Sheet n. 13

Doctor 22
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Navy: slides/ black: doctor's explanation/ underlined sentences: very important/blue: further explanation

- The word "lymphocytes" refers to B and T cells.

- B and T cells can recognize different antigens. Majority T cell receptors are limited, they canonly recognize linear (un3D shape and uncharged) peptides of a certain length that must be bound to the cleft of MHC molecules (There is an exception, α/β T and γδ T cells recognize lipids and carbs via MHC-like molecule. This is why T cells exhibit MHC restriction. B cells, however, can recognize a wider range of antigens. They can recognize macromolecules such as peptides, proteins, carbohydrates, lipids and metal ions.

- MHC II presents extracellular antigens to CD4 T cells, while MHC I present intracellular antigens to CD8 cells. There is a difference in the processing of these molecules and the antigens they present (another exception, the extracellular antigens that cross-present through APCs specially dendritic cells are presented via MHC I.

- The antigen goes to the lymphocyte - The number of lymphocytes is limited, therefore, it would not be efficient for lymphocytes to go and look for microbes that enter from the multiple portals of entry (skin, GI tract, respiratory tract) or are blood-borne. Instead, antigen presenting cells (APCs) can be found at these sites, and they capture the antigens (this is the first step of antigen presentation to lymphocytes). Then the antigen can be brought to the T cell

- There are five APCs – All express MHC II. The most efficient type is dendritic cells which express MHC II, MHC I, in case of viral infected antigen, and can activate naïve T lymphocytes. Macrophages and B cells activate effector T lymphocytes. There are also thymic epithelial cells (important for lymphocyte development) and vascular epithelial cells.

- Once the dendritic cell captures the antigen, it is no longer a resting/immature dendritic cell. The cells mature and start expressing co-stimulators and the homing receptor CCR7 (chemokine receptor 7). In T cell rich zones, CCL19 and CCL21 are secreted and they are ligands for the homing receptor. This will recruit dendritic cells to the T cell rich zone. During this process, the dendritic cells will complete their maturation and process the antigen.

- MHC I – Intracellular antigens such as viruses and mutated proteins of tumor cells they get tagged by ubiquitin, which sends them to the proteasome for degradation. At the same time, MHC I synthesis occurs in the ER. The degraded peptide attaches to theTAP transporter, which will translocate the peptide into the ER. The TAP has a high affinity for tapasin, which will allow the TAP to bring the peptide with it into the ER. Then, the peptide is loaded onto the MHC molecule, passed through the Golgi apparatus, and finally sent through an exocytotic vesicle to the cell surface

MHC II – Extracellular Antigens – The antigen enters the cell in a membrane bound vesicle known as an endosome. This endosome becomes a phagosome, then it fuses with a lysosome to become a phagolysosome. This phagolysosome has a very low pH and enzymes such as cathepsins which cause degradation of proteins into peptides. MHC II synthesis occurs in the ER and immediately a protein, known as the invariant chain, binds to the cleft. This ensures that peptides that are meant to bind to MHC I won't accidentally bind to MHC II. The MHC II molecule is then sent to the phagolysosome and that's where loading will occur.(EXTRA: The invariant chain associates with nascent MHC class II molecules and blocks the association of MHC class II molecules with endoplasmic reticulum (ER)-misfoled proteins**)**

-ANTIGEN RECOGNITION IN THE ADAPTIVE IMMUE SYSTEM AND LYMPHOCYTES DEVELOPMENT:

Although B and T cell receptors differ in many ways, they both have constant andvariable regions and antigens bind to the variable region.

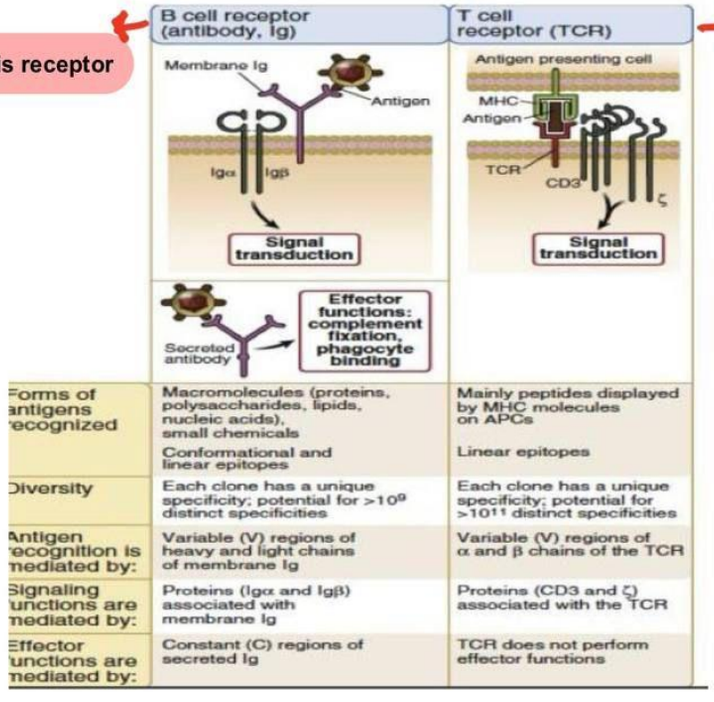
B and T cell receptors differ in many ways. This includes:

1. **Form of Antigens Recognized – As mentioned earlier, B cell receptors can recognize a larger variety of molecules, while T cellshave MHC restriction.**

2. **Location – B cell receptors may be membrane bound or in a secreted soluble form. T cell receptors are always membrane bound.**

3. **Effector Functions – T cell receptors do not have any effector functions but can recognize about 10^11 different epitopes without encountering with antigens, while B cell receptors have effector functions done by theFc portion of the antibody. Effector functions include complement fixation (activation) and phagocyte binding.**

4 polypeptide chains (2 heavy and 2 light chains). This receptor can be bound or Soluble



	B cell receptor (antibody, Ig)	T cell receptor (TCR)
Forms of antigens recognized	Macromolecules (proteins, polysaccharides, lipids, nucleic acids), small chemicals Conformational and linear epitopes	Mainly peptides displayed by MHC molecules on APCs Linear epitopes
Diversity	Each clone has a unique specificity; potential for $>10^9$ distinct specificities	Each clone has a unique specificity; potential for $>10^{11}$ distinct specificities
Antigen recognition is mediated by:	Variable (V) regions of heavy and light chains of membrane Ig	Variable (V) regions of α and β chains of the TCR
Signaling functions are mediated by:	Proteins (Igα and Igβ) associated with membrane Ig	Proteins (CD3 and ζ) associated with the TCR
Effector functions are mediated by:	Constant (C) regions of secreted Ig	TCR does not perform effector functions

2 polypeptide chains, alpha and beta . (Always membrane-bound)

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The diversity of both receptors is very huge. And this is due to the utilization of genetic–recombination

Antibodies

Antibodies = B cell Receptors (BCRs) = Immunoglobulins (Ig)

An antibody molecule is composed of four polypeptide chains—two identical heavy (H) Chains and two identical light (L) chains—with each chain containing a variable region and a constant region.

- **The light chain consists of 1 variable domain and 1 constant domain**

1. **The heavy chain consists of 1 variable domain and 3 (when secreted) or 4 (when membrane-bound) constant domains. So the constant domain can be longer or shorter.**

Each light chain is attached to a heavy chain and the two heavy chains are attached to each other by disulfide bonds.

Each variable domain also has hypervariable regions (also known as complementarity-determining regions {CDRs}). This is what gives the fine specificity of the receptor. The fine specificity of the antigen comes from the epitope (also known as the determinant).

The paratope is the functional antigen binding site.

Affinity maturation is a process that occurs during the immune response, ONLY in antibodies. It involves the enhancement of the binding strength (affinity) between antibodies and antigens over time. This process is important for the immune system to produce antibodies that are highly effective in recognizing and neutralizing specific pathogens, via somatic hyper mutation.

parts of the Antibody:

1. **Fab (fragment of antigen binding) region - The part of the antibody that binds to theantigen. Each antibody has two, identical Fab regions.**

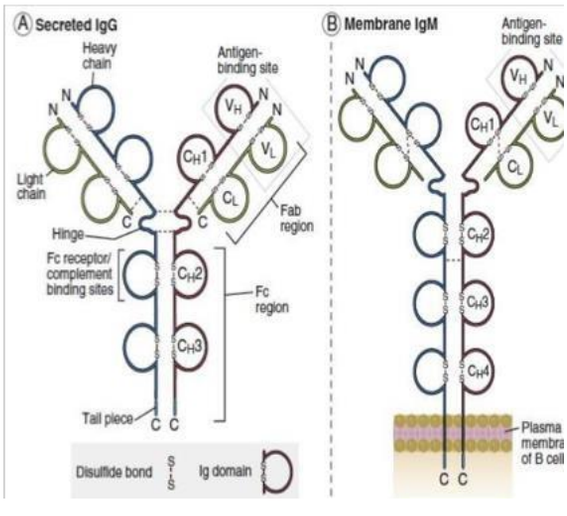
a. The Fab region is composed of the variable and constant domain of the light chain andthe variable and first constant domain of the heavy chain.

b. As a monomer, an antibody can bind to 2 similar epitopes of different antigens for the two Fab regions (or two epitopes of the same antigen). However, antibodies can also exist as dimers, trimers, or pentamers. Therefore, they can be bound to up to 10

2. **epitopes, for the 10 Fab regions present in an antibody that exists as a pentamer**

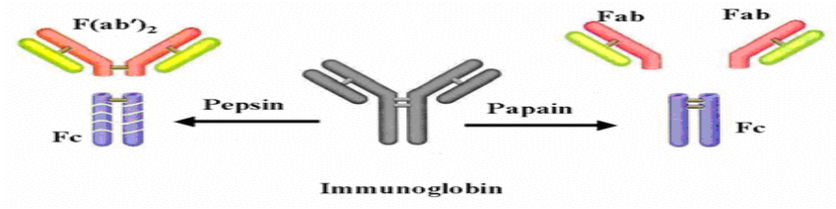
3. **Fc (fragment that crystallizes) region - Responsible for most of the biological activityand effector functions of the antibodies.**

4. **Hinge region – It is a flexible region between the Fab and Fc parts. It allows the twoantigen-binding Fab regions of each antibody to move independently of each other. This allows them to bind to different epitopes separately**



-In the heavy chain, the different forms of the Fab region are called IDIOTYPES
-The different forms of the heavy chain are called ISOTYPES.

• Examples on enzymes that can cleave antibodies are pepsin and papain, they cleave in different ways



Isotypes

There are five types of heavy chains, called μ, δ, γ, ε, and α, which differ in their constantregions. Antibodies that contain different heavy chains belong to different classes, or isotypes, and are named according to their heavy chains (IgM, IgD, IgG, IgE, and IgA).

The antigen receptors of naive B lymphocytes, which are mature B cells that have not encountered any antigens, are membrane-bound IgM and IgD. After stimulation by Antigen and helper T lymphocytes, the antigen-specific B lymphocyte clone may expandand

These same B cells may produce antibodies of other heavy-chain classes. This change inIg isotype production is called heavy-chain class (or isotype) switching. For example, a class switch may be from an IgM which recognizes antigen X to an IgG which also recognizes antigen X. The heavy chains (more specifically, the Fc portion) can change during the B cell life span, but the light chain (which can be of κ-kappa or λ-lambda types) doesn't.

The next table illustrates the different isotypes. While most of what the professor statedis in the table, there are a few additional points mentioned:

• Out of the four IgG subclasses, IgG1 and IgG3 are the most common in humans. IgG antibodies have multiple functions, including complement fixation (activation), opsonization, and neonatal immunity (immunity transferred from the mother to the fetussince IgG antibodies are the only antibodies that can pass through the placenta)

• IgM in its soluble form is a pentamer. Therefore, it can bind up to 10 different antigens or 10 different epitopes of the same antigen.

Isotype of antibody	Subtypes (H chain)	Serum concentration (mg/ml)	Serum half-life (days)	Secreted form	Functions
IgA - mainly surface of bacteria and viruses. found as "dimer and trimer", and found on → body fluid, secretion of GIT, RT, saliva, tears - found in serum in TFace amount as monomer	IgA1,2 (α1 or α2) found on mucosal prevent the attachment	3.5	6	Mainly dimer, also monomer, trimer Cα1 Cα2 Cα3 J chain	Mucosal immunity naive means pre-antigen exposure
IgD	None (δ)	Trace	3	Monomer	Naive B cell antigen receptor
IgE ↳FcεRI	None (ε)	0.05	2	Monomer Cε1 Cε2 Cε3 Cε4	Defense against helminthic parasites, immediate hypersensitivity "allergy"
IgG ↑most abundant	IgG1-4 (γ1, γ2, γ3 or γ4) IgG1, IgG3 are the best opsonins which bind to FcγR1	13.5	23	Monomer Cγ1 Cγ2 Cγ3 ↳10 binding sites of 10 epitopes of the same antigen or 10 similar epitopes of different antigens	Opsonization, fixation complement activation, antibody-dependent cell-mediated cytotoxicity, neonatal immunity, feedback inhibition of B cells, cross the placenta ADCC
IgM	None (μ)	1.5	5	Pentamer Cμ1 Cμ2 Cμ3 Cμ4 J chain	Naive B cell antigen receptor (monomeric form), complement activation, opsinization

(IgD is concerned with RNA splicing that is happening to the RNA encoding the constant domain of the Heavy chain).

Immature B lymphocytes express IgM only. IgD found on cytoplasm.

Mature NAÏVE B lymphocytes express IgM and IgD.
The same B cells may produce antibodies of other heavy-chain classes .This change in Ig isotype Production is called heavy-chain class (or isotype) switching. (This doesn't change specificity but Changes the function (effector mechanism)

The strength with which one antibody binds to one epitope of an antigen is called the Affinity of the interaction. Affinity can mature (affinity maturation).

The total strength of binding is much greater than the affinity of a single antigen- antibody bond and is called the avidity of the interaction. This considers the bindingof all the units of the antibody (so it takes into account whether the antibody is monomeric, dimeric, etc.)

Antibodies produced against one antigen may bind to other, structurally similarantigens. Such binding to similar epitopes is called a cross-reaction.

Cross-reaction measures the extent to which different antigens appear similar tothe immune system

Cross-reactivity **between antigens occurs when an antibody raised against one specific antigen has a competing high affinity toward a different antigen.**

These similar antigens can be self-antigens sometimes.

It's called also "molecular mimicry ":(the Ag of the microbe is similar to self-antigens)

Molecular mimicry is a hallmark of the pathogenesis of rheumatic fever where the streptococcal group A carbohydrate epitope, structurally mimics cardiac myosin in the human disease, rheumatic carditis (rheumatic).(3rd year Insha'Allah)

-Monoclonal Antibodies

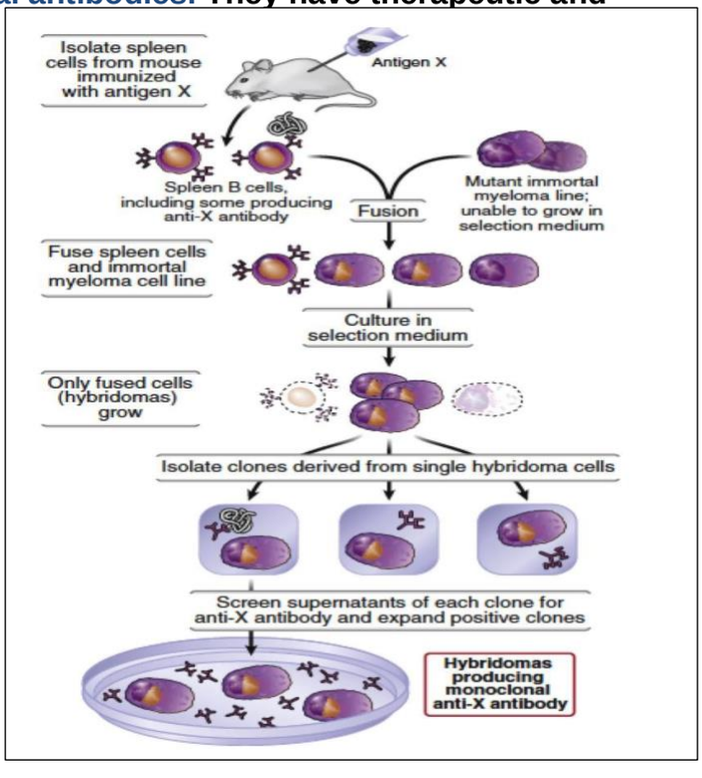
The realization that one clone of B cells makes an antibodyof only one specificity (recognizes one antigen) has been exploited to produce monoclonal antibodies. They have therapeutic and research uses.

Making Monoclonal Antibodies

1. Antigen X is injected into a mouse. Then, the B cell population of the spleen is taken, as the spleen has clones of the B cells that produce antibodies that are specific to antigen X.

2. B cells have a short life span in vitro. Therefore, they are fused with plasma myeloma cells, which are immortal, tumor cells. This fusion results in a hybridoma cell.

a. The plasma myeloma cell must be unable to produce its own antibodies. This is because we only want the antibodies produced by the B cell clones of the mouse.



3. Selection (screening) and expansion are done on the clones that produce Antibodies against antigen X.

The issue with using these antibodies for medical purposes is that they originate from a mouse, which the immune system will recognizeas foreign. Recently, monoclonal antibodies havebeen generated by using recombinant DNA technology to clone the DNA encoding human antibodies of the desired specificity.

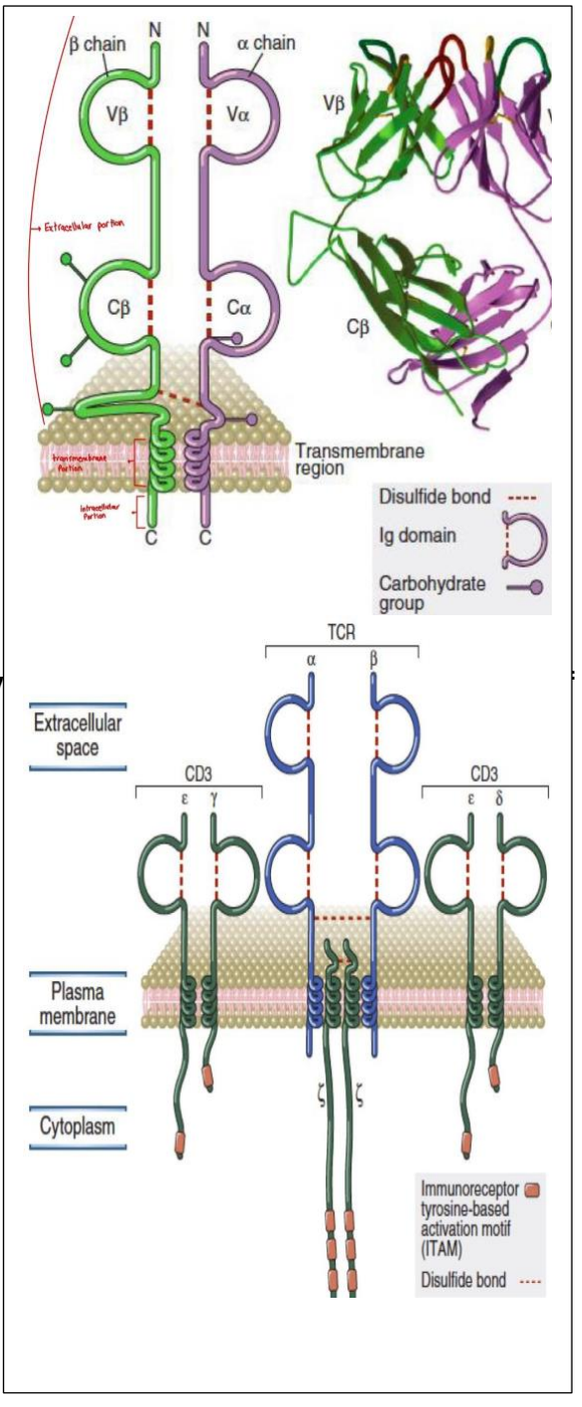
T Cell Receptors

The antigen receptor of MHC-restricted CD4+ helper T cells and CD8+ cytotoxic T lymphocytes(CTLs) is a heterodimer consisting of two transmembrane polypeptide chains, designated TCR α and β, covalently linked to each other by adisulfide bridge between extracellular cysteine residues.

Each chain is made up of a variable and constant domain.The variable domains of the α and β chains form the antigen binding site of T cell receptors. Of course, there are also hypervariable regions within the variable region. The chain also has a transmembrane sequence.

TCRs do not exhibit class switching or affinity maturation. While most T cells receptors are αβ (beta chain act as heav

γδ (gamma delta).



The T cell receptor complex is a complex ofthe TCR and associated signaling molecules. (Co-receptor)

These signaling molecules help in signal transduction after the antigen binds. For T cells these signaling molecules are CD3 andzeta (ζ).

- **Allylic exclusion occurs in both T+B cell receptors.**
- **In B cell receptor light chain(kappa and lambda)**

Antibodies also have signaling molecules,Igα and Igβ.

-B cell's markers: CD19, 20,21,40,43.

-T cell's markers: CD3, 4, 8. +-

Each gene segment encodes for alpha/ beta chain if we are talking about T cell receptor,heavy/ light chain if we are talking about B cell receptor, you have 2 copies on each chromosome one from your mother and the other from your father, so if the encodes thecope from your father and its functional and good then the other copy from your motherwill be excluded (shut off) this is called allylic exclusion. So only one specific antigen binding site would result.

The Immune Repertoire

As mentioned earlier, the CDRs give the fine specificity of the receptor. But why do they need this high specificity?

As adaptive immunity is very specific, the antigen receptors of lymphocytes must be able to bind to and distinguish between many, often closely related, chemical structures. So each lymphocyte clone must have a unique receptor that can differentiate between distinct antigens. The process of lymphocyte maturation first generates a very large number of cells (perhaps as many as 109) before any encounter with antigens. This large collection of distinct lymphocytes makes up the immune repertoire. However, not all these cells have useful receptors. The selection process now comes into play and promotes the survival of cells with receptors that can recognize antigens. Cells that cannot recognize antigens or have the potential to cause harm will be eliminated.

Remember that this specificity is for the sake of antigen binding. Therefore, although each clone of T lymphocytes recognizes a different antigen, the antigen receptors transmit biochemical signals that are fundamentally the same in all lymphocytes and are unrelated to specificity.

Lymphocyte Development

DEVELOPMENT OF IMMUNE REPERTOIRES

As the clonal selection hypothesis predicted, there are many clones of lymphocytes with distinct specificities, perhaps as many as 109, and these clones arise before an encounter with antigen.

• The process of lymphocyte maturation first generates a very large number of cells each with a different antigen receptor and then preserves the cells with useful receptors.

• Receptors are expressed on developing lymphocytes, and selection processes come into playthat promotes the survival of cells with receptors that can recognize antigens, such as microbial antigens, and eliminate cells that cannot recognize antigens in the individual or that have the potential to cause harm.

Lymphocyte Development

If we think about the receptors we need for all the antigens, microbes, fungi, and cancers we would need the earth's entire DNA to make a separate antigen binding site for every segment on T/Bcell receptors.

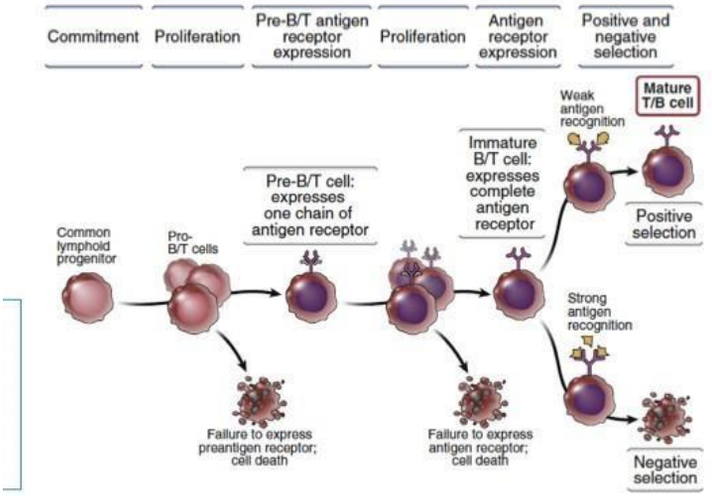
Site of development
for

B cells=>bone
marrow

• T cells=>thymus.

B cells also mature at the spleen but we
Wil not focus on this.

<u>The pic is important</u>



These lymphocytes possess a molecular mechanism and the centers of these mechanisms arecalled antigen receptor gene rearrangements, this has a critical role in the development of lymphocytes and generation of the diversity of T/B cell receptors so it is diverse and at the same time specific towards unknown antigens once they are encountered.

The first step in lymphocyte development is the commitment of are pluripotent stem cells to common lymphoid progenitorcells to either B cell lineages or T cell lineages. Then, the cells go through cycles of proliferation and expansion the most important step of development is antigen receptor gene rearrangement this serve for the whole diversity

The second step is selection either positive or negative selection before they become mature and Naïve (before meeting their foreign antigen).

In pro lymphocyte the state of antigen receptor gene rearrangement begins, it's the most crucial step of the generation of the diversity of B and T cells receptor, in B cell receptor the it Begins in the heavy chain while T cell receptor in beta chain.

Beta chain acts as heavy chain.

In pre B or T cell there will be expression for the heavy chain, then begins light chains in if it is B Cell and alpha chain if T cell.

- **The lymphocyte is committed to become either T or B cell, it either becomes pro-B cell and goes to the bone marrow or becomes proT cell and goes to the thymus.**

• In recombination step in pro there is combining of gene segments, so we have the heavy chain on B cell receptor or the beta chain of T cell receptor.

• In pre we continue with the light chain of B cell receptor or the alpha chain of T cell receptor.

The complete expression occurs after the pre expression, if it is immature B cell it will give igM And if it is mature will give IgM and IgD, and then starts the selection process.

Antigen receptors gene rearrangement is germline (encoded), inherited located on the chromosomes, each chromosome contains two copies.

-Immunoglobulin heavy chain is on chromosome 14

- K and lambda light chains can be on chromosome2 and 22

- Beta chain can be on chromosome 7

-Alpha chain is on chromosome 14

Between variable and constant there are joining segments (J), whether it is light or heavy chain. Beta chain and heavy chain contains short coding sequencing called diversity (D) numbering about 30-48 segments.

The first combination it could be D14, J2, V20, CM, this combination is random, The somatic recombination of V and J, or of V, D, and J, gene segments is mediated by a lymphoid-specific enzyme, the VDJ recombinase (RAG-1 and RAG-2) proteins, and additionalenzymes, most of which are not lymphocyte specific and are involved in repair of double- stranded DNA breaks introduced by the recombinase

RAG: recombination activating genes.

- **There are 2 D segments in beta chains and about 23 D segments in the heavy chain of antibodies.**
- **There are pluripotent stem cells in the bone marrow then we have cells that are committed to becoming B/T lymphocytes(called commitment), the common lymphoid progenitor cell will either continue in the bone marrow and become B lymphocytes or will go to the thymus and be T lymphocytes, then, there will be the progenitor for B or T cells in this stage antigen receptor gene rearrangement occurs (recombination), they are segments that encode for B/ T cell receptor ,they are germ-line encoded(inherited) so they are present but what happens is that the segments are relocated, one segment from your father and the next one from your mother and then from your mother and so on...**

-it will take the V region(variable region) and J region(joining region)(these are short coding segments) and then it binds them together by a constant region, this is <u>called combinatorial diversity</u>, enzymes do this called RAG enzymes(VDJ recombinase, RAG1,2) and these are in the bone marrow and the thymus, this will give so many possibilities.

Combinatorial diversity is limited to the number of segments.
Variable domains in heavy chain 30-45
<u>There is another enzyme TDT</u>: terminal deoxynucleotidyl transferase this E may make junction Between V, D, J (INSERTION OR DELETION nucleotides), this will make unlimited repertoire (It can add 10 and then remove 3, add 2 and remove 7...) This is called <u>junctional diversity.</u>

TDT adds/ deletes nucleotides from the DNA that resulted from the RAG enzyme (after relocating the segments) and this is called junctional diversity.

_So imagine the possibilities! Reorganizing segments gives us combinatorial diversity, and the pinpoints that are done by the TDT give us junctional diversity.

TDT doesn't work on the light chain of B cell.
RAG work on the light and heavy chain for both cells.
-So the repertoire of this B/T cell receptor to cover the cognate antigen is very properly
- -if we add/remove nucleotides without templates doesn't this result in mutations?
: There are DNA repair mechanisms working in lymphocytes, but they work randomly if there is a mutation.
- For example, a known mutation in the RAG enzyme results in severe combined immunodeficiency syndrome (lack of B as well as T cells).
-If there's a partial activity (nonsense mutation) it's called Omen syndrome.
If it is partial mutation the affected is B cell not Tcell, if it is complete both get affected.

- Early lymphoid progenitors contain Ig and TCR genes in their **inherited, or germline, configuration**. In this configuration, Ig heavy-chain and light-chain loci and the TCR α-chain and β-chain loci each contain multiple variable region (V) gene segments, numbering about 30-45, and one or a few constant region (C) genes.
- Between the V and C genes are groups of several short coding sequences called diversity (D) and joining (J) gene segments. (All antigen receptor gene loci contain V, J, and C genes, but only the Ig heavy-chain and TCR β-chain loci also contain D gene segments.
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- **The formation of functional genes that encode B and T lymphocyte antigen receptors is initiated by somatic recombination of gene segments that code for the variable regions of the receptors, and diversity is generated during this process.**
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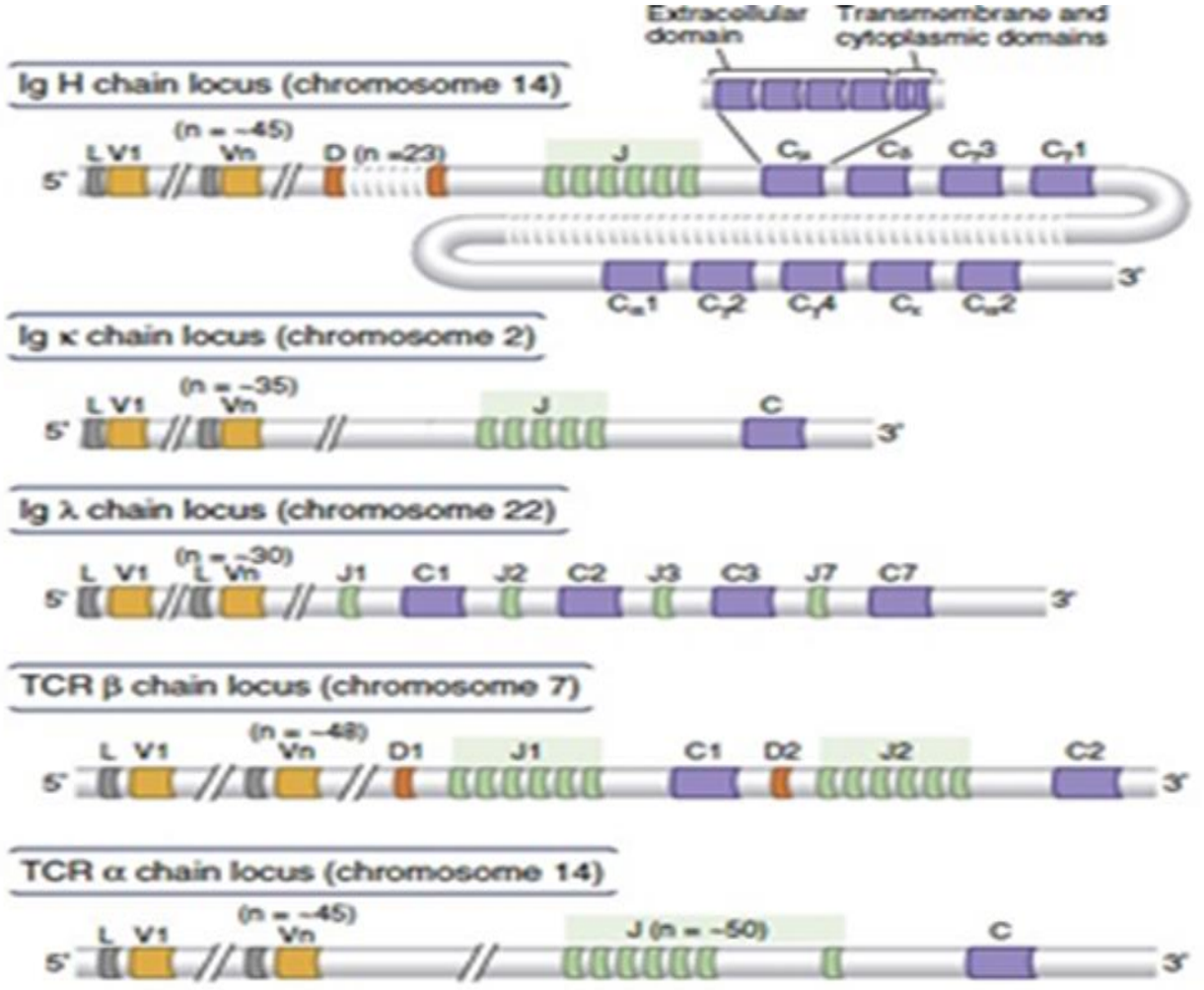
Germline organization of antigen receptor gene loci



Mechanisms of V (D) J Recombination and Generation of Ig and TCR Diversity

- **The somatic recombination of V and J, or of V, D, and J, gene segments ismediated by a lymphoid-specific enzyme, the VDJ breaks introduced by the recombinase.**
- **Diversity of antigen receptors is produced by the use of different combinations of V, D, and J gene segments in different clones of lymphocytes (called combinatorial diversity) and even more by changes in nucleotide sequences introduced at the junctions of the recombining V, D, and J gene segments (called junctional diversity)**

Recombination and expression of immunoglobulin (Ig)genes.

SOMATIC RECOMPINATION (D-J) Joining, then (V-D-J) joining, then take the first constant in ordered which is mew that's why the first is IgM, when it becomes mature it will transcript delta so when there is expression on immature IgM because it transcript delta it receives signals (feedback loop) signaling function (we have a functioning receptor intact receptor) it will shut down why??

Because of the specificity to have one TRANSCRIPTION of the specificity, this will make diverse receptor and distinct specificity ,a second reason(ALLELIC EXCLUSION) the chromosome 14 it is pair one from your father and on from your mother this will make two TRANSCRIPTIONS for the receptor ,it should be specific
Mew-delta-gamma

mRNA can undergo splicing which is an important step to form IgD on B cell receptors.

Alternative RNA splicing: IgM and IgD on receptor

Throughout the life cycle of the light chain they possess either Kappa or lambda even if there is affinity maturation or class switching
WHEN IT TRASCRIPT KAPPA IT WILL SHUT DOWN LEMBDA AND VICE VERSA

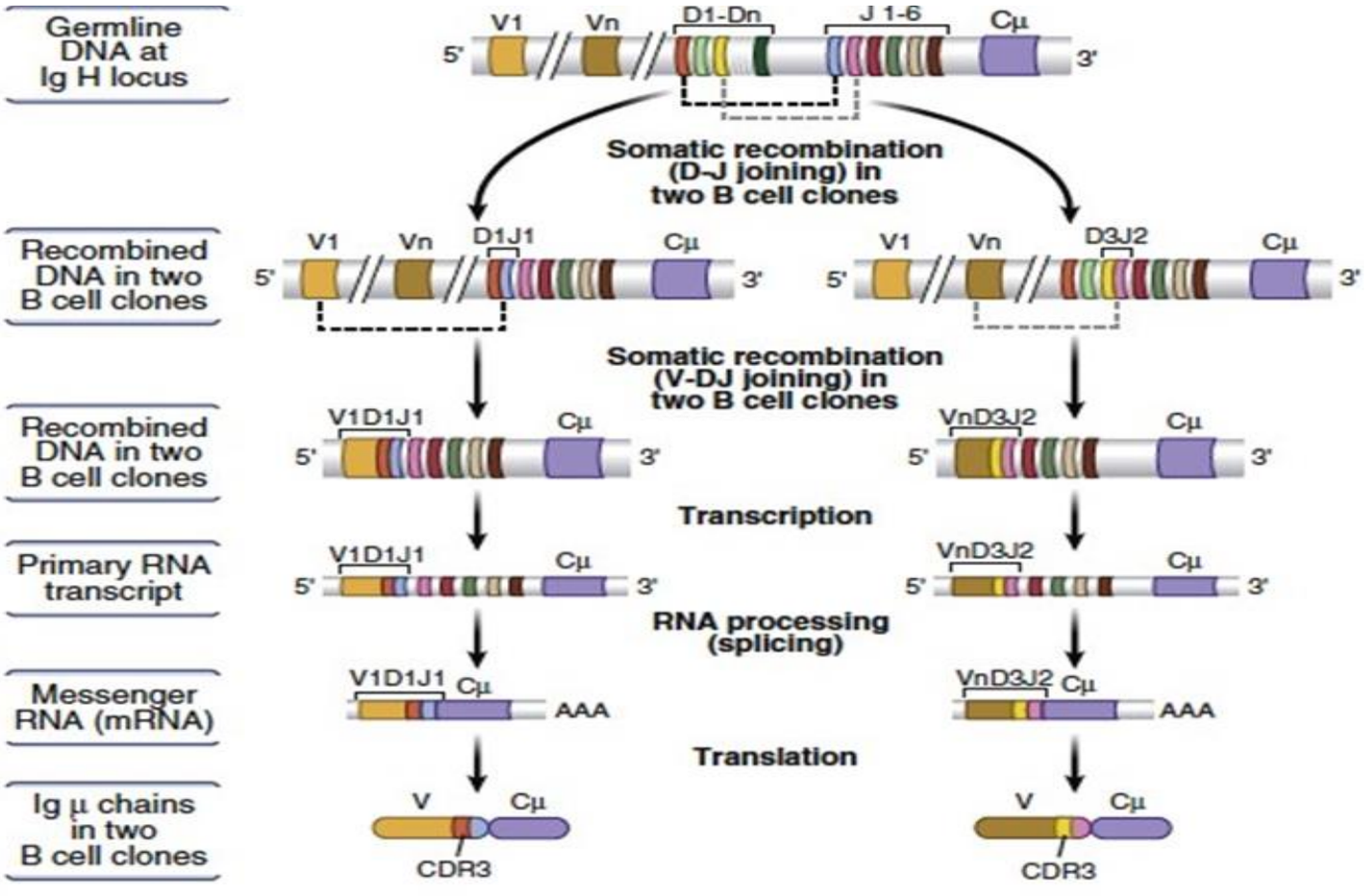
It starts transcript heavy chain then light chain in B cell or beta chain then alpha chain in TCR

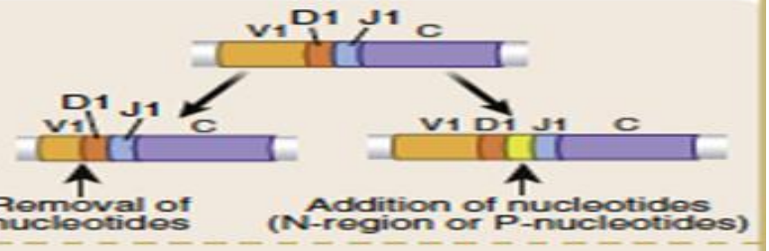
• Keep in mind: There is cytoplasmic expression before the surface expression.

• A frequently asked exam question: ☹

"What do immature/ mature B cells have on them?" Or "a group of cell has the following expression receptors are they mature? Immature? Pro-B cell?"

Immature naïve B cells have IgM **Mature B cells have IgM+IgD



	Immunoglobulin			T cell receptor	
	Heavy chain	κ	λ	α	β
Number of variable (V) gene segments	~45	35	30	45	48
Number of diversity (D) gene segments	23	0	0	0	2
Number of joining (J) gene segments	6	5	4	50	12
Mechanism					
Combinatorial diversity:					
Number of possible V(D)J combinations	Ig: ~3×10^6			TCR: ~6×10^6	
Junctional diversity:					
Total potential repertoire with junctional diversity	Ig: ~10^{11}			TCR: ~10^{16}	

- **Receptor gene rearrangement (recombining) starts from the middle of the diverse region (so if we were talking about the heavy chain in an antibody or beta chain in T cell receptor it starts from** the **D segment(diverse segment)that's present** only in Beta in T and Heavy chain in B **then it gets the variable** gene **then the joining**(at the level of DNA) **and** finally **the constant** region will be added at the level of mRNA, post transcription modification) This is basically what antigen receptor gene rearrangement is.

- **Remember: in the development steps between each step and the other there is proliferation things don't go smoothly always for example if something is not intact happened and not functional then this will signal for apoptosis, if it is normal, it will give survival signal(it will proliferate).**

- **B cell receptors will undergo somatic hyper mutation and affinity maturation, this happens after encountering the antigen (these are the steps during lymphocyte development until theyare naïve mature (and haven't been activated by an antigen yet).**

*Maturation and Selection of B Lymphocytes:

-B cell maturation occurs mostly in the bone marrow, but some maturation may also occur in the spleen.

1. Bone marrow progenitors committed to the B cell lineage proliferates, giving rise to manyprecursors of B cells, called pro-B cells. (Commitment = pro-B cells). IN THIS stage RAG and TDT both work on heavy chain of pro B cell ,then there will be recombination of the heavy chain

2. The Ig heavy-chain locus rearranges first,and only cells that are able to make an Ig μ (IgM) heavy-chain protein are selected to survive and become pre-B cells(cytoplasmic expression of the μ then the transcription and translation of the light chain)

3. The assembled pre-BCR serves essential functions in the maturationof B cells. The IgM-expressing B lymphocyte is the immature B cell.

4. The mature B cell expresses both IgM and IgD. How does this occur? The mRNA for the heavy chain gets spliced. The result of this splicing is that the mRNA once transcribed gives IgM and IgD. This cell is now able to respond to antigens in peripheral lymphoid tissues.

5. Now selection must occur. Positive selection occurs for B cells that express intact, functional BCRs. So developing B cells are positively selected, based mainly on the expression of complete antigen receptors and not on the recognition specificity of these cells. Negative selection occurs for B cells that react strongly with self-antigens.

Completion of B cell Maturation.

The IgM-expressing B lymphocyte is the immature B cell.

The IgM+ IgD+ cell is the mature B cell, able to respond to antigen in [illegible] lymphoid tissues.

Developing B cells are positively selected based mainly on expression of complete antigen receptors, and not on the recognition specificity of these [illegible]

The B cell repertoire is further shaped by negative selection against strong [illegible] of self-antigens.

Cells committed to becoming B cells (pro-B cell) so this common lymphoid progenitor will transform into pro cell and will start antigen receptor gene rearrangement, we're talking about B cell receptor so we will start with the heavy chain

• **If we were talking about T cells, then we start with the beta chain**

In the pre-B cell, there is a cytoplasmic new chain (cytoplasmic expression, translation + transcription occurred) through positive/negative selection will either become a memory lymphocyte or effector lymphocyte

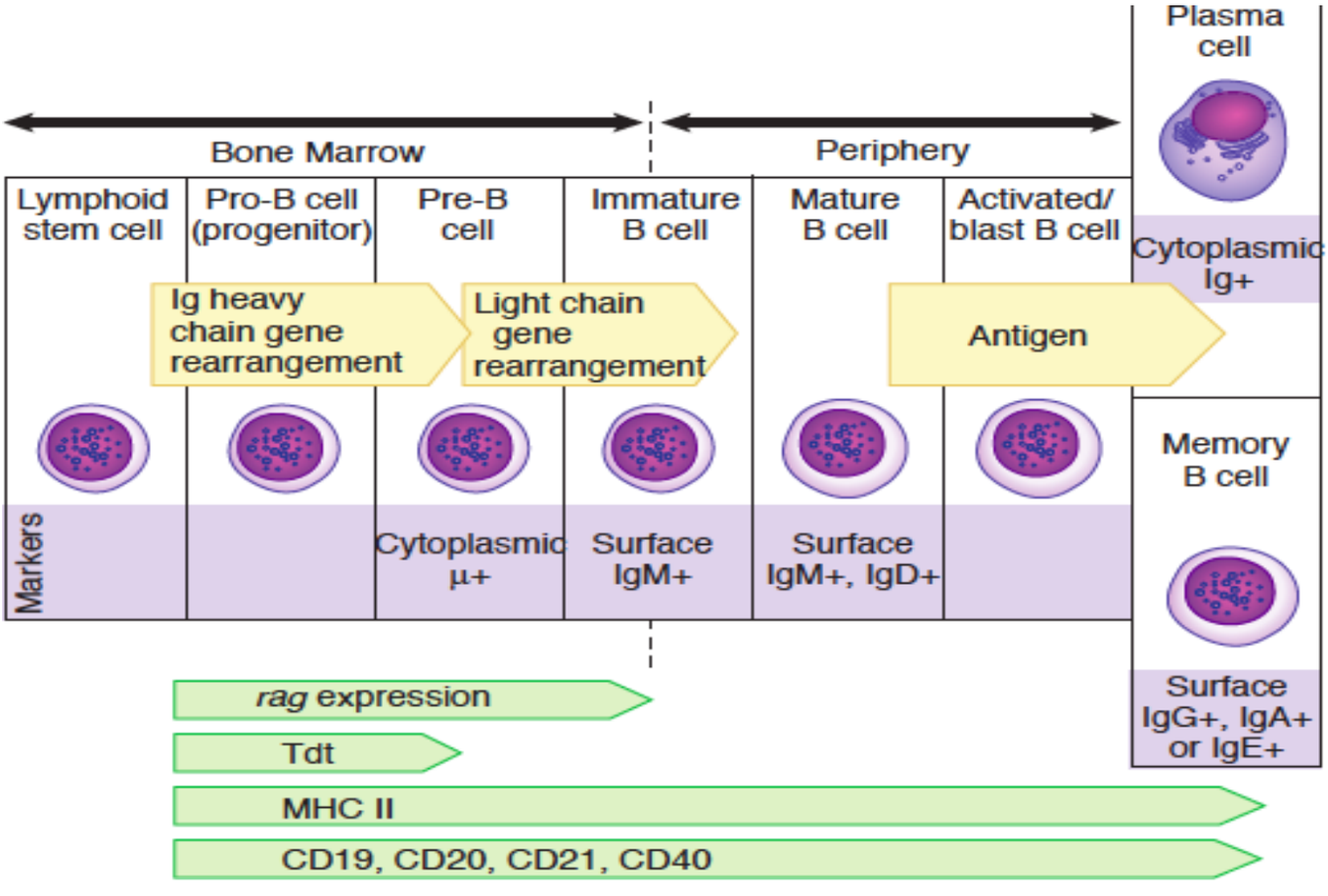
Effective=> IgM, Memory=> not IgM

• ***Cell surface IgM is immature B cell***

• ***IgM, IgD>mature B cell***

-RAG enzyme starts at the pro stage (where antigen receptor gene rearrangement occurs and continues until the MATURE stage)
-the surface markers:

-CD3=> T CELLS
-CD19, CD20, CD40, CD21=> B cells
-MHC class 2(APC) starts and stays with the cell until it dies



Maturation and selection of T lymphocyte

-T cell progenitors migrate from the bone marrow to the thymus, where the entire process of maturation occurs.

- -Double negative means they have neither CD4 nor CD8 on them.

-The least developed progenitors in the thymus cortex are called pro-T cells or double-negativeT cells (or double-negative thymocytes) because they do not express CD4 or CD8.

-TCR β gene recombination, mediated by the VDJ recombinase, occurs in some ofthese double-negative cells.

• If VDJ recombination is successful in one of the two inherited loci and a TCR βchain protein is synthesized, it is expressed on the cell surface in association with an invariant protein called pre-Tα, to form the pre-TCR complex of pre-T cells.

• If the recombination in one of the two inherited loci is not successful, recombination will take place in the other locus. If that fails too and a complete TCR β chain is not produced in a pro-T cell, the cell dies.

-When it comes out of the bone marrow and it's a committed lymphocyte to become a T cell they will become Pro-T cells, they get to the cortex of the thymus and we call them double negative T cells.

The pre-TCR complex delivers intracellular signals once it is assembled, similar to the signals from the pre-BCR complex in developing B cells.

• These signals promote survival, proliferation, and TCR α gene recombination and inhibit VDJ Recombination at the second TCR β-chain locus (allelic exclusion).

• Failure to express the chain and the complete TCR again results in death of the cell.

• The surviving cells express the complete αβ TCR and both the CD4 and CD8 co-receptors;these cells are called double-positive T cells (or double positive thymocytes).

At the same time, at the cortex of the thymus starts antigen receptor gene rearrangement and it starts at the beta chain, when they reach the medulla they are mature

They were double negative and then they become double positive(CD4+CD8 positive) then they will become single positive and after that either positive selection or negative selection, the cell that has CD4 interacts with MHC class 2 keeps CD4 and CD8 is gone

Positive selection occurs when react with self MHC molecule while negative selection react with self-antigen in thymus (it doesn't contain foreign antigen).

IF THE TCR react strongly with self-antigen it cause clonal deletion

CD4 AND CD8 Bind with non -polymorphic sites in MHC molecule

In organs transplantation if the organ refuse the body it is called graft versus host rejection the body start to deal with MHC In this organ as non self.

In double positive:

No encountering with an antigen=> inactive

Keep in mind they haven't encounter antigens yet primary lymphoid organs are sterile (they learn From self Ag)

If a cell with CD8 interacts with MHC class 1 then CD4 will be gone (will become single positive)

Selection of Mature T Cells

For a single positive cell high avidity (affinity) => negative selection

Low or medium avidity (affinity) =>positive selection

Let's compare:

The TDT starts at the heavy chain (receptor gene rearrangement) but stops at the beginning of the light chain here it continues till the end of selection

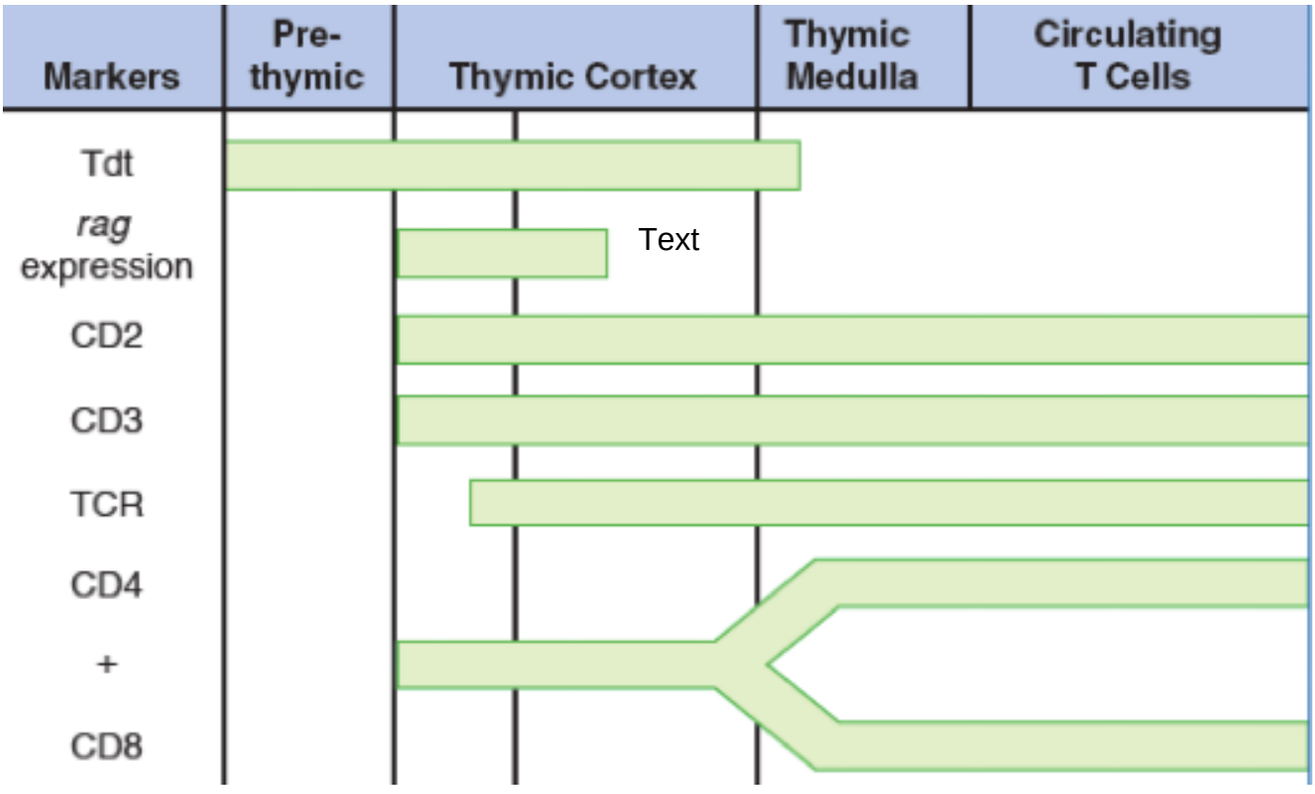
• If the TCR of a T cell recognizes an MHC molecule in the thymus, which must be a self MHC molecule displaying a self-peptide, and if the interaction is of low or moderate affinity, this T cell is selected to survive (positive selection).

• During this process, T cells whose TCRs recognize class I MHC–peptide complexes preserve the expression of CD8, the co-receptor that binds to class I MHC, and lose expression of CD4, the co-receptor specific for class II MHC molecules and the other way around. Single-positive Tcells.

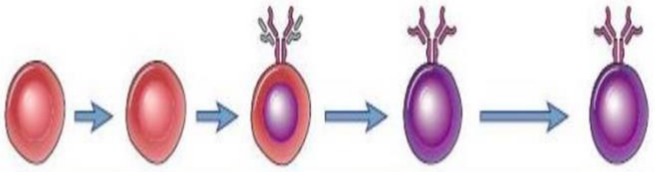
• Immature, double-positive T cells whose receptors strongly recognize MHC-peptide complexes in the thymus undergo apoptosis. This is the process of negative selection.

-At the same time, RAG expression (Combinatorial diversity) will define in the cortex so they will go fromPRO to PRE T cells

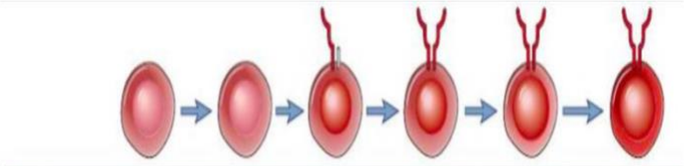
CD2+CD3 are surface markers for T cells.



They were double negative then double positive then will be single positive (either CD4 or CD8)



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	$CD43^+$	$CD43^+$ $CD19^+$ $CD10^+$	$B220^{lo}$ $CD43^+$	IgM^{lo} $CD43^-$	IgM^{hi}
Anatomic site	Bone marrow				Periphery
Response to antigen	None	None	None	Negative selection (deletion), receptor editing	Activation (proliferation and differentiation)



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)

FIGURE 8-19 Stages of T cell maturation. Events corresponding to each stage of T cell maturation from a bone marrow stem cell to

THE END ,GOOD LUCK 😀

لا تنسوا أهلنا في غزّة و سوريّا و السّودان و الإيغور و في كل مكان يذكر فيه اسمه الله من دعائكم