Inflammation, phagocytes, complement

(Milan Číž)

Inflammation

Inflammation

Body is against injury/invasion of microorganisms protected by physiological and physical barriers.

Skin – keratinocytes, sweat (washing, fatty acids)

GI tract – low pH in stomach, fatty acids, bile acids, natural microflora in intestines

Respiratory tract – cilia, mucosal coating

Nasopharynx and eye – mucosal coating, saliva, lysozyme in tears (peptidoglycan hydrolysis)

If these barriers are overcome → inflammation, immune reaction.

Inflammation

Definition:

Complex of immune and physological reactions to a disruption of organism integrity, which lead to an injury localisation, protection of damaged site and its healing.

Formation of inflammation:

- Antigenic stimulus (infection microorganisms)
- Chemical or physical injury
- Ischemia of organs and tissues

Typical signs of local inflammation:

- Rubor = redness
- Tumor = swelling
- Dolor = pain
- Calor = increased temperature

Types of inflammation:

- Acute – physiological defense reaction; passes without consequences and injured tissue is completely recovered
- Chronic = usually pathologic; tissue is to some extent replaced by connective tissue
- Sterile = without microorganisms

Inflammatory response process

First signals for inflammatory response development give by:

1. Degranulated tissue mast cells
2. Phagocytes
3. Mediators released from injured cells and extracellular matrix

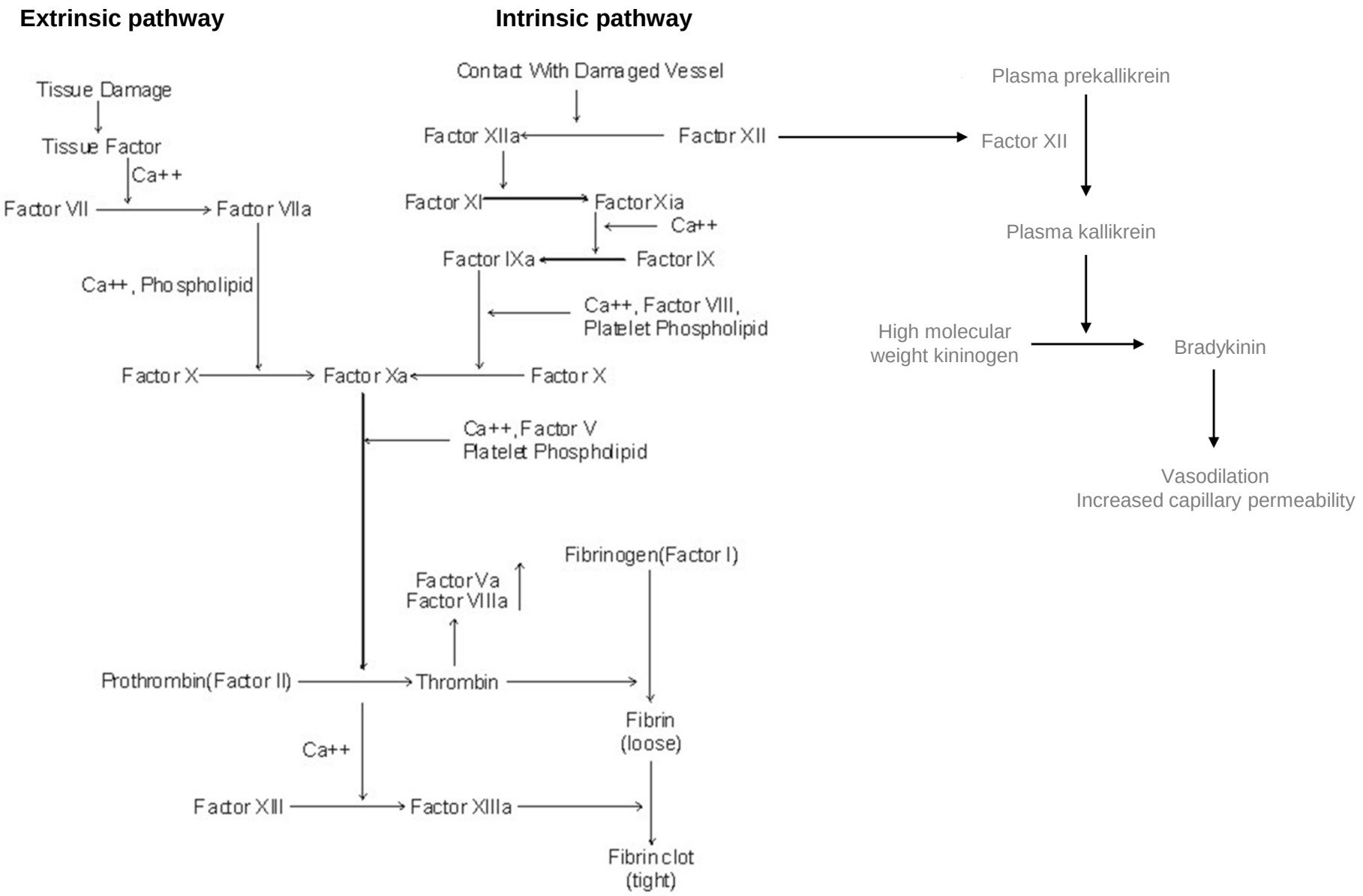
Processes accompanying inflammation

- **Increased vascular permeability**, leak of plasma into extravascular space and **swelling.**
- In the case of injury the first response is **hemocoagulation** – cessation of blood loss. Factors released from damaged cells activate coagulation. Major principle is conversion of soluble protein fibrinogen to insoluble fibrin.
- Activation of **fibrinolytic system** – prevention of blood clotting spreading outside the injury site. During fibrinolysis, enzyme plasmin is generated from inactive plasminogen. Plasmin cleaves fibrin to fibrin degradation products and reduces compactness of blood clot, eventually partially dissolves th blood clot.
- Activation of **kinin system** – activated coagulation factor XII acts on **prekallikrein**, **kallikrein** is created, followed by **bradykinin**, which increases capillary permeability.
- Increased expression of **adhesion molecules** on endothelium – attachment of phagocytes and later of lymphocytes.

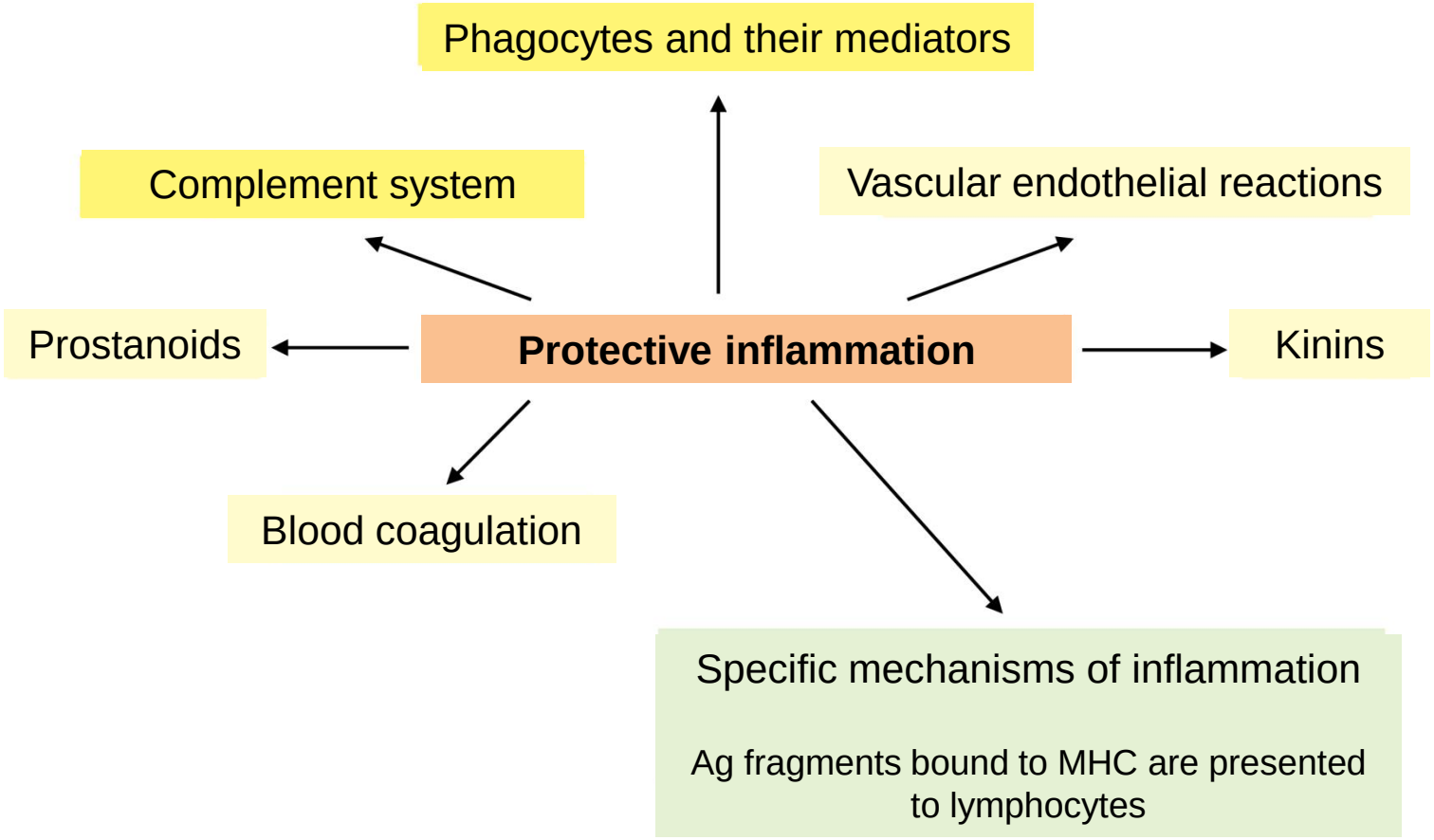
Processes accompanying inflammation

- Activation of **complement.**
- Activation of **phagocytes.**
- Influencing of **local nerve endings** (pain).
- Increased synthesis of **arachidonic acid metabolites** (TX, LT, PG).
- Changes in **temperature regulation** (fever). It is induced by stimulation of hypotalamic center by pro-inflammatory cytokines. This is followed by activation of tissue metabolism via mobilization of hypothalamus-hypophysis-adrenal axis (adrenal cortex– steroida, stress hormone cortisol). Increased temperature increase metabolism of immunocompetent cells. Expression of heat shock proteins (Hsp) is induced. They function as chaperones – they bind newly synthesised polypeptide chains and intracellular denatured proteins and help them to assemble into native conformations.
- **Liver** scavenge trace elements important for bacterial growth.
- **Cytokines** produced in inflammatory site stimulate production of acute phase proteins (C-reactive protein, CRP; complement components C3 and C4; serum amyloid P, SAP) in liver.

Coagulation cascade



Mechanisms of protective inflammation

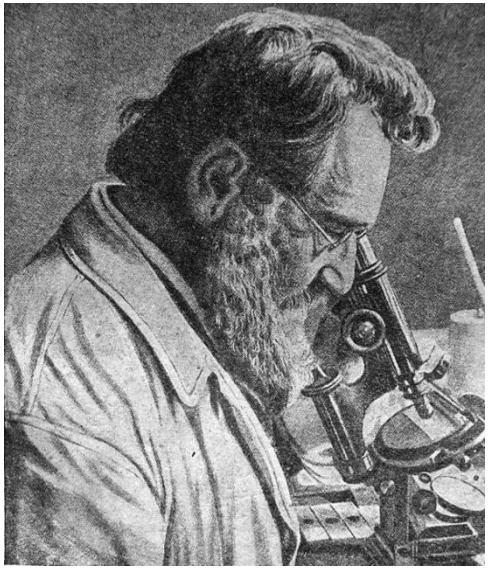


Phagocytes

History

I. Metchnikoff – Phagocytosis and innate immunity – Nobel prize in 1908

- Term „phagocyte“ used for the first time
- Phagocytes of starfish larvae engulfed a splinter; phagocytosis and digestion of bacteria by macrophages and PMNs
- From greek words „phagein“ = eat, „cytos“ = cell



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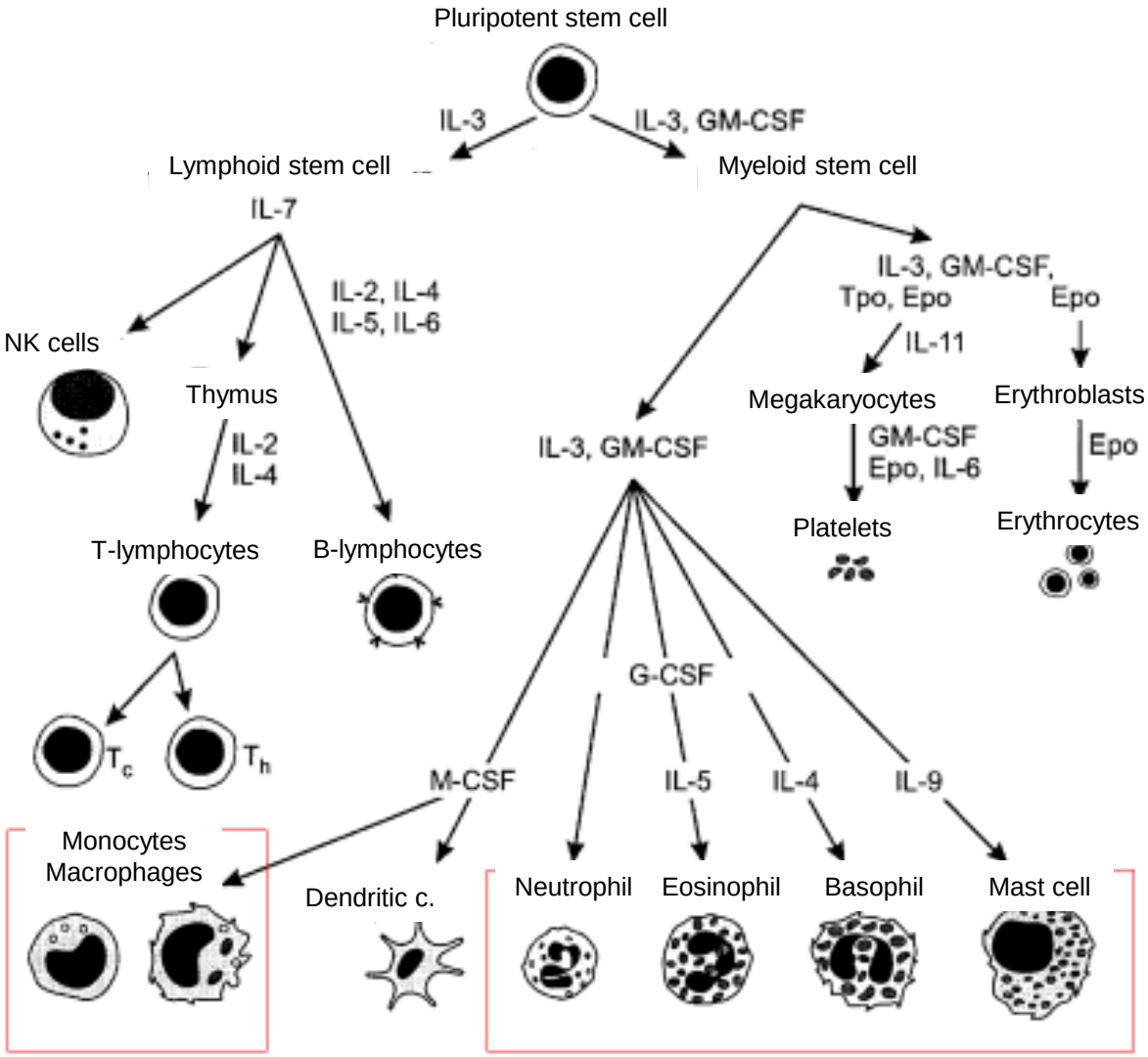
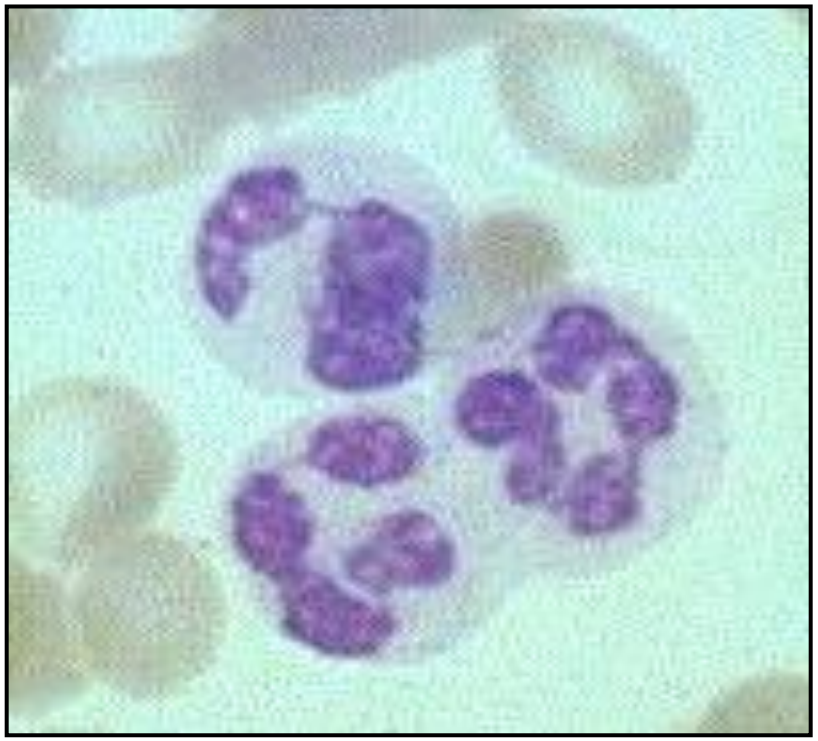
Cells of the immune system



Polymorphonuclear leukocytes (PMNs)

- 40-65% of all leukocytes (3-5 x 10^3 /µl of blood)
- short-living, abundant in blood, absent in healthy tissues
- characteristic nucleus
- granules and CD66 membrane marker
- the first line of defense against pathogenic microorganisms
- chemotaxis
- phagocytosis, intracellular killing
- generation of ROS and RNS
- degranulation
- inflammation, tissue damage



Polymorphonuclear leukocytes (PMNs)

- Primary granules
  - azurophilic
  - typical for young neutrophils
  - neutral proteases - cathepsin G, elastase, proteinase 3
  - lysozyme, defensins, phospholipase A2, myeloperoxidase
- Secondary granules
  - specific for mature neutrophils
  - lysozyme, NADPH oxidase, lactoferrin, elastase, colagenase
- Terciary granules
  - Gelatinase granules
  - in front end of migrating phagocytes
  - gelatinase (membrane destruction)
- Secretory vesicles
  - Reservoir of membrane components

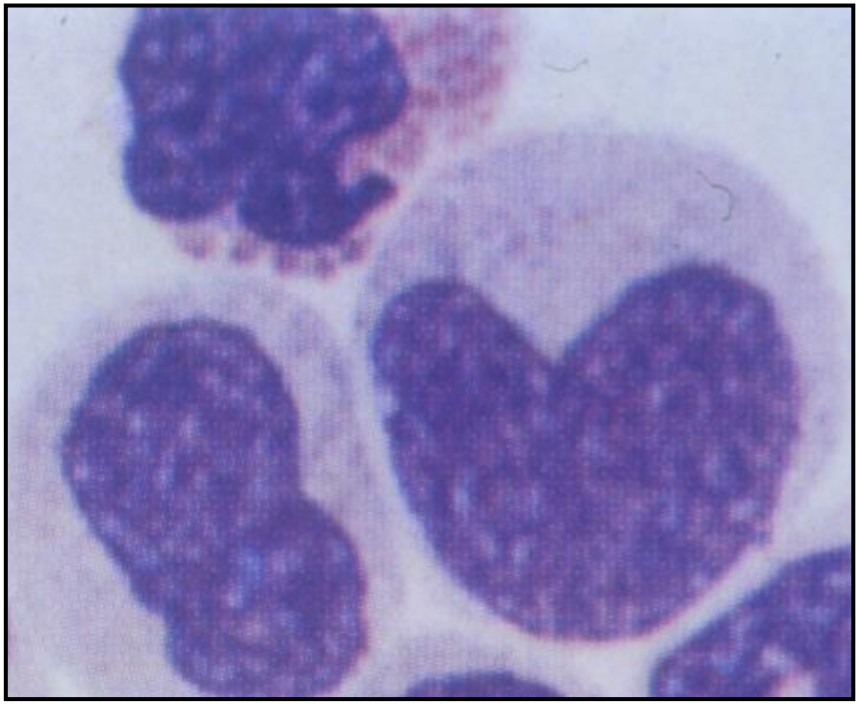
Eosinophils

Their role in an organism is still widely discussed and reassessed.
Originally – defense against parasites (worms)

- **NADPH oxidase** (similarly like in neutrophils)
- **Eosinophil peroxidase**
- **Major basic protein** and other granular proteins

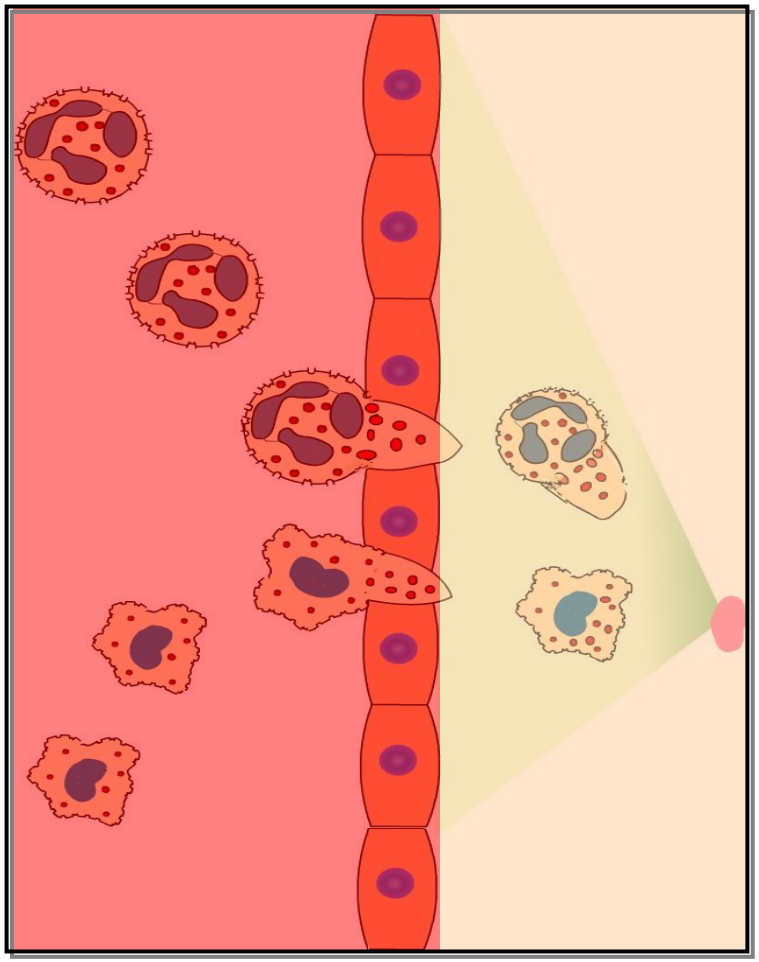
Monocytes / Macrophages

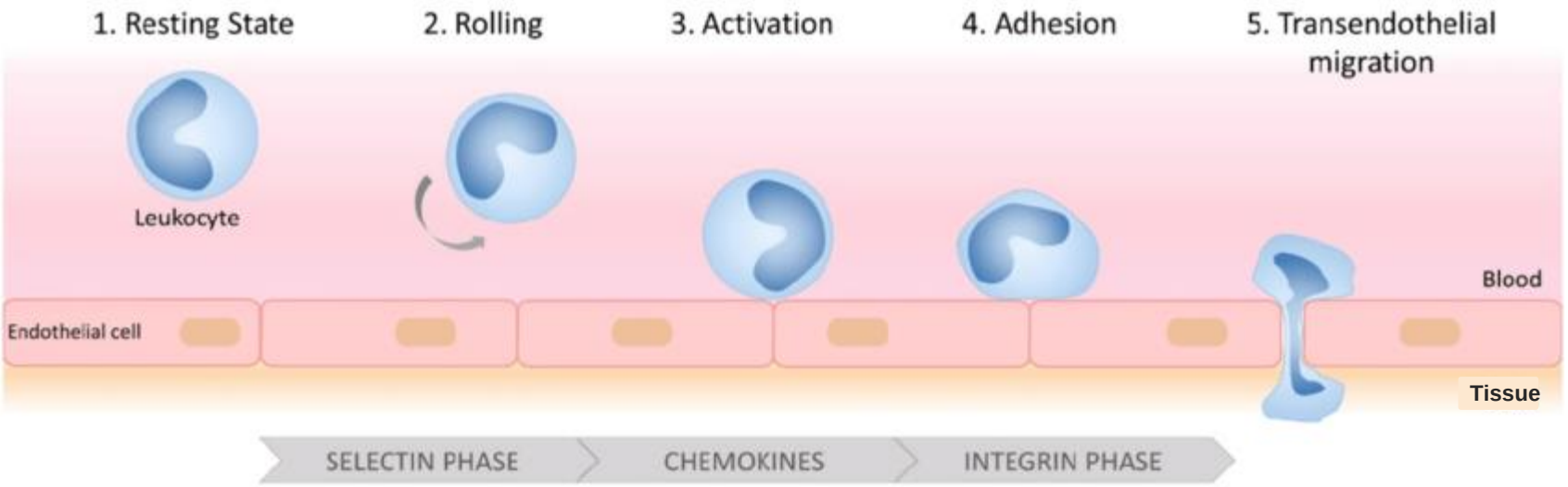
- phagocytosis, killing
- tissue renewal
- antigen presentation for specific immune response
- characteristic nucleus and CD14 membrane marker
- adher to plastic and glass
- activated by cytokines
- produce cytokines
- also eliminate malignant
  and altered self structures

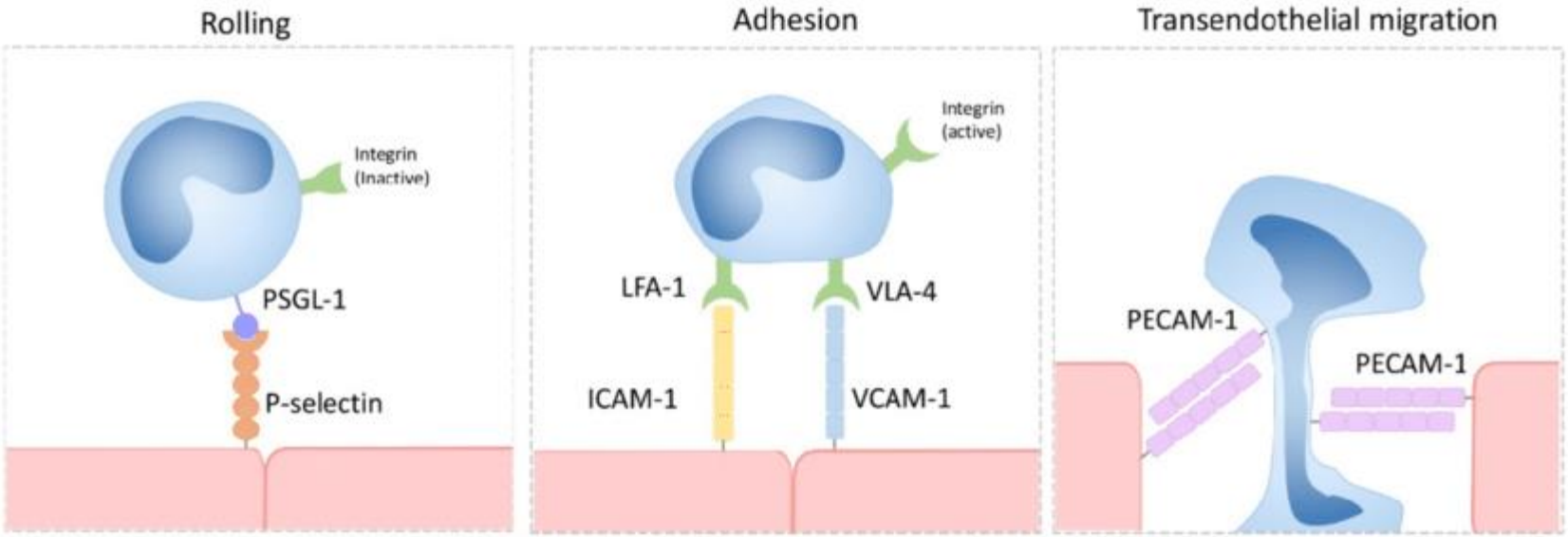


Activation of phagocytes in inflammation

- SOS signals
  - N-fMLP
  - PGs, LTs, PAF
  - Complement
  - Pro-inflammatory cytokines
- Response of phagocytes
  - chemotaxis
  - adherence
  - diapedesis
  - activation
  - peptides of hemocoagulation cascade
  - phagocytosis and killing

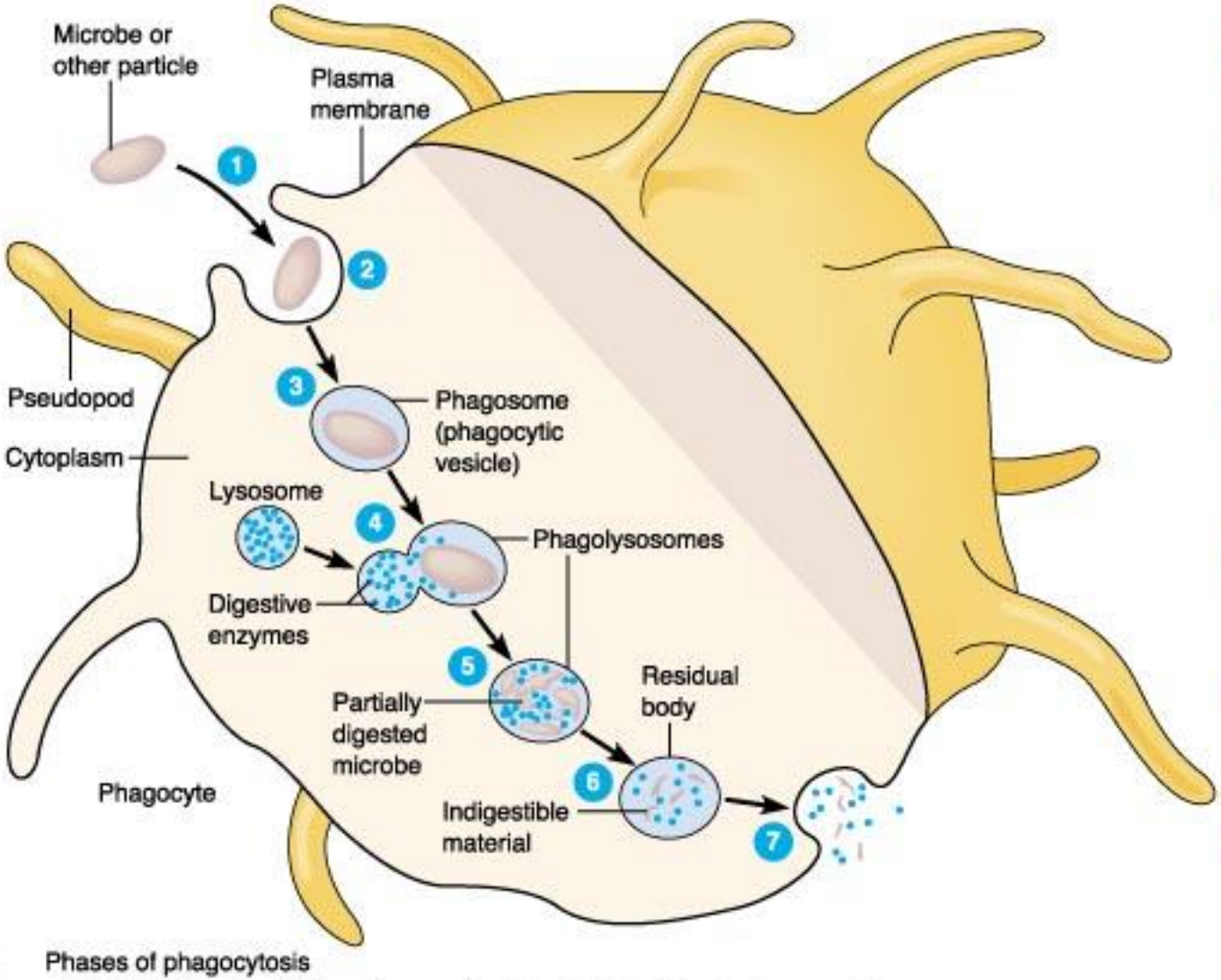



1. Resting State
2. Rolling
3. Activation
4. Adhesion
5. Transendothelial migration
Leukocyte
Blood
Endothelial cell
Tissue
SELECTIN PHASE
CHEMOKINES
INTEGRIN PHASE


Rolling
Integrin (Inactive)
PSGL-1
P-selectin
Adhesion
Integrin (active)
LFA-1
VLA-4
ICAM-1
VCAM-1
Transendothelial migration
PECAM-1
PECAM-1

Receptors on phagocytes

- complement receptors
- Fc receptors
- Toll-like receptors
- chemotactic receptors (fMLP)
- mannose receptors recognizing sugar structures on bacterial and viral surfaces
- scavenger receptors – recognize acetylated LDL



1. Chemotaxis and adherence of microbe to phagocyte.
2. Ingestion of microbe by phagocyte.
3. Formation of a phagosome.
4. Fusion of the phagosome with a lysosome to form a phagolysosome.
5. Digestion of ingested microbe by enzymes.
6. Formation of residual body containing indigestible material.
7. Discharge of waste materials.

Phases of phagocytosis

Killing mechanisms of phagocytes

Class of mechanism	Specific products
Acidification	pH=~3.5–4.0, bacteriostatic or bactericidal
Toxic oxygen-derived products	Superoxide O_2^-, hydrogen peroxide H_2O_2, singlet oxygen $^1O_2^{\bullet}$, hydroxyl radical $OH^{\bullet}$, hypohalite OCl^-
Toxic nitrogen oxides	Nitric oxide NO
Antimicrobial peptides	Defensins and cationic proteins
Enzymes	Lysozyme—dissolves cell walls of some Gram-positive bacteria. Acid hydrolases—further digest bacteria
Competitors	Lactoferrin (binds Fe) and vitamin B_{12}-binding protein

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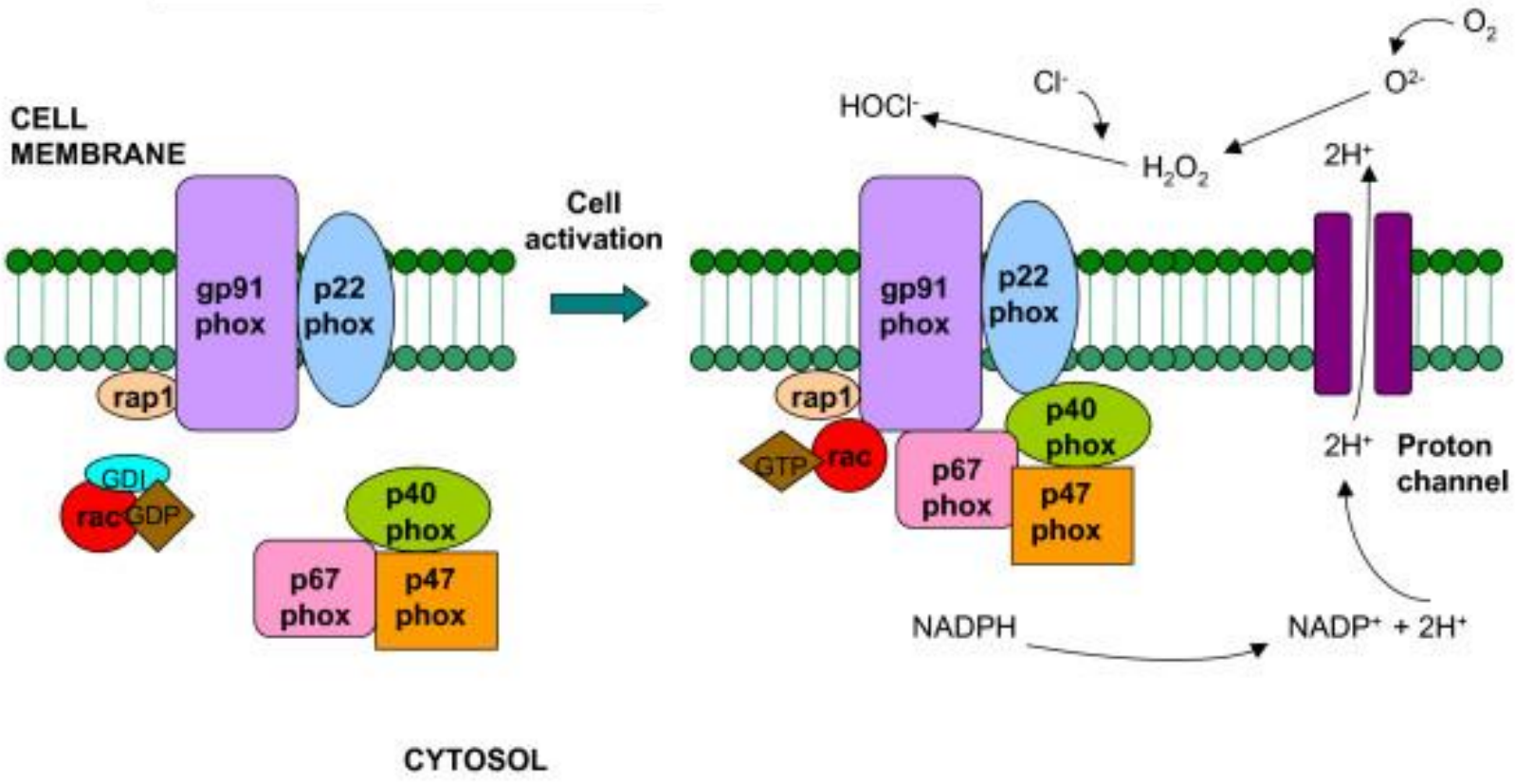
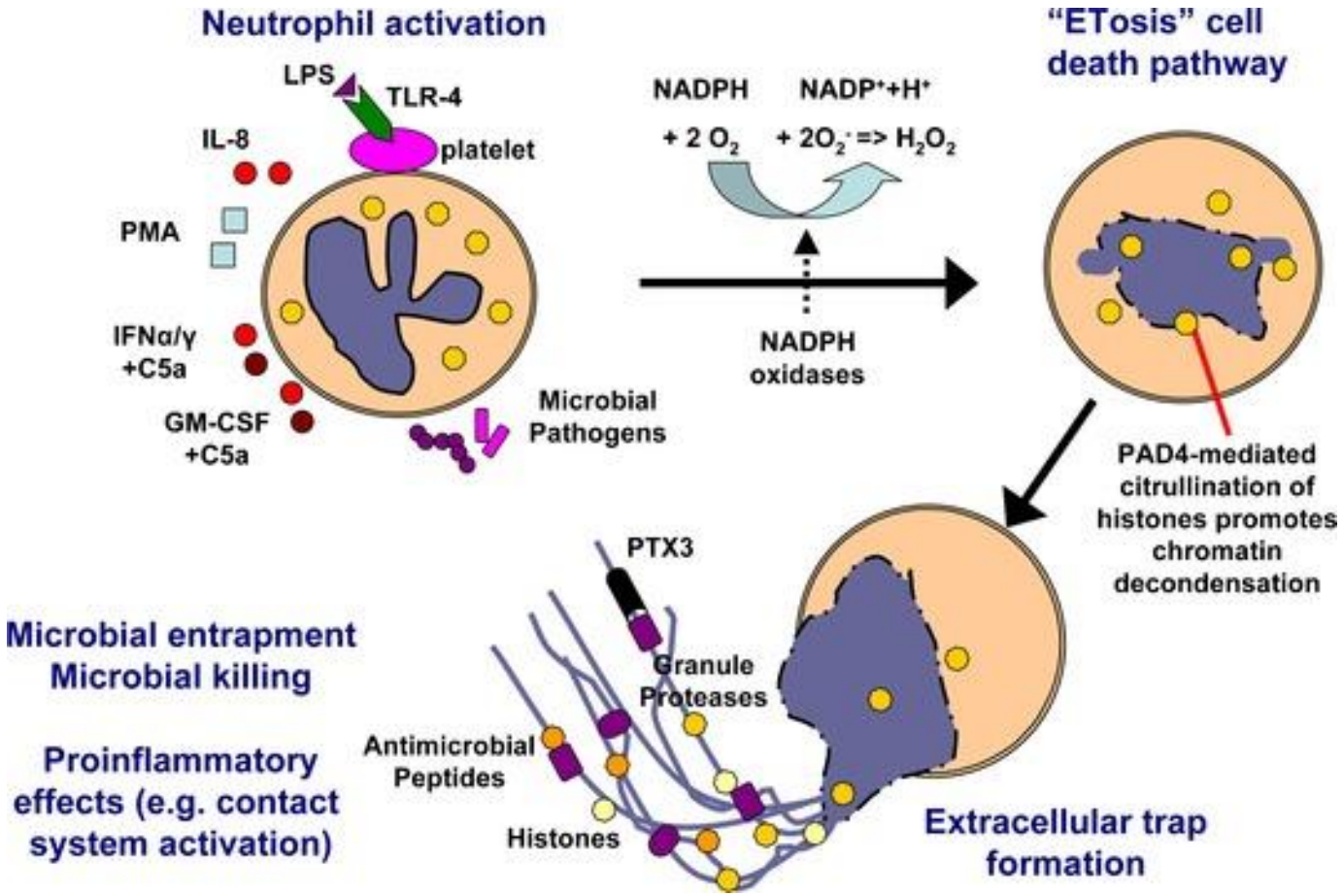
NADPH oxidase activation



Neutrophil extracellular trap (NETs) generation



Proper functioning of phagocytes is important for the organism

Deficiency in phagocyte functions = severe course of trivial infections

Example: CGD – defective NADPH oxidase

On the other hand, overactivated phagocytes – problems

Damage of neighbouring cells and tissues by reactive metabolites and proteolytic enzymes

Complement

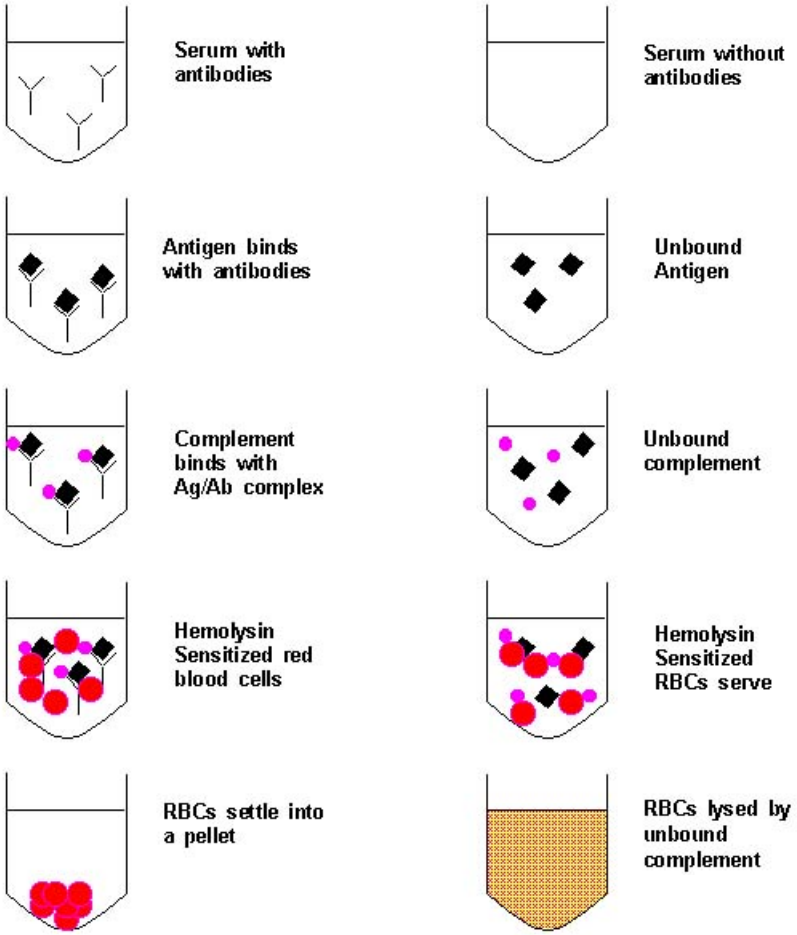
Komplement: historie

Discovered in 1894 – 1899

Jules Bordet (1870-1961)

Worked in Metchnikoff laboratory (Pasteur Institute, Paris)

Complement fixation test



Complement system

- composed from various plasma proteins (30)
- individual components interact
- pathogen opsonization
- induction of inflammatory response

Complement – basic terms

- C-activation: alteration of complement proteins and their interactions with other components
- C-fixation: complement utilization of Ag-Ab complexes
- Hemolytic activity: complement-mediated lysis of erythrocytes coated by antibody
- C-inactivation: heat denaturation

Proteins of complement system

Nomenclature:

- C1complex(qrs), C2, C3, C4, C5, C6, C7, C8, C9
- factors B, D, H and I, properdin (P)
- mannose-binding lectin (MBL), MBL-associated serine proteases (MASP-1 MASP-2)

Activated products of complement proteins (nomenclature)

Activated components are usually overlined: eg. $\overline{C1qrs}$

After being enzymatically cleaved, a larger fragment usually binds to activation complex or membrane and smaller peptide is released into surroundings.

Letter “b” is usually used for larger peptide and letter “a” for smaller peptide, eg. C3b/C3a, C4b/C4a, C5b/C5a.

General biochemical principle of complement activation cascade

Several complement proteins are **proteases**, which are themselves **activated by proteolytic cleavage**.

These enzymes are called zymogens.

Ways of complement activation

Complement cascade can be activated by one out of three ways:

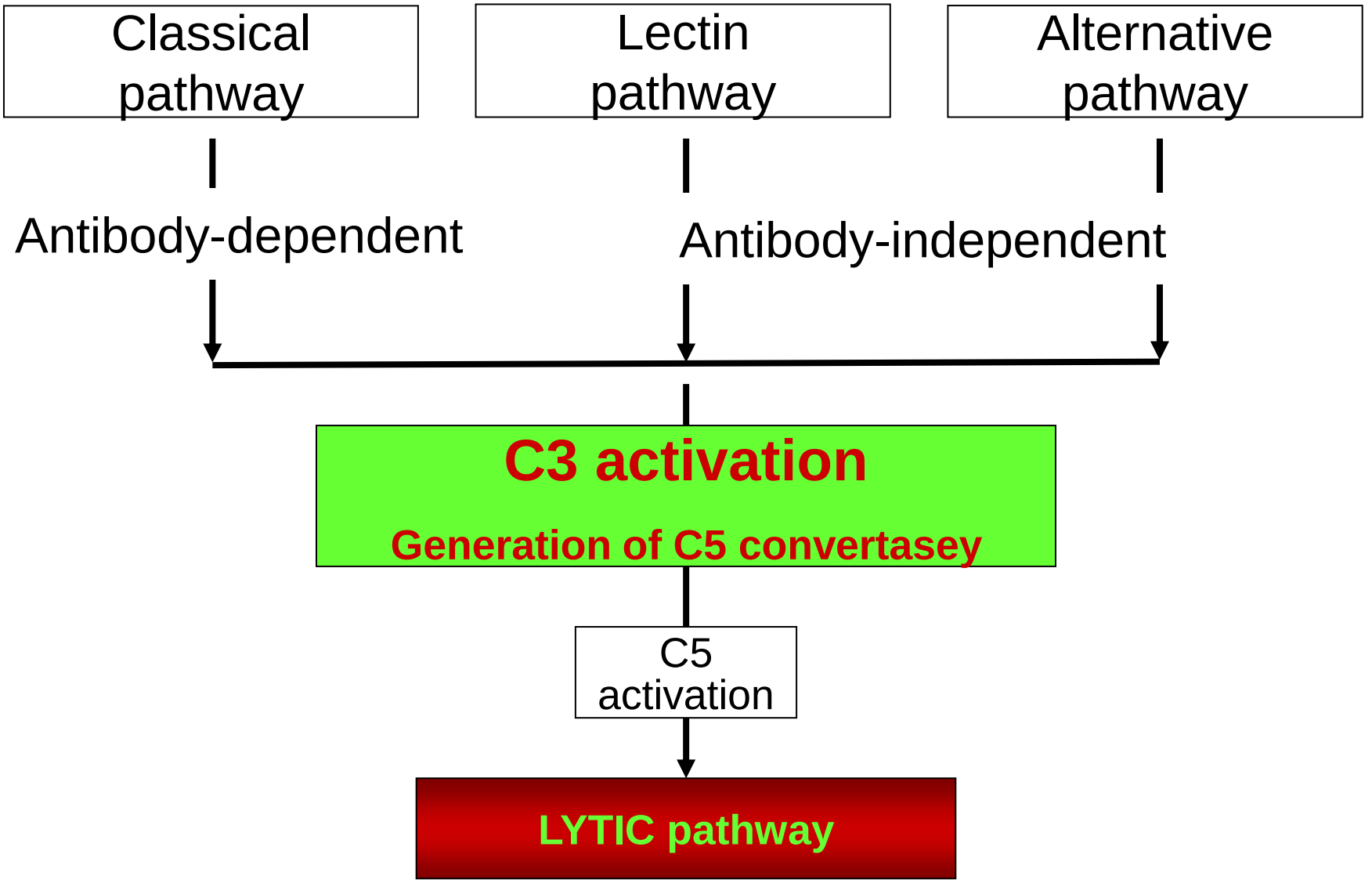
Classical pathway – initiated by C1q binding to:

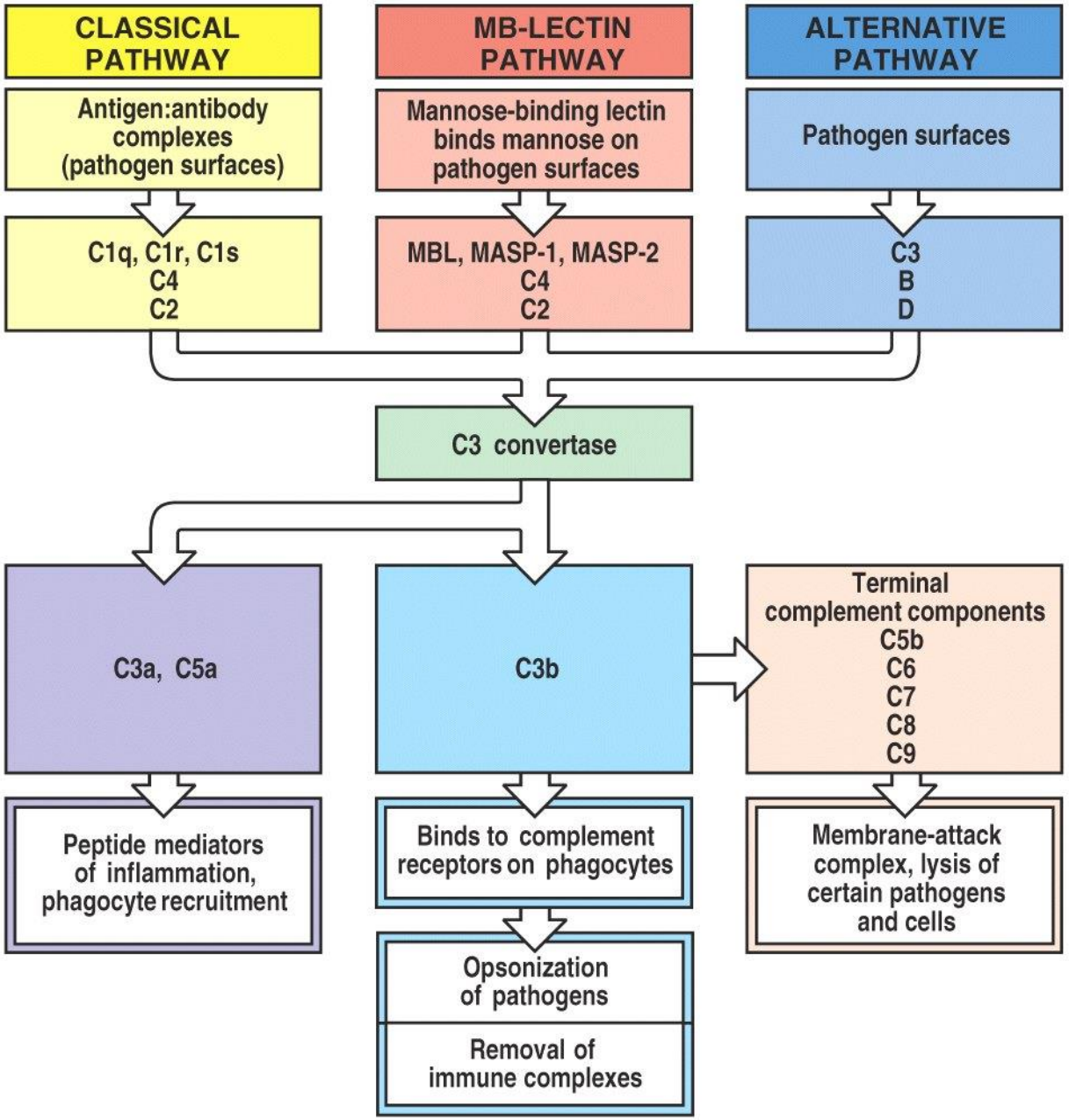
- Ab-Ag complex
- Surface components of bacteria (proteins, polyanionic structures)
- C-reactive protein (acute phase protein which binds to phosphocholin residues of bacterial polysaccharides)

Lectin pathway – initiated by binding of MBL (mannose-binding lectin) = serum protein, concentration of which increases during acute phase of immune response, to surface structures of bacteria and viruses, which contain mannose

Alternative pathway – initiated by binding of spontaneously activated C3 to the surface of pathogens

Ways of complement activation





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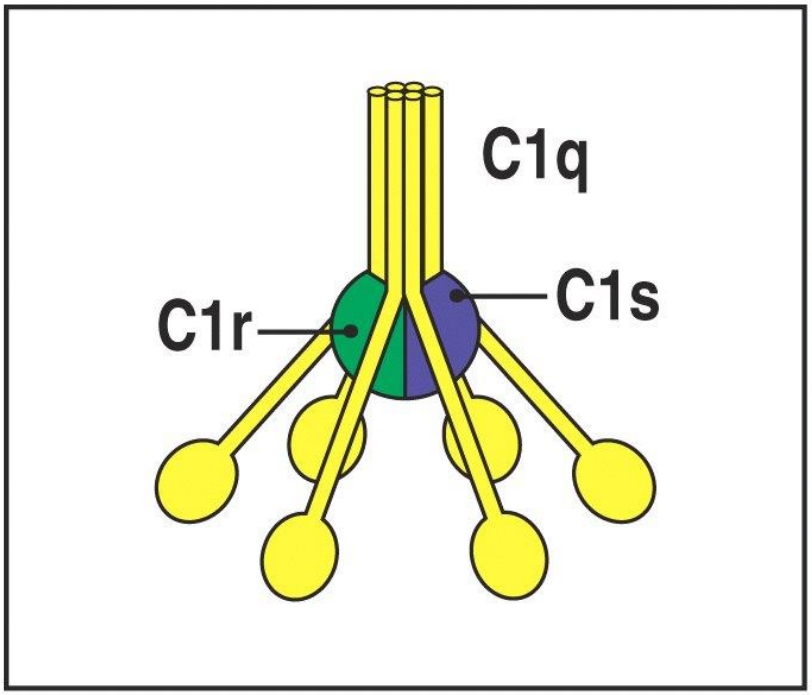
Classical pathway activation

C1q is a complex with 6 heads = binding site of C1q is associated with 2 molecules of C1r and C1s. Binding leads to activation of C1r, then of C1s and cleavage of C4.



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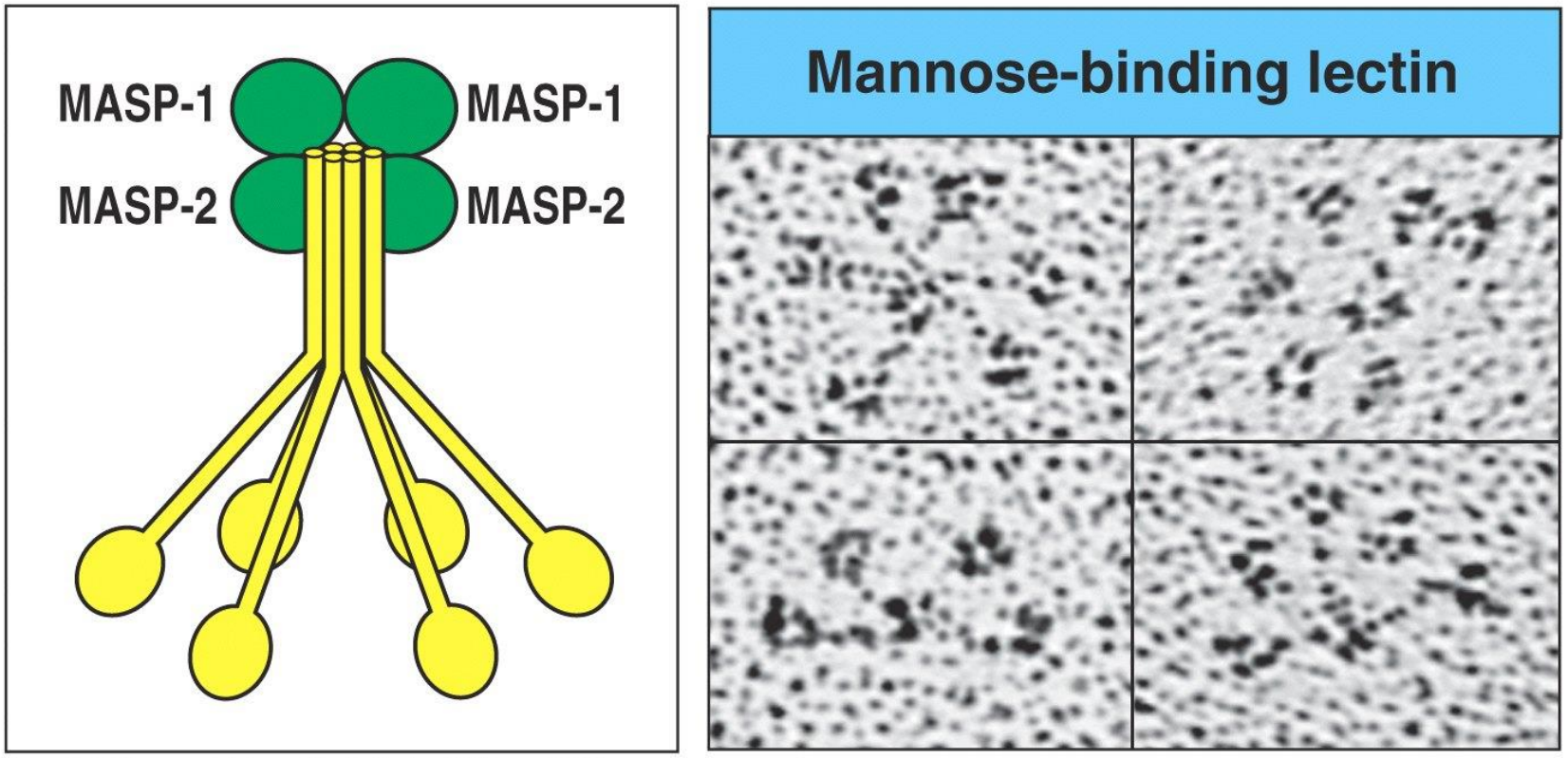
Lectin pathway activation

MBL (mannose binding lectin) resembles C1 complex. 6 heads with affinity to saccharide structures of pathogen surface are associated with 2 molecules of MBL-associated serine proteases (MASP-1 a MASP-2). Binding leads to activation of MASP and cleavage of C4.



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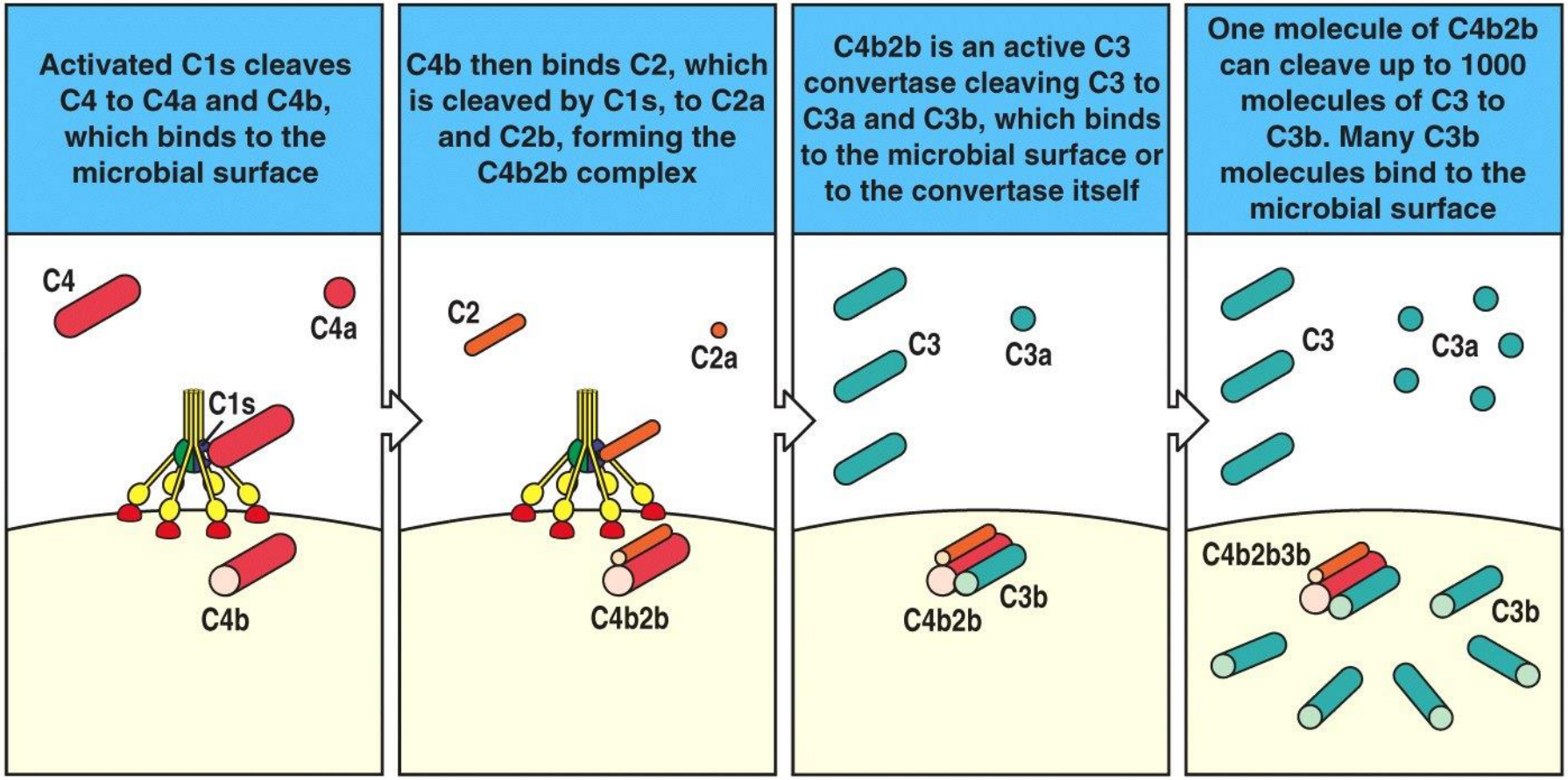
Classical (and lectin) pathway activation



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Alterntive pathway activation

C3 is spontaneously cleaved – formation of functionally active C3b.

C3b binds factor B, which cleaved by factor D.

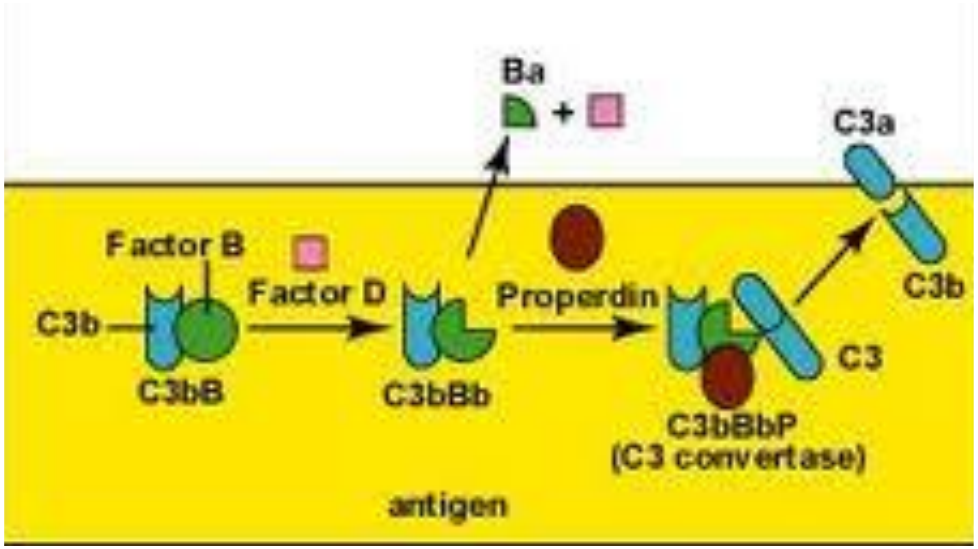
Soluble C3 convertase is formed, which cleaves more C3 to C3a and C3b.

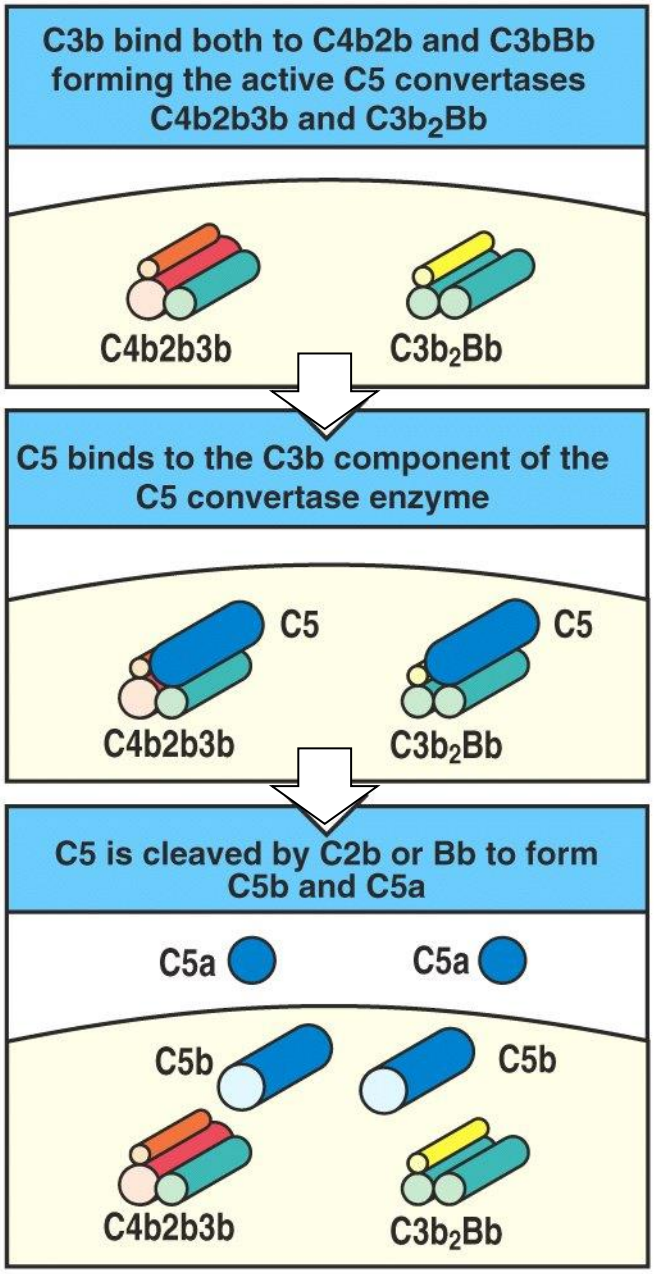
C3b binds to the surface of own cells or pathogens.

Rapidly inactivated on the surface of own cells – formation of iC3b.

C3bBb on the surface of pathogen – no regulátory proteins present. Binding of factor P (properdin), which stabilizes C3bBb convertase aktivity.

C3bBb is an equivalent of C4b2b of classical pathway.





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C4b2b3b from classical (lectin) pathway

or its equivalent $C3b_2Bb$ from alternative pathway

are C5 convertases

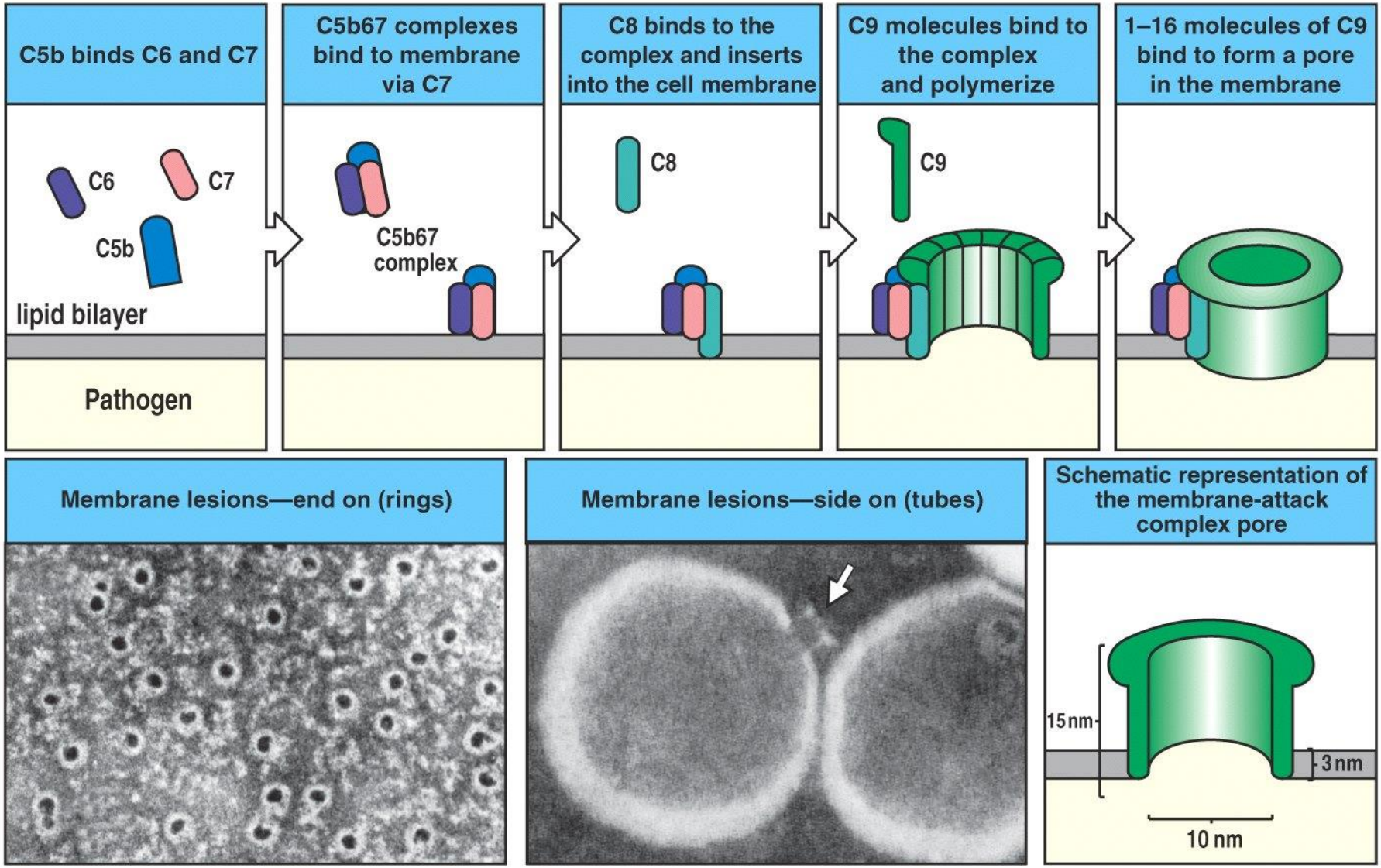


Figure 2-35 Immunobiology, 6/e. (© Garland Science 2005)

Results of complement activation

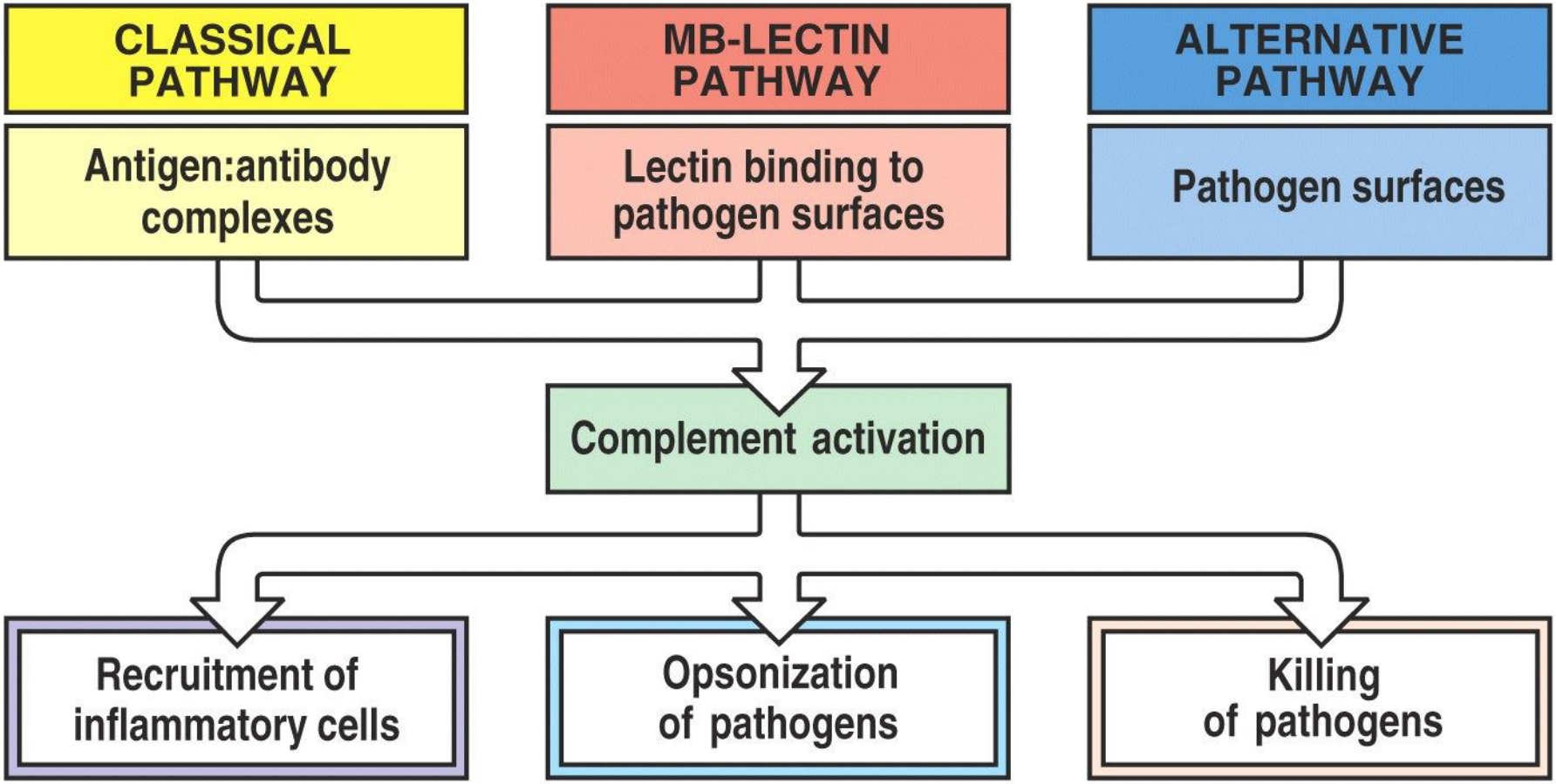


Figure 2-18 Immunobiology, 6/e. (© Garland Science 2005)

Functional protein classes in the complement system	
Binding to antigen:antibody complexes and pathogen surfaces	C1q
Binding to mannose on bacteria	MBL
Activating enzymes	C1r C1s C2b Bb D MASP-1 MASP-2
Membrane-binding proteins and opsonins	C4b C3b
Peptide mediators of inflammation	C5a C3a C4a

Functional protein classes in the complement system	
Membrane-attack proteins	C5b C6 C7 C8 C9
Complement receptors	CR1 CR2 CR3 CR4 C1qR
Complement-regulatory proteins	C1INH C4bp CR1 MCP DAF H I P CD59

Figure 2-20 Immunobiology, 6/e. (© Garland Science 2005)

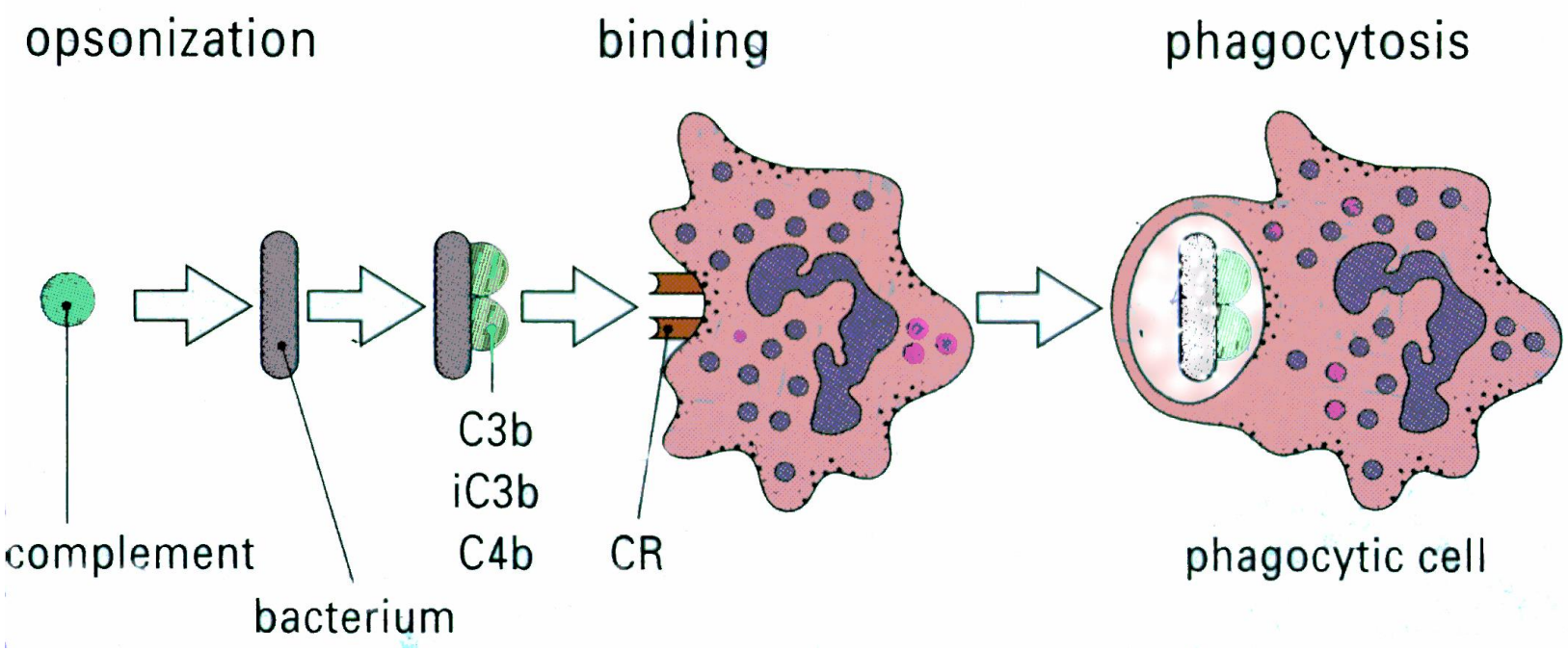
Receptor	Specificity	Functions	Cell types
CR1 (CD35)	C3b, C4b iC3b	Promotes C3b and C4b decay Stimulates phagocytosis Erythrocyte transport of immune complexes	Erythrocytes, macrophages, monocytes, polymorphonuclear leukocytes, B cells, FDC
CR2 (CD21)	C3d, iC3b, C3dg Epstein–Barr virus	Part of B-cell co-receptor Epstein–Barr virus receptor	B cells, FDC
CR3 (Mac-1) (CD11b/ CD18)	iC3b	Stimulates phagocytosis	Macrophages, monocytes, polymorphonuclear leukocytes, FDC
CR4 (gp150,95) (CD11c/ CD18)	iC3b	Stimulates phagocytosis	Macrophages, monocytes, polymorphonuclear leukocytes, dendritic cells
C5a receptor	C5a	Binding of C5a activates G protein	Endothelial cells, mast cells, phagocytes
C3a receptor	C3a	Binding of C3a activates G protein	Endothelial cells, mast cells, phagocytes

Figure 2-31 Immunobiology, 6/e. (© Garland Science 2005)

Biological effects of complement activation products

- C3a (anaphylatoxin) - degranulation of mast cells; increased vascular permeability; anaphylaxis
- C3b (opsonin) - opsonization; phagocyte activation
- C4a – anaphylaxis
- C5a - anaphylaxis similarly to C3, but more intensive; attractant and activator of neutrophils, elicits neutrophil aggregation, stimulation of oxidative metabolism and release of leukotrienes, degranulation of mast cells
- MAC – cytolysis

Opsonization and phagocytosis



Complement – summary of effets

- useful:
  - opsonization and facilitated phagocytosis
  - chemoattraction and phagocyte activation
  - Lysis of bacteria and infected cells
  - regulation of antibody response
  - clearance of immune complexes
  - clearance of apoptotic cells
- harmful:
  - inflammation, anaphylaxis