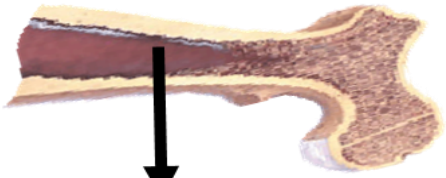
HLA

(Human Leucocyte Antigen)

Genes that provide Adaptive immunity

Hematopoietic Cells



Bone Marrow

Hematopoietic Stem Cell

Myeloid Progenitor Cell

Lymphoid Progenitor Cell

RBC

Platelet

Natural Killer Cell

Neutrophil

Eosinophil

Basophil

Monocyte

Dendritic Cell

Macrophage

Inflammation

Innate

T Lymphocytes

- Helper cells
- Regulatory cells
- Cytotoxic cells

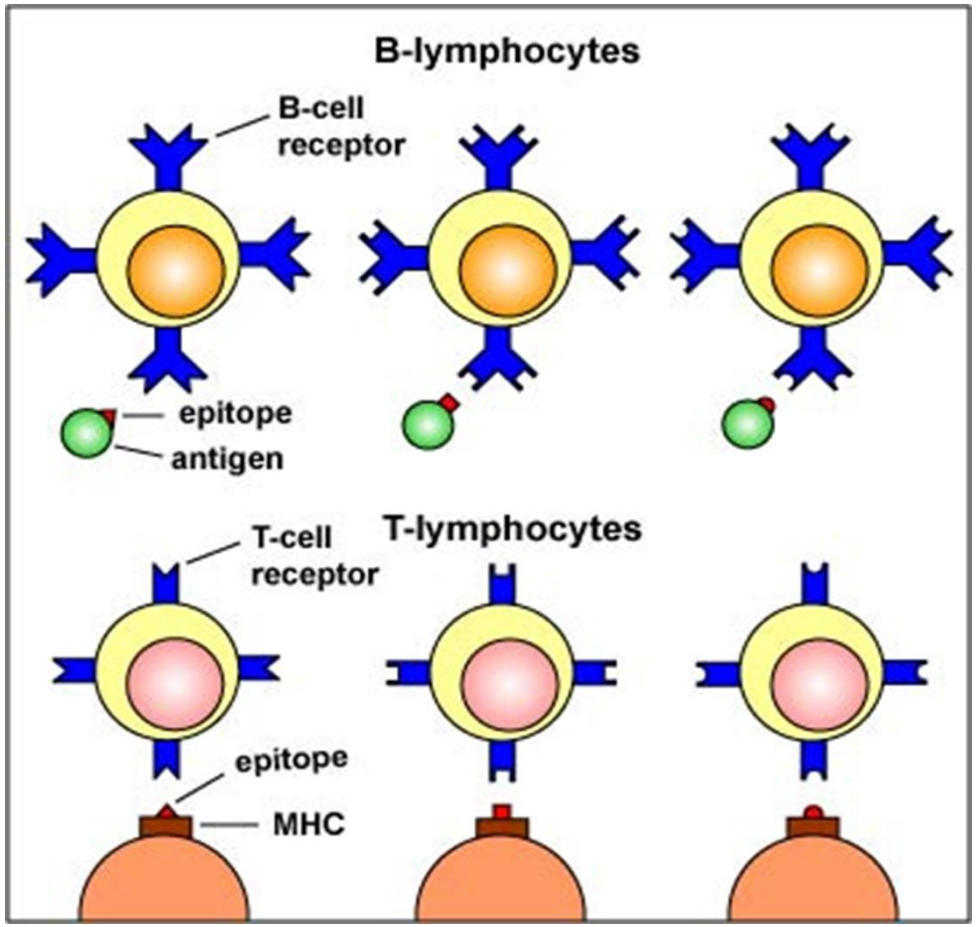
B Lymphocyte

Plasma Cell

Antibodies

Adaptive

The basis of adaptive immunity:



B and T lymphocytes express receptors on the surface that can recognize foreign antigens.

B lymphocyte receptors can recognize an antigen on their own.

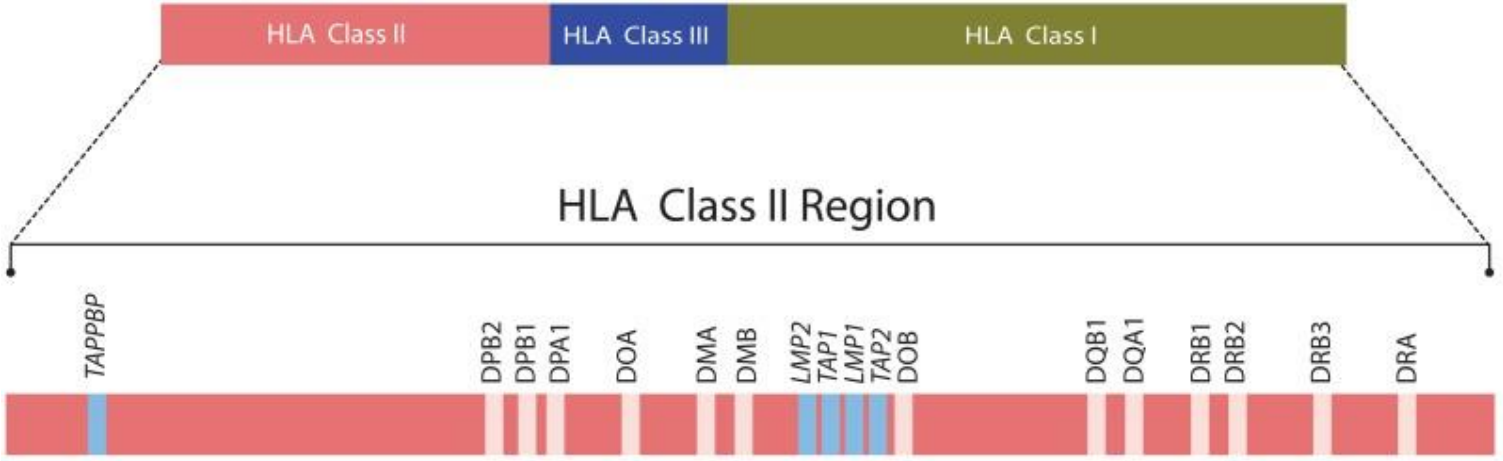
T-lymphocyte receptors recognize antigen (or antigen fragments = epitope) from foreign proteins if it is expressed on the surface of other somatic or immune cells. These antigen fragments must be inserted into the 'groove' of special molecules expressed on the surface of other somatic or immune cells, defined as molecules of the major histocompatibility complex (MHC).

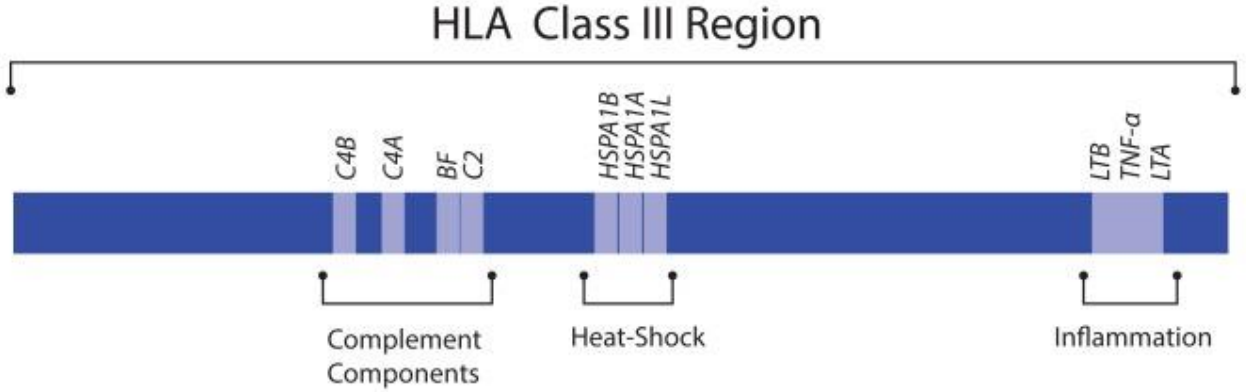
MHC I
MHC II

Major histocompatibility complex.

- **A term for highlighting a group of genes (300-400) of expression of various proteins, factors and antigen presentation molecules, which are engaged in the functional complex of the immune system.**

- **The HLA antigen (human leukocyte antigen) or HLA gene complex is an important fragment of the MHC gene complex and is justified as a synonym for MHC.**



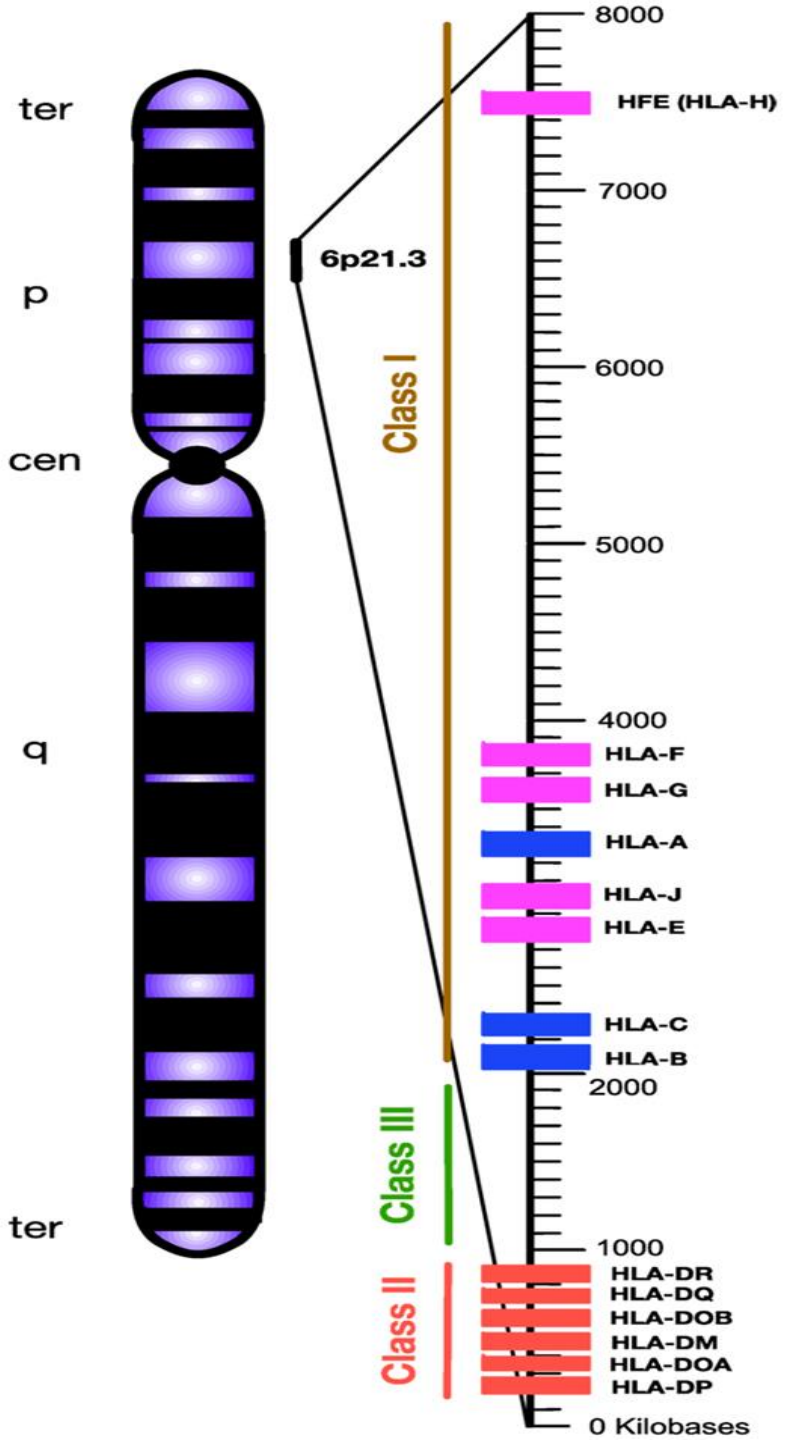


Jean Dausset,
Jan van Rood,
George Snell – giving name for HLA in 1954.

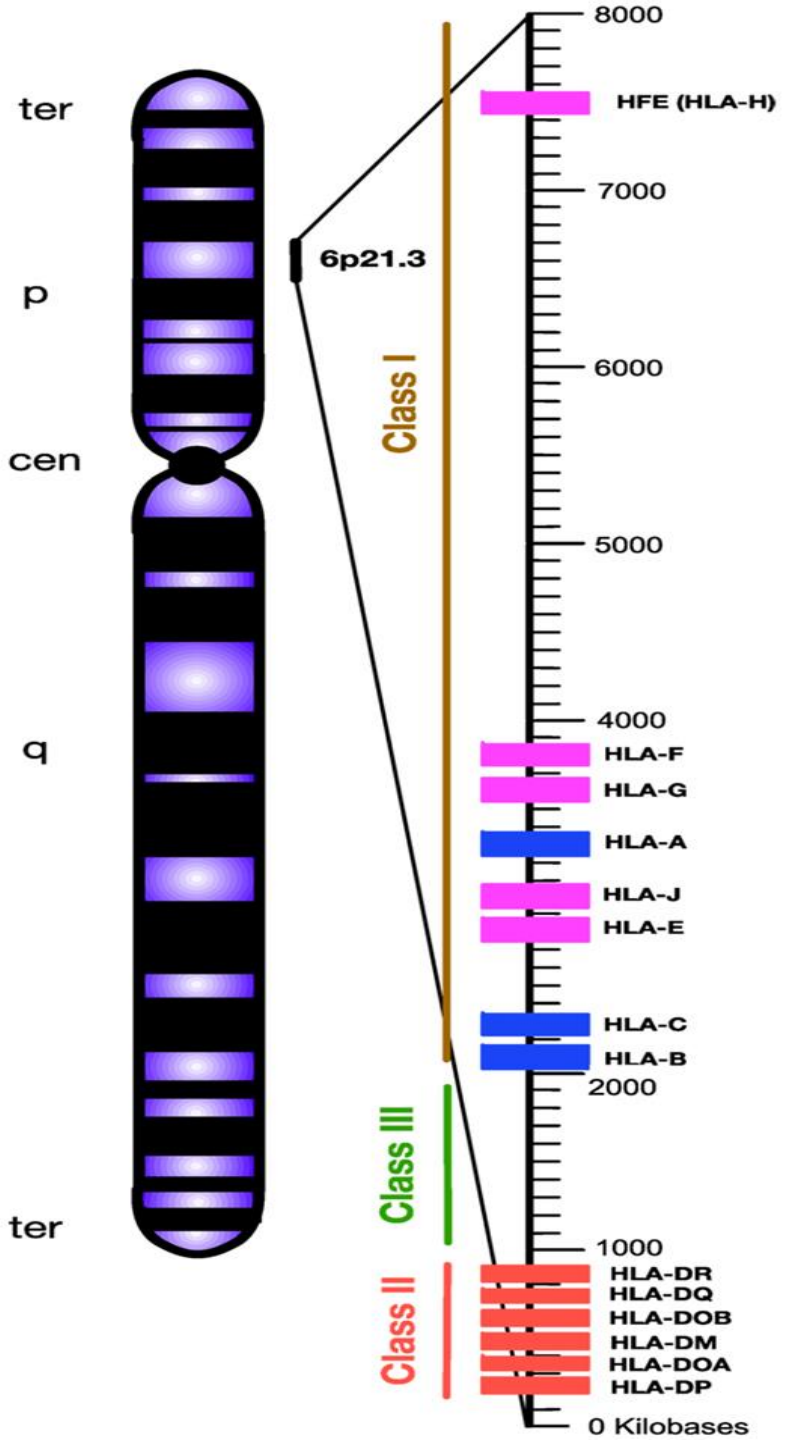
In 1980 – Noble Award for characterization of MHC and elucidation of transplant principles and risk of graft rejection.

- **HLA – antigens that express imminent factors of immunity on white blood cells (HLA-II), as on all other nuclear cells (HLA-I).**
- **HLA I is in the white cell and all other cells,**
- **HLA II – only in white blood cells.**
- **The HLA gene complex is identified on the short arm of chromosome 6 – 6p21.3.**
- **MHC genes contain an average of 3500 kilobases of nucleotides, the expression of which is attributed not only to the functions of the immune system, but also to other cellular functions.**

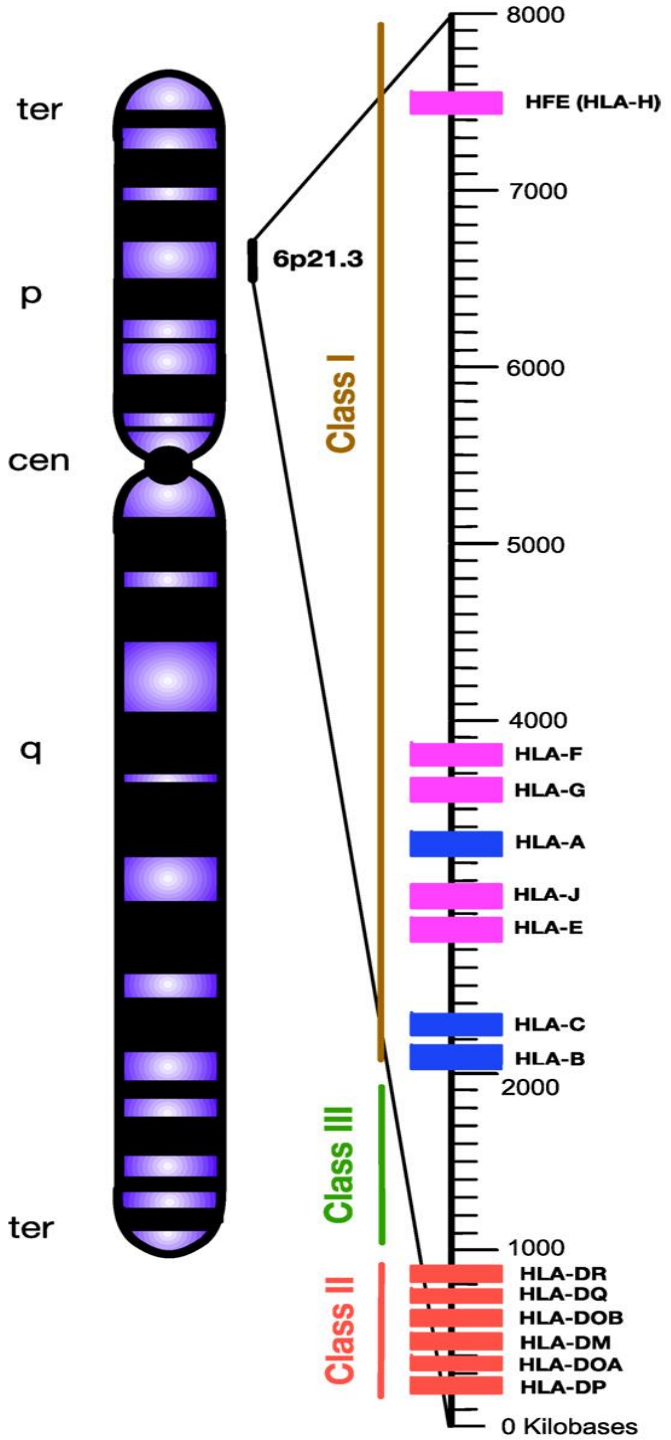
MHC genes



- **Class I and class II MHC genes are the most polymorphic genes present in any mammalian genome.**
- **In the population, the total number of HLA alleles with different amino acid sequences is estimated to be over 10,000, with more than 3,000 variants for the HLA-B locus alone.**
- **MHC polymorphism may have evolved because it ensures that human populations will be able to deal with the virtually unlimited diversity of microbes and will be protected from devastating loss of life from emerging infections.**



- **There are three class I MHC genes called HLA-A, HLA-B, and HLA-C, which encode three types of class I MHC molecules with the same names.**
- **HLA-E, HLA-F, and HLA-G genes encode class I–like molecules, many of which are recognized by NK cells.**
- **There are three class II HLA gene loci called HLA-DP, HLA-DQ, and HLA-DR. Each class II MHC molecule is composed of a heterodimer of α and β polypeptides. The DP, DQ, and DR loci on each chromosome contain separate genes designated A and B, encoding the α and β chains, respectively.**
- **Every individual has two HLA-DP genes (called DPA1 and DPB1), two HLA-DQα genes (DQA1, 2), one HLA-DQβ gene (DQB1), one HLA-DRα gene (DRA1), and one or two HLA-DRβ genes (DRB1 and DRB3, 4, or 5).**
- **The set of MHC alleles present on each chromosome is called an MHC haplotype. For instance, an HLA haplotype of an individual could be HLA-A2, B5, DR3, and so on (using the simpler nomenclature for HLA alleles).**



- **p – short arm of chromosome**
- **Q – long arm of the chromosome**
- **The HLA III or MHC III genes are responsible for the expression of proinflammatory cytokines such as TNF-α, and complement proteins, as C4, C2, factor B.**

Classical Rules

MHC I or MHC II molecules are expressed on the surface of somatic and immune cells to ensure continuous and perfect (exact) control by T lymphocytes (CD8+ and CD4+, respectively): ligand-receptor.

If these molecules are associated with the epitope of the foreign antigen, then CD8+ and CD4+ must ensure through different mechanisms the destruction of cells – the entity of the adaptive immune response.

General properties of the MHC structure

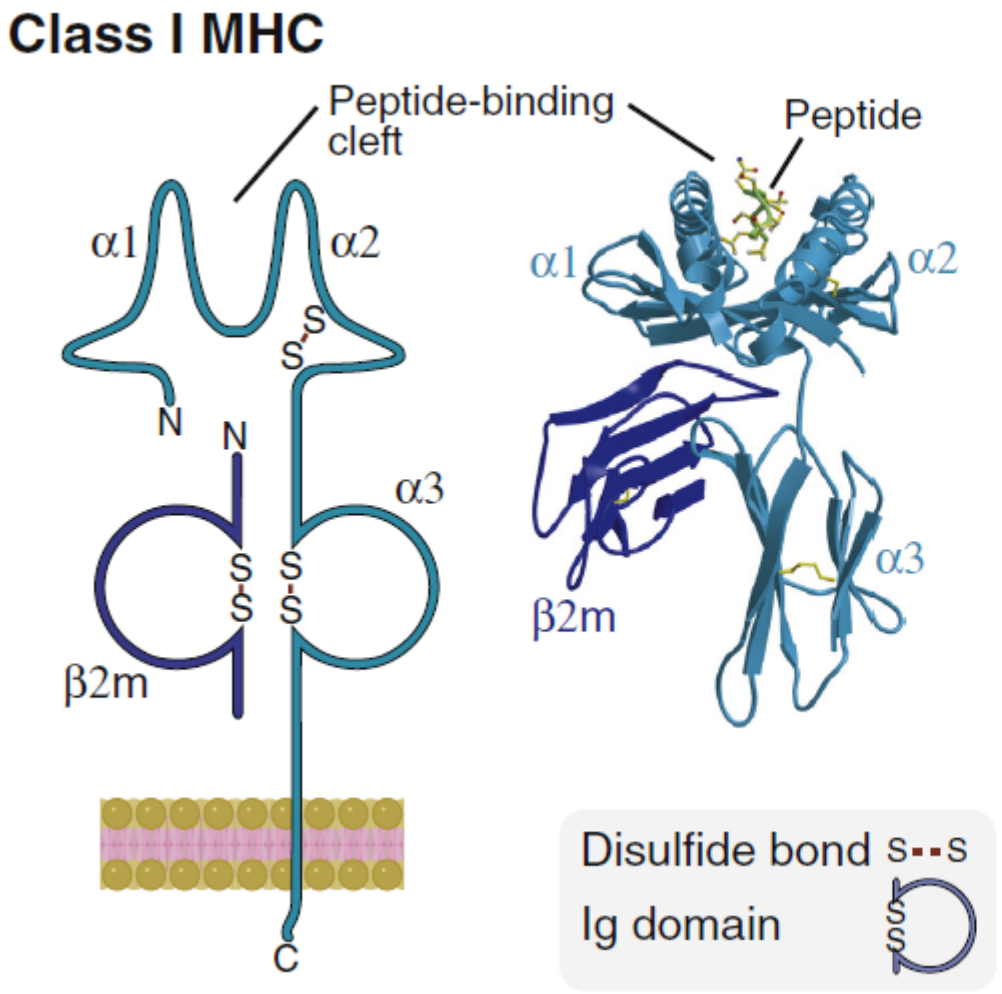
Each MHC molecule consists of 3 compartments:

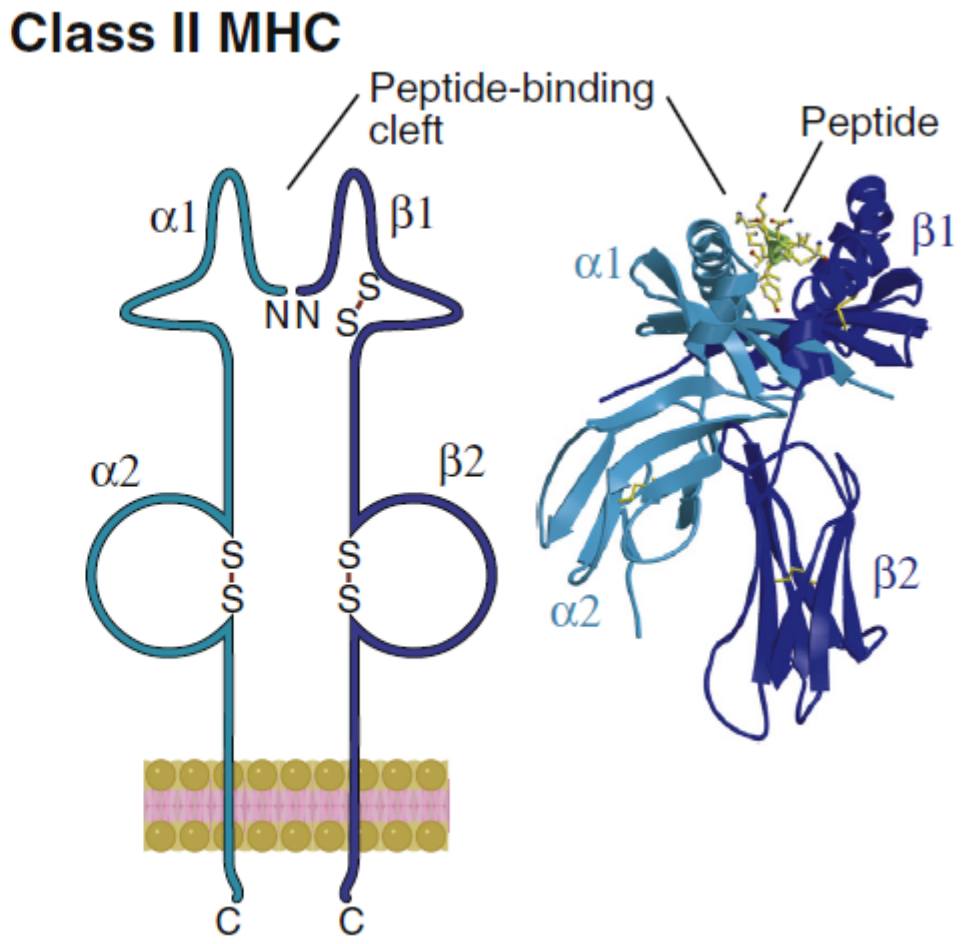
1. **The funnel-shaped extracellular fragment that binds foreign protein, supported by residues of various amino acids.**

2. **Transmembrane fragment.**

3. **Cytoplasmic fragment.**

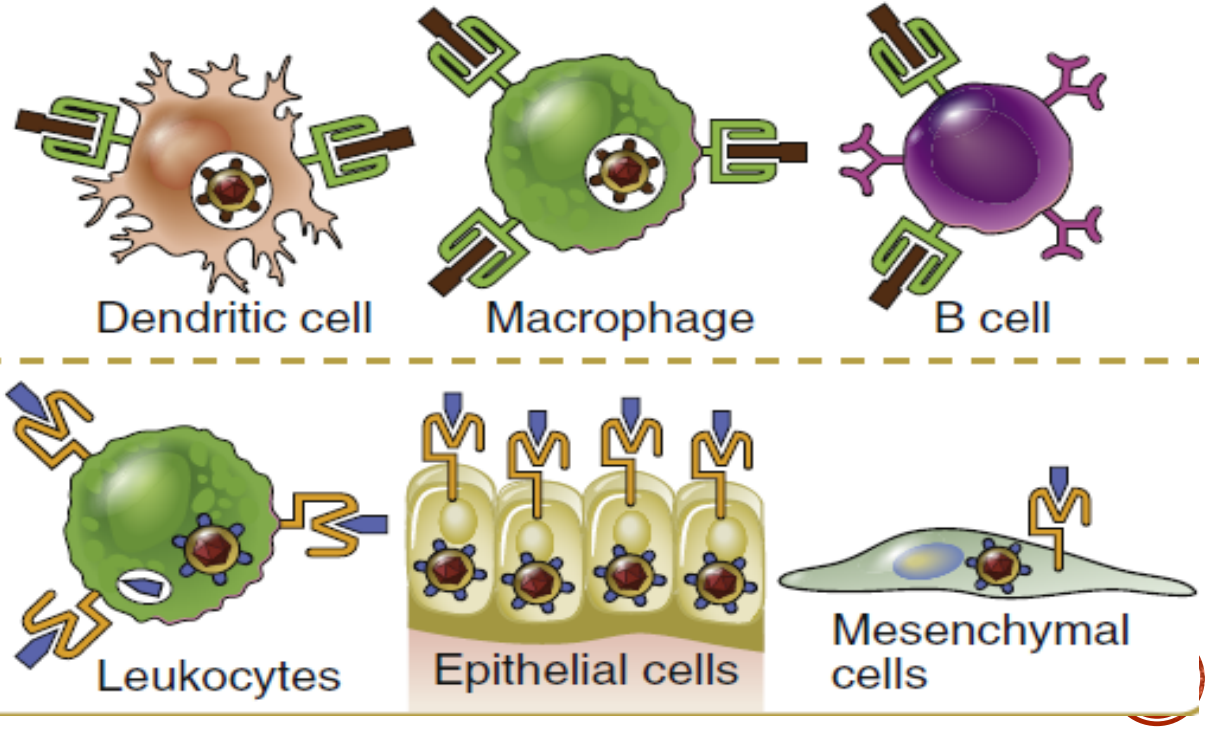
The MHC I molecule consists of the long hard chain alpha (α1-2-3) and the short chain beta which represents a β2-microglobulin.
The MHC II molecule consists of 2 similar chains: alpha and beta.





HLA I genes (HLA I – A, B, C) are expressed on the somatic cells (except central neurons), even platelets. They present antigen to CD8.
HLA II genes (HLA II – DP, DQ, DR) are expressed on the antigen-presenting cells (APC) to CD4 – specialized cells: B lymphocytes; Dendritic cells; Macrophages; Epithelial cells of the thymus; Langerhans cells; Endothelial cells

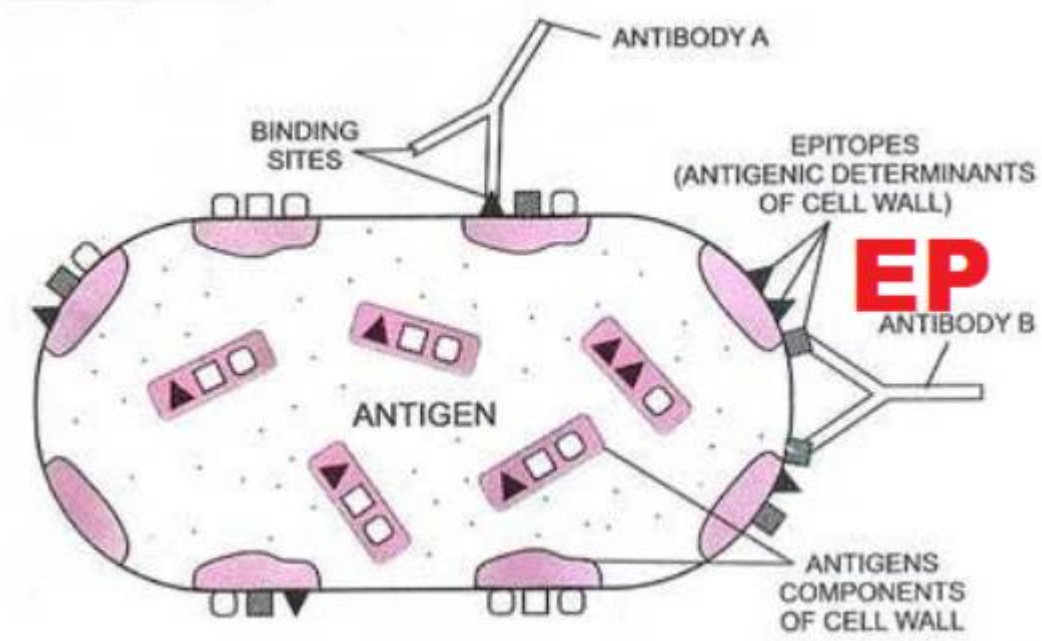
MHC-expressing cell types:	
Class II: Dendritic cells, macrophages, B cells	$CD4^+$ helper T lymphocytes interact with dendritic cells, macrophages, B lymphocytes
Class I: All nucleated cells	$CD8^+$ CTLs can kill any type of virus-infected cell



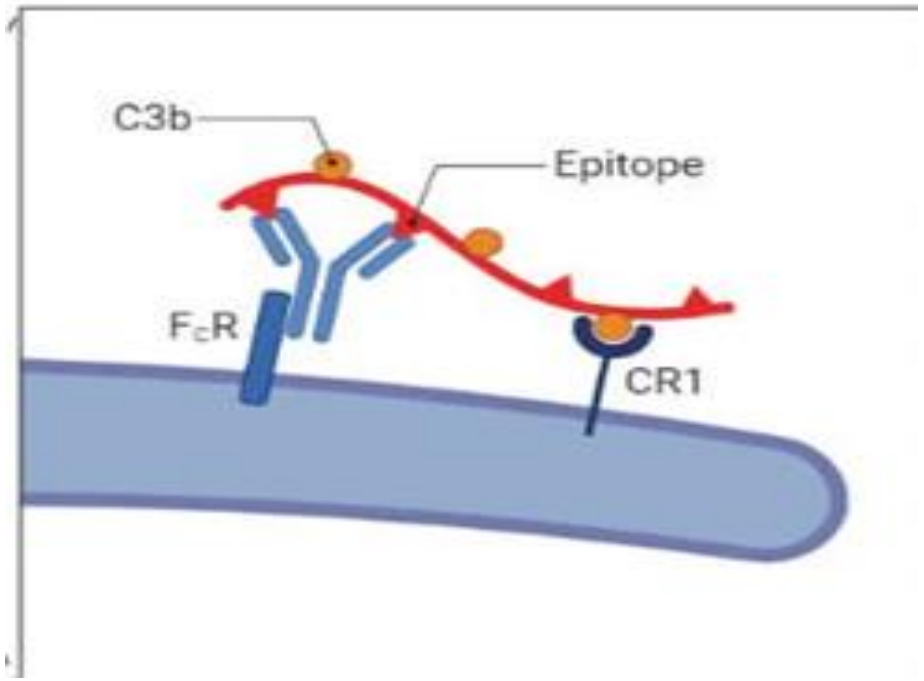
Main role of MHC I and MHC II:

- **MHC I - recognition of foreign endoantigen (proteins) in the cytosol of any nucleated cell, processing in proteolytic structures called proteasomes and displaying for CD8+**

- **MHC II - recognition of foreign exoantigen, processing in late endosomes and lysosomes and presentation of non-self antigen epitope to CD4+**



The epitope contains: 1-5 monosaccharides or 5-8 aminoacids.



C3b – opsonization complement
FcR – phagocyte receptor for Fc-Ac

3 general types of antigens:

1. **Exoantigens** or hetero-antigens – Ag produced outside the body and are foreign to the host immune system (e.g. infectious antigens).

2. **Endoantigens** – Ag produced in the cells of the host organism: e.g. Ag erythrocytes or Ag produced by cells infected with bacteria and viruses or by cancer cells.

3. **Autoantigens**- Ag formed by one's own cells, which normally would not cause an immune response, but a sensitized immune system will generate an attack and the development of an autoimmune disease.

Important features:

- **Exoantigens will enter the body through the oral, parenteral and inhaled routes (microorganisms, toxins, food, graft proteins, pollen, etc.)**

- **The viral exoantigen can become endoantigen when the virus infects the host cell.**

- **The endoantigens will be processed by MHC type I.**
- **The exoantigens will be processed by MHC type II.**

Important features:

- **Linear epitopes** are recognized by **T-lymphocytes**.

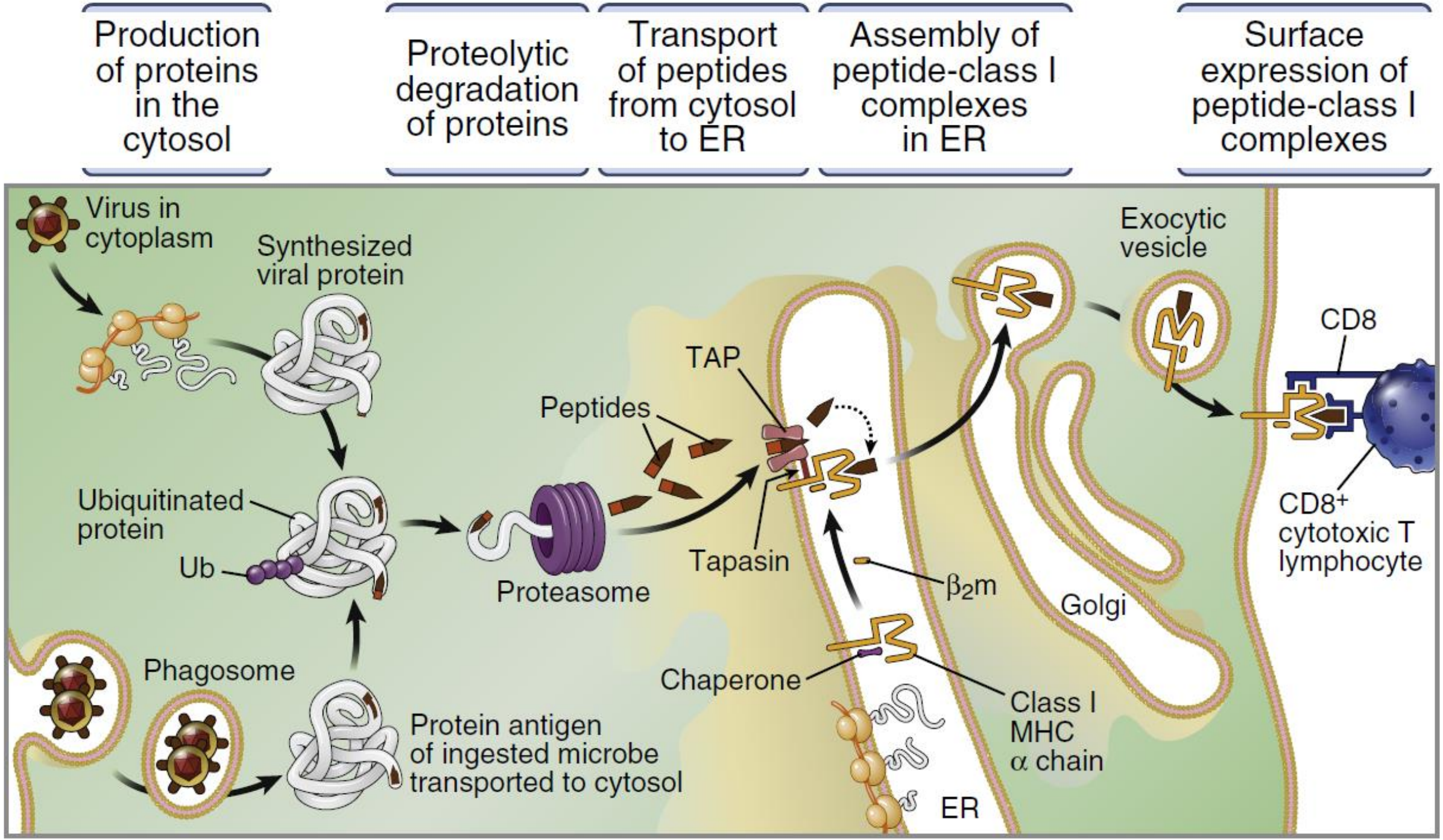
- **Conformational epitopes**, as a structure expressed by the flexible region of tertiary antigen by fusing amino acid residues (on average 10-16) from different loops of the protein, are recognized by **B lymphocytes**.

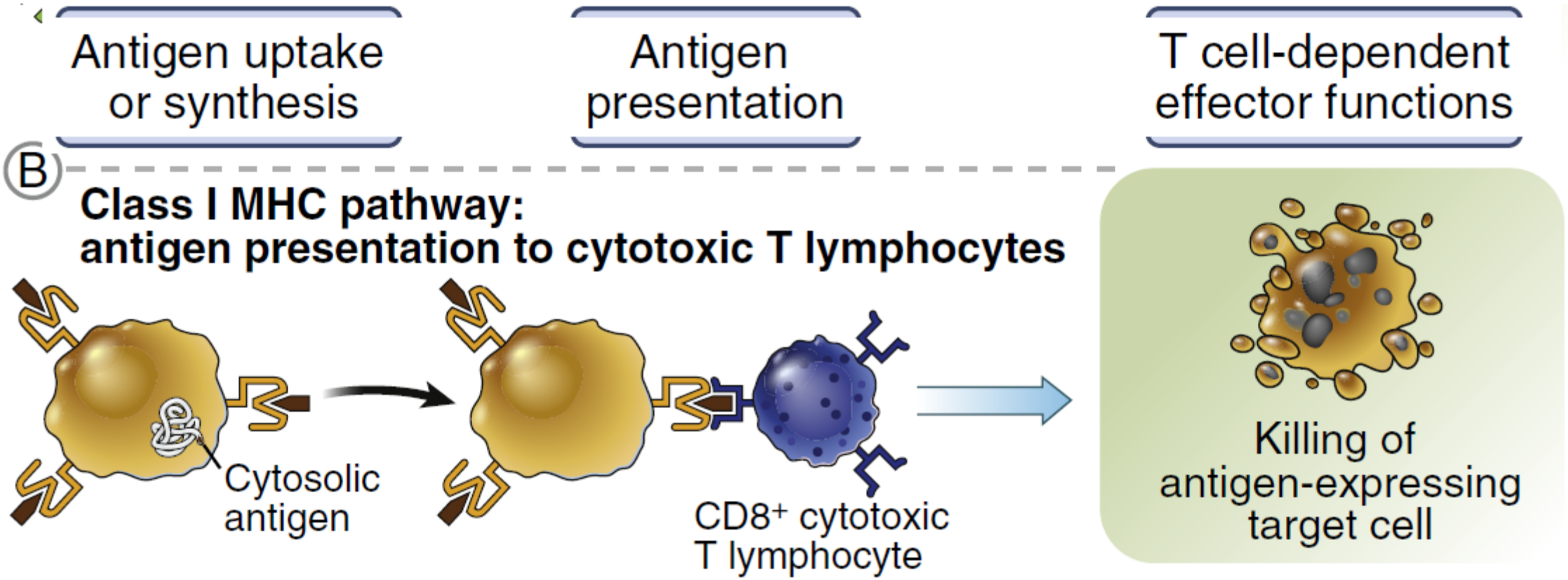
Presentation of antigen epitope by MHC I to CD8+ lymphocytes

Foreign antigens processed by MHC I are virus-derived intracellular antigens, abnormal proteins stored in the cytosol, abnormal proteins synthesized by ribosomes, or from microbes that are ingested but whose antigens are transported into the cytosol (the process of cross-presentation).
etc.
The assembly of class I molecules with antigenic peptides requires coordination of several processes:

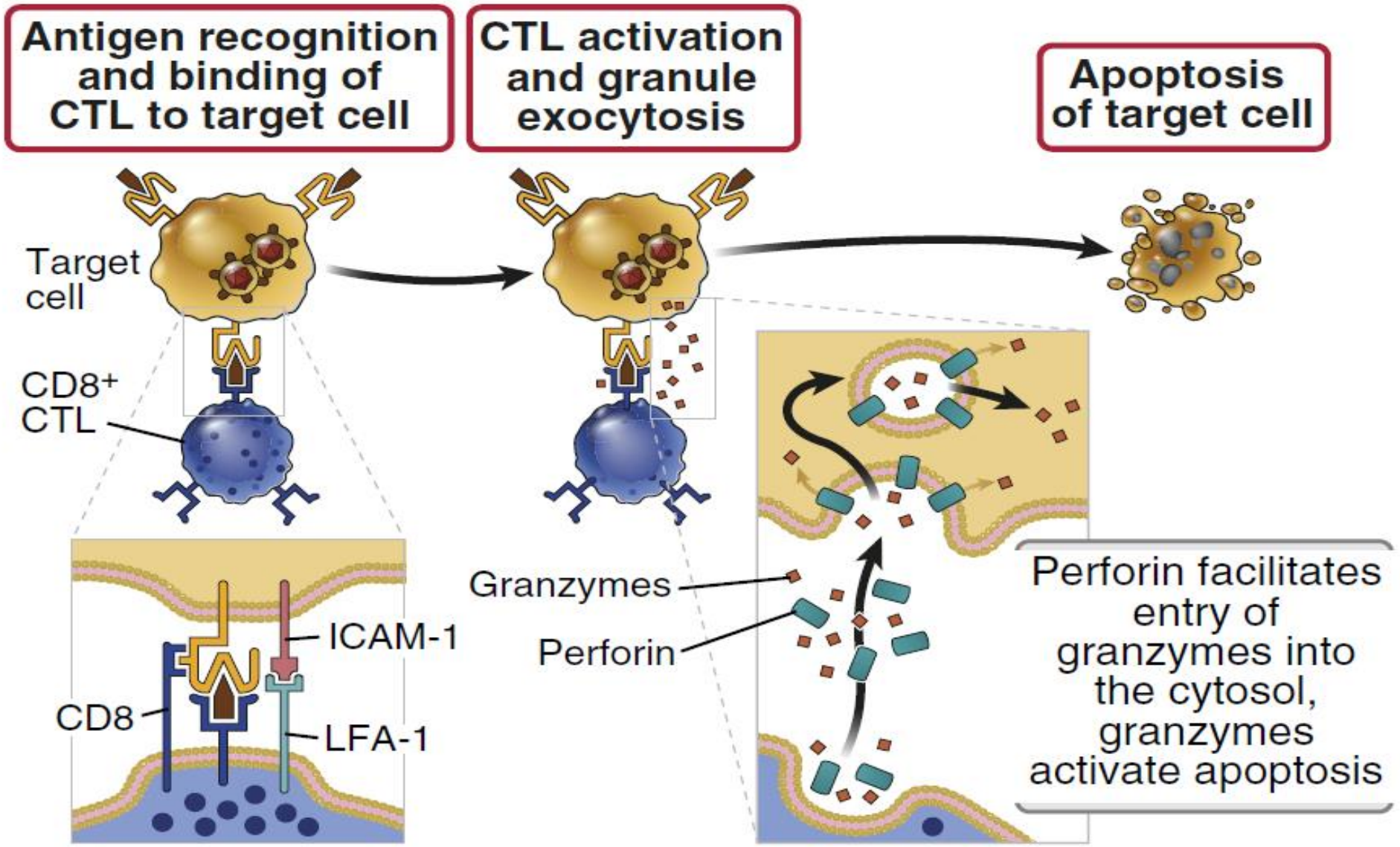
1. **Proteins enter the cytoplasm of cells;**
2. **Cytoplasmic proteins are unfolded, ubiquitinated, and degraded in proteasomes into peptides;**

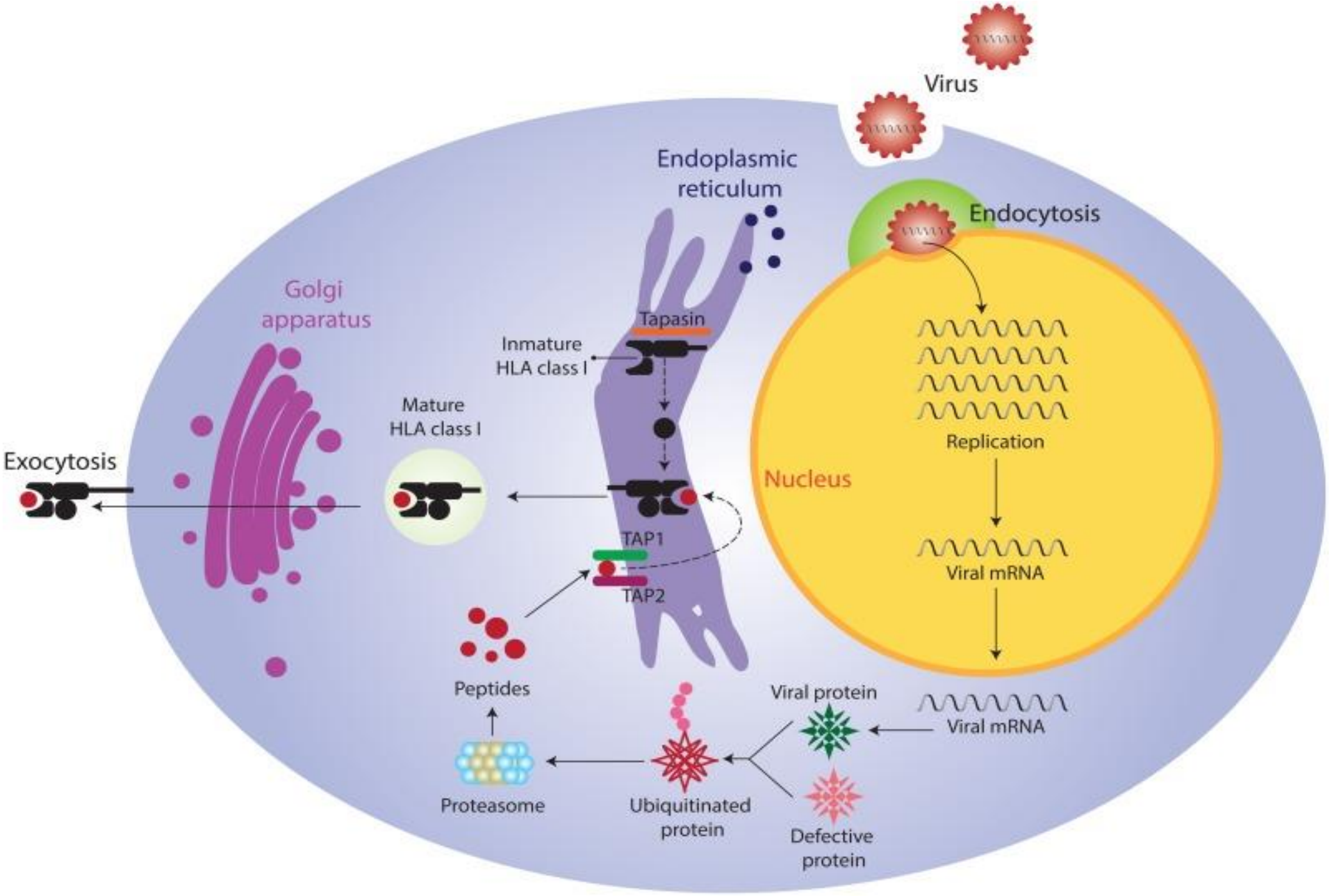
3. **The peptides that are produced are transported by the transporter associated with antigen processing (TAP) into the endoplasmic reticulum (ER), where the peptides may be further trimmed;**
4. **Newly synthesized class I MHC molecules are initially stabilized by chaperones and attached to TAP by a linker protein called tapasin;**
5. **The peptide–class I MHC complexes formed leave the ER and through the Golgi apparatus move to the membrane where through exocytosis they will be exposed and subsequently recognized by CD8+ lymphocytes.**


Production of proteins in the cytosol
Proteolytic degradation of proteins
Transport of peptides from cytosol to ER
Assembly of peptide-class I complexes in ER
Surface expression of peptide-class I complexes
Virus in cytoplasm
Synthesized viral protein
Ubiquitinated protein
Ub
Proteasome
Peptides
TAP
Tapasin
Exocytic vesicle
CD8
CD8+ cytotoxic T lymphocyte
β2m
Golgi
Chaperone
Class I MHC α chain
ER
Phagosome
Protein antigen of ingested microbe transported to cytosol


Antigen uptake or synthesis
Antigen presentation
T cell-dependent effector functions
B
Class I MHC pathway:
antigen presentation to cytotoxic T lymphocytes
Cytosolic antigen
CD8+ cytotoxic T lymphocyte
Killing of antigen-expressing target cell

Mechanisms of killing of infected cells by CD8+ cytotoxic T lymphocytes (CTLs)




Virus
Endoplasmic reticulum
Endocytosis
Golgi apparatus
Tapasin
Inmature HLA class I
Mature HLA class I
Exocytosis
Nucleus
Replication
Viral mRNA
TAP1
TAP2
Peptides
Viral protein
Viral mRNA
Proteasome
Ubiquitinated protein
Defective protein

Presentation of antigen epitope by MHC II CD4+ lymphocyte. Steps

1. **Foreign exoantigens (exogenous proteins – derived from extracellular pathogens) are through phagocytosis or internalization inserted into the antigen-presenting cells, which express special receptors, which by internalization will ensure the penetration of foreign protein into the cytosol.**

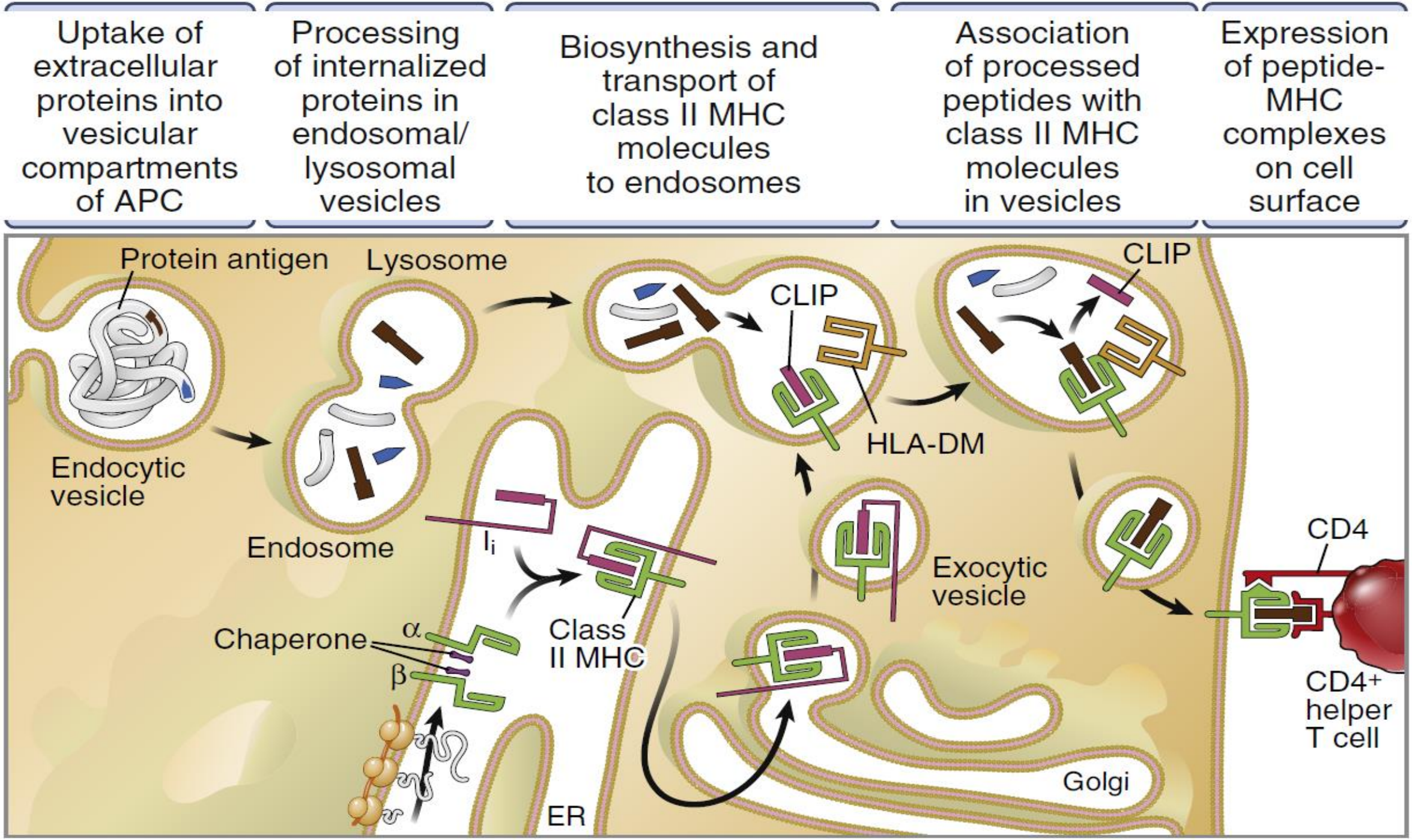
2. **After internalization into APCs, the microbial proteins enter into intracellular vesicles, called endosomes or phagosomes, which may fuse with lysosomes, where proteins are broken down by proteolytic enzymes, generating many peptides of varying lengths and sequences.**

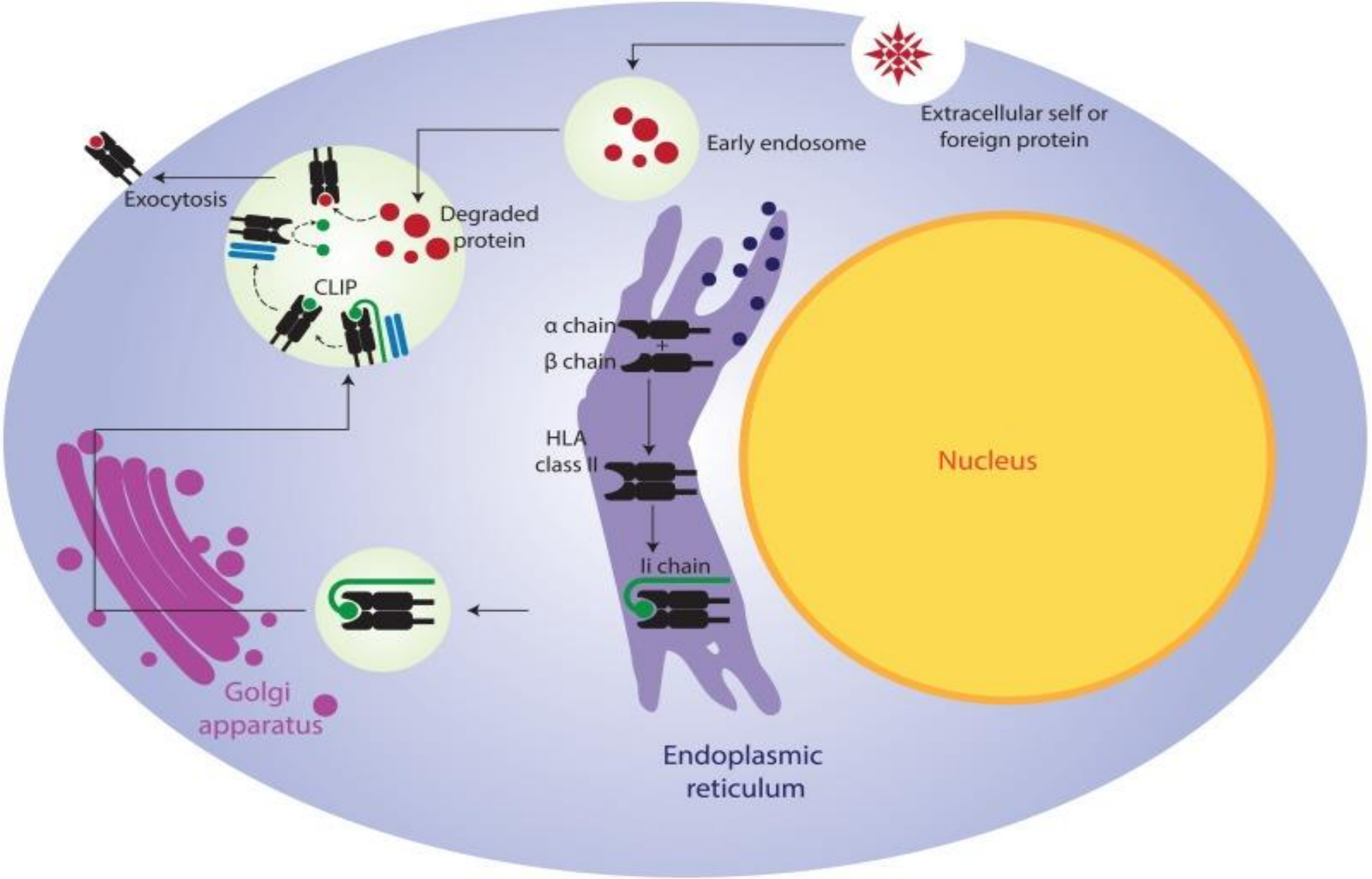
3. **Peptides bind to newly synthesized MHC molecules in specialized vesicles. Class II MHC–expressing APCs constantly synthesize these MHC molecules in the endoplasmic reticulum (ER). Each newly synthesized class II molecule carries with it an attached protein called the invariant chain (Li), which contains a sequence called the class II invariant chain peptide (CLIP) that binds to the peptide binding cleft of the class II molecule.**

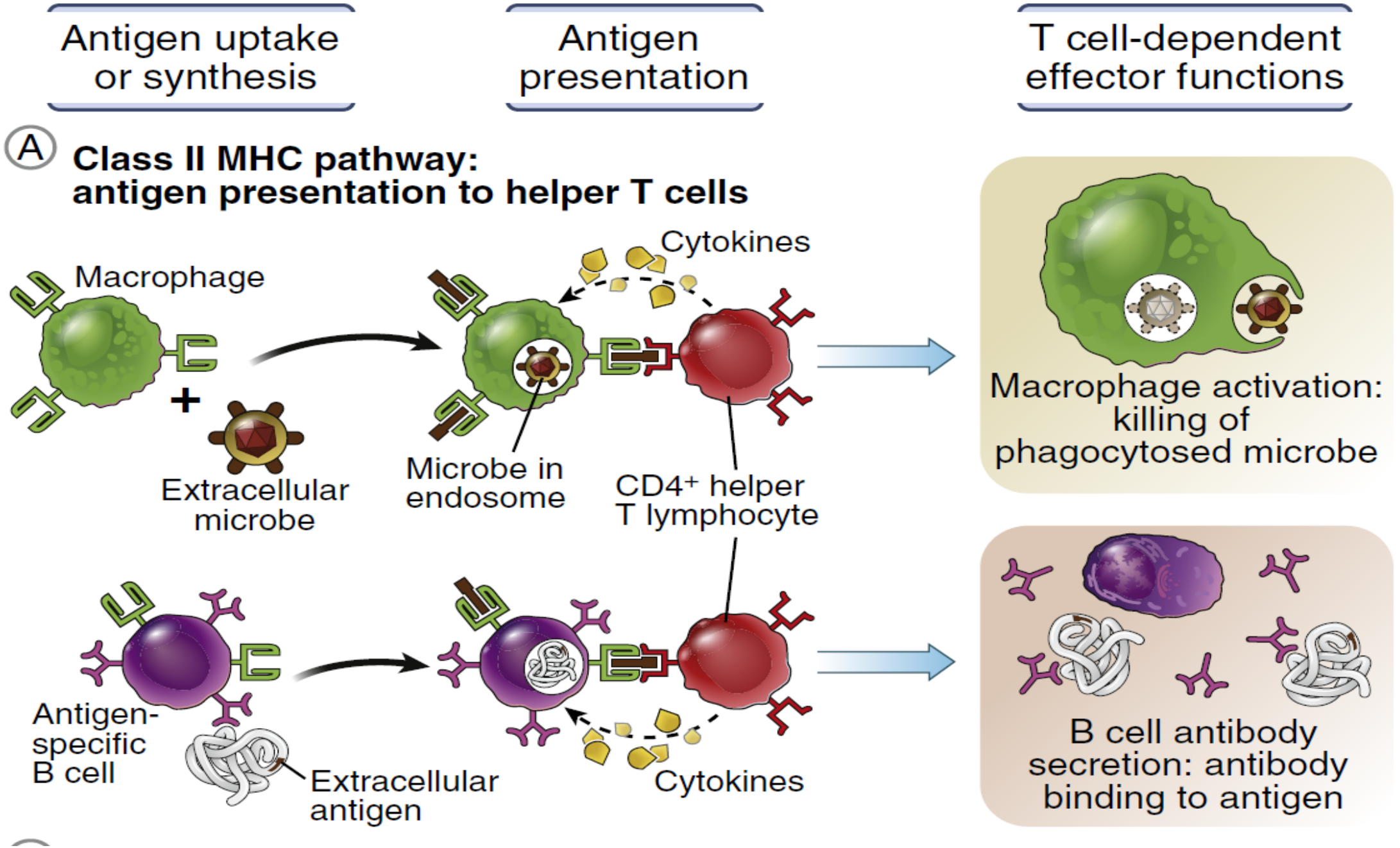
4. **This MHC II with its Li is targeted to the endosomal vesicles that contain peptides derived from ingested extracellular proteins. In these vesicles, the Li is degraded, leaving only CLIP in the peptide binding cleft. The degradation of the LI protein will allow the association of the MHC II molecule to the exogenous protein derived from the processing of exogenous antigen in endosome-lysosome.**

5. **Late endosomes/lysosomes also contain a class II MHC–like protein called DM, whose function is to exchange CLIP in the class II MHC and subsequent binding of the antigenic peptide.**
6. **Peptide loading stabilizes class II MHC molecules, which are exported to the cell surface. Once the class II MHC molecule binds tightly to one of the peptides generated from the ingested proteins, this peptide-MHC complex becomes stable and is delivered to the cell surface.**
7. **If the MHC molecule does not find a peptide it can bind, the empty molecule is unstable and is eventually degraded by lysosomal proteases.**

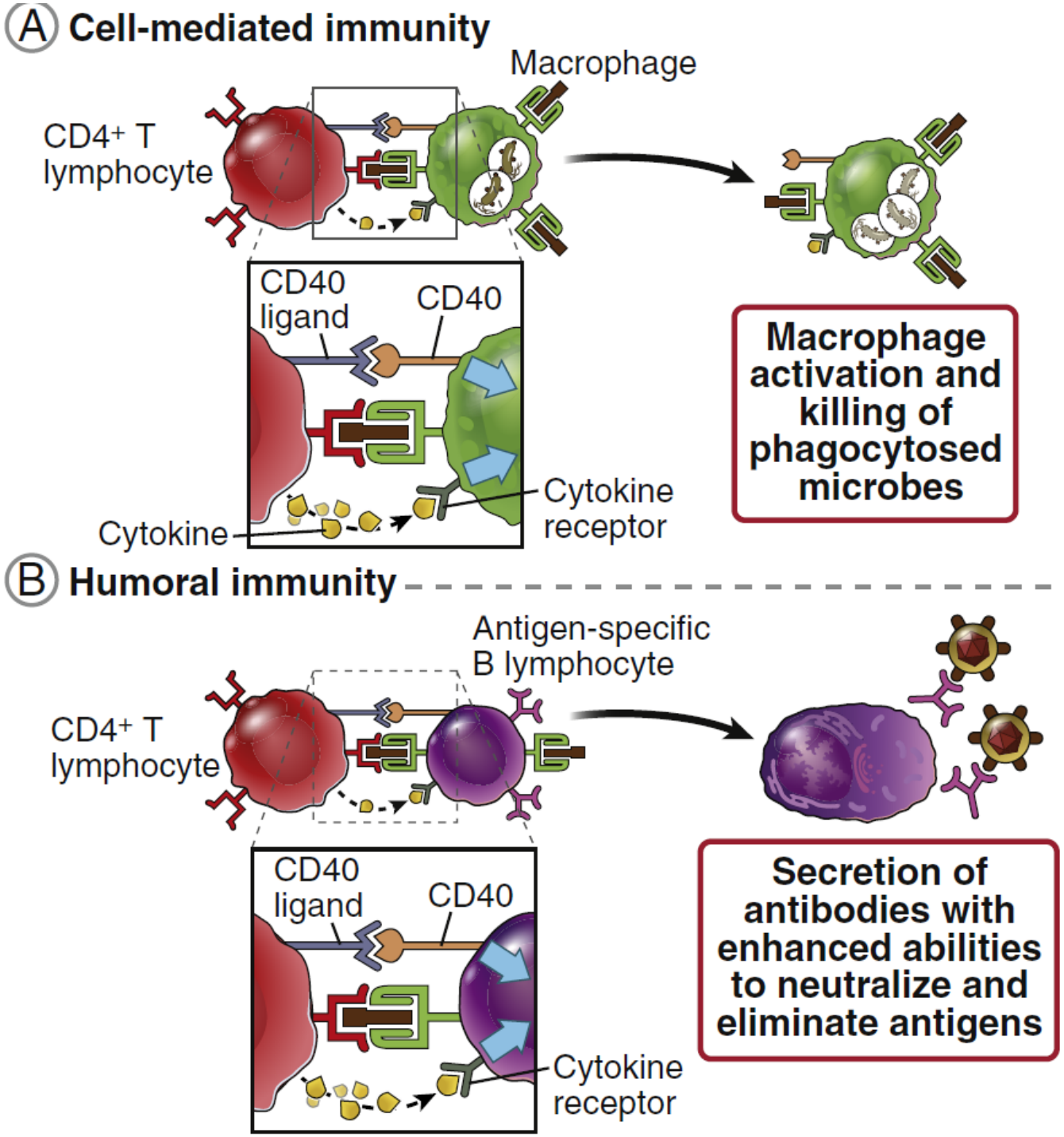
One protein antigen may give rise to many peptides, only a few of which (perhaps only one or two) can bind to the MHC molecules present in the individual and have the potential to stimulate immune responses in that individual.


Uptake of extracellular proteins into vesicular compartments of APC
Processing of internalized proteins in endosomal/ lysosomal vesicles
Biosynthesis and transport of class II MHC molecules to endosomes
Association of processed peptides with class II MHC molecules in vesicles
Expression of peptide-MHC complexes on cell surface
Protein antigen
Lysosome
CLIP
CLIP
Endocytic vesicle
HLA-DM
Endosome
I_i
Exocytic vesicle
CD4
Chaperone
α
β
Class II MHC
CD4+ helper T cell
Golgi
ER


Extracellular self or
foreign protein
Early endosome
Exocytosis
Degraded
protein
CLIP
α chain
β chain
HLA
class II
Ii chain
Nucleus
Golgi
apparatus
Endoplasmic
reticulum

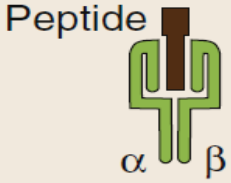
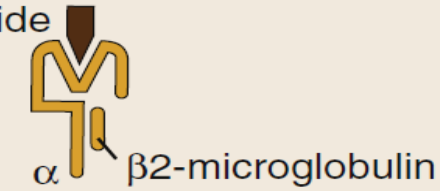
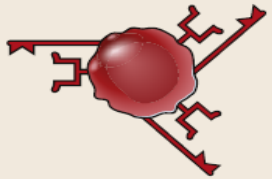
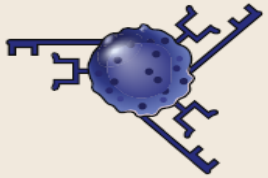


Antigen–presenting cells (APCs): monocytes, macrophages, dendritic cells, B cells



Roles of CD40L and cytokines in effector functions of CD4+ helper T cells

CD4+ T cells that have differentiated into effector cells express CD40L and secrete cytokines. CD40L binds to CD40 on macrophages or B lymphocytes, and cytokines bind to their receptors on the same cells. The combination of signals delivered by CD40 and cytokine receptors (arrows) activates macrophages in cell-mediated immunity (A) and activates B cells to produce high-affinity isotype-switched antibodies in humoral immune responses (B).

Feature	Class II MHC Pathway	Class I MHC pathway
Composition of stable peptide-MHC complex	Polymorphic α and β chains of MHC, peptide 	Polymorphic α chain of MHC, β2-microglobulin, peptide 
Cells that express that MHC	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)	Enzymatic components of cytosolic proteasome
Site of peptide loading of MHC	Late endosomes and lysosomes	Endoplasmic reticulum
Molecules involved in transport of peptides and loading of MHC molecules	Invariant chain, DM	TAP

Important:

1. Professional antigen presenting cells (APCs) (follicular and tissue dendrites, macrophages, B lymphocytes may have both on the membrane

MCH I and MCH II

2. Nonprofessional APC - all nucleated cells exhibit only Ag in association only with molecules

MCH class I

Important:

3. **Dendritic cells are the only professional APCs able of activating naïve T lymphocytes.**

4. **Tissue dendritic cells are DCs made of immature tissues and play the role of sentinel – they have a high capacity to capture antigens, but show low capacity to present Ag.**

Important:

5. **Follicular dendritic cells become mature following Ag uptake and migration to lymph nodes. They have the ability to present Ag and activate T lymphocytes, but lose the ability to capture Ag.**
6. **The presentation of Ag to B lymphocyte can be achieved through follicular dendritic cells in the spleen and lymph nodes.**

Summary

1. T-CD8 cells will activate if they recognize both foreign antigen through specific receptor and antigen MHC I molecules through CD8 molecules.
2. If MHC I molecules on the surface of somatic cells are destroyed, then activation of T-CD8 lymphocytes is compromised, which will affect the killing of infected cells and tumor cells. CD8 are molecules of T-CD8 with a co-stimulatory effect.
3. The same for T-CD4+ is performed the process of exoantigen recognition.
4. The CD4 binding with MHC II leads to the release of various cytokines by helper T lymphocytes (CD4+).

Cross-presentation

- **Some dendritic cells have the ability to capture and to ingest virus-infected cells or tumor cells and present the viral or tumor antigens to naive CD8+ T lymphocytes, this process is called cross-presentation, or cross-priming.**
- **The capture and processing made by 2 pathway: cytosolic and vacuolic.**
- **Under the action of interferon-gamma, some somatic cells may express MHC II**

Cross-presentation

Cytosolic pathway:

After phagocyte by APC, the exoantigen is processed (degraded) in the proteosome.

Subsequently, the antigen is transported to RE via TAP caries, where it is processed (split) under the action of aminopeptidases and then loaded for interaction with MHC I molecules.

Cross-presentation

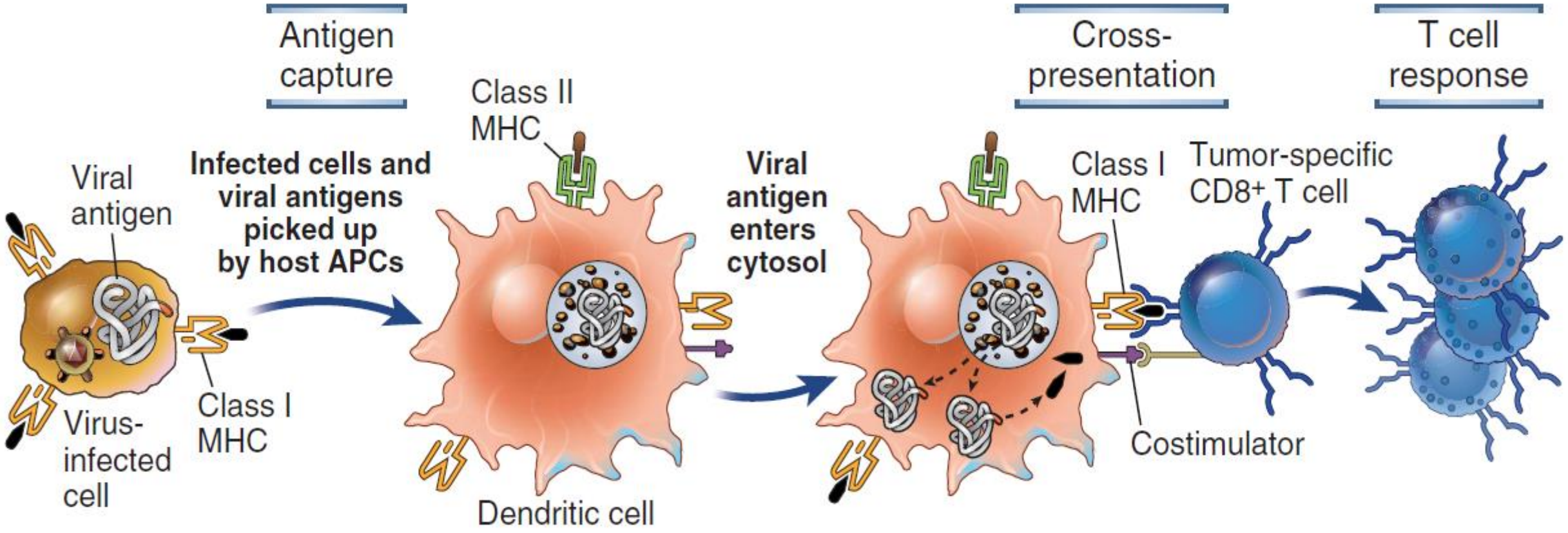
Vacuolic pathway:

The internalized antigens are degraded by cathepsin S in the phagosome and subsequently loaded into the HLA I complex.

Subsequently, the MHC I complex and epitope antigen is directed to the APC membrane through the vacuolic recycling mechanism, where Ag is recognized by T-CD8+.

In a one APC, both antigen presentation pathways may be involved.

This process is important for CD8+ T cell responses to viruses, other cytosolic microbes, and tumors. Defense against these pathogens and tumors requires CD8+ T cells, and the activation of these T cells is best induced by antigen presentation by DCs.



Natural Killer (NK) cells express special receptors to MHC I molecules:
NK-immunoglobulin-like receptor (KIR) – Killer Immunoglobulin-Receptor
NK cells process receptors that inhibit KIR activity to avoid eventual attack by NK on normal cells.

NK can attack abnormal cells (infected, tumor) that express MHC I molecules at a reduced level, thereby leading to incompetence of CD8.

Therefore, the cytolytic action of NK on the infected cell or tumor cell can compensate for decreased CD8 activity, when there is HLA I incompetence.

Organ transplantation (e.g. kidney, bone marrow) is a condition when the MHC I molecules of the donor graft are not recognized by CD8 and CD4 T lymphocytes, respectively, as their own, which can lead to the destruction of transplanted cells and graft rejection by cytolysis, apoptosis and triggering of the inflammatory response.

Adjustment between HLA-A, HLA-B and HLA-DR molecules is an important and decisive condition for avoiding transplant rejection.

Corneal grafts do not require this kind of adjustment, until in an inflammatory process conditions are created for access to the graft of T-lymphocytes (CD4).

Types of grafts:

Autographs (skin transplant from one region of the body to another).

Isographs (tissue transfer between people of identical genetic status, e.g. twins).

Allographs or homografts (transplantation of tissue from person to person of the same species).

Xenographs or **heterographs** (tissue transplantation between different species of biological organisms).

Graft destruction mechanisms and tipe:

1. **Active T-CD8 cells – type IV cellular hypersensitivity pattern.**

2. **B lymphocytes, which will produce antibodies - the model of humoral hypersensitivity type II (cytolytic reaction supported by antibodies, as well as classical complement activation and opsonization).**

- ► Hyperreactive rejection – the first few hours.
- ► Acute rejection – 10-30 days
- ► Chronic or delayed rejection – months and even years.

Mutations in HLA genes and incidence of various somatic diseases

HLA-MHC I (A, B, C)

HLA-B27 – a variant of the HLA-B gene (8% in the population)

HLA-B27 – 26 alleles encoding 24 protein subfamilies

HLA-B2701 – HLA-B2725

The HLA-B27 gene is associated with high incidence of rheumatic diseases such as:

- **Ankylosing spondylarthritis (men > women = 8:1) HLA-B27>80%**
- **Psoriatic arthritis**
- **Reactive arthritis**
- **For these diseases, HLA-B27 is a notable diagnostic marker!**
- **N.B. HLA-B27 gene is not found among Japanese, so other markers are used for this ethnic.**

HLA-B27

The HLA-B27 gene is also associated with uveitis (>70%) and Crohn's colitis.

The plausible pathogenetic theory:
The gene changes the structure of the gut microbiome (trillions of bacteria, viruses, fungi, etc.). It controls the immune system, and HLA-B27 can disrupt its functional nativity.
Patients with AS (ankylosing spondylarthritis) have a less diverse microbiome, with more harmful bacteria and an intestinal barrier that can allow toxins and microbes to translocate the intestinal barrier, prompting an inflammatory response.
Proof: Microbes that should only be found in the gut sometimes appear in the joints of SA patients.

HLA-B27

Pathogenetic:

Plausible theory – arthrogenic peptide hypothesis:

When HLA-B27 forms a bond with a peptide from a microbial source, it triggers a response of T-CD8 cells to an autopeptide with a structure similar to microbial peptide.

Evidence for this mechanism has developed in patients with reactive arthritis after salmonella or chlamydia infections, which trigger a specific response of HLA-B27 CD8 T cells.
IL-23 is elevated in patients with HLA-B27, this cytokine being involved in the immune-inflammatory response.

HLA-B27

Pathogenetic:

Plausible theory – molecular mimicry hypothesis:

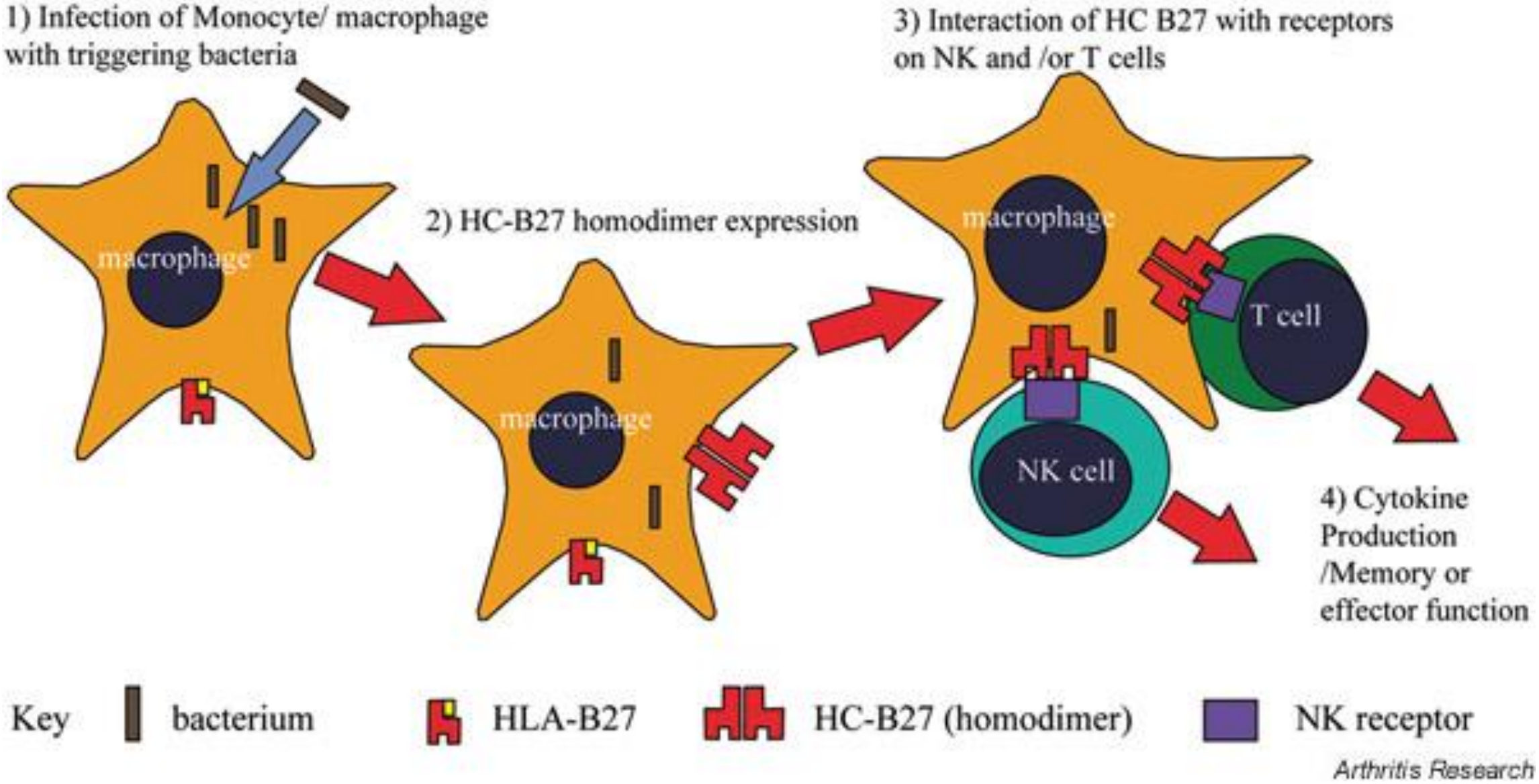
Structurally, the HLA-B27 molecule imposes itself through common amino acid sequences with different bacteria.
Thus, the antibodies formed can react not only with the epitope of microbial antigen, but also with the HLA-B27 molecule expressed on somatic cells.

HLA-B27

Pathogenetic:

The plausible theory – the receptor hypothesis:

The HLA-B27 molecule is used by microorganisms, which recognize it, as an entrance gate to the cell.

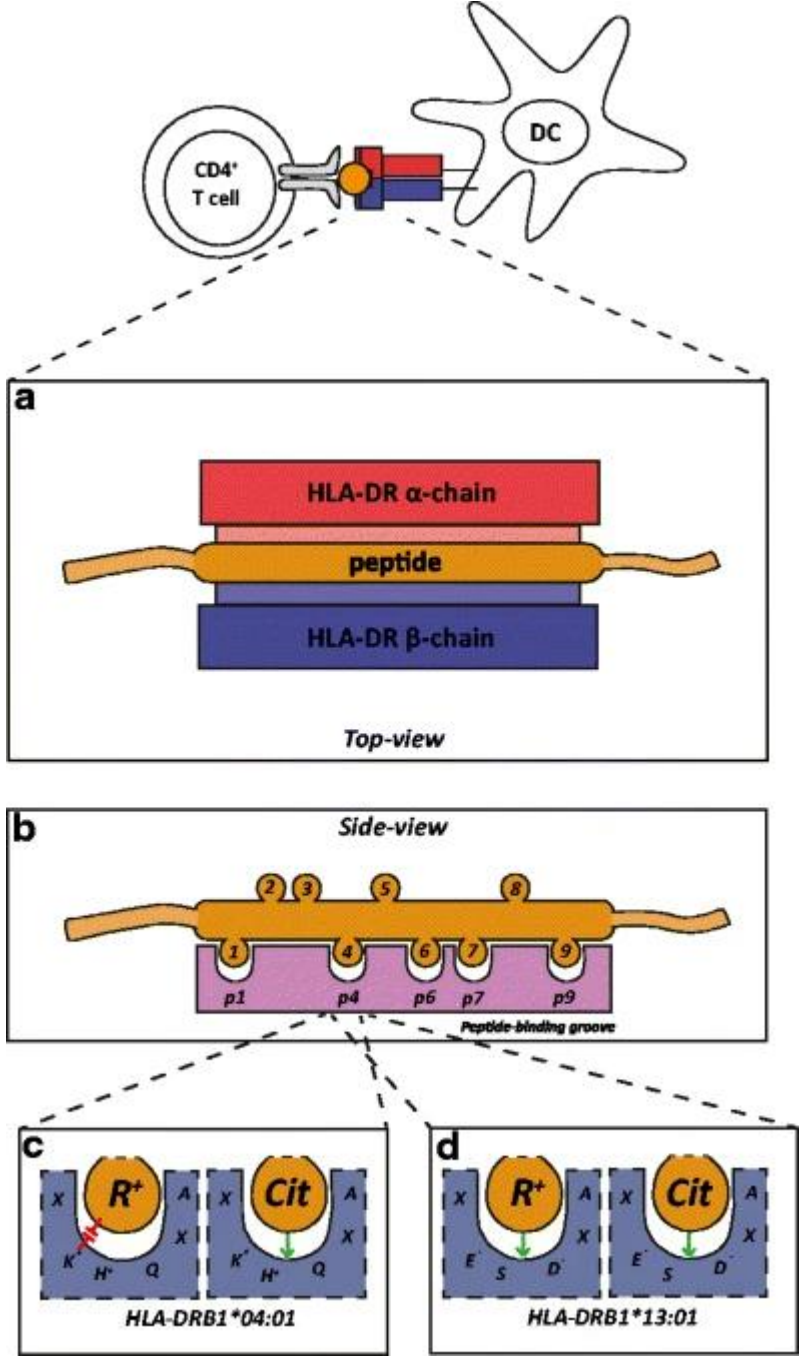

1) Infection of Monocyte/ macrophage with triggering bacteria
macrophage
2) HC-B27 homodimer expression
macrophage
3) Interaction of HC B27 with receptors on NK and /or T cells
macrophage
T cell
NK cell
4) Cytokine Production /Memory or effector function
Key
bacterium
HLA-B27
HC-B27 (homodimer)
NK receptor
Arthritis Research

Mutations in HLA genes and incidence of various somatic diseases

HLA-MHC II (DR, DP, DQ)

The genetic polymorphism of MHC II is > compared to MHC I

The presence of the MHC II-DRB1 variant is associated with rheumatoid arthritis.



In the case of HLA-DRB1, MCH II presents T-CD4 lymphocytes with the antigen of proteins with high citrulline content (amino acid obtained from arginine or ornithine) in the synovial fluid.
As a result, the inflammatory response intensifies.