Complement systems:

Complement system and their biological function

(Classical and Alternative pathway)

Objectives:

After carefully listening to this lecture, you will be able to:

- Define complement system
- Know the different complement system
- Understand the component of the complement system
- Explain how different pathways are activated
- Know the difference in the pathways of the different complement system

Introduction:

The **complement system** 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, promotes inflammation, and attacks the pathogen's plasma membrane. It is part of the innate immune system, which is not adaptable and does not change over the course of an individual's lifetime. It can be recruited and brought into action by the adaptive immune system. It consists of a number of small proteins found in the blood, in general synthesized by the liver, and normally circulating as inactive precursors (pro-proteins).It contributes to inflammation by inducing local changes in blood flow and the influx of inflammatory cells into the affected area. When stimulated by one of several triggers, proteases in the system cleave specific proteins to release cytokines and initiate an amplifying cascade of further cleavages. The pathology that accompanies uncontrolled activation or incomplete performance of complement's function is often the result of a deficiency or impairment of one of the components.

Overview:

Complement system are mainly activated by three main pathway i.e. classical complement pathway, alternative complement pathway and lectin pathway. Each complement pathway has unique proteins for the initiating steps, but shared the same or related proteins for the intermediate steps and uses the same components in the last step, culminating in the same activities. Each initiating pathway is triggered by a different type of activator, usually a cell, microbe, or molecular aggregate that presents charge patterns that are "recognized" by component of the individual initiating pathway, making complement one of the innate immune system's primary pattern-recognition mechanisms for detecting nonself. They are triggered by 1) the binding of one of their components to the activator, 2) a cascade of enzyme activation, and 3) generation of biological effects. There are physical and chemical mechanisms as well as specific regulators that prevent uncontrolled activation and damage to local cells and tissues. A network of fluid-phase and cell associated regulatory proteins and specific receptors interact with the component of complement and their split products, and are involved in controlling complement activation at the cell surface, as well as a wide variety of cell-signaling events.

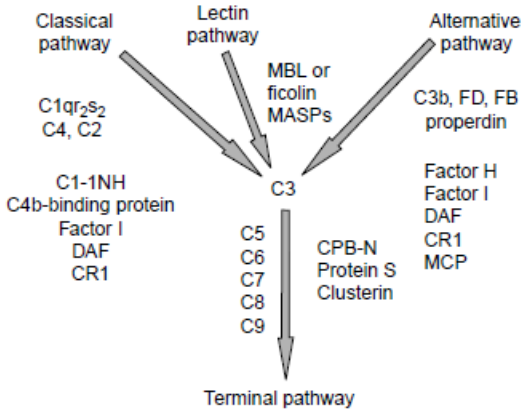


FIGURE 4.1 Schematic of the complement activation pathways. The three initiation pathways (CP, LP, and AP) generate C3 convertases that cleave C3 and allow activation of the terminal components.

Functions of complement:

After initial activation, the various complement components interact, in a highly regulated cascade, to carry out a number of basic functions including:

- Lysis of cells, bactera, and viruses
- Opsonization, which promotes phagocytosis of particulate antigens

- Binding to specific complement receptors on cells of the immune system, triggering specific cell functions, inflammation, and secretion of immunoregulatory molecules
- Immune clearance, which removes immune complexes from the circulation and deposits them in the spleen and liver

Complement components:

The components of complements (proteins and glycoproteins) are synthesized by liver hepatocytes, blood monocytes, tissue macrophages, and epithelial cells of the gastrointestinal and genitourinary tracts. They are circulated in the serum in the form of proenzymes, or zymogens. The complement-reaction sequences starts with an enzyme cascade.

Complement components are designated by numerals (C1-C9), by letter symbols (e.g. factor D), or by trivial names (e.g. homologous restriction factor). Peptide fragments formed by activation of a component are denoted by small letters. In most cases, the smaller fragments resulting from cleavage of a component is designated "a" and the larger fragment designated "b" (e.g. C3a, C3b but C2 is an exception: C2a is the larger fragment). The complement fragments interact with one another to form functional complexes. Those complexes that have enzymatic activity are designated by a bar over the number or symbol (e.g. C4b2a)

Complement activation:

There are three pathways that can activate the complement system, and different structural areas of the immunoglobulin molecules are involved in complement fixation by two of them. Complement is the name given to a complex series of some 20 proteins which, along with blood clotting, fibrinolysis and kinin formation forms one of the triggered enzymes system found in plasma. The final step of the three pathways is the formation of membrane attack complex (MAC) in all the pathways.

The classical pathway of complement activation:

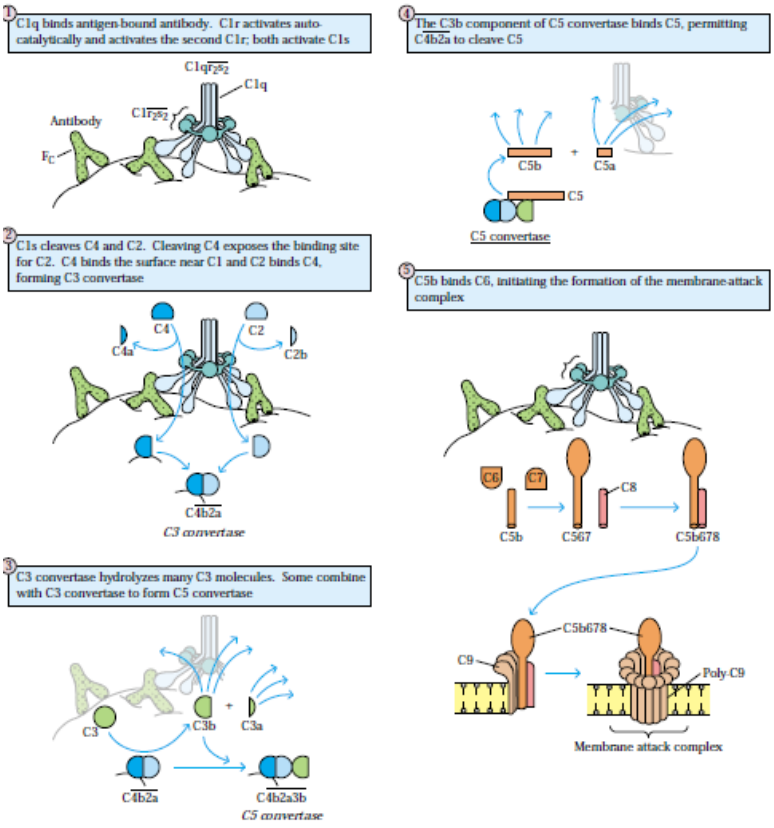


FIGURE 13-5 Schematic diagram of intermediates in the classical pathway of complement activation. The completed membrane-attack complex (MAC, bottom right) forms a large pore in the membrane.

Complement activation by the classical pathway commonly begins with the formation of soluble antigen-antibody complexes or with the binding of antibody to antigen on a suitable target, such as bacterial cell. The initial stage of activation involves C1, C2, C3 and C4 which are present in plasma in functionally inactive forms.

The formation of an antigen-antibody complex induces conformational changes in the F_c portion of the IgM molecules that expose a binding site for the C1 component of the complement system. C1 in serum is a macromolecular complex consisting of C1q and the two molecules each of C1r and C1s, held together in a complex($C1qr_2s_2$) stabilized by Ca^{2+} ions. Each C1r and C1s monomer contains a catalytic domain and an interaction domain, the latter facilitates interaction with C1q or with each other.

When the pentameric IgM is bound to antigen on a target surface it assumes the so called “staple” configuration, in which at least three binding sites for C1q are

exposed. Binding of C1q to Fc binding sites induces a conformational change in C1r that convert C1r to an active serine protease enzyme, C1r, which then cleaves C1s to a similar active enzyme, C1s. C1s has two substrates C4 and C2. The C4 component is a glycoprotein containing three polypeptide chain α,β and γ. C4 is activated when C1s hydrolyze a small fragment (C4a) from the amino terminus of the α-chain exposing a binding site on the larger fragment (C4b). The C4b fragment attaches to the target surface in the vicinity of C1 and the C2 proenzyme then attaches to the exposed binding site on C4b. where the C2 is then cleaved by the neighbouring C1s; the smaller fragment (C2b) diffuses away. The resulting C4b2a complex is called C3 convertase, referring to its role in converting the C3 into an active form. The smaller fragment from C4 cleavage, C4a, is an anaphylatoxin, or mediator of inflammation, which does not participate directly in the complement cascade.

The native C3 component consists of the two polypeptide chains, α and β. Hydrolysis of a short fragment (C3a) from the amino terminus of the α-chain by the C3 convertase generates C3b. some of the C3b binds to C4b2a to form a trimolecular complex C4b2a3b, called C5 convertage. The C3b component of this complex bind C5 and alters its conformation, so that the C4b2a component can cleave C5 into C5a, which diffuses away, and C5b, which attaches to C6 and initiates formation of the menbrane –attack complex(MAC).

Alternative pathway:

The alternative pathway of complement activation involves four serum proteins: C3, factor B, factor D and properdin. The alternative pathway is initiated in most cases by cell-surface constituents that are foreign to the host. Here serumC3, which contains an unstable thioester bond, is subject to slow spontaneous hydrolysis to yield C3a and C3b. The C3b component can bind to foreign surface antigens or even to the host's own cells. But the C3b bound to the bacterial cell wall remains active for a longer time. The C3b present on the surface of the foreign cells can bind another serum protein called factor B to form a complex stabilized by Mg^{2+}. Binding of C3b exposes a site on factor B that serves as the substrate for an enzymatically active serum protein called factor D. factor D cleaves the C3b-bound factor B, releasing a small fragment (Ba) that diffuses away and generating C3bBb. The C3bBb complex has C3 convertase activity and thus is analogous to

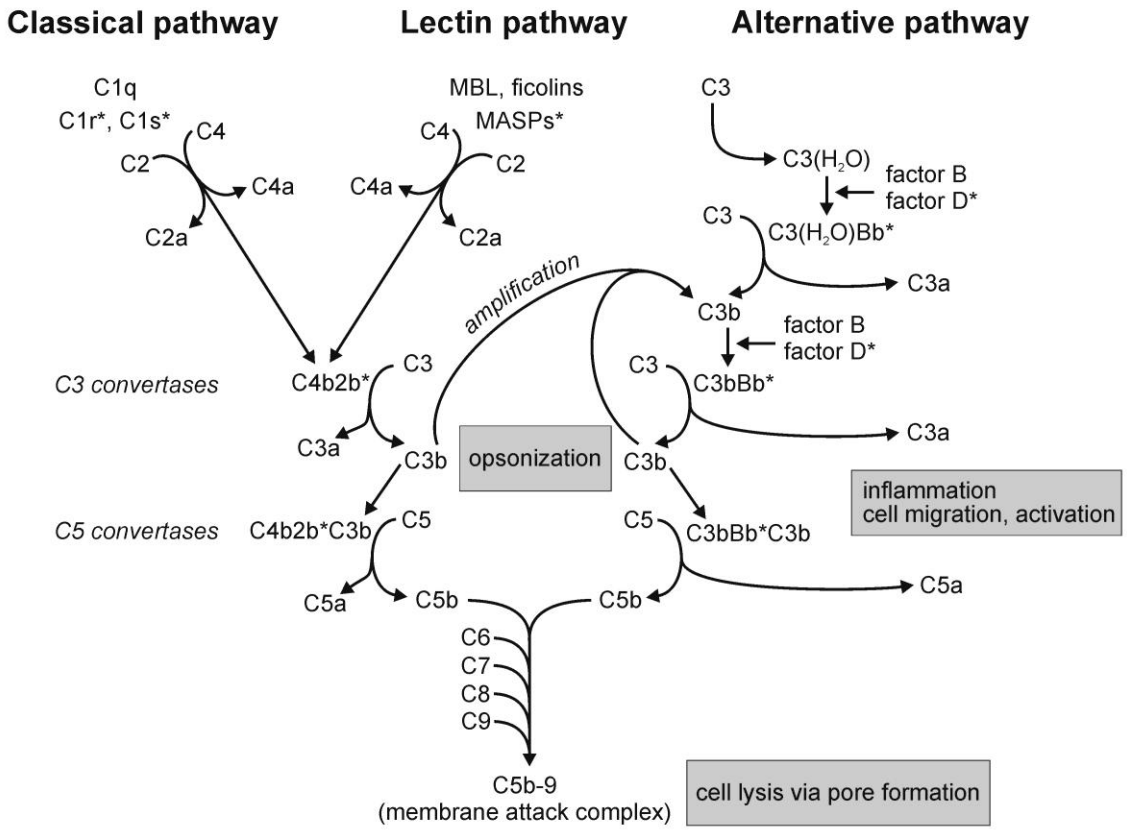
the C4b2a complex in the classical pathway. The C3 convertase activity of C3bBb has a half-life of only 5minutes unless the serum protein properdin binds to it, stabilizing it and extending the half –life of this convertase activity to 30 minutes.

The C3bBb generated in the alternative pathway can actiavate unhydrolyzed C3 to generate more C3b autocatalytically. As a result, the initial step are repeated and amplified, so that more than 2 X 10^6 molecules of C3b can be deposited on an antigenic surface in less than 5 minutes. The c3 convertase activity of C3bBb generates the C3bBb3b complex, which exhibit C5 convertase activity, analogous to the C4b2a3b complex in the classical pathway. The nonenzymatic C3b component binds C5, and the Bb component subsequently hydrolyzes the bound C5 to genetates C5a and C5b; the latter binds to the antigenic surfaces.

The lectin pathway:

Lectins are proteins that recognize and bind to specific carbohydrate targets. It does not depend on antibody for its activation. However, the mechanism is more like that of the classical pathway because after its initiation, it proceeds through the action of C4 and C2, to produce a C5 convertase.

The lectin pathway is activated by the binding of mannose binding lectin(MBL) to mannose residues on glycoproteins or carbohydrates on the surface of microorganisms. MBL is an acute phase protein produce in inflamatary responses. Its function in the complement pathway is similar to that of C1q, which it resembles in structure. After MBL binds to the surface of the cell or pathogen, MBL-associated serine-proteases, MASP-1 and MASP-2, bind to MBL. The active complex formed by this association causes cleavage and activation of C4 and C2. The MASP-1 and MASP-2 have structurally similar to C1r and C1s and mimic their activities. This means of activating the C2-C4 components to form a C5 convertase without need for specific antibody binding represents an important innate defence mechanism.



Differences between the complement pathways:

The classical pathway is activated, for example, by Ag–Ab complexes that react with activated C1q. The lectin pathway is initiated by either serum MBL or ficolins that recognize certain oligosaccharide moieties on microbial surfaces. The alternative pathway can be activated either by the presence of foreign surfaces such as LPSs or through C3b generated by spontaneous hydrolyses, the so-called “tick-over”. All three pathways merge at the level of C3, and activation of either pathway ultimately results in generation of the potent proinflammatory complement split products C3a and C5a as well as the terminal membrane attack complex (MAC).

Biological functions of complement system:

Complement serves as an important mediator of the humoral response by amplifying the response and converting it into an effective defense mechanism to destroy invading microorganisms. The MAC mediates cell lysis, while other complement components or split products participate in the inflammatory

response, opsonization of antigen, viral neutralization, and clearance of immune complexes.

The membrane-attack complex formed by complement activation can lyse gram-negative bacteria, parasites, viruses, erythrocytes, and nucleated cells. These pathways serve as an important innate immune defense against infectious microorganisms as the alternative and lectin pathways of activation generally occur without an initial antigen-antibody interaction. The requirement for an initial antigen-antibody reaction in the classical pathway supplements these nonspecific innate defenses with a more specific defense mechanism. The complement system is quite effective in lysing gram-negative bacteria but resistant in gram-positive bacteria because the thick peptidoglycan layer in their cell wall prevents insertion of the MAC into the inner membrane.

The cleavage products of complement components can mediate inflammation. The smaller fragments resulting from complement cleavage, C3a, C4a and C5a, called anaphylatoxins, bind to receptors on mast cells and blood basophils and induce degranulation, with release of histamine and other pharmacologically active mediators.

The complement system mediates viral neutralization by a number of mechanisms. The binding of antibody and/ or complement to the surface of a viral particle creates a thick protein coating which neutralizes viral infectivity by blocking attachment to susceptible host cells. The deposits of antibody and complement on viral particles also facilitate binding of viral particle to cells possessing Fc or type 1 complement receptor (CR1). Finally, complement is effective in lysing most, enveloped viruses resulting in fragmentation of the envelope and disintegration of the nucleocapsid.

Conclusion:

From the above study we learnt that complement system is an important immune system, which help us to degrade the antigen and protect our body from the harmful effects of antigens. They can eliminate foreign particles through various ways as we studied earlier. Their active participation in immune system is very important. Deficiency in any of the protein associated with the complement system can lead to various diseases such as asthma , glomerulonephritis, various forms of arthritis, autoimmune heart disease, multiple sclerosis, inflammatory bowel

disease, paroxysmal nocturnal hemoglobinuria, atypical hemolytic uremic syndrome and ischemia-reperfusion injuries, and rejection of transplanted organs.

The complement system is also becoming increasingly implicated in diseases of the central nervous system such as Alzheimer's disease and other neurodegenerative conditions such as spinal cord injuries.

Glossary:

1. **alternative pathway (of complement activation):** Activation pathway involving complement components C3, Factor B, Factor D, and Properdin which, in the presence of a stabilizing activator surface such as microbial polysaccharide, generates the alternative pathway C3 convertase C3bBb.
2. **anaphylatoxin**: A substance (e.g. C3a, C4a or C5a) capable of directly triggering mast cell degranulation.
3. **Complement:** A group of serum proteins, some of which act in an enzymatic cascade, producing effector molecules involved in inflammation (C3a, C5a), phagocytosis (C3b), and cell lysis (C5b-9).
4. **Classical pathway** (of complement activation): Activation pathway involving complement components Cl, C2 and C4 which, following fixation of Clq, e.g. by antigen-antibody complexes, produces the classical pathway C3 convertase C4b2a.
5. **Cytotoxic:** Kills cells.
6. **Innate immunity:** Immunity which is not intrinsically affected by prior contact with antigen, i.e. all aspects of immunity not directly mediated by lymphocytes.
7. **Lectins:** A family of proteins, mostly of plant origin, which bind specific sugars on glycoproteins and glycolipids. Some lectins are mitogenic (e.g. PHA, ConA).
8. **Macrophage:** Large phagocytic cell, derived from the blood monocyte, which also functions as an antigenpresenting cell and can mediate ADCC.
9. **Mannose binding protein: A** member of the collectin family of calcium-dependent lectins, and an acute phase protein. It functions as a stimulator of the classical pathway of complement activation, and as an opsonin for phagocytosis by binding to mannose, a sugar residue usually found in an exposed form only on the surface of microorganisms.

10. **Membrane attack complex** (MAC): Complex of complement components C5b-C9 which inserts as a pore into the membrane of target cells leading to cell lysis.

FAQ:

1. **What are the components of the membrane attack cpmplex in the complement pathway**

Ans: C5b, 6,7,8,9

2. **What is anaphylotoxin?**

Ans: Anaphylotoxin or complement peptides are fragments that are produced as part of the activation of the complement system. It causes the release of histamine and other mediators of immediate hypersensitivity from basophils and mast cells, thereby producing sign and symptoms of immediate hypersensitivity without involvement of IgE.

3. **How the lectin pathway is get activated?**

Ans: Lectin pathway is activated by the binding of mannose binding lectin(MBL) to mannose residues on glycoproteins or carbohydrates on the surface of microorganisms.

4. What is innate immunity?

Ans: Innate immunity refers to nonspecific defense mechanisms that come into play immediately or within hours of an antigen's appearance in the body.

Reference:

1. **Ivan M. Roitt & Peter J. Delves; Roitt's Essential Immunology; tenth edition**
2. Gabriel Virella; Medical Immunology; Fifth edition
3. Richard A. Goldsby, Thomas J. Kindt, Barbara A. Osborne, Janis Kuby; Immunology; Fifth edition

Links:

1. **http://www.complement-genetics.uni-mainz.de/**

2. http://www.cehs.siu.edu/fix/medmicro/cfix.htm
3. http://www.gla.ac.uk/Acad/Immunology/compsyst.htm