Stages of lymphocyte maturation (Abbas Chapter 8)
Hematopoietic stem cells (HSCs) give rise to many distinct progenitors, e.g. a common lymphoid progenitor (CLP). **CLPs** give rise mainly to **B** and **T** cells, but may also contribute to **NK** cells and some **DCs**. **Pro-B** cells differentiate to follicular (FO) **B** cells, marginal zone (MZ) **B** cells, and **B-1** B cells. **Pro-T** cells may commit to either the αβ or γδ **T** cell lineages.

**Commitment to the T lineage** depends on signals delivered by Notch-1, whose intracellular domain mediates transcriptional activation of T lineage genes in collaboration with other transcription factors such as GATA-3. **Commitment to the B lineage** is mediated by the EBF and E2A transcription factors and subsequently by Pax-5.

Transcription factors are indicated by italics.
Early B and T cell development is characterized by the proliferation of committed progenitors induced by cytokine-derived signals

IL-7 = “proliferation” cytokine, produced by stromal cells

IL-7 for B- and T cells in the mouse
IL-7 for only T cells in humans

IL-7 activity ceases before gene rearrangement

Human X-linked severe combined immunodeficiency disease (X-SCID)

• Mutation of the common γ-chain of IL-7
• Block of T cell and NK cell development
• Normal B cell compartment
Principle checkpoints in lymphocyte maturation

- **Proliferation**
  - Pre-B/T antigen receptor expression

- **Proliferation**
  - Antigen receptor expression

- **Positive and negative selection**
  - Weak antigen recognition
  - Positive selection
  - Strong antigen recognition
  - Negative selection

**Mature T/B cell**

- Pro-B/T cell: expresses one chain of antigen receptor
- Pre-B/T cell: expresses complete antigen receptor
- Immature B/T cell: expresses complete antigen receptor

Failure to express pre-antigen receptor; cell death
Failure to express antigen receptor; cell death

Positive and negative selection during maturation

„Positive“

• Building a correct receptor
• Low avidity for self

„Negative“

• Non-functional receptor
• High avidity for self

„Rescue mechanisms“

• Second allele
• Receptor editing
Germline organization of human Ig loci

H chain locus (1250 kb; chromosome 14)

κ chain locus (1820 kb; chromosome 2)

λ chain locus (1050 kb; chromosome 22)
Domains of Ig and TCR proteins

A. Ig

- Ig heavy (μ) chain (membrane form)
- Ig light chain

B. TCR

- TCR β chain
- TCR α chain
Germline organization of human TCR loci

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)
Southern blot of DNA from nonlymphoid (liver) cells and from a monoclonal population of B lymphocyte lineage origin (e.g., a B cell tumor) is shown in schematic fashion. The DNA is digested with a restriction enzyme (EcoRI as depicted), different-sized fragments are separated by electrophoresis, and the fragments are transferred onto a filter. The sites at which the EcoRI restriction enzyme cleaves the DNA are indicated by arrows. The size of the fragments containing the Jκ3 segment of the Ig κ light chain gene or the Vκ29 V region gene was determined by use of a radioactive probe that specifically binds to Jκ3 segment DNA or to Vκ29 DNA. In the hypothetical example shown, Vκ29 is part of a 5-kb EcoRI fragment in liver cells but is on a 3-kb fragment in the B cell clone studied. Similarly, the Jκ3 fragment is 8 kb in liver cells but 3 kb in the B cell clone.
Diversity of antigen receptor genes

From the same germline DNA, it is possible to generate recombined DNA sequences and mRNAs that differ in their V-D-J junctions. In the example shown, three distinct antigen receptor mRNAs are produced from the same germline DNA by the use of different gene segments and the addition of nucleotides to the junctions.
V(D)J recombination

RSS (recombination signal sequences) comprise:
- a heptamer (CACAGTG)
- a spacer (12 or 23 nucleotides)
- a nonamer (AT-rich)

RAG (recombination activating gene)
Rag-1/2 = V(D)J recombinase recognizes:
a DNA sequence at the junction between a heptamer and the coding sequence

Conserved hepta- and nonamers, and spacers of 12 or 23 base pairs ensure correct recombination
Transcriptional regulation of Ig genes

Recombination brings promotor sequences close to the enhancer
Sequential events during V(D)J recombination

1. Synapsis
2. Cleavage
3. Hairpin opening and end-processing
4. Joining

Unrearranged locus

V
5' 7 9 9 7 3'
J

1. Synapsis

Rag-1/Rag-2

5' 7 3'

2. Cleavage

Discarded loop

Rag-1/Rag-2

5' 7 3'

3. Hairpin opening and end-processing

Hairpins

Artemis/DNA-PK, exonucleases, TdT

5' 3'

4. Joining

N and P nucleotides

Ku70/Ku80/DNA-PK
XRCC4/DNA Ligase IV
Sequential events during V(D)J recombination

1. **Synapsis**: Portions of the chromosome are made accessible to the recombination machinery. Two selected coding segments and their adjacent RSSs are brought together by a chromosomal looping event and held in position for subsequent cleavage, processing, and joining.

2. **Cleavage**: Double-stranded breaks are enzymatically generated at RSS-coding sequence junctions by recombination via the Rag-1/Rag-2 complex. *Rag* genes are lymphoid specific and are expressed only in developing B and T cells.

3. **Hairpin opening and end-processing**: The broken coding ends (but not the signal/RSS ends) are modified by the addition or removal of bases, and thus greater diversity is generated. *Artemis* is an endonuclease that opens up the hairpins at the coding ends. A lymphoid-specific enzyme, called terminal deoxynucleotidyl transferase (TdT), adds bases to broken DNA ends.

4. **Joining**: The broken coding ends as well as the signal ends are brought together and ligated by a doublestranded break repair process found in all cells that is called nonhomologous end joining.
Creation of junctional diversity
### Stages of B cell maturation

<table>
<thead>
<tr>
<th>Stage of maturation</th>
<th>Stem cell</th>
<th>Pro-B</th>
<th>Pre-B</th>
<th>Immature B</th>
<th>Mature B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAG expression</td>
<td></td>
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<td></td>
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<tr>
<td>TdT expression</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ig DNA, RNA</strong></td>
<td>Unrecombined (germline) DNA</td>
<td>Unrecombined (germline) DNA</td>
<td>Recombined H chain gene (VDJ); μ mRNA</td>
<td>Recombined H chain gene (VDJ), κ or λ genes (VJ); μ or κ or λ mRNA</td>
<td>Alternative splicing of VDJ-C RNA (primary transcript), to form Cμ and Cλ mRNA</td>
</tr>
<tr>
<td><strong>Ig expression</strong></td>
<td>None</td>
<td>None</td>
<td>Cytoplasmic μ and pre-B receptor–associated μ</td>
<td>Membrane IgM (μ+ κ or λ light chain)</td>
<td>Membrane IgM and IgD</td>
</tr>
<tr>
<td><strong>Surface markers</strong></td>
<td>CD43+</td>
<td>CD43+</td>
<td>B220lo</td>
<td>IgMlo</td>
<td>IgMhi</td>
</tr>
<tr>
<td></td>
<td>CD19+</td>
<td>CD19+</td>
<td>CD43+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CD10+</td>
<td>CD10+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Anatomic site</strong></td>
<td>Bone marrow</td>
<td></td>
<td></td>
<td>Periphery</td>
<td></td>
</tr>
<tr>
<td><strong>Response to antigen</strong></td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Negative selection (deletion), receptor editing</td>
<td>Activation (proliferation and differentiation)</td>
</tr>
</tbody>
</table>
Ig H and L chain recombination and expression
Pre-B cell and pre-T cell receptors

**Pre-BcR**
μ chain + surrogate L chains (VpreB, Vλ5)

**Pre-TcR**
β chain + preTα

First checkpoints for „allelic exclusion“
B lymphocyte subsets (B1 – B2 B cells)

FL HSC = fetal liver hematopoietic stem cell

BM HSC = bone marrow hematopoietic stem cell
Coexpression of IgM and IgD

Alternative **RNA splicing** (of introns)
T cell maturation
# Stages of T cell maturation

<table>
<thead>
<tr>
<th>Stage of maturation</th>
<th>Stem cell</th>
<th>Pro-T</th>
<th>Pre-T</th>
<th>Double positive</th>
<th>Single positive (immature T cell)</th>
<th>Naive mature T cell</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proliferation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RAG expression</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>TdT expression</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TCR DNA, RNA</strong></td>
<td>Unrecombined (germline) DNA</td>
<td>Unrecombined (germline) DNA</td>
<td>Recombined β chain gene [V(D)J-C]; β chain mRNA</td>
<td>Recombined β, α chain genes [V(D)J-C]; β and α chain mRNA</td>
<td>Recombined β, α chain genes [V(D)J-C]; β and α chain mRNA</td>
<td>Recombined β, α chain genes [V(D)J-C]; β and α chain mRNA</td>
</tr>
<tr>
<td><strong>TCR expression</strong></td>
<td>None</td>
<td>None</td>
<td>Pre-T receptor (β chain/pre-T α)</td>
<td>Membrane αβ TCR</td>
<td>Membrane αβ TCR</td>
<td>Membrane αβ TCR</td>
</tr>
<tr>
<td><strong>Surface markers</strong></td>
<td>c-kit⁺ CD44⁺ CD25⁻</td>
<td>c-kit⁺ CD44⁺ CD25⁺</td>
<td>c-kit⁺ CD44⁻ CD25⁺</td>
<td>CD4⁺CD8⁺ TCR/CD3lo</td>
<td>CD4⁺CD8⁻ or CD4⁺CD8⁺ TCR/CD3hi</td>
<td>CD4⁺CD8⁻ or CD4⁺CD8⁺ TCR/CD3hi</td>
</tr>
<tr>
<td><strong>Anatomic site</strong></td>
<td>Bone marrow</td>
<td>Thymus</td>
<td>Thymus</td>
<td>Thymus</td>
<td>Thymus</td>
<td>Thymus</td>
</tr>
<tr>
<td><strong>Response to antigen</strong></td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Positive and negative selection</td>
<td>Activation (proliferation and differentiation)</td>
<td>Activation (proliferation and differentiation)</td>
</tr>
</tbody>
</table>
Maturation of T cells in the thymus
TCR α and β chain gene recombination and expression
CD4/CD8 expression and selection
Positive/negative selection in the thymus

**Positive selection**
the process in which thymocytes with low avidity TCRs to peptide–self MHC complexes are stimulated to survive

**Negative selection**
Thymocytes whose receptors recognize peptide-MHC complexes in the thymus with high avidity undergo apoptosis or differentiate into regulatory T cells

„Removal“ of self-reactivity in thymus = **central tolerance**
More aspects of central tolerance induction

**AIRE** *(autoimmune regulator)*

induces tissue-specific genes in the thymus

*How divers does the TcR repertoire need to be?*

*(or: how to balance between non-responder and autoimmunity)*
Other cells passing thymic education

**γδT cells**

- First come – first serve
  (if rearrangement of γ and δ is successful, the result will be a γδ TcR)
- Diversity is theoretically high, practically low

**NKT cells**

- Are not MHC restricted
- Do not recognize peptide antigens
- Express a NK-like surface marker
- The TcR of NKT cells recognizes lipid antigens on CD1
- Many NKT have an “invariant” TcR