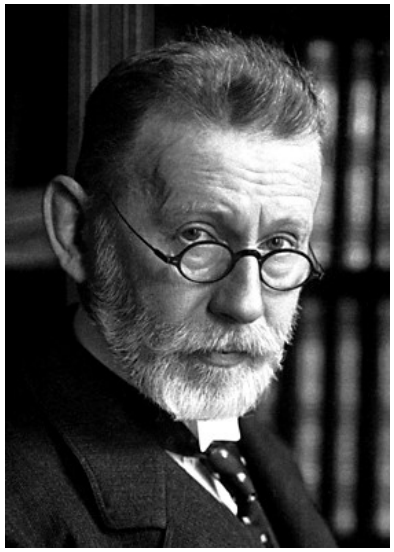
The complement system

Complement is defined as “the activity of blood serum that completes action of antibody”.

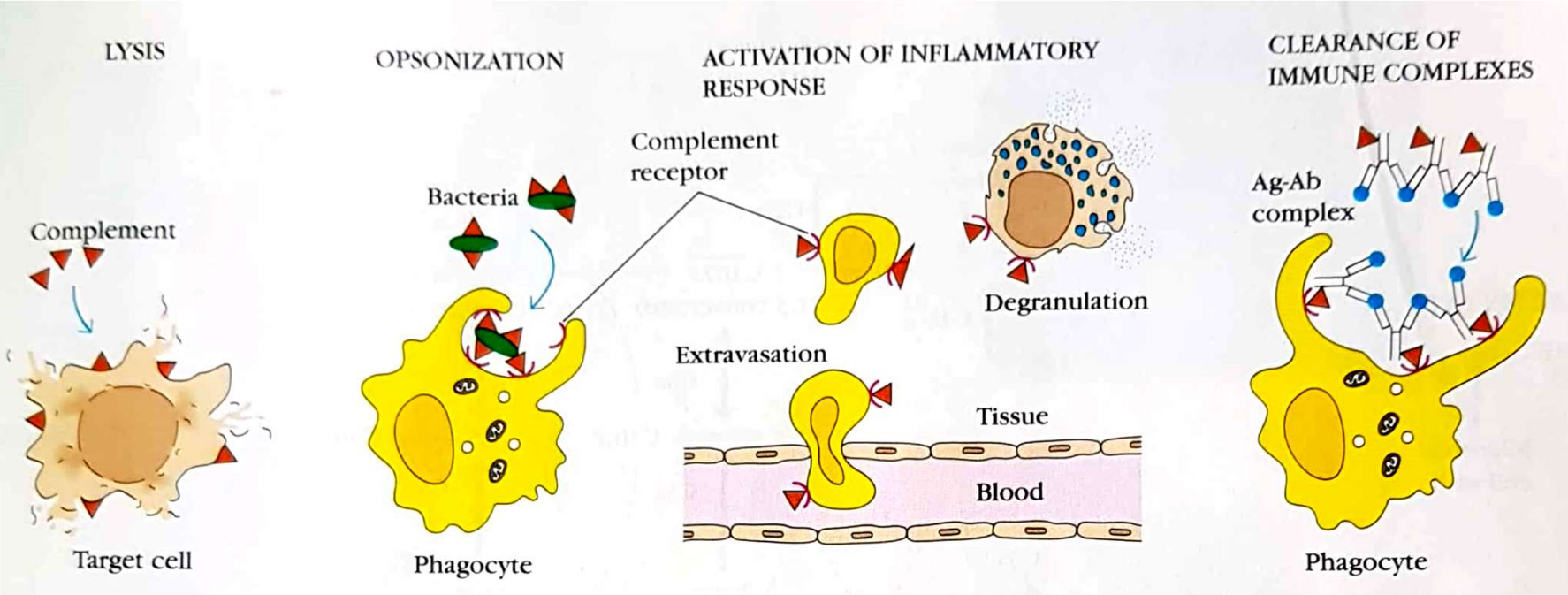
The term complement refers to a set of 50-plus serum proteins and glycoproteins that cooperates with both the innate and the adaptive immune systems to eliminate pathogens, dying cells, and immune complexes from the body.

The term was coined by Paul Ehrlich



The Nobel Prize in Physiology or Medicine in the year 1908
was awarded to **Paul Ehrlich** and Ilya Ilyich Mechnikov “in recognition of their work on immunity.“

Basic functions of activated complement components



After activation various complement components they interact in a highly regulated cascade to carry out a number of basic functions including

1. Lysis of cells, Bacteria and viruses.
2. Opsonization, which promotes phagocytosis of particular antigen.
3. Binding to specific complement receptors on cells of the immune system triggering specific cell functions, infalmmation, and secretion of immunoregulatory molecules.
4. Immune clearance, which removes complexes from the circulation and deposits them in the spleen and liver.

1. Complement system consists of soluble proteins and glycoproteins.

2. Most complement components are synthesized in the liver by hepatocytes.

3. Some are also produced by blood monocytes, tissue macrophages, fibroblasts, and epithelial cells of the gastrointestinal and genitourinary tracts.

4. Complement components constitute approximately 15% of the globulin protein fraction in plasma.

5. Several of the regulatory components of the system exist on cell membranes. So, the term complement now embraces proteins and glycoproteins distributed between the blood plasma and cell membranes.

6. Most circulate in the serum in functionally inactive form as proenzymes or zymogens.

7. Proteolytic cleavage removes an inhibitory fragment and exposes the active site of the molecule.

8. The complement reaction sequence starts with an enzyme cascade.

1. Complement factors are designated by numerals (C1-C9)

2. By letter symbols (Factor D)

3. Or by trivial names (homologous restriction factor)

4. Peptide fragments formed by activation of components are denoted by small letters.

5. Smaller fragments designated as "**a**" and larger as "**b**" (C3a, C3b).

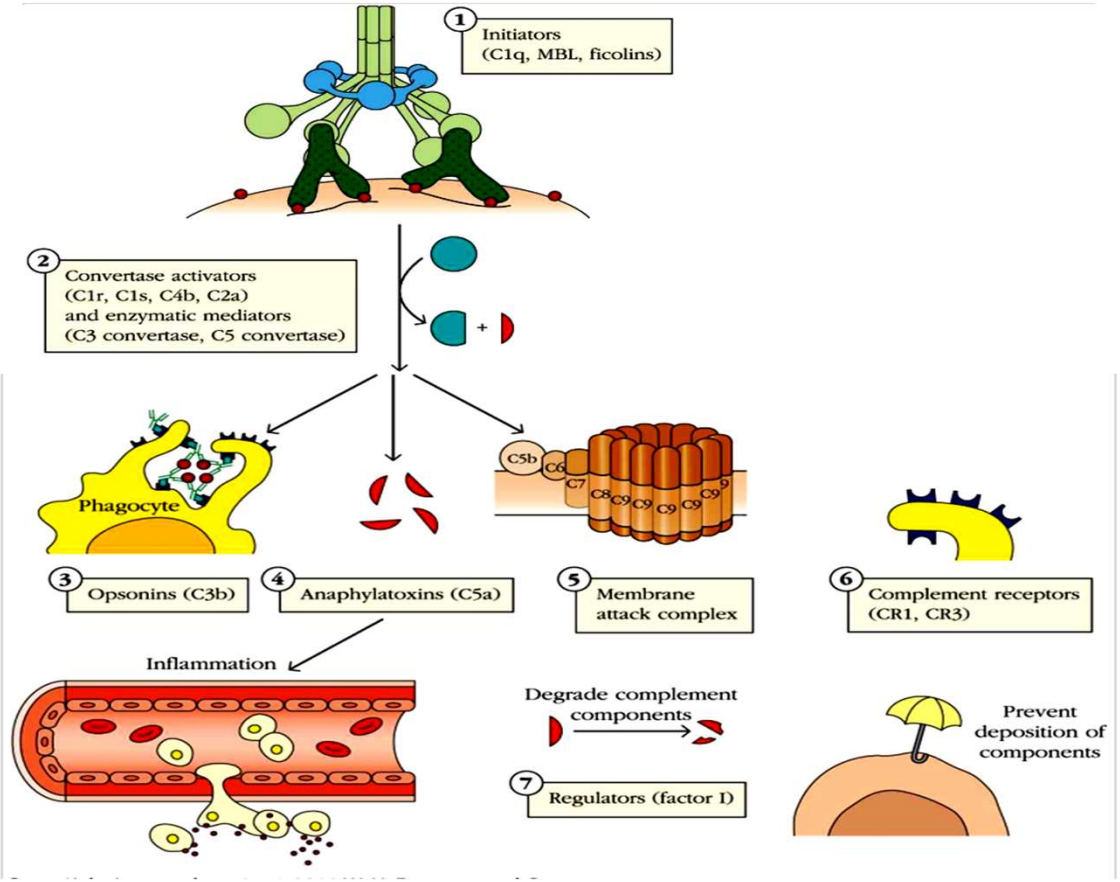
6. C2 is an exception where C2a is larger in size than C2b.

7. The larger fragments bind to the target while smaller fragments diffuse from the site and cause localized inflammatory reactions by binding to the specific receptors.

8. The complement fragments interact with one another to form one functional complex.

9. The complexes those have enzymatic activity are designated with a bar over the number or symbol ($C4\overline{b2a}$, $C3\overline{bBb}$).

Complement components can be classified into seven functional categories



(1) The complement pathways are initiated by proteins that bind to pathogens, either directly or via an antibody or other pathogen-specific protein. After a conformational change,

(2) enzymatic mediators activate other enzymes that generate the central proteins of the complement cascade, the C3 and C5 convertases, which cleave C3 and C5, releasing active components that mediate all functions of complement, including

(3) opsonization,

(4) inflammation

(5) generation of the membrane attack complex (MAC). Effector complement proteins can label an antibody-antigen complex for phagocytosis (opsonins), initiate inflammation (anaphylatoxins), or bind to a pathogen and nucleate the formation of the MAC. Often, these effectors act through

(6) complement receptors on phagocytic cells, granulocytes, or erythrocytes.

(7) Regulatory proteins limit the effects of complement by promoting their degradation or preventing their binding to host cells.

1. **Initiator complement** components initiate their respective complement reactions by binding to particular soluble or membrane-bound molecules. Once activated by their ligand, they undergo conformational alterations resulting in changes in their biological activity.

2. **Enzymatic mediators**. Several complement components are proteolytic enzymes that cleave and activate the next member of a complement reaction sequence. Proteins that are inactive until cleaved by proteases are called **zymogens.** Some complement proteases become active by binding to other macromolecules and undergoing a conformational change; others are zymogens themselves, inactive until cleaved by another "upstream" protease. The two enzyme complexes that cleave the complement components C3 and C5 are called the **C3 and C5 convertases**, respectively, and **occupy places of central importance in complement biology**. The sequence of proteins in a complement pathway from the initiator protein to the biological effector is referred to as a **"complement cascade."**

3. **Phagocytosis-enhancing components, or opsonins**. On activation of the complement cascade, several complement proteins are cleaved into two fragments, each of which then takes on a particular role. For C3 and C4, the larger fragments, **C3b and C4b, serve as opsonins**, binding covalently to microbial cells and serving as ligands for phagocytic cells with receptors for C3b or C4b.

4. **Inflammatory mediators**. Some small complement fragments act as inflammatory mediators. These fragments bind to receptors on the endothelial cells lining small blood vessels and induce an increase in capillary diameter, thus enhancing blood flow to the affected area. They also attract other cells to the site of tissue damage. Since these effects can be harmful (even lethal) in excess, these fragments are called **anaphylatoxins,** derived from the Greek phrase meaning "against protection**." C3a and C5a** are examples of anaphylatoxins.

5. **Membrane attack proteins.** Proteins of the membrane attack complex (MAC) insert into the cell membranes of invading microorganisms and punch holes that result in lysis of the pathogen. The MAC has been extensively imaged by electron microscopy. The complex itself forms a **ring-shaped multimer** of complement proteins with a central hole through which cytoplasmic contents can escape. MACs can also form on infected host cells, although the complement system must first overcome the regulatory mechanisms designed to protect host cells from complement attack.

6. **Complement receptor proteins**. Receptor molecules on cell surfaces bind complement proteins and signal specific cell functions. For example, some complement receptors such as CR1 bind to complement components such as C3b that have opsonized pathogens, triggering phagocytosis of the C3b-bound pathogen. Binding of the anaphylatoxin complement component C5a to C5a receptors (C5aRs) on neutrophils stimulates neutrophil degranulation and inflammation.

7. **Regulatory complement components.** Host cells are protected from unintended complementmediated damage by the presence of regulatory proteins. These regulatory proteins include factor I, which degrades C3b, and CD59 (protectin), which inhibits the formation of the MAC on host cells.

Three major pathways of complement activation.

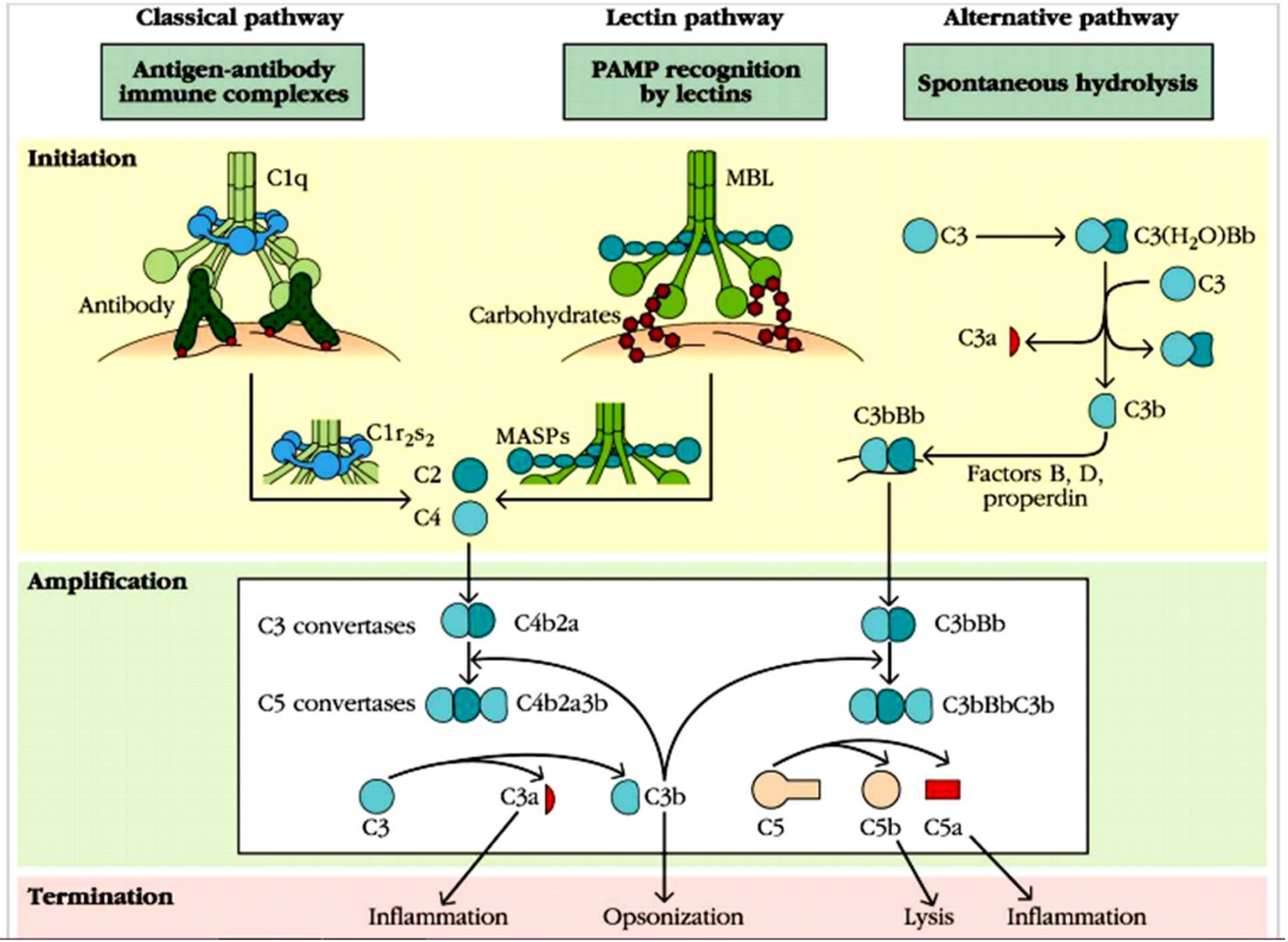


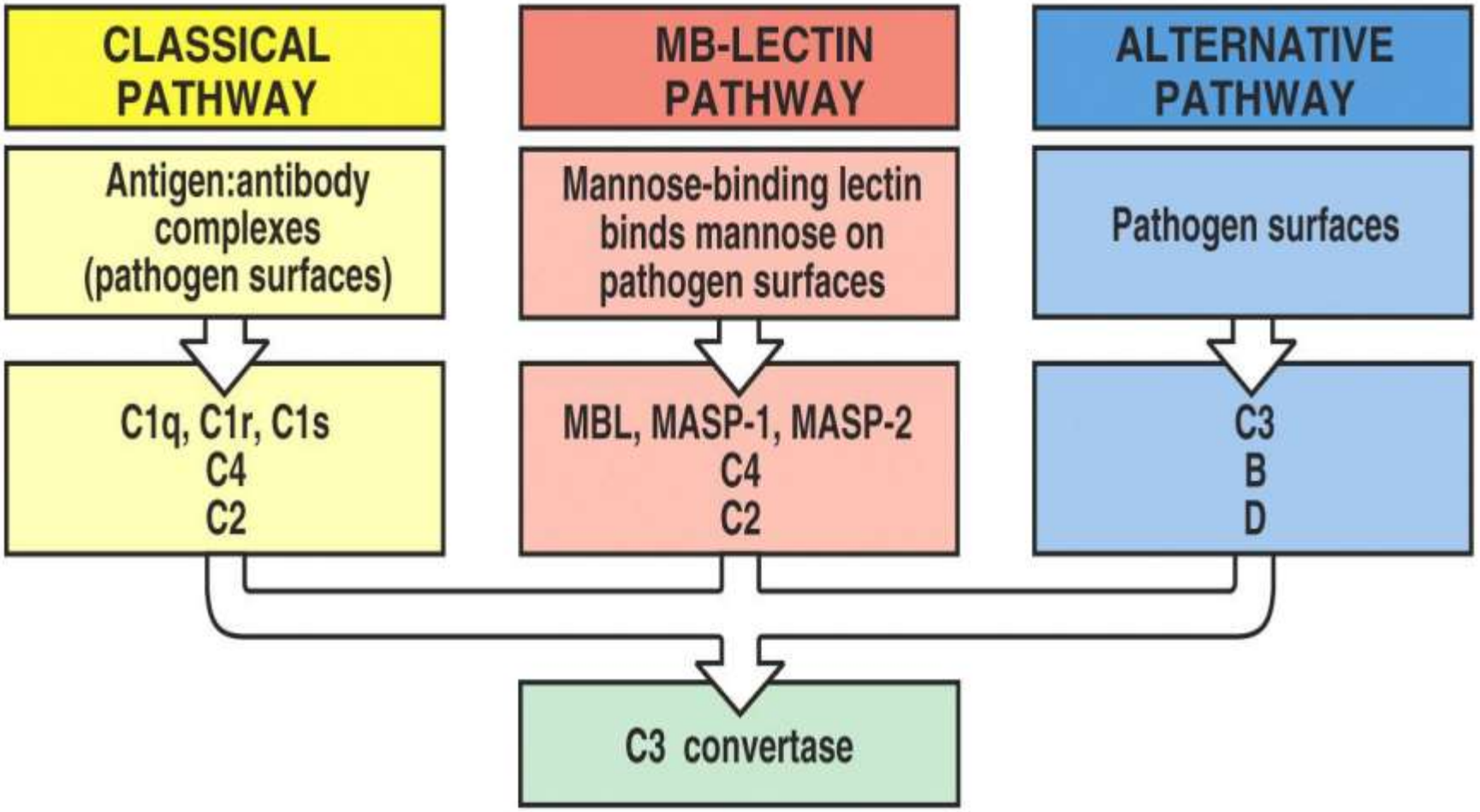
Fig. **Generation of C3 and C5 convertases by the three major pathways of complement activation**.

The classical pathway is initiated when C1q binds to antigen-antibody complexes. The antigen is shown here in dark red and the initiating antibody in green. The C1r enzymatic component of C1 (shown in blue) is then activated and cleaves C1s, which in turn cleaves C4 to C4a and C4b. C4b attaches to the membrane and binds C2, which is then cleaved by C1s to form C2a and C2b. (C2b is then acted on further to become an inflammatory mediator.) C2a remains attached to C4b, forming the classical pathway C3 convertase (C4b2a).

In the lectin pathway, mannose-binding lectin (MBL, green) binds specifically to conserved carbohydrate arrays on pathogens, activating the MBL-associated serine proteases (MASPs, blue). The MASPs cleave C2 and C4, generating the C3 convertase as in the classical pathway.

In the alternative pathway, C3 undergoes spontaneous hydrolysis to C3(H O), which binds serum factor B. On binding to C3(H O), B is cleaved by serum factor D, and the resultant C3(H O)Bb complex forms a fluid-phase C3 convertase. Some C3b, released after C3 cleavage by this complex, binds to microbial surfaces. There, it binds factor B, which is cleaved by factor D, forming the cell-bound alternative pathway C3 convertase, C3bBb. This complex is stabilized by properdin.

There is a second set of convertase enzymes generated in the early stages of complement activation.
The **C5 convertases** are formed by the addition of a C3b component to each of the two C3 convertases. C5 convertases cleave C5 into C5a, an inflammatory mediator, or anaphylatoxin, and C5b, which is the initiating factor of the membrane attack complex.


CLASSICAL PATHWAY
MB-LECTIN PATHWAY
ALTERNATIVE PATHWAY
Antigen:antibody complexes (pathogen surfaces)
Mannose-binding lectin binds mannose on pathogen surfaces
Pathogen surfaces
C1q, C1r, C1s
C4
C2
MBL, MASP-1, MASP-2
C4
C2
C3
B
D
C3 convertase

The Classical pathway is initiated by antibody binding to antigens

The classical pathway of complement activation is considered as part of the adaptive immune response since it begins with the formation of antigen-antibody complexes.

These complexes may be soluble, or they may be formed when an antibody binds to antigenic determinants, or epitopes, situated on viral, fungal, parasitic, or bacterial cell membranes.

Soluble antibody antigen complexes are often referred to as immune complexes. Only complexes formed by antigens with antibodies of the IgM class or IgG1, IgG2 and IgG3 subclasses of IgG antibodies are capable of activating the classical complement pathway.

The initial activation involves interaction of these antibody-antigen complexes with the complement components C1, C2, and C4, normally found in the plasma as inactive precursors or zymogens. The formation of an antigen-antibody complex induces conformational changes in the nonantigen-binding (Fc) portion of the antibody molecule.

This conformational change exposes a binding site on the antibody for the C1 component of complement. In serum, C1 exists as a macromolecular complex consisting of one molecule of C1q and two molecules each of the serine proteases C1r and C1s, held together in a Ca^{2+}-stabilized complex ($C1qr_2 s_2$). The C1q molecule itself is composed of 18 polypeptide chains that associate to form six collagen-like triple helical arms, the tips of which bind the C_H2 domain of the antigen-bound antibody molecule.

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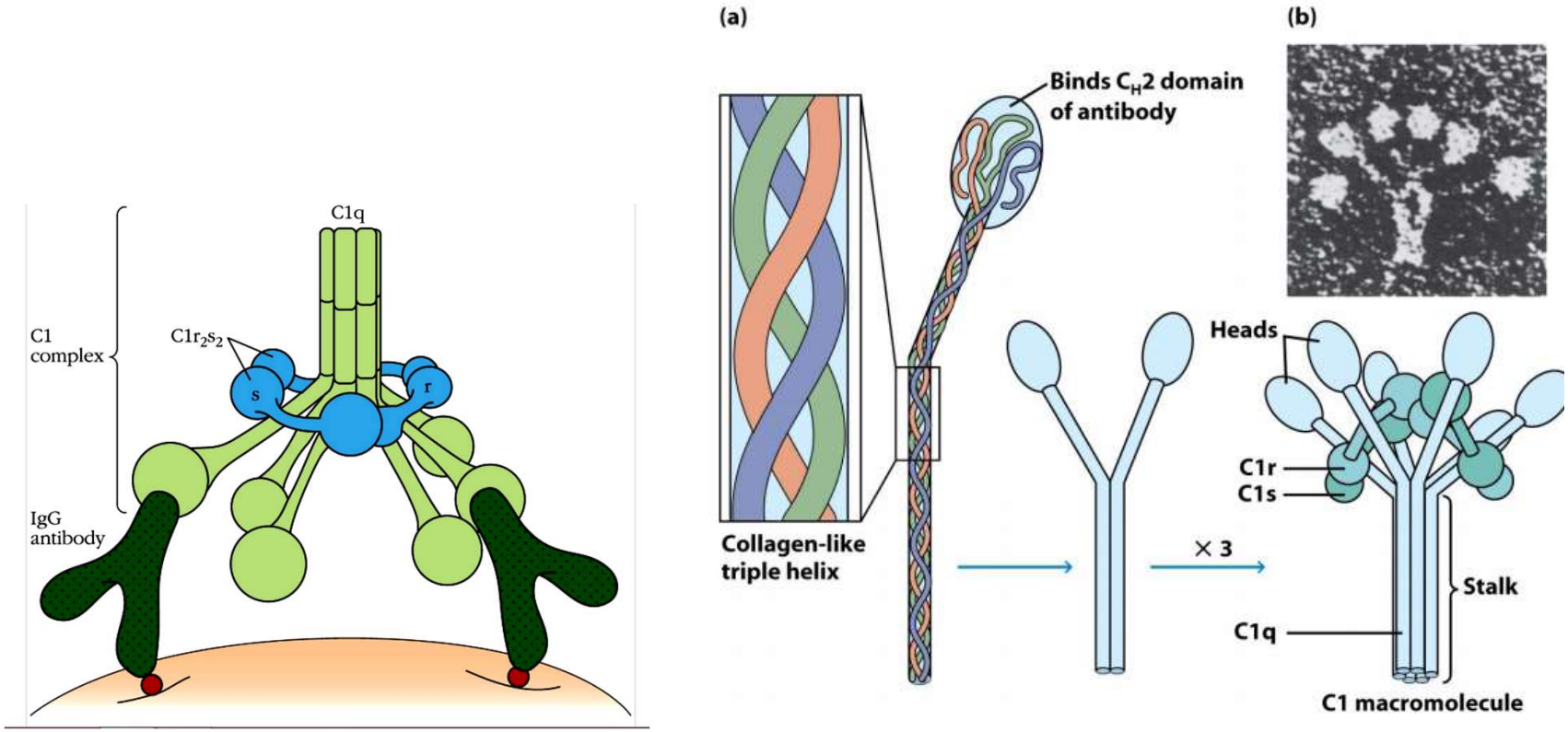
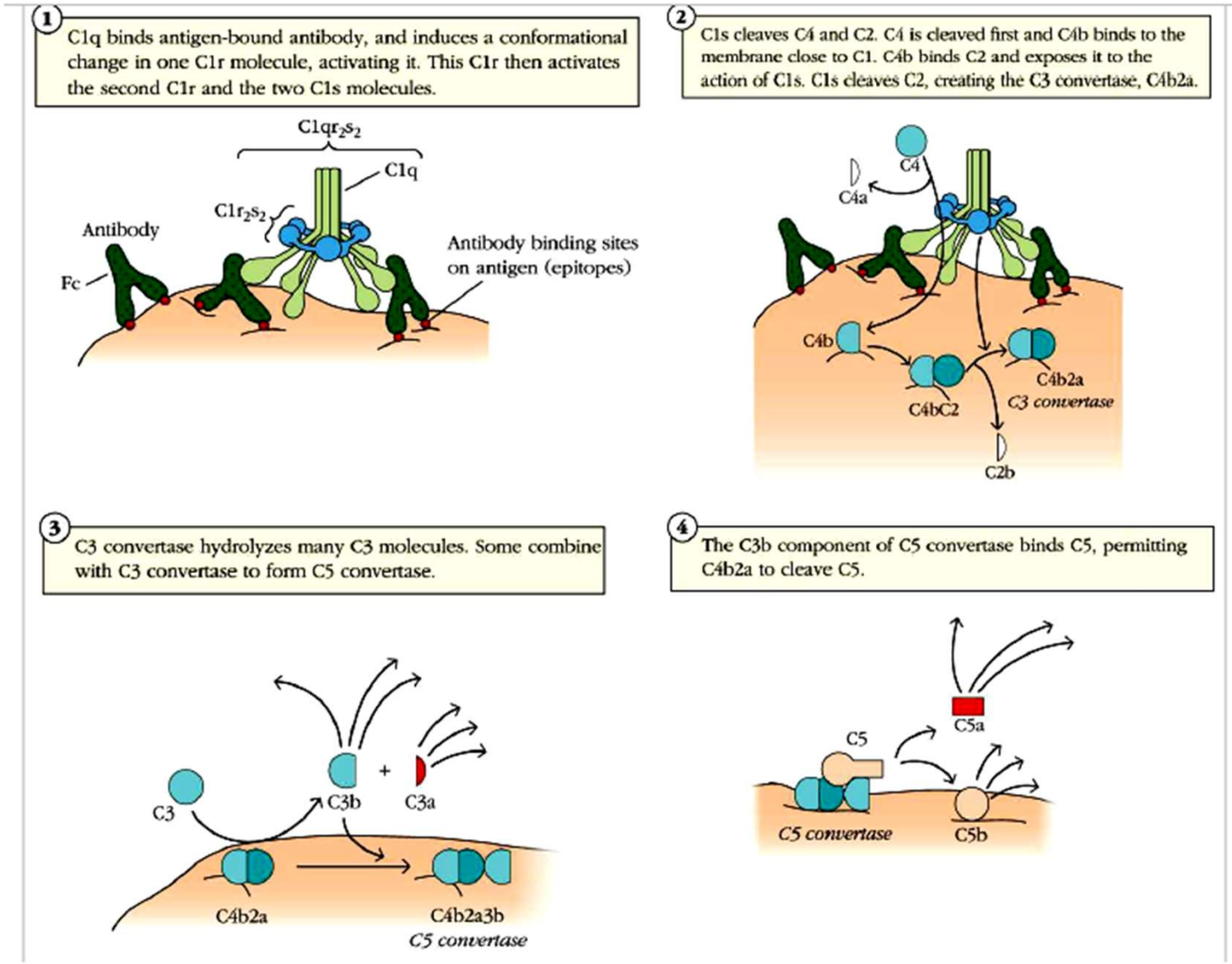


Structure of the C1 macromolecular complex. C1q interacts with two molecules each of C1r and C1s to create the C1 complex. The C1q molecule consists of 18 polypeptide chains in six collagen-like triple helices.



Antigenic determinants are shown in dark red, initiating components (antibodies and C1q) are shown in green, active enzymes are shown in blue, and anaphylatoxins in bright red.

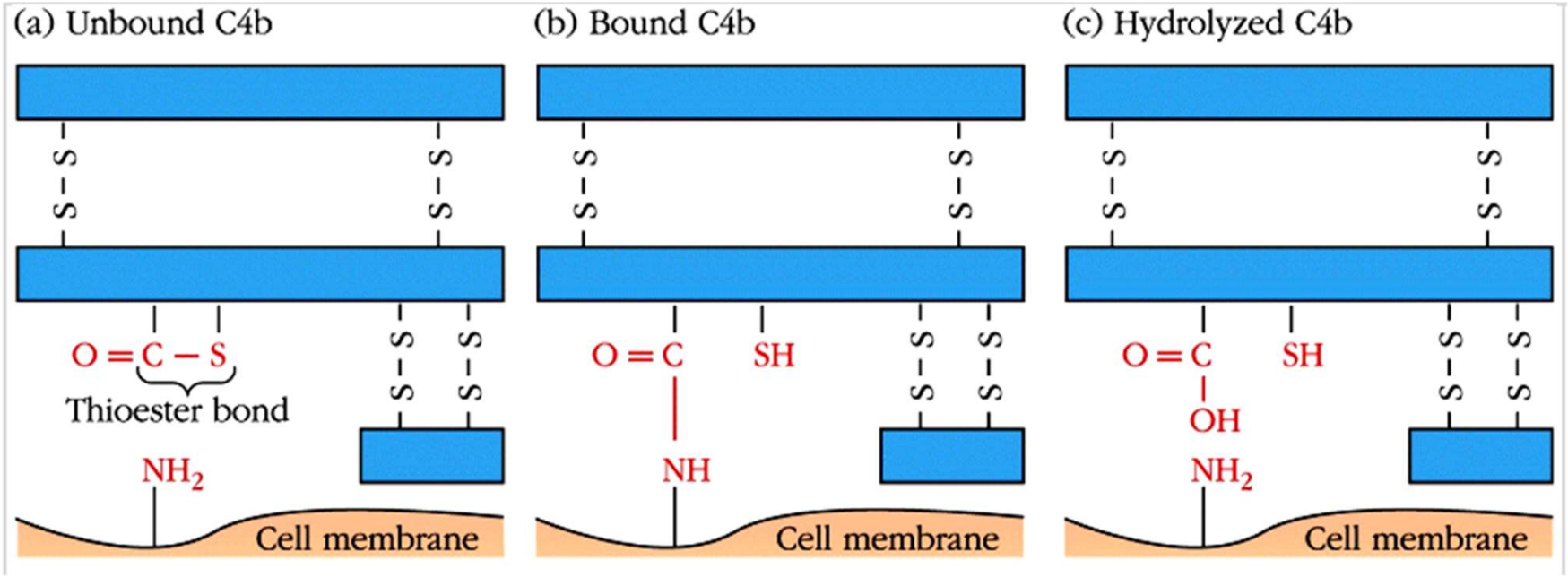
1. Binding of C1q to the C 2 domains of the Fc regions of the antigen-complexed antibody molecule induces a conformational change in one of the C1r molecules. This conformational change in the C1r molecule converts it to an active serine protease enzyme that then cleaves and activates its partner C1r molecule. The two C1r proteases then cleave and activate the two C1s molecules.

2. Activated C1s has two substrates, C4 and C2. C4 is activated when C1s hydrolyzes a small fragment (C4a) from the amino terminus of one of its chains.

3. The C4b fragment attaches covalently to the target membrane surface in the vicinity of C1, and then binds C2. C4b binding to the membrane occurs when an unstable, internal thioester on C4b is exposed on C4 cleavage and reacts with hydroxyl or amino groups of proteins or carbohydrates on the cell membrane.

4. This reaction must occur quickly, before the unstable thioester is further hydrolyzed and can no longer make a covalent bond with the cell surface .

5. Indeed, approximately 90% of C4b is hydrolyzed before it can bind the cell surface. C4b is also capable of forming covalent bonds with the constant regions of antibody molecules involved in antigenantibody complexes.



Binding of C4b to the microbial membrane surface occurs through a thioester bond via an exposed amino or hydroxyl group.

(a) Both C3b and C4b contain highly reactive thioester bonds, which are subject to nucleophilic attack by hydroxyl or amino groups on cell membrane proteins and carbohydrates.

(b) Breakage of the thioester bond leads to the formation of covalent bonds between the membrane macromolecules and the complement components.

(c) If this covalent bond formation does not occur quickly after generation of the C3b and C4b fragments, the thioester bond will be hydrolyzed as shown.

6. On binding C4b at the membrane surface or on an immune complex, C2 becomes susceptible to cleavage by the neighboring C1s enzyme. A smaller C2b fragment diffuses away, leaving behind an enzymatically active C4b2a complex. In this complex, C2a is the enzymatically active fragment, but it is active only when bound by C4b. This C4b2a complex, is the C3 convertase that converts C3 into its enzymatically active form.

7. The membrane-bound or immune complex–bound C3 convertase enzyme, C4b2a, now hydrolyzes C3, generating two unequal fragments: the small anaphylatoxin C3a, and the pivotally important fragment C3b. A single C3 convertase molecule can generate over 200 molecules of C3b, resulting in tremendous amplification at this step of the classical pathway.

8. The generation of C3b is an essential precursor to many of the subsequent reactions of the complement system. Deficiencies of complement components that act prior to C3 cleavage leave the host extremely vulnerable to both infectious and autoimmune diseases, whereas deficiencies of components later in the pathway are generally of lesser consequence.

9. In particular, patients with deficiencies in C3 itself are unusually susceptible to infections with both gram-positive and gram-negative bacteria. This is because C3b acts in three important and different ways to protect the host:

In a manner very similar to that of C4b, C3b binds covalently to microbial surfaces, providing a molecular "tag" that allows phagocytic cells with C3b receptors to engulf the tagged microbes. This process is called **opsonization.**

C3b, like C4b, can attach to the Fc portions of antibodies participating in soluble antigenantibody complexes. These C3b-tagged immune complexes are bound by C3b receptors on phagocytes or red blood cells, and are either phagocytosed, or conveyed to the liver where they are destroyed.

Some molecules of C3b bind the membrane-localized C4b2a enzyme to form the trimolecular, membrane-bound, C5 convertase complex C4b2a3b .

The C3b component of this complex binds C5, and the complex then cleaves C5 into the two fragments: C5b and C5a. C4b2a3b is therefore the C5 convertase of the classical pathway.

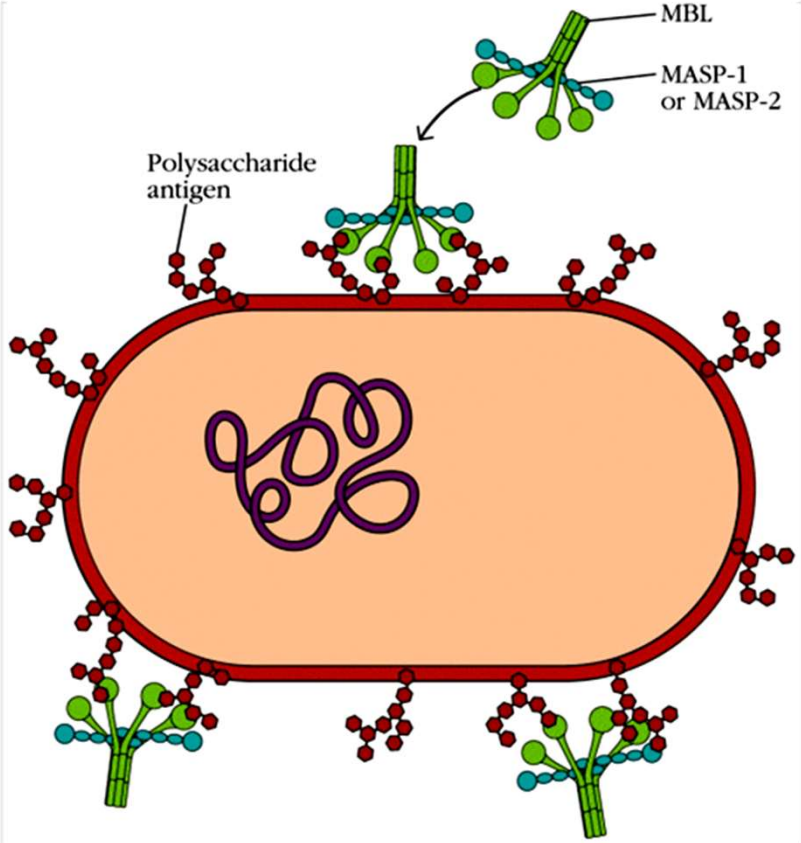
The trio of tasks accomplished by the C3b molecule places it right at the center of complement attack pathways. **C3b is thus a central component in all three complement activation pathways**.

The Lectin Pathway

This pathway is initiated when soluble proteins recognize microbial antigens . The lectin pathway of complement activation, like the classical pathway, proceeds through the activation of a C3 convertase composed of C4b and C2a.

This pathway uses **lectins**—proteins that recognize particular carbohydrate components—as its specific receptor molecules .

Because it does not rely on antibodies from the adaptive immune system, the lectin pathway is considered to be **an arm of innate, rather than adaptive, immunity**.



Initiation of the lectin pathway relies on lectin receptor recognition of microbial cell surface carbohydrates.

Lectin receptors, such as MBL (Mannose-binding lectin), bind microbial cell surface carbohydrates. They bind the (MBL-associated serine proteases) MASP family serine proteases, which cleave C2 and C4 to mediate formation of a lectin-pathway C3 convertase.

Mannose-binding lectin (MBL), the first lectin demonstrated to be capable of initiating complement activation, binds close-knit arrays of mannose (sugar) residues that are found on the surfaces of microbes such as *Salmonella, Listeria, and Neisseria* bacteria; *Cryptococcus* neoformans and *Candida albicans* fungi; and even on the membranes of some viruses such as HIV1 and respiratory syncytial virus (RSV).

Further characterization of MBL demonstrated that it also recognizes N-acetylglucosamine, Dglucose, and L-fucose polymers on microbial surfaces. All these sugars, including mannose, present their associated hydroxyl groups in defined three-dimensional arrays and thus MBL is acting as a classic pattern recognition receptor .

Individuals with low levels of MBL suffer from repeated, serious bacterial infections.

MBL is constitutively expressed by the liver and belongs to the subclass of lectins known as collectins.

Other lectin receptors have been recognized as initiators of the lectin pathway of complement activation. These include collectin-10 and collectin-11 as well as several members of the ficolin family: ficolin-1, ficolin-2, and ficolin-3.

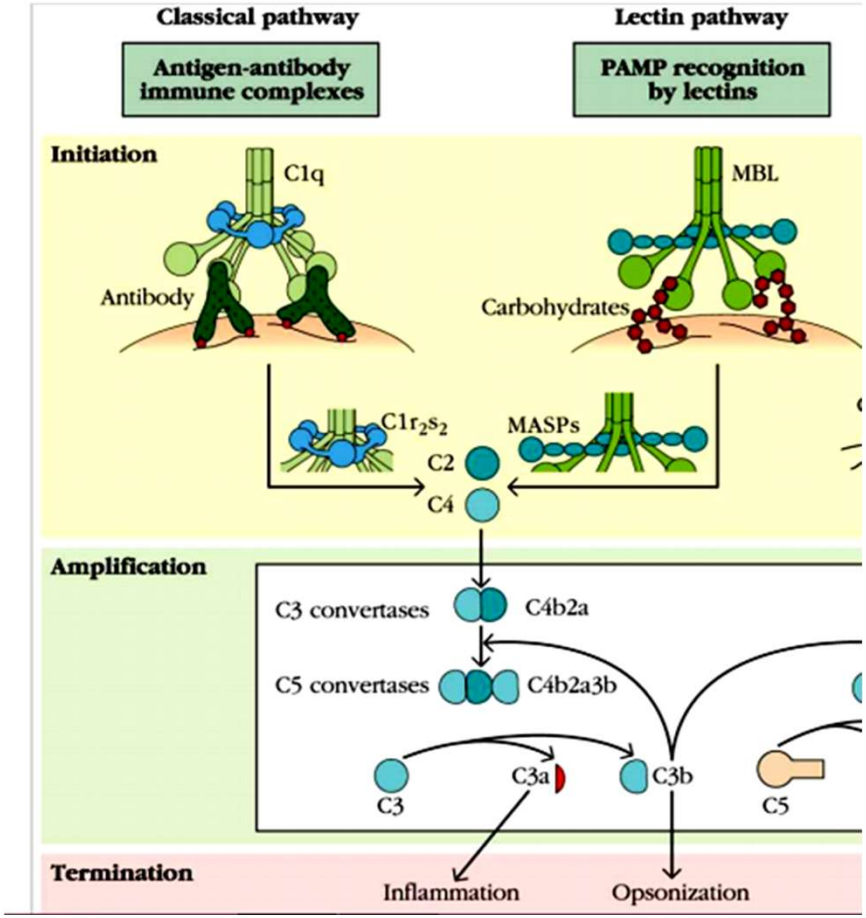
These proteins share a common “stalk” consisting of a collagen-like triple helix, coupled to a carbohydrate recognition structure. The nature of the recognition structure defines the lectin as belonging to the collectin or the ficolin family.

In the blood, MBL is associated with MBL-associated serine proteases, or MASP proteins.

Three MASP proteins—MASP-1, MASP-2, and MASP-3—have been identified. MASP-2 is the most active protein.

MASP-2 is structurally related to the serine protease C1s, and when MBL binds to a microbial surface, associated MASP-2 molecules cleave both C2 and C4, giving rise to the C4b2a which is known as C3 convertase . This is similar to the C3 convertase of the classical pathway.

From this point on, the lectin pathway uses all the same downstream components as the classical pathway.



The alternative pathway is initiated in three distinct ways

Initiation of the alternative pathway of complement activation, like the lectin pathway, is independent of antibody-antigen interactions. Therefore, this pathway is also considered to be part of the innate immune system.

However, unlike the lectin pathway, the alternative pathway uses a different set of C3 and C5 convertases .

The alternative pathway C3 convertase, **C3bBb,** is made up of one molecule of the C3b protein fragment and one molecular fragment unique to the alternative pathway, Bb.

A second C3b is then added to make the alternative pathway C5 convertase, C3bBbC3b.

Alternative pathway can be initiated in three distinct ways.

The first mode of initiation to be discovered, the "tickover" pathway, uses the four serum components C3, factor B, factor D, and properdin (or factor P) .

Two additional modes of activation for the alternative pathway have also been identified: one is initiated by **properdin**, and the other by proteases such as thrombin and **kallikrein**.

The Alternative (Tick over (slow speed or smooth) **Pathway**

The term tick over refers to the fact that C3 is constantly being made and spontaneously inactivated and is thus considered to be "ticking over."

The alternative tick over pathway is initiated when C3, which is at high concentrations in serum, undergoes spontaneous hydrolysis at its internal thioester bond, yielding the molecule known as hydrolyzedC3, **C3(H_2O).**

The conformation of C3(H_2O) is different from that of the C3 parent protein.

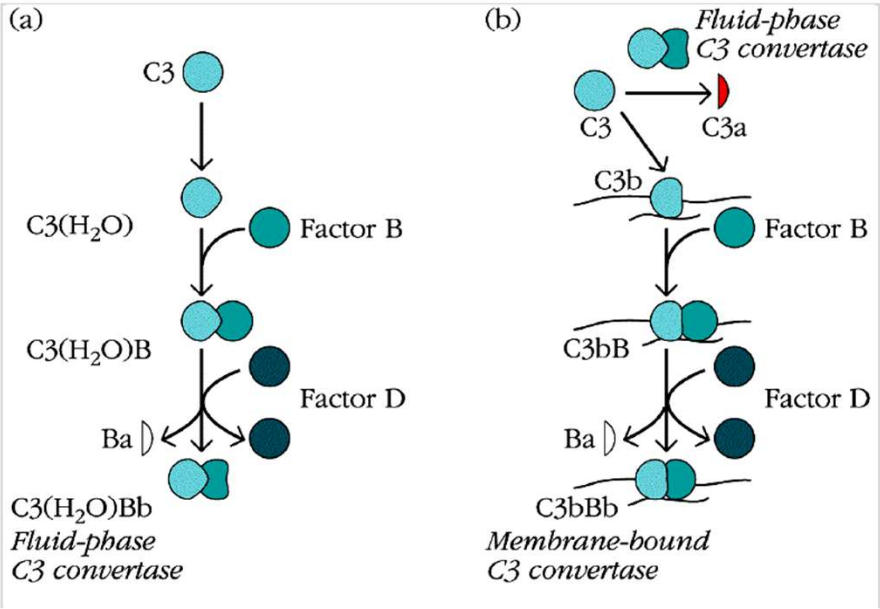
C3(H_2O) accounts for approximately 0.5% of plasma C3. In the presence of serum Mg , C3(H_2O) binds another serum protein, factor B .

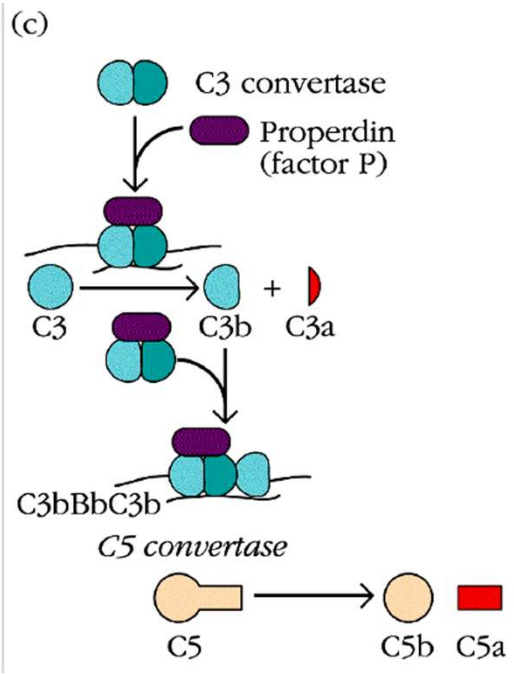
When bound to C3(H_2O), factor B becomes susceptible to cleavage by a serum protease, factor D.

Factor D cleaves factor B, releasing a smaller Ba subunit that diffuses away, leaving a catalytically active Bb subunit bound to C3(H_2O).

The C3(H_2O)Bb complex is referred to as the "fluid-phase C3 convertase" because it remains in the blood plasma **and is not bound to any cells.**

In the plasma, the fluid-phase convertase cleaves many molecules of C3 into C3a and C3b .
The C3(H_2O)Bb complex is not very stable in a healthy host and it is rapidly degraded, hence the term "tick over" for this pathway.





Initiation of the alternative tickover pathway of complement.

(a) Spontaneous hydrolysis of soluble **C3 to C3(H O)** allows the altered conformation of C3(H O) to bind factor B, rendering it susceptible to cleavage by factor D. The resulting complex C3(H O)Bb forms a fluid-phase convertase capable of cleaving C3 to C3a and C3b.

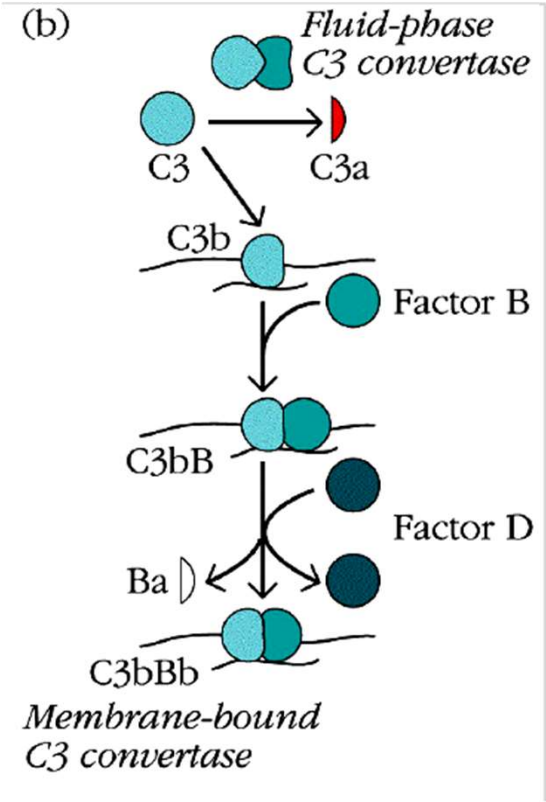
(b) Some of the C3b molecules formed by the fluidphase convertase bind to cell membranes. C3b, like C3(H O), binds factor B in such a way as to make B susceptible to factor D– mediated cleavage.

(c) The membrane-bound C3bBb is stabilized by **properdin** (factor P), which binds the C3bBb complex on the membrane. Addition of a second C3b molecule to the C3bBb complex forms the C5 convertase, which is also stabilized by properdin.

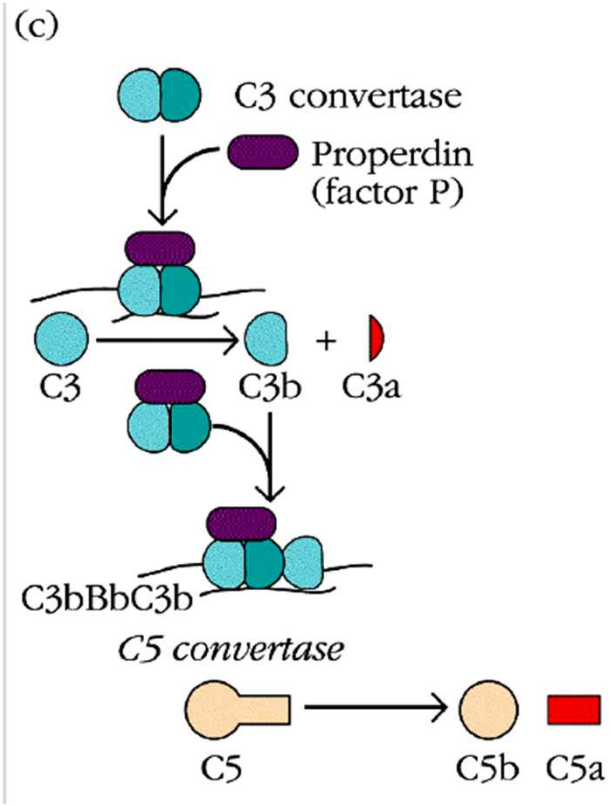
However, if there is an infection present, some of the newly formed C3b molecules bind nearby microbial surfaces via their thioester linkages .

In the presence of a microbial infection, factor B binds the newly attached C3b molecules on the microbial cell surface , and becomes susceptible to cleavage by factor D, with the generation of C3bBb complexes.

These C3bBb complexes are now located on the microbial membrane surface. Like the C4b2a complexes of the classical pathway, the cell-bound C3bBb complexes have C3 convertase activity, and this complex now takes over from the fluid-phase C3(H O)Bb as the predominant C3 convertase.



To be clear, there are two C3 convertases in the alternative tickover pathway:
a fluid-phase C3(H O)Bb, which initiates the pathway, and
a membrane-bound C3bBb C3 convertase that amplifies it and results in microbial destruction.
The cell-bound alternative pathway C3 convertase is unstable until it is bound by properdin (otherwise known as factor P), a serum protein. Once stabilized by properdin, these cell-associated, C3bBb C3 convertase complexes rapidly generate large numbers of C3b molecules on the microbial surface. Many of these then bind factor B, which is cleaved in turn by factor D, thus facilitating the cleavage of yet more molecules of C3 and amplifying the rate of C3b generation. This amplification pathway is rapid; once the alternative pathway has been initiated, more than 2 × 10 molecules of C3b can be deposited on a microbial surface in less than 5 minutes.

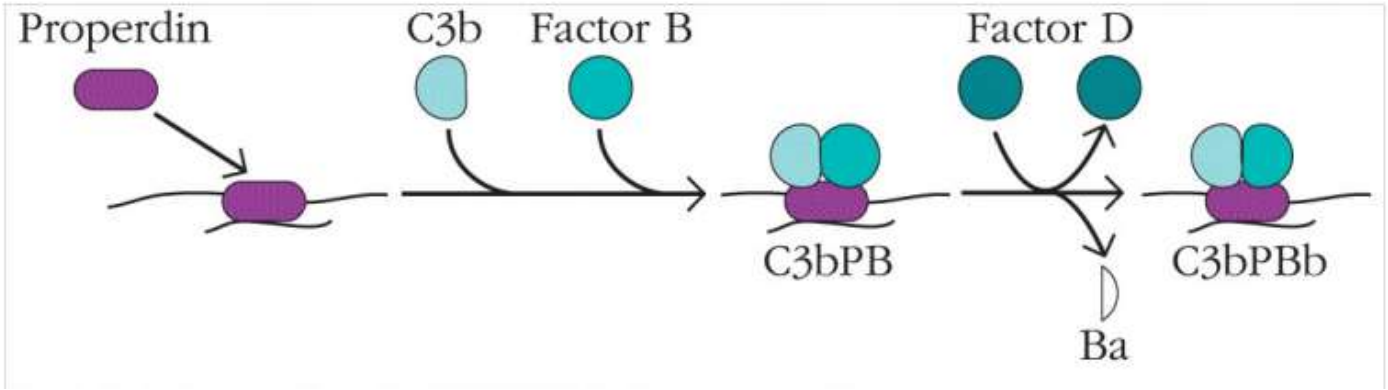


The alternative C5 convertase complex has the composition C3bBbC3b, and like the alternative C3 convertase, it is also stabilized by binding to properdin.

Like the classical and lectin pathway C5 convertase, C3bBbC3b cleaves C5, which goes on to form the MAC (membrane attack complex).

The Alternative Properdin-Activated Pathway

In addition to stabilizing the ongoing activity of the alternative pathway, properdin may also serve to initiate it. In vitro experiments demonstrated that if properdin molecules were attached to an artificial surface and allowed to interact with purified complement components in the presence of Mg , the immobilized properdin bound C3b and factor B . This bound factor B proved to be susceptible to cleavage by factor D, and the resultant C3bPBb complex acted as an effective C3 convertase, Thus, it seemed that properdin could initiate activation of the alternative pathway on an artificial substrate.



Initiation of the alternative pathway by specific, noncovalent binding of properdin to the target membrane. Properdin (factor P) binds to components of microbial membranes, and stabilizes the binding of C3bBb complexes of the alternative complement pathway. The difference between this and the tickover pathway is that properdin binds first and initiates complement deposition on the membrane.

The Alternative Protease-Activated Pathway

The biochemical pathway that leads to complement activation is similar in concept to the blood coagulation pathway. Both use protease cleavage and conformational alterations of key proteins to modify enzyme activities, as well as amplification of various steps of the pathways by feed-forward loops.

Several decades ago, it was shown that protein factors involved in blood clotting, such as **thrombin, could cleave the complement components C3 and C5 in vitro,** with the release of the active anaphylatoxins C3a and C5a. Since these cleavage reactions required relatively high thrombin concentrations, they were at first thought not to be physiologically meaningful.

More recently, however, it has been demonstrated in a mouse disease model that initiation of the **coagulation cascade may result in the cleavage of physiologically relevant amounts of C3 and C5 to produce C3a and C5a.**

Interestingly, when blood platelets are activated during a clotting reaction, they release high concentrations of ATP and Ca^{2+} along with serine/threonine kinases.

These enzymes act to phosphorylate extracellular proteins, including C3b.
Phosphorylated C3b is less susceptible to proteolytic degradation than its unphosphorylated form, and thus, by this route, **activation of the clotting cascade enhances all of the complement pathways.**

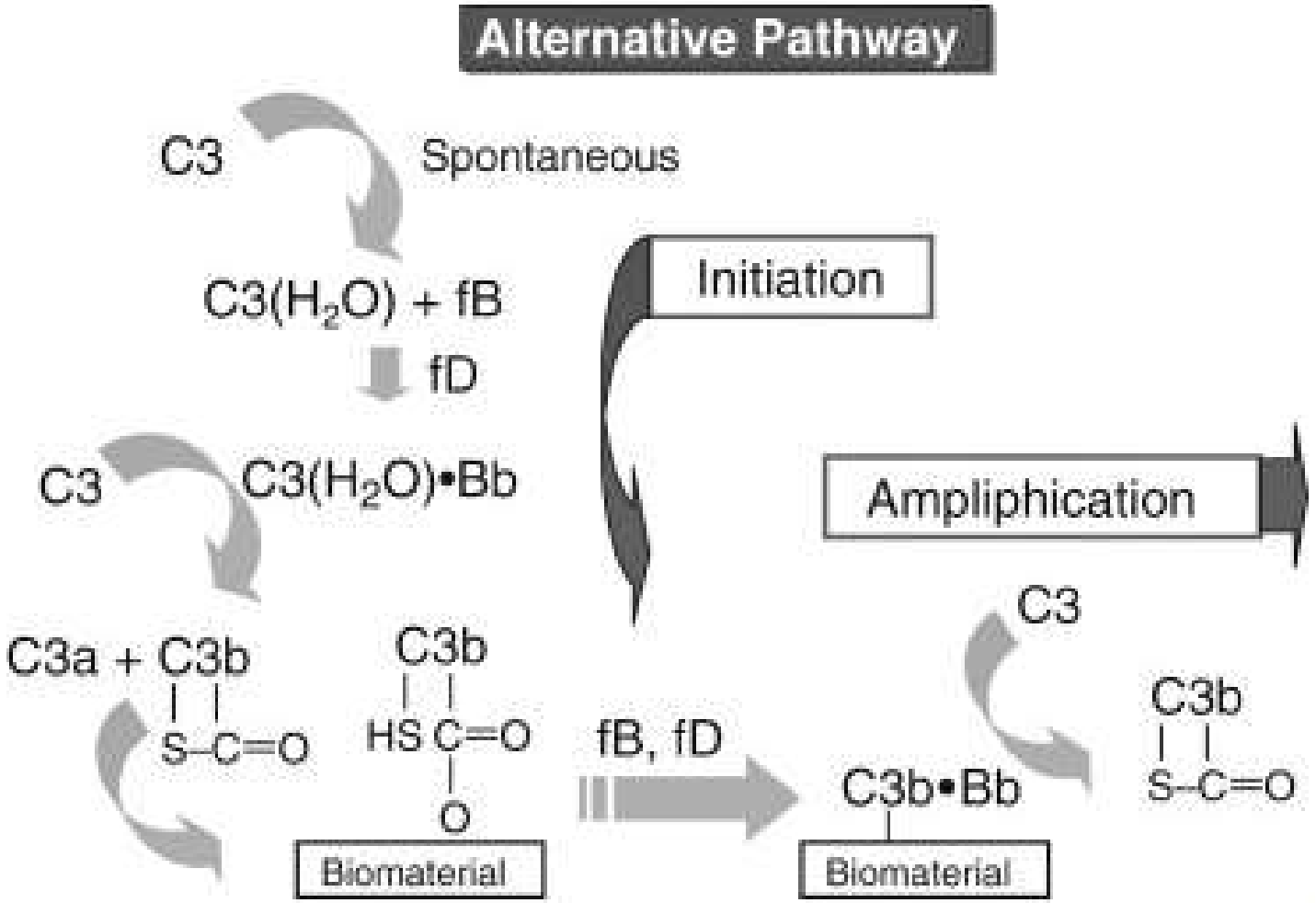
Summary of alternative pathway

The alternative tickover pathway is initiated when C3(H O) binds to factor B, which then becomes susceptible to cleavage by factor D into Ba and Bb. The Bb fragment continues to bind to the hydrolyzed C3(H O) and together they form a fluid-phase C3 convertase. Some of the C3b generated by this convertase adheres to microbial surfaces; there it binds factor B, which again, in the presence of factor D, is cleaved, resulting in the formation of the membrane-bound C3 convertase, C3bBb. This complex is stabilized by properdin.

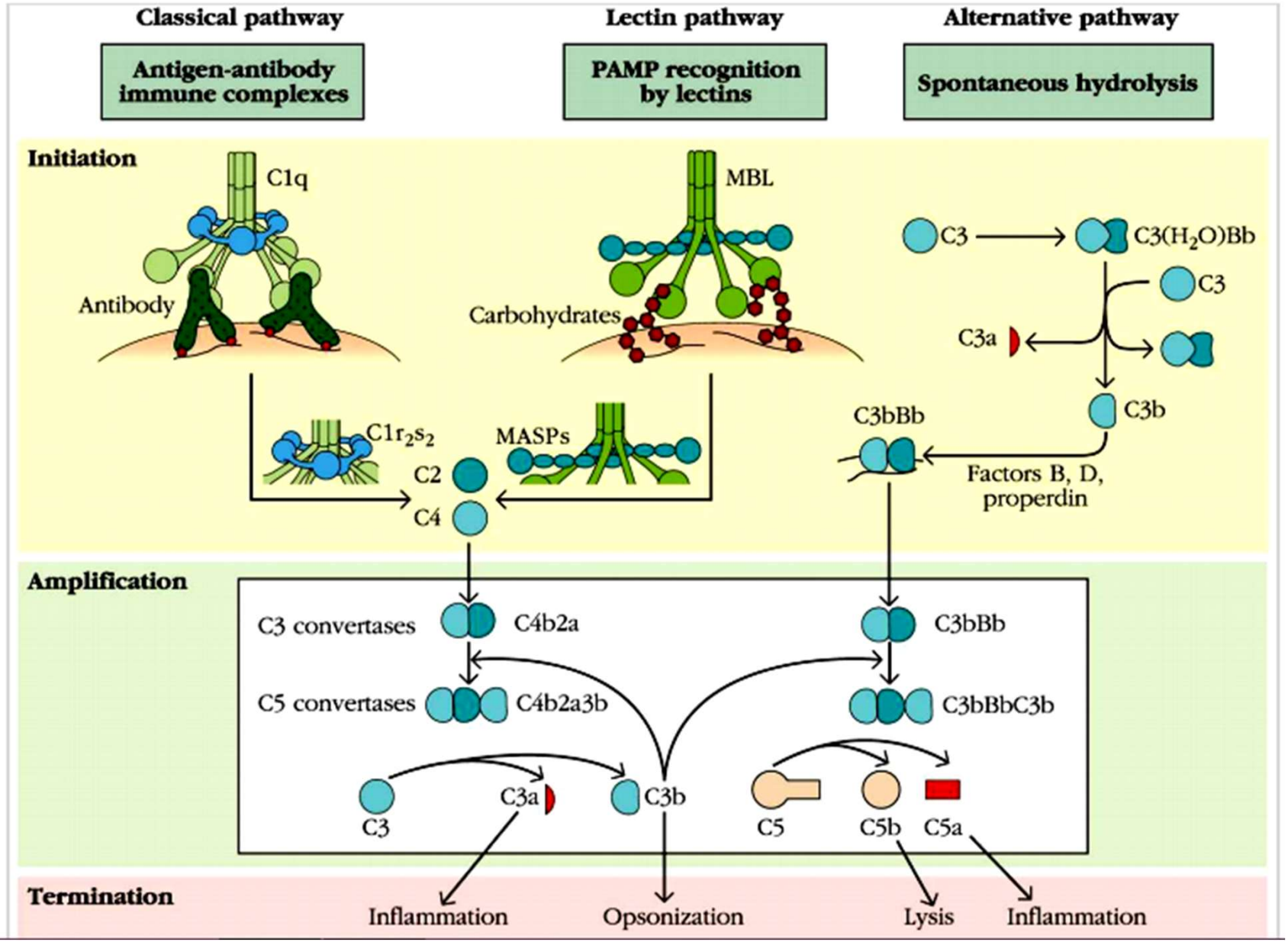
The alternative pathway may also be activated by the initial binding of properdin to a bacterial surface.

Generation of the anaphylatoxin C5a can also be effected by thrombin cleavage of C5, linking the coagulation and complement cascades.

The end result of the initiating sequence of the alternative pathway is the generation of enzymes that cleave C3 into C3a and C3b, and C5 into C5a and C5b.


Alternative Pathway
C3
Spontaneous
C3(H2O) + fB
fD
Initiation
C3
C3(H2O)•Bb
Ampliphication
C3a + C3b
S–C=O
C3b
HS C=O
O
Biomaterial
fB, fD
C3
C3b
S–C=O
C3b•Bb
Biomaterial

Three major pathways of complement activation.



The Three Complement Pathways Converge at the Formation of C5 Convertase and Generation of the membrane attack complex) MAC

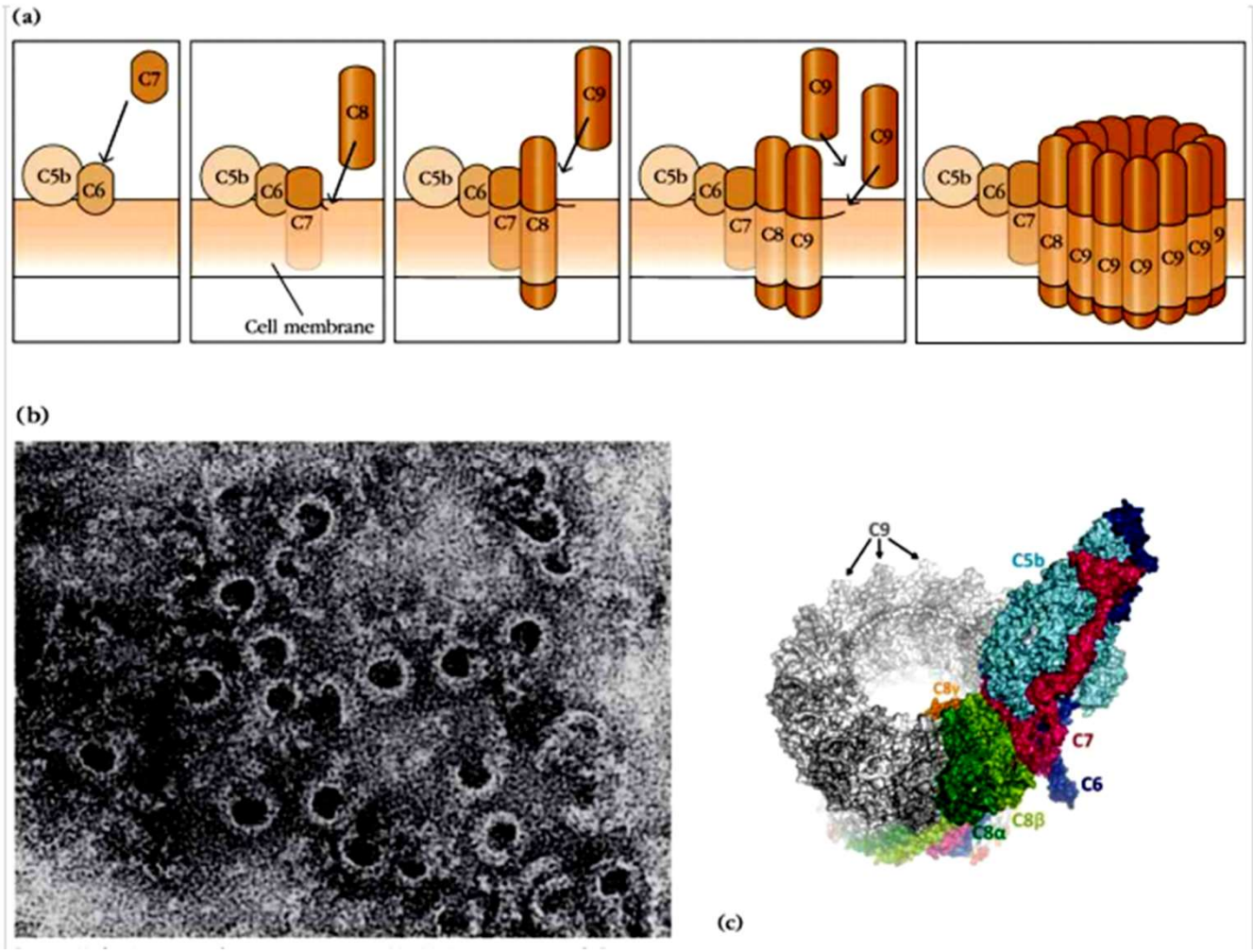
All three initiation pathways culminate in the formation of C5 convertase. For the classical and lectin pathways, C5 convertase has the composition C4b2a3b; for the alternative pathway, C5 convertase has the formulation C3bBbC3b. However, the end result of all types of C5 convertase activity is the same: cleavage of the C5 molecule into two fragments, C5a and C5b.

The large C5b fragment is generated on the surface of the target cell or immune complex and provides a binding site for the subsequent components of the membrane attack complex (MAC). However, the C5b component is extremely labile and is not covalently bound to the membrane, as are C3b and C4b. Therefore, it is rapidly inactivated unless it is stabilized by the binding of C6.

When C5b binds to the serum protein C6, the resulting complex interacts reversibly with the cell membrane via both ionic and hydrophobic bonds. Binding of C7 to C5bC6 induces a conformational change in C7 that exposes hydrophobic regions on its surface capable of inserting into the interior of the microbial membrane .

The insertion of C7 into the cell membrane is the triggering event for the formation of the membrane attack complex, which will ultimately cause cell death. If, however, C6 and C7 binding occurs on an antigen-antibody (immune) complex or other noncellular surface, then the hydrophobic binding sites will be unable to anchor the complex and it is released. Sometimes these complexes bind to C8 or even to C9 before being released.

Released membrane attack complexes can potentially insert into the membrane of nearby cells and mediate “innocent bystander” lysis. However, under physiologic conditions, such lysis is usually minimized by regulatory proteins; the “orphan” complexes are bound by the regulatory protein S (also called vitronectin) and then destroyed.



Formation of the membrane attack complex (MAC).

(a) Formation of the MAC, showing the addition of C6, C7, C8, and C9 components to the C5b component.

(b) Photomicrograph of poly-C9 complex formed by in vitro polymerization of C9 and complement-induced lesions on the membrane of a red blood cell. These lesions result from formation of membrane attack complexes.

(c) Relative locations of the members of the membrane attack complex: C5b, C6, C7, C8, and C9

C8 is made up of two peptide chains: C8β and C8αγ. Binding of C8β to the C5b67 complex induces a conformational change in the C8αγ dimer such that the hydrophobic domain of C8αγ can insert into the interior of the phospholipid membrane.

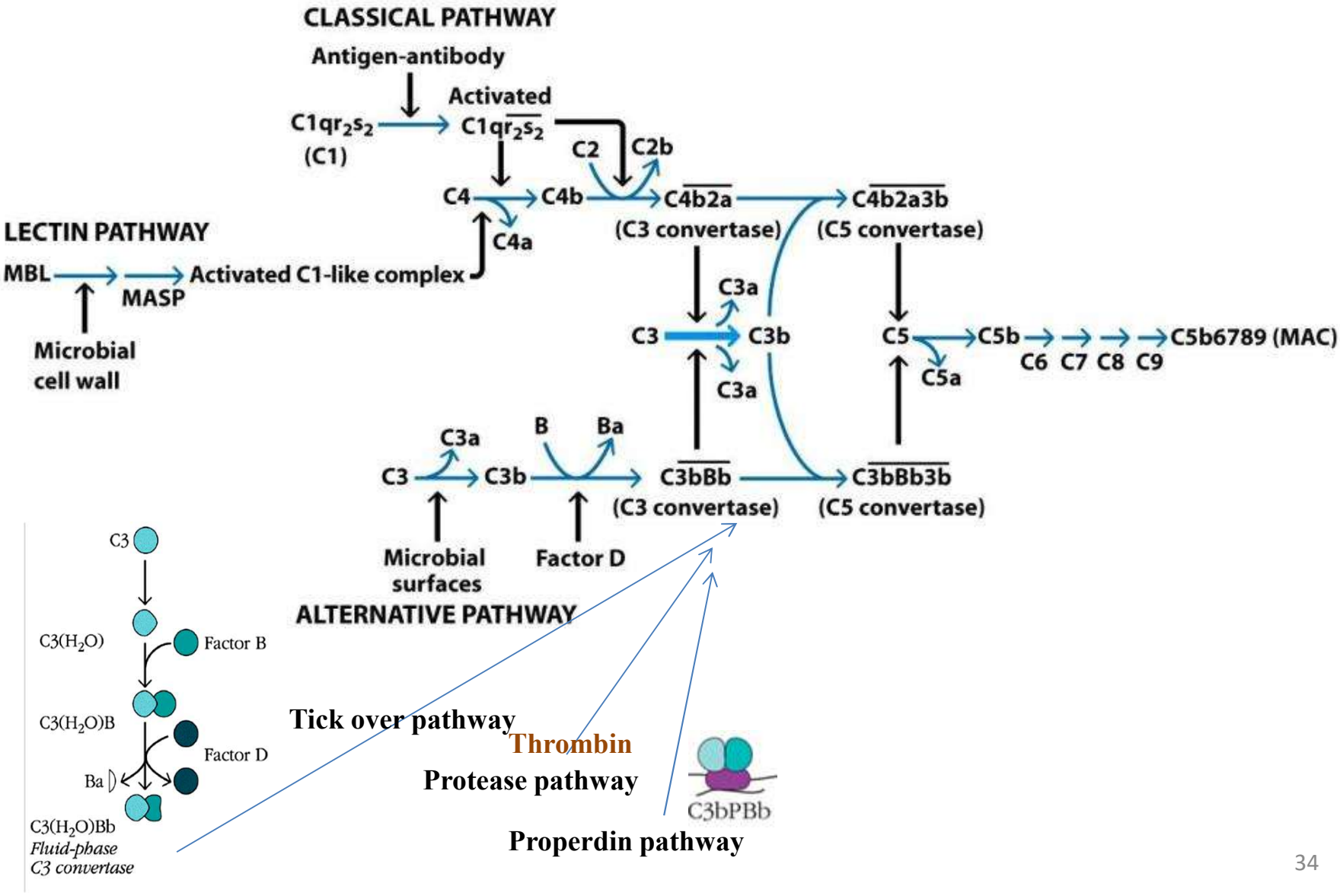
The C5b678 complex is capable of creating a small membrane pore, **10 Å in diameter**. The final step in the formation of the MAC is the binding and polymerization of C9 to the C5b678 complex. As many as 10 to 19 molecules of C9 can be bound and polymerized by a single C5b678 complex.

During polymerization, the C9 molecules undergo a conformational transition, so that they, too, can insert into the membrane.

The completed MAC, which has a tubular form and functional pore diameter of **70 to 100 Å**, consists of a C5b678 complex surrounded by a poly-C9 complex .

Loss of plasma membrane integrity leads irreversibly to cell death.

Complement activation pathways



Initiating and amplifying proteins of the classical and lectin-mediated complement pathways

Molecule	Biologically active fragments	Biological function	Active in which pathway
IgM, IgG		Binding to pathogen surface and initiating complement cascade	Classical pathway
Mannose-binding lectin (MBL), or ficolins		Binding to carbohydrates on microbial surface and initiating complement cascade	Lectin pathway
C1	C1q	Initiation of the classical pathway by binding Ig	Classical pathway
		Binding to apoptotic blebs and initiating phagocytosis of apoptotic cells	
	$(C1r)_2$	Serine protease, cleaving C1r and C1s	
	$(C1s)_2$	Serine protease, cleaving C4 and C2	
MASP-1		MBL-associated serine protease 1. MASP-2 appears to be functionally the more relevant MASP protein	Lectin pathway
MASP-2		Serine protease. In complex with MBL/ficolin, cleaves C4 and C2	Lectin pathway
C2	$C2a^{-}$	Serine protease. With C4b, is a C3 convertase	Classical and lectin pathways

Cont.

Initiating and amplifying proteins of the classical and lectin-mediated complement pathways

	C2b*	Inactive in complement pathway. Cleavage of C2b by plasmin releases C2 kinin, a peptide that stimulates vasodilation	
C4	C4b	Binds microbial cell membrane via thioester bond. With C2a, is a C3 convertase	Classical and lectin pathways
	C4c, C4d	Proteolytic cleavage products generated by factor I	
C3	C3a	Anaphylatoxin. Mediates inflammatory signals via C3aR	Classical and lectin pathways
	C3b	Potent in opsonization, tagging immune complexes, pathogens, and apoptotic cells for phagocytosis	
	iC3b and C3f	Proteolytic fragments of C3b, generated by factor I iC3b binds receptors CR3, CR4, and CRIg; CR2 binds weakly	
	C3d and C3dg	Proteolytic fragments of iC3b generated by factor I and trypsin like proteases including plasmin, thrombin etc. C3d and C3dg bind to CR2, and when they are bound to both antigen and to CR2, this facilitates antigen binding to B cells	
	C3c	Proteolytic fragment of iC3b generated by factor I and trypsin-like proteases. C3c binds CRIg on fixed tissue macrophages	

*C2a in this text refers to the larger, active fragment of C2

Initiating and amplifying proteins of the alternative complement pathways

Molecule	Biologically active fragments	Biological function
C3	C3a	Anaphylatoxin. Mediates inflammatory signals via C3aR
	C3b	Potent in opsonization, tagging immune complexes, pathogens, and apoptotic cells for phagocytosis With Bb, forms the C3 convertase With Bb and one more molecule of C3b (C3bBb3b), acts as a C5 convertase
	$C3(H_2O)$	C3 molecule in which the internal thioester bond has undergone hydrolysis With Bb, acts as a fluid-phase C3 convertase
	iC3b and C3f	Proteolytic fragments of C3b, generated by factor I iC3b binds receptors CR3, CR4, and CRIg; CR2 binds weakly
	C3d and C3dg	Proteolytic fragments of iC3b generated by factor I and trypsin-like proteases including plasmin, thrombin etc. C3d and C3dg both bind to CR2. When each is bound to both antigen and to CR2, this enhances the strength of antigen binding to B cells
	C3c	Proteolytic fragment of iC3b generated by factor I and trypsin-like proteases. C3c binds CRIg on fixed tissue macrophages

Cont.

Initiating and amplifying proteins of the alternative complement pathways

Factor B		Binds C3(H_2O) and is then cleaved by factor D into two fragments: Ba and Bb
	Ba	Smaller fragment of factor D–mediated cleavage of factor B May inhibit proliferation of activated B cells
	Bb	Larger fragment of factor D–mediated cleavage of factor B With C3(H_2O), acts as fluid-phase C3 convertase With C3b, acts as cell-bound C3 convertase With two molecules of C3b, acts as C5 convertase
Factor D		Proteolytic enzyme that cleaves factor B into Ba and Bb only when it is bound to either C3(H_2O) or to C3b
Properdin		Stabilizes the C3bBb complex on microbial cell surface

The proteins of the complement membrane attack complex (MAC)

Molecule	Biologically active fragments	Biological function
C5	C5a	Anaphylatoxin; binding to C5aR induces inflammation
	C5b	Component of membrane attack complex (MAC). Binds cell membrane and facilitates binding of other components of the MAC
C6		Component of MAC. Stabilizes C5b. In the absence of C6, C5b is rapidly degraded
C7		Component of MAC. Binds C5bC6 and induces conformational change allowing C7 to insert into interior of membrane
C8		Component of MAC. Binds C5bC6C7 and creates a small pore in membrane
C9		Component of MAC. Ten to 19 molecules of C9 bind C5bC6C7C8 and create large pore in membrane

The three main classes of complement activity in the service of host defense

Activity	Responsible complement component
Innate defense against infection	
Lysis of bacterial and cell membranes	Membrane attack complex (C5b-C9)
Opsonization	Covalently bound C3b, C4b
Induction of inflammation and chemotaxis by anaphylatoxins	C3a and C5a (anaphylatoxins) and their receptors on leukocytes
Interface between innate and adaptive immunity	
Augmentation of antibody responses	C3b and C4b and their proteolyzed fragments bound to immune complexes and antigen; C3 receptors on immune cells
Enhancement of immunologic memory	C3b and C4b and their fragments bound to antigen and immune complexes; receptors for complement components on follicular dendritic cells

Cont.

The three main classes of complement activity in the service of host defense

Enhancement of antigen presentation	MBL, C1q, C3b, C4b, and C5a
Potential effects on T cells	C3, C3a, C3b, C5a
Complement in the contraction phase of the immune response	
Clearance of immune complexes from tissues	C1, C2, C4; covalently bound fragments of C3 and C4
Clearance of apoptotic cells	C1q; covalently bound fragments of C3 and C4. Loss of CD46 triggers immune clearance
Induction of regulatory T cells	CD46

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Complement in the contraction phase of the immune response	
Clearance of immune	C1, C2, C4; covalently bound fragments of C3 and C4
Clearance of apoptotic cells	C1q; covalently bound fragments of C3 and C4. Loss of CD46 triggers immune clearance
Induction of regulatory T cells	CD46

Receptors that bind complement components and their breakdown products

Receptor	Other name(s)	Ligand	Cellular expression pattern	Function
CR1	CD35	C3b, C4b, C1q, iC3b	Erythrocytes, neutrophils, monocytes, macrophages, eosinophils, FDCs, B cells, some T cells	Clearance of immune complexes, enhancement of phagocytosis, regulation of C3 breakdown
CR2	CD21, Epstein-Barr virus receptor	C3d, C3dg C3d, iC3b	B cells, FDCs	Enhancement of B-cell activation, B-cell coreceptor, and retention of C3d-tagged immune complexes
CR3	CD11b/CD18, Mac-1	iC3b and factor H	Monocytes, macrophages, neutrophils, NK cells, eosinophils, FDCs, T cells	Binding to adhesion molecules on leukocytes, facilitates extravasation; iC3b binding enhances opsonization of immune complexes
CR4	CD11c/CD18	iC3b	Monocytes, macrophages, neutrophils, dendritic cells, NK cells, T cells	iC3b-mediated phagocytosis
CRIg	VSIG4	C3b, iC3b, C3c	Fixed tissue macrophages	iC3b-mediated phagocytosis and inhibition of alternative pathway
C1qRp	CD93	C1q, MBL	Monocytes, neutrophils, endothelial cells, platelets,	Induces T-cell activation; enhances phagocytosis

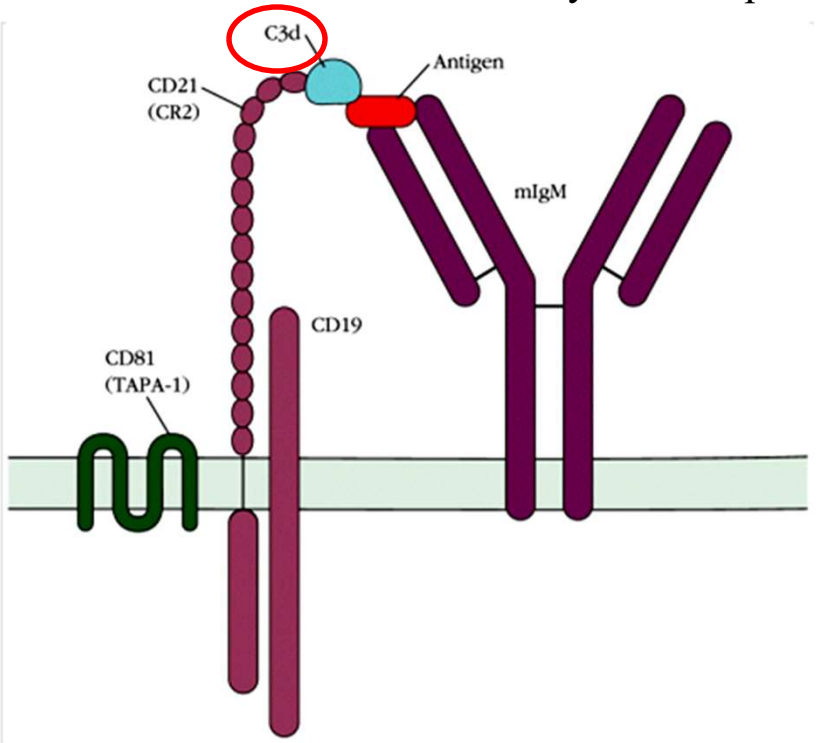
Cont

Receptors that bind complement components and their breakdown products

SIGN-R1	CD209	C1q	Marginal zone of spleen, lymph node macrophages	Enhances opsonization of bacteria by MZ macrophages
C3aR	(None)	C3a	Mast cells, basophils, granulocytes	Induces degranulation
C5aR	CD88	C5a	Mast cells, basophils, granulocytes, monocytes, macrophages, platelets, endothelial cells, T cells	Induces degranulation; chemoattraction; acts with IL-1β and/or TNF-α to induce acute-phase response; induces respiratory burst in neutrophils
C5L2	(None)	C5a	Mast cells, basophils, immature dendritic cells	Uncertain, but most probably down-regulates proinflammatory effects of C5a

The breakdown products of C3b—iC3b, C3d, and C3dg—are each bound specifically by the complement receptor CD21 (CR2), which is expressed on B cells in noncovalent association with the B-cell receptor.

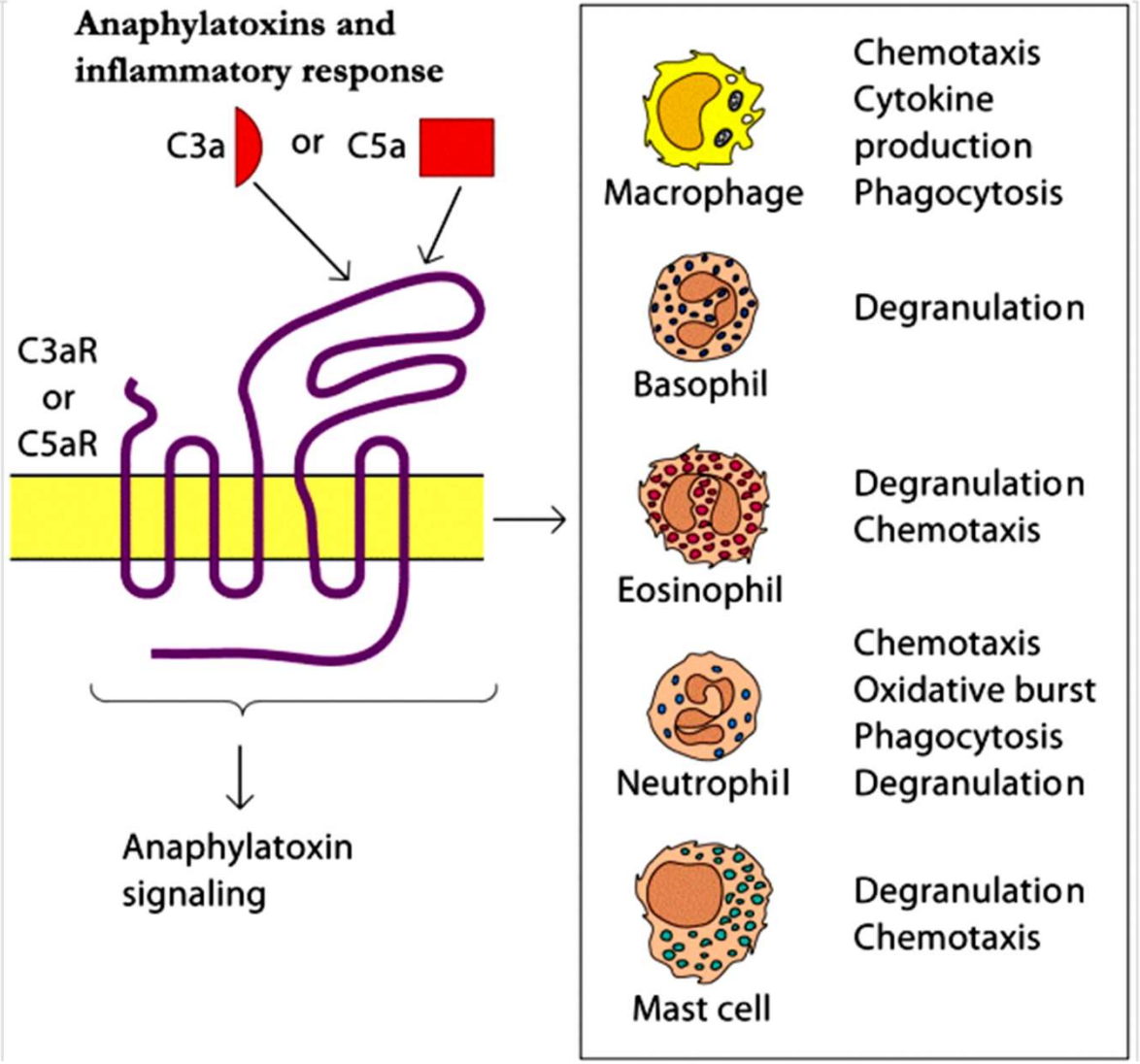
Since C3b can form covalent bonds with antigens, and these bonds are not affected by breakdown of C3b to iC3b, C3d, and C3dg, the close association of CD21 with the B-cell receptor enables the B cell to simultaneously bind antigen via both the B-cell receptor and CD21
.
This has the effect of reducing the antigen concentration necessary for B-cell activation by approximately 100-fold. Deficiencies in CD21 have been identified in patients suffering from autoimmune diseases such as systemic lupus erythematosus.



Coligation of antigen to B cells via the B-cell receptor and the B-cell coreceptor.

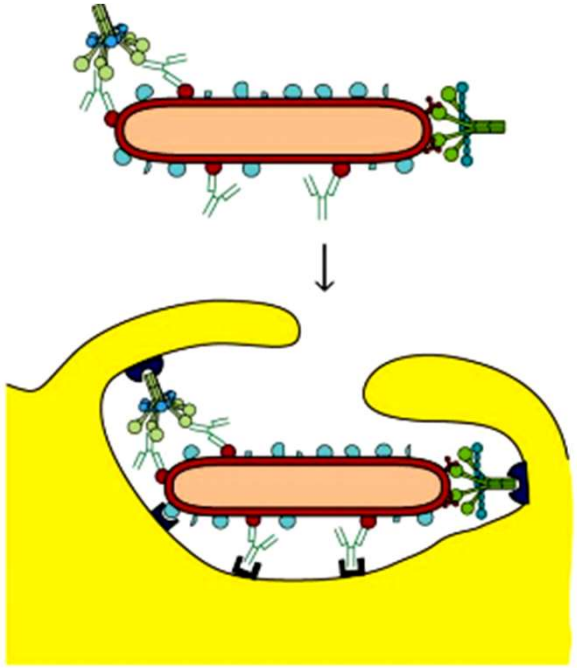
The B-cell coreceptor is a complex of three cell membrane molecules: CD21 (CR2), CD81 (TAPA-1), and CD19. Antigen that has been covalently bound to fragments of the C3 complement component is bound by both the immunoglobulin BCR and the CD21 complement receptor, thus significantly increasing the avidity of the cell receptors for the antigen and allowing lower concentrations of antigen to trigger B-cell activation. The CD19 component is important in B-cell signaling by antigen.

Release of proinflammatory mediators



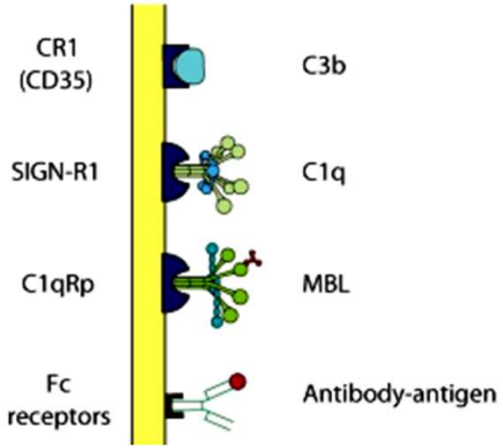
Binding of the anaphylatoxins C3a and C5a to the G protein–coupled receptors C3aR and C5aR. C3aR and C5aR are G protein–coupled receptors. Binding of the anaphylatoxins to these receptors stimulates the release of proinflammatory mediators from macrophages, neutrophils, basophils, eosinophils, and mast cells.

Opsonization of microbial cells by complement components and antibodies.



Opsonization with antibody and complement also provides critical protection against viral infection. Antibody and complement can create a thick protein coat around a virus that neutralizes viral infectivity by preventing the virus from binding to receptors on the host cell.

They then promote phagocytosis by activated macrophages via the Fc and complement receptors, followed by intracellular destruction of the digested particle.

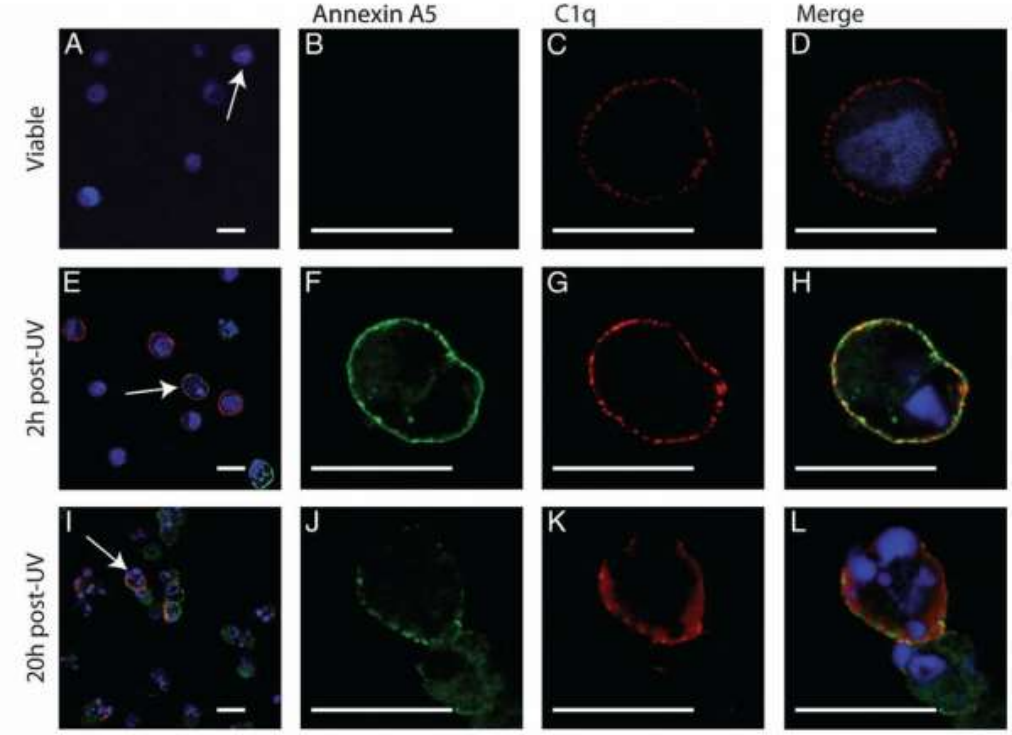


Phagocytosis is mediated by many different complement receptors on the surface of macrophages and neutrophils, including CR1, SIGN-R1, and C1qRp.

Phagocytes, using their Fc receptors, also bind to antigens opsonized by antibody binding.

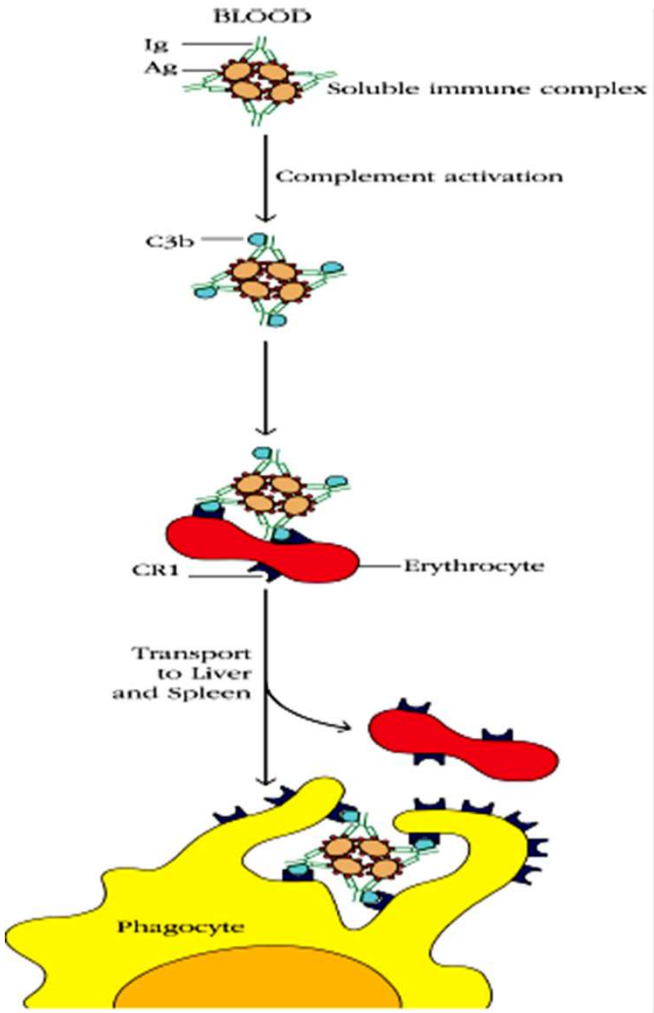
Disposal of Apoptotic Cells and Bodies

Apoptotic cells express the phospholipid phosphatidylserine on the exterior surface of their plasma membranes. In healthy cells, this phospholipid is normally restricted to the cytoplasmic side of the membrane, and the change in its location as the cell enters apoptosis serves to signal the immune system that the cell is dying. Exposed phosphatidylserine is then bound by the serum protein, annexin A5, which in turn is recognized by C1q. Nuclear fragmentation, DNA cleavage, and the expression of DNA on the cell surface are also hallmarks of apoptosis, and recent work has demonstrated that C1q binds specifically to DNA as well as to glycoproteins and phospholipids exposed on the surfaces of dying cells and apoptotic fragments . Once apoptosis begins, the dying cell is broken down into membrane-bound vesicles termed apoptotic bodies, which also express phosphatidylserine and/or DNA on their exterior membrane surfaces



C1q colocalizes with annexin A5 on the surface of apoptotic cells. This figure demonstrates that both C1q and annexin A5 are deposited on the surface of human HeLa cells treated with lethal doses of ultraviolet light. The three pictures on the left show the locations of cells, stained purple to highlight nuclear material; some of these cells can also be seen in the merged images on the far right. The cells were also stained green for annexin A5 (which binds to exposed phosphatidylserine residues) and red for C1q before receiving lethal doses of ultraviolet light. The second and third rows show similar staining 2 hours and 24 hours following treatment, respectively. At 2 hours, C1q and annexin A5 binding are clearly visible. At 20 hours, nuclear fragmentation and nuclear blebbing, hallmarks of apoptotic cell death, can be seen. Scale bars represent 20 μm.

Disposal of Immune Complexes



The coating of soluble immune complexes with C3b facilitates their binding by CR1 on erythrocytes.

Although red blood cells express lower levels of CR1 (100–1000 molecules per cell, depending on the age of the cell and the genetic constitution of the host) than do granulocytes (5×10^4 per cell), there are about 1000 erythrocytes for every white blood cell, and therefore erythrocytes account for about 90% of the CR1 in blood.

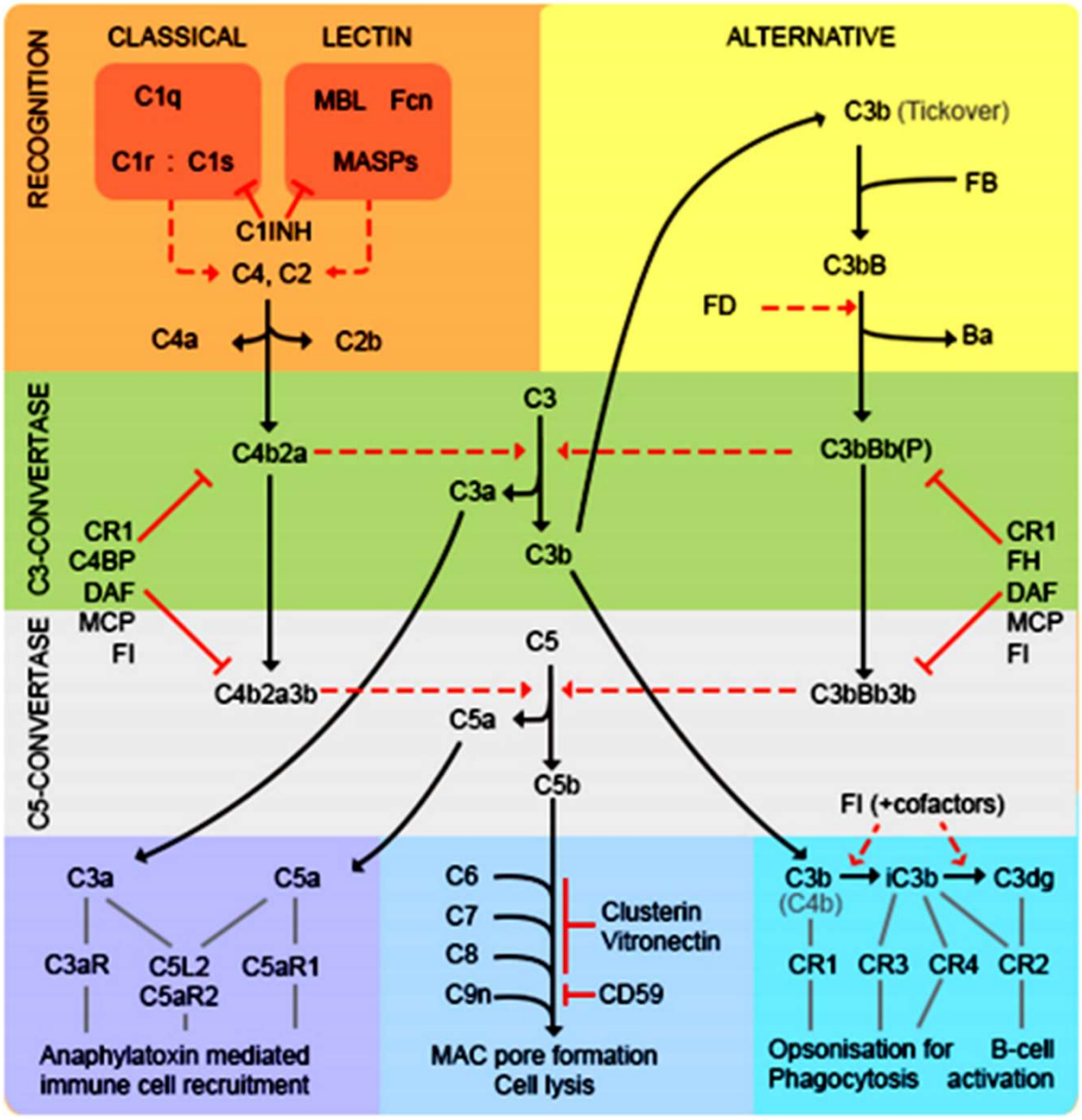
Erythrocytes also therefore play an important role in clearing C3b-coated immune complexes by conveying them to the liver and spleen, where the immune complexes are stripped from the red blood cells and phagocytosed

Clearance of circulating immune complexes by binding to erythrocyte complement receptors and subsequent stripping from these receptors by macrophage complement receptors in the liver and spleen. Because erythrocytes have fewer CR1 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.

Proteins involved in the regulation of complement activity

Protein	Soluble or membrane bound	Pathway(s) affected	Function
C1 inhibitor (C1INH)	Soluble	Classical and lectin	Induces dissociation and inhibition of $C1r_2s_2$ from C1q; serine protease inhibitor
Decay-accelerating factor (DAF; CD55)	Membrane bound	Classical, alternative, and lectin	Accelerates dissociation of C4b2a and C3bBb C3 convertases
CR1 (CD35)	Membrane bound	Classical, alternative, and lectin	Blocks formation of, or accelerates dissociation of, the C3 convertases C4b2a and C3bBb by binding C4b or C3b Cofactor for factor I in C3b and C4b degradation on host cell surface
C4BP	Soluble	Classical and lectin	Blocks formation of, or accelerates dissociation of, C4b2a C3 convertase Cofactor for factor I in C4b degradation
Factor H	Soluble	Alternative All pathways	Blocks formation of, or accelerates dissociation of, C3bBb C3 convertase Cofactor for factor I in C3b degradation
Factor I	Soluble	Classical, alternative, and lectin	Serine protease: cleaves C4b and C3b using cofactors shown in
Membrane cofactor of proteolysis, MCP (CD46)	Membrane bound	Classical, alternative, and lectin	Cofactor for factor I in degradation of C3b and C4b
S protein (vitronectin)	Soluble	All pathways	Binds soluble C5b67 and prevents insertion into host cell membrane
CD59 (protectin)	Membrane bound	All pathways	Binds C5b678 on host cells, blocking binding of C9 and the formation of the MAC
Carboxypeptidases N, B, and R	Soluble	Anaphylatoxins produced by all pathways	Cleave and inactivate the anaphylatoxins C3a and C5a

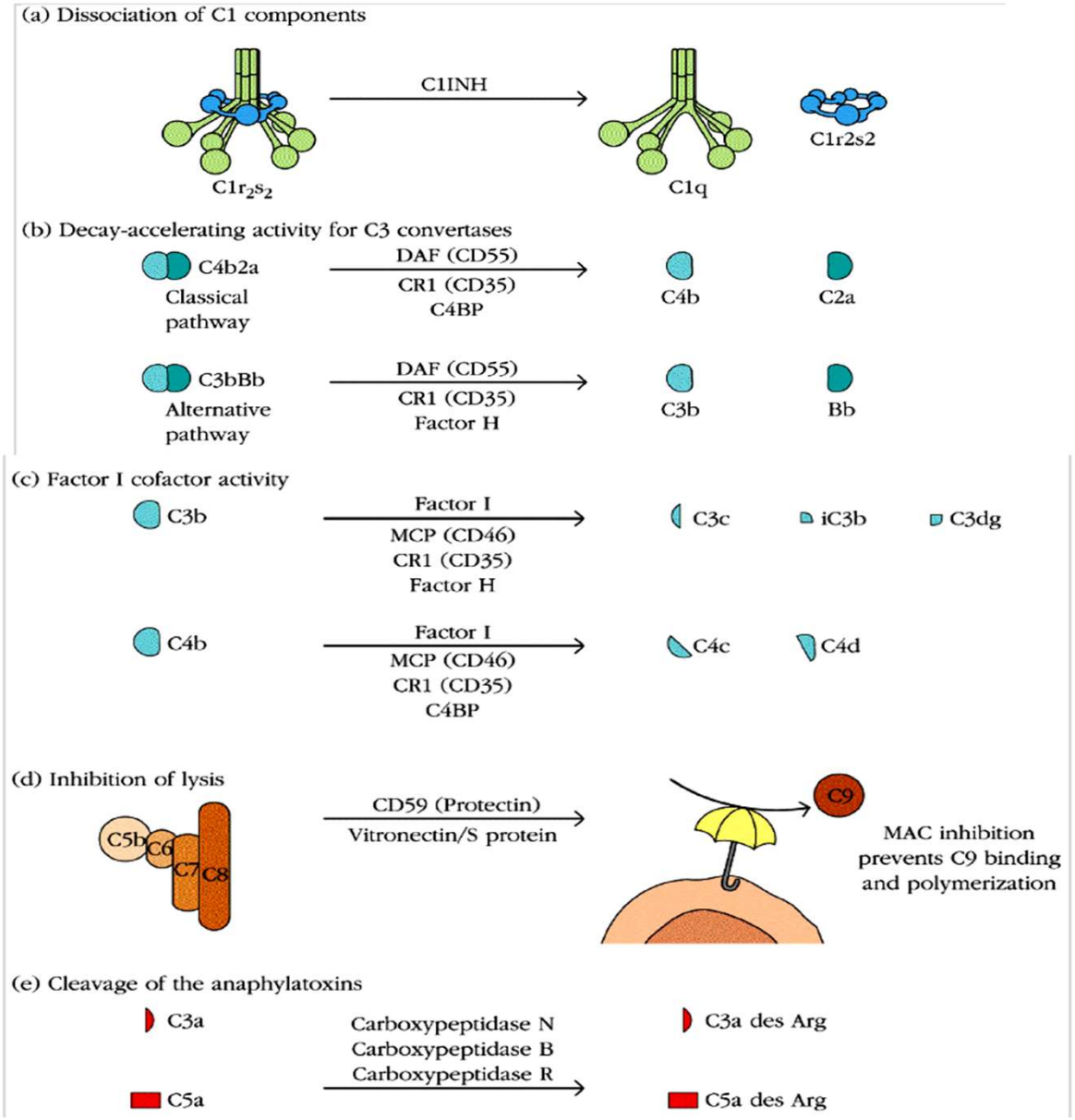


Complement Regulators

C1-inhibitor (C1INH) functions as a regulator to prevent excessive activation of both classical and lectin pathways

Other regulators
Complement receptor 1 (CR1),
C4 binding protein (C4BP),
decay accelerating factor (DAF),
membrane cofactor protein (MCP),
and factor I (FI)
factor H (FH)

The Regulation of Complement Activity

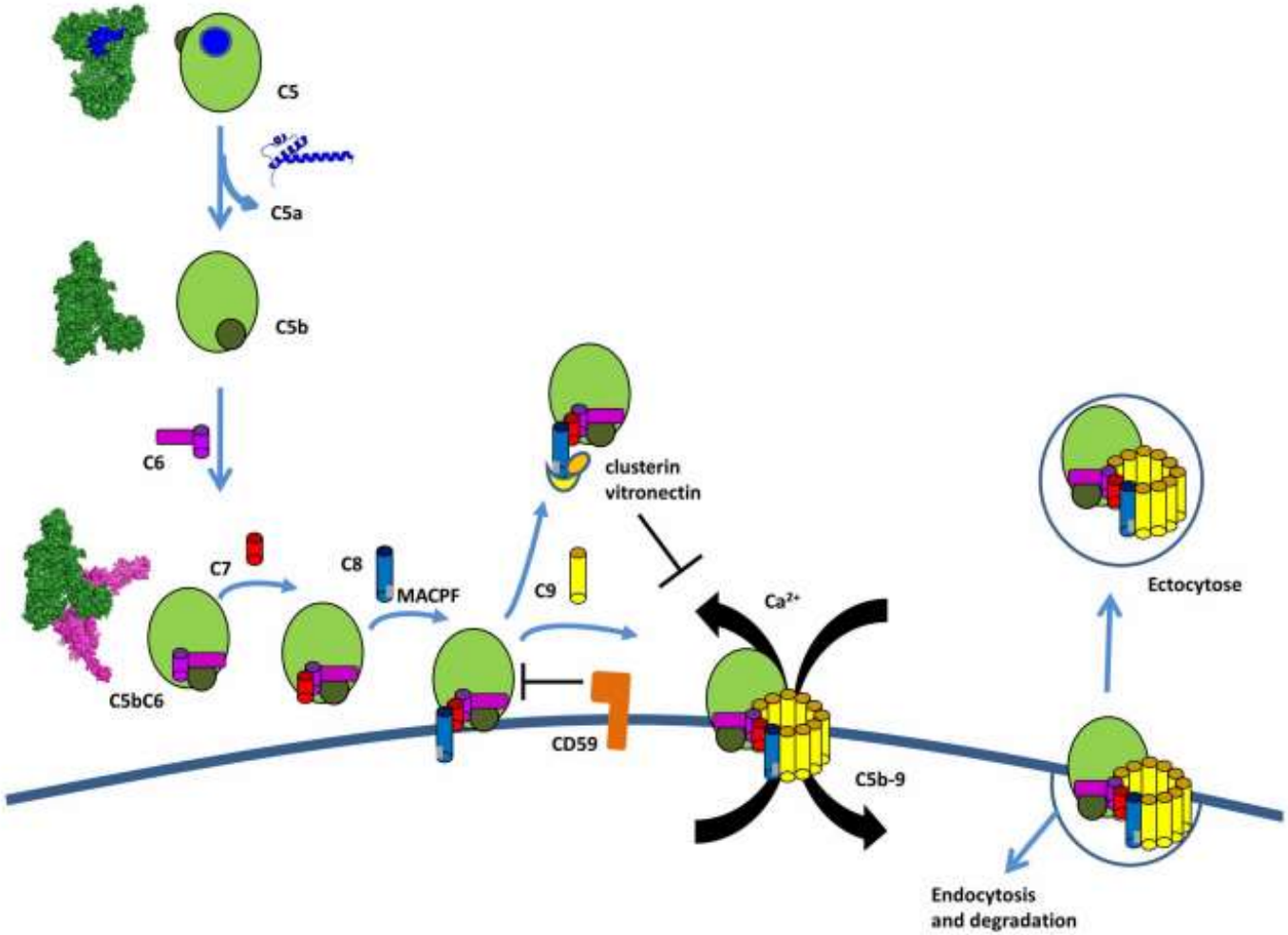


All biological systems with the potential to damage the host are subject to rigorous regulatory mechanisms.

Such a mechanism is essential to destroy the appropriate pathogen but not the healthy tissue.

By this activity damage to healthy host tissues is minimized.

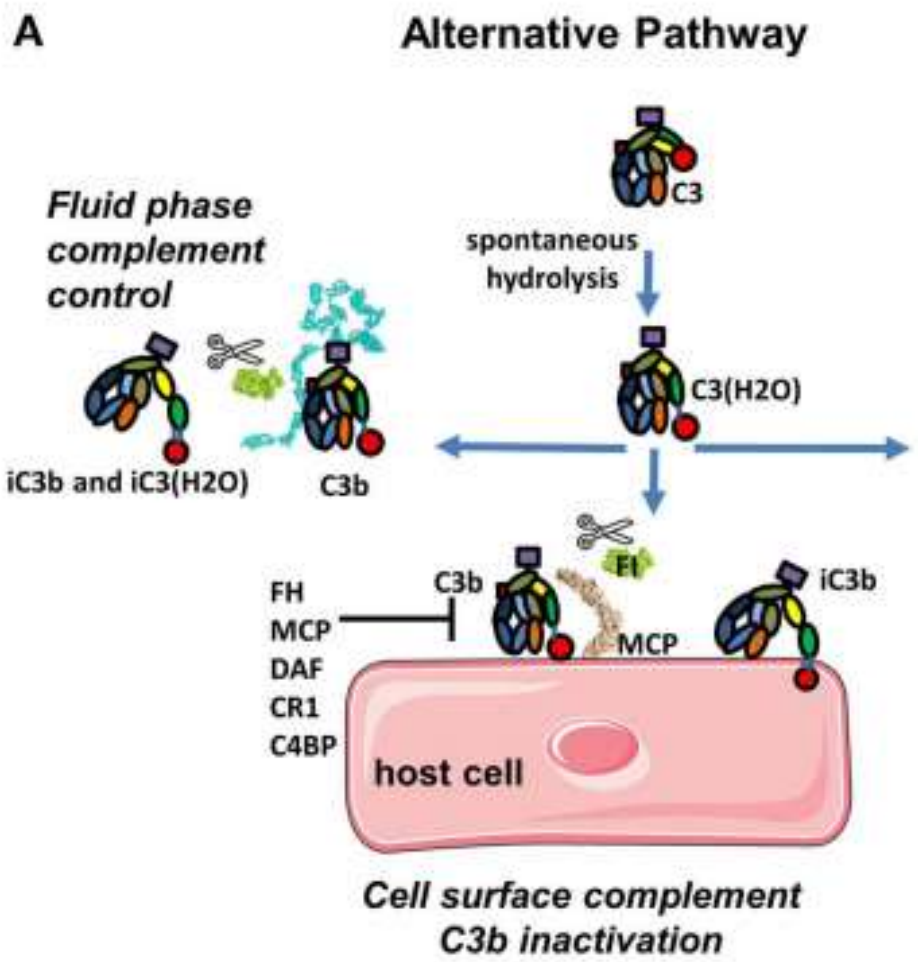
The various stages at which complement activity is subject to regulation is shown in the figure



The terminal complement pathway. The C5 convertase cleaves an inert molecule of C5 into a potent anaphylatoxin, C5a, and a bioactive fragment C5b. C5b interacts with C6, C7, C8, and multiple copies of C9 to form the membrane attack complex C5b-9 (MAC). C5b-8 inserts into the membrane and C9 polymerize to form a pore inducing Ca flux and pathogen lysis. Host cells are protected from lysis by expression of CD59, which prevents the insertion and by clusterin and vitronectin, which bind to C8 and prevents insertion in the membrane. If MAC is bound to the membrane, host cells can internalize it or remove it by ectocytose.

Complement is only fully activated in cases of **pathogen infection**. During an infection, complement leads to inflammation, opsonization, phagocytosis, and destruction of the pathogen and ultimately results in activation of the adaptive immune response.

In a healthy individual, the Alternate Pathway is permanently active at low levels to survey for presence of pathogens.
Healthy host cells are protected against complement attack and are resistant to persistent low-grade activation.
The three pathways are activated on the surface of apoptotic cells, which are constantly generated within the body during normal cellular homeostasis

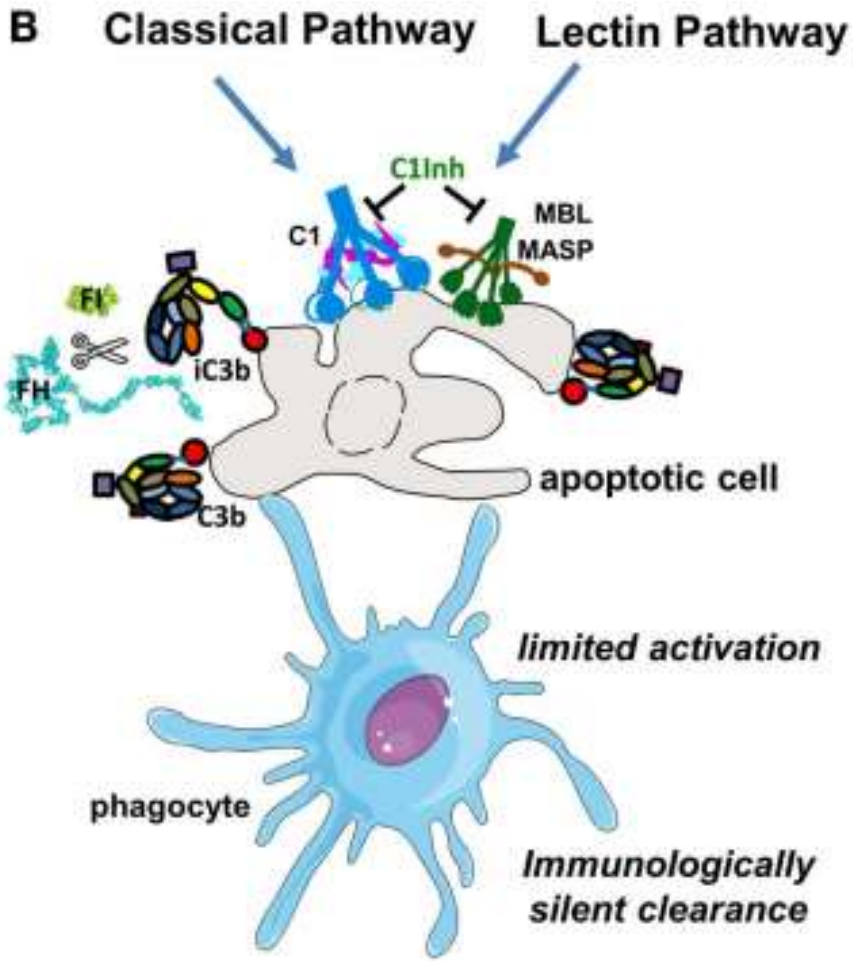


In the plasma, during normal physiological conditions, the dominant active complement pathway is the Alternative pathway

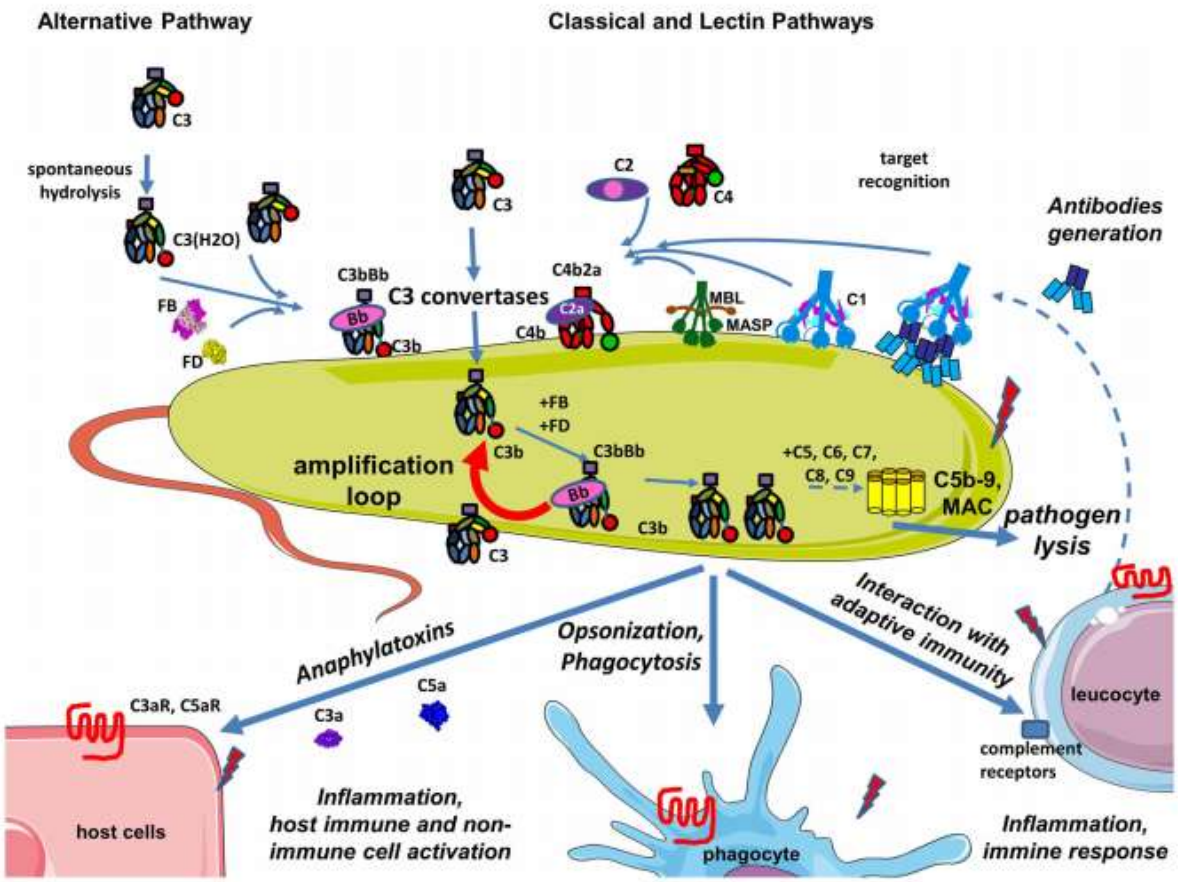
The rate of hydrolysis of C3 to C3(H2 O) can be **accelerated** by interactions between C3 and a number of biological and artificial interfaces,

including gas bubbles,
biomaterial surfaces, and
lipid surfaces and complexes

Complement activation in physiological conditions.
(A) The alternative pathway is permanently active due to spontaneous transformation of bio-inactive molecule C3 to bioactive C3(H_2 O). This allows generation of C3b, which is rapidly inactivated by FH and FI in fluid phase or is covalently bound to the surface and then inactivated on host cells.



(B) Classical and lectin pathway recognition molecules bind to apoptotic cells and together with C3b from the alternative pathway induce a low level of complement activation. Apoptotic cells are not lysed, but rapidly cleared by phagocytes in an immunologically silent manner. Host cells and plasma contain multiple regulatory proteins to assure that complement activation will be limited in physiological conditions



Complement during infection with a pathogen.

The permanent activity of the alternative pathway allows it to immediately identify pathogens that are not specifically protected against complement. Danger-associated molecular patterns on its surface of pathogens are recognized by C1q, MBL, and ficolins allowing classical and lectin pathway activation, C3 convertase, C4b2a generation, and C3 cleavage. Opsonization due to covalent binding of C3b to the target is accelerated by the amplification loop of the complement pathways. The effector functions resulting from this complement activation are: pathogen lysis by C5b-9 membrane attack complex, opsonization and phagocytosis of the pathogen, activation of host immune and non-immune cells by complement anaphylatoxins, inflammation, stimulation of an adaptive immune response, and antibody generation. Secreted antibodies will bind to the pathogen and create immune complexes that will be recognized by C1q and will activate the classical pathway. Altogether these mechanisms contribute to pathogen elimination