APC and Antigen Presentation
Contents

• Introduction
• Antigen-presenting cells
• Antigen processing and presentation
APCs are Required to Present Antigenic Peptide Fragments to T cells

Abbas & Lichtman. Cellular and Molecular Immunology, 5th ed. W. B. Saunders 2003
Induction of adaptive immune responses

Antigens (microbes) enter through interfaces with external environment (portals of entry: skin, GI tract, respiratory tract)

Antigens are transported to peripheral lymphoid organs (lymph nodes, spleen) by professional APCs (Dendritic Cells) or in soluble form

Naïve lymphocytes that re-circulate through the same organs locate the antigens and are activated
The capture and presentation of Protein antigens by dendritic cells

Mannose receptors, C-type Lectin receptors and Toll-like receptors

Antigen capture by Langerhans cells (LC) | Loss of LC adhesiveness | Migration of LC | Maturation of migrating LC | Activation of naive T lymphocytes in draining lymph nodes

and expression of CCR7, and upregulation of MHC I & II, and coreceptors
Antigen-presenting cell, APC

A variety of cell types specialized in the presentation of peptide-MHC to lymphocytes, causing either tolerance or immunity.

Antigen presentation

The process by which APC express peptide-MHC on their cell surface in a form recognizable by lymphocytes.
Professional Antigen Presenting Cells

**Antigen uptake**
- Dendritic cell

**Antigen presentation**
- Costimulator (e.g., B7)
- CD28

**Response**
- Naive T cell activation; clonal expansion and differentiation into effector T cells

**Peripheral lymphoid organ or nonlymphoid tissues**
- Macrophage

**Peripheral lymphoid organ**
- B cell

**Effector T cell activation; activation of macrophages (cell-mediated immunity)**

**Effector T cell activation; B cell activation and antibody production (humoral immunity)**
I. Dendritic cells

Dendritic cells (DCs) are immune cells which are able to process antigen material and present it on the surface to other cells of the immune system (known as antigen-presenting cells).

DCs are the most powerful APCs and the only APCs that can stimulate the activation of naïve T cells (priming).

They act as messengers between the innate and adaptive immunity.

Dendritic cells were first described by Paul Langerhans (Langerhans cells) in the late nineteenth century. It wasn't until 1973, however, that the term "dendritic cells" was coined by Ralph M. Steinman and Zanvil A. Cohn.
Surface Marker of DC:

**MHC class I/II molecules**

CD1a, **CD11c**, CD83 (human)

33D1, NLDC145 (mouse)

**Co-stimulatory molecules:**

B7.1(CD80)/B7.2(CD86), CD40, CD44, CD54

**Pattern recognition receptors:**

TLRs, C-type Lectin receptors
Classification of DC

- **Lymphoid tissue DC**:
  - follicular (滤泡样) DC (FDC),
  - interdigitating (并指状) DC (IDC),
  - thymic DC

- **Non-lymphoid tissue DC**
  - Langerhans (朗格汗斯) cell (skin)
  - interstitial (间质性) DC

- **Circulating DC**
  - peripheral blood DC,
  - veiled (隐蔽) cell (lymph fluid)
1) Interdigitating DC (IDC)

- Main APC to induce primary immune response;
- Derived from Langerhans cells;
- FcR- and C3bR-, MHC I and II\(^{\text{high}}\);
- Distributed mainly in the T cell area of secondary lymphoid tissue, present Ags to T cells.
2) Follicular DC (FDC)

- Main APC to induce secondary immune response;
- Derived from interstitial DC;
- Highly express FcR, CR1 and CR2 (retain antigen-antibody-complement complex for B cells to recognize);
- Located in lymph follicles which are rich in B cells;
- Involved in the generation and maintenance of memory B cells.
3) Langerhans cells (LC)

- Found in the epidermis (skin) and mucous membranes;
- MHC I and II\textsuperscript{high}, highly express FcR and C3bR, Birbeck particle (due to langerin expression);
- Powerful ability to capture and process Ags and migration to lymph node after activation.
# Conventional DC and plasmacytoid DC

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Secretion</th>
<th>Toll-like receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloid dendritic cell (mDC)</td>
<td>Similar to monocytes. Two subsets: (1) mDC-1, major stimulator of T cells (2) Rare mDC-2, fighting wound infection</td>
<td>IL-12</td>
<td>TLR 2, TLR 4</td>
</tr>
<tr>
<td>Plasmacytoid dendritic cell (pDC)</td>
<td>Look like plasma cells, but have certain characteristics similar to myeloid dendritic cells. Produce high amounts of IFN-α and thus became known as IPC (interferon-producing cells) before their dendritic cell nature was revealed.</td>
<td></td>
<td>TLR 7, TLR 9</td>
</tr>
</tbody>
</table>
Maturation of Dendritic Cells
Immature DC

Phenotype:
- **High expression** of receptors related to phagocytosis (FcR, CR, mannose receptor, DC-sign);
- **Low expression** of CD54, CD40, CD80, CD86 and MHC II

Function: 1) strong capacity to ingest and process Ags, but weak ability to present Ags
2) induction of immune tolerance
3) sensing of infectious agents by TLR (pattern recognition receptors)

Langerhans cells (LC)
Mature DC

Phenotype:

*low expression* of receptors related to phagocytosis (FcR, CR, mannose receptor); *high expression* of CD54, CD40, CD80, CD86 and MHC II

Function:

weak ability to capture and process MHC II presented Ags, powerful ability to present MHC I and II presented Ags.

Lymphoid DCs
Dendritic Cell Maturation

Immature Dendritic Cell (periphery) → Mature Dendritic Cell (lymphoid tissue)

- Microbial Products
- Inflammatory Cytokines

Ag Capture
Ag Processing
Costimulation
T Cell Activation
Why are dendritic cells the most efficient APCs for initiating immune responses I

• Location
  - At sites of microbe entry (epithelia), tissues
  - Express receptors (CCR1,2, and 5) that recognize chemokines.

• Receptors for capturing microbes
  - Such as mannose and C-type lectin receptors and Toll-like receptors (TLRs) which function as pattern recognition receptors and have very active endocytic machinery
Why are dendritic cells the most efficient APCs for initiating immune responses II

Migration to T cell zones of lymphoid organs:

Role of CCR7 (ligands are Mip-3b and SLC)
Co-localize with naïve T cells

Maturation during migration:

Increase levels of MHC molecules, induce costimulators (B7 molecules, CD40) and decrease endocytic capacity
Conversion from cells for antigen capture into cells for antigen presentation and T cell activation
DC can induce immune tolerance

- **Central tolerance**: induced by negative selection of T cells in the thymus.

- **Peripheral tolerance**: immature DC capture autoantigen when they migrate from non-lymphoid tissue to T cell area of secondary lymphoid tissue, and induce peripheral tolerance.
The cells of the mononuclear phagocyte system originate in the bone marrow, circulate in the blood, and mature and become activated in various tissues (Fig. 2-5). The first cell type that enters the peripheral blood after leaving the marrow is incompletely differentiated and is called the monocyte. Monocytes are 10 to 15 μm in diameter, and they have bean-shaped nuclei and finely granular cytoplasm containing lysosomes, phagocytic vacuoles, and cytoskeletal filaments. Once they enter tissues, these cells mature and become macrophages. Macrophages may assume different morphologic forms after activation by external stimuli, such as microbes.
Monocytes/Macrophages

Phagocytic vacuoles and cytoplasmic organelles

FIGURE 2-6 Morphology of mononuclear phagocytes. A. Light micrograph of a monocyte in a peripheral blood smear. B. Electron micrograph of a peripheral blood monocyte. (Courtesy of Dr. Noel Weidner, Department of Pathology, University of California, San Diego.) C. Electron micrograph of an activated tissue macrophage showing numerous phagocytic vacuoles and cytoplasmic organelles. (From Fawcett DW. Bloom & Fawcett's Textbook of Histology, 12th ed. Chapman & Hall, 1994. With kind permission of Springer Science and Business Media.)
Monocytes/Macrophages

Macrophages (MΦ): white blood cells within tissues, produced by the division of monocytes

Greek: big eaters, from makros "large" + phagein "eat";

Functions:

Phagocytosis
Antigen processing and presentation

Surface Markers:
MHC-I and II molecules; CAM: LFA-1, ICAM-1, B7, CD40; CKR: M-CSFR; FcR; CR: CR1, CR3, CR4; Pattern-recognition receptor (PRR): mannose receptor, scavenger receptor (CD91), Toll-like receptors
Macrophages

A majority of macrophages are stationed at strategic points where microbial invasion or accumulation of dust is likely to occur. Each type of macrophage, determined by its location, has a specific name:

<table>
<thead>
<tr>
<th>Name of cell</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dust cells/Alveolar macrophages</td>
<td>pulmonary alveolus of lungs</td>
</tr>
<tr>
<td>Histiocytes</td>
<td>connective tissue</td>
</tr>
<tr>
<td>Kupffer cells</td>
<td>liver</td>
</tr>
<tr>
<td>Microglia</td>
<td>neural tissue</td>
</tr>
<tr>
<td>Epithelioid cells</td>
<td>Granulomas (肉芽肿)</td>
</tr>
<tr>
<td>Osteoclasts</td>
<td>bone</td>
</tr>
</tbody>
</table>

Resource: BM cell stimulated by CSF-1, peritoneal: 2-4% thioglycollate
Functions of Macrophages

- Phagocytosis
  destroy phagocytosed microorganisms

- Inflammation - Secretion of soluble factors:
  cytokines: IL-1, IL-6, TNF, IL-12, IL-18, etc.

- Antigen processing and presentation
  phagocytosis/pinocytosis/receptor-mediated endocytosis
Immune regulation by Macrophage

**Up-regulation:** Ag-presentation; secretion of cytokines that up-regulate immune response, e.g. IL-12, IL-18

**Down-regulation:** inhibitory $M\phi$ produce IL-10, TGF-$\beta$, PG, etc.

**Clearance of dead cells in the body to prevent autoimmunity**
B cells

Antigen bound by B-cell surface receptor

Antigen internalized and degraded to peptide fragments

Fragments bind to MHC class II and are transported to cell surface
Non-professional APCs

- Endothelial cell (EC)
- Fibroblastic cell
- Activated T cell

Under some circumstances, they can express MHC II and present Ags (e.g. IFNγ stimulation)

- Target cells (such as tumor cell, virus infected cells) express MHC I and present endogenous Ag to CD8+ T cells
Antigen processing and presentation

• Conversion of native antigen (globular protein) into peptides capable of binding to MHC molecules and transport to cell surface.

• Deliver processed antigen to lymphoid organ where T cells resides.

• Determines which source of antigen generates peptides that are displayed by class I or class II MHC.
Presentation of **Extracellular and Cytosolic Antigens**

<table>
<thead>
<tr>
<th>Antigen presentation to:</th>
<th>Class II–restricted CD4+ helper T cells</th>
<th>Class I–restricted CD8+ cytolytic T cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong> Endocytosis of extracellular foreign protein antigen</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>+</td>
<td>Class II MHC</td>
</tr>
<tr>
<td><strong>B</strong> Endogenous synthesis of foreign protein antigen</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Ovalbumin gene</td>
<td>Transfection</td>
<td>Processed peptide bound to class I MHC</td>
</tr>
<tr>
<td><strong>C</strong> Artificial introduction of foreign protein antigen into cytoplasm</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>Osmotic shock</td>
<td>Antigen uptake and release into cytosol</td>
</tr>
</tbody>
</table>

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The six steps of antigen presentation

1. Acquisition of antigen
2. Tagging antigen for destruction
3. Proteolysis
4. Delivery of peptides to MHC molecule
5. Binding of peptide to MHC molecule
6. Display of MHC-peptide complex
The pathway of MHC I-associated endogenous Ag presentation

endogenous antigen (such as virus Ag, tumor Ag)
  \[ \xrightarrow{\text{degraded by proteasome (LMP2/7)}} \]
  \[ \xrightarrow{\text{in cytoplasm}} \]
antigen peptide \((8-10 \text{ aa})\)
  \[ \xrightarrow{\text{transported to endoplasmic reticulum by TAP}} \]
Peptide/MHC-I molecule complex
  \[ \xrightarrow{\text{to surface of APC}} \]
present to CD8+T
Acquisition of antigen

1. High failure rate of protein synthesis – misfolded proteins must be destroyed – up to 30% of translation products may never fold correctly.

2. For viral infected cells acquisition of viral antigen is constitutive.
Class I MHC pathway

Tagging proteins for destruction (The Ubiquitin-proteasome pathway)

MQIFVKTLTGKTITLEVEPSD
TIENVKAKIQDKEGIPPDQQR
LIFAGKQLEDGRTLSDNYIQK
ESTLHLVLRLRGG 76aa
Proteolysis

Proteasome LMP2, LMP7, MECL-1
Aminopeptidases
Thimet oligopeptidase
Bleomycin hydrolase
Trpeptidyl peptidase II
ER resident aminopeptidases

Proteolysis is rapid/constitutive/processive/can be regulated by IFN\textsubscript{\gamma}
Degradation in the proteasome

Cytoplasmic cellular proteins, including non-self proteins are degraded continuously by a multicatalytic protease of 28 subunits.

The components of the proteasome include MECL-1, LMP2, LMP7. These components are induced by IFN-γ and replace constitutive components to confer proteolytic properties.

LMP2 & 7 encoded in the MHC

Proteasome cleaves proteins after hydrophobic and basic amino acids and releases peptides into the cytoplasm.
Proteasome, the Cytosolic Meat Grinder That Chops Up Proteins
Delivery of peptide to MHC molecule

Transporter-associated with antigen processing (TAP1 & 2)

Transporter has preference for >8 amino acid peptides with hydrophobic C termini.
Maturation and loading of MHC class I

Endoplasmic reticulum

Calnexin binds to nascent class I chain stabilises until 2-M binds floppy MHC

2-M binds and stabilises until 2-M binds floppy MHC

Tapasin, calreticulin, TAP 1 & 2 form a complex with the floppy MHC

Cytoplasmic peptides are loaded onto the MHC molecule and the structure becomes compact
The class I MHC pathway of processing of endogenous cytosolic protein antigens

- Partly folded MHC class I α chains bind to calnexin until β_2_-microglobulin binds
- MHC class I α:β_2_-m complex is released from calnexin, binds a complex of chaperone proteins (calreticulin, Erp57) and binds to TAP via tapasin
- Cytosolic proteins and defective ribosomal products (DRiPs) are degraded to peptide fragments by the proteasome. TAP delivers peptides to the ER
- A peptide binds the MHC class I molecule and completes its folding. The MHC class I molecule is released from the TAP complex and exported to the cell membrane

Figure 5-5 Immunobiology, 7th ed. (© Garland Science 2008)
Fate of MHC class I

Exported to the cell surface

Sent to lysosomes for degradation
Show movie
The pathway of MHC II-associated exogenous Ag presentation

Exogenous antigen

- Phagocytosis, pinocytosis, FcR-phagocytosis

Endosome

- Ii binds in the groove of MHC class II

Endosome

- lysosome

Phagolysosome

- protease

Degrade into 12~15aa peptide

- protease

- DM

- M II C

- release the CLIP and allowing other peptide to bind

Ag peptide/MHC class II molecule complex

transport to the surface of APC, recognized by CD4+T
Acquisition of exogenous antigens

Membrane Ig receptor mediated uptake

Complement receptor mediated phagocytosis

Opsonization

Fc receptor mediated phagocytosis

Phagocytosis

Pinocytosis
2. tagging for destruction

Entirely by physico-chemical means:

- low pH: $4.5 < \text{pH} < 6.0$
- strongly reducing environment $[-\text{SH}]= \text{mM}$
- enzymatic assistance: GILT (oxidoreductase)
Exogenous pathway

Antigen is taken up from the extracellular space into intracellular vesicles

In early endosomes of neutral pH, endosomal proteases are inactive

Acidification of vesicles activates proteases to degrade antigen into peptide fragments

Vesicles containing peptides fuse with vesicles containing MHC class II molecules

Figure 5-8 Immunobiology, 7ed. (© Garland Science 2008)
Class II-associated invariant chain peptide (CLIP)

In the endoplasmic reticulum, the invariant chain (II) forms a complex with MHC class II molecule, blocking the binding of peptides and misfolded proteins. II is cleaved in an acidified endosome, leaving a short peptide fragment, CLIP, still bound to the MHC class II molecule. Endocytosed antigens are degraded to peptides in endosomes, but the CLIP peptide blocks the binding of peptides to MHC class II molecules. HLA-DM binds to the MHC class II molecule, releasing CLIP and allowing other peptides to bind. The MHC class II molecule then travels to the cell surface.

Figure 5-11 Immunobiology, 7ed. (© Garland Science 2008)
The functions of Ii:

- involve in the assembling and folding of MHC class II molecule;
- Block the groove of MHC class II molecule;
- Lead the assembled class II molecule to M II C.

**CLIP**: class II-associated invariant chain peptide
MHC class II maturation and invariant chain

In the endoplasmic reticulum

Need to prevent newly synthesised, unfolded self proteins from binding to immature MHC

Invariant chain stabilises MHC class II by non-covalently binding to the immature MHC class II molecule and forming a nonomeric complex
Surface expression of MHC class II-peptide complexes

Exported to the cell surface (t1/2 = 50hr)

Sent to lysosomes for degradation

MIIC compartment sorts peptide-MHC complexes for surface expression or lysosomal degradation
The class II MHC pathway of processing of internalized vesicular protein antigens

1. Uptake of extracellular proteins into vesicular compartments of APC
2. Processing of internalized proteins in endosomal/lysosomal vesicles
3. Biosynthesis and transport of class II MHC molecules to endosomes
4. Association of processed peptides with class II MHC molecules in vesicles
5. Expression of peptide-MHC complexes on cell surface

Abbas & Lichtman. Cellular and Molecular Immunology, 5th ed. W. B. Saunders 2003
Show movie
How class I- and class II-associated antigen presentation influence the nature of the host T cell response

A. Class II MHC–associated presentation of extracellular antigen to helper T cells

- Antigen uptake or synthesis
  - Macrophage
  - Extracellular antigen
  - Class II MHC
- Antigen presentation
  - CD4+ helper T lymphocyte
  - Cytokines
- T cell effector functions
  - Macrophage activation: destruction of phagocytosed antigen
  - B cell antibody secretion: antibody binding to antigen

B. Class I MHC–associated presentation of cystolic antigen to cytolytic T lymphocytes

- Antigen uptake or synthesis
  - Endogenously synthesized antigen
  - Class I MHC
- Antigen presentation
  - CD8+ cytolytic T lymphocyte
- T cell effector functions
  - Killing of antigen-expressing target cell
Class II MHC pathway of presentation of vesicular peptide antigens

• Proteins ingested into endosomes/lysosomes are processed and presented in association with class II molecules
• Most vesicular peptides are derived from extracellular proteins
• CD4 binds to class II MHC; therefore, CD4+ T cells recognize class II-displayed peptides (only some cell types express class II MHC)
• CD4+ T cells are helper cells that activate B lymphocytes and macrophages
• Antibodies and macrophages attack and destroy extracellular microbes
Class I MHC pathway of presentation of cytosolic peptide antigens

- **Cytosolic proteins** are processed into peptides and presented in association with **class I molecules**
- Most cytosolic peptides are derived from **endogenous** (e.g. viral, tumor) proteins; some may be from phagocytosed microbes, proteins enter cytosol
- **CD8 binds to class I MHC; therefore, CD8+ T cells** recognize class I-displayed peptides
- **CD8+ T cells** give rise to **cytotoxic T lymphocytes (CTLs)** that **kill** other cells that harbor infections or are transformed (all nucleated cells express class I MHC)
- **CTLs** destroy cells infected with intracellular microbes and eradicate reservoirs of infection
Significance of MHC-associated antigen presentation

• MHC molecules display foreign and self peptides from the extracellular and intracellular environment
  - T cells survey the body for foreign (microbial) peptides

• Different classes of MHC molecules present cytosolic (endogenous) and vesicular (ingested) peptides
  - Helper T cells and CTLs respond to the microbes that each is best able to combat

• T cell receptors only recognize MHC-peptide complexes, and MHC molecules are cell surface proteins
  - T cells interact with other cells and not with cell-free antigens

• Only peptides bind to MHC molecules
  - T cells recognize only proteins (natural source of peptides)

• Few peptides are presented even from complex proteins
  - Immunodominance: few peptides bind to any MHC molecule
The problem for CD8 T cells

- Viruses and tumors may be present in any nucleated cells; therefore, the immune system has to be able to generate CTL responses (class I-restricted) to any nucleated cell.

- Only some APCs, particularly DCs, are able to initiate the responses of naïve T cells.

- How are antigens from virus-infected or neoplastic non-APC cell types “transferred” to APCs?
Cross-presentation of antigens to CD8$^+$ T cells

- Class I MHC molecules present exogenous Ags to CD8$^+$ T cells
- Cross-presentation of Ags by DC plays an important role in anti-viral infection and anti-tumor immunity.

-explains how infections of non-professional APCs can lead to the initiation of a CD8 T cell response
Functions of APCs

- Capture antigens and take them to the “correct” place
  - To peripheral lymphoid organs, through where naïve lymphocytes circulate

- Display antigens in a form that can be recognized by specific lymphocytes
  - For T cells: MHC-associated peptides (cytosolic peptides to class I, vesicular peptides to class II)
  - For B cells: native antigens; APCs include macrophages, follicular dendritic cells in germinal centers

- Provide “second signals” for T cell activation
  - Costimulators and cytokines induced by microbes; ensure that T cells respond best to microbial antigens
Thank you!