The Complement system

Harsha Perera

Complements

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Introduction

- Oldest component in immune system
- Bathing fluids surrounding tissues
- Found in inactive form
- Activated sequentially / cascade manner
- Activation of one promotes the next protein
- Play an important role in host defense
- Inappropriate activation / deficiency lead to disease

The complement system

- A defensive system / innate immunity
- Over 30 proteins produced by the liver
- Found in circulating blood serum

- Complement kills microbes in

. Opsonization

Inflammation

Cytolysis

Overview

- Part of innate immune system
- It is named “complement system” because it was first identified as a heat-labile component of serum that “complemented” antibodies in the killing of bacteria
- 30 proteins /3 g/L to overall serum protein

A Cascade system

- Operates as a cascade system

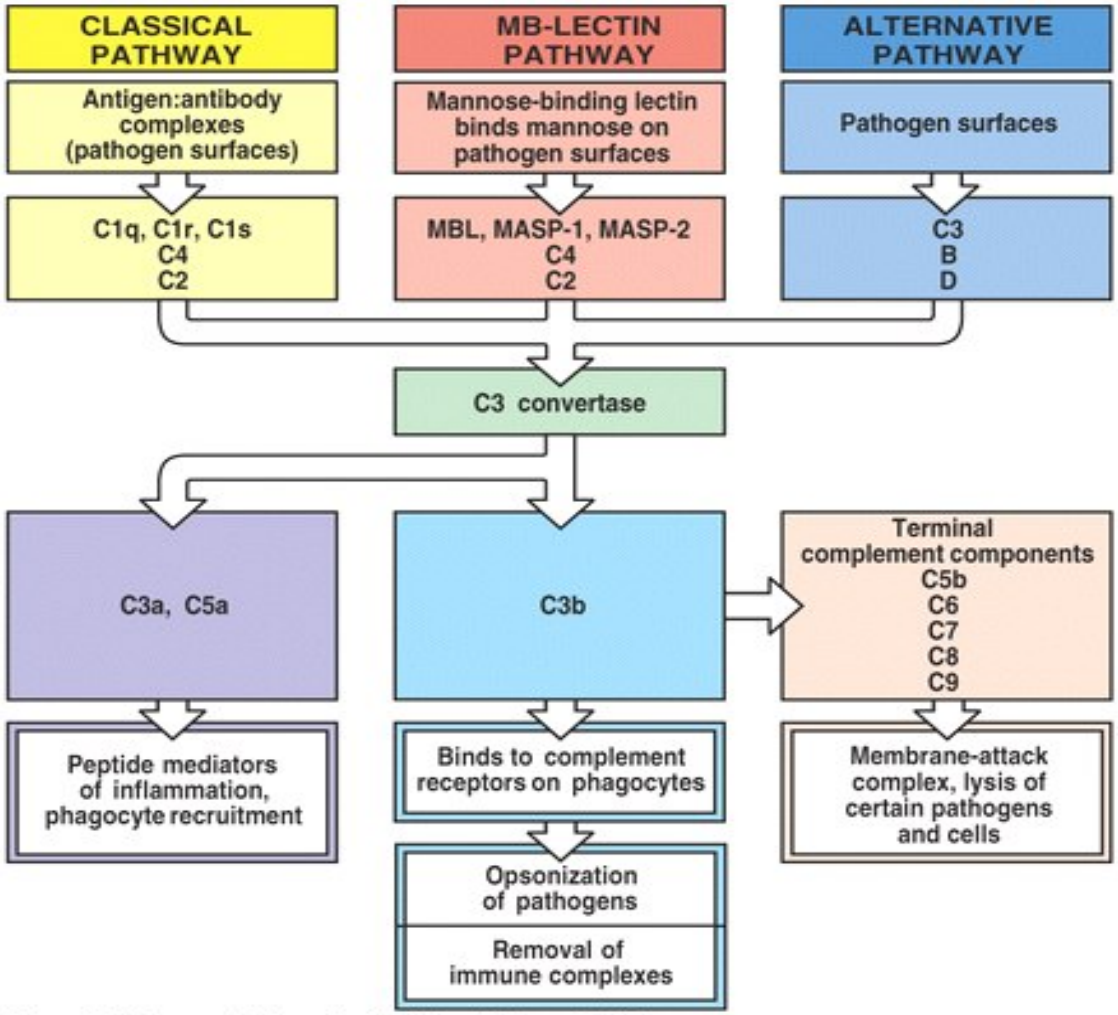


Figure 2-19 Immunobiology, 6/e. (© Garland Science 2005)

Cascade activation

- Inactive complement proteins designated by an uppercase letter C (un split)
  - Eg: C1
- Once split become active
- The active products designated with a lower case a or b
  - Eg: C1a and C1b

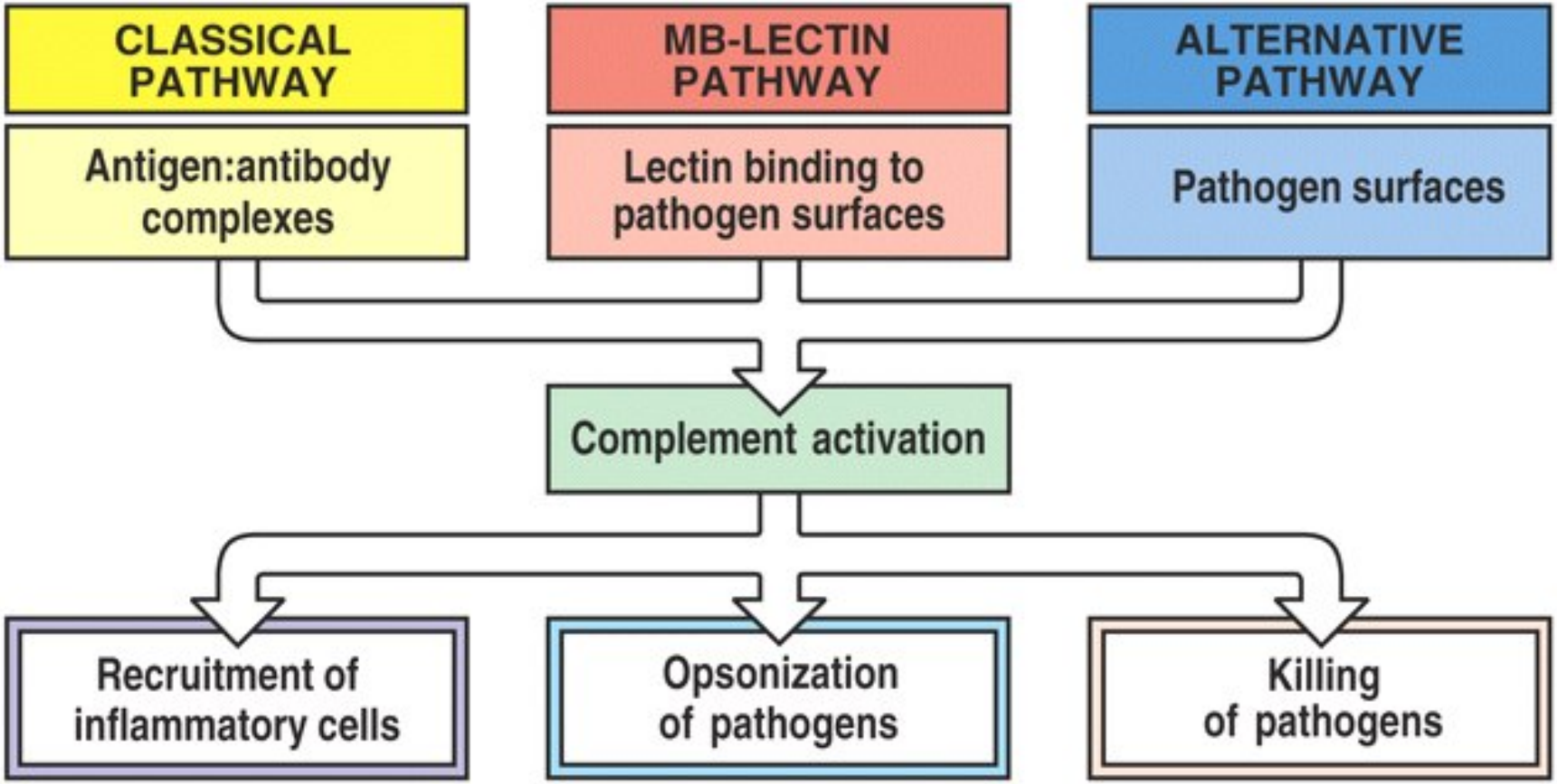


Figure 2-18 Immunobiology, 6/e. (© Garland Science 2005)

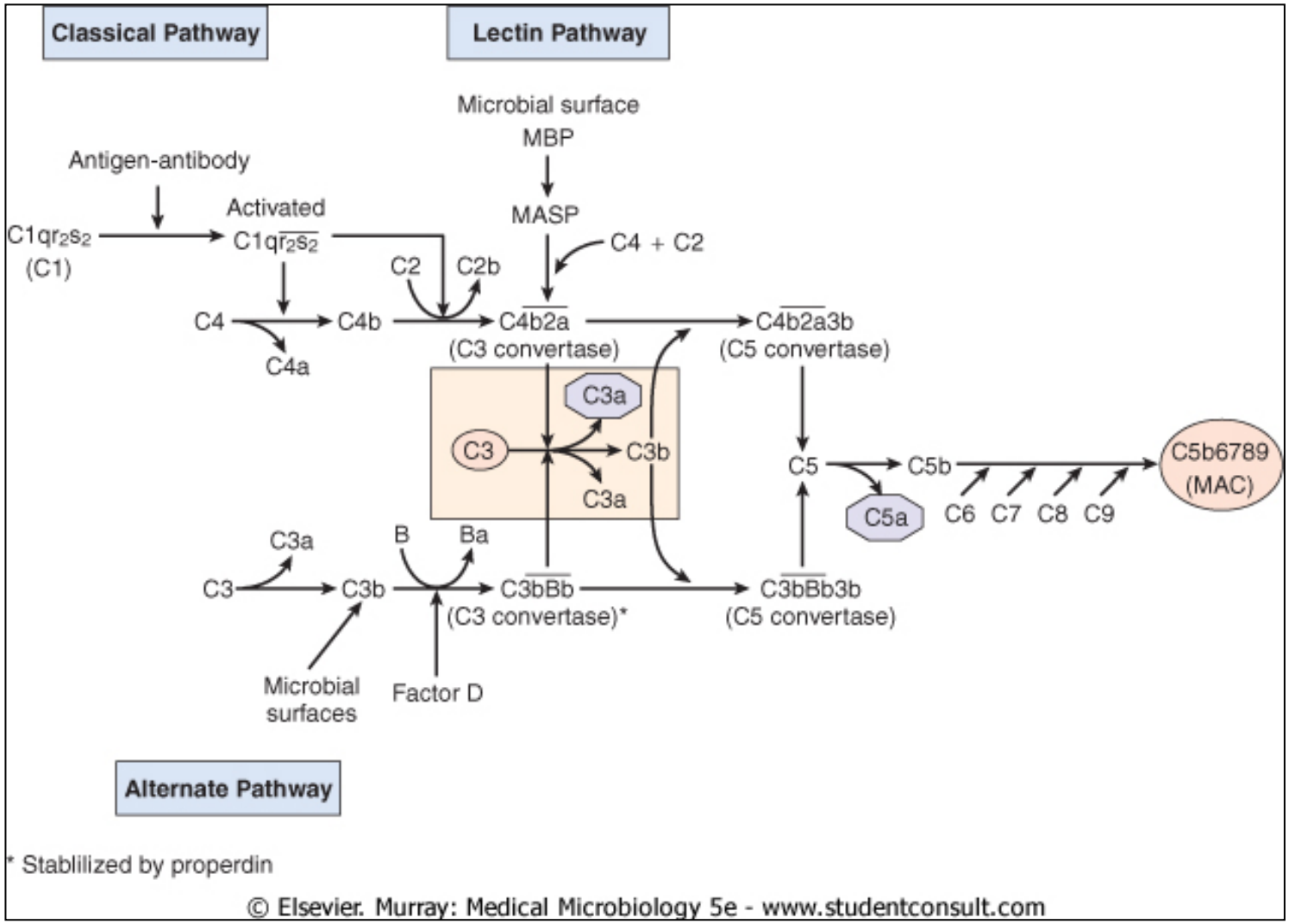


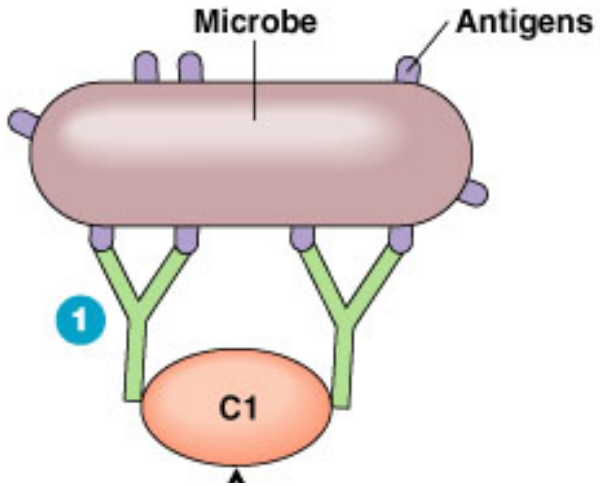
Figure 12-8 The classical, lectin, and alternate complement pathways. Despite different activators, the goal of these pathways is cleavage of C3 and C5 to provide chemoattractants and anaphylotoxins (C3a, C5a), an opsonin (C3b) that adheres to membranes, and to initiate the membrane attack complex to kill cells. MASP, MBP-associated serine protease; MBP, mannose-binding protein. (Redrawn from Rosenthal KS, Tan JS: Rapid review microbiology and immunology, St. Louis, 2002, Mosby.)

Three Pathways

- The complement pathway can be activated by either of three different pathways
  - Classical pathway (specific immune system)
  - alternative pathway (non-specific immune system)
  - Lectine binding pathway

The Classical Pathway

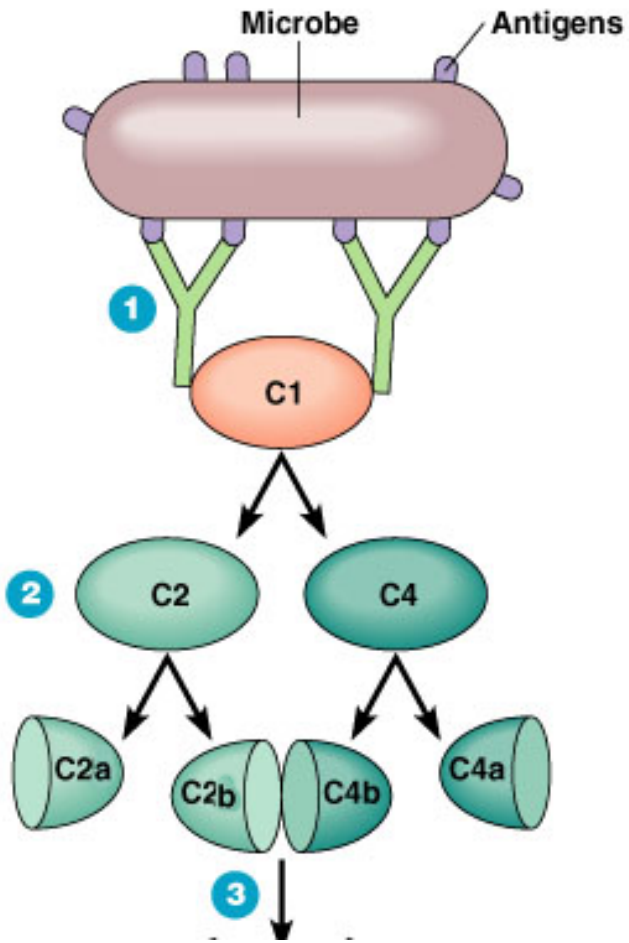
- Part of specific immune response (relies on antibodies to initiate the reaction)
- C1 becomes activated once it binds to the ends of antibodies



The building of a C3 activation complex

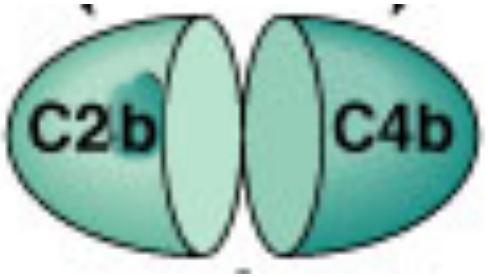
- Once C1 is activated
- Triggers 2 other complement proteins:
  - C2 and C4 by cutting them in half
- C2 → C2a and C2b
- C4 → C4a and C4b

- C2b and C4b on the surface of bacteria
- C2a and C4a diffuse away

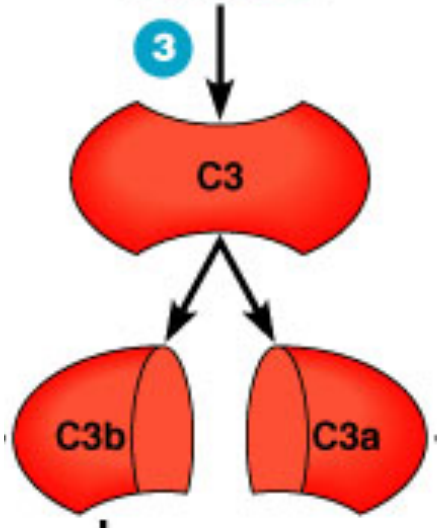


C3 Activation complex

- C2b and C4b bind on the surface
- Form C3 activation complex



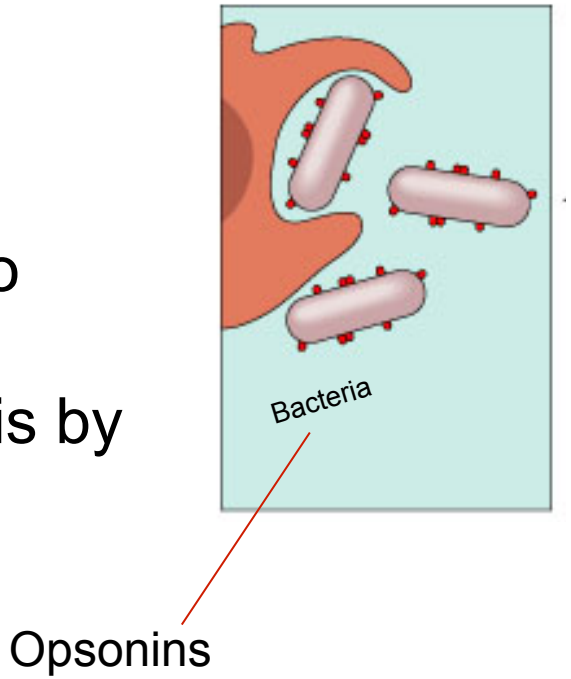
- The function of the C3 activation complex is to activate C3 proteins
  - Cleaving C3 into C3a and C3b

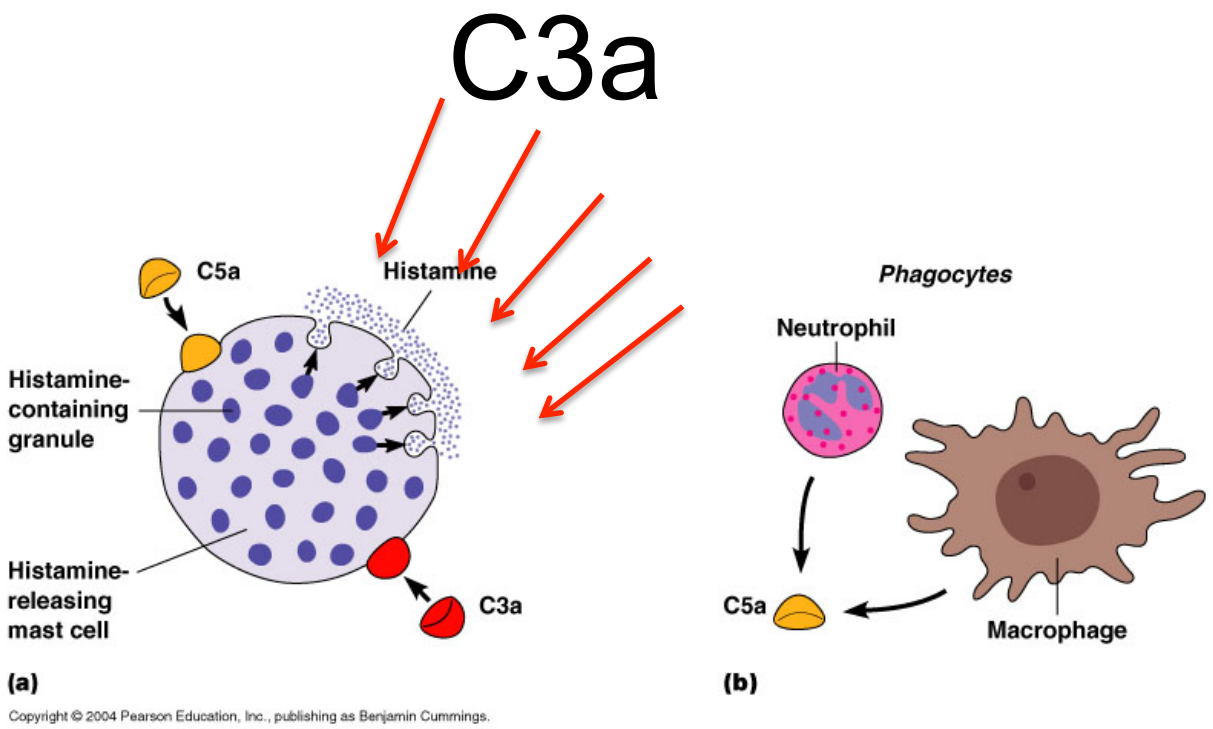


C3b

- The C3b bind to and coat the surface of the bacteria

- C3b is an opsonin
  - Opsonins are molecules bind both to bacteria and phagocytes
  - Opsonization increases phagocytosis by 1000 fold

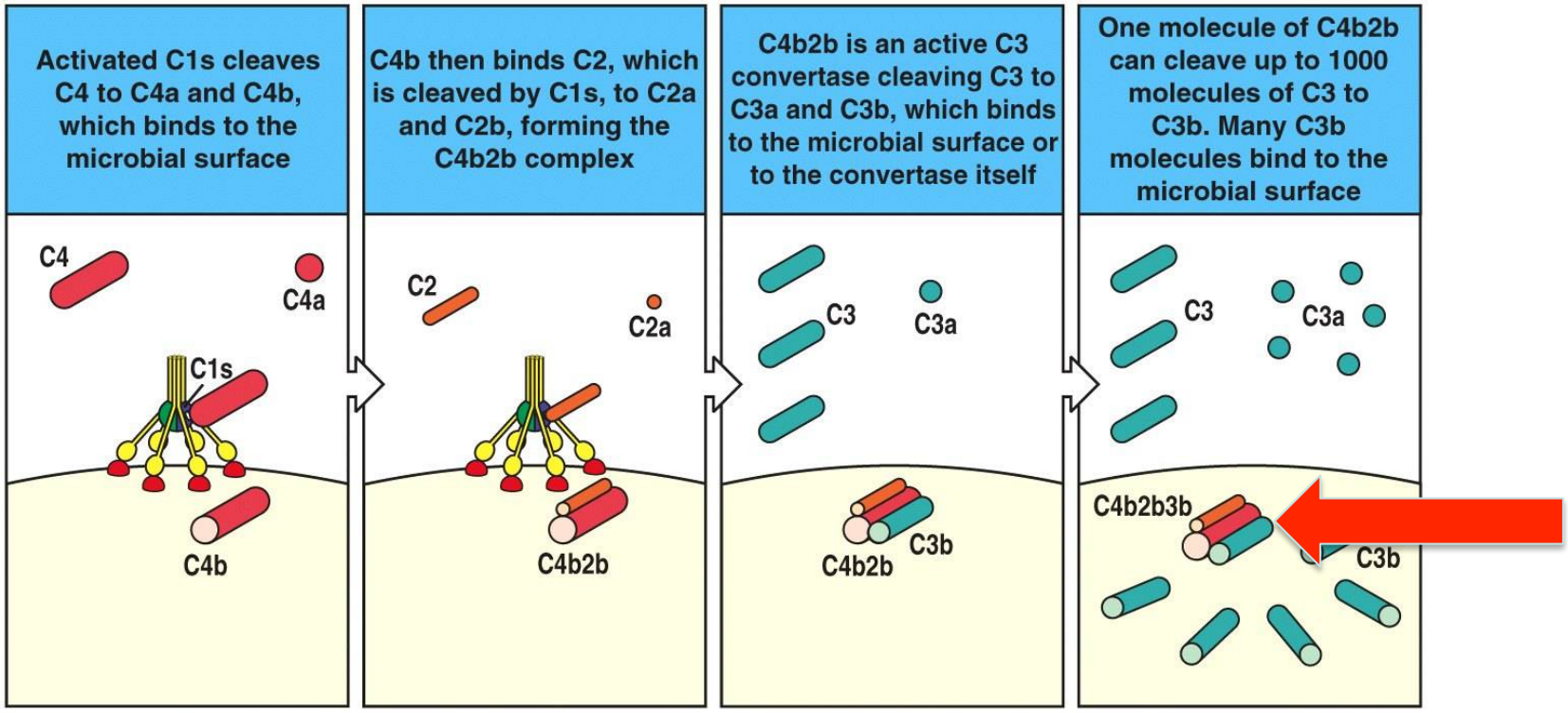




- C3a increases the inflammatory response
- Binding to mast cells and trigger to release histamine

Building the C5 activation complex

- C3b binds to C2b and C4b
- Forms a new complex:
  C5 activation complex (C4bC2bC3b)



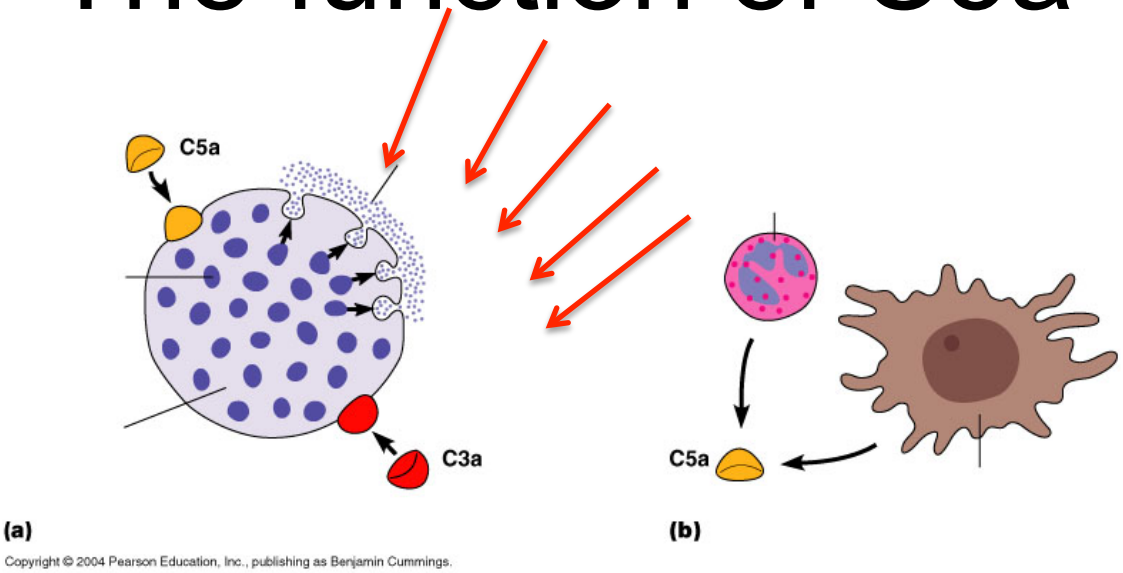
The C5 activation complex

- The C5 activation complex (C2b, C4b & C3b) activates C5 proteins

  C5 ⟶ C5a and C5b

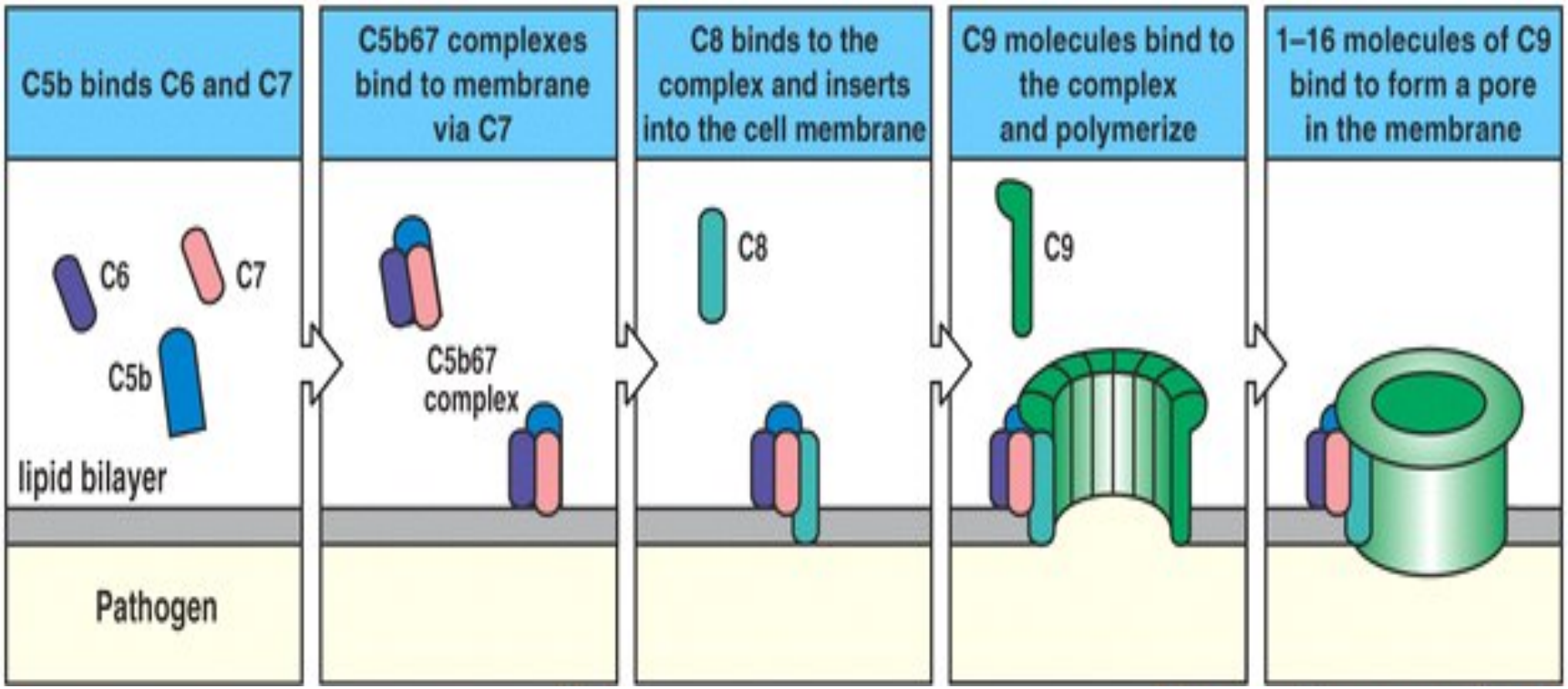
- Many C5b proteins are produced by the C5 activation complex
- C5b begin to coat the surface of the bacteria

The function of C5a



- C5a moves away from the bacteria
  - Binds to mast cells and increases inflammation
  - Most powerful chemotactic factor known for leukocytes

Common Terminal pathway



Building the Membrane Attack complex

- C5b on the surface of bacteria binds to C6
- The binding of C6 to C5b activates C6 so that it can bind to C7
- C7 binds to C8 which in turn binds to many C9’s
- Form a circular complex called Membrane attack complex (MAC)

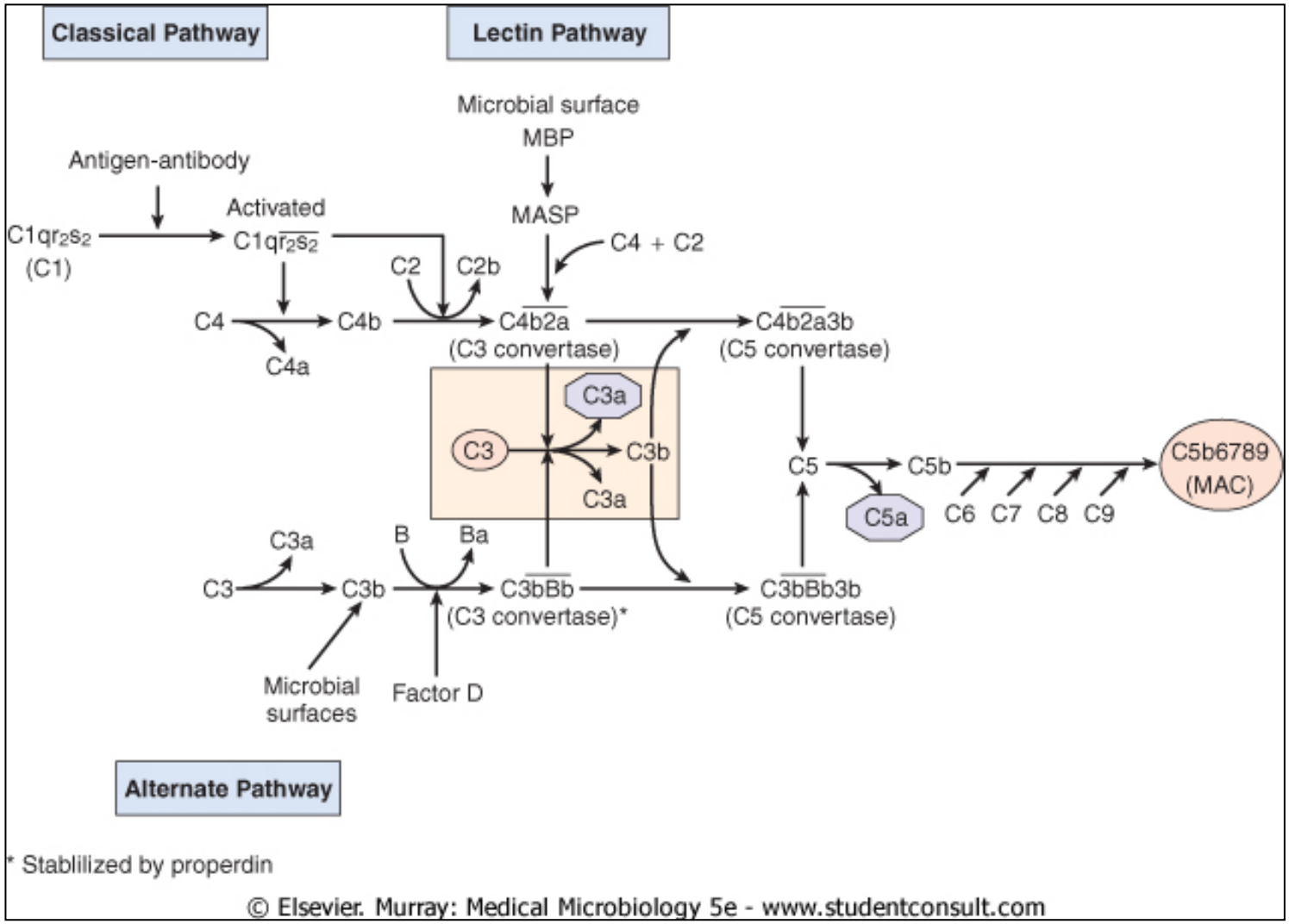
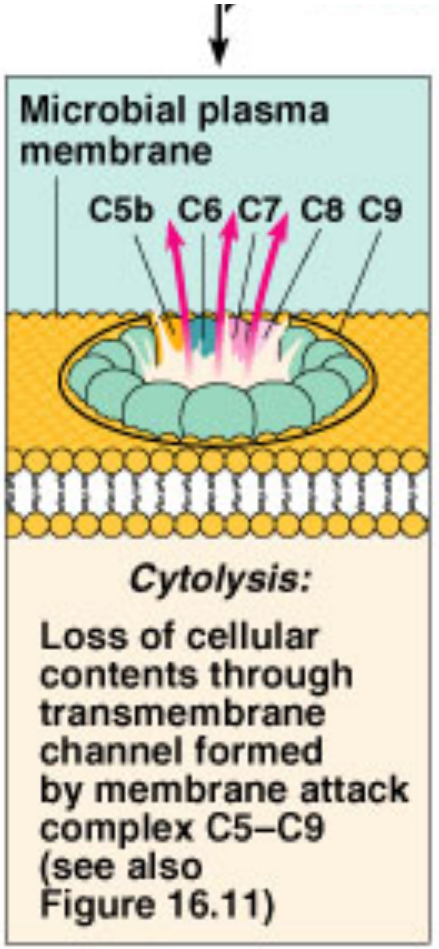


Figure 12-8 The classical, lectin, and alternate complement pathways. Despite different activators, the goal of these pathways is cleavage of C3 and C5 to provide chemoattractants and anaphylotoxins (C3a, C5a), an opsonin (C3b) that adheres to membranes, and to initiate the membrane attack complex to kill cells. MASP, MBP-associated serine protease; MBP, mannose-binding protein. (Redrawn from Rosenthal KS, Tan JS: Rapid review microbiology and immunology, St. Louis, 2002, Mosby.)

Membrane Attack complex

- The MAC causes Cytolysis
  - The circular membrane attack complex acts as a channel in which cytoplasm can rush out of and water rushes in
- The cells inner integrity is compromised and it dies
- Animation of the classical pathaway

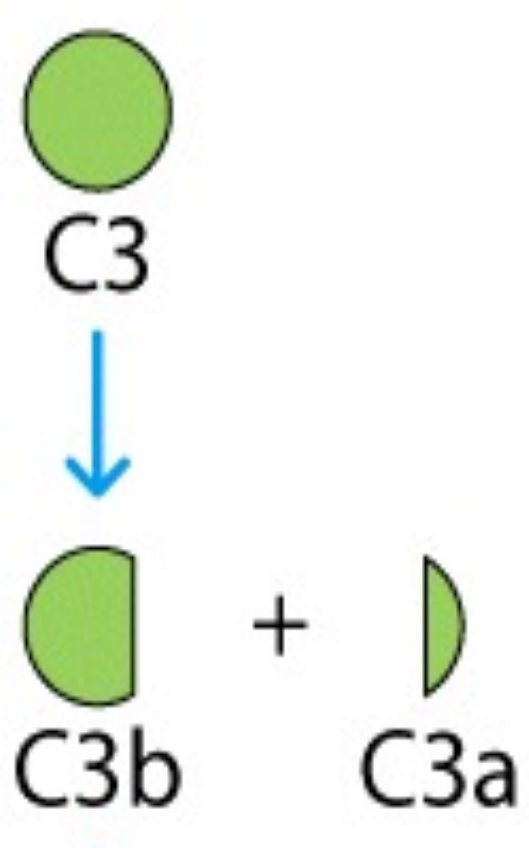


The alternative pathway

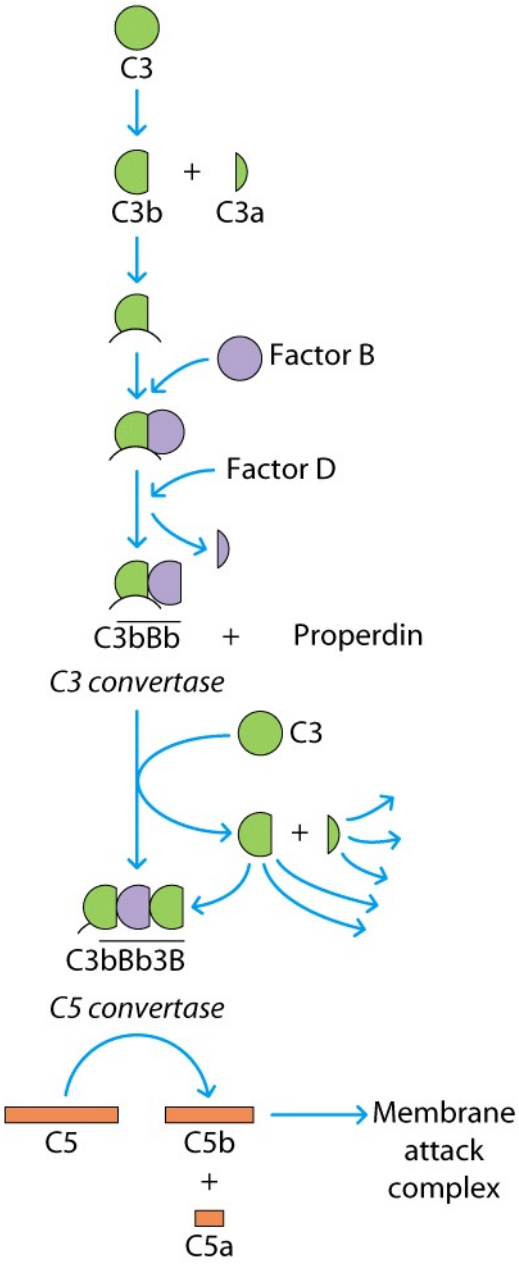
- Is part of the non-specific defense
- Not required antibodies to initiate
- Is slower than the Classical pathway

Initiation of Alternative pathway

- C3 contains in unstable thioester bond
- C3 subject to slow spontaneous hydrolysis to C3b and C3a
- The C3b is able to bind to foreign surface antigens

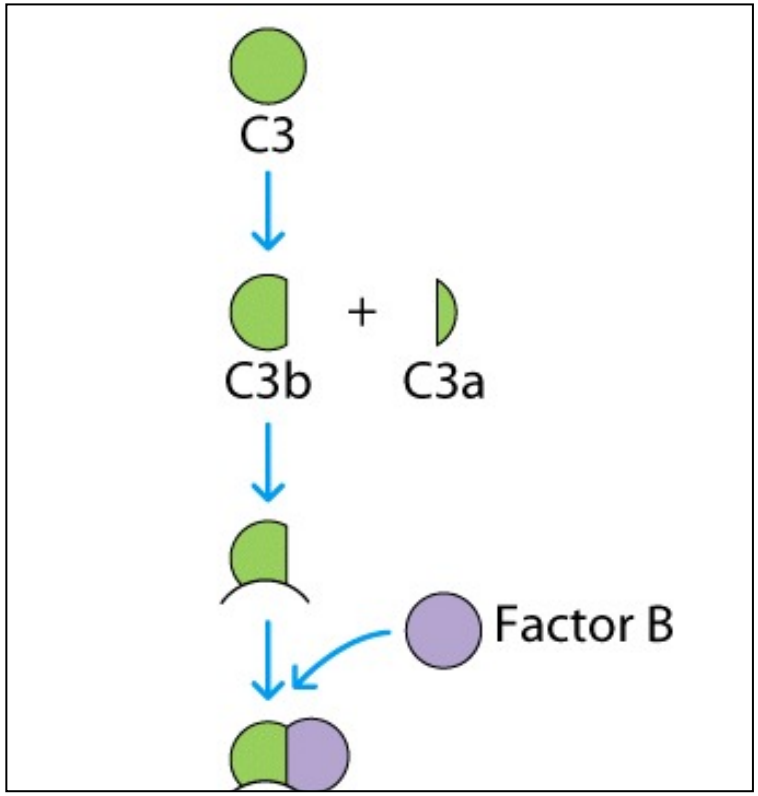


Alternative complement pathway



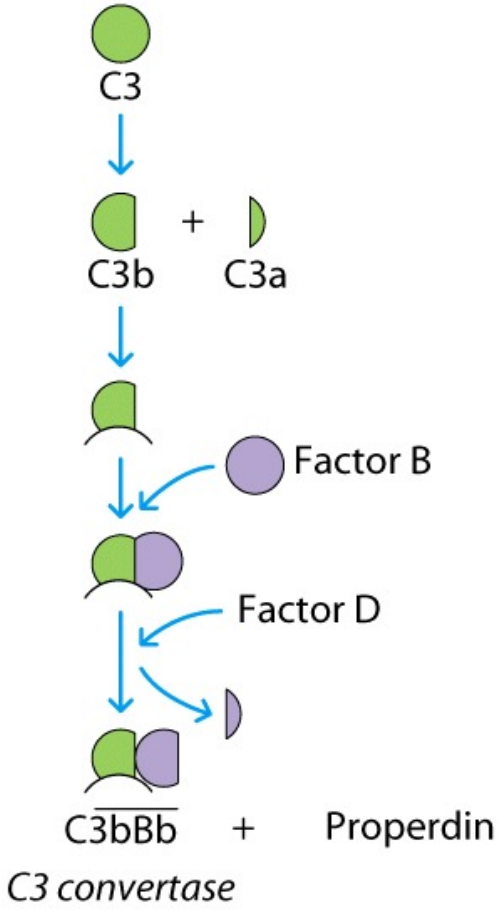
Factor B

- Activated C3b binds to another plasma protein called factor B

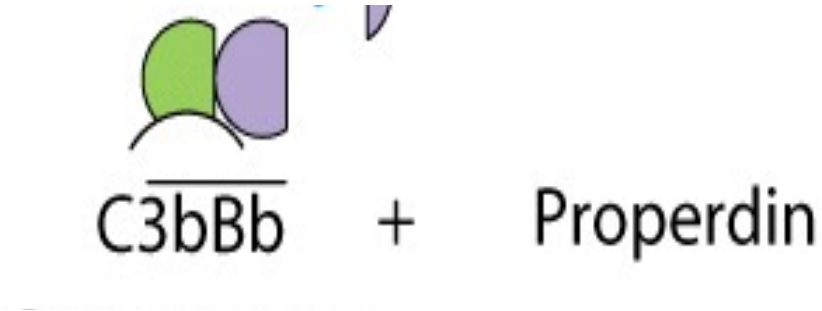


Factor D

- Protein enzyme called Factor D cleave Factor B
  - Ba and Bb
- Factor Bb remains bound to C3b
- Ba and Factor D disperse away



The C3 activation complex

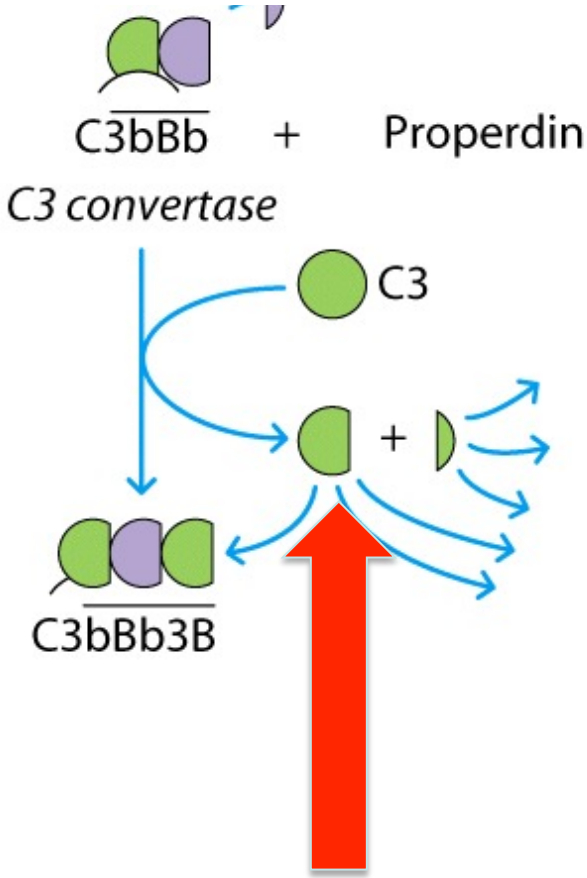


- Properdin (Factor P) binds to the C3bBb complex to stabilize it

- C3bBbP make up the C3 activation complex for the alternative pathway

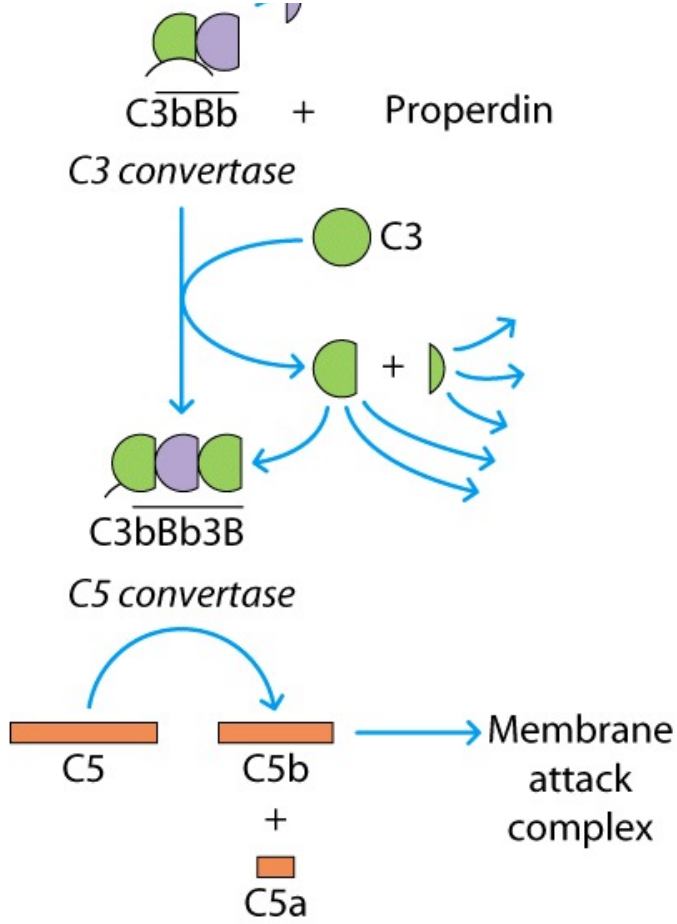
The C3 activation Complex

- The C3 activation complex produce more C3b
- This allows the initial steps of this pathway to be repeated and amplified



C5 activation complex

- Once additional C3b binds to the C3 activation complex it converts it into a C5 activation complex
- The C5 activation complex cleaves C5 into C5a and C5b
- C5b begins the production of the MAC



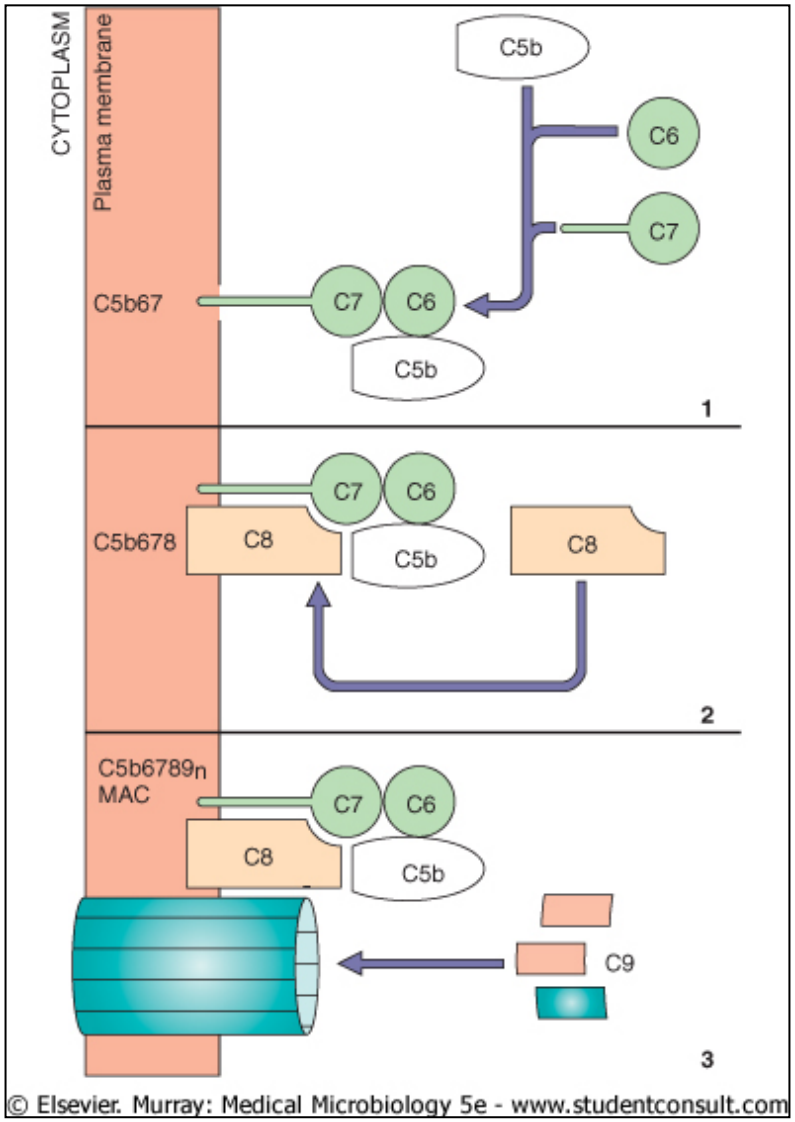


Figure 12-9 Cell lysis by complement. Activation of C5 initiates the molecular construction of an oil-well-like membrane attack complex (MAC).

Lectin Pathway

- **Mannose-binding protein** / large serum protein

- Binds to non-reduced mannosem and glucosamine on bacterial and other cell surfaces

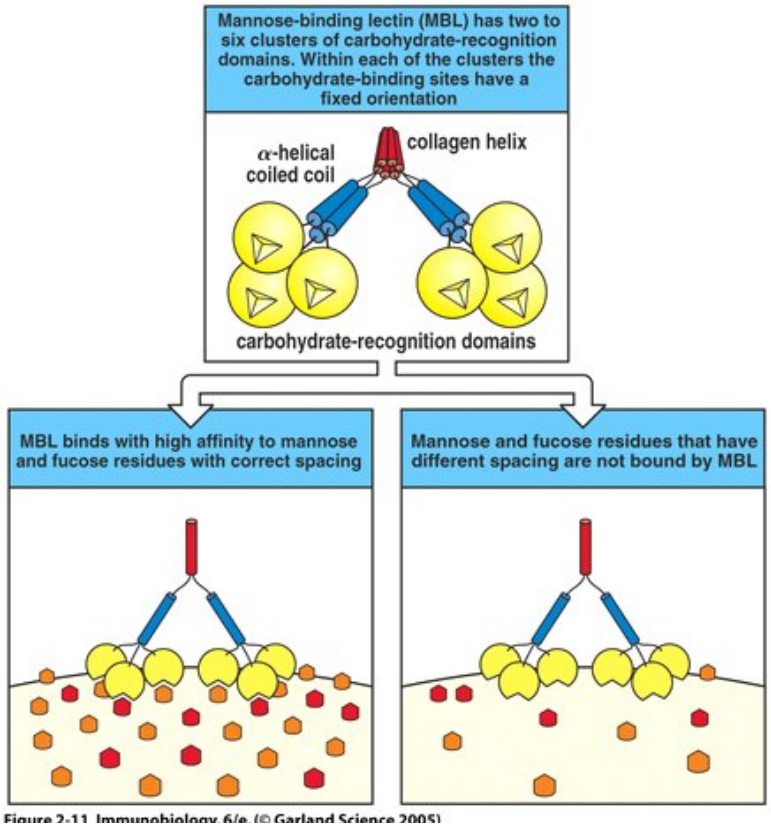
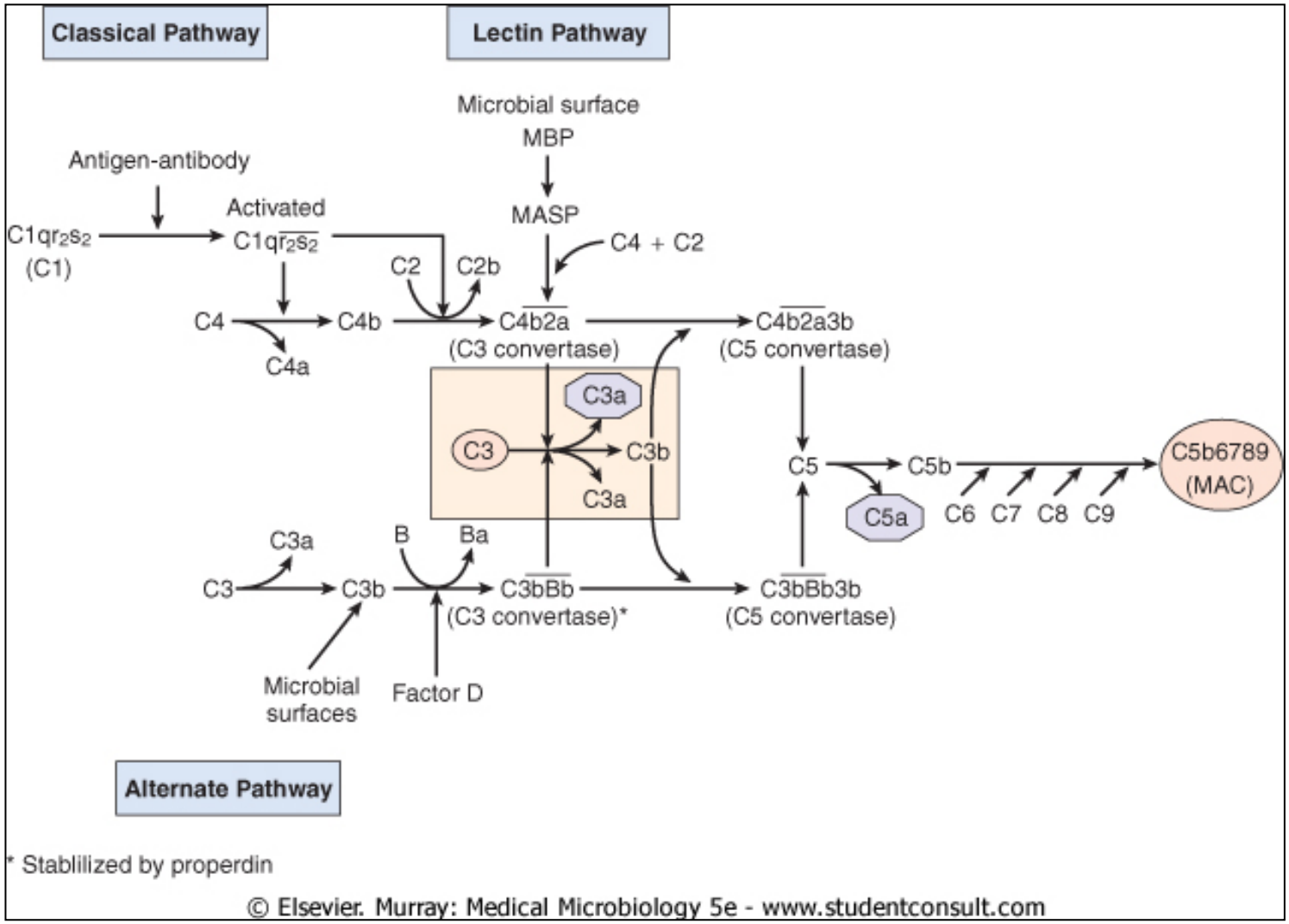


Figure 2-11 Immunobiology 6/e. (© Garland Science 2005)



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SUMMARY OF BIOLOGICAL EFFECTS MEDIATED BY COMPLEMENT PRODUCTS

Effect	Complement product mediating*
Cell lysis	C5b–9, the membrane-attack complex (MAC)
Inflammatory response	
Degranulation of mast cells and basophils†	C3a, C4a, and C5a (anaphylatoxins)
Degranulation of eosinophils	C3a, C5a
Extravasation and chemotaxis of leukocytes at inflammatory site	C3a, **C5a**, C5b67
Aggregation of platelets	C3a, C5a
Inhibition of monocyte/macrophage migration and induction of their spreading	Bb
Release of neutrophils from bone marrow	C3c
Release of hydrolytic enzymes from neutrophils	C5a
Increased expression of complement receptors type 1 and 3 (CR1 and CR3) on neutrophils	C5a
Opsonization of particulate antigens, increasing their phagocytosis	**C3b**, C4b, iC3b
Viral neutralization	C3b, C5b–9 (MAC)
Solubilization and clearance of immune complexes	C3b

*Boldfaced component is most important in mediating indicated effect.

†Degranulation leads to release of histamine and other mediators that induce contraction of smooth muscle and increased permeability of vessels.

Regulation of Complement Activation

- Several mechanisms for preventing generation of the C3 convertase to protect against inappropriate complement activation
  - C1 inhibitor
  - C4 binding protein
  - Factor H
  - Factor I
  - cell surface proteins
  - which are decay-accelerating factor (DAF) membrane cofactor protein
  - CD59 (protectin) prevents formation of the membrane attack complex

Immune system and diseases

- A genetic deficiency in these protection systems can result in disease

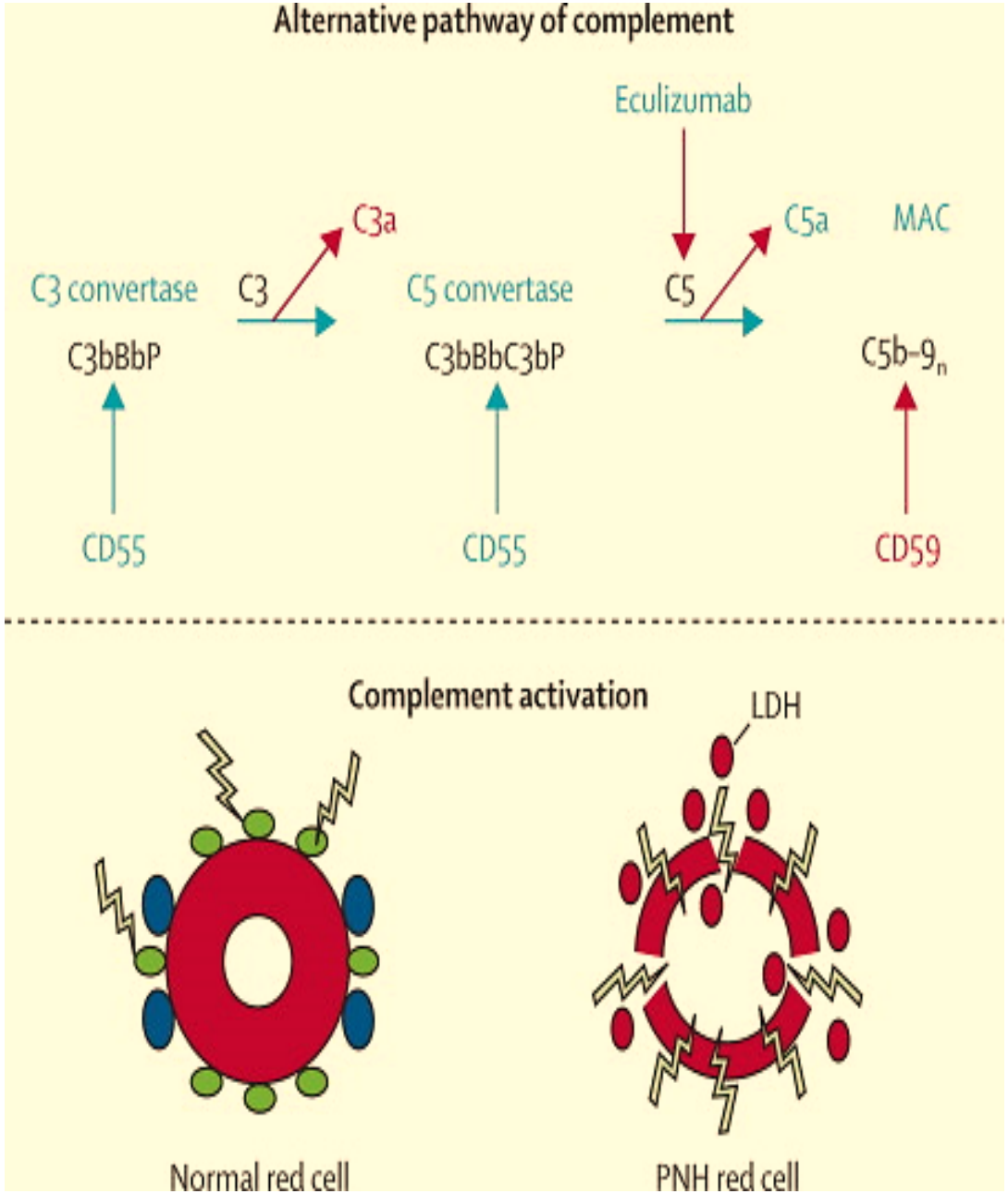
Control proteins of the classical and alternative pathways	
Name (symbol)	**Role in the regulation of complement activation**
C1 inhibitor (C1INH)	Binds to activated C1r, C1s, removing it from C1q
C4-binding protein (C4BP)	Binds C4b, displacing C2b; cofactor for C4b cleavage by I
Complement receptor 1 (CR1)	Binds C4b, displacing C2b, or C3b displacing Bb; cofactor for I
Factor H (H)	Binds C3b, displacing Bb; cofactor for I
Factor I (I)	Serine protease that cleaves C3b and C4b; aided by H, MCP, C4BP, or CR1
Decay-accelerating factor (DAF)	Membrane protein that displaces Bb from C3b and C2b from C4b
Membrane cofactor protein (MCP)	Membrane protein that promotes C3b and C4b inactivation by I
CD59 (protectin)	Prevents formation of membrane-attack complex on autologous or allogenic cells. Widely expressed on membranes

Complement system and diseases

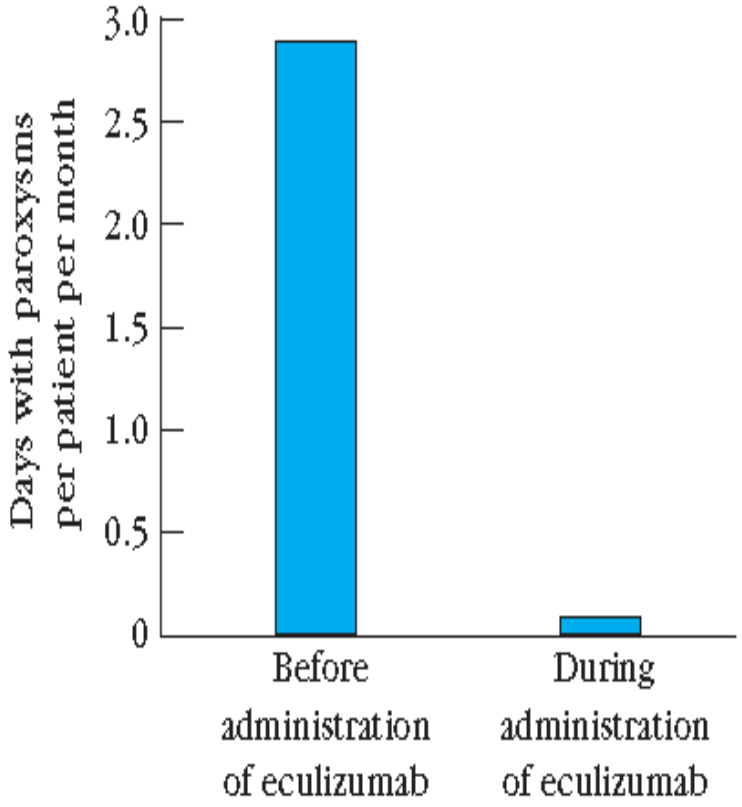
- SLE → low C3 and C4
- Terminal complement deficiency → low C5, C6, C7, C8, C9
- Properdin deficiency is an X-linked disorder that also causes susceptibility to neisserial infections.
- C1-inhibitor deficiency or hereditary angioedema → low C4 with normal C1 and C3 levels

Complement system as a therapeutic target

- Purified C1 esterase inhibitor
- Ecluzimab in PNH


Alternative pathway of complement
Eculizumab
C3a
C5a
MAC
C3 convertase
C3
C5 convertase
C5
C3bBbP
C3bBbC3bP
C5b-9n
CD55
CD55
CD59
Complement activation
LDH
Normal red cell
PNH red cell

Complement system as a therapeutic target



Summary - Activation

- Complement can be activated by the binding of antibody (Classical) or by the adherance of C3b to foreign material (Alternative)
- The two pathways converge at the formation of the C5 convertase (C4b2a3b or C3bBbC3b)
- The final common pathway is the formation of the membrane attack complex

Summary - Function

- **Opsonization** – C3b
- **Chemotaxis** – C5a (attracts neutrophils)
- **Increases vasodilation & permeability** of capillary beds via mast cell and basophil activation – C3a & C5a (Anaphylatoxins)
- **Cellular Lysis** via the MAC

Short Note

- Comprises a group of serum proteins exist in inactive forms
- Complement activation occurs by the classical, alternative, or lectin pathways, each of which is initiated differently
- The three pathways converge in a common sequence of events that leads to generation of a molecular complex that causes cell lysis
- The classical pathway is initiated by antibody binding to a cell target; reactions of IgM and certain IgG subclasses activate this pathway.
- Activation of the alternative and lectin pathways is antibody-independent. These pathways are initiated by reaction of complement proteins with surface molecules of microorganisms
- In addition to its key role in cell lysis, mediates opsonization of bacteria, activation of inflammation, and clearence of immune complexes
- Interactions of complement proteins and protein fragments with receptors on cells of the immune system control both innate and adaptive immune responses
- Clinical consequences of inherited complement deficiencies range from increases in susceptibility to infection to tissue damage caused by immune complexes.