ANTIGEN-RECEPTOR GENES & B CELL DEVELOPMENT

Date: Friday, March 18, 2011   Monday, March 21, 2011
10:30 AM – 11:30 AM   10:30 AM – 11:30 AM

LEARNING GOAL
You will be able to diagram and describe the stages of B cell development including somatic DNA rearrangements, and explain 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:

• State the approximate size of antibody repertoire needed for immune protection.
• Diagram the order of H and L chain gene rearrangements that occur during B cell development.
• 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, junctional diversity, and N segment addition.
• Describe how antibody diversity is generated in secondary lymphoid tissues
• Describe the maturation of B cells from stem cells, including the pre-B cell receptor.
• State the cell origin of CLL, Burkitt’s lymphoma, Hodgkin’s lymphoma, ALL, and multiple myeloma
• 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 (2008), Chapter 4; Chapter 7, pp 262-272; 308-312

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

Introduction
Genetic basis for the antibody repertoire

I. 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

II. Ig gene rearrangements - basic features
   B-cell development
   Order of rearrangements
   Heavy chain: DJ, VDJ
   Light chain: VJ
   ProB, PreB, Immature B and Mature B cells
   Allelic exclusion - only one V\(_{H}\) and one V\(_{L}\) rearranged/B cell
   Mechanism of Ig gene rearrangement
   Conserved heptamer/nonamer (RSS) recognition sequences
   Membrane IgM vs. secreted IgM
   Surface IgM and IgD

III. Generation of Antibody Diversity in bone marrow
   Combinatorial joining of V, D and J gene segments
   Junctional diversity- N-region addition and imprecise joining
   Combinations of H and L chain proteins
   Selection against self-reactive B cells

IV. Generation of antibody diversity in secondary lymphoid tissues
   Somatic mutation

V. B-lineage tumors: Leukemia and lymphoma
   Chromosomal translocations

VI. T cell antigen receptor
   Structure
   Gene organization and rearrangement
INTRODUCTION

Problem: One can estimate that an individual needs to be able to make antibodies to recognize about one million epitopes. What is the genetic basis for being able to generate so many different antibodies?

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. GERMLINE ORGANIZATION OF IMMUNOGLOBULIN GENES

Three groups of Genes: $\kappa$, $\lambda$, 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 $\kappa$, $\lambda$ and H chains are located on separate chromosomes. Each set of genes, $\kappa$, $\lambda$ and heavy chain, has a similar basic organization.

A. 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 ($\kappa$ or $\lambda$), 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.

B. 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 (Cβ) 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.

II. 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. B Cell Development

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.

**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 μ-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, μ-chain, Igα and Igβ.

**Immature B Cells:** At the next identifiable stage in B cell maturation, κ or λ light chains are also produced. These associate with μ 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 \( \mu \) and \( \delta \) heavy chains in association with the original \( \kappa \) or \( \lambda \) 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.

**B. 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.

**C. Mechanism of Ig gene rearrangement**

- Conserved recognition sequences
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 junctions are in CDR3.

Membrane IgM vs. Secreted IgM: Secreted and membrane forms of μ-chain result from alternative RNA splicing.
Co-expression of membrane IgM and membrane IgD on B cells: IgM and IgD on a given B cell have the same idiomorph (VH + VL). Given that one cell makes only one antibody, how can μ and δ heavy chains be produced simultaneously by the same B cell? The answer is, μ and δ chains with the same VH domain result from alternative splicing of primary transcripts (nuclear RNA).

III. GENERATION OF ANTIBODY DIVERSITY IN BONE MARROW

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

The germline contains multiple germline VH and VL 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.

Even the same set of germline V, D, and J gene segments can generate different amino acid sequences at the junctions. This junctional diversity can arise from imprecise joining or N-region addition.
B. **Imprecise joining**: or imprecise DNA rearrangement occurs because nucleotide sequences at the 3’ end of a light chain V gene segment and the 5’ end of a J gene segment or at the ends of V, D, and J gene segments in a heavy chain can each recombine at any of several nucleotides in the germline sequence.

C. **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 deoxyribonucleotide transferase (TdT).

Because of junctional diversity (due to imprecise joining and N-region addition), the number of different amino acid sequences present in CDR3 of antibody molecules is much greater than the number of germline V and D segments present in the genome (Table 1).

D. **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.

### Table 1. Mechanisms Contributing to the Generation of Antibody diversity in the Human

<table>
<thead>
<tr>
<th></th>
<th>H</th>
<th>κ</th>
<th>λ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germline genes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V gene segments</td>
<td>40</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>J segments</td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>D segments</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Combinatorial joining</td>
<td>6,000</td>
<td>200</td>
<td>120</td>
</tr>
<tr>
<td>V x J (X D)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-L chain associations</td>
<td></td>
<td>1.2 x 10^6</td>
<td></td>
</tr>
<tr>
<td>H x κ</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.
IV. GENERATION OF ANTIBODY DIVERSITY IN SECONDARY LYMPHOID TISSUES

A. Somatic hypermutation: Changes in antigen-binding specificity as a result of somatic mutations in V genes can generate additional diversity.

When does somatic hypermutation (diversification) occur?
- Pre B and early B cells utilize unmutated germline V genes.
- Primary antibody response utilizes mostly unmutated V genes.
- Secondary antibody response utilizes mostly V genes that have undergone somatic mutation.

B. The enzyme, AID, is required for somatic hypermutation. AID deficient patients have only IgM and the Ig genes are not somatically diversified.
V. B-LINEAGE TUMORS

Individual B-lineage cells in BM or periphery can undergo neoplastic transformation giving rise to leukemia or lymphoma.

<table>
<thead>
<tr>
<th>B-lineage tumors</th>
<th>Normal cell equivalent</th>
<th>Location</th>
<th>Status of Ig V genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute lymphoblastic leukemia</td>
<td>Lymphoid progenitor</td>
<td>Bone marrow and blood</td>
<td>Unmutated</td>
</tr>
<tr>
<td>Pre-B-cell leukemia</td>
<td>Pre-B cell</td>
<td>Pre-B receptor</td>
<td>Unmutated</td>
</tr>
<tr>
<td>Mantle cell lymphoma</td>
<td>Resting naive B cell</td>
<td></td>
<td>Unmutated</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia (CLL)</td>
<td>Activated or memory B cell</td>
<td></td>
<td>Usually unmutated</td>
</tr>
<tr>
<td>Follicular center cell lymphoma Burkitt’s lymphoma</td>
<td>Mature memory B cell</td>
<td>Peripheral</td>
<td>Mutated, intracranial variability</td>
</tr>
<tr>
<td>Hodgkin’s lymphoma</td>
<td>Germinal center B cell</td>
<td></td>
<td>Mutated +/- intracranial variability</td>
</tr>
<tr>
<td>Waldenström’s macroglobulinemia</td>
<td>IgM-secreting B cell</td>
<td></td>
<td>Mutated, no variability within clone</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>Plasma cell, Various isotypes</td>
<td>Bone marrow</td>
<td>Mutated, no variability within clone</td>
</tr>
</tbody>
</table>

Chromosomal translocation of c-myc into the IgH locus leads to B cell tumor.
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 δγ TCR is similar to αβ except that the polypeptide chains are designated γ and δ.

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.

**Comparison of Gene Rearrangements in B and T Cells**

<table>
<thead>
<tr>
<th>Event</th>
<th>Process</th>
<th>Nature of change</th>
<th>Process occurs in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B cells</td>
<td>T cells</td>
</tr>
<tr>
<td>V-region assembly</td>
<td>Somatic recombination of DNA</td>
<td>Irreversible</td>
<td>Yes</td>
</tr>
<tr>
<td>Junctional diversity</td>
<td>Imprecise joining, N-sequence insertion in DNA</td>
<td>Irreversible</td>
<td>Yes</td>
</tr>
<tr>
<td>Transcriptional activation</td>
<td>Activation of promoter by proximity to the enhancer</td>
<td>Irreversible but regulated</td>
<td>Yes</td>
</tr>
<tr>
<td>Switch recombination</td>
<td>Somatic recombination of DNA</td>
<td>Irreversible</td>
<td>Yes</td>
</tr>
<tr>
<td>Somatic hypermutation</td>
<td>DNA point mutation</td>
<td>Irreversible</td>
<td>Yes</td>
</tr>
<tr>
<td>IgM, IgD expression on surface</td>
<td>Differential splicing of RNA</td>
<td>Reversible, regulated</td>
<td>Yes</td>
</tr>
<tr>
<td>Membrane vs secreted form</td>
<td>Differential splicing of RNA</td>
<td>Reversible, regulated</td>
<td>Yes</td>
</tr>
</tbody>
</table>
STUDY QUESTIONS

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. State the number of C\textsubscript{H} genes and their relationship to Ig subclasses.
5. List the order of Ig H and L chain gene rearrangements.
7. What is the significance of allelic exclusion and describe the mechanism responsible for generating allelic exclusion.
8. 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.
9. Describe how the hepatamer/nonamer signal sequences (RSS) mediate V, D and J gene rearrangements.
10. State the role of RNA processing in synthesis of Ig L chains.
11. For the light chain genes, compare the intervening nucleic acid sequences that are lost during DNA recombination with those lost during RNA splicing.
12. Diagram the sequence of events in B cells that lead to the production of L chains; start with rearrangement of L chain genes.
13. State how \(\mu\) and \(\delta\) H chain can arise from the same primary transcript.
14. Compare the organization of TCR and Ig genes.
15. Compare the mechanism of rearrangements of TCR genes and Ig genes.
16. State the approximate number of antibody specificities needed for protection.
17. State the approximate number of germline V\textsubscript{H}, V\textsubscript{L}, D, J\textsubscript{H}, and J\textsubscript{L}, gene segments.
18. Explain the mechanisms by which CDR3 becomes more variable than CDR1 and CDR2.

23. Compare the contribution of somatic mutation to the diversity of Ig receptors with the diversity of T cell receptors.

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 hypervariable regions within each of the heavy and light chain variable regions.
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. VDJ_H gene rearrangement on one allele; VJ_L 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
LEARNING GOAL
Understanding in vitro assessment of immunologic responses that have contemporary clinical relevance

BACKGROUND READING
Janeway: Most of the concepts are developed in the text of the Notes, Dr Knight’s lectures, and also the Letter to the Editor of the New England J of Medicine posted on the HD site.

DEVELOPED BY
John A. Robinson, M.D.
I. Active and Passive Immunity

A. Introduction

1. Many life-threatening infections are caused by the release of protein toxins by bacteria after they have infected a patient. *Diphtheria* and *tetanus* are two prime examples. A patient will be protected against the toxin effects if specific antibodies against the toxin are present at the time of infection.

2. Protective antibodies arise during actual infection or can be induced by vaccines.

3. The following vignettes underscore the need to understand basic immune responses and their timing in order to successfully treat some serious infections.

4. Implementation of an immunologic treatment strategy must be based on understanding of how specific antibody responses are induced and how long they are effective.

5. **For the purposes of this Small Group, do not worry about the complex cellular interactions required for antibody formation - this comes later but be sure you understand the CONCEPTS of primary and secondary immunization and active versus passive immunization.**

B. The following graph illustrates the temporal sequence of antibody formation to an antigen (or infection).

![Fig. 1.20 The course of a typical antibody response](image)

*Fig. 1.20 The course of a typical antibody response.* First encounter with an antigen produced a primary response. Antigen A introduced at time zero encounters little specific antibody in the serum. After a lag phase, antibody against antigen A (blue) appears; its concentration rises to a plateau, and then declines. When the serum is tested for antibody against another antigen, B (yellow), there is none present demonstrating the specificity of the antibody response. When the animal is later challenged with a mixture of antigens A and B, a very rapid and intense secondary response to A occurs. This illustrates immunological memory, the ability of the immune system to make a second response to the same antigen more efficiently and effectively, providing the host with a specific defense against infection. This is the main reason for giving booster injections after an initial vaccination. Note that the response to B resembles the initial or primary response to A, as this is the first encounter of the animal with antigen B.
C. The temporal lag in appearance of specific antibody in the patient's peripheral blood during a primary immune response to an infectious pathogen is a critical problem. The early period of little, if any, significant antibody production is a time of extreme vulnerability. Antibiotics provide some protection while the immune system gears up for antibody synthesis which then provides active immunity.

D. The significant decline of serum antibody concentration over a 4-6 week period after an immune stimulus emphasizes the importance of memory in adaptive immune responses. The memory pool of immune cells that remember the first infection prime the system for a rapid, specific response to the same pathogen. This is designated a secondary immune response.

E. The graph also illustrates the rapidity of a secondary response to the same antigen that the individual had originally been vaccinated with. Not only is there is no "window" of opportunity for organism and its toxin to escape antibody neutralization after the second antigen exposure but serum antibody levels are maintained thereafter for a much longer period of time.

F. Vaccines are designed to mimic active immunity that would have been triggered by an actual infection. Use of a vaccine before the actual infection provides the host with the ability to respond with a rapid secondary response that abbreviates the vulnerable period of no antibody formation during early infection.

G. Modern biotechnology now provides a way for the physician to provide "instant" specific antibodies to an infected or potentially infected patient. In this case, the physician, using epidemiologic and clinical data, must be able to predict the organism that the patient is most likely infected with and then passively immunize the patient by infusing the specific, premade antibody into the patient at the time of the infection. The drawback to passive immunization is that the duration of protection against the infection is defined by the half-life of the infused antibody which is about 3 weeks. No active immunization occurs.

H. Apply the basic concepts of primary and secondary immune responses and active and passive immunity to the clinical examples below (You do not have to read about the specific diseases to understand the concepts). What type of immunity, if any, do is needed to treat the following 2 patients?

Rev 12/13/2010
a. A twenty year old on active military duty arrives at your office with a one inch long, very superficial laceration of the forearm caused by a dog bite. The dog belonged to his neighbor and was able to be confined and observed for clinical rabies. The real concern is whether the patient could die from tetanus toxin if a tetanus infection transpired. Tetanus bacteria only grow well in situations where there is reduced oxygen. The wound was easily cleaned and did not require sutures.

b. A 23 year old mountaineer, who arrived in town only last week, shows up in the ER with a lacerated, swollen and tender swelling of the right gluteus muscle. The wound has dirt and leafy debris embedded in it. The patient was injured "escaping from an angry bear". He lived in a “holler” his entire life and has never been to a doctor before. Knowing what you do about tetanus from the first clinical scenario, what immunologic concepts would have to be applied in this instance?

I. Now apply your general understanding of immune responses to a current problem. If the *anthrax organism* gains access to a host environment that allows it to move from the spore form to a toxin producing phase, the patient may die very rapidly because of the severe systemic effects of its toxins. Anthrax produces three exotoxins. Two of them, after gaining access to the interior of host macrophages and monocytes, kill them. The dead cells release massive amounts of molecules that have potent and ultimately deadly effects on coagulation and cardiovascular responses. The other toxin, called a shuttle vehicle, delivers the first 2 toxins to the host cells.

a. Predict and discuss what type of immunity must be generated and the most rational strategies to provide immunologic protection against anthrax.

**J.** A large group of African patients are known to have survived Ebola virus infection. This is a rapidly progressive infection with a very high fatality rate and currently there is no vaccine available for it. Your research lab in Kenya is studying antibody responses to Ebola and has large amounts of plasma from Ebola survivors.

a. If Ebola virus infection broke out in your lab, what type of immunity would have to be invoked and how could you do it?

**K.** The slide below depicts a real-life application of the concepts you have just discussed. The entire letter to the editor is posted on the Host Defense Website. The clinical problem was that a patient in southern China developed a life threatening case of avian influenza. The virus had caused similar cases in the region but they were not all fatal. Plasma (which will contain antibodies only, no leucocytes) from a survivor was infused into the patient. Interestingly, the antibody levels to the avian influenza virus remained high for 6 months after the patient recovered. *What type of immunity* was operative in
II. Immunologic Assays used for detection and assessment of antibody responses that students, residents and attending physicians have to interpret frequently

A. General Principles

1. What does the term “antibody titer” mean?
   a. Titer is an old but still frequently used term that serves a purpose because it provides a sense of the amount of antibody to the antigen measured in the patient’s serum or whatever biological fluid measured; for example, spinal fluid, urine, pleural fluid. Many new clinical antibody assays now express the titer concept in “units”.

   b. The principle is: the greater the amount of antibody in the serum, the further the serum can be diluted and antibody still be detected.

   c. How is a “titer” determined? The following is an example of the basic procedure.
Prepare serial twofold dilutions of the serum to be tested for antibody to the antigen ranging from 1:10 to 1:640 in 0.5ml amounts as shown below.

Begin with 1.0 ml of a 1:10 dilution of serum in well 1 and 0.5 ml of saline in wells 2 through 7. Remove 0.5 ml from well 1, place it in well 2, and mix. You now have 1.0 ml of a 1:20 dilution in well 2. Next, take 0.5 ml of the 1:20 dilution and mix it with the 0.5 ml of saline in well 3. This gives 1.0 ml of a 1:40 dilution of serum in well 3 and leaves 0.5 ml of a 1:20 dilution in well 2. Repeat this procedure for wells 4, 5, 6, and 7 to give serial twofold dilutions in the row of wells.

d. Study the response to an influenza virus infection shown below. The usefulness of the titer concept should be evident. At the time of infection, the patient had no specific antibody to the virus and became ill. The response of the immune system can be tracked by the rising titers to the virus. By 2 weeks the titer was ~1/100 and at 3 weeks it peaked at 1/500. A year later, the patient still had residual amounts of specific antibody but at a much lower titer.

e. Titers can be very helpful in several ways:

1. If, at the beginning of an infection, a patient already has IgG titers to the suspected virus, you know that the suspected virus is not the cause of the current illness but that the patient, sometime in the past, has been infected with it.
2. If, at the beginning or during the clinical expression of an infection, no anti-viral IgG can be detected but a titer of IgM specific for the virus is detected, it is very likely the patient has been infected very recently with the virus in question.

1. Questions

   a. Three patients (A, B, C) have the following symptoms: chills, fever, muscle aches and cough (all typical of a “flu-like” syndrome)
      i. “A” symptoms are markedly better but he came to clinic anyway about 18 days after he got sick.
      ii. “B” has had the symptoms for about 48’
      iii. “C” has been sick for 2 weeks

The physician suspects they all have (or had) Virus X and draws blood from them at the time of their visit (Day 1), 3 weeks later (Day 21) and then a year later (Day 365) and has them tested for IgM and IgG antibodies to Virus X. Appropriate positive (known serum positive for antibodies to X) and negative (known serum negative for X antibodies) controls were done.
Results:

<table>
<thead>
<tr>
<th></th>
<th>D1</th>
<th>D21</th>
<th>D365</th>
</tr>
</thead>
<tbody>
<tr>
<td>“A” IgM</td>
<td>1:10</td>
<td>1:2</td>
<td>Negative</td>
</tr>
<tr>
<td>IgG</td>
<td>1:100</td>
<td>1:300</td>
<td>1:20</td>
</tr>
<tr>
<td>“B” IgM</td>
<td>1:20</td>
<td>1:50</td>
<td>Negative</td>
</tr>
<tr>
<td>IgG</td>
<td>Negative</td>
<td>1:300</td>
<td>1:10</td>
</tr>
<tr>
<td>“C” IgM</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>IgG</td>
<td>1:50</td>
<td>1:50</td>
<td>1:50</td>
</tr>
</tbody>
</table>

Did everyone have virus X and, if they did, when?

B. The ELISA test has become a common way to screen sera for specific antibodies. As a general rule, it is highly sensitive to the detection of antibodies, but may have low specificity for a specific pathogen. Clinically, a negative ELISA is very helpful because it effectively excludes the presence of the infection being considered (assuming the patient is able to make antibodies). A positive ELISA however almost always prompts more specific immunologic testing to validate the ELISA result.

1. ELISA (enzyme linked immunosorbent assay) exploits the ability of certain enzymes to form color compounds after reacting with specific substrates. These enzymes can be linked to antibody probes. The enzyme tagged antibodies are then incubated in systems where the antigen in question has already been bound to a surface (usually a plastic well). If a specific reaction occurs, the colorless substrate specific for the enzyme that was used as a tag on the probe antibody is then added and a color reaction will develop if the tagged antibody had bound to its antigen and the color reaction can be quantitated.
2. The **ELISA method** is reaction of a specific antigen (viral, bacterial, etc.) bound to a plastic surface with an unknown serum to determine if the serum contains specific antibodies to the antigen in question. After excess serum is washed away, enzyme linked **antihuman Ig** is added to detect any bound patient Ig to the antigen. After washing again, a **substrate** specific for the enzyme that had been tagged onto the antihuman Ig is added. If specific antihuman Ig plus patient Ig plus antigen X is present, color develops. Several variations of this test can be used clinically but the principle remains the same.

3. An example of the ELISA “**problem**”: A medical student draws blood from a combative and agitated patient in the emergency department. During the procedure the patient knocked the syringe out of the student’s hand and the needle tip punctured her forearm. The patient’s blood was sent to Pathology for HIV testing. An ELISA assay was done (see below) and was “indeterminant.”
4. A Western Blot of the patient’s serum was then done. Be sure you understand why a Western Blot is necessary. To do this you must understand the concepts of sensitivity and specificity of laboratory assays.

5. IMMUNOBLOT - WESTERN BLOT ANALYSIS

   a. This test exploits the ability of an electrical charge to ‘pull’ a mixture of denatured antigens through a porous gel. Smaller antigens migrate rapidly through the gel; larger ones migrate slowly or not at all. After a fixed time under the influence of the electrical field, the migrated antigen bands are then transferred by direct apposition to a membrane (usually electrostatically) and can then be localized and identified with specific antiserum layered near the membrane. The antigen-antibody reactions are then visualized by using a developing agent that usually is antihuman Ig and a chromogenic substance. Substituting an unknown (patient serum) as the source of viral specific Ig and then comparing the specific bands to known antigen controls is a sensitive test for specific antibody.
b. Western blot analysis is used to confirm the presence of anti-HIV antibody in a patient who has a positive ELISA assay for HIV antigens. The sequence here would be: HIV antigen mixture plus electrophoresis results in migrated HIV proteins. Add patient sera to be tested and a positive anti-HIV control, incubate with antihuman Ig and a colorimetric compound, which then is developed for confirmation of a specific antibody response to HIV.
MAJOR HISTOCOMPATIBILITY COMPLEX

Date: 3/24/11

Figures: (Unless otherwise noted) Janeway’s Immunobiology, 7th 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 ($\beta_2$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.

Text Figure 3.16

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.

Text Figure 3.17
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.

![Diagram of MHC genes encoding MHC molecules on the surface of nucleated cells.](image)

**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 $\alpha$ 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**.

![Cytotoxic T cell recognizes complex of viral peptide with MHC class I and kills infected cell](image)

**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
Antigen Presentation and Cellular Activation

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Table I. Antigen Presenting Cells (APCs).

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Location</th>
<th>Phagocytic</th>
<th>Class II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mononuclear phagocytes</td>
<td>Blood, liver, spleen tissues</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>(Macrophages)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dendritic cells</td>
<td>Skin, lymphoid tissues</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>B lymphocytes</td>
<td>Lymphoid tissues, sites of immune reactions</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Liver-Kupffer cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain- Microglial cells</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cell | Activated function
--- | ---------------------
Macrophage | Phagocytosis and activation of bactericidal mechanisms, Antigen presentation
Liver-Kupffer cells
Brain- Microglial cells
Skin - Langerhans’ cells
T lymphocyte area of lymph nodes – interdigitating dendritic cells
B lymphocyte area of lymph nodes - follicular dendritic cells

B lymphocytes
Antigen Processing

Summarized

Antigen Processing, Class I Summarized

Antigen Processing, Class II Summarized

Summarized Class II Antigen Processing
MHC Class I Antigen Processing

Detailed Class I Antigen Processing

Peptides produced in the cytosol are transported into the endoplasmic reticulum

Cytosol

Endoplasmic reticulum

Transporter associated with Antigen Processing (TAP)

LMP2 and LMP7

Proteasome

Peptide fragments

Proteasome
Chaperones: calnexin, Erp57, calreticulin

Partly folded MHC class I α, chains bind to calnexin until β2-microglobulin binds

MHC class I α,chains in complex is released from calnexin, binds a complex of chaperone protein (β2-microg.) and TAP (transporter for antigen presentation) and binds to TAP via tapasin

Cytosolic proteins and defective ribosomal products (DRPs) are degraded to peptide fragments by the proteasome, TAP delivers peptides to the ER

A peptide binds the MHC class I molecule and completes its folding. The MHC class I molecule is released from the TAP complex and exported to the cell membrane

Virus
MHC Class II Antigen Processing

1. Antigen is taken up from the extracellular space into intracellular vesicles.
2. In early endosomes of neutral pH, endosomal proteases are inactive.
3. Acidification of vesicles activates proteases to degrade antigens into peptide fragments.
4. Vesicles containing peptide-antigen complexes bind with vesicles containing MHC class II molecules.

Intracellular events:

- Invariant chain (Ii) forms a complex with MHC class II molecule, blocking the binding of peptides and misfolded proteins.
- It is cleaved in an acidified endosome, leaving a short peptide fragment, CLIP, still bound to the MHC class II molecule.
- Endocytosed antigens are degraded in endosomes, but the CLIP peptide blocks the binding of peptides to MHC class II molecules.
- MHCII-DPB binds to the MHC class II molecule, releasing CLIP and allowing other peptides to bind. The MHC class II molecule then travels to the cell surface.
MHC Class II Restricted Presentation of Antigen to T Lymphocytes
CD4+ T lymphocytes do not recognize free or soluble antigens.

Rather, they recognize antigen on the surface of APCs in the context of class II molecules.

The interaction between T lymphocytes and antigen and the class II molecule is highly specific and will result in T cell proliferation and differentiation.

The CD4 subset of T lymphocytes contains effectors for:

- Macrophage activation - designated Th1.
- Antibody synthesis - designated Th2.
Activation of T Cells by Interaction with Antigen Presenting Cells That Do Not Express B7
Cellular Reactions Required for Production of Antibodies

- CD28
- CD28
- TCR
- TCR
- B7
- B7
- MHC II
- MHC II
- CD40
- CD40
- CD40 Ligation
- CD40 L expression, no ligation
- B cell proliferation and differentiation
- B cell proliferation and differentiation
- Plasma cells

Immune Synapse

page 13-14
Complex functions, such as T cell proliferation, require TCR engagement and signaling for many minutes or hours. The mechanism of sustained TCR engagement is the formation of a specialized contact, termed the immunological synapse. The formation of the immunological synapse provides a mechanism for sustained TCR engagement and signaling.
Immune Synapse

Inner circle (green)  Surrounding ring (red)

TCR, CD4, CD28  LFA-1::ICAM-1
STUDY QUESTIONS

In order for T-cell recognition and activation to occur, a T cell needs to encounter a foreign antigen in association with an MHC protein. It would seem feasible for a single type of MHC protein to suffice for this purpose; however, as transplant surgeons have found to their dismay, many different MHC proteins exist in humans. Why are MHC proteins so polymorphic; that is, why are there so many different MHC alleles?

Compare MHC restriction for T helper and cytotoxic T lymphocytes.

Compare the various types of antigen presenting cells.