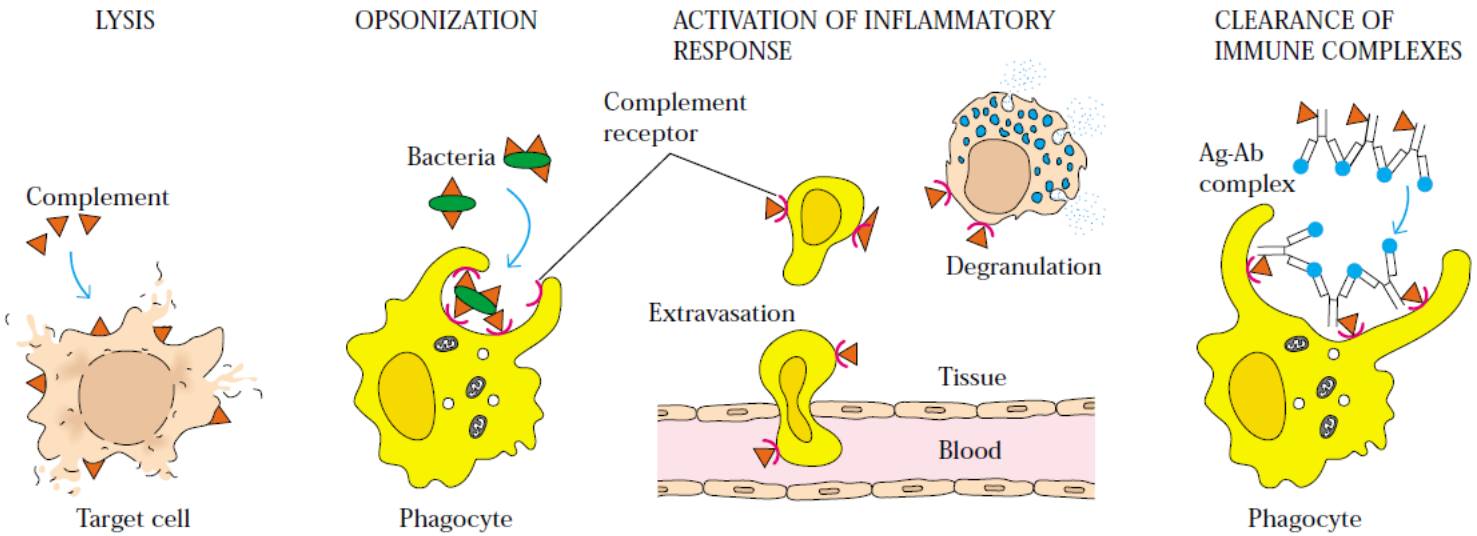
The Complement System

- In the 1890s, Jules Bordet found that sheep antiserum to the bacterium *Vibrio cholerae* caused lysis of the bacteria and that heating the antiserum destroyed its bacteriolytic activity.
- Surprisingly, the ability to lyse the bacteria was restored to the heated serum by adding fresh serum that contained no antibodies directed against the bacterium and was unable to kill the bacterium by itself.
- Bordet correctly reasoned that bacteriolytic activity requires two different substances: first, the specific antibacterial antibodies, which survive the heating process, and a second, heat-sensitive component responsible for the lytic activity.
- Bordet devised a simple test for the lytic activity, the easily detected lysis of antibody-coated red blood cells, called **hemolysis.**
- Paul Ehrlich in Berlin independently carried out similar experiments and coined the term *complement,* defining it as “the activity of blood serum that completes the action of antibody.”
- In subsequent years, researchers discovered that the action of complement was the result of interactions of a large and complex group of proteins.

The Functions of Complement

- Complement system includes more than 30 soluble and cell-bound proteins.
- It affects both innate and acquired immunity.
- Interaction of cellular receptors with complement proteins controls B-cell activities gives this system a role in the highly developed acquired immune system.
- Functions:
- Lysis of cells, bacteria, and viruses
- Opsonization, which promotes phagocytosis of particulate antigens
- Binding to specific complement receptors on cells of the immune system, triggering specific cell functions,
- inflammation, and secretion of immunoregulatory molecules
- Immune clearance, which removes immune complexes from the circulation and deposits them in the spleen and liver



The Complement Components

- The proteins and glycoproteins that compose the complement system are synthesized mainly by
  - ✓ Liver hepatocytes,
  - ✓ Blood monocytes,
  - ✓ Tissue macrophages, and
  - ✓ Epithelial cells of the gastrointestinal and genitourinary tracts
- These components constitute 5% (by weight) of the serum globulin fraction.
- Most circulate in the serum in functionally inactive forms as proenzymes, or zymogens, which are inactive until proteolytic cleavage, which removes an inhibitory fragment and exposes the active site.
- The complement-reaction sequence starts with an enzyme cascade.

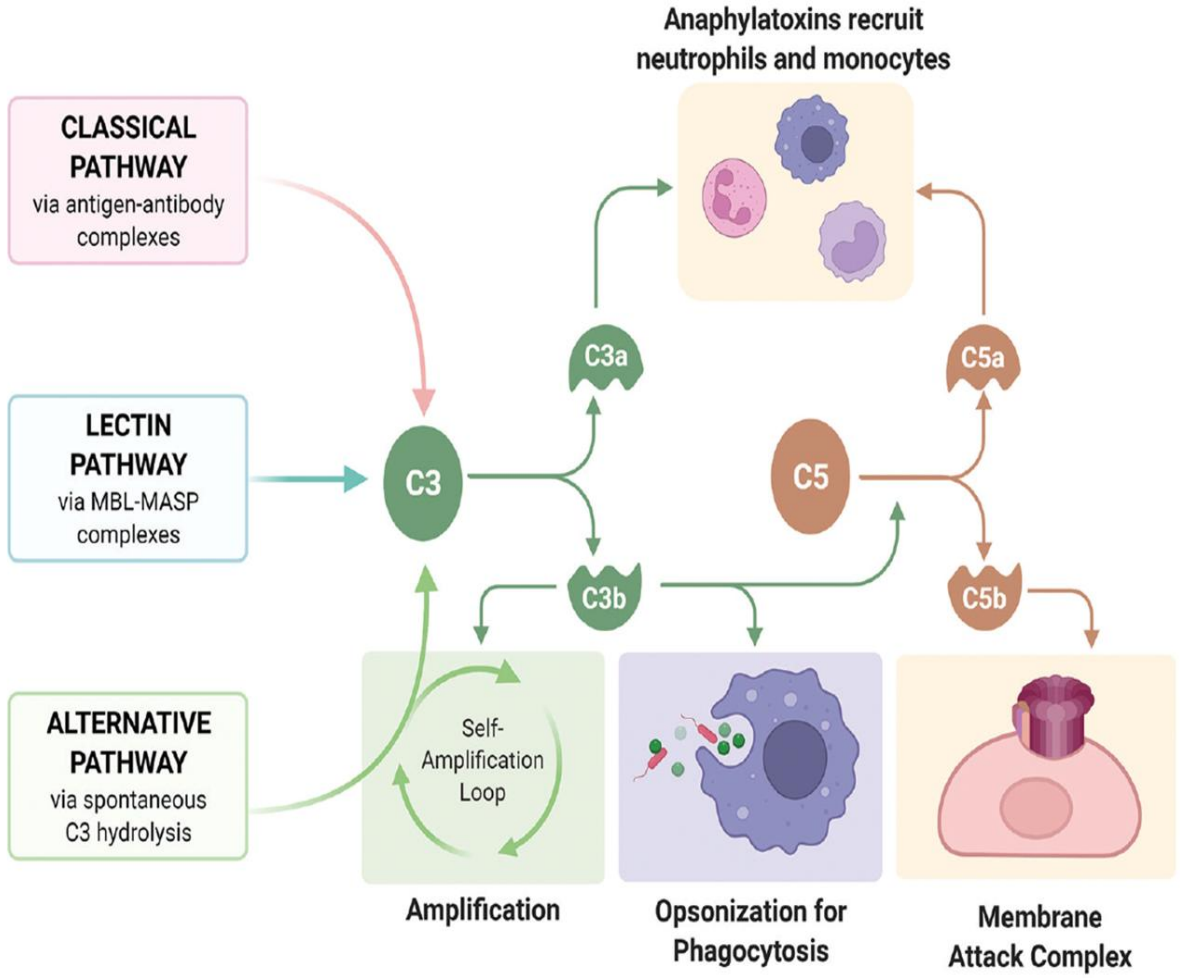
Naming Complement Components

- Complement components are designated by:
  - Numerals (C1–C9), or
  - Letter symbols (e.g., factor D), or
  - Trivial names (e.g., homologous restriction factor).
- Peptide fragments formed by activation of a component are denoted by small letters.
- In most cases cleavage results in:
  - Smaller fragment designated “a” and
  - Larger fragment designated “b” (e.g., C3a, C3b).
  - ❖ C2 is an exception: C2a is the larger cleavage fragment.
- Larger fragments bind to the target near the site of activation, and
- Smaller fragments diffuse from the site and can initiate localized inflammatory responses by binding to specific receptors.
- Complexes that have enzymatic activity are designated by a bar over the number or symbol (e.g., $\overline{C4b2a}$, $\overline{C3bBb}$).

Complement Activation

The Classical Pathway Begins with Antigen-Antibody Binding

- CP begins with the formation of soluble antigen-antibody complexes or with the binding of antibody to antigen on a suitable target, such as a bacterial cell.
- IgM and IgG can activate the classical complement pathway.
- The initial stage of activation involves C1, C2, C3, and C4, which are present in plasma in functionally inactive forms.
- The formation of an antigen-antibody complex ***induces conformational changes in the Fc portion of the IgM molecule*** that expose a binding site for the C1 component of the complement system.
- C1 in serum is a macromolecular complex consisting of **C1q** and **two molecules each of C1r** and **C1s,** held together in a complex **(C1qr2s2)** stabilized by **Ca^{2+} ions.**
- The C1q molecule is composed of 18 polypeptide chains that associate to form six collagen-like triple helical arms, the tips of which bind to exposed C1q-binding sites in the CH_2 domain of the antibody molecule.
- Each C1r and C1s monomer contains a catalytic domain and an interaction domain; the latter facilitates interaction with C1q or with each other.



- The C1q molecule is composed of 18 polypeptide chains that associate to form six collagen-like triple helical arms, forming head and stalk.
- The heads bind with CH_2 region of antibody.
- Each C1r and C1s monomer contains a catalytic domain and an interaction domain; the latter facilitates interaction with C1q or with each other.
- Each C1 molecule must bind by its C1q globular heads to at least two Fc sites for a stable C1-antibody interaction to occur.

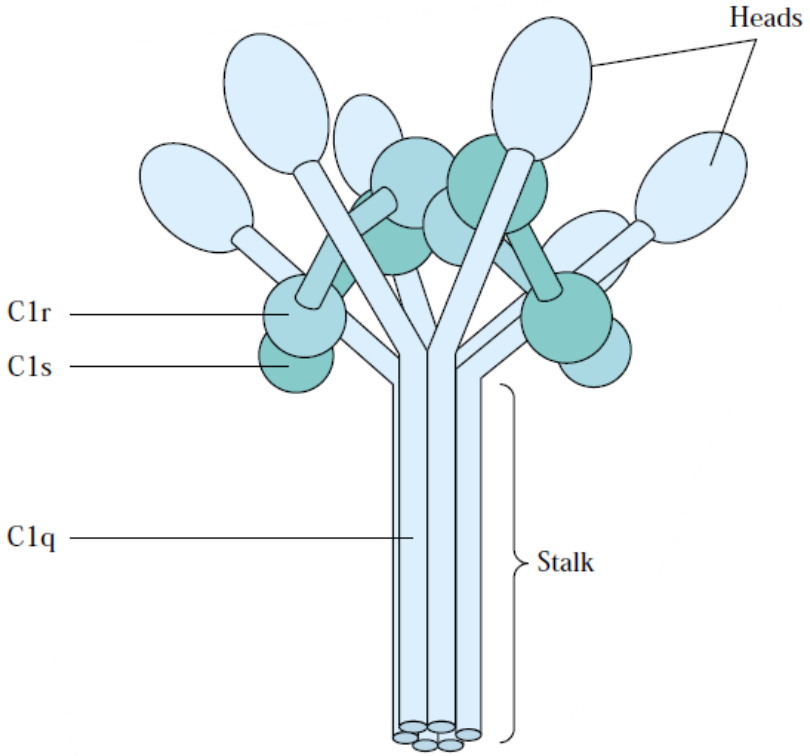
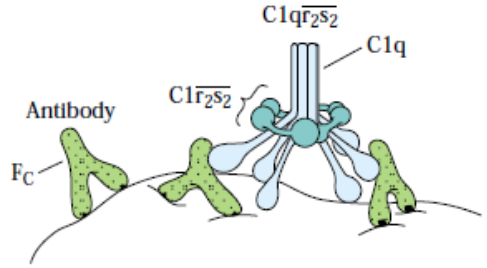
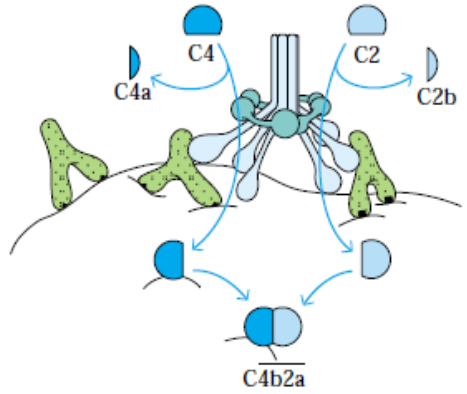


FIGURE 13-3 Structure of the C1 macromolecular complex. (a) Diagram of $C1qr_2s_2$ complex. A C1q molecule consists of 18 polypeptide chains arranged into six triplets, each of which contains one A, one B, and one C chain. Each C1r and C1s monomer contains a catalytic domain with enzymatic activity and an interaction domain that facilitates binding with C1q or with each other. (b) Electron micrograph of C1q molecule showing stalk and six globular heads. *[Part (b) from H. R. Knobel et al., 1975,* Eur. J. Immunol. ***5:****78.]*

1 C1q binds antigen-bound antibody. C1r activates autocatalytically and activates the second C1r; both activate C1s

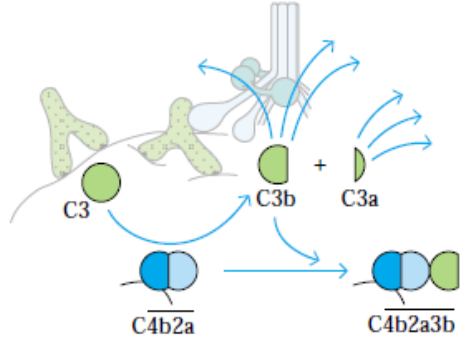


2 C1s cleaves C4 and C2. Cleaving C4 exposes the binding site for C2. C4 binds the surface near C1 and C2 binds C4, forming C3 convertase



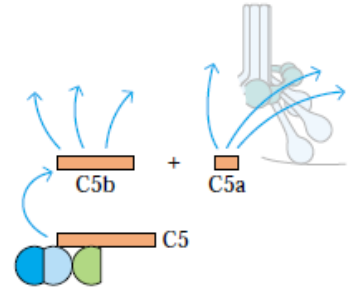
C3 convertase

3 C3 convertase hydrolyzes many C3 molecules. Some combine with C3 convertase to form C5 convertase



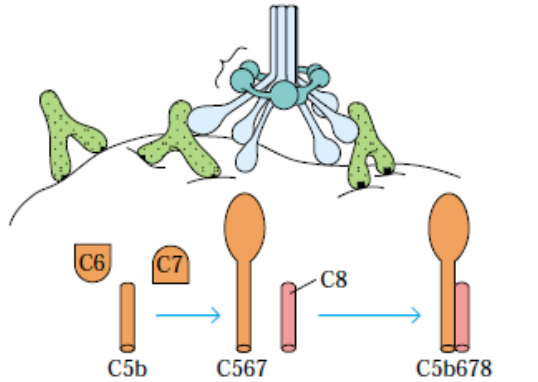
C5 convertase

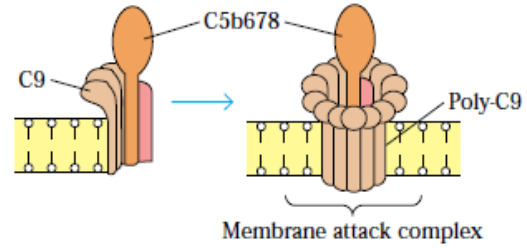
4 The C3b component of C5 convertase binds C5, permitting $\overline{C4b2a}$ to cleave C5



C5 convertase

5 C5b binds C6, initiating the formation of the membrane-attack complex





Membrane attack complex

The Alternative Pathway Is Antibody-Independent

- The alternative pathway is antibody-independent.
- The alternative pathway generates bound C5b, the same product that the classical pathway generates, but it does so without the need for antigen-antibody complexes for initiation.
- Because no antibody is required, the alternative pathway is a component of the innate immune system.
- This major pathway of complement activation involves four serum proteins: C3, factor B, factor D, and properdin.
- The alternative pathway is initiated in most cases by cell-surface constituents that are foreign to the host.
- For example, both gram-negative and gram-positive bacteria have cell-wall constituents that can activate the alternative pathway.

- In the alternative pathway, serum C3, which contains an unstable thioester bond, is subject to slow spontaneous hydrolysis to yield C3a and C3b.
- The C3b component can bind to foreign surface antigens (such as those on bacterial cells or viral particles) or even to the host's own cells.
- The membranes of most mammalian cells have high levels of sialic acid, which contributes to the rapid inactivation of bound C3b molecules on host cells; consequently this binding rarely leads to further reactions on the host cell membrane.
- Because many foreign antigenic surfaces (e.g., bacterial cell walls, yeast cell walls, and certain viral envelopes) have only low levels of sialic acid, C3b bound to these surfaces remains active for a longer time.
- The C3b present on the surface of the foreign cells can bind another serum protein called factor B to form a complex stabilized by Mg^{2+}.
- Binding to C3b exposes a site on factor B that serves as the substrate for an enzymatically active serum protein called factor D.
- Factor D cleaves the C3b-bound factor B, releasing a small fragment (Ba) that diffuses away and generating C3bBb.
- The C3bBb complex has C3 convertase activity and thus is analogous to the C4b2a complex in the classical pathway.
- The C3 convertase activity of C3bBb has a half-life of only 5 minutes unless the serum protein properdin binds to it, stabilizing it and extending the half-life of this convertase activity to 30 minutes.

- The C3bBb generated in the alternative pathway can activate unhydrolyzed C3 to generate more C3b autocatalytically.
- As a result, the initial steps are repeated and amplified, so that more than 2×10^6 molecules of C3b can be deposited on an antigenic surface in less than 5 minutes.
- The C3 convertase activity of C3bBb generates the C3bBb3b complex, which exhibits C5 convertase activity, analogous to the C4b2a3b complex in the classical pathway.
- The nonenzymatic C3b component binds C5, and the Bb component subsequently hydrolyzes the bound C5 to generate C5a and C5b ; the latter binds to the antigenic surface.

TABLE 13-1 Initiators of the alternative pathway of complement activation

PATHOGENS AND PARTICLES OF MICROBIAL ORIGIN
Many strains of gram-negative bacteria
Lipopolysaccharides from gram-negative bacteria
Many strains of gram-positive bacteria
Teichoic acid from gram-positive cell walls
Fungal and yeast cell walls (zymosan)
Some viruses and virus-infected cells
Some tumor cells (Raji)
Parasites (trypanosomes)
NONPATHOGENS
Human IgG, IgA, and IgE in complexes
Rabbit and guinea pig IgG in complexes
Cobra venom factor
Heterologous erythrocytes (rabbit, mouse, chicken)
Anionic polymers (dextran sulfate)
Pure carbohydrates (agarose, inulin)