Cytotoxic Cells: CD$^+$ CTLs, NK cells, CD4+ killers

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Lecture outline

• Overview CD8+ T cell mediated immunity
• CD8+ T cell activation and differentiation into CTLs
• CTL effector function
• CTL function and dysfunction in human diseases
• Viral evasion of CTLs
• NK cell overview
• NK cell activation and inhibition
Role of CD8 T cells in eradicating infection

- Phagocytosed bacteria in vesicles and cytoplasm
- CD4+ T cell
- CD40 CD40L
- Killing of bacteria in phagolysosome
- Viable bacteria in cytoplasm
- Killing of infected cell
- Infected cell with microbes or antigens in cytoplasm
- CD8+ T cells (CTLs)
- Killing of infected cell
Induction and effector phases of CD8+ T cell responses

- Antigen recognition and induction of response in lymphoid organs
- T cell proliferation and differentiation
- Differentiated CD8+ CTL cells enter circulation
- Migration of effector T cells to site of antigen

Dendritic cells carry microbes or their antigens to lymph nodes

Lymph node

Naive CD8+ T cell

Costimulator

CD8+ effector T cells (CTLs)

Naive CD8+ T cell

Microbe

Tissue site of infection

Dendritic cell

CTL killing of infected tissue cell
Cross presentation: Proteins taken into cell via endocytosis/phagocytosis are transported to cytosol where they enter the class I MHC processing pathway.
Prior to infection: 1 in 100,000 naïve CD8+ T cells specific for any viral peptide

After antigen stimulation: 15–20 divisions, 50,000-fold expansion

After infection is resolved: 90%–95% CTL undergo apoptosis

For up to 75 years: long-lived population of memory cells remain
Role of helper T cells in the differentiation of CD8+ T lymphocytes

Major role of CD4+ T cells in CD8+ T cell response is the generation of memory CD8+ T cells
Steps in cytotoxic T lymphocyte–mediated lysis of target cells

Antigen recognition and immune synapse formation

Granule exocytosis

Detachment of CTL

Target cell death
CTL Granule Proteins: PERFORIN and GRANZYMES

PERFORIN:
- Pore forming/membrane disruptive protein
- Works on cholesterol rich membranes (e.g. mammalian but not microbial cells)
- Homologous to Complement C9
- Required for delivery of granzymes into target cells

GRANZYMES
- Serine proteases that cleave a variety of substrates, including caspases.
- 5 human Gzms
- Gzms activate target cell apoptosis through caspase-dependent and independent pathways
- GzmB has the strongest pro-apoptotic function; most clearly implicated in CTL and NK induced apoptosis
Mechanisms of cytotoxic T lymphocyte–mediated killing of target cells

A. Perforin/granzyme–mediated cell killing

- Target cell
- CD8\(^+\) CTL
- CTL releases granule contents into immune synapse
- Perforin induces uptake of granzymes into target cell endosome and release into cytosol, activating caspases

Apoptosis of target cell
Granzyme B Delivery Cell Death

CAD = Caspase-activated DNase
CTLs are Serial Killers

One CTL can sequentially kill several target cells.

The CTL may protect itself by cathepsins that degrade released perforin that binds to the CTL membrane.

Perforin molecules that diffuse away are inhibited by plasma lipids.

The formation of an immune synapse between a CTL and target limits bystander cell damage.

Bystander cells (e.g. antigen presenting cells) may be protected from death by expressing specific and irreversible granzyme inhibitors (serpins).
CTL Granule Proteins: Granulysin

- Lipid-binding, cationic peptide
- Works on cholesterol-poor membranes on microbes
- Preferentially lyses microbial cells
- Has alarmin/pro-inflammatory properties

Clinical Evidence for Roles of CD8+ T cells

• CD8 T cell deficiency caused by homozygous mutations in \textit{CD8, TAP1, TAP2, DOCK8}
  • Chronic sinopulmonary infections
  • Severe cutaneous HSV and HPV infections

• Exhausted CD8\(^+\) T cells in cancer patients
  • Block PD-1, revive CTL, enhanced anti-tumor immunity and anti-viral responses

• A functional CTL (CD8+) response is required to clear the infection and
  • COVID-19 severity is increased as the CD8+ response becomes somehow diminished or exhausted
T cell exhaustion
Role of CTL/Perforin in Diseases

- Perforin plays a permissive role in malaria: attack on antigen-bearing brain endothelial cells
- Perforin plays a key role in the autoimmune destruction of insulin-producing $\beta$ cells in the pancreatic Islets leading to Type 1 diabetes mellitus
- CTL are major contributors to:
  - Viral myocarditis and dilated cardiomyopathy
  - Allograft rejection
  - Liver injury in acute viral hepatitis
Hemophagocytic lymphohistiocytosis

What Happens Without CTL of NK Killing Function?

• **Familial hemophagocytic lymphohistiocytosis** (HLH): mutations in perforin gene or other genes critical for degranulation of cytotoxic granules

• NK cells and CTL can be activated by infected cells to secrete interferon-γ, but cannot kill the infected cells, so excess interferon-γ keeps getting produced

• Uncontrolled activation and proliferation of CD4+ and CD8+ T cells, cytokine storm, macrophage activation and proliferation, pancytopenia, and anemia.

• Activated macrophages in the spleen and bone marrow are intensely phagocytic, removing erythrocytes, leukocytes, and platelets from the circulation
Viral evasion of CTL surveillance

- Inhibition of proteasomal activity: EBV, human CMV
- Block in MHC synthesis and/or ER retention: adenovirus, human CMV

Class I MHC pathway

- Block in TAP transport: HSV
- Removal of class I from ER: CMV
- Engagement of NK cell inhibitory receptors by "decoy" viral class I-like molecules: murine CMV

Cytosolic protein → Proteasome → ER → Virus

CD8+ CTL

NK cell
Natural Killer (NK) Cells

Like other ILCs, NK do not have highly diverse clonally distributed antigen receptors
NK Cell Function

- Functions of NK cells are similar to those of CD8+ cytotoxic T lymphocytes (CTLs)

- NK activation by infected cells results in release of perforin, granzymes which kill the target cell, and secretion of interferon γ, which activates macrophages.
NK Activation: Balance of Inhibitory and Activating Receptors

Inhibitory receptors:

- Recognize Class I MHC proteins (markers of normal self expressed by all nucleated cells)
- Signal via ITIM motifs
Antibody-dependent cellular cytotoxicity (ADCC)

1. Antibody-coated cell
2. Surface antigen
3. IgG
4. FcγRIII
5. NK cell
6. Killing of Antibody-coated cell
Clinical Evidence of Role of NK cell in Defense and Disease

Evidence that NK cells are important in viral immunity in humans and mice
• Severe Epstein-Barr virus infection in rare patients lacking NK cell function (MCM4, GATA2, IRF8 mutations)
• Severe herpesvirus infections in rare patients without Natural Killer cells.
• Natural Killer cell depletion enhances virus synthesis and virus-induced hepatitis in vivo.

NK cells in Hematopoietic Stem Cell Transplantation
• Detrimental effects – grafted allogeneic stem cell rejection by host NK cells
• Beneficial effects - in leukemia patients given an allogeneic hematopoietic stem cell graft ---- graft vs. leukemia activity – arising donor NK cells kill residual host leukemia cells