Lect. 5 HUMORAL FACTORS
Complement system

By :Dr.Agharid Ali Hussein

Learning objectives
This Lecture deals with

1. **Complement system and their role in immunity,**
2. **Different pathways of complement activation.**
3. **Function of complement system**

INTRODUCTION - HUMORAL FACTORS

Circulating effector blood proteins of innate immunity are
Complement
Opsonin
Properdin etc.

❑ Introduction :

The complement system is an essential innate defense system. Although its main role is to kill pathogens immediately when they enter the body, the complement system also alerts the immune systems to the presence of invaders, regulates inflammation, removes damaged or altered cells, and regulates adaptive immune responses.

the system cleave specific proteins to release cytokines and initiate an amplifying cascade of further cleavages. The end-result of this activation cascade is massive amplification of the response and activation of the cell-killing membrane attack complex.

❖ COMPLEMENT SYSTEM

1. **The complement system is one of the major effector mechanisms of humoral immunity and as well as of innate immunity. Complement components are synthesized at various sites like liver macrophages.**
2. **The complement system is composed of** at least 30 different complement proteins.**Heat labile (56°C in 30 minutes) serum (plasma) proteins and constitutes about 10% of the globular fraction of serum.**
3. **Molecular weight of the complement components varies from 24 KDa (factor D) to 460 KDa (C1q).**
4. **The complement proteins are labeled numerically with the prefix C (C1, C2, C3 --- C9) or designated by letters of the alphabet (B, D, P etc).**
5. **Peptide fragments formed by activation of a component are indicated by small letter (C3a, C3b etc).**
6. **The complement fragments interact with one another to form functional complexes.**
7. **Those complexes with enzymatic activity are indicated by a bar over the number or symbol.**

10. **Complement system is normally inactive but activated under certain condition like microbial infection and generates effector mechanism to destroy the activator (i.e the microbes).**
11. **Activation of complements involve the sequential proteolysis of proteins to generate enzymes with proteolytic activity.**
12. **Proteins that gain proteolytic enzymatic activity by the action of other proteases are called zymogens (proenzymes).**
13. **Zymogens are activated sequentially i.e. the product of first reaction catalyzes a second reaction and the product of second reaction catalyzes third reaction and so on. This types of chain of enzymatic reaction are known as cascade reaction.**
14. **The products of activated complement attach covalently to microbial cell surfaces or antibody coated microbes or other antigens and cause lysis of the target cells (e.g.microbe).**
15. **Complement activation is inhibited by regulatory proteins that are present in normal host cells in absence of microbes. Thus normal host is not affected..**

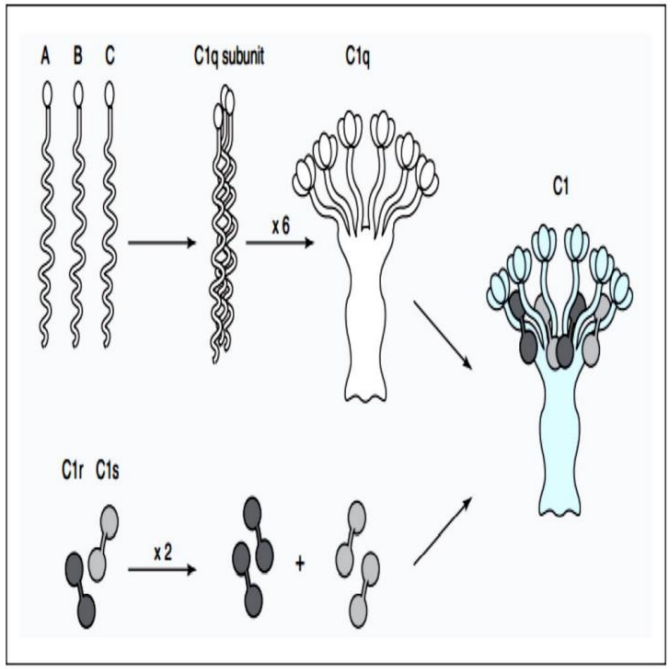
❑ Pathways of Complement Activation

There are three major pathways for complement activation:

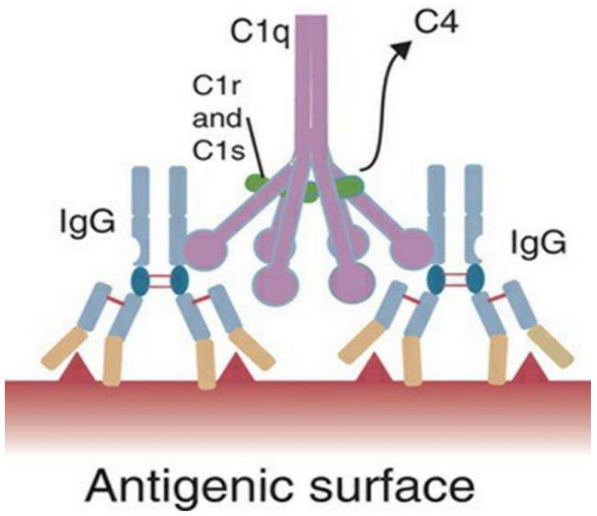
1. **The Classical Pathway – which is activated by certain antibodies bound to antigens.**
2. **The Alternative Pathway – which is activated by microbial cell in the absence of antibody.**
3. **The Lectin Pathway - which is activated by plasma lectin bound to mannose residues on microbes.**

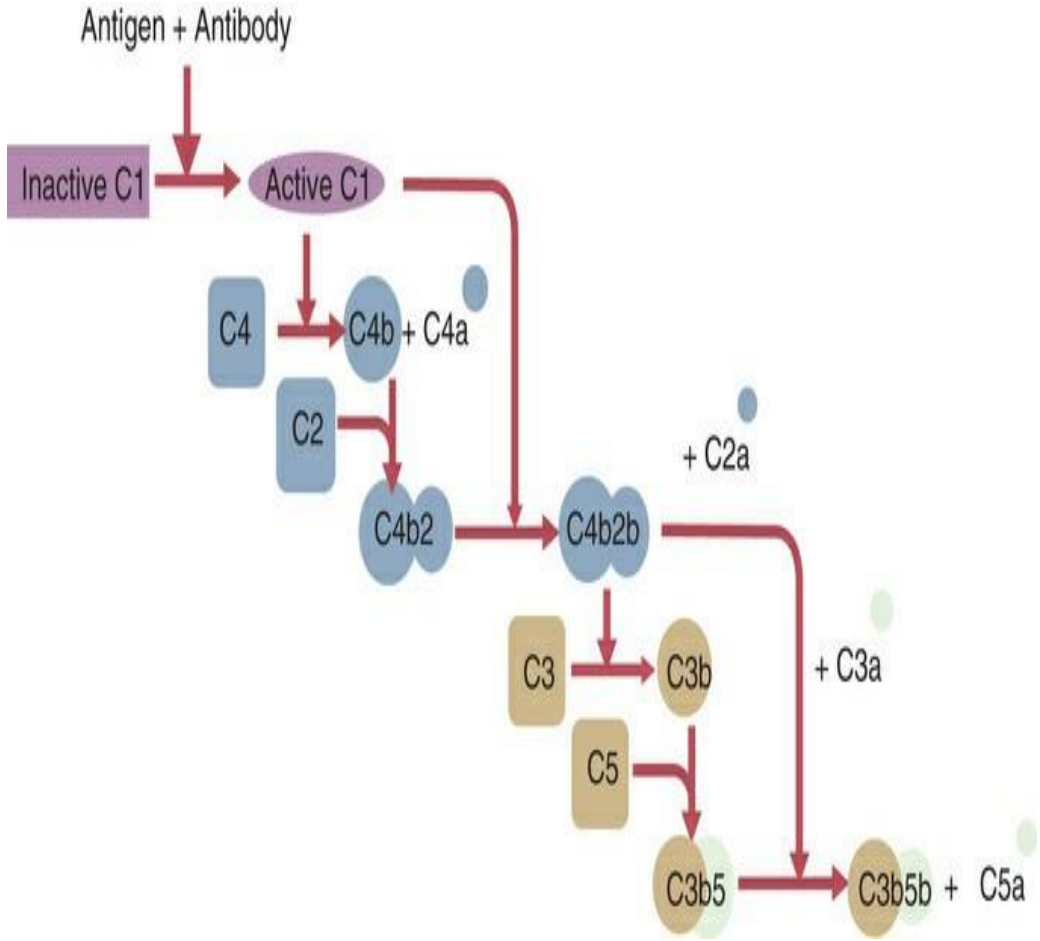
❖ Classical pathway

1. In classical pathway, first complement component is C_1 complex.
2. The C_1 molecule is composed of three separate proteins C_{1q}, C_{1r} and C_{1s} bound together by calcium (Ca++) dependent bonds.
3. The classical pathway is activated when the hexameric C1q(The C_{1q} subunit is made up of an umbrella like radial array of six chains that are connected to central stalk by a collagen like arm)and each chain has globular head, which recognizes and binds to Fc region of immunoglobulin heavy chain of IgG and IgM.
4. C_1r and C_1s are serine esterases and they form a tetramer complex containing two molecule of each and located between C_1q strands. Binding of C_{1q} to two or more Fc regions leads to enzymatic activation of C_1r that cleaves and activates C_1s.
5. Active C1s cleaves C4 into C4a and C4b. C2 then binds to C4b to form the complex C4b2.(C4bC2).
6. Activated C1s then splits the bound C2, generating a small peptide fragment C2a and the C4bC2b complex. C1s cannot act on soluble C2; the C2 must first be bound to C4b before it can be split .
7. The C4bC2b complex is a potent protease whose target is C3, and it is therefore called classical C3 convertase.
8. The newly generated C3b binds and activates C5. Subsequent reactions lead to formation of THE TERMINAL COMPLEMENT COMPLEX AND MICROBIAL KILLING.



C_1 complex

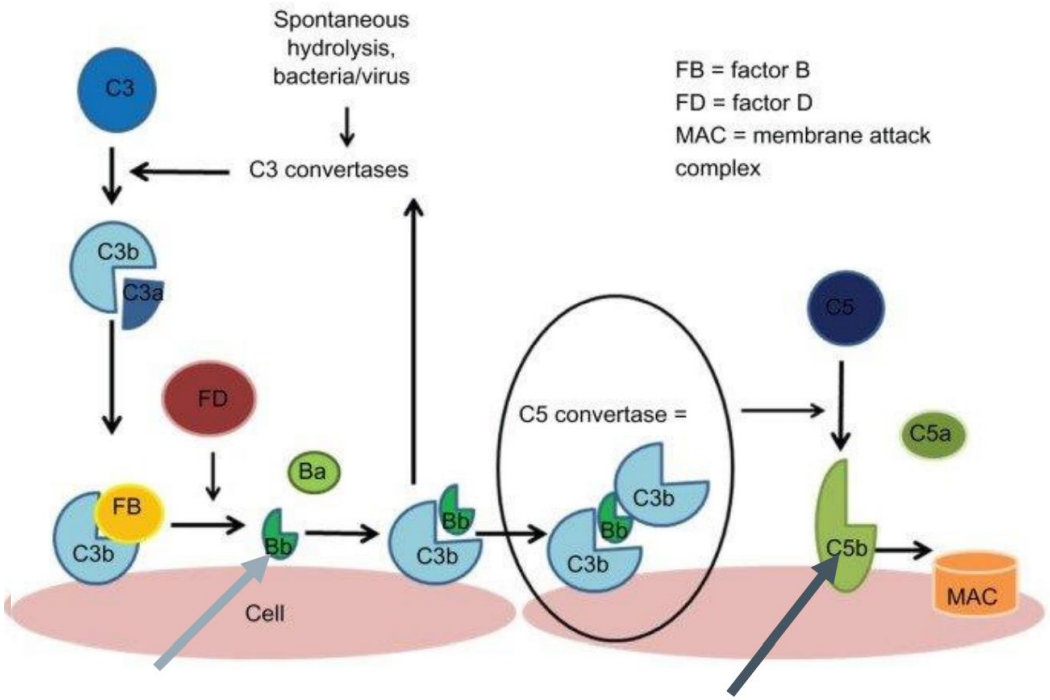




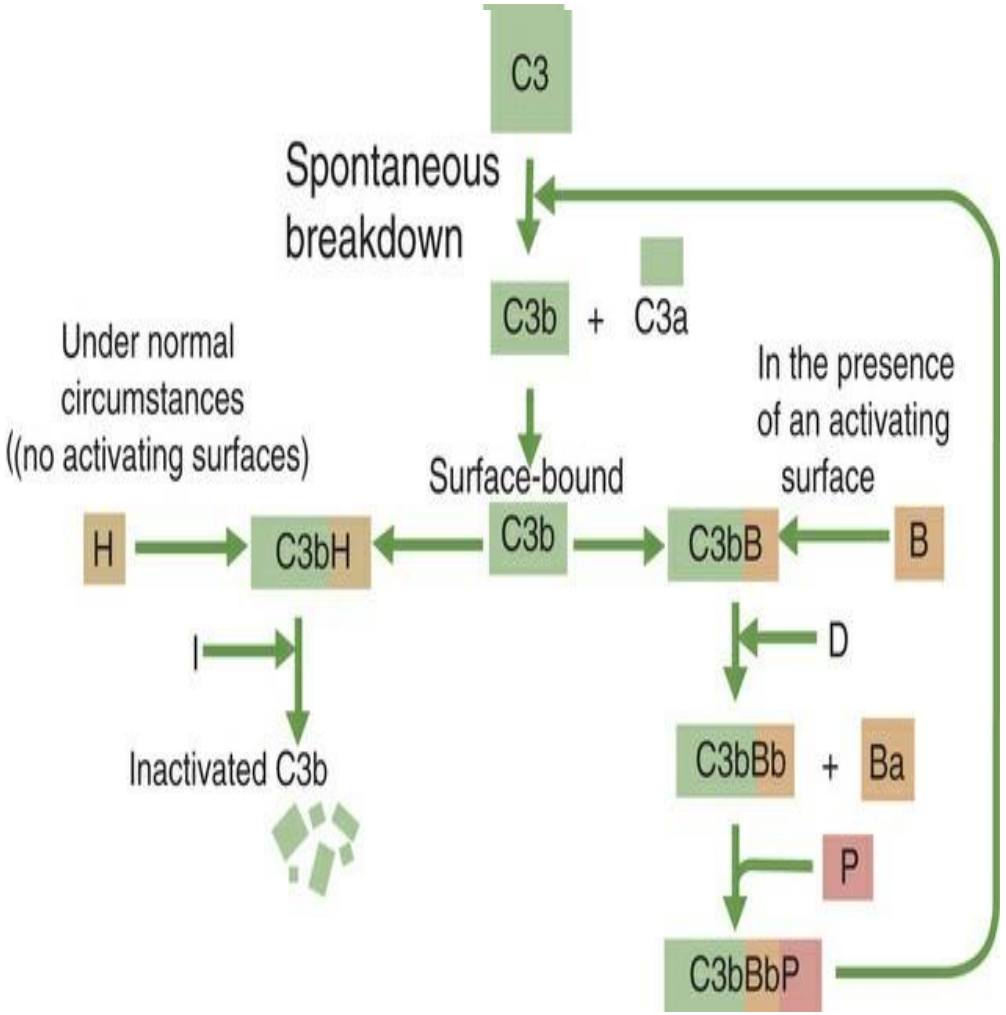
The basic features of the classical complement pathway.

❖ Alternative pathway

1. In alternative pathway C3 is activated and form a stable attachment of C3b to microbial cell surface without the involvement of antibody.
2. Normally C3 in plasma breaks down spontaneously into C3a and C3b. The newly formed C3b binds(another example of substrate modulation) covalently through thioester bonds to the surface of cells including microbes including bacterial cell walls, bacterial lipopolysaccharides(endotoxin), viruses, aggregated immunoglobulin (IgA), cobra venom etc. permit activation of C3b. Thus activation can occur by both immunologically and non immunologically.
3. The bound C3b binds to a plasma protein called B factor and once bound, factor B is cleaved by a plasma serine protease (called factor D) to generate a bound fragment called Bb (also a soluble fragment Ba).
4. Factor D acts only on B factor after it is bound to C3b (another example of substrate modulation).
5. The complex C3bBb is the alternative pathway C3 convertase and cleaves C3 to C3b and C3a. C3a is released and C3b remain attached to cells. Half-life of C3b is only 5 minutes.
6. Another protein called properdin (factor P) binds to the complex to form C3b BbP and increase the half-life to 30 minutes. Microbial cells favors the attachment of Properdin.
7. C3b may also be generated by other protease from activated phagocytic cells and there is generation of C3b at the site of inflammation.
8. Some C3b molecules generated by alternative pathway bind to C3 convertase itself and form C3bBb3b, which function as the alternative pathway C5 convertase and cleave C5 to initiate THE TERMINAL STEPS OF COMPLEMENT ACTIVATION.



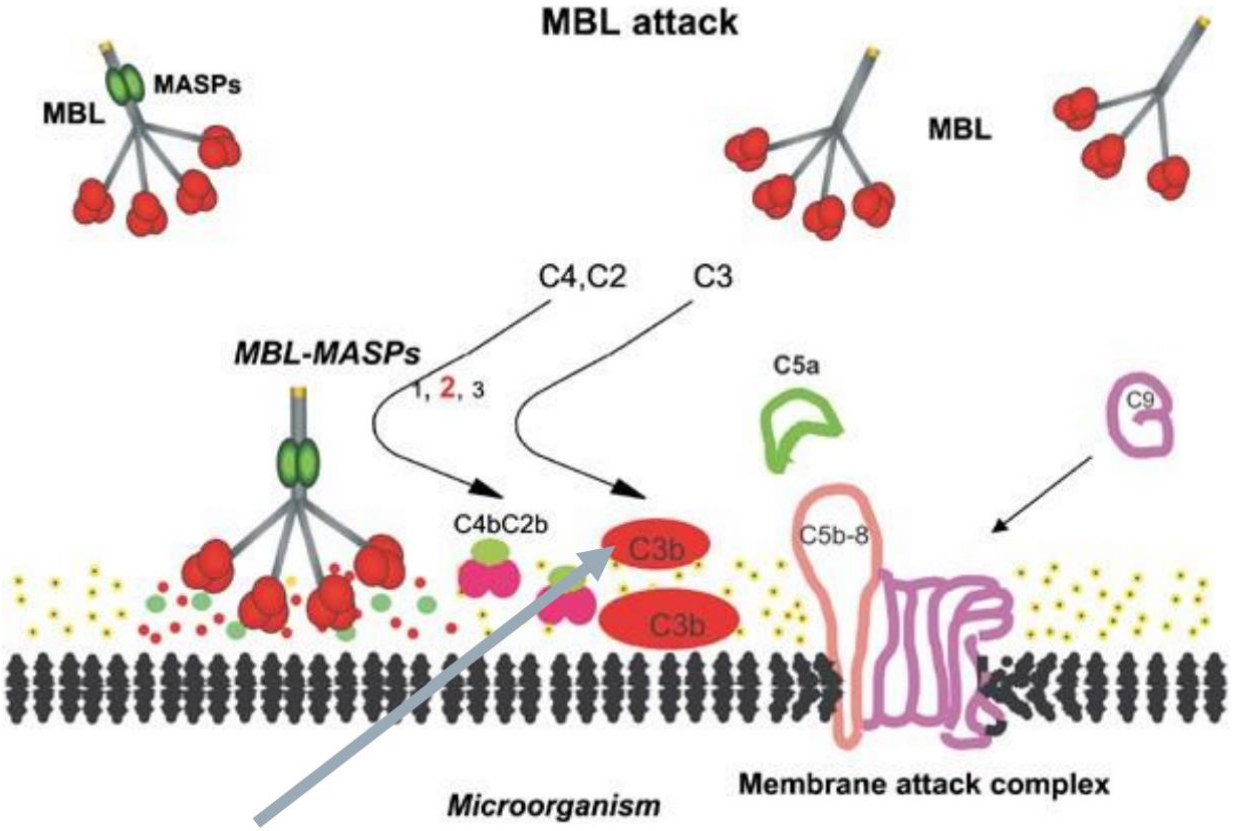
The alternative complement pathway.

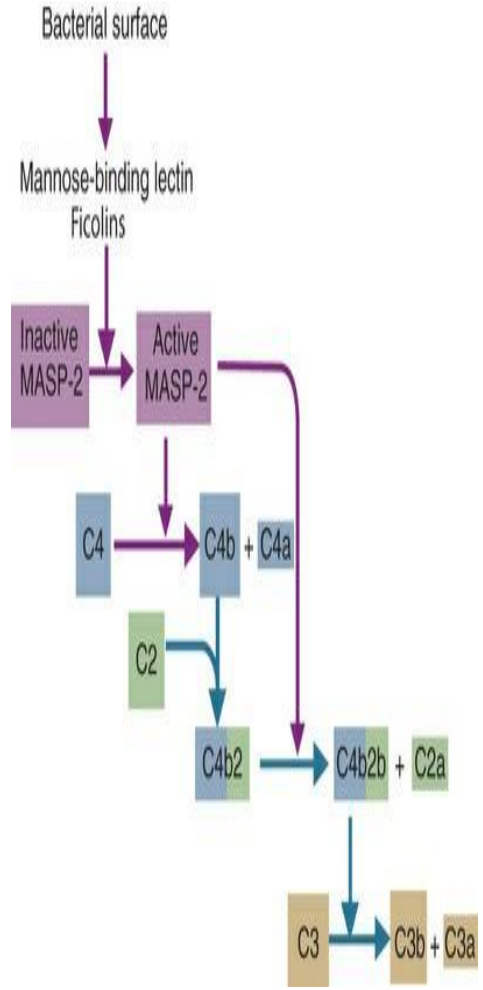


The alternative complement pathway.

The lectin pathway/mannose - binding pathway

1. When macrophages ingest bacteria or other foreign materials, they are stimulated to secrete IL-1, IL-6 and TNF- α Interleukins-cytokines) .
2. These three cytokines act on hepatocytes and stimulate them to secrete acute phase proteins, one such protein is Lectin (mannose binding protein).
3. Mannose is a major component of bacterial cell wall glycoproteins.
4. The mannose binding protein (MBP or MBL) binds to bacteria in blood stream and acts as opsonin.
5. The mannose binding protein or lectin pathway is homologous to the classical pathway, but with the opsonin, mannose-binding lectin (MBL), instead of C1q. This pathway is activated by binding mannose-binding lectin to mannose residues on the pathogen surface, which activates the MBL-associated serine proteases(MASP), very similar to C1r and C1s which can then split C4 into C4a and C4b and C2 into C2a and C2b. C4b and C2a then bind together to form the C3-convertase, as in the classical pathway.





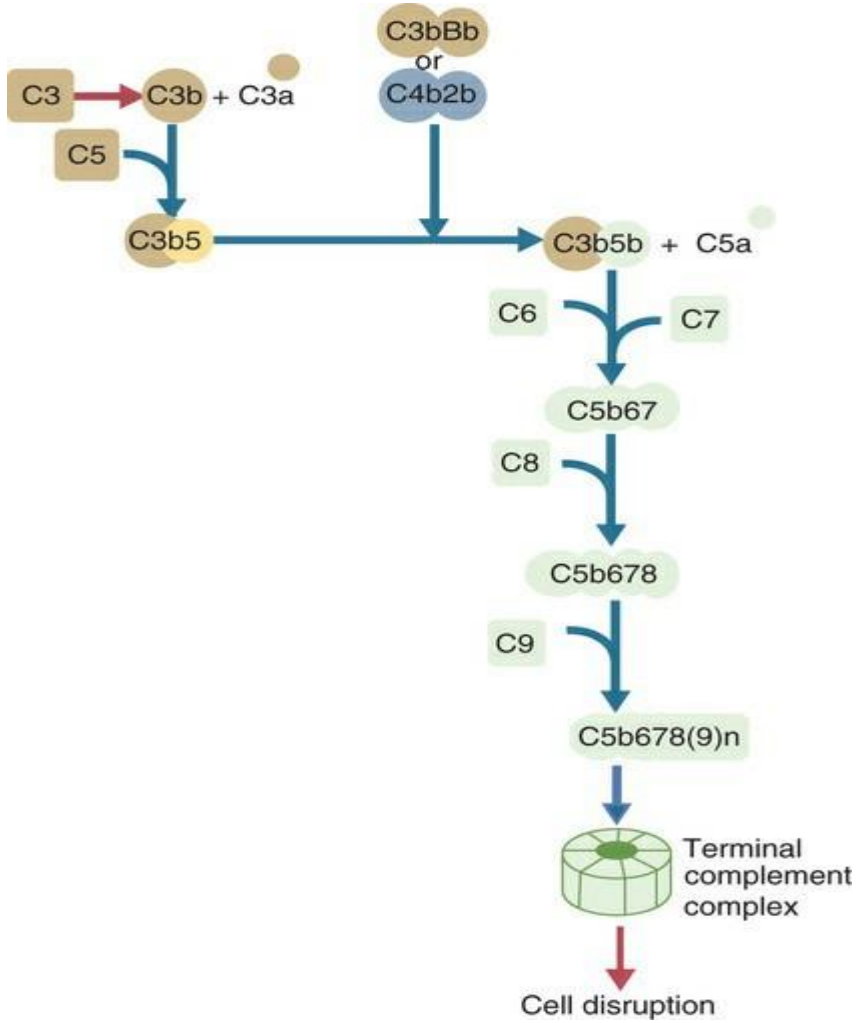
The lectin pathway/mannose - binding pathway

❖ Terminal pathway of complement activation

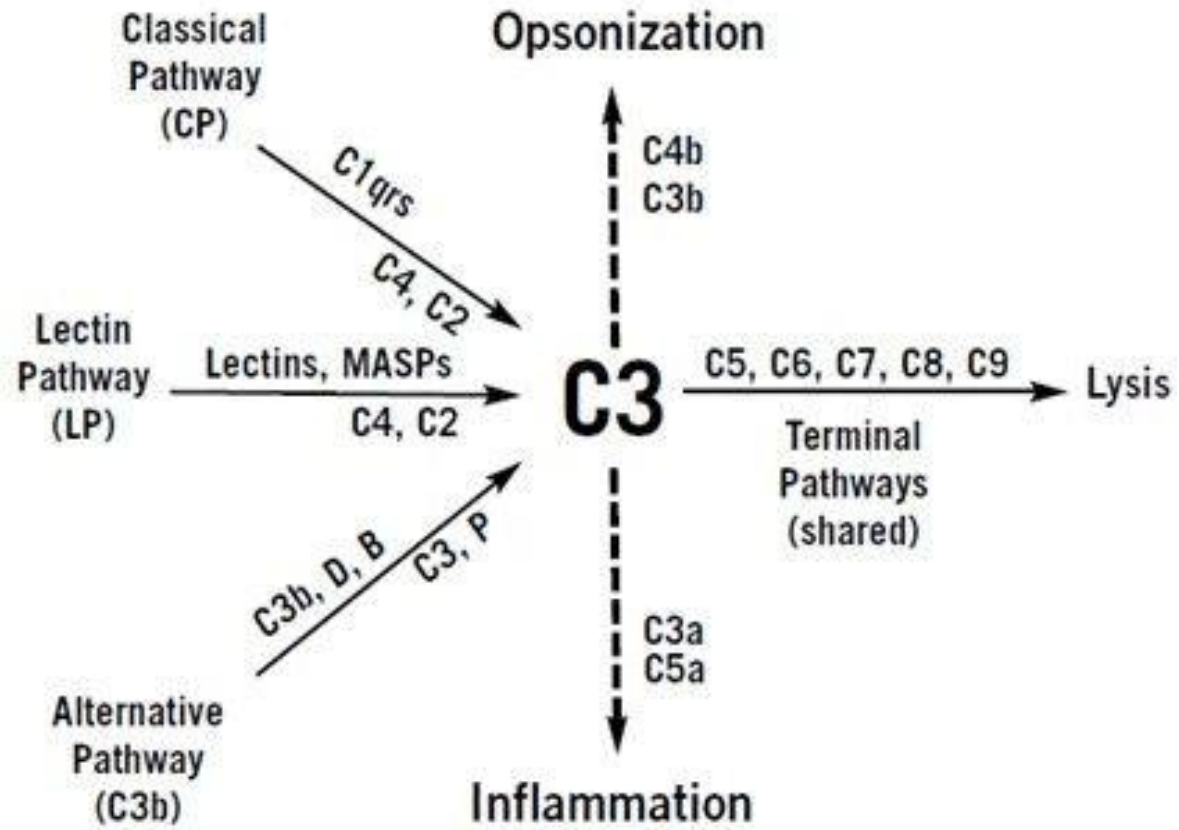
MBA : C5b initiates the membrane attack pathway, which results in the membrane attack complex (MAC), consisting of C5b, C6, C7, C8, and polymeric C9. MAC is the cytolytic endproduct of the complement cascade; it forms a transmembrane channel, which causes osmotic lyses of the target cell

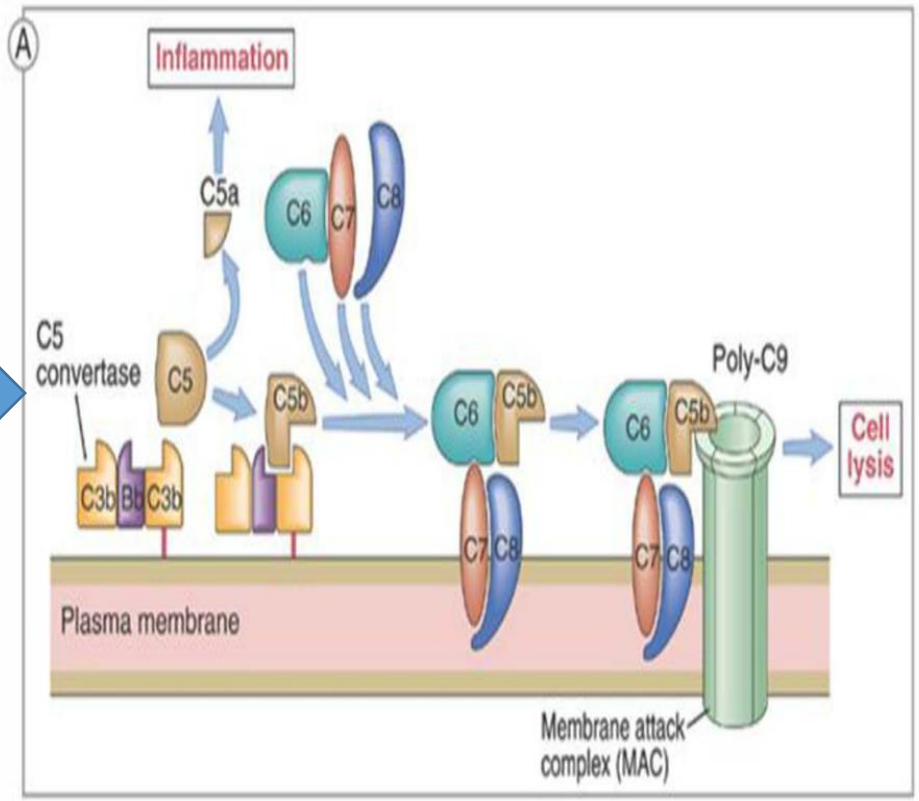
Mechanism of membrane attack pathway

1. **Once C5 binds to C3b, C5convertase generated by classical pathway (C4b2a), alternative pathway (C3bBb) or mannose binding pathway cleaves C5 to small peptide C5a (released) and C5b, which attach to C3b.**
2. **This cleavage exposed a site on C5b and binds C6 and C7 to form C5b67.**
3. **The C5b67 can remove itself from C3b and insert into the lipid bilayer of nearby cell or microbial membrane.**
4. **Once it is inserted into lipid bilayer, it binds to one C8 molecule and multiple C9 molecules (about 12 to 18) to form a complex C5b6789 of tubular transmembrane pore called the membrane attack complex (MAC).**
5. **The MAC form a large doughnut(دونات)shaped structure that inserts itself into a cell membrane and forms a transmembrane channel and cause osmotic lysis of the target cell.**



Terminal pathway of complement activation


Classical Pathway (CP)
C1qrs
C4, C2
Lectin Pathway (LP)
Lectins, MASPs
C4, C2
Alternative Pathway (C3b)
C3b, D, B
C3, P
Opsonization
C4b
C3b
C3
C5, C6, C7, C8, C9
Lysis
Terminal Pathways (shared)
C3a
C5a
Inflammation


A
Inflammation
C5a
C6
C7
C8
C5 convertase
C5
C5b
C3b
Bb
C3b
C6
C5b
C7
C8
C6
C5b
C7
C8
Poly-C9
Cell lysis
Plasma membrane
Membrane attack complex (MAC)

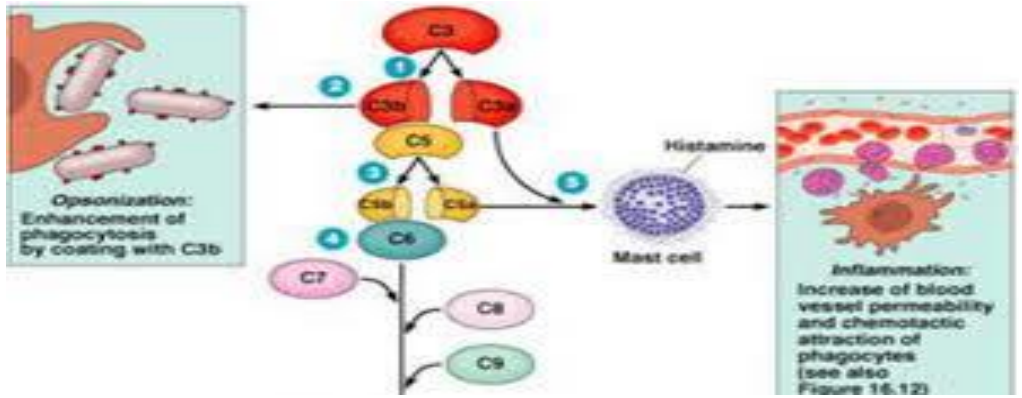
Complement Activation Pathways			
Feature	Classical	Alternative	Lectin
Detects	• Surfaces with bound antibodies (IgG and IgM) at Fc binding cite	• Surfaces without antigen and antibody complex	• Surfaces with mannose
Initiating factor	• C1q • C1r • C1s	• C3 spontaneous cleavage • Factor D and P	• Mannose binding lectin and associated proteases
C3 convertase	• C4b • C2b	• C3b • Factor Bb	• C4b • C2b

❑ Functions of the Complement

The complement system has five major antimicrobial functions.

1. **Lysis –rupturing membranes of foreign cells**
2. **Activation of inflammation – Several peptides produced by proteolytic cleavage of proteins bind to vascular endothelial cells and lymphocytes. These cells then produce cytokines which stimulate inflammation and enhances responses to foreign antigens. 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.**

3. Opsonization –enhancing phagocytosis of antigens, Opsonization of bacteria takes place when immunoglobulin G (IgG) molecules bind to specific epitopes on bacterial surface antigens through the antigen-binding site of the IgG molecule. In addition, microbial cell surface activates the complement system either directly through interaction with microbial polysaccharides through the alternate pathway, or indirectly through interaction with IgG or IgM bound to bacteria Fc region through the classic pathway. both IgG and C3b are important opsonins. Phagocytic cells, either neutrophil or macrophage, have specific surface receptors(CR1, CR3, and CR4) for the Fc region of the IgG molecule and C3b. The opsonized microbe is ingested through receptor-mediated phagocytosis.



4. **Chemotaxis** - attracting macrophages and neutrophils.
5. **Neutralizes virus-** For most viruses, the binding of serum antibody to the repeating subunits of the viral structural proteins creates particulate immune complexes ideally suited for complement activation by the classical pathway. Some viruses (e.g., retroviruses, Epstein-Barr virus, Newcastle disease virus, and rubella virus) can activate the alternative, lectin, or even the classical pathway in the absence of antibody.
6. **Solubilization of immune complexes** – Some virus infections that are not cytopathic – the virus does not kill cells – lead to the accumulation of antibody- virus complexes. When these immune complexes lodge in blood vessels they can cause damage. An example is glomerulonephritis caused by deposition of antibody-antigen complexes in the kidney. Some complement proteins can disrupt these complexes and facilitate their clearance from the circulatory system.

❑ Complement deficiency

1. It is thought that the complement system might play a role in many diseases with an immune component, such as, glomerulonephritis, various forms of arthritis, autoimmune heart disease, multiple sclerosis,and rejection of transplanted organs.
2. The complement system is also becoming increasingly implicated in diseases of the central nervous system such as Alzheimer's disease and other neurodegenerative conditions such as spinal cord injuries.
3. Deficiencies of the terminal pathway predispose to both autoimmune disease and infections .

Self-Test Questions

1. What is complement?
2. What are the 3 pathways of activation?
3. Which pathways are not activated by antibodies?
4. Which complement components stimulate inflammation?
5. Name 2 effects of complement.
6. What disease or infection may a person deficient in