# SCHOOL OF STUDIES IN ZOOLOGY
# JIWAJI UNIVERSITY, GWALIOR

# THE COMPLEMENT SYSTEM
## (A COMPLEX INNATE IMMUNE SURVEILLANCE SYSTEM)

**Subject :** Zoology

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**Unit :** III

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SOS in Zoology

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# HISTORY

Research on complement began in the **1890s**, when **Jules Bordet (Institute Pasteur in Paris)** observed:

1. Sheep antiserum to the bacterium *Vibrio cholerae* caused lysis of the bacteria.
2. Heating the antiserum destroyed its bacteriolytic activity.
3. Addition of fresh normal serum, that contained no Abs against the bacterium and was unable to kill the becterium by itself, restored the ability to lyse the bacteria by the heated antiserum.

- Bacteriolytic activity requires 2 different substances.

  a. The specific antibacterial Abs, which are resistant to the heating process.

  b. Heat-sensitive component responsible for the lytic activity.

- **Paul Ehrlich in Berlin** carried out similar experiments and named the substance complement, defining it as "the activity of serum that completes the action of Ab."

- **Bordet** won the **Nobel Prize in 1919** –

  **"Complement-mediated bacteriolysis"**

# COMPLEMENT PLAYS A KEY ROLE IN BOTH INNATE AND ADAPTIVE IMMUNITY

- The complement system is the major effector of the humoral immune system.
- Although the discovery of complement and most early studies were linked to the activity of complement following Ab binding, a major role for this system is the recognition and destruction of pathogens based on recognition of pathogen-associated molecular patterns, or PAMPs, rather than on Ab specificity.

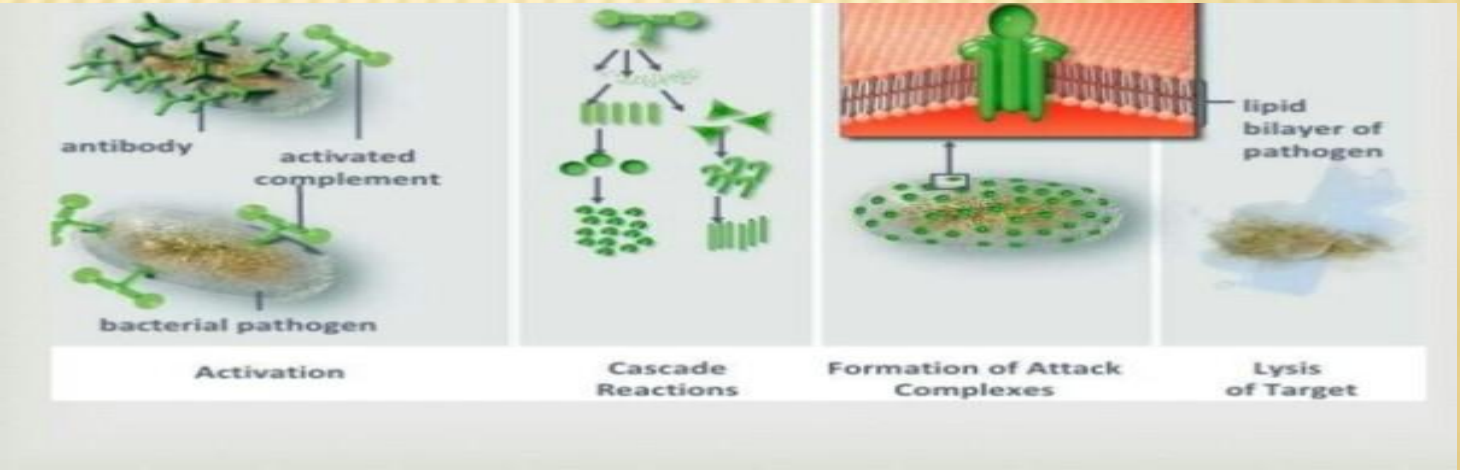
# THE COMPLEMENT COMPONENTS

- More than 30 soluble and cell-bound protein.
- Participate in both innate and adaptive immunity.
- Produced by hepatocytes (mainly), monocytes and epithelial cells of the gastrointestinal and genitourinary tracts.
- Constitute 5% (by weight) of the serum globulin fraction.
- Many components are proenzymens (zymogens), which are functionally inactive until proteolytic cleavage, which removes an inhibitory fragment and exposes the active site.
- Reaction starts with an enzyme cascade.

# THE FUNCTIONS OF COMPLEMENT

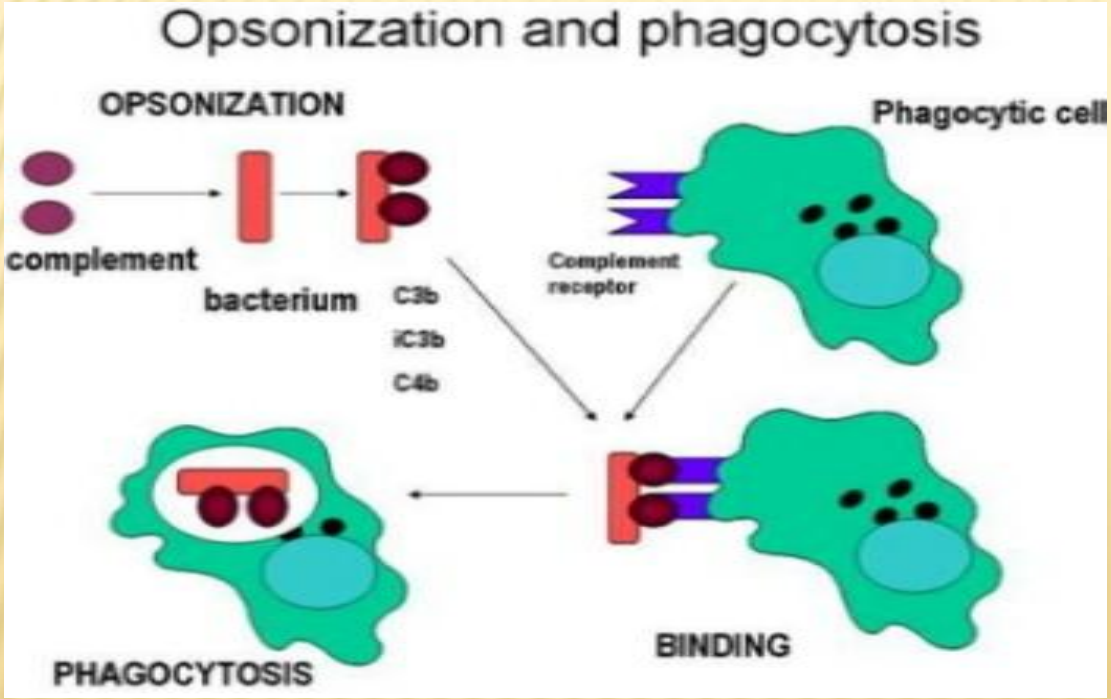
1. **Cytolysis :**

- Lysis of cells, bacteria, and viruses – the major effector of the humoral branch of the immune system.
- Disrupt the membrane & the entry of water and electrolytes into the cell.



# 2. Opsonization :

- C3b & C1q; promtes phagocytosis of particulate Ags.
- Extremely important when pathogen carries a capsule.



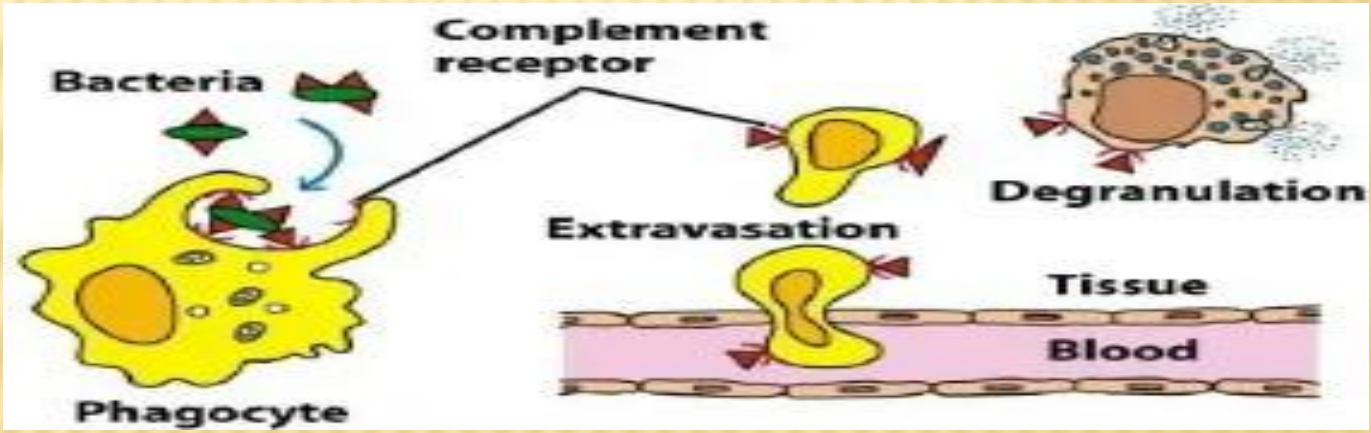

C3b is an opsonin

Opsonin are molecules that bind both to bacteria and phagocytes

Opsonin increses phagocytosis by 1,000 fold.

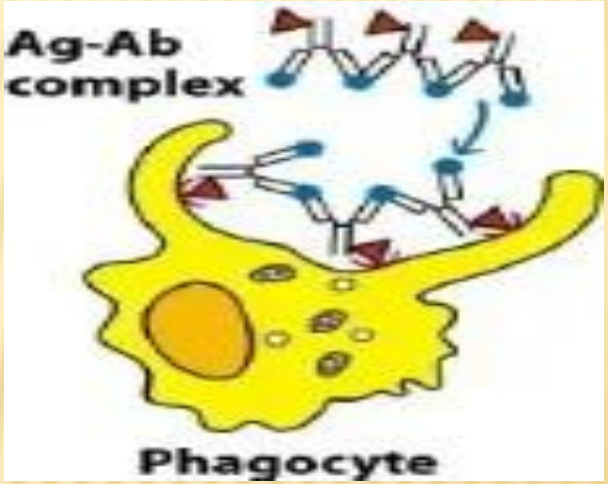
# 3. Activation of Inflammatory Response :

Binding to specific complement receptors on cells of the immune system, triggering specific cell functions, inflammations, and secretion of immunoregulatory molecules.



# 4. Clearance of Immune Complexes :

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

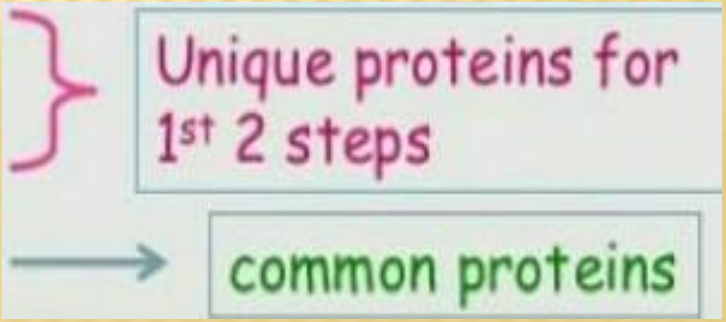


# THREE PATHWAYS FOR COMPLEMENT ACTIVATION :

- 1. Classical Pathway
- 2. Alternative Pathway
- 3. Lectin or MBL (mannose-binding lectin) Pathway.

- Pathway for activation require multiple steps categorized as :

- 1. Recognition
- 2. Enzyme activation
- 3. Biological activity



# COMPLEMENT ACTIVATION

1. Classical Pathway – Begins with the formation of Ag-Ab complex.

2. Alternative Pathway – is initiated by cell-surface constituesnts that are foreign to the host.

   - Ab-independent.

3. Lectin Pathway – is activated by the binding of mannose-binding lectin (MBL) to mannose residues on glycoproteins or carbohydrates on the surface of microorganisms.

   - Ab-independent.

# A CASCADE SYSTEM

- **The complement works as a cascade system.**

  - Cascade is when one reaction triggers another reaction which triggers others and so on. These types of systems can grow exponentially very fast.

# CASCADE ACTIVATION

- Complements proteins are often designated by an uppercase letter C and are inactive until they are split into products.

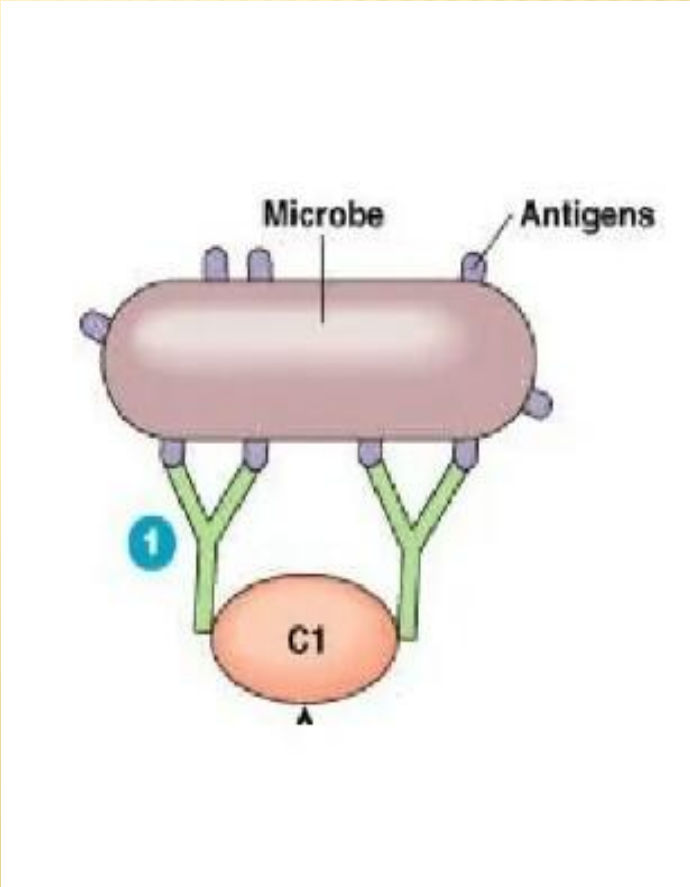
  - Example: C1

- When the products are split they become active. The active products are usually designated with a lowe case a or b.

  - Example: C1a and C1b

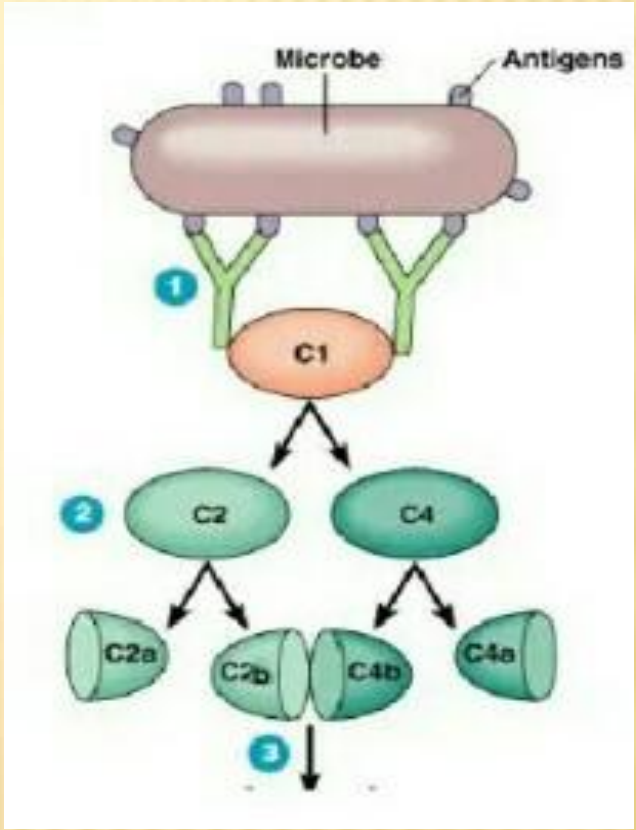
# THE CLASSICAL PATHWAY

- The classical pathway is considered to be part of the specific immune response because it relies on antibodies to initiate it.
- C1 becomes activated when it binds to the ends of antibodies.



# THE BUILDING OF A C3 ACTIVATION COMPLEX

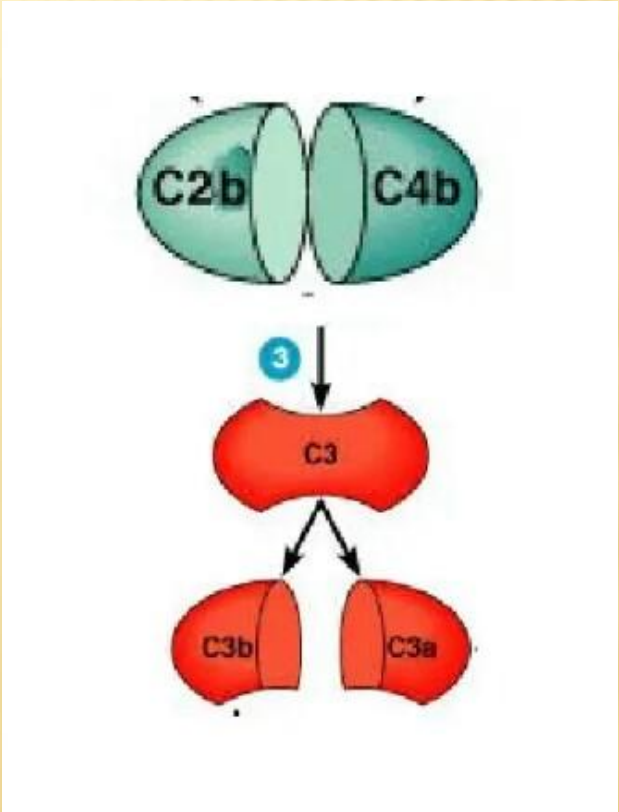
- Once C1 is activated, it activates 2 other complement proteins, C2 and C4 by cutting them in half.
- C2 is cleaved into C2a and C2b.
- C4 is cleaved into C4a and C4b.
- Both C2b and C4b bind together on the surface of the bacteria.
- C2a and C4a diffuse away.



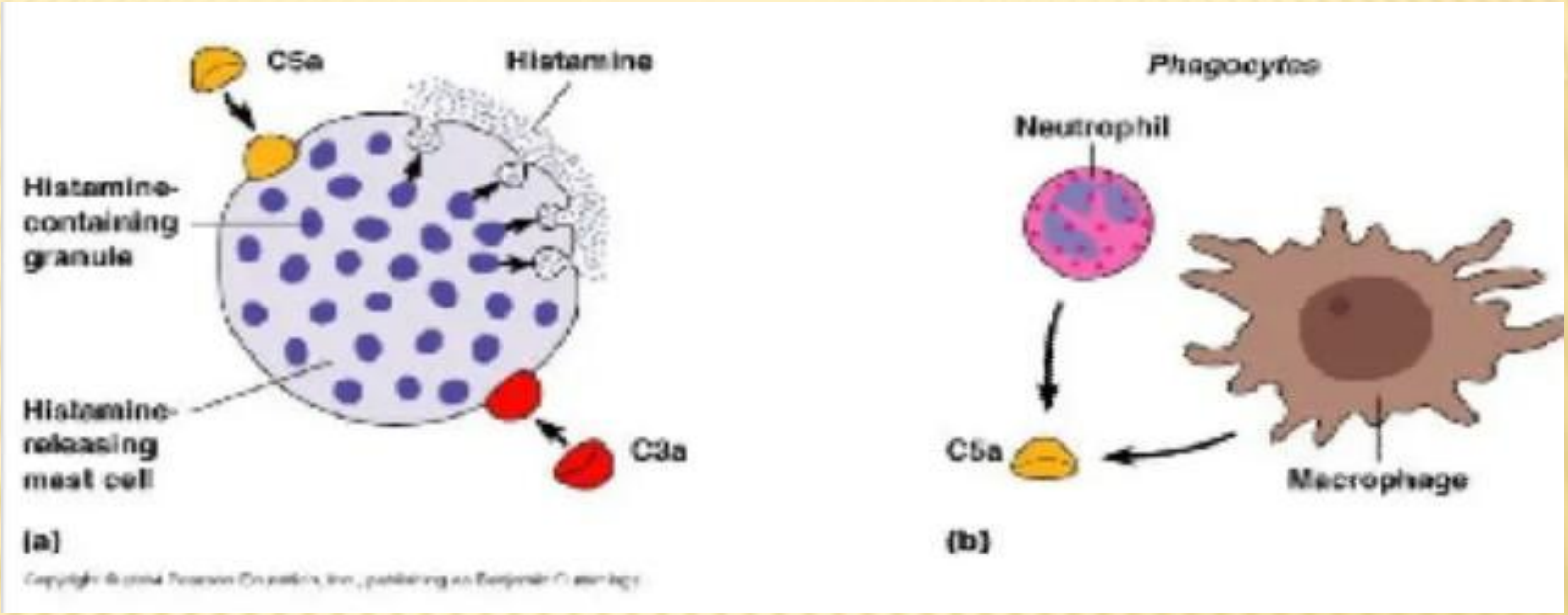
# C3 ACTIVATION COMPLEX

- C2b and C4b bind together on the surface to form a C3 activation complex.
- The function of the C3 activation complex is to activate C3 proteins.

- This is done by cleaving C3 into C3a and C3b.



# C3A




C3a increase the inflammatory response by binding to mast cells and causing them to release histamine.

# C3B

- Many C3b molecules are produced by the C3 activation complex.
- The C3b bind to and coat the surface of the bacteria.
- C3b is an opsonin :

- Opsonins are molecules that bind both to bacteria and phagocytes.
- Opsonization increases phagocytosis by 1,000 fold.

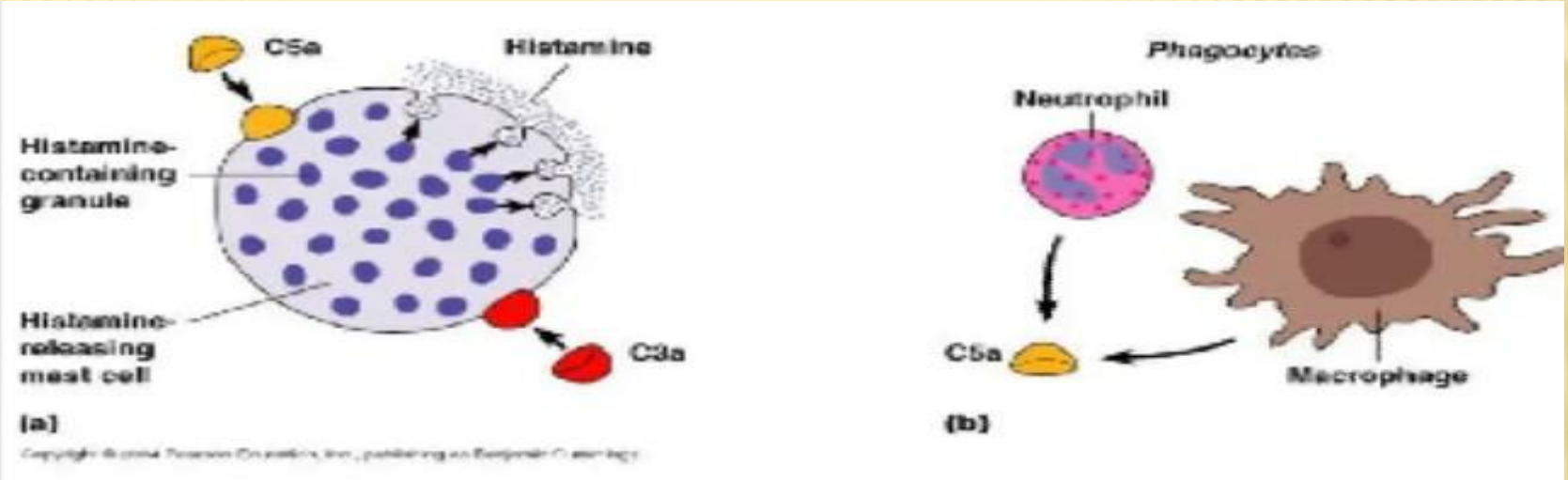
# BUILDING THE C5 ACTIVATION COMPLEX

- Eventually enough C3b is cleaved that the surface of the bacteria begins to become saturated with it.
- C2b and C4b which make up the C3 activation complex has a slightly affinity for C3b and C3b binds to them.
- When C3b binds to C2b and C4b it forms a new complex referred to as the C5 activation complex.

# THE C5 ACTIVATION COMPLEX

- The C5 activation complex (C2b,C4b,C3b) activates C5 proteins by cleaving them into C5a and C5b.
- Many C5b proteins are produced by the C5 activation complex. These C5b begin to coat the surface of the bacteria.

# THE FUNCTION OF C5A




- C5a disperses away from the bacteria.

  - Binds to mast cells and increases inflammation.

  - Most powerful chemotactic factor know for leukocytes.

# BUILDING THE MEMBRANE ATTACK COMPLEX

- C5b on the surface of bacteria binds to C6.
- The binding of C6 to C5b activates C6 so that it can bind to C7.
- C7 binds to C8 which in turn binds to many C9's.
- Together these proteins form a circular complex called the Membrane attack complex (MAC).

# FORMATION OF C5B6789, MEMBRANE-ATTACK COMPLEX

Cb5 attaches to C6, then to C7, and the C5b67 inserts into the membrane.

↓

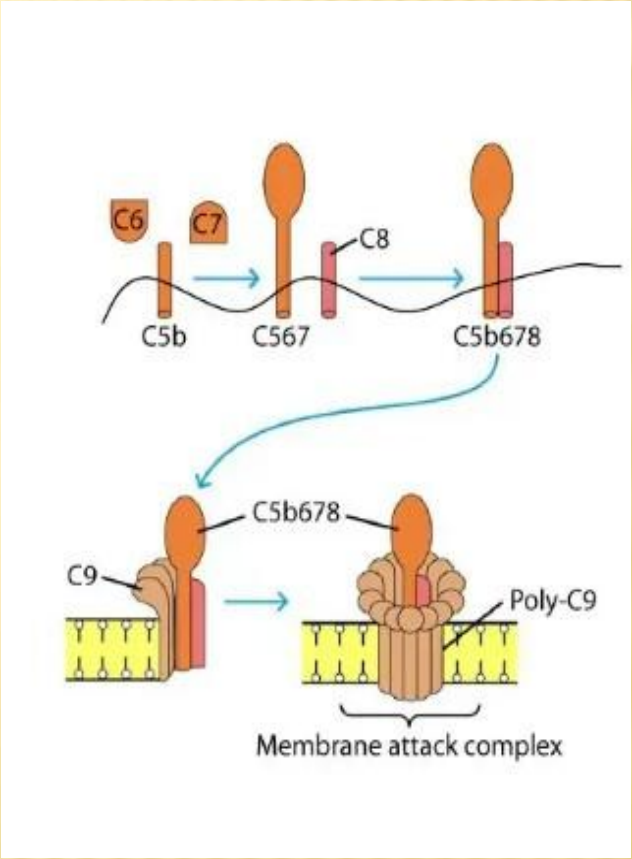
binding of C8 to membrane-bound C5b67 induces a 10 Å pore.

↓

binding and polymerization of C9, a perforin-like molecule, to C5b678.

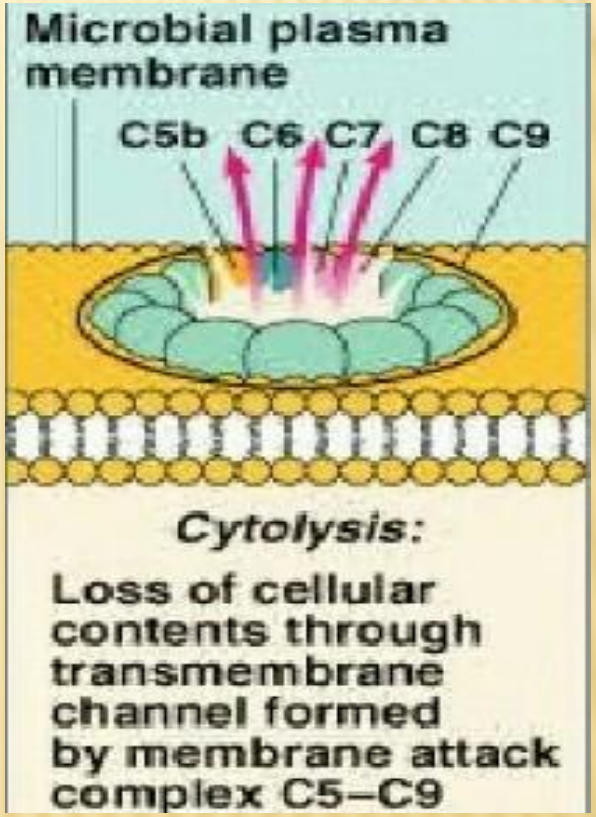
↓

The completed membrane-attack complex (MAC) has a tabular form and functional pore size of 70 – 100 Å.

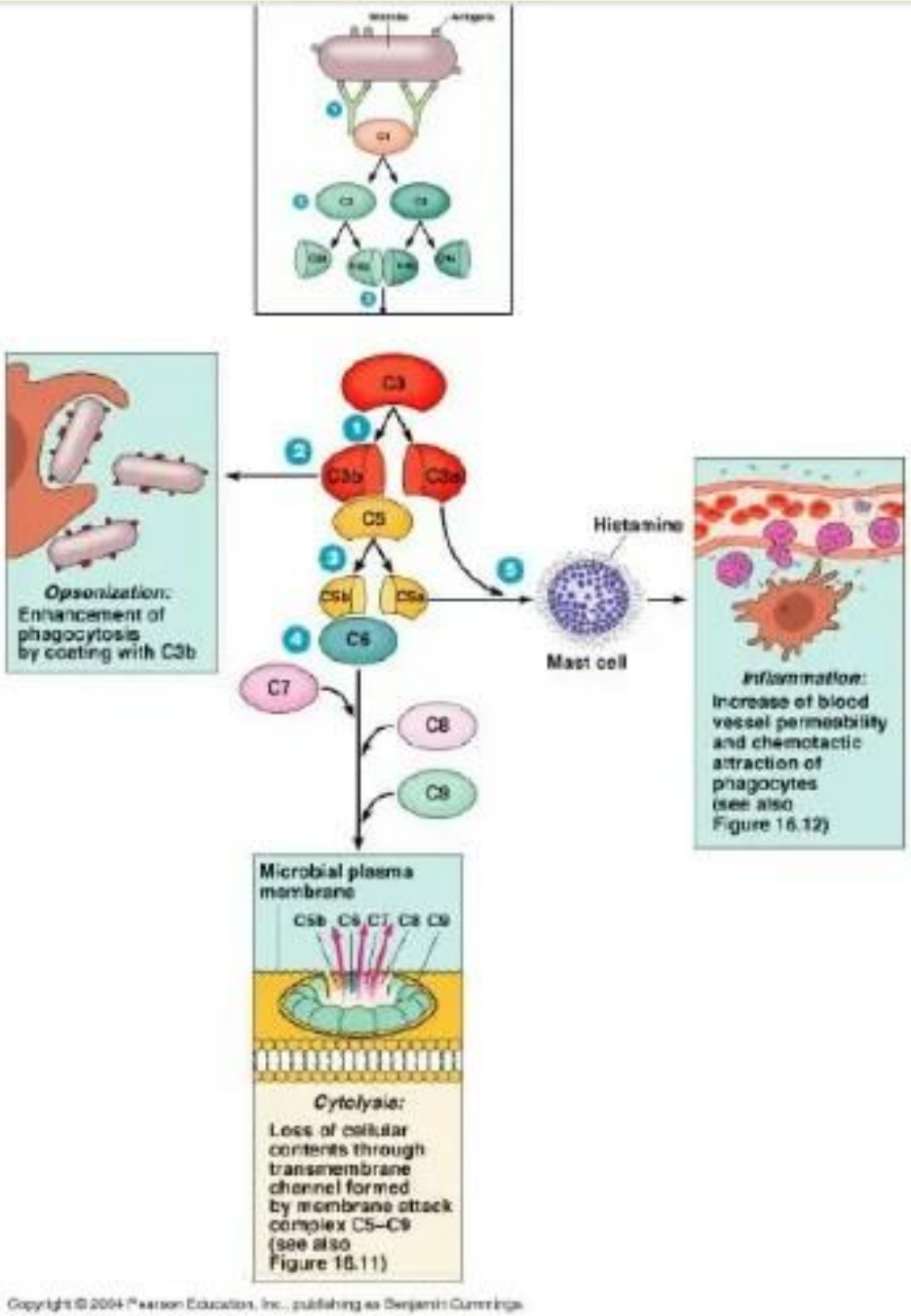


# MEMBRANE ATTACK COMPLEX

- The MAC cause Cytolysis.
  - The circular membrane attack complex acts as a channel in which cytoplasm can rush out of and water rushes in.
- The cells Inner integrity is compromised and it dies.
- Animation of the classical pathway.



# Overview



# THE ALTERNATIVE PATHWAY

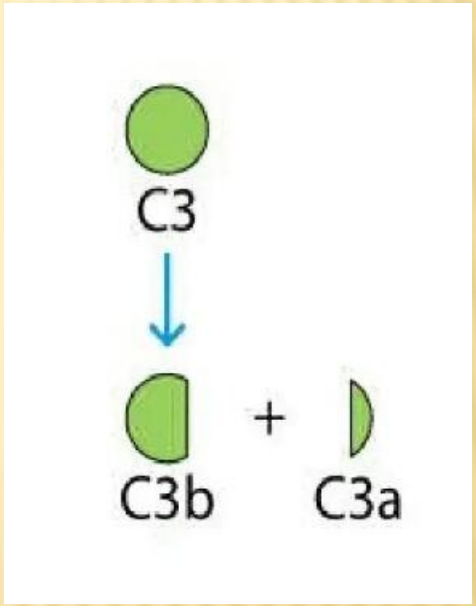
- The alternative pathway is part of the non-specific defense because it does not need antibodies to initiate the pathway.
- The alternative pathway is slower than the Classical pathway.

# THE ALTERNATIVE PATHWAY IS AB-INDEPENDENT

- The activation of alternative pathway doesn't need Ab; thus, It is a component of the innate immune system.
- It is initiated by cell-surface constituents that are foreign to the host, e.g., bacterial cell wall.
- C1, C4 and C2 are not involved in the alternative pathway.
- Four serum proteins, C3, factor B, factor D, and properdin, are involved in this pathway.

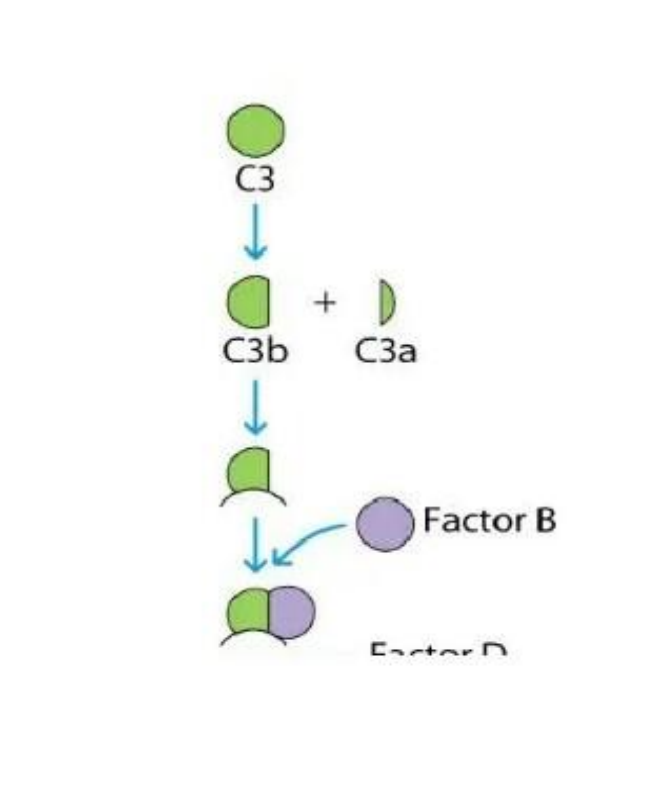
# INITIANTION OF THE ALTERNATIVE PATHWAY

- C3 contains in unstable thioster bond.
- This unstable bond makes C3 subject to slow spontaneous hydrolysis to C3b and C3a.
- The C3b is able to bind to foreign surface antigens.
- Mammalian cells contain sialic acid which inactivates C3b.



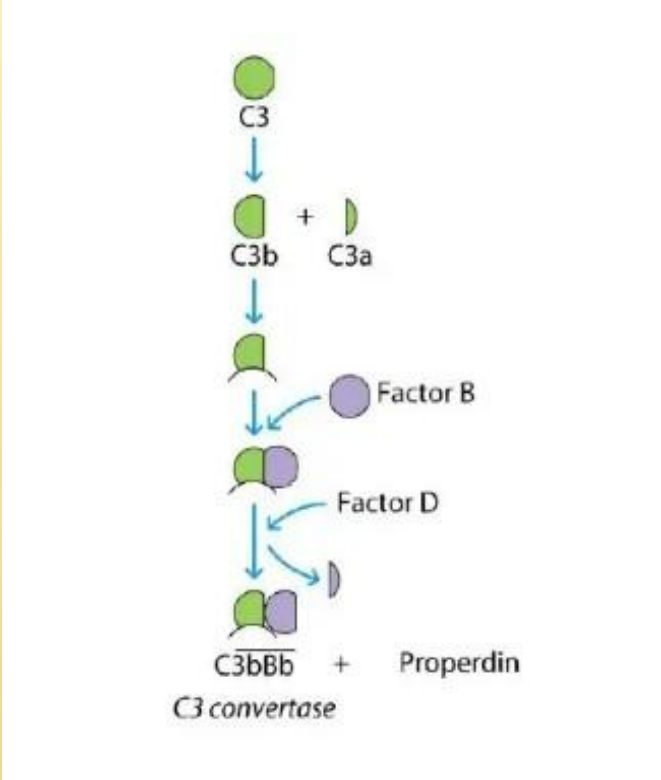
# FACTOR B

- C3b on the surface of a foreign cells binds to another plasma protein called factor B.

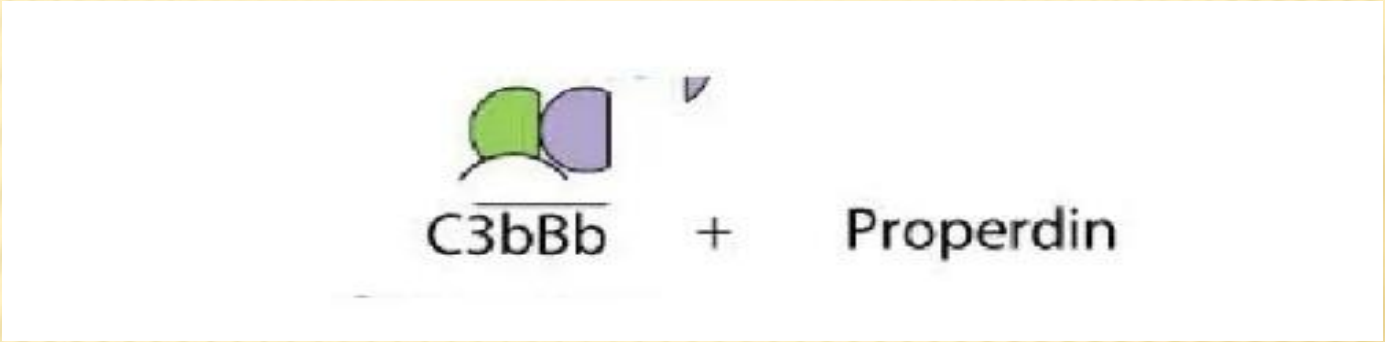


# FACTOR D

- The binding of C3b to factor B allows a protein enzyme called Factor D to cleave Factor B to Ba and Bb.
- Factor Bb remains bound to C3b while Ba and Factor D disperse away.



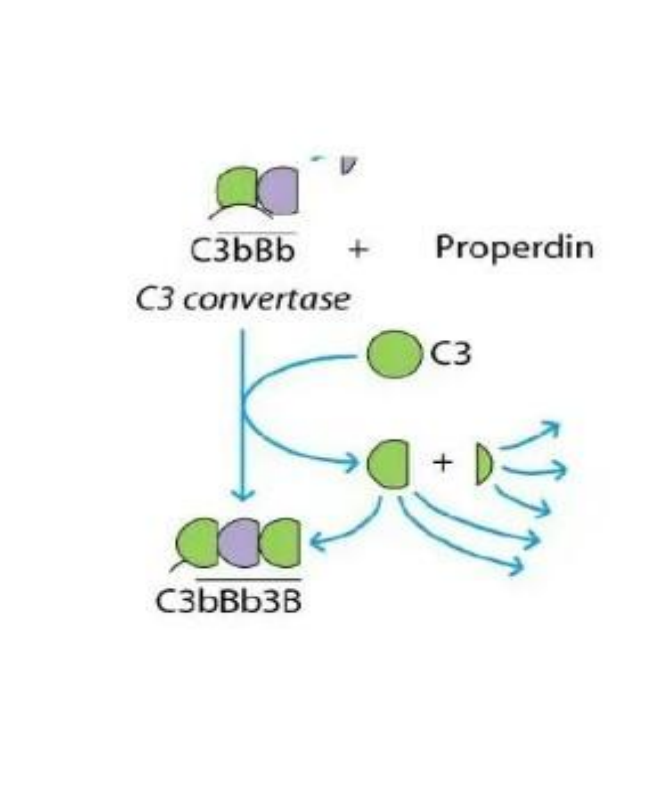
# THE C3 ACTIVATION COMPLEX




- Properdin, also called factor P, binds to the C3bBb complex to stablize it.
- C3bBbP make up the C3 activation complex for the alternative pathway.

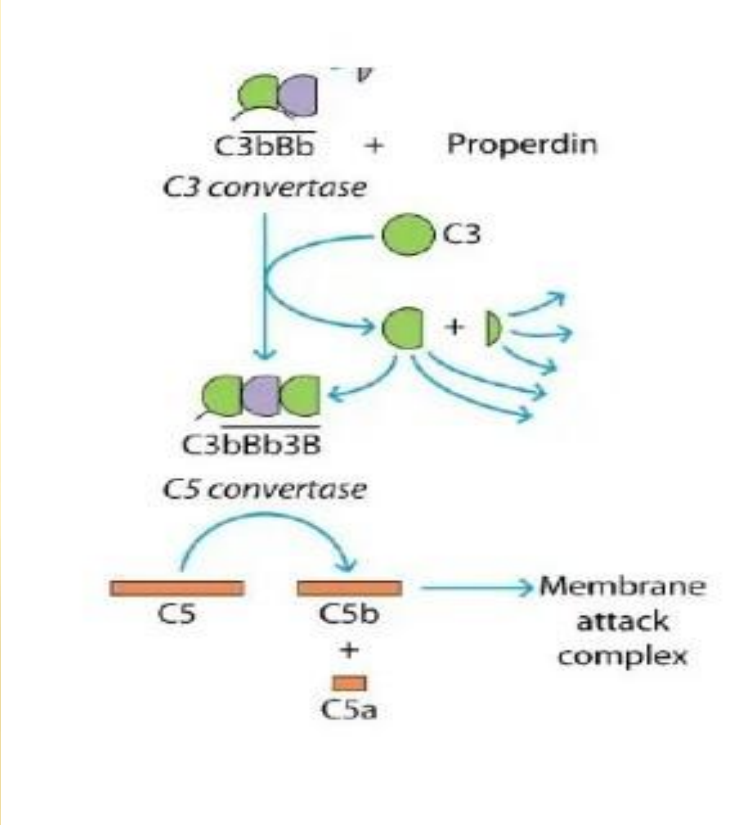
# THE C3 ACTIVATION COMPLEX

- The C3 activation complex causes the production of more C3b.
- This allows the initial steps of this pathway to be repeated and amplified.
- $2X10_6$ molecules can be generated in 5 minutes.

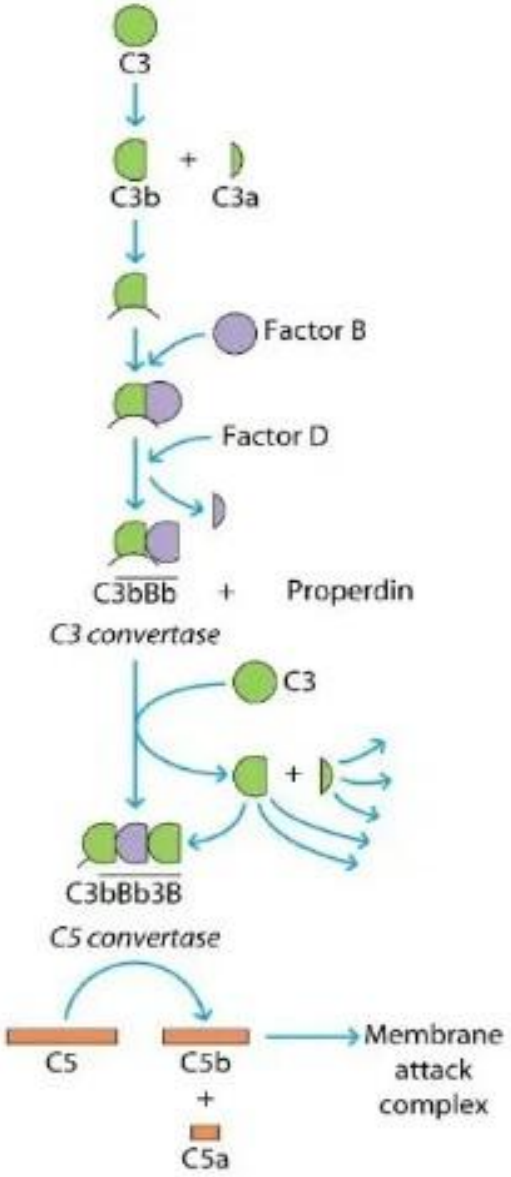


# C5 ACTIVATION COMPLEX

- When an additional C3b binds to the C3 activation complex it converts it into a C5 activation complex.
- The C5 activation complex cleaves C5 into C5a and C5b.
- C5b begins the production of the MAC.



# Alternative Pathway Overview



# THE LECTIN PATHWAY ORIGINATES WITH HOST PROTEINS BINDING MICROBIAL SURFACES

Lectin: Protein that bind to a carbohydrate.

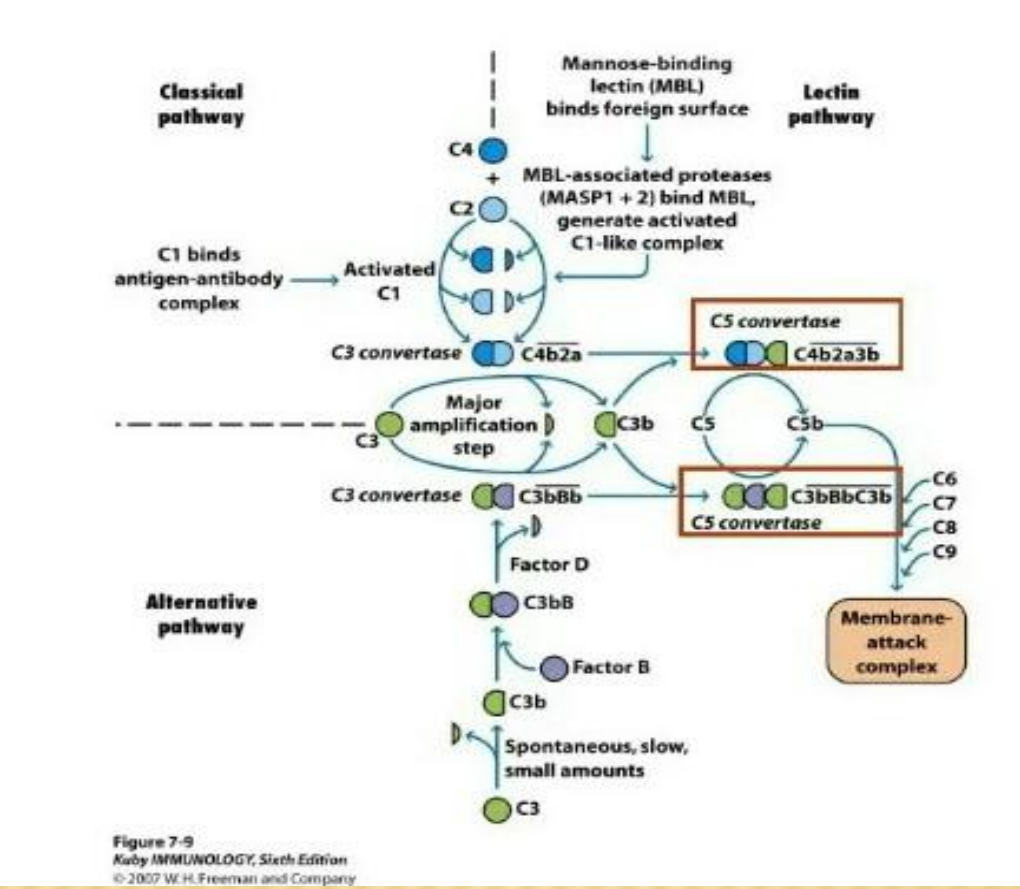
MBL (mannose-binding lectin):

- an acute phase protein which binds to mannose residues on glycoproteins or carbohydrates on the surface of microorganisms (structurally similar to C1q)

MASP-1 & MASP-2 : MBL-associated serine protease (structurally similar C1r and C1s)

- MBL is induced during inflammatory responses.
- After MBL binds to the surface of a microbe, MBL-associated serine proteases, MASP-1 and MASP-2, bind to MBL.
- The MBL-MASP-1/2 complex mimics the activity of the C1r and C1s, and causes cleavage and activation of C4 and C2.
- Thus, the lectin pathway is Ab-independent. It is an important innate defense mechanism comparable to the alternative pathway, but utilizing the elements of the classical pathway, except for the C1 proteins.

# THE THREE COMPLEMENT PATHWAYS CONVERGE AT THE MEMBRANE-ATTACK COMPLEX




The Three complement pathways converge at the production of an active C5 convertase

↓

C5b6789

membrane-attack complex (MAC)

# REGULATION OF COMPLEMENT SYSTEM

1. **C1 inhibitor :**

- Important regulator of classic pathway
- A serine protease inhibitor (serpin)
- Irreversibly binds to amd inactivates C1r and C1s, as well as MASP in lectin pathway

2. **Factor H :**

- Regulates alternative pathway
- Reduce amount of C5 convertase available
- With both cofactor activity for the factor I-mediated C3b cleavage, and decay accelerating activity against C3bBb (C3 convertase)

## 3. Properdin :

- Protects C3b and stabilizes C3 convertase.

## 4. Factor :

- Cleaves cell-bound or fluid phase C3b and C4b
  → Inactivates C3b and C4b.

## 5. Decay accelerating factor (DAF) :

- Glycoprotein on surface of human cells.
- Prevents assembly of C3bBb or accelerates disassembly of preformed convertase → no formation of MAC.
- Acts on both classical and alternative.

## 6. C4b-binding protein (C4BP) :

- Inhibits the action of C4b in classical pathway.
- Splits C4 convertase and is a cofactor for factor I.

## 7. Complement Receptor 1 (CR-1) :

- Co-factor for factor I, together with CD46

## 8. Protectin (CD59) and Vitronectin (S protein) :

- Inhibits formation of MAC by binding C5b678
- Presents on “self” cells to prevent complement from damaging them

# CLINICAL ASPECTS OF COMPLEMENT

## 1. Deficiency of C5-C8 & Mannan-binding lectin :

- Prodispose to serve *Neisseria* bacteremia.

## 2. Deficiency of C3 :

- Severe, recurrent pyogenic sinus & resp. tract infections.

## 3. Deficiency of C1 esterase inhibitor :

- Angiodema →inc. capillary permeability and edema.

## 4. Deficiency of DAF :

- Increased complement-mediated hemolysis → paroxysmal nocturnal hemoglobinuria

## 5. Transfusion mismatches :

- Activation of complement → generate large amounts of anaphylatoxins & MAC → red cell hemolysis.

## 6. Autoimmune diseases :

- Immune complexes bind complement → low complement levels + activate inflammation → tissue damage.

## 7. Severe liver disease :

- Deficient complement proteins → predispose to infection with pyogenic bacteria.

## 8. Factor I deficiency :

- Low levels of C3 in plasma due to unregulated activation of alternative pathway ⟶ recurrent bacterial infections in children.
- Mutation in factor I gene ⟶ implicated in development of Hemolytic Uremic Syndrome.