**General information**

455 LSA, Tuesday 11 to noon

Anytime after class

email: lcoscoy@berkeley.edu

Use MCB150 as subject line

Please only quick (yes/no) questions

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**Cell mediated immunity**

- **Thymus**
- **Naive T-cells (CD8 or CD4)**
- **Bone marrow**
- **Spleen**
- **Lymph nodes**

**Antigen**

**Infection**

**Dendritic cells**

**Effector T-cells**

**Memory T-cells**

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**Antigen Recognition by B and T lymphocytes**

- **B Cell**
- **T Cell**
- **APC**

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**MHC Expression**

<table>
<thead>
<tr>
<th>Cells</th>
<th>MHC Class I</th>
<th>MHC Class II</th>
</tr>
</thead>
<tbody>
<tr>
<td>T cells</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>B cells</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Macrophages</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Dendritic cells</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Thymic Epithelia</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Hepatocytes</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Kidney</td>
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<tr>
<td>Muscle</td>
<td>+/-</td>
<td>-</td>
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<tr>
<td>Red blood cells</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
A simple view of T-cell mediated immunity

After initial activation, T cells differentiate into effector cells and can be activated in lymphoid organs and periphery.

APCs still activate effector Th cells.

Target cells activate effector CD8+ Tc (CTLs). (Target cells can be any Class I MHC expressing cell.)

Antigen Processing and Presentation

Laurent Coscoy

MCB 150

Activation of T cells

- APCs determine which peptides will be presented on Class I and Class II MHC during initial activation.

- T cells need to be able to distinguish between external antigens (taken up by APCs) and internal antigens (infected cell).
What is Antigen Processing?
- Enzymatic process of degrading proteins through proteases into antigenic peptides.
- Antigen processing requires energy (ATP) and movement of endocytic vesicles.
- Cell fixation experiments demonstrated that antigen processing was an active process.

Cellular Compartments
Exogenous Antigens vs Endogenous Antigens
- Endogenous proteins in cytosol or in secretory vesicles.
- Exogenous proteins come in through endosomes.

Two Antigen Processing Pathways
- Endogenous antigens in cytosol presented on class I MHC molecules to CD8+ T cells.
- Exogenous antigens in endosomes presented on Class II MHC molecules to CD4+ T cells.

Endogenous Antigens
Cytosolic Pathway
- Endogenous antigens are from proteins produced inside the cell.
- These includes self protein antigens and foreign protein antigens.
- Class I MHC antigens activate cytotoxic CD8 T cells for killing infected cells and tumor cells.
**Endogenous Antigens**

**Proteasome**

The proteasome is a cylindrical shaped catalytic protease complex of 28 subunits for cytosolic protein degradation.

- The proteasome unfolds proteins and then cleaves proteins into peptides and amino acids.

Conserved throughout the eukaryotes and the archaeabacteria

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**Endogenous Antigens**

**LMP2 and LMP7**

- LMP2 and LMP7 are proteasome subunits that are encoded within the MHC.
- Interferons (IFNs) increase expression of LMP2 and LMP7.
- LMP2 and LMP7, along with LMP 10, appear to have specialized role for increasing the production of peptides that have the preferred sequence for Class I MHC binding. e.g. 7-10 amino acids with a hydrophobic carboxy residue.

How many peptides are generated in a cell?

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**Endogenous Antigens**

**Generation of Class I MHC Peptides**

- **TAP proteins** (Transporters associated with Antigen Processing)
- TAP 1 and TAP 2 form heterodimer in membrane of ER to facilitate selective transport of peptides from cytoplasm into lumen of ER.
- TAP pump preferentially transport peptides with a length of 8–15 amino acids.

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**Endogenous Antigens**

**Generation of Class I MHC Peptides**

- Calnexin is a chaperone protein that binds to newly synthesized α-chain Class I MHC and retains the Class I MHC until Beta 2-microglobulin binds.
Tapasin and Calreticulin both bind to the newly formed Class I MHC complexes. Tapasin forms a bridge between connecting the TAP proteins with the Class I MHC molecules.

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- Peptides bind to the Class I MHC molecules and allow release from tapasin and calreticulin.

- Peptide binding provides stability for Class I MHC to allow transfer to surface.

Do you remember how peptides bind MHC-I molecules

- Though a majority of Class I peptides are ready after leaving proteasome as much as 15% still need trimming.

- Cytosolic proteases have been identified that can trim NH₂ terminal after proteasome.

- Recent data indicate that peptides can be trimmed in ER to fit in Class I MHC pocket.

How do internal membrane proteins or secretory proteins enter Class I MHC pathway?

- Membrane or secreted proteins can be presented in Class I MHC but must be degraded in the cytosol.

- Membrane-bound proteins after synthesis directly into ER can return through retrograde translocation to the cytosol for degradation.

CD8+ Tc Activated by Endogenous or Intracellular Antigens

- Effector CD8+ Tc (CTLs) are primarily needed for killing in infected cells.

- CTLs can also be activated against cancer cells (tumor) targets.
CTL Killing of Infected Target Cells

- Viruses must replicate inside cells and many bacteria and parasites live inside host cells.
- Therefore antigens for stimulating CTLs come from inside the cell because they signal an intracellular infection.

RMA-S TAP Deficient Cell Line

- RMA-S Mouse tumor cell line found to have mutation in TAP and resultant decreased Class I MHC expression and resistance to CTL killing.
- In absence of TAP Class I MHC molecules are unstable and translocated back to cytosol and degraded.
- Class I MHC expression and killing restored by addition of exogenous peptide or transfection with TAP.

CTL Lysis of RMA-S Cells

Transfection of TAP back into RMA-S Cells (RMA-S + TAP) restores killing of RMA-S targets by tumor specific CTLs.

TAP Deficient Humans

Nonsense mutation in TAP-2 gene inherited with MHC haplotype.

Results in low Class I MHC expression.

"Tatiana" was homozygous for MHC alleles.

Immune Evasion

Viruses interfere with Class I MHC expression to escape killing by CTLs

- Herpes Simplex Virus (HSV) protein ICP47 can selectively bind to TAP and inhibit the transfer of peptides into ER.
- Transfection of ICP47 alone caused decreased Class I expression.

Exogenous Antigen Pathway

Endocytic processing pathway
Cellular Compartments
Exogenous Antigens vs Endogenous Antigens

- Endogenous proteins in cytosol.
- Exogenous proteins come in through endosomes.

How are peptides generated?

- Peptides bound to MHC Class II molecules are derived from engulfed pathogens (also self proteins and internalized TM proteins)
- Acidification of endocytic vesicles activates proteases that degrade proteins into fragments.
- These peptide fragments are loaded onto MHC class II molecules

Trafficking of MHC class II molecules

MHC class II α and β chains associate in the ER
In the trans-Golgi network, MHC class II is sorted into vesicles
These vesicles deliver MHC class II to specialized compartments where peptide loading occurs

What prevents MHC class II from binding peptides in the ER?

Exogenous Pathway
Invariant chain

- Invariant chain (Ii) binds to Class II MHC molecules in the ER to prevent endogenous peptide binding.
- Ii is cleaved to leave a peptide fragment (CLIP) in the binding groove.
- CLIP (Class II associate Invariant chain Peptide)

How is CLIP peptide removed?

Exogenous Pathway
HLA-DM

- HLA-DM (H-2M in mice) is a Class II like MHC molecule that binds to and stabilizes empty Class II molecules.
- HLA-DM helps in the release of CLIP fragment so that antigenic peptide can bind.
Ii Chain Prevents Newly synthesized self proteins from binding Class II MHC groove until Class II MHC is in endosomes.

What would an HLA-DM deficient cell look like?

CD4+ T<sub>h</sub> Activated by Exogenous Antigens

- Foreign antigens/extracellular pathogens need to be taken up by APCs to get noticed by immune system and activate Th cells.
- Thus, APCs serve as sentinels or extra barrier to get immune system activated.

Class II Deficiency in Humans

Class II deficiency is an autosomal recessive trait caused by defects in transcription factors controlling Class II expression (lack HLA-DR and DQ).

Class II deficiency will also reduce number of CD4+ T cells - Why?

And lower levels of antibodies Why?

Immune Evasion Class II MHC

Viral Inhibition of Class II MHC

- Adenovirus interferes with Class II upregulation in APCs.
- HSV binds Class II
- HIV interference with Class II processing.
- Pathogens that evade lysosomes
- Intracellular Leishmania & mycobacteria (tuberculosis) reside in their own vesicles.

Comparison of Pathways
How are T cell antigens kept apart?
Location, Location, Accessory Proteins

- Class I and Class II MHC molecules both traverse through ER to cell surface but load peptides in different cell compartments.

Control is through accessory proteins.
- Class I requires TAP Tapasin etc control.
- Class II requires low pH for removal of Ii.

CD1 Proteins
Class I Like MHC Molecules

- CD1a-e are nonpolymorphic Class I like molecules that associate with β2m.
- CD1 present a limited number of bacterial glycolipid antigens to T cells (e.g., mycolic acid from M. tuberculosis).
- Bacterial cell wall lipids make excellent innate immune targets because they are not easily mutated.

CD1 Antigen Presentation

- CD1 proteins have hydrophobic binding groove for hydrocarbon chains of lipid tails.
- Hydrophilic sugar end or lipid head of antigen available for T cell binding.

CD1 Antigen Processing
Lipid and glycolipid antigens still require processing.

CD1 proteins sample lipid antigens in different compartments.