

## Cell-mediated effector responses

Antibodies mainly eliminate **extracellular** bacteria and their products. The cell-mediated branch of the immune system targets **intracellular** pathogens like viruses and intracellular bacteria and fungi.

**There are two major categories of cell-mediated immunity:**

- 1-Killing by cytotoxic T lymphocytes (CTLs or TC), natural killer cells (NK cells) and antibody-dependent cell-mediated toxicity (ADCC);
- 2-Delayed-type hypersensitivity (DTH) mediated by TH1 cells.

The **effector molecules** that are produced by effector T cells, and we will talk about some of these individually later. Note that TH2 cells, which do **not** participate in cellular immunity but which help B cell immunity, are shown for comparison. Also note that all three cell types produce **both soluble and surface-displayed molecules** that contribute to the activity of the cell.

## Cell-mediated responses that result in direct killing by effector cells Cytotoxic T cells

There are two phases of activation for a CTL response:

Naïve CTL precursors (CTL-P cells) are activated to differentiate into CTLs;  
Effector CTLs recognize the target and destroy it.

In phase 1, which probably occurs on the surface of an APC, the TCR of the CTL-P binds to class I / peptide complex of stimulator cell, and CD28 of CTL-P binds to B7 of the APC . The now-activated CTL-P begins to express **TAC**, the  $\alpha$  chain of the IL-2 receptor, which converts its IL-2 receptor to the high-affinity form. IL-2 that **probably** is made by a TH1 cell interacting with the same or a nearby APC binds to the CTL-P's IL-2 receptor (paracrine stimulation) and causes it to proliferate and differentiate into an **effector** or **memory** CTL. Memory CTLs have visible **granules** that contain proteins they will use when they kill. As the amount of antigen in the body is gradually reduced by immune action, the actual effector CTLs and TH1 helper cells involved die by apoptosis, but memory cells survive in case the antigen returns again.

## Target cell binding and destruction by CTLs

The process of target cell binding and destruction by CTLs . When the effector CTL's TCRs encounter and successfully bind the class I/peptide complexes on a target cell, a signal is given that causes the adhesion molecule **LFA-1** on the CTL surface to undergo a conformational change and enter a **high affinity state** for a period of 5-10 minutes. This allows the CTL to adhere more tightly to the target, and during this period, the target cell is "programmed" to undergo apoptosis. LFA-1 then reverts to its normal low-affinity conformation, and the CTL dissociates from the target cell.

While it is bound to the target cell, the microtubule-organizing center (MTOC) Golgi stacks and cytoplasmic granules of the CTL re-orient toward the side of the nucleus where the junction has formed. Molecules of the protein, **perforin**, which has some homology to the pore-forming component of complement, **C9**, are released from the CTL's cytoplasmic granules into the space between the CTL and target cells. In the presence of sufficient  $Ca^{+2}$  ion, the perforin inserts into the target cell membrane, forming pores .Proteolytic enzymes called **granzymes**, also released from the CTL's cytoplasmic granules, enter the target cell through the perforin pores and initiate the apoptotic program in the target cell. This program involves activating **caspases**, proteases that pre-exist in the target cell in an inactive form. The result of the caspase cascade is DNA and nuclear fragmentation, blebbing of the cell membrane, and other hallmarks of apoptosis. CTLs can also initiate apoptosis in the target cell by a mechanism involving the **Fas ligand (Fas-L)** molecule, a member of the **tumor necrosis factor family**, that it carries on its cell surface. If the target cell has **Fas**, the receptor for Fas-L, on its surface, the trimeric Fas-L on the CTL causes the Fas on the target cell to trimerize. Associated with Fas is the protein, **FADD ( for Fas-associated protein with death domain)**, and trimerization causes another protein, **procaspase 8**, to associate with FADD via its own death domain. Procaspase 8 becomes activated to **caspase 8**, and a resulting caspase cascade leads to apoptosis of the cell.

## Differences between naïve and effector or memory CTLs

Naïve and effector or memory CTLs differ in a number of ways :

1. CD28/B7 interaction is required for naïve CTL-P activation but not for activation of effector or memory CTLs;
2. Effector or memory CTLs have approximately 2-4 times as much of the adhesion molecules, CD2 and LFA-1 (which interact with LFA-3 and ICAM, respectively, on target cells) as naïve CTL-P cells;
3. Effector and memory CTLs have cytoplasmic granules, whereas CTL-P cells do not;
4. Naïve CTL-P cells can leave the circulation only at the high endothelial venules (HEVs) in lymph nodes, whereas memory and effector CTLs can exit the circulation in the skin, mucosal tissues and sites of inflammation due to the presence of the necessary “homing receptors” on their surfaces.
5. Both naïve and effector or memory CTLs require for their activation the cell surface protein, CD45. The phosphatase activity of CD45's cytoplasmic domain dephosphorylates and thereby activates src-family kinase members such as Lck and Fyn. However, the isoform of CD45 expressed by effector and memory CTLs (called **CD45RO**) associates with the TCR/CD3/CD8 complex better than the form expressed on CTL-Ps (**CD45RA**). These isoforms result from **alternative splicing** of the CD45 mRNA. CD45RO is thought to be better than CD45RA at activating the src-family kinases.

## Natural killer (NK) cells

NK cells appear to develop from a progenitor that also gives rise to T cells. However, NK cells are negative for TCRs, CD4 and CD8, and they carry a surface molecule called **CD16** which is one of the Fc-receptors that bind IgG isotypes (**Fc $\gamma$ RIII**).

NK cells arise during a cell-mediated response earlier than CTLs. Virus-infected cells produce **IFN- $\gamma$  and IFN- $\alpha$**  which stimulate an increase in NK cell numbers and activity that peaks at 3 days post-infection. As the NK cell peak declines, CTLs appear to peak in activity at 7 days post-infection. So NK cells are a first line of defense. However, there is **no NK cell memory**, so if virus appears again, the response of NK cells is new.

Like CTLs, NK cells kill by means of perforin and granzymes stored in granules. And they also kill by means of **TNF- $\alpha$**  that is either secreted or displayed on their surface.

Since they do not have a self-restricted TCR on their surface, how do they know what targets to kill?

Over the past approximately 5 years, the activity of NK cells has been shown to be controlled by **opposing activation and inhibitory signals** mediated by several classes of cell surface receptors .

**Activation receptors (AR)** are C-type lectins like **NKR-P1** which require  $\text{Ca}^{+2}$  ion for activity and recognize carbohydrates on the surface of target cells. They may be specific for altered glycosylation patterns stimulated by viral infection. Other adhesion molecules like CD2 and CD28 may also contribute to a signal to kill a target. (CD16 can also participate, but it requires the presence of bound antibody). Recognizes self MHC molecules on the target cell, the **activation signal is vetoed** and the target cell is not killed. The inhibitory receptors are of two types:

**C-type lectin inhibitory receptors (CLIRs)** – heterodimeric CD94/NKG2 receptors fall into this class. They recognize a class I MHC molecule called **HLA-E** that cannot get to the cell surface unless it contains a peptide derived from HLA-A, HLA-B or HLA-C. The amount of HLA-E on the surface is an indication of how much of the classical Class I MHC molecules are being made by the cell. These receptors are **not** specific for which alleles of class I molecules are being produced.

In contrast, the second set of inhibitory receptors, called **killer cell inhibitory receptors (or KIR)** are specific for one or several allelic products of the HLA-A, HLA-B and/or HLA-C loci. There are at least 50 different members of the KIR family, and each NK cell can express several of these. KIR receptors are members of the immunoglobulin superfamily, and therefore are also called **ISIRs (for immunoglobulin superfamily inhibitory receptors)**.

**Either type of inhibitory receptor has veto power over AR receptors.**

The result of the opposing signals mechanism is that cells with low amounts of class I MHC molecules or low amounts of “normal self” class I MHC molecules are targeted for killing by NK cells. As we have seen earlier, viruses have evolved mechanisms for escaping killing by CTLs by reducing the expression of class I MHC molecules on the cell surface. NK cells will take note of the low level of class I MHC and kill the virus-infected cell.

## **Antibody-dependent cell-mediated cytotoxicity (ADCC)**

NK cells, macrophages, monocytes neutrophils and eosinophils all have **Fc receptors** that allow them to passively adsorb antibodies that have been secreted by plasma cells onto their cell surfaces. If those antibodies have specificity for a molecule on the surface of a target cell (e.g., an antigen expressed on a budding virus), the potential killer cell can use that antibody to establish contact with the virus-infected target . The cells then are activated to become **effector cells**, and the killing mechanism that develops depends upon the cell type whose Fc receptor bound the antibody:

- Neutrophils kill by secretion of lytic enzymes;
- NK cells kill by means of perforin, granzymes and TNF;
- Macrophages kill by release of lytic enzymes and TNF;
- Eosinophils kill by release of lytic enzymes and perforin.

## **Delayed-type hypersensitivity (DTH)**

DTH is initiated by TH1 cells recognizing class II MHC/peptide complexes on APCs. DTH is characterized by large influxes of **non-specific inflammatory cells**, principally **macrophages**, that are attracted to the area. Relatively few of the cells in such an inflammatory infiltrate are TH1 cells. Examples of DTH reactions are the **tuberculin reaction** observed in an individual who has been exposed to *Mycobacterium tuberculosis* and is injected intradermally with **PPD**, a **purified protein derivative** derived from the bacteria. A firm swelling is observed 24-48 hours after challenge due to the cellular infiltrate attracted by sensitized TH1 cells (also called **TDTH cells**). **Poison ivy** and **poison oak** are other examples of DTH responses. They occur in a sensitized person 24-48 hours after re-exposure to the oils produced by these plants.

The **sensitization phase** for DTH occurs during the 1-2 weeks following initial exposure to the antigen. If exposure is through the skin as for poison ivy, Langerhans cells (members of the dendritic cell family) carry the antigen to the draining lymph nodes where it activated TDTH cells whose TCRs recognize a complex of class II MHC and peptide derived from the antigen. The sensitization phase involves the co-receptor **CD28**, and the sensitized cells produce **IL-2 receptor** and **IL-2** which stimulates them in an **autocrine** fashion.

The **effector phase** of the response occurs upon secondary contact with the antigen. The sensitized TDTH cells secrete cytokines and chemokines that attract inflammatory cells to the area of re-exposure to antigen.



The peak of the response does not occur for 24-48 hours since it takes **time** to recruit the inflammatory cells to the area and activate them. Monocytes arrive from the blood and differentiate to macrophages which are activated by cytokines secreted by TDTH cells and by **TNF- $\alpha$**  on the TDTH cell surface. The macrophages then up-regulate their own **class II MHC molecules** and **TNF receptors** which contribute to further amplifying the reaction. They also **produce oxygen radicals and nitric oxide** which help to destroy the antigen. If the antigen is difficult to eliminate, like *Mycobacterium tuberculosis* that infect and live inside macrophages in the lung, the DTH response is prolonged and results in **granulomas** developing within the lung tissue . The activated macrophages effectively wall off the infected cells within a nodule called a **tubercle**. Unfortunately, release of destructive molecules such as reactive oxygen species damages healthy tissue as well as infected cells.

Besides *Mycobacterium tuberculosis*, a variety of other **intracellular-dwelling** organisms stimulate DTH responses.

Intracellular fungi such as *Pneumocystis carinii* and *Cryptococcus neoformans*;

Intracellular parasites such as *Leishmania*;

Viruses such as Herpes simplex, measles virus and smallpox virus (variola);

Contact antigens such as poison ivy and certain dyes.

In AIDS patients whose cell-mediated immune systems are devastated by the HIV virus, T<sub>H</sub>1 cells are all but absent. Their inability to mount DTH responses to intracellular fungi such as *Pneumocystis carinii* and *Candida albicans*—organisms that a healthy individual easily fights off by DTH—allows these organisms to establish fulminant infections, resulting in serious symptoms and sometimes death. These infections in AIDS patients are termed **opportunistic infections**, since the organisms use the opportunity of a depressed DTH responsiveness to establish themselves.