The Complement System

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INTRODUCTION

The Nobel Prize in Physiology or Medicine 1919
Jules Bordet

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The Nobel Prize in Physiology or Medicine 1919 was awarded to Jules Bordet *"for his discoveries relating to immunity"*.

- **The heat-labile (its lytic activity destroyed when heated at 56℃ for 30 mins) component of normal plasma**
- **Complement the lysing of bacteria by antibody**

Jules Bordet,1898

Immune serum

+

Bacteria

↓

lysis

Non-immune serum

+

Bacteria

↓

No lysis

Bacteria

↓

lysis

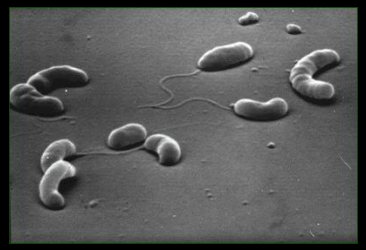
Heat-inactivated serum

+

Bacteria

↓

No lysis



tube	Normal serum	Immune serum	56℃,30' heated-Immune serum	comma bacillus	result	interpretation
1	+			+	No lysis	*Normal serum can not lyse comma bacillus.*
2		+		+	Lysis	*Immune serum can lyse comma bacillus.*
3			+	+	No lysis	*The component in the immune serum that lyse comma bacillus could be inactivated by heat (56℃,30').*
4	+		+	+	lysis	*Something in normal serum could complement the lysing activity of immune serum that had been destroyed by heat.*

experiment by Bordet, 1898

Nature and functions 1

- **Complement – A group of （approximately 30） plasma and cell membrane proteins involved in the effector role of the host defense process.**

- **Synthesized in the liver and by cells involved in the inflammatory response.**

Nature and functions 2

- Most proteins of the C are present in the circulation in an inactive form (**zymogen**)
- Undergo sequential activation **(Cascade)** to ultimately cause their biological effects.
- With **constant concentration** in serum
- **Three pathways** of complement activation
  - Classical pathway
  - Alternative pathway
  - Lectin pathway

Nomenclature 1

- **Classical pathway and terminal components: C1(qrs), C2, C3, C4, C5, C6, C7, C8, C9**
- **Alternative pathway: factors B, D, P (properdin)**
- **Controls proteins: factors H and I, C1 inhibitor (C1-INH), complement receptor 1 (CR1)...**

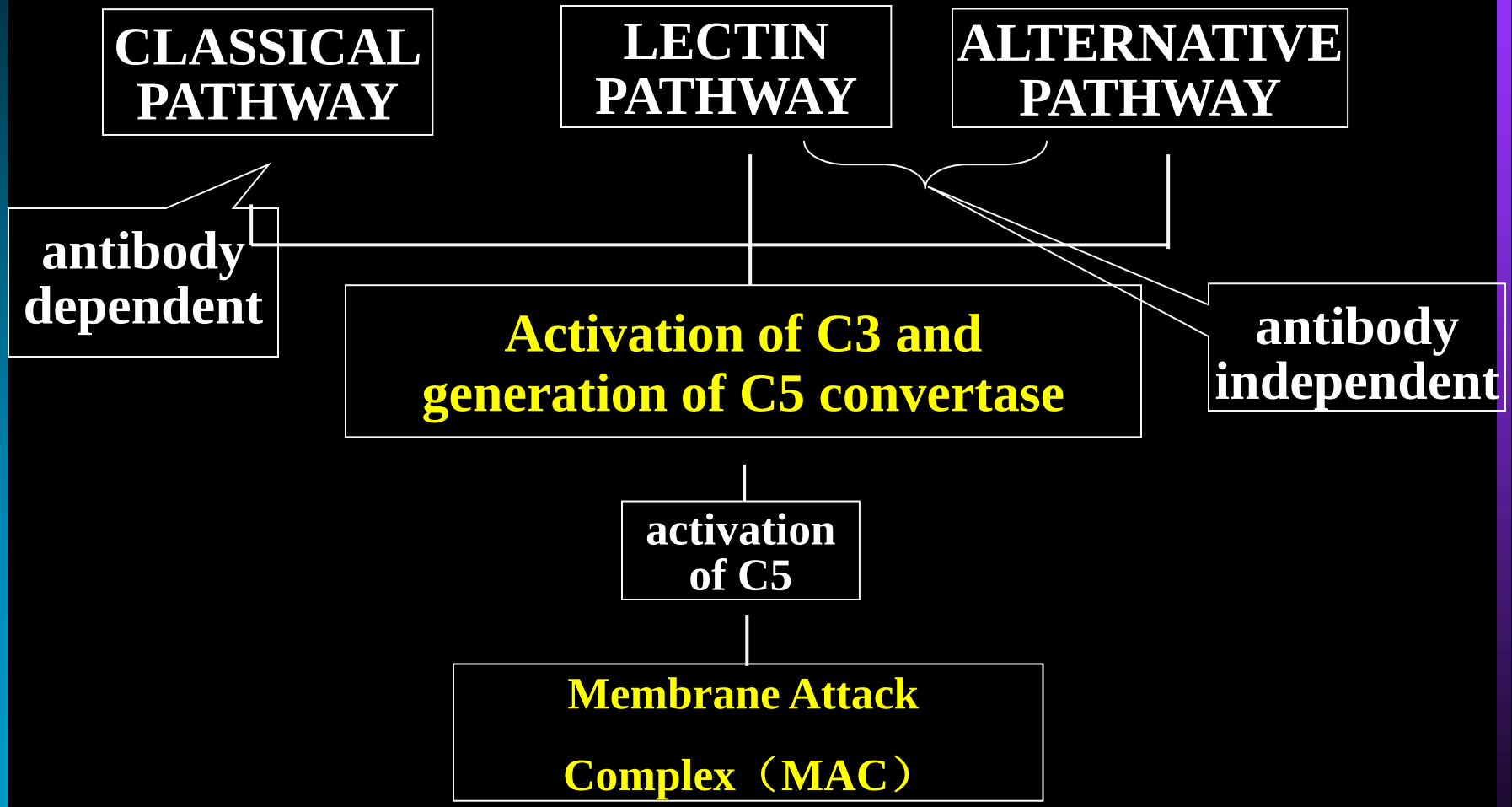
Nomenclature 2

- **When enzymatically cleaved, the larger moiety, binds to the activation complex or membrane and the smaller peptide is released in the microenvironment**

- **Letter "b" is usually added to the larger, membrane-binding, peptide and "a" to the smaller peptide (eg., C3b/C3a, C4b/C4a, C5b/C5a), *EXCEPT* C2 (the larger, membrane-binding moiety is C2a; the smaller one is C2b)**

- **Activated component are usually over-lined: *eg.* $\overline{\text{C1qrs}}$,$\overline{\text{C4b2a}}$**

Pathways of complement activation



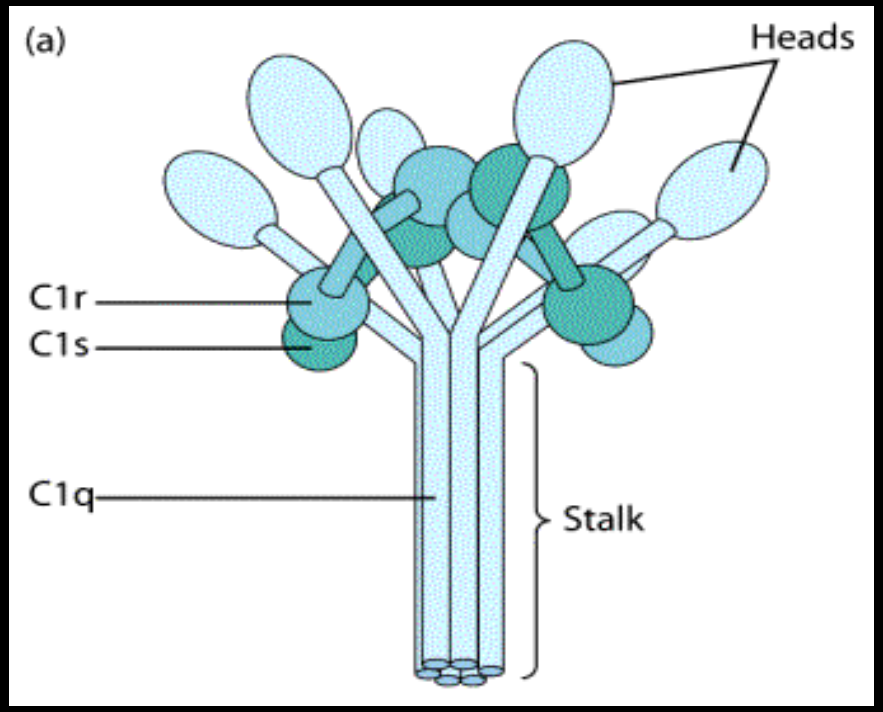
Pathways of complement activation

- **Classical pathway**: initiated by Ag-Ab complex
- **MBL (mannan-binding lectin) pathway**: triggered by MBL; microorganism complex mannan: repeating sugar patterns usually on the carbohydrate capsule of bacteria
- **Alternative pathway**: some foreign particles
- All three pathways lead to the formation of complex enzymes capable of binding and cleaving a key component,**C3**.
- Thereafter, the pathways proceed identical to form a membrane attack complex, which ultimately causes cell lysis.

The Classical Pathway

- **Ag-Ab complexes are the main activators of this pathway.**
- **Activated by the binding of Ab to Ag on a target cell.**
- **Only antibody classes of IgM, IgG1, IgG2, and IgG3 can activate classical pathway.**

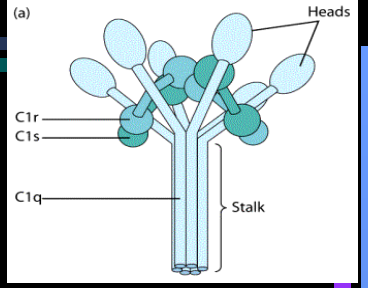
Component Protein Complex C1



C1 complex：C1q + 2 C1r + 2 C1s

The C1qrs complex

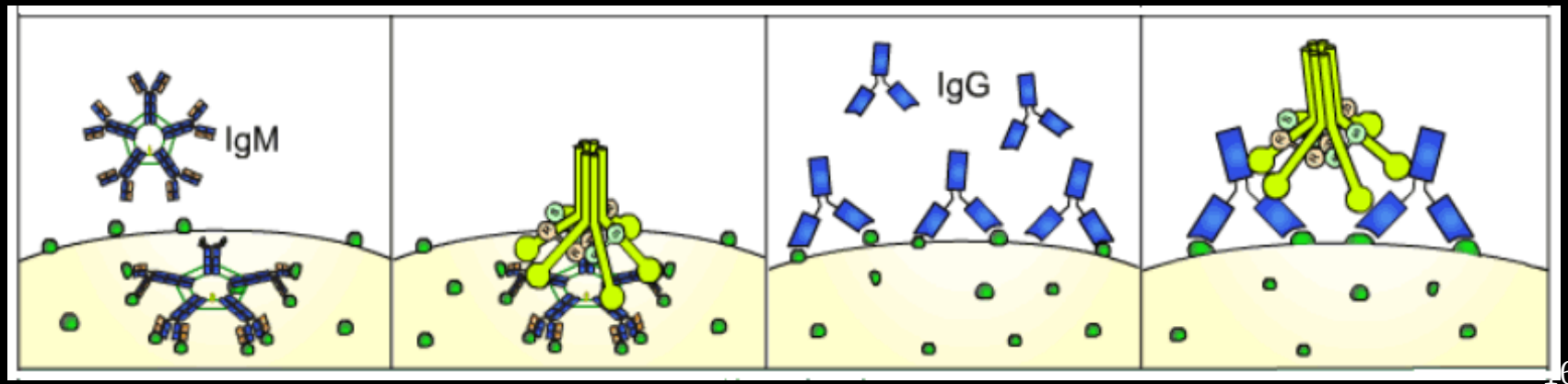
Activation of C1



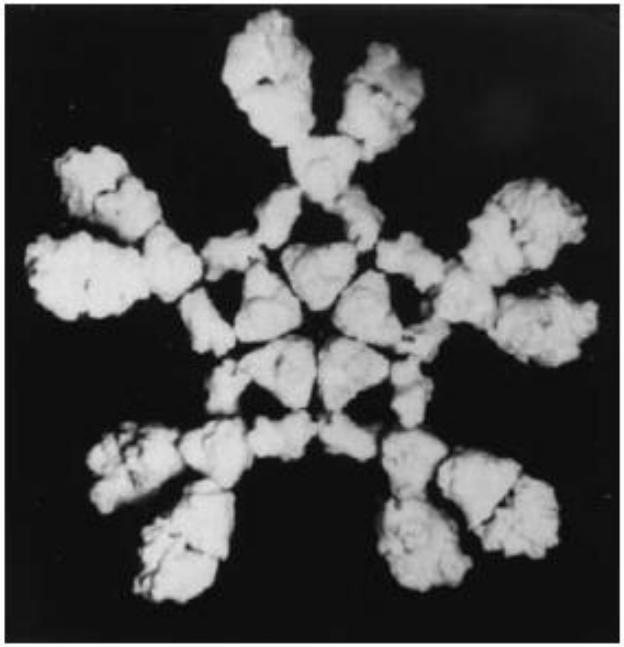
- Activation of C1 occurs when the **two globular head regions** of the subunit C1q bind to the Fc regions **simultaneously**.
- So one **single** IgM or **two closely spaced IgG** molecules bound to the antigen can activate C1
- When C1 binds to the Ab in an antigen-antibody complex it initiates the classical pathway and becomes enzymatically active and is referred to as $\overline{C1qrs}$

Activation Effectiveness

- C1q binds to the Fc region of Ig and requires at least two adjacent Fc regions
- If epitope on a target Ag are too low in density for proper arrangement of Ab molecules, C1 binding does not occur.
- IgM is more effective at activating complement than IgG



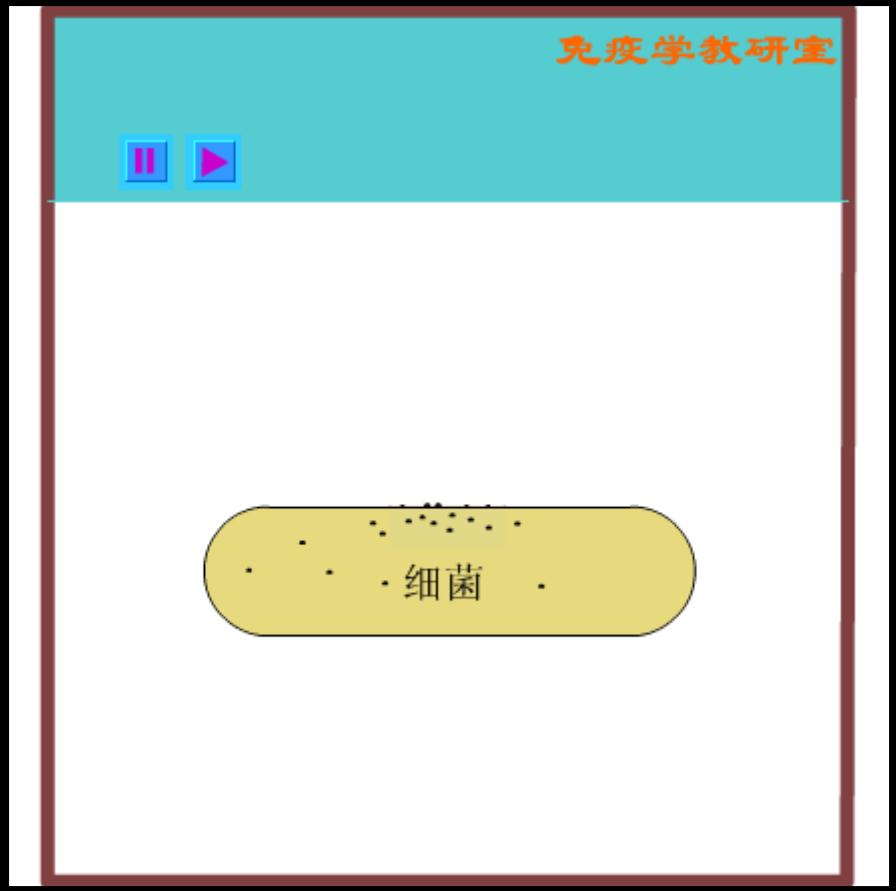
(a)



(b)

FIGURE 6-4 Models of pentameric IgM in planar form (a) and "staple" form (b). Several C1q-binding sites in the Fc region are accessible in the staple form, whereas none are exposed in the planar form. *[From A. Feinstein et al., 1981,* Monographs in Allergy ***17:****28, and 1981,* Annals of the New York Academy of Sciences ***190:****1104.]*

The activation of C1

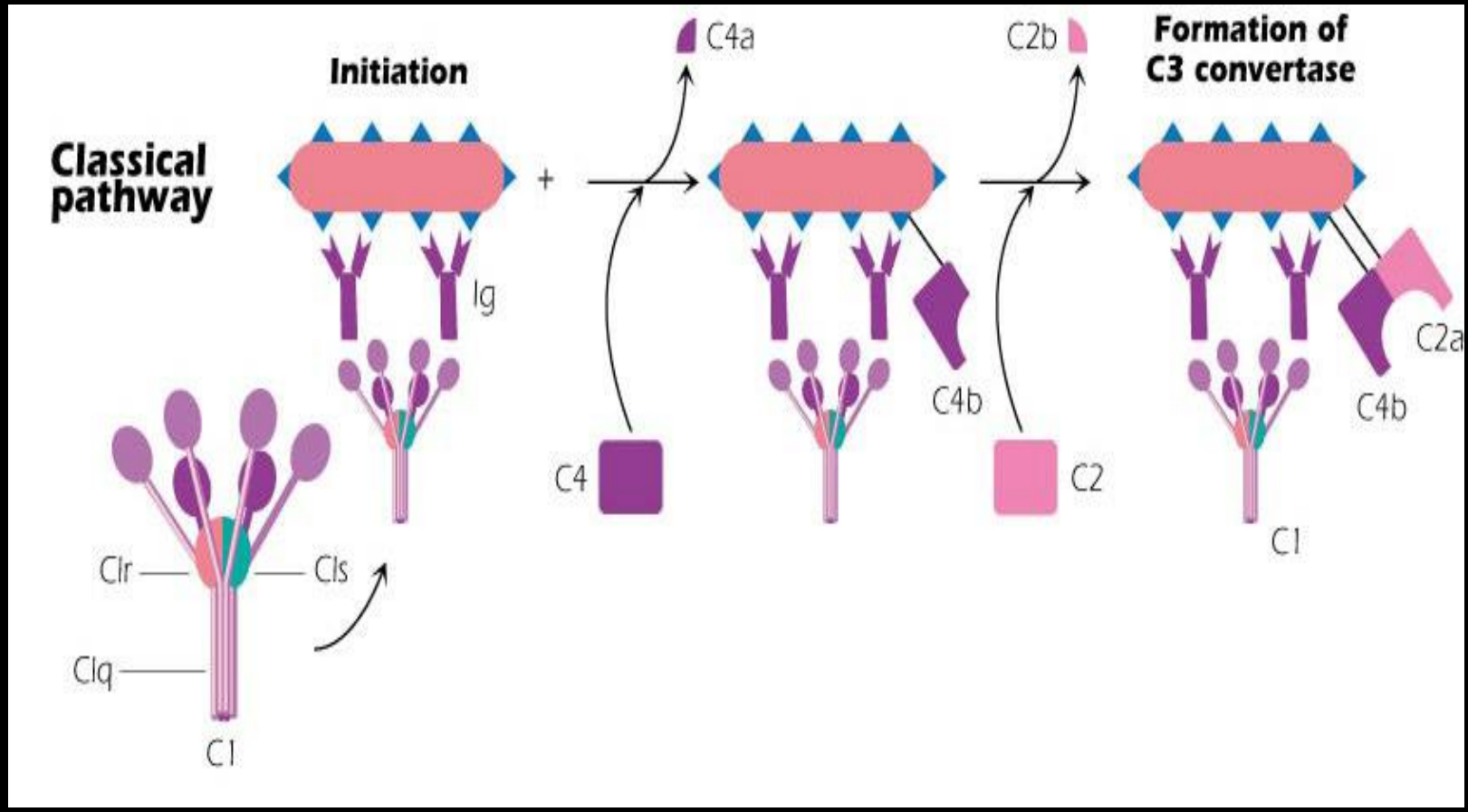


Activation of C1

- **Appropriate binding of C1q results in activation of the proteolytic enzyme activities of C1r and subsequently C1s**

- **C1s then cleaves the next component of the pathway, C4**

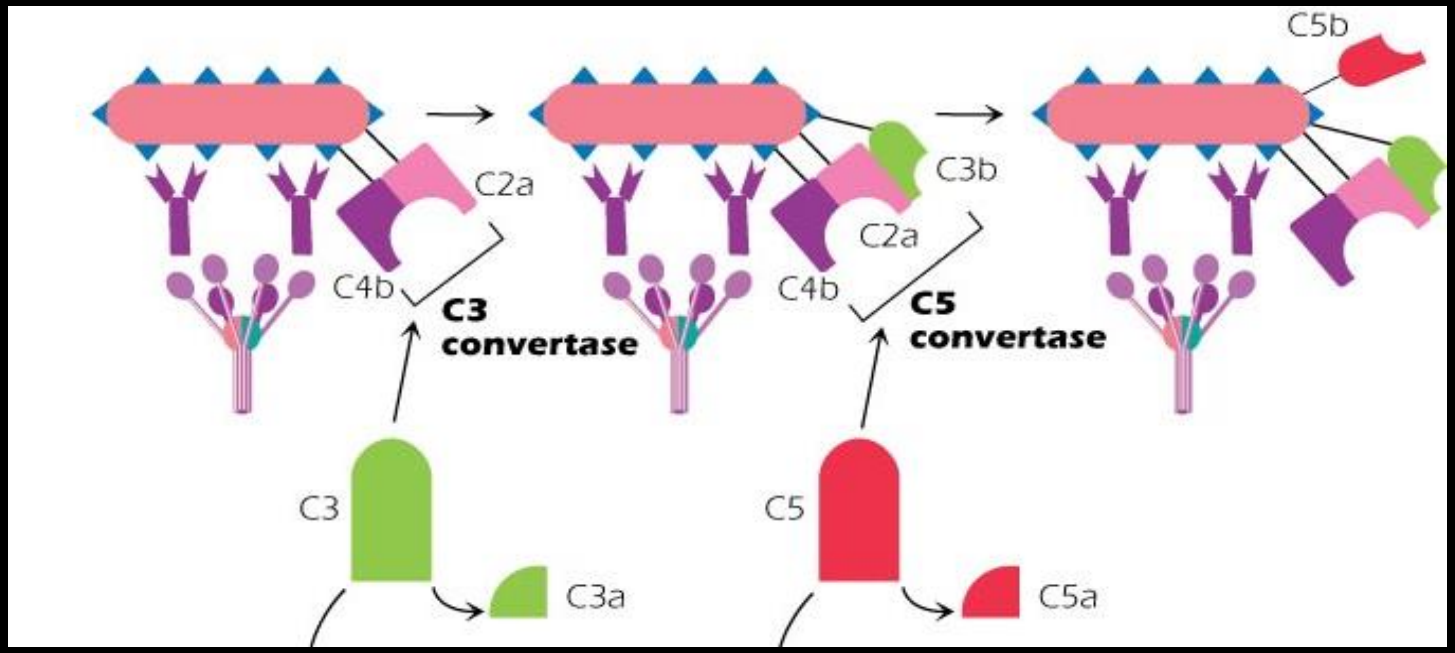
Classical Pathway



- **C4b2a** is the **C3 convertase** of classical pathway.

C3 and C5 convertases

- **C4b2a: Classical pathway C3 convertase**
- **C4b2a3b: Classical pathway C5 convertase**



- **Cleavage of C3 produces two fragments:**
  - **C3b: larger, continues the sequential activation of successive components**
  - **C3a: smaller, fluid-phase, anaphylatoxin**
- **Cleavage of C5 produces two fragments:**
  - **C5b: binds to the cell surface, nucleus for binding the terminal complement components**
  - **C5a: released into the fluid phase, most potent anaphylatoxin and chemotactic factor**

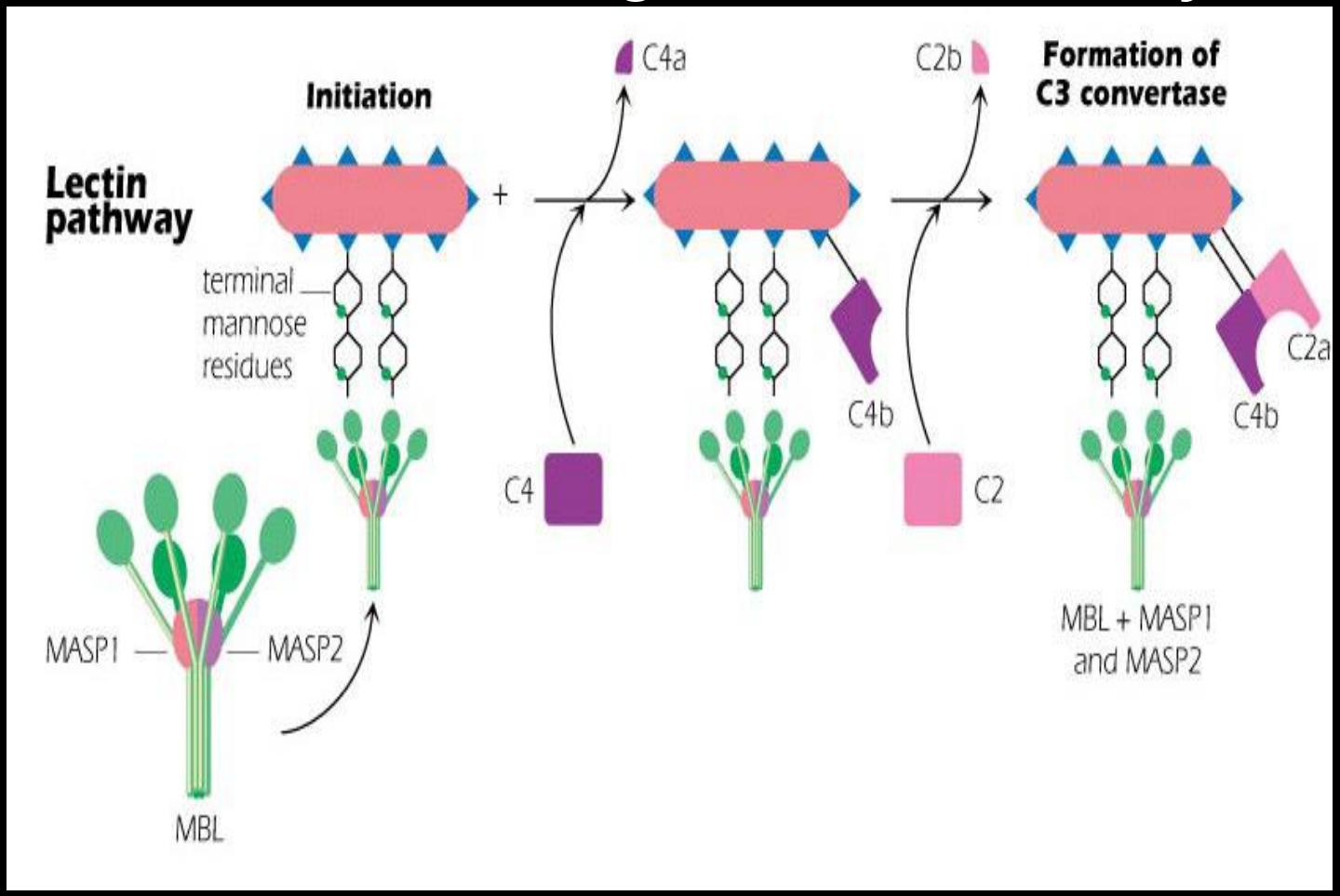
Non-immunologic classical pathway activators

- **Certain microbes**
- **Other structures**
  - **Urate crystals**
  - **Heparin**
  - …

The Lectin Pathway

- **Antibody-independent**
- mannan-binding lectin, **MBL** recognizes and binds to certain carbohydrates (mannan) on the surface of some microorganisms
- Activates ***MBL associated serine proteases*** (MASP-1 and MASP-2) that cleave and activate C4 and C2, which generate C3 convertase

Mannan-binding Lectin Pathway



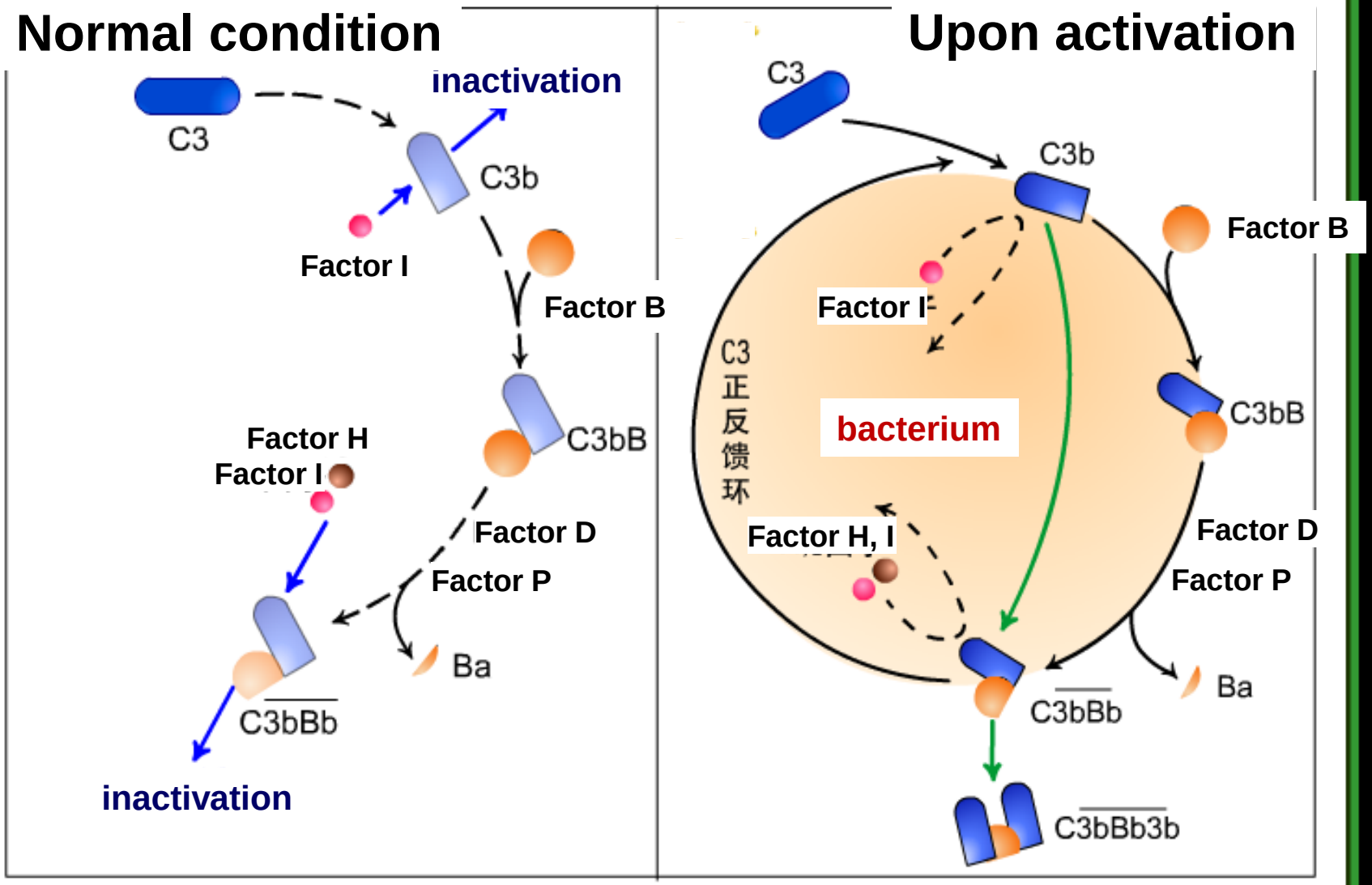
MBL, MASP-1 and MASP-2 complex is C1-like

The Alternative Pathway

- **Antibody-independent**
- **Initiated by foreign cell surface proteins**
  - **Lipopolysaccharides (endotoxins) from gram-negative bacteria;**
  - **Teichoic acid from gram-positive cell walls**
  - **parasites (trypanosomes); Fungal and yeast cell walls (zymosan)**
  - **Some tumor cell (Raji); Some viruses and viruses-infected cells**
  - **Nonpathogens: Cobra venom factor…**

The Alternative Pathway

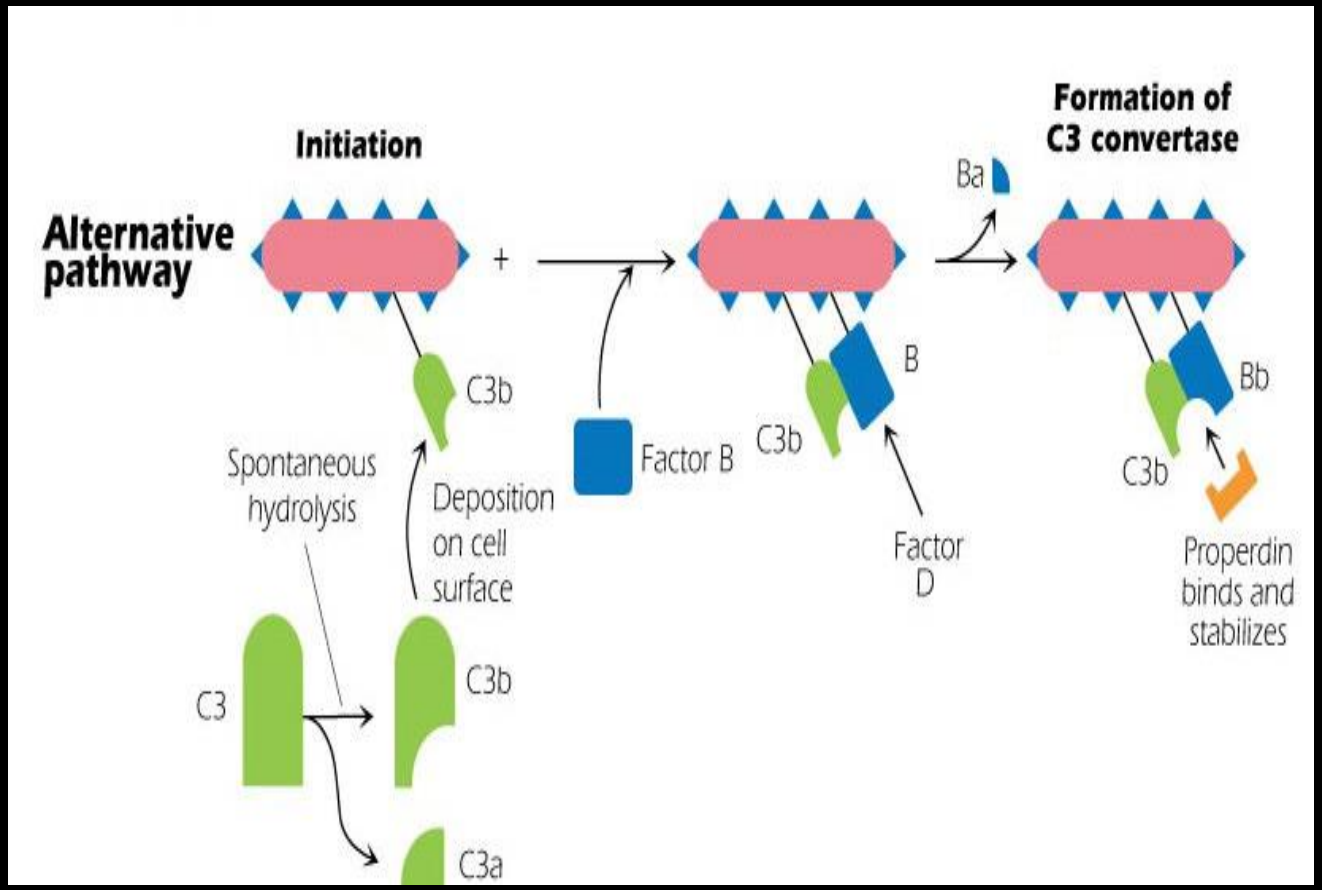
- **Circulating C3 undergoes spontaneously hydrolysis**
  - **C3b is continually generated at a low rate and is always circulating in the blood system.**
  - **The half-life of this active form of C3 in surrounding medium is roughly 30-60 ms, so it will be inactive very soon if it does not bind to appropriate surface**
  - **Other Stringent control mechanisms (in fluid phase and cell-associated) operate to limit the extent for the reaction**



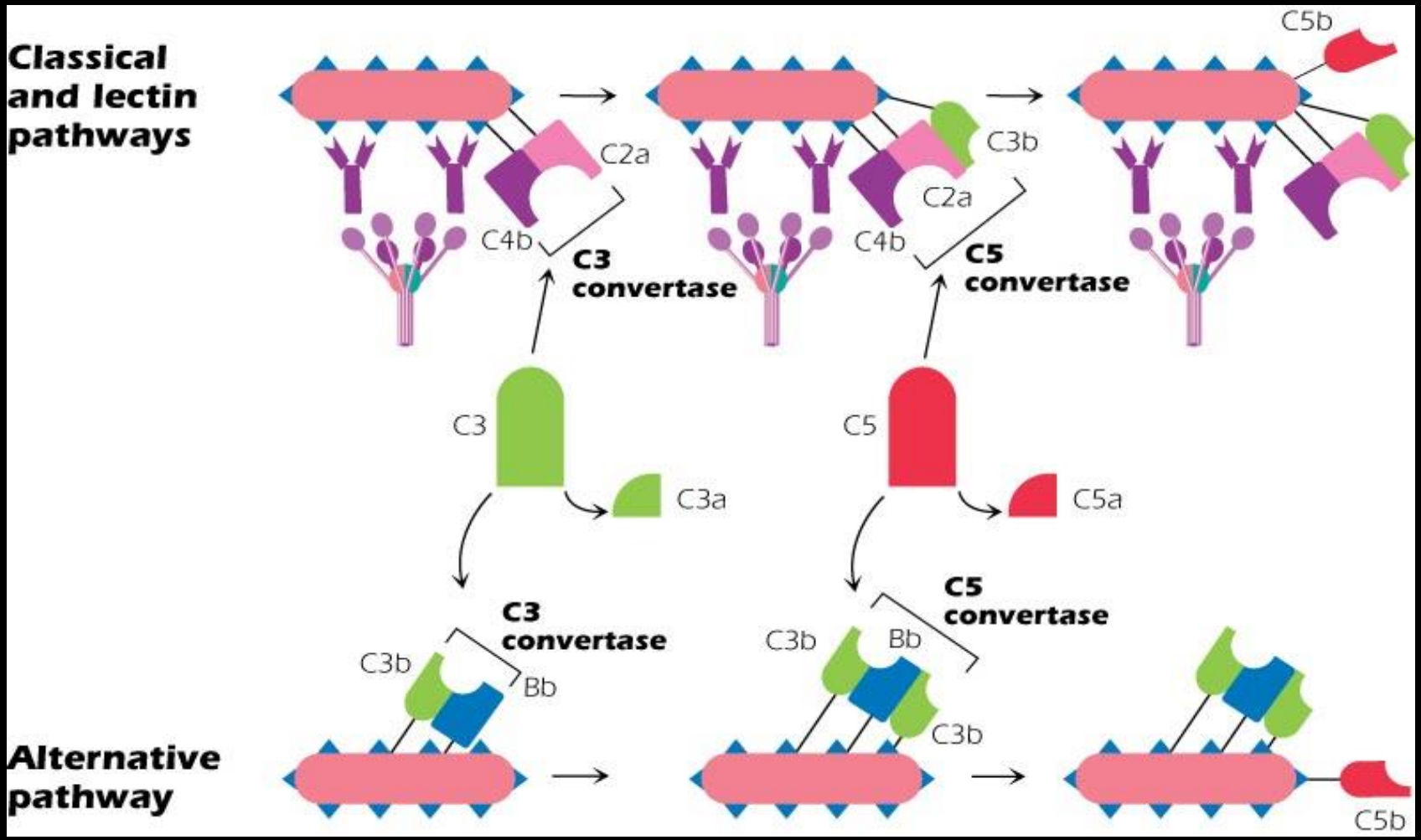
The Alternative Pathway

Immunology

Alternative Pathway



C3 and C5 convertases of each pathway



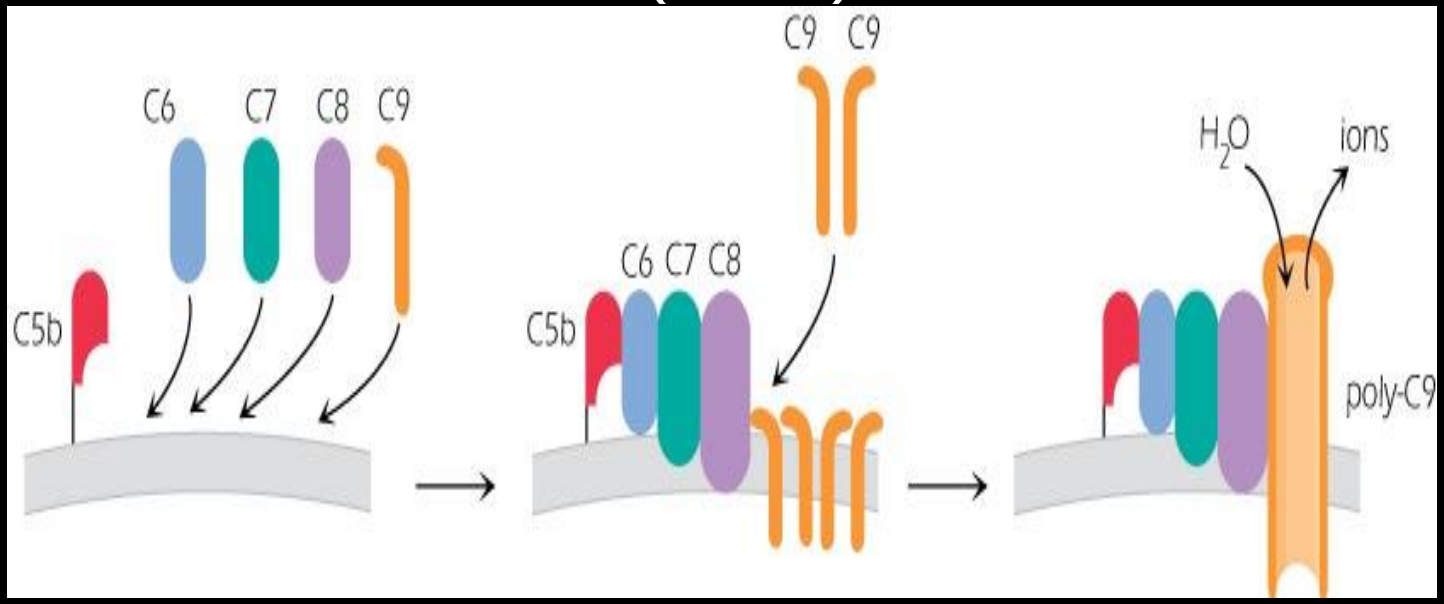
C3 and C5 convertases of each pathway

- **Classical and lectin pathway**
  - **C3 convertase: C4b2a**
  - **C5 convertase:C4b2a3b**
- **Alternative pathway**
  - **C3 convertase: C3bBb**
  - **C5 convertase: C3bBb3b**

The Terminal Sequence

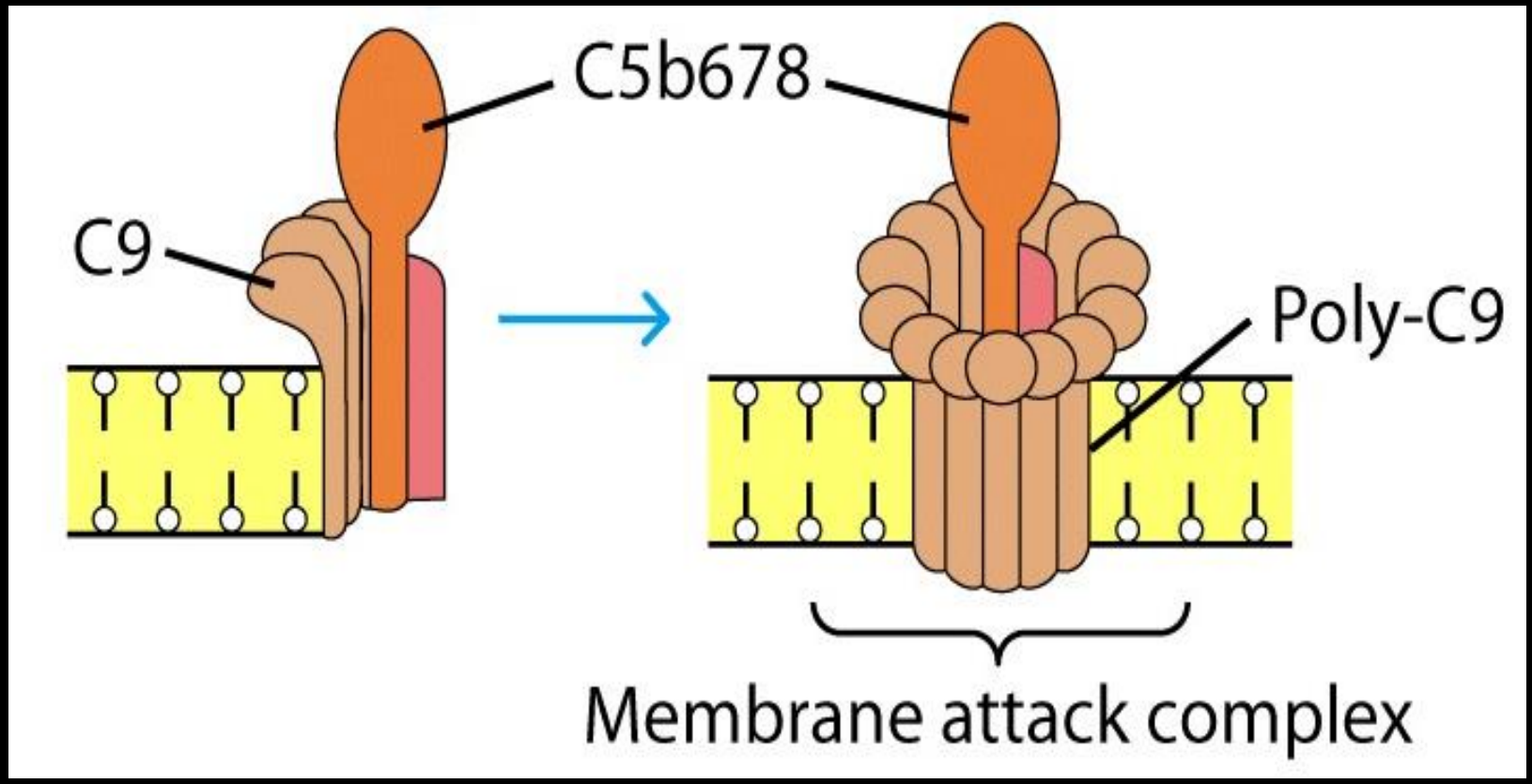
- **Terminal components of the complement cascade: C5b, C6, C7, C8, and C9**
- **Components are common to all pathways**
- **Bind to each other and form a MAC**
- **Results in cell lysis**

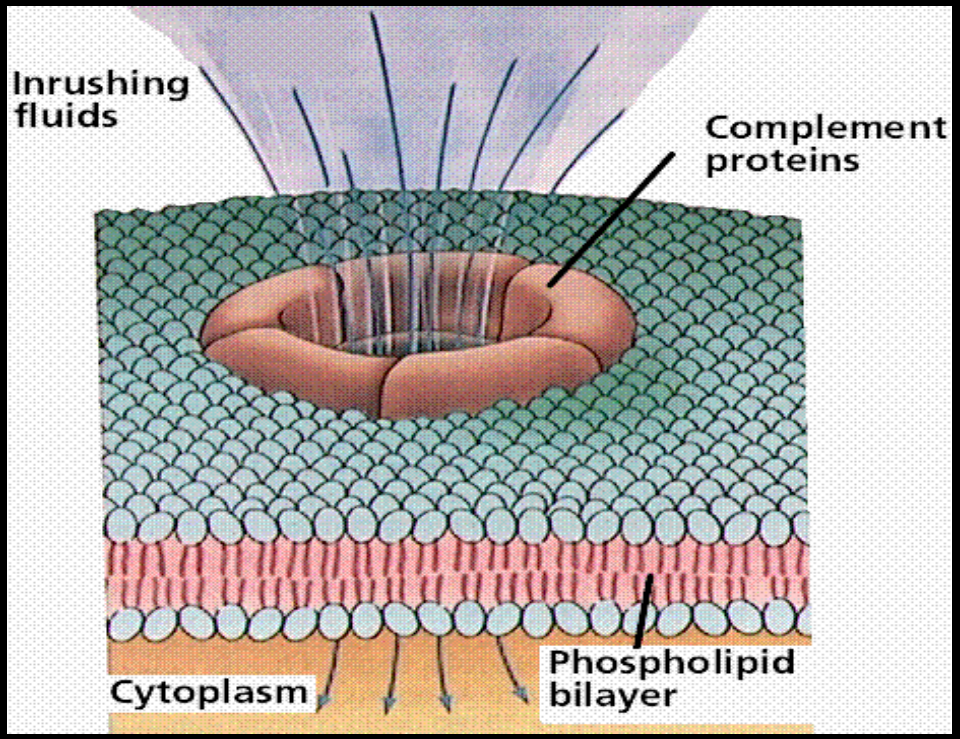
Formation of the membrane attack complex (MAC)



1. C6 binding to C5b on a cell surface.
2. C7 then binds to C5b and C6 and undergoes a hydrophobic structural transition.
3. C8 binding to C5bC6C7 creating a small pore.
4. 10-16 C9s can be polymerized by a single C5bC6C7C8 complex, forming C5678(9)n, termed MAC

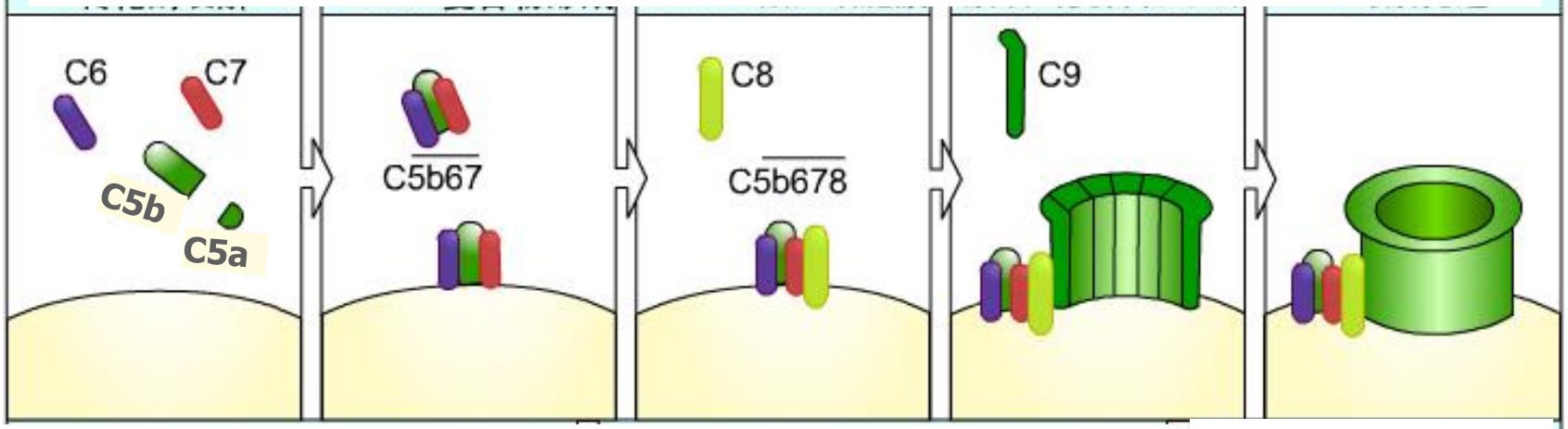
Formation of MAC





- A transmembrane channel is formed by MAC, which disturbs the osmotic equilibrium of the cell. Ions pass through the channel, and water enters the cell.
- Cell swells, membrane becomes permeable to macromolecules, which then escape from the cell.
- Result is cell lysis.

Membrane attacking



$\overline{C4b2b3b}$

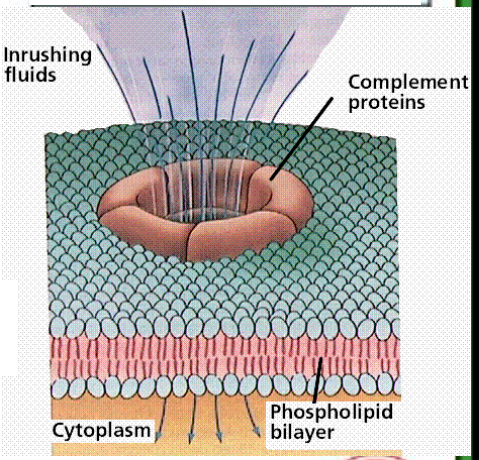
C5 convertase

C5 → C5b → C5b~9

C6 C7 C8 C9

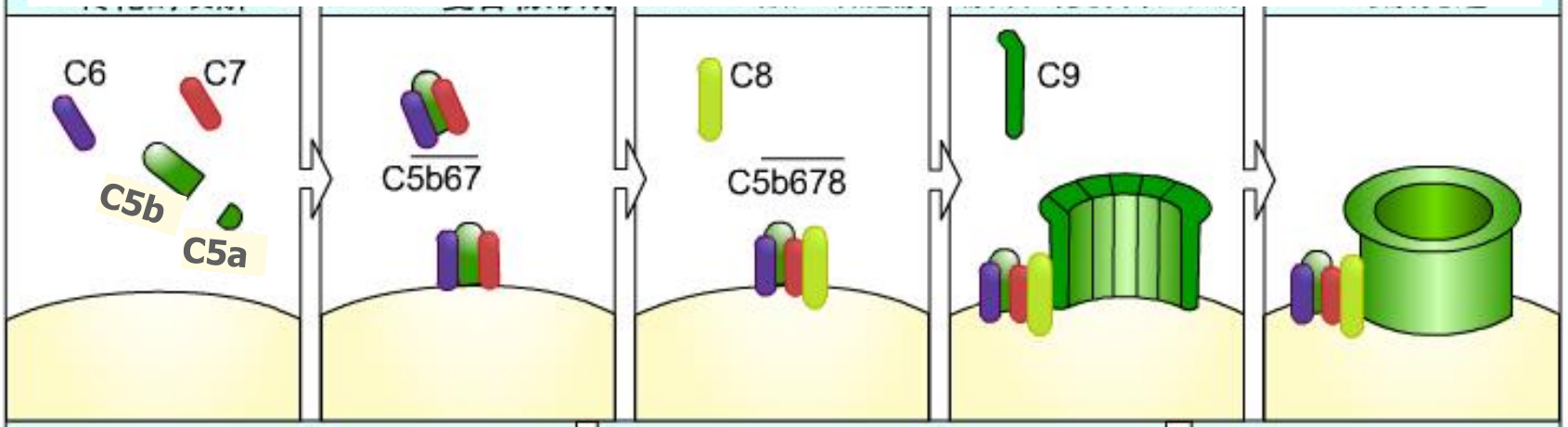
MAC

transmembrane channel

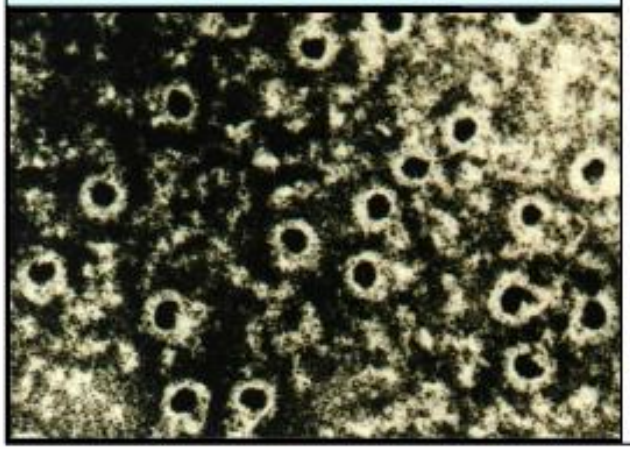


Immunology

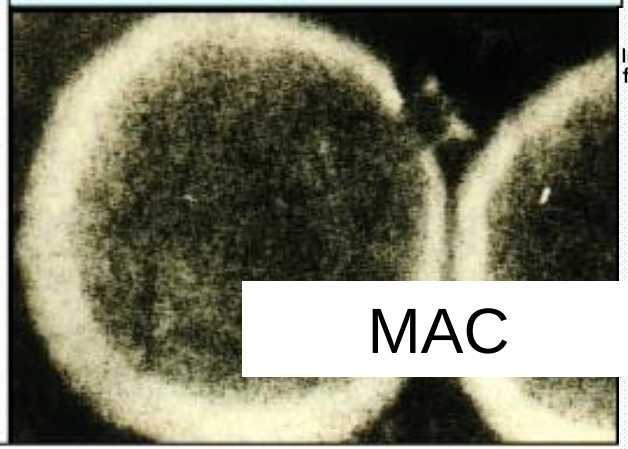
Membrane attacking



Membrane damage-Top view

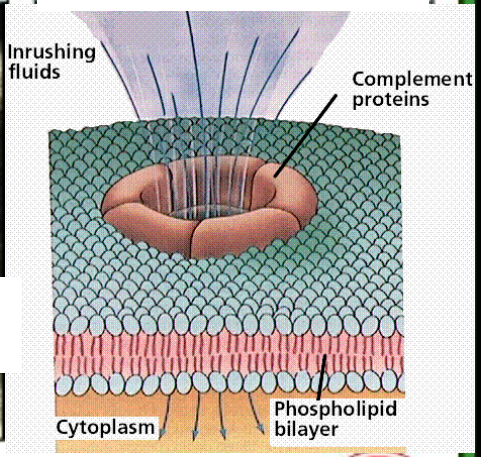


Membrane damage-side view



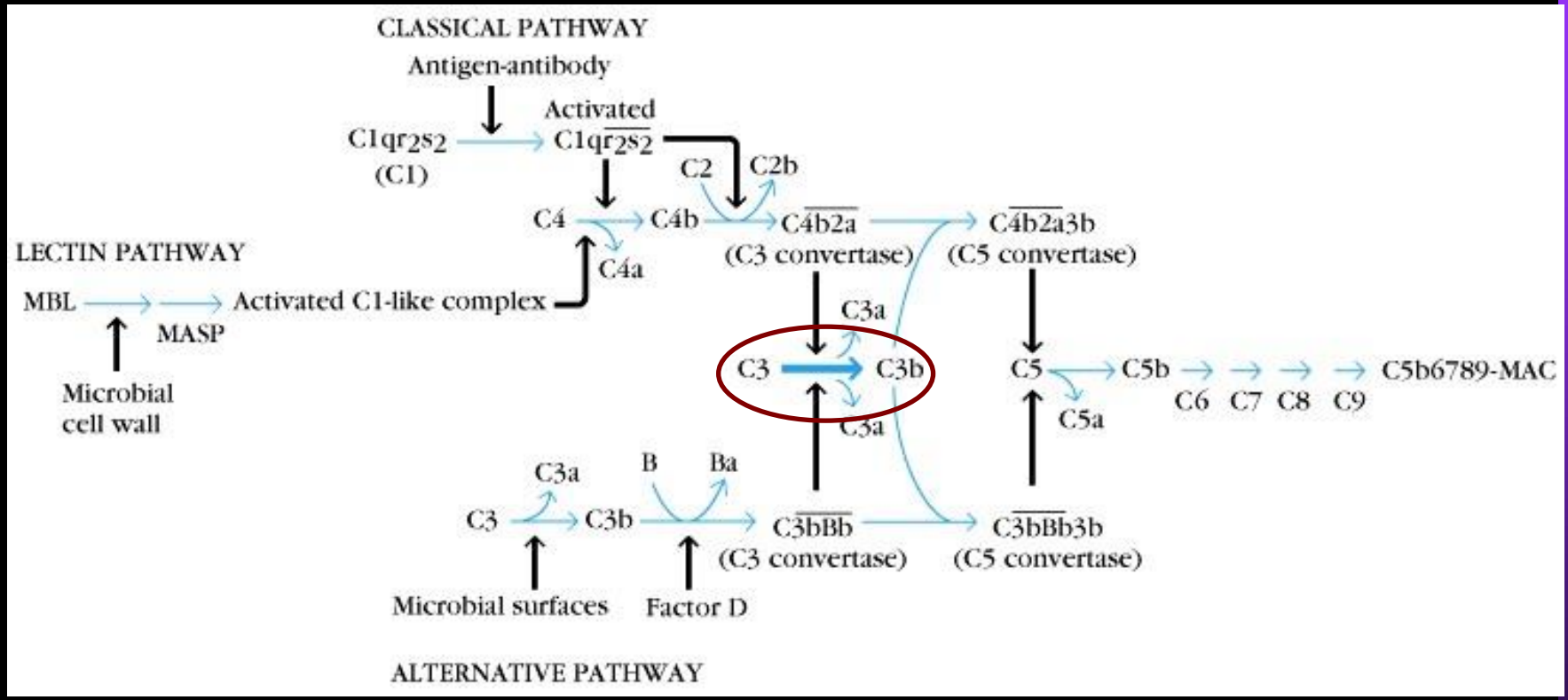
MAC

transmembrane channel



Immunology

Complement activation pathways

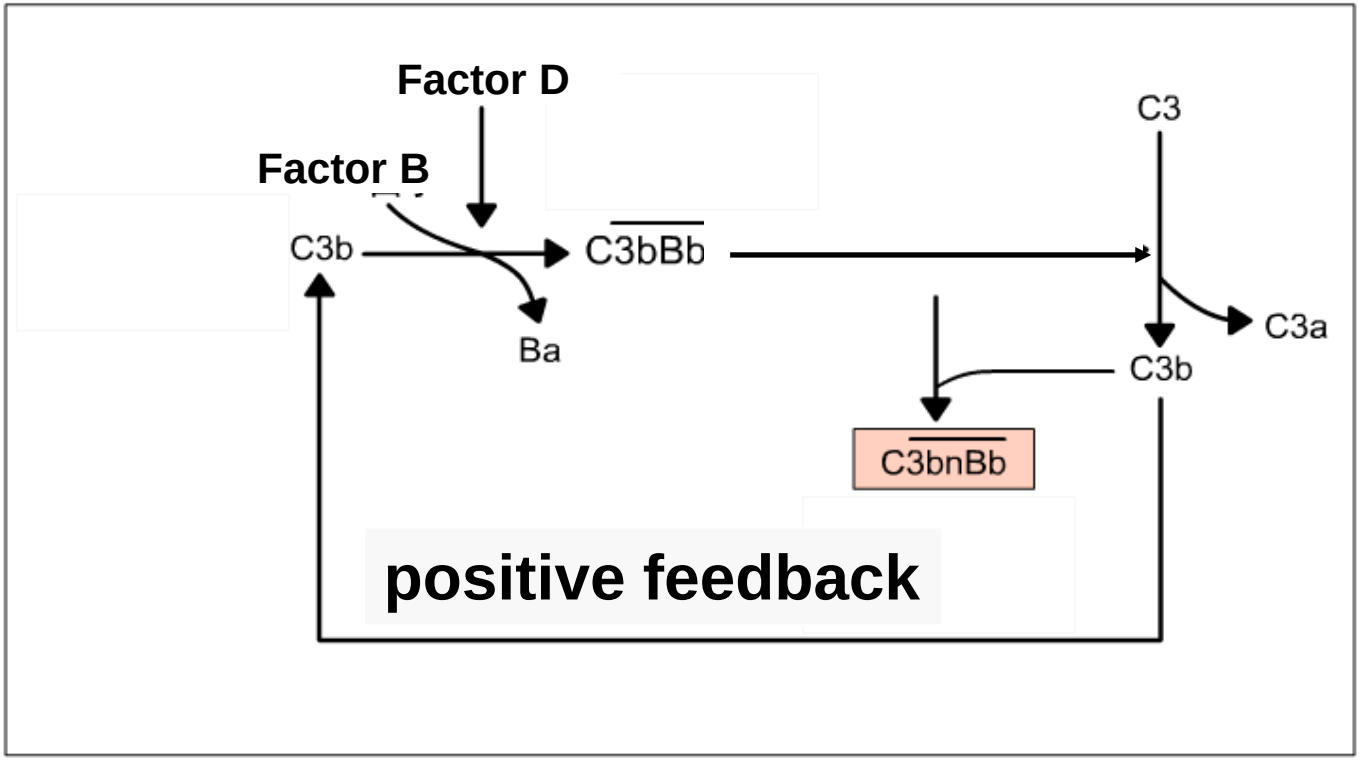


Amplification is a key feature of C activation

- One enzyme generate multiple products One molecule of C4b2a can cleave up to 1000 molecules of C3 to C3b

- There is positive feedback in C activation

Can generate a massive response from a single triggering event within a short period of time



Alternative pathway

Immunology

Amplification by positive feedback

The classic complement pathway is activated by antibody-antigen complexes. The antibody isotypes that activate include both

A. IgG and IgA

B. IgM and IgG

C. IgG and IgD

D. IgE and IgG

E. IgM and IgA

Which one of the following does not occur when the alternative complement pathway is activated?

- **A. Breakdown of C5 into C5a and C5b**
- **B. Breakdown of C4 into C4a and C4b**
- **C. Breakdown of C3 into C3a and C3b**
- **D. Activation of the membrane attack complex**
- **E. Generation of anaphylatoxins**

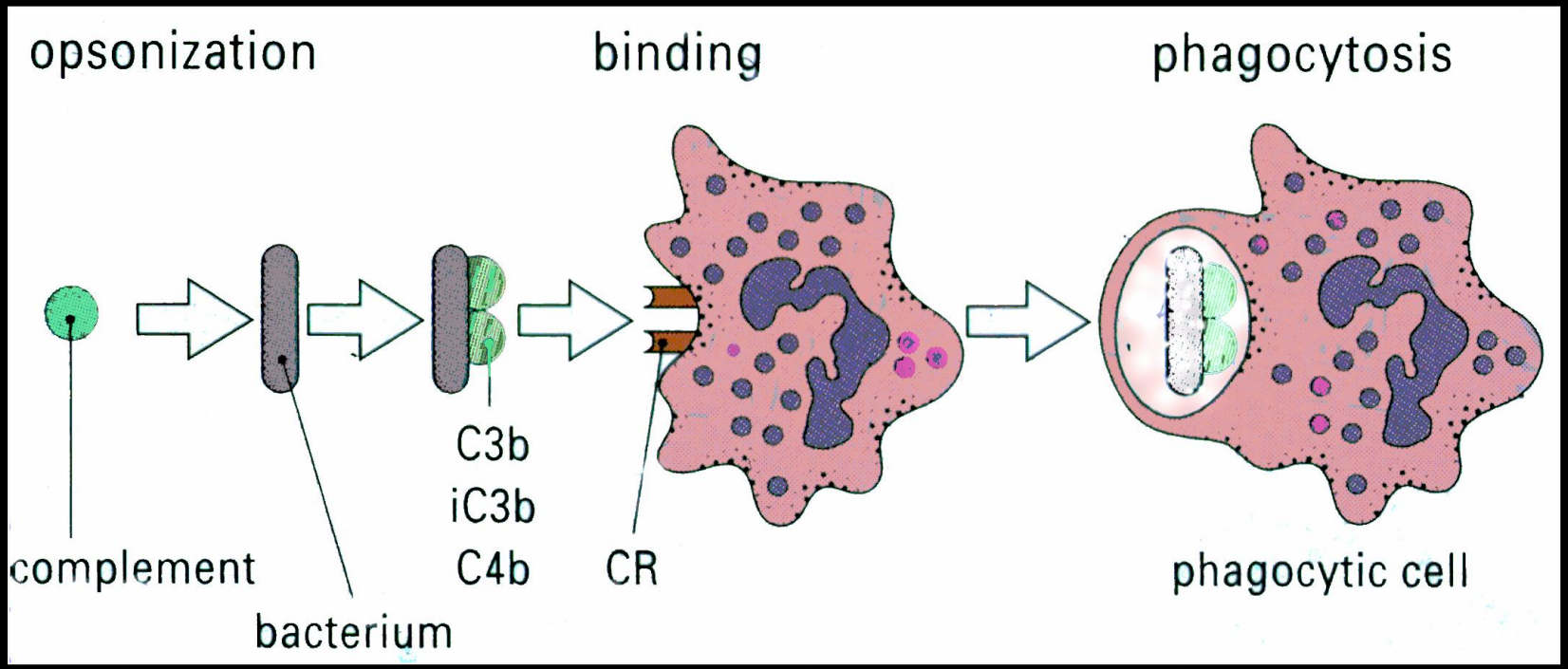
Biological consequences (function) of Complement Activation

1. **Cell lysis**
2. **Opsonization and phagocytosis**
3. **Viral neutralization**
4. **Inflammation**
5. **Clearance of Immune Complexes**

1. Cell lysis

- **Cells**
- **Bacteria**
- **Parasites**
- **Enveloped viruses**

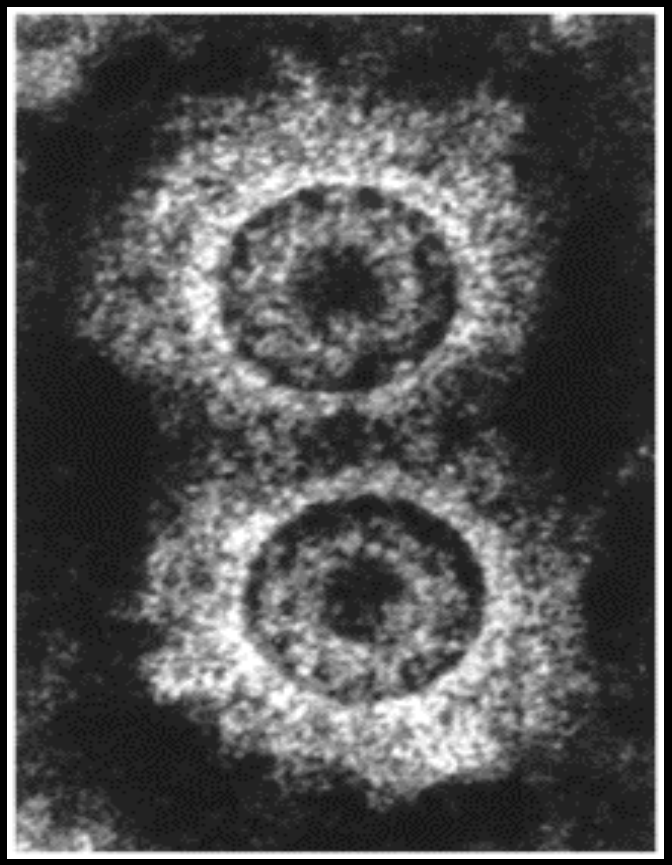
2. Opsonization and phagocytosis

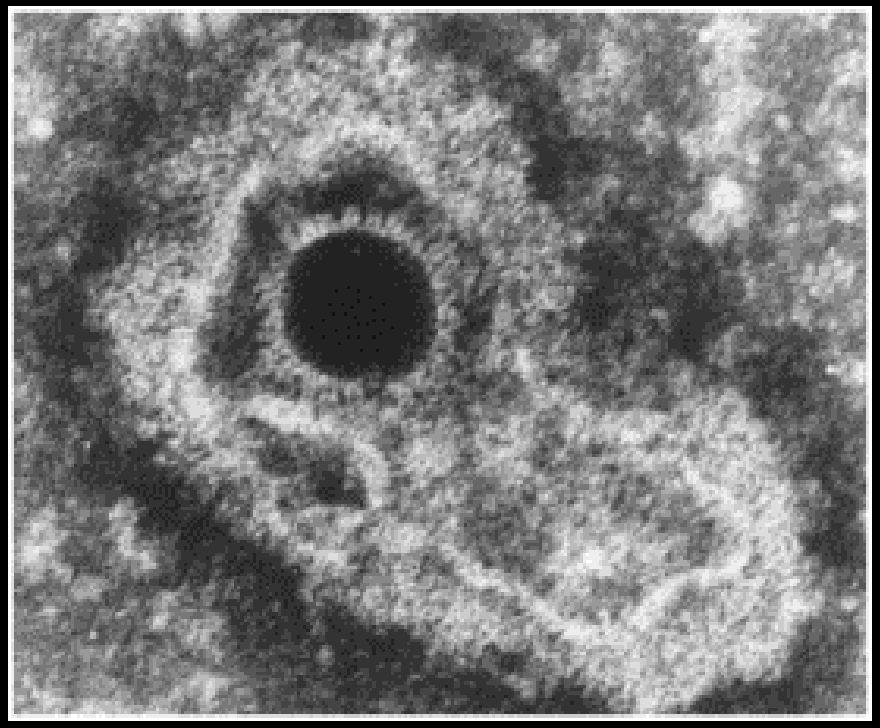


- **C3b is the major opsonin of the complement system**

3. Complement Proteins Neutralize Viruses

- **Creates a thick protein coating that can block attachment to susceptible host cells**

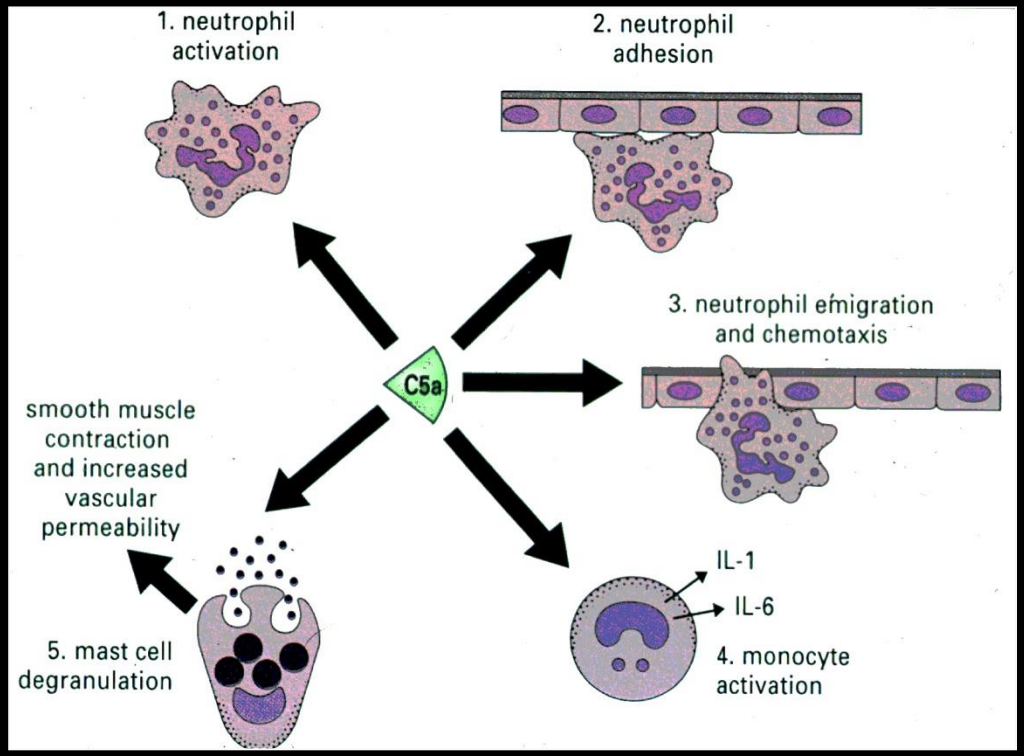


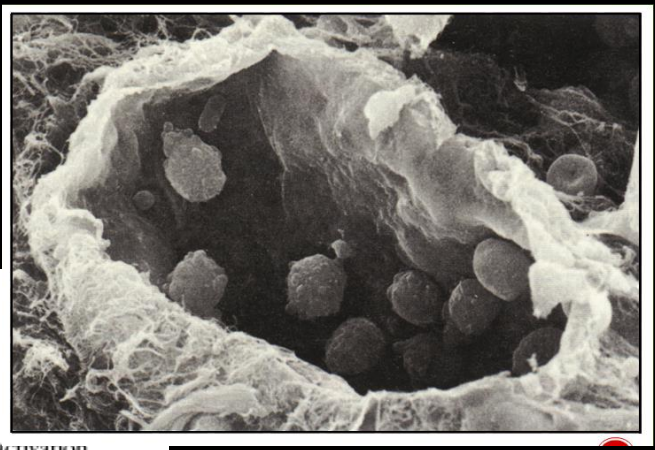


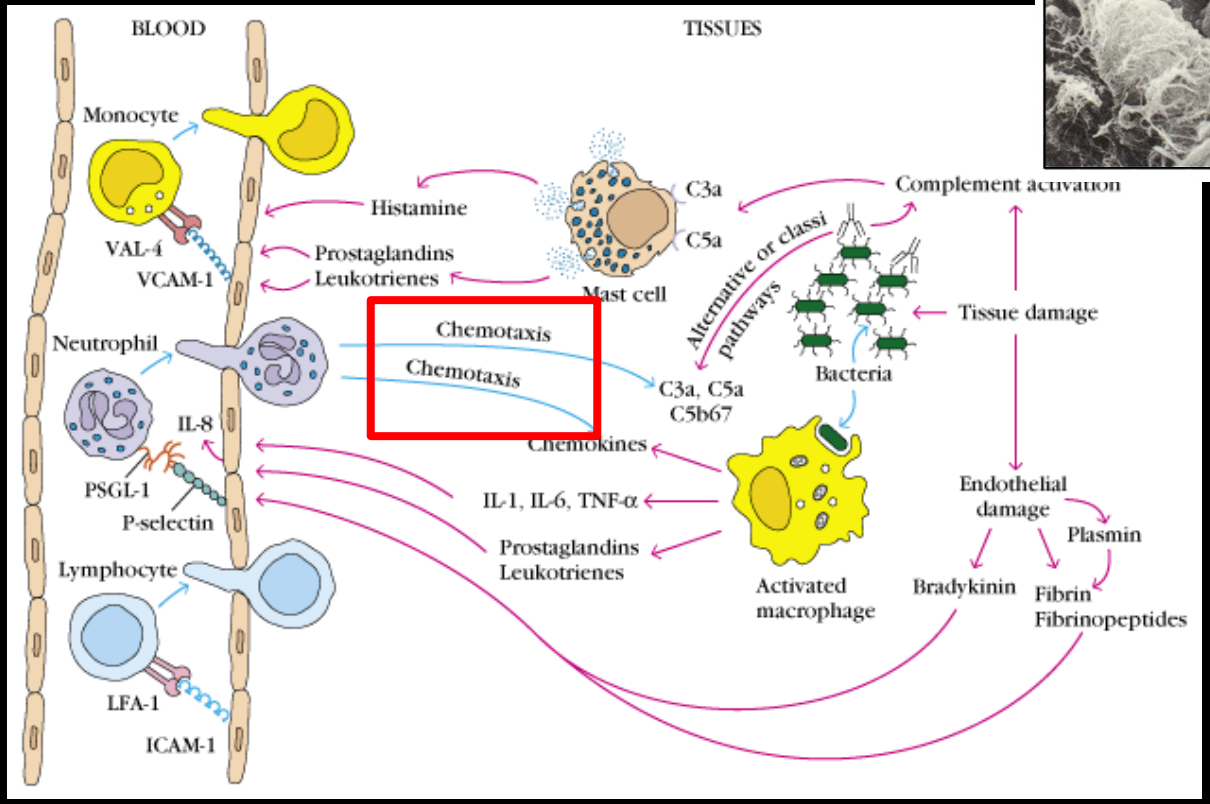
Coating of the **Epstein-Barr virus**

4. Inflammation

- **Mediated mainly by smaller fragments**
  - **Chemotactic for neutrophils and mononuclear phagocytes**
  - **Anaphylatoxins**






BLOOD
TISSUES
Monocyte
VAL-4
VCAM-1
Neutrophil
IL-8
PSGL-1
P-selectin
Lymphocyte
LFA-1
ICAM-1
Histamine
Prostaglandins
Leukotrienes
Mast cell
C3a
C5a
Chemotaxis
Chemotaxis
C3a, C5a
C5b67
Alternative or classi
pathways
Complement activation
Tissue damage
Bacteria
Chemokines
IL-1, IL-6, TNF-α
Prostaglandins
Leukotrienes
Activated
macrophage
Endothelial
damage
Plasmin
Bradykinin
Fibrin
Fibrinopeptides

chemotaxis

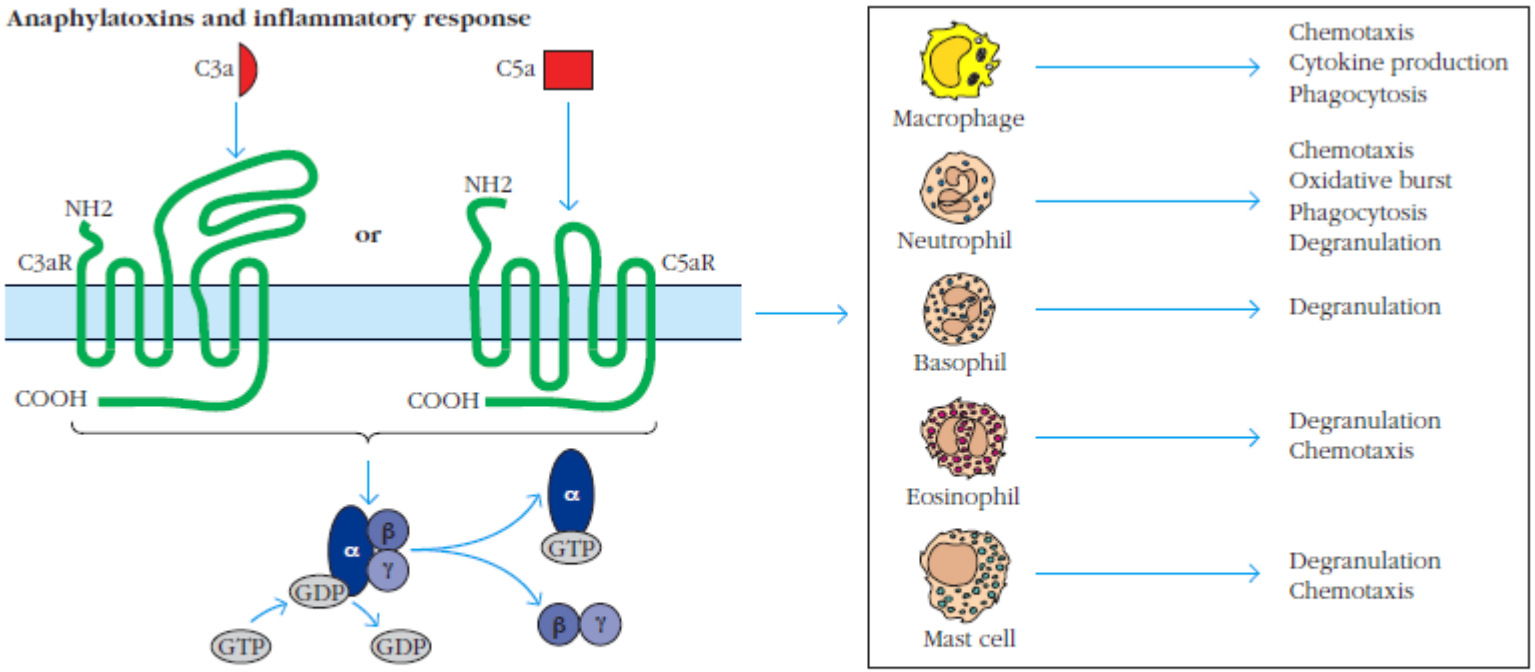
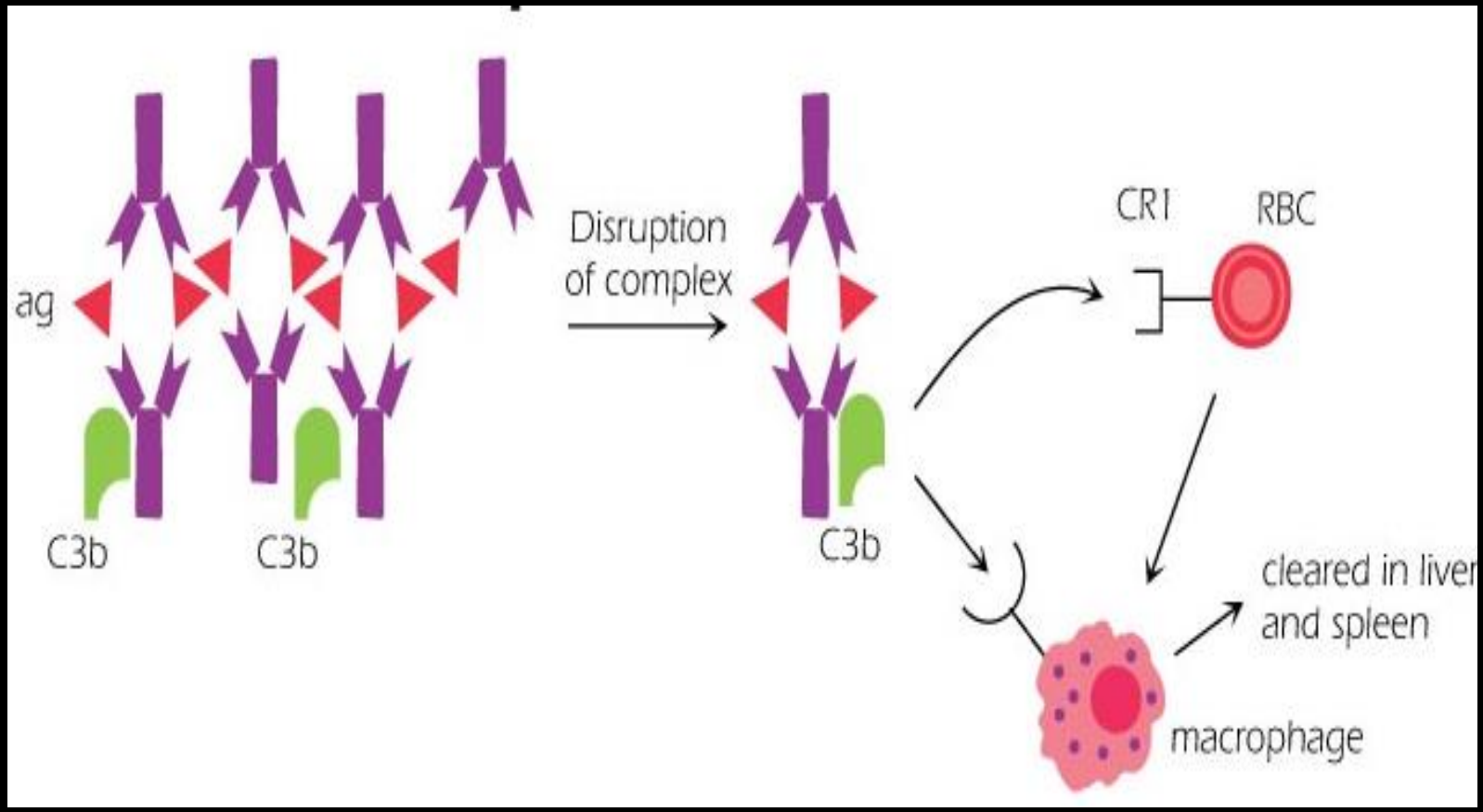
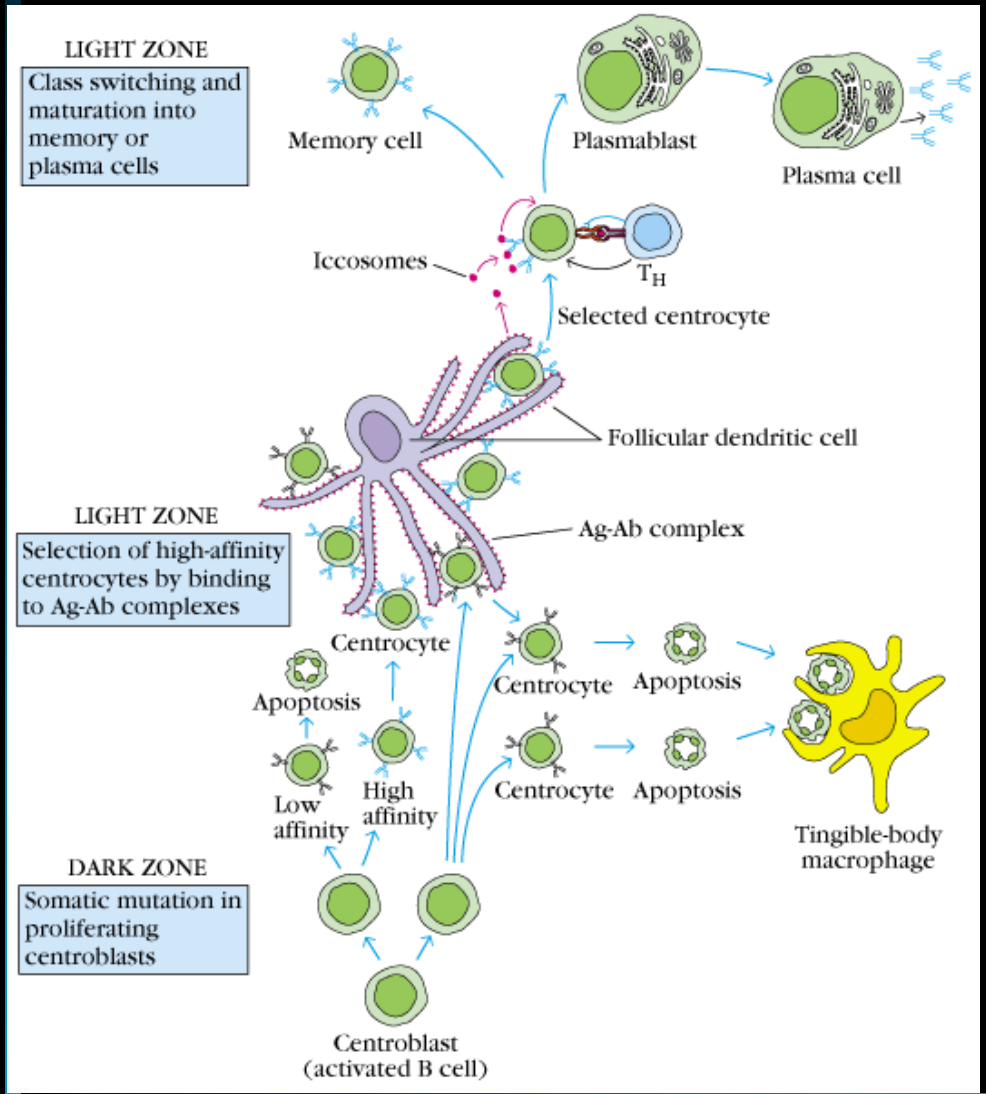


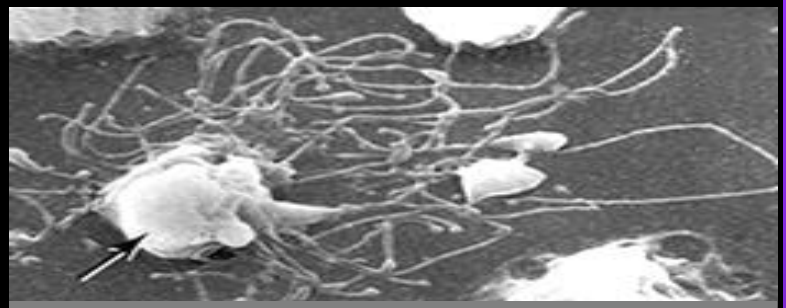
FIGURE 6-12 Binding of the anaphylatoxins C3a and C5a to the G-protein-coupled receptors C3aR and C5aR. The C3aR and C5aR receptors are members of the G-protein-coupled receptor family described in Chapter 4. Binding of the anaphylatoxins to these receptors stimulates the release of proinflammatory mediators from macrophages, neutrophils, basophils, eosinophils, and mast cells, as indicated. *[Adapted from J. R. Dunkelberger and W.-C. Song, 2010, Complement and its role in innate and adaptive immune responses,* Cell Research **20**:34–50, *Figure 3B.]*

5. Clearance of Immune Complexes

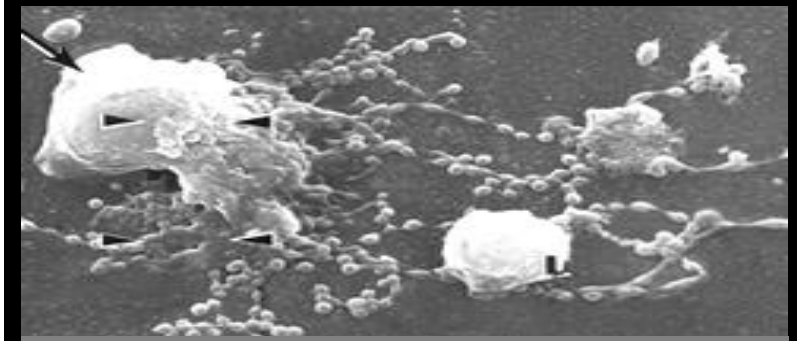


Mantaine immune memory





Filiform dendrites



iccosome formation on dendrites

FDC— immune complex IC— CD21/CR2、FcγR— iccosome

Deficiency of the complement protein C4 would inhibit which one of the following complement activities?

A. Formation of C3b for opsonization
B. Formation of C5a for chemoattractant for neutrophils
C. Formation of the membrane attack complex
D. Completion of the classical pathway to the splitting of C3
E. Formation of C5 convertase via the alternative pathway

Regulation Of Complement system Activation

- **To prevent complement-mediated destruction of the host's own tissues**
- **When causing disease**
  - **Usually misdirected**
  - **congenital defect**
- **Regulation**
  - **Self-regulation**
  - **Fluid-phase regulator**
  - **Solid-phase regulator**

Self-regulation

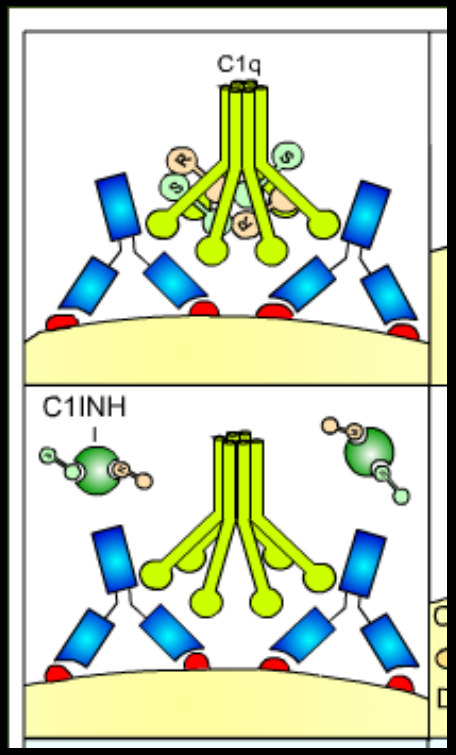
- **Many activated components are very unstable, including C4b, C4b2a, C3b, C4b2a3b, C3bBb, C5b, C5b-7…**

Regulators may

- **dissociate the convertase, C1INH**
- **cleave the complement component that is left on the cell surface: Factor I**
- **Act as a cofactor for this cleavage:C4bp**

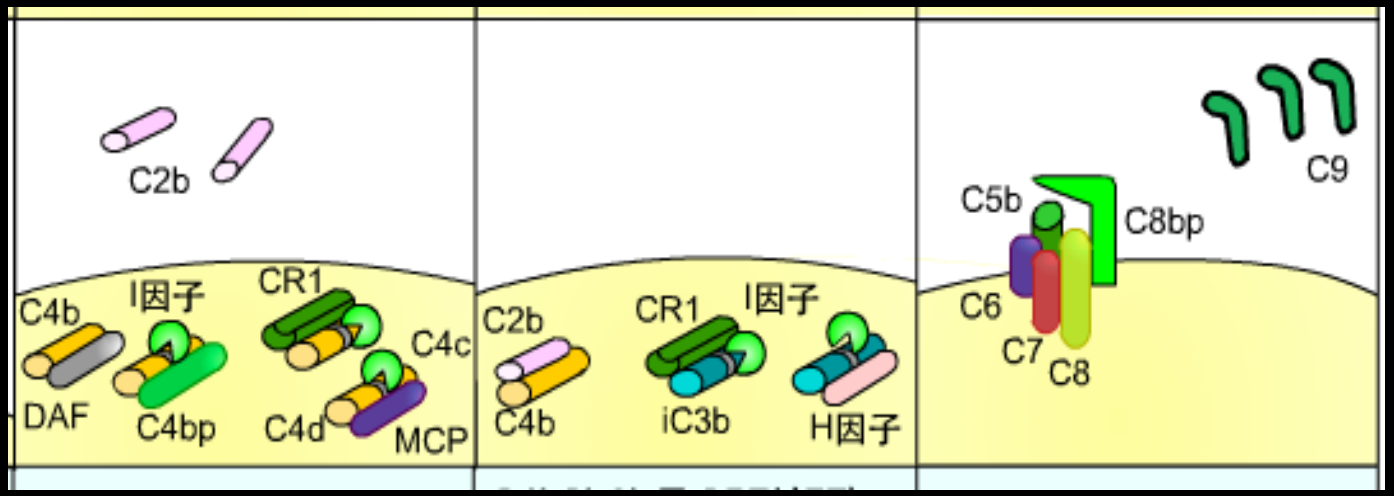
Fluid-phase regulator

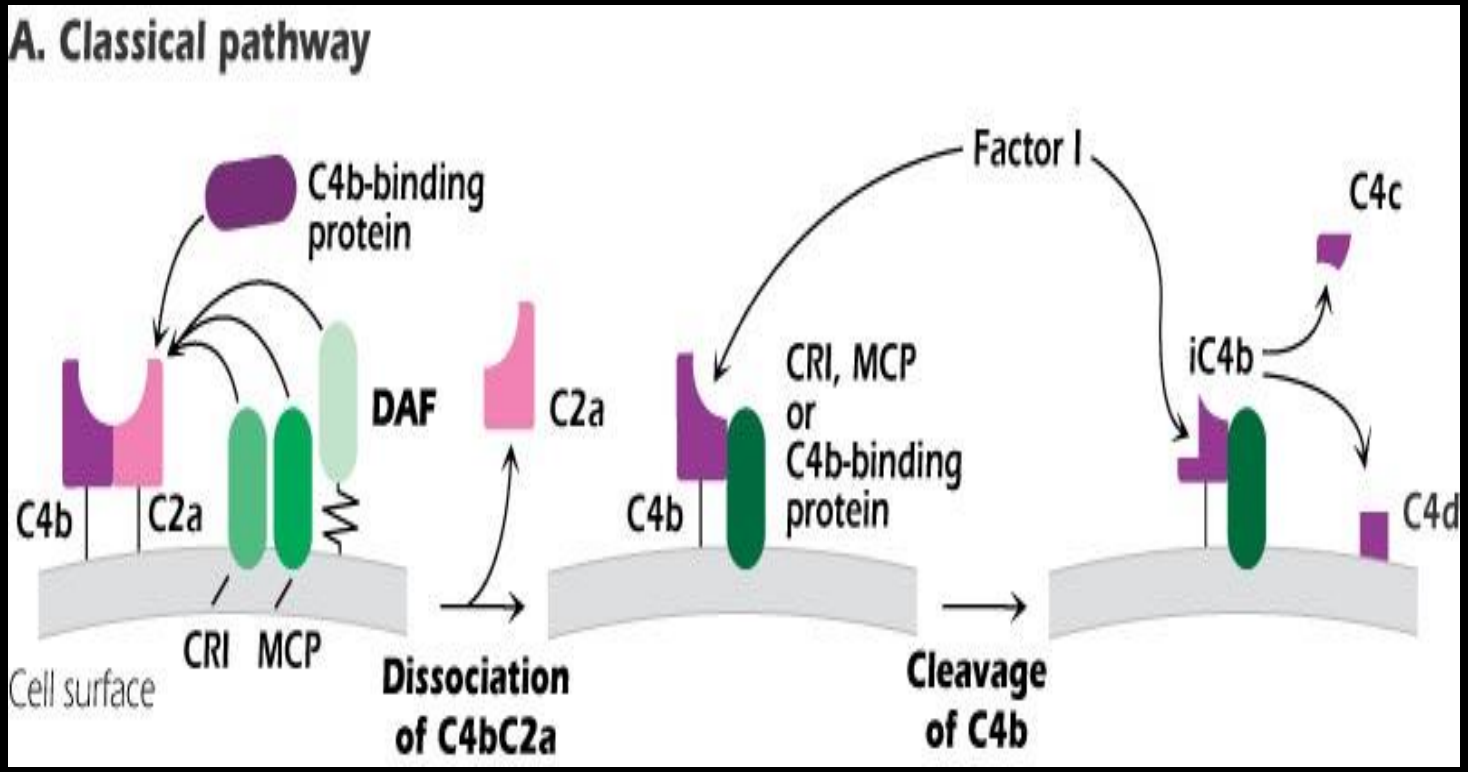
- **C1 inhibitor, CIINH**
- **C4 binding protein, (C4bp) + factor I regulate C4b**
- **Factor I + factor H regulate C3b**
- **S protein, clusterin, factor J…**



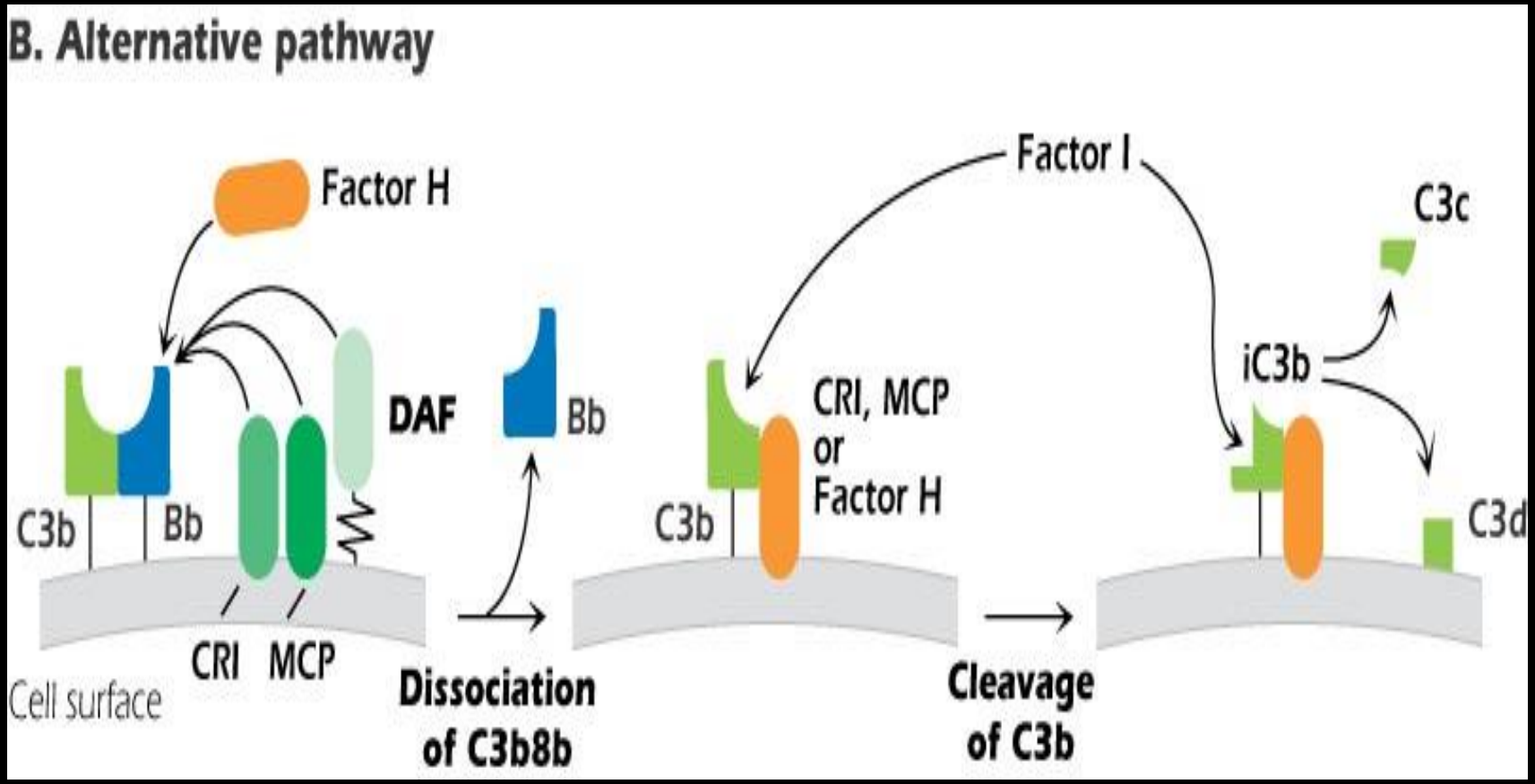
Solid-phase regulator

- **CR1**, complement receptor type 1, aims C3b, C4b, C3bBb, C4b2a
- **DAF**, Decay accelerating factor, accelerator of C3 convertase and C5 convertase decay
- C8-binding protein
- …



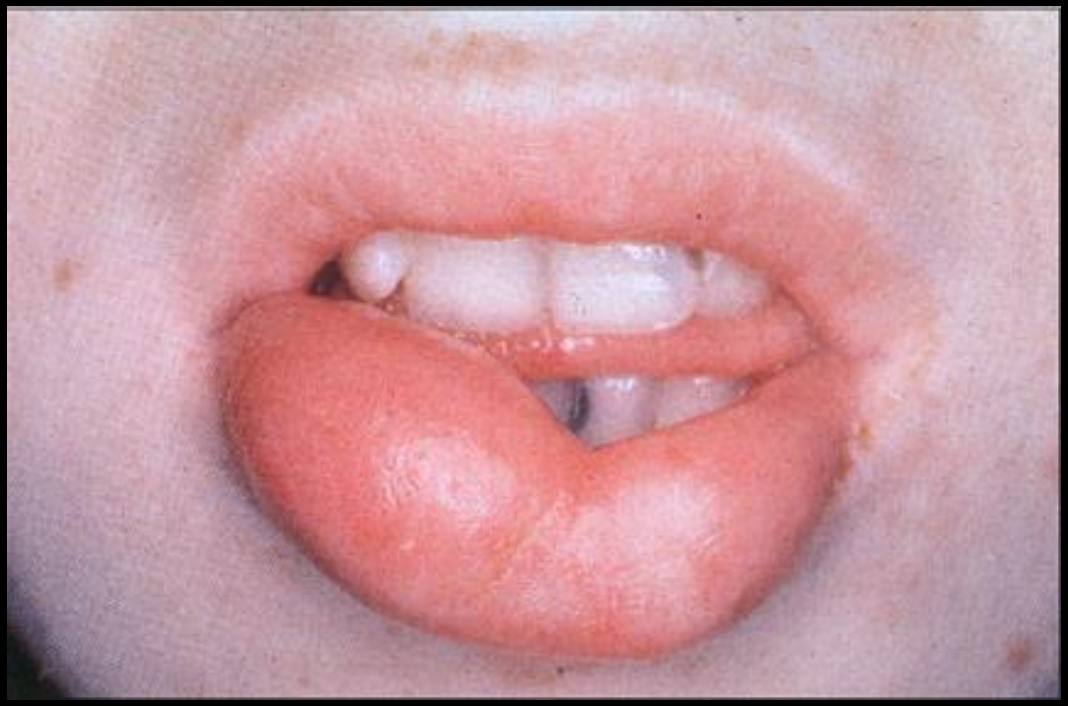


- several proteins regulate the classical pathway C3 convertase by binding to C4b and displacing C2a from the complex


B. Alternative pathway
Factor H
DAF
C3b
Bb
CRI
MCP
Cell surface
Bb
Dissociation
of C3b8b
Factor I
CRI, MCP
or
Factor H
C3b
iC3b
C3c
C3d
Cleavage
of C3b

If out of control…

C1-inhibitor deficiency: angioedema



- The skin of the face, normally around the mouth swell up over the period of minutes to several hours. The swelling can also occur elsewhere, typically in the hands. Hives may develop simultaneously.
- There is often no direct identifiable cause, although mild trauma, including dental work and other stimuli, can cause attacks
- Abdominal pain, usually accompanied by intense vomiting, weakness, and in some cases, watery diarrhea. These stomach attacks can last anywhere from 1–5 days on average, and can require hospitalization for aggressive pain management and hydration.

- Cobra venom factor functions like human C3b to activate C when added to human plasma, and not inhibited by human regulators

microbial complement evasion strategies

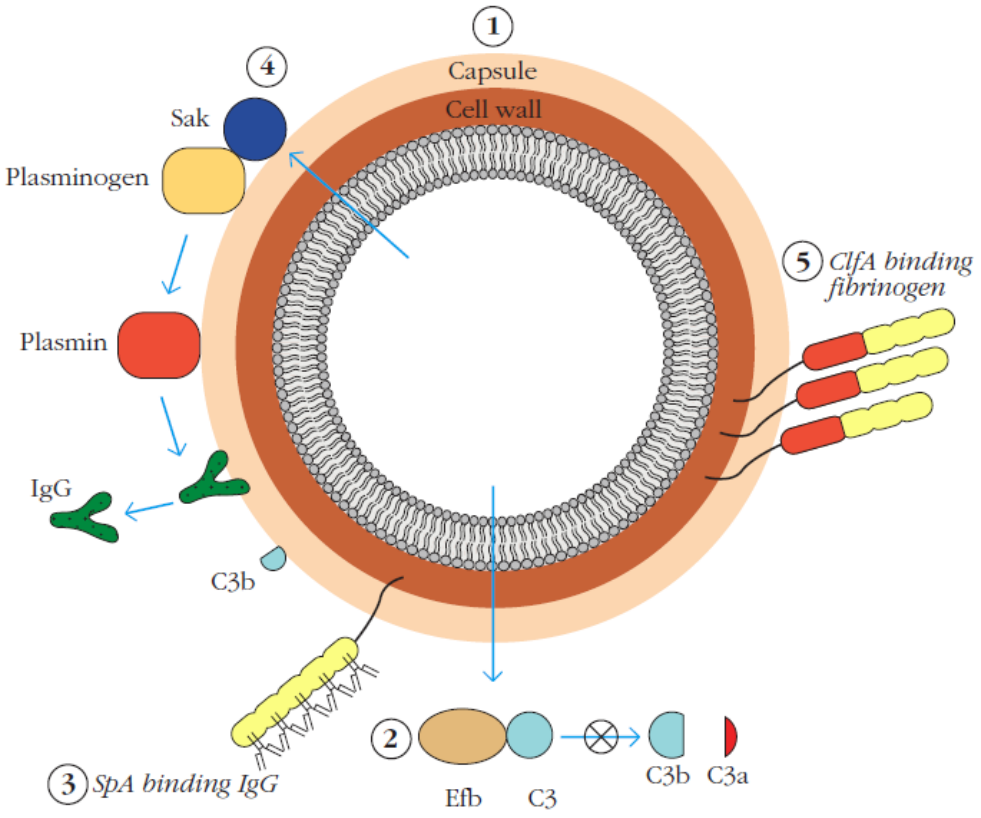


FIGURE 1
Mechanisms by which *S. aureus* avoids opsonophagocytosis. (1) The capsular polysaccharides denies access of neutrophils to opsonized bacteria. (2) The extracellular fibrinogen binding protein (Efb) binds C3, preventing it from reaching the cell surface and inhibiting further activation of the complement cascade. (3) Protein A (SpA) binds IgG in a conformation that does not permit Fc receptor binding. (4) Staphylokinase (Sak), secreted by the bacterium, activates plasminogen, a protease capable of cleaving and inactivating IgG and C3b. (5) Clumping factor A binds factor I and localizes it to the microbial surface, where it cleaves and inactivates any C3b that binds there. *[Adapted from Foster, T. J., 2005. Immune evasion by Staphylococci.* Nature Reviews Microbiology ***3:*** *948–958, Figure 3.]*

Summary

- **Definition and characteristics**
- **Major component**
  - **Structure of C1, MAC**
- **Three pathways**
  - **Activator**
  - **C3, C5 convertase**
- **Major Functions**

Complement activation pathways