**Adaptive immunity:**

**How T cells recognize antigen**

- Each clonal T cell receptor (TCR) is specific for a particular sequence of amino acids in a small peptide antigen (9-16 amino acids)

- The peptide is generated from proteins in antigen-presenting cells, where it bind to MHC (Major Histocompatibility Complex) molecules

- **Both the amino acids of the bound peptide and the presenting MHC molecule are recognized by the TCR: p-MHC**

---

**Generation of the repertoire of T cell clones**

in the adaptive immune system of each individual presents three challenges:

First

*The great number of pathogen peptides*

- Peptides of 10 amino acids in length
- 20 amino acids

\[ \text{# of different peptides} = 20^{10} \approx 10^{13} \]

Require \( > 10^{13} \) T cell clones each with different TCRs to recognize this array of peptides presented by different MHC molecules

Solution: a **somatic recombination mechanism** to generate the large number of structurally diverse clonal TCRs, not enough DNA in genome to encode this number of different TCR genes
Generation of the repertoire of T cell clones in the adaptive immune system of each individual presents three challenges:

Second

*Microbial pathogens can mutate around a stereotyped defense recognition system*

Solution: evolve many alternative forms of MHC molecules that bind completely different pathogen peptides

- This evolutionary strategy equips the species with a large number of alternative MHC molecules that differ in their binding pockets, and thus bind and present different peptides
- This results in MHC genes being extremely polymorphic

**P-MHC**

*(HLA)*

- Adaptive immune system based on differences of MHC molecules among individuals that confer specificity for different peptides
- The specificity of peptide binding is determined by pockets in the MHC molecules that only bind certain amino acid side chains

"Pocket" of MHC

Polymorphic residues of MHC

Peptide

Anchor residue of peptide

Polymorphic residue of MHC

T cell contact residue of peptide

MHC

T cell receptor

**Solution:**

- Evolve many alternative forms of MHC molecules that bind completely different pathogen peptides.
Strategy: duplicate loci and increase number of alleles

Number of alleles

<table>
<thead>
<tr>
<th>HLA-A</th>
<th>HLA-B</th>
<th>HLA-C</th>
<th>HLA-DR</th>
<th>HLA-DQ</th>
<th>HLA-DP</th>
</tr>
</thead>
<tbody>
<tr>
<td>451</td>
<td>238</td>
<td>789</td>
<td>522</td>
<td>34; 71</td>
<td>23; 124</td>
</tr>
</tbody>
</table>

Possible Combinations of alleles

- 84,689,682
- 304,360,151,838,000,000

Generation of the repertoire of T cell clones in the adaptive immune system of each individual presents three challenges:

Three

The adaptive immune system must develop T cell clones that specifically bind and recognize pathogen peptides prior to encountering the pathogen

Solution: Use self-peptides as a surrogate for pathogen peptides
Problem: the TCR of randomly generated T cell clones could either be incapable of recognizing one’s own MHC, or alternatively strongly recognize self-peptides presented in self MHC

This requires a clonal selection process centered in the thymus and driven by self p-MHC to select the repertoire of clones with TCR appropriate for the self-MHC and self-peptides of each individual

- Non-reactive against self  (“Tolerance”)  
- Reactive against non-self

The selection process (Thymic “education”) has two stages that occur during T cell development

- **First stage** selects T cell clones that recognize self-peptide in an individual’s own MHC molecules - *positive selection*

  ![Diagram](chart.png)

- **Second stage** negative selection eliminates overtly self reactive clones with high affinity for self-peptide- MHC …”central tolerance”

  (Self-peptides are used as a surrogate for foreign peptides)
Immunologic self is the set of self-peptides and self-MHC molecules that generates and is recognized by the individual’s adaptive immune T cell repertoire.

One of the major functions of the innate immune system natural killer (NK) cell population is to detect decreases in the expression of the MHC portion of “self” p-MHC.

The set of self-MHC molecules varies from individual to individual because of MHC polymorphism.

Accordingly, the total TCR repertoire selected on self peptide-self MHC is nearly unique for each individual:

- Major selective advantages to the species since there is essentially no set of stereotyped recognition structures shared by different individuals in the species.
- Other individuals of the same species inherit different MHC alleles and their cells and tissues are recognized as non-self and attacked as if they were pathogens…Histocompatibility.
- However because the adaptive immune system is patterned on self, it sets the stage for the development of autoimmune disease.
Primary immune response

The T cell clones generated by selection on self-peptides that recognize, but are relatively unresponsive to self (tolerance), are then used in each adaptive immune response to identify non-self peptides typically encoded by pathogens.

The non-self peptides are analogously presented by self-MHC molecules and are recognized by TCR of T cell clones as “not quite-self” (altered self) when triggered by innate immune signals, resulting in T cell activation.

Types of surveillance for pathogen peptides

*There are fundamentally two classes of pathogens that the immune system must recognize and respond to: viruses and bacteria*

- A viral peptide on a cell’s MHC molecules signifies to a T cell that it is infected and should be killed.
- A bacterial peptide on a phagocytic cell that ingested a bacterium signifies to a T cell the phagocyte has ingested a foreign substance and must be helped to eliminate the pathogen by the activated T cell.
Two different classes of MHC molecules direct the different immune responses to the two different pathogen types in this surveillance:

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Virus - or Pathogen infected cell</th>
<th>Bacteria or components of an extracellular pathogen that have been phagocytized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presenting cell</td>
<td>Any nucleated cell</td>
<td>Macrophage/DC</td>
</tr>
<tr>
<td>Peptide presented on</td>
<td>MHC class I molecules</td>
<td>B cell</td>
</tr>
<tr>
<td>MHC class II molecules</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The immune system makes this distinction by loading and recognizing peptides in either class I or class II MHC:

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Cytosolic Virus or Pathogen</th>
<th>Ingested Bacteria or Endocytic Pathogen</th>
<th>Extracellular Pathogen or Toxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presenting cell</td>
<td>Any cell</td>
<td>Macrophage/DC</td>
<td>B cell</td>
</tr>
<tr>
<td>Peptide degraded in</td>
<td>Cytosol</td>
<td>Endocytic vesicles</td>
<td>Endocytic vesicles</td>
</tr>
<tr>
<td>Peptides bind to</td>
<td>MHC class I</td>
<td>MHC class II (or I)</td>
<td>MHC class II</td>
</tr>
<tr>
<td>Presented to</td>
<td>CD8 T cells</td>
<td>CD4 T cells (or CD8)</td>
<td>CD4 T cells</td>
</tr>
<tr>
<td>Effect on presenting cell of T cell recognition</td>
<td>Death of cell presenting the viral antigen</td>
<td>Activation of cell to enhance pathogen killing</td>
<td>Provision of help to B cell for production of antibodies</td>
</tr>
</tbody>
</table>
Class I and II MHC molecules

Structural features that determine peptide binding

Structure of peptide-binding class I MHC domain
The ligand for the CD8 T cell TCR

MHC Class I Domains

The overall structure of class I and class II MHC is rather similar

Class I  Class II
MHC class I and II molecules have homologous domain organization, but different chain structure.

The Structure of MHC Molecules: MHC Class I

- The α chain is ~350 AA long
- Three globular domains, α1, α2 and α3, each ~90AA
- α1 and α2 form the antigen-binding cleft
- β2 microglobulin ~100AA, associates with the α3 domain, not MHC encoded
- ~70 AA transmembrane and cytoplasmic portion
The Structure of MHC Molecules: MHC Class II

- Composed of two similar membrane spanning proteins, the α-chain and β-chain both encoded within the MHC.
- Each chain is made of two globular domains, each ~90AA.
- α1 and β1 domains form the antigen-binding cleft.

How peptides bind

- Usually peptides are 9 amino acids in length.
- Always oriented with NH2 terminus to the left.
- Most often are anchored by interactions of the side chains of their 2nd (P2) and 9th (P9) amino acids to MHC pockets that confers specificity for amino acids with similar physical properties, e.g. size, charge, hydrophobicity, etc.

Rules for binding to MHC class I molecules
The bound peptide must be oriented in the same direction in the MHC to allow the TCR clone specific for the peptide to identify it.

Cluster of tyrosines recognize NH$_2$

COOH

+ charged amino acids interact with negative COOH

The bound peptide must be oriented in the same direction in the MHC to allow the TCR clone specific for the peptide to identify it.

Rules for peptide binding to MHC class I molecules

Role of side chains

3 Different Proteins yield 3 different peptides that can bind to the same MHC molecule

A MHC Class I molecule selects homologous peptides derived from different proteins that have P2 and P9 side chains composed of homologous amino acids, e.g. tyrosine and leucine or isoleucine.
MHC class II molecule binding a peptide

Class II MHC molecules are only constitutively expressed on “professional” antigen presenting cells: DC, macrophages and B cells.

How peptides bind

Rules for binding to MHC class II molecules

TCR

• Side chains in the middle of the peptide tether it to pockets via multiple hydrogen bonds, van der Waals and electrostatic forces
• The peptide ends are free and the peptide length is variable
• Interactions with the peptide backbone orient the peptide as in class I molecules
Different rules for peptide binding to class II MHC molecules

Peptides binding class II molecules vary in length, are anchored in the middle, but are also always oriented with NH2 termini to the left.

Genetic polymorphisms of MHC genes

HLA Genetics
Organization of the MHC

Two classes of peptide presenting MHC molecules are encoded by the HLA ABC and D genes

<table>
<thead>
<tr>
<th>Class I</th>
<th>Class III</th>
<th>Class II</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-A</td>
<td>C4A</td>
<td>HLA-DR</td>
</tr>
<tr>
<td>HLA-B</td>
<td>C4B</td>
<td>HLA-DQ</td>
</tr>
<tr>
<td>HLA-C</td>
<td>C2</td>
<td>HLA-DP</td>
</tr>
<tr>
<td></td>
<td>Bf</td>
<td>TNF-α</td>
</tr>
</tbody>
</table>

Diversity of MHC class I and II genes

Arises from two mechanisms:

- **Duplication of a gene locus in an individual** resulting in multiple loci, *polygeny*

  - HLA-A
  - HLA-B

  Isoforms in same person

- **Development of multiple alleles at a locus among individuals in the species**, *polyallelism*

  - HLA-A1
  - - A2
  - - A3
  - - A11

  - HLA-A1
  - HLA-B7

  - - A2
  - - B8
  - - A3
  - - B15
  - - A11
  - - B27

  Alleles in different individuals
MHC polymorphism is all about survival, it is an evolutionary response to the structural diversity and mutation potential of microorganisms

No practical biologic limit on the number of alleles for the species

Frequency-dependant selection- The individual with the rarest allele has the best chance to survive an infection

**Duplication of a locus incurs a risk**

• Each duplication results in a new set of antigen-presenting structures

• Each MHC type selects its own allele-specific TCR clonal repertoire capable of recognizing additional pathogen peptides

• However, each duplication increases the size of immune self and mandates more negative clonal selection across all repertoires during repertoire formation, reducing the size of the repertoire for each allele

**Practical maximum is ~ three loci each for class I and class II**

<table>
<thead>
<tr>
<th>HLA-DR</th>
<th>HLA-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-DQ</td>
<td>HLA-B</td>
</tr>
<tr>
<td>HLA-DP</td>
<td>HLA-C</td>
</tr>
</tbody>
</table>

(Remember both maternal and paternal alleles are expressed)
Nomenclature

Genotype: the collection of genes in an individual, usually referring to a small segment of a chromosome

Alleles: the alternative forms of a gene found in different individuals

Allotypes or allomorphs: the different protein forms encoded by alleles

Haplotype: the genes (alleles) contributed by one parent, usually referring to alleles of both class I and class II loci

Gene loci exhibit linkage, a measure of their genetic distance

Linkage disequilibrium: certain alleles in a haplotype are found together significantly more (or less) frequently than expected by chance

Nomenclature: The genetic “unit” of the HLA system is the allele, with each defined by its own DNA nucleotide sequence

E.g. HLA-B*0801

<table>
<thead>
<tr>
<th>Allele</th>
<th>“Specificity”</th>
</tr>
</thead>
<tbody>
<tr>
<td>*0801</td>
<td>HLA-B8</td>
</tr>
<tr>
<td>*0802</td>
<td></td>
</tr>
<tr>
<td>...</td>
<td></td>
</tr>
<tr>
<td>*0821</td>
<td></td>
</tr>
</tbody>
</table>

But to make things “simpler”, alleles can be grouped in families, e.g. HLA-B*27

“specificity”, is an old nomenclature used when human alloantibodies were used to first detect HLA serologic “specificities” or “antigens”
Codominant expression of MHC alleles

- Yellow = paternal haplotype
- Blue = paternal haplotype
- Green = maternal haplotype
- Pink = maternal haplotype

A graft is compatible only if there is a complete match at all MHC alleles, i.e. a two haplotype match for all MHC loci.

HLA genetics in transplantation

A graft is compatible only if there is a complete match at all MHC alleles, i.e. a two haplotype match for all MHC loci.

- Yellow = paternal haplotype
- Blue = paternal haplotype
- Green = maternal haplotype
- Pink = maternal haplotype

Note that in a family the parents always differ by one haplotype from the children, while children may share 0, 1 or 2 haplotypes.

In situations where a transplant is required, the family is first typed to find 2 haplotype matches, then unrelated individuals are studied.