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, even when not infected.

- Degradation of protein: by **proteasome**.

- Proteasome: \*A large multi-catalytic protease complex. (Fig. 5.17)

 \* Conserved from archaebacteria 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  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.)

- 2 -microglobulin binds the  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.

\*\* 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

Generation of peptides for MHC class II presentation (Fig. 5.20)

\*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. But some of their antigens can be released, processed and presented.

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.

*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 (a type called 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, catalyzed by **HLA-DM**, and peptides are loaded onto MHC class II in the late endosomal compartment

\*\* 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.) Class II molecules also bind self peptides; there is also excess of MHC class II over peptide.

\*\* 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.

MHC GENES, POLYMORPHISM AND IMMUNE RESPONSES

-Human MHC genes are generally given the prefix **HLA** (human leukocyte antigen). To distinguish from RBC antigens that determine the classical ABO blood groups for transfusion compatibility.

One mechanism of MHC diversity is due to **gene families**:

**-**Each individual has three genes encoding class I  chains that present Ag to CD8 T cells: HLA-A, HLA-B, HLA-C. (Fig. 5.24) Sometimes called class I isotypes. Other class I-related proteins associate with 2-mic. but have other functions (i.e. HLA-E).

-Each human has three pairs of genes encoding class II  and  chains that present Ag to CD4 T cells: HLA-DP, HLA-DR, HLA-DQ (Fig. 5.24) Sometimes called class II isotypes. Other class II-related proteins do not bind peptides but regulate class II presentation (i.e. HLA-DM, DO)

-Thus, each person has three genes for class I MHC and three pairs of genes ( and ) for class II MHC.

Definition: **Genetic polymorphism**: variation at a single genetic locus and its product within a species; the individual variant gene is called **allele**.

The other mechanism of MHC diversity is due to genetic polymorphism:

* There are multiple alleles for each MHC gene in the population (Fig. 5.25)
* Therefore, different individuals will have different sets, capable of binding different population of antigenic peptides.
* Each individual usually is heterozygous for most MHC isotypes because father and mother contribute different alleles
* Therefore, most individuals express 6 different class I and 6 different class II.
* The full array of alleles for the MHC molecules on chromosome 6 in an individual is called the **haplotype** (e.g. HLA-A2, B27, C16, DR1, etc.)

Peptide binding cleft (Fig. 5.15)

-Note that the main structural aspects of each MHC are similar, they just bind different peptides because the polymorphisms are primarily in the peptide-binding contact residues and TCR contact residues. (Fig. 5.29)

-A given MHC molecule can bind with high affinity a wide variety of peptides with some

common sequence features: **anchor residues** are identical or similar among set of peptides that bind with high affinity (Fig. 5.30).

#### TCR/MHC-peptide interaction (Fig. 5.22)

-involves CDR loops of TCR and  binding to the composite surface of MHC+peptide.

**MHC restriction** means that a given T cell receptor is specific not only for a given peptide, but for a unique combination of peptide plus a particular MHC molecule. (Fig. 5.31)

The advantage of MHC gene families and polymorphism (Fig. 5.32)

Each pathogen expresses many proteins with different possible peptides presented

* Multiple MHC isotypes and polymorphism allow an individual to present a larger pool of foreign peptides to T cells, thus increasing the chances of an effective adaptive immune response
* Heterozygosity at HLA alleles prolongs time to progression in HIV (Fig. 5.35)
* Having multiple combinations of alleles in the species population ensures that at least some individuals will survive an epidemic
* Certain alleles more prevalent in geographic areas where specific pathogens have long been present (e.g. malaria)

#### MHC genetic organization

- MHC genes are linked in a large cluster of genes ~200 (human chr. 6) (Fig. 5.26)

- Genes for class I MHC and class II MHC are in separate areas of the locus

- "Class III MHC" locus: some genes important for immune response including complement proteins and cytokines (*not all*). Usually not polymorphic.

- Genes for many other proteins involved in antigen peptide processing are in MHC class II locus. e.g. TAP1 and 2, tapasin, LMP2 and 7 proteasome subunits. (Fig. 5.28)

- When LMP2 and 7 are expressed, they change the proteasome specificity to generate peptides that are better fit for MHC class I binding

- Transcription of many of these genes is coordinated: **interferons** can increase transcription of many MHC genes via common transcriptional activators.

Expression of class I genes, 2-microglobulin, as well as proteosome and TAP subunits, is upregulated by interferon  and , factors induced by viral infection

Class II genes are turned on by interferon-, made by T helper cells in response to bacterial infection