The Development of B lymphocytes: Chap. 4 and 6

B cell development: Today’s subjects

- Phase I: Productive vs. non-productive rearrangement of immunoglobulin gene segments
- Phase II & III: Tolerance induction (= no self reactivity)
  - Receptor editing
  - Clonal deletion

Stages in development B cells

- Phase 1: Generation of diverse anti-densely expressed B-cell receptors in the bone marrow
- Phase 2: Generation of diversity in the bone marrow
- Phase 3: Promotion of a fraction of immature B cells to become mature B cells in the secondary lymphoid tissue
- Repertoire assembly
- Negative selection
- Positive selection

Stages of BCR development: Phase I

- Mature B cell
- Early pre-B cell
- Late pro-B cell
- Pro-B cell
- Immature B cell

- Germ-line
- V-D-J rearrangement
- Germ-line
- Germ-line
- Germ-line
- Germ-line
- U-J rearranging
- U-J rearranging
- U-J rearranging
- U-J rearranging
- α heavy chain, Ig light chain, light on surface

BM
SLN
Heavy chain rearrangement inefficient process

- In every Ig rearrangement there is 2/3 chance that you generate an out-of-the-frame sequence
- Many B cells die because they can not make a functional Ig
- Most loses are during heavy chain development
- Light chain: more trials!

Quality check pre-B cell receptor

- Is rearranged heavy chain capable of combine with light chain?
  - Use a template: surrogate light chain consisting of VpreB and \(\lambda 5\)
- IgB shuts down further rearrangement

Pre-B cells and allelic exclusion

- Eliminates B cells that do not make a functional heavy chain
- Prevents B cells from making more than one heavy chain
  - Successful rearrangement and expression of heavy chain
    - Stop transcription of RAG
    - Signal degradation of RAG
    - Chromatin is reorganized into a state that resists gene rearrangement

Pre-B cell to Immature B cell

- Cell expresses \(\mu\) and IgM
- Cell expresses \(\mu\) and IgM
- Apoptosis
Light chain rearrangement is more efficient

- Large pre-B cell with rearranged heavy chain divides yielding approx. 100 cells per B cell clone (i.e. with identical VDJ)
- Kappa light chain rearranges first, if unsuccessful followed by lambda
- Only V and J need to recombine, therefore higher success rate then with additional D
- If first recombination fails to give correct fusion, then other V region in upstream position will recombine with downstream J
- If again non-productive, then recombination of kappa locus on other chromosome
- If kappa fails, then lambda locus rearranges
- 85% of pre-B cells yield productive light chain rearrangement

Checkpoints during B cell development in bone marrow

<table>
<thead>
<tr>
<th>Early pre-B cell</th>
<th>Heavy chain rearrangement</th>
<th>First checkpoint</th>
<th>Light chain rearrangement</th>
<th>Second checkpoint</th>
<th>Mature B cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Several proteins play a role in B cell development

Checkpoints during B cell development

Checkpoints during B cell development

Stages in development B cells

Several proteins play a role in B cell development
Phase II in B cell development: Central Tolerance induction (Negative selection)

- Negative selection starts in bone marrow on self antigens presented by stromal and hematopoietic cells
- Potential multivalent self antigens are glycoproteins, proteoglycans and glycolipids on cells
- Immature B cells with self reactivity are maintained within bone marrow, others will go into circulation
- Immature B cells in the bone marrow that are self-reactive (up to 75%) need to deal with:
  - clonal deletion
  - Receptor editing (give them another chance)

Clonal activation of self reactive B cells (monovalent antigen): anergy

- Monovalent antigens are usually soluble proteins
- B cell becomes non-responsive:
  - Expresses IgD but hardly any IgM
  - Half-life: 1-5 days

Receptor editing to change self reactivity (multivalent antigens)

Genes that are involved in B cell tolerance

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>APA</td>
<td>Helper B cell tolerance</td>
</tr>
<tr>
<td>B220</td>
<td>Helper B cell tolerance</td>
</tr>
<tr>
<td>Bcl-6</td>
<td>Helper B cell tolerance</td>
</tr>
<tr>
<td>Blimp</td>
<td>Helper B cell tolerance</td>
</tr>
<tr>
<td>Bmi-1</td>
<td>Helper B cell tolerance</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Helper B cell tolerance</td>
</tr>
<tr>
<td>IgM</td>
<td>Helper B cell tolerance</td>
</tr>
<tr>
<td>IgD</td>
<td>Helper B cell tolerance</td>
</tr>
<tr>
<td>IgD</td>
<td>Helper B cell tolerance</td>
</tr>
<tr>
<td>IgM</td>
<td>Helper B cell tolerance</td>
</tr>
<tr>
<td>IgD</td>
<td>Helper B cell tolerance</td>
</tr>
<tr>
<td>IgM</td>
<td>Helper B cell tolerance</td>
</tr>
<tr>
<td>IgD</td>
<td>Helper B cell tolerance</td>
</tr>
<tr>
<td>IgM</td>
<td>Helper B cell tolerance</td>
</tr>
</tbody>
</table>

Nemazee, D. Nat Rev Immunol. 2015
**Maturing B cells**

- After leaving bone marrow, B cells are still immature. The final stage occurs when immature B cells enter a secondary lymph node.
- BCR stimulation dictates how B cell matures (positive selection).

**Mature B cells**

- They express both IgM and IgD.
- Recirculate between blood and secondary lymphoid organs.
- Transition via the lymph node to follicles.
  - CXCR5 is the homing receptor.
  - On average, they remain in the follicles for 16 hours.
After successful rearrangement, VH and VL low affinity antibody secreted in pentameric form of IgM; high avidity (5x2 binding units) compensates for low affinity of binding unit.

Somatic mutation increases affinity, therefore lower degree of avidity needed: isotype switch from IgM (pentamer) to other types (IgG, IgA or IgE; bivalent).

### Isotype switching

- Isotype switching ensures fusion of VH to other heavy chain isotype (no change in epitope recognition).
- Isotype light chain not changed.
- Initiated by Switch (S) sequences and catalyzed by activation-induced cytidine deaminase (AID).
- Looping out of DNA puts VH in juxtaposition of new isotype.

### Functions of isotypes

- Neutralization (e.g. blocking interaction virus with cellular receptor).
- Opsonization / activation complement system leading to lysis pathogen and ingestion by phagocytes.
- Activation NK cells by binding to Fc receptors.
- Activation mast cells by interaction with IgE receptor (discussed in Chapter 9).
Properties of isotypes

<table>
<thead>
<tr>
<th>Property</th>
<th>IgM</th>
<th>IgD</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
<th>IgA</th>
<th>IgE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transport across epithelium</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Transport across placenta</td>
<td>–</td>
<td>–</td>
<td>0.3</td>
<td>0.4</td>
<td>0.3</td>
<td>0.4</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Diffusion into extravascular sites</td>
<td>0.5</td>
<td>–</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Mean serum level (mg/ml)</td>
<td>1.5</td>
<td>0.03</td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>0.5</td>
<td>2.5</td>
<td>5 x 10^-8</td>
</tr>
</tbody>
</table>

- Transport across epithelium: secretion into lumen of gut, milk, saliva, sweat, and tears to combat parasites, microbes, and viruses outside the body.
- Transport across placenta to supply fetus with protective antibodies.
- Diffusion in extravascular sites of damaged or infected tissues (related to size of antibody).

Affinity Maturation

Summary B cell development

Movie by Jullian Kirk Elleker
Various B cell tumors reflect mistakes in different development stages of B cells

- Mistake in rearrangement can lead to activation of oncogenes (controlling cell growth, division and differentiation)

- B cell tumors are clonal, i.e. derived from single recombined B cell

- Tumors derived from naive B cells grow in follicles of lymph nodes
  => follicular center cell lymphoma

- Tumors derived from plasma cells grow in bone marrow
  => myeloma