Introduction to Immunology

B-Cell Development and Antibody Maturation

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B-Cell Biology

Antigen-Independent B-Cell Development

Antigen-Dependent B-Cell Development
2 Phases of B-Cell Development at 2 Locations

Bone marrow:
- B-cell development
  - Antigen-independent

Lymph nodes:
- B-cell maturation
  - Antigen-dependent

Spleen:
- B-cell maturation

Antigen-independent

B-cell development

B-Cell Biology

Antigen-Independent B-Cell Development

- Generation of B Cells in the Bone Marrow

Antigen-Dependent B-Cell Development

- Ig Class Switch, Somatic Hypermutation
- Germinal Center Reaction
3 Processes Establish Diversity of Pre-Immune Repertoire:

- Combinatorial diversity ($V_H$, $D_H$, $J_H$ & $V_L$, $J_L$)
- Junctional diversity
- Combinatorial diversity through HC and LC combinations

It is estimated that these processes could give rise to $10^{11}$ different antibody specificities that comprise the antigen receptor repertoire of naive B-cells.

Not All Rearrangements Lead to Suitable Antigen-Receptor:

- Some $V_H$ and $V_L$ gene segments are pseudogenes
- Junctional diversity can lead to reading frame shifts
- Junctional diversity can introduce translational stop codons

Additional Complexities:

- Each cells has 2 HC and 4 LC alleles
- Generation of autoreactive antibody receptors

It results a considerable cell wastage!

How is the generation of the pre-immune repertoire regulated?
Antigen-Independent B-Cell Development

Generation of B Cells in the Bone Marrow

1. DNA rearrangements establish the primary repertoire, creating diversity

2. Allelic exclusion ensures that each clone expresses a single antibody on the surface, establishing specificity

3. Deletion of self-reactive clones establishes immunological tolerance

Ordered Rearrangement of Ig Genes During B-Cell Development in the Bone Marrow

Phase 1: HC rearrangement

Phase 2: LC rearrangement
Pre-B Cell Receptor

Membrane $\mu$HC
$\lambda$5, VpreB (surrogate [or pseudo] LC)
Ig$\alpha$ and Ig$\beta$ signaling components

Tonic signaling - no known ligand
Ordered Rearrangement Gives Allelic Exclusion

Heavy chain rearrangement occurs first:
- DJ on both alleles
- V-DJ on one allele

Productive rearr. (1/9)
- \( \mu \)HC and preBCR

1. STOP HC rearrangement
2. Proliferation
3. Begin LC rearrangement

Non-productive rearr. (8/9)
- V-DJ on second allele
- \( \mu \)HC and preBCR

Ordered Rearrangement of Ig Genes During B-Cell Development in the Bone Marrow

Phase 1: HC rearrangement
Phase 2: LC rearr.
Rearrangement of Ig Alleles is Ordered and Regulated to Achieve Allelic Exclusion

TWO checkpoints which confer allelic exclusion: pre-BCR and BCR
Establishment of Allelic Exclusion

Signaling through pre-BCR or BCR:

- reduces expression of RAG-1 and RAG-2
- targets RAG-2 for proteasomal degradation
- reduces access of the HC locus to the recombinase machinery (mechanism unclear)

BCR (B Cell Receptor)

Membrane µ HC
Kappa or lambda LC
Igα and Igβ signaling molecules

Tonic and ligand signaling

- Stop LC rearr.
- Survival
- Can sample self antigens
B Cell Tolerance

Central tolerance
• tolerance to self antigens that is established in lymphocytes developing in central lymphoid organs;
• main mechanism: clonal deletion

Peripheral tolerance
• tolerance to self antigens that is established in lymphocytes in the peripheral tissues
• clonal deletion, anergy, clonal ignorance
Antigen-Independent B-Cell Development

Generation of B Cells in the Bone Marrow

1. DNA rearrangements establish the primary repertoire, creating diversity

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Antigen-Dependent B-Cell Development Occurs in the Germinal Center (GC) Reaction
Antigen-Dependent B-Cell Development

Generation of B cells with High-Affinity Antigen-Receptors in the Germinal Center (GC) Reaction

1. T-cell dependent activation of antigen-specific naïve B cells, the precursor cells of the GC-reaction

2. Somatic Hypermutation and Ig Class Switch during the GC-reaction generates high-affinity antigen-specific B cells with specialized effector functions

3. Differentiation of antigen-selected GC B cells into memory B cells and plasma cells, the carriers of antibody-dependent (humoral) immunity

Compartmentalization of Antigen-Dependent B-Cell Development

<table>
<thead>
<tr>
<th>Location</th>
<th>Process</th>
<th>T-cell zone</th>
<th>GC dark zone</th>
<th>GC light zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen recognition induces expression of effector molecules by the T cell, which activates the B cell</td>
<td>T-cell dependent activation</td>
<td>SHM</td>
<td>CSR; memory B &amp; plasma cell differentiation</td>
<td></td>
</tr>
<tr>
<td>B-cell proliferation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Differentiation to resting memory cells and antibody-secreting plasma cells</td>
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</tr>
</tbody>
</table>
Somatic Hypermutation (SHM)

- Random mutagenesis, mostly single base changes
- Limited to V(D)J (does not extend to C regions)
- Hypermutation is $10^6$ more than normal mutation rate ($10^{-3}$/bp/generation compared to $10^{-9}$/bp/generation)
- Occurs only in mature, antigen-activated B-cells
- Combined with selection, results in clones making antibodies with increased affinity for antigen: Affinity Maturation

Features of SHM

**SHM Introduces Mutations into the Rearranged V(D)J Gene Segments**

1. Distribution within ~2Kb from the promoter. Requires transcription
2. Requires cis-acting elements
3. Intrinsic characteristics
   - Mostly single bp substitutions
   - Predominance of Transitions over Transversions
   - Specific hotspots motifs: AAGTT / CAGCT = RGYW
4. Associated with DNA strand breaks
Pattern of V Gene Mutations Provides Evidence of Cyclical Mutation and Selection Events

- Random mutation combined with selection

![Diagram showing pattern of V gene mutations]
**Affinity Maturation:**

- Increase in the affinity for the specific antigen of the antibodies produced during a humoral immune response
- Particularly prominent in secondary (memory) immunizations

**Class Switch Recombination (CSR):**

- A DNA rearrangement that allows the same VDJ to be expressed with different heavy chain constant regions
Expression of Alternate Isotypes

Two mechanisms for expression of alternate isotypes:

- IgM to IgD (and membrane to secreted) via differential RNA processing
- IgM to IgG, IgA or IgE by DNA rearrangement

Secreted Antibodies Function in Various Ways To Eliminate Foreign Invaders

- Neutralization
  - Antibody prevents bacterial adherence
- Opsonization
  - Antibody promotes phagocytosis
- Complement activation
  - Antibody activates complement, which enhances opsonization and lysos some bacteria

Figure 6-12: Immunobiology, 7th ed. (C. Garland Science 2008)
The Germinal Center Microenvironment

**Dark Zone (Centroblasts)**

1. Proliferation (one cell cycle completed in 12 h)

2. Generation of antibody-variants by SHM
The Germinal Center Microenvironment

Light Zone (Centrocytes)

1. Selection
   - for high-affinity B cell clones
   - against newly generated self-reactive B cell clones

2. Generation of different antibody isotypes by CSR

3. Differentiation into memory B cells and plasma cells
Selection

The Germinal Center Microenvironment
Two Differentiated B Cell States Follow GC Reaction

Memory B cell: No Ig secretion, but rapid response to renewed antigen-encounter with high affinity and switched isotypes; circulate b/w lymphoid tissues through the blood

Plasma cell: Ig secretion of high affinity and switched isotypes; home to the bone marrow

Most B-Cell Lymphomas Derive from the GC

V(D)J recombination | IgV hypermutation | Ig isotype switch
---|---|---
Immature B cells | Naïve B cells | Antigen
Bone-marrow | | |

GERMINAL CENTER

| Dark Zone | Light Zone |
---|---|
Centroblasts | Centrocyes |

Germinal center

| Memory B cells |
---|
Plasma blasts |
Apoptosis |
Plasma cells |

unmutated IgV genes

| ALL |
---|
Mantle cell lymphoma |
B-cell chronic lymphocytic leukemia (CLL) |

hypermuted IgV genes

| Diffuse large cell lymphoma |
---|
Burkitt lymphoma |
Follicular lymphoma |

AIDS-lymphomas

Hodgkin lymphoma

Multiple Myeloma
Most B-Cell Lymphomas Derive from the GC

- Many GC-derived lymphomas are characterized by reciprocal balanced chromosome translocations (BCL2-IgH, c-MYC-IgH, and BCL6-IgH)

**t(14;18) Translocation in Follicular Lymphoma**
*(G-banded Karyotype)*
### Chromosomal Translocations in B-Cell Lymphoma

<table>
<thead>
<tr>
<th>Lymphoma</th>
<th>Translocation</th>
<th>Gene</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoplasmacytic</td>
<td>t(9;?) (p13;?)</td>
<td>PAX5</td>
<td>Transcription Factor</td>
</tr>
<tr>
<td>Mantle cell</td>
<td>t(11;14) (q13;q32)</td>
<td>BCL1</td>
<td>Cyclin D1</td>
</tr>
<tr>
<td>Follicular</td>
<td>t(14;18) (q32;q11)</td>
<td>BCL2</td>
<td>Anti-Apoptosis</td>
</tr>
<tr>
<td>MALT lymphoma</td>
<td>t(11;18) (q21;q21)</td>
<td>API2/MLT</td>
<td>Anti-Apoptosis</td>
</tr>
<tr>
<td>Diffuse large cell</td>
<td>t(3;x) (q27;x)^</td>
<td>BCL6</td>
<td>Transcription Factor</td>
</tr>
<tr>
<td>Burkitt</td>
<td>t(8:14) (q24;q32)</td>
<td>cMYC</td>
<td>Transcription Factor</td>
</tr>
</tbody>
</table>

^x=various chromosomal partners

### Reciprocal Translocations in B-Cell Lymphoma as Mistakes of the B-Cell Specific Ig-Locus Modifying Processes

**V(D)J Recomb.**
- RSS (recombination signal sequence)
- D\(\rightarrow\)J\(\rightarrow\)J
- in bone marrow

**CSR**
- S\(\mu\)
- C\(\mu\)
- S\(\gamma\)
- C\(\gamma\)

**SHM**
- V\(\rightarrow\)V
- in GC
Consequences of Chromosomal Translocations

- Fusion protein (Sarcomas and leukemias)
- Deregulated transcription (Lymphomas)
  - since IgH locus is expressed throughout B-cell development

Disruption of Normal Transcriptional Programs by Genetic Lesions Promotes Lymphomagenesis
Conclusions

- The GC-reaction generates memory B cells and plasma cells that produce high-affinity antibodies, which are necessary to protect against invading microorganisms

    There is a caveat, however...

- The beneficial role of the GC in immunity is somewhat counterbalanced by its detrimental role in lymphoma-ogenesis, as the majority of B-cell lymphomas originate from GC B cells