The Complement System

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About Complement

• The term ‘complement’ refers to a set of serum proteins that cooperates
with both innate and adaptive immune systems to eliminate blood and
tissue pathogens.

• Major effector of humoral branch of immunity.

Discovery

I. Jules Bordet (1890s) at Institut Pasteur in Paris began work on complement.

Experiment

- Injected bacterium *Vibrio cholerae* to sheep
- Antibodies were raised against this bacterium

Observation

- Sheep antiserum to bacterium caused bacterial lysis (membrane destruction).
- Heating the antiserum destroyed its bacteriolytic activity.
- Ability to lyse the bacteria was restored to the heated serum by adding fresh serum that contained no antibacterial antibodies.
- Fresh antiserum from a non-immunized sheep failed to lyse the bacteria.

Inference

Bacteriolysis required two different substances:

i. heat-stable specific antibodies that bound to the bacterial surface

ii. heat-labile (sensitive) component responsible for the lytic activity.

II.Paul Ehrlich carried out similar experiments in Berlin and coined the term complement, defining it as “the activity of blood serum that completes the action of antibody.”

III. Researchers later discovered that the action of complement is the result of interactions among a complex group of more than 30 glycoproteins.

Chemical nature of the complement proteins

- Composed of a plethora of soluble proteins and glycoproteins
- Constitute approximately 15% of globulin protein fraction in plasma (concentration ~3 mg/ml).
- Additionally, several regulatory components of the system on cell membranes are glycoproteins.

Distribution of the complement proteins

- Distributed among the blood plasma and cell membranes as inactive zymogen or proenzymes.
- Proteolytic activity of enzymes cleaves the inhibitory fragment exposing the active site of the molecule.

Site of synthesis

Complement components are synthesized in
- liver by hepatocytes.
- blood monocytes
- tissue macrophages
- fibroblasts
- epithelial cells of the gastrointestinal and genitourinary tracts.

Properties of the complement proteins

- Acute phase proteins.
- Possess pattern recognition capacity (Pathogen associated molecular patterns – PAMPs) or antibody (Ab) specificity.
- Undergo changes in concentration during inflammation.

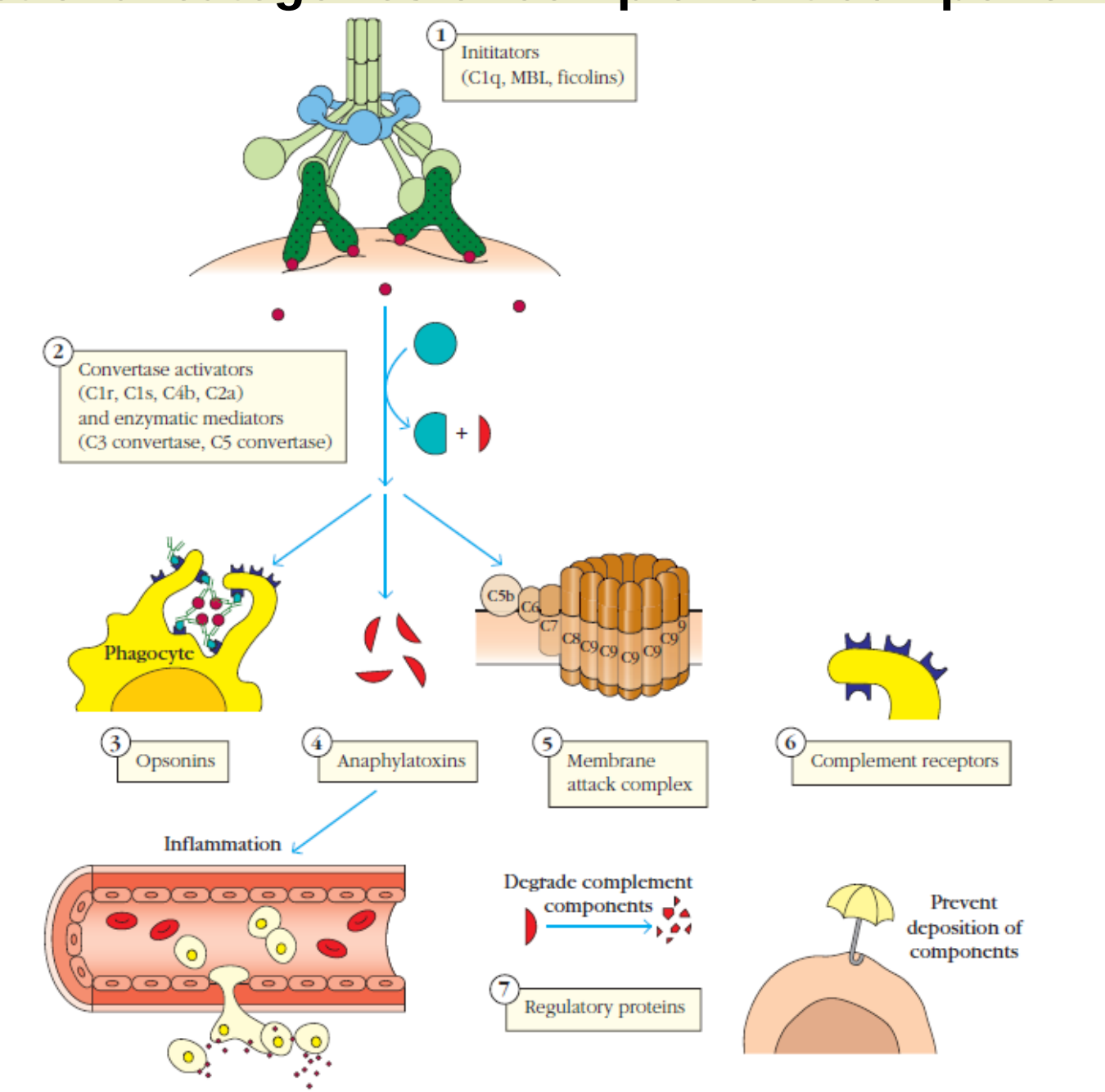
Thus various actions of complement system are triggered not only by antibody but also by components of innate immune system.

Function of the complement proteins

Complement proteins interact with one another in highly regulated catalytic cascades to execute the following functions –

i. Lysis of cells, bacteria and viruses by binding and opsonizing, rendering them susceptible to receptor-mediated phagocytosis by macrophages, which express membrane receptors for complement proteins.
ii. Elicit inflammatory responses, interface with components of the adaptive immune system.
iii. Immune clearance which clear immune complexes from the serum depositing them in the liver/spleen, and/or eliminate apoptotic cells.
iv. Finally, a Membrane Attack Complex (MAC) assembled from complement proteins directly kills some pathogens by creating pores in microbial membranes.

Functional categories of complement components



Major pathways of complement activation

There are three major pathways of complement activation –

Classical pathway
Alternate pathway
Lectin pathway

Initiating event of each of these pathways of complement activation is different

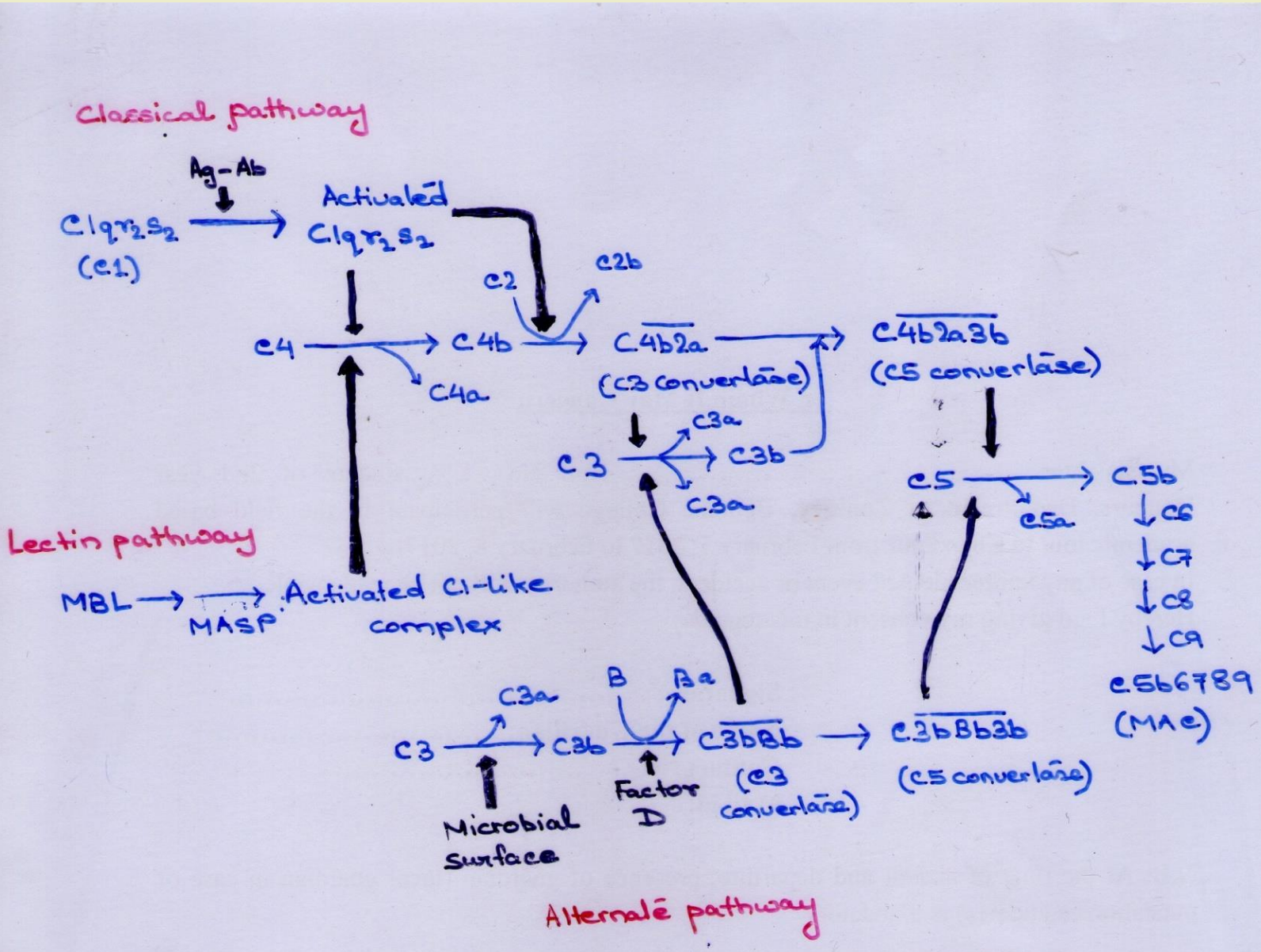
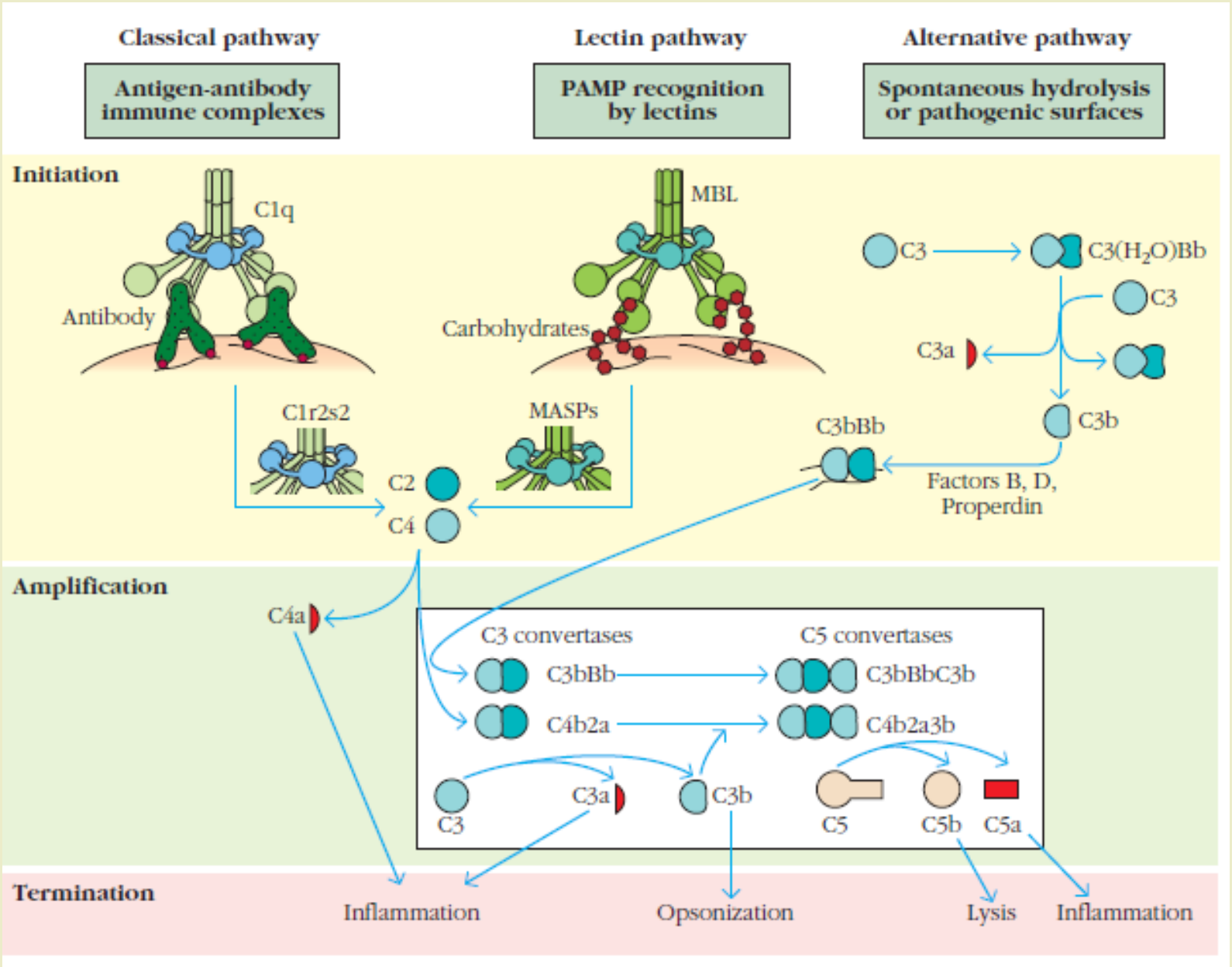


Fig : Overview of the Complement Activation Pathway

Some facts

- *Proteins of the classical pathway are numbered in the order in which the proteins were discovered, which does not quite correspond with the order in which the proteins act in the pathway.*
- *The binding of one component to the next always induces either a conformational change or an enzymatic cleavage, which allows for the next reaction in the sequence to occur.*
- *As a general rule, the larger fragment of a cleaved complement component is designated with the suffix "b," and the smaller with the suffix "a." However, there is one exception to this rule: the larger, enzymatically active form of the C2 component is named C2a.*

Initiation of major pathways of complement activation

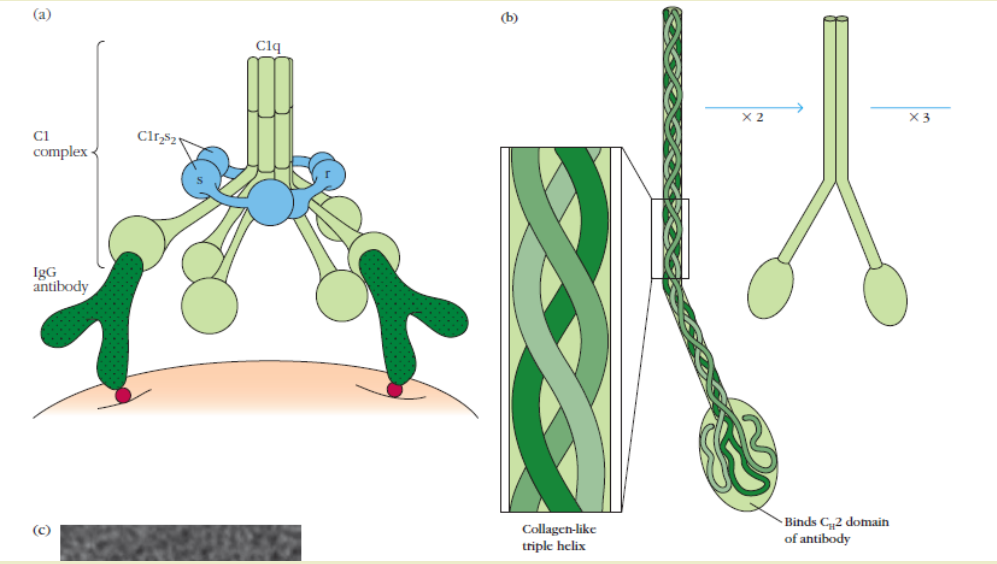


The Classical Pathway of complement activation

Initiated by antibody binding

- Begins with the formation of antigen-antibody complexes
- Considered to be part of the adaptive immune response.
- Complexes are soluble, or formed when an antibody binds to antigenic determinants, or epitopes, situated on viral, fungal, parasitic, or bacterial cell membranes.
- Soluble antibody-antigen complexes are termed immune complexes.
- Complexes formed by IgM or certain subclasses of IgG antibodies activate the classical complement pathway.
- Initial stage of activation involves C1, C2, C3, and C4, present in plasma as 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 for the C1 component of complement.

Structure of C1



- 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 (C1qr2s2).
- The C1q molecule 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.
- Each C1 macromolecular complex must bind by its C1q globular heads to at least two Fc sites for a stable C1-antibody interaction to occur.

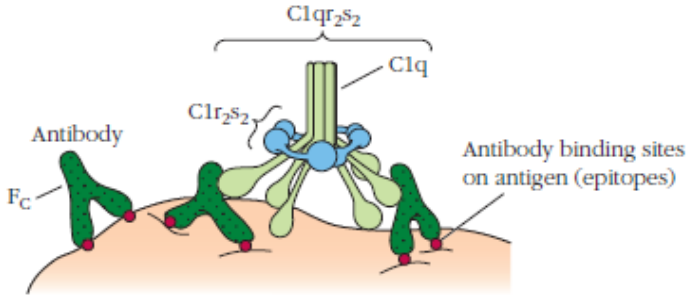
Reason behind difference in efficiency with which IgM and IgG can activate complement

- Serum IgM exists as pentamer of the basic four-chain Ig structure.
- Circulating, nonantigen-bound IgM adopts a planar configuration, where C1q binding sites are not exposed.
- Pentameric IgM bound to a multivalent antigen undergoes a conformational change, assuming the so-called “staple” configuration, where at least three binding sites for C1q are exposed.
- Thus, an IgM molecule engaged in an antibody-antigen complex can bind C1q, whereas circulating, nonantigen-bound IgM cannot.
- In contrast to pentameric IgM, monomeric IgG contains only one C1q binding site per molecule, and the conformational changes IgG undergoes on antigen binding are much more subtle than those experienced by IgM.
- There is therefore a striking difference in the efficiency with which IgM and IgG are able to activate complement.

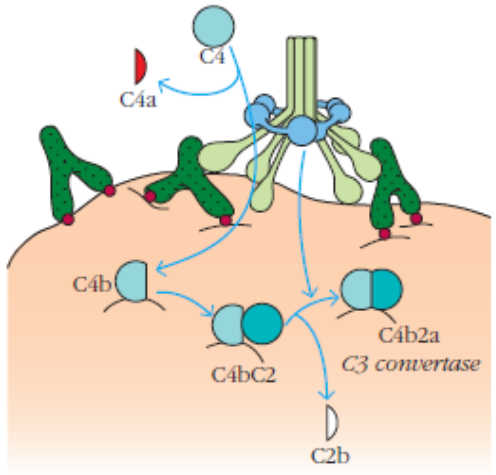
- **Less than 10 molecules of IgM bound to a red blood cell can activate the classical complement pathway and induce lysis, whereas some 1000 molecules of cell-bound IgG are required to ensure the same result.**

Intermediates in the classical pathway of complement activation

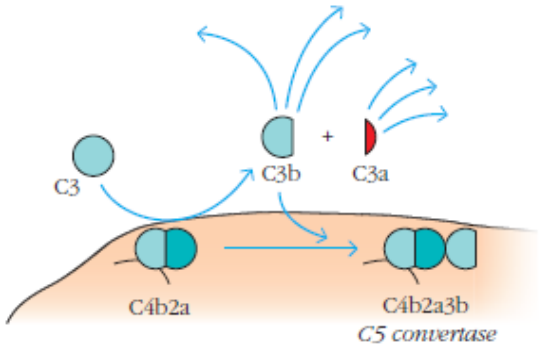
1 C1q binds antigen-bound antibody, and induces a conformational change in one C1r molecule, activating it. This C1r then activates the second C1r and the two C1s molecules.



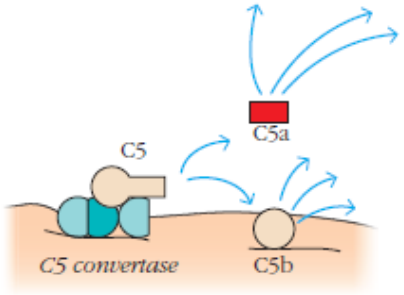
2 C1s cleaves C4 and C2. C4 is cleaved first and C4b binds to the membrane close to C1. C4b binds C2 and exposes it to the action of C1s. C1s cleaves C2, creating the C3 convertase, C4b2a.



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 C4b2a to cleave C5.



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.

Intermediates in the classical pathway of complement activation

- Binding of C1q to the C_H2 domains of the Fc regions of the antigen-complexed antibody molecule induces a conformational change in one of the C1r molecules that converts it to an active serine protease enzyme.
- This C1r molecule then cleaves and activates its partner C1r molecule.
- The two C1r proteases then cleave and activate the two C1s molecules.
- 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.
- 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, exposed upon C4 cleavage, reacts with hydroxyl or amino groups of proteins or carbohydrates on the cell membrane.
- This reaction must occur quickly, otherwise the thioester C4b is further hydrolyzed and can no longer make a covalent bond with the cell surface.
- Approximately 90% of C4b is hydrolyzed before it can bind the cell surface.
- On binding C4b, C2 becomes susceptible to cleavage by the neighboring C1s enzyme, and the smaller C2b fragment diffuses away, leaving behind an enzymatically active C4b2a complex.
- In this complex, C2a is the enzymatically active fragment, but it is only active when bound by C4b. This C4b2a complex is called C3 convertase, referring to its role in converting C3 into an active form.
- The smaller fragment generated by C4 cleavage, C4a is an anaphylatoxin.

- The membrane-bound C3 convertase enzyme, C4b2a, now hydrolyzes C3, generating two unequal fragments; the small anaphylatoxin C3a and the pivotal fragment C3b.
- A single C3 convertase molecule generates over 200 molecules of C3b, resulting in tremendous amplification at this step of the classical pathway.
- The generation of C3b is centrally important to many of the actions of complement as can be seen from deficiencies of

  i) complement components that act prior to C3 cleavage leave the host extremely vulnerable to both infectious and autoimmune diseases.

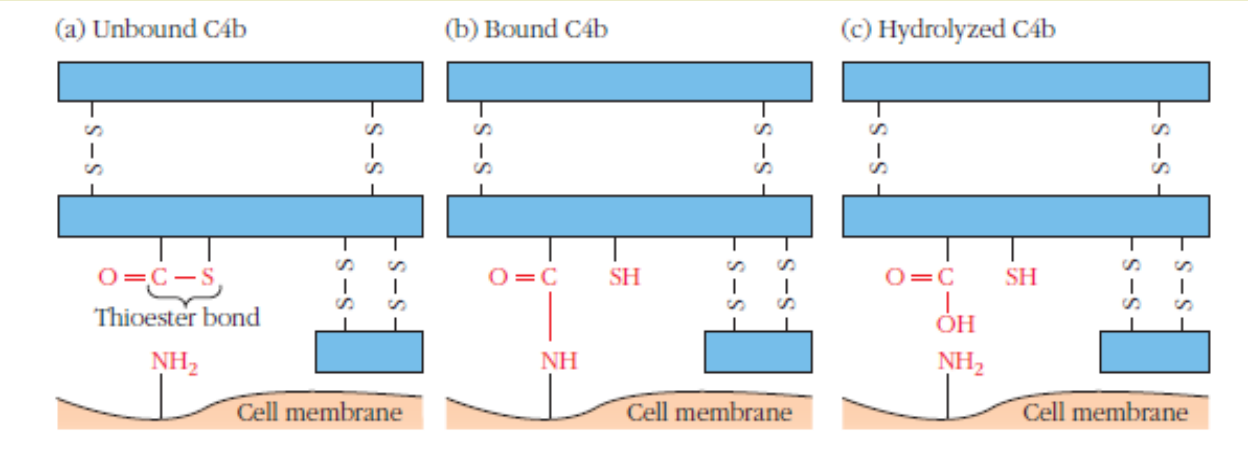
  ii) components later in the pathway are generally of lesser consequence.
- This is because C3b acts in three important and different ways to protect the host.

  i) Like C4b, C3b binds covalently to microbial surfaces, providing a molecular “tag” and thereby allowing phagocytic cells with C3b receptors to engulf the tagged microbes. This process is called opsonization.

  ii) C3b molecules can attach to the Fc portions of antibodies participating in soluble antigen-antibody 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.

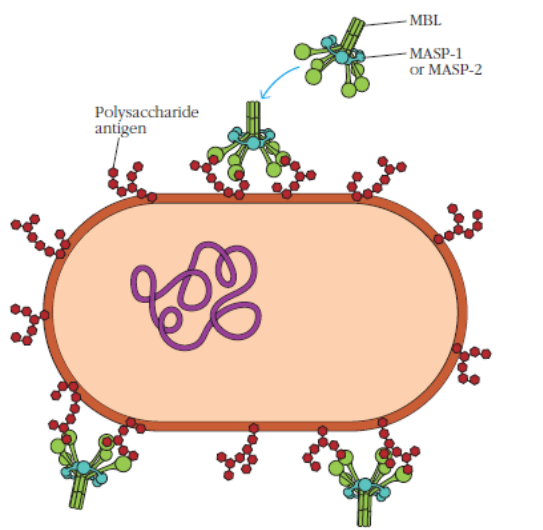
  iii) Some molecules of C3b bind the membrane localized C4b2a enzyme to form the trimolecular, membrane bound, C5 convertase complex C4b2a3b.
- The C3b component of this complex binds C5, and the complex then cleaves C5 into the two fragments: C5b and C5a.
- C4b2a3b is therefore the C5 convertase of the classical pathway.
- This trio of tasks accomplished by the C3b molecule places it right at the center of complement attack pathways.
- C5b goes on to form the MAC with C6, C7, C8, and C9.

Binding of C4b to the microbial membrane surface



- Binding of C4b to the microbial membrane surface occurs through a thioester bond via an exposed amino or hydroxyl group.
- Both C3b and C4b contain highly reactive thioesters, which are subject to nucleophilic attack by hydroxyl or amino groups on cell membrane proteins and carbohydrates.
- Breakage of the thioester leads to the formation of covalent bonds between the membrane macromolecules and the complement components.
- If this covalent bond formation does not occur quickly after generation of the C3b and C4b fragments, the thioester will be hydrolyzed to a nonreactive form

The Lectin Pathway of complement activation



Initiation

- Initiated when soluble proteins recognize microbial antigens.
- Instead of relying on antibodies to recognize microbial threat and to initiate the complement activation process (as in classical pathway), this pathway uses lectins – *proteins that recognize specific carbohydrate components primarily found on microbial surfaces* – as its specific receptor molecules.
- Component of innate immunity.
- Mannose-binding lectin (MBL), the first lectin demonstrated to be capable of initiating complement activation, binds close-knit arrays of mannose residues found on microbial surfaces of *Salmonella*, *Listeria*, and *Neisseria* strains of bacteria; *Cryptococcus neoformans* and *Candida albicans* strains of fungi; and on membranes of some viruses like HIV-1 and respiratory syncytial virus.
- The complement pathway that is initiated is the lectin pathway of complement activation.
- Like the classical pathway, it proceeds through the activation of a C3 convertase composed of C4b and C2a.

Other initiators of lectin pathway

- Another family of proteins structurally related to collectins termed ficolins act as additional initiators of the lectin pathway of complement activation.
- L-ficolin, H-ficolin, and M-ficolin each bind specific types of carbohydrates on microbial surfaces.

Intermediates in the lectin pathway of complement activation

- Intermediates are explained using MBL as the example.
- MBL is associated in the serum with MBL-Associated Serine Proteases, or MASP proteins.
- Three MASP proteins namely MASP1, MASP2 and MASP3 exist out of which MASP2 is most important factor in the next step of the MBL pathway.
- MASP-2 is structurally related to the serine protease C1s, and can cleave both C2 and C4, giving rise to the C3 convertase (C4b2a).
- Thus, lectin pathway utilizes the same components as classical pathway except the C1 complex.
- The soluble lectin receptor replaces the antibody as the antigen-recognizing component, and MASP proteins take the place of C1r and C1s in cleaving and activating the C3 convertase.
- Once C3 convertase is formed, the reactions of lectin pathway are the same as for classical pathway.
- The C5 convertase of the lectin pathway, like that of the classical pathway, is also C4b2a3b.

The Alternate Pathway of complement activation

Initiation

Initiated in three distinct ways

1. Initiation is independent of antibody-antigen interactions, hence this pathway is also considered to be part of the innate immune system (like the lectin pathway).
2. Unlike lectin pathway, this pathway uses its own set of C3 and C5convertases.
3. In the alternative pathway C3 convertase is made up of one molecule of C3b and one molecule unique to the alternative pathway, Bb.
4. A second C3bis then added to make the alternative pathway C5 convertase.

Intermediates in the alternate pathway of complement activation

i. C3 undergoes spontaneous hydrolysis to C3(H_2O), which binds serum factor B.
ii. On binding to C3(H_2O), B is cleaved by serum factor D, and the resultant C3(H_2O)Bb complex forms a fluid phase C3 convertase.
iii. Some C3b, released after C3 cleavage by this complex, binds to microbial surfaces.
iv. There, it binds factor B, which is cleaved by factor D, forming the cell-bound alternative pathway C3 convertase, C3bBb.
v. This complex is stabilized by properdin.
vi. C5 convertases are formed by addition of a C3b fragment to each of the C3 convertases.

Three complement pathways converge at the formation of C5 convertase

•The end result of the three initiation pathways is the formation of a C5 convertase.

•For the classical and lectin pathways, the C5 convertase has the composition C4b2a3b; for the alternative pathway, the C5 convertase has the formulation C3bBbC3b. (In the thrombin-initiated pathway, the anaphylatoxin C5a isformed by cleavage of C5 by the blood clotting enzymes, but functionally meaningful C5b concentrations are not generated by this route.)

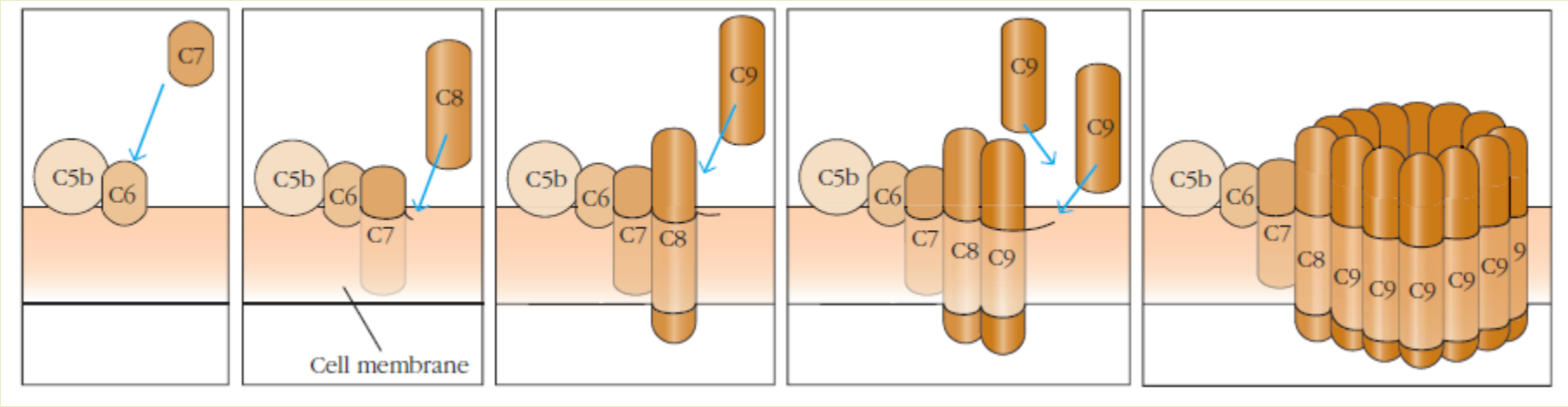
•However, the end result of all types of C5 convertase activity is the same: the cleavage of the C5 molecule into two fragments, C5a and C5b.

•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 MAC.

•However, the C5b component is extremely labile, is not covalently bound to the membrane like C3b and C4b, and is rapidly inactivated unless it is stabilized by the binding of C6.

C5 initiates the generation of MAC

•Till the formation of C5b, all reactions occur on the hydrophilic surfaces of microbes or on immune complexes in the fluid phase of blood, lymph, or tissues.
•In contrast, when C5b binds C6 and C7, the resulting complex undergoes a conformational change which exposes hydrophobic regions on the surface of the C7 component capable of inserting into the interior of the microbial membrane.
•C8 is made up of two peptide chains: C8β and C8αγ.
•Binding of C8β to the C5b67 complex induces a conformational change in the C8 dimer so that the hydrophobic domain of C8αγ can insert into the interior of the phospholipid membrane.
•The C5b678 complex can create a small pore, 10 Å in diameter, and formation of this pore can lead to lysis of red blood cells, but not of nucleated cells.
•The final step in the formation of the MAC is the binding and polymerization of C9 to the C5b678 complex.
•About 10 to 19 molecules of C9 can be bound and polymerized by a single C5b678 complex.
•During polymerization, C9 molecules undergo transition to insert into the membrane.
•The completed MAC, which has a tubular form and functional pore diameter of 70 Å to 100 Å, consists of a C5b678 complex surrounded by a poly-C9 complex .
•Loss of plasma membrane integrity leads irreversibly to cell death.



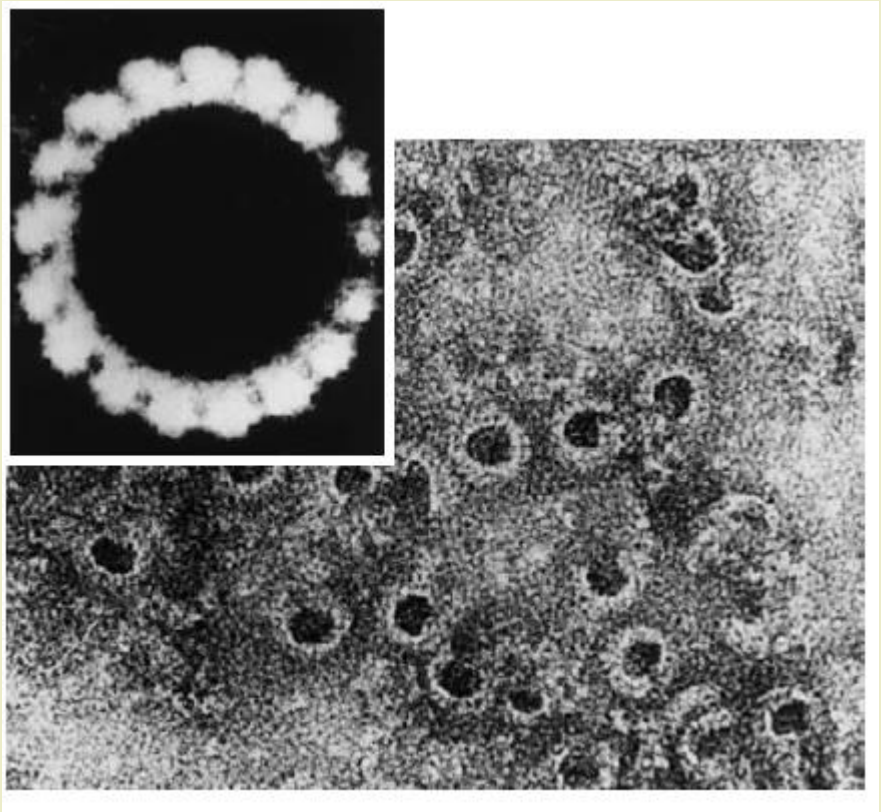


Figure 9: Formation of membrane attackcomplex (MAC) showing addition of C6, C7, C8, and C9 components to the C5b component.

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.

Under physiologic conditions, such lysis is usually minimized by regulatory proteins.

Complement receptors and function

Receptor	Alternative name(s)	Ligand	Cell surface binding or expression	Function
CR1	CD35	C3b, C4b, C1q, iC3b	Erythrocytes, neutrophils, monocytes, macrophages, eosinophils, FDCs, B cells, and some T cells	Clearance of immune complexes, enhancement of phagocytosis, regulation of C3 breakdown
CR2	CD21, Epstein-Barr virus receptor	C3d, C3dg (human), C3d (mouse) iC3b	B cells and 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, and C3c	Fixed-tissue macrophages	iC3b-mediated phagocytosis and inhibition of alternative pathway
$C1qR_p$	CD93	C1q, MBL	Monocytes, neutrophils, endothelial cell, platelets, T cells	Induces T-cell activation; enhances phagocytosis
SIGN-R1	CD209	C1q	Marginal zone and 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

Reference: Kuby Immunology, 3rdth , 6th and 7th edition

THANK YOU