**CLONAL SELECTION**

1. Each clone expresses one unique receptor.
2. Receptors form independent of antigen encounter.
3. Self-reactive clones are eliminated (tolerance).
4. Antigen encounter selects specific clones for proliferation and differentiation.

**CLONOTYPIC RECEPTORS**

- **B CELLS**: Antibody (immunoglobulin)
- **T CELLS**: T cell receptor

1. Protein Structure
2. Gene Organization
3. UNIQUE GENE REARRANGEMENT

**ANTIBODIES**

- **Ig CONSTANT DOMAIN**
- **Ig VARIABLE DOMAIN**
Variable Domains Have Hypervariable (HV) or Complementarity Determining Regions (CDRs)

HV regions occur at loops between beta sheets

Both Heavy and Light Chains Are Required to Form The Antigen Binding Site

Both Heavy and Light Chains Are Required to Form The Antigen Binding Site

Preparation/Preinpar

Preparation/Preinpar
T CELL RECEPTORS ARE SIMPLER THAN ANTIBODIES

Antigen Recognition

Antibodies:
Secreted or Transmembrane

TCR: Transmembrane

The Diversity Problem:
How are $10^8$ clonotypic antibodies encoded?

HYPOTHESIS #1: Germline genes encode everything
(Could there be $2 \times 10^4$ or more Ig genes?)

HYPOTHESIS #2: Somatic mutation of single germline genes
(How could the genome sustain such a high, and currently unknown, rate of somatic mutation?)

ANSWER LIES IN ORGANIZATION AND UNIQUE REARRANGEMENT OF Antibody and TCR GENES

Ig Polypeptides Are Encoded by LIGHT CHAIN Multiple Gene Segments

<table>
<thead>
<tr>
<th>Variable</th>
<th>Constant</th>
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<tr>
<td>V</td>
<td>J</td>
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HEAVY CHAIN

<table>
<thead>
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<th>Constant</th>
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<tr>
<td>V</td>
<td>D</td>
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</table>

A Prototype Ig Gene: Murine Kappa

About 10 0 $V_K$ gene segments 4 J Gene Segment s 1 $C_K$ Gene Segment

Multiple V gene segments, distant from J and C
A few J gene segments
One C gene segment

Murine Ig Heavy Chain Gene Organization

~120 V Gene Segments ~20 Ds 4 Js 8 Constant Gene Segments
TCR Alpha and Beta Loci

TCR Delta and Gamma Loci

IMMUNOGLOBULIN GENES UNDERGO TWO DNA REARRANGEMENTS

1. V(D)J Recombination: both light and heavy chains
2. Class switch recombination: heavy chains only

V(D)J Recombination in the Kappa Locus

About 10 V<sub>K</sub> gene segments
4 J Gene Segment s
1 C<sub>K</sub> Gene Segment

Random selection of V and J
DNA is deleted

Coding segments joined directly

Ig Heavy Chain VDJ Recombination—2 DNA Deletions, DJ and VD

VDJ Recombination Entails Deletion of Genomic DNA

VDJ joining occurs by DNA recombination
RAG PROTEINS are lymphocyte-specific and mediate precise DNA recognition and cutting.

**DNA repair enzymes, that are NOT lymphoid specific, rejoin the cut ends of DNA.**

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**Omenn Syndrome: Mutation in RAG-1 Gene**

An infant with a skin rash and recurrent bacterial and fungal infections

- Presented at two weeks with severe generalized dermatitis and diarrhea.
- Developed a life-threatening disseminated infection with Staphylococcus aureus.
- Diagnosis was suspected after noting absence of thymic shadow on X-ray, markedly reduced serum immunoglobulins, absent B cells and reduced numbers of T cells from peripheral blood.
- In vitro V(D)J recombination assay was 10% of normal. Sequencing of the RAG-1 gene revealed a missense mutation.
- Bone marrow transplantation is the only therapeutic option.

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**SUMMARY-PROTEIN STRUCTURE**

1. Antibodies are the clonotypic receptors for B cells.
2. T cell receptors are clonotypic receptors for T cells.
3. Antibodies are tetramers of 2 identical light chains and 2 identical heavy chains. Each chain has variable constant regions.
4. Antibody heavy chain constant regions eliminate antigen.
5. Antibody variable regions recognize antigen; antibody heavy chain constant regions eliminate antigen.
6. HV regions from both light and heavy chains are necessary to form an antigen binding site.
7. TCRs resemble two Ig light chains; their sole function is to recognize antigen.

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**SUMMARY-Ig and TCR GENE REARRANGEMENT**

1. Ig and TCR genes are encoded by 30-150 V gene segments, several J and D gene segments and few C gene segments.
2. Unrearranged Ig and TCR genes are inactive.
3. VDJ recombination forms functional Ig and TCR genes.
4. VDJ recombination involves deletion of DNA.
5. RAG1 and RAG2 genes are lymphoid specific components of VDJ recombination and are required for formation of Ig and TCR genes.
6. VDJ recombination provides a mechanism to generate huge diversity, primarily via combinatorial mechanisms.

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**CONSEQUENCES OF V(D)J RECOMBINATION**

**(in addition to formation of a functional gene)**

1. Combinatorial diversity: # of possible combinations is the product of the # of recombining segments i.e. for mouse h.c.: $120 \times 20 \times 4 = 10^4$
2. Junctional diversity at CDR3
   - Deletion of bases at junctions
   - N region additions at junctions
   - P region additions at junctions
3. Activates transcription of the rearranged gene
   - Juxtaposition of intronic enhancers with V region promoters.
4. Allows receptor editing to alter potentially self-reactive antibodies