

Reading Assignment: Janeway *et al.* Chap. 1 Section 1-14; Fig. 1.22; Chap. 5 Pg. 169-182
OMIT: HLA-DO

I. Antigen specificity of $\alpha:\beta$ T cells

A. CD4 and CD8 molecules are called co-receptors

1. CD4 binds class II molecules & CD8 binds class I molecules
2. No accident CD4⁺ T cells are class II restricted and CD8⁺ T cells are class I restricted.

B. **Antigenic determinants** are the part of protein antigen recognized by T cells. Also called **epitopes**.

C. T cells rarely recognize determinants on native protein antigens. Antigen is usually in a denatured or fragmented form = **Linear determinants**

1. Peptides of 8-11 amino acids for CD8⁺ T cells & ~13-17 amino acids for CD4⁺ T cells
2. Antibody blocking: Antibodies specific for TCR or MHC molecules inhibit T cell responses. But, antibodies specific for antigens rarely block $\alpha:\beta$ T cell responses.

D. A typical protein antigen has very few determinants recognized by T cells.

E. CD4⁺ T cells are usually specific for exogenous antigens. Example: bacterial peptides

F. CD8⁺ T cells are usually specific for antigens endogenously synthesized by antigen-presenting cells. Example: viral peptides

NOTE: **DO NOT** think CD4⁺ T cells respond to only bacteria & CD8⁺ T cells respond to only viruses.

II. Constraints on T Cell Activation

A. Presence of T cells specific for peptide = **T Cell Repertoire**. Depends on presence of V genes, thymic maturation of T cells, and their survival of selection.

Absence of such T cells is called "Hole in T Cell Repertoire".

(Presented in T Cell Development Lecture)

B. Ability of peptide to bind MHC molecules = **Antigenic Determinant Selection**

(Presented in MHC Class I & II Lecture)

C. Production of proper peptide = **Antigen Processing**

III. Common Features of Professional Antigen-Presenting Cells

A. All professional presenting cells express both MHC class I & class II molecules, and can stimulate both CD4⁺ & CD8⁺ T cells.

B. All types of presenting cells increase expression of MHC & costimulatory molecules after activation.

C. Activated presenting cells are more potent in stimulating T cells.

IV. CD4⁺ T Cells: Evidence for Antigen Processing

- A. Antigens become sequestered inside presenting cells.
- B. Fixed presenting cells pulsed with intact antigen will not stimulate T cells.
- C. Peptides can be presented by fixed presenting cells.
- D. Purified class II molecules inserted into liposomes can present peptides.
- E. Peptides bind directly to MHC molecules.

V. CD4⁺ T cells: Mechanism of Antigen Processing

- A. Antigens are internalized by endocytosis (molecules) or phagocytosis (particles).
- B. Protein antigens are cleaved by acidic proteases called **cathepsins**.
Various protease inhibitors block antigen processing.
- C. Cleavage occurs in **endosomes and/or lysosomes**, acidic organelles.
Lysosomotropic agents that neutralize intracellular acidic pH inhibit antigen processing.

Also called acidotropic agents.

D. MHC Class II Compartment (MIIC). Also called Compartment of Peptide Loading (CPL) or Class II Vesicle (CIIV).

1. Newly synthesized class II molecules associate with Ii chain that contains a sorting signal. Directs class II molecules to an endocytic compartment.
2. Ii chain dissociates after its proteolytic cleavage by cathepsins.
3. Peptides bind class II molecules in the MIIC, a specialized compartment. MIIC is **not** an endosome or a lysosome.

E. **Class II-like** human DMA - DMB & murine H-2 M heterodimers.

1. Ii chain peptide CLIP dissociates from class II molecules. DM enhances efficiency of peptide exchange and thus antigen processing.
2. DM stabilizes empty class II molecules
3. DM itself does **not** bind antigen peptides
4. If DM molecule is absent, class II molecules are unstable and retain CLIP.

F. Peptides bind newly synthesized or recycled class II molecules inside presenting cells.

Relative use of these class II molecules differs among various types of presenting cells.

1. Treatment of presenting cells with cycloheximide, a protein synthesis inhibitor, may or may not block antigen processing.
2. Brefeldin A prevents protein transport from RER to Golgi. Treatment of presenting cells with Brefeldin A may or may not interfere with antigen processing.

G. Professional antigen-presenting cells differ in their expression of cathepsins.

VI. CD8⁺ T Cells: Evidence for Antigen Processing

A. For decades, CD8⁺ T cells were thought to be specific for intact foreign plasma membrane protein antigens, and that idea is wrong.

B. After immunization with live virus, CD8⁺ T cells are specific for viral nuclear protein which is not found at plasma membrane (Townsend et al, 1985).

C. Target cells transfected with genes encoding truncated forms of viral proteins are lysed by T cells.

- D. Peptides of viral or allogeneic MHC proteins inhibit or stimulate T cell responses.
- E. T cells from mice immunized with peptides of viral proteins can kill virally infected cells.

VII. CD8⁺ T Cells: Mechanism of Antigen Processing

- A. Lysosomotropic reagents usually do **not** inhibit lysis of target cells.
- B. Viral proteins have to be synthesized by target cells.
 - 1. UV irradiated virus will not stimulate flu-specific CD8⁺ T cells.
 - 2. Target cells pulsed with exogenous native viral proteins are not killed.
- C. Brefeldin A blocks lysis of target cells.
 - D. Cleavage of antigens occur in cytoplasm mediated by a multiple subunit complex called **proteasome** with a variety of proteolytic activities.
 - 1. Different subpopulations of proteasome with different activities.
 - 2. *Lmp 2 and 7* genes encode subunits, map to MHC, and modify activities.
 - E. **Tap** is a peptide transporter in RER membrane.
 - 1. Tap translocates cytosolic peptides into ER lumen.
 - 2. Forms heterodimer and is encoded by *Tap 1 & Tap 2* genes of the MHC
 - F. Peptides bind newly synthesized class I molecules in RER.
 - G. If peptides too long to bind class I molecule, can be trimmed after inside ER lumen.

IMPORTANT CONCEPT: Antigen processing by both pathways is an integral part of the synthesis & assembly of MHC molecules.

NOTE: The two antigen processing pathways are **not** completely separated from each other. Reason why CD4⁺ T cells can respond to viruses, and CD8⁺ T cells can respond to bacteria.

DIFFERENCES BETWEEN ANTIGEN PROCESSING PATHWAYS

CD 4⁺ T CELLS

Class II Restricted
Exogenous Antigen
Sensitive to Lysosomotropic Reagents
In Acidic Organelles
Cathepsins
Variable Sensitivity to Brefeldin A
No Equivalent Involves Peptide Transporter = Tap
Complex Forms in Post-Golgi = MIIC
Class II Targeted by Ii Chain
Involves Class II-Like Molecules
Recycled & Newly Synthesized Class II

CD 8⁺ T CELLS

Class I Restricted
Endogenously Synthesized Antigen
Insensitive
In Cytoplasm
Proteasome
Sensitive to Brefeldin A

Complex Forms in RER
No Equivalent
No Equivalent
Newly Synthesized Class I