

THE COMPLEMENT SYSTEM: HISTORY, PATHWAYS, CASCADE AND INHIBITORS

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Since its discovery in the 19th century, the complement system has developed into a clinically significant entity. The complement system has been implicated in a variety of clinical conditions, from autoimmune diseases to ischemia–reperfusion injury in transplantation. This article charts the historical progress of our understanding of the complement system and provides a synopsis on the activation pathways and its inherent regulators.

Keywords: complement system, complement inhibitors, complement activation, complement history, complement cascade

Introduction

The complement system is an integral part of the innate immune response and acts as a bridge between innate and acquired immunity. It consists of a series of proteins that are mostly (although not exclusively) synthesised in the liver, and exist in the plasma and on cell surfaces as inactive precursors (zymogens). Complement mediates responses to inflammatory triggers through a co-ordinated sequential enzyme cascade leading to clearance of foreign cells through pathogen recognition, opsonisation and lysis [1]. Complement also possesses anti-inflammatory functions: it binds to immune complexes and apoptotic cells, and assists in their removal from the circulation and damaged tissues [2, 3]. The complement proteins are activated by, and work with IgG and IgM antibodies, hence the name ‘complement’. Many complement proteins exist in a ‘precursor’ form and are activated at the site of inflammation. The complement system is more complex than many enzymatic cascades as it requires the formation of sequential non-covalently associated activated protein fragments. These in turn become convertases and cleave components for the next enzymatic complex in the cascade, and the rapid dissociation of these complexes (and loss of enzymatic activity) forms an integral part of the elegant regulation of complement activity.

History

In the late 19th century, the focus of scientific research was on the human body’s defence against microbial in-

fection. The ‘Theory of Metchnikoff’ proposed that phagocytes in the blood were capable of ingesting and destroying the invading bacteria, thus providing the basis of innate cellular immunity. This phagocytic theory was challenged by many pathologists initially on the basis that the phagocytic leucocytes were ‘truly causal in the successful response to infection’ [4]. Buchner and colleagues (1891) found a heat labile factor in blood that was capable of killing bacteria, and named it ‘alexin’ (in Greek, means ‘to ward off’) [5, 6]. Jules Bordet supported this ‘humoral theory’ (immunity conferred due to antitoxic and bactericidal substances in body fluids) by demonstrating that immune lysis required the presence of two factors: a heat-labile lytic factor (similar to alexin) and a heat-stable factor, which he termed sensitiser (which we now know was antibody) [7]. Paul Ehrlich described the side-chain theory of antibody formation, especially the mechanisms of antibody neutralisation by toxins that induced bacterial lysis with the help of complement (which has replaced the historical term alexin). According to his theory, the immune cells contained receptors that could recognise antigens, and following immunisation, these receptors multiplied and were shed into the circulation as ‘amboceptors’ (now called antibodies). These antibodies attached not only to specific antigens but also to a heat-labile antimicrobial component called ‘complement’ [8, 9]. Ehrlich’s theory proposed that the antibody and complement combined to form a complex enzyme capable of attacking and killing cells and micro-organisms. In the ensuing years, this concept had a protagonist in the form of Bordet who argued that the antigen-antibody union was reversible, contradicting Ehrlich’s view that the antigen-antibody union was a

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firm and based on stereo chemical specificity [10]. Ehrlich's concept emphasised the presence of multiple antigens and complements in the serum, while Bordet's view revolved around a 'single complement' component that bound non-specifically to the antigen.

The concept that complement was not a single substance was provided by Ferrata and Brand, who demonstrated separation of complement into two fractions: midpiece (renamed as C1) and endpiece (renamed as C2) [11, 12]. They observed that the bactericidal activity of complement was only possible when both fragments were present. Von Dungern described a phenomenon whereby complement was inactivated by yeast cells, and a similar inactivation phenomenon was observed using cobra venom by Braun and Omorokow (both now known to activate the alternative activation pathway) [13, 14]. Coca demonstrated that the inactivation of the complement by yeast cells was due to the removal of a heat labile component, and yeast-treated complement activity could be reconstituted by the addition of normal guinea-pig serum which had been inactivated by heating for 30 minutes at 56°C [15]. This heat-stable component of the complement system was referred to as C3. Inactivation of another complement by ammonia led to isolation and characterisation of a new component referred to as C4 [16]. The complement components C1 to C4 were initially assigned names in order of their discovery, and not according to their role in the activation sequence.

Improved electrophoretic and ultracentrifugation techniques over the next few decades enabled characterisation of complement as 'proteins', contrary to the prevailing opinion that complement was serum lipoid/soap complex [7]. C3, originally described in the 1920s, was now shown to be made of six proteins and was initially termed C'3a–C'3f in order of their discovery. In the same era, other studies focused on the reaction sequence of complement components. Ueno and Mayer showed that it was possible to 'build up' the complement system by using purified components [7]. Mayer [17, 18] proposed the 'one-hit' theory that suggested a single 'complement hit' could cause lysis of an erythrocyte. This was later supported by Inoue et al. who showed that the complement could kill a bacterium by a single hit [19]. Using a reconstitution assay, Mayer and colleagues added partially purified components to antibody-sensitised sheep erythrocytes to unravel the reaction sequence of the classical pathway [20, 21]. Work by eminent scientists like Nilsson, Muller-Eberhard and colleagues led to isolation and characterisation of the various components of the complement system: C4 [22], C5 [23], C6 and C7 [24], C8 [25] and C9 [26]. Nelson and colleagues were able to isolate complement components in animals as well [27, 28]. These researchers determined the sequence of component activation for what we now refer to as the classical activation pathway as: C'1 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 nomenclatures and the new terminology being, in order of activation C1, C4, C2, C3, C5, C6, C7, C8 and C9.

Pathways of activation

There are three known pathways for complement activation: Classical, Alternative and Lectin pathway.

Classical pathway

The classical pathway is initiated by IgM or IgG antigen/antibody complexes binding to C1q (first protein of the cascade) leading to activation of C1r, which in turn cleaves C1s. This in turn activates the serine proteases that lead to cleaving of C4 and C2, leading to formation of C4b2a (C3 convertase), which in turn cleaves C3 into C3a and C3b [29]. While C3a acts as a recruiter of inflammatory cells (anaphylatoxin), C3b binds to the C4b2a complex to form C5 convertase (C4b2a3b). The C5 convertase initiates the formation of the Membrane Attack Complex (MAC), that inserts into membrane creating functional pores in bacterial membranes leading to its lysis [30]. The classical pathway can also be activated by other danger signals like C-reactive protein, viral proteins, polyanions, apoptotic cells and amyloid, thus providing evidence that classical pathway could be activated independent of antibodies [31–34].

Alternative pathway

Fifty years after the discovery of the classical activation pathway, Pillemer et al. [35, 36] proposed a highly controversial alternative activation pathway. Initially, this was rejected by the scientific community and only substantiated and accepted more than a decade later. Pillemer's hypothesis was based on observations that the complement system could be activated by direct binding of bacteria and yeast independent of antibody interaction [37]. It was originally named the 'properdin pathway' and is now known as the alternative pathway [33]. The alternative pathway is not so much an activation pathway, as it is a failure to regulate the low level continuous formation of a soluble C3 convertase. The internal thioester bond of C3 is highly reactive and undergoes spontaneous hydrolysis resulting in a molecule known as C3 (H₂O) which resembles C3b. This can then bind to factor B, and be processed into a short lived soluble C3 convertase that can generate more C3b. If this C3b binds to a nearby surface that is incapable of inactivating it (such as bacteria/yeast cells or damaged host tissues), this then leads to amplification of the alternative pathway [38–40]. The presence of complement regulators in healthy cells ensures the spontaneous hydrolysis of C3 is kept in check. C3 activation takes place when C3b binds to factor B and is then cleaved by factor D (a process which is stabilised by magnesium ions and properdin) [41]. The enzymatic action of factor D acts as the rate limiting step of the alternative pathway and cleaves factor B, the larger fragment of which remains bound to C3b to form the alternative pathway C3 convertase–C3bBb [29, 42]. C3b is able to create new C3 convertase in the presence of Factors

B and D, thus acting as an ‘amplification loop’ for other pathways, as well as the alternative pathway [33]. The alternative pathway omits the components C1, C2 and C4.

Lectin pathway

Forty years after the proposal of the alternative pathway, the MBL (mannose-binding lectin)/MASP (MBL-associated serine protease) pathway was discovered. This pathway was characterised by using proteins isolated from rabbit liver and serum, but its function remained unclear initially [43, 44]. Two forms of MBL (MBL-A and -C) are present in rodents compared to a single form in the humans. Studies linking the deficiency of MBL protein to immunodeficiencies in children led to its recognition as an important activator of the complement system [45, 46]. The initiating molecules for this pathway are collectins (MBL and ficolin), which are multimeric lectin complexes. These bind to specific carbohydrate patterns uncommon in the host, leading to activation of the pathway through enzymatic activity of MASP [41]. There are structural similarities shared between MBL and C1 complexes (MBL- with

C1q-associated serine proteases, MASP-1 and MASP-2 with C1r and C1s, respectively), leading to the belief that complement activation by MBL and C1 complexes are similar [47]. MASP-2 cleaves C4 and C2 to form C3 convertase, while MASP-1 may cleave C3 directly bypassing the C4b2a complex, albeit at a very slow rate [48, 49]. Another serine protease, MASP-3 was shown to down-regulate the C4 and C2 cleaving activity of MASP-2 [50]. Following the initial characterisation of MBL, 3 other lectins (known as ficolins) have been shown to interact with MASP: ficolin-1 (or M-ficolin), ficolin-2 (or L-ficolin) and ficolin-3 (or H-ficolin or Hakata antigen). The ficolins activate the lectin pathway by forming active complexes with MASP [51, 52]. More recently, a new C-type lectin (CL-11) was shown to interact with MASP-1 and/or MASP-3 and could activate the lectin pathway [53].

Other activators of the complement system

Various serine proteases belonging to the coagulation system have also been shown to activate the complement cascade independent of the established pathways. *In vitro*

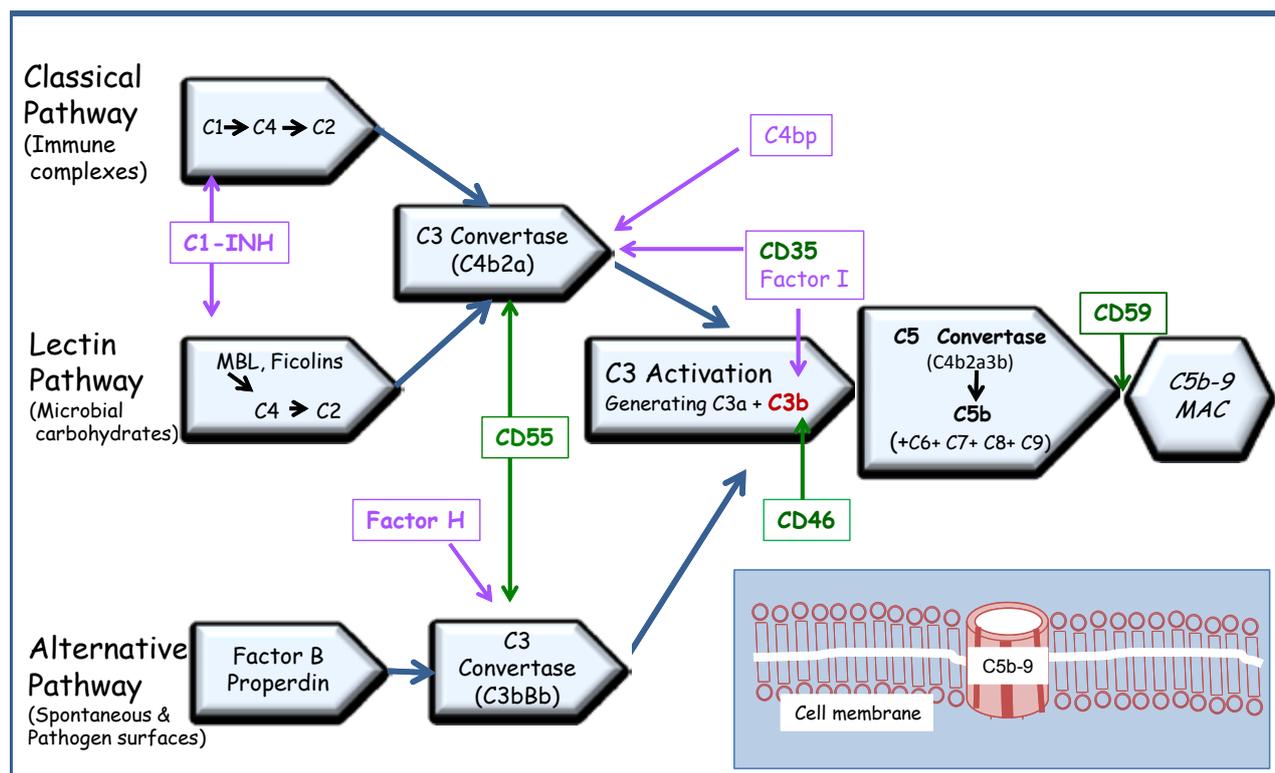


Fig. 1. Pathways of complement activation: classical, alternative and lectin pathway: IgM or IgG antigen/antibody complexes binding to C1q, the first protein of the cascade, initiates the classical pathway. The alternative pathway is not so much an activation pathway, as it is a failure to regulate the low level continuous formation of a soluble C3 convertase. The third pathway is known as MBL (Mannose-binding lectin)/MASP (MBL associated Serine Protease) pathway. The initiating molecules for the MBL pathway are multimeric lectin complexes that bind to specific carbohydrate patterns uncommon in the host, leading to activation of the pathway through enzymatic activity of MASP. The sites of action of the membrane bound complement regulators—CD35, CD46, CD55 & CD59 (green boxes) and the fluid phase regulators – C1-INH, Factor H, Factor I and C4bp (violet boxes) are represented with arrows

Insert: Membrane Attack Complex (MAC). The interaction of C5b with C6, C7, C8 and C9 leads to formation of C5b–9 or Membrane Attack Complex (MAC), a multimolecular structure that inserts into the membrane creating a functional pore leading to cell lysis

findings suggested that the coagulation factors FXa, FXIa and plasmin can cleave both C5 and C3, leading to generation of anaphylatoxins C5a and C3a [54]. Studies have documented FVIII and von Willebrand factor to possess lectin activity [55]. Vice versa, complement factors are also known to interact with the coagulation system. C1 inhibitor was shown to block the endogenous coagulation pathway [56], while C5a was shown to induce tissue factor (membrane glycoprotein that serves as a cofactor for blood coagulation factor VIIa) activity on endothelial cells [57]. Individual cells have also been implicated in activating certain elements of complement pathway. Huber-Lang et al. showed that phagocytic cells, especially lung macrophages could generate C5a from C5 independent of the plasma complement system using cell bound serine proteases [58]. C-reactive protein is an acute phase reactant that can activate the classical pathway of the complement system, and its role in the complement led ischemia–reperfusion injury (IRI) has been shown in intestinal and myocardial animal IRI models [34, 59]. Similarly, cross-talk between complement and toll-like receptors has shown to be possible due to mitogen activated protein kinases in renal IRI setting [60]. Cross-talk between complement system and other systems will exist, and future research will be aimed at evaluating these ‘communicators’ between systems.

Complement cascade

The principal function of the complement system is protection of the host from infection/inflammation by recruiting (chemotaxis) and enhancing phagocytosis by innate immune cells (opsonisation), leading to lysis of the target cells. All three pathways lead to the generation of C3 convertase that cleaves the C3 protein into C3a and C3b. While C3a acts as an anaphylatoxin, C3b covalently binds to the activating surface and participates in the self-activation loop of complement activation via the alternate pathway. C3b also associates with C3 convertases (C4b2a or C3bBb) to form the C5 convertase, which cleaves C5 complement into C5a and C5b [61]. Interaction of C5b with C6, C7, C8 and C9 leads to formation of C5b–9/MAC, a multimolecular structure that inserts into the membrane creating a functional pore leading to cell lysis [30]. MAC can cause lysis of some cells (e.g. erythrocytes) with a single hit, but some nucleated cells required multiple hits, or rather, multiple channel formation to cause cell lysis [62, 63]. However, studies have shown that when the number of channels assembled on the cells is limited, sublytic C5b–9 can activate transcription factors and signal transduction, leading to inhibition of apoptosis and cell homeostasis [64, 65]. The complement cascade with the inherent inhibitors is shown in *Figure 1*.

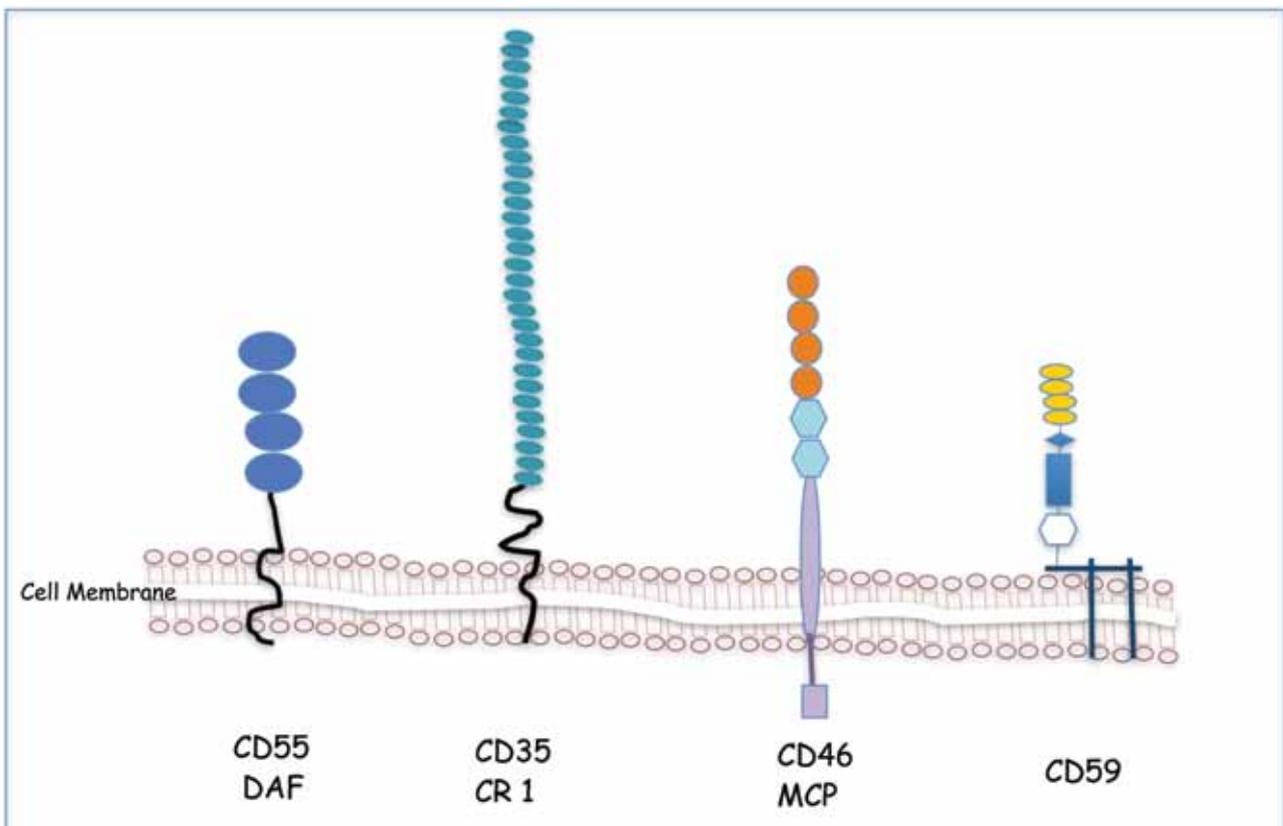


Fig. 2. Membrane Bound Complement Regulators: DAF, CR1 and MCP belong to a gene super family called as ‘regulators of complement activation’ (RCA)/complement control proteins (CCP) and share a common structural motif called short consensus repeat (SCR). The SCR structure (circles) consists of around 60 amino acids held together by two disulfide bridges formed by cysteine residues. CD59 is a GPI-anchored membrane complement that is expressed on almost all cells in the body. CD59 is the only well-characterised membrane inhibitor acting at the terminal step and prevents the assembly of the MAC

The anaphylatoxins (C3a and C5a) are key players in the recruitment of inflammatory cells and release of mediators that amplify the inflammatory response. C5a is probably the principal anaphylatoxin mediating inflammation. C5a binds to C5a receptor (C5aR or CD88) that is widely present on inflammatory and non-inflammatory cells [66, 67]. Apart from recruiting the neutrophils, C5a also increases neutrophil adhesiveness and aggregation. C5a causes secretion of pro-inflammatory cytokines and lysosomal enzymes from the macrophages and monocytes, thus leading to chemotaxis [29, 68, 69]. C5a also up-regulates adhesion molecules such as α -integrin and β 2-integrin; in particular, Mac-1, in polymorphonuclear leukocytes [70, 71]. C5a was shown to be an important inflammatory mediator for the early adhesive interactions between neutrophils and endothelial cells in the acute inflammatory response [71]. It is responsible for up-regulation of vascular adhesion molecules such as P-selectin, E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) [29, 72].

C3a does not act as a chemoattractant for neutrophils, but aids migration of eosinophils and mast cells [73, 74]. C3a and C5a also act on their receptors expressed on innate immune cells such as dendritic cells, thus playing a role in initiating and regulating T cell responses [75]. In the IRI setting, MAC has been shown to mediate IR injury, and its inhibition was shown to attenuate the IRI effect [76, 77].

Inherent regulation of pathways

To prevent inadvertent injury by activated complement, the host tissues have developed intricate and elaborate mechanisms in the form of soluble and membrane bound complement regulators that inhibit complement activation. The two main regulation mechanisms are: decay-acceleration activity (DAA) which increases the rate of dissociation of (C4b2a and C3bBb) C3 convertases, and factor I cofactor activity (CA), which results in the factor I-mediated cleavage of covalently bound C3b and C4b into inactive fragments incapable of reforming the C3 convertases [78, 79]. The pathways are regulated by both membrane-bound and fluid phase complement regulators that keep the complement system in check.

Membrane bound complement regulators

The membrane bound regulators—DAF, CR1 and MCP belong to a gene super family called as ‘regulators of complement activation’ (RCA)/Complement control proteins (CCP) and share a common structural motif called short consensus repeat (SCR). The SCR structure consists of around 60 amino acids held together by two disulfide bridges formed by cysteine residues [80]. The structural moiety of the membrane bound complement regulators are depicted in *Figure 2*.

CD35 {complement receptor 1 (CR1)}: classical, lectin and alternative pathway

CD35 is a transmembrane glycoprotein that facilitates the decay of C3/C5 convertase in both the classical and alternate pathways and acts as a co-factor for factor I in the degradation of C3b and C4b [81]. Human CR1 is found on B cells, follicular dendritic cells, erythrocytes, polymorphonuclear cells, phagocytic macrophages and on podocytes in the glomerular of the kidney [82]. Expression of CD35 on erythrocytes is believed to be crucial in handling circulating immune complexes and abating the development of autoimmunity.

CD46 {membrane cofactor protein (MCP)}: classical, lectin and alternative pathway

CD46 acts as a cofactor for factor I mediated cleavage of C3b and C4b. It is widely expressed in humans apart from the erythrocytes, while in the rodents, it is expressed only in the testis [83]. By regulating the production of interferon (IFN)- γ and interleukin (IL)-10 in the T helper cells, it is involved in the down-modulation of adaptive T helper type 1 immune responses [84]. Deficiency of CD46 is a predisposing factor for numerous disease conditions arising from complement-mediated ‘self-attack’.

CD55 {decay accelerating factor (DAF)}: classical and alternative pathway

CD55 is a glycosyl-phosphatidyl-inositol (GPI) anchored membrane protein that is widely expressed on vascular and non-vascular tissue cells. The main role of CD55 is the inhibition and acceleration of the decay of classical and alternative pathways C3 convertase [85]. Apart from complement regulation, human DAF is known to act as a receptor for infection by certain viruses (echovirus and coxsackie B virus) and serves as a ligand for activation-associated leukocyte antigen CD97 [82]. Like rodent CD46, rodent CD55 is limited in its tissue expression.

CD59 (protectin): membrane attack complex

CD59 is a GPI-anchored membrane protein that is expressed on almost all cells in the body [86]. CD59 is the only well-characterised membrane inhibitor acting at the terminal step, and prevents the assembly of the MAC by inhibiting the C5b-8 catalysed insertion of C9 into the lipid bilayer [87].

CrrY {Complement receptor 1-related gene/protein Y}

CrrY is a transmembrane protein specific to rodents and is widely expressed on rat and mouse cells to compensate

for the lack of rodent CD55 and CD46 expression. CrrY possesses both DAA and CA properties and mimics the activities of the human DAF and MCP which regulate C3 deposition on host cells [88].

Fluid phase or 'soluble' regulators:

C1-Inhibitor (C1-INH): classical and lectin pathway

In the fluid phase, the best-known regulatory protein is C1-Inhibitor (C1-INH), which is synthesised in the liver and by monocytes. It forms an irreversible complex with the serine proteases C1r and C1s, typical of serpin regulation, and inactivates them. This leads to the disassociation of C1r and C1s from C1q in the complex. C1-INH can also bind to MASP-1 and MASP-2 and inactivate them leading to disruption of the lectin pathway [89, 90]. Under physiologic conditions, activated C1 has a half-life of only 13 seconds in the presence of C1-INH (regulates nonspecific complement activation) [91].

C1-INH inhibits other serine proteases, like kallikrein, along with coagulation factors (XIa, XIIa and plasmin), thus playing a role in coagulation regulation [92]. Absence or low levels of C1-INH results in conditions like hereditary angioneurotic oedema where spontaneous activation of C1 and kallikrein leads to the manifestation of symptoms [93]. Various animal IRI models have shown that C1-INH can protect liver, intestine, heart and brain tissue from ischemia–reperfusion damage [94].

Factor I: classical, lectin and alternative pathway

Factor I cleaves C3b and C4b to form C3 and C4 fragments (iC3b, C3c, C3dg and C4c and C4d, respectively), thus blocking the formation of C3 and C5 convertase enzymes [89]. The cofactors supporting factor I cleavage are factor H, CD35, CD46 and C4b-binding protein [95]. Factor I is secreted by cells such as hepatocytes, macrophages, lymphocytes, endothelial cells and fibroblasts. The outcome after renal transplantation is poor in patients known to have either a complement factor H or complement factor I mutation, with approximately 80% of patients losing the graft to recurrent disease within 2 years [96]. Mutations in factor I are linked with occurrence of atypical hemolytic uremic syndrome (HUS) [97] in human and increases the susceptibility to pyogenic infections like meningitis and upper respiratory tract infections [98].

Factor H: alternative and classical pathway

Factor H possesses multiple binding sites for C3b and accelerates the decay of the alternative C3 convertase through 'competitive binding' for factor B [99]. It also facilitates the cleavage of C3b by supporting factor I activity. It plays an essential role in controlling the alternative

pathway in blood and on cell surfaces. Impaired recognition of factor H by host cell surfaces due to mutations and polymorphisms can lead to complement-mediated tissue damage and disease [100]. It is mainly synthesised in the liver, with minimal contributions from fibroblasts and endothelial cells. Genetic changes in factor H are linked to clinical conditions like HUS, membranoproliferative glomerulonephritis (dense deposit disease) and age-related macular degeneration [101].

C4bp (C4b-binding protein): classical and lectin pathway

C4bp is a regulator of the classical and lectin complement pathways. It binds to C4b and accelerates the decay of the C3 convertase [102, 103]. It also acts as a cofactor for the cleavage of C4b by factor I. It is predominantly synthesised in the liver and to a lesser extent by activated monocytes. C4bp has a complex structure, mainly composed of alpha-chains with a single copy of a beta-chain. C4bp possesses binding sites for heparin and C-reactive protein as well [104]. C4bp is up regulated in certain autoimmune diseases like lupus, but true deficiency state associated clinical conditions are yet to be reported.

Carboxypeptidase N: anaphylatoxin inactivator

Carboxypeptidase N was found to abolish the activity of the anaphylatoxins C3a and C5a and also those derived from bradykinin [105]. It is synthesised in the liver and cleaves carboxy-terminal arginines and lysines from peptides (such as complement anaphylatoxins, kinins and creatine kinase MM-skeletal muscle) found in the bloodstream [106]. Removal of the terminal arginine from C3a and C5a results in formation of C3a (desArg) and C5a (desArg), both of which have markedly lower ability to signal through receptor binding. Carboxypeptidase N plays an important role in protecting the body from excessive build up of potentially deleterious peptides that can act as local autocrine or paracrine hormones [107].

Conclusion

As evident by the historical progress, our understanding of the complement system is expanding. The complement system has been implicated in a variety of conditions: autoimmune diseases, sepsis, transplantation, ischemia-reperfusion injuries, traumatic brain injury, infections and bone biology [33]. Target areas for complement immunomodulation include blocking the activation pathways and developing specific complement inhibitors. Presently, rodent models dominate the complement research field, but whether findings from animal models can be potentially translated into human clinical application remains to be seen.

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