Immunology (AIM)

T cell receptors can only recognise pieces of antigen held by MHC.
The MHC molecule
↳ cell surface protein → coded for by MHC locus

→ MHC determines whether a transplanted tissue will be accepted or rejected → from the pioneering work of Benacerraf, Dausset & Snell

→ many alleles of the MHC gene → inherited allele determines susceptibility to disease.
→ two classes of MHC molecules

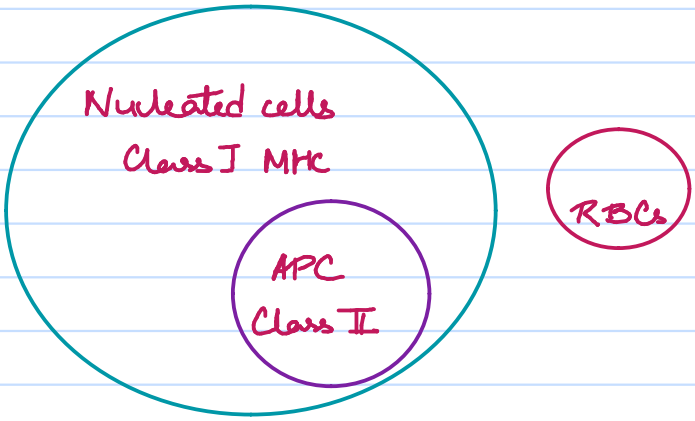
class I class II

very similar in their quaternary str, but differ in how they attain it from their primary structure

Class I MHC molecules
① present on all nucleated cells
② specialise in presenting antigens that originate from the cytosol, like viral proteins

Class II MHC molecules ① present only on APCs
② specialise in presenting antigens from extracellular spaces that have been engulfed by this cell

1. participate in both cellular & humoral responses
2. genes present in chr. 6 in human & 17 in mice



Structure & func. of MHC molecules

- three classes of MHC molecules – I, II, III
- Class I and Class II
  1. membrane-bound glycoproteins
  2. highly specialised antigen-presenting molecules with grooves to form unusually stable complexes with peptide ligands
- Class III
  1. unrelated molecules
  2. secreted proteins – component of complements & inflammatory molecules like cytokines

Class I MHC molecules

- 45 KDA α-chain + 12 KDA β_2-microglobulin
- α chain
  1. $\alpha_1, \alpha_2, \alpha_3$ – 90 amino acid long each
  2. transmembrane domain – 25 aa
  3. cytoplasmic anchor segment of 30 aa
- β_2-microglobulin
  1. Similar to α_3 domain
  2. no transmembrane region – non covalently bound to α-chain

α_1 & α_2 domains

- form a platform of eight antiparallel beta strands spanned by two long α-helical regions
- structure forms a deep groove or cleft with long α-helices as sides & β-strands of β-sheet at bottom
  ↳ enough to bind peptide of 8-10 amino acids

α_3 domain & β_2-microglobulin

- arranged into 2 β-plated sheets (immunoglobulin fold)
- α_3 is highly conserved among MHC class I molecules → binds to CD8

All of these molecules are essential to the proper folding and expression of the MHC peptide complex on cell surface. (Daudi cell experiment)

Class II MHC molecules

- two different polypeptide chains -
  - 33 KDa α-chain
  - 28 KDa β-chain
- has external domains, transmembrane & cytoplasmic region
- (α_1 & α_2) + (β_1 & β_2)
- α_2 & β_2 close to membrane; bear immunoglobulin groove like α_3/β_2 microglob
- α_1 & β_1 domains form peptide binding groove for processed antigen - thus similar, but still different because it is encoded by two different proteins instead of one
- class II molecules form an open pocket - because it lacks class I conserved sequences that bind to terminal amino acids of peptides
  ↳ binds peptides 13-18 aa long

Promiscuity of Class I & II molecules

- broad specificity of binding unlike TCR or antibody
- A single MHC molecule can bind several antigenic peptides & some peptides can bind to several different MHC molecules
- association of MHC-peptide is very stable under physiological conditions

Class I MHC-peptide interaction

- endogenous processing pathway → peptides derived from endogenous intracellular proteins
  ↓
  transported to ER
  ↓
  interaction with class I MHC
- each cell expresses several unique MHC class I molecules, each with different peptide promiscuity rules - set of MHC class I alleles inherited determines which specific peptide fragments from a larger protein will get expressed.
- characteristics of bound peptides isolated from MHC class I molecules:

1. 8-10 aa in length (mostly nonamers)
2. contain sp. amino acid at specific locations along the peptide (anchor residues)

side chains of anchor amino acids are complementary with the surface features of the binding groove, allowing diverse peptides with different sequences but with the anchor sites.

anchor sites are different for different class I variants

→ anchors are generally hydrophobic residues with a carboxy-terminal

How do they interact?
Anchor residues at both ends of the peptide are buried deep within the binding groove, holding the peptide firmly in place (nonamers bind preferentially)

The main contacts between amino acids & class I MHC molecules involve residue ② at the amino terminal end & residue ⑨ at the C-terminus end, rest arches away from the floor of the groove, allowing slightly longer or shorter peptides to be accommodated & direct interaction with TCR

MHC Class I – peptide binding

Class II MHC – peptide interaction

- class II MHC molecules bind and present peptides to CD4 T cells – can bind a variety of peptides
- peptides are typically derived from exogenous pathway – either from self-membrane bound proteins, or foreign proteins internalised via phagocytosis or receptor mediated endocytosis & then processed via exogenous pathway.

- Peptide-binding groove open at both ends
  ↓
  allows longer peptides (13-18 aa) to bind

- peptides bound to MHC class II molecules maintain roughly constant elevation on the floor of the binding groove

- central core of 13 aa necessary for binding
- conserved sequence motifs and not conserved anchor residues
  — hydrogen bonds b/w peptide backbone and binding groove distributed throughout binding site and not at the ends like in I.
- internal sequence of 7-10 amino acids - major contact points
- aromatic/hydrophobic sequence at N-terminus and 3 additional hydrophobic residues at C terminal end of the peptide

Points to remember:

1. Each MHC molecule has only 1 binding site
2. membrane-bound, T cell-interaction requires cell-cell contact
3. mature T cells must have a TCR that recognises peptide bound to MHC
4. cytokines (esp., interferon γ) increase level of expression of MHC
5. polymorphism of MHC is required for survival of species

MHC peptide binding assay

- detection of MHC-peptide
- Rate assays and cell assay

- measures how well a specific peptide binds to a MHC molecule (affinity of peptide)

Fluorescence Polarisation Assay - when fluorescently labelled peptide binds to MHC, its rotation slows, resulting in an increase in fluorescence polarisation

ELISA-Based assay - peptides are incubated with soluble MHC molecules, and binding is detected using antibodies specific to the peptide-MHC complex

- determines the proliferation of T cells or the cytokine-secreting by T cells in the presence of potentially immunogenic peptides

Cellular Expression of MHC molecules

MHC Class I expression

→ expressed constitutively on all nucleated cells of the body, however level of expression differs among cells
→ highest levels of class I molecules found on lymphocytes, while hepatocytes, fibroblasts, etc., express very low levels
→ low levels of expression facilitates relative success of liver transplants by reducing the likelihood of graft rejection

→ MHC I molecules in non-infected cells display self peptide, in virus-infected cells self-peptide + viral peptide

MHC Class II expression

→ found only on professional APCs, sometimes only after an inducing event
→ level of expression varies among APCs – depending on stage of differentiation or level of activation

B cells, macrophages, fibroblasts, astrocytes, endothelial cells & epithelial cells

Regulation of MHC expression

- class I and class II MHC genes: flanked by 5′ promoter sequences – that bind to sequence specific transcription factor
- Transcriptional regulation of MHC is mediated by both positive & negative elements
- ex, CIITA (A class II MHC transcriptional activator) and RFX-5 can bind to promoter region of MHC II
- separate exons encode a region of the class I and II proteins

Cytokines

- interferon (α, β, γ)
- TNFs increase the expression of class I MHC molecule
- IFN-γ → also induces expression of class I MHC molecules
  → induces CIITA indirectly to activate class II MHC expression on a variety of cells (deg with IL4)