Lymphocyte Development and Antigen Receptor Gene Rearrangement

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Steps of lymphocytes development

- First; Pluripotent stem cells in bone marrow known as hematopoietic stem cells (HSCs), give rise to a common lymphoid progenitor (CLP) then give rise to pro-B cells, pro- T .cells and NK cells
- Pro-T cells migrate to Thymus and may commit to either the αβ .or γδ T cell lineages
- Second; pro- B and pro-T cell proliferate in response to cytokines IL-7
- third; Functional antigen receptor genes start to be formed on pre-cells (when formed called immature cells)
- For pre-B cells in the bone marrow and in pre- T cells in the – .thymus by a process of gene rearrangement

- Pre- cells proliferate in response to signal transduction from formed receptor
- Fourth;1- Selection of pre-cells that start forming receptor
- Fifth; Formation of the whole receptor on cell and become immature cell, the receptors cover limitless repertoire of potential antigen binding specificities. And the process repeated millions of times through out the life

Selection events that preserve immature cells that have-produced functional antigen receptor proteins and eliminate potentially dangerous cells that strongly recognize self antigens, .cells that remain after selection called mature cells

- Sixth; Differentiation of mature B and T cells into functionally and phenotypically distinct subpopulations. T cells develop into CD4+ and CD8+ αβ T lymphocytes in thymus. Then cells .transported to peripheral LN

Stages of lymphocyte maturation

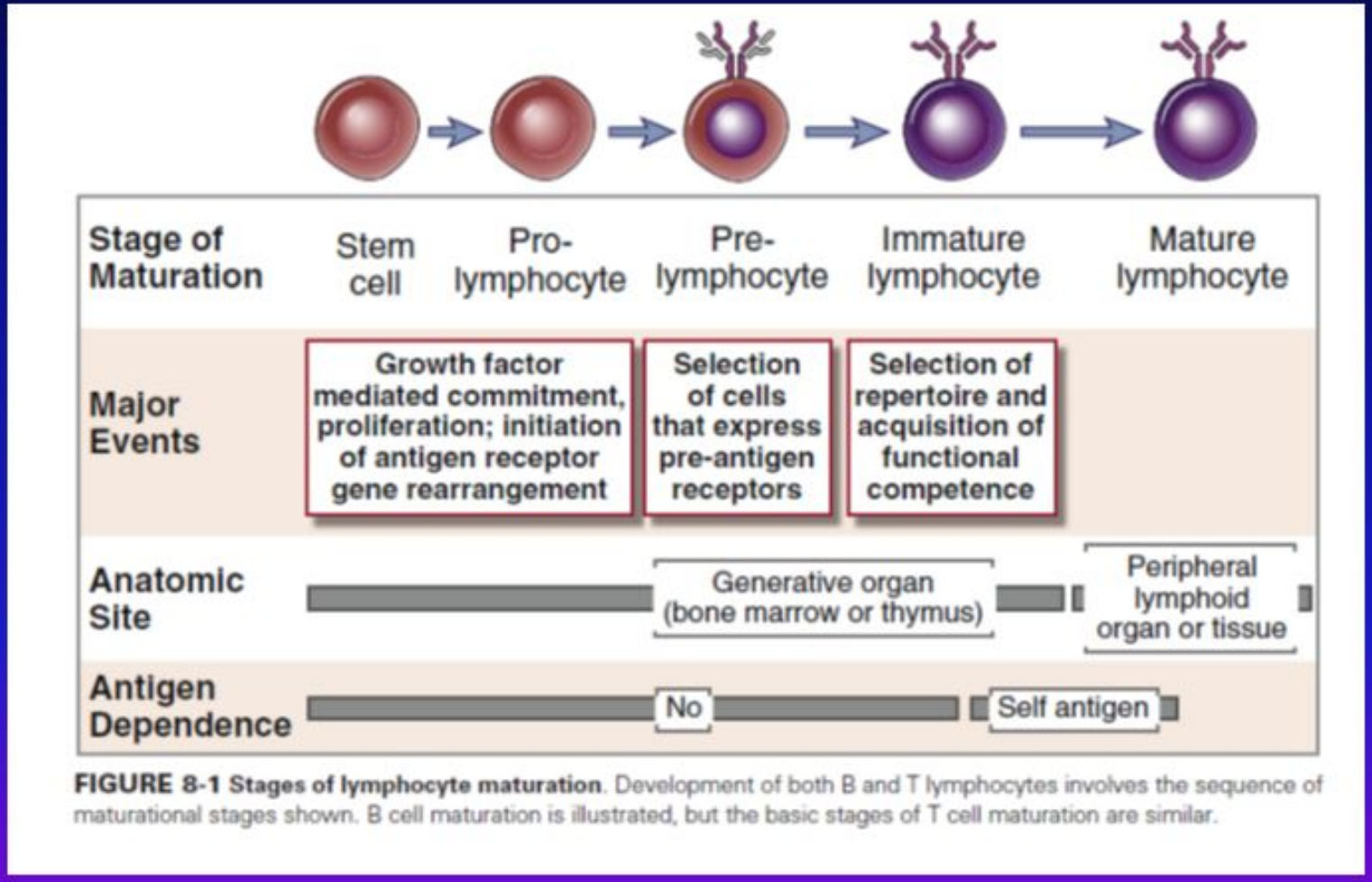
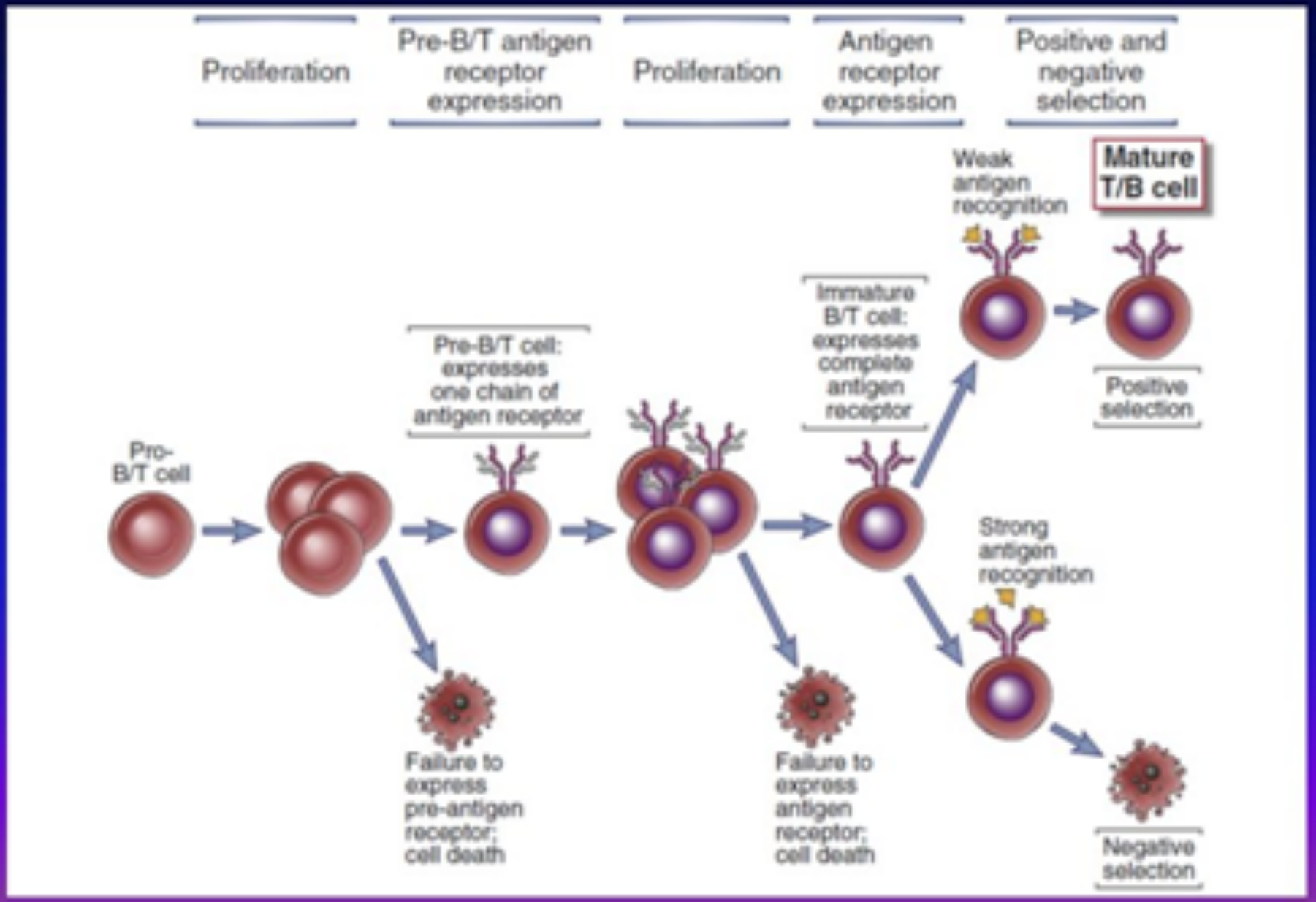


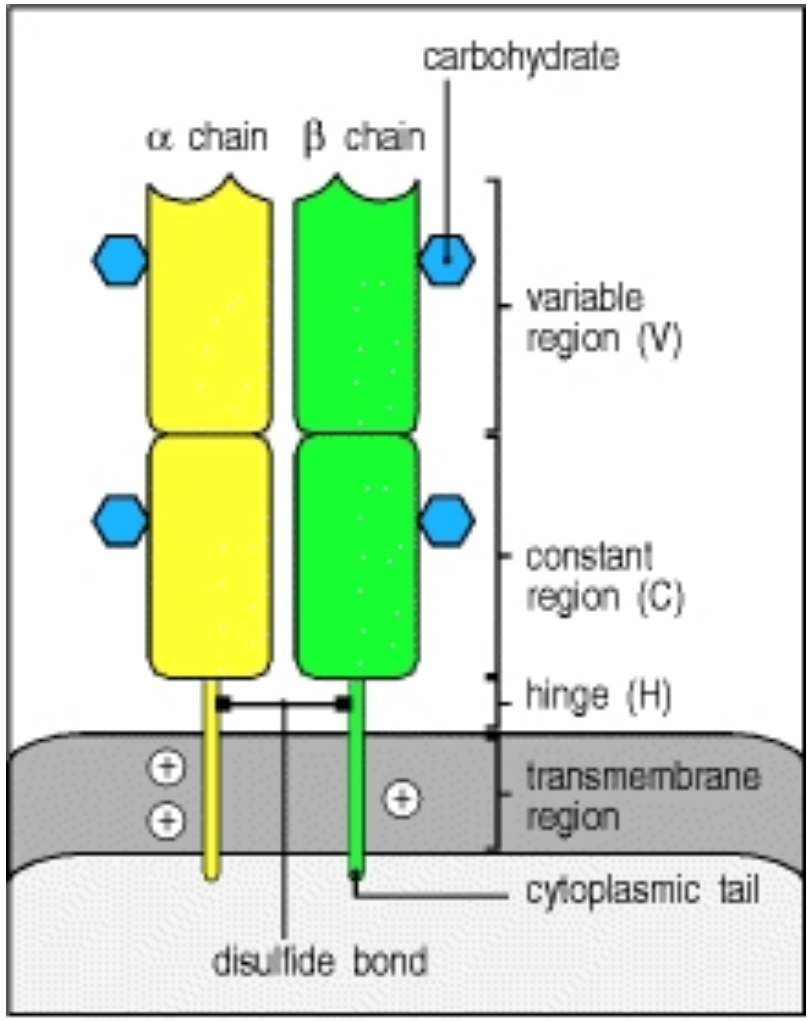
FIGURE 8-1 Stages of lymphocyte maturation. Development of both B and T lymphocytes involves the sequence of maturational stages shown. B cell maturation is illustrated, but the basic stages of T cell maturation are similar.

Checkpoints in lymphocyte maturation



- Functional receptor (TCR, BCR formation or antibody-formation and class switching) are created during maturation by somatic recombination
- VDJ recombination is the **process happen in early T,-B cell development** by which T cells and B cells randomly assemble different gene segments – known as variable (V), diversity (D) and joining (J) genes – in order to generate unique receptors (known as antigen receptors) that can collectively recognize many .different types of molecule
- The diversity is generated by random joining of different gene segments
- somatic recombination happens in BM for B cells-and in thymus for T cells

αß TCR

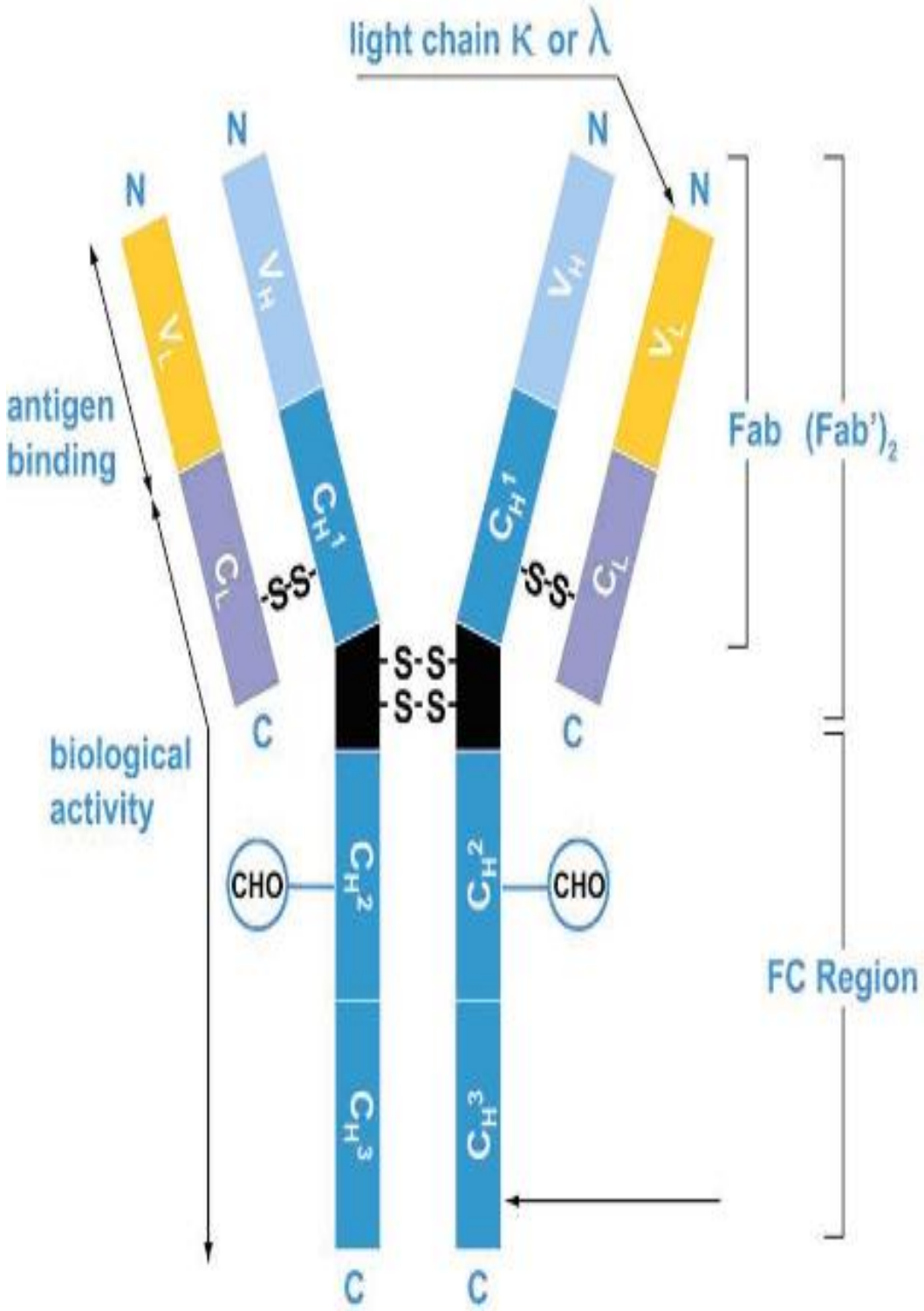


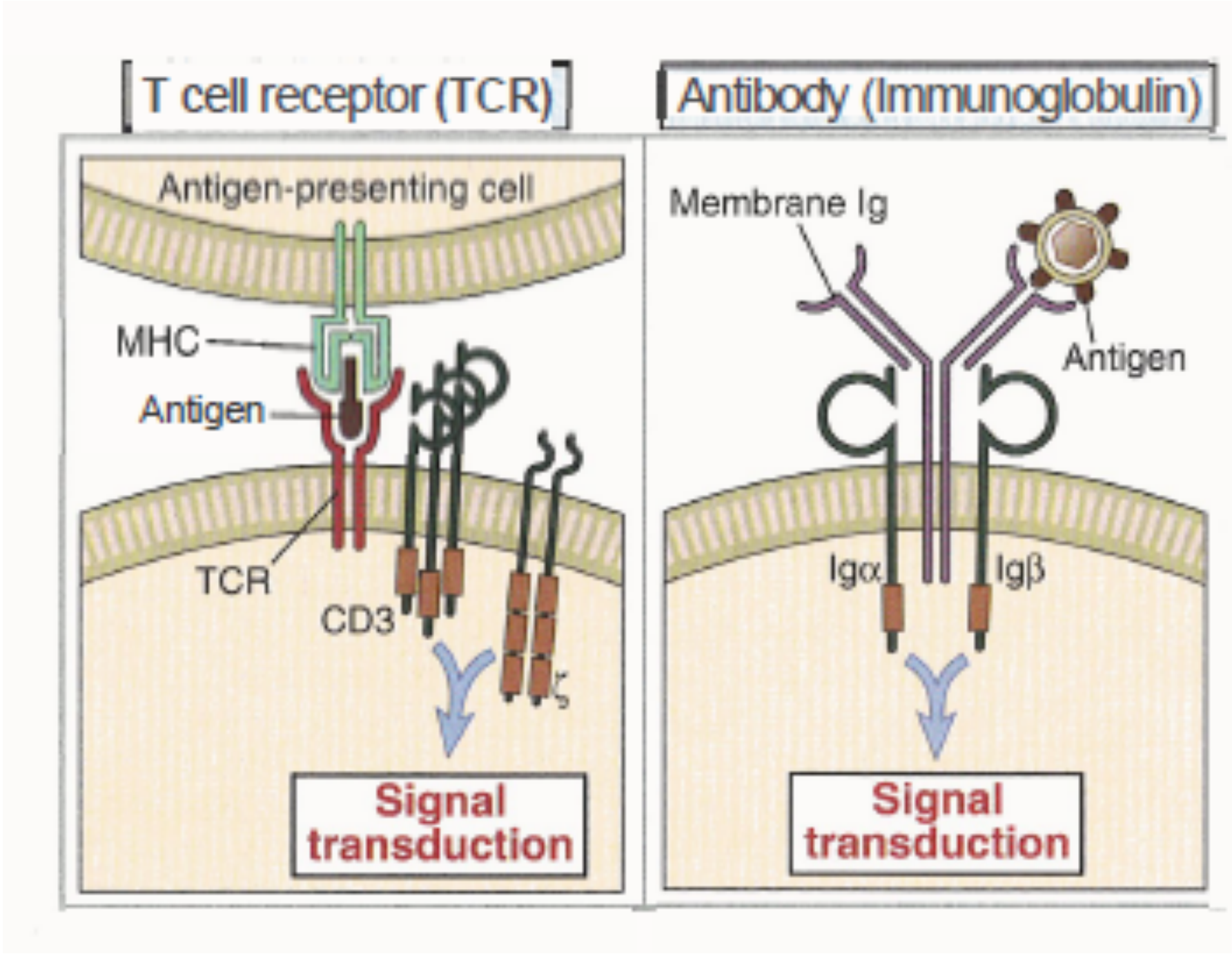
- TCR complex is the αß receptor plus the ζ chain and two CD3 signaling proteins
- Each chain constitute of one variable, one constant, hinge, transmembrane and cytoplasmic tail
- covalently linked to each other by a disulfide bridge between extracellular cysteine residues
- TCR that specifically recognizes peptide-MHC complexes
- Hypervariable regions on both Vα and Vß are the same as those of antibody located on Ag-binding site and called CDR and they are 3 sites for each

BCR

The B lymphocyte antigen receptor is a transmembrane antibody molecule (2 heavy and 2 light chains) associated with two signaling chains called Igα and Igβ

There is also hinge - region, transmembrane part




T cell receptor (TCR)
Antibody (Immunoglobulin)
Antigen-presenting cell
MHC
Antigen
TCR
CD3
ζ
Signal transduction
Membrane Ig
Antigen
Igα
Igβ
Signal transduction

Genetic rearrangement or somatic recombination

The T and B cell receptor gene segments are

V for variable, 65 segments

D for diversity, 6 segments

J for joining, 27 segments

C for constant, two types for BCR (kappa and lambda) light chain and C μ and Cδ for BCR heavy chain for receptor

Firstly the two BCR heavy chains and the TCR β, are – formed

- one of each DJ gene segments come together then one V gene (by RAG-1 and 2 enzyme) (antigen binding site)

- C μ for constant part of BCR and C β for TCR respectively, V(D)J-C gene segments result in pre- BCR and pre-TCR β

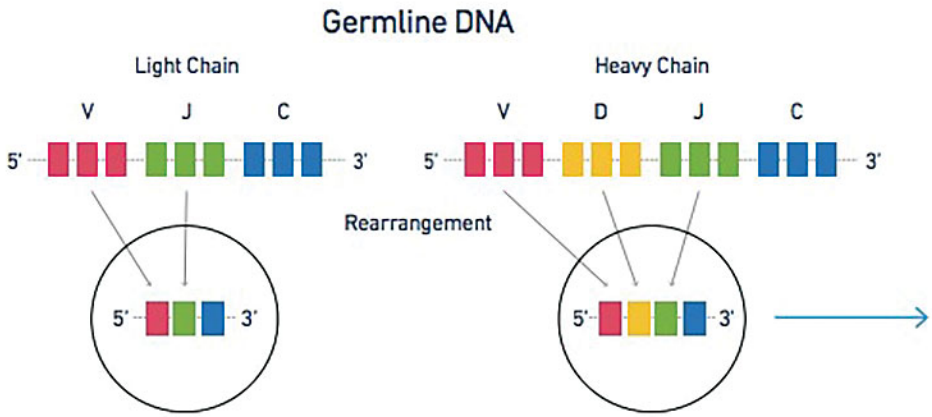
follow

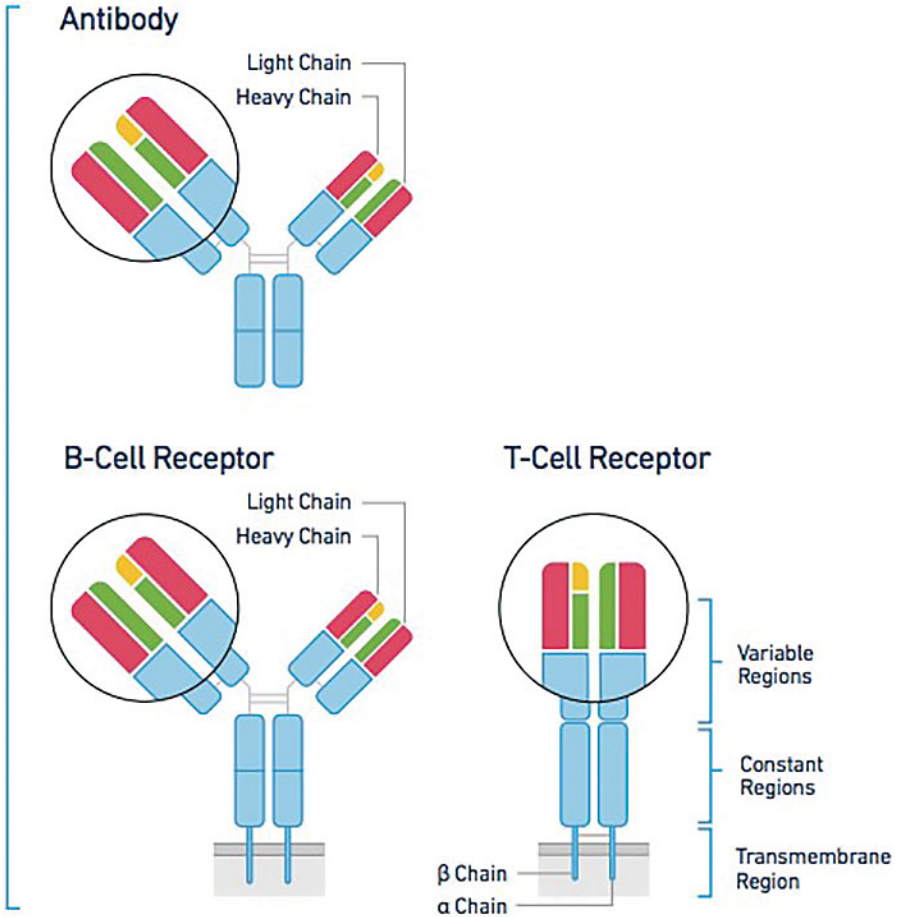
- Then the 2 BCR light chains and the TCR alpha chain are formed
  - VJ gene for BCR light chain and TCR α chain (antigen binding site) (by RAG-1 and 2 enzyme)
  - VJ-C kappa or lambda for BCR light chain and VJ-Cα for TCR α chain development, respectively
- Complete receptor formed on immature T & B cells

- Note, class switch in antibody happen later in 2nd lymph node and use the same process to get different antibodies (CϒC for (IGG, Cδ for IGD, Cε for IGE, Cμ for IGM and Cα for IGA

- Antigen binding site is on variable region on both BCR and TCR
- Variable region made of VDJ an heavy chain and VJ on light chain on BCR
- Variable region of TCR is made of VDJ on TCR beta chain and of VJ on TCR alpha chain

V(D)J Recombination

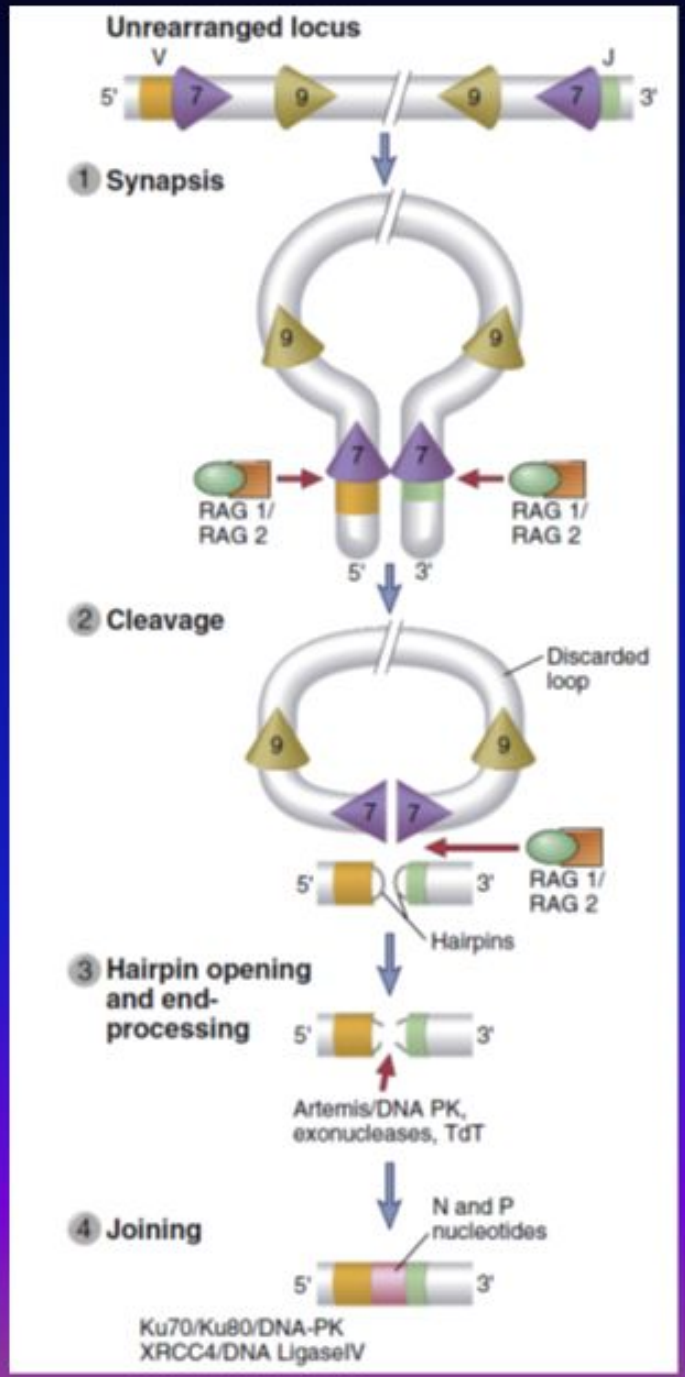


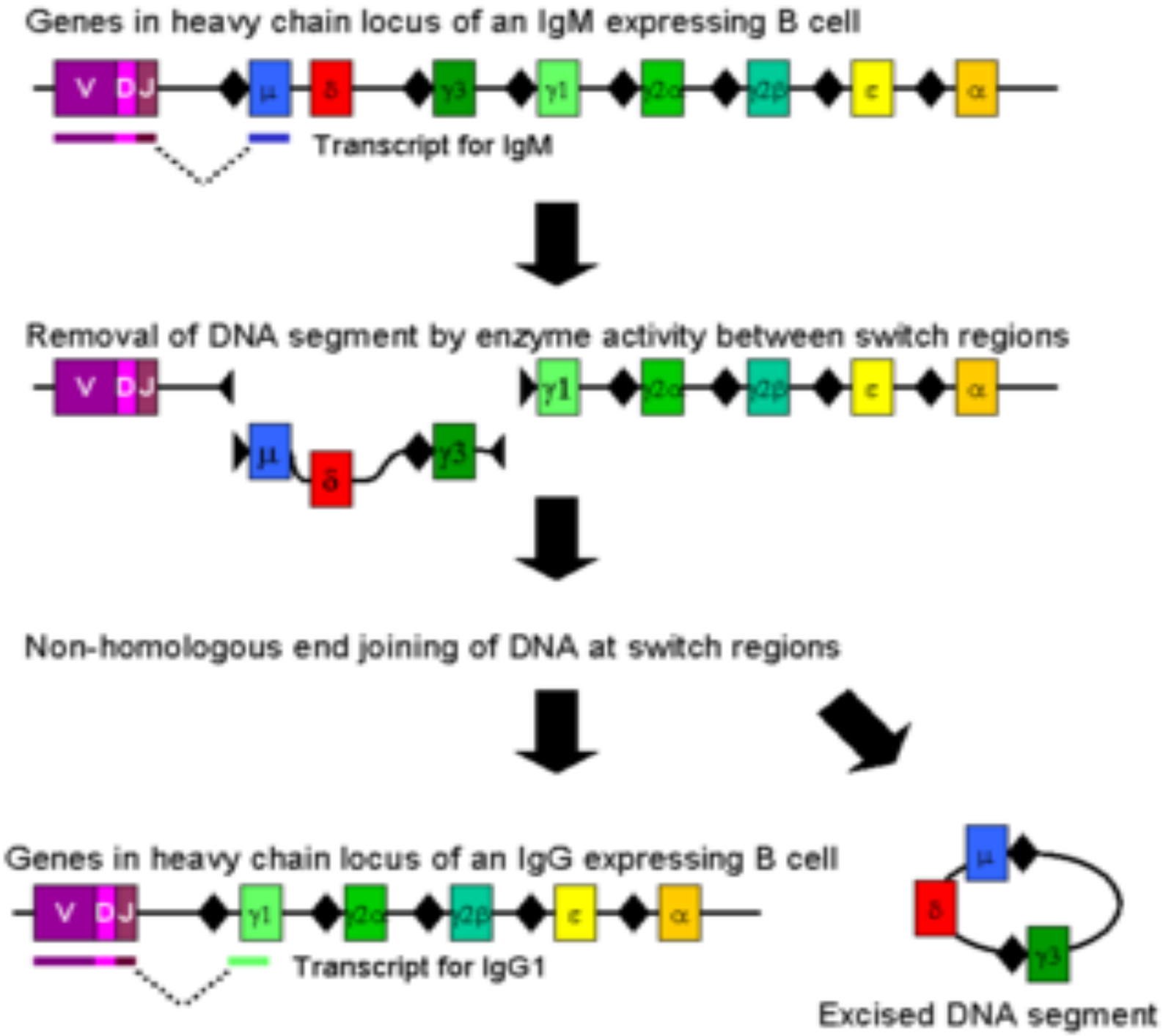


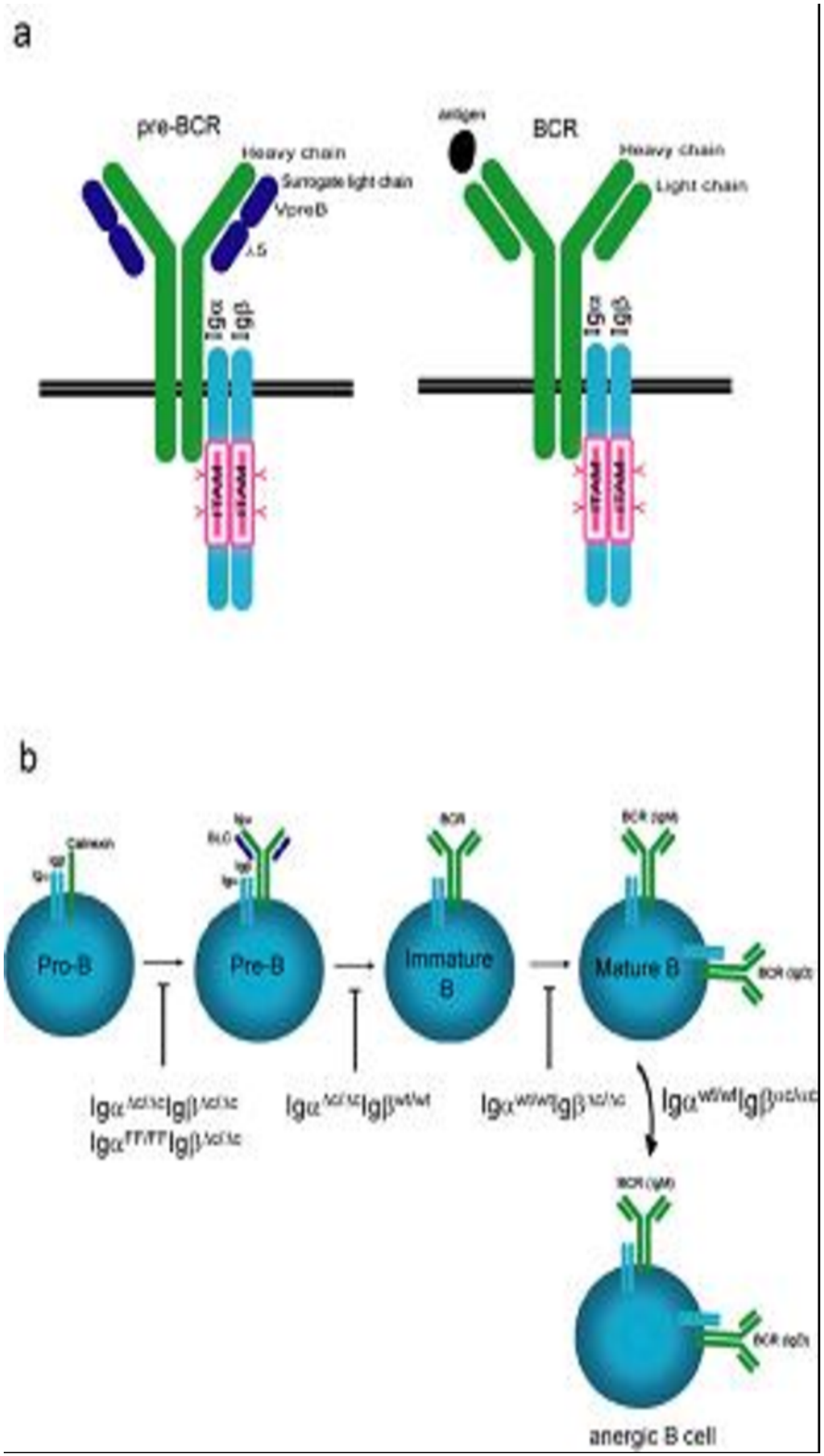
Genetic rearrangement or somatic recombination

- DNA Recombination include
  - Synapse, making chromosomal loop
  - Cleavage (RAG-1 and 2 called V-D-J recombinases)
  - Hairpin opening and end-processing(addition or removal of ,bases) mediated by Artemis endonuclease
  - Joining (Ligase) and addition of new nucleotides is mediated by the enzyme terminal deoxynucleotidyl .transferase (TdT)
- Constant gene segment c then attached
- Variability in binding sites Because of
  - .Combinatorial diversity
  - D segment, are more common in BCR and antibody **heavy chains and in TCR β** chains. This

Sequential events during V(D)J recombination




Genes in heavy chain locus of an IgM expressing B cell
V D J
μ
δ
γ3
γ1
γ2α
γ2β
ε
α
Transcript for IgM
Removal of DNA segment by enzyme activity between switch regions
V D J
γ1
γ2α
γ2β
ε
α
μ
δ
γ3
Non-homologous end joining of DNA at switch regions
Genes in heavy chain locus of an IgG expressing B cell
V D J
γ1
γ2α
γ2β
ε
α
Transcript for IgG1
μ
δ
γ3
Excised DNA segment


a
pre-BCR
Heavy chain
Surrogate light chain
VpreB
λ5
Igα
Igβ
ITAM
ITAM
Antigen
BCR
Heavy chain
Light chain
Igα
Igβ
ITAM
ITAM
b
Pro-B
Pre-B
Immature B
Mature B
BCR (IgD)
Igα+/+Igβ+/+
Igα+/+Igβ+/+
Igα+/+Igβwt/wt
Igαwt/wtIgβ+/+
Igαwt/wtIgβ+/+
BCR (IgM)
BCR (IgD)
anergic B cell

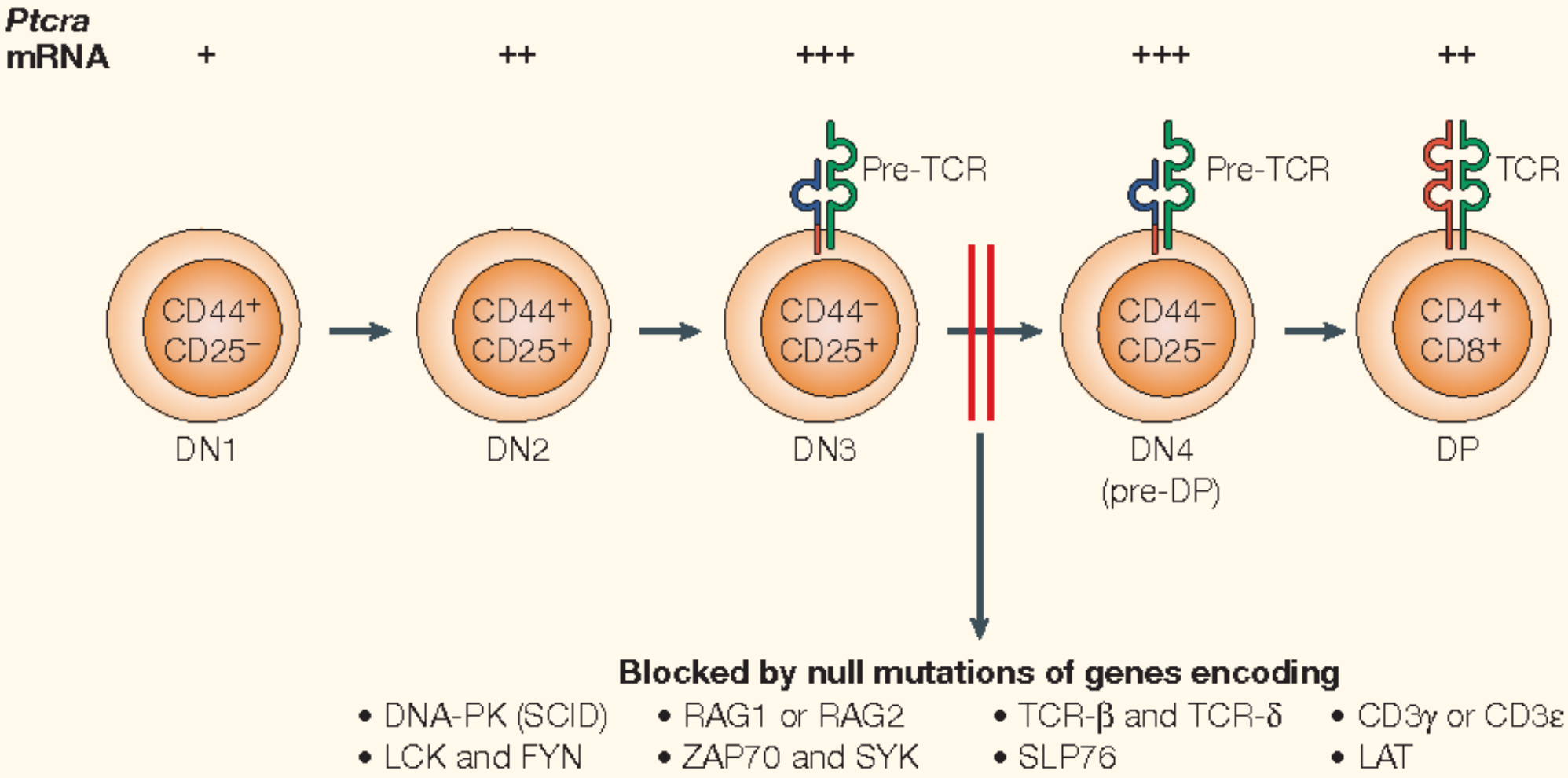


Figure 3 | **Early stages of development of CD4⁻CD8⁻ (double negative) and CD4⁺CD8⁺ (double**

T cell development

- T cell precursors (**prothymocytes**) are attracted to the thymus from the BM by a chemotactic .factor secreted by thymic epithelial cells
- The pro thymocytes are TCR - CD3+CD4-CD8- or "**double-negative" cells (in subcapsular .area)**
- Some Double-negative cells productively rearrange gamma and delta chain gene segments develop into gamma/delta T cels (γδ T cells 10%) The majority of double-negative cells will go on to rearrange alpha and beta chain .gene segments 90%

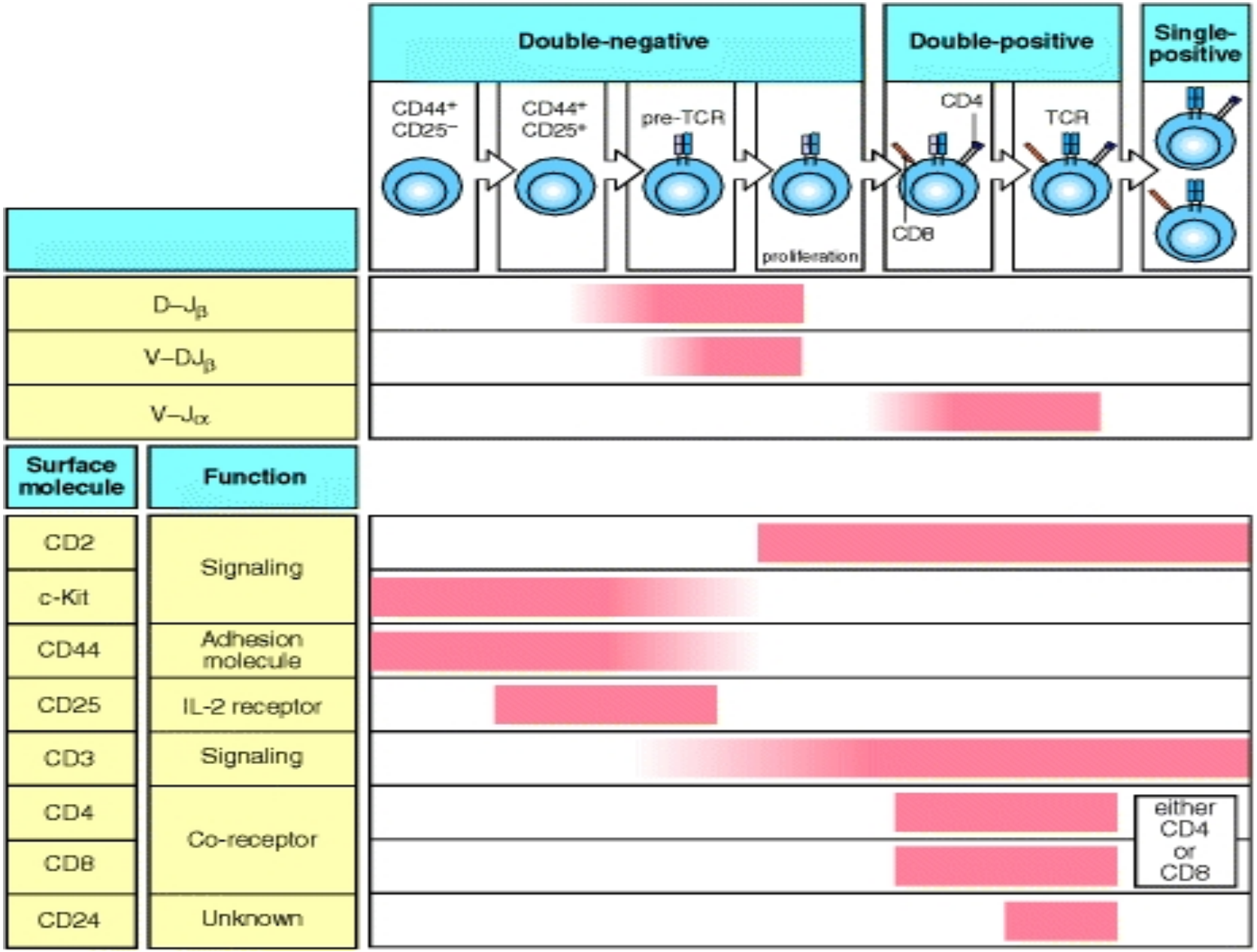
follow

- **in cortex;** The TCR β chain protein is expressed on the cell surface first (by DNA recombination of VDJ beta segments with beta constant segments) in association with an invariant protein called pre-Tα and with CD3 and ζ proteins to form the **pre-T cell receptor** (pre- TCR) complex
- **then** alpha chain gene rearrangement is enhanced (VJ alpha with constant alpha) forming complete T .cell receptor with CD3 (**Immature T cells**)
- At the same time both CD4 and CD8 are expressed and the cells called **double positive immature T cells**

Selection of immature T cells

- **Positive selection** of double positive cells (CD4+CD8+) is the process that preserves T cells that recognize self MHC (with self .peptides) with no binding
- **Negative selection** of double positive is the process in which thymocytes whose TCRs bind strongly to self peptide antigens in association with self MHC molecules are deleted or convereted to Treg
- Further check point for deletion self reactive T cells occurs In medulla, the thymic epithelial cells express a nuclear protein called AIRE (autoimmune regulator) that induces the expression of a number of tissue-specific genes in the thymus. These genes are normally expressed only in specific peripheral organs. Their AIRE-dependent expression in the thymus makes many tissue-specific peptides available for presentation to developing T cells, facilitating the deletion (negative selection) of these cells

- **Transforming into single positive (either CD4 or CD8) in medulla** because one co-receptor is shut-off randomly, or as a result of Positive Selection of Thymocytes: Development of the Self MHC–Restricted T Cell Repertoire). Those that bind MHC1 transformed into CD8, and those bind MHC2 transformed into CD4


Double-negative
Double-positive
Single-positive
CD44+ CD25−
CD44+ CD25+
pre-TCR
proliferation
CD4
CD8
TCR
D–Jβ
V–DJβ
Vα–Jα
Surface molecule
Function
CD2
c-Kit
CD44
CD25
CD3
CD4
CD8
CD24
Signaling
Signaling
Adhesion molecule
IL-2 receptor
Co-receptor
Unknown
either CD4 or CD8

γδ T cells

- CD4-, CD8-, CD3+ T cells, 5% in peripheral blood T cells
- Frequent in mucosal epithelium
- Can help in antibody class switch as alpha beta T cells
- Have a regulatory function, it sense tissue stress rather than antigen,and downregulate damaging immune response
- Help in innate immune because
  - sense Ag directly without processing or MHC restriction.--they help in viral infection
  - also help in early life when alpha beta T cells and antigen processing is immature
  - sense peptide and non-peptide Ag (mycobacterium)

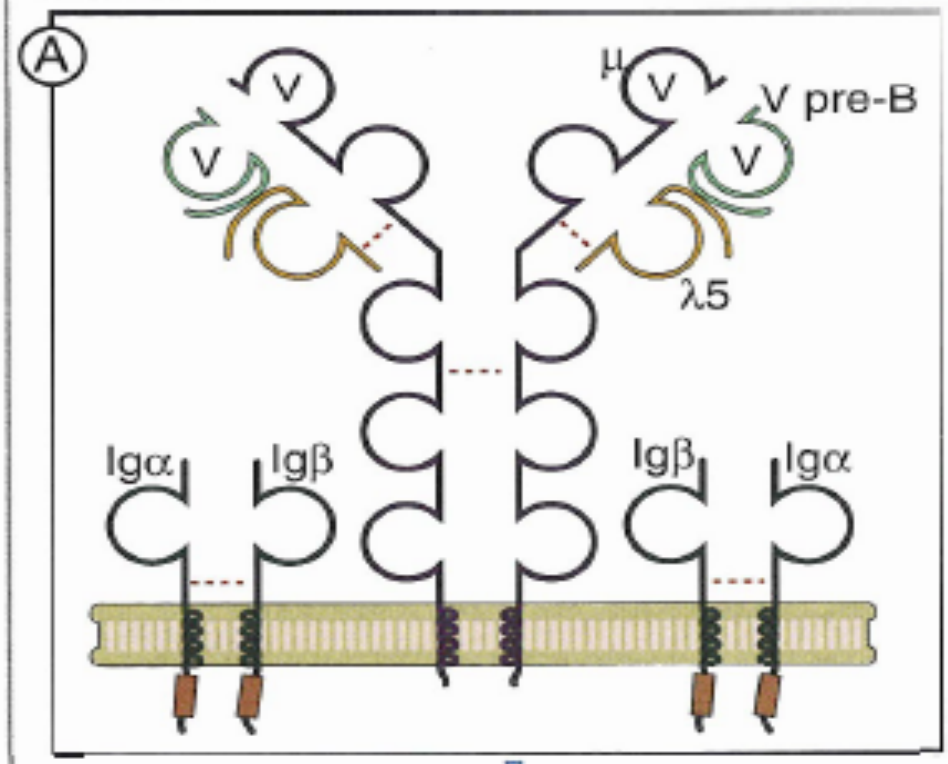
B cells

- Pro-B cells; the earliest stage in BM
- receptor expression is the first key to lymphocyte ;survival
- Early: 2 heavy chains formed (IgH (the 2 IGM heavy chains with surrogate light chains and ;immunoglobulin alpha and beta) = **pre-B cells**
- Later: completed Ag receptors formed by formation of light chains kappa type, if fail use lambda light chain

  Light chain contain just V-J segments

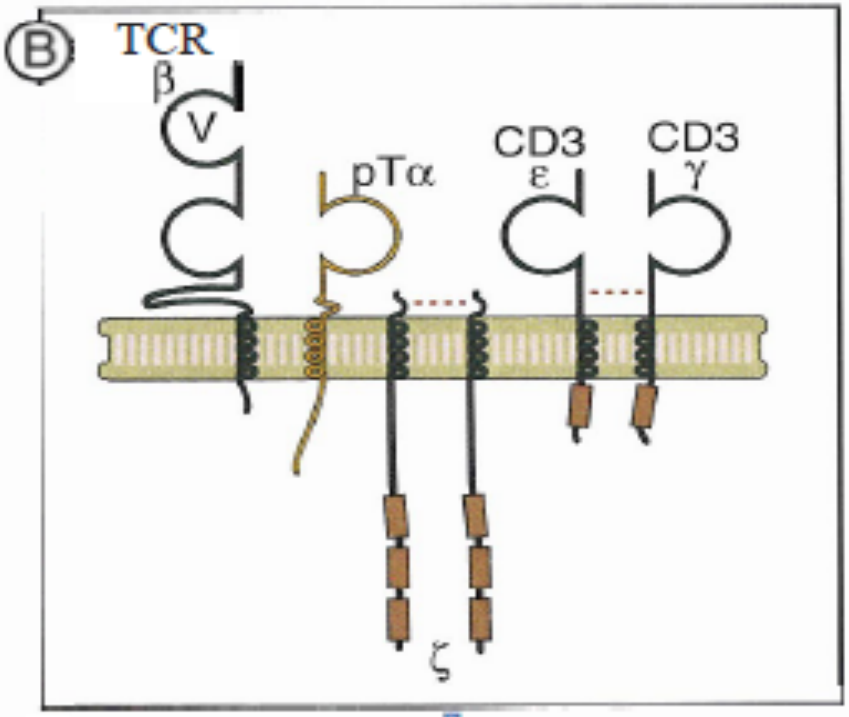
  immature B cells = complete IGM BCR

Pre–B cell receptor



- inhibition of H chain recombination (allelic exclusion)
- Proliferation of pre–B cells
- Stimulation of κ light chain recombination

Pre–T cell receptor



- Inhibition of β chain gene recombination
- Proliferation of pre–T cells
- Stimulation of a chain recombination
- Expression of CD4 and CD8

Selection of immature B cells

- **Selection follows initial survival after immature lymphocytes express antigen receptors (Best understood for T cells, but also occurs for B cells)**

- **Positive Selection (life, expansion, continued maturation) occurs if the Ig receptor binds self MHC. Cells that not binding die**

Negative selection

- **Negative selection in B cells not always occur as just receptor editing happen if the B cell receptor bind strongly .to self antigen**
- **Receptor editing is Changing the variable part on light chain; replacing VJ of light chain with new VJ Kappa or lambda . If editing in B cells fail; clonal deletion**
- **.only 5% of formed T cells and 10% of B cells selected**

Most B cells migrate to peripheral LN where maturation happens (mature B cell) by expressing IGD beside IGM

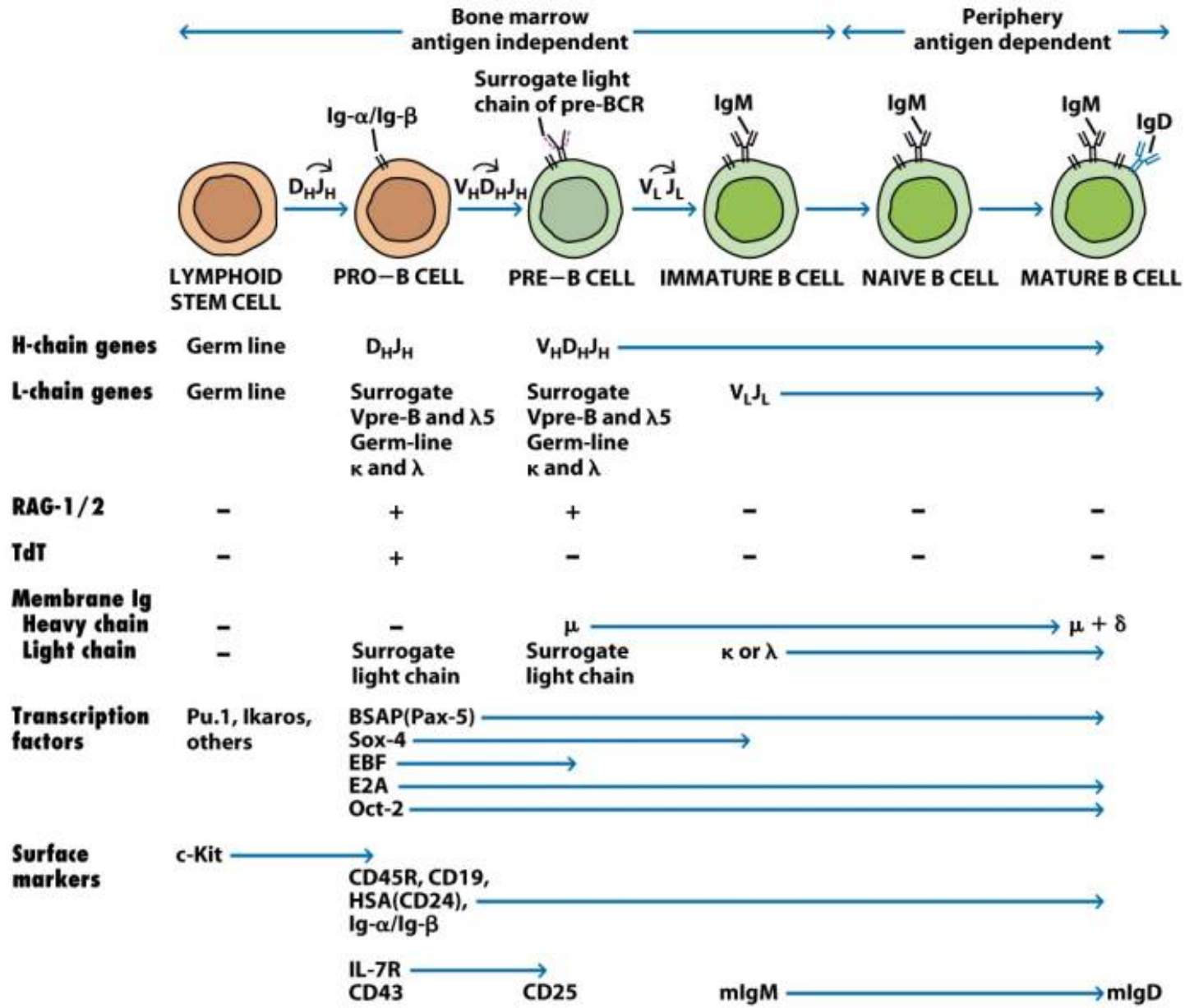


Figure 11-3
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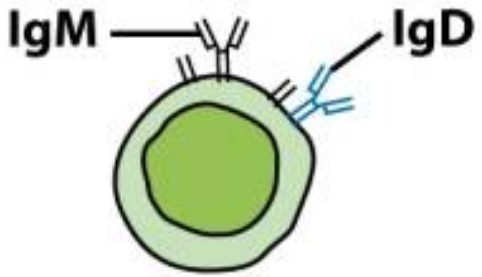
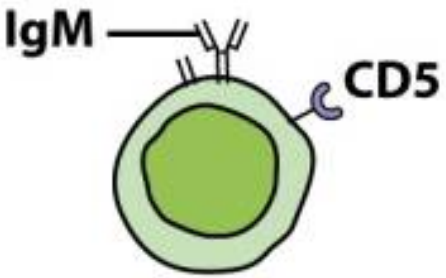
B1 cells (CD5+ B cells)

- B-1 cells 5-10% of blood B cells naturally found from fetal life, produce IGM, natural antibody present without immunization. has limited diversity give rapid antibody production against microbe. Act against carbohydrates, do not do isotype switch or do affinity maturation, no need to T cell help, and self renewing, present in the peritoneum .and in mucosal sites
- Marginal zone B cells are a distinct population of B cells that mainly respond to polysaccharides. After activation, these cells differentiate into short-lived plasma cells that .produce mainly IgM





Attribute	Conventional B cells (B-2 B cells)	B-1 B cells
Major sites	Secondary lymphoid organs	Peritoneal and pleural cavities
Source of new B cells	From precursors in bone marrow	Self-renewing (division of existing B-1 cells)
V-region diversity	Highly diverse	Restricted diversity
Somatic hypermutation	Yes	No
Requirements for T-cell help	Yes	No
Isotypes produced	High levels of IgG	High levels of IgM
Response to carbohydrate antigens	Possibly	Definitely
Response to protein antigens	Definitely	Possibly
Memory	Yes	Very little or none
Surface IgD on mature B cells	Present on naive B cells	Little or none

Figure 11-5
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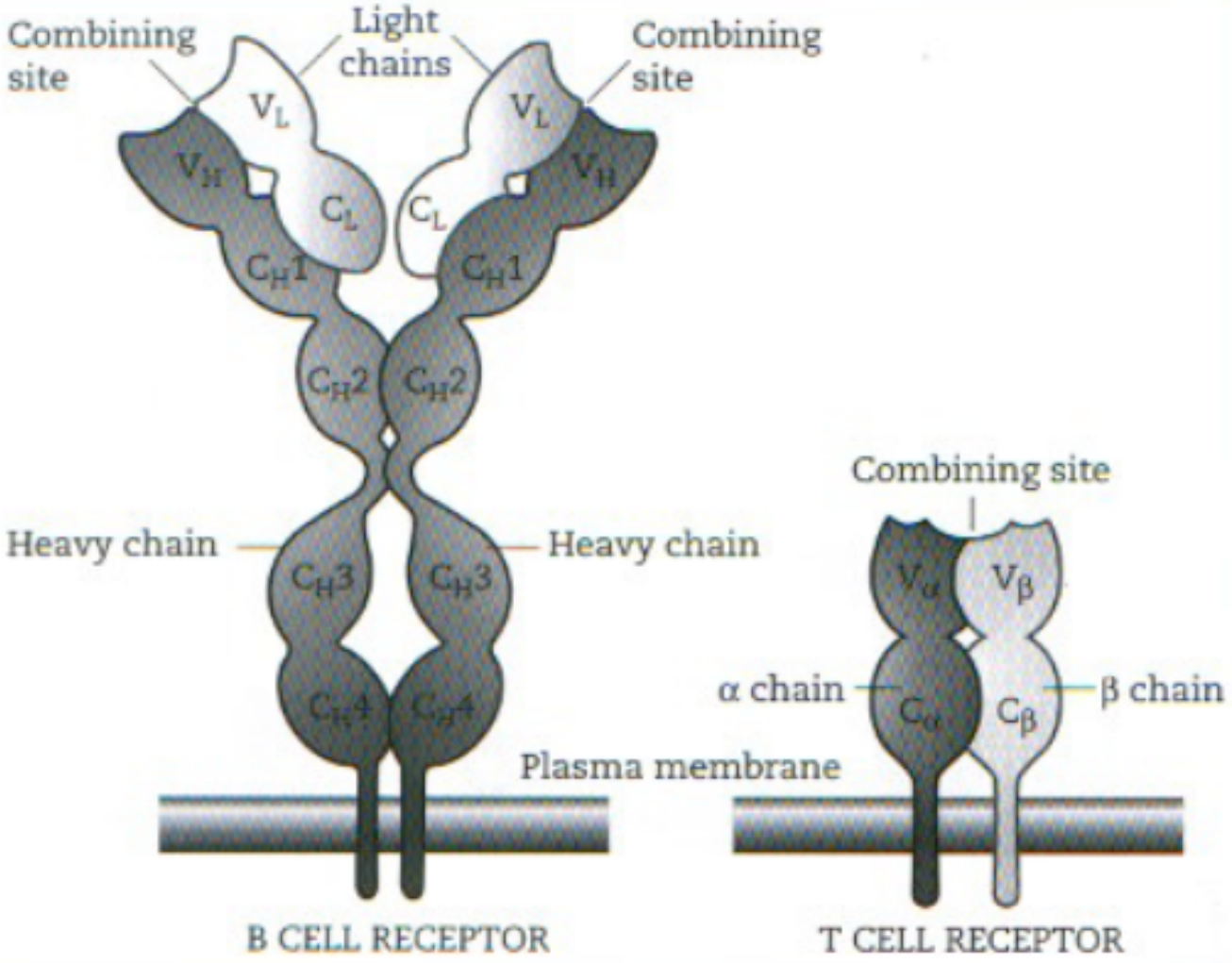
B & T CELL RECEPTORS



Allelic exclusion

- After a B cell produces a functional immunoglobulin gene during V(D)J recombination, it cannot express any other variable region (a process known as allelic exclusion) thus each B cell can produce antibodies containing only one kind of variable chain
- and it ensures that every B cell will express a single receptor, thus maintaining clonal specificity