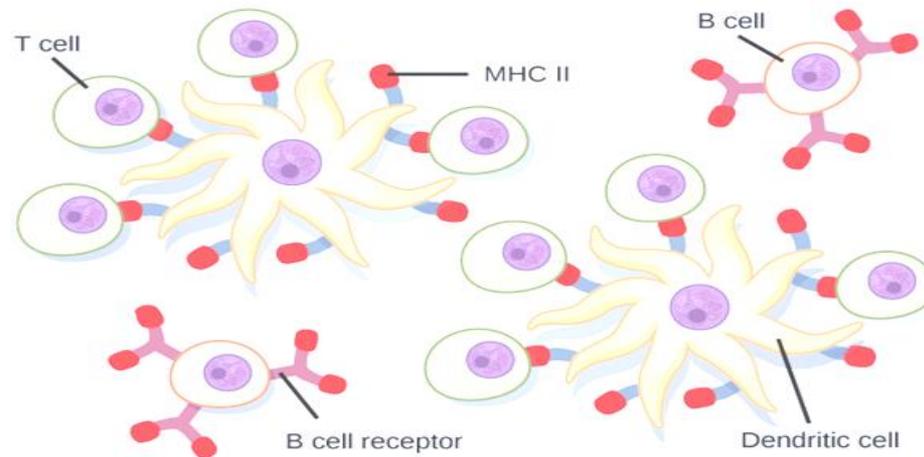
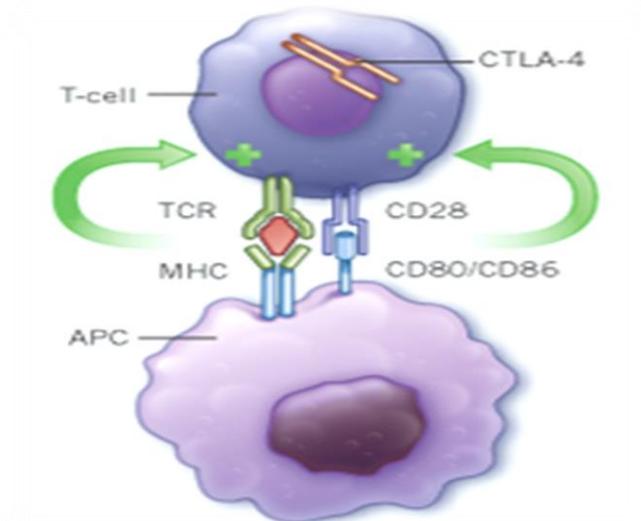


# Lecture 4



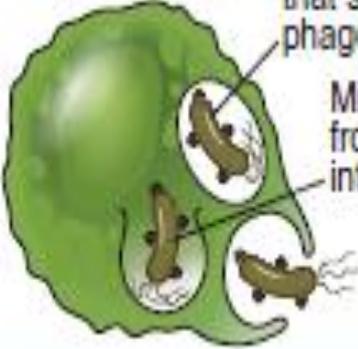
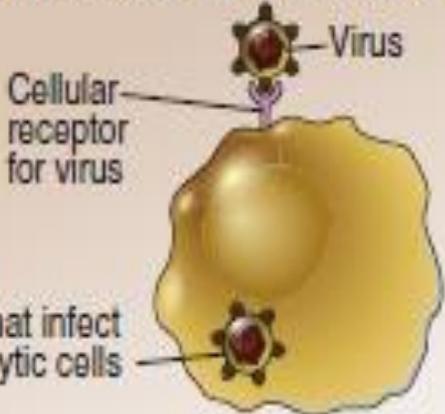
**Cellular immune response**  
**Effector mechanisms of cellular immunity**

# Cellular immune response



**Activation of T lymphocytes by intracellular microorganisms**

... let us remind ourselves

Intracellular microbes	Examples
<p><b>(A) Phagocyte</b></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>(B) Nonphagocytic cell (e.g., epithelial cell)</b></p> <p>Cellular receptor for virus</p> <p>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

# Phases of T-cell response

The response of T lymphocytes to antigens of intracellular microorganisms takes place in several successive stages.

During this response:

- ✓ The number of T lymphocytes **specific** for the given antigen increases.
- ✓ The transformation of **naive** into **effector** and **memory** T lymphocytes.

## *... let us remind ourselves*

Naive T lymphocytes...

...recirculate constantly...

...before eliminating antigens, they must additionally differentiate from naive to effector lymphocytes...

...that process begins with antigen recognition.

T lymphocytes **recognize** peptide fragments of protein antigens...

...and that as part of the products of the MHC on the APC that bring processed antigens from the periphery to the secondary lymphatic organs...

...the most effective in this process are dendritic cells because they provide an additional (second) signal for activation.

## *... let us remind ourselves*

...after activation of T lymphocytes (antigen-specific) they begin to synthesize and secrete cytokines  
... Cytokines also stimulate **clonal expansion**

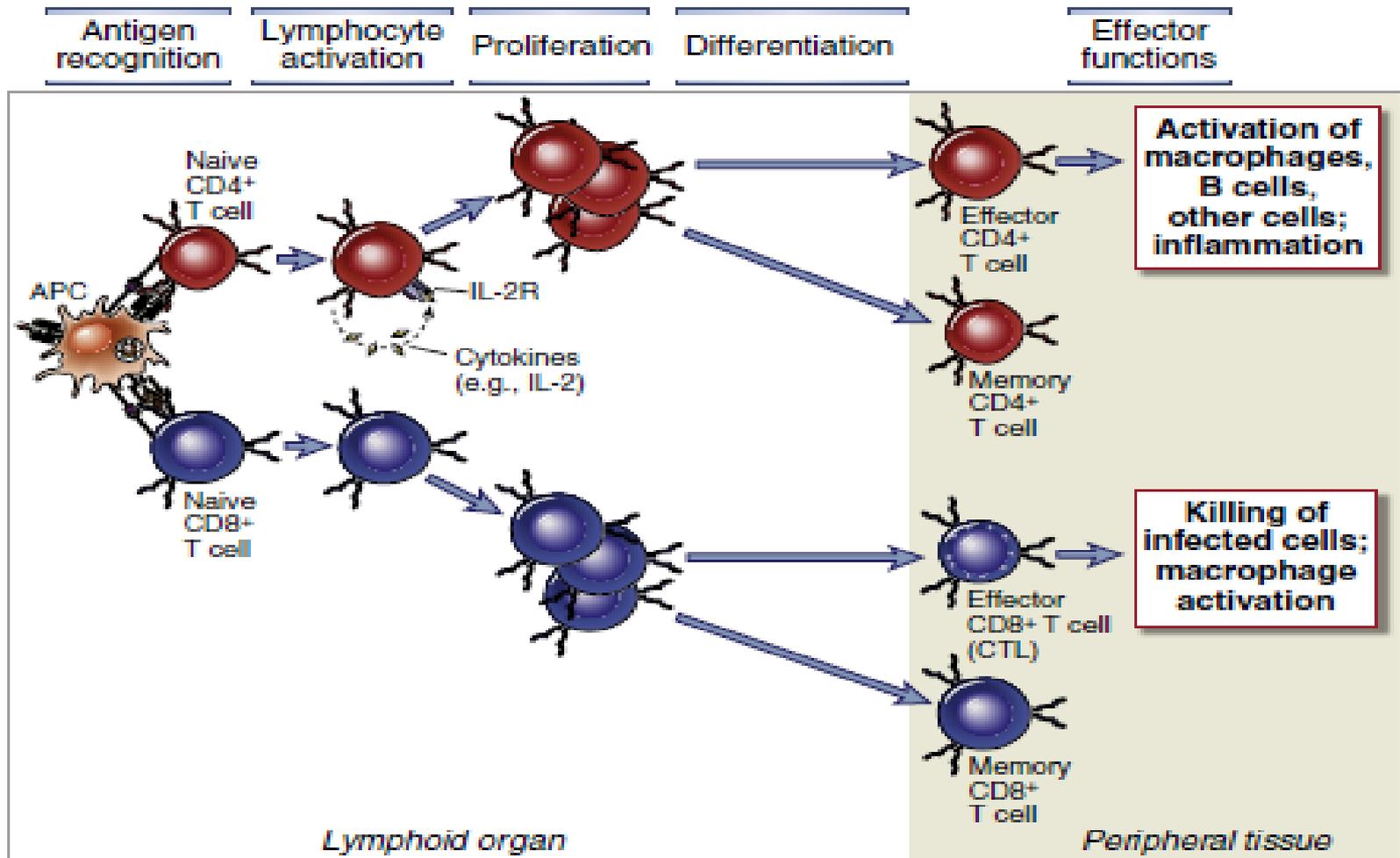
...lymphocytes activated in this way further **differentiate** into effector and memory lymphocytes

...some of these cells remain in the lymph node to participate in the elimination of the infected cells and to help B lymphocytes

...most other effector T lymphocytes migrate to the site of infection...

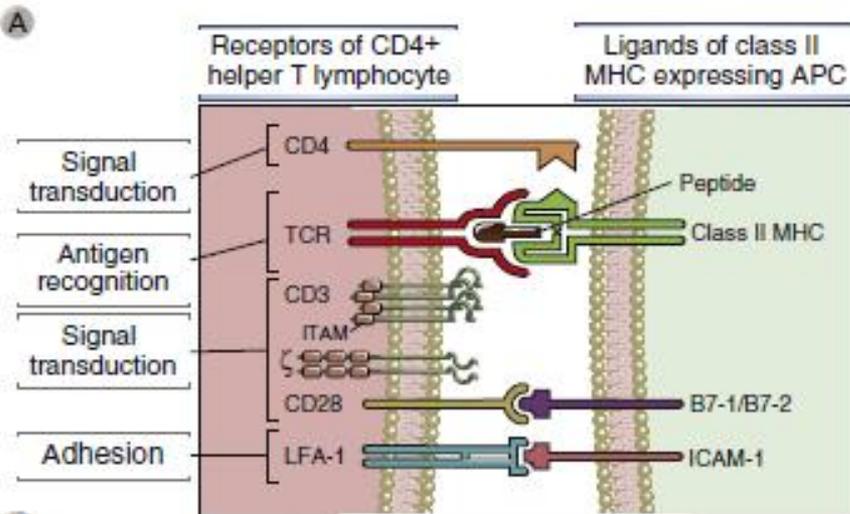
...after antigen elimination, some of these lymphocytes become memory T lymphocytes

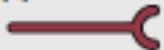
# Phases of T lymphocyte activation: from naive to effector T lymphocytes



# Antigen recognition and costimulation

Initiating a T-cell response requires multiple molecules on T lymphocytes to recognize the appropriate ligands on APC.



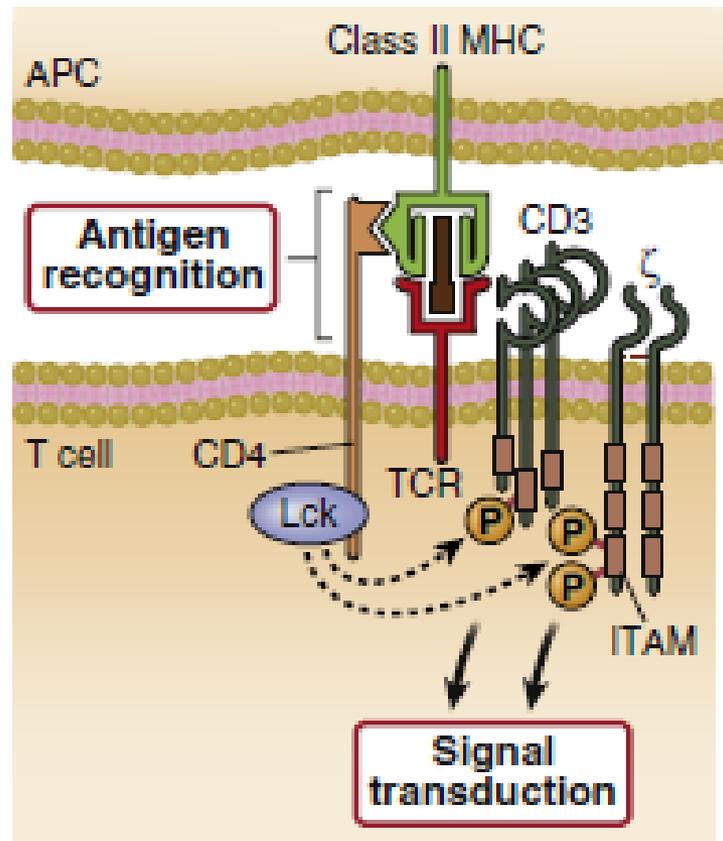
T cell accessory molecule	Function	Ligand	
		Name	Expressed on
CD3 	Signal transduction by TCR complex	None	
$\zeta$ 	Signal transduction by TCR complex	None	
CD4 	Signal transduction	Class II MHC 	Antigen presenting cells
CD8 	Signal transduction	Class I MHC 	All nucleated cells
CD28 	Signal transduction (costimulation)	B7-1/B7-2 	Antigen presenting cells
CTLA-4 	Signal transduction (negative regulation)	B7-1/B7-2 	Antigen presenting cells
PD-1 	Signal transduction (negative regulation)	PD-L1/PD-L2 	Antigen presenting cells, tissue cells, tumor cells
LFA-1 	Adhesion	ICAM-1 	Antigen presenting cells, endothelium

# 1. Recognition of peptides within the MHC molecule

This is the first signal for the activation of T lymphocytes. Receptor (TCR complex and coreceptors).

Coreceptors are **CD4** or **CD8** molecules.

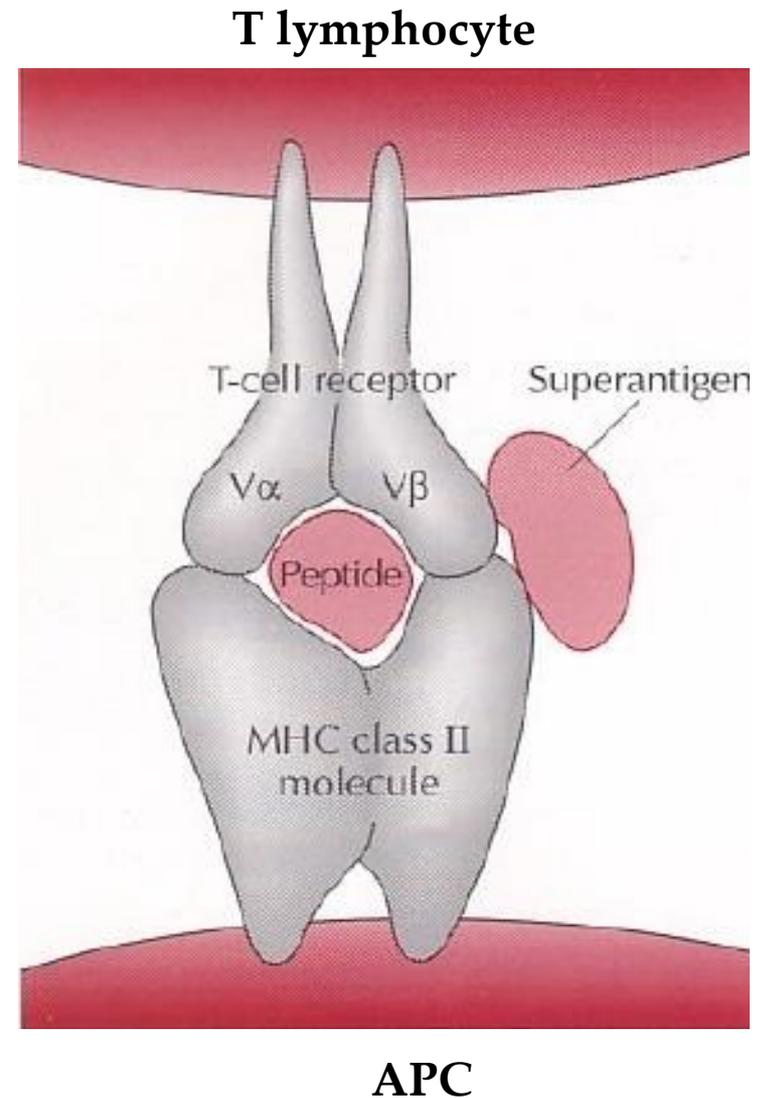
In the TCR complex, Recognition of antigens perform variable regions of  $\alpha$  and  $\beta$  chains of TCR molecules, while the invariable function of signalling perform proteins CD3 and  $\zeta$ .



# Superantigens

**Superantigens** - some exotoxins of Gram-positive bacteria (*S. aureus* and *S. pyogenes*) stimulate a large number of CD4+ T lymphocytes by directly binding to class II MHC molecules on APC and to regions of V $\beta$ TCR on T lymphocytes that are not part of active site.

By nonspecifically activating large numbers of CD4+ T lymphocytes, superantigens stimulate the production of large amounts of cytokines, resulting in a systemic reaction similar to septic shock.



## 2. Adhesive molecules in T lymphocyte activation

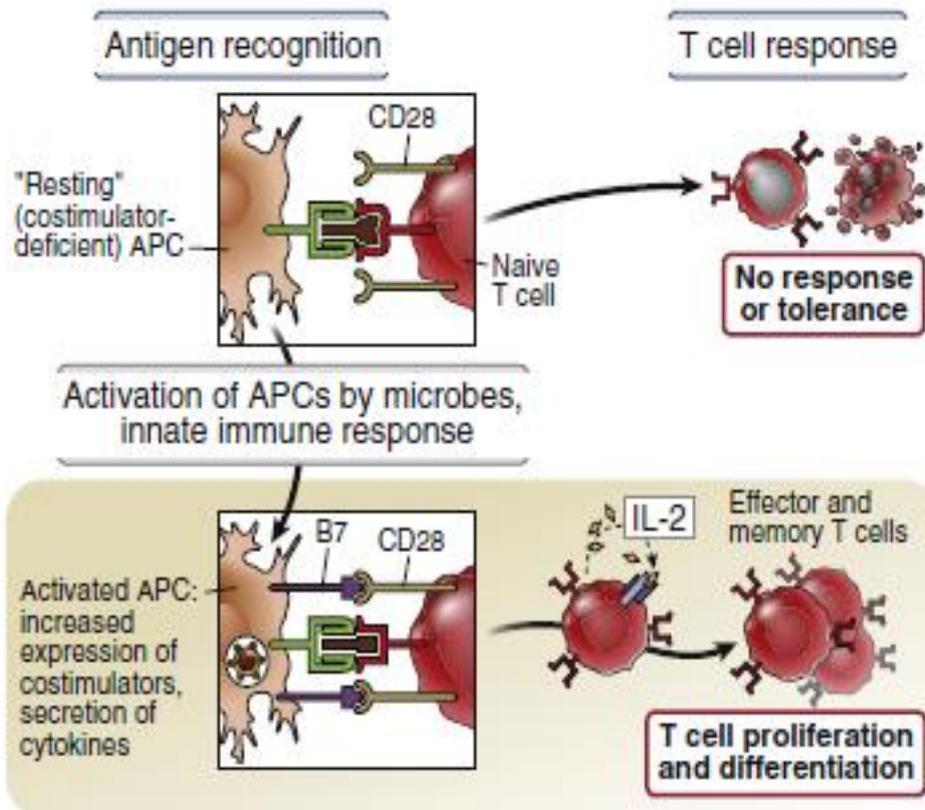
Adhesive molecules are expressed on T lymphocytes, recognize their ligands on APC and thus stabilize the binding of T lymphocytes to APC.

The most important adhesive molecules belong to a family of heterodimeric proteins called integrins. The main integrin on T cells is **LFA-1** (Leukocyte Function Associated Antigen -1). Its ligand is ICAM-1 (Intercellular adhesion molecule-1).

Integrins are also important in directing the migration of effector T lymphocytes to the site of infection.

### 3. Costimulators in the activation of T lymphocytes

Costimulators are molecules expressed on **APCs** and provide a **second signal**.



The best studied **B7-1(CD80)** and **B7-2(CD86)** are expressed on professional APC.

The expression of these molecules increases significantly when APC comes into contact with microorganisms.

The ligand for these molecules is **CD28** expressed on T lymphocytes.

In the absence of CD28 and B7 interaction, not only that there is no lymphocyte activation, but the lymphocyte can be disabled for a long time.

Another group of costimulatory molecules consists of **CD40** on APC and its **CD40 ligand** (CD154) on T lymphocytes.

The contact of these molecules does not directly enhance the activation of T lymphocytes. Instead, this binding increases the expression of B7 molecules on APCs and prompts them to secrete IL-12, which stimulates T lymphocyte differentiation.

Protein antigens (e.g. those used in vaccines) are inert and cannot induce a T cell immune response on their own, but it is necessary to give them substances that activate APCs (dendritic cells, macrophages, and probably also B lymphocytes). These substances are **adjuvants**.

Adjuvants work by inducing the expression of costimulators on APCs and prompting them to secrete activating cytokines.

Different members of the CD28 family participate in the activation, but also in the inhibition of T lymphocytes.

**To limit or end the immune response are important:**

...**CTLA4** which also binds to B7 on APC, but transmits an inhibitory signal and prevents the immune response to some tumors.

...**PD-1** which binds to similar ligands and inhibits the response to infection allowing chronicity.

**Receptor:** TCR recognizes the peptide within the APC

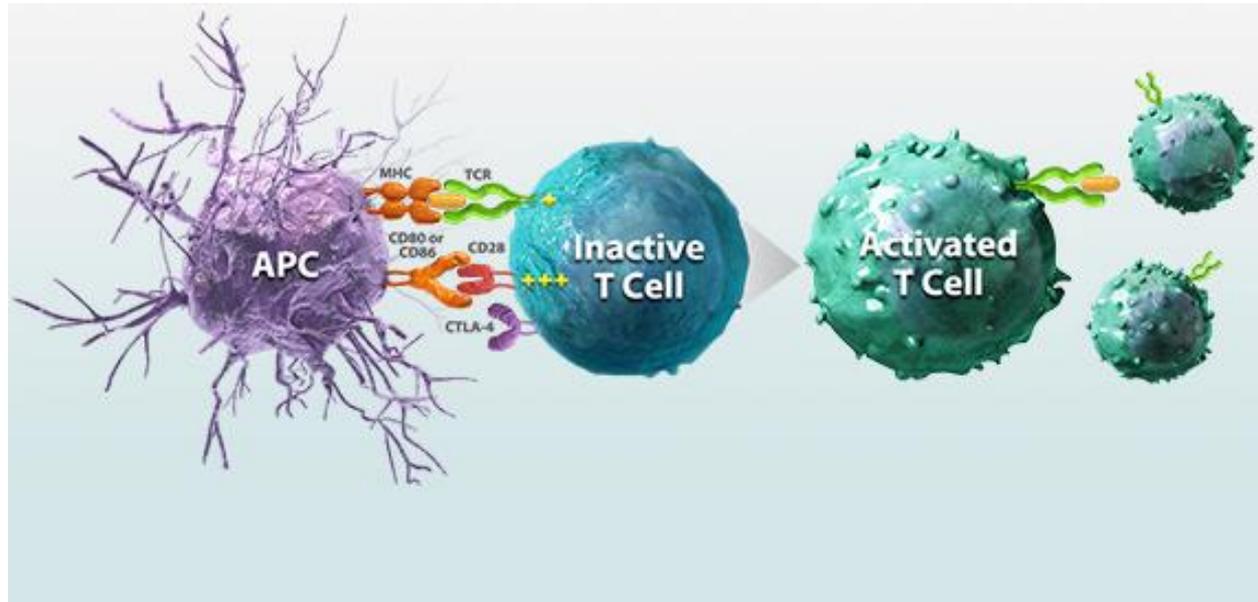
## **ACCESSORY MOLECULES :**

**CO-RECEPTORS** (expressed on T lymphocytes): CD4 and CD8

**ADHESIVE MOLECULES** (expressed on T lymphocytes): LFA-1

**COSTIMULATORS** (expressed on APC): B7-1, B7-2, CD40

After antigen recognition and costimulators bind to their ligands, gene transcription for cytokines, their receptors, cell cycle activators and effector molecules (eg CD40 ligand) begins.



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.

# Functional response of T lymphocytes to antigens and costimulation

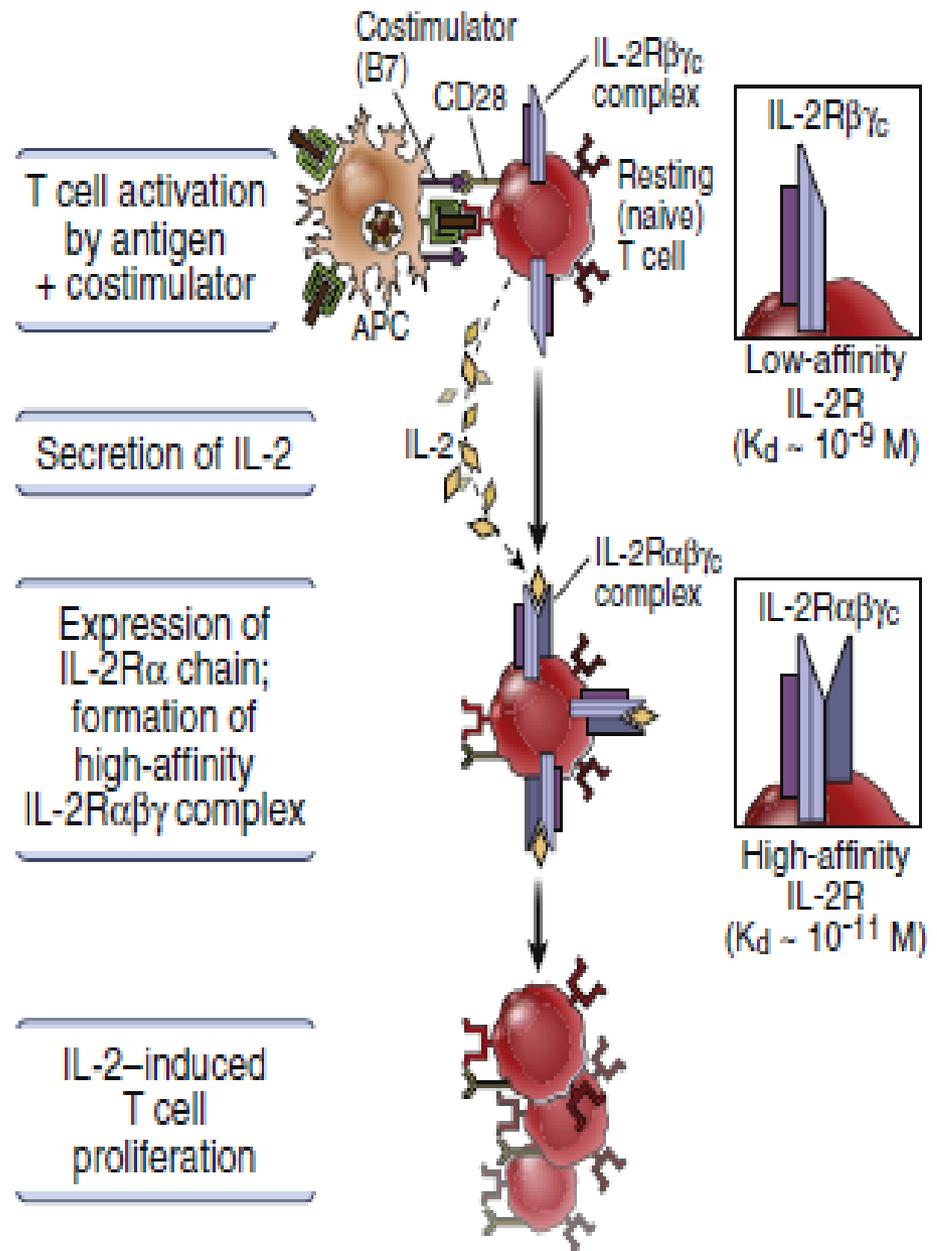
## 1. Cytokine secretion and expression of their receptors

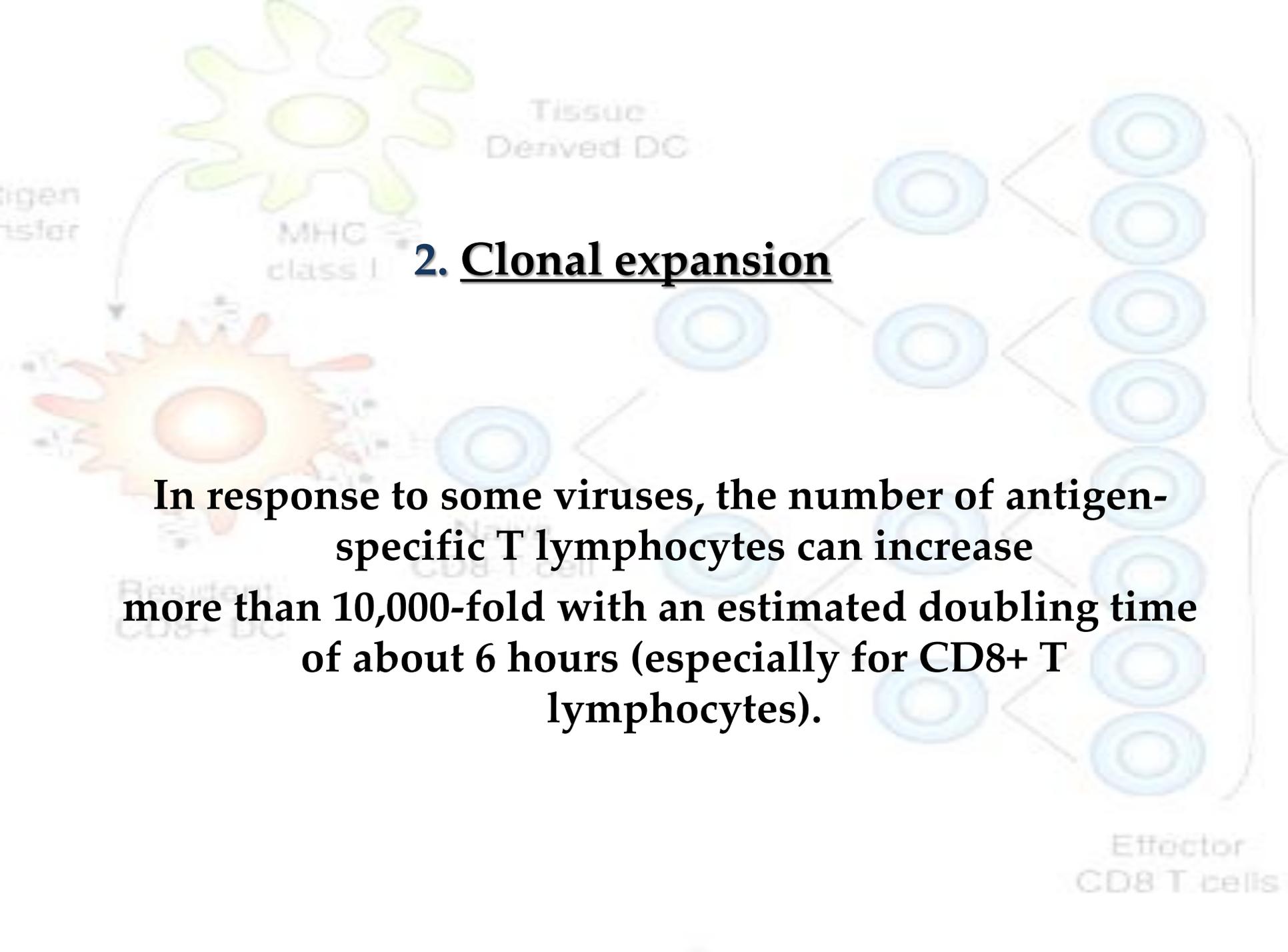
In a non-specific immune response, the main source of cytokines is the **macrophage**.

In the specific immune response it is **CD4+ T lymphocyte**.

**IL-2** is the first cytokine secreted immediately (one to two hours) after activation. Activation also stimulates **IL-2 receptor expression**.

**IL-2 is a growth (proliferation) and survival factor of T lymphocytes.**



The diagram illustrates the clonal expansion of CD8+ T cells. On the left, a 'Tissue Derived DC' (dendritic cell) is shown with 'MHC class I' molecules on its surface. An 'Antigen' is being presented to a 'CD8+ T cell'. The interaction leads to the clonal expansion of the T cell, shown as a single cell on the left that branches into two, then four, and finally a large group of 'Effector CD8 T cells' on the right. Labels include 'Antigen', 'MHC class I', 'Tissue Derived DC', 'CD8+ T cell', and 'Effector CD8 T cells'.

## **2. Clonal expansion**

**In response to some viruses, the number of antigen-specific T lymphocytes can increase more than 10,000-fold with an estimated doubling time of about 6 hours (especially for CD8+ T lymphocytes).**

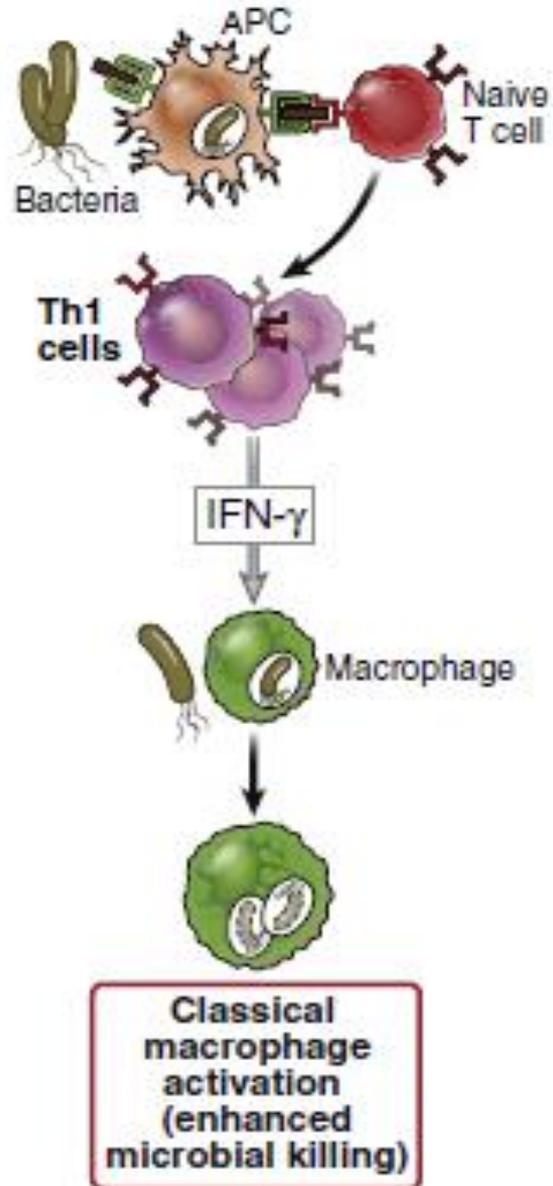
### 3. Differentiation of naive into effector T lymphocytes

Helper CD4+ T lymphocytes differentiate into **effector** lymphocytes that produce **membrane molecules** and **cytokines** in response to antigen.

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

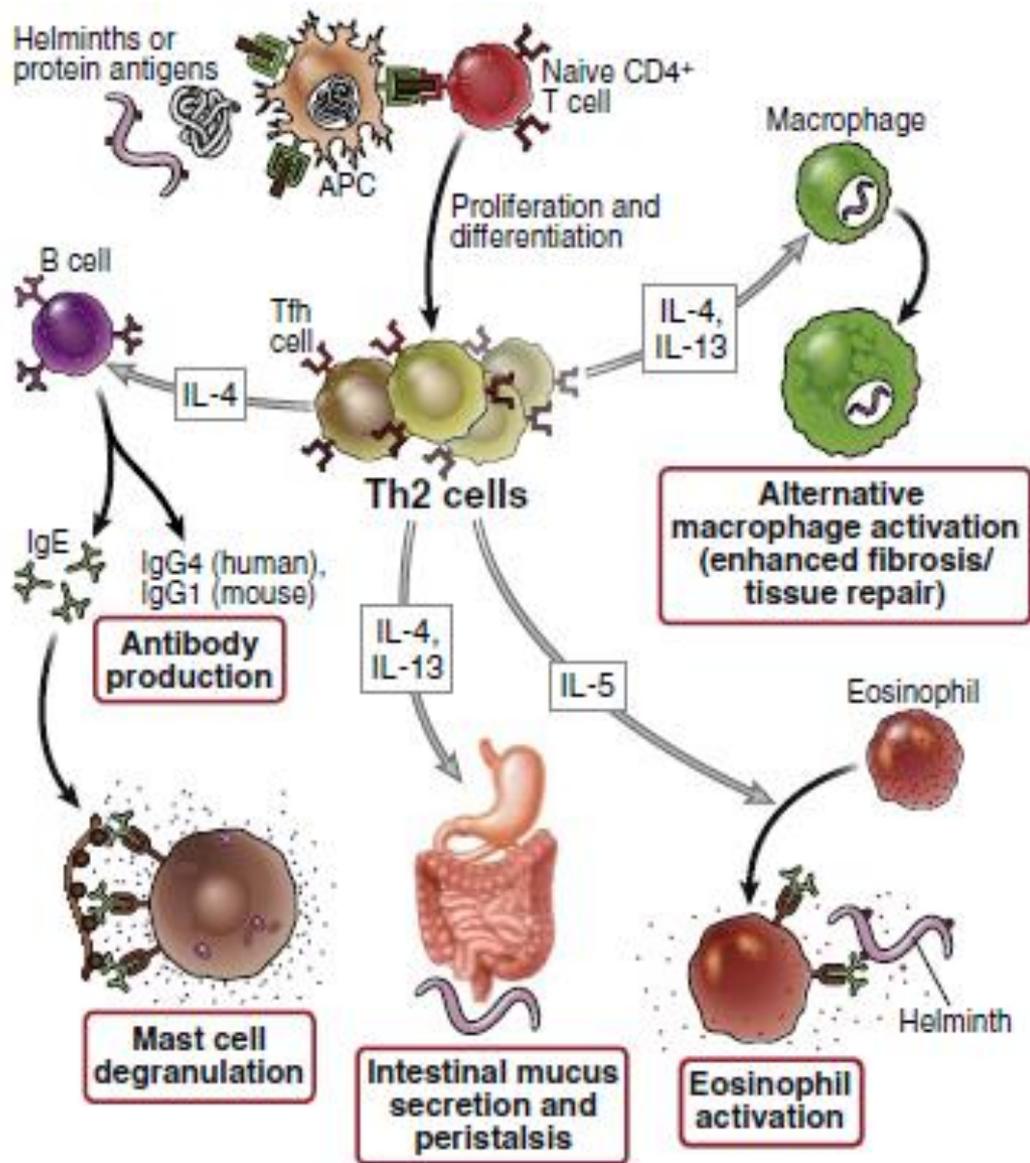
Naive CD4+T lymphocytes differentiate into **different effector cells** that secrete **different sets of cytokines** and perform **different functions**

Effector T cells	Defining cytokines	Principal target cells	Major immune reactions	Host defense	Role in disease
Th1 	IFN- $\gamma$	Macrophages 	Macrophage activation	Intracellular pathogens	Autoimmunity; chronic inflammation
Th2 	IL-4 IL-5 IL-13	Eosinophils 	Eosinophil and mast cell activation; alternative macrophage activation	Helminths	Allergy
Th17 	IL-17 IL-22	Neutrophils 	Neutrophil recruitment and activation	Extracellular bacteria and fungi	Autoimmunity; inflammation
Tfh 	IL-21 (and IFN- $\gamma$ or IL-4)	B cells 	Antibody production	Extracellular pathogens	Autoimmunity (autoantibodies)



Function of **Th1** lymphocytes

**IFN $\gamma$**

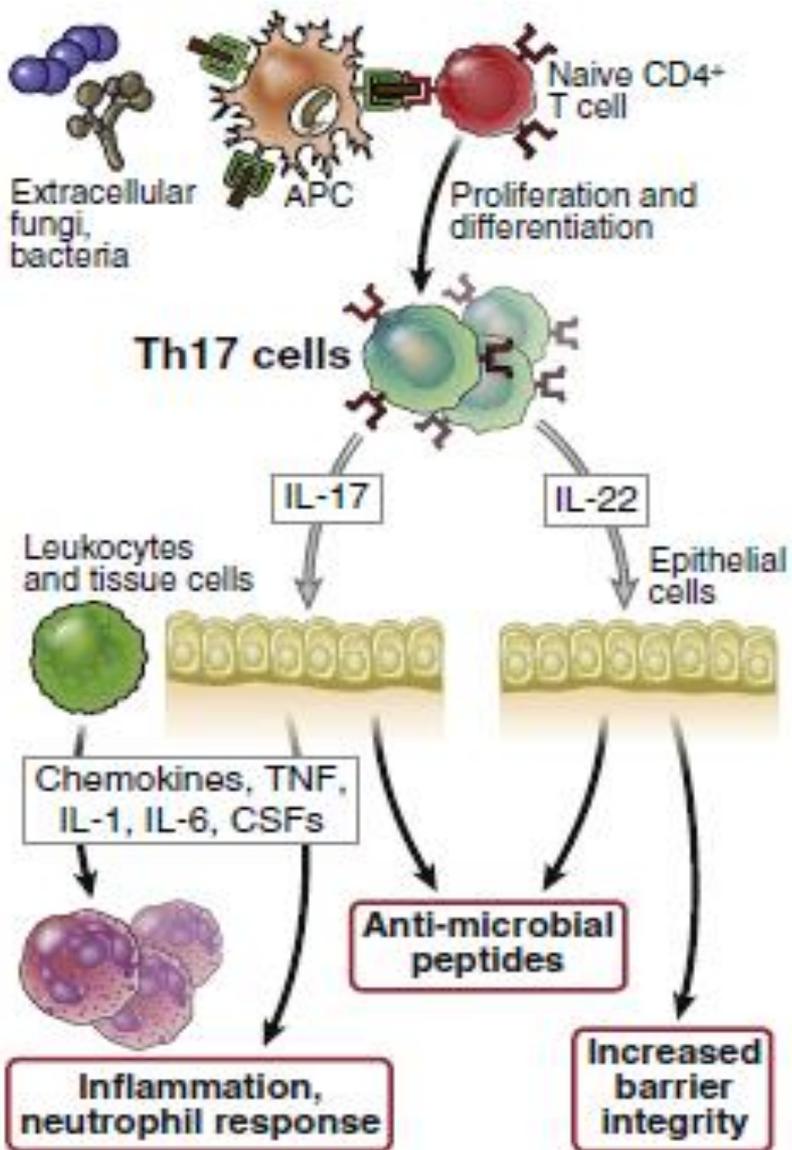


## Function of **Th2** lymphocytes

**IL-4**

**IL-5**

**IL-13**



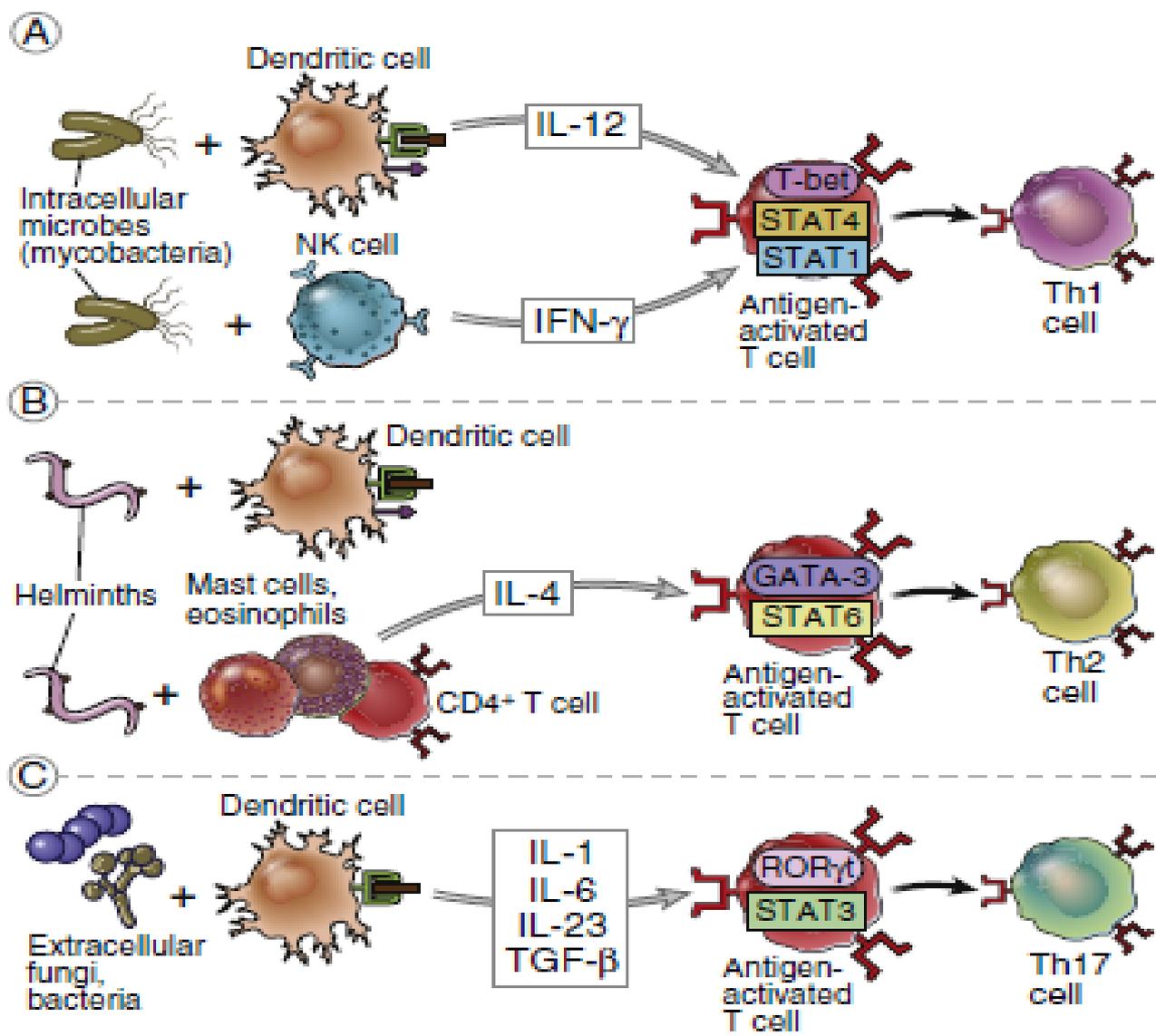
## Function of **Th17** lymphocytes

**IL-17**

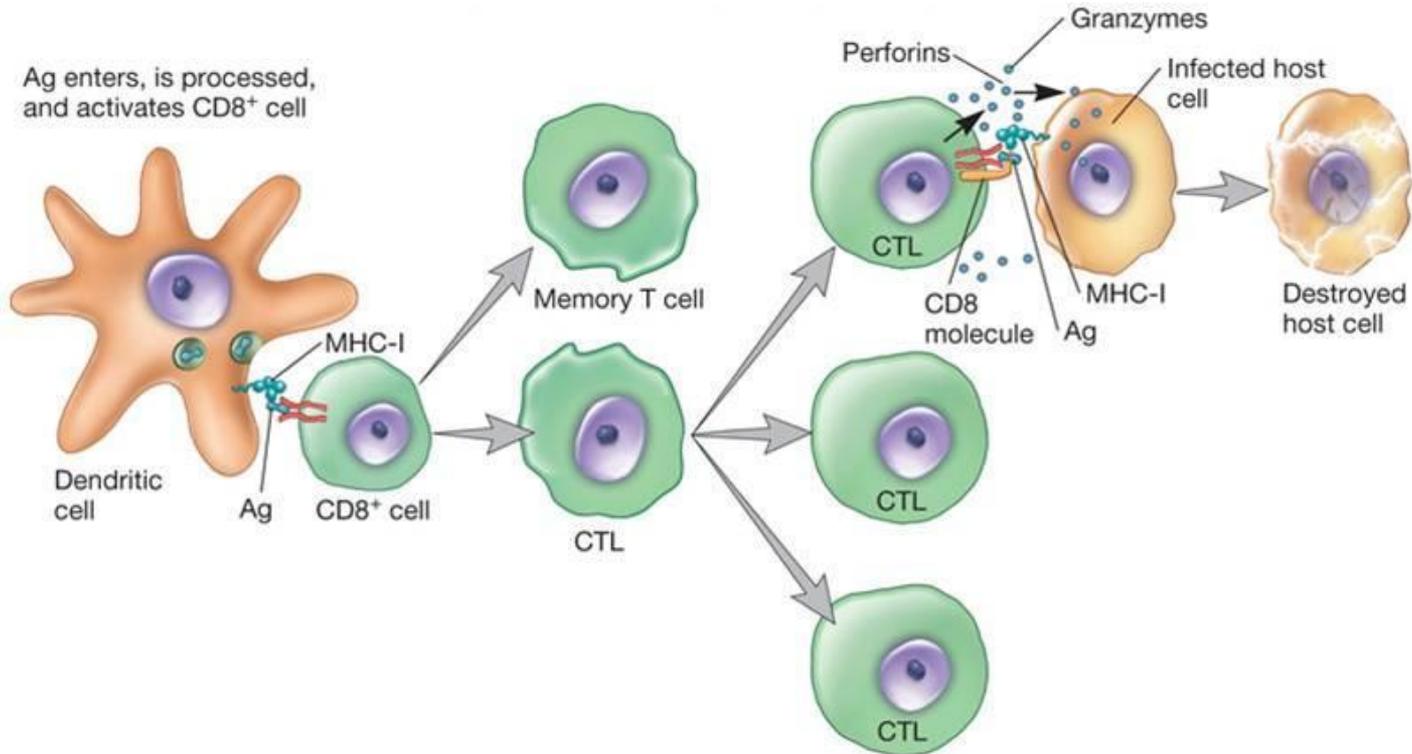
**IL-22**

**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.**

# The development of **Th1**, **Th2** and **Th17** effector lymphocytes



# 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

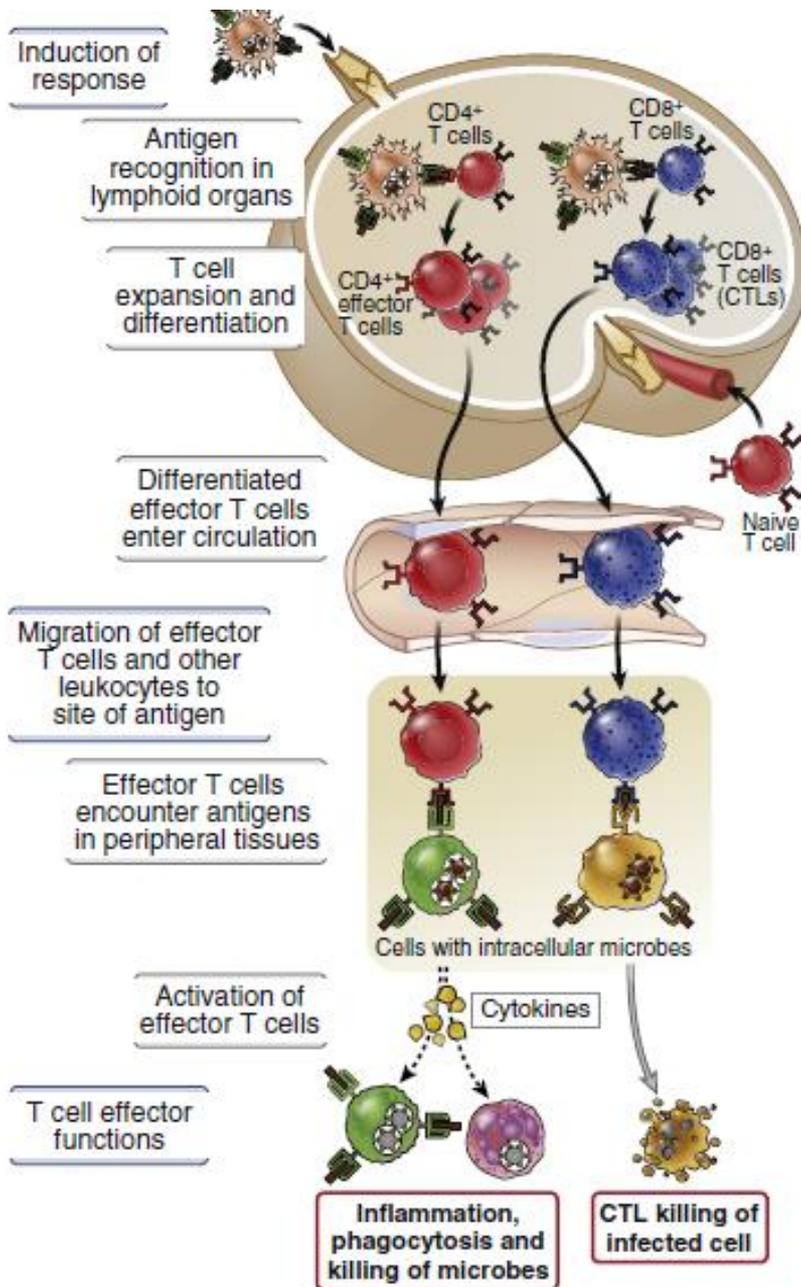
recognize the peptide in the context of MHC class I products.

Function: **cytotoxic T lymphocyte.**

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

# NAIVE T LYMPHOCYTES

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



# EFFECTOR T LYMPHOCYTE

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

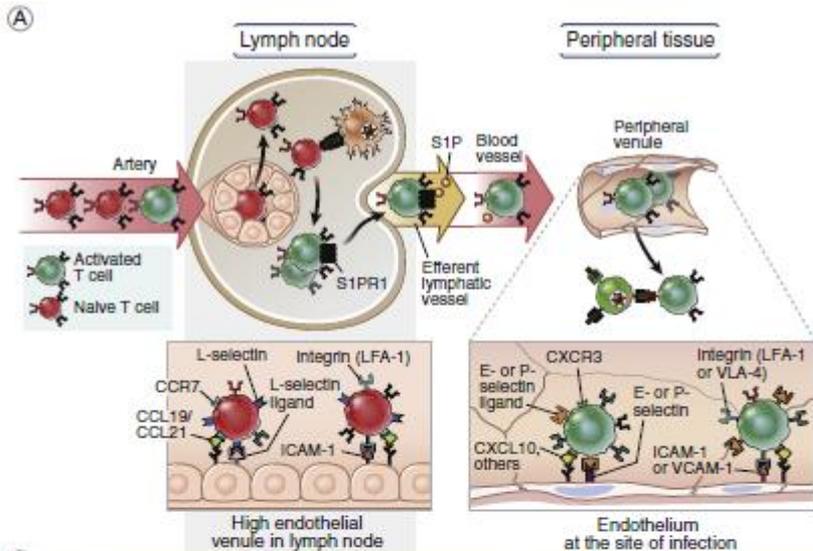
Effector CD8+T: CTL

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

Effector lymphocytes became that thanks to the **calling of new PROGRAMS:**

- programs needed **FOR FINDING THE SITE OF INFECTION** - applies to both CTL and Th1 and Th2
  - ✓ 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.
- programs needed **FOR ELIMINATION OF MICROORGANISMS** - specific for each type of effector lymphocytes

# Migration of effector lymphocytes to the site of infection



**B**

T cell homing receptor	Ligand on endothelial cell	Function of receptor: ligand pair
Naive T cells L-selectin	L-selectin ligand	Adhesion of naive T cells to high endothelial venule (HEV) in lymph node
LFA-1 ( $\beta_2$ -integrin)	ICAM-1	Stable arrest on HEV
CCR7	CCL19 or CCL21	Activation of integrins and chemotaxis
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 ( $\beta_2$ -integrin) or VLA-4 ( $\beta_1$ 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

- ✓ 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.
- ✓ Entry into infected tissues is regulated by the same mechanisms that regulate the migration of other leukocytes into tissues.
- ✓ 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).
- ✓ After activation, **lymphocytes** express **receptors for chemokines** produced by macrophages and endothelial cells located on the surface of the endothelium.

**Rolling - selectins**

**Tight binding – integrins**

**Motility - chemokines**

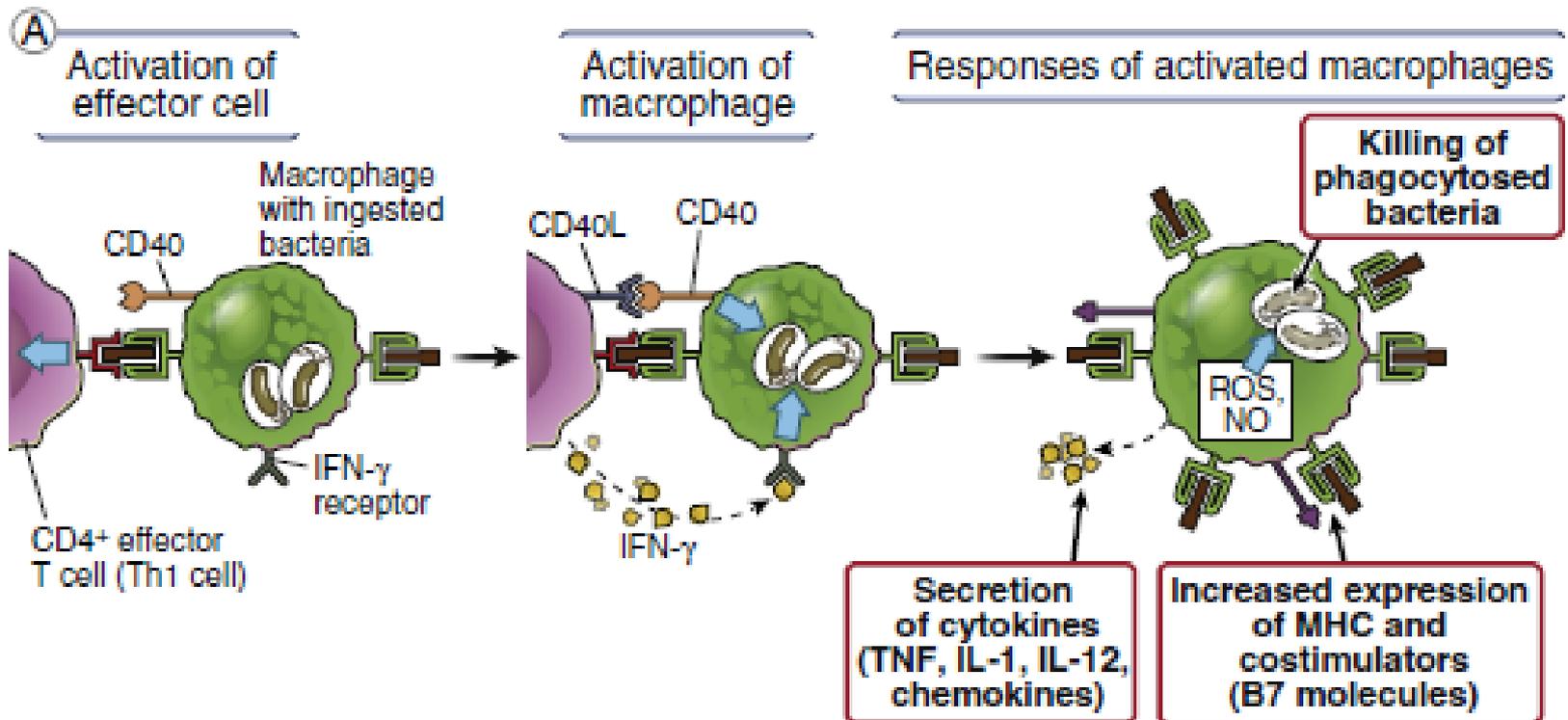
**Passage through the endothelium - PECAM-1 (CD31)**

- ✓ Settlement of T lymphocytes in infected tissues does not depend on specificity (antigen recognition) but on adhesive molecules and chemokines, so that all circulating effector T lymphocytes formed in response to other infectious agents enter the focus of any infection.
- ✓ Those that are specific for a given current infection recognize the antigen and are additionally activated.
- ✓ Thus, VLA integrins are also expressed more strongly, some of which enable adhesion to fibronectin and hyaluronic acid, which stops specific lymphocytes in the infected tissue, while the others continue on.

# Effector functions of the Th1 subpopulation of CD4+ lymphocytes

**The function of Th1 lymphocytes is the activation of macrophages that have phagocytosed microorganisms.**

**In addition, Th1 lymphocytes help CD8+ T lymphocytes to differentiate into CTLs, as well as B lymphocytes to develop into plasma cells.**



**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- $\gamma$ production
Increased expression of B7 costimulators, MHC molecules	Increased T cell activation (amplification of T cell response)

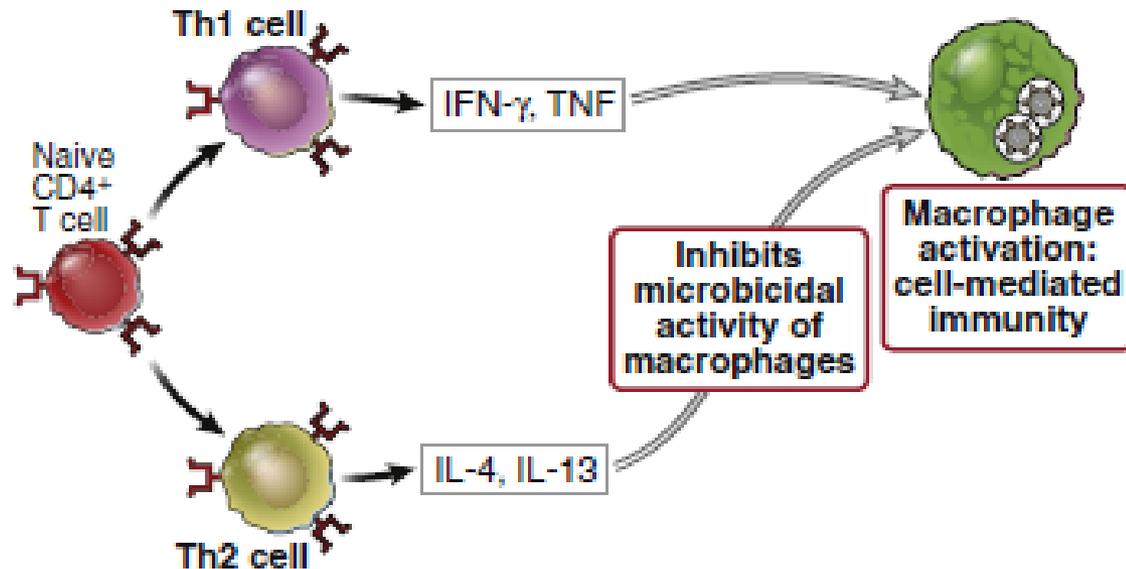
# Effector functions of the Th2 subpopulation of CD4+ lymphocytes

**Th2 lymphocytes recruit eosinophils, stimulate IgE synthesis, inhibit macrophages (alternative activation).**

# Effector functions of the Th17 subpopulation of CD4+ lymphocytes

**Th17 lymphocytes recruit neutrophils and to a lesser extent monocytes. In addition to inflammation, Th17 stimulate the production of defensins and maintain the functional integrity of epithelial barriers.**

# Pathogenesis of tuberculosis and leprosy



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

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

# Effector functions of CD8+ CTL

