COMPLEMENT SYSTEM

RAKESH SHARDA

Department of Veterinary Microbiology

NDVSU College of Veterinary Science & A.H.,

MHOW

Complement: History

Discovered in 1894 by Bordet

It represents lytic activity of fresh serum

Its lytic activity destroyed when heated at 56°C for 30 min

Complement

- A series of molecules present in serum which interact with the bacterial cell membranes and with each other so that, at completion, they punch a hole in the bacterial membrane.
- It is composed of a series of reactions which act as a cascade with the product of one active on the next in the chain.
- The products amplify the reactions, increase permeability of blood vessels and also act to attract cells to the site of the reaction. The products make the target more attractive to phagocytosis by immune cells (“opsonization”).
- 30+ components – 20 are soluble, rest attached to membranes

Complement

- It can be activated by interaction of one of the initial components with bacterial cell surface substances (“Lectin pathway”), bacterial products (the “Alternative pathway”), or by antibodies that have bound to antigens (the “Classical pathway”).
- There is a “recognition unit”, made up of some of the initial components.
- The final product is the “membrane attack complex”.

Complement functions

- Host benefit:
  - opsonization to enhance phagocytosis
  - phagocyte attraction and activation
  - lysis of bacteria and infected cells
  - regulation of antibody responses
  - clearance of immune complexes
  - clearance of apoptotic cells
- Host detriment:
  - Inflammation, anaphylaxis

Definitions

- C-activation: alteration of C proteins such that they interact with the next component
- C-fixation: utilization of C by Ag-Ab complexes
- Hemolytic units (CH50): dilution of serum which lyses 50% of a standardized suspension of Ab-coated r.b.c
- C-inactivation: denaturation (usually by heat) of an early C-component resulting in loss of hemolytic activity
- Convertase/esterase: altered C-protein which acts as a proteolytic enzyme for another C-component

Proteins of the complement system (nomenclature)

- C1(qrs), C2, C3, C4, C5, C6, C7, C8, C9
- factors B, D, H and I, properdin (P)
- mannose binding lectin (MBL), MBL associated serine proteases (MASP-1 MASP-2)
- C1 inhibitor (C1-INH, serpin), C4-binding protein (C4-BP), decay accelerating factor (DAF), Complement receptor 1 (CR1), protein-S (vitronectin)

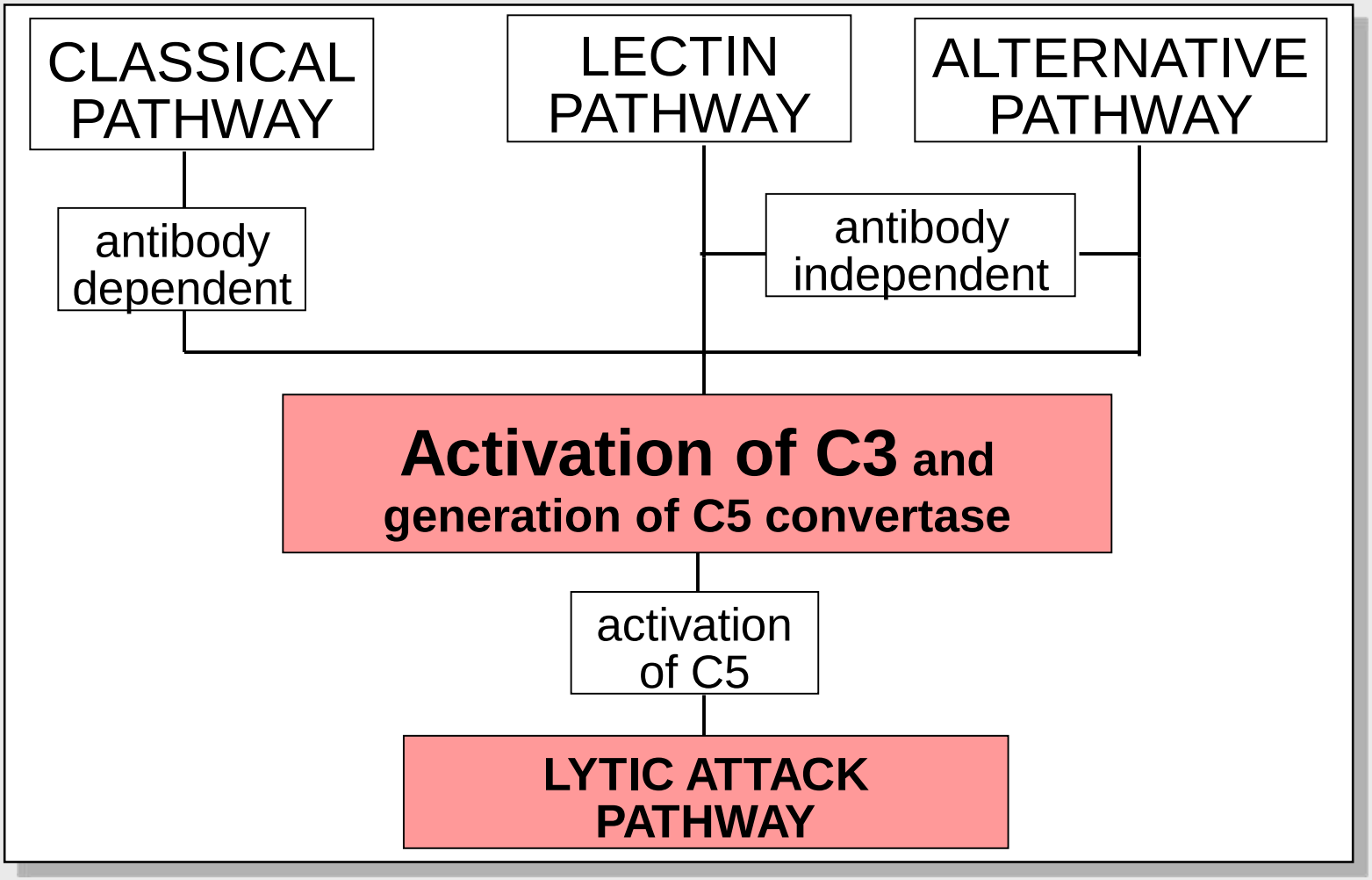
Activation product of complement proteins (nomenclature)

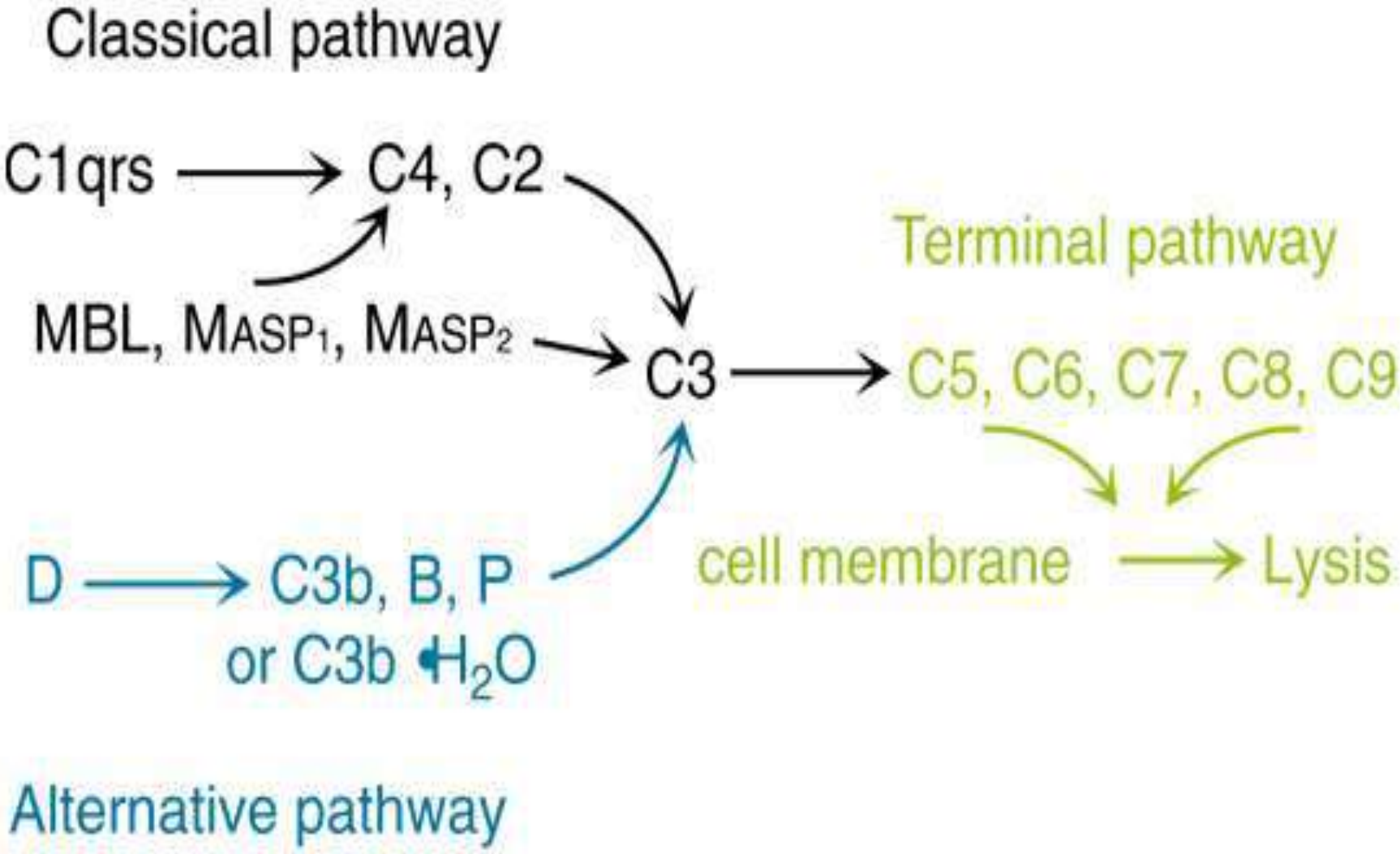
Activated component are usually over-lined: *e.g.* C1$\overline{qrs}$

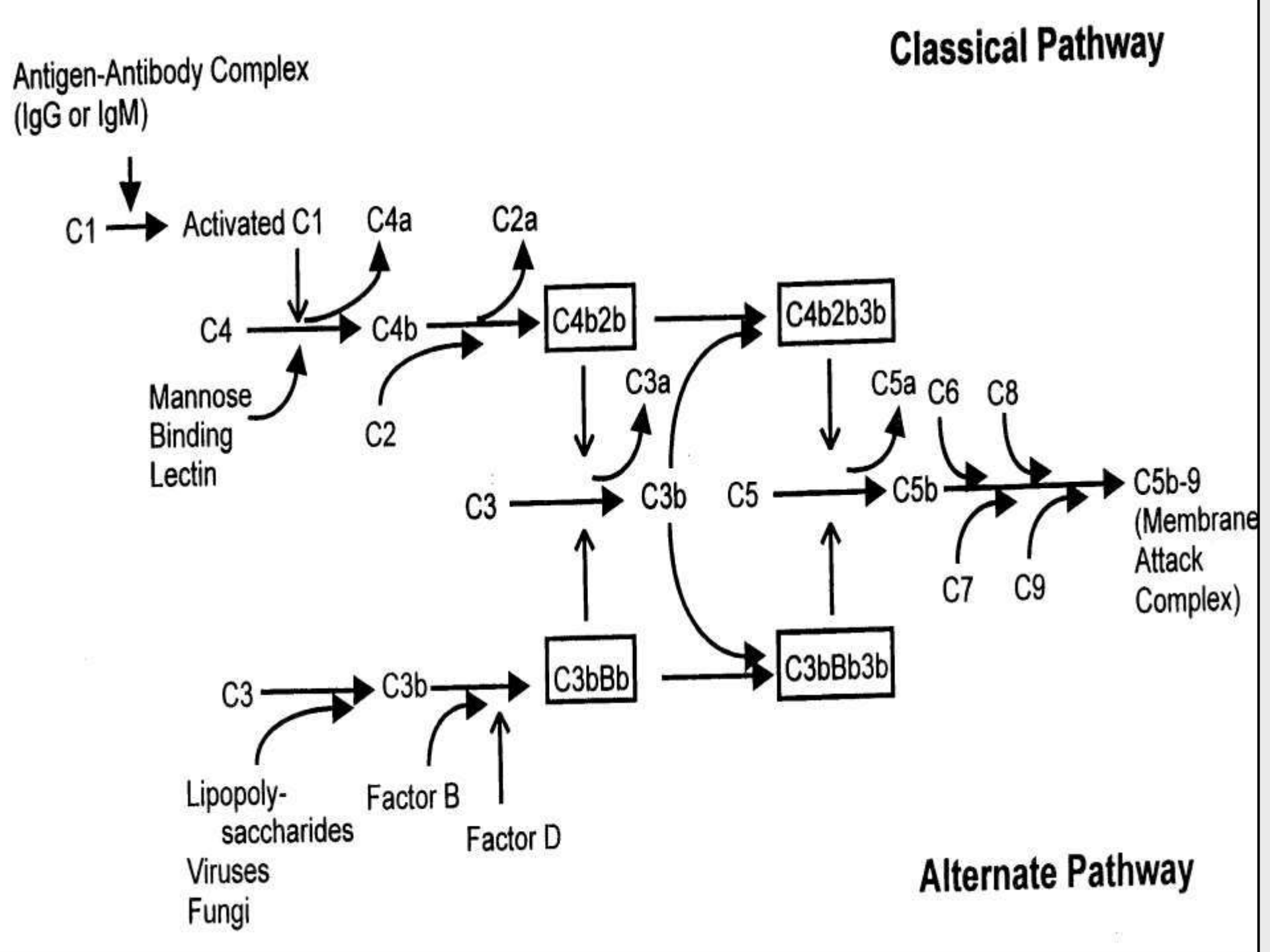
When enzymatically cleaved, the larger moiety, binds to the activation complex or membrane and the smaller peptide is released in the microenvironment

Letter “b” is *usually* added to the larger, membrane-binding, peptide and “a” to the smaller peptide (*e.g.*, C3b/C3a, C4b/C4a, C5b/C5a), ***EXCEPT*** C2 (the larger, membrane-binding moiety is C2a; the smaller one is C2b)

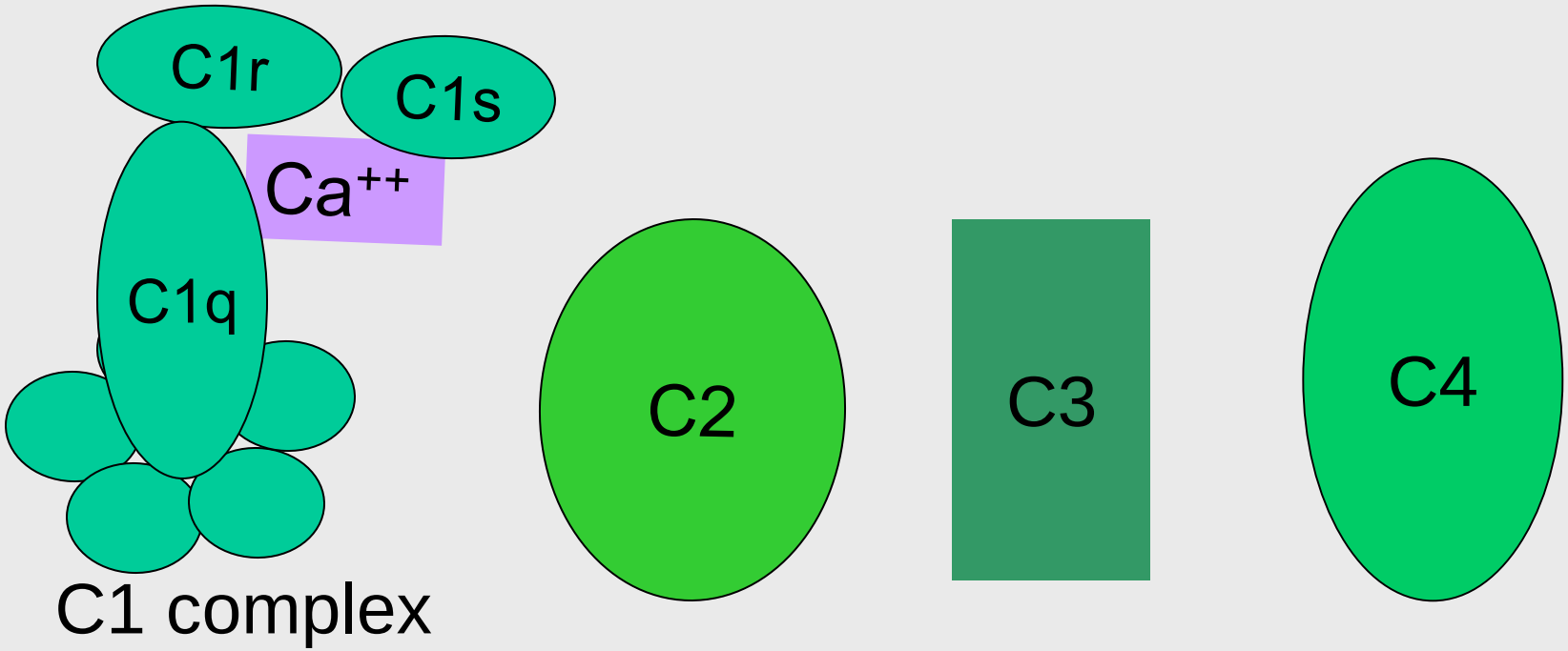
Pathways of complement activation






Classical Pathway
Antigen-Antibody Complex
(IgG or IgM)
C1
Activated C1
C4a
C2a
C4
C4b
C4b2b
C4b2b3b
Mannose
Binding
Lectin
C2
C3a
C5a
C6
C8
C3
C3b
C5
C5b
C5b-9
(Membrane
Attack
Complex)
C7
C9
C3
C3b
C3bBb
C3bBb3b
Lipopoly-
saccharides
Viruses
Fungi
Factor B
Factor D
Alternate Pathway

Components of the Classical Pathway



C1 a multi-subunit protein containing three different proteins, C1q, C1r and C1s, binds to the Fc region of IgG and IgM antibody molecules that have interacted with antigen.

Classical Pathway
Generation of C3-convertase

- **The binding of C1 to antibody is via C1q and C1q must cross link at least two antibody molecules before it is firmly fixed.**
- **The binding of C1q results in the activation of C1r which in turn activates C1s. The result is the formation of an activated "C1qrs", which is an enzyme that cleaves C4 into two fragments C4a and C4b.**
- **The C4b fragment binds to the membrane and the C4a fragment is released into the microenvironment.**
- **Activated "C1qrs" also cleaves C2 into C2a and C2b.**
- **C2a binds to the membrane in association with C4b and C2b is released into the microenvironment. The resulting C4bC2a complex is a C3 convertase, which cleaves C3 into C3a and C3b.**

Classical Pathway
Generation of C5-convertase

- C3b binds to the membrane in association with C4b and C2a
- C3a is released into the microenvironment.
- The resulting C4bC2aC3b is a C5 convertase.
- The generation of C5 convertase is the end of the classical pathway.

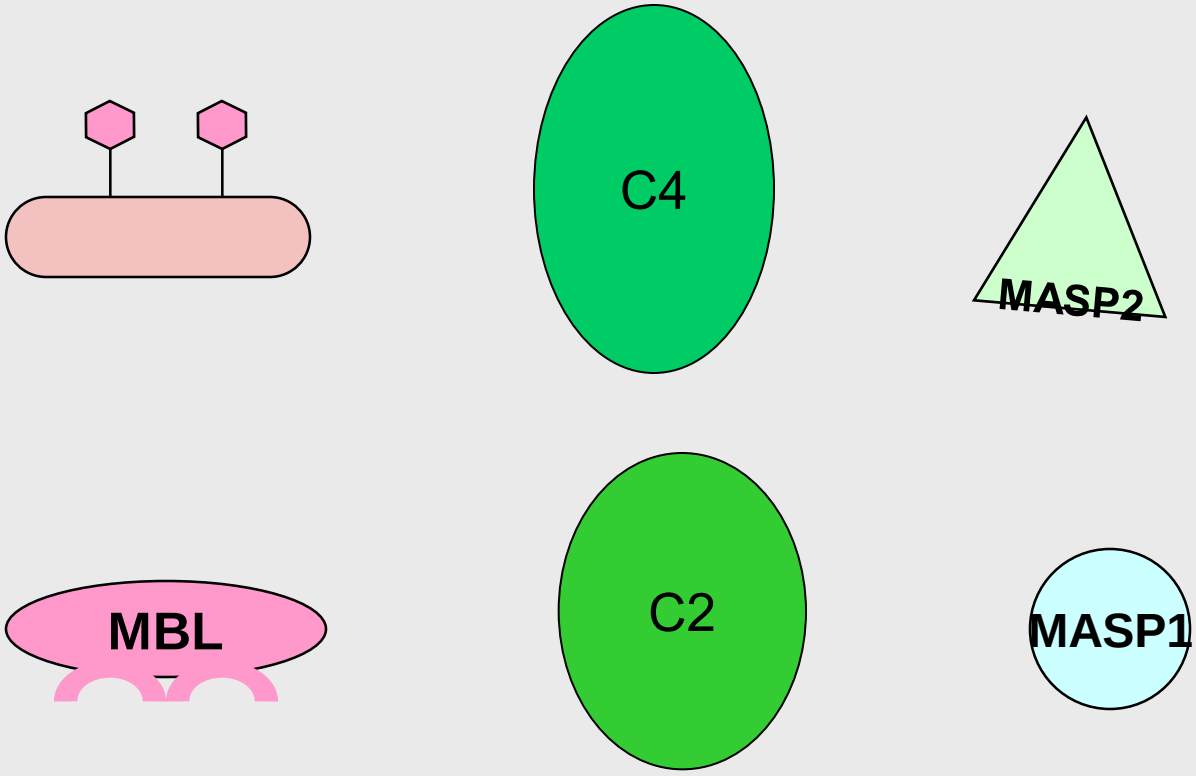
Biological Activities of Classical Pathway Components

Component	Biological Activity
C2b	Prokinin; cleaved by plasmin to yield kinin, which results in edema
C3a	Anaphylotoxin; can activate basophils and mast cells to degranulate resulting in increased vascular permeability and contraction of smooth muscle cells, which may lead to anaphylaxis
C3b	Opsonin Activation of phagocytic cells
C4a	Anaphylaotoxin
C4b	Opsonin

Control of Classical Pathway Components

Component	Regulation
All	C1-inhibitor (C1-INH); dissociates C1r and C1s from C1q
C3a	C3a-inactivator (C3a-INA; Carboxypeptidase B)
C3b	Factors H and I; Factor H facilitates the degradation of C3b by Factor I
C4a	C3a-INH
C4b	C4 binding protein (C4-BP) and Factor I; C4-BP facilitates degradation of C4b by Factor I; C4-BP also prevents the association of C2a with C4b thus blocking formation of C3 convertase

Components of mannose-binding lectin pathway



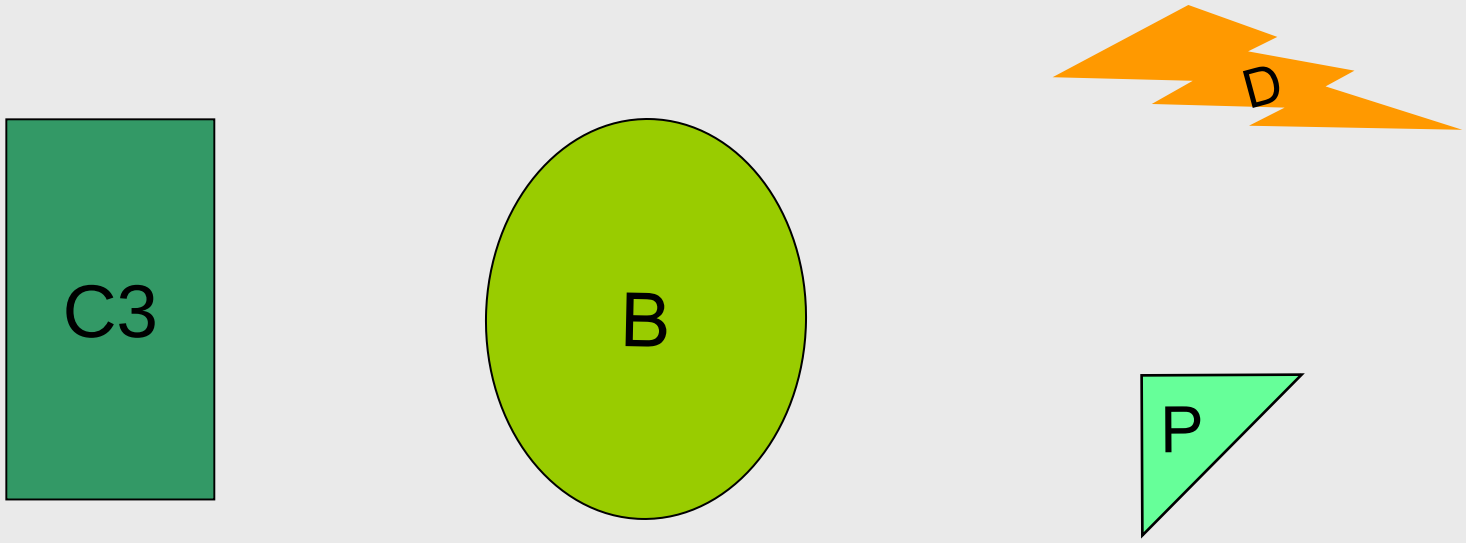
Mannose-binding lectin pathway

- Initiated by the binding of mannose binding lectin (MBL) to bacterial surfaces with mannose-containing polysaccharides
- Binding of MBL to a pathogen results in the association of two serine proteases, MASP-1 and MASP-2 (MBL-associated serine proteases).
- Formation of the MBL/MASP-1/MASP-2 tri-molecular complex results in the activation of the MASPs and subsequent cleavage of C4 into C4a and C4b.
- The C4b fragment binds to the membrane and the C4a fragment is released into the microenvironment.

Mannose-binding lectin pathway

- Activated MASPs also cleave C2 into C2a and C2b.
- C2a binds to the membrane in association with C4b and C2b is released into the microenvironment.
- The resulting C4bC2a complex is a C3 convertase, which cleaves C3 into C3a and C3b.
- C3b binds to the membrane in association with C4b and C2a and C3a is released into the microenvironment.
- The resulting C4bC2aC3b is a C5 convertase.
- The generation of C5 convertase is the end of the lectin pathway.

Components of the alternative pathway



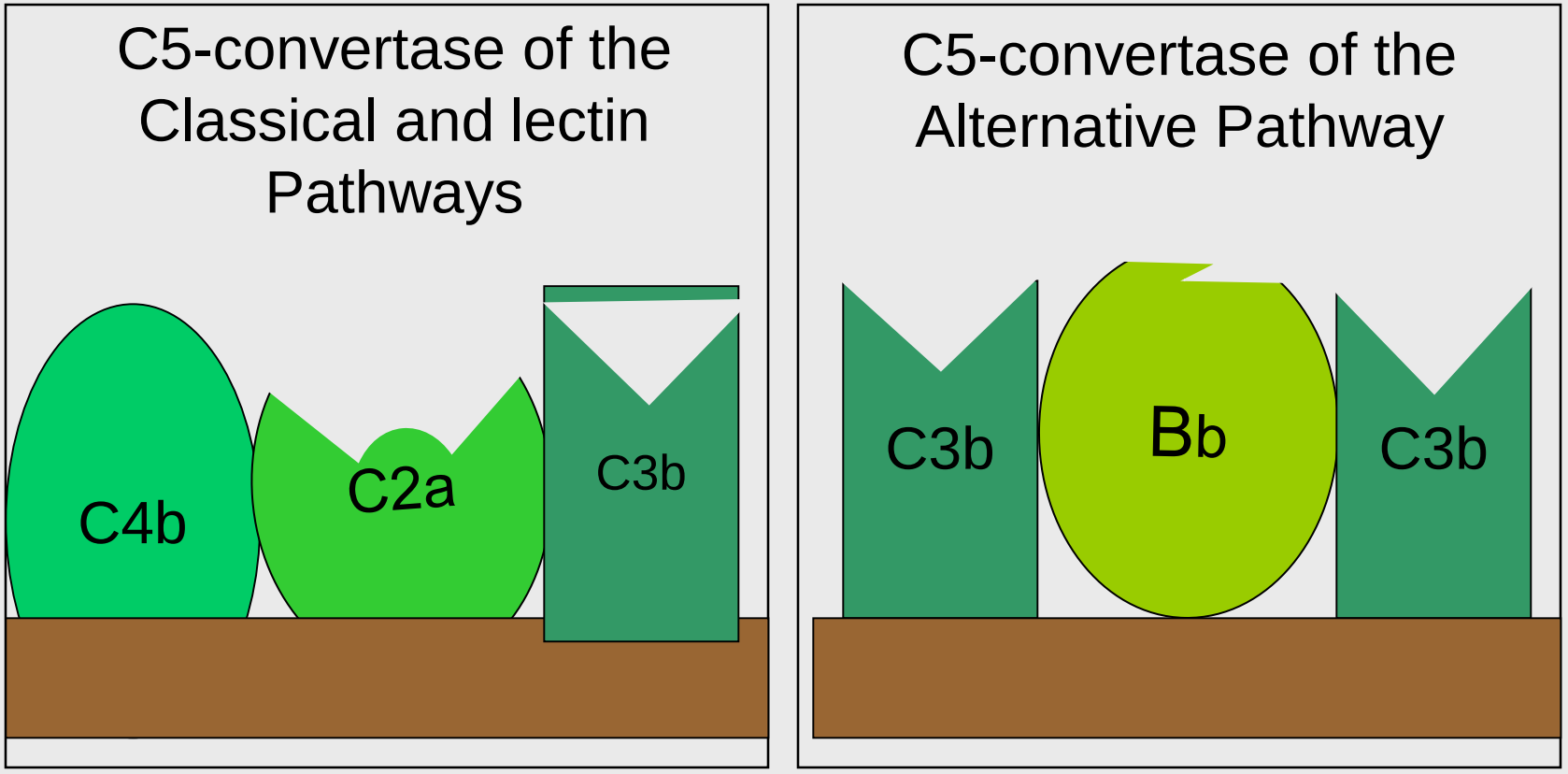
Spontaneous C3 activation

- In serum there is low level spontaneous hydrolysis of C3 to produce C3i.
- Factor B binds to C3i and becomes susceptible to Factor D, which cleaves Factor B into Bb.
- The C3iBb complex acts as a C3 convertase and cleaves C3 into C3a and C3b.
- C3iBb complex has a very short half life

C3-activation
the amplification loop

- **Once C3b is formed, Factor B will bind to it and becomes susceptible to cleavage by Factor D.**
- **The resulting C3bBb complex is an alternative C3 convertase that will continue to generate more C3b, thus amplifying C3b production.**

C5-convertase of the two pathways

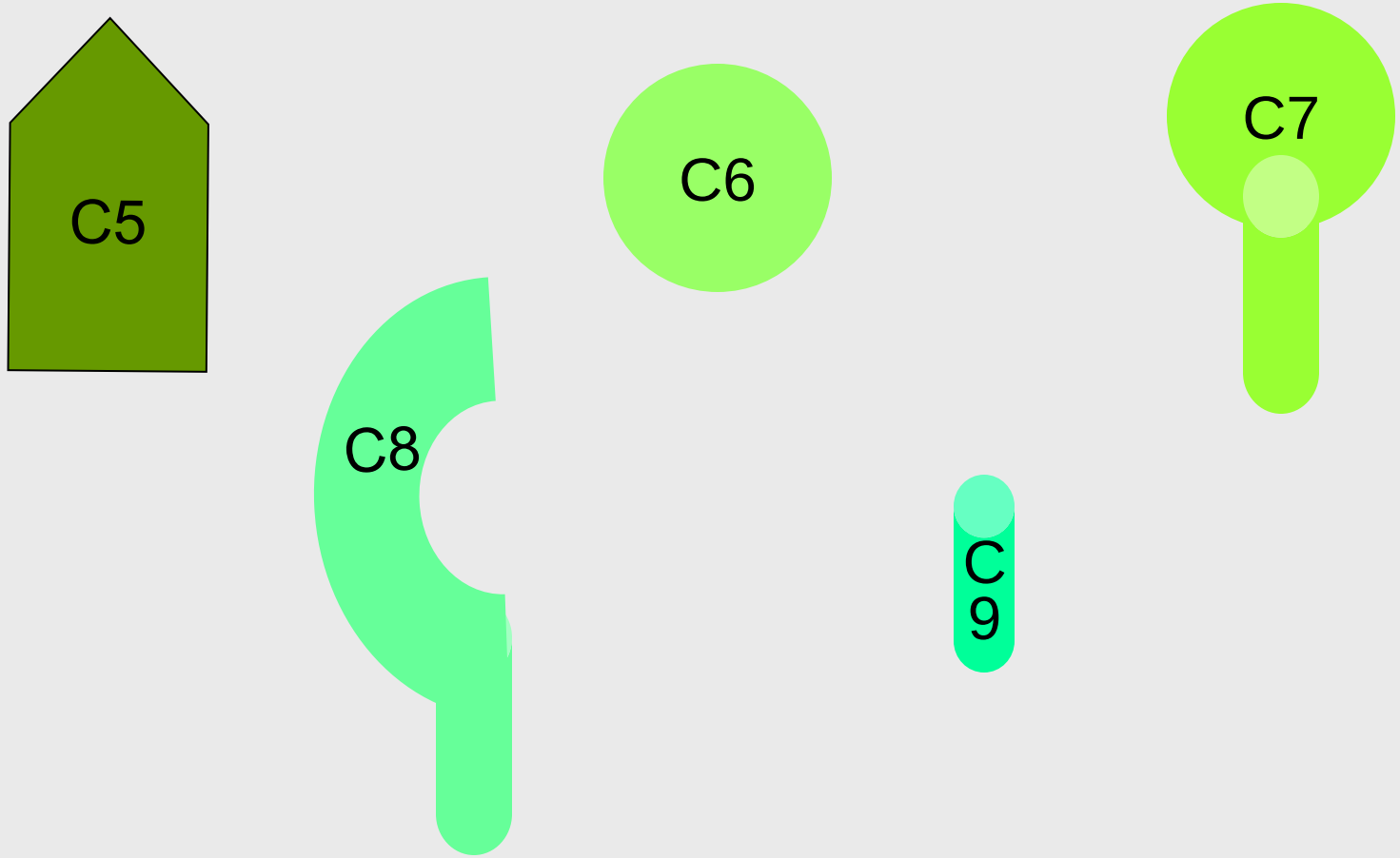


Lytic pathway

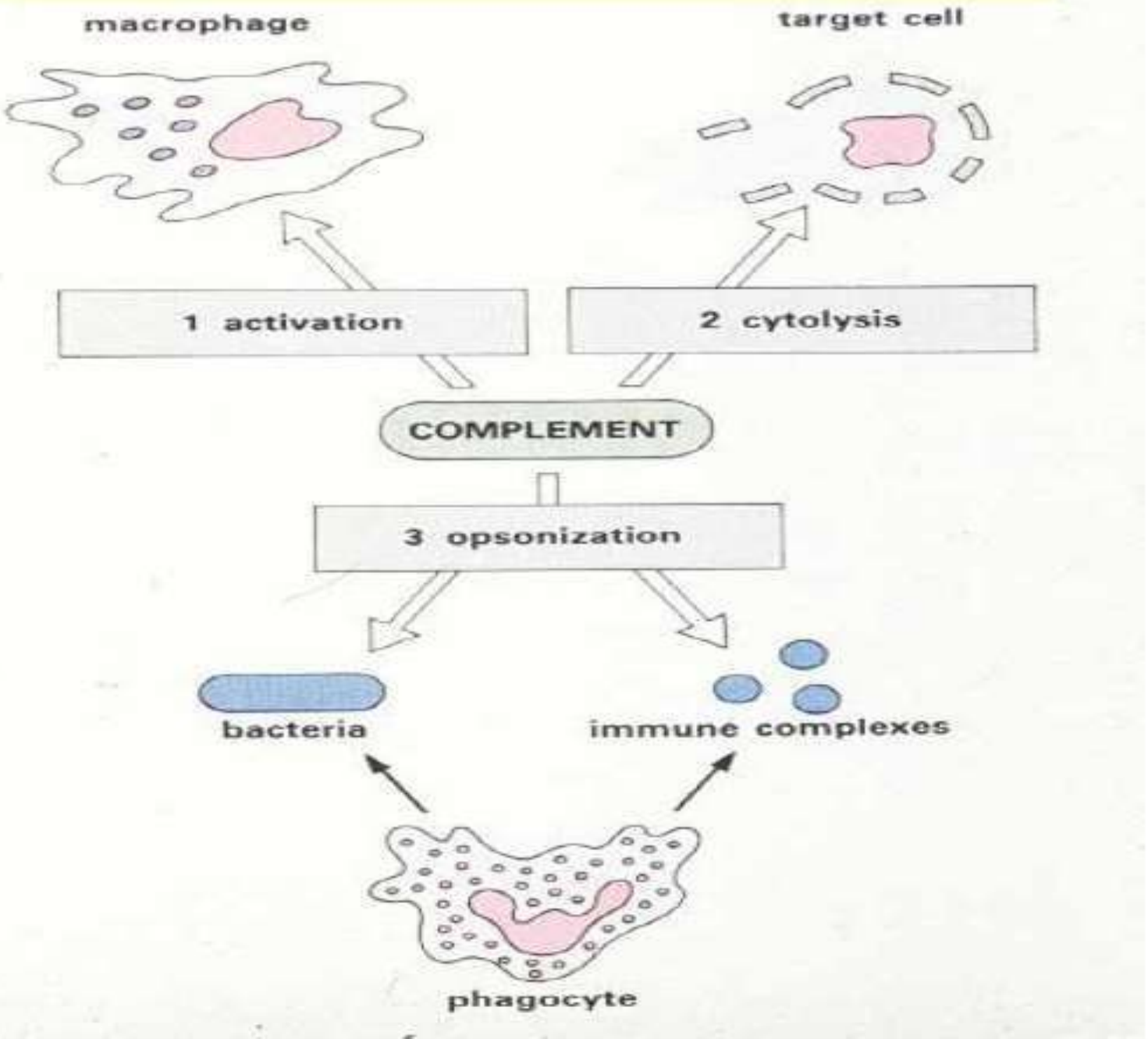
Generation of C5 convertase leads to the activation of the

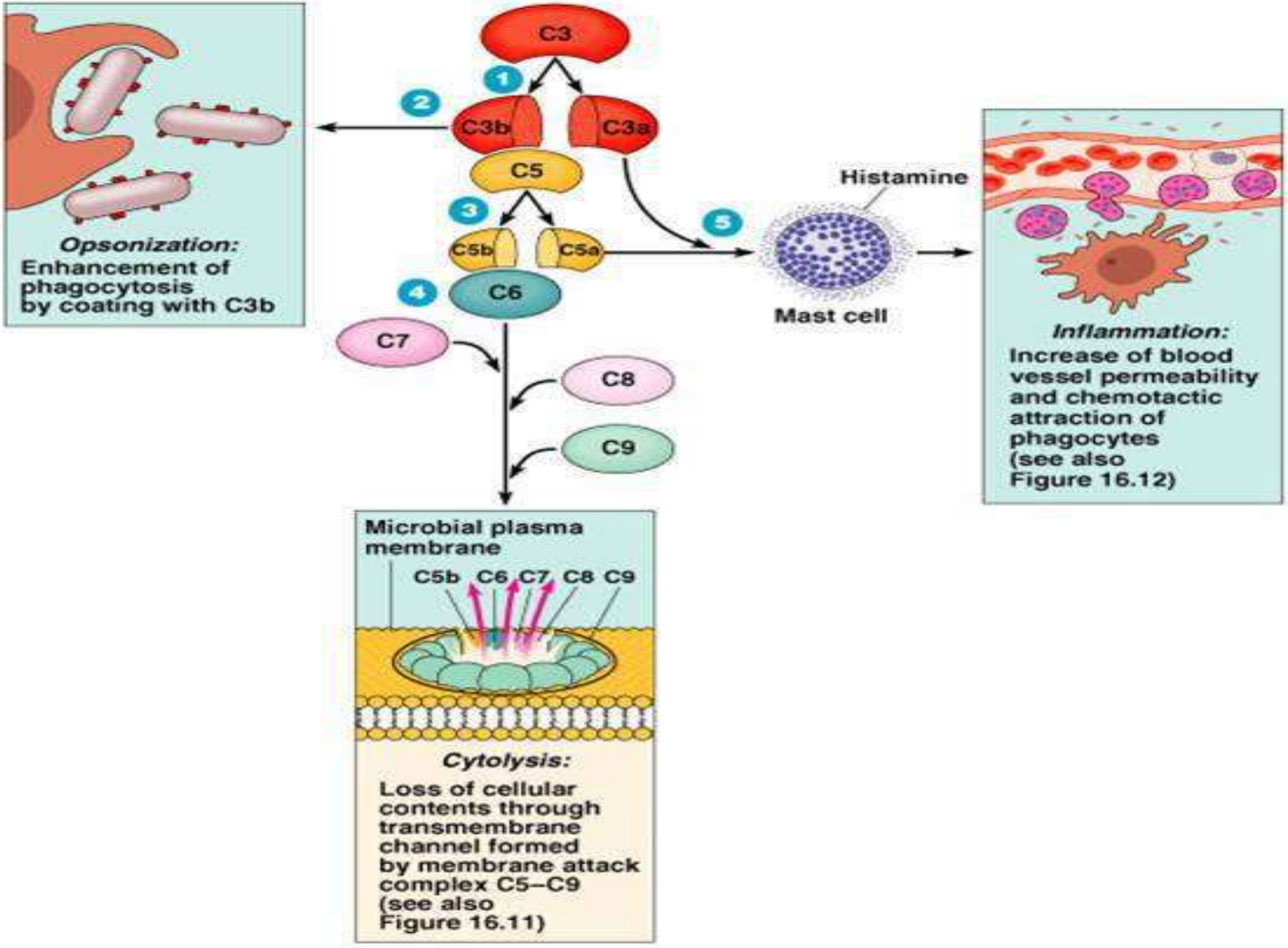
Lytic pathway

Components of the lytic pathway



Biological Activities of Complement





Biological properties of C-activation products

Product	Biological Effects	Regulation
C2b (prokinin)	edema	C1-INH
C3a (anaphylatoxin)	mast cell degranulation; enhanced vascular permeability; anaphylaxis	carboxy-peptidase- B (C3-INA)

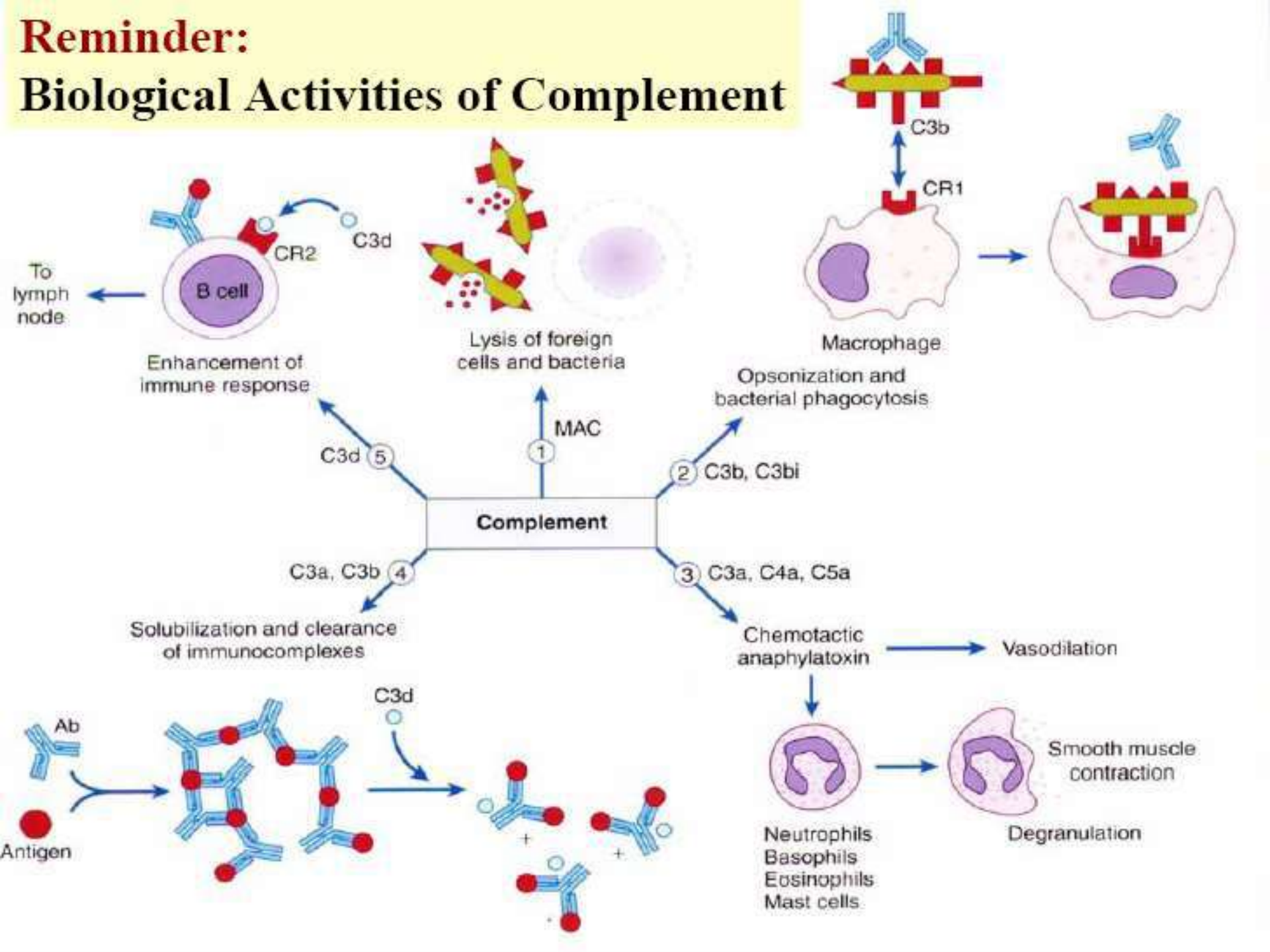
Biological properties of C-activation products

Product	Biological Effects	Regulation
C3b (opsonin)	opsonization; phagocyte activation	factors H & I
C4a (anaphylatoxin)	as C3, but less potent	(C3-INA)
C4b (opsonin)	opsonization; phagocytosis	C4-BP, factor I

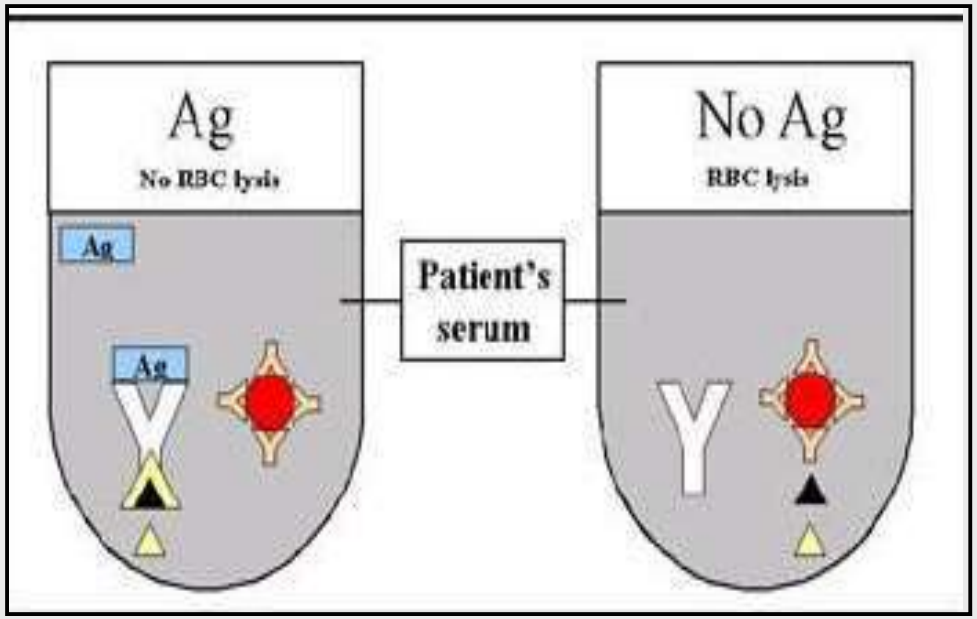
Biological properties of C-activation products

Product	Biological Effects	Regulation
C5a (chemotactic factor)	anaphylactic as C3, but much more potent; attracts & activates PMN causes neutrophil aggregation, stimulation of oxidative metabolism and leukotriene release	carboxy-peptidase-B (C3-INA)
C5b67	chemotaxis, attaches to other membranes	protein-S

Reminder:
Biological Activities of Complement



COMPLEMENT FIXATION TEST

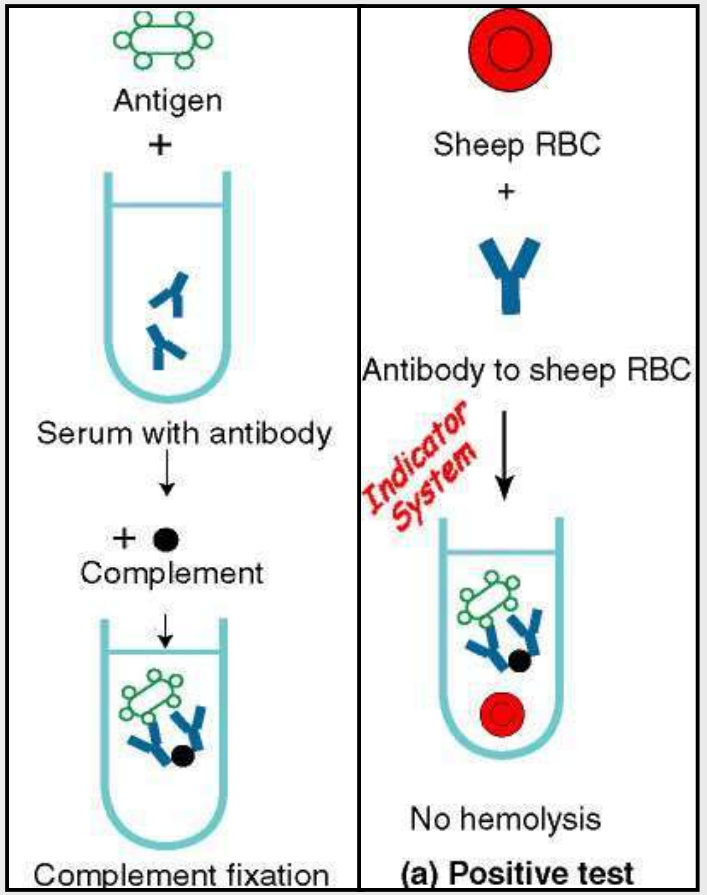


COMPLEMENT FIXATION TEST

The activation of the classical complement pathway by antibody bound to antigen results in the generation of membrane attack complexes that can disrupt the membranes. If the antibody is bound to red cells, these are ruptured and haemolyis occurs. This phenomenon can be used to measure serum antibody levels in a test called the complement fixation test. Complement fixation tests are most useful as an aid in the diagnosis of acute or recent viral infection, because they primarily detect IgM class of antibody. The test entails the use of viral antigens, guinea pig complement and an indicator system of "sensitized" sheep RBCs, ie.e sheep RBCs + anti-sheep RBC antibody (haemolysin).

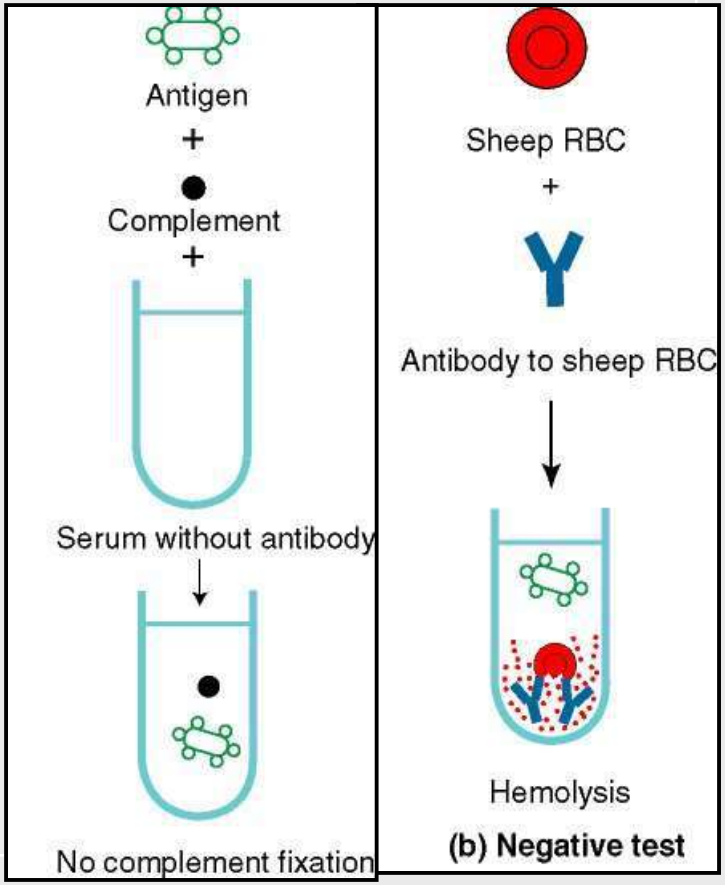
COMPLEMENT FIXATION TEST

- **The complement fixation assay indicator system uses sheep red blood cells (SRBC) and anti-SRBC antibody (haemolysin).**
- **If the ANTIBODY SPECIFIC FOR THE ANTIGEN IN THE ASSAY IS PRESENT in the patient's serum, then complement is completely consumed in the reaction and there is none left to bind to the SRBC/anti-SRBC complexes.**
- **A Test Positive For Ab = NO HEMOLYSIS**



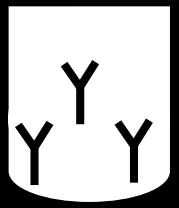
COMPLEMENT FIXATION TEST

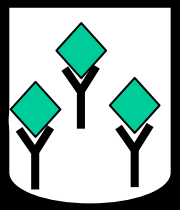
- If there is NO ANTIBODY PRESENT in the patient's serum the antigen is not bound, and the complement reagent does not have immune complexes with which to react.
- Complement is still present in the indicator reaction and binds strongly to the SRBC/anti-SRBC complexes. This causes the SRBCs to burst in a process called hemolysis.
- A Test Negative For Ab = LOTS OF HEMOLYSIS

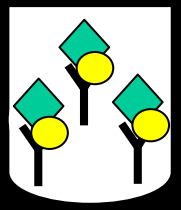


Complement Fixation

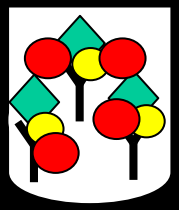
Day 1

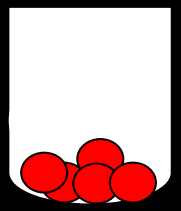

Serum with antibodies


Antigen binds to antibodies

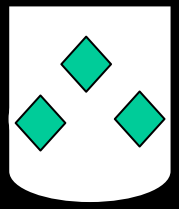

Complement binds to Ag/Ab complex

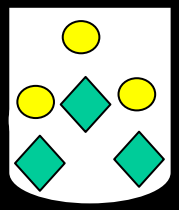
Day 2

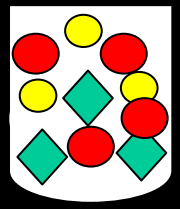

Hemolysin sensitized red blood cells serve as an indicator

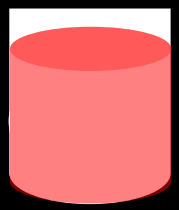

No lysis
Positive


Serum without antibodies


Unbound antigen


Unbound complement


Hemolysin sensitized red blood cells serve as an indicator


Lysis
Negative

Complement Fixation