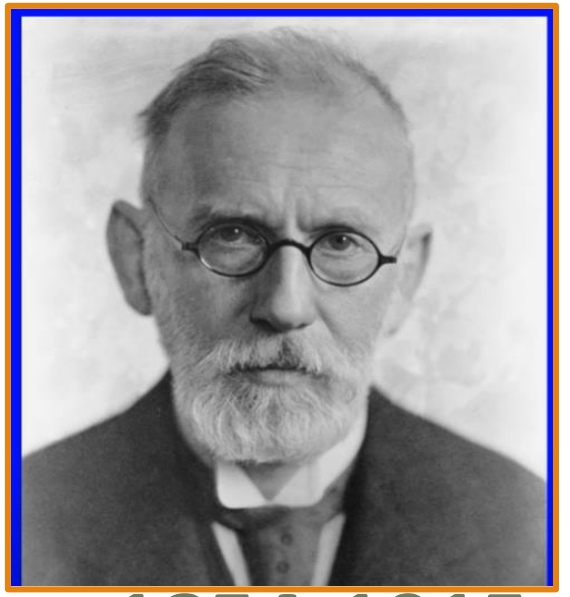
Complement system (CS)
Contribution to innate immunity
Bridge between innate immunity and adaptive immunity
Own pathological patterns of CS incompetence

Presenter: Titica Elena

Discovered in 1986 by Julles Bordet as a component of the innate immune system, CS was named and defined in such a way by Paul Ehrlich for its abilities to "complement", "help", "augment" the antibacterial defense made by antibodies formed during adaptive immune activation (specialized landmarks being T lymphocytes and B lymphocytes).

It is appreciated as a complex network of about 30 plasma proteins synthesized in the liver and associated with the cell membrane, which can trigger effective inflammatory and cytolytic immune responses directed against infectious agents (bacteria, viruses, parasites.

1870-1961



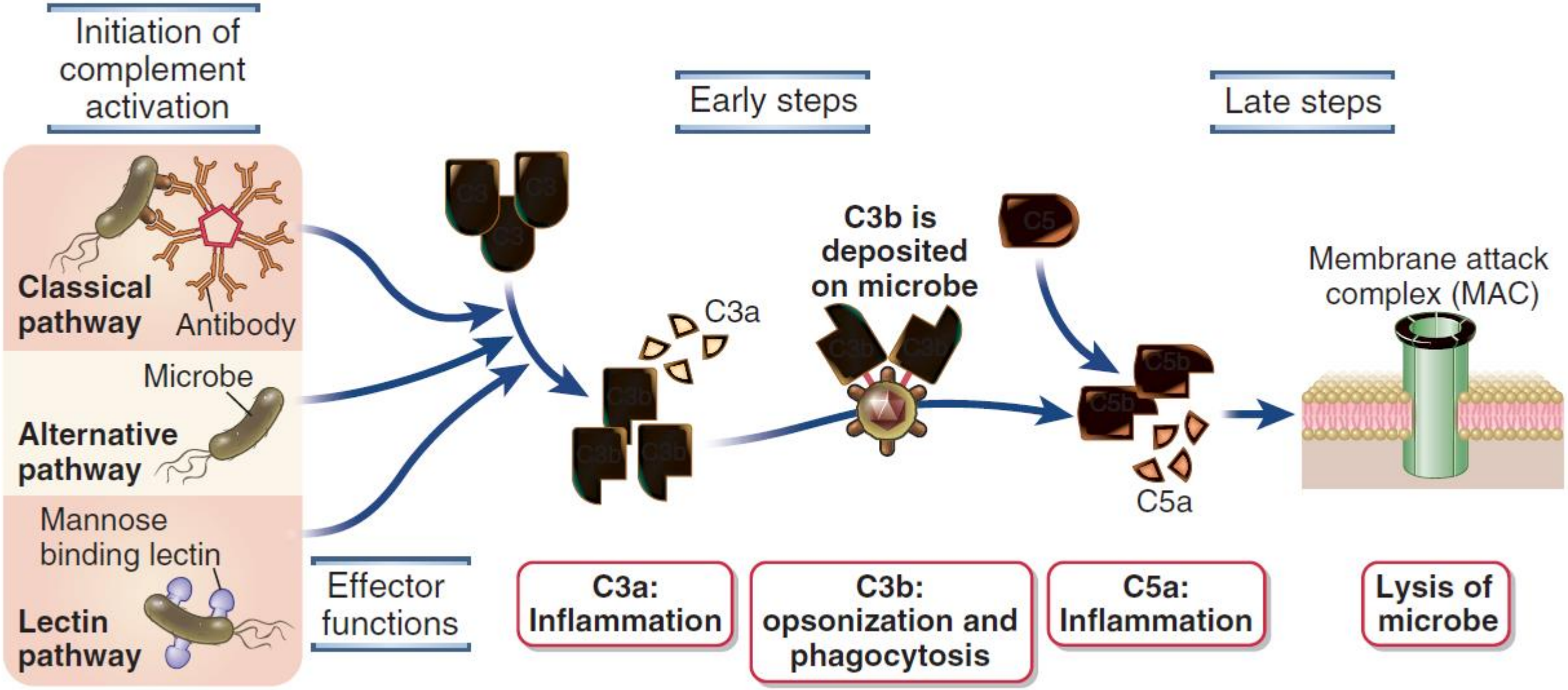
1854-1915

The approximately 30 proteins of SC are estimated to account for 15% of the globular protein fraction of plasma with an average cocktail of 3 g/L.

CS excels through cascading processes in 3 activation models: (classical, alternative and lecithin-assisted) resulting in:

1. **Generation of proinflammatory mediators (eg, anaphylatoxins).**
2. **Generation of phagocyte opsonization components (macrophages, neutrophils, eosinophils).**
3. **Proteolytic lysis of the pathogen by assembling the membrane attack complex, MAC (Membrane Attack Complex).**

Pathways of complement activation



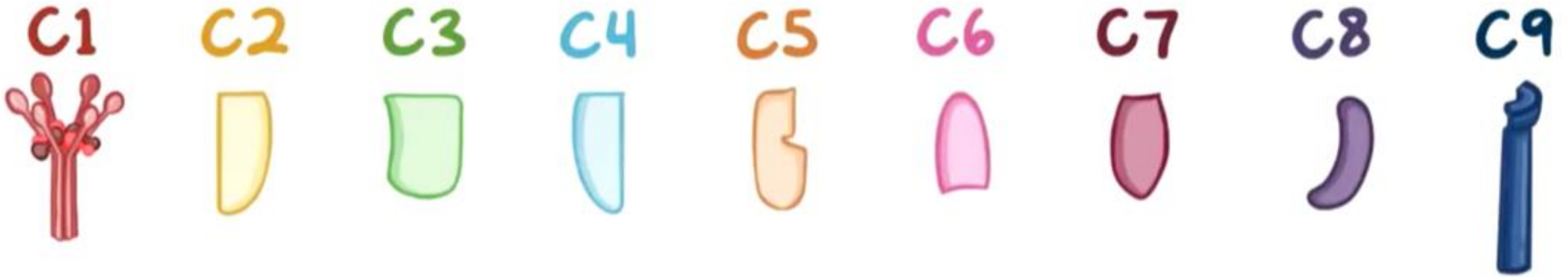
CS nomenclature

1. Components (C) found in plasma in zymogen form (therefore pro-enzymes activated by clivation) or expressed on the surface of different cells: C1, C2, C3,..... C9. The numbers correspond to the order of discovery.
2. Mutual activation cascade adjustment factors: factor B, factor D, factor H, factor I.... etc.
3. Receptors on the surface of cells that bind CS components.

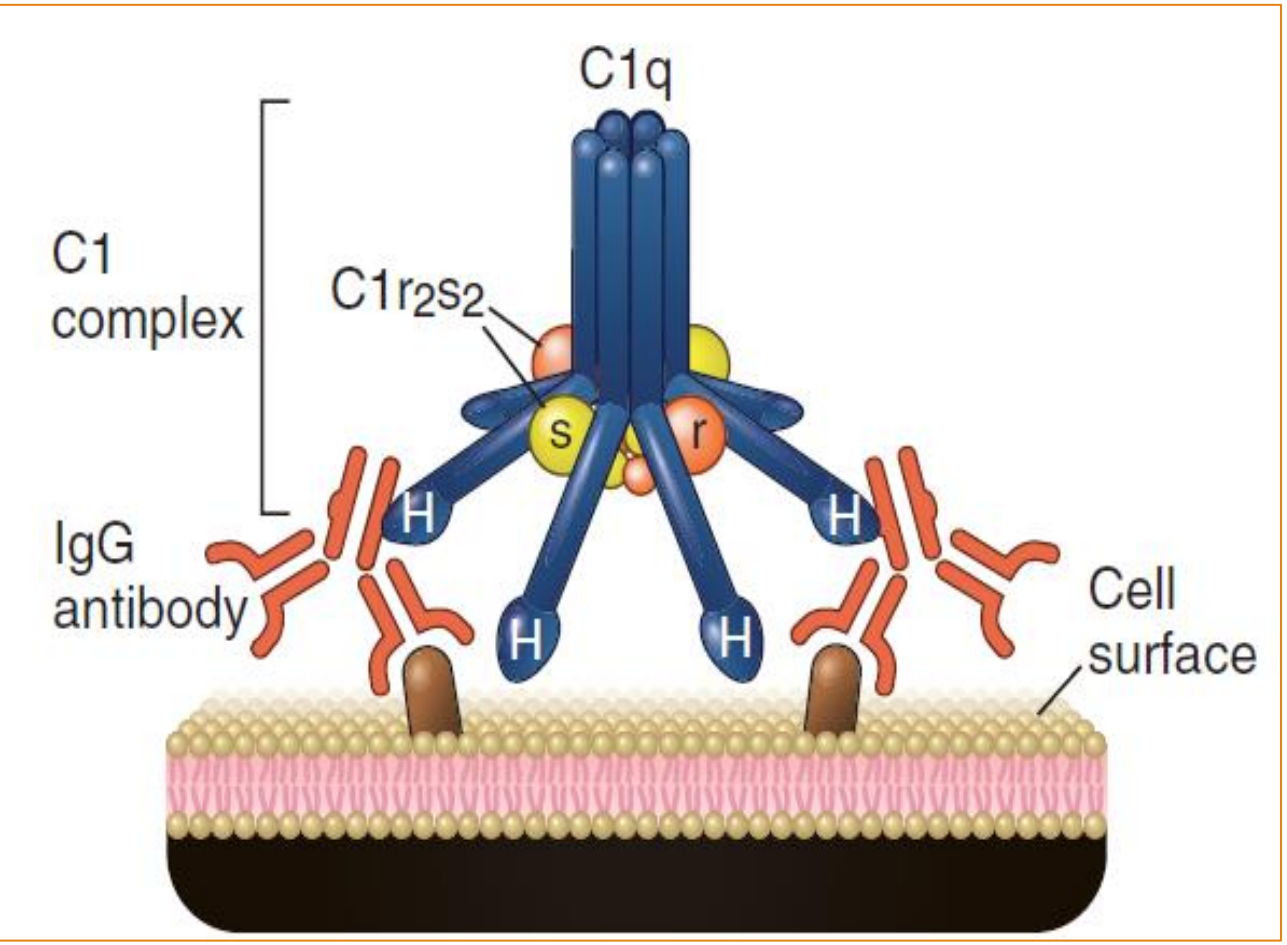
The classic CS activation path (discovered first)

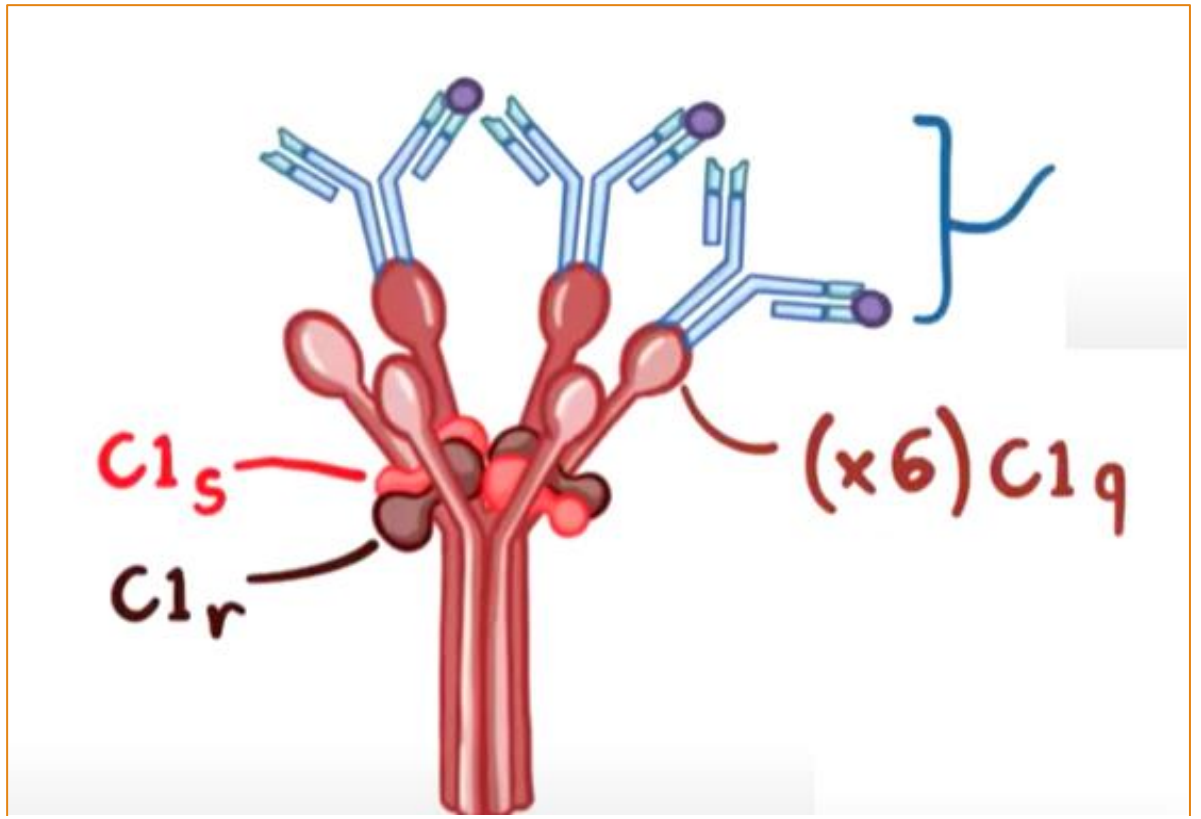
It is triggered by the soluble complex Ab+Ag or of Ab reacting with Ag on the cell surface (e.g. endotheliocyte).

All components participate, but is triggered by C1.

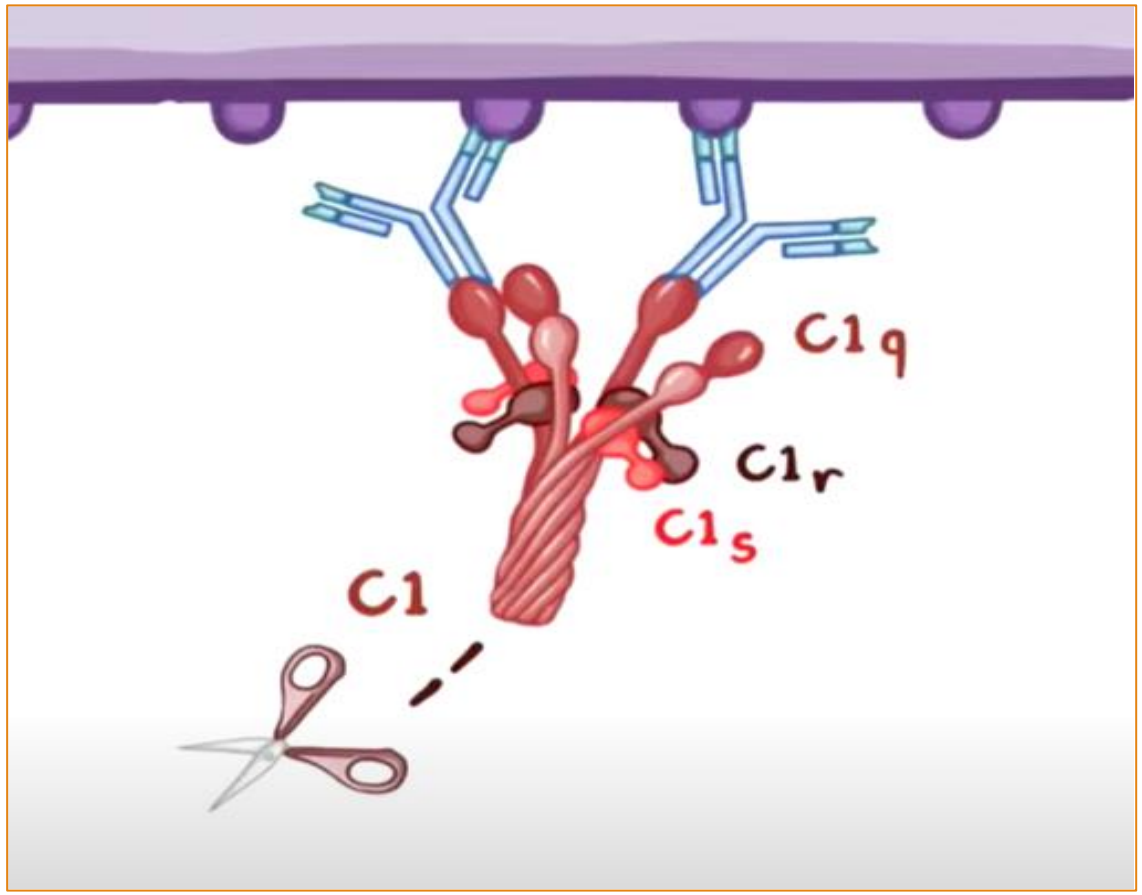


The classic activation starts with C1 consisting of C1q (binding site for Ab), C1s and C1r. C1r and C1s form a tetramer composed of two C1r and two C1s molecules. The ends of C1r and C1s contain the catalytic domains of these proteins. C1q has 6 subunits, which bind to the Fc fragment of the antibody (e.g. IgG or IgM)





Thus, each 1Cq connects an Ab, and per total one molecule of C1q will create 6 bonds with 6 Ab-Ag complexes

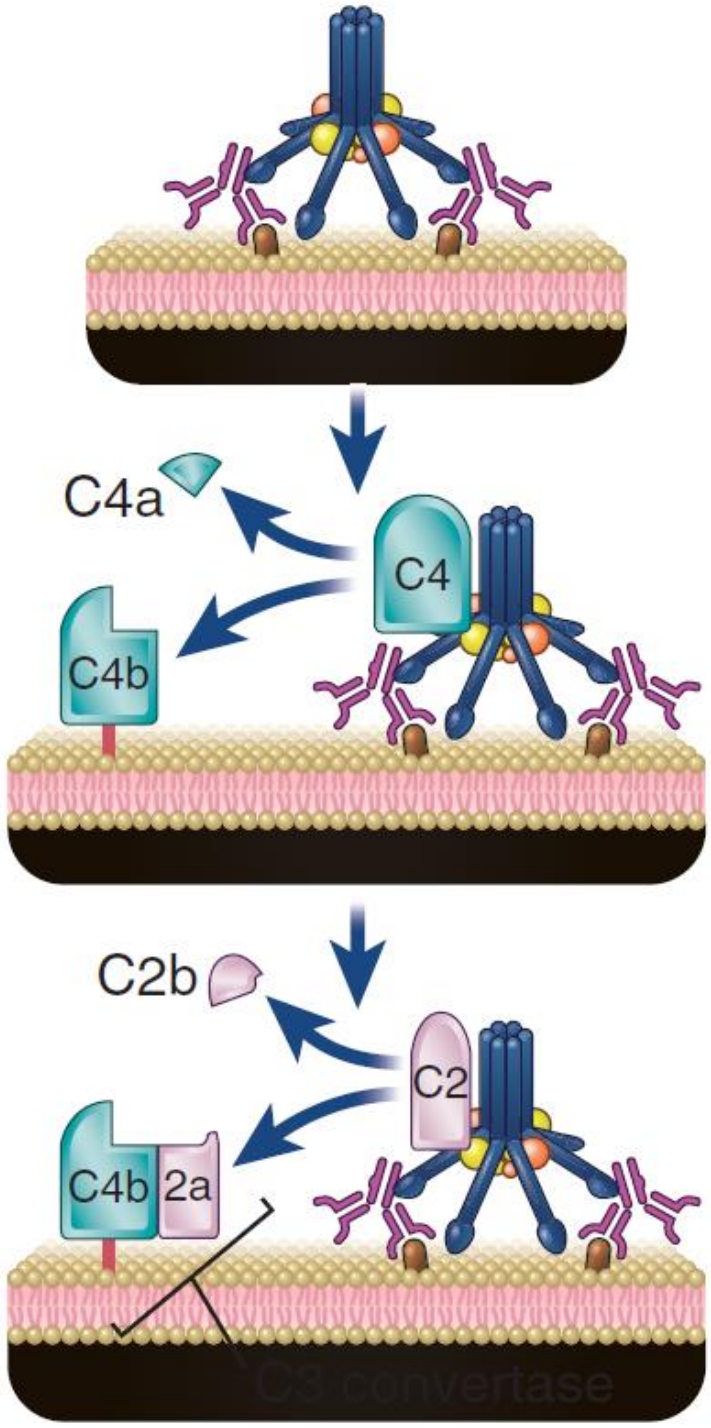


C1r and C1s are serine proteases, but they are hidden and cannot realize their enzymatic activity. When C1q binds 2 or more Ab, the C1 molecule wraps around the radial arms of the C1q complex in a manner that juxtaposes the catalytic domains of C1r and C1s, thereby exposing the enzyme-active sites of C1s and C1r.

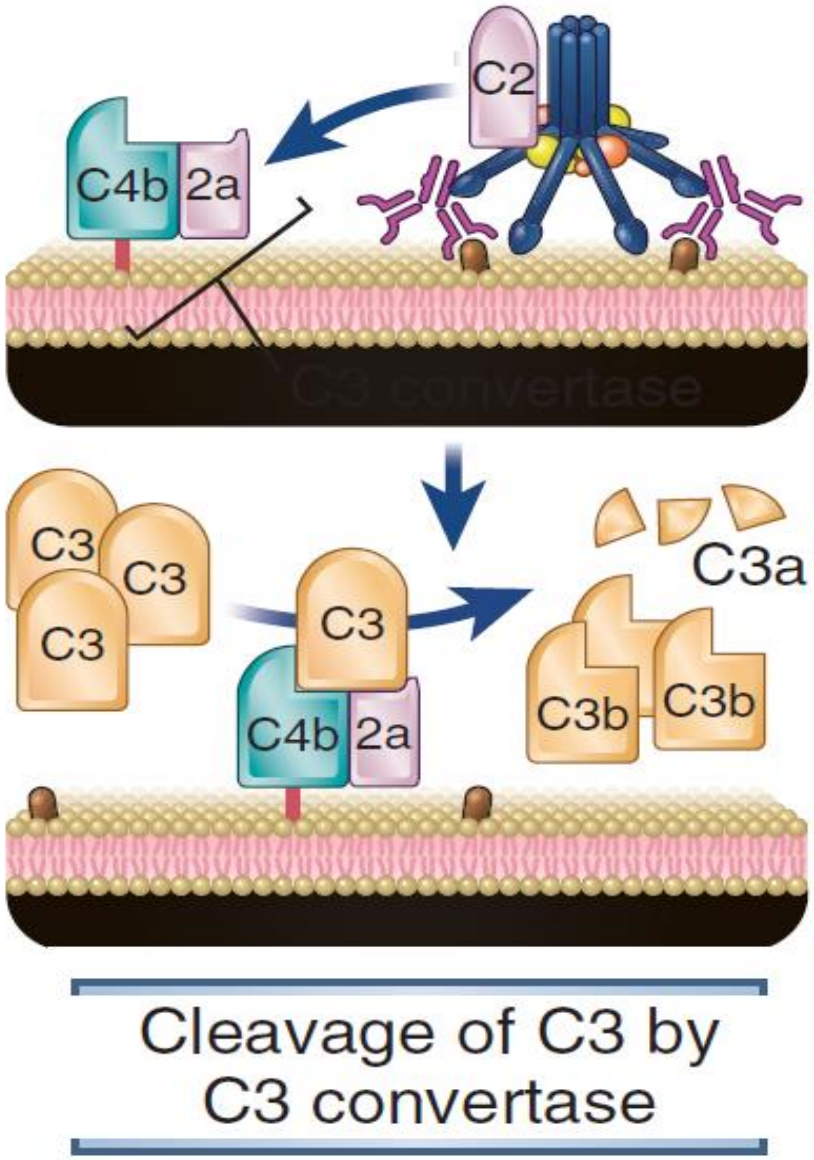
Binding of antibodies to multivalent antigen; binding of C1 to antibodies

Cleavage of C4 by $C1r_2S_2$ enzyme; covalent attachment of C4b to antigenic surface and to antibodies

Cleavage of C2; binding of C2a to C4b to form C4b2a complex (C3 convertase)



- **C1rs activates C4 by cleavage. C4=C4a+C4b.**
- **C4b subsequently binds the membrane of the pathogen.**
- **C1rs cleaves in the next stage C2. C2=C2a+C2b.**
- **C2a subsequently binds the pathogen membrane and together with C4b forms a proteolytic complex, defined as convertase 3.**



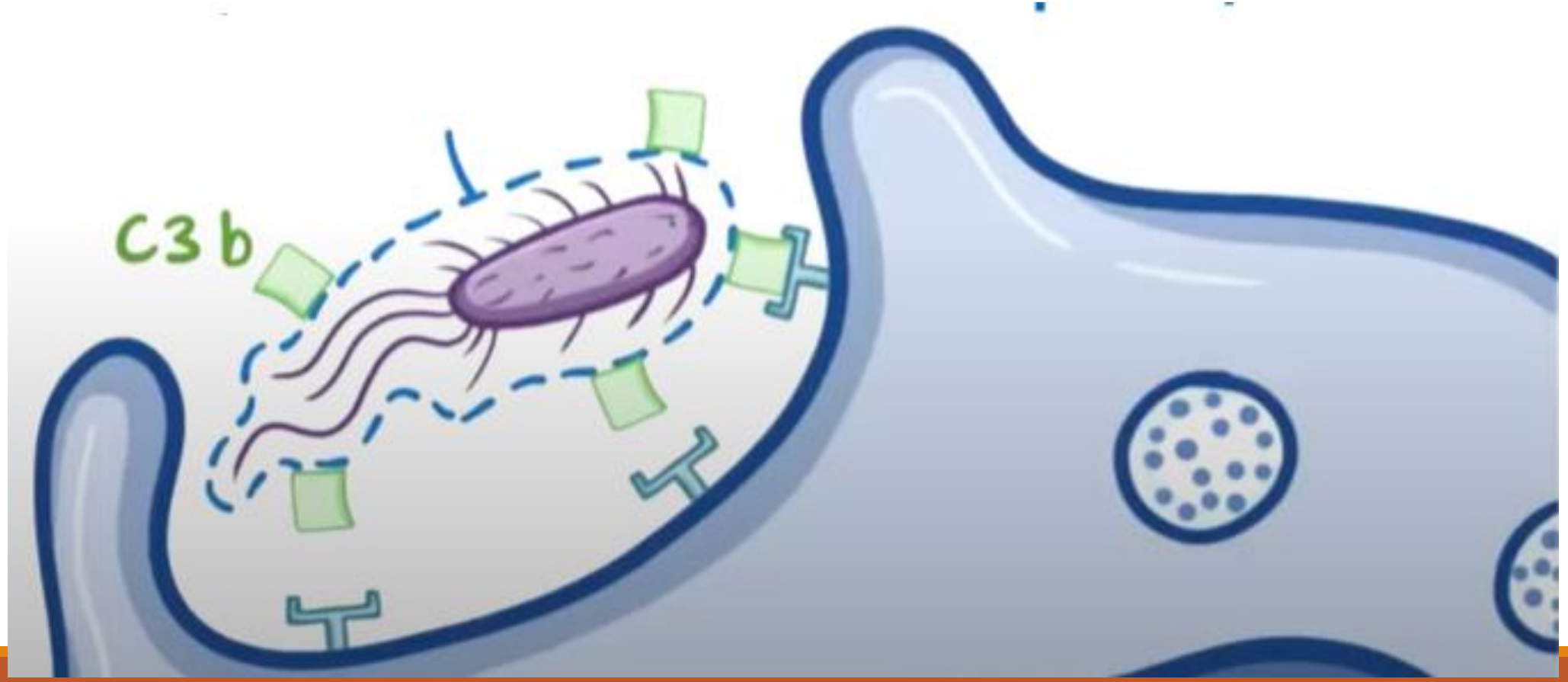
Cleavage of C3 by C3 convertase

One C1 = 10 convertase

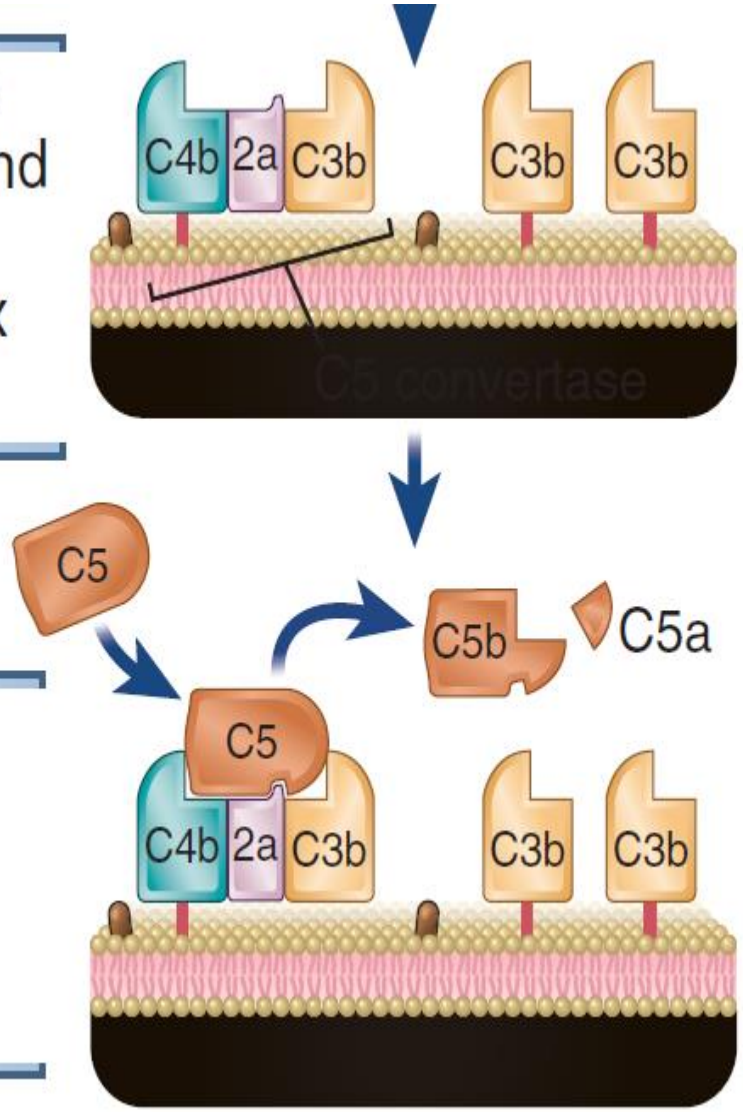
1000 C1 in sec

1. **Convertase 3 cleaves C3. C3 = C3a + C3b**
2. **One molecule of C1 forms 10 convertases 3.**
3. **One C3 convertase splits over 1000 C1 in 1 sec.**
4. **Thus, a large number of C3a (anaphylatoxins) and C3b (opsonins) are formed in a very short time.**

Opsonin C3b binds the pathogen on one side, and on the other hand binds to special receptors expressed on phagocytes (eg. macrophages, neutrophils, eosinophils), which results in facilitating the complex process of phagocytosis.



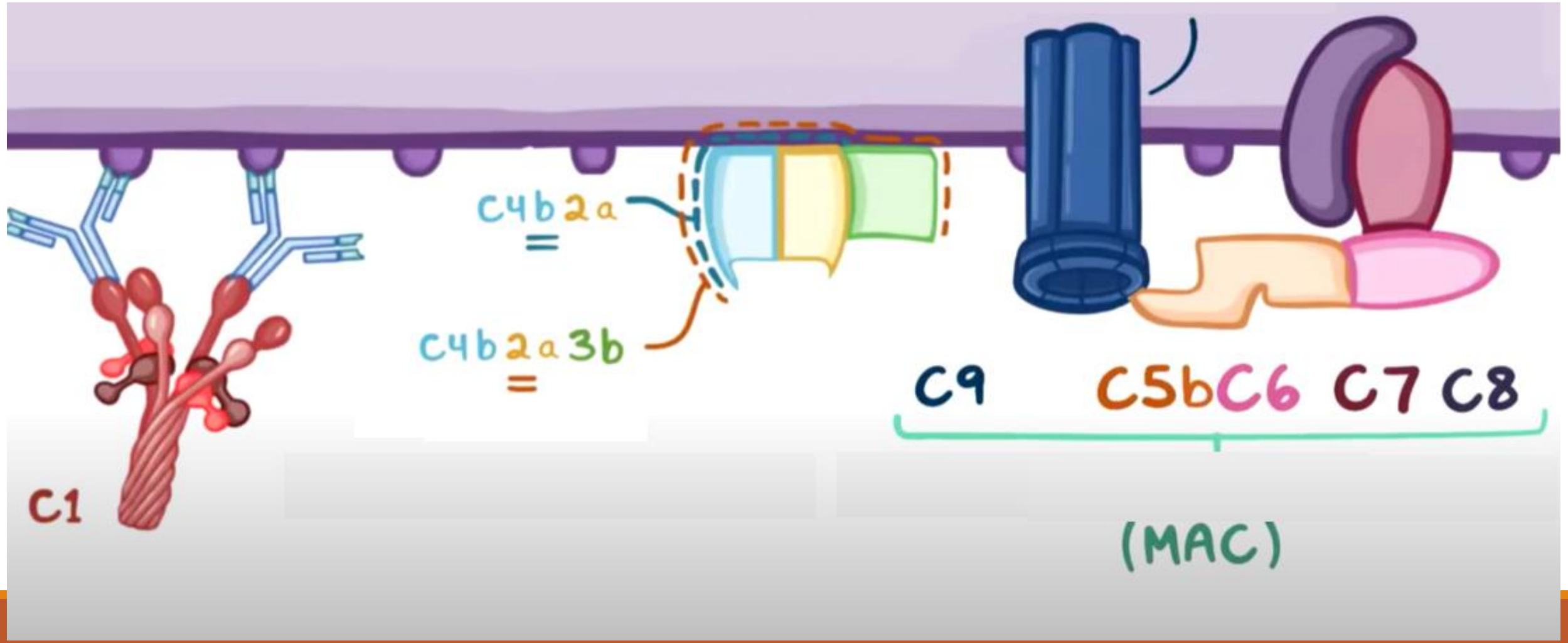
Binding of C3b to antigenic surface and to C4b2a to form C4b2a3b complex (C5 convertase)



Cleavage of C5; initiation of late steps of complement activation

- **C3b which not participate in opsonization will associate to convertase 3 (C4bC2a), forming convertase 5:**
- **C4bC2a + C3b = Convertase 5.**
- **Convertase 5 cleaves C5, forming: C5a (anaphylatoxin) and C5b (proteolytic enzyme).**

C5b is subsequently involved in the formation of a proteolytic complex C5bC6C7C8, which finally associates C9, forming the MAC complex – Membrane Attack Complex that will perforate the pathogen's membrane, that will lead to osmotic cell lysis.



Extrahepatic sources of C1q:

- Dendritic cells
- Monocytes
- Mast cells

Other functions of C1q:

- Stimulating dendritic cell maturation (DC)
- Increased expression of MHC-II molecules on DC surface
- Stimulation of migration into the lymphoid tissue of DC

Alternative complement activation pathway

The basic element of this pathway consists in the spontaneous cleavage of C3 without action of C3 convertase as in classical pathway (C4b + C2a), leading to the formation of C3a and C3b.

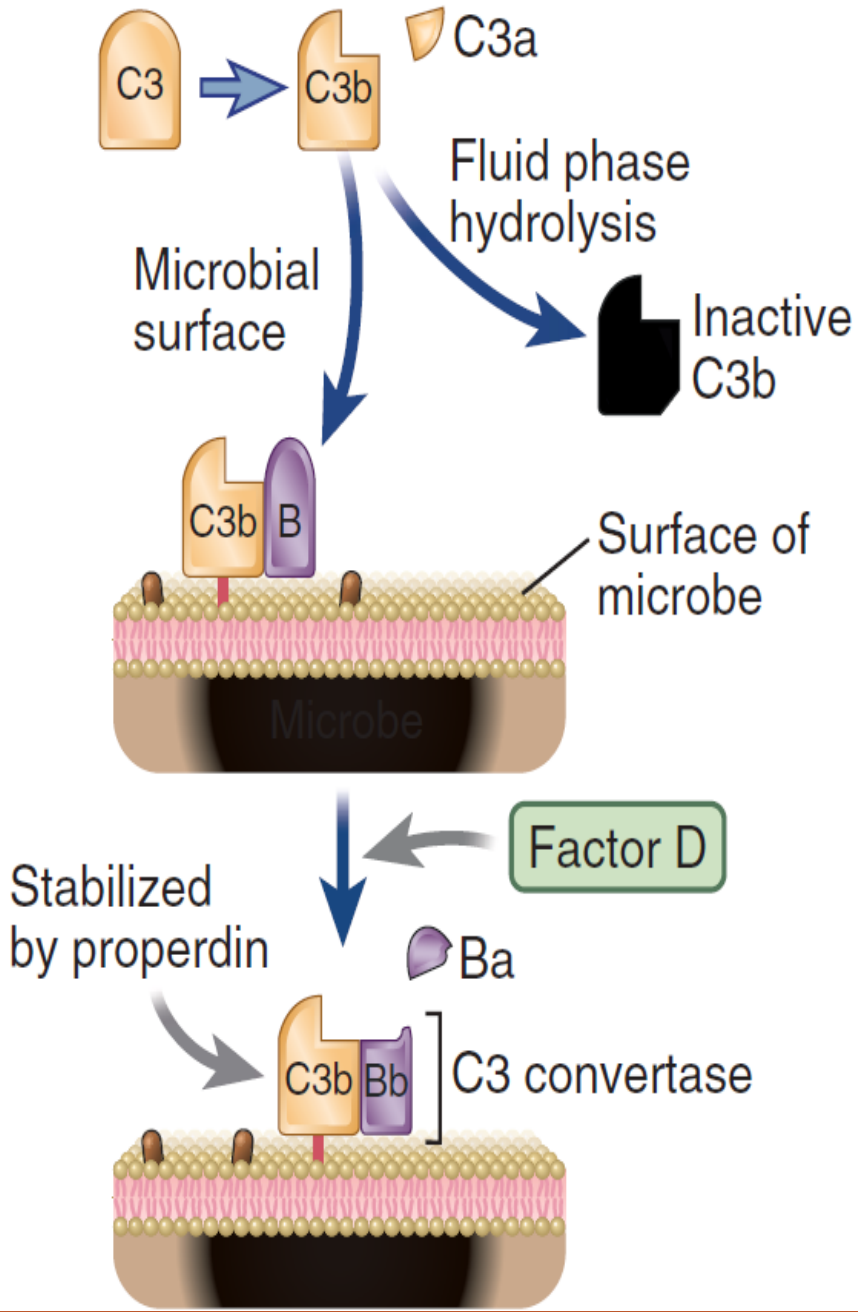
But this process is very slow and eventually small amounts of C3b are formed.

Normally, C3 in plasma is being continuously cleaved at a low rate (1% to 2% of the total plasma C3 per hour) to generate C3b.

Spontaneous cleavage of C3

C3b binds covalently to microbial surfaces, binds Factor B

Cleavage of Factor B by Factor D; stabilization by properdin

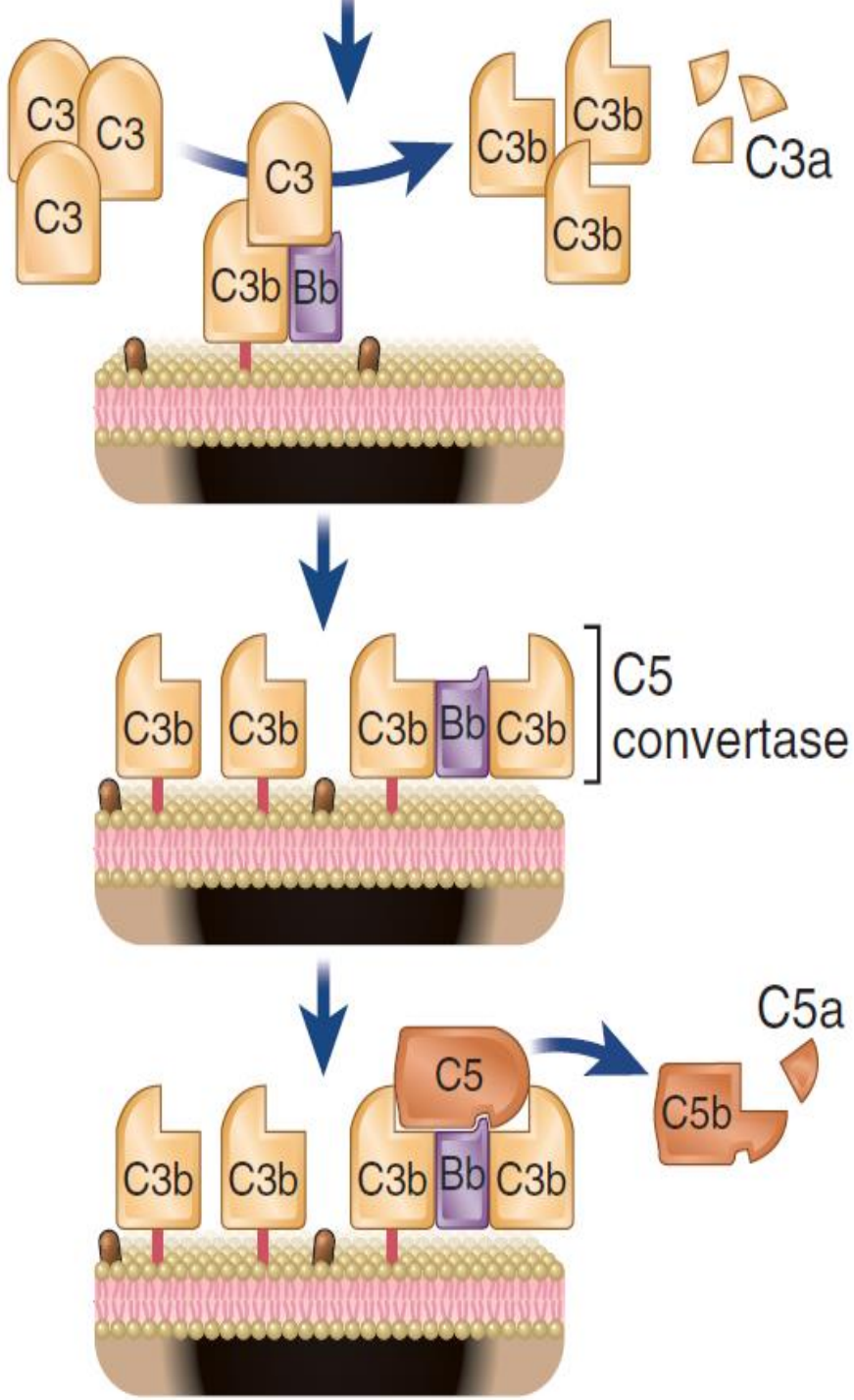


- **C3b binds to the membrane of the pathogen.**
- **Factor B in plasma binds to C3b.**
- **The D factor is always active and cleaves factor B, forming 2 factors: Bb and Ba.**
- **C3b+Bb = C3 convertase, which split C3 into C3a and C3b.**

Cleavage of additional C3 molecules by cell-associated C3 convertase

C3b covalently binds to cell surface, binds to C3bBb to form C5 convertase

Cleavage of C5; initiation of late steps of complement activation



- **C3b+Bb = C3 convertase, which split C3 into C3a and C3b.**
- **C3b+Bb+C3b= C5 convertase**
- **Subsequently, CS activation is similar to the classical path.**

Alternative complement activation pathway

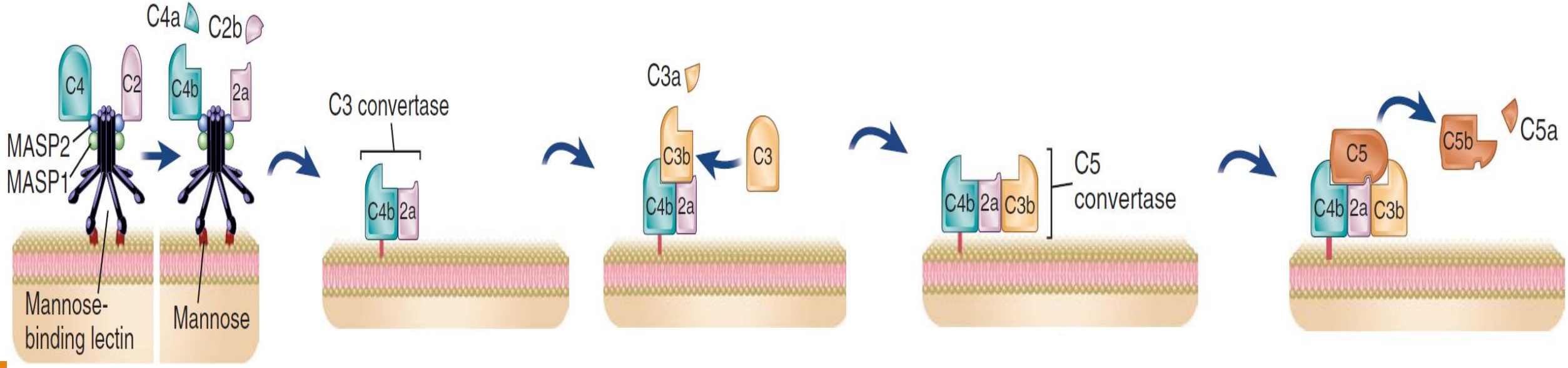
Properdin (factor P), a glycoprotein released by monocytes, and T lymphocytes encoded by the X chromosome activates the alternative CS activation pathway.

The mechanism consists in prolonging the life of convertase 3 and 5. The association to C3b increases the affinity of factor B to factor D.

At the same time, related to the Ab + Ag complex, properdin can trigger the alternative CS activation pathway.

Activation of complement by mannose-lectin complex

- **Lectin – is a protein complex similar to C1q.**
- **Mannose is a polysaccharide in the membrane of the pathogen, that activate lectin, which split C4 and C2**
- **C4b and C2a complex constitute convertase 3.**
- **From this point, CS activation is similar to the classical pathway.**



- **Lectin is formed in the liver, a process activated under the action of acute phase proteins (C-reactive protein, amyloid A, fibrinogen).**
- **Like C1q it has 6 heads and forms bonds with 2 proteases MASP-1 and MASP-2 (MBL-associated serine proteases) within the association of bacterial mannose.**
- **MSAP-1 and MASP-2 cleave C2 and C4 leading to the formation of convertase 3. Subsequently, complement activation proceeds similarly to the classical path.**
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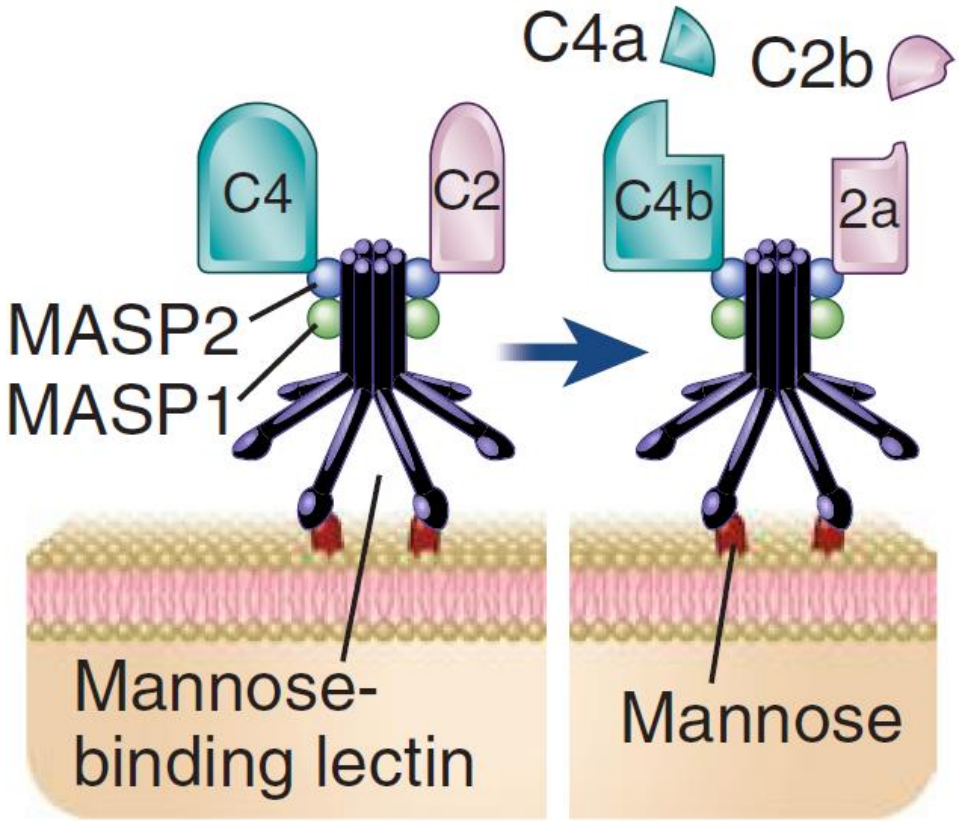
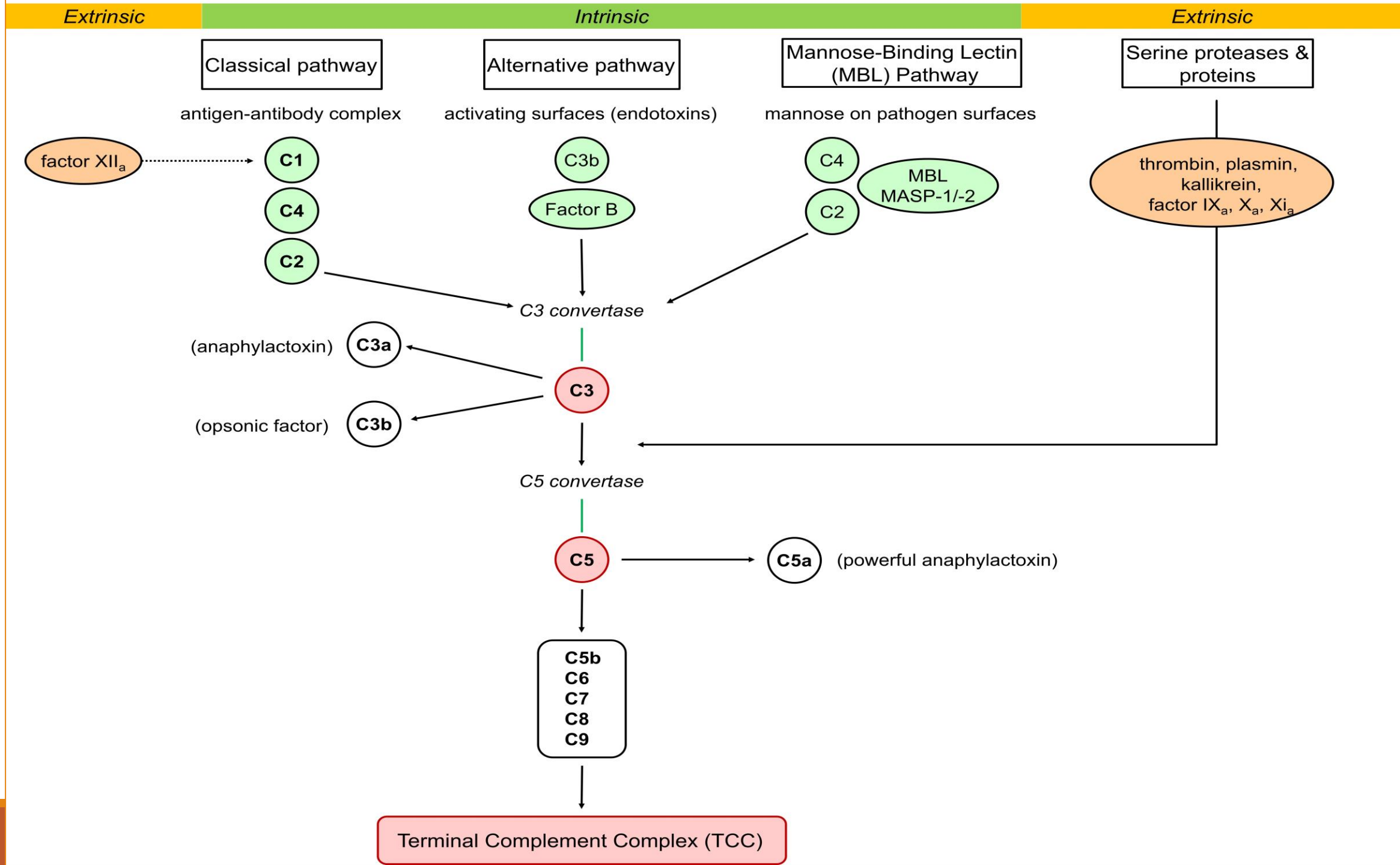
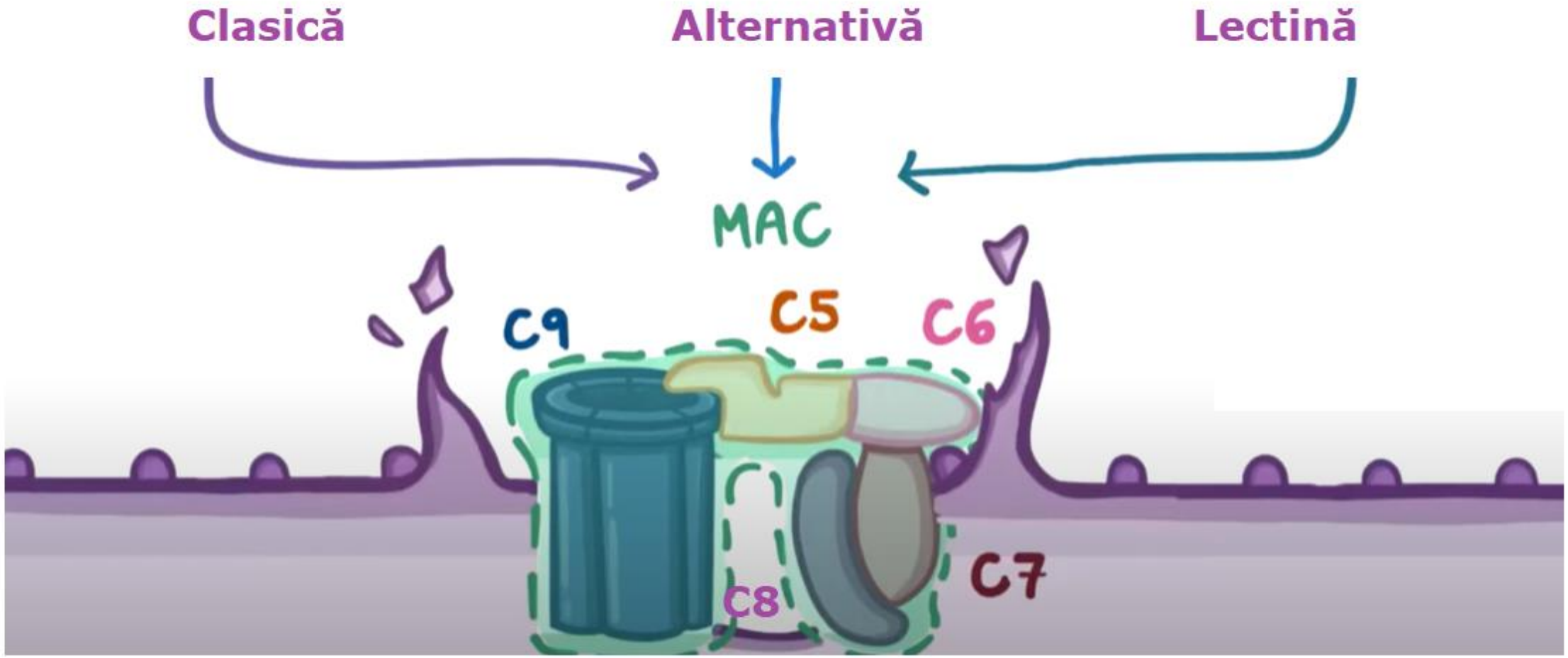



Extrinsic
Intrinsic
Extrinsic
Classical pathway
Alternative pathway
Mannose-Binding Lectin (MBL) Pathway
Serine proteases & proteins
antigen-antibody complex
activating surfaces (endotoxins)
mannose on pathogen surfaces
factor XII$_a$
C1
C4
C2
C3b
Factor B
C4
C2
MBL
MASP-1/-2
thrombin, plasmin, kallikrein, factor IX$_a$, X$_a$, Xi$_a$
C3 convertase
(anaphylactoxin)
C3a
C3
(opsonic factor)
C3b
C5 convertase
C5
C5a
(powerful anaphylactoxin)
C5b
C6
C7
C8
C9
Terminal Complement Complex (TCC)

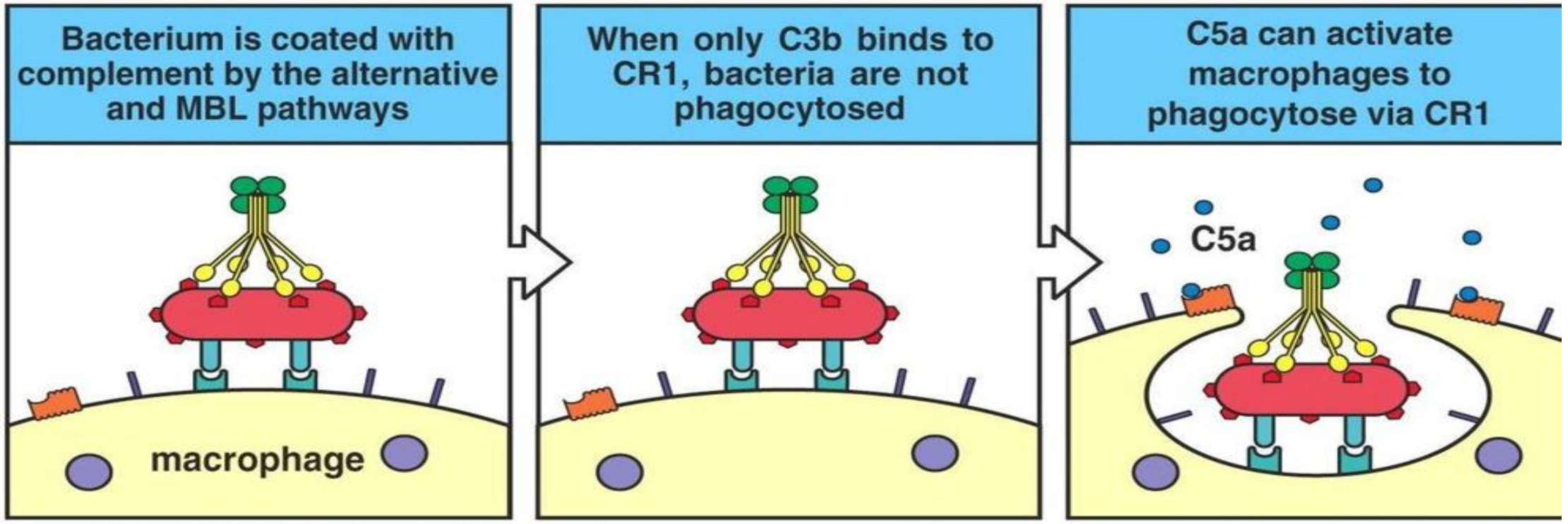
Thus, all 3 pathways of CS activation ultimately lead to a common phenomenon: the formation of the MAC complex – the biological entity of CS – the oldest component of the innate immune system – the destruction of gram negative bacteria.

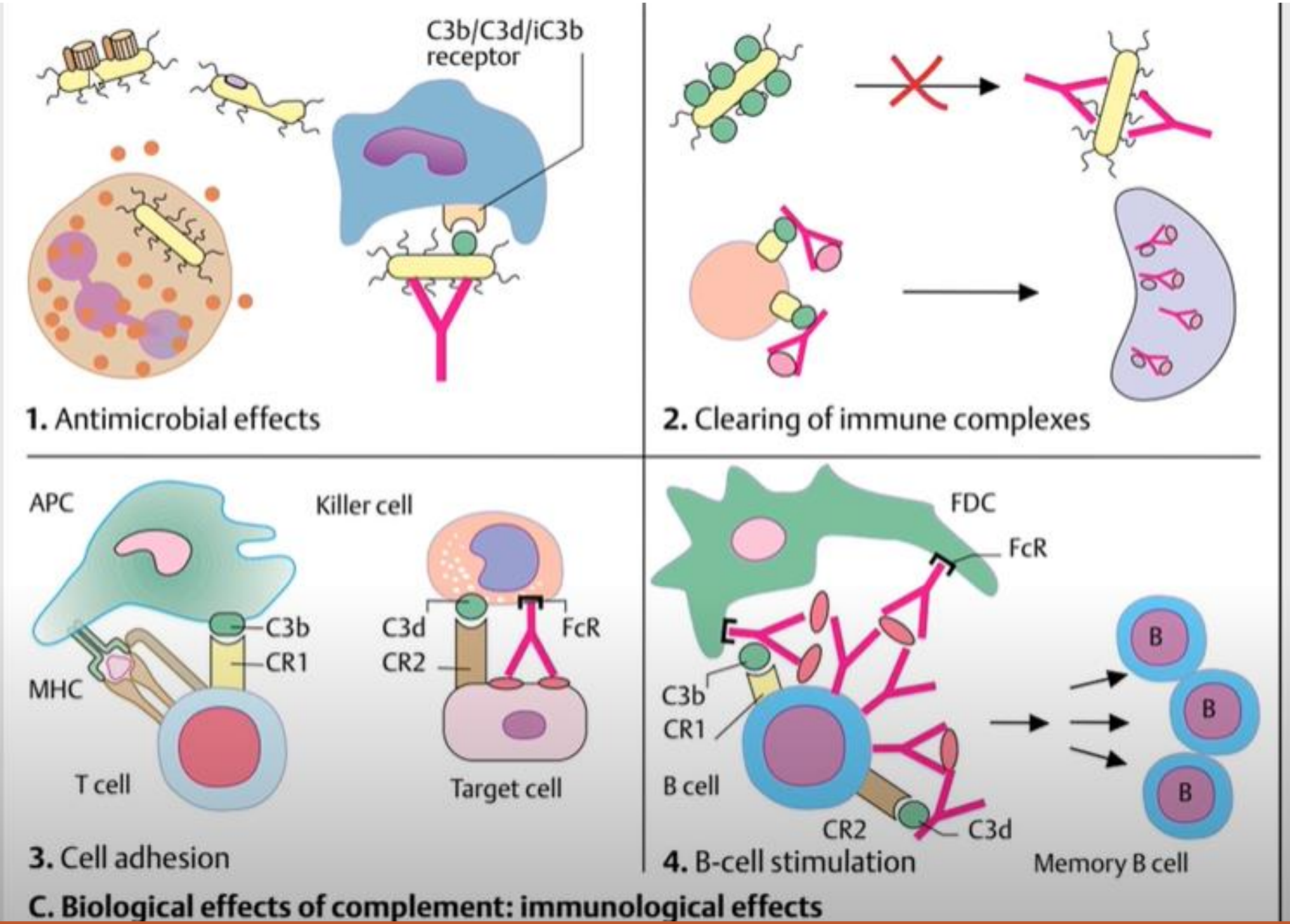


Another function of CS, other than MAC, is imposed by supporting the inflammatory response by C3a and C5a (anaphylatoxins).

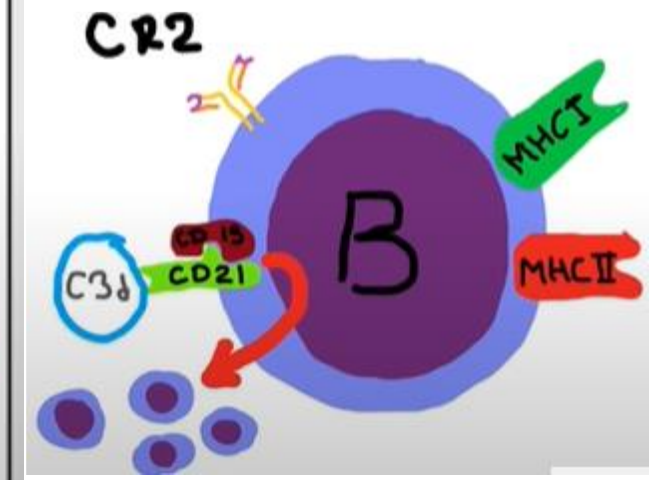
1. **Anaphylatoxins activate mast cells, inducing their degranulation with the release of a large number of allergy and inflammation mediators (e.g. histamine and heparin).**
2. **Anaphylatoxins also have chemo-attractant effects similar to chemokines, resulting in the recruitment of neutrophils, monocytes and eosinophils from the blood.**

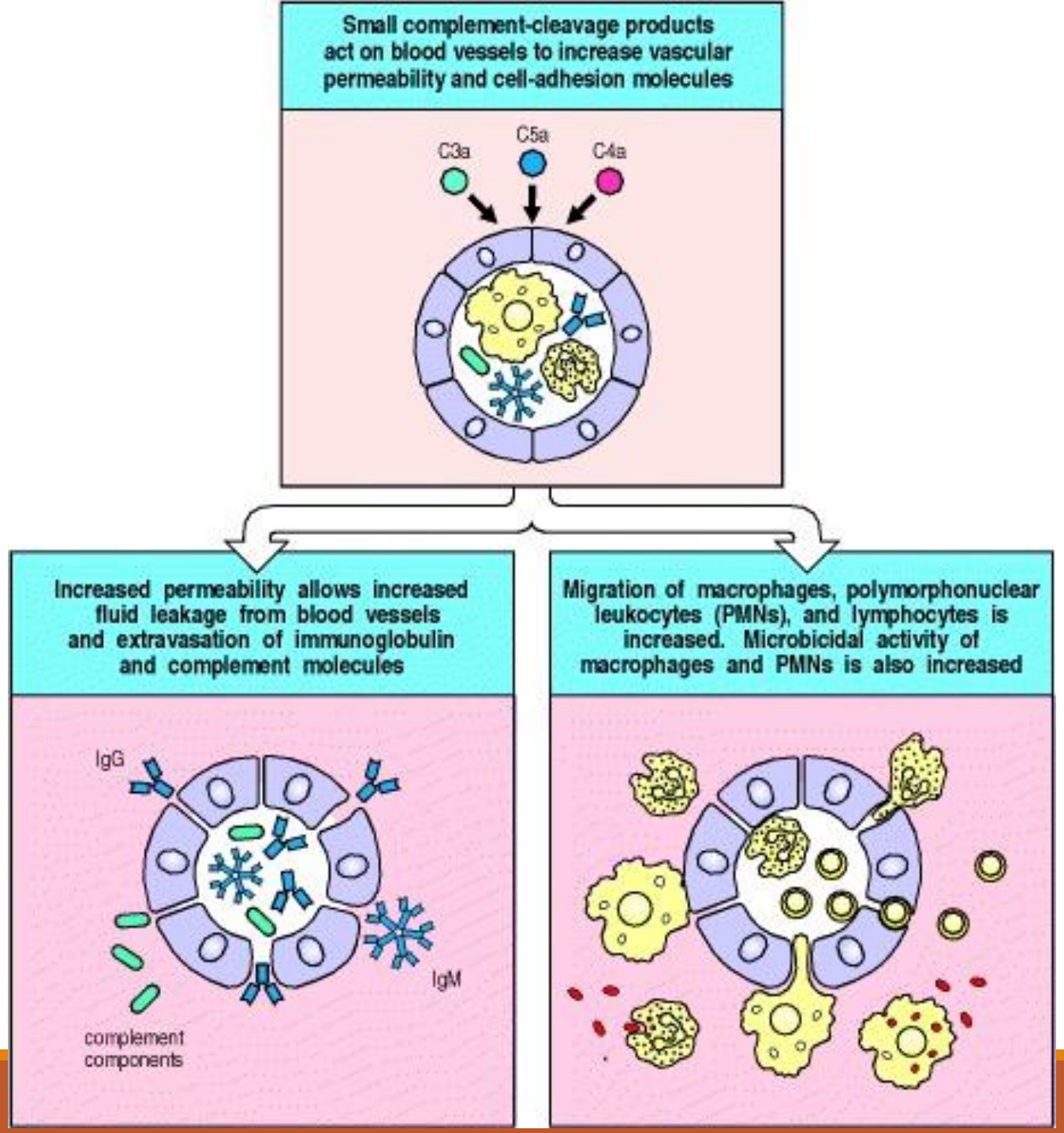
3. Anaphylatoxin C5a also activates the phagocytosis process of macrophages of the pathogen opsonized by C3b, formed by the alternating pathway or lectin-mannose and binding the CR1 receptor of the macrophage.





The C3d or CR2 or CD21 receptor is expressed on B lymphocytes.
Its activation leads to additional activation of LB like the action of IL-4 and their proliferation.





If the circulating Ab+Ag complex persists for a longer time, then there is a risk of inflammation triggering events:

1. **Anaphylatoxins increase the permeability of the vessel.**

2. **Subsequently, they have extravasation of complement components and Ig M and IgG.**

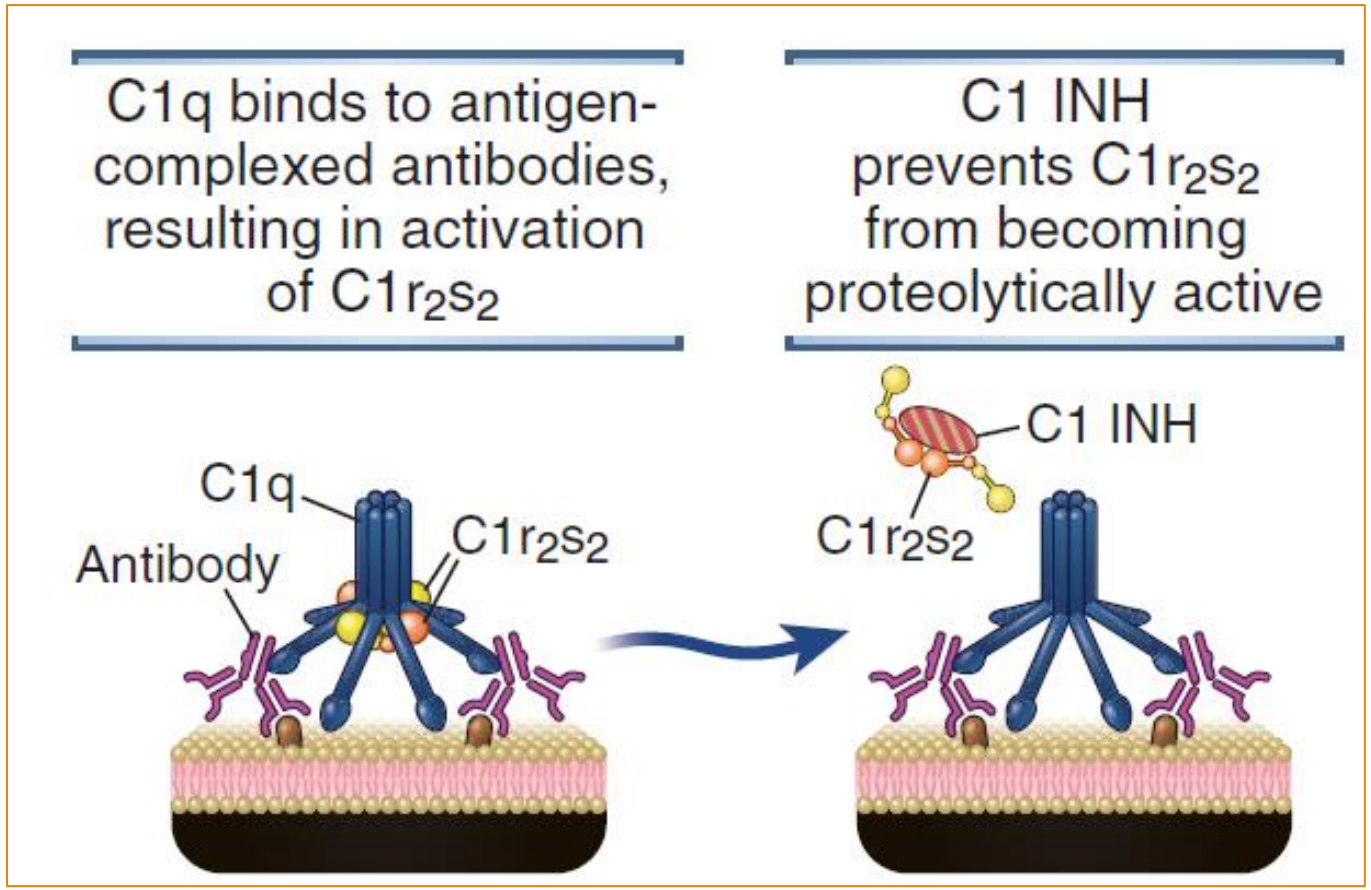
3. **By activating the chemoattraction systems, the migration of neutrophils and monocytes into the perivascular tissue and the aggravation of the inflammation process is accentuated.**

It is a scenario characteristic of the autoimmune process Systemic Lupus Erythematosus and Rheumatoid arthritis.

Systems of own cell defense

- Spontaneous formation of C3a and C3b complexes is dangerous in order to form convertase 3 and 5 and in turn activation of CS with its effects.
- Thus, it is necessary to control the activity of the compliment activation.
- This control is provided by various plasma proteins or expressed on the surface of cells.

Systems of own cell defense



System 1:

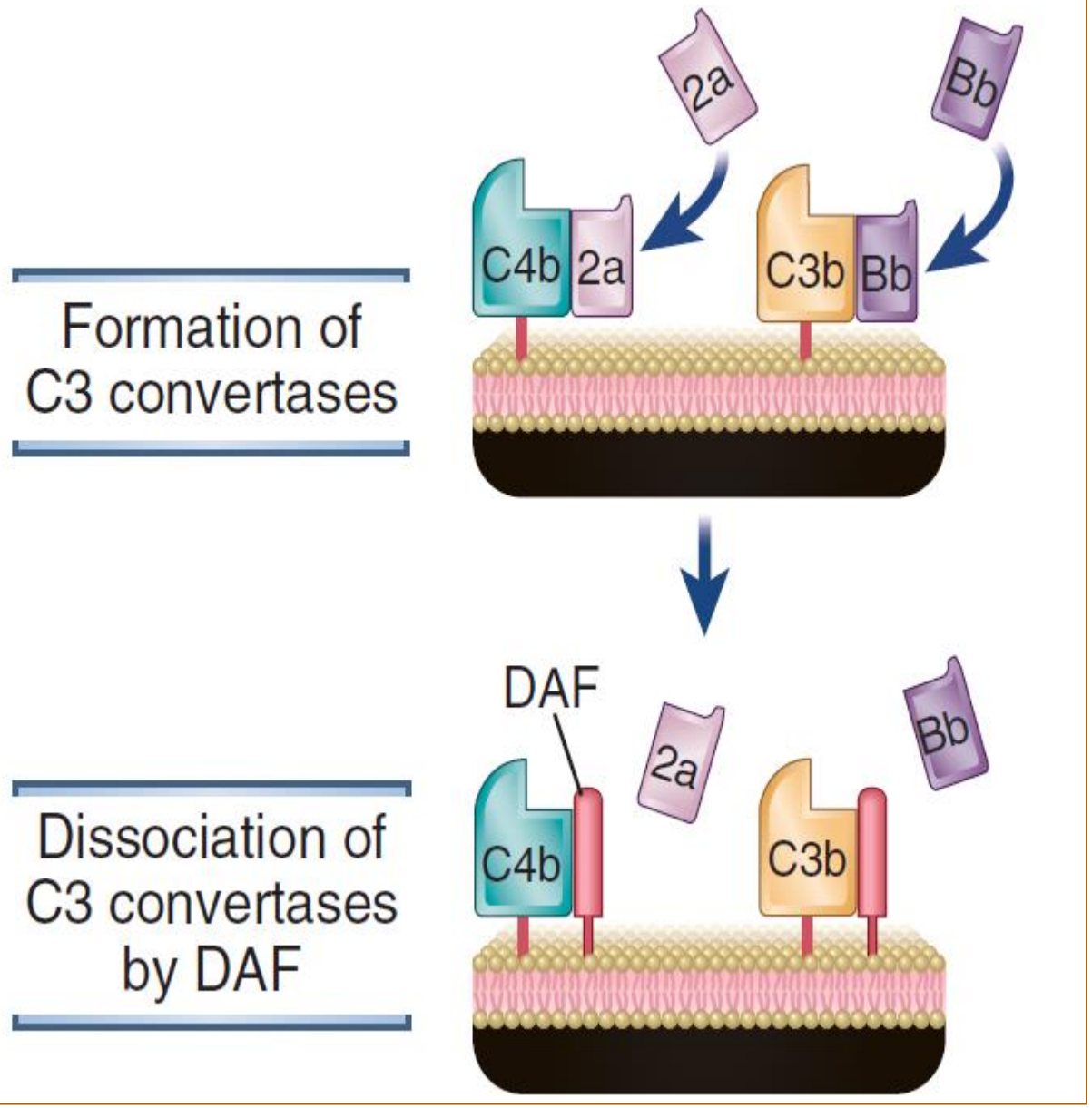
Plasma C1-inhibitor (C1 INH) is a serine protease inhibitor (serpin) that mimics the normal substrates of C1r and C1s, thus inhibiting activation of CS by classical pathway.

Systems of own cell defense

System 2:

- In addition to inhibitory factor C1 (plasma protease) there is also factor H, a plasma protein that acts as a cofactor for Factor I, cleaves C3b, forming factor C3Bi, which cannot make together with Bb alternative convertase 3.
- Thus, Factor H is a regulator of the alternative but not the classical pathway.
- However, C3Bi is also a powerful opsonin.

Systems of own cell defense



System 3:

- **CD55 factor or decay acceleration factor 3 (DAF) is a protein on the surface of cells that displaces C2a from C4b and Bb from C3b (dissociation of C3 convertases, thus inhibits the classical and alternative pathway of CS activation.**
- **CD55 factor is not expressed on the pathogen membrane, and alternative convertase 3 remains active long enough.**

Systems of own cell defense

System 4:

All nucleated cells express the CD46 receptor.
CD46 binds C3b and C4b and serves as a cofactor for Factor I - plasma serine protease, which facilitates their degradation.

Factor I–mediated cleavage of C3b (and 4b) generates the fragments called iC3b, C3d, and C3dg, which do not participate in complement activation but are recognized by receptors on phagocytes and B lymphocytes.

Systems of own cell defense

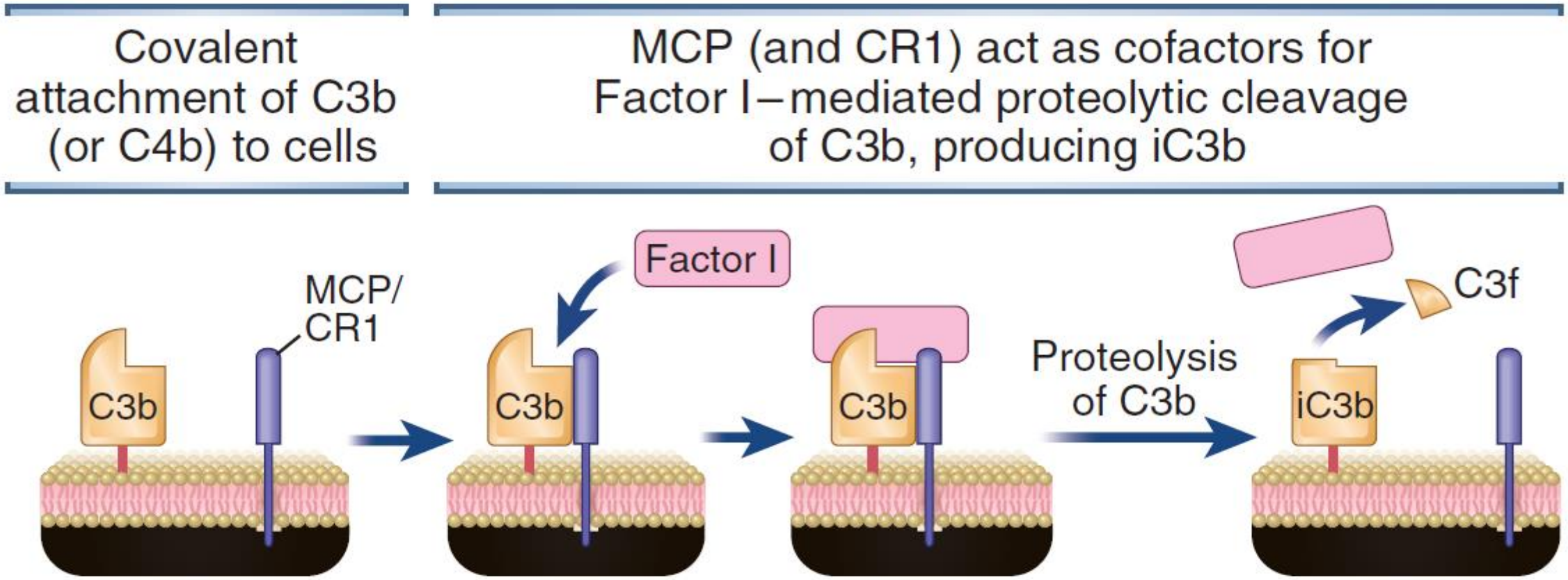
System 5:

To avoid MAC attack on the membrane of its own cells there are factors that have a protective effect in this regard:

1. **CD59 (expressed on leukocytes, erythrocytes, endotheliocytes, epitheliocyte, neurons, astrocytes) – inhibits C9 assembly (polymerization) in the C5b-C8 complex.**

2. **MCP (membrane cofactor protein) that binds C3b and C4b, thus leading to blocking the formation of convertase 3 and convertase 5, which results in diminishing the process of formation of the MAC complex.**

MCP, CR1 (complement receptor-1), and DAF are produced by mammalian cells but not by microbes. Therefore, these regulators of complement selectively inhibit complement activation on host cells and allow complement activation to proceed on microbes.



Complement and adaptive immune System

1. The receptor to C3a (C3aR – receptor) coupled with G protein is expressed on T and B lymphocytes. Its activation modulates their activity. In inactivated LT C3aR is expressed intracytosolic. In activated LT the receptor is translocated on the membrane, its activation increases the cytotoxicity of CD8.
2. The receptor to C5a expressed on LT and monocytes. Its activation increases the release of pro-inflammatory cytokines and chemokines.

Complement and adaptive immune system

3. Dendritic cells and macrophages can form the extrahepatic C1q component. By activating CD21 and CD35 receptors, C1q components activate the expression of CMH II molecules and facilitate the maturation and migration of dendritic cells.

4. Activation of CD21 (receptor to C3b) facilitates activation of B lymphocytes in the presence of a reduced number of antigens (2-4 times). Activation of CD21 also increases the efficiency of the signaling system between CD40-R on B lymphocytes and CD40-L (CD154) on the surface of T lymphocytes.

Pathological patterns of the complement system

1. Insufficiency C1q, C1r, C4, C2, and C3 leading to disturbance of removal of circulating complex Ab+Ag. This mechanism is characteristic of the pathogenesis of systemic lupus erythematosus, as a form of type III hypersensitivity.
2. Deficiencies in the terminal complement components, including C5, C6, C7, C8, and C9, lead to MAC dysfunction, which results in diminished defenses against gram-negative bacteria (e.g. Neisseria).

C9 insufficiency has no pathological consequences.

Pathological patterns of the complement system

Deficiencies in complement regulatory proteins, as:

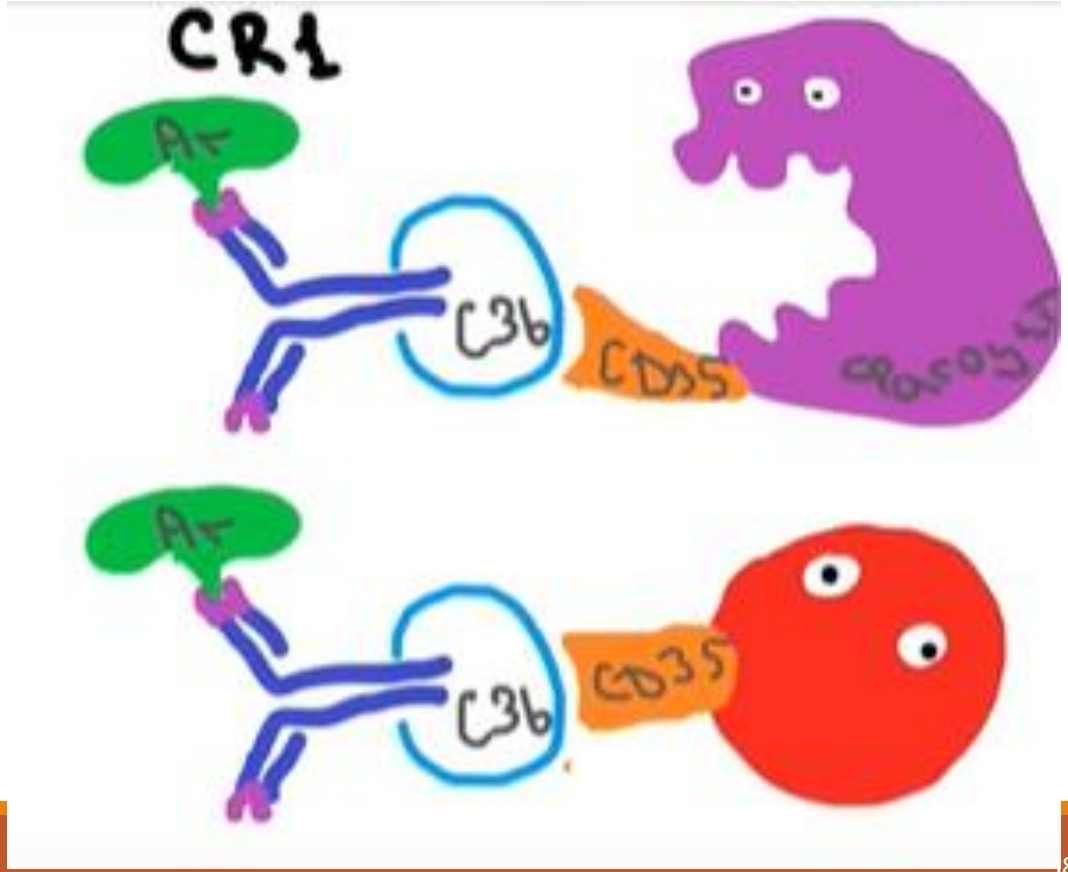
3. **In patients with Factor I deficiency, plasma C3 is depleted as a result of the unregulated formation of fluid-phase C3 convertase. The clinical consequence is increased infections with pyogenic bacteria.**

4. **Hereditary autosomal dominant deficiency of C1 INH lead to disease called hereditary angioedema which becomes covalently attached to the complement proteins as C1r2-C1s2 and, as a result, the dissociates them from C1q, thus stopping activation by the classical pathway. It is manifested by angioedema, which clinically presents itself with severe edema in different localities of the body (airways, abdominal cavity, face, arms). The mediators of edema formation in patients with hereditary angioedema include a proteolytic fragment of C2, called C2 kinin, and bradykinin. Excess bradykinin is considered the main pathogenetic mechanism of edema.**

Pathological patterns of the complement system

5. CR1 receptor (CD35) deficiency to C3b expressed on macrophages and erythrocytes. It results in slow and inefficient removal of the Ab+Ag complex:

- **by macrophages**
  - **including macrophages that phagocyte erythrocytes that through CD35 associate Ac + Ag + C3b.**



Systemic lupus erythematosus is the pathology evolving on this pathological pattern of SC.

Pathological patterns of the complement system

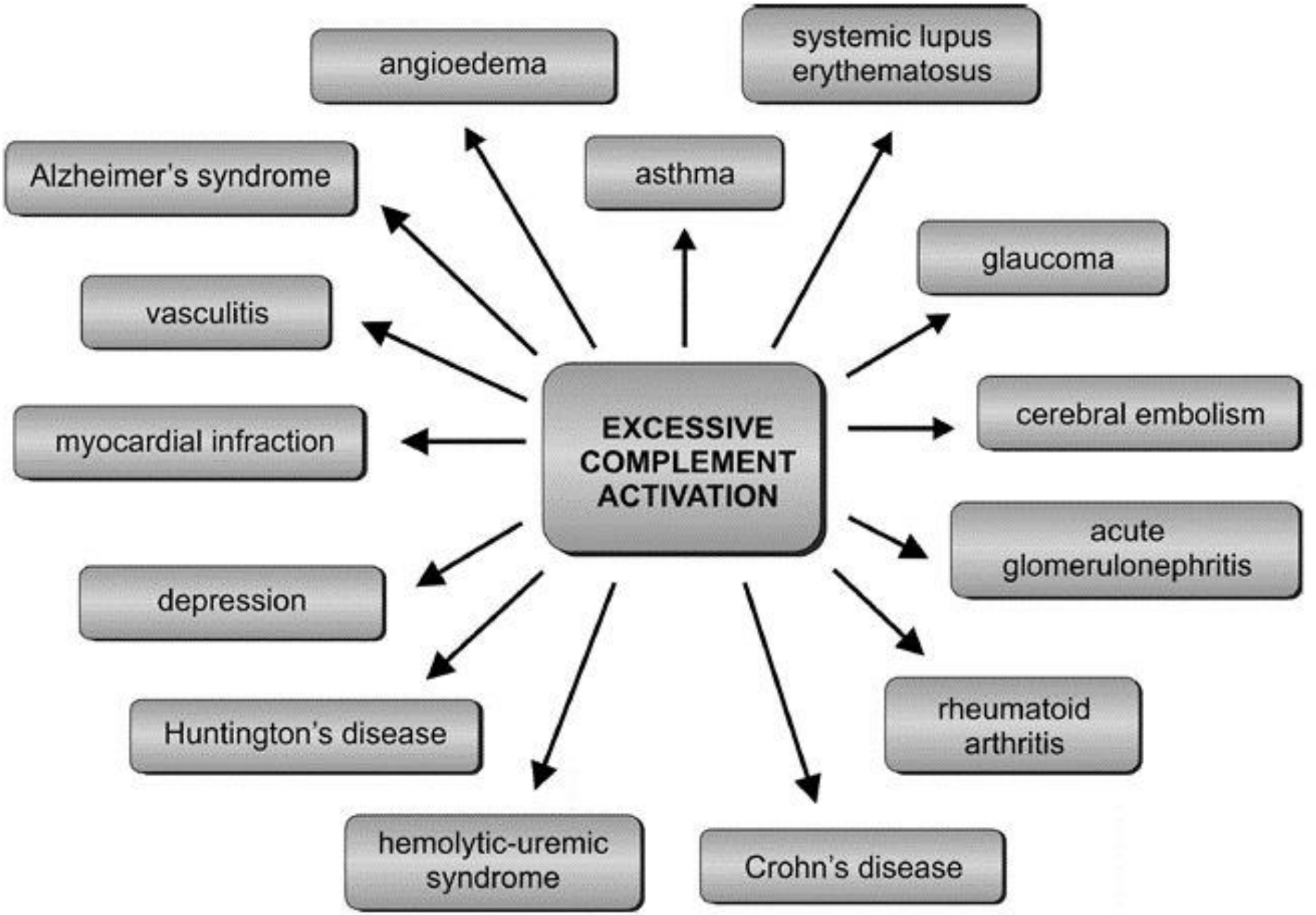
6. Properdin deficiency in X-linked mutations. Diminishing this glycoprotein in plasma reduces the activity of the alternative complement activation pathway. It results in activation of meningococcal impact and increases susceptibility to Neisseria infection.

7. Lectin deficiency. It affects antibacterial defenses in children, to whom the adaptive immune response is not yet perfect. This phenomenon confirms the role of the innate immune system in fighting pediatric infections.

Pathological patterns of the complement system

8. C3 deficiency. It leads to an impaired defense against gram-negative bacteria (e.g. Neisseria, Hemophilus influenza) and gram-positive (e.g. Streptococcus pneumoniae).

9. Deficiency of factors B, D, H and P in the alternative activation system increases the vulnerability and severity of Neisseria, Proteus, Pseudomonas infections.


EXCESSIVE COMPLEMENT ACTIVATION
angioedema
systemic lupus erythematosus
Alzheimer's syndrome
asthma
glaucoma
vasculitis
myocardial infraction
cerebral embolism
acute glomerulonephritis
depression
Huntington's disease
rheumatoid arthritis
hemolytic-uremic syndrome
Crohn's disease

CS and Alzheimer's disease

C1q and C3 bind amyloid beta that is not yet detached from the neuron in the initial stage of Alzheimer's disease.
As a result, the neuron becomes a target of attack by CS, resulting in lysis and phagocytosis of nerve cells.

In cerebral ischemia–reperfusion process, activation of the inflammatory response is supported by CS.


THANK YOU!