

Non-Specific Immunity

Innate (natural, native, non-specific) immunity

- Always present, ready to recognise and eliminate microbes. May be stimulated also by non-microbial agents.
- Frequently eliminates microbes before the specific immunity becomes active.
- Receptors are encoded in the germline, are not a product of recombination of genes.

Differences between the Innate and Acquired Immunity

- Innate Immunity

- Universal
- Rapid
- Lacks memory

- Acquired Immunity

- Not universal
- ‘Slow’ to develop
- Memory
- Specific but in some situations reacts to autoantigens
- ‘Plays to the tune of the innate immune system’

“Trained immunity”

It has recently been shown that some mechanisms of non-specific immunity have the ability to respond more intensively, effectively, after repeated stimulation by some (non-specific) stimuli.

It can be detected in NK cells, dendritic cells, myeloid cells as well as some stem cells.

Epigenetic mechanisms are involved

In addition to the body's defense, participation in the pathogenesis of several diseases accompanied by chronic inflammation is also being considered.

Signals of danger

- **EXOGENEOUS** (PAMPs)
- **ENDOGENEOUS** (e.g. STRESS PROTEINS
RELEASED FROM NECROTIC CELL)

Inborn immunity – activating signals (alarmins)

Pathogen-Associated Molecular Patterns (PAMPs)

(C. A. Jeneway, Jr, 1989)

microbial structures, motifs, present in large groups of microorganisms, necessary for their life

e.g. lipopolysachrides, lipopeptides, peptidoglycans, manose nonmetylated CpG present in bacterial DNA, dsRNA of RNA-vises



Danger, Damage-Associated Molecular atterns (DAMPs)

(Polly Celine Eveline Matzinger, 1994)

Molecular structures of the host macroorganism

e.g. HSP60, HSP70, fragments of fibrinogen, fibronectin, hyaluronan..



Differences between innate and specific immunity

	Innate immunity	Adaptive immunity
Specificity	<p>For structures shared by classes of microbes ("molecular patterns")</p> <p>Different microbes Identical mannose receptors</p>	<p>For structural detail of microbial molecules (antigens); may recognize nonmicrobial antigens</p> <p>Different microbes Distinct antibody molecules</p>
Receptors	<p>Encoded in germline; limited diversity</p> <p>Toll-like receptor N-formyl methionyl receptor Mannose receptor</p>	<p>Encoded by genes produced by somatic recombination of gene segments; greater diversity</p> <p>TCR Ig</p>
Distribution of receptors	<p>Nonclonal: identical receptors on all cells of the same lineage</p>	<p>Clonal: clones of lymphocytes with distinct specificities express different receptors</p>
Discrimination of self and nonself	<p>Yes; host cells are not recognized or they may express molecules that prevent innate immune reactions</p>	<p>Yes; based on selection against self-reactive lymphocytes; may be imperfect (giving rise to autoimmunity)</p>

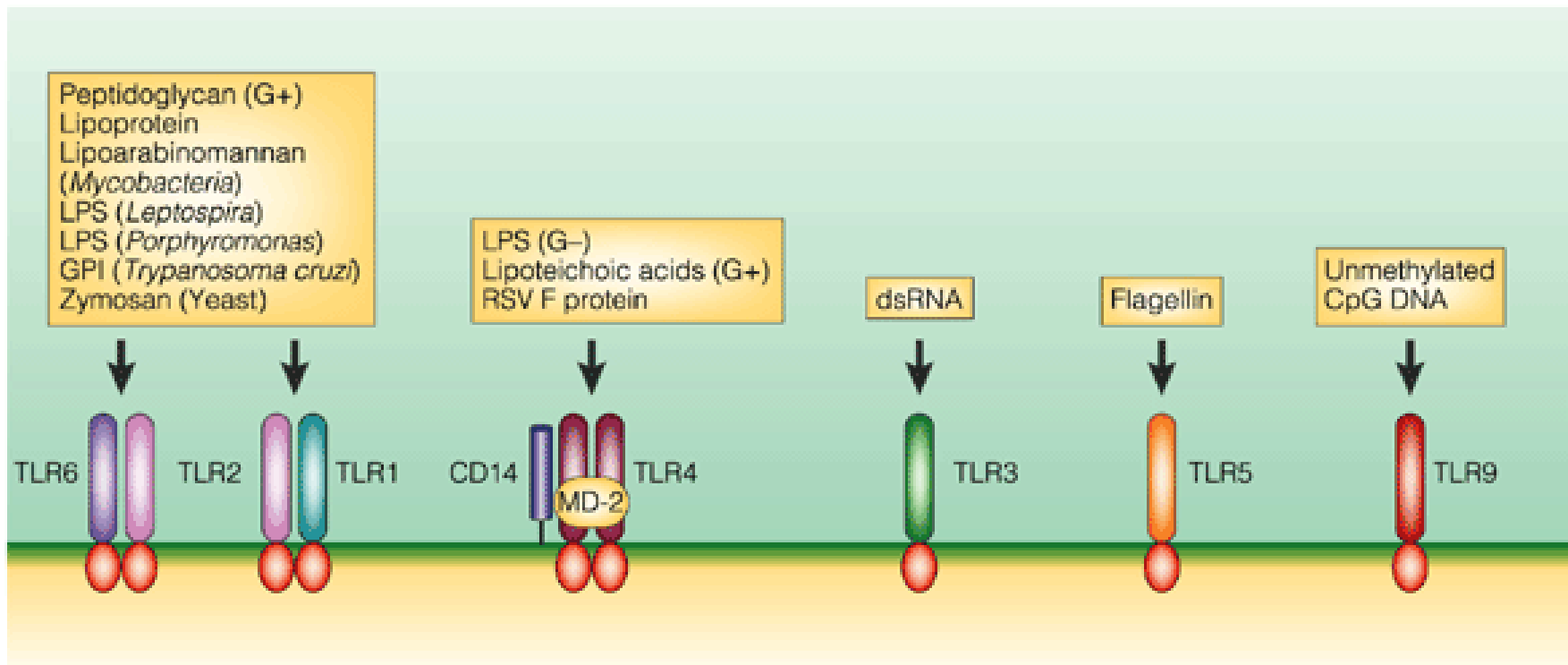
PAMPs – pathogen-associated molecular patterns exogenous substances activating non-specific immunity (Endotoxin, mannose, double-stranded RNA, unmethylated CpG nucleotides)

DAMPs – danger associated molecular patterns – endogenous substances stimulating non specific immunity – heat shock proteins, uric acid

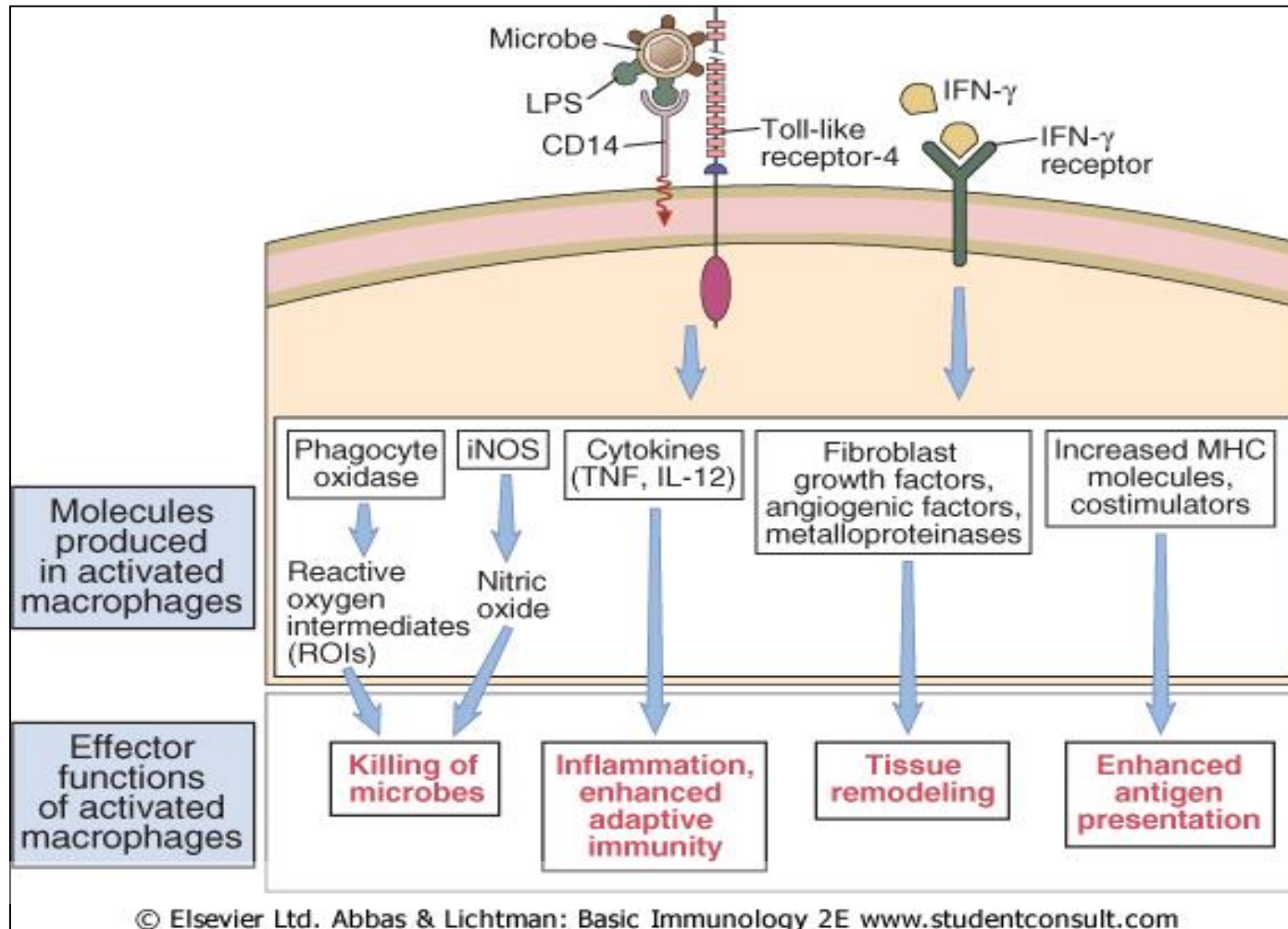
PRR- Pattern recognition receptors - recognize PAMPs, DAMPs.

TOLL-like receptors –surface or intracellular receptors recognizing various PAMPs. Expressed on dendritic cells, macrophages, granulocytes, epithelial cells.... They induce activation of these cells.

TOLL-LIKE RECEPTORS



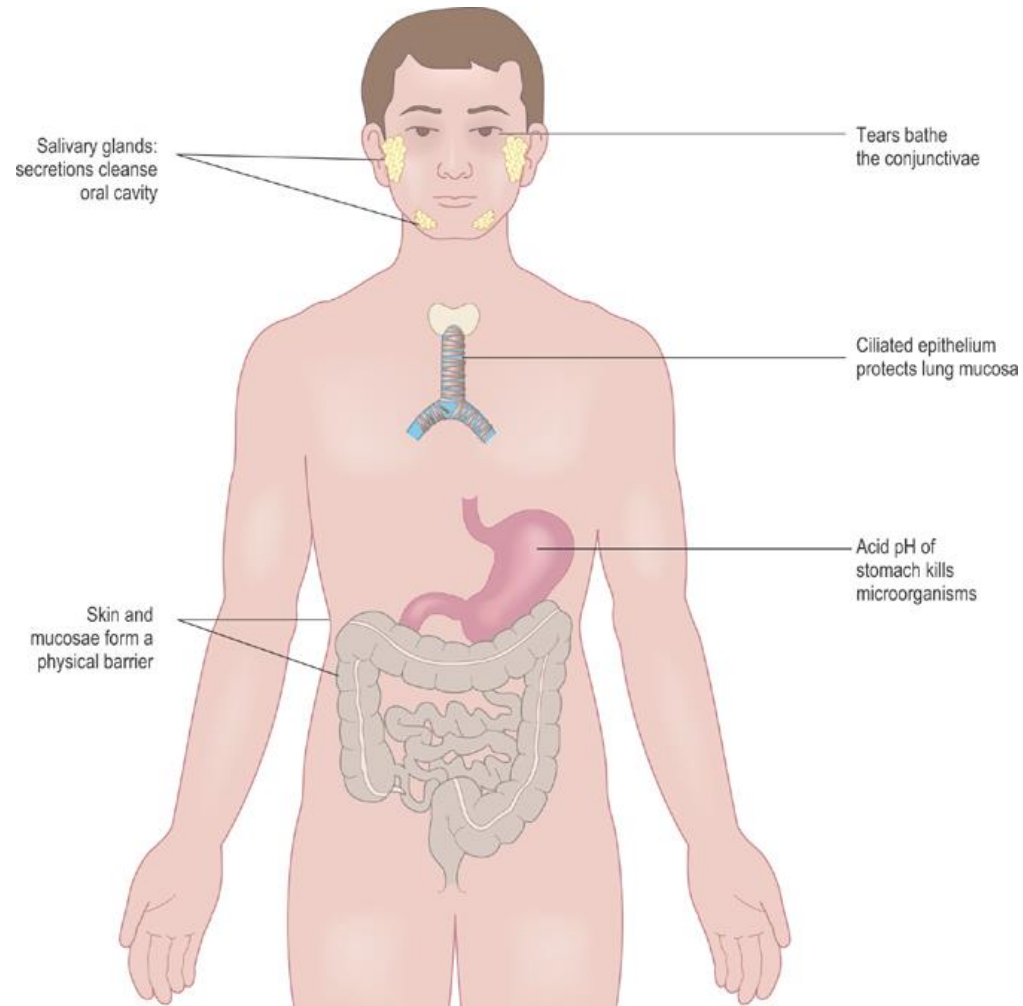
Activation by Toll-like Receptors and by Cytokine Receptors



Basic components of non-specific defence

- Non Specific barriers
 - Anatomical/Physiological
- Acute phase reactants and Inflammation
 - Complement/Interferons/CRP
- Innate cells
 - PMN/Macrophages/NK cells

Non-specific barriers of human body

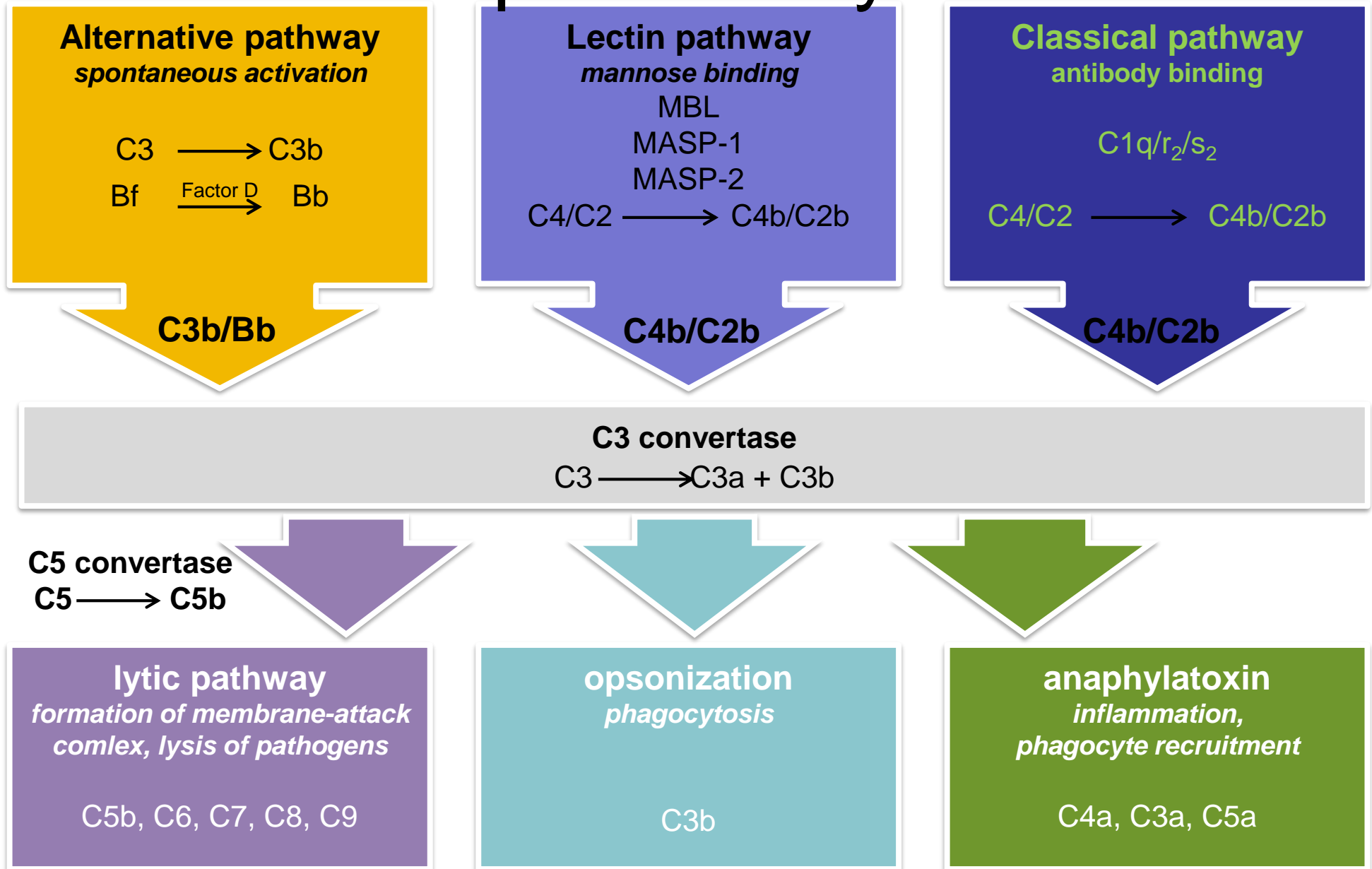


The Complement System

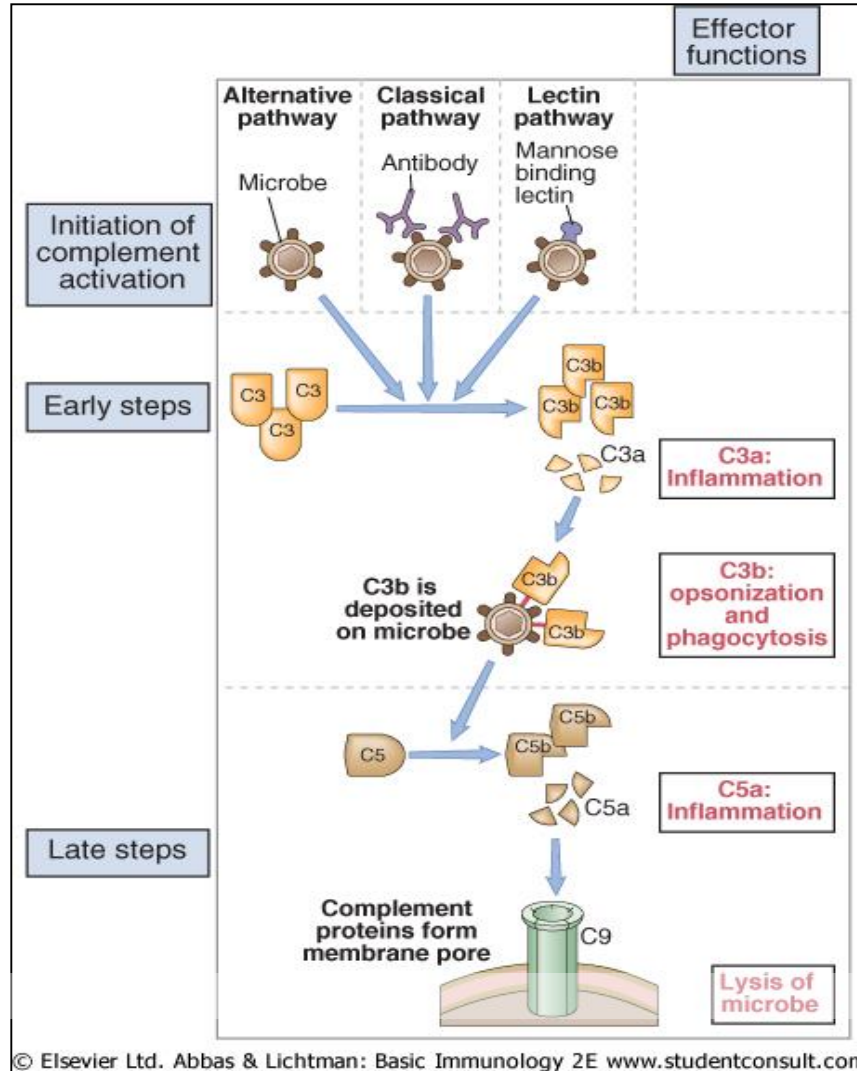
General features of the Complement System Activation

- Inactive, preformed protein is activated by the proteolytic cleavage.
- It is cleft into the smaller part (called a) and a bigger part (called b).
- Usually the bigger part has also proteolytic activity, while the smaller part has various other biological activities (chemotactic, anaphylatoxic).
- Component C6-C9 are activated without cleavage, they just „attach“ to the complex of the other complement components.

Complement system



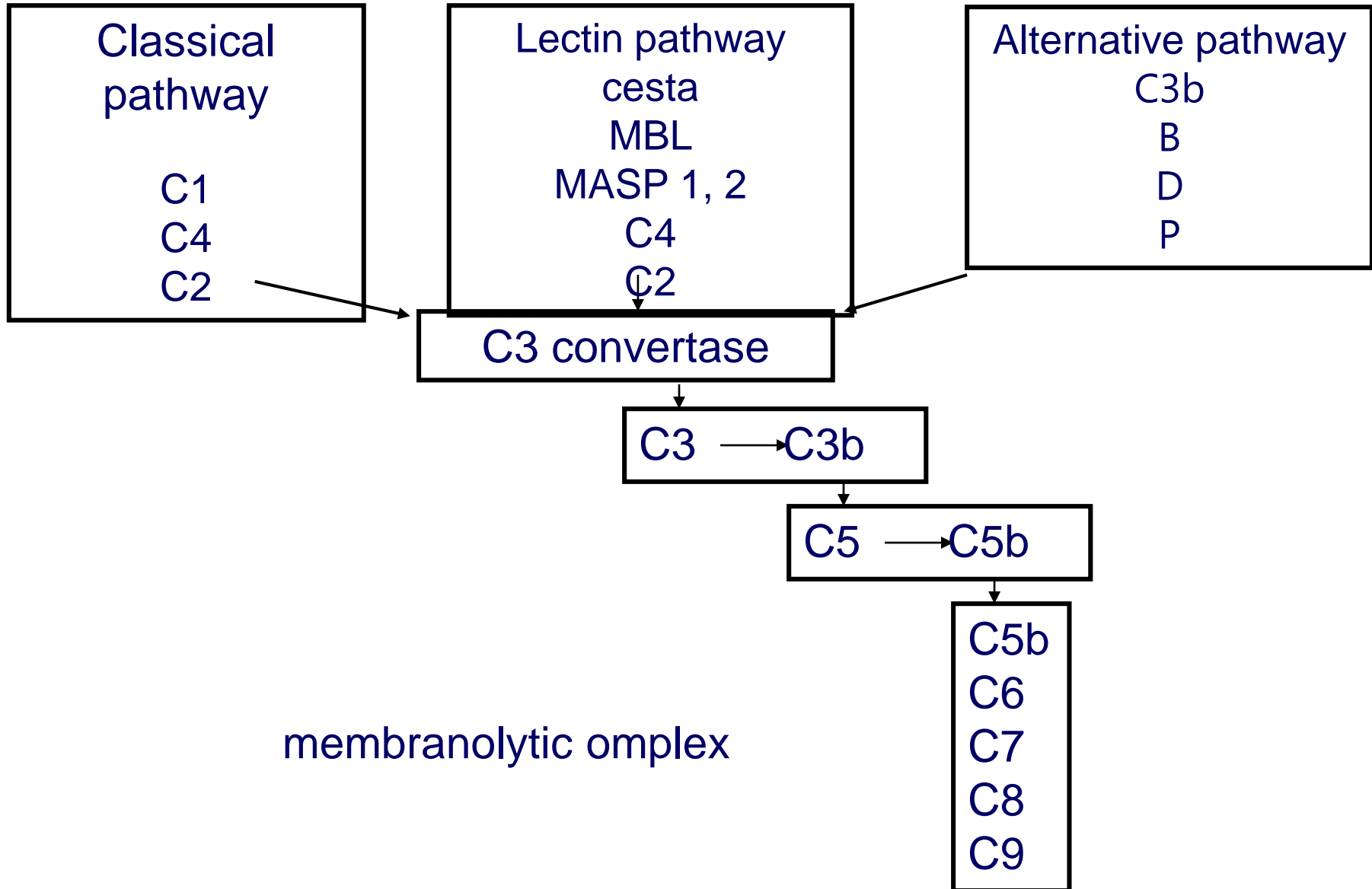
The Complement System



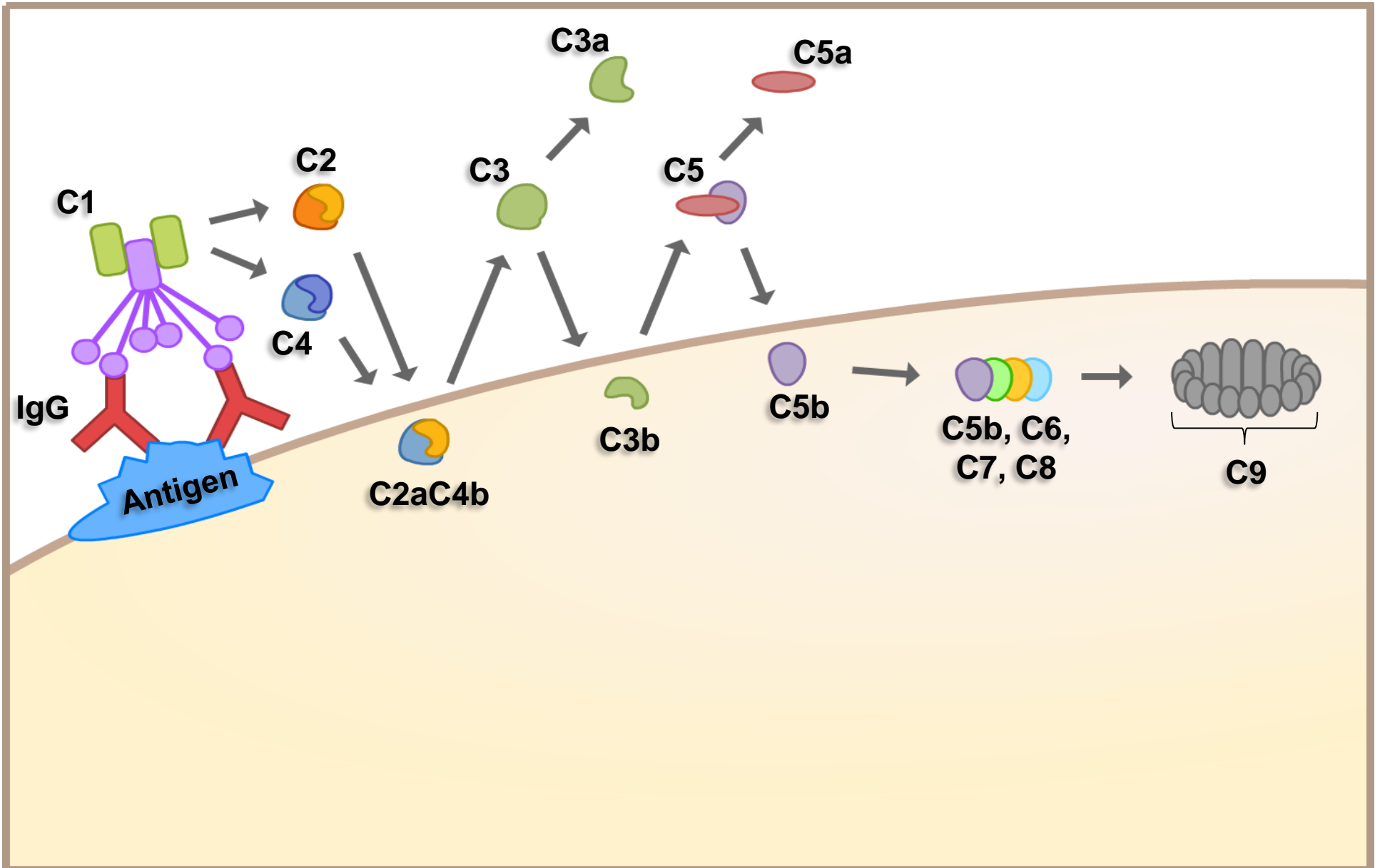
Complement system activation

- Classical pathway:
 - Complexes IgG-antigen, IgM-antigen,
 - C-reactive protein
- Alternative pathwas
 - Lipopolysaccharide of G- bacteria
 - Cell wall of some bacteria
 - Cell wall of the yeasts (zymozan)
 - Aggregated IgA
- Lectin pathway:
 - Mannose and other sacharides

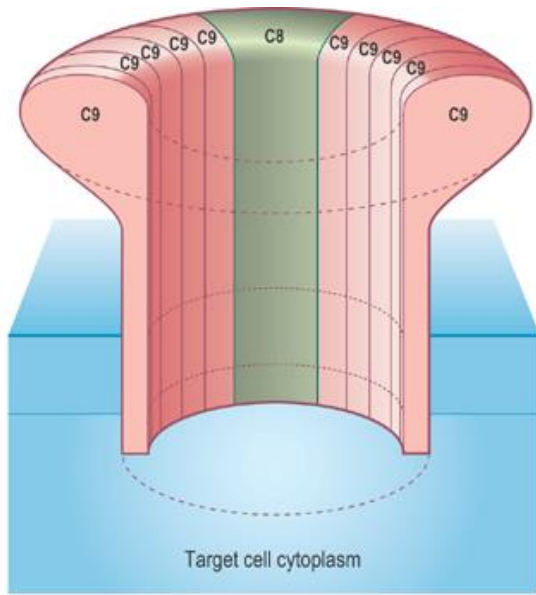
Complement system activation



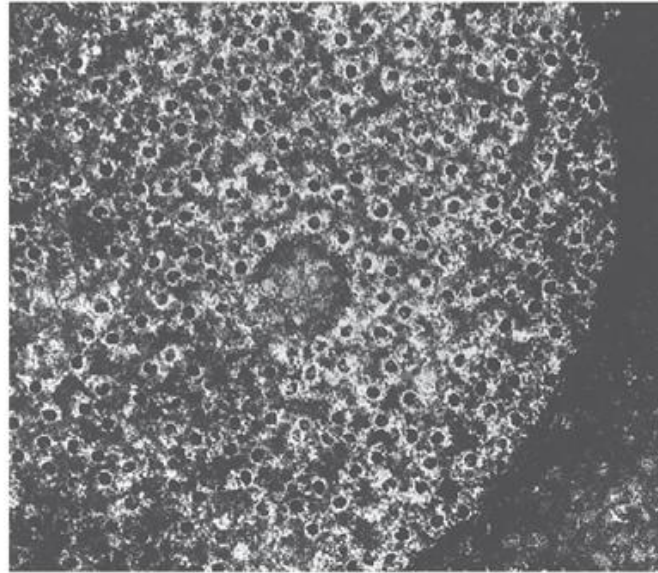
Classical pathway complement activation



Effect of C9



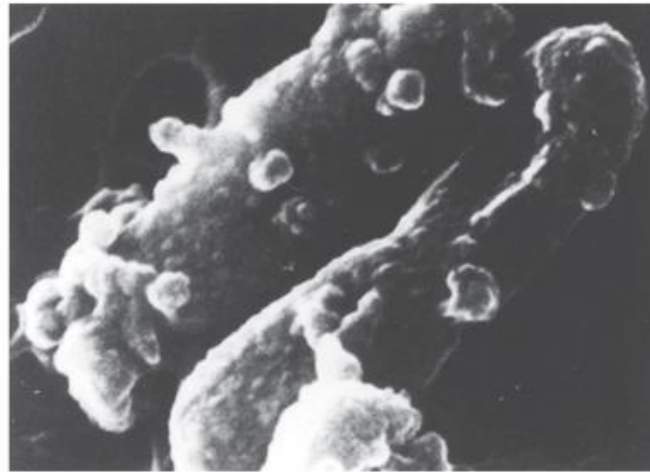
(a)



(b)

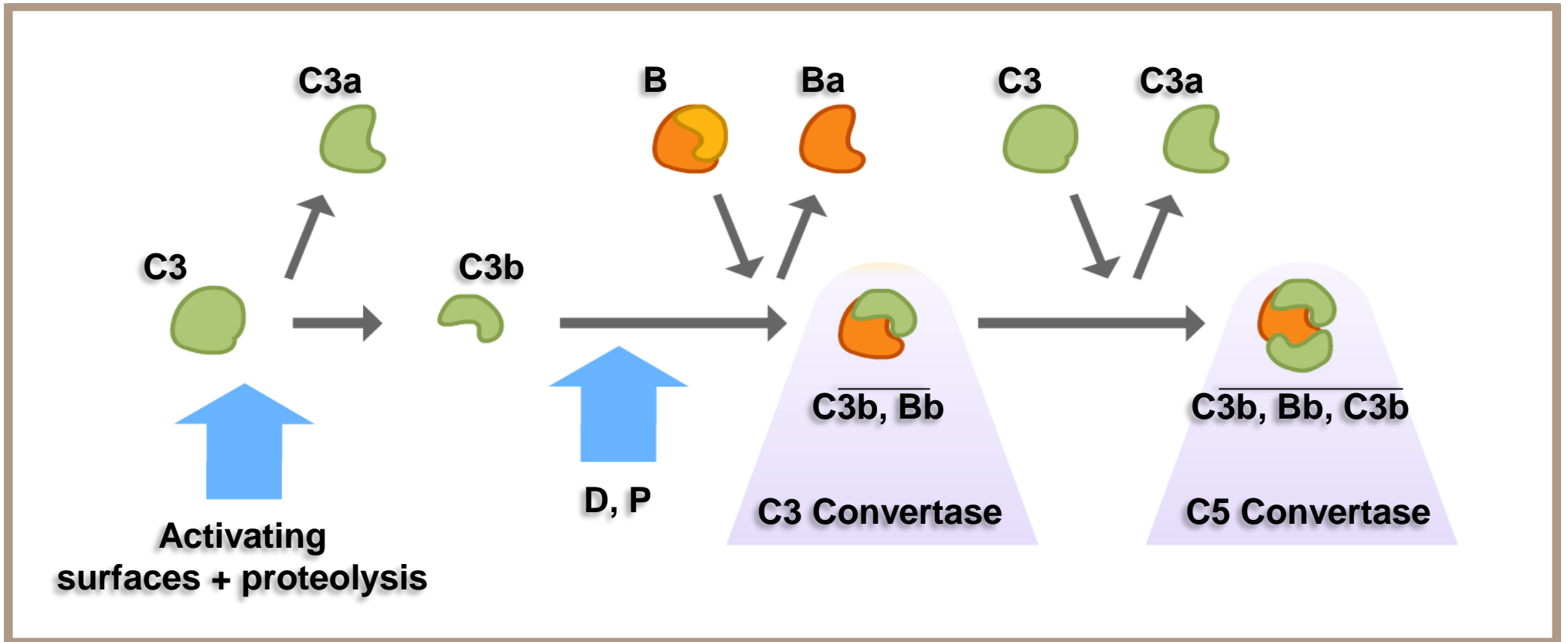


(c)



(d)

Actiation of Alternative Pathway of the Complement system



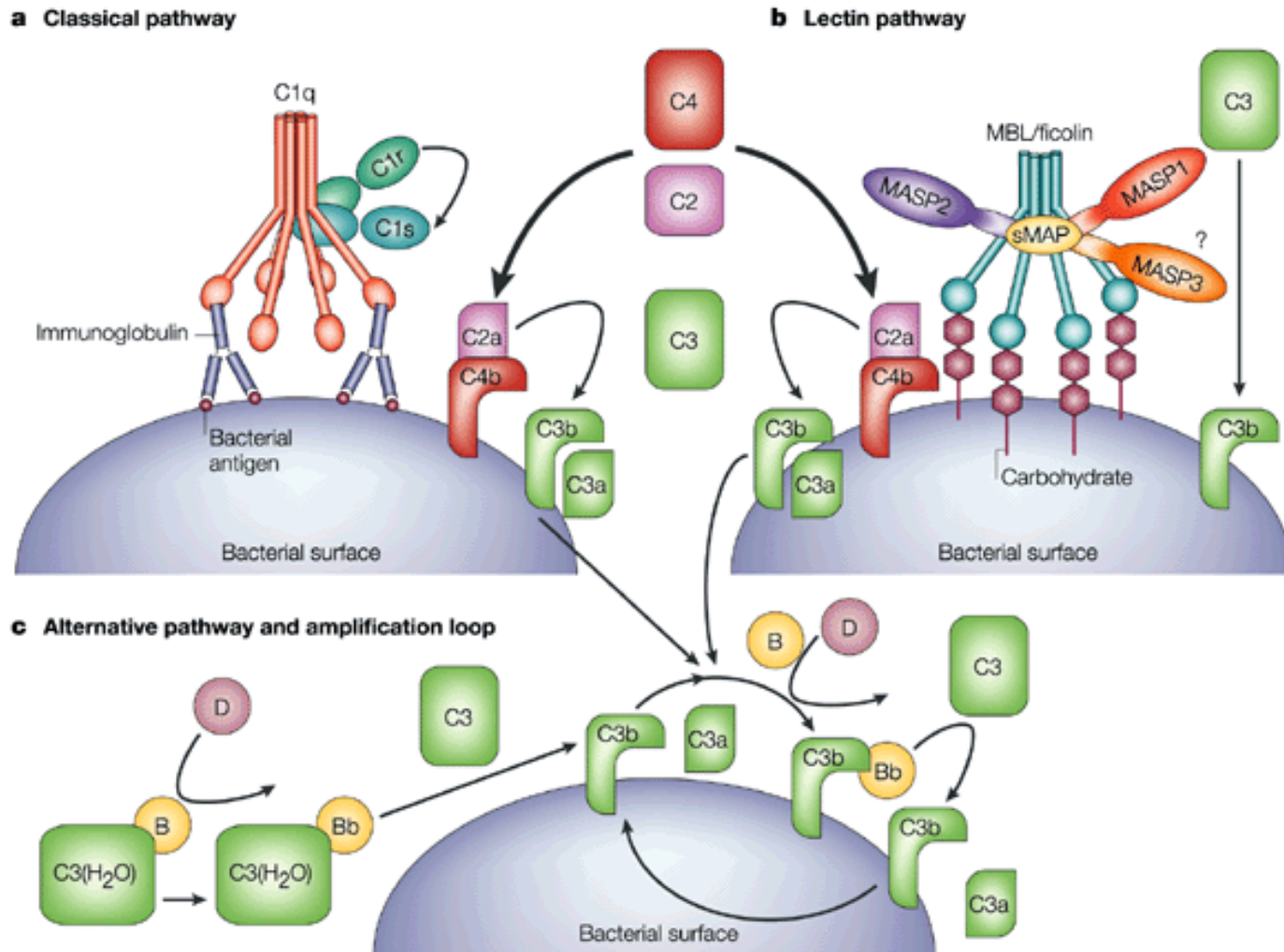
Alternative pathway of the complement system

- Initiated by spontaneous activation of C3 on the surface of some bacteria (eg due to LPS) or yeast (eg due to zymosan),
- Component B is activated
- Component D and P (properdin) participate in the stabilization of the complex.
- Alternative C3 convertase (C3bBb) is formed.

Lectin (third) pathway of the complement system activation.

- Mannose-binding lectin (MBL) binds to several cell surface polysaccharides.
- Activated MBL activates C2 and C4.
- MBL is a polymer composed of three subunits.
- Several exon and promoter polymorphisms lead to a very low level ("deficit") of MBL (5-10% of the population).
- Most people with MBL deficiency have no immunodeficiency symptoms.

Activation of classic and lectin complement pathways



Biological effects of activated complement system

- C9 - cytolytic effect
- C3b - opsonisation
- C3a, C5a – anaphylatoxins, liberation of histamine
- C5a - chemotaxin

Regulation of the Complement System

- Factors present in plasma
 - Classical pathway – mainly C1-inhibitor - C1-INH (deficiency leads to hereditary angioedema).
 - Alternative pathway – factor H, factor I
- Faktors on cell membranes:
 - CD59, CD55 (DAF - decay accelerating factor) - CD46

Pathogenetic Significance of the Complement System

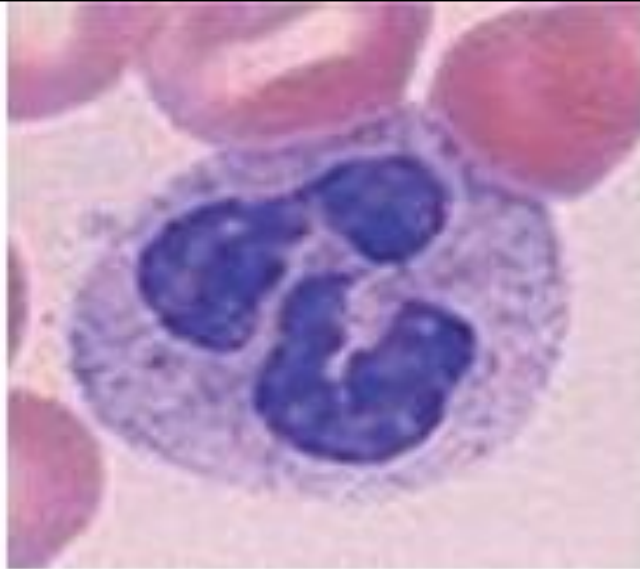
- **Deficit of components of the classical and alternative pathways** – proneness to bacterial infections and to systemic autoimmune diseases.
- **C1-INH deficiency**: hereditary angioedema.
- **Mutations of factor H** – atypical hemolytic-uremic syndrome.
- **Several polymorphisms of factor H** are linked to **senile macular degeneration**.
- Paroxysmal night hemoglobinuria – caused by mutations of the gene **PIG-A** (product of this gene binds CD 55 and CD 59 into cytoplasmatic membrane).

Phagocytosis

Phagocytic cells

- Polymorphonuclear granulocytes
- Monocytes + macrophages
- Dendritic cells mainly non-activated cells. After activation they lose most of their phagocytic activity.

Polymorphonuclear granulocyte



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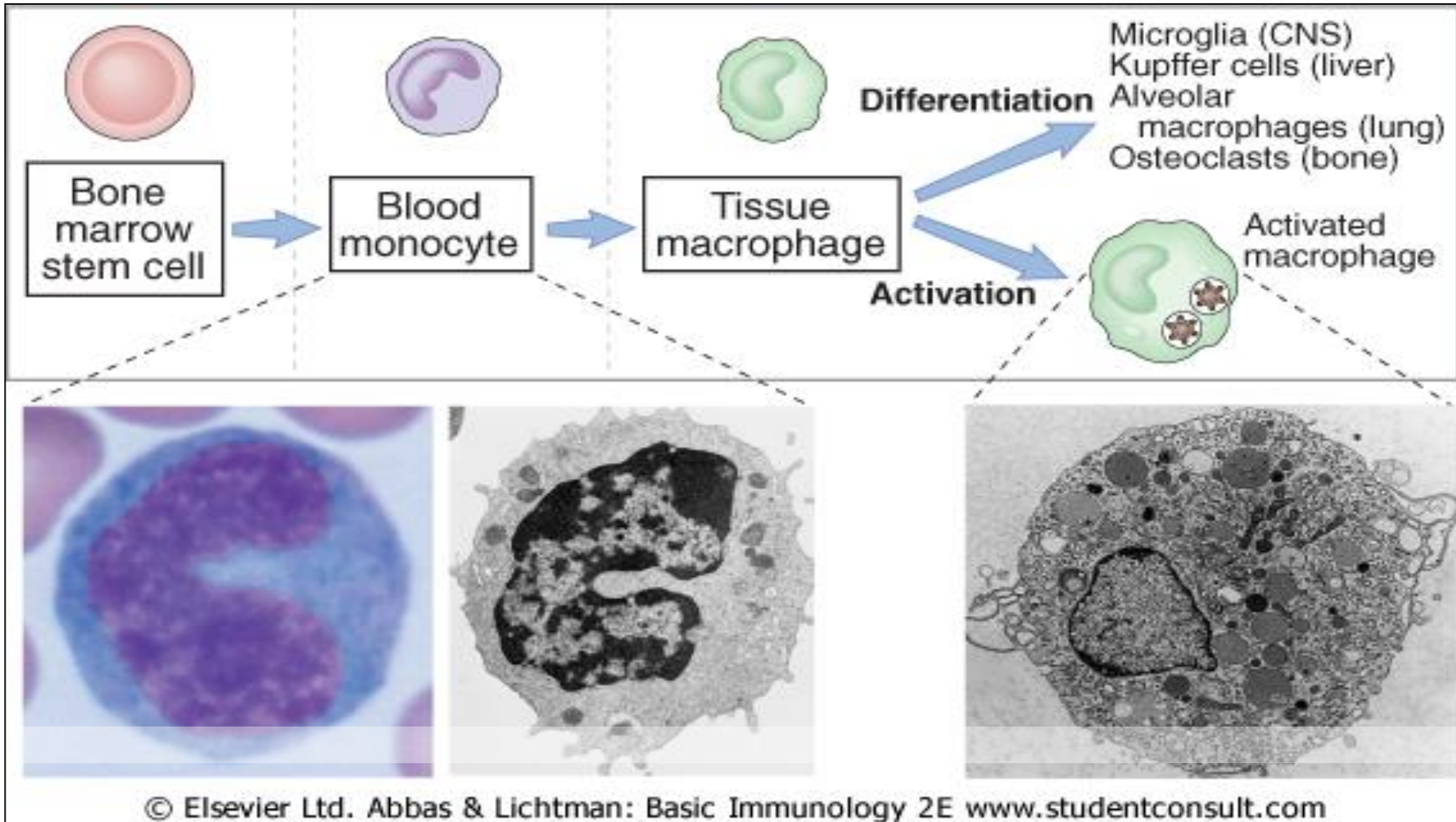
Normal blood count (in adults)

- Erythrocytes: $4-5 \times 10^{12}/l$
- Thrombocytes: $150-300 \times 10^9/l$
- Leukocytes: $4-9 \times 10^9/l$
 - **Granulocytes: 55-70%**
 - **Eosinophils: 1-4%**
 - **Basophils: 0-1%**
 - **Lymphocytes: 24-40%**
 - **Monocytes: 3-8%**

Macrophages

- Derived from blood monocytes.
- Connective tissue macrophages
 - Kupffer cells (liver)
 - Alveolar macrophages (lungs)
 - Microglia (CNS)
 - Osteoclasts (bone)
 - Peritoneal macrophages

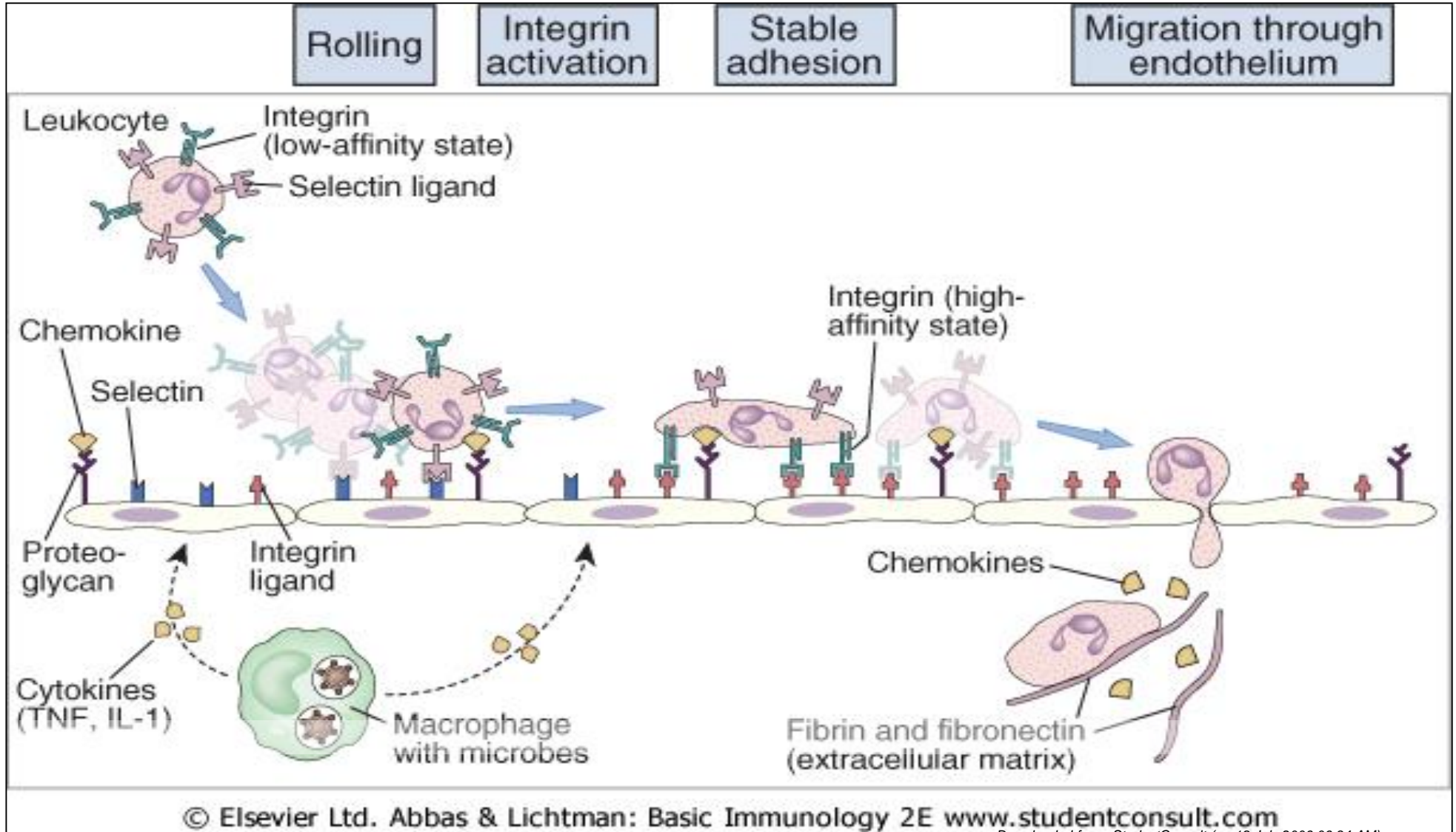
Development of macrophages



Two phases of interaction between phagocytic and endothelial cells

- **Rolling** - reversible interaction due to binding between endothelial selectins and their ligands on the surface of leukocytes (eg sialyl-LewisX).
- **Stable binding** - formed after activation by pro-inflammatory stimuli. It is given by the interaction between leukocyte integrins and their ligands on endothelium - eg ICAM-1.

Extravasation of leukocytes



Natalizumab

- A humanized monoclonal antibody that binds to the $\alpha 4$ integrin (which binds to the integrin receptor VCAM-1).
- Adhesion and subsequent transfer of leukocytes (mainly T-lymphocytes) to the extravascular spaces is blocked.
- Used in the treatment of multiple sclerosis, and recently also in Crohn's disease.

“Classical” phases of phagocytosis

- Chemotaxis - targeted movement of cells against the concentration gradient of chemotactic factors (chemotaxins).
- Adhesion
- Ingestion – formation of a phagosome, which consequently fuses with a lysosome to form a phagolysosome.
- Killing and Intracellular degradation of microbes

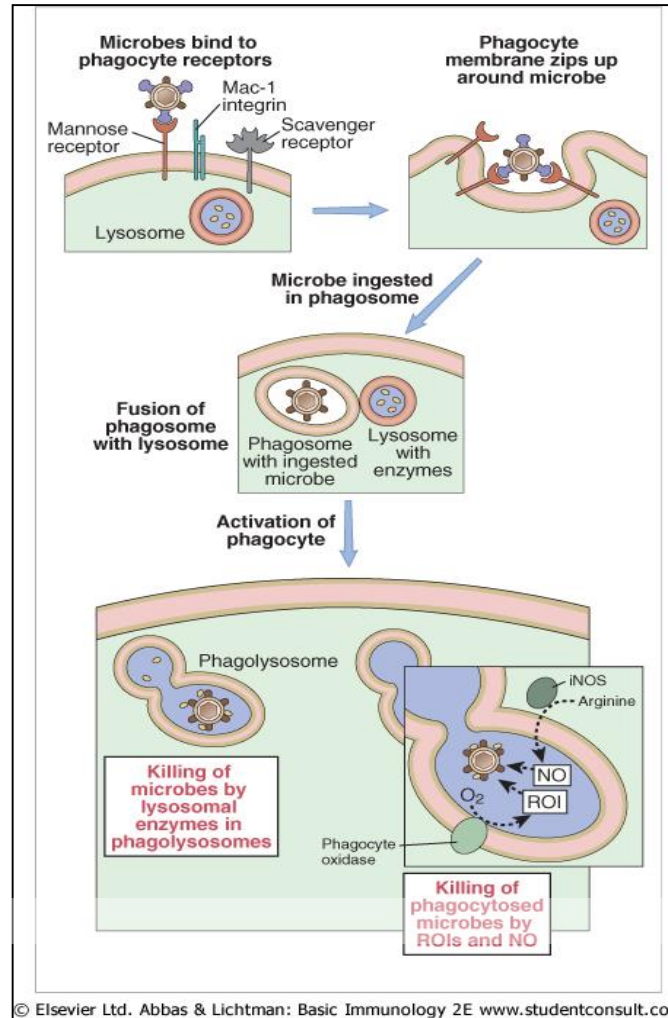
Chemotaxins

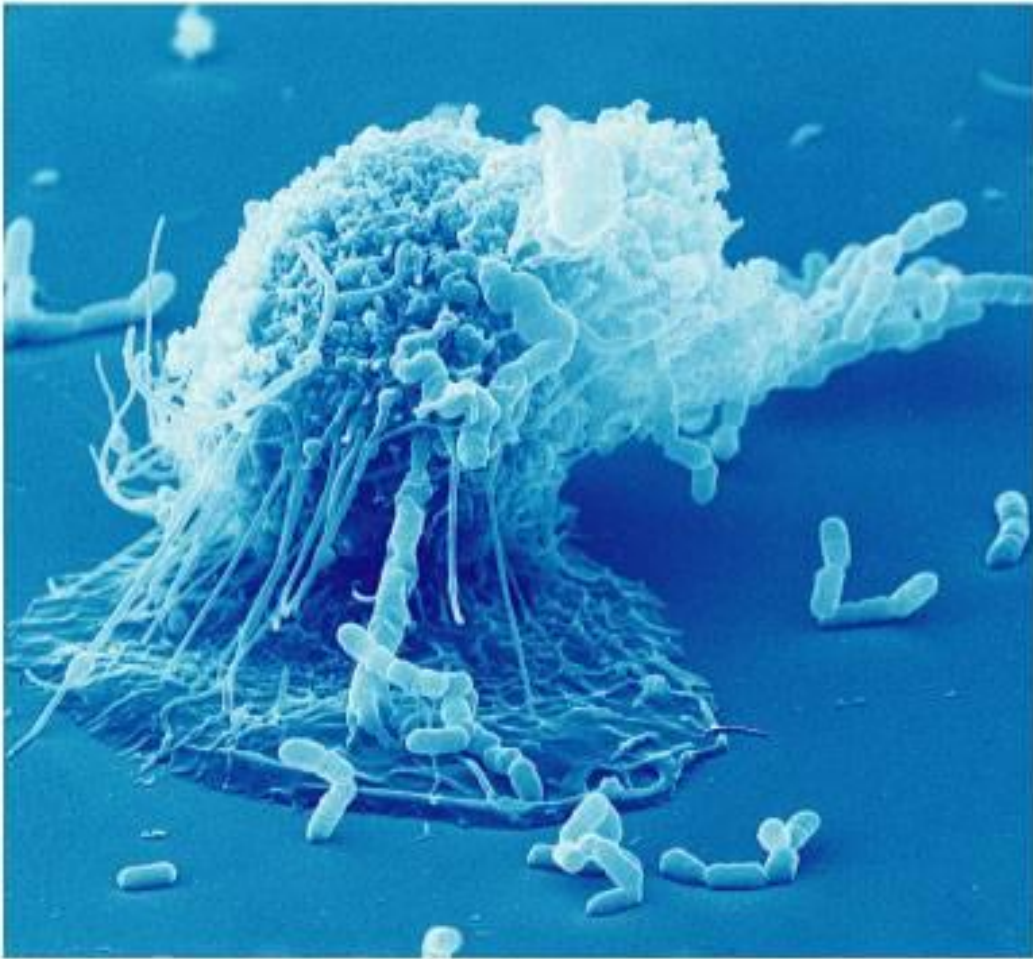
- Attract phagocytic cells
- Products of destroyed cells
- C5a
- IL-7, IL-1
- Leukotriens

Opsonins

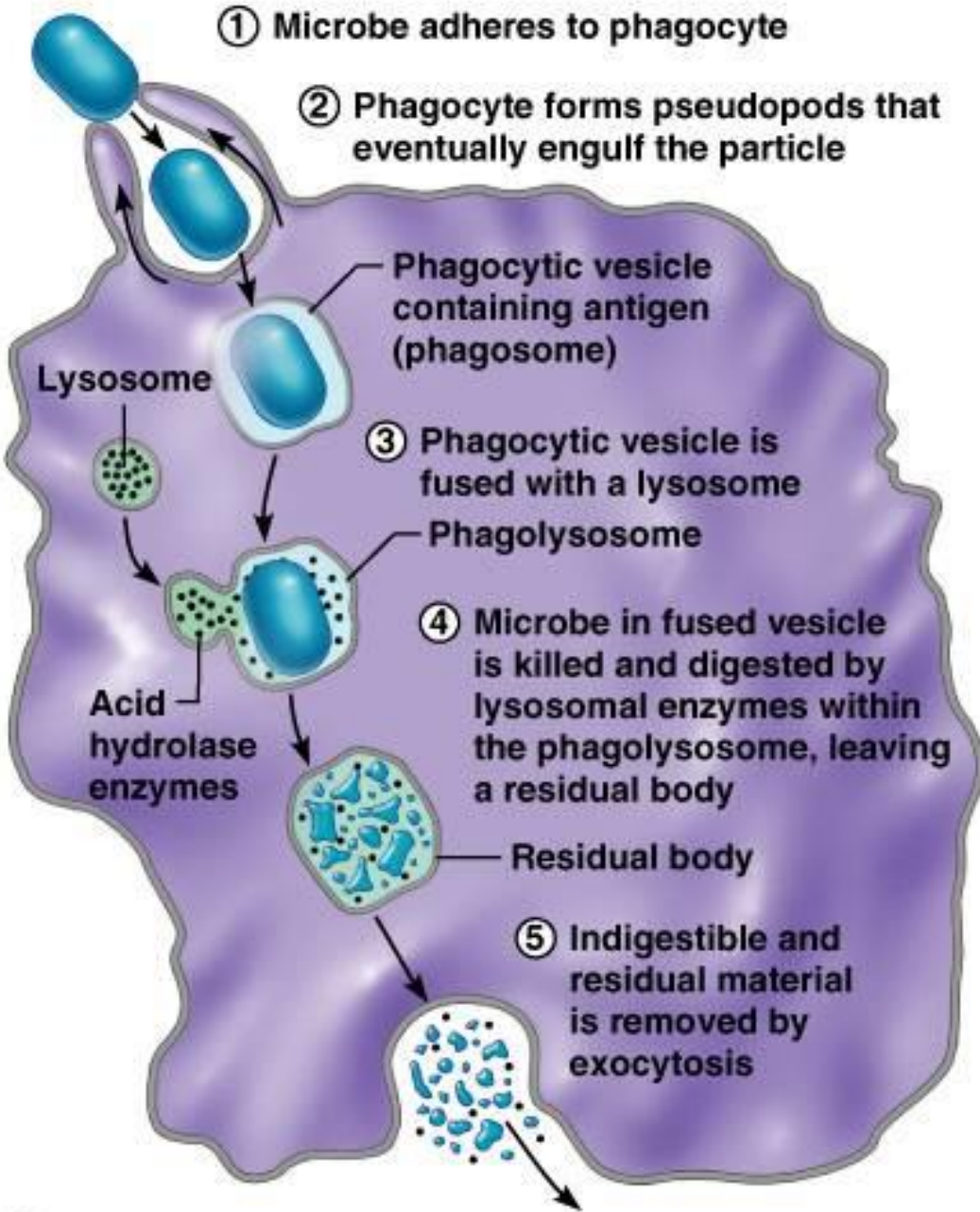
- Substances enhancing phagocytic process by improving adhesion of the particle to the phagocytic cell.
- Specific: IgG, (IgM only indirectly by activation of the complement system)
- Non- specific: C3b, fibronectin....

Steps of phagocytosis





(a)



(b)

Killing mechanisms of phagocytic cells

- Reactive metabolites of oxygen (H_2O_2 , hydroxyl radical ($\cdot\text{OH}$), superoxide anion (O_2^-), singlet oxygen ($\cdot\text{O}_2$)
- Reactive nitrogen intermediates (NO , NO_2)
- Hydrolases: protease, lipases, DNAses
- Low pH
- Lysozyme
- Lactoferrin
- Defensins – antimicrobial polypeptides

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

Lysozyme

- Cleaves cell walls of G+ bacteria
- Present in granules of neutrophil granulocytes, in plasma, secretions.

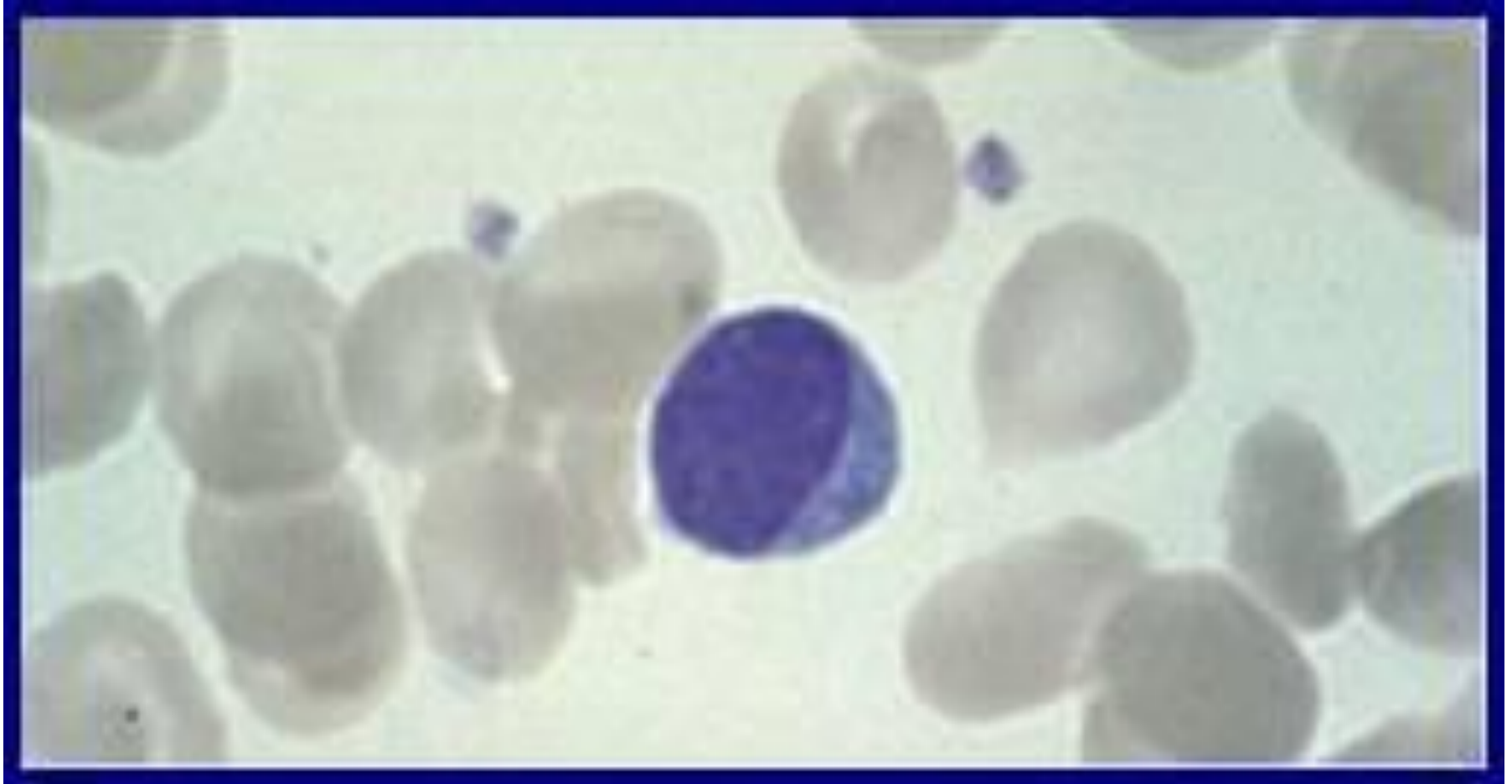
Defensins

- Polypeptides with antibiotic-like effect on bacteria and fungi.
- Produced mainly by granulocytes and by epithelial cells.
- Lead to desintegration of cell membranes, formation of pores in membranes .
- Main groups are α and β defensins

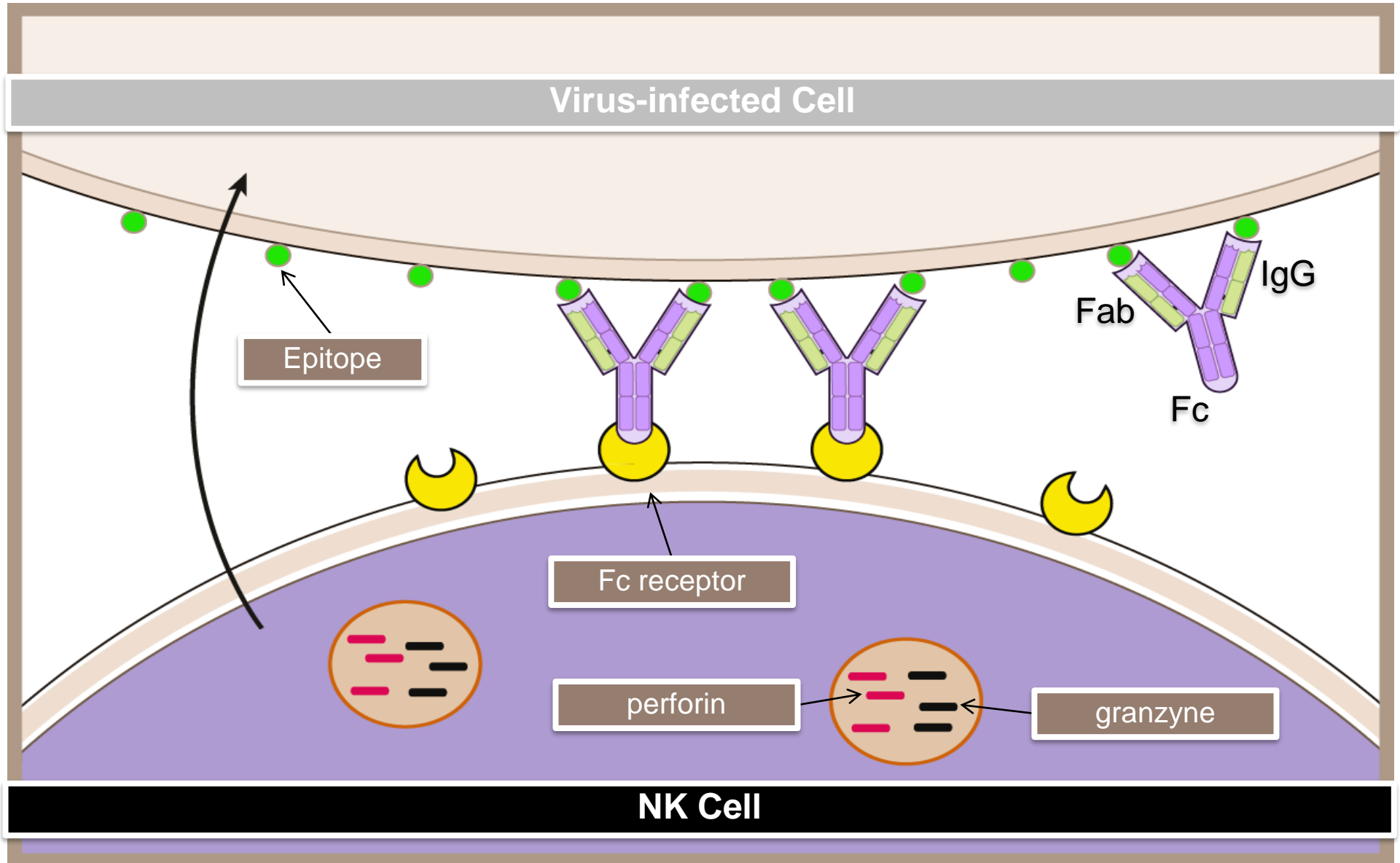
Natural killers (NK cells)

- Originate in non-T non-B lymphocyte lineage.
- Morphologically: large granulated lymphocytes (LGL).
- Recognition of target cells is antigen non-specific.
- Virus infected and tumor cells are the main targets.
- Target cells are recognised mainly by decreased HLA-I expression.
- Cytotoxic mechanisms are similar to Tc cells: perforin and induction of apoptosis.
- Target cells can be recognised by the Antibody Dependent Cellular Cytotoxicity (ADCC).
- Produce various cytokines, e.g. IFN- γ , IL-12.

Large granulated lymphocyte



Antibody dependent cellular cytotoxicity (ADCC)



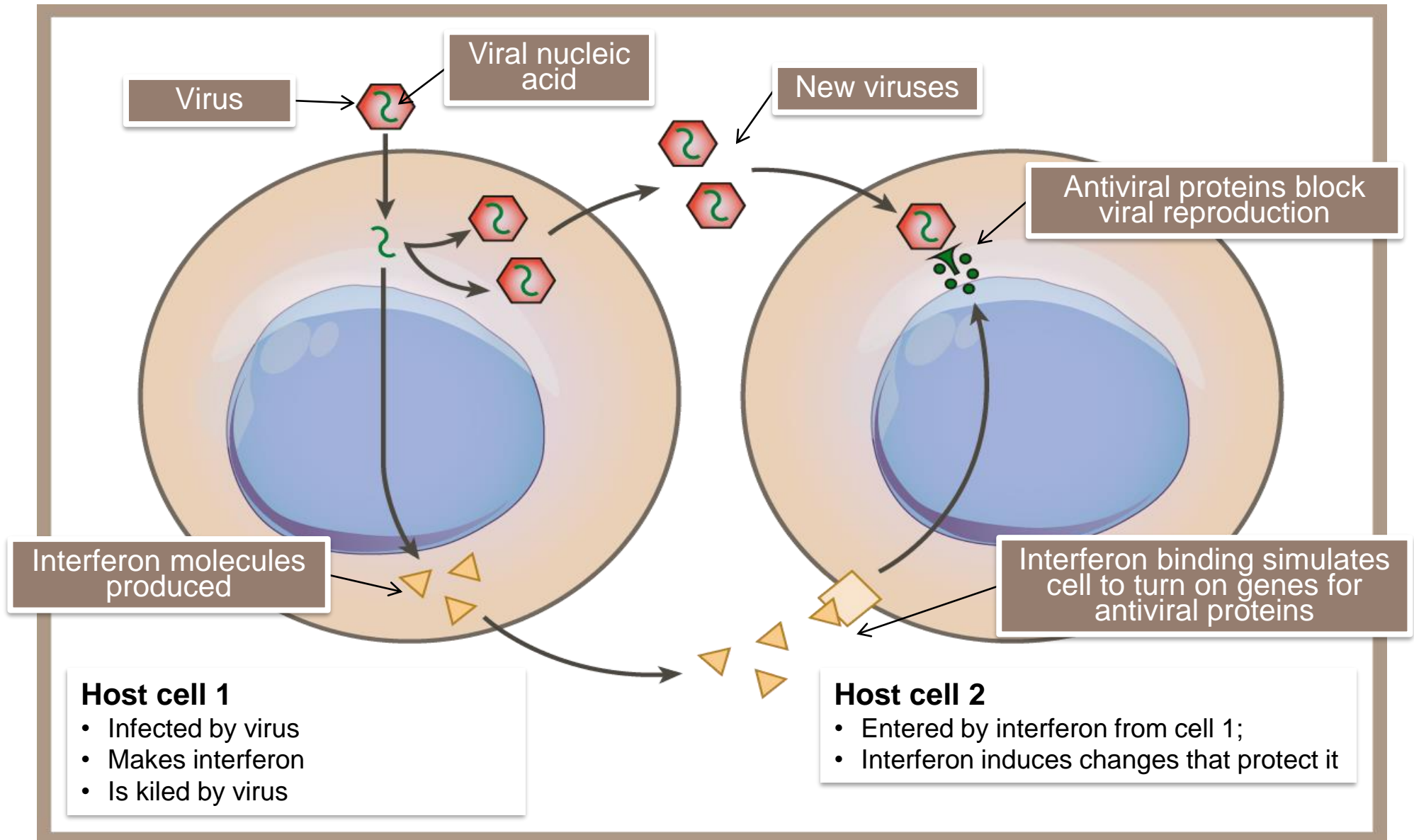
Interferons (IFN)

- Type 1: IFN α , IFN β – produced mainly by virus-infected cells (fibroblasts, granulocytes). Lead to inhibition of the virus replication in target cells.
- Type 2 - „Immune interferon“: IFN γ : is produced by activated TH1+ cells, NK cells . Induces activation of macrophages.
- Interferon type 3 - IFN λ (and other molecules) – similar to IFN-I

Antiviral effect of interferons

- Binds to a specific receptor (heterodimer) of infected and still uninfected cells.
- Activation of a number of anti-virus mechanisms:
 - IF-2 protein kinase - phosphorylates (thereby inactivates) the initiation factor IF-2 - necessary for proteosynthesis.
 - 2'5' oligoadenylate synthetase - activation of ribonucleases cleaving viral RNA.

The action of interferon (IFN)



Inflammation

- A genetically fixed, rapid response to a tissue damage.
- Acute inflammation plays a crucial role in the protection of the body, however chronic inflammation may lead to an organism damage.
- Components of the innate immunity play a crucial role.
- Local consequences of inflammation
 - Increased blood flow to affected area
 - Recruitment of phagocytes to affected area, particularly neutrophils and macrophages
 - Alteration of vascular permeability leading to entry of soluble molecules from the plasma

Inflammasome

It is a protein oligomer consisting of intracytoplasmic signaling molecules (caspases and others).

It is expressed in myeloid cells It is considered part of innate immunity.

The specific composition of the inflammasome is dependent on the activators that cause its assembly.

The inflammasome supports the maturation of pro-inflammatory cytokines.

Local mediators of inflammation

- Products of activation of the kinin, complement, and coagulation systems. **Usually C3a and C5a plays a significant role.**
- Vasoactive amines – histamin, serotonin - reased from the damaged cells or stimulated macrophages.
- Metabolites of arachidonic acid
- Platelet activating factor
- Produkty of monocytes and granulocytes: IL-1, TNF- α , IL-6, IL-18, chemokines, NO,
- Produkts of activated lymphocytes: TNF- α , IL-6, IFN- γ , chemokines

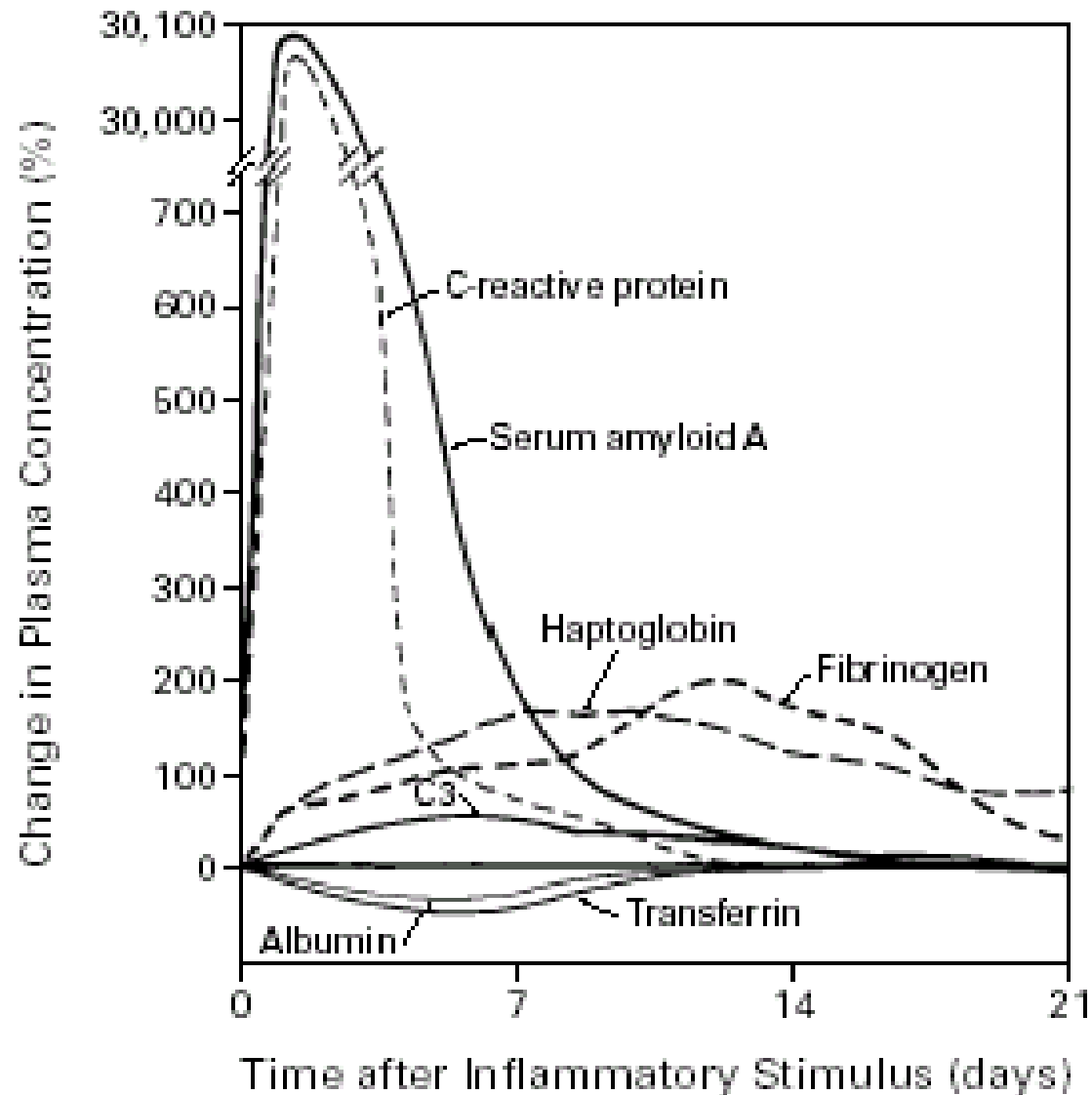
General symptoms and signs of inflammation

- Orchestrated mainly by IL-1, IL-6, TNF- α
- Fever (effect of cytokines on hypothalamic centers)
- Fatigue, somnolence
- Loss of appetite
- Laboratory signs: leukocytosis, increased ESR, increase in acute phase proteins, decreased levels of iron and zinc in serum.

Accute-phase proteins

- Serum levels are increased during inflammation
- Produced by the liver after stimulation by IL-1, IL-6, TNF- α
- Best known: C-reactive protein
- Others: Complement components, A1-AT, fibronectin..

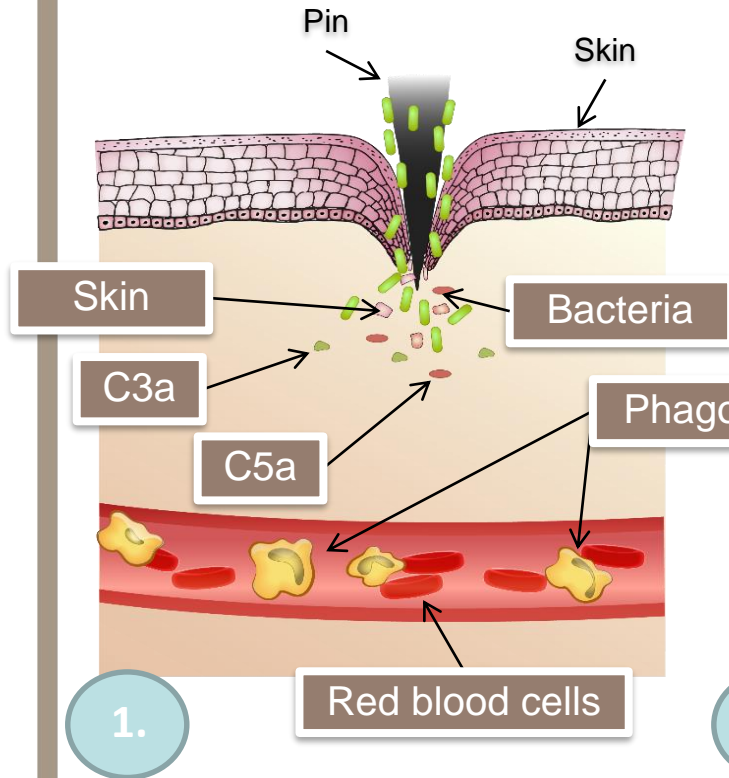
Accute phase response



Initiation of inflammatory response

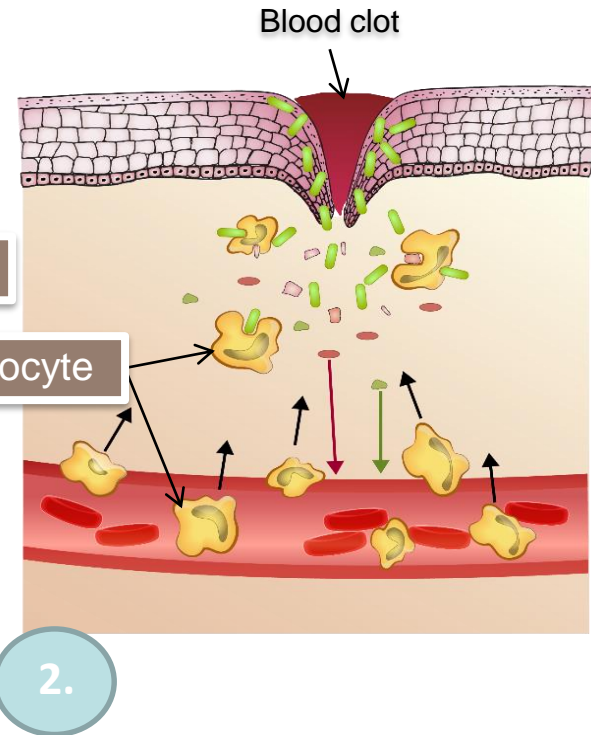
Step 1

Damaged tissues increasing blood flow to the area



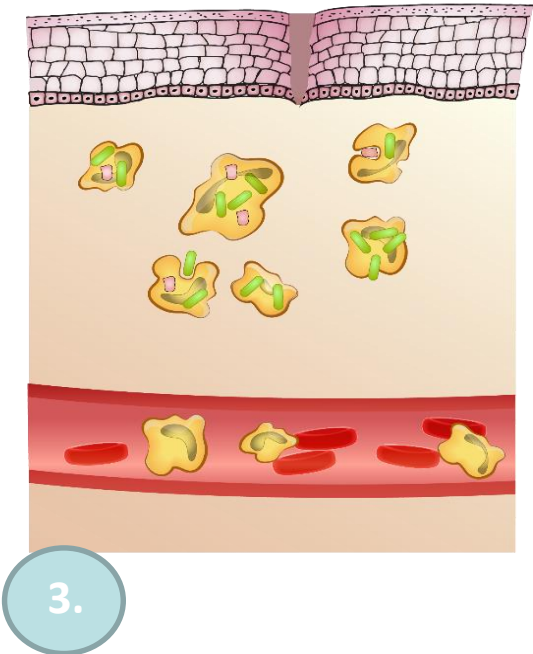
Step 2

Histamines cause capillaries to leak, releasing phagocytes and clotting factors into the wound



Step 3

Phagocytes engulf bacteria, dead cells, and cellular debris



Drugs modulating inflammatory process

- Non-steroidal anti-rheumatic (anti-phlogistic) drugs (acidosalicylic acid, paracetamole,...)
- Antimalarics
- Glucocorticoids
- Monoclonal antibodies against inflammatory cytokines and adhesion molecules
- Janus kinase inhibitors