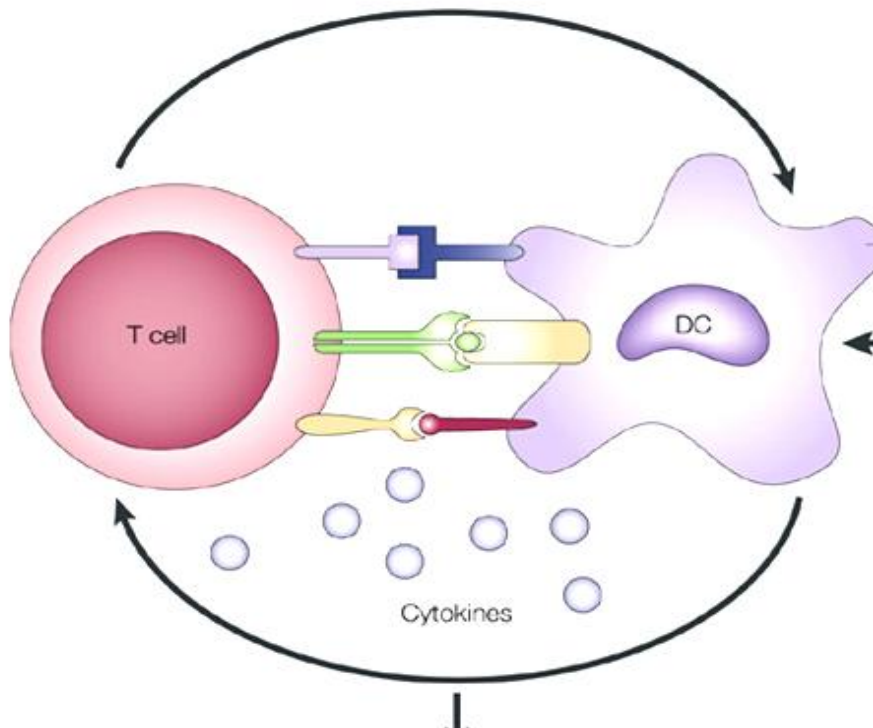


# Recognition of Antigen in Acquired Immunity



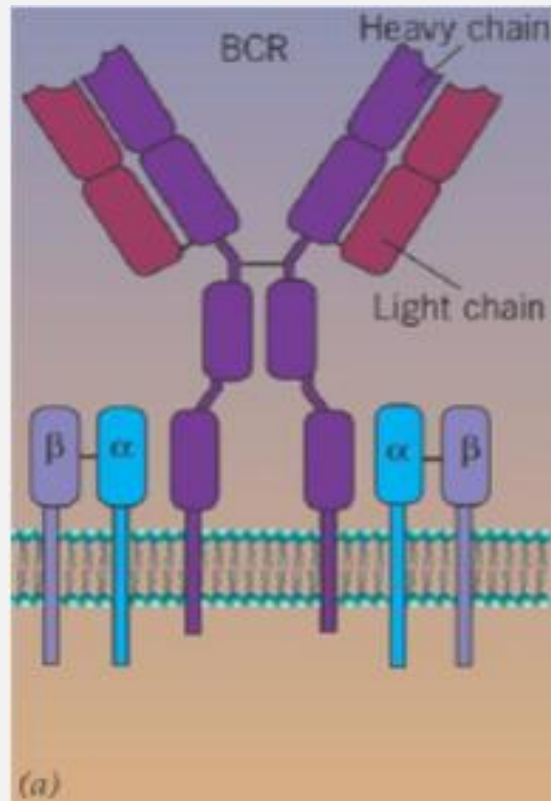
## **Adaptive (specific) immune response...**

... it starts when  
antigen receptors on lymphocytes  
recognize (see) antigens.

# Antigen Receptors of Lymphocytes

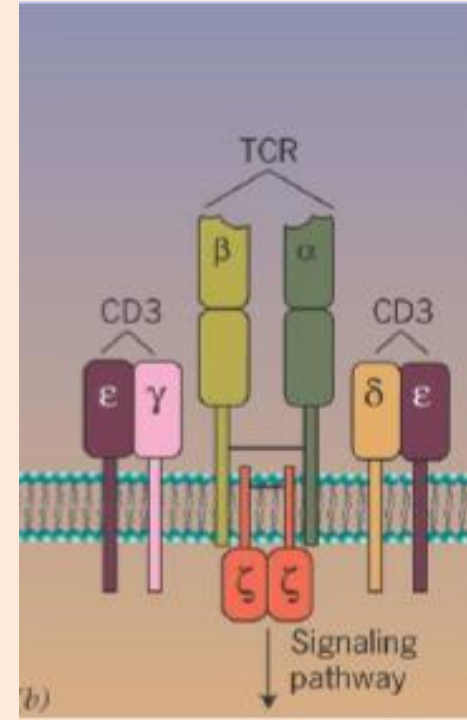
## B cell receptor (BCR)

- 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).

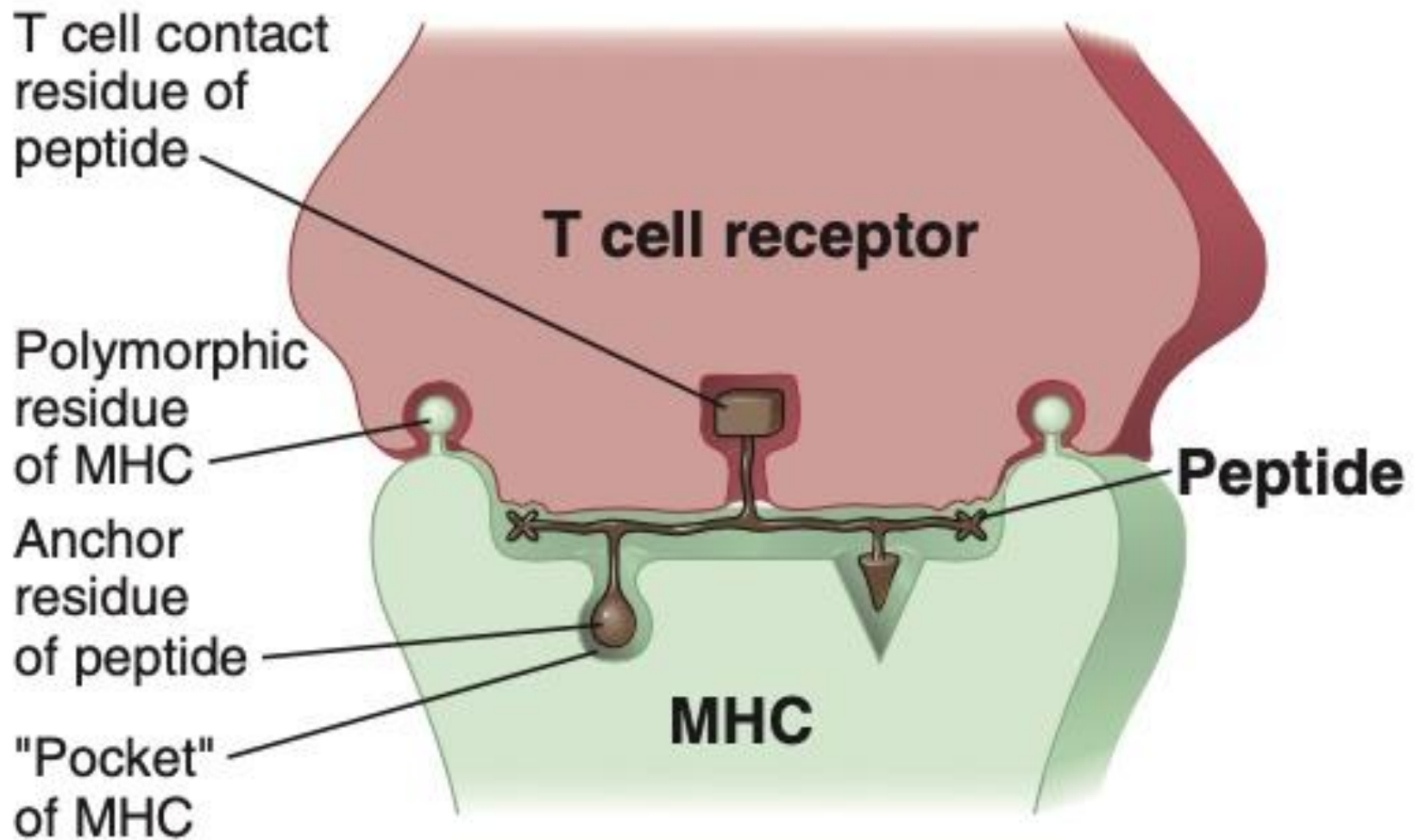


# What do T lymphocytes see?

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.*



# 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 always specific to the antigen that caused it because the activation of lymphocytes is due to specific antigen recognition.

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

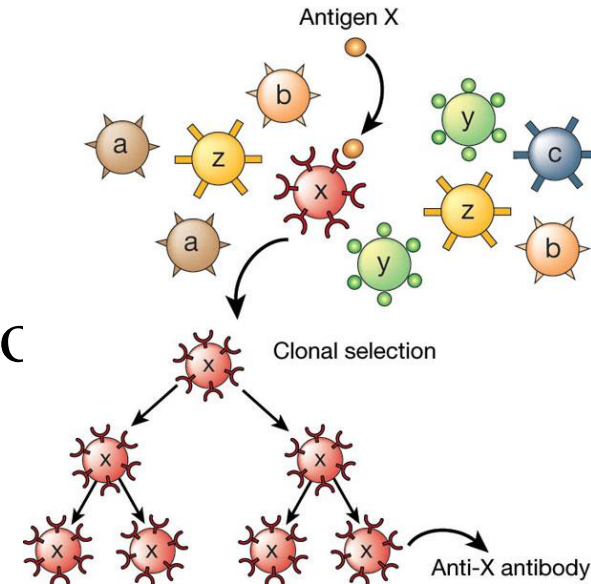
These receptors are able to recognize, distinguish and bind a large number of antigens.



...Let's remember

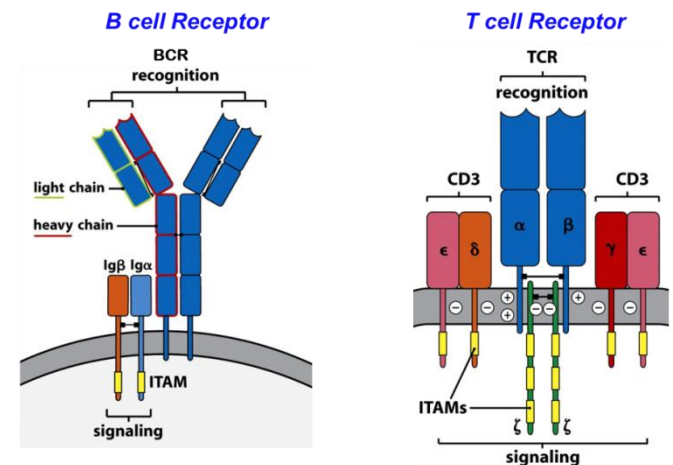
These receptors are clonally distributed, which means that each clone of lymphocytes has a unique receptor.

Which is different from the receptors for other lymphocyte clones.



Although every lymphocyte recognizes different antigen, antigen receptors carry out the same activation signals.

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

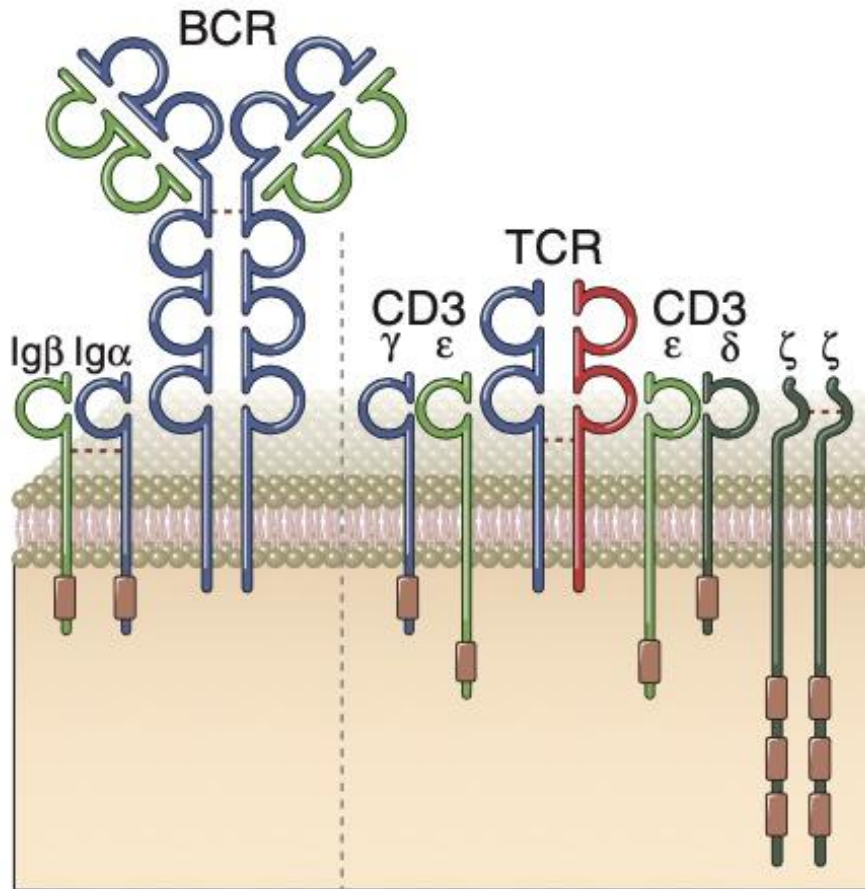


*The questions that need to be answered today...*

...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

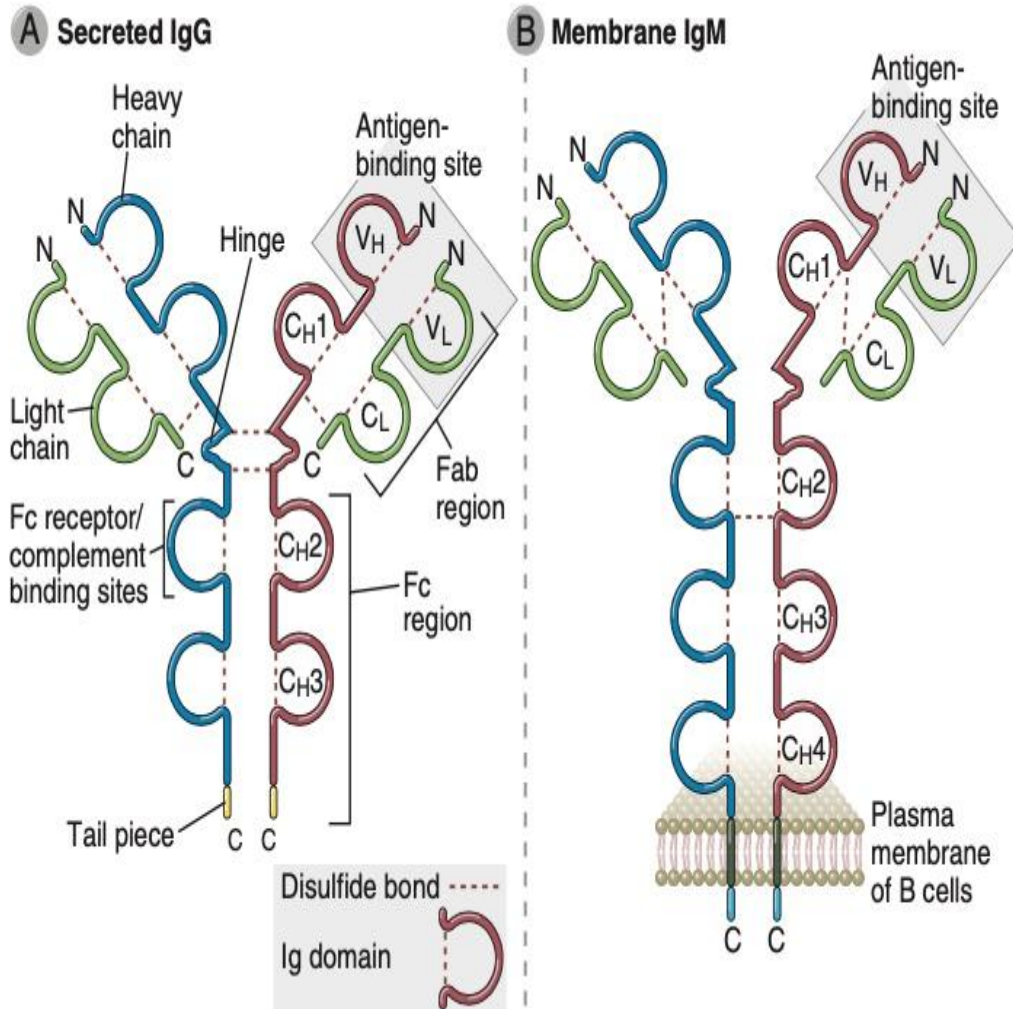
Receptors consist of **variable (V)** and conserved **constant regions (C)**. Within the V region there are hypervariable regions of **CDR** that are responsible for recognizing antigens, i.e. to connect with him.

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

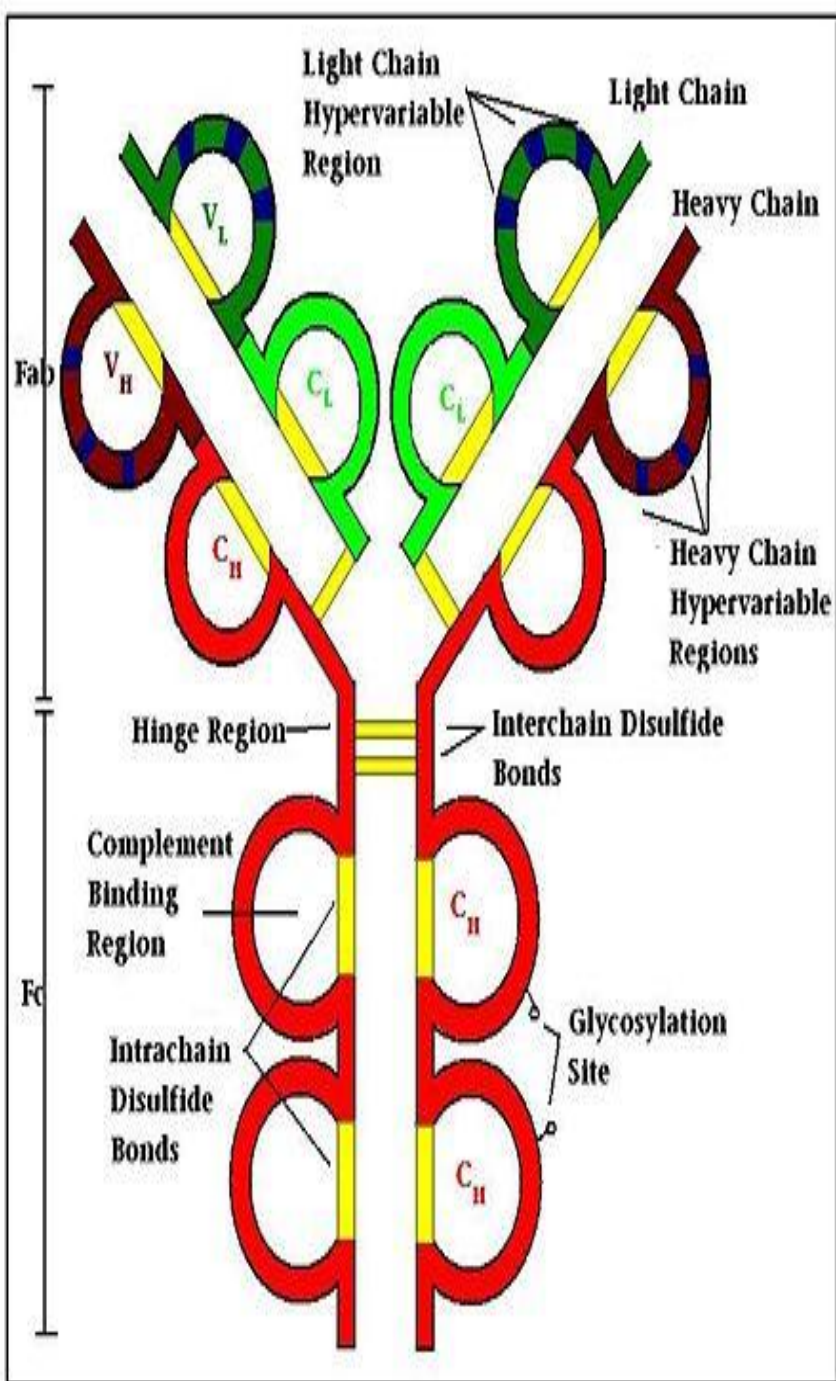
Antibody (At) = immunoglobulin (Ig) =  $\gamma$  globulin



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.

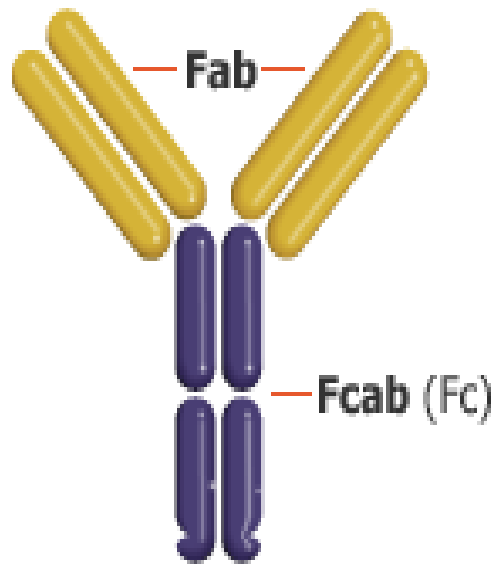




The variable heavy chain region (V<sub>H</sub>) as well as the variable light chain region (V<sub>L</sub>) contain three hypervariable regions each. (CDR\*).

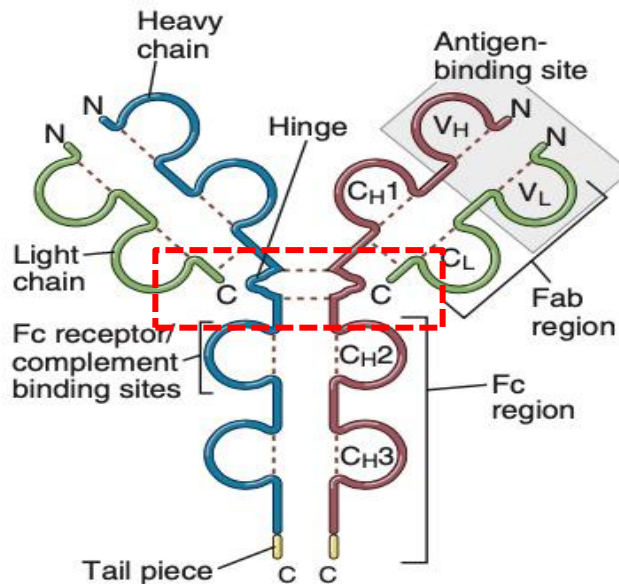
The highest variability is expressed by CDR3, which is located at the junction of the V and C regions. CDR3 is most involved in the binding of antibodies to the antigen.

*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*

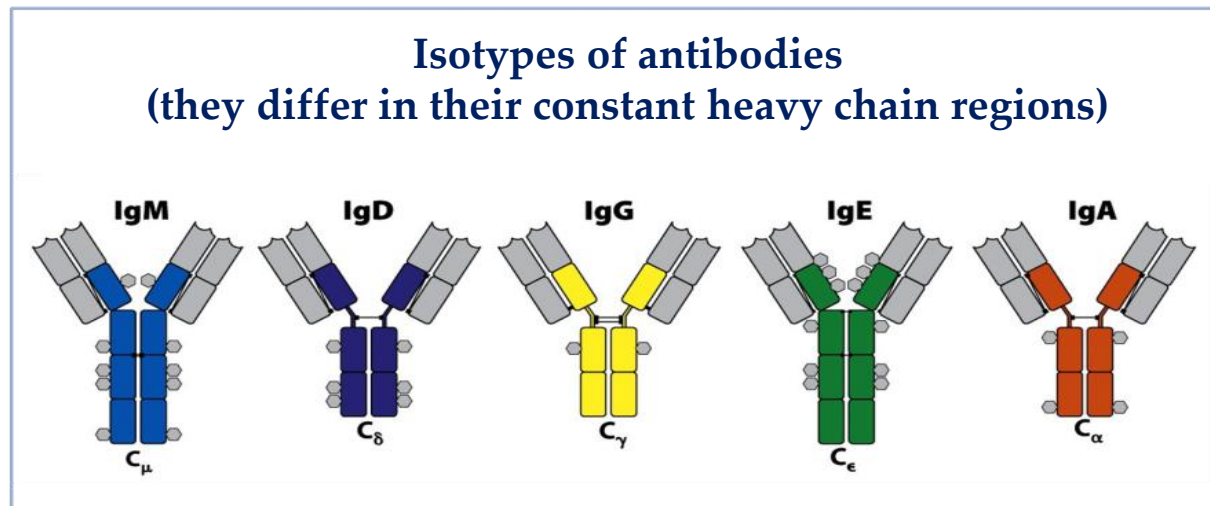
There are two types of light chains that differ in their C regions:  $\kappa$  and  $\lambda$ . One B lymphocyte synthesizes only  $\kappa$  or only  $\lambda$  never both.

There are 5 types of heavy chains: which also differ in the structure of the C region:  $\mu$ ,  $\delta$ ,  $\gamma$ ,  $\epsilon$  и  $\alpha$ .

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.**

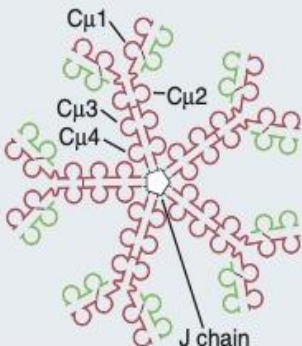
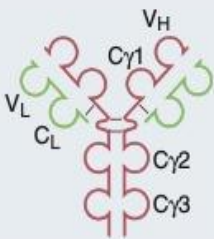
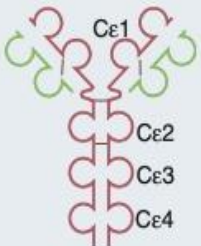
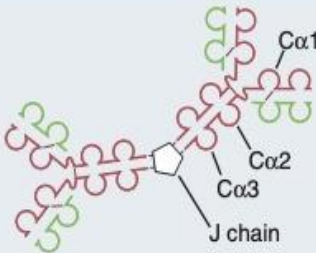
Isotypes of immunoglobulins differ from each other in physical and biological properties, as well as effector functions.





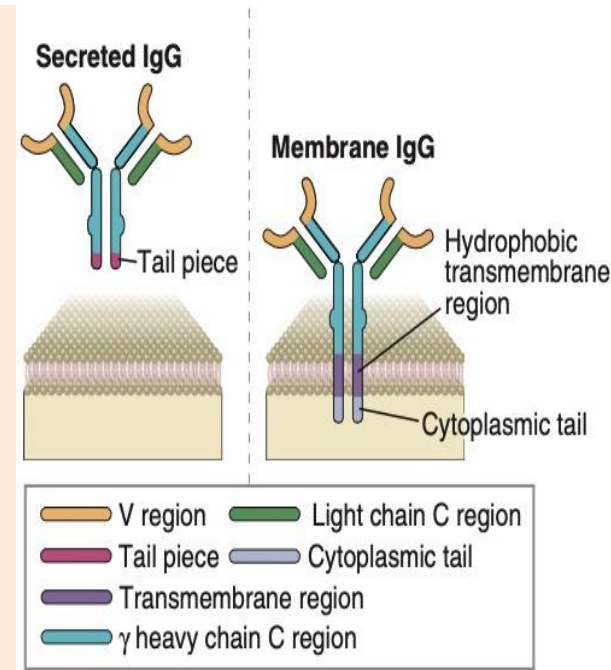
# Classes of antibodies

Isotope of Antibody	Subtypes (H Chain)	Serum Concentration (mg/mL)	Serum Half-life (days)	Secreted Form	Functions
IgA	IgA1,2 ( $\alpha$ 1 or $\alpha$ 2)	3.5	6	IgA (dimer) Monomer, dimer, trimer	Mucosal immunity
IgD	None ( $\delta$ )	Trace	3	None	Naive B cell antigen receptor
IgE	None ( $\epsilon$ )	0.05	2	IgE Monomer	Defense against helminthic parasites, immediate hypersensitivity
IgG	IgG1-4 ( $\gamma$ 1, $\gamma$ 2, $\gamma$ 3, or $\gamma$ 4)	13.5	23	IgG1 Monomer	Opsonization, complement activation, antibody-dependent cell-mediated cytotoxicity, neonatal immunity, feedback inhibition of B cells
IgM	None ( $\mu$ )	1.5	5	IgM Pentamer	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

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

Fusion

Mixture of fused and unfused cells

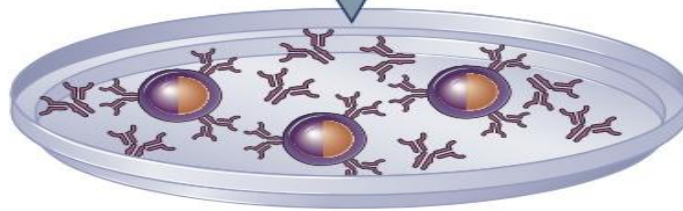
In vitro selection in HAT medium

Only fused cells (hybridomas) grow

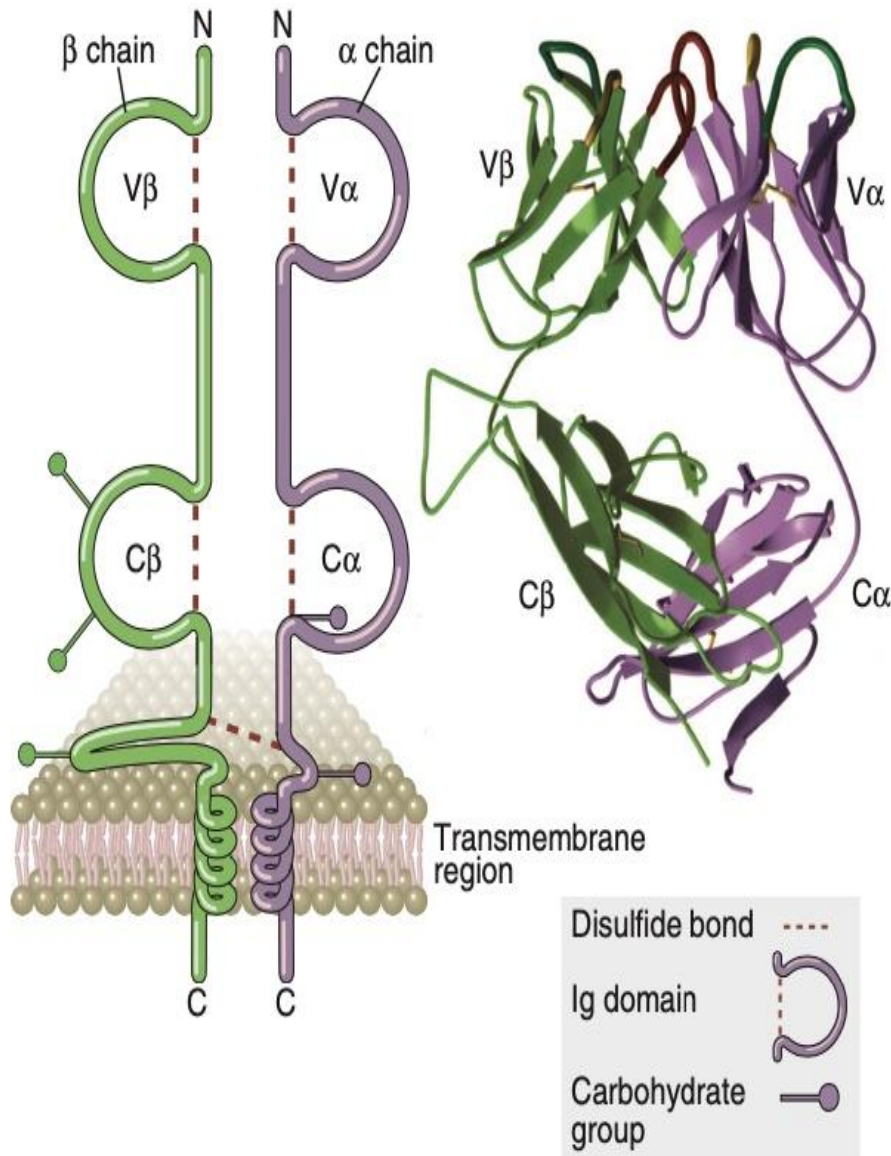
Isolate clones derived from single cells

Screen supernatants for each clone of anti-X antibody and expand positive clones

**Hybridomas producing monoclonal anti-X antibody**



# TCR (*T cell receptor*)



TCR recognizes peptide antigens within the MHC molecule.

TCR is a heterodimer composed of  $\alpha$  and  $\beta$  chain.

$\alpha$  and  $\beta$  chain have one V and C region each..

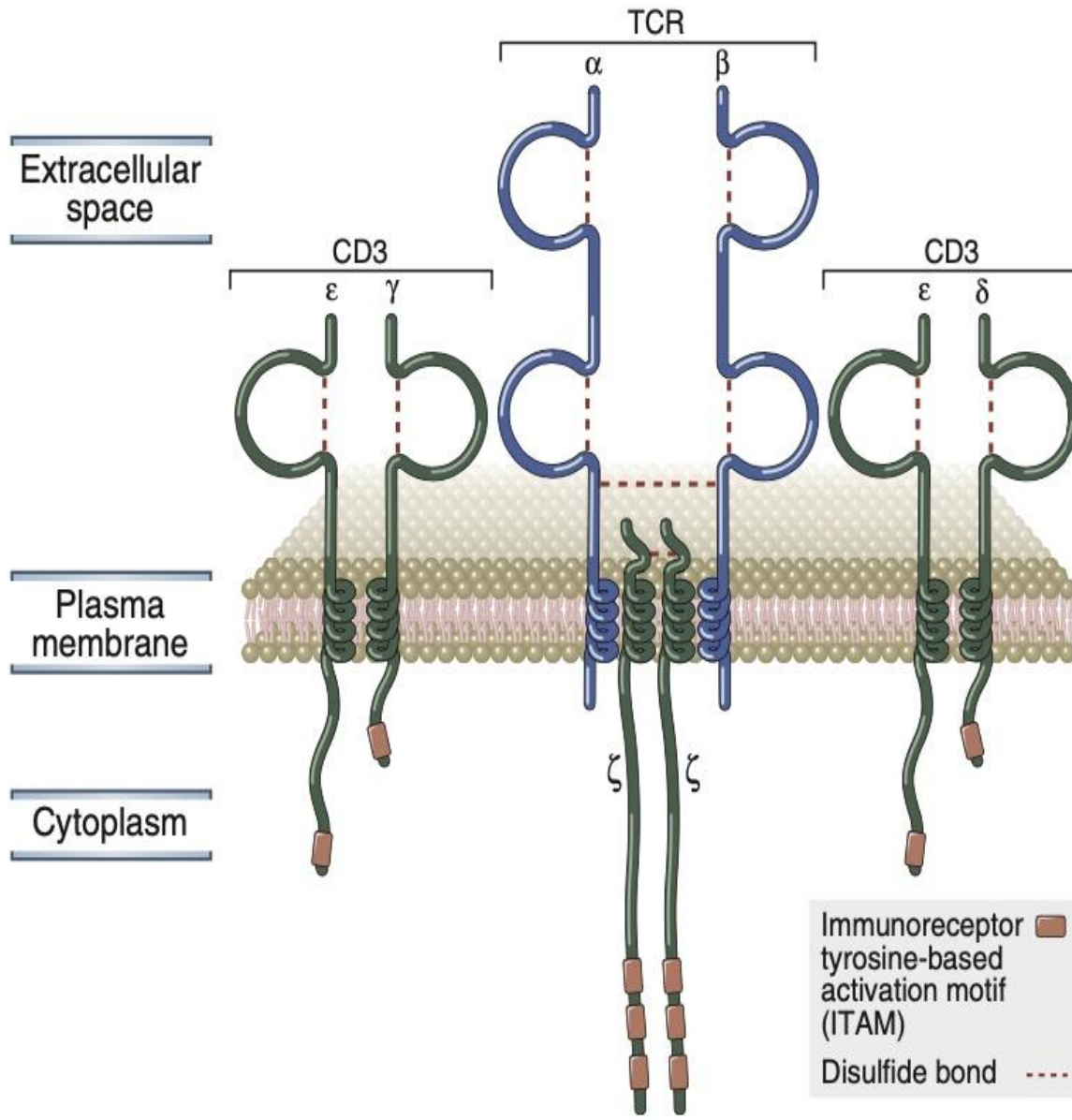
In the V regions of both chains there are three CDRs each.

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.**



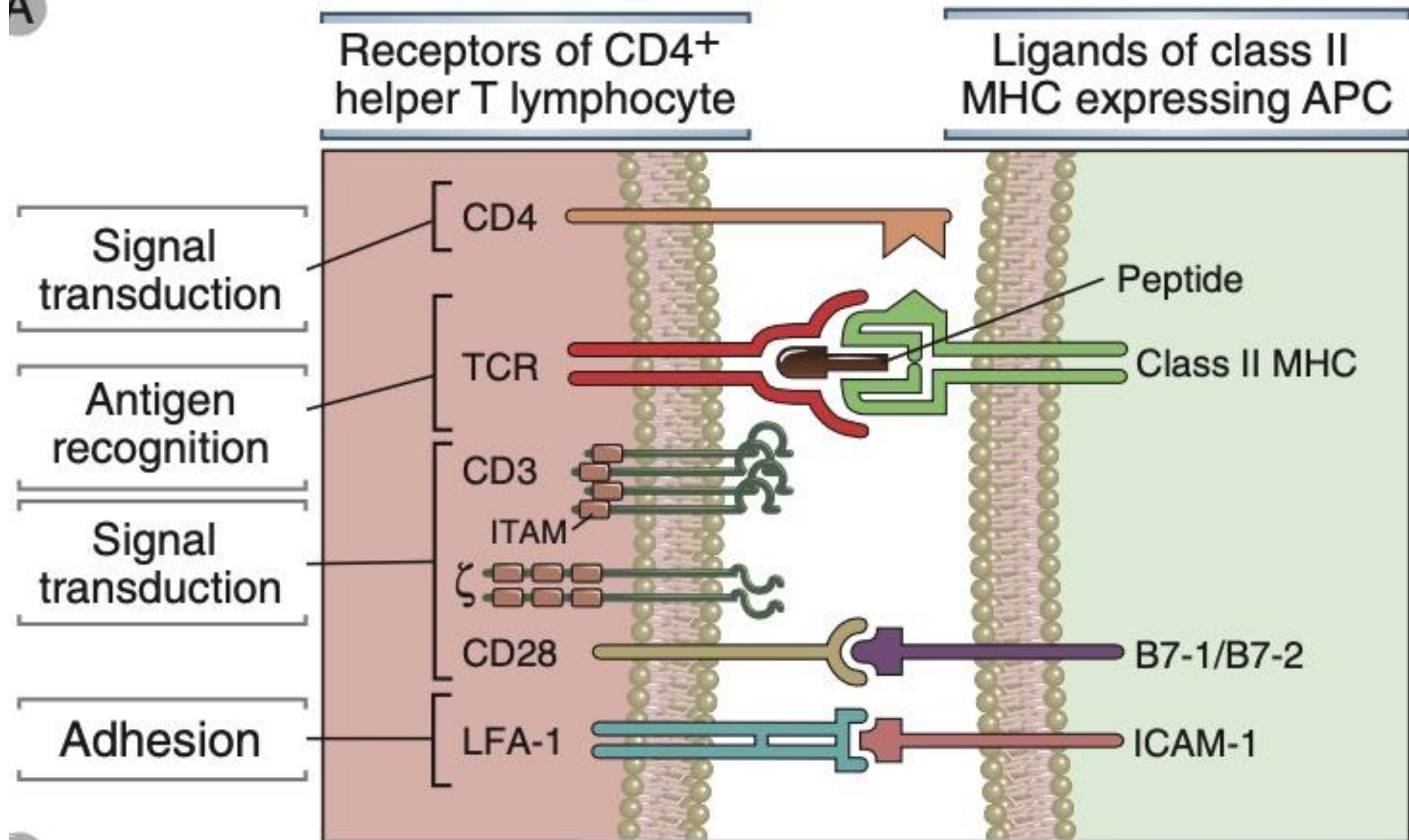
# TCR complex

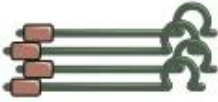







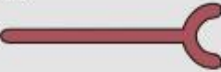
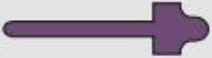






The TCR molecule is associated with a complex of proteins called **CD3**, as well as  **$\zeta$  (zeta) proteins**, which all together form the TCR complex.

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

A



T cell accessory molecule	Function	Ligand	
		Name	Expressed on
CD3 	Signal transduction by TCR complex	None	
	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



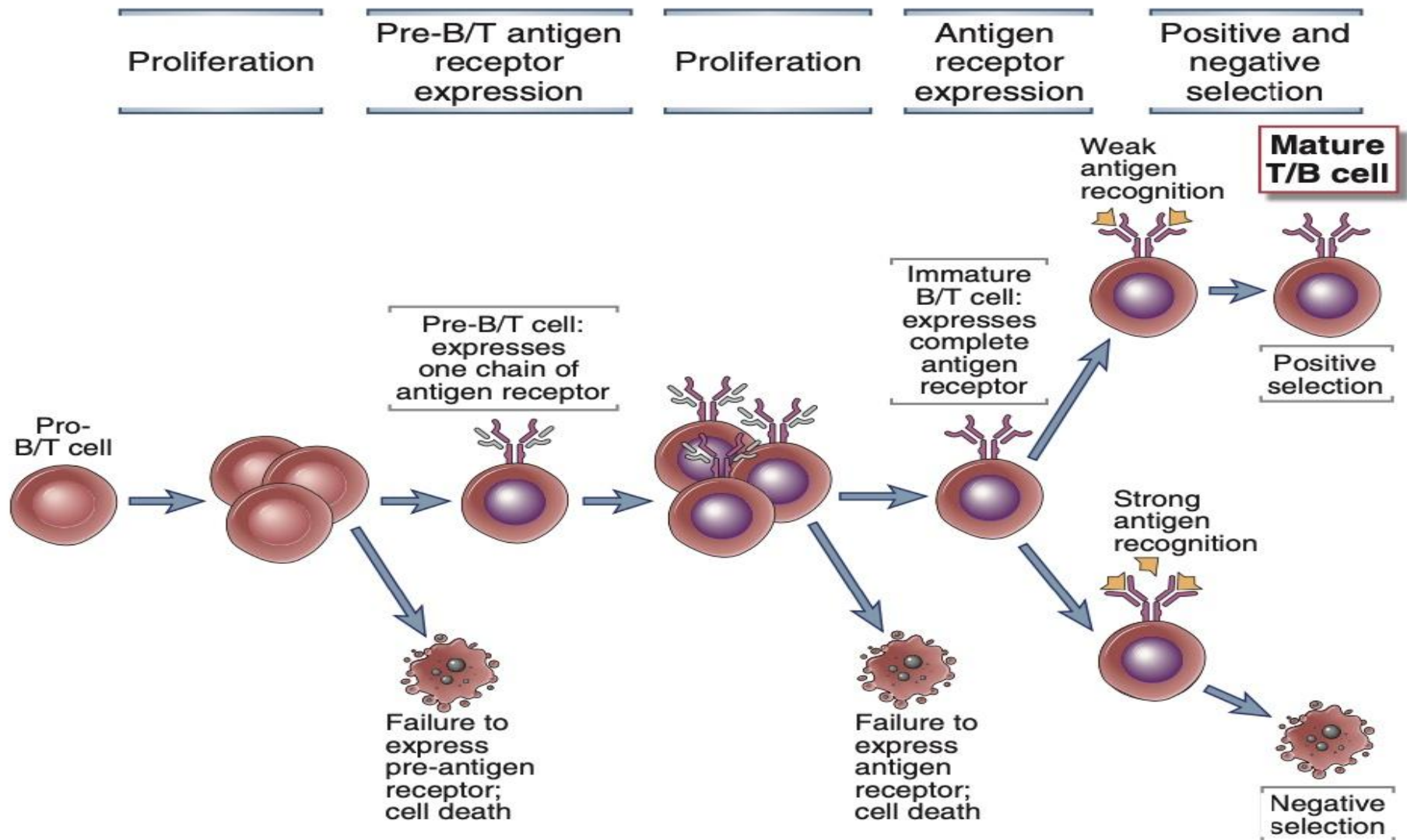
# Immune repertoire

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

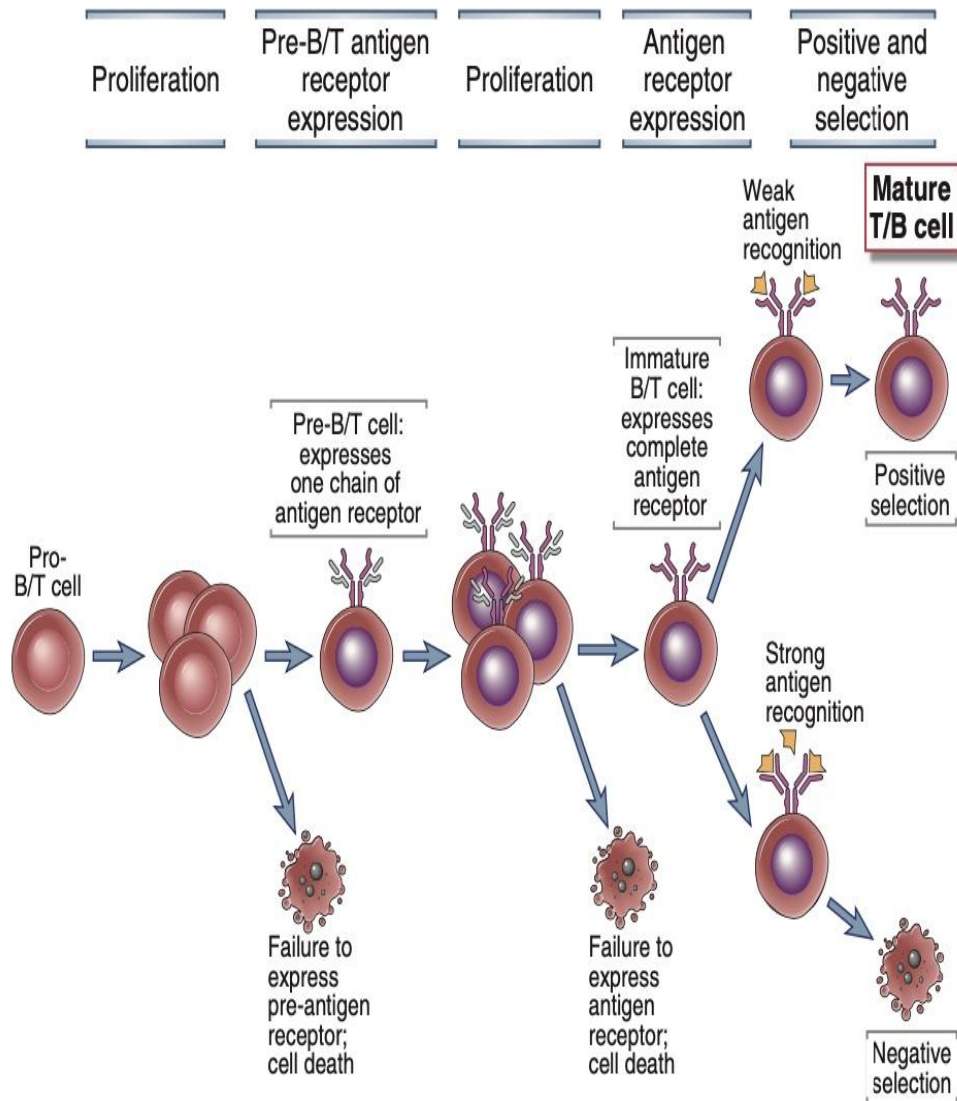
The formation of such different receptors occurs during the  
maturation process of lymphocytes...

# Lymphocyte maturation

Lymphocytes are derived from bone marrow stem cells.



# 1. Proliferation



Proliferation 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.

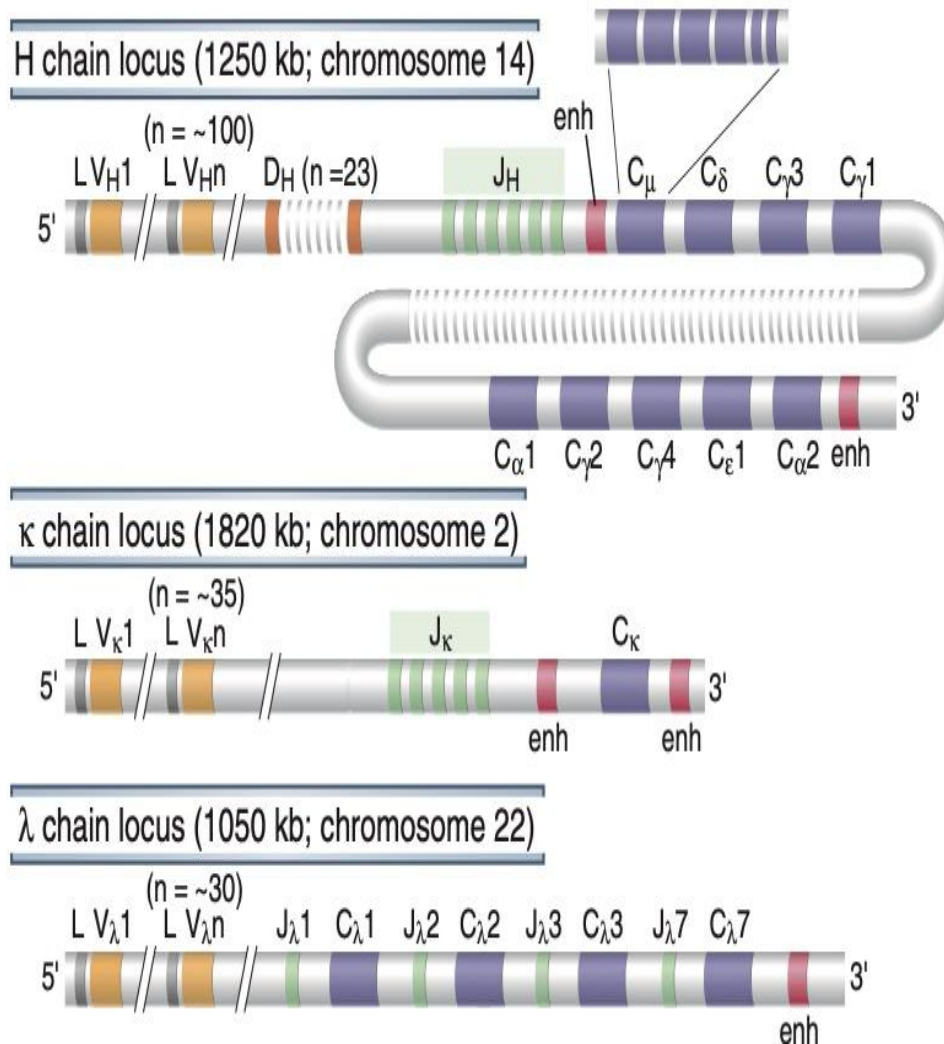
## 2. Differentiation

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

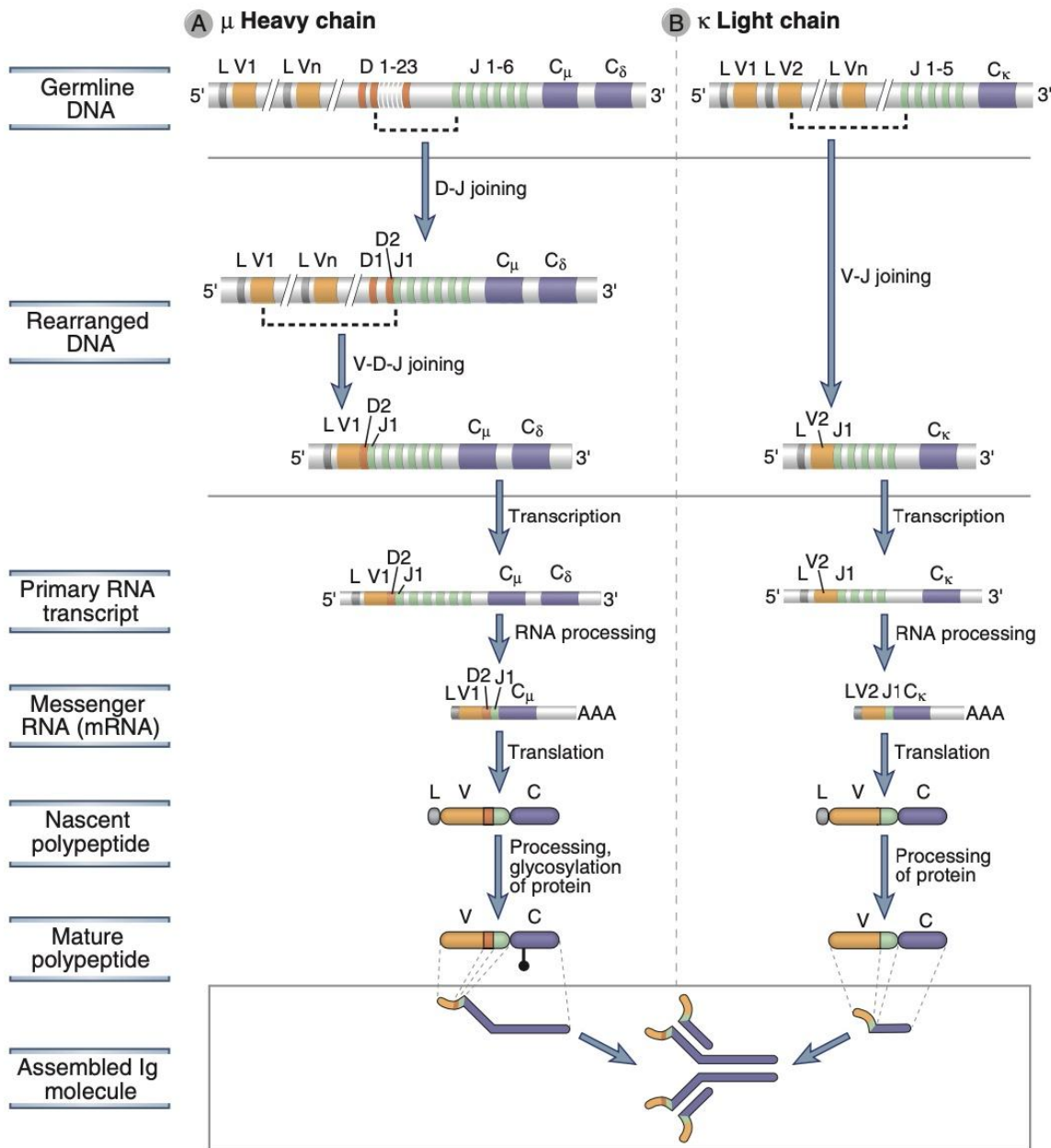
**D** diversity

**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 recombination 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)**

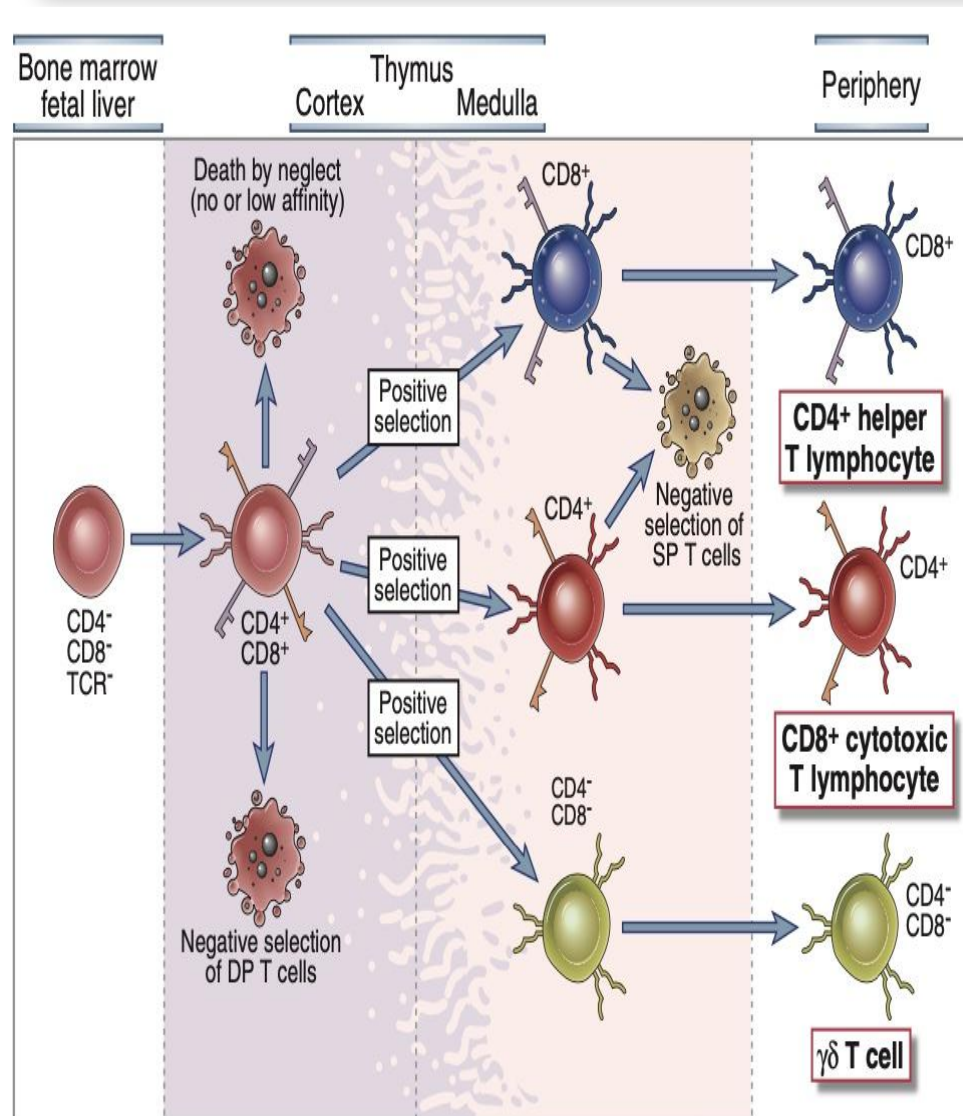
... and even more by

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.**

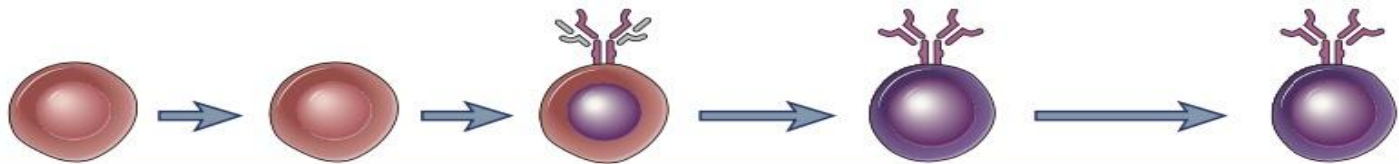
The biological purpose of selection is to preserve those lymphocytes whose antigenic specificity is potentially beneficial and not dangerous for antigens of their own tissues.

***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
<b>Proliferation</b>	[Bar]		[Bar]		
<b>RAG expression</b>		[Bar]		[Bar]	
<b>TdT expression</b>		[Bar]			
<b>Ig DNA, RNA</b>	Unrecombined (germline) DNA	Unrecombined (germline) DNA	Recombined H chain gene (VDJ); $\mu$ mRNA	Recombined H chain gene (VDJ), $\kappa$ or $\lambda$ genes (VJ); $\mu$ or $\kappa$ or $\lambda$ mRNA	Alternative splicing of VDJ-C RNA (primary transcript), to form $C_\mu$ and $C_\delta$ mRNA
<b>Ig expression</b>	None	None	Cytoplasmic $\mu$ and pre-B receptor-associated $\mu$	Membrane IgM ( $\mu + \kappa$ or $\lambda$ light chain)	Membrane IgM and IgD
<b>Surface markers</b>	CD43 <sup>+</sup>	CD43 <sup>+</sup> CD19 <sup>+</sup> CD10 <sup>+</sup>	B220 <sup>lo</sup> CD43 <sup>+</sup>	IgM <sup>lo</sup> CD43 <sup>-</sup>	IgM <sup>hi</sup>
<b>Anatomic site</b>	[Bar] Bone marrow			[Bar] Periphery	
<b>Response to antigen</b>	None	None	None	Negative selection (deletion), receptor editing	Activation (proliferation and differentiation)

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

$\mu$  chain and surrogate chain are associated with  $Ig\alpha$  and  $Ig\beta$  chains and form a pre-BCR complex, which is important for:

- delivery of proliferative and anti-apoptotic signals

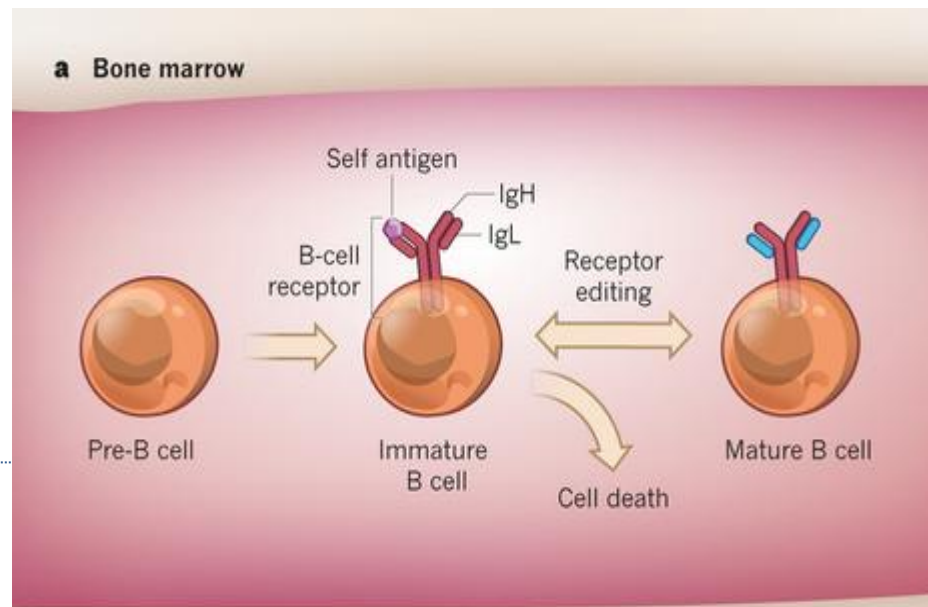
- Allelic exclusion (i.e. gene locking for heavy chains on the second chromosome)

- triggers gene recombination for light chains (first for  $\kappa$  and then for  $\lambda$  if it fails first)

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

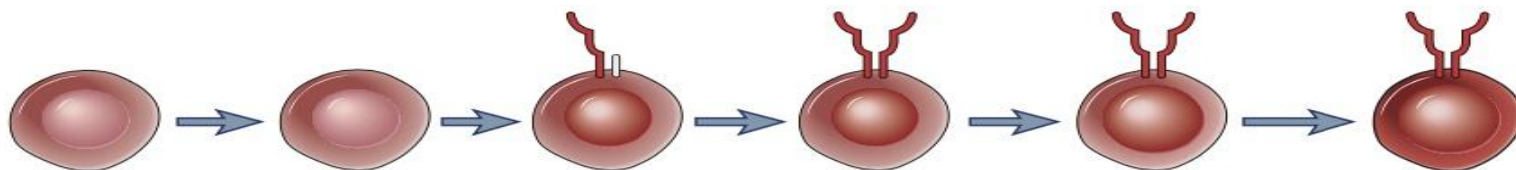
After the random process of B lymphocyte repertoire, those cells that express the complete receptors are positively selected, while those cells that strongly recognize their own antigens are selected negatively.

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.



# 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
<b>Proliferation</b>						
<b>RAG expression</b>						
<b>TdT expression</b>						
<b>TCR DNA, RNA</b>	Unrecombined (germline) DNA	Unrecombined (germline) DNA	Recombined $\beta$ chain gene [V(D)J-C]; $\beta$ chain mRNA	Recombined $\beta$ , $\alpha$ chain genes [V(D)J-C]; $\beta$ and $\alpha$ chain mRNA	Recombined $\beta$ , $\alpha$ chain genes [V(D)J-C]; $\beta$ and $\alpha$ chain mRNA	Recombined $\beta$ , $\alpha$ chain genes [V(D)J-C]; $\beta$ and $\alpha$ chain mRNA
<b>TCR expression</b>	None	None	Pre-T receptor ( $\beta$ chain/pre-T $\alpha$ )	Membrane $\alpha\beta$ TCR	Membrane $\alpha\beta$ TCR	Membrane $\alpha\beta$ TCR
<b>Surface markers</b>	$c\text{-kit}^+$ $\text{CD44}^+$ $\text{CD25}^-$	$c\text{-kit}^+$ $\text{CD44}^+$ $\text{CD25}^+$	$c\text{-kit}^+$ $\text{CD44}^-$ $\text{CD25}^+$	$\text{CD4}^+\text{CD8}^+$ $\text{TCR/CD3}^{\text{lo}}$	$\text{CD4}^+\text{CD8}^-$ or $\text{CD4}^-\text{CD8}^+$ $\text{TCR/CD3}^{\text{hi}}$	$\text{CD4}^+\text{CD8}^-$ or $\text{CD4}^-\text{CD8}^+$ $\text{TCR/CD3}^{\text{hi}}$
<b>Anatomic site</b>	Bone marrow	Thymus				Periphery
<b>Response to antigen</b>	None	None	None	Positive and negative selection		Activation (proliferation and differentiation)

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

**$\beta$  chain and pre-T $\alpha$  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  $\alpha$  chain

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