Lymphocyte Development and Antigen Receptor Gene Rearrangement

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Steps of lymphocytes development

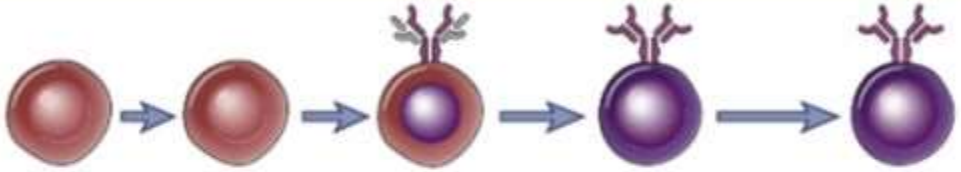
- First; Pluripotent stem cells in bone marrow known as hematopoietic stem cells (HSCs), give rise to a common lymphoid progenitor (CLP) then give rise to pro-B cells, pro-T cells and NK cells.
- Pro-T cells migrate to Thymus and may commit to either the αβ or γδ T cell lineages.
- Second; pro- B and pro-T cell proliferate in response to cytokines IL-7
- Third; Pre-B and pre- T cells formed by making half of the receptor

- Fourth;1- Selection of pre-cells that start forming receptor
- Fifth; Formation of the whole receptor on cell and become **immature cell**, 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 T cells into functionally and phenotypically distinct subpopulations. T cells develop into CD4+ and CD8+ αβ T lymphocytes in thymus.
- migration of mature cells to peripheral lymph nodes and they are activated by macrophages and DCs,.

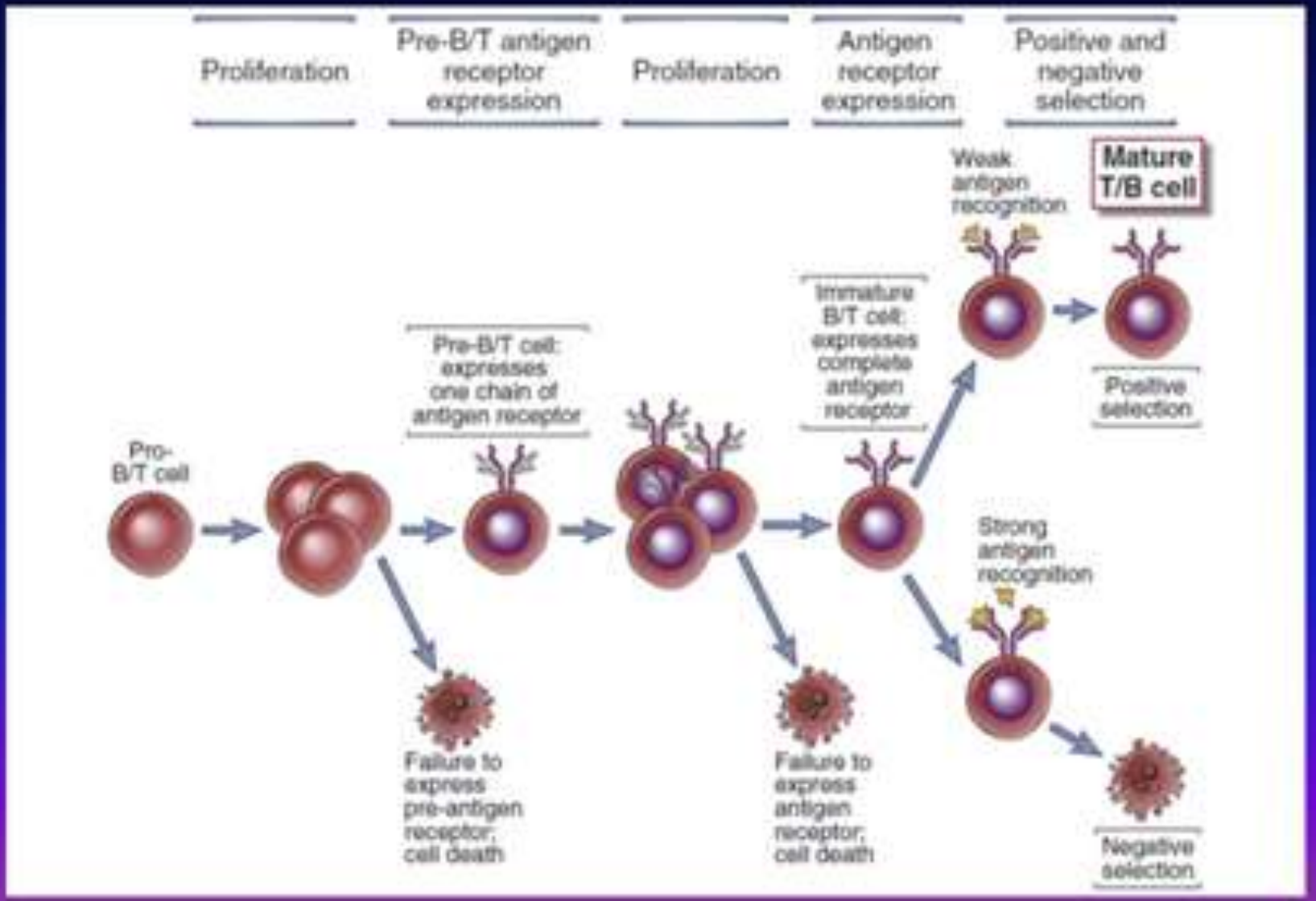
Stages of lymphocyte maturation



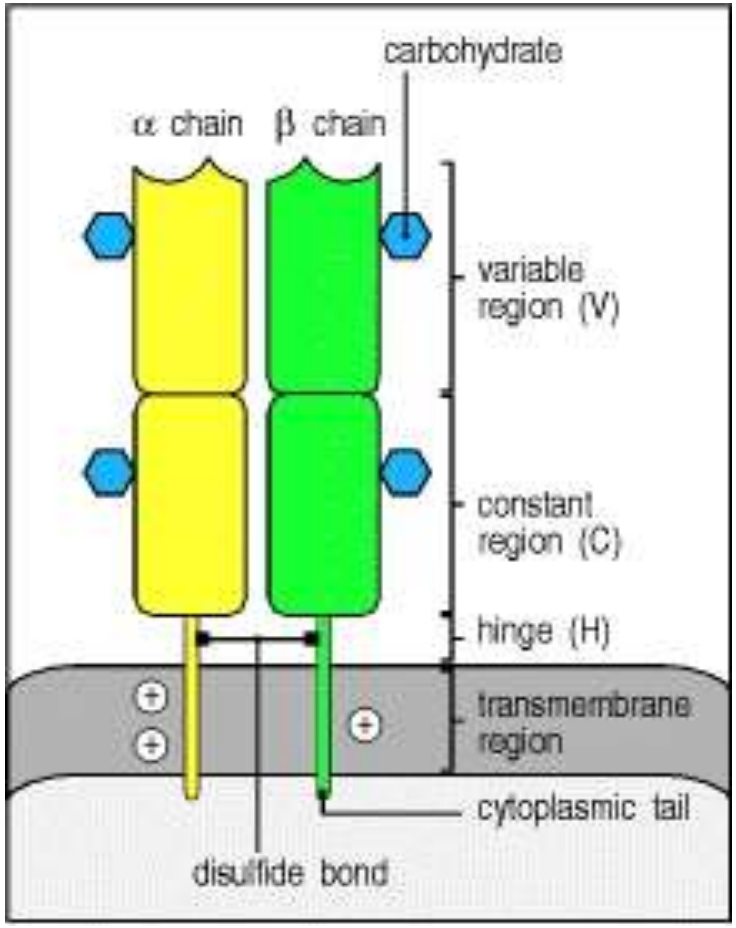
Stage of Maturation	Stem cell	Pro-lymphocyte	Pre-lymphocyte	Immature lymphocyte	Mature lymphocyte
Major Events	Growth factor mediated commitment, proliferation; initiation of antigen receptor gene rearrangement		Selection of cells that express pre-antigen receptors	Selection of repertoire and acquisition of functional competence	
Anatomic Site			Generative organ (bone marrow or thymus)		Peripheral lymphoid organ or tissue
Antigen Dependence			No		Self antigen

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



αß TCR

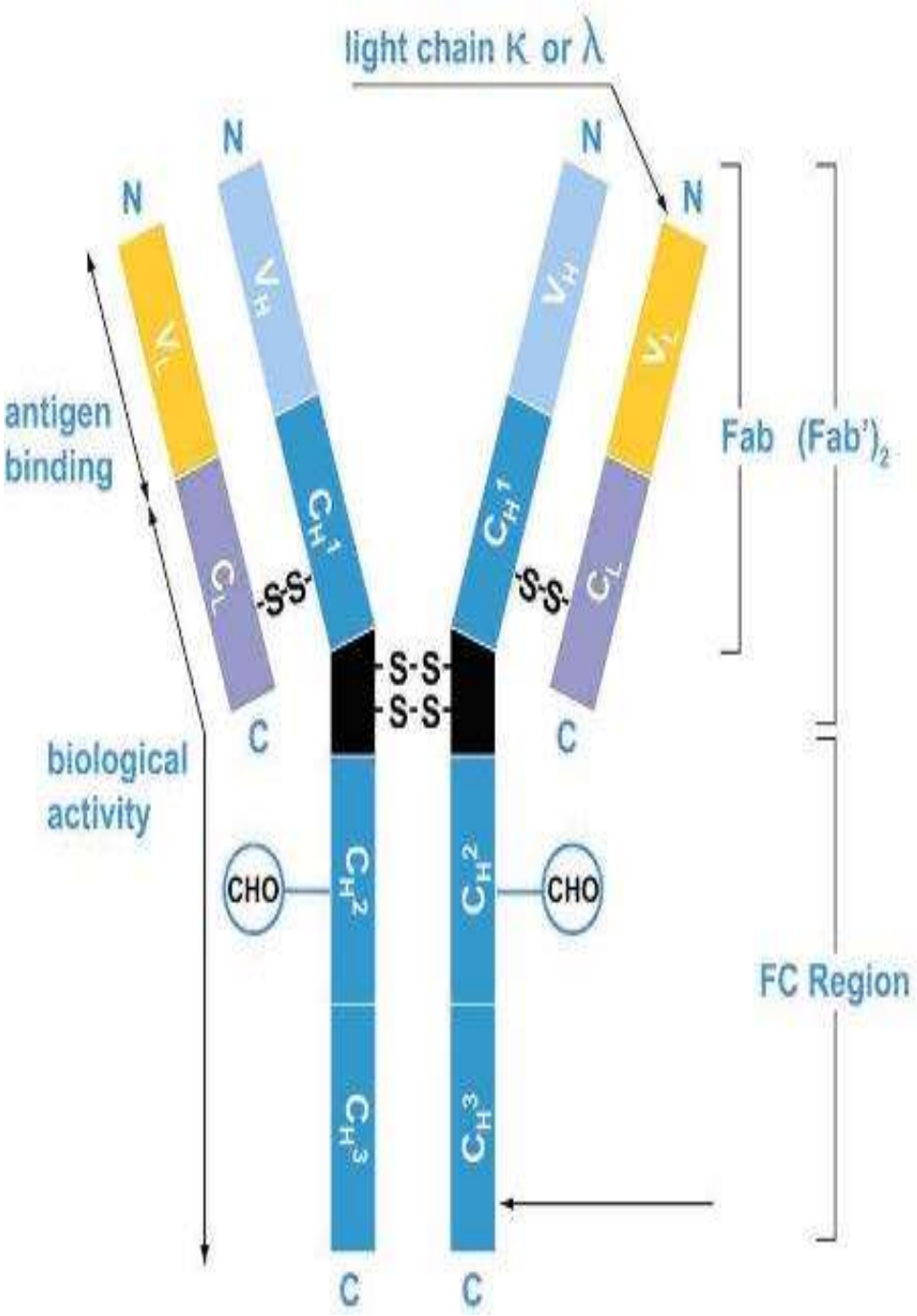


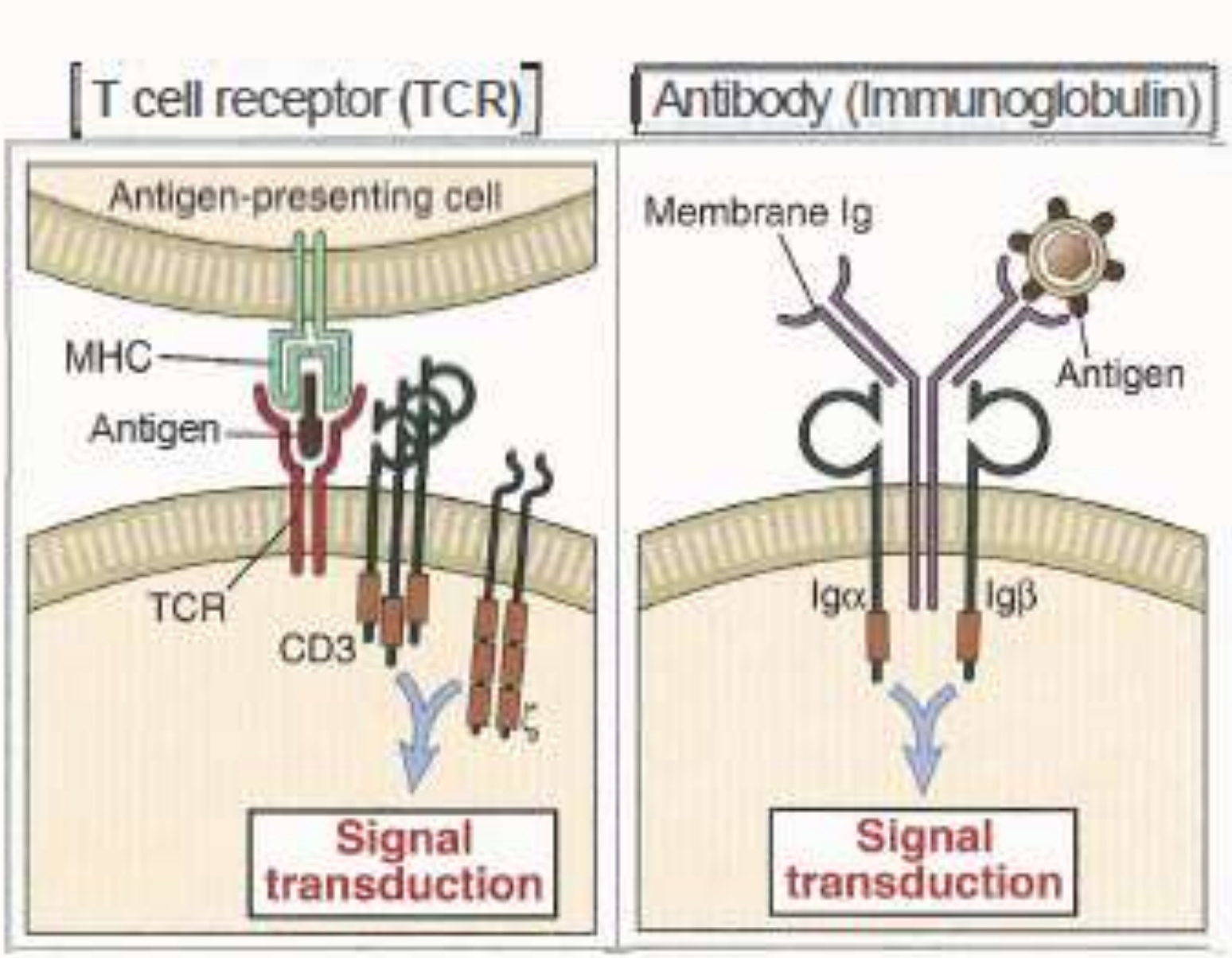
- TCR complex is the αß receptor plus the ζ chain and two CD3 signaling proteins
- Each chain constitute of one variable, one constant, hinge, transmembrane and cytoplasmic tail
- covalently linked to each other by a disulfide bridge between extracellular cysteine residues
- TCR that specifically recognizes 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

-Functional receptor (TCR, BCR formation or antibody - formation) are created during maturation by **somatic recombination or DNA rearrangement of 4 genetic segments**

- V for variable
- D for diversity
- J for joining
- C for constant C kappa or lambda for BCR and C β or C α for TCR β and alpha chains development -VDJ recombination is the **process happen in early T, B cell development** by which T cells and B cells randomly assemble different gene segments – VDJC– in order to generate unique receptors (known as antigen receptors) that can collectively recognize many different types of molecule.

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%.

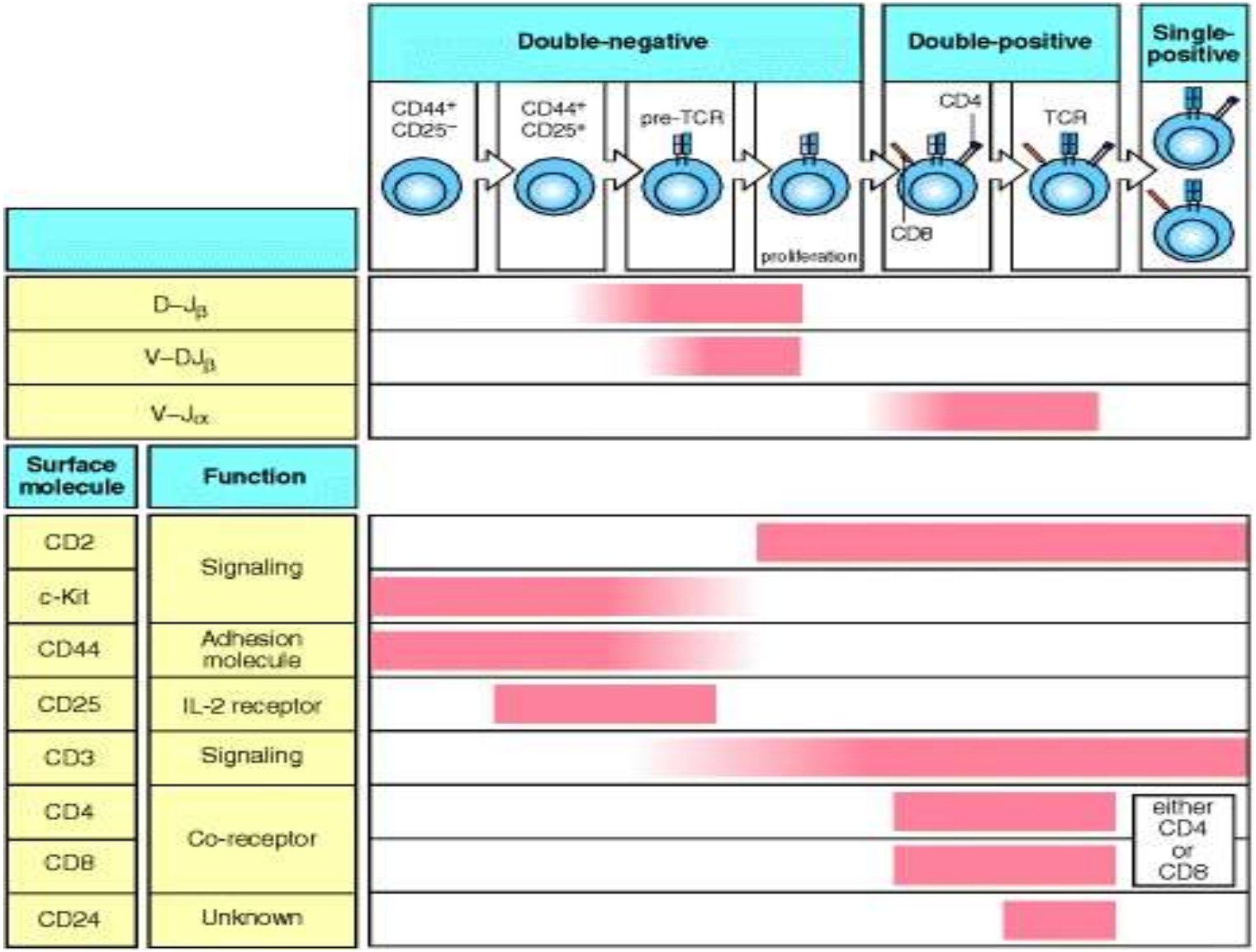
follow

- **in cortex;** The TCR β chain protein is expressed on the cell surface first (by DNA recombination of VDJ segments with beta constant segments) in association with an invariant protein called pre-Tα to form the **pre-T cell receptor** (pre- TCR) complex
- CD3 and ζ proteins are present on pre-T ell as coreceptors
- **then** alpha chain gene rearrangement is enhanced (VJ 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).
- **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 mature cells (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
V–Jα
Surface molecule
Function
CD2
c-Kit
Signaling
CD44
Adhesion molecule
CD25
IL-2 receptor
CD3
Signaling
CD4
CD8
Co-receptor
either CD4 or CD8
CD24
Unknown

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

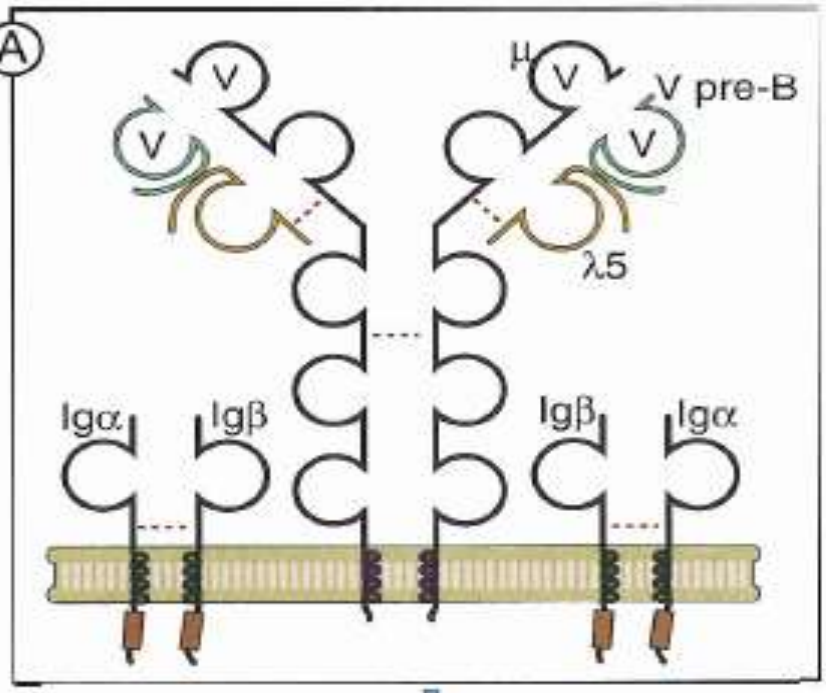
sense peptide and non-peptide Ag (mycobacterium)

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 (by DNA recombination of VDJ segments with lambda or cappa constant segments) with surrogate light chains = **pre-B cells**;
- immunoglobulin alpha and beta) are present in pre-B cells as coreceptors
- Later: completed Ag receptors formed by formation of light chains kappa type, if fail use lambda light chain (VJ with constant lambda or cappa)

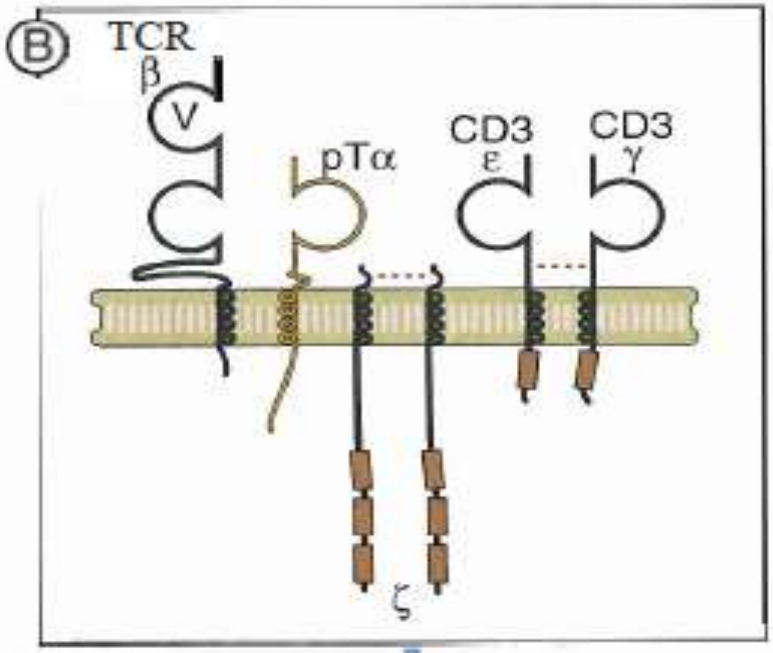
immature B cells = complete IGM BCR

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

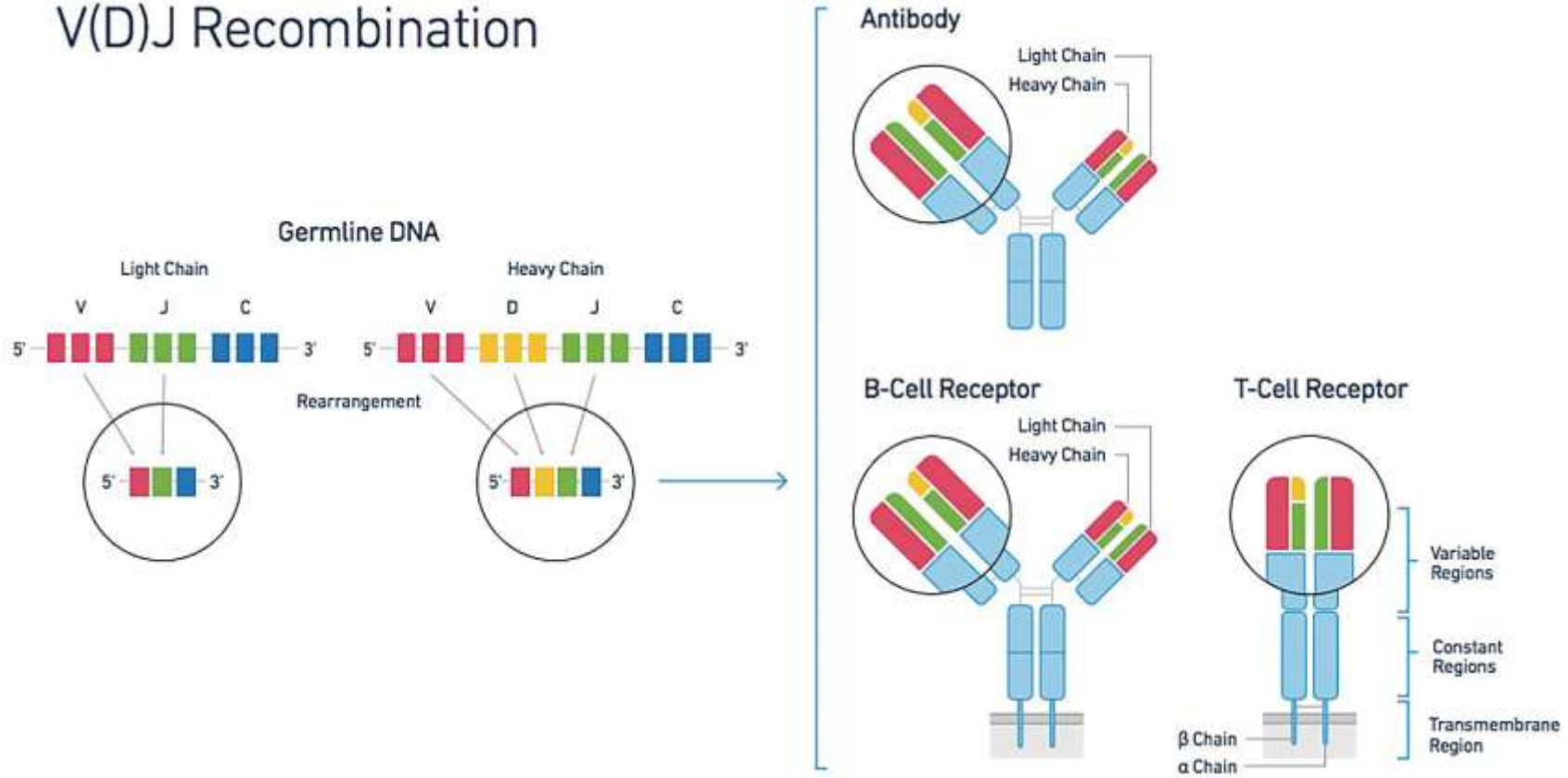
- Positive Selection (life, expansion, continued maturation) occurs if the Ig receptor binds self MHC. Cells that not binding die
- Negative selection in B cells not always occur as just receptor editing happen if the B cell receptor bind strongly to self antigen.
- Receptor editing is Changing the variable 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

Note, first class of AB produced is IGM class by binding C μ in bone marrow result in IGM receptor on B cells, then class switch in antibody happen late in 2nd lymph node and use the same process to get different antibodies (CϒIGG, Cδ for IGD, Cε for IGE, Cμ for IGM and Cα for IGA)

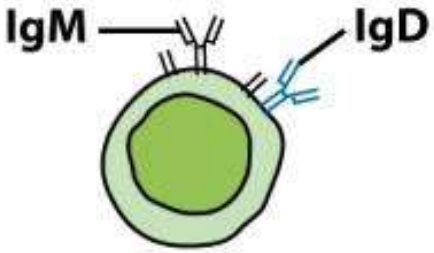
- Antigen binding site is on variable region on both BCR and TCR
- Variable region made of VDJ on heavy chain and VJ on light chain on BCR
- Variable region of TCR is made of VDJ on TCR beta chain and of VJ on TCR alpha chain

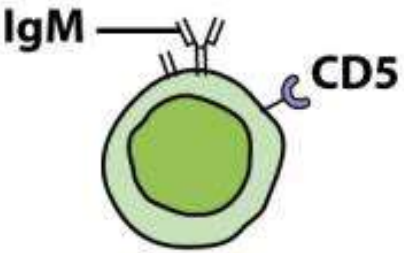
V(D)J Recombination



B1 cells (CD5+ B cells)

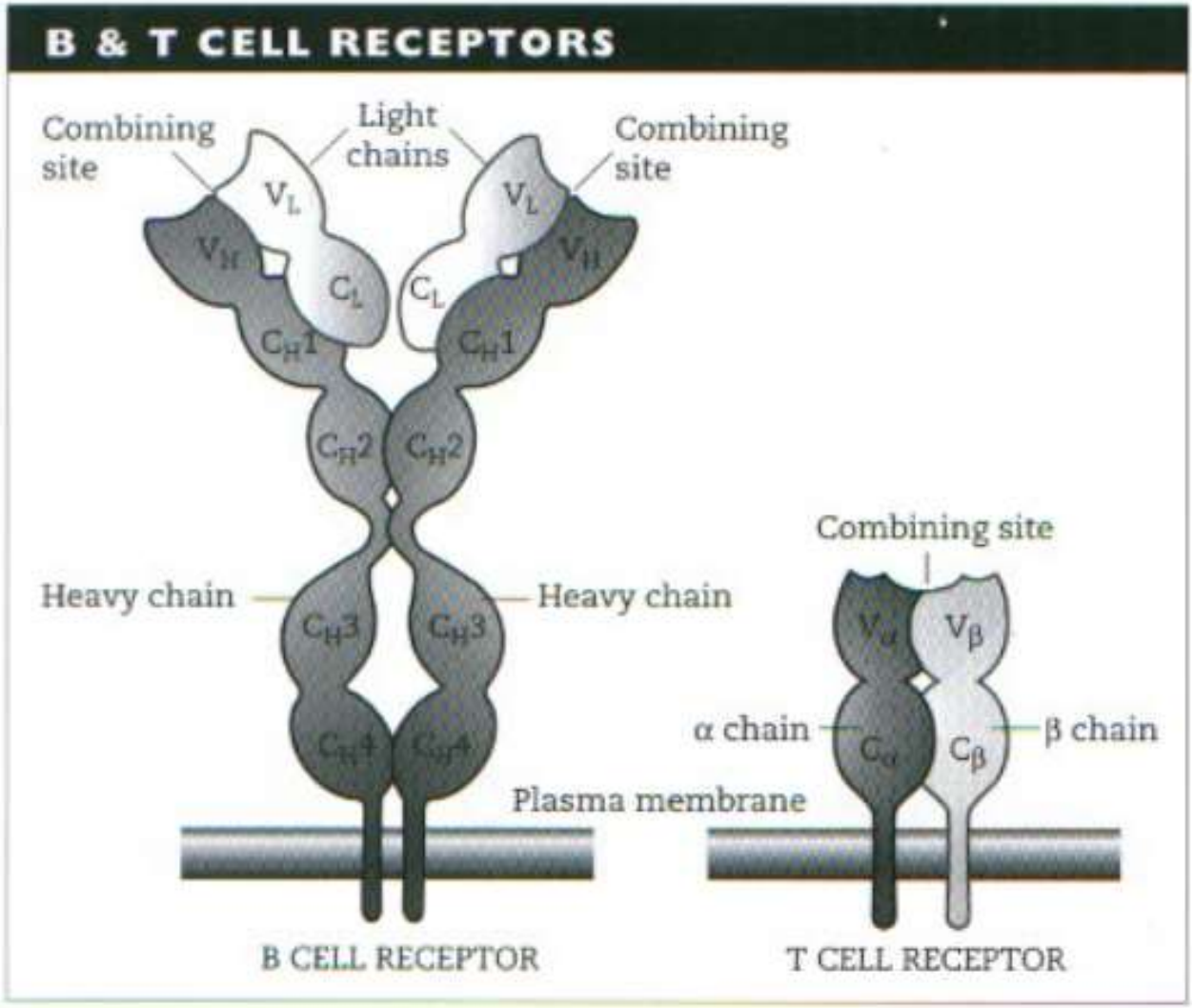
- B-1 cells 5-10% of blood B cells naturally found from fetal life, produce IGM, natural antibody present without immunization. has limited diversity give rapid antibody production against microbe. Act against carbohydrates, do not do isotype switch or do affinity maturation, no need to T cell help, 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
Kuby IMMUNOLOGY, Sixth Edition


B & T CELL RECEPTORS
Combining site
Light chains
Combining site
V_L
V_H
C_L
C_H1
C_H2
C_H3
C_H4
Heavy chain
Heavy chain
Plasma membrane
B CELL RECEPTOR
Combining site
V_α
V_β
α chain
β chain
C_α
C_β
T CELL RECEPTOR

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

Genetic rearrangement or somatic recombination

- DNA Recombination include enzymes as
  - 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).