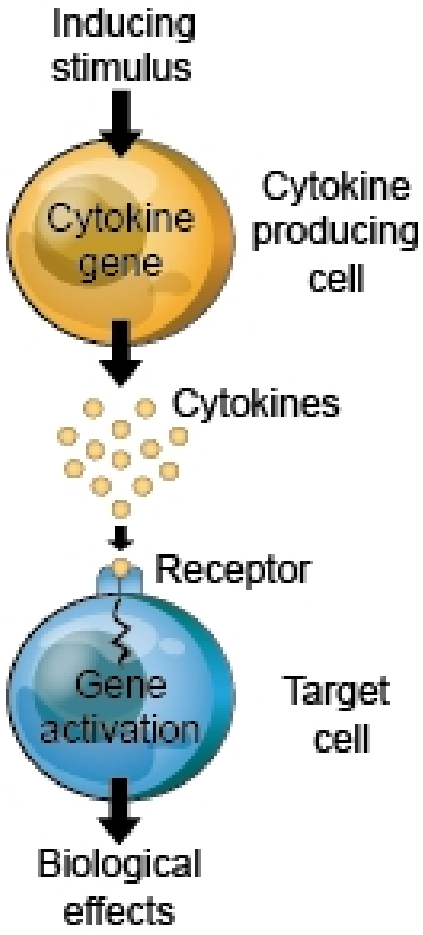
Cytokines and Cytokine storm

Cytokines

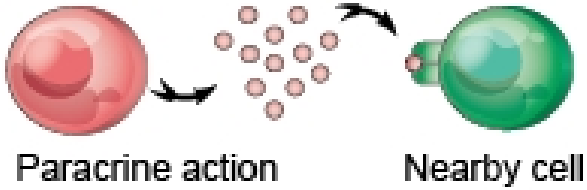
- A large family of proteins (more than 60) critical for the communication between immune and haematopoietic cells, as well as cells of the microenvironment
- Low-molecular-weight proteins (~25kDa) secreted by various types of immune as well as stromal cells in response to an activating stimulus
- Exert several biological activities through binding to cognate receptors on cell surface

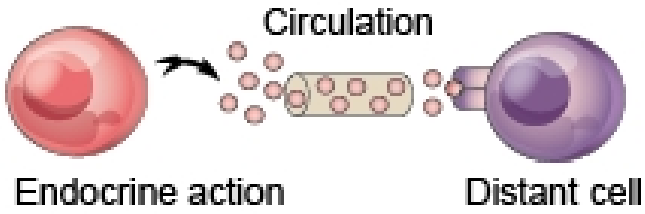


Action mode of cytokines

- Cytokines can act in an **autocrine** manner, affecting the behavior of the cell that releases the cytokine, or in a **paracrine** manner, affecting adjacent cells

- Some cytokines are stable enough to act in an **endocrine** manner, affecting distant cells (ability to enter the circulation and half-life in the blood)





Classification of cytokines

❖ Based on their structure cytokines are grouped into 5 families:

- the IL-1 family
- the hematopoietin superfamily
- the interferons
- the TNF family
- the chemokine family

❖ **IL-1 family**

- consists of 11 members (e.g. IL-1α, IL-β, IL-18)
- most members are produced as inactive proproteins
- proteolytic cleavage leads to the formation of the mature form
- bind to IL-1-family receptors and signal through the NFκB pathway

❖ Hematopoietin family

- non-immune-system growth and differentiation factors (e.g. erythropoietin), growth hormones, interleukins with roles in innate and adaptive immunity (IL-6, GM-CSF)
- bind to tyrosine kinase-associated receptors that form dimers upon ligand binding and intracellular signaling is initiated

❖ Interferons

- the first family of cytokines to be discovered
- named for their ability to "interfere" with viral replication
- classified into three major types : type I (IFN-α, IFN-β), type II (IFN-γ) and type III (IFN-λ)
- very important role in anti-viral defense
- interferon receptors signal through the JAK–STAT pathway

❖ TNF family

- contains more than 17 cytokines (TNF-α, lymphotoxins, CD40L)
- important functions in innate and adaptive immunity
- mainly transmembrane but also found in soluble form
- TNFRI and II receptors signal through the NFκB pathway

❖ Chemokine family

- chemoattractant cytokines with small molecular weight (8-10kDa)
- more than 50 known chemokines
- 4 subfamilies: CXC, CC, lymphotactin (XC) and fractalkine (CX_3C)
- recruitment of leukocyte subsets under homeostatic and pathological conditions
- signal through 7-transmembrane G-protein coupled receptors ⟶ changes in cell adhesiveness and in the cell's cytoskeleton ⟶ migration

TABLE 3.2 Selected Chemokines and Chemokine Receptors

Chemokine	Original Name	Chemokine Receptor	Major Function
CC Chemokines			
CCL2	MCP-1	CCR2	Mixed leukocyte recruitment
CCL3	MIP-1α	CCR1, CCR5	Mixed leukocyte recruitment
CCL4	MIP-1β	CCR5	T cell, dendritic cell, monocyte, and NK recruitment; HIV coreceptor
CCL5	RANTES	CCR1, CCR3, CCR5	Mixed leukocyte recruitment
CCL11	Eotaxin	CCR3	Eosinophil, basophil, and Th2 recruitment
CCL17	TARC	CCR4	T cell recruitment
CCL19	MIP-3β/ELC	CCR7	T cell and dendritic cell migration into parafollicular zones of lymph nodes
CCL21	SLC	CCR7	T cell and dendritic cell migration into parafollicular zones of lymph nodes
CCL22	MDC	CCR4	NK cell, T cell recruitment
CCL25	TECK	CCR9	Lymphocyte recruitment into intestine
CCL27	CTACK	CCR10	T cell recruitment into skin
CXC Chemokines			
CXCL1	GROα	CXCR2	Neutrophil recruitment
CXCL8	IL-8	CXCR1, CXCR-2	Neutrophil recruitment
CXCL9	Mig	CXCR3	Effector T cell recruitment
CXCL10	IP-10	CXCR3	Effector T cell recruitment
CXCL12	SDF1	CXCR4	B cell migration into lymph nodes; plasma cell migration into bone marrow
CXCL13	BCA-1	CXCR5	B cell migration into lymph nodes and into follicles; T follicular helper cell migration into follicles
C Chemokines			
XCL1	Lymphotactin	XCR1	T cell and NK cell recruitment
CX3C Chemokines			
CX3CL1	Fractalkine	CX3CR1	T cell, NK cell, and monocyte recruitment

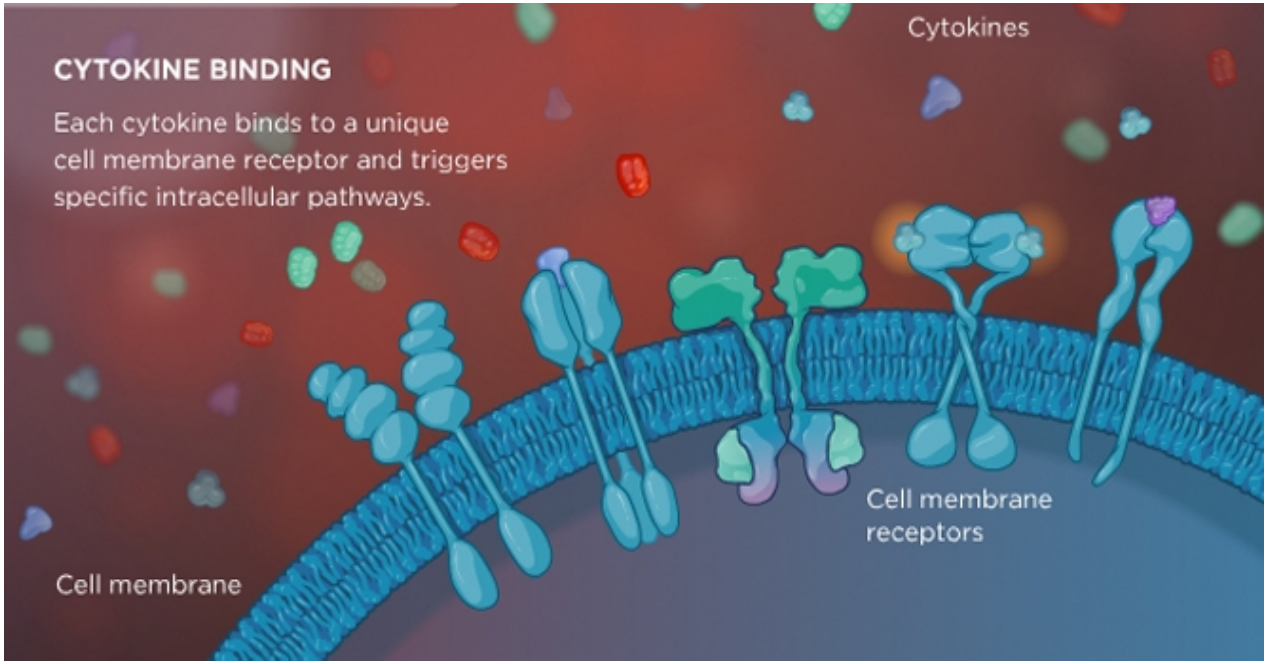
CTL, Cytotoxic T lymphocyte; *IL*, Interleukin; *NK*, natural killer cells.

Cytokine Receptors

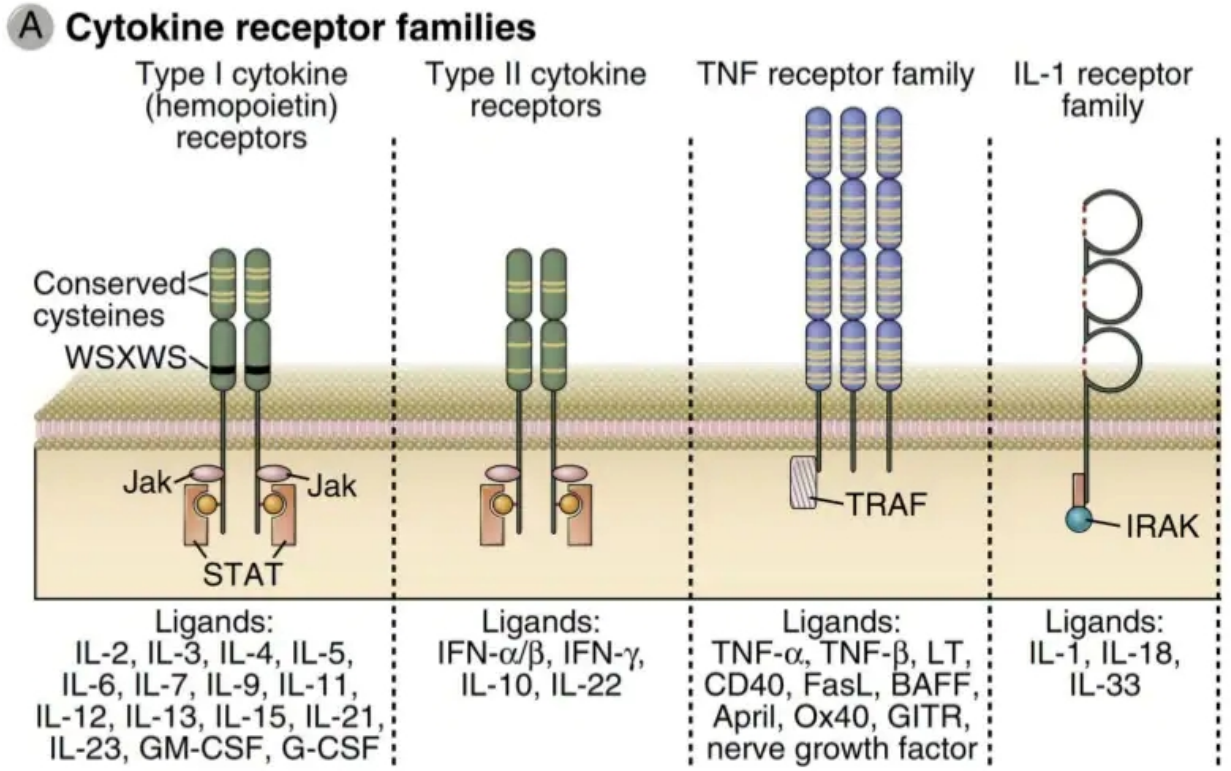
- Binding of cytokines with their receptors initiates complex intracellular molecular interactions that ultimately cause changes in cellular gene transcription

- The vast majority of known cytokine receptors are transmembrane proteins, consisting of extracellular, transmembrane, and cytoplasmic regions

- The extracellular membrane region is the site that recognizes binding cytokines and the cytoplasmic region initiates signal transduction after receptor activation



Classes of Cytokine receptors



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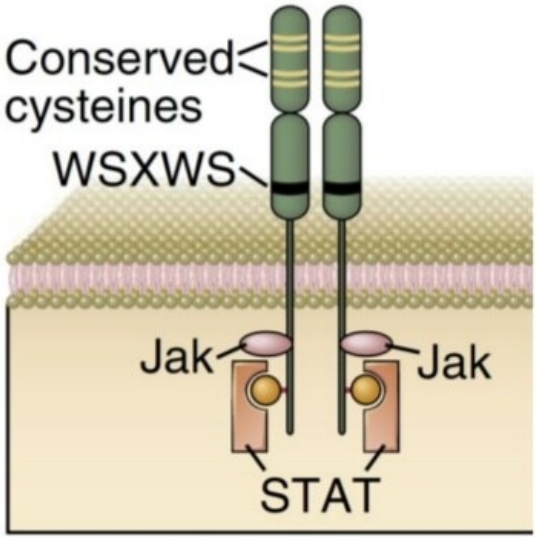
Type I Cytokine Receptors (Hematopoietin Receptor Family)



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

- Dimers or trimers
- Contain 1 or 2 domains with a conserved cysteines
- Proximal peptide stretch containing a tryptophan-serine-X-tryptophan-serine (WSXWS) motif (X = amino acid)

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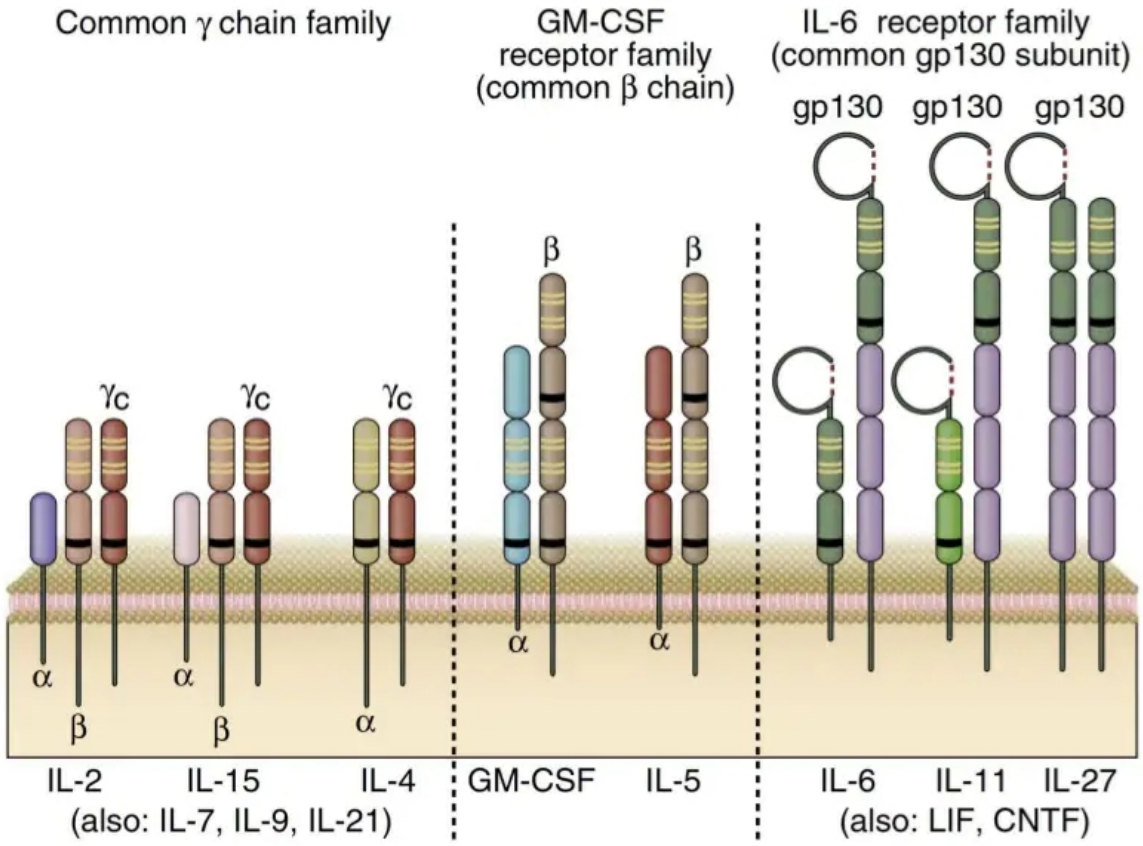
Subunit composition of cytokine receptors



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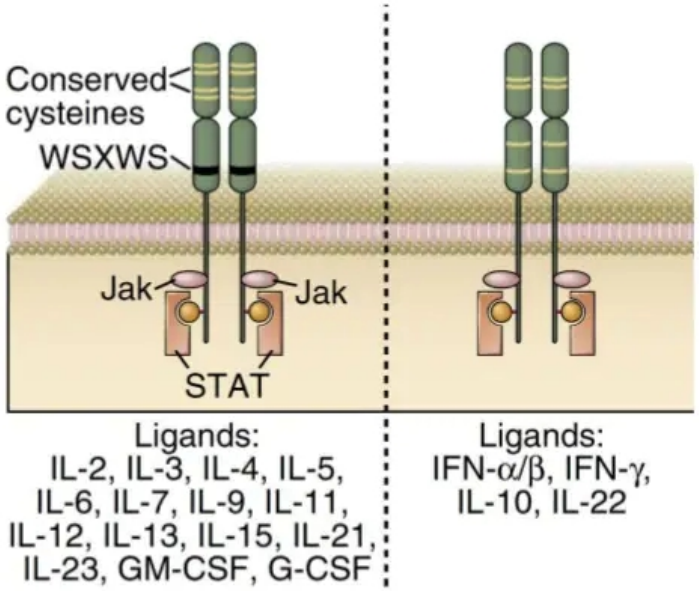
- Divided into subgroups based on structural homologies or use of shared signaling polypeptides
- Common γ chain (CD132)
  - Receptors for IL-2, IL-4, IL-7, IL-9, IL-15, IL-21
- Common β chain (CD131) subunit
  - Receptors for IL-3, IL-5, GM-CSF
- Common gp130 signaling component
  - For IL-6, IL-11, IL-27, LIF, CNTF

Type II Cytokine Receptors (IFN Receptor Family)

- 2 extracellular domains with conserved cysteines
- Do not contain WSXWS motif
- Signaling through type I, type II cytokine receptor: JAK-STAT signaling



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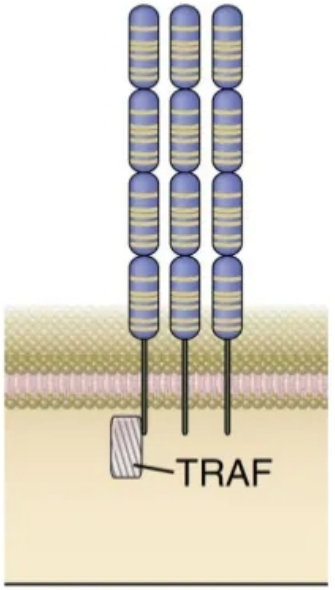
TNF Receptor Family

- Preformed trimers
- Conserved cysteine-rich extracellular domains
- Shared intracellular signaling mechanisms
- TNFRI & TNFRII, CD40 protein, Fas, lymphotoxin receptor & BAFF receptor family



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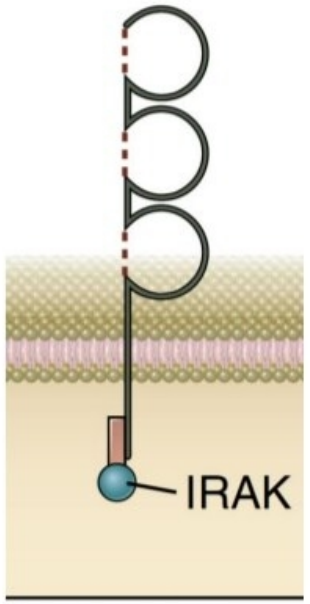
IL-1/TLR Family

- Share a conserved cytosolic sequence, called Toll-like/IL-1 receptor (TIR) domain → engage similar signal transduction pathways → induce new gene transcription
- Adaptors link TLRs to different IRAK (IL-1R–associated kinase)



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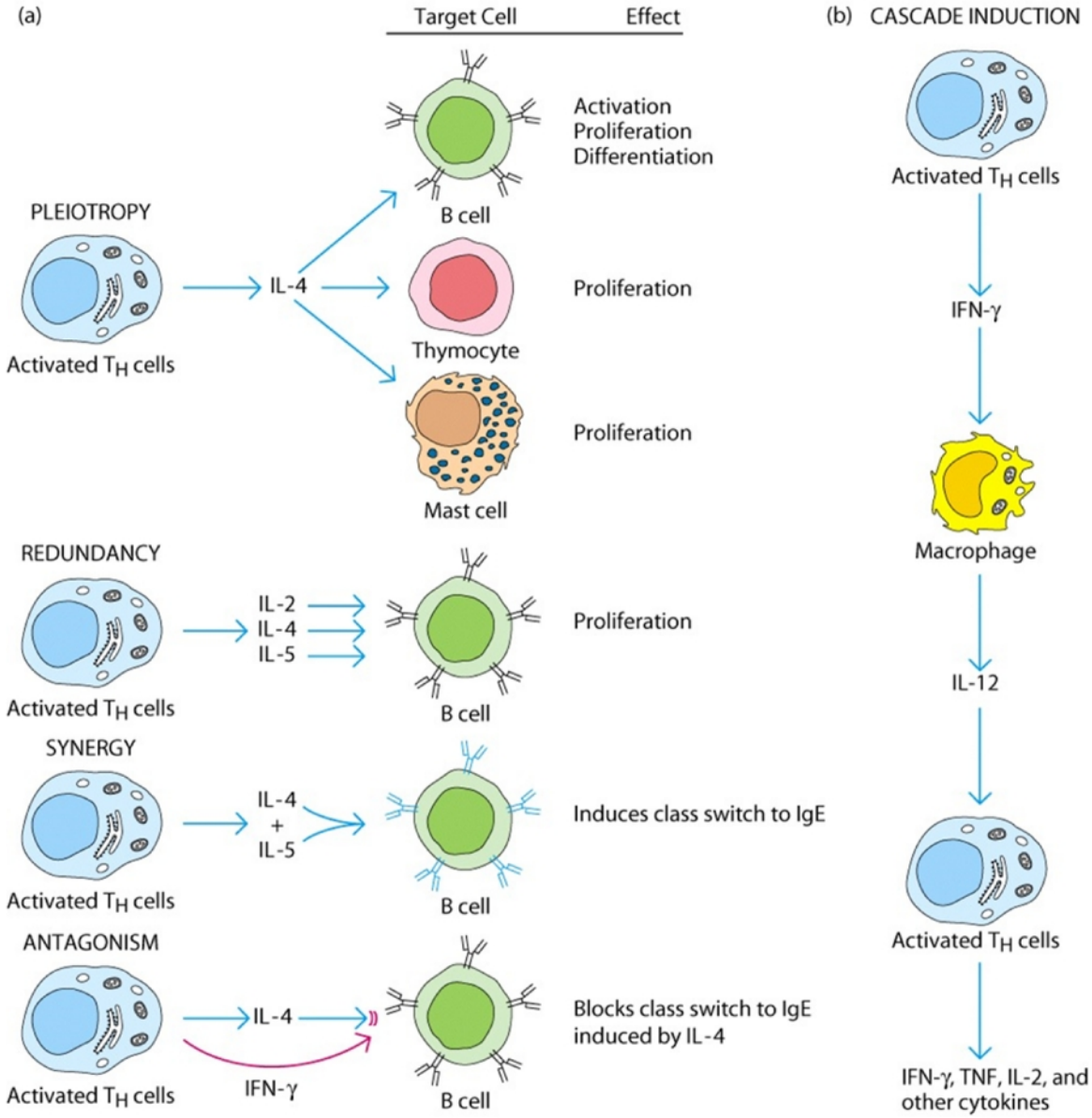
Action of Cytokines

- **Pleiotropy**
  - Affects multiple cell types
- **Redundancy**
  - Multiple cytokines affect cells of the same type
- **Synergy**
  - Cytokines acting in concert on the same cell
- **Antagonism**
  - Competing actions
- **Cascading**
  - Cytokines acting sequentially


(a)
Target Cell
Effect
PLEIOTROPY
Activated TH cells
IL-4
B cell
Activation
Proliferation
Differentiation
Thymocyte
Proliferation
Mast cell
Proliferation
REDUNDANCY
Activated TH cells
IL-2
IL-4
IL-5
B cell
Proliferation
SYNERGY
Activated TH cells
IL-4
+
IL-5
B cell
Induces class switch to IgE
ANTAGONISM
Activated TH cells
IL-4
IFN-γ
B cell
Blocks class switch to IgE
induced by IL-4
(b) CASCADE INDUCTION
Activated TH cells
IFN-γ
Macrophage
IL-12
Activated TH cells
IFN-γ, TNF, IL-2, and
other cytokines

SHARED CYTOKINE RECEPTORS SUBUNITS

Shared Cytokine-Receptor Subunits	
Shared receptor chain	**Cytokines recognized**
γ_c	IL-2, -4, -7, -9, -15, -21
IL-2Rβ	IL-2, IL-15
IL-4Rα	IL-4, -13
IL-13Rα1	IL-4, -13
β_c	IL-3, -5, GM-CSF
gp130	IL-6, -11, -27, -31, LIF, OSM, CNTF, CT-1, CLC
IL-12Rβ1	IL-12, -23
IL-10R2	IL-10, -22
IL-20R2	IL-20, -19, -24
IL-22R	IL-22, -24, -20

- Sharing of signal transducing molecules explains the redundancy and antagonism exhibited by some cytokines

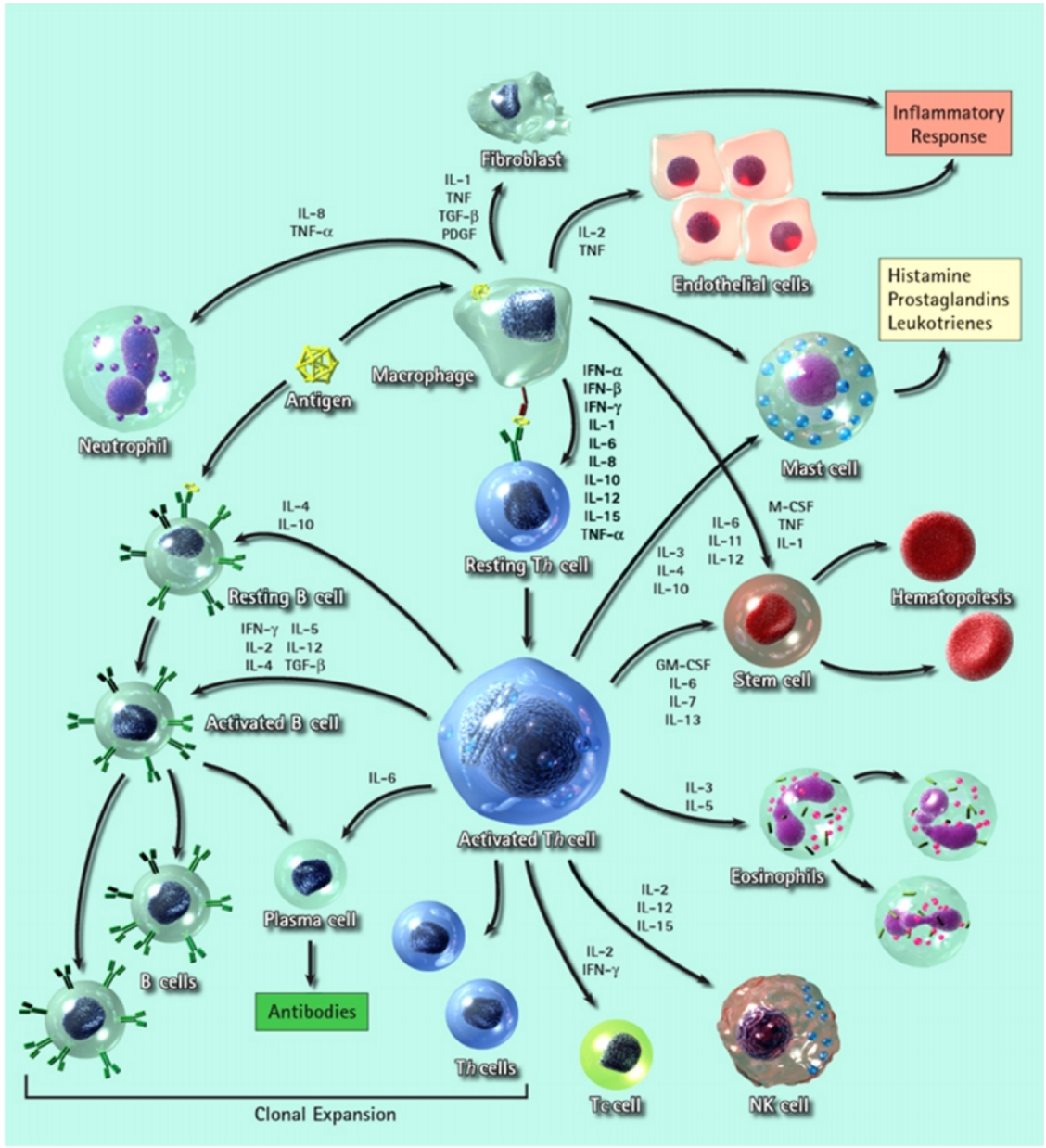
TABLE 12-1 Functional groups of selected cytokines[1]

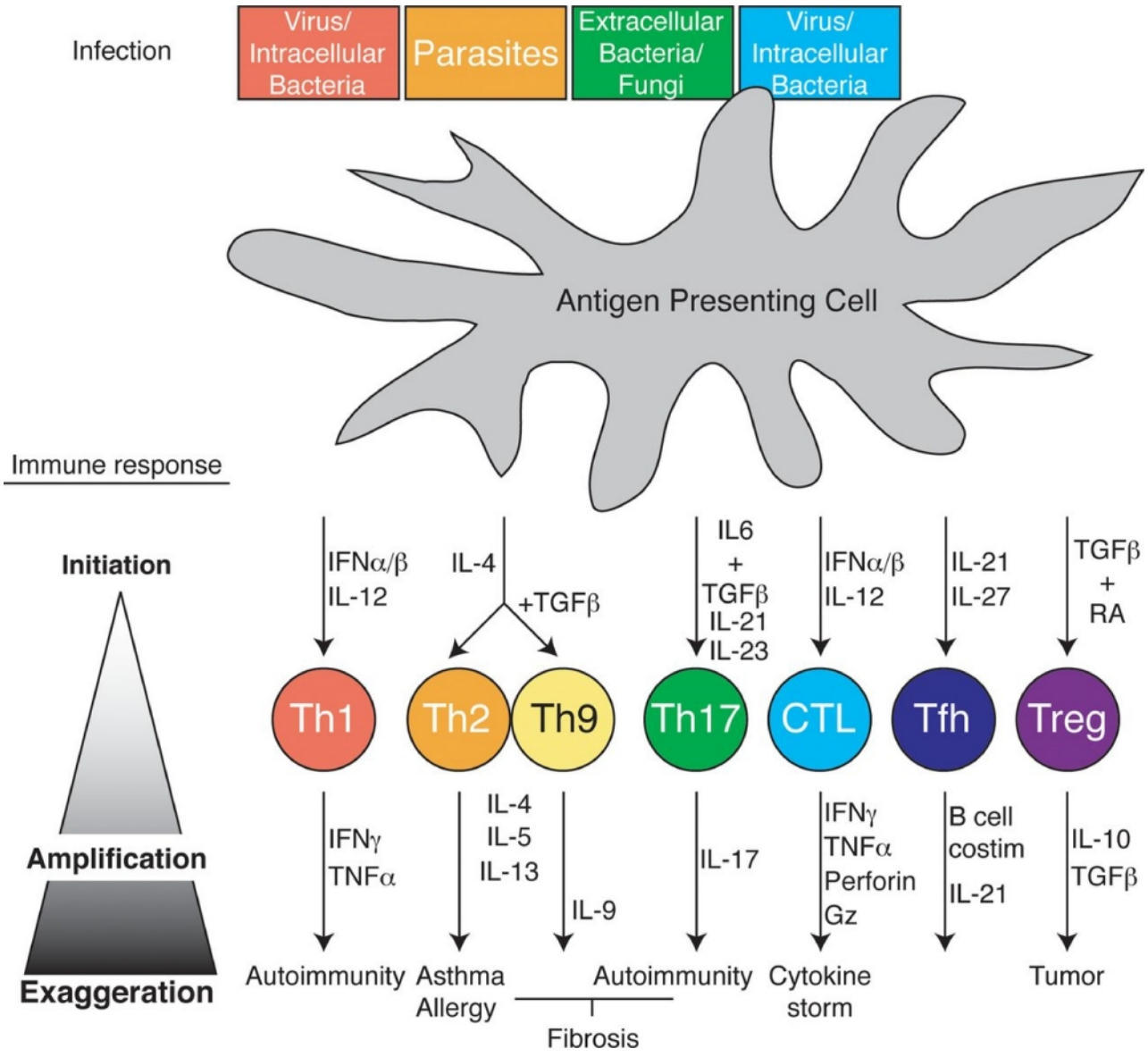
Cytokine*	Secreted by**	Targets and effects
SOME CYTOKINES OF INNATE IMMUNITY		
Interleukin 1 (IL-1)	Monocytes, macrophages, endothelial cells, epithelial cells	Vasculature (inflammation); hypothalamus (fever); liver (induction of acute phase proteins)
Tumor necrosis factor-α (TNF-α)	Macrophages	Vasculature (inflammation); liver (induction of acute phase proteins); loss of muscle, body fat (cachexia); induction of death in many cell types; neutrophil activation
Interleukin 12 (IL-12)	Macrophages, dendritic cells	NK cells; influences adaptive immunity (promotes T_H1 subset)
Interleukin 6 (IL-6)	Macrophages, endothelial cells	Liver (induces acute phase proteins); influences adaptive immunity (proliferation and antibody secretion of B cell lineage)
Interferon α (IFN-α) (this is a family of molecules)	Macrophages	Induces an antiviral state in most nucleated cells; increases MHC class I expression; activates NK cells
Interferon β (IFN-β)	Fibroblasts	Induces an antiviral state in most nucleated cells; increases MHC class I expression; activates NK cells
SOME CYTOKINES OF ADAPTIVE IMMUNITY		
Interleukin 2 (IL-2)	T cells	T-cell proliferation; can promote AICD. NK cell activation and proliferation; B-cell proliferation
Interleukin 4 (IL-4)	T_H2 cells; mast cells	Promotes T_H2 differentiation; isotype switch to IgE
Interleukin 5 (IL-5)	T_H2 cells	Eosinophil activation and generation
Interleukin 25 (IL-25)	Unknown	Induces secretion of T_H2 cytokine profile
Transforming growth factor β (TGF-β)	T cells, macrophages, other cell types	Inhibits T-cell proliferation and effector functions; inhibits B-cell proliferation; promotes isotype switch to IgE; inhibits macrophages
Interferon γ (IFN-γ)	T_H1 cells; $CD8^+$ cells; NK cells	Activates macrophages; increases expression MHC class I and class II molecules; increases antigen presentation

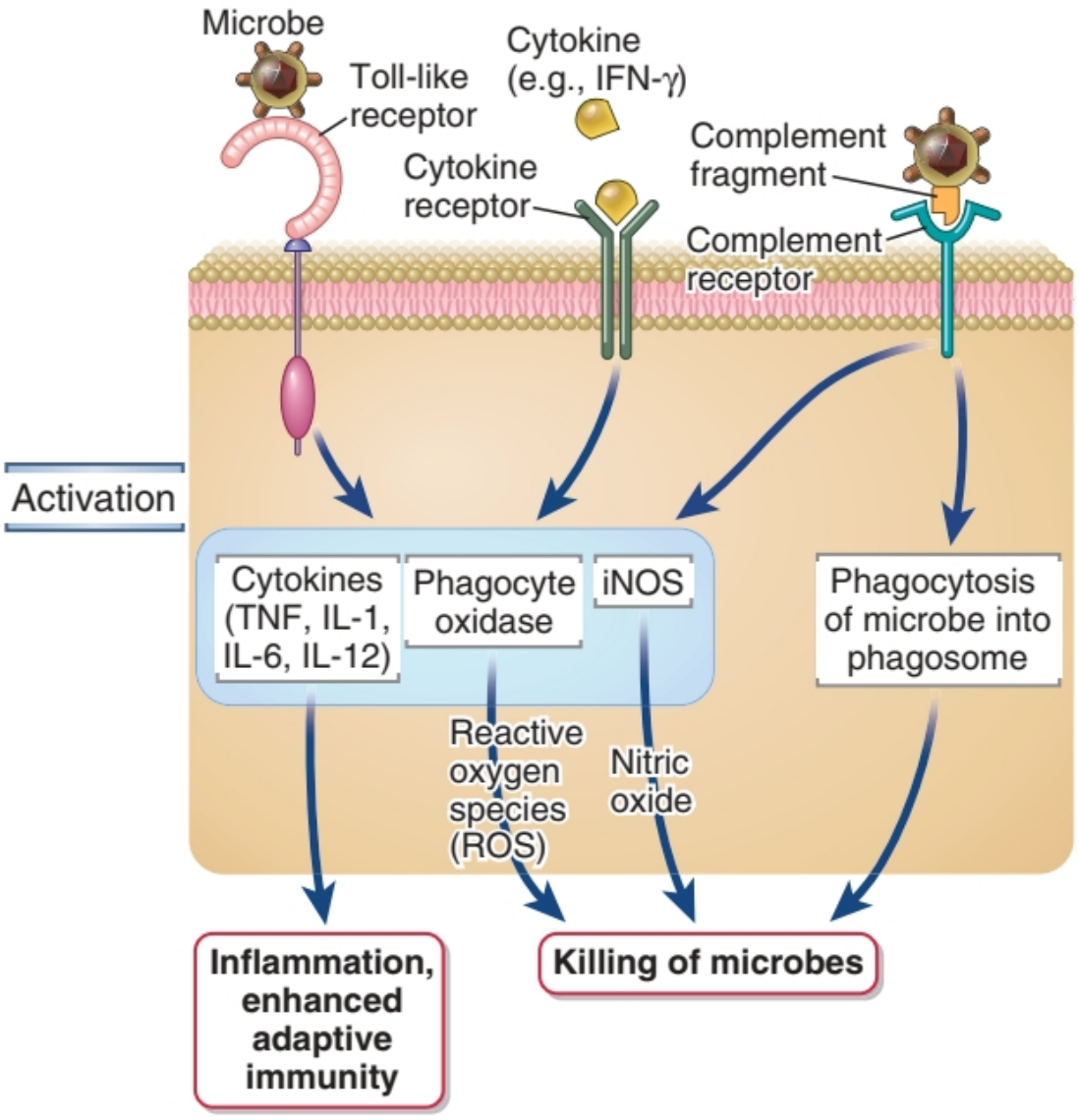
[1]Many cytokines play roles in more than one functional category.

*Only the major cell types providing cytokines for the indicated activity are listed; other cell types may also have the capacity to synthesize the given cytokine.

**Also note that activated cells generally secrete greater amounts of cytokine than unactivated cells.


Fibroblast
Inflammatory Response
IL-1
TNF
TGF-β
PDGF
IL-8
TNF-α
IL-2
TNF
Endothelial cells
Histamine
Prostaglandins
Leukotrienes
Neutrophil
Antigen
Macrophage
IFN-α
IFN-β
IFN-γ
IL-1
IL-6
IL-8
IL-10
IL-12
IL-15
TNF-α
Mast cell
IL-4
IL-10
Resting Th cell
IL-6
IL-11
IL-12
M-CSF
TNF
IL-1
IL-3
IL-4
IL-10
Hematopoiesis
Resting B cell
IFN-γ IL-5
IL-2 IL-12
IL-4 TGF-β
Stem cell
GM-CSF
IL-6
IL-7
IL-13
Activated B cell
IL-6
Activated Th cell
IL-3
IL-5
Eosinophils
Plasma cell
IL-2
IL-12
IL-15
B cells
IL-2
IFN-γ
Antibodies
Th cells
Tc cell
NK cell
Clonal Expansion


Infection
Virus/ Intracellular Bacteria
Parasites
Extracellular Bacteria/ Fungi
Virus/ Intracellular Bacteria
Antigen Presenting Cell
Immune response
Initiation
Amplification
Exaggeration
IFNα/β
IL-12
IL-4
+TGFβ
IL6
+
TGFβ
IL-21
IL-23
IFNα/β
IL-12
IL-21
IL-27
TGFβ
+
RA
Th1
Th2
Th9
Th17
CTL
Tfh
Treg
IFNγ
TNFα
IL-4
IL-5
IL-13
IL-9
IL-17
IFNγ
TNFα
Perforin
Gz
B cell costim
IL-21
IL-10
TGFβ
Autoimmunity
Asthma
Allergy
Fibrosis
Autoimmunity
Cytokine storm
Tumor



- ✓ The immune system is expected to recognize foreign invaders, respond proportionally to the pathogen burden and <u>then return to homeostasis</u>

- ✓ A balance is required between sufficient cytokine production to eliminate the pathogen and avoidance of a hyperinflammatory response which causes clinically significant collateral damage.

Cytokine Storm

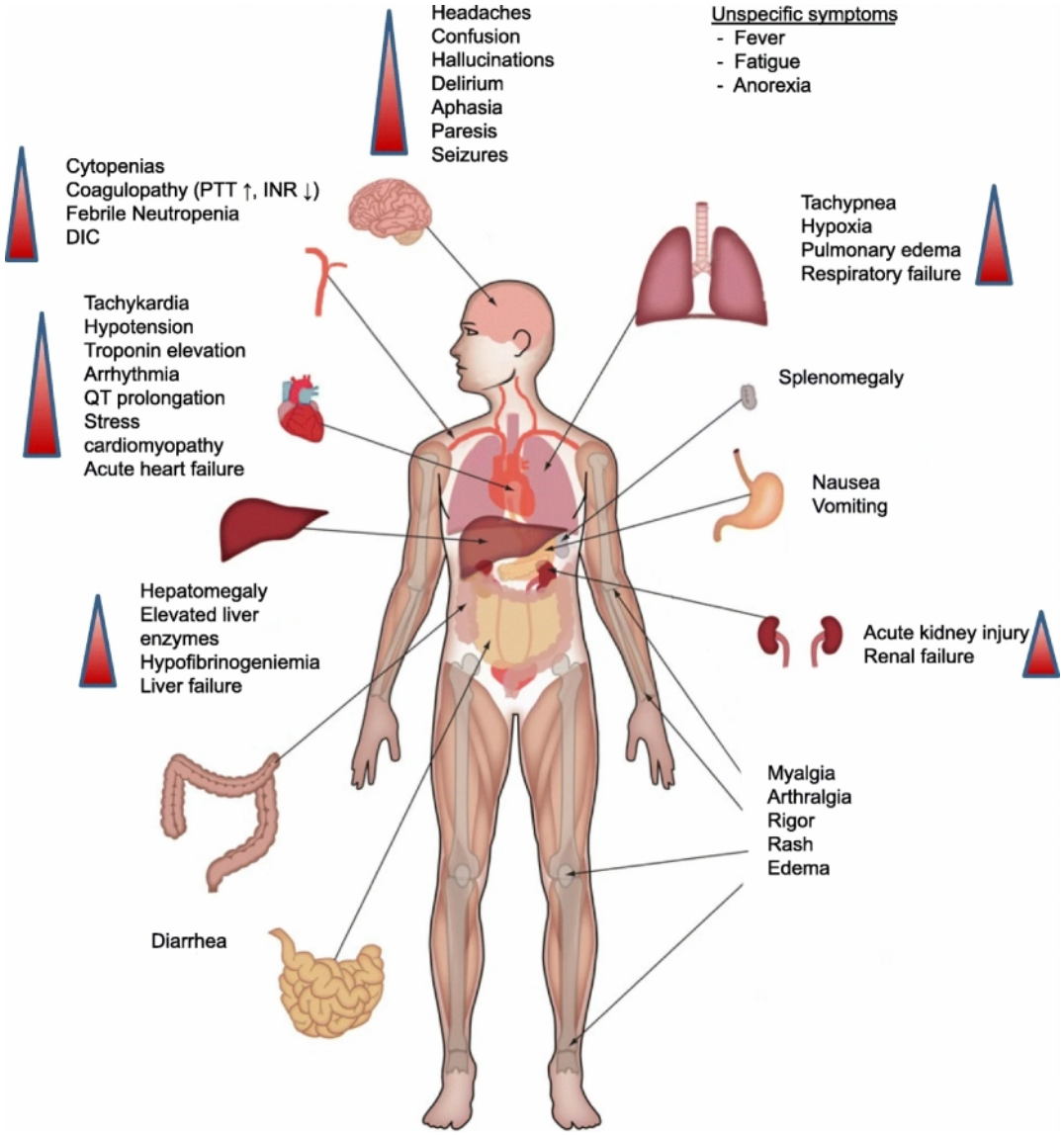
- **Unifying definition based on 3 criteria:**

1. elevated circulating cytokine levels
2. acute systemic inflammatory symptoms
3. secondary organ dysfunction beyond that which could be attributed to a normal response to a pathogen

- Cytokine storm is a life-threatening systemic inflammatory syndrome that can be triggered by various therapies, pathogens, cancers, autoimmune conditions, and monogenic disorders.

- Important for the clinician to recognize cytokine storm as it has prognostic and therapeutic implications.

Clinical Symptoms



- The onset and duration of cytokine storm vary, depending on the cause and treatments administered.

- Although the initial drivers may differ, late-stage clinical manifestations of cytokine storm converge and often overlap.

Potential triggers and initiators of cytokine storm

- Iatrogenic cytokine storm: CAR T-cell therapy, other T-cell engaging immunotherapies, bi-specific antibodies, immune checkpoint inhibitors

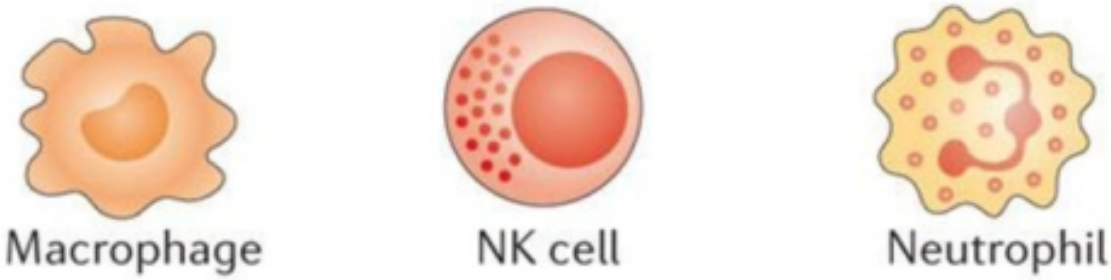
- Pathogen-induced cytokine storm: bacterial infections *(streptococcus species, Staphylococcus aureus),* viral infections *(Epstein-Barr Virus, cytomegalovirus, coronavirus..)*

- Monogenic (single gene-associated) cytokine storm: rare diseases with mutations in genes regulating the innate immune system, inflammasomes or the cytotoxic capacity of NK cells or CTLs

Table 1. Soluble Mediators in Cytokine Storm.*

Mediator	Main Cell Source	Type and Function
Cytokines and growth factors		
Interleukin-1	Macrophages, epithelial cells; pyroptotic cells	Proinflammatory alarmin cytokine; pyrogenic function, macrophage and Th17 cell activation
Interleukin-2	T cells	Effector T-cell and regulatory T-cell growth factor
Interleukin-6	Macrophages, T cells, endothelial cells	Proinflammatory cytokine; pyrogenic function, increased antibody production, induction of acute-phase reactants
Interleukin-9	Th9 cells	Protection from helminth infections, activation of mast cells, association with type I interferon in Covid-19[26]
Interleukin-10	Regulatory T cells, Th9 cells	Antiinflammatory cytokine; inhibition of Th1 cells and cytokine release
Interleukin-12	Dendritic cells, macrophages	Activation of the Th1 pathway; induction of interferon-γ from Th1 cells, CTLs, and NK cells; acting in synergy with interleukin-18
Interleukin-17	Th17 cells, NK cells, group 3 innate lymphoid cells	Promoting neutrophilic inflammation, protection from bacterial and fungal infections
Interleukin-18	Monocytes, macrophages, dendritic cells	Proinflammatory alarmin cytokine; activation of Th1 pathway, acting in synergy with interleukin-12
Interleukin-33	Macrophages, dendritic cells, mast cells, epithelial cells	Proinflammatory alarmin cytokine; amplification of Th1 and Th2 cells, activation of NK cells, CTLs, and mast cells
Interferon-γ	Th1 cells, CTLs, group 1 innate lymphoid cells, and NK cells	Proinflammatory cytokine; activation of macrophages
Tumor necrosis factor	Macrophages, T cells, NK cells, mast cells	Increasing vascular permeability; pyrogenic function
GM-CSF	Th17 cells	Proinflammatory cytokine
VEGF	Macrophages	Angiogenesis
Chemokines		
Interleukin-8 (CXCL8)	Macrophages, epithelial cells	Recruitment of neutrophils
MIG (CXCL9)	Monocytes, endothelial cells, keratinocytes	Interferon-inducible chemokine; recruitment of Th1 cells, NK cells, plasmacytoid dendritic cells
IP-10 (CXCL10)	Monocytes, endothelial cells, keratinocytes	Interferon-inducible chemokine; recruitment of macrophages, Th1 cells, NK cells
MCP-1 (CCL2)	Macrophages, dendritic cells, cardiac myocytes	Recruitment of Th2 cells, monocytes, dendritic cells, basophils
MIP-1α (CCL3)	Monocytes, neutrophils, dendritic cells, NK cells, mast cells	Recruitment of macrophages, Th1 cells, NK cells, eosinophils, dendritic cells; pyrogenic function
MIP-1β (CCL4)	Macrophages, neutrophils, endothelium	Recruitment of macrophages, Th1 cells, NK cells, dendritic cells
BLC (CXCL13)	B cells, follicular dendritic cells	Recruitment of B cells, CD4 T cells, dendritic cells†
Plasma proteins		
CRP	Hepatocytes	Monomeric CRP increases interleukin-8 and MCP-1 secretion; interleukin-6 increases CRP expression
Complement	Hepatocytes, other cells	Complement activation contributes to tissue damage in cytokine storm; complement inhibition can reduce immunopathologic effects of cytokine storm
Ferritin	Ubiquitous	Primary site of iron storage in cells

Participation of innate immune cells in cytokine storm

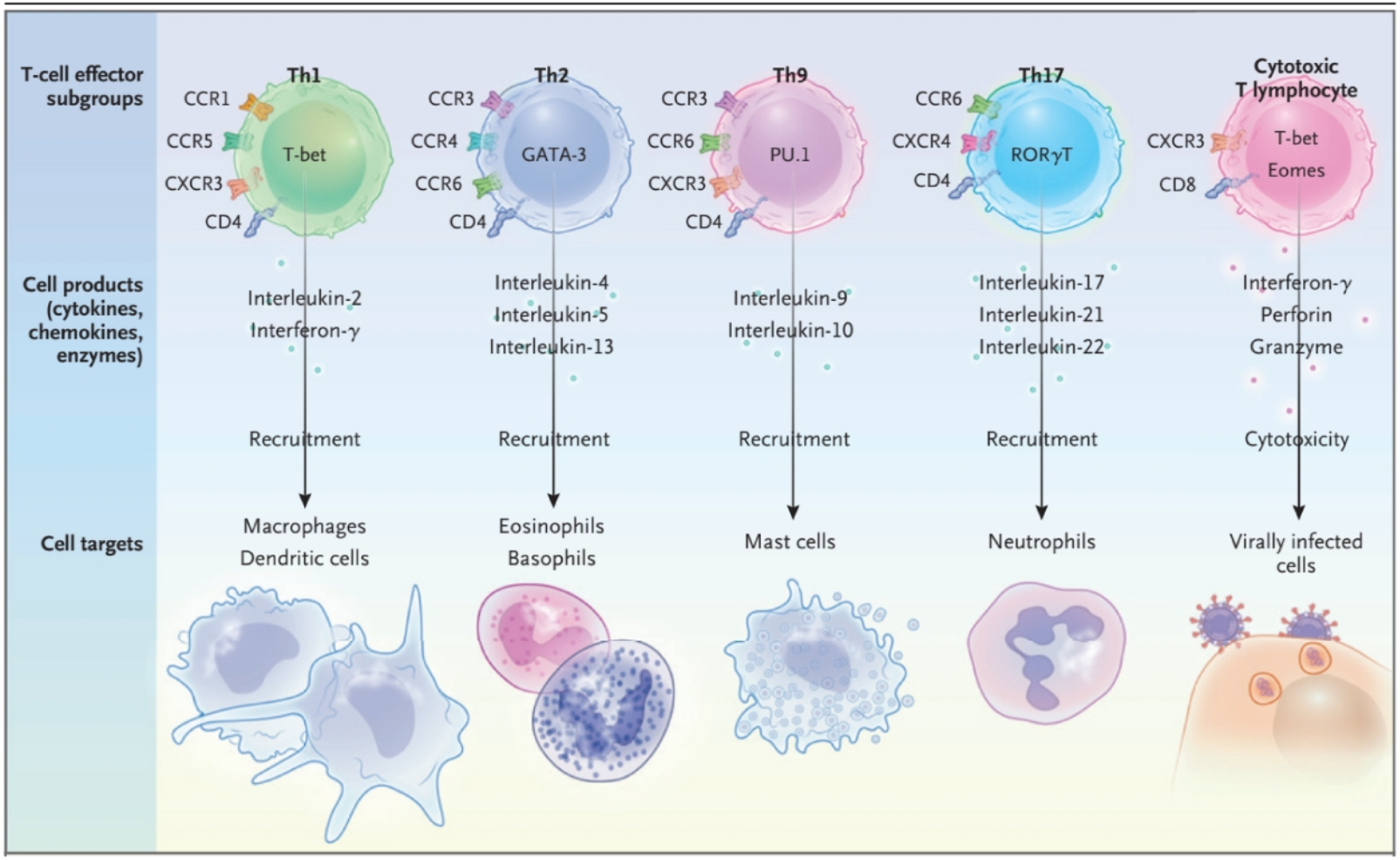


(a) secrete excessive amounts of cytokines (severe tissue damage and organ failure)
(b) Interferon-γ-induced hemophagocytosis by macrophages (cytopenias)

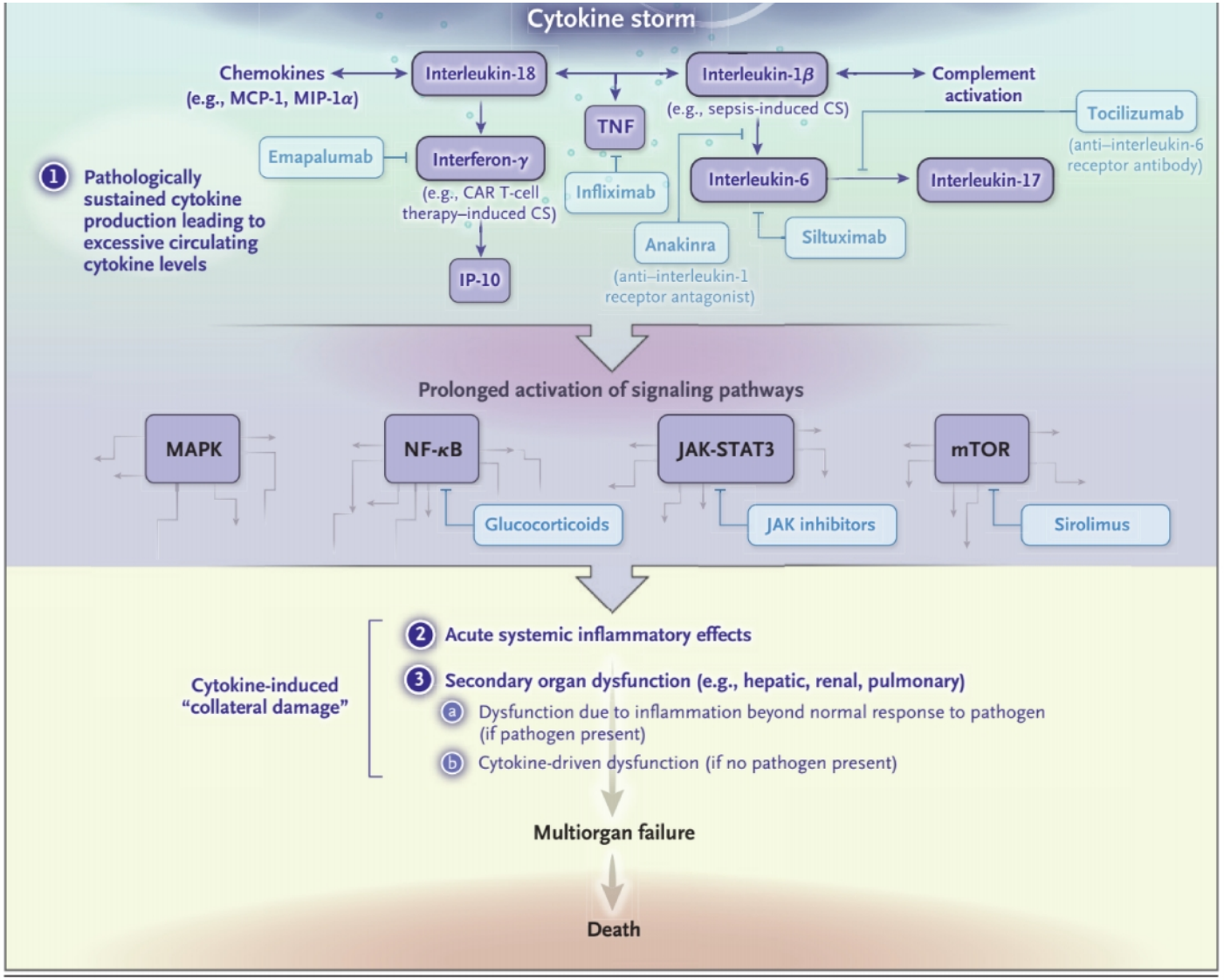
(a) secretion of excessive amounts of cytokines
(b) difficulty to resolve inflammation due to IL-6-induce impairment of the cytolytic function of NK cells

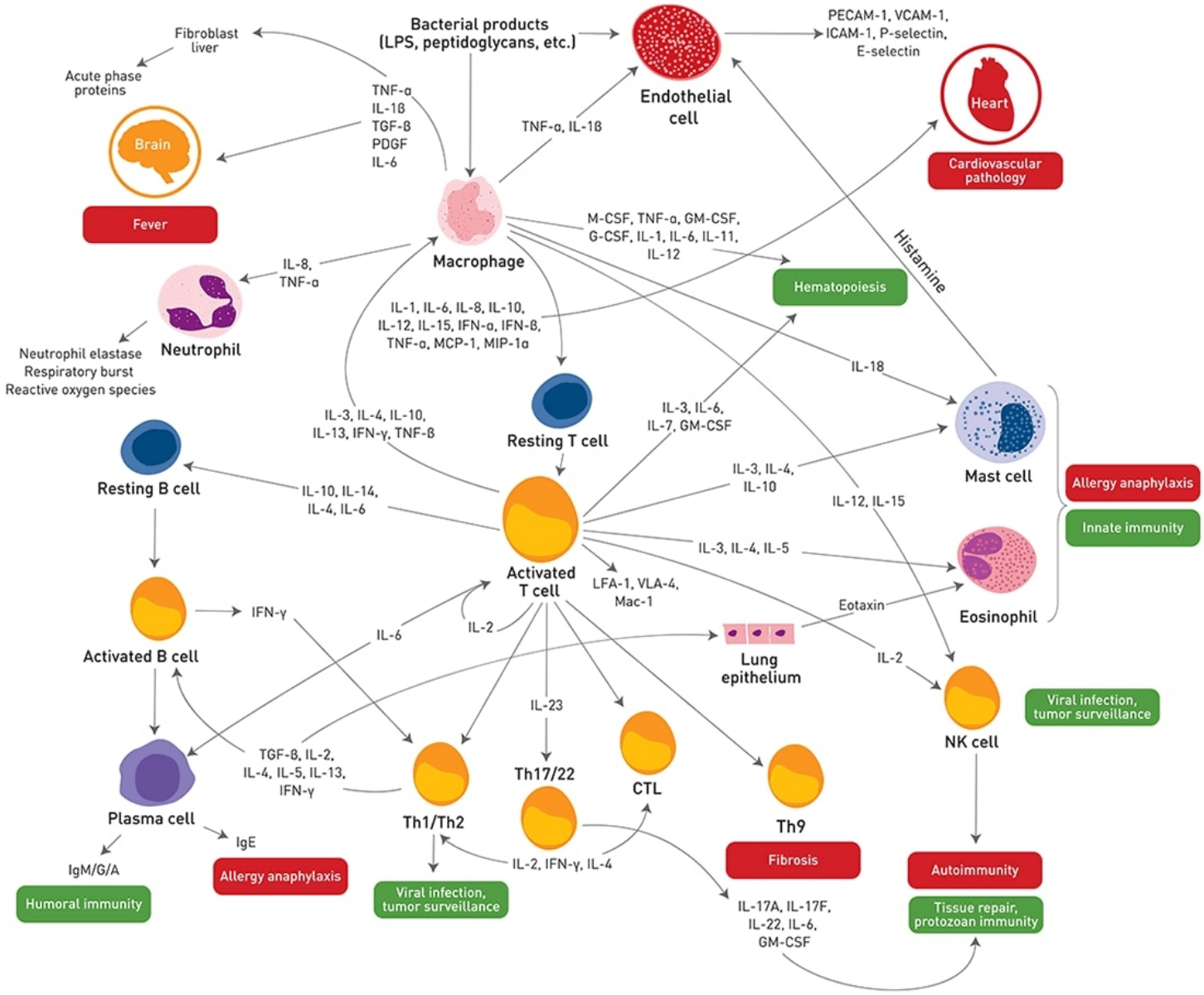
(a) secretion of excessive amounts of cytokines
(b) produce neutrophil extracellular traps, a network of fibers that contribute to thrombi formation and amplify cytokine production during cytokine storm

Participation of adaptive immune cells in cytokine storm



Therapeutic regimes for cytokine storm




Fibroblast
liver
Acute phase
proteins
Bacterial products
(LPS, peptidoglycans, etc.)
Endothelial
cell
PECAM-1, VCAM-1,
ICAM-1, P-selectin,
E-selectin
Heart
Cardiovascular
pathology
TNF-α
IL-1β
TGF-β
PDGF
IL-6
TNF-α, IL-1β
Brain
Fever
Macrophage
M-CSF, TNF-α, GM-CSF,
G-CSF, IL-1, IL-6, IL-11,
IL-12
Hematopoiesis
Histamine
IL-8,
TNF-α
Neutrophil
Neutrophil elastase
Respiratory burst
Reactive oxygen species
IL-1, IL-6, IL-8, IL-10,
IL-12, IL-15, IFN-α, IFN-β,
TNF-α, MCP-1, MIP-1α
IL-18
Resting T cell
IL-3, IL-4, IL-10,
IL-13, IFN-γ, TNF-β
IL-3, IL-6,
IL-7, GM-CSF
Mast cell
Resting B cell
IL-10, IL-14,
IL-4, IL-6
IL-3, IL-4,
IL-10
IL-12, IL-15
Allergy anaphylaxis
Innate immunity
Activated
T cell
LFA-1, VLA-4,
Mac-1
IL-3, IL-4, IL-5
Eosinophil
Eotaxin
IFN-γ
IL-6
IL-2
Activated B cell
Lung
epithelium
IL-2
NK cell
Viral infection,
tumor surveillance
IL-23
TGF-β, IL-2,
IL-4, IL-5, IL-13,
IFN-γ
Th17/22
CTL
Th9
Plasma cell
Th1/Th2
IgE
IgM/G/A
Allergy anaphylaxis
Humoral immunity
Viral infection,
tumor surveillance
IL-2, IFN-γ, IL-4
Fibrosis
Autoimmunity
IL-17A, IL-17F,
IL-22, IL-6,
GM-CSF
Tissue repair,
protozoan immunity


Thank
you