Innate immunity

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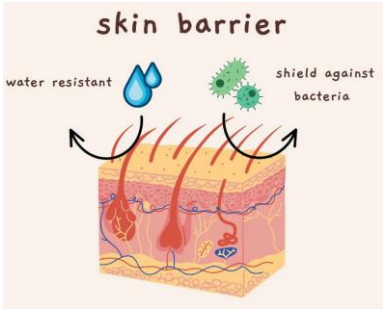
Molecular Immunology

Agenda

- First line of defense
- Cells of innate immunity
- Phagocytosis
- Receptors
- Communication: cytokines and chemokines

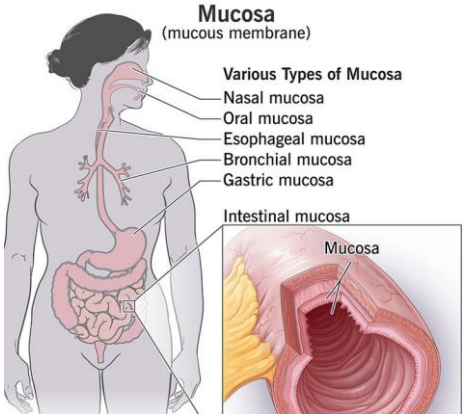
First line of defense: Physical barriers

- Skin

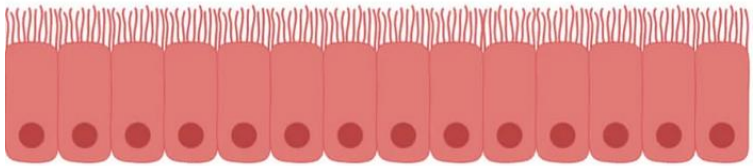


- Mucosal epithelia

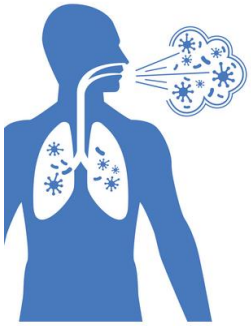
  Mucins (glycoproteins) in mucus



- Cilia

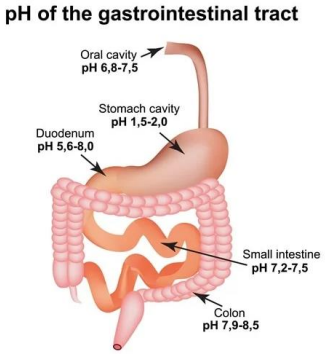


- Coughing, sneezing, vomiting, diarrhea

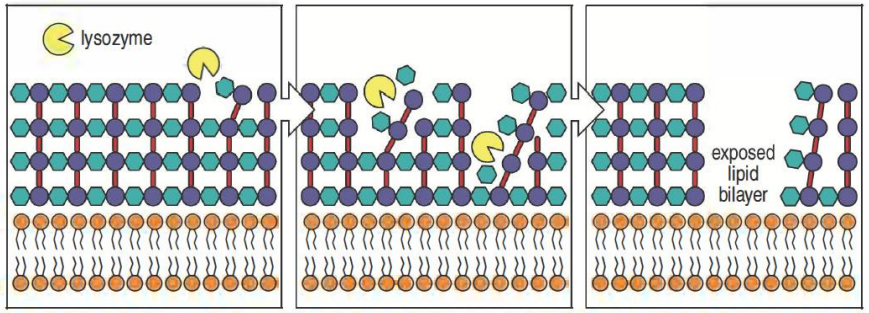


First line of defense: Chemical barriers

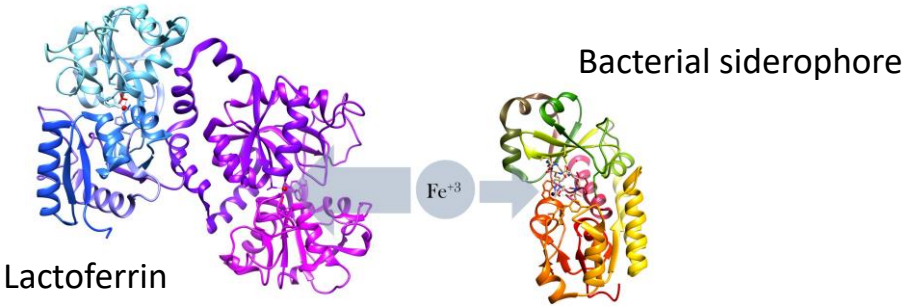
- Digestive fluids



- Lysozyme & Phospholypase A2
  - Tears
  - Saliva
  - Paneth cells



- Lactoferrin



First line of defense: Chemical barriers

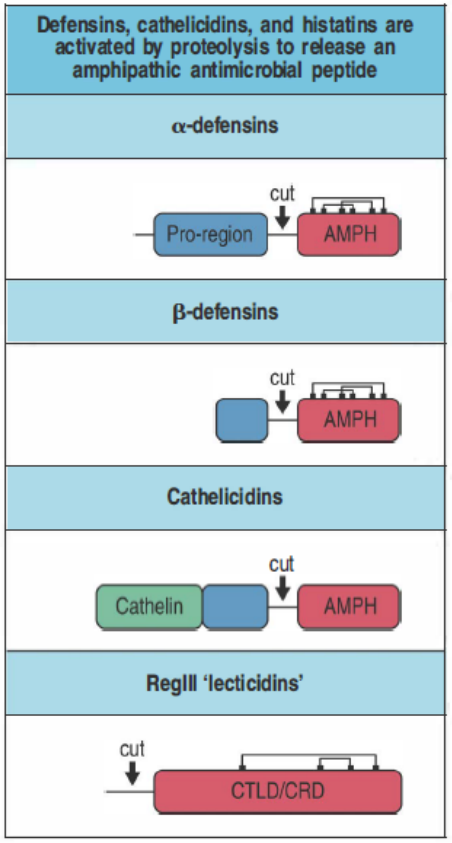
- Antimicrobial pepties (secreted by epithelial cells and phagocytes)

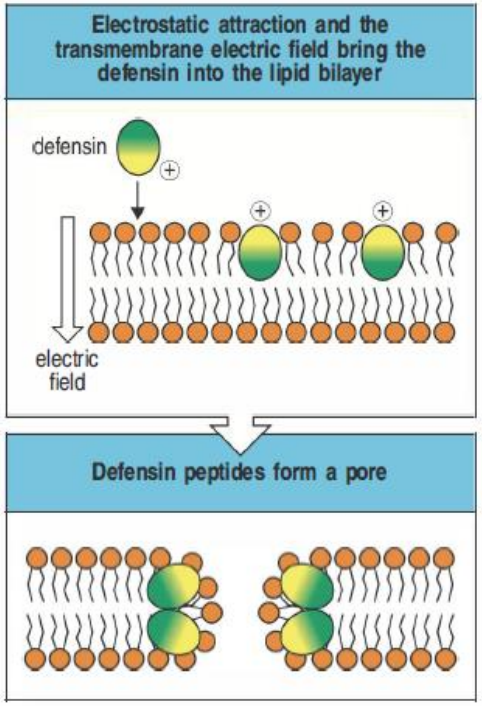
❖ Defensins

❖ Cathelicidins

❖ Histatins

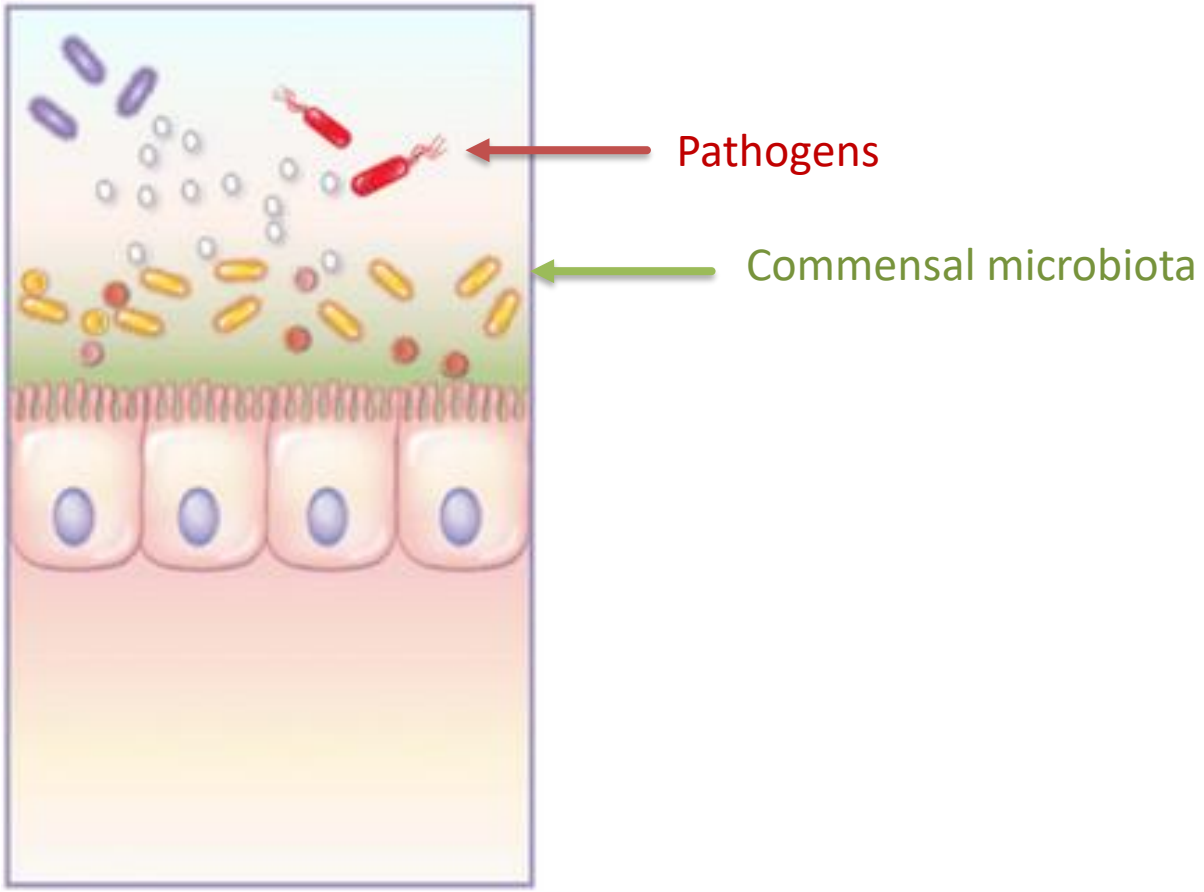
❖ Lectins

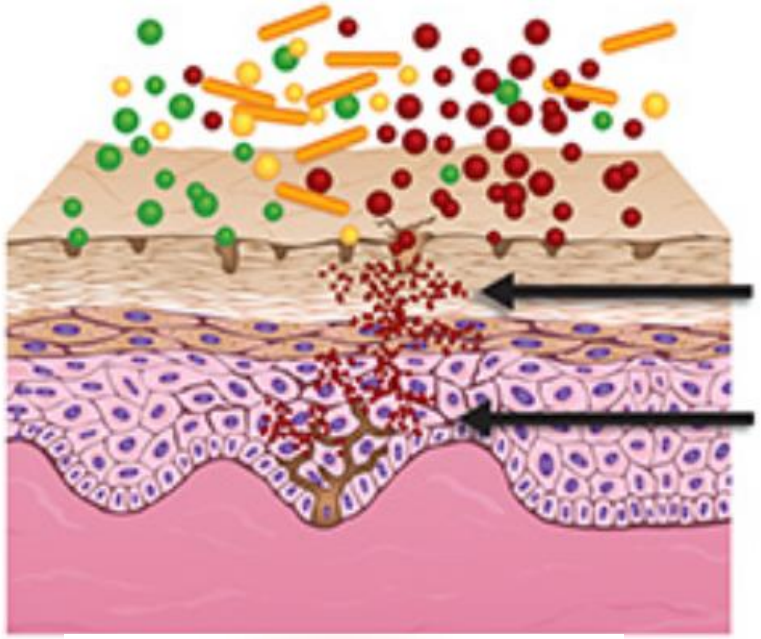




First line of defense: Commensal microbiota

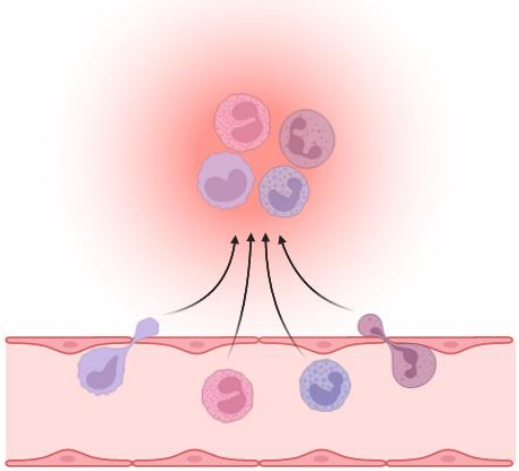
Competition of nutrients and epithelial attachment sites

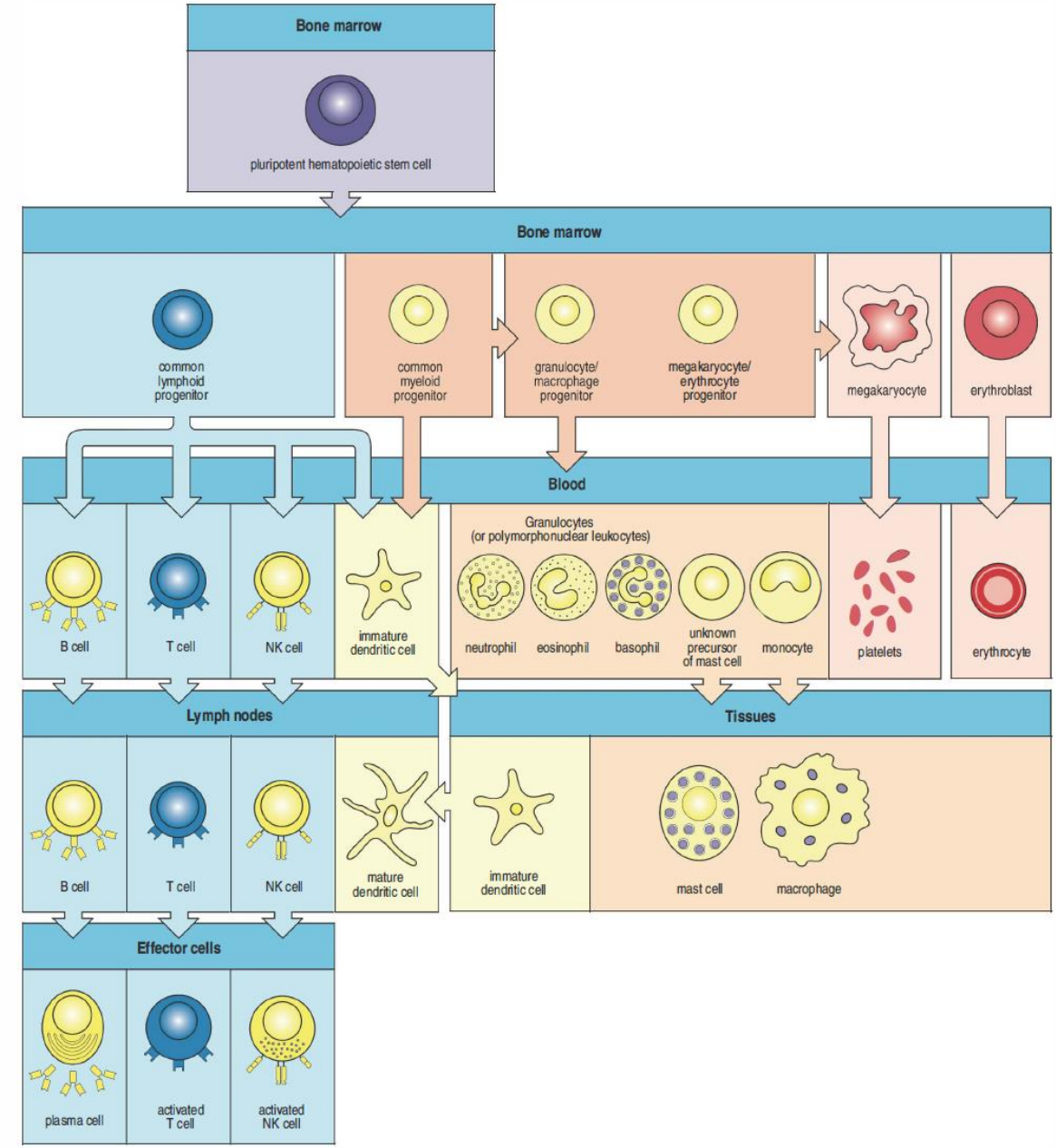




Barrier disruption

Pathogen invasion




Bone marrow
pluripotent hematopoietic stem cell
Bone marrow
common lymphoid progenitor
common myeloid progenitor
granulocyte/ macrophage progenitor
megakaryocyte/ erythrocyte progenitor
megakaryocyte
erythroblast
Blood
B cell
T cell
NK cell
immature dendritic cell
Granulocytes (or polymorphonuclear leukocytes)
neutrophil
eosinophil
basophil
unknown precursor of mast cell
monocyte
platelets
erythrocyte
Lymph nodes
Tissues
B cell
T cell
NK cell
mature dendritic cell
immature dendritic cell
mast cell
macrophage
Effector cells
plasma cell
activated T cell
activated NK cell

Innate Immunity

- Fast reaction

- No memory (but trained immunity)

- Pattern recognition receptors (PRR)

Pathogen-associated molecular patters (PAMP)

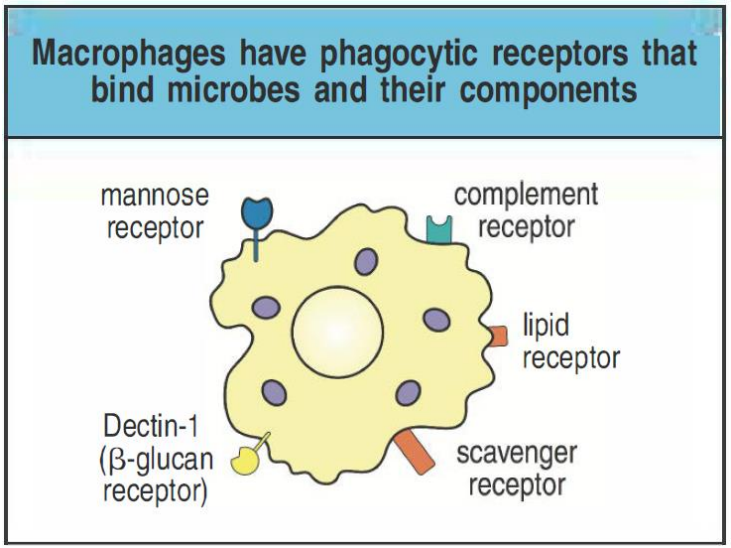
Phagocytes

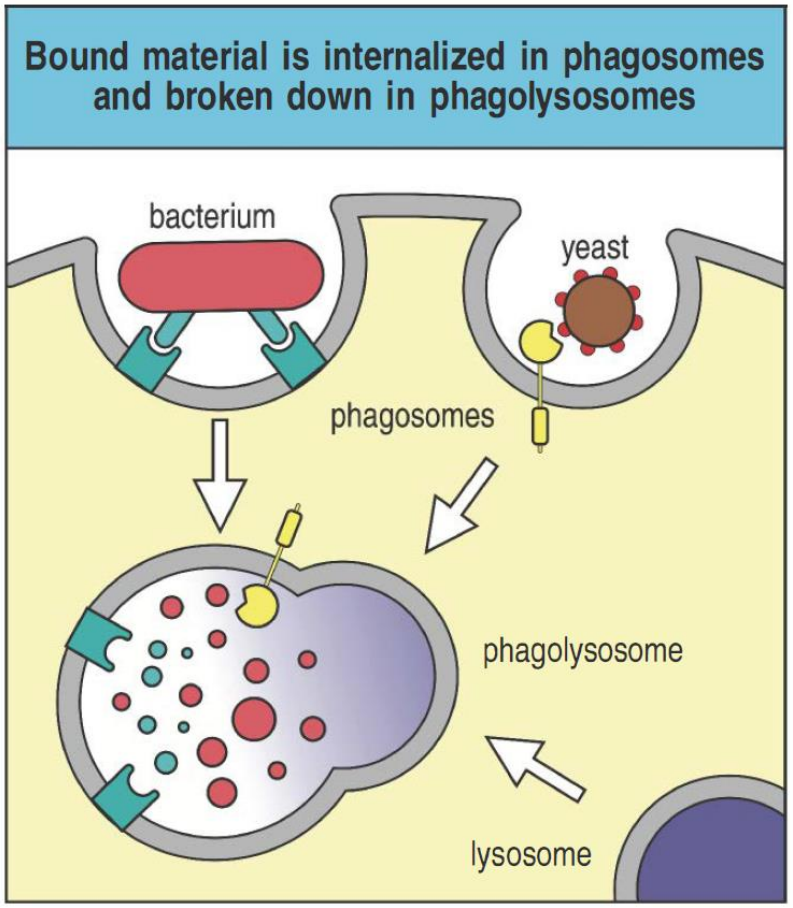
- Monocytes/Macrophages

- Dendritic cells

- Neutrophils (Granulocytes)

Phagocytosis

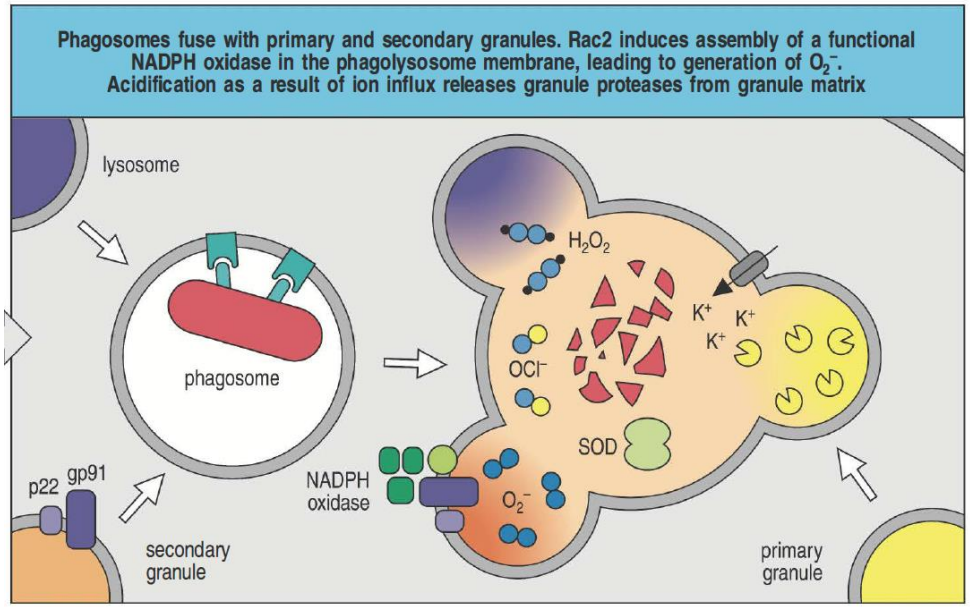




Alternatives:

- Receptor mediated endocytosis (clathrin coat)
- Macropinocytosis

Phagocytosis



Antimicrobial mechanisms of phagocytes		
Class of mechanism	**Macrophage products**	**Neutrophil products**
Acidification	pH=~3.5–4.0, bacteriostatic or bactericidal	
Toxic oxygen-derived products	Superoxide O_2^-, hydrogen peroxide H_2O_2, singlet oxygen $^1O_2^*$, hydroxyl radical $^*OH$, hypohalite OCl^-	
Toxic nitrogen oxides	Nitric oxide NO	
Antimicrobial peptides	Cathelicidin, macrophage elastase-derived peptide	α-Defensins (HNP1–4), β-defensin HBD4, cathelicidin, azurocidin, bacterial permeability inducing protein (BPI), lactoferricin
Enzymes	Lysozyme: digests cell walls of some Gram-positive bacteria Acid hydrolases (e.g. elastase and other proteases): break down ingested microbes	
Competitors		Lactoferrin (sequesters Fe^{2+}), vitamin B_{12}-binding protein

Respiratory burst: Superoxide anion transformed into H_2O_2 by superoxide dismutase.

Neutrophils have also primary and secondary granules with enzymes and antimicrobial peptides

Granulocytes

- Neutrophils: Short lived cells. Highly abundant in blood.
  - Pathogen elimination (Phagocytosis and NETs)
  - Inflammation regulation
  - Tissue repair (clearing debris)

- Eosinophils: Parasitic infections. Allergy.

- Basophils: Parasitic infections. Release of pro-inflammatory substances

NETosis (Neutrophils)

- NETs (Neutrophil extracellular traps): Modified chromatin decorated with bactericidal proteins from granules and cytoplasm

- Release of:
  - Granule components into the cytosol
  - Modification of histones leading to chromatin decondensation

- Destruction of the nuclear envelope and formation of pores in the plasma membrane

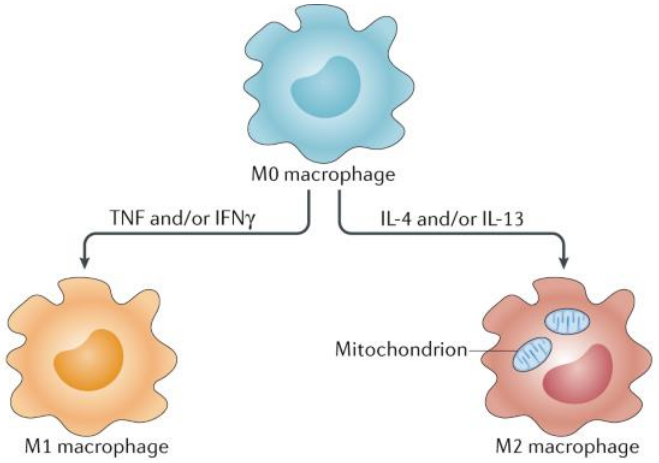
- Induced by pathogens, antibodies and immune complexes or cytokines.

- Dependent on Reactive oxigen species (ROS): NADPH oxidase

Monocytes/Macrophages

Monocytes → Macrophages:
(Blood) (Tissue)

Kuppfer cells (Liver)
Microglia (Neural tissue)
Alveolar macrophage (Lung)



Antimicrobial properties (Proinflammatory)

Tissue repair properties (Antiinflammatory)

Dendritic cells (DC)

- Arise from myeloid and lymphoid progenitors
- Tissue-resident cells

- Conventional DC (cDC):
  - Process ingested microbes to generate antigens that activate T cells
  - Cytokine secretion

- Plasmacytoid DCs (pDC):
  - Antiviral interferon production

Detection (Receptors)

Receptor characteristic	Pattern Recognition Receptor Innate immunity	TCR/BCR Adaptive immunity
Specificity inherited in the genome	Yes	No
Expressed by all cells of a particular type (e.g. macrophages)	Yes	No
Triggers immediate response	Yes	No
Recognizes broad classes of pathogens	Yes	No
Interacts with a range of molecular structures of a given type	Yes	No
Encoded in multiple gene segments	No	Yes
Requires gene rearrangement	No	Yes
Clonal distribution	No	Yes
Able to discriminate between even closely related molecular structures	No	Yes

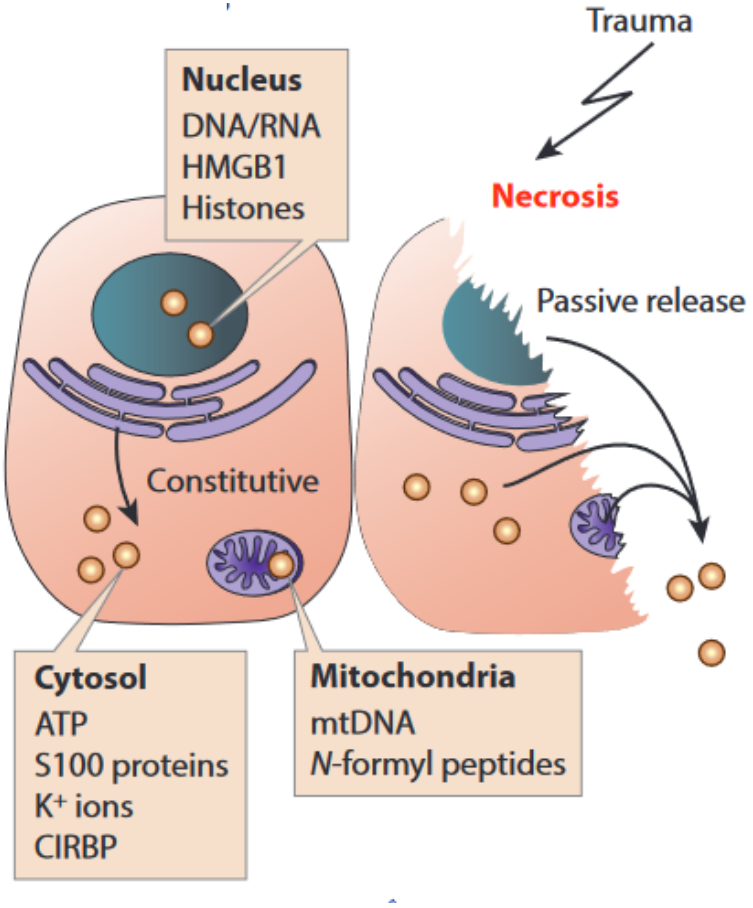
Types of Pattern Recognition Receptors (PRR)

- Free receptors in the serum
  - Acute-phase proteins: C-reactive protein, MBL
- Membrane-bound phagocytic receptors
- Membrane-bound signaling receptors
- Cytoplasmic signaling receptors

Pathogen-associated molecular patterns (PAMPs)

- Endotoxin (Lipopolysaccharide - LPS)
- Peptidoglycan
- Bacterial lipoprotein
- 1→3β-D-glucan
- Lipoteichoic acid
- Bacterial flagellin
- HSPs (heat-shock proteins)
- viral dsRNA
- bacterial CpG nucleotides

Damage-associated molecular patterns (DAMPs)

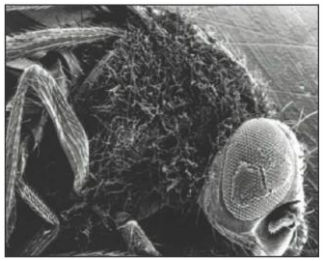


DAMP-sensing receptors	Expression pattern	DAMPs	Pro-inflammatory functions	Major inflammatory diseases
TLRs				
TLR2	Ubiquitous, high in DCs, monocytes, macrophages and neutrophils	HMGB1, several HSPs, SNAPIN, versican, biglycan, decorin, eosinophil-derived neurotoxin, surfactant protein A/D, β-defensin 3, histone, SAA, Aβ, β2-glycoprotein I	Promotes the production of pro-inflammatory cytokines and chemokines	IRI, RA, cancer and other inflammatory diseases
TLR3	Ubiquitous, high in DCs, monocytes, macrophages and NK cells	mRNA	Promotes the production of pro-inflammatory cytokines, chemokines and IFN-I	IRI and other inflammatory diseases
TLR4	Ubiquitous, high in DCs, monocytes, macrophages, neutrophils and endothelial cells	HMGB1, tenascin-C, several HSPs, S100s, HMGN1, biglycan, decorin, heparin sulfate, hyaluronic acid, fibrinogen, fibronectin, β-defensin 2, surfactant protein A/D, lactoferrin, neutrophil elastase, peroxiredoxin, histone, SAA, ox-LDL	Promotes the production of pro-inflammatory cytokines, chemokines and IFN-I	IRI, RA, cancer and other inflammatory diseases
TLR7	Ubiquitous, high in pDCs, monocytes, macrophages and B cells	IgG–ribonucleoprotein complex, microRNAs	Promotes the production of IFNα and other cytokines and chemokines	SLE and other inflammatory diseases
TLR9	Ubiquitous, high in pDCs, monocytes, macrophages and B cells	IgG–chromatin complex, mtDNA, HMGB1	Promotes the production of IFNα and other cytokines and chemokines	SLE and other inflammatory diseases
CLRs				
DNGR1	Mainly in DCs	F-actin	Promotes DC antigen cross-presentation, inhibits IL-10 production	Cancer, atherosclerosis
MINCLE	Monocytes, macrophages, DCs, neutrophils and B cells	Sin3A-associated protein 130, β-glucosylceramide	Promotes pro-inflammatory cytokine production	Stroke, traumatic brain injury, Gaucher disease
Dectin-1	Monocytes, macrophages, DCs, neutrophils, mast cells, T and B cells	N-glycans	Promotes IRF5-dependent gene expression	Cancer
NLRs				
NLRP3	DCs, neutrophils, monocytes and macrophages	MSU, glucose, cholesterol crystals, Aβ, ATP, oxPAPC, Alu-RNA	Promotes IL-1β and IL-18 secretion and initiates pyroptosis	T2D, NASH, gout, atherosclerosis, AD, IRI
RLRs				
RIG-I	Ubiquitous, highly expressed in epithelial cells and myeloid cells	Endogenous 5′ppp RNA	Promotes the production of IFN-I and other cytokines and chemokines	SLE, cancer
MDA5	Ubiquitous, highly expressed in epithelial cells and myeloid cells	Unedited long self-dsRNA, endogenous retroviral RNA	Promotes the production of IFN-I and other cytokines and chemokines	AGS, cancer
CDSs				
cGAS	Ubiquitous, highly expressed in epithelial cells, DCs, monocytes, macrophages and T cells	Cytoplasmic DNA	Promotes the production of IFN-I and other cytokines and chemokines	AGS, AT, cancer, senescence
AIM2	Ubiquitous, highly expressed in epithelial cells, DCs, monocytes, macrophages, B cells and NK cells	Cytoplasmic DNA, damaged DNA in the nucleus	Promotes IL-1β and IL-18 secretion and initiates pyroptosis	Cancer, chronic kidney disease
RAGE				
RAGE	Ubiquitous	AGEs, HMGB1, S100s, Aβ, DNA	Promotes the expression of pro-inflammatory genes, as well as cell migration, proliferation and apoptosis	Diabetic vascular complications, CVD, AD, cancer and other inflammatory diseases

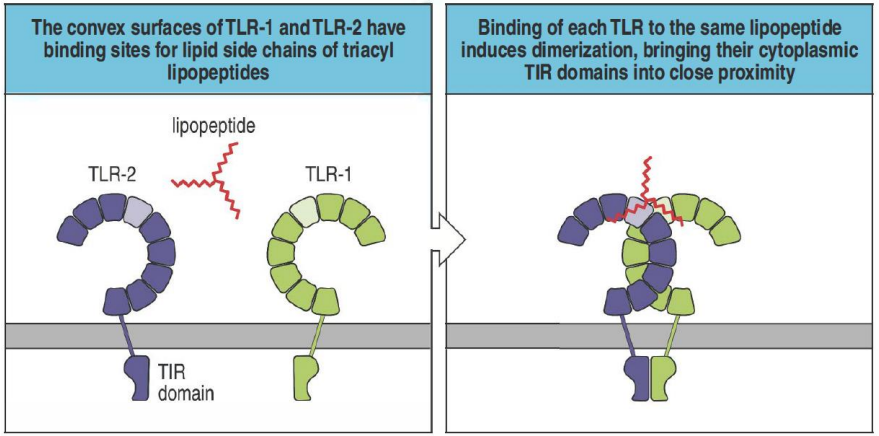
https://doi.org/10.1038/s41577-019-0215-7

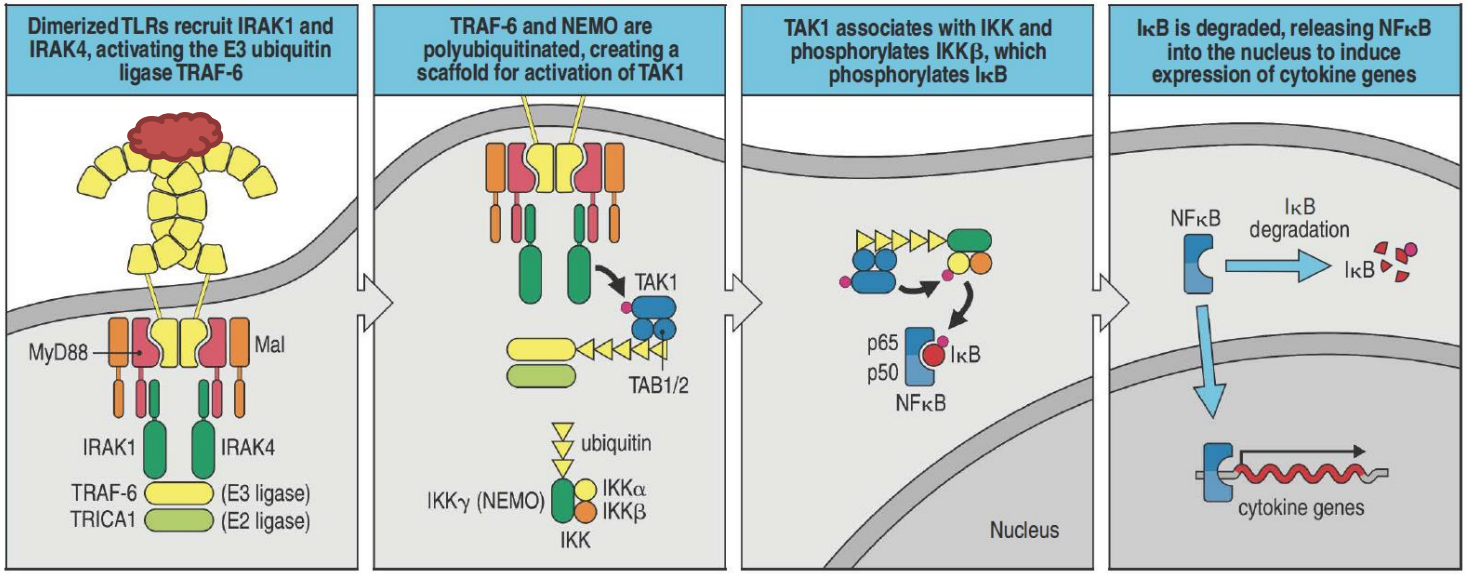
DAMP-sensing receptors	Expression pattern	DAMPs	Pro-inflammatory functions	Major inflammatory diseases
TREMs				
TREM1	Myeloid cells, epithelial cells, endothelial cells and fibroblasts	HMGB1, HSP70, PGLYRP1, actin	Promotes pro-inflammatory cytokine and chemokine secretion	Myocardial infarction, atherosclerosis, RA, ureteral obstruction, cancer
TREM2	Myeloid cells, highly expressed in DCs, monocytes, macrophages and neutrophils	PA, PC, PE, PG, PI, PS, CL, SF, SM, APOA1, APOA2, APOB, APOE, APOJ, LDL, HDL, VLDL, Lp(a), HSP60	Modulates cell differentiation, survival, phagocytosis, chemotaxis	AD, NHD and other neurodegenerative diseases
GPCRs				
FPR1	Ubiquitous, high in neutrophils, monocytes and macrophages	N-formylated peptides, cathepsin G, FAM19A4, annexin 1	Promotes chemotaxis of neutrophils and monocytes/macrophages	Hepatic injury, sterile lung injury, emphysema, SIRS
FPR2	Ubiquitous, high in neutrophils, monocytes and macrophages	Aβ42, SAA, oxLDL, LL-37 and other peptides	Promotes chemotaxis of neutrophils and monocytes/macrophages	–
P2Y2R	Ubiquitous, high in epithelial cells, neutrophils, DCs, monocytes and macrophages	ATP, UTP	Promotes migration and activation of various immune cells	Chronic lung disease, asthma, hepatitis, atherosclerosis
P2Y6R	Ubiquitous, high in stromal cells, neutrophils, monocytes, macrophages and T cells	UDP	Promotes proliferation and cytokine and chemokine production in stromal cells	Asthma, atherosclerosis, chronic lung disease
P2Y12R	Mainly in platelets, also in DCs, monocytes, macrophages and T cells	ADP	Promotes platelet activation and Th17 differentiation	Atherosclerosis, EAE and other autoimmune diseases
CaSR	Ubiquitously expressed	Ca^{2+}	Promotes monocyte/macrophage recruitment and NLRP3 activation	–
GPRC6A	Ubiquitous	Ca^{2+}	Promotes NLRP3 activation	–
Ion channels				
TRPM2	Ubiquitous	ROS	Promotes chemokine production and NLRP3 activation	Colitis and other inflammatory diseases
Other TRPs	Ubiquitous	ROS	Promotes the production of inflammatory neuropeptides	Gout and other inflammatory diseases
P2X7R	Ubiquitous	ATP	Promotes cytokine and chemokine production, NLRP3 inflammasome activation and T cell activation	Respiratory tract, gut, liver, cardiovascular, renal diseases, neurodegenerative diseases and diabetes

https://doi.org/10.1038/s41577-019-0215-7

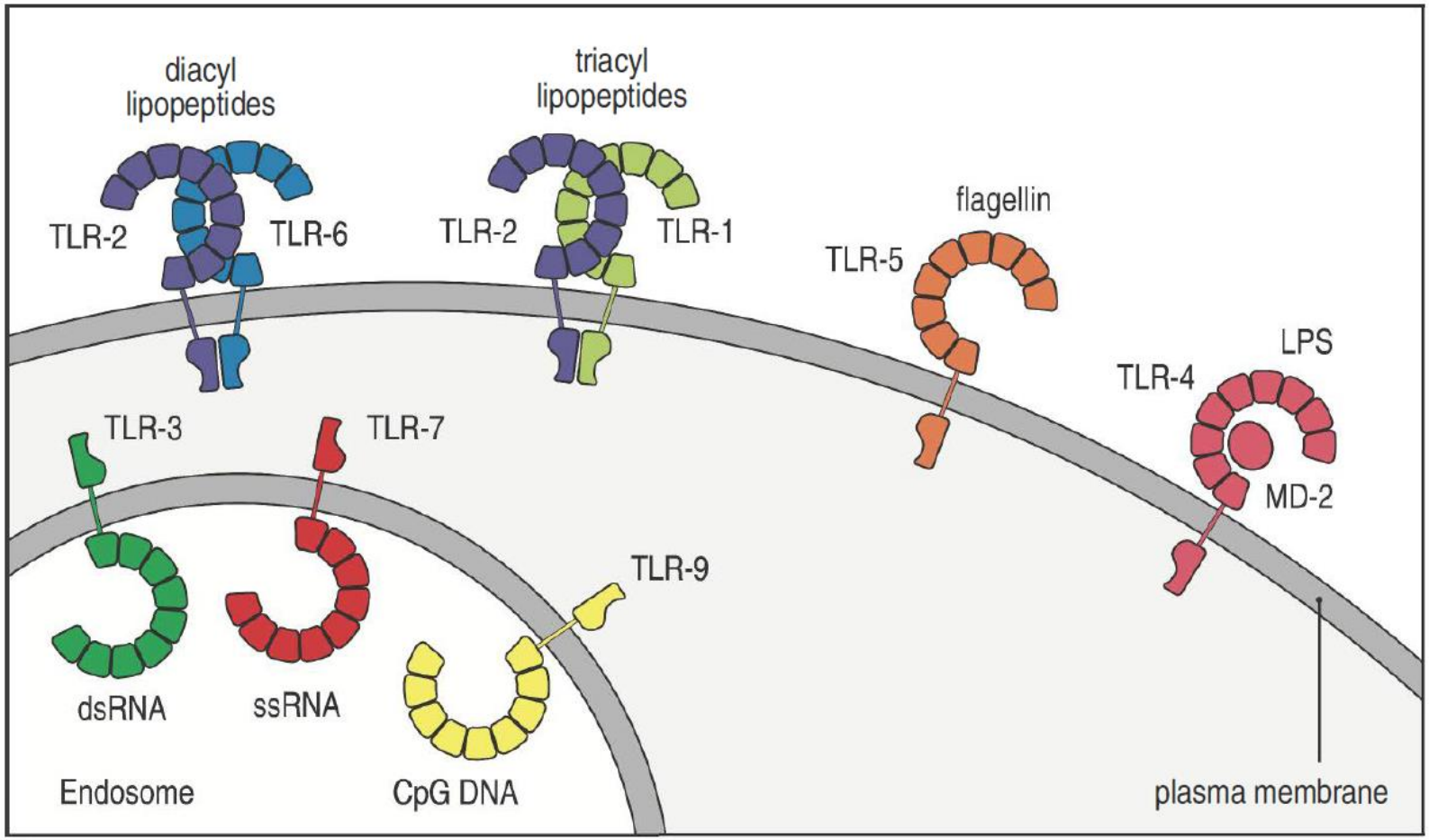


Toll-like receptors (TLR)





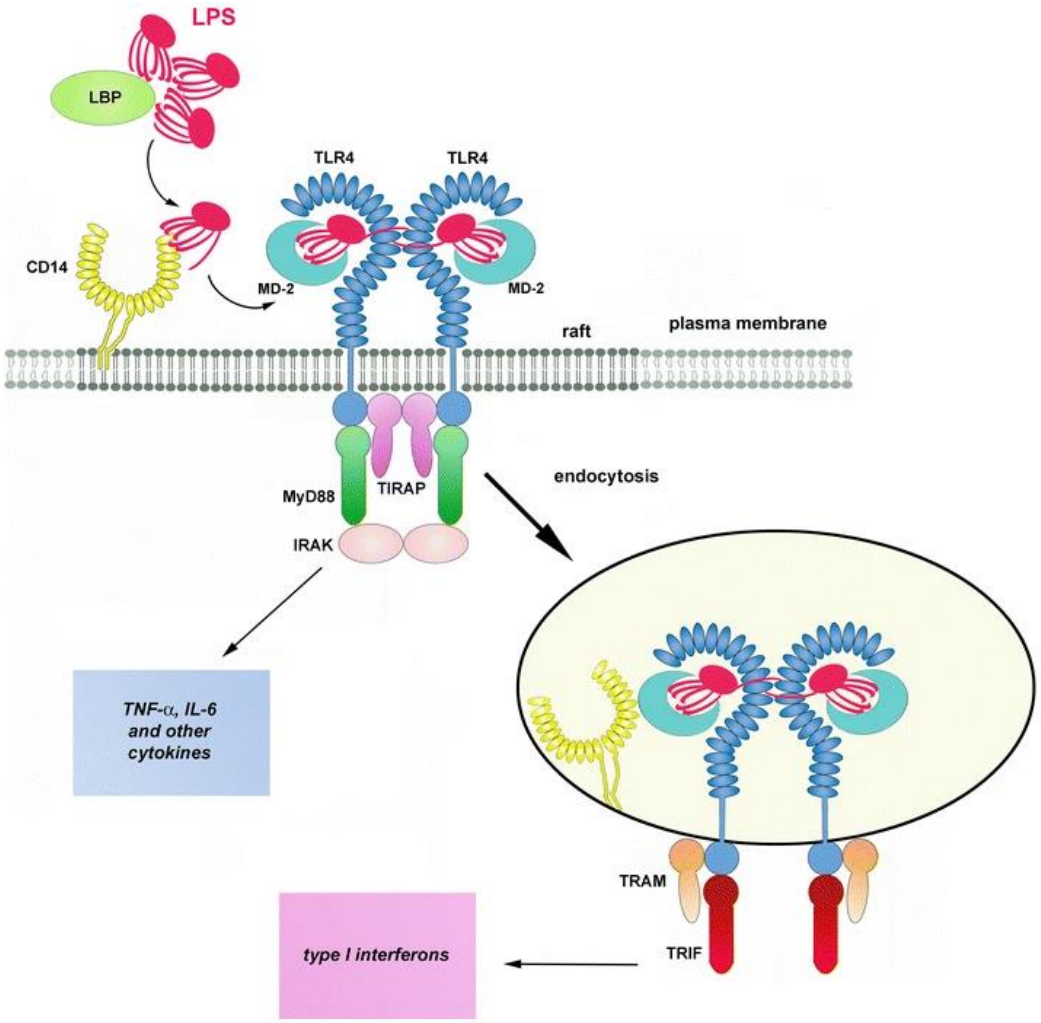
Toll-like receptors



Toll-like receptors

Innate immune recognition by mammalian Toll-like receptors		
Toll-like receptor	**Ligand**	**Cellular distribution**
TLR-1:TLR-2 heterodimer	Lipomannans (mycobacteria) Lipoproteins (diacyl lipopeptides; triacyl lipopeptides) Lipoteichoic acids (Gram-positive bacteria) Cell-wall β-glucans (bacteria and fungi) Zymosan (fungi)	Monocytes, dendritic cells, mast cells, eosinophils, basophils
TLR-2:TLR-6 heterodimer		
TLR-3	Double-stranded RNA (viruses)	NK cells
TLR-4 (plus MD-2 and CD14)	LPS (Gram-negative bacteria) Lipoteichoic acids (Gram-positive bacteria)	Macrophages, dendritic cells, mast cells, eosinophils
TLR-5	Flagellin (bacteria)	Intestinal epithelium
TLR-7	Single-stranded RNA (viruses)	Plasmacytoid dendritic cells, NK cells, eosinophils, B cells
TLR-8	Single-stranded RNA (viruses)	NK cells
TLR-9	DNA with unmethylated CpG (bacteria and herpesviruses)	Plasmacytoid dendritic cells, eosinophils, B cells, basophils
TLR-10	Unknown	Plasmacytoid dendritic cells, eosinophils, B cells, basophils
TLR-11 (mouse only)	Profilin and profilin-like proteins (*Toxoplasma gondii*, uropathogenic bacteria)	Macrophages, dendritic cells, liver, kidney, and bladder epithelial cells

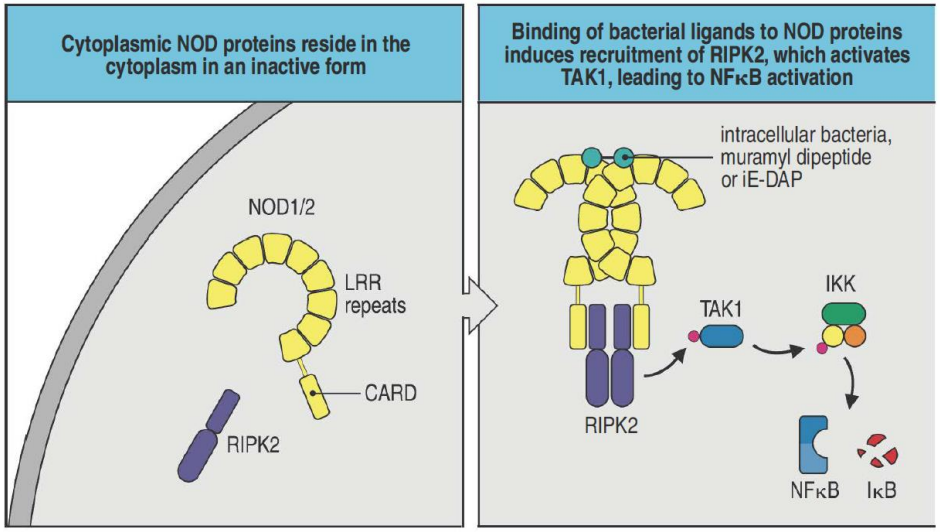
TLR-4



Płóciennikowska, A., Hromada-Judycka, A., Borzęcka, K. *et al*.. *Cell. Mol. Life Sci.* **72**, 557–581 (2015).

NOD-like receptors (NLR)

- NOD1 sensor for γ-glutamyl diaminopimelic acid (iE-DAP)
  - Product of peptidoglycans of Gram-negative bacteria
- NOD2 sensor for muramyl dipeptide
  - Common bacterial peptidoglican



Inflammatory response

- Triggered by the recognition of pathogens and tissue damage

1) Reinforcement

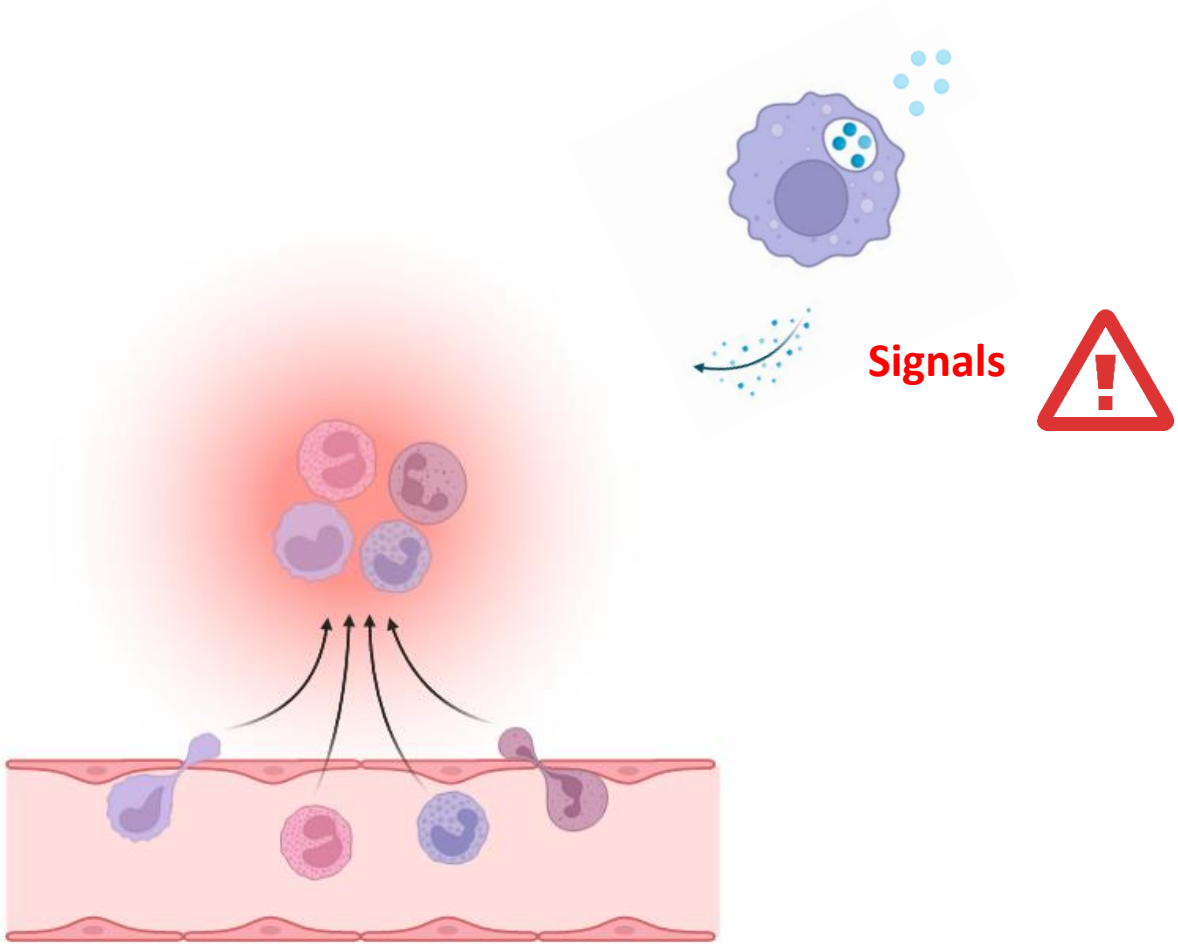
2) Creation of physical barrier to avoid pathogen dissemination

3) Promoting tissue healing

❖ Characteristics:

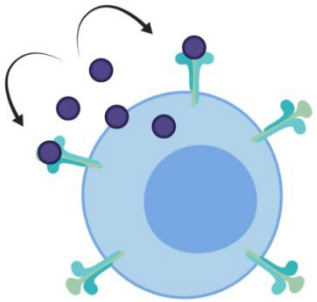
Redness, heat, swelling, pain and release of signals

Communication
(Chemokines and Cytokines)

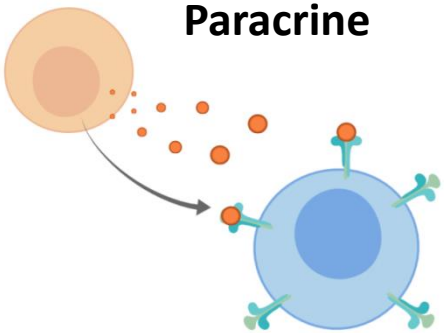


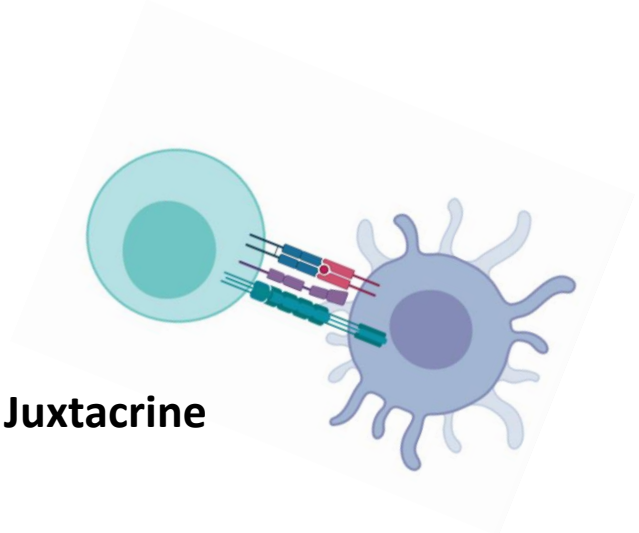
Communication
(Chemokines and Cytokines)

Autocrine

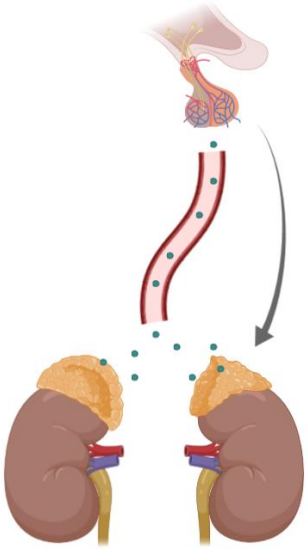


Paracrine



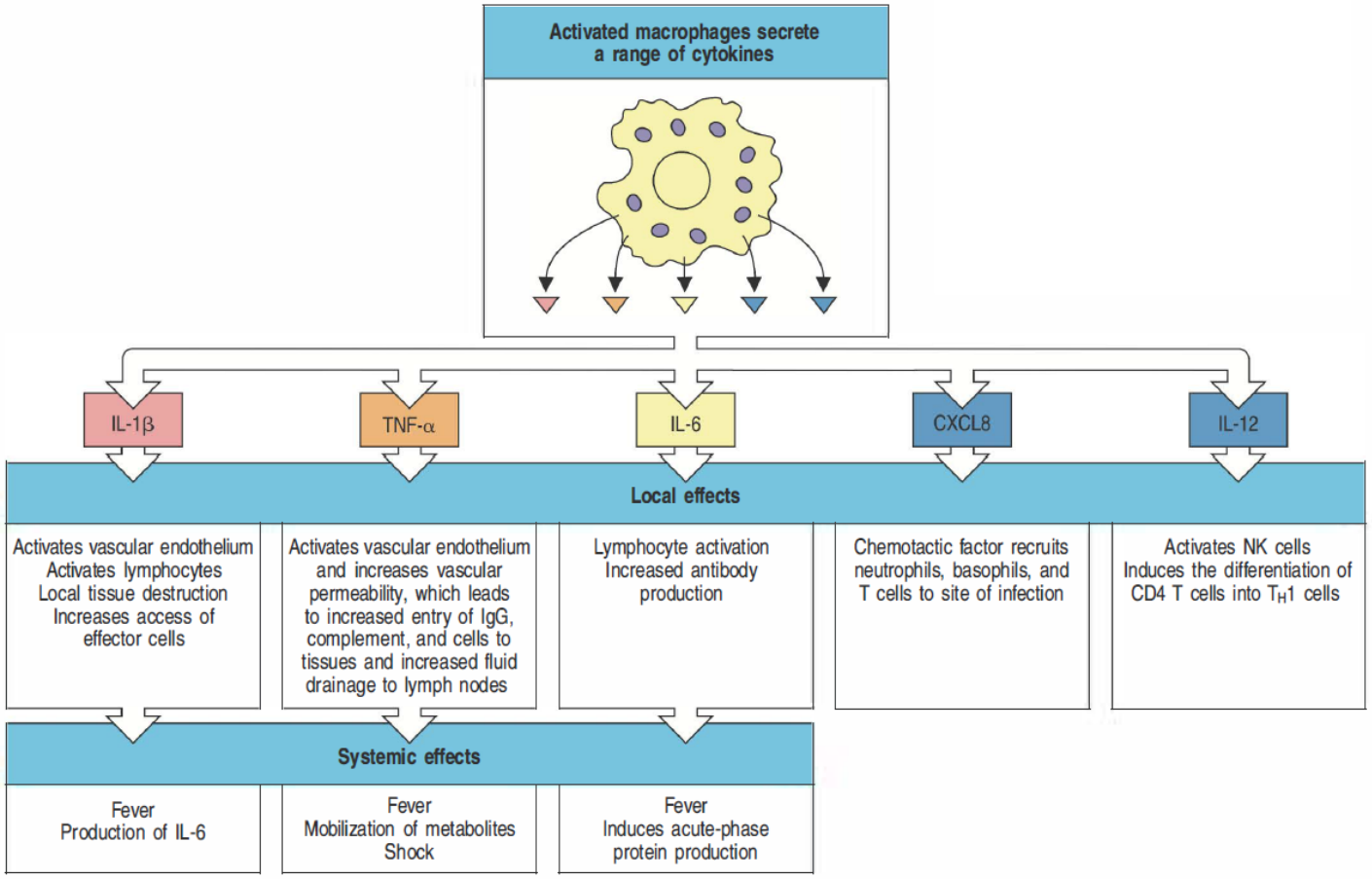


Juxtacrine

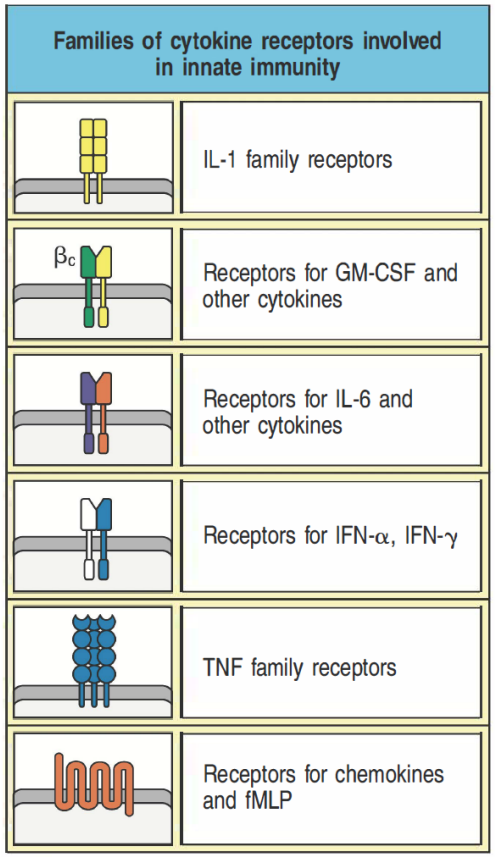


Endocrine

Communication (Chemokines and Cytokines)



Communication (Chemokines and Cytokines)



Class	Chemokine	Produced by	Receptors	Cells attracted	Major effects
CXC	CXCL8 (IL-8)	Monocytes Macrophages Fibroblasts Epithelial cells Endothelial cells	CXCR1 CXCR2	Neutrophils Naive T cells	Mobilizes, activates and degranulates neutrophils Angiogenesis
	CXCL7 (PBP, β-TG, NAP-2)	Platelets	CXCR2	Neutrophils	Activates neutrophils Clot resorption Angiogenesis
	CXCL1 (GROα) CXCL2 (GROβ) CXCL3 (GROγ)	Monocytes Fibroblasts Endothelium	CXCR2	Neutrophils Naive T cells Fibroblasts	Activates neutrophils Fibroplasia Angiogenesis
CC	CCL3 (MIP-1α)	Monocytes T cells Mast cells Fibroblasts	CCR1, 3, 5	Monocytes NK and T cells Basophils Dendritic cells	Competes with HIV-1 Antiviral defense Promotes T_H1 immunity
	CCL4 (MIP-1β)	Monocytes Macrophages Neutrophils Endothelium	CCR1, 3, 5	Monocytes NK and T cells Dendritic cells	Competes with HIV-1
	CCL2 (MCP-1)	Monocytes Macrophages Fibroblasts Keratinocytes	CCR2B	Monocytes NK and T cells Basophils Dendritic cells	Activates macrophages Basophil histamine release Promotes T_H2 immunity
	CCL5 (RANTES)	T cells Endothelium Platelets	CCR1, 3, 5	Monocytes NK and T cells Basophils Eosinophils Dendritic cells	Degranulates basophils Activates T cells Chronic inflammation
CXXXC (CX_3C)	CX3CL1 (Fractalkine)	Monocytes Endothelium Microglial cells	CX_3CR1	Monocytes T cells	Leukocyte–endothelial adhesion Brain inflammation

Communication (Cytokines)

- MW: ~25 kDa
- Released by different cell types
- Bind to specific receptors

- Structural families:
  - Hematopoietic family
  - TNF family
  - Chemokine family

Cytokines

- IL-1 : Fever, T cell & macrophage activation
- IL-2: Proliferation of T cells
- IL-3: Hematopoiesis (Basophils)
- IL-4: Th2 cell polarization, B cell activation, IgE switch
- IL-5: Proliferation and differentiation of eosinophils
- IL-6: Acute phase proteins, Th17 helper polarization
- IL-8: T cell and neutrophil chemotaxis
- IL-9: Mast cell activation
- IL-10: Inhibition of macrophages, T and B cells
- IL-12: Activation of NK cells. Th1 polarization
- IL-13: B cell activation. Macrophage inhibition.

- TNFα: Local inflammation
- IFNγ: Antiviral, macrophage activation, MHC upregulation
- IFNα/β: Antiviral, MHC upregulation
- MCP-1: Monocyte chemotaxis
- MIP1α: Monocyte, T cell and eosinophil chemotaxis

Chemokines

- MW: 8-14 kDa
- Attraction and migration of immune cells

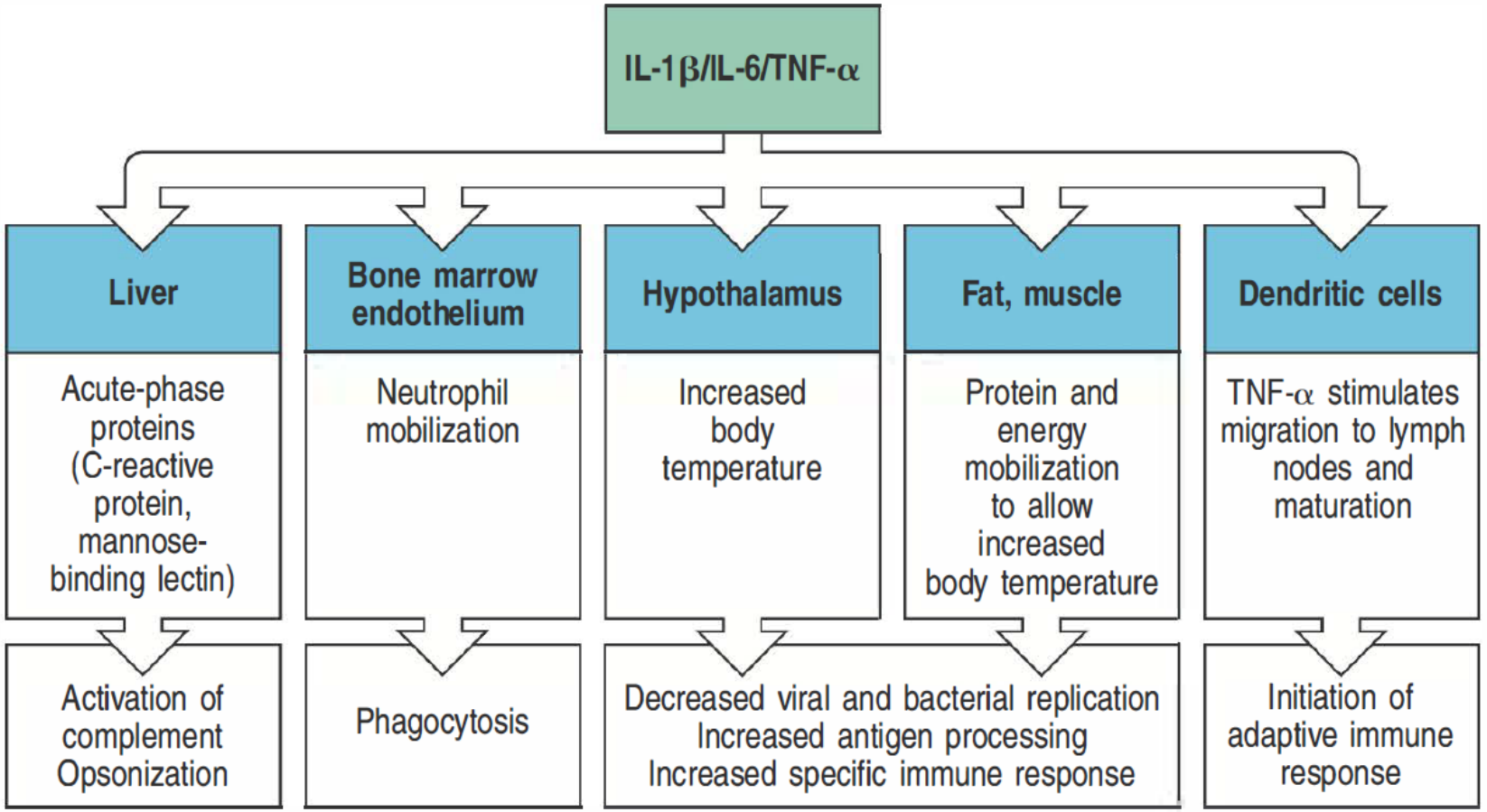
- Bind to chemokine receptors which are integral membrane proteins (belong to the family of the G-protein-coupled receptors)

- Conserved feature of chemokines is a **group of cysteine residues** that can form one or two disulfide bridges

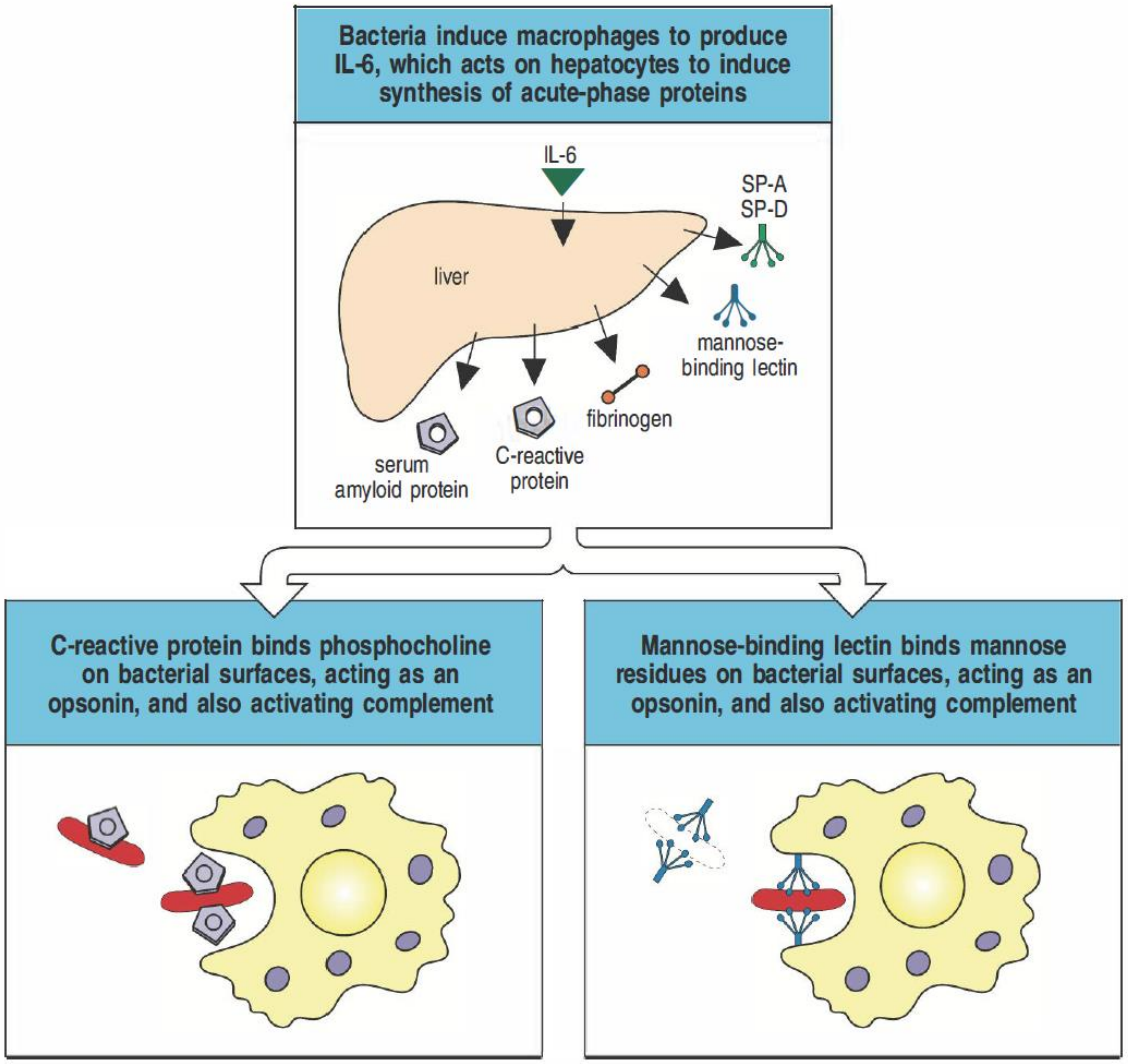
- Chemokine superfamilies:
  - C-C –Chemokine: two consecutive conserved cysteines in the amino terminus.
    - CCL-2: monocyte chemoattractant
  - C-X-C-chemokine: one aminoacid between the 2 conserved cysteines in the amino terminus.
    - CXCL-8: Neutrophil chemoattractant
  - C : only one conserved cyteine in the amino terminus.
  - CX3C: cysteines separated by 3 amino acids.

*L or R at the end indicates Ligand or receptor

Communication (Chemokines and Cytokines)



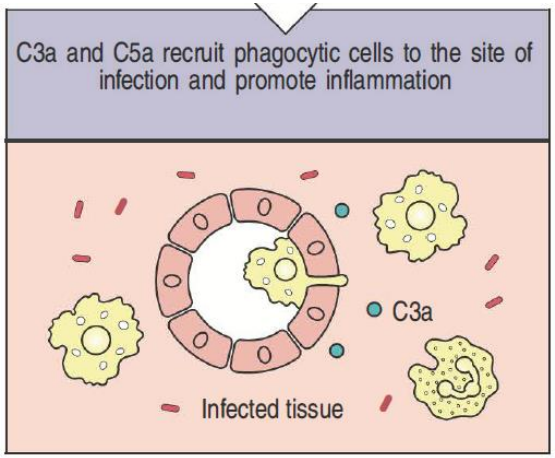
Communication (Acute-phase proteins)



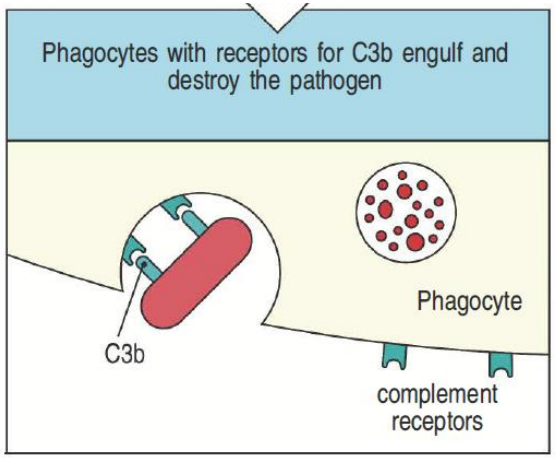
Complement system

Proteins present in plasma as zymogen: Activated by proteolytic cleavage

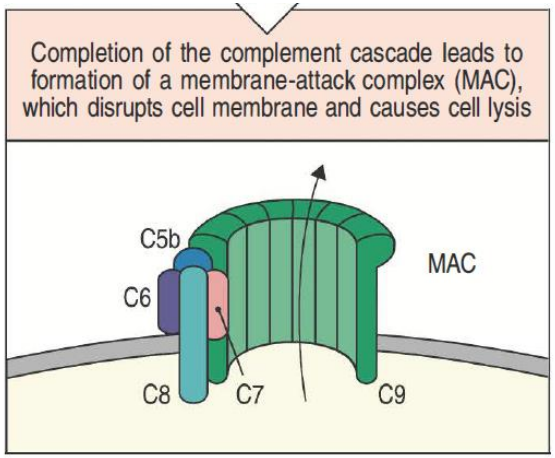
Pattern recognition receptors (PRR) or antibodies activate complement



Recruitment

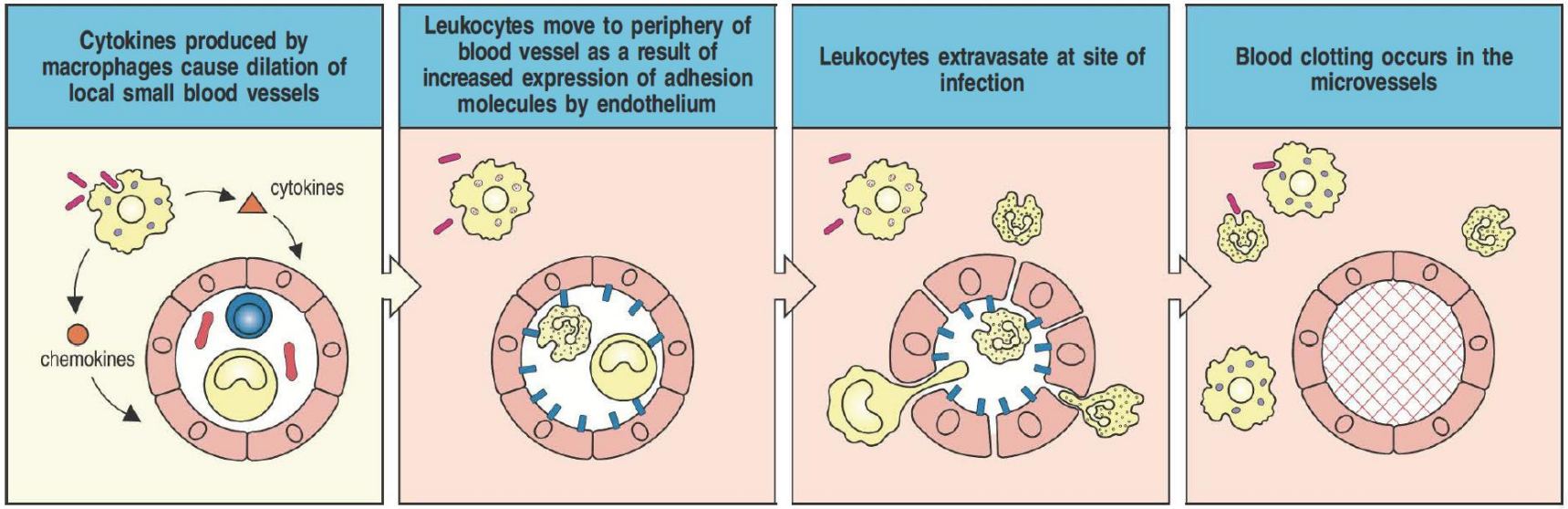


Phagocytosis

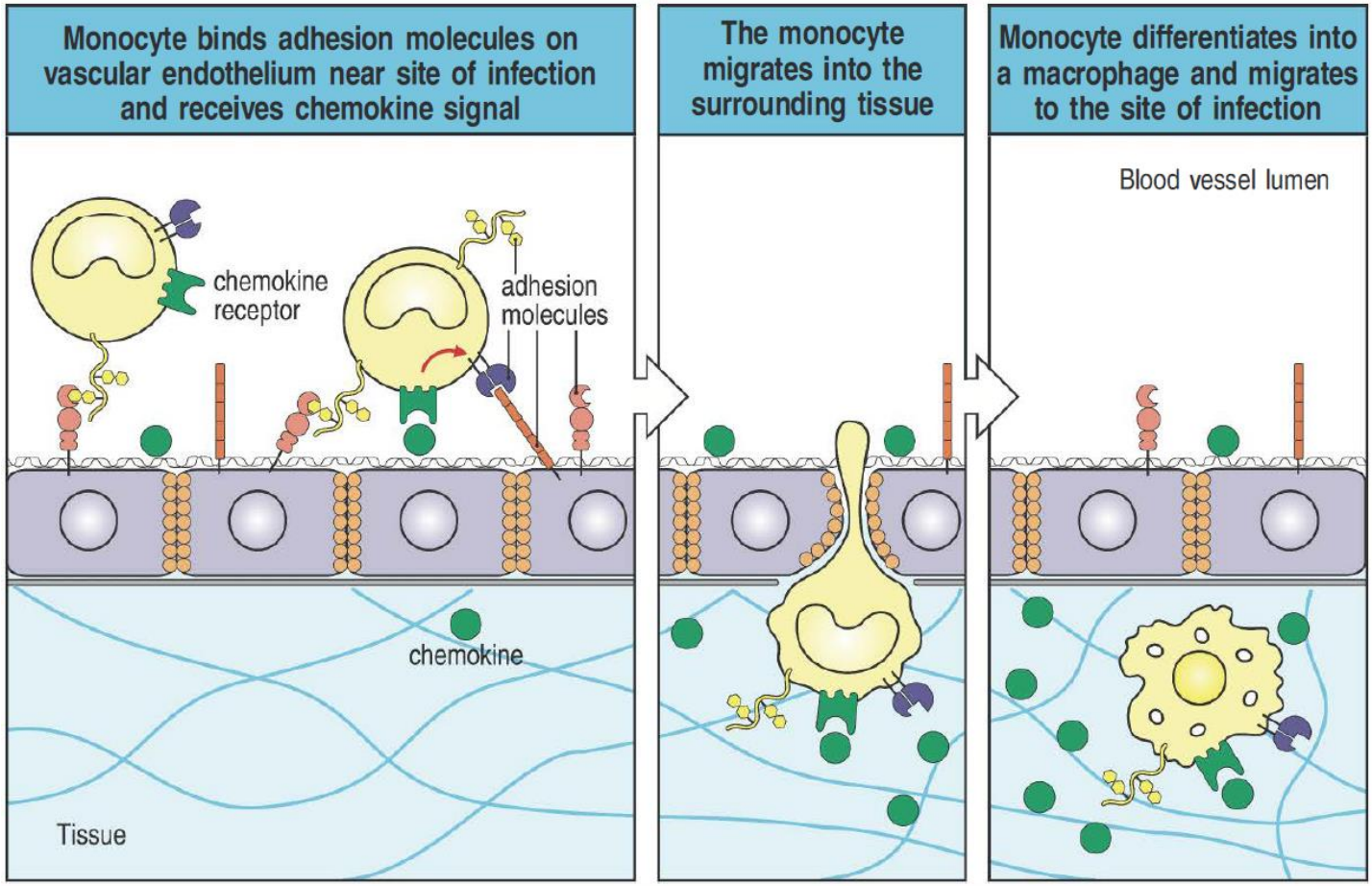


Cell lysis

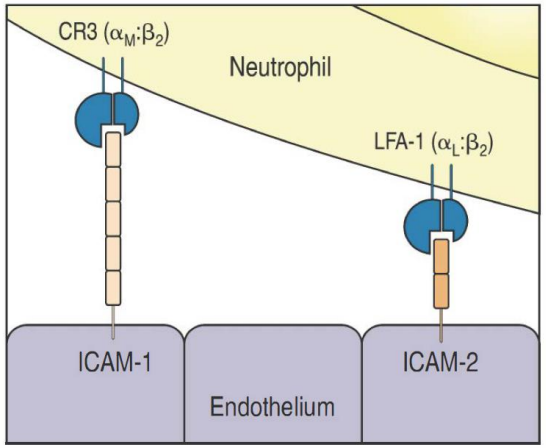
Recruitment

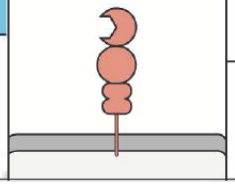
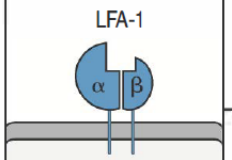
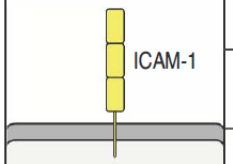


Recruitment

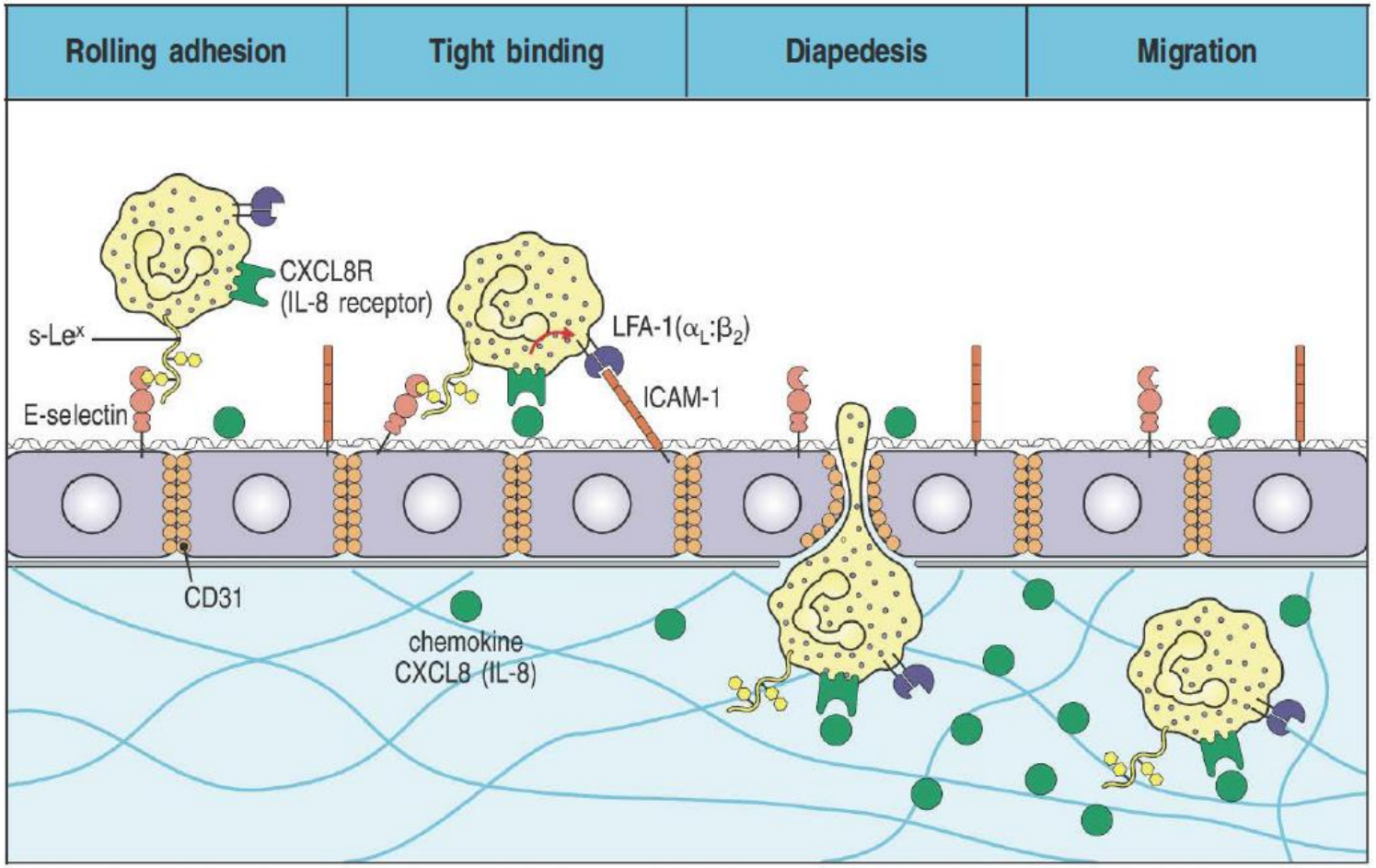


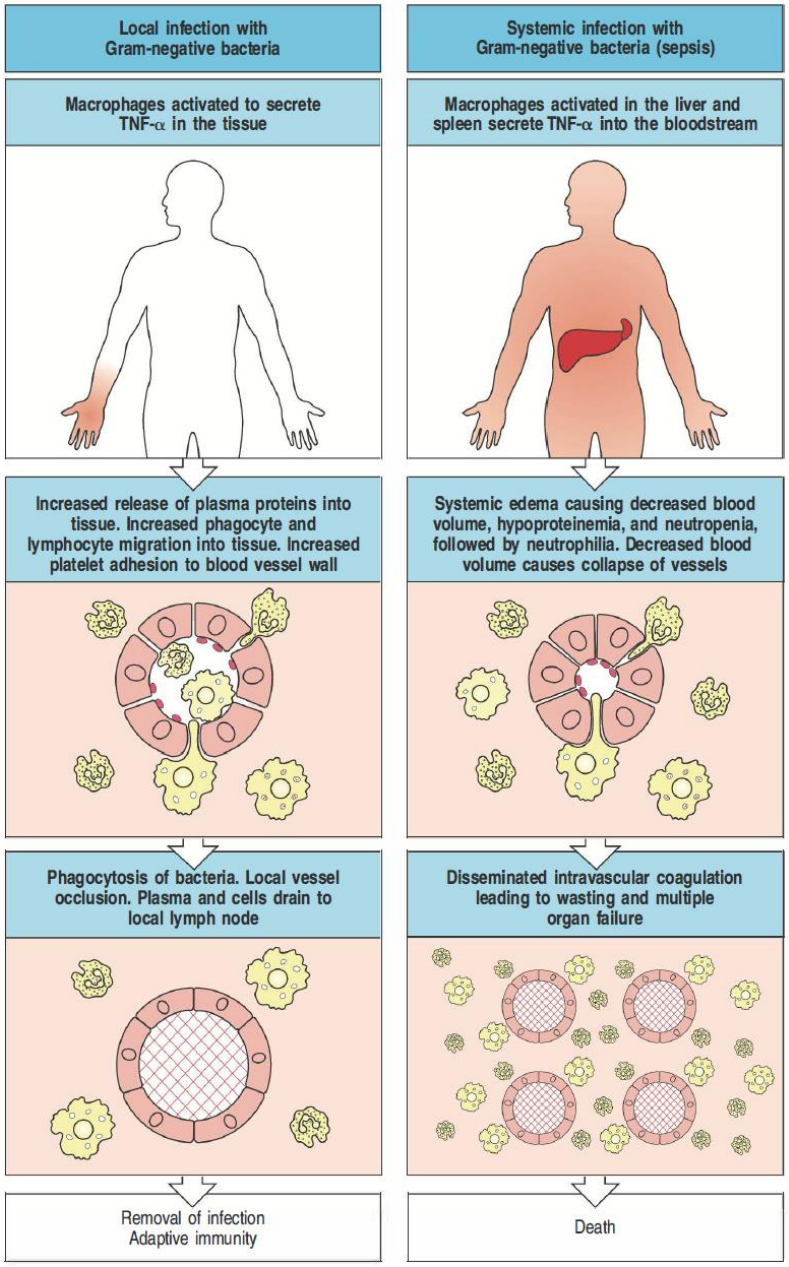
Adhesion molecules



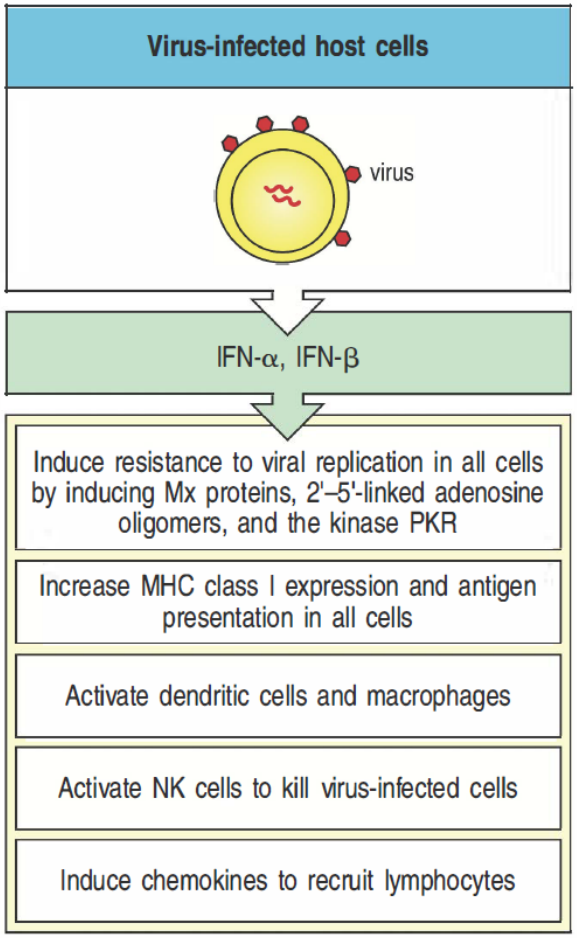
		Name	Tissue distribution	Ligand
Selectins Bind carbohydrates. Initiate leukocyte–endothelial interaction		P-selectin (PADGEM, CD62P)	Activated endothelium and platelets	PSGL-1, sialyl-Lewis^x
		E-selectin (ELAM-1, CD62E)	Activated endothelium	Sialyl-Lewis^x
Integrins Bind to cell-adhesion molecules and extracellular matrix. Strong adhesion		α_L:β_2 (LFA-1, CD11a:CD18)	Monocytes, T cells, macrophages, neutrophils, dendritic cells, NK cells	ICAMs
		α_M:β_2 (CR3, Mac-1, CD11b:CD18)	Neutrophils, monocytes, macrophages, NK cells	ICAM-1, iC3b, fibrinogen
		α_X:β_2 (CR4, p150.95, CD11c:CD18)	Dendritic cells, macrophages, neutrophils, NK cells	iC3b
		α_5:β_1 (VLA-5, CD49d:CD29)	Monocytes, macrophages	Fibronectin
Immunoglobulin superfamily Various roles in cell adhesion. Ligand for integrins		ICAM-1 (CD54)	Activated endothelium, activated leukocytes	LFA-1, Mac1
		ICAM-2 (CD102)	Resting endothelium, dendritic cells	LFA-1
		VCAM-1 (CD106)	Activated endothelium	VLA-4
		PECAM (CD31)	Activated leukocytes, endothelial cell–cell junctions	CD31

Adhesion molecules

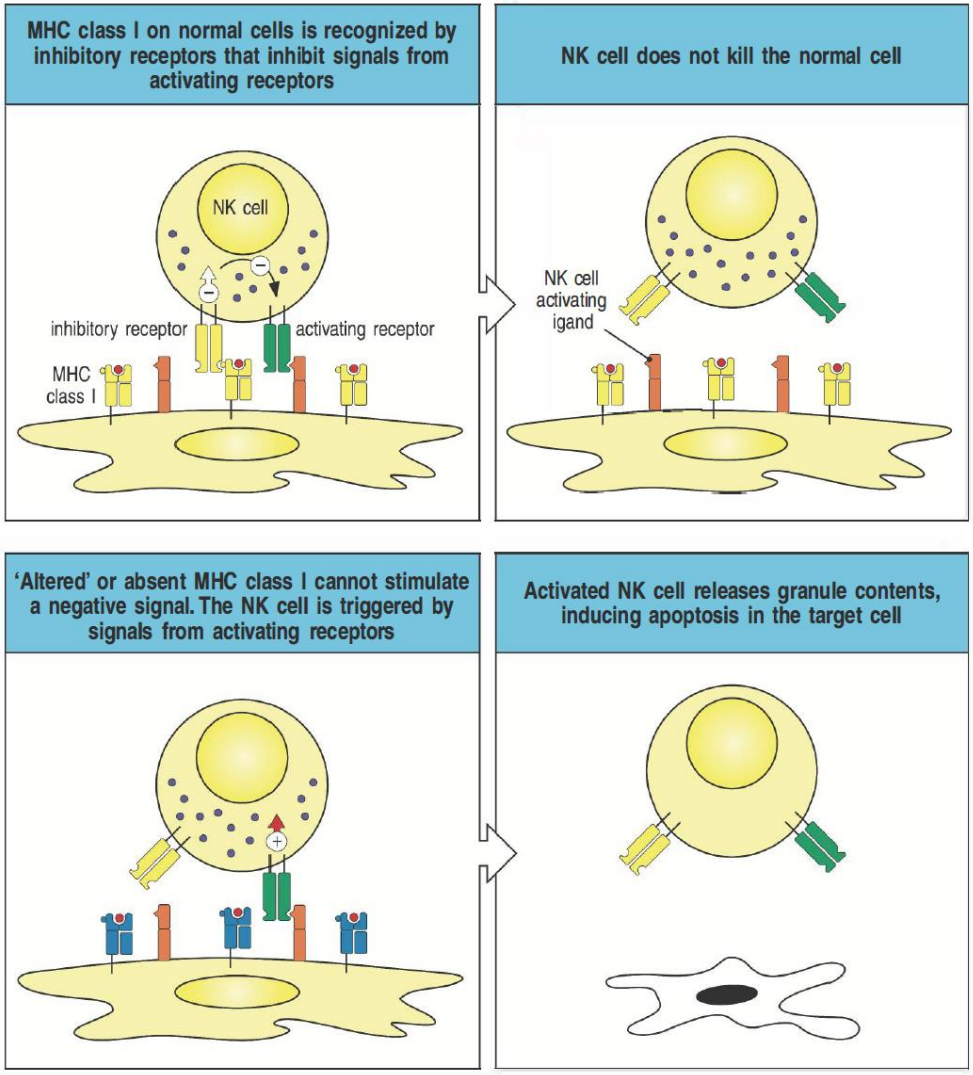



Local infection with Gram-negative bacteria
Systemic infection with Gram-negative bacteria (sepsis)
Macrophages activated to secrete TNF-α in the tissue
Macrophages activated in the liver and spleen secrete TNF-α into the bloodstream
Increased release of plasma proteins into tissue. Increased phagocyte and lymphocyte migration into tissue. Increased platelet adhesion to blood vessel wall
Systemic edema causing decreased blood volume, hypoproteinemia, and neutropenia, followed by neutrophilia. Decreased blood volume causes collapse of vessels
Phagocytosis of bacteria. Local vessel occlusion. Plasma and cells drain to local lymph node
Disseminated intravascular coagulation leading to wasting and multiple organ failure
Removal of infection
Adaptive immunity
Death

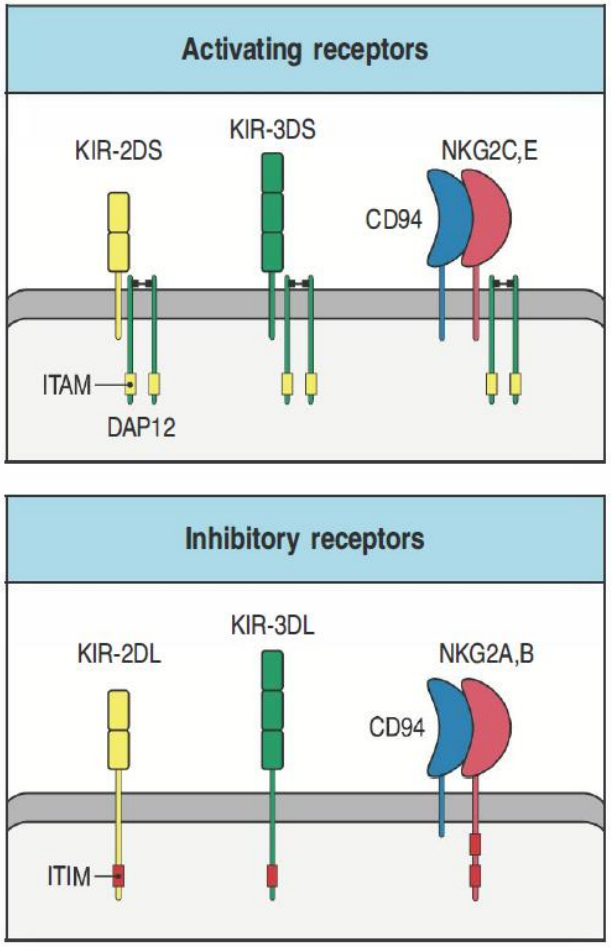
Natural-killer (NK) cells

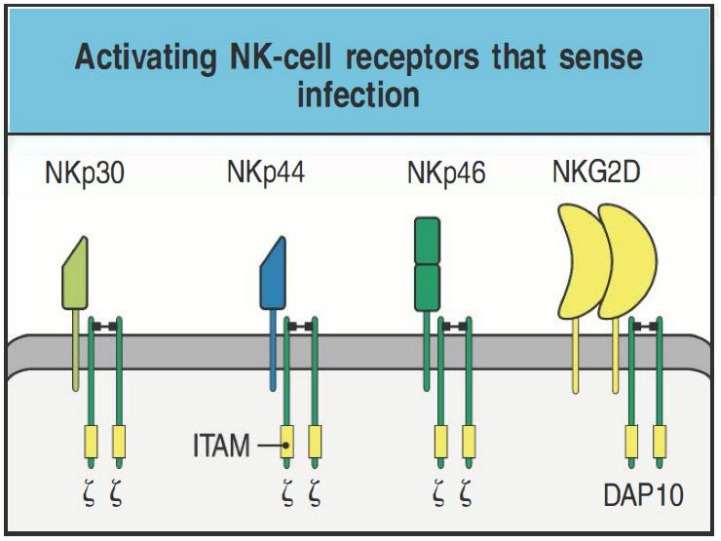


Natural-killer (NK) cells

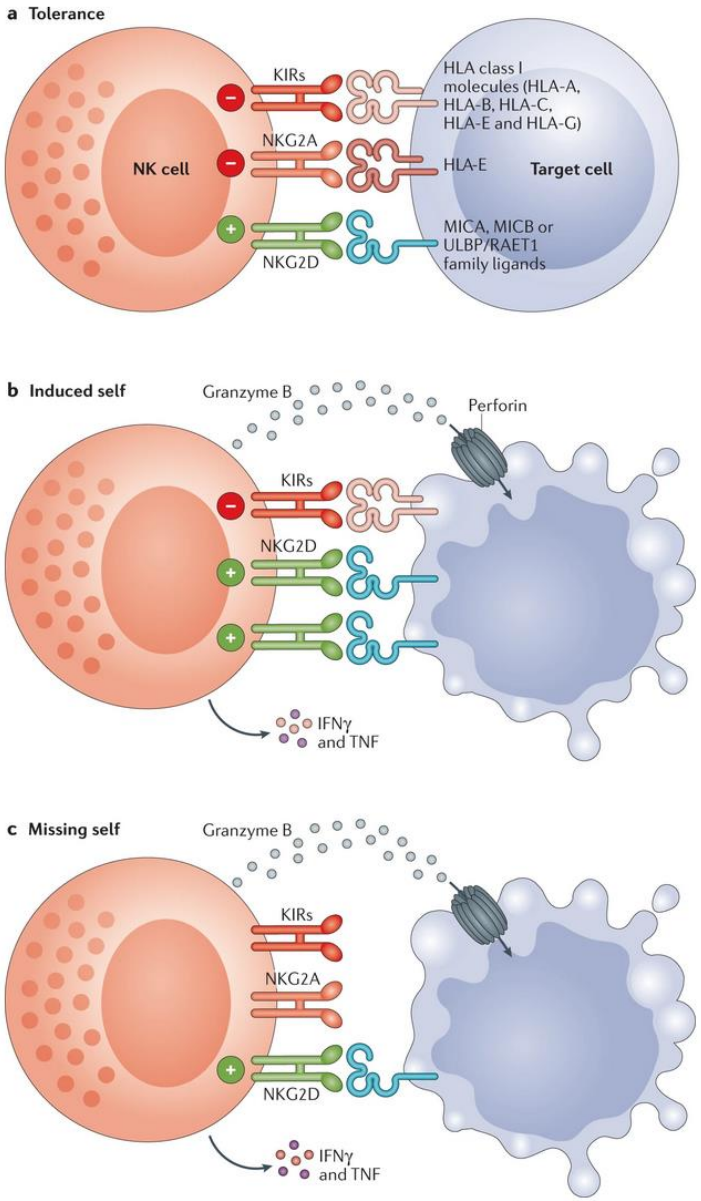


Natural-killer (NK) cells



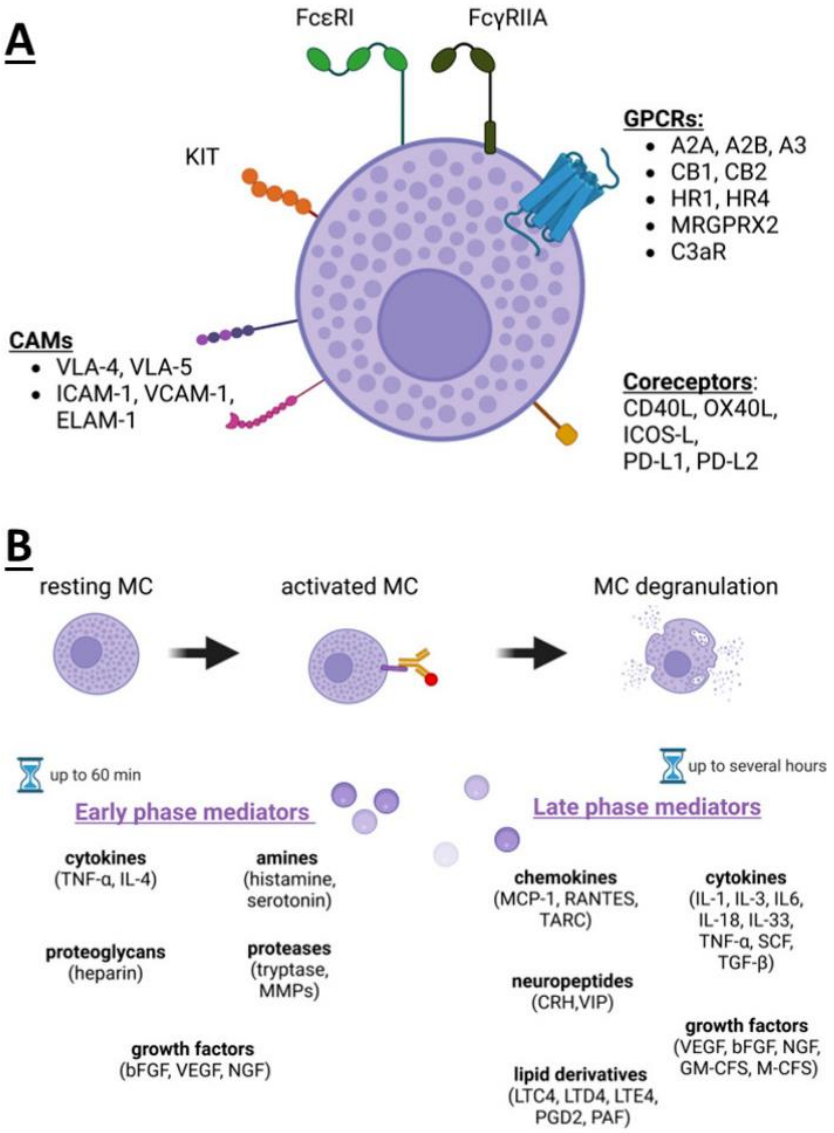


Natural-killer (NK) cells



Mast cells

- First described by Ehrlich in his 1878 doctoral thesis on the basis of their unique staining characteristics and large granules.
- Tissue-resident cells, often adjacent to blood vessels and close to epithelial surfaces, creating a barrier for pathogens.
- Release potent inflammatory mediators, such as **histamine, proteases, chemotactic factors, cytokines and metabolites of arachidonic acid** that act on the vasculature, smooth muscle, connective tissue, mucous glands and inflammatory cells.
- Play a central role in inflammatory and immediate allergic reactions.



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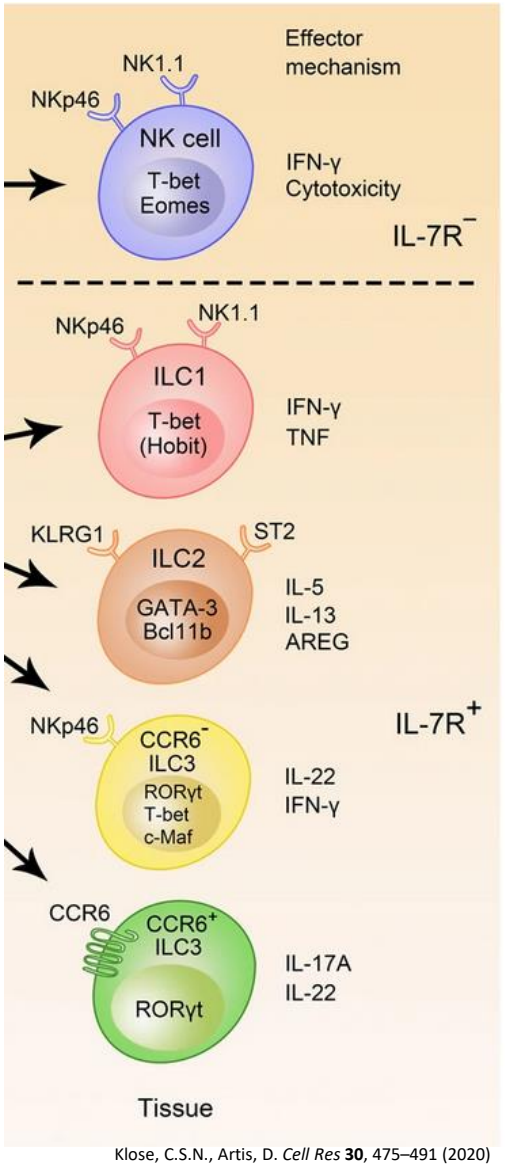
Innate lymphoid cells (ILCs)

- Resident in non-lymphoid organs (mucosal surfaces)
  - Rare in blood circulation or lymphoid organs
- Can develop in the absence of recombination-activating genes (Rag-1 and Rag-2). Do not develop antigen-receptors.
- Rely on PRRs and receptors for host-derived signals like dietary metabolites, microbial products, hormones, neuropeptides, and cytokines.
- Early source of cytokines
- T cell counterparts



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Thank you for your attention!

Questions?

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