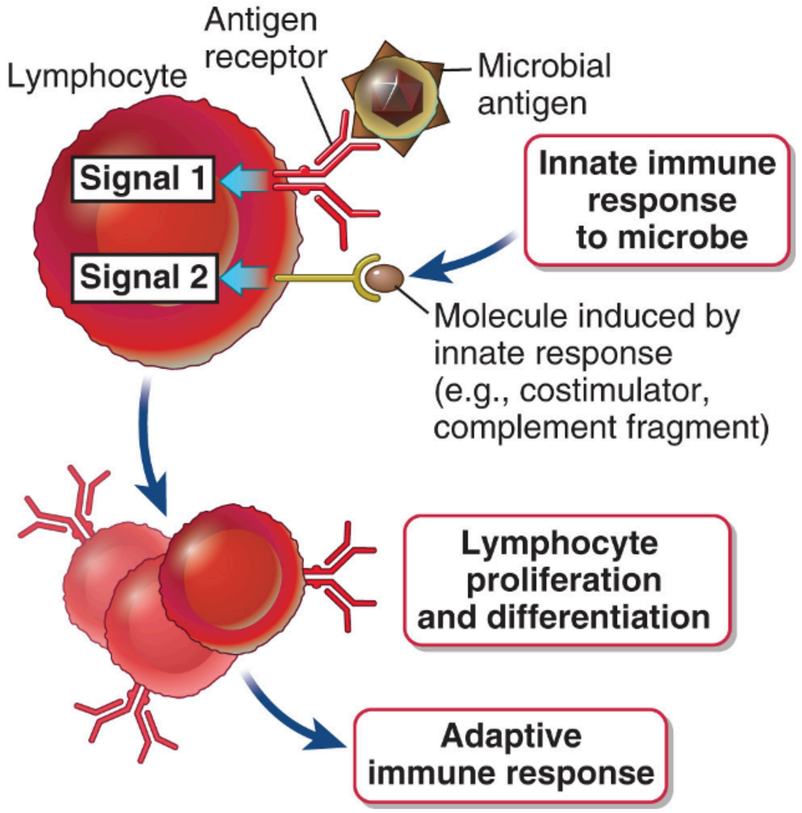
Antigen presentation to T cells and the functions of MHC molecules

Prof. Andrea Ablasser

Stimulation of Adaptive Immunity



Antigen recognition by T cells

Key principles for AG recognition by T cells

The odds of a naive T cell to recognise a specific AG is $1:10^6$

How does the immune system ensure that a specific AGs “finds” its corresponding T cell?

Enabled via **antigen capture** from the site of entry/production by **antigen-presenting cells** (APCs) that bring it to lymphoid organs where T cells circulate

Key principles for AG recognition by T cells

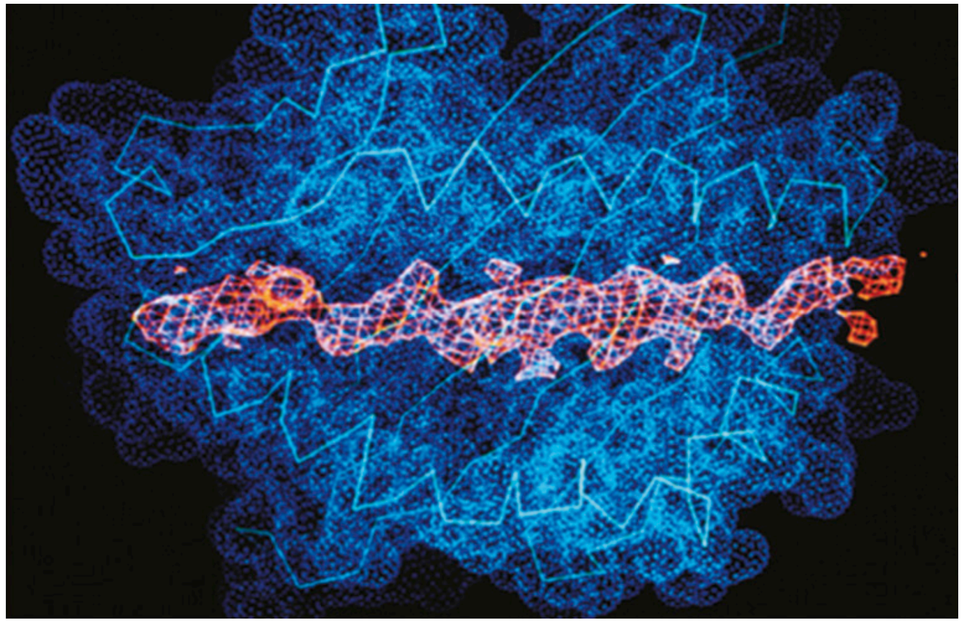
Major function of T cells is to eradicate intracellular microbes and to activate other cells (macrophages, B cells)

How does the immune system ensure that a T cell is only activated by cell-associated AGs?

Specialized proteins called *major histocompotbility complex (MHC)* are expressed on the surfaces of host cells and display host-associated antigens

Antigens (AG) recognised by T cells

How do T cells see AG?

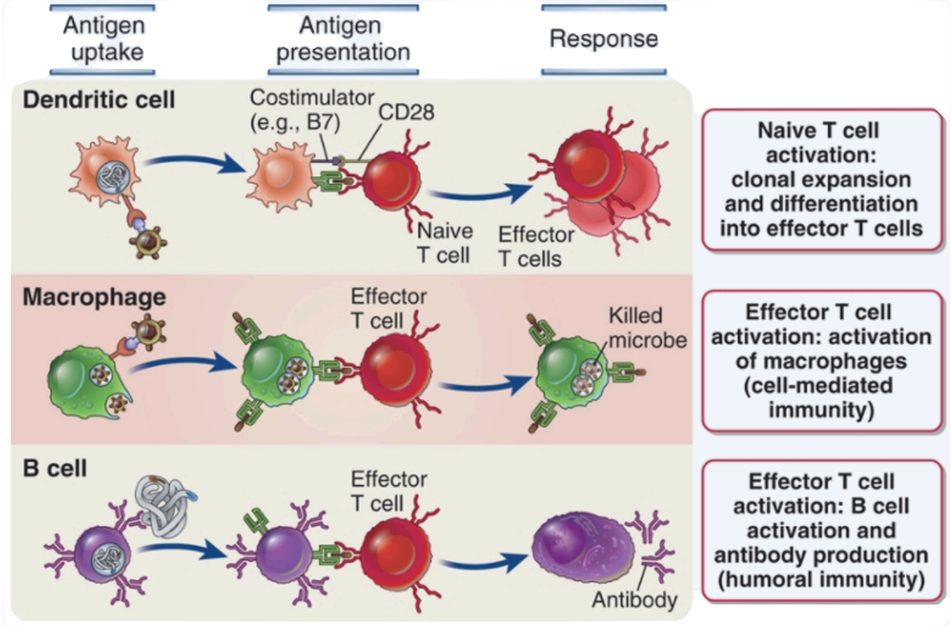


- Short, mostly linear *peptide* sequence (from foreign protein antigen)
- Specific *MHC* molecules with bound antigens (only one combination)

How do APCs capture AG and how do they transport this to T cells?

General features of (professional) APCs

- Activate naive T cells or previously differentiated effector T cells
- **DCs** are the most effective for activating *naive T cells*
- **Macrophages and B lymphocytes** mostly activate *CD4+ helper T cells*
- Display *peptide-MHC complexes* for recognition by T cells (**first signal**)
- Provide additional *costimulators* required for full responses of T cells (**second signals**)
- Secrete *cytokines* important for T cell differentiation
- Exposure to microbes increases their antigen presenting functions
- Receive s*ignals back from lymphocytes* that enhance their antigen-presenting functions



Dendritic cells (DCs)

Classical DCs: present in most *epithelia* that interface the external environment

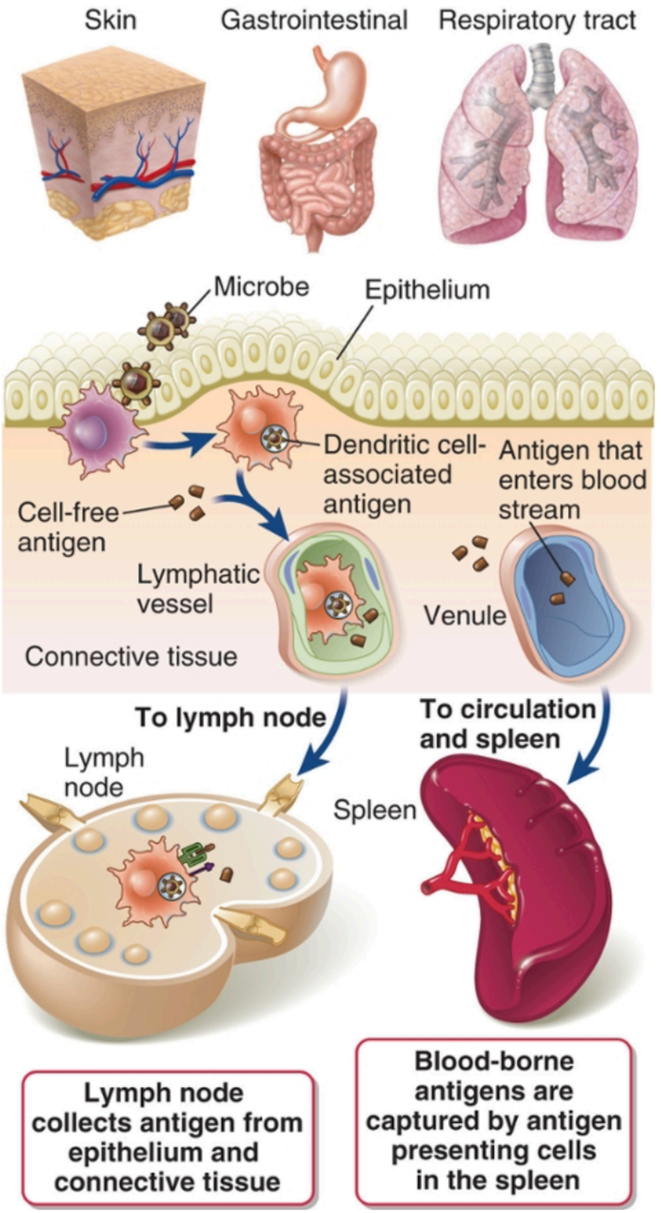
➔ transport antigens to the *lymph* (also present in cell-free form), where they get concentrated in *lymph nodes* (= filters sampling the lymph before reaching the blood)

Plasmacytoid DCs: *circulating*

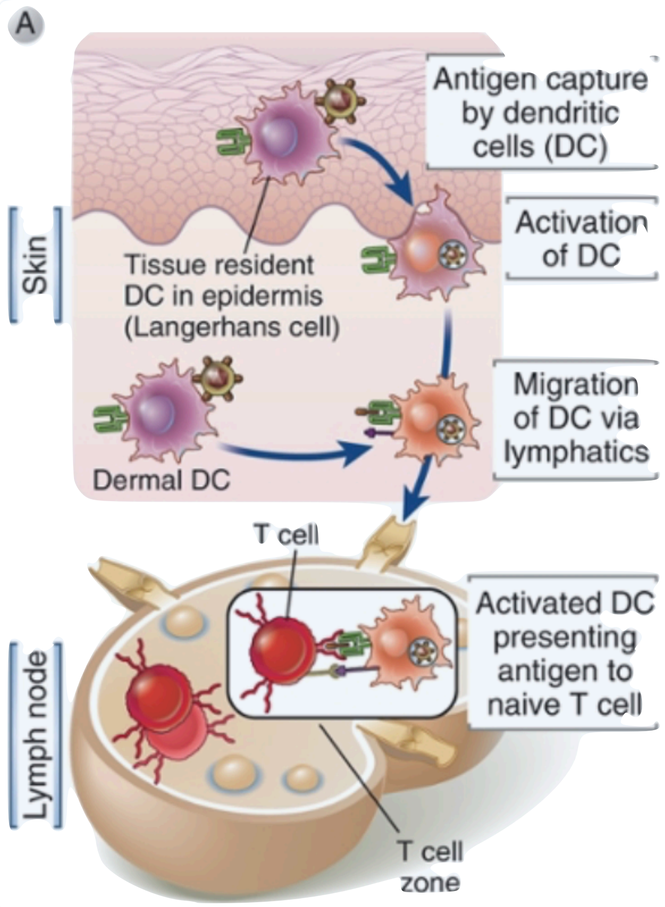
➔ sample antigens in the *bloodstream* (also spleen resident DCs) and taken them to the *spleen*

Most efficient for initiating *primary T cell responses*:

- Strategical location
- Receptors: capture and respond to microbes
- Migrate via lymphatics into lymph node T cell zone
- High levels of peptide-MHC complexes, costimulatory and cytokines



Maturation of DCs



B

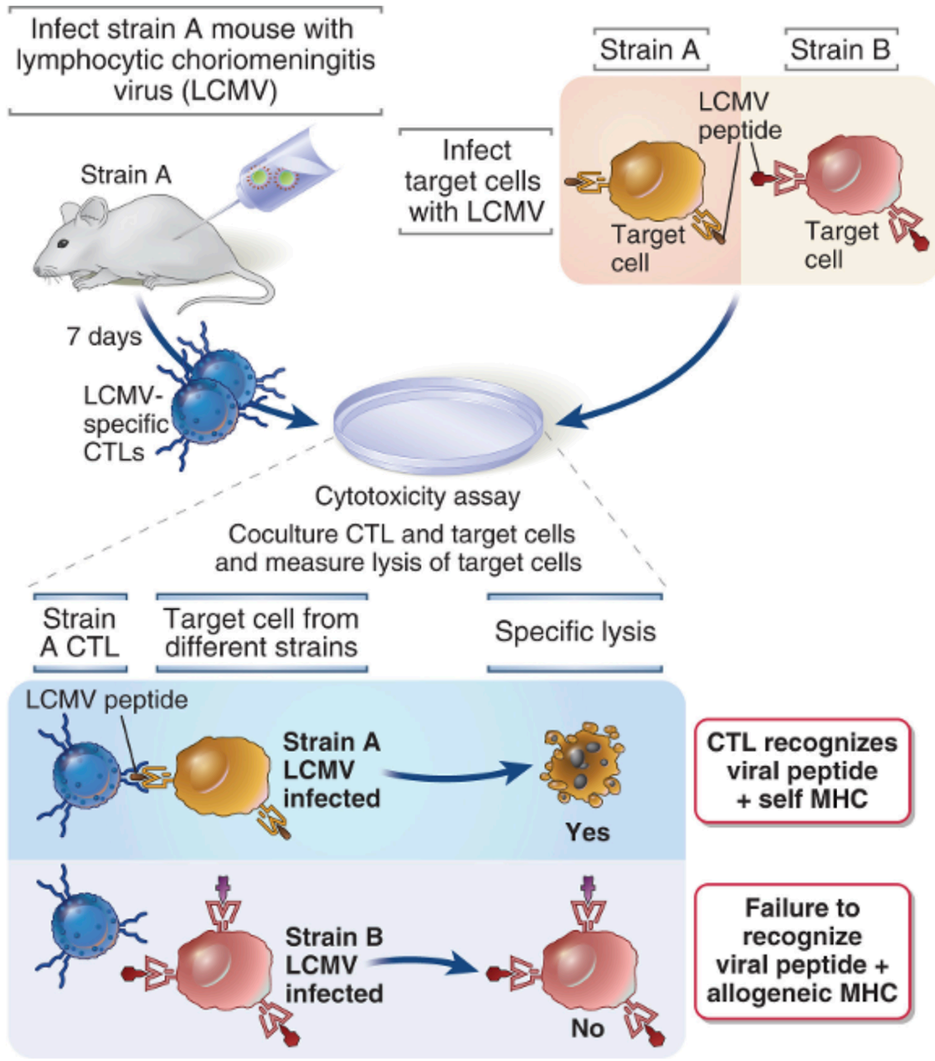
	Tissue resident resting dendritic cell	Activated dendritic cell
Principal function in T cell activation	Antigen capture	Antigen presentation to T cells
Expression of Fc receptors, mannose receptor	++	–
Expression of molecules involved in T cell activation: B7-1, B7-2, ICAM-1, IL-12	– or low	++
Class II MHC molecules		
Half-life	~10 hr	>100 hr
Number of surface molecules	~10^6	~7×10^6

Functions of other antigen-presenting cells

- **Cell-mediated immunity**: antigen promotion of phagocytes microbes by macrophages to effector T cells (enhances *microbicidal activities of macrophages*)
- **Humoral immunity:** B lymphocytes internalise antigens and present peptides to helper T cell (essential for *T cell-dependent antibody production*)
- **All nucleated cells**: present peptides from cytosolic protein antigens to CD8+ *CTLs* (all susceptible to viral infections and cancer)
- **Some endothelial and epithelial cells** can express class II MHC and may present antigen to T cells (e.g. thymic epithelial cells constitutively express MHC molecules and play a critical role in presenting complexes *to maturing T cells in the thymus*)

The Major Histocompatibility Complex

Discovery of MHC restriction



The human major histocompatibility complex (MHC)

"Genes that determine the *fate of grafted tissue*, present in *all mammalian species.*"

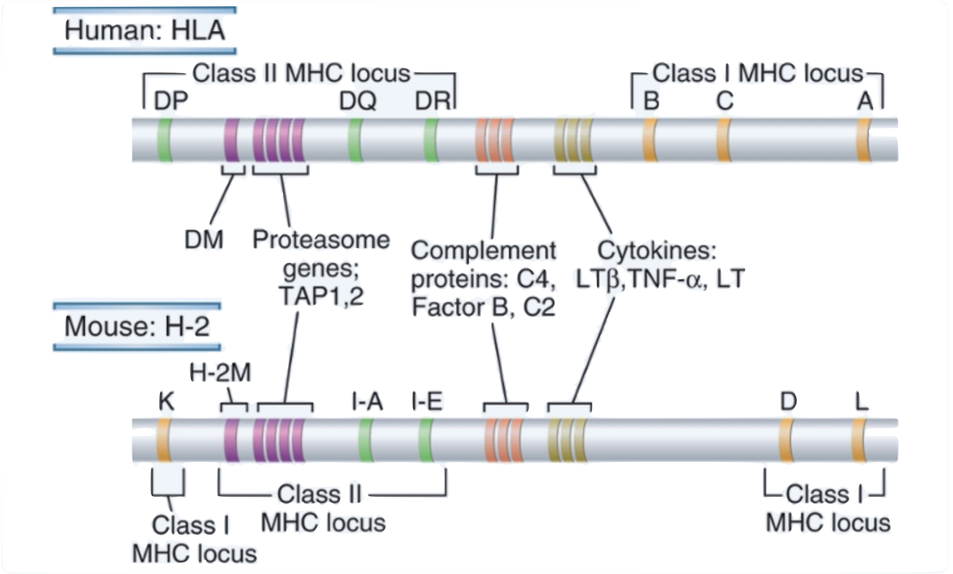
- **MHC restriction**: T cells specific not only for antigen but also for MHC molecules
- **MHC locus:** contains 2 types of polymorphic *MHC genes class I and class II* + other nonpolymorphic genes involved in antigen presentation (TAP1/2)
- MHC genes are the *most polymorphic genes* in any mammalian genome (> 10000 alleles for class I and 3000 for class II)
- **Variations** result from *inheritance* of distinct DNA sequences
- The products of different MHC molecules *bind and display different peptides*
- MHC polymorphism may have evolved to ensure that human populations will be able to *deal with highly diversified microbes* and to be protected from devastating loss of life from emerging infections

The human major histocompatibility complex

MHC genes are *co-dominantly* expressed in each individual (alleles from both parents are expressed, maximising th
molecules available to bind peptides).

Three class I MHC genes: *HLA-A, HLA-B, HLA-C*
Three class II HLA loci: *HLA-DP, HLA-DQ, HLA-DR*
Each class II MHC: composed heterodimer of a and b polypeptides

MHC haplotype: set of MHC alleles present on each chromosome



MHC class I and class II

- Class I MHC:
  - expressed on *all nucleated cells*
  - display endogenous (*viral and tumor)* antigens for recognition by CTLs
  - constitutive expression is increased by *type I interferons* (IFN-a and IFN-b) and IFN-y

- Class II MHC:
  - expressed on *DCs, B lymphocytes, macrophages*, thymic epithelia cells and few others
  - regulated mainly by *IFN-y* produced early by NK cells and later by activated T cells
  - increased expression in response to *TLR* signalling

- **Class II transcription activator (CIITA):** master regulator of MHC gene expression

Tissue	MHC class I	MHC class II
Lymphoid tissues		
T cells	+++	+*
B cells	+++	+++
Macrophages	+++	++
Dendritic cells	+++	+++
Epithelial cells of thymus	+	+++
Other nucleated cells		
Neutrophils	+++	–
Hepatocytes	+	–
Kidney	+	–
Brain	+	–†
Nonnucleated cells		
Red blood cells	–	–

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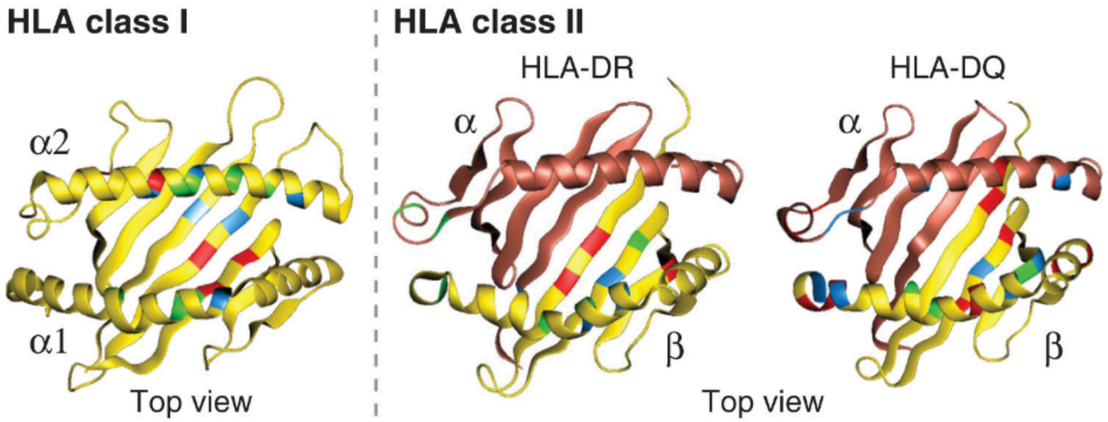
Structure of MHC molecules

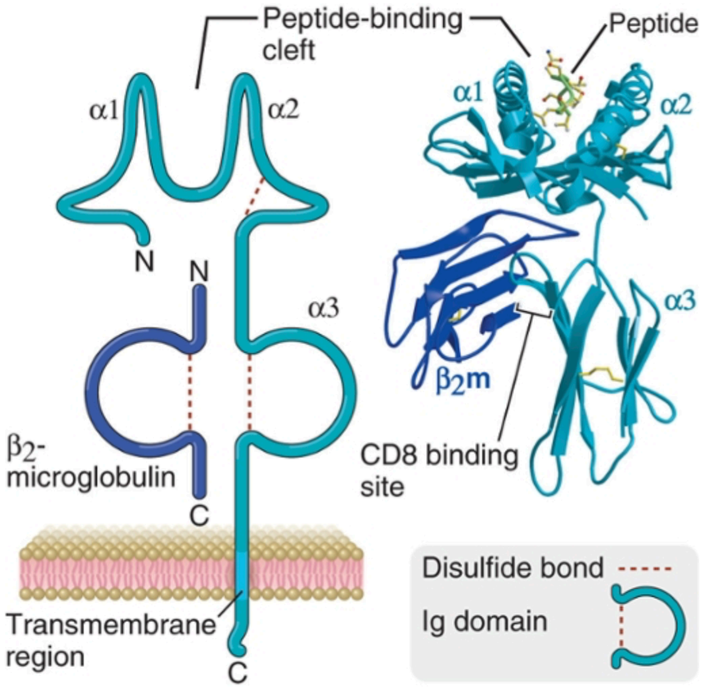
General properties of MHC molecules

- Each MHC molecule comprises an extracellular peptide-binding cleft, an Ig-like domain, a transmembrane domain, and cytoplasmic domains
- The polymorphic amino acid residues are located in the area of the peptide binding cleft
- The non-polymorphic Ig-like domains interact with T cell co-receptors (CD4/CD8)



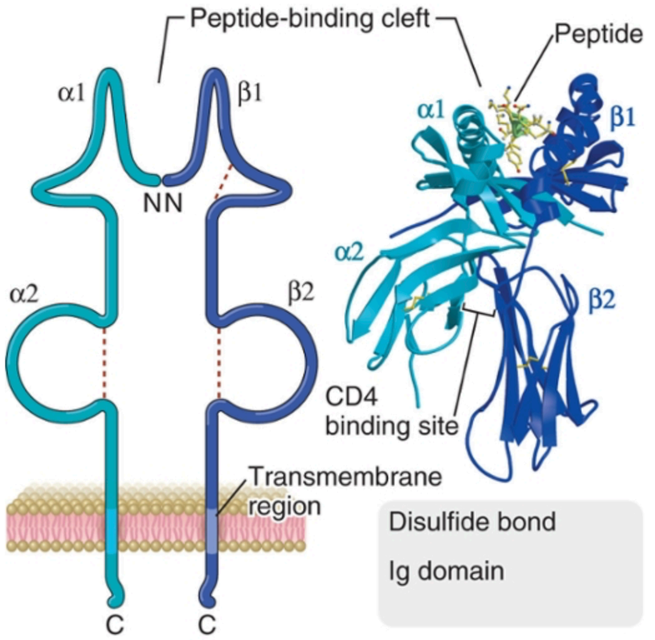
Class I MHC molecules

- 2 non-covalently linked chains: a *alpha chain* + non-MHC encoded *beta2-microglobulin (B2M)*
- b2m = is encoded outside MHC gene, structurally homologous to Ig-like domain and *invariant*.
- Polymorphic residues around peptide-binding cleft contribute to variations among alleles
- Hydrophobic sequence at C-terminus that spans the membrane
- Trimeric complex requires presence of *all three components* (a chain, b2m and bound peptide) to ensure only *useful peptide-loaded MHC* molecules are expressed on cell surfaces
- Most individuals are heterozygous for MHC genes ➔ express *6 class I molecules per cell* with a chains encoded by 2 inherited allele.



Class II MHC molecules

- 2 non-covalently associated chains (a + b), which are *both polymorphic* genes
- N-termini *interact* to form the peptide binding cleft where polymorphic residues reside
- *Stable expression* on cell surface requires *presence of all components* of the trimer (both chains + bound antigen peptide)
- Individuals inherit from each parents one DPA and one DPB gene encoding a and b chains and an HLA-DP molecules one DQA and DQB one DRA and one or two DRB genes ➔ 6-to-8 pairs of class II HMC molecules



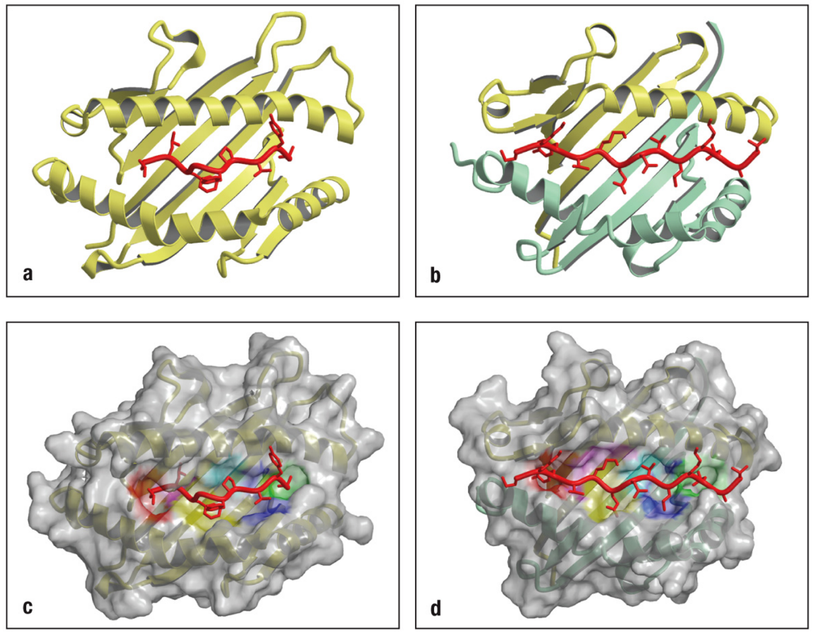
Stabilization of MHC molecule by binding of the peptide

Special feature of MHC molecules: should be able to bind to a *variety of distinct AGs*

➔ *Different from other peptide receptors!*

Binding of peptides:

1. MHC molecules are **unstable if not bound to peptide** (important: the binding of a particular AG to MHC is kept and cannot be exchanged)
2. Peptide is an **integral part of the MHC molecule**!



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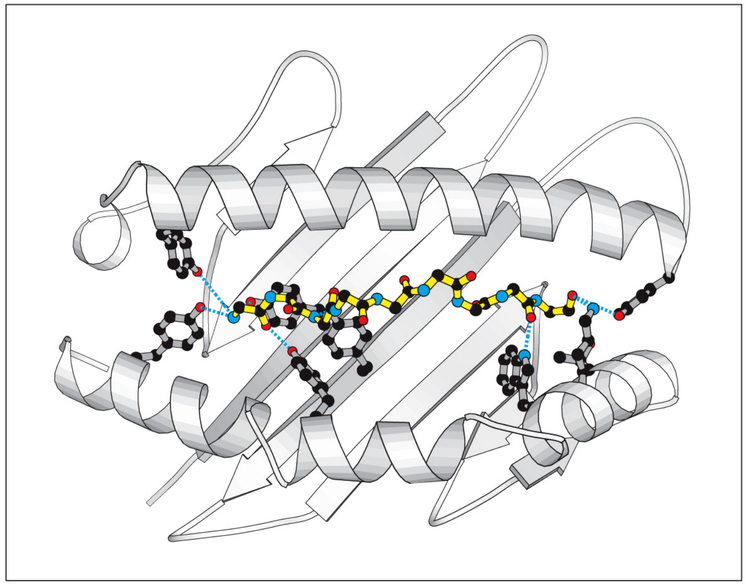
Specificities of MHC class I molecules

Length of peptide for MHC class I: usually *8-10 AS* long

- **Invariant sites** at the end of the cleft: bind to free groups at the N- and C-terminal part of the peptide

Two important features that result from the interaction mechanism:

1. Individual MHC class I molecules can bind a *broad spectrum of peptides*
2. *Allelic variants* of MHC class I bind to a *distinct spectrum* of peptides



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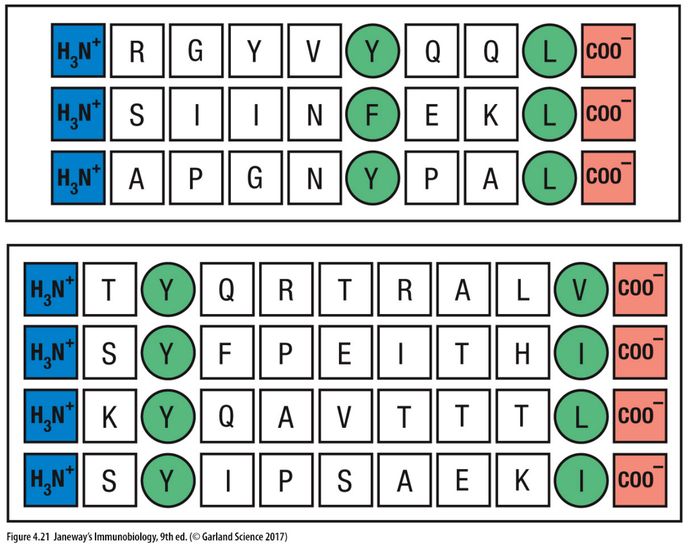
Specificities of MHC class I molecules

Polymorphic site of MHC molecules within the *interaction regions* with the peptides!

Peptides that can bind to a given MHC class I molecule share specific AS in their sequence ➔ *anchor residues*.
Other important residues also exist, called “secondary anchors”

Position + identity of these residues varies in between different MHC molecules



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Anchor residues shown as green circles

Specificities of MHC class II molecules

MHC class II molecules are *unstable* if not bound by peptide

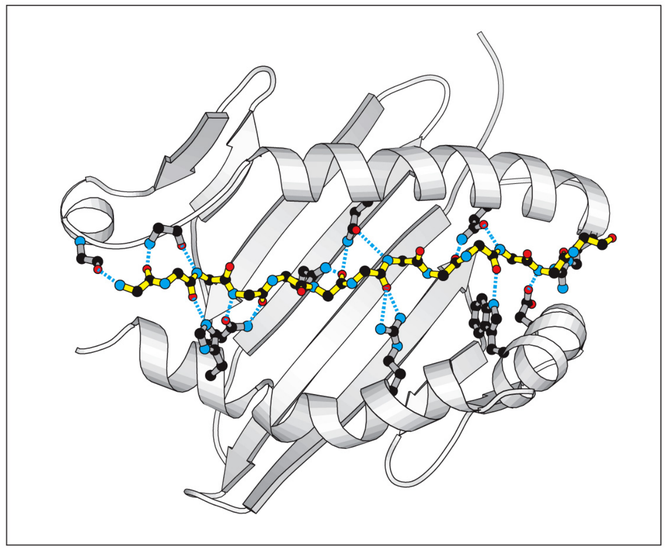
⚠ Interaction different from MHC class I: binding of peptides with minimal length of 13 AS, potentially even longer (17AS,..).

Peptides are *not fixed* at their end, but can emerge *out of the binding cleft*.

There are **no clear anchor residues**, instead binding accomplished by interaction with peptide backbone.

➔ MHC class II molecules can interact with a variety of distinct peptides.

Ex: during synthesis in the ER, MHC class II loaded with *invariant chain* (protein)



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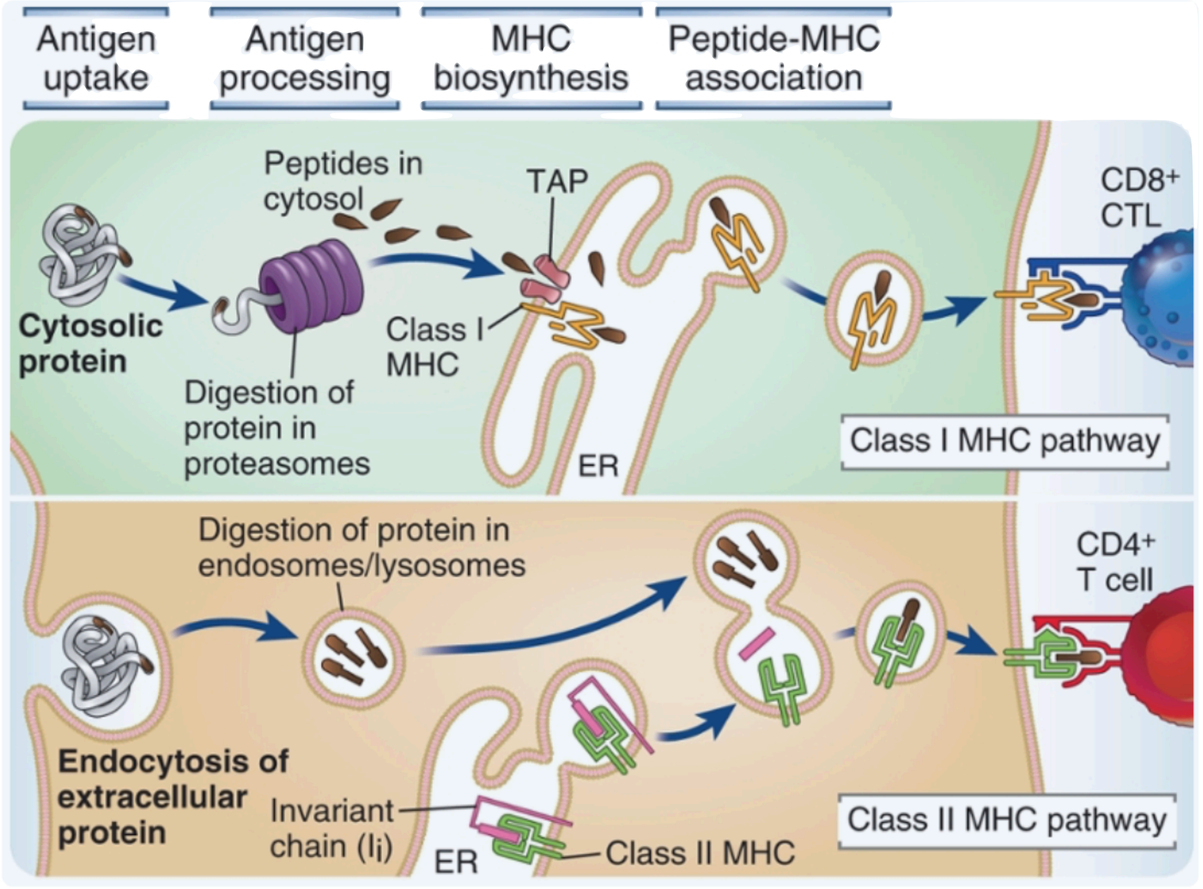
Processing of Protein Antigens

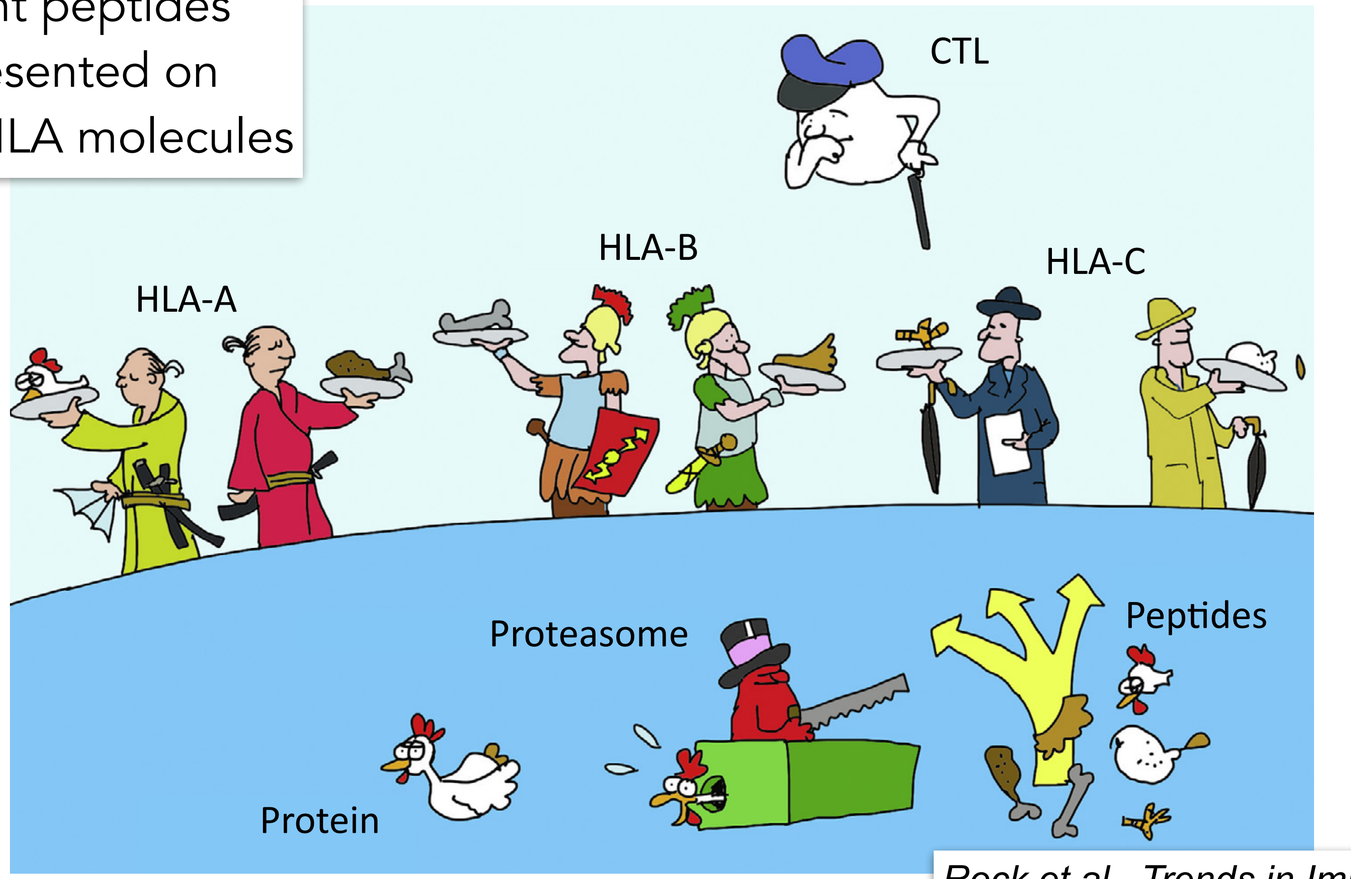
Processing of protein antigens

Antigen processing: conversion of proteins *antigens into peptides* with structural characteristics required for their l
molecules during biosynthesis, which is requires for stable complex assembly.



Class I MHC processing and presentation of cytosolic proteins

Different peptides are presented on different HLA molecules



Rock et al., Trends in Immunology 2016

Source of protein antigens: *viral* proteins, proteins from *internalised microbes* that escaped into the cytosol, proteins that are secreted into the cytosol, endogenous misfolded proteins (stressed cells, tumor cells)

Degradation of proteins by the proteasome

Cytosolic protein degradation: carried out by a large multi-catalytic protease complex = ***proteasome***

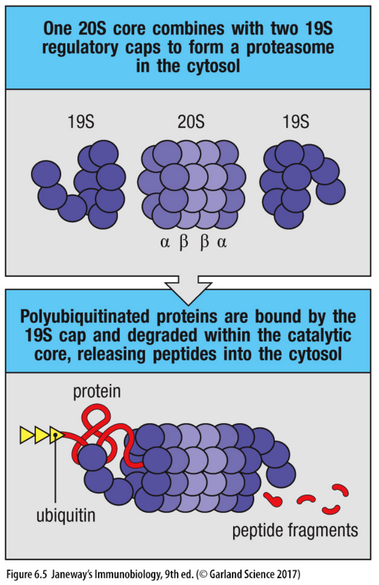
Structure: one 20S catalytic core and two 19S regulatory caps.

Ubiquitin-proteasome system = *UPS* responsible for protein degradation. Proteins tagged by *ubiquitin chains* = min. 4 ubiquitin molecules linked together via K48 residues

K48-linked ubiquitin chains recognized by the *19S* subunit of proteasome ➔ Protein **unfolded** and the *20S* subunit **degrades** it in *sequence-independent* manner.

⇓

Peptide fragments generated = *ligands* for **MHC class I** molecules!



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Degradation of proteins by the proteasome

Special proteasomes

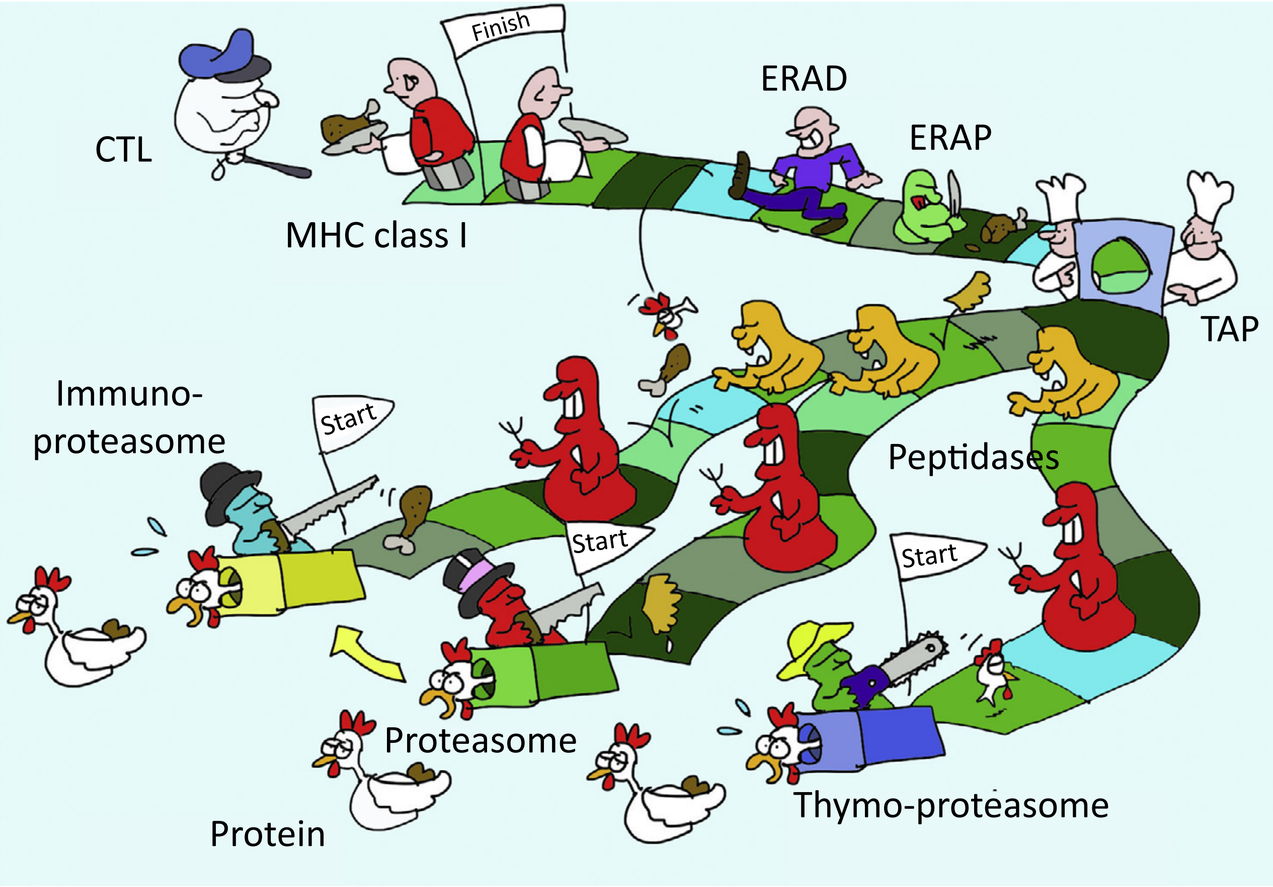
- **Interferons** can *induce* specific *subunits* of proteasome (PSMB9/PSMB8 etc)
  ➔ IFN-induced proteasome = **immunoproteasome**

  Preferences for **cleaving** after *hydrophobic residues*, thus preparing "better" suited ligands for **MHC class I**
- **Thymus**, another specific proteasome = **thymoproteasome**
  Presumably for the **development** of *CD8+ T* cells.
- IFN-γ induce **PA28 proteasome-activator complex:** increases the flux of *peptide processing*

Defective ribosomal products

DRiPs presented by **MHC class I**
➔ Include peptides translated from *introns*, translation of *frameshift* and *improperly folded* proteins.

Peptide processing (I)



Transport of peptides through TAP into ER

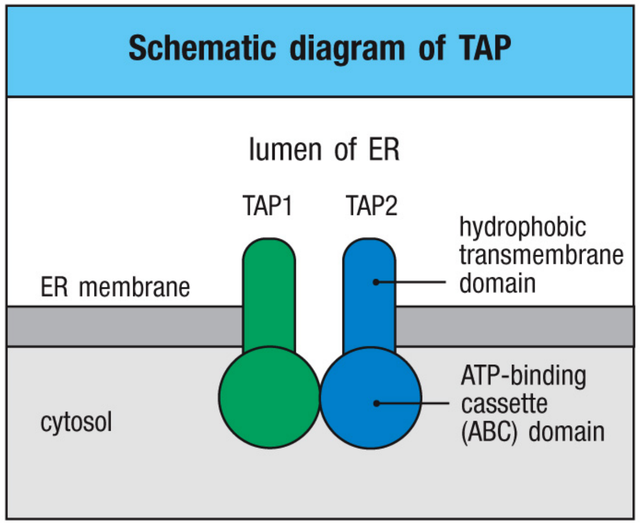
Peptides produced in the cytosol must be transported into the **ER lumen**

➔ Accomplished by two **ATP-binding cassette** = *ABC* proteins named TAP1 and TAP2 (= **transporter associated with antigen processing 1** and **2**)

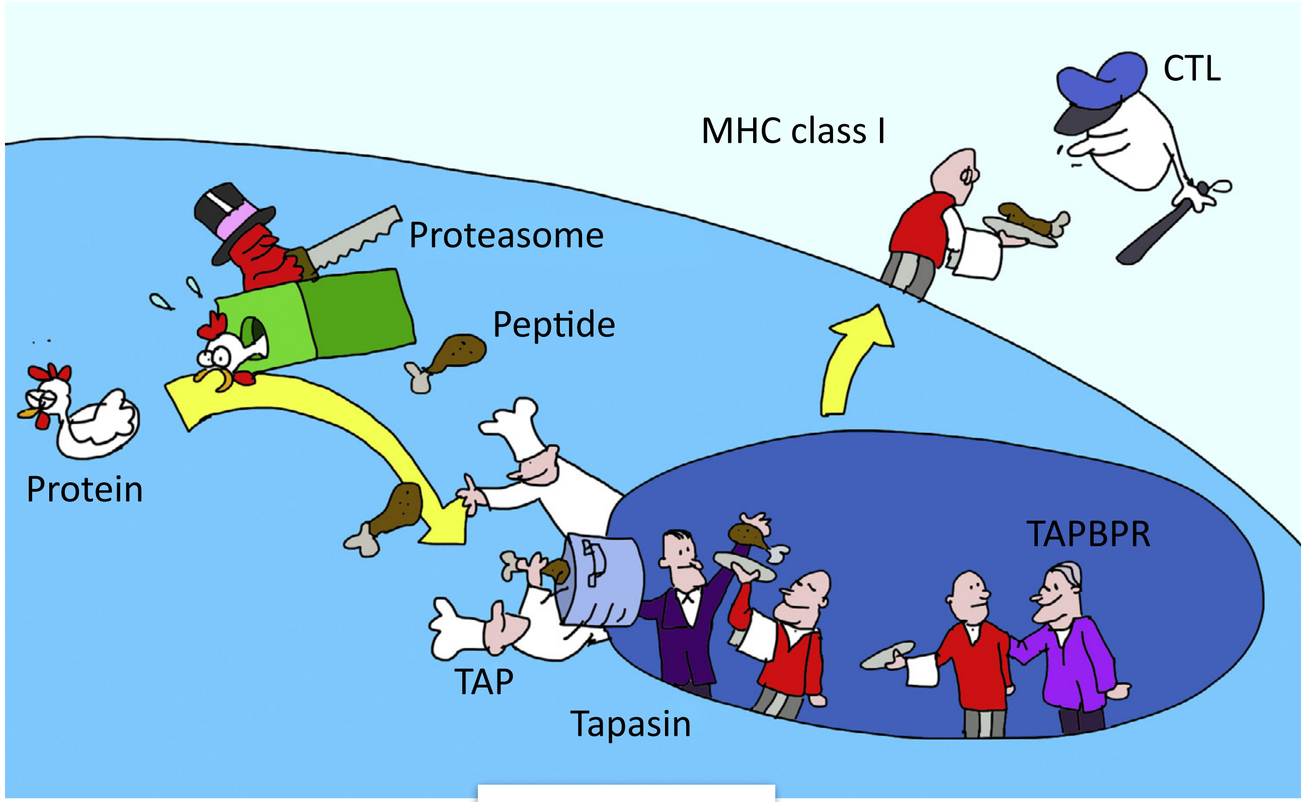
TAP proteins = **heterodimers**!

Transport capacity: peptides ~ *8-16* aa, but in general lack and sequence-specificity (preference for hydrophobic C-termini)

Peptides too long trimmed at *N-terminal* end by enzyme **endoplasmic reticulum aminopeptidase associated with antigen processing** = *ERAAP*



Loading of MHC I complex in the ER



Trimming by ERAAD

MHC I “chaperoned”

Loading of MHC I complex in the ER

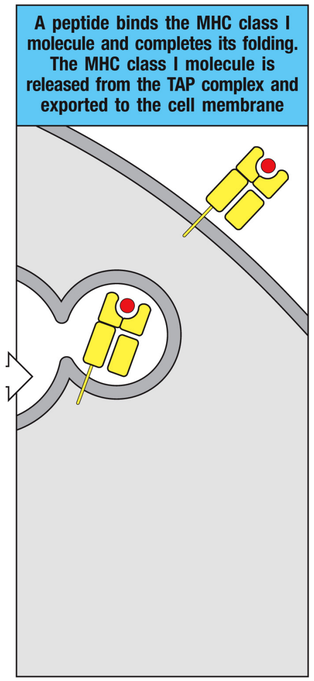
Once **peptide is bound**: MHC class I-peptide complexes transported to the *cell surface*

Most **peptides not bound** ➔ transported back into the *cytosol* involving **SEC61**

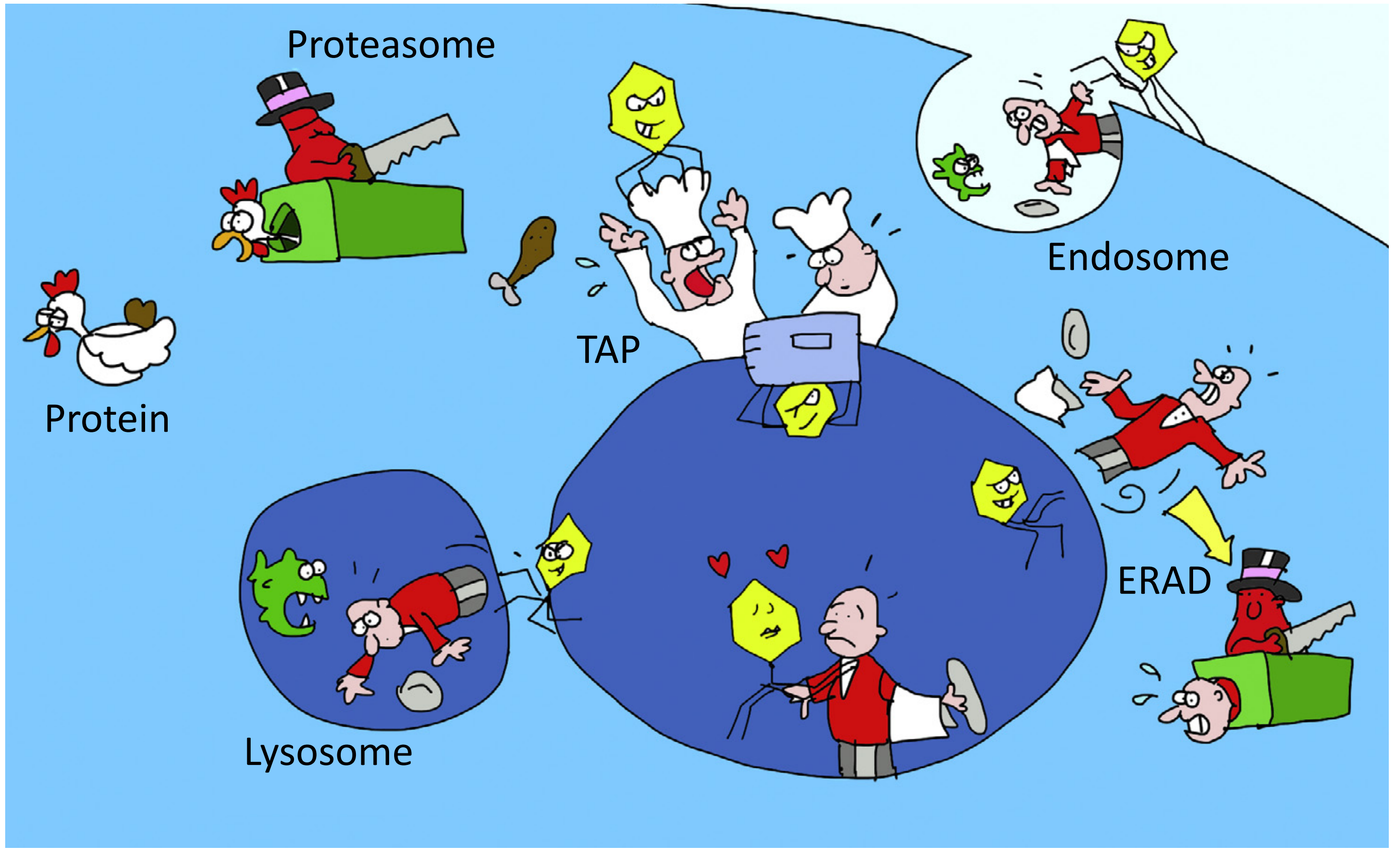
If MHC I class I molecule does not bind peptide ➔ transported back into the *cytosol* via **endoplasmic reticulum-associated protein degradation** = *ERAD*

↳ Retrotransport involves **ubiquitination** and **UPS** ➔ *degrades* misfolded proteins in cytosol.

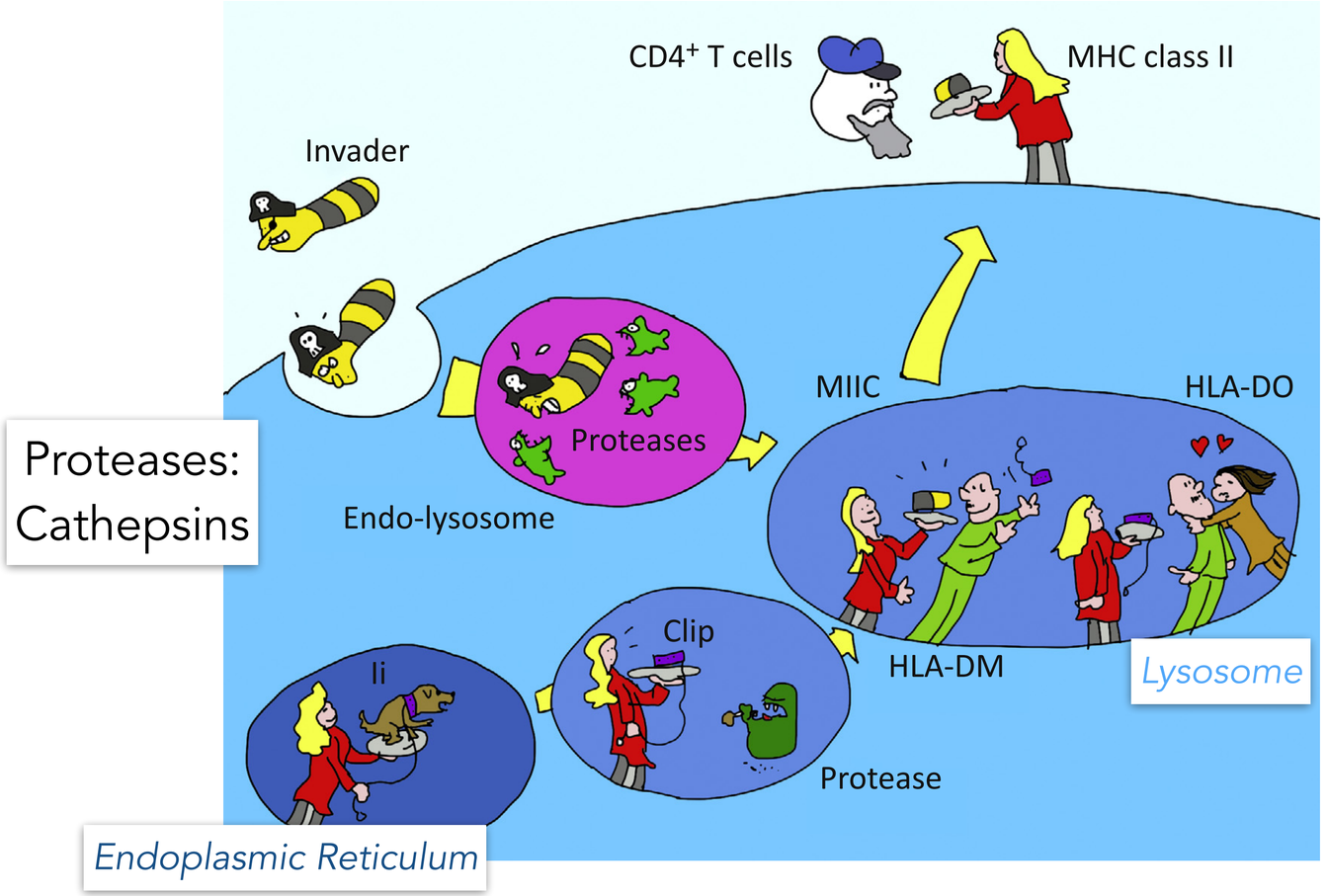
In uninfected cells: MHC class I molecule present in **“free” form** ➔ ensures that these can readily bind to peptides in case of an *infection*



Viral Immune Evasion strategies target MHC I AG presentation



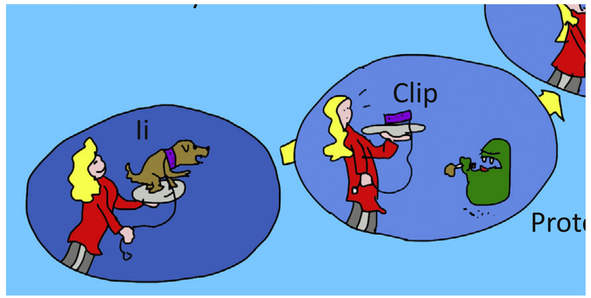
Class II MHC processing and presentation of exogenous proteins



Proteases: Cathepsins

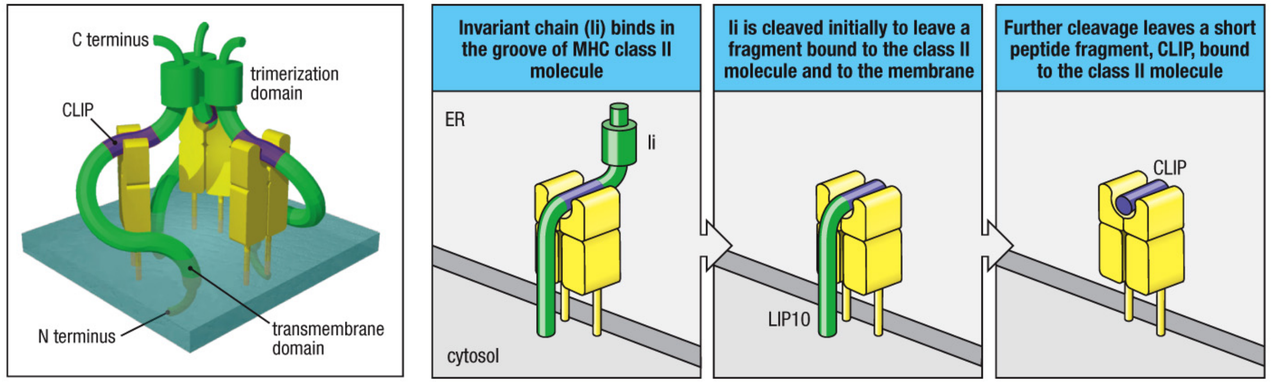
Source of protein antigens: extracellular proteins, cell surface sorted/endocytosed, intracellular proteins that may be membrane-bound, vesicular or involved in autophagy

The invariant chain



Function:

- Ii binds **non-covalently** to MHC class II molecules through the **CLIP** (class II-associated invariant chain peptide) domain
- >>> **prevents peptide binding to MHC II in the ER**
- Ii **directs MHC II molecules to acidic compartments**



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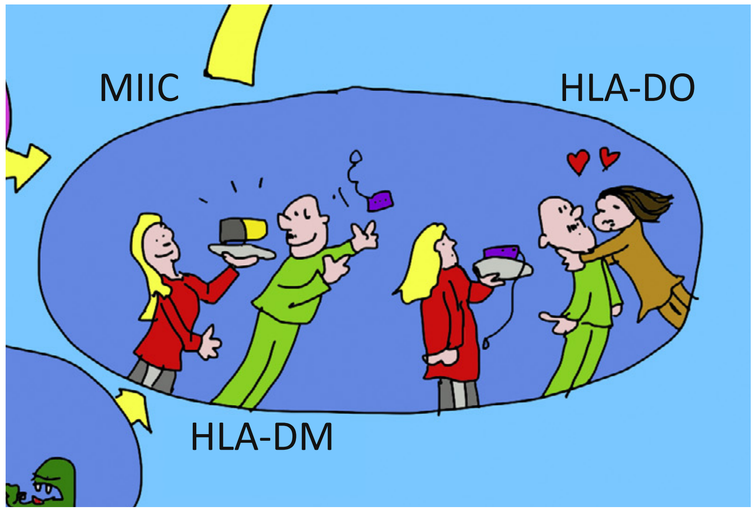
Regulation of CLIP exchange by HLA-DO and HLA-DM



Inhibitory equivalent of HLA-DM: *HLA-DO*

HLA-DM

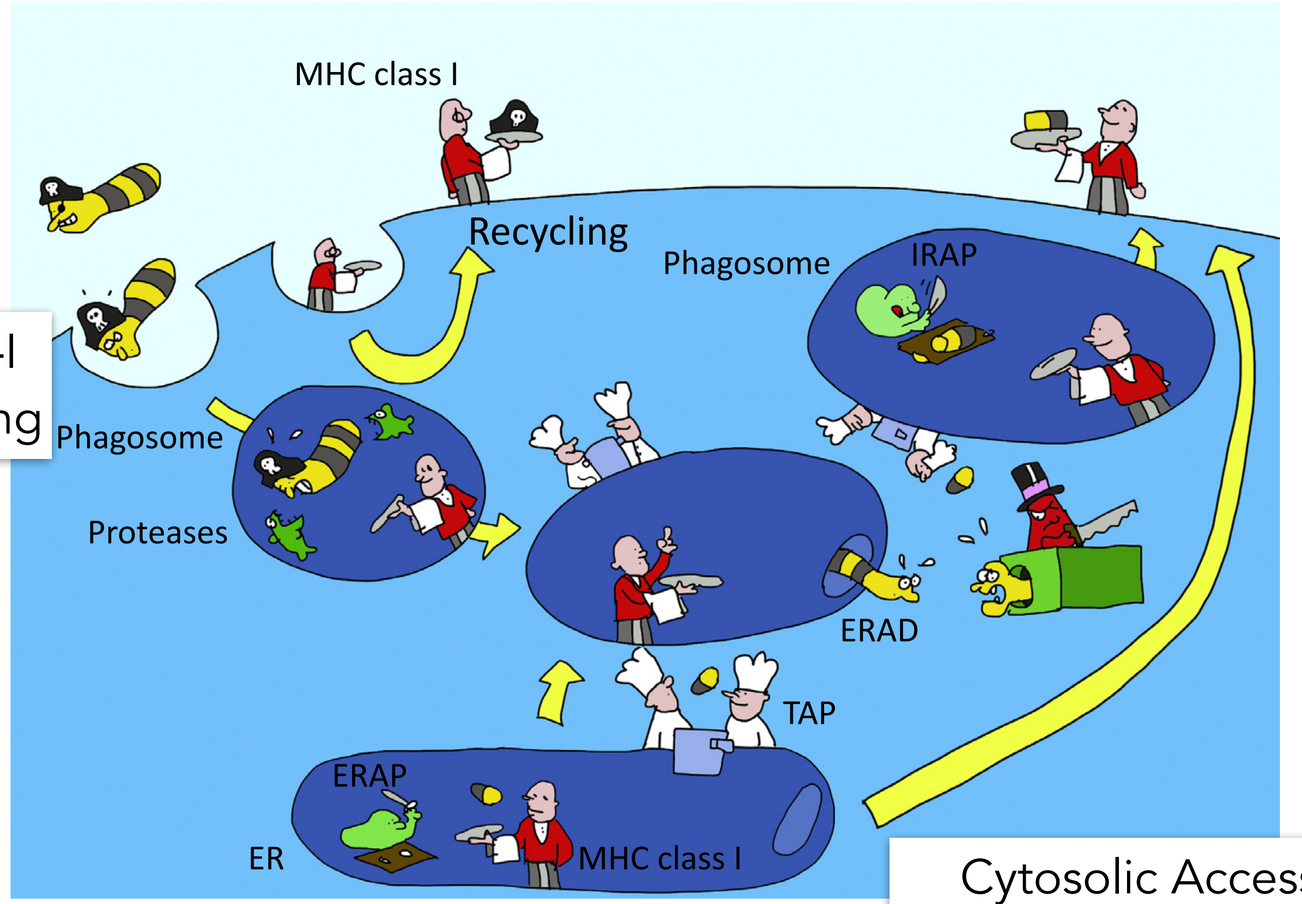
MHC class II-like molecule, containing α- and β-chain

Predominantly found in the *endosomal compartment:*

➔ **stabilize** empty MHC class II molecules + catalyses **release** of **CLIP**!

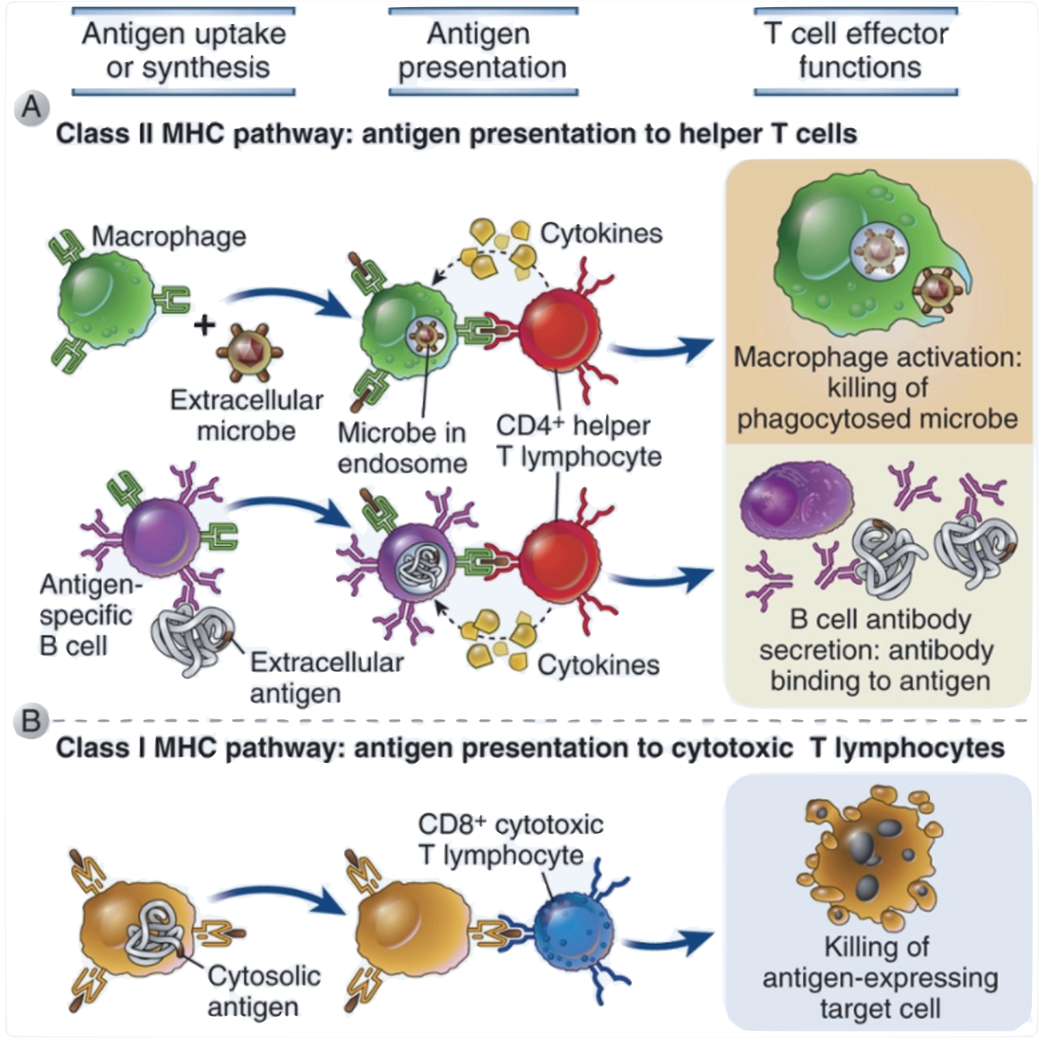
➔ Other function: *peptide editing*

Cross-presentation



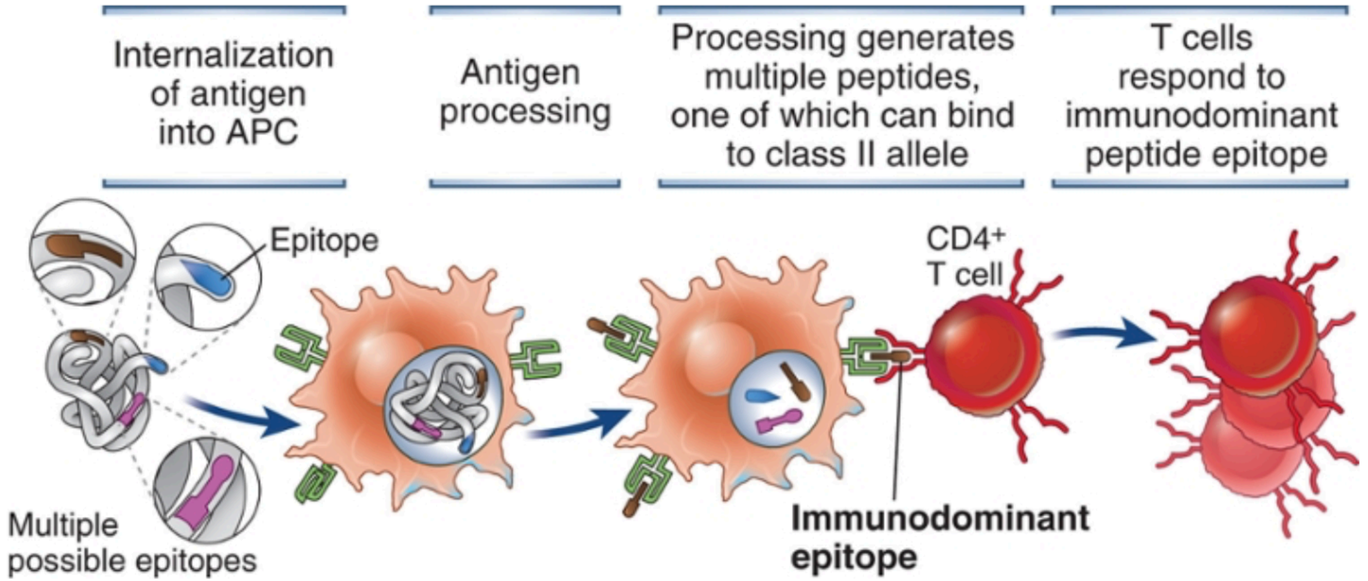
Nature of T cell responses

Determination of the *subset of T cells* response to antigens are linked to the *functions* of these T cells. Antigens from microbes in *different cellular locations* selectively activate T cells that are *most effective* at elimination that *particular type* of microbe.



Immunogenicity of protein antigens

- Peptides generated by proteolysis in APCs and bind most avidly to MHC elicit the strongest T cell responses (*immunodominant epitopes*), important knowledge in the design of *vaccines*
- The expression of *particular class II MHC alleles* determines the ability to respond to particular antigens



Presentation of nonprotein antigens to T cells

Chemicals, small molecules and metal ions can activate T cells in a *MHC-restricted* manner.

NKT, yd T cells are smaller populations that recognise nonprotein antigens *without involvement of MHC molecules*

- **NKT cells** express T receptors with *limited diversity* recognising *lipids and glycolipids* displayed by class-I like nonclassical MHC molecule *CD1*
- **Yd T cells** express antigen receptors similar but not identical to CD4+ and CD8+ T cells and recognise *different types of antigens*, including some proteins and lipids, small phoshphorylated molecules and alkyl amines (not MHC restricted)

Response of T cells to superantigens

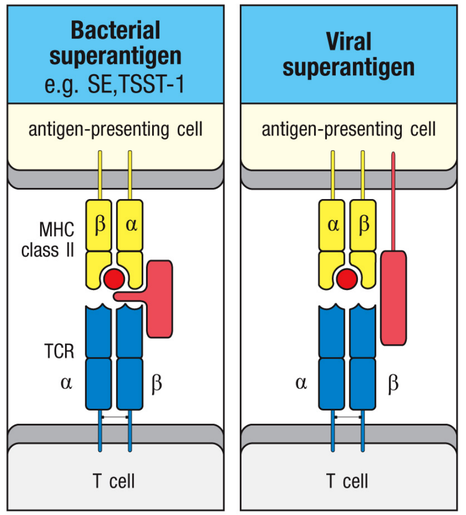
Superantigens

- Distinct set of antigens stimulating *primary T cells responses* ➔ similar response than for allogeneic MHC molecules.
- Produced by many different pathogens: responses = **helpful** for pathogen

Suppression of *adaptive* immune response

Systemic *toxicity*

- Recognized by T cells *without* being *processed*
- Biological activity of superantigens depends on binding as **intact protein** to outside surface of MHC class II molecule that has **already bound peptides**
  Instead of triggering a specific adaptive immune Response, SAGs cause massive production of cytokines by CD4 T cells



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