COMPLEMENT SYSTEM

Complement was discovered by Jules Bordet many years ago as a heat-labile component of normal plasma that augments the opsonization of bacteria by antibodies and allows antibodies to kill some bacteria. This activity was said to '**complement**' the antibacterial activity of **antibody**, hence the name. Although first discovered as an effector arm of the antibody response, complement can also be activated early in infection in the absence of antibodies. It seems clear that complement first evolved as part of the **innate immune system**, where it still plays an important role.

The **complement system** is made up of a large number of distinct plasma proteins that react with one another to opsonize pathogens and induce a series of inflammatory responses that help to fight infection.

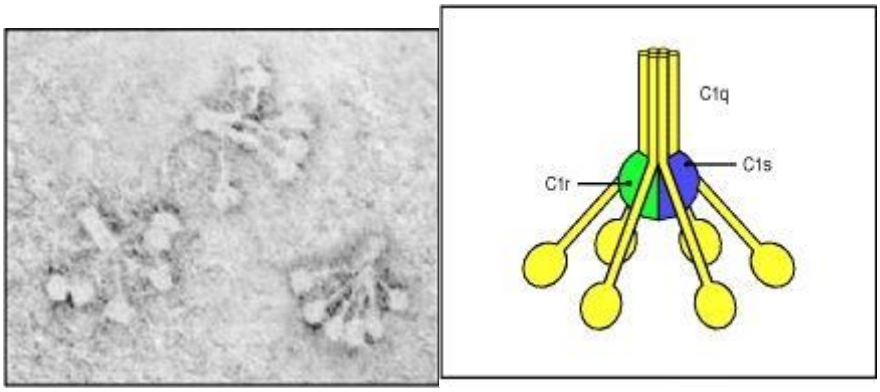
The proteins and glycoproteins that compose the complementsystem are synthesized mainly by liver hepatocytes, although significant amounts are also produced by blood monocytes, tissue macrophages, and epithelial cells of the gastrointestinal and genitourinary tracts
Most circulate in the serum in functionally 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), 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.Complexes with enzymatic activity have bar on top $\overline{C4}\overline{b2}a$

Complementpathways:

1- Classical pathway

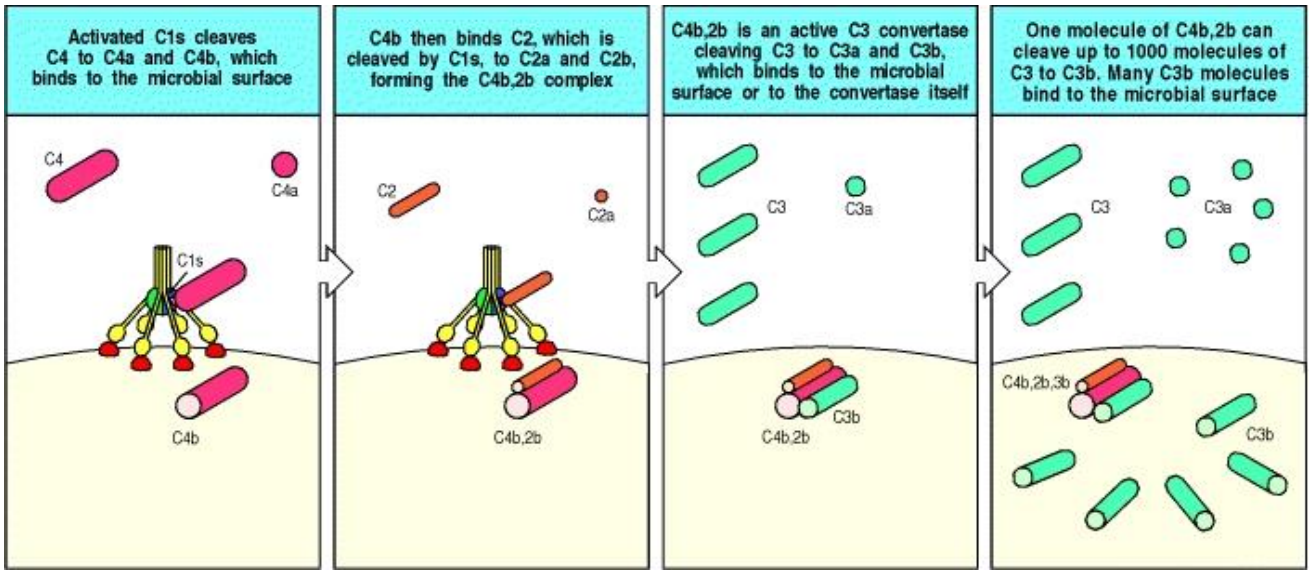
- Complement activation by the classical pathway commonly begins with the formation of soluble antigen-antibody complexes (immune complexes) or with the binding of antibody to antigen on a suitable target, such as a bacterial cell.
- IgM and certain subclasses of IgG (human IgG1, IgG2, and IgG3) can activate the classical complement pathway.
- The initial stage ofactivation involves C1, C2, C3, and C4, which are present in plasma in functionally inactive forms.

- The formation of an antigen-antibody complex inducesconformational changes in the Fc portion of the IgM moleculethat expose a binding site for the C1 component of thecomplement system
- C1 in serum is a macromolecular complexconsisting of C1q and two molecules each of C1r andC1s, held together in a complex (C1qr2s2)
- When pentamericIgM 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, IgG molecule, on the other hand, contains only a single C1q-binding site
- Binding ofC1q to Fc binding sites induces a conformational change in C1r that converts C1r to an active serine protease enzyme, $\mathbf{\bar{C}1r}$, which then cleaves C1s to a similar active enzyme, $\mathbf{\bar{C}1S}$
- $\mathbf{\bar{C}1S}$ has two substrates, C4 and C2
- C4 is activated when C1s hydrolyzes a small fragment, exposing a bindingsite on the larger fragment (C4b).
- The C4b fragment attachesto the target surface in the vicinity of C1, and the C2 proenzymethen attaches to the exposed binding site on C4b
- C2 is then cleaved by the neighboring C1s; the smallerfragment (C2b) diffuses away.
- The resulting C4b2a complexis called C3 convertase, referring to its role in converting theC3 into an active form.
- The smaller fragment from C4 cleavage, C4a, is an anaphylatoxin, or mediator of inflammation
- C3compenents hydrolysis by C3 convertase into C3a and C3b
- Some of the C3bbinds to $\bar{C}$4b2a to form a trimolecular complex $\bar{C}$4b2a3b,called C5 convertase.
- The C3b component of this complex binds C5 and alters its conformation, so that the C4$\bar{b}$2a component can cleave C5 into C5a, which diffuses away, and C5b, which attaches to C6 and initiates formation of the membraneattack complex
- Some of the C3b generated by C3 convertase activity does not associate with C4$\bar{b}$2a; instead it diffuses away and then coats immune complexes and particulate antigens, functioning as an opsonin



The first protein in the classical pathway of complement activation is C1, which is a complex of C1q, C1r, and C1s

Diagram of classical pathway



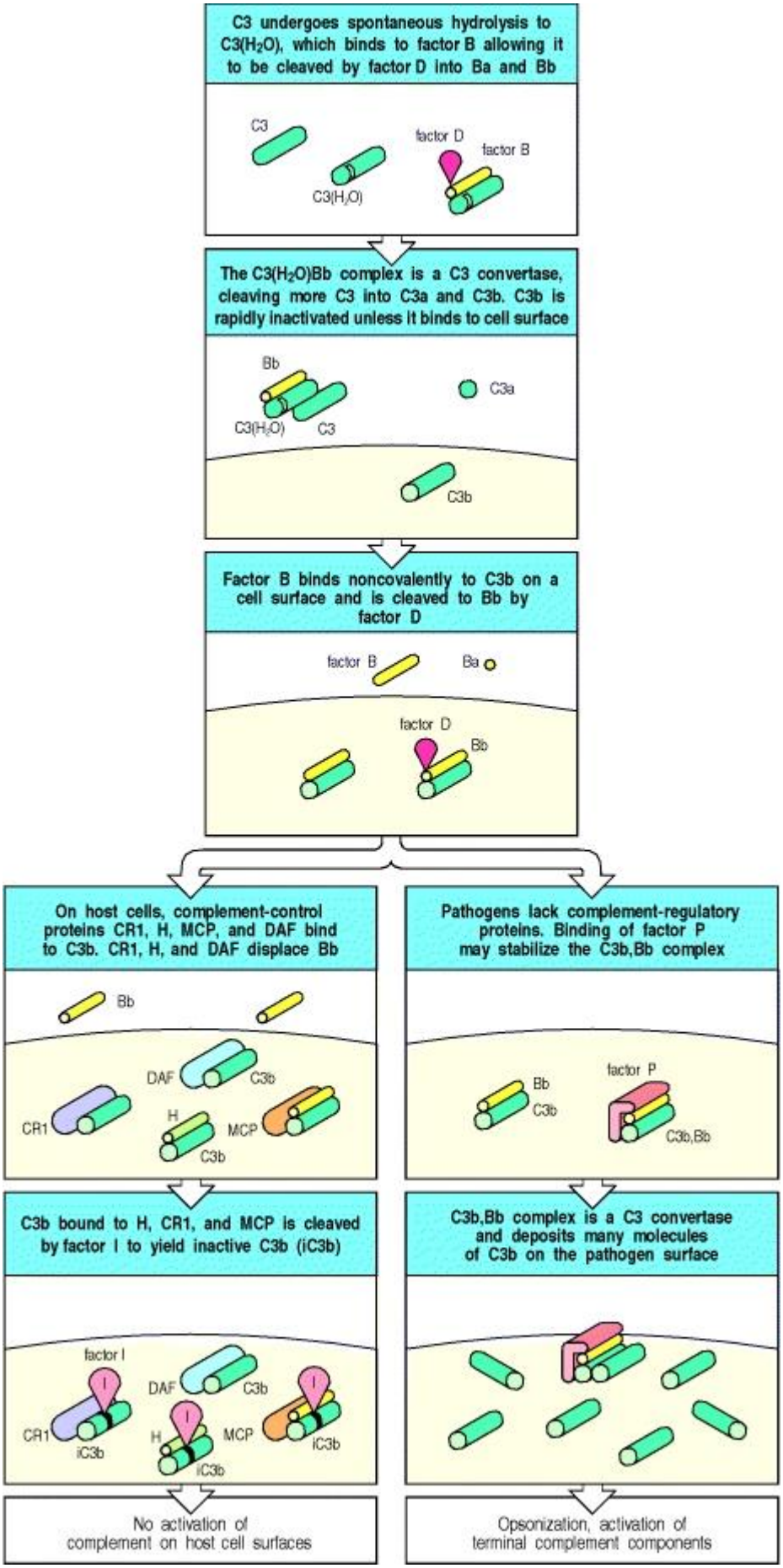
2- The mannan-binding lectin pathway is homologous to the classical pathway

- The MB-lectin pathway uses a protein very similar to C1q to trigger the complement cascade. This protein, called the mannan-binding lectin (**MBL**), is a collectin, like C1q.
- Mannan-binding lectin binds specifically to mannose residues, and to certain other sugars, which are accessible and arranged in a pattern that allows binding on many pathogens.
- mannan-binding lectin is able to initiate complement activation by binding to pathogen surfaces.
- Mannan-binding lectin, like C1q, is a six-headed molecule that forms a complex with two protease zymogens, which in the case of the mannanbindinglectin complex (MBL complex) are MASP-1 and **MASP-2** .

- When the MBL complex binds to a pathogen surface, MASP-1 and MASP-2 are activated to cleave C4 and C2. Thus the MB-lectin pathway initiates complement activation in the same way as the classical pathway, forming a C3 convertase from C2b bound to C4b.
- People deficient in mannan-binding lectin experience a substantial increase in infections during early childhood, indicating the importance of the MB-lectin pathway for host defense.

3- alternative pathway of complement

- The third pathway of complement activation is called the alternative pathway because it was discovered as a second, or 'alternative,' pathway for complement activation after the classical pathway had been defined.
- This pathway can proceed on many microbial surfaces in the absence of specific antibody, and it leads to the generation of a distinct C3 convertase designated C3b,Bb.
- In contrast to the classical and MB-lectin pathways of complement activation, the alternative pathway does not depend on a pathogen-binding protein for its initiation; instead it is initiated through the spontaneous hydrolysis of C3.
- both gram-negative and gram-positive bacteria have cell-wall constituents that can activate the alternative pathway.
- serum C3, which contains an unstablethioester bond, is subject to slow spontaneous hydrolysis toyield C3a and C3b. The C3b component can bind to foreignsurface antigens (such as those on bacterial cells or viral particles)
- The C3b present on thesurface of the foreign cells can bind another serum proteincalled factor B.
- Binding to C3b exposes a site on factor B that serves as the substrate for an enzymatically active serum protein called factorD.
- Factor D cleaves the C3b-bound factor B, releasing a smallfragment (Ba) that diffuses away and generating C3$\overline{b}$Bb.
- TheC3$\overline{b}$Bb complex has C3 convertase activity and thus is analogousto the C4$\overline{b}$2a complex in the classical pathway.
- The C3convertase activity of C3$\overline{b}$Bb has a half-life of only 5 minutesunless the serum protein properdin binds to it, stabilizingit and extending the half-life of this convertase activity to30 minutes.
- The nonenzymaticC3b component binds C5, and the Bb componentsubsequently hydrolyzes the bound C5 to generate C5a and C5b, the latter binds to the antigenic surface.

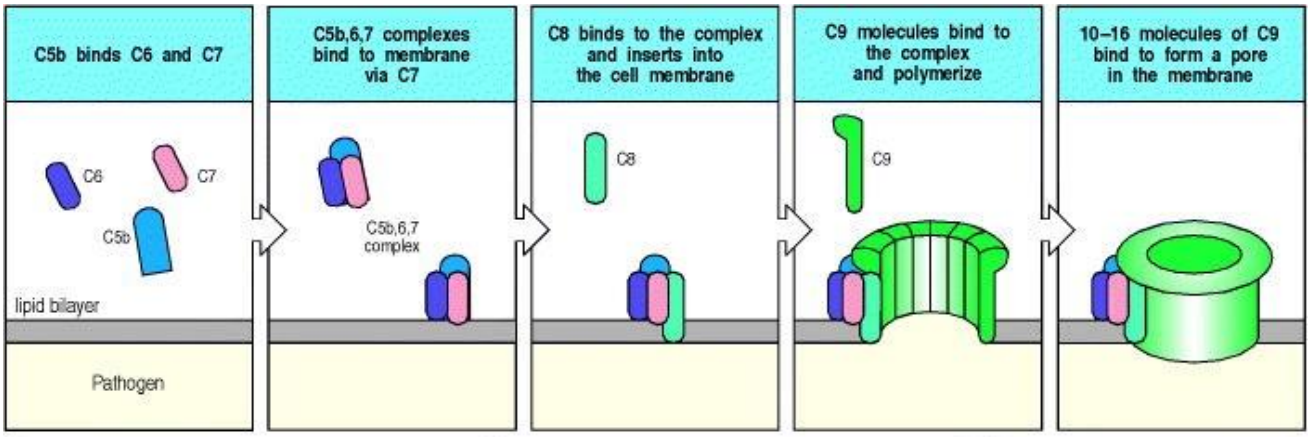

C3 undergoes spontaneous hydrolysis to C3(H2O), which binds to factor B allowing it to be cleaved by factor D into Ba and Bb
C3
factor D
factor B
C3(H2O)
The C3(H2O)Bb complex is a C3 convertase, cleaving more C3 into C3a and C3b. C3b is rapidly inactivated unless it binds to cell surface
Bb
C3a
C3(H2O)
C3
C3b
Factor B binds noncovalently to C3b on a cell surface and is cleaved to Bb by factor D
factor B
Ba
factor D
Bb
On host cells, complement-control proteins CR1, H, MCP, and DAF bind to C3b. CR1, H, and DAF displace Bb
Bb
DAF
C3b
CR1
H
MCP
C3b
Pathogens lack complement-regulatory proteins. Binding of factor P may stabilize the C3b,Bb complex
Bb
C3b
factor P
C3b,Bb
C3b bound to H, CR1, and MCP is cleaved by factor I to yield inactive C3b (iC3b)
factor I
DAF
C3b
CR1
iC3b
H
iC3b
MCP
iC3b
C3b,Bb complex is a C3 convertase and deposits many molecules of C3b on the pathogen surface
No activation of complement on host cell surfaces
Opsonization, activation of terminal complement components

❖ **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-attackcomplex (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
- 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.
- 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
- Binding of C8 to membrane-bound C5b67 induces a conformational change in C8, so that it too undergoes a hydrophilic-amphiphilic structural transition, exposing ahydrophobic 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 bloodcells 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.
- 10–17 molecules of C9 can be bound and polymerized by

a single C5b678 complex.

- The completed MAC, which has a tubular form and functional pore size of70–100 Å, consists of a C5b678 complex surrounded by apoly-C9 complex.



❖ Regulation of complement system

- The activation of C1 is controlled by a plasma serine proteinase inhibitor or **serpin**, the C1 inhibitor (C1INH). C1INH binds the active enzyme C1r:C1s, and causes it to dissociate from C1q, which remains bound to the pathogen.
- C4b is bound by a cofactor known as the C4b-binding protein (C4BP), CR1 (complement receptor 1) and MCP (membrane cofactor protien) which bind to C4b and prevent its association with C2a.
- Factor H is an important complement regulator at cell membranes, Factor H has affinity for the terminal sialic acids of host cell membrane glycoproteins and this increases the binding of factor H to any C3b deposited on host cells.
- A number of proteins competitively inhibit the binding of C2 to cell-bound C4b and of factor B to cell-bound C3b, thereby inhibiting convertase formation
- Several RCA proteins also act on the assembled C3 convertasecausing it to dissociate; these include C4bBP, CR1, and factor H. In addition, decayacceleratingfactor (DAF or CD55)
- There are also inhibitory mechanisms that prevent the inappropriate insertion of the membrane-attack complex into membranes, as S protein, homologus restriction factor(HRF) and CD59 bind C5b67 MAC preventing assembly of poly 9 and blocking formation MAC.

❖ The Functions of Complement

- Lysis of cells, bacteria, and viruses: The end result of complement activation is a pore in the lipid bilayer membrane that destroys membrane integrity. This is thought to kill the pathogen by destroying the proton gradient across the pathogen cell membrane
- **Opsonization, which promotes phagocytosis of particulate antigens**:
  This occurs by the specific recognition of bound complement components by complement receptors (CRs) on phagocytes. These complement receptors bind pathogens opsonized with complement components: opsonization of pathogens is a major function of C3b and its proteolytic derivatives. C4b also acts as an opsonin but has a relatively minor role, largely because so much more C3b than C4b is generated.

- **Binding to specific complement receptors** on cells of the immune system, triggering specific cell functions, inflammation, and secretion of immunoregulatory molecules, The small complement fragments C3a, C4a, and C5a act on specific receptors to produce local inflammatory responses, C5a also acts directly on neutrophils and monocytes to increase their adherence to vessel walls, their migration toward sites of antigen deposition, and their ability to

ingest particles, as well as increasing the expression of CR1 and CR3 on the surfaces of these cells

- **Immune clearance**, which removes immune complexesfrom the circulation and deposits them in the spleen and liver.

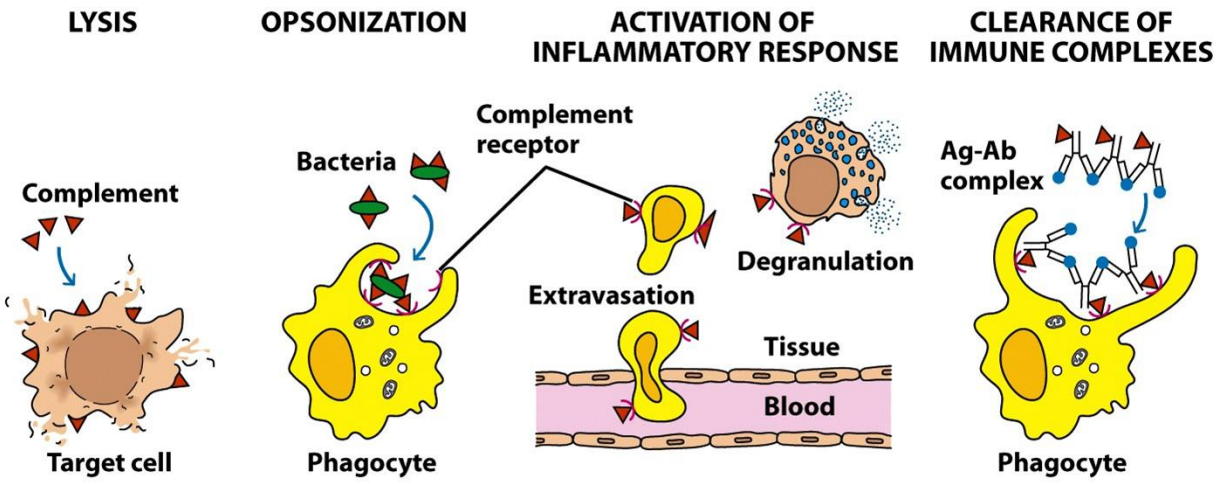


Figure 7-1
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