The complement system

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Introduction

- The complement system is one of the major effector mechanisms of humoral and innate immunity.

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

- It is activated by microbes and by antibodies attached to microbes and other antigens.

- Activation of complement involves sequential proteolysis of the proteins to generate enzyme complexes with proteolytic activity.
- The products of complement activation become covalently attached to microbial surfaces or to antibodies bound to microbes and other antigens.
- 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** : activated by certain isotypes of antibodies bound to antigens.
- **The alternative pathway** : activated on microbial surfaces in the absence of the antibody
- **The lectin pathway** : activated by a plasma lectin that binds to mannose residues on microbes

- The complement activation pathways differ in how they are initiated, all of them result in the generation of enzyme complexes that are able to cleave the most abundant complement protein , C3.

- Hence, the proteolysis of C3 to generate biologically active products and the subsequent covalent attachment of a product of C3 called C3b to microbial surfaces is the central event in complement activation.

- Complement activation depends on the generation of two proteolytic complexes: the C3 convertase which cleaves C3 into two proteolytic fragments (C3a and C3b).

- The second proteolytic complex is the C5 convertase which cleaves C5 into C5a and C5b.
- The pathways of complement activation differ in how C3b is produced but follow a common sequence of reactions after cleavage of C5.
- Complement activation promote phagocytosis as phagocytes express receptors for C3b and contributes in inflamation by peptides produced by C3 cleavage.
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- The C5 convertase assemble after the prior generation of C3b and this contributes to both inflammation (C5a) and formation of pores in the membranes of target microbes.

The Alternative pathway

- It is one of the effector mechanisms of innate immunity. Complement activation through the alternative pathway results in the proteolysis of C3 and the stable attachment of its breakdown product C3b to microbial surfaces, without the role for the antibody.

- Normally, C3 in plasma (the fluid phase) is being continuously cleaved at a low rate to generate C3b in a process called C3 tickover. As C3 contains a reactive thoiester domain that exposed after cleavage.

- A small amount of C3b may become covalently attached to cell surfaces , including microbes through this thioester bond.

- If these bonds are not formed the C3b remainds in the fluid Phase and the thioester bond is hydrolysed , rendering the protein inactive and further complement activation is prohibited.

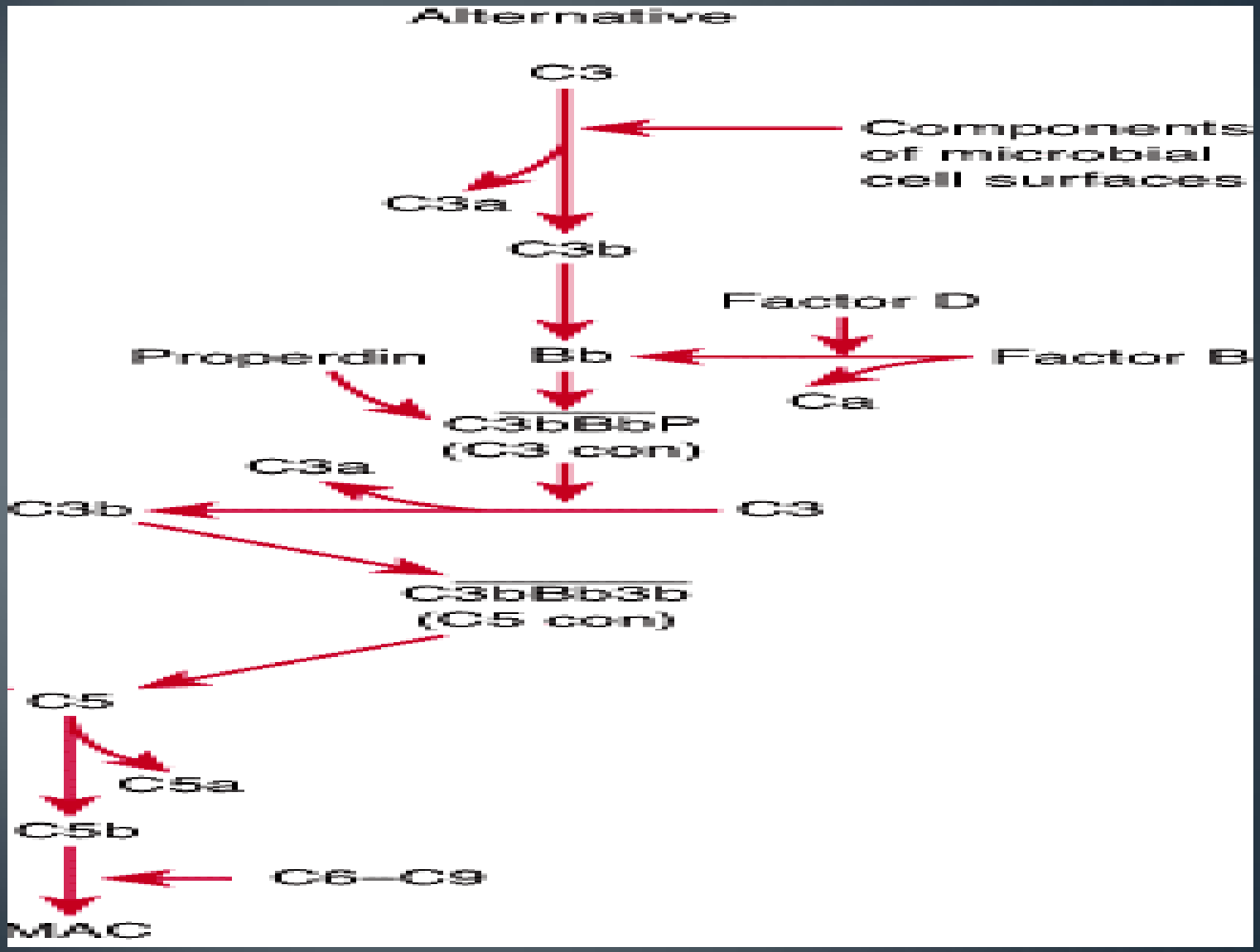
- When C 3b bind to the surface of microbes , it undergoes a conformational change , and a plasma protein factor called factor B binds to the C3b forming C3bB.

- The bound factor B ids cleaved by a plasma serine protease called factor D and releasing a big fragment called Bb that remained attached to C3b forming C3bBb. This comlex is the alternative pathway C3 convertase and it cleaves more C3 mol. And hence setting an amplification sequence.

- Properdin , is an alternative pathway protein that binds to and stabilizes the C3bBb complex. Its attachment is favored on microbial as opposed to normal host cells.

- Some of the C3b molecules formed by the alternative pathway convertase binds to the convertase itself, this results in the formation of the alternative pathway C5 convertase (C3bBbC3b).

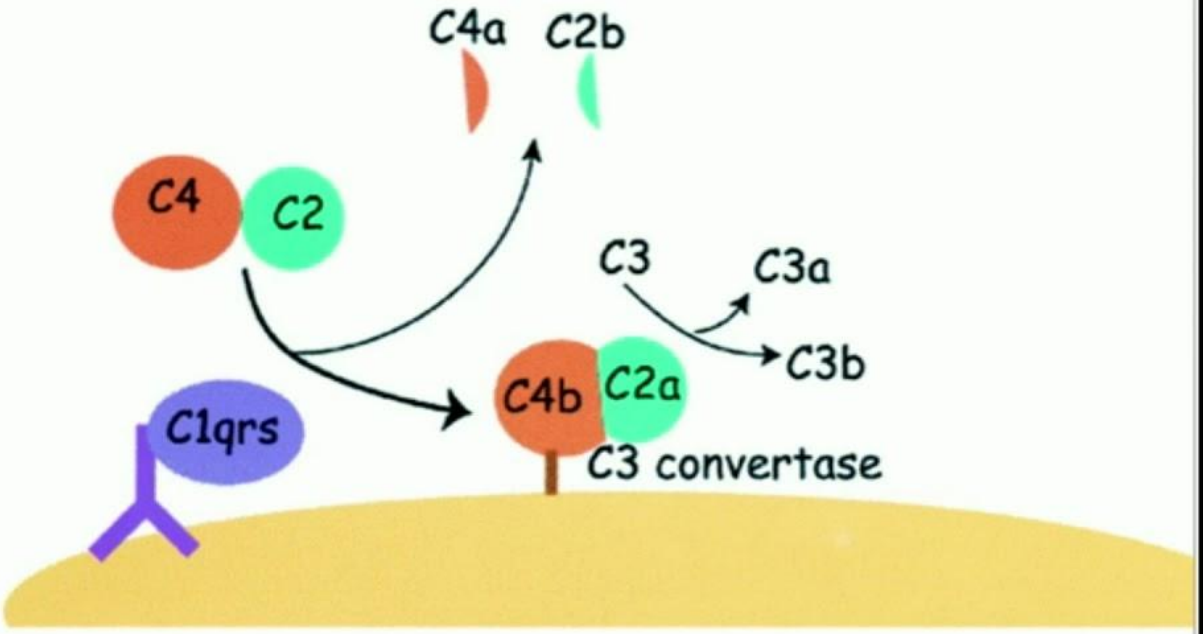
- The C5 convertase cleaves C5 and initiates the late steps of complement activation.

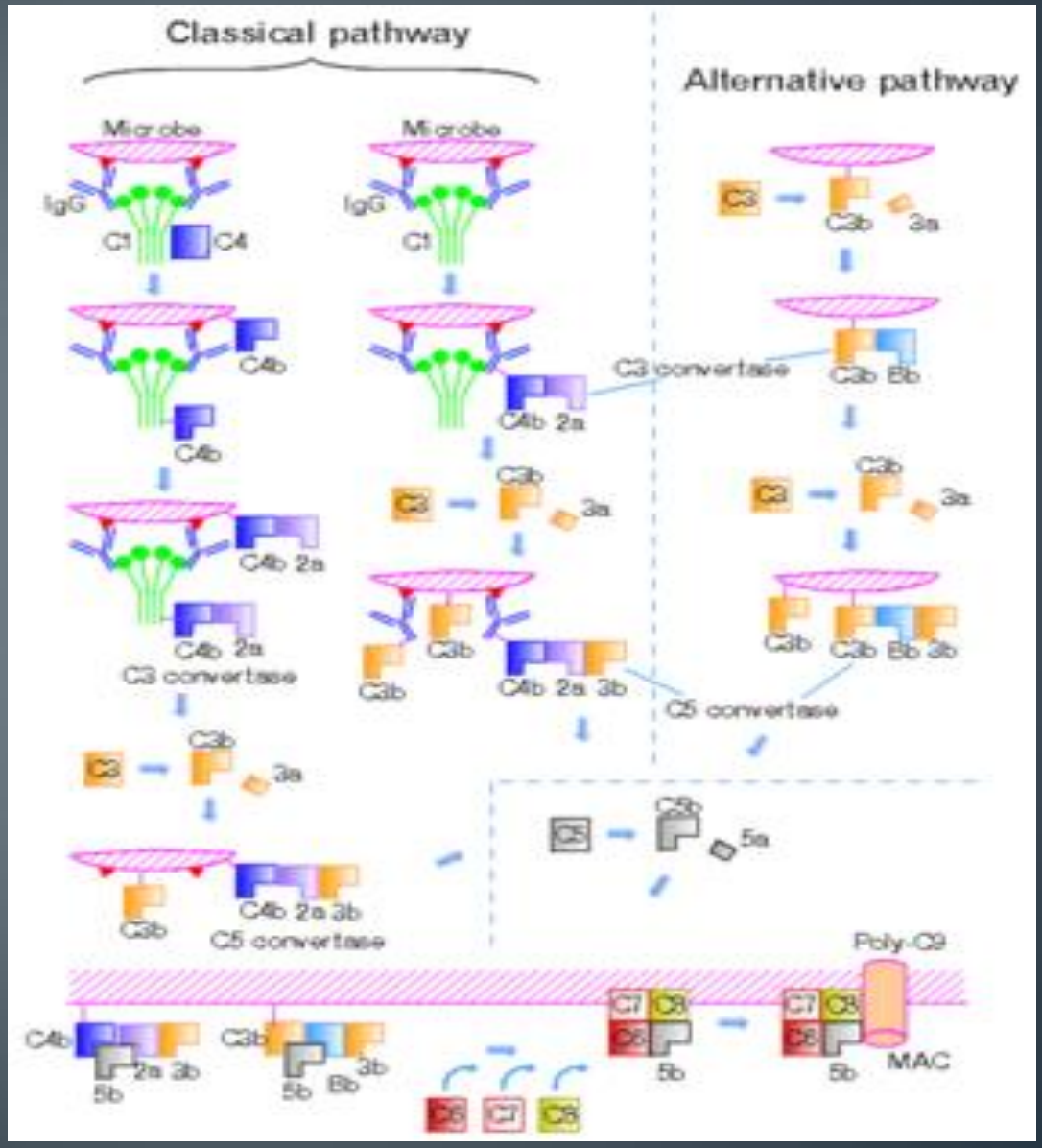

Alternative
C3
Components of microbial cell surfaces
C3a
C3b
Factor D
Properdin
Bb
Factor B
Ca
C3bBbP
(C3 con)
C3a
C3b
C3
C3bBb3b
(C5 con)
C5
C5a
C5b
C6—C9
MAC

The Classical Pathway

- The classical complement activation pathway is on of the effector mechanisms of the humeral immunity.
- It is initiated by binding of the complement protein C1 the CH2 domain of IgG or the CH3 domain of IgM.
- C1 is a large metameric protein composed of C1q,C1s and C1r subunits. C1q bind to the antibody and C1s and C1r are proteases.
- Each Ig Fc region has one binding site for C1q and each C1q molecule must bind at least 2 Ig heavy chains to be activated.

C3 Convertase (C3-Activating Complex)




Classical pathway
Alternative pathway
Microbe
IgG
C1
C4
C4b
C4b 2a
C3 convertase
C3
C3b
3a
C4b 2a 3b
C5 convertase
C5
5a
Poly-C9
C7
C8
C6
5b
MAC
Bb
2a
3b

- Binding of 2 or more of the globular heads of C1q to the Fc region of IgG or IgM leads to enzymatic activation of C1r which cleaves and activates C1s.

- Activated C1s cleaves the next protein in the complement cascade C4(homologous to C3 of alternative P.) to generate C4a (small fragment which is released) and C4b which binds to the Ag- Ab complex or to the adjacent cell surface to which the antibody is bound.

- C2 then complexes with C4b and is cleaved by a near by C1s to generate C2b(unknown importance) and C2a fragment which remains physically associated to the C4b.

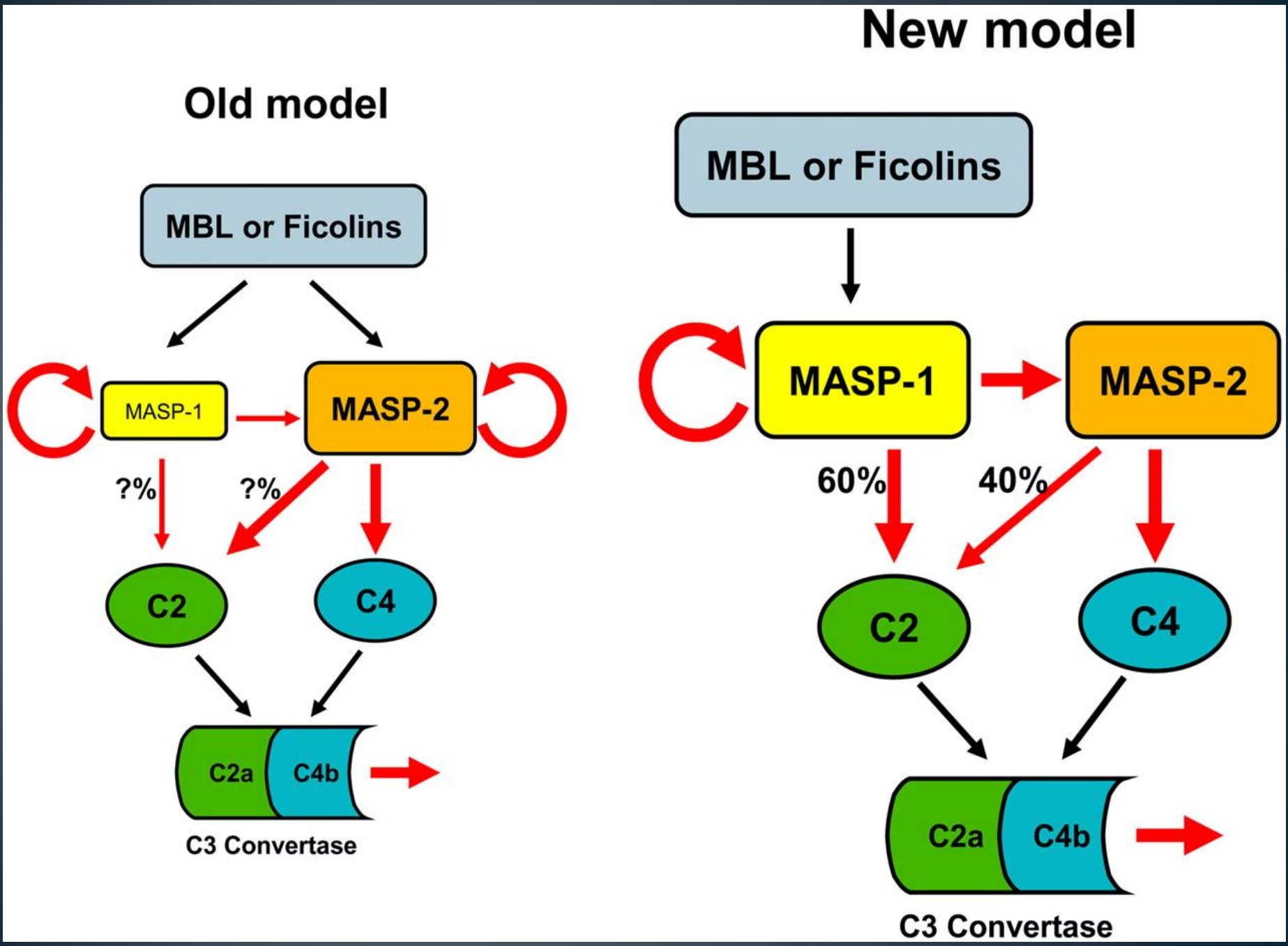
- The resulting C4b2a is the classical pathway C3 convertase, it cleaves and cleaves C3(C4b binds and 2a cleaves), yielding C3b which binds to the Ig complex or cell surface.

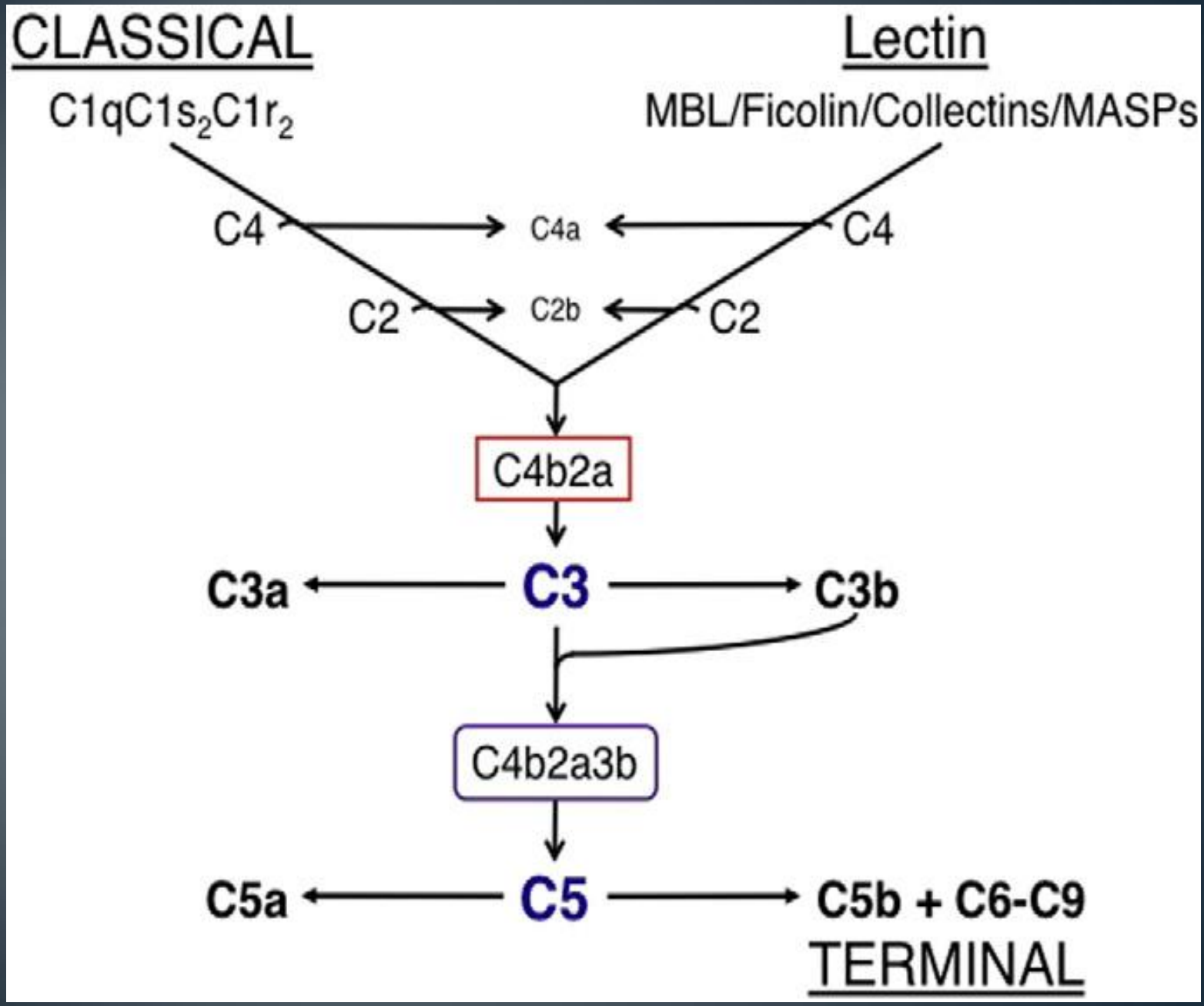
- Once C3b is bound it binds to factor B and generates more C3 convertase by the alternative pathway. The process of activation then proceeds as discussed in the alternative pathway.

N.B: An antibody independent variant of Classical pathway which is activated by carbohydrate binding to cell surface lectin (SIGN-R1 expressed by splenic marginal zone macrophages occurs in pneumococcal infections.

The Lectin Pathway

- The lectin pathway is one of the effector mechanisms of the innate immunity.
- The lectin pathway is triggered by binding of the microbial polysaccharide to circulating Lectins as mannose (mannan) binding lectins or to ficolins.
- These soluble lectins are collagen-like proteins that structurally resemble C1q.
- MBL and ficolins associate with MBL-associated serine proteases (MASPs) which are structurally homologous C1r and C1s proteases and serve similar functions (cleavage and activation of C4 and C2).
- Subsequent events in this pathway are identical to classical pathway.


Old model
MBL or Ficolins
MASP-1
MASP-2
?%
?%
C2
C4
C2a
C4b
C3 Convertase
New model
MBL or Ficolins
MASP-1
MASP-2
60%
40%
C2
C4
C2a
C4b
C3 Convertase


CLASSICAL
Lectin
C1qC1s2C1r2
MBL/Ficolin/Collectins/MASPs
C4
C4a
C4
C2
C2b
C2
C4b2a
C3a
C3
C3b
C4b2a3b
C5a
C5
C5b + C6-C9
TERMINAL

Late Steps of complement activation

- **C5 convertase** generated by the classical , alternative or lectin pathway activate the late components of the complement system which culminate in the formation of the membrane attack complex (MAC).

- C5 convertase cleaves C5 into C5a(released) and C5b remains bound to the complement proteins deposited on the cell surface.

- C6, C7,C8 and C9 are structurally related proteins with no enzymatic activity.

- The C7 component of the C5b,6 and 7 complex is hydrophobic and inserts into the lipid bilayer of the cell membranes and becomes a high affinity receptor of the next component C8.

- The C8 component has 3 chains , two chains bind to the C5b-C7 complex and the third bins to the lipid bilayer.

- This stable binding complex (C5b-C8) has limited ability to lyse cells . The active MAC is formed by the binding of C9 , that forms pores in the plasma membrane and form channels that results in entry of water and rupture of cells to which the MAC is inserted.

Function of the complement

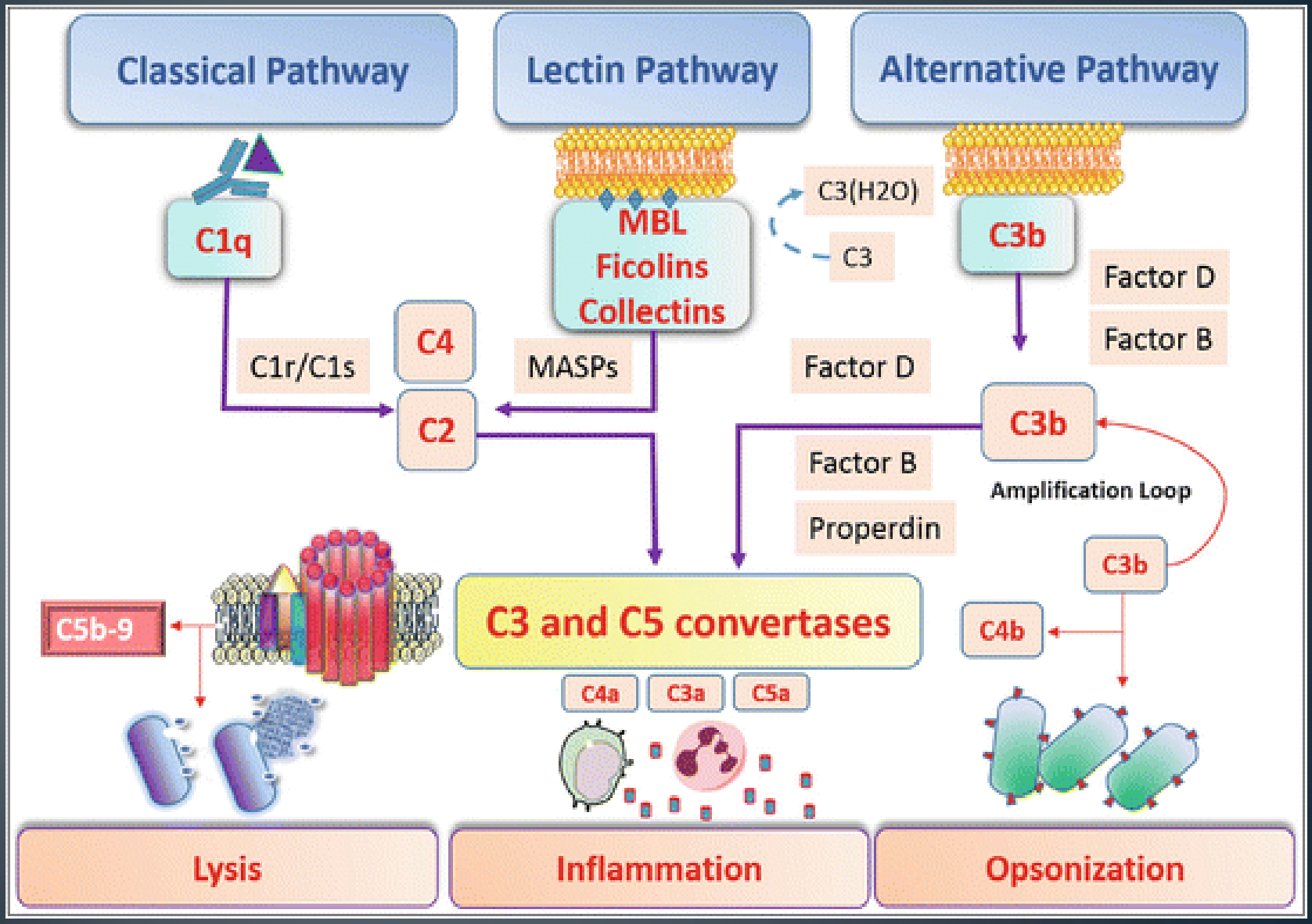
- The principle effector functions of the complement system in innate immunity and specific humoral immunity are:

  1- promote phagocytosis of microbes on which complement is activated.

  2- stimulate inflammation

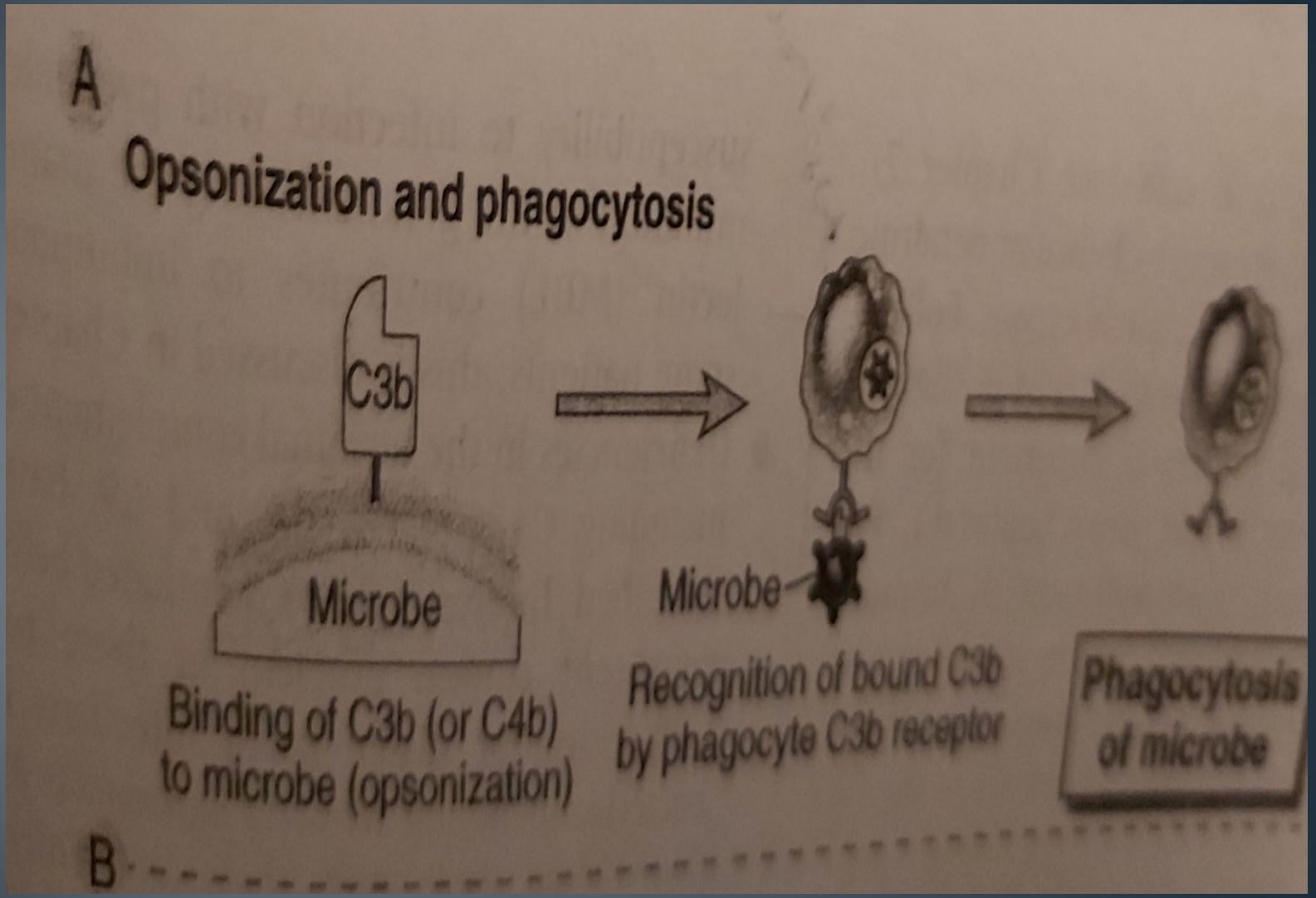
  3-Stimulate lyses of microbes

- In addition products of complement activation facilitate the activation of B lymphocytes and the production of antibodies.


Classical Pathway
Lectin Pathway
Alternative Pathway
C1q
MBL
Ficolins
Collectins
C3(H2O)
C3
C3b
Factor D
Factor B
C1r/C1s
C4
C2
MASPs
Factor D
C3b
Factor B
Properdin
Amplification Loop
C3b
C5b-9
C3 and C5 convertases
C4b
C4a
C3a
C5a
Lysis
Inflammation
Opsonization

1- Opsonisation and phagocytosis

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


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

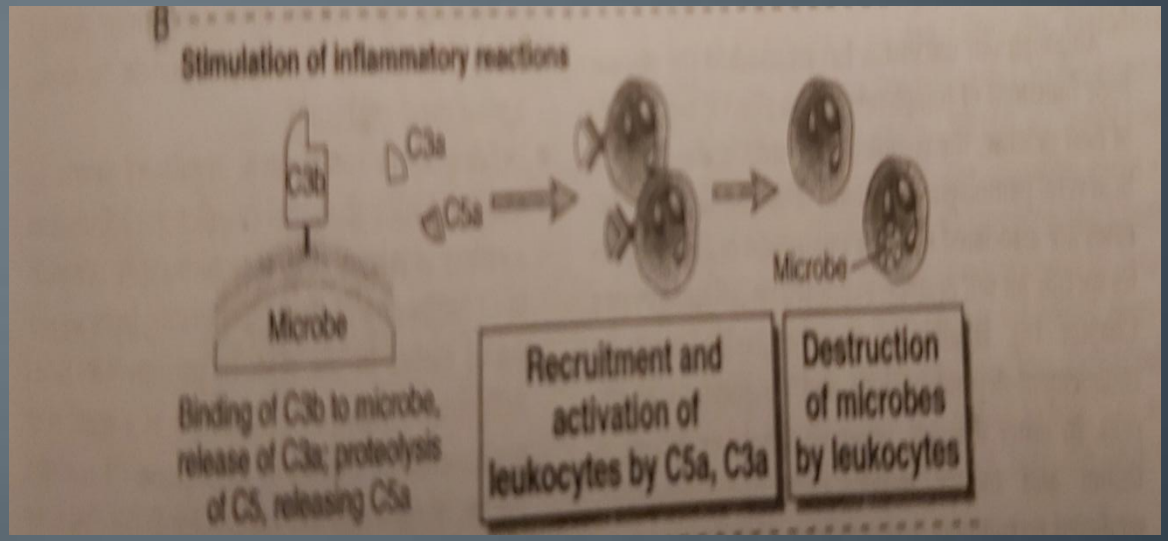
2- Stimulation of inflammatory responses

- The proteolytic components of the complement fragments C5a, C4a, and C3a induce acute inflammation by activating mast cells and neutrophils (they induce their proinflammatory action by binding to specific receptors)

- All three peptides (anaphylatoxins) bind to mast cells and induce degranulation, with the release of vasoactive mediators such as histamine.

- In neutrophils C5a , stimulate motility , firm adhesion to the endothelial cells and at high doses stimulates respiratory burst and production of reactive O2 species.

- C5a may act directly on vascular endothelial cells and induce vascular permeability and expression of P-selectin, which promotes neutrophil binding


B
Stimulation of inflammatory reactions
C3b
C3a
C5a
Microbe
Microbe
Binding of C3b to microbe, release of C3a; proteolysis of C5, releasing C5a
Recruitment and activation of leukocytes by C5a, C3a
Destruction of microbes by leukocytes

3-Complement-mediated Cytolysis

- Complement mediated cytolysis is mediated by the MAC.
- Most pathogens have evolved thick walls that impede the access of the MAC to their membranes.
- Complement mediated cytolysis appears to be important for the defense against only a few pathogens that are unable to resist MAC insertion such as genus Neisseria.

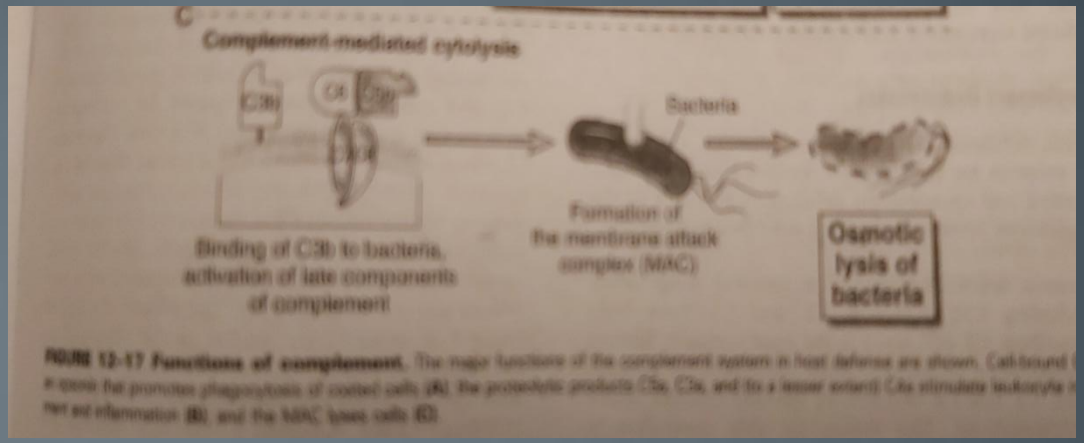


FIGURE 12-17 Functions of complement. The major functions of the complement system in host defense are shown. Cell-bound
e opsonic that promotes phagocytosis of coated cells (**A**), the proteolytic products C3a, C5a, and (to a lesser extent) C4a stimulate leukocyte
rapid acute inflammation (**B**), and the MAC lyses cells (**C**).

Other functions of the complement system

- 1-On mounting an Ag-Ab response against a circulating antigen, if the immune complexes accumulate in the blood, they may be deposited in the vessel walls and lead to inflammatory reactions that damage the vessels and surrounding tissues.

The complement binds to these complexes and facilitate their stabilization and clearance by phagocytes.

- The C3d protein from C3 binds to Cr2 on B cells and facilitate B cell activation and the initiation of the humoral immune response.

Regulation of Complement Activation

Activation of the complement cascade and the stability of active complement proteins are tightly regulated.

Complement activation needs to be regulated for two reasons:

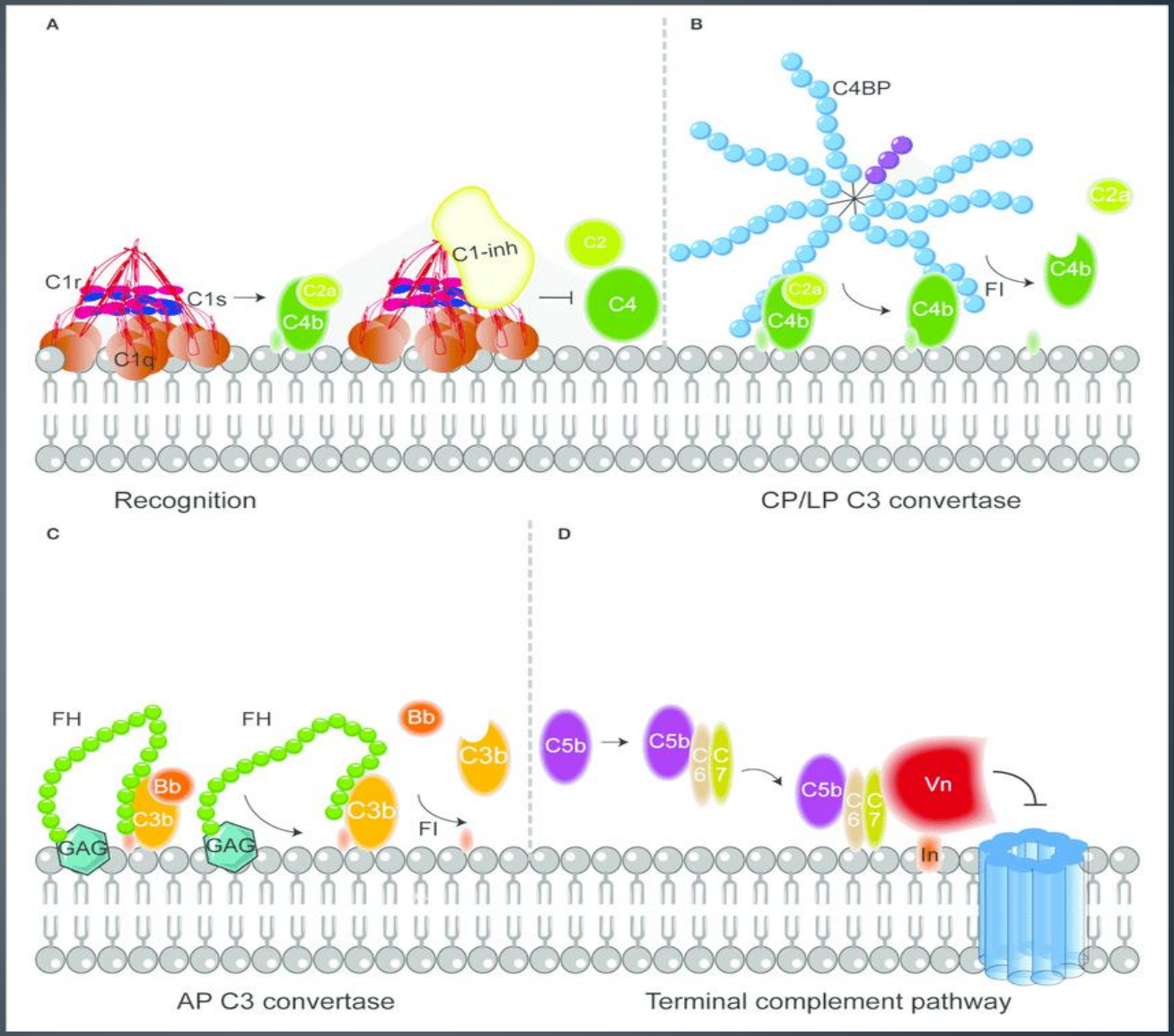
- 1-low level complement activation goes on spontaneously, and if such activation is allowed to proceed, it can result in damage to the normal cells and tissues.

- 2-when complement is activated on microbial cells or Ag-Ab complexes, it needs to be controlled because degradation products of the complement can diffuse to adjacent cells and injure them.

1- the proteolytic activity of Cr1 and C1s is inhibited by a plasma protein called C1 inhibitor (C1INH):

- C1INH is a serine protease inhibitor that mimics C1r and C1s. If C1q binds to an antibody and starts the process of complement activation, C1INH is cleaved via the enzymatic activity of C1r and C1s and becomes covalently attached to them and as a result C1r2-C1s2 dissociates from C1q, thus stopping the activation by the classical pathway.

2- assembly of the components of C3 and C5 coonvertases is inhibited by binding of regulatory proteins to C3b and C4b


A
B
C1r
C1s
C1q
C2a
C4b
C1-inh
C2
C4
C4BP
C2a
C4b
C2a
C4b
FI
C4b
Recognition
CP/LP C3 convertase
C
D
FH
FH
Bb
C3b
Bb
C3b
GAG
GAG
C3b
FI
C5b
C5b
C6
C7
C5b
C6
C7
Vn
In
AP C3 convertase
Terminal complement pathway

- C3b on mammlian cells is bound by membrane cofactor protein
- (MCP,or CD 46), CR1, decay accelerating factor(DAF) and factor H.
- C4bon cell surfaces is bound by DAF, CR1, C4-binding protein (C4BP).

These proteins inhibit the complement activation via classical or alternative pathway via competitive binding.

3- Cell associated C3b is proteolytically degraded by a plasma serine protease called factor I which is active only in presence of regulatory proteins

- MCP, factor H, C4BPand CR1 serve as cofactors for factor –I mediated cleavage of C3b and C4b.

4- Formation of MAC is inhibited by a membrane protein called CD59 (a glycophosphatidyleinositol-linked protein expressed on many cell types and by plasma protein s as protein S.

- It incorporates itself into the assembling MAC after the membrane insertion of C5b-8 and hence inhibiting the addition of C9 (it is present on normal host cells but not on the microbes).

- Protein S binds to C5b,6,7 complexes and inhibiting their insertion into cell membranes near the site of the initiation of the complement cascade.

Complement Deficiencies

Genetic deficiencies of the complement proteins and regulatory proteins are the causes of various human diseases.

1- Genetic deficiencies in the classical pathway components (C1q, C1r, C4, C2 and C3):

- Fifty percent ofC2 (most common human deficiency) and C4 deficiencies develop SLE (unknown cause) but possibly due to:

a- Defects in compolemnt activation leads to failure to clear circulting immune complexes and hence are deposited in the circulation and leads to local inflammtion

b-complement play an important role in clearance of apoptotic bodies containing fragmented DNA and these apoptotic are likely the source of nuclear antigens that trigger autoantibody response.

c-complement regulates antigen- mediated signals received by B cells and in their absence B – cell tolerance may not be induced and auto antibody results.

2- Deficiencies in components of the alternative pathway (Properdin and factor D) results in increased susceptibility to infection with pyogenic bacteria.

3- Mutation in the gene encoding MBL results in immunodeficiency in some patients.

4- Deficiency in the terminal complement components (C5-C9) results in the propensity for disseminated infections by Niesseria bacteria.

5-Deficiency in complement regulatory proteins are associated with abnormal complement activation and a variety of related clinical abnormalities:

a-C1INH deficiency: causes an inherited autosomal dominent disease called Heridetary angioneurotic edema.

- Mechanism: C1INH is reduced(<20 or 30% of normal) that activation by C1 complexes is not controlled and increased break down of C2and C4
- Clinically : acute accumulation of edema fluid in shin and mucosa ---→abdominal pain, vomiting, diarrhea and life threatening air way obstruction.
- The mediators of edema are proteolytic products of C2 called C2 kinin and bradykinin.

b-Genetic deficiency of DAF: causes a disease called Paroxysmal nocturnal hemoglobinuria

- CCC: recurrent bouts of intravascular hemolysis partly due to unregulated complement activation of the surface of erythrocytes.
- Clinically: recurrent hemolysis that results chronic hemolytic anemia and venous thrombosis.
- Mechanism: acquired mutation in hematopoietic stem cells.

c-Factor I deficiency:

- Plasma C3 is depleted as result of unreguleted formation of fluid phase C3 convertase (Tickover mechanism)-→ pyogenic infections.

d- Factor H deficiency: rare

- CCC: excessive alternative pathway activation an C3 consumption-→ glomerulonephritis due to inadquate clearance of IC and their renal deposition .

6- Deficeincy in complement receptors (CR3 and CR4)--→ leucocyte adhesion deficiency.

- CCC: recurrent pyogenic infections caused by inadequate adherence of neutrophils to the endothellium at sites of infection

THANK YOU