

The Complement System

- **Definition**
 - **The Classical Complement Pathway**
 - **The Lectin Pathway**
 - **The Alternative Complement Pathway**
- **Ways in which Microorganisms can Resist Body Defenses by Circumventing the Complement Pathways**

Introduction..

- The complement system refers to a series of proteins circulating in the blood and bathing the fluids surrounding tissues.
- The proteins circulate in an inactive form, but when activated they act in concert in an orderly sequence to exert their biological effects.
- The term “Complement” refers to the ability of these proteins to complement i.e. augment the effects of other components of immune system eg. Antibody



Discovery of complement

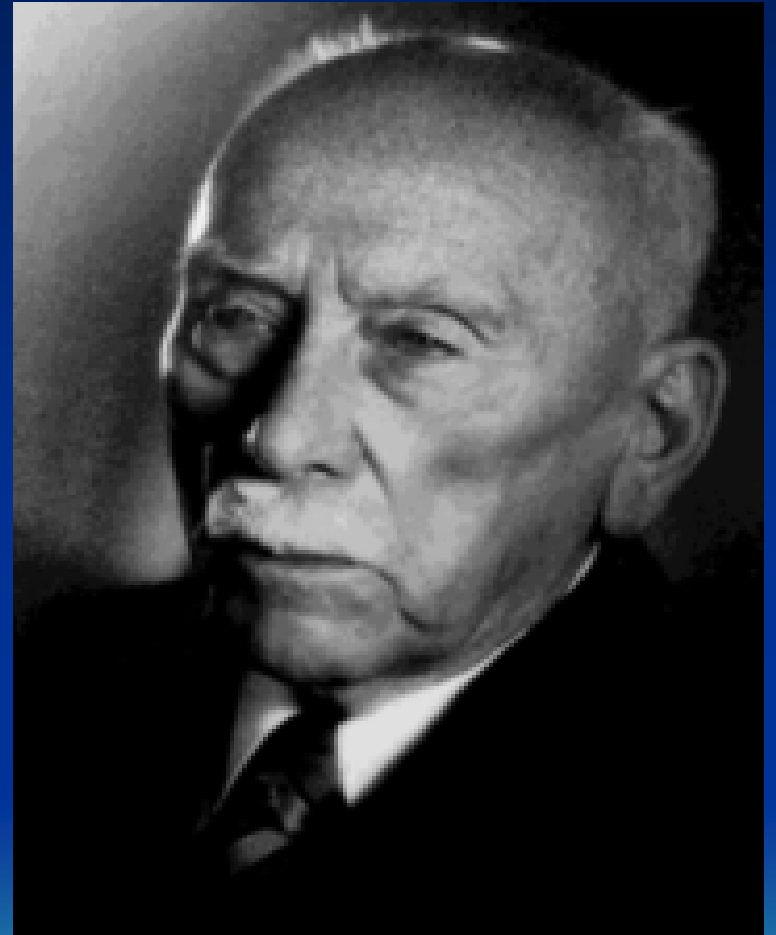
- **In 1984, Pfeiffer**, demonstrated that some *cholera vibrios* can be lysed by guineapig anti-cholera serum.
- Heating of the serum at 56°C for 30 minutes abolished this activity but heating did not abolish the Ab activity, because heated serum serum could still transfer immunity to another guineapig.
- Addition of normal fresh serum to the heat treated antiserum restored its lytic activity.
- He concluded that antibodies to the bacilli, plus a heat labile component present in immune as well as normal serum, were necessary for the lysis of *V. cholerae* in vitro.

Subsequently **Bordet and Ehrlich** confirmed that the same result could be obtained in vitro, suspending vibrios in the presence of fresh immune serum.



History

- **Jules Jean Baptiste Vincent Bordet**
- (Belgium) 13 June 1870 – 6 April 1961)
- Won noble prize in 1919 for discovery in immunity



Outcome of complement activation

- Three main effects
 1. **Lysis of cells** such as bacteria, allografts and tumor cells
 2. **Generation of mediators** that participate in inflammation and attract neutrophils
 3. **Opsonization** ie. Enhancement of phagocytosis



Beneficial effects of complement activation

6 beneficial effects

1. Trigger inflammation
2. Chemotactically attract phagocytes to the infection site;
3. Promote the attachment of antigens to phagocytes (enhanced attachment or **opsonization**)
4. Cause **lysis of gram-negative** bacteria and human cells displaying foreign epitopes
5. Plays a role in the **activation of naive B-lymphocytes** and
6. **Remove harmful immune complexes** from the body.



Components of the complement system

There are more than 20 components of which followings are important.

- C1, C2, C3, C4, C5, C6, C7, C8, C9
- Factor B, Factor D, Factor H, Factor I,
- Properdin, C1 inhibitor, C4 binding protein, S protein

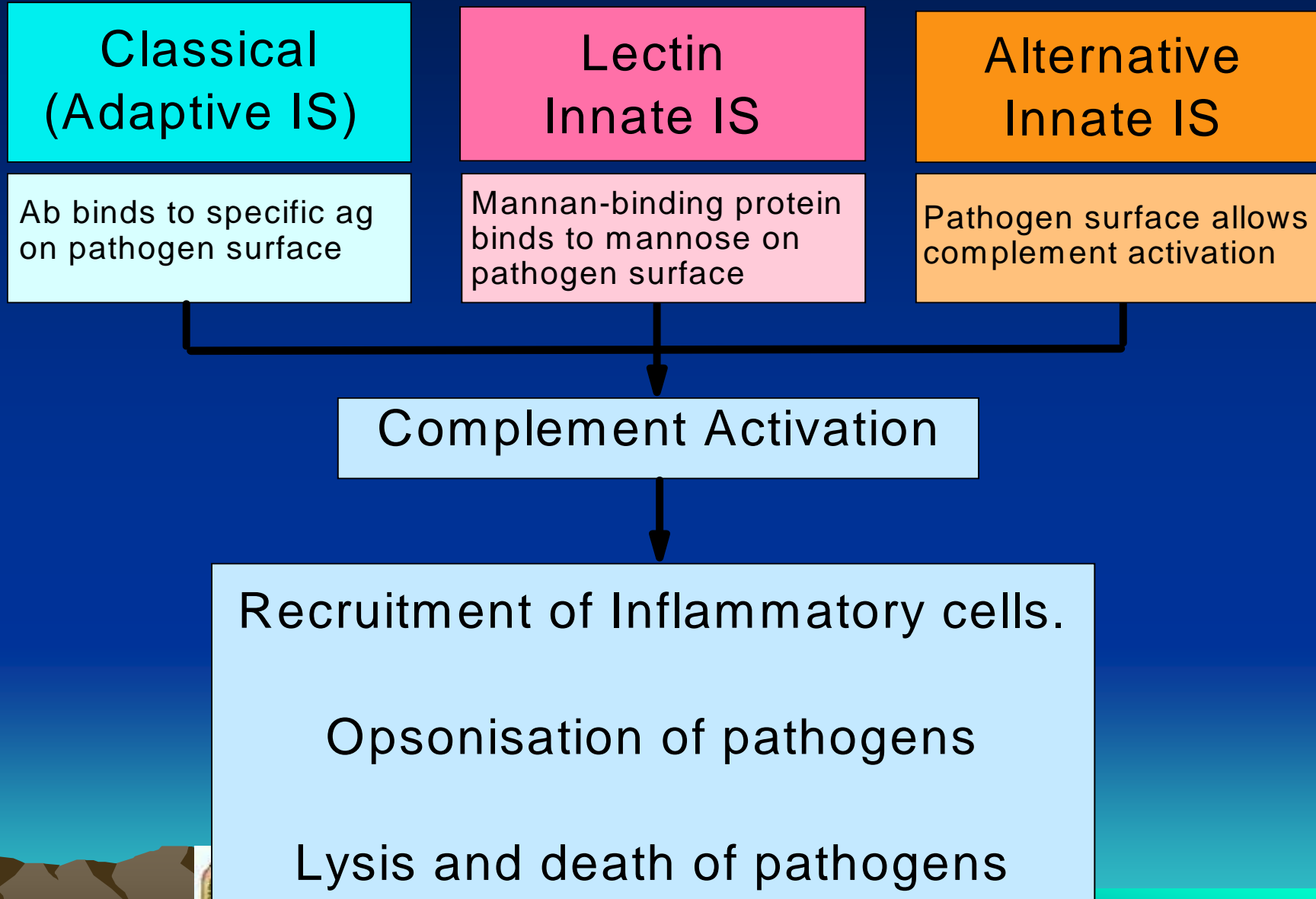
Activation of complements

Why it needs to be activated?

Several complement components are proenzymes, which must be cleaved to form active enzymes.

Activation of the complement can be initiated either by antigen –antibody complexes or by variety of nonimmunologic molecules eg. Endotoxin.

Pathways of Complement Activation



CLASSIC PATHWAY

ALTERNATIVE PATHWAY

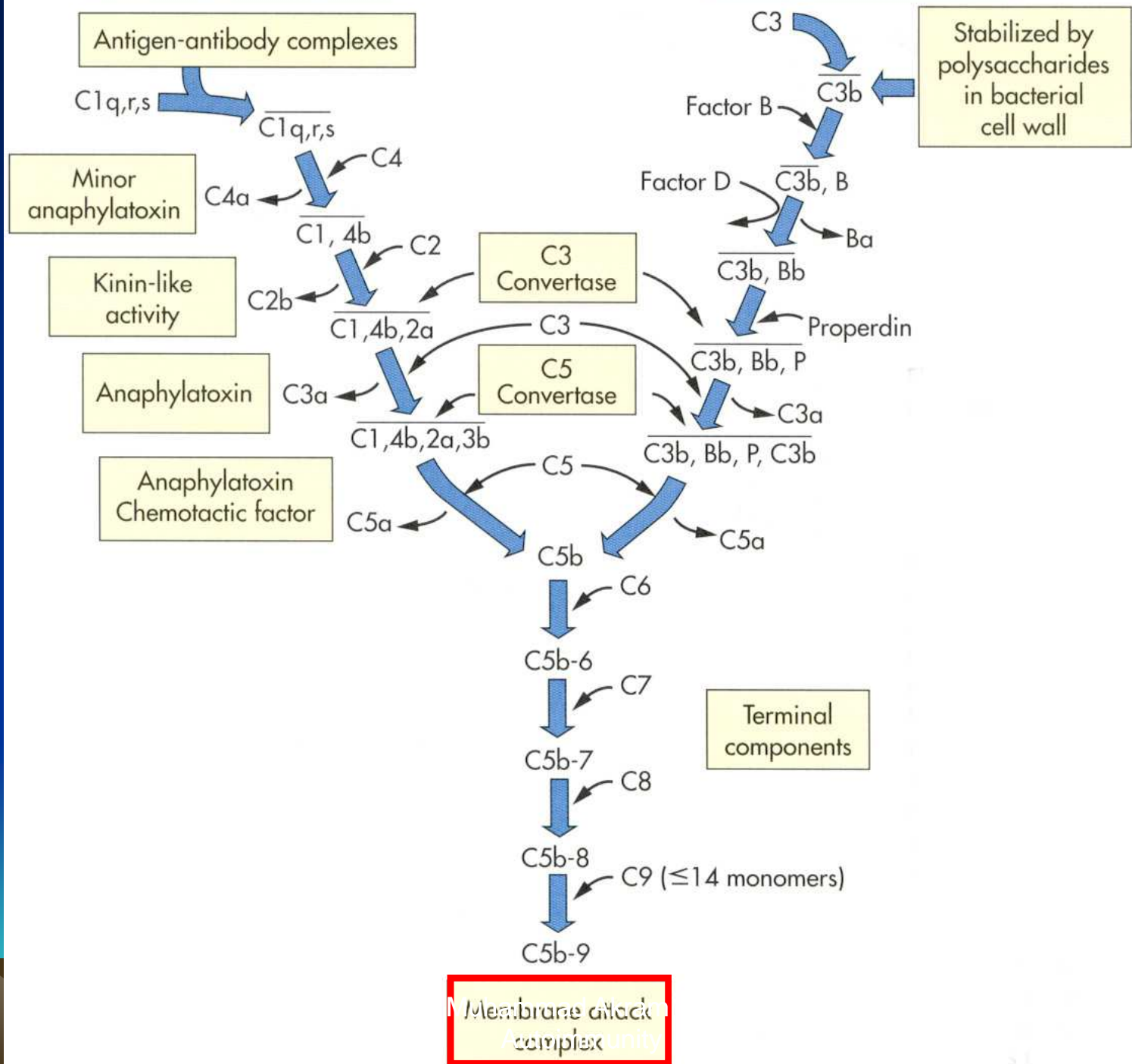
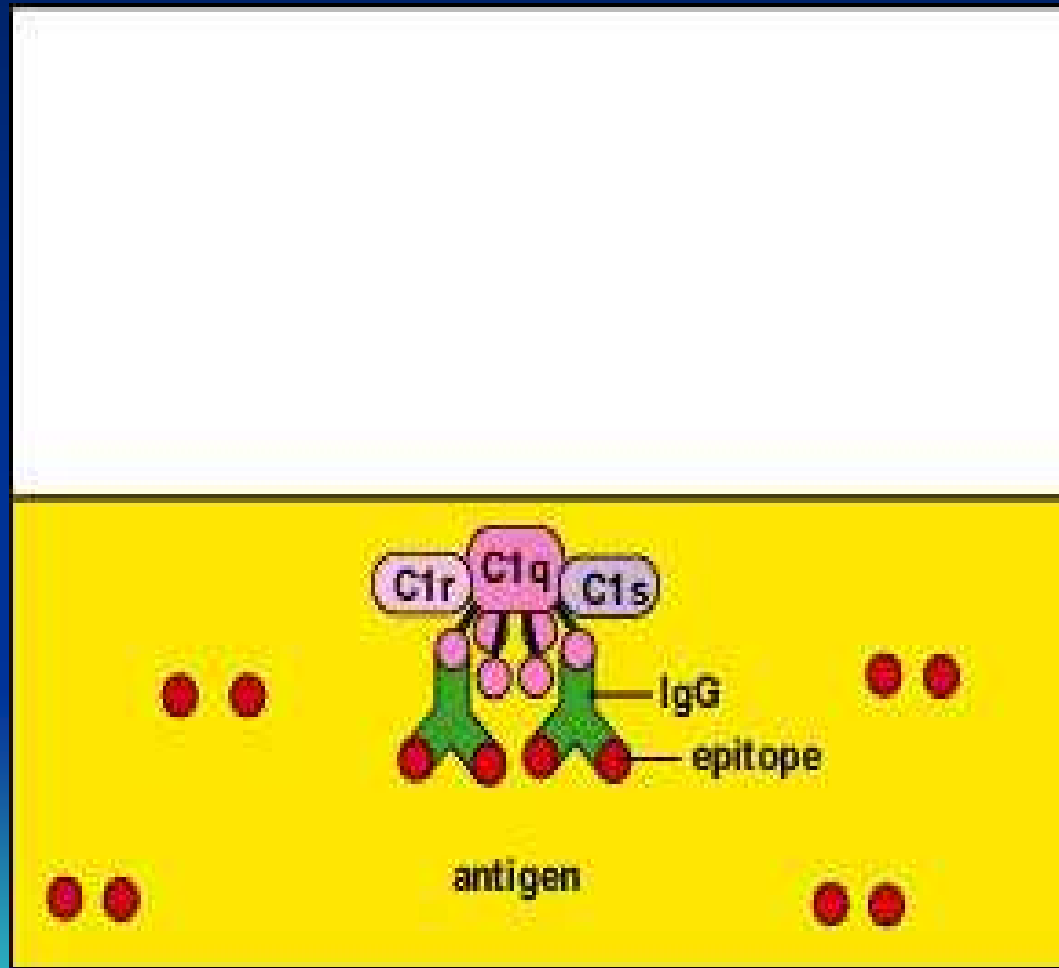


Fig. 2: Assembly of C1 during the Classical Complement Pathway

The Fab of IgG or IgM bind to epitopes on an antigen. C1q, C1r, and C1s then assemble on the Fc portion of the antibodies to form C1, the first enzyme of the classical complement pathway. The enzyme C1 is able to cleave C4 into C4a and C4b, as well as C2 into C2a and C2b.



Animation of Assembly of C1 during the Classical Complement Pathway

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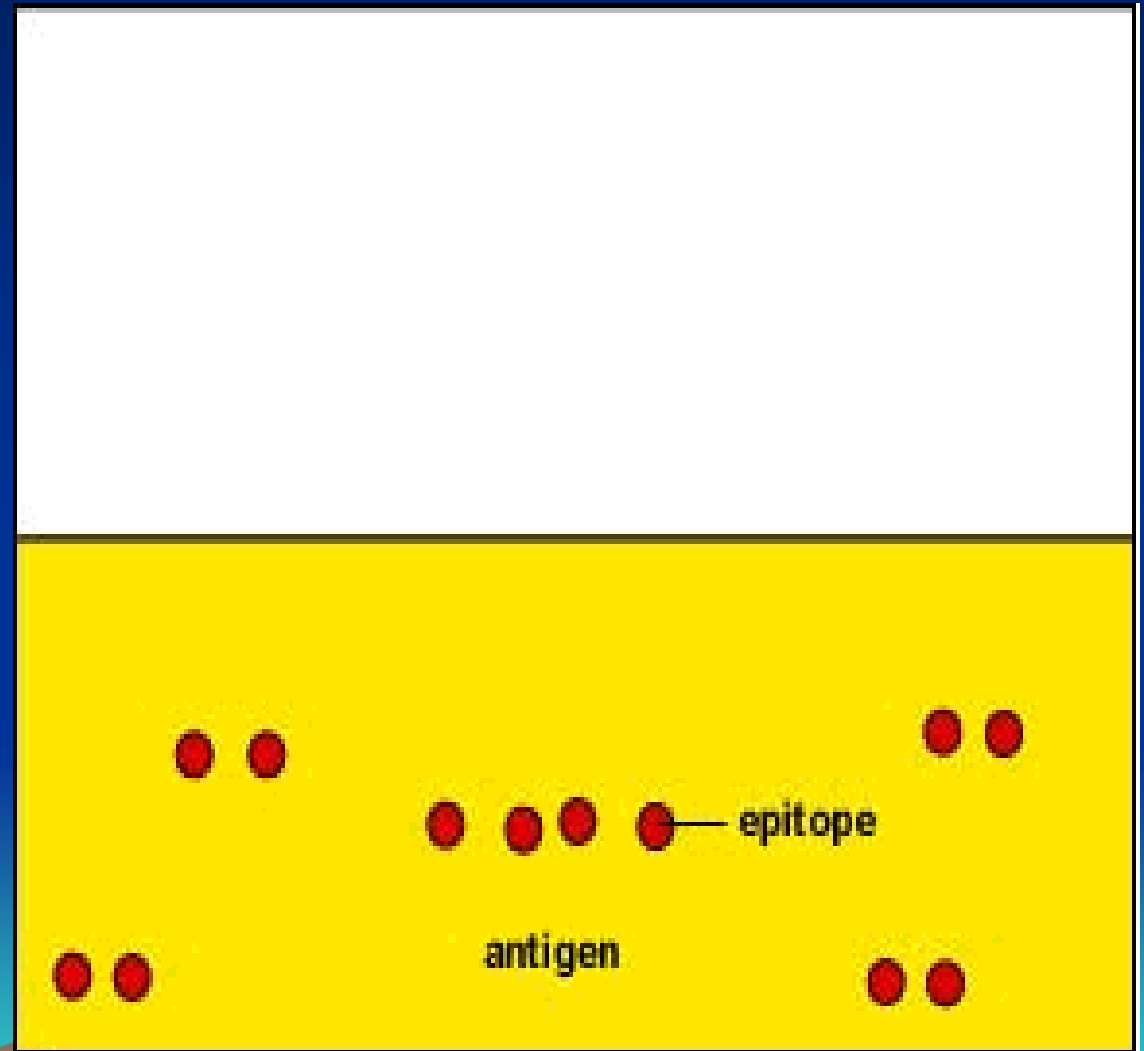
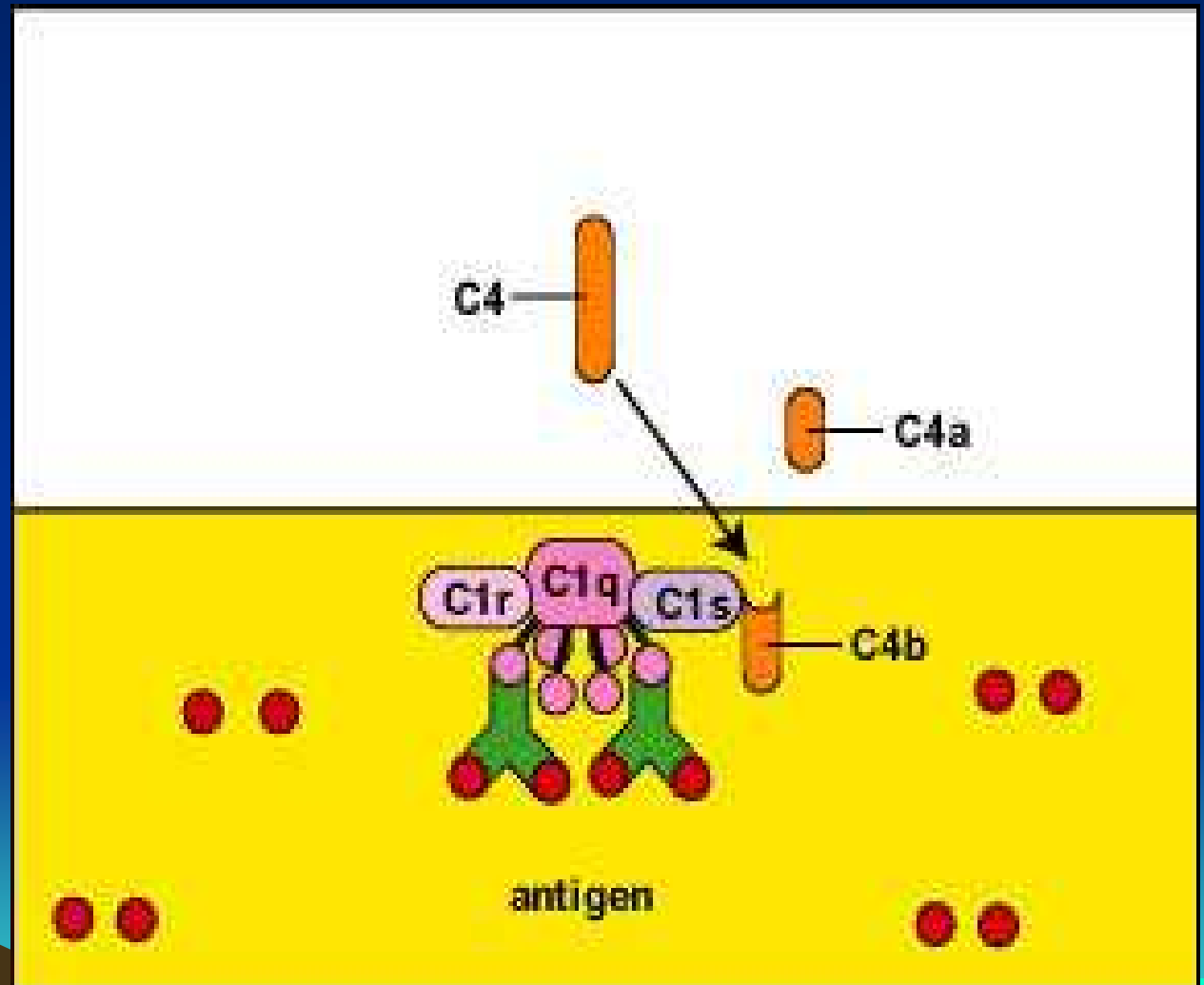


Fig. 3: Cleavage of C4 by C1 during the Classical Complement Pathway

The enzyme C1 is able to cleave C4 into C4a and C4b. The C4b then binds to adjacent proteins and carbohydrates on the surface of the antigen.



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Fig. 4: Cleavage of C2 and Formation of C3 Convertase during the Classical Complement Pathway

C2 binds to the C4b and the enzyme C1 subsequently cleaves C2 into C2a and C2b. The classical complement pathway is now activated. The C4b2a functions as a C3 convertase that can enzymatically cleave hundreds of molecules of C3 into C3a and C3b.

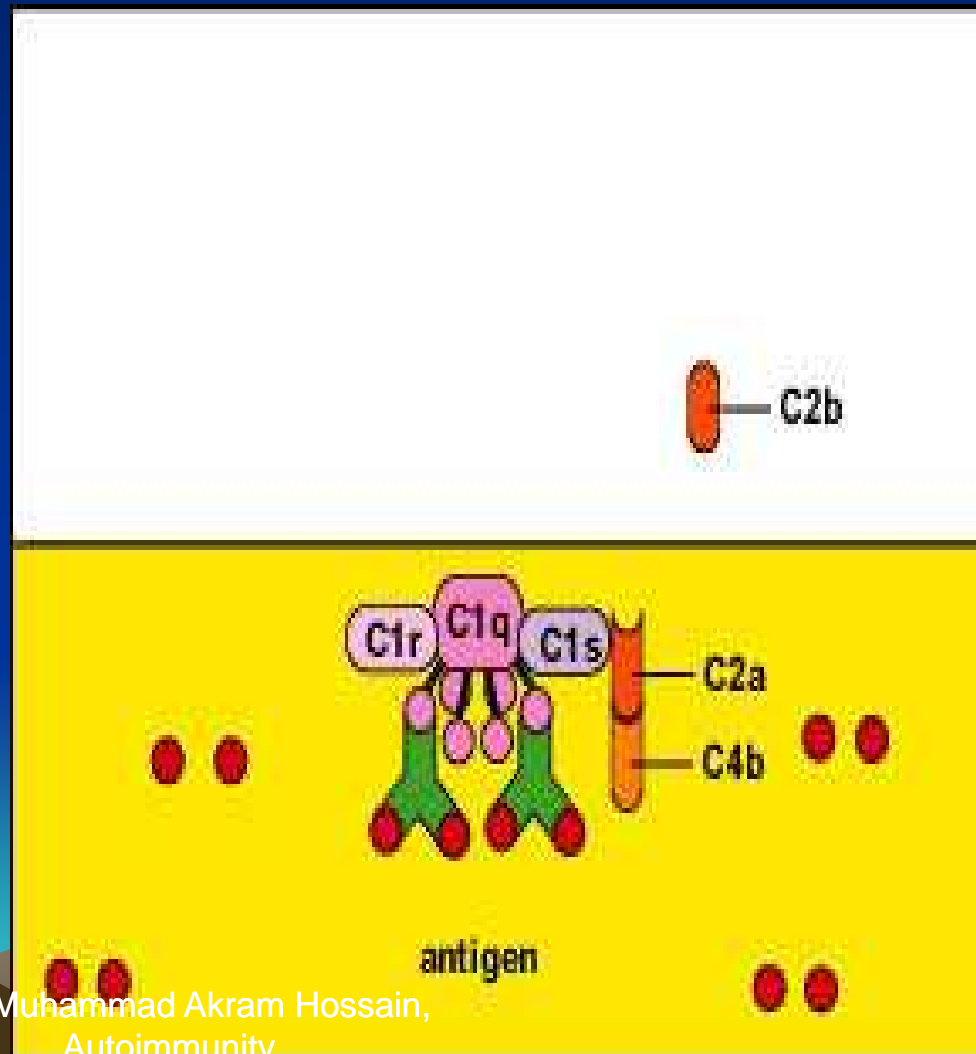


Fig. 5: Cleavage of C3 by C3 Convertase during the Classical Complement Pathway

The C4b2a functions as a C3 convertase that can enzymatically cleave hundreds of molecules of C3 into C3a and C3b. C3b, and to a lesser extent C4b, attaches antigens to phagocytes for opsonization (enhanced attachment). One portion of the C3b binds to proteins and polysaccharides on microbial surfaces; another portion binds to CR1 receptors on phagocytes, B-lymphocytes, and dendritic cells. This results in improved phagocytosis. C3a can promote inflammatory responses that enable body defense cells and defense chemicals to leave the blood and enter the tissues.

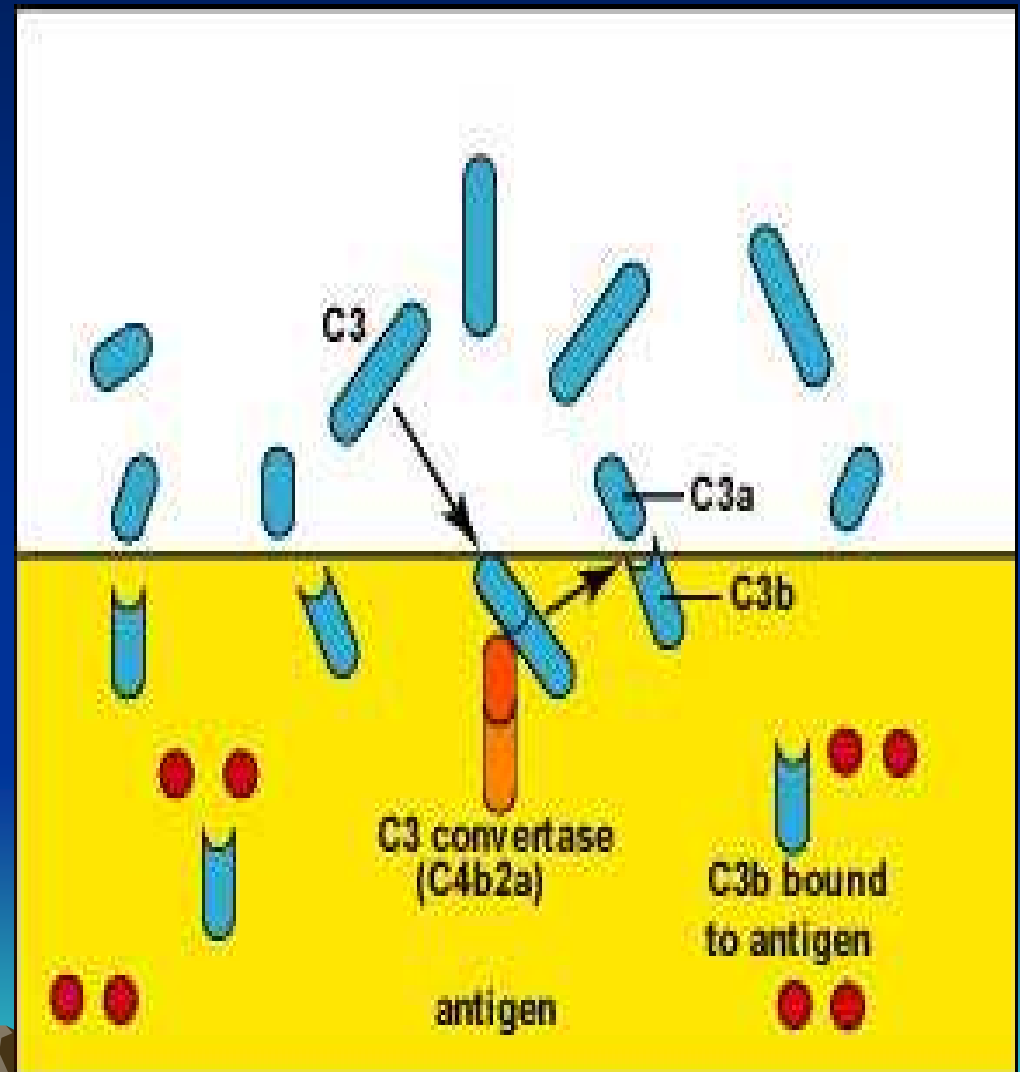


Fig. 6: Formation of C5 Convertase during the Classical Complement Pathway

Some molecules of C3b bind to C4b2a, the C3 convertase, to form C4b2a3b, a C5 convertase that cleaves C5 into C5a and C5b.

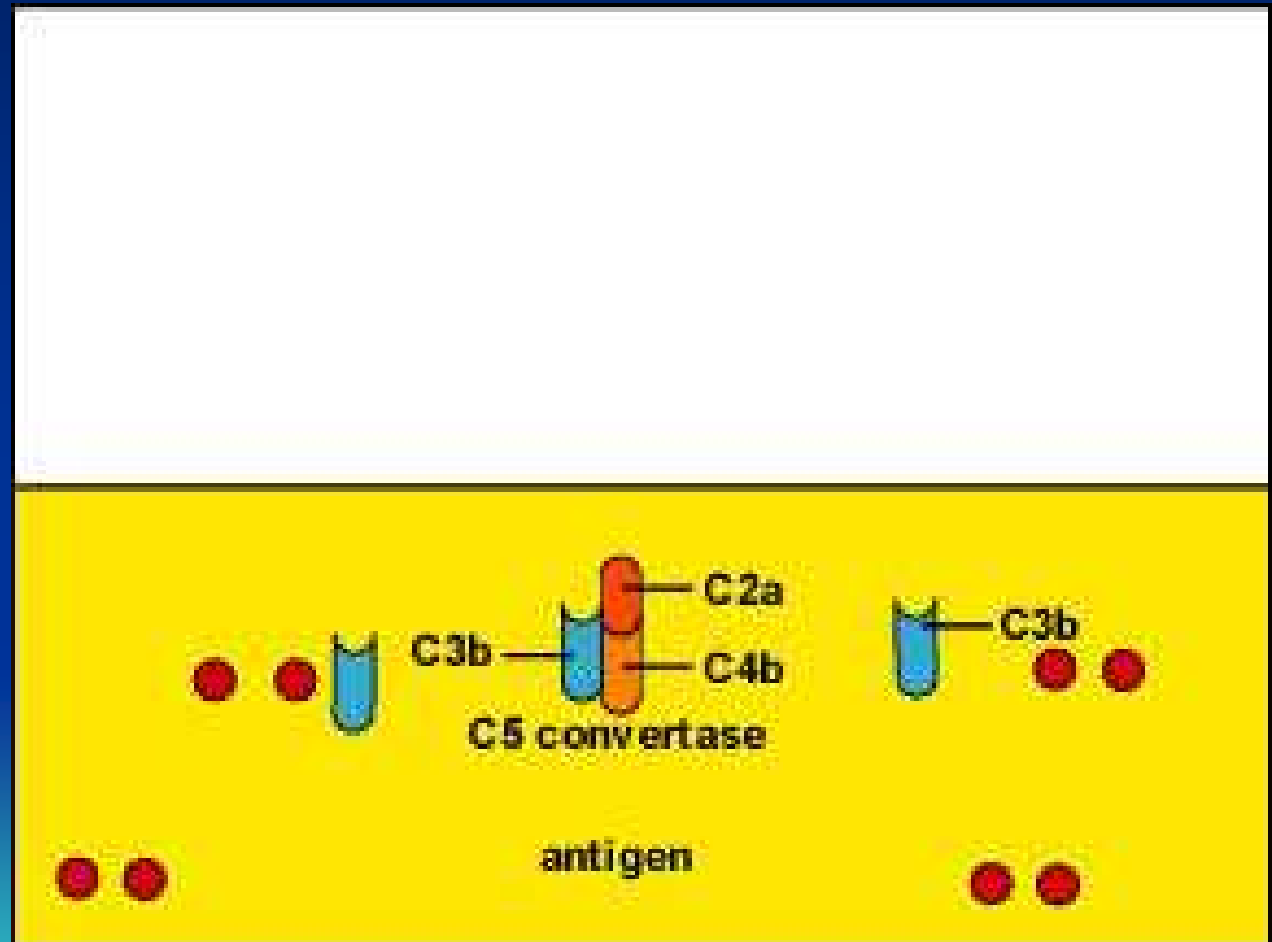


Fig. 7: Formation of C5 Convertase during the Classical Complement Pathway

C4b2a3b functions as a C5 convertase that cleaves C5 into C5a and C5b. C5a is the most potent complement protein triggering inflammation. It causes mast cells to release vasodilators such as histamine so that blood vessels become more permeable; it increases the expression of adhesion molecules on leukocytes and the vascular endothelium so that leukocytes can squeeze out of the blood vessels and enter the tissue (diapedesis); it causes neutrophils to release toxic oxygen radicals for extracellular killing; and it induces fever. C5a also functions as a chemoattractant for phagocytes. Leukocytes will move towards increasing concentrations of C5a. C5b becomes part of the Membrane Attack Complex (MAC).

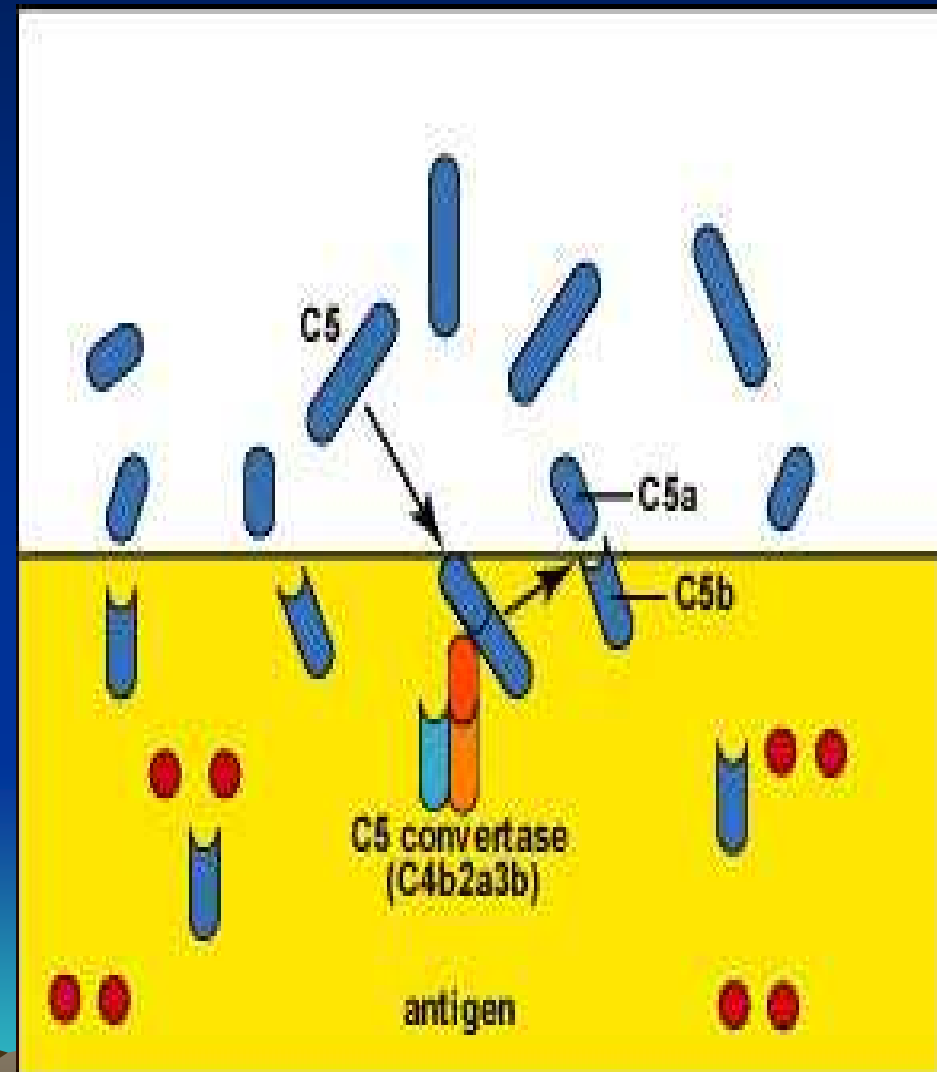
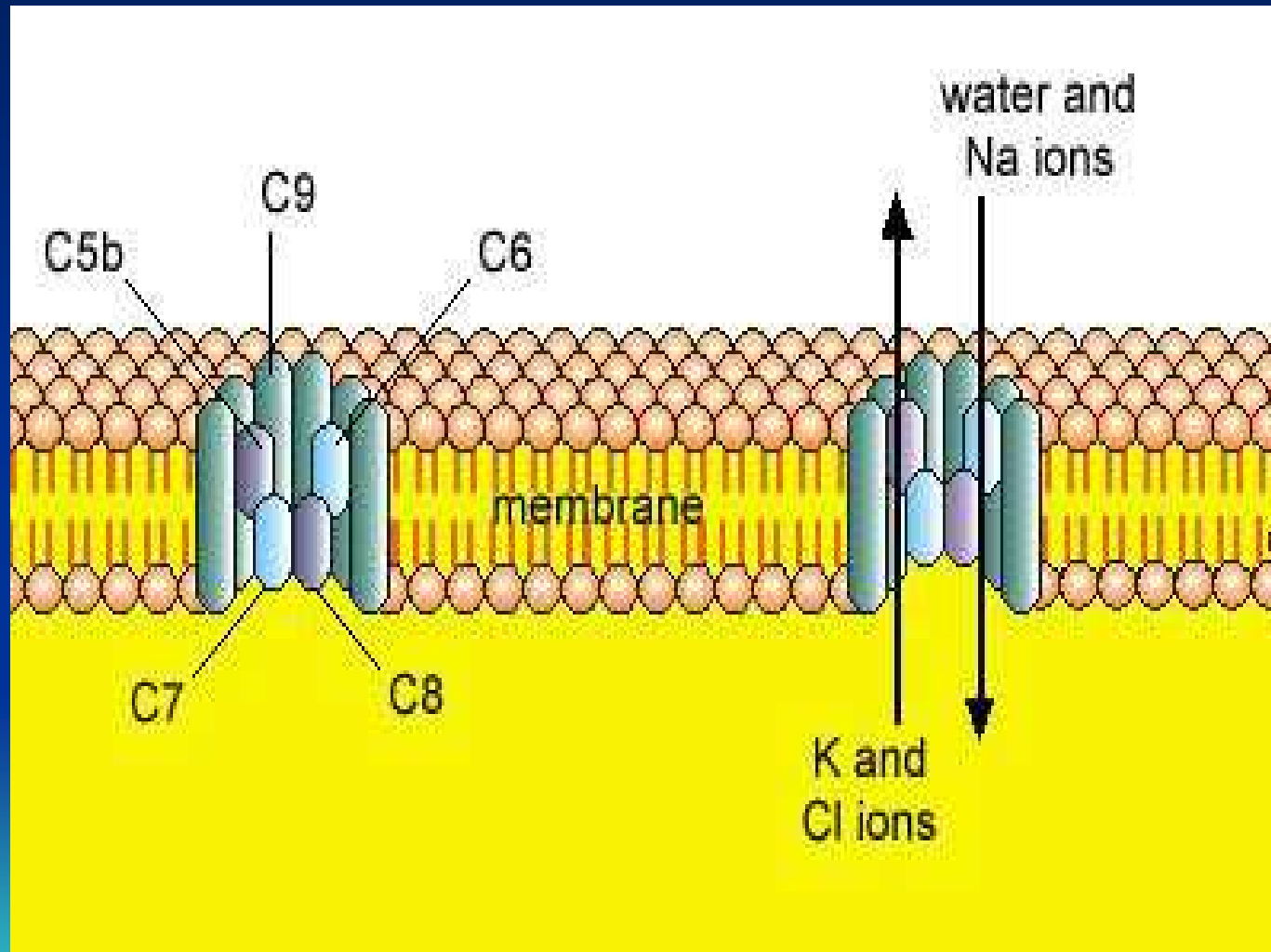


Fig. 8: Formation of the Membrane Attack Complex (MAC) during the Classical Complement Pathway



- Multiple molecules of C9 combine with C5b, C6, C7, and C8 to form the Membrane Attack Complex (MAC).

The Alternative Complement Pathway

- Activation of the alternative complement pathway begins when **C3b (or C3i) binds to the cell wall and other surface components of microbes.** C3b can also bind to IgG antibodies. **Factor B** then combines with the cell-bound C3b to form **C3bB.** **Factor D** then splits the bound Factor B into Bb and Ba, forming **C3bBb.**
- **properdin** then binds to the Bb to form **C3bBbP** that functions as a **C3 convertase** ([see Fig. 1](#)) capable of enzymatically splitting hundreds of molecules of C3 into C3a and C3b.



Alternative Pathway

- C3 is an unstable molecule continually undergoing hydrolysis in the blood and lymph. This rate of hydrolysis can be accelerated in the presence of some pathogens. C3b then binds to Factor B making it susceptible to cleavage by Factor D. This creates the **C3 convertase** of the Alternative Pathway, C3bBb.

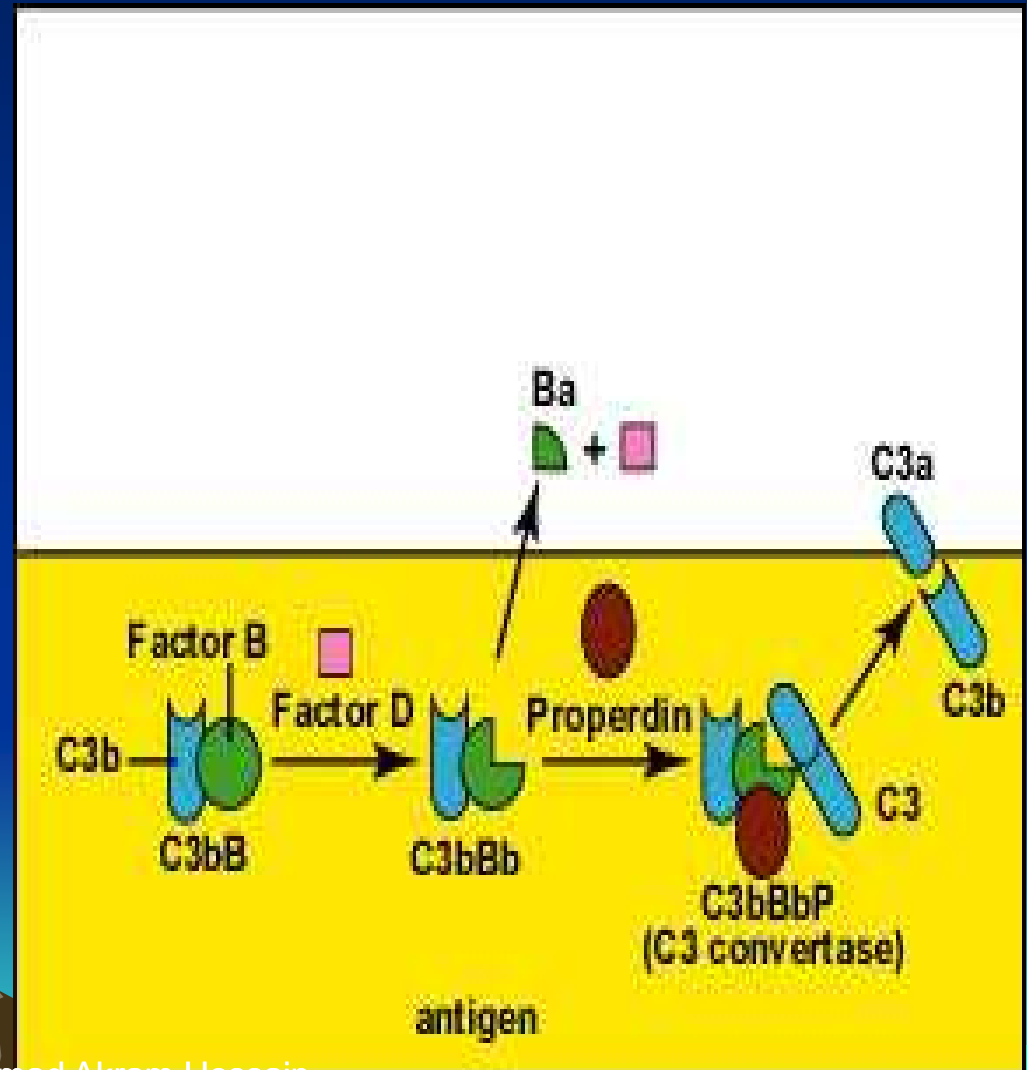


C3

- Central molecule in the Complement System.
- Highly labile
- Present in large amounts in serum (0.7-1.5g/l)
- After action by the C3 convertases C3b is covalently bound to the surface of the target cell.

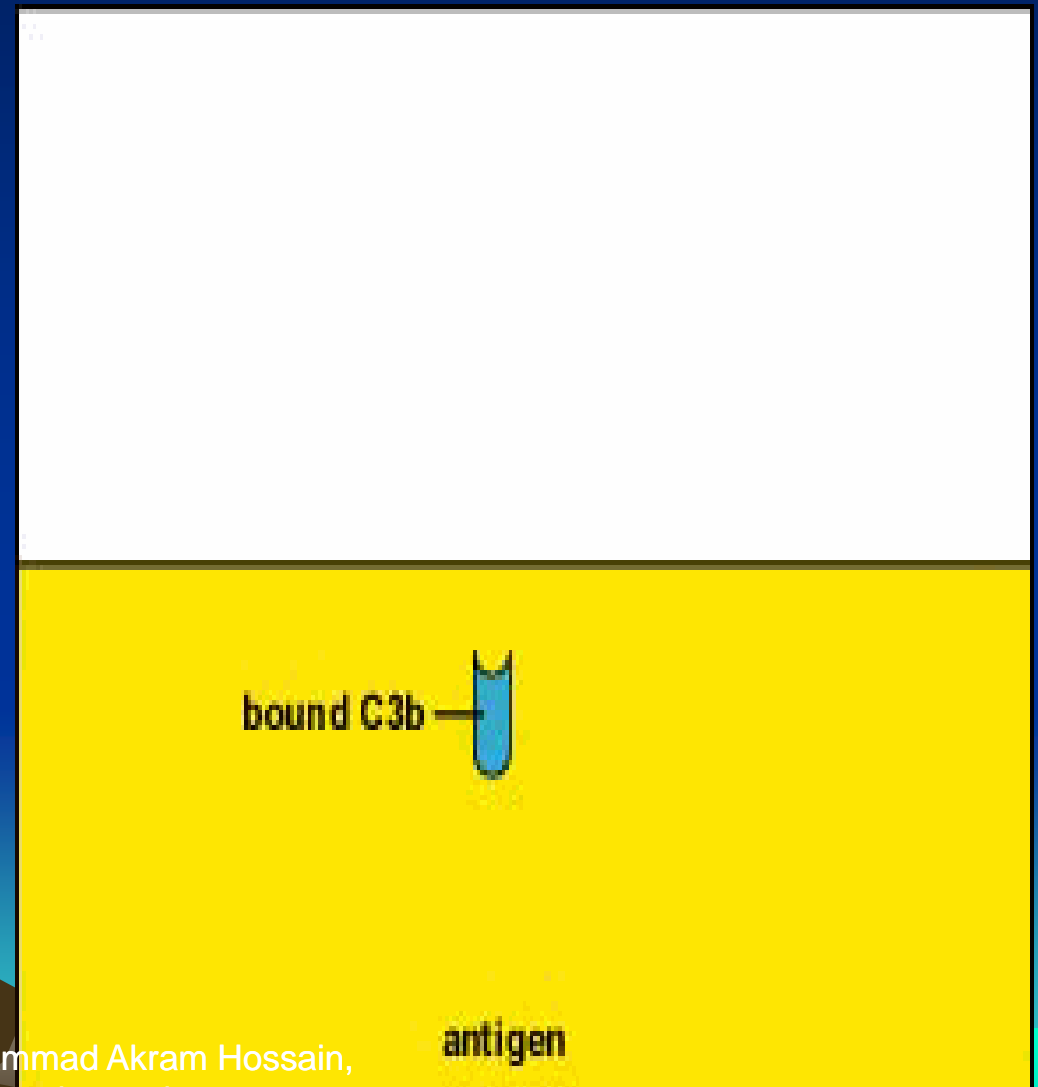
Fig. 1: Activation of the Alternative Complement Pathway and Formation of C3 Convertase

Activation of the alternative complement pathway begins when C3b (or C3i) binds to the cell wall and other surface components of microbes. Alternative pathway protein Factor B then combines with the cell-bound C3b to form C3bB. Factor D then splits the bound Factor B into Bb and Ba, forming C3bBb. A serum protein called properdin then binds to the Bb to form C3bBbP. C3bBbP functions as a C3 convertase that can enzymatically split hundreds of molecules of C3 into C3a and C3b.



Animation of Activation of the Alternative Complement Pathway and Formation of C3 Convertase

Activation of the alternative complement pathway begins when C3b (or C3i) binds to the cell wall and other surface components of microbes. Alternative pathway protein Factor B then combines with the cell-bound C3b to form C3bB. Factor D then splits the bound Factor B into Bb and Ba, forming C3bBb. A serum protein called properdin then binds to the Bb to form C3bBbP that functions as a C3 convertase capable of enzymatically splitting hundreds of molecules of C3 into C3a and C3b.



Formation of C5 Convertase during the Alternative Complement Pathway

Some of the C3b subsequently binds to some of the C3bBb to form C3bBb3b, a C5 convertase capable of enzymatically splitting hundreds of molecules of C5 into C5a and C5b.



Fig. 2: Formation of C5 Convertase during the Alternative Complement Pathway

Some of the C3b subsequently binds to some of the C3bBb to form **C3bBb3b**, a **C5 convertase** capable of splitting molecules of C5 into C5a and C5b

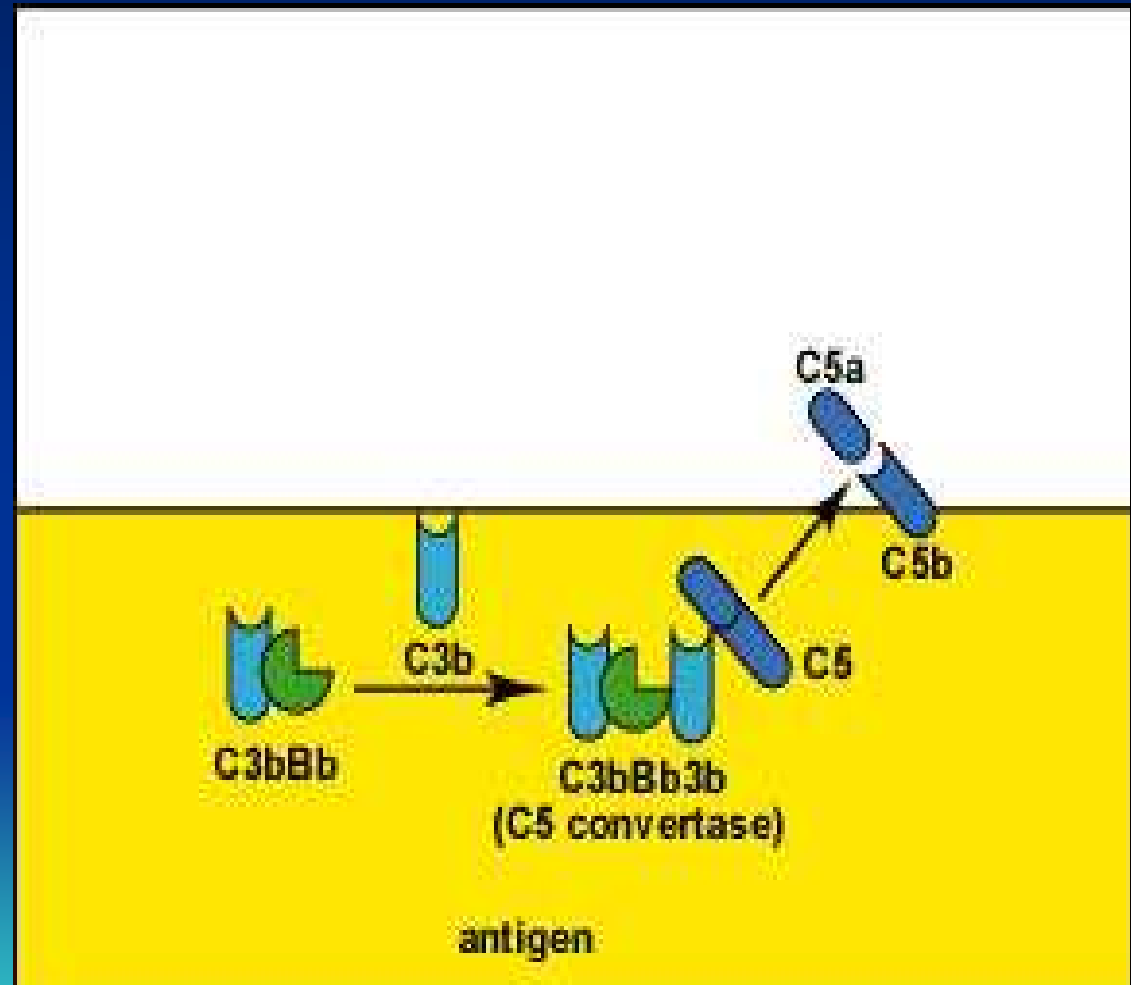
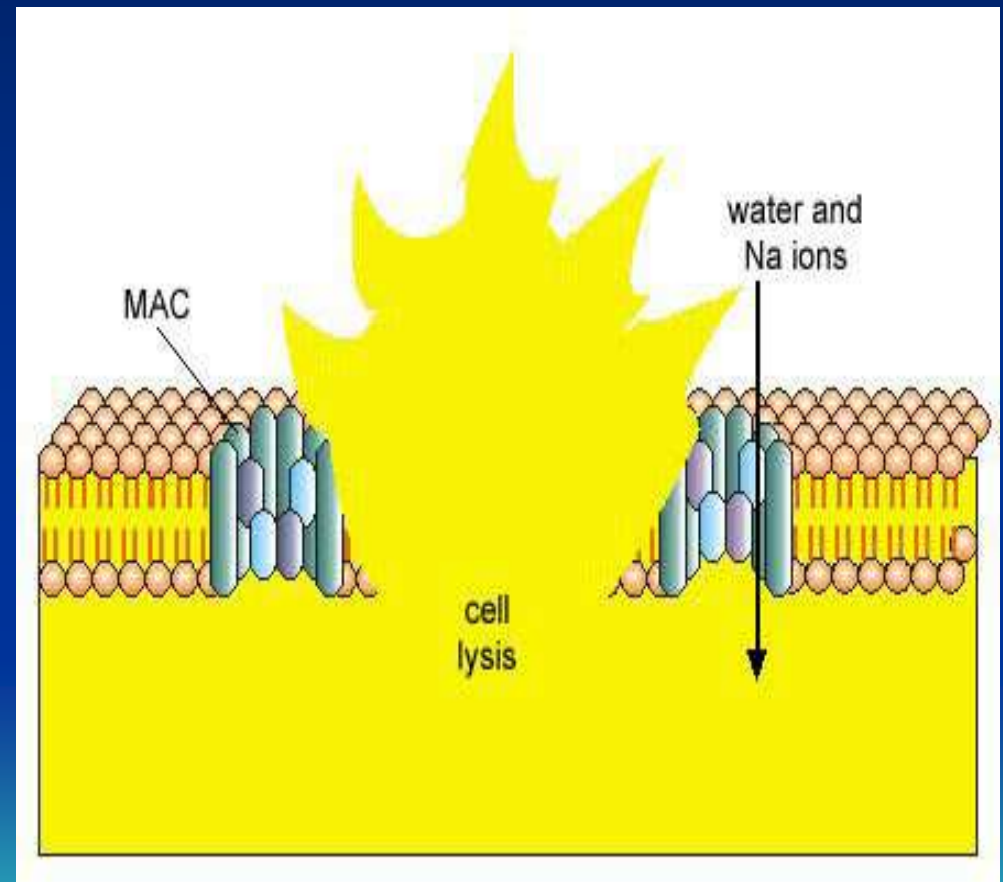


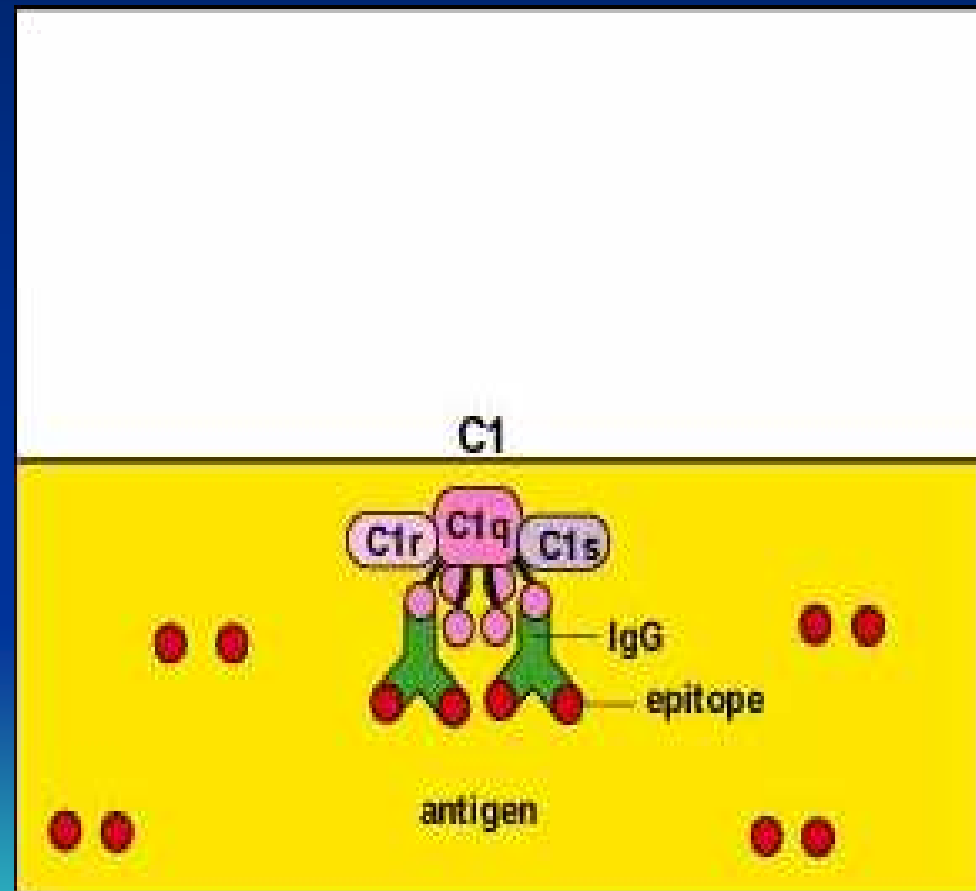
Fig. 9: The Membrane Attack Complex (MAC) Causing Cell Lysis

- This C5b6789 Membrane Attack Complex (MAC) puts pores into lipid bilayer membranes of human cells to which antibodies have bound. This results in cell lysis. MAC can also damage the envelope of enveloped viruses and put pores in the outer membrane and cytoplasmic membrane of gram-negative bacteria causing their lysis.



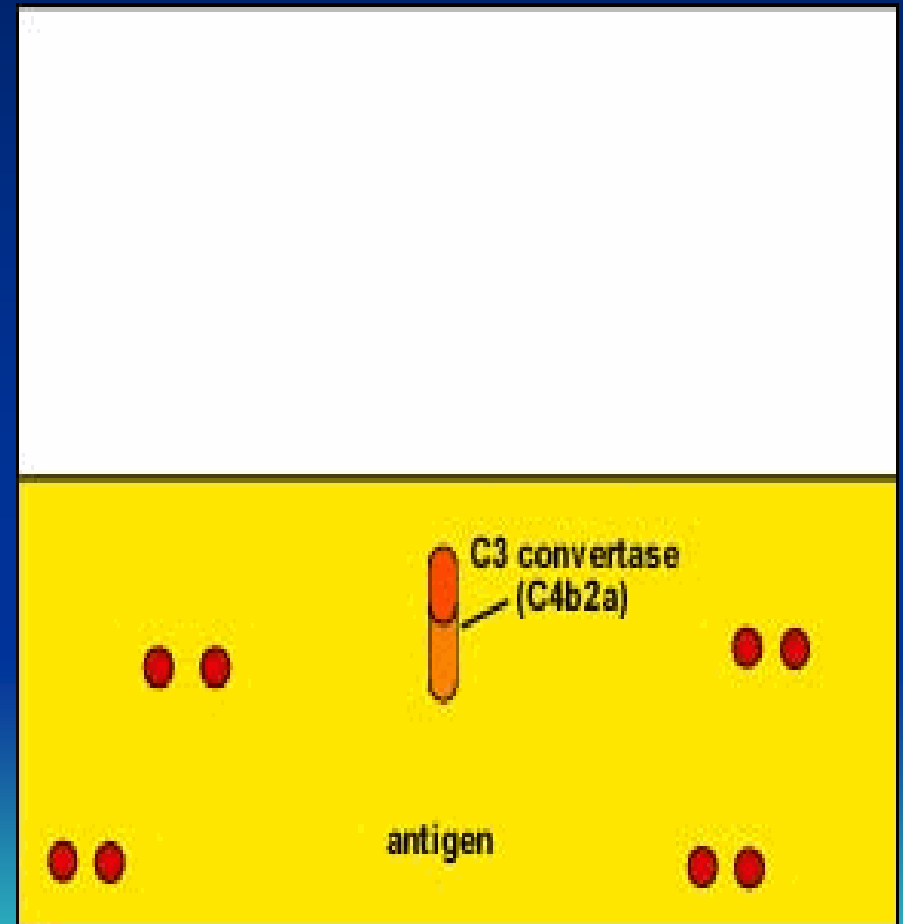
Animation of Formation of C3 Convertase during the Classical Complement Pathway

The enzyme C1 is able to cleave C4 into C4a and C4b. The C4b binds to adjacent proteins and carbohydrates on the surface of the antigen. C2 then binds to the C4b and C1 cleaves C2 into C2a and C2b. The C4a2b functions as a C3 convertase that can subsequently cleave hundreds of molecules of C3 into C3a and C3b.



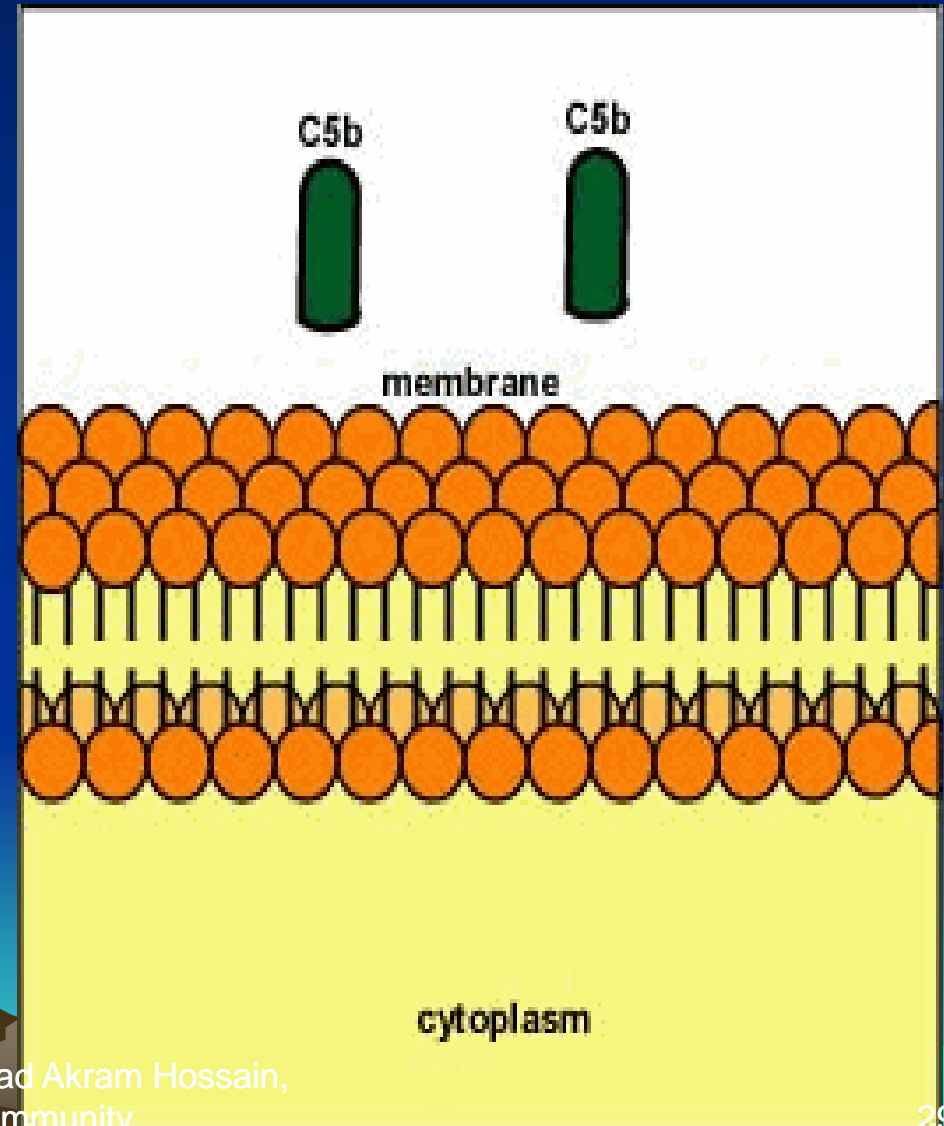
Animation of Formation of C5 Convertase during the Classical Complement Pathway

- The C4a2b functions as a C3 convertase that can subsequently cleave hundreds of molecules of C3 into C3a and C3b. Much of the C3b binds to adjacent proteins and carbohydrates on the antigen to participate in opsonization while C3a can stimulate inflammatory responses. Some of the C3b binds to C4b2a to form C4b2a3b, a C5 convertase that can cleave C5 into C5a and C5b.



Animation of The Membrane Attack Complex (MAC) Causing Cell Lysis

This C5b6789n, or membrane attack complex (MAC), puts pores into lipid bilayer membranes of human cells to which antibodies have bound. This results in cell lysis. MAC can also damage the envelope of enveloped viruses and put pores in the outer membrane and cytoplasmic membrane of gram-negative bacteria causing their lysis.



The Lectin Pathway

- The lectin pathway is mediated by **mannan-binding lectin (also known as mannan-binding protein or MBP)**. MBP is a protein that binds to the mannose groups found in many microbial carbohydrates but not usually found in the carbohydrates of humans. The MBP is equivalent to C1q in the classical complement pathway.
- Activation of the lectin pathway begins when **mannan-binding protein (MBP) binds to the mannose groups of microbial carbohydrates**. This forms an enzyme similar to C1 of the classical complement pathway that is able to cleave C4 and C2 to form **C4bC2a, the C3 convertase** capable of enzymatically splitting hundreds of molecules of C3 into C3a and C3b.



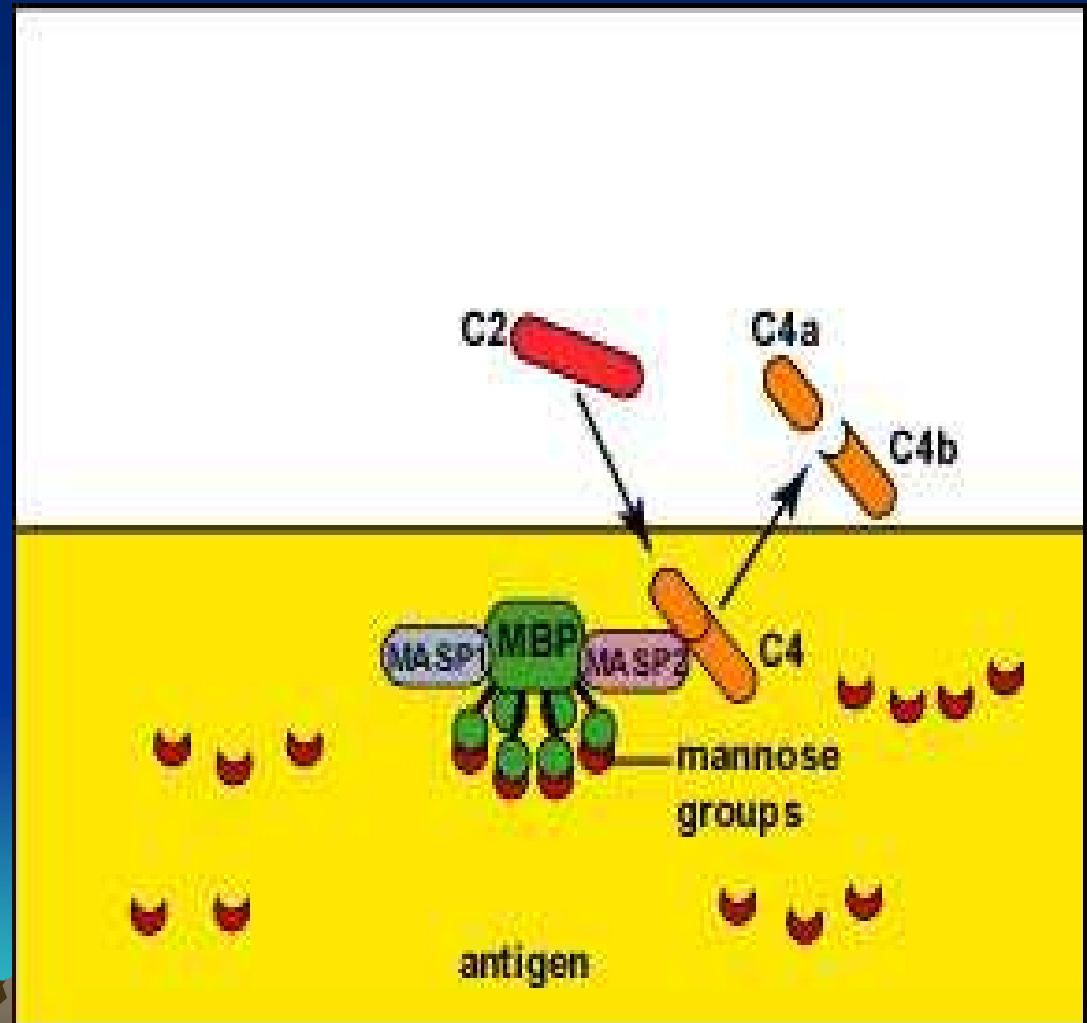
Lectin Pathway

- Mannan Binding Protein is structurally very similar to C1q but it can combine directly to any mannose groups on the bacterial cell wall without the need for specific antibodies.
- MASP is a protease capable of reacting with C4 & C2 resulting in the formation of C4bC2b, the same **C3 convertase** that is formed by the action of the Classical Pathway.



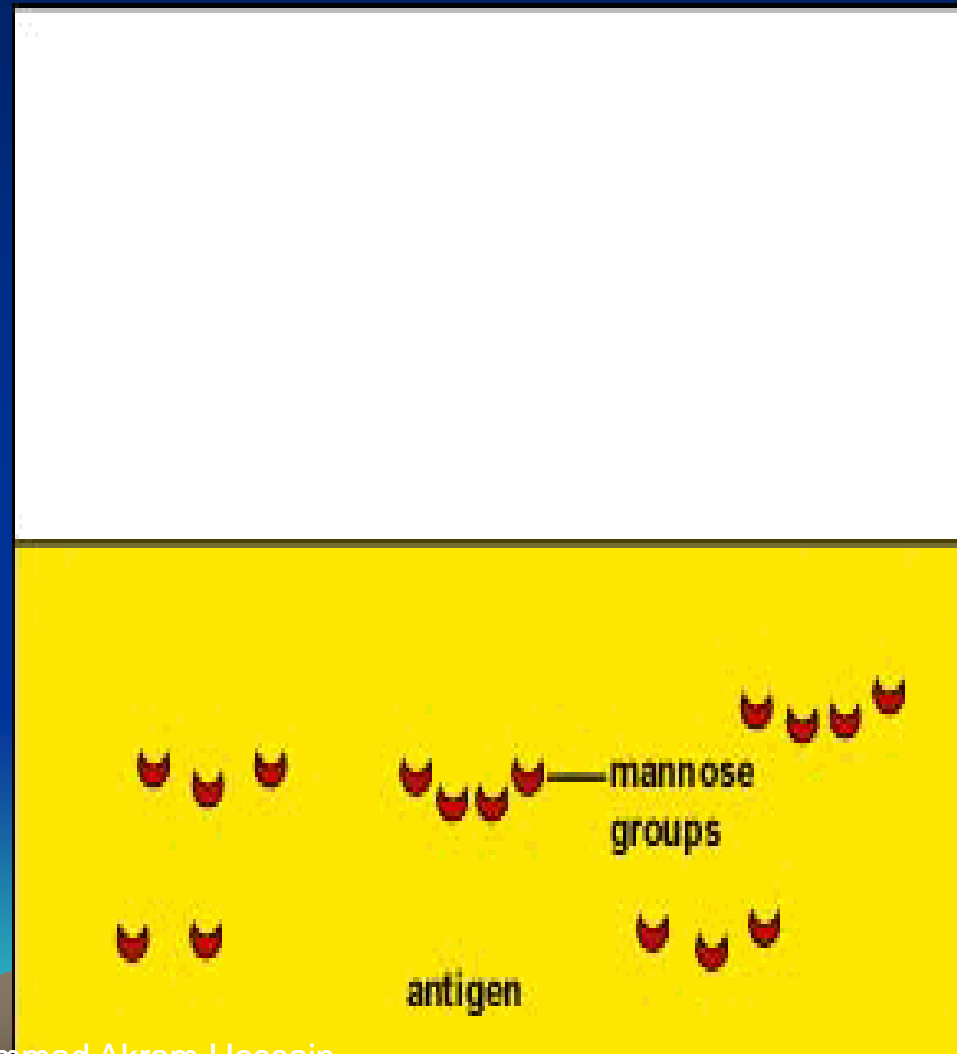
Fig. 1: Activation of the Lectin Pathway

Activation of the lectin pathway begins when mannan-binding protein (MBP) binds to the mannose groups of the carbohydrates on microorganisms. Two more lectin pathway proteins called MASP1 and MASP2 (equivalent to C1r and C1s of the classical pathway) now bind to the MBP. This forms an enzyme similar to C1 of the classical complement pathway that is able to cleave C4 and C2 to form C4bC2a, the C3 convertase that is able to enzymatically split hundreds of molecules of C3 into C3a and C3b.



Animation of Activation of the Lectin Pathway

Activation of the lectin pathway begins when mannan-binding protein (MBP) binds to the mannose groups of the microbial carbohydrates. Two more lectin pathway proteins called MASP1 and MASP2 (equivalent to C1r and C1s of the classical pathway) now bind to the MBP. This forms an enzyme similar to C1 of the classical complement pathway that is able to cleave C4 and C2 to form C4bC2a, the C3 convertase capable of enzymatically splitting hundreds of molecules of C3 into C3a and C3b.



Small Complement Fragments

- The fragments C3a, C4a & C5a can
 - act as chemotaxins to attract inflammatory cells such as neutrophils to the site of Complement Activation i.e. of infection
 - act as anaphylatoxins and induce degranulation of mast cells that releases histamine and other vasoactive substances that increase vascular permeability

Triggering inflammation

C5a is the most potent complement protein triggering inflammation.

- Release histamine from mast cells – **vasodilatation** & increase permeability
- it increases the expression of **adhesion molecules** on leukocytes and the vascular endothelium so that **leukocytes can squeeze out of the blood vessels and enter the tissue (diapedesis)**
- it causes **neutrophils to release toxic oxygen radicals for extracellular killing**
- and it **induces fever.**
- To a lesser extent **C3a** and **C4a** also promote inflammation.

Chemotaxis

C5a also functions as a **chemo attractant** for phagocytes.

Phagocytes will move towards increasing concentrations of C5a and subsequently attach, via their CR1 receptors to the C3b molecules attached to the antigen.

Opsonization

**Promoting the attachment of antigens to phagocytes
(enhanced attachment or opsonization)**

- **C3b** and to a lesser extent, **C4b** can function as **opsonins**, that is, they can attach antigens to phagocytes. One portion of the C3b binds to proteins and polysaccharides on microbial surfaces; another portion **attaches to CR1 receptors on phagocytes, B-lymphocytes, and dendritic cells for enhanced phagocytosis.** [\(see Fig. 10\).](#)

Opsonization...

How human cells escape from opsonization?

- Actually, C3b molecule can bind to pretty much any protein or polysaccharide.
- **Human cells**, however, produce **Factor H** that binds to C3b and **allows Factor I to inactivate the C3b**.
- On the other hand, substances such as **LPS on bacterial cells facilitate the binding of Factor B to C3b** and this **protects the C3b from inactivation by Factor I**. In this way, C3b does not interact with our own cells but is able to interact with microbial cells.
- C3a and C5a increase the expression of C3b receptors on phagocytes and increase their metabolic activity.



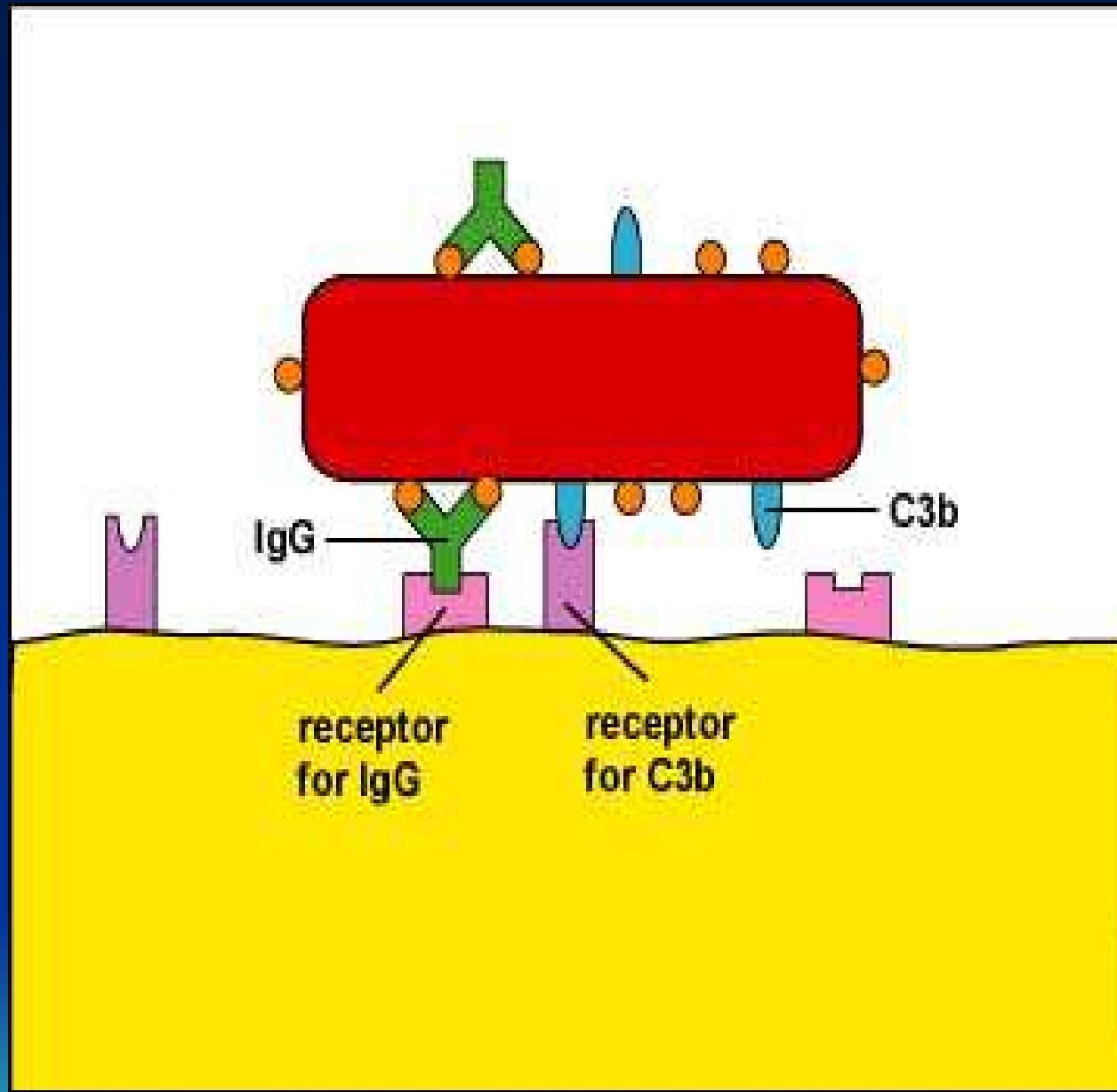
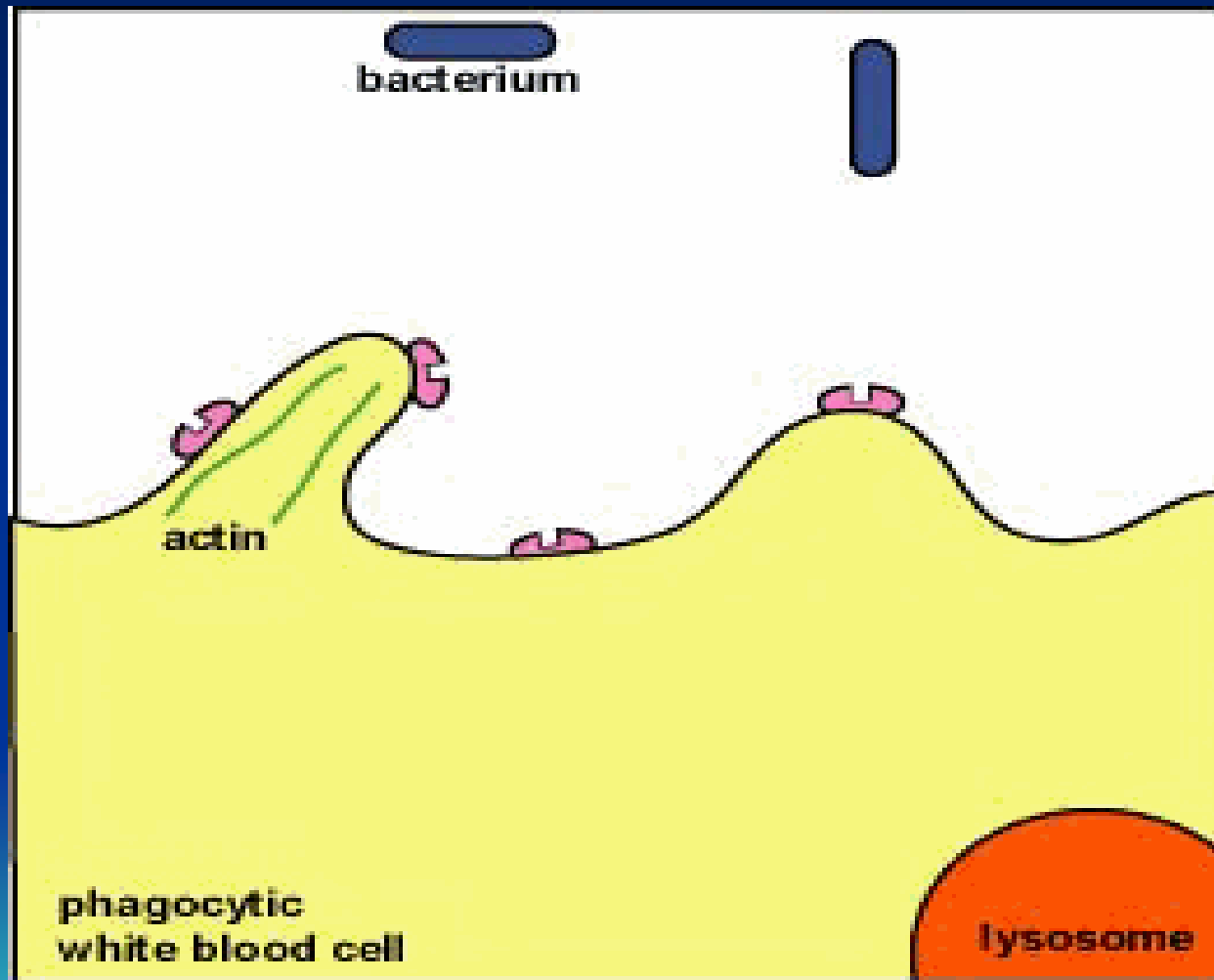


Fig. 2: Enhanced Attachment of Bacteria to Phagocytes

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Animation of Enhanced Attachment of Bacteria to a Phagocyte



Opsonins such as the antibody IgG and the complement protein C3b stick microbes to the phagocyte.

Cytolysis

C5b6789n, functions as a Membrane Attack Complex (MAC) . This helps to destroy gram-negative bacteria as well as human cells displaying foreign antigens (virus-infected cells, tumor cells, etc.) by causing their lysis; and. It can also damage the envelope of enveloped viruses.



Activation of B lymphocyte

- **Serving as a second signal for activating naive B-lymphocytes**
- **Some C3b is converted to C3d. C3d binds to CR2 receptors on B-lymphocytes. This serves as a second signal for the activation of B-lymphocytes whose B-cell receptors have just interacted with their corresponding antigen.**

Removing harmful immune complexes from the body

- **C3b** and to a lesser extent, **C4b** help to remove harmful immune complexes from the body.
 - The C3b and C4b attach the immune complexes to CR1 receptors on erythrocytes. The erythrocytes then deliver the complexes to fixed macrophages within the spleen and liver for destruction.

Production & regulation of complements

- Synthesized by
 - Liver
 - Spleen
 - Macrophages
 - Intestinal epithelial cells
- Regulated by
 - Various inactivators
 - C1 esterase inhibitor, C3b inhibitor, anaphylatoxin inhibitor
 - Metabolic degradation



Complement mediated diseases

- Deficiency of C5-C8 & MBL
 - Enhances susceptibility to Neisseria infections
- Deficiency of C3-
 - Severe recurrent pyogenic and respiratory tract infections.
- Inherited Deficiency of C1 esterase inhibitors
 - Angioedema
- Acquired Deficiency of Decay accelerating factor
 - Paroxysmal nocturnal hemoglobinuria
- Mismatched ABO transfusion
 - Hemolysis, Shok
- Immune complex mediated disease –
 - Glomerulonephritis

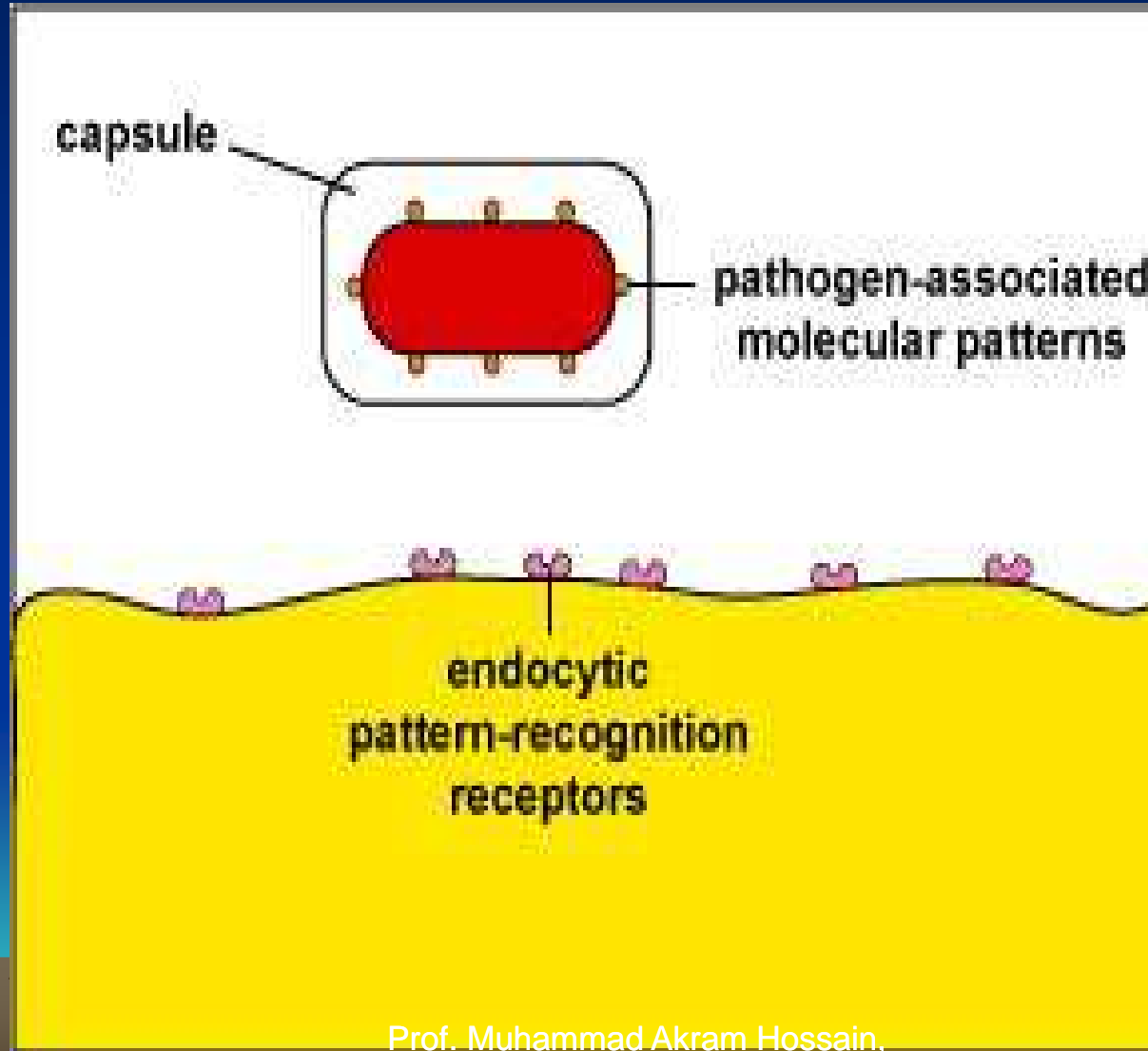


Summary

- Complement consists of serum proteins in inactive form, which when activated they act in concert, in orderly sequence to exert their biological effects.
- The biological effects of complement activation include
 - Cytolysis of target
 - Opsonization
 - Immune adherence
 - Release of mediators of inflammation
 - Chemotaxis
- There 3 pathways for activation
 - Classical
 - Alternate
 - Lectin mediated
- Deficiency of complement is associated with many diseases like SLE, increased susceptibility to infections (gonococcal, nephritis).



Fig. 14: Capsules Blocking the Unenhanced Attachment of Bacteria to Phagocytes



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Review questions

1. What are the complements? Where they remain?
2. How complements are activated? What are the pathways for complement activation?
3. What is the final effect of complement activation? What are the other biological effects?
4. Name some complement mediated diseases.
5. Name some complement components. Which one is central and from where complements are produced?
6. What are anaphylatoxins, chemotaxins?
7. Why complements need to be activated?
8. Why antibodies present in the blood don not activate complements?
9. Which classes of antibodies can activate complement? Which one is most efficient and why?

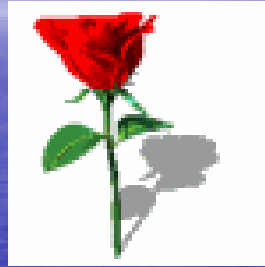


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