Chapter 2
Antibody Structure and the Generation of B-Cell Diversity

- Molecular and structural basis of antibody diversity
- How B cells develop and function in the body
- How B cells are activated and participate in adaptive immunity

Location and production of Immunoglobulins
1. Antibodies are specific for individual epitopes
2. Membrane bound form is present on a B-cell
3. Ag binding to B cell stimulates it to secrete Ab

Antibody structure
- Heavy (5 classes), Light (2 classes), Constant and Variable
- Disulfide bonds
- Skip figure 2.3 (Fab- fragment antigen binding)
  (Fc- fragment crystallizable)
Immunoglobulin Isotypes or Classes

Monomers/dimers

pentamers

IgG

γ

IgM

μ

IgA

α

IgD

δ

IgE

ε

Immunoglobulin domain

Figure 2-5: Immunoglobulin domain (Figure courtesy of Garlands Scarcity 2005)

Light-chain C domain

Light-chain V domain

Figure 2-6: Immunoglobulin domain (Figure courtesy of Garlands Scarcity 2005)
**Hypervariable (CDR) Regions of Antibodies**

**Amino Acid Sequence Variability in the V domain**

**Mechanisms of Epitope Recognition**

- Linear and discontinuous epitopes
- Multivalent Antigens
- Polymeric Antibodies
- Epitope binding mechanisms
Physical properties of Antigens

Epitope - part of the antigen bound by Ab

Mulivalent - antigen with more than one epitope, or more than one copy of an epitope

Variety of structures and sizes recognized by Ab’s

Linear vs Discontinuous epitopes

Affinity - binding strength of an antibody for its epitope

Poliovirus VP1 - blue, contains several epitopes (white) that can be recognized by humans

Figure 2-9

Figure 2-26 part 1 of 2
Poliovirus
VP1 - blue, contains several epitopes (white) that can be recognized by humans.

Antibodies bind a range of structures:
- Pockets
- Grooves
- Extended surfaces
Antibody Structure Summary

- Produced by B-cells
- Y shape, Four polypeptide chains, Ig domains
- Constant and Variable regions
- 5 classes - IgG, IgM, IgD, IgA, IgE
- CDR, Hypervariable regions
- Epitope recognition

Generation of Ig diversity in B cells

Unique organization
- Only B cells can express Ig protein
- Gene segments
  - κ, λ - Light chain
  - α, δ, ε, γ, μ - Heavy Chain
- present on three chromosomes

The Gene Rearrangement Concept

- Germline configuration
- Gene segments need to be reassembled for expression
- Sequentially arrayed
- Occurs in the B-cells precursors in the bone marrow (soma)
- A source of diversity BEFORE exposure to antigen
Gene rearrangements during B-cell development

V-variable, J-joining, D-diversity gene segments; L-leader sequences

λ - 30 V & 4 pairs J & C (light chain) chs22
κ - 40 V & 5 J & 1 C (50% have 2x V) (light chain) chs2
H - 65 V & 27 D & 6 J chs14

<table>
<thead>
<tr>
<th>Immunoglobulin heavy- and light-chain loci</th>
</tr>
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<tbody>
<tr>
<td>L</td>
</tr>
<tr>
<td>L_1</td>
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J-joining

<table>
<thead>
<tr>
<th>Light chain</th>
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<tbody>
<tr>
<td>germline DNA</td>
</tr>
<tr>
<td>somatic recombination</td>
</tr>
<tr>
<td>VJ-joined rearranged DNA</td>
</tr>
<tr>
<td>CDR1 and CDR2</td>
</tr>
<tr>
<td>CDR3</td>
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</table>

D-diversity

<table>
<thead>
<tr>
<th>Heavy chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>germline DNA</td>
</tr>
<tr>
<td>somatic recombination</td>
</tr>
<tr>
<td>DJ joined DNA</td>
</tr>
<tr>
<td>CDR1 and CDR2</td>
</tr>
<tr>
<td>CDR3</td>
</tr>
</tbody>
</table>
Random Recombination of Gene segments is one factor contributing to diversity

200 x 120 x 10,530 = 3,369,600 Ig molecules

Mechanism of Recombination
- Recombination signal sequences (RSSs) direct recombination
  - V and J (L chain)
  - V D J (H chain)

Mechanism of Somatic Recombination
- V(D)J recombinase: all the protein components that mediate the recombination steps
- RAG complex: Recombination Activating Genes (RAG-1 and RAG-2) encode RAG proteins only made in lymphocytes, plus other proteins
- Recombination only occurs through two different RSS bound by two RAG complexes (12/23 rule)
- DNA cleavage occurs to form a single stranded hairpin and a break at the heptamer sequences
- Enzymes that cut and repair the break introduce Junctional Diversity
Junctional Diversity

- Nucleotides introduced at recombination break in the coding joint corresponding to CDR3 of light and heavy chains
  - V and J of the light chain
  - (D and J) or (V and DJ) of the heavy chain
- P nucleotides generate short palindromic sequences
- N nucleotides are added randomly - these are not encoded
- Junctional Diversity contribute \(3 \times 10^7\) to overall diversity!
Generation of BCR (IgD and IgM)

• Rearrangement of VDJ of the heavy chain brings the gene’s promoter closer to Cµ and Cδ

• Both IgD and IgM are expressed simultaneously on the surface of the B cell as BCR - ONLY isotypes to do this

• Alternative splicing of the primary transcript RNA generates IgD and IgM

• Naïve B cells are early stage B cells that have yet to see antigen and produce IgD and IgM

Alternative Splicing of Primary Transcript to generate IgM or IgD

Summary Biosynthesis of IgM in B cells
Mature B cell

- Long cytoplasmic tails interact with intracellular signaling proteins
- Disulfide-linked
- Transmembrane proteins - invariant
- Dual-function
  1) help the assembled Ig reach the cell surface from the ER
  2) signal the B cell to divide and differentiate

Principle of Single Antigen Specificity

- Each B cell contains two copies of the Ig locus (Maternal and Paternal copies)
- Only one is allowed to successfully rearrange - Allelic Exclusion
- All Igs on the surface of a single B cell have identical specificity and differ only in their constant region
- Result: B cell monospecificity means that a response to a pathogen can be very specific

DNA hybridization of Ig genes can diagnose B-cell leukemias
Generation of B cell diversity in Ig’s before Antigen Encounter

1. Random combination of V and J (L chain) and V, D, J (H chain) regions
2. Junctional diversity caused by the addition of P and N nucleotides
3. Combinatorial association of Light and Heavy chains (each functional light chain is found associated with a different functional heavy chain and vice versa)

Developmental stages of B cells

1. Development before antigen
   - Immature B cell
   - Mature naïve B cell (expressing BCR - IgM and IgD)
2. Development after antigen
   - Plasma cell (expressing BCR and secretes Antibodies)

Processes occurring after B cells encounter antigen

- Processing of BCR versus Antibody
  1. Plasma cells switch to secreted Ab
  2. Difference occurs in the c-terminus of the heavy chain
  3. Primary transcript RNA is alternatively processed to yield transmembrane or secreted Ig’s

- Somatic Hypermutation
  1. Point mutations introduced to V regions
  2. 10^6 times higher mutation rate
  3. Usually targets the CDR

- Affinity maturation - mutant Ig molecules with higher affinity are more likely to bind antigen and their B cells are preferentially selected

- Isotype switching
RNA processing to generate BCR or Antibody

1. IgM is the first Ab that is secreted in the IR
2. IgM is pentameric and each H chain can bind complement proteins
3. Isotypes with better effector functions are produced by activated B cells
4. Rearrangement of DNA using SWITCH regions
   - all C genes preceded by switch sequence (except δ)
   - start from the µ gene and any other C gene (plus sequential)
5. Regulated by cytokines secreted by T cells
Immunoglobulin classes

1. C regions determine the class of antibody and their effector function
2. Divided into Subclasses based on relative abundance in serum
3. Each class has multiple functions

1. IgM and IgG can bind complement
2. IgG crosses placenta
3. Receptors for constant regions (Fc Receptors)
   - IgG (FcG receptors): macrophages, neutrophils, eosinophils, NK cells, others
   - IgE (FcE receptors): mast cells, basophils, others

<table>
<thead>
<tr>
<th>Immunoglobulin class or subclass</th>
<th>IgM</th>
<th>IgD</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
<th>IgA1</th>
<th>IgA2</th>
<th>IgE</th>
</tr>
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<tbody>
<tr>
<td>Heavy chain</td>
<td>p</td>
<td>δ</td>
<td>γ1</td>
<td>γ2</td>
<td>γ3</td>
<td>α1</td>
<td>α2</td>
<td>ε</td>
<td></td>
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<tr>
<td>Molecular weight (kDa)</td>
<td>970</td>
<td>184</td>
<td>146</td>
<td>146</td>
<td>165</td>
<td>146</td>
<td>160</td>
<td>160</td>
<td>188</td>
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<tr>
<td>Serum level (mean adult mg ml⁻¹)</td>
<td>1.5</td>
<td>0.03</td>
<td>0.3</td>
<td>0.3</td>
<td>0.5</td>
<td>2.0</td>
<td>0.5</td>
<td>8 x 10⁴</td>
<td></td>
</tr>
<tr>
<td>Half-life in serum (days)</td>
<td>10</td>
<td>3</td>
<td>21</td>
<td>20</td>
<td>7</td>
<td>21</td>
<td>6</td>
<td>6</td>
<td>2</td>
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</table>
Initial Immune Response mediated by IgM

IgM (plasma cells in lymph nodes, spleen, and bone marrow and circulate in blood/lymph)

- Low affinity binding to antigen via multiple binding sites
- Exposure of constant region
- Activation of complement
- Phagocytose
- Kill directly
- Hypermutation and affinity maturation
- Two binding sites sufficient for strong binding
- Isotype switching to IgG

IgG

- Circulates in blood and lymph (most abundant Ab in internal fluids)
- Extravasation, Higher affinity binding to antigen
- Recruit phagocytes
- Neutralize antigens
- Activate complement
- Multiple effector functions

Monomeric IgA

- Plasma cells in lymph nodes, spleen, bone marrow
- Secreted into Blood
- Effector functions
  1. Mainly neutralization
  2. Minor opsonization and activation of complement

Dimeric IgA

- Lymphoid tissue associated with mucosal surfaces
- Secreted into Gut lumen & body secretions
IgE
Plasma cells in lymph nodes or germinal centers
Bind strongly to Mast cells
Cross-linking of receptor bound Ab releases histamine and other activators

Inflammation
- Expulsion of large pathogens
- Allergies

### Table: Functions of IgM, IgD, IgG1, IgG2, IgG3, IgG4, IgA, IgE

<table>
<thead>
<tr>
<th>Function</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>
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<tbody>
<tr>
<td>Neutralization</td>
<td>++</td>
<td>++</td>
<td>--</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td>Opsonization</td>
<td>----</td>
<td>----</td>
<td>++++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sanitization for killing by NK cells</td>
<td>--</td>
<td>--</td>
<td>++</td>
<td>--</td>
<td>++</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Sanitization of mast cells</td>
<td>--</td>
<td>--</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Activation of complement system</td>
<td>+++</td>
<td>--</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>--</td>
<td>+</td>
<td>--</td>
</tr>
<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>++++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Diffusion into extravascular sites</td>
<td>++</td>
<td>--</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++</td>
<td>+</td>
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<tr>
<td>Mean serum level (mg ml⁻¹)</td>
<td>1.5</td>
<td>0.03</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>0.3</td>
<td>21</td>
<td>Cell³</td>
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</tbody>
</table>

### Changes in Immunoglobulin genes during a B cell’s life

<table>
<thead>
<tr>
<th>Event</th>
<th>Mechanism</th>
<th>Permanence of change to the B cell’s genome</th>
</tr>
</thead>
<tbody>
<tr>
<td>V region assembly from gene fragments</td>
<td>Somatic recombination of genomic DNA</td>
<td>Irreversible</td>
</tr>
<tr>
<td>Generation of functional diversity</td>
<td>Immunization in joining rearranged DNA segments adds non-gene nucleotides (P and N) and deletes germline nucleotides.</td>
<td>Irreversible</td>
</tr>
<tr>
<td>Assembly of transcriptional controlling elements</td>
<td>Promoter and enhancer are brought closer together by V-region assembly</td>
<td>Irreversible</td>
</tr>
<tr>
<td>Transcription activated with compression of surface IgM and IgG</td>
<td>Two patterns of splicing and processing RNA are used</td>
<td>Reversible and regulated</td>
</tr>
<tr>
<td>Synthesis changes from membrane Ig to secreted antibody</td>
<td>Two patterns of splicing and processing RNA are used</td>
<td>Reversible and regulated</td>
</tr>
<tr>
<td>Somatic hypermutation</td>
<td>Point mutation of genomic DNA</td>
<td>Irreversible</td>
</tr>
<tr>
<td>Isotype switch</td>
<td>Somatic recombination of genomic DNA</td>
<td>Irreversible</td>
</tr>
</tbody>
</table>
Summary: Generation of B-cell diversity

- Diversity before Antigen exposure (Antigen Independent)
  - Random Recombination
  - Junctional Diversity
  - Combinatorial association

- Diversity after Antigen exposure (Antigen Dependent)
  - Switch to secreted Ab
  - Somatic Hypermutation
  - Affinity Maturation
  - Isotype Switching

- Immunoglobulin Classes
  - Properties
  - Effector functions