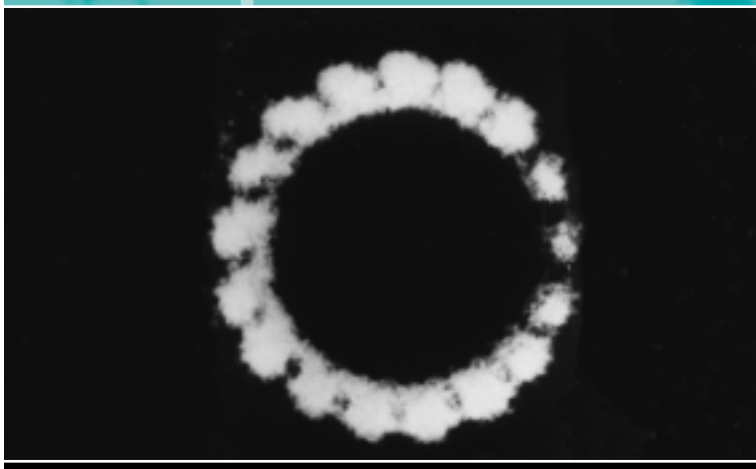
chapter 13

The Complement System



Poly-C9 Complex

THE COMPLEMENT SYSTEM IS THE MAJOR EFFECTOR of the humoral branch of the immune system. 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. 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 ensuing years, researchers discovered that the action of complement was the result of interactions of a large and complex group of proteins.

This chapter describes the complement components and their activation pathways, the regulation of the complement system, the effector functions of various complement components, and the consequences of deficiencies in them. A Clinical Focus section describes consequences of a defect in proteins that regulate complement activity.

The Functions of Complement

Research on complement now includes more than 30 soluble and cell-bound proteins. The biological activities of this system affect both innate and acquired immunity and reach far beyond the original observations of antibody-mediated lysis of bacteria and red blood cells. Structural comparisons of the proteins involved in complement pathways place the origin of this system in primitive organisms possessing the most rudimentary innate immune systems. By contrast, the realization that interaction of cellular receptors with complement proteins controls B-cell activities gives this system a role in the highly developed acquired immune system. Thus we have a system that straddles innate and acquired immunity, contributing to each in a variety of ways.

After initial activation, the various complement components interact, in a highly regulated cascade, to carry out a number of basic functions (Figure 13-1) including:

- 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

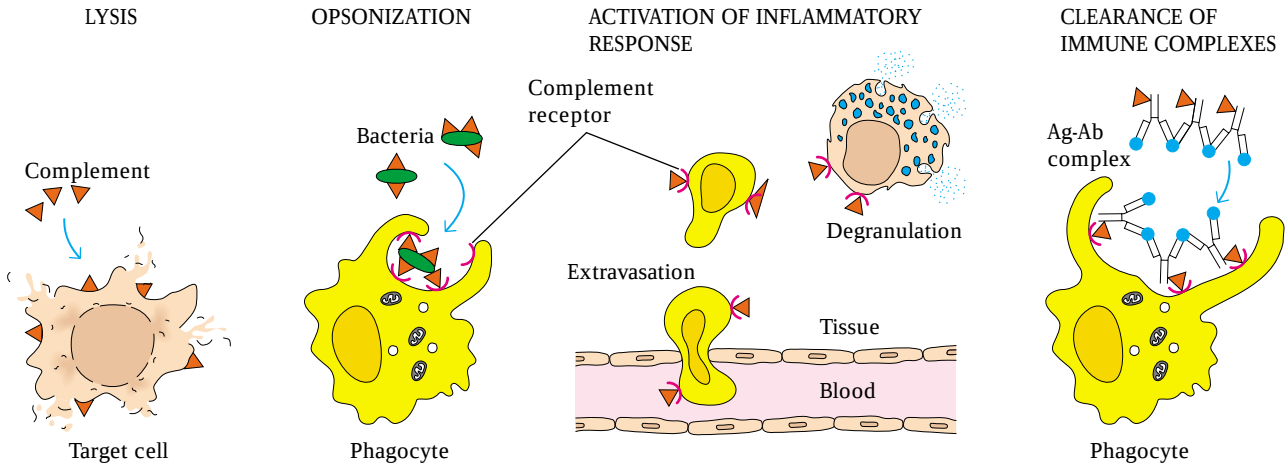


FIGURE 13-1 The multiple activities of the complement system. Serum complement proteins and membrane-bound complement receptors partake in a number of immune activities: lysis of foreign cells by antibody-dependent or antibody-independent pathways; opsonization or uptake of particulate antigens, including bacteria, by phagocytes; activation of inflammatory responses; and clearance of circulating immune complexes by cells in the liver and spleen. Soluble complement proteins are schematically indicated by a triangle and receptors by a semi-circle; no attempt is made to differentiate among individual components of the complement system here.

The Complement Components

The proteins and glycoproteins that compose the complement system are synthesized mainly by liver hepatocytes, although significant amounts are also produced by 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.

Complement components are designated by numerals (C1–C9), by letter symbols (e.g., factor D), or by trivial names (e.g., homologous restriction factor). Peptide fragments formed by activation of a component are denoted by small letters. In most cases, the smaller fragment resulting from cleavage of a component is designated "a" and the larger fragment designated "b" (e.g., C3a, C3b; note that C2 is an exception: C2a is the larger cleavage fragment). The larger fragments bind to the target near the site of activation, and the smaller fragments diffuse from the site and can initiate localized inflammatory responses by binding to specific receptors. The complement fragments interact with one another to form functional complexes. Those complexes that have enzymatic activity are designated by a bar over the number or symbol (e.g., $C\overline{4b2a}$, $C\overline{3bBb}$).

Complement Activation

Figure 13-2 on page 301 outlines the pathways of complement activation. The early steps, culminating in formation of C5b, can occur by the **classical pathway,** the **alternative pathway,** or the **lectin pathway.** The final steps that lead to a membrane attack are the same in all pathways.

The Classical Pathway Begins with Antigen-Antibody Binding

Complement activation by the classical pathway commonly begins with the formation of soluble antigen-antibody complexes (immune complexes) or with the binding of antibody to antigen on a suitable target, such as a bacterial cell. IgM and certain subclasses of IgG (human IgG1, IgG2, and IgG3) 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. Because the components were named in order of their discovery and before their functional roles had been determined, the numbers in their names do not always reflect the order in which they react.

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 ($C1qr_2s_2$) 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 C_H2 domain of the antibody molecule (Figure 13-3, on page 302). 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

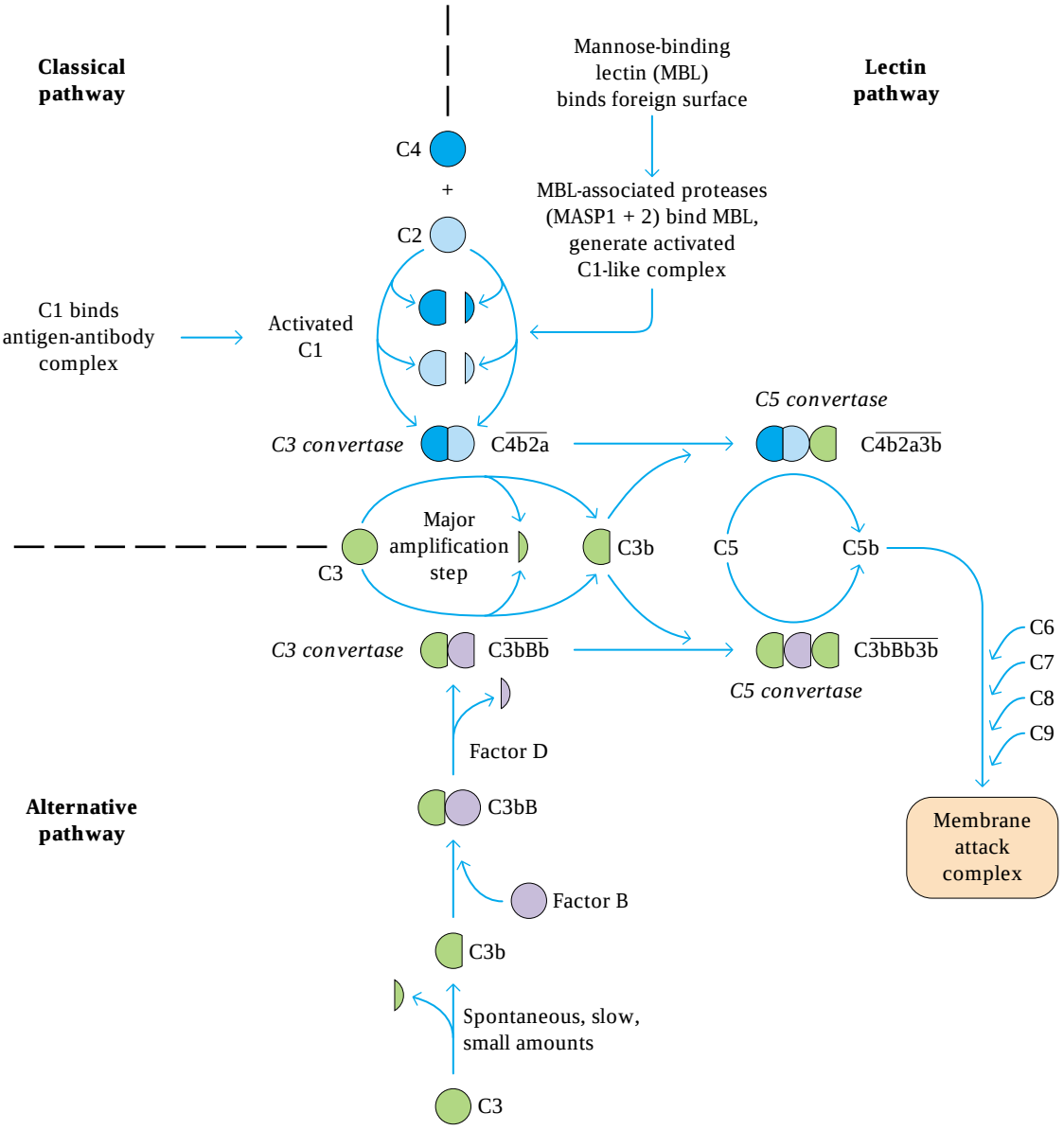


FIGURE 13-2 Overview of the complement activation pathways. The classical pathway is initiated when C1 binds to antigen-antibody complexes. The alternative pathway is initiated by binding of spontaneously generated C3b to activating surfaces such as microbial cell walls. The lectin pathway is initiated by binding of the serum protein MBL to the surface of a pathogen. All three pathways generate C3 and C5 convertases and bound C5b, which is converted into a membrane-attack complex (MAC) by a common sequence of terminal reactions. Hydrolysis of C3 is the major amplification step in all pathways, generating large amounts of C3b, which forms part of C5 convertase. C3b also can diffuse away from the activating surface and bind to immune complexes or foreign cell surfaces, where it functions as an opsonin.

occur. When pentameric IgM is bound to antigen on a target surface it assumes the so-called "staple" configuration, in which at least three binding sites for C1q are exposed. Circulating IgM, however, exists as a planar configuration in which the C1q-binding sites are not exposed (Figure 13-4, on page 302) and therefore cannot activate the complement cascade. An IgG molecule, on the other hand, contains only a single C1q-binding site in the C_H2 domain of the Fc, so that firm C1q binding is achieved only when two IgG molecules are within 30–40 nm of each other on a target surface or in a complex, providing two attachment sites for C1q. This difference accounts for the observation that a single molecule of IgM bound to a red blood cell can activate the classical complement pathway and lyse the red blood cell while some 1000 molecules of IgG are required to assure that two IgG molecules are close enough to each other on the cell surface to initiate C1q binding.

The intermediates in the classical activation pathway are depicted schematically in Figure 13-5 (page 303). Binding of C1q to Fc binding sites induces a conformational change in

(text continues on page 304)

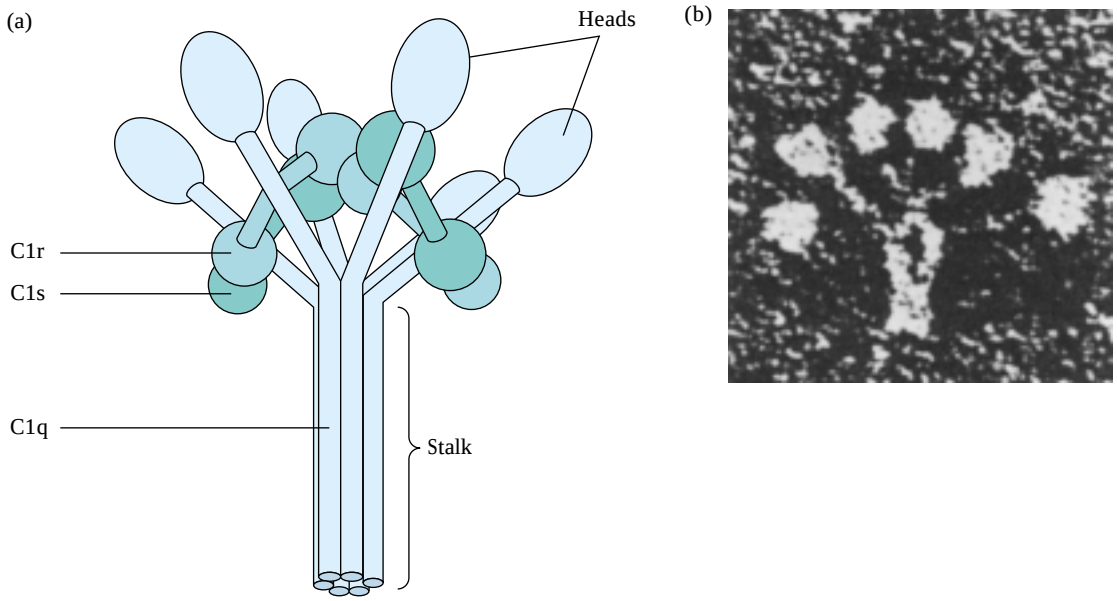


FIGURE 13-3 Structure of the C1 macromolecular complex. (a) Diagram of $C1qr_2s_2$ complex. A C1q molecule consists of 18 polypeptide chains arranged into six triplets, each of which contains one A, one B, and one C chain. Each C1r and C1s monomer contains a catalytic domain with enzymatic activity and 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.]*

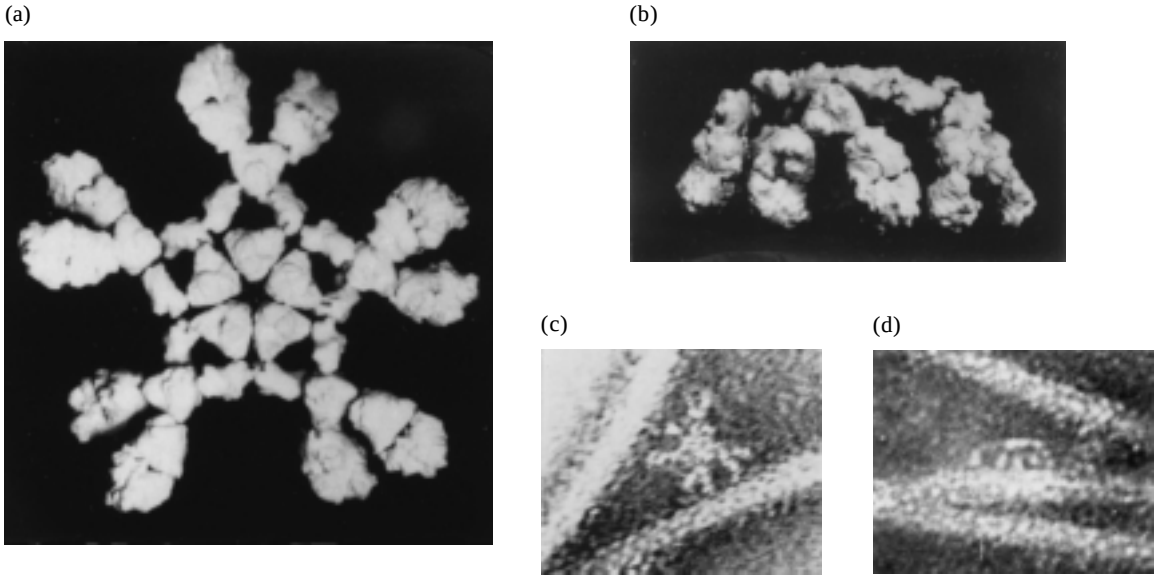
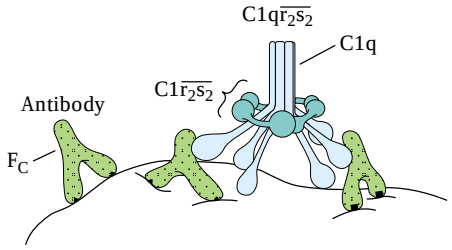


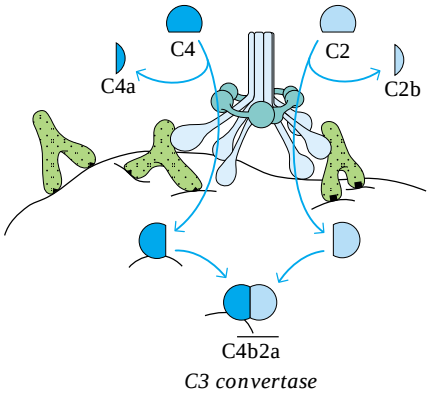
FIGURE 13-4 Models of pentameric IgM in planar form (a) and "staple" form (b). Several C1q-binding sites in the Fc region are accessible in the staple form, whereas none are exposed in the planar form. Electron micrographs of IgM antiflagellum antibody bound to flagella, showing the planar form (c) and staple form (d). *[From A. Feinstein et al., 1981,* Monogr. Allergy, ***17:****28, and* 1981, Ann. N.Y. Acad. Sci. ***190:****1104.]*

VISUALIZING CONCEPTS

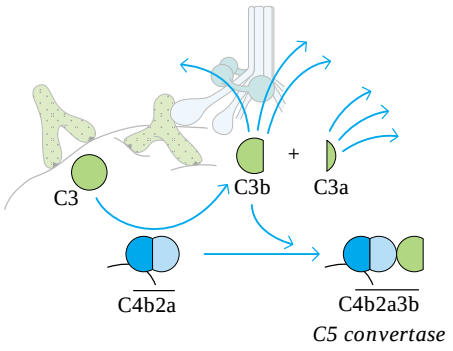
1 C1q binds antigen-bound antibody. C1r activates autocatalytically and activates the second C1r; both activate C1s



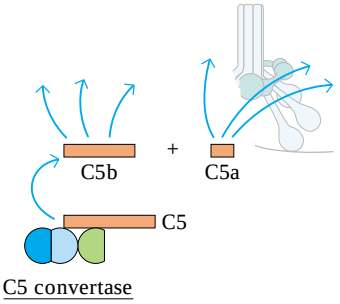
2 C1s cleaves C4 and C2. Cleaving C4 exposes the binding site for C2. C4 binds the surface near C1 and C2 binds C4, forming C3 convertase



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



4 The C3b component of C5 convertase binds C5, permitting C4b2a to cleave C5



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

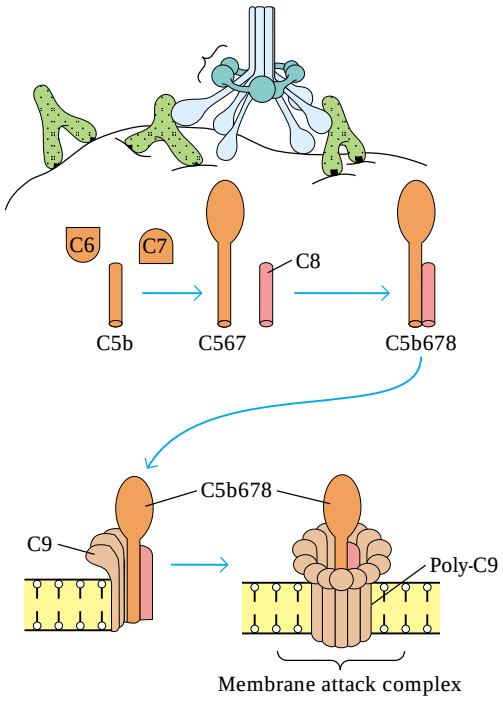


FIGURE 13-5 Schematic diagram of intermediates in the classical pathway of complement activation. The completed membrane-attack complex (MAC, bottom right) forms a large pore in the membrane.

C1r that converts C1r to an active serine protease enzyme, $C\overline{1r}$, which then cleaves C1s to a similar active enzyme, $C\overline{1s}$. $C\overline{1s}$ has two substrates, C4 and C2. The C4 component is a glycoprotein containing three polypeptide chains α, β, and γ. C4 is activated when $C\overline{1s}$ hydrolyzes a small fragment (C4a) from the amino terminus of the α chain, exposing a binding site on the larger fragment (C4b). The C4b fragment attaches to the target surface in the vicinity of C1, and the C2 proenzyme then attaches to the exposed binding site on C4b, where the C2 is then cleaved by the neighboring $C\overline{1s}$; the smaller fragment (C2b) diffuses away. The resulting $C\overline{4b2a}$ complex is called C3 convertase, referring to its role in converting the C3 into an active form. The smaller fragment from C4 cleavage, C4a, is an anaphylatoxin, or mediator of inflammation, which does not participate directly in the complement cascade; the anaphylatoxins, which include the smaller fragments of C4, C3, and C5 are described below.

The native C3 component consists of two polypeptide chains, α and β. Hydrolysis of a short fragment (C3a) from the amino terminus of the α chain by the C3 convertase generates C3b (Figure 13-6). A single C3 convertase molecule can generate over 200 molecules of C3b, resulting in tremendous amplification at this step of the sequence. Some of the C3b binds to $C\overline{4b2a}$ to form a trimolecular complex $C\overline{4b2a3b}$, called C5 convertase. The C3b component of this complex binds C5 and alters its conformation, so that the $C\overline{4b2a}$ component can cleave C5 into C5a, which diffuses away, and C5b, which attaches to C6 and initiates formation of the membrane-attack complex in a sequence described later. Some of the C3b generated by C3 convertase activity does not associate with $C\overline{4b2a}$; instead it diffuses away and then coats immune complexes and particulate antigens, functioning as an opsonin as described in the Clinical Focus. C3b may also bind directly to cell membranes.

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 (Table 13-1). For example, both gram-negative and gram-positive bacteria have cell-wall constituents that can activate the alternative pathway. The intermediates in the alternative pathway for generating C5b are shown schematically in Figure 13-7 (page 306).

In the classical pathway, C3 is rapidly cleaved to C3a and C3b by the enzymatic activity of the C3 convertase. 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 (see Figure 13-6c). 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 sub-

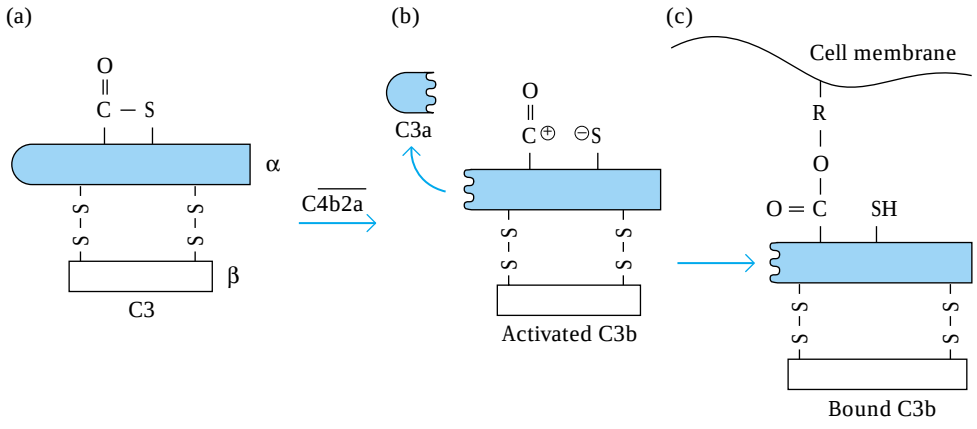


FIGURE 13-6 Hydrolysis of C3 by C3 convertase $C\overline{4b2a}$ (a) Native C3. (b) Activated C3 showing site of cleavage by $C\overline{4b2a}$ resulting in production of the C3a and C3b fragments. (c) A labile internal thioester bond in C3 is activated as C3b is formed, allowing the C3b fragment to bind to free hydroxyl or amino groups (R) on a cell membrane. Bound C3b exhibits various biological activities, including binding of C5 and binding to C3b receptors on phagocytic cells.

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)

SOURCE: Adapted from M. K. Pangburn, 1986, in *Immunobiology of the Complement System,* Academic Press.

strate 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 $C\overline{3bBb}$. The $C\overline{3bBb}$ complex has C3 convertase activity and thus is analogous to the $C\overline{4b2a}$ complex in the classical pathway. The C3 convertase activity of $C\overline{3bBb}$ 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 $C\overline{3bBb}$ 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 $C\overline{3bBb}$ generates the $C\overline{3bBb3b}$ complex, which exhibits C5 convertase activity, analogous to the $C\overline{4b2a3b}$ 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 (see Figure 13-7); the latter binds to the antigenic surface.

The Lectin Pathway Originates With Host Proteins Binding Microbial Surfaces

Lectins are proteins that recognize and bind to specific carbohydrate targets. (Because the lectin that activates complement binds to mannose residues, some authors designate this the MBLectin pathway or mannan-binding lectin pathway.) The lectin pathway, like the alternative pathway, does not depend on antibody for its activation. However, the mechanism is more like that of the classical pathway, because after initiation, it proceeds, through the action of C4 and C2, to produce a C5 convertase (see Figure 13-2).

The lectin pathway is activated by the binding of mannose-binding lectin (MBL) to mannose residues on glycoproteins or carbohydrates on the surface of microorganisms including certain *Salmonella*, *Listeria*, and *Neisseria* strains, as well as *Cryptococcus neoformans* and *Candida albicans.* MBL is an **acute phase protein** produced in inflammatory responses. Its function in the complement pathway is similar to that of C1q, which it resembles in structure. After MBL binds to the surface of a cell or pathogen, MBL-associated serine proteases, MASP-1 and MASP-2, bind to MBL. The active complex formed by this association causes cleavage and activation of C4 and C2. The MASP-1 and -2 proteins have structural similarity to C1r and C1s and mimic their activities. This means of activating the C2–C4 components to form a C5 convertase without need for specific antibody binding represents an important innate defense mechanism comparable to the alternative pathway, but utilizing the elements of the classical pathway except for the C1 proteins.

The Three Complement Pathways Converge at the Membrane-Attack Complex

The terminal sequence of complement activation involves C5b, C6, C7, C8, and C9, which interact sequentially to form a macromolecular structure called the **membrane-attack complex (MAC).** This complex forms a large channel through the membrane of the target cell, enabling ions and small molecules to diffuse freely across the membrane.

The end result of activating the classical, alternative, or lectin pathways is production of an active C5 convertase. This enzyme cleaves C5, which contains two protein chains, α and β. After binding of C5 to the nonenzymatic C3b component of the convertase, the amino terminus of the α chain is cleaved. This generates the small C5a fragment, which diffuses away, and the large C5b fragment, which binds to the surface of the target cell and provides a binding site for the subsequent components of the membrane-attack complex (see Figure 13-5, step 5). The C5b component is extremely labile and becomes inactive within 2 minutes unless C6 binds to it and stabilizes its activity.

Up to this point, all the complement reactions take place on the hydrophilic surface of membranes or on immune complexes in the fluid phase. As C5b6 binds to C7, the resulting complex undergoes a hydrophilic-amphiphilic structural transition that exposes hydrophobic regions, which serve as binding sites for membrane phospholipids. If the reaction occurs on a target-cell membrane, the hydrophobic binding sites enable the C5b67 complex to insert into the phospholipid bilayer. If, however, the reaction occurs on an immune

VISUALIZING CONCEPTS

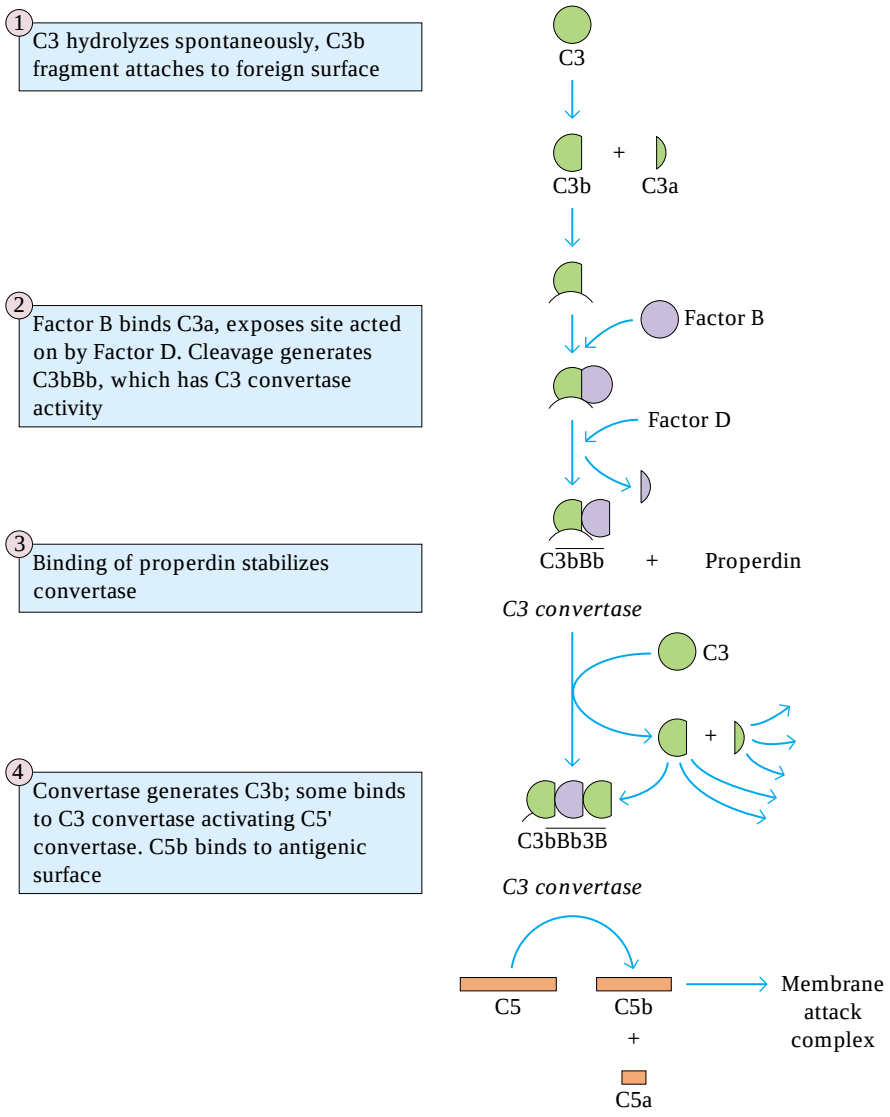


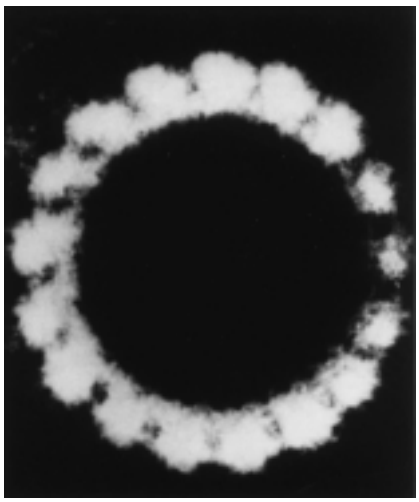
FIGURE 13-7 Schematic diagram of intermediates in the formation of bound C5b by the alternative pathway of complement activation. The $\overline{C3bBb}$ complex is stabilized by binding of properdin. Conversion of bound C5b to the membrane-attack complex occurs by the same sequence of reactions as in the classical pathway (see Figure 13-5).

complex or other noncellular activating surface, then the hydrophobic binding sites cannot anchor the complex and it is released. Released C5b67 complexes can insert into the membrane of nearby cells and mediate "innocent-bystander" lysis. Regulator proteins normally prevent this from occurring, but in certain diseases cell and tissue damage may result from innocent-bystander lysis. A hemolytic disorder resulting from deficiency in a regulatory protein is explained in the Clinical Focus section and an autoimmune process in which immune complexes mediate tissue damage will be considered in Chapter 20.

Binding of C8 to membrane-bound C5b67 induces a conformational change in C8, so that it too undergoes a hydrophilic-amphiphilic structural transition, exposing a hydrophobic region, which interacts with the plasma membrane. The C5b678 complex creates a small pore, 10 Å in diameter; formation of this pore can lead to lysis of red blood cells but not of nucleated cells. The final step in formation of

the MAC is the binding and polymerization of C9, a perforin-like molecule, to the C5b678 complex. As many as 10–17 molecules of C9 can be bound and polymerized by a single C5b678 complex. During polymerization, the C9 molecules undergo a hydrophilic-amphiphilic transition, so that they too can insert into the membrane. The completed MAC, which has a tubular form and functional pore size of 70–100 Å, consists of a C5b678 complex surrounded by a poly-C9 complex (Figure 13-8). Since ions and small molecules can diffuse freely through the central channel of the MAC, the cell cannot maintain its osmotic stability and is killed by an influx of water and loss of electrolytes.

(a)



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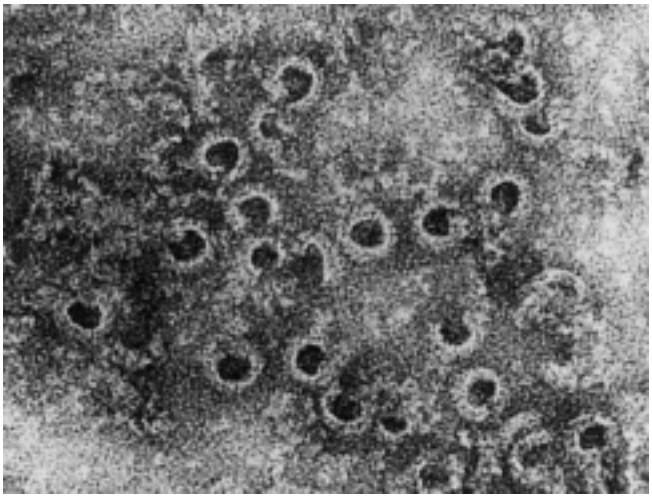


FIGURE 13-8 (a) Photomicrograph of poly-C9 complex formed by in vitro polymerization of C9. (b) Photomicrograph of complement-induced lesions on the membrane of a red blood cell. These lesions result from formation of membrane-attack complexes. *[Part (a) from E. R. Podack, 1986,* in Immunobiology of the Complement System, *Academic Press; part (b) from J. Humphrey and R. Dourmashkin, 1969,* Adv. Immunol. ***11:****75.]*

Regulation of the Complement System

Because many elements of the complement system are capable of attacking host cells as well as foreign cells and microorganisms, elaborate regulatory mechanisms have evolved to restrict complement activity to designated targets. A general mechanism of regulation in all complement pathways is the inclusion of highly labile components that undergo spontaneous inactivation if they are not stabilized by reaction with other components. In addition, a series of regulatory proteins can inactivate various complement components (Table 13-2). For example, the glycoprotein C1 inhibitor (C1Inh) can form a complex with $C1r_2s_2$, causing it to dissociate from C1q and preventing further activation of C4 or C2 (Figure 13-9a(1)).

The reaction catalyzed by the C3 convertase enzymes of the classical, lectin, and alternative pathways is the major amplification step in complement activation, generating hundreds of molecules of C3b. The C3b generated by these enzymes has the potential to bind to nearby cells, mediating damage to the healthy cells by causing their opsonization by phagocytic cells bearing C3b receptors or by induction of the membrane-attack complex. Damage to normal host cells is prevented because C3b undergoes spontaneous hydrolysis by the time it has diffused 40 nm away from the $\overline{C4b2a}$ or $\overline{C3bBb}$ convertase enzymes, so that it can no longer bind to its target site. The potential destruction of healthy host cells by C3b is further limited by a family of related proteins that regulate C3 convertase activity in the classical and alternative pathways. These regulatory proteins all contain repeating amino acid sequences (or motifs) of about 60 residues, termed *short consensus repeats* (SCRs). All these proteins are encoded at a single location on chromosome 1 in humans, known as the *regulators of complement activation* (RCA) gene cluster.

In the classical and lectin pathways, three structurally distinct RCA proteins act similarly to prevent assembly of C3 convertase (Figure 13-9a(2)). These regulatory proteins include soluble C4b-binding protein (C4bBP) and two membrane-bound proteins, complement receptor type 1 (CR1) and membrane cofactor protein (MCP). Each of these regulatory proteins binds to C4b and prevents its association with C2a. Once C4bBP, CR1, or MCP is bound to C4b, another regulatory protein, factor I, cleaves the C4b into bound C4d and soluble C4c (Figure 13-9a(3)). A similar regulatory sequence operates to prevent assembly of the C3 convertase $\overline{C3bBb}$ in the alternative pathway. In this case CR1, MCP, or a regulatory component called factor H binds to C3b and prevents its association with factor B (Figure 13-9a(4)). Once CR1, MCP, or factor H is bound to C3b, factor I cleaves the C3b into a bound iC3b fragment and a soluble C3f fragment. Further cleavage of iC3b by factor I releases C3c and leaves C3dg bound to the membrane (Figure 13-9a(5)). The molecular events involved in regulation of cell-bound C4b and C3b are depicted in Figure 13-10 (page 310).

TABLE 13-2 Proteins that regulate the complement system

Protein	Type of protein	Pathway affected	Immunologic function
C1 inhibitor (C1Inh)	Soluble	Classical	Serine protease inhibitor: causes $C1r_2s_2$ to dissociate from C1q
C4b-binding protein (C4bBP)*	Soluble	Classical and lectin	Blocks formation of C3 convertase by binding C4b; cofactor for cleavage of C4b by factor I
Factor H*	Soluble	Alternative	Blocks formation of C3 convertase by binding C3b; cofactor for cleavage of C3b by factor I
Complement-receptor type 1 (CR1)* Membrane-cofactor protein (MCP)*	Membrane bound	Classical, alternative, and lectin	Block formation of C3 convertase by binding C4b or C3b; cofactor for factor I-catalyzed cleavage of C4b or C3b C3bBb
Decay-accelerating factor (DAE or CD55)*	Membrane bound	Classical, alternative, and lectin	Accelerates dissociation of $C4\overline{b2a}$ and $C3\overline{bBb}$ (classical and alternative C3 convertases)
Factor-I	Soluble	Classical, alternative, and lectin	Serine protease: cleaves C4b or C3b using C4bBP, CR1, factor H, DAE, or MCP as cofactor
S protein	Soluble	Terminal	Binds soluble C5b67 and prevents its insertion into cell membrane
Homologous restriction factor (HRF) Membrane inhibitor of reactive lysis (MIRL or CD59)*	Membrane bound	Terminal	Bind to C5b678 on autologous cells, blocking binding of C9
Anaphylatoxin inactivator	Soluble	Effector	Inactivates anaphylatoxin activity of C3a, C4a, and C5a by carboxypeptidase N removal of C-terminal Arg

*An RCA (regulator of complement activation) protein. In humans, all RCA proteins are encoded on chromosome 1 and contain short consensus repeats.

Several RCA proteins also act on the assembled C3 convertase, causing it to dissociate; these include the previously mentioned C4bBP, CR1, and factor H. In addition, decay-accelerating factor (DAF or CD55), which is a glycoprotein anchored covalently to a glycophospholipid membrane protein, has the ability to dissociate C3 convertase. The consequences of DAF deficiency are described in the Clinical Focus section. Each of these RCA proteins accelerates decay (dissociation) of C3 convertase by releasing the component with enzymatic activity (C2a or Bb) from the cell-bound component (C4b or C3b). Once dissociation of the C3 convertase occurs, factor I cleaves the remaining membrane-bound C4b or C3b component, irreversibly inactivating the convertase (Figure 13-9b).

Regulatory proteins also operate at the level of the membrane-attack complex. The potential release of the C5b67 complex poses a threat of innocent-bystander lysis to healthy cells. A number of serum proteins counter this threat by binding to released C5b67 and preventing its insertion into the membrane of nearby cells. A serum protein called S protein can bind to C5b67, inducing a hydrophilic transition and thereby preventing insertion of C5b67 into the membrane of nearby cells (Figure 13-9c(1)).

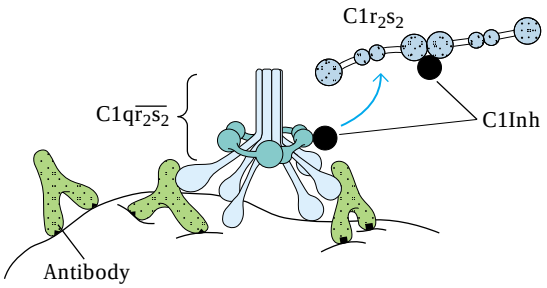
Complement-mediated lysis of cells is more effective if the complement is from a species different from that of the cells being lysed. This phenomenon depends on two membrane proteins that block MAC formation. These two proteins, present on the membrane of many cell types, are *homologous restriction factor* (HRF) and *membrane inhibitor of reactive lysis* (MIRL or CD59). Both HRF and MIRL protect cells from nonspecific complement-mediated lysis by binding to C8, preventing assembly of poly-C9 and its insertion into the plasma membrane (Figure 13-9c(2)). However, this inhibition occurs only if the complement components are from the same species as the target cells. For this reason, MIRL and HRF are said to display homologous restriction, for which the latter was named. As discussed in Chapter 21, homologous restriction poses a barrier to the use of organs from other species for clinical transplantation.

VISUALIZING CONCEPTS

Regulation of the Complement System

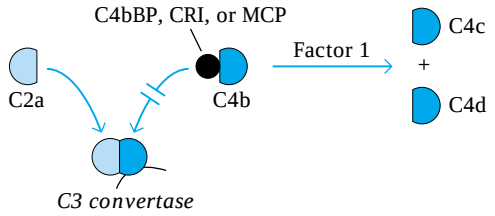
(a) Before assembly of convertase activity

1. C1 inhibitor (C1Iab) binds $C1r_2s_2$, causing dissociation from C1q



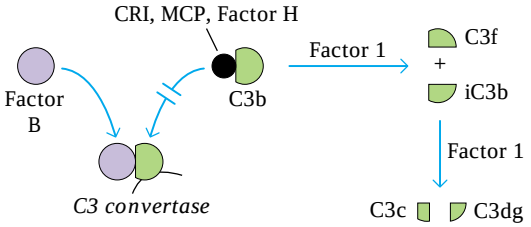
2. Association of C4b and C2a is blocked by binding C4b-binding protein (C4bBP), complement receptor type I, or membrane cofactor protein (MCP)

3. Inhibitor-bound C4b is cleaved by Factor 1



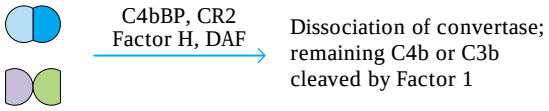
4. In alternative pathway, CR1, MCP, or Factor H prevent binding of C3b and Factor B

5. Inhibitor-bound C3b is cleaved by Factor 1



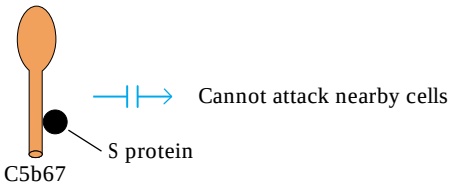
(b) After assembly of convertase

C3 convertases are dissociated by C4bBP, CR1, Factor H, and decay-accelerating Factor (DAF)



(c) Regulation at assembly of membrane-attack complex (MAC)

1. S protein prevents insertion of C5b67 MAC component into the membrane



2. Homologous restriction factor (HRF) and membrane inhibitor of reactive lysis (MIRL or CD59) bind $C8_1$, preventing assembly of poly-C9 and blocking formation of MAC

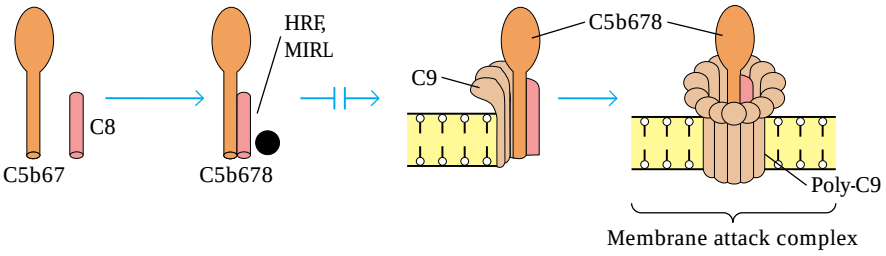


FIGURE 13-9 Regulation of the complement system by regulatory proteins (black).

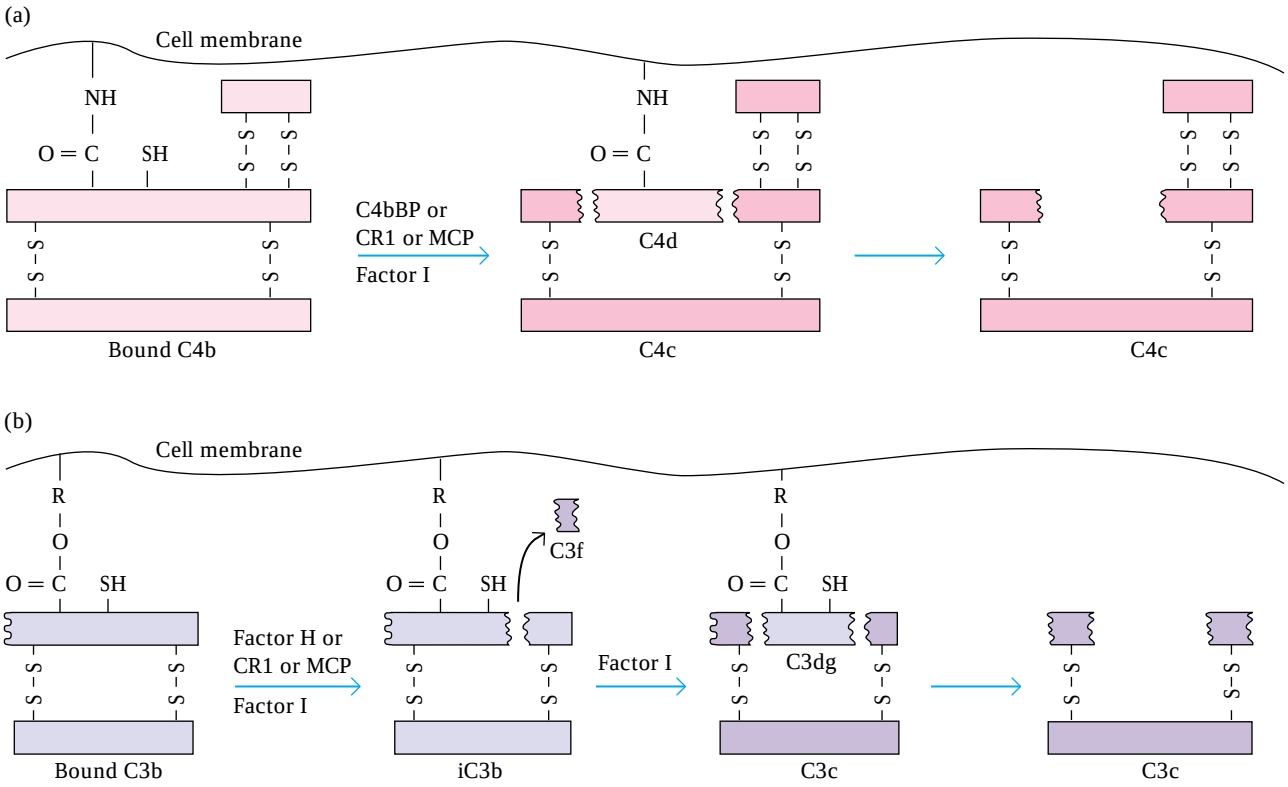


FIGURE 13-10 Inactivation of bound C4b and C3b by regulatory proteins of the complement system. (a) In the classical pathway, C4bBP (C4b-binding protein), CR1 (complement receptor type 1), or MCP (membrane cofactor protein) bind to C4b and act as cofactors for factor I–mediated cleavage of C4b. (b) In the alternative pathway, factor H, CR1, or MCP bind to Ccb and act as cofactors for factor I–mediated cleavage of C4b. Free diffusible fragments are shown in dark shades; membrane bound components in light shades.

Biological Consequences of Complement Activation

Complement serves as an important mediator of the humoral response by amplifying the response and converting it into an effective defense mechanism to destroy invading microorganisms. The MAC mediates cell lysis, while other complement components or split products participate in the inflammatory response, opsonization of antigen, viral neutralization, and clearance of immune complexes (Table 13-3, page 312).

Many of the biological activities of the complement system depend on the binding of complement fragments to complement receptors, which are expressed by various cells. In addition, some complement receptors play an important role in regulating complement activity by binding biologically active complement components and degrading them into inactive products. The complement receptors and their primary ligands, which include various complement components and their proteolytic breakdown products, are listed in Table 13-4 (page 312).

The Membrane-Attack Complex Can Lyse a Broad Spectrum of Cells

The membrane-attack complex formed by complement activation can lyse gram-negative bacteria, parasites, viruses, erythrocytes, and nucleated cells. Because the alternative and lectin pathways of activation generally occur without an initial antigen-antibody interaction, these pathways serve as important innate immune defenses against infectious microorganisms. The requirement for an initial antigen-antibody reaction in the classical pathway supplements these nonspecific innate defenses with a more specific defense mechanism. In some instances, the requirement for antibody in the activating event may be supplied by so-called natural antibodies, which are raised against common components of ubiquitous microbes.

The importance of cell-mediated immunity in host defense against viral infections has been emphasized in previous chapters. Nevertheless, antibody and complement do play a role in host defense against viruses and are often crucial in containing viral spread during acute infection and in protecting against reinfection. Most—perhaps

CLINICAL FOCUS

Paroxymal Nocturnal Hemoglobinuria: a Defect in Regulation of Complement Lysis

Common conditions associated with deficiency in the complement components include increased susceptibility to bacterial infections and systemic lupus erythematosus which is related to the inability to clear immune complexes. Deficiency in the proteins that regulate complement activity can cause equally serious disorders. An example is paroxymal nocturnal hemoglobinuria, or PNH, which manifests as increased fragility of erythrocytes, leading to chronic hemolytic anemia, pancytopenia (loss of blood cells of all types) and venous thrombosis (formation of blood clots). The name PNH derives from the presence of hemoglobin in the urine, most commonly observed in the first urine passed after a night's sleep. The cause of PNH is a general defect in synthesis of cell-surface proteins, which affects the expression of two regulators of complement, DAF (decay accelerating factor or CD55) and MIRL (membrane inhibitor of reactive lysis or CD59).

DAF and MIRL are cell-surface proteins that function as inhibitors of complement-mediated cell lysis, but act at different stages of the process. DAF inhibits cell lysis by causing dissociation and inactivation of the C3 convertases of the classical, lectin, and alternative pathways (see Figure 13-9b). MIRL acts later in the pathway by binding to the C5b678 complex, which inhibits C9 binding and prevents formation of the pores that destroy the cell under attack. Both proteins are expressed on erythrocytes as well as a number of other hematopoetic cell types. Deficiency in these proteins leads to highly increased sensitivity of host cells to the lytic effects of the host's complement activity. PNH, the clinical consequence of deficiency in DAF and MIRL, is a chronic disease with a mean survival time between 10 and 15 years. The most common causes of mortality in PNH are venous thrombosis affecting hepatic veins and progressive bone-marrow failure.

An obvious question about this rare but serious disease concerns the fact that two different proteins are involved in its pathogenesis. The simultaneous occurrence of a genetic defect in each of them would be rarer than the 1 in 100,000 incidence of PNH. The answer is that neither protein itself is defective in PNH; the defect lies in a posttranslational modification of the peptide anchor that binds them to the cell membrane. While most proteins that are expressed on the surface of cells have hydrophobic sequences that traverse the lipid bilayer in the cell membrane, some proteins are bound by glycolipid anchors (glycosyl phosphatidylinositol, or GPI) attached to amino acid residues in the protein. Without the ability to form GPI anchors, proteins that attach in this manner will be absent from the cell surface, including both DAF and MIRL.

The defect identified in PNH lies early in the enzymatic path to formation of a GPI anchor and resides in the *pig-a* gene (phosphatidylinositol glycan complementation class A gene). Transfection of cells from PNH patients with an intact *pig-a* gene restored the cells' resistance to host complement lysis. Examination of *pig-a* sequences in PNH patients reveals a number of different defects in this X-linked gene, indicating somatic rather than genetic origin of the defect. This description of PNH underscores the fact that the complement system is a powerful defender of the host but also a dangerous one. Complex systems of regulation are necessary to protect host cells from the activated complement complexes generated to lyse intruders.

all—enveloped viruses are susceptible to complement-mediated lysis. The viral envelope is largely derived from the plasma membrane of infected host cells and is therefore susceptible to pore formation by the membrane-attack complex. Among the pathogenic viruses susceptible to lysis by complement-mediated lysis are herpesviruses, orthomyxoviruses, paramyxoviruses, and retroviruses.

The complement system is generally quite effective in lysing gram-negative bacteria (Figure 13-11). However, some gram-negative bacteria and most gram-positive bacteria have mechanisms for evading complement-mediated damage (Table 13-5). For example, a few gram-negative bacteria can develop resistance to complement-mediated lysis that correlates with the virulence of the organism. In *Escherichia coli* and *Salmonella*, resistance to complement is associated with the smooth bacterial phenotype, which is characterized by the presence of long polysaccharide side chains in the cell-wall lipopolysaccharide (LPS) component. It has been proposed that the increased LPS in the wall of resistant strains may prevent insertion of the MAC into the bacterial membrane, so that the complex is released from the bacterial cell rather than forming a pore. Strains of *Neisseria*

TABLE 13-3 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, C**5**a
Extravasation and chemotaxis of leukocytes at inflammatory site	C3a, C**5**a, 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	C**3**b, 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.

gonorrheae resistant to complement-mediated killing have been associated with disseminated gonococcal infections in humans. Some evidence suggests that the membrane proteins of resistant *Neisseria* strains undergo noncovalent interactions with the MAC that prevent its insertion into the outer membrane of the bacterial cells. These examples of resistant gram-negative bacteria are the exception; most gram-negative bacteria are susceptible to complement-mediated lysis.

Gram-positive bacteria are generally resistant to complement-mediated lysis because the thick peptidoglycan layer in their cell wall prevents insertion of the MAC into the inner membrane. Although complement activation can occur on the cell membrane of encapsulated bacteria such as *Streptococcus pneumoniae*, the capsule prevents interaction between C3b deposited on the membrane and the CR1 on phagocytic cells. Some bacteria possess an elastase that inactivates C3a and C5a, preventing these split products from inducing an

TABLE 13-4 Complement-binding receptors

Receptor	Major ligands	Activity	Cellular distribution
CR1 (CD35)	C3b, C4b	Blocks formation of C3 convertase; binds immune complexes to cells	Erythrocytes, neutrophils, monocytes, macrophages, eosinophils, follicular dendritic cells, B cells, some T cells
CR2 (CD21)	C3d, C3dg,* iC3b	Part of B-cell coreceptor; binds Epstein-Barr virus	B cells, follicular dendritic cells, some T cells
CR3 (CD11b/18)	iC3b	Bind cell-adhesion molecules on neutrophils, facilitating their extravasation; bind immune complexes, enhancing their phagocytosis	Monocytes, macrophages, neutrophils, natural killer cells, some T cells
CR4 (CD11c/18)			
C3a/C4a receptor	C3a, C4a	Induces degranulation of mast cells and basophils	Mast cells, basophils, granulocytes
C5a receptor	C5a	Induces degranulation of mast cells and basophils	Mast cells, basophils, granulocytes, monocytes, macrophages, platelets, endothelial cells

*Cleavage of C3dg by serum proteases generates C3d and C3g.

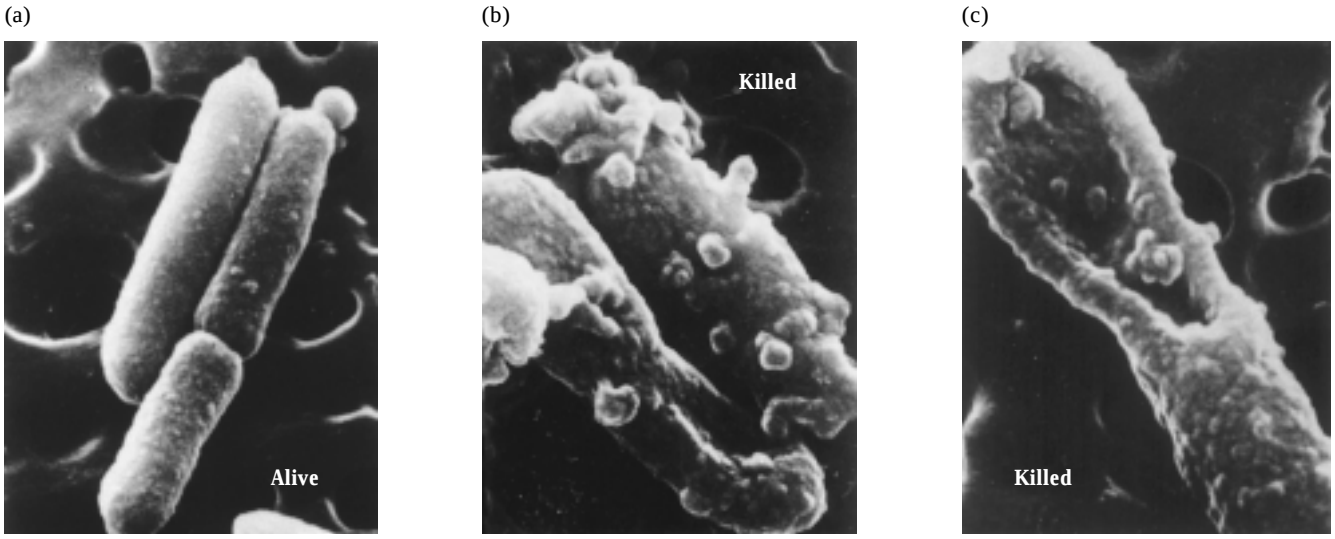


FIGURE 13-11 Scanning electron micrographs of *E. coli* showing (a) intact cells and (b, c) cells killed by complement-mediated lysis. Note membrane blebbing on lysed cells. *[From R. D. Schreiber et al., 1979,* J. Exp. Med. ***149:****870.]*

inflammatory response. In addition to these mechanisms of evasion, various bacteria, viruses, fungi, and protozoans contain proteins that can interrupt the complement cascade on their surfaces, thus mimicking the effects of the normal complement regulatory proteins C4bBP, CR1, and DAF.

Lysis of nucleated cells requires formation of multiple membrane attack complexes, whereas a single MAC can lyse a red blood cell. Many nucleated cells, including the majority of cancer cells, can endocytose the MAC. If the complex is removed soon enough, the cell can repair any membrane

TABLE 13-5 Microbial evasion of complement-mediated damage

Microbial component	Mechanism of evasion	Examples
	GRAM-NEGATIVE BACTERIA	
Long polysaccharide chains in cell-wall LPS	Side chains prevent insertion of MAC into bacterial membrane	Resistant strains of *E. coli* and *Salmonella*
Outer membrane protein	MAC interacts with membrane protein and fails to insert into bacterial membrane	Resistant strains of *Neisseria gonorrhoeae*
Elastase	Anaphylatoxins C3a and C5a are inactivated by microbial elastase	*Pseudomonas aeruginosa*
	GRAM-POSITIVE BACTERIA	
Peptidoglycan layer of cell wall	Insertion of MAC into bacterial membrane is prevented by thick layer of peptidoglycan	*Streptococcus*
Bacterial capsule	Capsule provides physical barrier between C3b deposited on bacterial membrane and CR1 on phagocytic cells	*Streptococcus pneumoniae*
	OTHER MICROBES	
Proteins that mimic complement regulatory proteins	Protein present in various bacteria, viruses, fungi, and protozoans inhibit the complement cascade	Vaccinia virus, herpes simplex, Epstein-Barr virus, *Trypanosoma cruzi, Candida albicans*

KEY: CR1 = type 1 complement receptor; LPS = lipopolysaccharide; MAC = membrane-attack complex (C5b–9).

damage and restore its osmotic stability. An unfortunate consequence of this effect is that complement-mediated lysis by antibodies specific for tumor-cell antigens, which offers a potential weapon against cancer, may be rendered ineffective by endocytosis of the MAC (see Chapter 22).

Cleavage Products of Complement Components Mediate Inflammation

The complement cascade is often viewed in terms of the final outcome of cell lysis, but various peptides generated during formation of the MAC play a decisive role in the development of an effective inflammatory response (see Table 13-3). The smaller fragments resulting from complement cleavage, C3a, C4a, and C5a, called **anaphylatoxins,** bind to receptors on mast cells and blood basophils and induce degranulation, with release of histamine and other pharmacologically active mediators. The anaphylatoxins also induce smooth-muscle contraction and increased vascular permeability. Activation of the complement system thus results in influxes of fluid that carries antibody and phagocytic cells to the site of antigen entry. The activities of these highly reactive anaphylatoxins are regulated by a serum protease called carboxypeptidase N, which cleaves an Arg residue from the C terminus of the molecules, yielding so-called *des-Arg* forms. The des-Arg forms of C3a and C4a are completely inactive while that of C5a retains about 10% of its chemotactic activity and 1% of its ability to cause smooth muscle contraction.

C3a, C5a, and C5b67 can each induce monocytes and neutrophils to adhere to vascular endothelial cells, extravasate through the endothelial lining of the capillary, and migrate toward the site of complement activation in the tissues. C5a is most potent in mediating these processes, effective in picomolar quantities. The role of complement in leukocyte chemotaxis is discussed more fully in Chapter 15.

C3b and C4b Binding Facilitates Opsonization

C3b is the major **opsonin** of the complement system, although C4b and iC3b also have opsonizing activity. The amplification that occurs with C3 activation results in a coating of C3b on immune complexes and particulate antigens. Phagocytic cells, as well as some other cells, express complement receptors (CR1, CR3, and CR4) that bind C3b, C4b, or iC3b (see Table 13-4). Antigen coated with C3b binds to cells bearing CR1. If the cell is a phagocyte (e.g., a neutrophil, monocyte, or macrophage), phagocytosis will be enhanced (Figure 13-12). Activation of phagocytic cells by various agents, including C5a anaphylatoxin, has been shown to increase the number of CR1s from 5000 on resting phagocytes to 50,000 on activated cells, greatly facilitating their phagocytosis of C3b-coated antigen. Recent studies indicate that complement fragment C3b acts as an adjuvant when coupled with protein antigens. C3b targets the antigen directly to the phagocyte, enhancing the initiation of antigen processing and accelerating specific antibody production.

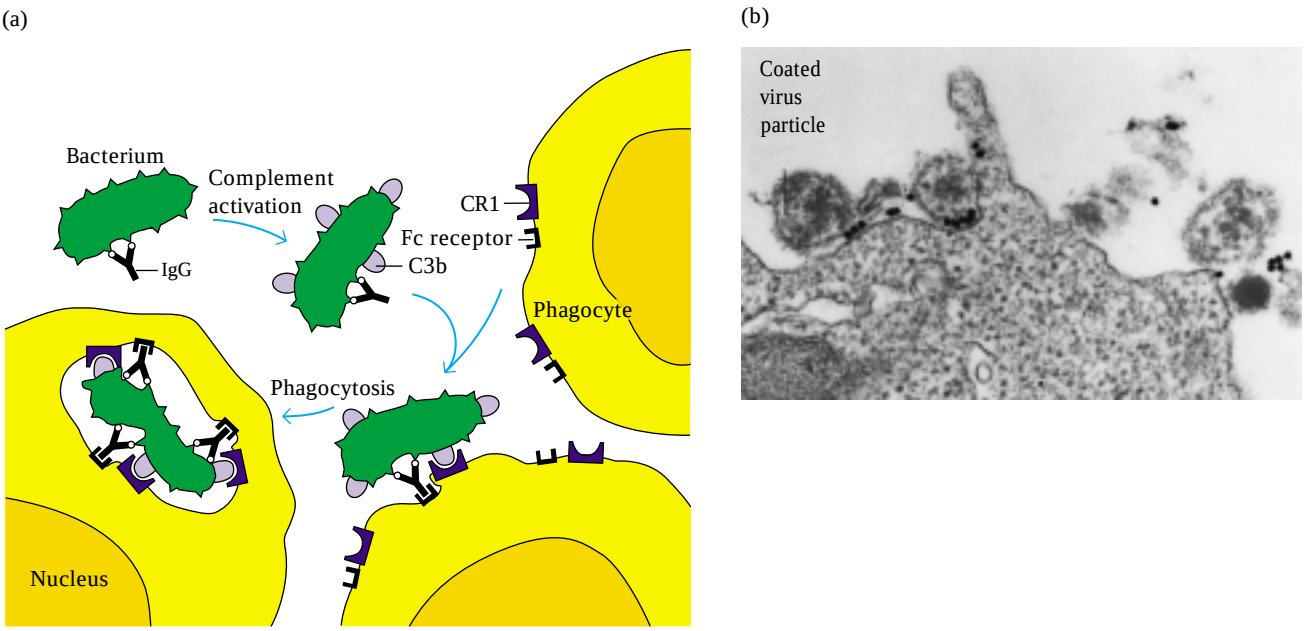


FIGURE 13-12 (a) Schematic representation of the roles of C3b and antibody in opsonization. (b) Electron micrograph of Epstein-Barr virus coated with antibody and C3b and bound to the Fc and C3b receptor (CR1) on a B lymphocyte. *[Part (b) from N. R. Cooper and G. R. Nemerow, 1986, in* Immunobiology of the Complement System, *Academic Press.]*

The Complement System Also Neutralizes Viral Infectivity

For most viruses, the binding of serum antibody to the repeating subunits of the viral structural proteins creates particulate immune complexes ideally suited for complement activation by the classical pathway. Some viruses (e.g., retroviruses, Epstein-Barr virus, Newcastle disease virus, and rubella virus) can activate the alternative, lectin, or even the classical pathway in the absence of antibody.

The complement system mediates viral neutralization by a number of mechanisms. Some degree of neutralization is achieved through the formation of larger viral aggregates, simply because these aggregates reduce the net number of infectious viral particles. Although antibody plays a role in the formation of viral aggregates, in vitro studies show that the C3b component facilitates aggregate formation in the presence of as little as two molecules of antibody per virion. For example, polyoma virus coated with antibody is neutralized when serum containing activated C3 is added.

The binding of antibody and/or complement to the surface of a viral particle creates a thick protein coating that can be visualized by electron microscopy (Figure 13-13). This coating neutralizes viral infectivity by blocking attachment to susceptible host cells. The deposits of antibody and complement on viral particles also facilitate binding of the viral particle to cells possessing Fc or type 1 complement receptors (CR1). In the case of phagocytic cells, such binding can be followed by phagocytosis and intracellular destruction of the ingested viral particle. Finally, complement is effective in lysing most, if not all, enveloped viruses, resulting in fragmentation of the envelope and disintegration of the nucleocapsid.

The Complement System Clears Immune Complexes from Circulation

The importance of the complement system in clearing immune complexes is seen in patients with the autoimmune disease systemic lupus erythematosus (SLE). These individuals produce large quantities of immune complexes and suffer tissue damage as a result of complement-mediated lysis and the induction of type II or type III hypersensitivity (see Chapter 16). Although complement plays a significant role in the development of tissue damage in SLE, the paradoxical finding is that deficiencies in C1, C2, C4, and CR1 predispose an individual to SLE; indeed, 90% of individuals who completely lack C4 develop SLE. The complement deficiencies are thought to interfere with effective solubilization and clearance of immune complexes; as a result, these complexes persist, leading to tissue damage by the very system whose deficiency was to blame.

The coating of soluble immune complexes with C3b is thought to facilitate their binding to CR1 on erythrocytes. Although red blood cells express lower levels of CR1 ($\sim 5 \times 10^2$ per cell) than granulocytes do ($\sim 5 \times 10^4$ per cell), there are about 10^3 red blood cells for every white blood cell; therefore, erythrocytes account for about 90% of the CR1 in the blood. For this reason, erythrocytes play an important role in binding C3b-coated immune complexes and carrying these complexes to the liver and spleen. In these organs, immune complexes are stripped from the red blood cells and are phagocytosed, thereby preventing their deposition in tissues (Figure 13-14). In SLE patients, deficiencies in C1, C2, and C4 each contribute to reduced levels of C3b on immune complexes and hence inhibit their clearance. The lower levels of CR1 expressed on the erythrocytes of SLE patients also may interfere with the proper binding and clearance of immune complexes.

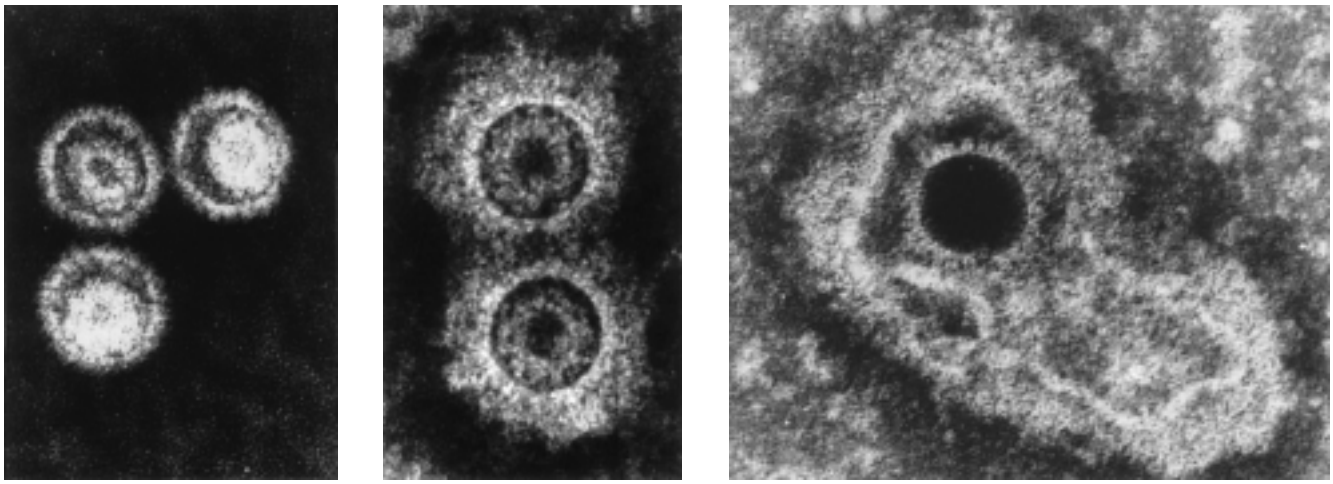


FIGURE 13-13 Electron micrographs of negatively stained preparations of Epstein-Barr virus. (a) Control without antibody. (b) Antibody-coated particles. (c) Particles coated with antibody and complement. *[From N. R. Cooper and G. R. Nemerow, 1986, in* Immunobiology of the Complement System, *Academic Press.]*

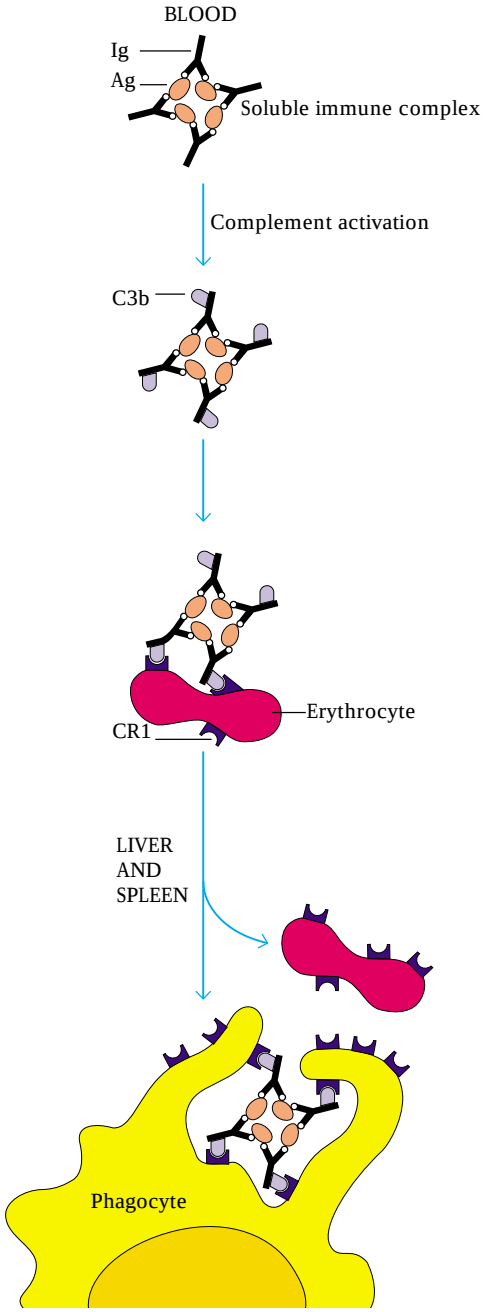


FIGURE 13-14 Clearance of circulating immune complexes by reaction with receptors for complement products on erythrocytes and removal of these complexes by receptors on macrophages in the liver and spleen. Because erythrocytes have fewer receptors than macrophages, the latter can strip the complexes from the erythrocytes as they pass through the liver or spleen. Deficiency in this process can lead to renal damage due to accumulation of immune complexes.

Complement Deficiencies

Genetic deficiencies have been described for each of the complement components. Homozygous deficiencies in any of the early components of the classical pathway (C1q, C1r, C1s, C4, and C2) exhibit similar symptoms, notably a marked increase in immune-complex diseases such as systemic lupus erythematosus, glomerulonephritis, and vasculitis. These deficiencies highlight the importance of the early complement reactions in generating C3b, and the critical role of C3b in solubilization and clearance of immune complexes. In addition to immune-complex diseases, individuals with such complement deficiencies may suffer from recurrent infections by pyogenic (pus-forming) bacteria such as streptococci and staphylococci. These organisms are gram-positive and therefore resistant to the lytic effects of the MAC. Nevertheless, the early complement components ordinarily prevent recurrent infection by mediating a localized inflammatory response and opsonizing the bacteria. Deficiencies in factor D and properdin—early components of the alternative pathway—appear to be associated with *Neisseria* infections but not with immune-complex disease.

Patients with C3 deficiencies have the most severe clinical manifestations, reflecting the central role of C3 in activation of C5 and formation of the MAC. The first patient identified with a C3 deficiency was a child suffering from frequent severe bacterial infections and initially diagnosed as having agammaglobulinemia. After tests revealed normal immunoglobulin levels, a deficiency in C3 was discovered. This case highlights the critical function of the complement system in converting a humoral antibody response into an effective defense mechanism. The majority of patients with C3 deficiency have recurrent bacterial infections and may have immune-complex diseases.

Individuals with homozygous deficiencies in the components involved in the MAC develop recurrent meningococcal and gonococcal infections caused by *Neisseria* species. In normal individuals, these gram-negative bacteria are generally susceptible to complement-mediated lysis or are cleared by the opsonizing activity of C3b. MAC-deficient individuals rarely have immune-complex disease, which suggests that they produce enough C3b to clear immune complexes. Interestingly, a deficiency in C9 results in no clinical symptoms, suggesting that the entire MAC is not always necessary for complement-mediated lysis.

Congenital deficiencies of complement regulatory proteins have also been reported. The C1 inhibitor (C1Inh) regulates activation of the classical pathway by preventing excessive C4 and C2 activation by C1. Deficiency of C1Inh is an autosomal dominant condition with a frequency of 1 in 1000. The deficiency gives rise to a condition called hereditary angioedema, which manifests clinically as localized edema of the tissue, often following trauma, but sometimes with no known cause. The edema can be in subcutaneous tissues or within the bowel, where it causes abdominal pain, or in the upper respiratory tract, where it causes obstruction of the airway.

Studies in humans and experimental animals with homozygous deficiencies in complement components have

been the major source of information concerning the role of individual complement components in immunity. These findings have been greatly extended by studies using knockout mice genetically engineered to lack expression of specific complement components. Investigations of in vivo complement activity in these animals has allowed dissection of the complex system of complement proteins and the assignment of precise biologic roles to each.

SUMMARY

- The complement system comprises a group of serum proteins, many of which 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, the complement system mediates opsonization of bacteria, activation of inflammation, and clearance of immune complexes.
- Interactions of complement proteins and protein fragments with receptors on cells of the immune system control both innate and acquired immune responses.
- Because of its ability to damage the host organism, the complement system requires complex passive and active regulatory mechanisms.
- Clinical consequences of inherited complement deficiencies range from increases in susceptibility to infection to tissue damage caused by immune complexes.

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USEFUL WEB SITES

http://www.complement-genetics.uni-mainz.de/

The Complement Genetics Homepage from the University of Mainz gives chromosomal locations and information on genetic deficiencies of complement proteins.

http://www.cehs.siu.edu/fix/medmicro/cfix.htm

A clever graphic representation of the basic assay for complement activity using red blood cell lysis, from D. Fix at University of Southern Illinois, Carbondale.

http://www.gla.ac.uk/Acad/Immunology/compsyst.htm

Notes from D. F. Lappin at University of Glasgow, UK, on the complement system. The site includes a listing of all complement proteins and their molecular properties.

Study Questions

CLINICAL FOCUS QUESTION Explain why complement disorders involving regulatory components such as PNH may be more serious than deficiencies in the active complement components.

1. Indicate whether each of the following statements is true or false. If you think a statement is false, explain why.

   a. A single molecule of bound IgM can activate the C1q component of the classical complement pathway.

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Review and quiz of key terms

b. C3a and C3b are fragments of C3.
c. The C4 and C2 complement components are present in the serum in a functionally inactive proenzyme form.
d. Nucleated cells tend to be more resistant to complement-mediated lysis than red blood cells.
e. Enveloped viruses cannot be lysed by complement because their outer envelope is resistant to pore formation by the membrane-attack complex.
f. C4-deficient individuals have difficulty eliminating immune complexes.

2. Explain why serum IgM cannot activate complement by itself.

3. Would you expect a C1 or C3 complement deficiency to be more serious clinically? Why?

4. Some microorganisms produce enzymes that can degrade the Fc portion of antibody molecules. Why would such enzymes be advantageous for the survival of microorganisms that possess them?

5. Complement activation can occur via the classical, alternative, or lectin pathway.

a. How do the three pathways differ in the substances that can initiate activation?
b. Which portion of the overall activation sequence differs in the three pathways? Which portion is similar?
c. How do the biological consequences of complement activation via these pathways differ?

6. Enucleated cells, such as red blood cells, are more susceptible to complement-mediated lysis than nucleated cells.

a. Explain why the red blood cells of an individual are not normally destroyed as the result of innocent-bystander lysis by complement.
b. Under what conditions might complement cause lysis of an individual's own red blood cells?

7. Briefly explain the mechanism of actionÏ of the following complement regulatory proteins. Indicate which pathway(s) each protein regulates.

a. C1 inhibitor (C1Inh)
b. C4b-binding protein (C4bBP)
c. Homologous restriction factor (HRF)
d. Decay-accelerating factor (DAF)
e. Factor H
f. Membrane cofactor protein (MCP)

8. For each complement component(s) or reaction (a–l), select the most appropriate description listed below (1–13). Each description may be used once, more than once, or not at all.

Complement Component(s)/Reactions

a. _____C3b
b. _____C1, C4, C2, and C3
c. _____C9
d. _____C3, factor B, and factor D
e. _____C1q
f. _____$C\overline{4b2a3b}$
g. _____C5b, C6, C7, C8, and C9
h. _____$C3 \rightarrow C3a + C3b$
i. _____C3a, C5a, and C5b67
j. _____C3a, C4a, and C5a
k. _____$C\overline{4b2a}$
l. _____$C3b + B \rightarrow C3\overline{bBb} + Ba$

Descriptions

(1) Reaction that produces major amplification during activation
(2) Are early components of alternative pathway
(3) Compose the membrane-attack complex
(4) Mediates opsonization
(5) Are early components of classical pathway
(6) Has perforin-like activity
(7) Binds to Fc region of antibodies
(8) Have chemotactic activity
(9) Has C3 convertase activity
(10) Induce degranulation of mast cells (are anaphylatoxins)
(11) Has C5 convertase activity
(12) Reaction catalyzed by factor D
(13) Reaction catalyzed by $C1qr_2s_2$

9. You have prepared knockout mice with mutations in the genes that encode various complement components. Each knockout strain cannot express one of the complement components listed across the top of the table below. Predict the effect of each mutation on the steps in complement activation and on the complement effector functions indicated in the table below using the following symbols: NE = no effect; D = process/function decreased but not abolished; A = process/function abolished.

	Component knocked out						
	C1q	C4	C3	C5	C6	C9	Factor B
COMPLEMENT ACTIVATION							
Formation of C3 convertase in classical pathway							
Formation of C3 convertase in alternative pathway							
Formation of C5 convertase in classical pathway							
Formation of C5 convertase in alternative pathway							
EFFECTOR FUNCTIONS							
C3b-mediated opsonization							
Neutrophil chemotaxis							
Cell lysis							