Complement system:

The complement system, also known as complement cascade, is a part of the immune system that enhances (complements) the ability of antibodies and phagocytic cells to clear microbes and damaged cells from an organism, promote inflammation, and attack the pathogen's cell membrane. It is a part of the innate immune system, the complement system can, however, be recruited and brought into action by antibodies generated by the adaptive immune system.

The complement system consists of approximately 30 serum molecules consisting nearly 10% of the total serum proteins and forming one of the major defense systems of the body. Most of the complement glycoproteins are synthesized predominantly by the liver; but macrophages and many other cell types are also sources of various complement components, especially at sites of infection and/or inflammation.

The complement system can be activated by classical, alternative and lectin pathways. The proteins of the system act in enzyme cascades. Where each step generates enzyme, which act in the following step of the cascade and all the three pathways generate enzymes which cleave C3 into two fragments, C3a and C3b as a central step in the process of complement activation.

1. The classical pathway:

Immunoglobulins and native complement components are normally found in the serum and in the lymph, but these molecules do not interact with each other until the antibodies interact with their corresponding antigens and undergo the necessary secondary and tertiary conformational changes. The classical pathway is activated by immune complexes and the activation is initiated by the binding of C1 to domains in IgG (CH2-domain) or IgM (CH3-domain) which are complexes with Ag. Detailed chemical studies have revealed that C1 is actually a complex of three different types of molecules [C1q, dimerized C1r (C1r2), and dimerized C1s (C1s2)] held loosely together through noncovalent bonds and requiring a physiological Ca2 concentration for their proper association.

The recognition unit of the complement pathway is the C1q which is a molecule with a six globular head group and when a specific Ab (IgG or IgM) interacts with its corresponding Ag, binding sites for the globular head group of the C1q are exposed on the Fc-region of the Ab molecule. At least two molecules of IgG or CRP or one molecule of IgM are required for binding C1q. after binding to Fc-region of Ab molecule, a conformational change occurs in C1q and this C1q change causes the pro-enzyme C1r to become the enzymatically active C1r. the substrate for the enzyme C1r is C1s, which is then cleaved to become the serine esterase (C1s).
C1q contains several distinct portions; there is a collagen-like stalk/stem region that branches into a six-branched umbrella-shape. Within the umbrella portion of the stem, an association with the C1r2 and C1s2 proenzymes occurs. Each of the six collagen-like branches of C1q terminates in a globular head region.

The activation unit, the active enzyme of C1s cleaves two proteins, C4 (into C4a & C4b) and C2 (into C2a & C2b), in a magnesium-dependent reaction. C4b and C2a

combine to form an active enzyme, C4b2a, which is the classical pathway, C3-convertase that cleaves many molecules of C3 (into C3a & C3b).

The C3b is either form a covalent bond with an Ag or with bystander surfaces (e.g. erythrocytes) in immune adherence or can bind to C4b2a to form C4b2a3b, a C5-convertase, an enzyme cleaved C5 (into C5a & C5b). C5b binds to a molecule of C6 to form a stable bimolecular complex which bind to C7 to form a trimolecular complex (C5b67), this trimolecular complex binds hydrophobically to a membrane since the complex is amphiphilic, this allow it to insert into cell membranes. C8 now joins the complex and unwinds into the cell membrane. Thus, forming a functional trans-membrane channel and itself cause disruption and lysis of membranes, an effect which is greatly enhanced by the incorporation of C9 and if more than six molecules of C9 enter the complex, typically doughnut-shaped pores are formed. C9 is not essential for the lytic event, but it does accelerate lysis.

2. **The alternative pathway**

The alternative pathway is activated near 'protected' surfaces such as bacterial or fungal cell walls, bacterial lipopolysacharide, some virus-infected cells and rabbit RBCs. It has been shown that the (activating surfaces) is actually a protective surface, protecting spontaneously hydrolyzed C3 (non-enzymatically cleaved into C3a and C3b) from being inactivated by the control proteins.

In presence of factor D and magnesium, C3b-like molecule can cleave factor B (into Ba and Bb), Bb binds to the C3b to form an alternative pathway C3-convertase C3bBb. The C3bBb is a very unstable and quickly inactivated by control proteins, unless it's bound to activating surface and stabilized by P (properdin), the C3bBbP enzymatic complex can cleave additional molecules of C3 and if a second C3b is inserted into the C3-convertase, it becomes C3bBb3bP, this becomes a C5-convertase that can cleave C5 into C5a and C5b. the membrane attack unite for the alternative pathway begins with C5b and progresses through C6,7,8 and C9 in exactly the same sequence as it does for the classical pathway.

3. The lectin pathway:

The newest discovered pathway for activating the second, fourth, and third complement components is the lectin complement pathway, which involves a serum MBL (mannan-binding lectin) and other serum lectins called ficolins. The activation of the lectin pathway does not involve antigen-antibody interactions.

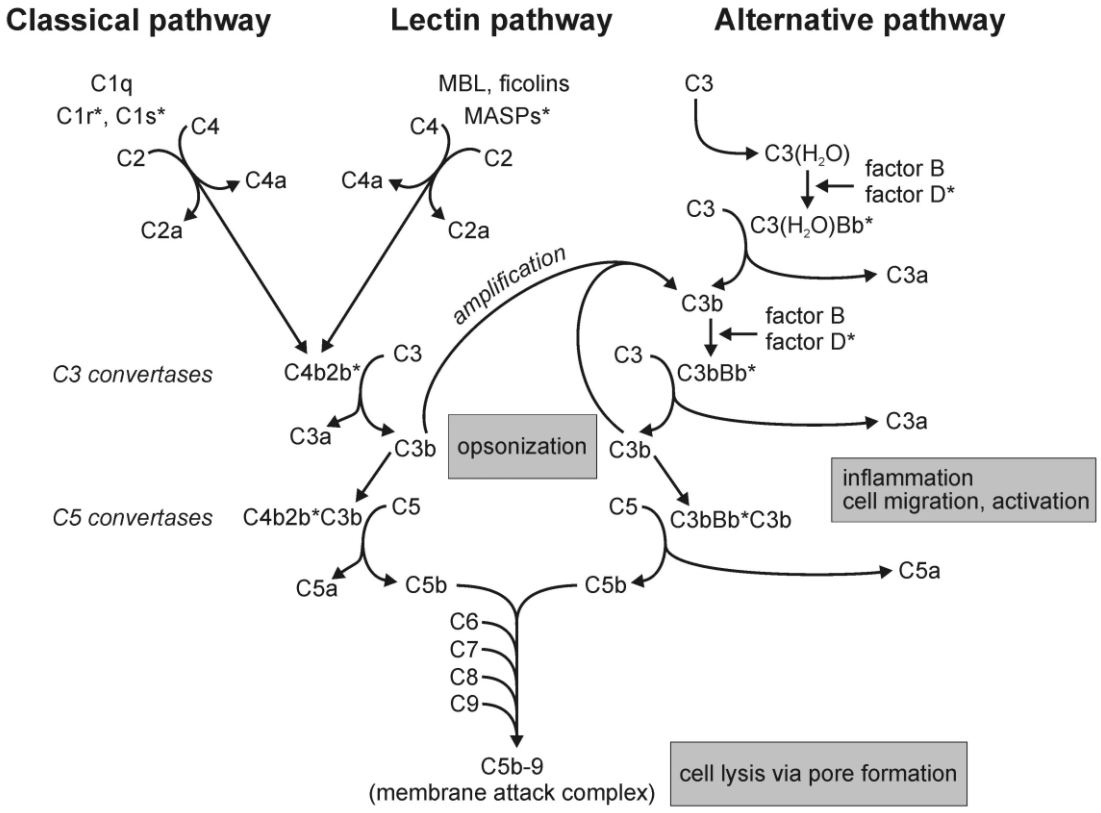
Lectin pathway is activated by bacterial carbohydrates, the molecule which initiates the pathway mannan-binding lectin (MBL), the MBL is associated with two proenzymes MASP-1 and MASP-2 MBL-associated serine proteases). When MBL binds to terminal mannose group on bacterial carbohydrates it activates MASP-1&2 which go on to activate the classical pathway in an Ab-independent fashion. But it has been shown that activated MASP-2 cleaves C4 and C2 while activated MASP-1 cleave C3 and C2.

Note: C1q itself is also able to bind directly to some microorganisms, including mycoplasma and retroviruses, in an Ab-independent fashion).

Primary Function of the Complement System

The primary function of the complement system is to bind and neutralize any foreign substance that activates it, and then to effectively cause those neutralized complement-coated substances to tightly adhere to phagocytes, thereby enhancing phagocytosis. In this regard, the third complement component (C3) is a major factor due to its position in the classical, alternative, and lectin pathways and because of its relatively high concentration in serum.

Activation pathway	Classic	Alternative	Lectin
Activator	Ag–Ab Complex	spontaneous hydrolysis of C3	MBL-Mannose Complex
C3-convertase	C4b2a	C3bBb	C4b2a
C5-convertase	C4b2a3b	C3bC3bBb	C4b2a3b
MAC development	C5b+C6+C7+C8+C9		



<u>The biological consequences of complement activation are:</u>

1. ***opsonization*:** the complement component coating the surface of a target such as bacteria. Phagocytic cells carrying receptors for these complement components are

there able to bind to the bacteria, and once it become opsonized, more effective phagocytosis occur.

2. ***Chemotaxis:*** C5a is a potent chemotactic factor and it induces the directed migration of neutrophils and monocytes into the area of inflammation.
3. ***Anaphylaxis:*** C3a, C5a a biologically active peptides and these anaphylatoxin mediate inflammation by inducing the release of mediators from basophiles and mast cells, causing smooth muscle contraction and increase vascular permeability.

4. ***Immune adherence:*** it's the covalent bonding between C3b and nearby soluble immune complexes or particulate surfaces. Since C3b has receptors on human erythrocyte, B-lymphocytes, monocytes, glomerular epithelial cells and mast cells. One biologic purpose for immune adherence is to facilitate removal of soluble immune complexes and immune adherence provides a mechanism for the soluble complexes to bind to erythrocytes, facilitating removal of these complexes by the reticuloendothelial system.

5. ***Kinin activation:*** The fragment of C2b interacts with plasmin to produce kinin-like activity. The biologic activity of C2b results in smooth muscle contraction, mucous gland secretion, increased vascular permeability, and pain.

6. ***Lysis of target cells:*** the final step in complement activation causes the assembly of a membrane attack complex (MAC), which can insert itself into lipid bilayers, such as the outer membrane of gram-negative bacteria or a viral envelope, and then osmotic disruption of the target cell causing lysis.

Complement components are synthesized in the liver, with the exception of C1, which is synthesized in the epithelial cells of the intestine. Limited quantities of complement components, including C1q can be synthesized by activated macrophage-monocyte.

Control of complement activation

The first mean of control is if activated complement (enzyme) does not combine with its substance within milliseconds, the activity is lost.

Several proteins serve as inhibitors or inactivators of specific reactions or products involved in the complement cascade like:

- C1-inhibitor (C1INH): form irreversible complex with both C1r and C1s and block their enzyme activities and dissociate them from C1q.
- Factor H & I: serve to control tightly the enzyme that cleave C3 and C5 (factor I: Inactivates C3b and C4b. Whereas, factor H accelerates the decay of alternative C3-convertase C3bBb3b by dissociating Bb from the enzyme.).
- C4-binding protein (C4BP): bind to and inactivate C4.
- Anaphylatoxin inactivators (carboxypeptidase) effect C4a, C3a, C5a by removal of a single amino acid (carboxyterminal arginine).

- ❖ MAC inhibitor: a serum protein can bind to fluid phase C5b67, prevent its attachment to membrane protein or C8.
- ❖ Inhibition of assembly: like the (protectin, CD59) and (delay accelerating factors, DAF).

Clinical diseases associated with complement activity:

1. C1INH-deficiency or angioneurotic edema:

This is a rare genetic disorder due to a genetically inherited C1-INH deficiency, of which two main variants are known. Deficiency of C1INH leads to uncontrolled activation of the classical PW. In the most common, the genetic inheritance of a silent gene results in a significantly lower level of C1-INH. The second variant is characterized by normal levels of C1-INH protein, but 75% of the molecules are dysfunctional, that is, will not inhibit activated C1r2 or C1s2 because of an aberrant amino acid substitution.

Thus, C1s continues to cleavage C4 and C2 resulting in C2a release leading to kinin like activity and C4a release leading to anaphylactic reaction Activity. Both producing swelling (extra-ordinary amount of fluid in tissue spaces as a result of increase vascular Permeability and smooth muscle contraction). No immunodeficiency, and decreased C2 & C4, with Normal C3 (downstream C4BP works). The hereditary form called hereditary angioedema (HAE), its autosomal dominant trait. Whereas, acquired from associated with lympho-proliferative diseases.

2. Paroxysmal nocturnal hemoglobinuria (PNH)

It is a clonal disorder of hemopoietic cells, in which there is increased susceptibility to damage by complement due to deficiency of glycosyl phosphatidyl inositol (GPI) which binds delay accelerating factors (DAF or CD 55) and **protectin** (CD59) to membrane. The clinical feature of this illness is the episodic hemoglobinuria mainly at morning, hemolytic anemia, increased susceptibility to infection due to decreased number or function of WBC.

3. Nephritic factors (NF)

- ❖ It is a pathological enhancing protein.
- ❖ It is IgG with specific to alternative PW C3-convertase.
- ❖ It prevents inactivation of C3 - convertase by H and I control proteins.
- ❖ Presence of this factor leads to C3 deficiency and recurrent infection, in addition to lipodystrophy and emaciation.