

Cell mediated effector responses: cytotoxic T cells

Humoral and cellular immunity

- The cell mediated and humoral responses **play different roles** in protecting host.
- The humoral responses mediated by antibodies can bind and neutralize antigens on the surface of cells and in the extracellular spaces. Thus **primary domain of antibodies lies outside the cells**.
- The principal role of **cell-mediated immunity** is to detect and eliminate cells that harbor **intracellular pathogens** such as viruses and mycobacteria, or **altered-self cells such as tumor cells**.

Cell-mediated Immunity (CMI)

- Just as B cells differentiate into plasma cells to become “effective”, so **resting T cells differentiate into memory/effector cells**.
- Effects of cells involved in CMI include **cytokine secretion (help) and direct cytotoxicity** to affected cells (CTLs)
- These effector cells may be
 - (a) **Helper T cells**
 - (b) **Cytotoxic Cells**
- **Both antigen specific and antigen-nonspecific cells can contribute to the cell-mediated immune response.**

Cells of CMI

- **Antigen specific cells of cellular immunity**
 - **Cytokine-secreting CD4+ T_H cells** which mediate **delayed type hypersensitivity (will not be discussed in detail here)**.
 - **CD8+ cytotoxic T lymphocyte (T_C cells or CTLs)**
- **Antigen-nonspecific cells of cellular immunity**
 - **Natural Killer (NK) Cells**
 - **Nonlymphoid cell types such as macrophages, neutrophils, and eosinophils** .
- **Cellular immune responses are not completely independent of antibodies** as macrophages, neutrophils and eosinophils use antibodies in **Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)**.

Effector responses

- **The importance of cell mediated immunity becomes evident when immune system is defective.**
- Children in **DiGeorge syndrome**, who are born without a thymus generally are able to control extracellular bacteria but **can not effectively eliminate intracellular pathogens (virus, intracellular bacteria and fungi)**.
- **In these children even the attenuated virus present in a vaccine can cause life-threatening infections.**

Naïve and Effector T cells

- **In particular, effector T cells are characterized by their less stringent activation requirements, increased expression of adhesion molecules, and production of both membrane bound and soluble effector molecules.**

TABLE 14-1 Comparison of naïve and effector T cells

Property	Naïve T cells	Effector T cells
Co-stimulatory signal (CD28-B7 interaction)	Required for activation	Not required for activation
CD45 isoform	CD45RA	CD45RO
Cell-adhesion molecules (CD2 and LFA-1)	Low	High
Trafficking patterns	HEVs* in secondary lymphoid tissue	Tertiary lymphoid tissues; inflammatory sites

*HEV = high endothelial venules; sites in blood vessel used by lymphocytes for extravasation.

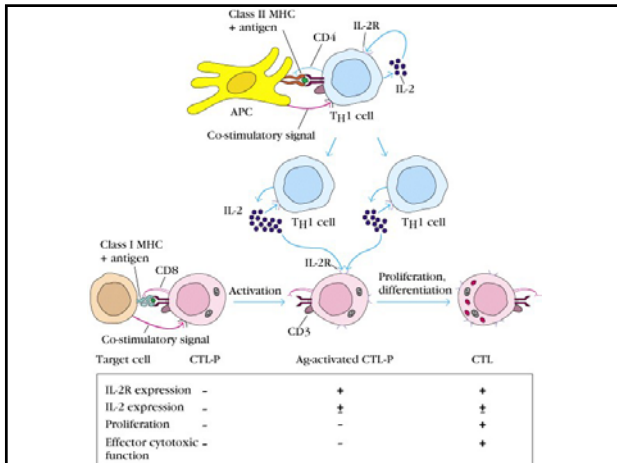
Effector molecules of effector T cells

TABLE 14-2 Effector molecules produced by effector T cells

Cell type	Soluble effectors	Membrane-bound effectors
CTL	Cytotoxins (perforins and granzymes), IFN- γ , TNF- β	Fas ligand (FASL)
T _H 1	IL-2, IL-3, TNF- β , IFN- γ , GM-CSF (high)	Tumor necrosis factor β (TNF- β)
T _H 2	IL-3, IL-4, IL-5, IL-6, IL-10, IL-13, GM-CSF (low)	CD40 ligand

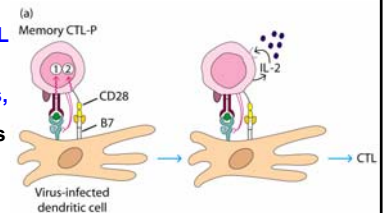
Cytotoxic T Cells

- Naive Cytotoxic T cells (CTLs) are **incapable of killing target cells** and are, therefore, referred to as CTL-precursors (CTL-Ps)
- The effector cytotoxic T cells (CTLs) which **have cytolytic activity** are generated after activation of naive CTLs.
- Stimulation of CTL-Ps is similar to T_H cells and require **3 sequential signals**
 - Signal 1 - Via the TCR and MHC-Class I
 - Signal 2 - Via CD28 and B-7 on APCs
 - A third cell division signal, via IL-2, is required for proliferation and differentiation of antigen activated CTL-P into effector CTLs.
- Note: In general, the amount of IL-2 secreted by an antigen activated CTL-Ps may be not sufficient to induce their proliferation and differentiation and require additional IL-2 produced by proliferating TH1 cells.



Memory CTL-P and effector CTL

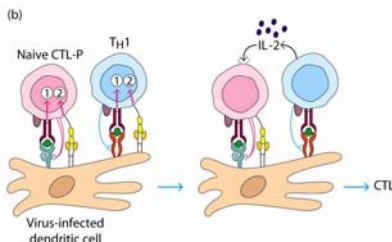
- In contrast to naive CTL-Ps, memory CTL-Ps by themselves may produce sufficient amount of IL-2 required their proliferation and differentiation into effector CTLs and do not require IL-2 from TH1 cells.
- Thus memory CTL-Ps have lower activation requirement than naive CTL-Ps.
- Unlike immature CTLs (CTL-Ps), activated effector CTLs do not require Signal 2 or IL-2 to kill target cells.
- Note: this means that **PROFESSIONAL APCs are still required for initial activation of CTL-Ps**, but that **ALL CELLS which express Class I can be killed by mature CTLs**.



Role of TH1 cells in effector CTL generation

- The role of TH1 cells in the generation of effector CTL is not fully understood and it unlikely that CTL and TH1 cell interact directly.
- IL-2 and costimulation are essential for the activation of naive CTL into effector CTL and TH1 cells can be the mediators in providing these signals to CTL.

- In addition to providing IL-2, TH1 cells can induce the upregulation of co-stimulatory molecules on the APCs and thereby help in the CTL-P cells activation and differentiation.



Mature CTLs

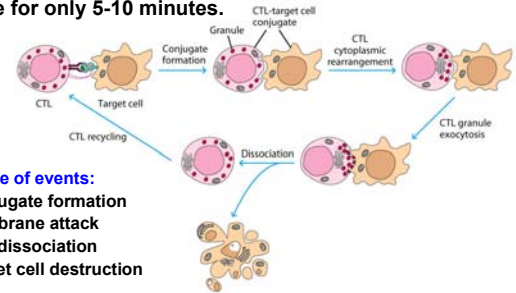
- The proliferation and differentiation of both antigen activated TH1 cells and CTL-Ps depend on IL-2. So IL-2 is one of the most important cytokine for T cells activation and differentiation.
- In IL-2 knockout mice, the absence of IL-2 abolish the CTL-mediated cytotoxicity.
- After clearance of antigen, the level of IL-2 declines and this induces TH1 cells and CTL to undergo programmed cell death by apoptosis and immune response is rapidly terminated.
- In this way, this lessens the likelihood of nonspecific tissue damage from the inflammatory response.

Effector CTLs

- The **effector CTLs** have the capability to lyse cells and are critical in the recognition and elimination of **altered self cells** (e.g. virus infected cells and tumor cells) and in the **graft-rejection reactions**.
- In general **CTLs** are **CD8+** cells and are **MHC-1 restricted** although in rare circumstances **CD4+ Class-II MHC** restricted T cells have been shown to function as CTLs
- The CTL mediated immune responses can be divided into two phases
 - **First phase** activates and differentiates naive T cells into functional effector T cells
 - In **second phase**, **effector CTLs** recognize antigen-class 1 MHC complex and **destroy the target cells**.

Stages in CTL-mediated killing of target cells

- After antigen specific recognition, **LFA-1** on CTL binds to **ICAMs** on the target cell membrane forming a **conjugate**.
- Antigen mediated CTL activation converts **LFA-1** from a **low-avidity state to a high-avidity state** and persist in this stage for only **5-10 minutes**.



Sequence of events:

1. Conjugate formation
2. Membrane attack
3. CTL dissociation
4. Target cell destruction

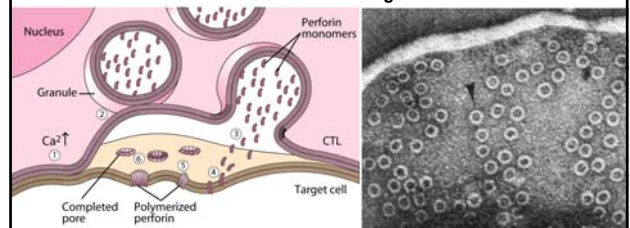
Cell Death induced by CTLs

- Effector CTLs** induce cell death in target cells in **two ways**
 1. **Directional delivery of cytotoxic proteins** (perforin and granzymes) by CTLs to target cells
 2. **Fas mediated cytotoxicity**
- Cell death by locally secreted granules:**
 - **A) Perforin**-which forms **Complement-like pores** in the membranes of target cells
 - **B) Granzymes/Fragmentins** – serine proteases which digest the intracellular contents of the target cell and induce **apoptosis/DNA fragmentation in the target**.

Note: **CTL-Ps** lack **cytoplasmic granules** (granzymes and perforins); They appear in effector CTL only after activation of CTL-Ps .

Perforin and granzyme mediated killing

- Perforin** exhibits some **sequence homology with the terminal C9 component of complement system**.
- The **membrane pores** formed by perforin are similar to those observed in **complement-mediated lysis**.
- Perforin mediates granzyme entry in two ways**
 1. **Pore formation** in the cell membrane of the target
 2. **Perforin assisted pathway:** Perforin is necessary for release of granzyme B from the vesicle into the cytoplasm of target cell.



Fas mediated cytotoxicity of CTLs

- Some CTL lines lack perforin and granzymes. In these, **cytotoxicity is mediated by Fas**.
- Fas** is a **transmembrane protein** present on the target cells.
- Fas can deliver a death signal** (apoptosis of target cells) when crosslinked by its natural ligand called '**Fas ligand**' (FasL) present on the membrane of activated CTLs.
- A feature of cell death by apoptosis is the involvement of the '**caspase**' family of **cysteine proteases**.
- CTLs use '**perforin/granzymes**' and '**Fas ligand**' to initiate '**caspase**' cascade in their targets to induce apoptosis.

Next Lecture

- Chapter 14:** Cell mediated effector responses
 - NK cells
 - ADCC
 - Assessment of cell mediated cytotoxicity