Soluble components of innate defense

Soluble effector molecules of innate immunity

- Soluble molecules recognize microbes and promote innate defense
- The soluble effector molecules function in two major ways:
  - By binding to microbes, they act as **opsonins** >> enhance the capability of phagocytes to ingest pathogens
  - Promote an inflammatory response (e.g., recruitment innate immune cells)

Major components: complement system, collectins, pentraxins, ficolins

The Complement System

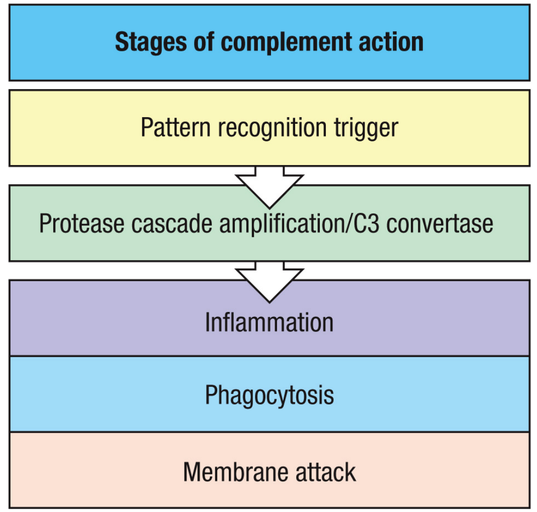
Discovered in the *1890's* by Jules Bordet the complement system is a collection of *soluble proteins* (**>** 30 different proteins mainly produced in the *liver*) present in the blood or other body fluids.

Together these factors

- Augment *opsonization* and killing of bacteria = to „*complement*" the action of antibodies
- Promote the recruitment of phagocytes to the site of infection
- Directly kill microbes

In absence of infection, proteases in inactive form = *zymogens* (pro-enzymes)

When encounter pathogen ➔ activated via *proteolysis*



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The recognition of pathogen by the complement system

Recognition of molecules specifically on microbial surfaces

↓

Activation of initial zymogen

↓

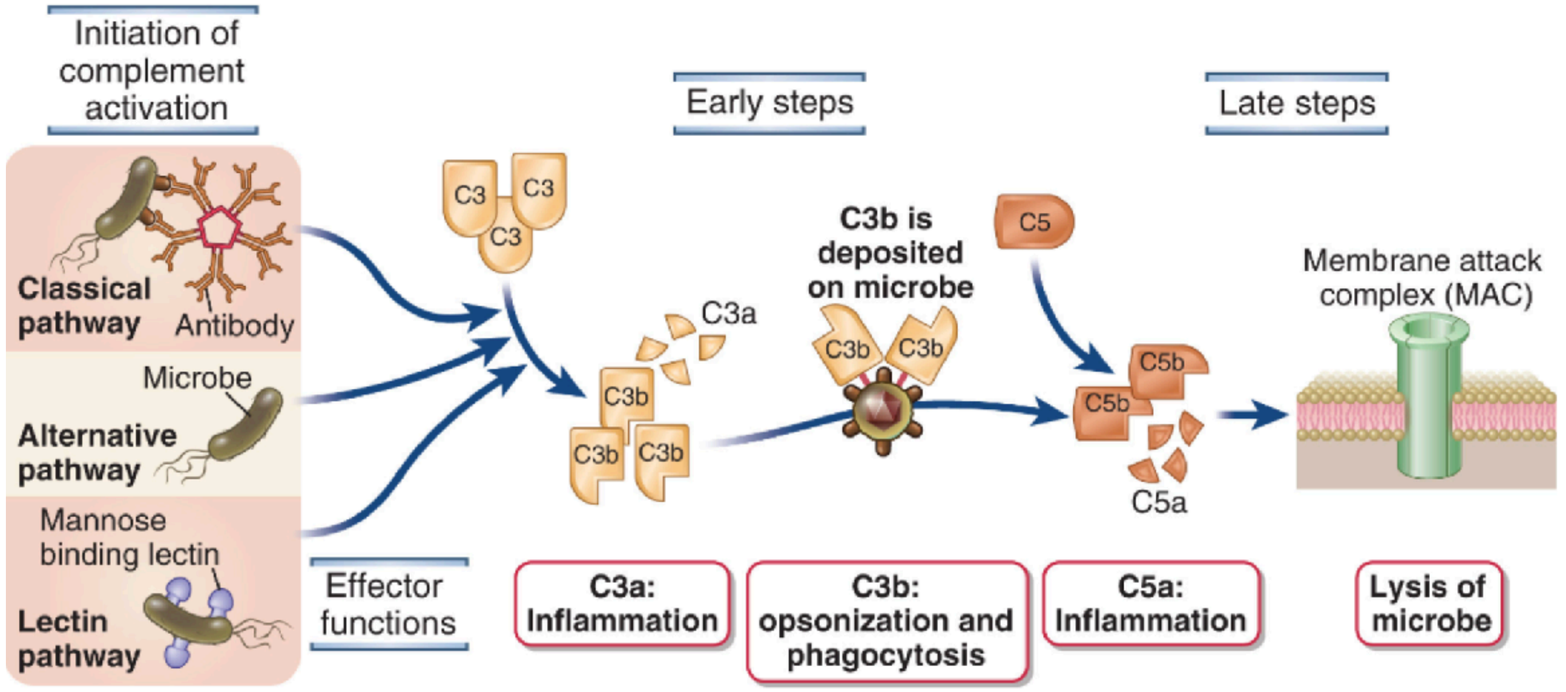
Cascade of proteolysis

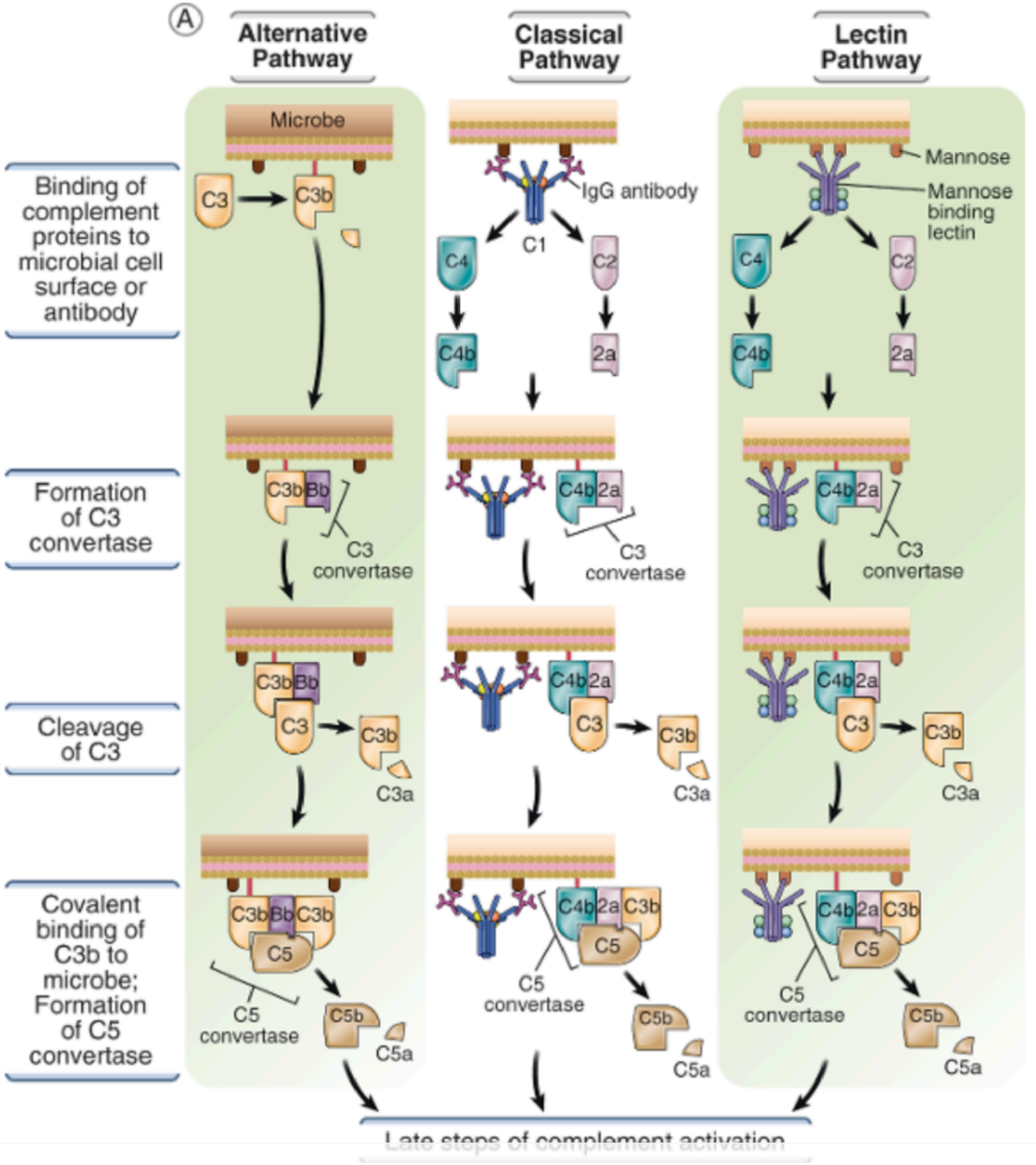
↓

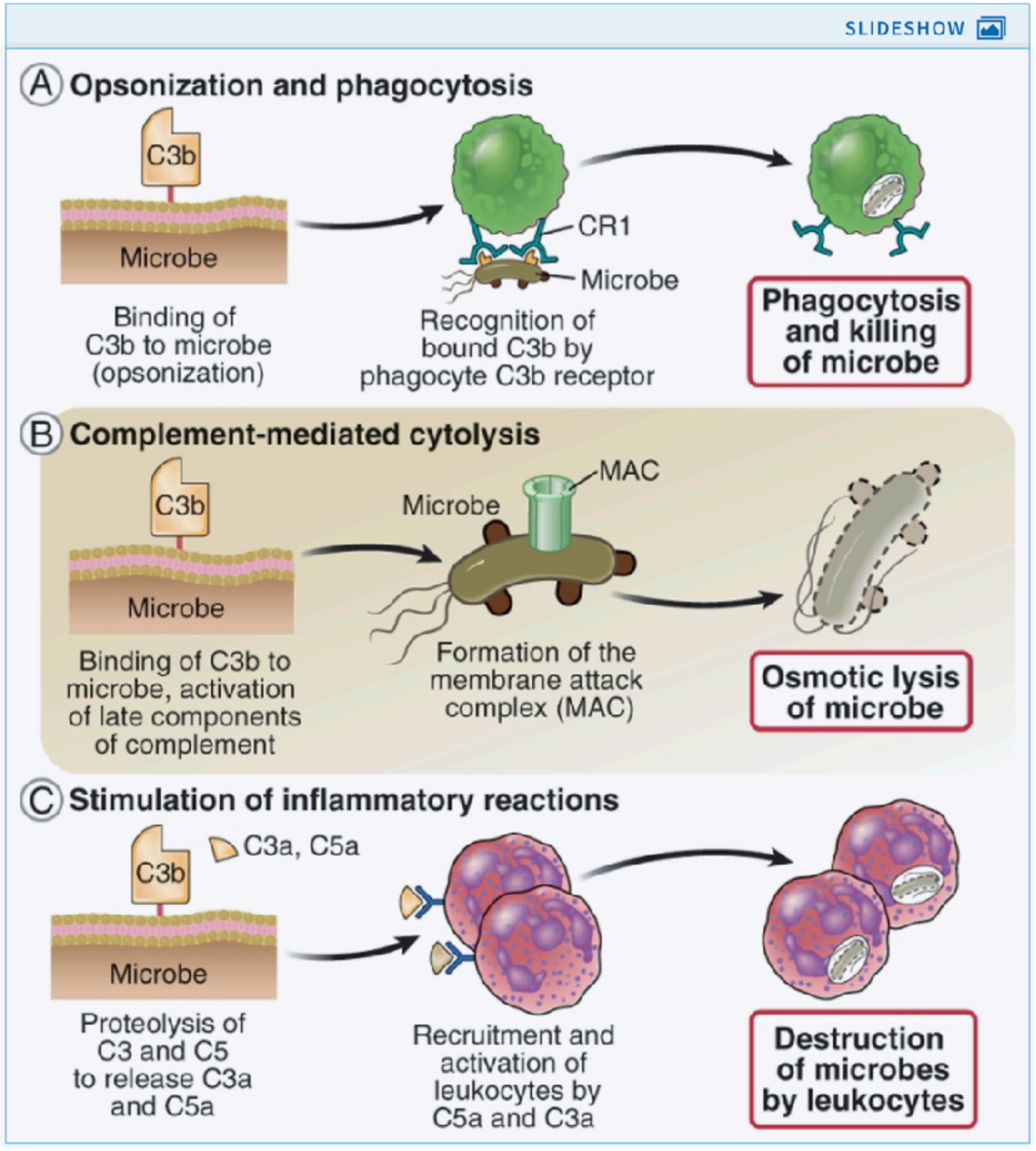
Activation of the effector complement components

Classical pathway	**Alternative pathway**	**Lectin pathway**
Antibody-triggered	Pathogen-triggered	Carbohydrate-triggered
C1q	spontaneous hydrolysis	spontaneous hydrolysis

The 3 pathways of the complement system




A
Alternative Pathway
Classical Pathway
Lectin Pathway
Binding of complement proteins to microbial cell surface or antibody
Formation of C3 convertase
Cleavage of C3
Covalent binding of C3b to microbe; Formation of C5 convertase
Microbe
C3
C3b
IgG antibody
C1
C4
C2
C4b
2a
Mannose
Mannose binding lectin
C3b Bb
C3 convertase
C4b 2a
C3a
C5
C5 convertase
C5b
C5a
Late steps of complement activation

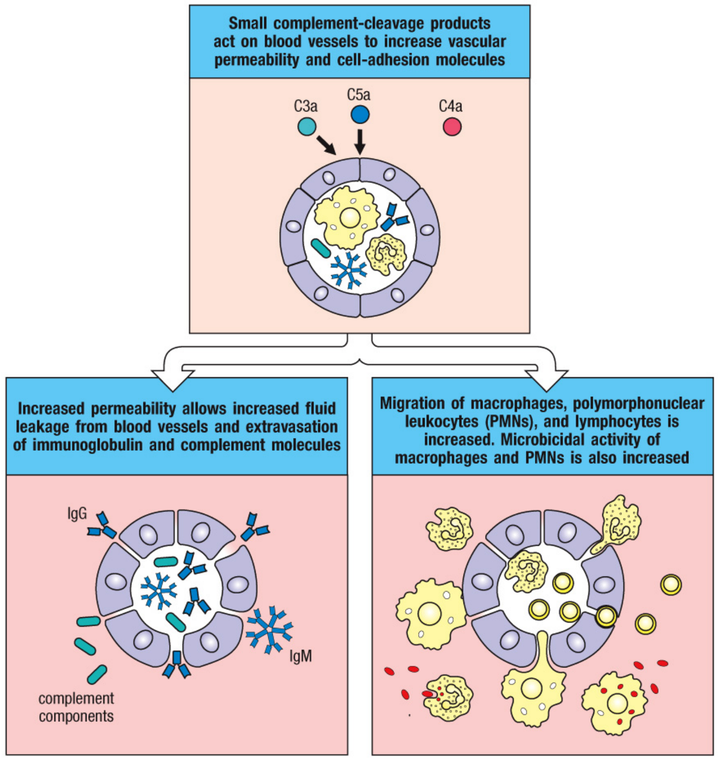

SLIDESHOW
A Opsonization and phagocytosis
C3b
Microbe
Binding of C3b to microbe (opsonization)
CR1
Microbe
Recognition of bound C3b by phagocyte C3b receptor
Phagocytosis and killing of microbe
B Complement-mediated cytolysis
C3b
Microbe
Binding of C3b to microbe, activation of late components of complement
MAC
Microbe
Formation of the membrane attack complex (MAC)
Osmotic lysis of microbe
C Stimulation of inflammatory reactions
C3b
C3a, C5a
Microbe
Proteolysis of C3 and C5 to release C3a and C5a
Recruitment and activation of leukocytes by C5a and C3a
Destruction of microbes by leukocytes

Initiation of local inflammatory responses by small fragments of complement proteins

C3a, C3b and C5a = *anaphylatoxins,* inducing generalized circulatory collapse ➔ **shock-like symptoms**

Effects

- Contraction of *smooth muscles*
- Increasing *vascular permeability*
- Synthesis of *adhesion molecules* on endothelial cells (C3a, C5a)
- Activation of *mast cells*
- C5a activates *phagocytic cells* (increasing expression of CRs)



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The inflammatory response and antiviral immunity

Induced innate responses to infection

The innate immune system eliminates microbes mainly by inducing the **acute inflammatory response** and by **antiviral defense mechanisms**.

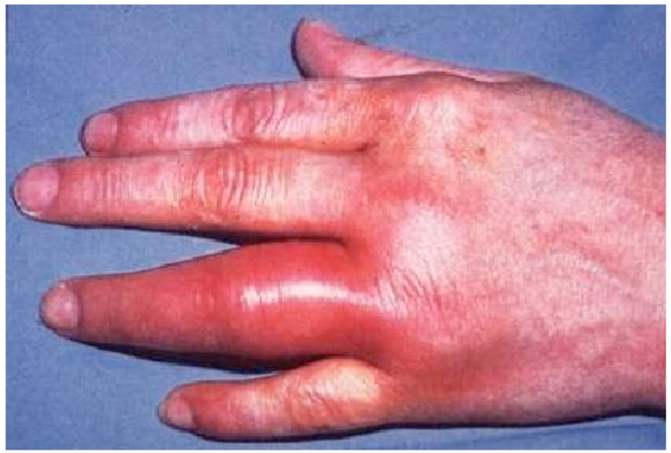
Different type of microbes elicit distinct innate immune reactions:

Extracellular bacteria and fungi >> Neutrophils, Monocytes, Complement

Intracellular bacteria >> Phagocytes

Viruses >> Type I interferons, NK cells

Inflammation



Characteristic features of inflammation:

redness, warmth, swelling, pain

The inflammatory reaction recruits cells to the site of infection, leads to an increase in the permeability of blood vessels and is accompanied with activation of cells and proteins in the extravascular space and irritation of nerve ends.

Initiation of an acute inflammatory reaction

MAMPs & DAMPs

↓

Sentinel cells (DCs, macrophages, endothelial cells, etc)

↓

Cytokines & small-molecule mediators & chemokines

↓

Changes in blood vessels

Increased blood flow

Increased adhesiveness

Increased permeability

↓

Inflammatory reaction

Proinflammtory cytokines

Cytokines

Small proteins (~ 25kDa): autocrine, paracrine or endocrine!

Different subfamilies based on structural features

➔ Important roles in local + systemic effects that contributes to *innate and adaptive* immunity.

Recognition of distinct types of *pathogens*

↓

distinct set of *PRRs*

↓

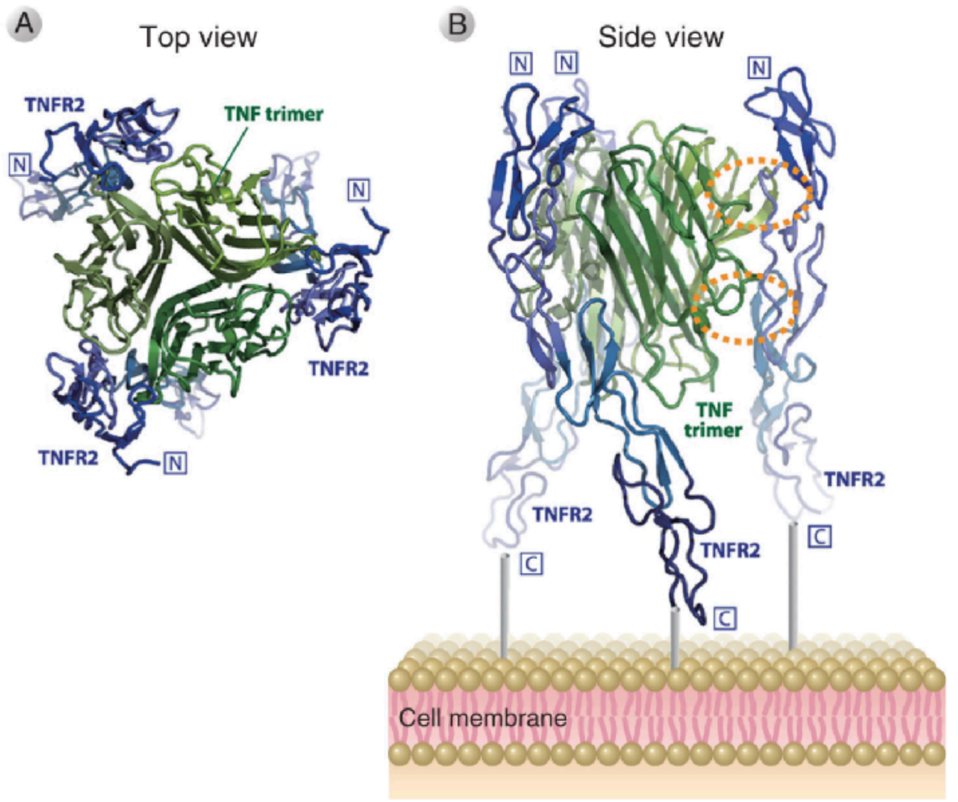
different set of *cytokines*

↓

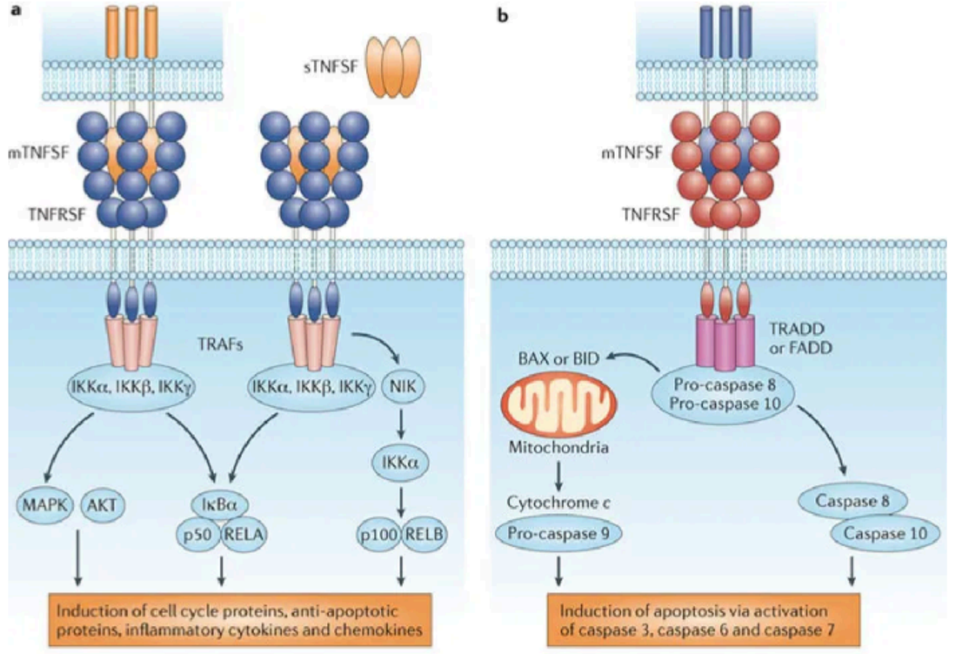
Pathogen-adapted response

Tumor Necrosis Factor

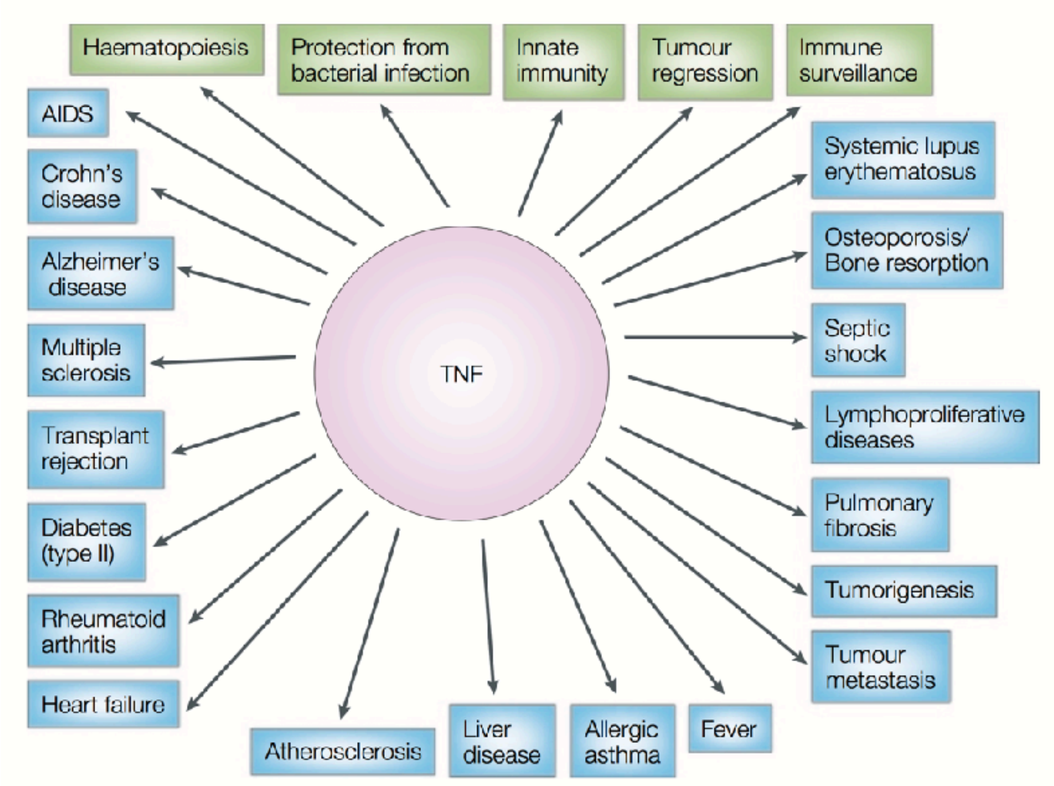
- Originally identified as a factor that causes tumour cell death
- TNF (alpha) synthesized as a membrane-bound proform, which is subsequently cleaved
- TNFR is present on most cells



Various signaling pathways of the TNF superfamily



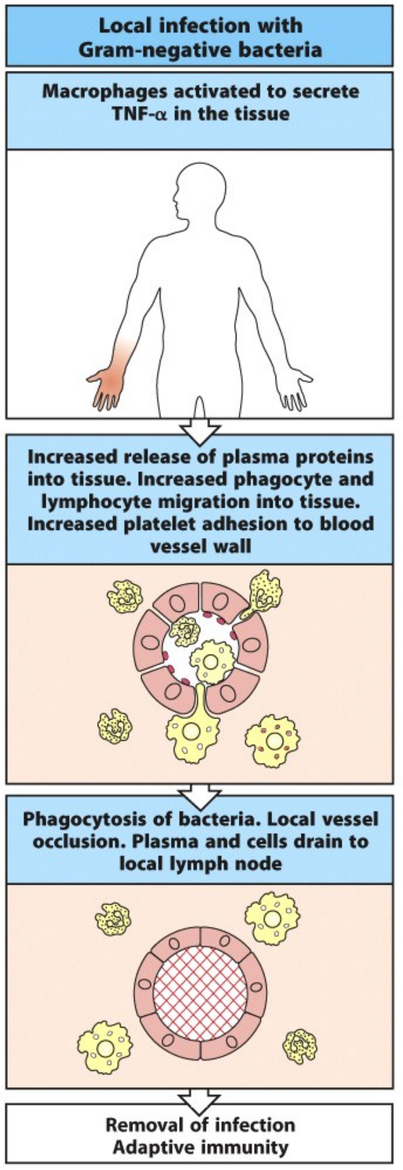
Tumor Necrosis Factor



The dual roles of TNF-alpha (1)

Some TNF-a is good…

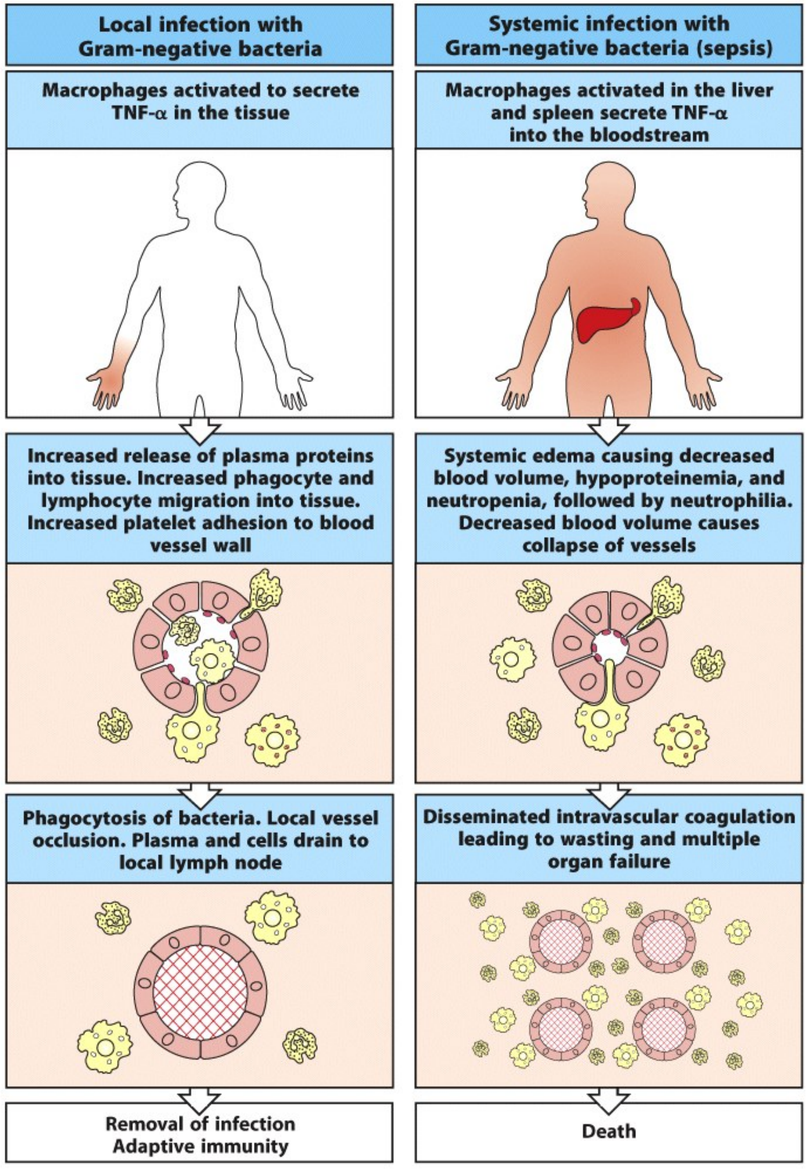
- stimulates extravasation of cells
- stimulates blood clotting in local small vessels
- when blocked, pathogen spreading is increased
- stimulation of DC migration to lymph node and maturation of DCs



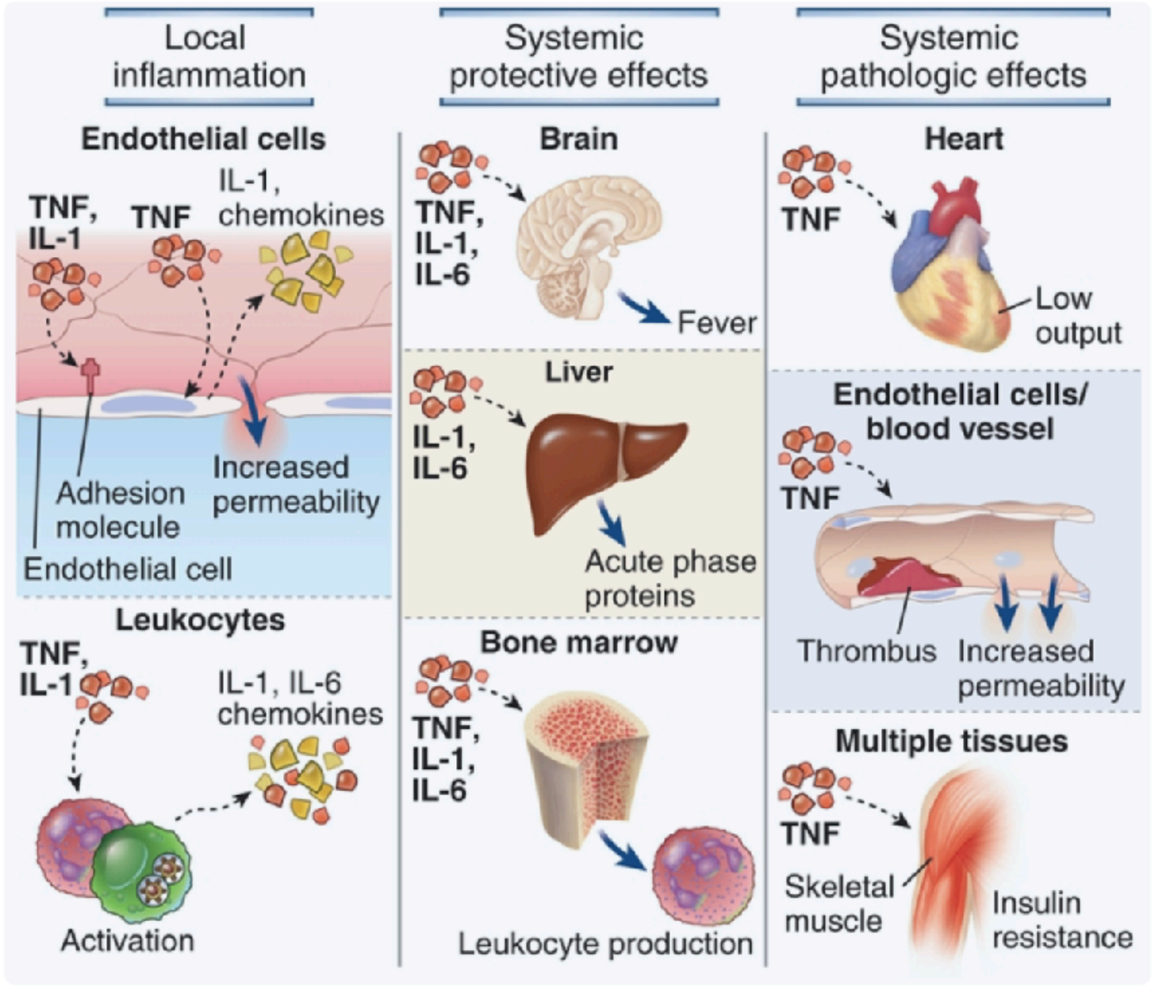
The dual roles of TNF-alpha (2)

…but too much is bad

- systemic TNFa (soluble cytokine form produced through cleavage by TACE/ADAM17)
- causes vasodilation > **septic shock**
- causes blood clotting > **DIC** (disseminated intravascular coagulation; consumption of clotting proteins)



Important cytokines and chemokines and their effector function



The acute-phase response

= production of acute-phase proteins in the liver

- acute-phase proteins have functional properties of antibodies, but are less specific allowing the recognition of a broad range of diverse pathogens
- likewise their synthesis is not specifically induced, but instead upon stimulation via several cytokines

1) **CRP**

- C-reactive protein (pentraxin family)
- CRP: multipronged PR molecule, that binds to bacterial/fungal cell wall components

> opsonizing of pathogens

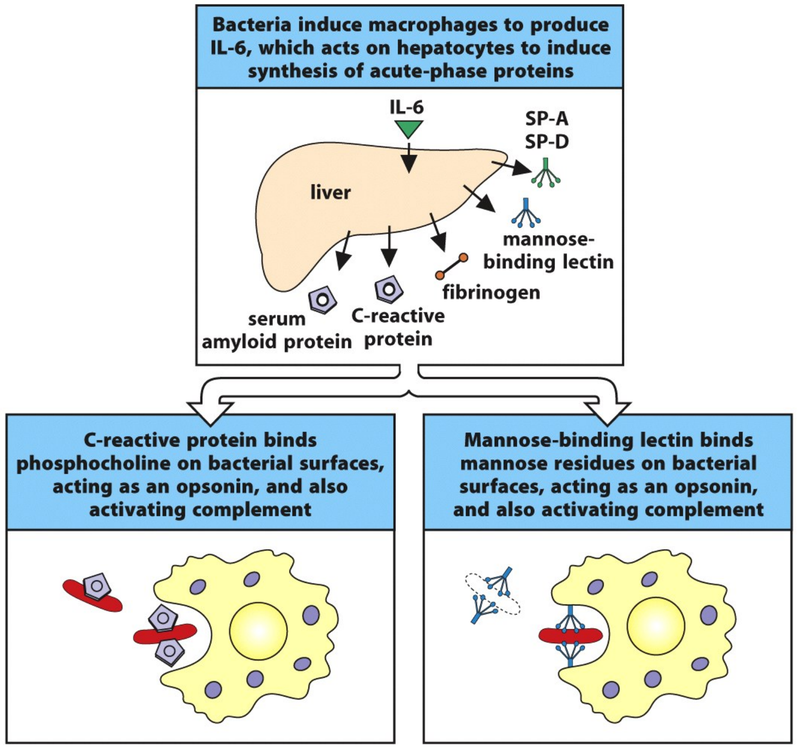
> activation of the complement

2) **MBL (mannose-binding lectin)**

- opsonin, activation of the complement cascade

3) **SP-A/SP-D (surfactant proteins)**

- opsonin for Pneumocystis jirovecii



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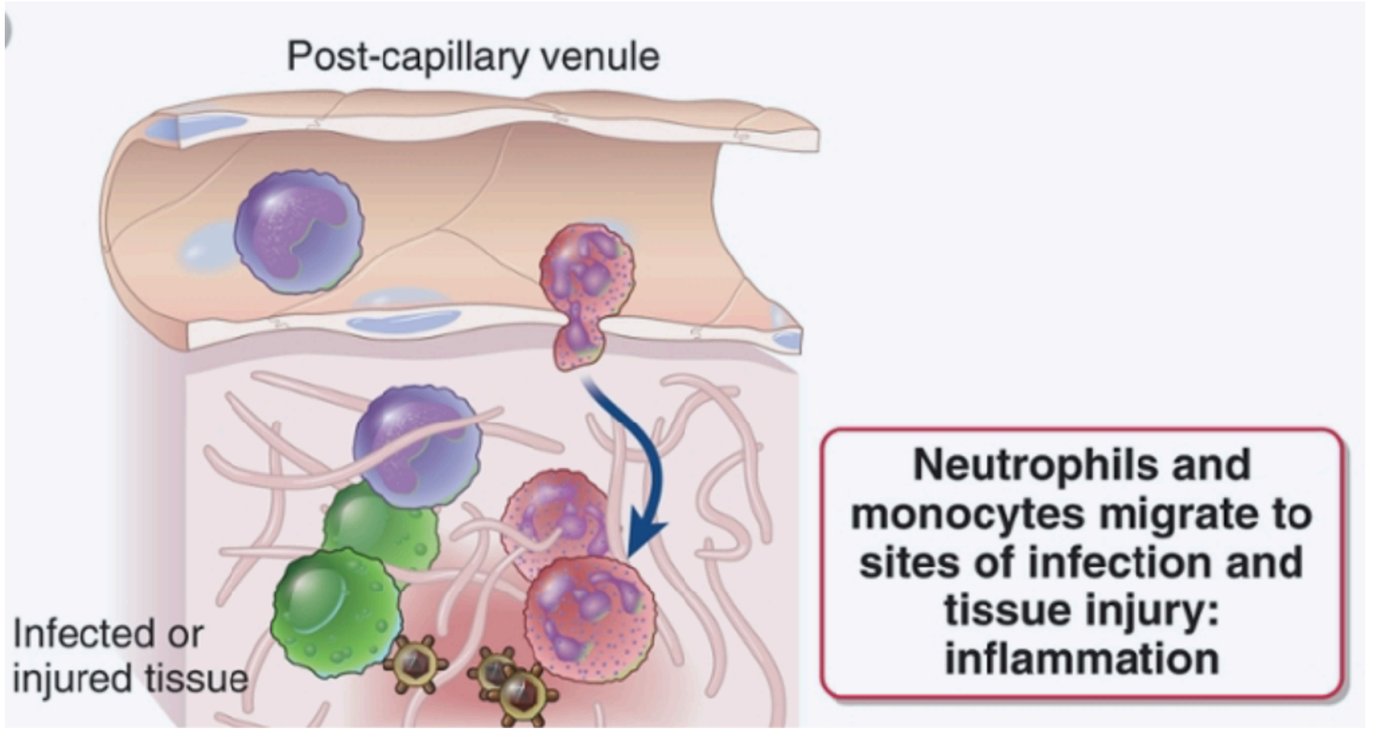
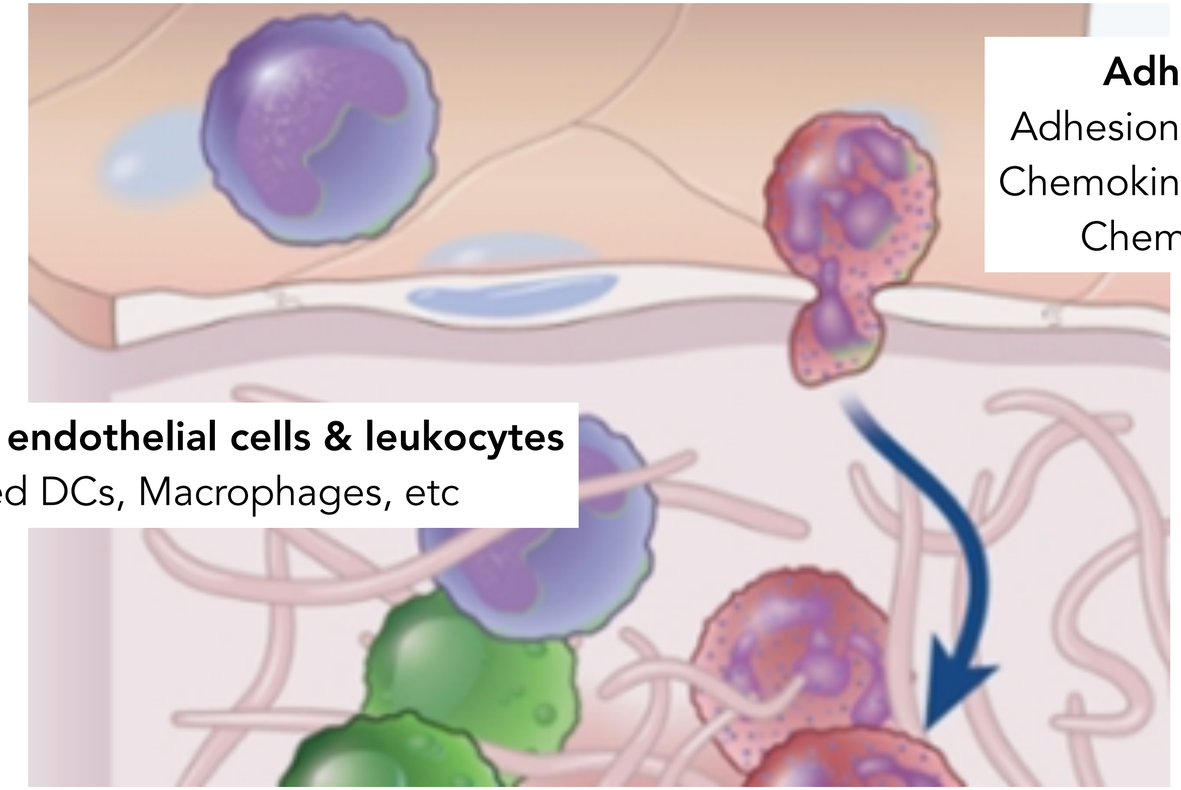
Leukocyte migration



Delivery of leukocytes of myeloid lineage from the circulation into tissue sites of infection or injury, where the cells perform their protective functions of eliminating infectious pathogens, clearing dead tissues, and repairing the damage

Some general principles on leukocyte migration



Adhesion
Adhesion molecules
Chemokine receptors
Chemokines

Activation of endothelial cells & leukocytes
Activated DCs, Macrophages, etc

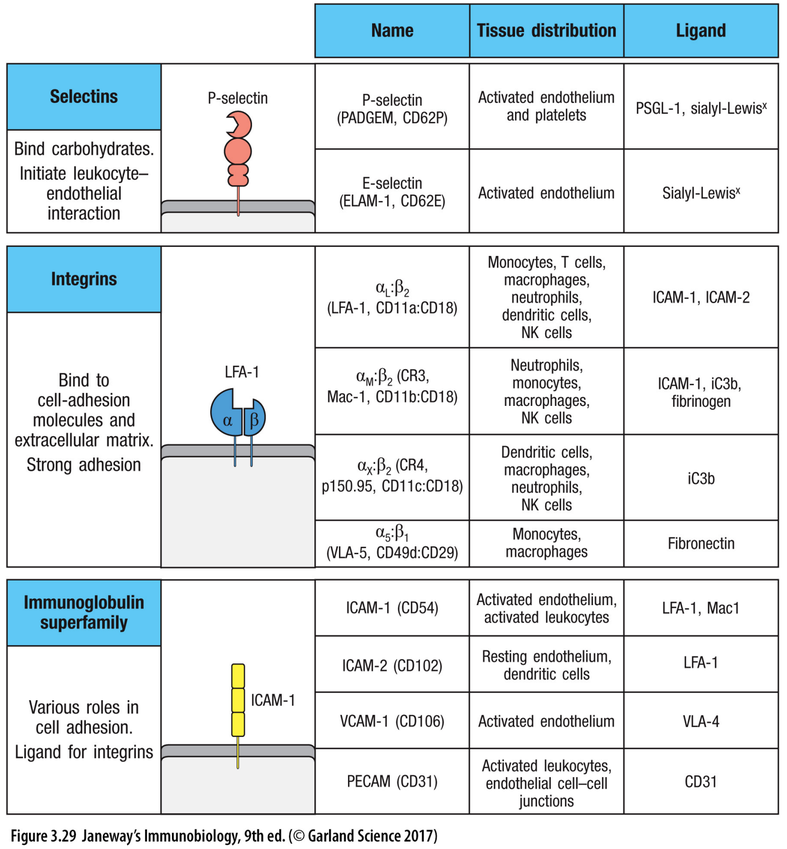
Cell-adhesion molecules

Adhesion of leukocytes to vascular endothelial cells is mediated by two classes of molecules, selectins and integrins, and their ligands.

1) Selectins

2) Integrins

3) Intercellular adhesion molecules (ICAM)



	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	ICAM-1, ICAM-2
	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

Figure 3.29 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Selectins and Selectin ligands

- Expressed on *endothelial cells*
- Membrane *glycoproteins* that bind to carbohydrate groups
- Expression is induced by *cytokines* secreted during an infection (e.g., TNF-a)
- P-Selectin and E-Selectin bind to *sulfated sialyl-Lewis^X* (displayed on neutrophils)
- Establish a weak interaction, which is *crucial for the rolling of the leukocytes* on the endothelium

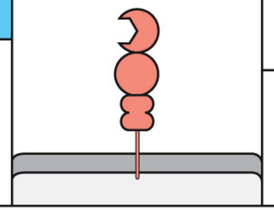
		Name	Tissue distribution	Ligand
Selectins	P-selectin 	P-selectin (PADGEM, CD62P)	Activated endothelium and platelets	PSGL-1, sialyl-Lewis^x
Bind carbohydrates. Initiate leukocyte–endothelial interaction		E-selectin (ELAM-1, CD62E)	Activated endothelium	Sialyl-Lewis^x

Figure 3.29 (part 1 of 3) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Integrins and Integrin Ligands

- Composed of two transmembrane protein chains - alpha and beta
- Two distinct "states": inactive and active
- Active state = *strong interaction* with its ligands and can be induced by chemokine signaling
- LFA-1 and CR3: important leukocytes integrins that mediate *extravasation*
- Bind to *ICAM* molecules on the endothelium, induced during infection
- Continuous *recruitment of monocytes* from the blood into the tissue
  ➔ develop into tissue-resident macrophages (homeostatic function)

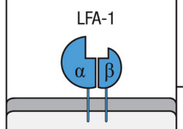
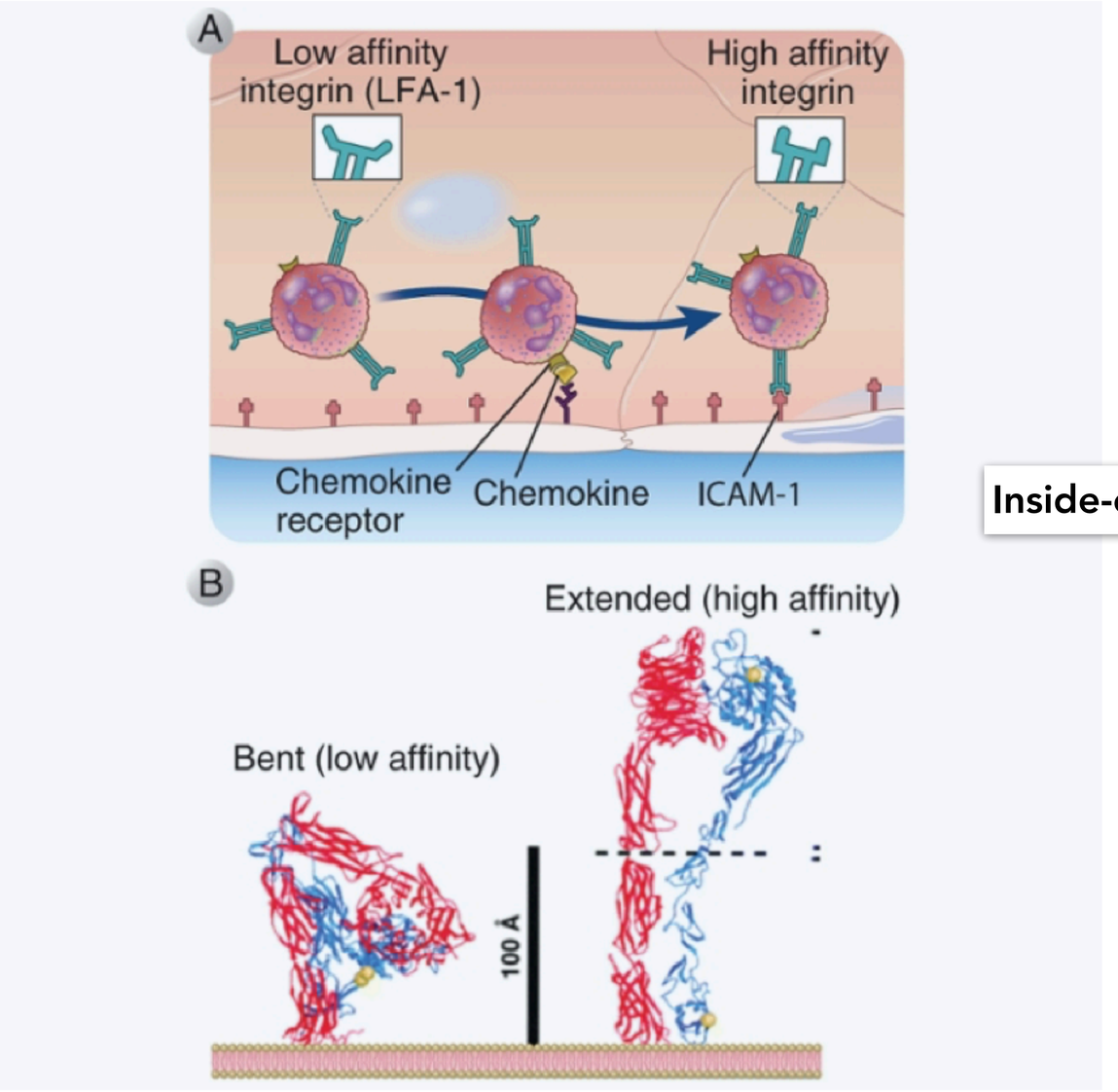
	Name	Tissue distribution	Ligand
Integrins Bind to cell-adhesion molecules and extracellular matrix. Strong adhesion	α_L:β_2 (LFA-1, CD11a:CD18)	Monocytes, T cells, macrophages, neutrophils, dendritic cells, NK cells	ICAM-1, ICAM-2
	α_M:β_2 (CR3, Mac-1, CD11b:CD18)	Neutrophils, monocytes, macrophages, 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

Figure 3.29 (part 2 of 3) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

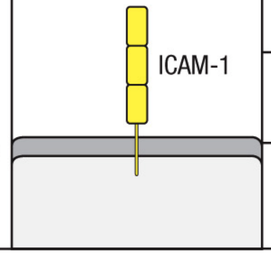


Inside-out-signaling

Intercellular adhesion molecule (ICAM)

- Contain *immmunoglobulin-like* domains in the extracellular part
- Are expressed on the *endothelium* after an infection (ICAM-1 and ICAM-2)
- Are *ligands* for integrins

	Name	Tissue distribution	Ligand
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



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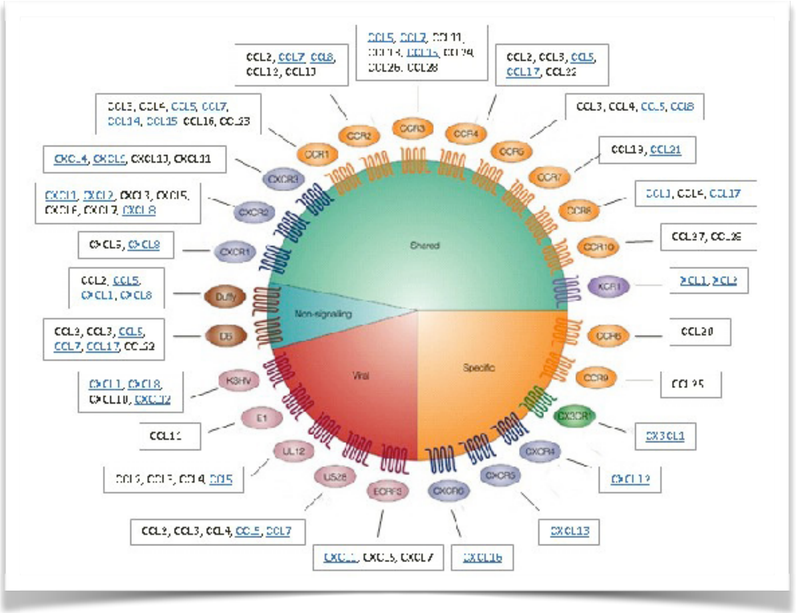
Chemokines and Chemokine Receptors

Two major classes of chemokines

1) CC (e.g. CCL2 acts on monocytes)

2) CXC (e.g. CXCL8 acts on neutrophils)

NB: "C" stands for cysteine residue in the N-term part



Chemokine receptors GPCR superfamily

G proteins stimulate signalling events that result
In cytoskeletal changes, increased cell motility, and integral activation.

Biological Actions of Chemokines

Recruitment of circulating leukocytes from blood vessels into extravascular sites:

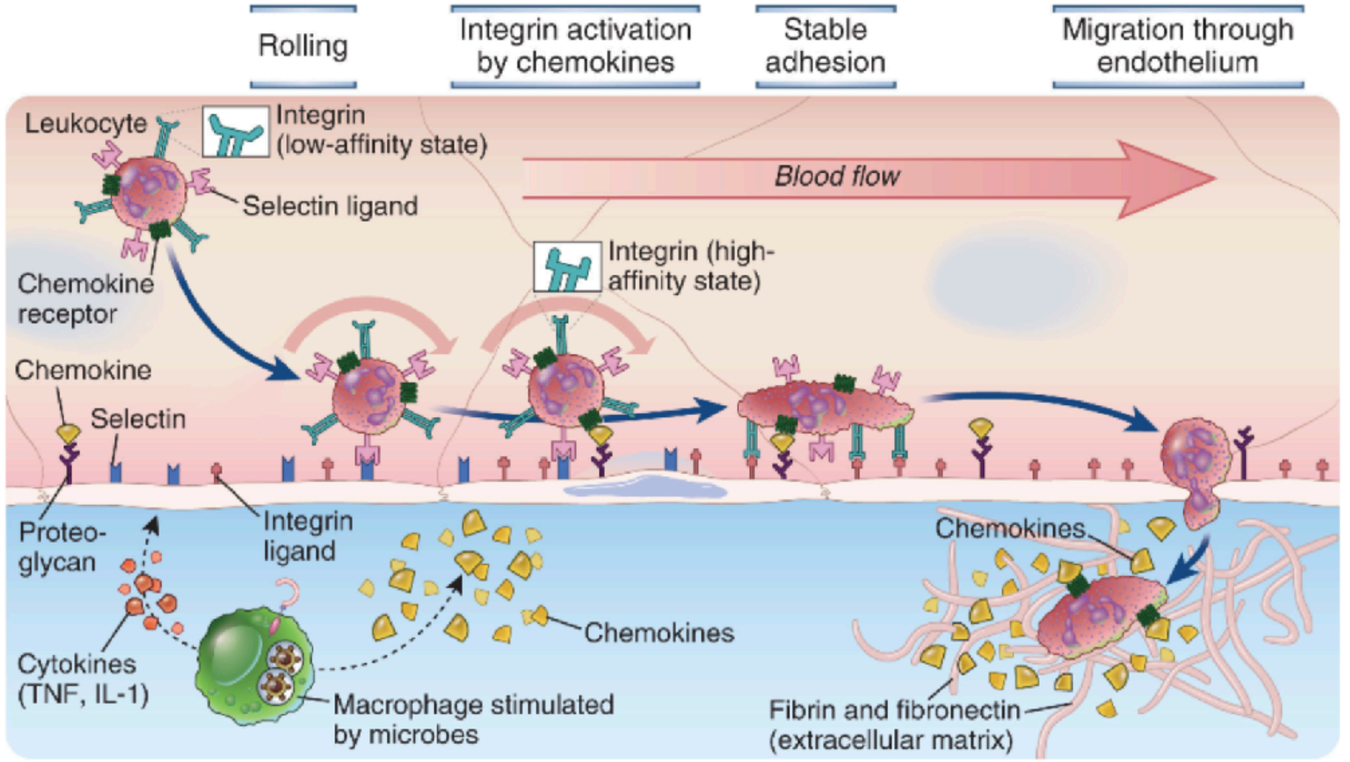
- Increased adhesion of leukocytes to endothelium
- Migration of leukocytes through blood vessels and towards the site of infection or tissue damage.
- Guiding of lymphocytes through their developmental processes
- Regulation of angiogenesis

Chemokines do not act alone ➔ require other vasoactive substances to work efficiently

Crossing the blood vessel wall

Site of infection: vasodilatation through vasoactive substances

➔ Slow-down in blood flow + facilitation of interactions between blood cells (neutrophils, macrophages) and endothelium



Crossing the blood vessel wall

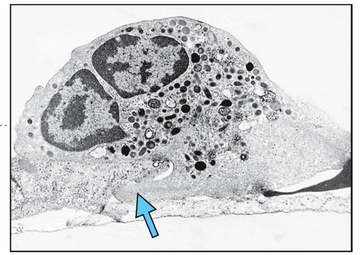


Figure 3.31 (part 3 of 3) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

4 steps of extraversion into inflamed tissue:

1) Rolling adhesion

P-Selectin and E-Selectin expressed on endothelium and interact with *sulfated sialyl-Lewis^X* on neutrophils and monocytes ➔ weak interaction that enables "rolling" along the endothelium

2) Firm Adhesion

Tight interaction mediated by *integrin-ICAM interactions*

- Integrins, expressed on neutrophils or macrophages, become activated during rolling due to chemokine signaling (CXCL8 attached to endothelium)
- ICAM-1 induced on endothelium cells through action of TNF-alpha and other cytokines

➔ *Stops the rolling* process of leukocytes!

3) Diapedesis

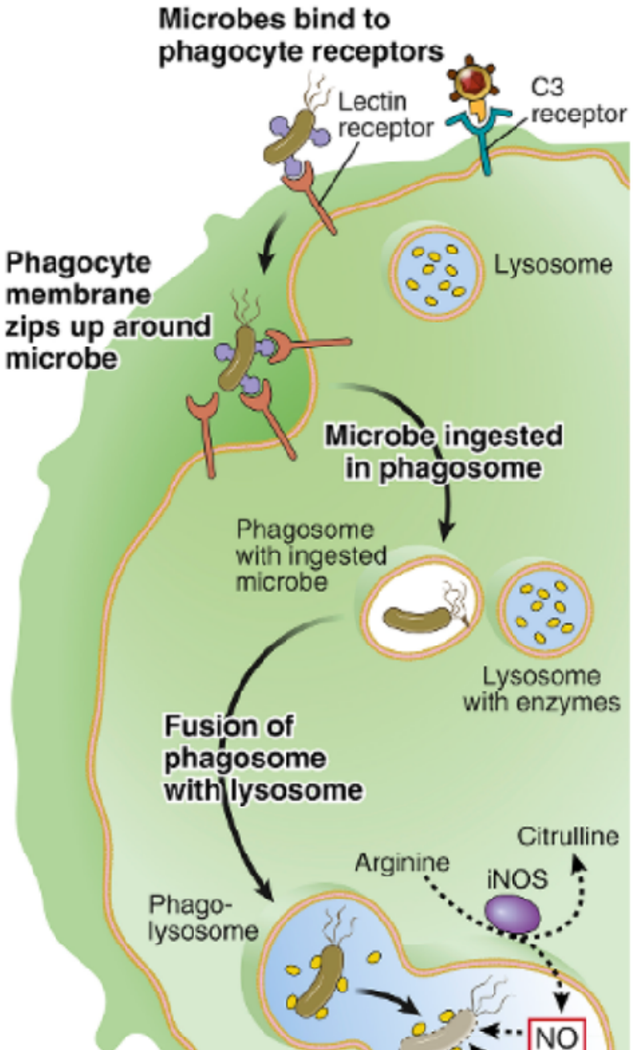
Cells squeeze through the *intercellular space*, facilitated by adhesion molecules (CD31). Extracellular matrix metalloproteinases help to break basal membrane down.

4) Migration

Cells migrate along *chemokine gradient* towards site of infection ➔ Chemokines (CXCL8, CCL2) bind to proteoglycans in extracellular space.

Phagocytosis (1)

- Binding to microbial surface structures
- Internalization into phagosome
- Fusion with lysosome —> phagolysosome
- *neutrophils: fusion with primary and secondary granules*



Phagocytosis Receptors

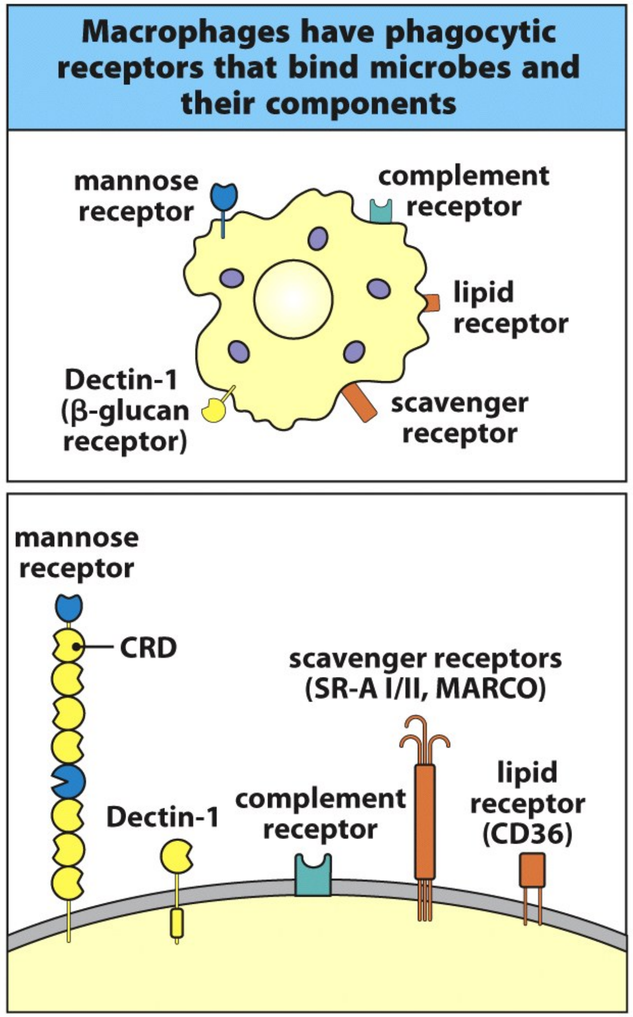
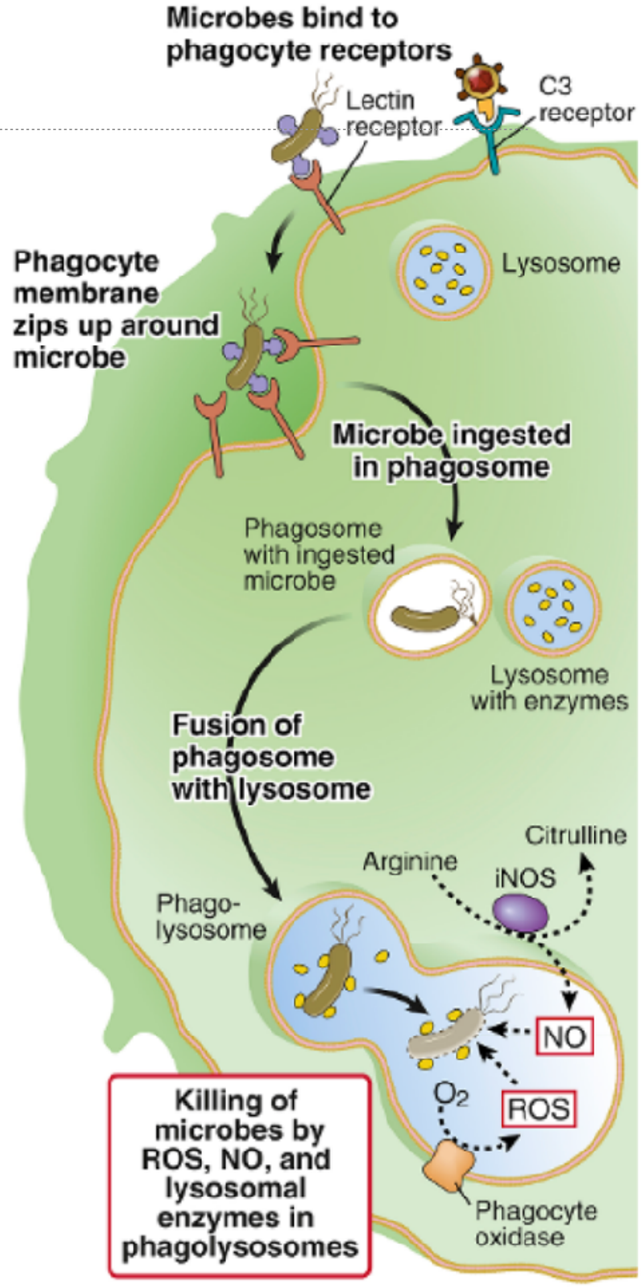


Figure 3.2 part 1 of 2 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Intracellular killing mechanisms

- Receptor activation triggers the assembly of a multimeric protein complex that produces **ROS** (reactive oxygen species)
- Inducible nitric oxide synthase (iNOS) catalyzes **NO** production
- **Lysosomal proteases** (e.g., Elastase, Cathepsin G) break down microbial proteins
- Neutrophils can also kill microbes by neutrophil extracellular traps (**NETs**)



Respiratory burst

- Reactive oxygen species (O2-; H2O2)
- generated by NADPH oxidase (phagocyte oxidase)
- multicomponent enzyme:
- membrane-associated cytochrome b558 complex (2nd granules and lysosomes)
- cytoplasmic components p67,p40,p47
- inactive in resting phagocytes:
- Receptor activation assembly of multicomponent enzyme —> **respiratory burst**

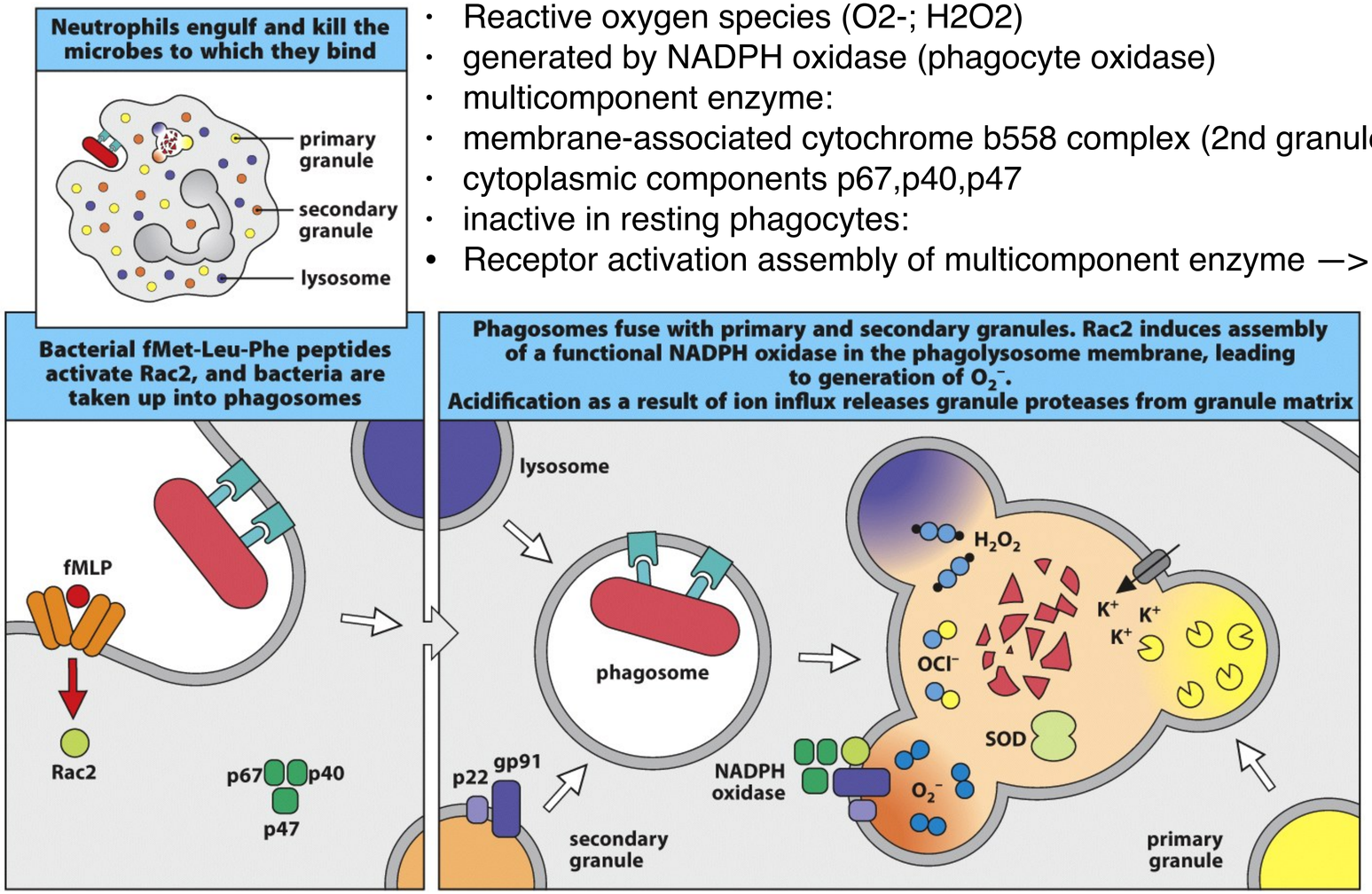
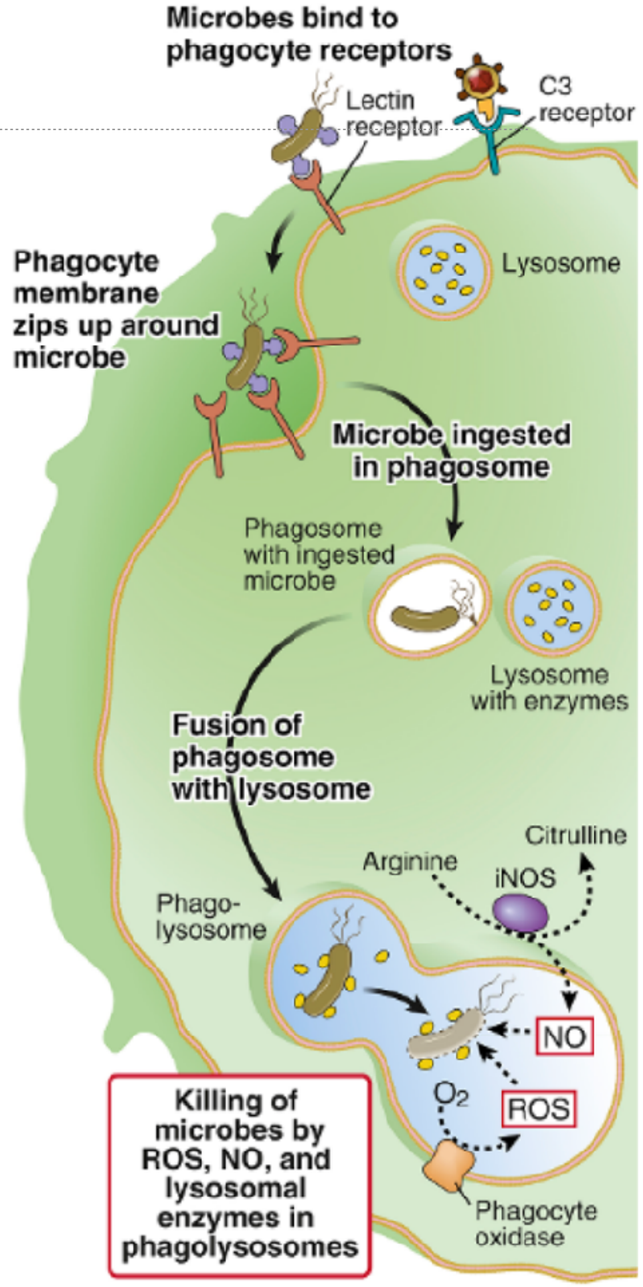


Figure 3.5 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Intracellular killing mechanisms

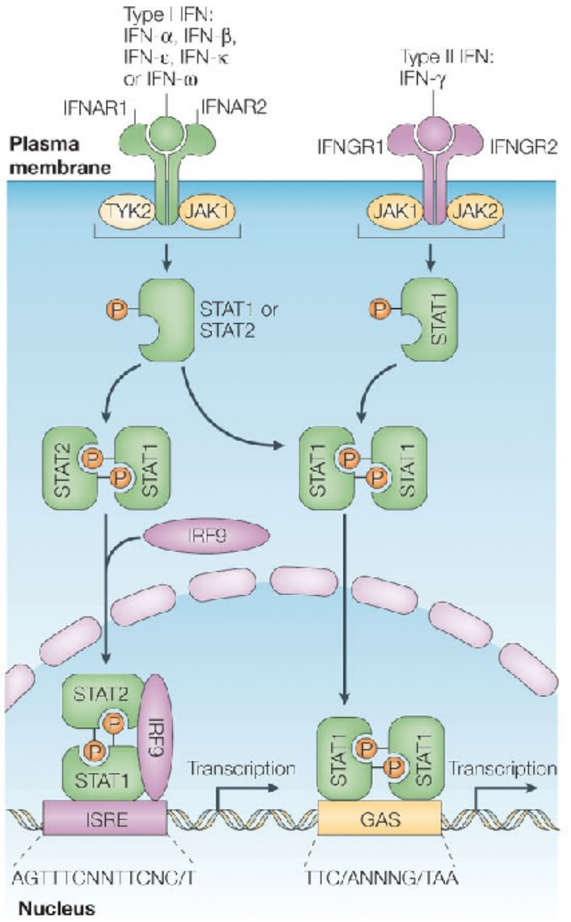
- Receptor activation triggers the assembly of a multimeric protein complex that produces **ROS** (reactive oxygen species)
- Inducible nitric oxide synthase (iNOS) catalyzes **NO** production
- **Lysosomal proteases** (e.g., Elastase, Cathepsin G) break down microbial proteins
- Neutrophils can also kill microbes by neutrophil extracellular traps (**NETs**)



Interferons - critical antiviral cytokines

"Interfere with viral spread"

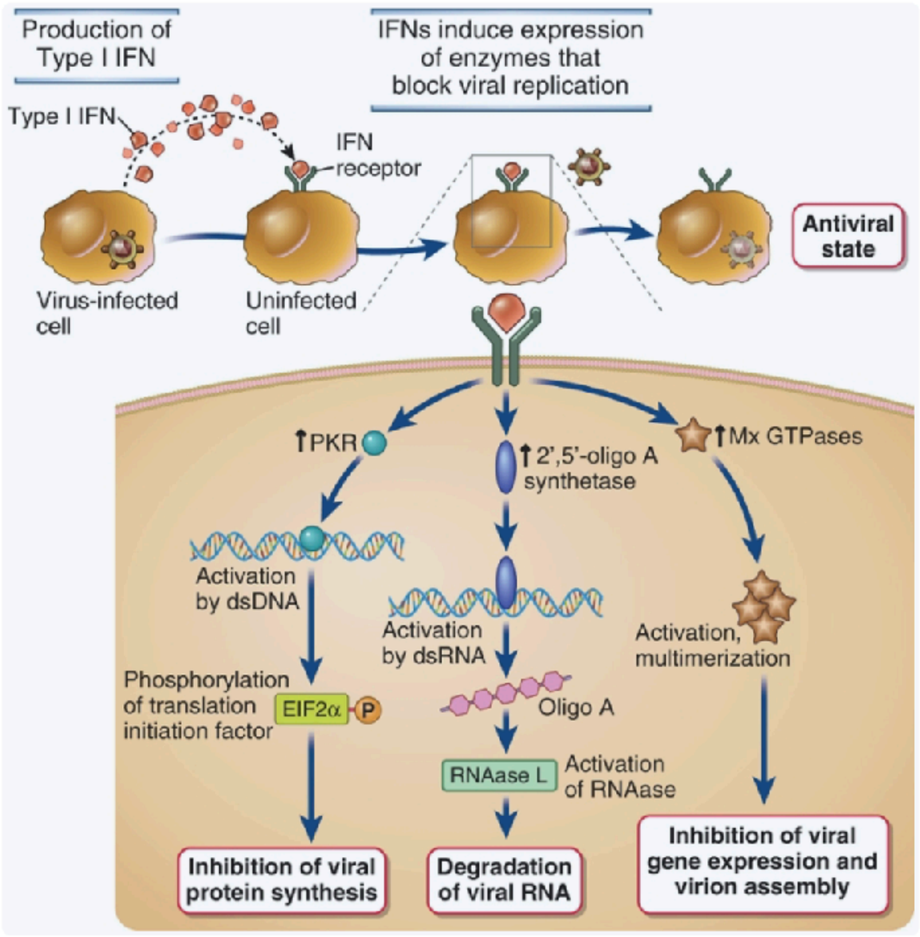
- IFN-alpha (several related proteins) and IFN-beta (one gene)
- IFN-beta (less IFN-alpha) synthesized by many cell types upon viral infection
- TLRs and intracellular nucleic sensors majorly contribute to the production of type I IFNs
- Plasmacytoid DCs a major producers of type I IFNs



Nature Reviews | Immunology

The biological effects of type I interferons

- Type I IFN trigger a battery of interferon-stimulated genes in their target cells, which confers an “antiviral state” via multiple mechanisms
- Type I IFNs increase the cytotoxicity of NK cells and CD8+ T cells and promote differentiation of naive T cells into the Th1 subset of helper T cells
- Type I IFNs up-regulate the expression of class I MHC molecules
- Type I IFNs can sensitive cells towards pro-apoptotic signals



Mechanisms that limit innate immune responses

IL-10 is an anti-inflammatory cytokine, produced by many immune cells and has important immunoregulatory functions