IMMUNO TECHNOLOGY

LECTURE 04: COMPLIMENTS

The complement system

The Complement Components

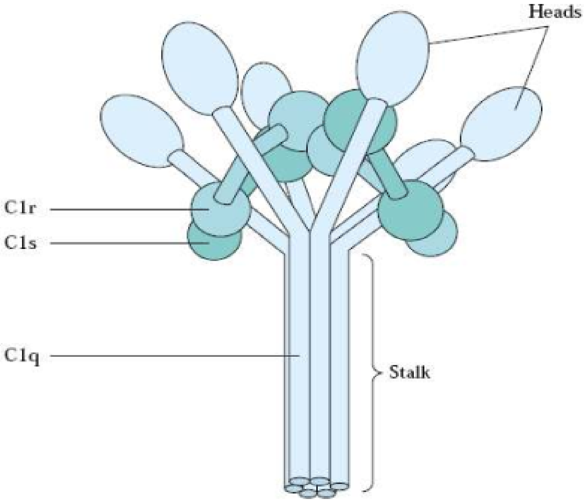
- The **proteins** and **glycoproteins** that compose the complement system are synthesized mainly by **liver hepatocytes**, although significant amounts are also produced by **blood monocytes, tissue macrophages** and **epithelial cells** of the gastrointestinal and genitourinary tracts. These components 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).

The complement pathways

The complement system can be activated by three pathways as:

1. The Classical Pathway – Needs antigen antibody complex formation (Acquired immunity)
2. The Alternative Pathway – doesn't need antigen antibody complex (Innate immunity)
3. The Lectin Pathway – doesn't need antigen antibody complex (Innate immunity)

The Classical Pathway Begins with Antigen-Antibody Binding



Activation of classical pathway of complements

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.

Involvement of Igs and complements in the classical pathway

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, which are present in plasma in functionally inactive forms. Because the components were named in order of their discovery and before their functional roles had been determined, the numbers in their names do not always reflect the order in which they react. 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.

Structure of C1

C1 in serum is a macromolecular complex consisting of **C1q** and **two molecules** each of **C1r** and **C1s**, held together in a complex **(C1qr2s2** forms the head**)** stabilized by Ca^{2+} ions. The C1q molecule (stalk) 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 C_H2 domain of the antibody molecule. Each C1r and C1s monomer contains a catalytic domain and an interaction domain; the latter facilitates interaction with C1q or with each other.

Interaction of C1 with IgM and IgG

Each C1 molecule must bind by its C1q globular heads to at least two Fc sites for a stable C1-antibody interaction to occur. 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**. **Circulating IgM,** however, exists as a **planar configuration** in which the **C1q-binding sites are not exposed** and therefore cannot activate the complement cascade.

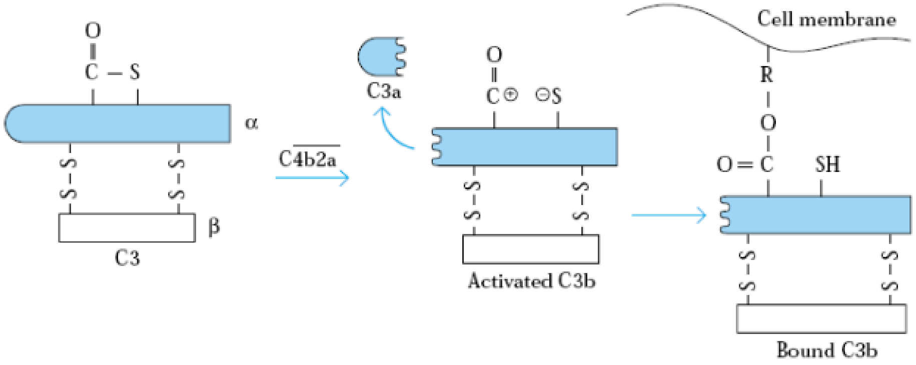
An IgG molecule, on the other hand, contains only a single C1q-binding site in the C_H2 domain of the Fc, so that firm C1q binding is achieved only when **two IgG molecules** are within **30–40 nm** of each other on a target surface or in a complex, providing two attachment sites for C1q. This difference accounts for the observation that 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.

Cascade reaction of classical pathway

Binding of C1q to Fc binding sites induces a conformational change in C1r that converts C1r to an active serine protease enzyme, C1r, which then cleaves C1s to a similar active enzyme, C1s. C1s has two substrates, C4 and C2.

The C4 component is a glycoprotein containing three polypeptide chains α, β, and γ. C4 is activated when C1s hydrolyzes a small fragment (C4a) from the amino terminus of the α chain, exposing a binding site on the larger fragment (C4b). The C4b fragment attaches to the target surface in the vicinity of C1, and the C2 proenzyme then attaches to the exposed binding site on C4b, where the C2 is then cleaved by the neighboring C1s; the smaller fragment (C2b) diffuses away. The resulting **C$\overline{\text{4b2a}}$ complex** is called **C3 convertase**, referring to its role in converting the C3 into an active form.

The native C3 component consists of two polypeptide chains, α and β. Hydrolysis of a short fragment (C3a) from the amino terminus of the α chain by the C3 convertase generates C3b (see below figure).



A single **C3 convertase** molecule can generate over **200 molecules** of **C3b**, resulting in tremendous amplification at this step of the sequence. Some of the C3b binds to C$\overline{4b2a}$ to form a trimolecular complex **C$\overline{4b2a3b}$,** called **C5 convertase**. The C3b component of this complex binds C5 and alters its conformation, so that the C4b2a component can cleave C5 into C5a, which diffuses away, and C5b, which attaches to C6 and initiates formation of the membrane attack complex in a sequence described later.

The end result of activating the classical pathway is production of an active C5 convertase. This enzyme cleaves C5, which contains two protein chains, α and β. After binding of C5 to the nonenzymatic C3b component of the convertase, the amino terminus of the α chain is cleaved. This generates the small C5a fragment, which diffuses away, and the large C5b fragment, which binds to the surface of the target cell and provides a binding site for the subsequent components of the membrane-attack complex.

The **C5b** component is extremely **labile** and **becomes inactive** within **2 minutes unless C6 binds to it** and stabilizes its activity. Up to this point, all the complement reactions take place on the **hydrophilic surface** of **membranes** or on **immune complexes** in the fluid phase. As **C5b6 binds to C7**, the resulting complex undergoes a **hydrophilic-amphiphilic structural transition** that exposes hydrophobic regions, which serve as binding sites for membrane phospholipids. If the reaction occurs on a target-cell membrane, the hydrophobic binding sites enable the C5b67 complex to insert into the phospholipid bilayer.

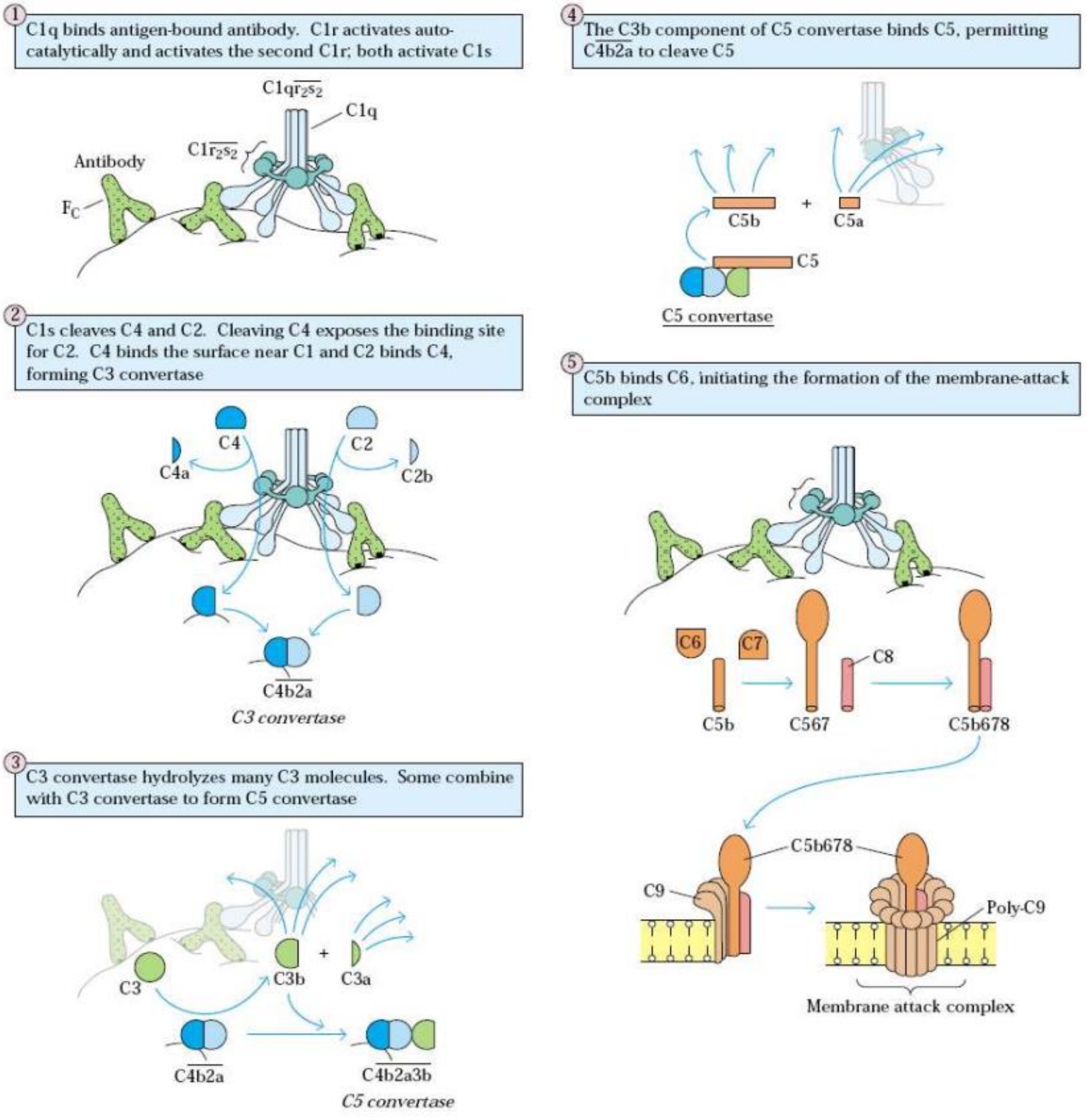
Innocent-bystander lysis

If, however, the reaction occurs on an immune complex or other noncellular activating surface, then the hydrophobic binding sites **cannot anchor the complex** and it is released. Released **C5b67 complexes** can insert into the membrane of nearby cells and mediate **"innocent-bystander" lysis**. Regulator proteins normally prevent this from occurring, but in certain diseases cell and tissue damage may result from innocent- bystander lysis. Eg: A hemolytic disorder resulting from deficiency in a regulatory protein results in an autoimmune process in which immune complexes mediate tissue damage.

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.**

Generation of Membrane Attack Complex (MAC)

The final step in formation of the MAC is the binding and polymerization of C9, a perforin- like molecule, to the C5b678 complex. As many as 10–17 molecules of C9 can be bound and polymerized by a single C5b678 complex. During polymerization, the C9 molecules undergo a hydrophilic-amphiphilic transition, so that they too can insert into the membrane. The completed MAC, which has a tubular form and functional pore size of **70–100 Å**, consists of a **C5b678 complex** surrounded by a **poly-C9 complex**. Since ions and small molecules can diffuse freely through the central channel of the MAC, the cell cannot maintain its osmotic stability and is killed by an influx of water and loss of electrolytes.


① C1q binds antigen-bound antibody. C1r activates autocatalytically 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

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

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

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

The Alternative Pathway is Antibody-Independent

The alternative pathway generates bound C5b, the same product that the classical pathway generates, but it does so without the need for antigen-antibody complexes for initiation. Because no antibody is required, the alternative pathway is a component of the **innate immune system**. This major pathway of complement activation involves **four serum proteins: C3, factor B, factor D** and **properdin**.

The alternative pathway is initiated in most cases by **cell-surface constituents** that are foreign to the host (see Table). For example, both **gram-negative** and **gram-positive bacteria** have **cell-wall constituents** that can activate the alternative pathway.

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

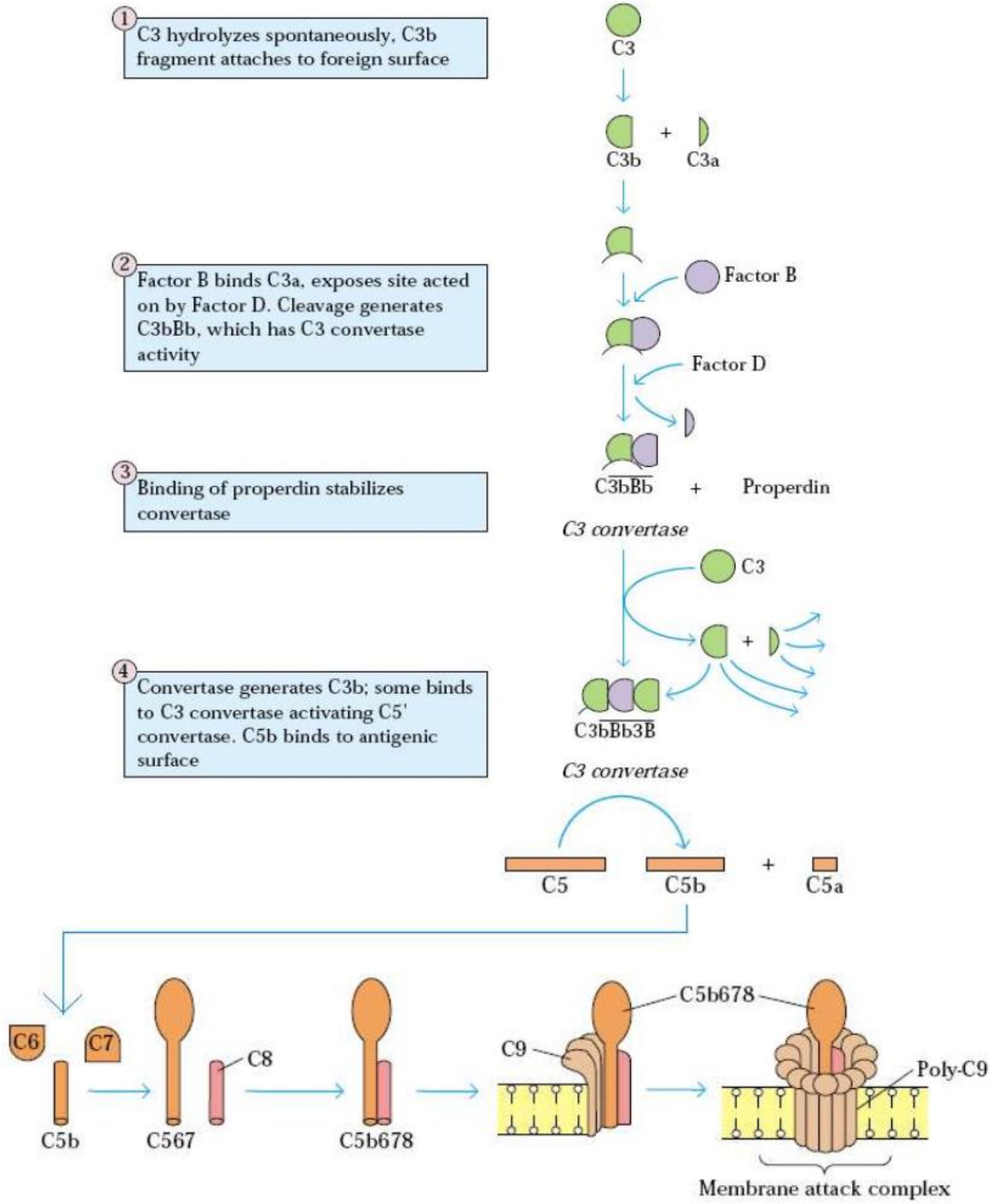
In the classical pathway, C3 is rapidly cleaved to C3a and C3b by the enzymatic activity of the C3 convertase. In the alternative pathway, **serum C3,** which contains **an unstable thioester bond,** is subject to **slow spontaneous hydrolysis** to yield C3a and C3b. The C3b component can bind to foreign surface antigens (such as those on bacterial cells or viral particles) or even to the host's own cells (see Figure 13-6c).

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

The C3b present on the surface of the foreign cells can bind another **serum protein** called **factor B** to form a complex stabilized by Mg^{2+}. Binding to C3b exposes a site on factor B that serves as the sub strate for an enzymatically active serum protein called **factor D**. Factor D cleaves the C3b-bound factor B, releasing a small fragment (Ba) that diffuses away and generating C3bBb. The **$\overline{\text{C3bBb}}$ complex** has **C3 convertase** activity and thus is **analogous** to the **$\overline{\text{C4b2a}}$ complex** in the **classical** pathway. The C3 convertase activity of C3bBb has a half-life of only **5 minutes** unless the serum protein **properdin** binds to it, stabilizing it and extending the **half-life** of this convertase activity to **30 minutes**.

The C3bBb generated in the alternative pathway can activate **unhydrolyzed C3** to generate more **C3b autocatalytically**. As a result, the initial steps are repeated and amplified, so that more than **$2x10^6$** molecules of **C3b** can be deposited on an antigenic surface in **less than 5 minutes**. The C3 convertase activity of C3bBb generates the **$\overline{\text{C3bBb3b}}$ complex**, which exhibits **C5 convertase** activity, **analogous** to the **C4b2a3b complex** in the **classical** pathway. The nonenzymatic C3b component binds C5, and the Bb component subsequently hydrolyzes the bound C5 to generate C5a and C5b (see Figure); the latter binds to the antigenic surface.

Formation of MAC complex from this point is same as that of the classical pathway. C5b binds to C6, C7, C8 and poly-C9 to form the MAC. The intermediates in the alternative pathway for generating C5b are shown schematically in the following figure.



① C3 hydrolyzes spontaneously, C3b fragment attaches to foreign surface

② Factor B binds C3a, exposes site acted on by Factor D. Cleavage generates C3bBb, which has C3 convertase activity

③ Binding of properdin stabilizes convertase

④ Convertase generates C3b; some binds to C3 convertase activating C5' convertase. C5b binds to antigenic surface

The smaller fragment from C4 cleavage, **C4a,** is an **anaphylatoxin**, or **mediator of inflammation**, which does not participate directly in the complement cascade; **C3a** and **C5a** as well acts as **anaphylatoxins** apart from C4a.

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

The Lectin Pathway originates with host proteins binding microbial surfaces

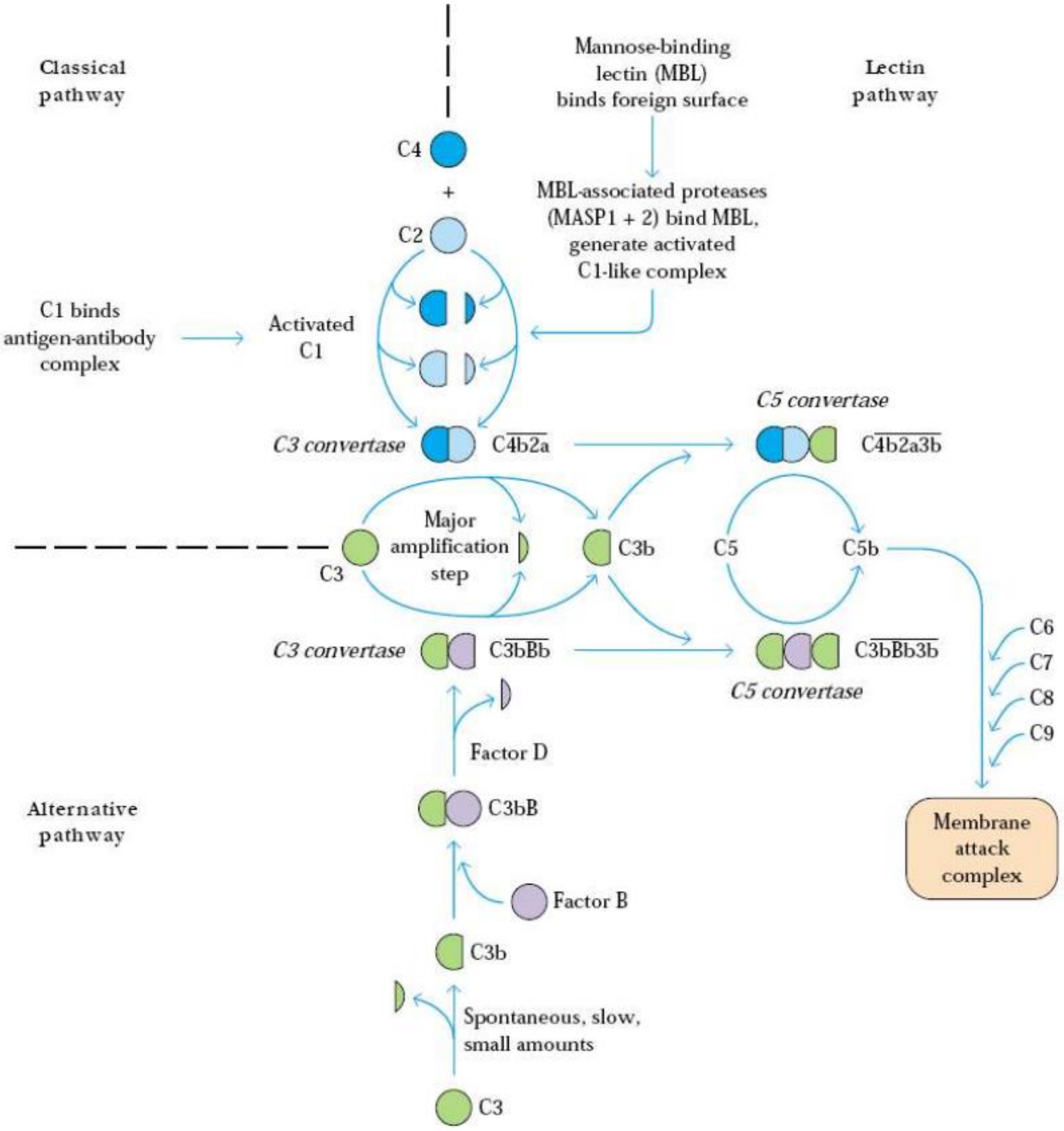
Lectins are **proteins** that recognize and bind to **specific carbohydrate targets**. (Because the lectin that activates complement binds to **mannose residues**, some authors designate this as the MBLectin pathway or mannan-binding lectin pathway).

The lectin pathway, like the alternative pathway, **does not depend on antibody** for its activation. However, the mechanism is **more like** that of the **classical** pathway, because after initiation, it proceeds, through the action of **C4** and **C2**, to produce a **C5 convertase**. 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***.

MBL is an **acute phase protein** produced in inflammatory responses. Its function in the complement pathway is similar to that of C1q, which it resembles in structure. After **MBL binds** to the **surface** of a **cell** or **pathogen**, **MBL-associated serine proteases, MASP-1** and **MASP-2** 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 without need for specific antibody binding represents an important innate defense mechanism comparable to the alternative pathway, but utilizing the elements of the classical pathway except for the C1 proteins.

Overview of the complement activation pathways

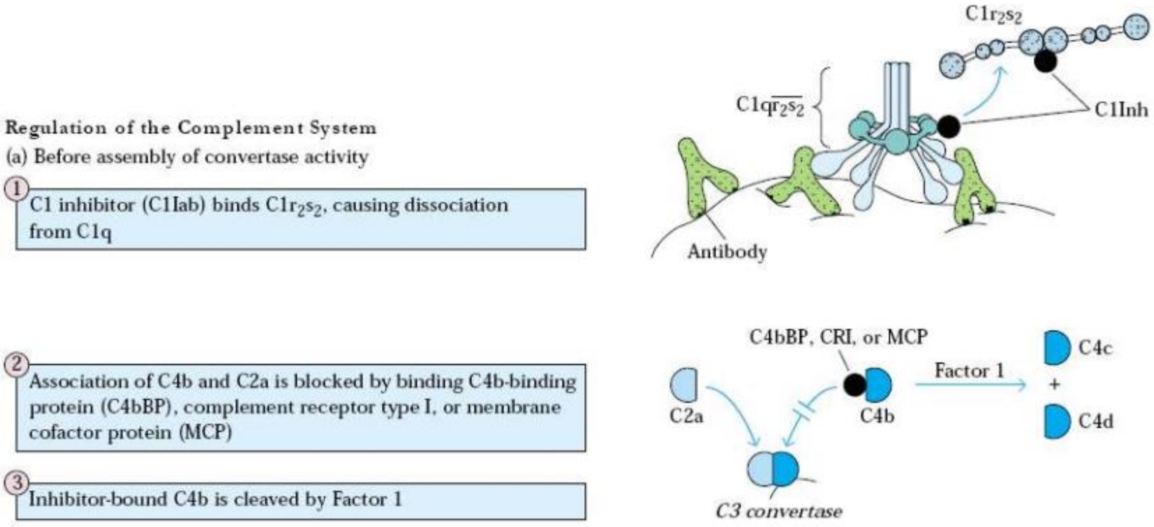
The classical pathway is initiated when C1 binds to antigen-antibody complexes. The alternative pathway is initiated by binding of spontaneously generated C3b to activating surfaces such as microbial cell walls. The lectin pathway is initiated by binding of the serum protein MBL to the surface of a pathogen. All three pathways generate C3 and C5 convertases and bound C5b, which is converted into a membrane- attack complex (MAC) by a common sequence of terminal reactions. Hydrolysis of C3 is the major amplification step in all pathways, generating large amounts of C3b, which forms part of C5 convertase. C3b also can diffuse away from the activating surface and bind to immune complexes or foreign cell surfaces, where it functions as an opsonin.



Regulation of the complement system

Protein	Type Of Protein	Pathway Affected	Immunological Functions
C1 inhibitor (C1Inh)	Soluble	Classical	Serine protease inhibitor: causes C1r2s2 to dissociate from C1q
C4b-binding protein	Soluble	Classical and lectin	Blocks formation of C3 convertase by (C4bBP) 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-cofactor protein (MCP)*	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
Decay-accelerating factor (DAE or CD55)*	Membrane bound	Classical, Alternative and Lectin	Accelerates dissociation of C4b2a and C3bBb (classical and alternative C3 convertases)
Factor-I	Soluble	Classical, Alternative and Lectin	Serine protease: cleaves C4b or C3b and lectin 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 inhibitor of reactive lysis (MIRL)	Membrane bound	Terminal	Bind to C5b678 on autologous cells, blocking binding of C9
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.



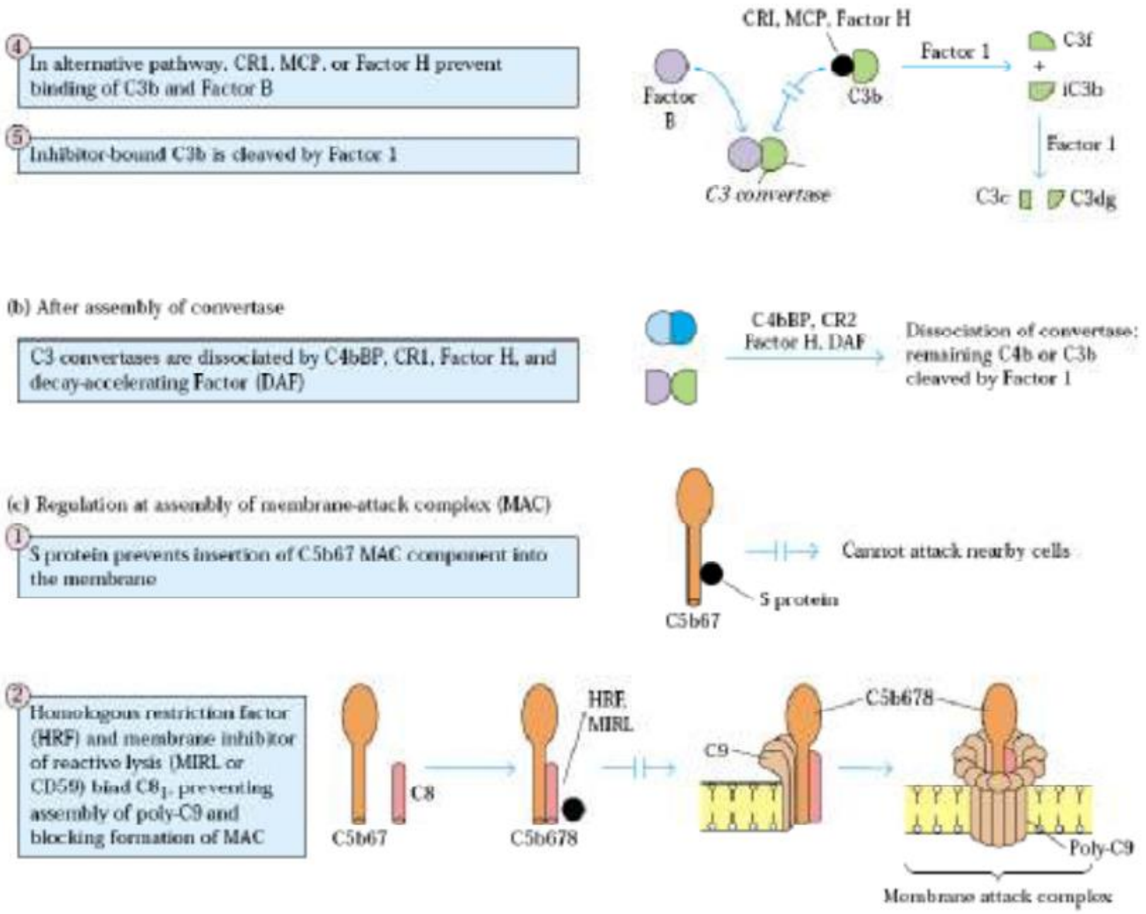
Regulation of the Complement System

(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)

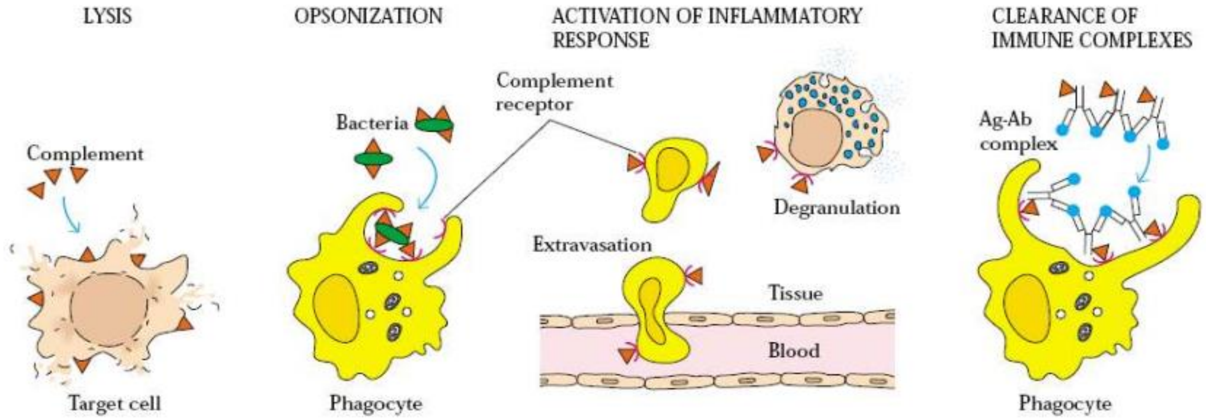
③ Inhibitor-bound C4b is cleaved by Factor 1



Biological role of complement activation

Biological role / Effector function of complements	Complements products
Cell lysis	C5b–9, 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

The multiple biological activities of the complement system



Serum complement proteins and membrane-bound complement receptors partake in a number of immune activities:

1. Lysis of foreign cells by antibody-dependent or antibody-independent pathways;
2. Opsonization or uptake of particulate antigens, including bacteria, by phagocytes;
3. Activation of inflammatory responses; and
4. Clearance of circulating immune complexes by cells in the liver and spleen.

Soluble complement proteins are schematically indicated by a triangle and receptors by a semi-circle; no attempt is made to differentiate among individual components of the complement system here.

Microbial evasion of complement mediated damage

Certain microorganisms evade the complement system and causes infection. The following are the examples:

Gram negative bacteria		
Examples of Microbes	**Microbial components**	**Mechanism of evasion**
Resistant strains of *E. coli* and *Salmonella*	Long polysaccharide chains in cell-wall LPS	Side chains prevent insertion of MAC into bacterial membrane
Resistant strains of *Neisseria gonorrhoeae*	Outer membrane protein	MAC interacts with membrane protein and fails to insert into bacterial membrane
Pseudomonas aeruginosa	Elastase	Anaphylatoxins C3a and C5a are inactivated by microbial elastase
Gram positive bacteria		
Examples of Microbes	**Microbial components**	**Mechanism of evasion**
Streptococcus	Peptidoglycan layer of cell wall	Insertion of MAC into bacterial membrane is prevented by thick layer of peptidoglycan
Streptococcus pneumoniae	Bacterial capsule	Capsule provides physical barrier between C3b deposited on bacterial

		membrane and CR1 on phagocytic cells
Other microbes		
Examples of Microbes	**Microbial components**	**Mechanism of evasion**
Vaccinia virus, herpes simplex virus, Epstein-Barr virus, *Trypanosoma cruzi, Candida albicans*	Proteins that mimic complement regulatory proteins	Protein present in various bacteria, viruses, fungi, and protozoans inhibits the complement cascade

Complement deficiencies

Genetic deficiencies lead to diseases as well infections have been described for each of the complement components.

Deficiencies of Components of the classical pathway (c1q, c1r, c1s, C4, and C2)	A marked increase in immune-complex diseases such as systemic lupus erythematosus, Glomerulonephritis, and vasculitis
	Recurrent infections by pyogenic (pus forming) Bacteria such as *streptococci* and *staphylococci*
Deficiencies in factor D and properdin - early components of The alternative pathway	Appear to be associated with *Neisseria* infections but not with immune-complex disease.
C3 deficiency	Have recurrent bacterial infections and may have immunecomplex Diseases.