**T CELL ANTIGEN RECOGNITION (TCR & MHC)**

**PURPOSE:** To provide a basic understanding of the T cell Receptor (TCR), the Major Histocompatibility Complex (MHC), and MHC restricted antigen recognition.

**EDUCATIONAL GOALS:** by the end of this lecture you should be able to:

1. Draw the TCR and the CD3 complex. Locate and label the following structures: the TCR, CD3, the α and β-chains, the variable region, and the constant regions.
2. Describe the role of the TCR and CD3 in T cell signaling and activation.
3. Outline TCR gene rearrangement, and its role in the generation of TCR diversity
4. Draw the basic structures of class I and class II MHC molecules. Locate and label the following structures: the MHC chains (α/β2m or α/β), the peptide binding groove, and the CD4/CD8 binding site. Be able to identify the site where the greatest allele polymorphism exists.
5. Explain the consequences of MHC allelic polymorphism in transplantation.

**Supporting readings:** Parham (2015) pg 113-120 (TCR) and 120-126; 135-145 (MHC) or Abbas (2014) pgs. 71-73; 78-88 (TCR) and 49-69 (MHC)

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I. THE T CELL AND THE T CELL RECEPTOR (TCR)

A. B and T cells recognize different types of antigens.

1. BCR recognize (general rules)
   - 3D epitopes of macromolecules (proteins, carbohydrates, lipids, nucleic acids and even small chemicals)
   - ag directly binds/cross-links the BCR
   - antigens can be soluble or membrane-bound and in their native or a denatured form
   - for defense the antigen needs to be accessible

2. TCR recognize (general rules)
   - peptides from protein antigens
   - peptide must be presented (bound) by major histocompatibility complex (MHC) on host cells
   - NOT free antigen (e.g., free virions)

*T cell recognition requires the TCR to recognize BOTH (1) self-MHC and (2) peptide antigen. This is MHC-restricted antigen recognition.*
B. T cell Receptor Complex

1. Formed by a disulfide-linked heterodimer
   a. $\alpha\beta$ (alpha/beta chains) - most common T cell type
   b. $\gamma\delta$ (gamma/delta chains) - a minority of T cells, found at predominantly mucosal sites
   c. T cells either $\alpha\beta$ OR $\gamma\delta$ TCR but NEVER both

2. Structure
   a. The TCR subunits are transmembrane bound proteins with very short cytoplasmic tails
   b. TCR is noncovalently linked to a nonvariable complex of transmembrane proteins known collectively as CD3 and $\zeta$ proteins
   c. The TCR is only found associated with CD3 and vise versa
   d. Roles
      i. The variable region of the $\alpha\beta$ subunits contact the MHC and antigen complex
      ii. The CD3 and $\zeta$-chains transmit the signal from the TCR into the cell
   e. Clinical note: T cells can be identified by CD3 expression

![Diagram of TCR and CD3 structure]

C. TCR diversity
   - the genes encoding the TCR rearrange in different combinations during T cell development
   - the $\alpha$ and $\beta$ loci are unique from the heavy and light chain loci involved in Ig production (the Ig loci remain in their germ line conformation during T cell development)
   - The TCR polypeptides ($\alpha/\beta$ and $\gamma/\delta$) consist of two major domains
     - constant region
     - variable region – interacts with the antigen and MHC; composed of multiple gene segments assembled by gene rearrangement
       - $\alpha$ (and $\gamma$ genes) - V and J gene segments
       - $\beta$ (and $\delta$ genes) - V, D, and J gene segments

D. Allelic exclusion prevents both loci and two different TCRs from being effectively expressed in a given cell.
It is important to recognize that:

1. these events are **random** (Note: there may be some bias; however, this is still under investigation).
2. gene rearrangement events occur early in T cell development in the **absence of antigen**.

### Mechanisms of diversity

1. Multiple V, D, and J segments
2. Combinatorial diversity
   a. V, (D), and J gene segment combinations (Note: there may be some bias in arrangement.)
   b. α and β chains combinations
3. Diversity gene segments (D) code in all three reading frames.
4. Junctional diversity
   a. Exonucleases
   b. TdT

Note: T cells do NOT undergo somatic mutation as B cells do following **ACTIVATION**.

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**Focus question:** Do all mature T cells express CD3? Is CD3 a good marker for a T cell? What is the role of the TCR? CD3?

**Thought question:** Is the α locus more like the light or heavy chain in Ig? Is the β locus more like the light or heavy chain in Ig?
II. THE MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

The HLA locus is composed by a number of linked genes on a relatively small area on the short arm of chromosome 6. Because they are so closely positioned, they are usually inherited as a group. The class I and II MHC genes in humans are encoded by the HLA (Human Leukocyte Antigens). The MHC/HLA genes were originally known as transplant antigens, as they were largely responsible for graft rejection.

A. Class I MHC genes
1. Antigen presenting complexes encoded by HLA-A, HLA-B, and HLA-C genes
2. Expressed on almost all nucleated cells (not RBCs)
3. Absent or barely detectable on brain, sperm, placental trophoblasts and during early stages of embryogenesis
4. Expression upregulated by type 1 IFNs (IFN-α, IFN-β)

B. Class II MHC genes
1. Antigen presenting complexes encoded by HLA-DR, HLA-DP, and HLA-DQ (α and β-chains)
2. Expressed on
   a. Professional antigen presenting cells such as mature B cells, activated macrophages, and dendritic cells
   b. Thymic epithelial cells
3. Inducible on activated macrophages, some activated epithelial and endothelial cells, and other activated cell types
4. Expression upregulated by type 2 IFNs (IFN-γ)

C. Class III MHC genes
1. Are NOT antigen presenting complex genes
2. Encode several secreted proteins associated with immune process; e.g., some soluble serum proteins, components of the complement system and tumor necrosis factors (TNF)
D. MHC Alleles

- Human MHC loci are **highly polymorphic** (each locus has approximately 30-150 alleles)
- Individuals express two alleles at each locus (one from each parent)
- HLA alleles are **codominantly expressed** (both the maternally and paternally derived alleles are expressed on the surface of each cell)
- A single cell expresses multiple class I and II protein products on its cell surface
- The MHC/HLA genes are generally inherited as a group or a block called a **haplotype** (set of alleles carried on a single chromosome)
- ¼ of siblings will share the same haplotype

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<thead>
<tr>
<th></th>
<th>HLA genes</th>
<th>Individuals X’s HLA alleles</th>
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<tbody>
<tr>
<td><strong>Class I genes (human)</strong></td>
<td>HLA-A</td>
<td>A- 2 / A-24</td>
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<td>HLA-B</td>
<td>B-15 / B-27</td>
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<td></td>
<td>HLA-C</td>
<td>C-2 / C-16</td>
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<tr>
<td><strong>Class II genes (human)</strong></td>
<td>HLA-DR</td>
<td>DR1 / DR4</td>
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<td>HLA-DP</td>
<td>DP3 / DP 7</td>
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<td>HLA-DQ</td>
<td>DQ4 / DQ 17</td>
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E. Structure of Class I and Class II Molecules (protein level)

1. Protein structures of class I and class II MHC are very similar
   a. Four immunoglobulin domains
   b. Peptide binding groove large enough to accommodate small antigenic peptides
   c. A binding site for CD4 or CD8 to promote the interaction between that antigen presenting cell and the T cell

2. Class I MHC
   a. Each class I MHC molecule (HLA-A, HLA-B, and HLA-C) is noncovalently attached to a nonpolymorphic protein, \( \beta \)-2-microglobulin
   b. CD8 is able to bind a conserved region of the molecule

3. Class II MHC
   a. Class II MHC molecules (HLA-DR, DP, DQ) are composed of both an \( \alpha \)-chain and a \( \beta \)-chain
   b. These molecules are held together noncovalently
   c. CD4 is able to bind a conserved region of the molecule

F. The Peptide-Binding Groove and the Peptide

1. Structure
   a. Both class I and class II binding grooves are formed by eight anti-parallel \( \beta \) strands with two \( \alpha \)-helical bridges.
   b. The peptide-binding groove is critical for binding short 8-18 amino acid chains (the T cell epitope) from the antigen
   c. Most of MHC diversity exists in the peptide binding groove
   d. MHC restriction of antigen recognition - when the TCR engages both the MHC’s \( \alpha \)-helical bridges and the peptide lying in the groove
2. Binding
   a. Dilemma:
      · Despite the presence of the several hundred different allelic variants of class I and II MHC molecules in humans, an individual has only one allele from each parent for each class I gene (HLA-A, B, and C) and class II gene (HLA-DR, DP, and DQ)
      · This limited number of genes must be able to present all necessary antigenic peptides to the T cell for activation.
   b. Solution:
      · NOT gene rearrangement like TCR and BCR
      · Peptide display is not as specific as the antigen recognition systems used by the TCR and BCR
      · Promiscuous binding – allows each MHC molecule to present multiple peptides
      · Requirements for binding
        - length
        - anchor residues – conserved peptide resides that secure the peptide into the peptide-binding groove

<table>
<thead>
<tr>
<th>Peptide Binding by Class I and Class II MHC Molecules</th>
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<tbody>
<tr>
<td><strong>Class I Molecules</strong></td>
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<tr>
<td>Peptide-binding domain</td>
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<tr>
<td>General size of the peptide-binding cleft</td>
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<tr>
<td>Nature of peptide-binding cleft</td>
</tr>
<tr>
<td>Peptide motif involved in binding of MHC molecule</td>
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</tbody>
</table>

You do NOT need to memorize this table. You need to recognize that there are differences in class I MHC and class II MHC binding, but you do NOT need to know the specific details.

**Focus question:** How many different class I MHC molecules are expressed on the surface of an activated B cell? An RBC? What do class I and class II MHC structures look like? What cells express each MHC class? Where does the peptide bind? How does TCR and BCR antigen binding differ from MHC binding?

**Thought question:** Do you think that these differences in binding might explain why some individuals have different courses with infectious organisms or develop autoimmune disease?
Study Questions:

The T cell and the TCR

1. Indicate whether each of the following questions is true or false. If it is false, explain why.
   a. T cells like B cells are able to react against most antigens including proteins, polysaccharide, lipids and haptens.
   b. CD4+ cells play a major role in immunity by directing and modulating the immune system.
   c. A single T cell has thousands of receptors that allow it to react with multiple antigens.
   d. There are two types of TCRs, the \( \alpha \gamma \) (alpha/gamma) and the \( \beta \delta \) (beta/delta).
   e. During T cell Receptor engagement with MHC/peptide, the TCR provides the specificity of the reaction while the CD3 complex transmits the signal into the cell.
   f. TCR diversity is due in part to the gene rearrangement of multiple gene segments to form the TCR constant region.
   g. All mature T cells have exactly the same genome.
   h. The \( \alpha \beta \) TCR is bivalent and has two antigen-binding sites.
   i. Each \( \alpha \beta \) T cell expresses only one TCR type.
   j. A naïve helper T cell expresses class II MHC on its cell surface.
   k. A monoclonal antibody against human \( \beta_2 \)-microglobulin is able to identify HLA-DR, DQ, and DP molecules on the surface of the cells.
   l. Professional antigen presenting cells express both class I and class II MHC molecules simultaneously.
   m. In addition to the transplantation antigens, the human MHC locus encodes components of the complement system and tumor necrosis factors (TNF).
   n. Your patient needs a bone marrow transplant. His/ her parents are more likely to be a perfect match than his/her sibling.
   o. CD4+ T cells express class I MHC on their cell surface.

2. Helper and cytotoxic T cells can be distinguished from each other by function, cell surface receptors and MHC restriction. Please list these differences.

3. Describe the principles of TCR rearrangement.

4. List and describe the different mechanisms of TCR diversity.

5. List the cells that usually express MHC class I molecules. List the cells that usually express MHC class II molecules.

6. Diagram the general structure of both the class I and II MHC molecules (from the side). Remember the immunoglobulin domains and each of the components: the transmembrane domain (how it binds to the cell), the peptide binding groove, peptide, and the binding sites of CD4 or CD8. Diagram the TCR interaction.

7. A patient (daughter) is going to receive a kidney transplant from her mother. The mother and patient (daughter) exhibit the following genotype at the MHC (note: HLA-DP and DQ were excluded for simplicity):

   Mother: A1/A5 B7/B8 C7/C9 DR4/DR5  

   a. Is this the biological mother of your patient?
   b. What are the patient’s class I MHC molecules? What are the patient’s class II MHC molecules?
   c. Despite class I being present on almost all nucleated cells, it has been discovered that matching class II MHC is more critical in determining the long-term outcome of transplantation (both solid organ or bone marrow). Would either of the following cadaver donors be a better choice for transplantation?

   Donor #1: A9/A14 B4/B11 C9/C9 DR2/DR9  
   Donor #2: A6/A26 B8/B9 C9/C9 DR4/DR15

   Note: we will revisit this topic in more depth during the transplantation lectures (in Failure to Thrive).

8. An individual expresses a limited number of class I MHC molecules and class II MHC molecules. How is it possible that this small number of MHC molecules is able to present a large variety of antigenic peptides to T cells?
SELECTED ANSWERS

The T cell and the TCR

1. a. False. While B cells are able to react against these potential antigens, T cell antigens are generally confined to proteins that can be presented via the MHC (general concept).
   b. True.
   c. False. While a TCR has thousands of TCRs on its cell surface, all of the TCRs are from a single recombination event and recognize only a single antigen/MHC complex.
   d. False. The two types of TCRs are αβ (alpha-beta) and γδ (gamma-delta).
   e. True.
   f. False. TCR diversity is due in part to the gene rearrangement of multiple gene segments to form the TCR variable region.
   g. False. Gene rearrangement leads to minor changes in the DNA of all T and B cells. These changes will be slightly different in all clonally unrelated cells.
   h. False. All TCRs have a single binding site composed by the combination of the αβ or γδ-chains. A single immunoglobulin molecule has two binding sites.
   i. True.
   j. False. A naïve T cell does not have class II MHC on its cell surface. It recognizes class II MHC + peptide on a professional antigen presenting cell. Note: some activated helper T cells express class II MHC.
   k. False. These are class II MHC molecules; they do not contain β2-microglobulin. Class I MHC molecules (HLA-A, B and C) all do.
   l. True.
   m. True.
   n. False. Offspring inherit one allele from each parent; therefore, they usually match only 50% with either parent. Siblings have a one in four chance of being a perfect match (histocompatible) throughout the MHC loci.
   o. True. Naïve CD4+ T cells are nucleated and they express class I MHC and not class II MHC on their cell surface unless it was appropriately stimulated. They are activated by class II MHC + peptide.

2. |                | **Helper T cell** | **Cytotoxic T cell** |
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<tbody>
<tr>
<td><strong>Function</strong></td>
<td>Activates the immune system with cytokines</td>
<td>Kill cells</td>
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<tr>
<td><strong>Cell surface markers</strong></td>
<td>CD4+</td>
<td>CD8+</td>
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<tr>
<td><strong>MHC restriction</strong></td>
<td>Class II</td>
<td>Class I</td>
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5. Class I MHC most nucleated cells. Protein is absent or barely detectable on brain, sperm, placental trophoblasts, and during early stages of embryogenesis.

7. a. No. They fail to share an allele both at the HLA-A and HLA-DR loci.
   b. Class I MHC  HLA-A2/A2, HLA-B8/B9, and HLA-C6/C9
   Class II MHC  HLA-DR2/DR9
   c. Cadaver #1

8. A given MHC can bind to a wide variety of peptides. A class I MHC binds a peptide that is 8-10 amino acids long. Since the class I MHC only contacts the peptide at the anchor residues, a given MHC can bind a wide variety of peptides with the appropriate residues at these sites. The peptides can differ at other sites allowing a wide variety of antigenic peptides to be presented. A similar situation is seen in class II MHC.
ANTIGEN PROCESSING AND T CELL ONTOGENY

PURPOSE: To provide a basic understanding of the exogenous and endogenous antigen processing pathways and T cell ontogeny.

EDUCATIONAL GOALS: by the end of this lecture you should be able to:

1. Compare and contrast the two types of antigen processing with regard to the origin of the antigen being processed, host antigen recognition molecules involved, and fate of antigen within intracellular compartments.
2. Predict the role of each pathway in immune function.
3. Describe T lymphocyte differentiation with regard to anatomical sites, receptor-ligand interactions, and the evolution of CD markers.
4. Explain T cell education, and its roles of positive and negative selection in MHC restriction and tolerance.

Supporting readings: Parham (2015) pg. 122-135 (antigen processing), 177-197 (T cell development) OR Abbas (2014) pg. 49-69 (antigen processing), 88-91 w/a focus on (T cell development)

I. Introduction

Target Cells and APCs
All cells that express either class I or II MHC molecules can present peptides to T cells; therefore, all could be designated antigen-presenting cells. While most immunologist call cells that display peptide in association with class II professional antigen-presenting cells (APCs), they call cells that present peptides in the context of class I to CD8+ cytotoxic T cells target cells.
II. Antigen Processing Pathways

There are 2 major antigen processing pathways—the endogenous pathway and the exogenous pathway. The endogenous pathway generally presents antigens from cytosolic sources while the endocytic pathway presents antigens from the extracellular milieu. Segregating antigens by their source allows different classes of T cells to recognize the antigen. These different T cell classes allow cellular immunity to target its response more effectively.

A. Cytosolic pathway presents endogenous antigens

Class I MHC molecules are receptors that are capable of binding fragments of proteins that have been synthesized in the cytoplasm of the cell (most intracellular antigens). Hypothetically, any protein (self, viral, bacteria, tumor, etc.) that was synthesized in cytoplasm could be expressed on a class I MHC molecule. If the protein fragment is seen as ‘non-self’, this peptide:MHC complex on the cell’s surface is recognized by CD8+ cytotoxic T cells (CTL). Once activated through their antigen receptors, CTLs will kill the non-self-bearing cell.

Pathway
1. Endogenous antigen (e.g., a viral component from a virus replicating within the cell, a tumor antigen, etc.) is degraded within the cytoplasm by a proteasome
2. Cytosolic peptides are transported by TAP (transporter associated with antigen processing) into the rough endoplasmic reticulum
3. The Class I MHC α-chain is appropriately folded and associated with β2-microglobulin through chaperone-mediated interactions
4. The class I MHC is loaded with peptide and moves from the rough endoplasmic reticulum (RER) through the Golgi to the plasma membrane
B. Endocytic pathway presents exogenous antigens

MHC class II molecules are receptors that are capable of binding fragments of proteins that have been phagocytized or endocytosed from the extracellular milieu (extracellular antigens). Hypothetically, any protein (self, viral, bacteria, tumor, etc.) that was synthesized elsewhere in the body and was endocytosed/phagocytosed by a professional APC could be expressed on a class II MHC. If the protein fragment is seen as ‘non-self’, this complex on the cell’s surface can be recognized by CD4+ helper T cells (Th). Once activated through their antigen receptors, Th cells will synthesize a series of cytokines that regulate both cellular and humoral immunity.

Pathway
1. Exogenous antigen is derived from extracellular milieu
2. Antigen uptake occurs by phagocytized, endocytosed, or pinocytosed depending on the APC
3. MHC class II α- and β-chains are synthesized in the RER and associate with the invariant (Ii)-chain
4. The αβ Ii complexes are transported through the Golgi complex to endosomes and lysosomes
5. The Ii-chain is progressively degraded as the proteolytic activity increases in each successive compartment ultimately leaving only CLIP (class II-associated invariant chain peptide) in the peptide binding groove
6. CLIP is removed and an exogenous peptide from the endosome or lysosome is added
7. The peptide-loaded class II MHC is transported to the plasma membrane

C. Messages from each pathway
1. The message to the CD8+ T cell: If a peptide is recognized as foreign on class I MHC, there is a problem WITHIN THIS cell→Kill the cell.
2. The message to the CD4+ T cell: If a peptide is recognized as foreign on class II MHC, there is a problem WITHIN THE BODY→Produce cytokines to help activate /modulate the immune system.

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<thead>
<tr>
<th>Summary of Antigen Processing Pathways</th>
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<td><strong>Humans</strong></td>
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<td><strong>Encoded by</strong></td>
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<td><strong>Antigen source</strong></td>
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<td><strong>Special components in pathway</strong></td>
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<td><strong>Recognition and action</strong></td>
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Note: these are broad generalizations. There are exceptions to these categorizations.
Focus question: Why are self-peptides bound to MHC molecules (class I and class II)? What would the consequence be of an exogenous peptide being bound to class I MHC molecules? Would it be beneficial to the body?

F.Y.I. There is experimental evidence that this does occur in some situations, but this is beyond the scope of this course.

C. An exception (to the division of extracellular versus intracellular antigen sources)

- Some microbes have evolved to resist the microbicidal activities of phagocytes
  - Many pathogenic intracellular bacteria and protozoa are able to survive and replicate in the vesicles (phagolysosome) of phagocytes
- Because these pathogens exist in the compartment used as the source of antigen for class II MHC, their antigenic peptides are loaded onto class II MHC.
- IFN-\(\gamma\) generation promotes macrophage activation and defense against many of these pathogens
- Some of these microbes may enter the cytoplasm of infected cells and multiply in this compartment allowing peptides from the cytoplasm to be loaded onto class I MHC

D. The Implications

The inability of MHC to discriminate between foreign antigens and self-antigens raises the following questions:

1. The concentration of self-proteins will outnumber microbial or “altered self” antigens. How does foreign antigen recognition occur if most MHC molecules are occupied by self-antigens?
2. MHC molecules are constantly displaying self-antigens. Why don’t most people develop a response against them and develop autoimmunity?

III. T CELL ONTOGENY (DEVELOPMENT)

A. General scheme of a T cell’s life

1. bone marrow progenitor cells migrate to the thymus gland (primary lymphoid organ)
2. immature cells enter the cortex and mature as they travel toward the medulla
3. mature naive T cells leave the thymus through the peripheral blood circulation and travel to the secondary lymphoid organs (e.g., spleen and lymph nodes)
4. cells populate the secondary lymphoid organs where they can encounter antigen and exert their effector function

B. The thymus

- primary lymphoid organ for T cells
- shrinks after puberty or corticoid exposure (illness, drugs)
- a triangular bi-lobed structure composed of histologically and functionally distinct cortex and the medulla
- As thymocytes (developing T cells) move down the web created by the thymic stroma from the cortex towards the medulla, **they become more mature and express or lose different cell surface markers.**

- Direct contact with
  - non-lymphoid thymic stromal cells (e.g., thymic epithelial cells) – soluble and membrane-bound factors
  - bone marrow derived **interdigitating dendritic cells** - aids **thymic education (selection)**
- The thymic microenvironment directs T cell development through both direct cell-cell contact and soluble protein mediators (cytokines).

**C. T cell differentiation in the Thymus**

T cell differentiation in the thymus is complex and involves multiple steps. In summary,

1. Pro-thymocytes (CD2+, CD7+; **pro-T cells**) migrate from the bone marrow to the **thymic cortex.**
2. The **TCR undergoes rearrangement** leading to the low level expression of both the TCR and CD3 and the formation of **pre-T cells.** This step determines the antigenic specificity of the T cell. Because pre-T cells do not yet express either CD4 or CD8, they are referred to as **double negative cells** (TCR+lo, CD3+, CD4- and CD8-).
3. Following TCR expression, both CD4 and CD8 are coexpressed to produce **double positive cells** (TCR+lo, CD3+, CD4+ and CD8+). It is during this stage that **thymic education occurs.** CD4+CD8+ cells undergo the processes of **positive and negative selection.**
4. The CD4+ and CD8+ cells surviving selection down regulate one or other of the co-receptor molecules they express and become **single positive cells** expressing either CD4+/CD8- or CD4-/CD8+. These cells upregulate the expression of their αβ TCR molecules and are either αβhiCD3+CD4+ T cells or αβhiCD3+CD8+ T cells.

**D. Selection**

It is thought that only CD4+CD8+ T cells that bind with a crucial affinity to the epithelial cell’s MHC molecules survive selection. Thymic selection of αβ+ cells can be divided into two phases, positive and negative selection.

1. **Positive selection** ensures that the T cell **CAN** identify peptides restricted by the individual’s MHC.
   
   During this step, the T cell becomes “**educated**” to self-MHC. This means that the T cell can only recognize antigen when it is bound to the same MHC that was encountered in the thymus. This leads to **MHC restriction.**

2. **Negative selection** removes cells that recognize self-peptides in context of self-MHC. Failure to remove T cells capable of recognizing and reacting to self-antigens in the context of self-MHC would result in autoimmunity.
IV. MHC polymorphism in health and disease

The allelic forms of class I and class II MHC molecules, which are expressed by an individual, determine the repertoire of peptides that can be presented. This would suggest that there is a genetically predetermined capacity to react immunologically against a given immunogen. This could play a role in individual immune responses leading to resistance or susceptibility to infection or the development of autoimmunity.

In addition, the allelic forms of class I and class II MHC molecules determine which T cells survive positive and negative selection. Whether certain T cells are selected for or deleted by the MHC repertoire, it could have significant consequences on both health and disease.

A. Immune defense. Because of the relatively small number of ‘foreign’ proteins in a typical pathogen and the MHC imposed constraints on peptide binding, usually only a few epitopes from any antigen (or pathogen) can be presented effectively to a T cell.
Differences in MHC within the population influence the antigens to which an individual’s TH or T CTL cells can respond. This can occur by the MHC affecting the peptides presented or by the T cell repertoire that survives thymic education. This means that some individuals may be more susceptible to certain threats than others are.

The expression of multiple MHC genes increases the likelihood that at least one of each person’s MHC molecules could effectively present antigen and select a set of effector T cells in each individual. For example, each individual expresses six different class I genes (3 loci, 2 alleles at each loci). The great polymorphism probably also provides protection for the species at large.

**B. Autoimmunity.** Statistically, MHC alleles have been linked to autoimmune diseases. These diseases occur more frequently among persons who express a particular MHC/HLA. While there are multiple hypothesized mechanisms of this association, it must be noted that no HLA allele is associated with disease in 100% of the cases. Therefore, MHC/HLA is probably only one of the factors involved in disease development.

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<thead>
<tr>
<th>Disorder</th>
<th>Associated HLA</th>
<th>Risk</th>
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<tr>
<td>Ankylosing spondylitis</td>
<td>B27</td>
<td>80-fold</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Dw14 / DR4</td>
<td>47-fold / 26-fold</td>
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<tr>
<td>Insulin-dependent diabetes mellitus</td>
<td>DQw8</td>
<td>32-fold</td>
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<td>Sjogren’s syndrome</td>
<td>DR3</td>
<td>6-fold</td>
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<tr>
<td>Grave’s disease</td>
<td>DR3</td>
<td>4-fold</td>
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<tr>
<td>Systemic lupus erythematosus</td>
<td>DR2</td>
<td>3-fold</td>
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Please note that these are examples. During this module, it is important to recognize that certain diseases are HLA-associated, but not the details (e.g., the actual disorders, HLA, or risk.)
STUDY QUESTIONS

1. Indicate whether each of the following questions is true or false. If it is false, explain why.
   a. The majority of all peptides presented by class I MHC molecules are self-proteins.
   b. The role of the invariant chain is to prevent binding of endogenous peptides to class II MHC molecules prior to loading in the endosome/lysosome.
   c. Viruses are intracellular pathogens; therefore, they will only be presented on class I MHC and not class II MHC.
   d. It is advantageous to kill a cell expressing a foreign exogenous peptide.
   e. T cells are thymically derived; therefore, T cell function and numbers would NOT be affected during bone marrow ablative therapy.
   f. In the thymus, developing T cells interact with the thymic epithelium and interdigitating cells which aid in thymic education and/or selection.
   g. The term Double Positive T cells refers to the expression of two cell surface markers: TCR and CD3.
   h. T cell antigen specificity is determined pre-thymically.

2. Indicate whether each of these cellular components is involved in the processing, presentation or recognition of exogenous antigens (EX), endogenous antigens (END), both (B) or neither (N).
   a. ______ Class I MHC molecules
   b. ______ Class II MHC molecules
   c. ______ α and β-chains of the MHC
   d. ______ β2-microglobulin
   e. ______ CD4+ T cells
   f. ______ CD8+ T cells
   g. ______ invariant chain
   h. ______ transport from the RER => Golgi
   i. ______ proteasomes
   j. ______ TAP proteins
   k. ______ phagocytosis or endocytosis
   l. ______ endosomes or lysosomes
   m. ______ CD3+ T cells

3. Diagram both the exogenous and endogenous antigen pathways.

4. What is the difference between a target cell and an antigen presenting cell? What is the significance of this difference? Why is it important that they express different antigen types (exogenous versus endogenous) and that they stimulate different T cell subclasses (CD4+ helper T cells versus CD8+ cytotoxic T cells)?

5. Products of TAP-1 and TAP-2 genes
   a. Are part of the proteasome.
   b. Bind β2-microglobulin.
   c. Prevent peptide binding to MHC molecules.
   d. Transport peptides into the endoplasmic reticulum for binding to class I MHC molecules.
   e. Transport peptides into the endoplasmic reticulum for binding to class II MHC molecules.

6. What are the changes in cell surface marker expression during the development of a helper T cell? Of a cytotoxic T cell? Include in your answer the following stages: pro-T cell, pre-T cell, immature T cell, and mature T cell.

7. If patient X was missing his or her class II MHC molecules what would X’s T cell composition look like? Would there be normal numbers of CD4+ and CD8+ T cells? Why?
8. Where is each of the following T cell types found (options: in transit to the thymus, the thymus, the periphery, predominantly in mucosal tissues and/or nowhere)?

a. $\alpha\beta^+, CD3^+, CD4^+, CD8^+$
b. $\alpha\beta^+, CD3^-, CD4^+, CD8^+$
c. $\alpha\beta^+, CD3^+, CD4^-, CD8^-$
d. $\gamma\delta^+$
e. $\alpha\beta^+, CD3^+, CD4^-, CD8^+$
f. $\alpha\beta^+, \gamma\delta^+, CD3^+, CD4^+, CD8^-$
g. $\alpha\beta^+, CD3^+, CD4^+, CD8^-$
h. $\alpha\beta^-, CD3^-, CD4^-, CD8^-$

9. Describe briefly the degrees of specificity and mechanisms that generate the diversity of the interaction in the following:
   a. TCR binding to antigenic peptide
   b. TCR binding to MHC
   c. MHC binding to antigenic peptide

10. Will cytotoxic T cells generated following infection with virus A kill the following target cells?
   a. Virus A infected target cells with an unrelated MHC.
   b. Target cells with the identical class I MHC as the host from which the cytotoxic cells were isolated infected with Virus A.
   c. Target cells with the identical class II MHC as the host from which the cytotoxic cells were isolated infected with Virus A.
   d. Target cells with the identical class I MHC as the host from which the cytotoxic cells were isolated infected with Virus B.
   e. Target cells with the identical class II MHC as the host from which the cytotoxic cells were isolated infected with Virus B.

11. a. If you were to infect a potential target cell with virus and then incubate these cells with cytotoxic T cells specific for the virus + the target cell’s MHC, what would happen?
   b. What would happen in the situation above if the virus were heated killed prior to addition to the target cells?

**SELECTED ANSWERS**

1. a. True.
   b. True.
   c. False. While viral peptides will be expressed on class I MHC via the cytosolic pathway, they will also be presented on class II MHC via endocytic pathway. Viral particles can be bound by the BCR and processed on the B cell’s class II. In addition, virally infected cells and antibody-virus complexes can be phagocytosed by macrophages or Langerhan cells and presented on their class II MHC.
   d. False. If the peptide comes from an exogenous source, it most likely came from a neighboring cell. It does not indicate that there is something wrong with THAT cell. The appropriate response would be to upregulate the immune response, not kill the cell. This is the concept behind the two “independent” pathways of antigen processing.
   e. False, pro-T cells migrate from the bone marrow to the thymus. Because of this, T cell numbers are affected during certain therapies that effect hematopoiesis.
   f. True.
   g. False, while Double Positive T cells express both TCR and CD3, the term refers to the presence of both CD4 and CD8 on these immature T cells.
   h. False, T cell antigen specificity is determined by TCR rearrangement and production. This occurs in the thymus during T cell development.
2. a. END Class I MHC molecules  h. B transportation from the RER => Golgi
    b. EX Class II MHC molecules  i. END proteasomes
    c. EX α and β-chains  j. END TAP proteins
    d. END β2-microglobulin  k. EX phagocytosis or endocytosis
    e. EX CD4+ T cells (helper cells)  l. EX endosomes or lysosomes
    f. END CD8+ T cells (cytotoxic cells)  m. B CD3+ T cells (all T cells)
    g. EX the invariant chain

5. d. The products of the TAP-1 and –2 genes selectively transport peptides 8-9 amino acids in length from the cytoplasm into the ER where they bind to MHC class I molecules.

7. Individuals lacking class II MHC would have near normal levels of CD8+ T cells; however, CD4+ T cells would be significantly reduced. During thymic T cell education, the absence of class II MHC would discourage the positive selection of CD4+ T cells.

8. a. in the thymus  
    b. nowhere (the TCR and CD3 are always coexpressed on the cell surface)
    c. in the thymus
    d. predominantly in the mucosal tissues
    e. in the thymus and in the periphery
    f. nowhere (αβ and γδ are not coexpressed)
    g. in the thymus and in the periphery
    h. in transit to the thymus and perhaps for a short time in the thymus (note: this would also be the profile of cells outside of the T cell lineage)

9. a. TCR binding to Antigenic peptide is very specific. Generally, each TCR recognizes only a single peptide antigen. The diversity of the receptor comes from TCR rearrangement including 1) Multiple V, D and J segments (on both chains), 2) Random combination of VDJ segments, 3) Random combination of α and β-chains, and 4) Functional and insertional variability.
    b. TCR binding to MHC is very specific. Each TCR generally recognizes only a single MHC molecule. This interaction is moderately diverse (predominantly due to diversity in the V-gene segment).
    c. MHC binding to antigenic peptide is NOT very specific. Each type of MHC molecule can bind a unique set of peptides. The diversity generated by the MHC molecule results from polymorphisms (multiple alleles at a given locus within a species). Through population polymorphisms, the diversity of the MHC for a given species is an astronomical number, 10^{12}. This would create a major obstacle if a perfect match were required for organ transplantation. In addition, diversity of MHC:peptide binding comes through the ‘promiscuity’ of the MHC:peptide interaction. Peptide binding is mediated through the interaction of the MHC with specific anchor residues on the peptide. The other residue sites on the peptide generally do not effect peptide binding and allow a multitude of antigens from a pathogen to be presented to the immune system.

10. a. Will NOT lyse these cells. Matches antigen (virus) but does not match MHC.
    b. Will lyse these cells. Matches class I MHC and antigen (virus).
    c. Will NOT lyse these cells. Matches antigen (virus) but does not match class I MHC.
    d. Will NOT lyse these cells. Matches class I MHC but does not match antigen (virus).
    e. Will NOT lyse these cells. Matches neither class I MHC nor antigen (virus).
11. a. Lysis. Upon infection, viral peptides would be expressed within the target cell. These would be processed via the endogenous antigen pathway and presented in the context of class I MHC. Recognition by the CD8+ cytotoxic T cell would lead to lysis. You have met the requirements of T cell activation 1) matching peptide antigen and 2) matching MHC.

b. No lysis. While viral proteins would be present, heat killed virus cannot replicate within the cell. Therefore, viral proteins are neither made within the cell nor present in the cytoplasm. Therefore, they will not be processed via the endogenous pathway for recognition by a CD8+ T cell. Note: the viral proteins could be phagocytosed, processed via the exogenous antigen pathway, and presented by class II MHC to a CD4+ T cell.
**T CELL ACTIVATION AND EFFECTOR FUNCTIONS**

**PURPOSE:** to provide a basic understanding of T cell activation, clonal proliferation, and introduce T cell effector functions.

**EDUCATIONAL GOALS:** by the end of this lecture you should be able to:

1. Diagram the two signals required for activation of naïve CD4+ T cells including the role of each ligand interaction
2. Describe anergy including why it occurs and its potential role in immune tolerance
3. Compare and contrast the professional antigen presenting cells (APCs) including mechanism of antigen capture and the expression patterns of class II MHC and B7
4. Outline the steps in T cell proliferation. Include the roles of antigen, antigen processing, cytokine, and cytokine receptors in this process (you will need to incorporate information from previous lectures)
5. Describe mechanisms involved in down regulating the immune response and generating memory

**Supporting readings:** Parham (2015) pg. 199-221 – OR – Abbas (2014) pg. 93-116. * I find the Abbas text better at presenting the important concepts in this lecture. *

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**I. T CELL ACTIVATION**

A. Naïve Tc and Th cells require two signals for activation

1. Naïve T cells “sample” MHC + peptide combinations through low affinity interactions mediated by adhesion molecules
2. Engagement of the TCR results in increased binding affinity of the adhesion molecules
3. Naïve T cells need **two signals** for activation
   
   #1 TCR engagement – TCR : peptide (antigen) + self-MHC
   
   #2 Costimulatory signal – CD28 (T cell): B7 (APC)
4. Cells that receive only signal #1 are **anergized** (role in peripheral tolerance?)

B. Effector and memory T cells require only signal #1 for function
Focus question: Why is it important that effector T cells can be triggered by a single signal through the TCR complex?

II. Antigen presenting cells (APC) and antigen sources

A. Sources
1. Immature dendritic cells and macrophages phagocytose the antigen in the tissue and carry it to the draining lymph node
2. Draining interstitial fluids carry antigen to dendritic cells, macrophages and B cells in lymph node

B. Comparison of professional APCs

<table>
<thead>
<tr>
<th></th>
<th>Macrophage</th>
<th>Dendritic cells</th>
<th>B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen uptake</td>
<td>Phagocytosis +++</td>
<td>Phagocytosis by tissue dendritic cells +++</td>
<td>Antigen-specific receptor (Ig)-mediated endocytosis +++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Viral infection +++</td>
<td>In lymph node +/-</td>
</tr>
<tr>
<td>MHC expression</td>
<td>Inducible by bacteria and cytokines - to +++</td>
<td>Constitutive on mature cells +++</td>
<td>Constitutive Increases on activation + to +++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ on immature cells</td>
<td></td>
</tr>
<tr>
<td>Co-stimulator delivery</td>
<td>Inducible - to +++</td>
<td>Constitutive in mature non-phagocytic lymphoid dendritic cells</td>
<td>Inducible - to +++</td>
</tr>
</tbody>
</table>

Focus question: Could the lack of expression of B7 in the periphery promote a tolerizing environment? Could inflammatory cytokines and the upregulation of B7 promote an activating environment?

Hint: This is a mechanism of peripheral tolerance that we will discuss during Immune Regulation later in the course.
III. T cell signaling and proliferation (common to both CD4 and CD8 T cells)

- T cell receptor engagement
- Clustering of multiple TCRs. CD4/CD8 molecules, CD28 and other cell surface receptors
- Transphosphorylation / activation of Lck and Fyn (protein tyrosine kinases)
- Phosphorylation of the ITAMs (Immunoreceptor tyrosine-based activation motifs) on the CD3 and ζ (zeta) chains
- ZAP70 (zeta-associated protein tyrosine kinase-70) docks via the phosphorylated ITAMs
- Lck phosphorylates ZAP70 leading to its activation
- ZAP70 phosphorylates numerous proteins including phospholipase Cγ (PLCγ) and guanine nucleotide exchange factors (GEFs)
- PLCγ and the GEFs both initiate a series of cascades
- Production of transcription factors (i.e., NFAT, NFκB, and AP-1)
- Induce the transcription of many genes including IL-2 and the IL-2 receptor α-chain

While this is a complicated series of events, the following points you should be familiar with:

- Clustering of multiple receptors on the cell surface leads to the activation of the tyrosine kinases Lck and Fyn that phosphorylate ITAMs found on the cytoplasmic tails of subunits of CD3 that allow additional proteins to associate with the complex.
- ZAP70 binds the ITAMs and is phosphorylated and activated by Lck.
- ZAP70 phosphorylates PLCγ and GEFs, which initiate a series of cascades that result in the production of transcription factors.
- Transcription factors induce IL-2 and IL-2R α-chain production that promotes T cell proliferation and differentiation.
- Certain immunosuppressive drugs (e.g., cyclosporin and FK506) block the production of transcription factors by blocking calcineurin function.
IV. CLONAL PROLIFERATION

As you remember, TCR diversity is very important to assure that one can mount a cell-mediated response against nearly any invader. Because of the **great diversity very few T cells actually react against a single antigen** (one in $10^4$-$10^6$ TCRs will react with a given peptide (antigen) + MHC). Because of this, clonal proliferation needs to occur before there is a significant T cell response.

**Mechanism of antigen-specific T cell amplification**
- the IL-2R is a **trimeric receptor** ($\alpha$, $\beta$, and $\gamma$-chains)
- the $\beta$ and $\gamma$-chains are constitutively expressed on mature T cells
- the $\alpha$-chain is expressed on **ACTIVATED but not resting T cells**
- the trimeric complex is needed for IL-2 **binding and signaling** at cytokine levels normally produced by T cells in the body (physiological concentrations)
  - low affinity receptor – $\alpha$
  - intermediate affinity receptor – $\beta\gamma$
  - high affinity receptor – $\alpha\beta\gamma$

- IL-2 binding and IL-2R signaling lead to T cell proliferation. **This significantly increases the number of cells reactive to the threat/pathogen.** This allows for greater effector activity within the body.
V. T CELL MIGRATION (Where do these processes tend to occur?)

A. Naïve T cells circulate through lymph nodes

Naïve T cells recirculate
1. enter the lymph node via the arterial blood, through the postcapillary endothelial vascular lining of the **high endothelial venules (HEVs)**
2. search for its MHC + peptide match in the LN
3. exit via the lymphatics → circulation → another LN to maximize the opportunity for antigen + MHC recognition

**Recruitment of naïve T cells in the lymph node:**
1. **rolling** is mediated by **L-selectin molecules** on T cells that bind to special **glycoprotein ligands** that contain sialyl-Lewisx (i.e., CD34) on the high endothelium in LN
2. naïve T cells home to lymph nodes because of L-selectin binding to its ligand on high HEVs in lymph nodes
3. **chemokine** stimulation of the T cell → a structural alteration of the **LFA-1** (an integrin) molecule → an increase in its affinity for its ligand, **ICAM-1**
4. **firm attachment** (LFA-1 on T cells binds to ICAM-1 on the HEV) → **diapedes**

**Activation in the LN**
1. naïve T cells sample APCs (dendritic cells) for MHC + peptide match
2. **signal #1 and #2** → phosphorylation of ITAMs on CD3 → docking of tyrosine kinases that activate the second messenger systems → transcription factor production/activation → synthesis of IL-2 and the IL-2R α-subunit
3. Clonal proliferation and differentiation into **effector T cells**
4. Movement of many effector cells in the peripheral blood for migration to the site of infection while some cells remain in the LN
Recruitment of activated T cells into inflamed/infected tissues

Following activation proliferation, and differentiation, effector T cells are free to migrate out of the lymph nodes. As T cells differentiate into effector T cells they no longer express L-selectin on their cell surface, and they up regulate other homing molecules to promote migration to the site of infection.

1. Effector T cell rolling - mediated by P and E-selectin on the activated endothelium (L selectin is down regulated on effector T cells)
2. chemokine stimulation of the T cell $\rightarrow$ structural alteration of the LFA-1 and VLA-4 molecules $\rightarrow$ an increase in its affinity for their ligands, ICAM-1 and VCAM-1
3. firm attachment is mediated by LFA-1/VLA-4 on T cells binding to endothelial ICAM-1/VCAM-1

Stimulation and effector functions at the site of infection

1. Sample cells for MHC + peptide match
2. Signal #1 through TCR $\rightarrow$ effector functions (effector T cells only require signal #1 for stimulation)
3. Effector activities
   a. CD4+ T cells – cytokine production
      - CD8 cell proliferation $\rightarrow$ defense against viruses, malignant cells
      - Macrophage activation $\rightarrow$ intracellular bacteria
      - NK cell function $\rightarrow$ viruses, malignant cells
      - B cell proliferation / isotype switching $\rightarrow$ viruses (extracellular stages), bacteria (intracellular), helminthic parasites
   b. CD8+ T cells – killing

Focus questions: Describe the life of a T cell once it leaves the thymus. Where does it go, how does it circulate, what does it need for activation and where is this most likely to occur?
VI. DOWN REGULATING THE IMMUNE RESPONSE

T cell activation and proliferation is dependent upon antigen stimulation.

- clearance of the threat → loss of T cell activation
  → stop of IL-2 and IL-2R synthesis
  → loss of survival factors
  → death by apoptosis

- down modulatory cytokines (e.g., TGF-β) also inhibit the immune response.

Within a week or two of clearance, the only remaining component of the immune response is a pool of memory cells.

*Focus questions:* How does the loss of antigen lead to apoptosis of the effector T cells? What is the role of TGF-β?

VII. MEMORY

Immunological memory allows the immune system to respond more rapidly and effectively to previously encountered threats or pathogens.

Goal: provide protective immunity (immunity to reinfection) so that no, unapparent (subclinical), or mild infection occurs upon subsequent exposure.
VIII. LIFE OF A T CELL

Naïve T cells wait in the periphery for specific antigen stimulation. Naïve T cells can recirculate for a year or so waiting for activation. The actual fate of these cells is unknown.

**STUDY QUESTIONS**

1. Indicate whether each of the following questions are true or false. If they are false, explain why.
   a. Because all professional antigen presenting cells (e.g., macrophages, B cells and dendritic cells) express class II MHC, they are all equally effective in antigen presentation to CD4+ T cells.
   b. Professional antigen presenting cells (e.g., B cells, macrophages and dendritic cells) constitutively express class II MHC, but rarely express class I MHC molecules.
   c. Clonal proliferation increases the number of T cells reactive to a specific immunogen.
   d. The goal of immunological memory is to provide long-term protective immunity through expansion of reactive cell populations and increased effector function.
   e. The IL-2 receptor becomes functional when the β− and γ-chains are expressed following T cell activation.
   f. Immunological memory occurs by the accumulation of large numbers of naïve T cells waiting years in the periphery for antigen stimulation.
2. Fill in the blank(s) in each statement below with the most appropriate term(s) from the following list.

<table>
<thead>
<tr>
<th>cytokine</th>
<th>CD4 T cells</th>
<th>class I</th>
<th>B7</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>CD8 T cells</td>
<td>class II</td>
<td>CD28</td>
</tr>
<tr>
<td>IL-4</td>
<td>exogenous</td>
<td>native</td>
<td></td>
</tr>
<tr>
<td>IFN-γ</td>
<td>endogenous</td>
<td>processed</td>
<td></td>
</tr>
</tbody>
</table>

a. Dendritic cells express _______ constitutively while B cells must be activated before they express this membrane bound molecule.

b. Activation of Th cells results in the secretion of _______ and expression of its high affinity receptor, leading to T cell proliferation and differentiation.

c. In addition to the TCR : peptide MHC interaction, naive CD4+ T cells require the interaction of _______ on the T cell and _______ on the APC for full activation.

d. Individuals lacking class II MHC molecules fail to produce _______ T cells. These cells are responsible for _______ production.

e. CD8+ T cells recognize _______ antigen in the context of _______ MHC antigen.

f. CD4+ T cells recognize _______ antigen in the context of _______ MHC antigen.

g. B cells can recognize _______ antigen while T cells are only able to recognize _______ antigen in the context of self-MHC.

3. Explain the process, goal and mechanism of clonal proliferation. Include information on IL-2 production, IL-2 receptor expression and proliferation.

4. Describe the process of creating effector and memory cells. Use information from previous lectures as well as today’s lecture. Include information on thymic development, selection, activation, clonal proliferation and differentiation into Tc and Th cells. Include the role of antigen in these processes. (The highlights of this were presented in the candy bar exercise.)

5. The acquired immune response is said to have four characteristics: antigenic specificity, diversity, memory and self/nonself recognition. Explain how the processes of T cell ontogeny and clonal selection are responsible for these properties.

SELECTED ANSWERS

1. a. False, CD4 T cells recognize BOTH peptide + MHC and the costimulatory molecule B7. Not all professional antigen presenting cells express B7; therefore, not all APCs are equally effective at T cell activation.

b. False, like other nucleated cells professional antigen presenting cells express class I constitutively.

c. True.

d. True.

e. False, β− and γ-chains are expressed on resting T cells. On mature T cells, the α-chain is only expressed following activation.

f. False, naive T cells generally survive a few months to a year in the periphery without antigen stimulation. Clonal proliferation and memory cells account for immunological memory.

2. a. B7

b. IL-2

c. CD28, B7

d. CD4 T cells, cytokine

e. Endogenous, class I

f. Exogenous, class II

g. Native, processed
Quick questions (do not reflect quiz or exam content instead they provide a quick reflection of content)

1. MHC class I molecules (HLA-A, B, and C) are important because they
   A. Bind processed antigen and present it to helper T cells
   B. Have binding sites for CD8 molecules
   C. Present exogenous antigens effectively
   D. Present native viral peptides to killer T cells

2. Explain why each of the following ligand : receptor pairs is important in B cell synthesis of IgG against viral antigen Q.
   A. Viral antigen Q on class II MHC : TCR on a viral A specific Th cell
   B. CD40 (B cell) : CD40L (T cell)
   C. B7 (B cell) : CD28 (T cell)
   D. ICAM-1 : LFA-1
   E. Antigen Q binding to the BCR

3. Go through each of the following statements and determine if they are true or false and explain why.
   A. Th 2 cells make cytokines that promote allergic responses.
   B. Cytokines synthesized by Th1 cells inhibit the generation of Th2 cells
   C. Cytokines synthesized by Th1 and Th2 cells inhibit the generation of Th17 cells
   D. T reg cells are inhibited by Th1 and Th2 cells
   E. Newly activated CD8+ T cells will rapidly kill the virus infected APCs that entered the lymph node to stimulate it.
   F. CTLs kill via necrotic death
   G. Th2 cells promote macrophage function
   H. Th1 cells do not promote B cell functions
**Brief answers**

1. B. Class I MHC molecules (HLA-A, B, and C) have a CD8 binding site on their side that facilitates CTL recognition. Class I MHC molecules present antigens that were processed via the endogenous pathway.

2. Explain why each of the following ligand:receptor pairs is important in B cell synthesis of IgG against viral antigen Q.
   
   A. Viral antigen Q on class II MHC : TCR on a viral A specific Th cell
      *Provides T cell activation allowing effective T cell help (cytokines, CD40L)*
   
   B. CD40 (B cell) : CD40L (T cell)
      *Prompts isotype switching to IgG*
   
   C. B7 (B cell) : CD28 (T cell)
      *While effector T cell do not need CD28 ligation for activation or function, binding of CD28 helps to enhance that process.*
   
   D. ICAM-1 : LFA-1
      *Just like adhesion molecules are needed for neutrophil firm adhesion and the binding of CTLs to their target cells, Th cells need adhesion modules to bind to APCs (B cells in this case) to deliver effective signals both into the B cell and the T cell for collaboration.*
   
   E. Antigen Q binding to the BCR
      *Antigen binding to the BCR activates the B cell prompting antibody production. The antigen that binds to the BCR also becomes the antigen source of the exogenous antigen processing pathway in the B cell leading to the viral antigen being presented on class II MHC (HLA-DR, DP, and DQ).*

3. Go through each of the following statements and determine if they are true or false and explain why.
   
   A. Th 2 cells make cytokines that promote allergic responses.
      *True, IL-4 promotes IgE and mast cell production. IL-5 promotes eosinophil production.*
   
   B. Cytokines synthesized by Th1 cells inhibit the generation of Th2 cells
      *True, IFN-γ and IL-12 inhibit Th2 generation and promote the development of Th1 cells.*
   
   C. Cytokines synthesized by Th1 and Th2 cells inhibit the generation of Th17 cells
      *True, Th17 cells are induced by the presence of TGF-β and proinflammatory cytokines IL-23 and IL-6 as well as the lack of cytokines that promote the Th1 and Th2 pathways. This new T cell subset, Th17 cells, makes IL-17 that prompts the recruitment of neutrophils.*
   
   D. T reg cells are inhibited by Th1 and Th2 cells
      *False, while the generation of Treg cells is inhibited by proinflammatory, Th1 and Th2 cells, the role of Treg cells is to inhibit the function of Th1 and Th2 cells. Treg cells are best generated under “tolerogenic states” where there are high levels of TGF-β, and low levels of IL-6, IL-23, and other proimmune cytokines. The exception to this is IL-2. Treg cells use IL-2 to grow.*
   
   E. Newly activated CD8+ T cells will rapidly kill the virus infected APCs that entered the lymph node to stimulate it.
      *False, while dendritic cells are often infected by virus when they enter a lymph node during a viral infection, newly activated CD8+ T cells need to undergo differentiation and proliferation before they become functional effector cells. This can take many days, and allows the infected dendritic cell to induce further T cell stimulation before its demise.*
   
   F. CTLs kill via necrotic death
      *False, CTLs kill target cells via apoptosis. This “neat and tidy” death helps to ensure that the pathogen isn’t released and is cleaned up by macrophages.*
   
   G. Th2 cells promote macrophage function
      *False, while Th2 cells do express CD40L which stimulates macrophage function, Th2 cells generate potent macrophage inhibitors like IL-4 and IL-10 that ultimately downregulate macrophage function during Th2 reactions. Th2 cells are known for their ability stimulate antibody production and allergic reactions.*
   
   H. Th1 cells do not promote B cell functions
      *False, while Th2 cells make potent B cell activators like IL-4, IL-5, and IL-6, Th1 cells do have stimulatory effects on B cells through the generation of IL-2, IFN-γ, and CD40L. The differences between the Th1 and Th2 is both quantitative (Th2 cells promote more antibody than Th1 cells) and qualitative (Th1 promotes some IgG subclasses while Th2 promotes other IgG subclasses, IgA, and IgE). It does fit that Th1 cells would make IgG that is able to promote macrophage and NK cell function.*
Learning Objectives:

1. Identify the different arms/processes of the immune system and know how they are selectively activated by specific signals given by different classes of microorganisms.
2. Compare and contrast the immune mechanisms used to control intracellular versus extracellular pathogens.
3. Delineate how the immune system can cause tissue damage in the process of eliminating pathogens.
4. Identify general mechanisms by which pathogens evade immune system defenses.

References: Parham, The Immune System. Chapter 8   Abbas, Basic Immunology Chapter 6: p.118 and Chapter 8

I. Introduction

Immune System Function – resist pathogens (microbes that cause disease), foreign bodies and abnormal cells. The immune system is an intricate network of tissues, cells and molecules which function through various mechanisms to achieve resistance or immunity to disease caused by pathogenic microorganisms. Resistance to pathogens is the primary function of the immune system. This immunity can be acquired by natural infection with these microbes or by deliberate immunization with microbes or pieces of them (vaccines). The response of the immune system may prevent infection entirely or keep infections self limiting. When the immune system fails to develop (congenital deficiencies) or becomes severely compromised (AIDS), the result is death unless there is outside intervention (e.g.,. antibiotics, immunotherapy etc..).

II. The Organisms - Classes of Pathogenic Microorganisms

A. Bacteria – two major classes based on cell wall composition which can be visualized by special stain (Gram stain) Gram positive organism – thick peptidoglycan layer, Gram negative organisms – thin peptidoglycan layer plus the presence of lipopolysaccharide (LPS).

Extracellular Bacteria – these organisms replicate outside of host cells, primarily in the airways connective tissue, gut lumen and the blood. They include the majority of bacteria (gram positive cocci, gram negative enteric rods). They tend to cause pathology using one of two mechanisms; causing inflammation or producing toxins.

Intracellular Bacteria - facultative intracellular organisms include bacteria that can survive and replicate both outside and inside host cells. Many are uniquely adapted to survive within macrophages. Frequently these organisms cause pathology by causing chronic infections which activate and sustain immune response against host tissue. Obligate intracellular bacteria (very few bacteria) MUST replicate within cells.
B. **Viruses** – these organisms are obligate *intracellular* pathogens that can only replicate inside host cells, using the synthetic machinery of the cell. They invade a large variety of cells types by using normal host cell molecules as receptors for attachment and cell entry. They come in two forms: naked and enveloped. Viruses cause disease a number of different ways including; direct cell lysis, inciting host immune response and transformation of host cells.

C. **Fungi** – fungi are *eucaryotic* organisms and some replicate extracellularly and others intracellularly. Some do both. Fungi usually cause only mild disease in healthy individuals. Those who have underlying illness, or are immunodeficient may acquire severe life threatening fungal infections.

D. **Parasites** – unicellular and *multicellular eucaryotic* organisms that account for more morbidity and mortality that any other class of microorganism, particularly in developing countries. Some replicate intracellularly, some extracellularly. Others have complex life cycles that contain both intra- and extra – cellular stages.

### III. GENERAL IMMUNOLOGY CONCEPTS RELATED TO PATHOGENS

The point of entry and focus of infection as well as the site of replication (intra vs extracellular) frequently dictates the type and magnitude of immune response against the organism.

A. **INNATE (NON-SPECIFIC) IMMUNITY** – provides early defense against pathogens

1. **Exterior defenses**- first line of defense against microorganisms; skin, mucosal epithelium, lysozyme in secretions, acidic environment of the stomach.

2. **Interior defenses** - tissue damage either by infection or trauma causes *inflammation*, a non specific immune response aimed at clearing the tissue of foreign or dead material and tissue regeneration. The primary mediators of inflammation are the phagocytes: *neutrophils and macrophages*. Acute inflammation is characterized by vascular changes including vasodilation and increased permeability which allows cells and fluid to accumulate in the affected tissue. Neutrophils and macrophages, while both phagocytic play different roles in the response to infection.

Innate defense mechanisms are **present prior to exposure to infectious agents**, are not enhanced by repeated exposure (no memory), and do not discriminate among individual pathogens (although different classes of organisms activate different innate components i.e., viruses activate NK cells)

**Receptors of Innate Immunity**

a. **Pattern Recognition Molecules** – receptors located on phagocytes that recognize repeating molecular patterns located on the surfaces of microorganisms. This is a mechanism by which the immune system can be non-specifically activated by many different microorganisms. Examples include: the mannin binding lectin that initiates the lectin Complement pathway, and the macrophage mannose receptor which mediates receptor mediated endocytosis.

b. **Toll-Like Receptors** (TLR) – signaling molecules located on phagocytes and dendritic cells that recognize structure on classes of pathogens and that induce responses that lead to innate and acquired immune responses. They were first discovered in the fruit fly, where they are called Toll proteins. Currently, we know the most about TLR-4, which is activated by the binding of
LPS-LPS binding protein complex to CD14 on macrophages. The activated CD14 molecule associates with TLR-4 on the surface of the macrophage resulting in signaling through TLR-4. Signaling through TLR-4 leads to the activation of NFkB (transcription factor) in the nucleus resulting in pro-inflammatory cytokine release.

**B. ACQUIRED (specific, adaptive) IMMUNITY** - these immune responses are **highly specific** for each particular pathogen. The responses **improve** with each encounter (magnitude and speed of responses increases) because the immune system **remembers** the pathogen. Mediated by T cells (cellular) and B cells (humoral).

### IV. General Mechanisms of Anti-Microbial Immunity

**A. Immunity to Extracellular Bacteria**

**Innate** – principal mechanisms are complement activation (alternative pathway), phagocytosis (neutrophils) and inflammation.

**Acquired** – humoral immunity functions to eliminate the bacteria or to neutralize their toxins.

1. **opsonizing antibody** – (IgG) enhances phagocytosis with and without C3b (encapsulated bacteria, gram positive bacteria)

2. **neutralizing antibody** -  inhibits attachment to receptors on host tissue, binds toxins and inactivates them (toxin producers)

3. **complement activation** - classical pathway, lysis of gram negative bacteria, C3b opsonization of gram positives, inflammation.

**B. Immunity to Intracellular Bacteria**

**Innate** – phagocytosis by macrophages (inactivated), may result in limited killing or inhibition of replication, ultimately it will not control the infection.

**Acquired** – cell-mediated immunity (CD4 and CD8 mediated)

1. **CD4+ T cell** activation, with the differentiation into **Th1 effectors** under the influence of IL-12. Th1 cells secrete **IFN-γ** which activate macrophages to kill intracellular bacteria. (most intracellular bacteria)

2. **CD8+ T cell** differentiation into **cytotoxic T cells** which lyse infected macrophages. This allows the bacteria to be phagocytized by activated macrophages which can kill them (some intracellular bacteria).

3. **Activated macrophages** – macrophages activated by **IFN-γ** in association with other cytokines (TNF-α, TNF-α, GM-CSF) become effector cells which kill via variety of mechanisms including the elaboration of ROI and RNI, secretion of lysozyme and other antimicrobial peptides), secretion of cytokines (which activate other effector cells)
The macrophage activation that occurs as a result of Th1 activation is also capable of causing tissue damage. Because intracellular bacteria are particularly good at resisting the anti-microbial activities of macrophages, these organisms often persist for a long time resulting in a chronic struggle for the immune system. This frequently results in **granuloma** formation, an attempt to wall off the organisms. Extensive granuloma formation results in massive tissue destruction and loss of function. Example – **tuberculosis**.

Differences among individuals in the strength and character of the immune response to an organism may directly affect disease progression and clinical outcome. Example, **leprosy** – Th1 response to the organism results in the tuberculoid form (restricted growth of organisms, tissue destruction by the immune system), less severe form, responds to treatment – Th2 response to the organism results in the lepromatous form (unrestricted growth of the organisms, extensive damage) may require life-long treatment.

**Evasion Strategies**

- a) exotoxins – leukocidins that kill or impair phagocytes
- b) IgA protease – inactivates IgA
- c) capsules or slimes – that prevent phagocytosis
- d) prevention of phagosome-lysosome fusion
- e) escape from the phagosome

**C. Immunity to Viruses**

**Innate** – principal mechanisms include 1) inhibition of infection by the production of Type 1 interferons (IFN-α and IFN-β) by infected cells which produce a local and transient anti-viral effect on neighboring cells and 2) Natural Killer cell mediated lysis of virally infected cells.

**Acquired** – both humoral and cell mediated immunity is involved in the resolution and resistance to viral infections.

1. **Humoral** – *neutralizing* antibodies (IgG) prevent viral attachment and entry into host cells. Antibodies are only effective during the extracellular stage of viral infection i.e., prior to entry into host cells or in the case of cytopathic (lytic) viruses after release from the cell but before entry into another neighboring cell. IgA antibodies may neutralize viruses that enter via the respiratory or intestinal mucosa. **Complement** activation also participates in antibody mediated immunity by promoting phagocytosis and by directly lysing enveloped viruses.

2. **Cell mediated** – virus specific CTLs are CD8+ T cells which recognize cytosolic endogenously processed viral antigens in association with MHC Class I molecules. The infected cells are lysed by the CTLs. In order to fully activate CTLs, they require co-stimulation by cytokines secreted from activated CD4+ T cells. Only cell mediated but not humoral immune mechanisms can eradicate an established viral infection.

**Evasion Strategies**

1. downregulation of MHC Class I proteins
2. virokines and viroreceptors
D. Immunity to Fungi

**Innate** - phagocytes are the most important innate defense against fungi. They liberate ROI, RNI, lysosomal enzymes.

**Acquired** – Cell mediated immunity, specifically Th1 mediated granulomatous responses control many of the opportunistic and systemic fungal infections. This frequently causes host cell injury in the form of granulomas.

E. Immunity to Parasites – these include a wide range of pathogens from intracellular organisms to worms that can be seen with the naked eye. The immune responses that control them are diverse as well.

**Acquired** – for the intracellular pathogens of macrophages, Th1 responses are most important. To control worm (helminth) infections, the activation of Th2 cells resulting in IgE and the activation of eosinophils is important. For one of the most important parasitic infections, malaria, the combination of immune response required to eliminate this organism is still not fully understood.

**Evasion strategies**

1. cuticle formation
2. antigenic variation

V. Conclusion

Many pathogens have evolved with their human hosts for thousands of years. Remember, the goal of the pathogen is to survive and replicate – not to kill its host. The better adapted a pathogen – the less likely that it will kill its host or at least do so very slowly. The goal of the host is to eliminate the pathogen quickly so as to incur the least amount of damage. Over time, both humans and the microbes have adapted strategies for success. These strategies constantly evolve. Ultimately, it is the balance between the microbe’s ability to resist host immunity (*stayed tuned until next year in Microbiology*) and the power and nature of the host’s immune responses that determines the outcome of infection.