ANTIGEN RECOGNITION BY B LYMPHOCYTES: IMMUNOGLOBULIN STRUCTURE

Date: Thursday, March 19, 2013
10:30am – 11:25am

LEARNING GOAL
You will be able to compare and contrast the structure, effector functions and antigenic features of the five classes of immunoglobulins. You will also be able to diagram somatic DNA rearrangements required for generation of B and T lymphocytes.

OBJECTIVES
To attain the goal for these lectures you will be able to:

● List the classes of immunoglobulins (Ig).
● Diagram the basic structure of Ig (including polymeric Ig) including H and L chains, antigen binding site, Fab and Fc.
● Describe the major biological characteristics of each Ig class/subclass.
● State the approximate half-life of antibody titers after tetanus vaccination and after infection-caused measles.
● Diagram the IgH and Igκ chain gene loci.
● Diagram the order of H and L chain gene rearrangements that occur during B cell development.

READING ASSIGNMENT
Janeway et. al., 2012, Chapter 4, pp. 127-133
Chapter 5, pp. 157-161

LECTURER
Katherine L. Knight, Ph.D.
CONTENT SUMMARY

I. Classes of Immunoglobulins: IgG, IgA, IgM, IgD, IgE

II. Basic Immunoglobulin Structure

   Core Structure
   Heavy chains
   Light chains
   Variable and constant regions
   Fab and Fc fragments

III. Major characteristics of immunoglobulin classes (antibody binding and effector functions)

   IgM, IgG, IgD, IgE, IgA

IV. Half-life of serum antibody titers following disease or immunization

V. Germline organization of Ig genes

   Three groups of genes; \( \kappa, \lambda \), and H chain
   V, D and J gene segments
   \( C_\kappa \) and \( C_H \) region genes

VI. Immunoglobulin gene rearrangements- basic feature

   A. Order of rearrangement
IMMUNOGLOBULIN STRUCTURE

I. CLASSES OF IMMUNOGLOBULINS

- IgG - predominant Ab induced in secondary response
- IgA - predominant Ig in external secretions
- IgM - predominant Ab induced in primary response
- IgD - found mainly on surface of B cells
- IgE - involvement in allergic hypersensitivities

II. BASIC IMMUNOGLOBULIN STRUCTURE

- CORE STRUCTURE

All antibodies have a common core structure of two identical light chains (about 24 kilodaltons [kD]) and two identical heavy chains (about 55 or 70 kD) (Fig. 1). One light chain is attached to each heavy chain, and the two heavy chains are attached to each other. Both the light chains and the heavy chains contain a series of repeating, homologous units, each about 110 amino acid residues in length, which fold independently in a common globular motif, called an immunoglobulin domain.
HEAVY CHAINS

The H chains determine the Ig isotype, IgA, IgG, IgM, IgD, or IgE.

<table>
<thead>
<tr>
<th>Ig Class</th>
<th>Heavy Chain Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td>mu (µ)</td>
</tr>
<tr>
<td>IgG</td>
<td>gamma (γ)</td>
</tr>
<tr>
<td>IgE</td>
<td>epsilon (ε)</td>
</tr>
<tr>
<td>IgA</td>
<td>alpha (α)</td>
</tr>
<tr>
<td>IgD</td>
<td>delta (δ)</td>
</tr>
</tbody>
</table>

LIGHT CHAINS

All antibody light chains fall into one of two classes or isotypes, κ and λ. Each member of a light-chain isotype shares complete amino acid sequence identity of the carboxy terminal C region with all other members of that isotype. In man, antibodies with κ and λ light chains are present in about equal number.

VARIABLE AND CONSTANT REGIONS

Studies of myeloma proteins resulted in identification of variable (V) and constant (C) regions.

The N-terminal region of both H and L chains are considered variable regions; the remainder of H and L chains are considered the constant regions.

- **Variable Region:** The combination of the variable region of the H chain and the variable region of the L chain make up the antigen binding site of an immunoglobulin.

- **Constant Region:** Other effector functions of Ig are carried out by the constant domains. These include the ability to cross the placenta, sites for attachment to Fc receptors of macrophages, monocytes, mast cells and sites for binding complement.
Fab and Fc FRAGMENTS

III. MAJOR CHARACTERISTICS OF THE FIVE IMMUNOGLOBULIN CLASSES

![Diagram of Immunoglobulin Classes]

Figure 4-20. Immunochemistry, 6th ed. (© Garland Science 2008)
### Immunoglobulin Structure

<table>
<thead>
<tr>
<th>Heavy chain</th>
<th>Heavy chain</th>
<th>Heavy chain</th>
<th>Heavy chain</th>
<th>Heavy chain</th>
<th>Heavy chain</th>
<th>Heavy chain</th>
<th>Heavy chain</th>
<th>Heavy chain</th>
<th>Heavy chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ1</td>
<td>γ2</td>
<td>γ3</td>
<td>γ4</td>
<td>μ</td>
<td>α1</td>
<td>α2</td>
<td>δ</td>
<td>ε</td>
<td></td>
</tr>
<tr>
<td>Molecular weight (kDa)</td>
<td>146</td>
<td>146</td>
<td>165</td>
<td>146</td>
<td>970</td>
<td>160</td>
<td>160</td>
<td>184</td>
<td>188</td>
</tr>
<tr>
<td>Serum level (mean adult mg ml⁻¹)</td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>0.5</td>
<td>1.5</td>
<td>3.0</td>
<td>0.5</td>
<td>0.03</td>
<td>5 x 10⁻⁵</td>
</tr>
<tr>
<td>Half-life in serum (days)</td>
<td>21</td>
<td>20</td>
<td>7</td>
<td>21</td>
<td>10</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

- Classical pathway of complement activation
- Alternative pathway of complement activation
- Placental transfer
- Binding to macrophage and phagocyte Fc receptors
- High-affinity binding to mast cells and basophils
- Reactivity with staphylococcal Protein A

**Figure 4-16 Immunobiology, 7th ed. (© Garland Science 2008)**

### Functional activity

<table>
<thead>
<tr>
<th>Functional activity</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>
</tr>
</thead>
<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>Sensitization 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>Sensitization of mast cells</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Activates complement system</td>
<td>++</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

**Figure 3-19 Immunobiology, 7th ed. (© Garland Science 2008)**

### Distribution

<table>
<thead>
<tr>
<th>Distribution</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>
</tr>
</thead>
<tbody>
<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>
</tr>
<tr>
<td>Diffusion into extravascular sites</td>
<td>+/-</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Mean serum level (mg ml⁻¹)</td>
<td>1.5</td>
<td>0.04</td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>0.5</td>
<td>2.1</td>
<td>3 x 10⁻⁵</td>
</tr>
</tbody>
</table>
### IgM
- Ab of 1° response for protein Ag administered parenterally.
- Major class of Ab elicited by T-independent antigen (polysaccharide).
- Pentameric in its secreted form, m.w. = ~ 900,000 daltons.
- J chain (mw = 15,000 daltons) joins the subunits
- Expressed on the surface of B-cells as a monomer
- Effective complement fixation - classical pathway.
- Does not cross the placenta of man or other species.
- Serum concentration = ~ 100 mg/dl in adult.
- Part of B cell receptor (BCR)

### IgG
- Ab class characteristic of 2° response for most protein (T-dependent) Ag.
- Monomeric in its secreted or membrane form; m.w. = ~150,000 daltons.
- Variability in complement fixation according to subclass.
- Crosses the human placenta due to the presence of FcRn on the placenta.
- Serum concentration = ~1200 mg/dl in adult.

### IgD
- Usual form is the membrane form; very few IgD plasma cells.
- Functions as B-cell Ag receptor along with IgM
- Serum concentration of adults = ~3 mg/dl

### IgE
- Class of antibody involved in allergic hypersensitivities, such as ragweed allergy.
- IgE Ab(s) fix to mast cells and basophils via an Fc receptor on the cells
- Secreted as a monomer; m.w. ~190,000 daltons; CHO rich.
- Very low concentrations in serum of normal adults (~30 µg/dl).

### IgA
- Predominant Ig class in external secretions; occurs as a dimer.
- J chain joins the two subunits
- Predominant form in serum is monomeric; serum IgA is ~160,000 daltons.
- Function of serum IgA is not clear.
- Does not fix complement by classical pathway; can usually fix complement by alternative pathway.
- Does not cross placenta.
- Serum concentration = ~300 mg/dl in adult.
- Key role in mucosal immunity

**Immunoglobulin levels in human serum.**

The neonate is protected by passively transferred maternal IgG for the first few months after birth. Adult levels of IgM are reached at about 10 months of age; IgG at 4 years; IgA at about 10 years of age.

![Immunoglobulin levels in human serum](image)

**IV. Half-life of serum antibody titers after immunization or disease.**
Serum spanning a 20 year period was analyzed to determine the half-life of antibody to infectious agents following immunization or infection.

Result: The half-life of serum antibody titers ranged from 11 yrs for tetanus to 3014 years for measles.

![Graphs showing antibody half-life for tetanus and measles](image)

**Taken from: Amanna et al. (2007) N Engl J Med 357:1903**

### V. Ig GENE REARRANGEMENTS REQUIRED FOR SYNTHESIS OF ANTIBODIES

**GERMLINE ORGANIZATION OF IMMUNOGLOBULIN GENES**

Three groups of Genes: κ, λ and H Chain

The genes for the immunoglobulin polypeptide chains (and for the T-cell receptor chains) are split, Ig genes undergo a process of somatic DNA recombination (rearrangement) during B cell ontogeny.

Each of the 3 gene families, the kappa light chain family, the lambda light chain family and the heavy chain family can be divided into V-region genes and C-region genes. The κ, λ and H chains are located on separate chromosomes. Each set of genes, κ, λ and heavy chain, has a similar basic organization.
V, D and J Gene Segments

The Ig heavy and light chain loci are composed of multiple genes that give rise to the V and C regions of the proteins, separated by stretches of non-coding DNA. At the 5' end of each Ig locus are the **V region exons**, each about 300 base-pairs (bp) long, separated from one another by non-coding DNA of varying lengths. Downstream of the V genes are additional coding sequences, 30 to 50 bp long, which make up the **joining (J) segments** and, in the H chain locus only, the **diversity (D) segments**. The J and D gene segments code for the carboxy terminal ends of the V regions, including the third hypervariable (complementarily-determining) regions of antibody molecules. Thus, in an Ig light chain protein (κ or λ), the variable region is encoded by the V and J exons and the constant region by a C exon. In the heavy chain protein, the variable region is encoded by the V, D, and J exons. The constant region of the protein is derived from the multiple C exons and, for membrane-associated heavy chains, the exons encoding the transmembrane and cytoplasmic domains.

C Region Genes

At varying distances 3' of the V genes are the **C region genes**. In both mouse and man, the κ light chain locus and a single Cκ gene and the genes for heavy chain C regions (C\(\text{H}\)) of different isotypes are arranged in a tandem array. Each heavy chain C region gene actually consists of three to four exons (each similar in size to a V region exon) that make up the complete C region, and smaller exons that code for the carboxy terminal transmembrane (TM) and cytoplasmic domains of the heavy chains.

VI. IMMUNOGLOBULIN GENE REARRANGEMENTS - BASIC FEATURE

All cells except B-lineage, including plasma cells contain Ig genes in the germline configuration. The Ig genes are expressed only in B-lineage cells. Rearrangements of Ig genes are the essential first steps in the production of antibodies.
A. **Order of Ig gene rearrangement and B cell development**

DNA rearrangements occur in a precise order and occur independent of antigen stimulation.

1. **Heavy chain - DJ.** The first Ig gene rearrangement involves the heavy chain locus and leads to joining of one D and one J gene segment with deletion of the intervening DNA.

2. **Heavy chain - VDJ.** Following the DJ rearrangement, one of the many V genes is joined to the DJ complex, giving rise to a rearranged VDJ gene. At this stage, all D segments 5’ of the rearranged D are also deleted. *This VDJ recombination occurs only in cells committed to become B lymphocytes and is a critical control point in Ig expression because only the rearranged V gene is subsequently transcribed.* The C region genes remain separated from this VDJ complex by an intron.

3. **Light chain - VJ.** The next somatic DNA recombination involves a light chain locus. One V segment is joined to one J segment, forming a VJ complex, which remains separated from the C region by an intron, and this gives rise to the primary RNA transcript. Splicing of the intron from the primary transcript joins the C gene to the VJ complex, forming an mRNA that is translated to produce the κ protein. The light chain assembles with the previously synthesized μ to form the complete membrane IgM molecule, which is expressed on the cell surface, and the cell is now the immature B lymphocyte.
STUDY QUESTIONS

1. List Ig classes and their respective heavy chains.

2. Define V and C regions.

3. Draw a schematic diagram of IgG, IgD, IgE, monomeric IgM, polymeric IgM, monomeric IgA and secretory IgA.

4. Compare the similarity of heavy and light chain domains of antibodies of different specificities (for example, anti-salmonella versus anti-pneumococcus antibodies).

5. Identify the regions of IgG that comprise Fab and Fc fragments.

6. Identify major effector function(s) of each immunoglobulin isotype.

7. Compare the relative levels of each Ig class in serum and in secretions.


9. Given a B cell with a VDJ gene rearrangement describe the fate of:
   1. V_{H} gene segments upstream of the VDJ gene.
   2. D gene segments that were not used in the VDJ gene rearrangement.

10. Describe how the hepatamer/nonamer signal sequences (RSS) mediate V, D and J gene rearrangements.

11. State the role of RNA processing in synthesis of Ig L chains.

12. For the light chain genes, compare the intervening nucleic acid sequences that are lost during DNA recombination with those lost during RNA splicing.

13. Diagram the sequence of events in B cells that lead to the production of L chains; start with rearrangement of L chain genes.

ADDITIONAL QUESTIONS

1. _____ is the predominant serum immunoglobulin.

2. _____ is the predominant secretory immunoglobulin.

3. Most B lymphocytes have membrane Ig of what class(es)?

4. There is approximately _______ times more IgM than IgE in serum of normal individuals.
5. Antibody fragment with a single combining site is designated ________

ANSWERS TO ADDITIONAL QUESTIONS

1. IgG; 2. IgA; 3. IgD, IgM; 4. 1,000; 5. Fab

EXAMPLE OF TEST QUESTION

Ig classes can be distinguished on the basis of:

A. Their H chains.
B. Their L chains.
C. Both their H and L.
D. J chain.
E. V Regions.

CORRECT ANSWER TO ABOVE QUESTION: A
BASIC Ig STRUCTURE

**Immunoglobulin Isotypes**
**(Classes and Subclasses)**

**HEAVY CHAINS**
The H chains determine the Ig isotype, IgA, IgG, IgM, IgD, or IgE.

<table>
<thead>
<tr>
<th>Ig Class</th>
<th>Heavy Chain Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td>mu (α)</td>
</tr>
<tr>
<td>IgG</td>
<td>gamma (γ)</td>
</tr>
<tr>
<td>IgE</td>
<td>epsilon (ε)</td>
</tr>
<tr>
<td>IgD</td>
<td>delta (δ)</td>
</tr>
<tr>
<td>IgA</td>
<td>alpha (α)</td>
</tr>
</tbody>
</table>

**LIGHT CHAINS**

- Heavy chain: μ, γ, δ, or ε
- Light chain: κ or λ
STRUCTURE OF SECRETORY IgA

**Immunoglobulin Classes**

<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>
</thead>
<tbody>
<tr>
<td>Heavy chain</td>
<td>μ</td>
<td>δ</td>
<td>γ1</td>
<td>γ2</td>
<td>γ3</td>
<td>γ4</td>
<td>α1</td>
<td>α2</td>
<td>ε</td>
</tr>
<tr>
<td>Molecular weight (kDa)</td>
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<td>148</td>
<td>185</td>
<td>160</td>
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<td>188</td>
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</tr>
<tr>
<td>Serum level (mean adult mg ml⁻¹)</td>
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<td>5x10⁻⁶</td>
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<td>7</td>
<td>21</td>
<td>6</td>
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<td>2</td>
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</table>

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**Immunoglobulin Functions**

<table>
<thead>
<tr>
<th>Functional activity</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>
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<td>++</td>
<td>++</td>
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<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>Neutralization for killing by NK cells</td>
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<td>–</td>
<td>++</td>
<td>++</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>Neutralization of target cells</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Activates complement system</td>
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<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
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<td>IgD</td>
<td>IgG1</td>
<td>IgG2</td>
<td>IgG3</td>
<td>IgG4</td>
<td>IgA</td>
<td>IgE</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>
</tr>
<tr>
<td>Diffusion into extravascular sites</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mean serum level (mg ml⁻¹)</td>
<td>1.5</td>
<td>0.04</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>0.5</td>
<td>2.1</td>
<td>1</td>
</tr>
</tbody>
</table>

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Cross-linking of IgE on mast cell leads to degranulation

SERUM Ig LEVELS DURING DEVELOPMENT

Half-life of Serum Antibody Titers

Germline Organization of Ig Genes

Ig Constant Region Genes

Ig Gene Rearrangements
B CELL DEVELOPMENT AND GENERATION OF PRIMARY ANTIBODY REPERTOIRE

Date: Wednesday March 20, 2013
8:30 am – 9:30 am; 9:30 am – 10:30 am

LEARNING GOAL
You will be able to explain the process of B cell development in the bone marrow, and how a large antibody repertoire is developed. You will also be able to diagram the structure and rearrangement of T cell receptor (TCR) genes and explain how a large TCR repertoire develops.

OBJECTIVES
To attain the goal for these lectures you will be able to:

- Describe the mechanism by which individual B cells make only one antibody (allelic exclusion).
- Diagram the sequence of events leading from rearrangements of germline Ig genes to production of an Ig molecule.
- Describe how antibody diversity is generated through combinatorial joining and N segment addition.
- Describe the maturation of B cells from stem cells, including the pre-B cell receptor.
- Draw the structure of αβ and γδ T cell receptors.
- Diagram the mechanism by which T cells express a single antigen receptor.

READING ASSIGNMENT
Janeway’s Immunobiology (2012), Chapter 5; Chapter 8, pp 275-290; 301-305

LECTURER
Katherine L. Knight, Ph.D.
CONTENT SUMMARY

I. B cell development in bone marrow
   ProB, PreB, Immature B and Mature B cells
      A. Allelic exclusion - only one $V_H$ and one $V_L$ rearranged/B cell
      B. Mechanism of Ig gene rearrangement
         Conserved heptamer/nonamer (RSS) recognition sequences
      C. Membrane IgM vs. secreted IgM
      D. Surface IgM and IgD

II. Generation of antibody diversity in bone marrow
    A. Combinatorial joining of V, D and J gene segments
    B. Junctional diversity- N-region addition and imprecise joining
    C. Combinations of H and L chain proteins

III. B cell selection: Tolerance

IV. T cell antigen receptor
   A. Structure
   B. Gene organization and rearrangement
B CELL DEVELOPMENT IN BONE MARROW

**Major rule of antibody synthesis:** A single B cell makes only one kind of antibody specificity (one $V_H$ and one $V_L$), i.e., allelic exclusion occurs. Also, a single plasma cell makes only one kind of antibody; i.e., 1 kind of H chain & 1 kind of L chain; B cells may violate this rule & synthesize two or more heavy chain isotypes simultaneously for the cell surface, eg. IgM and IgD.

I. SEQUENTIAL REARRANGEMENT OF Ig GENES IN B CELL PRECURSORS

<table>
<thead>
<tr>
<th>Stem cell</th>
<th>Early pro-B cell</th>
<th>Late pro-B cell</th>
<th>Large pre-B cell</th>
<th>Small pre-B cell</th>
<th>Immature B cell</th>
<th>Mature B cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-chain genes</td>
<td>Germline</td>
<td>D-J rearranging</td>
<td>V-DJ rearranging</td>
<td>VDJ rearranged</td>
<td>VDJ rearranged</td>
<td>VD rearranged</td>
</tr>
<tr>
<td>L-chain genes</td>
<td>Germline</td>
<td>Germline</td>
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</tr>
<tr>
<td>Surface Ig</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>$\mu$ chain expressed on cell surface</td>
<td>$\mu$ chain expressed on cell surface</td>
<td>$\mu$ chain made from alternatively spliced H-chain transcripts</td>
</tr>
</tbody>
</table>

**ProB Cells:** These cells are precursors of PreB cells. They have IgH DJ gene rearrangements and no light chain gene rearrangements.

**Pre B Cells:** All B lymphocytes arise in the bone marrow from a stem cell that does not produce Ig. The earliest cell type that synthesizes a detectable Ig gene product contains cytoplasmic $\mu$-heavy chains composed of variable (V) and constant (C) regions. This cell is called the *pre-B lymphocyte* and is found only in hematopoietic tissues, such as the bone marrow and fetal liver. The pre-B receptor is comprised of surrogate light chain, $\mu$-chain, Ig$\alpha$ and Ig$\beta$

**Immature B Cells:** At the next identifiable stage in B cell maturation, $\kappa$ or $\lambda$ light chains are also produced. These associate with $\mu$ heavy chains and then the assembled IgM molecules are expressed on the cell surface, where they function as specific receptors for antigens. IgM-bearing B cells that are recently derived from bone marrow precursors are called *immature B lymphocytes* because they do not proliferate and differentiate in response to antigens. Once a B cell expresses a complete heavy or light chain, it cannot produce another heavy or light chain containing a different V region.

**Mature B Cells:** Having acquired a complete Ig and, therefore, an antigen specificity, B cells migrate out of the bone marrow and can be found in the peripheral circulation and
lymphoid tissues. They continue to mature, even in the absence of antigenic simulation. Mature B cells co-express μ and δ heavy chains in association with the original κ or λ light chain and, therefore, produce both membrane IgM and IgD. Both classes of membrane Ig have the same V region and hence the same antigen specificity. Such cells are responsive to antigens.

A. **Allelic Exclusion:** Only one IgH and one IgL allele are productively rearranged.

Each B cell clone and its progeny are specific for only one antigenic determinant. It is, therefore, necessary for each B cell to express only one set of Ig heavy and light chain V genes throughout its life. This occurs because only one functional heavy chain VDJ and one functional VJ gene rearrangement occur in each cell. The expression of only one allele in a cell is termed allelic exclusion.

B. **Mechanism of Ig gene rearrangement**

- Conserved recognition sequences

![Diagram of Ig gene rearrangement](image-url)
The recombination of V, D and J gene segments is mediated by specific DNA recognition sequences (RSS) located in the intervening DNA 3' of each V exon and 5' of each J segment and flanking both sides of each D segment. The RSS are highly conserved stretches of seven or nine nucleotides separated by non-conserved 12 or 23 nucleotide spacers. In a light chain gene, each heptamer or nonamer adjacent to a V exon recognizes a complementary stretch adjacent to a J exon. This allows recombinase to bring the two exons together, forming a loop of intervening DNA.

Enzymes then excise the intervening DNA in this loop and anneal the ends of the V and J exons. Recombination is mediated by recombinase enzymes RAG1 and RAG2. Not all rearrangements are functional.

The other product of V, D and J gene segments is the excised DNA which circularizes via signal joints. PCR of these excision circles, designated B cell recombination excision circle (BREC) can be used to demonstrate that B cells are developing in the bone marrow, e.g., after bone marrow transplantation.
C. Membrane IgM vs. Secreted IgM: Secreted and membrane forms of $\mu$-chain result from alternative RNA splicing

D. Co-expression of membrane IgM and membrane IgD on B cells: IgM and IgD on a given B cell have the same $V_H + V_L$. Given that one cell makes only one antibody, how can $\mu$ and $\delta$ heavy chains be produced simultaneously by the same B cell? The answer is, $\mu$ and $\delta$ chains with the same $V_H$ domain result from alternative splicing of primary transcripts (nuclear RNA).

II. GENERATION OF ANTIBODY DIVERSITY

A. Combinatorial Joining of V, D and J Gene Segments

The germline contains multiple germline $V_H$ and $V_L$ genes that have different sequences and produce Ig molecules with different specificities. D and J gene segments also contribute to diversity.

The somatic recombination of Ig DNA participates in the generation of antibody diversity in several ways. The combinatorial associations of different V, D, and J gene segments lead to a large potential for generating different antibody specificities. The maximum
possible number of combinations is the product of the number of V, D (if present), and J gene segments at each locus. Every clone of B cells and its progeny express a unique combination of V, D, and J genes.

B. **N-region addition:** Nucleotides, called N sequences, which are not present in the germline, can be added to the junctions of rearranged VDJ genes during rearrangement. This addition of new nucleotides is a random process mediated by an enzyme called terminal deoxyribonucleotidyl transferase (TdT).

C. **Combinations of H and L Chain Proteins**

In addition to these mechanisms operative at the level of Ig genes, the combination of different H and L chain proteins also contributes to diversity because the V region of each chain participates in antigen recognition.

II. **B cell selection: Tolerance**

What happens to B lineage cells in the bone marrow that have non-functional V(D)J gene rearrangements or that express self-reactive antibody?

B cells with non-functional V(D)J genes are deleted. B cells with anti-self reactivity can become anergic, can be deleted, or they can be rescued by receptor editing.
V. POLYCLONAL VS MONOCLONAL ANTIBODIES

**EPITOPE:** antigenic determinant; many associated with each protein antigen molecule

Polyclonal antibody is found in the serum of immunized individuals. Each of the different antibodies present in the serum, directed against different epitopes, are made by separate clones of B lymphocytes (plasma cells). Single B cells that make a single kind of antibody (monoclonal) can be immortalized in vitro and used as a continual source of a specific monoclonal antibody.

Anti-μ heavy chain (anti-IgM) can be used to identify B lymphocytes

VI. T CELL ANTIGEN RECEPTORS (TCR)

Most T cells have αβ TCR; ~5% of T cells have γδ TCR

A. **STRUCTURE**

- The αβ TCR is a disulfide linked heterodimer of α and β chains (α = 45 kD; β = 40 kD).
- Each chain has 2 Ig-like domains; The N-terminal domains are variable (V) regions; The C-terminal domains are polypeptide constant regions.
- The overall structure of $\delta\gamma$ TCR is similar to $\alpha\beta$ except that the polypeptide chains are designated $\gamma$ and $\delta$.

B. GENE ORGANIZATION AND REARRANGEMENT

- The overall organization of TCR genes is similar to that of Ig genes.
  - The V regions of $\alpha$ and $\gamma$ chains are encoded by V and J gene segments.
  - The V, D and J gene segments are associated with the same conserved heptamer and monomer nucleotide sequences found in Ig genes. Thus, TCR genes rearrange by the same mechanism as Ig genes.
  - Most importantly - as in B cells, only one VDJ and one VJ gene rearrangement occur in each T cell. Therefore, each T cell expresses a single TCR, specific for one particular antigenic determinant.
1. Identify the approximate number of heavy chain V, D and J gene segments in the germline.

2. Identify the approximate number of light chain V and J gene segments in the germline.

3. Diagram the organization of germline V, D and J heavy chain gene segments and V and J light chain gene segments. (Be sure to identify the exons and introns within each diagram).

4. List the order of Ig H and L chain gene rearrangements.

5. What is the significance of allelic exclusion and describe the mechanism responsible for generating allelic exclusion.

6. State how μ and δ H chain can arise from the same primary transcript.

7. Compare the organization of TCR and Ig genes.

8. Compare the mechanism of rearrangements of TCR genes and Ig genes.

**ADDITIONAL QUESTIONS**

**Indicate whether each of the following statements is true or false:**

1. Immunoglobulins are encoded by genes located on one chromosome.

2. Within one immunoglobulin molecule there may be two types of light chain.

3. The immunoglobulin combining site (for antigen) is contributed by the variable regions of the heavy and light chains.

4. IgG and IgM molecules are distinguished by differences in their heavy chain constant region sequences.

5. Myeloma proteins are the result of polyclonal B cell activation.

6. V gene segments are not joined with J gene segments in cells other than lymphoid cells.

7. Allelic exclusion refers to the phenomenon where only the heavy or light chain is produced by the cell but not both.

8. In pre-B cells, both heavy and light chain genes are rearranged.
ANSWERS TO THE ADDITIONAL QUESTIONS

1. False. Immunoglobulins are encoded by 3 gene families: for heavy chain, kappa and lambda light chains. The 3 gene families each reside on a separate chromosome.

2. False. The light chains of a single antibody are identical and the heavy chains are identical also. Therefore, one immunoglobulin may be either lambda or kappa.

3. True.

4. True.

5. False. Myeloma proteins are homogenous and result from 1 B cell becoming concerous.

6. True.

7. False. Allelic exclusion means that only one allele of each gene (H, and kappa or lambda) is expressed at the protein level - i.e., either the maternal or paternal heavy chain allele is expressed - as well as that for either kappa or lambda. The result of allelic exclusion is the expression by the B cell of only one immunoglobulin and one immunoglobulin specificity (clonal expression).

8. False. Light chain genes are in germline configuration.

EXAMPLES OF TEST QUESTIONS

1. Somatic hypermutation is most active during:
   A. Differentiation of pre-B cells into mature B cells
   B. Differentiation of pre-T cells into mature T cells
   C. Generation of memory B cells
   D. V_{H}, D, J_{H} gene rearrangements
   E. A primary immune response

Match the following Ig gene rearrangements with the appropriate cell type:
A. DJ gene rearrangement on one allele; VDJ gene rearrangement on the other allele; no VJ gene rearrangement

B. VJ gene rearrangements on both kappa-chain alleles.

C. VDJH gene rearrangement on one allele; VJL gene rearrangement on one allele.

D. No VDJ, DJ or VJ Ig gene rearrangements.

1. Plasma cell.
2. Pre-B lymphocyte.
3. T-lymphocyte.

Answers to above questions: 1-C 2-A 3-D
Germline Organization of Ig Genes

Ig Gene Rearrangements

Ig Gene Rearrangements
B Cell Development in Bone Marrow

HSC = hematopoietic stem cell (CD34+)

B cell Development in Bone Marrow

B cell Development in Bone Marrow

B cell Development in Bone Marrow
How Does Ig Gene Rearrangement Occur?

Conserved Recombination Recognition Sequences for RAG-1 and RAG-2

Mechanism of Ig Gene Rearrangement
How to Obtain Secreted IgM and Membrane IgM with the Same VDJ region? & How to Obtain IgM and IgD with the Same VDJ region?

Ans: Alternative RNA Splicing
Co-expression of Membrane IgM and IgD

IgM  IgD

Generation of Antibody Diversity

How large a repertoire do we need?

How, genetically, is such a large antibody repertoire generated?

Generation of the Antibody Repertoire

1. Combinatorial V(D)J gene joining
2. N nucleotide addition by TdT
3. Combinatorial association of H and L chains
What if V(D)J gene rearrangements are non-functional?

Ans: They die or encode self-reactivity?

Elimination of Self-reactive B cells

<table>
<thead>
<tr>
<th>Immature B cell (bone marrow)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multivalent self molecule</td>
</tr>
<tr>
<td>Downregulated or receptor editing</td>
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<tr>
<td>Cleaved B cell</td>
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<th>Soluble self molecule</th>
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<td>Migrates to periphery</td>
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<td>Mature B cell</td>
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<tr>
<th>No self reaction</th>
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<tbody>
<tr>
<td>Migrates to periphery</td>
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<tr>
<td>Mature B cell</td>
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</tbody>
</table>

A Second Chance for Self-reactive B cells:

- Receptor Editing
  - Strong ligation of IgM by self antigen
  - Arrest of B-cell development and continued light-chain rearrangement; low-cell surface IgM
Immature B Cells Leave Bone Marrow to Periphery

Immune Response to the Antigen

POLYCLONAL vs MONOCLONAL ANTIBODIES

- Protein Antigens have many epitopes
- B cells make Ab to a single epitope
- Different clones of B cells make Ab to different epitopes

H-chains Distinguish Ig Isotypes

Anti-μ, Anti-γ, Anti-α, etc
Structural Similarity of TCR and Ig

Organization of TCR Genes

Rearrangement of TCR Genes
Germline Organization of Ig Genes

Fig 4.4 © 2001 Garland Science

Ig Gene Rearrangements

Ig Gene Rearrangements

Ig Gene Rearrangements
B Cell Development in Bone Marrow

HSC = hematopoietic stem cell (CD34+)

B cell Development in Bone Marrow

HSC

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A Second Chance for Self-reactive B cells: Receptor Editing
Receptor Editing of Anti-self VJ gene rearrangements

B Cell Development in Bone Marrow
Immature B Cells Leave Bone Marrow to Periphery

Periphery

Antigen

Immune Response to the Antigen

POLYCLONAL vs MONOCLONAL ANTIBODIES

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H-chains Distinguish Ig Isotypes

Anti-μ, Anti-γ, Anti-α, etc
Structural Similarity of TCR and Ig

Organization of TCR Genes

Rearrangement of TCR Genes

T cell Rearrangement Excision Circles (TRECS)
MAJOR HISTOCOMPATIBILITY COMPLEX

Date: 3/21/13
Reading Assignment: Janeway’s Immunobiology, 8th Edition, pp. 141-146, 217-223

Figures: (Unless otherwise noted) Janeway’s Immunobiology, 8th Edition, Murphy et al., Garland Publishing.

KEY CONCEPTS AND LEARNING OBJECTIVES

You will be able to describe the major histocompatibility complex and to identify the structural and genetic nature of the complex.

To attain competence for this lecture you will be able to:

a. Identify the loci of the human major histocompatibility complex.
b. Compare and contrast the structure of the class I and class II loci molecules.
c. Identify the tissue distribution of the class I and class II MHC loci antigens.
d. Describe the genetic inheritance of MHC genes.
e. Identify the HLA loci important in cell mediated cytotoxicity.
f. Define MHC restriction.
CONTENT SUMMARY

Introduction

HLA Complex
  Class I Loci
  Class II Loci

Structure of MHC Products

Cell Surface Expression of MHC Class I and II Molecules

Co-Dominant Expression of MHC Antigens

Cellular Interactions

T Cytotoxic Lymphocyte Reactions with Target Cells
INTRODUCTION

- The Major Histocompatibility Complex (MHC) is a set of closely linked genetic loci (genes of the MHC) that have been found to be overwhelmingly important in determining the fate of engrafted tissue. This set of linked loci is highly polymorphic and plays a central role in control of the cellular interactions responsible for physiological immune responsiveness.

- There are two main types of MHC gene products (designated Class I and Class II molecules) and their physiological function is to present peptides to T lymphocytes. They accomplish this by sampling intracellular pools of peptides and presenting these peptides, at the cell surface, to T lymphocytes.

HLA COMPLEX

Humans (and other mammals) have a tightly linked gene cluster of cell surface glycoproteins that regulate immune cell interactions and evoke intense allograft rejection. In humans, the human leukocyte antigens (HLA) consist of three types of genetic loci: (See Figure 1.)

Class I Loci

- HLA-A
- HLA-B
- HLA-C

These molecules are present on virtually all nucleated cells and present antigen to CD8+ T lymphocytes.

Class II Loci

- HLA-D Subset loci: (DR, DQ, DP)

These molecules are found on dendritic cells, B-lymphocytes, and macrophages and present antigen to CD4+ lymphocytes.

Class III Loci

- These are genes that happen to reside in the MHC region but do not present antigen to T lymphocytes.
Figure 1. The HLA complex is found on the short arm of chromosome 6. The murine MHC gene structure is presented solely for reference purposes.

STRUCTURE OF MHC (MAJOR HISTOCOMPATIBILITY COMPLEX) PRODUCTS

Class I Loci Molecules (A, B, C of humans).

- Each of the loci codes for a polypeptide chain of about 44,000 daltons.
- On cell membranes these chains are associated non-covalently with a lighter chain, $\beta_2$ microglobulin (12,000 daltons), which is specified by a gene on another chromosome.
- The resulting cell surface molecule has one alpha chain, the Class I polypeptide, and one light chain $\beta_2$ microglobulin (see Figure 2).
Figure 2. Structure of an MHC Class I Molecule.

Figure 2. Legend.

a. Space filling model of a Class I human MHC (HLA) molecule.

b. Diagrammatic crystalline structure of a Class I HLA molecule (side view). The extracellular part of the molecule is depicted in side view with the portion distal to the cell membrane on top and the portion proximal to the membrane at the bottom. The transmembrane and intra-cytoplasmic domains are not shown. The \( \beta_2 \) microglobulin (\( \beta_2 \text{m} \)) and \( \alpha_3 \) domains support an interactive structure formed by the \( \alpha_1 \) and \( \alpha_2 \) domains. This interactive structure consists of a \( \beta \)-pleated sheet platform supporting two \( \alpha \) helices,
which form a cleft that binds antigenic peptide fragments. β strands are shown as broad arrows: α helices are shown as ribbon-like structures. N indicates the amino terminus.

c. Schematic representation of a view from above of the peptide binding cleft.

d. The molecule consists of a MW 44,000 polymorphic transmembrane glycoprotein termed the α chain, which bears the antigenic determinant in non-covalent association with a MW 12,000 non-polymorphic protein termed β2 microglobulin. The α chain has three extracellular domains termed α1, α2, and α3.

- β2 microglobulin is the same in all of these molecules, but α chains, specified by different alleles, differ in amino acid sequence.
- Carbohydrates constitute approximately 10% of the weight of the α chain.
- The C-terminal fragment of the α chain traverses the cell membrane.
- Parts of the α chain and β2 microglobulin resemble immunoglobulin chains, in particular the amino acid sequence of C_H domain of immunoglobulin chains.

Class II Loci molecules (D of humans)

Each of the loci codes for a polypeptide chain of about 60,000 daltons with two polypeptide chains per molecule:

- (34,000) - α chain
- (29,000) - β chain

A model of a Class II molecule is depicted in Figure 3.

MHC class I molecules bind short peptides of about 9 amino acids in length (variability is 8-10 amino acids).

MHC class II molecules bind longer peptides of 13-17 amino acids in length.

Comparison of peptides bound by MHC class I and class II molecules in Figures 4, 5, and 6.
Figure 3. Structure of an MHC Class II Molecule.

Figure 4. Peptides bound to MHC class I and MHC Class II Molecules. Panels a, c = MHC class I. Panels b, d = MHC class II.
The anchor residues (circled) that bind a particular MHC class I molecule in Figure 5 do not need to be identical but are always related. For example, valine (V), leucine (L), and Isoleucine (I) are all hydrophobic amino acids. Peptides also bind class I MHC molecules through their amino and carboxy termini.

The anchor residues (circled) that bind a particular MHC class II molecule in Figure 6 do not need to be identical but are always related. In this case, the first anchor residue (circled and on the left of the figure) is hydrophobic, the next anchor residue is negatively charged, the next
exhibits a tendency to be a basic amino acid, and for the last anchor residue a hydrophobic amino acid (on the right of the figure).

**CELL SURFACE EXPRESSION OF MHC CLASS I AND II MOLECULES**

MHC class I α chains and MHC class II α chain and β chains are encoded by separate genes of the MHC locus. (β₂ microglobulin is not encoded within the MHC region of chromosome 6.) These genes encode for proteins that associate in the endoplasmic reticulum and shuttle to the cell surface where they are membrane glycoproteins. See Figure 7.

**Figure 7.** MHC genes encoding MHC molecules on the surface of nucleated cells.

**CO-DOMINANT EXPRESSION OF MHC ANTIGENS**

- Each individual expresses in a co-dominant fashion the class I and class II genes of both chromosomes 6.

- Thus each individual expresses 3 maternal and 3 paternal class I molecular types, as well as 3 maternal and 3 paternal class II molecular types (on cells that express both class I and class II).
Each individual has two “half sets” (haplotypes) of genes. One haplotype is inherited from each parent. Both of these haplotypes are expressed equally (see Figure 8).

**Figure 8.** Codominant expression of MHC alleles.

With the exception of the DR α locus the number of different alleles (the variant genes that can occupy the locus) for class I and class II MHC genes is very large. See Figure 9.

The high degree of polymorphism in nucleotide sequence results in a high degree of polymorphism in amino acid sequence. The polymorphisms are located at specific sites within the MHC molecules. See Figure 10. The differences in amino acid sequence allow for peptide binding and for T lymphocyte recognition.
Figure 9. Number of alleles identified for MHC genes.

Figure 10. Site localization of allelic variation with MHC molecules.
CELLULAR INTERACTIONS

- A specific T cell response to antigen (Ag) on cell surfaces depends not on recognition of the Ag alone but on recognition of an antigenic peptide in the groove of an MHC molecule on the cell surface.

- Cytolytic T lymphocytes (CD8+) are specific for foreign Ag plus products of class I loci, whereas T helper (CD4+) lymphocytes are specific for foreign Ag plus products of the class II loci.

- The dependence of the T cells specific reactivity on foreign Ag plus MHC products rather than on foreign Ag alone is called MHC Restriction.

MHC Restriction - Lymphocytes typically interact with foreign antigen recognized by the lymphocyte in the context of host (self)-MHC molecules. Cellular reactions in which such MHC interactions are important include:

- The cytolysis of target cells by cytolytic T lymphocytes.

- T lymphocyte-antigen presenting cell (macrophages, dendritic cells, B lymphocytes) interactions associated with T lymphocyte production of cytokines.

T CYTOTOXIC LYMPHOCYTE REACTIONS WITH TARGET CELLS

- Cytotoxic T lymphocytes (CD8+) are elicited by host cells that carry foreign Ags, (e.g., virus infected cells). See Figure 11.

- The specificity of the T killer cells is for the viral peptide in the context of a host cell's class I determinant. See Figure 12.

Figure 11. Cytotoxic T lymphocytes recognize antigen in the context of class I MHC molecules.
STUDY QUESTIONS

1. Describe the genes that constitute the major histocompatibility complexes.

2. Each person has 2 haplotypes, with codominant genetic expression. Explain the statement.

3. Compare class I and class II gene products.

4. What is MHC restriction?
EXAMPLE OF TEST QUESTION

The human leukocyte antigen complex (HLA):

A. Elicits graft rejection.
B. Restricts immune responses.
C. Is the major histocompatibility complex for humans.
D. Functions physiologically to present antigen.
E. All of the above.

CORRECT ANSWER TO ABOVE QUESTION: E
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Class III Loci
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Codominant Expression of MHC Alleles

Maternal Chromosome Gene structure of the human MHC

Paternal Chromosome Gene structure of the human MHC

Human MHC Genes are Highly Polymorphic

Figure 6.10: Immunobiology, 6th (Garland Science 2012)
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What is MHC restriction?