Basic Immunology

Dentistry

Genetics of immunoglobulins, organization and expression of antigen receptor genes.

Central B-cell development.

Central (thymic) T cell development.

Ferenc Boldizsar

Cells of the lymphoid lineage

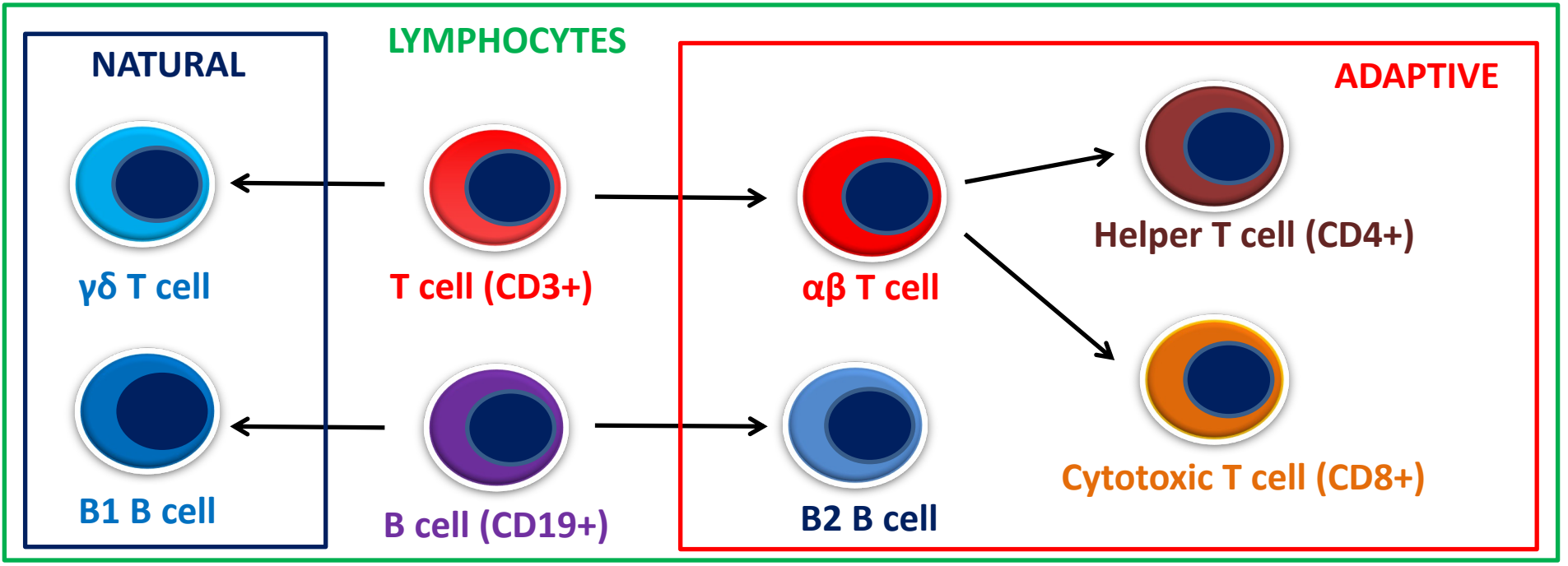
Innate lymphoid cells (ILC)

HAVE NO ANTIGEN-RECOGNITION RECEPTORS

There is no difference in the morphology!

Lymphocyte

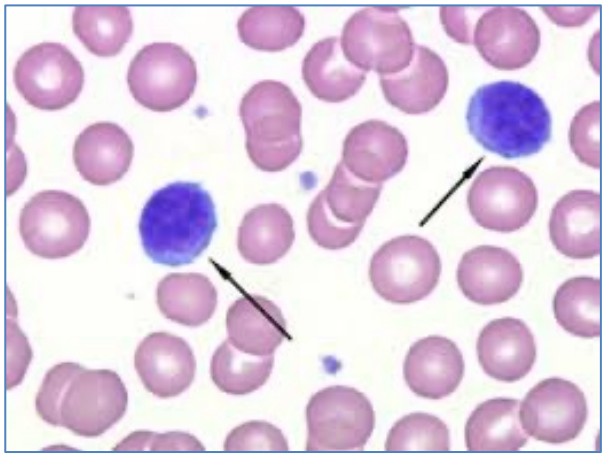
HAVE ANTIGEN-RECOGNITION RECEPTORS



Lymphocytes

Leukocyte %:	25-40*
Main function:	ADAPTIVE IMMUNITY
Recognition	Antigen-specific receptors (TCR, BCR)

* Including NK cells



B cell (CD19+)

↓

Antibody production

Cytotoxic T cell (CD8+)

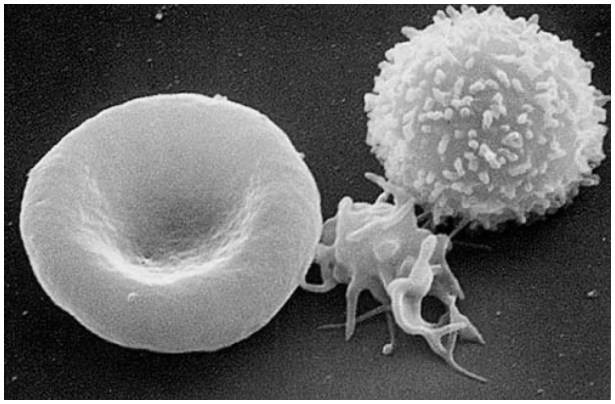
↓

Direct killing of target cell (infected or cancerous)

Helper T cell (CD4+)

↓

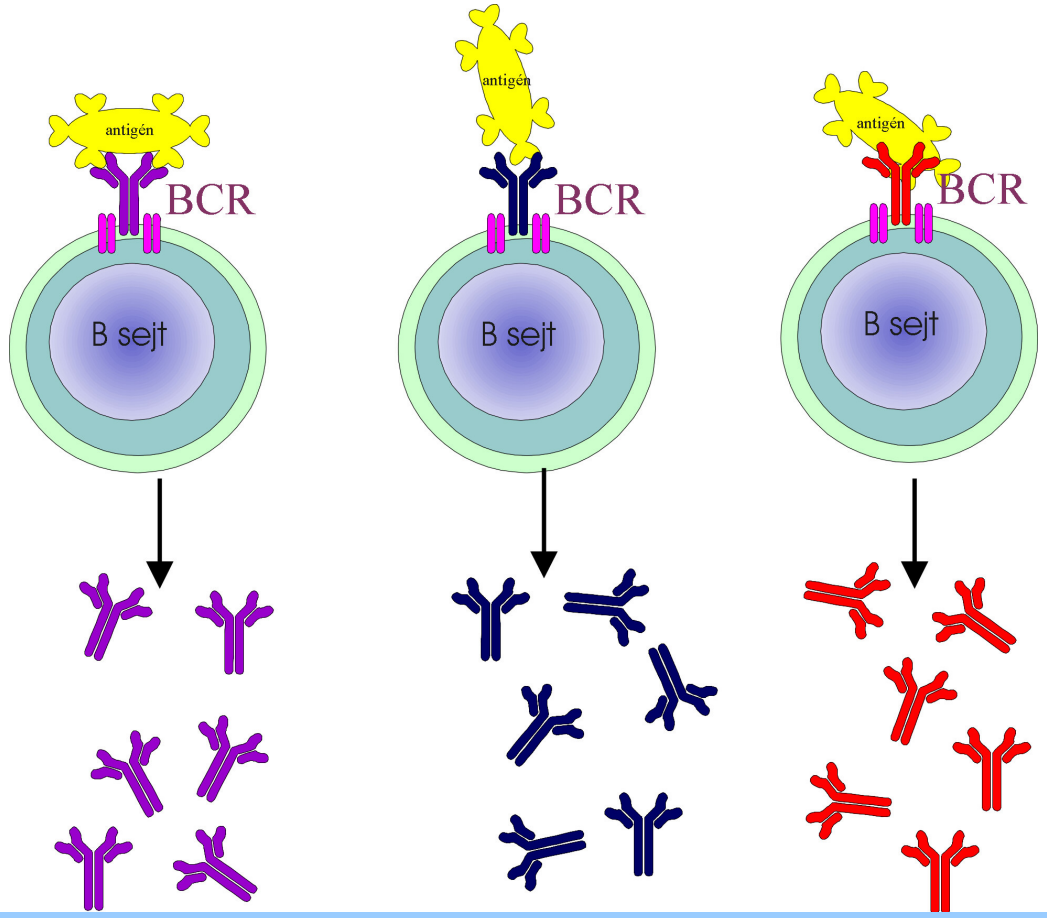
Regulation of the immune response



A red blood cell, a platelet and a lymphocyte (SEM image)

All of the above are done in an ANTIGEN-SPECIFIC manner!

Antibody – B-cell-Repertoire: 10^{11}



Tonegawa (Nobel prize:1987)

During B cell differentiation Immunoglobulin genes are rearranged and somatic Hypermutations take place.

Compared to the large repertoire relatively few Ig V genes are inherited.

Aim of lymphocyte differentiation

- Expression of Antigenreceptors with different specificitites

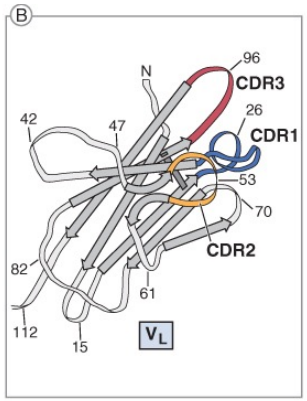
- Production of B- and T cell repertoire = Number of antigen recognition molecules: 10^9-10^{11} BcR, 10^{15}-10^{16} TcR;

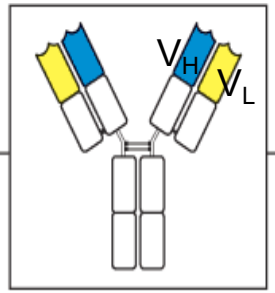
„Lymphocyte production = Glove factory" – Jan Klein.

The immune system produces antigen receptors for all potential antigens and is therefore ready to recognize those structures.

The genetic background of B- and T cell receptor production is the gene **rearrangement of Ig- and TcR genes in the progenitor cells.**

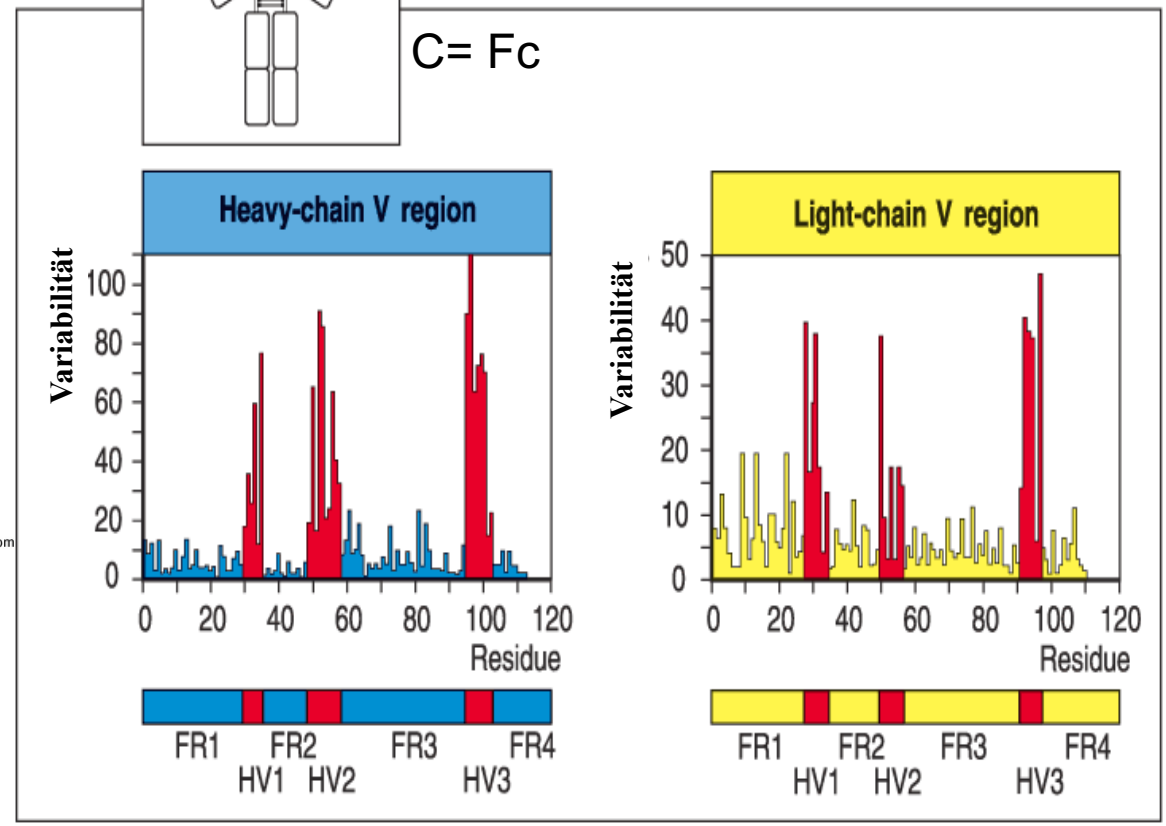
The antigen binding parts of the Immunoglobulins contain hypervariable (CDR) regions





V = Fab

C= Fc



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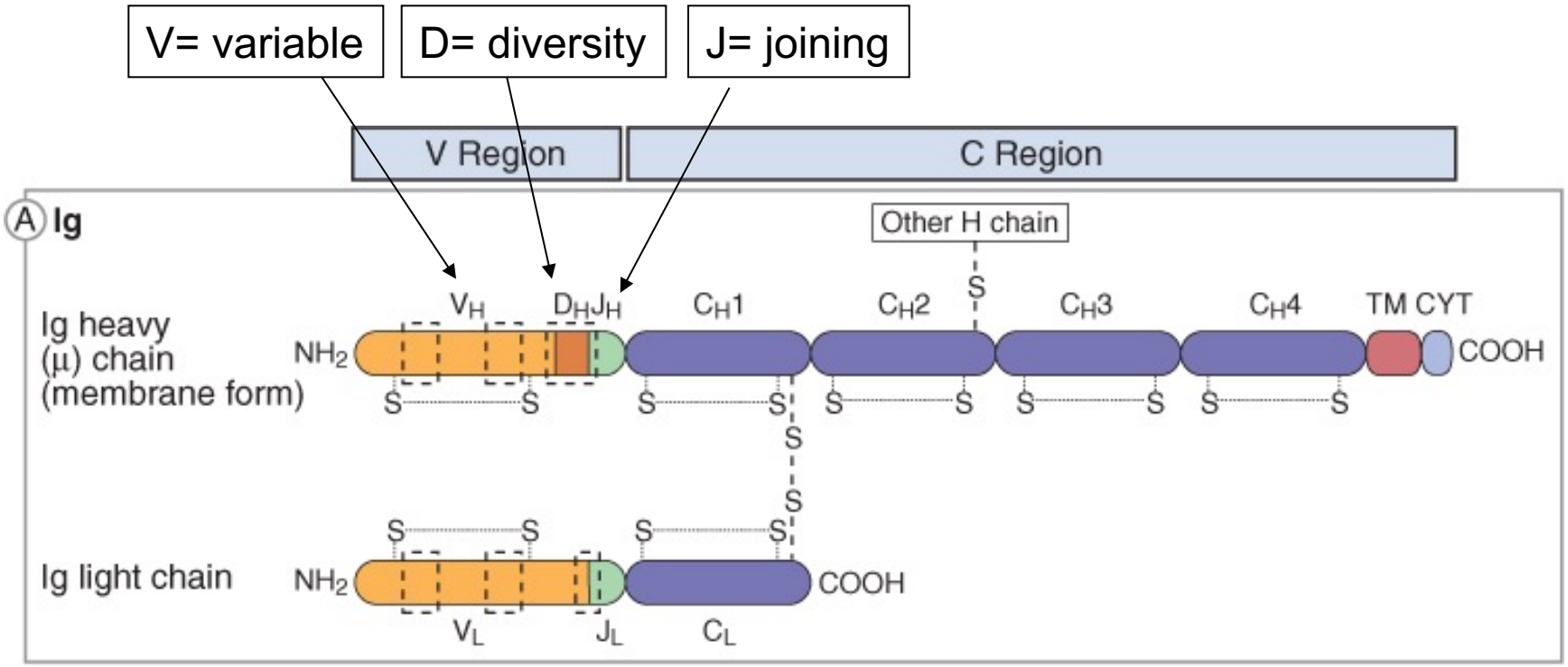
Domains of the immunglobulin heavy- and light chains

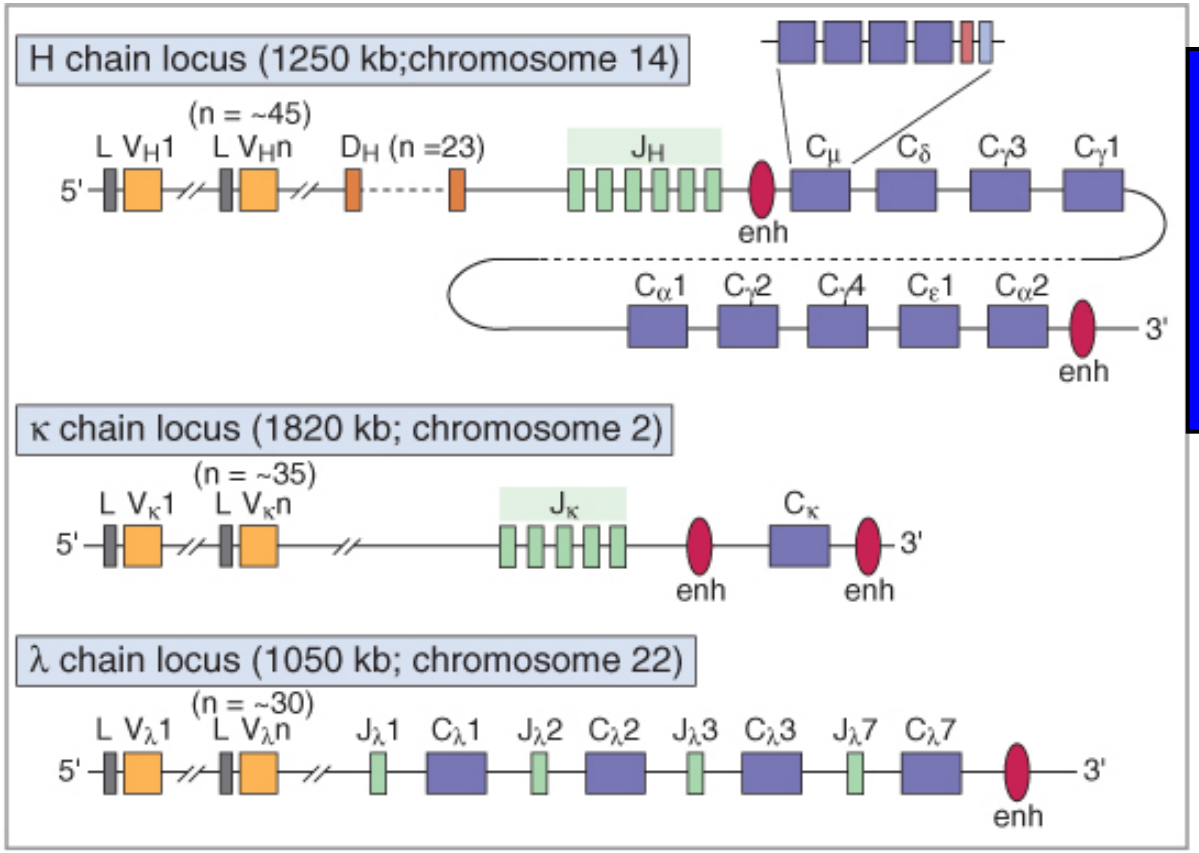


- The **variable (V)** and **constant (C)** **domains (units)** of the heavy- and light polypeptide chains are encoded by different **gene segments**.
- The genes of the Ig heavy- and light polypeptide chains are located in different chromosomes.

Gene organisation of the immunglobulin heavy- and light chain loci

V-Region:
V = Variable
D = Diversity
J = Joining
Gensegmente

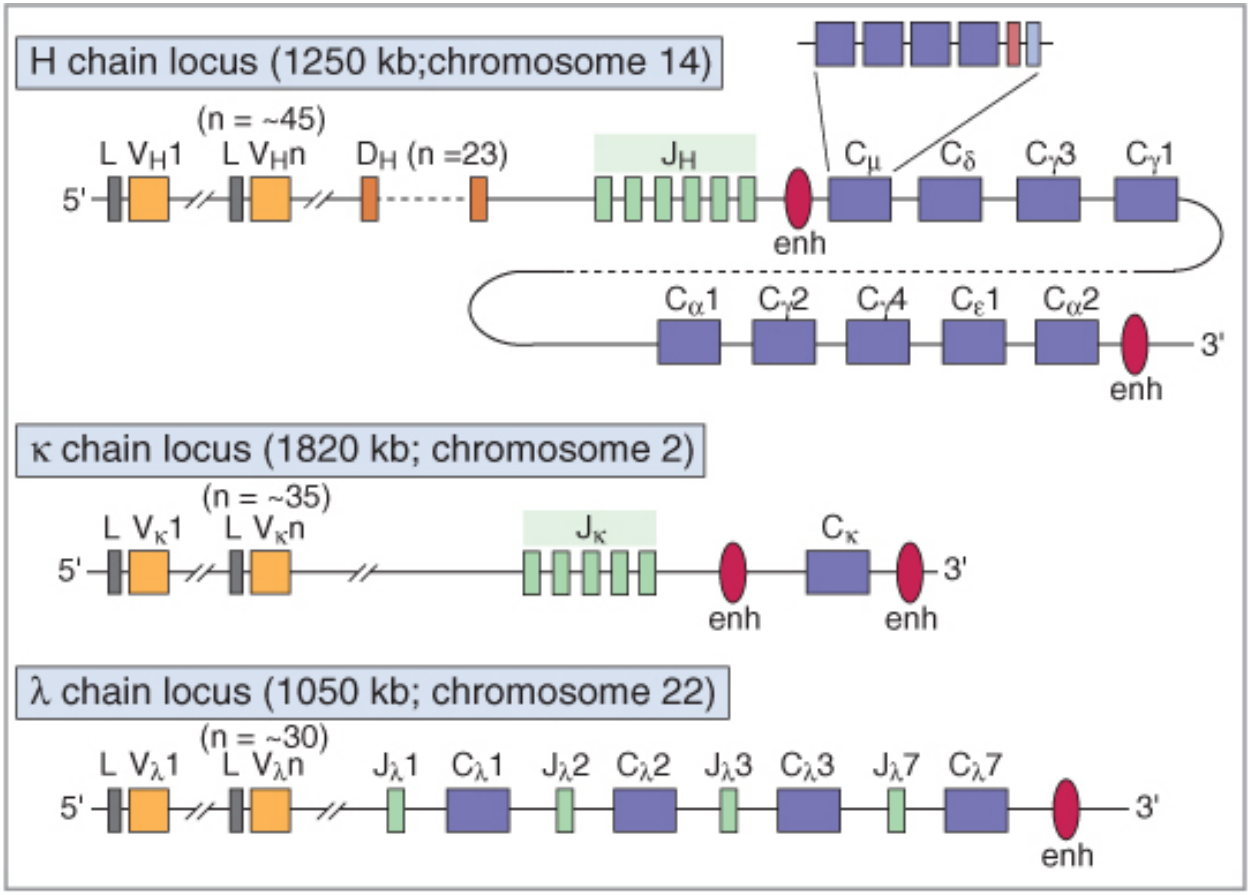
C-Region:
C = Constant
Gensegmente



Cμ - IgM
Cδ – IgD
Cγ – IgG
Cα – IgA
Cε - IgE

The **germline-DNA** → the basic, not-recombined form of the immunoglobulin genes

The germline Ig DNA: number of V-D-J-gene segments



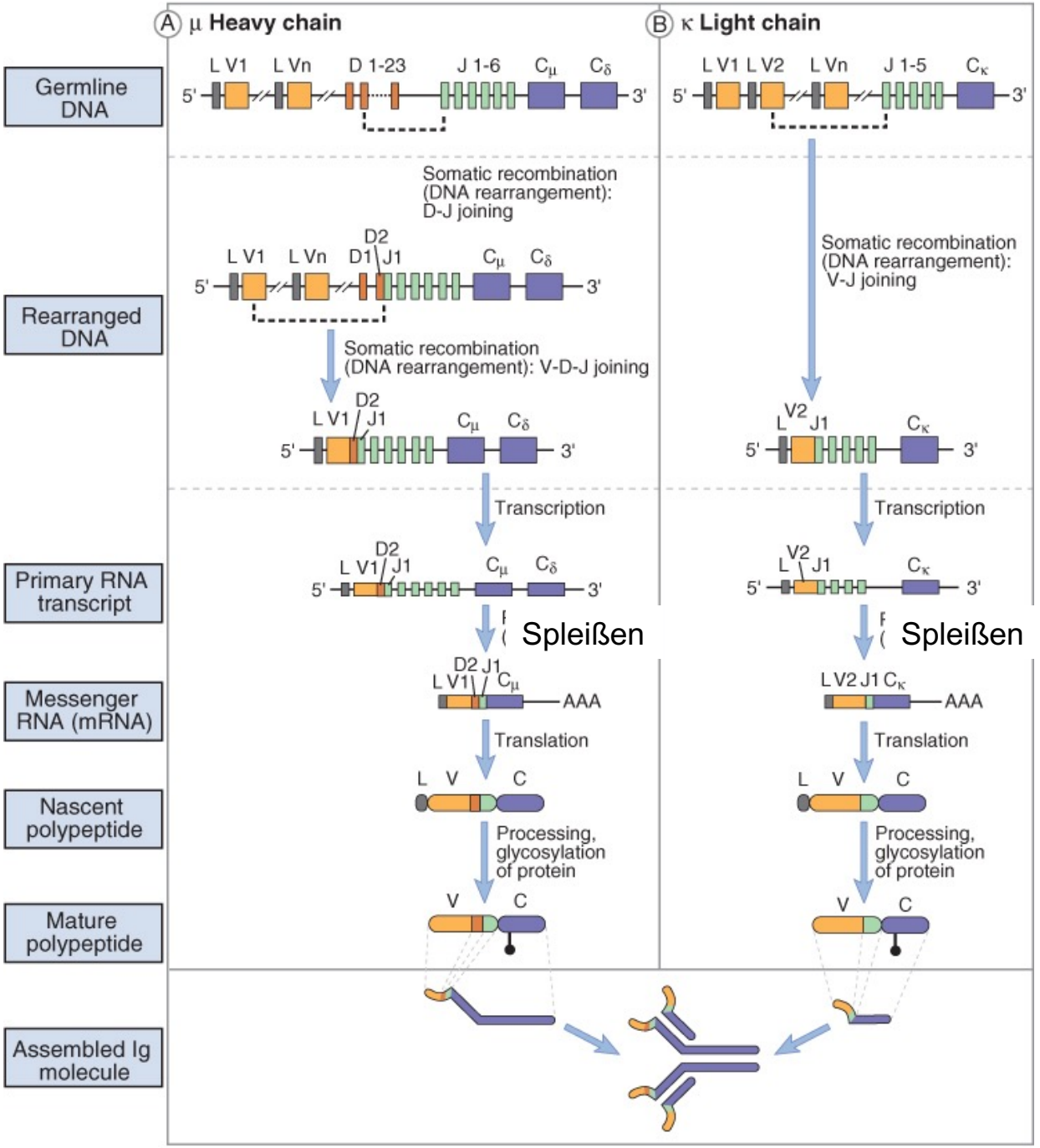
V- Segment: 45
D- Segment: 23
J - Segment: 6
C - Segment (8):
Cμ, Cδ, Cγ1-4,
Cα, Cε

V- Segment: 35
J - Segment: 5
C - Segment: 1

V-Segment: 30
J - Segment: 4
C - Segment: 4

In lymphocyte precursors the germline DNA will be rearranged by somatic recombination. = **Rearrangement**

Steps of the gene rearrangement



Molecular mechanism of the gene rearrangement

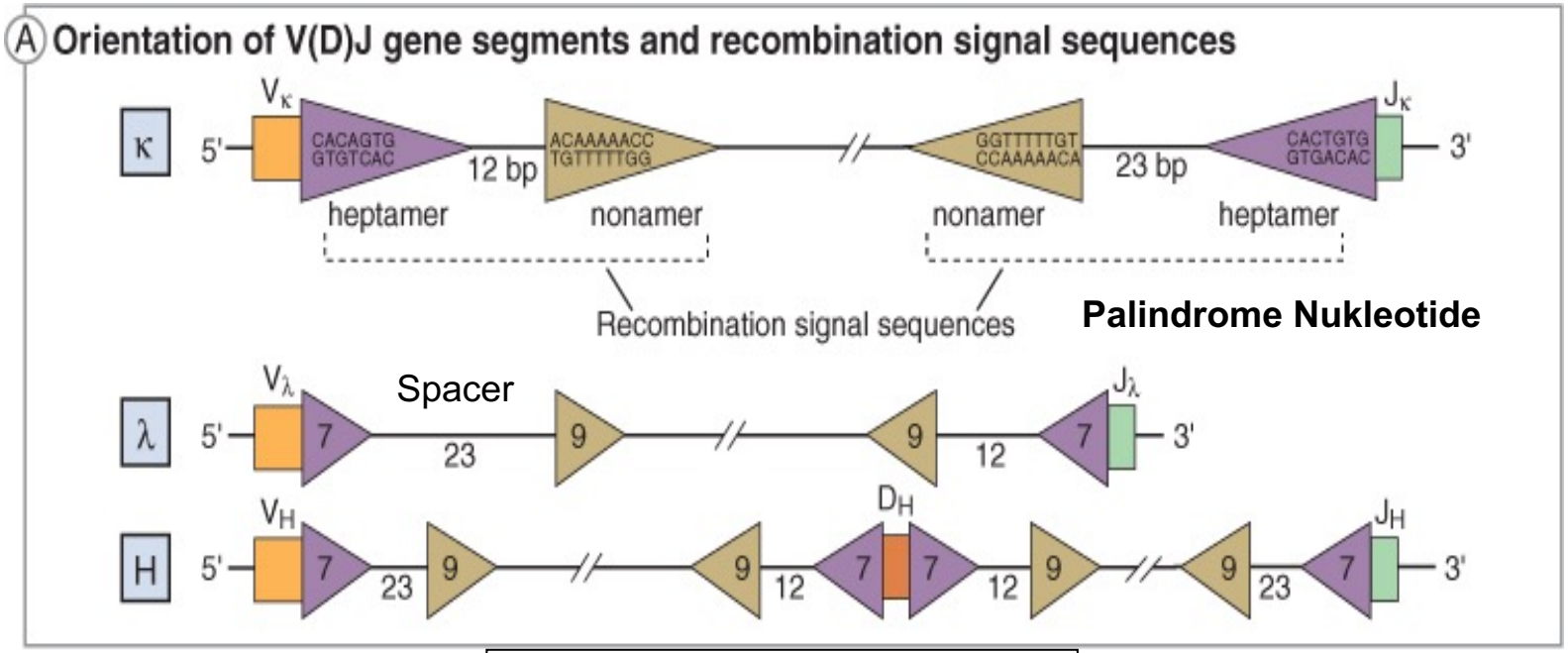
1. DNA loop formation
2. DNA cutting - Deletion
3. Ligation of the free DNA ends

Enzymes:

- VDJ-Recombinase: **RAG1 and -2**
- Heteromeric Proteincomplex: **DNA-Ligase, DNA-PK, Artemis-Protein**
- Terminale Deoxynukleotidyl-Transferase (**TdT**): →

N-Nukleotide-addition – random addition of nucleotides

The 12/23-base-pair rule during the recombination of Ig gene segments:

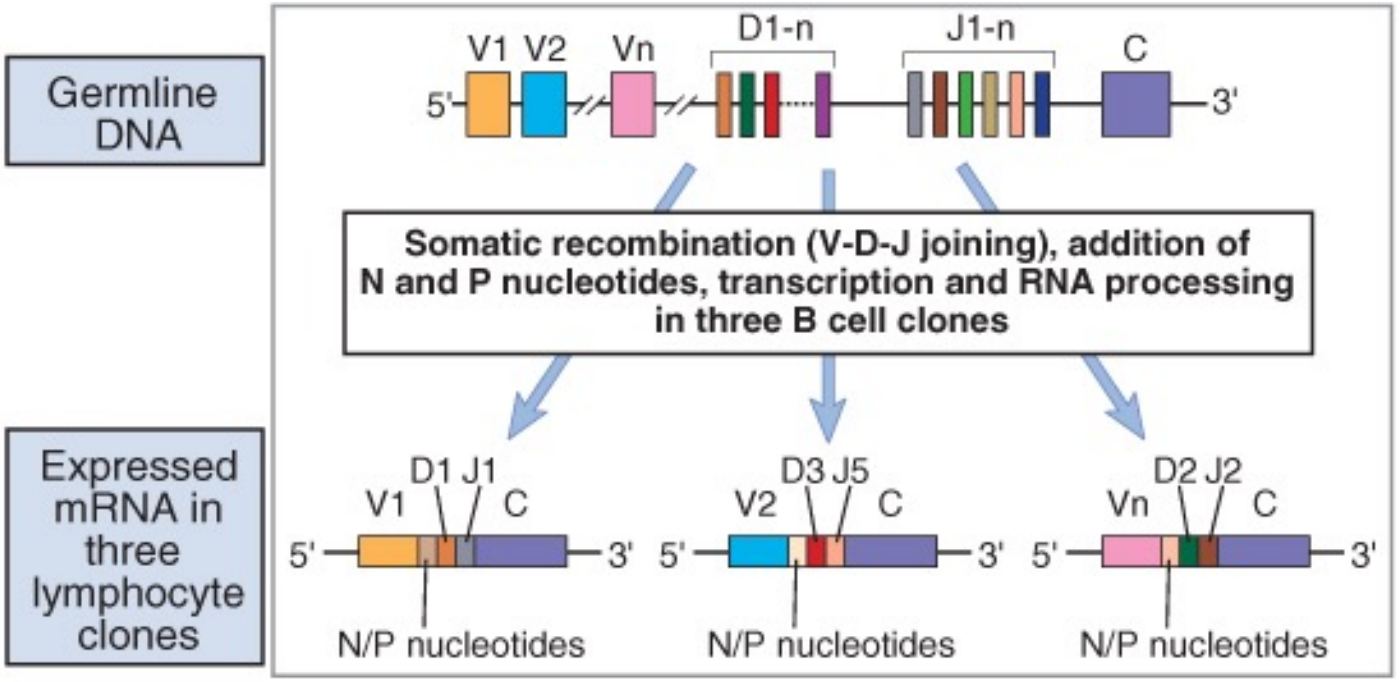


RSS= Rekombinations Signal Sequenz

Recombination-Signal-Sequence (RSS):

Contains a conserved heptamer and nonamer sequences which are divided by a non-conserved spacer sequence of either 12 or 23 basepairs.

Heavy chain gene rearrangement in three pro-B-cells



Random gene rearrangement → **Diversity**

T-cell-receptor (TcR)

T-cell-types:

1. $\alpha\beta$ **TcR+**

2. $\gamma\delta$ **TcR+**

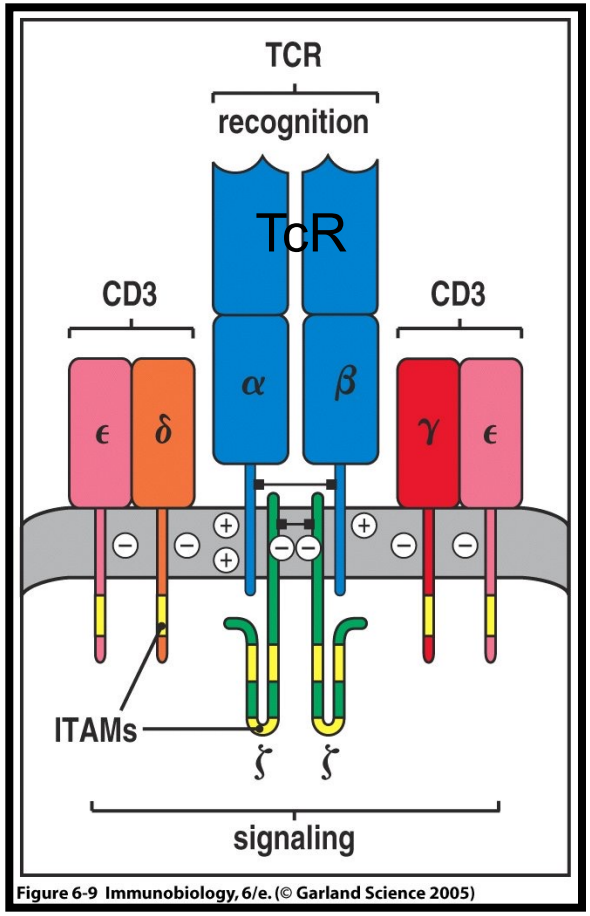
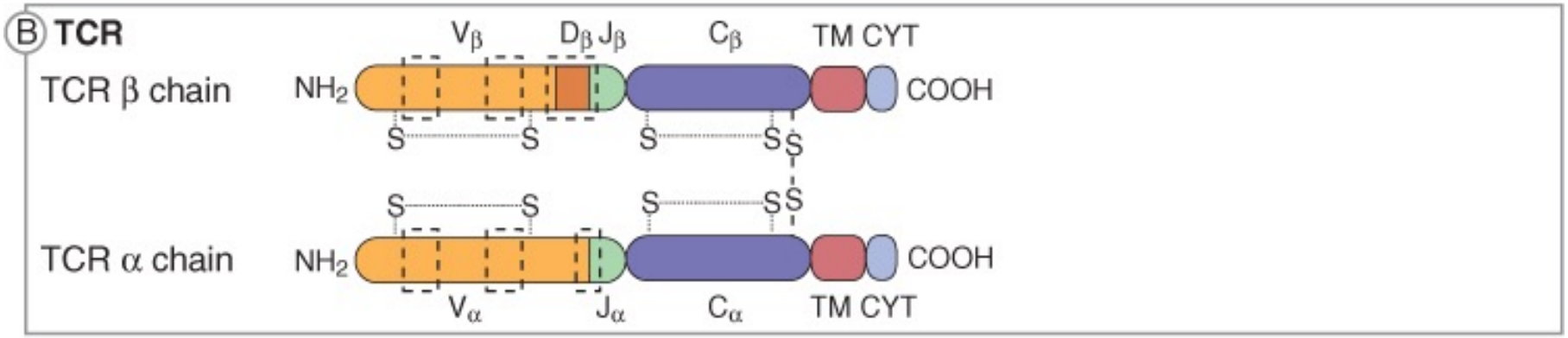


Figure 6-9 Immunobiology, 6/e. (© Garland Science 2005)

TcR α-β chains



Human TCR encoding genes

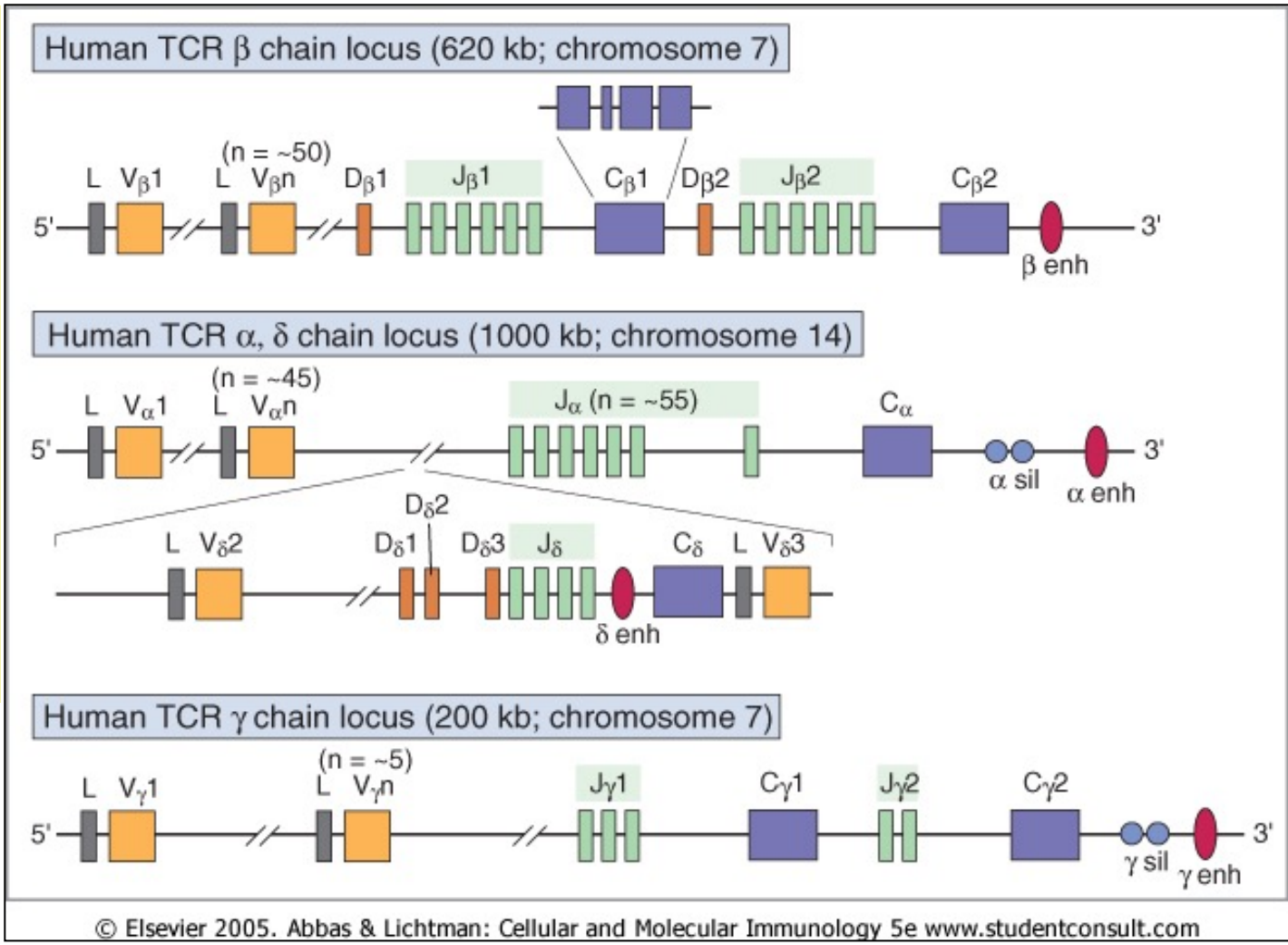
V,J,D and C genes

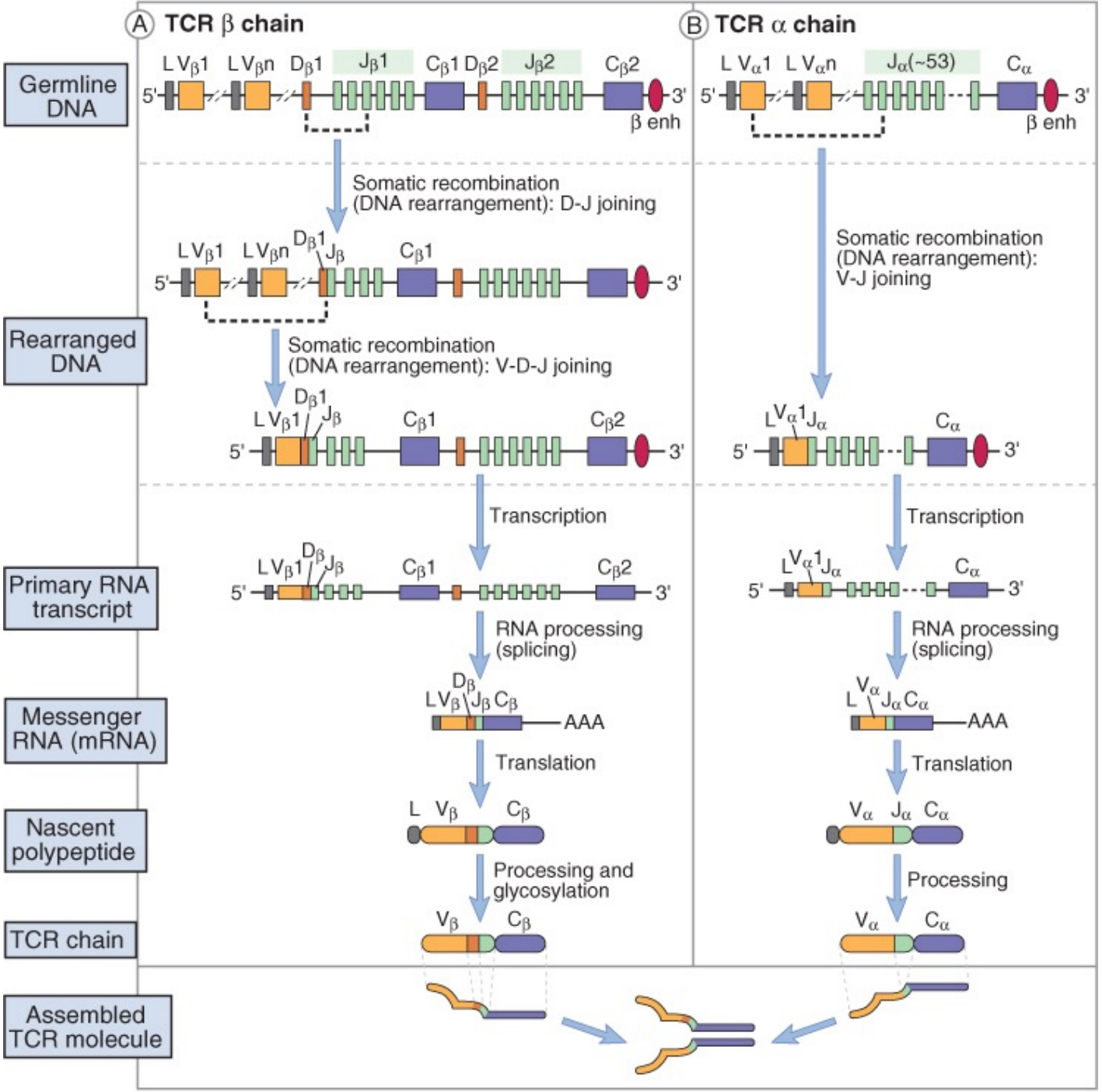
β locus:
50 –100 V, 1D,
6 J, 1 C genes

α locus:
45 V, 55 J genes

inserted: δ **locus:**
V,D,J and C genes

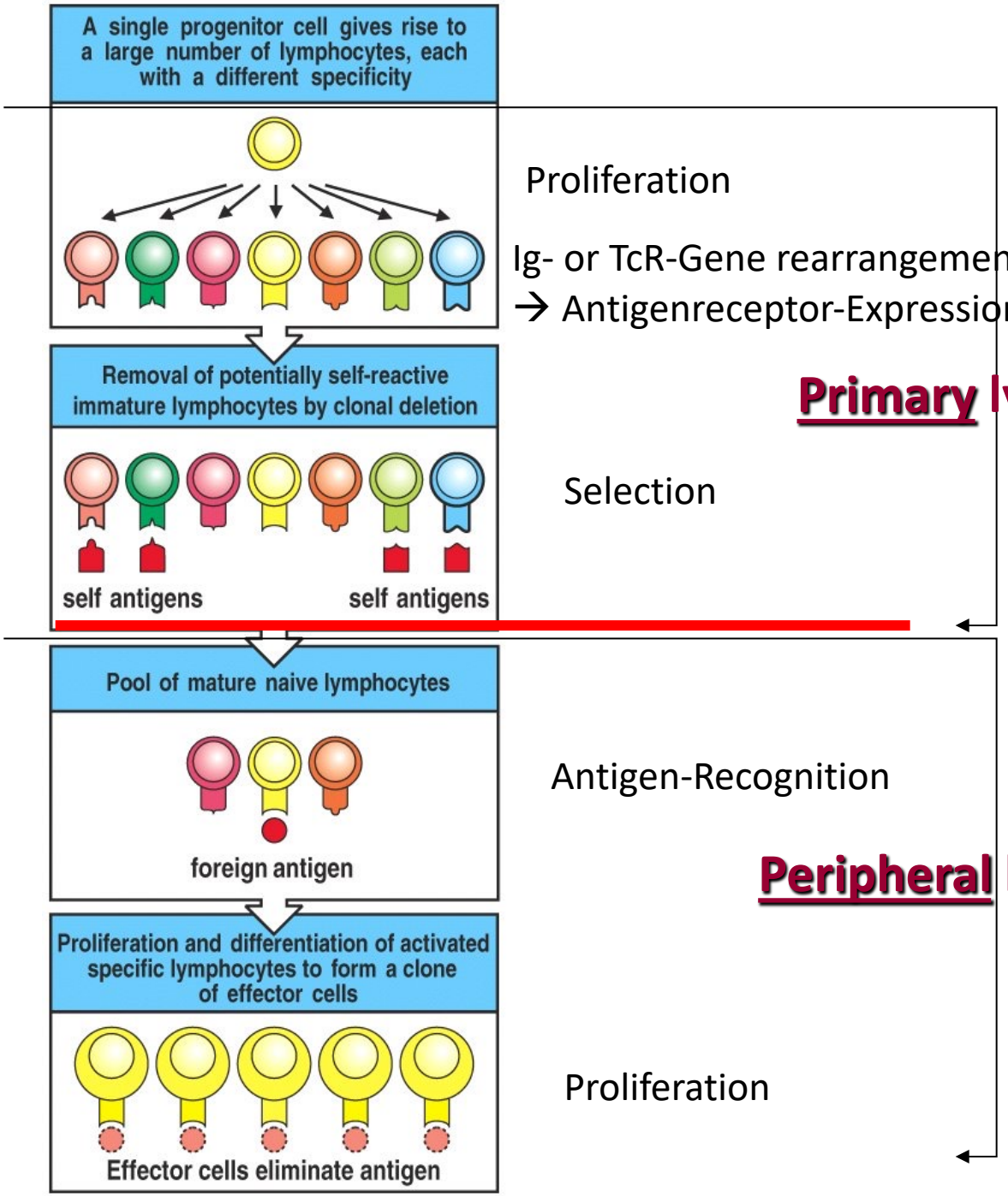
γ locus: V, J and C





The basis of TcR and BcR diversity

- The genes encoding the TcR α/β and γ/δ chains have similar structure (multiple V, D, J and C segments) than that of the Ig genes and the steps of the gene rearrangement is also the same (role of RAG1 and RAG2)
- The large number of V, D and J segments and their free recombination
- The effect of TdT **(terminal deoxynucleotidyl transferase)** during recombination → CDR3 variability is higher
- Random combination of TcR α/β and γ/δ chains (like Ig H/L chains)



Primary lymph organs

Peripheral lymph organs

Figure 1-14 Immunobiology, 6/e. (© Garland Science 2005)

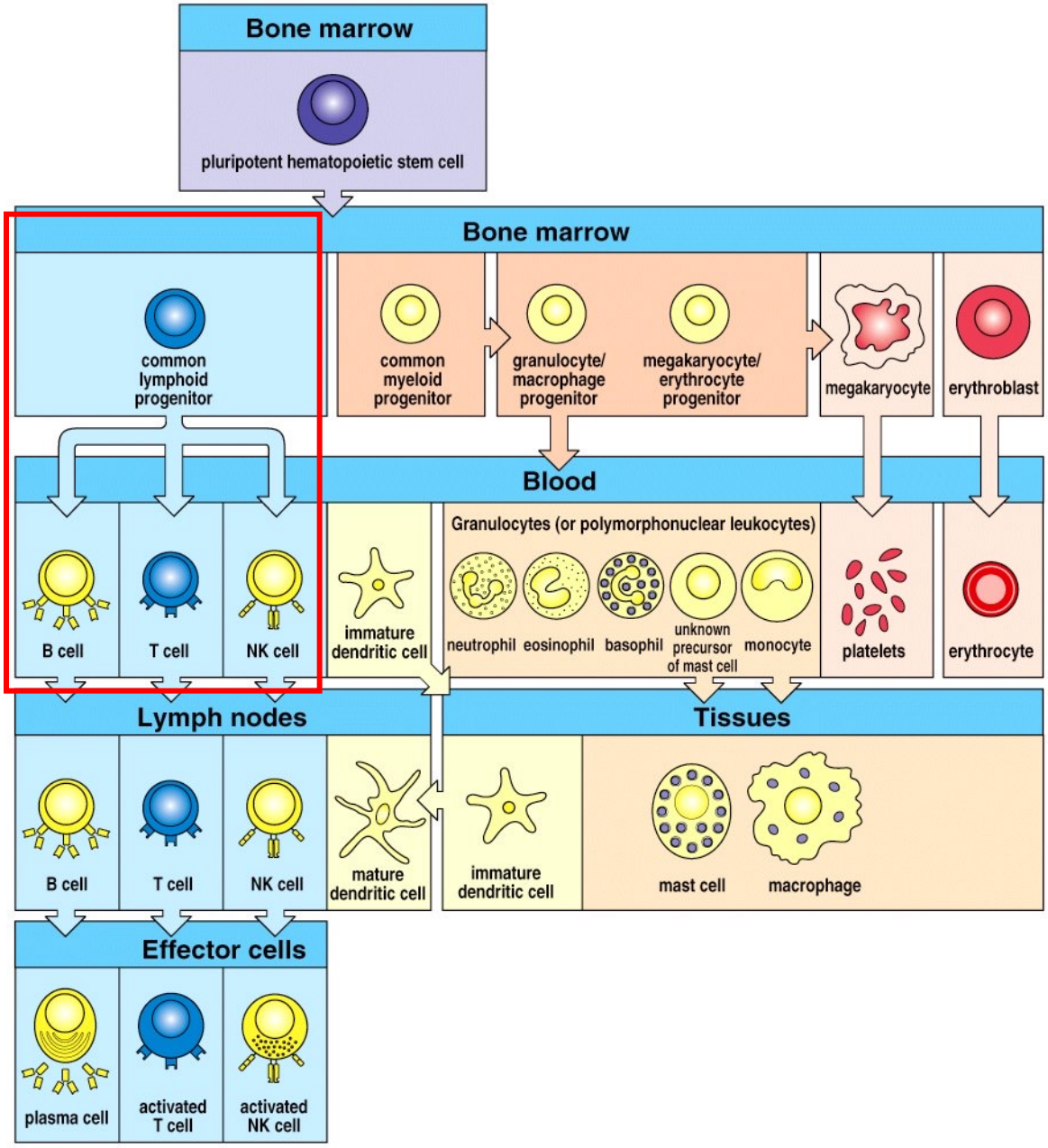
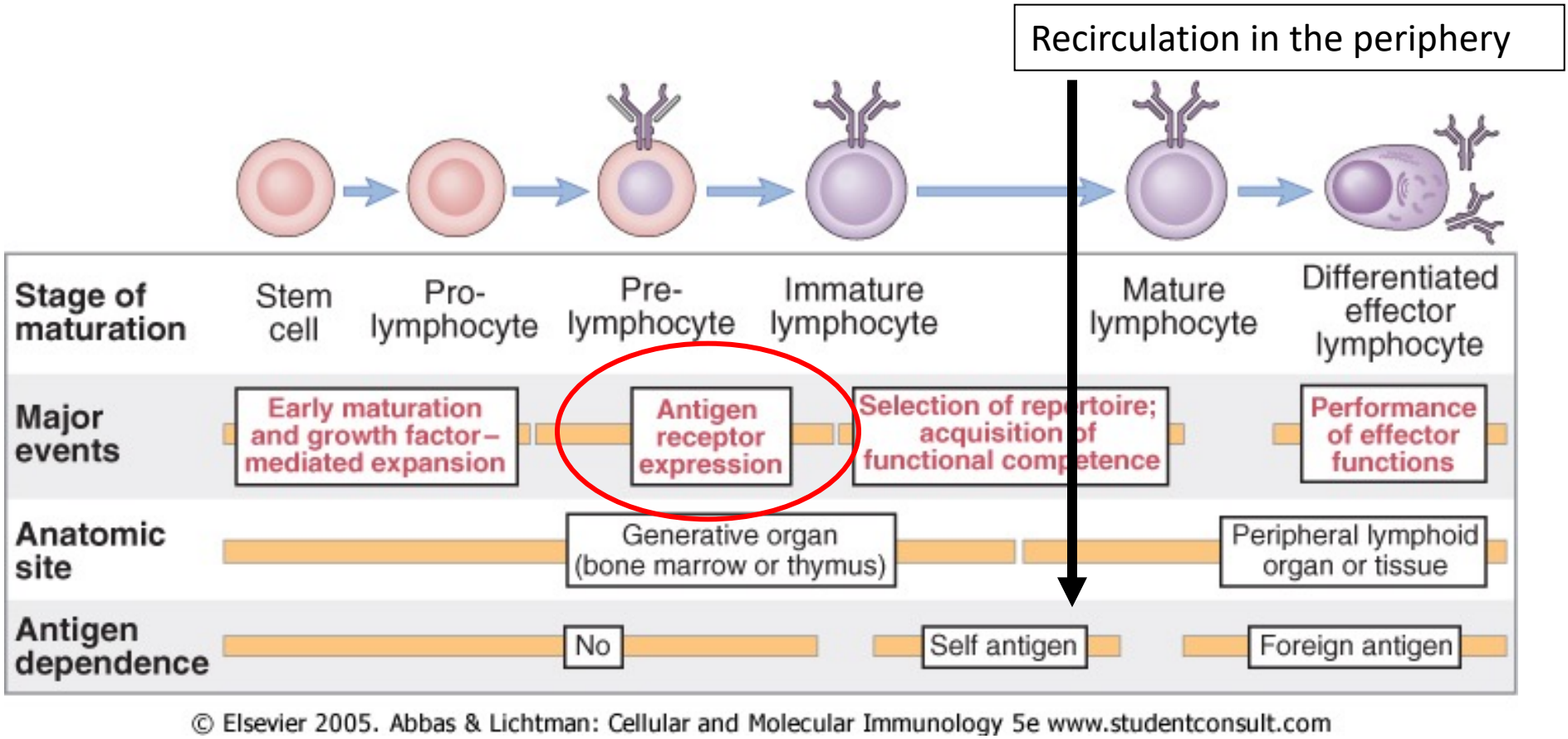


Figure 1-3 Immunobiology, 6/e. (© Garland Science 2005)

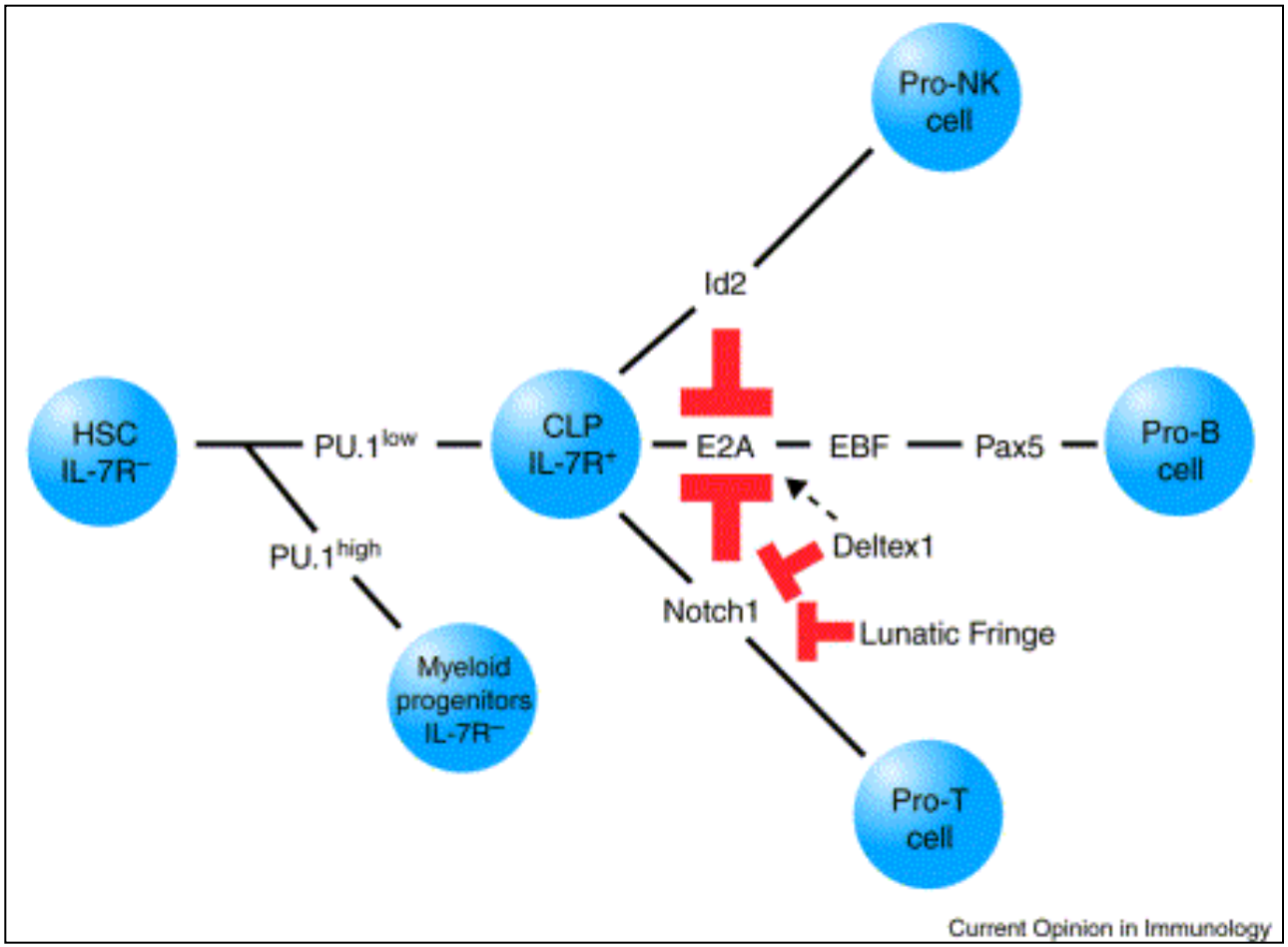
General characteristics of lymphocyte differentiation

1. Proliferation
2. Receptor-Gene rearrangement
3. Migration
4. Selection
5. Apoptosis

Steps of lymphocyte development



B/T/NK commitment – default E2A (B) path overruled by Notch (T) and/or Id2 (NK) signals



Role of BM stroma

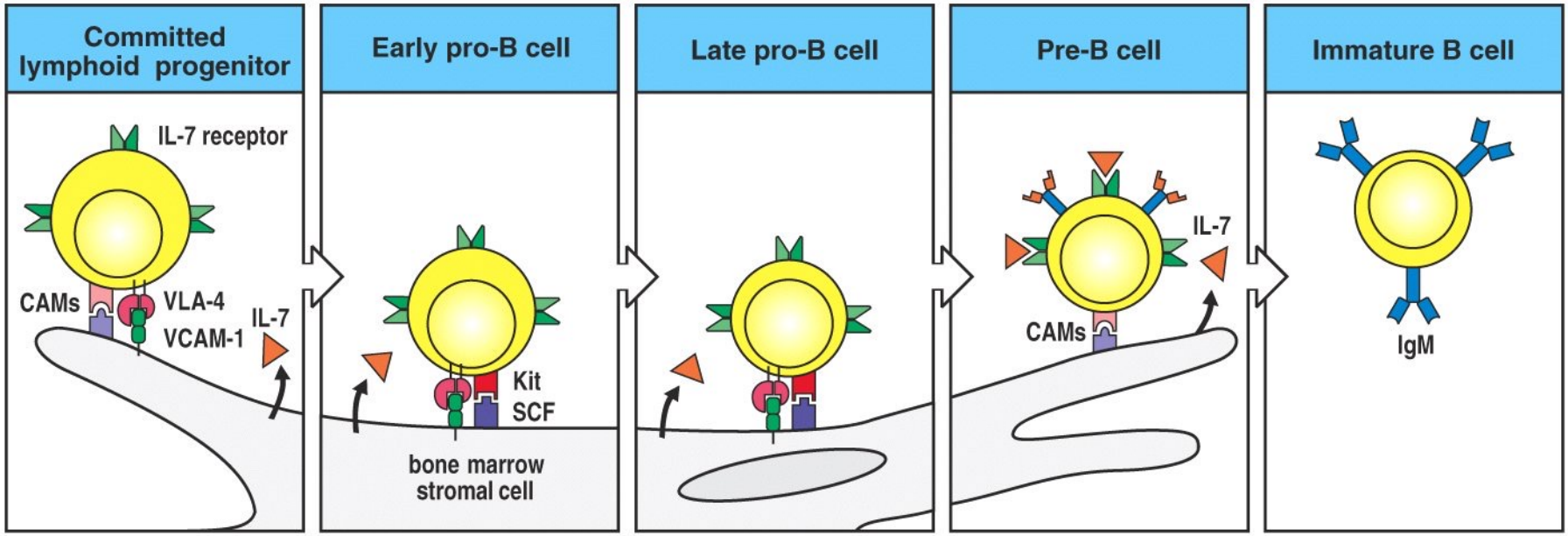
1. **Adhesion: –** CD44, VCAM-1

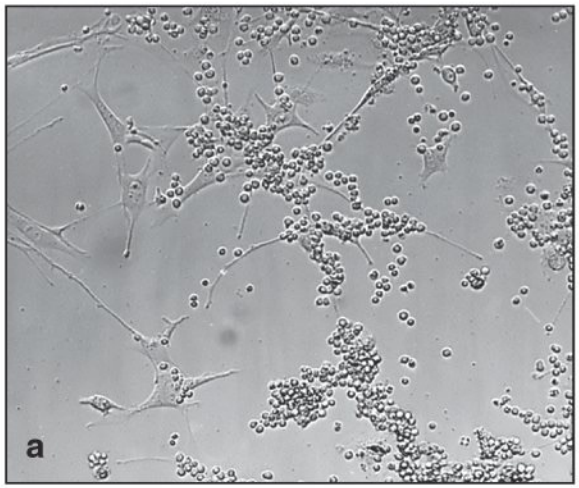
2. **Growth factors:** IL-7, IL-3, SCF**.**

3. **Response modifiers:** Wnt factors, ECM components**.**

4. **Chemokine-production:** SDF-1/CXCR4 ligand**.**

Elements of B:stromal interactions





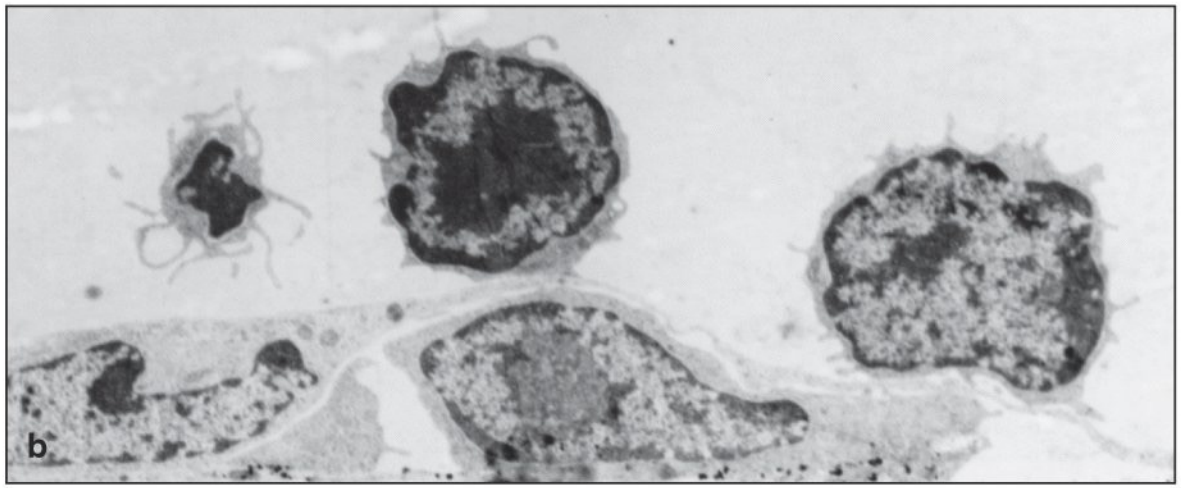


Figure 7-4 Immunobiology, 6/e. (© Garland Science 2005)

B-cell development I: HSC > "Large pre-B"

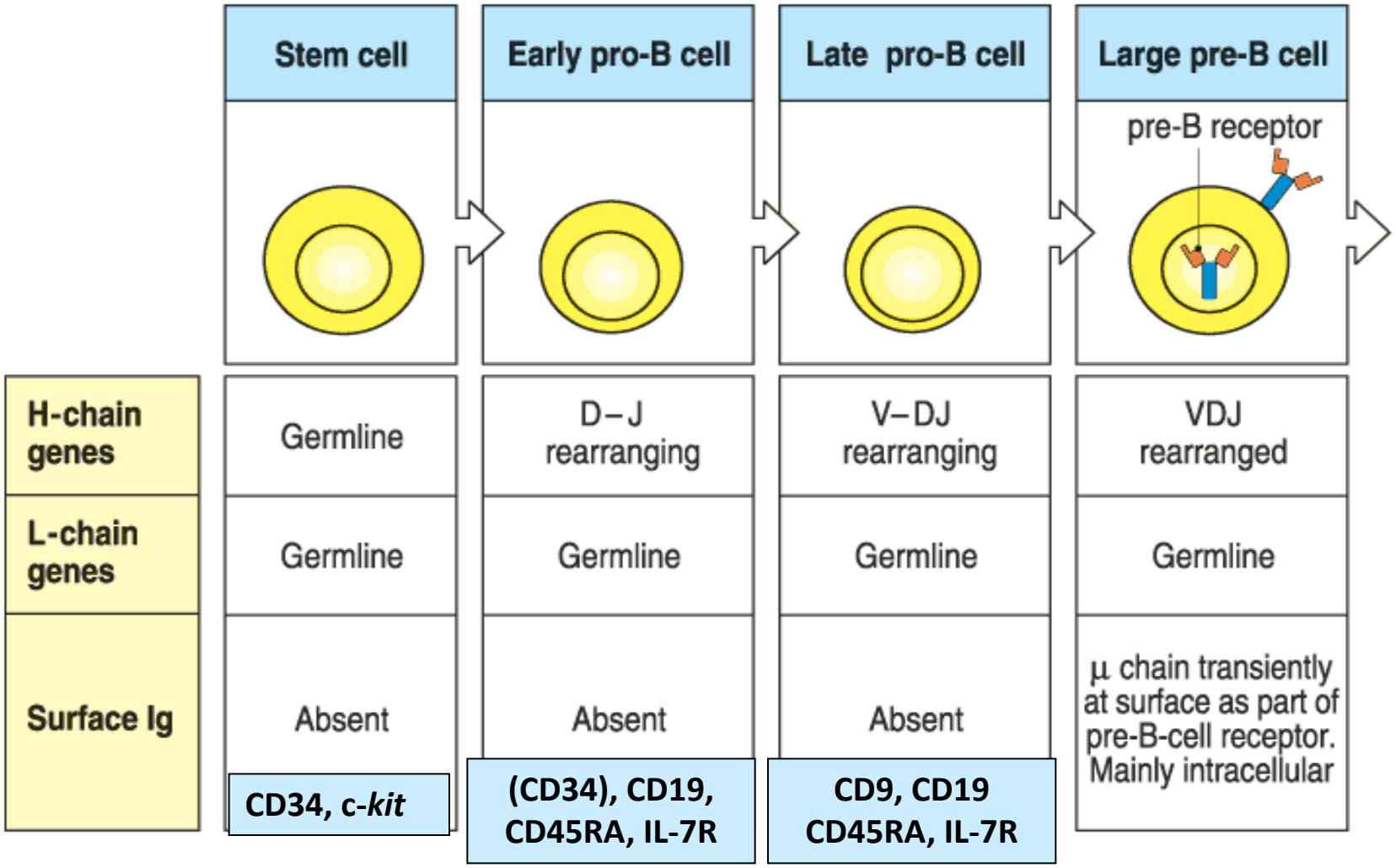
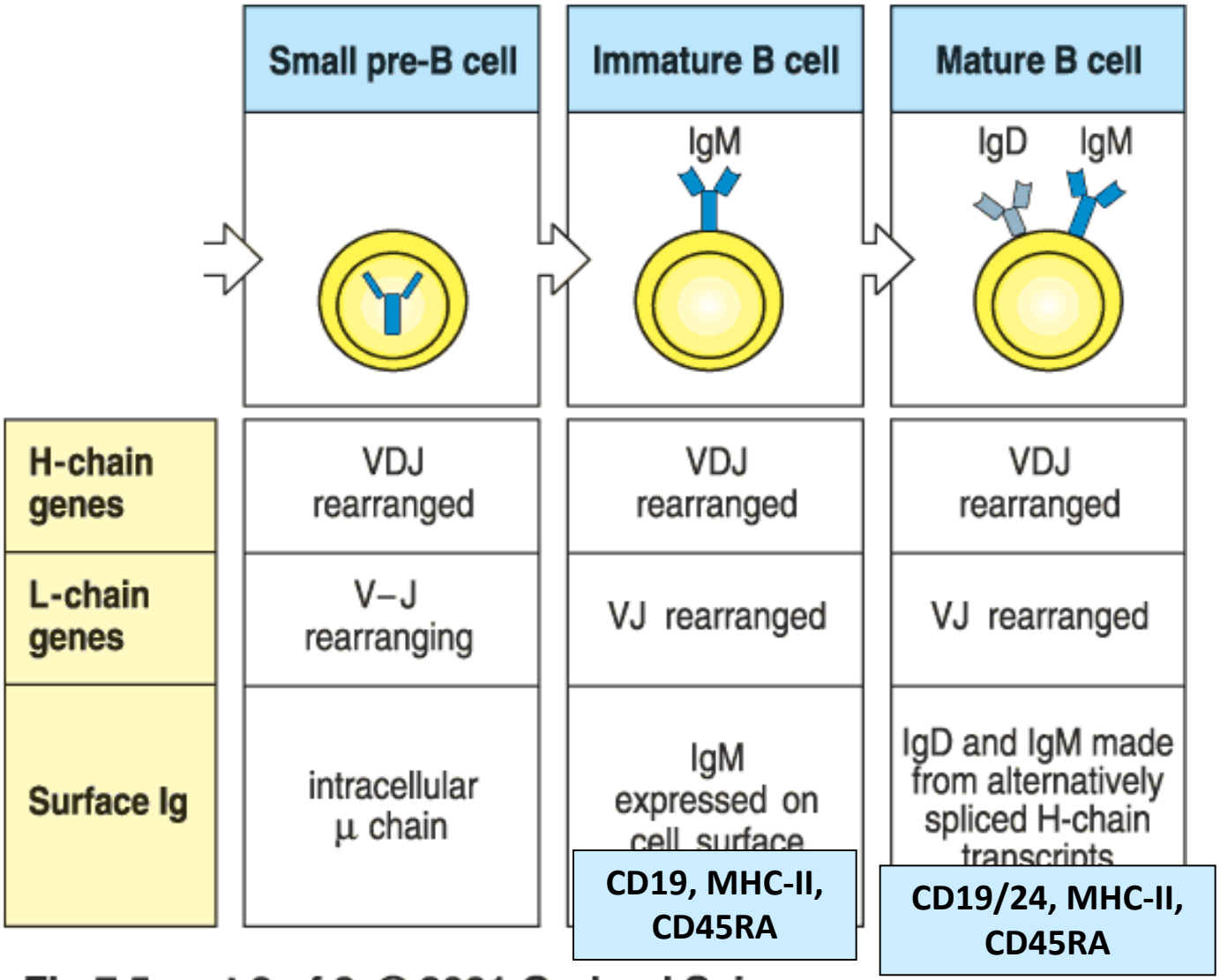


Fig 7.5 part 1 of 2 © 2001 Garland Science

B-cell development II “Small pre-B” > “mature B”



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Ontogenic differences between B-cell subsets

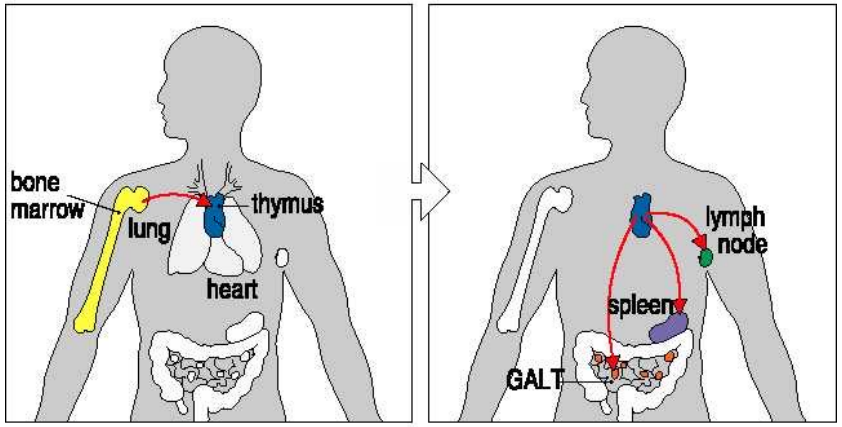
- **B-1 B cell subsets:** fetal origin, self-renewal, low-affinity autoantibody production, dominance in neonates and CLL, located in body cavities.

  (CD5+, CD43+, IgM++/IgD+)
- **Marginal zone B cells**: Ig phenotype similar to B-1 B cells, adult BM origin, distinct developmental regulation to Fo B cells, relatively sessile.

  (IgM++/IgD+, CD21++, CD23+/-)
- **Conventional follicular B cells.** (IgM+/IgD++, CD21+, CD23++, recirculating).

T-cell development in the thymus.

Figure 5.1



Production of T cell repertoire

Total repertoire:
TCR α, β: 10^{15}
TCR γ, δ: 10^{16}

T cell precursors are produced in the **bone marrow** from the common haemopoietic stem cell
They migrate through the blood circulation to the thymus

In the thymus: T cell maturation, educational steps
„double recognition" (MHC and peptide)

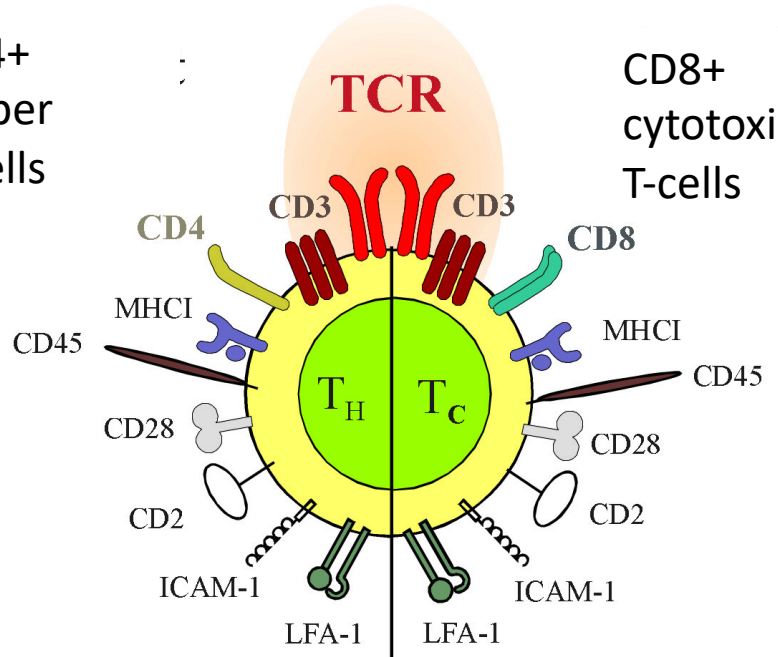
Periphery: mature, TCR expressing, CD4 or CD8 positive T cells → **Self-MHC restricted**

Self-tolerant T cells

Two different T cell lines with different receptor types (TcR)

T-lymphocytes with αβ TcR

CD4+ helper T cells



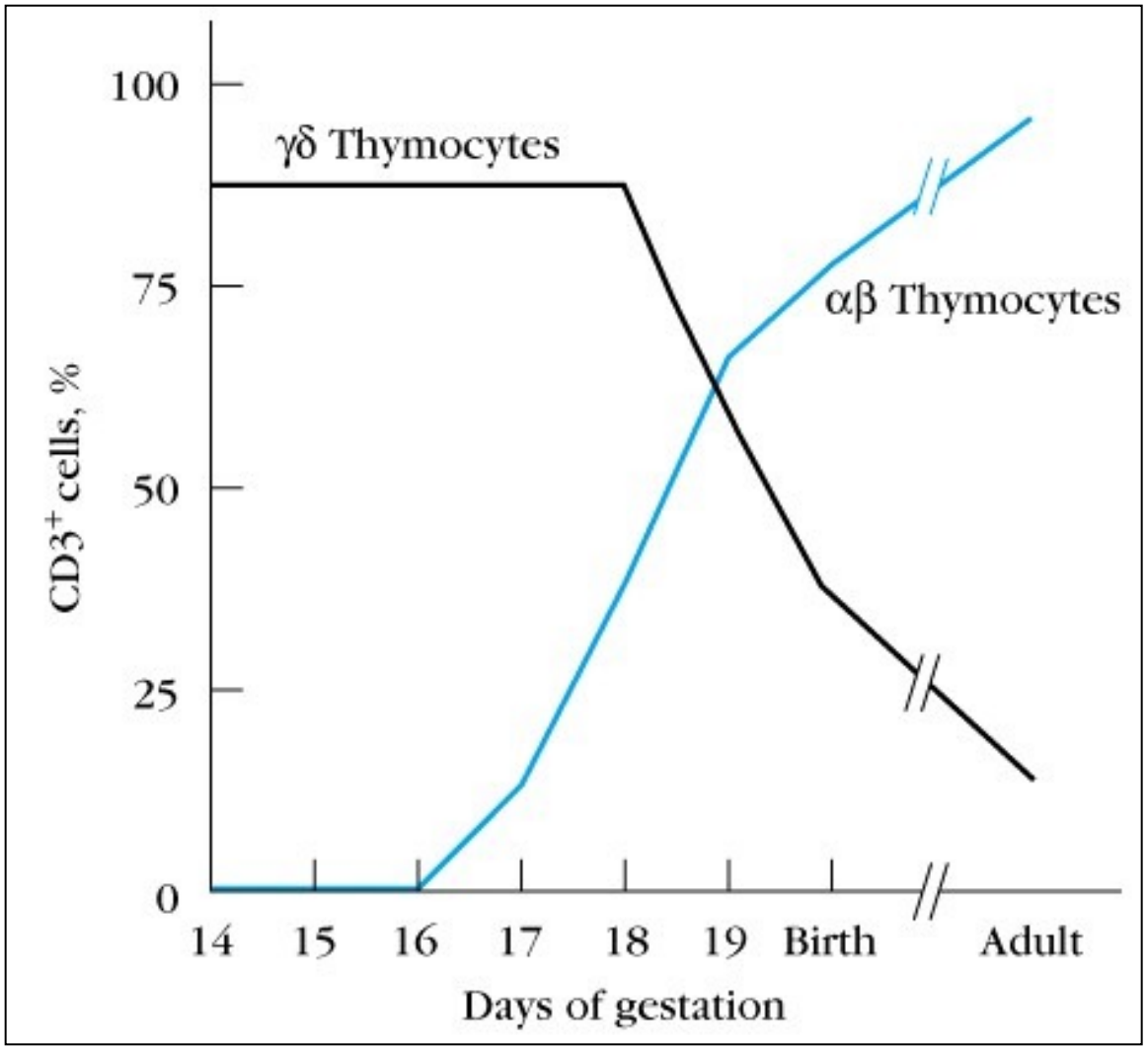
CD8+ cytotoxic T-cells

T-lymphocytes with γδ TcR

- CD4-CD8- cytotoxic T-cells

Intraepithelial – with lower TcR diversity

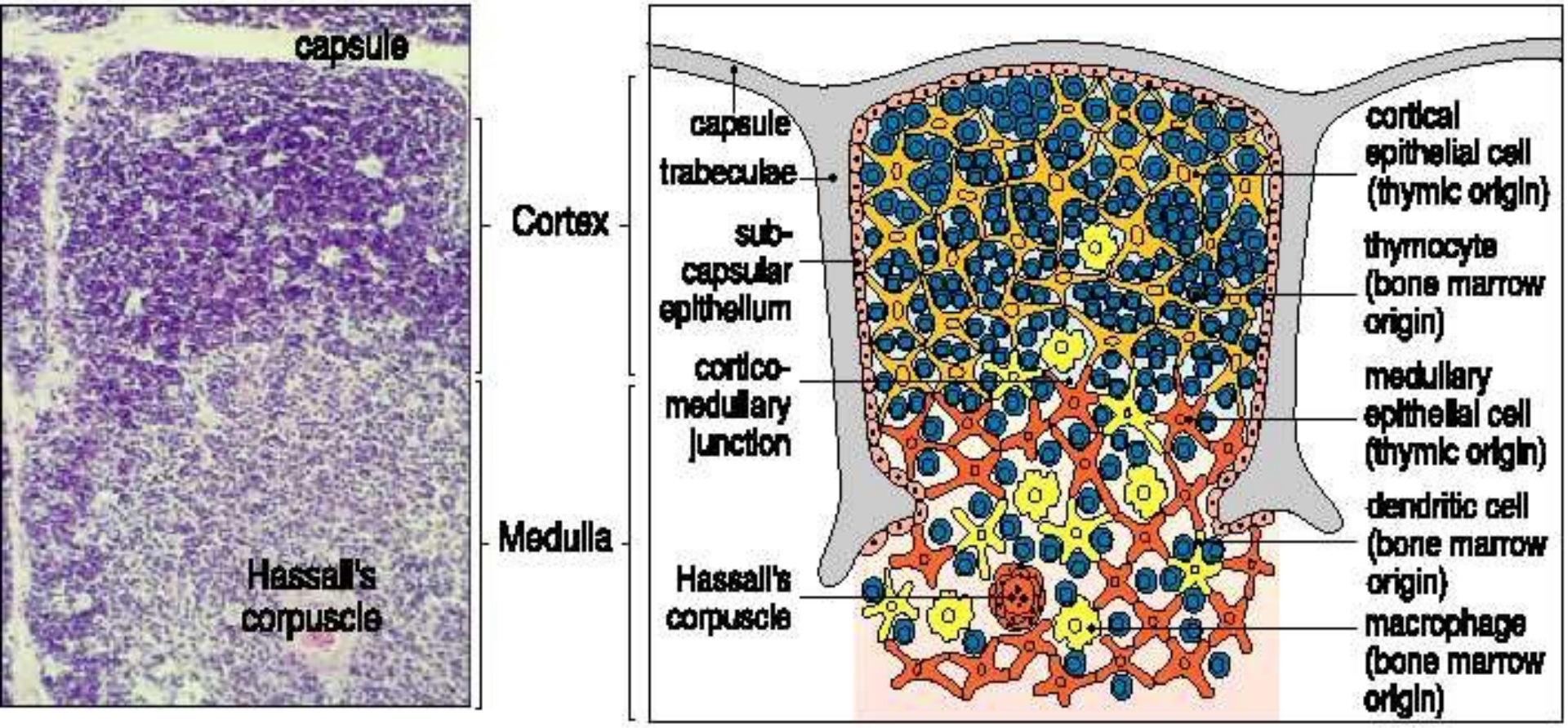
Lymphoid tissues – highly diverse receptors
Regulatory cytokine production



Full repertoir:
TCR α, β: 10^{15}
TCR γ, δ: 10^{16}

Structure of the thymus

Figure 5.3



The thymic stoma creates the microenvironment that is essential for T-cell development

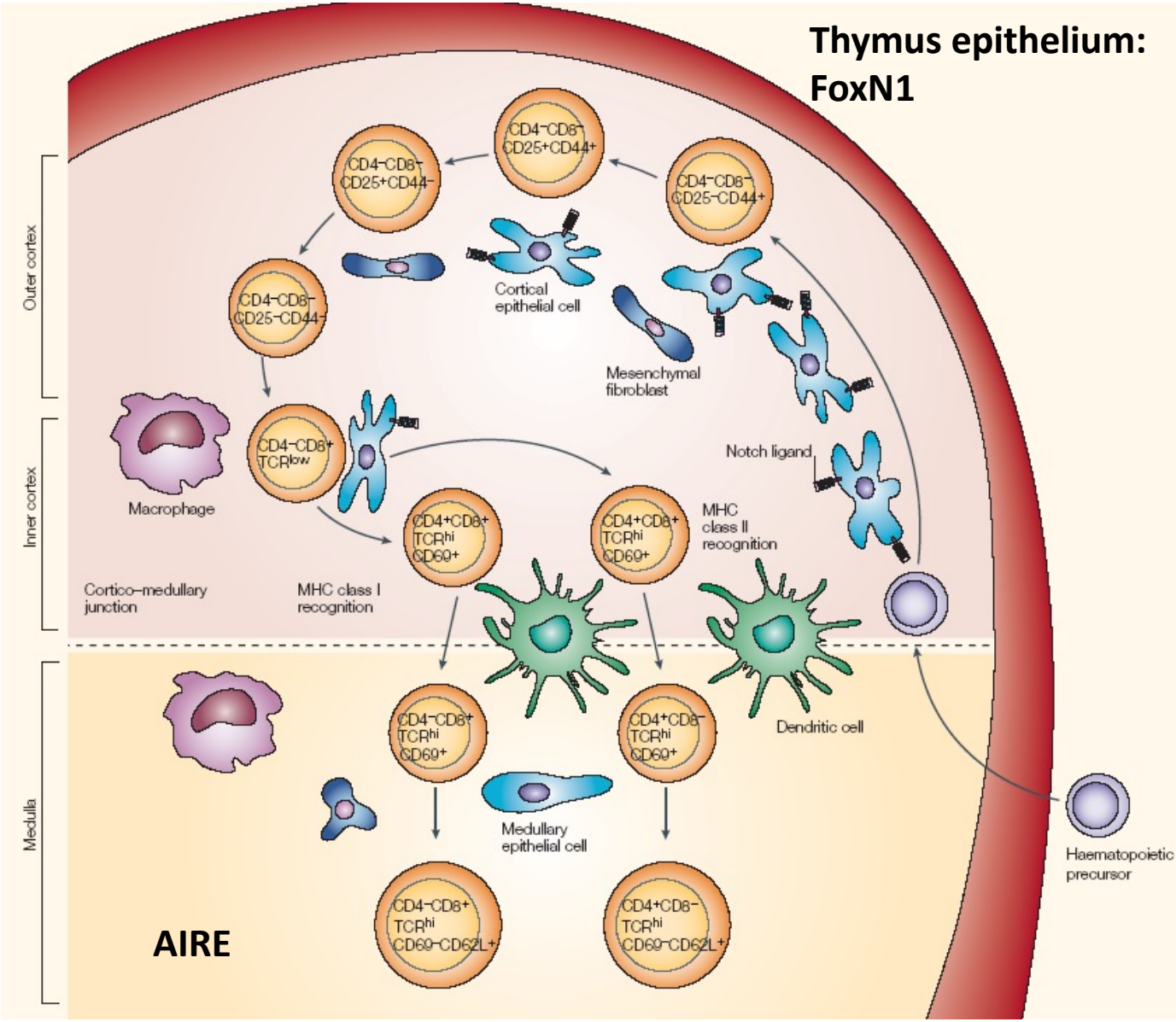
Thymic Microenvironment and T-cell Development

1. **Migration:**
   Chemokine effect

2. **Proliferation**
   IL-7

3. **Differentiation**
   - TcR-rearragement
   - Phenotypes

4. **Selection**
   Apoptosis



T-cell development in the thymus

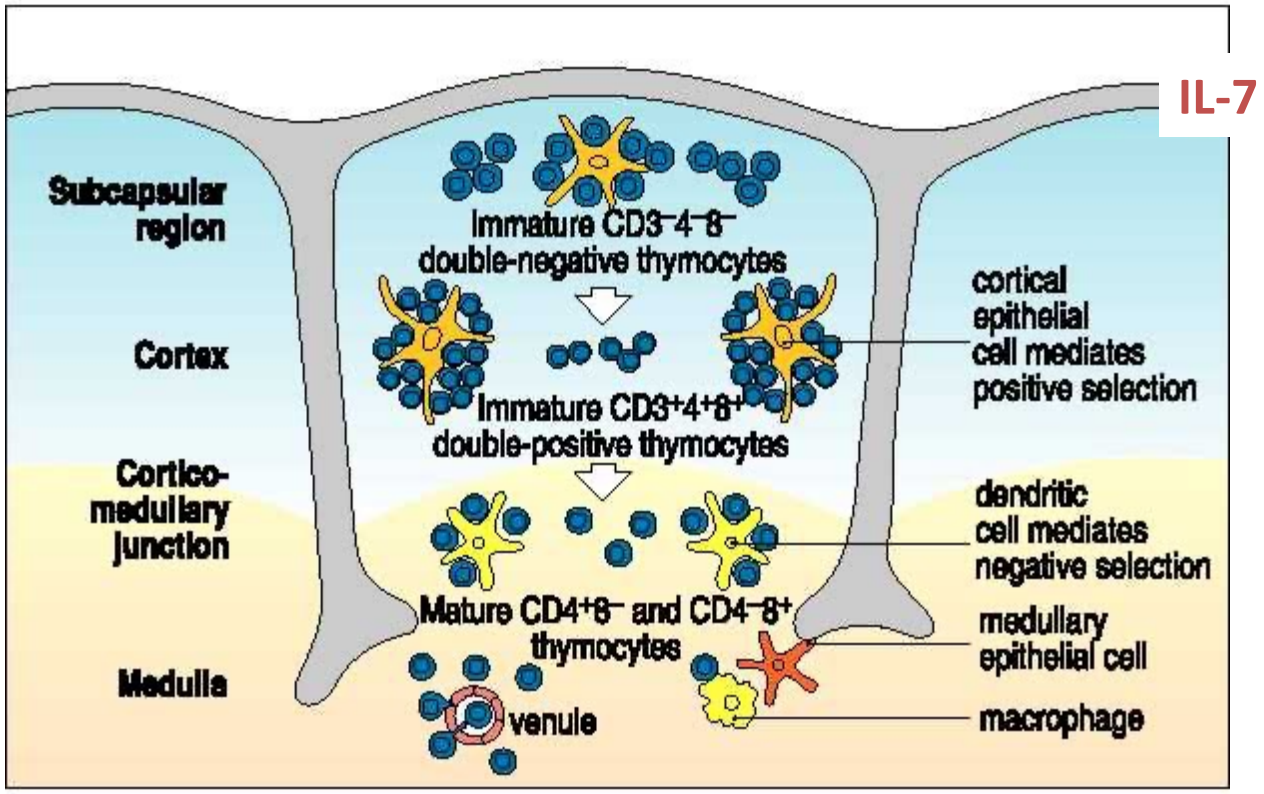
Thymocytes:

DN: 2-5 %

DP: 70-80%

CD4 SP: 10-15%
CD8 SP: 5-8%

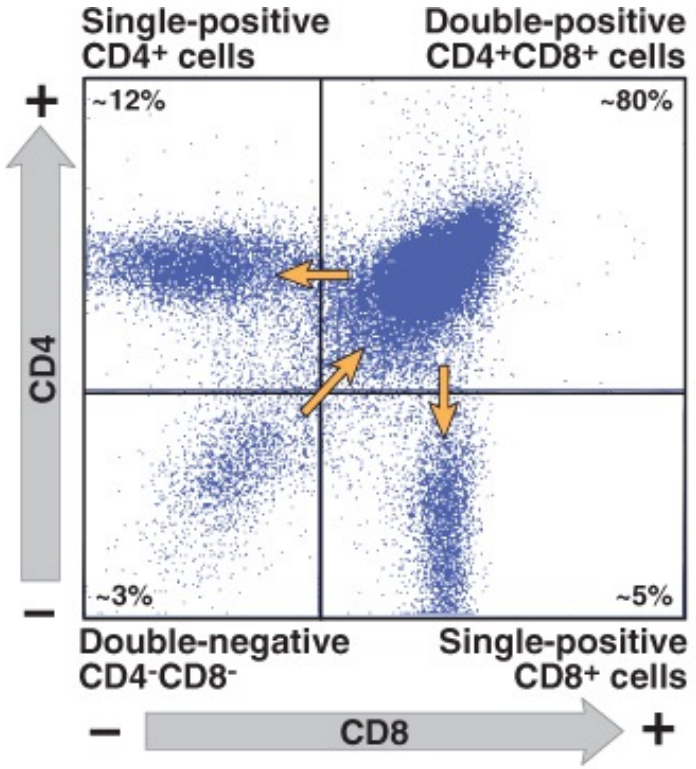
Figure 5.14

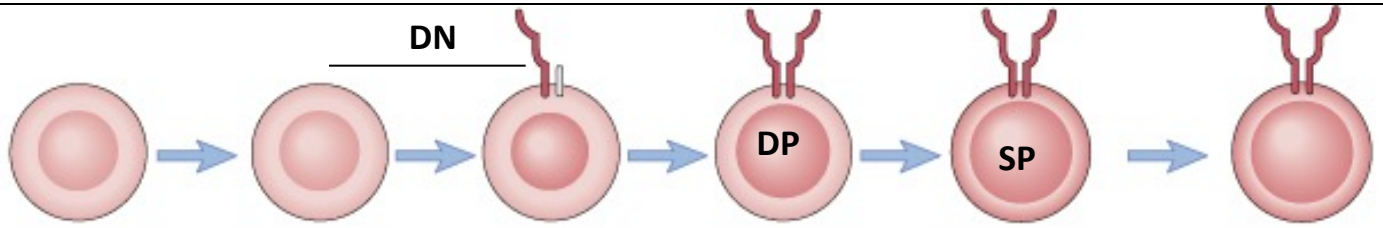


Young mouse: $5x10^7$ T-cells dayly
During selection 98 % of thymocytes die by apoptosis

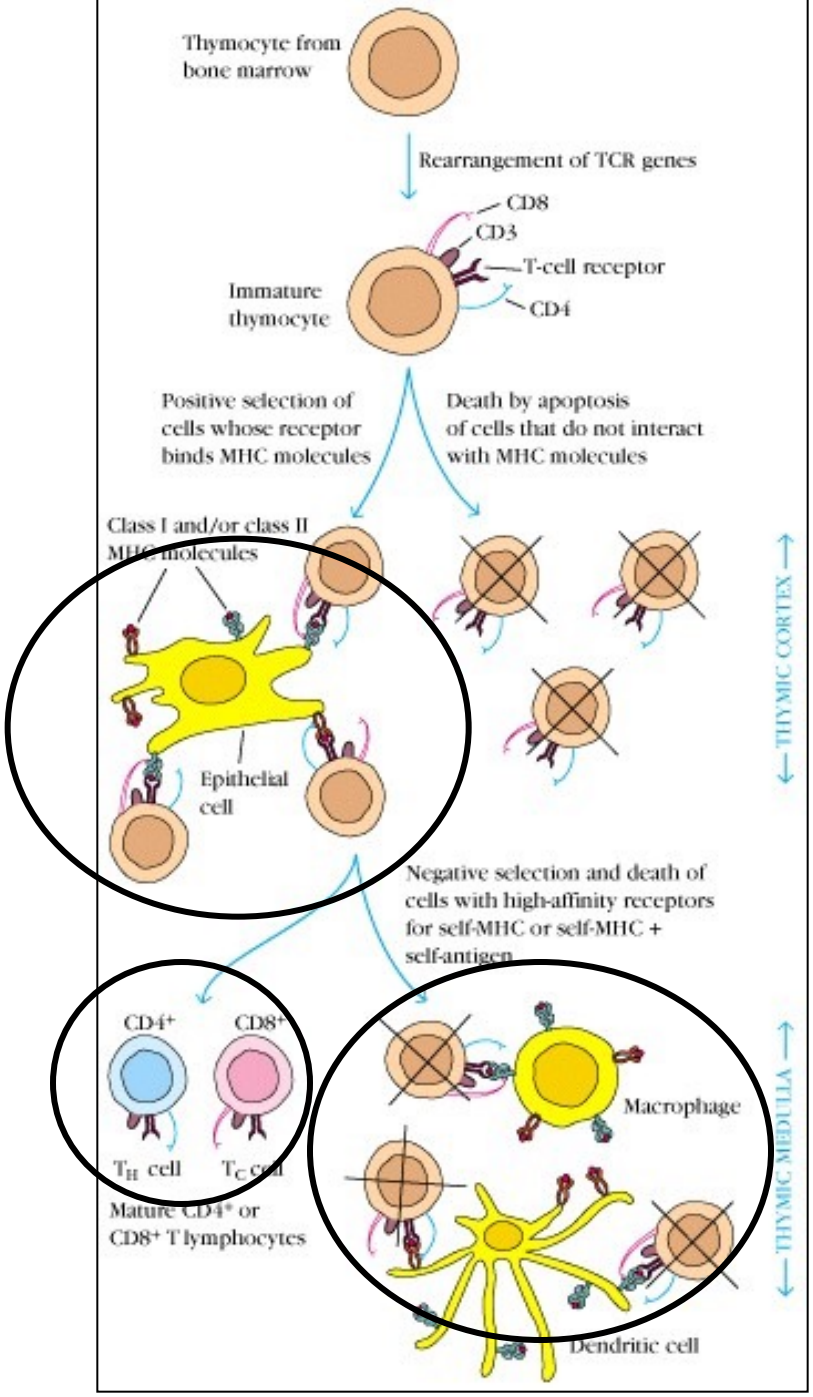
Dayly 1-2 x 10^6 mature T-cell migrate to the periphery

Thymocyte populations based on their cell surface markers





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	Negative selection	Activation (proliferation and differentiation)



Positive selection:

Epithelial cell - thymocyte interaction in the thymus cortex

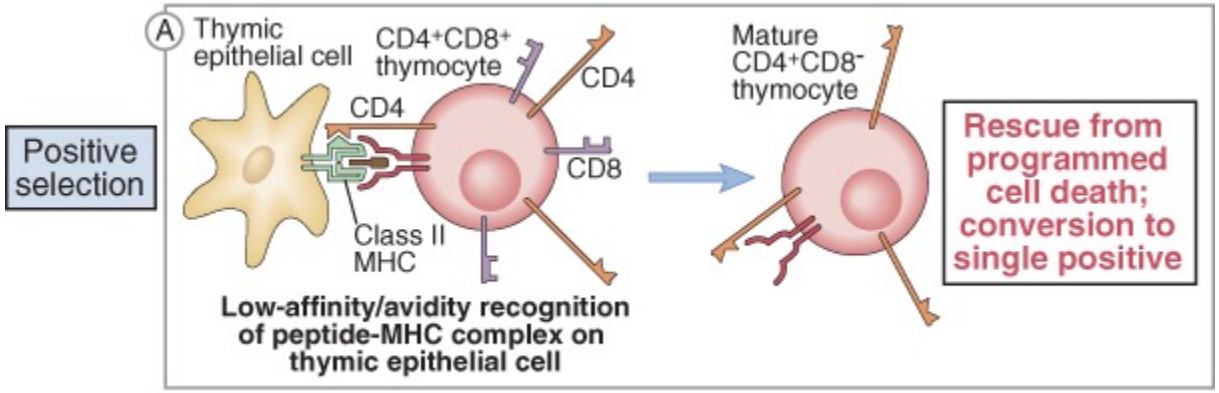
Survival of DP cells whose TcR is appropriate for self MHC recognition

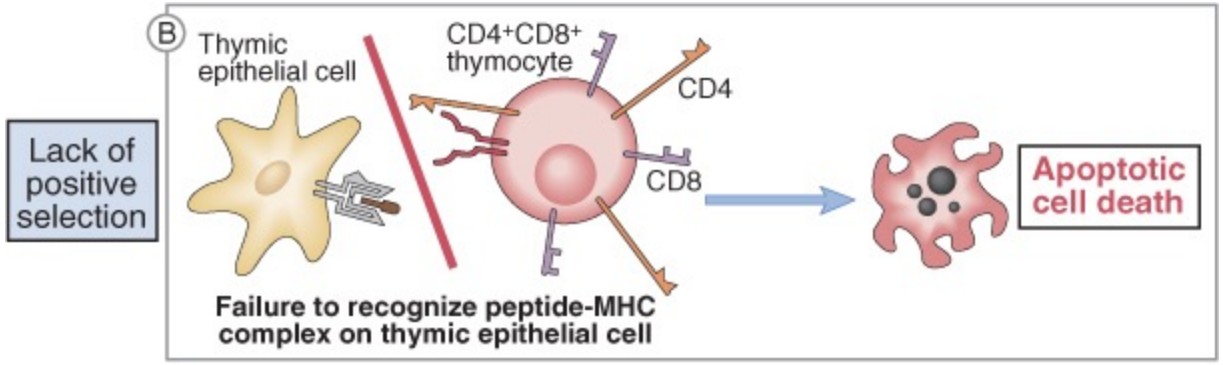
Negative selection:

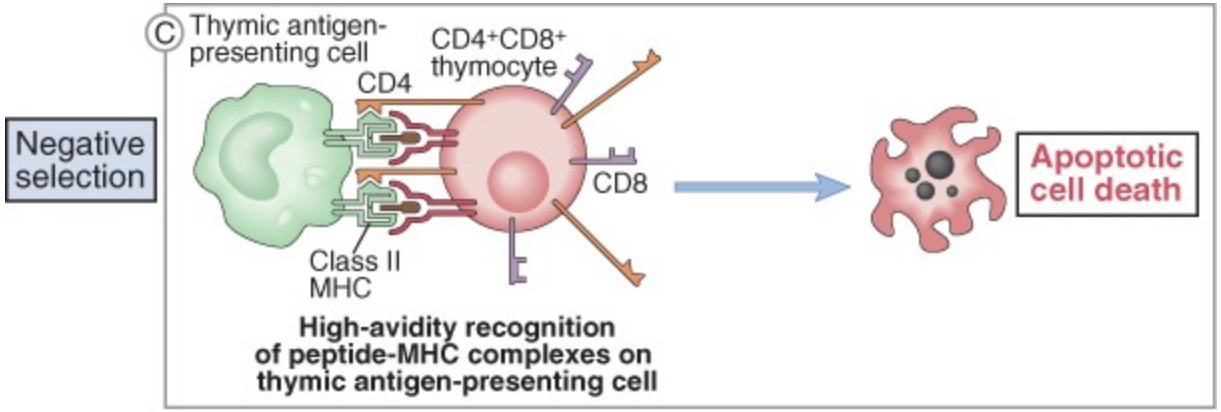
APC (macropahge or DC) – thymocyte Interaction in thymus medulla

Death of DP cells with high affinity TcR for self MHC + self peptide recognition

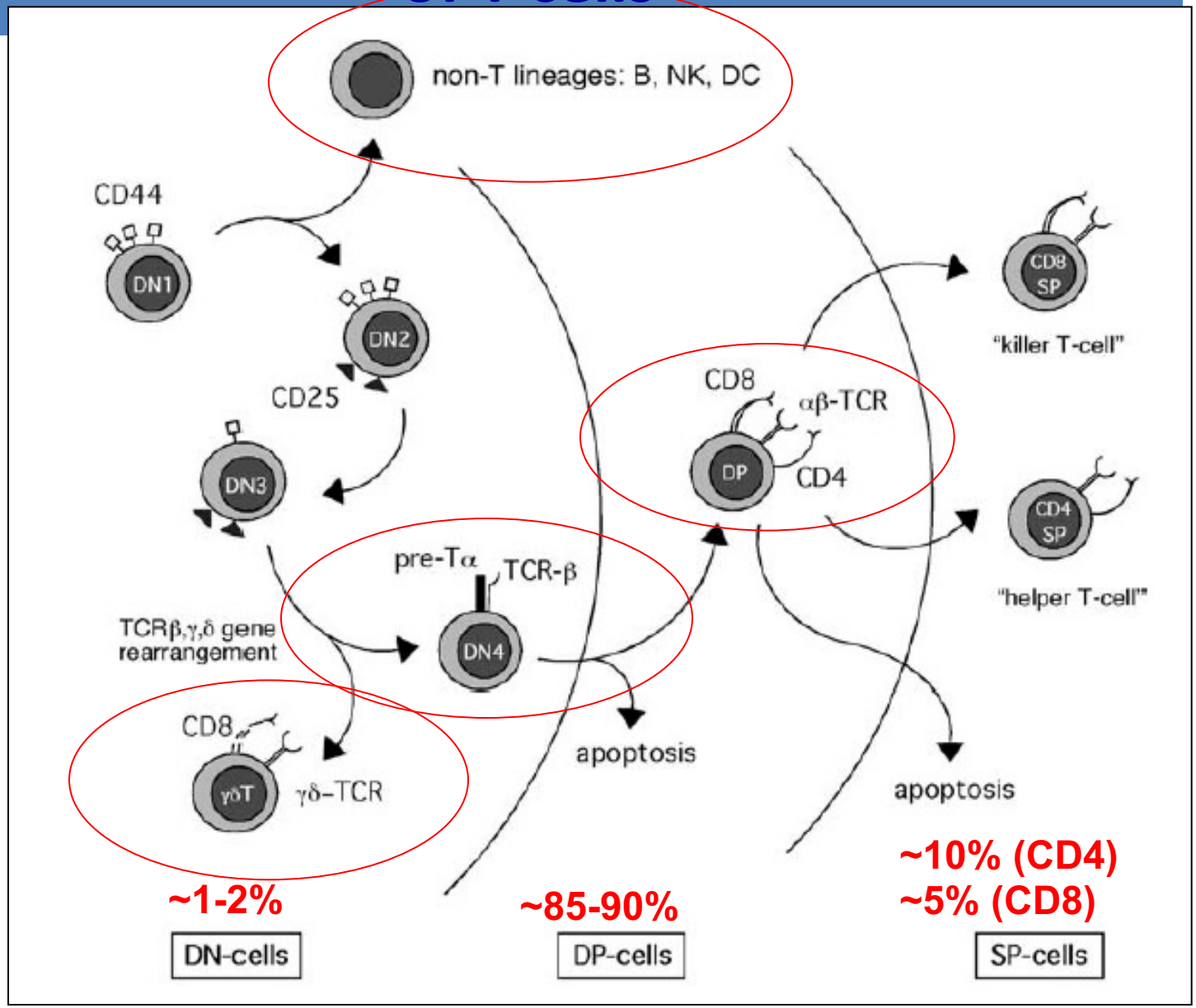
Differentiation into SP stage







Decision-making during the development of T cells



Phases of T-cell maturation in the thymus.

1. Initiation of either TCR β or γ/δ chain gene rearrangement.
2. Formation of pTα/TCRβ/CD3 (pTCR), allelic exclusion, IL-7-dependent proliferation - *β-selection.*
3. Initiation of TCRα gene rearrangement.
4. Completion of TCR α/β gene rearrangement, co-expression of CD4/CD8 molecules.
5. Recognition of MHC/peptide complexes displayed by thymic cortical epithelium – *positive selection*.
6. Binding to MHC/peptide complex displayed by thymic APC/medullary epithelial cells – *negative selection.*
7. Influence of stronger/more persistent signal: commitment towards CD4 or Treg (CD4/CD25+) subset.

„Checkpoints” in central B/T-lymphocyte development