Effector Mechanisms of Cellular Immunity

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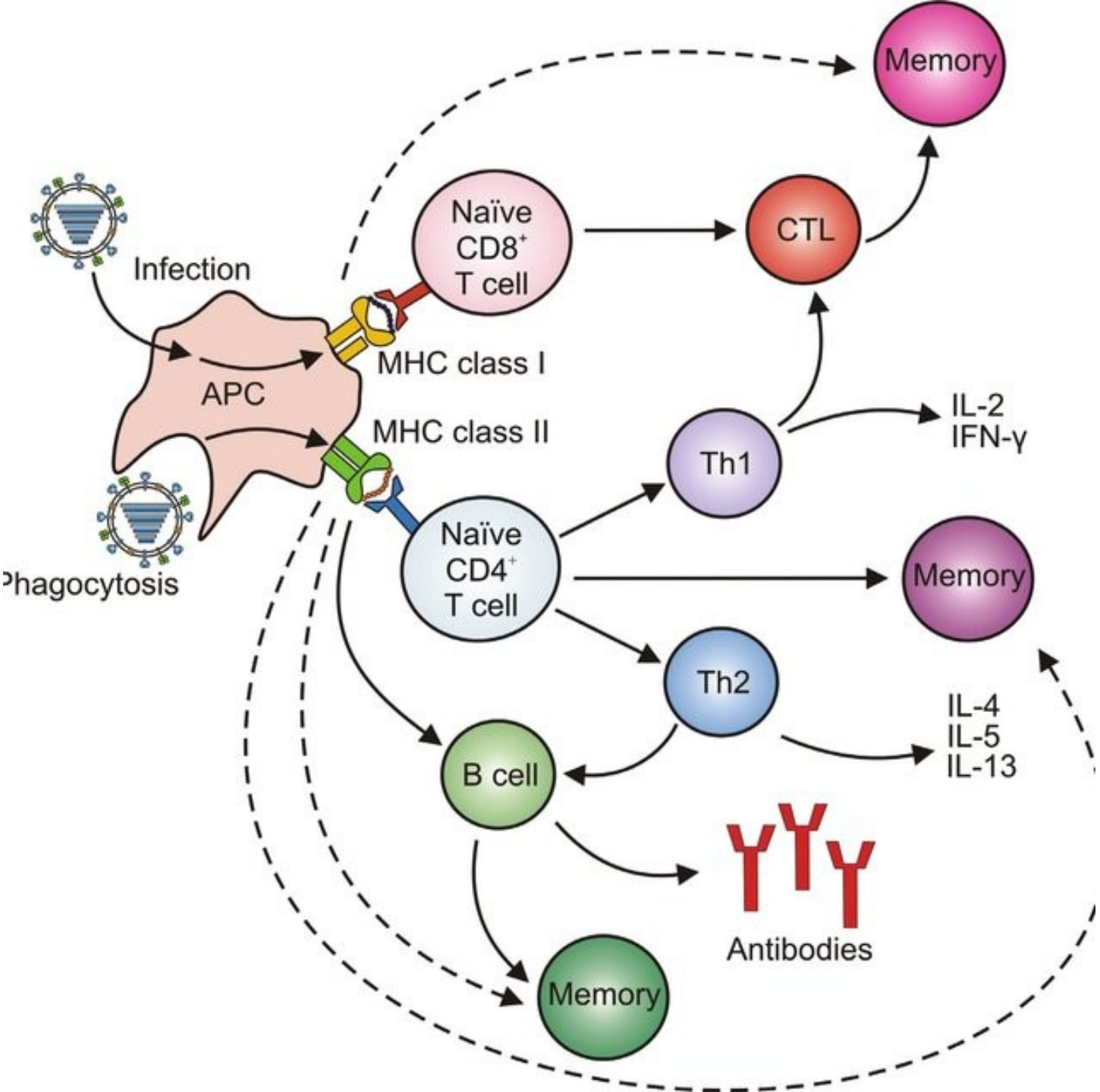
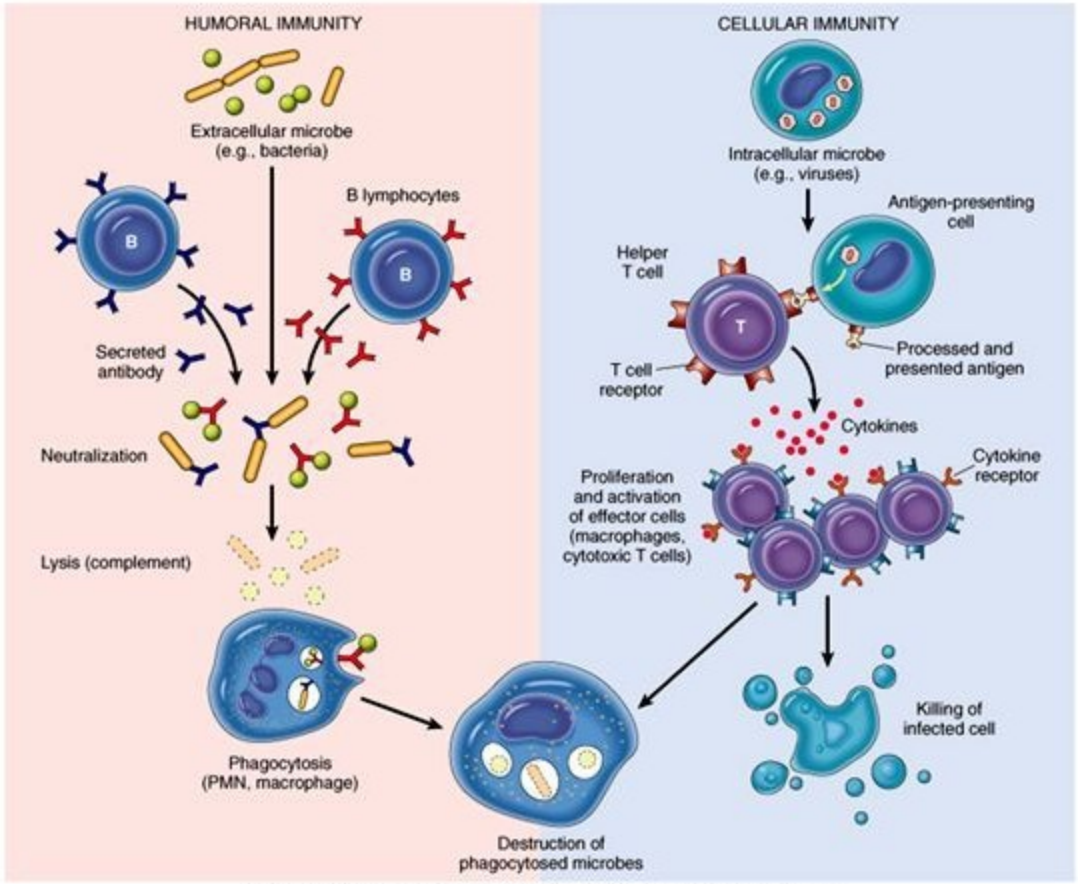
Cellular Immune System

- Cellular immune system plays a role in the fight against infections caused by **intracellular microbes**.
- It is a branch of the acquired immune response.
- **It is carried out by means of T lymphocytes.**


Memory
Naïve
CD8+
T cell
CTL
Infection
MHC class I
APC
MHC class II
IL-2
IFN-γ
Th1
Naïve
CD4+
T cell
Memory
Phagocytosis
Th2
IL-4
IL-5
IL-13
B cell
Antibodies
Memory

Humoral and Cell-mediated Immunity



Humoral Immunity

Involves B-cell production of antibodies that bind antigens resulting in either:

1. Neutralization
2. lysis (by the complement system), or
3. phagocytosis and destruction

Cell-mediated Immunity

Involves T-cell recognition of abnormal antigens on the surface of host cells (indicating viral infection or tumorigenic change) and the killing of infected cells.

Intracellular microbes can infect in 2 ways

- First, microbes are taken into the cell by **phagocytosis.**
- However, some of these microbes have developed resistance to the killer (microbicidal) activities of phagocytes.
- Most of the pathogenic intracellular bacteria and protozoa are able to survive and even reproduce in the vesicles of phagocytes.

Intracellular microbes can infect in 2 ways

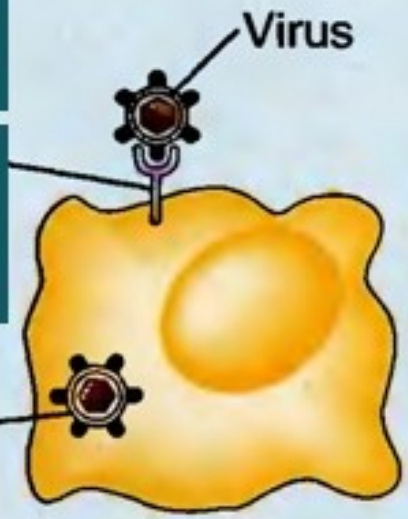
- **Some of the phagocyted microbes enter the cytoplasm of the infected cell where they can reproduce using the nutrients of the infected cell.**
- Cytoplasmic microbes are protected from microbicidal mechanisms.
- Because these mechanisms are limited by vesicular compartments.

Intracellular microbes can infect in 2 ways

- **Second, viruses can bind to receptors in many cells.**

- Viruses that bind to the receptor can multiply by causing infection in the cytoplasm of these cells.
- These cells do not have intrinsic mechanisms to destroy viruses.

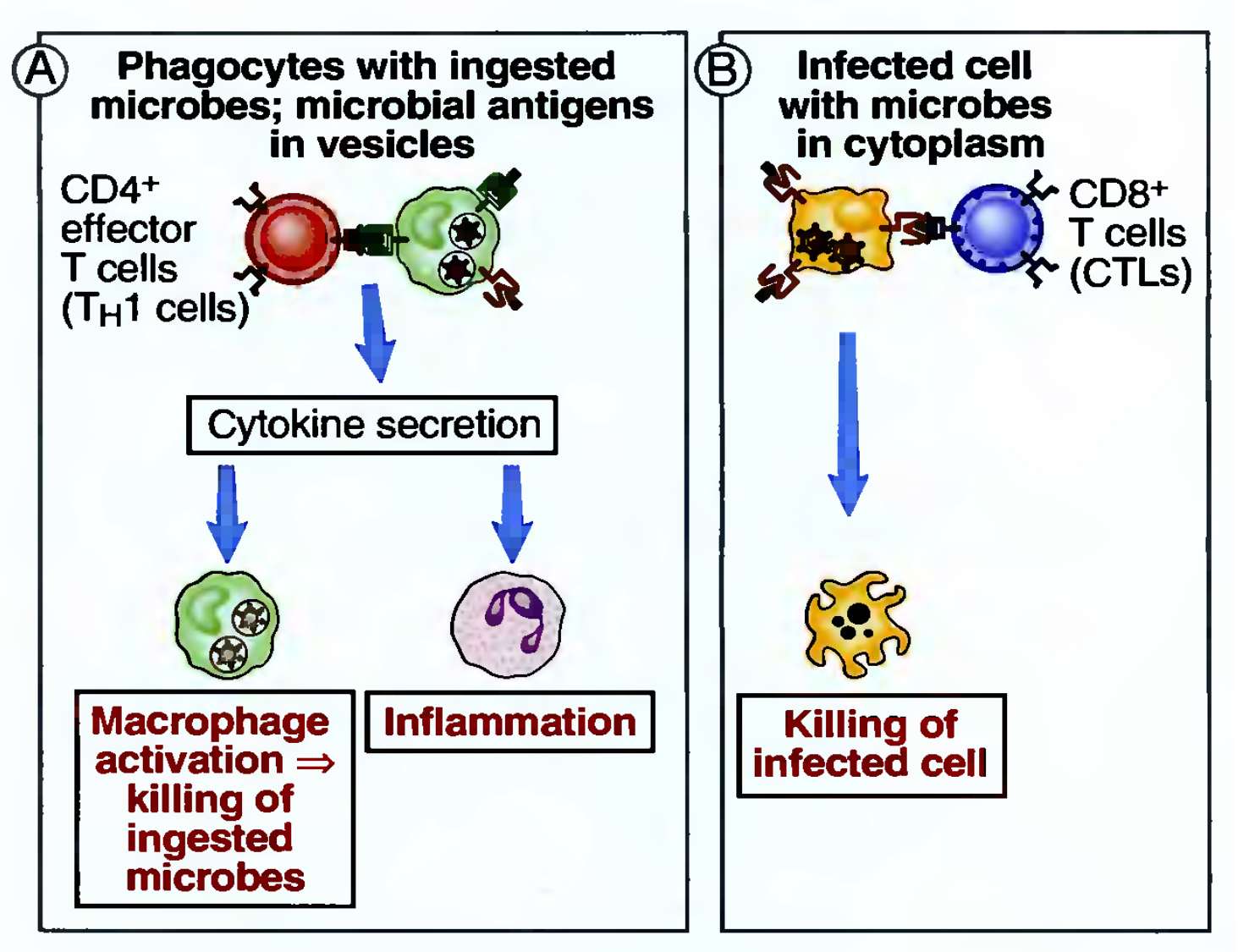
Hücre içi mikroplar	Örnekler
A Fagosit Fagosite olan mikroplar fagolizozomlar içinde yaşar Fagolizozomlardan kaçan mikroplar sitoplazmaya geçer	Intracellular bacteria: Mycobacteria *Listeria monocytogenes* *Legionella pneumophila* Fungi: *Cryptococcus neoformans* Protozoa: *Leishmania* *Trypanosoma cruzi*
B Non-fagositik hücre (örn epitelyal) Virus Virus için hücresel reseptör Non-fagositik hücreleri enfekte eden mikroplar	Viruses: All Rickettsiae: All Protozoa: *Plasmodium falciparum* *Cryptosporidium parvum*

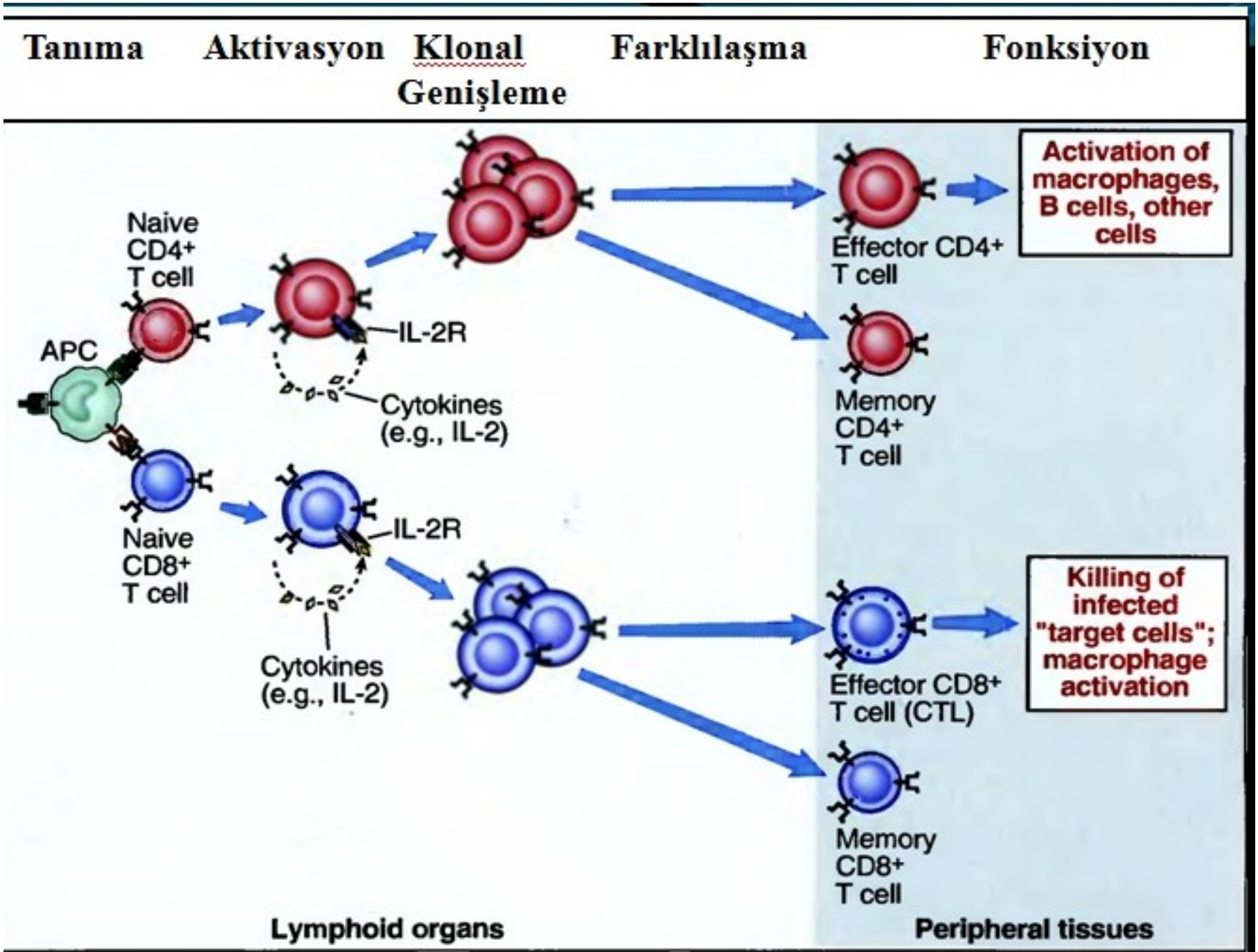


Cellular Immunity

- There are two types of cellular immunity reactions to eliminate intracellular microorganisms.
- **CD4 + T cells** enable the destruction of microbes in the vesicles of these phagocytes by activating phagocytes.
- **CD8 + T cells** eliminate any infection by killing any cell that contains microbes or microbial proteins in their cytoplasm.

Cellular immunity against intracellular microbes




Tanıma
Aktivasyon
Klonal Genişleme
Farklılaşma
Fonksiyon
Naive CD4+ T cell
APC
IL-2R
Cytokines (e.g., IL-2)
Effector CD4+ T cell
Activation of macrophages, B cells, other cells
Memory CD4+ T cell
Naive CD8+ T cell
IL-2R
Cytokines (e.g., IL-2)
Effector CD8+ T cell (CTL)
Killing of infected "target cells"; macrophage activation
Memory CD8+ T cell
Lymphoid organs
Peripheral tissues

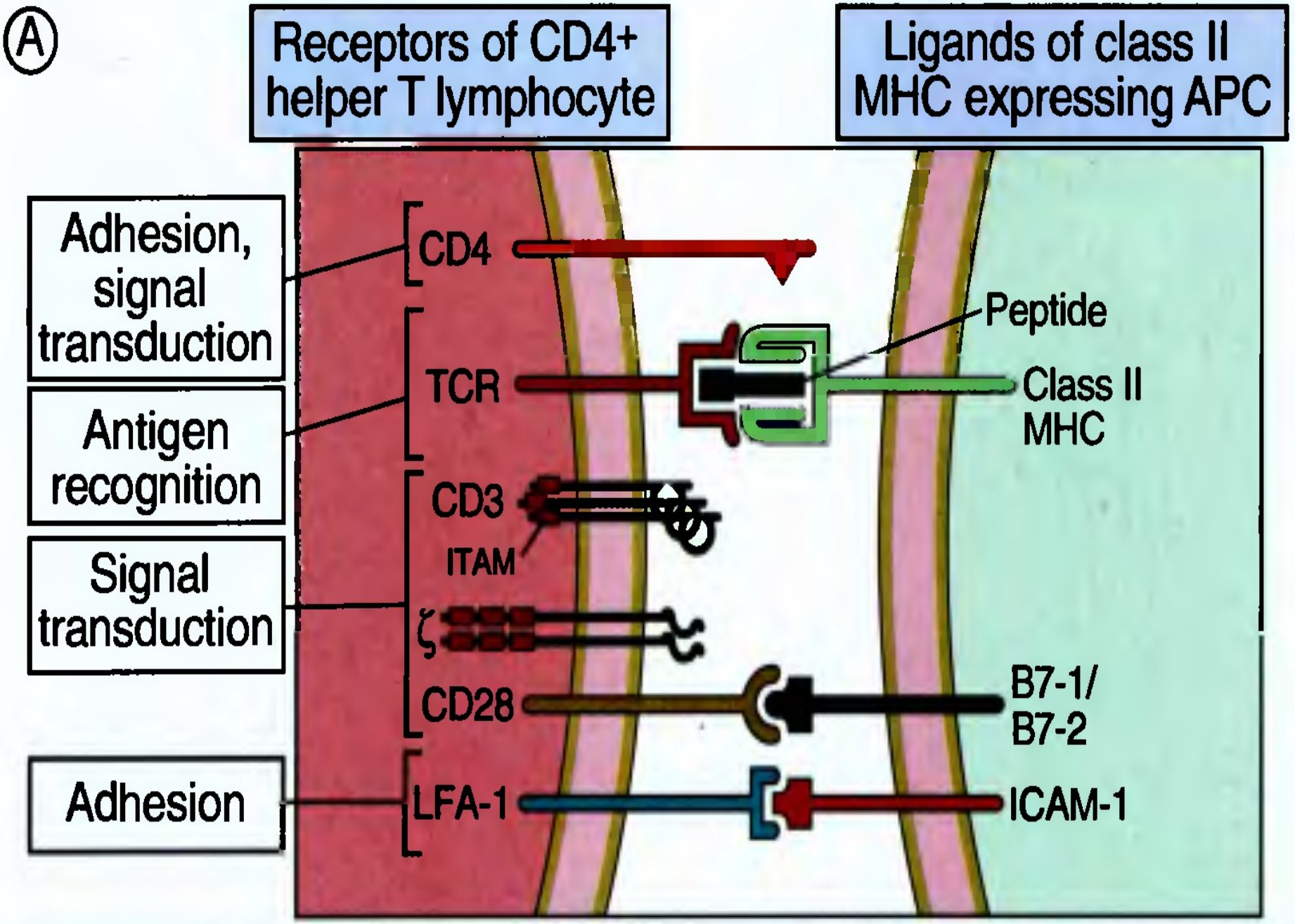
- Effector T cells, which are responsible for destroying microbes, are produced in the lymph nodes and spleen from undifferentiated T cells stimulated by microbial antigens.
- The differentiated T cells then migrate to the site of infection.

- Phagocytes in the infection area, which swallow microorganisms and take them into vesicles inside the cell, bind the peptide fragments of microbial proteins to class II MHC molecules and present them to the T cells of the CD4 + subgroup.

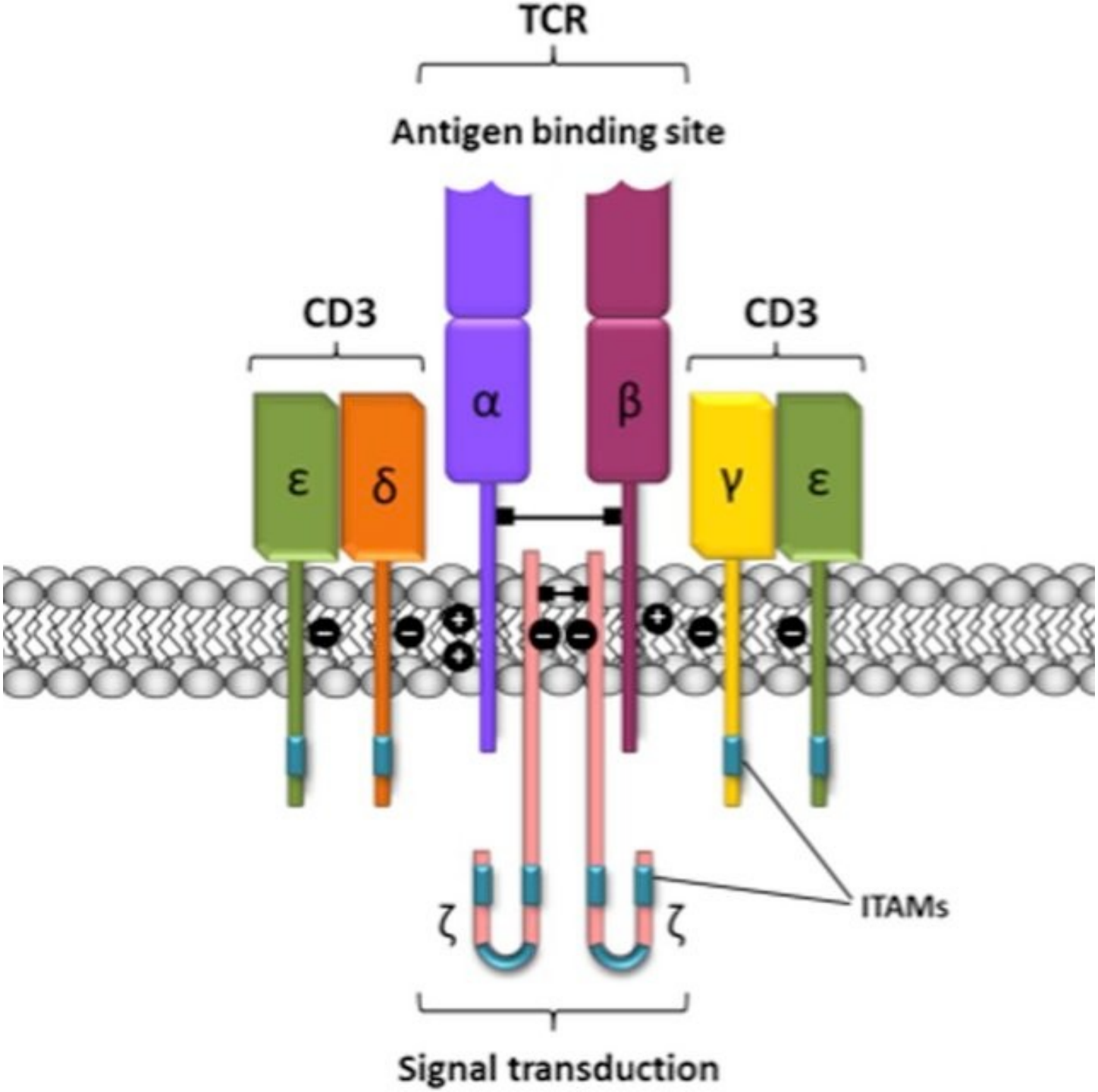
- Peptide antigens of microbes living in the cytoplasm of infected cells are presented to CD8 + T cells by class I MHC molecules.
- Recognition of antigens by effector T cells activates these cells in the direction of their main task of removing infectious pathogens.

Accessory Molecules

- Accessory molecules are invariant molecules for all T cells.
- Their functions are;
  - **recognition**
  - **signal transduction**
  - **adhesion**
- Different accessory molecules bind to different ligands
- **Each binding plays a separate and complementary role in T cell activation.**


A
Receptors of CD4+ helper T lymphocyte
Ligands of class II MHC expressing APC
Adhesion, signal transduction
Antigen recognition
Signal transduction
Adhesion
CD4
TCR
CD3
ITAM
ζ
CD28
LFA-1
Peptide
Class II MHC
B7-1/ B7-2
ICAM-1

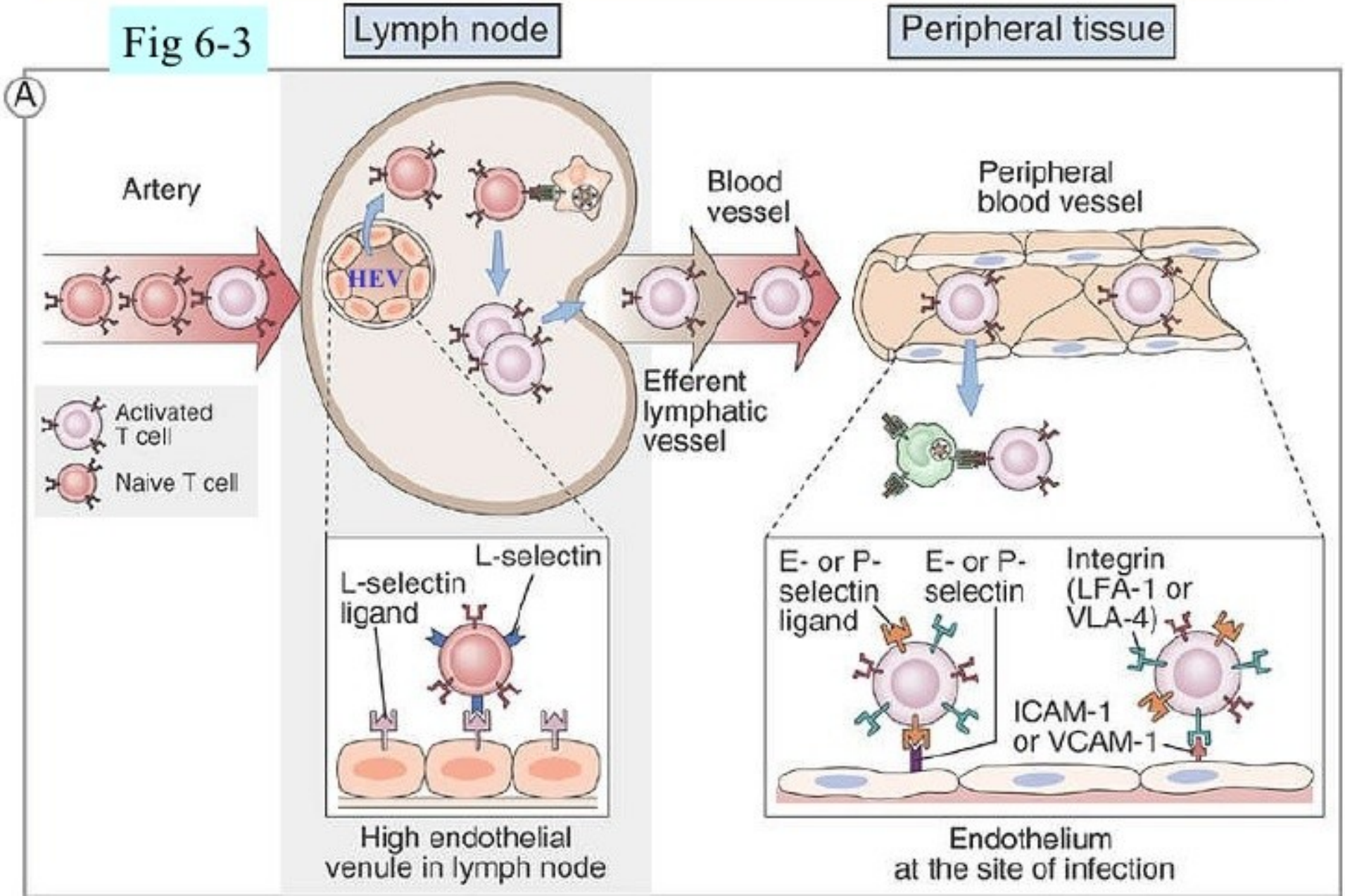
T hücre aksesuar molekülü	Fonksiyon	Ligand	
		Name	EKSPRESYON
CD3	THR kompleksi ile sinyal iletimi	None	
ζ	THR kompleksi ile sinyal iletimi	None	
CD4	Adezyon ve sinyal iletimi	Class II MHC	Antijen Sunan Hücreler
CD8	Adezyon ve sinyal iletimi	Class I MHC	Antijen Sunan Hücreler, CTL hedef hücreler
CD28	sinyal iletimi (eş uyarım)	B7-1/B7-2	Antijen Sunan Hücreler
CTLA-4	sinyal iletimi (negatif düzenleme)	B7-1/B7-2	Antijen Sunan Hücreler
LFA-1	Adezyon	ICAM-1	Antijen Sunan Hücreler Endotel
VLA-4	Adezyon	VCAM-1	Antijen Sunan Hücreler

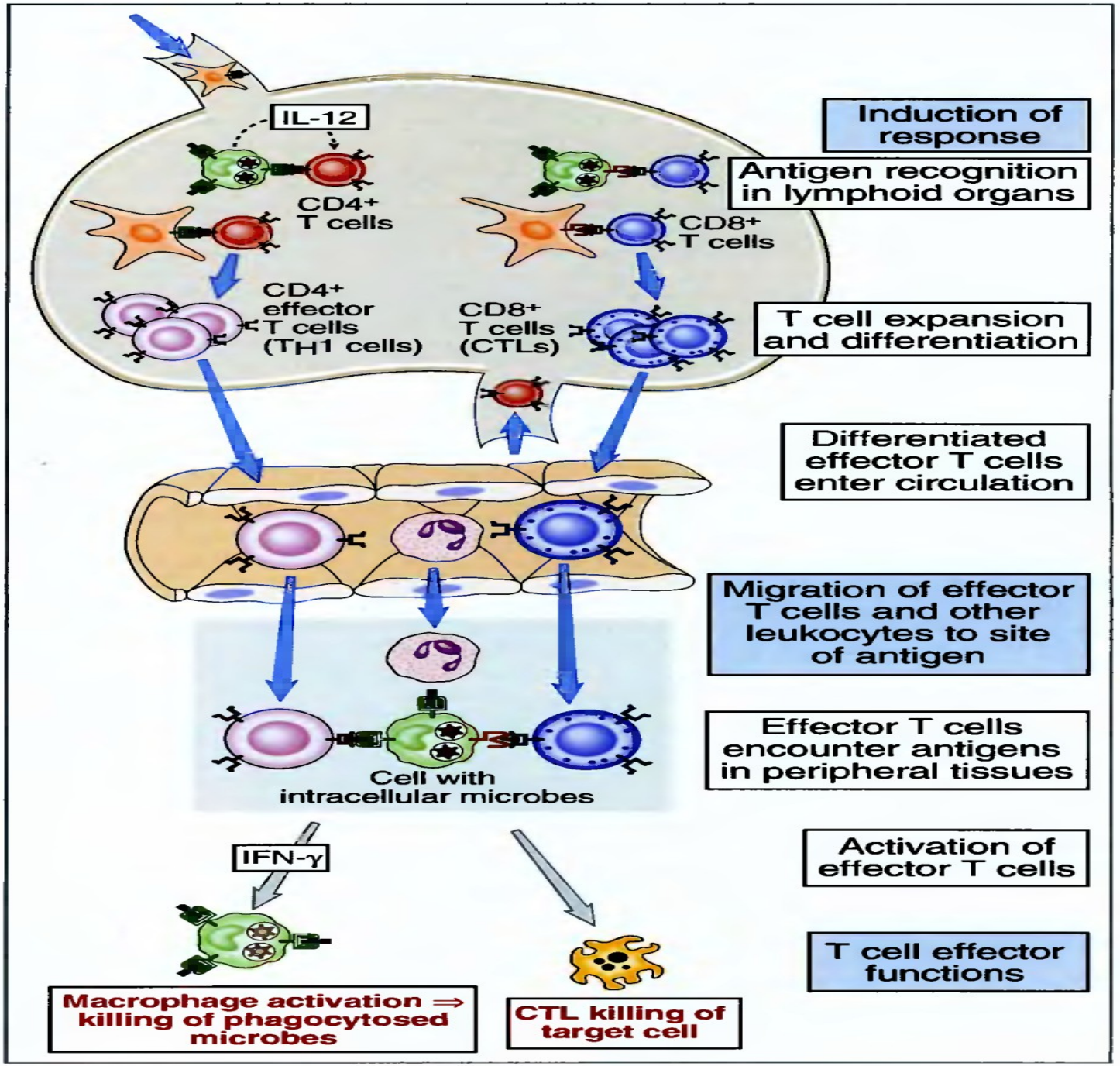

TCR
Antigen binding site
CD3
CD3
α
β
ε
δ
γ
ε
ζ
ζ
ITAMs
Signal transduction

Cellular Immunity

- In cellular immunity, T cells recognize protein antigens in two stages; While **naive T cells recognize antigens in lymphoid tissues and respond to proliferation and differentiation to effector cells**, **effector cells recognize the same antigens anywhere in the body and continue their function by destroying these microorganisms.**

Migration of naive and effector T cells

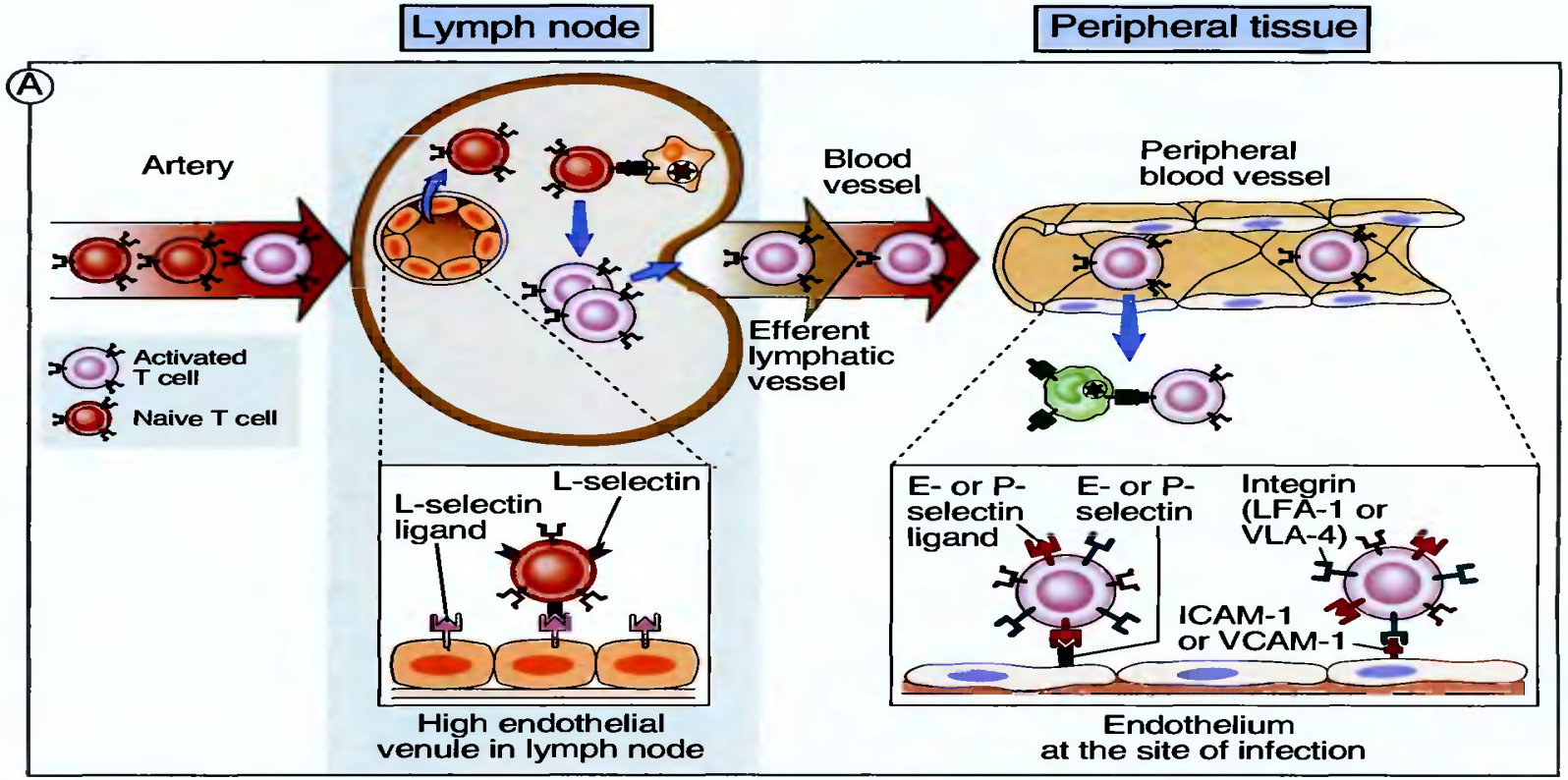



Induction of response
Antigen recognition in lymphoid organs
IL-12
CD4+ T cells
CD8+ T cells
CD4+ effector T cells (TH1 cells)
CD8+ T cells (CTLs)
T cell expansion and differentiation
Differentiated effector T cells enter circulation
Migration of effector T cells and other leukocytes to site of antigen
Effector T cells encounter antigens in peripheral tissues
Cell with intracellular microbes
Activation of effector T cells
IFN-γ
T cell effector functions
Macrophage activation ⇒ killing of phagocytosed microbes
CTL killing of target cell

Migration of Effector T Lymphocytes to the Infection Site

- Effector T cells migrate to the infection site; because **these lymphocytes express a large number of adhesion molecules that bind to the ligands in endothelium exposed to microbes.**

- **Another reason is chemoattractant cytokines produced at 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 in lymph node
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

Migration of Effector T lymphocytes

- Once activated, T cells can leave the lymph nodes.
- **T cell activation also leads to an increase in the expression of adhesion molecules that bind to molecules** in the endothelium of peripheral tissues stimulated by microbes or cytokines.

Migration of Effector T lymphocytes

The most significant T cell adhesion molecules are;

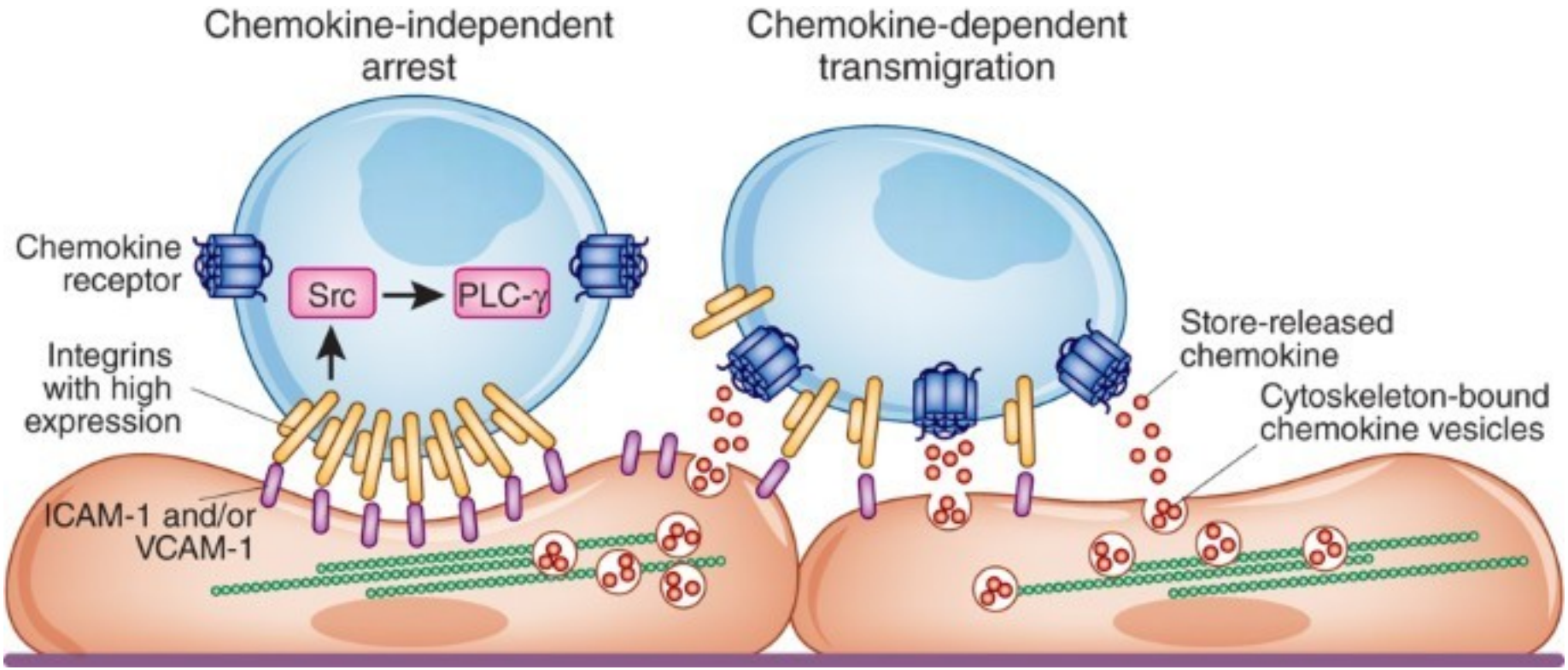
- E and P selectin
- LFA-1 (leukocyte function- associated antigen)
- VLA-4 (very late activation molecule)

Migration of Effector T lymphocytes

- At the same time, as one of the innate immune mechanisms for infection, cytokine secretion is observed from phagocytes that respond to the pathogen at the site of infection.
- **tumor necrosis factor (TNF)**
- **interleukin-1 (IL-1)**

Migration of Effector T lymphocytes

- TNF and IL-1 stimulate
- **E-selectin**
- **P-selectin**
- **ICAM-1** (intracellular adhesion molecule-1, LFA ligand)
- **VCAM-1** (vascular cell adhesion molecule-1, VLA-4 ligand) expression


Chemokine-independent arrest
Chemokine-dependent transmigration
Chemokine receptor
Src
PLC-γ
Integrins with high expression
ICAM-1 and/or VCAM-1
Store-released chemokine
Cytoskeleton-bound chemokine vesicles

- During activation, T cells not only increase the expression of adhesion molecules that help them adhere to the walls of the vessels at the site of infection, but also **reduce the expression of the L-selectin molecule, which regulates the migration of undifferentiated T cells to lymph nodes.**
- **Therefore, activated T cells tend to stay outside of normal lymph nodes.** This is because undifferentiated T cells need to enter the lymph node to localize microbes and protein antigens and initiate the immune response, **but once activated it is not needed.**

Migration of Effector T lymphocytes

- As effector cells reach the endothelium, macrophages and endothelial cells produce another cytokine called **chemokine.**
- **The main task of chemokines is to attract leukocytes to the area and stimulate their motility.** Chemokines are present in endothelial cells depending on cell surface proteoglycans and thus create high regional concentrations in infection area.

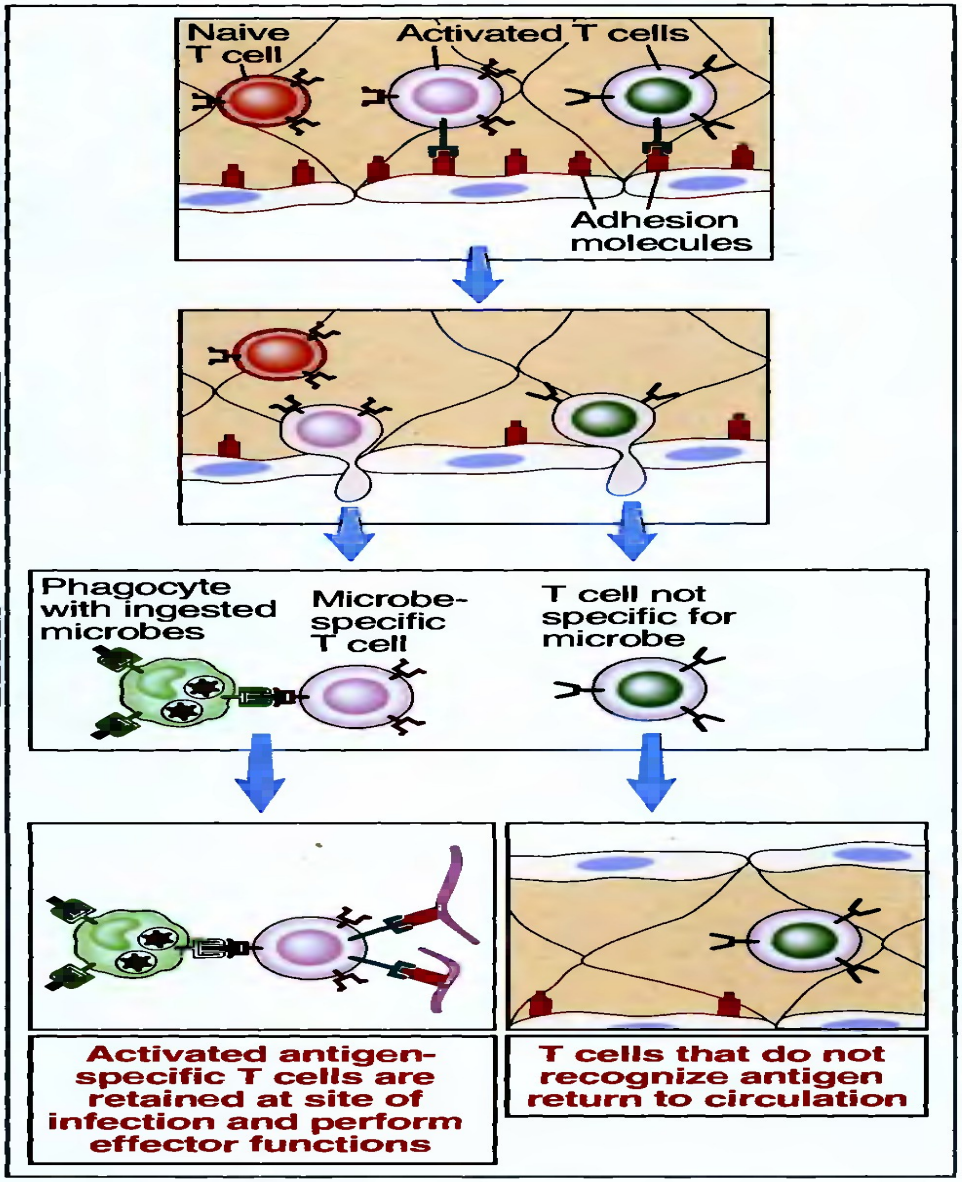
- Endothelial cell-associated **chemokines** act on loosely attached T cells, **increasing the affinity of integrins to endothelial ligands.**
- Chemokines stimulate **the motility of tightly bound T cells, and the resulting concentration difference causes the T cells to pass from the vessel walls to the infection site.**

Migration of Effector T lymphocytes

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



$CD4^+$ T ymphocyte Effector Functions

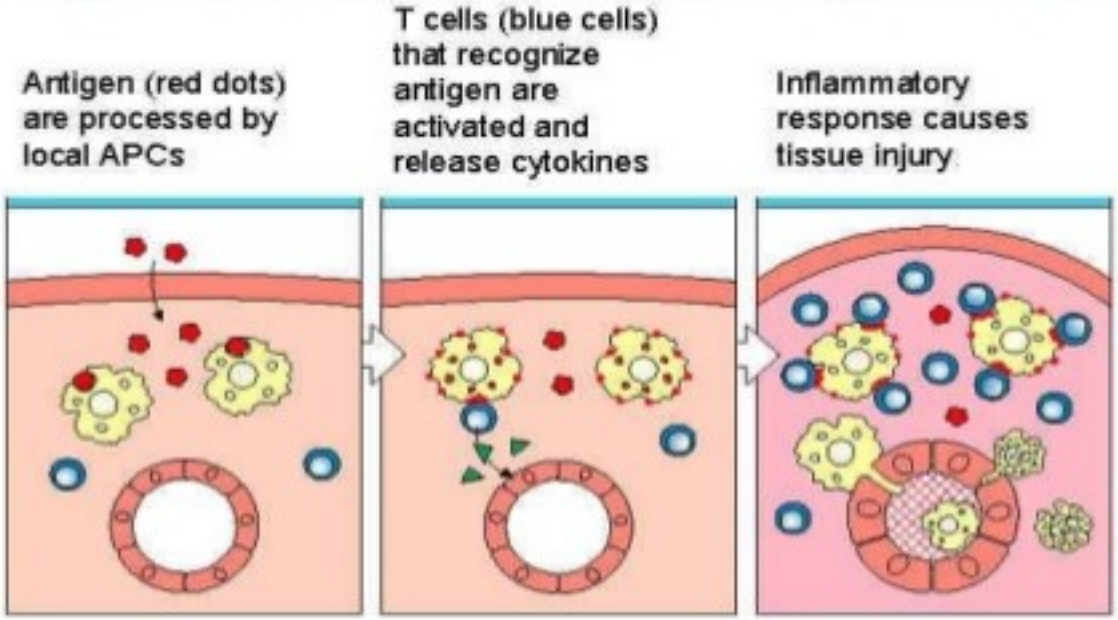
- Cellular immunity is a type of immunity that occurs against intracellular bacterial infections that can be transported through T lymphocytes.
- In cellular immunity, **TH1 CD4 + T lymphocytes activate macrophages** containing phagocyted microbes, thereby **increasing the microbicidal activity of phagocytes and the rate at which ingested microbes are killed.**

Delayed type Hypersensitivity (DTH)

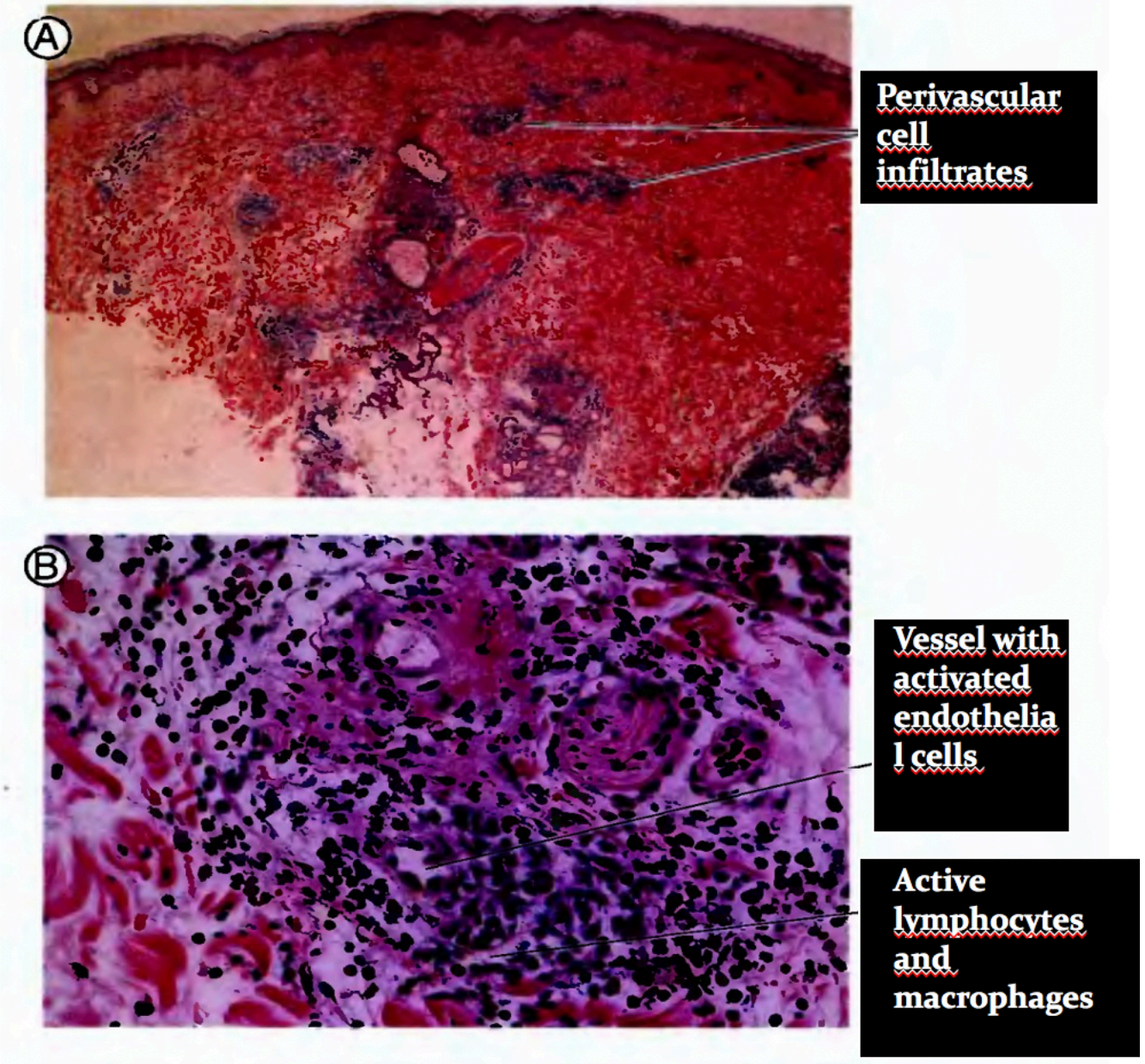
Delayed type hypersensitivity occurs **when a protein belonging to a microbe is injected into the skin of an individual who has previously encountered or immunized against this microbe**

introduction

Type IV hypersensitivity – delayed-type or contact



Antigen is presented by APCs to antigen-specific memory T cells that become activated and produce chemicals that cause inflammatory cells to move into the area, leading to tissue injury. Inflammation by 2-6 hours; peaks by 24-48 hours.


A
Perivascular cell infiltrates
B
Vessel with activated endothelia l cells
Active lymphocytes and macrophages

DTH reactions

1-infiltrations of T cells and monocytes

2- Edema due to increased vascular permeability as a result of the effects of cytokines released by CD4 + T cells.

3-Damage caused by products released into the environment as a result of fibrin storage and macrophage activation

- DTH reactions are performed to determine whether the individual has encountered the same antigen before. Ex. PPD test.

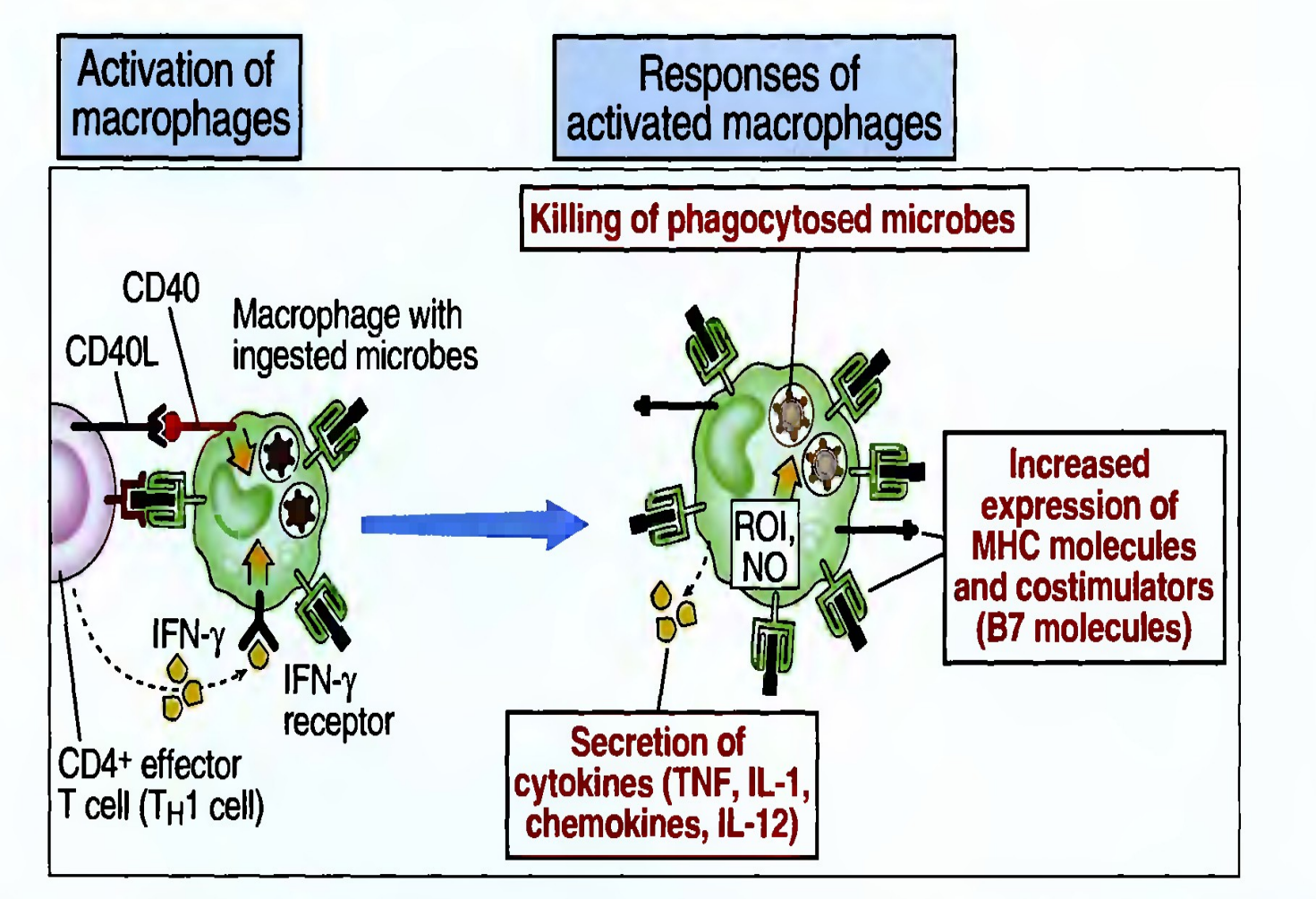
T-cell Mediated Macrophage Activation

- Effector T lymphocytes of the TH1 subgroup, which recognize macrophage-related antigens, activate macrophages by secreting macrophage activating cytokine (IFN- γ) via **CD40 ligand - CD40 interaction**

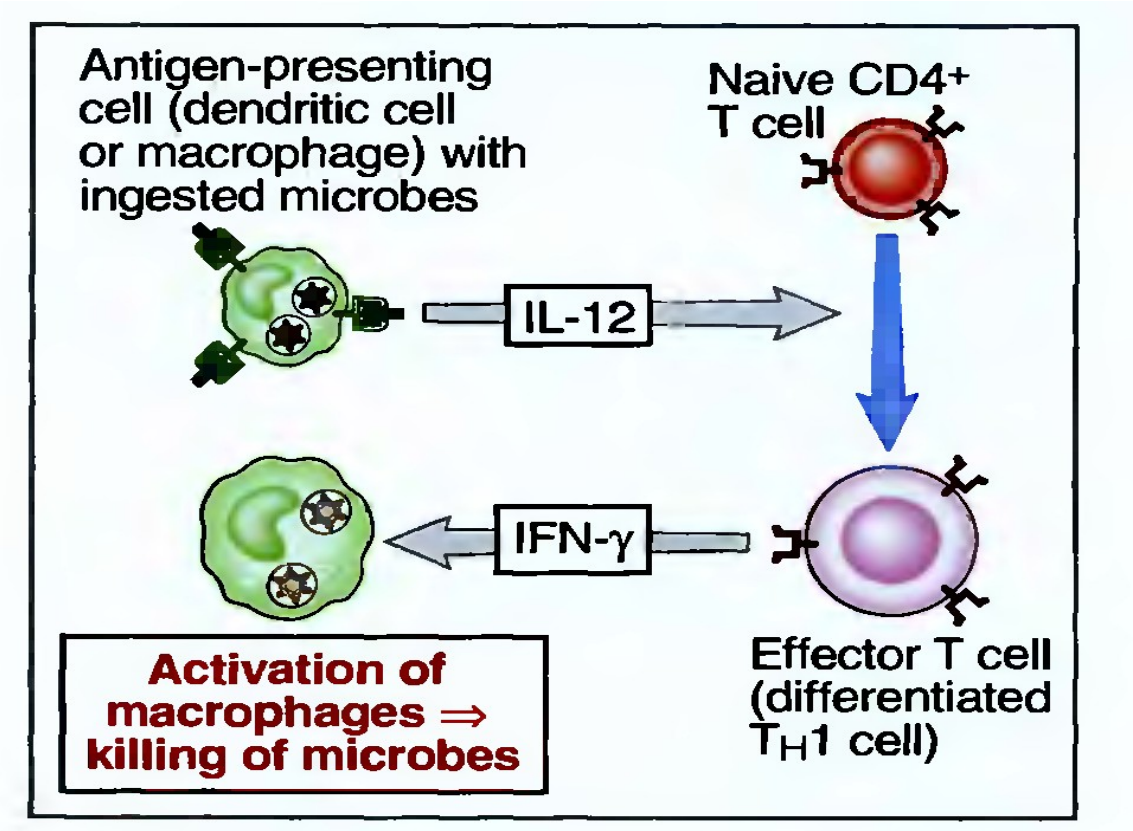
T-cell Mediated Macrophage Activation

- Macrophages associated with T cells are also macrophages that present antigens of phagocyted microbes, and these are phagocytes that need to be activated.
- **Secreted IFN-y accelerates macrophage activation and increases the severity of the response.**

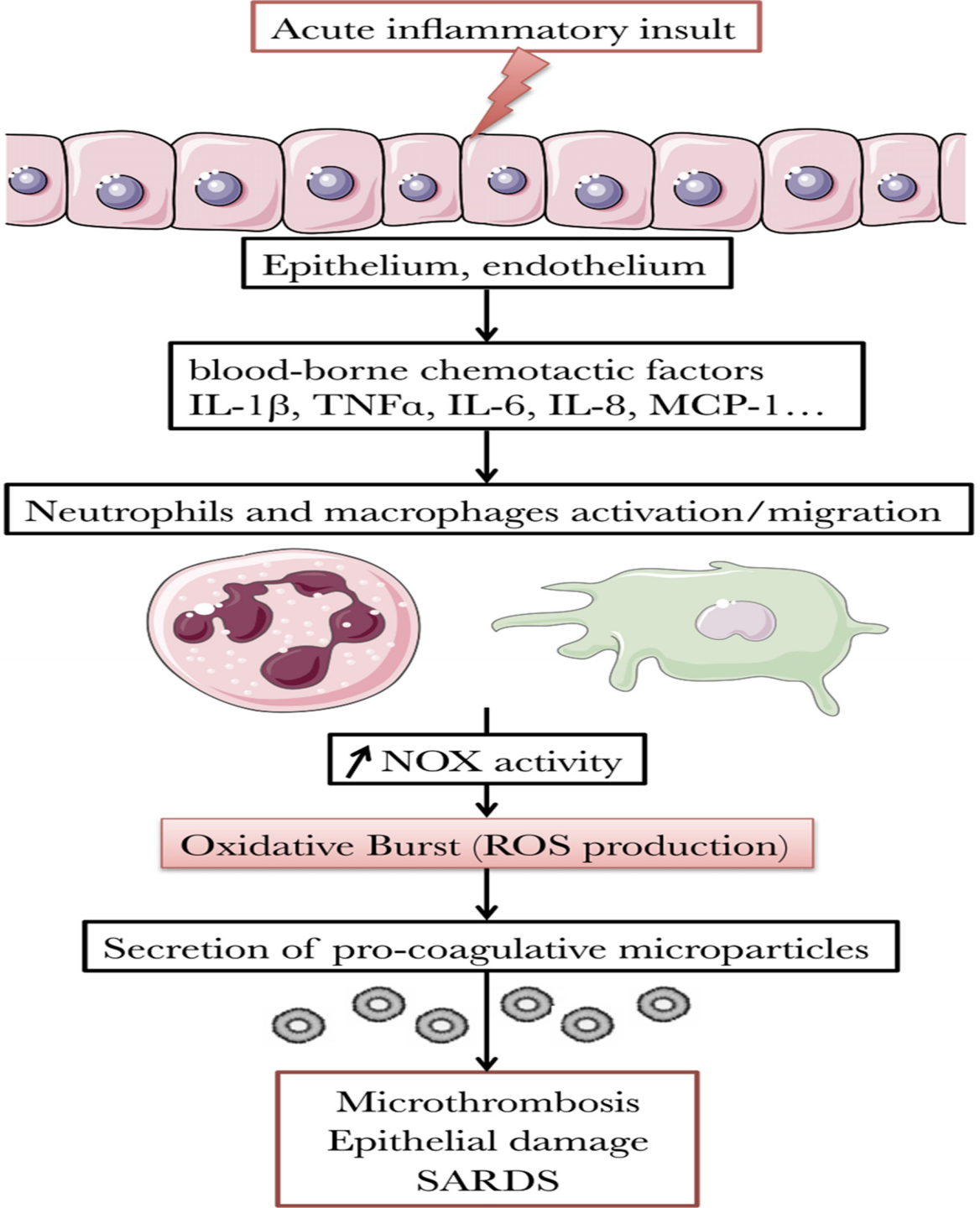
T-cell Mediated Macrophage Activation



Macrophages encountering microbes secrete IL-12 cytokine that stimulates the transformation of undifferentiated T cells into TH1 cells secreting IFN-and increase IFN-production. **IFN-y** activates macrophages to kill ingested microbes.



- CD4 + T lymphocytes also play a role in cellular immunity other than macrophage activation.
- **Antigen-stimulated CD4 + T cells secrete cytokines such as TNF that increase the expression of adhesion molecules and chemokine production by affecting the vascular endothelium.**

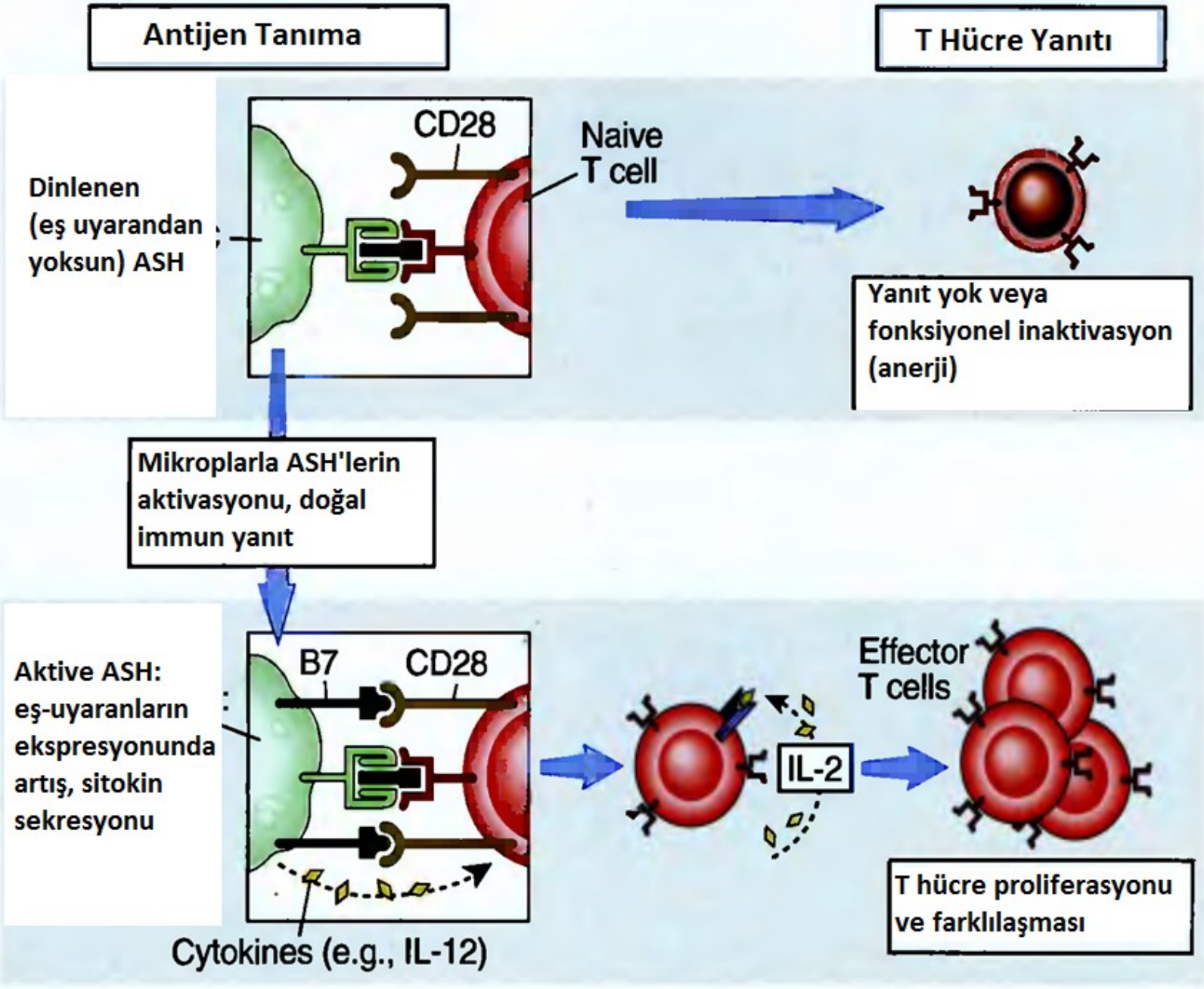

Acute inflammatory insult
Epithelium, endothelium
blood-borne chemotactic factors
IL-1β, TNFα, IL-6, IL-8, MCP-1…
Neutrophils and macrophages activation/migration
↗ NOX activity
Oxidative Burst (ROS production)
Secretion of pro-coagulative microparticles
Microthrombosis
Epithelial damage
SARDS

Elimination of Microbes with Activated Macrophages

- Macrophage activation leads to the expression of enzymes that catalyze the production of microbicidal substances in the phagosomes and phagolysosomes.
- The most important microbicidal substances produced in the lysosomes of macrophages; **reactive oxygen intermediates (ROI), nitric oxide (NO) and proteolytic enzymes.**
- When macrophages encounter microbes, these mechanisms are activated in innate immunity.

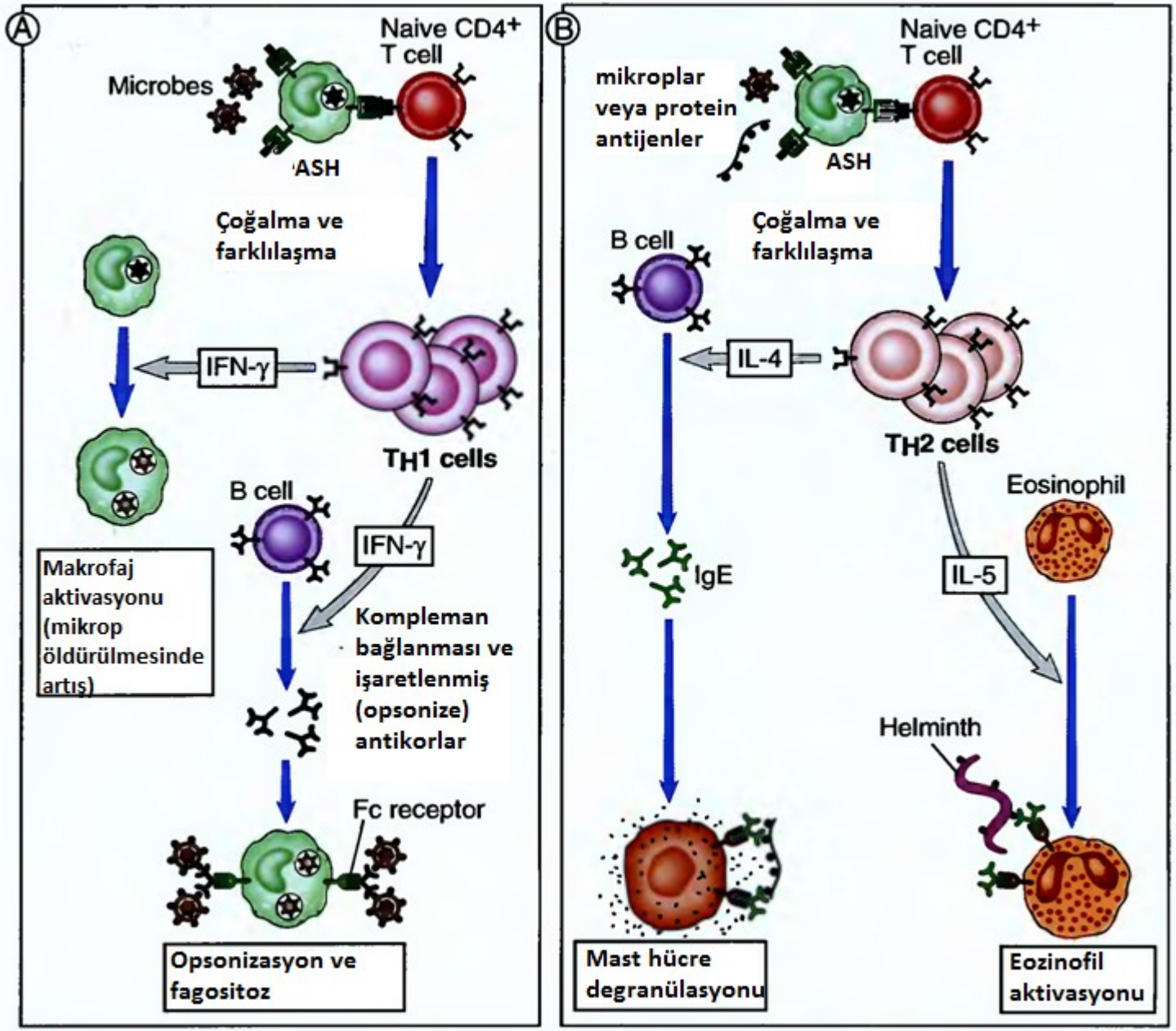
Elimination of Microbes with Activated Macrophages

- Activated macrophages play many important roles in cellular immunity, apart from killing microbes.
- Activated macrophages secrete cytokines such as **TNF, IL-1 and chemokines,** which stimulate the migration of neutrophils, monocytes and effector T lymphocytes to the infection site.

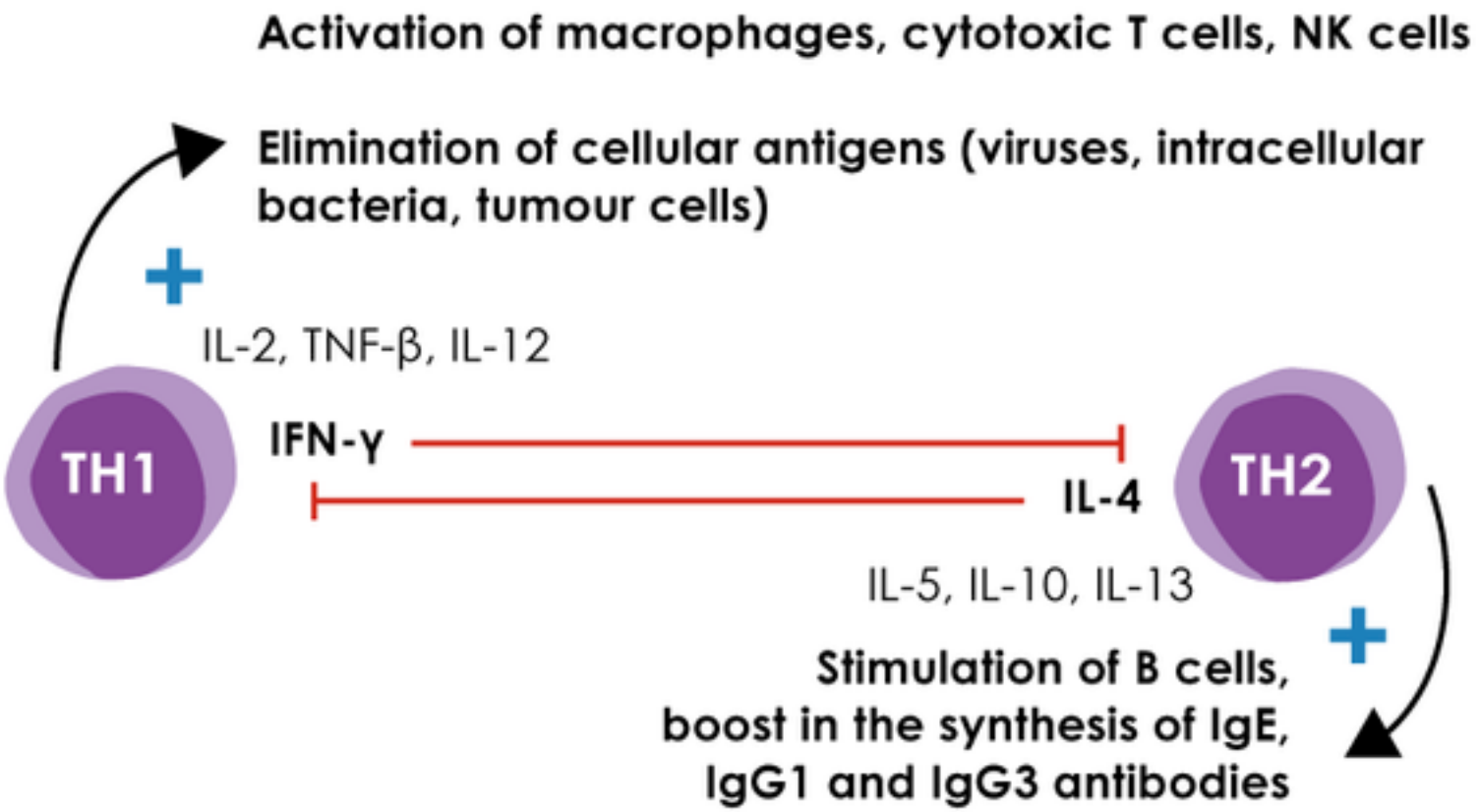

Antijen Tanıma
T Hücre Yanıtı
Dinlenen (eş uyarandan yoksun) ASH
CD28
Naive T cell
Yanıt yok veya fonksiyonel inaktivasyon (anerji)
Mikroplarla ASH'lerin aktivasyonu, doğal immun yanıt
Aktive ASH: eş-uyaranların ekspresyonunda artış, sitokin sekresyonu
B7
CD28
IL-2
Effector T cells
T hücre proliferasyonu ve farklılaşması
Cytokines (e.g., IL-12)

Cytokine	Primary Cell Sources	Key Functions in Inflammation
IL-1	Macrophages Endothelial cells	Synthesis of acute phase proteins by hepatocytes; Local and systemic inflammatory effects
IL-2	Activated T cells Th1 cells	Proliferation of T cells, B cells; Proliferation and activation of NK cells
IL-6	Macrophages Th2 cells Endothelial cells Adipocytes Myocytes Osteoblasts	Synthesis of acute phase proteins by hepatocytes; Proliferation of B cells; Down-regulation of IL-1 and TNF production; Activation of immune cells, osteoclasts, endothelial cells; Hypothalamic Pituitary Axis—fever & hormone release
IL-10	MacrophagesTh2 cells	Resolution of inflammation; Inhibition of Th1 inflammatory cytokine synthesis; Inhibition of activated macrophages and dendritic cells
IL-12	Macrophages Dendritic cells	Promotion of Th1 differentiation; Stimulation of IFN-γ production by T cells, NK cells
TNF-α	Macrophages T cells NK cells Lymphoid cells Endothelial cells Adipocytes Cardiac myocytes Fibroblasts Neuronal cells	Synthesis of acute phase proteins by hepatocytes; Recruitment and activation of neutrophils and monocytes at sites of infection; Stimulation of CRP release from liver; Activation of NF-κB pathway; Induction of insulin resistance
TGF-β	Macrophages T cells	Resolution of inflammation; Limit production of IL-2, IFN-γ, and TNF; Inhibition of proliferation/activation of B cells, T cells, macrophages.
IFN-γ	Th1 cells NK cells	Activation of macrophages; Suppression of Th2 cell activity; Promotion of leukocyte migration

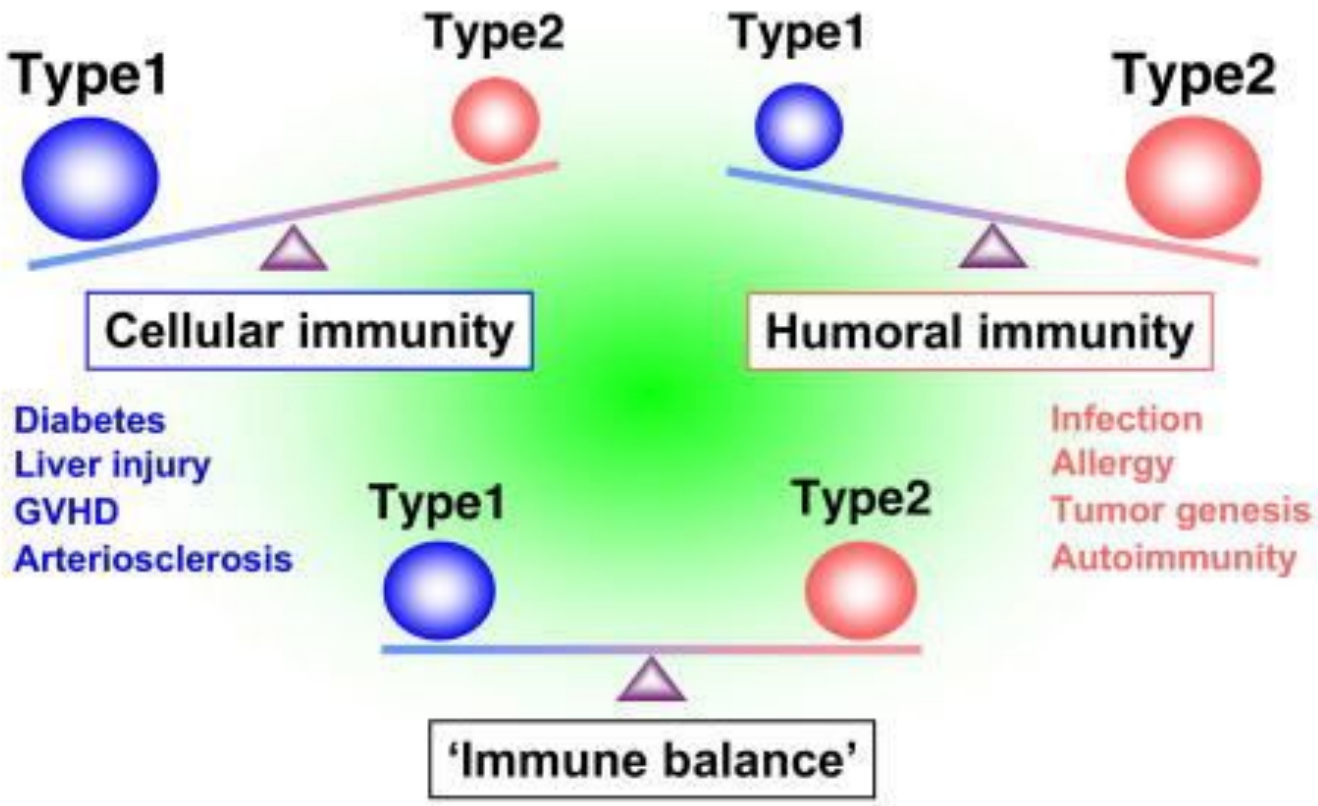
Abbreviations: IFN, interferon; IL, interleukin; NK, natural killer; NF-κB, nuclear factor-kappaB; Th, T helper;


A
Naive CD4+ T cell
Microbes
ASH
Çoğalma ve farklılaşma
IFN-γ
TH1 cells
B cell
IFN-γ
Makrofaj aktivasyonu (mikrop öldürülmesinde artış)
Kompleman bağlanması ve işaretlenmiş (opsonize) antikorlar
Fc receptor
Opsonizasyon ve fagositoz
B
Naive CD4+ T cell
mikroplar veya protein antijenler
ASH
Çoğalma ve farklılaşma
B cell
IL-4
TH2 cells
Eosinophil
IL-5
IgE
Helminth
Mast hücre degranülasyonu
Eozinofil aktivasyonu

Th1/Th2 Balance

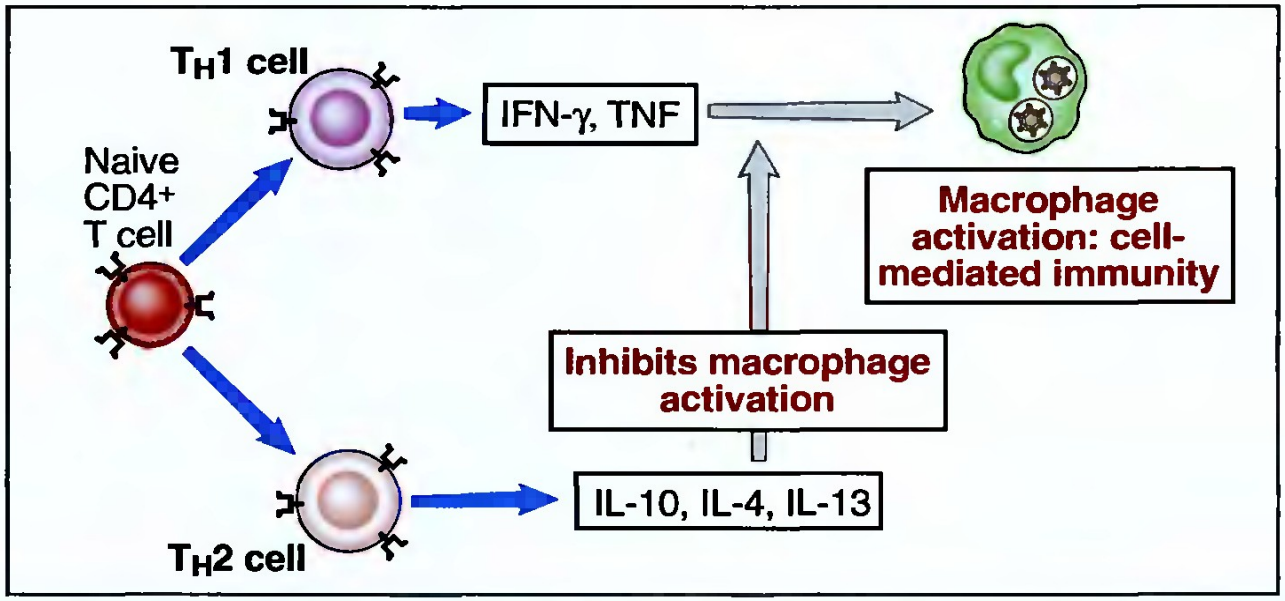


Regulation of 'Immune balance' is critical for our health



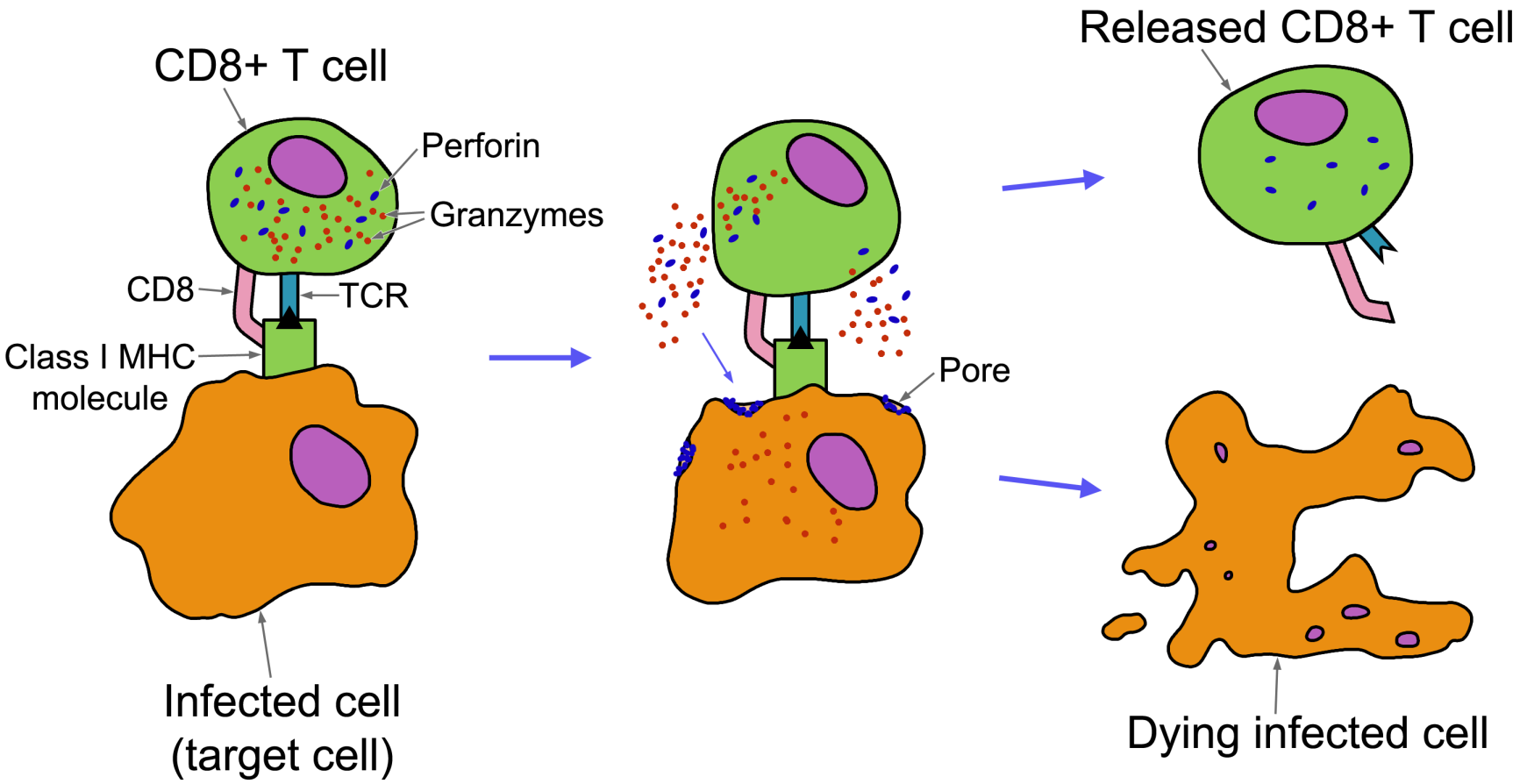
The role of Th2 Cells in Cellular İmmunity

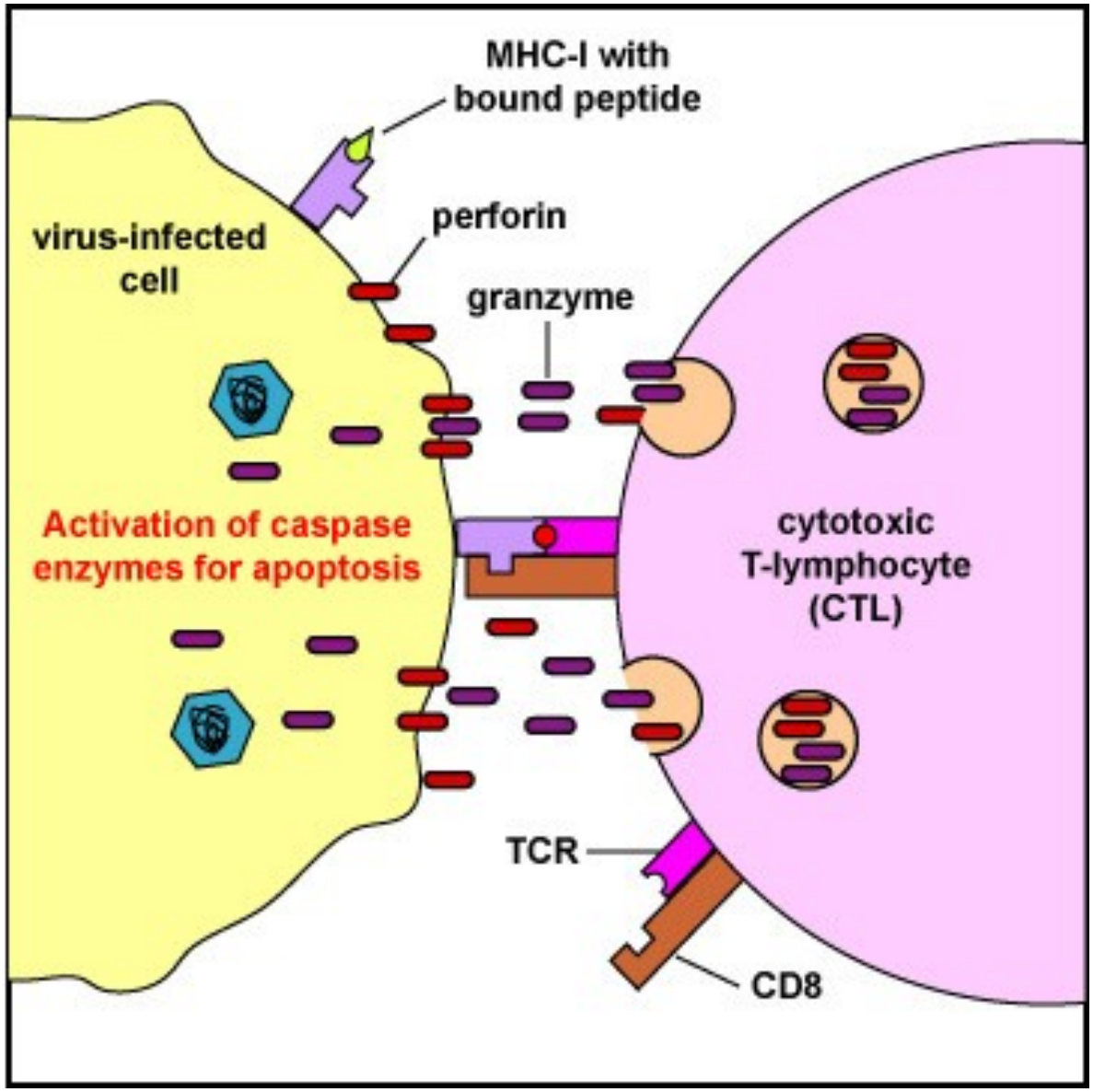
- The proportional activation of TH1 and TH2 cells against an infectious microbe can determine the outcome of the infection.
- For example, the protozoal parasite Leishmania major survives in the macrophage and the macrophages needs to be activated by TH1 cells.
- Many cobreed mice strains elicit a strong TH1 response to the parasite and thus manage to eradicate the infection.
- TH2 cells are more effective in responding to L. major in some species of mice, and these mice are not able to resist infection.

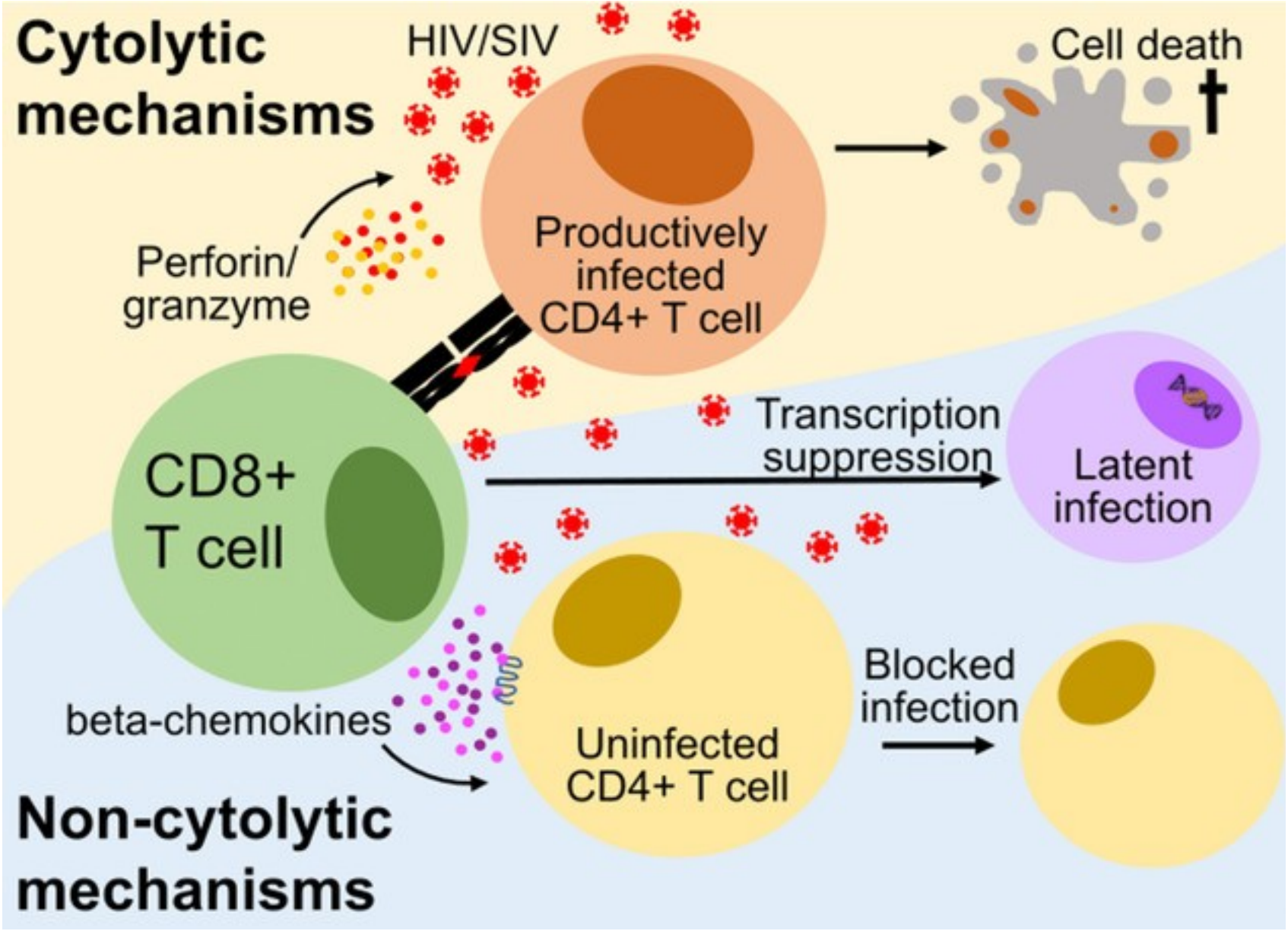


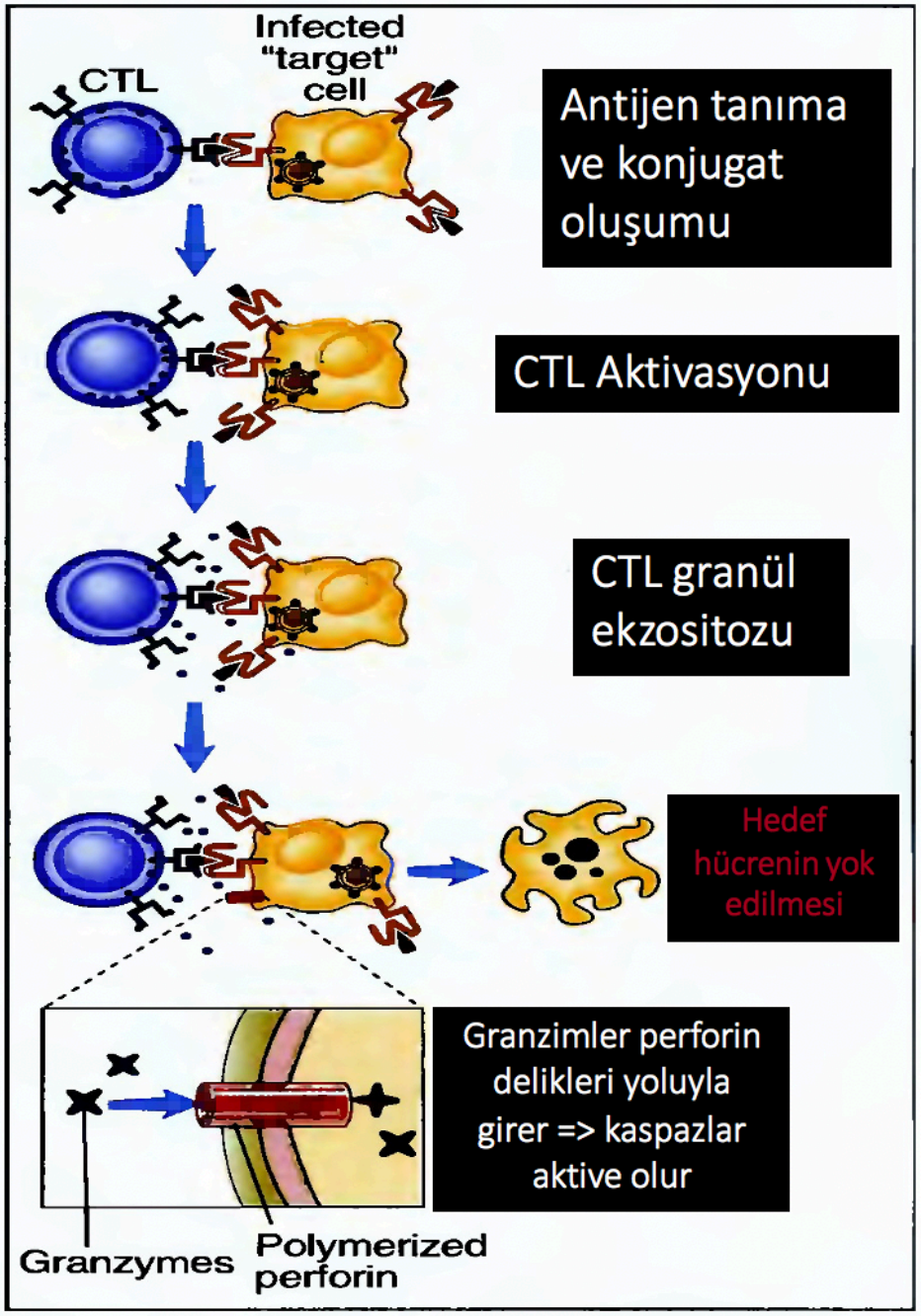
Infection	Response	Outcome
Leishmania major	Many mice: TH1 => BALB/c fmice : TH2 =>	Resolution Wide infection
Mycobacterium leprae	Some patients: TH1 => Defective TH1 or dominant TH2 =>	Tuberculoid lepra Lepramatose lepra

Effector Functions of CD8+ Cytolytic T Lymphocytes

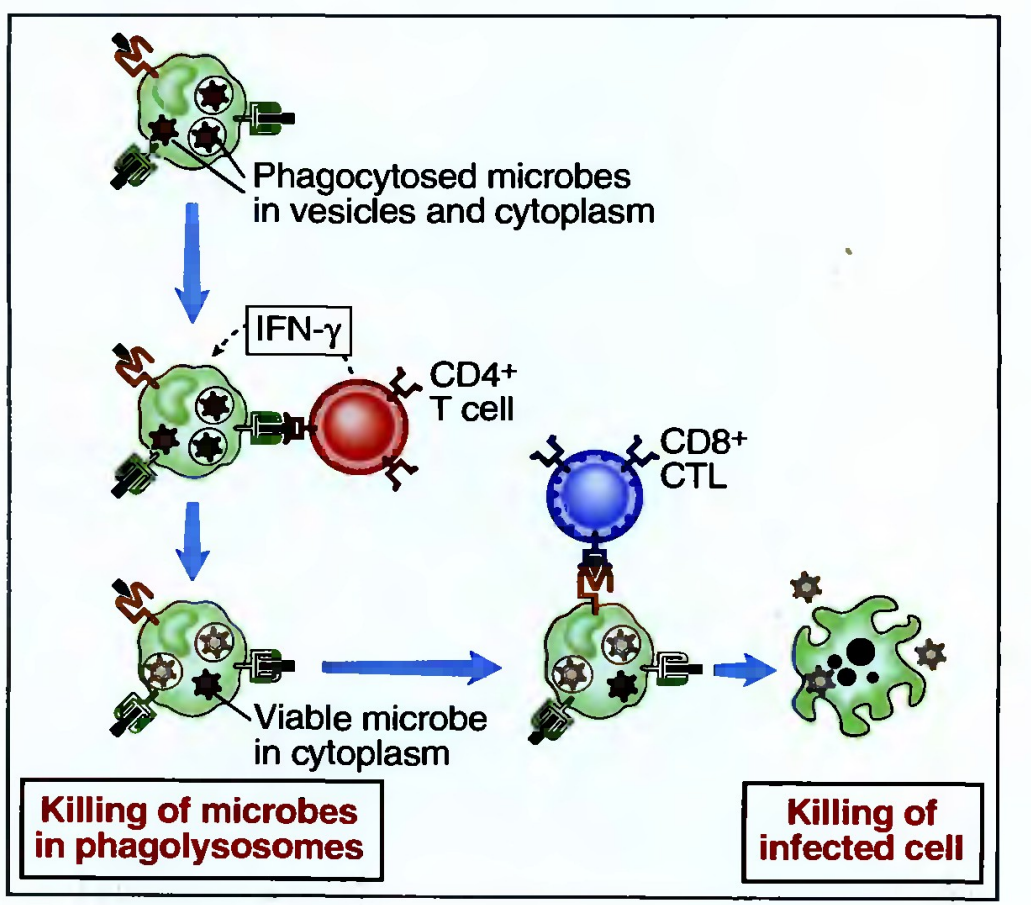



MHC-I with
bound peptide
perforin
virus-infected
cell
granzyme
Activation of caspase
enzymes for apoptosis
cytotoxic
T-lymphocyte
(CTL)
TCR
CD8


Cytolytic mechanisms
HIV/SIV
Cell death
Perforin/ granzyme
Productively infected CD4+ T cell
CD8+ T cell
Transcription suppression
Latent infection
beta-chemokines
Uninfected CD4+ T cell
Blocked infection
Non-cytolytic mechanisms


Infected "target" cell
CTL
Antijen tanıma ve konjugat oluşumu
CTL Aktivasyonu
CTL granül ekzositozu
Hedef hücrenin yok edilmesi
Granzimler perforin delikleri yoluyla girer => kaspazlar aktive olur
Granzymes
Polymerized perforin

Coordination of CD 4 and CD8 T Cells



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