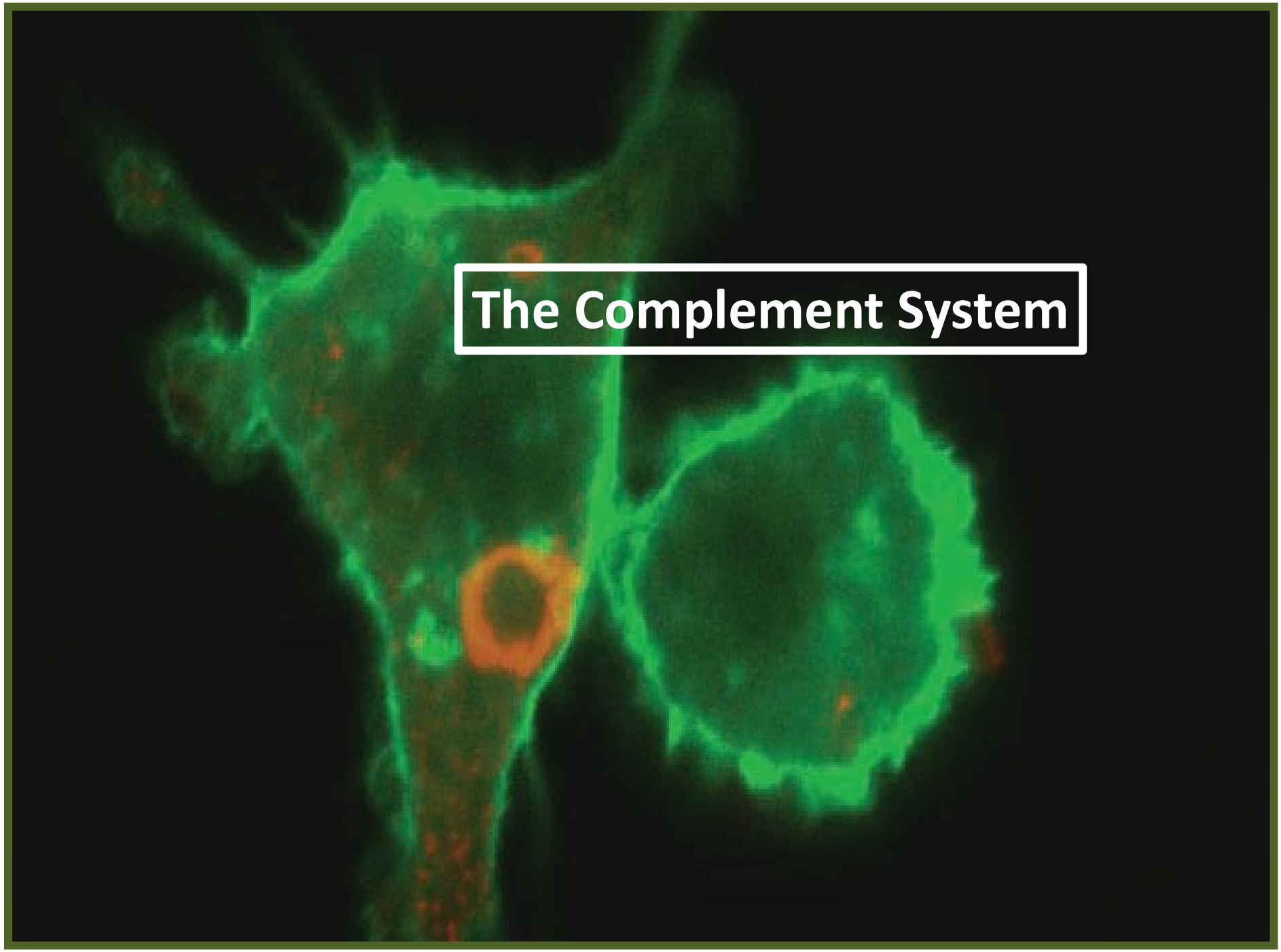
The Complement System

THE COMPLEMENT SYSTEM

The complement system consists of serum and cell surface proteins that interact with one another and with other molecules of the immune system in a highly regulated manner to generate products that function to eliminate microbes.

Several features of complement activation are essential for its normal function

- ***The complement system is activated by microbes and by antibodies that are attached to microbes and other antigens.***
- ***Activation of complement involves the sequential proteolysis of proteins to generate enzyme complexes with proteolytic activity***
- ***The products of complement activation become covalently attached to microbial cell surfaces, to antibodies bound to microbes and to other antigens, and to apoptotic bodies***
- ***Complement activation is inhibited by regulatory proteins that are present on normal host cells and absent from microbes***

Pathways of Complement Activation

- ***There are three major pathways of complement activation: the classical pathway, which is activated by certain isotypes of antibodies bound to antigens; the alternative pathway, which is activated on microbial cell surfaces in the absence of antibody; and the lectin pathway, which is activated by a plasma lectin that binds to mannose residues on microbes.***
- ***The central event in complement activation is proteolysis of the complement protein C3 to generate biologically active products and the subsequent covalent attachment of a product of C3, called C3b, to microbial cell surfaces or to antibody bound to antigen***.
- Complement activation depends on the generation of two proteolytic complexes: the **C3 convertase,** which cleaves C3 into two proteolytic fragments called C3a and C3b; and the **C5 convertase, which cleaves** C5 into C5a and C5b.
- Complement activation promotes phagocytosis because C3b becomes covalently linked to microbes, and phagocytes (neutrophils and macrophages) express receptors for C3b.
- Peptides produced by proteolysis of C3 (and other complement proteins) stimulate inflammation.
- The C5 convertase assembles after the prior generation of C3b, and this convertase contributes both to inflammation (by generation of the C5a fragment) and to the formation of pores in the membranes of microbial targets.

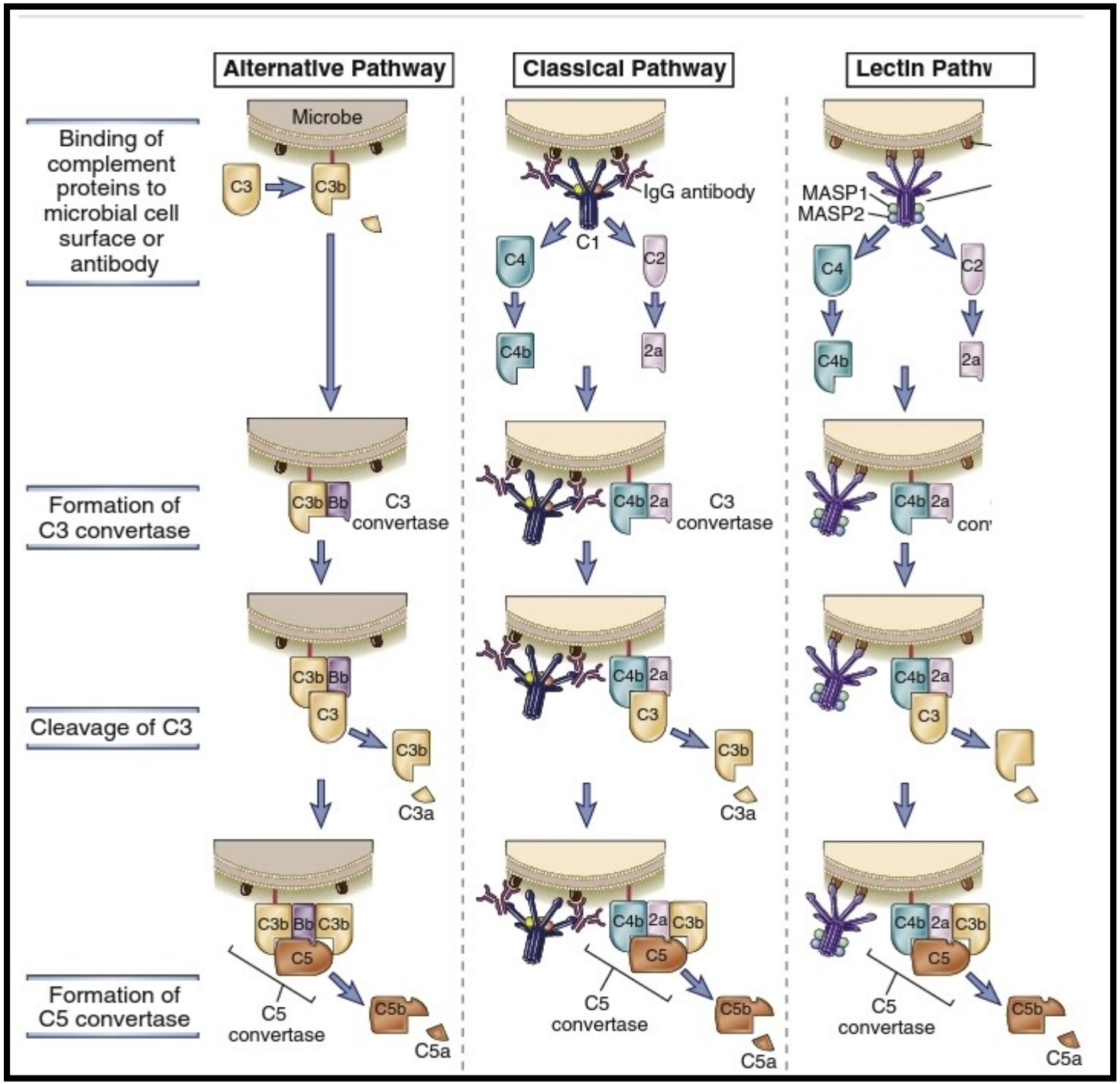

Fig:The early steps of complement activation by the alternative, classical, and lectin pathways.

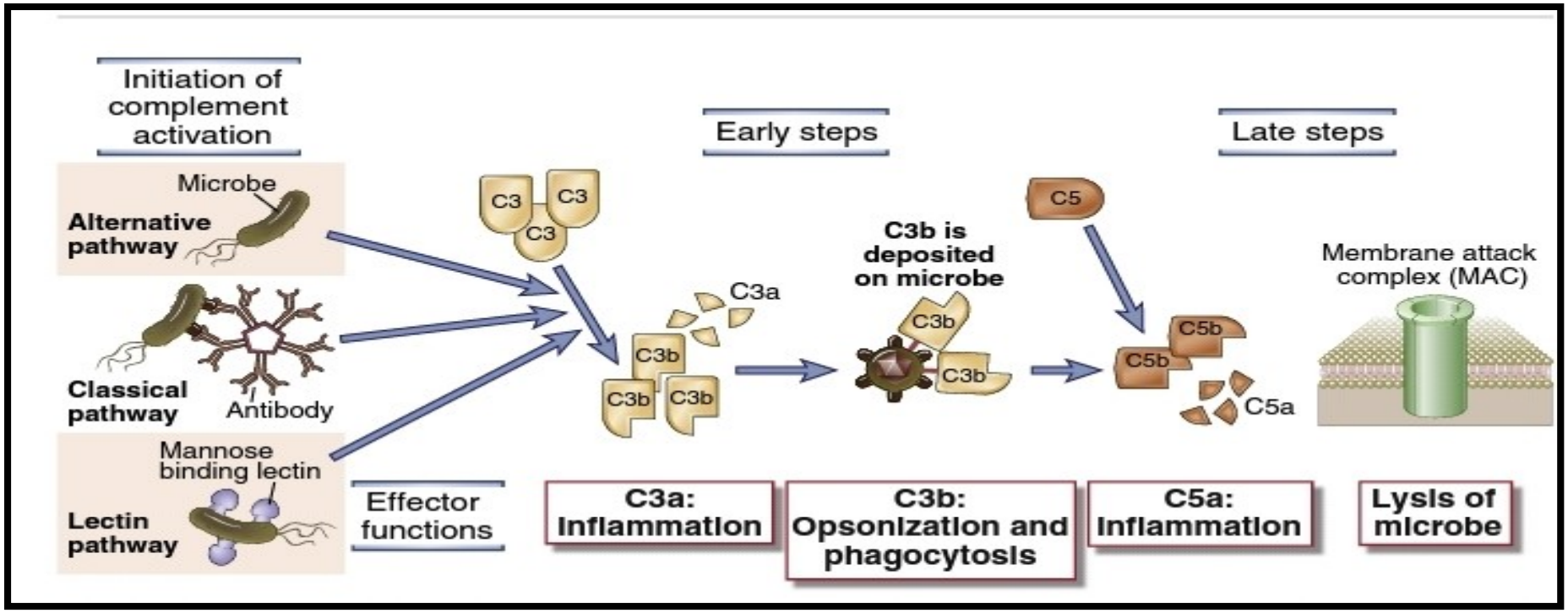

Fig:Pathways of complement activation. The activation of the complement system may be initiated by three distinct pathways, all of which lead to the production of C3b (the early steps). C3b initiates the late steps of complement activation, culminating in the production of peptides that stimulate inflammation (C5a) and polymerized C9, which forms the membrane attack complex, so called because it creates holes in plasma membranes. The principal functions of major proteins produced at different steps are shown.

The Alternative Pathway

The alternative pathway of complement activation results in the proteolysis of C3 and the stable attachment of its breakdown product C3b to microbial surfaces, without a role for antibody.

Spontaneous hydrolysis of plasma C3 leads to the formation of a fluid-phase C3 convertase and the generation of C3b. If the C3b is deposited on the surfaces of microbes, it binds Factor B and forms the alternative pathway C3 convertase.

This convertase cleaves C3 to produce more C3b, which binds to the microbial surface and participates in the formation of a C5 convertase. The C5 convertase cleaves C5 to generate C5b,the initiating event in the late steps of complement activation.

Alternative pathway activation readily occurs on microbial cell surfaces and not on mammalian cells.

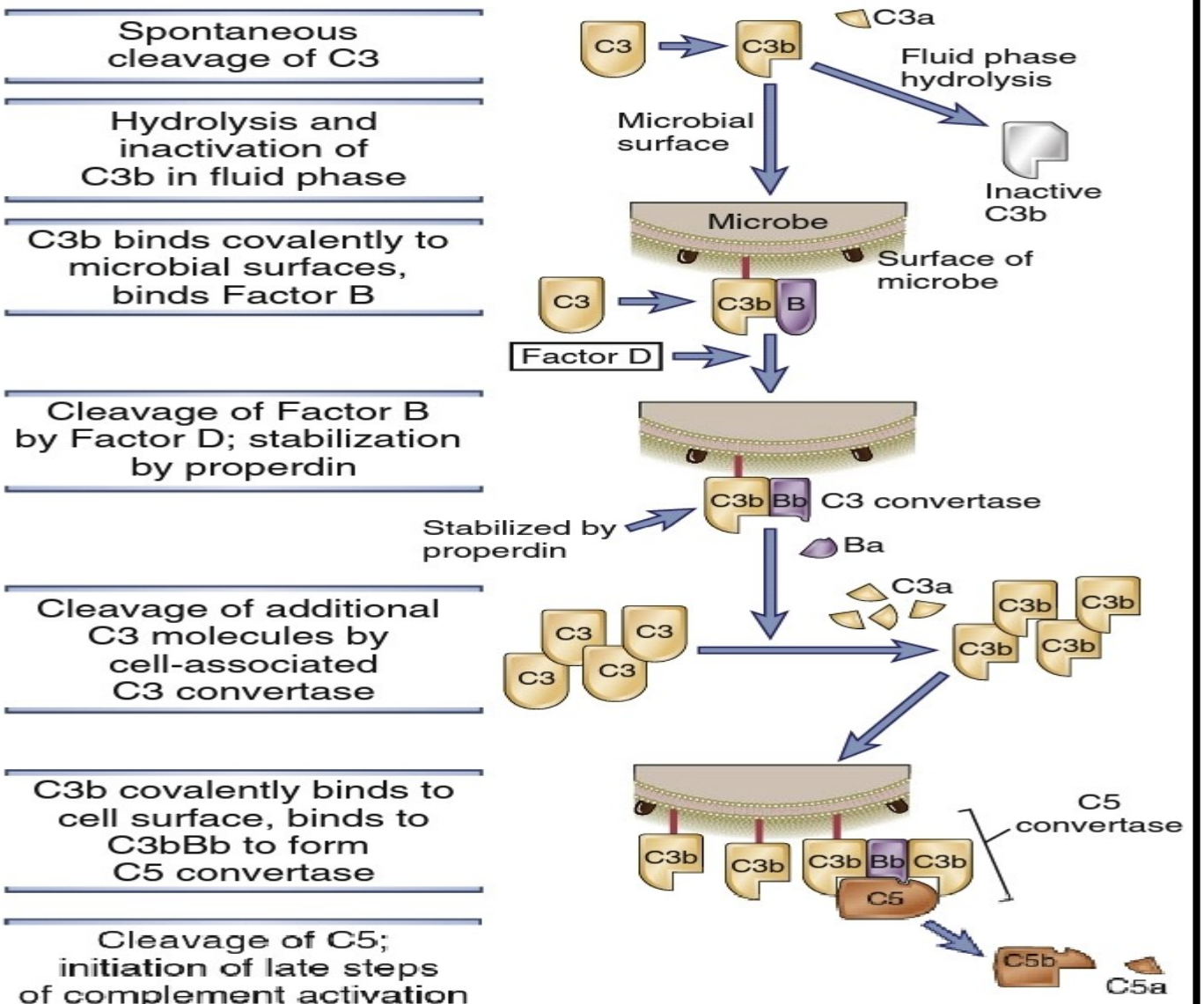

Fig:The alternative pathway of complement activation

TABLE 13-4 Proteins of the Alternative Pathway of Complement

Protein	Structure	Serum Concentration (μg/mL)	Function
C3	185 kD (α subunit, 110 kD; β subunit, 75 kD)	1000-1200	C3b binds to the surface of the microbe, where it functions as an opsonin and as a component of C3 and C5 convertases. C3a stimulates inflammation (anaphylatoxin).
Factor B	93-kD monomer	200	Bb is a serine protease and the active enzyme of the C3 and C5 convertases.
Factor D	25-kD monomer	1-2	Plasma serine protease cleaves factor B when it is bound to C3b.
Properdin	Composed of up to four 56-kD subunits	25	Properdin stabilizes C3 convertases (C3bBb) on microbial surfaces.

The Classical Pathway

The classical pathway is initiated by binding of the complement protein C1 to the CH2 domains of IgG or the CH3 domains of IgM molecules that have bound antigen. Only antibodies bound to antigens, and not free circulating antibodies, can initiate classical pathway activation.

Antigen-antibody complexes that activate the classical pathway may be soluble, fixed on the surface of cells , or deposited on extracellular matrices. The classical pathway is initiated by the binding of C1 to antigen-complexed antibody molecules, which leads to the production of C3 and C5 convertases attached to the surfaces where the antibody was deposited. The C5 convertase cleaves C5 to begin the late steps of complement activation.

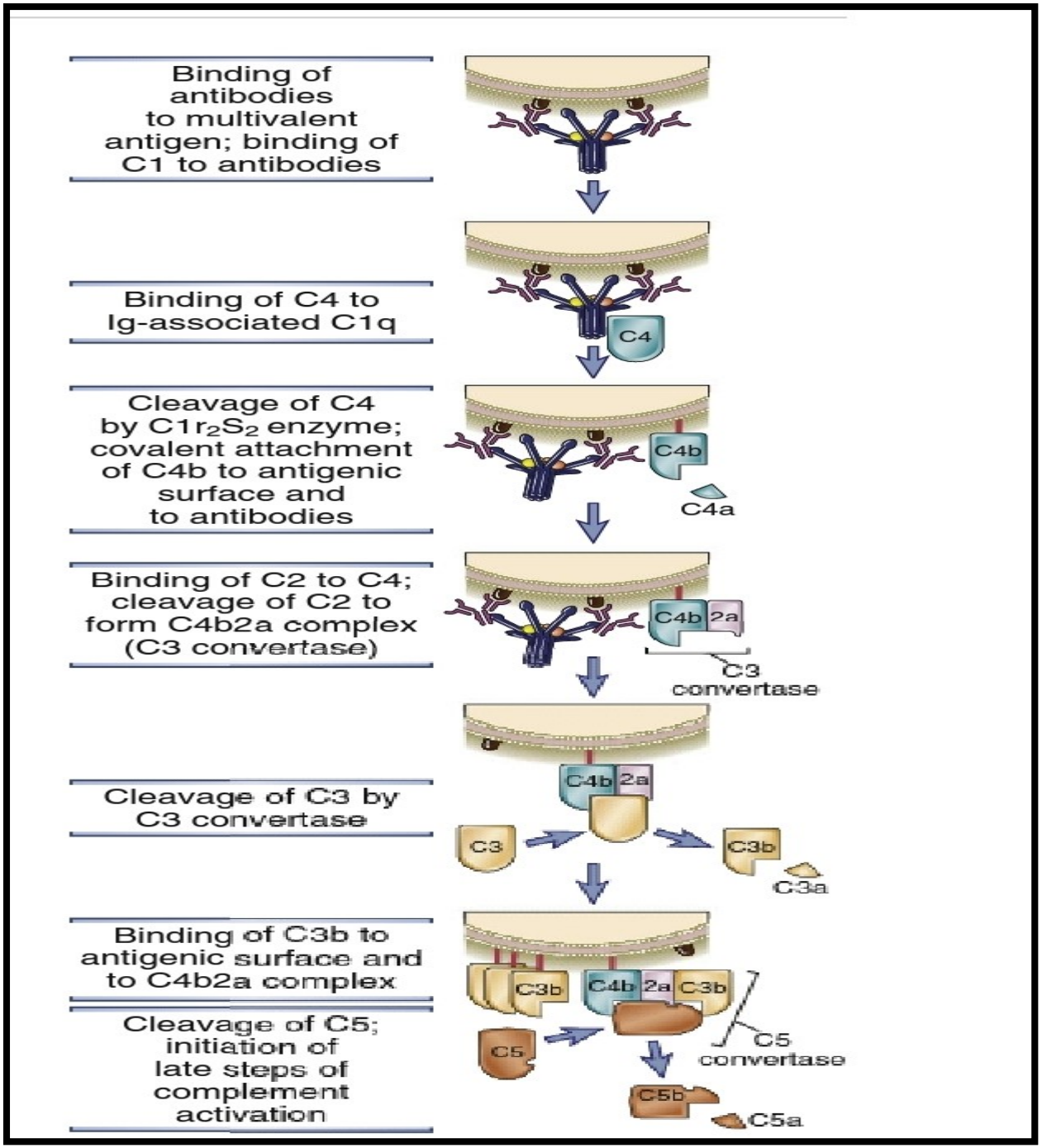

Fig:The classical pathway of complement activation.

Structure of C1

Structure of C1. C1q consists of six identical subunits arranged to form a central core and symmetrically projecting radial arms. The globular heads at the end of each arm, designated H, are the contact regions for immunoglobulin. C1r and C1s form a tetramer composed of two C1r and two C1s molecules. The ends of C1r and C1s contain the catalytic domains of these proteins. One C1r2s2 tetramer wraps around the radial arms of the C1q complex in a manner that juxtaposes the catalytic domains of C1r and C1s.

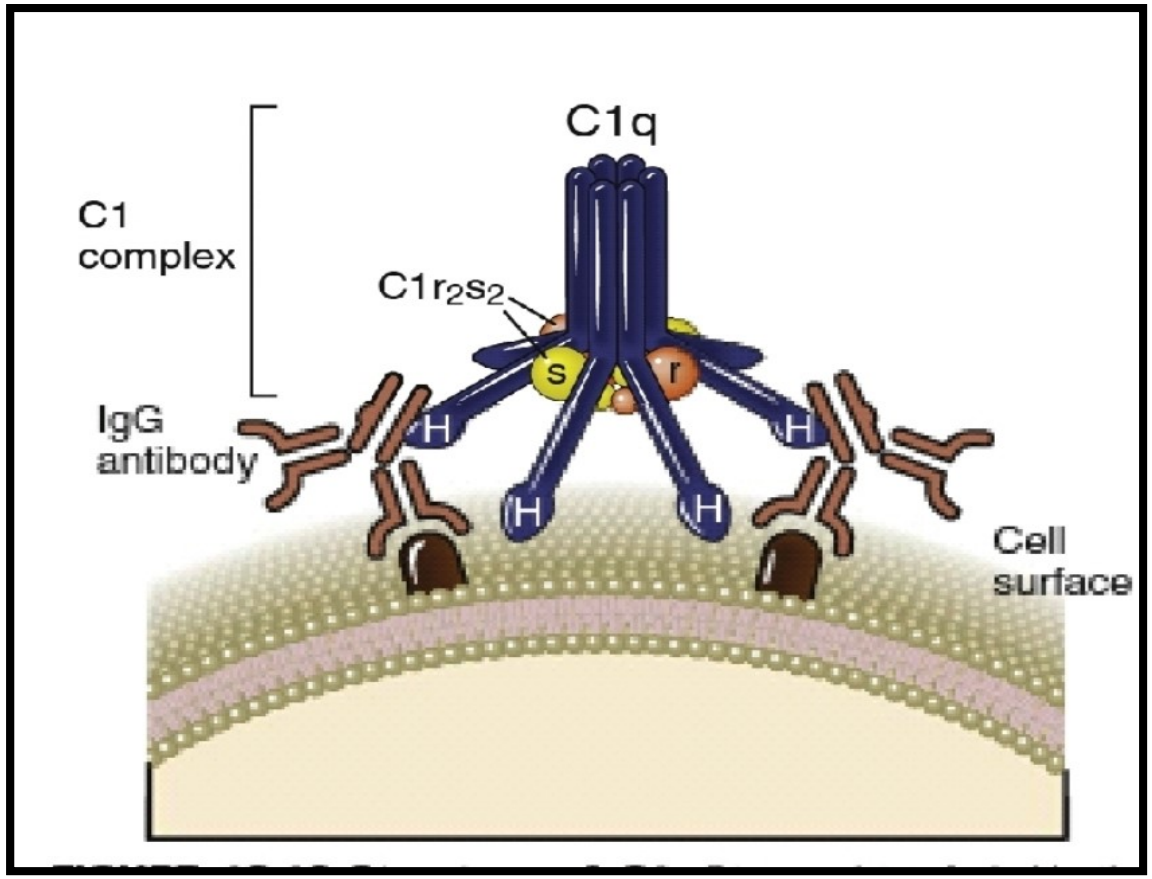

Fig:Structure of C1q

TABLE 13-5 Proteins of the Classical Pathway of Complement

Protein	Structure	Serum Concentration (μg/mL)	Function
C1 (C1qr2s2)	750 kD		Initiates the classical pathway
C1q	460 kD; hexamer of three pairs of chains (22, 23, 24 kD)	75-150	Binds to the Fc portion of antibody that has bound antigen, to apoptotic cells, and to cationic surfaces
C1r	85-kD dimer	50	Serine protease, cleaves C1s to make it an active protease
C1s	85-kD dimer	50	Serine protease, cleaves C4 and C2
C4	210 kD, trimer of 97-, 75-, and 33-kD chains	300-600	C4b covalently binds to the surface of a microbe or cell, where antibody is bound and complement is activated. C4b binds C2 for cleavage by C1s. C4a stimulates inflammation (anaphylatoxin).
C2	102-kD monomer	20	C2a is a serine protease and functions as the active enzyme of C3 and C5 convertases to cleave C3 and C5.
C3	See Table 13-4		

The Lectin Pathway

The lectin pathway of complement activation is triggered in the absence of antibody by the binding of microbial polysaccharides to circulating lectins, such as plasma mannose (or mannan)–binding lectin (MBL),or to ficolins.

- These soluble lectins are collagen- like proteins that structurally resemble C1q . MBL, L-ficolin, and H-ficolin are plasma proteins.
- The collagen-like domains present in these plasma proteins help assemble basic triple helical structures that can form higherorder oligomers.
- MBL binds to mannose residues on polysaccharides; the ficolin fibrinogen-like domain binds *N-acetylglucosamine–containing* glycans.
- MBL and ficolins associate with MBL-associated serine proteases (MASPs) including MASP1, MASP2, and MASP3.
- The MASP proteins are structurally homologous to the C1r and C1s proteases and serve a similar function, namely, the cleavage of C4 and C2 to activate the complement pathway. Higher-order oligomers of MBL associate with MASP1 and MASP2, although MASP3/MASP2 complexes may also be found.
- MASP1 (or MASP3) can form a tetrameric complex with MASP2 similar to the one formed by C1r and C1s, and MASP2 is the protease that cleaves C4 and C2.

Subsequent events in this pathway are identical to those that occur in the classical pathway

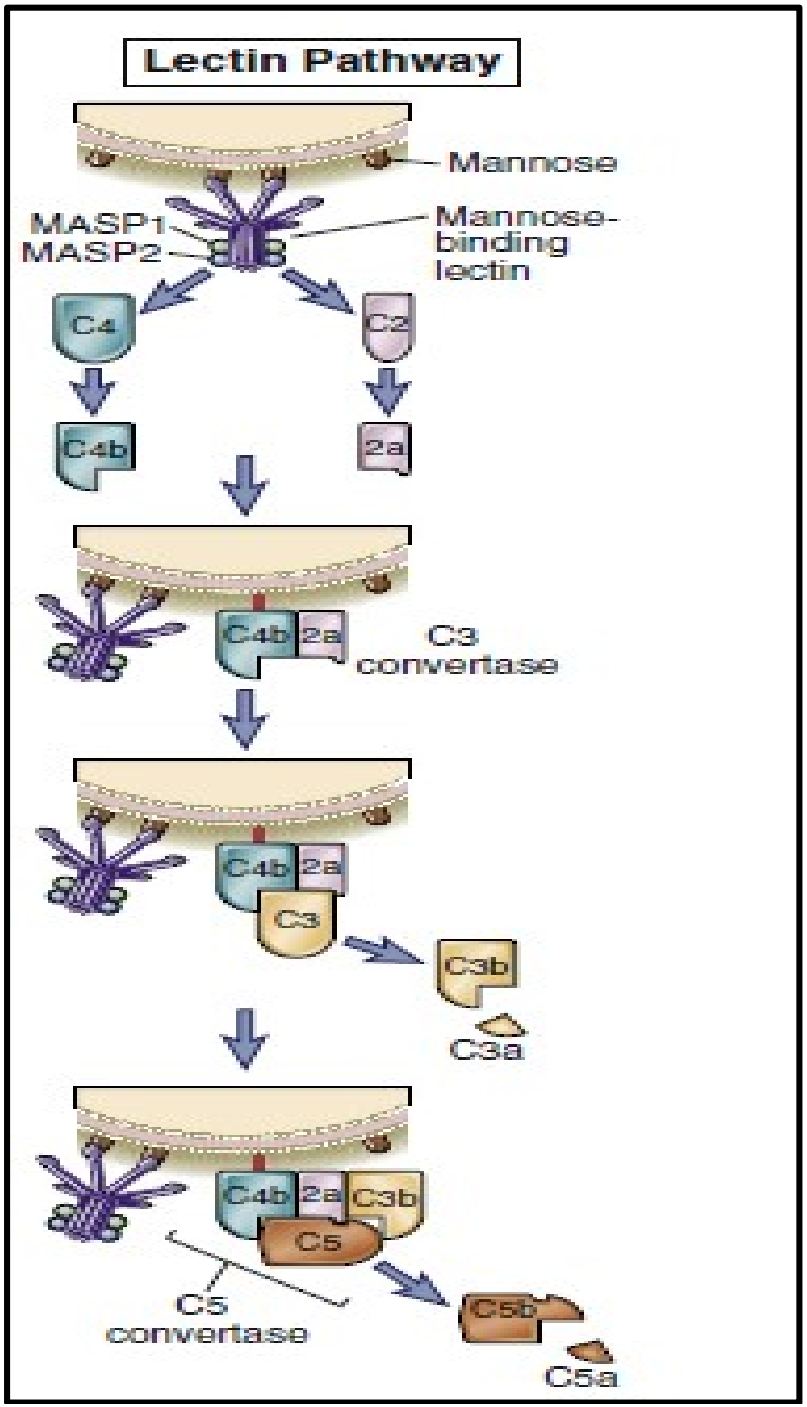
Lectin Pathway
Mannose
MASP1
MASP2
Mannose-binding lectin
C4
C2
C4b
2a
C4b 2a
C3 convertase
C4b 2a
C3
C3b
C3a
C4b 2a C3b
C5
C5 convertase
C5b
C5a

TABLE 13-6 Proteins of the Lectin Pathway of Complement

Protein	Structure	Serum Concentration (μg/mL)	Function
Mannose-binding lectin	Helical trimer of 32-kD chain and dimers to hexamers of this triple helix	1-8	Agglutinin, opsonin, complement fixing
M-ficolin (ficolin-1)	Helical trimer of 34-kD chain and a tetramer of this triple helix	Undetectable	Agglutinin, opsonin, complement fixing
L-ficolin (ficolin-2)	Helical trimer of 34-kD chain and a tetramer of this triple helix	1-7	Agglutinin, opsonin, complement fixing
H-ficolin (ficolin-3)	Helical trimer of 34-kD chain and a tetramer of this triple helix	6-83	Agglutinin, opsonin, complement fixing
MASP1	90-kD homodimer; homology to C1r/C1s	2-13*	Forms complex with MASP2 and collectins or ficolins and activates MASP3
MASP2	110-kD homodimer; homology to C1r/C1s	2-13	Forms complex with lectins, especially ficolin-3
MASP3	76-kD homodimer; homology to C1r/C1s	0.02-1.0	Associates with collectins or ficolins and MASP1 and cleaves C4

*Published concentrations may have been influenced by cross-reactivity of antibodies with MASP3; concentrations of the latter are derived by use of specific monoclonal antibodies. Most of these are plasma proteins, except M-ficolin, which is secreted by activated macrophages.

Late Steps of Complement Activation

- C5 convertases generated by the alternative, classical, or lectin pathway initiate activation of the late components of the complement system, which culminates in formation of the cytocidal membrane attack complex (MAC).
- C5 convertases cleave C5 into a small C5a fragment that is released and a two-chain C5b fragment that remains bound to the complement proteins deposited on the cell surface.
- The remaining components of the complement cascade, C6, C7, C8, and C9, are structurally related proteins without enzymatic activity.
- C5b transiently maintains a conformation capable of binding the next proteins in the cascade, C6 and C7.
- The C7 component of the resulting C5b,6,7 complex is hydrophobic, and it inserts into the lipid bilayer of cell membranes, where it becomes a high-affinity receptor for the C8 molecule.
- The C8 protein is a trimer composed of three distinct chains, one of which binds to the C5b,6,7 complex and forms a covalent heterodimer with the second chain; the third chain inserts into the lipid bilayer of the membrane. This stably inserted C5b,6,7,8 complex (C5b-8) has a limited ability to lyse cells.
- The formation of a fully active MAC is accomplished by the binding of C9, the final component of the complement cascades to the C5b-8 complex.

- C9 is a serum protein that polymerizes at the site of the bound C5b-8 to form pores in plasma membranes.
- These pores are about 100 Å in diameter, and they form channels that allow free movement of water and ions.
- The entry of water results in osmotic swelling and rupture of the cells on whose surface the MAC is deposited

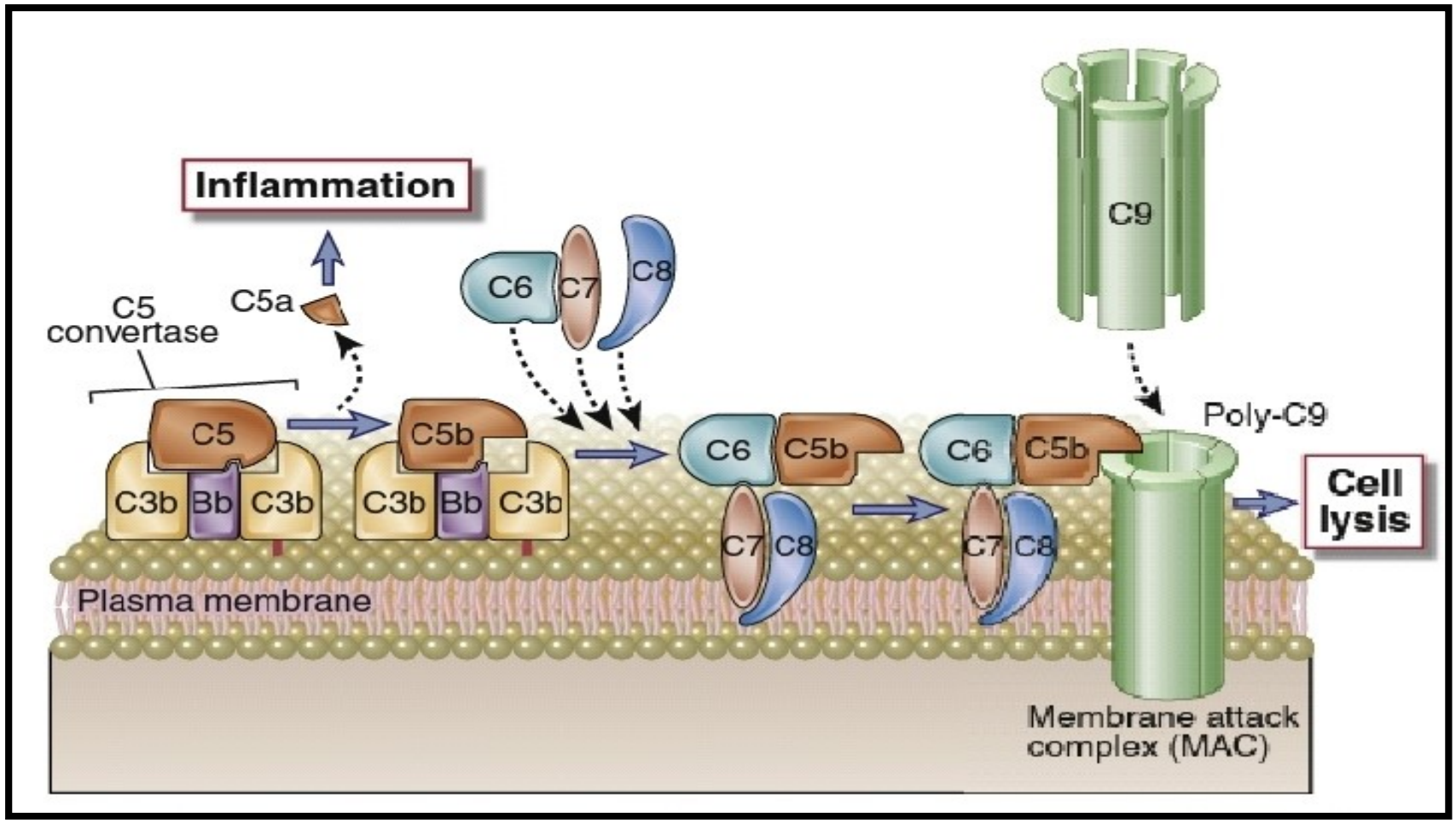

Fig:Late steps of complement activation and formation of the MAC

Receptors for Complement Proteins

Many of the biologic activities of the complement system are mediated by the binding of complement fragments to membrane receptors expressed on various cell types. The best characterized of these receptors are specific for fragments of C3 and are described in Table 13-8. Other receptors include those for C3a, C4a, and C5a, which stimulate inflammation, and some that regulate complement activation.

TABLE 13-8 Receptors for Fragments of C3

Receptor	Structure	Ligands	Cell Distribution	Function
Type 1 complement receptor (CR1, CD35)	160-250 kD; multiple CCPRs	C3b > C4b > iC3b	Mononuclear phagocytes, neutrophils, B and T cells, erythrocytes, eosinophils, FDCs	Phagocytosis Clearance of immune complexes Promotes dissociation of C3 convertases by acting as cofactor for cleavage of C3b, C4b
Type 2 complement receptor (CR2, CD21)	145 kD; multiple CCPRs	C3d, C3dg > iC3b	B lymphocytes, FDCs, nasopharyngeal epithelium	Coreceptor for B cell activation Trapping of antigens in germinal centers Receptor for EBV
Type 3 complement receptor (CR3, Mac-1, CD11bCD18)	Integrin, with 165-kD α chain and 95-kD β2 chain	iC3b, ICAM-1; also binds microbes	Mononuclear phagocytes, neutrophils, NK cells	Phagocytosis Leukocyte adhesion to endothelium (via ICAM-1)
Type 4 complement receptor (CR4, p150,95, CD11cCD18)	Integrin, with 150-kD α chain and 95-kD β2 chain	iC3b	Mononuclear phagocytes, neutrophils, NK cells	Phagocytosis, cell adhesion?

CCPRs, complement control protein repeats; *EBV*, Epstein-Barr virus; *FDCs*, follicular dendritic cells; *ICAM-1*, intercellular adhesion molecule 1.

Functions of Complement:

The principal effector functions of the complement system in innate immunity and adaptive humoral immunity are to promote phagocytosis of microbes on which complement is activated, to stimulate inflammation, and to induce the lysis of these microbes. In addition, products of complement activation facilitate the activation of B lymphocytes and the production of antibodies. Phagocytosis, inflammation, and stimulation of humoral immunity are all mediated by the binding of proteolytic fragments of complement proteins to various cell surface receptors, whereas cell lysis is mediated by the MAC.

- **Opsonization and Phagocytosis:**

Microbes on which complement is activated by the alternative or classical pathway become coated with C3b, iC3b, or C4b and are phagocytosed by the binding of these proteins to specific receptors on macrophages and neutrophils.

- **Stimulation of Inflammatory Responses:**

The proteolytic complement fragments C5a, C4a, and C3a induce acute inflammation by activating mast cells, neutrophils and endothelial cells.

- **Complement-Mediated Cytolysis:**

Complement-mediated lysis of foreign organisms is mediated by the MAC.

- **Other Functions**:

By binding to antigen-antibody complexes, complement proteins promote the solubilization of these complexes and their clearance by phagocytes.

The C3d protein generated from C3 binds to CR2 on B cells and facilitates B cell activation and the initiation of humoral immune responses.

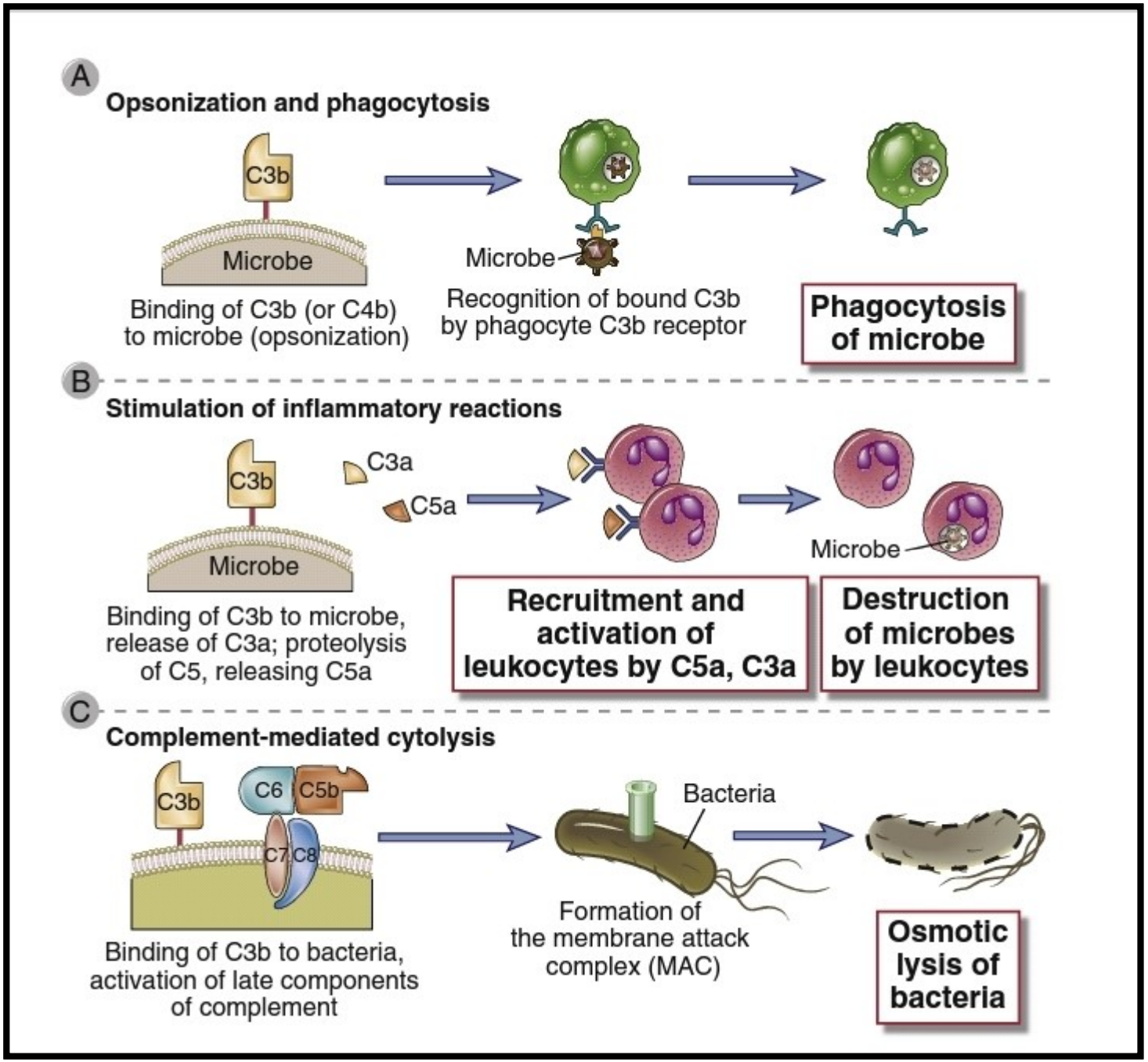
A
Opsonization and phagocytosis
C3b
Microbe
Binding of C3b (or C4b) to microbe (opsonization)
Microbe
Recognition of bound C3b by phagocyte C3b receptor
Phagocytosis of microbe
B
Stimulation of inflammatory reactions
C3b
Microbe
C3a
C5a
Binding of C3b to microbe, release of C3a; proteolysis of C5, releasing C5a
Recruitment and activation of leukocytes by C5a, C3a
Microbe
Destruction of microbes by leukocytes
C
Complement-mediated cytolysis
C3b
C6
C5b
C7
C8
Binding of C3b to bacteria, activation of late components of complement
Bacteria
Formation of the membrane attack complex (MAC)
Osmotic lysis of bacteria

Fig:Functions of complement. The major functions of the complement system in host defense are shown. Cell-bound C3b is an opsonin that promotes phagocytosis of coated cells (A); the proteolytic products C5a, C3a, and (to a lesser extent) C4a stimulate leukocyte recruitment and inflammation (B); and the MAC lyses cells(C)