ANTIGEN PROCESSING AND PRESENTATION

T cells recognize fragments (peptides) of pathogen proteins, presented by MHC (Fig. 5.10)

Two classes of T cell and two types of MHC molecule, for two types of danger

T cell types (Fig. 5.11, 5.12)
Two types of T cells distinguished by expression of CD4 or CD8 co-receptors
- CD8 T cells respond to intracellular sources of infection (e.g. viruses inside cells) and kill those cells (CD8 T cells = CTLs)
- CD4 T cells respond to extracellular sources of infection (e.g. bacteria, free viruses, parasites) and help cells that are presenting Ag from those pathogens
- CD4 T cells = helper T cells
- Helper T cells subdivided into Th1 and Th2 subclasses.
- Th1 help MΦ to phagocytose and kill extracellular pathogens
- Th2 help B cells make Ab

MHC types
- There are two classes of MHC, class I and II. They are expressed on different sets of cells, present peptides from different sources, and bind to (and activate) different types of T cells.
  - **Class I MHC** molecules are expressed on essentially all cells in your body (all are capable of being infected by viruses) (Fig. 5.23) and bind peptides derived from intracellular proteins. CTLs recognize peptides bound to class I MHC and lyse cells presenting foreign peptides.
  - **Class II MHC** molecules are expressed on “professional” APCs (dendritic cells, macrophages, B cells) (Fig. 5.23) and bind peptides derived from extracellular proteins that are endocytosed by the APC. CD4 T cells recognize peptides bound to class II MHC and “help” the APCs and neighboring cells with their immune function.

Unusual aspect of MHC molecules is **polymorphism** = many genetic variants in population. This is main reason for rejection of organ transplants (donor tissue is seen as “foreign”) and is the origin of the name MHC.

Structure of MHC molecules (Fig. 5.13)
- The two MHC molecules are cell surface glycoproteins closely related in structure.
- They both have a region that resembles Ig domains, and also a region that folds to form a cleft where peptides bind.
- Purified MHC and peptide molecules have been crystallized and analyzed.

**Class I**
- MHC class I is composed of two polypeptide chains, α and β2-microglobulin.
  - Only the α chain is transmembrane.
  - The two chains are associated in a non-covalent manner.
  - β2-microglobulin is not encoded by a gene in the MHC locus nor is it **polymorphic**.
  - The α3 domain and β2-microglobulin are similar to Ig fold; CD8 binds here (Fig. 5.14)
  - the α1 and α2 domains fold to make up the cleft where peptides bind.
Class II
- Also non-covalent complex of two transmembrane chains.
  - α and β chains, both encoded by MHC genes, and both are polymorphic (Fig. 5.13).
  - The parts of α and β close to cell surface are Ig-like domains (CD4 binding site- Fig. 5.14), whereas the top parts of α and β form the cleft where peptides bind.

Coreceptors strengthen TCR/MHC interaction and response
Affinity of TCR for MHC+Ag is lower than that of Ab for Ag; needs additional help from accessory molecules.
  - The two ligands for MHC class I: TCR and CD8 (Fig. 5.14)
  - The two ligands for MHC class II: TCR and CD4
  - TCR binds the region of MHC that is associated with antigenic peptides.
  - CD4 and CD8 bind to MHC molecules in a region closer to the APC membrane, away from the peptide-binding portions (Fig. 5.14).
CD8 and CD4 are “longer” extended molecules so they can interact with the same MHC/peptide complex as the TCR (Fig. 5.14)
CTLs express CD8, accounting for their specific recognition of class I MHC
T helper cells express CD4→ recognize class II MHC
Co-engagement of CD4 or CD8 has two functions
  1. Strengthen adhesion
  2. Amplify signaling to inside of cell

Peptide binding cleft (Fig. 5.15)
- Long peptide binding groove formed by a β-sheet “floor” and α-helical “walls”.
- Many different peptides can bind in cleft, but some length/sequence requirements
- Class I binds peptides 8-10aa long. Free N- and C-termini interact with conserved MHC residues at end of cleft and contribute much to binding.
- Class II binds longer peptides of varying length (13-25aa) and can hang out the end.
- Some of binding energy for MHC-peptide is provided by contacts between conserved MHC residues and peptide “backbone”, in addition to polymorphic residues binding to side-chains of aa’s in peptide
  - Stability of MHC-peptide binding is an essential adaptation to prevent exchange at the surface; you want to see peptides that accurately represent proteins in the target cell.

Why present Ag? Different rationale for different cells:
  1) Virally infected cell: need to be killed by CTL
  2) DC activated by PAMP receptor: need to prime resting T cells
  3) Macrophage phagocytosing pathogen: need to receive help from effector T cells and to destroy vesicular pathogens
  4) B cells: need to receive help from effector T cells for Ab responses/CSR/SHM
  5) Thymic epithelial cells (preview): need to shape T cell repertoire and self-tolerance
Review of the structure of cell (Fig. 5.16)
* cytosol
* nucleus: continuous with the cytosol through nuclear pores in nuclear membrane
  Has a double membrane - the outer one is contiguous with ER
* vesicular system: "continuous" with the extracellular fluid
  - ER: transmembrane proteins and secreted proteins are synthesized by ribosomes on the surface of ER membrane and transported into lumen of ER where they can fold correctly. Proteins are then transported to Golgi.
  - Golgi: post-translational modification (i.e. glycosylation) and secretion/trafficking of proteins.
  - Endosome: takes up extracellular material into vesicular system
  - Lysosome: vesicles containing proteolytic enzymes and low pH
  - Secretory vesicles: vesicles containing proteins to be secreted.

MHC CLASS I PEPTIDE PROCESSING
- Class I MHC presents internally-derived peptides (such as host cell proteins and viral proteins).
- All proteins are made in the cytosol by ribosomes: whereas cytoplasmic proteins are synthesized by free ribosomes in the cytoplasm, proteins destined for cell surface (such as MHC) are synthesized by ribosomes on ER membrane and are translocated into the lumen of ER, where the proteins fold. How does class I in ER meet up with peptides from cytosolic proteins?

Generation of peptides for MHC class I presentation from cytosolic proteins
- Proteins are continually synthesized and degraded in the cytosol.
- Degradation of protein: by proteasome.
- Proteasome: *A large multi-catalytic protease complex. (Fig. 5.17)
  * Conserved from archaeabacteria to humans
  * Large cylindrical complex with ~ 28 subunits
  * Protein is introduced into the core and digested
  * degrades both host and viral proteins
  * modified during viral infection to produce peptides optimal for class I MHC binding

Transport of peptide from cytosol to lumen of ER
- TAP = Transporters associated with Antigen Processing. (Fig. 5.17)
- TAP-1 and TAP-2 transport peptides from cytosol into lumen of ER where MHC class I molecules are located.
- TAP-1 and TAP-2 form a heterodimer; mutation in either gene can abolish antigen processing by class I MHC.

Assembly of MHC class I and peptide complex (Fig. 5.18)
Chaperone proteins = proteins that participate in protein folding and prevent degradation or aggregation.
- The partially-folded α chain of MHC class I binds the chaperone protein calnexin in lumen of ER. (Calnexin has an important role for immunologic protein assembly: also associates with partially-folded TCR, Ig, and MHC class II.)
- β2-microglobulin binds the α chain and the MHC class I heterodimer now dissociates from the chaperone and binds another chaperone complex that includes tapasin.
  * Tapasin binds TAP proteins and therefore forms a bridge between a MHC class I molecule and the peptide transport machinery.
- Binding of a peptide (transported from cytoplasm to ER by TAPs) to the peptide-binding groove of a MHC class I allows dissociation of the now fully assembled MHC class I molecule from chaperone proteins --> transport to cell surface.

**MOVIE**

** Excess of MHC class I molecules over peptides: in normal, uninfected cells MHC class I molecules bind degraded self proteins, and there is always more MHC class I proteins ready to bind peptides in ER. (If there were excess of peptides over MHC I and you had a viral infection, the viral peptides will have to fight with other self peptides to get onto MHC.)
** many viruses have gene products that interfere with function or stability of TAP, MHC class I, etc. ➔ to obstruct class I Ag presentation
But NK cells can recognize cells with absent class I and kill those cells

**MHC CLASS II PEPTIDE PROCESSING**

*Proteins from pathogens that replicate in the intracellular vesicles of macrophages (i.e. mycoplasma that causes TB or leprosy) are not accessible to proteasomes.
* Proteins that are endocytosed by B cells (after binding to surface Ig), macrophages or dendritic cells are also in the endosomal compartment inaccessible to proteasomes.

- Proteins in these vesicle compartments are degraded by lysosomal or endosomal proteases.  
- Endosomes become increasingly acidic as they progress inside the cell, and this change in pH activates acid proteases in the endosomal compartment.
- released peptides associate with class II molecules in these vesicles
- some pathogens, esp. mycobacteria, survive in endosomes by preventing fusion with lysosomes

**Assembly of MHC class II and peptide complex**  (Fig. 5.21)

- MHC class II proteins are transported into the lumen of ER during normal transmembrane protein synthesis, but the peptides for class II molecules won't be joining them until in the endosomes.
  
- how are MHC class II molecules prevented from binding peptides (destined for class I MHC) in the lumen of ER?

**Process**
- **Invariant chain** binds to MHC class II and blocks the peptide-binding groove during protein folding.
(Calnexin also associates with a class II MHC molecule during protein folding)

- Once the MHC class II-invariant chain complex is formed the chaperone protein disassociates and the MHC-invariant chain complex moves toward Golgi.
  * Without invariant chain MHC class II is retained in ER.
  * Invariant chain also escorts MHC class II to the endosomal compartment known as the MIIC (MHC class II compartment).
- In the MIIC compartment invariant chain goes through a series of peptide cleavages by proteases (cathepsins) that leave only the CLIP (class II-associated invariant-chain peptide) fragment bound to the peptide-binding groove of class II.
- Vesicle containing MHC class II with CLIP fuses with a vesicle containing peptides generated by acid proteases.
- CLIP is released and peptides are loaded onto MHC class II in the late endosomal compartment with the help of HLA-DM.
  - HLA-DM is a MHC class II-like protein but lacks a peptide-binding groove.
  - It is found predominantly in the late endosomal compartment.
  - It interacts with MHC class II and facilitates release of CLIP and binding of peptide to class II MHC.
- Class II molecules also bind self peptides; there is also excess of MHC class II over peptide.

**MOVIE**

** For both class I and class II, stable association with peptides is essential: if the association is too weak, pathogens can escape detection. It is also possible that peptide can fall off of one cell and become associated with MHC of another (uninfected) cell, thereby possibly destroying an innocent bystander or sending T cell help to the wrong target cell.