

IMMUNOLOGY

Components of the Complement System and Pathways of Complement Activation

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CONTENTS

[What is the complement system? History and Nomenclature](#)

[Overview of the function of complements](#)

[The Lectin pathway](#)

[The Classical pathway](#)

[Binding of immune complex to C1](#)

[Assembly of C3 convertase](#)

[Activation of C3 convertase via the classical pathway](#)

[Activation of C5 convertase via the classical pathway](#)

[Regulation of the Classical Pathway](#)

[The Alternate pathway](#)

[Formation of the initial fluid phase C3 convertase](#)

[Formation of the cell bound C3/C5 convertase](#)

[Amplification loop of the alternative pathway](#)

[Regulation of the alternative pathway](#)

[Formation of the MAC complex](#)

[Regulation of MAC activation](#)

[The Complement receptors](#)

[Biological effects of complements](#)

[Complement deficiencies and their pathogenesis](#)

[Types of complement disease and their genetic basis](#)

[Complement molecules and pathophysiological condition of the diseases](#)

[Complement activity in hypersensitivity and inflammation](#)

[Deficiencies of complement components](#)

[Clinical presentation of diseases caused by complement deficiencies](#)

Keywords

Complement, Classical pathway, lectin, alternate pathway, inflammation, opsonization, C3b, Factor I, MAC, C3 convertase, C5 convertase, autoimmune diseases

What is the Complement System?

The complement system plays a major role in the host defense system to combat microorganisms and other infectious agents, including the inflammatory response. The complement system comprises of a number of plasma proteins which function together to initiate a cascade which helps the host immune response. The complement system also includes those specific receptors for these complement plasma protein which are present on cells of the immune system and the inflammatory cells. The complement system is activated by the classical pathway which is antibody dependent or the alternative pathway which is antibody independent.

Historical significance

In the late 19th century, Nuttall and Bordet described a 'heat-labile' property of serum in which lysis of bacteria occurred when the serum was combined with a specific antibody. Ferrata made an important contribution to complement studies when in 1907, he reported that complements could be separated into two components by dialyzing with acidified water. This resulted in a 'euglobulin' precipitate fraction and a water-soluble albumin fraction. He observed that complement activity of lysing bacteria could only be possible when both these fragments were present and he subsequently named the fractions, C1 and C2. Sachs and Omorokow further showed that cobra venom inactivated another component and they named it, C3. When Gordon observed that ammonia could inactivate yet another component, it was promptly named, C4. since the nomenclature given by these scientist is only based on order of discovery and not on the order of reaction. During the 1930s it was shown that trypanosomes treated with antibody and complement bound to erythrocyte from primates but this phenomenon which forms the basis of the complement system, mediation of adherence of microorganism to leucocytes, was largely ignored till after the 1950s. Advancement in the techniques used for protein purification and analyses, led to the partial fractionation of the C1 protein by Pillemar, Ecker and co-workers in 1941. During the 1950s, the C1 was shown to have esterase activity by Becker as well as Pillemar and Lepow. The one-hit theory which suggests that a single complement lesion could cause lysis of an erythrocyte was given by Mayer and colleagues after a detailed analysis of the erythrocytic hemolysis. Pillemar and his colleagues were also the first to suggest that an antibody independent pathway existed on identifying properdin in yeast and certain bacteria. This basis of the alternative pathway was largely rejected by other scientists at the time. In the 1960s, Muller-Eberhard purified a major plasma protein B1c and also determined that it corresponded to C3. Subsequently the two groups of Linscott and Nishioka, and Nelson and his colleagues were able to purify nine active components of the complement pathway.

Nomenclature of the components

Complement is not a single substance but consisted of multiple proteins. Separated serum components, water-soluble pseudoglobulin and insoluble euglobulin, had no bactericidal activity. But when mixed together, complement activity was restored. The sequential actions of the serum fractions demonstrated that the lytic activity requires at least two factors one present in insoluble fraction which was termed as midpiece, which reacted with antibody and the other present in soluble fraction named endpiece, which reacted after midpiece to complete the lytic reaction. These serum factors subsequently renamed as C'1 and C'2 respectively. Application of the fractionation techniques available at that time identified additional factors named in order of their discovery C'3 and C'4. Although the four factors had already been demonstrated to be

required for the complement activation, the molecular nature of these factors were completely unknown. Complement was still thought of as a serum lipid or soap complex with the ability to dissolve membranes, or as a physicochemical state or colloidal attribute of fresh serum. The evidence that the complements are proteins was established later when electrophoretic and ultracentrifugation techniques were applied in their purification. These techniques revealed that C'3 is composed of six (C'3a-C'3f) different proteins. Using partly purified components, initially by Ueno and then by Mayer showed the reaction sequence of the complement components: C'1 is bound first followed sequentially by C'4, C'2, C'3a, C'3b, C'3e, C'3f, C'3c and C'3d. In 1968, the WHO Committee modified these nomenclature and the new terminology being, in order of activation C1, C4, C2, C3, C5, C6, C7, C8, and C9.

The complement proteins of the classical pathway and membrane attack complex are denoted by C1q, C1r, C1s, C4, C2, C3, C5, C6, C7, C8 and C9. This is also the order in which they react. Most of the proteins of the complement system exist as zymogens or pro-enzymes and become active on proteolytic cleavage. The active form is represented by a bar on top of the protein in question for example C1 \bar{r} . The cleavage products from of complement proteins are denoted by a suffix letter a, b etc to distinguish them from the parent protein. The 'a' is normally used for the smaller cleaved product of the parent molecule, whereas, the 'b' represents the bigger product. The exception to this nomenclature is the cleaved products of the C2 protein. In this case, the C2b represents the smaller fragment and the C2a is the larger fragment.

The proteins of the alternative pathway are represented by a prefix of 'F' for factor. However, in many cases the prefix is simply omitted and the protein may simply be referred to as 'B' instead of 'Factor B'.

Complement receptors are named according to either the ligand they represent for example C3a receptor or may also be denoted using the Cluster of differentiation (CD system. The receptor C3 is also classified according to their fragments and is known as CR1-CR4. Due to this variable nomenclature a protein like C3 has three names, C3b receptor, CR1 and CD35. This can often be a source of confusion.

Overview of the Function of Complements

The major function of the complement system is in host defence against infectious agents and in the inflammatory process. The complement proteins are a battery of about twenty plasma proteins that function either as enzymes or as binding proteins. Many distinct cell-surface receptors are also a part of the complement system that exhibit specificity for the physiological fragments of complement proteins and that occur on inflammatory cells and cells of the immune system. The pathways of the complement are regulated by various regulatory membrane proteins which prevent autologous complement activation and protect host cells from accidental complement attack (Fig 1).

Individuals with genetic deficiencies pertaining to deficiencies of one or more complement components show an increased susceptibility to recurrent infections by abscess-forming bacteria and production of autoimmune complexes.

There are three major biological activities of complement system:

- 1) Opsonization : the target molecule gets coated with the complement proteins and the phagocytes carrying complement receptors bind to these opsonized target cells and endocytose the opsonized particle.
- 2) activation of leucocytes and
- 3) lysis of target cells

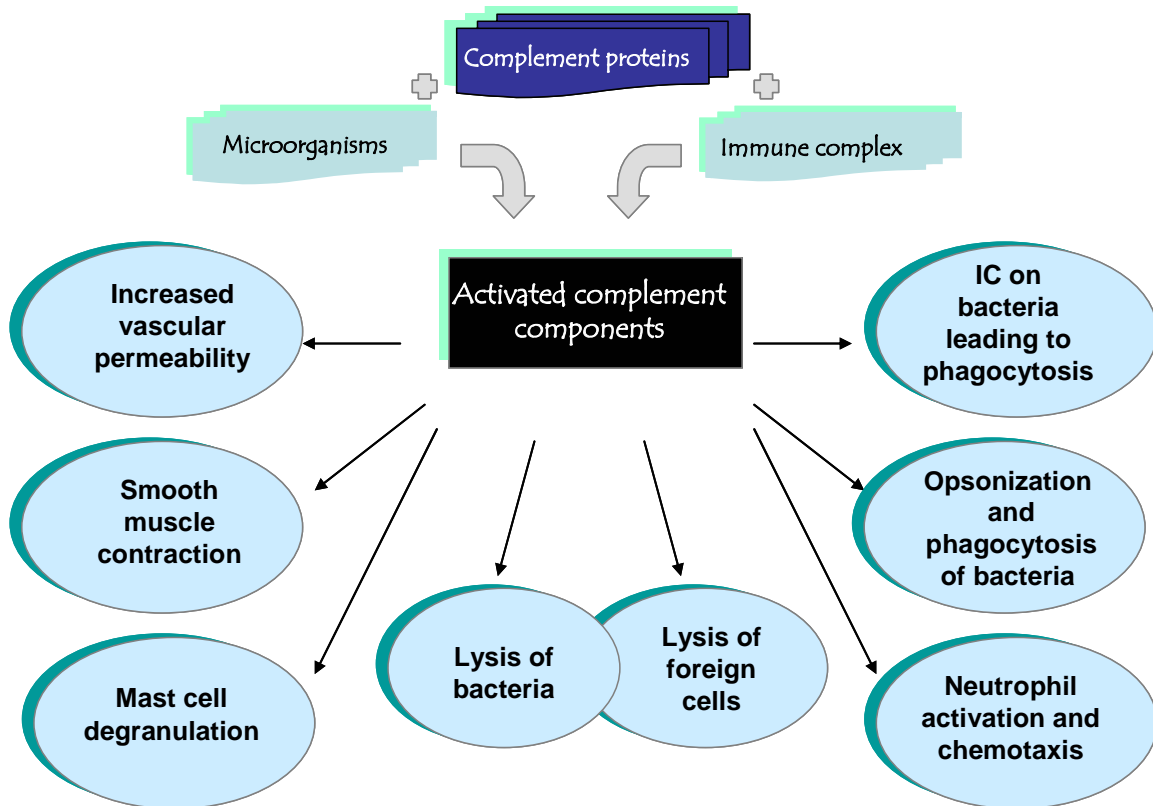


Fig 1: Functions of the complement proteins

How is the Complement Pathway Activated?

The complement can be activated by three pathways (Fig 2). The classical pathway is antibody dependent and the binding of antibody molecules (specifically IgM and IgG1, 2 and 3) to the foreign particle triggers the sequence of events which results in formation of the C3/C5 convertase.

The proteins of the alternative pathway, on the other hand, directly recognize the micro-organism and initiate the cascade of events which culminate towards formation of the c3/c5 convertase. The alternative pathway appears to be the major defense of the host to invading micro-organisms and bacteria. The alternative pathway constitutes a humoral component of natural defence against infections, which can operate without antibodies. The six proteins C3, B, D, H, I, and P together perform the functions of initiation, recognition and activation of this pathway which results in the formation of activator-bound C3/C5 convertase.

An overview of the three pathways activating the complement system

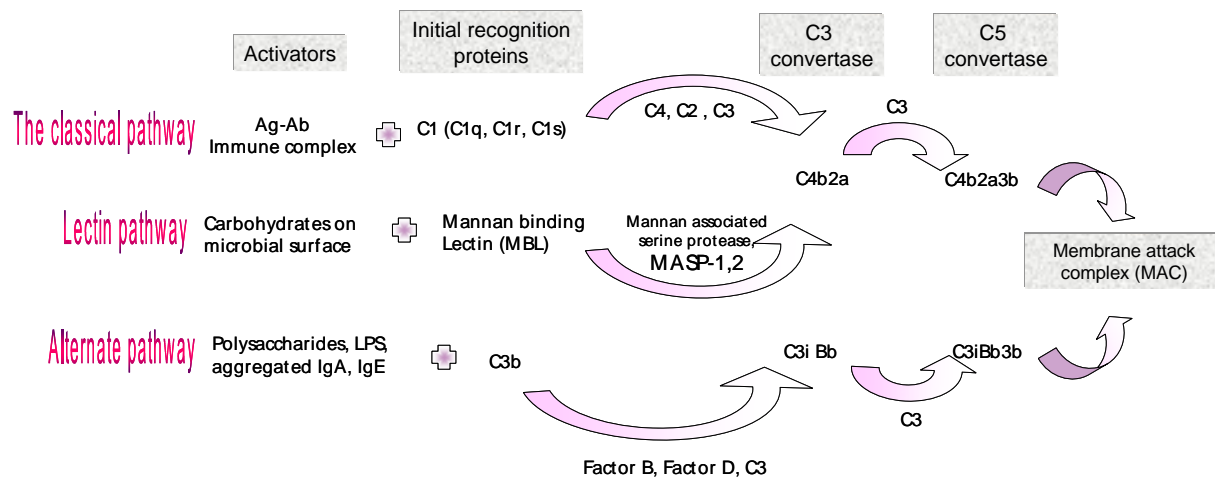


Fig 2: An overview of the three pathways activating the complement system

The Lectin Pathway

The lectin pathway is closely homologous with the classical pathway but is activated in an antibody-independent fashion. Instead of C1 complex, lectin pathway utilises MBL (mannan-binding lectin found in serum) which can interact with two serine proteinases MASP and MASP2 (mannan-binding lectin associated serine proteinases). The interaction of MBL, MASP, and MASP2 is analogous to the interaction of C1q, C1r, and C1s. Remaining steps of complement cascade in classical and lectin pathways are same.

The formation of the C3 convertase which in turn leads to the formation of the C5 convertase and activation of the C5 protein, finally leads to the activation of the membrane attack complex (MAC) which consists of a number of complement proteins. Once the MAC is activated by either of the two pathways, the final events of C9 oligomerisation and polymerization lead to the membrane lysis and opsonisation of the cell. The complement mediated lysis occurs in a number of cells which are recognized as foreign by the host body, such as, erythrocytes, platelets, bacteria, virus with a lipoprotein envelope and lymphocytes. In this chapter, all these mechanisms will be given in detail.

The Classical Pathway

The classical pathway of complement activation mediates the specific antibody response and this antibody dependent pathway is initiated with the binding of antibody molecules to the antigen. It can be said that the classical pathway follows a link to the adaptive immunity as its initiation occurs only on the binding of antibody to the antigen. The recognition and binding of C1 to the antibody complexed with the antigen initiate the cascade of events.

Binding of immune complex to C1

C1 is composed of C1q (410 kD) and doublets of C1r (85 kD) and C1s (85 kD) linked by Ca^{++} ions. This association of C1q and C1r2s2 is reversible and almost 70% of C1 exists in this form (Fig. 3).

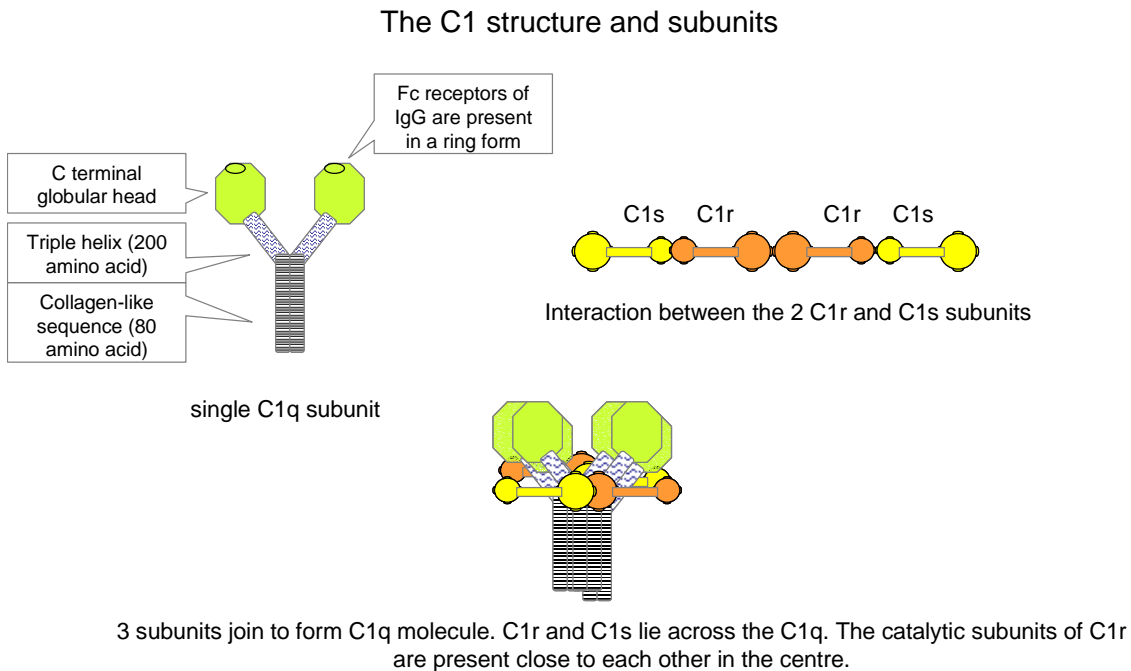


Fig. 3: The C1 structure and subunits

The C1 proenzyme present in the serum also has a tendency to undergo auto-activation and is tightly regulated by C1 inhibitor (C1-In). C1 (900 kD) binds to the Fc portion of either a single antibody molecule of IgM or to a pair of antibody molecules IgG1, IgG2, or IgG3, in apposition on the surface of the antigen. The subunit C1q effectively has the ability to interact with antibodies. When this binding occurs, the inhibitory action of C1-In is overcome and the conformational change occurs in C1r, followed by the proteolytic cleavage and activation of the four polypeptide chains of C1r2s2. The C1q on its own does not have an intrinsic catalytic activity but it effectively leads to the activation of C1r2s2, C1r and C1s are converted to catalytically active species, namely, C1r* and C1s*. This first step of the classical pathway, thus, occurs on the binding of immune complexes to C1q and activation of the associated C1r and C1s subunits into catalytically active species even in the presence of C1-In. The activation of the two C1s subunits finally lead to the next step of the classical pathway i.e the assembly of the C3 convertase or C4b2a, formed by the association of C4 and C2.

Assembly of the C3 convertase

The C3 convertase is composed to two subunits, the C4 and C2. Both are encoded within the MHC complex.

C4, a 210kDa fragment consists of three polypeptide chain, namely the 93kDa Alpha, a 75kDa Beta and the 33kDa Gamma. C4 is also known to exist in two isoforms, C4A and C4B. These two isoforms differ in their antigenicity, function and reactivity. Activated C4A preferentially binds to amine groups on proteins, whereas, C4B binds to hydroxyl groups on carbohydrates.

C4 is a substrate of the C1s* and undergoes cleavage with the loss of the small C4a (6kDa) fragment. The large fragment, C4b, is generated with a labile binding site for antigens and can directly attach to the cell membrane. If the association does not take place, the binding sites are not generated and C4 becomes C4bi as a result of a reaction of a thioester site with H₂O, which is incapable of taking the complement cascade forward. This inactivation occurs in almost 95% of C4. Active C4 forms a complex with C1, C14b. This complex binds to C2 (110kD), and on binding C2 can be cleaved by the C1 complex or other proteolytic enzymes, to release a small 35kDa fragment, C2b into solution and a large 75kDa fragment which dutifully attaches to the complex to form the C14b2a complex. So, a hemolytically active C4b provides the binding site for C2 as C2 is also a substrate for C1q although its affinity is less than that of C4. Upon cleavage by C1s*, the C2a fragment becomes firmly associated with C4b, a reaction dependent on the presence of magnesium ions, and the C3 convertase, C4b2a, is generated. The formed C4b2a complex is now able to cleave the next component of the cascade, C3. In contrast to the C4bC2 complex, the newly formed C4b2a complex is no longer dependent on magnesium ions. The enzymatic site of the C3 convertase is located in the C2a molecule and has substrate specificity for C3.

Activation of the C3 via the classical pathway

C3 is an extremely bulky protein. Native C3 molecule contains an internal thioester bond within which is metastable and contains an electrophilic carbonyl group susceptible to nucleophilic groups like hydroxyl and amine groups of neighbouring proteins and carbohydrates. There is always a steady low level of C3 activation due to the reaction of this thioester bond with water molecules.

The activated C3 results in non-specific binding and deposition. The C3 convertase breaks the C3 protein into C3a, a smaller fragment which is known to be involved in a number of inflammatory responses and C3b, the larger fragment which can covalently attach to cell surface via a short lived binding thioester site. Cleavage of the C3a peptide by C3 convertase makes the internal thioester bond on C3b extremely unstable and the thioester bond can either be hydrolysed with H₂O (as occurring in 95% of C3b molecules) and result in an inactive soluble form or it can bind to the surface of the cell or bacteria covalently by an ester or amide group. C3b is hence known to be important in the process of opsonization. The binding of C3b is the key step in distinction of self from non-self. Bound C3b functions as an opsonin. After the binding of the C3b component to the C4b component, the C3 convertase, C4b2a, becomes the C5 convertase, C4b2a3b.

Activation of C5 convertase by the classical pathway

The target protein of the C5 convertase, C5, is activated by the C3b component of the C4b2a3b convertase. C5 binding sites are present C3b4b dimers and the C5 binds to these dimers before being cleaved by the C5 convertase. C5 binding to C3b is enhanced when C3b is part of the C5 convertase complex and bound to C4b. The C5 is thus cleaved into two fragments C5a, a small

fragment which does not associate with cell surface and C5b, the larger fragment which is critical in initiating the lytic sequence of reaction. It is known to be important in the association of late-acting components of the complement system which produce a lesion on the bacterial surface leading to its death. C5a, on the other hand, although not involved in directing the complement mechanism, has been found to be an inflammatory mediator and is known to act on target cells through G_i proteins. C5 is further a part of the Membrane attack complex or MAC which is common to both the classical as well as the alternate pathway and is discussed later.

Regulation of the Classical Pathway

Classical pathway is regulated both by proteins in the fluid phase as well as the membrane regulatory proteins. In the fluid phase, regulation is by two mechanisms. The first one is by a serine proteinase inhibitor (serpin), C1 inhibitor, which binds and inactivates C1r and C1s. The second mechanism is blocking the formation of C3 convertase itself. C3 convertase or C4b2a is formed at low levels in the fluid phase due to the presence of plasma proteins which catabolize C4b, e.g. Factor I (a glycosylated, disulphide-linked, two-chain serine protease of high substrate specificity found in serum and plasma as an active enzyme) and C4 binding protein (C4b). Factor I is a C3b inactivator and cleaves C3b into C3c and α-2D resulting in the inactivation of C3b (Fig. 4).

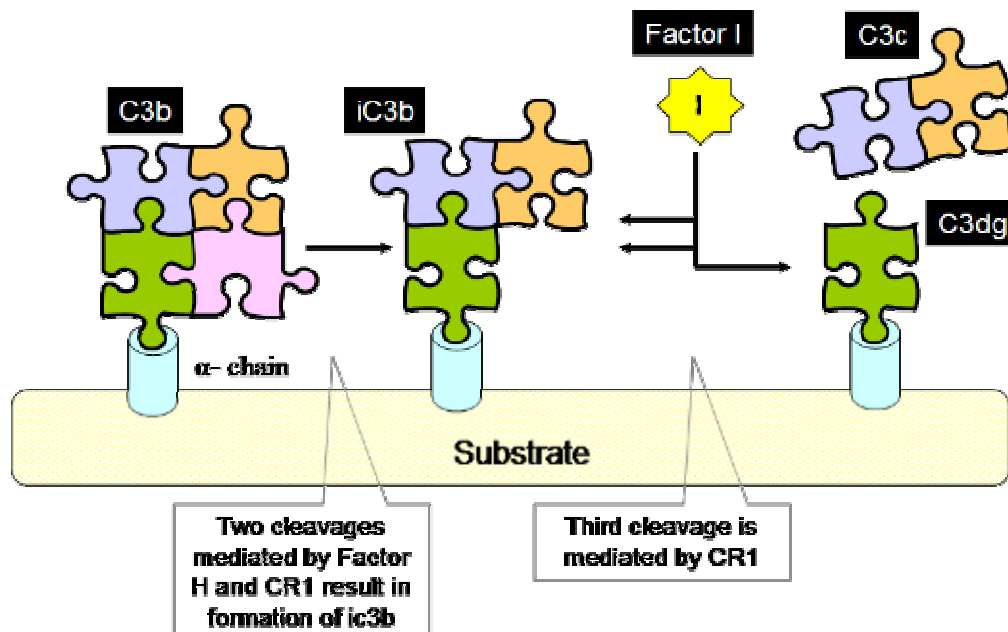


Fig 4: The C3b Regulation

The normal concentration of the C4bp in serum is greater than the single-site dissociation constant of the C4bp-C4b interaction. Thus, under most conditions the concentration of the C4bp greatly exceeds the small amounts of C4b generated by the activation of the classical pathway and C4b is therefore inactivated. C4bp is also capable of causing disassociation of C2a from the C3 convertase. Regulation at the membrane level takes place with the help of a number of cell

surface bound proteins or complement control proteins (CCP) which are members of the Regulators of Gene Clusters (RCA) and these are responsible for tight regulation of the complement pathway. These include the decay accelerating factor (DAF), CR1 (C3b receptor) and membrane cofactor protein (MCP). The function of CR1 is similar to the C4bp and prevents binding of C2 to C4b and also promotes disassociation of C2a from C4b. DAF shows activity towards C3b and C4b2a by both preventing the assembly of the complexes as well as promoting the disassociation of the same. DAF shows affinity to C3b and can be inhibited by C3b located on the same cell. MCP is a surface membrane C3 binding protein and acts as a co-factor for C3/C4 cleavage into their inactive forms, C3bi and C4bi. However, these membrane proteins are not found on the erythrocytes. MCP and CR1 both promote catabolism of C4b by Factor I. A C3 membrane proteinase or p57 has also been identified on complement resistant melanoma cell-lines with the ability to cleave and inactivate C3b. Complement regulation is also effected with the help of matrix proteins like Decorin which bind to C1q with high affinity and modulate its activity.

The Alternative pathway

The alternative pathway plays a major role in bacterial infection because, unlike the classical pathway, it is activated by invading micro-organisms and does not require antibody. The alternative pathway constitutes a humoral component of natural defence against infections, and can operate without antibodies. The six proteins C3, B, D, H, I, and P together perform the functions of initiation, recognition and activation of this pathway which results in the formation of activator-bound C3/C5 convertase.

Similar to the classical pathway, an initial enzyme is present that catalyses the formation of the target cell bound C3 convertase which in turn generates the C5 convertase. This results in the cleavage and activation of C5, therefore, in the assembly of the membrane attack complex (MAC).

Particulate polysaccharides, for example, bacterial (LPS), yeast (zymosan), or plant (inulin) polysaccharides, fungi, bacteria, viruses, certain mammalian cells, and aggregates of immunoglobulins, such as, the Fab portions of IgA or IgE are known to be activators of this pathway. It is not yet known which structure is commonly recognized by the complement proteins in these activators. It is known, however, that recognition involves C3b. The microenvironment of the particle-bound C3b determines whether C3b prefers the binding of B, which causes activation of the pathway, or of H, which cancels the progression of the reaction. C3b is covalently attached to receptive surfaces, and interaction between the putative recognition site in C3b and the recognised structure in the immediate environment may be quite weak. This strategy would allow a wide spectrum of different but related substances to be recognised by C3b. The degree of specificity of the pathway is low, but not non-specific. As described previously, C3 has been shown to contain a thioester bond which undergoes a low level of hydrolysis with water. The spontaneous slow hydrolysis of the thioester converts inactive native C3 to a functionally active C3b-like molecule called C3i. This continuous production is the chemical basis of what has been called the "tick-over" phenomenon. C3b, the product of the reaction catalysed by the C3 convertase, forms a subunit of the enzyme itself. The second

property is the cause of positive feedback, which is the driving force of amplification of the pathway

Formation of the initial fluid phase C3 convertase

In the alternative pathway the reactions are not driven by enzymes, the spontaneously generated supply of C3i due to the steady rate of hydrolysis of C3 determines the progression of the pathway. The binding of factor B to C3i is Mg²⁺-dependent and this proenzyme complex is activated by factor D. the binding of Factor D to C3i is similar to the binding of C2 to C4 observed in the classical pathway. Factor D cleaves the bound factor B to release Ba, a 30kD fragment and forms the C3iBb (Bb being the second fragment of 60kD), the initial C3 convertase which stays in the fluid phase. C3iB is regulated by properdin (P). P binds to cell-bound C3b and stabilizes the C3/C5 convertase. P is negatively regulated by factors H and I.

Formation of the cell bound C3/C5 convertase

C3iBb is a reversible trimolecular complex. Fluid phase C3iBb is a C3 convertase enzyme that further cleaves its C3 to C3b*. The surface bound C3b* acts as a binding site for more factor B and initiates an amplification loop. Electron microscopy studies have shown that Bb has two sites and only one is used for binding to C3b while the other may be the catalytic domain. The C3 convertase is stabilized by seven folds when its Mg²⁺ is replaced by Ni²⁺.

The C3 convertase can function as C5 convertase when another C3b molecule is present in its vicinity. The second C3b molecule modifies C5 for making it compatible for binding and cleavage by Bb and forming the C5 convertase of the alternative pathway, C3bBbC3b. the C3/C5 convertase is stabilized by properdin.

Regulation of the alternative pathway

Similar to the classical pathway, the alternate pathway is also tightly regulated with the help of both fluid phase and membrane proteins. Factor H, a fluid phase protein has two distinct enzymatic activities. A slow and spontaneous decay of C3b results in Bb, this phenomenon is accelerated with the help of Factor H, resulting in the disassociation of Bb and C3 convertase. The other activity of Factor H is a co-factor activity for factor I to mediate cleavage of C3b into a hemolytic inactive form C3bi on bacteria, immune complexes and free soluble C3b-Bb complex. Factor H recognizes polyanionic structures on the self surface, such as sialic acid and the glycosaminoglycan (GAG) chains of proteoglycan (eg heparin sulfate and dermatan sulfate) and thus inhibits complement activation on host surface. The membrane regulatory proteins of the alternative pathway are the decay accelerating factor (DAF) and CR1 (Fig 5). These proteins accelerate the disassociation of C3bBb-C3 convertase complex. Similar to the regulatory activities of the classical pathway, CR1 and MCP act as co-factors of Factor I for C3b cleavage.

Amplification loop of the alternative pathway

A C3b molecule bound to the cell surface can either enter the amplification loop in which it binds to Factor B to form a convertase enzyme and amplifies further deposition of more molecules of C3b on the cell surface; or it can be broken down by factor H with the help of any of the three co-factors, the fluid-phase factor H or the membrane bound CR1 or MCP. The outcome of C3b is determined by the nature of its interacting surface. A self surface has intrinsic

molecules like DAF, CR1 and MCP on its surface which are likely to inhibit the formation of C3 convertase enzyme (Fig 5).

At the same time, the presence of microorganism and a non-self surface acts as a protected site for c3b binding since the actor B has a higher binding affinity for C3b and it immediately forms a stable complex with the C3b (even a few) bound to the bacterial surface, C3bBbP-C3 convertase enzyme. This complex results in further deposition of C3b in the vicinity.

C3b formed by either the classical or the alternate pathway can trigger the alternate pathway again with the help of this positive feed-back loop. However, this amplification is not constant and presence of Factor H and factor I in the plasma together limit the alternate pathway activation.

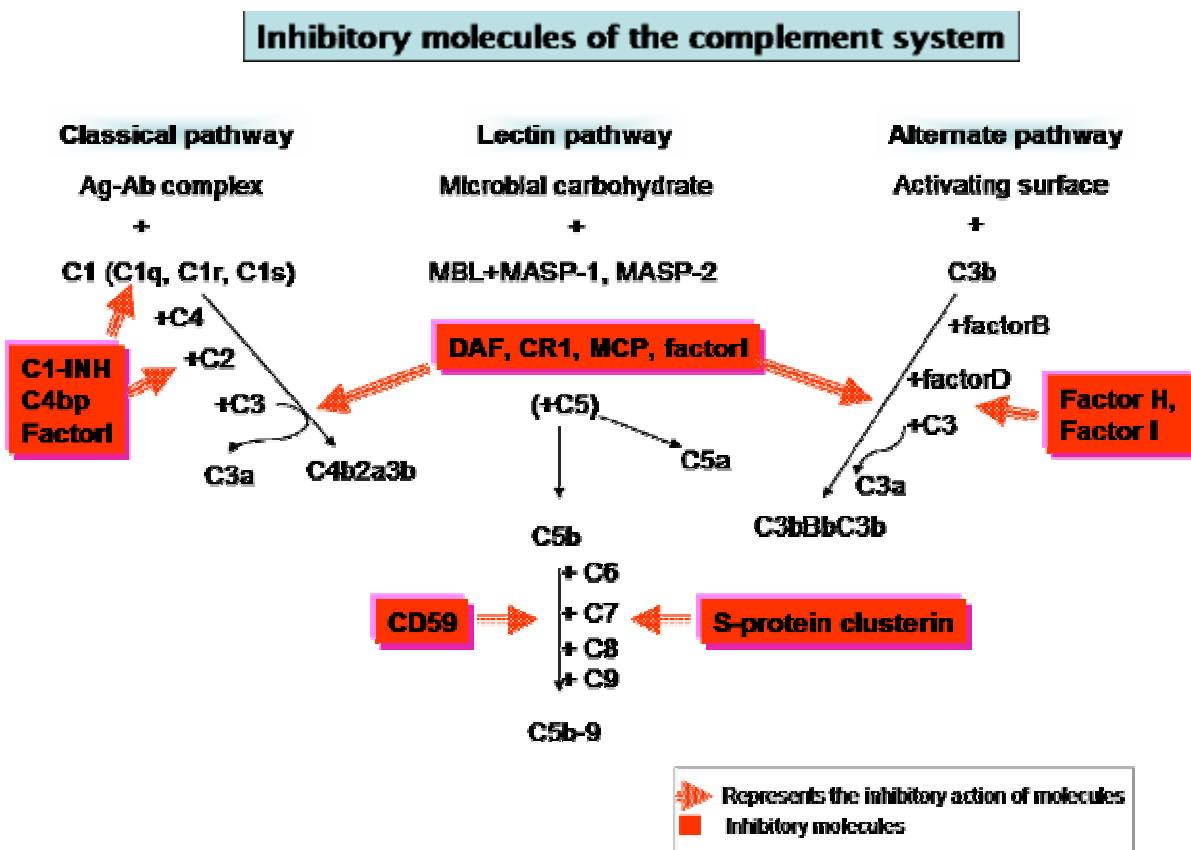


Fig 5: Inhibitory molecules of the complement system

Formation of the MAC complex

The MAC constitutes a supramolecular organisation that is composed of approximately twenty protein molecules and has a molecular weight of approx. 1.7 million. The fully assembled MAC contains one molecule each of C5b, C6, C7, and C8 and one or more molecules of C9 (Fig. 6). These are the five precursors present in the MAC, all of which are hydrophilic glycoproteins. Evidence suggests that the proteins participating in the transmembrane channel formation are structurally interrelated. The steps towards formation of MAC are non-enzymatic. When the C5

convertase cleaves C5 to produce nascent C5b (C5b*) then the assembly of MAC takes place. The binding of C7 to the C5b6 complex exposes certain hydrophobic sites on the now trimolecular (C5b67 or C5b-7) complex, making it feasible for the molecule to insert itself into the lipid bilayer of cells when present in the vicinity of the cell. Further, C8 and C9 bind sequentially to this complex forming the lytic plug or pore-forming molecule (Fig 6). The binding of C8 to C5b-7 causes a small amount of lysis. The C9 forms a polymeric complex of upto 14 C9 molecules and it is this oligomerization reaction which causes a majority of the lysis. When about 12 molecules accumulate there, a single transmembrane channel through which water and electrolytes may pass is formed. The Poly(C9) is a cylinder with inner and outer diameters of 9 nm and 15 nm respectively, tube length 15 nm, rimmed by a 4.6 nm thick torus with inner and outer diameters of 11 nm and 22 nm on one end. This polymerization of hydrophobic molecules may be a common mechanism for cellular death. All five proteins, C6, C7, C8, C9, and C9 related protein or C9RP share certain antigenic properties. C9-related protein (C9RP) is the protein responsible for pore-forming activity and has been isolated from murine and human cells. Because the protein interacts with C9, it has been called C9-related protein, a term synonymous with cytolysin, perforin (an effector molecule of killer T-cells and NK cells), and pore forming protein. Although C9 and C9RP are similar, probably homologous proteins, and may be analogous in their function, they differ in that isolated C9RP is cytotoxic by itself, whereas isolated C9 is not. Under conditions promoting homopolymerisation, C9RP kills cells without the participation of other proteins. For C9 to exert its cytotoxic effect it requires cell-bound C5b-8.

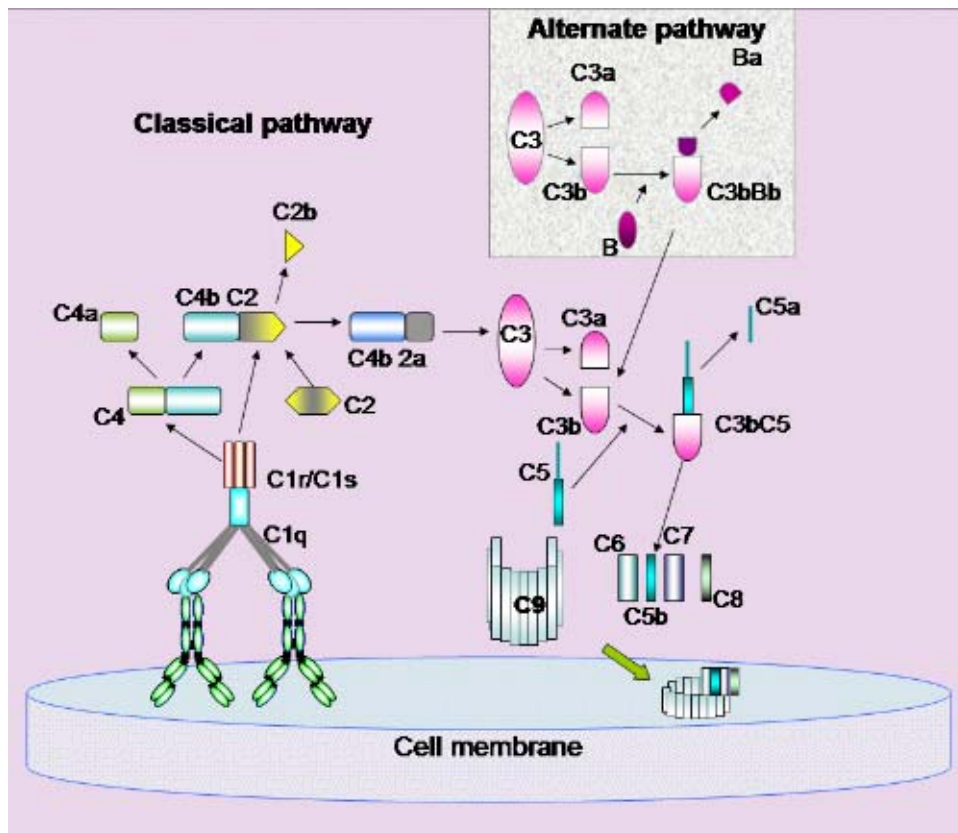


Fig 6: MAC formation through the classical and alternate pathway

For example, T-lymphocytes kill target cells by inserting perforin. Bacterial toxin, streptolysin O, are also pore forming molecules. Therefore the end product consists of the tetramolecular C5b-8 complex (approximately 550kD) and tubular poly-C9 (approximately 1100kD). This form of the MAC, once inserted into the cell membranes, creates complete transmembrane channels leading to osmotic lysis of the cell. The transmembrane channels formed vary in size depending on the number of C9 molecules incorporated into the channel structure. Whereas the presence of poly-C9 is not absolutely essential for the lysis of red blood cells or of nucleated cells, it may be necessary for the killing of bacteria.

Regulation of MAC activation

The C5b67 hydrophobic complex can insert itself into cell surfaces in the vicinity of the original surface of complement activation. In such a case, uncontrolled 'reactive lysis' can result in unwanted damage to the cell membrane of host tissue or 'self'. This is checked by a number of proteins which bind to the fluid phase C5b67 before it can attach to host membrane. The most abundant of these proteins is the plasma protein, S protein or vitronectin. S protein is the primary inhibitor of serum. It competes with membrane lipids for the metastable binding sites of C5b-7 and allows the binding of C8 and C9, but prevents C9 polymerization; in fact, by binding to the complex, it prevents the attachment of C5b-7 to the cell surface. The interaction results in the SC5b67 complex which cannot insert into lipid bilayers. Its function appears to be to protect the cells adjacent to sites of complement activation from accidental attack. The hydrophilic complex, SC5b-7, binds C8 and three C9 molecules to form SC5b-8 and SC5b-9. All three complexes contain neoantigens that are not detectable in the precursor proteins and that are distinct from the neoantigens of poly-C9. In addition to blocking the membrane binding site, S protein, as mentioned earlier, also prevents polymerisation of C9. These functions allow S protein to control the formation of the MAC. Alongwith the S protein, the multifunctional protein, SP-40,40, has been discovered to modulate the soluble membrane attack complex (SMAC, SC5b-9) of complement, causes cell aggregation, and accelerates immune complex formation. SP-40,40 was discovered as a soluble protein present in serum, seminal plasma and cerebrospinal fluid. It was reported that SP-40,40 modulated the formation of the MAC of complement and was incorporated into SMAC at the stage of SC5b-7. When S protein or SP-40,40 binds to C5b-7 before C5b-7 binds to the membrane, SC5b-7, which is hydrophilic is formed. Finally, soluble SC5b-9 is formed via the bindings of C8 and C9. In the bindings of both S protein and SP-40,40 to C5b-7, the hydrophobic interactions seem to be a major force. In addition to S protein and SP-40,40, lipoproteins have been reported as inhibitors of MAC formation.

The cell surface antigen, CD59, has been found to be an inhibitor of complement-mediated lysis. CD59 is a membrane protein which is present on erythrocytes, monocytes, granulocytes, platelets, endothelial cells as well as on cells of reproductive and nervous tissue. The function of CD59 was first suggested by the finding that the purified antigen inhibits complement-mediated lysis by binding in a glycosylation-dependent manner to C5b-8 and/or C5b-9 preventing the formation of the MAC. Hence MAC is not assembled and inhibition of the lytic pore formation occurs. Comparisons of CD59 with other complement inhibitors, decay accelerating factor, and membrane cofactor protein, indicate that CD59 is the most potent inhibitor of complement-mediated lysis of human endothelial cells. In addition to inhibiting complement, CD59 can transmit activating signals to T cells and form a part of a signal-transducing complex on the

surface of these cells. It has been proposed that CD59 is also a second ligand for the human T lymphocyte adhesion molecule, CD2, but this is controversial. In the short period since its discovery, considerable progress has been made in understanding the function of CD59. While it is clear that CD59 protects tissues from attack by the complement system, the molecular basis of this process remains to be elucidated.

Binding of C8 and low density lipoprotein (LDL) also forms a complex which cannot cross the lipid bilayer. The Homologous restriction factor (HRH) or C8 binding protein is responsible for deposition of C8 and C9 on the membrane during MAC assembly. This 65kD protein which, like DAF, is anchored to cell membranes via a glycan linkage to phosphatidylinositol. Being a membrane protein, HRF is insoluble in aqueous solvents. HRF is detectable on human RBCs, platelets, neutrophils, monocytes, and lymphocytes. HRF shows affinity towards C9 isolated from membranes of human red blood cells (RBCs). It is capable of incorporating into the lipid bilayer of liposomes, and actively inhibits channel formation by C5b-8, C5b-9, and also inhibits the polymerization of C9. Inhibition of HRH by antibody did not affect C5b-7 uptake but led to an enhanced C9 binding to the target cell and also resulted in a 20 fold increase in reactive lysis.

Another protein that controls MAC channel formation has been isolated from human RBC membranes. The "membrane inhibitor of reactive lysis" (MIRL) is a 18- to 20kD protein and capable of restricting the assembly of C5b-9 on target cell membranes. MIRL is identical with the leukocyte antigen CD59 and is expressed on endothelial cells. It is released, in part, from cells after treatment with phosphatidylinositol-specific phospholipase C. Antibodies to MIRL are known to make normal cells susceptible to reactive lysis.

The Complement receptors

The surface of immune cells exhibits various receptors capable of binding complement proteins to facilitate the uptake of particles for opsonization and activation the cell bearing receptor. The most important receptors are those belonging to the family of C3 receptors. The C3 fragments, C3b, C3i and c3dg share complement receptor types 1 to 4, CR1-CR4) (Table 1).

The CR1 or CD35 is an opsonic receptor on neutrophils, monocytes and macrophages mediating endocytosis. It is also a co-factor to Factor I for cleavage of c3b to iC3b and its subsequent cleavage to C3c and C3dg. Even though factor H plays the major role for cleavage of C3b, but CR1 is probably the sole effector of iC3b cleavage. This role helps in protecting self cells from cleavage. CR1 present on erythrocytes and platelets helps in locating opsonized immune complex or bacteria and transport to phagocytes. CR1, in tandem with CR2 works on B lymphocytes.

The CR2 or CD21 is located on B lymphocytes, follicular dendritic cells and certain epithelial cells. It binds to iC3b, C3dg, Epstein barr virus (EBV) and IFNalpha. On B cells, the CR2 appear to bind to c3b and stimulate the antibody response. The EBV response appears to be more direct and apparently the binding of EBV to CR2 is independent of complement activation.

The CR3 or CD18/11b is found on descendants of myeloid cells and is an important receptor and adhesion molecule. It mediates phagocytosis of particles opsonized with iC3b. A number of microorganisms bind directly to CR3 without complement mediation and CR3 functions as a

lectin by binding to carbohydrates. CR3, along with three cell surface receptors, the LFA-1 (lymphocyte function associated antigen type 1, CD18/11a) and CR4 (p150-95, CD18/CD11c) belong to the leucocyte integrins family. The leucocyte integrins have their own type of β chain, $\beta 2$ which combines with a specific α chain. The integrin superfamily comprises of structurally related cell surface receptors and adhesion molecules including the fibronectin and vitronectin (S protein) receptor and fibrinogen receptor on platelets

The CR4 or CD18/11c binds to iC3b in the presence of calcium. It is present on cells from both the myeloid and lymphoid lineage. It is especially strongly expressed on tissue macrophages for binding to particles opsonized with iC3b.

Receptors for C3a and C5a, the anaphylatoxin receptors, are known to cause degranulation of mast cells on binding of C3a and C5a.

Table 1
Complement Receptors and their function

Complement receptors	Ligand	Cells harbouring the receptors	Function
CR1	C3b, C4b (iC3b to a much lesser extent)	B-cells, neutrophils, monocytes, follicular dendritic cells, macrophages, erythrocytes, glomerular epithelial cells	<ol style="list-style-type: none"> 1) Opsonic receptor for neutrophils, monocytes and macrophages 2) Co-factor for Factor I in cleavage of C3b to iC3b and further to C3c and C3dg. Acts to protect self 3) On RBCs, transports opsonized IC on bacteria to fixed mononuclear phagocytes 4) On B-cells, acts along with CR2
CR2	iC3b, C3dg, Epstein-barr virus iFN-alpha	B-cells, follicular dendritic cells, epithelial cells of cervix and nasopharynx	<ol style="list-style-type: none"> 1) On B cells acts as accessory receptor for C3b to stimulate antibody response 2) Localization of immune complexes to antigen presenting cells 3) Receptor for EBV, virus enters directly without activation of complement pathway
CR3	iC3b, zymosan, certain bacteria	Monocytes, macrophages, neutrophils, NK cells, follicular dendritic cells	<ol style="list-style-type: none"> 1) Mediates phagocytosis for iC3b bound cells 2) As a lectin also binds carbohydrates. Some yeast bind CR3 directly without activating complement 3) Role as adhesion molecule (superfamily leucocyte integrin)
CR4	iC3b	Neutrophils, monocytes, tissue macrophages	Binds to iC3b in Ca dependent mechanism. Found on cells of myeloid and lymphoid lineage

Role of antibody in alternative pathway

Some recent reports have implicated a role of antibody in the alternative pathway independent of the classical pathway. The metastable C3b is capable of binding to IgG, the preferred binding site being the Fd portion of the heavy chain. The bound C3b is not as easily inactivated by the regulatory Factor H and Factor I as compared to the free C3b. Aggregated IgG and aggregated IgM both activate the pathway, as well as some aggregated IgA myeloma proteins and some IgE myelomas; although the immunoglobulin concentration required for such activation is actually relatively high.

What Determines Whether or Not Complement Will Be Activated

1. Nature of antibody coating the cell
2. IgM antibodies are the best because they have more antigen-binding sites. They can achieve binding of two adjacent antigens by single IgM molecule. Only certain IgG subclasses are capable of activating complement: IgG subclasses 1, 2, and 3. Of these IgG subsets, IgG 3 is the best. IgG subclass 4 does not activate complement.
3. Number of antigen sites on red cell
4. The more antigen sites found on the red blood cell, the more likely two adjacent ones are bound by antibody.

Biological effects of complements

Each individual complement component performs different functions (Table 2).

Table 2

Biological activity of the complement proteins

Complement components	Functional activity
C14, C1423	Virus neutralization
C3a, C5a	Anaphylatoxin (histamine release, vascular dilation)
C3, C5 fragments	Chemotaxis of PMN, monocytes
C567	Eosinophils activation
C3b	Opsonization, Enhanced induction of Antibody formation, Stimulation of B-cell, Ab dependent cellular cytotoxicity
C3d	Enhanced induction of Antibody formation
C3 cleavage product	Granulocytosis
C5 cleavage product (C5a)	Neutropenia
C1-9	Bacteriolysis, Virolysis, lysis of virus infected and tumor cells, lysis of mycoplasma and protozoans

The functional aspects of the complements in the immune system can be broadly divided into five categories.

1. Opsonization and phagocytosis

The target organism on being bound by C3 molecules is ingested by macrophages and neutrophils (Fig. 7). Phagocytosis is further mediated by CR1, CR3, CR4 and also on binding to IgG on matrix proteins. It is also enhanced by some cytokines.

The mechanism of opsonization and phagocytosis

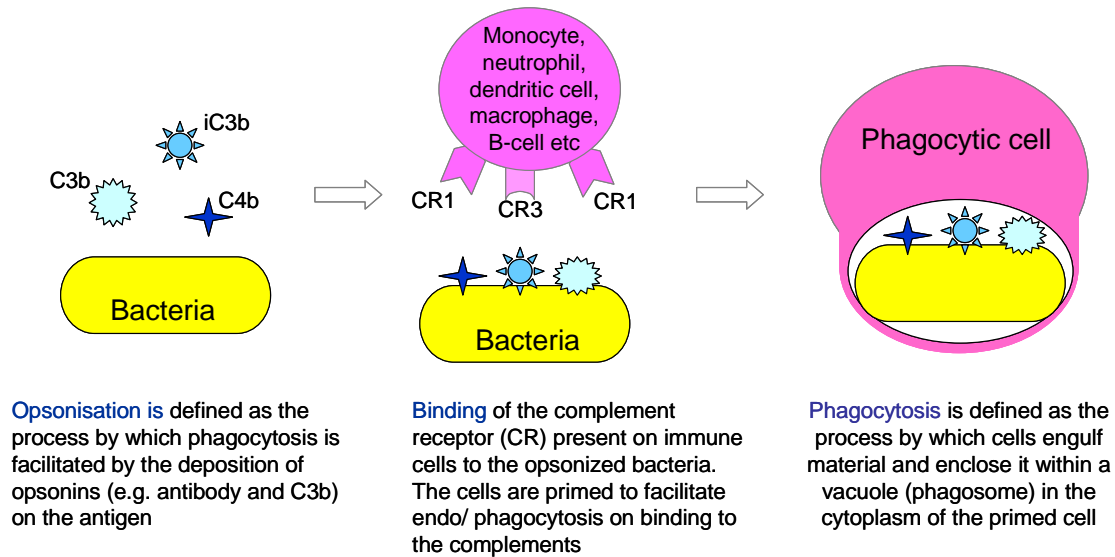


Fig 7: Mechanism of opsonization and phagocytosis

2. MAC formation and cytotoxicity in tissue

Formation of the MAC complex leads to reactive lysis. This lysis is achieved by osmotic gradient disruption of non-nucleated target cells. Whereas in nucleated cells, the effect of complements is vastly different. The nucleated cell is first activated by the complements and recovers from the MAC. Before the lysis occurs, there is an influx of Ca^{2+} ions in the cells, this is followed by PKC activation and generation of DAG and ceramide as by-products. Arachidonic acid, other eicosanoid species, reactive oxygen species and IL-1 β are also by-products generated by this mechanism. The microorganism tries to escape from being phagocytosed by removing the MAC from the surface either through vesiculation (in neutrophils or erythrocyte) or through endocytosis. Thereafter, the secondary phenotypic changes occur which are cell type specific. For example, glomerular epithelial cells respond to C5b-8 and C5b-9 by increasing the collagen synthesis which induces glomerular sclerosis associated with complement deposition. Certain proteases are also known to be activated when C5b-9 insertion into the membranes.

3. Inflammatory response; Anaphylatoxins and chemotaxis

The cleavage products of C3 and C5 are C3a and C5a respectively possess anaphylatoxin activity. They bind to the receptors present on mast cells and basophils membranes resulting in degranulation in the cells. These granules contain vasoactive amines like histamine. Release of histamine is associated with increased vascular permeability. A preferred action of anaphylatoxins is contraction of smooth muscle cells. The chemotactic properties of C3a, C5a and C5b67 are of relevance in polymorphonuclear leukocytes and macrophages. (Fig. 8)

Function of some key components of the complement system

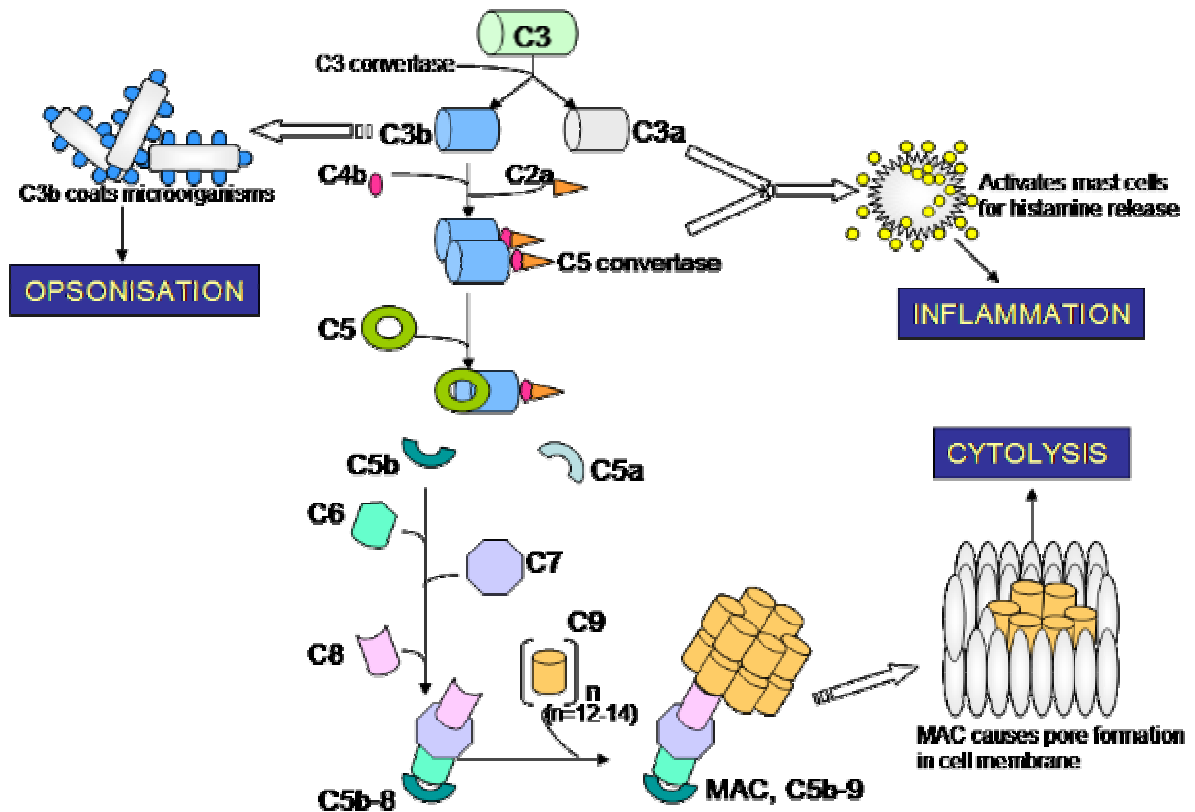


Fig 8: Function of some key components of the complement system

4. Immune response: adherence to immune complexes

The complements contribute heavily to the immune response due to their interaction with the immune complexes. This interaction alters the function of the complexes. The presence of C3b or C4b on immune complexes permits their interaction with their specific receptors on the surface of cells such as human erythrocytes, mononuclear phagocytes, polymorphonuclear leukocytes and lymphocytes.

5. Antiviral activity of complements

Reports show that herpes-type viral neutralization occurs by antibody and this phenomenon is augmented by C1, C4 and C2. RNA tumor viruses have also been shown to possess C1q receptor, which on binding to C1q leads to C1 activation leading to virolysis. The measles virus, when coated with antibody, is lysed via the alternative pathway.

Complement deficiencies and pathogenesis of the associated diseases

The diseases and disorders associated with the complement system are known to cause increased susceptibility to infections. In general, any deficiency of the immune state of the body will make

the individual prone to microbial infection, and this holds true for complement deficiencies also. Complement deficiencies are not very common and the clinical findings suggest that the immunodeficiency state of such patients are very similar to those with immunoglobulin deficiency. A common condition is that of recurrent bacterial infections from microorganisms which under a normal condition would have been opsonized or lysed by complement.

Early reports on complement deficiency were in total contrast to the current literature and suggested that complement did not play a protective role in the immune system. For example, C2 deficient humans and C6 deficient rabbits were observed to be healthy whereas C1 inactivator deficiency was found to be the cause of hereditary angio-edema associating the importance of complement activation for developing an inflammatory response. A report even downplayed the importance of complement saying that the complement might hold no more survival value to man than mid-digital hair'.

It was only in the early 1970s a variety of hereditary and acquired complement deficiency states have been reported in conjunction with severe bacterial infections and, perhaps more surprisingly, in patients with disease conditions associated with autoimmunity and immune complex formation. The complement components were recognized as protective of clinical symptoms. C3 deficiency syndromes in patients susceptible to infection, with findings resembling those of hypogammaglobulinemia, the demonstration of hereditary C2 deficiency in patients with SLE (systemic lupus erythematosus)-like disease , and studies of acquired C3 deficiency due to C3 nephritic factors in relation to glomerulonephritis were some of the novel findings at the time.

Detection of complement deficiency by ELISA

It was important to establish a working technique for the detection of complements which would further help in detecting deficiency of complements in the diseased state.

Simple assays for assessment of complement function are required in diagnostic work. Mostly C3 and C4 were measured and other complement proteins were often overlooked. Researchers have mostly used haemolytic assay systems for this purpose, and simplified procedures suitable for relatively large scale investigations of patients have been described.

To be useful in diagnostic work, screening procedures for the detection of complement deficiencies, should be simple and rapid, and should clearly distinguish between defects within the functional units of the complement system, i.e. the classical activation pathway (C1, C2, C4), the alternative activation pathway (C3, factor B, factor D, properdin), and the terminal sequence (C5-C9). Furthermore the assays should not be influenced by rheumatoid factors, and the reagents should be commercially available. One of the most standard ELISA procedure for complements was described by Fredrikson et al. and appears to meet all requirements.

In this report, the sera were incubated in microtitre plates with solid phase complement activators. Human polyclonal IgG or monoclonal IgM were used for classical activation pathway assays and Salmonella typhosa LPS for alternative activation pathway assays. This particular analysis focused on deposition of C9 and properdin as detected with enzyme-conjugated antibodies. In an attempt to avoid spurious results due to rheumatoid factors in patient sera,

monoclonal mouse and chicken antibodies were unsuccessfully tested as indicator reagents in the assay with solid-phase IgG. However, the use of solid-phase IgM as an activator completely circumvented the influence of rheumatoid factors. With solid-phase IgG or IgM, properdin deposition occurred in the absence of factor D. A combination of assays is suggested for diagnostic purposes: IgM-coated plates with detection of bound C9 and properdin for the classical pathway; and LPS-coated plates with detection for bound properdin for the alternative pathway. Therefore, the procedure distinguishes between defects of the classical activation pathway, the alternative activation pathway, and the terminal complement components. This analytical approach may be useful for the detection of inherited complement deficiency and assessment of complement function in acquired complement deficiency states.

For diagnosis, a combination of two screening assays, i. e. the IgM-ELISA with detection of C9 deposition and the LPS-ELISA with detection of properdin deposition would comprehensively reveal all known types of complement deficiency.

Several forms of acquired complement deficiency could produce difficulties of interpretation unless the analysis is combined with ordinary determinations of proteins, such as C3 and C4.

ELISA, using standard and commercially available reagents, appears to be a simple, rapid, and reliable method for the assessment of complement function, particularly the detection of complement deficiency states.

Currently the assessment of complement deficiency involvement in causing a pathophysiological condition relies on four observations

1. detection of hypocomplementaemia to the degree of disease activity
2. deposition of complement deposition during tissue injury
3. hypersynthesis and hypercatabolism of complements in disease state
4. comparison between the lesion of complement mediated tissue lesion in patient and experimental animals.

Types of complement disease and their genetic basis

The complement deficiencies can either be inherited in which genetic defect occurs either in the form of gene deletion loss of splicing sites or generation of stop codons in the coding region of the gene leading to an inactive product if any; or the deficiency may be acquired, ei the level of complement in the sera is decreased either due to increased consumption due to increased activity of inhibitory molecules, such as in systemic lupus erythemteous or due to deficiency of regulatory proteins.

Wherever, homozygous deficiency occurs, it is normally associated with recurrent bacterial infection. Properdin deficiency states are X-linked. Chromosome 1 contains the genes for C1q, C8, C4bp, factor H, CR1, CR2, and DAF, and chromosome 12 contains the genes for C1r and C1s. The gene for C3 has been localized to chromosome 19, factor I to chromosome 4, and C1 (IA) to chromosome 11. Four complement proteins, the C4A isotype, the C4B isotype, C2 and factor B, are encoded by genes within the major histocompatibility complex (MHC) on chromosome 6.

Those patients which have a partial defect show the acquired phenomenon. For example, hereditary angio-edema is due to heterozygous deficiency or dysfunction of the C1 inhibitor protein. In addition, homozygous C4A and C4B deficiencies may have pathogenic importance. The prevalence of these conditions almost certainly varies in different populations, but has been estimated to be in the order of 0.03%.

Complement molecules and pathophysiological condition of the diseases

Autoimmune diseases and complement proteins

Complement may protect against autoimmune disease by normalizing apoptosis, the natural process by which cells self-destruct. This natural self-destruction process is an important part of the normal way in which the body keeps house in its tissues, eliminating damaged or no longer useful cells. Natural autoantibodies (the autoantibodies of healthy people) may also play a role in regulating apoptosis by triggering complement activation and rapid destruction of these self-destructive cells so that dangerous autoantigens are not left in the bloodstream long enough to trigger a more global (dysregulated) immune response. In the absence of an effective complement system, then, apoptosis goes awry and leads to persistent exposure of autoantigens to the immune system. These autoantigens sometimes take the form of nucleosomes (collections of DNA and protein) that trigger the destructive forms of SLE autoantibodies.

Immune complexes are mostly cleared by the monocyte phagocyte system, especially in the liver and spleen. In case of primates which have CR1 receptor for C3b on erythrocytes. With a presence of almost 700 receptors per RBC, the avidity is high and allows binding to large complexes. The CR1 is exhausted frequently in cases of continuous production of complexes as the CR1 directs them to liver and spleen to be degraded. In the case of SLE, the receptors are almost halved. Further, in case of complement deficiencies, this debris (of immune complexes) accumulates and is deposited in the kidneys, blood vessels, joints, and other sites where it might further incite the immune system to produce inflammation and tissue damage. Two of the diseases are given in little more detail.

Systemic lupus erythematosus (SLE)

SLE is an example of autoimmune disease which is further complicated to result in an immune complex disease. In an autoimmune disease, the self is recognized as non-self and this leads to the formation of immune complex of the antibody to the self. All the components responsible for the removal of immune complexes like the mononuclear phagocyte, erythrocyte and complement system proteins, hence, become overloaded with the continuously produced immune complexes. These complexes are then deposited in the tissues. In the case of SLE, the deposition is in the vascular system and kidneys.

Rheumatoid arthritis

B cell, antibody and complements play an important role in mediating synovial inflammation. Complement is activated locally in synovial fluid and joint tissue. The functional activity in patients with rheumatoid arthritis was determined to be low. the complement components in RA were observed to be functionally inactive indicating that the complex had previously been

engaged in some activity. C3a, C5a, C4b, C3b, C3bi, C3d, C5b, C5b-C9 were observed to be increased in RA. Rheumatoid factors were present in joint fluid and in serum. Immune complexes containing IgG, rheumatoid factor and complement fragments were also observed. The phenomenon is understood as follows, an autoantibody IgG binds to antigen (which may be an anti-DNA released from cells as a result of damage by endotoxins) and forms an immune complex and this activated immune complex further binds to rheumatoid factor. The rheumatoid factor is essentially an aggregated anti-IgM autoantibody released by polyclonal activation of B-cells (Fig 9).

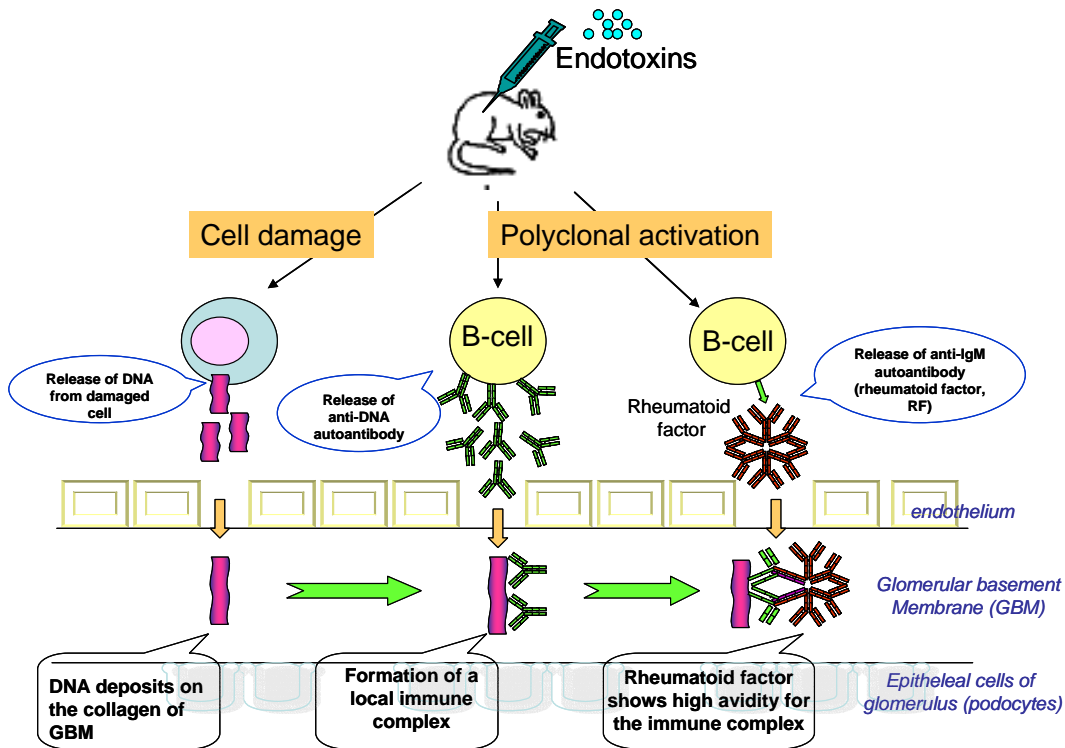


Fig 9: Autoantibody and Rheumatoid arthritis

Complement activity in hypersensitivity and inflammation

Antibody and antigen combine to form immune complexes. These complexes act on complements to release C3a and C5a. The complement activation acts on basophils to release vasoactive amines. The immune complexes can also directly act on basophils and platelets to release these amines. These amines, histamine and 5-hydroxytryptamine cause endothelial cell retraction and increased vascular permeability. The increase in vascular permeability leads to deposition of the immune complexes on the wall of the blood vessel. Platelet aggregation and complement activation follows and the aggregating platelets form microthrombi on the exposed collagen of the basement membrane of the endothelium. Neutrophils which are attracted by complement products cannot ingest the complexes and hence they simply release their lysosomal enzymes, further damaging the vessel wall.

Deficiencies of complement components

Deficiency in components of classical pathway

These result in impairment of functions such as phagocytic killing with opsonization, serum bactericidal activity, and removal of immune complexes. The homozygous C2 deficiency is associated with rheumatologic disease. The affected person exhibits a syndrome consistent with systemic lupus erythematosus. The affiliation of rheumatologic disease with complement deficiency is also seen in the rare C1 and C4 deficiencies. A review of all reported persons with a classical component deficiency reveals that about 66% have manifestations of a collagen vascular disease. In general, such individuals exhibit increased susceptibility to infection. Recurrent infections and meningitis caused by encapsulated organisms, especially *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae*, are the common diseases associated with complement deficiencies. Deficiencies of C4a and C4b vary in their disease association. While, C4a deficiency shows a higher prevalence in SLE and other immunological diseases, the C4b shows a greater incidence of association with bacterial meningitis caused by *H. influenzae* in children. This is reflective of the role of C4a in immune complex handling and the efficient binding of C4b to polysaccharides such as those present on bacteria. Homozygous C1r deficiency is shown to co-exist many a times with a partial defect of C1s. Some patients with hereditary angio-edema develop immune complex disease. The C2 and C3 deficiencies are given in further detail

C2 deficiency

C2 deficiency is quite common amongst Caucasians. C2 deficiency is often complicated by immunological disorders such as systemic lupus erythematosus (SLE), rheumatoid arthritis and vasculitis. This is found associated with human lymphocyte antigen (HLA) B18 and since, HLA B18 is not seen in the Japanese population, it is considered that C2 deficiency does not exist in this race. Only a couple of cases have been reported which show the existence of C2 deficiency in Japan. In Caucasians, the deficiency is associated with manifestations such as SLE, vasculitis, ankylosing spondylitis, rheumatoid arthritis, and type I diabetes. However, this mechanism by which these occur is not well defined. A possible explanation could be due to the genetic localization of the C2 gene at the MHC class III region. The ORF (open reading frames) in this region exist which on expression may produce certain proteins involved in the development and regulation of immune defence mechanisms, however, these are yet to be characterised. Therefore, it can be speculated that a possible genetic change of MHC class III region linked with C2 deficiency may alter immune regulation mechanisms and thereby cause autoimmune diseases. This hypothesis finds support by findings which show that IgA deficiency and common variable immunodeficiency are related with polymorphisms of MHC class III. It is also likely that this may be related to chronic idiopathic neutropenia.

C3 deficiency

As the focal point in the complement system, C3 participates in numerous effector mechanisms responsible for inflammation and host defence. C3 and its split products have a central role not only in the effector phase of the immune response but also in its initiation. Consequently it is not surprising that C3 deficiency leads to multiple, severe derangements including immune complex disease, impaired immune responses, impaired chemotaxis, reduced opsonophagocytosis and abnormal serum bactericidal activity. As with classical component deficiencies, the affected

individuals are at increased risk for rheumatologic disease, 79% demonstrating a lupus-like syndrome or systemic vasculitis. These individuals have a striking disposition to severe and recurrent infections caused primarily by encapsulated organisms, which first manifests itself at an early age. The common clinical symptoms are of sinopulmonary infection, bacteremia or meningitis.

Secondary C3 deficiency may also arise as a result of deficiency in the alternative pathway components, factor I or factor H. Factors H and I are primarily responsible for the down regulation of the fluid phase, alternative pathway C3 convertase. Deficiency of either of these proteins leads to an unchecked spontaneous formation of the C3 convertase, leading to C3 consumption and depletion.

Deficiency in components of alternative pathway

Inherited deficiencies have been described for properdin and factor D. Properdin is known to stabilize the alternative pathway C3 convertase through non-covalent interaction. Individuals with properdin deficiency exhibit an increased disposition for infections with encapsulated organisms, especially *N.meningitidis*. However, they do not show the same affiliation with rheumatologic disease as do individuals with either classical component or C3 deficiency states.

The role of factor D is to cleave factor B to generate the alternative pathway C3 convertase, C3bBb. Its deficiency has been reported in a male patient with recurrent neisserial infections. The disease has also been shown to be X-linked. Incomplete factor D deficiency has previously been found in monozygous twins who were adult females with a record of repeated upper and lower respiratory tract infections from childhood.

Deficiencies of MAC components

The membrane attack complex is responsible for direct complement-dependent serum bactericidal activity and is the major lytic effector mechanism of the complement cascade. Recent evidence from late complement component deficiency (LCCD) patients showing immune complex or rheumatologic disease suggests that MAC may also participate in tissue injury in a wide range of diseases.

Recurrent systemic infection caused by *N.meningitidis* or *N.gonorrhoeae* is the only clinical manifestation clearly associated with homozygous deficiency of C5, C6, C7, or C8. The C5 deficiency does not show symptoms markedly different from the other MAC components in spite of holding a key position in the cascade. The association between C9 deficiency and neisserial infection is not as strong as in C5-C8 deficiency. Individuals with total C9 deficiency exhibit delayed, but present, serum bactericidal and haemolytic activities in vitro. The C8 molecule is composed of two subunits, the alpha-gamma subunit and the beta subunit, and hereditary deficiencies may involve either subunit. C8 alpha-gamma deficiency is found among patients of black or Hispanic origin, while all well documented cases of C8 beta deficiency have been seen in the Caucasian population. C9 deficiency is remarkably common in the Japanese population, and various findings suggest the deficiency to be a susceptibility factor for development of meningococcal disease.

Deficiencies of Complement receptors and membrane proteins

Decreased expression of CR1 on erythrocytes and other cell types has been described in patients with SLE, and there are a large number of reports investigating the CR1 function in relation to pathogenic events in immune complex disease. The level of CR1 has also been reported to be suppressed in other disease conditions such as reactive arthritis caused by *Yersinia enterocolitica*. Patients with SLE also show decreased expression of CR2 on B-lymphocytes. The weight of evidence suggests that these deficiencies are acquired through disease-related mechanisms.

Table 3

Complement and the associated diseases

Complement component	Clinical association
C1s	SLE like syndrome
C1r	Recurrent bacterial infection, SLE like disease, chronic glomerulonephritis
C4	SLE like syndrome, DLE
C2	Glomerulonephritis, SLE, Discoid LE, chronic vasculitis, anaphylactoid purpura
C3	Recurrent infection skin eruptions, fever arthralgias
C5	SLE and recurrent infection, gonococcal infection
C6	repeated gonococemia, meningococcal meningitis
C7	Raynaud's phenomenon
C8	Repeated gonococemia, LE like syndrome
C1 inhibitor	Hereditary angioedema, SLE, DLE, progressive glomerulonephritis
C3b inactivator	Recurrent infection

Deficiency of DAF and other phospholipid anchored membrane proteins are known to cause a certain level of complement mediated lysis in patients of paroxysmal nocturnal hemoglobinuria. The mechanism for this is reported to be defect caused by somatic mutation of blood-forming cells.

Clinical presentation of diseases caused by complement deficiencies

The diseases caused by complement deficiencies can be broadly grouped into three categories

- Those that result in inadequate opsonisation (coating of cells to allow phagocytosis) - Opsonization defects.
- Those with defective cell lysis - Cell lysis defects.
- Those that lead to associated immune complex diseases.

Opsonisation defects

These deficiencies are present at a young age with sepsis. Sepsis is also recurrent. Component C3b is a potent opsoniser and any defect in the alternative, classical or lectin pathways that results in its deficiency may cause opsonisation failure. The same is true of a primary deficiency of C3. Patients with classical pathway deficiencies (C1qrs, C2 or C4) present with a pattern of disease similar to primary immunoglobulin deficiencies. They suffer frequent infections with *Strep. pneumoniae*, particularly affecting the lungs and sinuses and *H. Influenzae* and *N. Meningitidis* may also cause recurrent respiratory and meningeal infections. Those with alternative pathway deficiencies of Factor B, Factor D and Properdin can suffer low C3b activity, and again are prone to pneumococcal and meningococcal infections. Deficiency of MBL increases susceptibility to *Saccharomyces cerevisiae* infection along with pneumococcal and meningococcal disease. Primary C3 deficiency tends to present in early life with overwhelming infection with encapsulated organisms and a tendency to collagen vascular disease. Deficiencies of Factor H and Factor I which inhibit C3 production can lead to its exhaustion and cause a secondary C3 deficiency, with symptoms very similar to primary C3 deficiency.

Cell lysis defects

These conditions tend to present in late childhood or teenage/early-adult years with less severe sequelae resulting from infection. Deficiencies of C5–C9 result in an inability to lyse bacterial cells but humoral (antibody-based) immunity is unaffected. Recurrent infections are a particular feature of these complement deficiencies. *N. Meningitidis* is the usual infecting organism, causing meningeal and occasionally respiratory infection. Unusual serotypes of the organism such as Y and W135 tend to cause infection, rather than the A,B and C types that predominate in the general population.

Associated immune complex disease

Deficiencies of the classical pathway (C1qrs, C2 and C4) are prone to immune complex disease presenting as SLE. It is unclear exactly how the deficiency of complement leads to the condition. It is thought that an inability to clear circulating immune complexes leads to their deposition in tissues and an associated inflammatory response. Glomerulonephritis, systemic sclerosis (scleroderma) and vasculitis may also be associated with deficiencies of these complement factors.

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