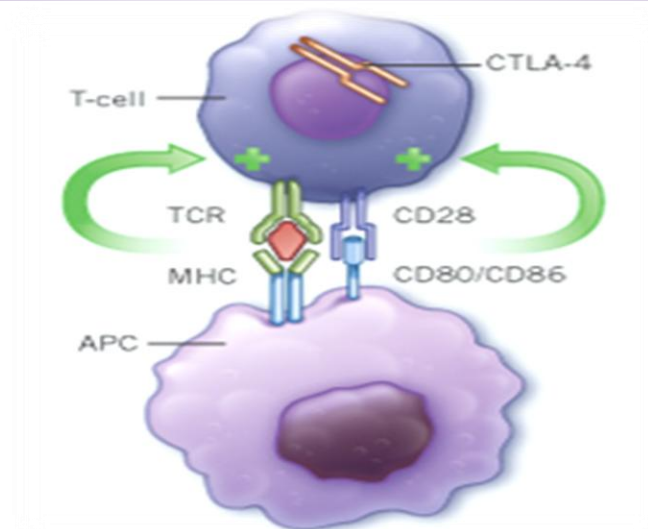


Teaching unit 06




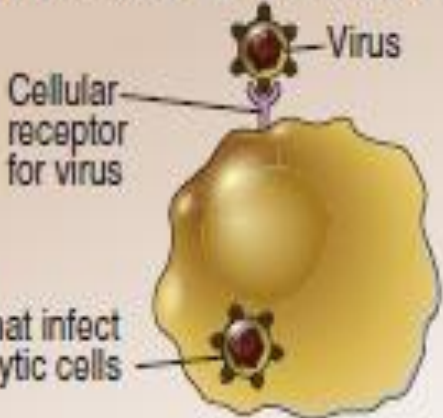
Effector mechanisms of cellular immunity

Cellular immune response



Activation of T lymphocytes by intracellular microorganisms

... let's repeat

Intracellular microbes	Examples
<p>(A) Phagocyte</p>  <p>Phagocytosed microbes that survive within phagolysosomes</p> <p>Microbes that escape from phagolysosomes into cytoplasm</p>	<p>Intracellular bacteria: <i>Mycobacteria</i> <i>Listeria monocytogenes</i> <i>Legionella pneumophila</i></p> <p>Fungi: <i>Cryptococcus neoformans</i></p> <p>Protozoa: <i>Leishmania</i> <i>Trypanosoma cruzi</i></p>
<p>(B) Nonphagocytic cell (e.g., epithelial cell)</p>  <p>Virus</p> <p>Cellular receptor for virus</p> <p>Microbes that infect nonphagocytic cells</p>	<p>Viruses: All</p> <p>Rickettsiae: All</p> <p>Protozoa: <i>Plasmodium falciparum</i> <i>Cryptosporidium parvum</i></p>

Cellular immunity protects us from intracellular microorganisms

T lymphocytes play a major role in this type of acquired immunity

There are two types of intracellular infections

The life history of T lymphocytes

Precursors mature in the thymus



Naïve CD4⁺ and CD8⁺ T cells enter the circulation



Naïve T cells circulate through lymph nodes
and find antigens



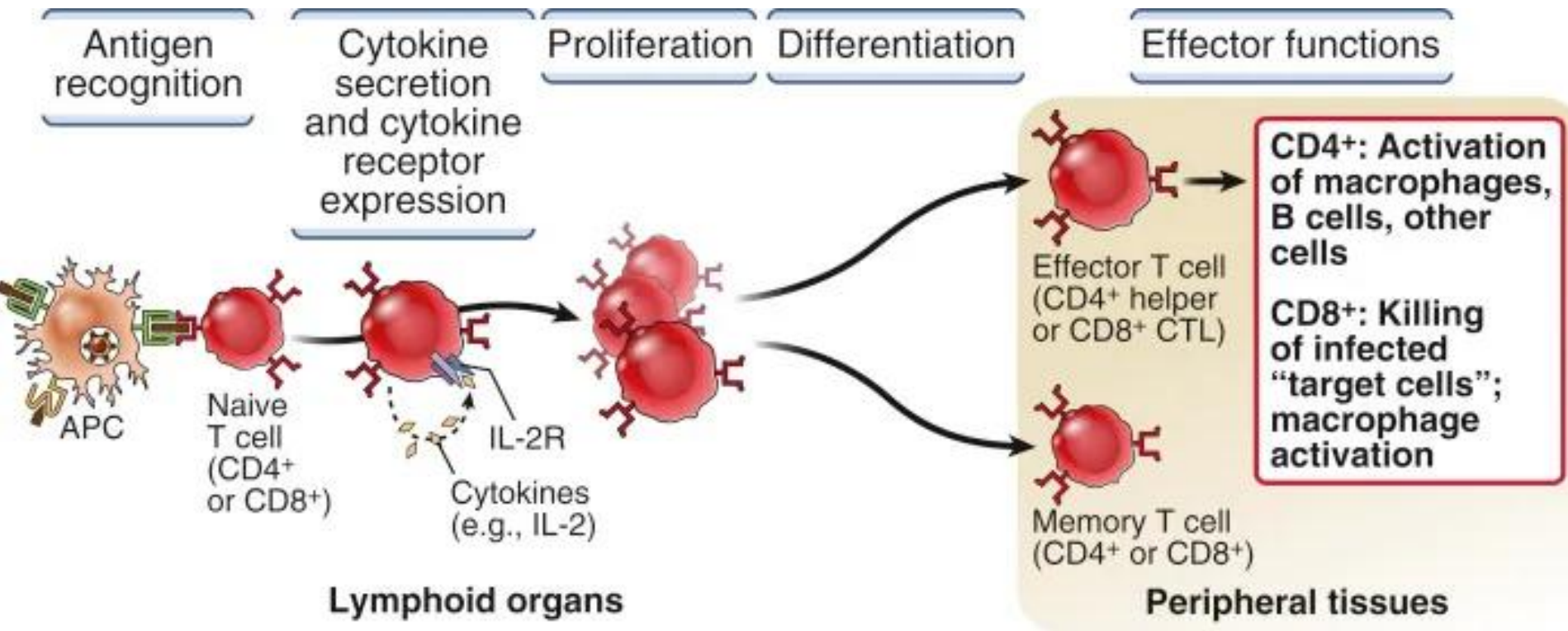
Clonal expansion;
differentiation into effector and memory cells



Effector T cells migrate to sites of infection

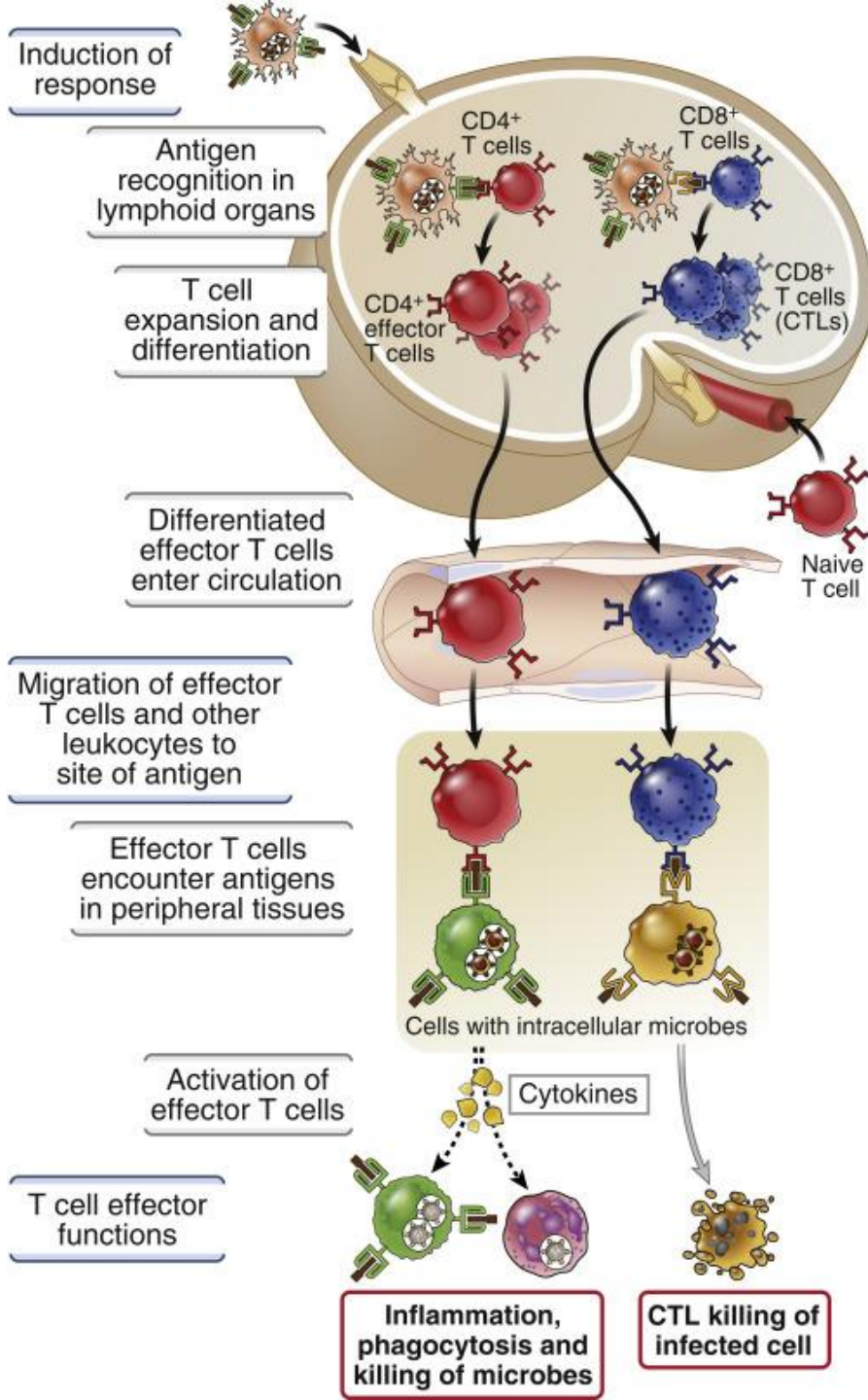


Eradication of infection

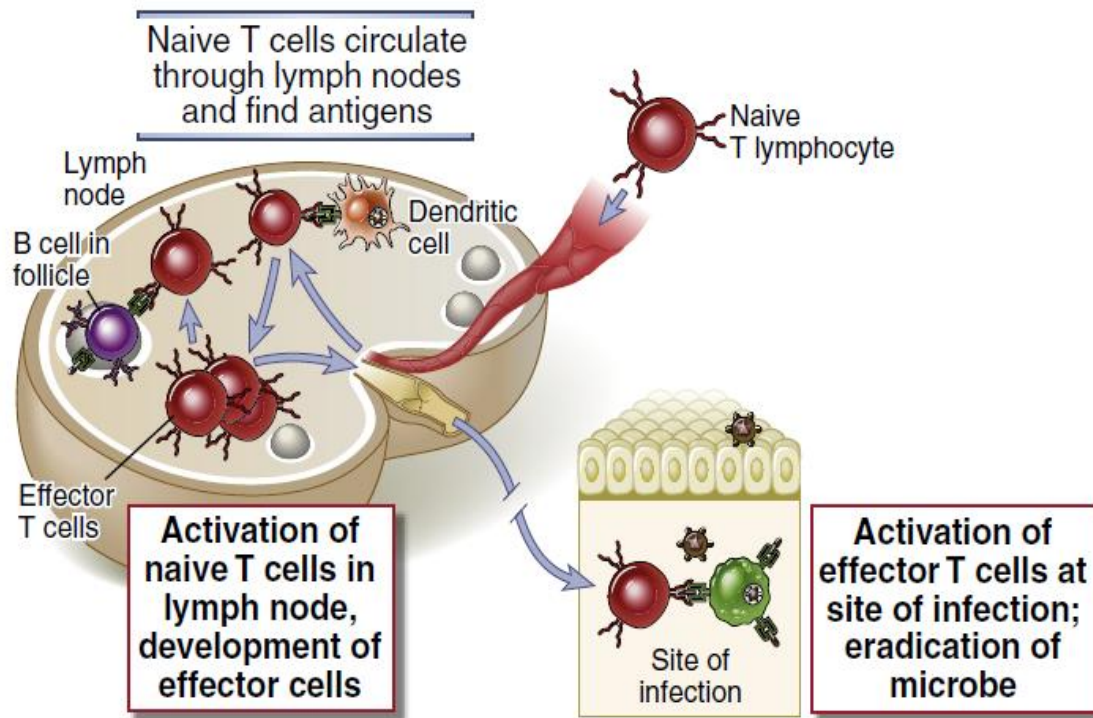


The final result of the activation of T lymphocytes is the **proliferation** (expansion) of the antigen-specific clone and the **differentiation** of naive into effector lymphocytes.

Initiation and effector phases of cellular immunity



Differentiation of naive into effector T lymphocytes

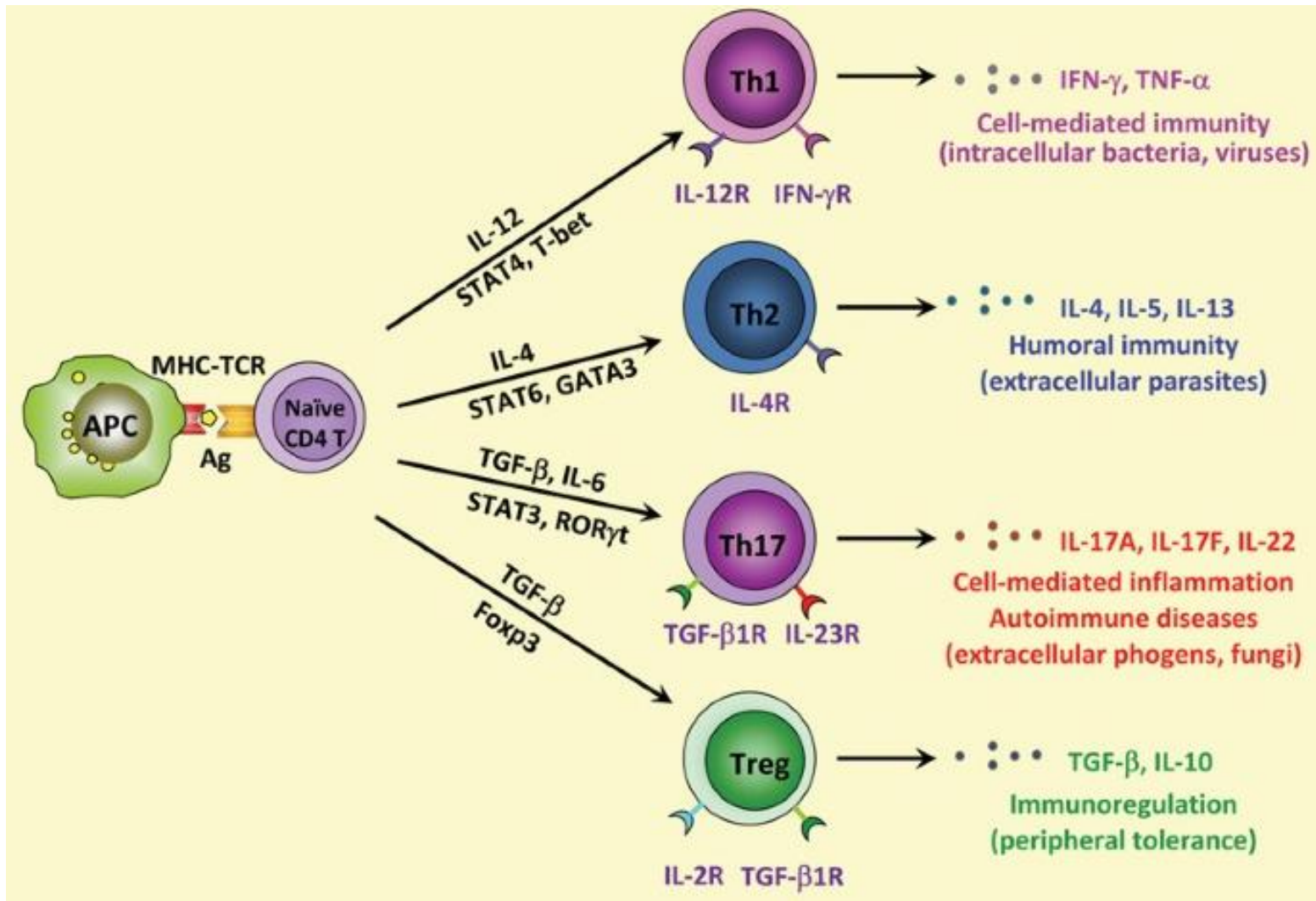


- After antigen recognition T cells activate and differentiate into effector cells, which may remain in the lymphoid organs to help B lymphocytes or migrate to sites of infection, macrophage activation.

These **effector** lymphocytes produce **membrane molecules** and **cytokines** in response to antigen.

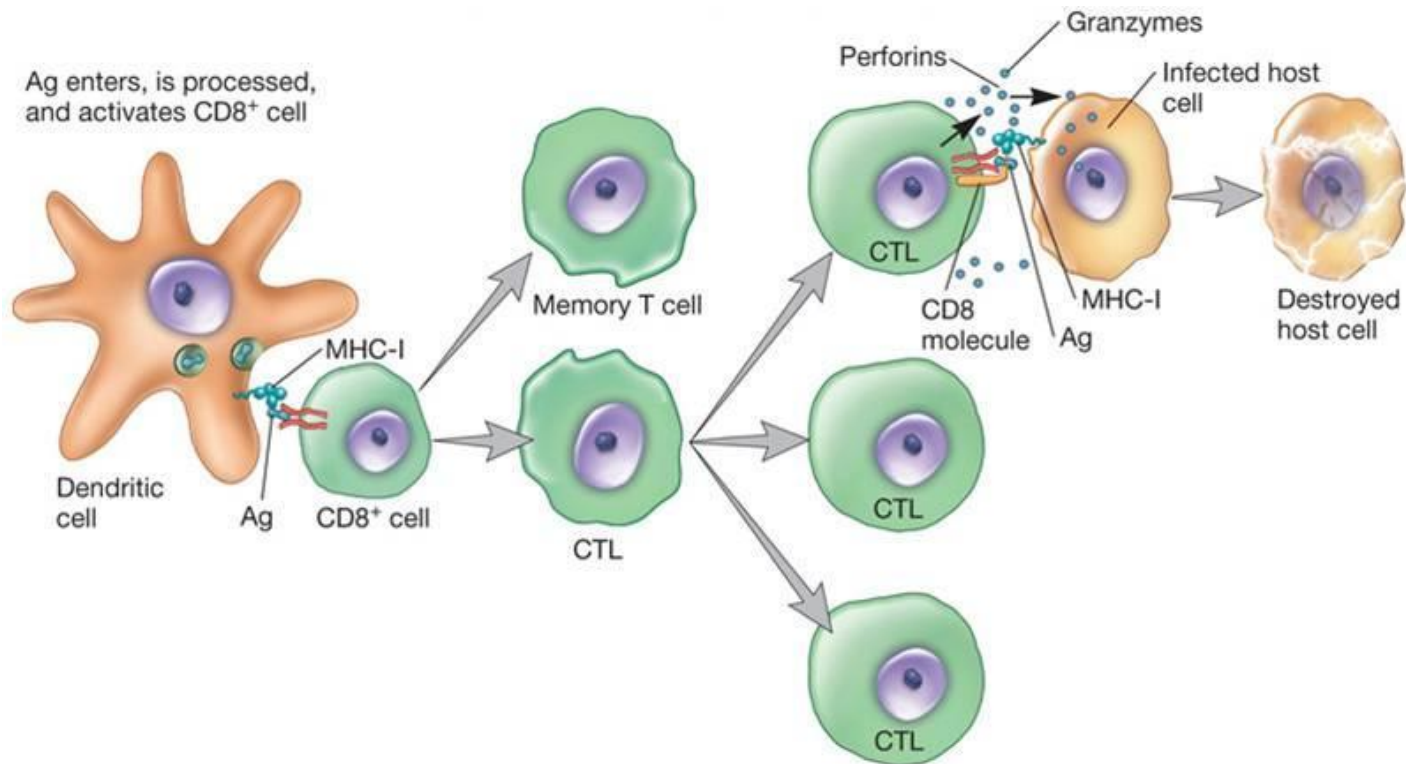
These products mainly activate **macrophages** and **B lymphocytes**.

Naive CD4⁺T lymphocytes differentiate into **different effector cells** that secrete **different sets of cytokines** and perform **different functions**



The emergence of effector Th1, Th2, Th17 from naive CD4+ T(Th0) lymphocytes is not a random process, but the direction of differentiation depends on the signals that arise after the contact of Th0 with the antigen. And the type of signal will depend on the characteristics of the pathogen, as well as on the genetic predisposition.

After activation, CD8+ T lymphocytes differentiate into **CTL**



Effector mechanisms of cellular immunity

Elimination of intracellular microorganisms

It remains for us to learn :

How do effector T lymphocytes find infected cells (intracellular microorganisms) anywhere in the body?

How do T lymphocytes eliminate intracellular infections?

Microorganisms:

Extracellular: they multiply outside our cells

Staphylococcus, Streptococcus, Escherichia, Clostridium...

Intracellular: they multiply inside our cells

- in APC:

Mycobacterium spp. (M. tuberculosis, M. leprae...), Listeria monocytogenes, Legionella pneumophila...

Leishmania spp, Tripanosoma spp, ...

Cryptococcus neoformans,...

- in other cells:

Vuruses

Rickettsiae

Plasmodium, Cryptosporidium

Types of cellular immunity

CD4+ T lymphocytes

recognize the peptide in the context of MHC class II products. They are the main source of interleukins.

Function: **helper T lymphocyte.**

They activate macrophages to efficiently destroy phagocytosed microbes.

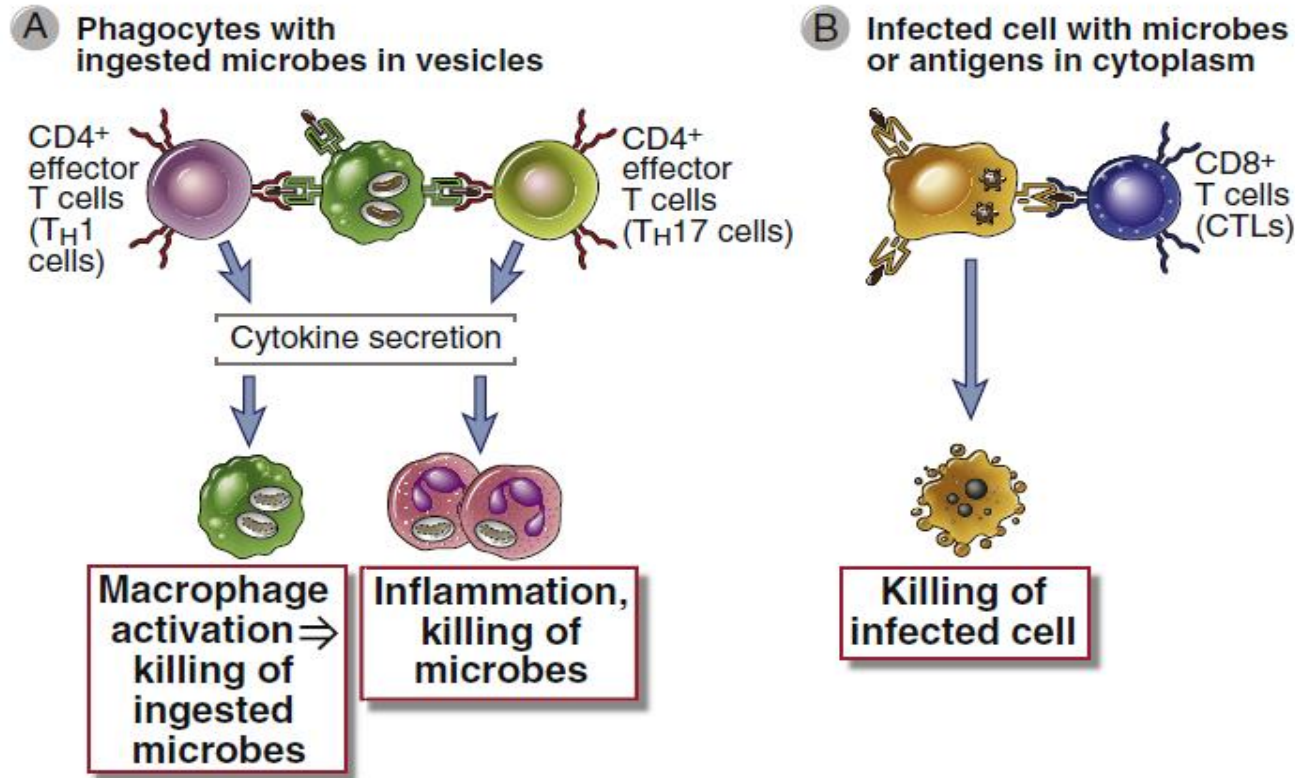
CD8+ T lymphocytes

recognize the peptide in the context of MHC class I products.

Function: **cytotoxic T lymphocyte.**

They kill all cells that contain microbes or their proteins in the cytoplasm.

Role of T cells in eradicating infections

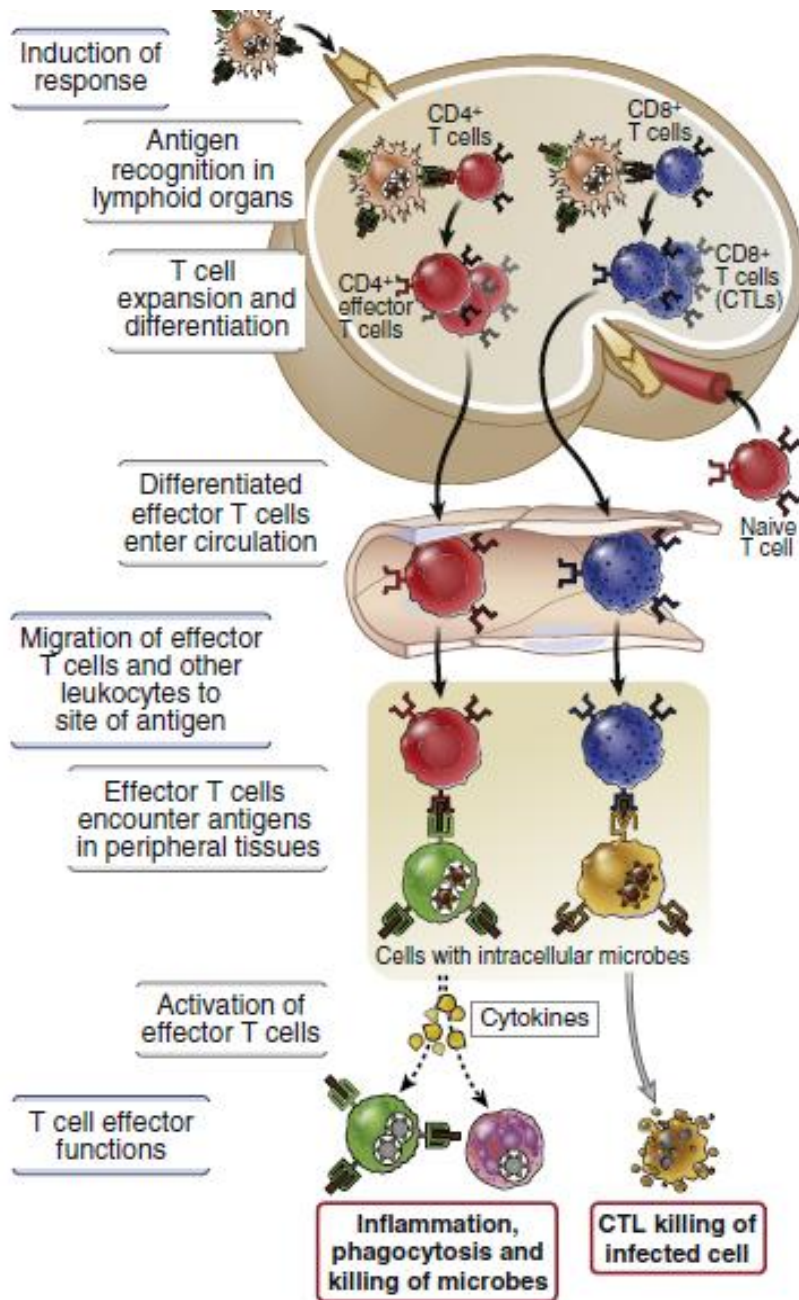


CD4⁺ T cells recognize antigens of phagocytosed and extracellular microbes and produce cytokines that activate the phagocytes to kill the microbes and stimulate inflammation.

CD8⁺ cytotoxic T lymphocytes (CTLs) recognize antigens of microbes residing in the cytosol of infected cells and kill the cells.

NAIVE T LYMPHOCYTES

- ✓ recognition,
- ✓ activation,
- ✓ proliferation and
- ✓ differentiation in



EFFECTOR T LYMPHOCYTE

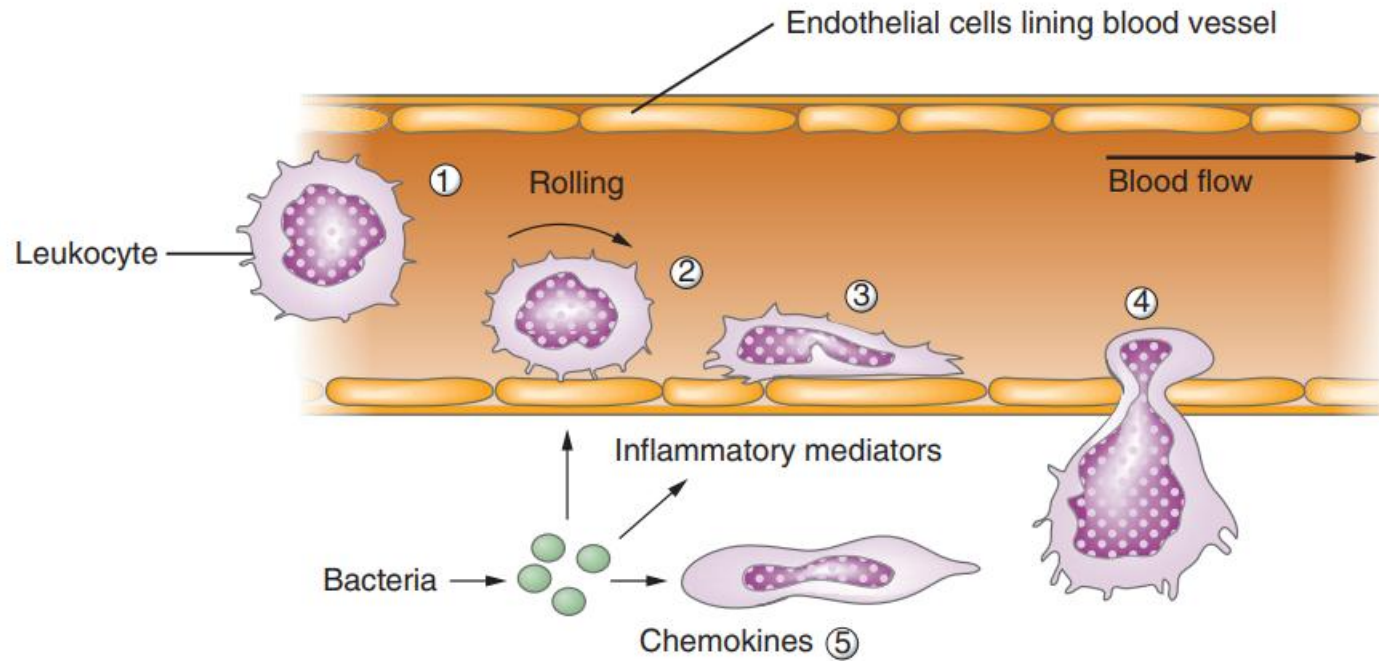
- ✓ migration,
- ✓ recognition,
- ✓ activation and
- ✓ effector functions

Effector CD4+T: Th1, Th2, Th17, Treg

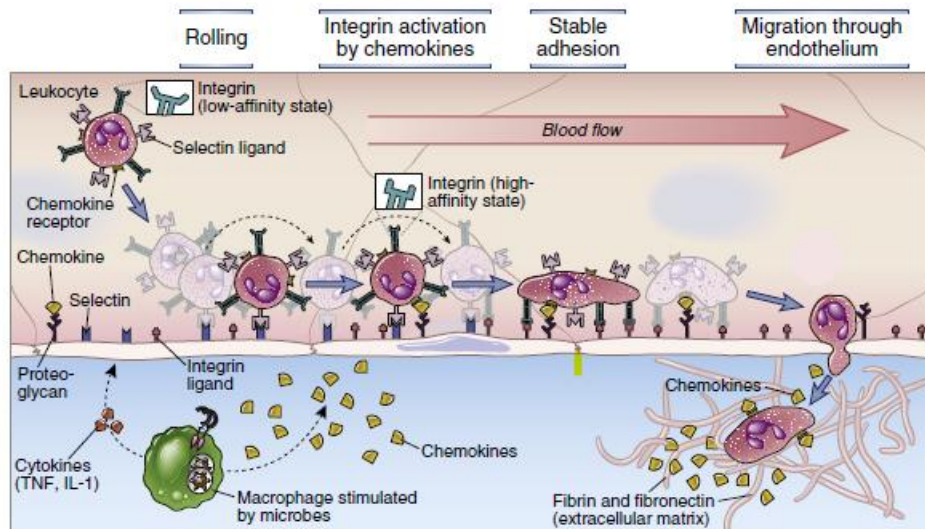
Effector CD8+T: CTL

- Interactions between leukocytes and endothelial cells that facilitate the recruitment of leukocytes into tissues occur through multiple steps.
- expression of adhesive molecules and chemokine receptors. Those molecules need to find ligands on the endothelial cells of the infected tissue. These ligands are expressed only on the endothelium of the infected tissue and are the result of a new program of these endothelial cells programmed by cytokines of non-specific immunity.

Leukocyte adhesion, extravasation and migration

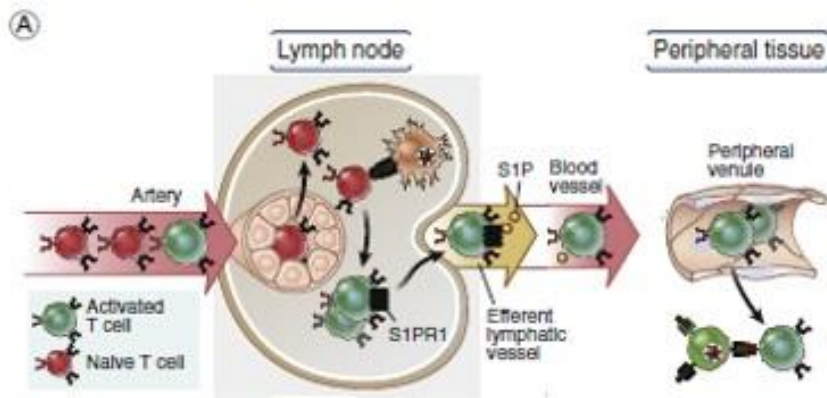


1. Free circulating leukocytes that are not attached to endothelial cells.
2. Leukocytes are attached to the endothelium and roll under the influence of blood flow, mediated by selectins (a process that takes seconds).
3. Leukocytes are firmly attached to the endothelium and migrate, mediated by integrins.
4. Leukocyte extravasation from the blood vessel, facilitated by PECAM (Platelet Endothelial Cell Adhesion Molecule), occurring over minutes.
5. Leukocytes migrate toward the source of infection or damage, directed by chemokines.

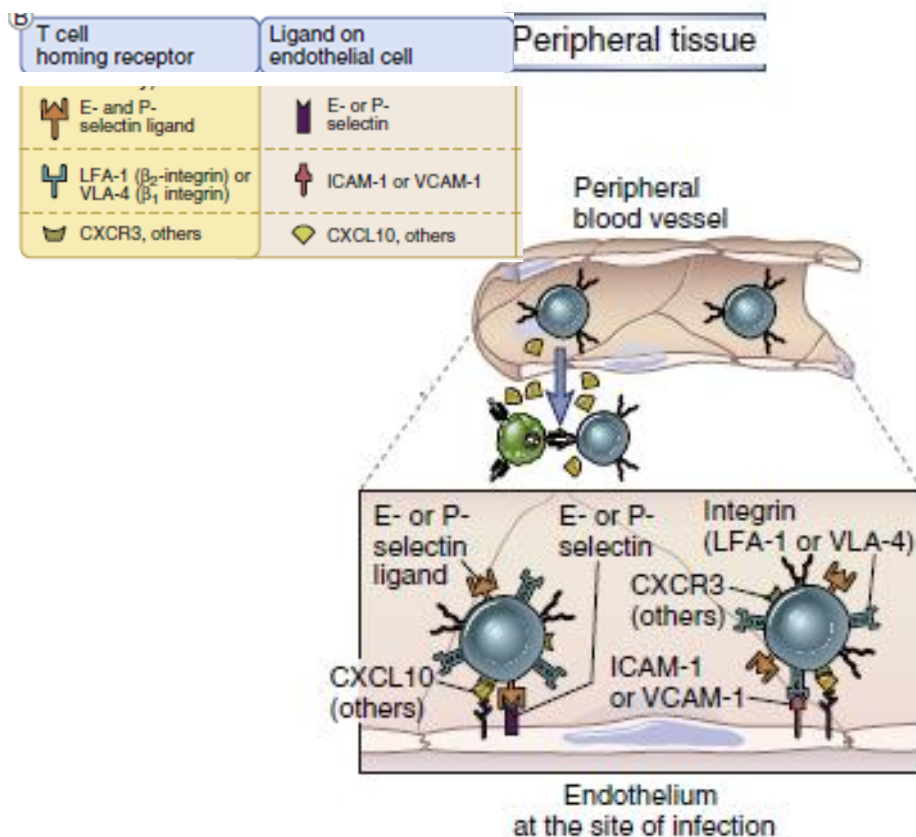


Neutrophils, monocytes, and T lymphocytes use similar mechanisms to migrate from the bloodstream to the tissues.

- At infection sites, **macrophages** that have ingested microbes release cytokines, such as TNF and IL-1. These cytokines **activate the endothelial cells** of nearby venules, prompting them to produce selectins, ligands for integrins, and chemokines.
- **Selectins** facilitate the initial weak binding of blood leukocytes to the endothelium, allowing the force of blood flow to cause leukocytes to **roll** along the endothelial surface.
- Once rolling occurs, **integrins** bind more firmly to their ligands on the endothelial cells, leading to the **stable adhesion** of leukocytes.
- Following this adhesion, leukocytes move between endothelial cells and migrate through the venular wall.



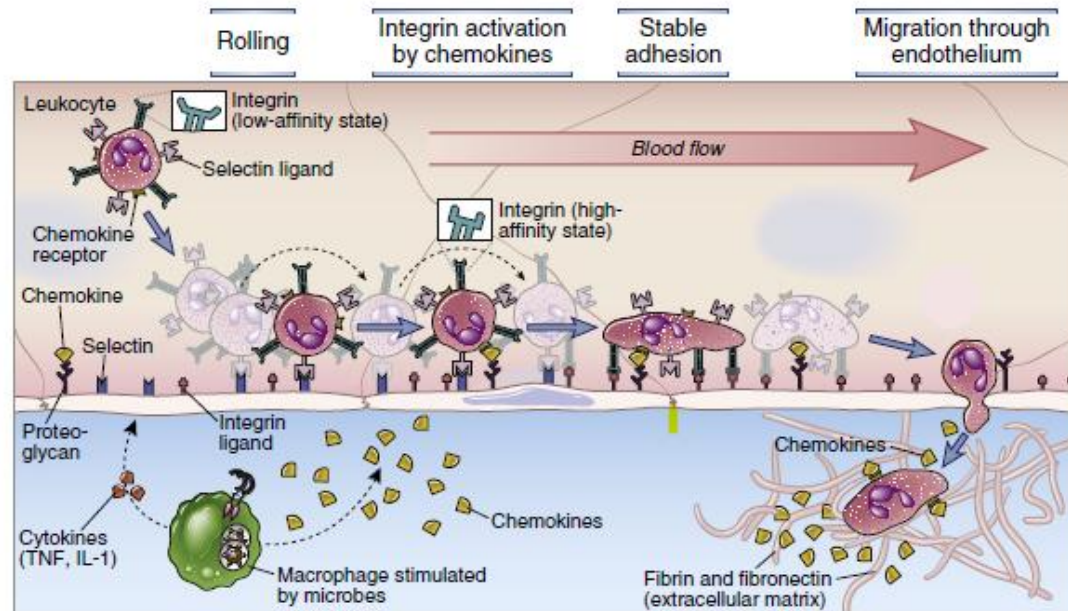
✓ Activated T lymphocytes reduce the expression of receptors for chemokines that are created in the T cell zones of lymph nodes, and increase the expression of receptors for chemokines present in the circulation. That's how they leave lymph node and reach the circulation.



✓ Activated lymphocytes increase the expression of ligands for **E or P selectins**, followed by high-affinity forms of integrins **LFA-1 and VLA-4**.

✓ At the same time, the endothelium at the site of infection is exposed to high concentrations of TNF and IL-1 and under this effect increases the expression of **E- and P-selectin**, as well as **ligands for integrins ICAM-1** (ligand for LFA-1) and **VCAM-1** (ligand for VLA -4).

Migration of effector lymphocytes to the site of infection



T cell homing receptor	Ligand on endothelial cell	Function of receptor: ligand pair
Activated (effector and memory) T cells E- and P-selectin ligand	E- or P-selectin	Initial weak adhesion of effector and memory T cells to cytokine-activated endothelium at peripheral site of infection
LFA-1 (β_2 -integrin) or VLA-4 (β_1 integrin)	ICAM-1 or VCAM-1	Stable arrest on cytokine-activated endothelium at peripheral site of infection
CXCR3, others	CXCL10, others	Activation of integrins and chemotaxis

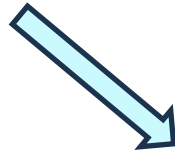
Rolling - **selectins**

Tight binding – **integrins**

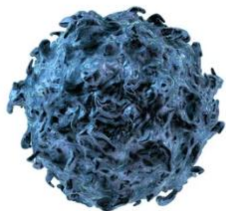
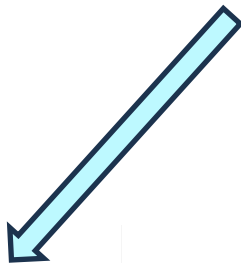
Motility - **chemokines**

Passage through the endothelium - **PECAM-1 (CD31)**

- ✓ All circulating effector T lymphocytes generated in response to different infectious agents migrate to the site of infection.
- ✓ T lymphocytes that are specific to the current infection recognize the antigen and begin to express VLA integrins, which help them remain in the infected tissue.
- ✓ At the same time, the non-specific lymphocytes continue to circulate throughout the body



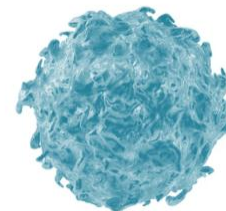
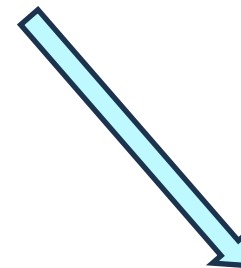
CD4 cells



Th1

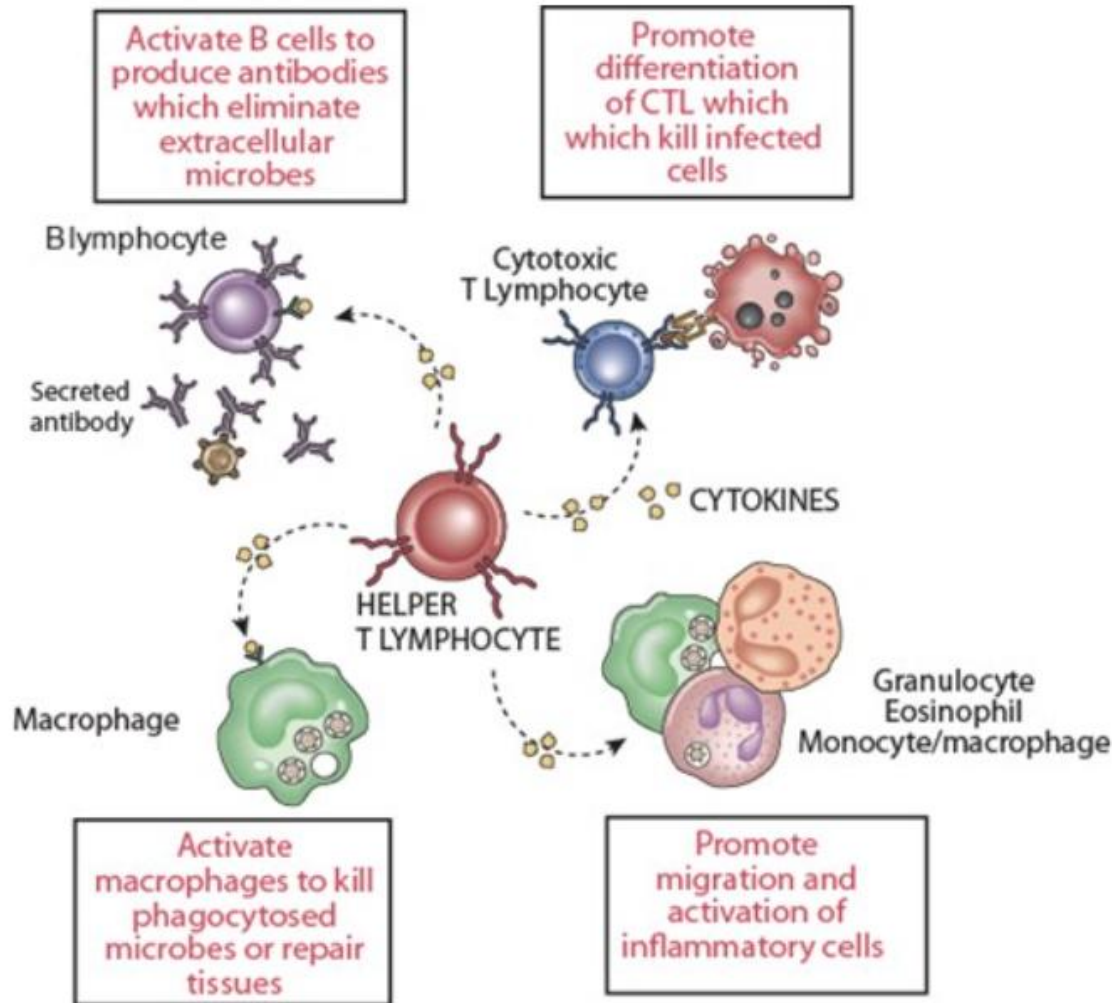


Th2

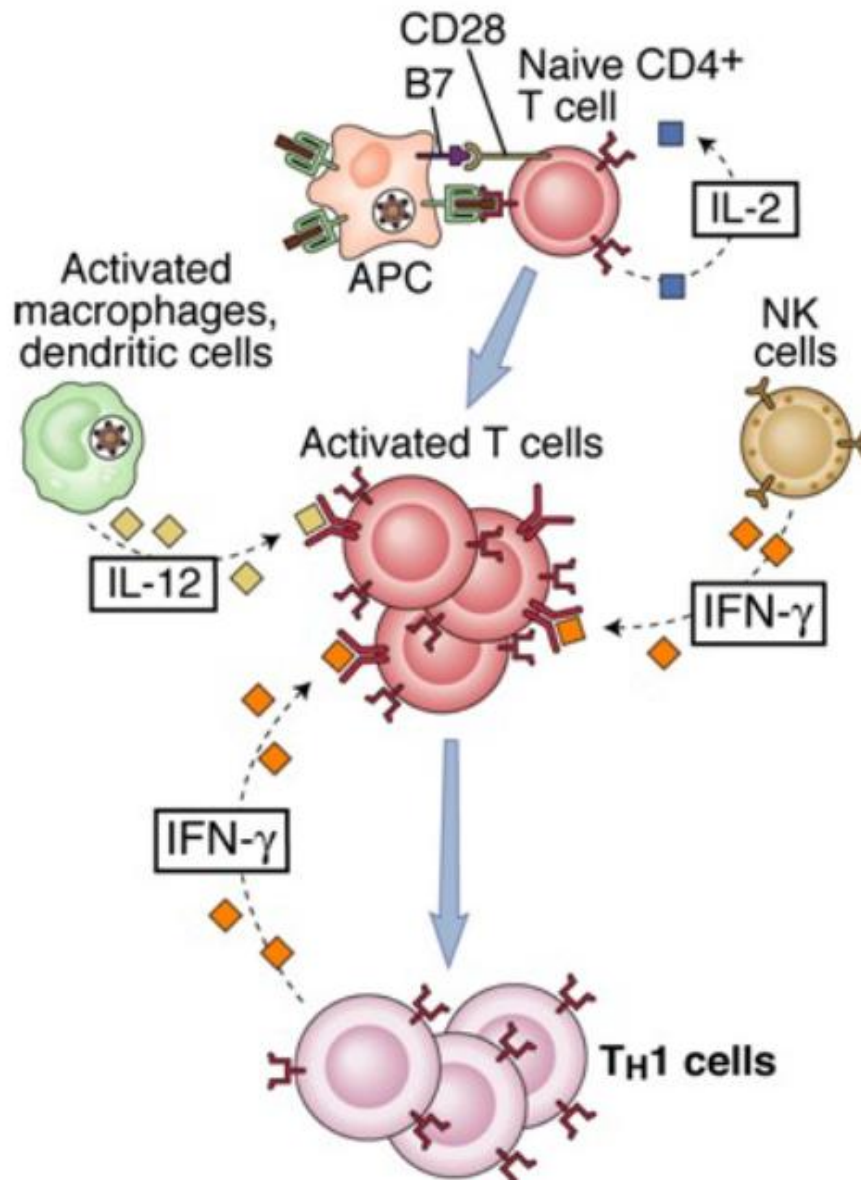


Th17

The role of CD4 helper T cells, which are essential for immune responses, is regulated by cytokines.

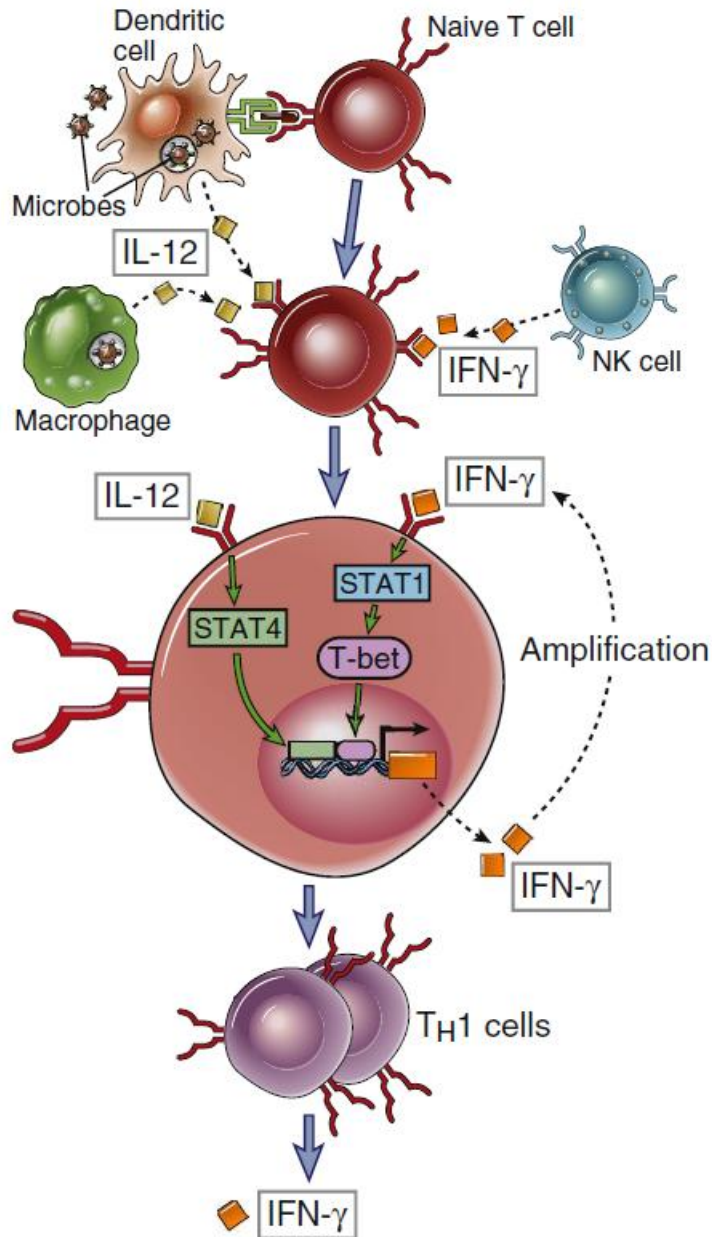


Development of T_H 1 cells



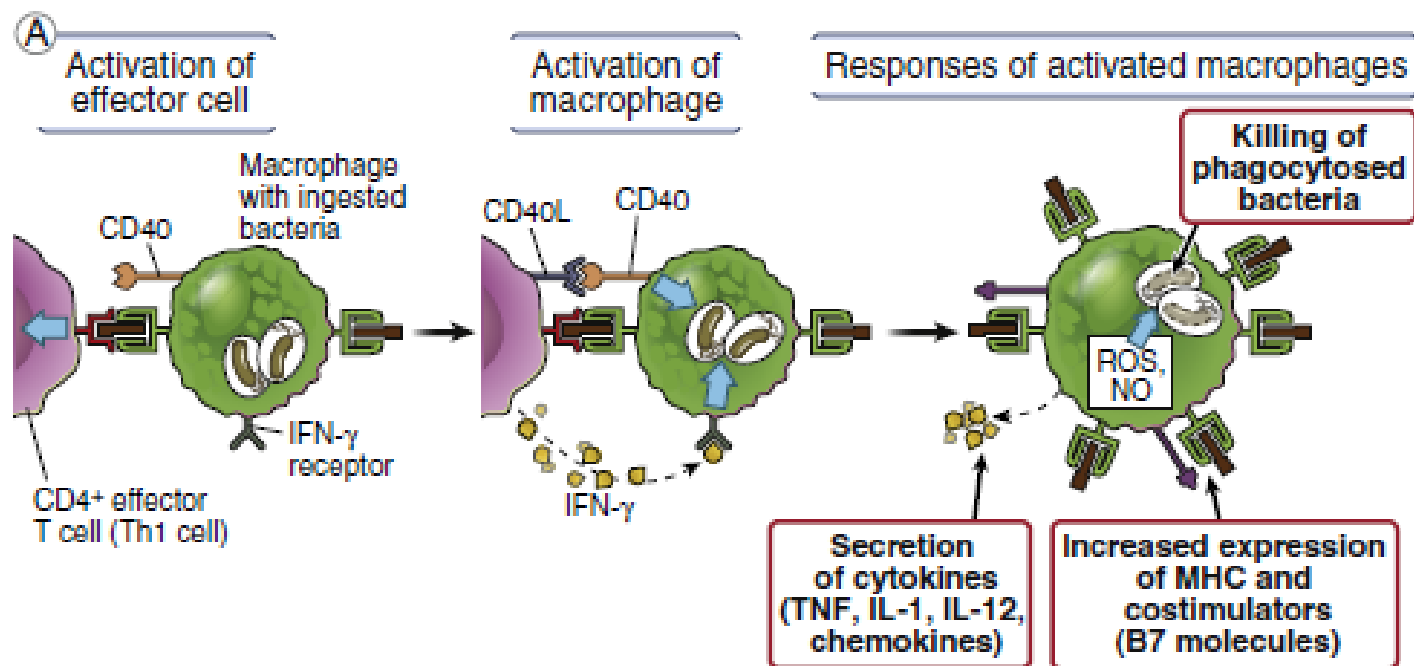
Th1 cells develop in response to microbes that activate dendritic cells and macrophages (most ingested bacteria).

Th1 cells stimulate phagocyte-dependent host defense, the defense mechanism that works against most bacteria that enter tissues and are ingested by phagocytes



Development of Th1 cells.

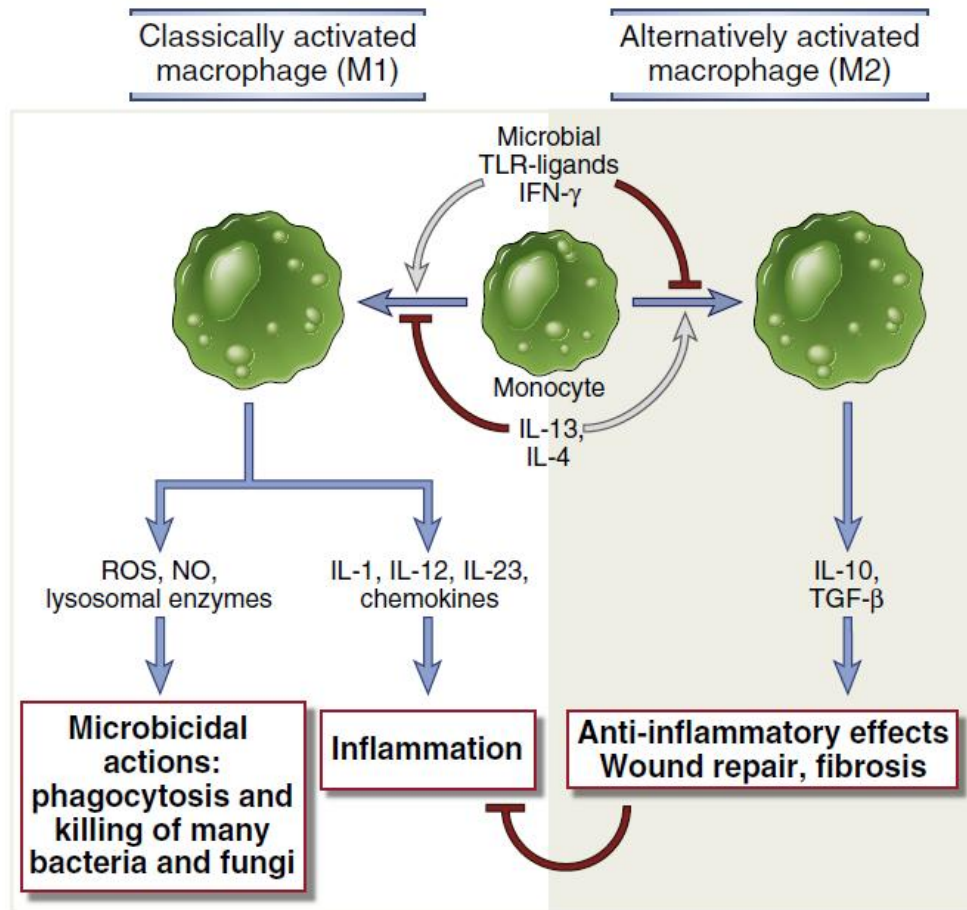
IL-12, produced by dendritic cells and macrophages in response to microbes, along with **IFN- γ** produced by NK cells, activates the transcription factors **T-bet**, **STAT1**, and **STAT4**. These factors stimulate the differentiation of naive CD4⁺ T cells into the **Th1 subset**.



B	Macrophage response	Role in cell-mediated immunity
	Production of reactive oxygen species, nitric oxide, increased lysosomal enzymes	Killing of microbes in phagolysosomes (effector function of macrophages)
	Secretion of cytokines (TNF, IL-1, IL-12) and chemokines	TNF, IL-1, chemokines: leukocyte recruitment (inflammation) IL-12: Th1 differentiation, IFN- γ production
	Increased expression of B7 costimulators, MHC molecules	Increased T cell activation (amplification of T cell response)

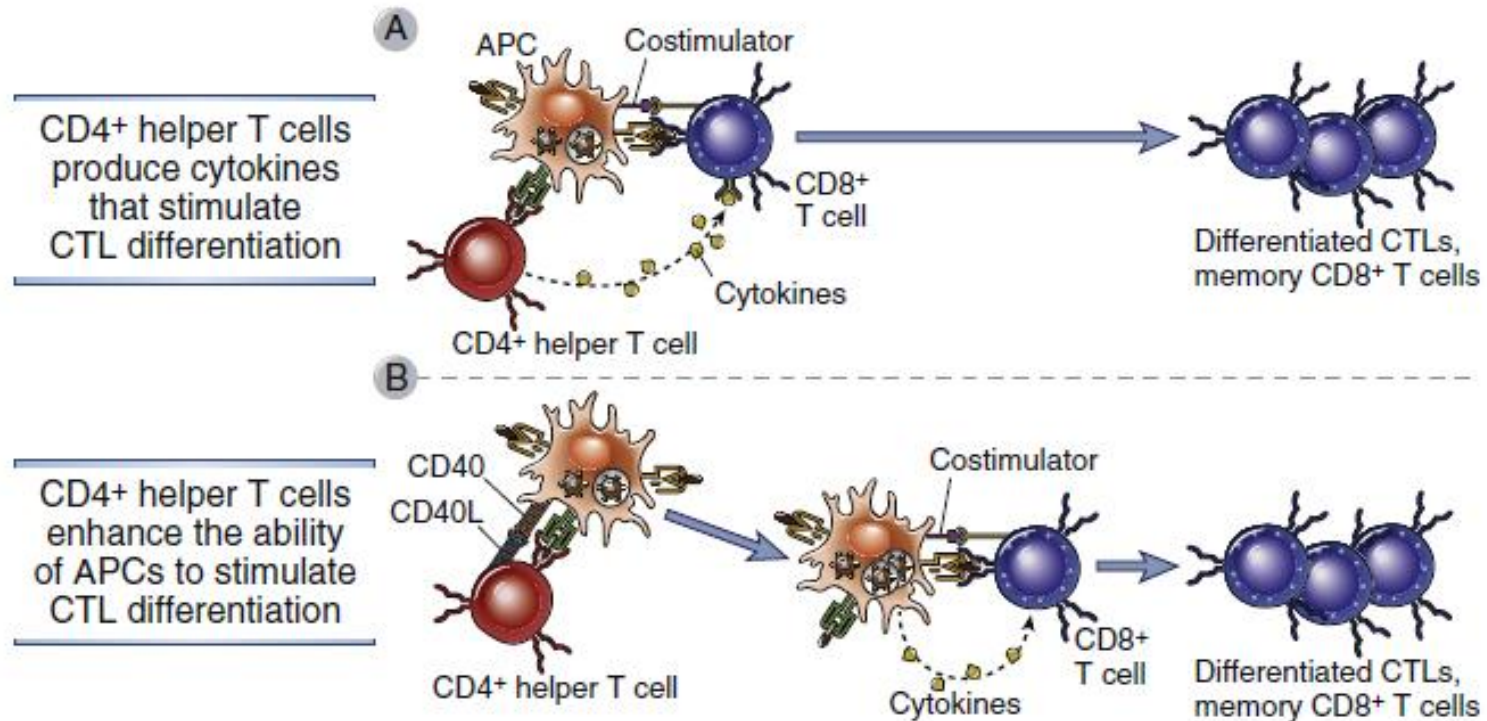
Macrophage activation by TH1 cells Macrophages are activated by CD40L-CD40 interactions and by IFN- γ expressed by TH1 cells and perform several functions that kill microbes, stimulate inflammation, and enhance the antigen-presenting capacity of the cells.

Classical and alternative macrophage activation



- Different stimuli activate monocytes-macrophages to develop into functionally distinct populations.
- Classically activated macrophages are induced by microbial products and cytokines (IFN- γ). They are microbicidal and involved in inflammation.
- Alternatively activated macrophages are induced by IL-4 and IL-13 produced by TH2 cells. They control inflammation and promote tissue repair and fibrosis.

Role of helper T cells in the differentiation of CD8+ T lymphocytes.



CD4⁺ helper T cells promote the development of CD8⁺ CTLs and memory cells by secreting cytokines that act directly on the CD8⁺ cells or by activating APCs to become more effective at stimulating the differentiation of the CD8⁺ T cells

Th2 cells

Th2 cells are involved in the elimination of **helminths**, protect **mucosal barriers**, and play a role in **allergic reactions**.

Function of **Th2** lymphocytes

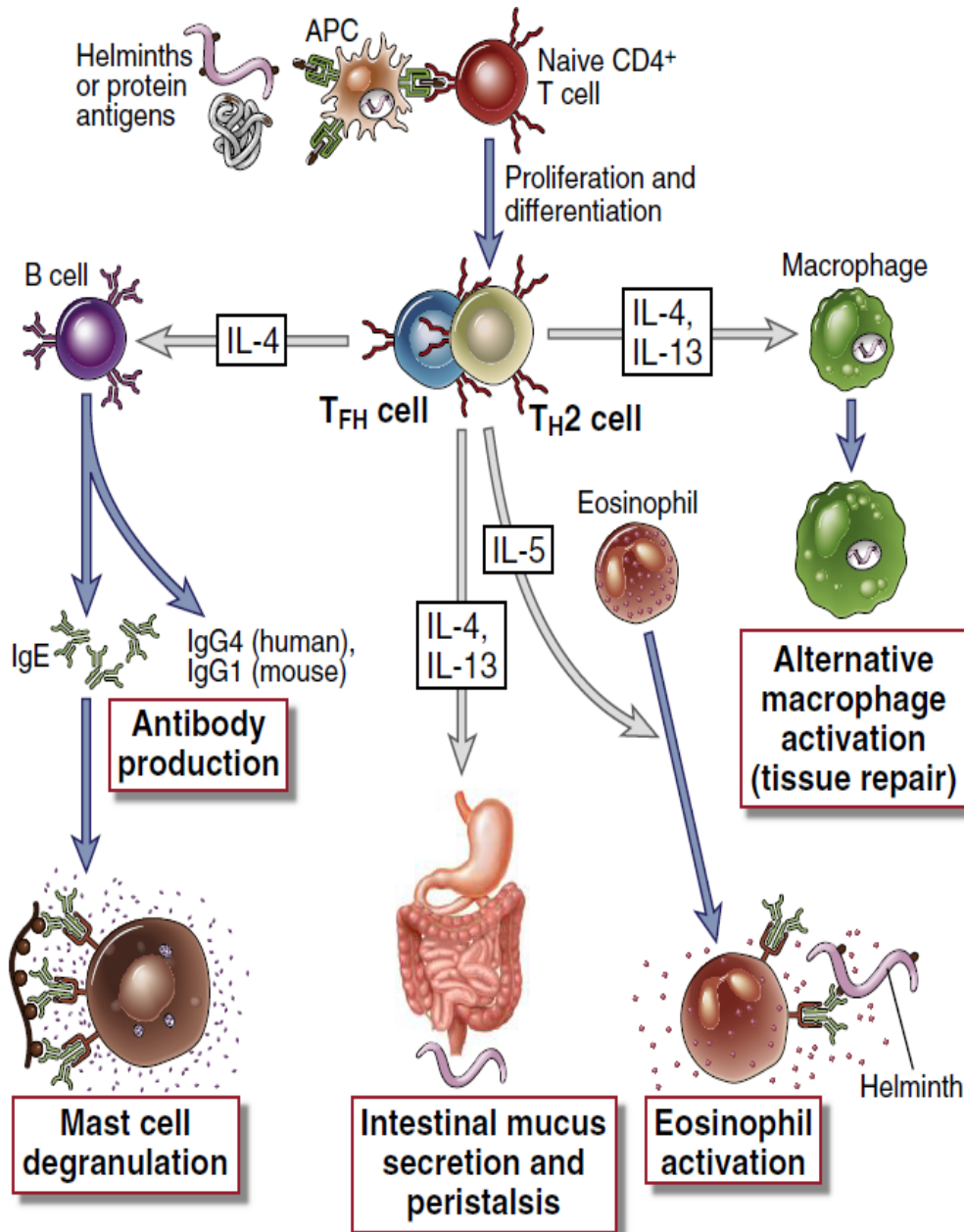
Th2 cells secrete **IL-4**, **IL-5**, and **IL-13**.

IL-4 acts on B cells to **stimulate production** of antibodies that bind to mast cells, such as **IgE**.

IL-4 is also an **autocrine growth and differentiation cytokine** for Th2 cells.

IL-5 activates **eosinophils**

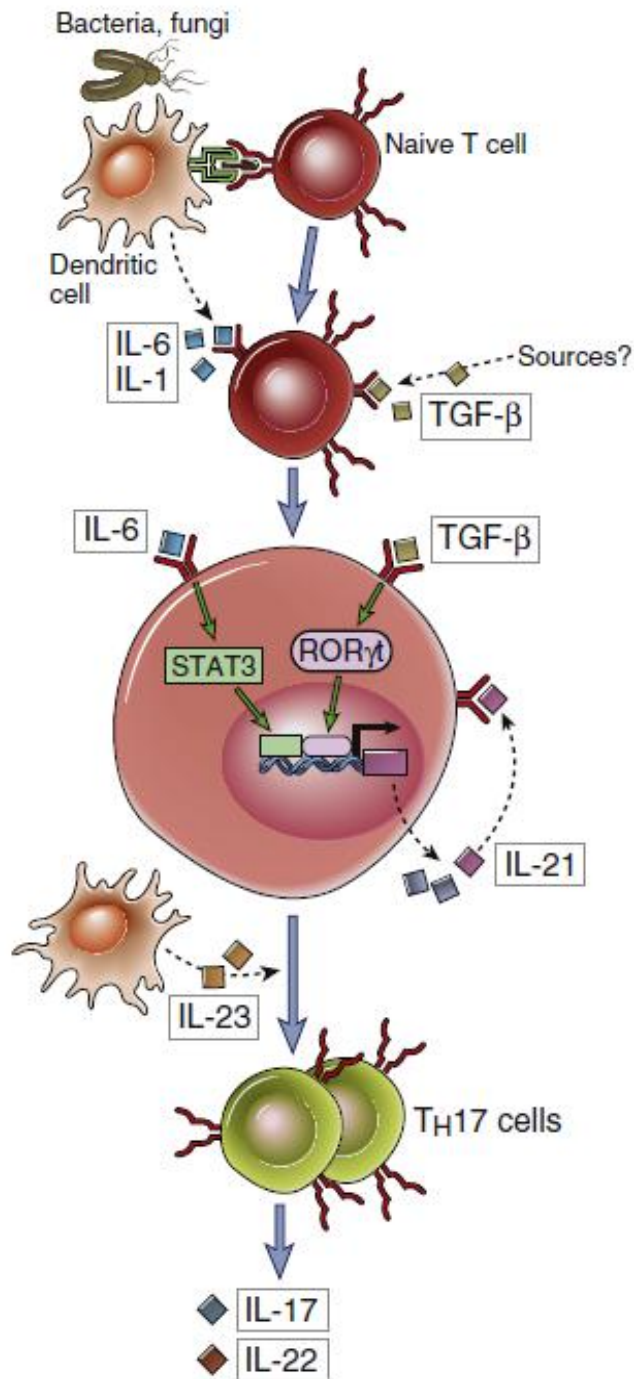
IL-4 and **IL-13** are involved in immunity at mucosal barriers, induce an alternative pathway of macrophage activation, and inhibit classical Th1-mediated macrophage activation.



Th17 cells

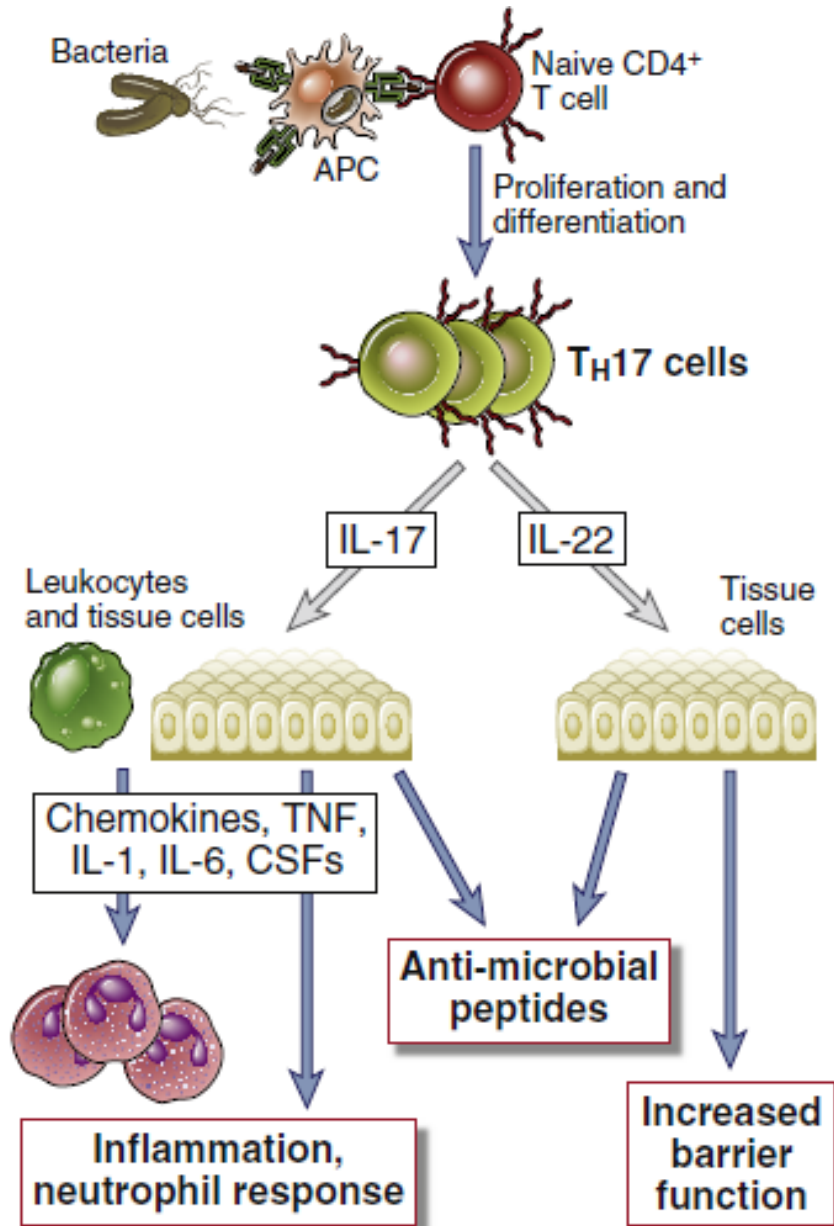
Th17 cells play a crucial role in the elimination of **extracellular bacteria** and **fungi**. These microbes activate dendritic cells (DCs) to produce cytokines that induce Th17 differentiation. Additionally, Th17 cells are implicated in various **inflammatory diseases**.

Development of Th17 cells.



IL-1 and **IL-6**, produced by antigen-presenting cells (APCs), along with transforming growth factor-beta (**TGF-β**) produced by various cells, activate the transcription factors **RORγt** and **STAT3**. This activation promotes the differentiation of naïve CD4⁺ T cells into the **Th17** subset.

Additionally, **IL-23**, also produced by APCs, stabilizes the Th17 cells. **IL-21**, which is produced by Th17 cells, enhances the immune response.

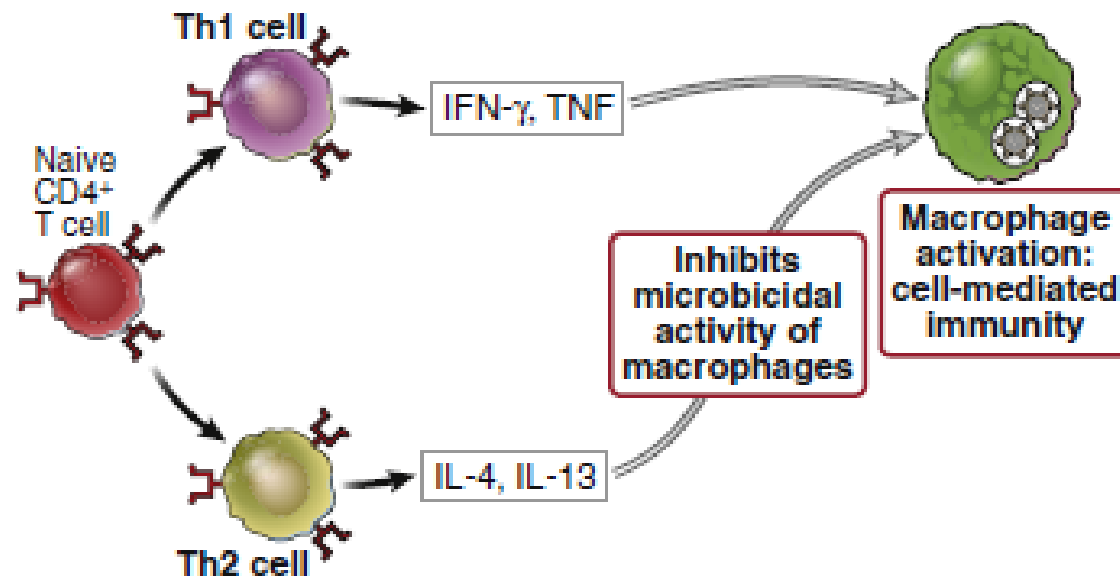


Function of **Th17** lymphocytes

Cytokines produced by Th17 cells:

- stimulate local production of chemokines that recruit neutrophils and other leukocytes
- increase production of antimicrobial peptides (defensins)
- promote epithelial barrier functions.

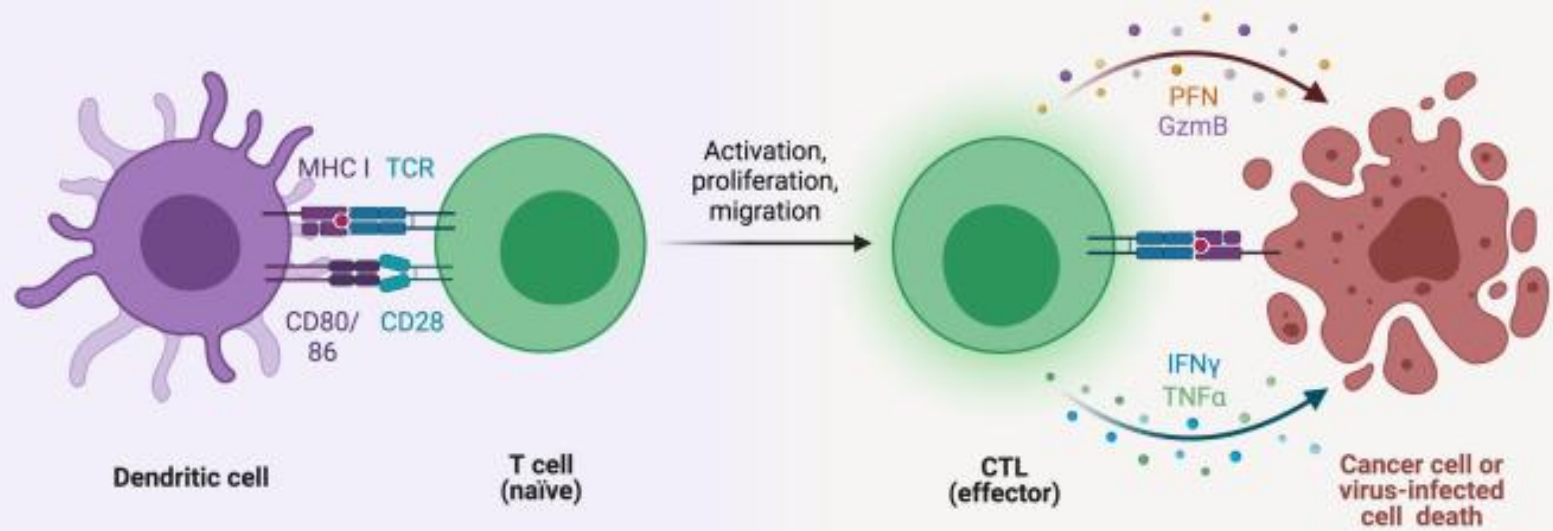
Pathogenesis of tuberculosis and leprosy



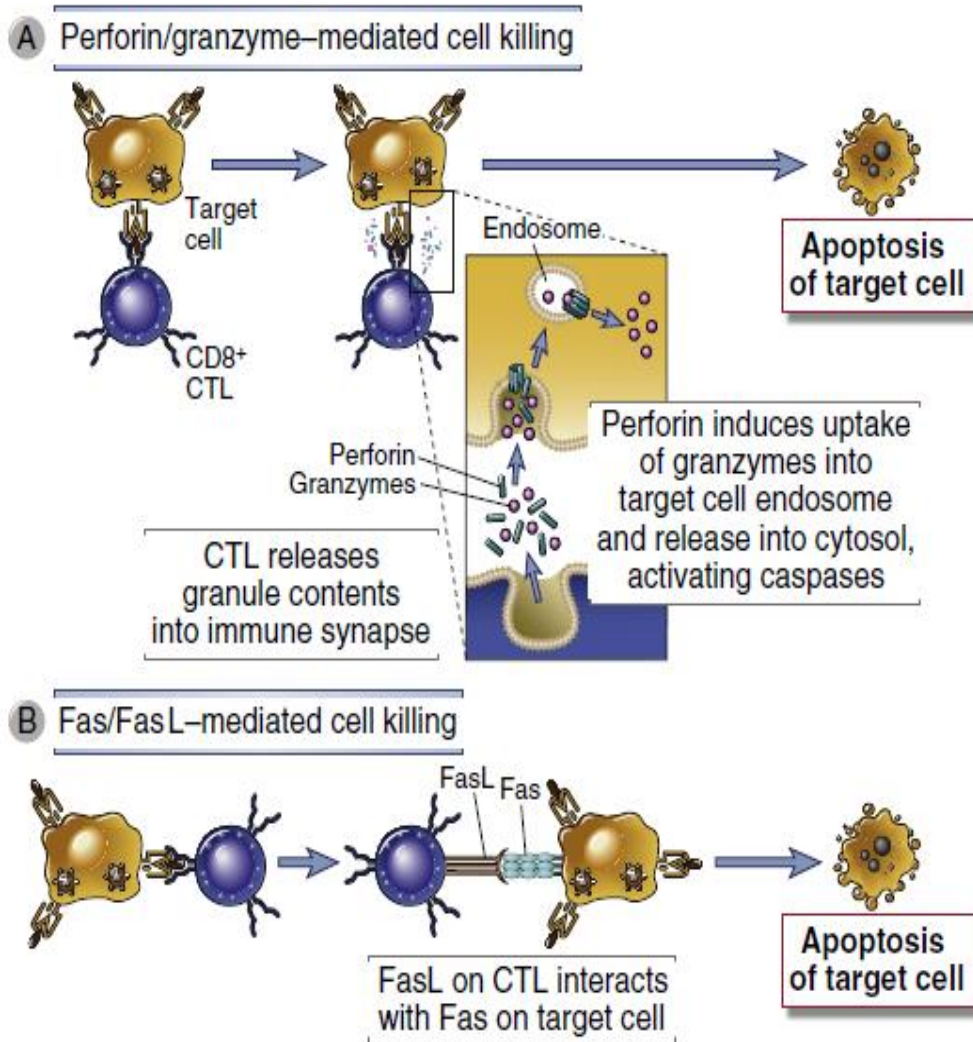
Infection	Response	Outcome
<i>Leishmania major</i>	Most mouse strains: Th1 \Rightarrow BALB/c mice: Th2 \Rightarrow	Recovery Disseminated infection
<i>Mycobacterium leprae</i>	Some patients: Th1 \Rightarrow Some patients: Defective Th1 or dominant Th2 \Rightarrow	Tuberculoid leprosy Lepromatous leprosy (high bacterial count)

The ratio between the activation of Th1 and Th2 lymphocytes determines the outcome of the infection

CD8+ cells



Effector functions of CD8+ CTL



Mechanisms of CTL-mediated killing of target cells.

CTLs kill target cells by two main mechanisms.

A, Complexes of perforin and granzymes are released from the CTL by granule exocytosis and enter target cells. The granzymes are delivered into the cytoplasm of the target cells by a perforin-dependent mechanism, and they induce apoptosis.

B, FasL is expressed on activated CTLs, engages Fas on the surface of target cells, and induces apoptosis

Cooperation between CD4⁺ and CD8⁺ T cells in the eradication of intracellular infections

- In a **macrophage** infected with an intracellular bacterium, some of the bacteria are sequestered in vesicles (**phagosomes**) and others may escape into the **cytoplasm**
- **CD4⁺ T** cells **recognize** antigens derived from the **vesicular microbes** and **activate the macrophage** to kill the microbes in the vesicles
- **CD8⁺ T** cells **recognize** antigens derived from the **cytoplasmic bacteria** and are needed to kill the infected cell, thus **eliminating the reservoir** of infection

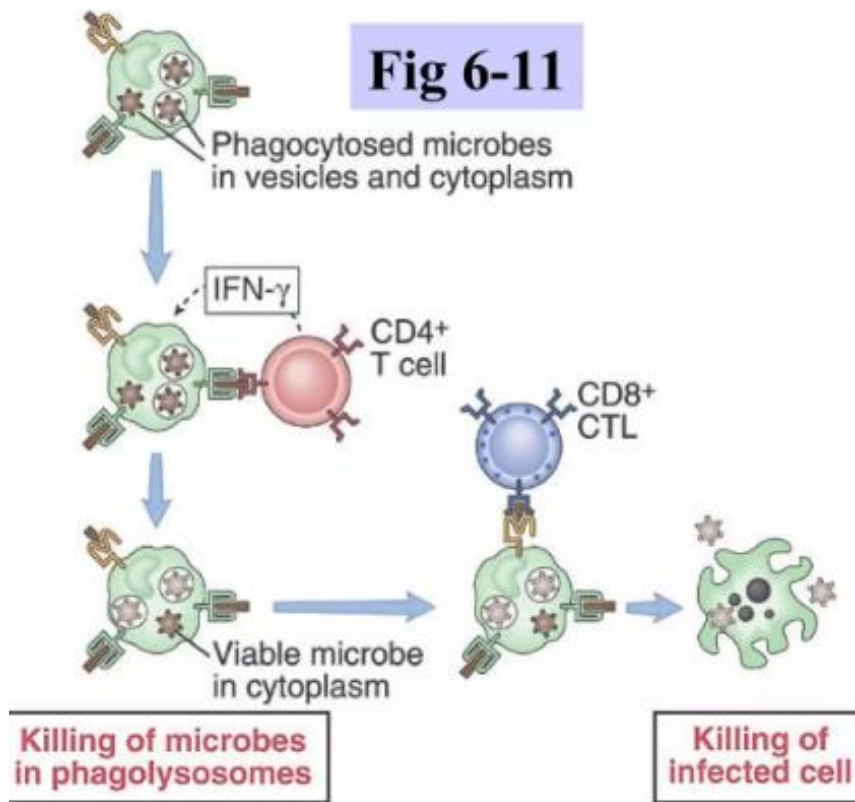


TABLE 16-3 Mechanisms of Immune Evasion by Viruses

Mechanism of Immune Evasion	Examples
Antigenic variation	Influenza, rhinovirus, HIV
Inhibition of antigen processing Blockade of TAP transporter Removal of class I molecules from the ER	Herpes simplex virus (HSV) Cytomegalovirus (CMV)
Production of "decoy" MHC molecules to inhibit NK cells	Cytomegalovirus (murine)
Production of cytokine receptor homologues	Vaccinia, poxviruses (IL-1, IFN- γ) Cytomegalovirus (chemokine)

Mechanism	Parasite example(s)
Antigenic variation	<i>Trypanosoma brucei</i> <i>Plasmodium</i> merozoites
Evasion from macrophages	
Prevention of lysosome-phagosome action	<i>Toxoplasma gondii</i>
Prevention of lysosomal toxic action	<i>Leishmania</i> amastigotes
Escape into cytoplasm	<i>Trypanosoma cruzi</i>
Resistance to complement lysis	<i>Leishmania</i> , <i>T. brucei</i> , <i>T. cruzi</i> , <i>Taenia solium</i>
Immune suppression	Filariae
Surface and secreted antioxidant enzymes	Parasitic nematodes, schistosomes

Mechanism of Immune Evasion	Examples
Extracellular bacteria	
Antigenic variation	<i>Neisseria gonorrhoeae</i> , <i>Escherichia coli</i> , <i>Salmonella typhimurium</i>
Inhibition of complement activation	Many bacteria
Resistance to phagocytosis	Pneumococcus
Scavenging of reactive oxygen species	Catalase-positive staphylococci
Intracellular bacteria	
Inhibition of phagolysosome formation	<i>Mycobacterium tuberculosis</i> , <i>Legionella pneumophila</i>
Inactivation of reactive oxygen and nitrogen species	<i>Mycobacterium leprae</i> (phenolic glycolipid)
Disruption of phagosome membrane, escape into cytoplasm	<i>Listeria monocytogenes</i> (hemolysin protein)