

TEACHING OBJECTIVES:

1. Understand different pathways of complement activation.
2. Know the enzymatic and nonenzymatic mechanisms of C activation.
3. Know the biological properties of C activation products.
4. Know the significance of C system in host resistance, inflammation and damage to self.
5. Understand the mechanisms of regulating C activation and its products.

SUPPLEMENTAL READING:

Male *et al.* Immunology (7th ed.), Chapter 4.

COMPLEMENT

I. COMPLEMENT FUNCTIONS, DEFINITIONS AND NOMENCLATURE

Historically the term **complement** (C) was used to refer to a heat labile serum component that was able to lyse bacteria. However, complement is now known to contribute to host defenses in other ways as well. Complement can **opsonize bacteria** for enhanced phagocytosis; it can **recruit and activate various cells** including PMNs and macrophages, it can **participate in regulation of antibody responses** and it can aid in the clearance of immune complexes and apoptotic cells. Complement can also have **detrimental effects** for the host; it contributes to inflammation and tissue damage and it can trigger anaphylaxis.

Complement is actually composed of over 20 different serum proteins (see Table 1) that are produced by a variety of cells including, **hepatocytes, macrophages and gut**

epithelial cells. Some complement proteins bind to immunoglobulins or to membrane components of cells. Others are proenzymes that when activated cleave one or more other complement proteins. Upon cleavage some of the complement proteins yield fragments that activate cells, increase vascular permeability or opsonize bacteria.

Table 1. Complement proteins

C1(qrs), C2, C3, C4, C5, C6, C7, C8 and C9
Factors B, D, H, I and Properdin (P)
Mannose binding lectin (MBL), MBL-associated serine proteases (MASP-1 and MASP-2)
C1 inhibitor (C1-INH, serpin), C4-binding protein (C4-BP), decay accelerating factor (DAF)
Complement receptor 1 (CR1) protein S (vitronectin)

The following are some definitions of terms commonly used in discussing complement:

C-activation: alteration of C proteins, enabling them to interact with another component

C-fixation: utilization of C by antigen-antibody complexes

Hemolytic unit (CH₅₀): dilution of serum that will lyse 50% of a standard amount of antibody-coated erythrocytes

C-inactivation: denaturation (usually by heat) of an early complement component resulting in loss of hemolytic activity

Convertase/esterase: altered C protein which acts as a **proteolytic enzyme** for another C component

The following are some conventions of complement nomenclature:

Activated components of complement are over-lined (*e.g.* C1^{qrs})

When enzymatically cleaved, the larger fragment binds to the activation complex or membrane and the smaller fragment is released into the microenvironment. The letter “b” is usually added to name of the larger membrane-binding fragment and the letter “a” to the smaller fragment (*e.g.* C3^b and C3^a). The exception is

for C2 in which the larger membrane-binding fragment is given the “a” designation and the smaller fragment is given the “b” designation.

II. COMPLEMENT PATHWAYS

The complement system is divided into four pathways each of which requires different protein components (Figure 1). The classical pathway, named because it was the first described, is dependent upon antibody to be operational. The lectin and alternative pathways are antibody independent for their function. All three of these pathways ultimately result in the activation of C3 and the formation of a C5 convertase, which leads to the activation of C5 and the lytic pathway. Each of these pathways is a series of sequential steps that proceed in a cascading fashion. Since some of the steps are enzymatic in nature there is amplification as the pathways proceed.

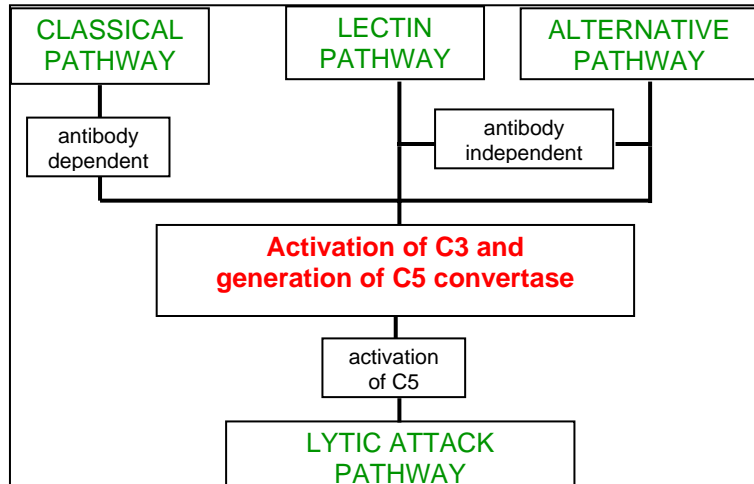


Figure 1. Pathways of complement activation

A. Classical Pathway (Figures 2 and 3)

C1 a multi-subunit protein containing three different proteins, C1q, C1r and C1s, binds to the Fc region of IgG and IgM antibody molecules that have interacted with antigen. C1 binding does not occur to antibodies that have not complexed with antigen and binding requires calcium and magnesium ions. (N.B. In some cases C1 can bind to aggregated immunoglobulin [e.g. aggregated IgG] or to certain pathogen surfaces

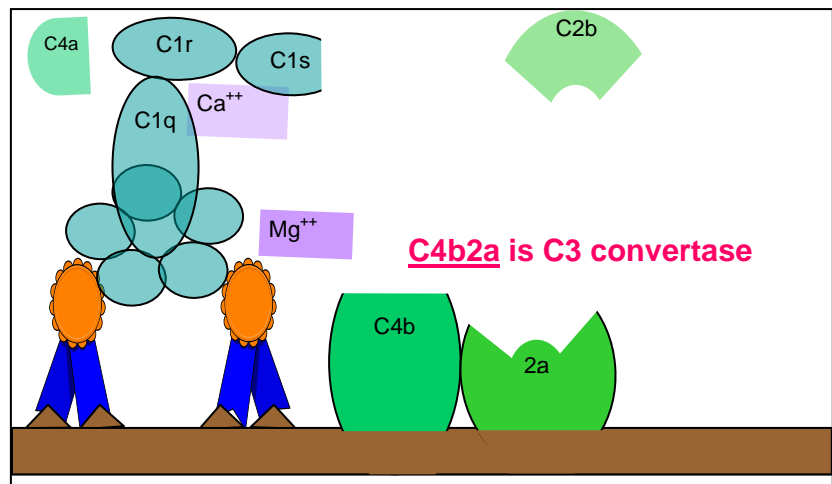


Figure 2. Generation of C3 convertase in the classical pathway

in the absence of antibody). The binding of C1 to antibody is via C1q and C1q must cross link at least two antibody molecules before it is firmly fixed. The binding of C1q results in the activation of C1r which in turn activates C1s. The result is the formation of an activated “C1qrs”, which is an enzyme that cleaves

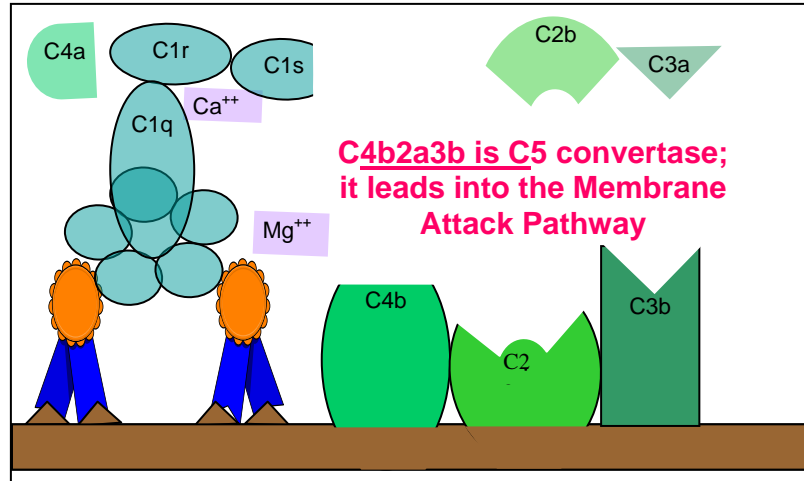


Figure 3. Generation of C5 convertase in the classical pathway

C4 into two fragments C4a and C4b. The C4b fragment binds to the membrane and the C4a fragment is released into the microenvironment. Activated “C1qrs” also cleaves C2 into C2a and C2b. C2a binds to the membrane in association with C4b and C2b is released into the microenvironment. The resulting C4bC2a complex is a C3 convertase, which cleaves C3 into C3a and C3b. C3b binds to the membrane in association with C4b and C2a and C3a is released into the microenvironment. The resulting C4bC2aC3b is a C5 convertase. The generation of C5 convertase is the end of the classical pathway.

Several of the products of the classical pathway have potent biological activities that contribute to host defenses. Some of these products may also have detrimental effects if produced in an unregulated manner. Table 2 summarizes the biological activities of classical pathway components.

Table 2. Biological Activity of classical pathway products

Component	Biological Activity
C2b	Prokinin ; cleaved by plasmin to yield kinin, which results in edema
C3a	Anaphylotoxin ; can activate basophils and mast cells to degranulate resulting in increased vascular permeability and contraction of smooth muscle cells, which may lead to anaphylaxis
C3b	Opsonin ; promotes phagocytosis by binding to complement receptors Activation of phagocytic cells
C4a	Anaphylotoxin (weaker than C3a)
C4b	Opsonin ; promotes phagocytosis by binding to complement receptors

If the classical pathway were not regulated there would be continued production of C2b, C3a, and C4a. Thus, there must be some way to regulate the activity of the

classical pathway. Table 3 summarizes the ways in which the classical pathway is regulated.

Table 3. Regulation of the Classical Pathway

Component	Regulation
All	C1-INH ; dissociates C1r and C1s from C1q
C3a	C3a inactivator (C3a-INA; Carboxypeptidase B) ; inactivates C3a
C3b	Factors H and I ; Factor H facilitates the degradation of C3b by Factor I
C4a	C3-INA
C4b	C4 binding protein(C4-BP) and Factor I ; C4-BP facilitates degradation of C4b by Factor I; C4-BP also prevents association of C2a with C4b thus blocking the formation of C3 convertase

The importance of C1-INH in regulating the classical pathway is demonstrated by the result of a deficiency in this inhibitor. C1-INH deficiencies are associated with the development of **hereditary angioedema**.

B. Lectin Pathway (Figures 4 and 5)

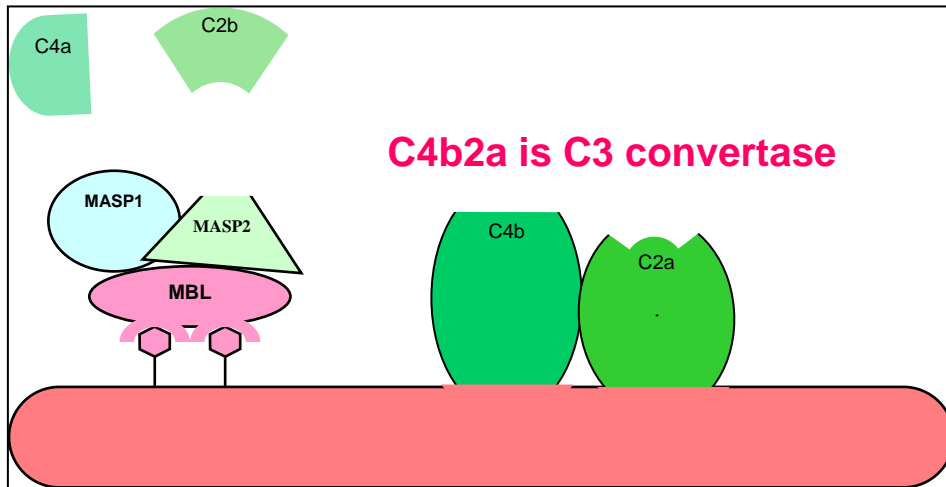


Figure 4. Generation of C3 convertase in the lectin pathway

The lectin pathway is very similar to the classical pathway. It is initiated by the binding of mannose binding lectin (MBL) to bacterial surfaces with mannose-containing **polysaccharides**. Binding of MBL to a pathogen results in the association of two serine proteases, MASP-1 and MASP-2 (MBL-associated serine proteases). MASP-1 and MASP-2 are similar to C1r and C1s, respectively and MBL is similar to C1q. Formation of the MBL/MASP-1/MASP-2 tri-molecular complex results in the activation of the MASPs and subsequent cleavage of C4 into C4a and C4b. The C4b fragment binds to the membrane and the C4a fragment is released into the

microenvironment. Activated MASPs also cleave C2 into C2a and C2b. C2a binds to the membrane in association with C4b and C2b is released into the microenvironment. The resulting C4bC2a complex is a C3 convertase, which cleaves C3 into C3a and C3b. C3b binds to the membrane in association with C4b and C2a and C3a is released into the microenvironment. The resulting C4bC2aC3b is a C5 convertase. The generation of C5 convertase is the end of the lectin pathway.

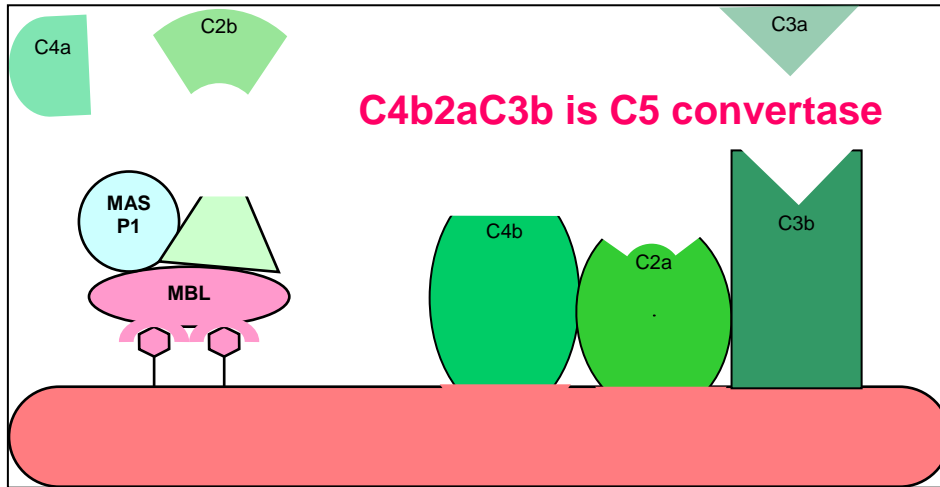


Figure 5. Generation of C5 convertase in the lectin pathway

The biological activities and the regulatory proteins of the lectin pathway are the same as those of the classical pathway.

C. Alternative Pathway

1. Amplification loop of C3b formation (Figure 6)

In serum there is low level **spontaneous hydrolysis of C3** to produce C3i. Factor B binds to C3i and becomes susceptible to Factor D, which cleaves Factor B into Bb. The **C3iBb** complex acts as a C3 convertase and cleaves C3 into C3a and C3b. Once C3b is formed, Factor B will bind to it and becomes susceptible to cleavage by Factor D. The resulting **C3bBb** complex is a C3 convertase that will continue to generate more C3b, thus amplifying C3b production. If this process continues unchecked the result

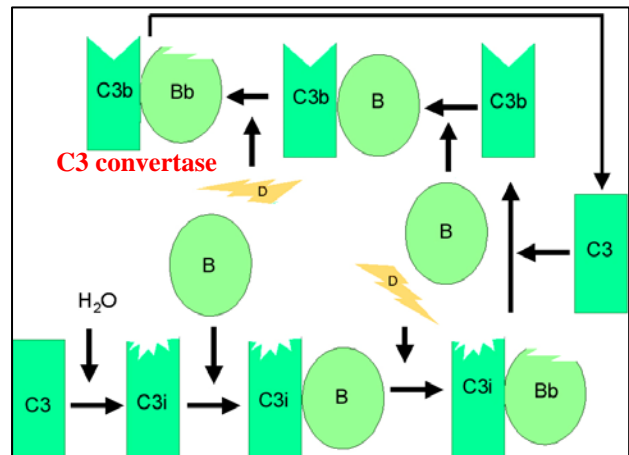


Figure 6. Spontaneous activation of C3

would be the consumption of all C3 in the serum. Thus, the spontaneous production of C3b is tightly controlled.

2. Control of the amplification loop (Figures 7 and 8)

As spontaneously produced C3b binds to autologous host membranes it interacts with DAF (decay accelerating factor), which blocks the association of Factor B with C3b thereby preventing the formation of additional C3

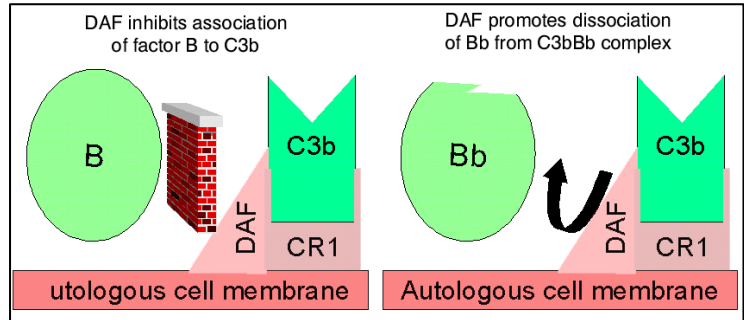


Figure 7. Regulation of activated C3 by DAF

convertase. In addition, DAF accelerates the dissociation of Bb from C3b in C3 convertase that has already formed, thereby stopping the production of additional C3b. Some cells possess complement receptor 1 (CR1). Binding of C3b to CR1 facilitates the enzymatic degradation of C3b by Factor I. In addition binding of C3 convertase (C3bBb) to CR1 also dissociates Bb from the complex. Thus, in cells possessing complement receptors, CR1 also plays a role in controlling the amplification loop. Finally, Factor H can bind to C3b bound to a cell or in the in the fluid phase and facilitate the enzymatic degradation of C3b by Factor I. Thus, the amplification loop is controlled by either blocking the formation of C3 convertase, dissociating C3 convertase, or by enzymatically digesting C3b. The importance of controlling this amplification loop is illustrated in patients with genetic deficiencies of Factor H or I. These patients have a C3 deficiency and increased susceptibility to certain infections.

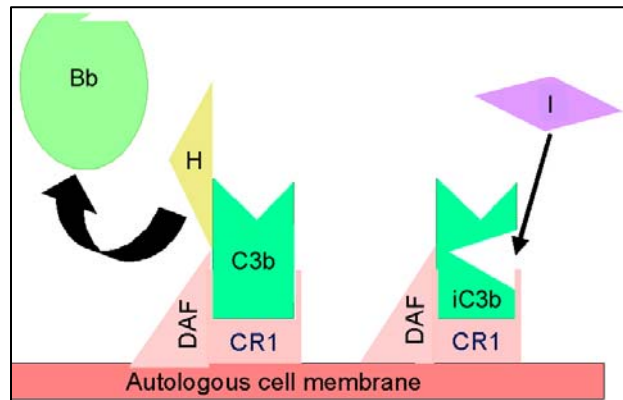


Figure 8. Regulation by CR1, Factor H and Factor I

3. Stabilization of C3 convertase by activator (protector) surfaces (Figure 9)

When bound to an appropriate activator of the alternative pathway, C3b will bind Factor B, which is enzymatically cleaved by Factor D to produce C3 convertase (C3bBb). However, C3b is resistant to degradation by Factor I and the C3 convertase is not rapidly degraded, since it is stabilized by the activator surface. The complex is further stabilized by properdin binding to C3bBb. Activators of the alternate pathway are components on the surface of pathogens and include: LPS of Gram⁻ bacteria and the cell walls of some bacteria and yeasts. Thus, when C3b binds to an activator surface, the C3 convertase formed will be stable and continue to generate additional C3a and C3b by cleavage of C3.

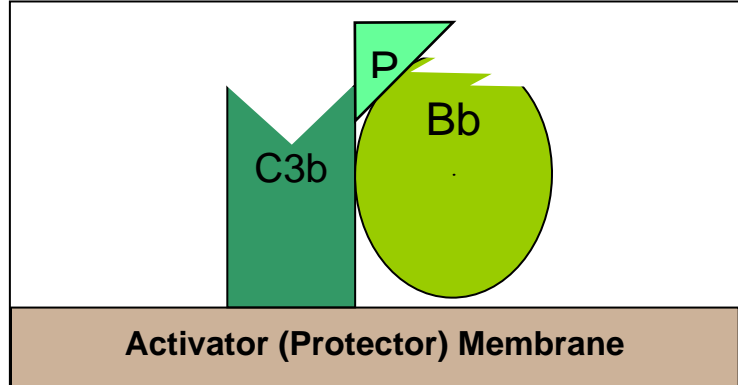


Figure 9. Stabilized C3 convertase of the alternative pathway

4. Generation of C5 convertase (Figure 10)

Some of the C3b generated by the stabilized C3 convertase on the activator surface associates with the C3bBb complex to form a C3bBbC3b complex. This is the C5 convertase of the alternative pathway. The generation of C5 convertase is the end of the alternative pathway. The alternative pathway can be activated by many Gram-negative (most significantly, *Neisseria meningitidis* and *N. gonorrhoea*), some Gram-positive bacteria and certain viruses and parasites, and results in the lysis of these organisms. Thus, the alternative pathway of C activation provides another means of protection against certain pathogens before an antibody response is mounted. A deficiency of C3 results in an increased susceptibility to these organisms. The alternate pathway may be the more primitive pathway and the classical and lectin pathways probably developed from it.

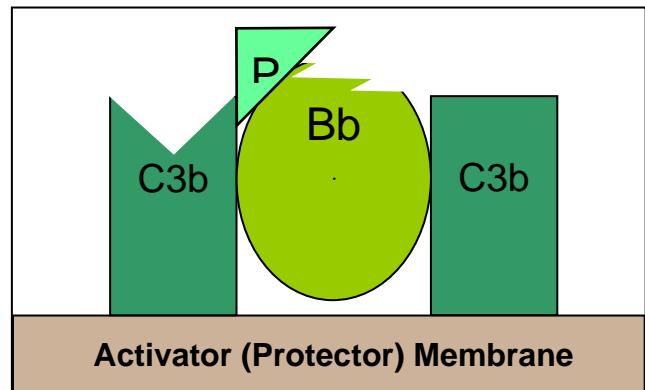


Figure 10. Stabilized C5 convertase of the alternative pathway

III. Membrane attack pathway (Figure 11)

C5 convertase from the classical (C4b2a3b), lectin (C4b2a3b) or alternative (C3bBb3b) pathway cleaves C5 into C5a and C5b. C5a remains in the fluid phase and the C5b rapidly associates with C6 and C7 and inserts into the membrane. The **C5b67** complex is referred to as the membrane attack complex (MAC). Subsequently C8 binds, followed by several molecules of C9. The C9 molecules form a pore in the membrane through which the cellular contents leak and lysis occurs. **Lysis is not an enzymatic process it is thought to be due to physical damage to the membrane.**

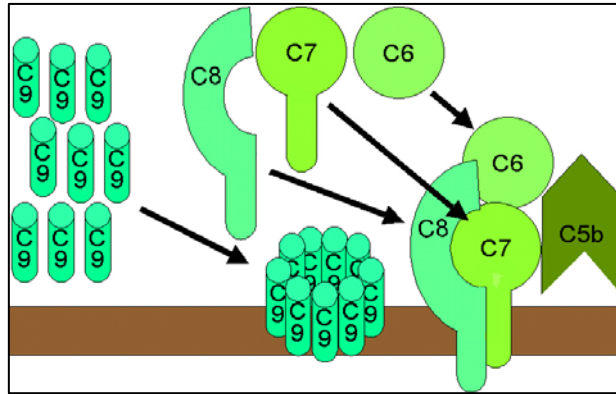


Figure 11. The lytic pathway

C5a generated in the lytic pathway has several potent biological activities. It is the **most potent anaphylotoxin**,. **In addition, it is a chemotactic factor for neutrophils and stimulates the respiratory burst in them and it stimulates inflammatory cytokine production by macrophages.** Its activities are controlled by inactivation by carboxypeptidase B (C3-INA).

Some of the C5b67 complex formed can dissociate from the membrane and enter the fluid phase. If this were to occur it could then bind to other nearby cells and lead to their lysis. The damage to bystander cells is prevented by **Protein S (vitronectin)**. Protein S binds to soluble C5b67 and prevents its binding to other cells.

Tables 4 and 5 summarize some of the important features of the complement system and Table 6 summarizes the association of diseases with complement deficiencies.

Table 4. Comparison of Classical, Lectin and Alternative Pathways

	Classical Pathway	Lectin Pathway	Alternative Pathway
Components	C1, C4, C2, & C3	MBL, MASP-1, MASP-2, C4, C2, & C3	C3, Factor B, Factor D & Properdin
Antibody initiated	Yes	No	No
Initiated by pathogen surfaces	Yes (in some cases)	Yes	Yes
Divalent cation required	Yes	Yes	Yes
Prokinin generation	Yes	Yes	No
Anaphylotoxin generation	Yes	Yes	Yes
C3 convertase	C4bC2a	C4bC2a	C3bBb
C5 convertase	C4bC2aC3b	C4bC2aC3b	C3bBbC3b
Feeds into membrane attack pathway	Yes	Yes	Yes
Regulatory Components	C1-INH, C4-BP, Factor I	C1-INH, C4-BP, Factor I	Factor H, Factor I, DAF, CR1

Table 5. Activities of Complement Activation Products and their Control Factors

Fragment	Activity	Effect	Control Factor (s)
C2b	Prokinin, accumulation of fluids	Edema	C1-INH
C3a	Basophil and mast cells degranulation; enhanced vascular permeability, smooth muscle contraction	Anaphylaxis	C3a-INA
C3b	Opsonin, phagocyte activation	Phagocytosis	Factors H and I
C4a	Basophil and mast cells degranulation; enhanced vascular permeability, smooth muscle contraction	Anaphylaxis (least potent)	C3a-INA
C4b	Opsonin	Phagocytosis	C4-BP and Factor I
C5a	Basophil and mast cells degranulation; enhanced vascular permeability, smooth muscle contraction	Anaphylaxis (most potent)	C3a-INA
	Chemotaxis, stimulation of respiratory burst, activation of phagocytes, stimulation of inflammatory cytokines	Inflammation	
C5bC6C7	Chemotaxis	Inflammation	Protein S (vitronectin)
	Attaches to other membranes	Tissue damage	

Table 6. Complement deficiencies and disease.		
Pathway/Component	Disease	Mechanism
Classical Pathway		
C1INH	Hereditary angioedema	Overproduction of C2b (prokinin)
C1, C2, C4	Predisposition to SLE	Opsonization of immune complexes help keep them soluble, deficiency results in increased precipitation in tissues and inflammation
Lectin Pathway		
MBL	Susceptibility to bacterial infections in infants or immunosuppressed	Inability to initiate the lectin pathway
Alternative Pathway		
Factors B or D	Susceptibility to pyogenic (pus-forming) bacterial infections	Lack of sufficient opsonization of bacteria
C3	Susceptibility to bacterial infections	Lack of opsonization and inability to utilize the membrane attack pathway
C5, C6, C7 C8, and C9	Susceptibility to Gram-negative infections	Inability to attack the outer membrane of Gram-negative bacteria
Properdin (X-linked)	Susceptibility meningococcal meningitis	Lack of opsonization of bacteria
Factors H or I	C3 deficiency and susceptibility to bacterial infections	Uncontrolled activation of C3 via alternative pathway resulting in depletion of C3