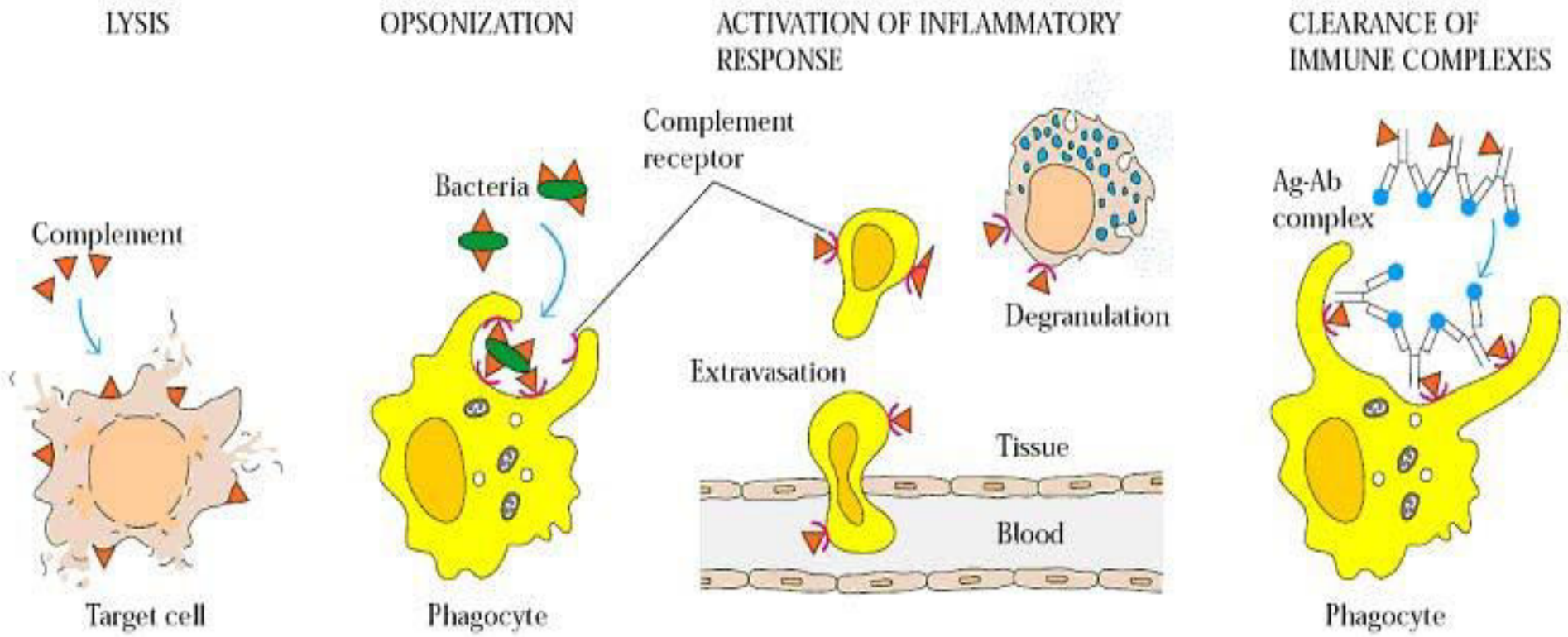
The Compliment System

- The compliment system is the major effector of the humoral branch of the immune system.
- Research on compliment started in 1890s.
- Julus Bordet at the Institute Pasteur observed that sheep antiserum to the bacterium *Vibrio cholerae* caused lysis of the bacteria & heating the antiserum destroyed its bacteriolytic ability.
- Surprisingly, the ability to lyse the bacteria was restored to the heated serum by adding fresh serum that contained no antibodies directed against the bacterium & by itself was unable to kill it.

- Bordet accurately concluded that bacteriolytic activity requires two different substances:
- i) the specific antibacterial antibodies, which survive the heating process &
- ii) heat sensitive component responsible for the lytic activity.
- Bordet devised a simple test for the lytic activity, the easily detected lysis of antibody coated RBCs, called haemolysis.
- Shortly afterwards in Berlin, Paul Ehrlich independently carried out similar experiments & coined the term complement, defining it as 'the activity of blood serum that completes the action of antibody'.

- The functions of complement:
- Research on complement now includes more than 30 soluble & cell bound proteins.
- After initial activation, the various complement components interact in a highly regulated cascade, to carry out a number of basic functions, including,
- - Lysis of cells, bacteria & viruses.
- - Opsonization, which promotes phagocytosis of particulate antigen.
- - Binding to specific complement receptors on cells of the immune system, triggering activation of immune responses.

- - Immune clearance, which removes immune complexes from the circulation & deposits them in the spleen & liver.



- The complement components:
- The proteins & glycoproteins of the complement system are synthesized mainly by liver hepatocytes.
- Significant amount of them are also produced by blood monocytes, tissue macrophages & epithelial cells of & the GI & urinogenital tracts.
- These constitute 5% of the serum globulin fraction & most circulate in the serum in the free inactive form.
- Many components are proenzymes or zymogens, which are inactive until proteolytic cleavage, which removes an inhibitory fragment & exposes the active site.

- The reaction sequence starts with an enzyme cascade.
- Several of the activated components become inactivated shortly if they do not react with the next component in the sequence.
- Complement components are designated by -
- - numerals c1 – c9.
- - letter symbols, eg. Factor D.
- - or by names, eg. Homologous restriction factor.
- The peptide fragments formed by activation of component are denoted by small letters.

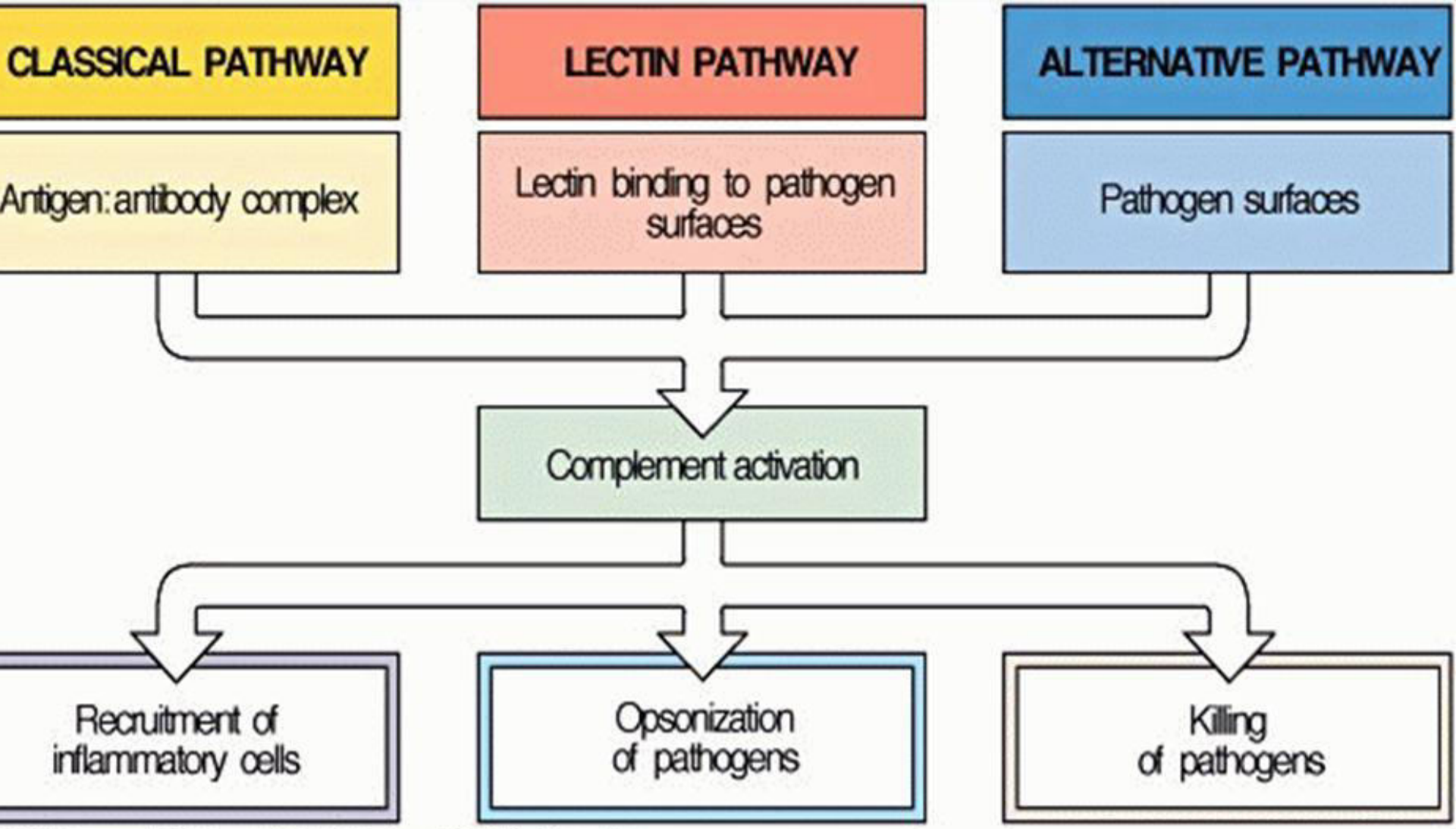
- In most cases, the smaller fragment is designated ‘a’ & a larger one ‘b’, eg. c3a, c3b.
- c2 is an exception where c2a is the larger fragment.
- The larger fragment bind to the target near the site of activation.
- The smaller fragment diffuse from the site & can initiate localized inflammatory responses by binding to specific receptors.
- The complement fragments interact with one another to form functional complexes.
- Complexes having enzymatic activity are designated by a bar over the number or symbol, eg. c4b2a, c3bBb.

- The model traditionally used to explain c activity is the lysis of erythrocyte sensitized by its antibody.
- The erythrocyte (E) – antibody (A) complex is called as EA.
- When c components are attached to EA, the product is called EAC, followed by the component that have reacted, eg. EAC12345 or EAC1-5.

- Complement activation:
- Complement is normally present in the body in an inactive form.
- When its activity is induced by Ag-Ab stimuli, c component react in a specific sequence as a cascade.
- C cascade is a series of reaction in which the preceding component act on succeeding component- cleaving them into dissimilar fragments.
- The c cascade can be triggered by three parallel but independent mechanisms or pathways which differ only in the initial steps.

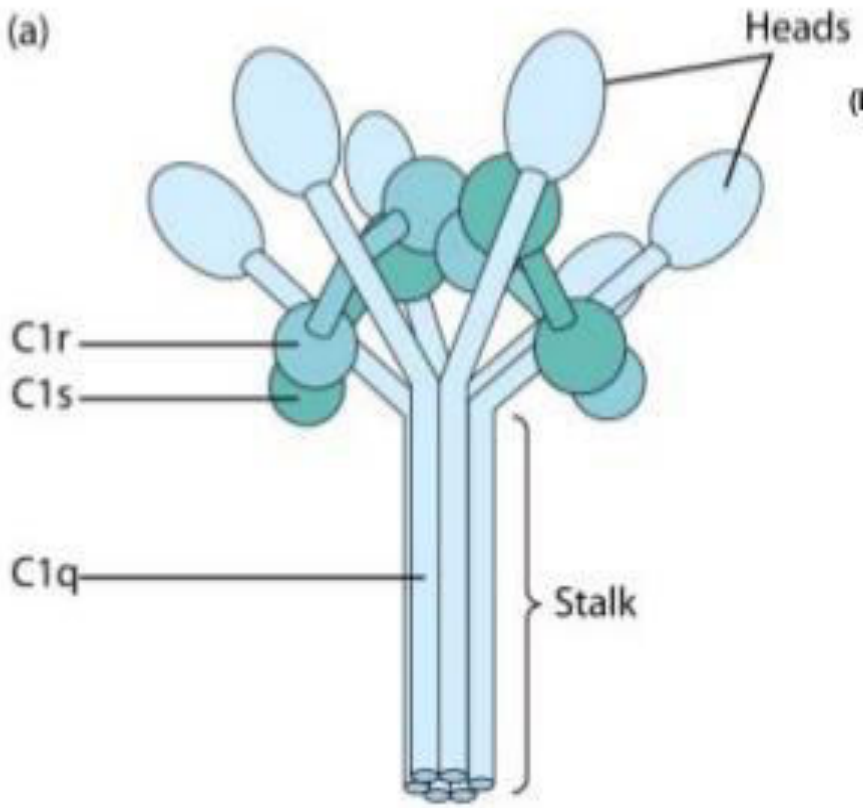
- Once c3 activation occurs, the subsequent steps are common in all pathways,
- - the classical pathway &
- - the alternative pathway or properdin pathway
- - the lectin pathway.

Complement System: Overview

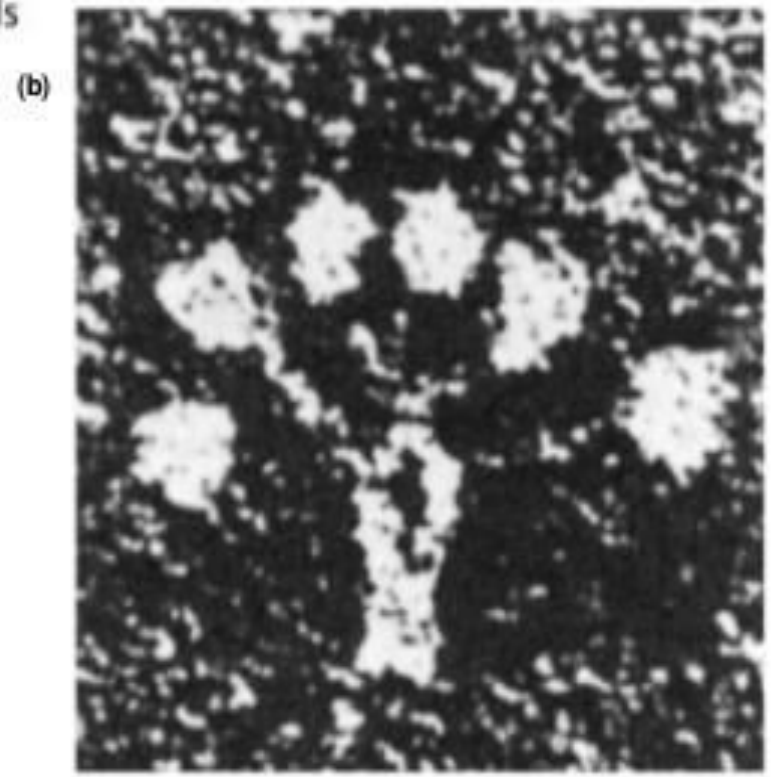


- The classical pathway:
- The chain of events in which c components react in a specific sequence following activation of c1 & typically culminates in cytolysis is known as the classical pathway.
- It consists of the following steps:
- 1) The first step is the binding of c1 to the Ag-Ab complex, traditionally represented EA.
- c1 in serum is a macromolecular complex consisting of c1q & two molecules each of c1r & c1s, held together in a complex (c1qr2s2) stabilized by calcium ions.

Structure of the C1 macromolecular complex

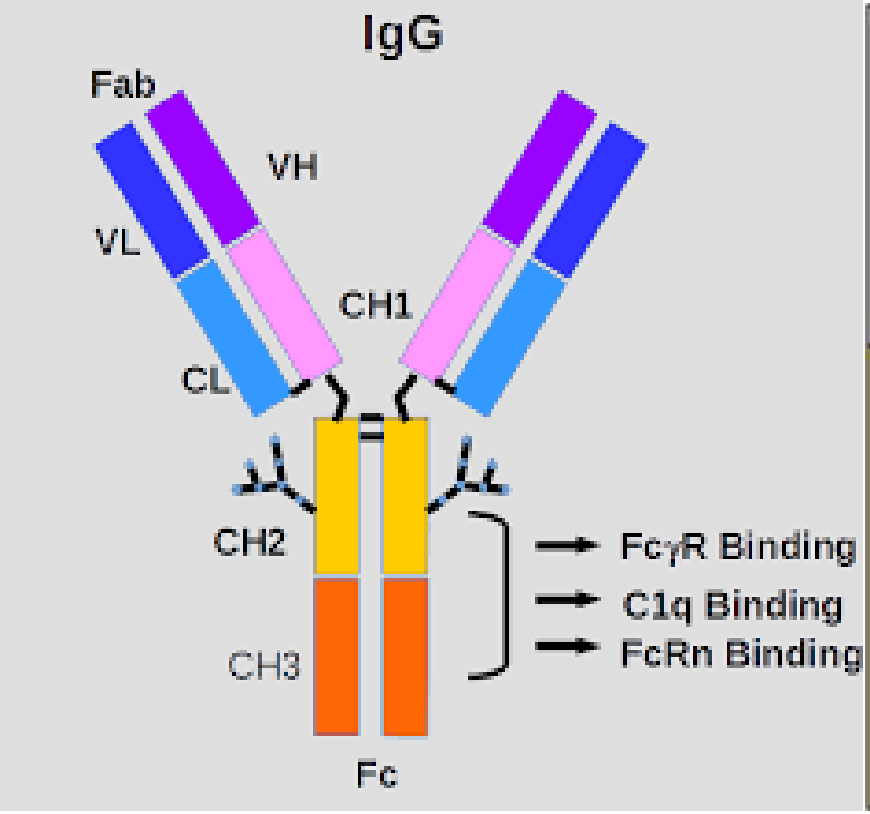


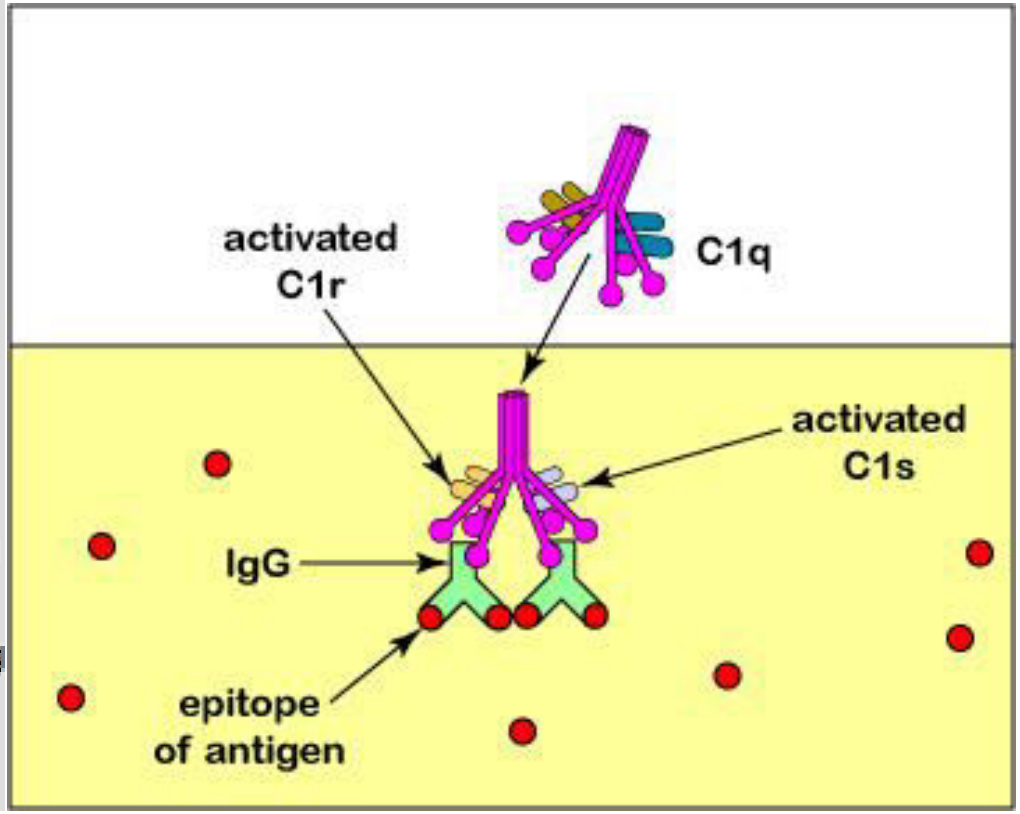
C1q molecule



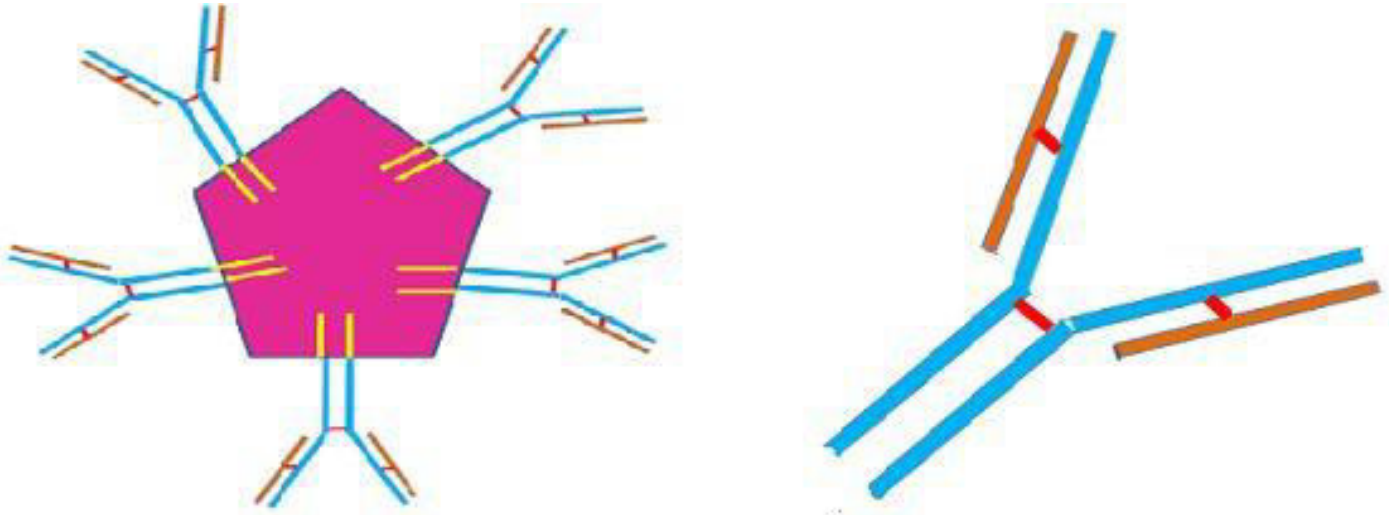
Kuby J et al., Immunology 2003

- c1q is the recognition unit of c1 & reacts with the Fc piece of IgM or IgG.

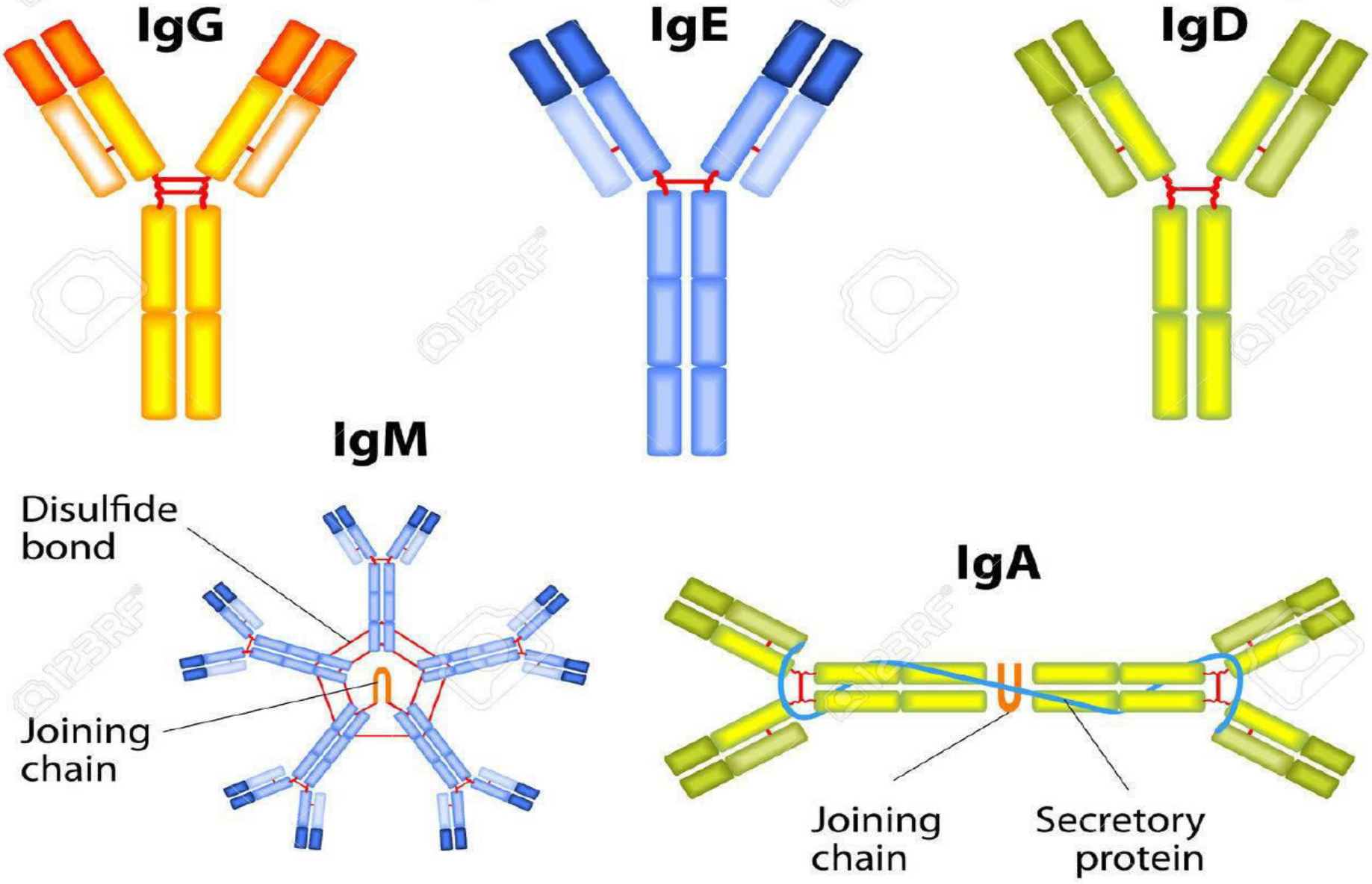


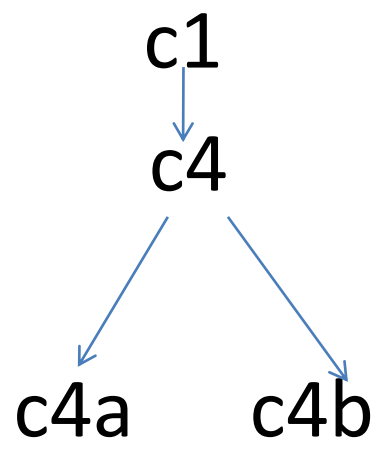


- c1q has six combining sites.
- Effective activation occurs only when c1q is attached to immunoglobulins by at least two of its binding sites.
- One molecule of IgM or two molecules of IgG can therefore initiate the process.

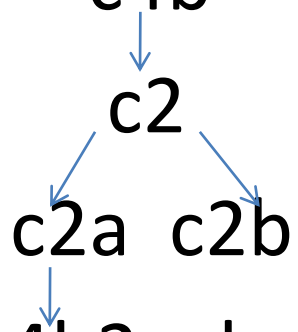


ANTIBODY CLASSIFICATION



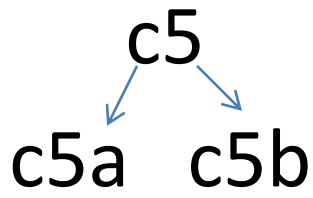
- c1q binding in the presence of calcium ions leads to sequential activation of c1r & c1s.
- 2) Activated c1s is an esterase, one molecule of which can cleave several molecules of c4.
- c4 is split into c4a which is an anaphylatoxin & c4b which binds to cell membrane along with c1.
- 

- 3) c4b in the presence og magnesiun ions cleave c2 into c2a which remains linked to cell bound c4b & c2b which is released into fluid phase.



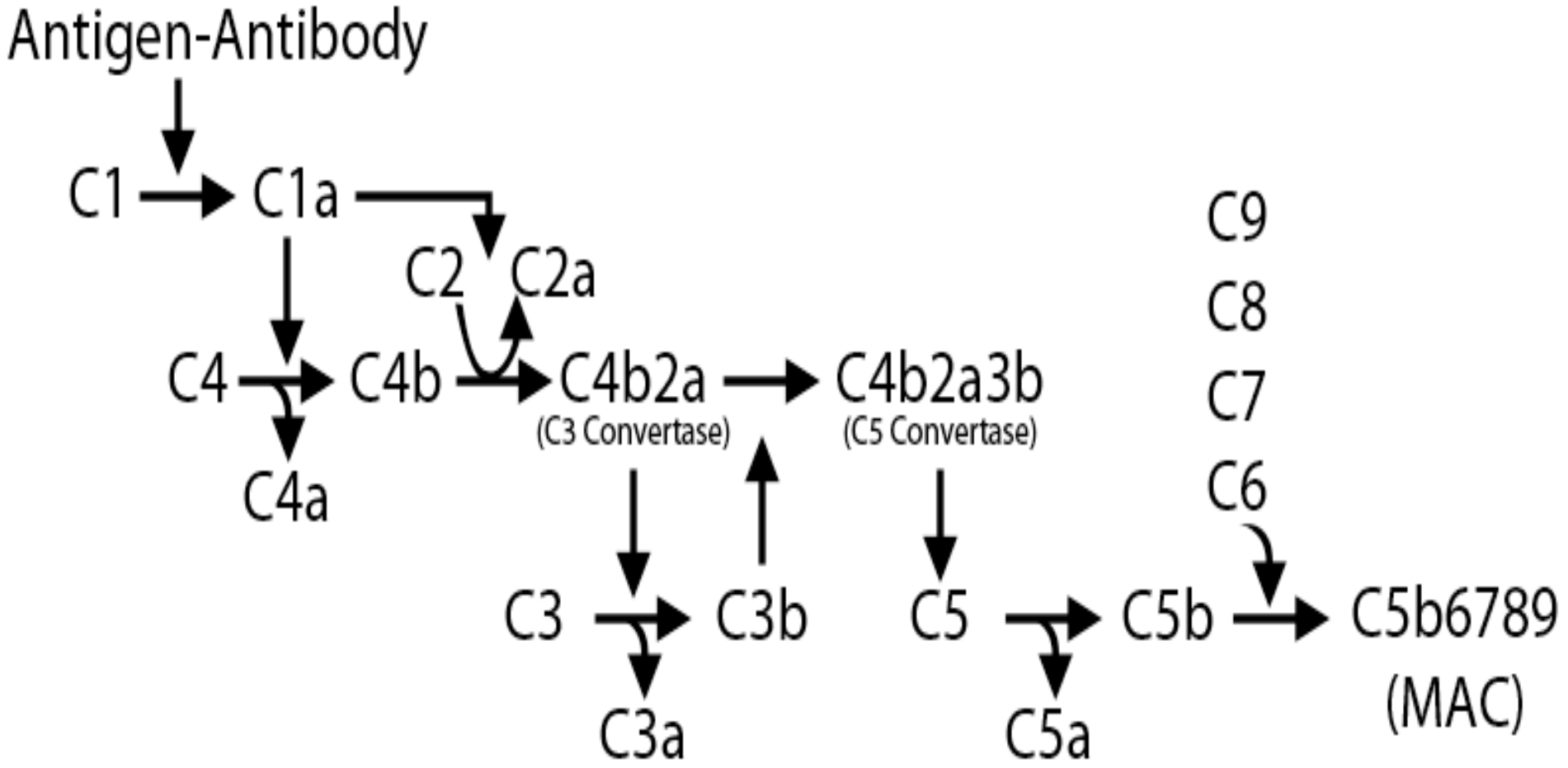
- c4b2a has enzymatic activity & is known as classical pathway c3 convertase.

- 4) c3 convertase splits c3 into two fragments,
- c4b2a
- c3a c3b
- c3a is an anaphylatoxin which is released into the medium.
- c3b which remains cell bound along with c4a2b to form a trimolecular complex c4b2a3b has enzymatic activity & is called c5 convertase.

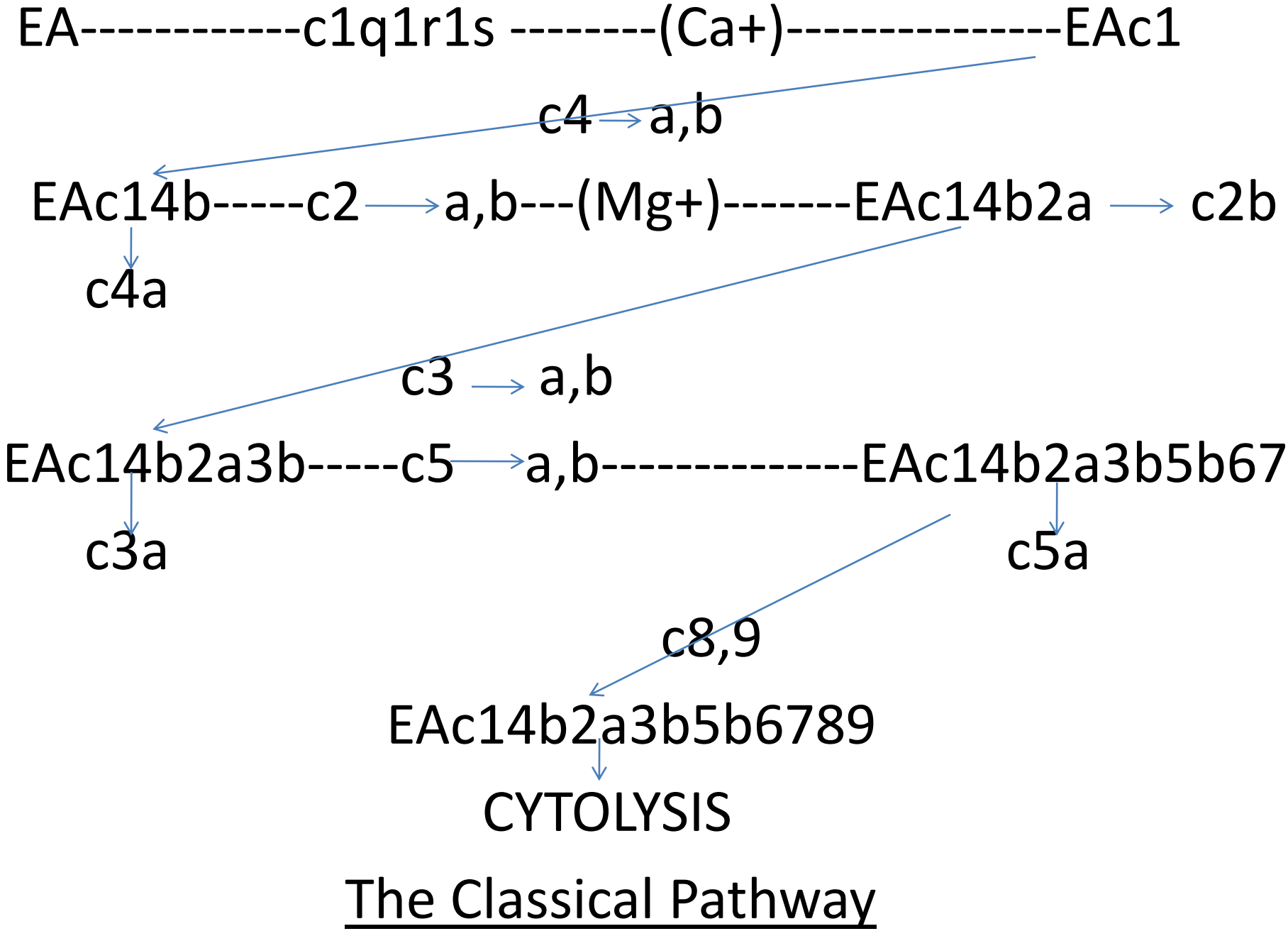
- 5) The membrane attack phase of complement activity begins at this stage with c5 convertase, converting c5 into,
- 

- c5a is an anaphylatoxin which is released into the medium.
- c5b continues the cascade.
- c6 & c7 then join together.
- A heat stable trimolecular complex is formed c5b67.

- A part of c5b67 binds to the cell membrane & prepare it for lysis by c8 & c9 which join the reaction subsequently.
- Most of the c5b67 escape & serve to amplify the reaction by absorbing unsensitized cells & rendering them susceptible to lysis by c8 & c9.
- The mechanism of compliment mediated cytolysis



The Classical Pathway

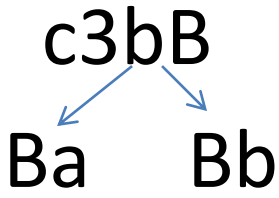


The Classical Pathway

- Alternative pathway:
- The central process in the complement cascade is the activation of c3, which is the major component of c.
- In the classical pathway, activation of c3is achieved by c42 (classical c3 convertase).
- The activation of c3 without prior participation of c142 is known as the alternative pathway.
- The first example of the alternative pathway was the demonstration by Pillemer (1954).
- He demonstrated properdin system as a group of serum proteins contributing to antibacterial defence without requiring specific antibodies.

- The activator in this system was zymosan, a polysaccharide from the yeast cell wall, but many other substances can also activate the pathway.
- These activators include bacterial endotoxins, IgA & D, the cobra venom factor & the nephritis factor (a protein present in the serum of glomerulonephritis patients.
- 1) The first step in the alternative pathway is the binding of c3b to an activator.
- c3b is continuously generated in small quantities in the circulation.
- But in free state, it is rapidly inactivated by the serum protein factors H & I.

- Bound c3b is protected from such inactivation.
- 2) It interacts with a serum protein called factor B (also known as c3 proactivator) to form a magnesium dependent complex c3bB.
- This complex is cleaved by another serum protein factor D (also known as c3 proactivator convertase) into two fragments Ba & Bb.



- Fragment Ba is released into the medium.

- 3) Fragment Bb remains bound to c3b, forming the esterase c3bBb complex, which is the alternative pathway c3 convertase.
- This enzyme c3bBb is extremely labile.
- The function of properdin (also called factor P) is to stabilize the c3 convertase.
- c3bBb hydrolyses c3 leading to further steps in the cascade as in the classical pathway.

- C3b in circulation

Free c3b inactivated by factors H & I

Stabilized when combined with activator, eg. Zymosan

c3b+ Factor B

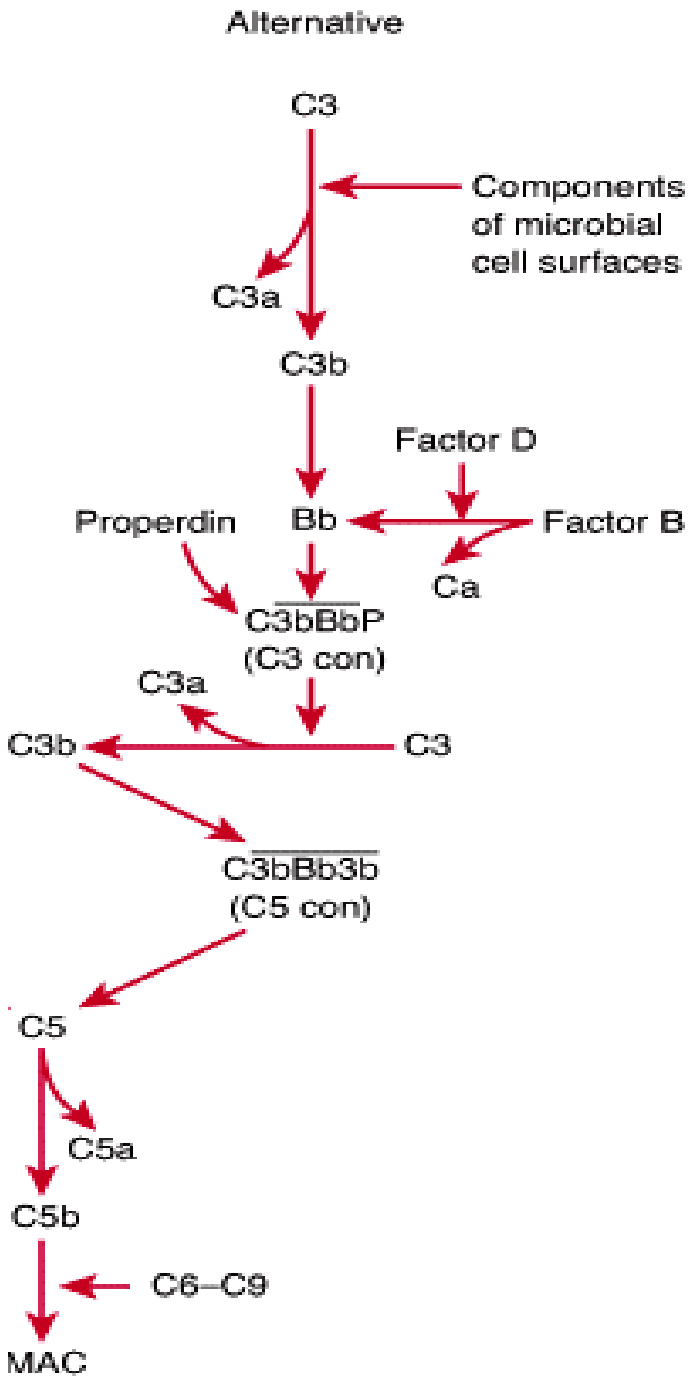
c3b B + (Factor D)

Ba

c3bBb + (Properdin)

c3b Bb P + c3

The Alternative pathway Cascade


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

Initiators of the alternative pathway of complement activation:

PATHOGENS AND PARTICLES OF MICROBIAL ORIGIN:

i) Many strains of gram-negative bacteria
ii) Lipopolysaccharides from gram-negative bacteria
iii) Many strains of gram-positive bacteria
iv) Teichoic acid from gram-positive cell walls
v) Fungal and yeast cell walls (zymosan)
vi) Some viruses and virus-infected cells
vii) Some tumor cells (Raji)
viii) Parasites (trypanosomes)

NONPATHOGENS:

i) Human IgG, IgA, and IgE in complexes
ii) Rabbit and guinea pig IgG in complexes
iii) Cobra venom factor
iv) Heterologous erythrocytes (rabbit, mouse, chicken)
v) Anionic polymers (dextran sulfate)
vi) Pure carbohydrates (agarose, inulin)

- The Lectin pathway:
- Recently, an additional means by which the complement cascade can be activated was described.
- This is known as lectin pathway.
- Lectins are proteins that bind to a carbohydrateo.
- 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 & c2, to produce a c5 convertase.

- The pathway is activated by the binding of mannose-binding lectin (MBL) to mannose residued on glycoproteins or carbohydrates on the surface of microorganisms.
- MBL is an acute phase protein produce in inflammatory responses.
- Its function in the complement pathway is similar to that of c1q, which it resembles in structure.
- 1) First MBL binds to the surface of a cell or pathogen.
- 2) Then, MBL associated serine protease (MASP) binds to it.

- 3) The active complex formed by this association causes cleavage & activation of c4.
- MASP has structural similarity to c1r & c1s & mimics their activities.
- Details of the lectin pathway remain obscure, but this way of activating the c2 – c4 components to form a c5 convertase without need for specific antibody binding certainly represents an important innate defence mechanism comparable to the alternative pathway.

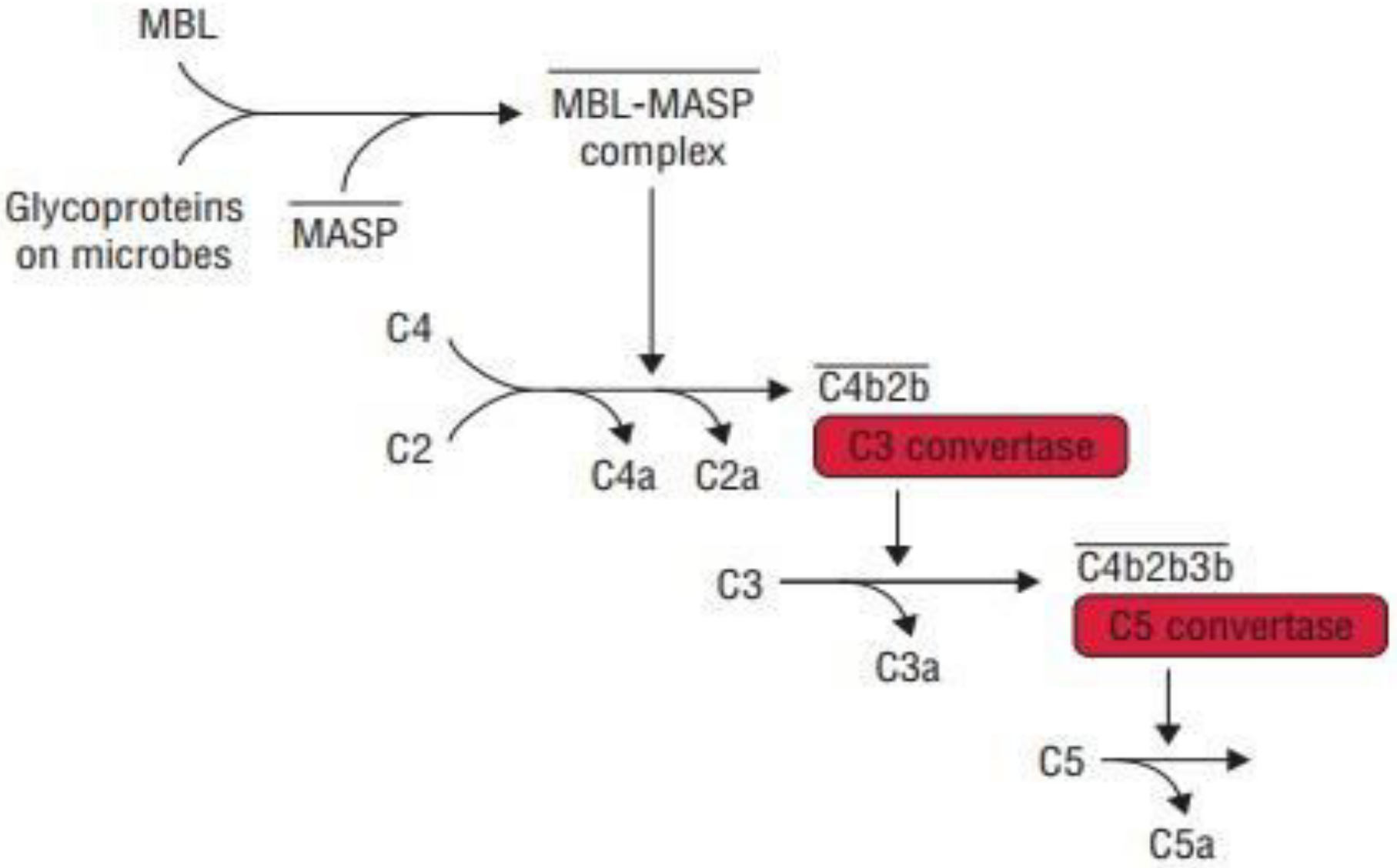
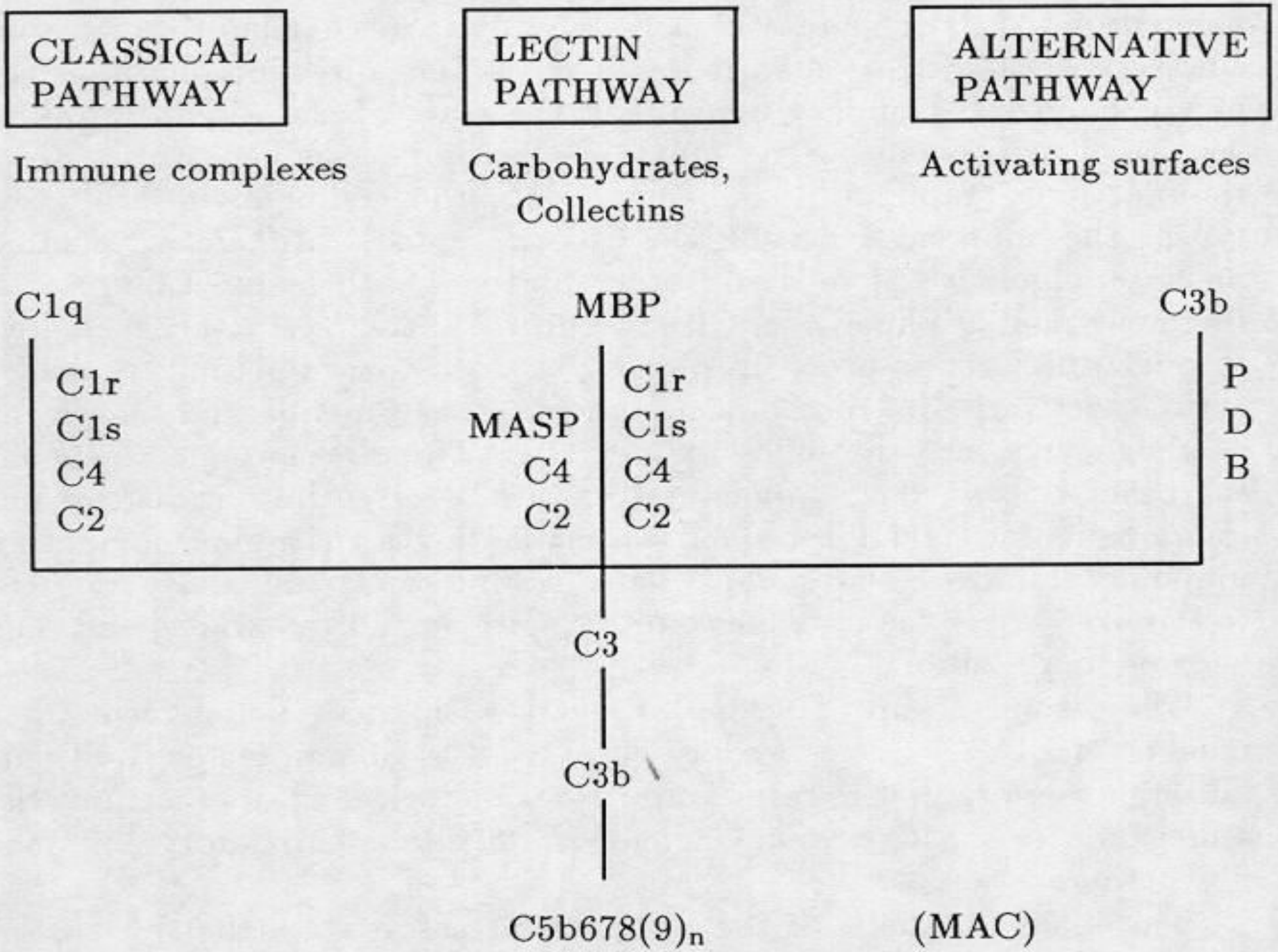
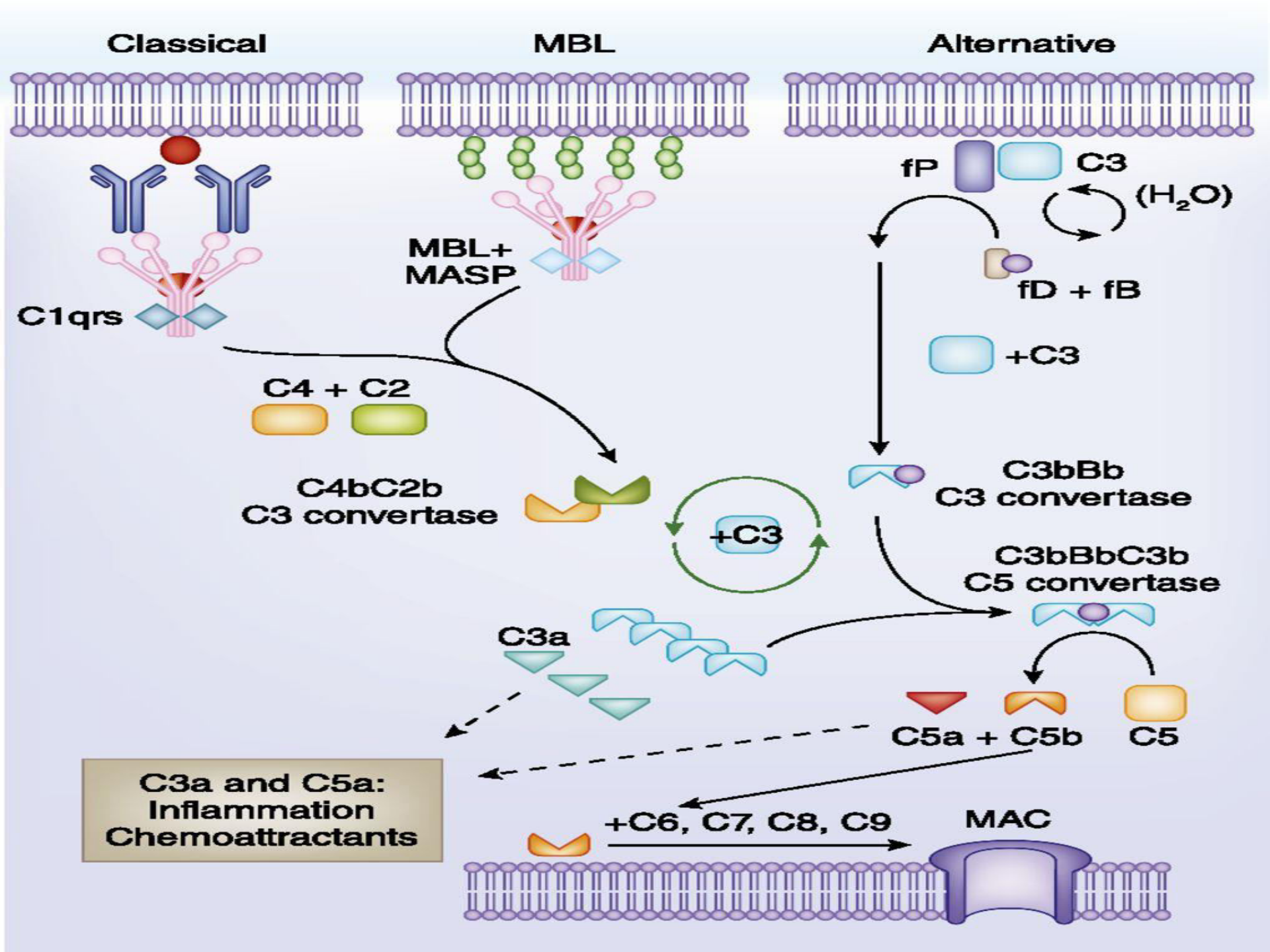


FIG. 15-5. Lectin pathway of activation of the complement.


CLASSICAL PATHWAY
LECTIN PATHWAY
ALTERNATIVE PATHWAY
Immune complexes
Carbohydrates, Collectins
Activating surfaces
C1q
MBP
C3b
C1r
C1s
C4
C2
MASP
C4
C2
C1r
C1s
C4
C2
P
D
B
C3
C3b
C5b678(9)n
(MAC)


Classical
MBL
Alternative
C1qrs
MBL+
MASP
fP
C3
(H2O)
fD + fB
+C3
C4 + C2
C4bC2b
C3 convertase
+C3
C3bBb
C3 convertase
C3bBbC3b
C5 convertase
C3a
C5a + C5b
C5
C3a and C5a:
Inflammation
Chemoattractants
+C6, C7, C8, C9
MAC

- Terminal sequence- Formation of Membrane Attack Complex:
- The terminal sequence of complement activation involves c5b, c6, c7, c8 & c9.
- These interact sequentially to form a macromolecular structure called the membrane attack complex (MAC).
- This complex displaces the membrane phospholipids, forming a large transmembrane channel that disrupts the membrane of the target cell & enables ions & small molecules to diffuse through it freely.

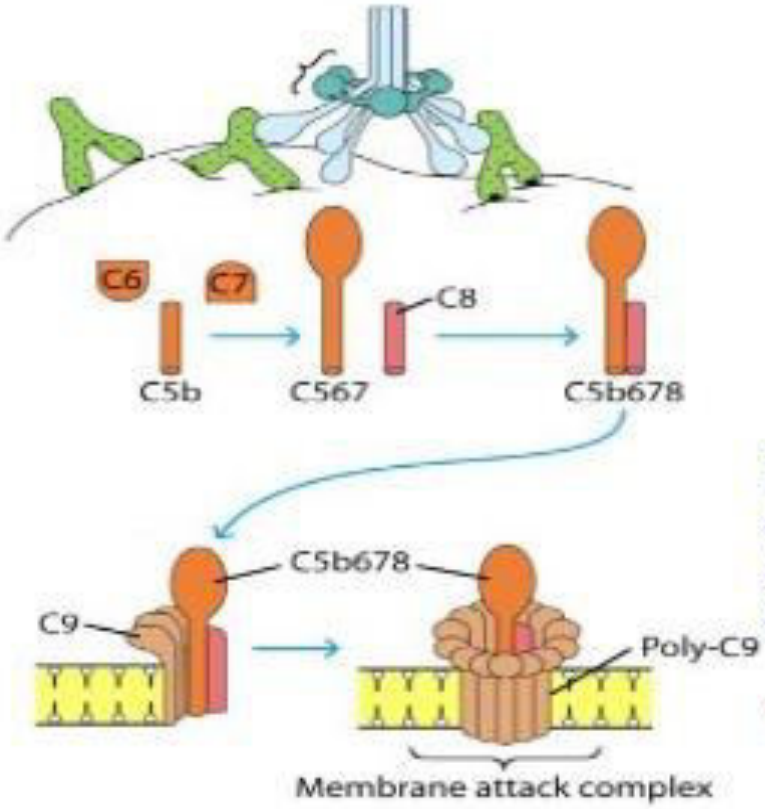
- The c5b component is extremely labile & is inactivated within two minutes unless c6 binds to it & stabilizes its activity.
- Up to this level, all complement reactions take place on the hydrophilic surface of membrane or on immune complexes in the humoral system.
- As c5b6 binds to c7, the resulting complex undergoes a hydrophilic- amphiphilic structural transition that exposes hydrophobic regions.
- These serve as binding sites for membrane phospholopids.
- This site enable c5b67 complex to insert into the phospholipid bilayer.

- Binding of c8 to this c5b67 complex induces a conformational change in c8.
- It also undergoes the hydrophilic- amphiphilic structural transition exposing a hydrophobic region which interacts with the plasma membrane.
- The c5b678 complex creates a small pore, 10 A^0 in diameter.
- Formation of this pore can lead to lysis of RBCs but not of nucleated cells.
- Final step is the binding & polymerization of c9 to the c5b678 complex.
- As many as 10-17 molecules of c9 can be bound & polymerized by a single c5b678 complex.

- During polymerization, the c9 molecules also undergo hydrophilic- amphiphilic transition so that they can be inserted into the membrane.
- The completed MAC has a tubular form & function pore size of 70-100 A^0.rough this
- It consists of a c5b678 complex surrounded by a poly c9 complex.
- Since ions & small molecules can diffuse freely this, the cell cannot maintain its osmotic stability & is killed by an influx of water & loss of electrolytes.

Schematic diagram of intermediates in the classical pathway of complement activation (5).

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

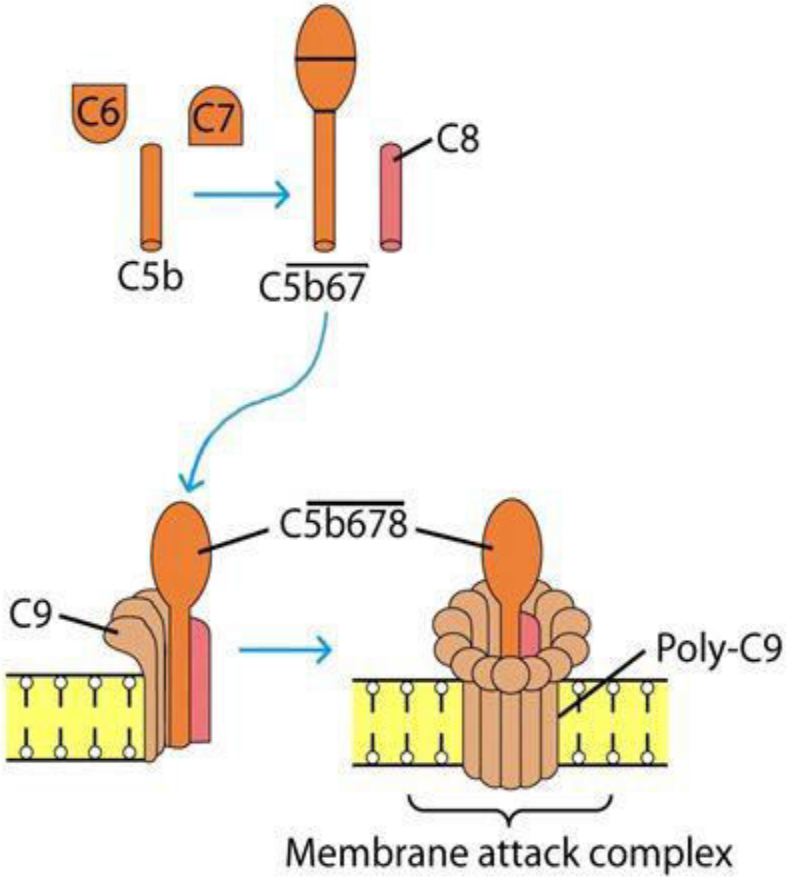


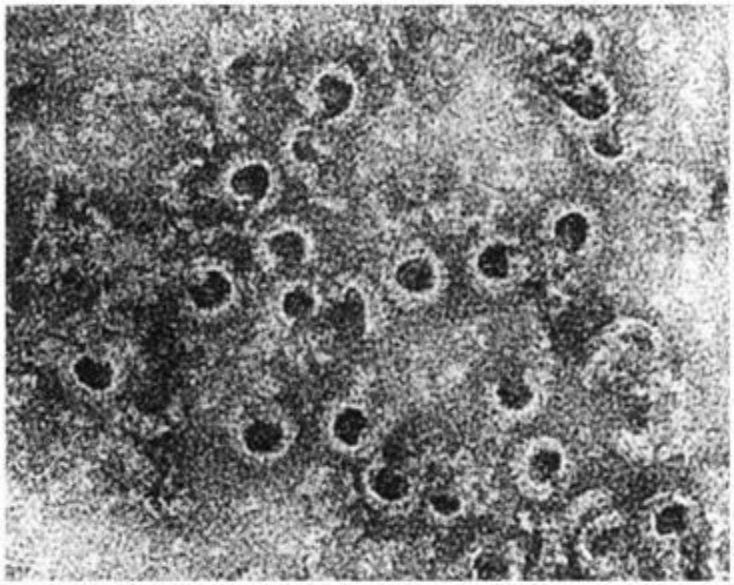
The MAC complex forms a large channel through the membrane of the target cell, enabling ions and small molecules to diffuse freely across the membrane.

C9: a perforin-like molecule

Kuby J et al., Immunology 2003

C5b triggers formation of the Membrane Attack Complex





MAC is most effective against

- Gram-negative bacteria
- Nucleated cells
- Enveloped viruses

CD59 prevents assembly of the membrane attack complex on human cells

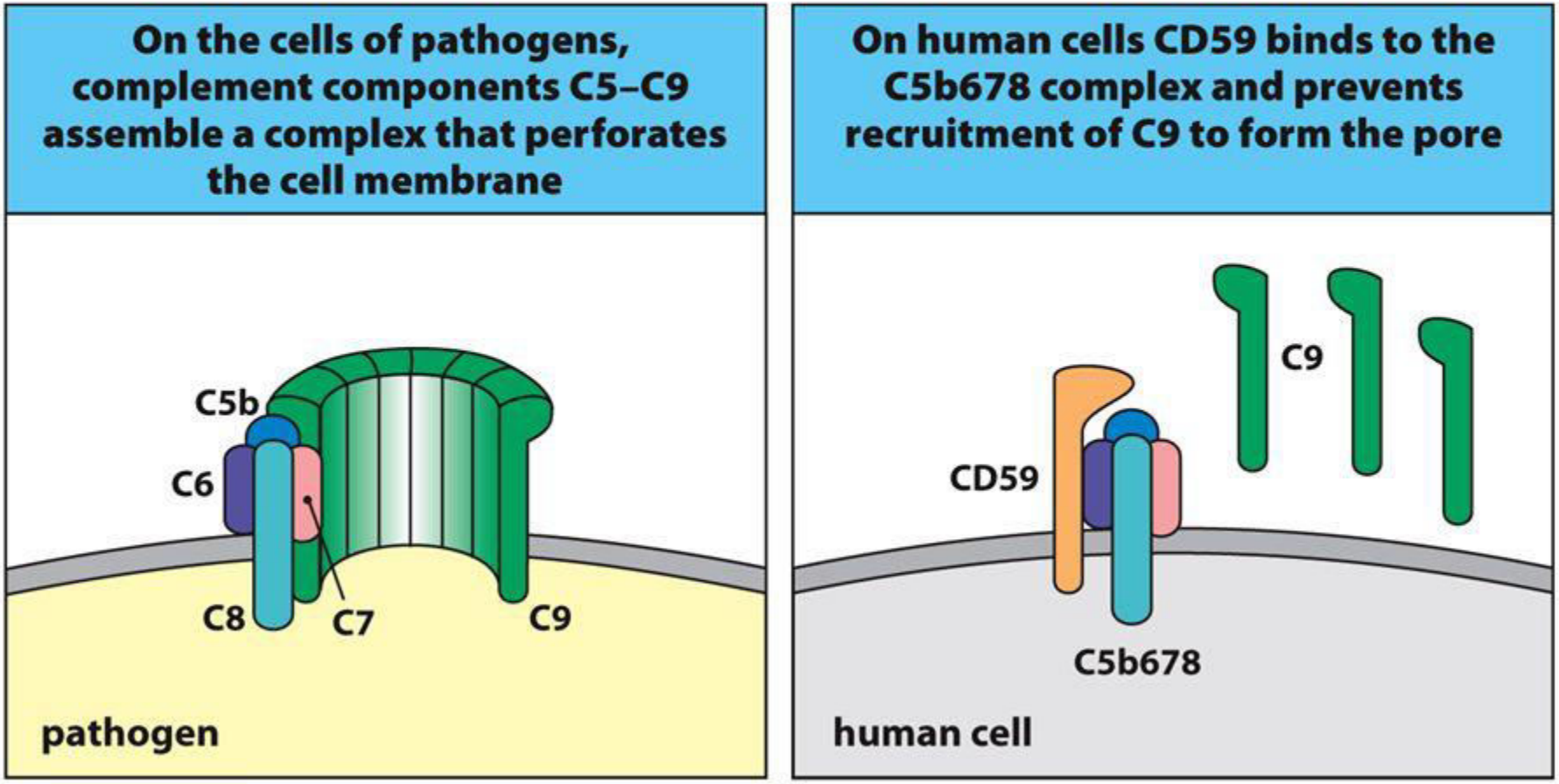


Figure 2.14 The Immune System, 4th ed. (© Garland Science 2015)