



## COMPLEMENT

The term "complement" was coined by Paul Ehrlich to describe the activity in serum, which could "complement" the ability of specific antibody to cause lysis of bacteria. Complement historically refers to fresh serum capable of lysing antibody-coated cells.

Complement system is composed of more than 25 different proteins produced by hepatocytes, macrophages and intestinal epithelial cells. Fibroblasts and intestinal epithelial cells make C1, while the liver makes C3, C6, and C9. They are present in the circulation as inactive molecules. Though some components are resistant to heat, heating serum at 56°C for 30 minutes destroys complement's activity. Serum complement levels, especially C3, often drop during infection as complement is activated faster than it is produced.

Several complement proteins are zymogens (proenzymes). When activated, they become proteases that cut peptide bonds in other complement proteins to activate them in turn. Complement proteins work in a cascade, where the binding of one protein promotes the binding of the next protein in the cascade. Complement components are numbered in the order in which they were discovered. During activation, some complement components are split into two parts. The larger part of the molecule called "b" while the smaller fragment called "a" may diffuse away. In most cases it is the "b" fragment binds to the surface of the cell to be lysed (the fragments of C2 are an exception to this rule: C2a binds to the membrane while C2b is freed into serum or tissue spaces). Inactivated fragments are indicated by a small "i". Enzymatically active forms are symbolized by a bar over the letter or number.

Activation of complement results in the production of several biologically active molecules, which contribute to non-specific immunity and inflammation. Complement is not antigen-specific and it is activated immediately in the presence of pathogen, so it is considered part of innate immunity. Since antibody also activates some complement proteins, complement activation is also part of humoral immunity. Their activation proceeds via different pathways in a cascade fashion leading to lysis.

Complement proteins can be quantified directly by ELISA, and complement activity can be measured by the complement fixation test.

### PATHWAYS OF COMPLEMENT ACTIVATION:

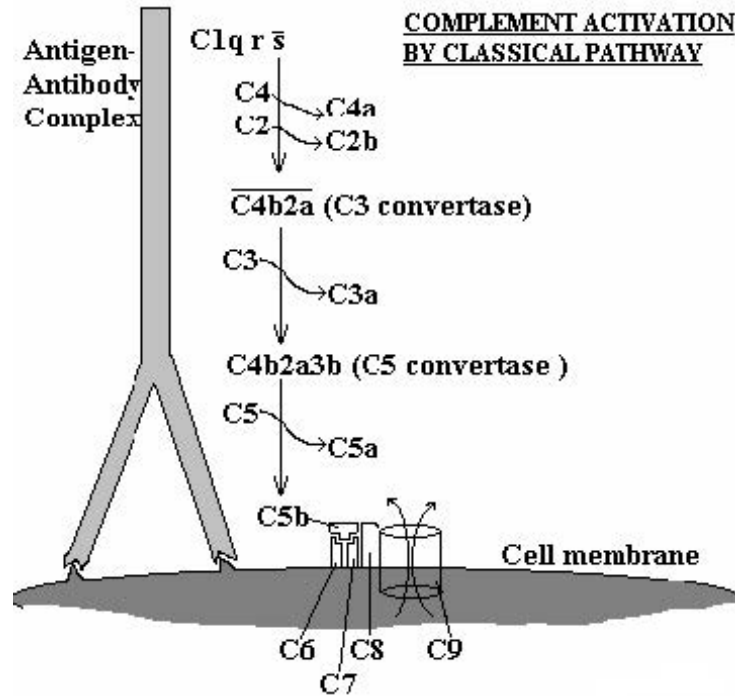
The complement activation can be divided into three pathways, classical, lectin (mannose binding protein) and alternative, all of which result in the activation of C5 and lead the formation of the membrane attack complex (MAC). The three pathways differ in the way C5 is broken down but after that the formation of MAC is essentially the same.

#### **CLASSICAL PATHWAY**

The classical pathway is triggered primarily by immune complexes (containing antigen and IgG or IgM) in the presence of complement components 1, 4, 2, 3, Ca<sup>++</sup> and Mg<sup>++</sup> cations. They react in the order C1q, C1r, C1s, C4, C2, C3, C5, C6, C7, C8 and C9. While IgG1, IgG2 and IgG3 (most effective) can activate complement, IgG4 is not able to activate at all.

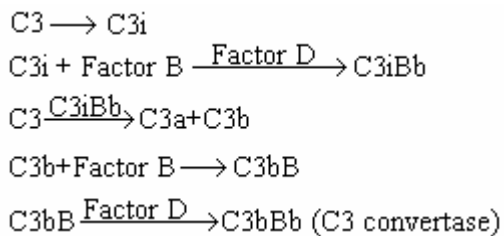
C1 is the first complement component to participate in classical pathway. It is composed of C1q, C1r and C1s. Binding of C1q to Ag-Ab complexes results in autocatalysis of C1r. The altered C1r cleaves C1s and this cleaved C1s is capable of cleaving both C4 and C2. Activated C1s enzymatically cleaves C4 into C4a and C4b. C4b binds to the Ag-bearing particle or cell membrane while C4a remains a biologically active peptide at the reaction site. C4b binds C2, which becomes susceptible to C1s and is cleaved into C2a and C2b. C2a remains complexed with C4b whereas C2b is released. C4b2a complex is known as C3 convertase. C3 convertase, in the presence of Mg<sup>++</sup>, cleaves C3 into C3a and C3b. C3b binds to the membrane to form C4b2a3b complex whereas C3a remains in the microenvironment. C4b2a3b complex functions as C5 convertase, which cleaves C5 into C5a and C5b. Generation of C5 convertase marks the end of the classical pathway. C5b initiates the formation of membrane attack complex.

C1qrs can also bind to a number of agents including some retroviruses, mycoplasma, poly-inosinic acid and aggregated IgG, and initiate the classical pathway



### ALTERNATIVE PATHWAY:

Alternate pathway is so called because it bypasses the requirement of antigen-antibody complex, C1, C2 and C4 components. It begins with the spontaneous activation of C3 in serum and requires Factors B and D and  $Mg^{++}$ , all present in normal serum. A C3b-like molecule (C3i) is generated by slow hydrolysis of native C3. C3i binds factor B, which is cleaved by Factor D to produce C3iBb. C3iBb cleaves native C3 into C3a and C3b. C3b binds factor B, which is again cleaved by Factor D to produce C3bBb (now C3 convertase).

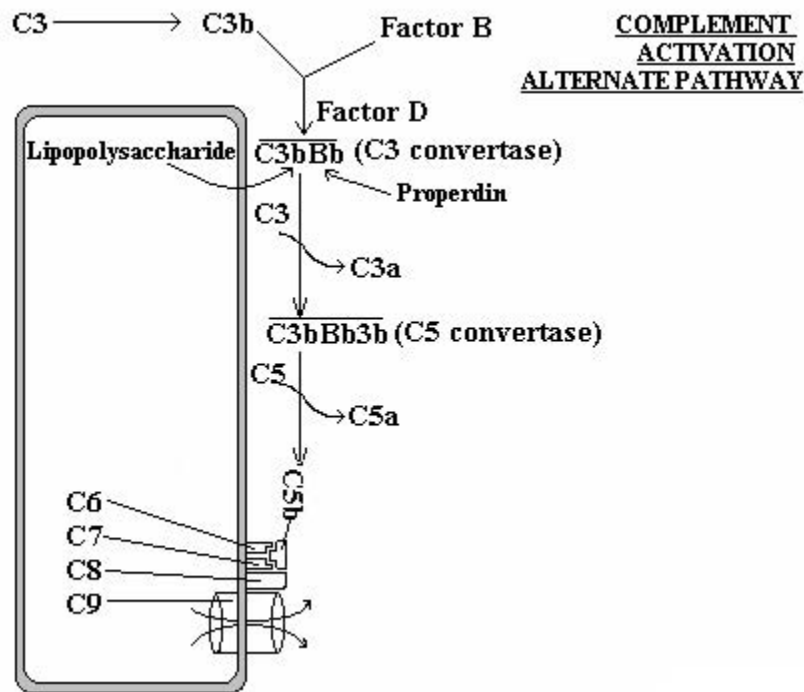


**Note:** If the generation of C3 convertase is difficult to follow, one may simply assume that C3 is spontaneously broken in serum to C3b, which is acted upon by Factor B to form C3bB. This is then cleaved by Factor D to form C3bBb.

C3b has very short life and unless stabilized by membrane or molecule present on many pathogens, it is quickly inactivated. In the absence of such a molecule, it binds quickly to RBCs via the C3b receptor (CR1), and decay accelerating factor (DAF) prevents the binding of Factor B. Another serum protein, factor H, can displace factor B and bind to C3b. Binding of factor H makes C3b more susceptible to factor I, which then cleaves it into many fragments (iC3b, C3d, C3e). C3 convertase generated in the classical pathway is also regulated by DAF, CR1 and Factor I.

Certain bacteria or their products such as peptidoglycan and polysaccharides can stabilise C3b. Thus, C3b bound to such a surface becomes relatively resistant to the action of factor I. Binding of another protein, properdin, further stabilizes this complex. Hence the alternative pathway is also known as the properdin pathway. Stabilized C3 convertase cleaves more C3 and produces C3bBb3b complex (C5 convertase), which cleaves C5 into C5a and C5b. C5b initiates the formation of membrane attack complex.

The alternative pathway provides a means of non-specific resistance against infection without the participation of antibodies and hence provides a first line of defence against a number of infectious agents. Some of the microbial components, which can activate the alternative complement cascade, include lipopolysaccharide (LPS) from Gram negative outer membranes (mainly *Neisseria*), teichoic acid from Gram positive cell walls, certain viruses, parasites, heterologous red cells, zymosan from fungal and yeast cell walls and some parasite surface molecules. Aggregated immunoglobulins (particularly IgA), cobra venom factor (CVF) and other proteins (e.g. proteases, clotting pathway products) also can activate the alternative pathway.



### **LECTIN PATHWAY:**

C4 activation can be achieved without antibody and C1 participation via the lectin pathway. Three proteins initiate this pathway namely a mannan-binding lectin/protein (MBL), and two mannan-binding lectin-associated serine proteases (MASP and MASP2), all present in normal serum. MBL binds to certain mannose residues on many bacteria and subsequently interacts with MASP and MASP2. The MBL-MASP-MASP2 complex is similar to Ab-C1qrs complex (of classical pathway) and leads to activation of C4, C2 and C3. The rest follows as in classical pathway.

### **FORMATION OF MAC:**

C5 convertase, generated by any of the pathways described above, cleaves C5 into C5a and C5b. C5b instantaneously binds C6 and subsequently C7 to yield a hydrophobic C5b67 complex, which attaches quickly to the plasma membrane. Subsequently, C8 binds to this complex and causes the insertion of several C9 molecules. The insertion of membrane attack complex causes formation of a hole (100 Å) in the membrane thus lysing the cell. If complement is activated on an antigen without a lipid membrane to which the C567 can attach, the C567 complex may bind to nearby cells and initiate bystander lysis.

### **REGULATION OF COMPLEMENT ACTIVATION:**

C1 Inhibitor (also known as serpin) inhibits the production of C3b by combining with and inactivating Cq1rs complex. This prevents formation of the C3 convertase, C4b2b.

Protein H inhibits the production of C3b by inhibiting the binding of Factor B to membrane-bound C3b, thereby preventing cleavage of B to Bb and production of the C3 convertase, C3bBb. Factor I inhibits the production of C3b by cleaving C3b into inactive forms.

Regulatory proteins promote or inhibit complement activity and protect self cells from lysis. C5b67 can bind indiscriminately to any cell membrane leading to their lysis. Such an indiscriminate damage to by-standing cells is prevented by protein S (vitronectin) that binds to C5b67 complex and blocks its indiscriminate binding to cells other than the primary target.

Decay accelerating factor (DAF) accelerates breakdown of C3 convertase.

Homologous restriction factor (HRF) prevents insertion of C8 and C9 into membranes.

## **SIGNIFICANCE OF COMPLEMENT ACTIVATION:**

### **Lysis of cells:**

The MAC that inserts itself into the membrane causes the cell to lyse. It may also cause the bystanding cells to lyse. Gram positive bacteria, which are protected by their thick peptidoglycan layer, bacteria with a capsule or slime layer around their cell wall, or non-enveloped viruses are less susceptible to lysis.

### **Inflammation and Phagocytosis:**

- C2b generated during the classical pathway of C activation causes vascular permeability and oedema.
- C4a, C3a and C5a are all anaphylotoxins, which cause basophil/mast cell degranulation and smooth muscle contraction.
- C5a and MAC (C5b67) are both chemotactic.
- C5a is also a potent activator of neutrophils, basophils and macrophages and thus amplifies inflammation.
- It also causes induction of adhesion molecules on vascular endothelial cells and hence promotes diapedesis.
- C3b and C4b act as opsonins on the surface of microorganisms and promote phagocytosis.

### **Other:**

Complement also helps to rid the body of antigen-antibody complexes. C3b receptors are present on RBCs, which pick up complement-coated immune complexes and deliver them to the Kupffer cells in the liver. This is called Immune adherence. The classic complement pathway appears to be important in the dissolution of immune complexes.

Certain viruses are neutralized after interaction with antibody and complement components.

C2a can be converted to C2 kinin, which regulates blood pressure by causing blood vessels to dilate.

They contribute to Type II hypersensitivity (incompatible blood transfusion, haemolytic disease of the newborn, autoimmune haemolytic anaemia and hyperacute graft rejection) and Type III hypersensitivity (Arthus reaction, Serum sickness, Poststreptococcal glomerulonephritis, Autoimmune disease and Rheumatoid arthritis)

Complement	Biological activity	Effect
C2b	Accumulation of body fluids	Oedema
C3a, C4a	Degranulation of mast cells and basophils, increases vascular permeability, smooth muscle contraction	Immunoregulation
C3b	Opsonisation and activation of phagocytes	Phagocytosis
C4b	Opsonisation	Phagocytosis
C5a	Basophil and mast cell activation, enhanced vascular permeability, smooth muscle contraction, chemotaxis, neutrophil aggregation, Oxidative metabolism stimulation, stimulation of leukotriene release and induction of helper T-cells.	Inflammation, anaphylaxis, Immunoregulation.
C5b67	Chemotaxis; attachment to other cell membranes.	Inflammation and lysis of bystander cells.

## **DEFICIENCY OF COMPLEMENT:**

Deficiency defects are transmitted as phenotypically autosomal-recessive traits. Genetic deficiencies in the alternative pathway are very rare. Deficiency of properdin is X-linked. Deficiency of the C1 inhibitor is inherited as an autosomal dominant. All patients with complement deficiency are more or less unduly susceptible to infection and to development of immune complex disease.

DEFICIENCY	CLINICAL CORRELATION
C3 (any pathway)	Autoimmune collagen vascular disease, susceptibility to infectious diseases
<b>Classical pathway</b>	
C1q, C1r, C1s, C4, C2	Autoimmune collagen vascular disease, susceptibility to infectious diseases
<b>Alternate pathway</b>	
Factor B	Meningococemia
Factor D	Recurrent pyogenic infections
Properdin	Recurrent pyogenic infections, Meningococemia

<b>Lectin pathway</b>	Recurrent infections
<b>Membrane attack complex</b>	
C5 C6, C7, C8 C9	Recurrent disseminated Neisseria infections, SLE Recurrent disseminated Neisseria infections Susceptibility to infectious diseases
<b>Regulatory proteins</b>	
C1 inhibitor Factor I, Factor H CR1 DAF, HRF	Hereditary angionurotic oedema (Quincke's disease) Autoimmune collagen vascular disease, Recurrent pyogenic infections SLE Paroxysmal nocturnal hemoglobinuria

### SUMMARY OF COMPLEMENT ACTIVATION BY THREE PATHWAYS

