COMPLEMENT SYSTEM

- Observations about complement were carried out by Nuthall Pfeiffer and Bordet in the 1800's.

- Researchers compared cholera vibrio with immune fresh serum in the test tube and;

- Cholera vibrio culture + fresh guinea pig immune serum against cholera→ **bacteriolysis (+)**
- Cholera vibrio culture + **heated** immune serum → bacteriolysis (-)
- Cholera vibrio culture + **heated** immune serum + non-immune fresh guinea pig serum → **bacteriolysis (+)**
- Typhoid bacilli + Typhoid Immune fresh guinea pig serum → **bacteriolysis (+)**
- Cholera vibrio culture + immunized fresh guinea pig serum **against typhoid bacilli**→ bacteriolysis (-)
- Cholera vibrio culture + **heated** typhoid immune guinea pig serum + fresh guinea pig serum → bacteriolysis (-)

- In the experiment, when the fresh serum containing the specific antibody was added to bacteria suspension at 37 °C, the lysis of these cells was observed; But if the serum is heated at 56 ° C, it is determined that the lytic characteristic has disappeared.

- Since loss of lytic activity can not be associated with heat-resistant antibodies, It has been recognized that there is a substance in the serum that is thermosensitive and has complementary activity to the functions of the antibodies, and this substance is called complement.

- Substance complexes which are found in serum, inactivate by staling or heating at 56 ° C for 30 minutes, does not increasing of amount with immunization, can bind to the appropriate antigen-antibody complex, lead to cytolysis in this way, are called as complement.

- COMPLEMENT is not an ANTIBODY, it binds to the antigen-antibody complex.

- The complement is composed of 11 components, C1(q, r, s) C2, C3, C4, C5, C6, C7, C8 and C9. Their molecular weight, amounts in serum, chemical structures are different.

Complement System

- The Complement system, which is a complex protein group at low concentration in normal serum, consists of approximately 30 different and inactive constituents with chemical weights ranging from 25 to 500 kDa, react able with each other, have chemical and immunological differences.

- At normal conditions they are present inactive and soluble in the blood serum and only become active in special cases where the products that mediate the various effector functions of the complement swing.

The Activation of Complement

- The complementary system activate in three ways.

- Classical pathway
- Alternative pathway
- Lectin pathway

- In three pathways, the first components are also different. Although the stimulants are different, the goal in all activation ways; Activation of C5 and Finally activation of the membrane attack complex.

Classical Pathway

- In this way C1q, C1r, C1s, C4, C2, C3, C5, C6, C7, C8, and C9 components are involved in this order.
- Almost all of these proteins are found in the form of proenzymes. To gain enzyme activity, they have to be degraded by the previous enzyme.
- The classical pathway complex activation is usually initiated by the IgG or IgM antibody complex which binds to the specific antigen.

- The classical pathway begins with the complement protein C1 and ends with cell fusion at C9.
- Antigen is usually in the form of cells (bacteria, erythrocytes, etc.) Antibodies are those that can bind complement. (Ig M and Ig G1, IgG2, IgG3)
- IgG4, IgA, IgD, IgE can not bind the complement.

- On the classic pathway, C1q initiates activation. C1r activates C1s. C1s cleaves C4 which is free in the serum and cause to form C4a and C4b.
- C4b binds to surface and pull toward C2 to itself. forms C2a and C2b by breaking C2.
- When C2b is free, C2a remains on the surface adhesively to C4b. The resulting C4b2a complex gains C3 convertase activity. It breaks C3 into C3a and C3b.

- C3b binds C4b2a molecule to form C4b2a3b complex. The resulting new complex wins C5 convertase activity and separates C5 to two molecules as C5a and C5b
- C3b binds C4b2a molecule to form C4b2a3b complex. The resulting new complex wins C5 convertase activity and separates C5 to two molecules as C5a and C5b
- C5b bind to C6, C7, C8, C9 form form MAC (Membrane Attack Complex), and cell lysis occurs.

Alternative Pathway

- The alternative pathway of the complement system is an innate component of the immune system's natural defense against infections. The alternative pathway opsonize and kill pathogens. The pathway is triggered when the C3b protein directly binds a microbe. It can also be triggered by foreign materials and damaged tissues. **There is no need for antigen-antibody complex** in alternative pathway activation.

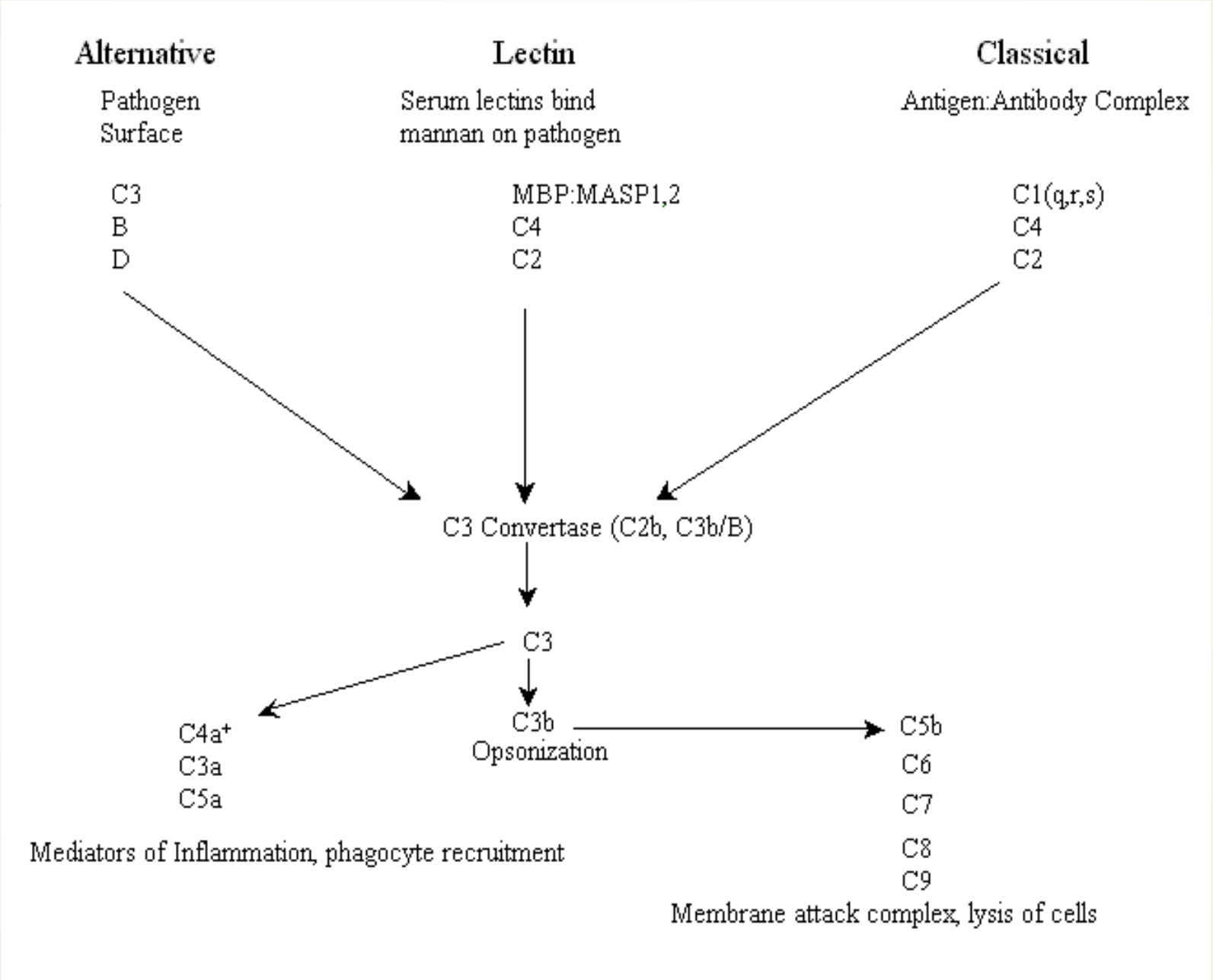
- The alternative pathway starts with C3 and ends at C9. The path also includes components such as factor B, factor D and properdine.

- It is initiated by the spontaneous hydrolysis of C3, which is abundant in the blood plasma.
- This change in shape allows the binding of plasma protein Factor B, which allows Factor D to cleave Factor B into Ba and Bb.
- Bb binds to C3b and forms C3bBb complex. This complex has convertase activity.
- C3bBb creates the new C3a and C3b and maintains the alternative pathway.
- The newly formed C3b binds to the C3bBb complex to form the C3bBb3b complex, which is an alternate C5 convertor. This continues C5 after the parts like the classic pathway.

Lectin pathway

- The third pathway that plays a role in the stimulation of the complement system is the lectin pathway and shows many similarities in the classical way. The lectin activation pathway is activated by the attachment of microorganism polysaccharides to the lectins. This activation mechanism plays a similar role in the classical pathway in later stages bacuse of the similarity between lektin and C1.

- In this pathway, mannose-binding lectin binds to mannose, glucose, or other sugars with 3- and 4-OH groups in terminal positions on carbohydrate or glycoprotein components of microorganisms including bacteria such as Salmonella, Listeria, and Neisseria strains. Fungal pathogens such as *Candida albicans* and *Cryptococcus neoformans* as well as some viruses such as HIV-1 and Respiratory syncytial virus (RSV) are bound by MBL.


Alternative
Lectin
Classical
Pathogen Surface
Serum lectins bind mannan on pathogen
Antigen:Antibody Complex
C3
B
D
MBP:MASP1,2
C4
C2
C1(q,r,s)
C4
C2
C3 Convertase (C2b, C3b/B)
C3
C4a+
C3a
C5a
C3b
Opsonization
C5b
C6
C7
C8
C9
Mediators of Inflammation, phagocyte recruitment
Membrane attack complex, lysis of cells

- Whichever pathway they act, the stimulation of the complement system exhibits simila

- The resulting C5 convertase activation, firstly C5 separated into C5a and C5b fragments. Then, C5b, which sticks to the cell wall, binds the rest of the system's constituents (C6, C7, C8 and C9); and C5-C6,7,8,9 (Membrane Attack Complex)complex forms.

- The MAC makes the membrane semipermeable; After the passage of some substances from the extracellular medium into the cell, such as water and calcium; Eventually the cell swells and explodes.

- Serum complement proteins and membrane-bound complement receptors partake in a number of immune activities**: lysis of foreign cells** by antibody-dependent or antibody-independent pathways; opsonization or uptake of particulate antigens including bacteria, by **phagocytosis**; **activation of inflammatory responses**; and **clearance of circulating immune complexes** by cells in the liver and spleen.

Complement Fixation Test

- The complement fixation test is an immunological medical test that can be used to detect the presence of either specific antibody or specific antigen in a patient's serum, based on whether complement fixation occurs. It was widely used to diagnose infections, particularly with microbes that are not easily detected by culture methods, and in rheumatic diseases. However, in clinical diagnostics labs it has been largely superseded by other serological methods such as ELISA and by DNA-based methods of pathogen detection, particularly PCR.

- This test includes two systems. The first one is **antigen** that suspected of being infection agent in patient and **patient serum.**

- The second system (indicator system)
  - Sheep erythrocytes
  - Anti-sheep erythrocyte antibody (hemolysin) against sheep erythrocytes,
  - The compliment (at a critical amount)

- The substances used in this experiment are;
- Antigen: the agent of the suspected disease
- Antibody: patient serum
- Complement: fresh guinea pig serum
- Sheep erythrocytes
- Hemolytic serum: Serum containing antibody against sheep erythrocytes
- Saline

- Firstly, add the patient's serum on the suspected antigen, then add a limited amount of complement to the solution. If the patient's serum contains antibodies against this antigen, the antigen-antibody complex that will form will bind the entire complement (Antigen + patient serum + complement). The critical amount of compliment is very important and it should be just enough for a system.

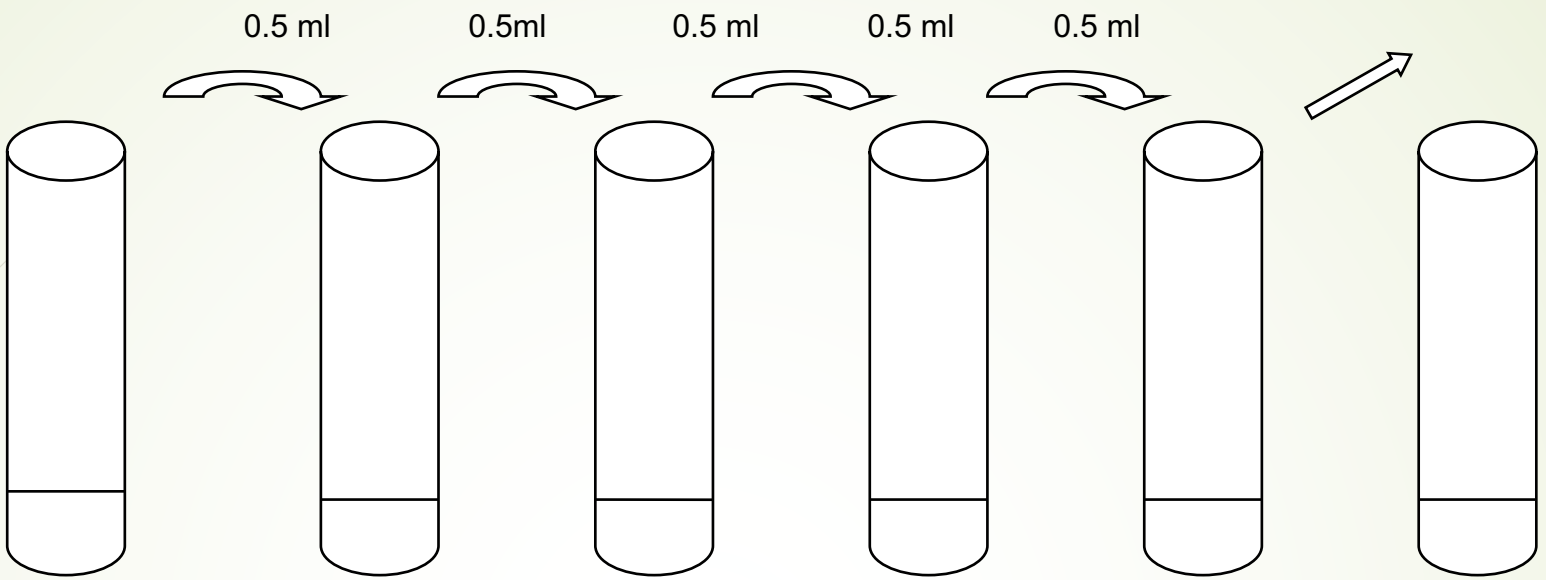
- In the next step, the indicator system consisting of sheep erythrocytes and hemolysin is included in the reaction.
- (Antigen + patient serum + complement + indicator system)
- If the complement can not bind to the complex formed by the antibody from the patient's serum, the exposed complement will bind to the sheep erythrocyte-hemolysin complex and a lysis will occur.

- Therefore, the lysis of the indicator cells is indicative of the absence of antibodies in the patient's serum and a negative result.

- The presence of complement -binding antibodies in the patient's serum, the absence of lysis of erythrocytes in the indicator system is a sign of a positive result.

HEMOLITHIC SERUM TITERS

- It is performed to determine the minimal hemolysis dose of serum.
- If an animal cell are injected to the other animal, the antibodies are formed against that cell. Such serum containing antibodies against erythrocytes are termed hemolytic serum or hemolysin
- Hemolytic serum is rabbit serum that containing antibody against sheep erythrocytes used in the complement fixation test.



0.9 ml S.F + 0.1ml (1/25 lik) hemolytic serum	0.5 ml SF	0.5 ml SF	0.5 ml SF	0.5ml SF	0.5ml SF
1 / 250 + 0.5 ml erythrocytes	1 / 500 + 0.5 ml	1 / 1000 + 0.5 ml	1 / 2000 + 0.5 ml	1 / 4000 + 0.5 ml	+ 0.5 ml (10' at room temperature.)
1 / 500 -	1 / 1000 + 0.5 ml complement	1 / 2000 + 0.5 ml comp.	1 / 4000 + 0.5 ml comp.	+ 0.5 ml comp	1 / 8000 + 0.5 ml comp. (at 37 °C for 30')
	1/1500	1/3000	1/6000	1/12000	

- The tube with the highest dilution of hemolysis is detected, the hemolytic serum dilution in this tube being called " a minimal hemolytic dose" (MHD) or " a hemolytic unit". In the complement fixation test, 3-fold intense dilution or 3 MHD will be used