Antigen Processing and Presentation
Introduction

• Traditional view:
  – MHC class II molecules present peptides derived from exogenous proteins to CD4\(^+\) T cells.
  – MHC class I molecules present peptides derived from cytosolic, nuclear, ER and mitochondrial proteins to CD8\(^+\) T cells.
**CYTOSOLIC PATHWAY**

Endogenous antigens $\rightarrow$ ATP $\rightarrow$ Cytoplasmic proteasome complex $\rightarrow$ Peptides $\rightarrow$ TAP $\rightarrow$ Endoplasmic reticulum $\rightarrow$ Peptide–class I MHC complex

$\pm$ Ubiquitin

**ENDOCYTIC PATHWAY**

Exogenous antigens $\rightarrow$ Exocytosis or phagocytosis $\rightarrow$ Endocytic compartments $\rightarrow$ Peptides $\rightarrow$ Peptide–class II MHC complex

Exopeptidases $\rightarrow$ Amino acids

*Figure 8-17
Kuby IMMUNOLOGY, Sixth Edition
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Endogenous pathway (class I MHC)

1. Endogenous antigen is degraded by proteasome.

2. Peptide is transported to RER via TAP.

3. Class I MHC α chain binds calnexin, then β₂ microglobulin. Calnexin dissociates. Calreticulin, tapasin, and ERP57 bind. MHC captures peptide, chaperones dissociate.

4. Class I MHC-peptide is transported from RER to Golgi complex to plasma membrane.

Exogenous pathway (class II MHC)

1. Class II MHC α and β bind invariant chain, blocking binding of endogenous antigen.

2. MHC complex is routed through Golgi to endocytic pathway compartments.

3. Invariant chain is degraded, leaving CLIP fragment.

4. Exogenous antigen is taken up, degraded, routed to endocytic pathway compartments.

5. HLA-DM mediates exchange of CLIP for antigenic peptide.

6. Class II MHC-peptide is transported to plasma membrane.
Introduction

• Updated view:
  – MHC class II molecules present peptides derived from cytosolic and nuclear proteins
    • 20-30% of peptides eluted from MHC class II are from intracellular sources.
    • Some viral antigens are intracellularly processed for MHC class II molecules.
  – MHC class I molecules present peptides derived from exogenous proteins via cross presentation.
MHC Class II Presentation of Intracellular Proteins

• How?
  – Uptake of material from dead cells
  – Endosomal TAP like transporters?
  – No evidence for either pathway.

• Autophagy ("self-eating")
  – Microautophagy
  – Macroautophagy
  – Chaperone-mediated autophagy
Autophagy

• Characterized by
  – Formation of double membrane vesicles
  – Induced by various stimuli: starvation, cytokines, caspase inhibition and chemicals

• Types:
  – Microautophagy
    • Internalization of small portions of the cytosol via lysosomal membrane invagination
    • Primarily studied in yeast and cell-free systems.
  – Pexophagy
    • Degradation of peroxisomes
    • Poorly defined in mammalian cells
  – Mitophagy
    • Degradation of mitochondrial
    • Poorly defined in mammalian cells
  – Cytoplasm-to-Vacuole Targeting
    • Yeast only
  – Macroautophagy
    • Constitutive process for degradation of long-lived proteins and organelles via formation of autophagosomes and fusion with lysosomes
  – Chaperone-mediated autophagy
    • Chaperone assisted transport of specific proteins into lysosomes for degradation
Macroautophagy

• Delivery of cell organelles and protein aggregates to the lysosome for degradation.
• Formation of a double membrane (from the ER?) known as the autophagosome, which closes around the target.
• Autophagosome fuses with late endosomes to form amphisomes and with lysosomes to form autolysosomes.
Macroautophagy

Menendez-Benito and Neefjes, 2007
Inhibition of Autophagosome Leads to Reduced Cross-presentation

(B) mRNA levels of glyceraldehyde-3-phosphate dehydrogenase and Atg12 were analyzed on day 4 after knockdown with GFP- or Atg12-siRNAs (Atg12.1 and Atg12.2) (C) IFN-γ ELISAs on supernatants of the EBNA1-specific CD4+ T cell clone P3-B7 and the EBNA3A-specific CD8+ T cell clone MS.B11, co-cultured with cognate target cells (P3-B7 and MS.B11). Where indicated, targets were electroporated with 10 μM siRNA twice in 4 days before overnight culture with T cells. (D) HLA-DR surface levels after treatment with 10 mM 3-methyladenine (3-MA) and 1 μM lactacystin for EBNA1-transfected L428 Hodgkin’s lymphoma cells (L428E1PC5, left) or with GFP- and Atg12-siRNAs (Atg12.1 and Atg12.2) for the EBV-transformed B cell line Ag876 (right).

Paludén et al, 2005
Co-Localization of Autophagosome and MIIC

Schmid et al., 2007
Targeting Ag to Autophagosome Increases Presentation

CD4 T cell Response

CD8 T cell Response

Schmid et al., 2007
Chaperone-mediated Autophagy

• Does not involve double membrane vesicles.
• Chaperone molecular complex of Hsc70, Hsp90 and other chaperones bind proteins with a KFERQ motif.
• Complex associates with Lamp2 on the lysosome and is transported into the lysosome.
Inhibition of LAMP-2 Diminishes MHC Class II Presentation

Zhou et al., 2005
Over Expression of LAMP-2 Increases MHC Class II Presentation

Zhou et al., 2005
MHC Class II Antigen Acquisition

Crotzer and Blum, 2009
Cross Presentation

• DCs are the main cross presenting cell type \textit{in vivo}.
  – Many cell types can cross present antigens \textit{in vitro}.
• DCs are more efficient at cross presentation
  – DC phagocytic pathway acidifies slowly
  – DCs have low levels of lysosomal proteases
  – Results in lower proteolysis and higher export of intact proteins and polypeptides to the cytosol.
Cross Presentation

• Source of peptides/Loading site
  – TAP Dependent Pathway
    • Phagosome-to cytosol-to phagosome
      – May involve Sec61 to move protein from phagosome to cytosol
      – Proteosome degradation
      – Loading occurs in the phagosome
    • Phagosome-to-cytosol
      – Once in cytosol, proteins behave as endogenous proteins
      – Loading occurs in the ER
    • Endosome/ER fusion-to cytosol
      – Once in cytosol, proteins behave as endogenous proteins
      – Loading occurs in the ER
  – TAP Independent Pathway
    • Vacuolar Pathway
    • Direct loading of peptides generated in the phagosome
TAP Dependent Cross Presentation

- Uty epitope is expressed only in males and is recognized by CD8 T cells.
- Generation of the Uty epitope is dependent upon immunoproteosomes.
- CBA splenocytes were injected into either B6 or LMP7−/− (B6 bkgd) mice. Measured CD8+ response.
- Implies a need for cytosolic processing

Palmowski et al., 2006
TAP Independent Processing

• Peritoneal macrophages were incubated with Ag after treatment with either lactacystin (proteosome inhibitor) or DMSO (carrier)

• CD8$^+$ T cell response was measured.

Belizaire et al., 2009
Cross Presentation Questions

• Where does ubiquitination take place?
  – In the cytosol or endosome/phagosome?
  – If in the cytosol, how do internalized proteins compete for proteosome access?

• Where does peptide loading take place?
  – Most evidence supports loading occurring in phagosomes that contain ER proteins.

• How do phagosomes acquire ER content?
SNARE Proteins

- SNARE family of proteins control intracellular membrane fusion.
  - Expressed at the membrane and in the cytosol.
- ER-SNAREs Sec22b and syntaxin (Stx) 18 are involved in phagocytosis.
- Sec22b controls recruitment of ER and ER-Golgi intermediate compartment (ERGIC) proteins to phagosomes.
Sec22b Recruits ER Proteins to Phagosomes

- DC cells were incubated with magnetic beads.
- Magnetic bead containing phagosomes were purified and examined for protein expression
- Ykt6=ER SNARE
- Lamp1=late endosome-lysosome marker
- TCL=total cell lysate

Cebrain et al, 2011
Sec22b Recruits Regulates Cross Presentation

- Sec22b KD inhibits cross presentation.
- Sec22b does not effect antigen uptake.
- Sec22b does not effect MHC class II or endogenous class I presentation.

Cebrain et al, 2011
Cross Presentation

Cebrain et al, 2011
Cross Presentation

• Splenic CD8αα⁺ DCs appear to be required for cross presentation. CD8αα⁻ are more efficient at class II presentation.
  – Due to differences in endocytic and phagocytic pH levels?

• Cross presentation also appears to be restricted to CD103⁺, Langerin⁺ DCs that are present in lungs, skin and LN, but not spleen

Hildner et al., 2009
Effect of DC Maturation on Cross Presentation: Effect of PRR

• Original studies:
  – Systemic administration of TLR ligands inhibit cross presentation
  – Prolonged treatment of DCs in vitro inhibits cross presentation
  – Explanations:
    • Decreased Ag uptake
    • Inhibition of Ag access to the cytosol

• Recent studies:
  – Some TLR ligands had no effect on cross presentation or enhanced cross presentation.
  – Antigen uptake is still functional in mature DCs