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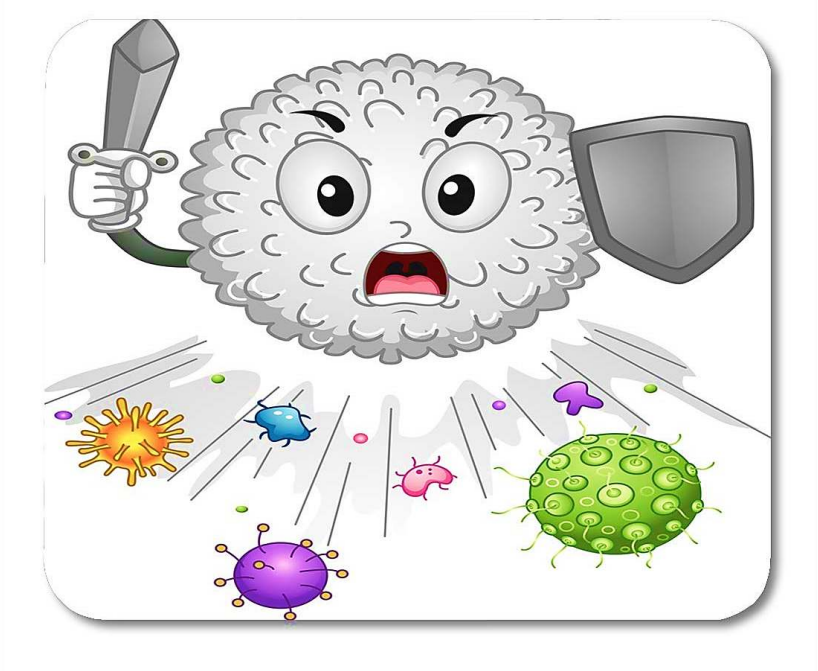
Complement system:
Immune effector mechanism



What is complement system???

- **Humoral branch** of the immune system.
- Complement includes **more than 30 soluble and cell-bound proteins.**
- After initial activation, the various complement components interact, in a highly regulated cascade, to carry out a number of **basic functions including:**

❖ **Lysis** of cells, bacteria, and viruses.

❖ **Opsonization,** which promotes phagocytosis of particulate antigens.

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

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

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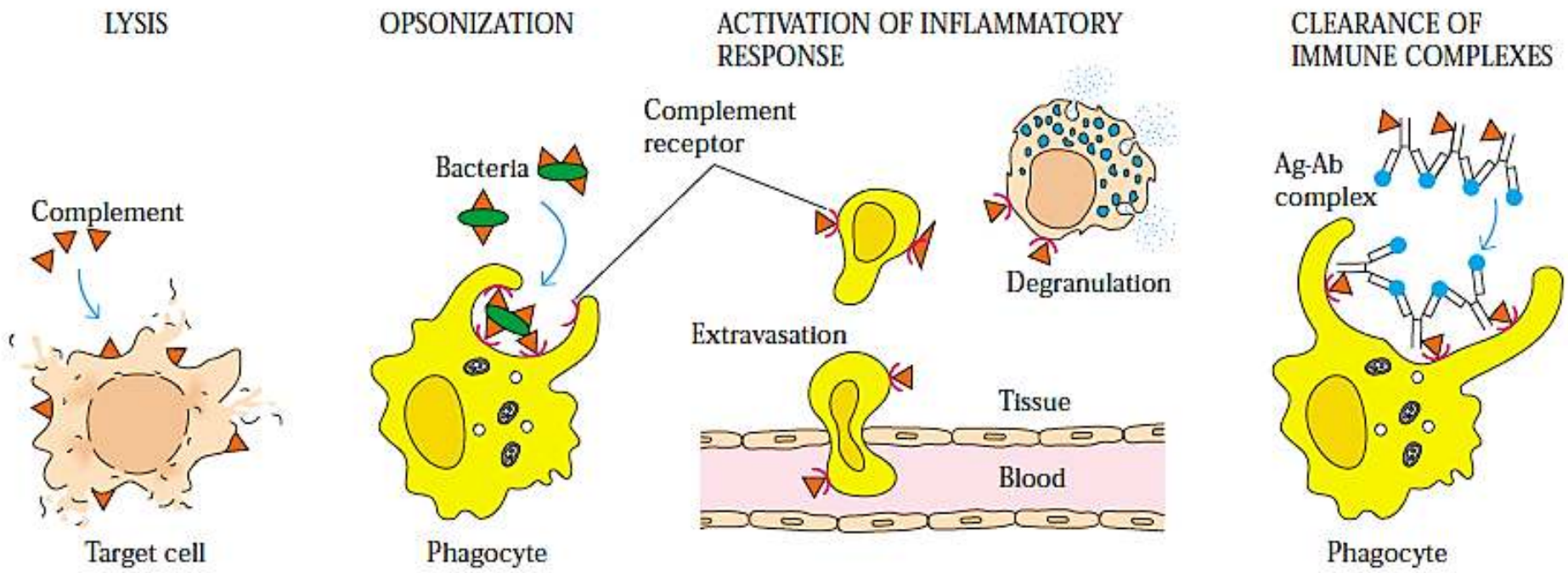
Basic Functions



The complement components

- The proteins and glycoproteins that compose the complement system 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.**
- These components constitute **5% (by weight) of the serum globulin fraction.** 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), or by trivial names (e.g., homologous restriction factor).**
- 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., C4b2a, C3bBb).**

Types of Complement pathways

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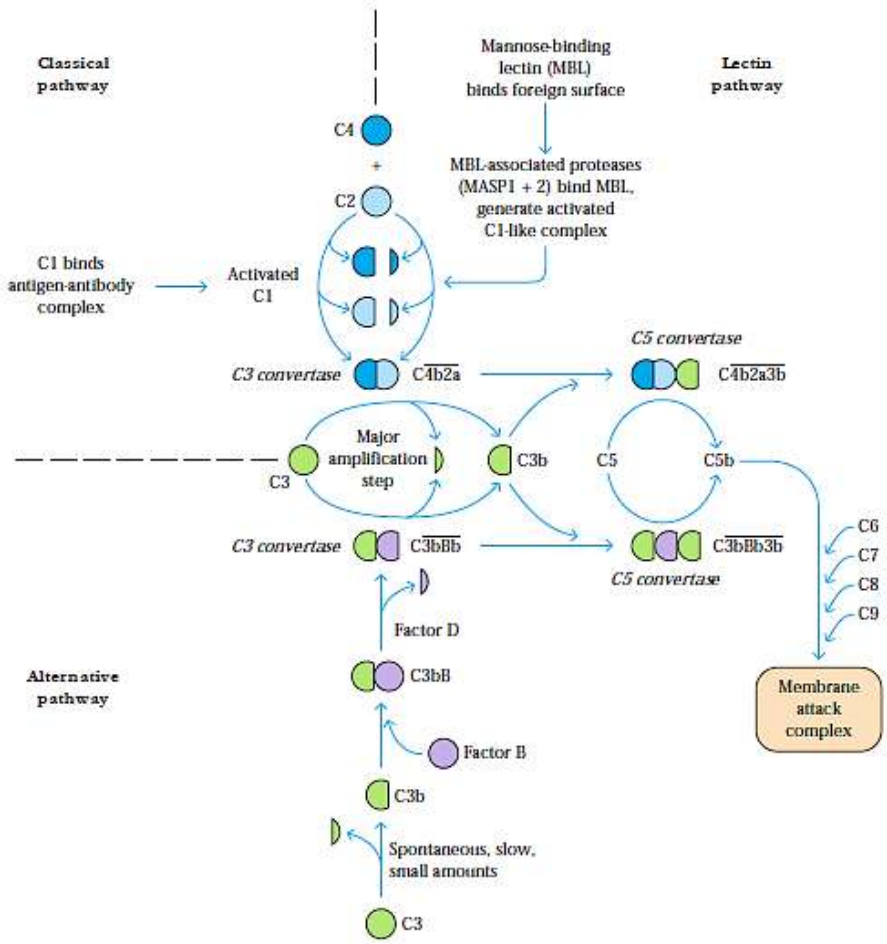
Classical pathway and Alternative pathway and Lectin pathway



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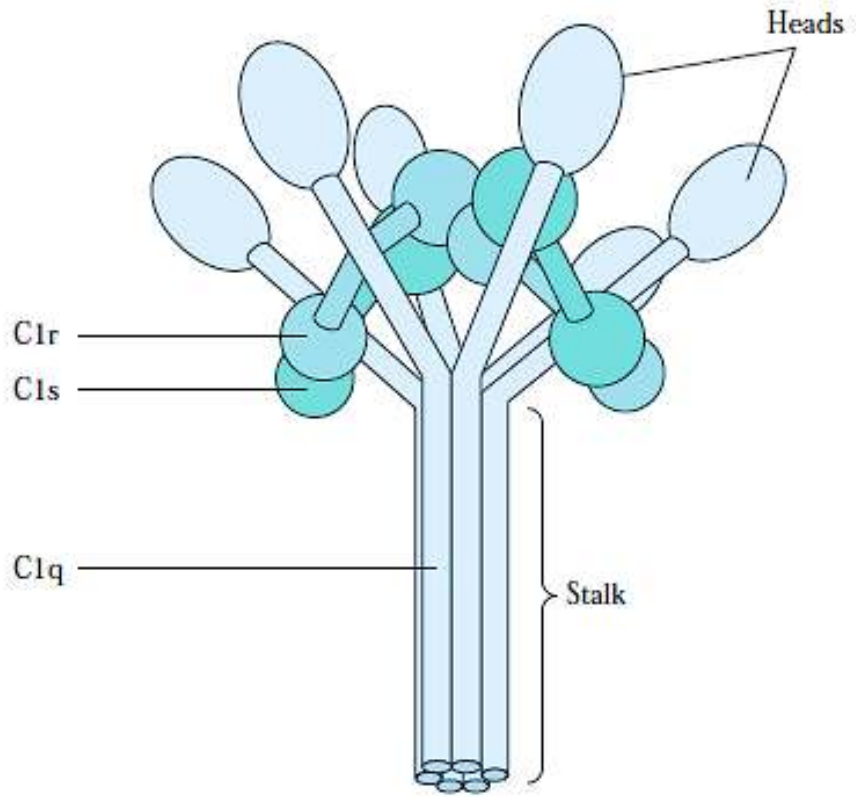
Classical pathway

- 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** of activation involves **C1, C2, C3, and C4.**
- 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.
- **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 $Ca2^{+}$ 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.**
- When **pentameric IgM** 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.**
- An **IgG molecule**, on the other hand, contains **only a single C1q-binding site** in the CH2 domain of the Fc, so that firm C1q binding is achieved only when **two IgG molecules are within 30–40 nm of each other** on a target surface or in a complex providing two attachment sites for C1q.

A single molecule of IgM bound to a red blood cell can activate the classical complement pathway and lyse the red blood cell while some 1000 molecules of IgG are required to assure that two IgG molecules are close enough to each other on the cell surface to initiate C1q binding.

Structure of C1 protein



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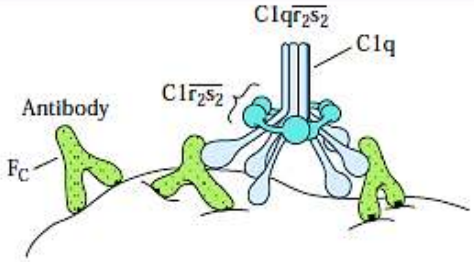
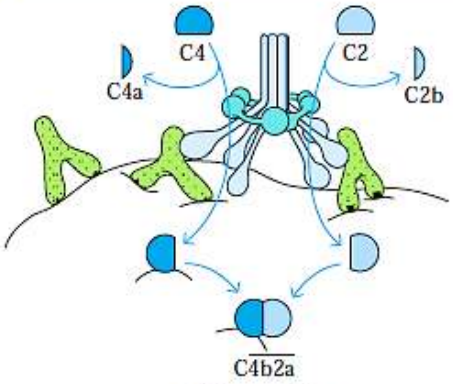
Steps of Classical Pathway

① C1q binds antigen-bound antibody. C1r activates auto-catalytically and activates the second C1r; both activate C1s

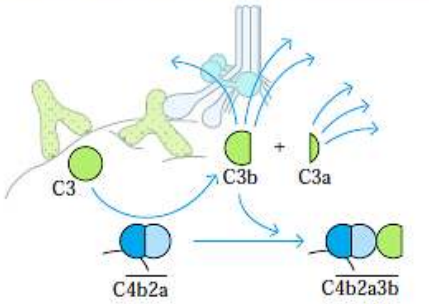


② C1s cleaves C4 and C2. Cleaving C4 exposes the binding site for C2. C4 binds the surface near C1 and C2 binds C4, forming C3 convertase



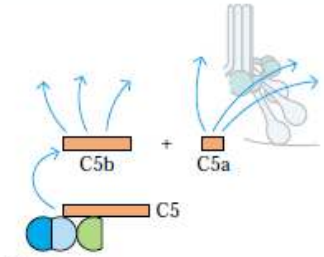
C3 convertase

③ C3 convertase hydrolyzes many C3 molecules. Some combine with C3 convertase to form C5 convertase



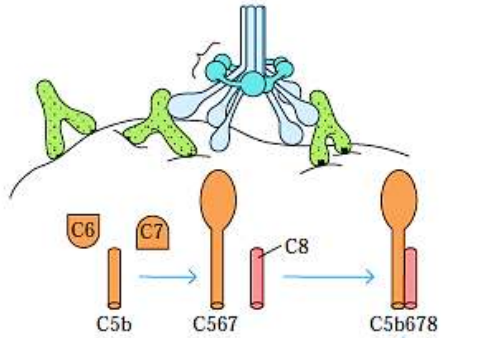
C5 convertase

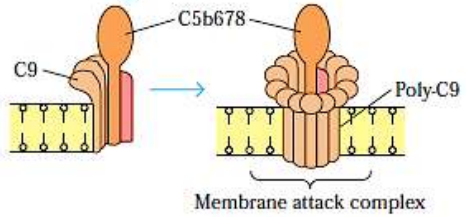
④ The C3b component of C5 convertase binds C5, permitting $\overline{C4b2a}$ to cleave C5



C5 convertase

⑤ C5b binds C6, initiating the formation of the membrane-attack complex





Membrane attack complex

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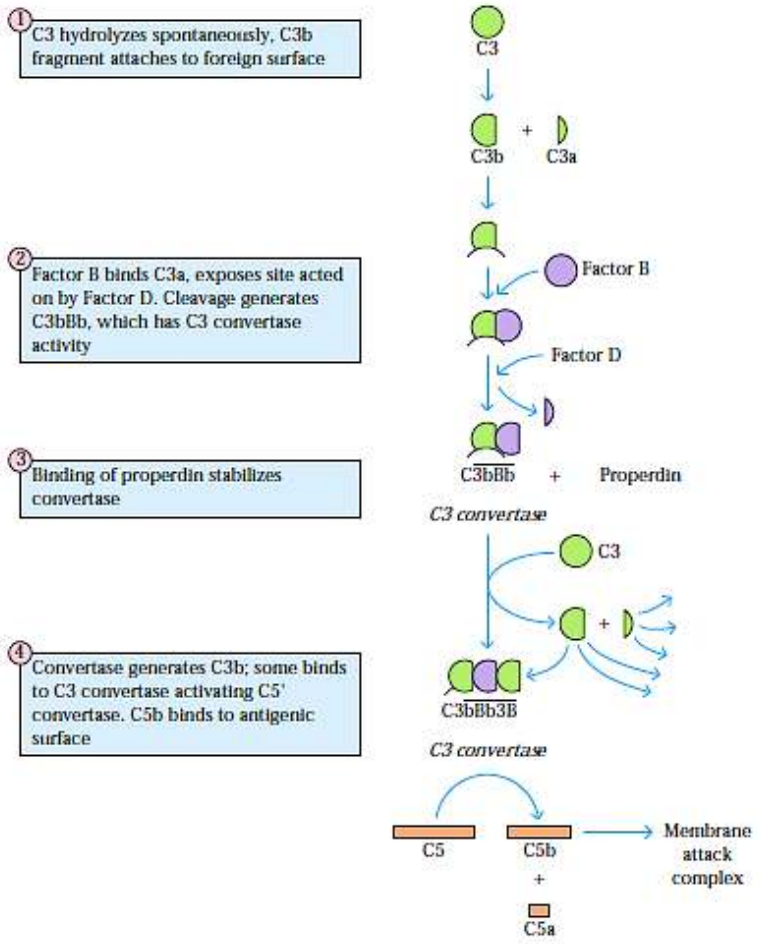
The Alternative pathway: Antibody-independent pathway



The Alternative pathway

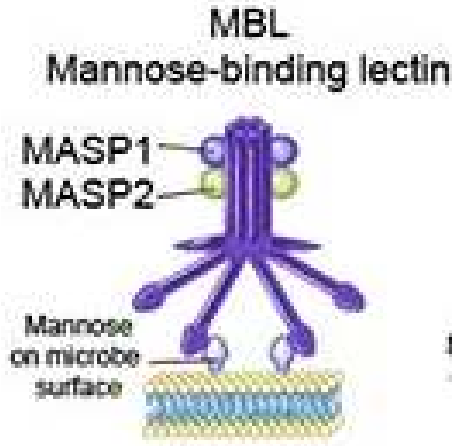
- Because no antibody is required, the alternative pathway is a component of the **innate immune system.**
- This major pathway of complement activation involves four serum proteins: **C3, factor B, factor D, and properdin.**
- The alternative pathway is initiated in most cases by **cell-surface constituents that are foreign to the host.**
- 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.**

Initiators of the alternative pathway of complement activation

PATHOGENS AND PARTICLES OF MICROBIAL ORIGIN
Many strains of gram-negative bacteria
Lipopolysaccharides from gram-negative bacteria
Many strains of gram-positive bacteria
Teichoic acid from gram-positive cell walls
Fungal and yeast cell walls (zymosan)
Some viruses and virus-infected cells
Some tumor cells (Raji)
Parasites (trypanosomes)
NONPATHOGENS
Human IgG, IgA, and IgE in complexes
Rabbit and guinea pig IgG in complexes
Cobra venom factor
Heterologous erythrocytes (rabbit, mouse, chicken)
Anionic polymers (dextran sulfate)
Pure carbohydrates (agarose, inulin)

Lectin 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.*
- After MBL binds to the surface of a cell or pathogen, **MBL-associated serine proteases,MASP-1 and MASP-2, bind to MBL.**
- The active complex formed by this association **causes cleavage and activation of C4 and C2.**
- The MASP-1 and -2 proteins have **structural similarity to C1r and C1s and mimic their activities.**
- This means of activating the C2–C4 components **to form a C5 convertase.**



The Three Complement Pathways Converge at the Membrane-Attack Complex (MAC)

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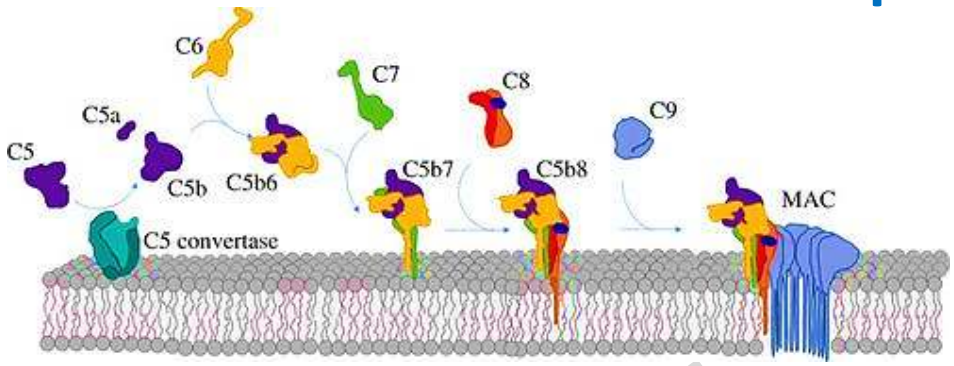

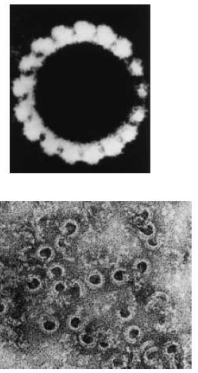

- A macromolecular structure forms a large channel through the membrane of the target cell, enabling ions and small molecules to diffuse freely across the membrane.
- Large C5b fragment, which binds to the surface of the target cell and provides a binding site for the subsequent components of the membrane-attack complex.
- The **C5b component is extremely labile and becomes inactive within 2 minutes unless C6 binds to it and stabilizes its activity.**
- 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.**
- 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, 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 blood cells but not of nucleated cells.
- Final step is polymerization of **C9, a perforin- like molecule (70–100 Å).**
- **Cell can not maintain osmotic stability and loss of electrolytes.**

Regulation of the Complement System

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A general mechanism of regulation in all complement pathways is the **inclusion of highly labile components that undergo spontaneous inactivation** if they are not stabilized by reaction with other components.

Examples

- A series of **regulatory proteins** can inactivate various complement components, the glycoprotein C1 inhibitor **(C1Inh)** can form a **complex with C1r2s2**, causing it to **dissociate from C1q** and preventing further activation of C4 or C2.
- The C3b generated by these enzymes has the potential to bind to nearby cells, mediating damage to the healthy cells by causing their opsonization by phagocytic cells bearing C3b receptors or by induction of the membrane attack complex. Damage to normal host cells is **prevented because C3b undergoes spontaneous hydrolysis** by the time it has diffused 40 nm away from the C4b2a or C3bBb convertase enzymes, so that it can no longer bind to its target site.

Proteins that regulate the complement system

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Protein	Type of protein	Pathway affected	Immunologic function
C1 inhibitor (C1Inh)	Soluble	Classical	Serine protease inhibitor: causes $C1r_2s_2$ to dissociate from C1q
C4b-binding protein (C4bBP)*	Soluble	Classical and lectin	Blocks formation of C3 convertase by binding C4b; cofactor for cleavage of C4b by factor I
Factor H*	Soluble	Alternative	Blocks formation of C3 convertase by binding C3b; cofactor for cleavage of C3b by factor I
Complement-receptor type 1 (CR1)*	Membrane bound	Classical, alternative, and lectin	Block formation of C3 convertase by binding C4b or C3b; cofactor for factor I-catalyzed cleavage of C4b or C3b C3bBb
Membrane-cofactor protein (MCP)*			
Decay-accelerating factor (DAE or CD55)*	Membrane bound	Classical, alternative, and lectin	Accelerates dissociation of $\overline{C4b2a}$ and $\overline{C3bBb}$ (classical and alternative C3 convertases)
Factor-I	Soluble	Classical, alternative, and lectin	Serine protease: cleaves C4b or C3b using C4bBP, CR1, factor H, DAE, or MCP as cofactor
S protein	Soluble	Terminal	Binds soluble C5b67 and prevents its insertion into cell membrane
Homologous restriction factor (HRF)	Membrane bound	Terminal	Bind to C5b678 on autologous cells, blocking binding of C9
Membrane inhibitor of reactive lysis (MIRL or CD59)*			
Anaphylatoxin inactivator	Soluble	Effector	Inactivates anaphylatoxin activity of C3a, C4a, and C5a by carboxypeptidase N removal of C-terminal Arg

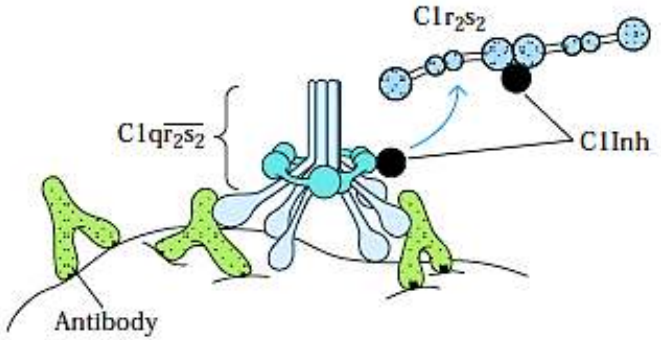
*An RCA (regulator of complement activation) protein. In humans, all RCA proteins are encoded on chromosome 1 and contain short consensus repeats.

Regulation of the complement system by regulatory proteins

Regulation of the Complement System

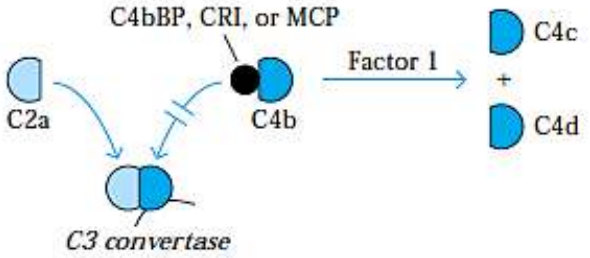
(a) Before assembly of convertase activity

1. C1 inhibitor (C1Iab) binds $C1r_2s_2$, causing dissociation from C1q



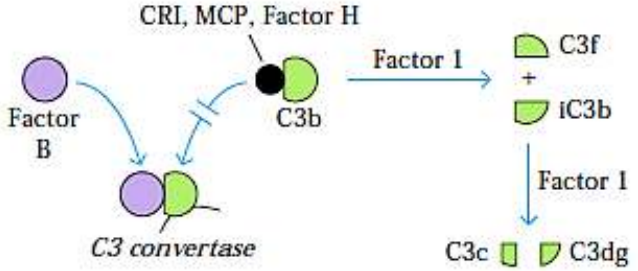
2. Association of C4b and C2a is blocked by binding C4b-binding protein (C4bBP), complement receptor type I, or membrane cofactor protein (MCP)

3. Inhibitor-bound C4b is cleaved by Factor 1



4. In alternative pathway, CR1, MCP, or Factor H prevent binding of C3b and Factor B

5. Inhibitor-bound C3b is cleaved by Factor 1



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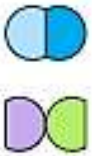
(b) After assembly of convertase

C3 convertases are dissociated by C4bBP, CR1, Factor H, and decay-accelerating Factor (DAF)

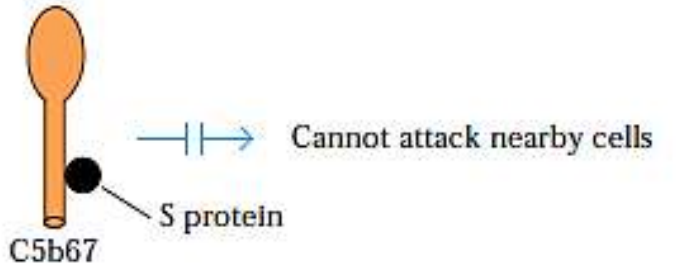


C4bBP, CR2
Factor H, DAF

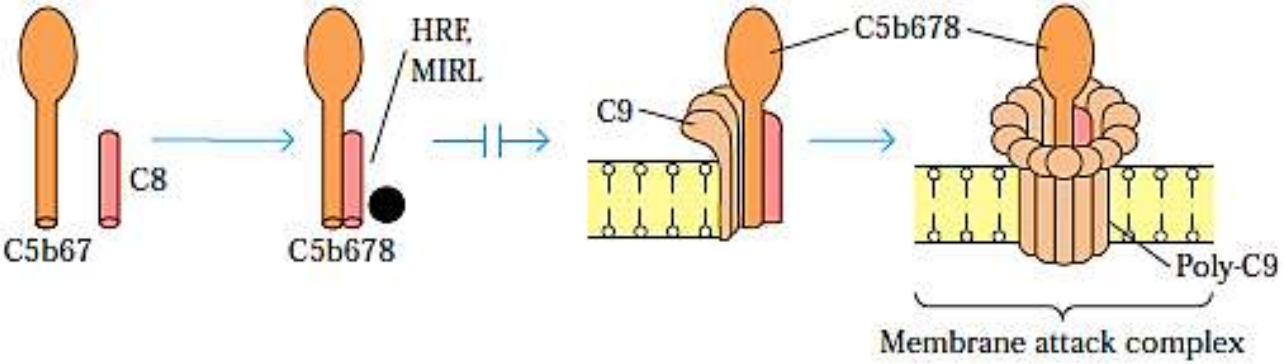
Dissociation of convertase; remaining C4b or C3b cleaved by Factor 1

(c) Regulation at assembly of membrane-attack complex (MAC)

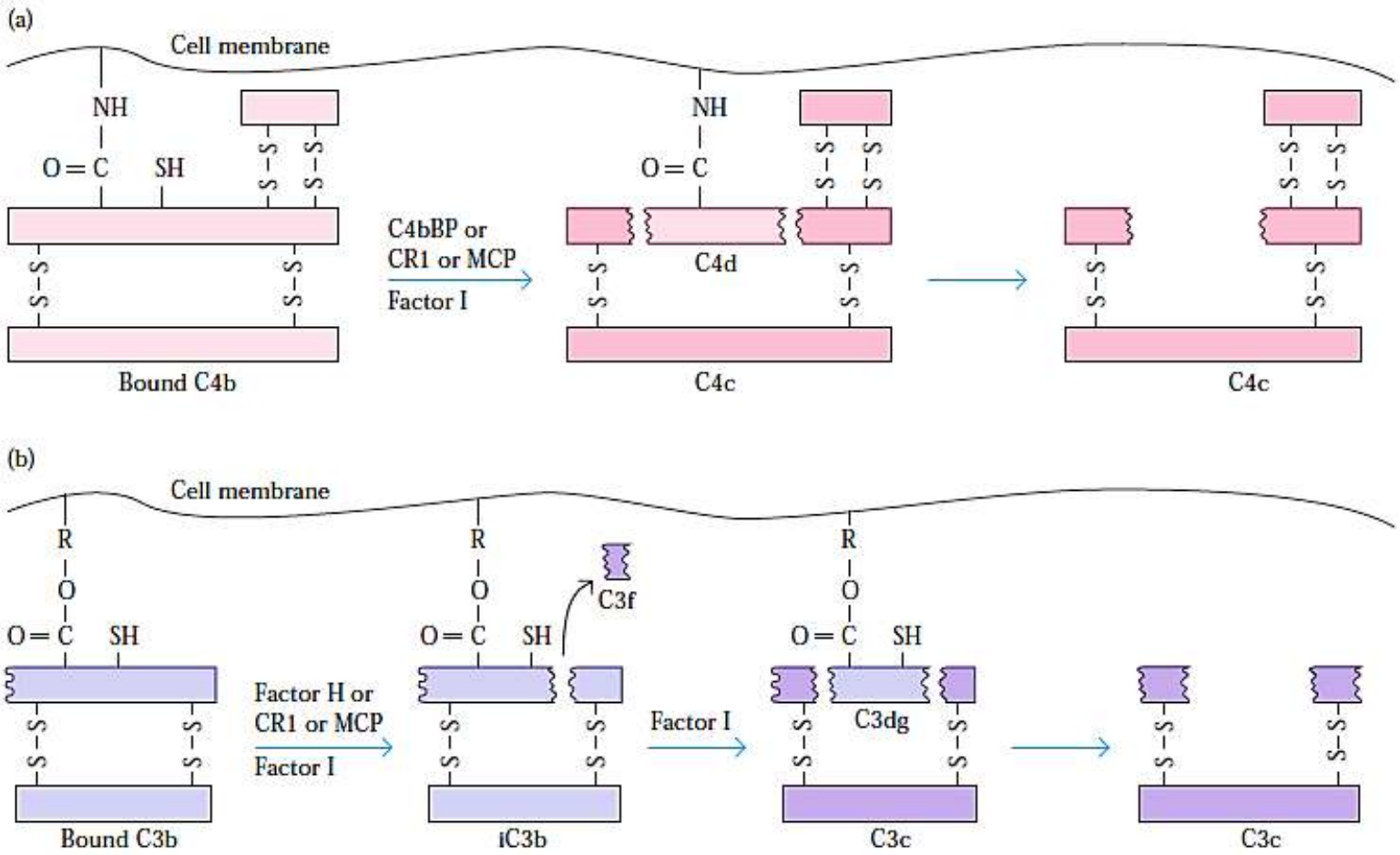
1. S protein prevents insertion of C5b67 MAC component into the membrane



2. Homologous restriction factor (HRF) and membrane inhibitor of reactive lysis (MIRL or CD59) bind $C8_1$, preventing assembly of poly-C9 and blocking formation of MAC



Inactivation of bound C4b and C3b by regulatory proteins of the complement system



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Biological Consequences of Complement Activation

Effect	Complement product mediating*
Cell lysis	C5b–9, the membrane-attack complex (MAC)
Inflammatory response	
Degranulation of mast cells and basophils†	C3a,C4a, and C5a (anaphylatoxins)
Degranulation of eosinophils	C3a, C5a
Extravasation and chemotaxis of leukocytes at inflammatory site	C3a, C5a, C5b67
Aggregation of platelets	C3a, C5a
Inhibition of monocyte/macrophage migration and induction of their spreading	Bb
Release of neutrophils from bone marrow	C3c
Release of hydrolytic enzymes from neutrophils	C5a
Increased expression of complement receptors type 1 and 3 (CR1 and CR3) on neutrophils	C5a
Opsonization of particulate antigens, increasing their phagocytosis	C3b, C4b, iC3b
Viral neutralization	C3b, C5b–9 (MAC)
Solubilization and clearance of immune complexes	C3b

*Boldfaced component is most important in mediating indicated effect.

†Degranulation leads to release of histamine and other mediators that induce contraction of smooth muscle and increased permeability of vessels.

The Membrane-Attack Complex can lyse a broad spectrum of cells

- The membrane-attack complex formed by complement activation **can lyse gram-negative bacteria, parasites, viruses, erythrocytes, and nucleated cells.**
- **Enveloped viruses** are susceptible to complement mediated lysis. The viral envelope is largely derived from the plasma membrane of infected host cells and is therefore susceptible to pore formation by the membrane attack complex.
- In ***Escherichia coli*** **and** ***Salmonella*****, resistance to complement**, which is characterized by the presence of **long polysaccharide side chains in the cell-wall lipopolysaccharide (LPS) component**. Increased LPS in the wall of resistant strains **may prevent insertion of the MAC into the bacterial membrane**, so that the complex is released from the bacterial cell rather than forming a pore.
- Strains of ***Neisseria gonorrheae*** **resistant** to complement-mediated killing have been associated with disseminated gonococcal infections in humans.
- **Gram-positive bacteria are generally resistant to complement- mediated lysis** because the thick peptidoglycan layer in their cell wall prevents insertion of the MAC into the inner membrane.

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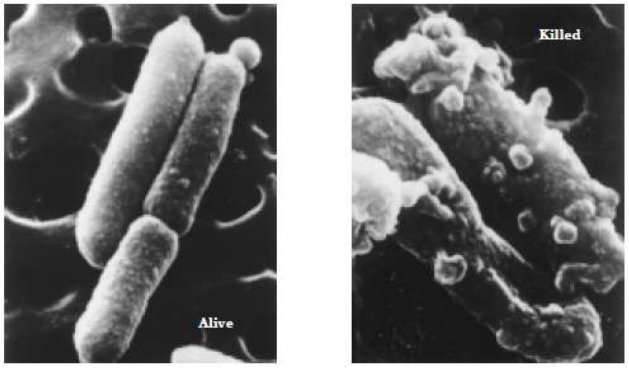

- Although complement activation can occur on the cell membrane of encapsulated bacteria such as ***Streptococcus pneumonia.***
- Lysis of **nucleated cells requires formation of multiple membrane attack complexes**, whereas a **single MAC can lyse a red blood cell.** Many nucleated cells, including the majority of cancer cells, can endocytose the MAC.

Microbial evasion of complement-mediated damage

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Microbial component	Mechanism of evasion	Examples
	GRAM-NEGATIVE BACTERIA	
Long polysaccharide chains in cell-wall LPS	Side chains prevent insertion of MAC into bacterial membrane	Resistant strains of *E. coli* and *Salmonella*
Outer membrane protein	MAC interacts with membrane protein and fails to insert into bacterial membrane	Resistant strains of *Neisseria gonorrhoeae*
Elastase	Anaphylatoxins C3a and C5a are inactivated by microbial elastase	*Pseudomonas aeruginosa*
	GRAM-POSITIVE BACTERIA	
Peptidoglycan layer of cell wall	Insertion of MAC into bacterial membrane is prevented by thick layer of peptidoglycan	*Streptococcus*
Bacterial capsule	Capsule provides physical barrier between C3b deposited on bacterial membrane and CR1 on phagocytic cells	*Streptococcus pneumoniae*
	OTHER MICROBES	
Proteins that mimic complement regulatory proteins	Protein present in various bacteria, viruses, fungi, and protozoans inhibit the complement cascade	Vaccinia virus, herpes simplex, Epstein-Barr virus, *Trypanosoma cruzi*, *Candida albicans*

KEY: CR1 = type 1 complement receptor; LPS = lipopolysaccharide; MAC = membrane-attack complex (C5b–9).

Cleavage products of complement components mediate inflammation

- Various peptides generated during formation of the MAC play a decisive role in the development of an effective **inflammatory response.**
- The **smaller fragments** resulting from complement cleavage, **C3a, C4a, and C5a**, called **anaphylatoxins, bind to receptors on mast cells and blood basophils and induce degranulation, with release of histamine and other pharmacologically active mediators.**
- The anaphylatoxins also induce **smooth-muscle contraction and increased vascular permeability.**
- Activation of the complement system thus results in **influxes of fluid that carries antibody and phagocytic cells to the site of antigen entry.**
- The activities of these highly reactive anaphylatoxins are **regulated by a serum protease called carboxypeptidase N, which cleaves an Arg residue from the C terminus of the molecules, yielding so-called *des-Arg* forms.**
- The **des-Arg forms of C3a and C4a are completely inactive** while that of C5a retains about 10% of its chemotactic activity and 1% of its ability to cause smooth muscle contraction.
- **C3a, C5a, and C5b67 can each induce monocytes and neutrophils to adhere to vascular endothelial cells, extravasate through the endothelial lining of the capillary, and migrate toward the site of complement activation in the tissues**.
- **C5a is most potent i**n mediating these processes, effective in picomolar quantities.

C3b and C4b binding facilitates opsonization

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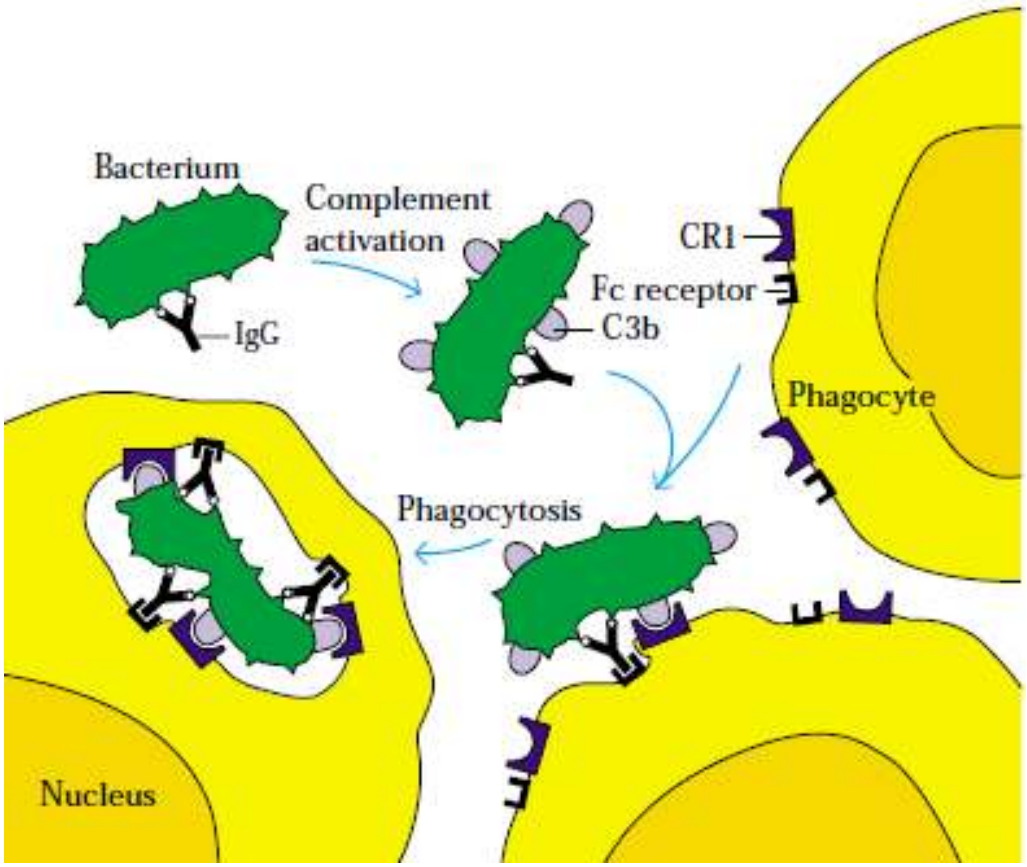
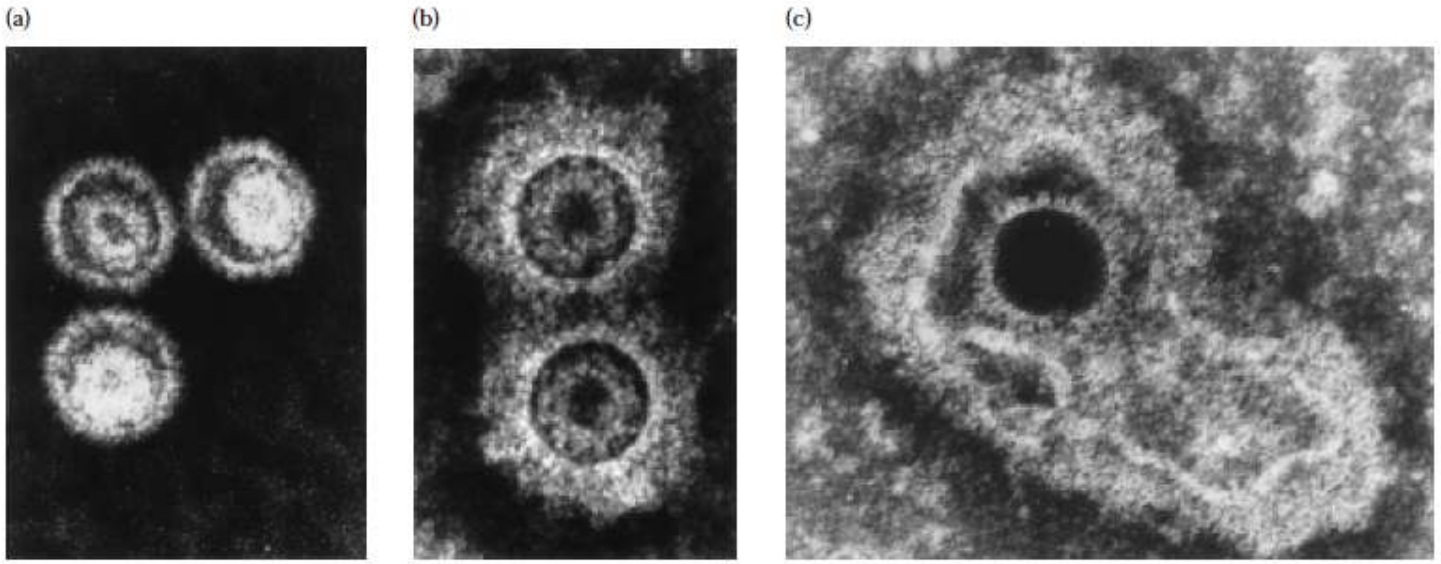


- **C3b is the major opsonin** of the complement system, although
- **C4b and iC3b** also have opsonizing activity.
- **Phagocytic cells**, as well as some other cells, express **complement receptors** (CR1, CR3, and CR4) that bind C3b, C4b and iC3b.
- Antigen coated with C3b binds to cells bearing CR1. If the cell is a phagocyte (e.g., a neutrophil, monocyte, or macrophage), **phagocytosis will be enhanced.**
- Activation of phagocytic cells by various agents, including **C5a anaphylatoxin**, has been shown **to increase the number of CR1s from 5000 on resting phagocytes to 50,000 on activated cells,** greatly facilitating their phagocytosis of C3b-coated antigen.

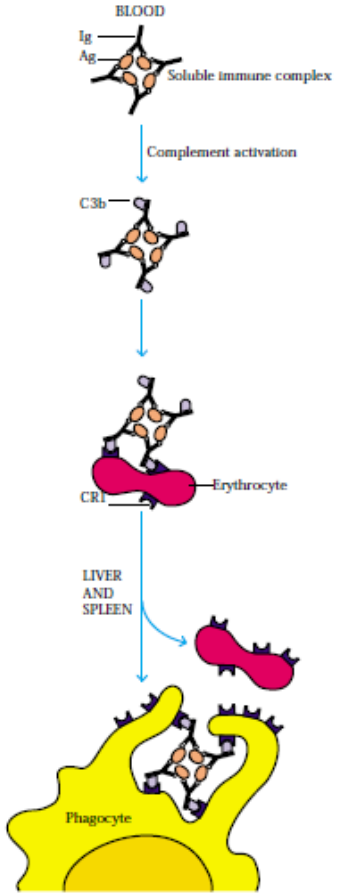
The complement system also neutralizes viral infectivity



Electron micrographs of negatively stained preparations of Epstein-Barr virus. (a) Control without antibody. (b) Antibody coated particles. (c) Particles coated with antibody and complement.

Some degree of neutralization is achieved through the formation of **larger viral aggregates**, Although **antibody plays a role in the formation of viral aggregates**. The **binding of antibody and/or complement to the surface of a viral particle creates a thick protein coating** that can be visualized by electron microscopy. This **coating neutralizes viral infectivity by blocking attachment to susceptible host cells.** In the case of **phagocytic cells, such binding can be followed by phagocytosis and intracellular destruction of the ingested viral particle**. Finally, complement is effective in lysing most, if not all, enveloped viruses, resulting in fragmentation of the envelope and disintegration of the nucleo-capsid.

The complement system clears immune complexes from circulation



C3b-coated immune complexes and carrying these complexes **to the liver and spleen**. In these organs, immune complexes are stripped from the red blood cells and are **phagocytosed, thereby preventing their deposition in tissues.**

The importance of the complement system in clearing immune complexes is seen in patients with the autoimmune disease **systemic lupus erythematosus (SLE).** These individuals produce **large quantities of immune complexes and suffer tissue damage** as a result of complement-mediated lysis and the induction of type II or type III hypersensitivity