# Presentation of Antigen and Recognition of Antigen in Acquired Immunity
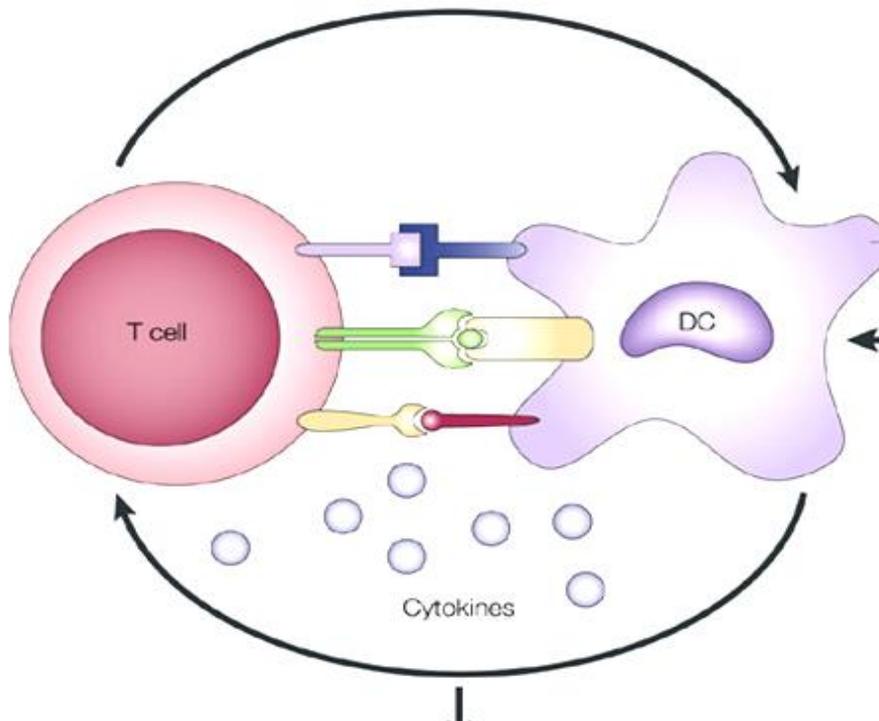
Antigen uptake and antigen presentation to lymphocytes

Recognition of antigens in acquired immunity

Presentation of Antigens

What do lymphocytes see?

APC division; APC function

Function of MHC molecules

Processing and presentation of protein antigens

Adaptive (specific) immune response...

… it starts when

antigen receptors on lymphocytes

recognize (see) antigens.

Antigen Receptors of Lymphocytes

B cell receptor (BCR)

- BCR (membrane antibody) recognizes macromolecules (proteins, lipids, polysaccharides, lipopolysaccharides, nucleic acids), as well as small molecules in solution or on the surface of the corpuscular antigen.
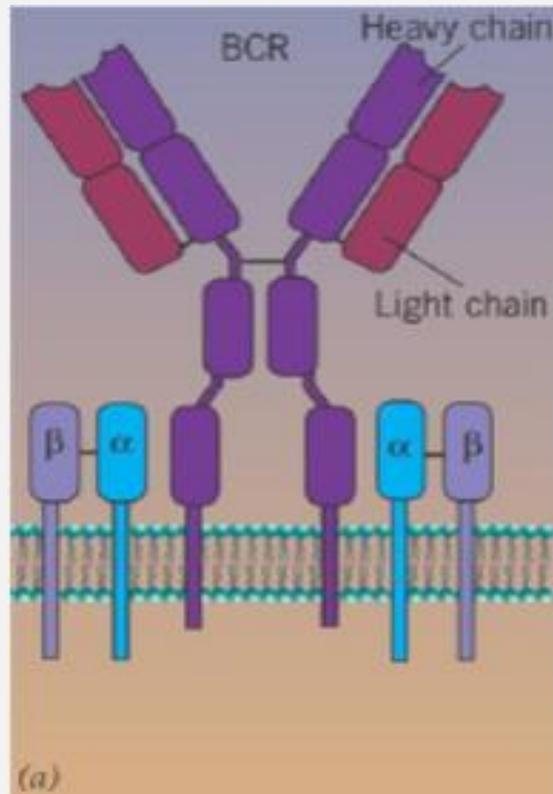
T cell receptor (TCR)

- Most of the TCR **recognizes only peptide fragments of protein antigens when displayed on the surface of the APC** (*Antigen Presenting Cells*).
- These peptides are displayed on the membrane as part of special molecules specializing in peptide rendering (MHC).
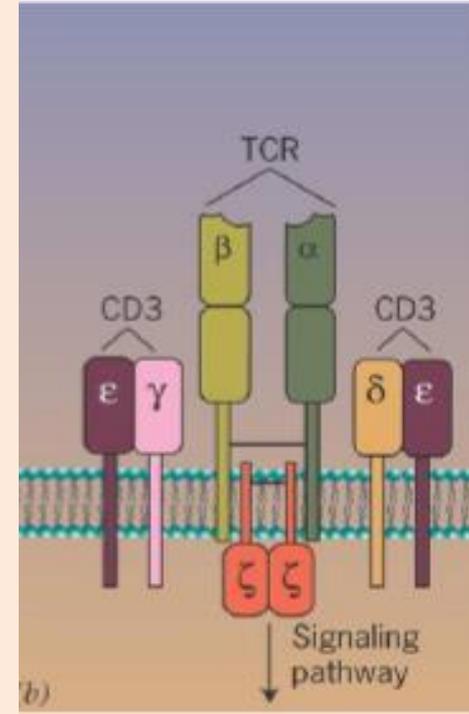
What do T lymphocytes see?

T lymphocytes only see (recognize) peptides attached to MHC

molecules (Major Histocompatibility Complex) on the surface of the host cell.

In other words: T lymphocytes see peptide parts of antigens only if they are presented in the context of MHC products that are expressed on our cells.

T lymphocytes of one individual recognize peptides only if they are presented within the MHC molecules characteristic of that individual – MHC restriction.

T cell contact residue of peptide

T cell receptor

Polymorphic residue of MHC

Peptide

Anchor residue of peptide

MHC

"Pocket" of MHC
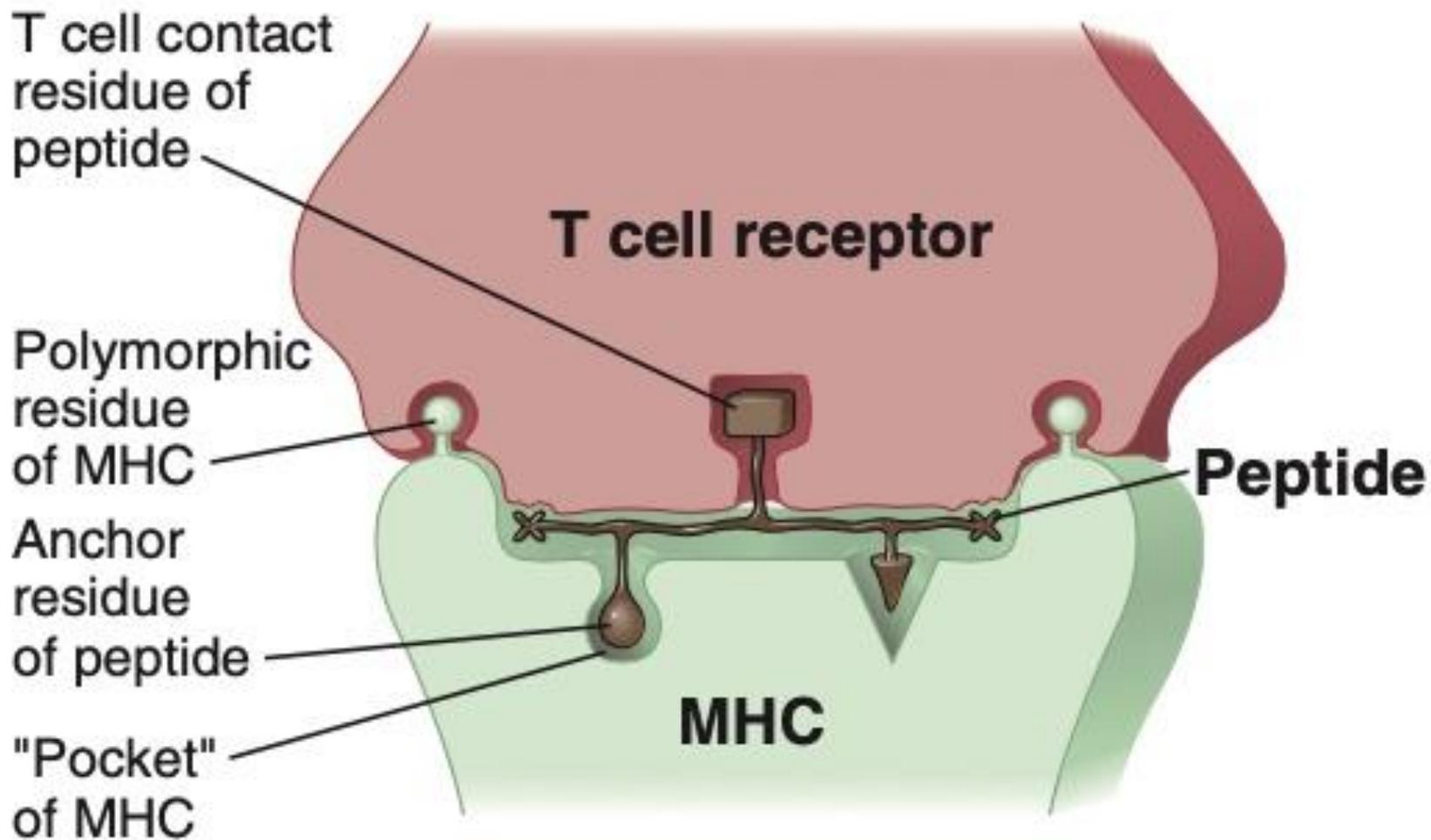
Cells That Display Antigen
(APC, *Antigen Presenting Cells)*

T_H1 cell.. Over here. Antigen found.

Immense Immunology Insight
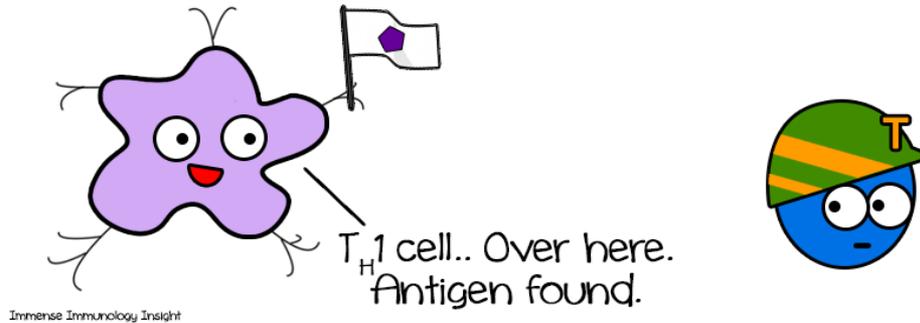
* **Professional APC (APC <u>in a narrow sense)</u>**
* Show peptide antigens in the MHC **II** class, and also in the MHC **I** class.

   These are: **Dendritic cells, Mo/Mf Cells, B lymphocytes**.

* **Non-professional APC (APC <u>in a broad sense)</u>**
   Display peptide antigens, as part of class <u>I MHC</u> molecules effector <u>CD8 + T lymphocytes.</u>
   Exception: erythrocytes, sperm cells and trophoblast.

Antigen Presentation

- To initiate immune responses, antigens are captured from their site of entry and concentrated in secondary (peripheral) lymphoid organs through which naïve T cells circulate constantly.

- T lymphocytes recognize and respond only to cell-associated antigens and not to soluble, cell-free antigens

Skin
Gastrointestinal
Respiratory tract

Microbe   Epithelium

Cell-free antigen

Dendritic cell-associated antigen

Lymphatic vessel

Antigen that enters blood stream

Venule

Connective tissue

To lymph node

To circulation and spleen
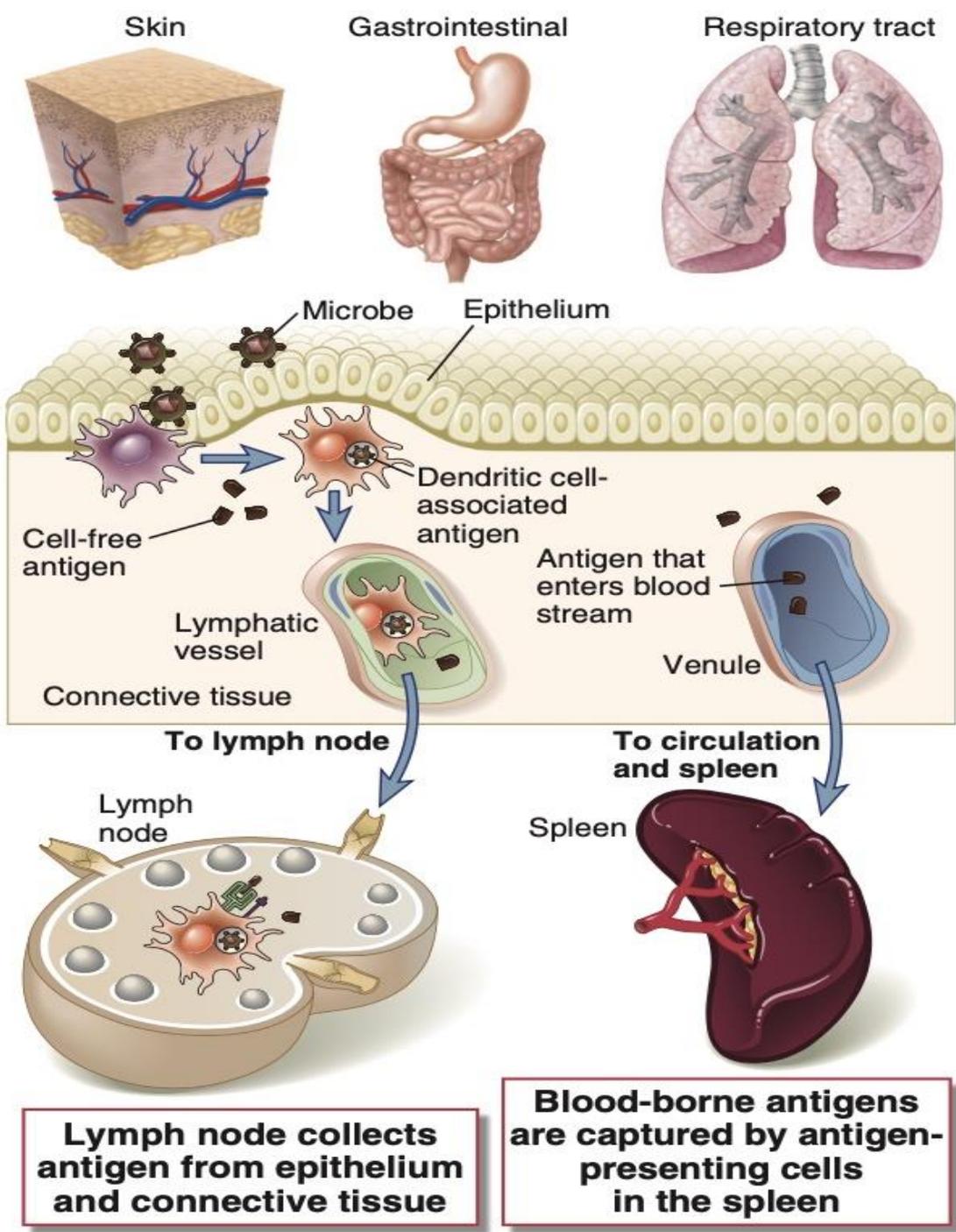
Lymph node

Spleen

Lymph node collects antigen from epithelium and connective tissue

Blood-borne antigens are captured by antigen-presenting cells in the spleen
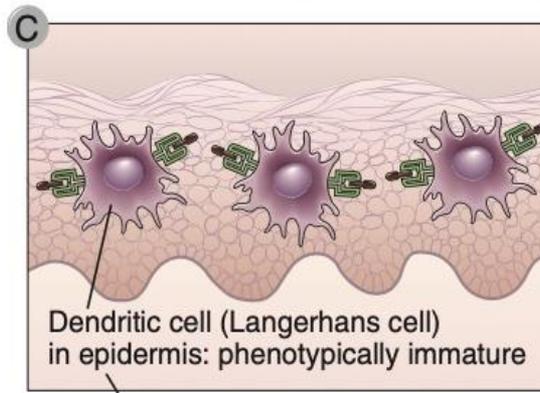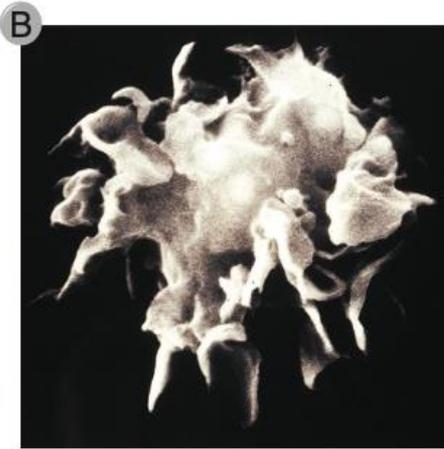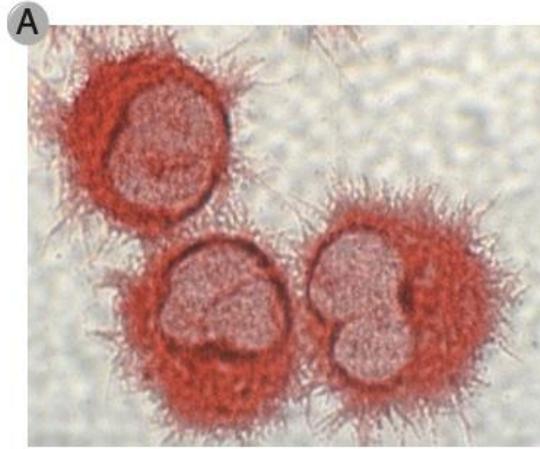
C Dendritic cell (Langerhans cell) in epidermis: phenotypically immature
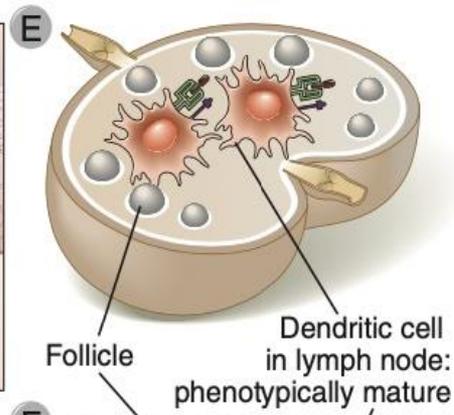
E Follicle

Dendritic cell in lymph node: phenotypically mature

Antigen capture by dendritic cells (DC)

Activation and maturation of dendritic cells

Antigen capture

Immature DC in epidermis (Langerhans cell)

Afferent lymphatic vessel

Migration of DC

Dermal DC

T cell

Antigen presentation

Mature dendritic cell presenting antigen to naive T cell

Lymph node

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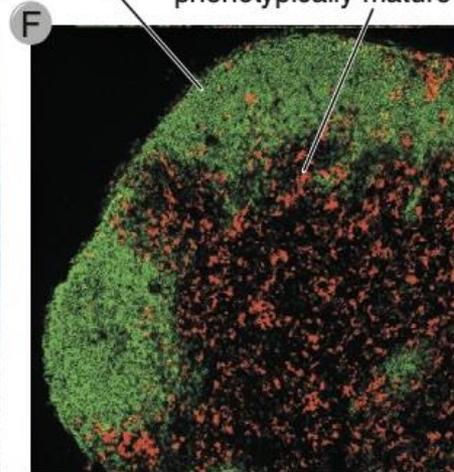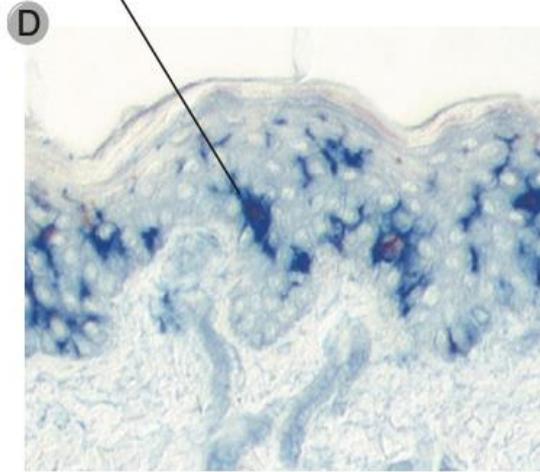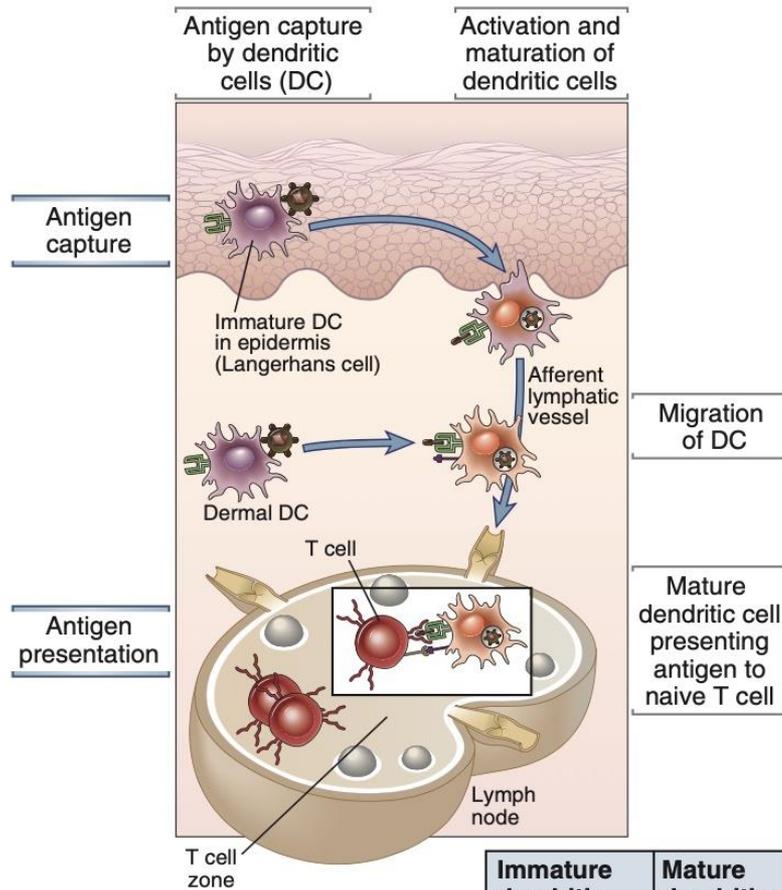
	Immature dendritic cell	Mature dendritic cell
Principal function	Antigen capture	Antigen presentation to T cells
Expression of Fc receptors, mannose receptors	++	−
Expression of molecules involved in T cell activation: B7, ICAM-1, IL-12	− or low	++
Class II MHC molecules		
Half-life	~10 hr	>100 hr
Number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

| Antigen uptake | Antigen presentation | Response |

Dendritic cell

Costimulator (e.g., B7) — CD28

Naive T cell — Effector T cells

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

Macrophage

Effector T cell — Killed bacteria

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

B cell

Effector T cell

Antibody

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

How APC take protein Antigens?

- ✓ Microorganisms, which have entered the body, are taken over by professional APCs. And they put them in the endosomes..
  -This event (as well as cytokines innate immunity – TNF и IL-1), activates dendritic cells.

- ✓ **Activated dendritic cells** they lose the ability to adhere to the epithelium and express receptors for those chemokines that are produced in lymph nodes (LN).
  -This leads them towards the regional LN In the paracortex. (T lymphocyte zone).

- ✓ **During migration, dendritic cells change further: they become mature** (they show co-stimulating molecules) and capable of activating T lymphocytes.

Let's remember...

In addition to dendritic cells, there are other professional APC!!!

Professional APC

1. Dendritic cells are most potent in activating naïve CD4+ T lymphocytes.

They can also direct the differentiation of naïve CD4+ T lymphocytes in different directions.

Dendritic cells display peptide antigens to CD8 + T lymphocytes also as part of Class I MHC molecules – CROSS PRESENTATION.

2. **Mo/Mf** are important APC **in the effector phase of cellular immune response.**

They present antigens to effector CD4+ T lymphocytes.

3. **B Lymphocytes are the dominant APC in the humoral immune response.**

Other Functions of APC

In addition to the presentation of antigens, APC provide T lymphocytes so-called The second activation signal...

...The second signal is provided by **co-stimulating membrane proteins and cytokines**.

| Features of Antigens Recognized by T Cells	Explanation
Most T cells recognize peptides and no other molecules.	Only peptides bind to MHC molecules.
T cells recognize linear peptides and not conformational determinants of protein antigens.	Linear peptides bind to clefts of MHC molecules, and protein conformation is lost during the generation of these peptides.
T cells recognize cell-associated and not soluble antigens.	Most T cell receptors recognize only peptide-MHC complexes, and MHC molecules are membrane proteins that display stably bound peptides on cell surfaces.
CD4$^+$ and CD8$^+$ T cells preferentially recognize antigens ingested from the extracellular environment into vesicles and antigens present in the cytosol, respectively.	Pathways of assembly of MHC molecules ensure that class II MHC molecules display peptides that are proteolytically degraded in vesicles in APCs and class I MHC molecules present peptides from cytosolic proteins that are degraded by cytosolic proteasomes.

Genes and Products of MHC

Class I MHC

Peptide-binding cleft

Peptide

α1   α2

α1   α2

N   N

α3

β2-microglobulin

β2m

α3

C

Transmembrane region

Disulfide bond -----

Ig domain

C

Class II MHC

Peptide-binding cleft

Peptide

α1   β1

α1   β1

NN

α2

α2   β2

β2

Transmembrane region

Disulfide bond -----
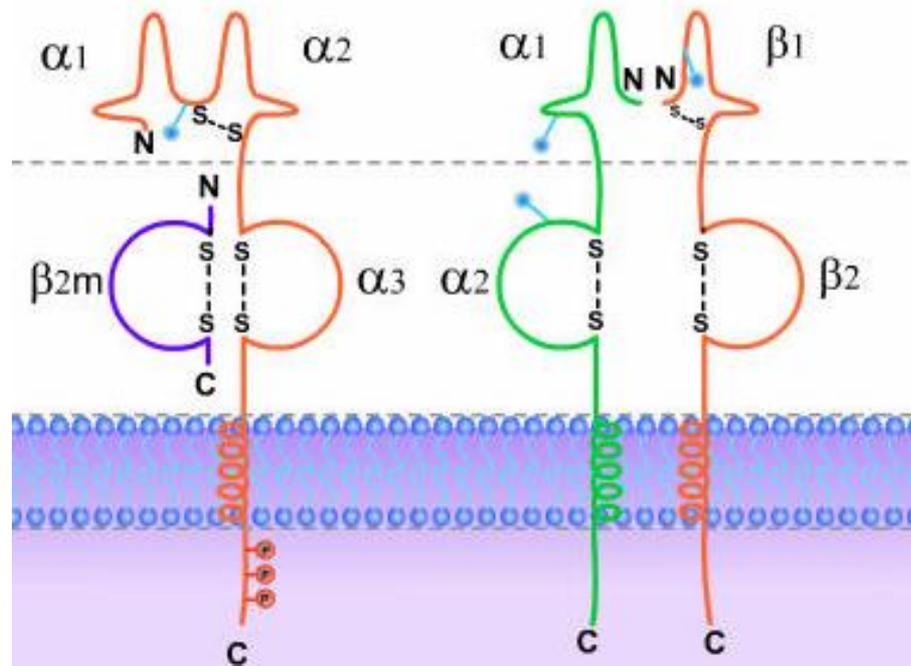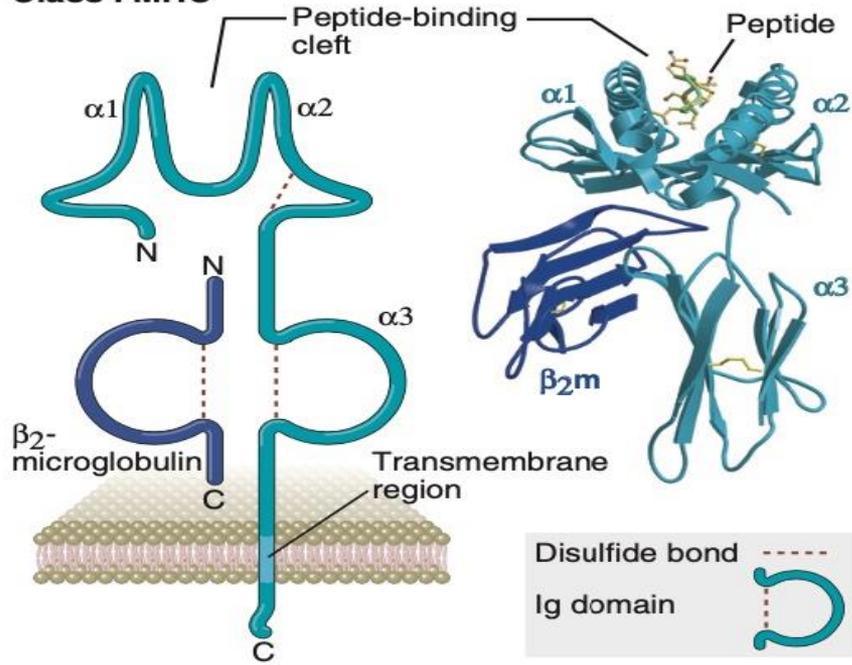
Ig domain

C   C
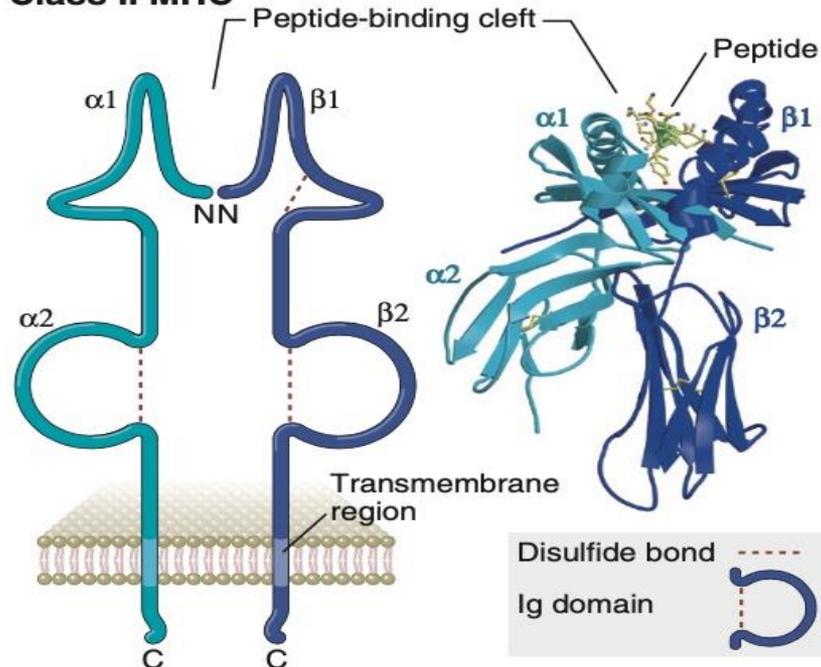
The molecules of MHC are membrane proteins whose function is to display peptides to the T lymphocytes.

A

HLA-A2 (Class I) HLA-DR1 (Class II)

B

Peptide

Pockets in floor of peptide binding groove of class II MHC molecule

Anchor residue of peptide
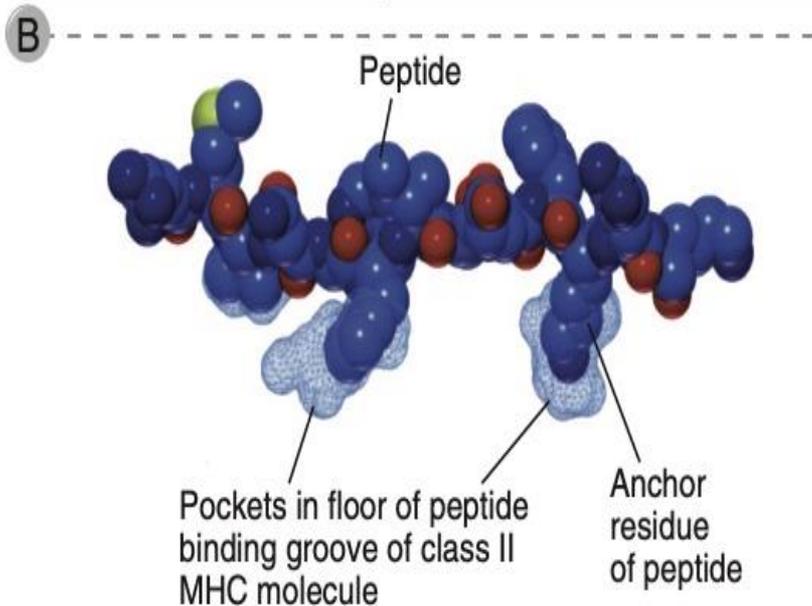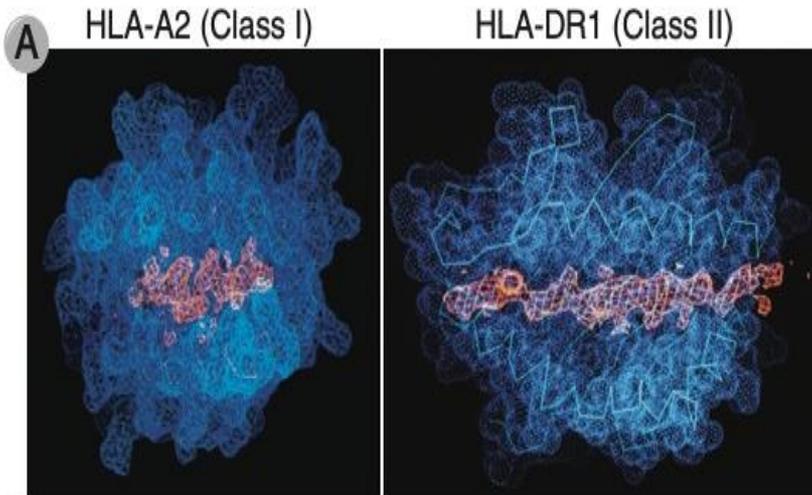
MHC molecules at their N-end have a dent to the bottom of which peptides bind

| **Products of the I class MHC**	**Products of the II class MHC**
• **α1 and α2 domains* build an active site, that is, a dent whose bottom is formed by the most polymorphic parts of molecules, to which a peptide-sized peptide is bound by terminal amino acids** 8-11 a. a.	• **the α1 and β1 domains make up the walls of the dent large enough to accommodate a peptide size** 10 – 30 a. a.
	• **The bottom of the dent makes up the most polymorphic parts of the molecule**.
• At the top of the dent there are less polymorphic parts of molecules that recognize T lymphocytes (Characteristic of each allele).	• At the top of the dent there are less polymorphic parts of molecules recognized by T lymphocytes (characteristic of each allele).
• the α3 domain is non-polymorphic (the same in alleles) and is the binding site of the T cell coreceptor CD8.	• β2 is a non-polymorphic and is a place of binding of the T cell coreceptor CD4.

* The domain (loop, globule) consists of amino acids twisted around one S-S bond.

Important!!!

**(1) Class I of MHC present antigens to CD8 + T lymphocytes**
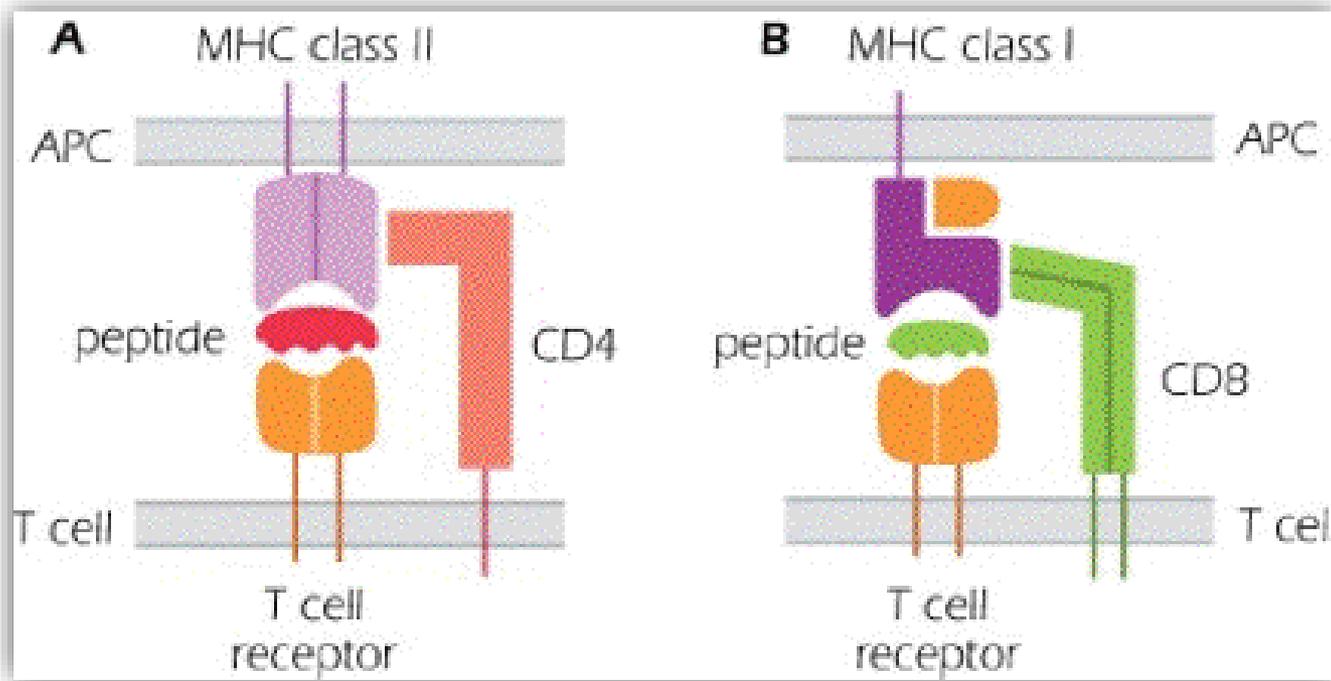
(2) Class II of MHC present antigens to CD4 + T lymphocytes

MHC Genes

- Polymorphism
- Coupled inheritance (in block)
- Codominant expression

- **MHC** locus (a set of coupled genes on one chromosome)
    - MHC = **HLA**

- **Genes of I and Class II MHC**
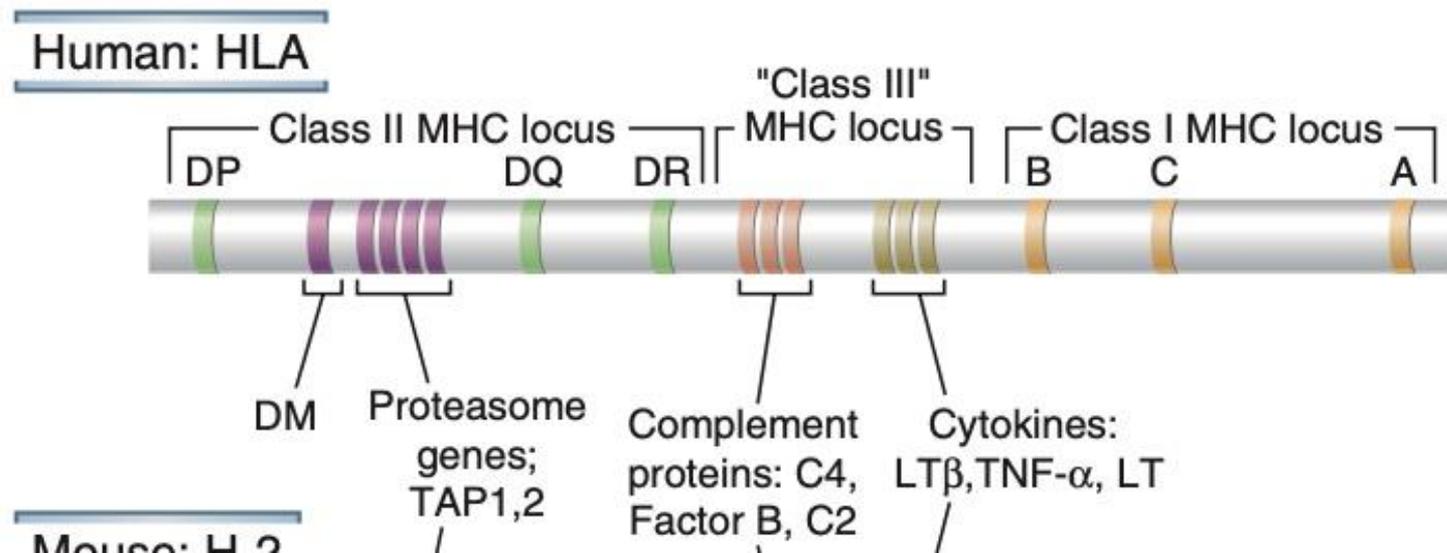
Characteristics of HLA(MHC) Genes

1. Codominant expression...

... the simultaneous expression of inherited alleles from the paternal and maternal chromosomes is mandatory.

a combination of alleles on one of the chromosomes called The MHC haplotype.

NUMBER of products reported:

✓ There are three genes – HLA-A, HLA-B, HLA-C **for α chain I class on each of the chromosomes:**

3x2 = 6 class I MHC in/on each cell..

✓ There are three genes –HLA-DP, HLA-DQ, HLA-DR for α chain and three or four genes for β chain (which can be combined) II class on both Chromosomes:

3x3x2 = 18 class II MHC products on every professional APC
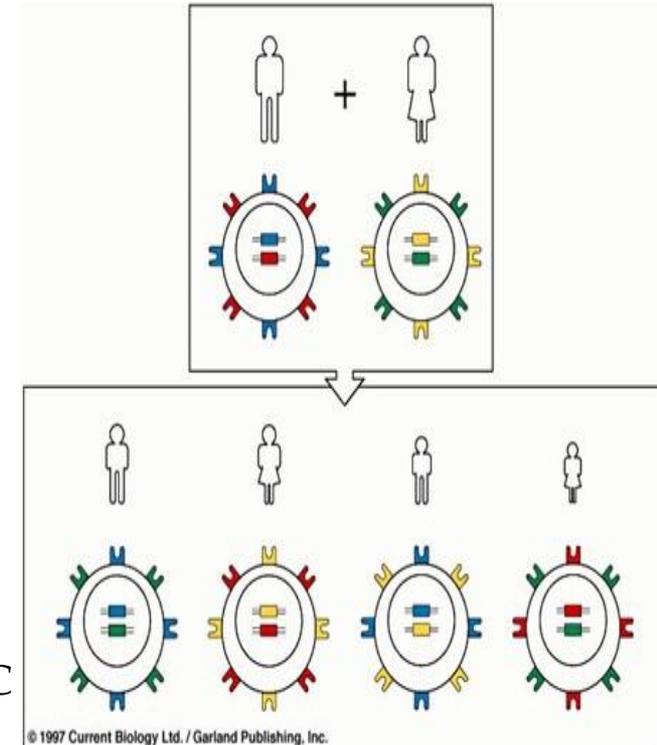
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2. The Inheritance...

3. Polymorphism...
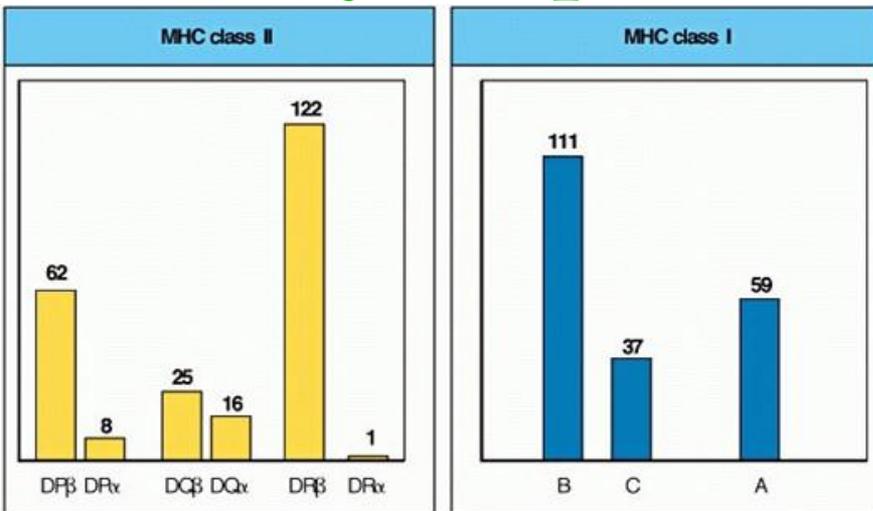
... **It has a large number of alleles.**
Each of these genes
At the level of the population.
It's not a result of genes.
recombination

they are already encoded by inherited genes.
It is important for selection within the population..

Characteristics of MHC molecules

TABLE 6–5 Comparative Features of Class I and Class II MHC Pathways of Antigen Processing and Presentation

Feature	Class I MHC Pathway	Class II MHC Pathway
Composition of stable peptide-MHC complex	Polymorphic α chain, β_2-microglobulin, peptide Peptide α β2-microglobulin	Polymorphic α and β chains, peptide Peptide α β
Types of APCs	All nucleated cells	Dendritic cells, mononuclear phagocytes, B lymphocytes; endothelial cells, thymic epithelium
Responsive T cells	$CD8^+$ T cells	$CD4^+$ T cells
Source of protein antigens	Cytosolic proteins (mostly synthesized in the cell; may enter cytosol from phagosomes)	Endosomal and lysosomal proteins (mostly internalized from extracellular environment)
Enzymes responsible for peptide loading of MHC	Cytosolic proteasome	Endosomal and lysosomal proteases (e.g., cathepsins)
Site of peptide loading of MHC	Endoplasmic reticulum	Specialized vesicular compartment
Molecules involved in transport of peptides and loading of MHC molecules	Chaperones, TAP in ER	Chaperones in ER; invariant chain in ER, Golgi and MIIC/CIIV; DM
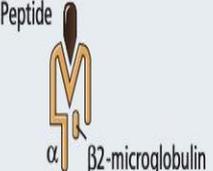
APC, antigen-presenting cell; CIIV, class II vesicle; ER, endoplasmic reticulum; MHC, major histocompatibility complex; MIIC, MHC class II compartment; TAP, transporter associated with antigen processing.

Each MHC molecule binds peptides derived from protein antigens and displays them to the T lymphocytes that recognize them with their TCR.

Each MHC molecule can display only one peptide at a time, but it is capable of displaying a large number of different peptides at different times (it is widely specific).

MHC molecules are constantly synthesized.

MHC molecules bind peptides during their synthesis and assembly within the cell.

MHC molecules are extremely unstable – they stabilize, survive and can reach the membrane of the cell only when they bind the peptide.
.

At any given moment, a huge number of MHC molecules are expressed on the cells.

MHC molecules also show peptides that originate from the proteins of that individual itself.

Processing and Presentation of Protein Antigens

Depending on the origin of the protein, there are two pathways of its processing for displaying T lymphocytes:

- Protein antigens synthesized outside our cells, and then introduced into the vesicles of professional APCs, are processed and presented to the CD4 + T lymphocytes as part of class II products of MHC.
.
- Protein antigens synthesized in any of our cells are presented to the CD8 + T lymphocytes in the context of class I products of MHC.
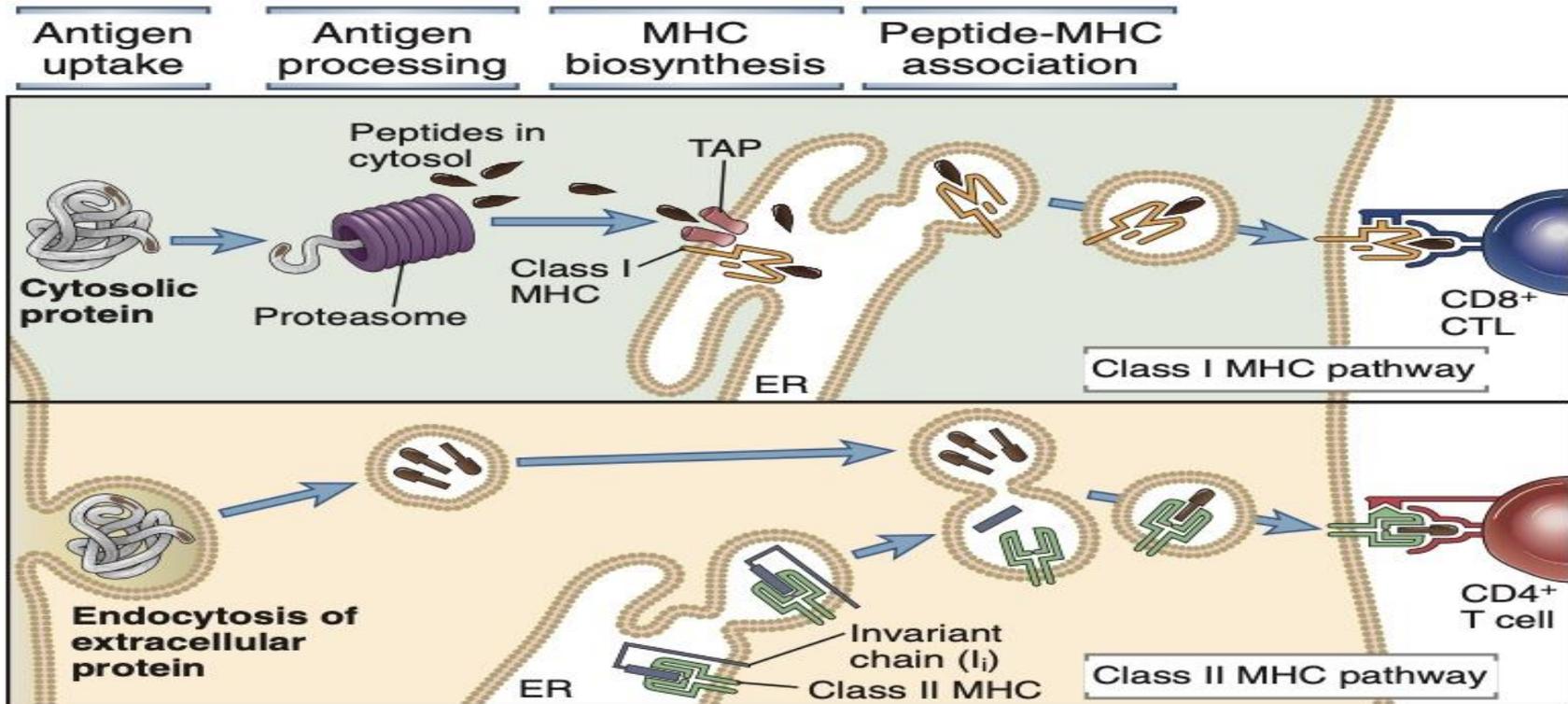
Processing and Presentation of Antigens Within I Class Molecules of MHC

Protein antigens in the cytoplasm are derived from:
viruses that multiply in the cytoplasm;
phagocytic microbes escaped from vesicles;
oncoproteins derived from altered genes.
All these proteins (as well as the aging and worn-out proteins of the cell itself) are proteolytically decomposed...
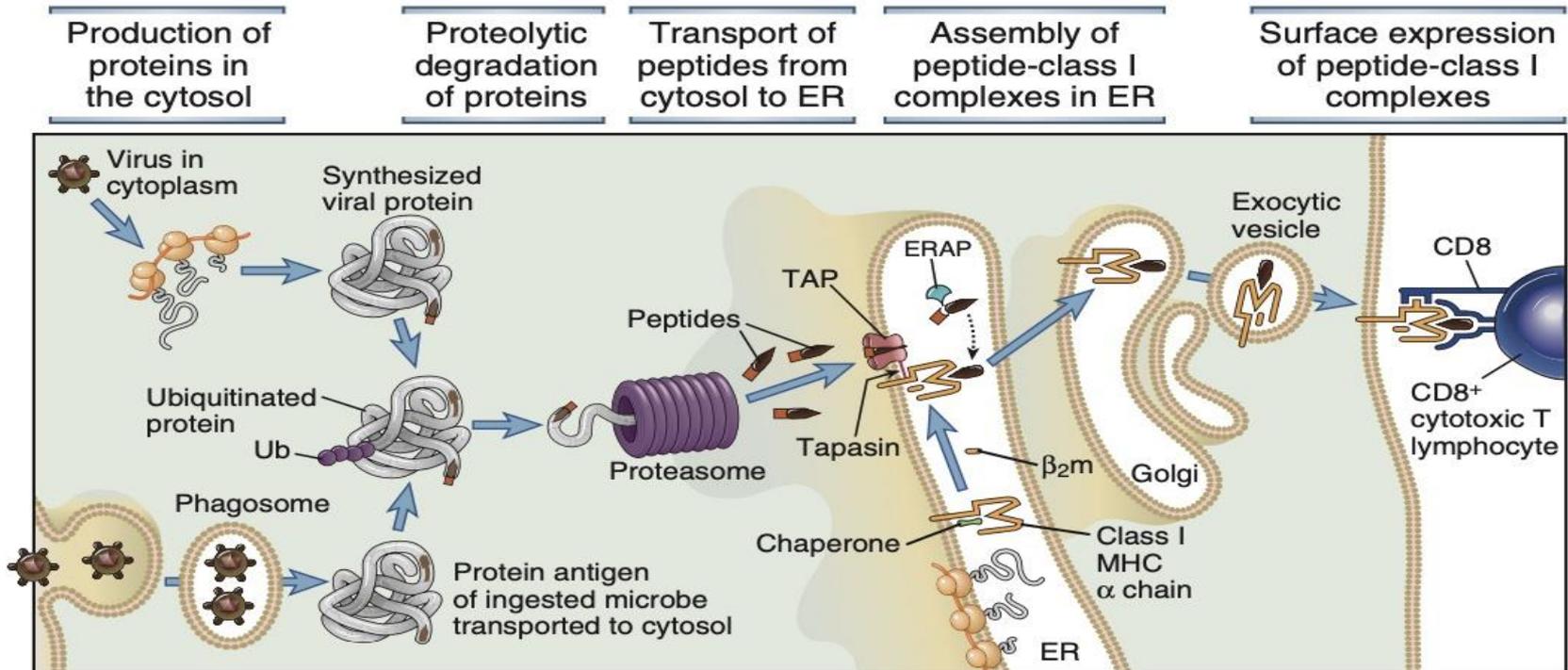
.... And that's by first unravelling, then connecting with ubiquitin and then running through the proteasome.

A proteasome is a proteolytic organelle in which proteins are cut into peptides suitable for bonding with class I molecules of MHC.

- **TAP** (*Transporter associated with Antigen Processing)* the active transport inserts peptides into the ER.

- MHC Class I molecules bind peptides in this way in the ER.

- **The exocytosis vesicle is transported to the membrane where they exhibit the bound peptide**.
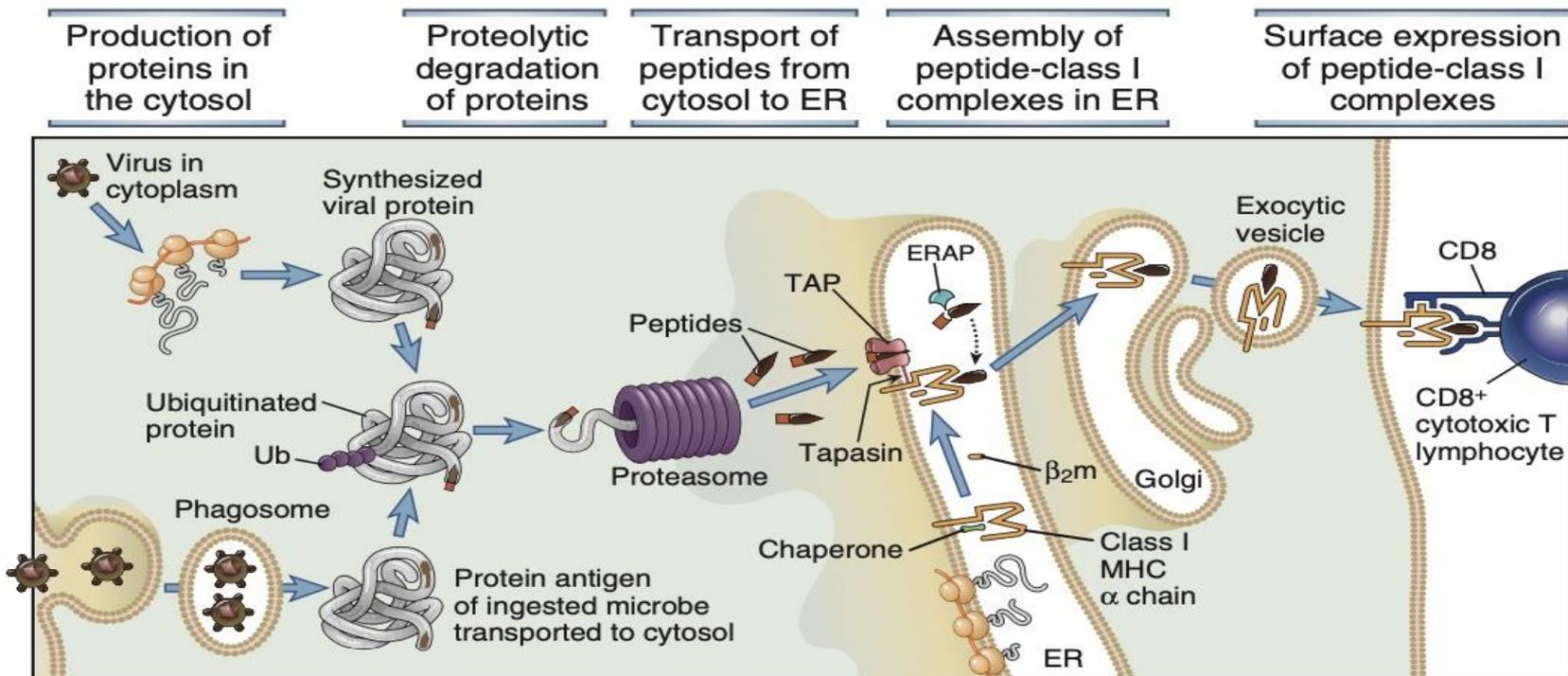
# Processing and Presentation of Antigens Within II Class Molecule MHC

Professional APCs intake microorganisms into endosomes and phagosomes by various mechanisms, which then merge with lysosomes in which the proteins of microorganisms are enzymatically decomposed to peptides of different lengths.

| 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
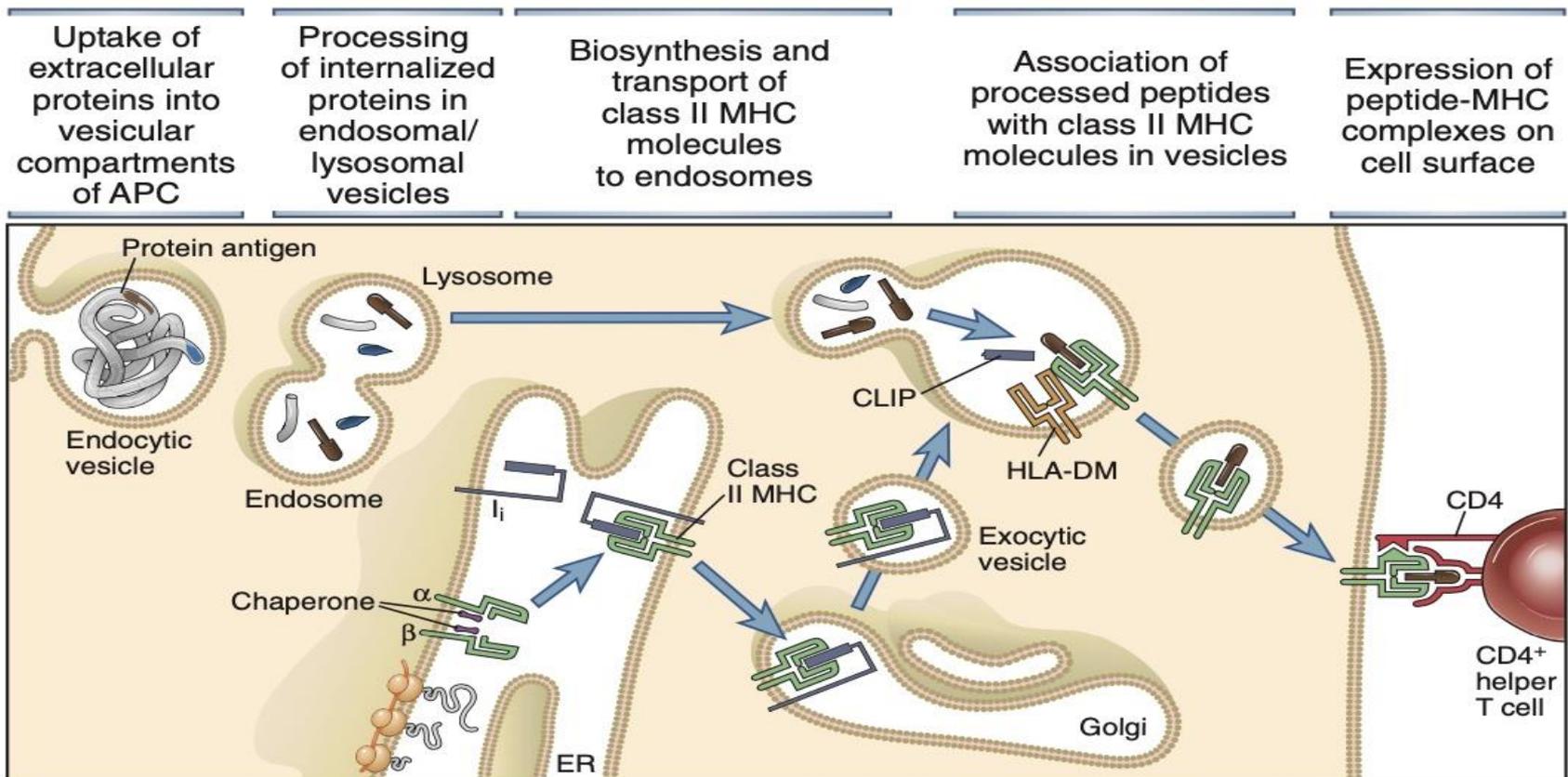
- MHC they are constantly synthesized in the ER in which they connect active place of MHC and **the unchangeable chain (CLIP** – *class II invariant chain peptide*). CLIP temporarily stabilize MHC in a newly formed exosome that merges with an endosome. In the endosome there are peptides formed by decomposition.
- In the endosomal vesicles there is **DM –** a molecule similar to a class II product of MHC which now takes the CLIP and frees up the place for peptide binding, it permanently stabilizes the complex that is expressed on the membrane.

Synthesis of class II MHC in ER	Transport of class II MHC and Iᵢ to vesicle	Binding of processed peptides to class II MHC	Transport of peptide-Class II MHC complex to cell surface	Expression of peptide-MHC complex on cell surface

HLA-DM–catalyzed removal of CLIP — HLA-DM

Peptide antigens

Iᵢ

Class II MHC

CLIP

Proteolytic degradation of Iᵢ

Endosome (MIIC/CIIV)

ER
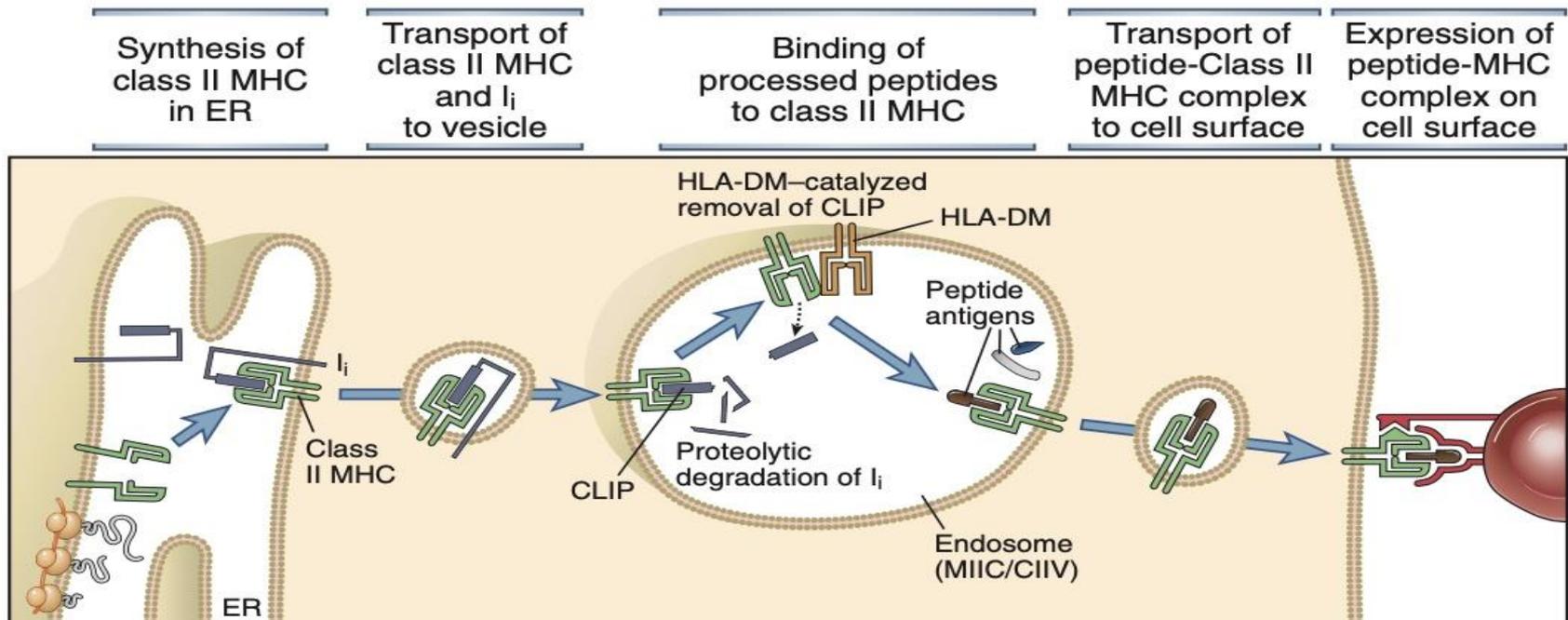
Cross Presentation
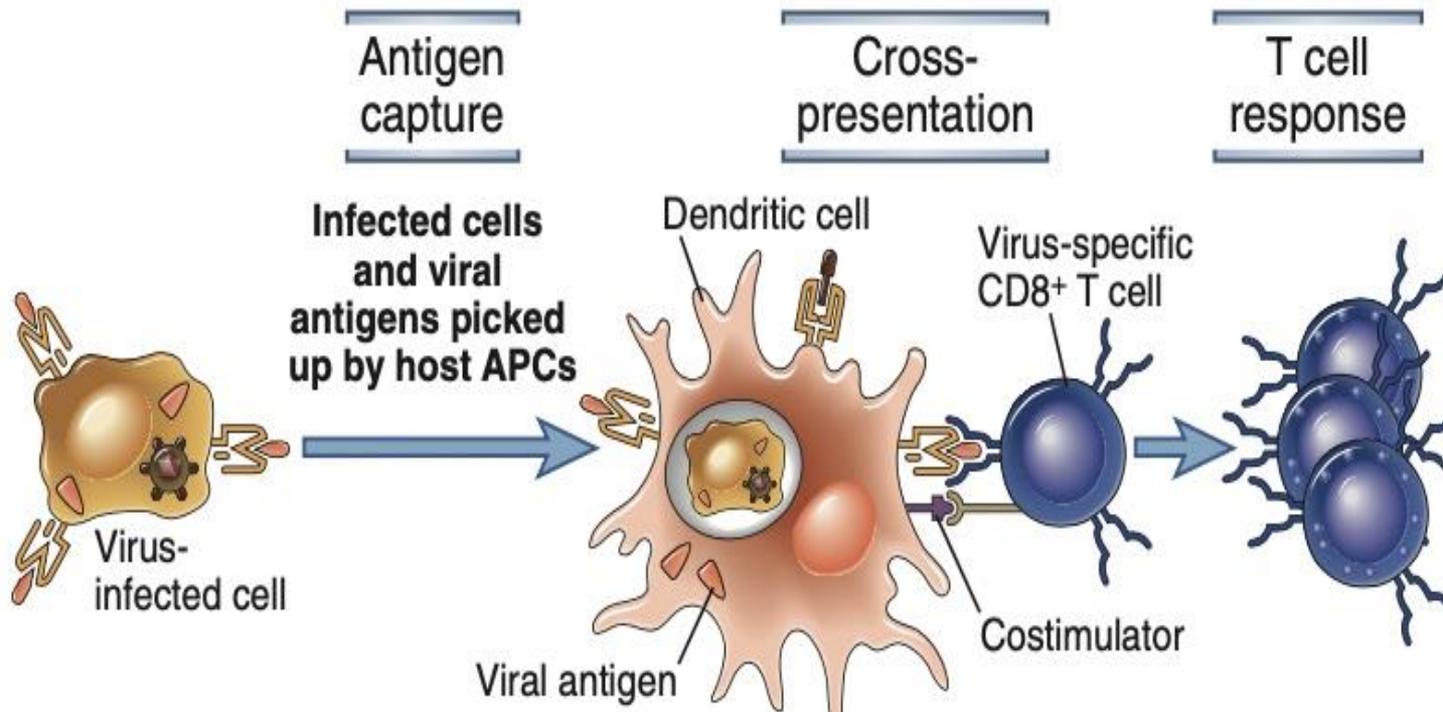
Physiological Significance of Antigen Presentation Within MHC

Antigen uptake or synthesis — Antigen presentation — T cell effector functions

A Class I MHC–associated presentation of cytosolic antigen to cytotoxic T lymphocytes

Cytosolic antigen — CD8+ cytotoxic T lymphocyte — Killing of antigen-expressing target cell

B Class II MHC–associated presentation of extracellular antigen to helper T cells

Macrophage — Antigen in endosome — Extracellular antigen — CD4+ helper T lymphocyte — Cytokines — Macrophage activation: destruction of phagocytosed antigen

Antigen-specific B cell — Extracellular antigen — B cell antibody secretion: antibody binding to antigen

Extracellular microorganisms are taken over by professional APC and show their peptides within molecules II class **MHC**. This complex is recognized by CD4 + T lymphocytes.

CD4 + T lymphocytes with their cytokines mediate:

- B lymphocytes to produce antibodies
- Phagocytes to ingest and destroy the microorganism.

In this way, two of the most effective mechanisms for the elimination of extracellular and phagocytic microbes are activated.

Antigen uptake or synthesis — **Antigen presentation** — **T cell effector functions**

A. Class I MHC–associated presentation of cytosolic antigen to cytotoxic T lymphocytes

Cytosolic antigen → CD8+ cytotoxic T lymphocyte → Killing of antigen-expressing target cell

B. Class II MHC–associated presentation of extracellular antigen to helper T cells

Macrophage + Extracellular antigen → Antigen in endosome / CD4+ helper T lymphocyte → Cytokines → Macrophage activation: destruction of phagocytosed antigen

Antigen-specific B cell — Extracellular antigen → Cytokines → B cell antibody secretion: antibody binding to antigen

...However:

Neither of these two mechanisms is effective.

against microorganisms (mainly viruses), which parasitize in the cytoplasm of infected cells.

Peptides of these cytoplasmic microorganisms are shown by class I molecules of MHC.

This is recognized by CD8 + T lymphocytes that differentiate into CTL capable of killing the infected cell and thus removing the infection.

What do B lymphocytes see?

- B lymphocytes recognize antigens on the surface of microorganisms or soluble antigens.

- These antigens B lymphocytes see in their native form..

Recognition of antigens in specific immunity

Antigen receptors of B- and T- cells

Maturation and selection of lymphocytes

...Let's remember

The acquired immune response is <u>always specific to the antigen that caused it</u> because the activation of lymphocytes is due to specific antigen recognition.

This <u>recognition is performed by antigen receptors</u>, which detect signals (antigens) and activate the response of the cell on which they are expressed.

These receptors are able to recognize, distinguish and bind a <u>large number of antigens</u>.

...Let's remember

These receptors are clonal distributed, which means that <u>each clone of lymphocytes have a unique receptor.</u> Which is different from the receptor fro other lymphocyte clones.

Antigen X

Clonal selection

Anti-X antibody
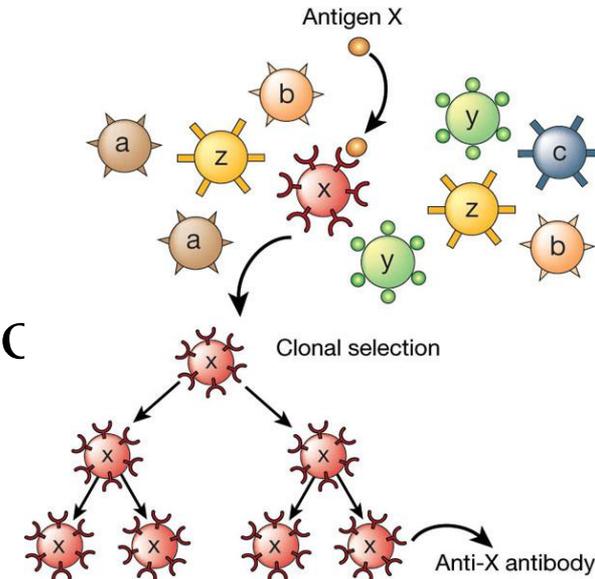
Although every lymphocyte recognizes different antigen, antigen receptors carry out the same activation signals.

T Cell and B Cell Antigen Receptors
(TCR and BCR)

B cell Receptor

BCR recognition

light chain
heavy chain

Igβ Igα

ITAM

signaling

T cell Receptor

TCR recognition

CD3   CD3

α   β

ε   δ   γ   ε

ITAMs   ζ   ζ

signaling
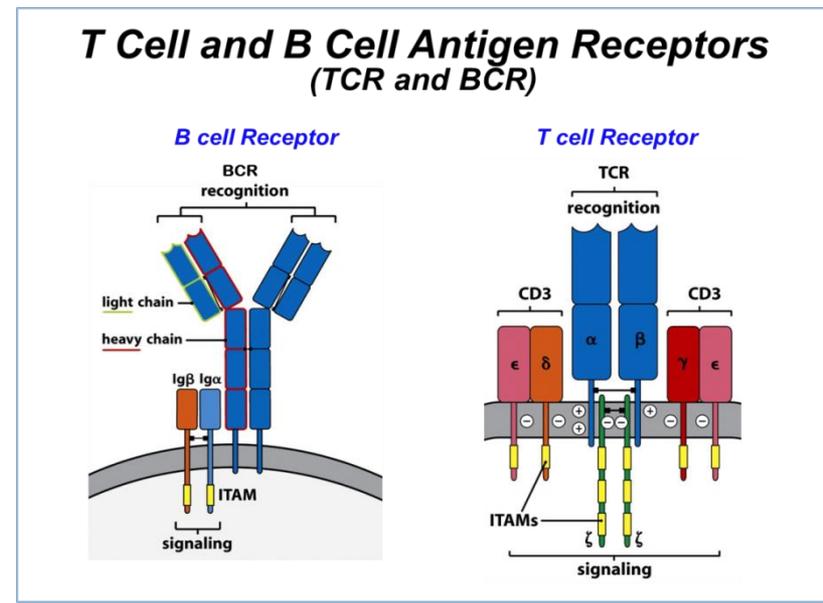
The questions that need to be answered today...

...How antigen receptors of lymphocytes manage to recognize so much different antigens while delivering the same activation signals to the cell?

How a huge variety of antigen receptors is formed?

Features of BCR and TCR
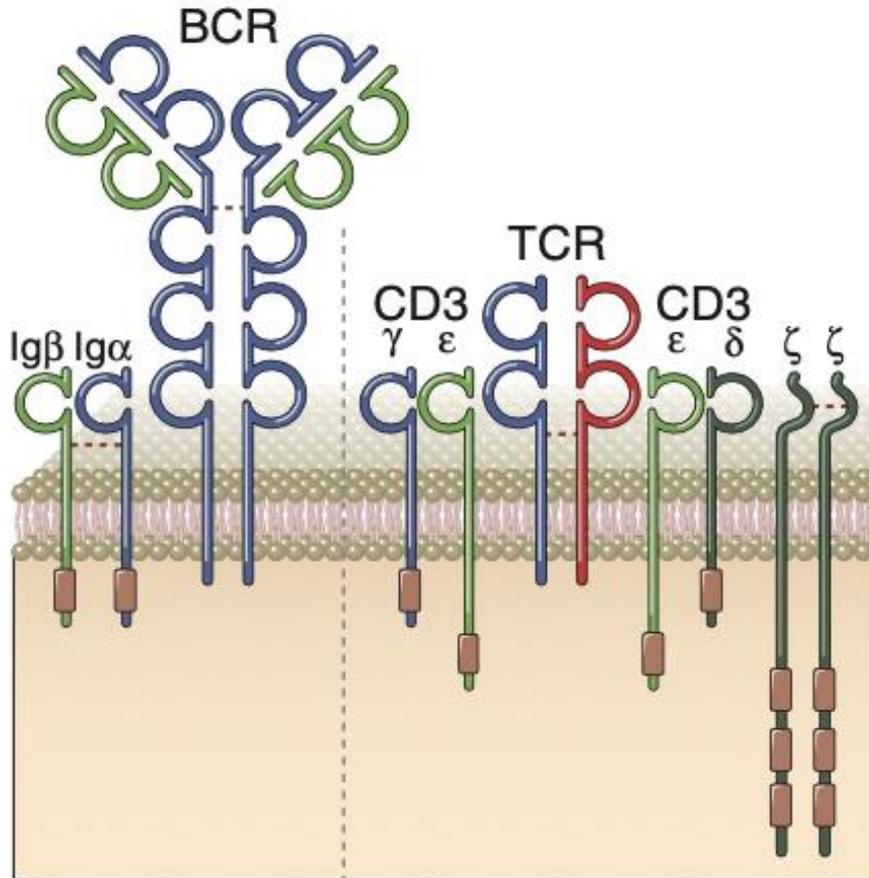
Antigen receptors of B and T lymphocytes recognize chemically the same or different structures.

B lymphocytes predominantly recognize conformational antigen determinants (different macromolecules and chemical groups).

T lymphocytes recognize linear antigenic determinants (peptides).

Features of BCR and TCR

Receptors consist of variable (V) and conserved constant regions (C). Within the V region there are hypervariable regions of CDR that are responsible for recognizing antigens, i.e. to connect with him.

Receptors are non-covalently bound to other immutable molecules whose task is to deliver signals initiated by antigen recognition to the cell – together with the receptor these molecules are called receptor complexes (BCR and TCR complex).

Antibodies exist as membrane (BCR) and soluble, while TCR exists only as a membrane receptor.

Antibodies

Antibody **(At) = immunoglobulin (Ig) = γ globulin**
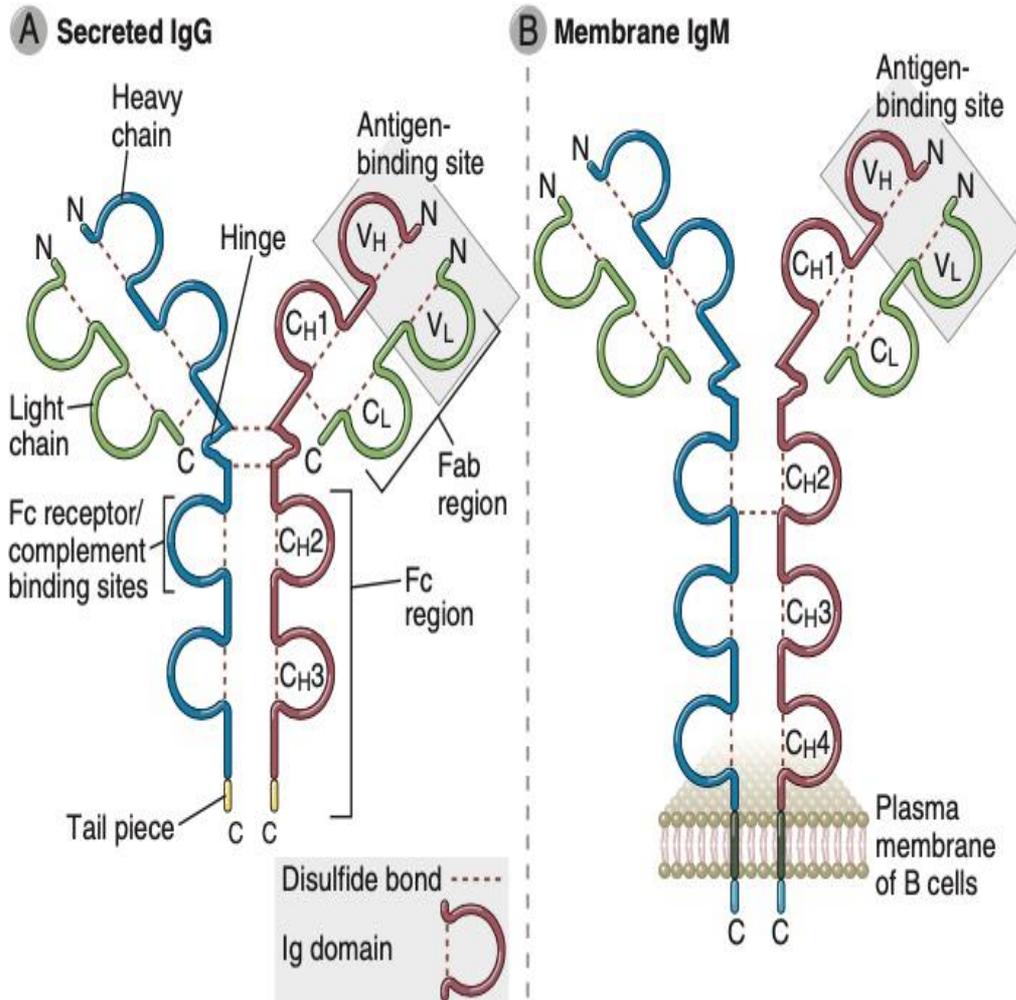
Molecule At it consists of **two identical heavy chains (H)** and **two identical light chains (L)**.

Each chain has **one variable (V)** and **one constant (C)** region.

The L chain consists of one V and one C domain.

The H chain has one V and three or four C domains.
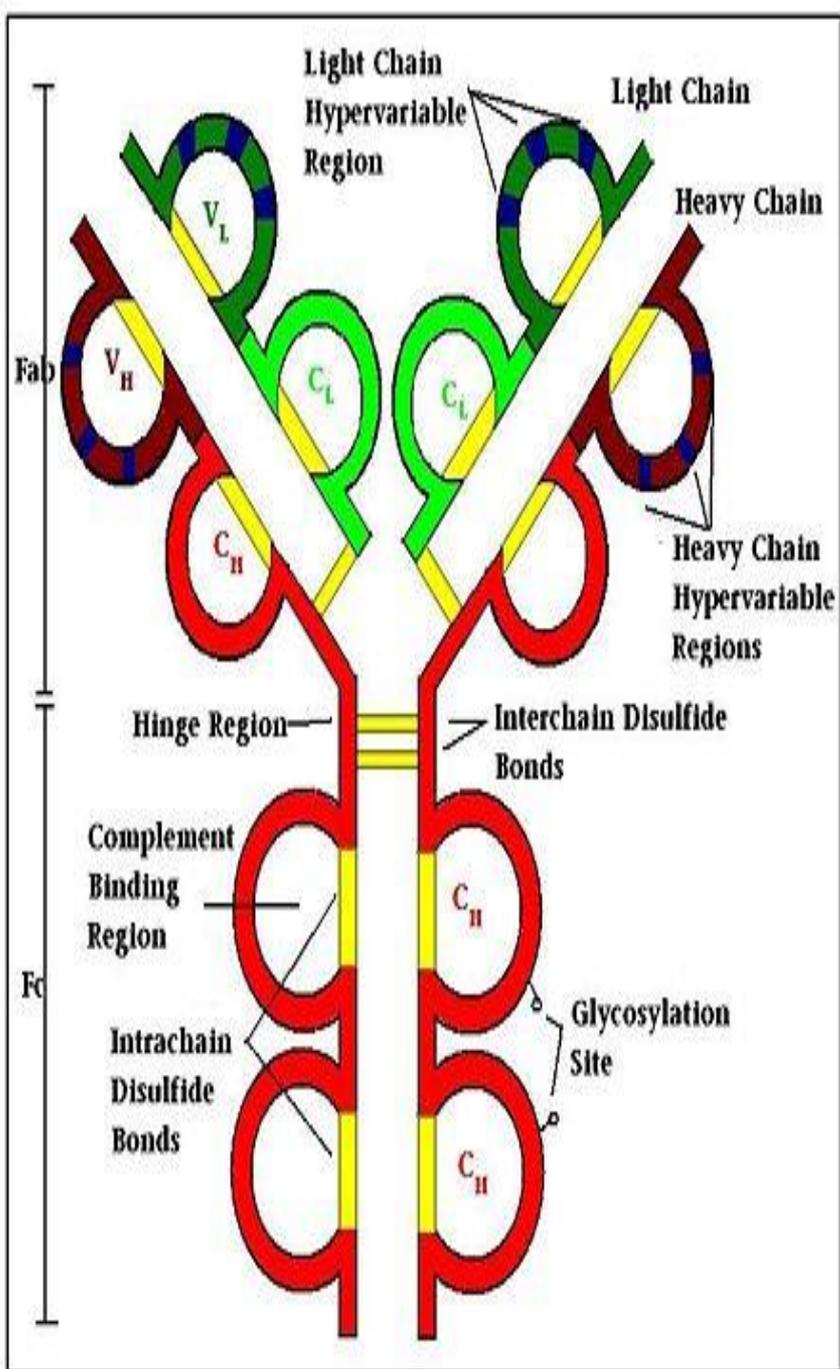
The variable heavy chain region (VH) as well as the variable light chain region (VL) contain three hypervariable regions each. **(CDR*)**.

The highest variability is expressed by CDR3, which is located at the junction of the V and C regions. CDR3 is most involved in the binding of antibodies to the antigen.

Complementarity Determining Region
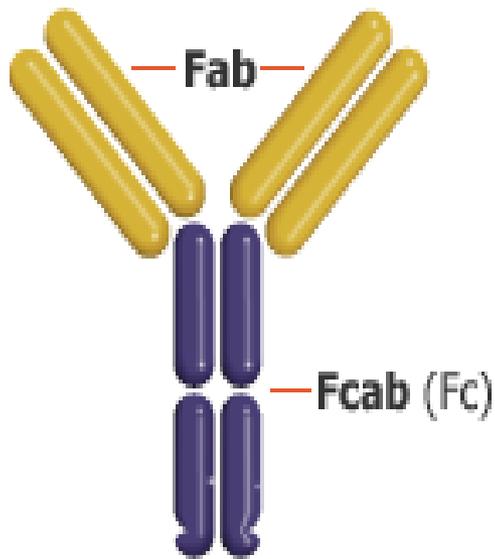
The Fab** region is responsible for binding antigens.

The Fc*** region is responsible for biological activities and effector functions.
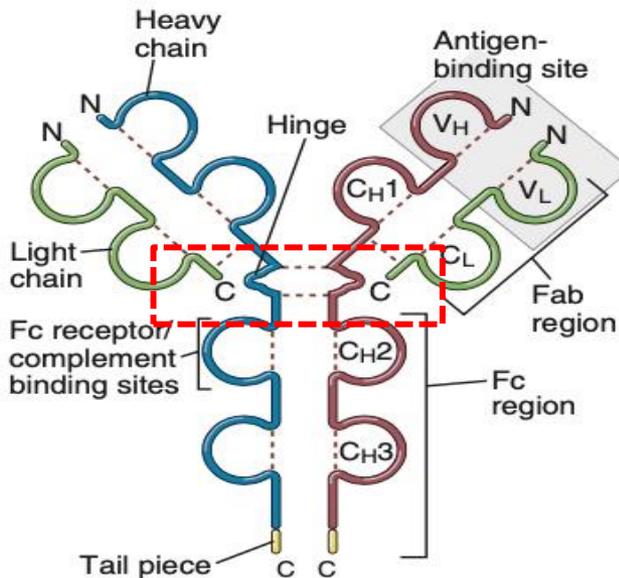
Between Fab and FC is the region of the joint or hinge. The joint allows the Fab regions of each antibody molecule to move independently of each other and thus bind antigens that can be located at different distances from each other..

** *Antigen Binding*
*** *Crystalline*

There are two types of light chains that differ in their C regions: κ and λ. One B lymphocyte synthesizes only κ or only λ never both.

There are 5 types of heavy chains: which also differ in the structure of the C region: μ, δ, γ, ε и α.

Each light chain can be combined with any heavy chain.
The heavy chain class defines isotype i.e. class of antibodies (immunoglobulins):
IgM, IgD, IgG, IgE, IgA.

Isotypes of immunoglobulins differ from each other in physical and biological properties, as well as effector functions.

Isotypes of antibodies
(they differ in their constant heavy chain regions)
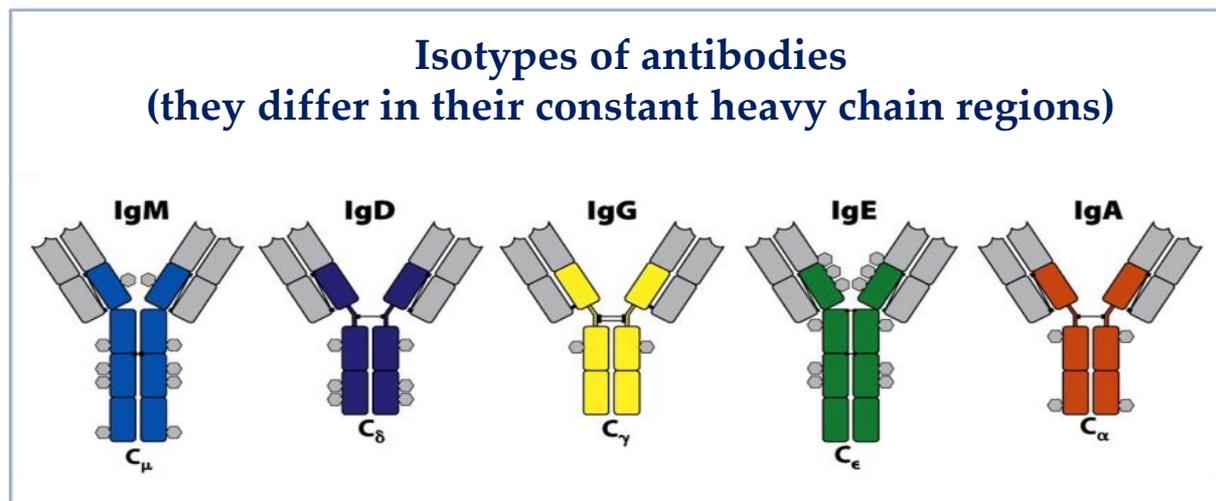
Classes of antibodies

Isotope of Antibody	Subtypes (H Chain)	Serum Concentration (mg/mL)	Serum Half-life (days)	Secreted Form		Functions
IgA	IgA1,2 (α1 or α2)	3.5	6	IgA (dimer) Monomer, dimer, trimer	Cα1 Cα2 Cα3 J chain	Mucosal immunity
IgD	None (δ)	Trace	3	None		Naive B cell antigen receptor
IgE	None (ε)	0.05	2	IgE Monomer	Cε1 Cε2 Cε3 Cε4	Defense against helminthic parasites, immediate hypersensitivity
IgG	IgG1-4 (γ1, γ2, γ3, or γ4)	13.5	23	IgG1 Monomer	V$_H$ Cγ1 V$_L$ C$_L$ Cγ2 Cγ3	Opsonization, complement activation, antibody-dependent cell-mediated cytotoxicity, neonatal immunity, feedback inhibition of B cells
IgM	None (μ)	1.5	5	IgM Pentamer	Cμ1 Cμ2 Cμ3 Cμ4 J chain	Naive B cell antigen receptor, complement activation
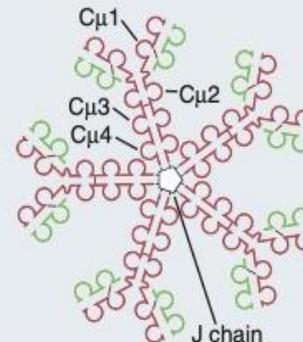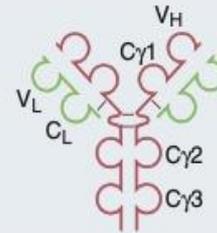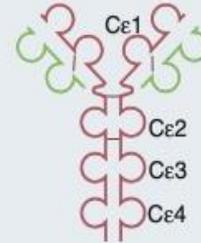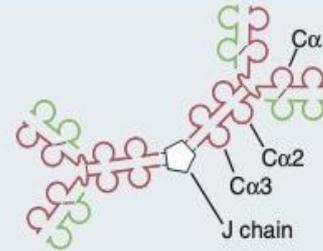
BCR of naïve B lymphocytes are antibodies of the class: IgM and IgD

BCR complex
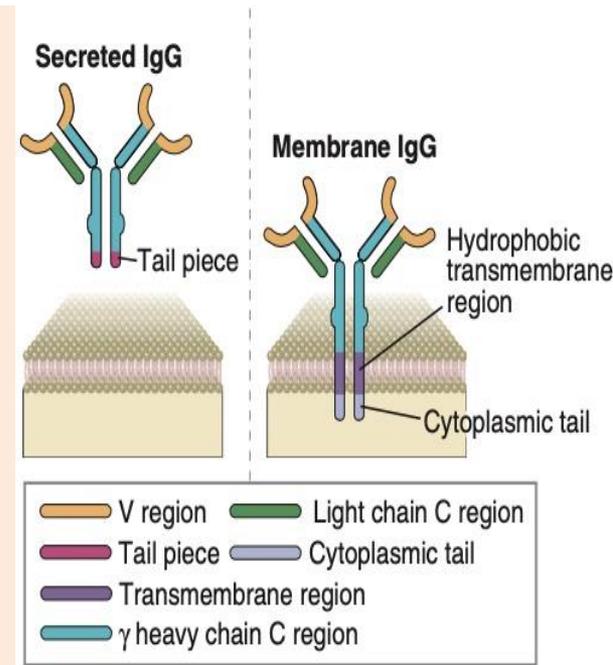
After stimulation by antigen and cytokines originating from helper T lymphocytes, a specific clone of B lymphocytes expands and differentiates into antibody secretion cells...

... Secreted antibodies can be classes of IgM while descendants of the same cells can secrete antibodies of other classes. This phenomenon is described as **changing or switching a class.** In fact, only the class (only the C region) of the heavy chain changes, while the type of light chain remains unchanged.

The most important thing is that the specificity for the antigen remains unchanged because the V regions do not change

The parts of the antigen that antibodies recognize are called epitopes or antigenic determinants.

There are linear (antibodies recognize them by the amino acid sequence) and conformational determinants.

The bond strength of one bound surface of antibodies and one epitope is **affinity.**

The total bond strength of one molecule of antibodies and antigens is **avidity.**

Isolate spleen cells from mouse immunized with antigen X

Antigen X
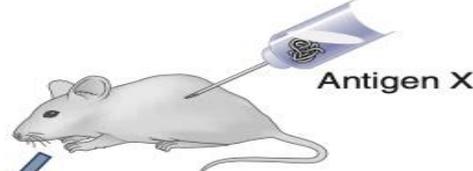
Mixture of spleen cells, including some producing anti-X antibody

Fusion

Mutant myeloma line; unable to grow in HAT selection medium; does not produce antibody

Mixture of fused and unfused cells

In vitro selection in HAT medium

Only fused cells (hybridomas) grow
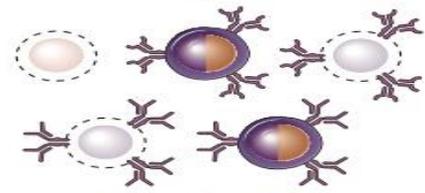
Isolate clones derived from single cells
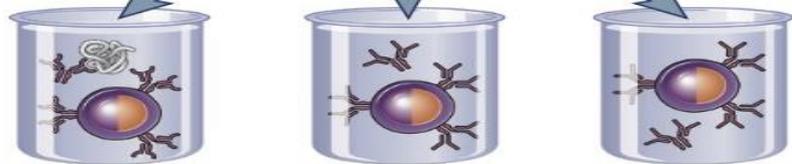
Screen supernatants for each clone of anti-X antibody and expand positive clones
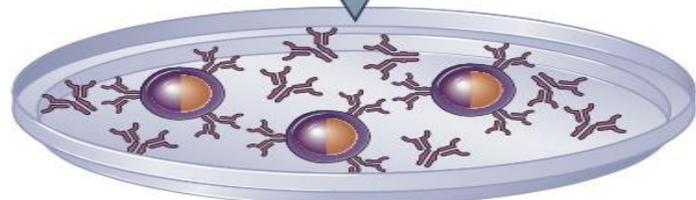
Hybridomas producing monoclonal anti-X antibody

TCR (*T cell receptor*)

β chain — N — N — **α chain**

Vβ   Vα

Cβ   Cα

Transmembrane region

C   C

Vβ   Vα
Cβ   Ca

Disulfide bond ----
Ig domain
Carbohydrate group
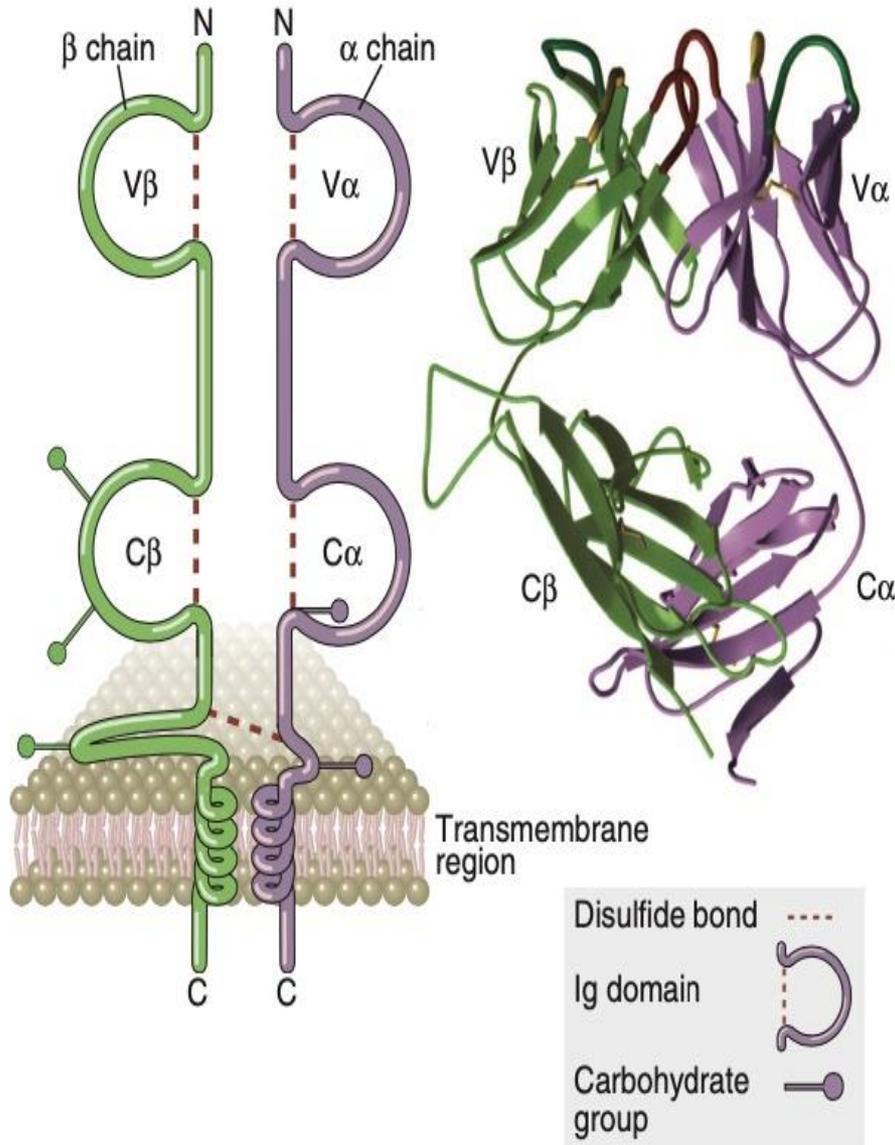
TCR recognizes peptide antigens within the MHC molecule.

TCR is a heterodimer composed of α and β chain.

α and β chain have one V and C region each..

In the V regions of both chains there are three CDRs each.

The biggest differences between the different TCR molecules are in their CDR3 regions.

Both chains of TCR molecules are anchored in the cell membrane and TCR is not produced in secreted form. During the life of a T-cell clone, TCR does not undergo class changes and maturation of affinity.

TCR complex
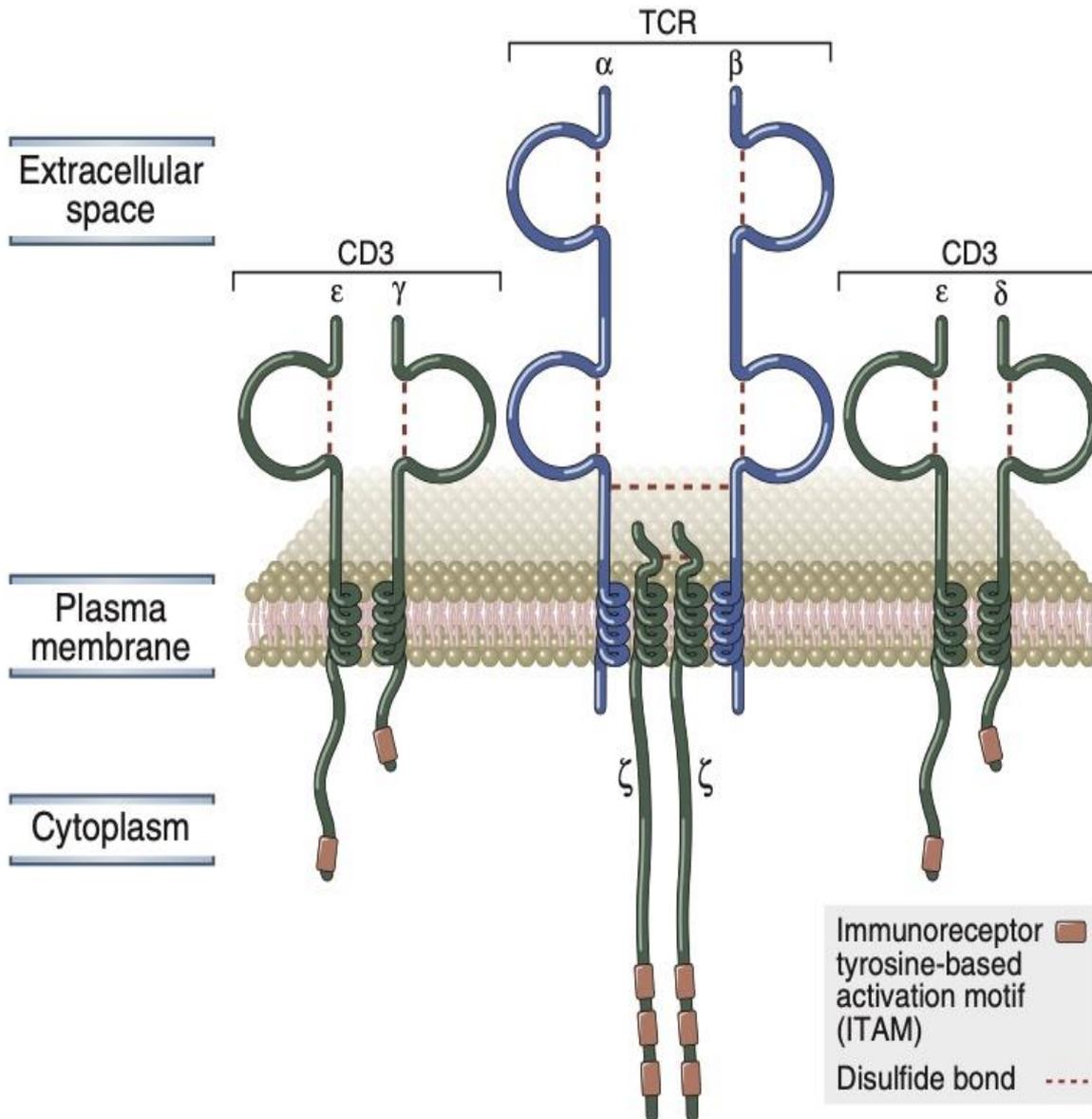
The TCR molecule is associated with a complex of proteins called CD3, as well as ζ (zeta) proteins, which all together form the TCR complex.

While TCR recognizes the antigen, CD3 and ζ chains participate in the conduction of signals important for the activation of T lymphocytes.

Receivers of CD4+ helper T lymphocyte — Ligands of class II MHC expressing APC

Signal transduction
- CD4

Antigen recognition
- TCR
- Peptide
- Class II MHC

Signal transduction
- CD3
- ITAM
- ζ
- CD28
- B7-1/B7-2

Adhesion
- LFA-1
- ICAM-1
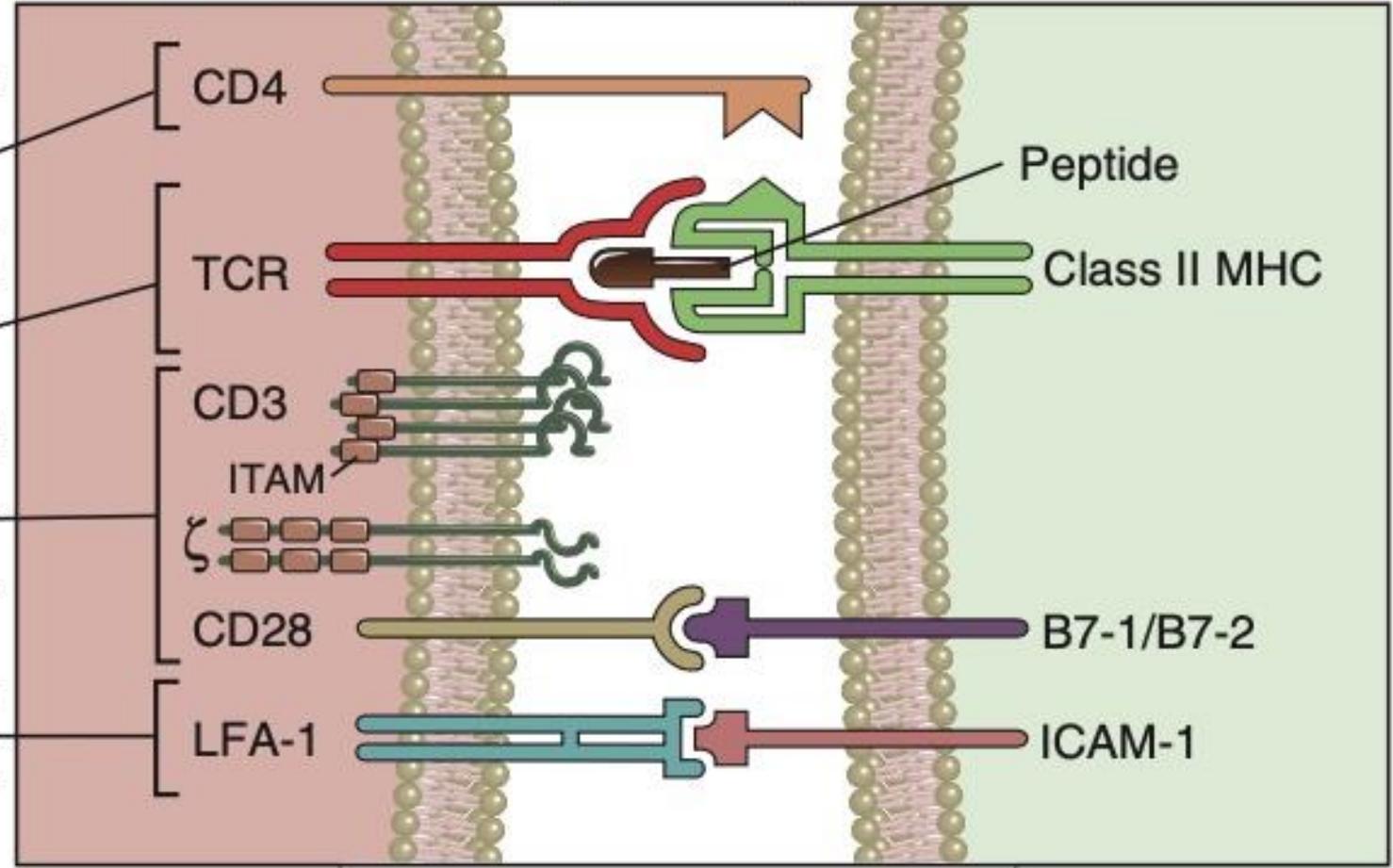
| T cell accessory molecule	Function	Ligand	
		Name	Expressed on
CD3	Signal transduction by TCR complex	None	
ζ	Signal transduction by TCR complex	None	
CD4	Signal transduction	Class II MHC	Antigen presenting cells
CD8	Signal transduction	Class I MHC	Antigen presenting cells, CTL target cells
CD28	Signal transduction (costimulation)	B7-1/B7-2	Antigen presenting cells
CTLA-4	Signal transduction (negative regulation)	B7-1/B7-2	Antigen presenting cells
LFA-1	Adhesion	ICAM-1	Antigen presenting cells, endothelium
VLA-4	Adhesion	VCAM-1	Endothelium
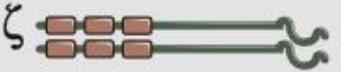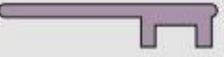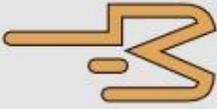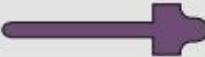
Immune repertoire

There are many lymphocyte clones of different specifics, and all of these clones are formed before the antigen encounters.

The formation of such different receptors occurs during the maturation process of lymphocytes...

Lymphocyte maturation

Lymphocytes are derived from bone marrow stem cells.
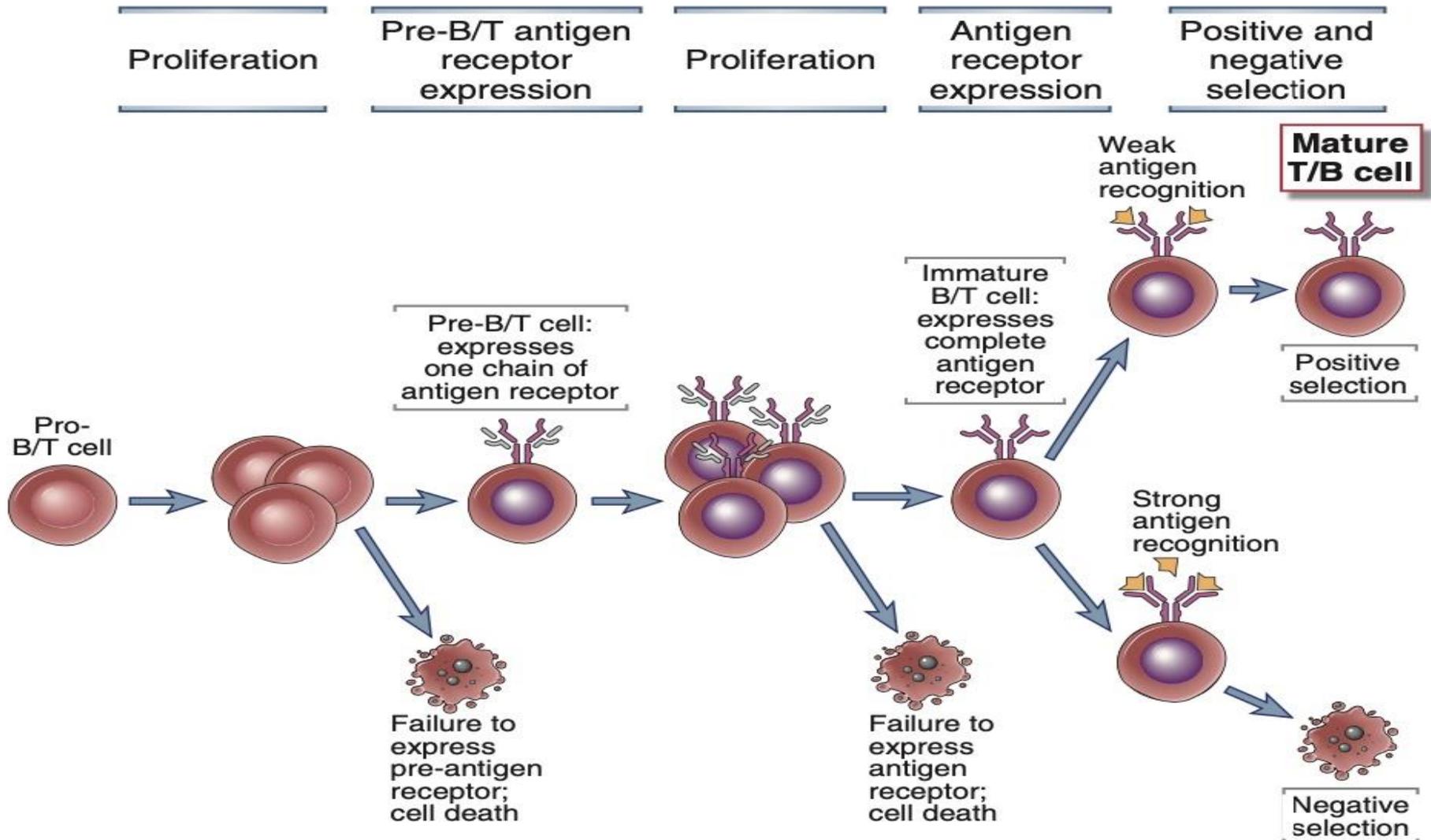
1. Proliferation

Proliferation | Pre-B/T antigen receptor expression | Proliferation | Antigen receptor expression | Positive and negative selection

Pre-B/T cell: expresses one chain of antigen receptor

Pro-B/T cell

Immature B/T cell: expresses complete antigen receptor

Weak antigen recognition

Mature T/B cell

Positive selection

Strong antigen recognition

Negative selection

Failure to express pre-antigen receptor; cell death

Failure to express antigen receptor; cell death

Proliferation at the stage of the earliest precursor cells depends on IL-7. It is a growth factor produced by bone marrow stromal cells that promotes the proliferation of both T- and B-precursors.

When expressed, antigen receptors assume the function of conducting proliferative signals, which ensures the expansion of only those clones that have expressed functional receptors.

2. Differentiation

Antigen receptors are encoded by several gene segments separated by introns (germ line).

During maturation, these parts of the genome are subject to reorganization (rearrangement) i.e. the fusion of exons (functional genes are created).

Diversity occurs during recombination because by random process, different exons from gene groups are merged. This is the central event in the maturation of lymphocytes.

Generating diversity of the receptors (GOD, Generation Of Diversity)
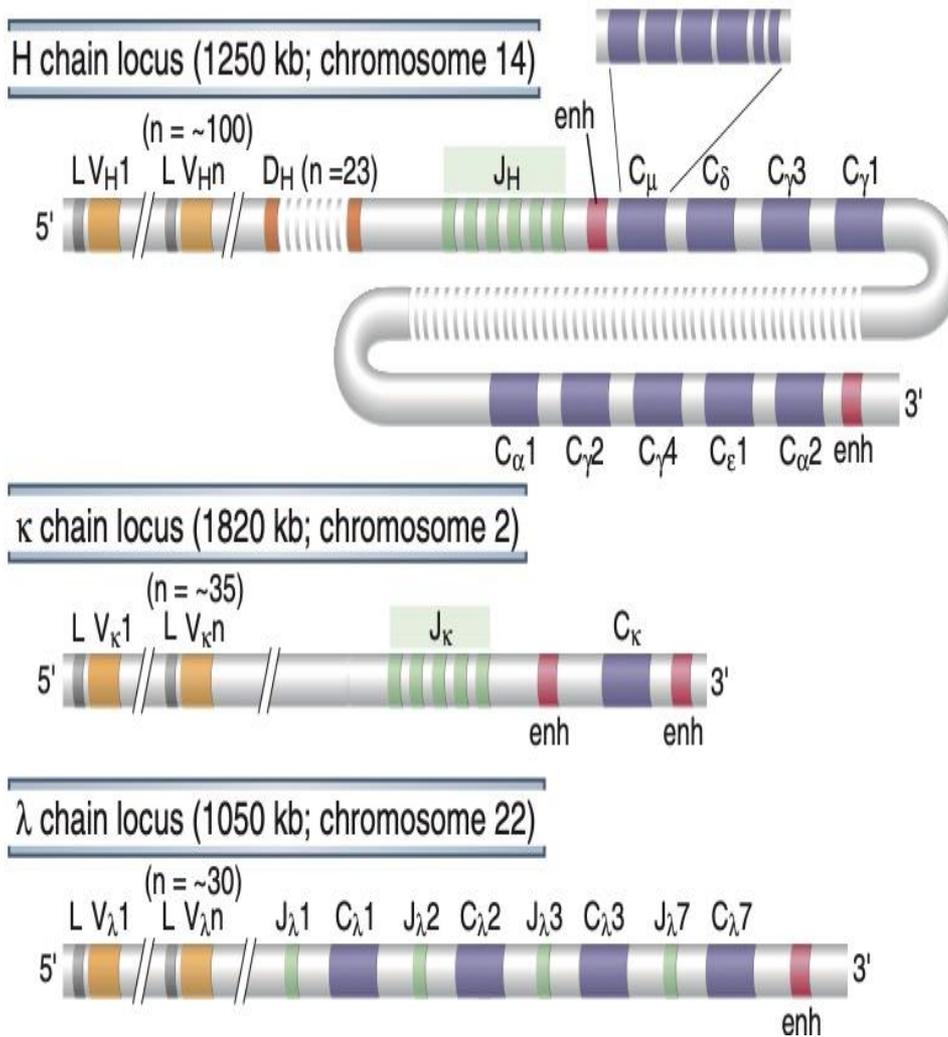
Dysfunctional genes

Germ line formation (germ line)

V variable

J joining

D diversity

C constant

Genes for variable region

Genes for a constant region

Recombination and expression of genes for immunoglobulins
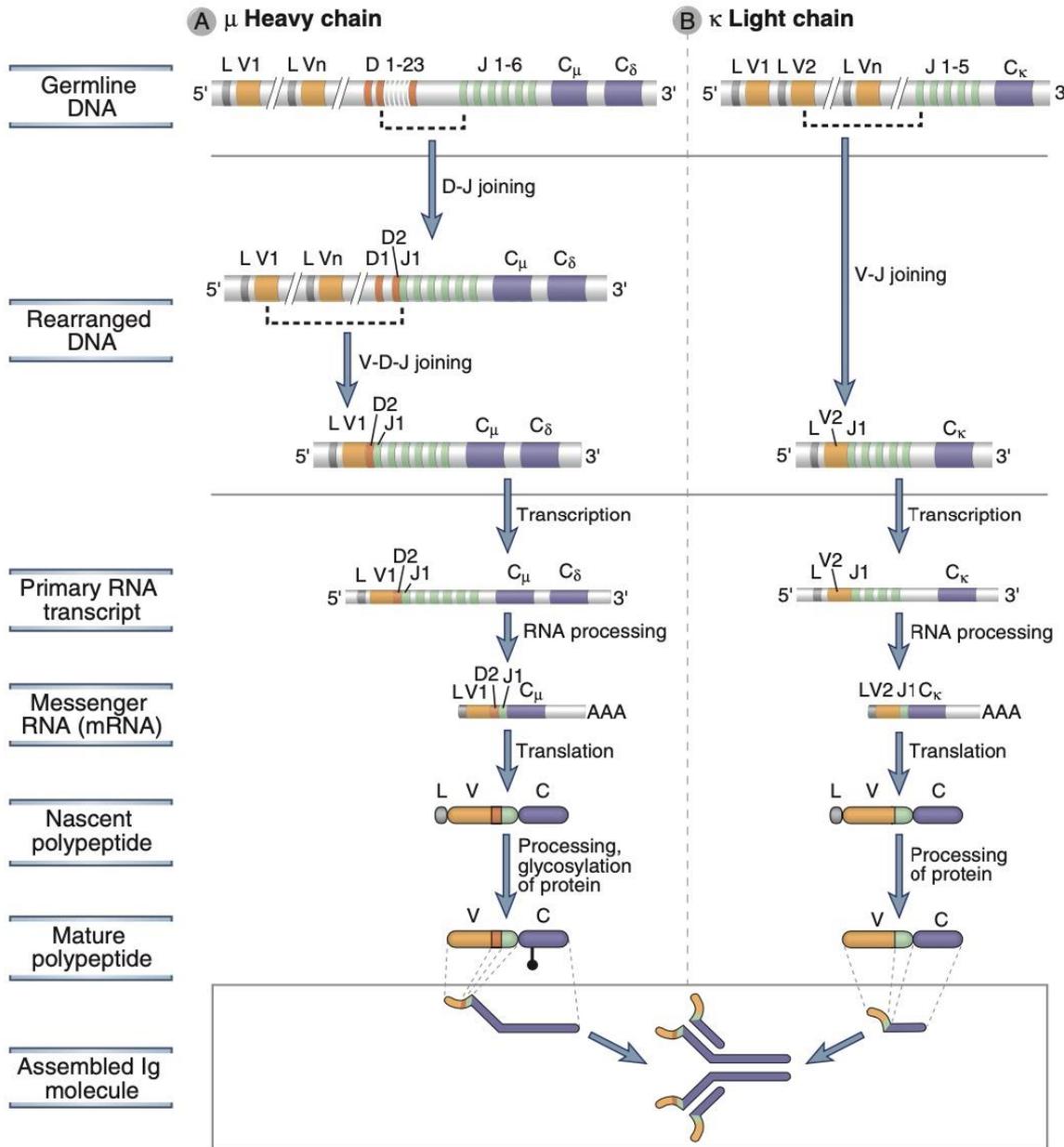
The formation of functional genes for heavy chains

Somatic recombination is performed by a group of enzymes, VDJ recombinases. The components of VDJ recombinance are RAG1 and RAG2.

Mechanisms of creating a variety of receptors

The variety of antigen receptors is formed:

1. using different V-D-J combinations in different lymphocytes
(COMBINATORY DIVERSITY)

... and even more by

2. Errors in V-D-J compounds when the order of nucleotides is changed
(CONNECTIVITY DIVERSITY)

3. Selection

Lymphocytes during maturation go through several stages of selection:

Positive and negative selection of T and B lymphocytes.

The biological purpose of selection is to preserve those lymphocytes whose antigenic specificity is potentially beneficial and not dangerous for antigens of their own tissues.

The requirement for selection is the expression of functional antigen receptor
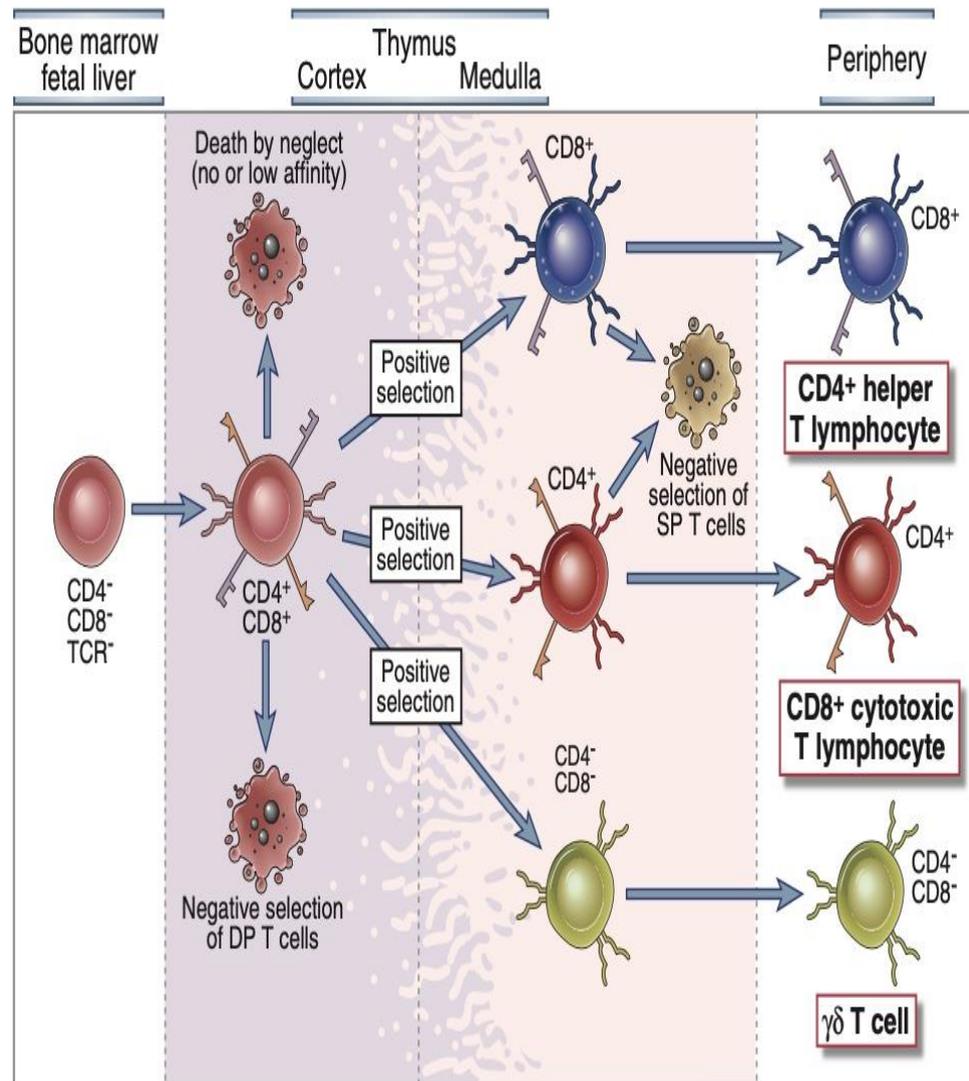
Maturation and selection of B lymphocytes

BONE MARROW
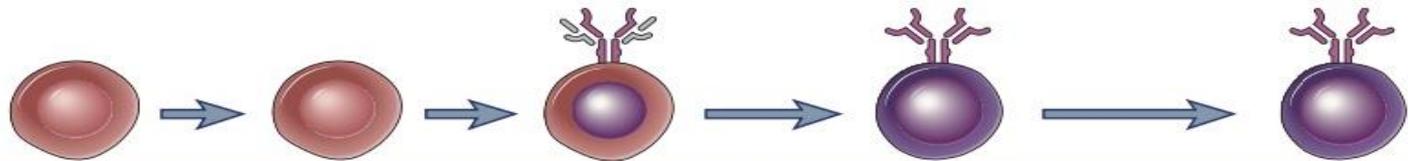
Stage of maturation	Stem cell	Pro-B	Pre-B	Immature B	Mature B
Proliferation					
RAG expression					
TdT expression					
Ig DNA, RNA	Unrecombined (germline) DNA	Unrecombined (germline) DNA	Recombined H chain gene (VDJ); μ mRNA	Recombined H chain gene (VDJ), κ or λ genes (VJ); μ or κ or λ mRNA	Alternative splicing of VDJ-C RNA (primary transcript), to form C_μ and C_δ mRNA
Ig expression	None	None	Cytoplasmic μ and pre-B receptor–associated μ	Membrane IgM (μ+ κ or λ light chain)	Membrane IgM and IgD
Surface markers	CD43+	CD43+ CD19+ CD10+	B220lo CD43+	IgMlo CD43-	IgMhi
Anatomic site	Bone marrow			Periphery	
Response to antigen	None	None	None	Negative selection (deletion), receptor editing	Activation (proliferation and differentiation)

In pre-B cells, an intracellular μ chain is expressed. Some of these chains are manifested on a membrane with constant surrogates of light chains on the membrane.

μ chain and surrogate chain are associated with Igα and Igβ chains and form a pre-BCR complex, which is important for:

-delivery of proliferative and anti-apoptotic signals

-Allelic exclusion (i.e. gene locking for heavy chains on the second chromosome)

-triggers gene recombination for light chains (first for κ and then for λ if it fails first)

When a cell with alternative splicing manages to synthesize both IgM and IgD becomes mature lymphocytes.

After the random process of B lymphocyte repertoire, those cells that express the complete receptors are positively selected, while those cells that strongly recognize their own antigens are selected negatively.

If a B lymphocyte with high affinity recognizes an antigen in the bone marrow, then it undergoes apoptosis or rearranges the receptor (receptor editing) By activating recombination, it synthesizes a new light chain. Thus , negative selection removes potentially dangerous cells that can recognize their own antigens and react against them.

a **Bone marrow**

Self antigen

IgH
IgL

B-cell receptor

Receptor editing

Pre-B cell

Immature B cell

Mature B cell

Cell death
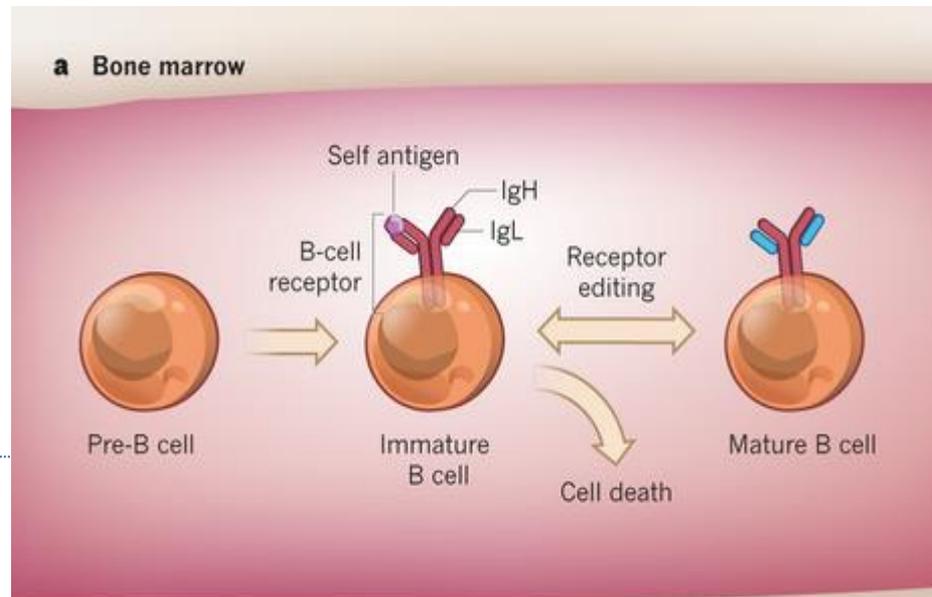
Maturation and selection of T lymphocytes

THYMUS
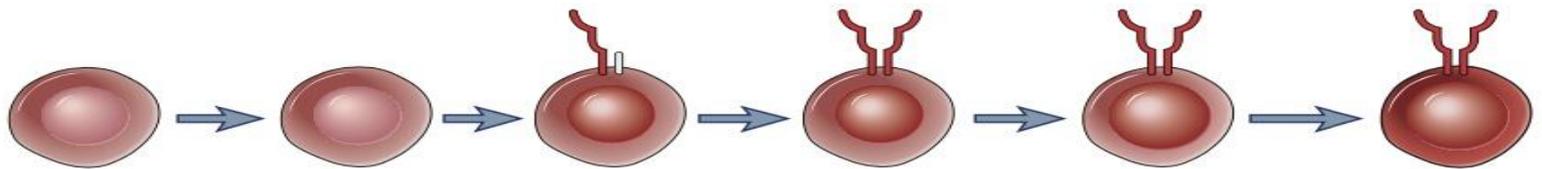
Stage of maturation	Stem cell	Pro-T	Pre-T	Double positive	Single positive (immature T cell)	Naive mature T cell
Proliferation						
RAG expression						
TdT expression						
TCR DNA, RNA	Unrecombined (germline) DNA	Unrecombined (germline) DNA	Recombined β chain gene [V(D)J-C]; β chain mRNA	Recombined β, α chain genes [V(D)J-C]; β and α chain mRNA	Recombined β, α chain genes [V(D)J-C]; β and α chain mRNA	Recombined β, α chain genes [V(D)J-C]; β and α chain mRNA
TCR expression	None	None	Pre-T receptor (β chain/pre-T α)	Membrane αβ TCR	Membrane αβ TCR	Membrane αβ TCR
Surface markers	c-*kit* $^+$ CD44$^+$ CD25$^-$	c-*kit* $^+$ CD44$^+$ CD25$^+$	c-*kit* $^+$ CD44$^-$ CD25$^+$	CD4$^+$CD8$^+$ TCR/CD3^{lo}	CD4$^+$CD8$^-$ or CD4$^-$CD8$^+$ TCR/CD3^{hi}	CD4$^+$CD8$^-$ or CD4$^-$CD8$^+$ TCR/CD3^{hi}
Anatomic site	Bone marrow	Thymus				Periphery
Response to antigen	None	None	None	Positive and negative selection		Activation (proliferation and differentiation)

In pre-T lymphocytes, a β chain of TCR is expressed on the membrane.

β chain and pre-Tα form the pre-TCR complex, which is important for:

- delivery of proliferative and anti-apoptotic signals
- Allelic exclusion (i.e. gene locking for heavy chains on the second chromosome)
- initiating gene recombination for α chain

- **Double positive CD4 + CD8 + T lymphocytes**
- **Positive and negative selection**