Basic Immunology

Dentistry

Lymphocyte groups.
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

NATURAL

γδ T cell

B1 B cell

T cell (CD3+)

B cell (CD19+)

ADAPTIVE

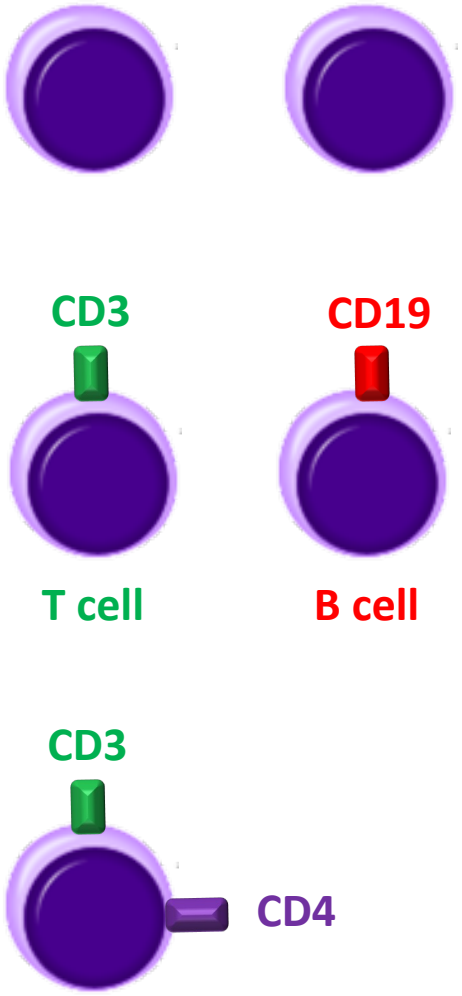
αβ T cell

B2 B cell

Helper T cell (CD4+)

Cytotoxic T cell (CD8+)

CD markers



Certain cells (e.g. lymphocytes) cannot always be distinguished based on their morphology.

Different cells can be identified and distinguished by the molecules they express on the cell surface or in the cytoplasm.
IMMUNOPHENOTYPE: The characteristic molecular pattern of a cell type determined with the use of antibodies.

Such SURFACE MOLECULES were given a standardized nomenclature:

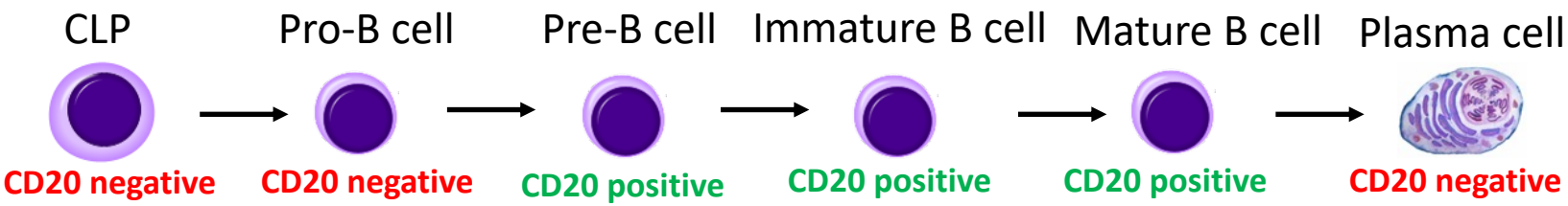
CD = **Cluster of differentiation**, usage: CD+number, e.g.: CD1, CD2, CD3, CD4, etc…

The structure and function of CD marker **varies**!

Example for immunophenotype:
CD3+/CD4+/CD8- → Helper T cell

Types of CD markers

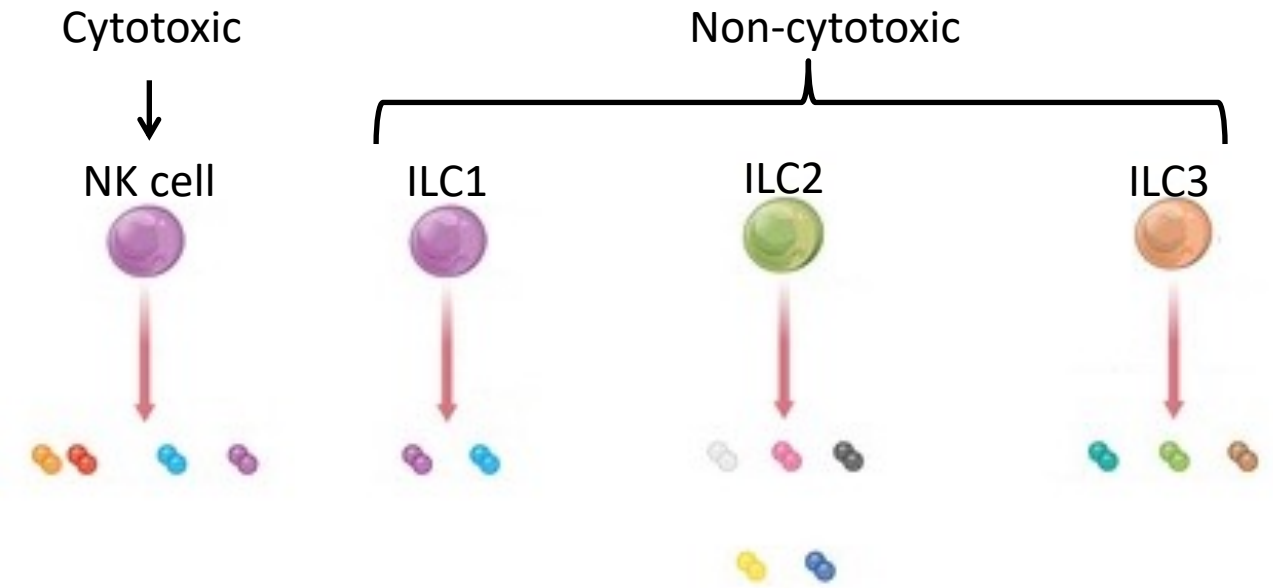
- **Lineage markers**: Molecules expressed exclusively on certain cell lineages.
  - E.g.: CD3 → found on all T cells CD19 → found on all B cells
- **Maturation markers:** The immunophenotype might differ in the phases of cell maturation, certain molecules are only expressed on immature cells, others on mature, fully functioning cells, etc.
  - E.g.: CD20 (It is also a lineage marker of B cells, cannot be found on any other cells)



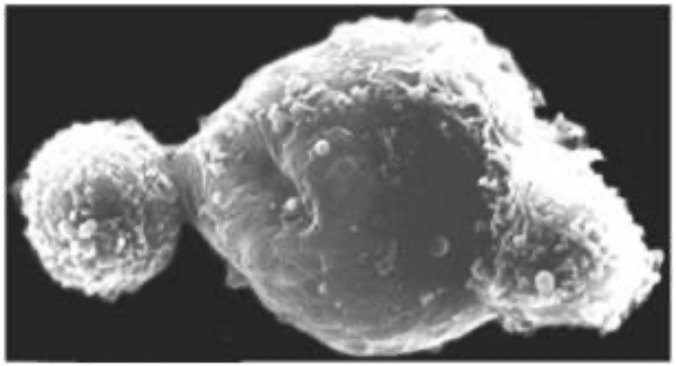
- **Activation markers:** Molecules expressed by activated cells, whereas resting cells either lack them completely or express them at low levels, e.g.:
  - CD25 (The alpha chain of the interleukin-2 receptor, IL-2Rα, see later)
  - CD80 and CD86 (B7-1 and B7-2, so-called costimulatory molecules expressed by activated antigen presenting cells, see later)

Innate lymphoid cells (ILC)

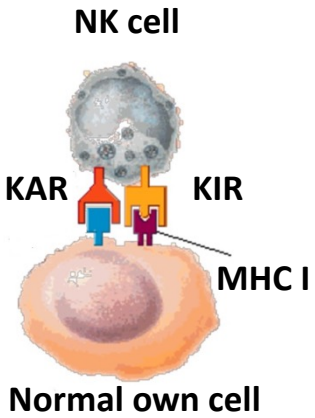
- They cannot be distinguished from lymphocytes based on their morphology but unlike adaptive lymphocytes they cannot recognize antigens. → **They have no antigen recognition receptors.**
- They are classified based on the cytokines they produce and the transcripition factors that are necessary for their formation. (see in the lectures):
  - Group 1 ILCs:
    - **NK cells**
    - ILC1
  - Group 2 ILCs:
    - ILC2
  - Group 3 ILCs:
    - ILC3/LTi



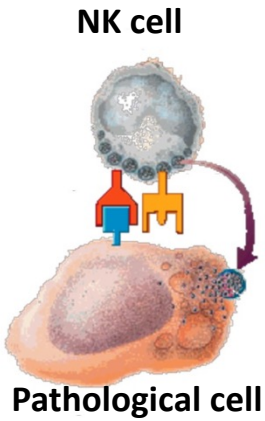
Natural killer cells (NK cells)



Two NK cells kill a cancerous cell.
(Scanning electron microscopy image)



CELL IS LEFT ALIVE



CELL IS KILLED

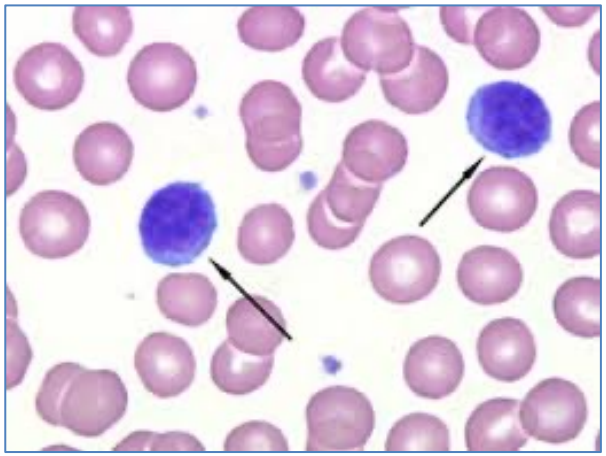
Blood lymphoid cells %:	**≈ 10**
Main function:	**Killing cells infected with intracellular pathogens, Killing cancer cells**
Recognition:	**KAR → killing the target KIR → sparing the target Fc receptor, Complement receptor**
Cytotoxicity:	**Fas-FasL, Perforin, Granzymes**
Produced mediators:	**Cytokines**
Fc receptor:	**FcγR (binds IgG)**
Characteristic marker:	**CD56**

Red: Only possible after the activation of the adaptive immunity

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+)

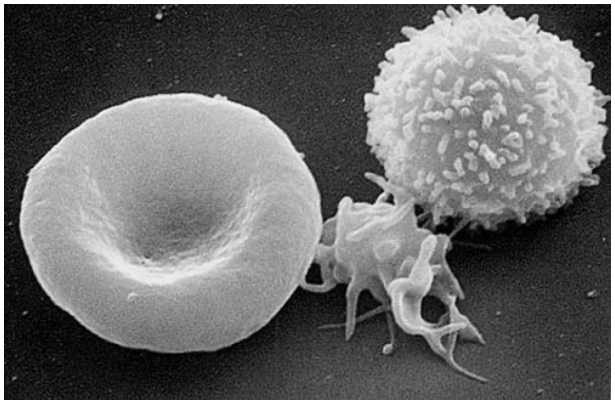
↓

Direct killing of target cell (infected or cancerous)

Helper T cell (CD4+)

↓

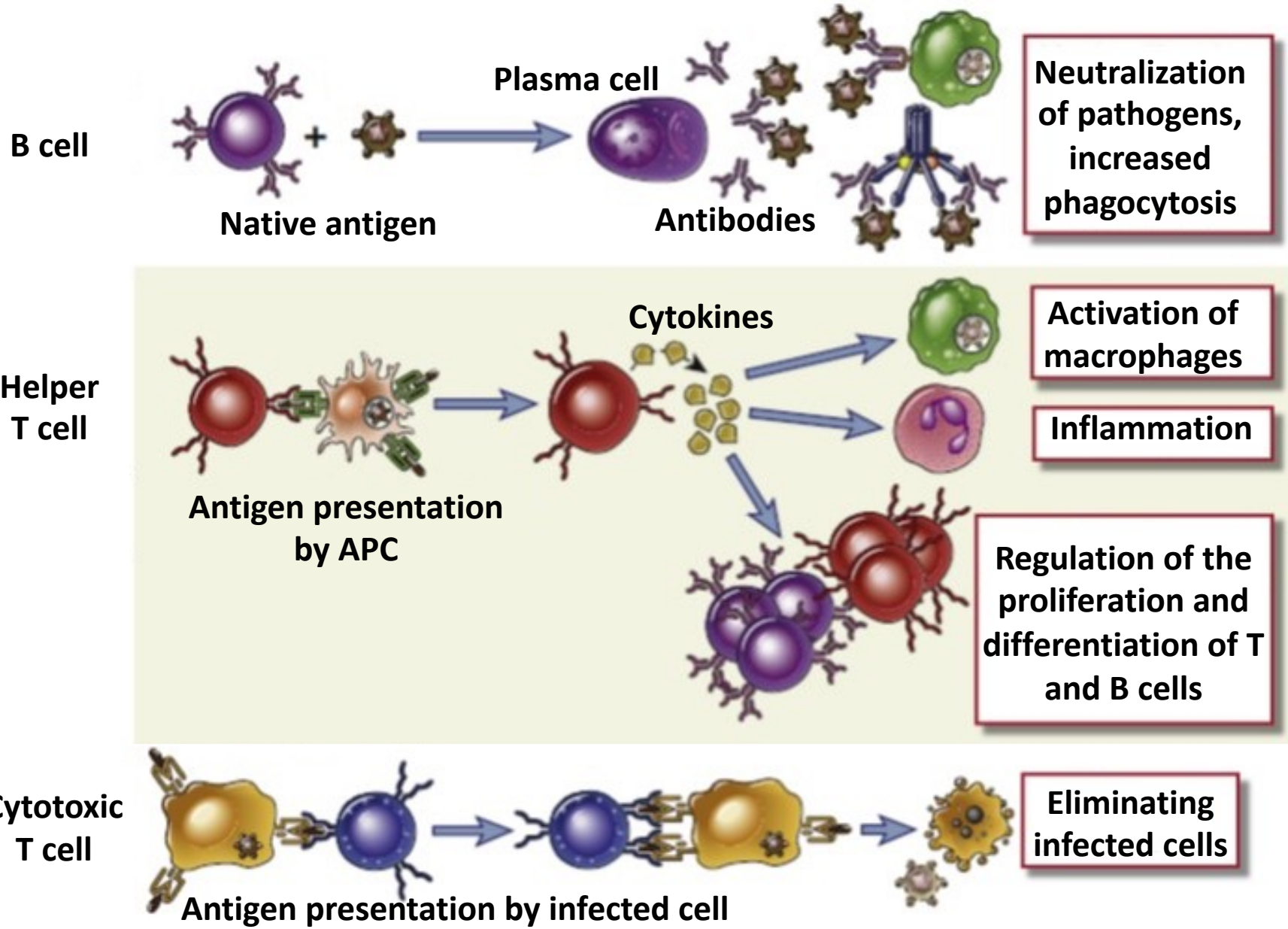
Regulation of the immune response



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

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

Main groups of lymphocytes

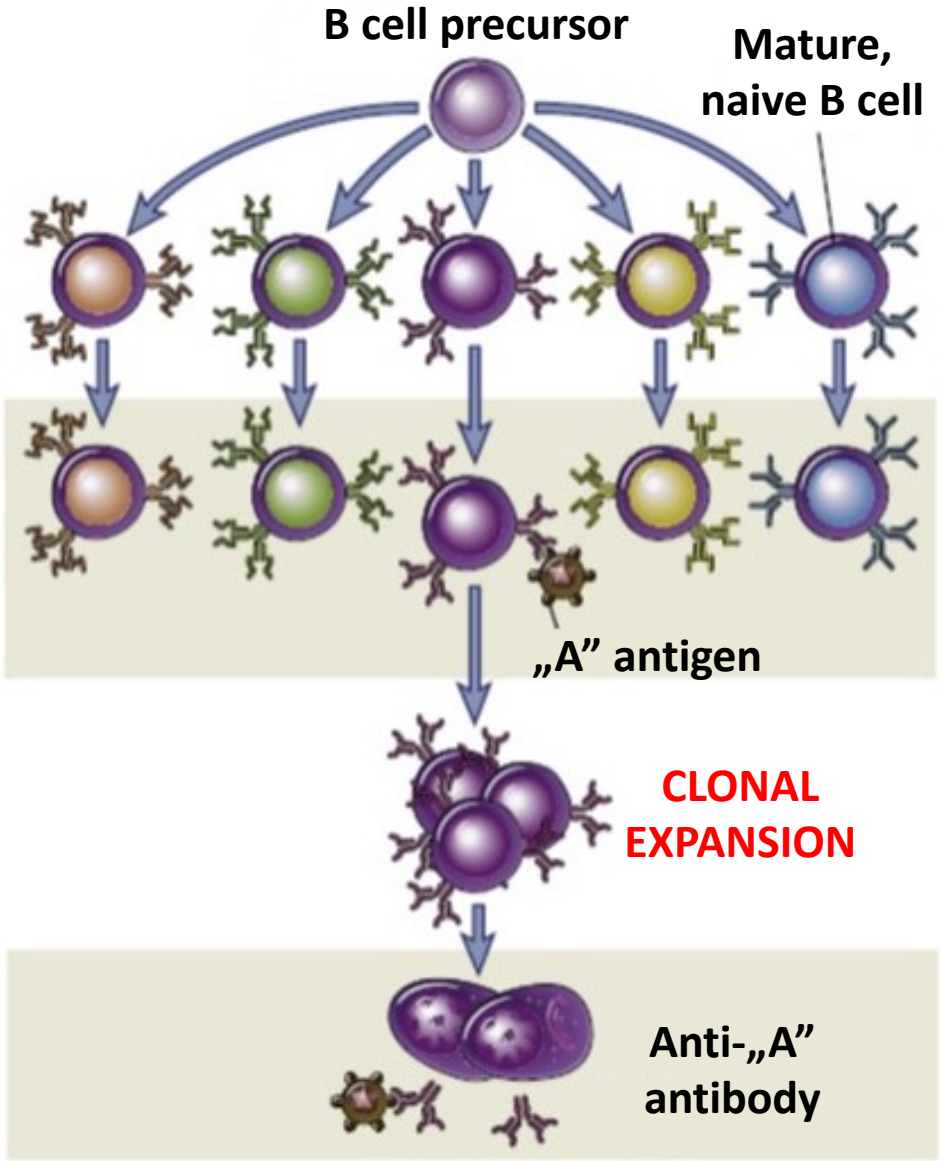


Clonality

1. Each newly produced lymphocyte expresses a **unique antigen-binding receptor**.

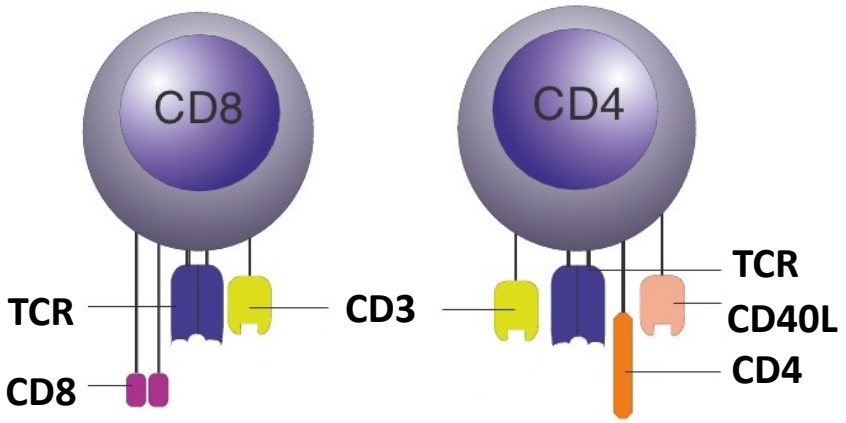
2. **Only those lymphocytes will become activated which recognize an antigen**. These selected cells will proliferate and produce **clones** of themselves with each sister cell having the same antigen-recognition receptor.

3. These clones will differentiate into **effector cells** which will participate in the immune response. (e.g. effector plasma cells produce antibodies)



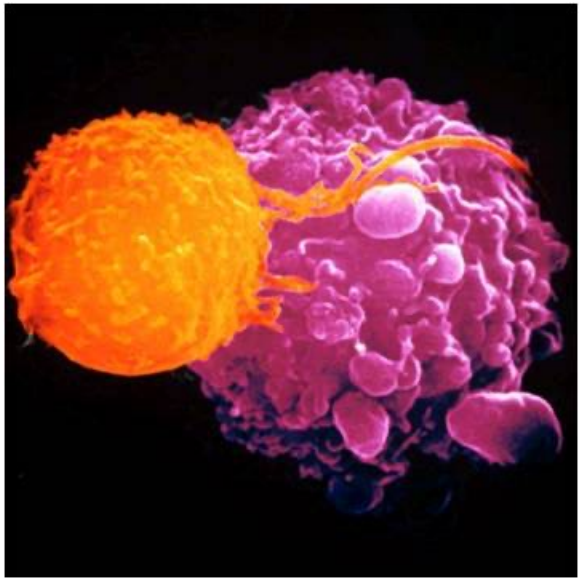
T cells

Main function:	**Antigen-specific killing of target cell (CD8+), Regulation of the immune response through cytokines (CD4+)**
Recognition:	**Through MHC, antigen-specific TCR**
Possible type of TCR:	**αβ and γδ**
Produced mediators:	**Cytokines**
Main types of αβ T cells:	**CD4+ Helper CD8+ Cytotoxic**
Site of production:	**Bone marrow, thymus**
Characteristic marker:	**CD3 (Makes a complex with the TCR)**



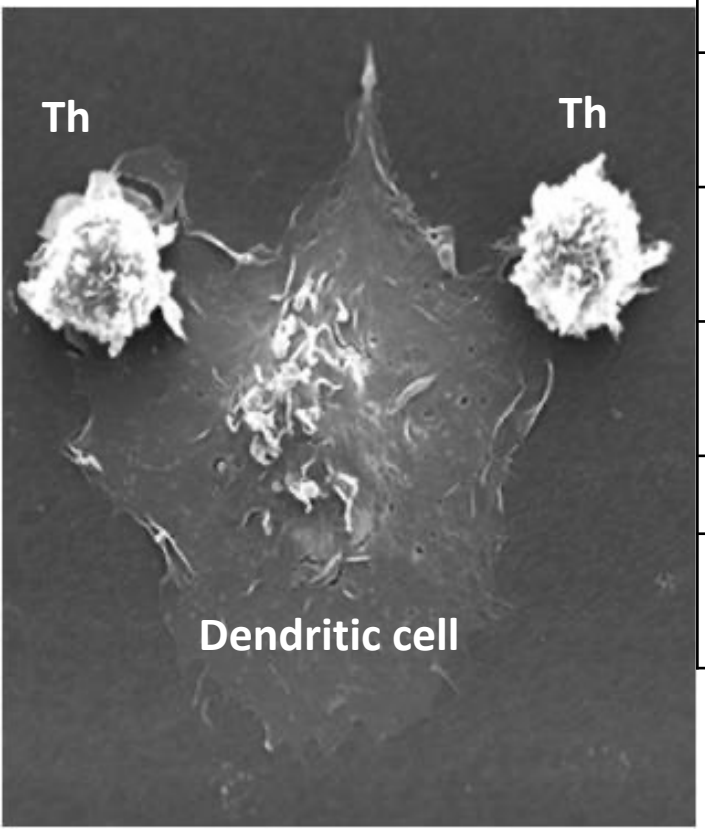
Cytotoxic T cells (Tc or CTL)

Blood T cells:	**1/3**
Main function:	**Effector cell of the cellular immunity**
Recognition:	**Through MHC I, antigen-specific TCR**
Target cells to kill:	**Infected with IC pathogens, Cancerous, Foreign (transplantations!)**
Recognized antigens:	**Endogenous (from the cytoplasm of the target cell)**
Cytotoxicity:	**Fas-FasL, Perforin, Granzyme**
Immunophenotype:	**CD3+/CD8+/CD4-**



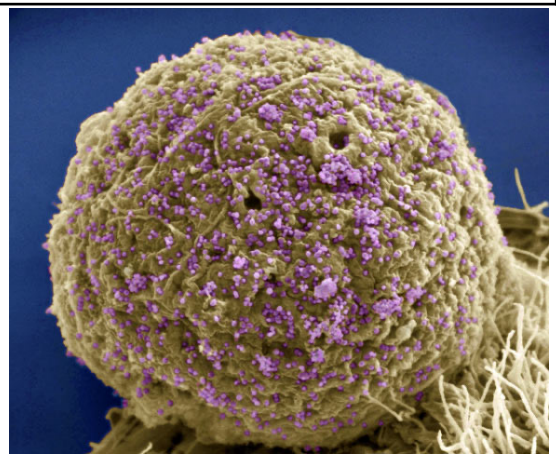
A cytotoxic T cell kills a cancer cell. (SEM image)

Helper T cells (Th)



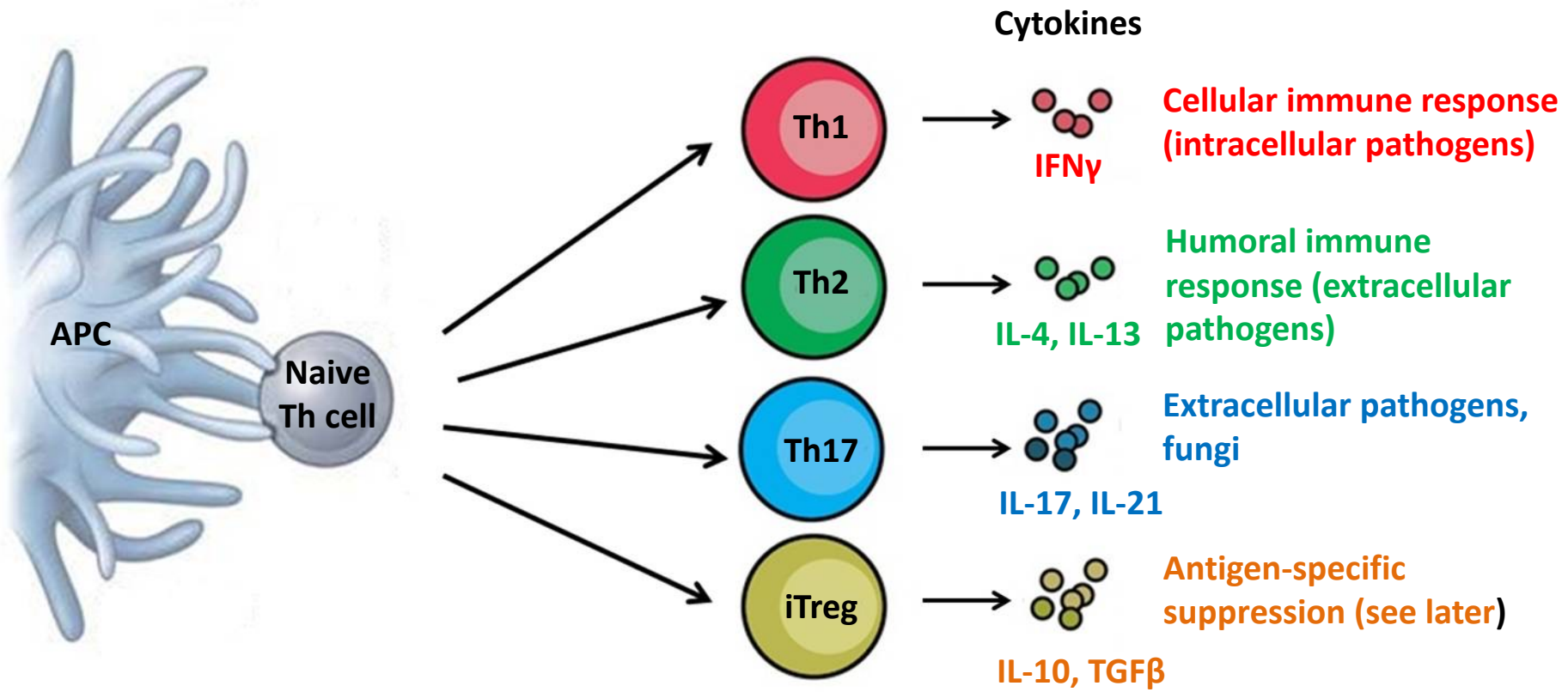
Two helper T cells attached to a dendritic cell. (Scanning electron microscopy image)

Blood T cells:	**1/3**
Main function:	**Regulation of immune response**
Recognition:	**Through MHC II, antigen-specific TCR**
Recognized antigens:	**Exogenous (degraded in phagolysosomes)**
Immunophenotype:	**CD3+/CD4+/CD8-**
Role in diseases:	**Autoimmunity, HIV infection**



Yellowish-brown: Th cell
purple: **HIV** virions
(SEM image)

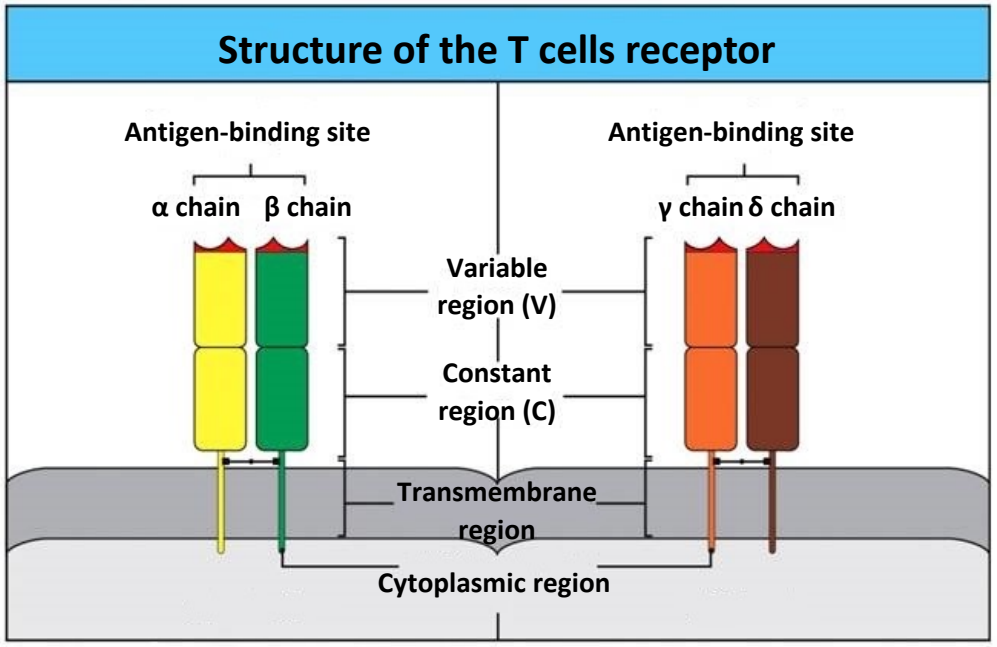
Main subtypes of Th cells



- Th17 cells play an important role in **inflammatory disorders**. (see later)
- **Regulatory T cells** (Treg): They can inhibit other immune cells (**suppression**, see later), their immunophenotype is: **CD4+/CD25+/Foxp3+**

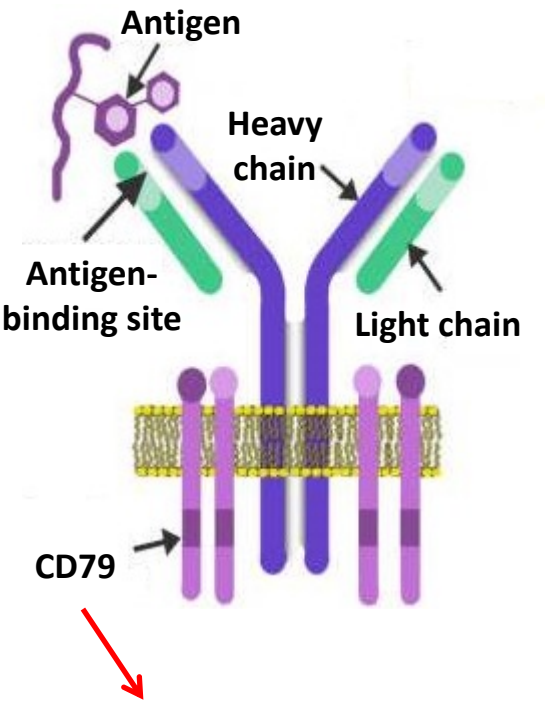
γδ T cells

- They express TCRs that consist of γ and δ chains.
- They are **innate-like lymphocytes**, they are not as well-characterized as αβ T cells.[17.]
- They are mainly found in the **skin** and the **mucosa**; usually as intraepithelial lymphocytes (IELs). They can be detected in the peripheral blood in low numbers.
- They participate in the early phases of the immune response against invasive pathogens.
- Their antigen-recognition is **MHC-independent**.
- They mainly recognize **lipid antigens**.



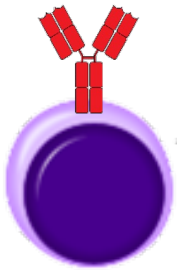
B cells

B cell receptor (BCR)



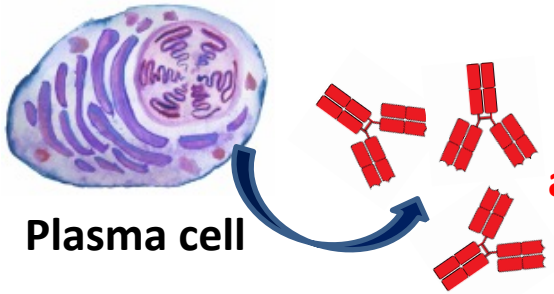
Signal transduction in the case of antigen binding

Blood lymphoid cells %:	**10-15**
Main functions:	**Antibody production, Antigen presentation**
Recognition:	**Native antigens with antigen-specific BCR**
Main types:	**B1 and B2**
Site of production:	**Bone marrow**
Characteristic marker:	**CD19 (makes a complex with BCR)**



BCR = surface immunoglobulin

B cell

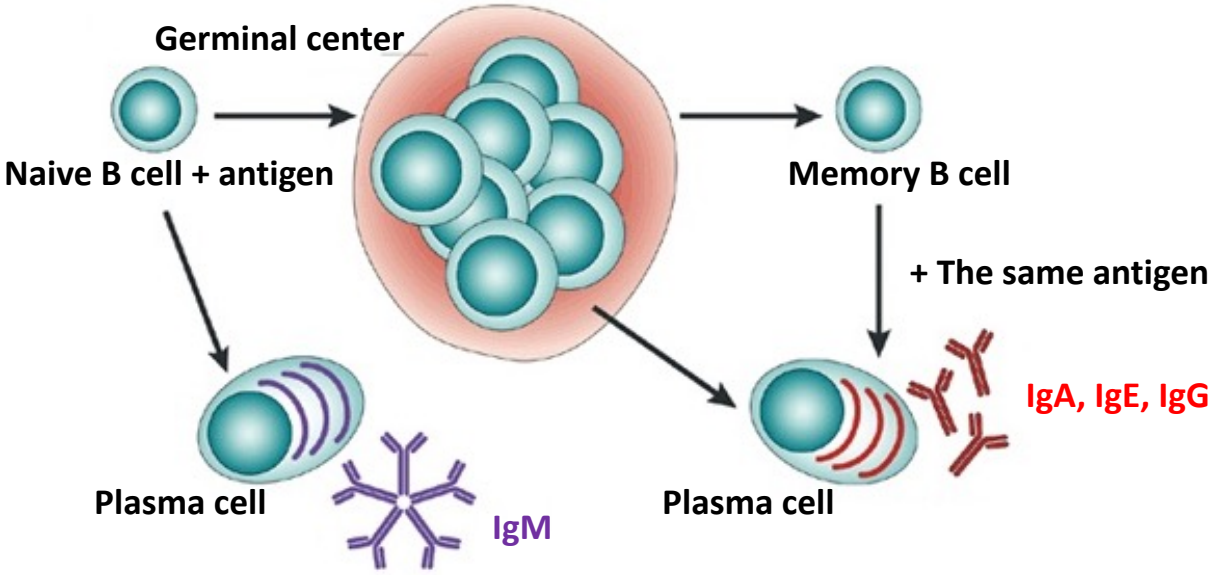


Plasma cell

Antibody against the same antigen recognized by the BCR (secreted immunoglobulin)

B2 B cells

Found in:	**Follicles in secondary lmyphoid organs, blood**
Main functions:	**Antibody production, Antigen presentation**
Recognition:	**Native antigens with antigen-specific BCR**
Site of primary maturation:	**Bone marrow**
Site of antigen-dependent maturation:	**Germinal center**
Produced antibodies:	**Monospecific, high-affinity, with varying isotype**

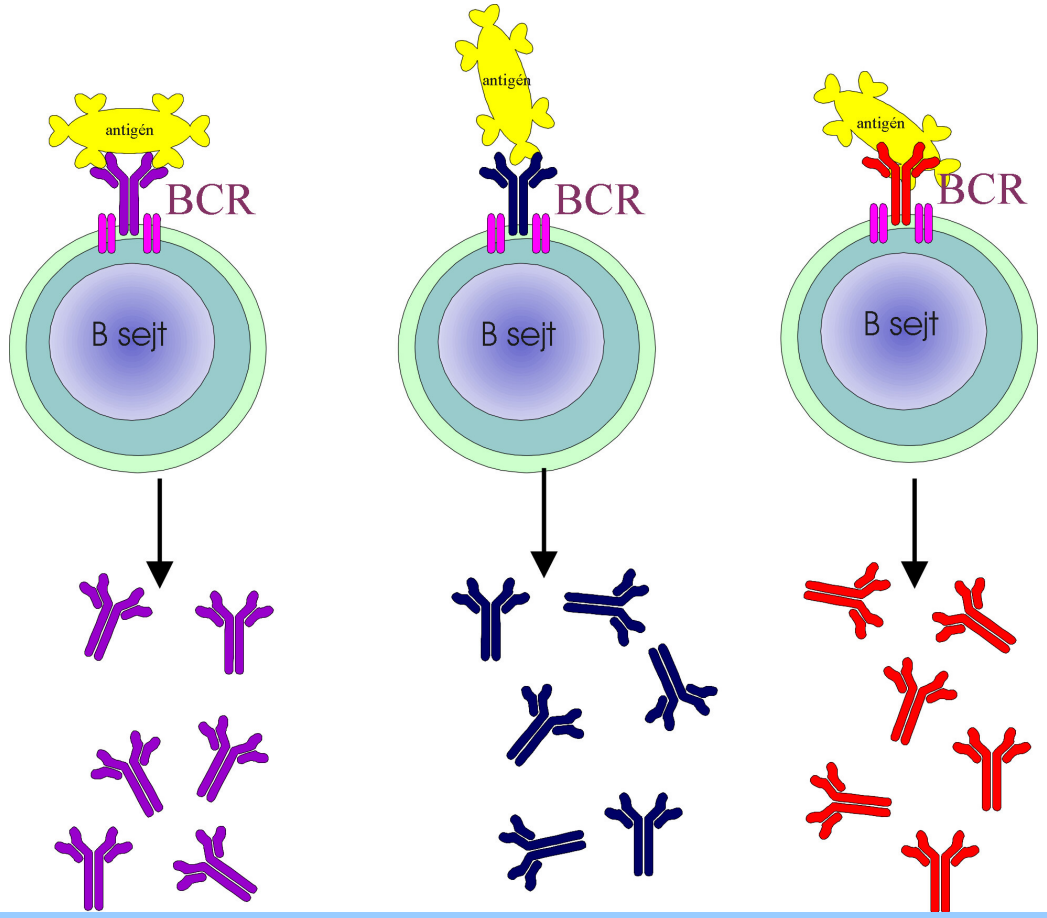


B1 B cells

- Only few can be found in the peripheral blood.
- **They are innate-like lymphocytes**, most of them reside on serous membranes. (e.g. peritoneum, pleura, pericardium)
- They are first produced in the fetus and later undergo self-renewal in the periphery, not in the bone marrow, as B2 cells do.
- They produce **natural autoantibodies** that can bind that can bind evolutionarily **conserved self-antigens**.
- They were first described as CD5+ B cells in mice.
- The immunophenotype of the human B1 cells is still controversial.

	B1 cells	B2 cells
Spontaneous antibody production	Significant	Minimal
Isotype of produced antibodies	**IgM**	IgM/IgG/IgA/IgE
Affinity and specificity of antibodies	**Polyspecific with low affinity**	Monospecific with high affinity
Affinity maturation, memory	No	Yes

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

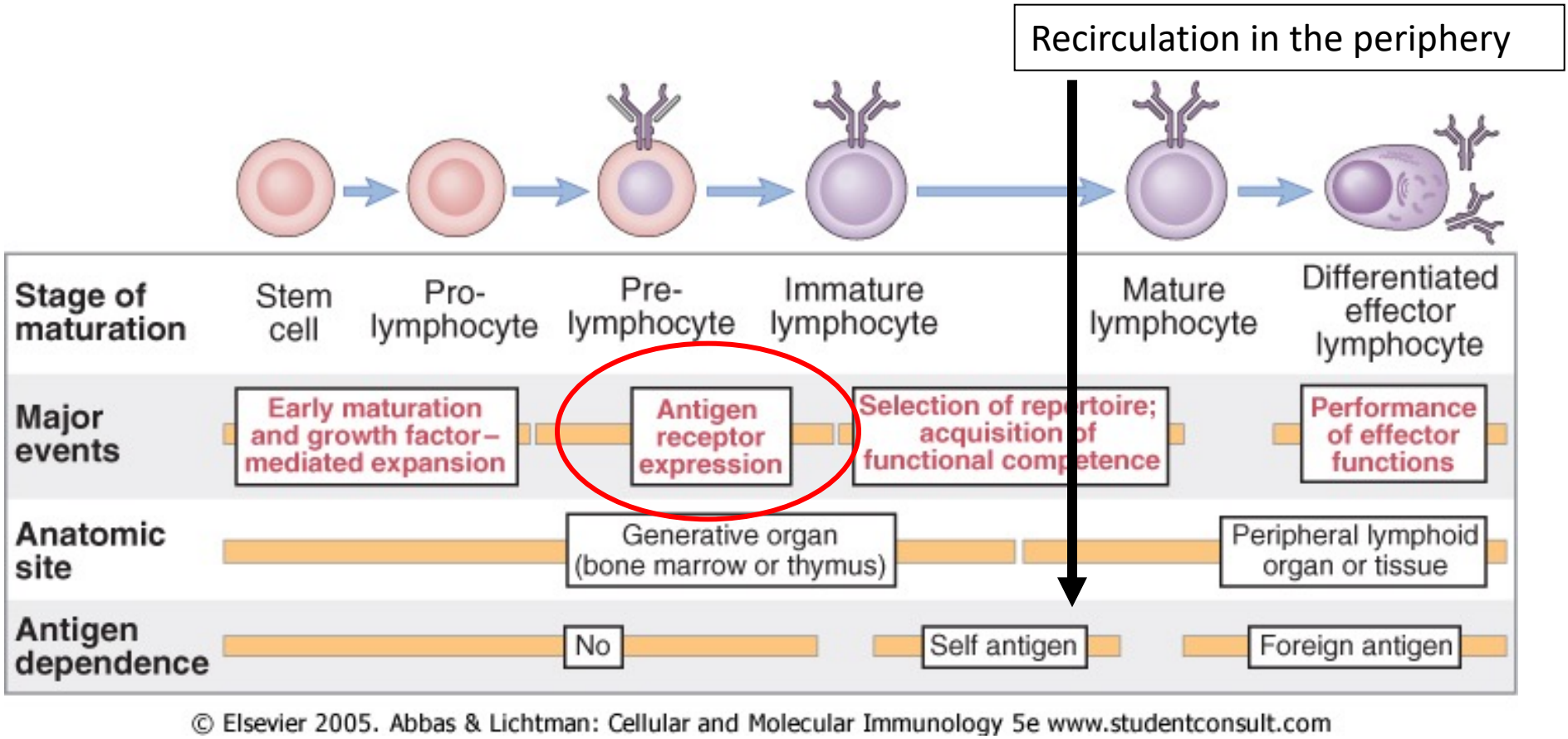
- Production of B- and T cell repertoire = Number of antigen recognition molecules: 10^9-10^{11} BcR, 10^{15}-10^{16} TcR;

„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.**

Steps of lymphocyte development



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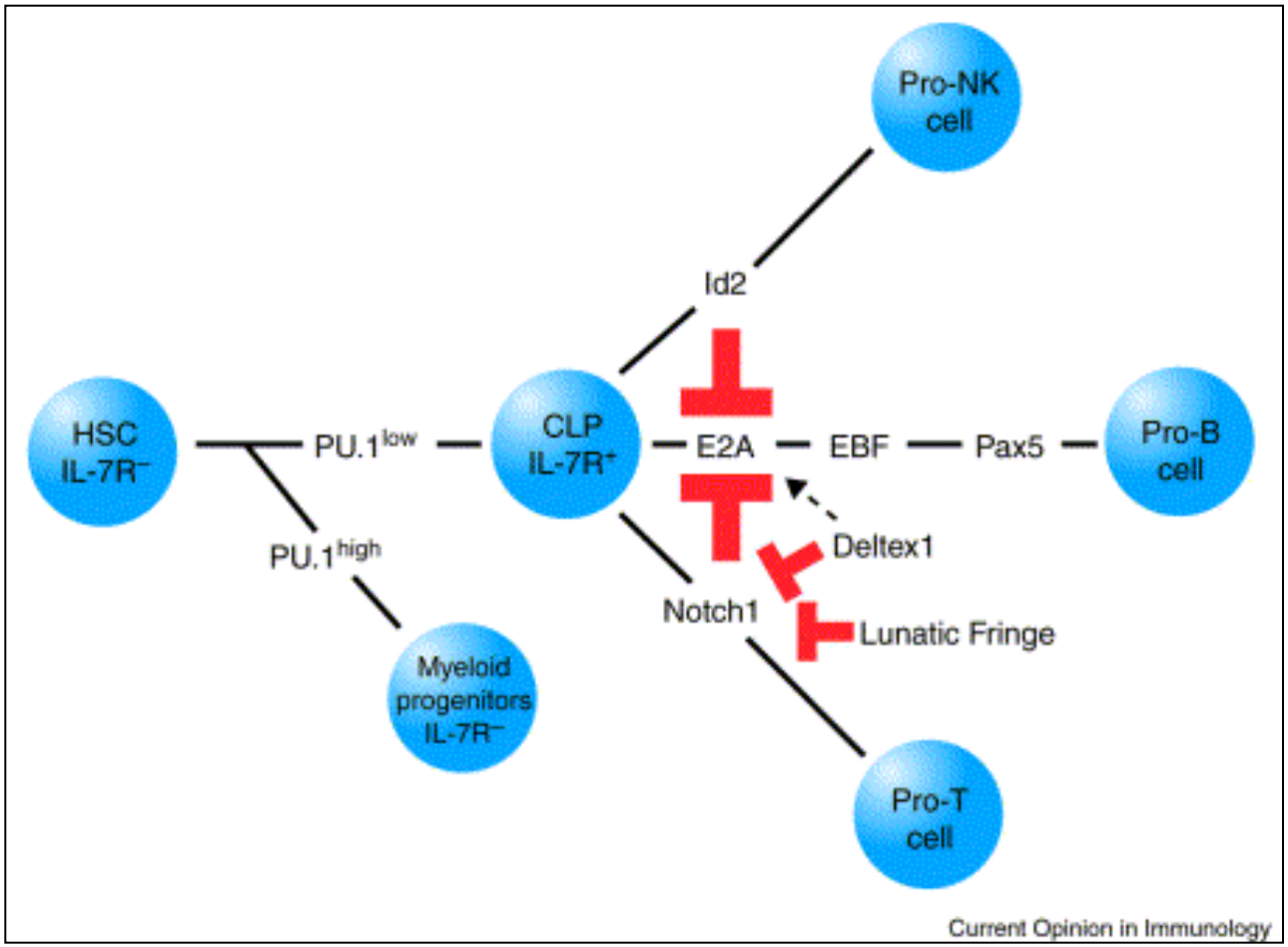
B/T/NK commitment – default E2A (B) path overruled by Notch (T) and/or Id2 (NK) signals



Role of BM stroma

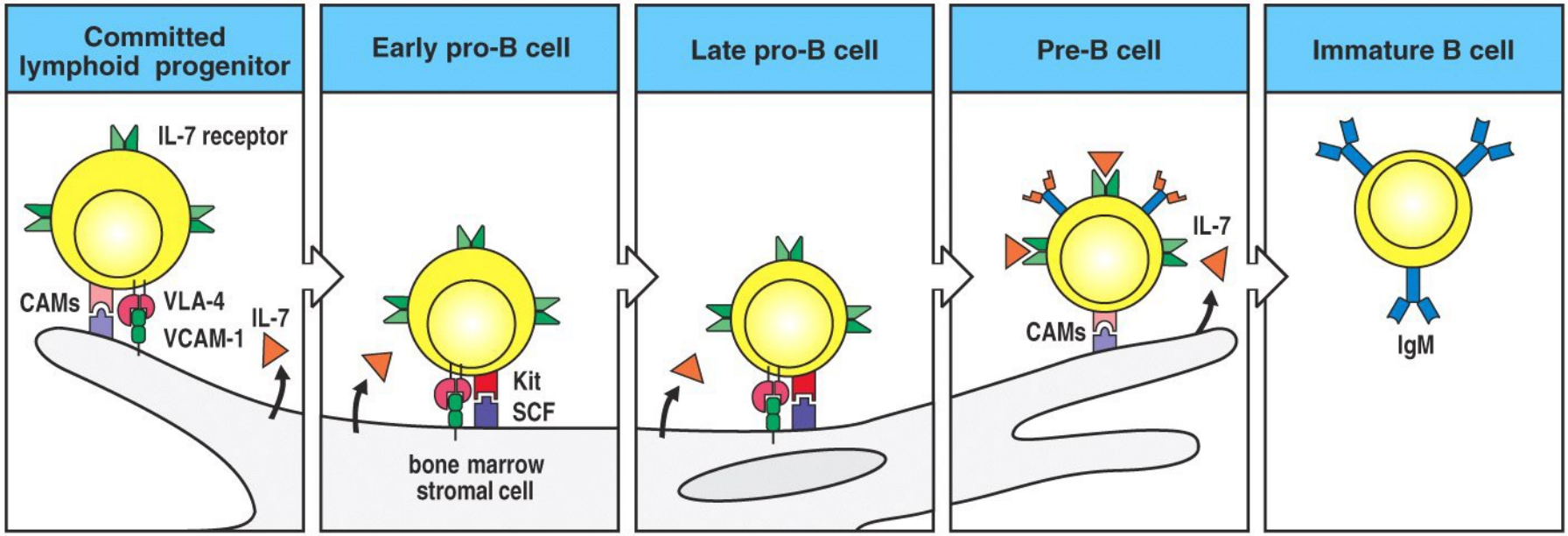
1. **Adhesion: –** CD44, VCAM-1

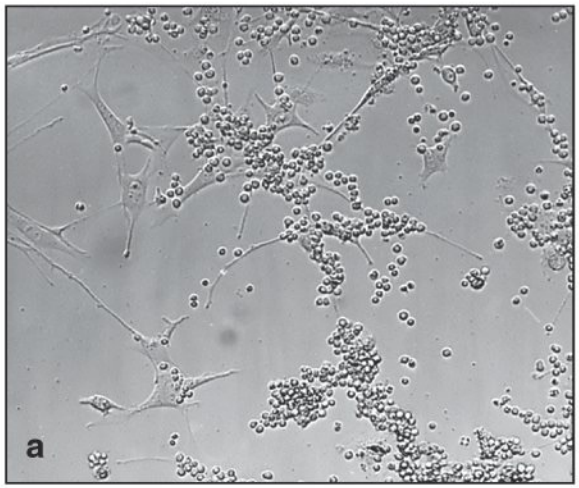
2. **Growth factors:** IL-7, IL-3, SCF**.**

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

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

Elements of B:stromal interactions





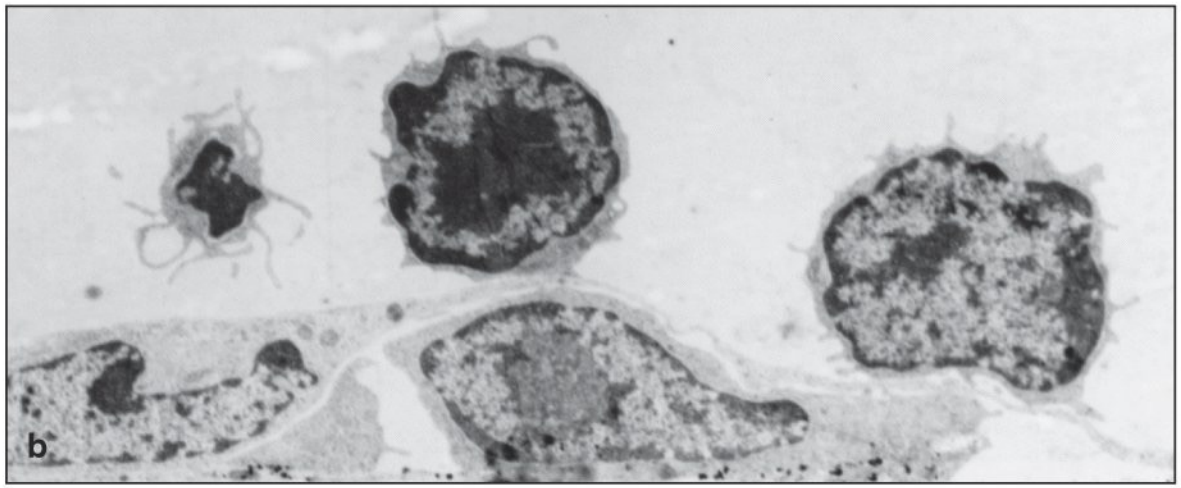
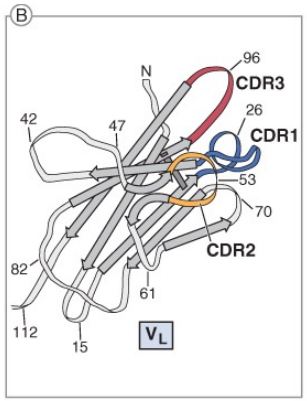


Figure 7-4 Immunobiology, 6/e. (© Garland Science 2005)

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



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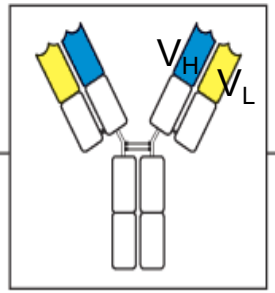


V = Fab

C= Fc

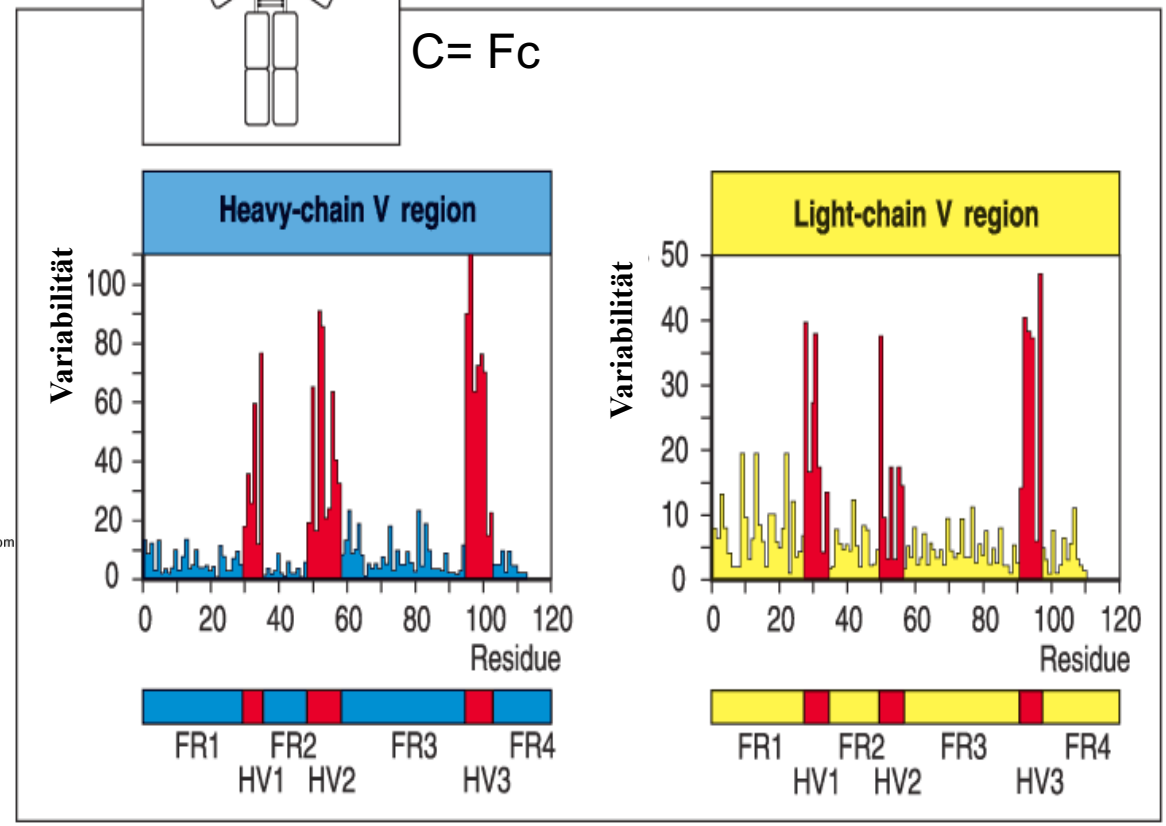
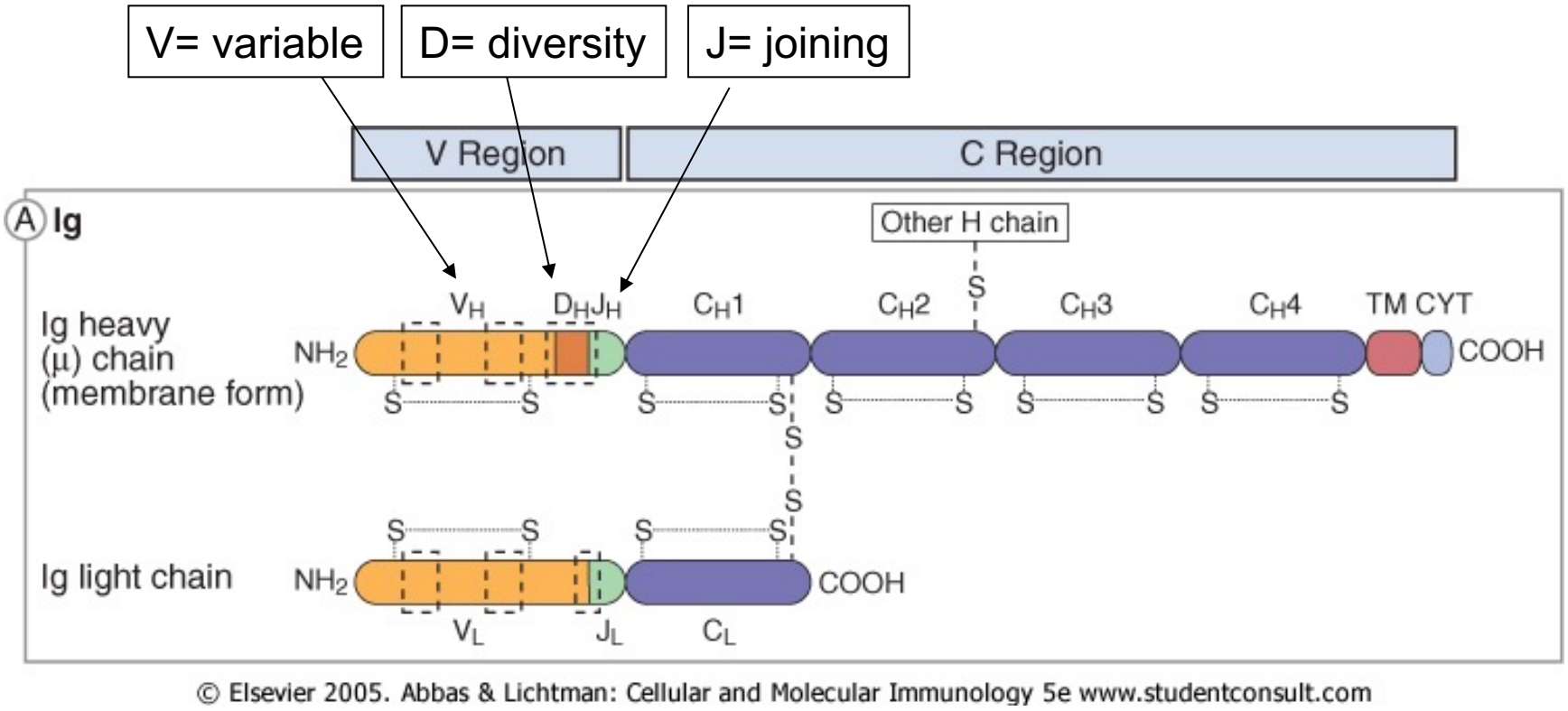


Fig 3.6 © 2001 Garland Science

Domains of the immunglobulin heavy- and light chains



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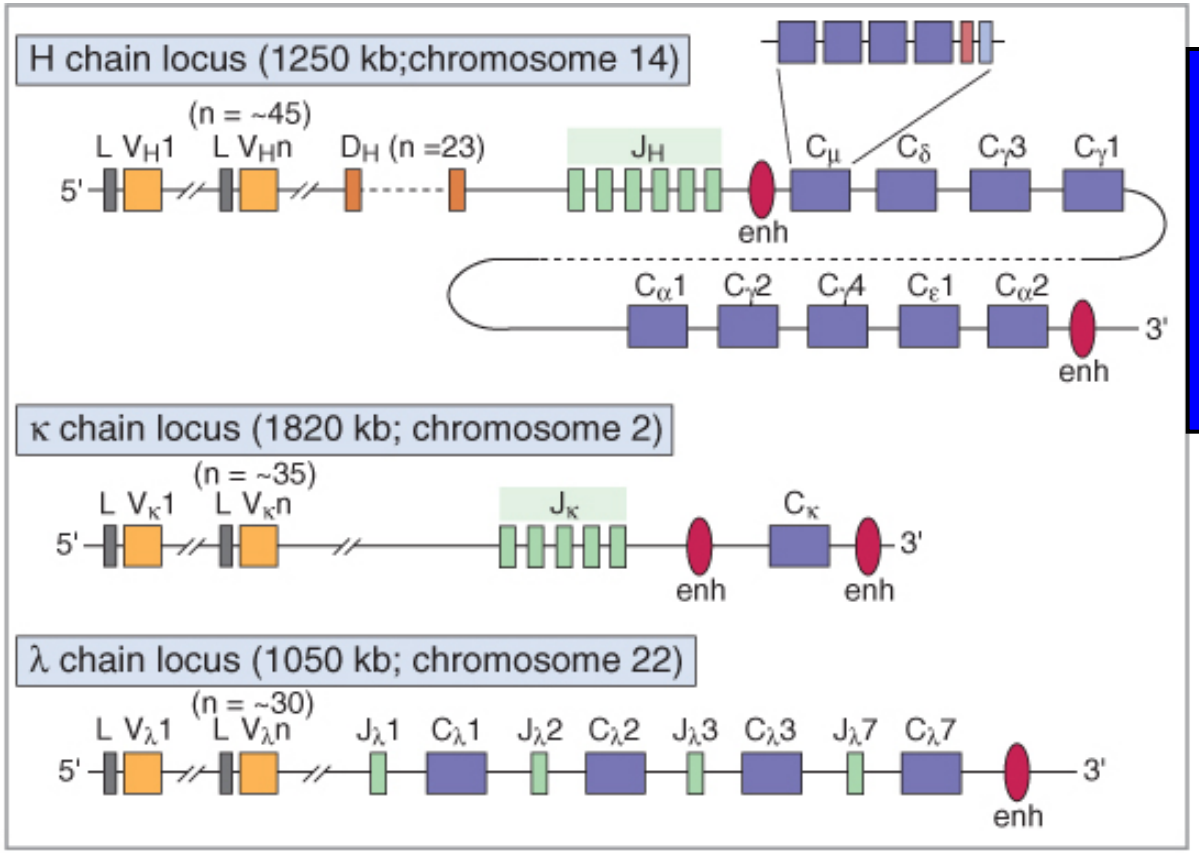
- The **variable (V)** and **constant (C)** **domains (units)** of the heavy- and light polypeptide chains are encoded by different **gene segments**.
- The genes of the Ig heavy- and light polypeptide chains are located in different chromosomes.

Gene organisation of the 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

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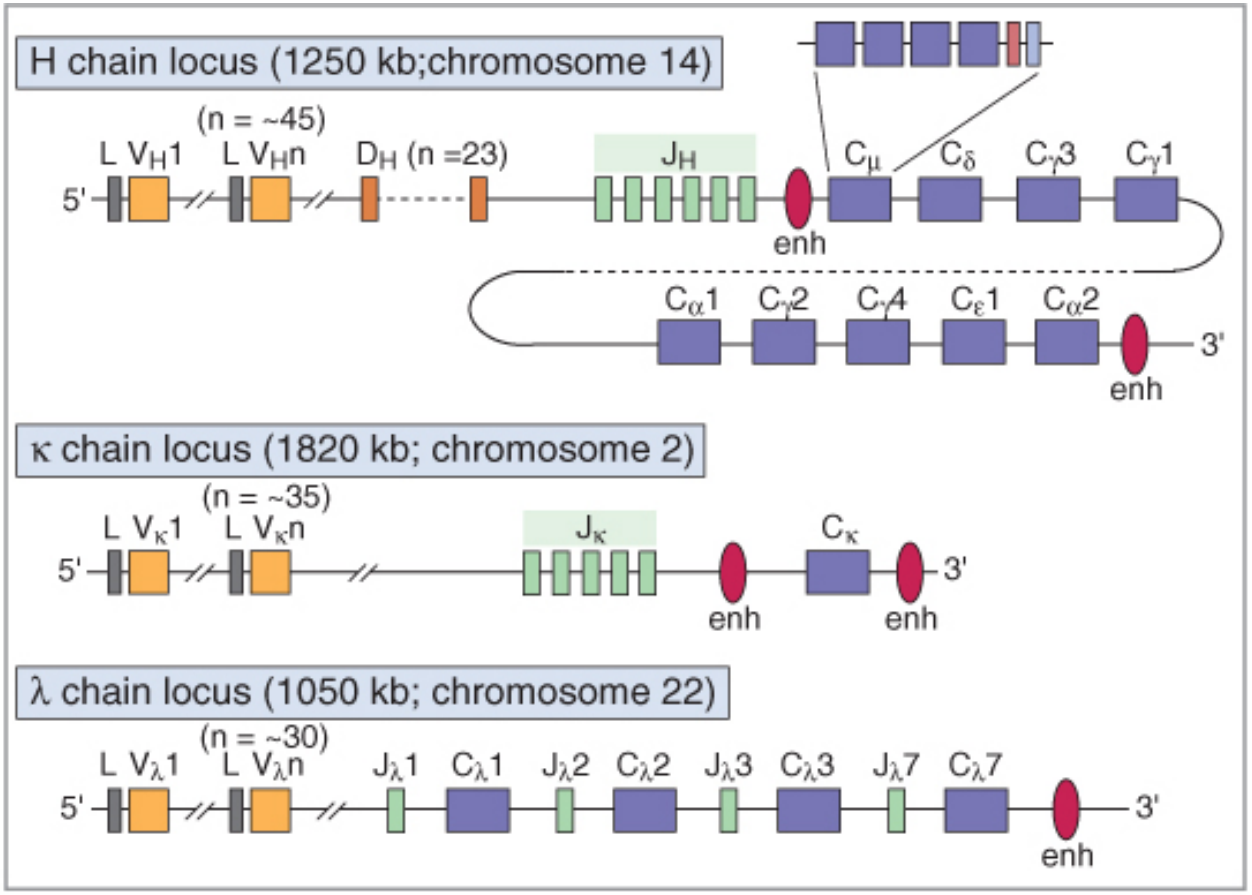
The **germline-DNA** → the basic, not-recombined form of the immunoglobulin genes

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



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

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

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

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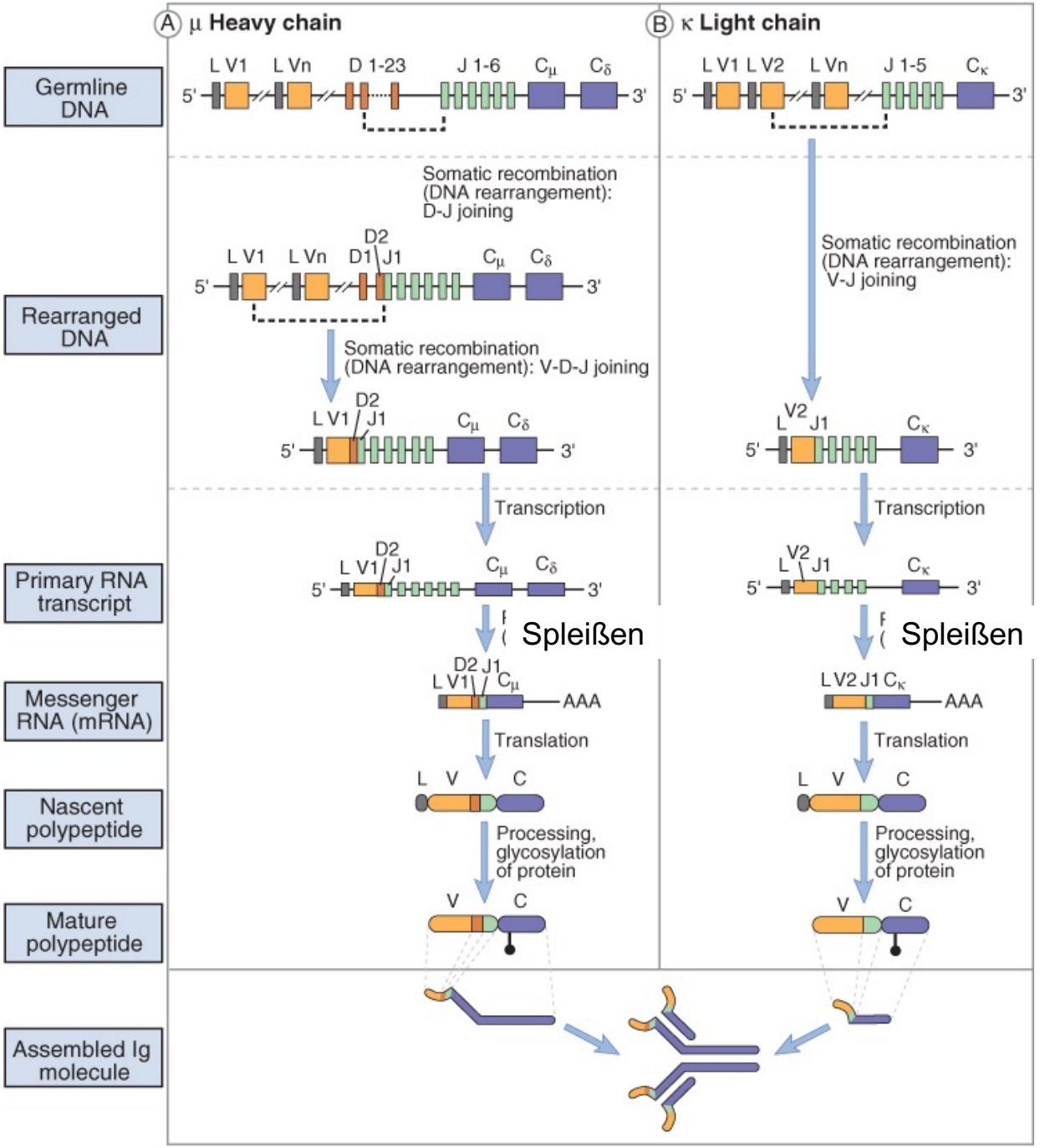
In lymphocyte precursors the germline DNA will be rearranged by somatic recombination. = **Rearrangement**

Steps of the gene rearrangement



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Molecular mechanism of the gene rearrangement

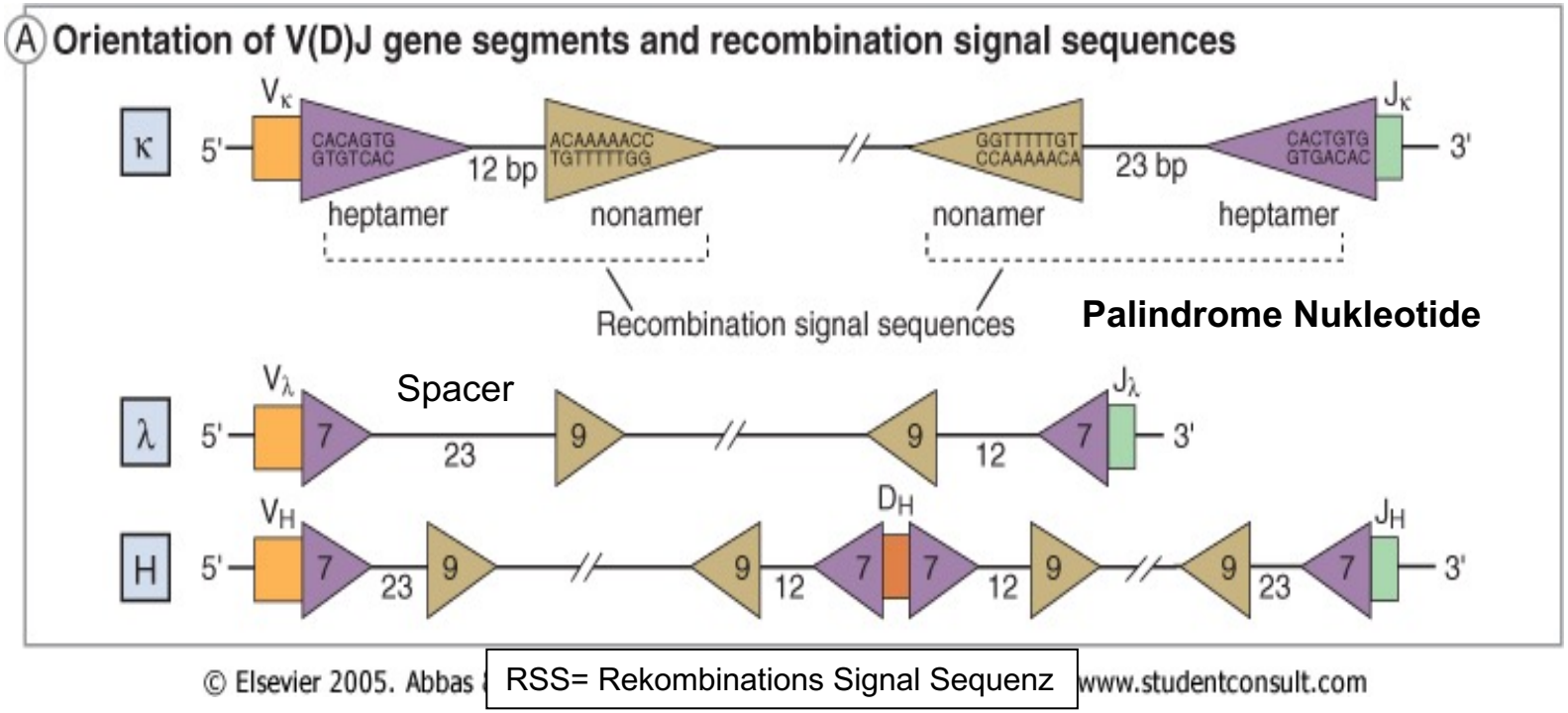
1. DNA loop formation
2. DNA cutting - Deletion
3. Ligation of the free DNA ends

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:

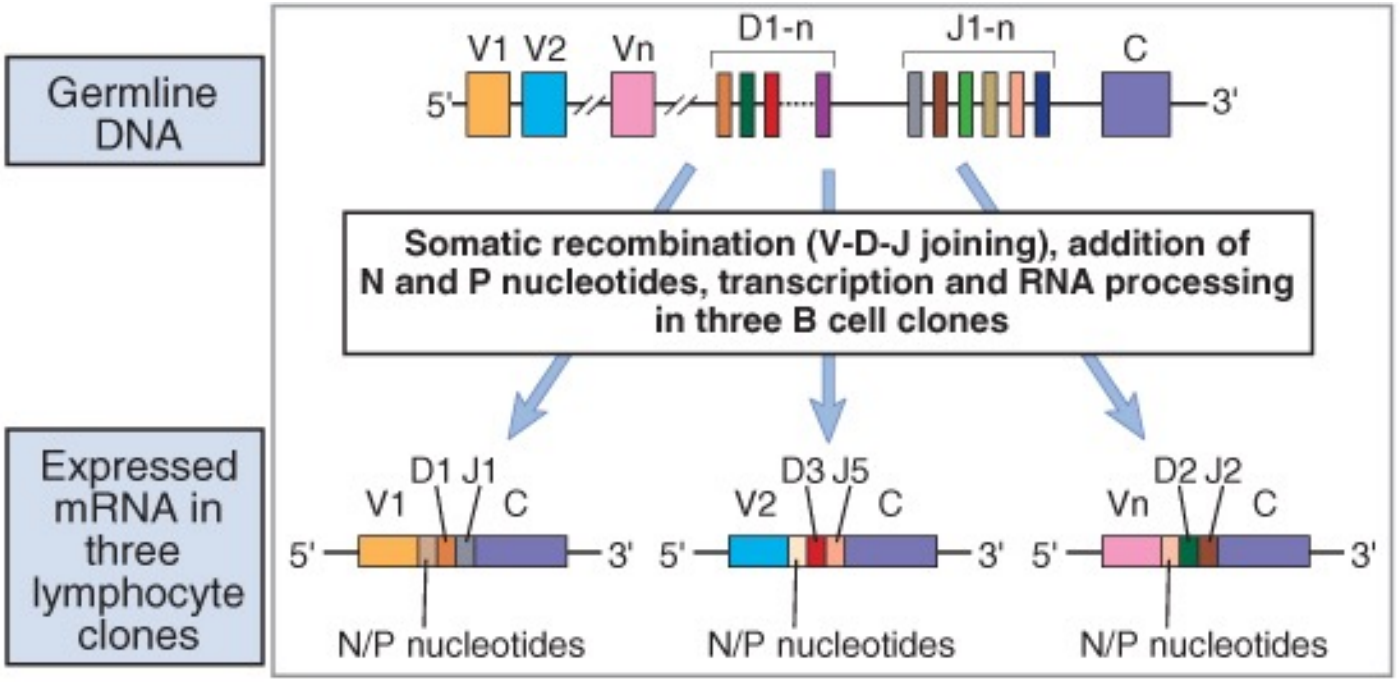


RSS= Rekombinations Signal Sequenz

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



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Random gene rearrangement → **Diversity**

T-cell-receptor (TcR)

T-cell-types:

1. αβ TcR+

2. γδ TcR+

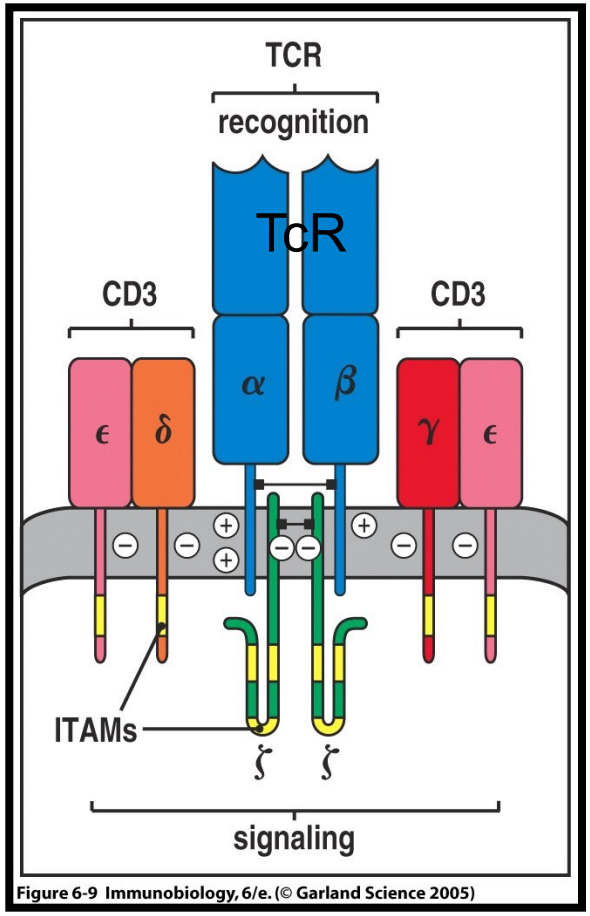
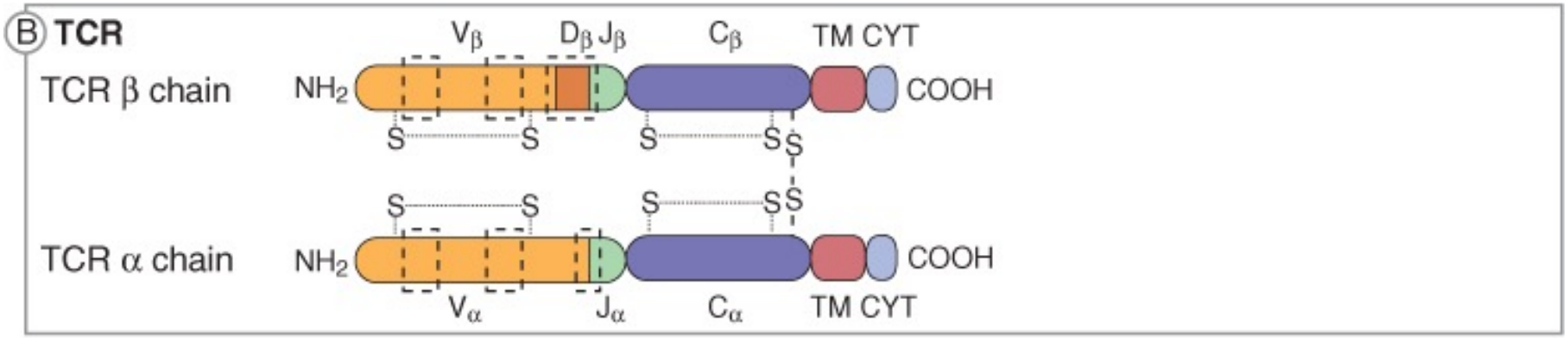


Figure 6-9 Immunobiology, 6/e. (© Garland Science 2005)

TcR α-β chains



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Human TCR encoding genes

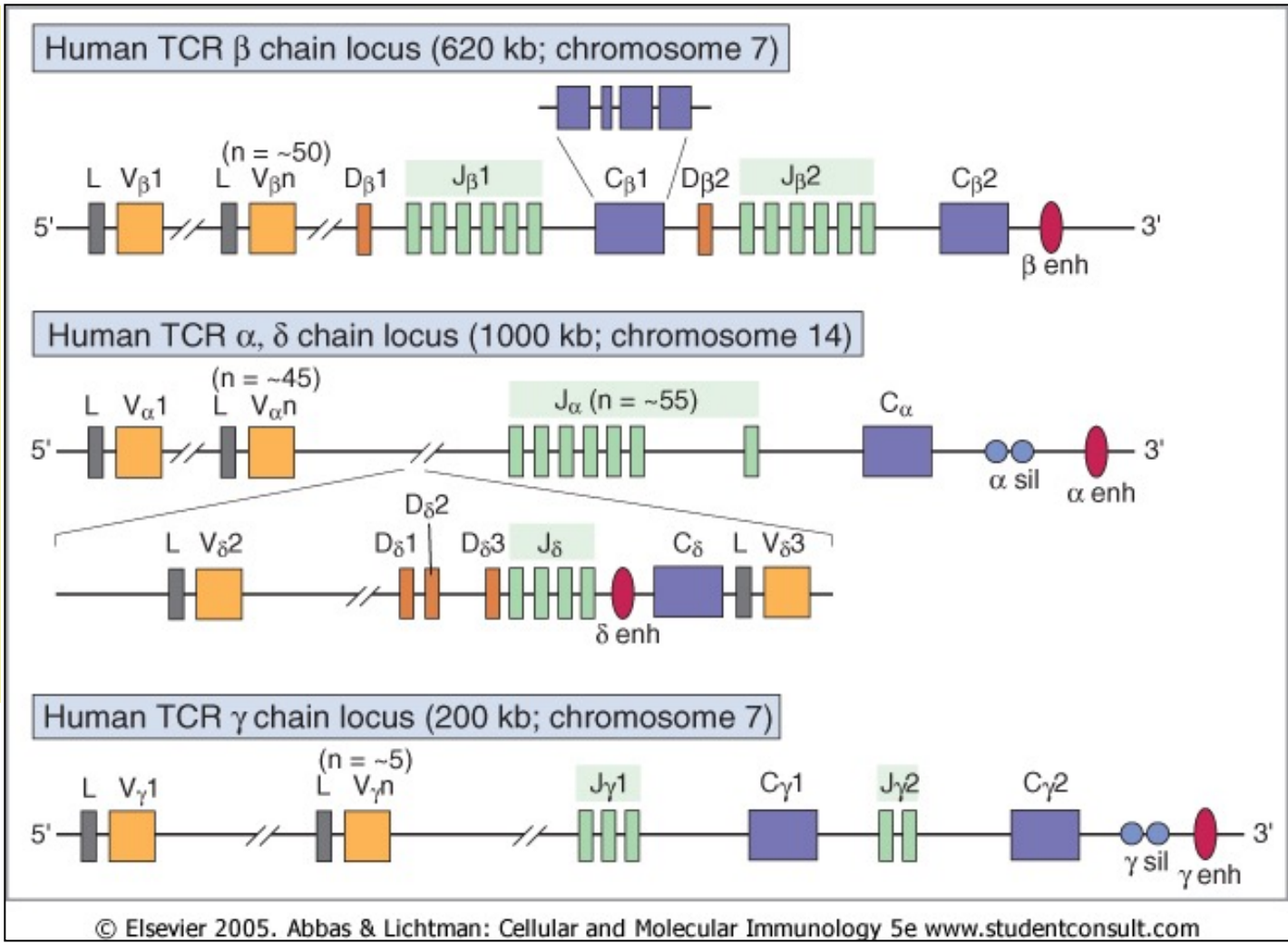
V,J,D and C genes

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



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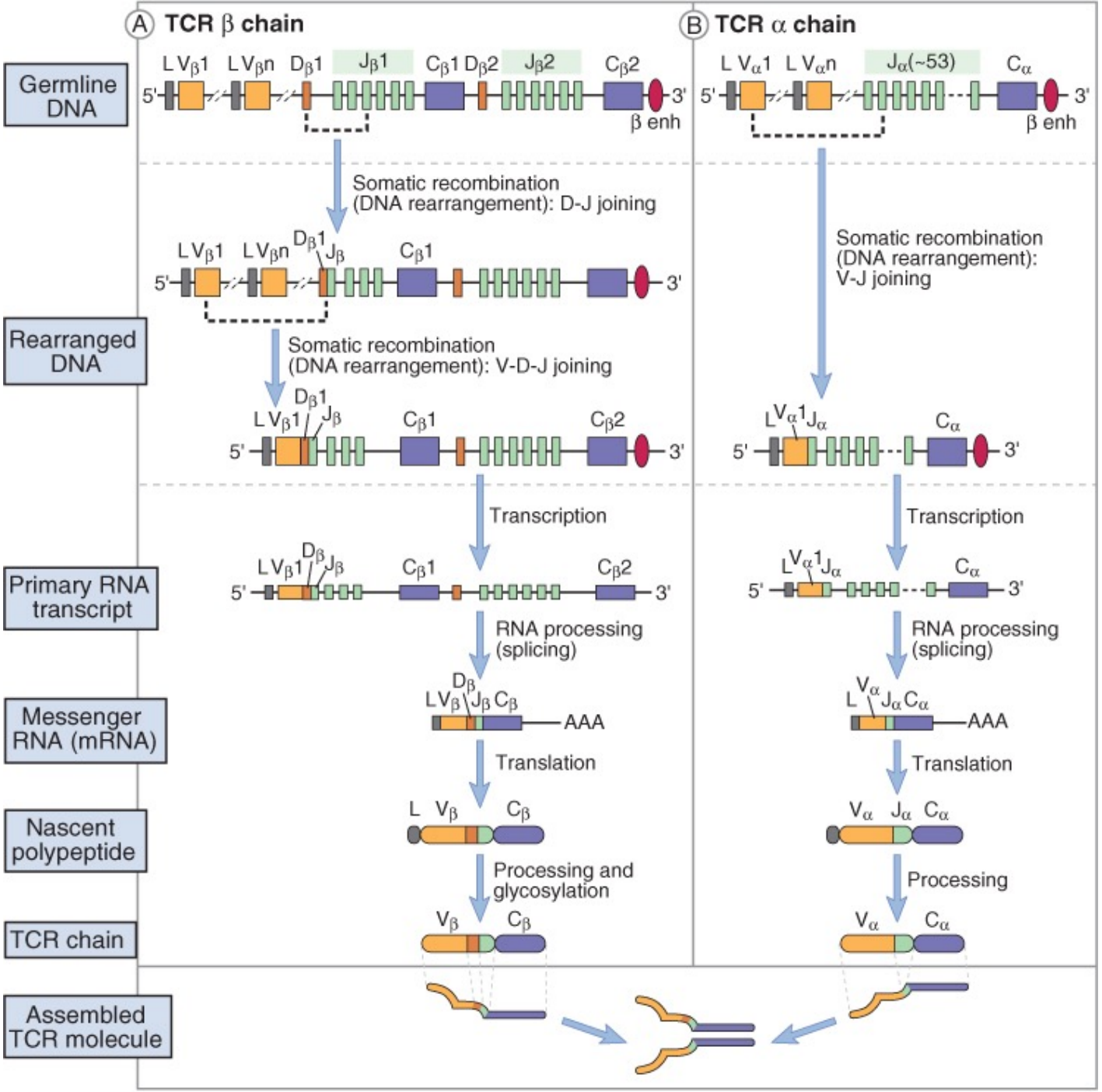

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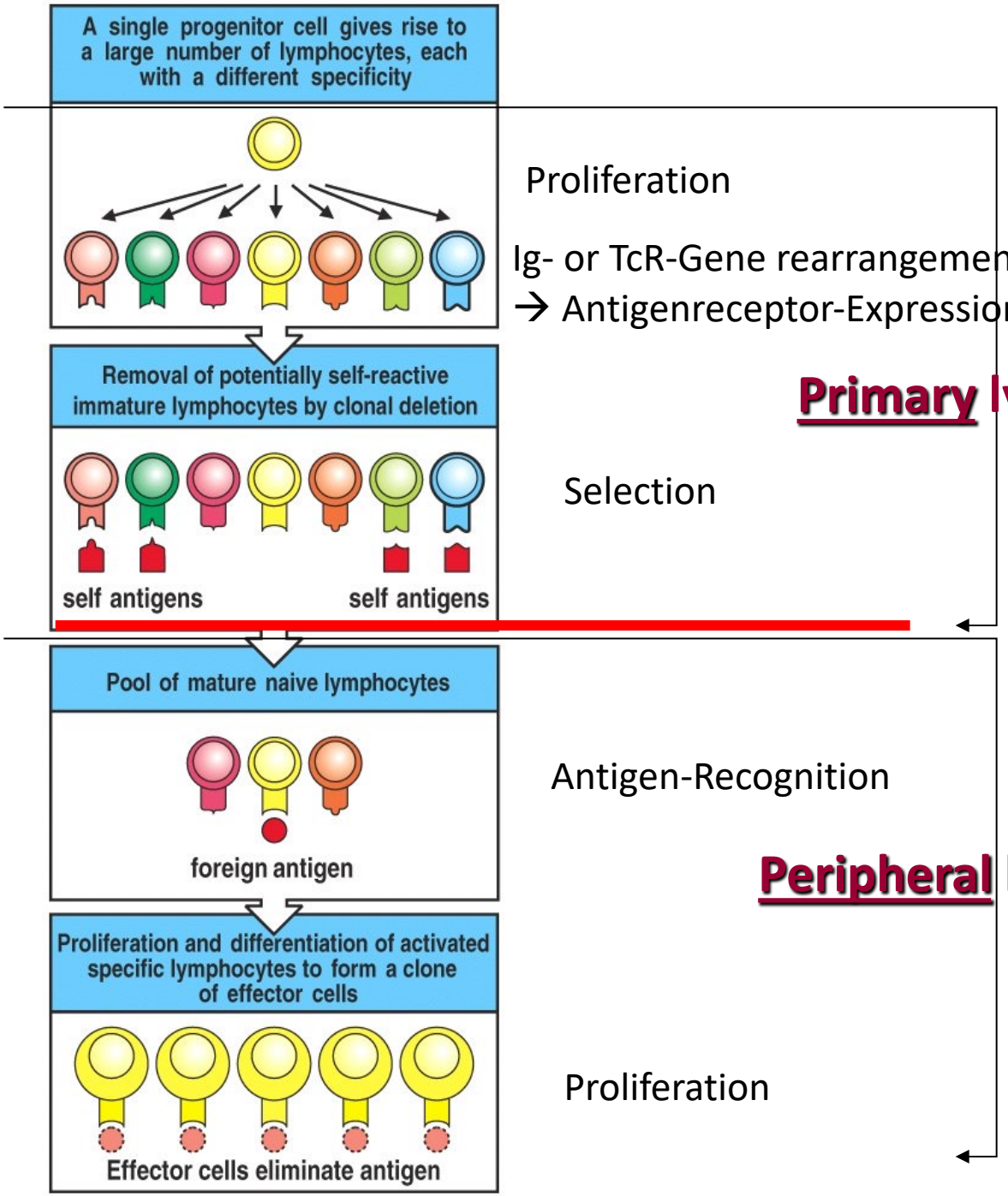
The basis of TcR and BcR diversity

- 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)

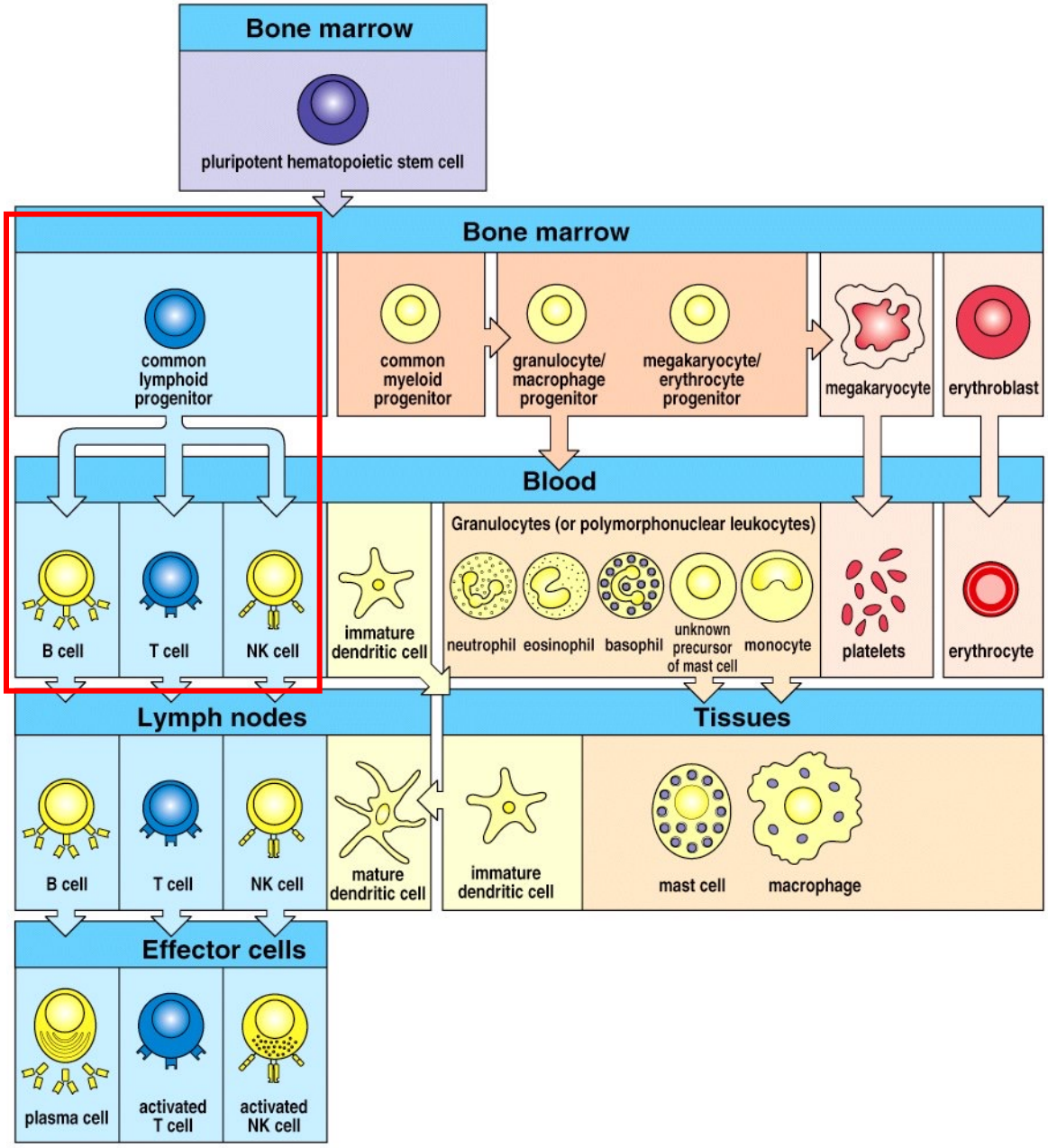
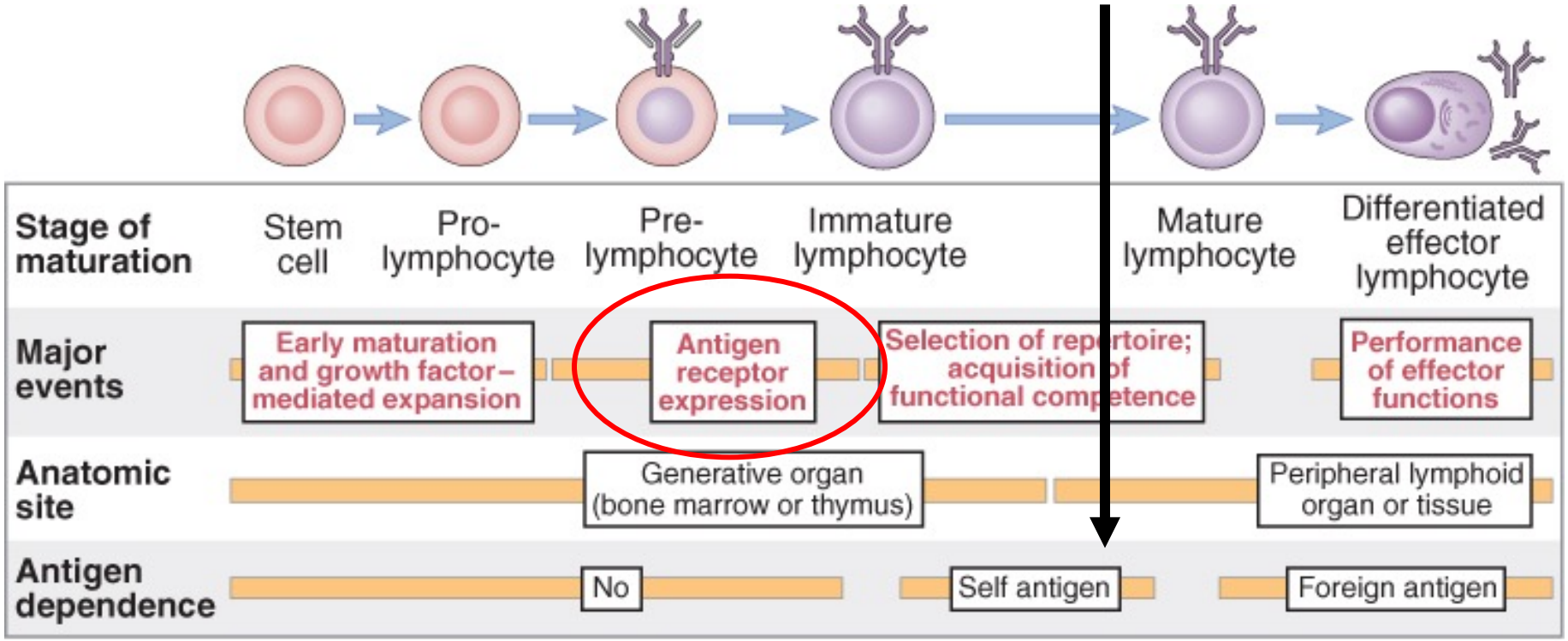


Figure 1-3 Immunobiology, 6/e. (© Garland Science 2005)

General characteristics of lymphocyte differentiation

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

Stages of Lymphocyte differentiation



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

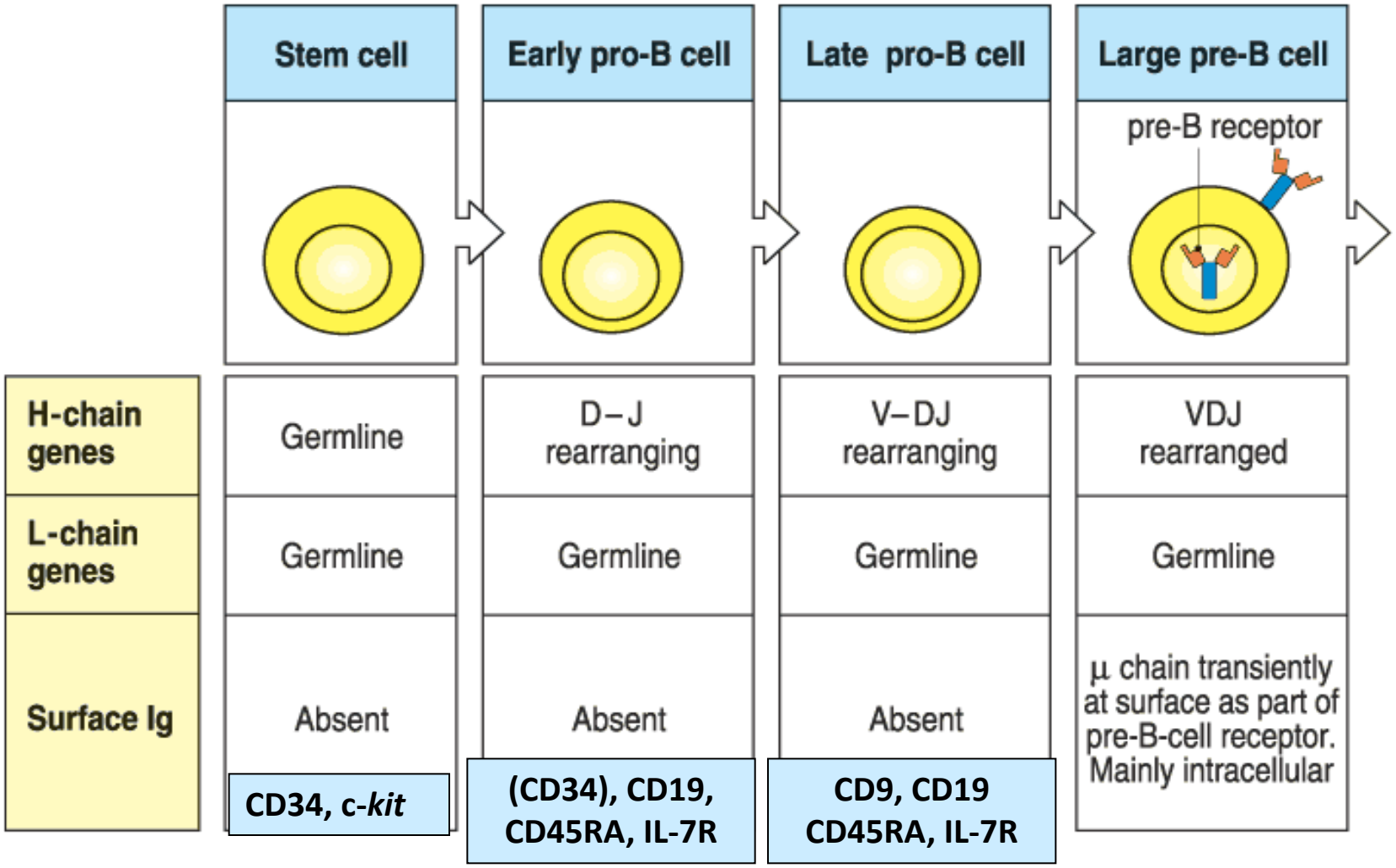


Fig 7.5 part 1 of 2 © 2001 Garland Science

B-cell development II "Small pre-B" > "mature B"

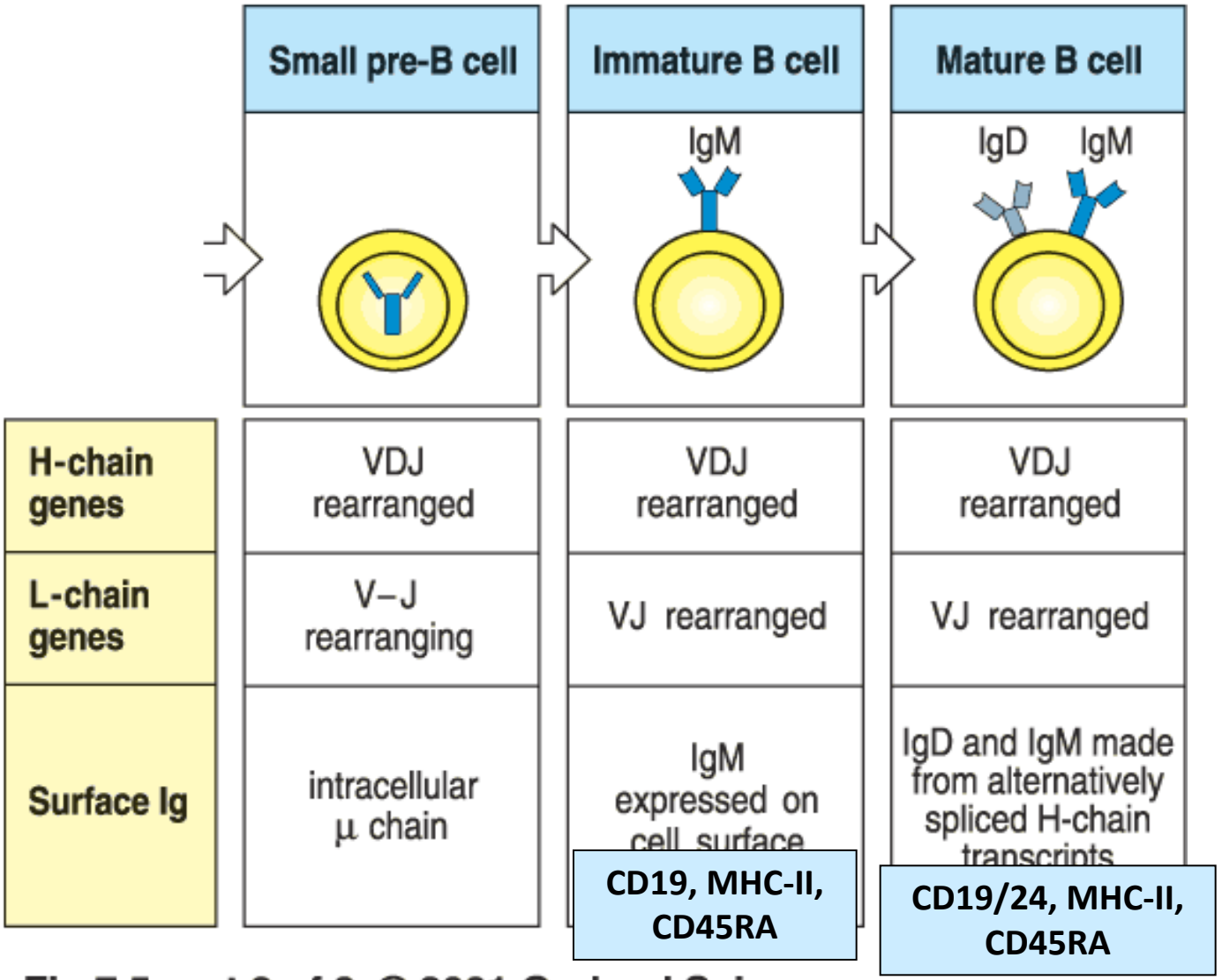


Fig 7.5 part 2 of 2 © 2001 Garland Science

Ontogenic differences between B-cell subsets

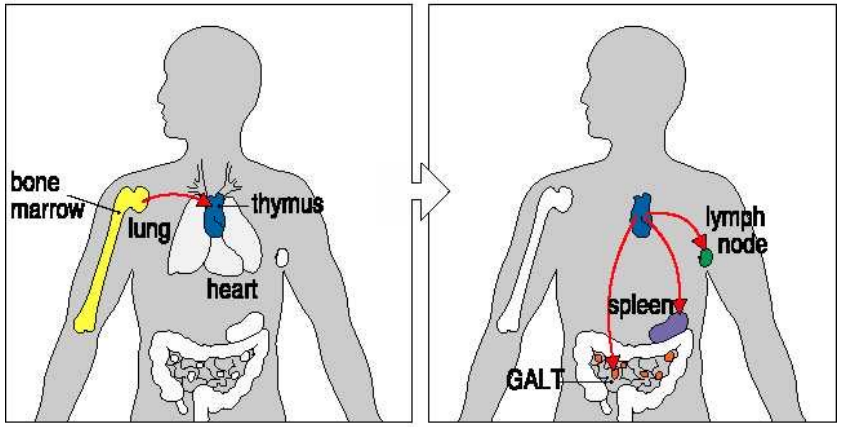
- **B-1 B cell subsets:** fetal origin, self-renewal, low-affinity autoantibody production, dominance in neonates and CLL, located in body cavities.

  (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

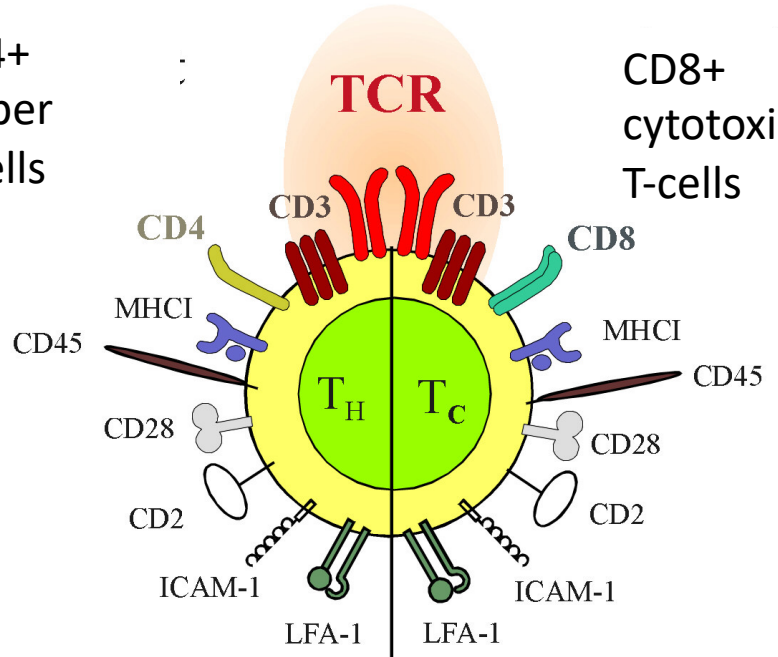
→ **Self-MHC restricted**

Self-tolerant T cells

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

T-lymphocytes with αβ TcR

CD4+ helper T cells



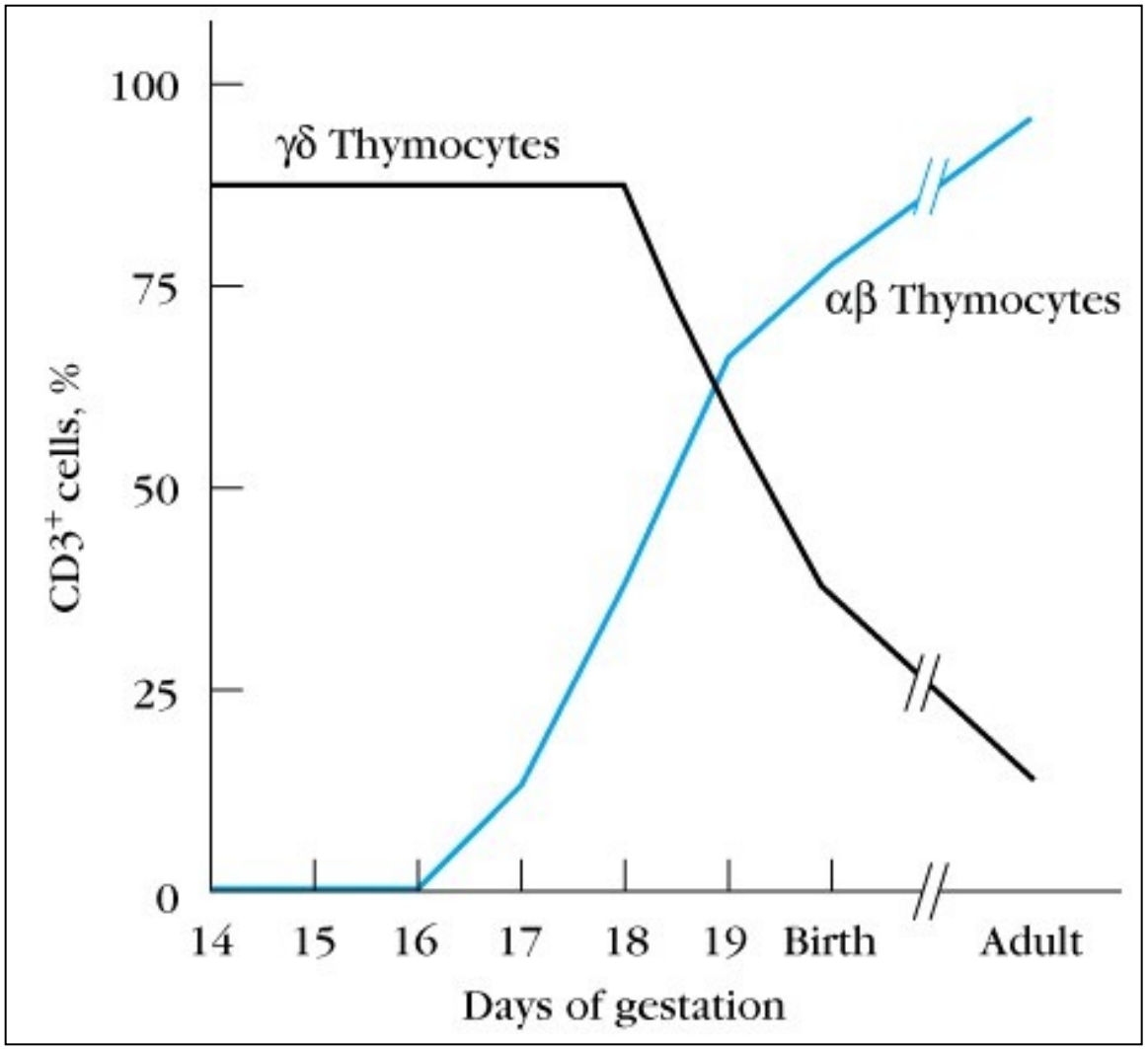
CD8+ cytotoxic T-cells

T-lymphocytes with γδ TcR

- CD4-CD8- cytotoxic T-cells

Intraepithelial – with lower TcR diversity

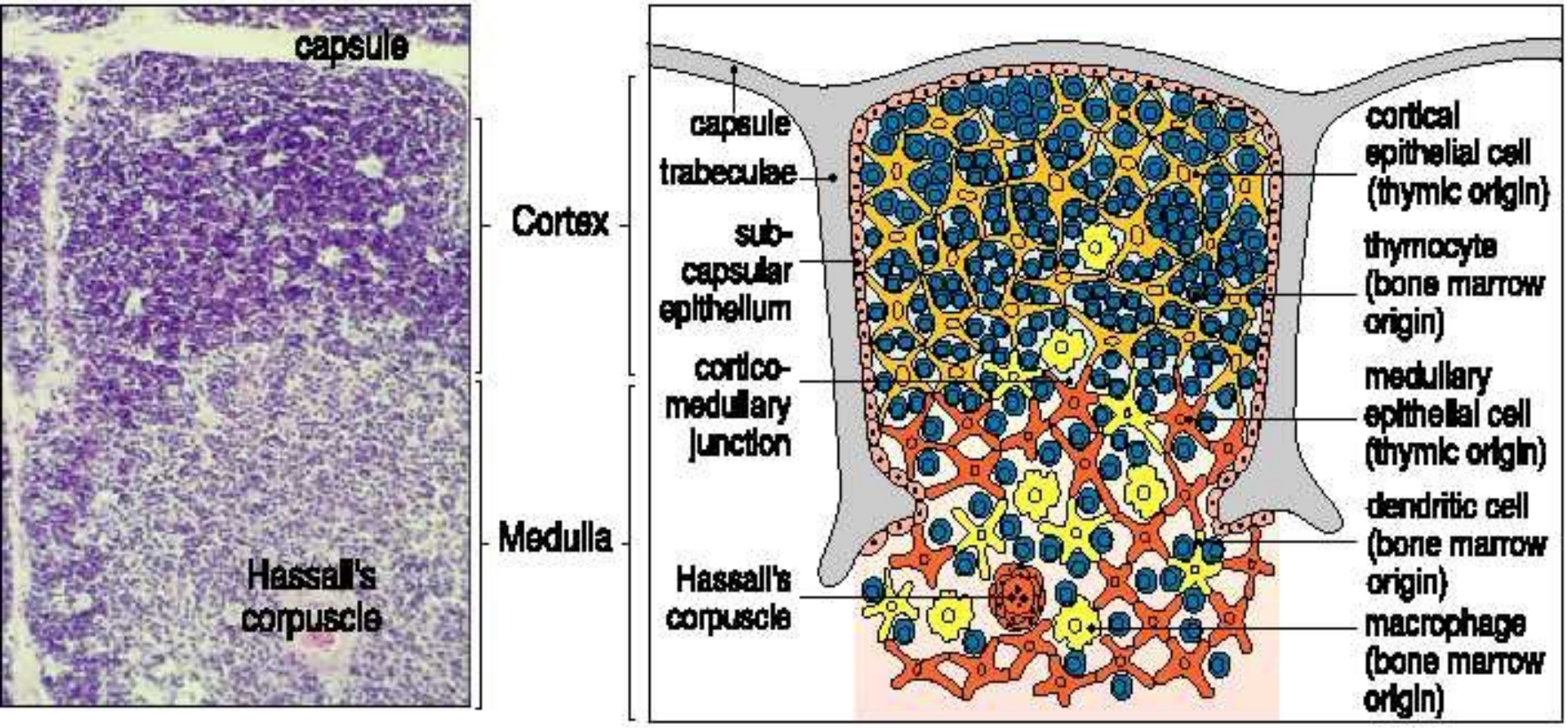
Lymphoid tissues – highly diverse receptors
Regulatory cytokine production



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

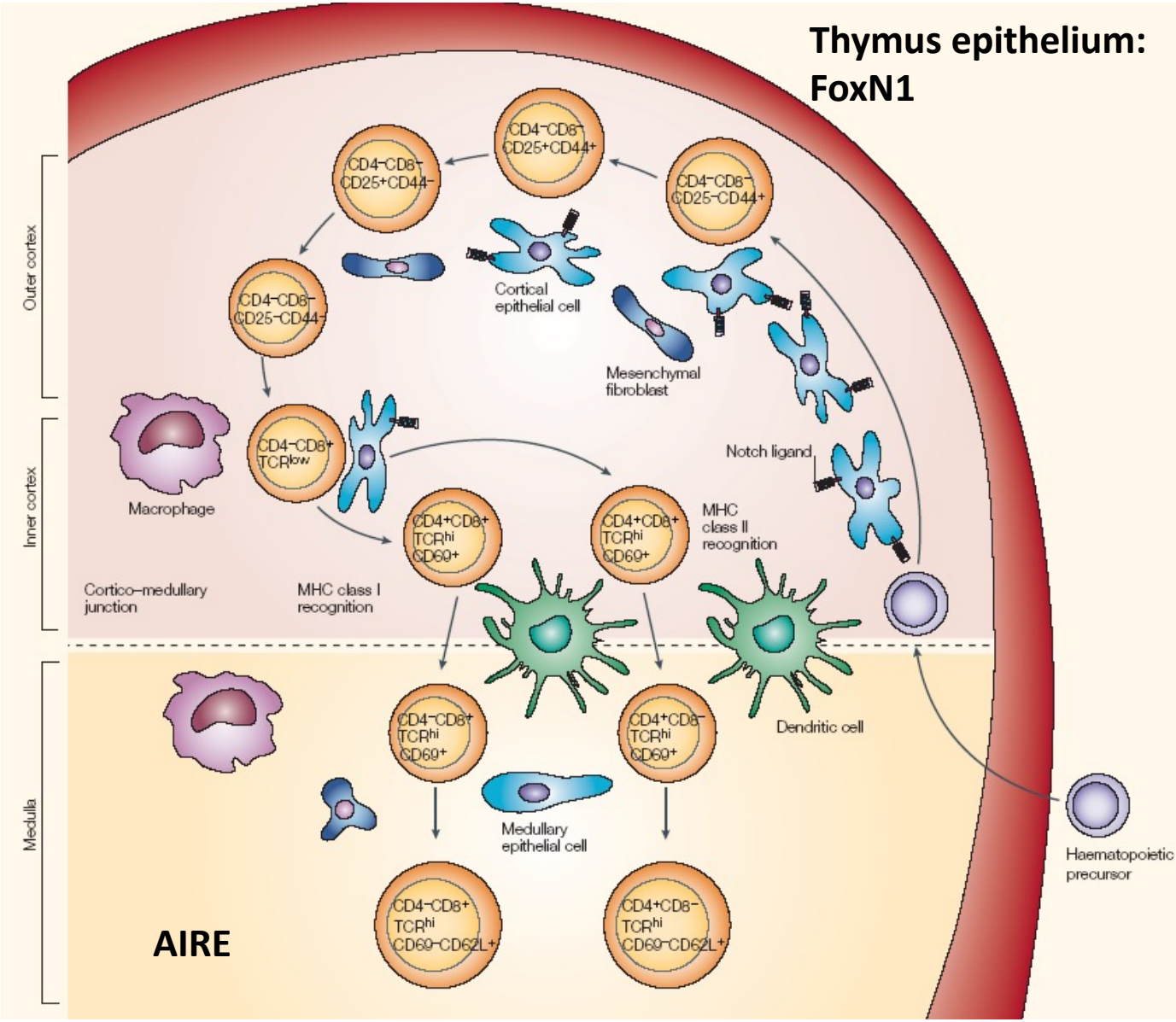
Thymic Microenvironment and T-cell Development

1. **Migration:**
   Chemokine effect

2. **Proliferation**
   IL-7

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

4. **Selection**
   Apoptosis



T-cell development in the thymus

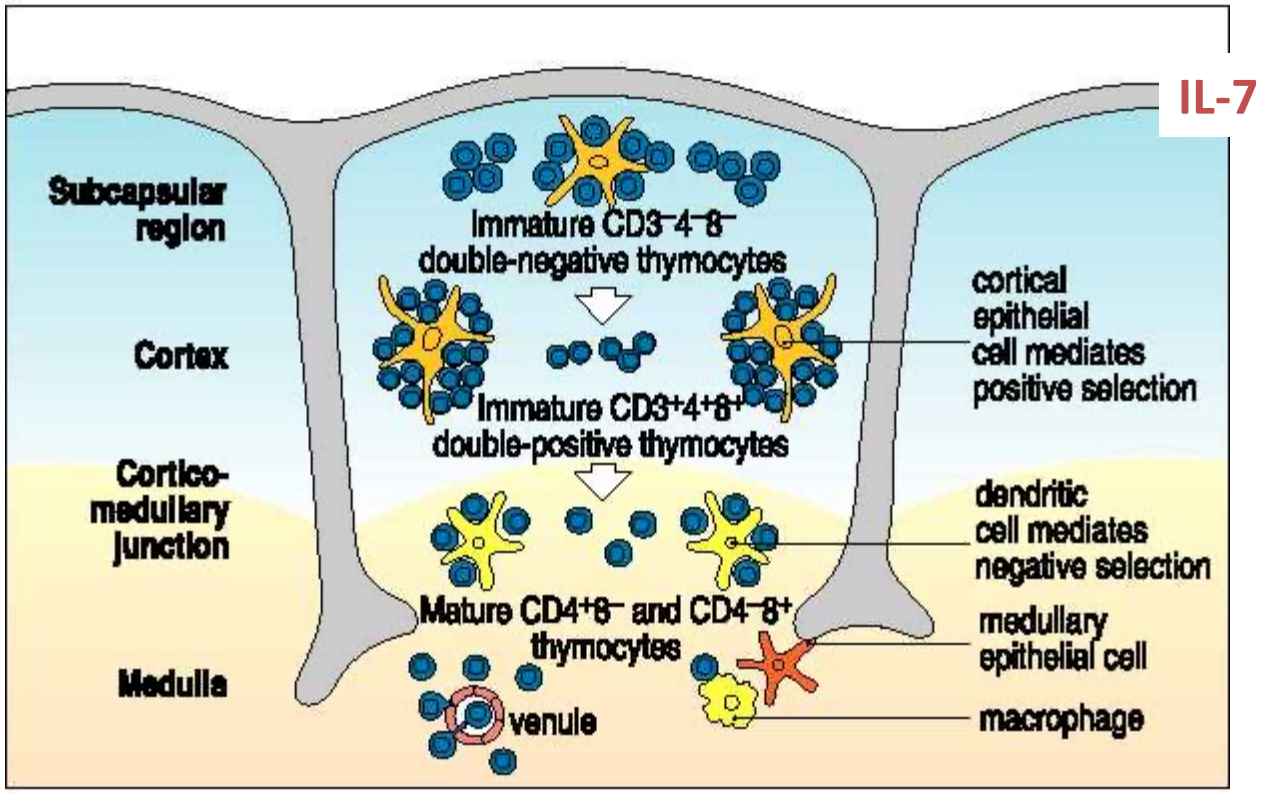
Thymocytes:

DN: 2-5 %

DP: 70-80%

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

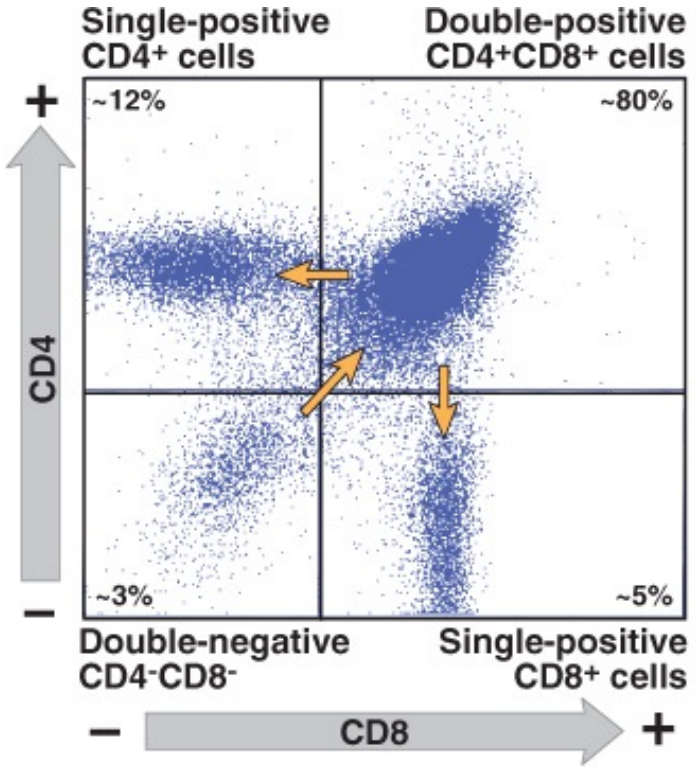
Figure 5.14



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



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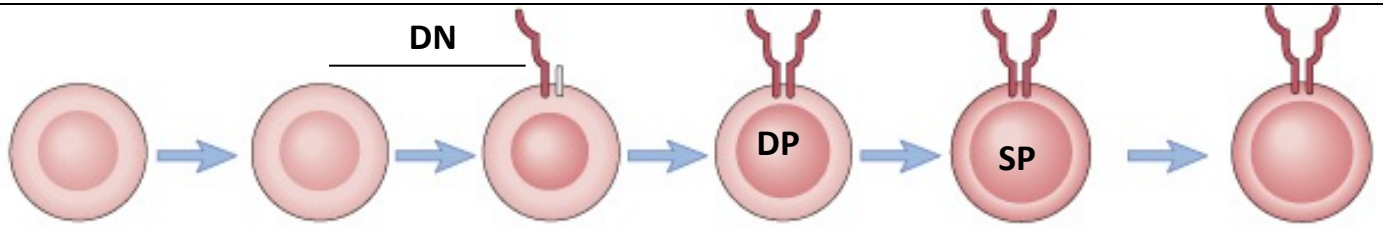

Stage of maturation	Stem cell	Pro-T	Pre-T	Double positive	Single positive (immature T cell)	Naive mature T cell
Proliferation						
RAG expression						
TdT expression						
TCR DNA, RNA	Unrecombined (germline) DNA	Unrecombined (germline) DNA	Recombined β chain gene [V(D)J-C]; β chain mRNA	Recombined 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)

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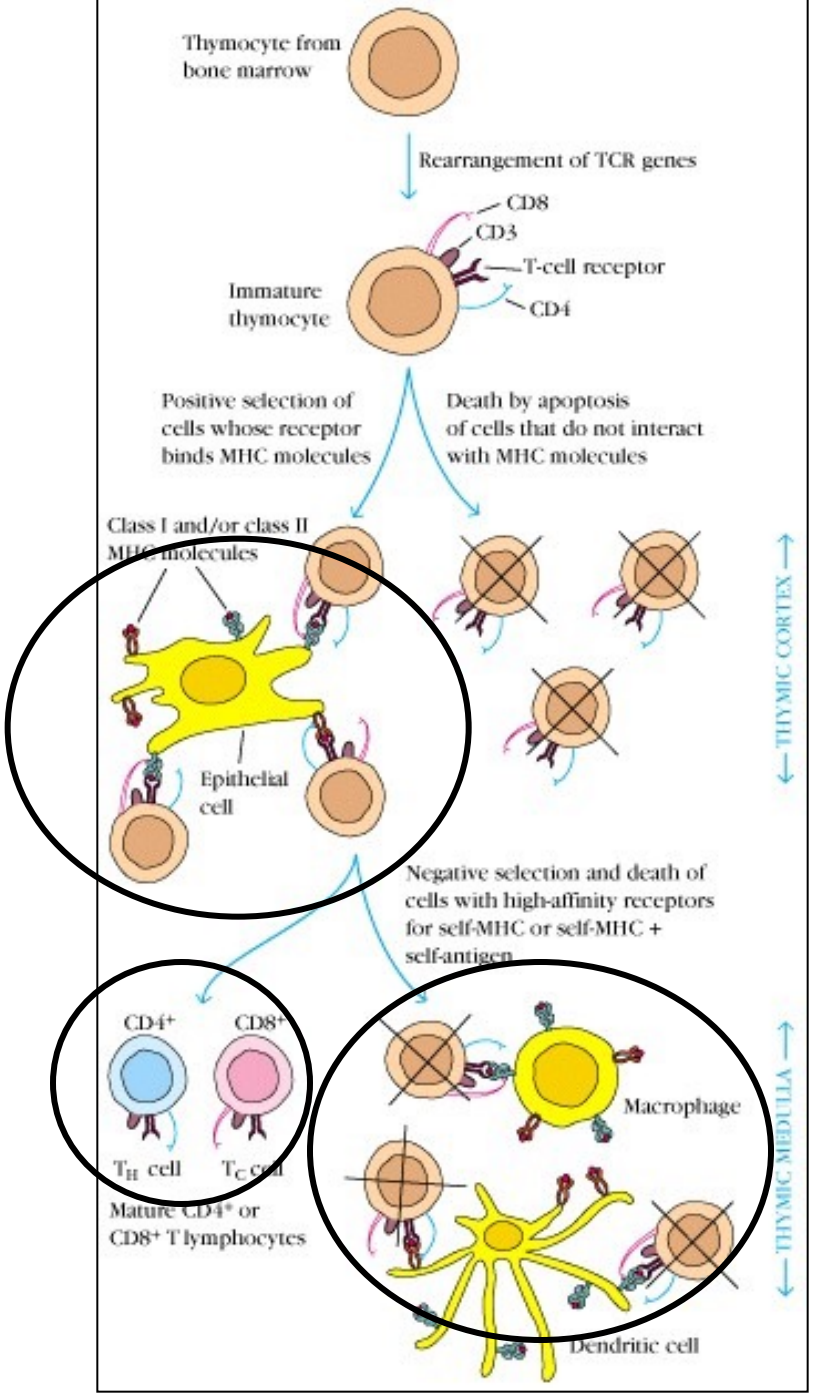


Positive selection:

Epithelial cell - thymocyte interaction in the thymus cortex

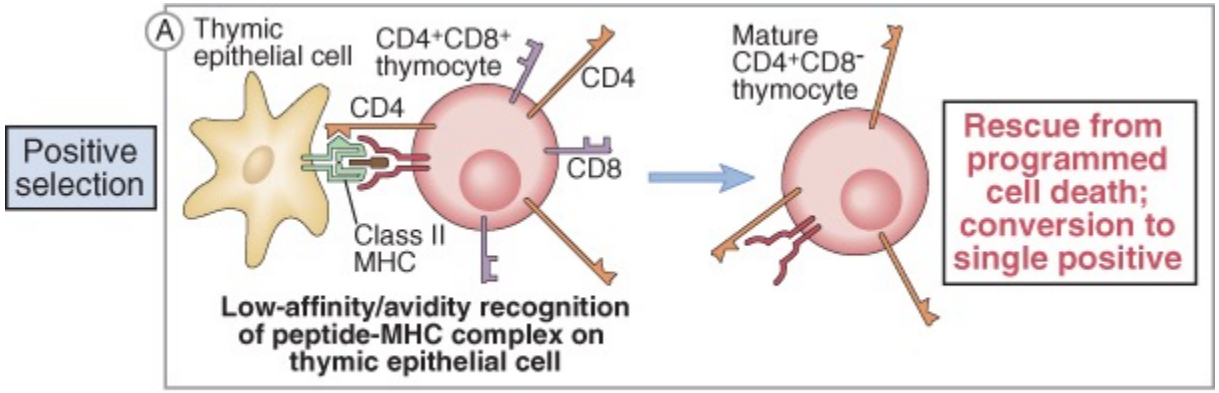
Survival of DP cells whose TcR is appropriate for self MHC recognition

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



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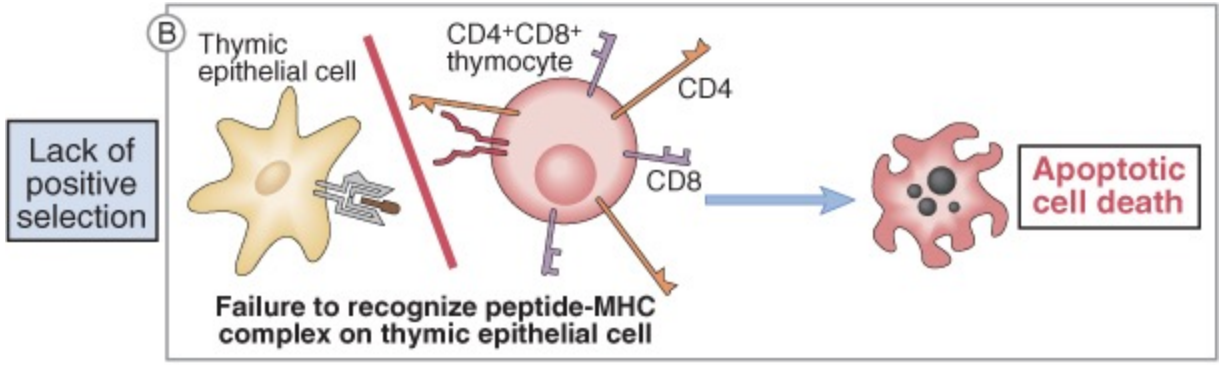

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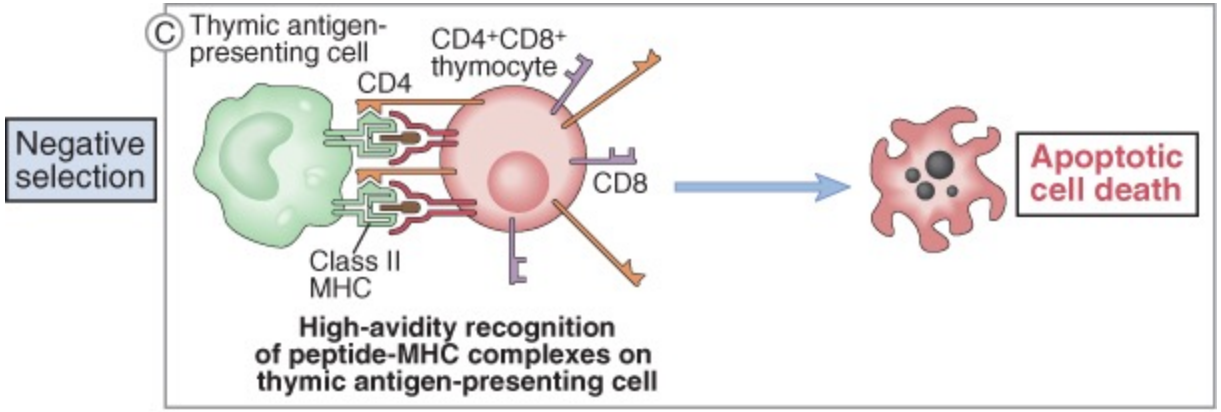

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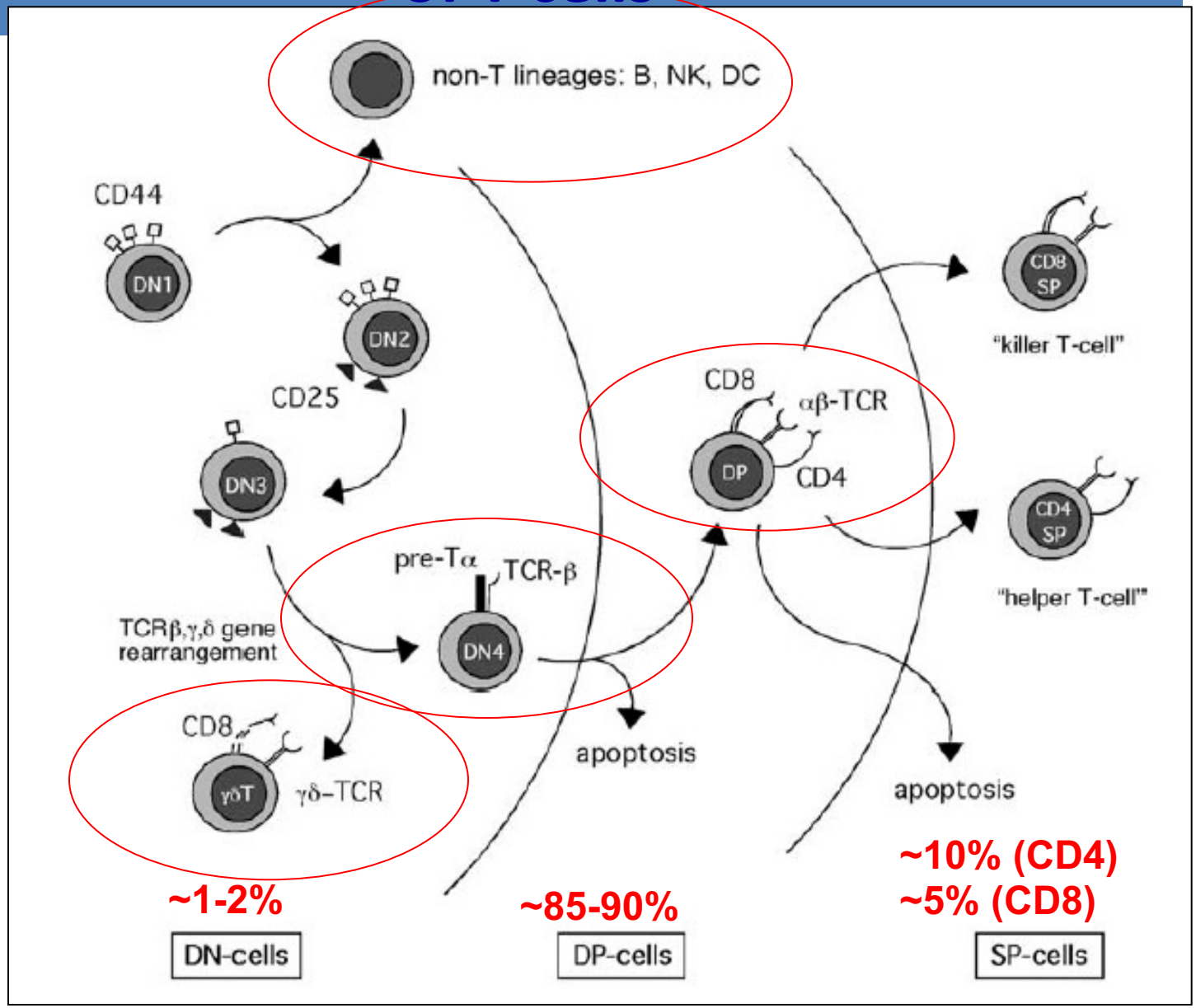
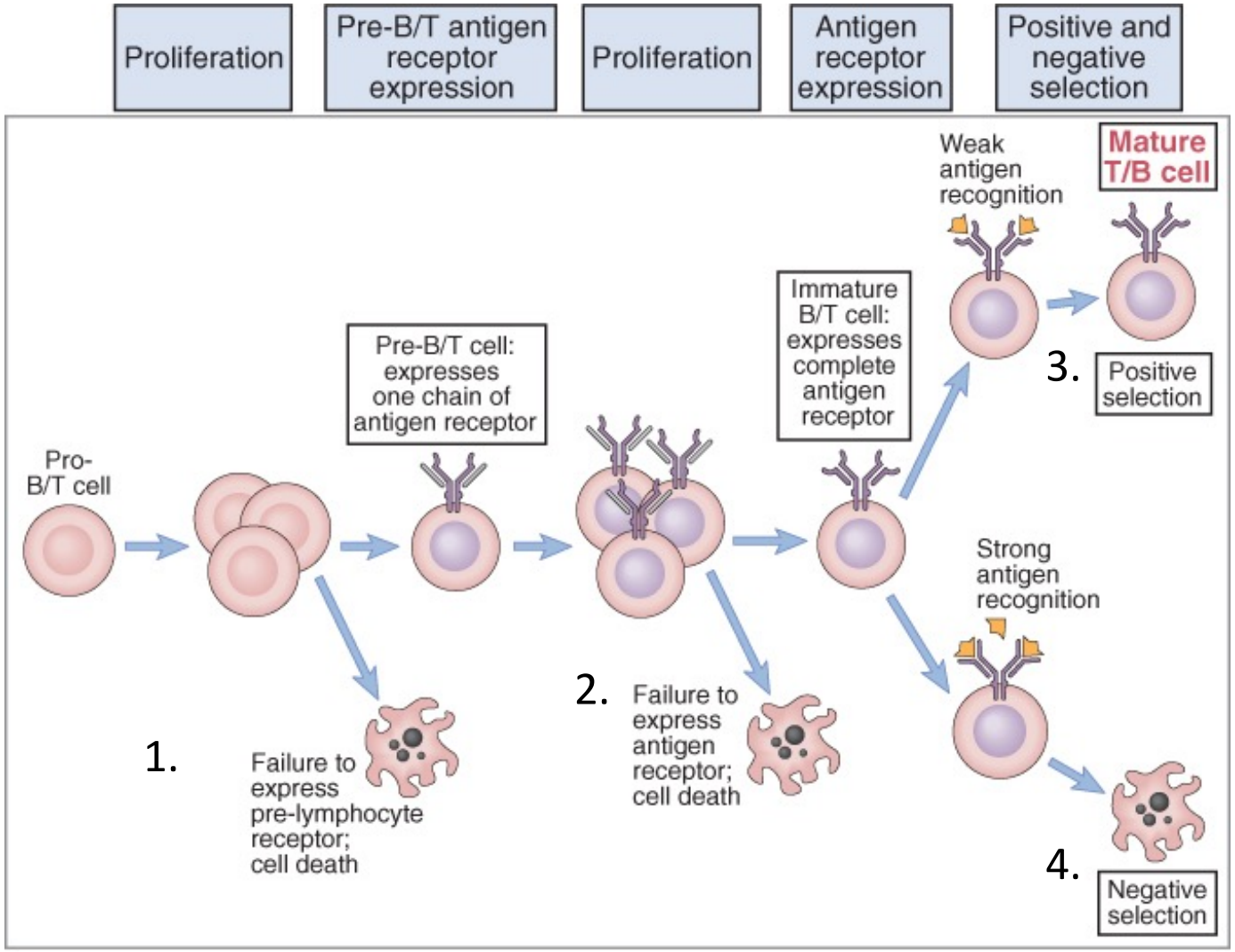
Decision-making during the development of T cells



Phases of T-cell maturation in the thymus.

1. Initiation of either TCR β or γ/δ chain gene rearrangement.
2. Formation of 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



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