**HLA Genetics**

Different MHC alleles confer different functional properties on the adaptive immune system

**Consequences for regulation of adaptive immunity:**

- The large number of MHC alleles means each individual has a nearly unique set of peptide-presenting allotypic MHC molecules
- These molecules present self-peptides during thymic development of the T cell repertoire and select a nearly unique repertoire of T cell clones that differs among individuals

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**Consequences for Transplantation**

The differences MHC between individuals means that the cells of a donor who differs from the recipient by any of the MHC alleles are recognized as non-self by the T cells of the recipient and are attacked as if they were a foreign substance

This difference is the origin of the name “major histocompatibility complex” that reflects the role for these molecules as the primary genetically determined targets for graft rejection or compatibility

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**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 at the same locus in different individuals

Allotypes or allomorphs: 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

**Methods used to detect MHC alleles “HLA typing” vary in their resolution to detect specific alleles or only groups of similar alleles**

- Pregnancy or transplant sera
- Complement mediated cytotoxic or immunofluorescence reactions on living lymphocytes
- Recognize broad “serologic specificities”, e.g. HLA-B27

**DNA probes or primers**

- DNA hybridization or sequencing
- Specific alleles (or defined families of related alleles), e.g. HLA-B*2705

But to make things “simpler”, alleles are grouped in families as “specificities”, e.g. HLA-B8 or HLA-B27, reflecting an old nomenclature used when human alloantibodies were used to first detect HLA “specificities”
Codominant expression of MHC alleles

- a = paternal
- b = paternal
- c = maternal
- d = maternal

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

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

If an immune response to a peptide is determined by a particular haplotype, e.g. “a” it exhibits codominant inheritance.

How does polymorphism influence binding of different peptides and what is its immunologic significance?

**HLA alleles act as Immune Response genes by determining which peptides are presented to a T cell**

The precise size, shape and charge of each peptide binding pocket in an MHC allotype are determined by particular amino acids coded for by polymorphisms that distinguish each of the MHC class I alleles.

These change the type of peptide that is bound and the interaction of the MHC with the TCR.

Polymorphic Amino acids that distinguish alleles of MHC class I molecules are found primarily in pockets that determine peptide binding or on the surface that interacts with the TCR.

Primary Anchors P2, P9
Secondary anchors P3, P6, P7
Location of MHC class I pockets termed “B” and “F” that bind P2 and P9 amino acid side chains of the peptide.

Example

<table>
<thead>
<tr>
<th>Position</th>
<th>B pocket</th>
<th>Allele</th>
<th>Amino acid bound at P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>Glu</td>
<td>B*2705</td>
<td>Lys/Arg</td>
</tr>
<tr>
<td></td>
<td>Lys</td>
<td>B*4001</td>
<td>Asp/Glu</td>
</tr>
</tbody>
</table>

Amino acids of the MHC molecule that form the B and F pockets confer the main specificity for binding peptides through complementary interactions with amino acids at position P2 and P9 in the peptide.

What peptides are found in MHC molecules?

- Elution of peptides from MHC molecules reveals that class I molecules typically bind 2000-10,000 different peptides per cell.
- Each of these peptides has the dominant motif reflecting the relatively conserved anchor residues, e.g., for HLA-B27: Motif XRXXXXX[KRYL]
- Most peptides are fragments of conventional cell proteins, e.g.:
  - HRAQVIYTR 40S ribosomal protein
  - RREEKERYK Heat shock protein 89
  - RRFFPYVYY Proteasome subunit C5
  - GRWPSSL Lamin B receptor
- Even the most abundant peptide species accounts for only 1% of the total peptides bound, so the T cell has its work cut out.

What accounts for the large number of HLA alleles?

- Homogeneity in a population allows a pathogen to adapt to a molecularly stereotyped adaptive immune response = disadvantage.
- Heterozygosity results in a more vigorous T cell response with more clones recognizing more peptides.
- Epidemics favor individuals with rare allotypes = frequency dependent selection.
- Selection for alleles depends on particular pathogen environment.
- Small populations loose alleles by chance = drift.
- Tribal amalgamation results in a large population with many alleles.

HLA alleles influence the number of peptides in a protein that can be recognized (Example HIV envelope protein), and thus the number of different T cell clones responding.

<table>
<thead>
<tr>
<th>HLA alleles</th>
<th>Peptides in HIV env able to bind each HLA allotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-B*2705</td>
<td>IRGKVQKEY KRRVYRREK  DFNPQAVVL</td>
</tr>
<tr>
<td>HLA-B*3501</td>
<td>IRPVVSTQL ARIVALERY  KPCVLTPL</td>
</tr>
<tr>
<td>HLA-B*0702</td>
<td>TRFENNTREK ERDRDSRIR  RPVSTQGL</td>
</tr>
<tr>
<td></td>
<td>DFFQGDRVLR LRSLGFSY  SPLSFQTHL</td>
</tr>
<tr>
<td></td>
<td>SRAKNWTRL TRVEVLGR  IFPRIRQGL</td>
</tr>
<tr>
<td></td>
<td>LREQQGGNMR CRAIRHMVR  PRRQGDEM LRQGQMRL</td>
</tr>
<tr>
<td></td>
<td>WRRSELYKVX</td>
</tr>
<tr>
<td># of peptides</td>
<td>15</td>
</tr>
</tbody>
</table>

**MHC molecule expression**

**Assembly of class I MHC molecules**
Organization of the MHC

Class II
- HLA-DR
- HLA-DQ
- HLA-DP

Class III
- TNF-α

Class I
- HLA-A
- HLA-B
- HLA-C

Maximum number of different types of MHC class I molecules (allotypes) expressed on the cell surface

<table>
<thead>
<tr>
<th>MHC class I loci</th>
<th>Specificity (Antigen)</th>
<th>Allele designation</th>
<th># of alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-A α-chain</td>
<td>A1, A2,...</td>
<td>A*0101,...</td>
<td>303</td>
</tr>
<tr>
<td>HLA-B α-chain</td>
<td>B7, B8,...</td>
<td>B*0702,...</td>
<td>559</td>
</tr>
<tr>
<td>HLA-C α-chain</td>
<td>Cw1, Cw2...</td>
<td>C*0101,...</td>
<td>150</td>
</tr>
</tbody>
</table>

Codominant expression of MHC genes yields 6 different class I molecules (allomorphs) on the cell

<table>
<thead>
<tr>
<th>Maternal haplotype</th>
<th>Paternal haplotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4mb</td>
<td>4mb</td>
</tr>
</tbody>
</table>

Each of these 6 MHC molecules selects its own T cell repertoire that only recognizes peptides presented by that particular type of MHC molecule - MHC restriction

What does the T cell see?

T cell receptors interact both with the MHC molecule and with the bound peptide

CDR3 regions of TCR α-chain and β-chain primarily, but variably, interact with the antigenic peptide
T cell receptors interact both with the MHC molecule and with the bound peptide

However each TCR contacts peptide and MHC slightly differently

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What are the immunologic consequences of the dual specificity of TCR for peptide and MHC?

MHC restriction of T cell recognition

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Because the TCR recognizes both peptide and MHC molecule, T cell recognition of MHC-peptide is both MHC restricted and specific for the immunizing peptide

In each of the 3 experiments the T cell is from a HLA-B7 person who recovered from infection by virus "X". The APC target cell is either infected with virus X or Y and is from an individual who is either HLA-B7 or HLA-B27

Target killed: Yes No No

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The recent sequencing of the MHC as part of the genome project has revealed a wealth of knowledge about this gene complex

Apart from the role of class I and class II MHC genes in antigen presentation, many of the other genes comprising the entire MHC appear to have been selected for antigen processing and other roles in the immune response
The MHC class I region contains additional class I genes other than HLA-A, B and C

**MHC class IB genes**
- **HLA-G** the only MHC class I expressed on trophoblast and inhibits NK cell killing of fetus
- **HLA-E** binds leader peptide of classic class I molecules, HLA-A, B and C and inhibits NK cell killing of cell by engagement of CD94 / NKG2A receptor

To escape recognition, an obvious strategy for a pathogen to take is to have a gene or genes that interferes with peptide processing or presentation on MHC class I (or II) molecules

To counter this, the NK cell lineage has evolved a set of receptors that detect "missing self", a decrease in the expression of class I MHC molecules

**Problem: how is the absence of a molecule recognized?**
- The NK cell is capable of "spontaneous" killing
- But it expresses an inhibitory receptor that binds an MHC ligand
- Normal cells are protected from spontaneous killing when they appropriately express this MHC ligand
- Loss of MHC ligand releases the NK cell from its tonic inhibition via engagement of the inhibitory receptor, resulting in its activation

**Mechanisms pathogens use to subvert adaptive immune recognition**

**Promiscuous triggering of TCR**
- Staphlococcal enterotoxin or Toxic Shock Syndrome
  - Toxin are superantigens that bind MHC II and one or a few Vβ families
  - The massive release of cytokines blocks an adaptive response and causes systemic toxicity

**Block immunosurveillance function**
- Inhibition of class I expression
  - Cytomegalovirus, HIV, Herpes simplex, Adenovirus
- Inhibition of TAP transport of peptides
  - Herpes simplex
- Proteasomal blockade
  - Epstein Barr virus EBNA