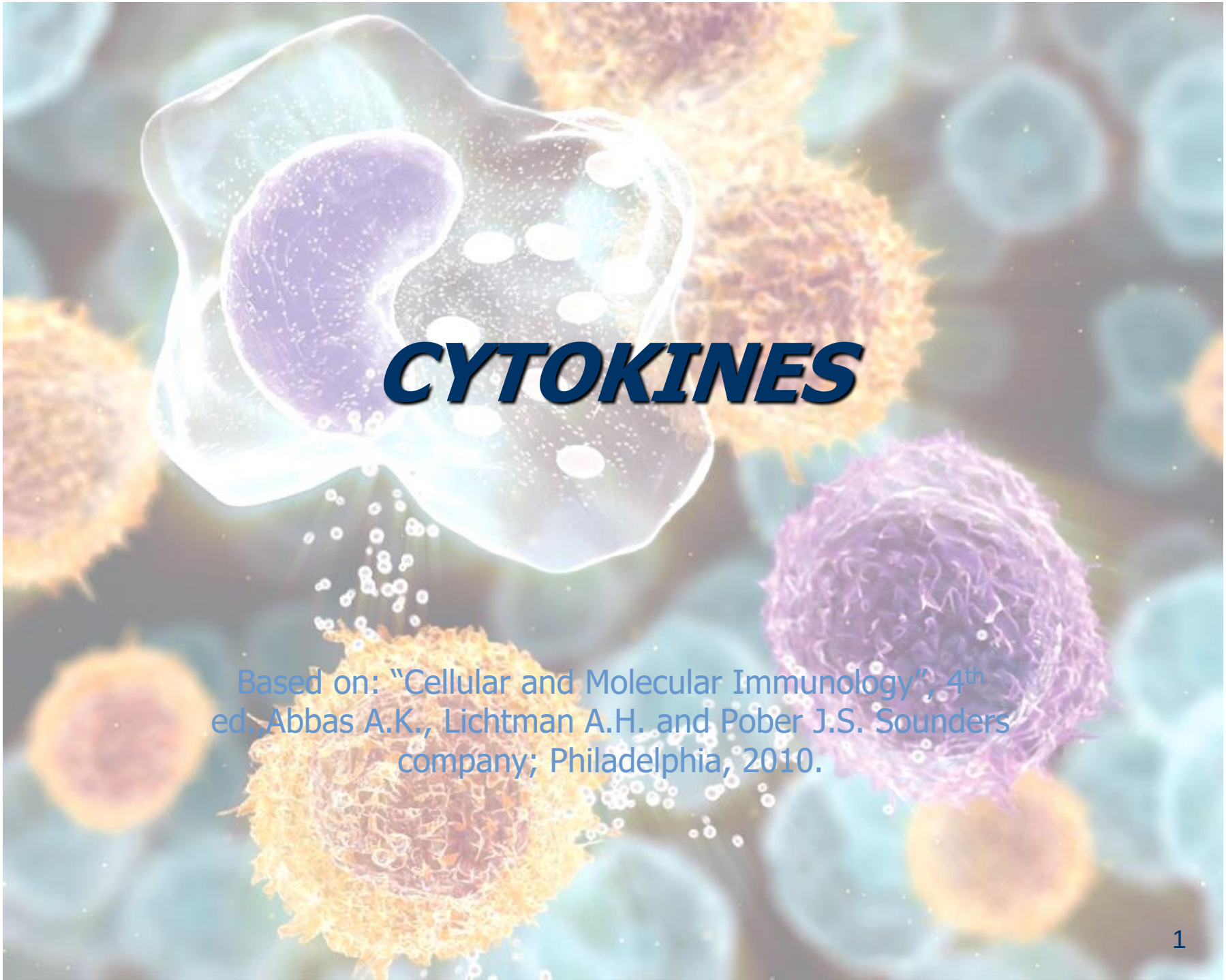
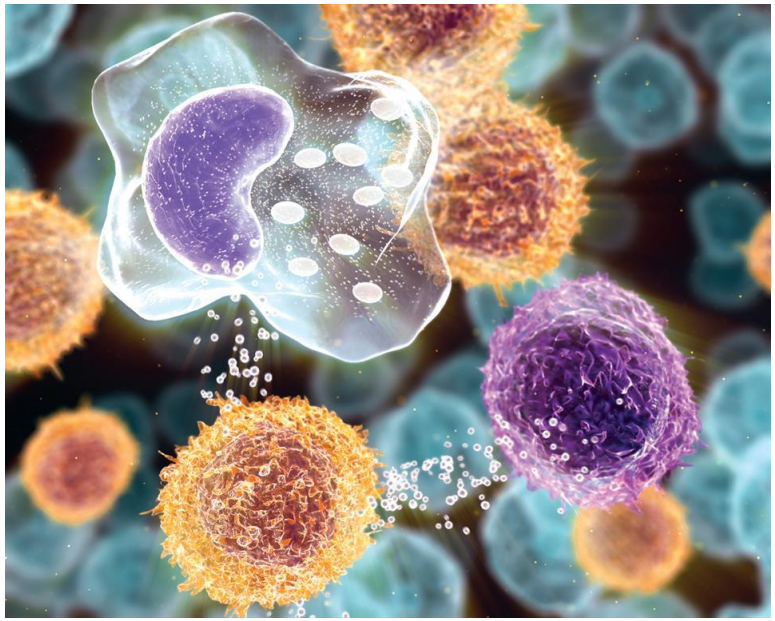


CYTOKINES

Based on: "Cellular and Molecular Immunology", 4th ed., Abbas A.K., Lichtman A.H. and Pober J.S. Sounders company; Philadelphia, 2010.

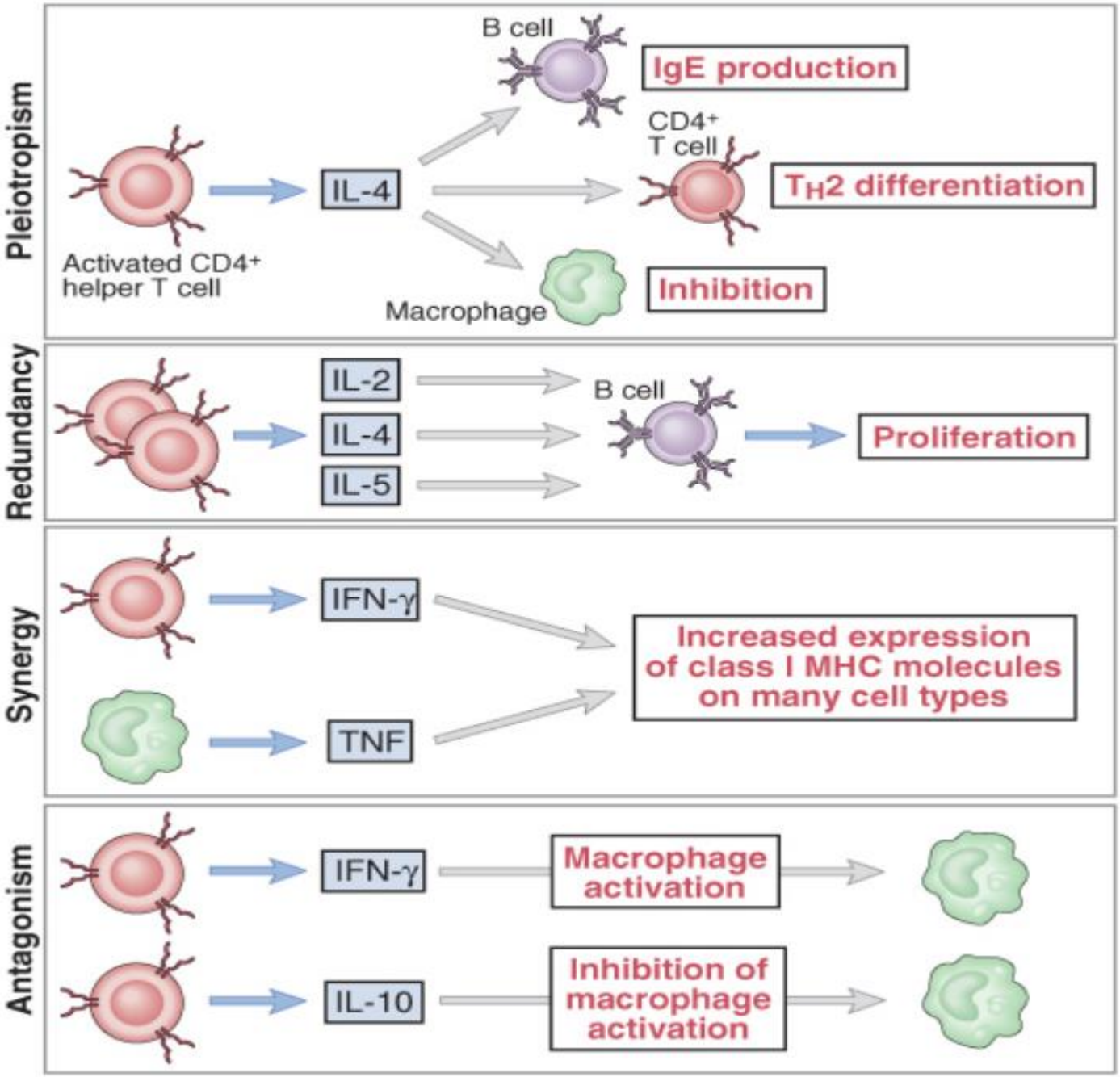
What are cytokines?



- Glycoproteins (15 – 25 kDa):
  - Interleukins (IL 1-35)
  - Interpherons (IF type 1,2,3)
  - Tumor necrosis factor family (TNF α i β)
  - Colony stimulating factors (CSF)
  - Others (TGF- β, LIF, MIF)

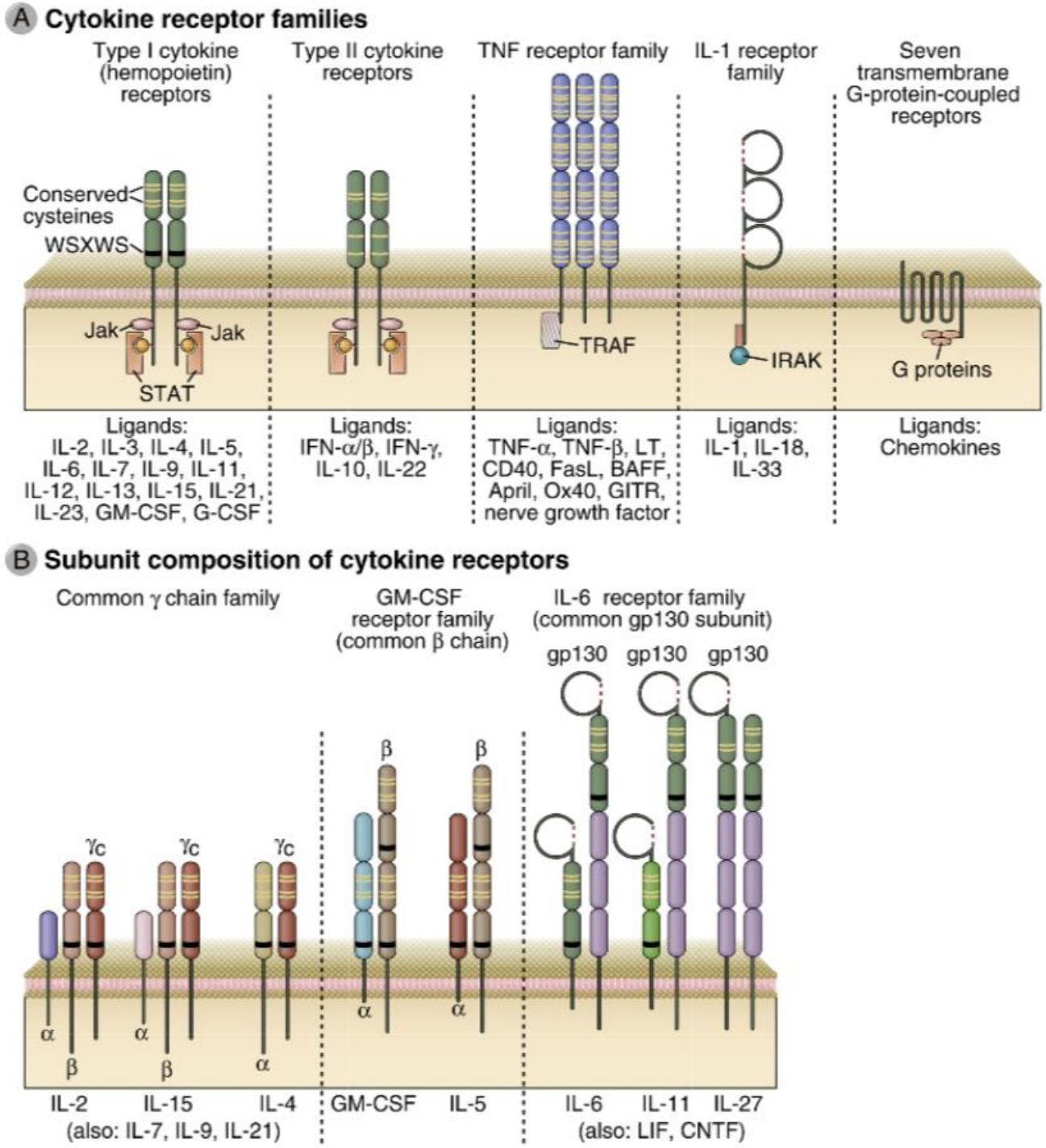
  - Chemokines

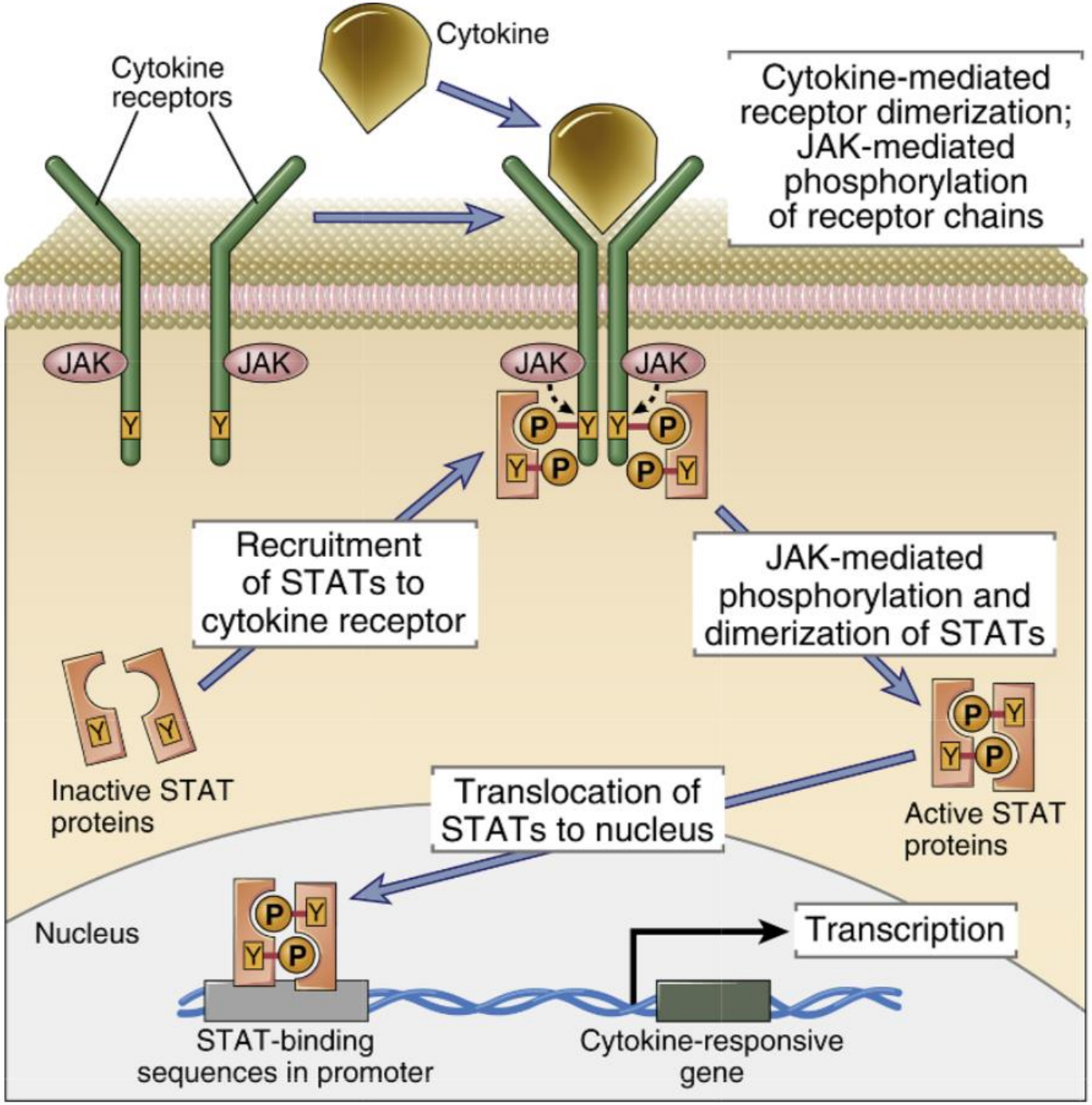
General properties of cytokines I

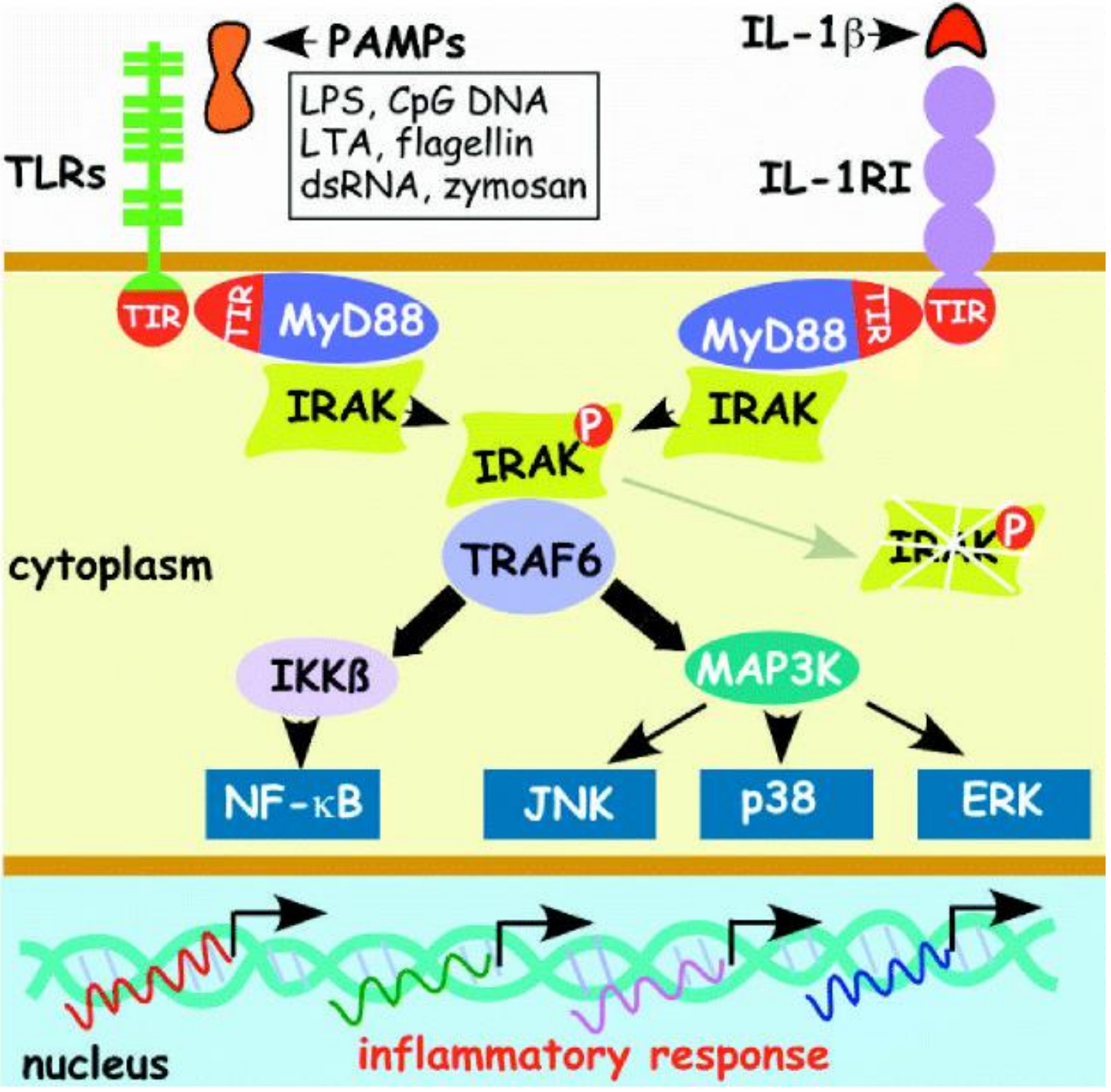


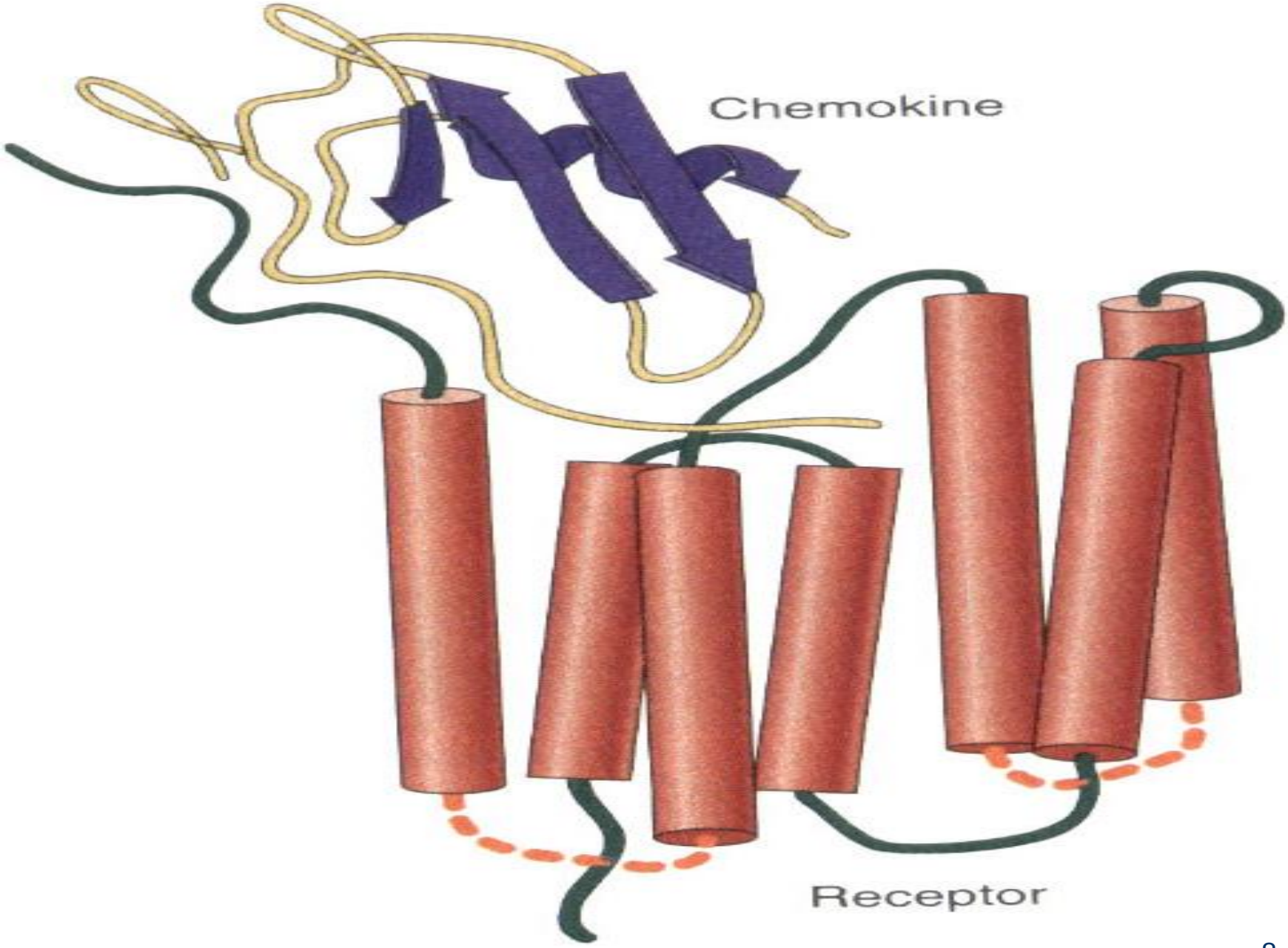
General properties of cytokines II

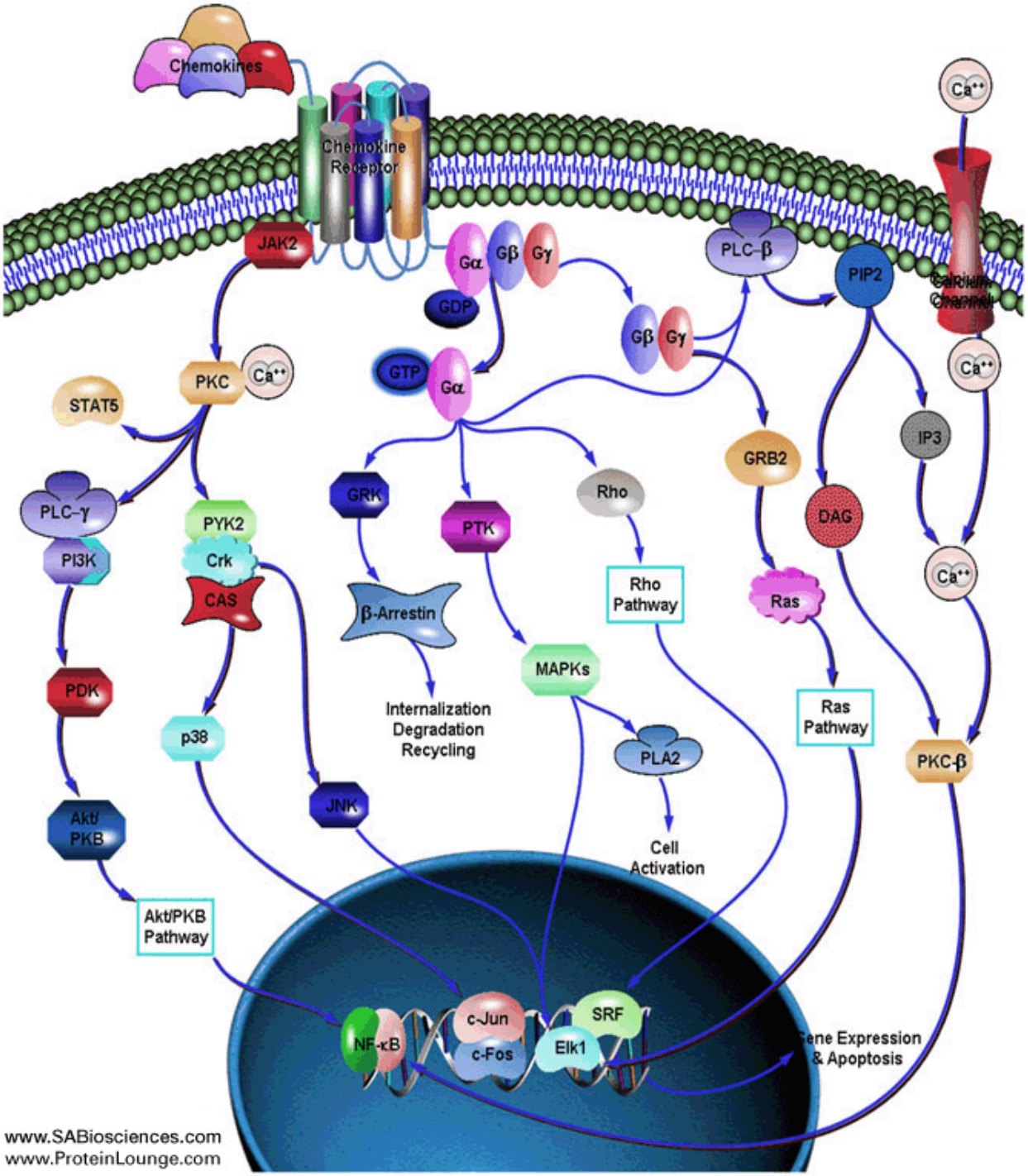
- NOT STORED
- ACT ON AUTOCRINE, PARACRINE (LOCAL) AND SYSTEMIC LEVELS
- ONE STIMULATES SECRETION OF ANOTHER
- HIGH RECEPTOR AFFINITY
- CHANGE THE LEVEL OF GENE TRANSCRIPTION (except chemokines, and sometimes TNF)


A Cytokine receptor families
Type I cytokine (hemopoietin) receptors
Type II cytokine receptors
TNF receptor family
IL-1 receptor family
Seven transmembrane G-protein-coupled receptors
Conserved cysteines
WSXWS
Jak
Jak
STAT
TRAF
IRAK
G proteins
Ligands: IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-12, IL-13, IL-15, IL-21, IL-23, GM-CSF, G-CSF
Ligands: IFN-α/β, IFN-γ, IL-10, IL-22
Ligands: TNF-α, TNF-β, LT, CD40, FasL, BAFF, April, Ox40, GITR, nerve growth factor
Ligands: IL-1, IL-18, IL-33
Ligands: Chemokines
B Subunit composition of cytokine receptors
Common γ chain family
GM-CSF receptor family (common β chain)
IL-6 receptor family (common gp130 subunit)
gp130
gp130
gp130
γc
γc
γc
α
β
α
β
α
β
β
α
α
IL-2
IL-15
IL-4
(also: IL-7, IL-9, IL-21)
GM-CSF
IL-5
IL-6
IL-11
IL-27
(also: LIF, CNTF)

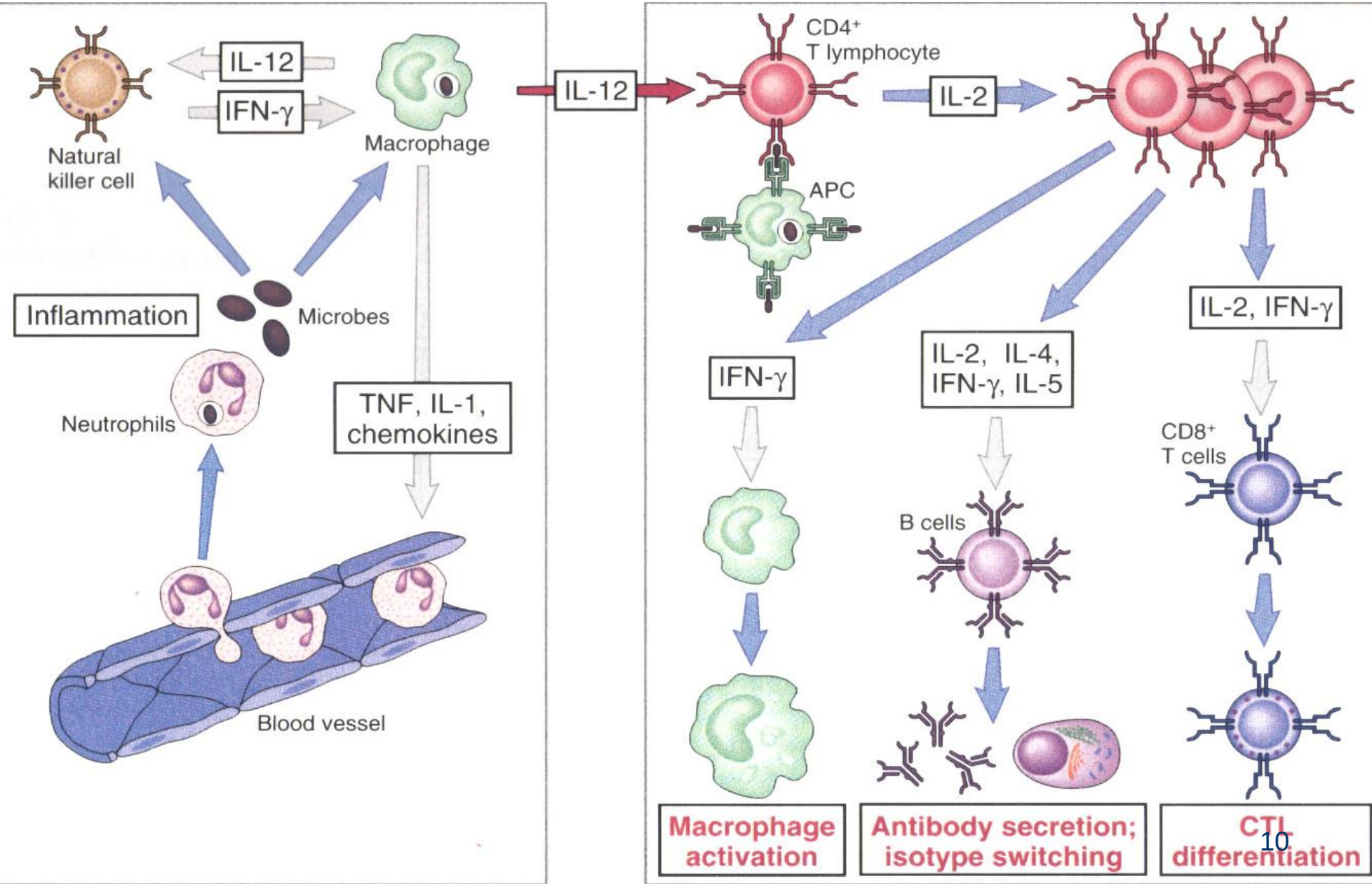

Cytokine
Cytokine receptors
Cytokine-mediated receptor dimerization; JAK-mediated phosphorylation of receptor chains
JAK
JAK
JAK
JAK
Y
Y
P
Y
Y
P
Y
P
P
Y
Recruitment of STATs to cytokine receptor
JAK-mediated phosphorylation and dimerization of STATs
Inactive STAT proteins
Active STAT proteins
Translocation of STATs to nucleus
Nucleus
Transcription
STAT-binding sequences in promoter
Cytokine-responsive gene


PAMPs
LPS, CpG DNA
LTA, flagellin
dsRNA, zymosan
TLRs
IL-1β
IL-1RI
TIR
MyD88
IRAK
P
cytoplasm
TRAF6
IKKß
MAP3K
NF-κB
JNK
p38
ERK
nucleus
inflammatory response


Chemokine
Receptor


Chemokines
Chemokine Receptor
JAK2
Gα
Gβ
Gγ
GDP
GTP
PLC-β
PIP2
Ca++
PKC
STAT5
PLC-γ
PI3K
PYK2
Crk
CAS
GRK
β-Arrestin
PTK
Rho
Rho Pathway
GRB2
Ras
DAG
IP3
PDK
Akt/ PKB
Akt/PKB Pathway
p38
JNK
Internalization
Degradation
Recycling
MAPKs
PLA2
Cell Activation
Ras Pathway
PKC-β
NF-κB
c-Jun
c-Fos
SRF
Elk1
Gene Expression & Apoptosis
www.SABiosciences.com
www.ProteinLounge.com

Innate and adaptive immunity



Cytokine classification

- **INNATE IMMUNITY CYTOKINES**
- **ADAPTIVE IMMUNITY CYTOKINES**
- **HEMATOPOIETIC CYTOKINES**
- **IMMUNOSUPPRESSIVE CYTOKINES**

INNATE IMMUNITY CYTOKINES

- Secreted by macrophages
- Stimmulated by LPS, nucleic acids, immunocomplexes, …

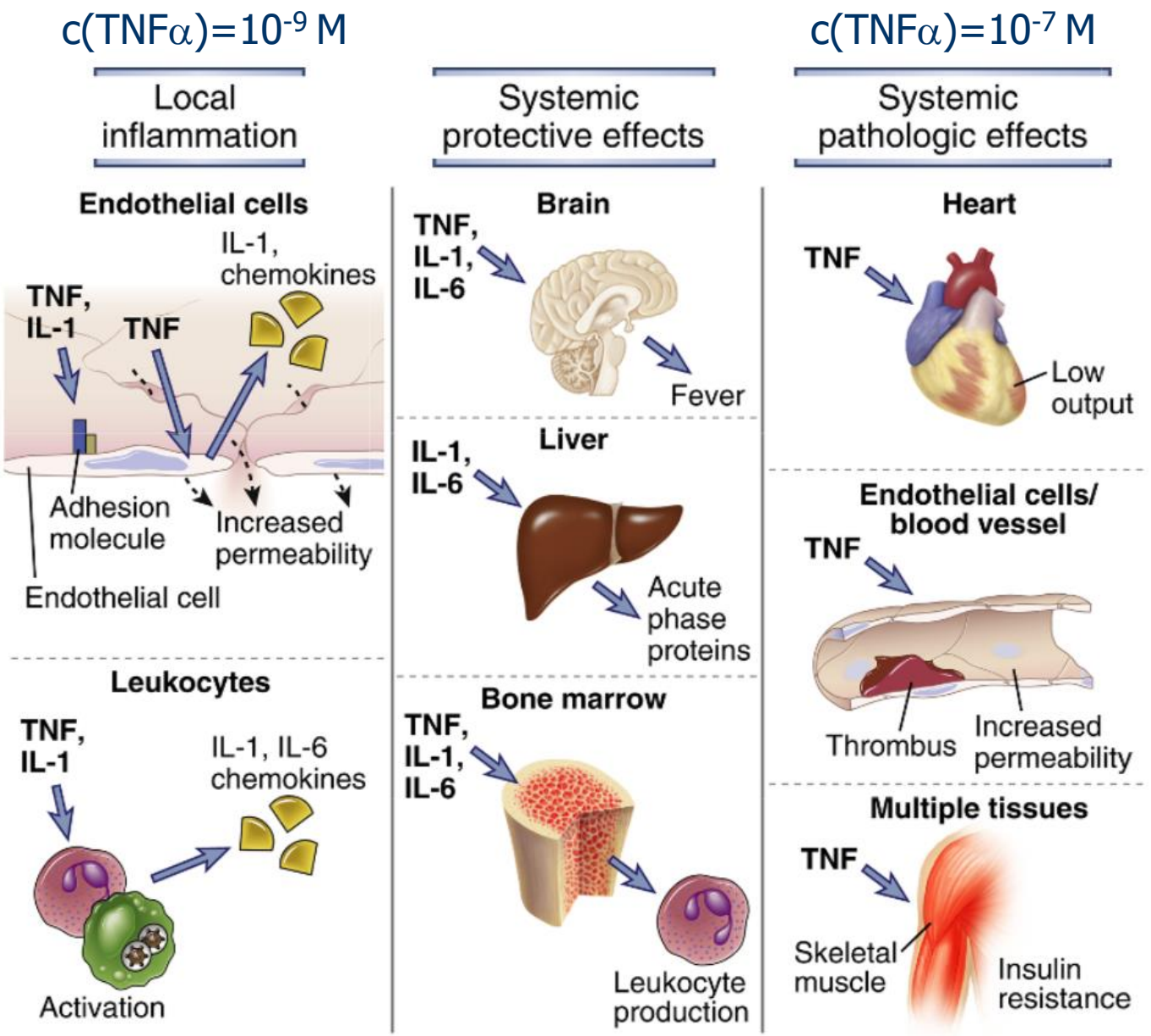
Those are:

- **TNF-α, IL-1, IL-12, IFα i β**
- IL-6, IL-8, IL-18, IL-23, IL-27,…

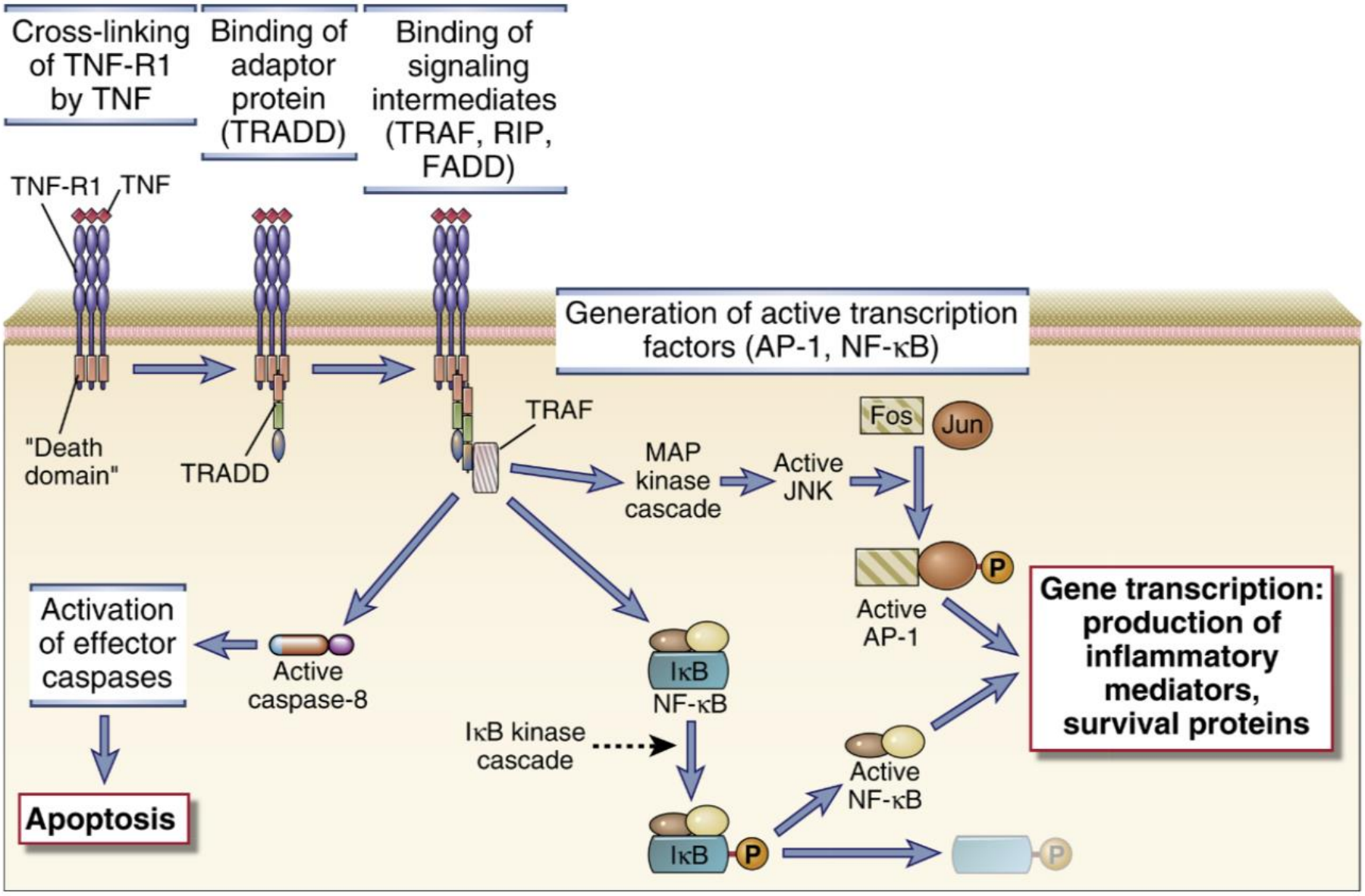
TNF-α

- - produced by macrophages
- - recruits neutrophils
- - stimulates endothelial cells to:
  - express selectins (E and P)
  - secrete chemokines
- Large amounts cause systemic effects (high body temperature, acute phase proteins production, appetite reduction)
- Very large amounts cause the development of cardiovascular shock

TNF α


c(TNFα)=10-9 M
Local inflammation
Endothelial cells
IL-1, chemokines
TNF, IL-1
TNF
Adhesion molecule
Increased permeability
Endothelial cell
Leukocytes
TNF, IL-1
IL-1, IL-6 chemokines
Activation
Systemic protective effects
Brain
TNF, IL-1, IL-6
Fever
Liver
IL-1, IL-6
Acute phase proteins
Bone marrow
TNF, IL-1, IL-6
Leukocyte production
c(TNFα)=10-7 M
Systemic pathologic effects
Heart
TNF
Low output
Endothelial cells/ blood vessel
TNF
Thrombus
Increased permeability
Multiple tissues
TNF
Skeletal muscle
Insulin resistance

TNF receptor cell sygnaling

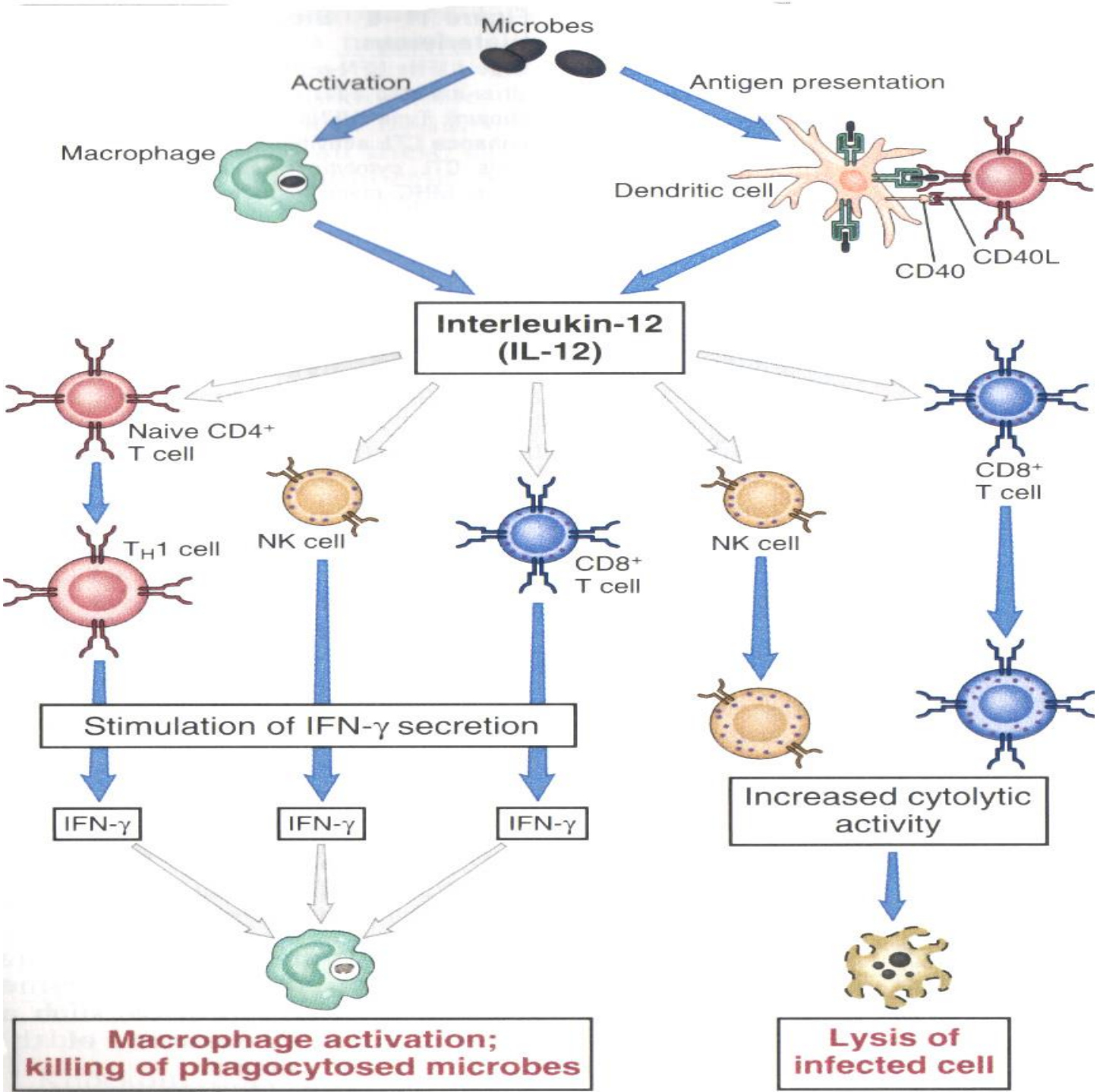


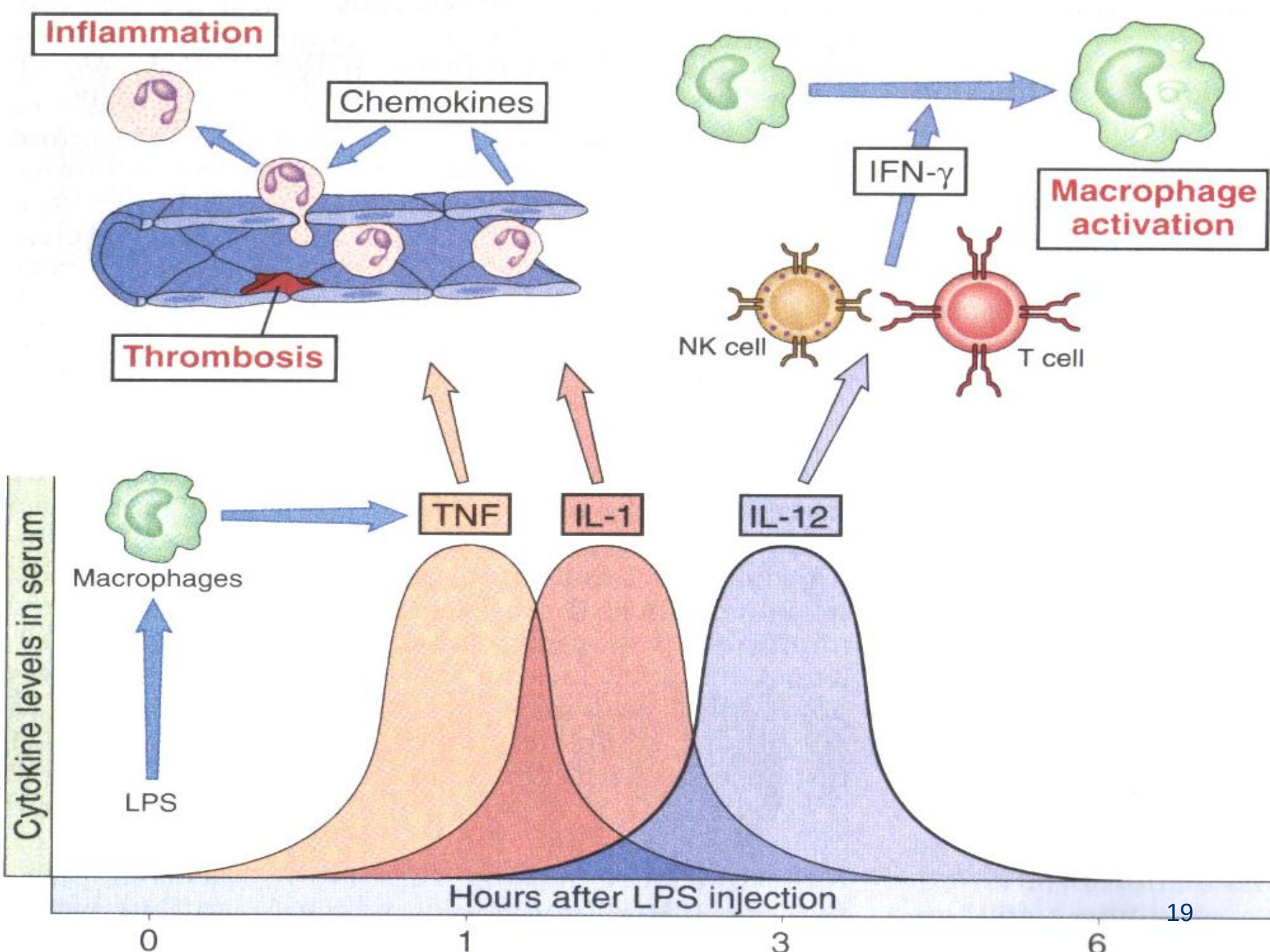
IL-1 family

- **Produced by macrophages**
- A complex network of proinflamatory cytokines
- **Group of 11 cytokines** (IL-1α, IL-1β, IL-1Ra, IL-18, IL-35...)
- **Function simmilar to TNF** (but does NOT lead to apoptosis or septic shock)
- **Syntesized from a stored precursor protein** (inflammasome → caspaze 1 → IL-1 cleavage)

IL-12

- Secreted by macrophages and dendritic cells
- Activated by intracellular microbes
  - Activates NK cells (cytolytic activity)
  - Stimulates IF-γ production by NK cells
  - Promotes Th1 differentiation

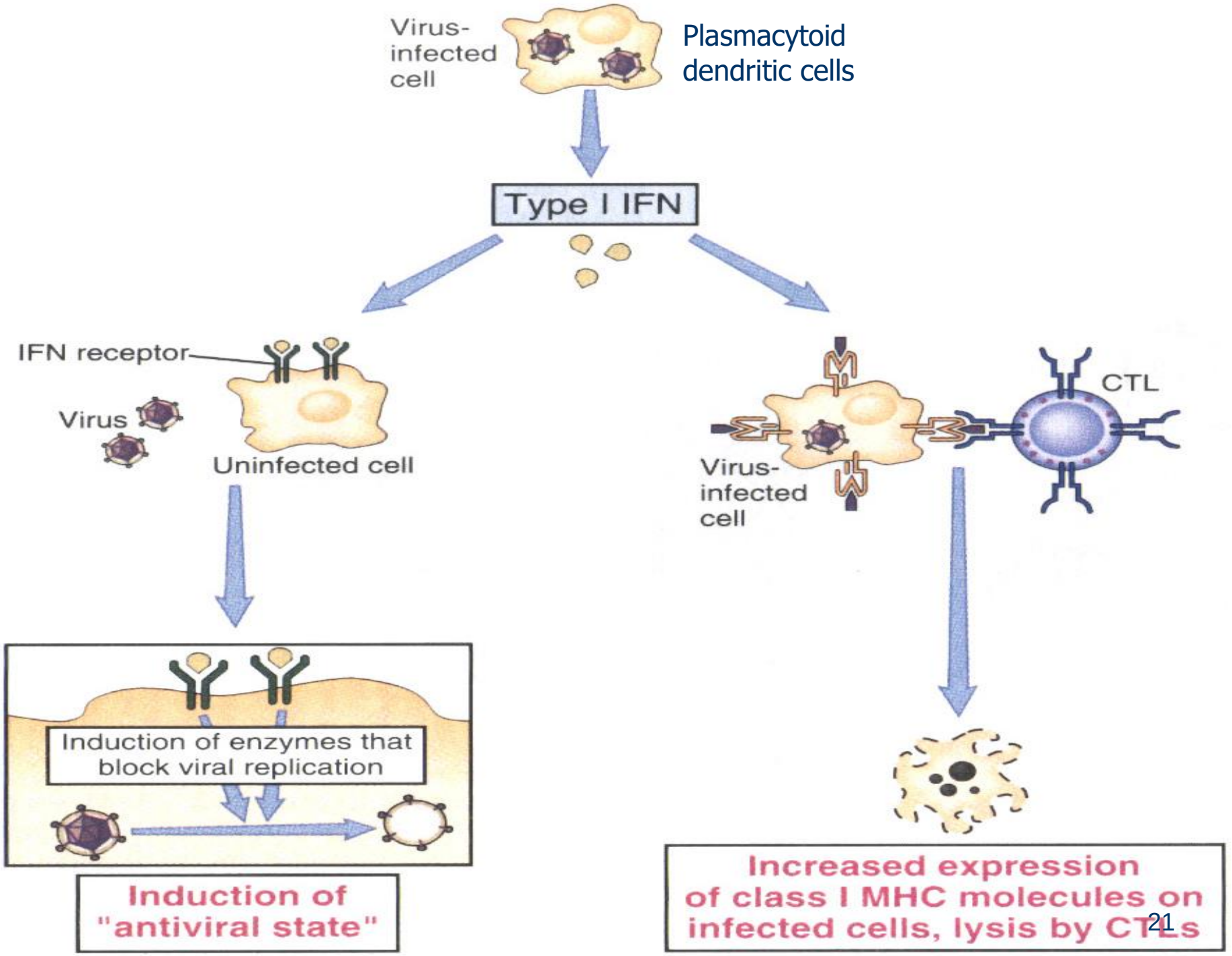

Microbes
Activation
Antigen presentation
Macrophage
Dendritic cell
CD40
CD40L
Interleukin-12
(IL-12)
Naive CD4+
T cell
TH1 cell
NK cell
CD8+
T cell
NK cell
CD8+
T cell
Stimulation of IFN-γ secretion
IFN-γ
IFN-γ
IFN-γ
Increased cytolytic
activity
Macrophage activation;
killing of phagocytosed microbes
Lysis of
infected cell


Inflammation
Chemokines
Thrombosis
IFN-γ
Macrophage activation
NK cell
T cell
TNF
IL-1
IL-12
Macrophages
LPS
Cytokine levels in serum
Hours after LPS injection
0
1
3
6

Interferons

- **Type 1 (**several α and one β**):**
  - Antiviral defense
  - Produced by macrophages and infected cells
  - Increase **MHC-I** expression, inhibit **viral** replication (inhibit protein synthesis, destroys RNA), activate NK cells, promote **Th1** differentiation
  - Anti-tumor defense

- **Type 2 – IF-γ** (innate and adaptive cytokine):
  - Produced by T lymphocytes and NK cells.
  - The most potent macrophage activator


Virus-infected cell
Plasmacytoid dendritic cells
Type I IFN
IFN receptor
Virus
Uninfected cell
CTL
Virus-infected cell
Induction of enzymes that block viral replication
Induction of "antiviral state"
Increased expression of class I MHC molecules on infected cells, lysis by CTLs

IL-6

- **Proinflamatory effects**
  - produced by macrophages, T-cells, smooth muscle cells, fibroblasts, endothelia, osteoblasts, adipocytes
  - Stimulates lymphocite T/B growth (adaptive immunity)
  - Stimulates the production of acute phase proteins, fever
  - Stimulates development of Th17
  - Elevated in many autoimmune diseases (tocilizumab)
  - Cancer: elevated IL-6 = poor survival

- **Anti-inflamatory myokin**
  - inhibits TNFa and IL-1 effects, stimulates IL-10 (produced by myocytes during contraction)
  - exercise-associated metabolic changes, tissue repair

Other innate immunity cytokines

- **IL-10**
  - Produced by macrophages and helper cells
  - Inhibits IL-12 and TNF-α production, as well as MHC – class II (**immunosuppression**)

- **IL-15**
  - Produced in response to viral infection
  - Stimulates NK cell proliferation

- **IL-15, IL-16, IL-23, IL-27, …**

ADAPTIVE IMMUNITY CYTOKINES

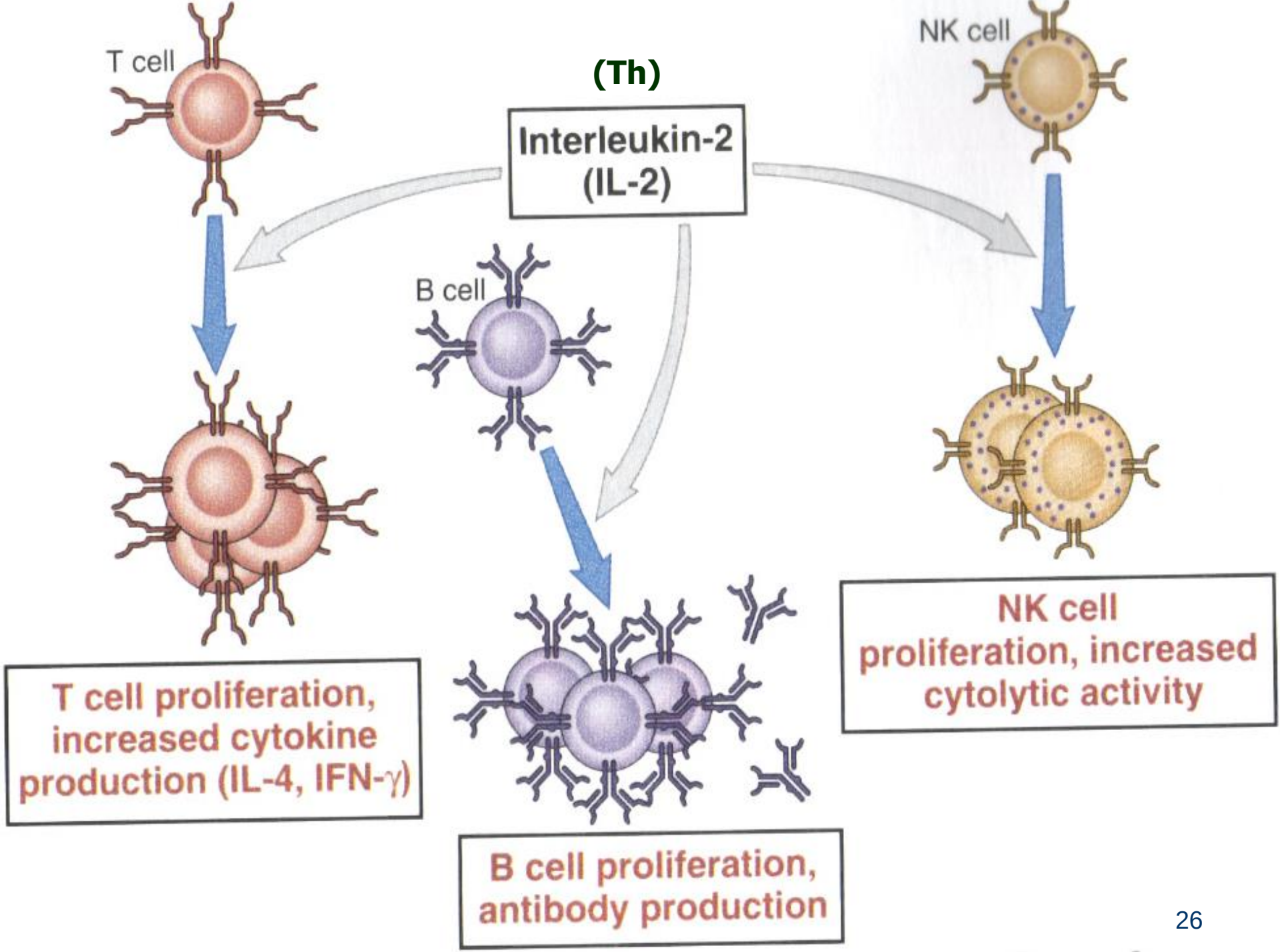
- Secreted by T-lymphocytes
- Stimulated by antigenes

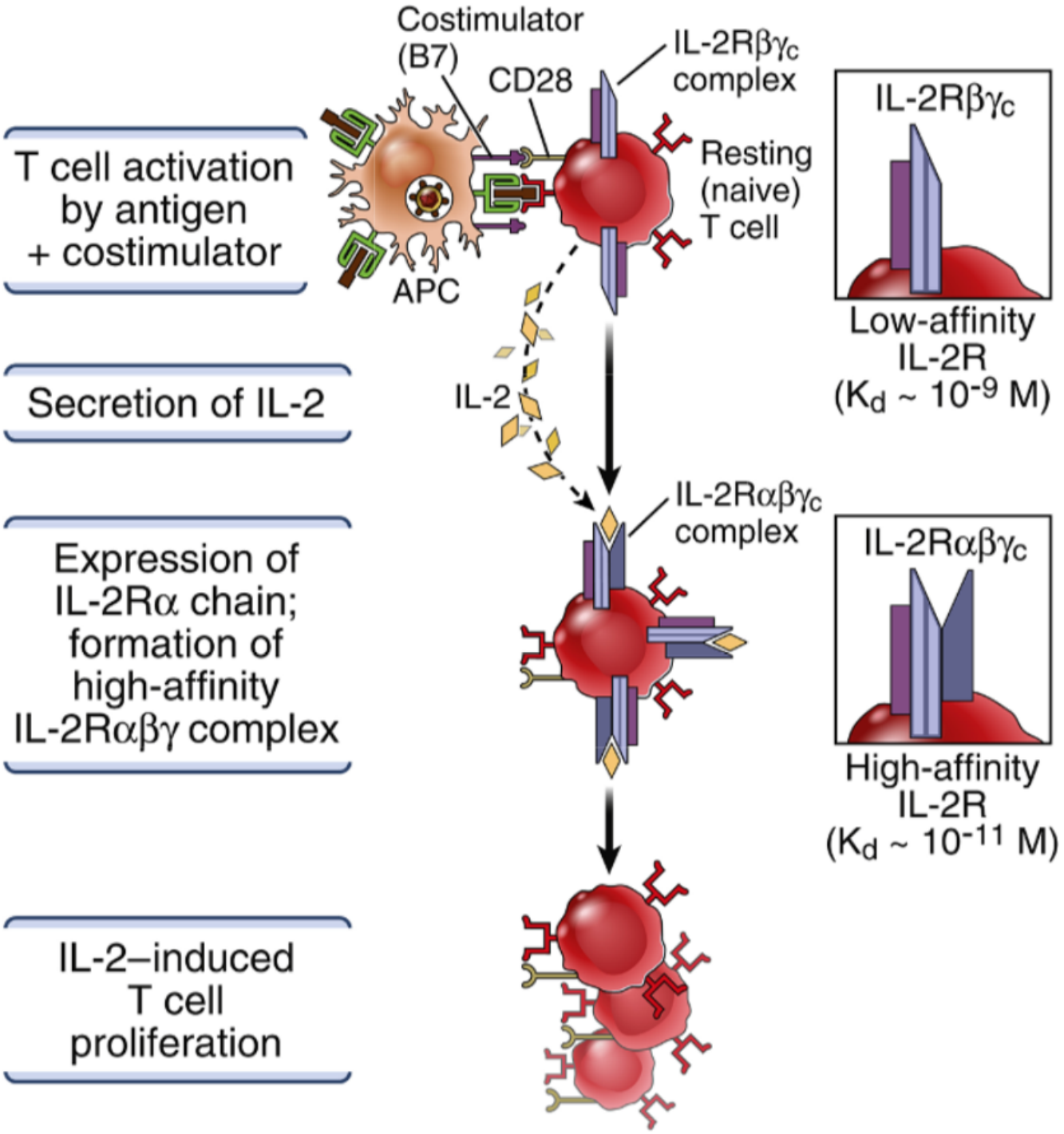
Those are:

- **IL-2, IL-4, IL-5, IF-γ**
- IL-6, IL-11, IL-13, IL-17,…

IL-2

- - Secreted by activated T-cells (autocrine and paracrine action)
- - The most potent lymphocyte proliferator
- - Promotes proliferation and differentiation of:
  - T – lymphocytes
  - B – lymphocytes
  - NK cells

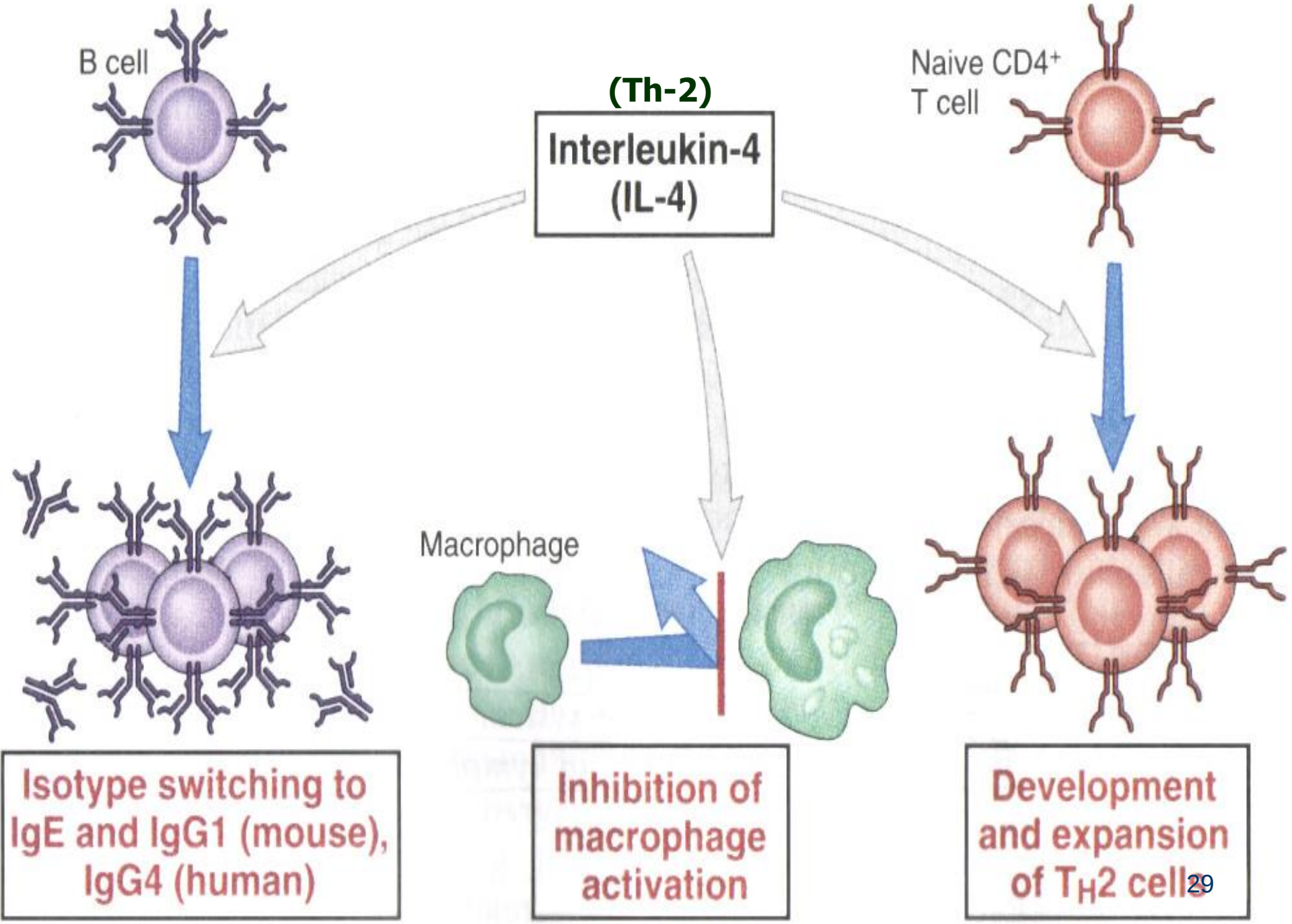

T cell
(Th)
Interleukin-2
(IL-2)
NK cell
B cell
T cell proliferation,
increased cytokine
production (IL-4, IFN-γ)
B cell proliferation,
antibody production
NK cell
proliferation, increased
cytolytic activity

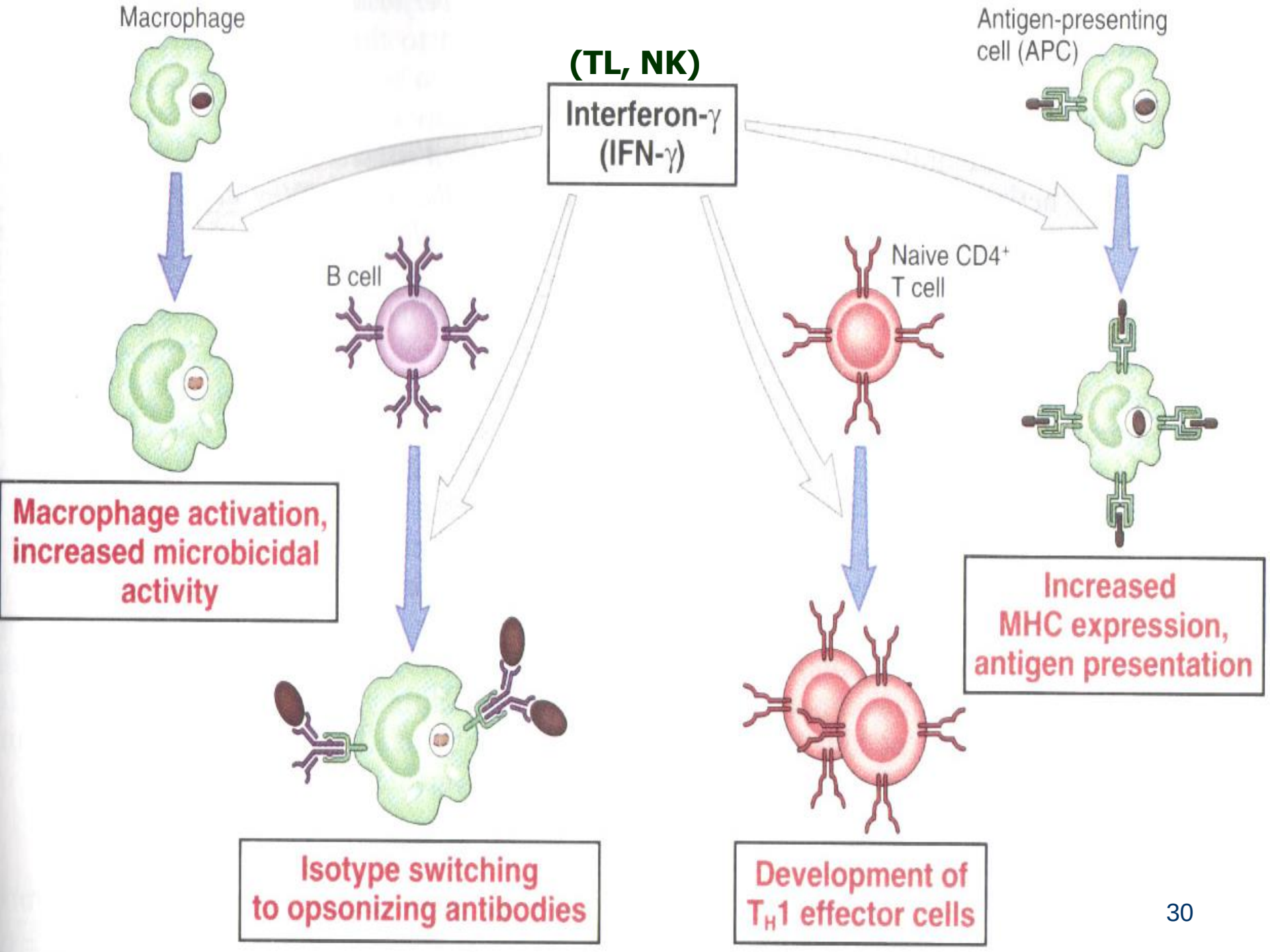

T cell activation by antigen + costimulator
Secretion of IL-2
Expression of IL-2Rα chain; formation of high-affinity IL-2Rαβγ complex
IL-2–induced T cell proliferation
Costimulator (B7)
CD28
IL-2Rβγc complex
Resting (naive) T cell
APC
IL-2
IL-2Rαβγc complex
IL-2Rβγc
Low-affinity IL-2R
(Kd ~ 10-9 M)
IL-2Rαβγc
High-affinity IL-2R
(Kd ~ 10-11 M)

- **IL-4:**
  - Produced by Th2
  - Stimulates the development of Th2
  - Stimulates IgE production
  - Supresses IFγ macrophare activation

- **IL-5:**
  - Produced by Th2 and basophiles
  - Activates eosinophiles
  - Stimulates differentiation of Th2

- **IL-6, IFγ**


B cell
(Th-2)
Interleukin-4
(IL-4)
Naive CD4+
T cell
Macrophage
Isotype switching to IgE and IgG1 (mouse), IgG4 (human)
Inhibition of macrophage activation
Development and expansion of TH2 cells


Macrophage
(TL, NK)
Antigen-presenting cell (APC)
Interferon-γ (IFN-γ)
B cell
Naive CD4+ T cell
Macrophage activation, increased microbicidal activity
Increased MHC expression, antigen presentation
Isotype switching to opsonizing antibodies
Development of TH1 effector cells

IMMUNOSUPPRESSIVE CYTOKINES

- produced by T-CD4+ (T_{reg}), macrophages
- Suppress macrophages and Th1

Those are:

- TGF-β, IL-10
- IL-4, IL-13,…

TGF-ß

- Inhibits lymphocyte T and macrophages, tissue
- Stimulates IgA secretion
- Stimulates production of extracellular matrix (healing and reparation)

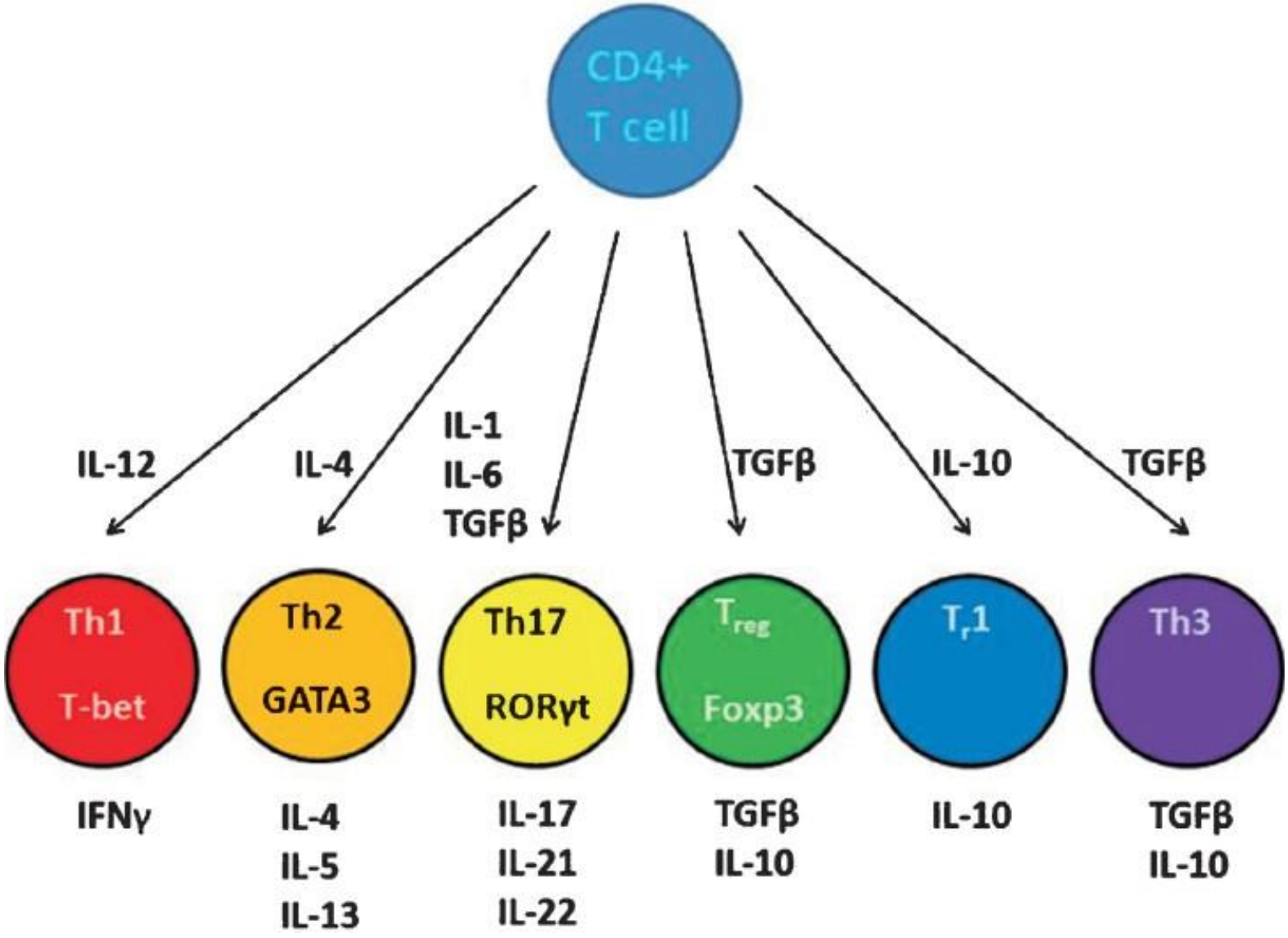
IL-10

- Inhibits macrophages

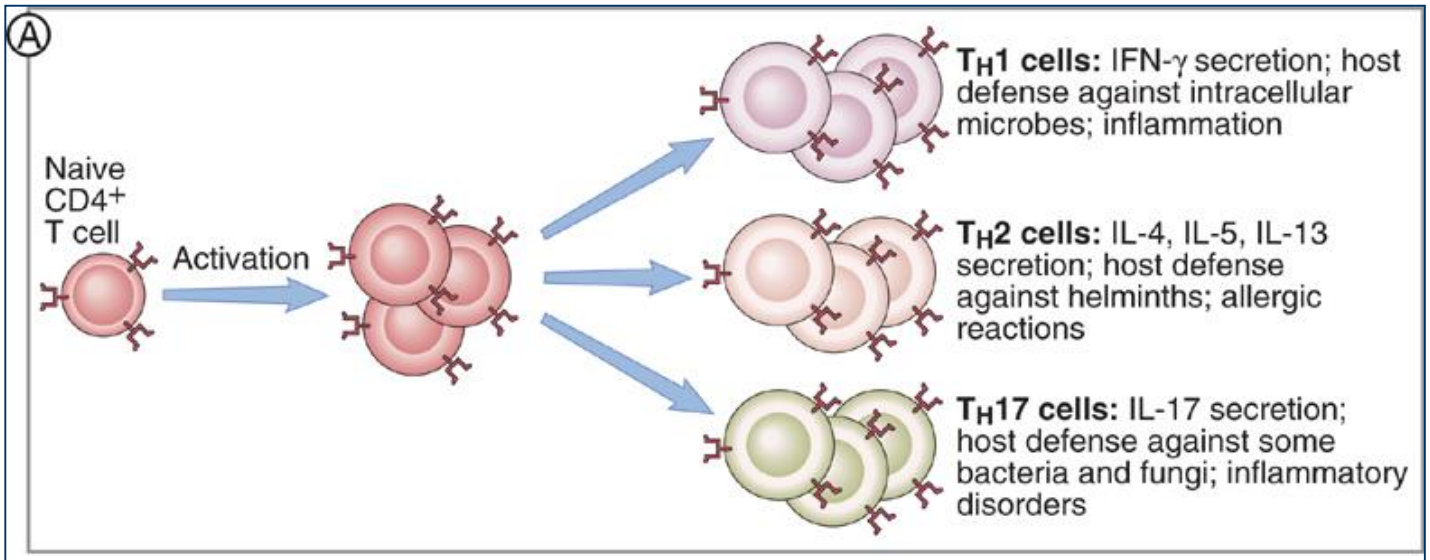
IL-4 and IL-13

- Inhibit cell-mediated and stimulate humoral immunity

T helper cell differentiation



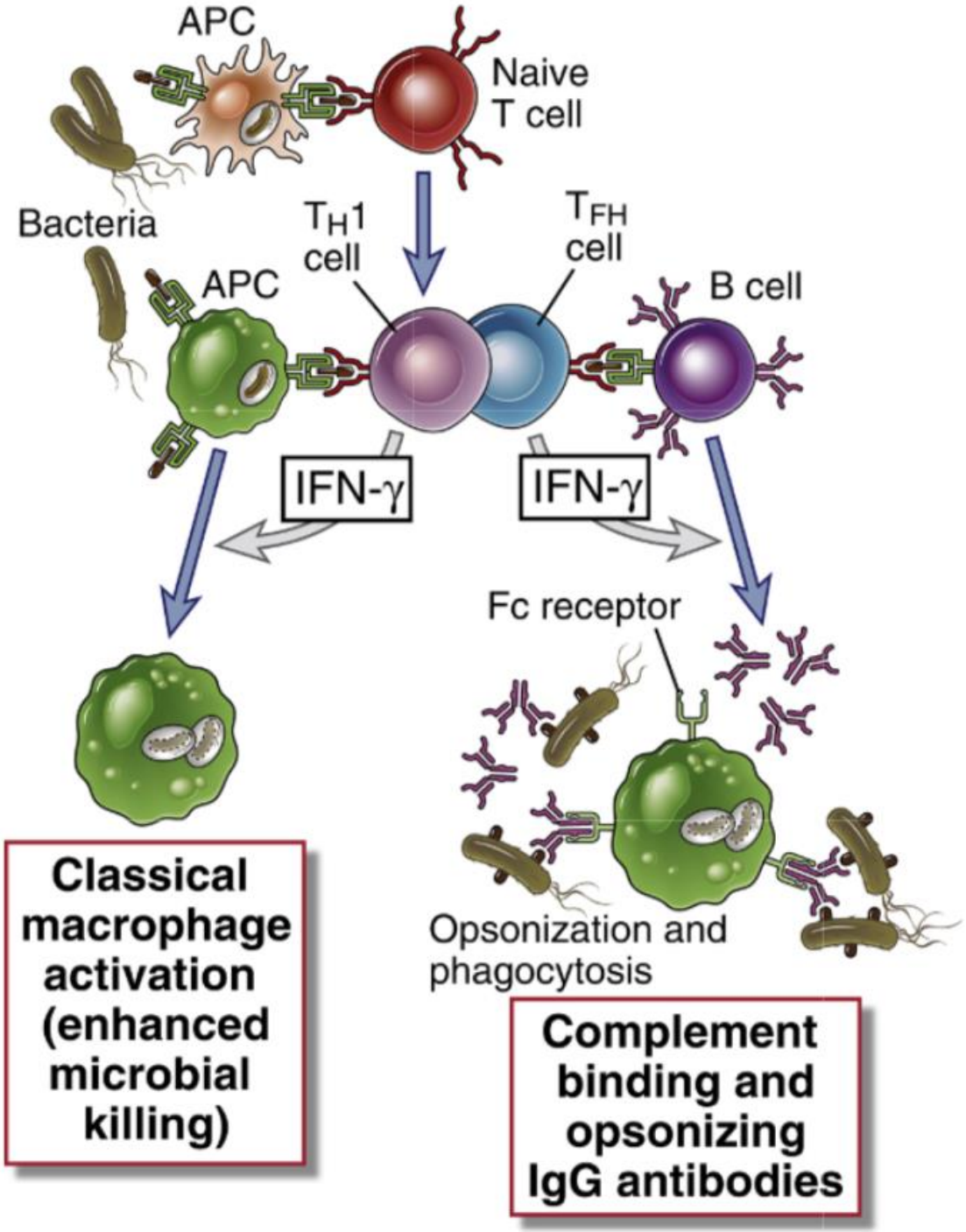
Th lymphocytes

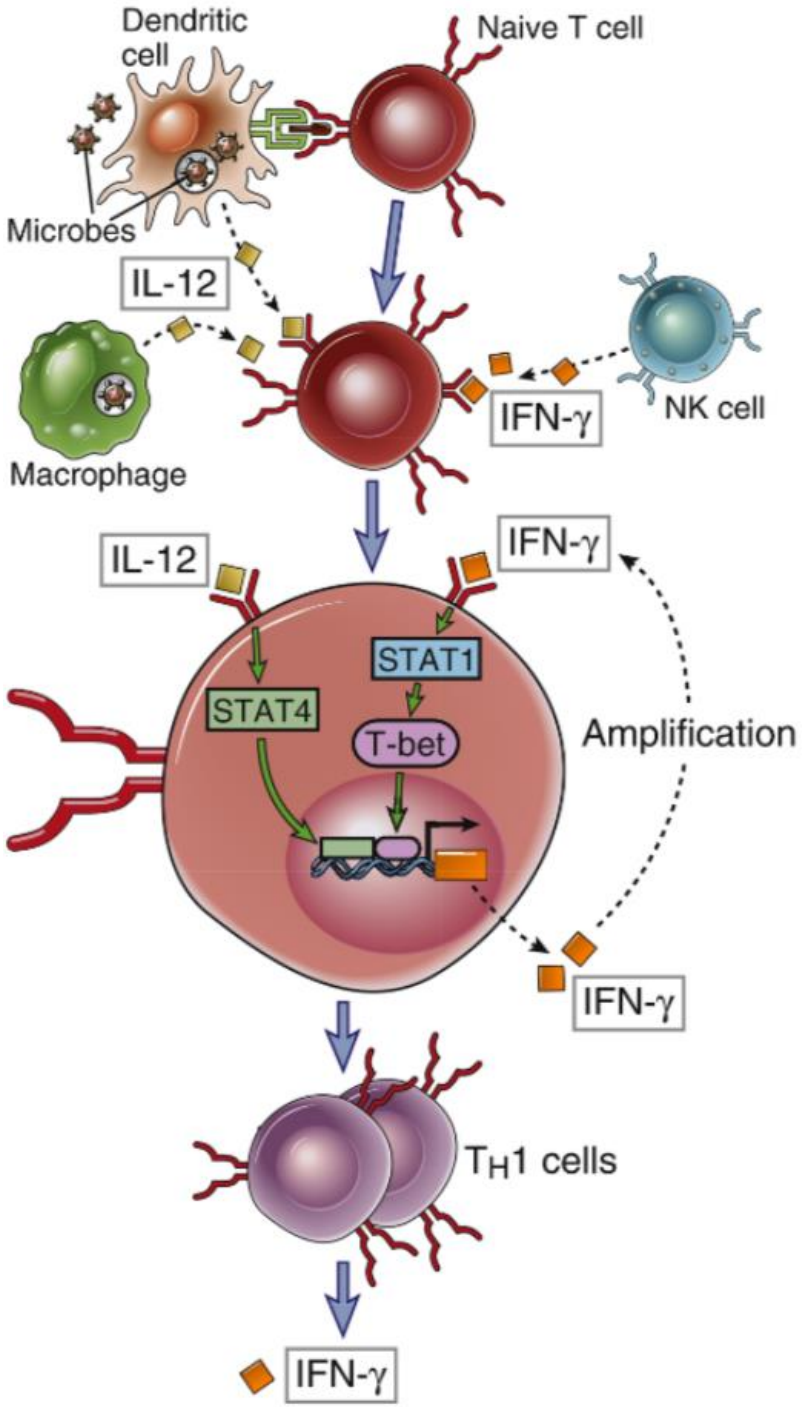


B

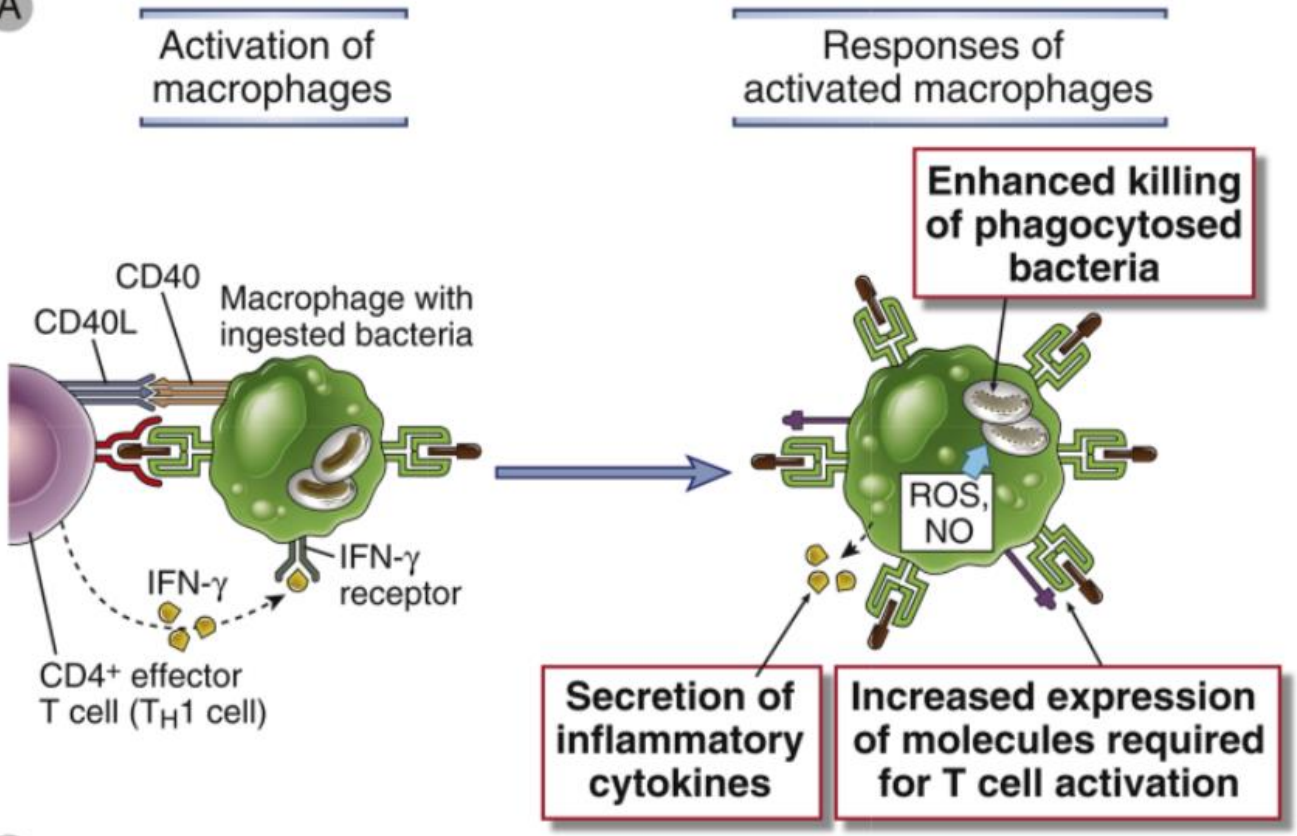
Property	T_H1	T_H2	T_H17
Principal cytokines produced	IFN-γ	IL-4, IL-5, IL-13	IL-17, IL-22
Antibody isotypes stimulated	Complement and Fc receptor-binding IgG subclasses such as IgG2a (mouse)	IgE; IgG1 (mouse), IgG4 (humans)	?
Macrophage activation	Classical (microbial killing)	Alternative (tissue repair)	?
Dominant leukocytes recruited	Monocytes	Eosinophils	Neutrophils, monocytes

Th1 cell function




Dendritic cell
Naive T cell
Microbes
IL-12
Macrophage
IFN-γ
NK cell
IL-12
IFN-γ
STAT1
STAT4
T-bet
Amplification
IFN-γ
TH1 cells
IFN-γ

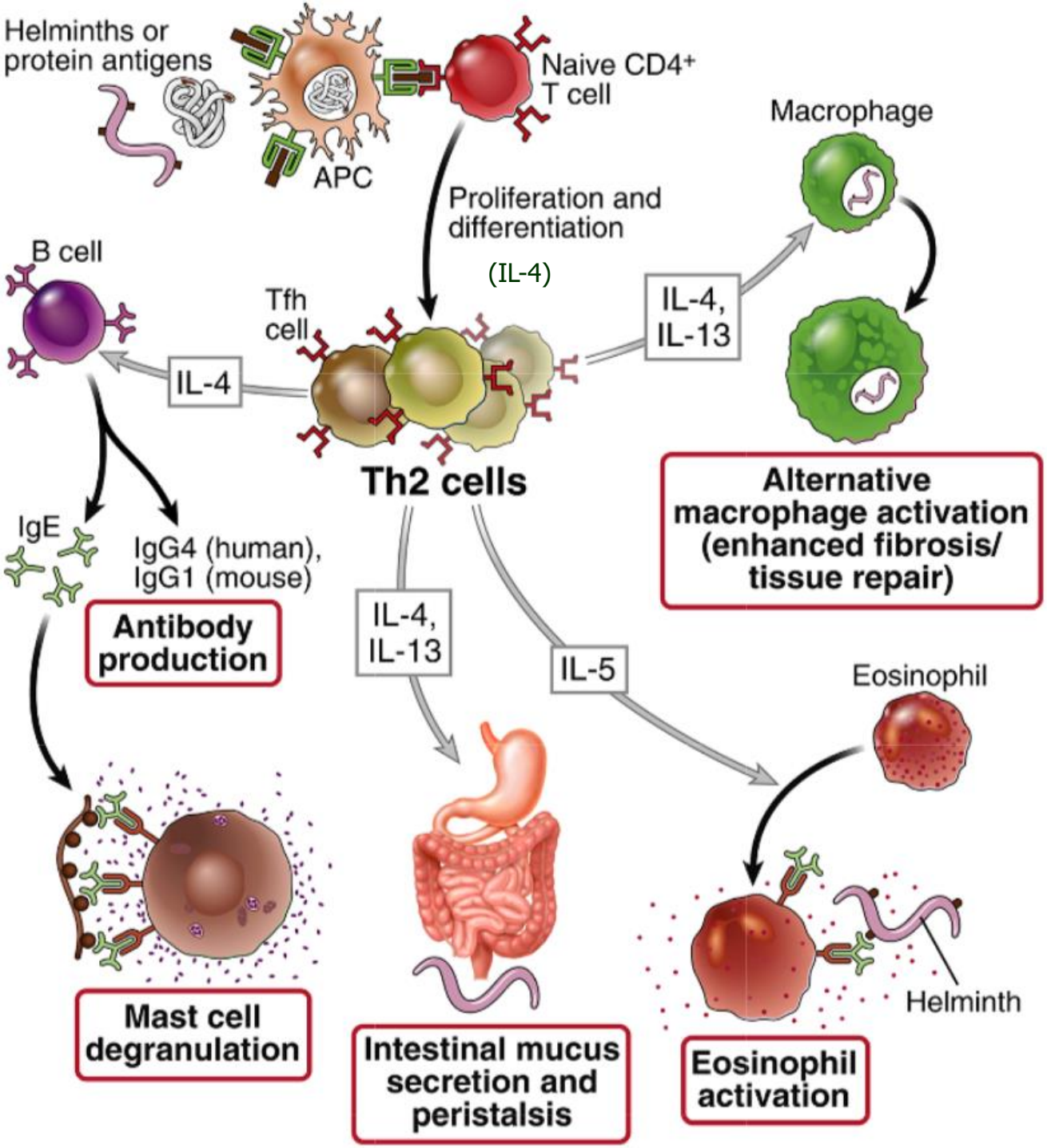
A

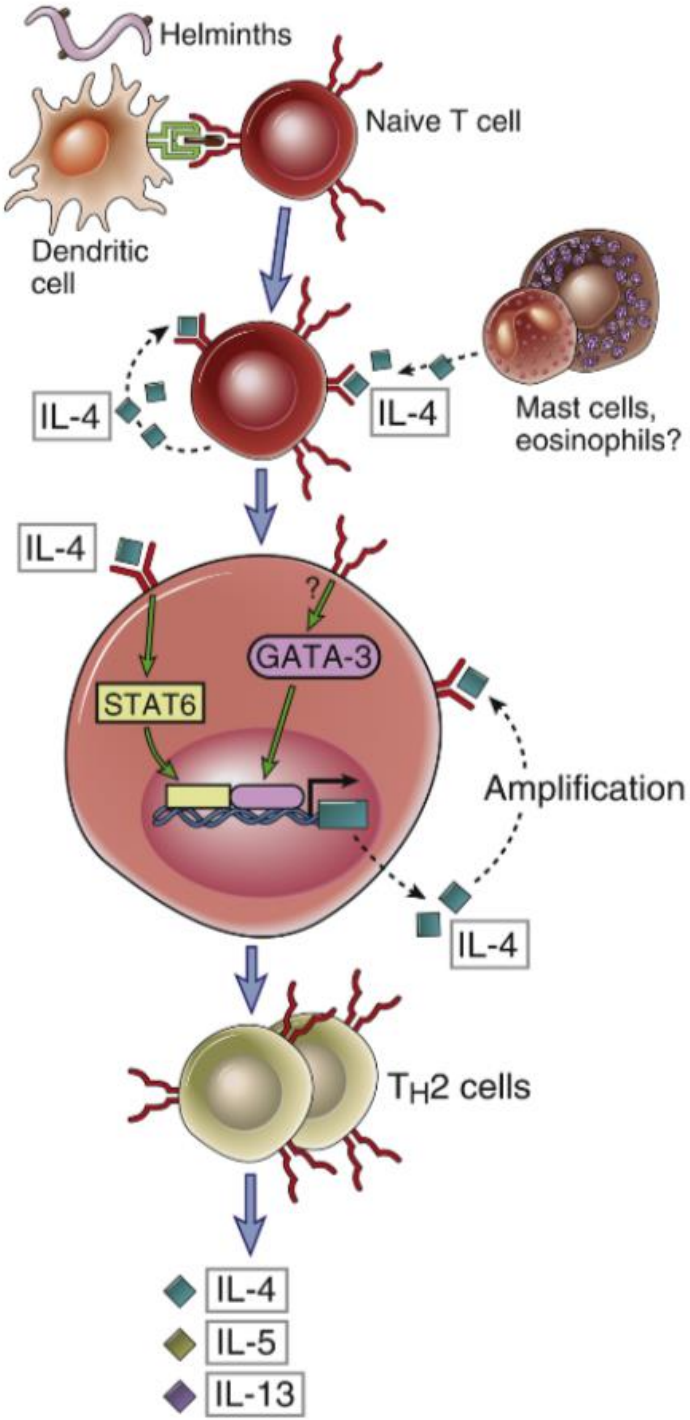


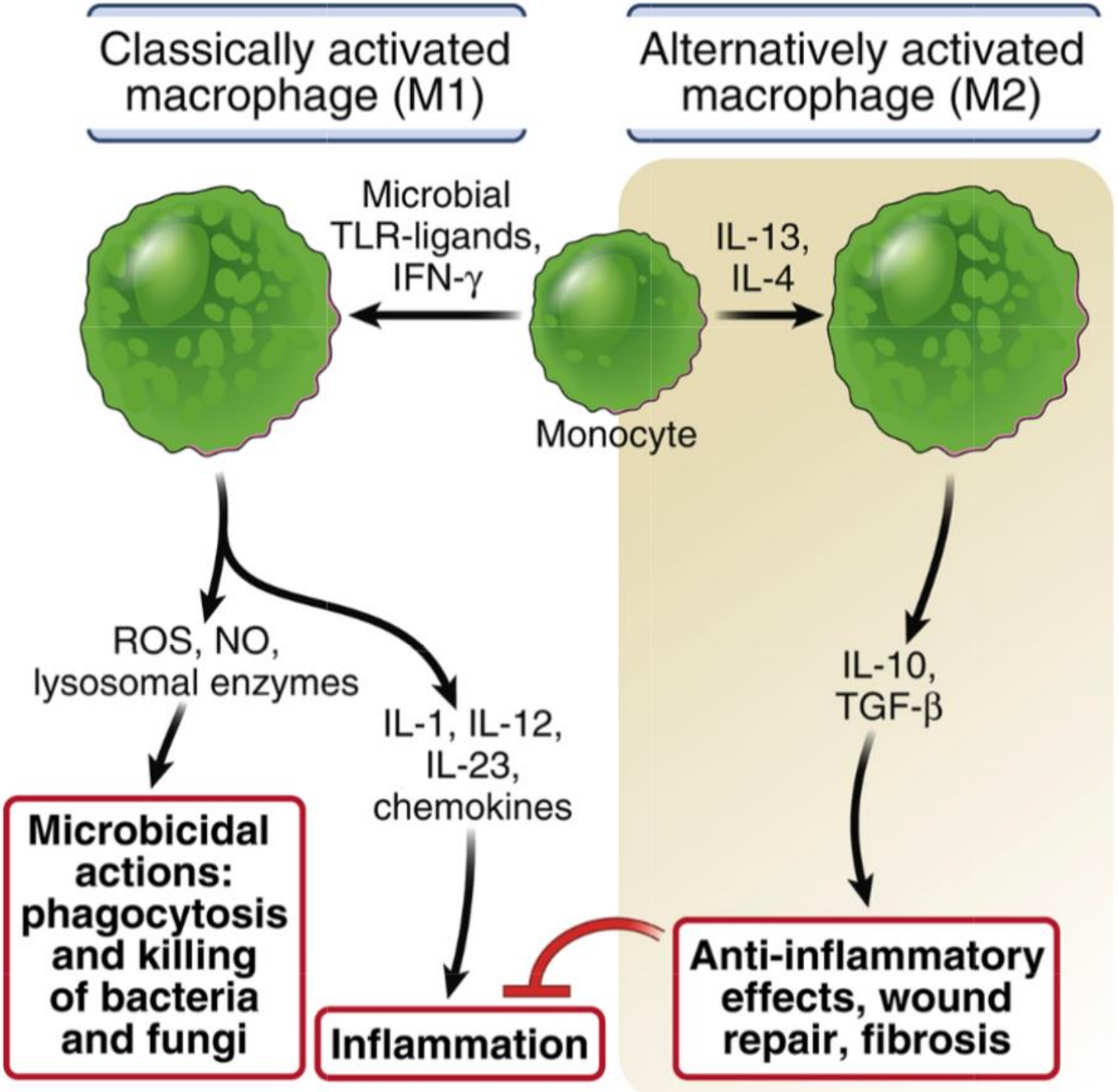
B

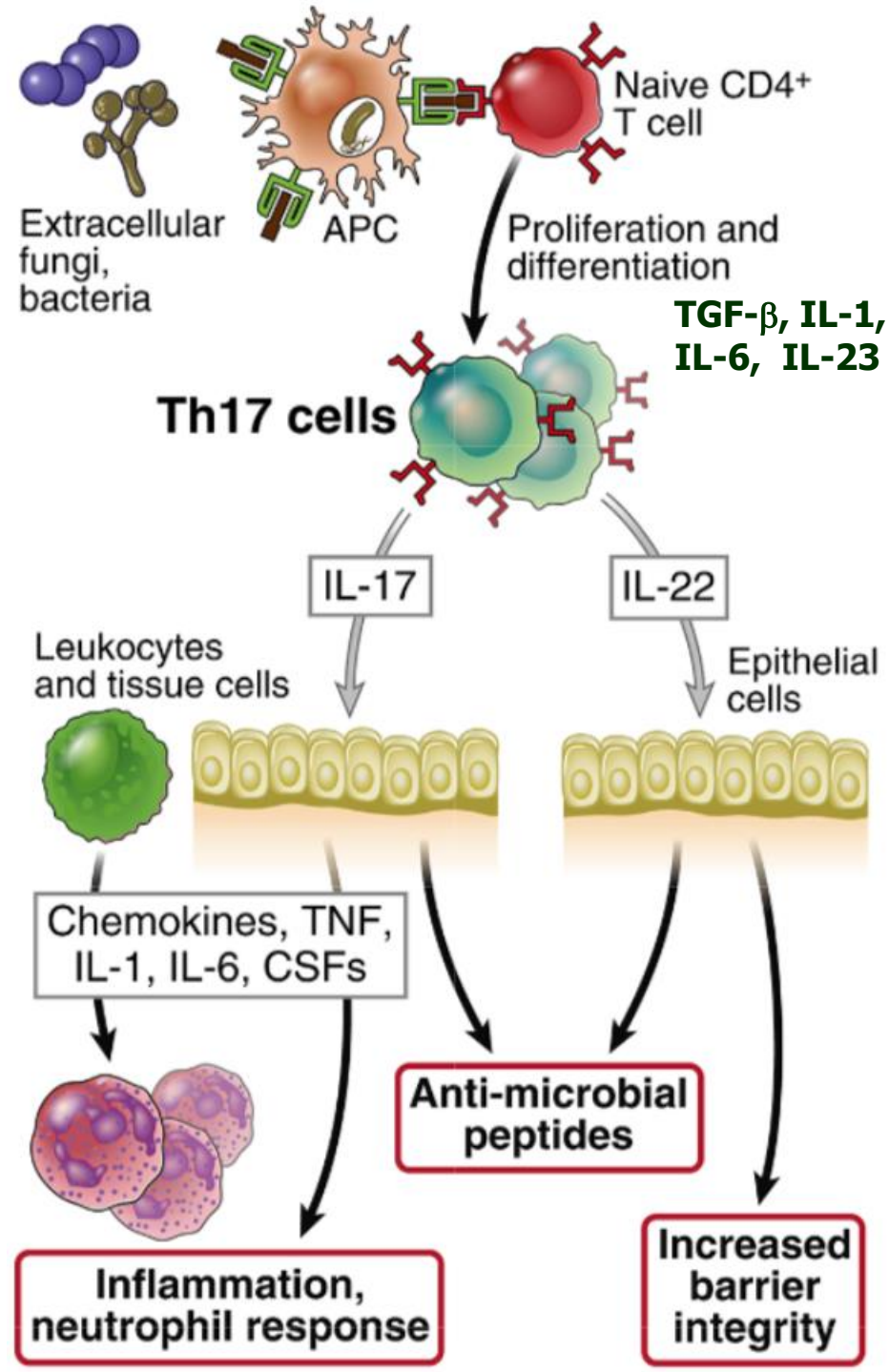
Macrophage response	Role in cell-mediated immunity
Production of reactive oxygen species, nitric oxide, increased lysosomal enzymes	Killing of microbes in phagolysosomes (effector function of macrophages)
Secretion of cytokines (TNF, IL-1, IL-12) and chemokines	TNF, IL-1, chemokines: leukocyte recruitment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

Th2 cell function




Helminths
Naive T cell
Dendritic cell
IL-4
IL-4
Mast cells, eosinophils?
IL-4
?
GATA-3
STAT6
Amplification
IL-4
TH2 cells
IL-4
IL-5
IL-13


Classically activated macrophage (M1)
Alternatively activated macrophage (M2)
Microbial TLR-ligands, IFN-γ
IL-13, IL-4
Monocyte
ROS, NO, lysosomal enzymes
IL-1, IL-12, IL-23, chemokines
IL-10, TGF-β
Microbicidal actions: phagocytosis and killing of bacteria and fungi
Inflammation
Anti-inflammatory effects, wound repair, fibrosis



Th-17 lymphocytes

Discovered in 2009.

Role in:

-host deffense against patogene

-(mucose and epithelia)

-tissue inflammation

-autoimmune diseases

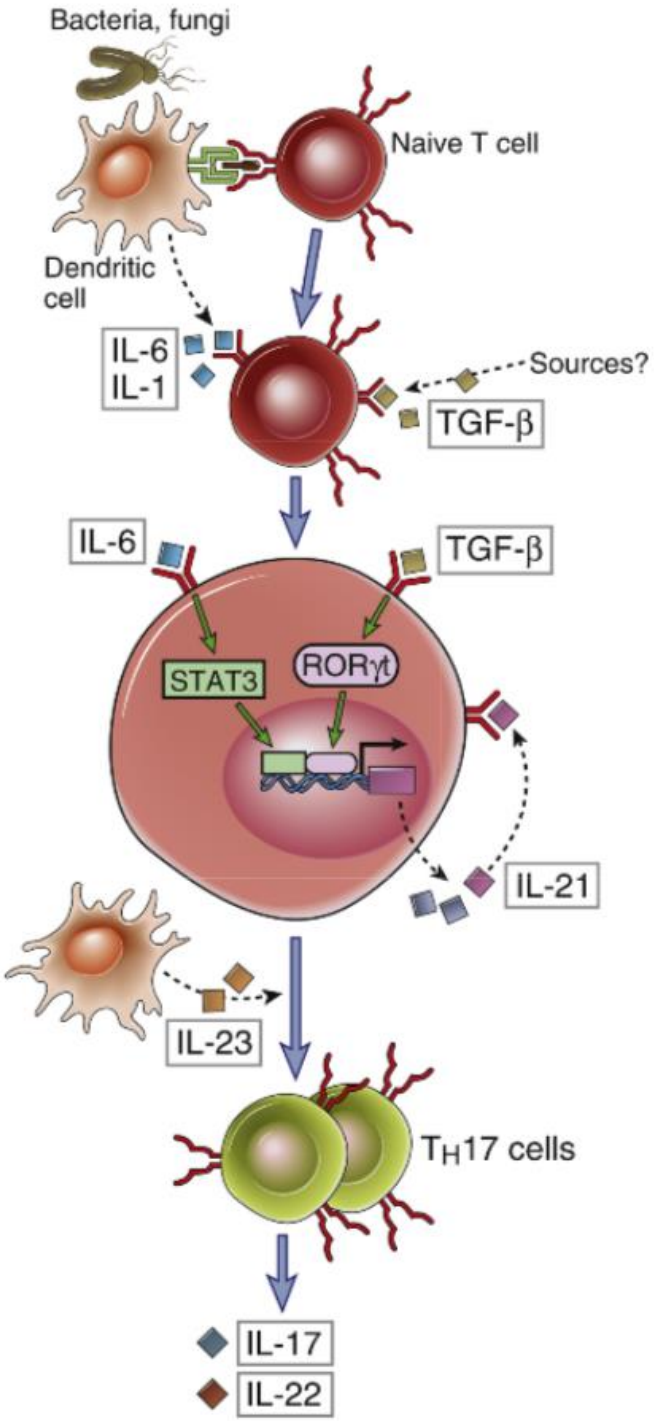
-Th17 insufficiency = oportunistic infections

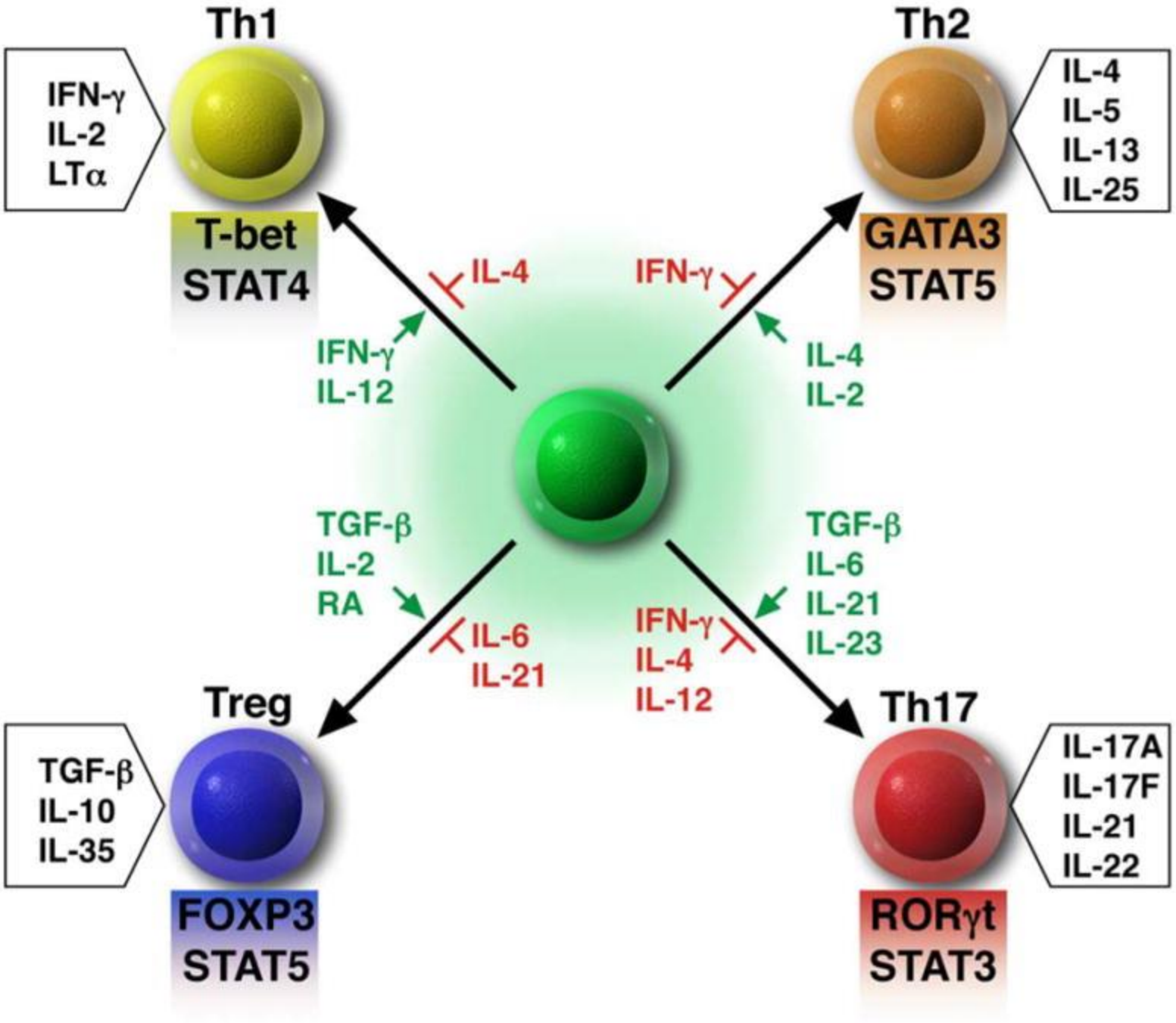
-IL-22 is a member of IL-10 family

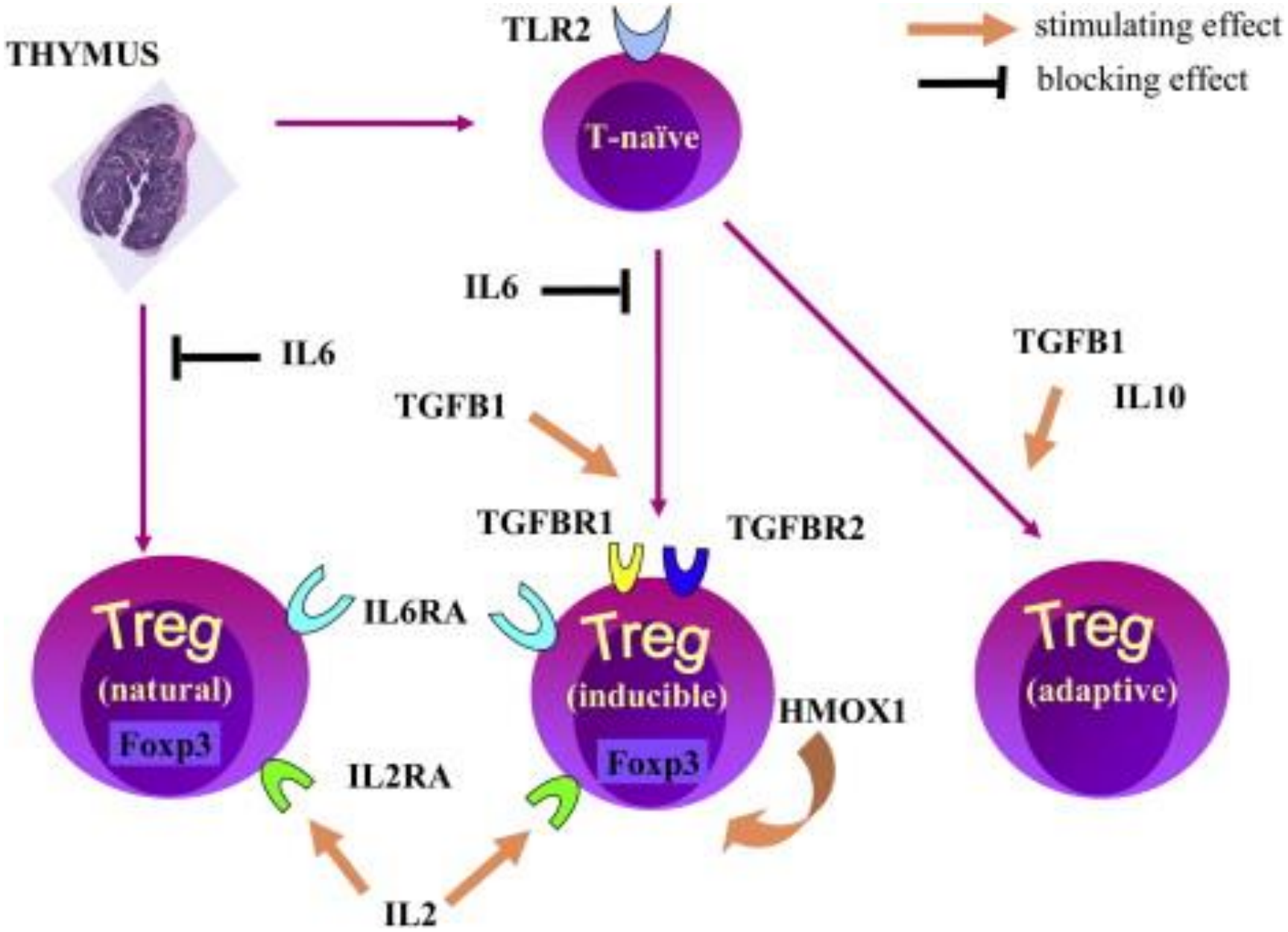
- **IL-17** + **IL-22** = proinflamatory and/or tissue protective function

-Th17 cells transdifferentiate into regulatory T cells during resolution of inflammation

(Nature 523, 221–225 (09 July 2015) doi:10.1038/nature14452)


Bacteria, fungi
Naive T cell
Dendritic cell
IL-6
IL-1
Sources?
TGF-β
IL-6
TGF-β
STAT3
RORγt
IL-21
IL-23
TH17 cells
IL-17
IL-22


Th1
IFN-γ
IL-2
LTα
T-bet
STAT4
Th2
IL-4
IL-5
IL-13
IL-25
GATA3
STAT5
IL-4
IFN-γ
IL-12
IFN-γ
IL-4
IL-2
TGF-β
IL-2
RA
IL-6
IL-21
TGF-β
IL-6
IL-21
IL-23
IFN-γ
IL-4
IL-12
Treg
TGF-β
IL-10
IL-35
FOXP3
STAT5
Th17
IL-17A
IL-17F
IL-21
IL-22
RORγt
STAT3



DOI: http://dx.doi.org/10.1016/j.jaci.2010.04.024

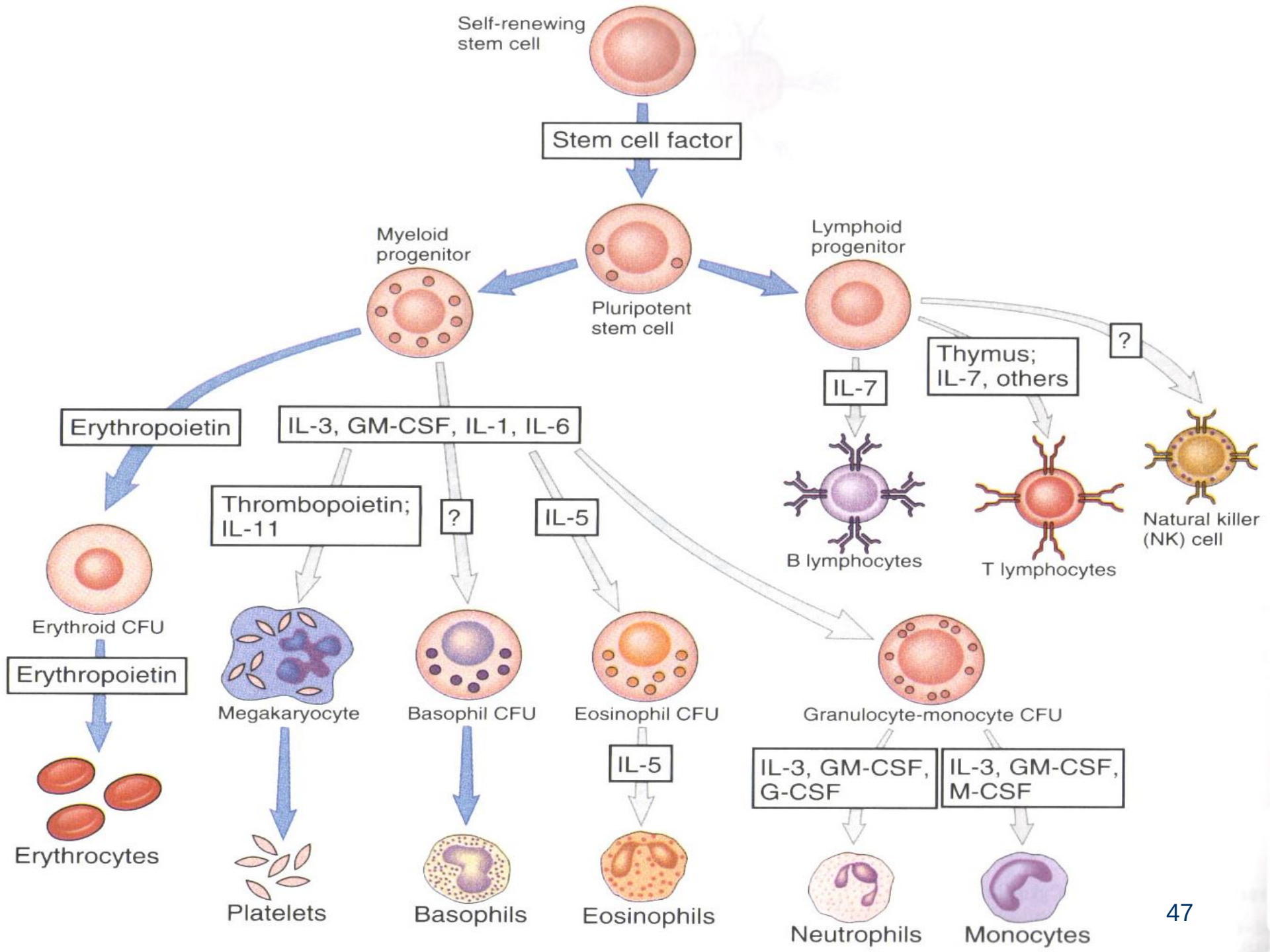
IPEX syndrome

(immunodysregulation polyendocrinopathy enteropathy X-linked *syndrome)*

- Disfunction of the FOXP3 transcription factor
- autoimmune enteropathy, psoriasiform or eczematous dermatitis, nail dystrophy, autoimmune endocrinopathies
- Autoimmune diseases (attacks from immune system against the body's own tissues and organs)

HEMATOPOIETIC CYTOKINES (CSF)

- **G-CSF, GM-CSF**
  - Produced by T-cells, macrophages and endothelial cells from infected tissue
- **IL-7**
  - Produced by bone marrow stromal cells
  - Stimulates proliferation of pre-T and B cells
- **IL-3 (multi CSF)** stimulates all progenitor cells
  - Produced by T helper
- **IL-1, IL-5, IL-6, erythropoietin,** …


Self-renewing stem cell
Stem cell factor
Pluripotent stem cell
Myeloid progenitor
Lymphoid progenitor
Erythropoietin
IL-3, GM-CSF, IL-1, IL-6
IL-7
Thymus; IL-7, others
?
Thrombopoietin; IL-11
?
IL-5
Erythroid CFU
Erythropoietin
Erythrocytes
Megakaryocyte
Platelets
Basophil CFU
Basophils
Eosinophil CFU
IL-5
Eosinophils
Granulocyte-monocyte CFU
IL-3, GM-CSF, G-CSF
IL-3, GM-CSF, M-CSF
Neutrophils
Monocytes
B lymphocytes
T lymphocytes
Natural killer (NK) cell