Complement System

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Complement system:

1. It is a major effetor of the humoral branch of the immune system.

2. In the 1890s, when Jules Bordet at the Institute Pasteur in Paris showed that sheep antiserum to the bacterium Vibrio cholera 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.

3. Paul Ehrlich in Berlin independently carried out similar experiments and coined the term complement.

The complement components :

Research on complement now includes more than **30 soluble and cell-bound protein** synthesized mainly by liver hepatocytes, also produced by blood monocytes, tissue macrophages, and epithelial cells of the gastrointestinal and genitourinary tracts.

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.

Complement components are designated by numerals **(C1–C9).** 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., $\overline{C4b2a}$, $\overline{C3bBb}$).

The Functions of Complement:

After initial activation, the various complement components interact, in a highly regulated cascade, to carry out anumber of basic functions including:

1. Lysis of cells, bacteria, and viruses.

2. Opsonization, which promotes phagocytosis of particulate antigens.

3. Binding to specific complement receptors on cells of the immune system, triggering specific cell functions, inflammation, and secretion of immunoregulatory molecules.

4. Immune clearance, which removes immune complexes from the circulation and deposits them in the spleen and liver.

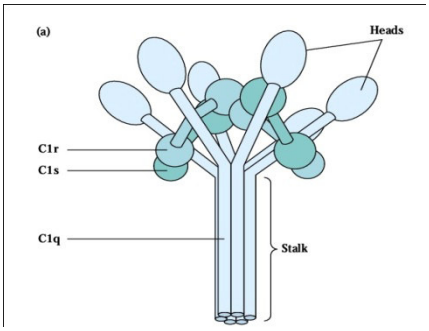
Complement Activation:

There are two steps in 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 complex (MAC)** are the same in all pathways.

Classical Pathway:

1. Complement activation by the classical pathway commonly begins with the formation of soluble antigen-antibody complexes (immune complexes) such as a bacterial cell, IgM and certain subclasses of IgG (human IgG1, IgG2, and IgG3). The initial stage of activation involves C1, C2, C3, and C4, which are present in plasma in functionally inactive forms. The formation of an antigen-antibody complex induces conformational changes in the Fc portion of the IgM molecule that expose a binding site for the C1 component of the complement system.

2. **Structure of C1:** C1 in serum is a macromolecular complex consisting of C1q and two molecules each of C1r and C1s, held together in a complex (C1qr2s2) stabilized by Ca^{++} 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 CH2 domain of the antibody molecule. Each C1r and C1s monomer contains a catalytic domain and an interaction domain; the latter facilitates in interaction with C1q or with each other.



3. Each C1 molecule must bind by its C1q globular heads to at least two Fc sites for a stable C1-antibody interaction to occur. It is found that a pentameric IgM is more efficient in activating classical pathway than IgG molecule. Binding of C1q to Fc binding sites induces a conformational change in C1r that converts C1r to an active serine protease enzyme, $\overline{C1r}$, which then cleaves C1s to a similar active enzyme, $\overline{C1s}$.

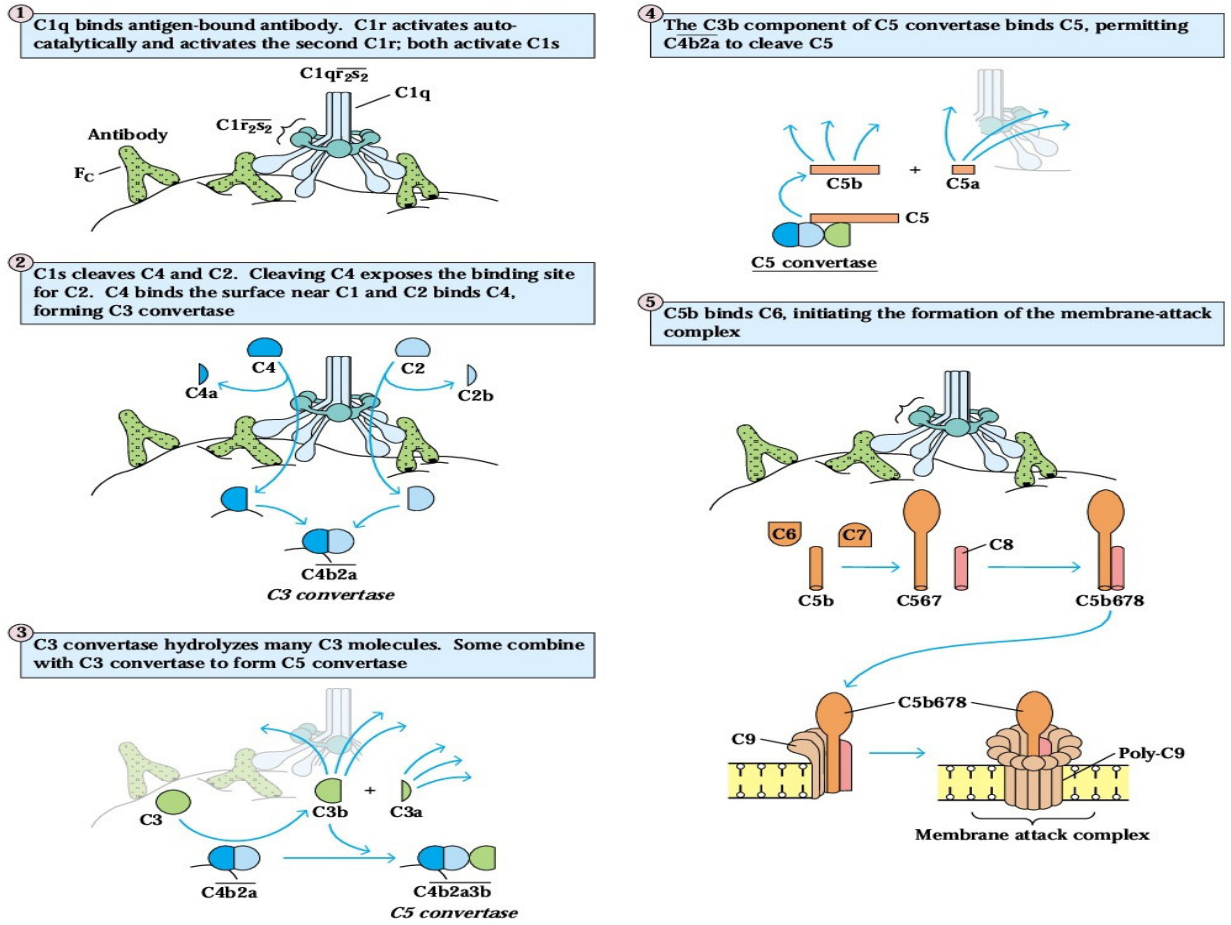
4. $\overline{C1s}$ has two substrates, C4 and C2. The C4 component is a glycoprotein containing three polypeptide chains α, β and γ . C4 is activated when $\overline{C1s}$ hydrolyzes a small fragment (C4a) from the amino terminus of the α chain, exposing a binding site on the larger fragment (C4b). 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 $\overline{C1s}$. The smaller fragment (C2b) diffuses away. The resulting

$\overline{C4b2a}$ complex is called C3 convertase, referring to its role in converting the C3 into an active form. A single C3 convertase molecule can generate over 200 molecules of C3b, resulting in tremendous amplification at this step of the sequence.

5. The native C3 component consists of two polypeptide chains, α and β. Hydrolysis of a short fragment (C3a) by the C3 convertase generates C3b. Some of the C3b binds to $\overline{C4b2a}$ to form a trimolecular complex $\overline{C4b2a3b}$, called C5 convertase. The C3b component of this complex binds C5 and alters its conformation, so that the $\overline{C4b2a}$ component can cleave C5 into C5a, which diffuses away, and C5b, which attaches to C6 and initiates formation of the membrane attack complex.

6. Some of the C3b generated by C3 convertase activity does not associate with $\overline{C4b2a}$; instead it diffuses away and then coats immune complexes and particulate antigens, functioning as an **opsonin**. C3b may also bind directly to cell membranes.

7. The smaller fragment from C4,C3 and C5 cleavage i.e C4a, C3a and C5a are **anaphylatoxin**, or mediator of inflammation, which does not participate directly in the complement cascade.



Alternative pathway :

The alternative pathway is initiated in most cases by cell-surface constituents that are foreign to the host. The alternative pathway like classical pathway generates bound C5b, 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**.

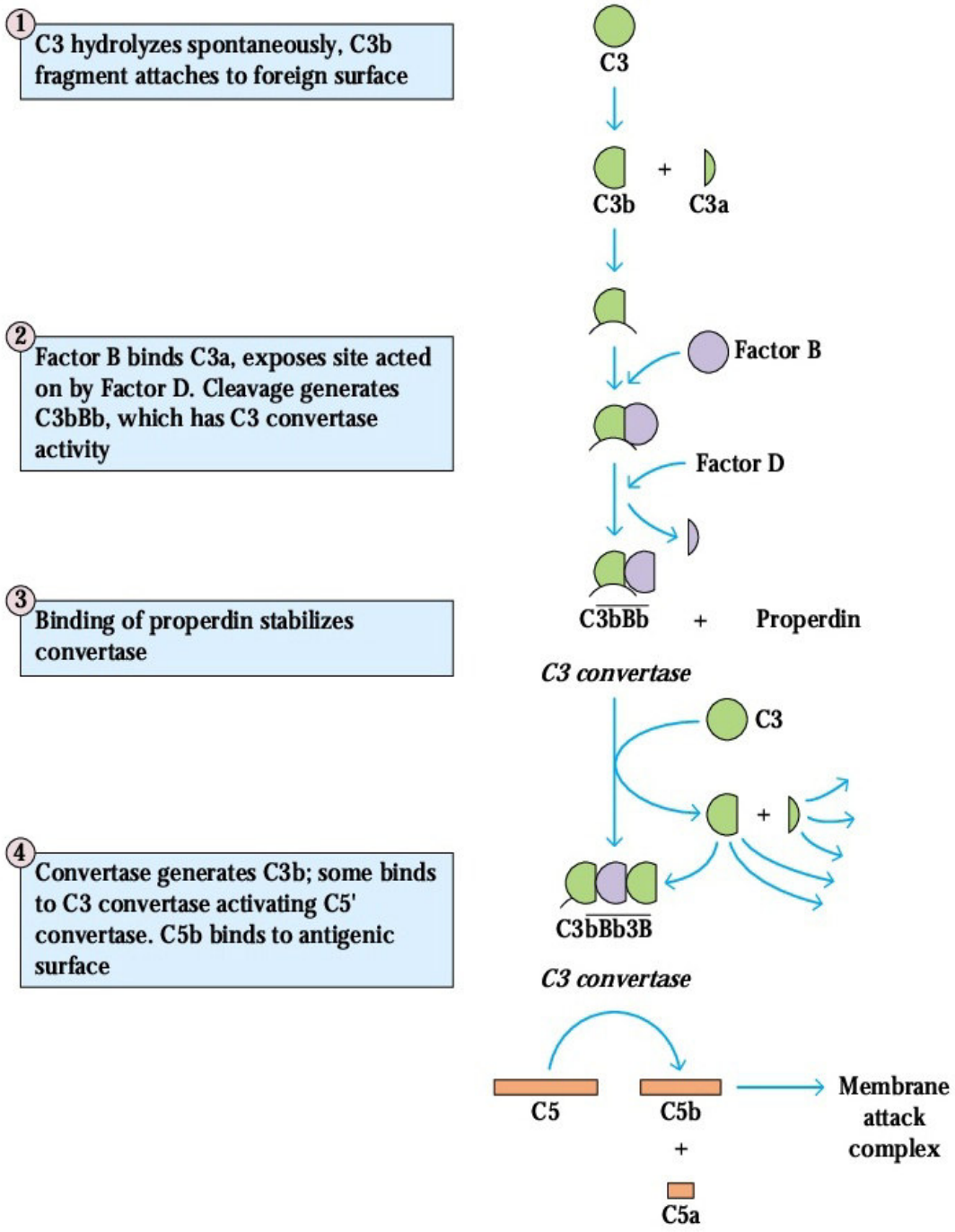
This major pathway of complement activation involves four serum proteins**: C3, factor B, factor D, and properdin**. Serum C3, which contains an unstable thioester bond, is subject to slow spontaneous hydrolysis to yield C3a and C3b. The C3b component can bind to foreign surface antigens (such as those on bacterial cells or viral particles or even to the host's own cells).

The membranes of most mammalian cells have high levels of **sialic acid**, which contributes to the rapid inactivation of bound C3b molecules on host cells; consequently this binding rarely leads to further reactions on the host cell membrane. Because many foreign antigenic surfaces (e.g., bacterial cell walls, yeast cell walls, and certain viral envelopes) have only low levels of sialic acid, C3b bound to these surfaces remains active for a longer time.

The C3b present on the surface of the foreign cells can bind another serum protein called factor B to form a complex stabilized by Mg^{++}. Binding to C3b exposes a site on factor B that serves as the substrate for an enzymatically active serum protein called factor D. Factor D cleaves the C3b-bound factor B, releasing a small fragment (Ba) that diffuses away and generating $\overline{C3bBb}$. The $\overline{C3bBb}$ complex has C3 convertase activity and thus is analogous to the $\overline{C4b2a}$ complex in the classical pathway.

The C3 convertase activity of $\overline{C3bBb}$ has a half-life of only 5 minutes unless the serum protein **properdin** binds to it, stabilizing it and extending the half-life of this convertase activity to 30 minutes.

The $\overline{C3bBb}$ generated in the alternative pathway can activate unhydrolyzed C3 to generate more C3b auto catalytically. As a result, the initial steps are repeated and amplified, so that more than 2x 10^6 molecules of C3b can be deposited on an antigenic surface in less than 5 minutes. The C3 convertase activity of $\overline{C3bBb}$ generates the $\overline{C3b\ Bb3b}$ complex, which exhibits C5 convertase activity, analogous to the $\overline{C4b2a3}$b complex in the classical pathway. The nonenzymatic C3b component binds C5, and the Bb component subsequently hydrolyzes the bound C5 to generate C5a and C5b; the latter binds to the antigenic surface.



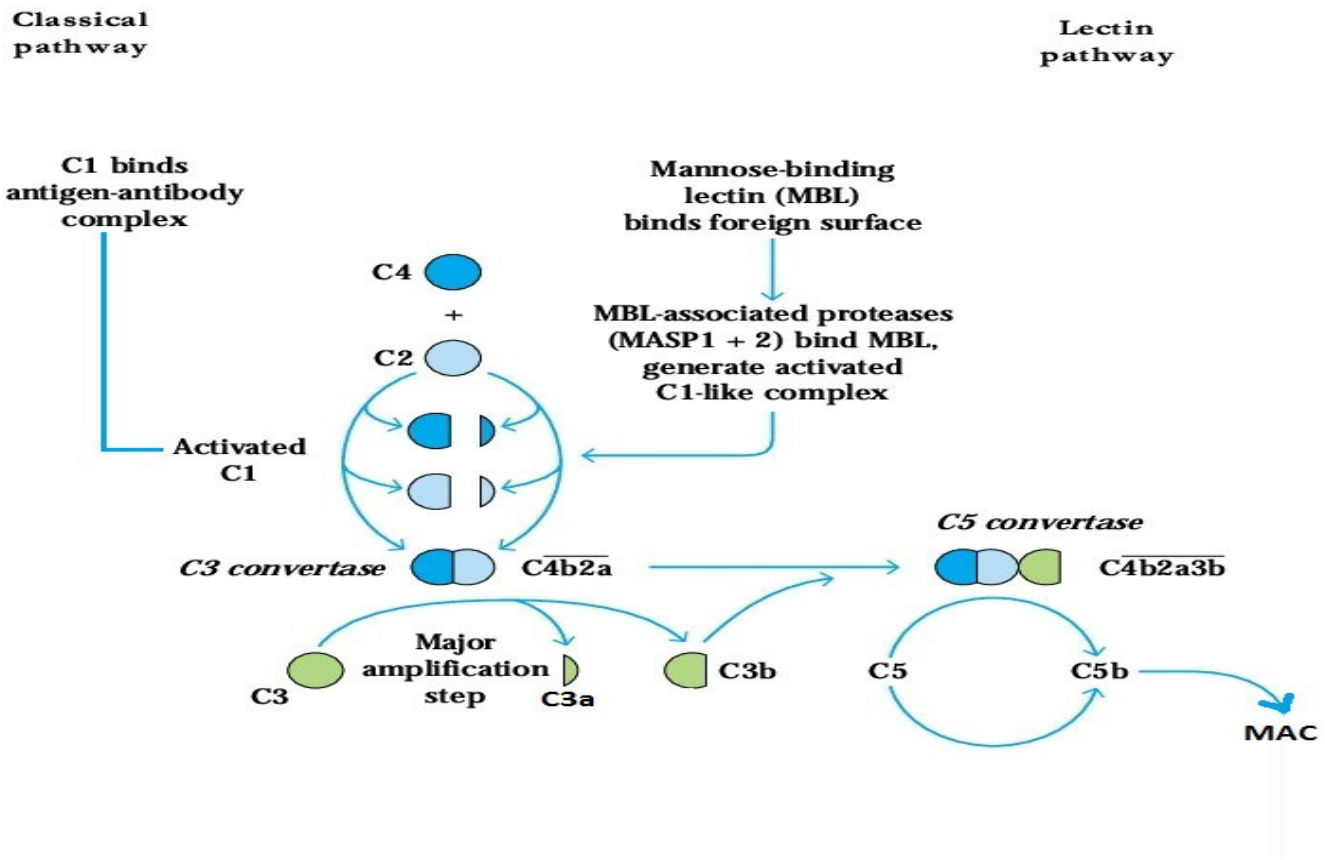
Alternative pathway

The Lectin Pathway:

Lectins are proteins that recognize and bind to specific carbohydrate targets. The lectin that activates comple ment binds to mannose residues, thus some authors designate this MBLectin pathway or mannan-binding lectin pathway. MBL is an acute phase protein produced in inflammatory responses.

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. 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.

Function of MBL 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 (structurally similar to C1r and C1s and mimic their activities) , bind to MBL. The active complex formed by this association causes cleavage and activation of C4 and C2. Then ultimately leads to formation of C5 convertase like classical pathway. I.e. lectin pathway utilize the elements of the classical pathway for complement activation except for the C1 proteins.



Lectin pathway

Formation of Membrane-Attack Complex :

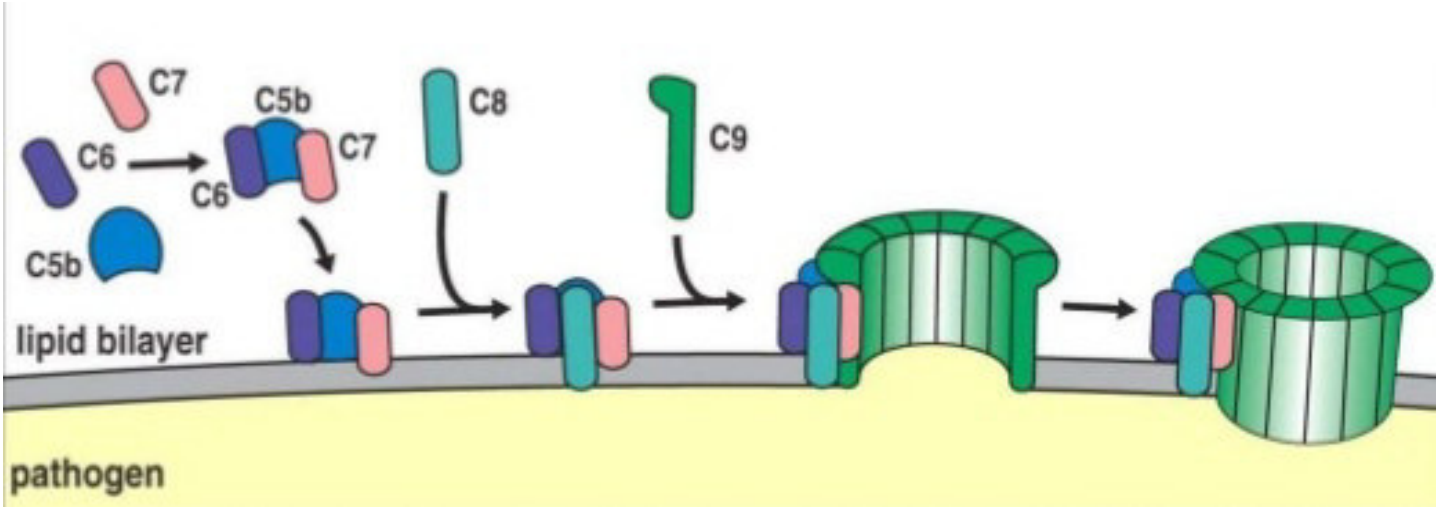
The Three Complement Pathways Converge at the membrane attack complex. The terminal sequence of complement activation involves C5b, C6, C7, C8, and C9, which interact sequentially to form the membrane-attack complex (MAC). This complex forms a large channel through the membrane of the target cell, enabling ions and small molecules to diffuse freely across the membrane. The cell cannot maintain its osmotic stability and is killed by an influx of water and loss of electrolytes.

The active C5 convertase cleaves C5, at the amino terminus of the α chain. 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.

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 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.

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. 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 10-17 perforin-like molecule, C9, to the C5b678 complex. During polymerization, the C9 molecules undergo a hydrophilic-amphiphilic transition, so that they too can insert into the membrane. The completed MAC has a functional pore size of 70–100 Å, consists of a C5b678 complex surrounded by a poly-C9 complex.



Related questions:

1. What are the different pathways of complement activation?

2. Describe the classical pathway of complement activation

3. How is the membrane attack complex (MAC) formed?

References:

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