Notes

58

COMPLEMENT

58.1 INTRODUCTION

The complement (C) system is part of the immune system called the innate immune system. The term complement was used to refer to a heat-labile serum component that was able to lyse bacteria. Today, complement is known to contribute to host defences in other ways as well. Complement can opsonize bacteria for enhanced phagocytosis; activate various cells including polymorphonuclear cells (PMNs) and macrophages; it can participate in regulation of antibody responses and it can aid in the clearance of immune complexes and apoptotic cells. Complement can also have detrimental effects for the host, it contributes to inflammation and tissue damage and it can trigger anaphylaxis.

In the late 19th century, Hans Ernst August Buchner found that blood serum contained a "factor" capable of killing bacteria. In 1894, Richard Pfeiffer, a German scientist, had discovered that when cholera bacteria were injected into the peritoneum of a guinea pig immunized against the infection, the pig would rapidly die. This bacteriolysis, Bordet discovered, did not occur when the bacteria was injected into a non-immunized guinea pig, but did so when the same animal received the antiserum from an immunized animal. Moreover, the bacteriolysis did not take place when the bacteria and the antiserum were mixed in a test tube unless fresh antiserum was used. However, when Bordet heated the antiserum to 55 degrees centigrade, it lost its power to kill bacteria. Finding that he could restore the bacteriolytic power of the antiserum if he added a little fresh serum from a non immunized animal, Bordet concluded that the bacteria-killing phenomenon was due to the combined action of two distinct substances, an antibody in the antiserum, which specifically acted against a particular kind of bacterium, and a non-specific substance, sensitive to heat, found in all animal serums, which Bordet called "alexine" (later named "complement").

The term "complement" was introduced by Paul Ehrlich in the late 1890s, according to him, the immune system consists of cells that have specific receptors on their surface to recognize antigens. Upon immunisation with an antigen, more of these receptors are formed, and they are then shed from the cells to circulate in the blood. These receptors, which we now call "antibodies," were called by Ehrlich "amboceptors" to emphasise their bi-functional binding capacity: They recognise and bind to a specific antigen, but they also recognise and bind to the heat-labile antimicrobial component of fresh serum. Ehrlich, therefore, named this heat-labile component "complement," because it is something in the blood that "complements" the cells of the immune system.

OBJECTIVES

After reading this lesson, you will be able to:

- describe the complement system.
- describe various Components of complement system
- explain the causes Complement activation
- explain Various Pathways of complement system
- describe how is C activation regulated
- describe Quantification of complement activity
- explain the effects of Deficiencies of complement system.

58.2 GENERAL PROPERTIES OF COMPLEMENT

It forms the part of normal serum protein of all humans, animals, birds, amphibians and fishes alike. The complement is non specific meaning the complement from one species can react with the antibodies from another species. It is heat labile, thus serum can be deprived of its complement activity by heating it at 56 C for 30 minutes. It ordinarily does not bind to free antigen but only with antibodies which has combined with the antigen. Only IgM, IgG3, 1and 2 fix complement. The complement constituents are not influenced by immunisation.

58.3 COMPONENTS OF COMPLEMENT SYSTEM

The **complement system** comprises a large number of plasma proteins that mediate several functions of the inflammatory process. These circulate as **proenzymes,** i.e. in an inactive form. Complement activation arises through a cascade process, whereby activation of one proenzyme results in activation of

Notes

the next proenzyme in the pathway, and so on. The two most important mechanisms for activation of the early complement components are the classical pathway, initiated by antigen-antibody complexes, and the alternate pathway, initiated by microbial surface molecules. These pathways result in the generation of enzymes capable of splitting component C3. Once C3 is split into C3a and C3b, the subsequent pathway of activation of the terminal components is identical, regardless of the initiating stimulus. The end result is the generation of several active molecules which mediate distinct biological properties:

The serum proteins of complement system are produced by a variety of cells including, hepatocytes, macrophages and gut epithelial cells. Some complement proteins bind to immunoglobulins or to membrane components of cells. Others are proenzymes that, when activated, cleave one or more other complement proteins. Upon cleavage some of the complement proteins yield fragments that activate cells, increase vascular permeability or opsonize bacteria.

Table 58.1: Proteins of the Complement system

Classical Pathway	Lectin Pathway	Alternative Pathway
Activation Proteins: C1qrs, C2, C3, C4 Control Proteins:C1-INH, C4-BP	Mannan binding protein (MBP), mannan-decay asociated serine protease (MASP,MASP2)	C3, Factors B & D*, Properdin (P) Factors I* & H, accelerating factor (DAF), Complement receptor 1 (CR1),*etc*.

Components underlined acquire enzymatic activity when activated.

Components marked with an asterisk have enzymatic activity in their native form.

58.4 PATHWAYS OF COMPLEMENT ACTIVATION

Complement activation can be divided into four pathways, the classical pathway, the lectin pathway, and the alternative pathways. Both classical and alternative pathways lead to the activation of C5 convertase and result in the production of C5b which is essential for the activation of the membrane attack pathway.

58.4.1 Classical Pathway

The chain of events in which C components react in a specific sequence following activation of C1 and typically culminate in immune cytolysis is known as the classical pathway. The steps involved in classical pathway are as follows

C1 activation

C1, a multi-subunit protein containing three different proteins (C1q, C1r and C1s), binds to the Fc region of IgG and IgM antibody molecules that have interacted with antigen. C1 binding does not occur to antibodies that have not complexed with antigen and binding requires calcium and magnesium ions. (*N.B.* In some cases C1 can bind to aggregated immunoglobulin [e.g. aggregated IgG] or to certain pathogen surfaces in the absence of antibody). The binding of C1 to antibody is via C1q and C1q must cross link at least two antibody molecules before it is firmly fixed. The binding of C1q results in the activation of C1r which in turn activates C1s. The result is the formation of an activated "C1qrs", which is an enzyme that cleaves C4 into two fragments C4a and C4b.

C4 and C2 activation (generation of C3 convertase)

The C4b fragment binds to the membrane and the C4a fragment is released into the microenvironment. Activated "C1qrs" also cleaves C2 into C2a and C2b. C2a binds to the membrane in association with C4b, and C2b is released into the microenvironment. The resulting C4bC2a complex is a C3 convertase, which cleaves C3 into C3a and C3b.

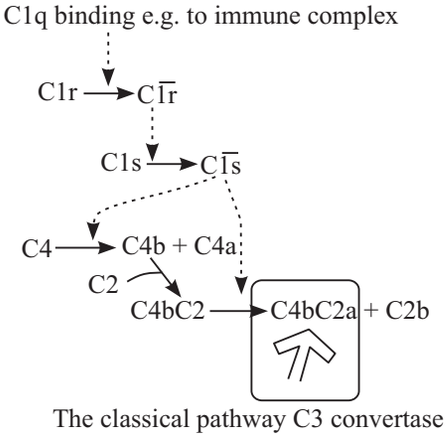

Fig. 58.1: Activation of the Classical Pathway

C3 activation (generation of C5 convertase)

C3b binds to the membrane in association with C4b and C2a, and C3a is released into the microenvironment. The resulting C4bC2aC3b is a C5 convertase. The generation of C5 convertase is the end of the classical pathway.

Several of the products of the classical pathway have potent biological activities that contribute to host defenses. Some of these products may also have detrimental effects if produced in an unregulated manner. Table summarizes the biological activities of classical pathway components.

Table 58.2: Biological Activity of classical pathway products

Component	Biological Activity
C2b	**Prokinin**; cleaved by plasmin to yield kinin, which results in edema
C3a	**Anaphylotoxin**; can activate basophils and mast cells to degranulate resulting in increased vascular permeability and contraction of smooth muscle cells, which may lead to**anaphylaxis**
C3b	**Opsonin**; promotes phagocytosis by binding to complement receptorsActivation of phagocytic cells
C4a	**Anaphylotoxin** (weaker than C3a)
C4b	**Opsonin**; promotes phagocytosis by binding to complement receptors

If the classical pathway were not regulated there would be continued production of C2b, C3a, and C4a. Thus, there must be some way to regulate the activity of the classical pathway. Table 58.3 summarizes the ways in which the classical pathway is regulated.

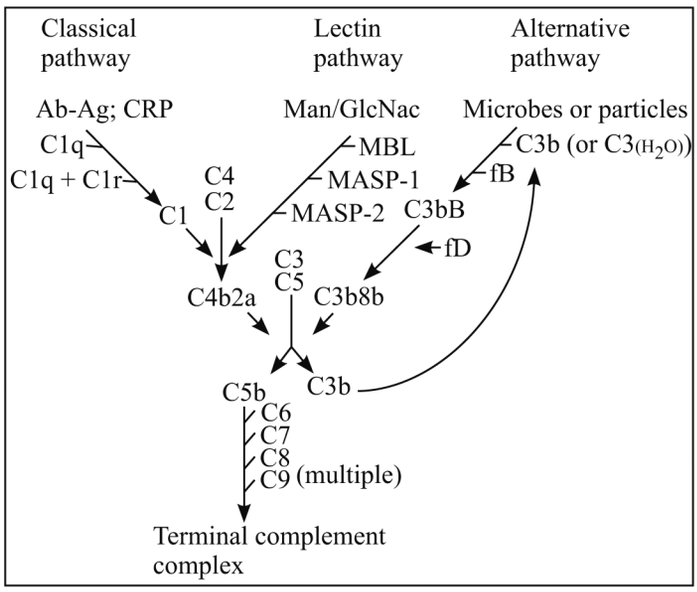

Fig. 58.2

Table 58.2: Regulation of the Classical Pathway

Component	Regulation
All	**C1-INH**; dissociates C1r and C1s from C1q
C3a	**C3a inactivator (C3a-INA;Carboxypeptidase B)**; inactivates C3a
C3b	**Factors H and I;** Factor H facilitates the degradation of C3b by Factor I

C4a	**C3-INA**
C4b	**C4 binding protein(C4-BP) and Factor I**; C4-BP facilitates degradation of C4b by Factor I; C4-BP also prevents association of C2a with C4b thus blocking the formation of C3 convertase

The importance of C1-INH in regulating the classical pathway is demonstrated by the result of a deficiency in this inhibitor. C1-INH deficiencies are associated with the development of hereditary angioedema.

58.4.2 Lectin Pathway

The lectinpathway is very similar to the classical pathway. It is initiated by the binding of mannose-binding lectin (MBL) to bacterial surfaces with mannose-containing polysaccharides (mannans). Binding of MBL to a pathogen results in the association of two serine proteases, MASP-1 and MASP-2 (MBL-associated serine proteases). MASP-1 and MASP-2 are similar to C1r and C1s, respectively and MBL is similar to C1q. Formation of the MBL/MASP-1/ MASP-2 tri-molecular complex results in the activation of the MASPs and subsequent cleavage of C4 into C4a and C4b. The C4b fragment binds to the membrane and the C4a fragment is released into the microenvironment. Activated MASPs also cleave C2 into C2a and C2b. C2a binds to the membrane in association with C4b and C2b is released into the microenvironment. The resulting C4bC2a complex is a C3 convertase, which cleaves C3 into C3a and C3b. C3b binds to the membrane in association with C4b and C2a and C3a is released into the microenvironment. The resulting C4bC2aC3b is a C5 convertase. The generation of C5 convertase is the end of the lectin pathway.

The biological activities and the regulatory proteins of the lectin pathway are the same as those of the classical pathway.

58.4.3 Alternative Pathway

The alternative pathway begins with the activation of C3 and requires Factors B and D and Mg^{++} cation, all present in normal serum.

1. Amplification loop of C3b formation

In serum there is low level spontaneous hydrolysis of C3 to produce C3i. Factor B binds to C3i and becomes susceptible to Factor D, which cleaves Factor B into Bb. The C3iBb complex acts as a C3 convertase and cleaves C3 into C3a and C3b. Once C3b is formed, Factor B will bind to it and becomes susceptible to cleavage by Factor D. The resulting C3bBb complex is a C3 convertase that will continue to generate more C3b, thus amplifying C3b production. If this process continues unchecked, the result would be the consumption of all C3 in the serum. Thus, the spontaneous production of C3b is tightly controlled.

2. Control of the amplification loop

As spontaneously produced C3b binds to autologous host membranes, it interacts with DAF (decay accelerating factor), which blocks the association of Factor B with C3b thereby preventing the formation of additional C3 convertase. In addition, DAF accelerates the dissociation of Bb from C3b in C3 convertase that has already formed, thereby stopping the production of additional C3b. Some cells possess complement receptor 1 (CR1). Binding of C3b to CR1 facilitates the enzymatic degradation of C3b by Factor I. In addition, binding of C3 convertase (C3bBb) to CR1 also dissociates Bb from the complex. Thus, in cells possessing complement receptors, CR1 also plays a role in controlling the amplification loop. Finally, Factor H can bind to C3b bound to a cell or in the in the fluid phase and facilitate the enzymatic degradation of C3b by Factor I. Thus, the amplification loop is controlled by either blocking the formation of C3 convertase, dissociating C3 convertase, or by enzymatically digesting C3b. The importance of controlling this amplification loop is illustrated in patients with genetic deficiencies of Factor H or I. These patients have a C3 deficiency and increased susceptibility to certain infections.

3. Stabilization of C convertase by activator (protector) surfaces

When bound to an appropriate activator of the alternative pathway, C3b will bind Factor B, which is enzymatically cleaved by Factor D to produce C3 convertase (C3bBb). However, C3b is resistant to degradation by Factor I and the C3 convertase is not rapidly degraded, since it is stabilized by the activator surface. The complex is further stabilized by properdin binding to C3bBb. Activators of the alternate pathway are components on the surface of pathogens and include: LPS of Gram-negative bacteria and the cell walls of some bacteria and yeasts. Thus, when C3b binds to an activator surface, the C3 convertase formed will be stable and continue to generate additional C3a and C3b by cleavage of C3.

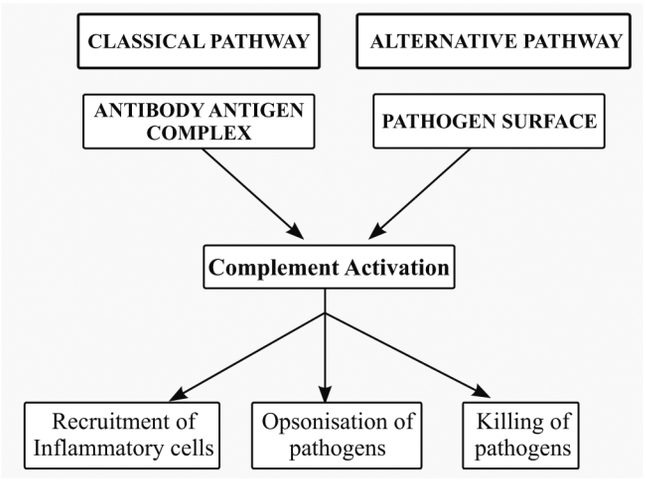

Fig. 58.3

4. Generation of C5 convertase

Some of the C3b generated by the stabilized C3 convertase on the activator surface associates with the C3bBb complex to form a C3bBbC3b complex. This is the C5 convertase of the alternative pathway. The generation of C5 convertase is the end of the alternative pathway. The alternative pathway can be activated by many Gram-negative (most significantly, *Neisseria meningitidis* and *N. gonorrhoea*), some Gram-positive bacteria and certain viruses and parasites, and results in the lysis of these organisms. Thus, the alternative pathway of C activation provides another means of protection against certain pathogens before an antibody response is mounted. A deficiency of C3 results in an increased susceptibility to these organisms. The alternate pathway may be the more primitive pathway and the classical and lectin pathways probably developed from it.

Remember that the alternative pathway provides a means of non-specific resistance against infection without the participation of antibodies and hence provides a first line of defense against a number of infectious agents.

Many gram negative and some gram positive bacteria, certain viruses, parasites, heterologous red cells, aggregated immunoglobulins (particularly, IgA) and some other proteins (e.g. proteases, clotting pathway products) can activate the alternative pathway. One protein, cobra venom factor (CVF), has been extensively studied for its ability to activate this pathway.

58.5 BIOLOGICALLY ACTIVE PRODUCTS OF COMPLEMENT ACTIVATION

Activation of complement results in the production of several biologically active molecules which contribute to resistance,anaphylaxis and inflammation.

Kinin production

C2b generated during the classical pathway of C activation is a prokinin which becomes biologically active following enzymatic alteration by plasmin. Excess C2b production is prevented by limiting C2 activation by C1 inhibitor (C1-INH) also known as serpin which displaces C1rs from the C1qrs complex (Figure 10). A genetic deficiency of C1-INH results in an overproduction of C2b and is the cause of hereditary angioneuroticedema. This condition can be treated with Danazol which promotes C1-INH production or with å-amino caproic acid which decreases plasmin activity.

Anaphylotoxins

C4a, C3a and C5a (in increasing order of activity) are all anaphylotoxins which cause basophil/mast cell degranulation and smooth muscle contraction.

Notes

Undesirable effects of these peptides are controlled by carboxypeptidase B (C3a-INA).

Chemotactic Factors

C5a and MAC (C5b67) are both chemotactic. C5a is also a potent activator of neutrophils, basophils and macrophages and causes induction of adhesion molecules on vascular endothelial cells.

Opsonins

C3b and C4b in the surface of microorganisms attach to C-receptor (CR1) on phagocytic cells and promote phagocytosis.

Other Biologically active products of C activation

Degradation products of C3 (iC3b, C3d and C3e) also bind to different cells by distinct receptors and modulate their functions.

Regulation of C activation

Left unchecked the complement activity can cause not only exhaustion of complement system but also serious damage to tissues. Several inbuilt control mechanisms regulate the complement cascade at different levels.

Table 58.4: Activities of Complement Activation Products and their Control Factors

Fragment	Activity	Effect	Control Factor (s)
C2a	Prokinin, accumulation of fluids	Edema	C1-INH
C3a	Basophil and mast cells degranulation; enhanced vascular permeability, smooth muscle contraction	Anaphylaxis	C3a-INA
C3b	Opsonin, phagocyte activation	Phagocytosis	Factors H and I
C4a	Basophil and mast cells degranulation; enhanced vascular permeability, smooth muscle contraction	Anaphylaxis (least potent)	C3a-INA
C4b	Opsonin	Phagocytosis	C4-BP and Factor I

C5a	Basophil and mast cells degranulation; enhanced vascular permeability, smooth muscle contraction	Anaphylaxis (most potent)	C3a-INA
	Chemotaxis, stimulation of respiratory burst, activation of phagocytes, stimulation of inflammatory cytokines	Inflammation	
C5bC6C7	Chemotaxis	Inflammation	Protein S (vitronectin)
	Attaches to other membranes	Tissue damage	

In summary, the complement system takes part in both specific and non-specific resistance and generates a number of products of biological and pathophysiological significance

Quantitation of Complement and its components

Complement activity of serum is measured by estimating the highest dilution of serum lysing sheep erythrocytes sensitised by antierythrocytic antibody. C components can also be quantified by radial immunodiffusion in agar.

Deficiencies of complement system

There are known genetic deficiencies of most individual C complement components, but C3 deficiency is most serious and fatal. Complement deficiencies also occur in immune complex diseases (e.g., SLE) and acute and chronic bacterial, viral and parasitic infections.

Table 58.5: Complement deficiencies and disease

Pathway/Component	Disease	Mechanism
Classical Pathway		
C1INH	Hereditary angioedema	Overproduction of C2b (prokinin)
C1, C2, C4	Predisposition to SLE	Opsonization of immune complexes help keep them soluble, deficiency results in increased precipitation in tissues and inflammation

Notes

Lectin Pathway		
MBL	Susceptibility to bacterial infections in infants or immunosuppressed	Inability to initiate the lectin pathway
Alternative Pathway		
Factors B or D	Susceptibility to pyogenic (pus-forming) bacterial infections	Lack of sufficient opsonization of bacteria
C3	Susceptibility to bacterial infections	Lack of opsonization and inability to utilize the membrane attack pathway
C5, C6, C7 C8, and C9	Susceptibility to Gram-negative infections	Inability to attack the outer membrane of Gram-negative bacteria
Properdin (X-linked)	Susceptibility meningococcal meningitis	Lack of opsonization of bacteria
Factors H or I	C3 deficiency and susceptibility to bacterial infections	Uncontrolled activation of C3 via alternative pathway resulting in depletion of C3

INTEXT QUESTIONS 58.1

1. Heat labile serum component that lyse bacteria is .....
2. The inactive form of complement are .....
3. The classical complement pathway is initiated by ..... complexes
4. Alternate pathway is initiated by ..... molecules
5. Major biologically active products of complement activation are ....., ..., ... and .....
6. Overproduction of Prokinin causes .....
7. Deficiency of Opsonin predisposes to .....

WHAT YOU HAVE LEARNT

The complement system is a set of serum proteins that play a major role in the immune response

- Some lyse foreign cells
- Some mediated inflammation and attract and activate phagocytic cells

- Some amplify the effects of antibodies Complement acts in a cascade fashion; the complement proteins are inactive, and the activation of one leads to the sequential activation of others There are three pathways of complement activation
- Classical pathway-results form antigen-antibody interactions that occur during specific immune responses (discussed in chapter 32)
- Alternative complement pathway-occurs in response to intravascular invasion by bacteria and some fungi; involves interaction of complement with the surface of the pathogen
- Lectin complement pathway-occurs when macrophages release mannose-binding protein (a lectin), which then can activate complement via the alternative pathway or the classical pathway

Overview of complement activation and immune responses

- Gram-negative bacteria at local tissue site interact with components of alternative pathway
- If bacteria persist or invade a second time, antibody responses activate the classical pathway
- Generation of C3a and C5a complement fragments leads to:
- Activation of mast cells, which release their contents, causing hyperemia
- Release of neutrophils from bone marrow into circulation, and their chemotaxis to injury site
- Ultimately neutrophils and phagocytes ingest and destroy the bacteria

TERMINAL QUESTIONS

1. Who gave the term “complement”?
2. What do you understand by the term “cascade process” ?
3. What is the classical pathway of complement system?
4. Enlist the regulatory components of classical pathway ?
5. How is the quantitation of complement done?
6. What is the role of complement system in immunity?
7. What are various pathways by which the complement system functions?
8. Draw a flow chart explaining the classical pathway of the complement system?
9. How can we measure the complement?

Notes

10. Give differences between various pathways of the complement systems?
11. What are the effects of deficiency of complement system?

ANSWERS TO INTEXT QUESTIONS 58.1

1. Complement
2. Proenzyme
3. Antigen-antibody
4. Microbial surface
5. Kinin, Anaphlotoxins, Chemotactic factor, Opsonins
6. Angioedema
7. SLE