte; MHC, major histocompatibility complex; TAP, transporter associated with antigen processing. *Adapted from Abbas, Basic Immunology.*

Learning objectives

- Explain the differences between professional and non-professional APCs
- Describe how antigens are sampled in the skin, at mucosal surfaces and from blood
- Describe how antigens are processed for presentation to helper CD4 T lymphocytes
- Describe how antigens are processed for presentation to cytotoxic CD8 T lymphocytes
- Explain the differences between the MHC class I and class II pathways
- Identify the signals that up regulate the co-stimulatory molecules on professional APCs
- Describe the properties of immature and mature dendritic cells