

Basic Immunology

Dentistry

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

Central B-cell development.

Central (thymic) T cell development.

Ferenc Boldizar

Cells of the lymphoid lineage

Innate lymphoid cells (ILC)



Lymphocyte



There is no difference
in the morphology!

HAVE NO ANTIGEN-RECOGNITION
RECEPTORS

HAVE ANTIGEN-RECOGNITION
RECEPTORS

NATURAL



$\gamma\delta$ T cell



B1 B cell

LYMPHOCYTES



T cell (CD3+)



B cell (CD19+)

ADAPTIVE



$\alpha\beta$ T cell



B2 B cell



Helper T cell (CD4+)

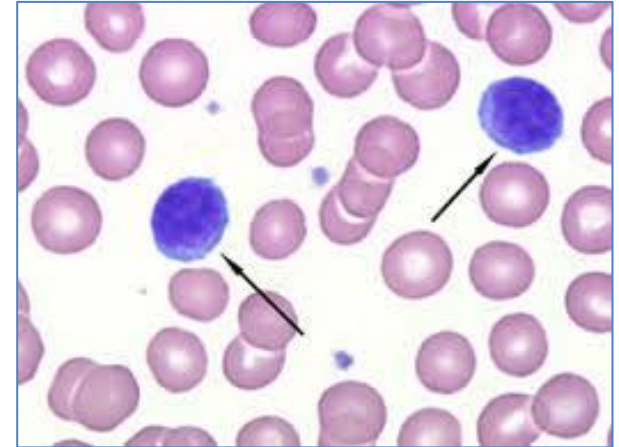


Cytotoxic T cell (CD8+)

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+)



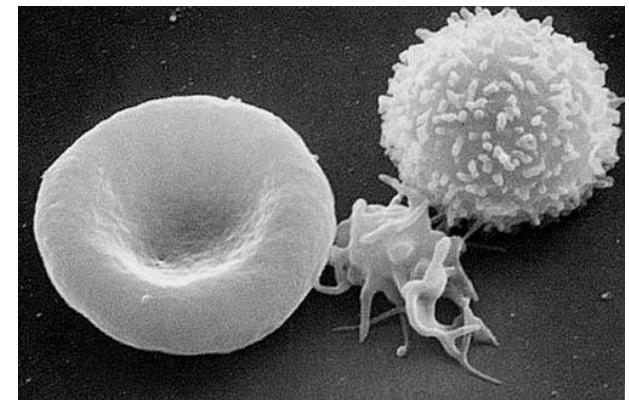
Direct killing of
target cell (infected
or cancerous)



Helper T cell (CD4+)



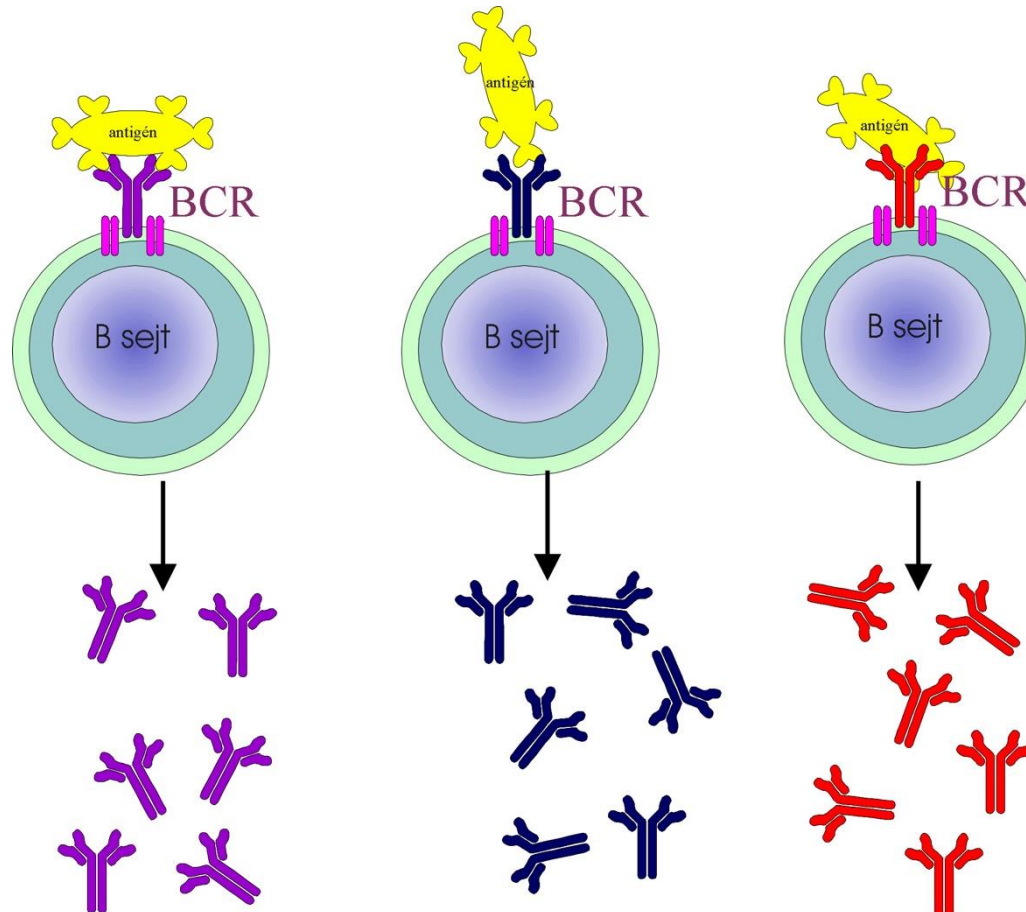
Regulation of the
immune response



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

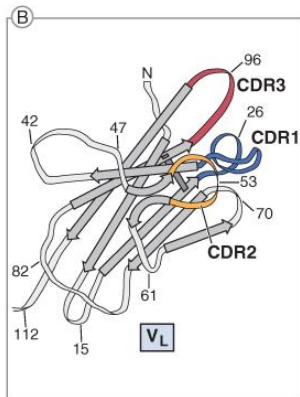
- Expression of Antigen receptors with different specificities
- Production of B- and T cell repertoire = Number of antigen recognition molecules: 10^9 - 10^{11} BcR, 10^{15} - 10^{16} TcR;

„Lymphocyte production = Glove factory” – Jan Klein.

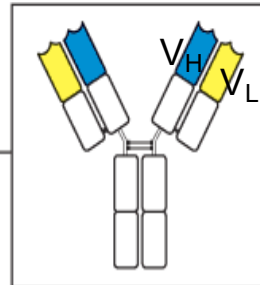
The immune system produces antigen receptors for all potential antigens and is therefore ready to recognize those structures.

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



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V = Fab

C = Fc

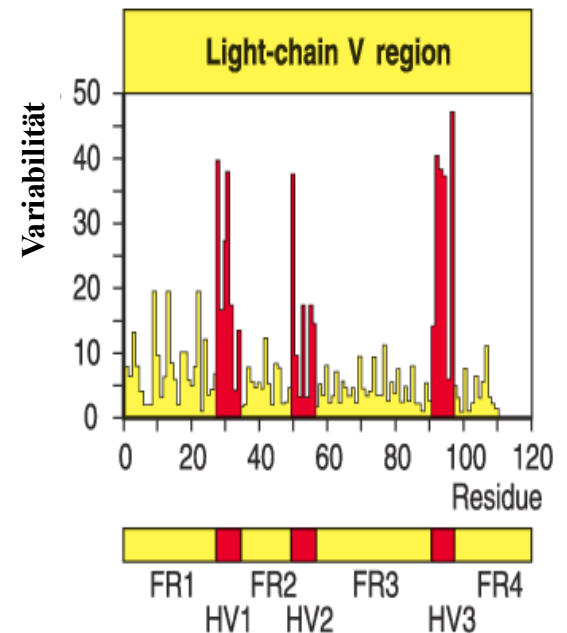
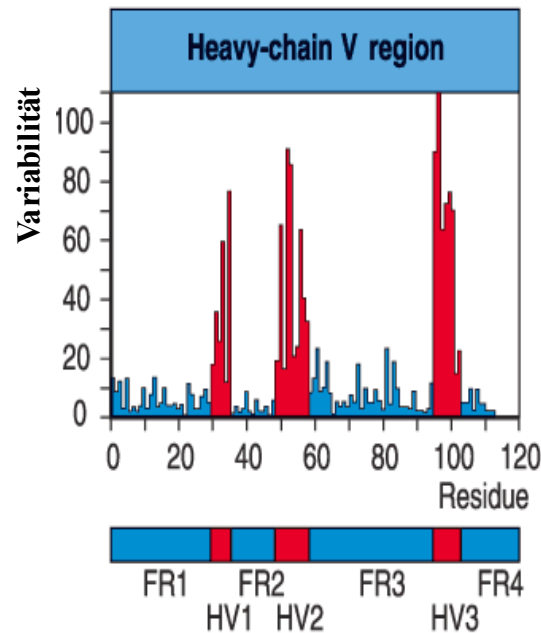
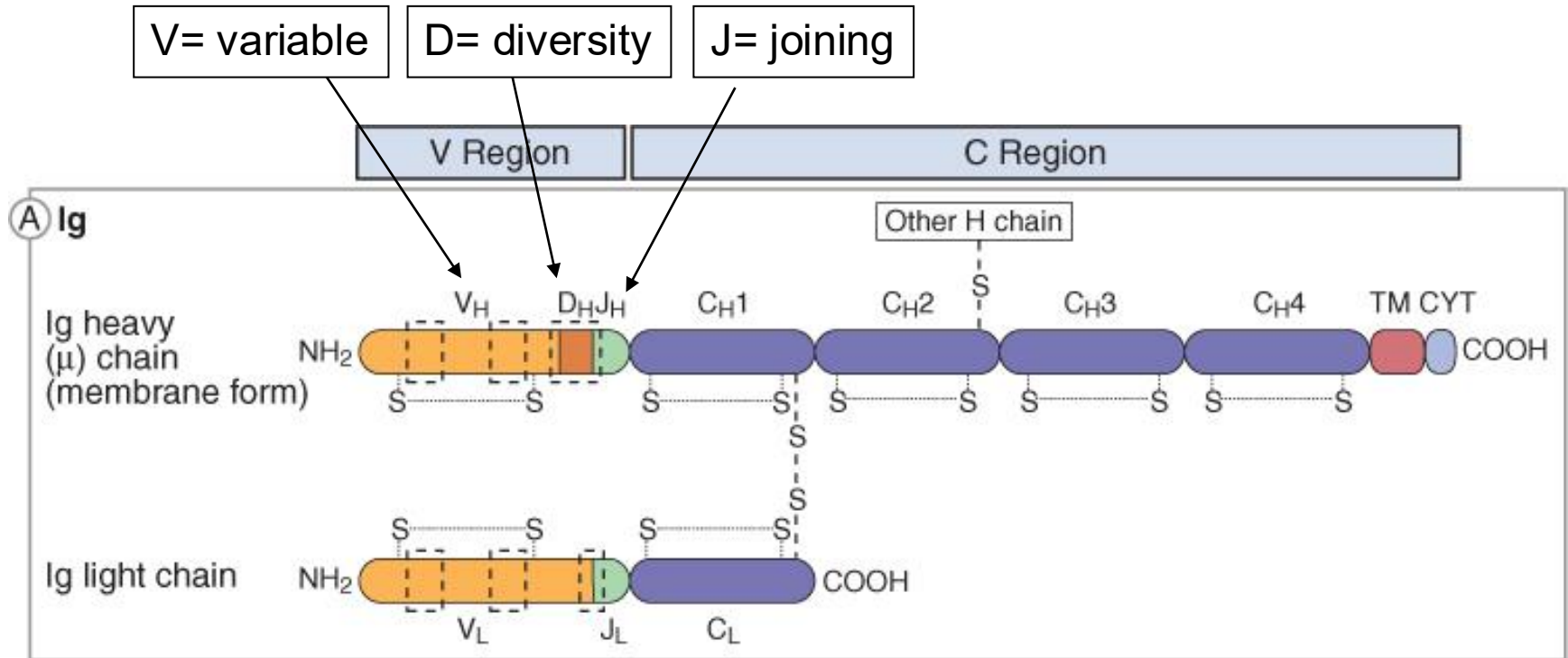


Fig 3.6 © 2001 Garland Science

Domains of the immunoglobulin heavy- and light chains



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- 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.

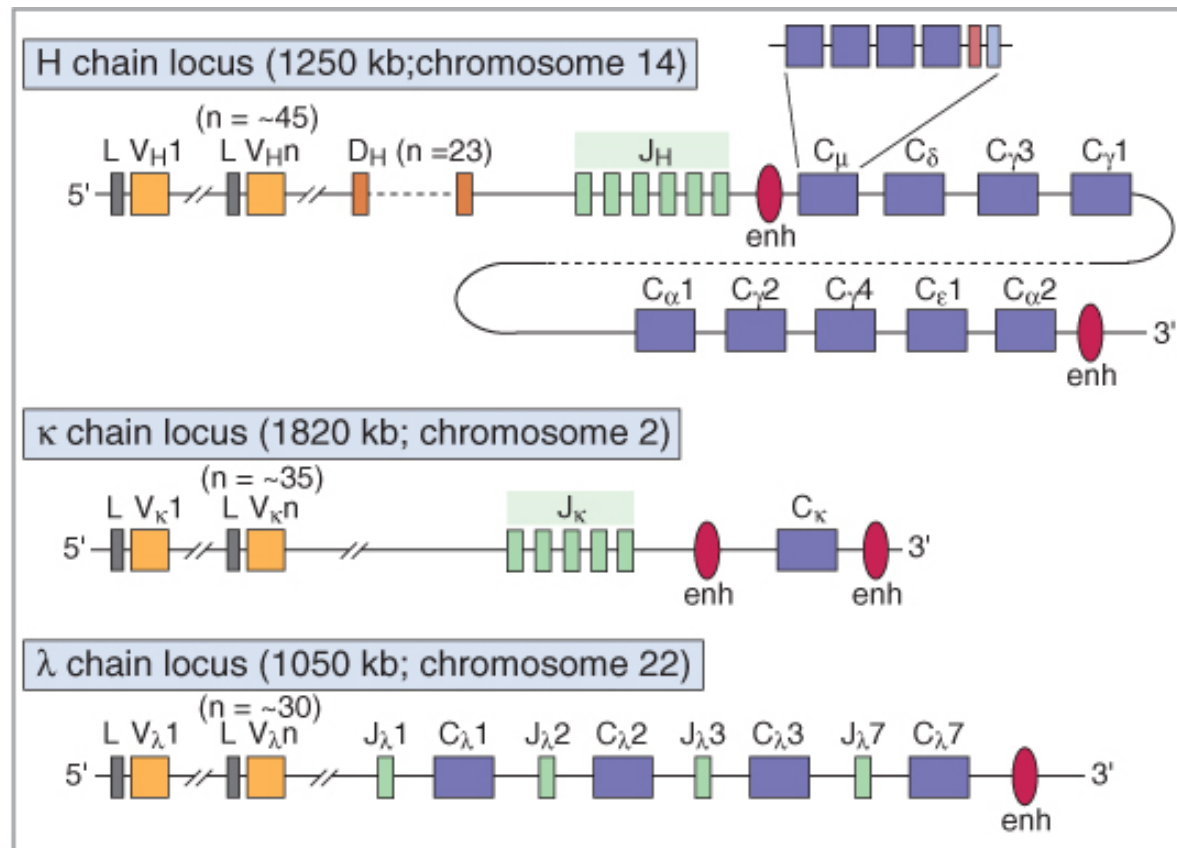
Gene organisation of the immunoglobulin heavy- and light chain loci

V-Region:

V = Variable
D = Diversity
J = Joining
 Gensegmente

C-Region:

C = Constant
 Gensegmente

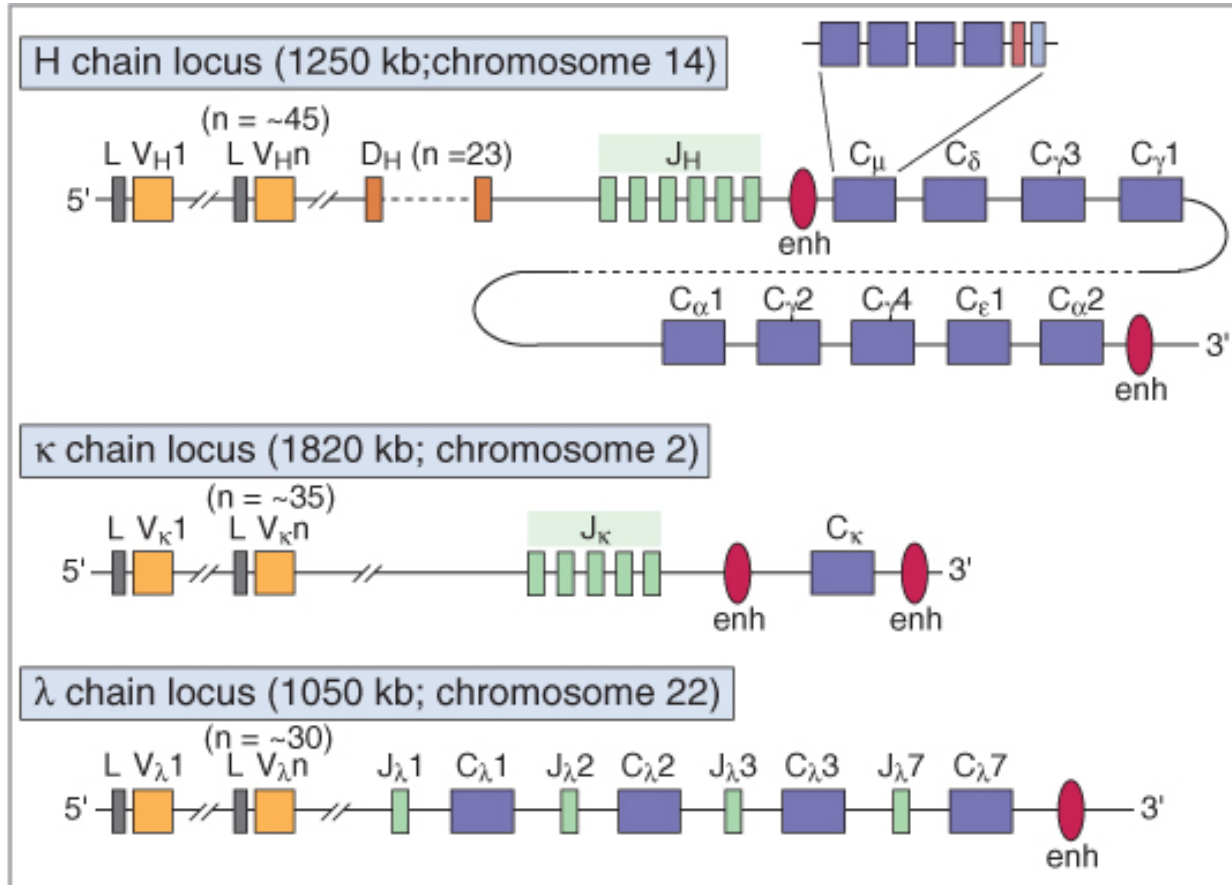


C_μ - IgM
 C_δ - IgD
 C_γ - IgG
 C_α - IgA
 C_ε - IgE

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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):
C_μ, C_δ, C_{γ1-4},
C_α, C_ε

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

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

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

Steps of the gene rearrangement

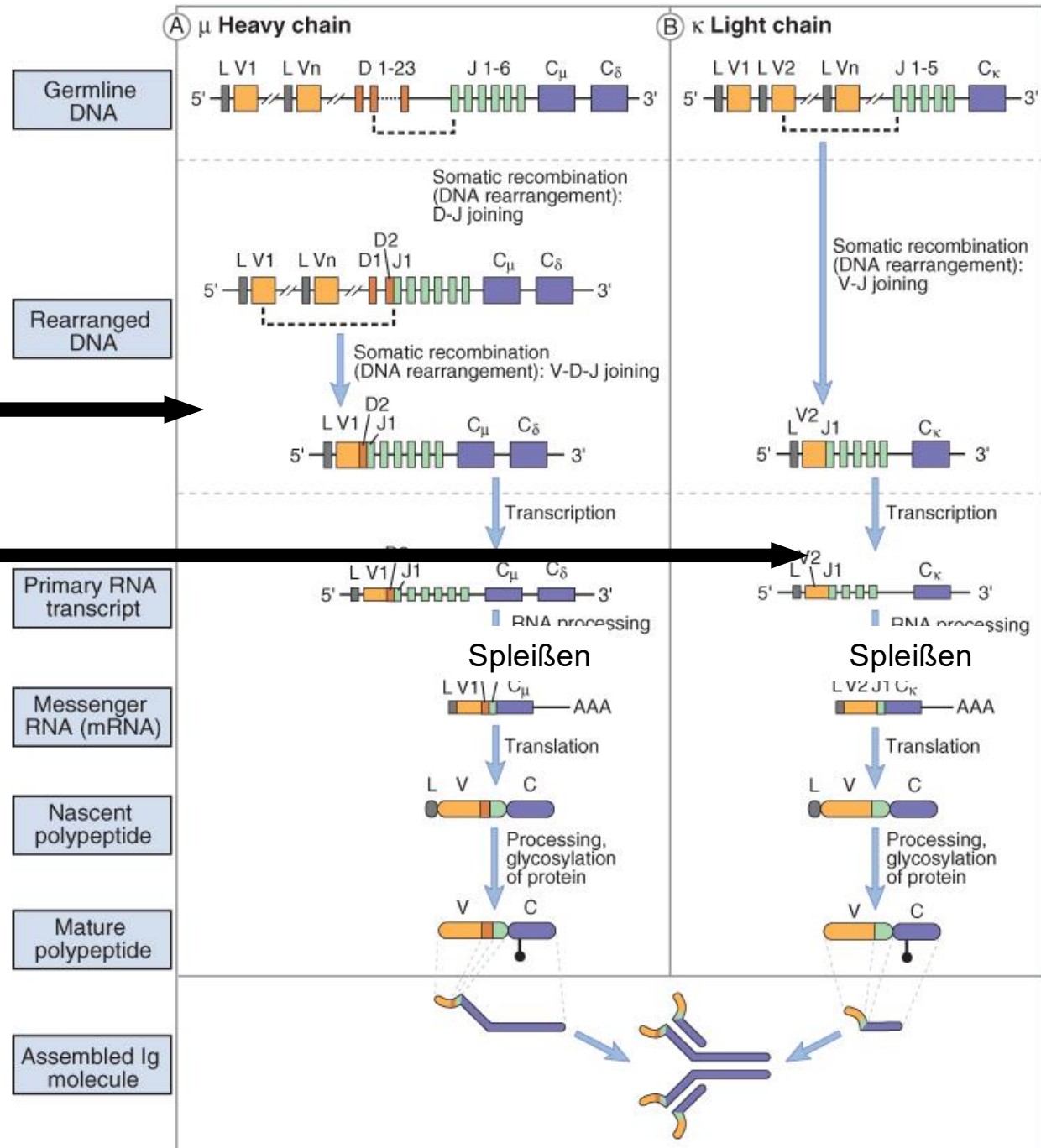
Allelic exclusion:

The succesful(productive) rearrangement of one heavy chain allele inhibits the recombination of the H locus on the other chromosome

Isotype exclusion:

The rearrangement of the κ light chain inhibits the λ Gene rearrangement and vice versa

All B cells express one single specific immunoglobulin (BcR).



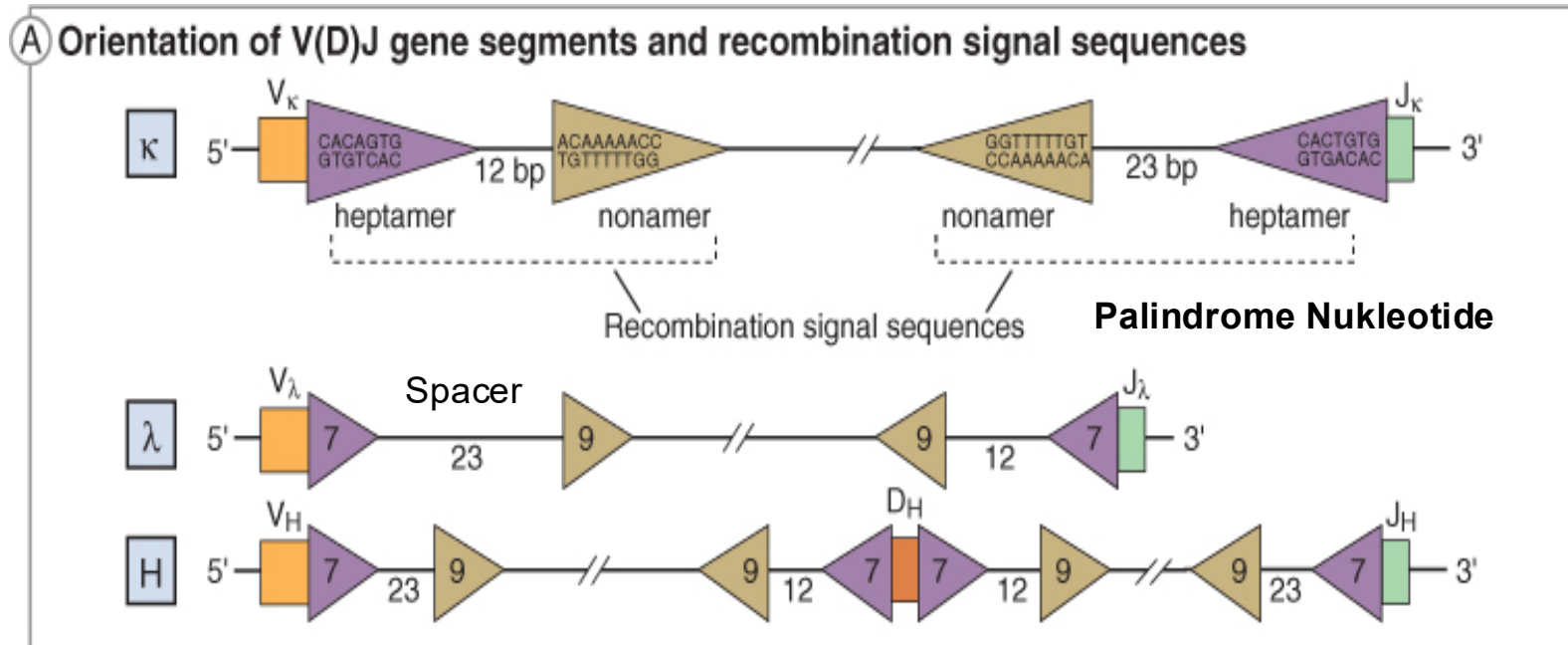
Molecular mechanism of the gene rearrangement

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:

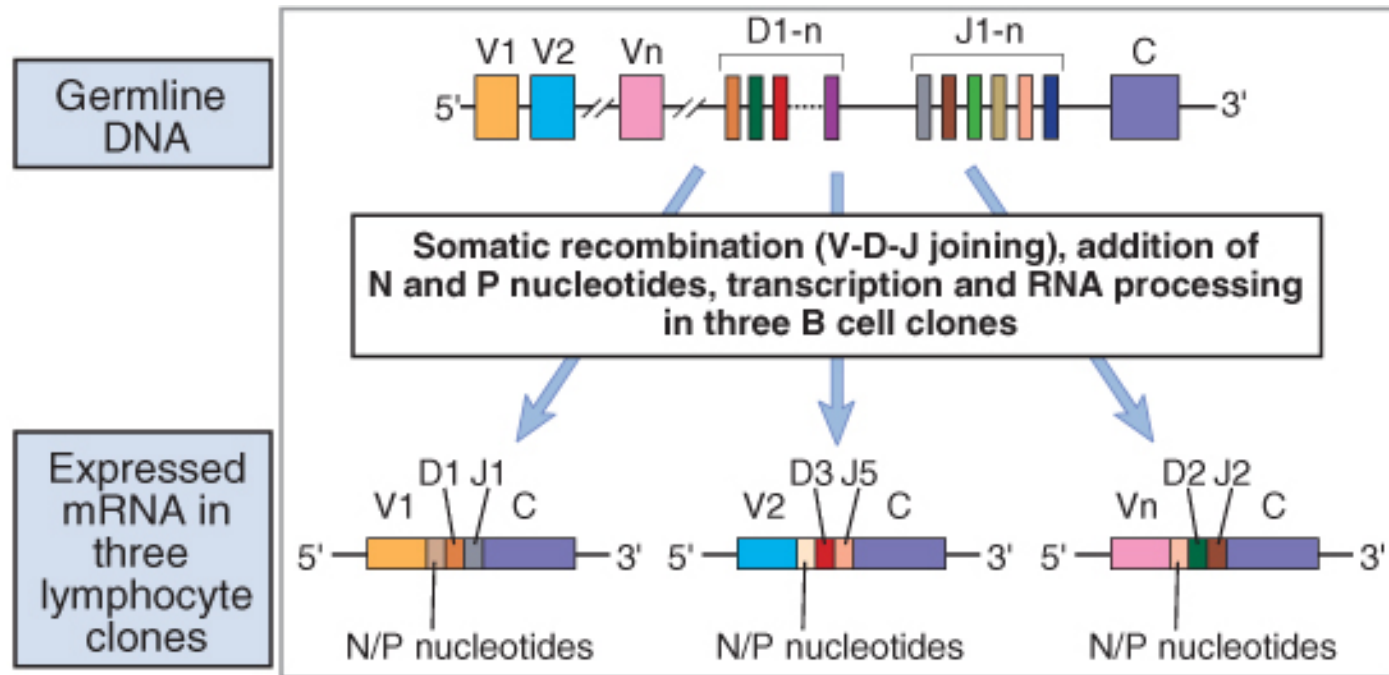


© Elsevier 2005. Abbas | RSS= Rekombinations Signal Sequenz | www.studentconsult.com

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



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Random gene rearrangement

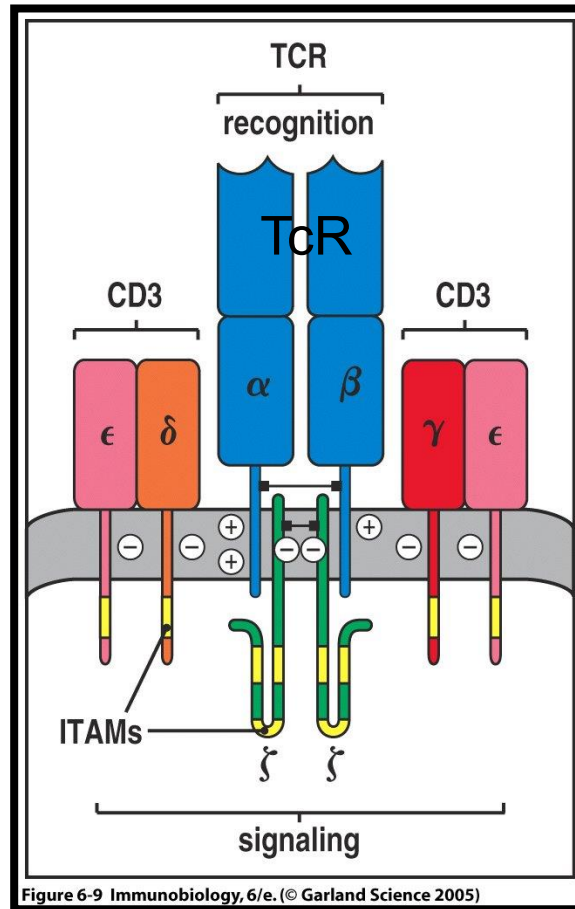
Diversity

T-cell-receptor (TcR)

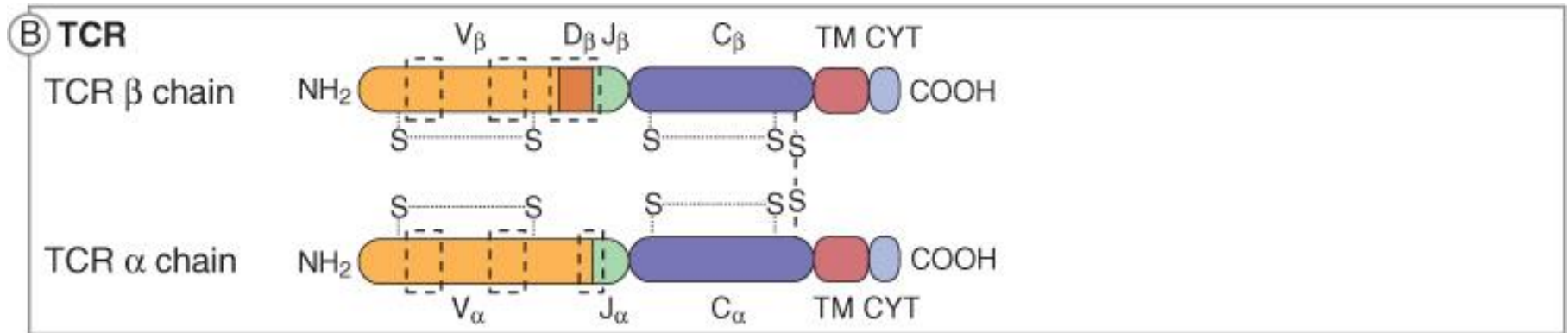
T-cell-types:

1. $\alpha\beta$ TcR+

2. $\gamma\delta$ TcR+



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:

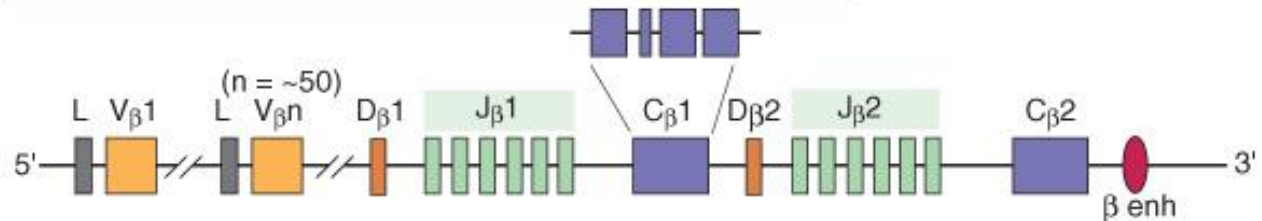
45 V, 55 J genes

inserted: δ locus:

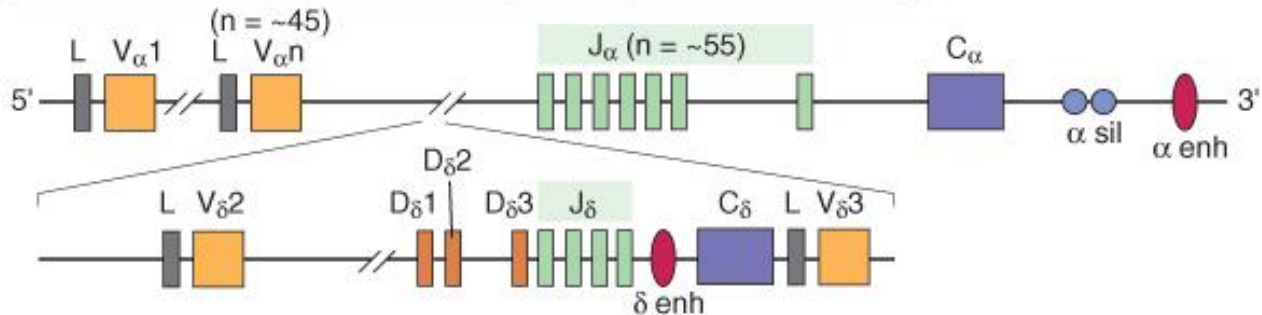
V, D, J and C genes

γ locus: V, J and C

Human TCR β chain locus (620 kb; chromosome 7)

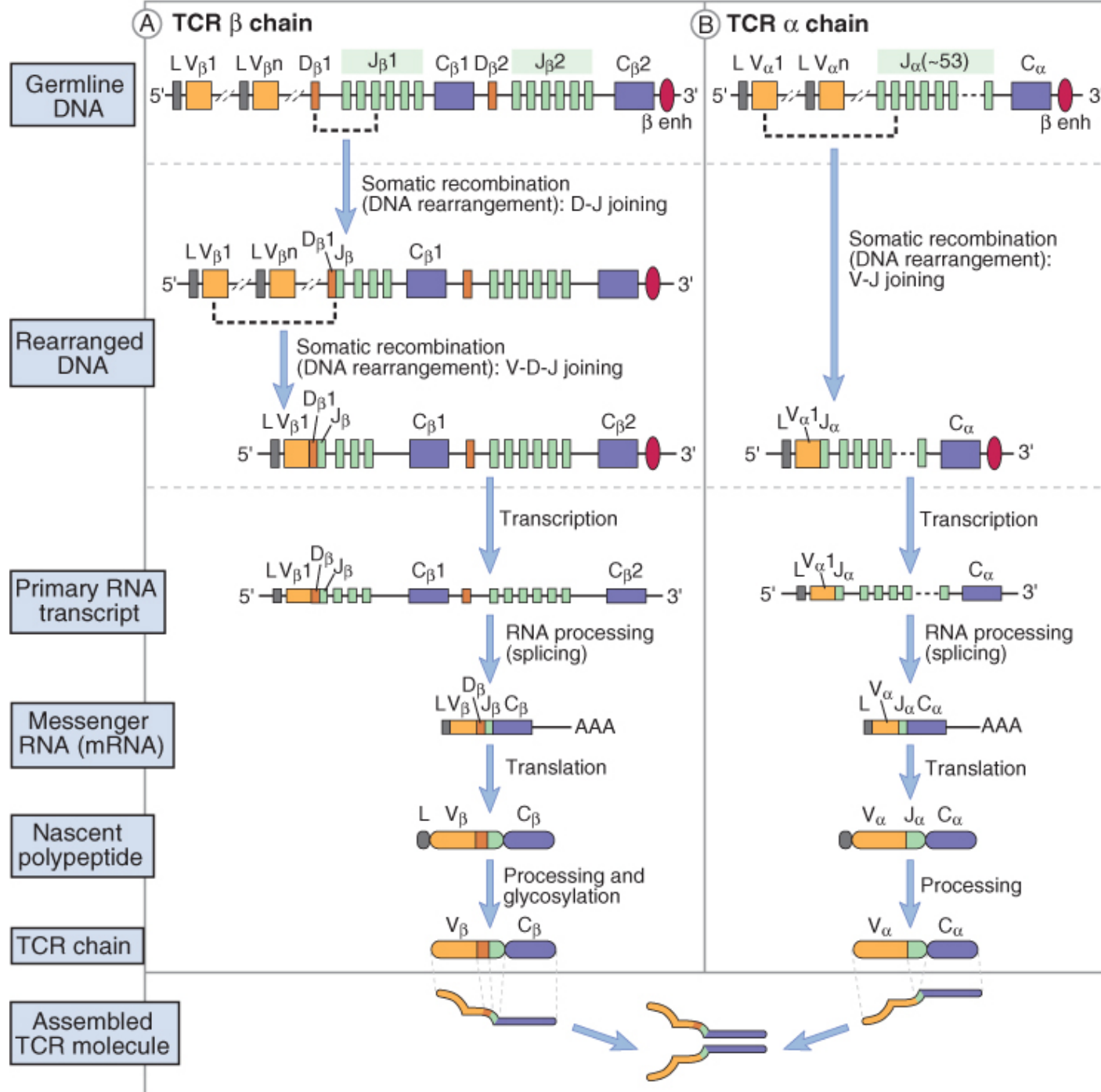


Human TCR α , δ chain locus (1000 kb; chromosome 14)



Human TCR γ chain locus (200 kb; chromosome 7)





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)

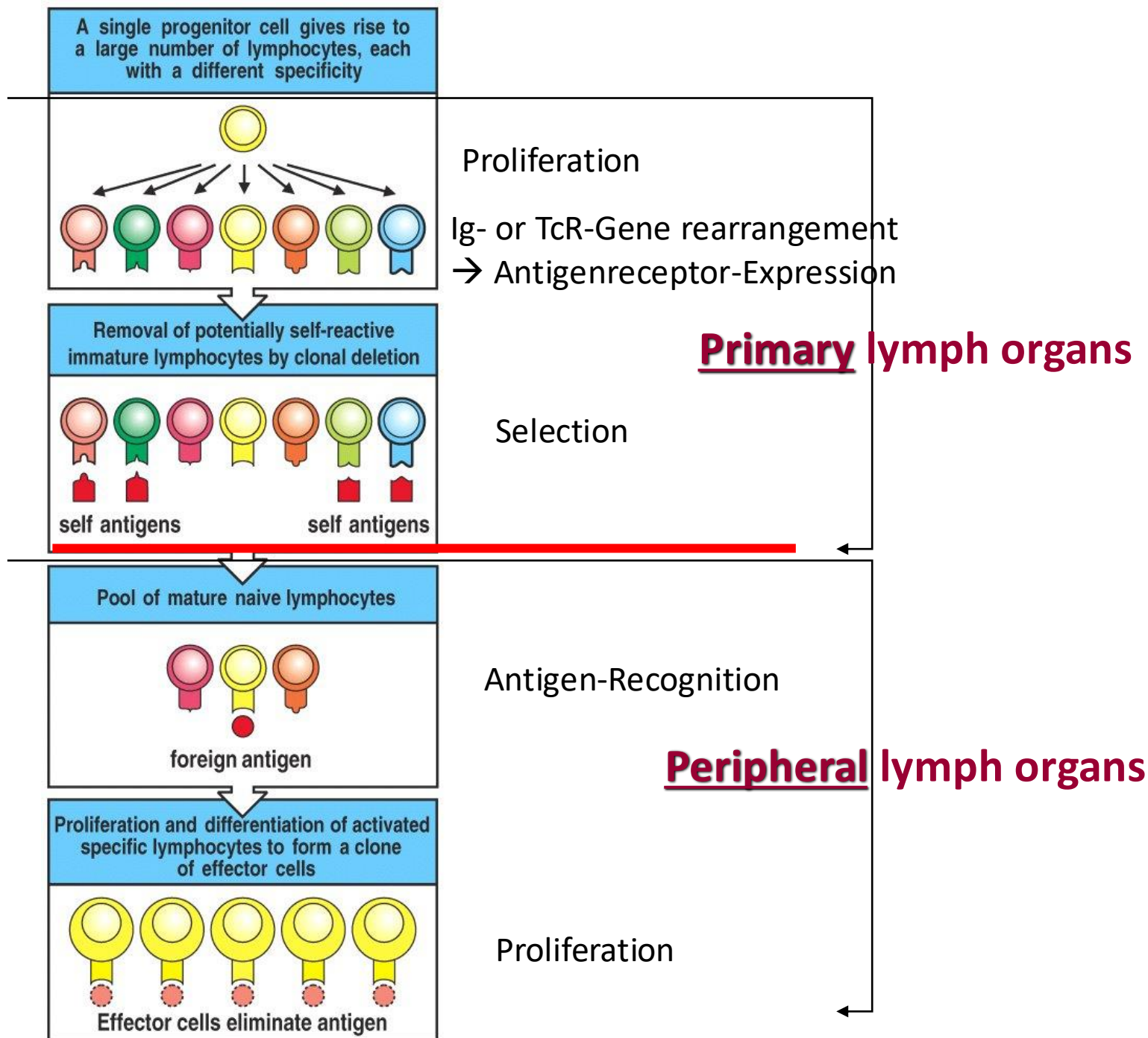


Figure 1-14 Immunobiology, 6/e. (© Garland Science 2005)

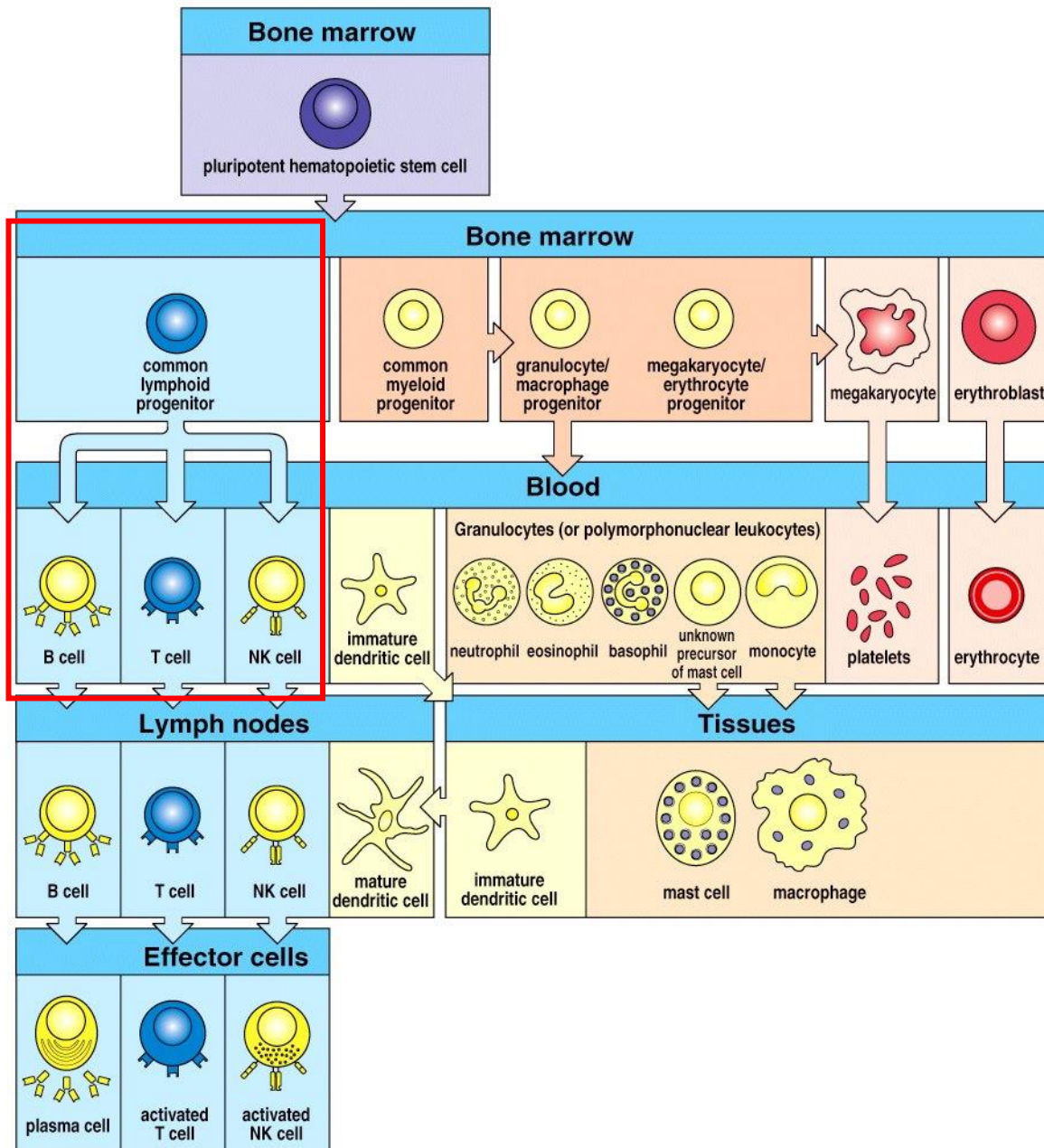
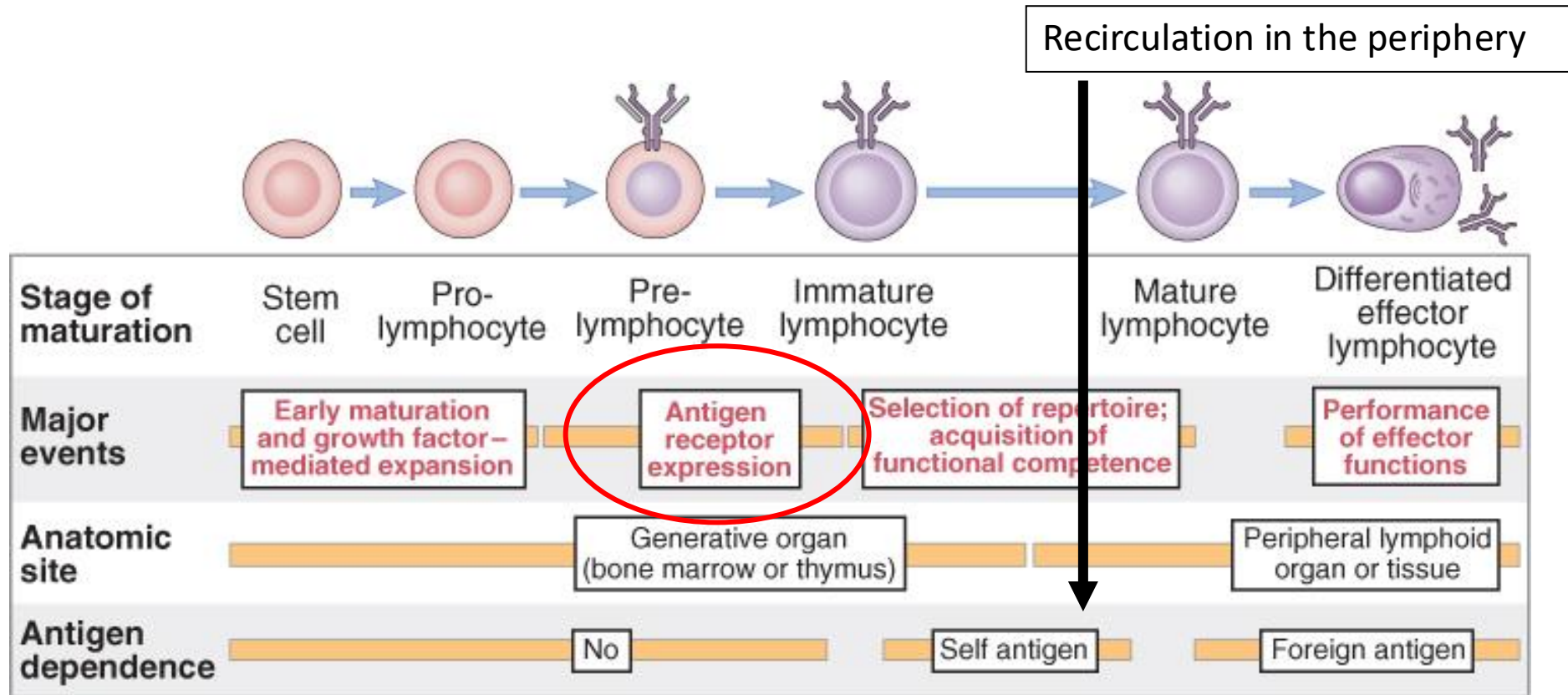


Figure 1-3 Immunobiology, 6/e. (© Garland Science 2005)

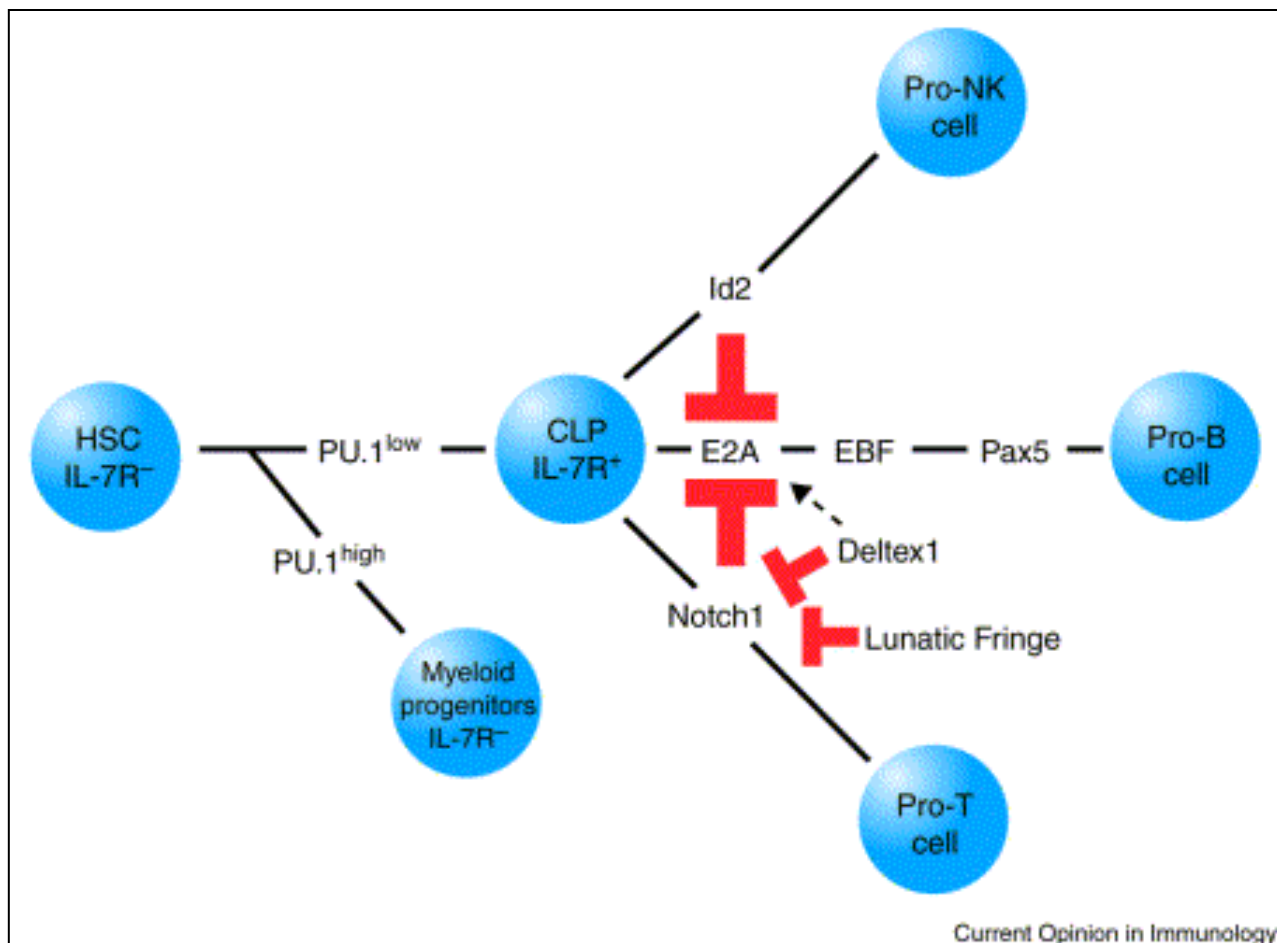
General characteristics of lymphocyte differentiation

- 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

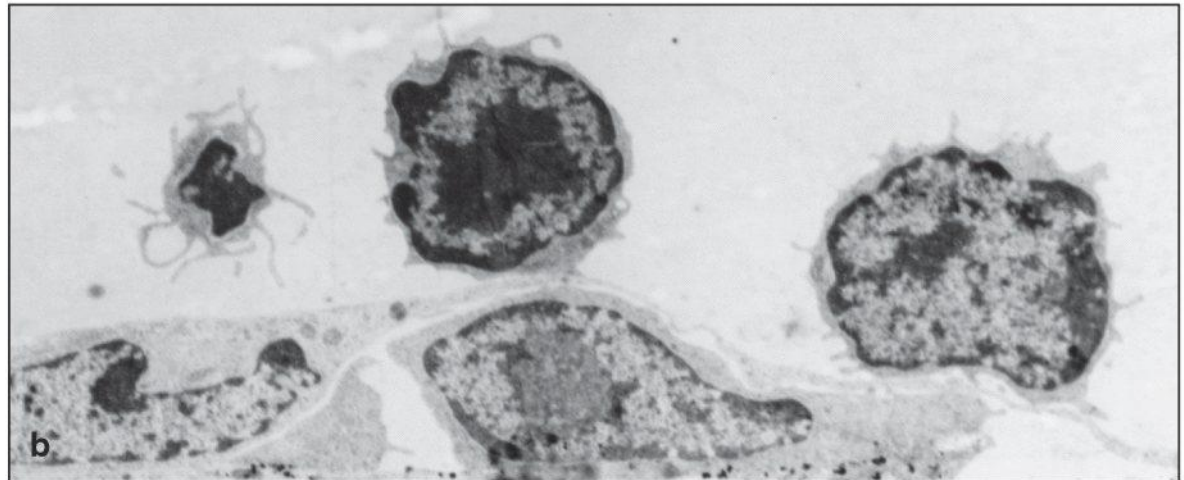
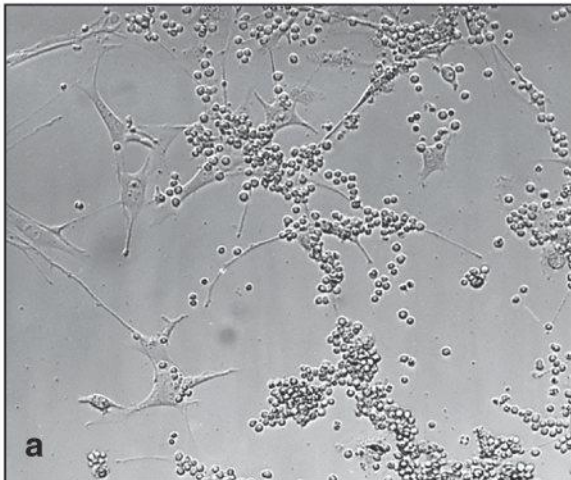
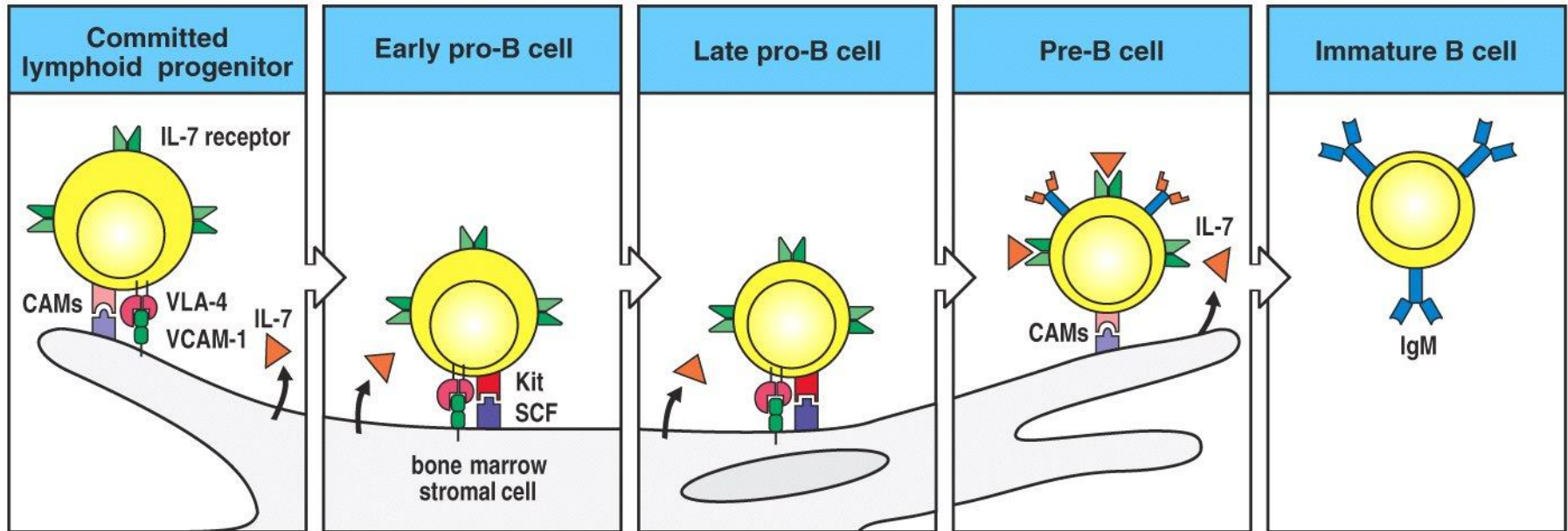


Figure 7-4 Immunobiology, 6/e. (© Garland Science 2005)

B-cell development I: HSC > “Large pre-B”

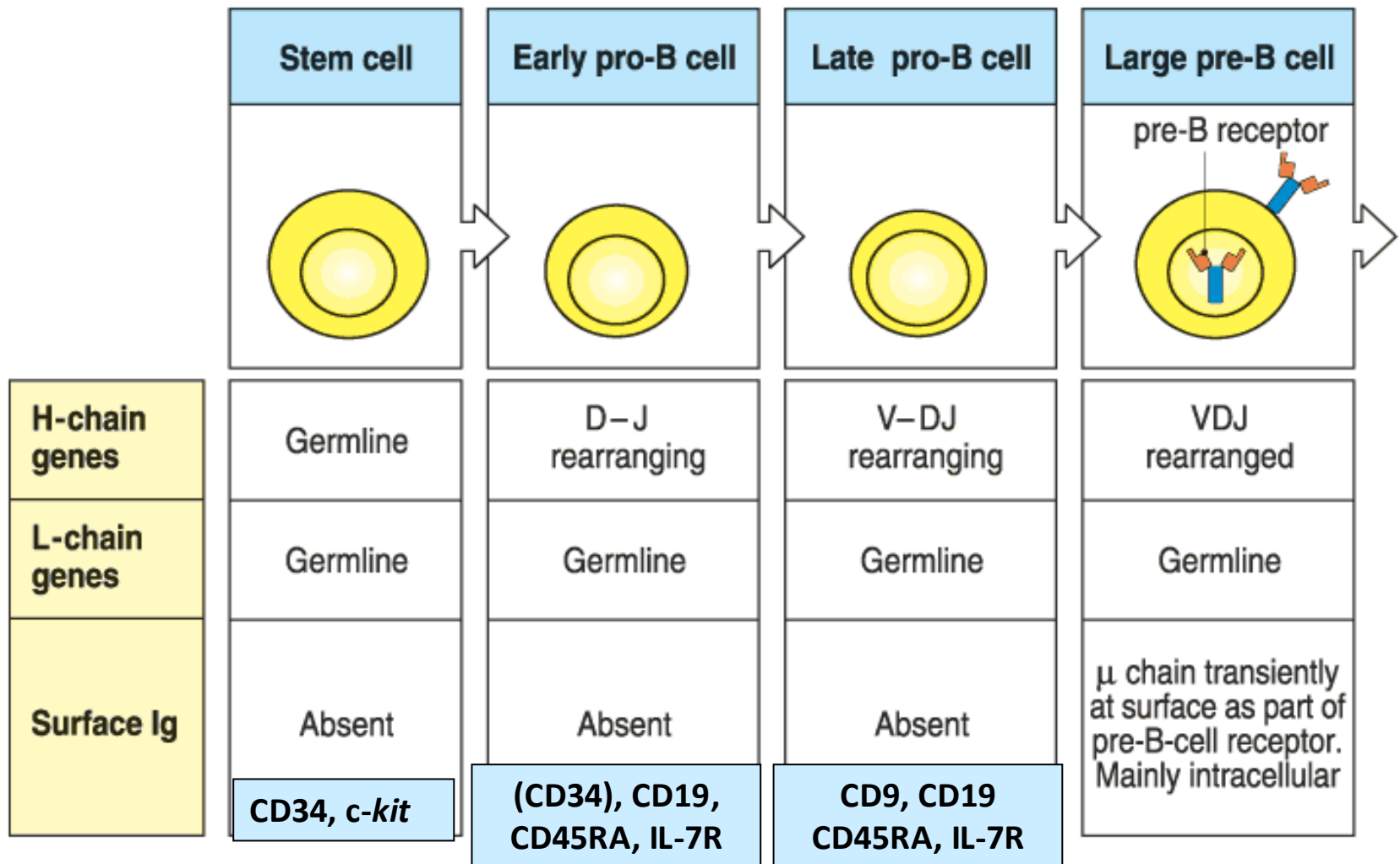


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B-cell development II “Small pre-B” > “mature B”

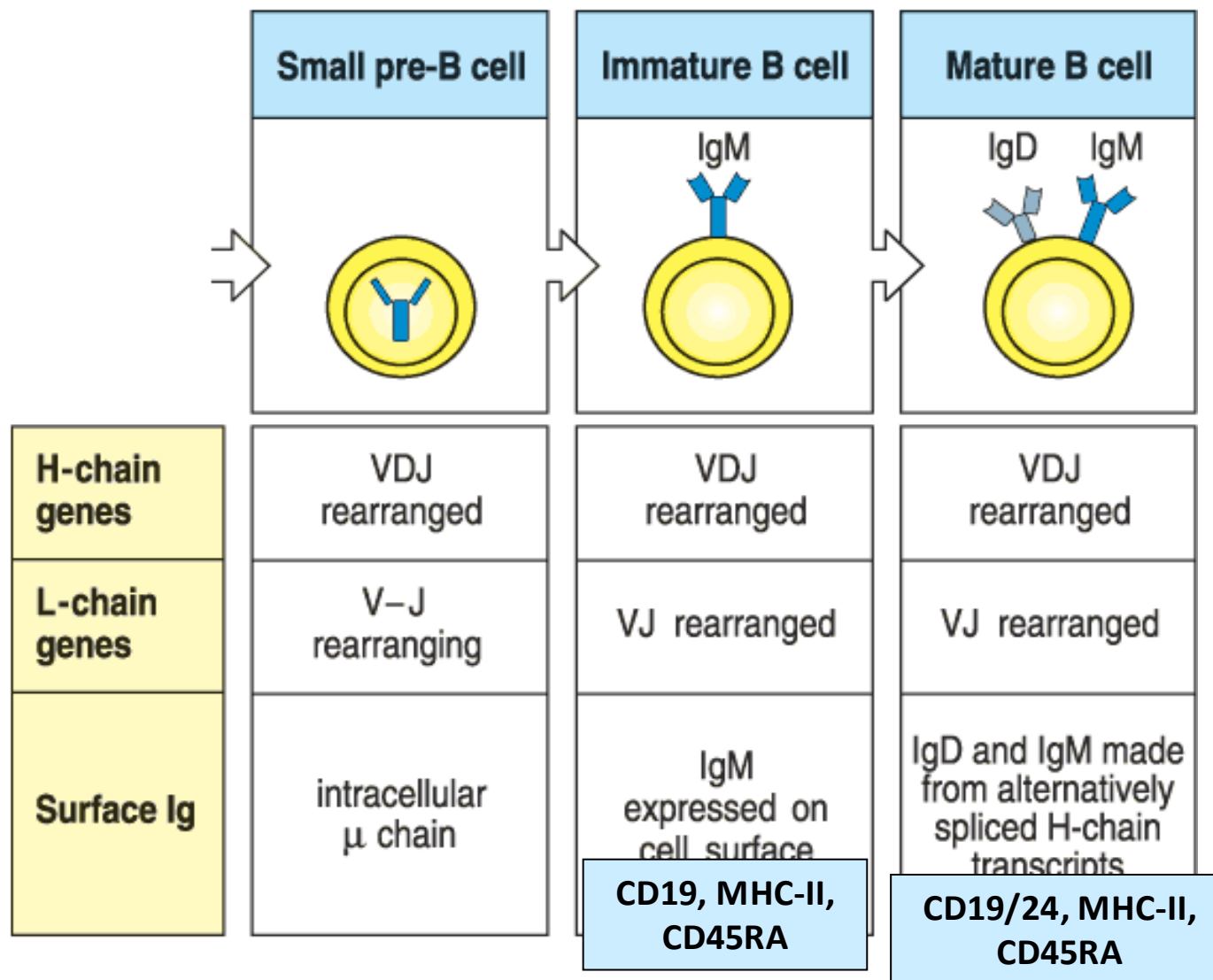


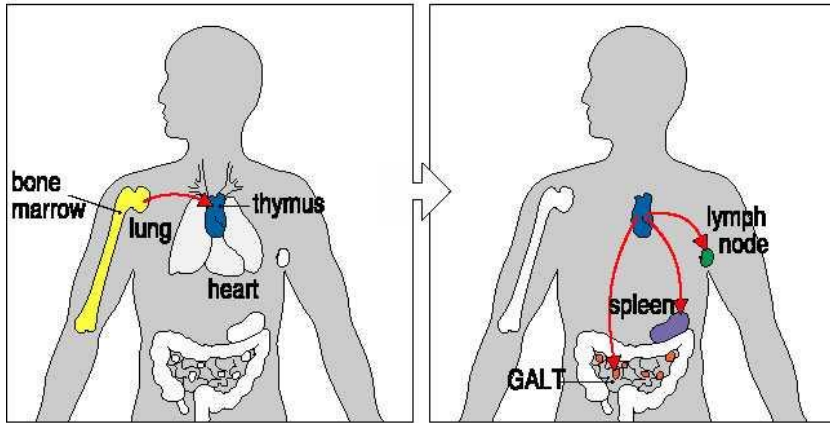
Fig 7.5 part 2 of 2 © 2001 Garland Science

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



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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)

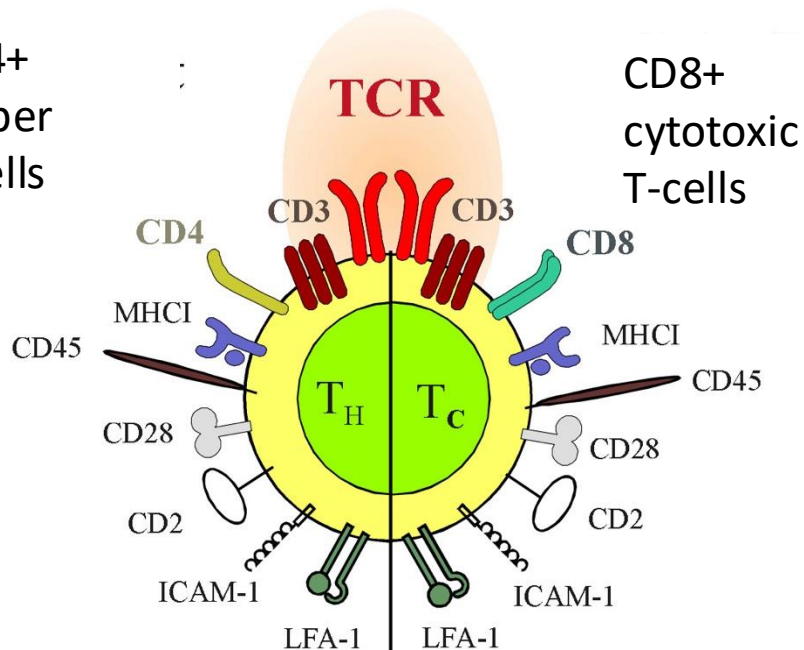
Periphery: mature, TCR expressing,
CD4 or CD8 positive T cells

Self-MHC restricted
Self-tolerant T cells

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

T-lymphocytes with $\alpha\beta$ TcR

CD4+
helper
T cells

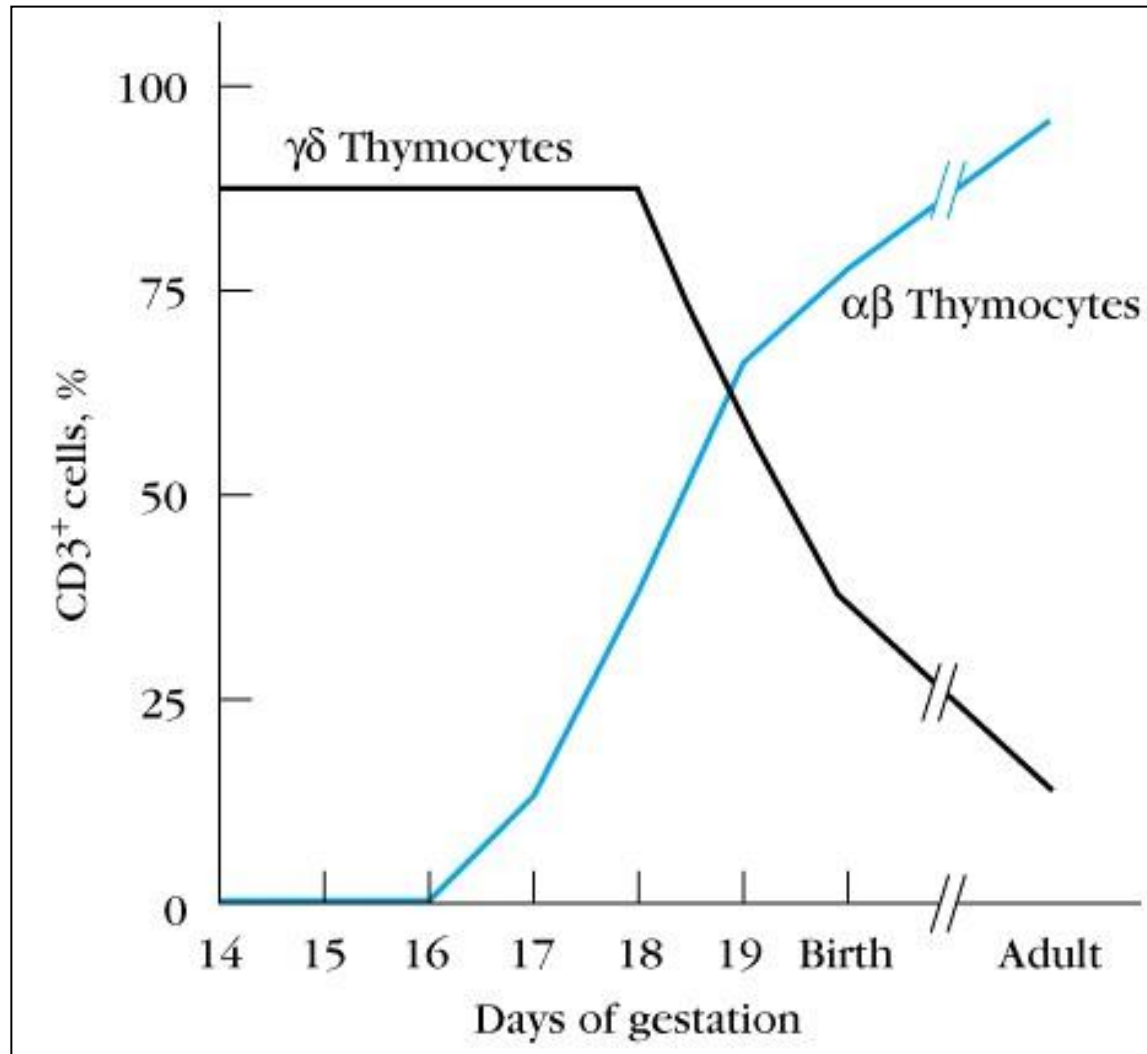


T-lymphocytes with $\gamma\delta$ TcR

- CD4-CD8- cytotoxic T-cells

Intraepithelial – with lower TcR diversity

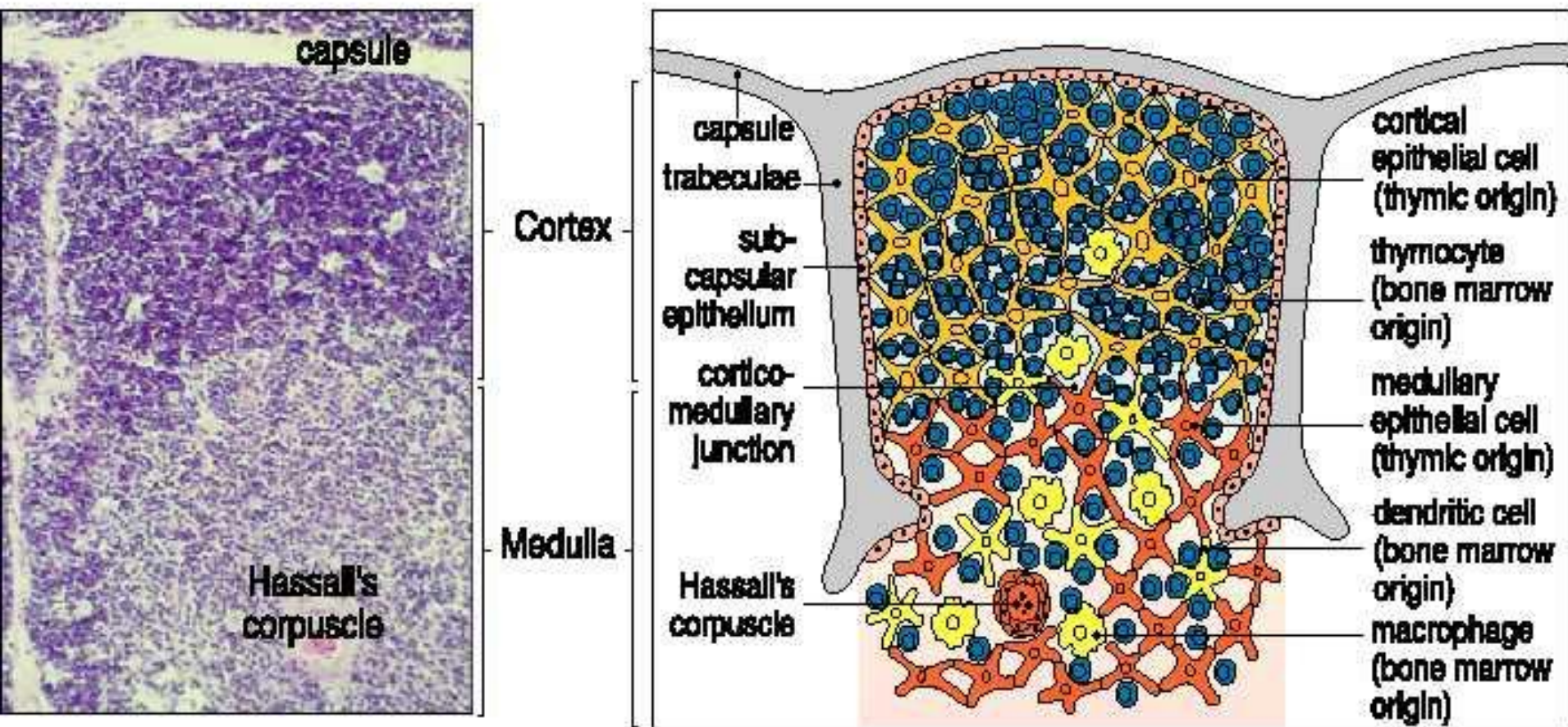
Lymphoid tissues – highly diverse receptors
Regulatory cytokine production



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

Structure of the thymus

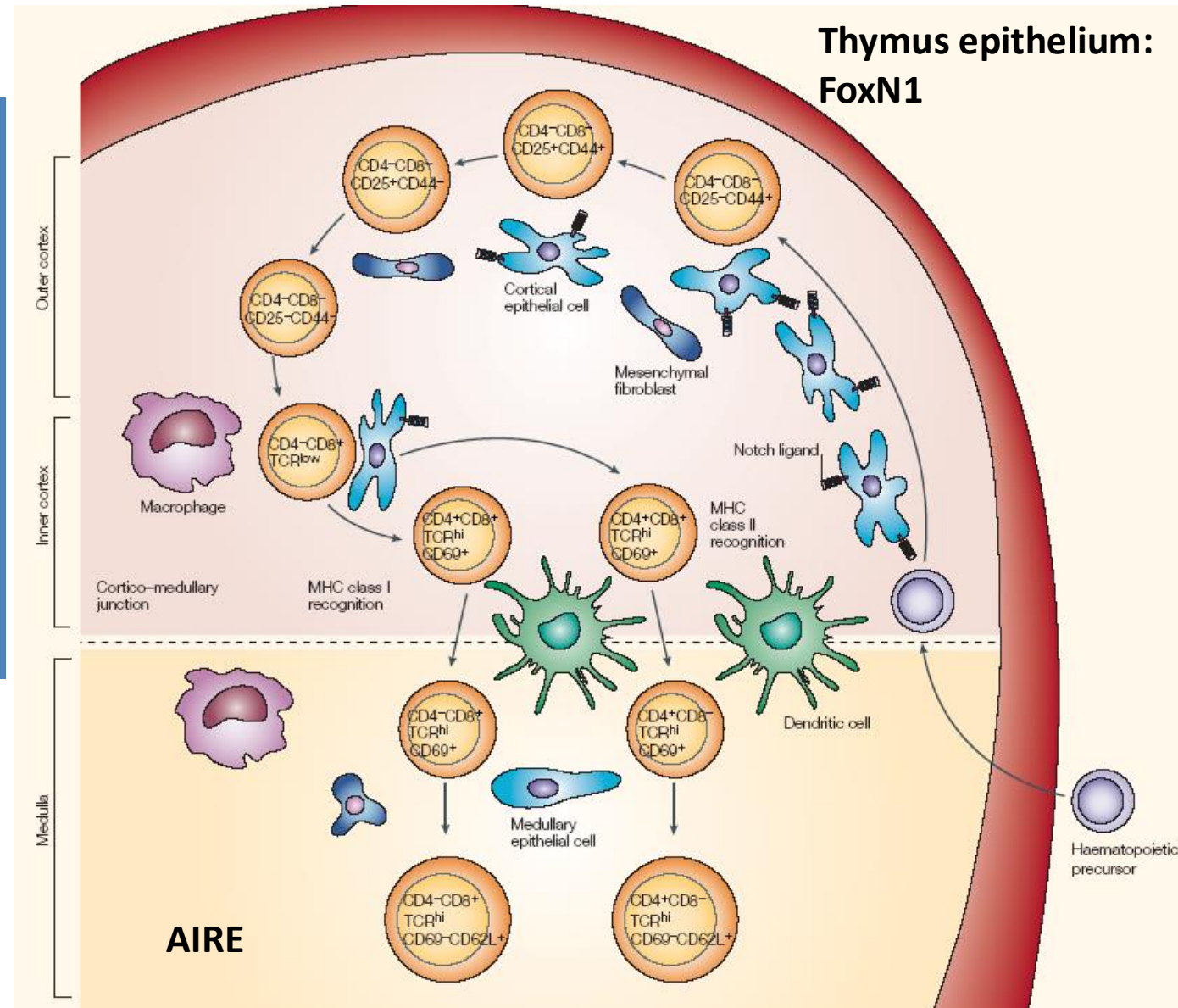
Figure 5.3



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

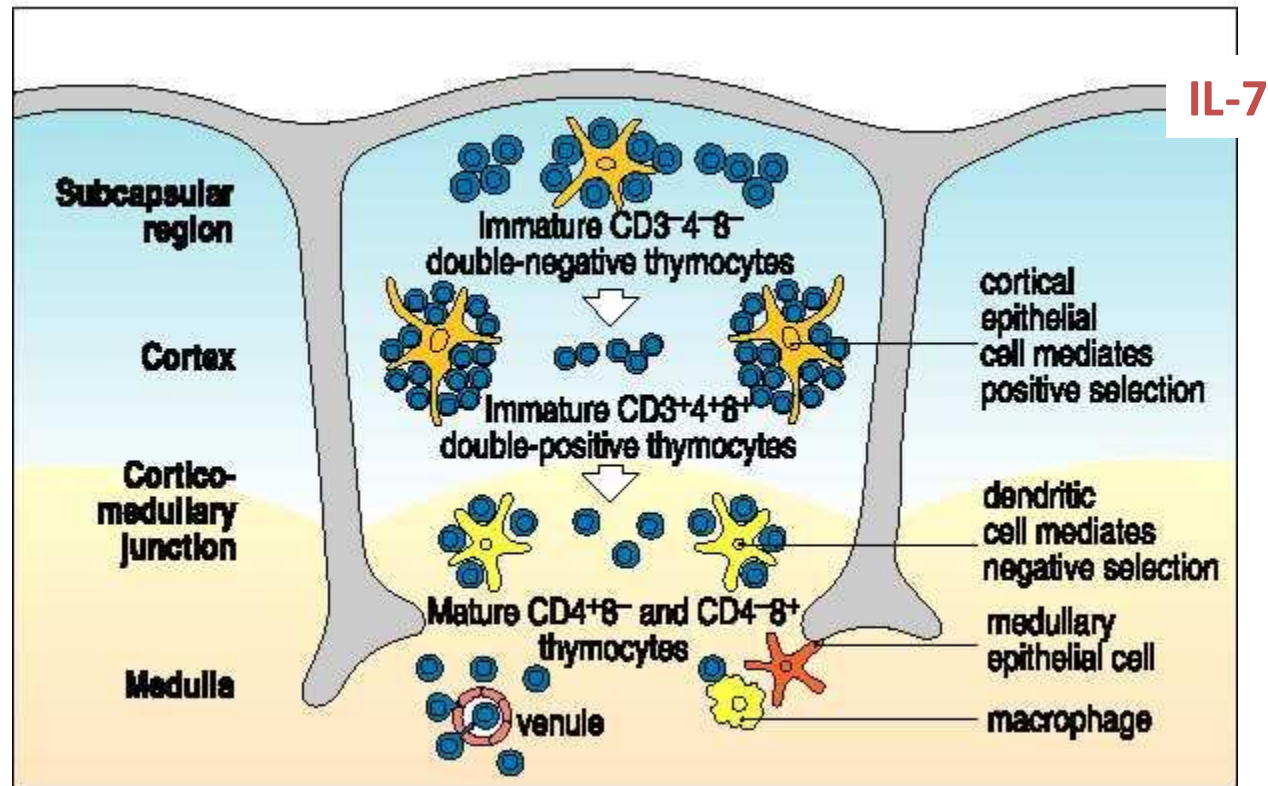
Thymic Microenvironment and T-cell Development

1. Migration:
Chemokine effect
2. Proliferation
IL-7
3. Differentiation
 - TcR-rearrangement
 - Phenotypes
4. Selection
Apoptosis



T-cell development in the thymus

Figure 5.14



Thymocytes:

DN: 2-5 %

DP: 70-80%

CD4 SP: 10-15%

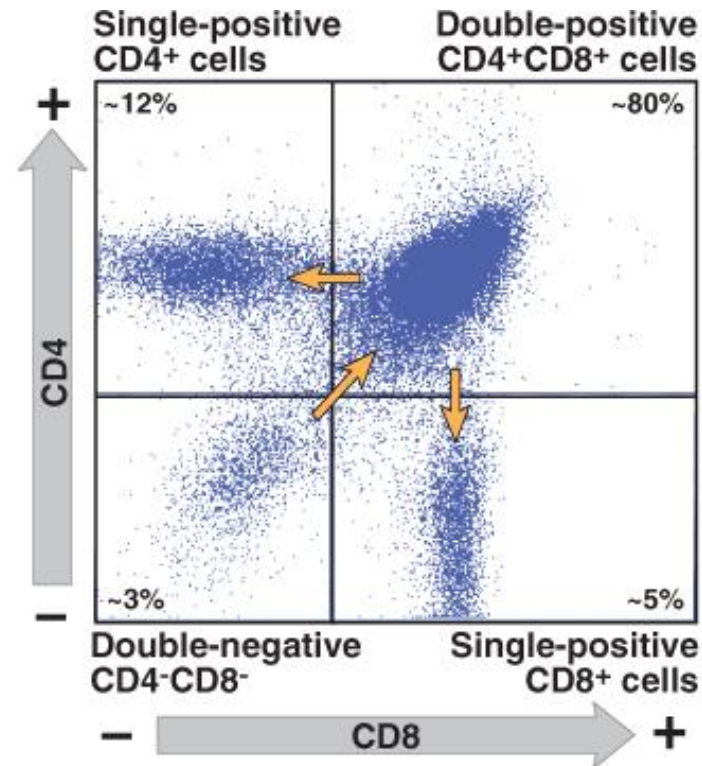
CD8 SP: 5-8%

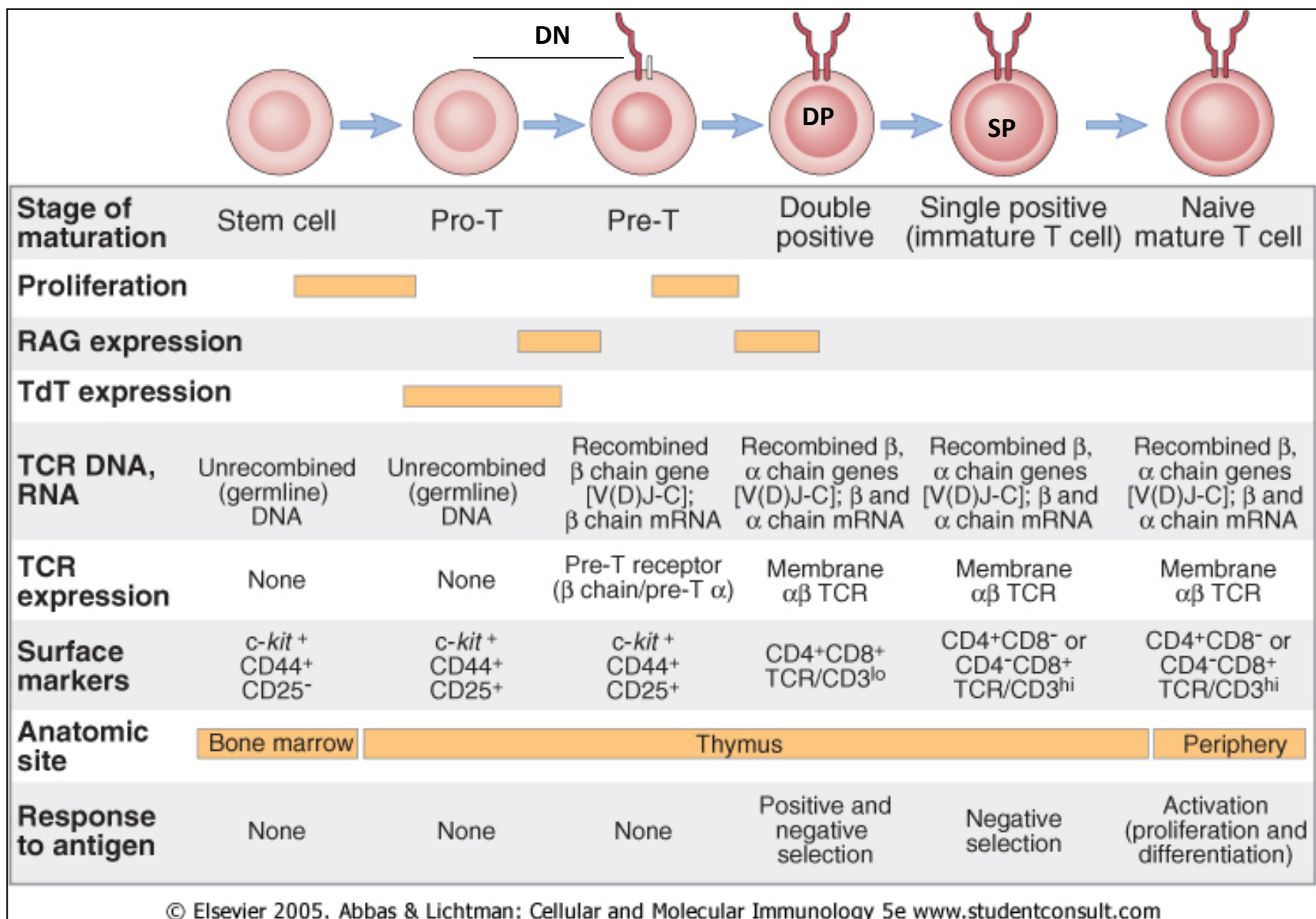
Young mouse: 5×10^7 T-cells daily

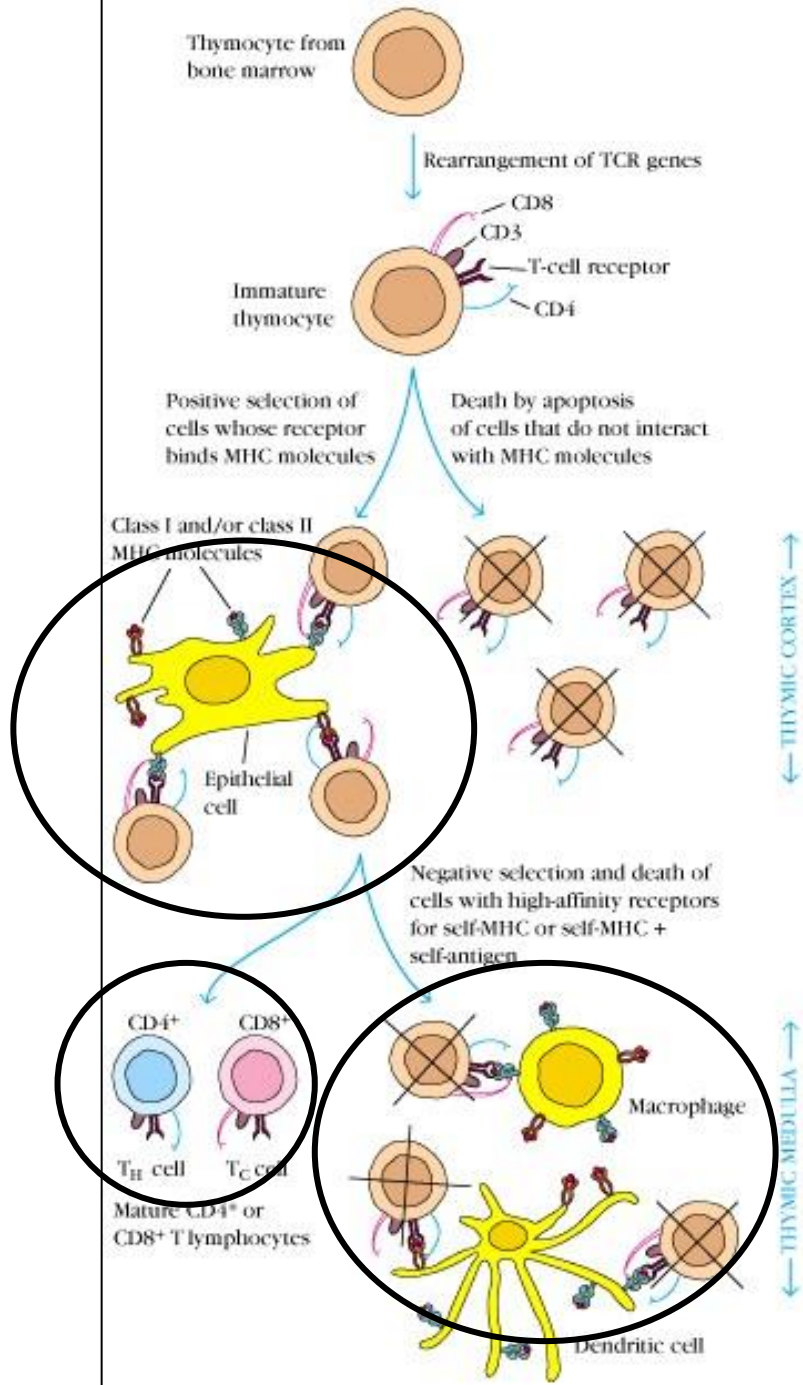
During selection 98 % of thymocytes die by apoptosis

Daily $1-2 \times 10^6$ mature T-cell migrate to the periphery

Thymocyte populations based on their cell surface markers







Positive selection:

Epithelial cell - thymocyte interaction in the thymus cortex

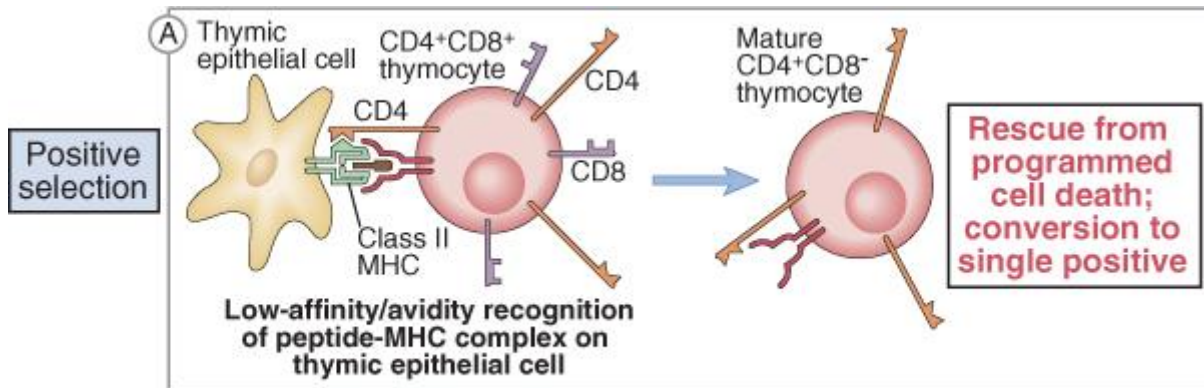
Survival of DP cells whose TcR is appropriate for self MHC recognition

Negative selection:

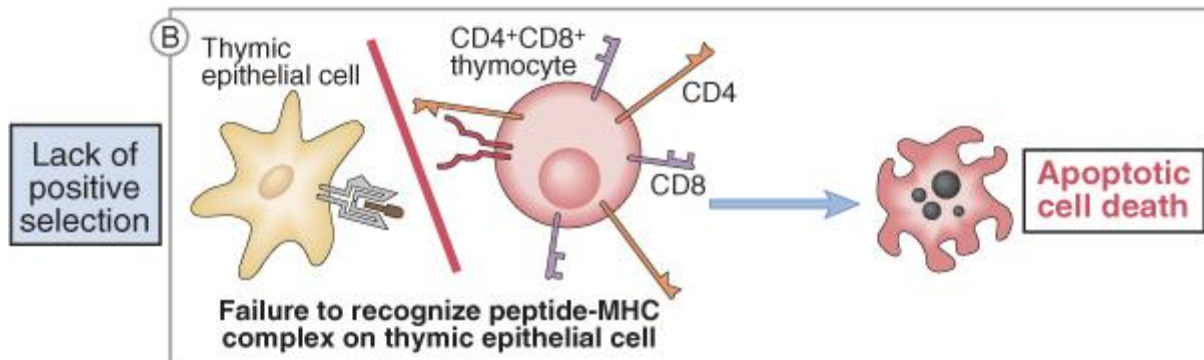
APC (macrophage or DC) – thymocyte Interaction in thymus medulla

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

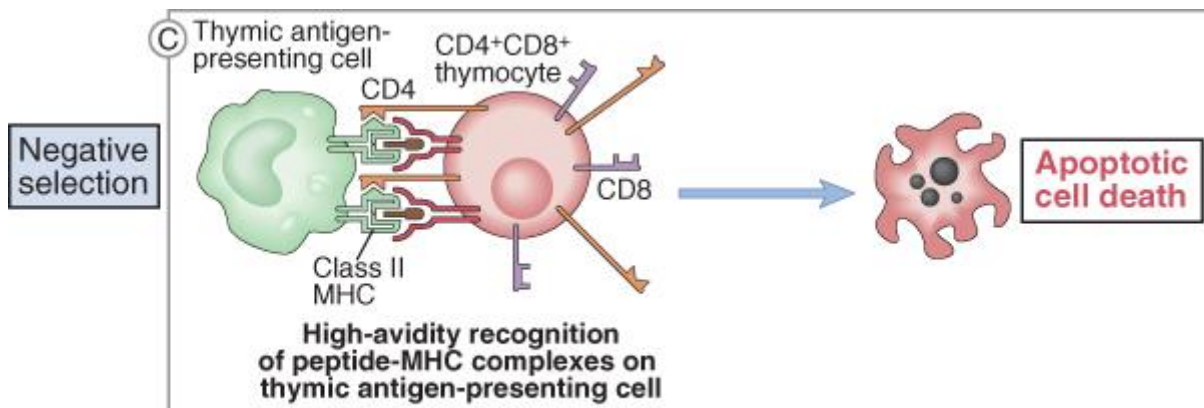
Differentiation into SP stage



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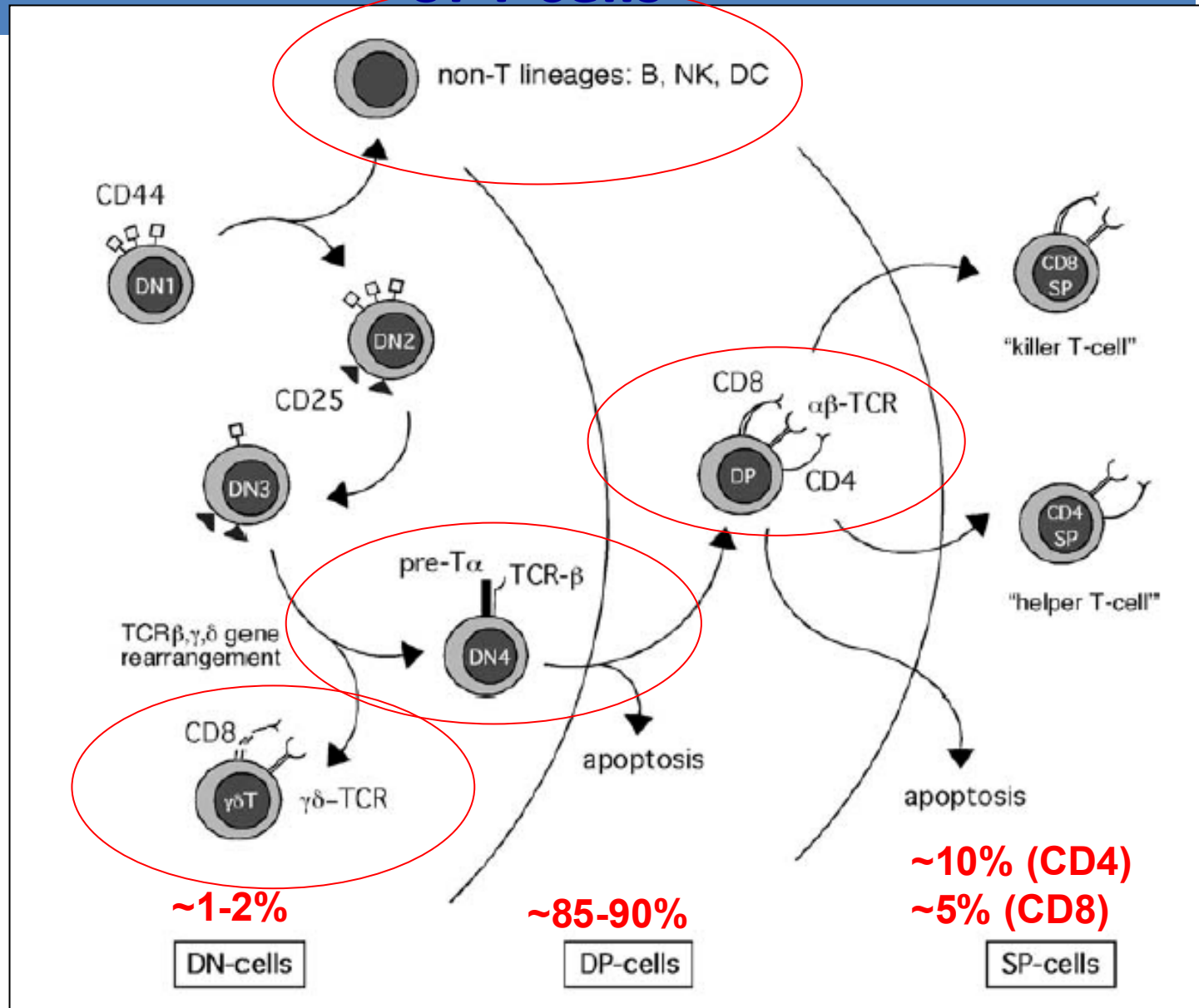


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Decision-making during the development of T cells



Phases of T-cell maturation in the thymus.

1. Initiation of either TCR β or γ/δ chain gene rearrangement.
2. Formation of pT α /TCR β /CD3 (pTCR), allelic exclusion, IL-7-dependent proliferation - *β -selection*.
3. Initiation of TCR α 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

