COMPLEMENT SYSTEM

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UG SEM 4 (H)
Immunology

History

•Research on complement began in the 1890s, when **Jules Bordet at the Institut Pasteur in Paris** showed that sheep antiserum to the bacterium *Vibrio cholerae* caused lysis of the bacteria and that heating the antiserum destroyed its bacteriolytic activity.

• He named those substances as Alexins.

• Paul Ehrlich coined the term complement.

Introduction

- It is named "complement system" because it was first identified as a heat-labile component of serum that ***"complemented or augment"*** antibodies in the killing of bacteria.

- Consists of serum and cell surface proteins involved in defense against pathogens and tissue damage mediated by antibodies.

- The Complement system is the major effector of cellular and humoral branch of immune system.

- Plays major role in both innate and adaptive immunity.

Introduction

- Complement system represents a group of about 30 proteins which augment or complement the immune response.
- Most of these proteins are found in serum or on cell surfaces.
- Synthesized in liver as inative precursors and are activated by proteolysis durig their interaction in a sequential manner.
- Also produced by blood monocytes, tissue macrophages and epithelial cells of he gastrointestinal and genitourinary tract.

General Properties

- Present in serum of all animals but its concentration is maximum in serum of guinea pig.
- Complement of one species are able to react with antibodies of other species but not to the same extent.
- C- proteins constitute about 5% of normal serum protein.
- Are glycoproteins.

General Properties

- Are synthesized rapidly in inflammatory responses – hence are called acute phase proteins.

- Heat labile and lost activity at 56^0 C for 30 mins and inactivated. Immunoglobulins are not inactivated at this temperature.

- Binds with Fc potion of immunoglobulns .

Effects Of Complement

1. Lysis of cells (bacteria, allografts, tumor cells)

2. Generation of mediators of inflammation

3. Opsonization – enhancement of phagocytosis

❑Complement components

•Components are designated by numbers (E.g. ; C1 – C9) or latters (E.g. : Factor D)

•(in serum inactive, activated sequentially as a cascade)

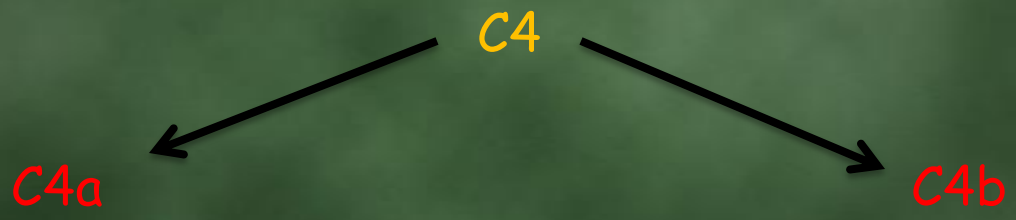
❑Complement receptors

•cell surface, recognize activated components

❑Regulatory proteins of complement

•both in serum and cell surface, inhibit activated components)

Complement Activation By Cleavage



a = smaller fragment.
-Diffusion

b= larger fragment.
-remains bound to microbe

Exception: C2:
C2a = large fragment
C2b = small fragment

Complement Pathway

Classical pathway:-

Is antibody dependent pathway and triggered by formation of soluble antigen-antibody complex or by binding of the antibody to the antigen present on the target cell surface.

Alternative pathway:-

Is antibody independent pathway stimulated by antigen directly eg. Bacterial cell surface components.

Lectin Pathway:-

Also antibody independent but resembles classical pathway.

Stages of Complement Activation

Three main stages in the activation of complement by any pathway are

- **Formation of C3 convertage**
- **Formation C5 convertage**
- **Formation of membrane attack complec(MAC)**

The initiation and formation of C3 convertage are different in classical and alternative pathway. These then follow the parralel route to merge at C5 convertage stage and finally generate the MAC by a common route.

Complement Activation

Sequential activation of complement components occurs via one of three pathways:

- the classic pathway,
- the lectin pathway, and
- the alternative pathway.

Of these pathways, the **lectin and the alternative pathways are more important the first time** we are infected by a microorganism because the antibody required to trigger the classic pathway is not present.

The lectin pathway and the alternative pathway are, therefore, participants in the innate arm of the immune system.

Complement Activation

All three pathways lead to the production of **C3b, the central molecule** of the complement cascade.

The presence of C3b on the surface of a microbe marks it as foreign and targets it for destruction. C3b has two important functions:

1. It combines with other complement components to generate C5 convertase, the enzyme that leads to the production of the membrane attack complex

2. It opsonizes bacteria because phagocytes have receptors for C3b on their surface.

Complement Activation

- Part of acquired immunity.

- In the **classic** pathway, **antigen–antibody complexes** activate **C1 to form a protease**, which cleaves **C2 and C4** to form a **C4bC2a** complex, C2a and C4b split off.

- The **C4bC2a is C3 convertase**, which cleaves **C3** molecules into two fragments, **C3a and C3b.**

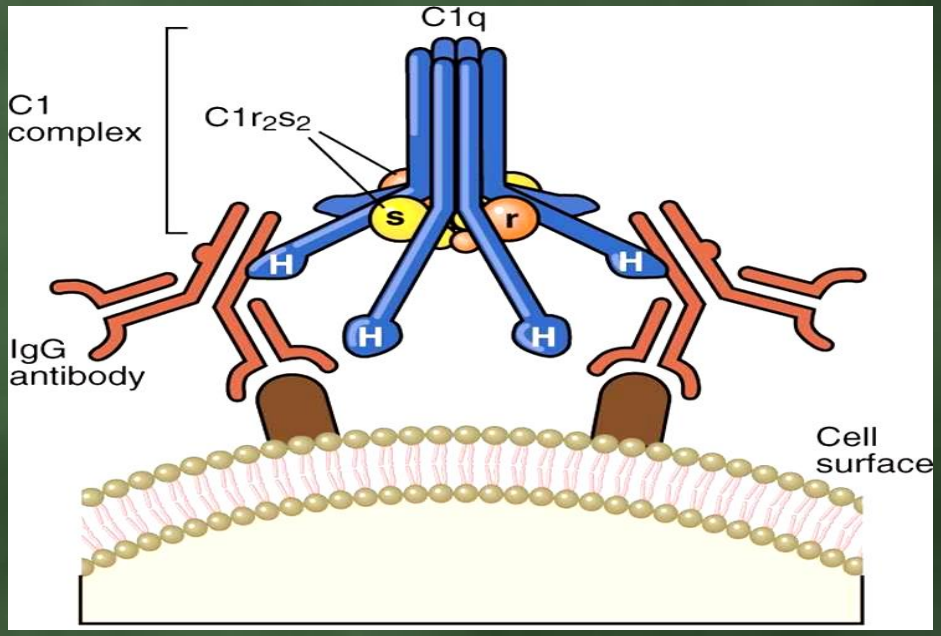
- C5b binds to **C6 and C7** to form a complex that interacts **with C8 and C9** to produce the **membrane attack** complex **(C5b,6,7,8,9**), which causes cytolysis.

Complement Activation

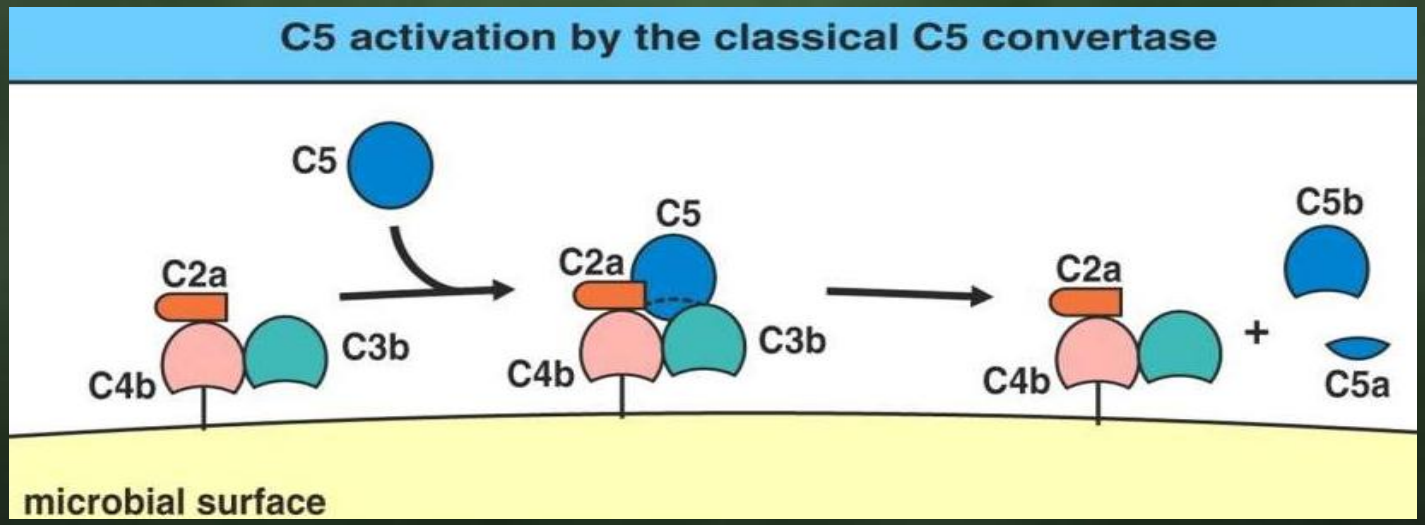
- Only IgM and IgG fix complement.
- One molecule of IgM can activate complement; however, activation by IgG requires two cross-linked IgG molecules.
- Of the IgGs, only IgG1, IgG2, and IgG3 subclasses fix complement; IgG4 does not.
- C1 is bound to a site located in the Fc region of the heavy chain.

Complement Activation

- C1 is composed of three proteins, C1q, C1r, and C1s.
- C1q is an aggregate of 18 polypeptides that binds to the Fc portion of IgG and IgM.
- It is multivalent and can cross-link several immunoglobulin molecules.
- C1s is a pro-enzyme that is cleaved to form an active

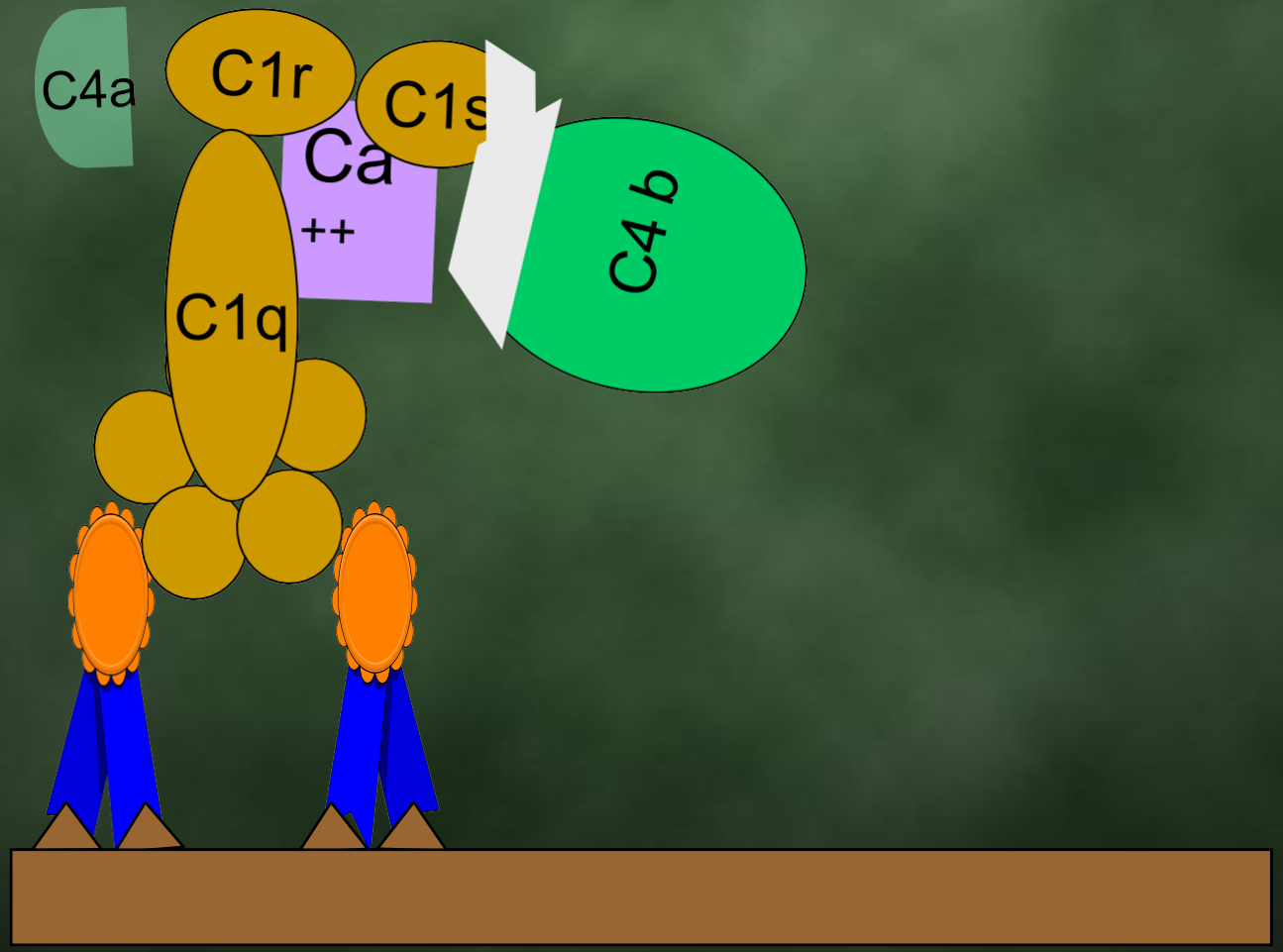


C5 convertase

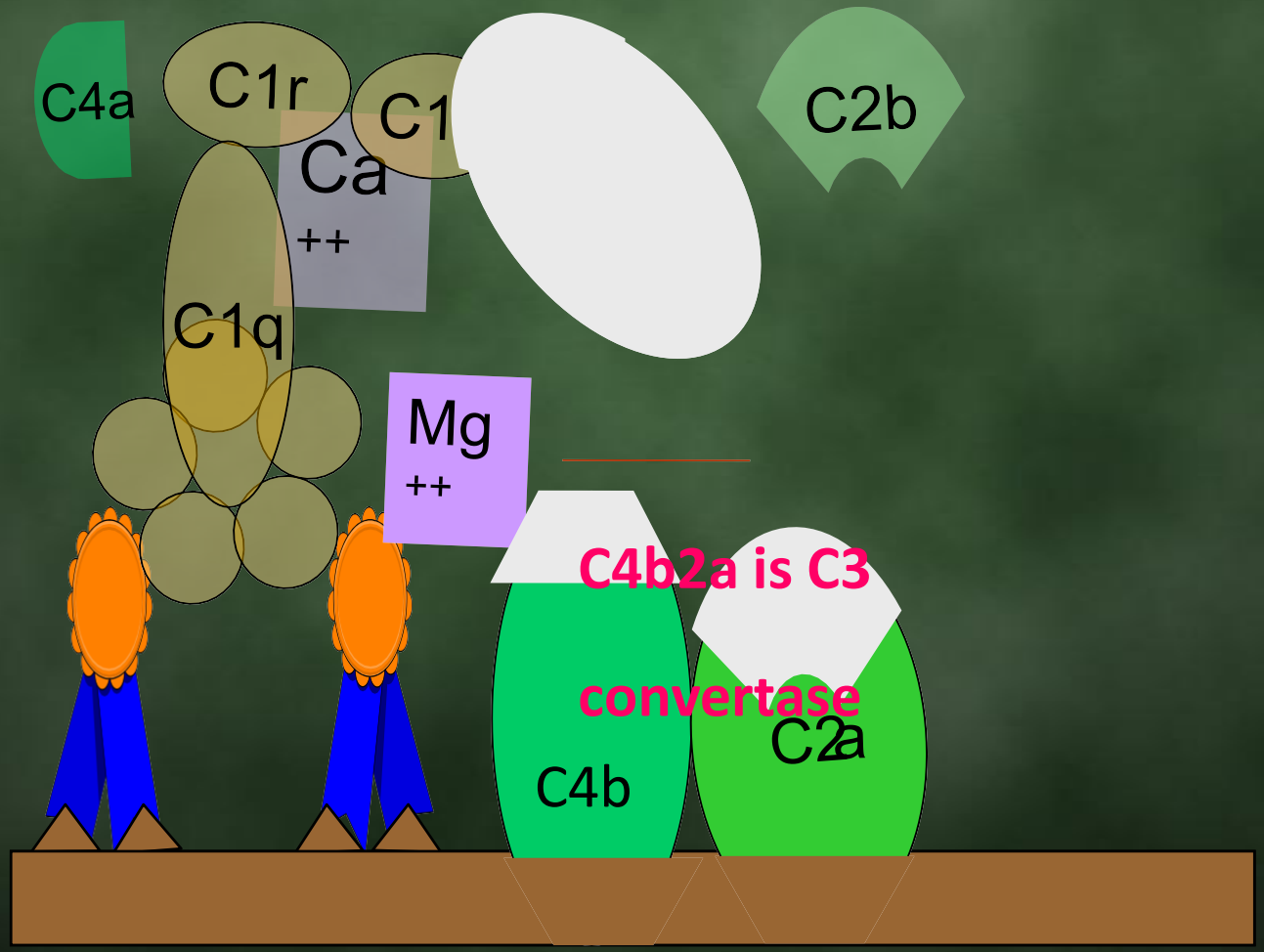


C4b-2a-3b functions as the classical

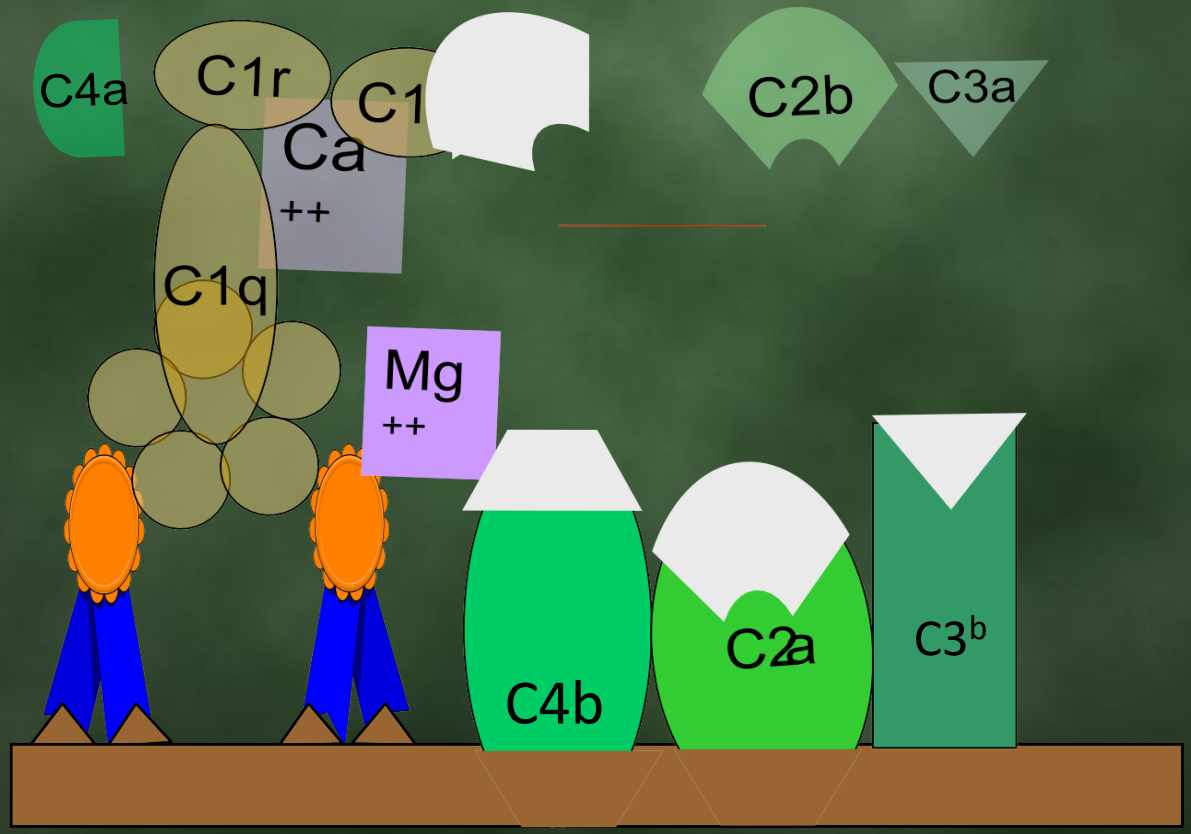
Classical Pathway Generation of C3-convertase



Classical Pathway Generation of C3-convertase

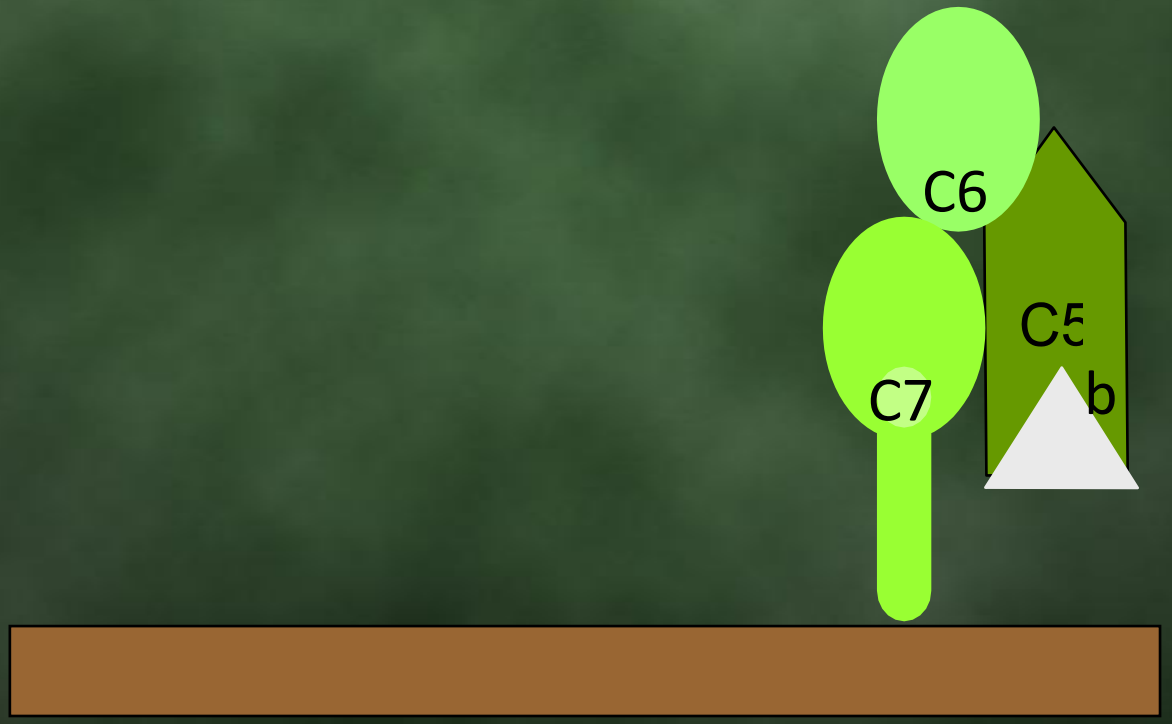


Classical Pathway Generation of C5-convertase

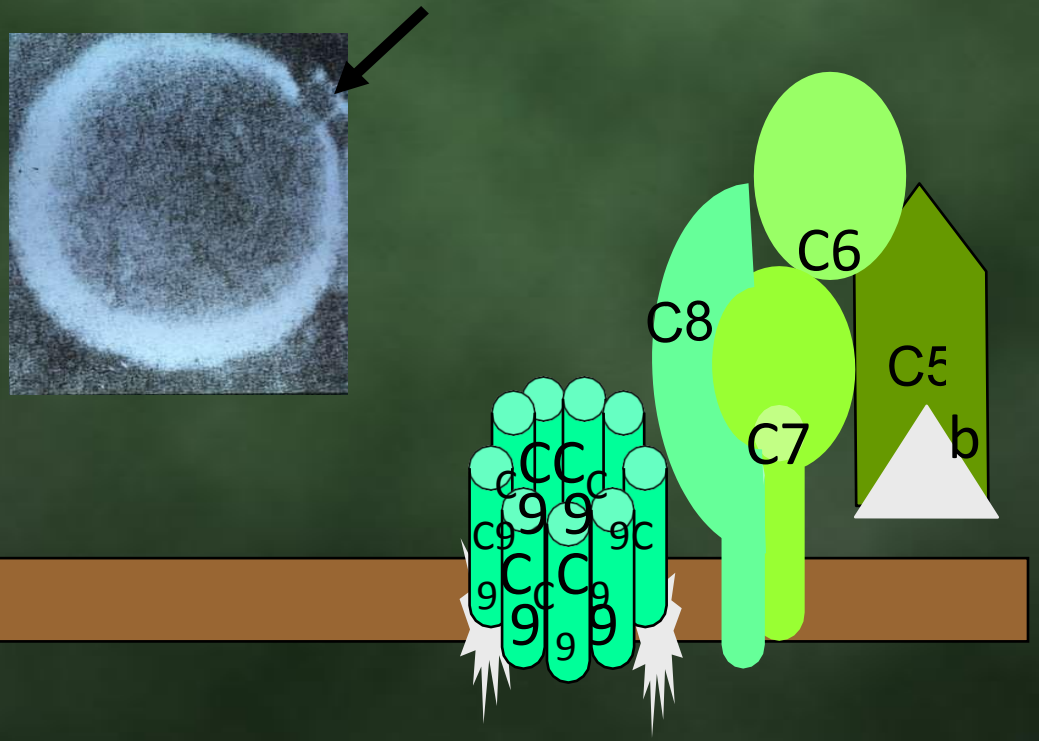


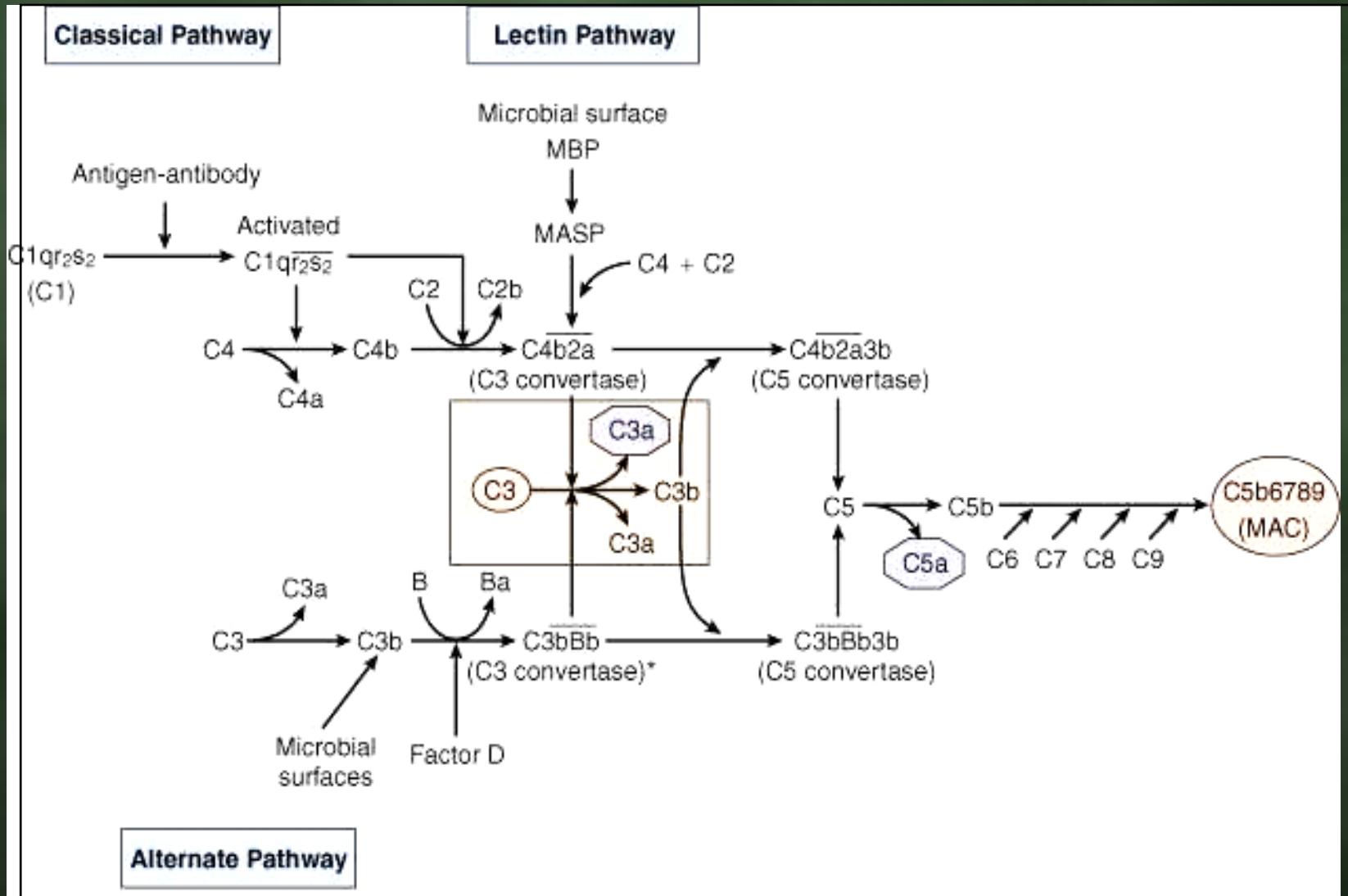
C4b2a3b is C5 convertase; it leads into the Membrane Attack Pathway

Lytic pathway assembly of the lytic complex



Lytic pathway insertion of lytic complex into cell membrane





Component Of the Classical Pathway

Native component	Active component(s)	Function(s)
C1(q,r,s)	C1q	Binds to antibody that has bound antigen, activates C1r.
	C1r	Cleaves C1s to activate protease function.
	C1s	Cleaves C2 and C4.
C2	C2a	Unknown.
	C2b	Active enzyme of classical pathway; cleaves C3 and C5.
C3	C3a	Mediates inflammation; anaphylatoxin.
	C3b	Binds C5 for cleavage by C2b. Binds cell surfaces for opsonization and activation of alternate pathway.
C4	C4a	Mediates inflammation.
	C4b	Binds C2 for cleavage by C1s. Binds cell surfaces for opsonization.

Components of the Membrane-Attack Complex

Native component	Active Component(s)	Functions
C5	C5a	Mediates inflammation; anaphylatoxin, chemotaxin.
	C5b	Initiates assembly of the membrane-attack complex (MAC).
C6	C6	Binds C5b, forms acceptor for C7.
C7	C7	Binds C5b6, inserts into membrane, forms acceptor for C8.
C8	C8	Binds C5b67, initiates C9 polymerization.
C9	C9n	Polymerizes around C5b678 to form channel that causes cell lysis.

Alternative Pathway

- Ab independnt pathway.
- In the **alternative** pathway, many unrelated cell surface substances, e.g., bacterial lipo-polysaccharides (endotoxin), fungal cell walls, and viral envelopes, can initiate the process by binding C3 and factor B.
- This complex is cleaved by a protease, factor D, to produce **C3bBb**.
- This acts as a **C3 convertase** to generate more C3b.
- **Alternative pathways are more important the first time** we are infected by a microorganisms.
- **Usually activated by products of micro-organisms like endo-toxin.**

Alternative Pathway

Other activators include:

- **Complexes containing IgA**
- **Some virus-infected cells (e.g. EBV)**
- **Many gram negative and gram positive organisms**
- **Parasites – Trypanosomes, Leishmania**
- **Erythrocytes**
- **Carbohydrates (agarose)**

Components Of Alternative Pathway

Native component	Active component(s)	Function(s)
C3	C3a	Mediates inflammation; anaphylatoxin.
	C3b	Binds cell surfaces for opsonization and activation of alternate pathway.
Factor B	B	Binds membrane bound C3b. Cleaved by Factor D.
	Ba	Unknown.
	Bb	Cleaved form stabilized by P produces C3 convertase.
Factor D	D	Cleaves Factor B when bound to C3b.
Properdin	P	Binds and stabilizes membrane bound C3bBb.

Lectin Pathway/ MBL Pathway

- In the lectin pathway, mannan-binding lectin (MBL) (also known as mannose-binding protein) binds to the surface of microbes bearing mannan (a polymer of the sugar, mannose).

- Binding causes activation of MASP (MBP- associated serine proteases) à cleave C2 and C4 and activate the classic pathway.

- Note that this process bypasses the antibody-requiring step and so is protective early in infection before antibody is formed.

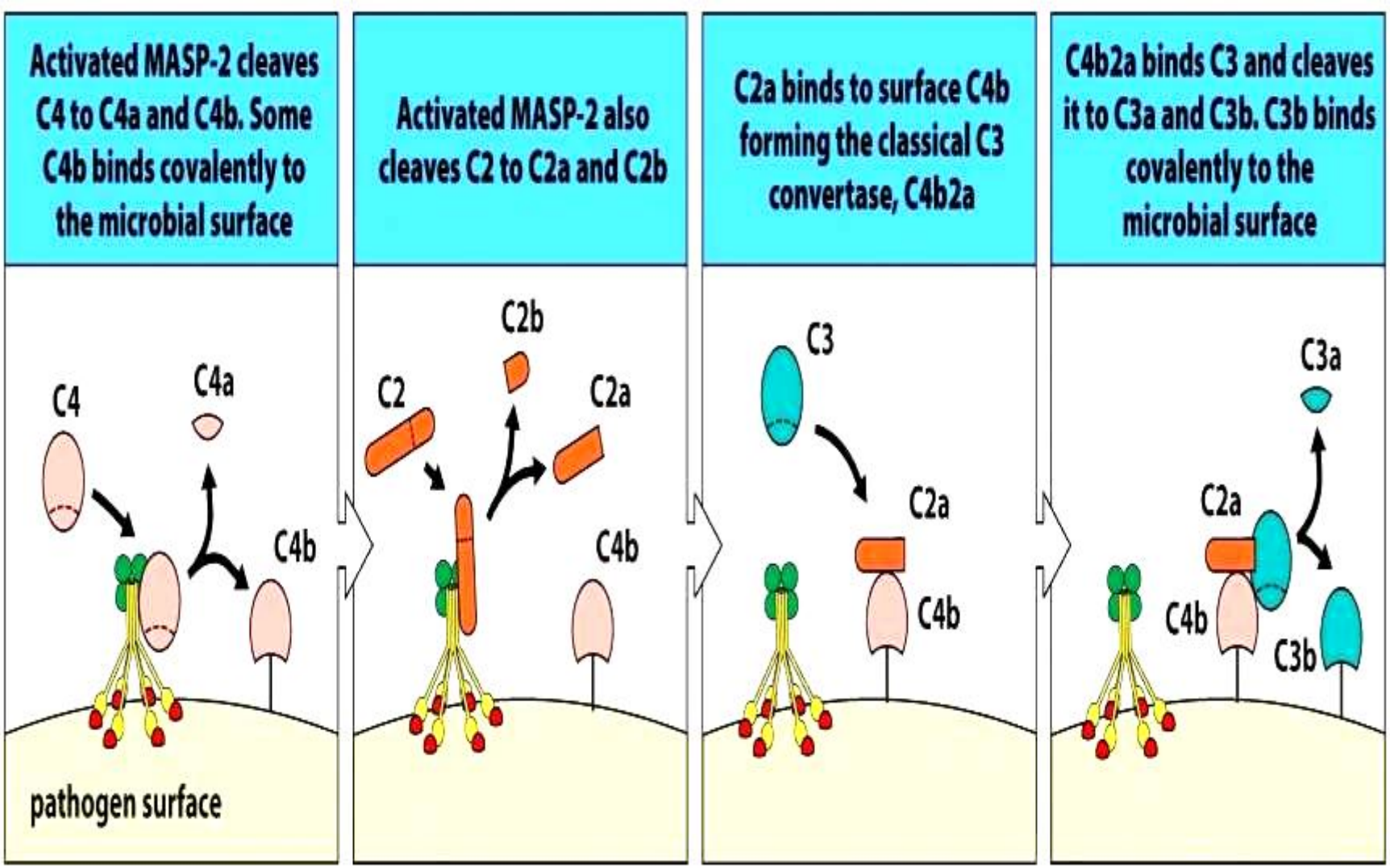
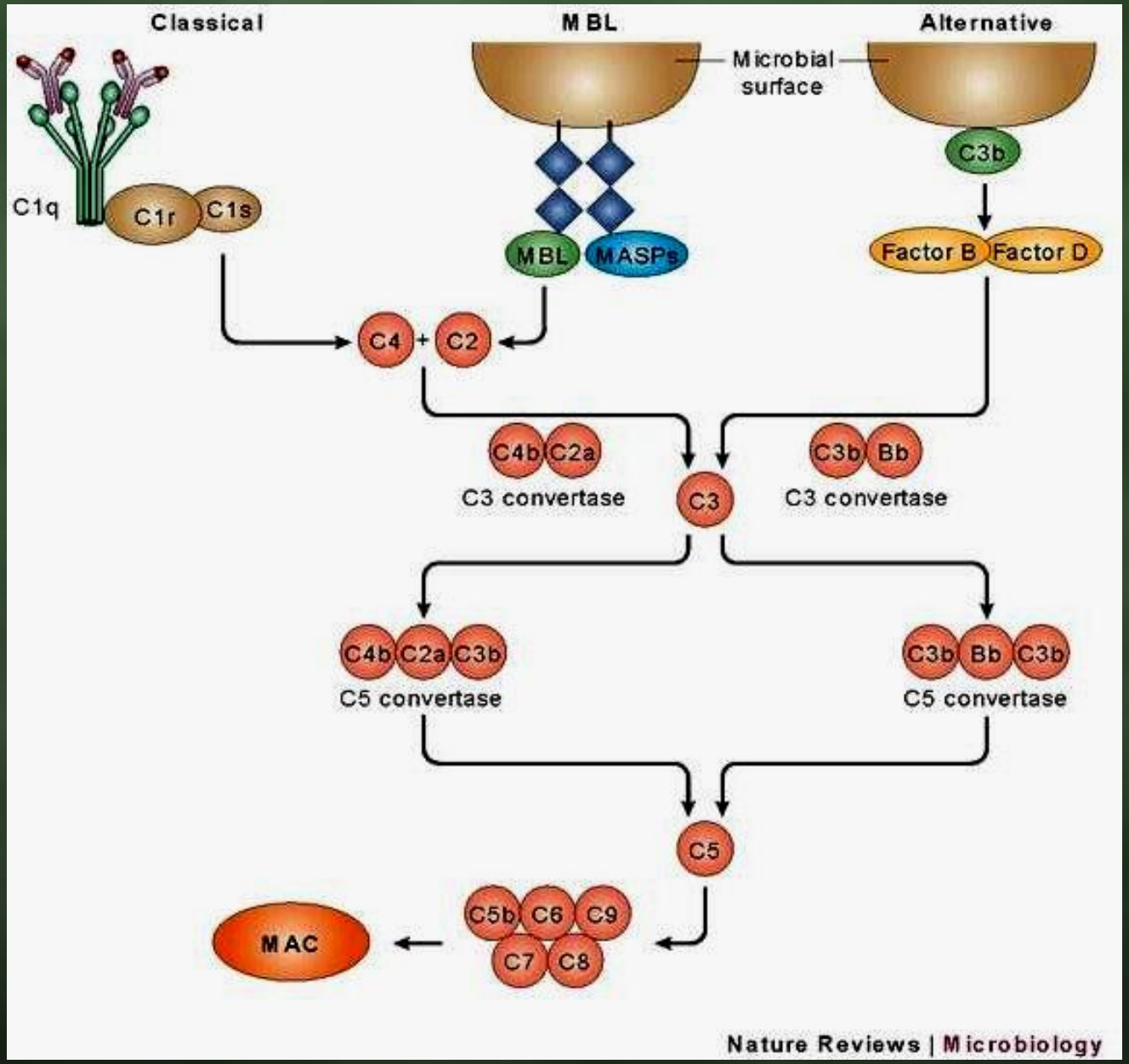


Figure 2.40 The Immune System, 3ed. (© Garland Science 2009)


Classical
MBL
Alternative
Microbial surface
C1q
C1r
C1s
MBL
MASPs
C3b
Factor B
Factor D
C4
+
C2
C4b C2a
C3 convertase
C3
C3b Bb
C3 convertase
C4b C2a C3b
C5 convertase
C3b Bb C3b
C5 convertase
C5
C5b C6 C9
C7 C8
MAC
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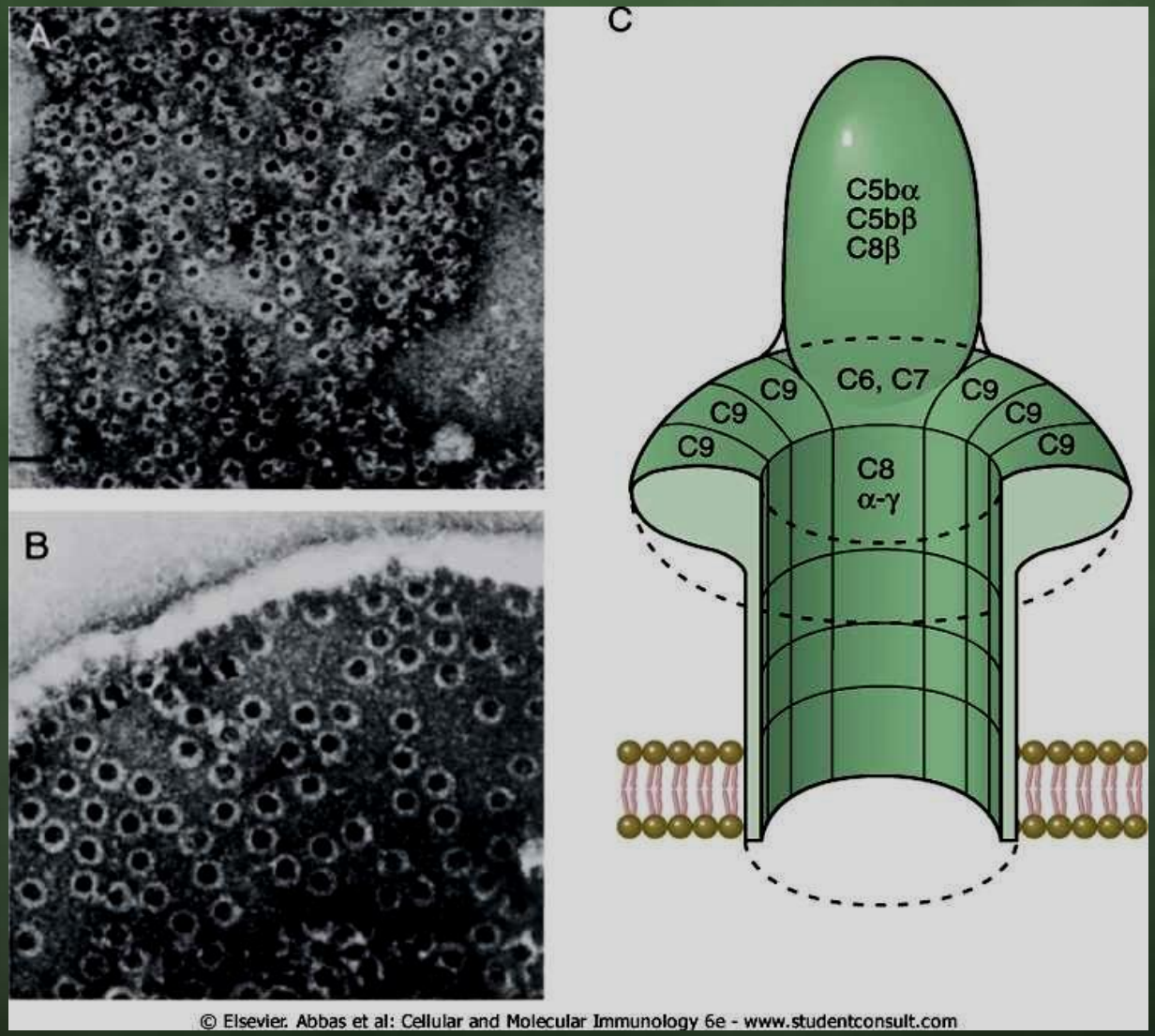
Membrane attack Pathway

- Cleavage of C5 into C5a and C5b.
- C5 (structurally homologous to C3 and C4, lacks internal thioester bond).
- C5b initiates formation of MAC (complex of C5b, C6, C7, C8 and multiple C9 molecules) binds to C6, and C7 , recruits C8 and complex penetrates more deeply into the membrane.
- C9, a pore-forming molecule with homology to perforin. The complex of C5b678 forms a nidus for C9 binding and polymerization.
- Penetrates membrane bilayers to form pores Disrupt the osmotic barrier, leading to swelling and lysis of susceptible cells

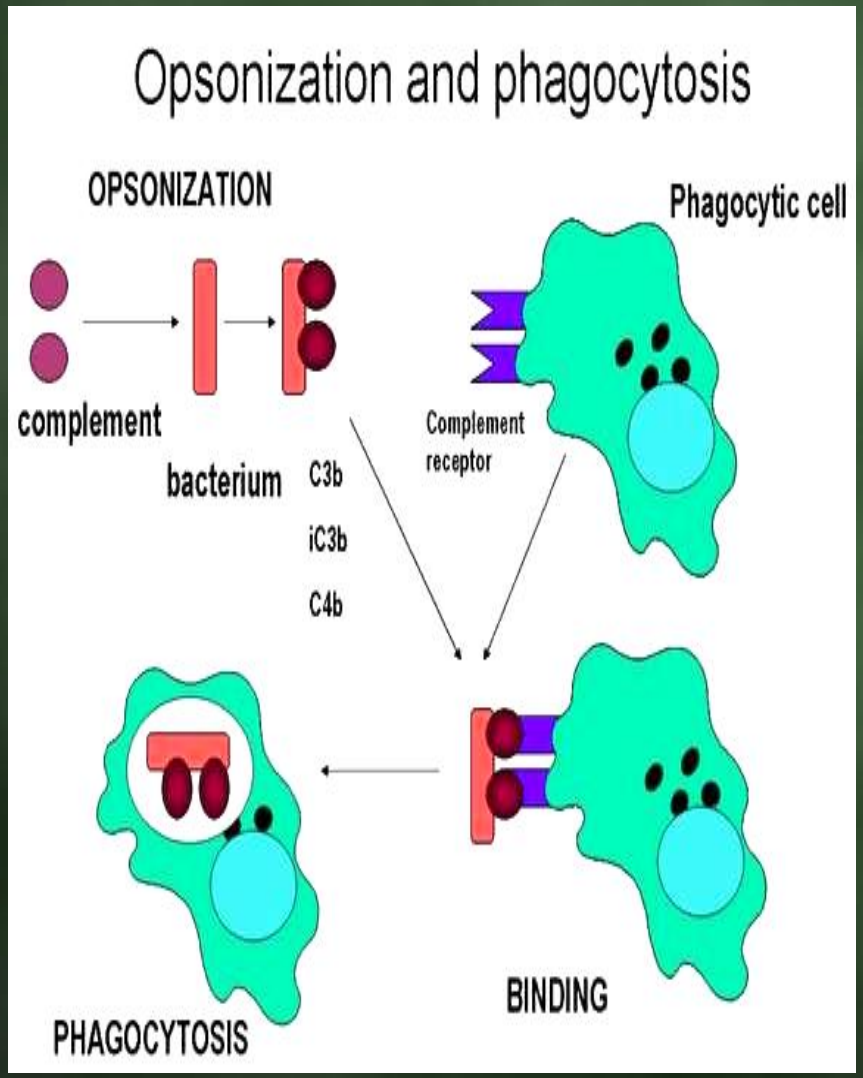


Biologic Effect Of Complement

1. **Opsonization**
   - **C3b & C1q; enhance phagocytosis.**

2. **Chemotaxis**
   - **C5a and C5,6,7 complex → attract neutrophils**
   - **C5a – enhance adhesiveness of neutrophils to the Endothelium.**

3. **Anaphylatoxin (C3a, C4a, C5a)**
   - **Cause degranulation of mast cells**
   - **Bind directly to smooth muscles of bronchioles →bronchospasm**



4. Cytolysis (MAC)

- **Disrupt the membrane & the entry of water and electrolytes into the cell**

5. Enhancement of antibody production

- **Binding of C3b to its receptors on the surface of activated B cells → enhanced antibody production**

❑**C3b is an opsonin.** Opsonins are molecules that bind both to bacteria and phagocytes. Opsonization increases phagocytosis by 1,000 fold.

Regulation Of Complement System

1. **C1 inhibitor**

- Important regulator of classic pathway
- A serine protease inhibitor (serpin)
- Irreversibly binds to and inactivates C1r and C1s, as well as MASP in lectin pathway

2. **Factor H**

- Regulate alternative pathway
- Reduce amount of C5 convertase available
- With both cofactor activity for the factor I- mediated C3b cleavage, and decay accelerating activity against C3bBb (C3 convertase)

3. Properdin

- Protects C3b and stabilizes C3 convertase

4. Factor I

- Cleaves cell-bound or fluid phase C3b and C4b → inactivates C3b and C4b

5. Decay accelerating factor (DAF)

- Glycoprotein on surface of human cells
- Prevents assembly of C3bBb or accelerates disassembly of preformed convertase à no formation of MAC
- Acts on both classical and alternative

4. C4b-binding protein (C4BP)

- Inhibits the action of C4b in classical pathway
- Splits C4 convertase and is a cofactor for factor I

5. Complement Receptor 1 (CR-1)

- Co-factor for factor I, together with CD46

6. Protectin (CD59) and Vitronectin (S protein)

- Inhibits formation of MAC by binding C5b678
- Present on "self" cells to prevent complement from damaging them