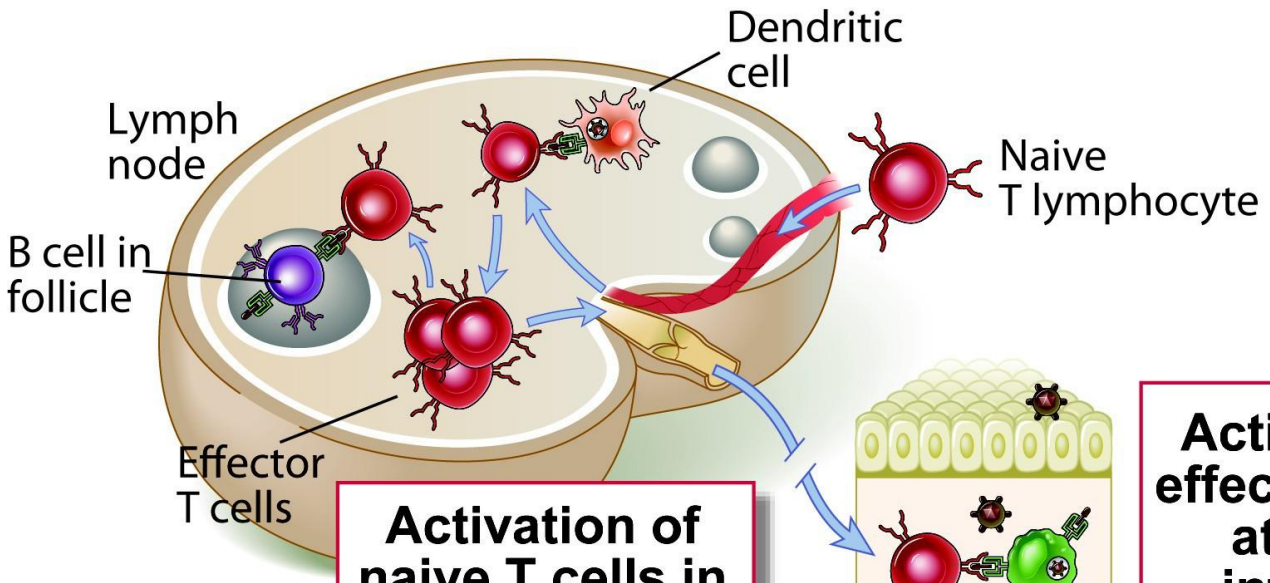
Cell mediated immunity

Activation of Naive and Effector T Cells

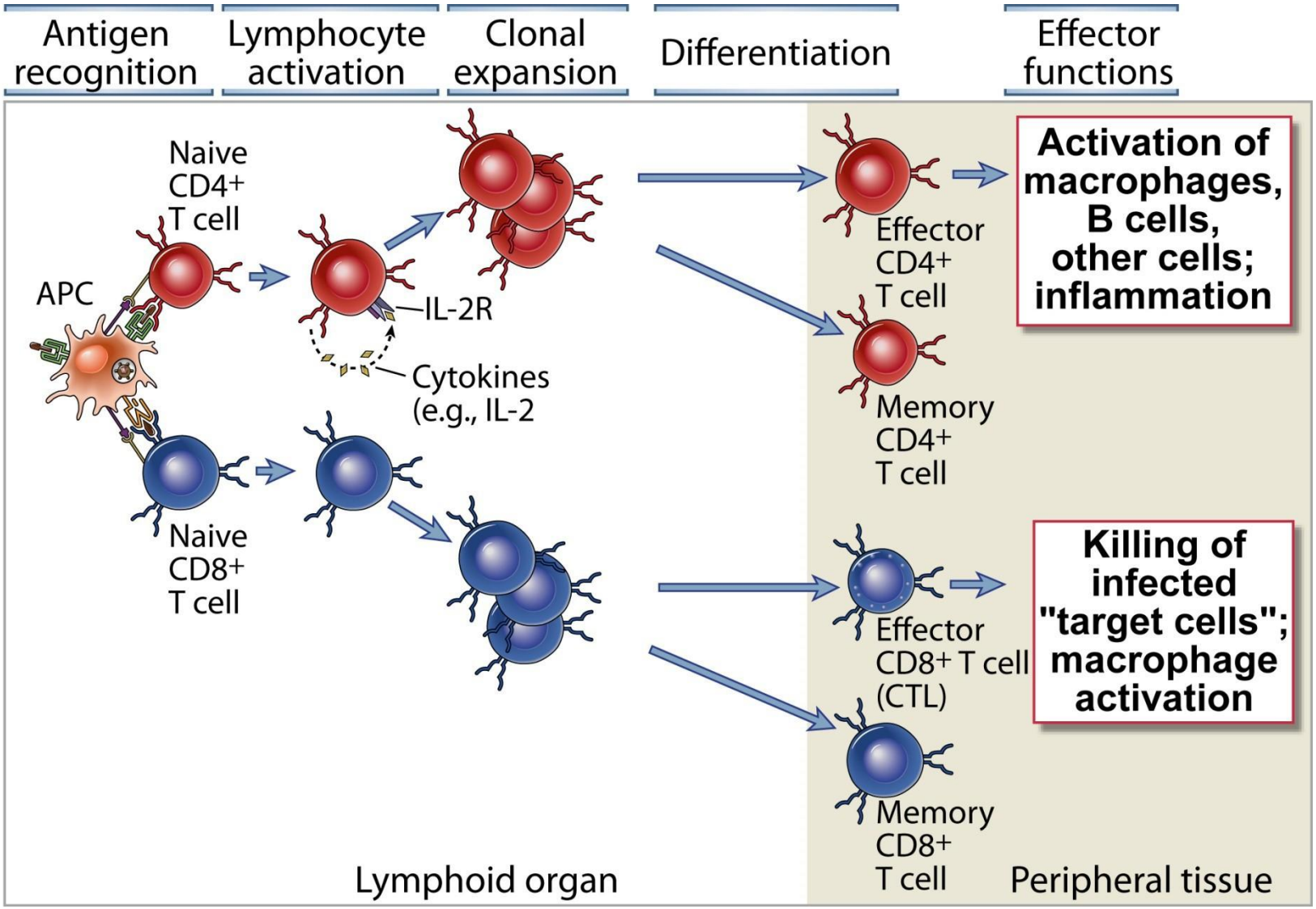
Niave T cells circulate through lymph nodes and find antigens



Activation of naive T cells in lymph node, development of effector cells

Activation of effector T cells at site of infection; eradication of microbe

Phases of T Cell Responses



Phases of T-Cell Response

- Sequential steps that result in increase in the **numbers of Ag-specific T-cells** and conversion of naïve T-cells to effector cells
- Naïve T lymphocytes circulate looking for Ag, express the receptor but can't perform the effector function
  - **must be stimulated to differentiate** into effector cell)**T-helper/CTL)** –initiated by Ag recognition
  - done in peripheral lymphoid tissue
  - also gets signals from microbes and/or innate immune system

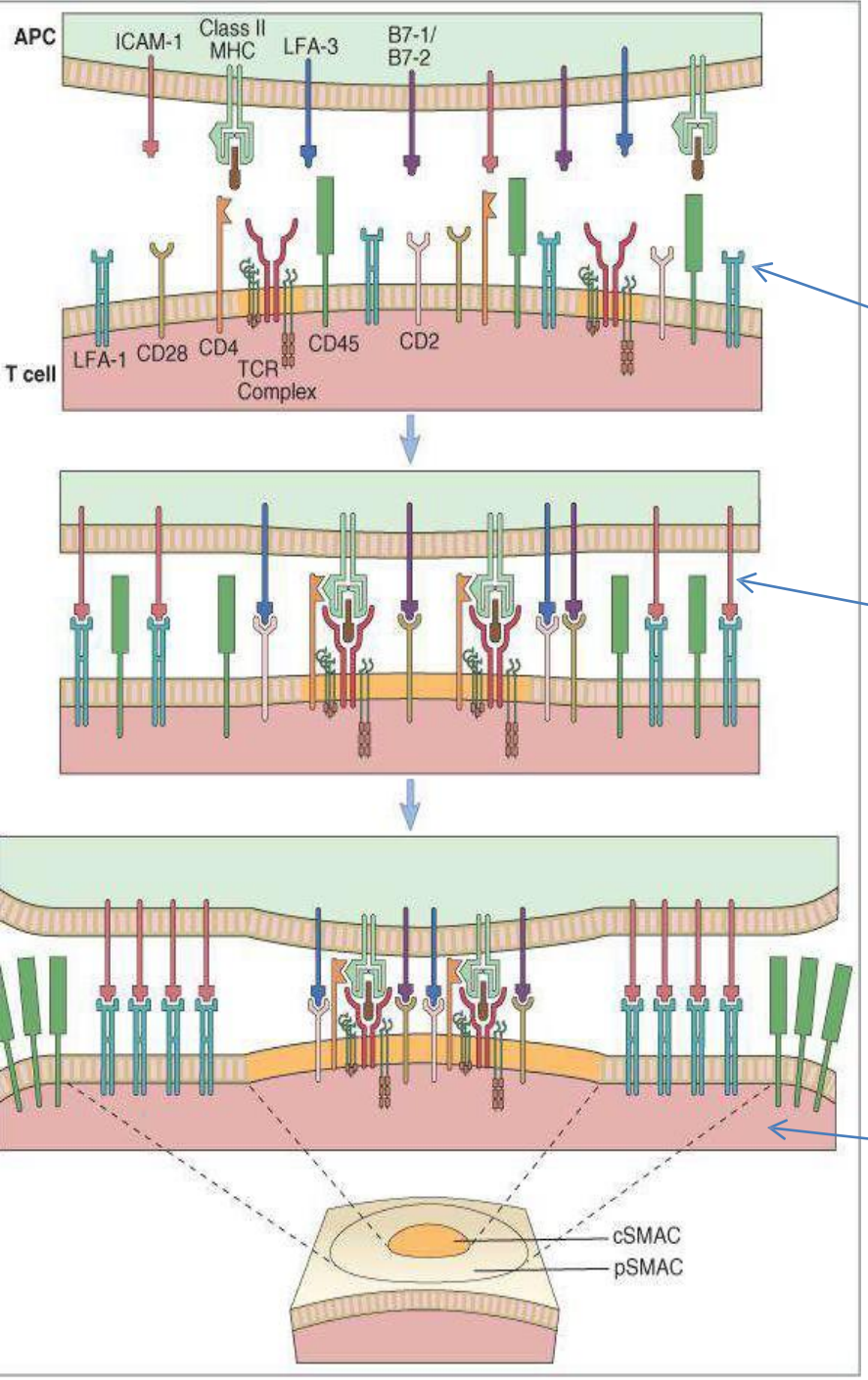
Phases of T-Cell Response

Combination of all signals cause Ag-specific T-cells to secrete **cytokines**

- happens to **CD4+ and CD8+ cells**
- cytokine and Ag and microbe act **as 2nd signal** that causes proliferation to increase the numbers of Ag-specific T-cells
  - called CLONAL EXPANSION
- fraction undergoes differentiation and switch functions from recognizing Ag to effector T-cells to eliminate microbes
- as microbe is eliminated, **effector T-cells** die and return to basal level of lymphocytes

MHC-Associated Peptide Recognition

- Initiation signal –TCR and CD8+ or CD4+ receptor recognize **peptide-MHC on APC**
  - cytosolic proteins –MHC I –CD8+ CTL
  - vesicular proteins –MHC II –CD4+ helper
  - **TCR recognizes peptide** and AA residues on MHC around peptide binding cleft
  - CD4 and CD8 are **co-receptors on MHC-restricted** T-cell – help to **bind TCR to MHC**
    - recognize at sites separate from the peptide binding site that helps to ensure the correct T-cell response
- Must get 2 or **more TCR and co-receptors** to bind MHC-peptides to initiate the signaling pathways
  - must encounter an array of **Ag for a long time or multiple times** to begin the activation –threshold needed to initiate response



Formation of the immunological synapse

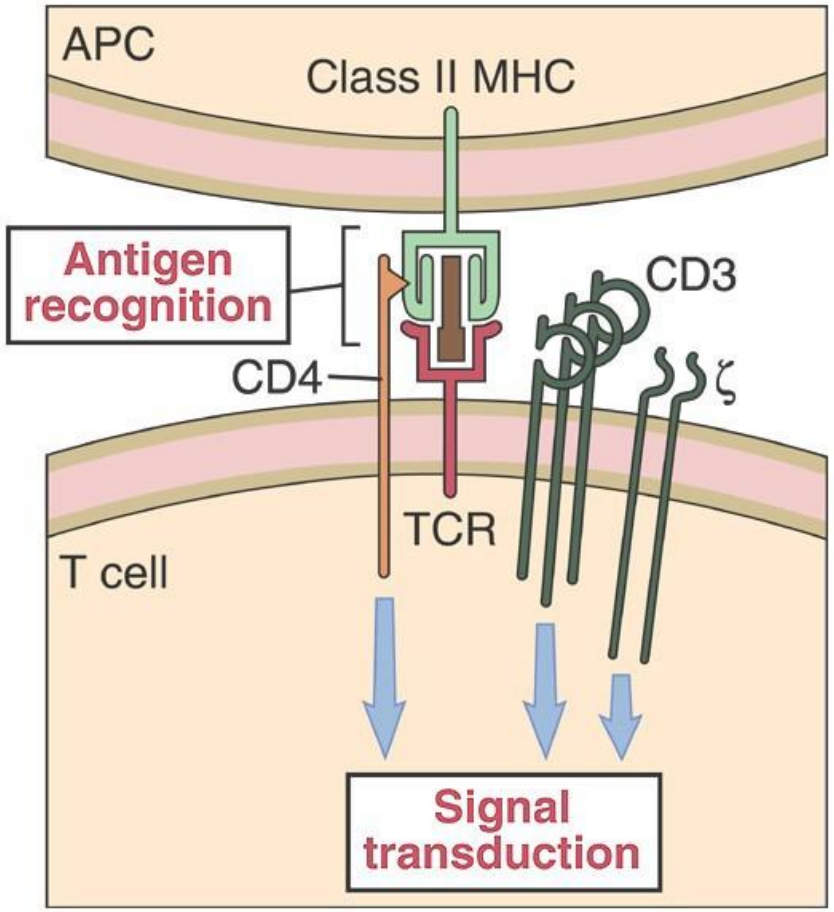
This schematic diagram illustrates the steps in the formation of the immunological synapse.

Before antigen recognition, various receptors on T cells and their ligands on APCs are dispersed in the plasma membranes of the two cells.

When the T cell recognizes antigen presented by the antigen-presenting cell (APC), selected receptors on the T cell and their respective ligands are redistributed to a defined area of cell-cell contact, forming the synapse.

The molecules in the central portion of the synapse form the central supramolecular activation cluster (cSMAC), and the molecules in the periphery form the peripheral supramolecular activation cluster (pSMAC)

Biochemical Signals



- T-cell activation requires proteins linked **to TCR to form TCR complex** and to CD4or CD8 coreceptor
- 2 set of molecules
  - **Ag receptors with much diversity**
  - those that are conserved signals
- TCR recognizes the Ag but can't pass on the signal so TCR associates with CD3 (**complex of 3 proteins**) and with a homodimer of ζ chain
  - now signal can be passed to the interior of the T-cell
  - TCR, CD3 and ζ make up TCR complex

Role of Costimulators

- Full T-cell activation requires costimulators on the surface of APC – provide 2nd stimuli to T-cell

- 2best defined are **B)7-1** CD80) and B7-2 (CD(86
  - both are on **professional APC** and their expression is increased when in contact with microbe

- B7 molecules recognize **CD28** expressed on nearly all T-cells and along with **TCR:MHC-peptide/co-receptor – essential for activating T-cells**
  - ensures full activation of naïve T-cells
  - absence of B7 and CD28 interactions =no activation

- **CD40L)ligand)** on T-cells and **CD)40 receptor) on APC** –not a direct enhancement of T-cell activation
  - causes the expression of more B7 molecules and activates APC to secrete cytokines
    - **IL-12 enhances T-cell proliferation** and makes APC better at stimulating T-cells

Response

- Response of T-lymphocytes to Ag and constimulation are **mediated by cytokines** that are secreted by the T-cell and acts on T-cells as well as other cells of the immune system

-IL-2 and T-Cell Activation

-2 hours after CD4+ cells IL-2 is secreted which enhances the ability of T-cells to respond to IL-2 by way of regulation of IL-2 receptor expression

-IL-2 receptor has **3 protein chains**, naïve T-cells express 2 of the 3 proteins ($\beta\gamma$) which can't bind IL-2 with high affinity

-3rd chain (α) gets expressed after activation to complete receptor

---now have receptor that **can strongly bind IL-2**, preferentially on the same T-cell

---IL-2 stimulates **T-cell proliferation** – forces cells into cell cycle

Thus, IL-2 acts as a **T-cell "growth hormone"**

-CD8+ T-cell –doesn't appear to make large amounts IL-2may depend on CD4+ helper cells to provide IL-2

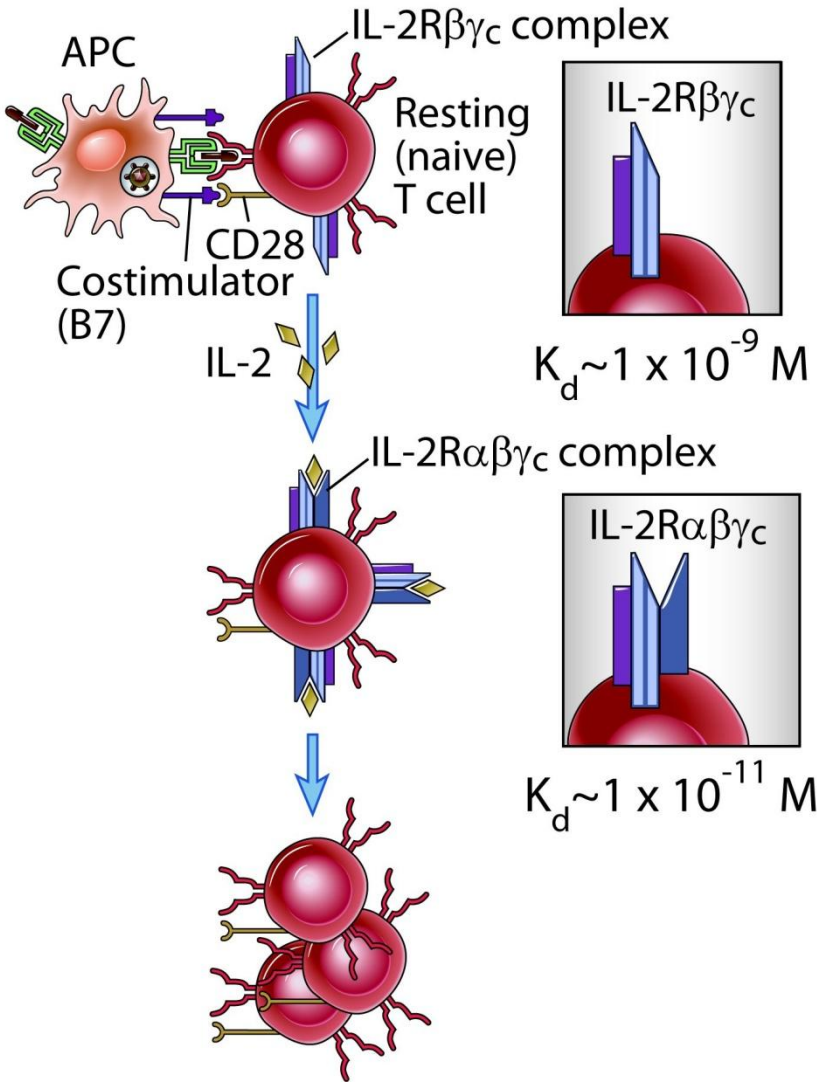
Regulation of IL-2 Receptor Expression

T cell activation by antigen + costimulator

Secretion of IL-2

Expression of IL-2Rα chain; formation of high-affinity IL-2Rαβγ complex

IL-2–induced T cell proliferation



Clonal Expansion

Within 1-2 days after activation, T-cells begin to proliferate and start expansion of Ag specific lymphocytes CD8+ T-cells

- before infection 1in 10^5 to 10^6 is specific for Ag
- after viral infections, 10-20% of all lymphocytes in lymphoid organs may be specific for that virus, an increase of 100,000 fold increase in Ag-specific for that virus

CD4+ T-cells

- see only a 100 to 1000 fold increase

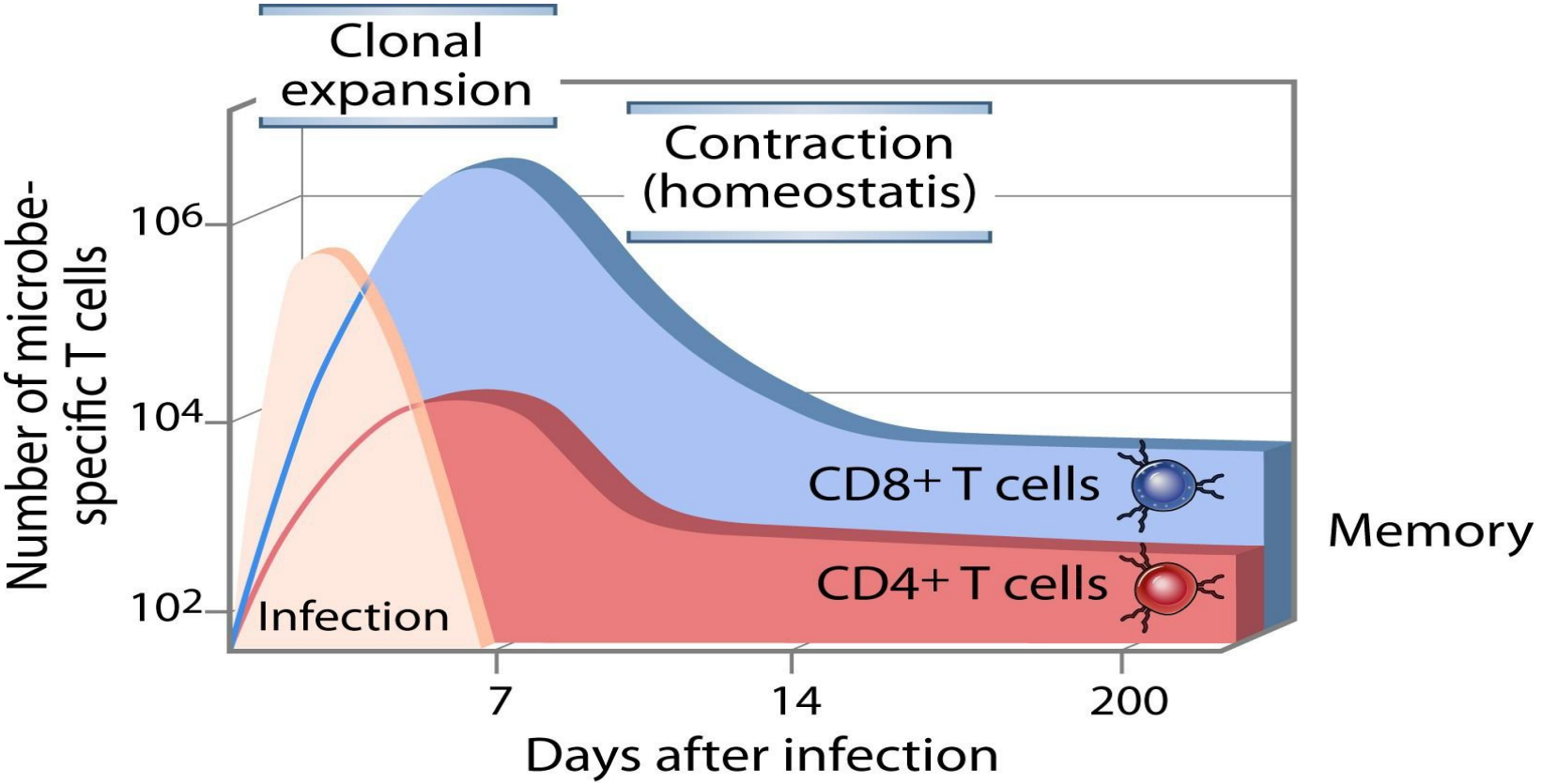
Difference may be due to difference in function

- CD8+ are effector cells themselves – kill infected cells
- CD4+ effector cells secrete cytokines that activated other effector cells; small amount of cytokine needed to cause action

Clonal Expansion Surprises

-Expansion is in specific T-cells for Ag but not other T-cells that do not recognize Ag

-Even though microbe may be complex usually> 5 immunodominant peptides used for expansion



Differentiation of Naïve Cells

- Need to become effector cells to eradicate the infection
- Changes in **gene expression –cytokines in CD4+ and CD8+ cells** or cytolytic proteins in CD8+ CTLs
- Appear **in 4-3days** and leave lymphoid tissue to move to the site of infection
- CD4+ and CD8+ perform different functions so differentiation is also different

CD4+Effector Cells

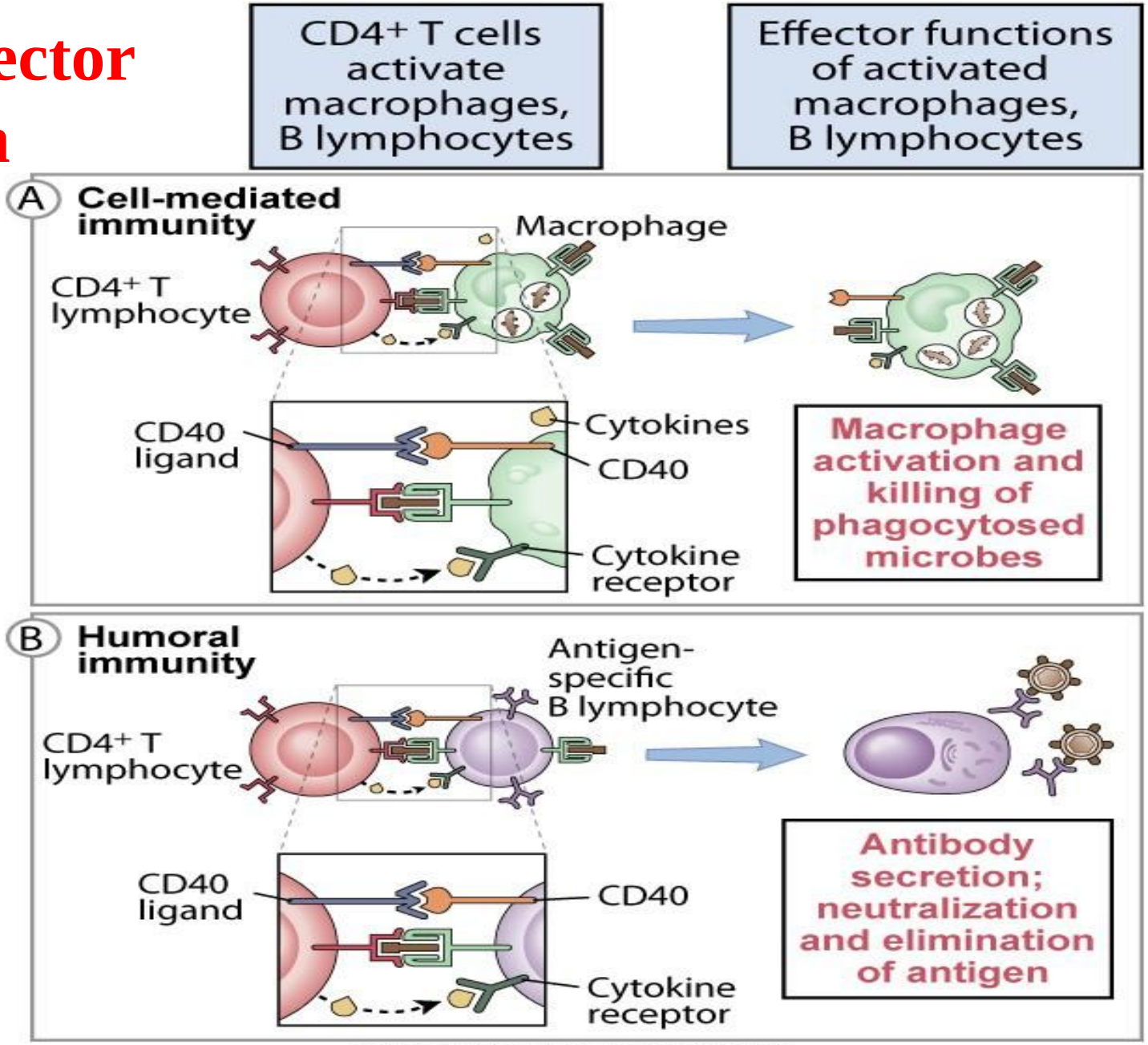
Respond to Ag by making surface molecules and cytokines to mainly **activate macrophages and B-cells**

CD40L (ligand) is the most important – expressed after Ag recognition and costimulation

- binds to CD40, mainly on macrophages, B-cells or dendritic cells
  - binding activates macrophages and B-cells
  - binding of dendritic cells cause **expression of costimulators** and T-cell activating cytokines

- interaction produces T-cells activating cytokine causing positive feedback on APC-induced positive cell activation

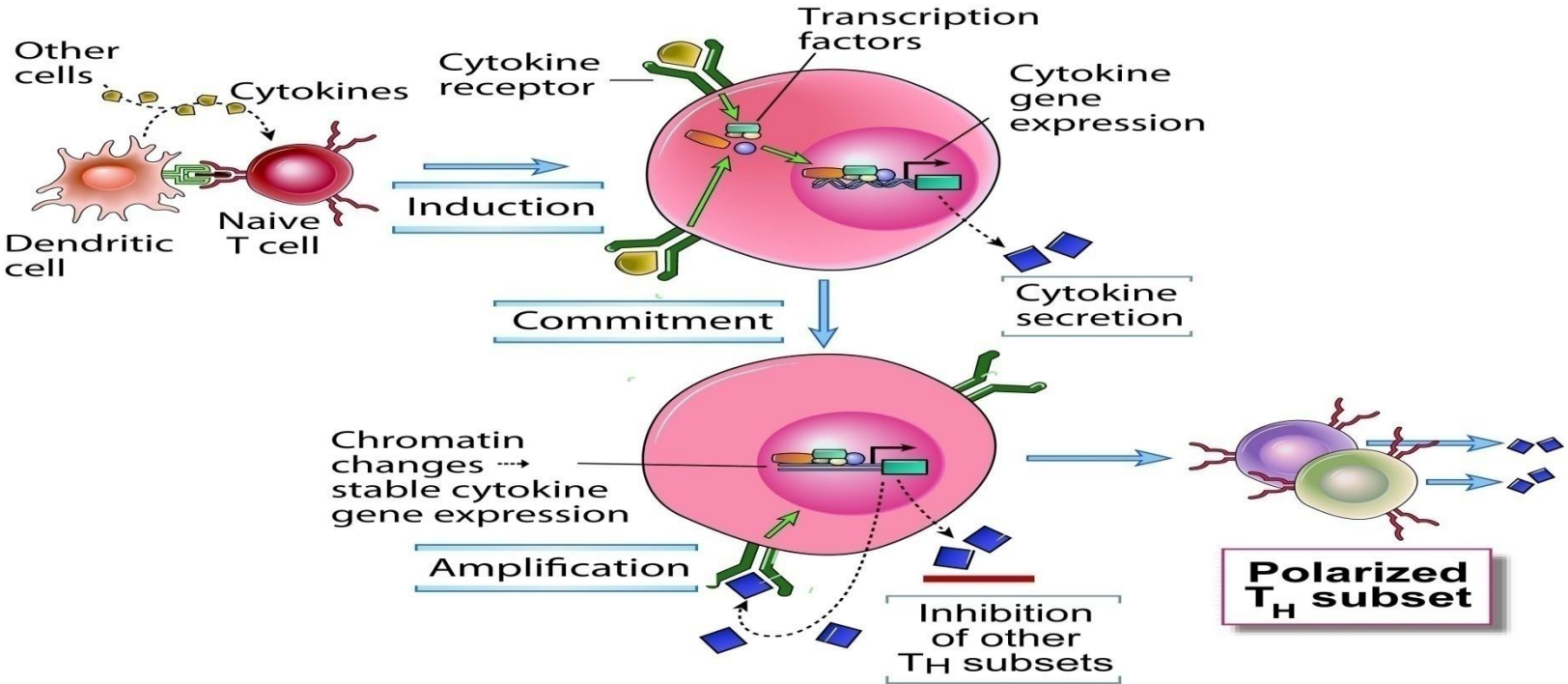
CD4 Effector Function



Helper T-Cell Subsets

- Different types of CD4+ effector T-cells with distinct function
- Subsets of effector cells produce distinct sets of cytokines that perform different functions
  - T_H1 and T_H2 cells

  --recently identified group called T_H17 because of signature cytokine – IL-17
  - regulatory T-cells is also subset



Differentiation of T_H1, T_H2 and T_H17

--Not a random process but is regulated by stimuli that naïve CD4+ T-cell receive when they encounter microbial Ag

--Macrophage and dendritic cells make IL-12 in response to many bacteria and viruses; NK cells make IFNγ

--T cell encounters Ag on APC also get exposed to IL-12

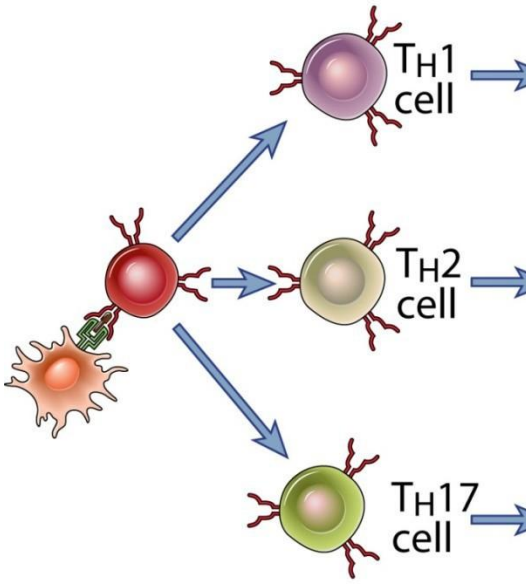
- promotes T_H1 proliferation and release of IFNγ to activate macrophages
- innate immune response of IL-12 by APC will drive the formation of $T_H1$helper cells (adaptive immune response(

If there is no IL-12 as in helminth infection, then the T-cell will secrete IL-4 and get a T_H2 response

- may also be influenced by type of dendritic cell that can dictate the T_H response

Differentiation of one type will inhibit the development of the other one to make most effective response to microbe

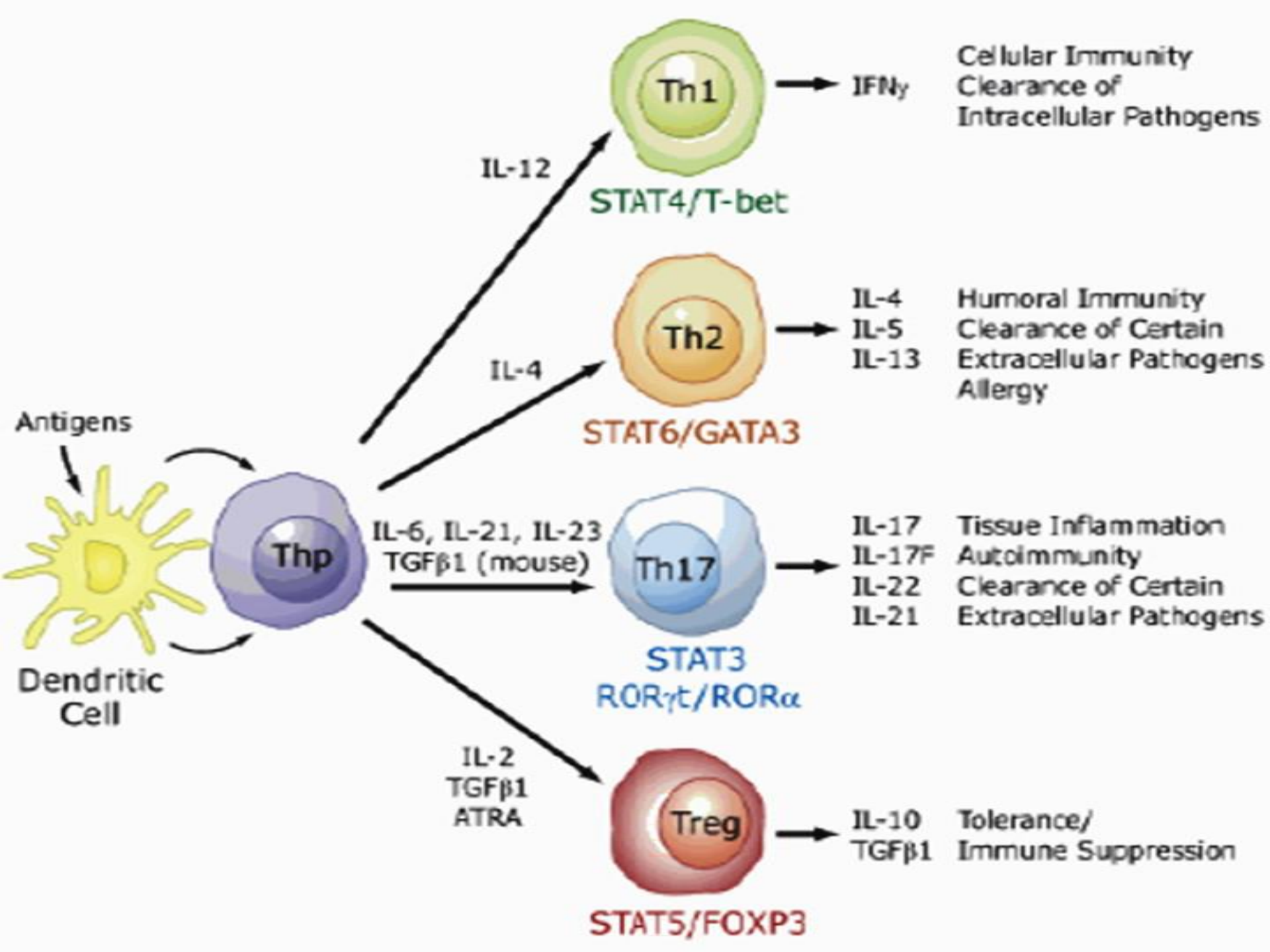
Types of CD4+ Cells and Cytokines



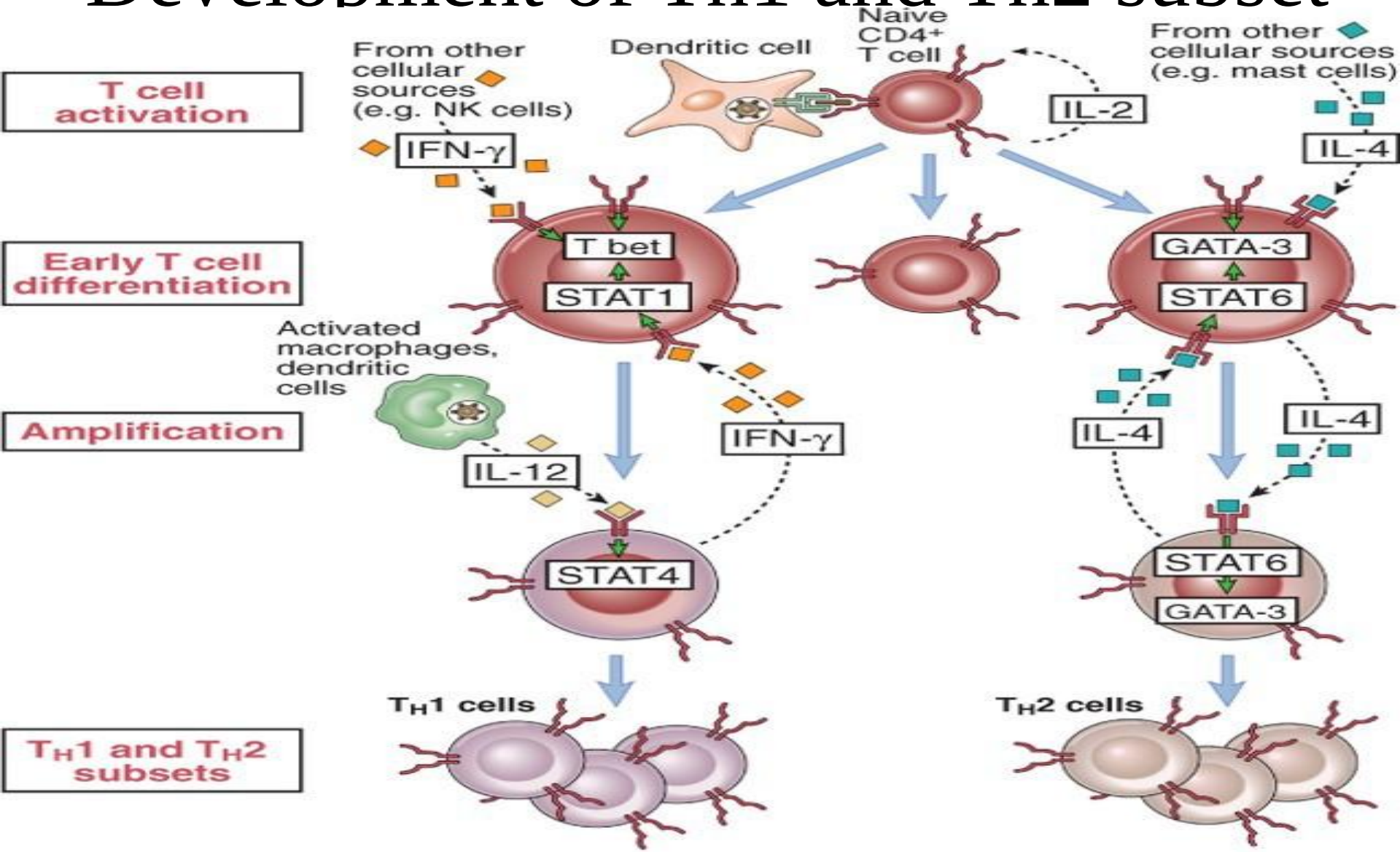
	Signature cytokines	Immune reactions	Host defense	Role in diseases
T_H1 cell	IFNγ	Macrophage activation; IgG production	Intracellular microbes	Autoimmune diseases; tissue damage associated with chronic infections
T_H2 cell	IL-4 IL-5 IL-13	Mast cell, eosinophil activation; IgE production; "alternative" macrophage activation	Helminthic parasites	Allergic diseases
T_H17 cell	IL-17A IL-17F IL-22	Neutrophilic, monocytic inflammation	Extracellular bacteria; fungi	Organ-specific autoimmunity

-T_H1 and T_H2 cells are distinguished by cytokines but also by cytokine receptors and adhesion molecules they express

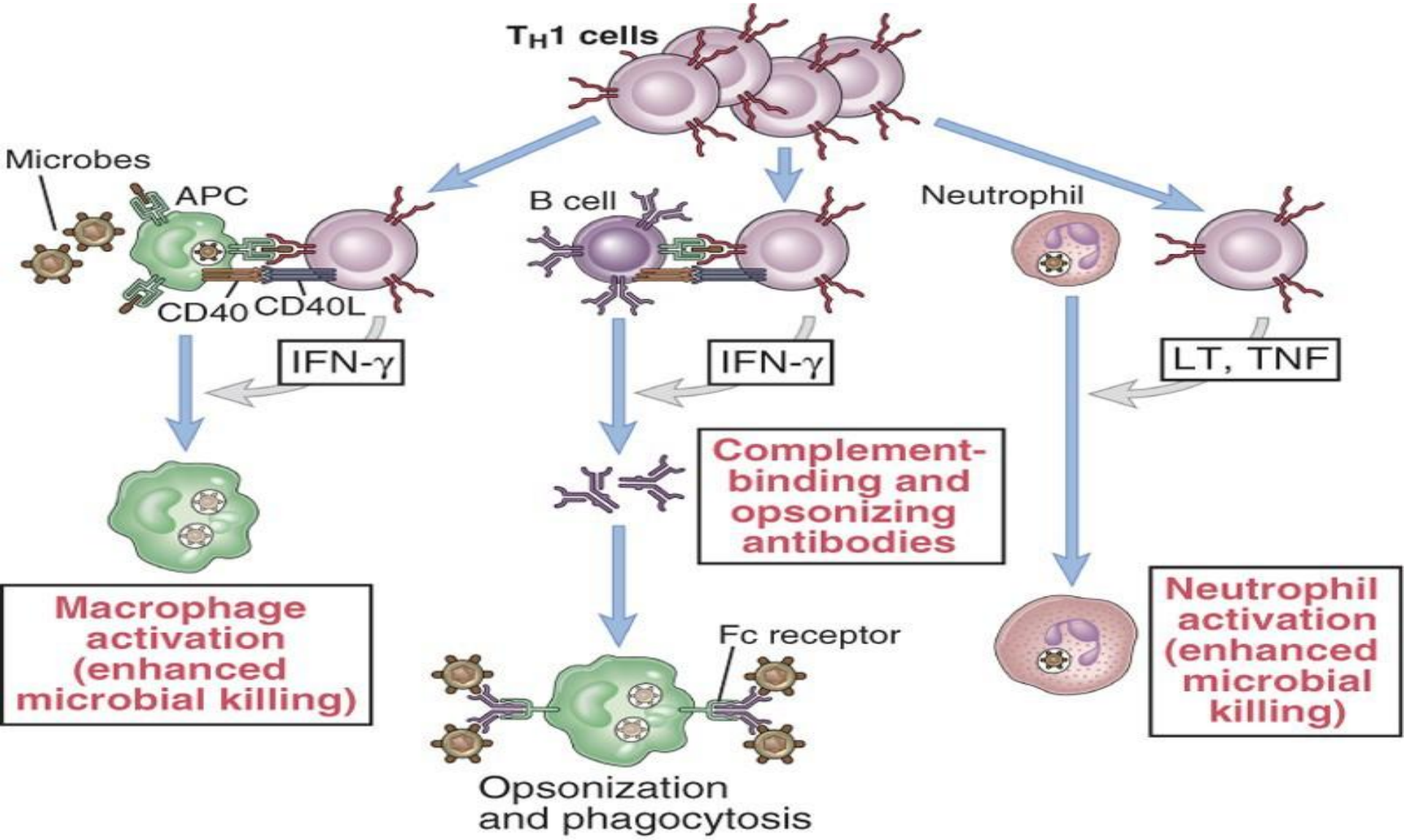
-Other $CD4^+$ cells produce various mixtures of cytokines – not readily classified

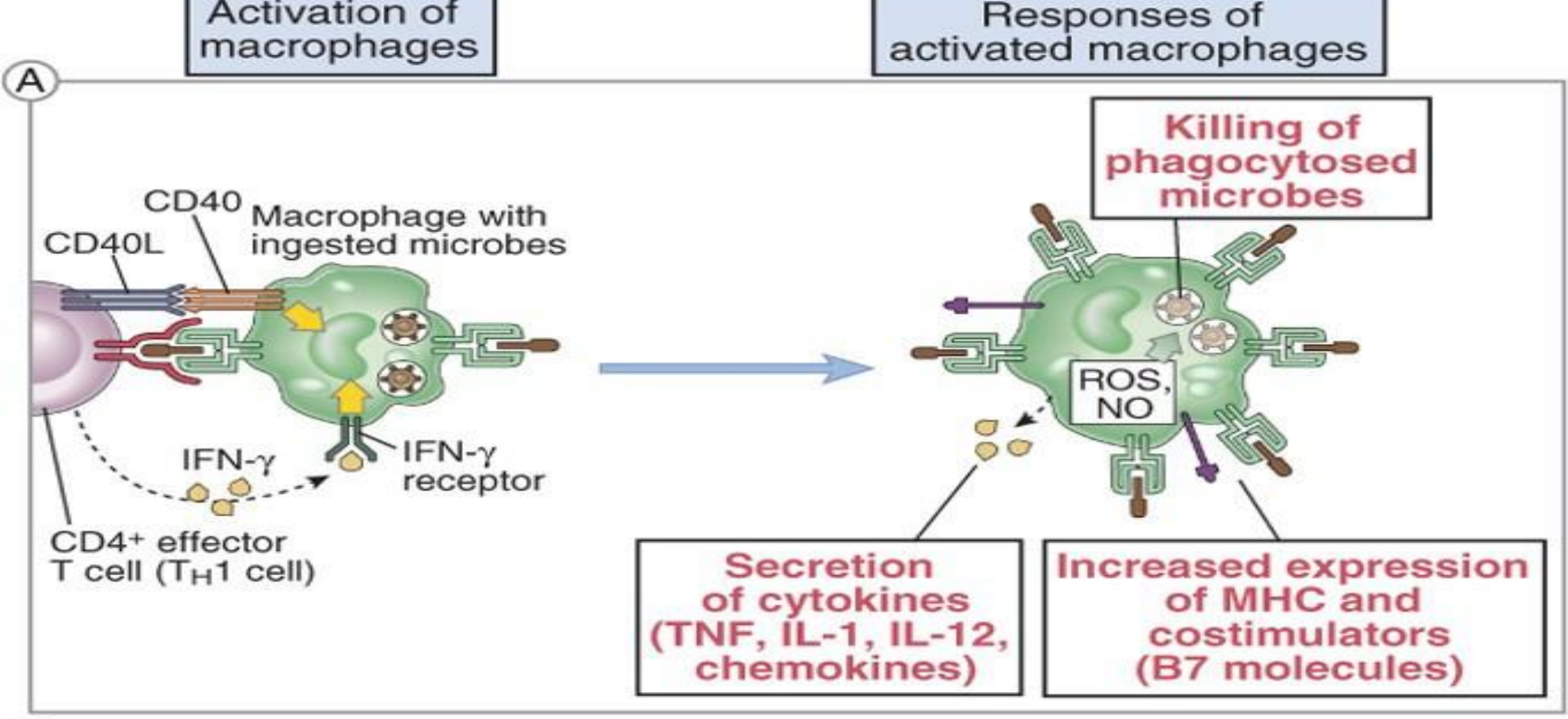

Th1
IFNγ
Cellular Immunity
Clearance of
Intracellular Pathogens
IL-12
STAT4/T-bet
Th2
IL-4
IL-5
IL-13
Humoral Immunity
Clearance of Certain
Extracellular Pathogens
Allergy
IL-4
STAT6/GATA3
Antigens
Thp
IL-6, IL-21, IL-23
TGFβ1 (mouse)
Th17
IL-17
IL-17F
IL-22
IL-21
Tissue Inflammation
Autoimmunity
Clearance of Certain
Extracellular Pathogens
STAT3
RORγt/RORα
Dendritic
Cell
IL-2
TGFβ1
ATRA
Treg
IL-10
TGFβ1
Tolerance/
Immune Suppression
STAT5/FOXP3

Development of Th1 and Th2 subset



Effector function of Th1 cells

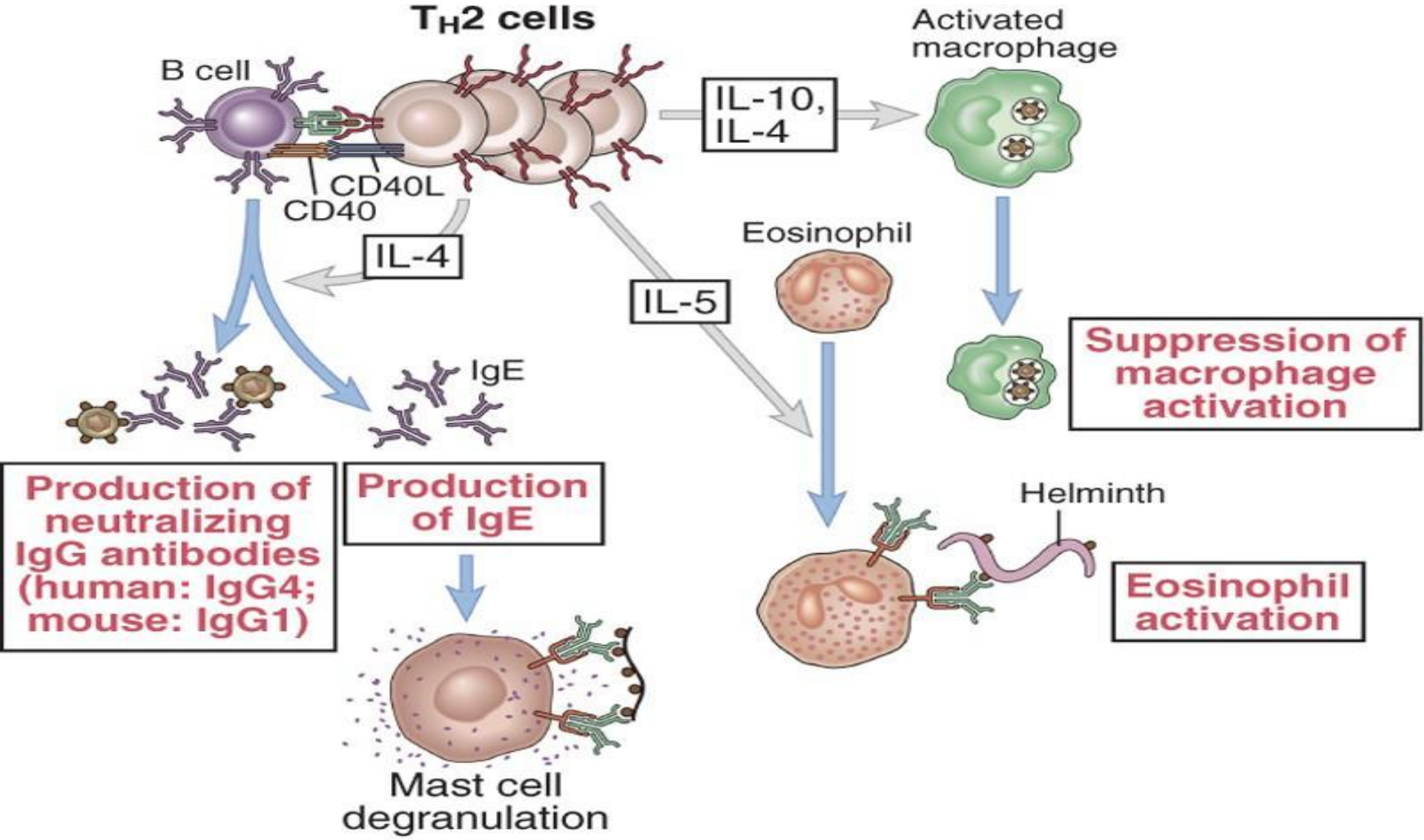




B

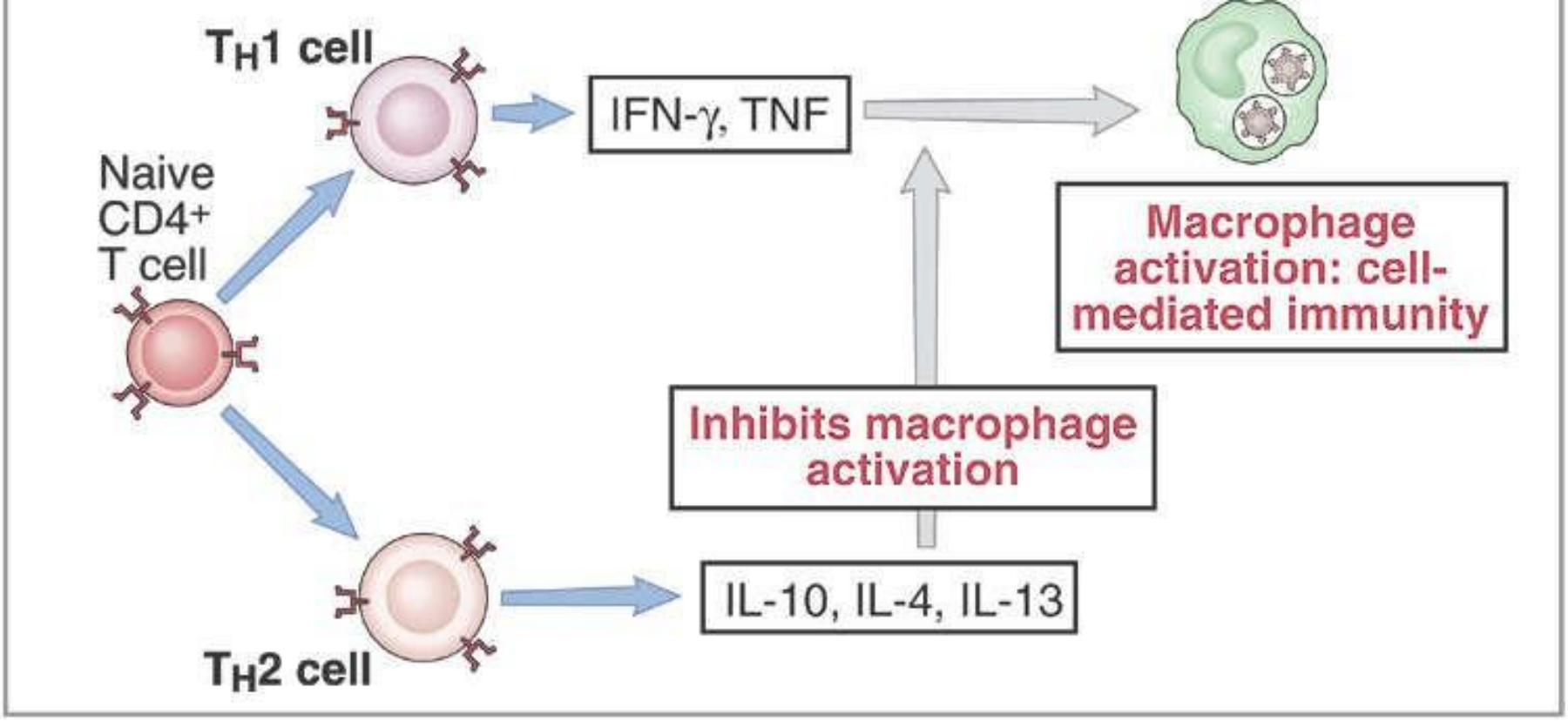
Macrophage response	Role in cell-mediated immunity
Production of reactive oxygen species, nitric oxide, increased lysosomal enzymes	Killing of microbes in phagolysosomes (effector function of macrophages)
Secretion of cytokines (TNF, IL-1, IL-12)	TNF, IL-1: leukocyte recruitment (inflammation) IL-12: T_H1 differentiation, IFN-γ production
Increased expression of B7 costimulators, MHC molecules	Increased T cell activation (amplification)

Effector function of Th2 cells



The balance between T_H1 and T_H2 cell activation determines the outcome of intracellular infections

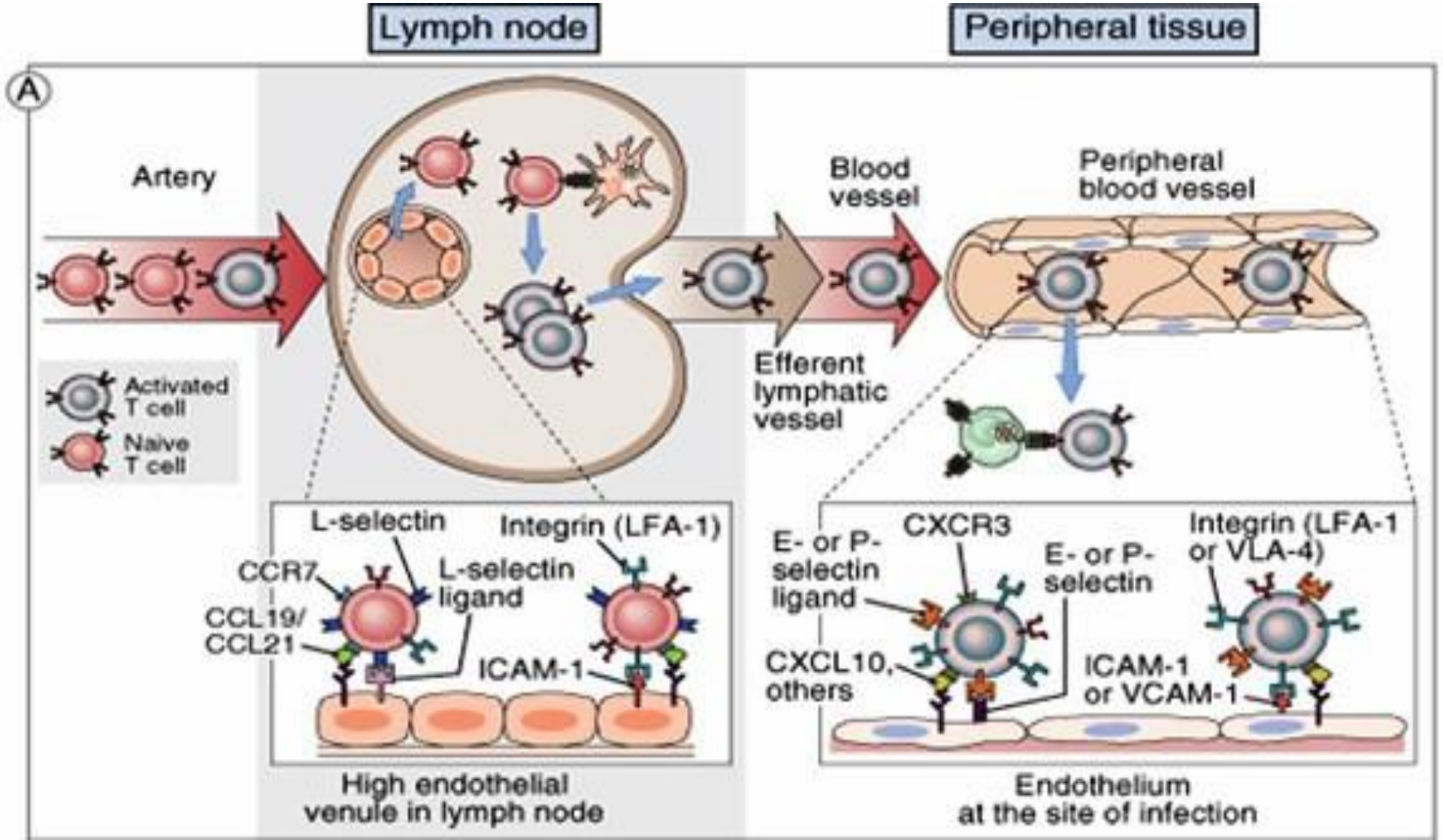
- **Naive $CD4^+$ T** lymphocytes may **differentiate into**
- **T_H1 cells**, which **activate phagocytes to kill ingested microbes**, and
- **T_H2 cells**, which **inhibit macrophage activation**
- **The balance between these two subsets may influence the outcome of infections**, as illustrated by ***Leishmania*** **)parasite) infection** in mice &**leprosy**)***Mycobacterium leprae***) in humans
- Both agents are **intracellular** → need **cell-mediated immunity**



Infection	Response	Outcome
Leishmania major	Most mouse strains: T_H1 ⇒ BALB/c mice: T_H2 ⇒	Recovery Disseminated infection
Mycobacterium leprae	Some patients: T_H1 ⇒ Some patients: Defective T_H1 or dominant T_H2 ⇒	Tuberculoid leprosy Lepromatous leprosy (high bacterial count)

Migration and retention of effector T cells at site of infection

Migration of naïve and effector T lymphocytes

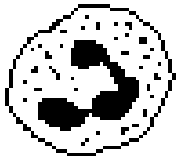


Migration of naive and effector T cells

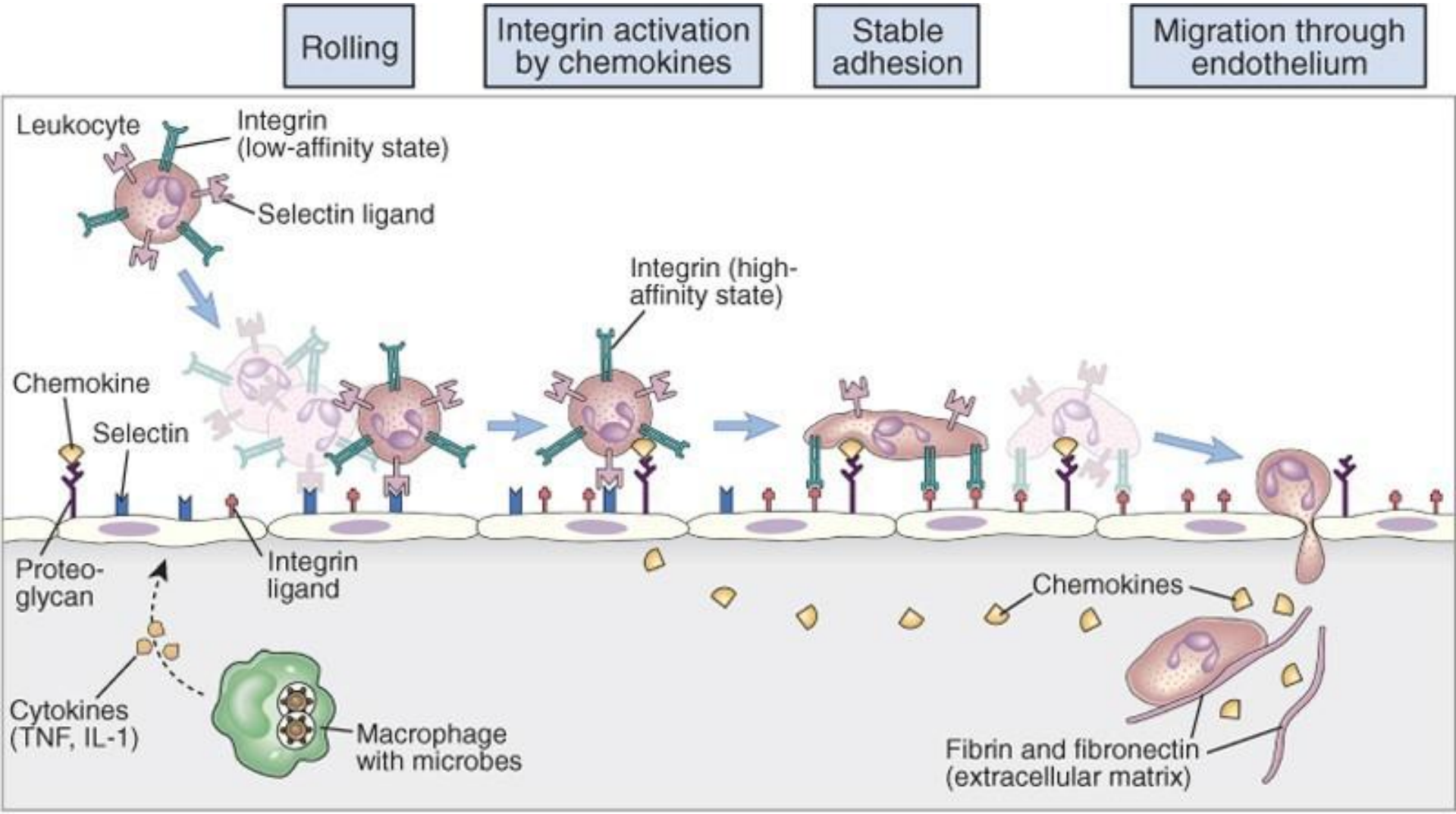
A. **Naive T lymphocytes home to lymph nodes** as a **result of L-selectin binding** to its ligand on high endothelial venules)**HEV**s), which are **present only in lymph nodes**

- **Activated T lymphocytes**, including effector cells, **home to sites of infection in peripheral tissues**, and this migration is facilitated by:
  - **E-selectin Ligand ←→ E-selectin**
  - **P-selectin Ligand ←→ P-selectin**
  - **Integrins:**
    - **LFA1 ←→ ICAM-1 (ligand(**
    - **VLA4 ←→ VCAM-1 (Ligand(**
- **Chemokines** that are produced in lymph nodes and sites of infection also **participate in the recruitment of T cells** to these sites

MARGINATION



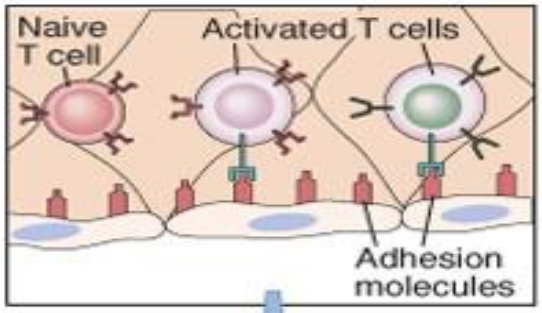
Recruitment of leukocyte at infection site



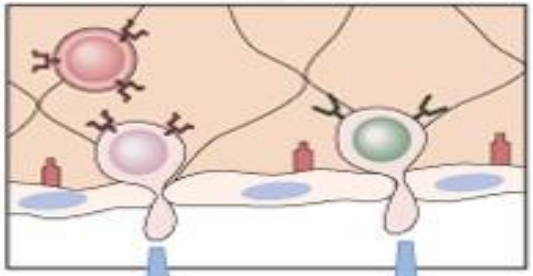
T cell homing receptors and their ligands

Ⓑ T cell molecules involved in homing	Endothelial cell molecules	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 (β2-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 (β2-integrin) or VLA-4 (β1 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

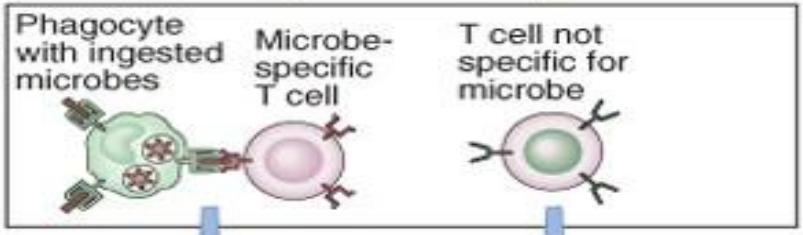
Increased expression of adhesion molecules on endothelium at site of infection ⇒ stable binding of activated T cells

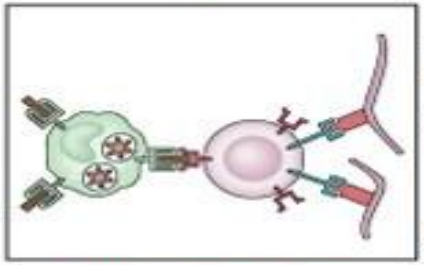


Effector T cells enter peripheral tissues

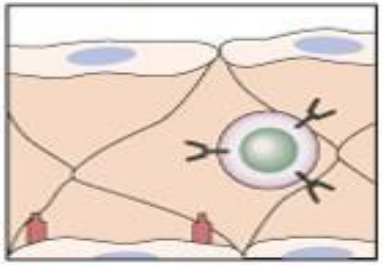


Antigen recognition by T cells specific for microbe





Activated antigen-specific T cells are retained at site of infection and perform effector functions



T cells that do not recognize antigen return to circulation

Development of Memory

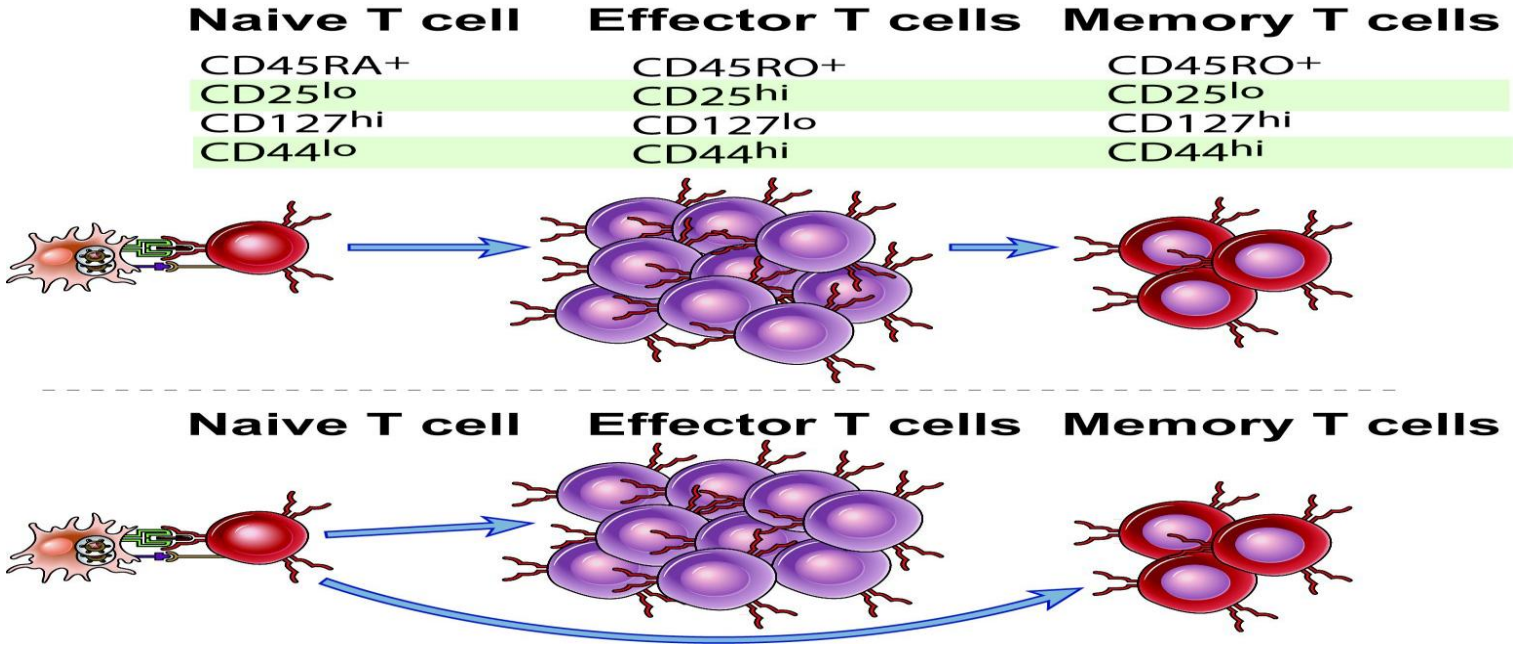
Fraction of Ag-activated T-cells differentiate into long-lived memory T-cells –even after infection is eradicated and innate immune reaction to infection pathogen is over

Subsets of memory –not known how it happens

- in mucosal barrier is effector memory =rapid effector functions
- in lymphoid tissue is central memory cells =populate lymphoid tissue and responsible for rapid clonal expansion after re-exposure

Memory T-cells do not produce cytokine or kill cells

- rapidly do so when encounter Ag again



Decline of the Immune Response

- Clonal expansion and differentiation occurs in peripheral lymphoid organs
- As infection is cleared and stimuli for lymph activation disappears and the response will decline
- Cells are deprived of survival factors –die by apoptosis; 2-1wks after infection cleared

–only thing that remains is the memory T-cells

There is need to down modulate T cell response. Otherwise, patient may die due to uncontrolled immune responses (immunopathology

Limiting and Terminating Immune Response

- Proteins homologous to CD28 are critical for limiting and terminating immune responses
- Inhibitory receptor **CTLA-4 is like CD28 recognizes B7** on APC and **PD-1** recognizes different but related ligands on many cell types
  - induced in activated T-cells
  - CTLA-4 involved **in inhibitory response to some tumors**
  - PD-1 inhibits **responses to some infection** and infection becomes chronic

CTLA-4 (Cytotoxic T-Lymphocyte Antigen 4)

CTLA-4 is found on the surface of T cells which is also known as CD152 (Cluster of differentiation 152).

It is a member of the immunoglobulin superfamily, which is expressed on the surface of T cells and transmits an inhibitory signal to T cells.

Function: CTLA-4 is a protein receptor that downregulates the immune response.

CTLA-4 is similar to the T-cell co-stimulatory protein.

The T cell attack can be turned on by stimulating the CD28 receptor on the T cell.

The T cell attack can be turned off by stimulating the CTLA-4 receptor, which acts as an "off" switch.

Mutation in CTLA-4 is associated with different autoimmune diseases.

T cell activation In Brief…

T cell responses are initiated by signals provided by clustering of TCR complexes through recognition of antigen on the surface of an APC and through signals provided by costimulators expressed on APCs.

The response of the T cell varies with the nature of the antigen, the APC that presents the antigen, and the stage of maturation and differentiation of the T cells.

The best defined costimulators for T cells are the B7 proteins, which are recognized by CD28 on T cells.

B7 molecules are expressed on professional APCs, and their expression is enhanced by microbes and by cytokines produced during innate immune reactions to microbes.

Cont…

The requirement for costimulation, especially for activation of naive T cells, ensures that T cell responses are induced in lymphoid organs, where professional APCs are concentrated, and against microbes and microbial products

T cell responses to antigen and costimulators include synthesis of cytokines , cellular proliferation, differentiation into effector and memory cells, and performance of effector functions.

Clustering of TCRs on antigen recognition triggers intracellular signaling pathways that result in the production of transcription factors, which activate a variety of genes in T cells.

Intracellular signaling may be divided into

membrane events,
cytoplasmic signaling pathways ,and
nuclear transcription of genes.

Cont…

Membrane events include the recruitment and activation of protein tyrosine kinases into the TCR complex ;the phosphorylation of TCR complex constituents)e.g., the ζ chains) ; and the recruitment of protein tyrosine kinases, especially ZAP- ,70 and adapter proteins.

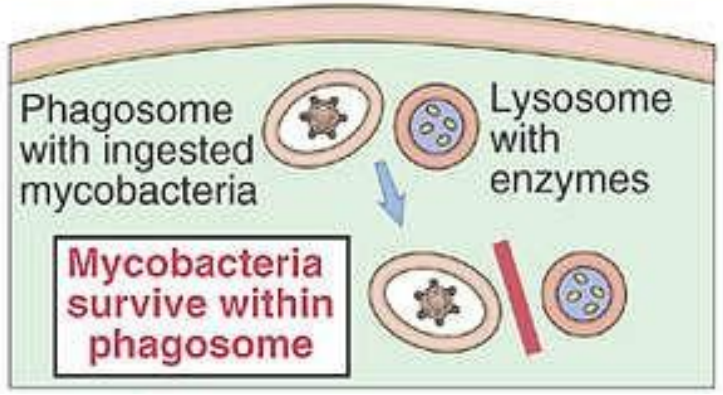
Cytoplasmic signaling pathways lead to the activation of effector enzymes, such as the kinases ERK, JNK, and PKC, and the phosphatase calcineurin.

These enzymes contribute to the activation of **transcription factors** such as NF-AT, AP-1, and NF-κb, which function to enhance gene expression in antigen-stimulated T cells.

Some peptides in which the TCR contact residues are altered may induce partial T cell responses or inhibit T cell activation by poorly understood biochemical mechanisms

Evasion of cell-mediated immunity by microbes

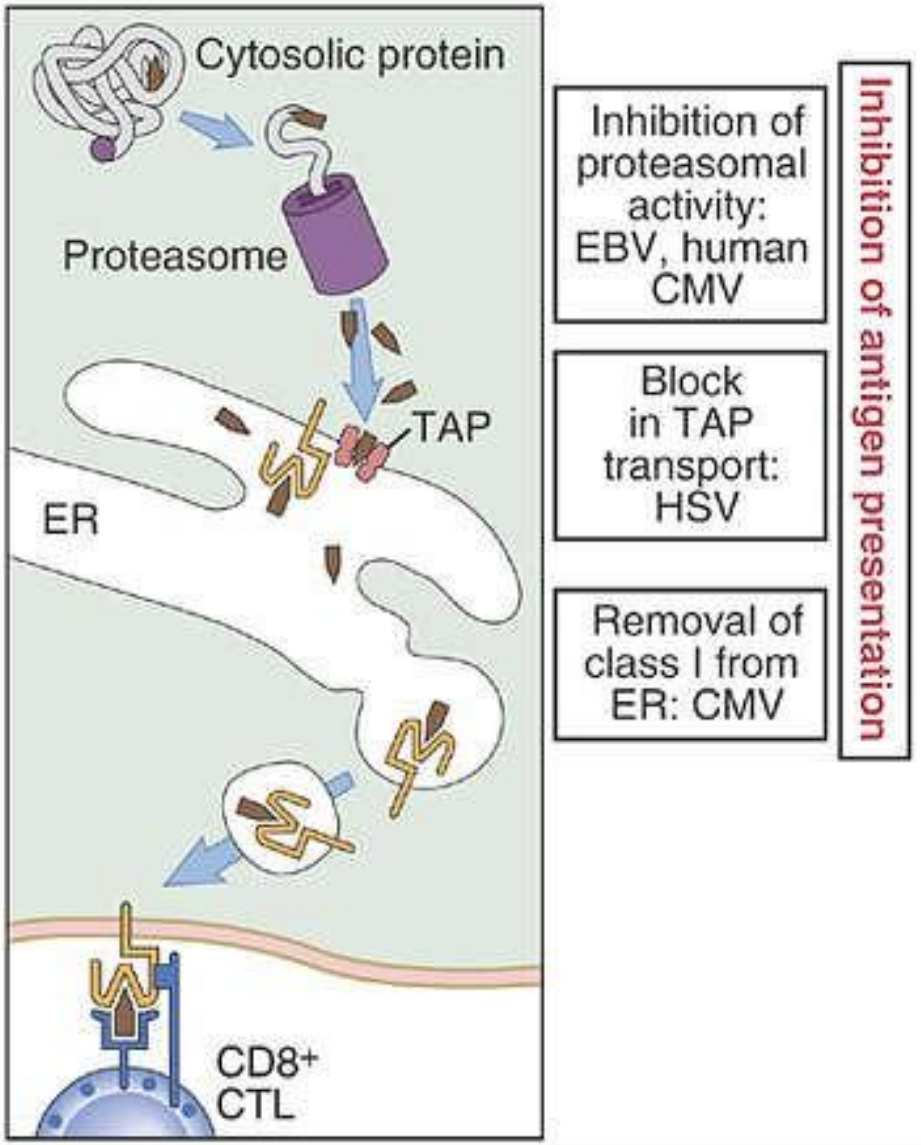
- Different bacteria &viruses resist the effector mechanisms of cell-mediated immunity by different mechanisms:
  - Inhibition of phagosome-lysosome fusion)*Mycobacterium tuberculosis*(
  - Inhibition of Ag presentation
    - HSV peptide **interference with TAP transporter**
    - **Inhibition of proteasomal activity** (CMV, EBV(
    - **Removal of MHC I from ER** (CMV(

Microbe	Mechanism	
Mycobacteria	Inhibition of phagolysosome fusion	

Evasion of cell-mediated immunity by microbes

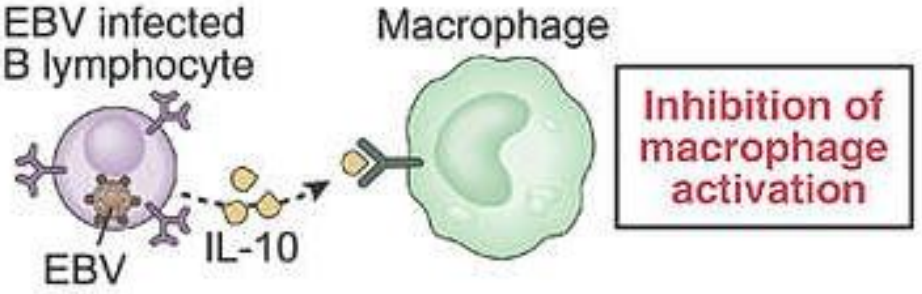
Microbe	Mechanism
Herpes simplex virus (HSV)	Inhibition of antigen presentation: HSV peptide interferes with TAP transporter
Cytomegalovirus (CMV)	Inhibition of antigen presentation: inhibition of proteasomal activity; removal of class I MHC molecules from endoplasmic reticulum (ER)
Epstein-Barr virus (EBV)	Inhibition of antigen presentation: inhibition of proteasomal activity

Fig 6-12



Evasion of cell-mediated immunity by microbes

- **IL-10 production** → inhibition of MΦ activation (EBV)
- Inhibition of effector cell activation by **soluble cytokine receptor** (Pox virus)

Microbe	Mechanism	
Epstein-Barr virus (EBV)	Production of IL-10, inhibition of macrophage activation	
Pox virus	Inhibition of effector cell activation: production of soluble cytokine receptors