Lymphocyte Development, migration and Antigen Receptor Gene Rearrangement

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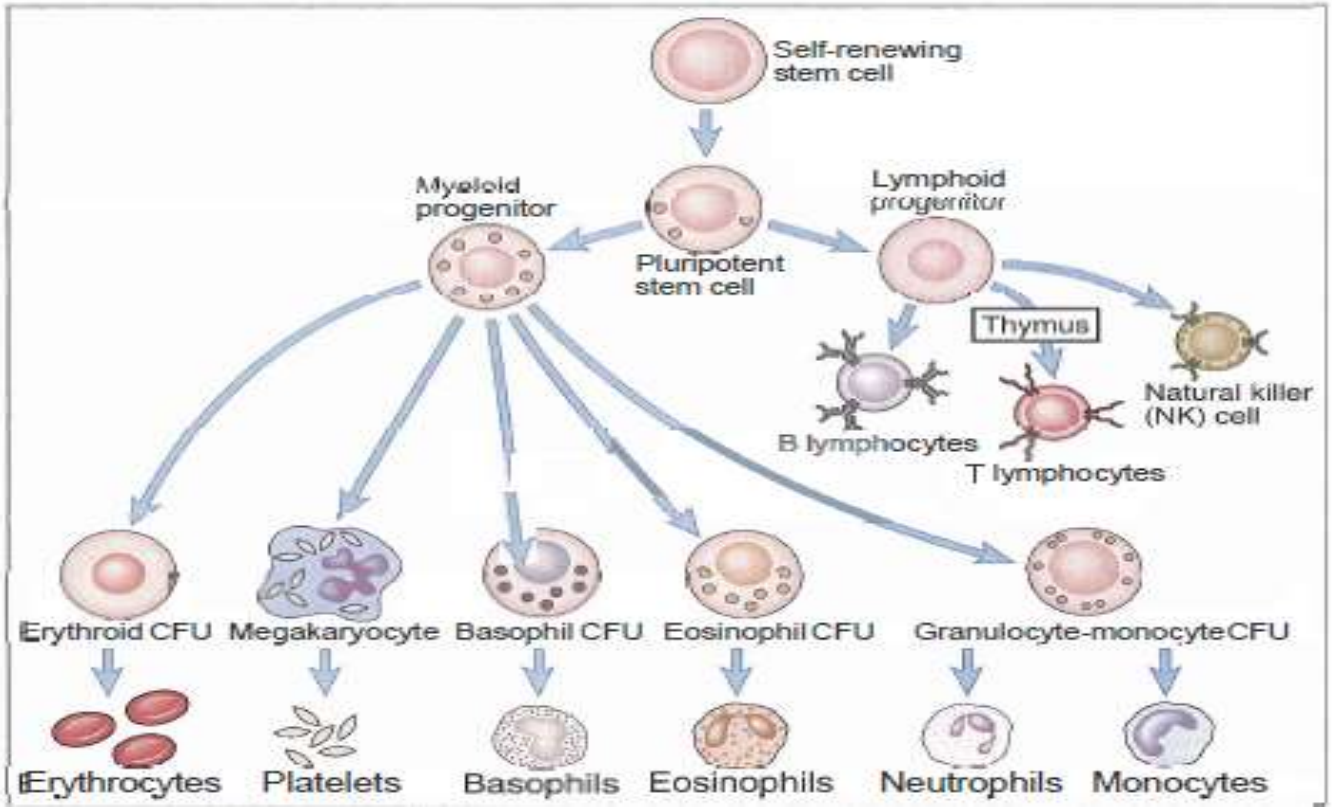
Receptors of acquired immune- cells

- Expressed on B and T (BCR and TCR) cell surfaces
  - Determined the antigen specificity of an individual lymphocyte
  - A pool of different cells of variable specificities produced in all individuals from early life and formed continuously through out life,
  - Expansion of one type of B or T lymphocytes (clonal expansion) is antigen dependent and each clone has cells with the same antigen-binding specificity.
  - Each human has its won clones of cells depending on what antigens invade his body
  - The total pool of receptors are capable of recognizing more than 10^{10} different structures.

- Hematopoietic stem cells (HSCs) give rise to two kinds of multipotent progenitor cells, one that generates lymphoid and another that produces myeloid cells,
- The common lymphoid progenitor gives rise to committed precursors of T cell, B cell
- The common myeloid progenitors give rise to committed precursors of the erythroid, megakaryocytic (platelets), granulocytic (Neutrophils, eosinophils, and basophils), and monocytic lineages,
- Stem cells express 2 main proteins, CD34 and stem cell antigen-1
- Hematopoietic Cytokines called Colony stimulating factors are the influencing factors for stem cell differentiation and maturation e.g; G-CSF, M-CSF and GM-CSF

Blood cells precursors



Steps of lymphocytes development

- First; 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.
- Pro-B cells stay in BM
- 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 good 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**.

-Differentiation of mature T cells into functionally and phenotypically distinct subpopulations. T cells develop into CD4+(Th) and CD8+(Tc) αβ T lymphocytes in thymus.

- Migration of mature cells to peripheral lymph nodes and they are activated by macrophages and DCs presenting antigen on MHC

- Binding of lymphocytes to antigen leads to
  - proliferation (clonal expansion) and differentiation to effector T helper (Th) and cytotoxic T cells (CTL) and plasma cells, and memory cells

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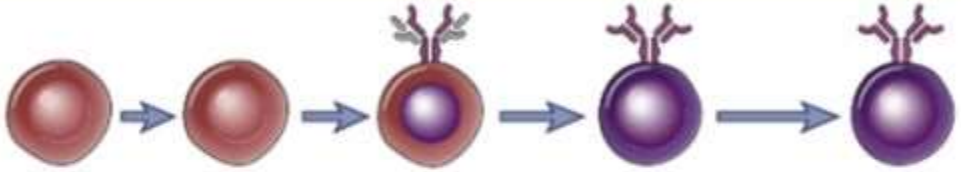
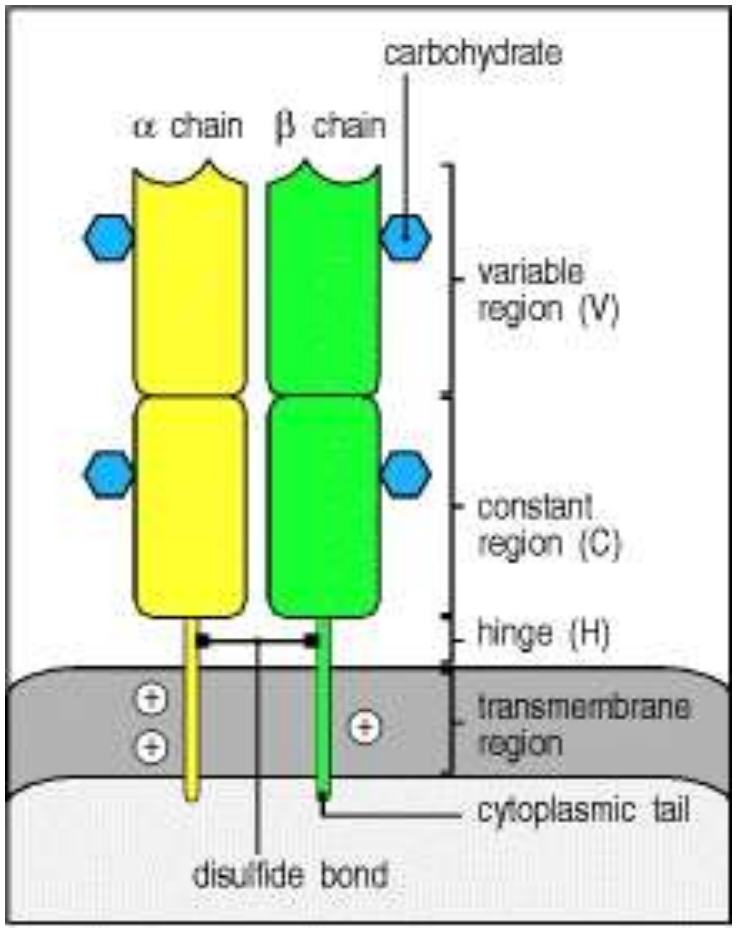
Stages of lymphocyte maturation



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.

αß TCR

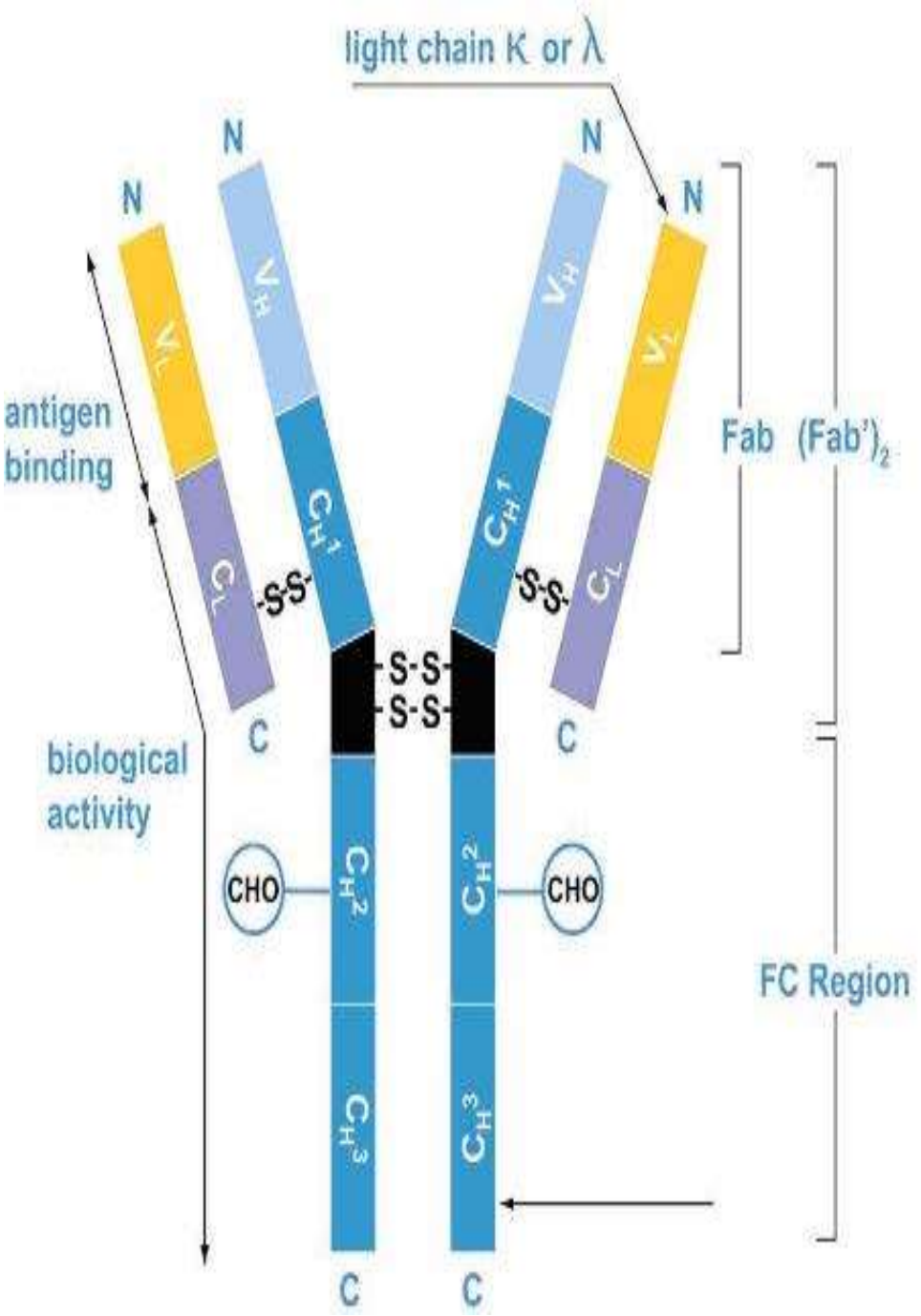


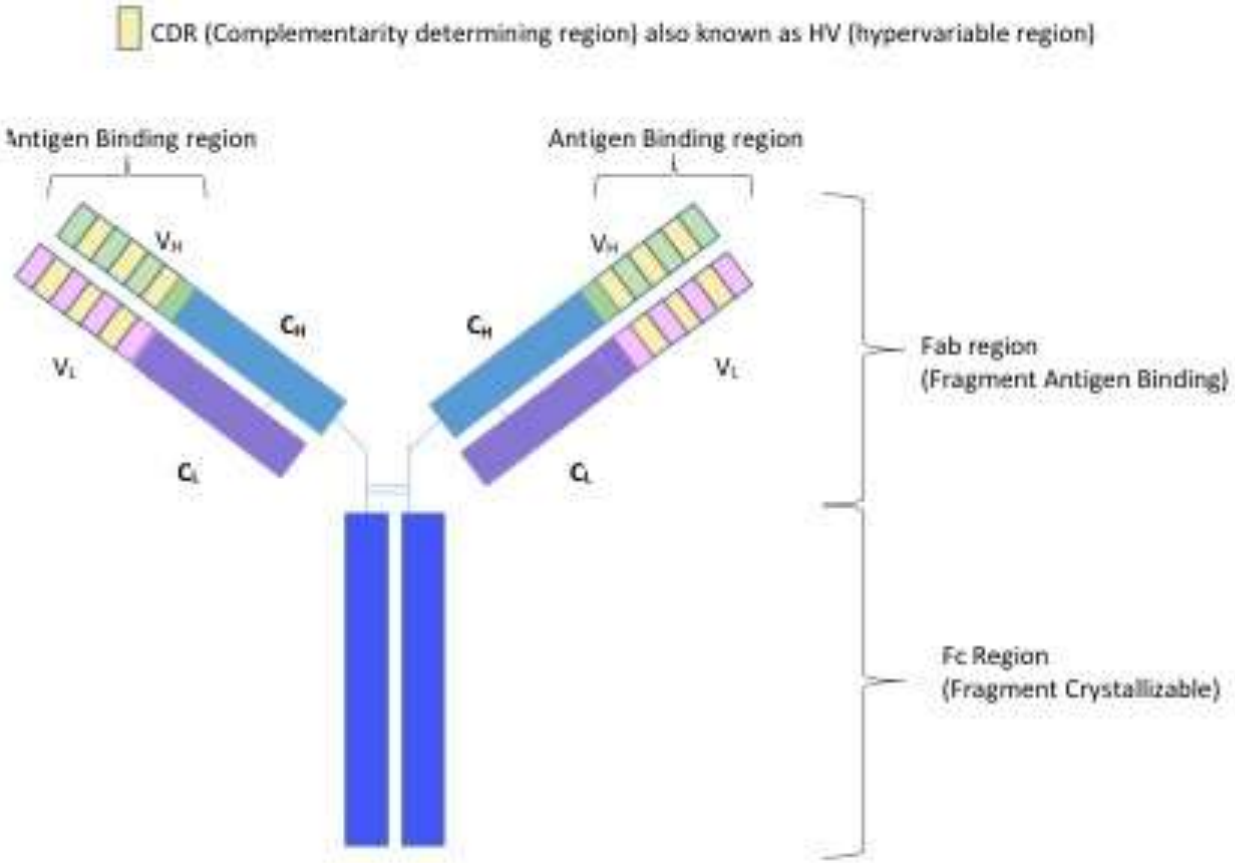
- The complete TCR is the αß receptor plus the ζ chain and two CD3 signaling proteins
- Each chain constitute of one variable, one constant alpha or beta, hinge, transmembrane and cytoplasmic tail
- TCR that specifically recognizes peptide-MHC complexes
- Single Ag binding site
- Lower affinity than Ab
- Ag must be bound to MHC
- CD4 (TH) or CD8 (TC) also binds MHC
- Hypervariable regions on Ag-binding site on top of both Vα and Vß and they are 3 sites

B Cell Receptor (BCR)

The complete 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
- Variable region has antigen binding site
- Hypervariable regions or (CDR) on Ag-binding are 3 sites
- Constant of light chain is either both kappa or lambda protein type
- Constant of heavy chain(there are 5 constant regions (CϒIGG, Cδ for IGD, Cε for IGE, Cμ for IGM and Cα for IGA),
- For BCR the constant is Cμ for IGM




CDR (Complementarity determining region) also known as HV (hypervariable region)
Antigen Binding region
Antigen Binding region
V_H
V_H
C_H
C_H
V_L
V_L
C_L
C_L
Fab region
(Fragment Antigen Binding)
Fc Region
(Fragment Crystallizable)

-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 kappa or lambda for BCR light chain and constant for IGM for heavy chain,
- C β or C α for TCR β and alpha chains
- development of VDJ-C recombination is the **process happen early to form T beta chain, B cell heavy chain development**
- Then followed by VJ-C formation to form light chain in B cells and alpha chain in T cells

Summary: Ig vs TCR

Element	Immunoglobulin		α:β T-cell receptors	
	H	κ+λ	β	α
Variable segments (V)	40	70	52	~70
Diversity segments (D)	25	0	2	0
D segments read in three frames	rarely	–	often	–
Joining segments (J)	6	5(κ) 4(λ)	13	61
Joints with N- and P-nucleotides	2	50% of joints	2	1
Number of V gene pairs	1.9×10^6		5.8×10^6	
Junctional diversity	$\sim 3 \times 10^7$		$\sim 2 \times 10^{11}$	
Total diversity	$\sim 5 \times 10^{13}$		$\sim 10^{18}$	

Figure 4-13 Immunobiology, 6/e. (© Garland Science 2005)

Diversity in the TCR gene locus

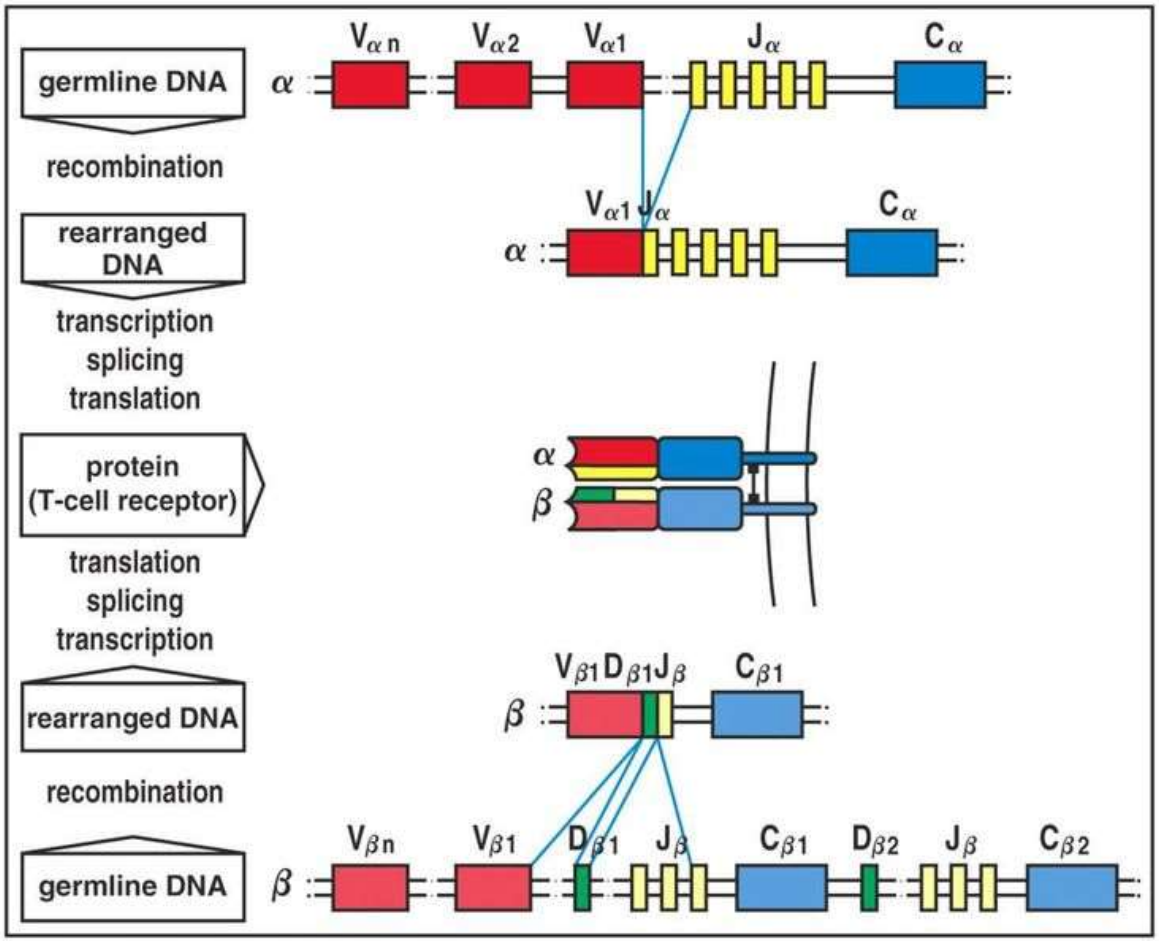


Figure 4-12 Immunobiology, 6/e. (© Garland Science 2005)

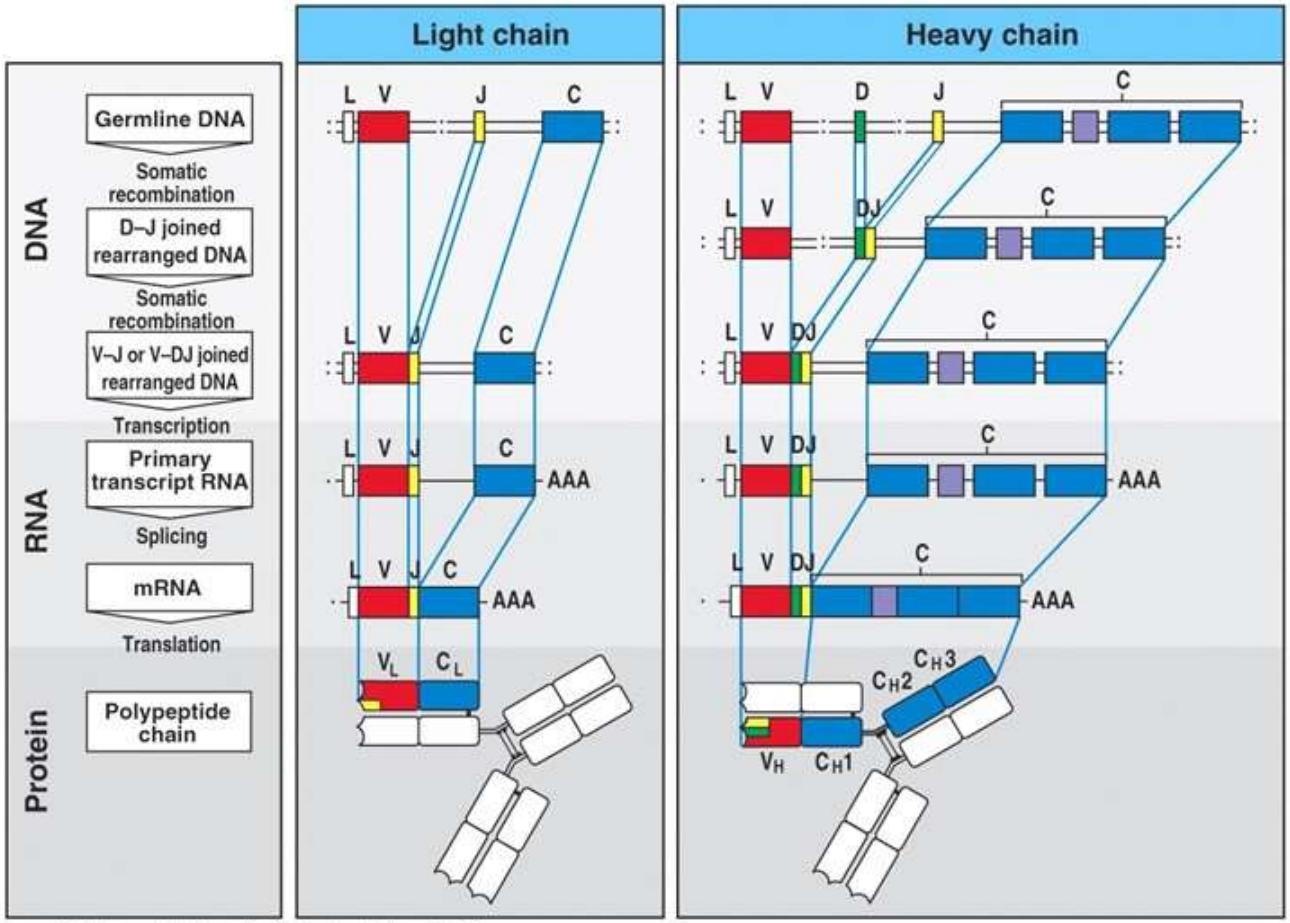


Figure 4-2 Immunobiology, 6/e. (© Garland Science 2005)

Summary: mechanisms that generate diversity in lymphocyte receptors

Event	Process	Nature of change	Process occurs in: B cells	Process occurs in: T cells
V-region assembly	Somatic recombination of DNA	Irreversible	Yes	Yes
Junctional diversity	Imprecise joining, N-sequence insertion in DNA	Irreversible	Yes	Yes
Transcriptional activation	Activation of promoter by proximity to the enhancer	Irreversible but regulated	Yes	Yes
Switch recombination	Somatic recombination of DNA	Irreversible	Yes	No
Somatic hypermutation	DNA point mutation	Irreversible	Yes	No
IgM, IgD expression on surface	Differential splicing of RNA	Reversible, regulated	Yes	No
Membrane vs secreted form	Differential splicing of RNA	Reversible, regulated	Yes	No

Figure 4-25 Immunobiology, 6/e. (© Garland Science 2005)

Genetic rearrangement or somatic recombination

- DNA Recombination include enzymes mainly Recombination Activating Genes (encode RAG-1 and RAG-2)
  - Synapse, making chromosomal loop
  - Cleavage
  - 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).

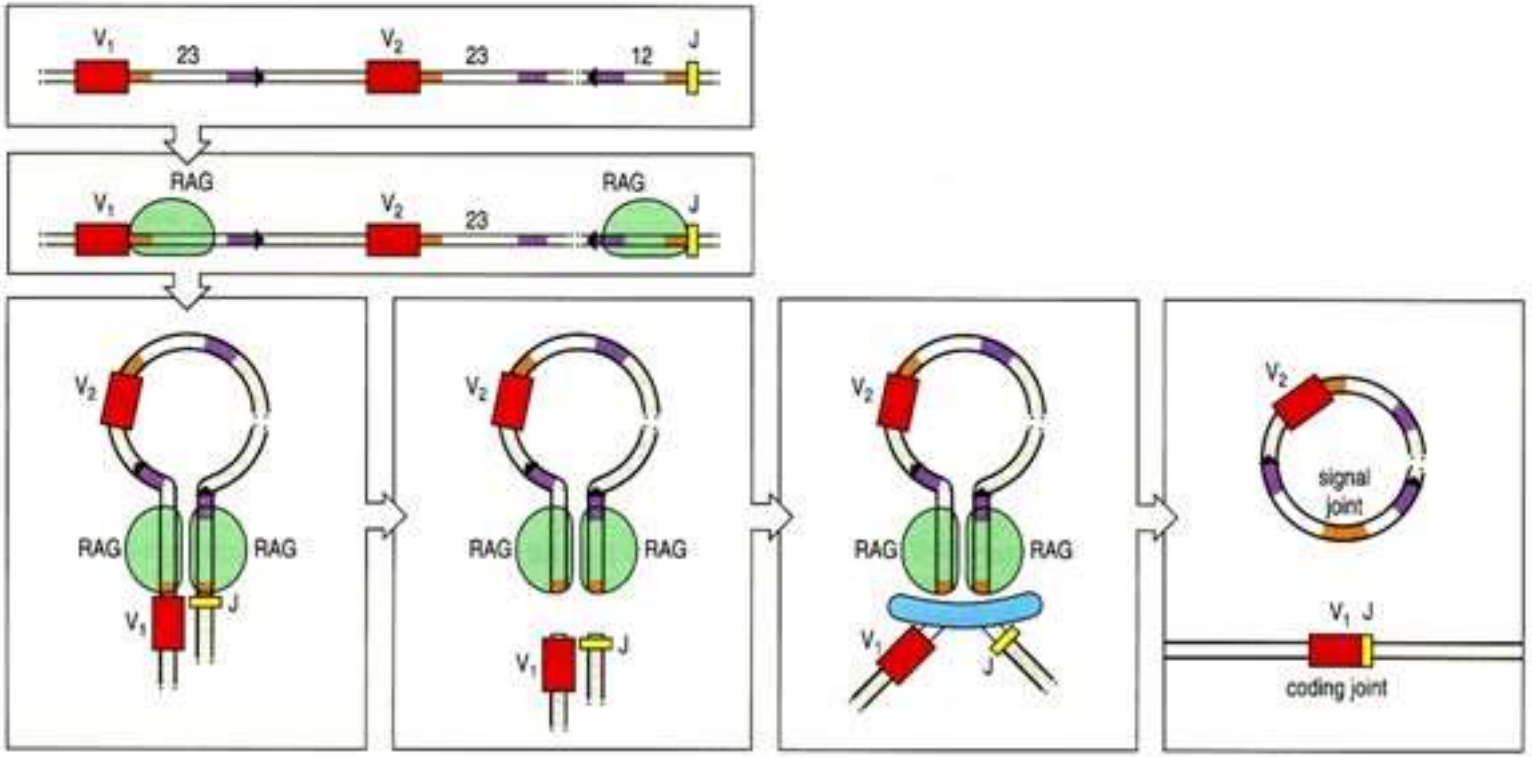


Figure 2.18 Gene segments encoding the variable region are joined by recombination at recombination signal sequences. The recombination between a V (red) and a J (yellow) segment of a light-chain gene is shown here. A RAG complex binds to the 23-bp spacer and another to the 12-bp spacer, so that a recombination signal sequence (RSS) containing a 12-bp spacer is brought together with that containing a 23-bp spacer. This is known as the 12/23 rule and ensures that gene segments are joined in the correct order. The DNA molecules are broken at the ends of the heptamer sequences (orange) and are then joined together with different topologies. The region of DNA that was originally between the V and J segments to be joined is excised as a small circle of DNA that has no function. The joint made in forming this circle is called the signal joint. Within the chromosomal DNA, the V and J segments are joined to form the coding joint. Formation of this joint involves opening up of hairpins that were formed at the original point of cleavage at one end of each V and J segment and then repairing the DNA in a way that introduces additional variability into the nucleotide sequence around the joint. The additional enzymes involved in these processes are represented in blue. Nonamers are shown in purple, heptamers in orange, spacers in white.

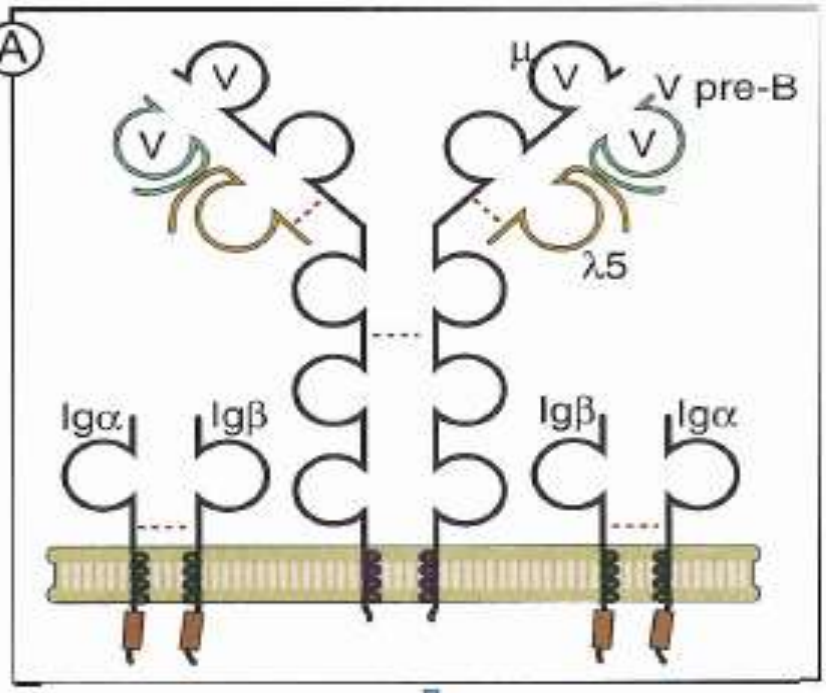
T cell development

- 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 of thymus)**.
- 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

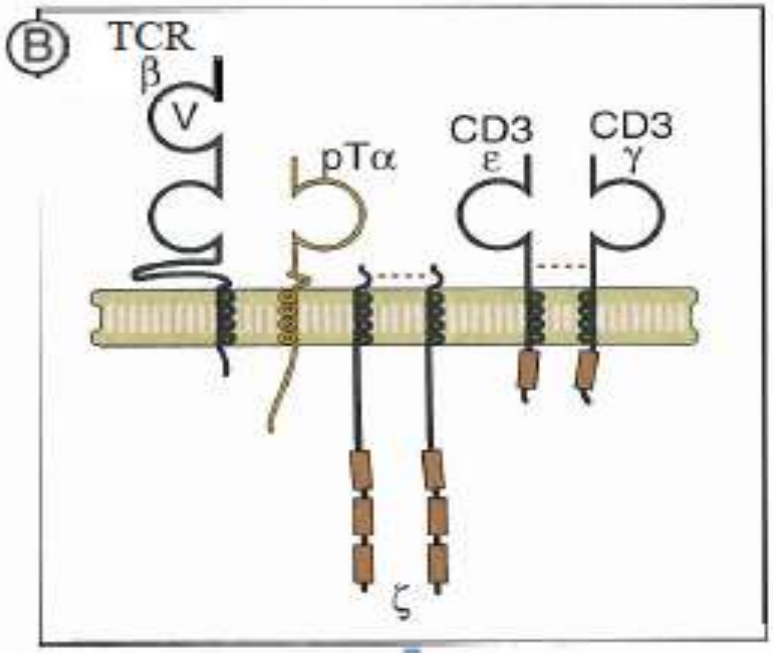
- **in cortex;** The TCR β chain protein is expressed on the cell surface first (by DNA recombination of VDJ - beta constant segments) in association with an invariant protein called pre-Tα to form the **pre-T cell receptor** (pre- TCR) complex
- CD3 and ζ proteins are present on pre-T ell as signaling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

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 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

γδ T cells (Intra-epithelial lymphocyte) (IEL)

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

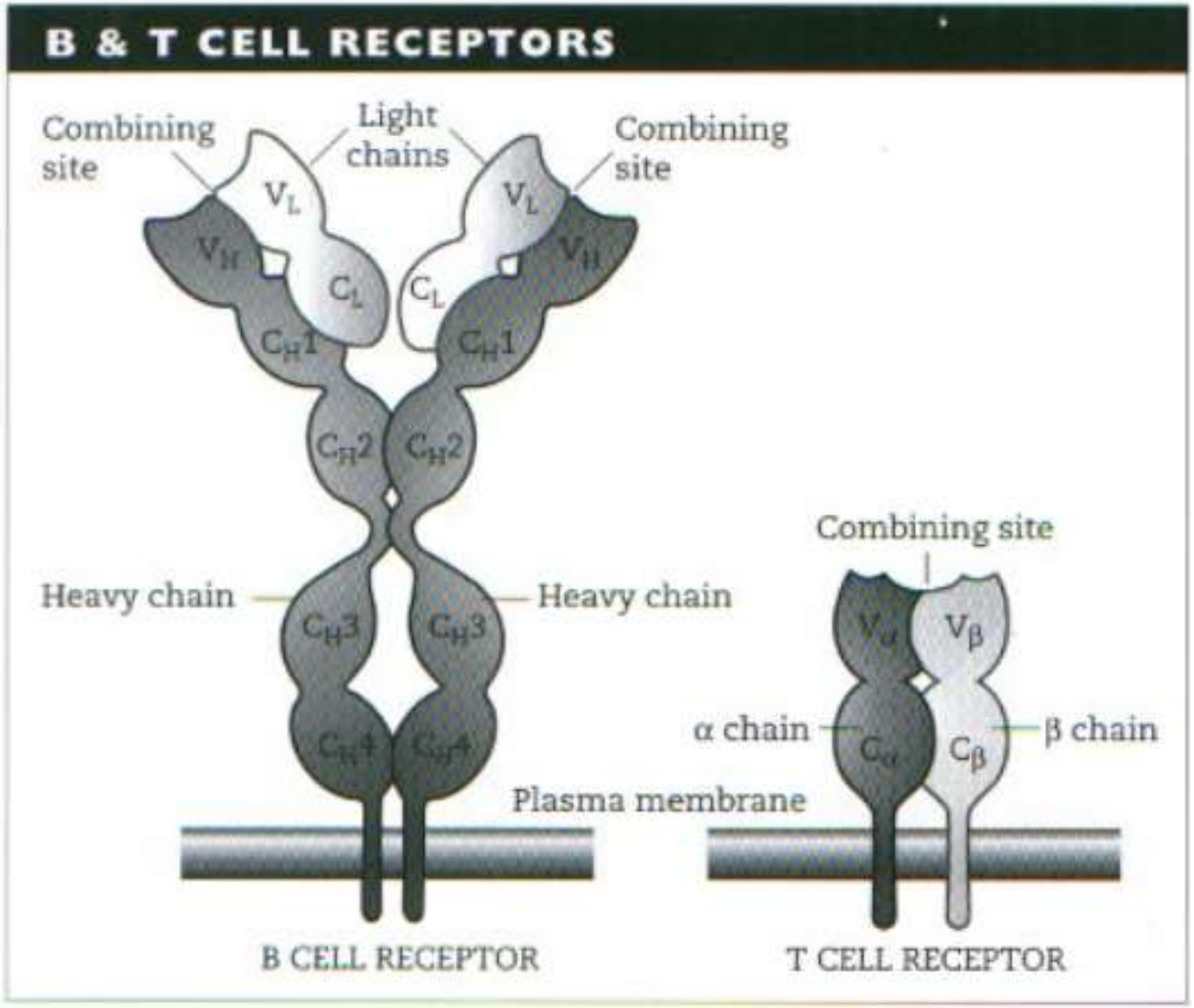
immature B cells = complete IGM BCR

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)


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

Innate and T cells migration

- Lymph from tissues (mainly innate cells carrying antigens macrophage and DC) passes into the nearby node through the afferent lymphatic vessel
- In the node they activate T and B cells
- Then the innate cells go into the cortical sinuses then they leave via the efferent lymphatic
- Naïve B and T cells migrate to secondary lymphoid tissues through High endothelial venules ;HEVs,
- If the T cells recognize antigen, they become effector cells, and they return to the circulation through the efferent lymphatics, If not activated recirculate to 2nd LN

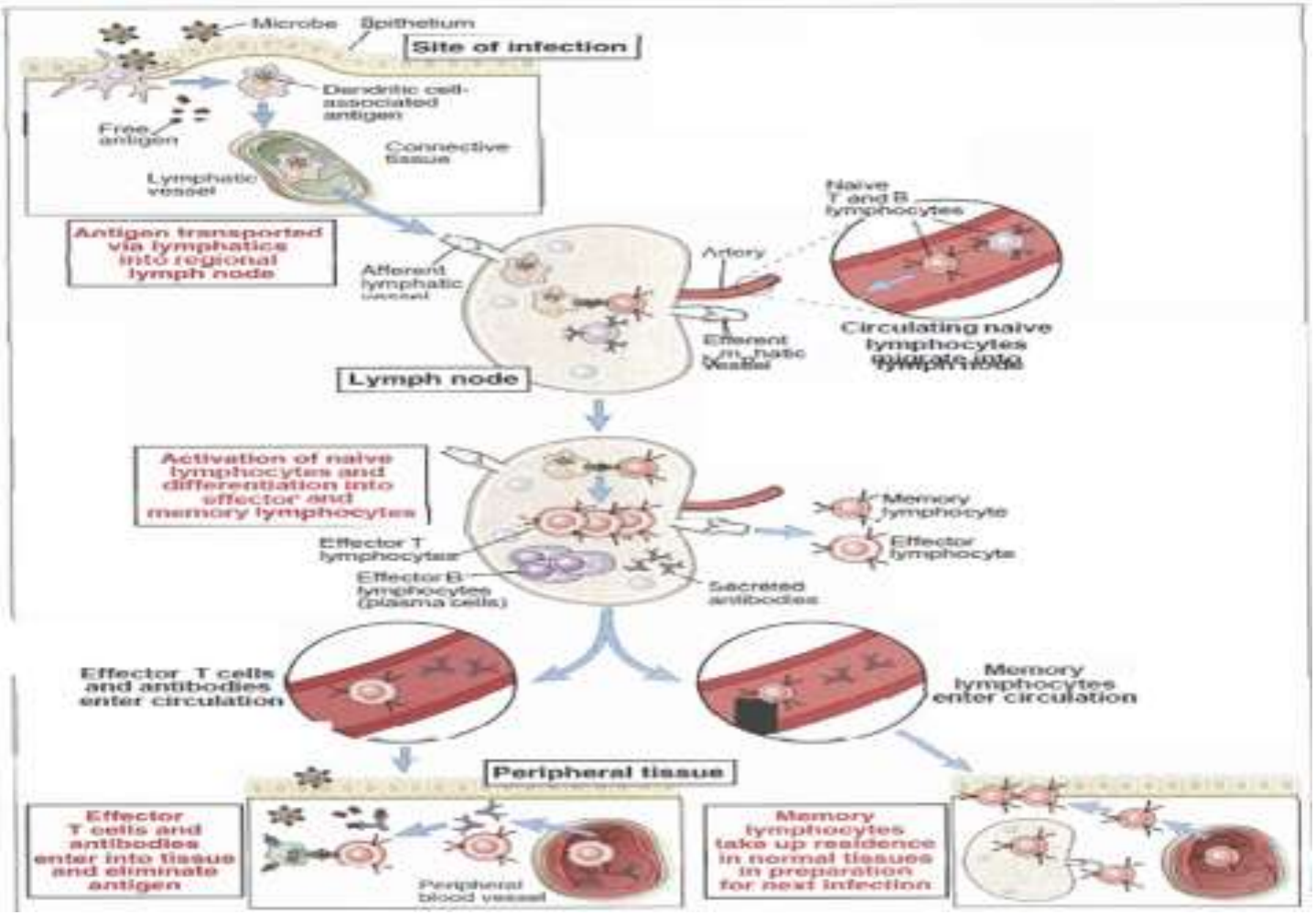
- Effector (Effector T cells include CD8+ cytotoxic T cells and CD4+ helper T cells) leave the blood and enter peripheral tissues through venules at sites of inflammation.
- the two molecules needed for selective entry of T cells into secondary lymphoid organs through HEV (CCR7 and L-selectin) high in naïve cells
- And are reduced on effector T cells therefore these cells do not readily reenter lymphoid tissues.

Memory T cells

- Central memory T cells were defined as human T cells that express high levels of CCR7 and L-selectin;
- effector memory T cells were defined as T cells that express low levels of CCR7 and L-selectin
- So Central cells recirculate back to peripheral node and reside there
- And the effector cell reside in tissues

B cells migration

- B cells migrate into follicles in 2nd LN, the site where they may encounter antigen and become activated.
- After B cell activated they reside in germinal center where they differentiate to antibody-producing plasma cells, they reside in medulla.
- Some memory cells reside in LN some in tissues as T cells


Microbe
Epithelium
Site of infection
Dendritic cell-associated antigen
Free antigen
Connective tissue
Lymphatic vessel
Antigen transported via lymphatics into regional lymph node
Afferent lymphatic vessel
Artery
Efferent lymphatic vessel
Lymph node
Naive T and B lymphocytes
Circulating naive lymphocytes
Activation of naive lymphocytes and differentiation into effector and memory lymphocytes
Effector T lymphocytes
Effector B lymphocytes (plasma cells)
Secreted antibodies
Memory lymphocyte
Effector lymphocyte
Effector T cells and antibodies enter circulation
Memory lymphocytes enter circulation
Peripheral tissue
Effector T cells and antibodies enter into tissue and eliminate antigen
Peripheral blood vessel
Memory lymphocytes take up residence in normal tissues in preparation for next infection

Immunity against certain microbe

- Immunity can be active or passive
  - Active; induced by previous exposure to an antigen and host immune cells respond and form antibodies and memory cells
  - Passive, transfer serum or lymphocytes from specifically immunized individual to not-exposed person (naïve). Maternal Ab to fetus