The recent sequencing of the MHC as part of the genome project revealed numerous genes that function in antigen processing and other roles in the immune response, apart from the class I and class II MHC genes.

The MHC is a unique multigenic and polymorphic gene complex that regulates the adaptive immune response.

Genes with immune functions other than class I and II MHC

**MHC class IB genes**
- MICA, MICB
- Expressed in fibroblasts and intestinal epithelium
- Upregulated by cellular injury and stress, not γ-IFN
- Engage stimulatory NK receptor NKG2D, activating NK cells
- Physiologic means of removing damaged/stressed cells

MHC class IB genes (Non-classic) Innate Immunity
- **HLA-G** the only MHC class I expressed on trophoblast and inhibits NK cell killing of fetus that would otherwise occur
- **HLA-E** binds leader peptide of classic class I molecules, HLA-A, B and C and inhibits NK cell killing of target cell by engagement of inhibitory CD94 / NKG2A receptor

Limited polymorphism, restricted tissue expression

Classical and non-classical MHC class II genes

Four intraspecies duplications resulted in four regions

- **Classical MHC class II genes**
  - HLA-DR (DRA, DRB1)
  - HLA-DQ (DQA1, DQB1)
  - HLA-DP (DPA1, DPB1)

- **Non-classical MHC class II genes**
  - DM primarily expressed in macrophages and DC
  - DO primarily expressed in B cells
Distinctive features of peptide binding to class II MHC molecules

- Peptide binding groove open and no binding of N or C termini
  - Peptide antigen length variable: 12-24 amino acids and length not critical
  - Pockets tether central portions of peptide and the pockets used vary considerably for loci and alleles
- Class II is a two chain molecule with both chains encoded in MHC and each contributes to peptide binding groove
  - Trans combinations of chains from different HLA-DQ or DP haplotypes generate additional post translational diversity
  - Polymorphism influences chain pairing and only certain gene pairs found – linkage disequilibrium

Peptide-MHC class II complex interactions with the CD4 T cell TCR

Multiple hydrogen bonds, van der Waals and electrostatic interactions with the peptide backbone or side chains tether the middle of the peptide to MHC class II molecules, the ends are free, with length varying from 12-24 amino acids

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

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MHC class II molecule binding a peptide

Multiple bonds exist between peptide and MHC II.

Contrasting mode of binding of CD8 and CD4 TCR to MHC I or II

The CD8 or CD4 TCR β-chain interacts with different portions of the MHC-peptide complex.

Two strategies for polymorphism in MHC class II genes

- HLA-DR loci only have polymorphisms in β-chain, with DRA locus nearly monomorphic, similar concept to class I MHC
- HLA-DQ and -DP have polymorphisms in the genes encoding the α-chain and the β-chain and the resulting products are more intricate

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>3</td>
</tr>
<tr>
<td>HLA-DRB1 β-chain</td>
<td>DR1, 2,…</td>
<td>DRB1*0101,… 440</td>
<td></td>
</tr>
</tbody>
</table>

Gene content here varies among haplotypes, too complex for now.
Codominant expression of MHC genes results in the presence of 2 different HLA-DR molecule allotypes on the cell surface.

**HLA-DR Polymorphisms**

NND alleles of DRB1 locus

**MHC class II region**

**Nomenclature:** Each allele is defined by its unique DNA nucleotide sequence.

- E.g. HLA-DRB1*0101
  - *0102 HLA-DR1
  - ... *0112
  - *0401
  - *0402
  - *0403 HLA-DR4
  - ...
  - *0424

But to make things “simpler”, structurally similar alleles are grouped in families that resemble those defined as “serologic specificities”, e.g. HLA-DR1 or HLA-DR4, reflecting an old nomenclature when human alloantibodies were used to detect HLA “antigens”.

**Alleles are often generated by a patchwork process of gene conversion that alters pockets en bloc**

**Polymorphic residues of the MHC class II β-chain alleles of HLA-DR are predominantly in the antigen-binding cleft of the molecule**

The most important difference among the HLA-DR4 alleles is that their P4 pockets differ.
What the actual sequence of some DRB1 alleles looks like

<table>
<thead>
<tr>
<th>DRB1 Allotype</th>
<th>Pocket in HLA-DR molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td>*0101</td>
<td>VFVL, LAIVM, AGST, LAIV</td>
</tr>
<tr>
<td>*0301</td>
<td>LFVM, DNQT, KR, L, YLF</td>
</tr>
<tr>
<td>*0401</td>
<td>FYVL, DFVIL, NSTQHR, NQST</td>
</tr>
<tr>
<td>*0402</td>
<td>VILM, KRHY, NSTQK, NQST</td>
</tr>
<tr>
<td>*0701</td>
<td>FILV, NST</td>
</tr>
<tr>
<td>*1501</td>
<td>LVI, FYI, ILV</td>
</tr>
</tbody>
</table>

(Hydrophobic Hydrophilic Negative Positive)

Pocket specificity and use varies with HLA-DR allotype

Residues in peptide available for TCR interaction vary

Unrooted tree relationship of HLA-DR alleles

Frequencies of HLA alleles differ markedly in different population groups
A polymorphism at position 57 of the DQB1 β-chain encodes either Asp or Ala or Val, a portion of pocket 9. When 57β is Asp, it can form a salt bridge with Asn at position 79 on the α-chain. DQB1 alleles with 57βAla/Val confer susceptibility to type 1 diabetes mellitus, while 57βAsp confers no susceptibility or resistance.

Codominant expression of HLA-DQ genes results in 4 different class II HLA-DQ molecules (allotypes) on the cell.

Only certain combinations of DQA1 and DQB1 alleles are found. 20 linkage disequilibrium haplotypes. Expect 107 pairs if random combination of DQA1 and DQB1 alleles.
Polymorphism of DQA1 and DQB1 influences chain pairing and only certain gene pairs are found together. In some instances gene pairs not represented fail to produce an intact HLA-DQ molecule. This linkage disequilibrium is centrally involved in determining the genetic basis of susceptibility to celiac disease and to type 1 diabetes mellitus. The linkage disequilibrium extends throughout the class II region and sometimes across the entire MHC.

Alleles of major DQ-DR haplotypes that exhibit linkage disequilibrium:

<table>
<thead>
<tr>
<th>DQB1</th>
<th>DQA1</th>
<th>DRB1</th>
<th>HLA-DR</th>
<th>T1DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>*0602</td>
<td>*0102</td>
<td>*1501</td>
<td>DR2</td>
<td>-</td>
</tr>
<tr>
<td>*0601</td>
<td>*0103</td>
<td>*1502</td>
<td>DR2</td>
<td>N</td>
</tr>
<tr>
<td>*0201</td>
<td>*0501</td>
<td>*0301</td>
<td>DR3</td>
<td>++</td>
</tr>
<tr>
<td>*0302</td>
<td>*0301</td>
<td>*0401</td>
<td>DR4</td>
<td>++</td>
</tr>
<tr>
<td>*0302</td>
<td>*0301</td>
<td>*0402</td>
<td>DR4</td>
<td>+</td>
</tr>
<tr>
<td>*0301</td>
<td>*0301</td>
<td>*0401</td>
<td>DR4</td>
<td>N</td>
</tr>
<tr>
<td>*0303</td>
<td>*0301</td>
<td>*0401</td>
<td>DR4</td>
<td>N</td>
</tr>
<tr>
<td>*0303</td>
<td>*0201</td>
<td>*0701</td>
<td>DR7</td>
<td>-</td>
</tr>
</tbody>
</table>

T1DM susceptibility also influenced by DQA1 and DRB1 alleles.

Some clinically important instances of linkage disequilibrium relevant to disease reflect a variable duplication in class III genes. The haplotype HLA-A1-B8-DR3-DQA1*0501-DQB1*0201 has a deletion of 25kb of DNA including the C4B gene, which decreases the C4 level by ~half.

CYP21B(A2) encodes steroid 21 hydroxylase and its absence results in congenital adrenal hyperplasia, a recessive disease due to a failure to synthesize cortisol; this results in androgenic precursor over-production. Androgen excess leads to ambiguous genitalia in females, rapid somatic growth during childhood in both sexes with premature closure of the epiphyses and short adult stature (Infant Hercules). Mispairing of class III regions that have one or two modules of C4 during cross over can result in an additional loss of CYP21B(A2).

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Some clinically important instances of linkage disequilibrium relevant to disease reflect a variable duplication in class III genes. The haplotype HLA-A25-B18-DR2 contains a defective gene for C2 with a 28bp deletion. No hemolytic activity in classic pathway. Associated with autoimmune disease (SLE).
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 (actually more)</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><strong>Total</strong></td>
<td><strong>6</strong></td>
<td><strong>16</strong></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.