Three "Limbs" of the Immune Response
Genetic Basis of Antibody Diversity

OUTLINE

PROBLEM OF ANTIBODY GENE DIVERSITY

GERMLINE vs. SOMATIC THEORIES

THREE GENE FAMILIES (H-chains, kappa, lambda)

GENE ORGANIZATION
V/J/D REARRANGEMENT
CLASS SWITCHING
SOMATIC MUTATION

SOUTHERN BLOT ANALYSIS OF GENE ORGANIZATION
How to Account for Antibody Diversity?

- An Ab combining site is made up of one $V_L$ and one $V_H$
- The specificity of any combining site is determined by its amino acid sequence.
- There exist many unique combining sites – let’s say at least $10^6$

How many $V$-region genes must exist?

If each $V$-region requires a unique gene, we might need only $10^3 L + 10^3 H$-chains (combinatorial association of $V_L$ and $V_H$)
How to Account for Antibody Diversity?

Germ-Line versus Somatic Theories

**Germ-line**: One germ-line gene exists for every V-region
  *i.e.* Need *thousands* of genes

**Somatic**: One germ-line gene diversifies during development.
  *i.e.* Need *as few as 3* genes ($V_H$, $V_K$, $V_\lambda$)

So...who’s right?

*Everybody is!*

1) *Many germ-line genes exist* (Germ-Line diversity)

2) *These genes are diversified B-cell development* (Somatic diversity)
L-chain

H-chain

COOH-terminus

NH₂-terminus

Constant Region

Variable Region

CDR's

κ-chain

protein

genesis

C-region

V-region

C-kappa

V-kappa

J-kappa

genes

C-kappa

V-kappa
**Organization of Human Immunoglobulin Genes**

**kappa:**

\[
\begin{array}{c}
\text{VK}\,1 & \text{VK}\,2 & \text{VK}(n) & J\kappa & \text{CK} \\
\end{array}
\]

**lambda:**

\[
\begin{array}{c}
\text{VL}\,1 & \text{VL}\,2 & \text{VL}(n) & J\,C\lambda\,1 & J\,C\lambda\,2 & J\,C\lambda\,3 & J\,C\lambda\,7 \\
\end{array}
\]

**heavy:**

\[
\begin{array}{c}
\text{Vh}\,1 & \text{Vh}\,2 & \text{Vh}(n) & Jh & \text{C\mu} & \text{C\delta} & \text{C\gamma}\,3 & \text{C\gamma}\,1 & \text{C\alpha}\,1 & \text{C\gamma}\,2 & \text{C\gamma}\,4 & \text{C\epsilon} & \text{C\alpha}\,2 \\
\end{array}
\]

**Human chromosome #**

\[
\begin{array}{c}
2 \\
22 \\
14 \\
\end{array}
\]

**Three Ig Gene Families:**

- H-Chains, κ-Chains, λ-Chains

Each family has V-(D)-J-C, varying numbers of each gene and differing details of organization.
MOLECULAR BASIS OF KAPPA GENE EXPRESSION

Germ-line configuration:

Following DNA rearrangement:

Following transcription:

Following RNA splicing:

Following translation:

Following peptide processing:
Organization of Human Immunoglobulin Genes

kappa:

Vκ1  Vκ2  Vκ(n)  JK  CK

lambda:

Vλ1  Vλ2  Vλ(n)  J Cλ1  J Cλ2  J Cλ3  J Cλ7

heavy:

Vh1  Vh2  Vh(n)  Dh  Jh  Cμ  Cδ  Cγ3  Cγ1  Cα1  Cγ2  Cγ4  Cε  Cα2

Some B-cells express both membrane IgM and IgD
SIMULTANEOUS SYNTHESIS OF IgM AND IgD IN B-CELLS BY ALTERNATE RNA SPLICING

common to many other genes
Organization of Human Immunoglobulin Genes

**kappa:**

\[ VK_1 \quad VK_2 \quad VK(n) \quad JK \quad CK \]

**lambda:**

\[ VL_1 \quad VL_2 \quad VL(n) \quad JC_\lambda 1 \quad JC_\lambda 2 \quad JC_\lambda 3 \quad JC_\lambda 7 \]

**heavy:**

\[ Vh_1 \quad Vh_2 \quad Vh(n) \quad Dh \quad Jh \quad C_\mu \quad C_\delta \quad C_\gamma 3 \quad C_\gamma 1 \quad C_\alpha 1 \quad C_\gamma 2 \quad C_\gamma 4 \quad C_\epsilon \quad C_\alpha 2 \]

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Memory: Ab-secreting cells switch from IgM to IgG...
Transcript before switch: \textit{IgM}

DNA rearrangement ("switch"): unique to \textit{Ig}

Transcript after switch: \textit{IgG3}

MOLECULAR BASIS OF CLASS SWITCHING
Summary: Sources of Antibody Diversity

1) \(~50\) germline \(V_k\)-genes

2) \(5\) germline \(J_k\)-segments
   “Combinatorial Joining” of \(V_k\) & \(J_k\)
   \(50\ V_k \times 5\ J_k = 250\)

3) \(DNA\ rearrangement\ is\ imprecise\)
   \(250 \times 10 = 2,500\)

4) \(Somatic\ mutation\ of\ rearranged\ V\)-genes
   \(2,500 \times 10 = 25,000\) different \(V_k\)-regions

5) \(Heavy\ and\ light\ chains\ associate\ randomly\)
   “Combinatorial Association”
   \(25,000 \times 25,000 = \sim 6 \times 10^8\) combining sites.
The Immune system as a defence organization

1. Its function is selective destruction.
2. It is large, complicated and elaborate.
3. It is expensive.
4. It is wasteful.
5. It has distinct components performing apparently identical functions.
6. It is slow to react.
7. It is prepared for events that never happen.
8. It fights today’s threats with the solutions of past problems.
9. It is susceptible to corruption.
10. It can destroy that which it protects.

Peter Parham, 1990
**T-CELL ANTIGEN RECEPTOR**

another rearranging DNA...

- **Members of Ig Superfamily**
- **α/β chains** (homologous to Ig L & H-chains)
- **Similar gene structure/rearrangement**
  (VVV-[DDD]-JJJ-C...)

**However:**
1. TCR is only membrane-bound
2. Monovalent
3. No somatic mutation
Genetic Events in B-Cell Differentiation

Surface markers: IgM/IgD...

DNA markers – Southern Blotting
Analysis of Kappa Gene Rearrangements by Southern Blotting

#1: germline

#2: rearranged (V36/J3)

#3: rearranged (V17/J2)
- Cut DNA with EcoRI
- Separate fragments by agarose gel electrophoresis (size)
- Blot & hybridize with C-kappa specific probe

**Autoradiograph of Southern blot hybridized with Ck probe**

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#1: germline band
#2,3: single *monoclonal* rearrangements (+ unrearranged allele)
#4: *polyclonal* rearrangements (+ unrearranged allele)
MONDAY
Immunoglobulin Biosynthesis,
Chapter 9

TUESDAY
ABO & Rh Blood Groups,
Chapter 10