K-RITH Immunology Lecture Series
An Intensive Course in Basic Immunology

18-21 January 2010
University of KwaZulu Natal, Durban
Nelson R. Mandela School of Medicine

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Lecture 5: Antigen Recognition by B Cell Receptors
(based on Lecture by Dr. Matthew Scharff, Einstein)
Questions to Consider

- How can we make antibody to every possible pathogen-i.e. Diversity?
- How do we avoid making autoantibodies-i.e. Specificity?
- How do we rapidly increase amount of antibody-i.e. Mobilization?
- How do we switch from making IgM to IgG- i.e. Isotype Switching?
- How do we increase the the affinity of antibody-i.e. Affinity maturation?
- How do we generate memory?
Antibody Molecules Use Different Mechanisms to Prevent Infection
Antibody Levels and Affinity Are Increased by Immunization

Figure 10-31 Immunobiology, 6/e. (© Garland Science 2005)
Structure of the Antibody Molecule

Figure 3-1 Immunobiology, 6/e. (© Garland Science 2005)
Clonal Selection of Antigen-specific Lymphocytes

A single progenitor cell gives rise to a large number of lymphocytes, each with a different specificity

Removal of potentially self-reactive immature lymphocytes by clonal deletion

self antigens

Pool of mature naive lymphocytes

foreign antigen

Proliferation and differentiation of activated specific lymphocytes to form a clone of effector cells

Effector cells eliminate antigen

Figure 1-11 Immunobiology, 7ed. (© Garland Science 2008)
V Domains of Heavy and Light Chains Contain Discrete Hypervariable Regions.

Figure 3-6 Immunobiology, 6/e. (© Garland Science 2005)
Hypervariable Regions Lie in Loops That Are Brought Together in the Folded Molecule
Clonal Selection of Antigen-specific Lymphocytes

A single progenitor cell gives rise to a large number of lymphocytes, each with a different specificity.

Removal of potentially self-reactive immature lymphocytes by clonal deletion:
- self antigens
- self antigens

Pool of mature naive lymphocytes:
- foreign antigen

Proliferation and differentiation of activated specific lymphocytes to form a clone of effector cells:
- Effector cells eliminate antigen

Figure 1-11 Immunobiology, 7ed. (© Garland Science 2008)
V-region Gene Are Constructed From Discrete Gene Segments
Germline Organization of the Immunoglobulin Heavy and Light-Chain Loci in the Human Genome

Figure 4-4 Immunobiology, 6/e. (© Garland Science 2005)
V-region Gene Segments Are Joined by Recombination
The Number of Functional Gene Segments for the V region Heavy and Light-Chains

<table>
<thead>
<tr>
<th>Segment</th>
<th>Light chains</th>
<th>Heavy chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable (V)</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>Diversity (D)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Joining (J)</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

Number of functional gene segments in human immunoglobulin loci

Figure 4-3 Immunobiology, 6/e. (© Garland Science 2005)
Germline Organization of the Immunoglobulin Heavy and Light-Chain Loci in the Human Genome

Figure 4-4 Immunobiology, 6/e, (© Garland Science 2005)
Gene Segments Encoding the V Regions Are Flanked by Conserved Heptamer and Nonamer Sequences

Recombination signal sequence (RSS) with 23-base-pair spacer

<table>
<thead>
<tr>
<th>Heptamer</th>
<th>Nonamer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CACAGTG</td>
<td>ACAAAAAACC</td>
</tr>
<tr>
<td>GTGTCAC</td>
<td>TGGTTTGG</td>
</tr>
</tbody>
</table>

Recombination signal sequence (RSS) with 12-base-pair spacer

<table>
<thead>
<tr>
<th>Heptamer</th>
<th>Nonamer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CACTGTG</td>
<td>GGGTTTTTGT</td>
</tr>
<tr>
<td>CCAAAACACA</td>
<td>GTGACAC</td>
</tr>
</tbody>
</table>

\[ \lambda \text{ chain} \]  
\[ V_\lambda \quad 23 \rightarrow \quad \text{RSS} \quad \text{RSS} \quad \begin{array}{c} <12 \quad J_\lambda \end{array} \]

\[ \kappa \text{ chain} \]  
\[ V_\kappa \quad 12 \rightarrow \quad \text{RSS} \quad \text{RSS} \quad \begin{array}{c} <23 \quad J_\kappa \end{array} \]

\[ \text{H chain} \]  
\[ V_H \quad 23 \rightarrow \quad \quad \begin{array}{c} <12 \quad D_H \quad 12 \quad \quad \begin{array}{c} <23 \quad J_H \end{array} \end{array} \]

Figure 4-5 Immunebiology, 7th ed. © Garland Science 2008
Introduction of P- and N-nucleotides at the Joints Between Gene Segments During Ig Gene Rearrangement
## Contribution of Factors to Antigen Receptor Diversity

<table>
<thead>
<tr>
<th>Element</th>
<th>Immunoglobulin</th>
<th>α:β T-cell receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
<td>κ+λ</td>
</tr>
<tr>
<td>Variable segments (V)</td>
<td>40</td>
<td>70</td>
</tr>
<tr>
<td>Diversity segments (D)</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>D segments read in three frames</td>
<td>rarely</td>
<td>–</td>
</tr>
<tr>
<td>Joining segments (J)</td>
<td>6</td>
<td>5(κ) 4(λ)</td>
</tr>
<tr>
<td>Joints with N- and P-nucleotides</td>
<td>2</td>
<td>50% of joints</td>
</tr>
<tr>
<td>Number of V gene pairs</td>
<td>1.9 x 10^6</td>
<td></td>
</tr>
<tr>
<td>Junctional diversity</td>
<td>~3 x 10^7</td>
<td></td>
</tr>
<tr>
<td>Total diversity</td>
<td>~5 x 10^{13}</td>
<td></td>
</tr>
</tbody>
</table>

*Figure 4-13 Immunobiology, 6/e. (© Garland Science 2005)*
Clonal Selection of Antigen-specific Lymphocytes

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Effector cells eliminate antigen.

Figure 1-11 Immunobiology, 7ed. (© Garland Science 2008)
Neutralizing Antibodies Prevent Viral Infection of Cells

- Virus binds to receptors on cell surface
- Receptor-mediated endocytosis of virus
- Acidification of endosome after endocytosis triggers fusion of virus with cell and entry of viral DNA
- Antibody blocks binding to virus receptor and can also block fusion event

Figure 9-25 Immunobiology, 6/e. (© Garland Science 2005)
Somatic Mutation Increases Affinity

Abbas, *Cellular and Molecular Immunology*
Activation-induced Cytidine Deaminase (AID)

Deficiency causes Hyper IgM type II

- AID is a cytidine deaminase whose *in vitro* substrate is ssDNA
- AID may associate with RPA, RNAP II &? others
- Transcription is required for somatic SHM and CSR

AID deaminates C to U followed by UNG and APE1.
Somatic Hypermutation of Antibody V Regions

- Replication
  - Short-patch BER
  - Long-patch BER
  - PCNA recruits error-prone polymerases (Pol η)

- Phase 1a
  - Mutations at G:C basepairs
  - ~40% of total mutations

- Phase 1b
  - Mutations at A:T basepairs
  - ~60% of total mutations

- Phase 2
  - UNG
  - MMR

Somatic Hypermutation

Error prone Polymerases → High fidelity Polymerases → Proofreading → Mismatch repair

10^-1 10^-3 10^-6 10^-9 10^-11 Mutation Frequency

dead death cancer cancer health

and yet also fitness

After Philip Coffino

Mutation Frequency = Mutations/bp/cell division
After T-cell-dependent Activation, B cells Undergo Rounds of Mutation and Selection That Generates High Affinity Memory B Cells
Humoral Immune Response

Class Switch Recombination

IgM → IgG / IgE / IgA

Antigen-binding site

Effector arm

Somatic Hypermutation
Low affinity → high affinity

Janeway and Travers, Immunobiology
Questions to Consider

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