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The multifunctional role of C3, the third component of complement

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The third component of complement, C3, is an important participant in immune surveillance and immune response pathways. It is probably one of the most versatile and multifunctional molecules known, interacting with numerous serum proteins, cell surface molecules and foreign proteins. Here, John D. Lambris reviews the multiple interactions of C3 emphasizing recent work on these interactions and addresses some of the unanswered questions and controversies.

The number of serum proteins, cell surface receptors and foreign proteins found to react with the third component of complement (C3), as well as the biological functions mediated by the different C3 fragments, have increased in the past ten years. This, together with the facts that the different fragments of C3 can interact simultaneously with various receptors or with other complement proteins, the various serum C3-binding proteins are synthesized by cells of the immune system, and the C3 fragments are processed during cell culture, leads to ambiguities when interpreting the biological effects ascribed to C3. The numerous responses mediated by the various C3 fragments indicate that the same fragments of C3 can exert markedly different effects, including stimulation or inhibition of cell proliferation as well as effects unrelated to proliferation. Some aspects of the structure and function of complement receptors and their ligands have been addressed in previous reviews[1–6]. The purpose of this review is to summarize C3–'ligand' interactions, emphasizing the recent findings pertaining to the structural features involved in these interactions and their relationship to the different functions of C3.

The C3 molecule

C3 is one of 30 complement proteins recognized to date; it has been the focus of intensive studies due to its functional versatility and its pivotal role in both the classical and alternative pathways of complement activation. This 185 kDa protein is the most abundant complement protein present in serum (1–2 mg ml^{-1}), and is comprised of two polypeptide chains (a 110 kDa α chain and a 75 kDa β chain) linked by one disulfide bond and by noncovalent forces. It contains two *N*-linked carbohydrate moieties at residues 63 and 917, with 8–9 and 5–6 mannose residues, respectively[7].

Cleavage of C3 between residues 77 and 78 (Arg–Ser) of the α chain, by either the classical (C4b,2a) or the alternative (C3b,Bb) pathway C3 convertases, leads to the generation of the C3b and C3a fragments. The C3b molecule transiently acquires the ability to be fixed covalently, through an ester or amide bond, to the hydroxyl or amino groups present on cell surfaces, complex carbohydrates, or immune complexes[8,9]. This covalent binding of C3b is a function of its thiolester group and involves the sulfur of Cys 988 and the carbonyl group of Glu 990, both of which are located in the C3d fragment of C3 (Ref. 9).

In contrast to native C3, C3b expresses multiple binding sites for other complement components, including C5, properdin (P), factors H, B and I, C4-binding protein (C4bp), CR1 (C3b receptor) and the membrane cofactor protein (MCP). Binding of these proteins to C3b leads either to amplification of the C3 convertase (by B and P in the presence of factor D) and initiation of the membrane attack complex (C5), or to the inactivation of C3b (MCP, CR1, H, C4bp by factor I). Whether amplification or inactivation occurs depends on the nature of the surface to which C3b is fixed.

The inactivation of C3b by factor I proceeds in three steps[10–14] and requires one of the several cofactor molecules (MCP, CR1, CR2, H, C4bp) that induce conformational changes in C3b necessary for I to exert its action. The cleavage of the α chain of C3b, first between residues 1281–1282 (Arg–Ser) and second between residues 1298–1299 (Arg–Ser) of C3, liberates the 2 kDa C3f fragment and yields iC3b (Refs 10–14). In addition, it has been suggested that factor I mediates a third cleavage between residues 932–933 (Arg–Glu), thus generating C3dg with concomitant liberation of C3c (Refs 11–14). However, the presence in the human C3 sequence of Arg–Glu in this position instead of the Arg–Ser, and the presence of Gln–Gly in mouse[15] and rabbit[16] C3 sequences, questions the cleavage of iC3b by factor I at this site. This suggests that enzymes other than factor I may mediate this cleavage. The contention that factor I does not make this third cleavage has been supported by studies where the factor I cleavages were carried out in the presence of protease inhibitors[17]; therefore, further studies are required to clarify which enzyme is responsible for this third cleavage. Even though the physiological enzyme(s) is not yet known, further proteolysis of C3dg by trypsin, elastase or plasmin generates C3d (Ref. 11). The C3 fragments, both soluble and/or surface bound, generated during complement activation can bind specifically to several cell surface receptors, known as CR1, CR2, CR3, CR4, CR5 and C3a receptors (Table 1); these C3–receptor interactions then lead to various biological responses.

Biosynthesis and genetics of C3

Although 90% of C3 synthesis is in the liver, its synthesis in monocytes, astrocytes, B lymphocytes and several tumor cell lines of different origins has been described[18,19]. C3 is synthesized as a single chain precursor, and after postsynthetic modification the native two-chain molecule is generated. The synthesis of C3 as well as of other complement components in tissues other than the liver may play a very important role in local inflammatory processes.

Studies on the allelic variants of C3 indicate that 22

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Table 1. Summary of cell surface proteins reacting with C3

Protein	Specificity	Cell type
C3aR	C3a, C4a	Mast cells, neutrophils, monocytes, basophils, macrophages, T lymphocytes, eosinophils
CR1	C4b, C3b, iC3b, C3c	Erythrocytes, Langerhans cells, neutrophils, monocytes, eosinophils, B and some T lymphocytes, follicular dendritic cells, milk and some tonsil macrophages, glomerular podocytes, also in gut Schwann's cells
CR2	iC3b, C3dg	B lymphocytes, follicular dendritic cells
	C3d, gp350	Thymocytes, B- and T-cell lines
CR3	iC3b, C3d	Neutrophils, monocytes, macrophages, follicular dendritic cells, natural killer (NK) and ADCC effector lymphocytes
CR4	iC3b	Monocytes, neutrophils, macrophages, NK and ADCC effector lymphocytes
CR5	C3dg/C3d	Neutrophils, platelets
DAF	C3bBb	Erythrocytes, all leucocytes, platelets
	C4bC2a	Epithelial cells, lymphocytes
MCP	C3b, C4b	Neutrophils, monocytes, most lymphocytes, platelets, reticulocytes
Factor H	C3b	Monocytes, tonsil B cells, Raji and U937 cells

different variants exist, the most common of which are C3F and C3S[18]. Even though initial studies in mice suggested a possible linkage of the C3 gene to the major histocompatibility complex (MHC), recent studies have shown that the mouse C3 gene (chromosome 17) is located some distance from the MHC. The human C3 gene is on chromosome 19. Exon mapping of the C3 gene indicates that the α′ chain of C3 is encoded by 24 different exons[19]. Taken together with the location of C3 ligand-binding sites (Fig. 1), this implies that the CR1-, CR2-, CR3-, H- and properdin-binding sites in C3 are encoded by separate exons.

C3 deficiencies

Deficiencies of C3 have been described so far in humans, guinea pigs and dogs. Patients with C3 deficiency have an increased susceptibility to infections that lead to purulent lesions. Although the antibody response to routine immunizations is normal, an impaired switch from IgM to IgG was observed in two C3-deficient patients immunized with limited doses of the T-dependent antigen, bacteriophage ϕX174 (Ref. 20). A similar defective antibody response was mounted in C3-deficient guinea pigs[21] and in dogs[22]. These findings and those made using pharmacologically C3-depleted animals suggest that C3 plays an important role in the generation of a normal immune response.

C3 in different species

C3 purified from humans, rabbits, mice, chickens, quail, *Xenopus*, rainbow trout and cobra has a similar molecular mass and chain structure. Cloning of human, mouse, rabbit and *Xenopus* C3 cDNA and cDNAs of other homologous proteins such as C4, C5 and α_2-macroglobulin allowed the analysis of their genes and the localization of these genes on different chromosomes. In addition, comparison of the deduced amino acid sequences of the above proteins, with the conservation of C3–'ligand' interactions between different species has been instrumental in elucidating some of the details of the C3–'ligand' interactions[23].

In contrast to C3 from the species indicated above, a protein called 'C3' purified from lamprey serum was found to consist of 84 kDa, 74 kDa and 32 kDa chains[24]. The amino acid sequence of a fragment containing the thiolester site showed a high degree of homology with the corresponding fragment of mammalian C3. Since the chain structure of this molecule is similar to C4, and the reported functions for this molecule are also properties of C4, it is not clear if it represents lamprey C3 or C4. These similarities, in view of the idea that both C3 and C4 evolved from a common molecule and in conjunction with the evolutionary antiquity of cyclostome species, suggest that this molecule may be the ancestor of mammalian C3 and C4.

Cell surface molecules interacting with C3

C3b/C4b receptor (CR1)

The complement receptor type one (CD35) was first isolated by Fearon in 1978 from human erythrocytes by its ability to control the amplification of C3 convertase[25]. It is specific for the C3b, iC3b, C3c and C4b complement fragments and exhibits an unusual genetic polymorphism with four different allotypes (160 kDa (C), 190 kDa (A), 220 kDa (B) and 250 kDa (D) forms). It is present on a variety of cell types (Table 1)[1,26,27]. The affinity of CR1 for the different fragments of C3 depends on the type of the fragment reacting with the receptor and whether they are in fluid phase or surface bound. Dimeric C3b has an affinity for CR1 ($K_a = 7 \times 10^7\ M^{-1}$) that is approximately 50–100 times greater than that of monomeric C3b, and 150 times greater than that of iC3b (Refs 26, 27). Recently, we localized the CR1-binding site within the 42 amino-terminal amino acids of the α′ chain of C3b (residues 727–768 of the C3 sequence)[28] (Fig. 1). A synthetic peptide covering this area of C3b, and the corresponding anti-peptide antibody both inhibited CR1 binding to C3b and C3c. The same region of C3b was previously suggested to be involved in the binding of factors H and B to C3b (Ref. 29).

The biological effects of the interaction between CR1 and the different C3 fragments are diverse, and depend on the nature of ligands interacting with the receptor as well as on the cell types involved[1,26,27]. Among the CR1-mediated functions are (1) the inhibition of complement activation, by either increasing the decay/dissociation of the C3 convertase in both complement pathways or by acting as a cofactor for the factor-I-mediated inactivation of C3b, (2) the processing of immune complexes, (3) the promotion of binding and phagocytosis of C3b-coated particles by phagocytic cells, and (4) its participation in several types of immune responses.

C3d/EBV receptor (CR2)

Complement receptor type 2 (CR2) (CD21) is a 140 kDa glycoprotein[30] that binds to iC3b and C3dg/C3d fragments of C3. Although initially thought to be present only on B lymphocytes, CR2 expression by other

cell types has recently been established[1,26,27] (Table 1). Its presence on immature thymocytes[31] is intriguing, as it could play a role in thymocyte differentiation and function.

As well as being the receptor for the different fragments of C3, CR2 also serves as a receptor for Epstein–Barr virus (EBV). The CR2-binding site in C3 (C3d) was localized within residues 1205–1214 of C3 (Fig. 1)[32], and these residues were subsequently found to be similar to a stretch of amino acids in the gp220/350 protein of EBV. A peptide corresponding to this segment (residues 19–31 of gp350) could bind to CR2 and inhibit the ability of C3 to support the growth of Raji cells[33]. This finding suggests that this segment of gp350 is involved in the interaction of EBV with CR2.

The involvement of CR2 in the modulation of the immune response is well established, even though the mechanism by which this occurs is unclear. Cross-linking of the receptor on the cell surface by anti-CR2 antibodies or particle-bound C3d enhances B-cell proliferation in the presence of T-cell-derived factors. In addition, C3 or monoclonal anti-CR2 antibodies support the growth of Raji cells in serum-free medium (reviewed in Ref. 27).

Due to the multifunctional role of C3, the nature of the ligands interacting with CR2 and stimulating B-cell activation are difficult to characterize. The C3d fragment of C3 thought to bind exclusively to CR2 has now also been shown to react with factor H (Ref. 34) and CR3 (Ref. 35). Since C3d can react with different receptors, synthetic peptides were used that mimic C3d in its ability to bind CR2; the effect of C3d on B-cell growth can be replaced by soluble peptides spanning residues 1201–1214 of human C3 (Refs 36, 37). In addition, synthetic peptides corresponding to the CR2-binding site in C3d supported the growth of $CR2^+$ human B-lymphoblastoid lines in serum-free medium. The growth-supporting effect of tetrameric P13 (residues $(1202–1214)_4$) but not monomeric P14 (1201–1214), and the inhibition of tetrameric P13-driven growth by monomeric P14, indicate that cross-linking of CR2 is a necessary signal for B-cell activation[33].

iC3b receptor (CR3)

Complement receptor type 3 (CR3) is a heterodimer found on the cell surface of monocytes, macrophages, granulocytes, follicular dendritic cells and large granular lymphocytes that bind the iC3b fragment of C3 (Refs 1, 26, 27). In addition to the binding site for iC3b, a second binding site on CR3 for polysaccharides has been identified by its ability to bind to lipopolysaccharide and to zymosan, the latter being inhibited by *N*-acetyl-D-glucosamine[26,27]. CR3, in addition to functioning in the phagocytosis of iC3b-coated particles, plays a role as an adhesive molecule in cellular interactions. CR3 is comprised of noncovalently associated 170 kDa α (CD11b) and 95 kDa β (CD18) chains. The β chain of CR3 is identical to the β chain of two other leukocyte receptors, LFA-1 and gp150/95, both of which are highly homologous to other cell adhesive molecules, termed integrins[38–41]. Other receptors in this superfamily are the fibronectin and vitronectin receptors, platelet glycoprotein IIb/IIIa, and the VLA family of leukocyte markers[42]. The ligands that bind to these integrins possess a domain containing the RGD (Arg-Gly-Asp) sequence, which has been implicated as a 'recognition site' in ligand–receptor

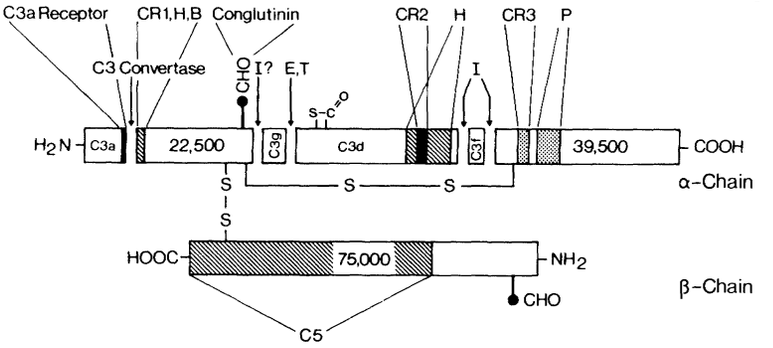

Fig. 1. *A schematic representation of the C3 molecule. Sites of C3 cleavage by C3 convertases, factor I (I), elastase (E) and trypsin (T) are indicated. The localization of the C3-binding sites for CR1 (Ref. 28), CR2 (Ref. 32), CR3 (Ref. 43), C3a receptor[47], H[29,34], properdin[57], C5 (Ref. 60), B[29] and conglutinin[64] is described in the references indicated. The position of the residues (see text) corresponding to these sites is based on the predicted[76] amino acid sequence of C3 after subtracting the signal peptide sequence.*

interactions. An RGD sequence was also found in the α chain of C3, and a synthetic peptide from this region (residues 1361–1380) could bind to CR3 (Fig. 1)[43]. However, it is unlikely that the RGD sequence alone is responsible for CR3 binding, since this sequence is replaced, in mouse[15] and rabbit[16] C3, by LGD and in *Xenopus* C3 by LKN (D. Grossberger, L. Du Pasquier and J.D. Lambris, unpublished). In addition to its binding to the RGD-containing site, CR3 has also been reported[35] to bind to C3d which suggests a two-site interaction between CR3 and iC3b.

p150/95 (CR4)

The p150/95 glycoprotein (CD11c), a member of the family of leucocyte adhesion receptor molecules, is expressed on myeloid cells and on some activated lymphocytes (Table 1). It binds iC3b in a divalent cation-dependent manner[44,45]. The physiological role of p150/95 is not yet clear but it appears to have similar properties to CR3.

'CR5'

Binding of the C3dg fragment of C3 to neutrophils and platelets (independent of divalent cation) suggested the presence of a receptor distinct from those mentioned above[44,46]. The nature as well as the function of this receptor remains speculative. A 90 kDa molecule purified from neutrophils and spleen cells has been suggested to be 'CR5'. This molecule reacted with iC3b, C3dg and C3d fragments of C3 (Ref. 44). The term CR5 and the designation of p150/95 as CR4 is not established and is often interchanged in the literature.

C3a receptor

Although the C3a fragment of C3 has been studied in detail, assisted by its crystallization and the use of synthetic peptides, the existence of a C3a receptor is based mainly on the ability of C3a to induce diverse effects on various cell types. Extensive studies on the function of C3a using synthetic peptides that span its carboxy-terminal 21 amino acids showed that the minimum length of peptide that elicits similar effects as C3a consists of the five carboxy-terminal amino acids of C3a (reviewed in Ref. 47). In addition to C3a, the C3a receptor reacts with C4a, but not with C5a; the latter

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Table 2. Summary of serum C3-binding proteins

Protein	Specificity	Function within complement activation
Factor H	C3b, iC3b, C3d	Cofactor for factor I Accelerates the decay of C3/C5 convertase
Factor B	C3b	Zymogen of Bb that forms the C3/C5 convertase
Factor I	C3b, iC3b	Serine protease degrading C3b
Properdin	C3b, iC3b, C3c	Stabilizes the C3/C5 convertase
C5	C3b	Initiates the formation of the membrane attack complex
Conglutinin	iC3b, C3c	Unknown

two anaphylatoxins are also generated during complement activation from the corresponding C4 and C5 complement components. Besides its effects on the induction of smooth muscle contraction and the increase of vascular permeability, C3a has also been found to mediate histamine release from mast cells, eosinophils, neutrophils and basophils, thromboxane A release from macrophages, and the secretion of lysosomal enzymes from neutrophils. The participation of C3a in the immune response has also been suggested due to the C3a-mediated inhibition of certain lymphocyte functions, including both polyclonal and specific antibody production and mitogenesis induced by concanavalin A, pokeweed mitogen and phytohemagglutinin[47].

Membrane cofactor protein (MCP) and decay-accelerating factor (DAF)

MCP (gp45-70) is a highly glycosylated molecule found on various cell types (Table 1) that functions as a surface cofactor for the inactivation of C3b, iC3b and C4b by factor I (Ref. 48). DAF[49], in addition to the cells that express MCP, is also found on erythrocytes. Its function is to accelerate the decay of the classical and the alternative pathway C3 convertases. Although DAF interacts primarily with the catalytic subunits of C3 convertases, a low-affinity interaction (apparent K_a 45 nM^{-1}) with C3b has been demonstrated[50].

Serum C3-binding proteins (Table 2)

Factor H

Factor H is a single chain 150 kDa polypeptide present in serum at a concentration of 500 $\mu g\ ml^{-1}$. Its effects on complement activation, via the alternative pathway, are exerted by binding to C3b and acting as an antagonist of factor B binding to C3b, as a decay-accelerating factor for the C3/C5 convertases, and as a cofactor for the factor-I-mediated cleavage of C3b (reviewed in Ref. 51). In addition to its presence in serum, factor H is also found on the cell surface of lymphocytes and monocytes, although its role here is unclear. A factor H cell-surface receptor has been found on B cells and monocytes, and it has been implicated in several cell responses[26,27].

Factor H and several other C3-binding complement molecules – including CR1, CR2, MCP, DAF and C4-binding protein (C4bp) – belong to a family of proteins that contain homologous repeats of about 60 amino acids[4–6]. These proteins also share functional similarities with respect to complement activation: all, except CR2, have decay-accelerating activity, and all, except DAF, serve as cofactors with different efficiencies for the cleavage of C3b by factor I. The structural and functional similarities of these molecules led to the speculations that a conserved structure in these molecules mediates their binding to C3b and these molecules may share similar binding sites within C3. This has recently been shown by the localization of both the H (Ref. 34) and CR2 (Ref. 32) binding sites in C3d within a region spanning residues 1192–1249 of C3, and the second H (Ref. 29) site in C3b within the same area as the CR1-binding site[28].

The cleavage of C3b by factor I at different sites suggested that these cofactor molecules may interact with C3 via multiple sites. In the α chain of C3, three different interaction sites for factor H have been described; two of these sites reside in the C3c fragment[29,52] and one in the C3d fragment[34] of C3. The C3d-binding site for H appears to be a discontinuous binding site that spans residues 1187–1249 of C3. The second H site in C3b, also involved in the C3b–factor B interaction, has been reported to be in the amino-terminal 42 amino acids of the α'-chain of C3b (residues 727–768 of C3)[29]. This segment of C3b was recently found to contain the CR1-binding site[28].

Factor B

The initiation of the alternative pathway has been proposed to start with the binding of factor B to hydrolysed C3 ($C3(H_2O)$). This initial interaction, via the Ba domain of factor B, enables factor D to cleave B to Bb and Ba, which then leads to the formation of the $C3(H_2O)Bb$ complex. This complex, thought to be the initial C3 convertase of the alternative pathway, cleaves C3 to C3b which, in the presence of factors B and D, can form the C3bBb convertase. The formation of the convertase is Mg^{2+} dependent and can be stabilized by properdin (reviewed in Ref. 50). The formation of the C3bBb complex suggests that a second site, in addition to the Ba site[53], exists in factor B for C3b. A 33 kDa carboxy-terminus Bb fragment (also containing the enzymatic site) binds to C3b with higher affinity than Bb or native factor B[54]; although it is not yet proven, this fragment may contain the Bb site for C3b. The interactions between Bb and C3b imply that at least two sites exist in factor B for C3b and suggest that C3b may also have two sites for the B fragments. Although the use of monoclonal anti-C3c or anti-C3d antibodies suggested that this may be the case, further investigation is required on this, as well as on the possible relationship between these sites and the cofactor-binding sites.

Factor I

Factor I, an 88 kDa two chain serine protease, mediates the cleavage of $C3(H_2O)$, C3b and C4b after modification of these molecules by the different cofactor molecules. Although direct binding of factor I to C3b has not been shown[55], factor I associates with the C3b–H complex with an apparent K_a of $1 \times 10^8\ M^{-1}$.

Properdin

Properdin (P) is a plasma glycoprotein that participates in the regulation of the alternative pathway of complement activation by binding to and stabilizing the C3bBb convertase[50]. Studies on P deficiencies have emphasized its critical role in the formation of an efficient C3 convertase, and in host defense against

meningococci[56]. The site in C3 responsible for properdin binding was localized within residues 1402–1435 of C3 (Fig. 1)[57]. A 34 amino acid synthetic peptide corresponding to this region binds to P and inhibits both P binding to C3b and the activation of the alternative pathway by rabbit erythrocytes or zymosan.

The precise mechanism of convertase stabilization by P, as well as the structural requirements for the P–C3bBb interaction, are unknown. It is not clear if P must bind to both C3b and Bb to exert its effects or if it is sufficient for P to bind only to C3b, thus changing the conformation of C3b and increasing its affinity for Bb. The use of synthetic peptides such as the one described, and the recent cloning of P cDNA[58], may facilitate studies addressing these questions.

C5

The interaction of C5 with C3b serves to bridge the alternative pathway C3 convertase with the initiation of the membrane attack complex. The interaction of C5 with the two C3b molecules present in the C5 convertase ($C3b_2Bb$)[59] leads first to its cleavage by Bb and concomitant release of C5a anaphylatoxin, and second to the initiation of the membrane attack complex[50]. The segment of C3b responsible for C5 binding was localized within the β chain of C3b (Ref. 60). Thus far, C5 is the only ligand known to bind to the β chain of C3.

C4b-binding protein

The C4bp, in addition to its cofactor activity for the factor-I-mediated cleavage of C4b and its decay-accelerating activity for the classical pathway convertase (C4bC2a), forms a complex with C3b and mediates its cleavage by factor I (Ref. 61). The efficiency of C4bp in this reaction is low compared to H, and its physiological significance has not been established.

Conglutinin

Conglutinin, until its recent purification from human serum[62], was believed to exist only in bovine serum[63]. It is a lectin-like molecule comprised of six 48 kDa (bovine) or 66 kDa (human) polypeptide chains with iC3b-binding activity. This binding is calcium dependent and is inhibited by carbohydrates, especially *N*-acetyl-D-glucosamine. Direct binding studies[64] with different fragments of C3 have localized the conglutinin interaction site on C3 to the carbohydrate moiety linked to residue 917 of C3 (Ref. 64). The significance of the iC3b–conglutinin interaction in complement regulation or in other functions that it may have is not known.

Other C3-binding proteins (Table 3)

Interleukin 2

The enhancement of IL-2-dependent proliferation of helper T-cell lines by aggregated C3 or by C3b-like C3 (Ref. 65), and the suppression of IL-2-dependent proliferation of T cells by the C3dk fragment of C3 (Ref. 66), suggest that these C3 fragments may interact with IL-2. Recently, IL-2 was shown to bind the C3b and C3c fragments of C3, and to inhibit the binding of C3b to CR1 (A. Erdei, unpublished). The interaction site on IL-2 for C3b was found to be different from that for the IL-2 receptor. In view of the recent finding[28] that CR1 binds to the amino-terminal 40 amino acids of the α′-chain of C3, these results suggest that IL-2 binds to the same region on C3b. This appears to contradict the finding that the IL-2-dependent proliferation of T cells is suppressed by C3dk or C3d, unless different mechanisms are assumed to operate in the stimulation and enhancement of T-cell proliferation by C3 fragments or that differences in experimental conditions account for these discrepancies.

Table 3. Summary of other C3-binding proteins

Protein	Specificity	Function within complement activation
Laminin	C3b, C3d	Unknown
Fibronectin	C3b, C3c, C3d	Unknown
HSV gpC	C3b	Acts as decay accelerating factor
C. albicans	iC3b, C3d	Unknown
EBV	C3b, iC3b	Acts as cofactor of factor I, and decay-accelerating factor
IL-2	C3b, C3c	Unknown

Basement membrane proteins

The detection of C3 fragments in the basement membranes of different tissues suggested that proteins in this region may interact specifically with these fragments. The C3d fragment of C3 has recently been shown to bind to laminin, fibronectin and type IV collagen[67]. The binding to laminin was completely inhibited by fluid phase laminin and partially by fibronectin, suggesting that the binding sites on C3d for these two proteins may be related. Earlier studies on the interaction of fibronectin with C3 fragments showed that C3c, as well as C3d, bound fibronectin[68]. Even though the significance of these interactions has to be established, if they do operate in human disease they may play an important role in the complement-mediated pathological processes in the kidney glomerulus.

Candida albicans

Candida albicans and *C. stellatoidea*, but not the less pathogenic *C. tropicalis*, *C. parapsilosis* and *C. krusei*, rosette with sheep erythrocytes coated with different fragments of C3 (EAC3bi and EAC3d cells)[69], suggesting that C3 receptors exist on the surface of the more pathogenic species of *Candida*. This observation was further substantiated by the ability of anti-CR3 antibodies to bind *C. albicans* and of anti-CR2 antibodies to inhibit rosetting with erythrocytes coated with C3 fragments[70]. These data suggested that different structures exist on *C. albicans* that can react with C3 fragments. One of these structures (70 kDa), recently purified from whole yeast cell extracts on a C3d affinity column, inhibited rosetting of *C. albicans* with EAC3d (Ref. 71); monoclonal antibodies against this protein had a similar inhibitory effect. The possible relationship between this molecule and mammalian complement receptors has not yet been explored at the molecular level. Further investigation of such a relationship and of the specificity of the *C. albicans* C3 receptors could provide information on the pathogenesis of *C. albicans*.

gC of herpes simplex virus

Herpes simplex virus type 1 (HSV-1) encodes at least six viral proteins that, when expressed on the surface of infected cells, serve as targets for the host's humoral and

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cellular responses[72]. One of these proteins, glycoprotein C (gC), is a C3b-binding protein[73]. The gC protein is expressed on HSV-1-infected endothelial, epithelial and fibroblast cell lines. The binding of gC to C3b diminishes the ability of complement to neutralize the virus and inhibits the complement-mediated lysis of HSV-1-infected cells. Purified gC possesses decay-accelerating activity for the alternative pathway C3 convertase similar to that of the other regulators of the C3 convertase formation.

Although cells infected with HSV-2 cannot rosette with C3b-coated erythrocytes, it was recently shown[74] that gC from HSV-2 binds to Sepharose-bound iC3 and provides protection against complement-mediated neutralization similar to that of gC from HSV-1[74].

Epstein–Barr virus

Epstein–Barr virus, besides binding to CR2, was recently shown to regulate the alternative pathway. In addition to serving as a cofactor for the factor-I-mediated cleavage of C3b, purified EBV can also accelerate the decay of the alternative, but not the classical, C3 convertase[75]. While the molecule(s) on EBV responsible for the above effects remains to be characterized, it is clear that EBV and gC influence the activation of the alternative pathway differently, since the latter does not exhibit cofactor activity for cleavage of C3b by factor I.

Conclusions

Many questions regarding the multiple interactions of C3 remain to be answered. The studies reviewed here highlight the importance of C3 not only in complement activation but also in the immune response. It is clear from the observed multireactivities of C3 that it is necessary to study the molecular features of these C3 interactions in detail and to reinvestigate some of the effects exerted by C3 as better tools become available. Understanding C3 interactions at the molecular level will help us to determine how it exerts its multiple functions and may facilitate the design of specific complement inhibitors.

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