Nomenclature

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

Allotypes or allomorphs: the different protein forms encoded by alleles

Genotype: the collection of genes in an individual, often referring to the two alleles of a locus

Haplotypes: 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 making up a haplotype are found together significantly more (or less) frequently than expected by chance. Ancestral or Extended haplotypes

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
    *0802
    ...
    *0821

*2701
*2702
*2703
...
*2725

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

“Specificity”, an old nomenclature used when human alloantibodies were used to first detect HLA serologic “specificities” or “antigens”

Codominant expression of MHC alleles

Relationships within a family

During pregnancy the mother tolerates the expression of paternal MHC molecules in the fetus (Fetal allograft)

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

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

HLA genetics in autoimmunity

Autoimmune diseases are determined by certain HLA alleles that present particular self-peptides

If a disease was determined by a gene on haplotype “d”

Only the individuals with haplotype “d” would be at high risk for its development (Used in children with T1 diabetes mellitus)
Different MHC alleles confer different functional properties on the adaptive immune system by specifying molecules that have different peptide binding abilities.

MHC alleles regulate immune responsiveness by influencing the number of peptides in a protein that can be recognized (Example HIV envelope protein)

<table>
<thead>
<tr>
<th>Allele</th>
<th>HLA-B*27052</th>
<th>HLA-B*3501</th>
<th>HLA-B*0702</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motif</td>
<td>XRRRRRRR[KRYL]</td>
<td>XPXXXXXXY</td>
<td>XRRRRRRR[KRYL]</td>
</tr>
<tr>
<td>Peptides able to bind each allelic HLA molecule</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRGKVQKEY</td>
<td>DFRPQEQVUL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRPVVSTQL</td>
<td>KPCVXZFL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRPNNTKX</td>
<td>RPVSTQLL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRQQGRG</td>
<td>SFLSQYTHL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SRAKNNTTL</td>
<td>IPRREIQGL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LREQQXNK</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRPGGQXK</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WRSELYKK</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td># of peptides</td>
<td>15</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

Location of MHC class I pockets termed “B” and “F” that bind P2 and P9 amino acid side chains of the peptide

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 a dominant motif reflecting the relatively conserved anchor residues, e.g. for HLA-B27
  
  Motif XRRRRRRR[KRYL]
  
- Most peptides are fragments of conventional cell proteins, e.g.
  
  - HRAQVYTR: 40S ribosomal protein
  - RRIKEIVKK: Heat shock protein 89
  - ARLFGIRAK: Breast basic conserved protein
  - RRFPYYYV: Proteasome subunit C5
  - GRWPGLSL: Lam 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

6. How do peptides get loaded onto the proper kind of MHC molecule?

How do cytosolic viral peptides synthesized within virally infected cells get loaded only on class I, but not class II molecules, to trigger killing by CD8T cells?

How do peptides from endocytosed bacteria get loaded on class II, but not class I molecules, to elicit macrophage activation and B cell help?
The endocytic and synthetic pathways are usually quite separate.

**Class II peptide loading from peptides produced by lysosomal degradation of endocytosed organism**

**Class I peptide loading from peptides synthesized in the cytosol**

**Proteosome- digests ubiquitin-tagged cytosolic proteins**

The proteasome is composed of the proteasome catalytic core and regulatory complexes that bind and unfold ubiquitinylated substrates.

3 of the 7 subunits in each ring of the 20s core confer the proteolytic activity.

Peptide processing changes in an immune response:

IFN-γ, made by NK, CD8 and some CD4 T cells, upregulates the synthesis of three new proteasome immunosubunits

LMP2, LMP7 and MECL-1 replace the constitutive β1, β5 and β2 subunits, and change proteasome specificity to make hydrophobic peptides with greater affinity for MHC pockets.

The peptide boronate, Bortezomib, reversibly inhibits the chymotrypsin-like activity of the β5 subunit.

**Loading class I MHC molecules with cytosolic peptides**

MHC class I molecules are synthesized and bind peptides derived from cytosolic molecules during assembly within the ER.

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The peptide boronate, Bortezomib, reversibly inhibits the chymotrypsin-like activity of the β5 subunit.
Production of 9 AA peptides for class I MHC

The proteasome makes precise cuts only at the C termini of the peptides

Other peptidases, some in the e.r., nibble back the N termini until the peptide fits exactly, e.g. the IFN-γ-inducible leucine aminopeptidase (LAP)

The peptide production system is not coordinated with the peptide binding specificity of the individual’s MHC class I molecules

Class II loading is centered in the vesicular system

Acidic endosomal proteases digest ingested proteins into peptides that will load MHC class II molecules

This process does not require the precise proteolysis needed in the class I system, since the peptide termini are not constrained by MHC class II

Peptide and β2 microglobulin subunit are required to stabilize the MHC class I molecule

Empty MHC class I molecules are unstable

This prevents “friendly fire” killing of bystander cells by the uptake of random peptides by empty MHC molecules

Invariant chain (Ii)

Class II MHC molecule peptide loading depends on the synthesis of Ii

• A chaperone that complexes with MHC class II molecules during their synthesis in the endoplasmic reticulum

• Ii Blocks the class II peptide binding groove of the newly synthesized MHC class II molecule in the e.r. and prevents loading by peptides destined for class I molecules

• A recognition sequence on the Ii transmembrane portion redirects the nascent MHC II molecule to traffic to the acidic endosomal compartment where it will be loaded with the degraded ingested exogenous peptides

Within the acidic endosome, Ii is first degraded to CLIP (Class II-associated invariant chain peptide) by specific endosomal acidic cysteine proteases (cathepsins)
HLA-DM, an ancient but non-classical class II molecule catalyzes the release of CLIP and the binding of high affinity peptides via interaction of peptide amino acid side-chains with MHC pockets. Without Ii, the MHC class II molecule now is free to traffic to the cell membrane.

**7. Expression of MHC molecule on the cell surface, how it all works**

A review...

**Organization of the MHC**

<table>
<thead>
<tr>
<th>Class</th>
<th>MHC class I loci</th>
<th>Specificity</th>
<th>Allele designation</th>
<th># of alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>HLA-A α-chain</td>
<td>A1, A2,…</td>
<td>A*0101,…</td>
<td>673</td>
</tr>
<tr>
<td></td>
<td>HLA-B α-chain</td>
<td>B7, B8,…</td>
<td>B*0702,…</td>
<td>1077</td>
</tr>
<tr>
<td></td>
<td>HLA-C α-chain</td>
<td>Cw1, Cw2…</td>
<td>Cw*0101,…</td>
<td>360</td>
</tr>
</tbody>
</table>

β₂ microglobulin encoded on chromosome 15

10-20,000 molecules of each type are present on most cells.

During generation of the T cell repertoire each of these sets of 6 class I molecules bound its own set of self-peptides and independently selected its own repertoire of T cells.

Each can be separately loaded with different pathogen peptides.
Polygenic human MHC class II (HLA-DR) genes

<table>
<thead>
<tr>
<th>MHC class II loci</th>
<th>Specificity (Antigen)</th>
<th>Allele designation</th>
<th># of alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-DRA α-chain</td>
<td></td>
<td>DRA*0101</td>
<td>4</td>
</tr>
<tr>
<td>HLA-DRB1 β-chain</td>
<td>DR1, 2,…</td>
<td>DRB1*0101, …</td>
<td>669</td>
</tr>
</tbody>
</table>

Maximum number of different types of HLA molecules expressed on the cell surface

<table>
<thead>
<tr>
<th></th>
<th>Nucleated cells</th>
<th>Antigen presenting cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I (HLA-A)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Class I (HLA-B)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Class I (HLA-C)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Class II (HLA-DR)</td>
<td>0</td>
<td>2*</td>
</tr>
<tr>
<td>Class II (HLA-DQ)</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Class II (HLA-DP)</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>16</td>
</tr>
</tbody>
</table>

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

8. Recognition of p-MHC by the TCR

The classic Zinkernagel & Doherty experiment

How T cell responses differ in two unrelated individual with different MHC genes that are infected with the same virus

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

MHC I-CD8 TCR CDR3 hypervariable regions of TCR α and β-chain primarily contact peptide
Person A is infected by virus x e.g. influenza, and makes a T cell response

Isolate the responding T cell clone

Infect target cells of person A (HLA-B7) and person B (HLA-B27) with the same virus

As a control infect target cells of person A with another virus, e.g. herpes

In each experiment 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

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

The HLA-B27 person responds to the same virus and viral proteins, but selects different peptides to bind to the MHC molecule

9. When “Self” goes missing

One viral survival stratagem is to inhibit expression of class I MHC molecules, also seen in malignant cells

Several families of receptor, the natural killer (NK) receptors, exist to recognize the reduced expression of self-MHC

NKR are highly expressed on A special lymphocyte lineage, “NK cells” Effector CD8 T cells

Summary points

• During development ~16 T cell repertoires are separately selected on self-peptides presented by 3 types of class I and 3 types of class II MHC molecules; The T cell recognizes peptide-MHC

• Later during an immune response these same T cells recognize “not quite self”/non self peptides presented on these MHC molecules and the T cells then clonally expand

• MHC molecules are codominantly expressed, with class I molecules found on the surface of all nucleated cells and class II molecules on professional antigen presenting cells

• The alleles of the MHC genes specify different amino acids in MHC pockets that bind peptide side chains, and this confers specificity on MHC molecules to bind different peptides

• As a consequence individuals vary markedly in what particular peptides their T cells recognize…this results in allograft rejection

• Class I and class II MHC molecules differ markedly in the details of how they bind peptides and the biochemical pathways the peptides take to be loaded on the MHC. These differences assure that the correct CD4 or CD8 adaptive immune response is made to a peptide

• The fact that class I MHC molecules bind the CD8 molecule and class II MHC molecules bind the CD4 molecules assists in the discrimination

• The presence of a “not quite self”/non self peptide on a MHC class I molecule renders the cell a target of a cytotoxic CD8 T cell, while a peptide in a class II molecule evokes macrophage activation and B cell help

• Since the entire system is generated on self-peptides there is a potential for pathologic self-recognition and autoimmune disease