Paths reunited: initiation of the classical and lectin pathways of complement activation

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Abstract

Since its isolation almost fifty years ago, considerable progress has been made towards understanding the structural organisation and mode of action of C1, the initiating complex of the classical pathway of complement activation. Nevertheless, knowledge is still incomplete, especially with regard to the interactions between subcomponents, C1q, C1r and C1s, that trigger activation. Recent studies have provided new insights into these interactions, and have revealed unexpected parallels with initiating complexes of the lectin pathway of complement: MBL-MASP and ficolin-MASP. Here, we develop and expand these concepts and delineate their implications towards the key aspects of complement activation via the classical and lectin pathways.

Introduction

The classical and lectin pathways of complement activation are central combatants in the fight against disease. Both are able to lyse pathogens directly via antibody-independent mechanisms, providing immediate response against invasion (Fujita et al., 2004; Schwaeble et al., 2002). They also stimulate key cellular and humoral interactions within the innate immune system, including chemotaxis, phagocytosis and cell adhesion. Crucially, complement activation promotes B-cell differentiation and maintenance of immune tolerance, building vital bridges with adaptive immunity (Carroll, 2004). Once established, activation on antibody-antigen complexes further facilitates directed killing (Porter & Reid, 1978). More recently it has emerged that complement components not only target non-self but also altered-self structures, and early complement components are key players in tissue homeostasis through clearance of apoptotic and necrotic cells (Botto, 1998; Stuart et al., 2005; Taylor et al., 2000).

As expected from their important biological roles, deficiencies in complement components are associated with increased risk of acute, severe infections (Summerfield, 2003). For example, lack of C1q increases susceptibility to encapsulated bacteria such as *Streptococcus pneumonia* (Colten & Rosen, 1992). It is also linked with defective clearance of immune complexes and cellular debris, leading to glomerulonephritis (Botto et al., 1998), and increased risk of autoimmune disease, particularly systemic lupus erythematosus (Lewis & Botto, 2006). Whilst normally protective, complement can inadvertently contribute to disease pathogenesis. For example, C1q is involved in the pathology of several amyloid diseases, such as Alzheimer's disease and readily binds to prion proteins to initiate complement activation and exacerbate disease progression (Yasojima et al., 1999). Reperfusion of tissues (e.g. heart and kidney) following ischemia, triggers lectin pathway-mediated host damage, therefore specific inhibitors of complement have considerable therapeutic potential (Møller-Kristensen et al., 2003; Mollnes et al., 2002). Hence, understanding the molecular interactions triggering complement activation is of great interest from biochemical, immunological and medical perspectives and will form a basis for rational drug design.

2

Befitting its biological importance, the study of the composition and structure of the C1 complex has a long history. As early as 1963, the initiating complex of the classical pathway, C1, was shown to comprise three subcomponent proteins: C1q, C1r and C1s (Lepow et al., 1963). It is now well established that C1q functions as the recognition subcomponent of the complex by binding to a wide variety of targets including microorganisms, immune complexes, apoptotic and necrotic cells and amyloids to initiate the stepwise activation of the associated serine proteases, C1r and C1s. C1q is a hexamer of subunits, each assembled from three different polypeptide chains, A, B and C (Reid & Porter, 1976) (Figure 1). N-terminal collagenous tails (subsequently referred to as stalks) splay apart at an interruption in this domain, called the kink, and terminate in C-terminal globular heads to form large structures (~530 kDa) that resemble bouquets (Strang et al., 1982). Subcomponents C1r and C1s are modular proteins, each consisting of two CUB domains separated by an EGF-like domain, followed by two CCP modules and a serine protease domain (SP) (Schwaeble et al., 2002; Sim & Tsiftsoglou, 2004). C1r dimerizes through interactions between the SP domain of one polypeptide and the CCP domains of its partner (Budayova-Spano et al., 2002). In the presence of Ca^{2+}, each chain binds to a C1s polypeptide via the CUB1-EGF domains to form a C1rs tetramer (C1s-C1r-C1r-C1s; ~340 kDa). The interaction with C1q is also Ca^{2+}-dependent and forms the basis for this review. When C1 binds to a target via its globular heads, C1r activates through auto-cleavage. C1r then cleaves and activates C1s. To ensure only localised activation, the SP domains stay attached to C1 via single disulfide bonds to their respective N-terminal fragments. The first substrate of C1s is C4. Once cleaved it rapidly attaches to the pathogen surface via a reactive thioester bond. C2 then binds to the C4b fragment and is also cleaved by C1s to form the C3 convertase (C4b2a), the next component of the pathway. Thus, C1 activation and subsequent steps are limited to the surfaces of pathogens and other targets, a key regulatory aspect of the pathway.

The lectin pathway of complement was discovered comparatively recently (in 1987) compared to the classical pathway, but parallels were immediately apparent (Ikeda et al., 1987). MBL and ficolins resemble C1q in electron micrographs and bind to homologues of C1r and C1s called MBL-associated serine proteases (MASPs) (Figure 1). Initially upon the discovery of MASP-1 (Ji et al., 1993; Matsushita & Fujita, 1992) and later MASP-2 (Thiel et al., 1997), it was believed that initiating complexes would function in the same way as C1, i.e. involving stepwise activation of separate MASP components. However, subsequent studies revealed that this was not the case; rather MASPs bind independently to MBLs and ficolins, predominantly as 1:1 complexes (i.e one MASP dimer to each MBL/ficolin oligomer, (Chen & Wallis, 2001)) and both autoactivate. Differences became even more apparent, upon discovery of a third MASP, MASP-3, (Dahl et al., 2001) and a non-enzymatic protein that binds to MBL/ficolins called MAp-19 or sMAP, (Iwaki et al., 2006; Stover et al., 1999). It is now well accepted that MBL-MASP-2 and ficolin-MASP-2 complexes are able to activate complement independently of MASP-1, MASP-3 and MAp-19 and the biological roles of the latter

components are still largely undetermined. MASP-2 has a similar catalytic specificity to C1s, cleaving C2 and C4 (Rossi et al., 2001). The proteolytic activities of C1r/s and the MASPs have been recently covered in more detail in a recent excellent review (Gal et al., 2009).

Despite the considerable progress that has been made in elucidating the structure of C1 and MBL-MASP/ficolin-MASP complexes, the mechanism by which subcomponents combine to initiate complement cascade activation has remained elusive. Until recently, it was generally believed that subcomponents had evolved distinct strategies to associate with their partner proteins and activate complement (Gaboriaud et al., 2007). Principally, the C1q binding site on the C1rs tetramer was proposed to lie at the C1r-C1s interface (Gaboriaud et al., 2004), whilst the MBL binding site was thought to be localised on the CUB domains of MASPs. However, two recent papers by Phillips *et al.* and Bally *et al.* have challenged this dogma, highlighting the similarities between the classical and lectin complexes, thus uniting their likely mode of action once more (Bally et al., 2009; Phillips et al., 2009). In this review, we have focussed on how this recent knowledge helps us to address the key requirements for a functional model of C1, recently set out by Gal *et al.* (Gal et al., 2009). Specifically, any model should answer the following questions: (1) How C1q, C1r and C1s readily associate to form C1. (2) How the activation signal is transmitted from C1q to the C1r zymogens. (3) How zymogen C1r autoactivates after receiving the activation signal (and what prevents spontaneous autoactivation). (4) How C1r activates C1s inside the complex. (5) How C1s is able to cleave C4 and C2 in the fluid phase. (6) How C1-inhibitor gains access to both C1r and C1s in the complex to block their activity and disassemble the complex. None of the previous models of C1 have been able to satisfactorily answer all of these questions. Here we set out how our recent model provides insight into each question in turn.

Assembly of C1

Recent surface plasmon resonance studies, using purified wild-type and deletion components has allowed the kinetics of C1 formation to be dissected and has illustrated a number of key points that impact on structure of C1 (Bally et al., 2009; Phillips et al., 2009): (1) that C1r and C1s can both bind C1q individually; (2) that the C1rs tetramer binds tighter than C1r or C1s alone indicating that they both bind within the context of the tetramer; (3) that deletion of the CCP-CCP-SP domains does not prevent binding of C1r or C1s, implying that they do not interact with C1q significantly; and (4) most importantly that non-cognate (i.e., MBL-C1rs and C1q-MASP) complexes readily assemble, but with weaker affinities than cognate ones. This latter striking observation thus shows that the physical basis for assembly is shared between both complexes and is incompatible with the previous most widely accepted C1 models (Gaboriaud et al., 2007; Gaboriaud et al., 2004). Instead, the binding data indicate that C1rs tetramers present six separate binding sites to C1q that are located on the CUB domains (two from each C1r polypeptide and one from each C1s polypeptide). Both groups have

independently proposed that each site binds to a separate collagenous stalk of C1q (Bally et al., 2009; Phillips et al., 2009). Residues forming the binding sites are conserved in the C1r/C1s/MASP family and equivalent protease-binding motifs are present on the collagenous stalks of C1qs/MBLs and ficolins (Figure 2). These similarities explain the early observation that purified MBL oligomers of the lectin pathway are able to bind and activate C1rs tetramers of the classical pathway (Lu et al., 1990). The main difference between the C1 and MBL-MASP complexes is in their stoichiometries: in the lectin pathway, each recognition subcomponent binds to a single MASP dimer, whereas C1q binds to a C1rs tetramer (the equivalent of two MASP dimers; Figure 2) (Chen & Wallis, 2001). This distinction accounts for differences in the oligomeric structures of MBLs (dimers to hexamers of stalks in human MBL, but predominantly tetramers (Teillet et al., 2005)) and ficolins (mainly tetramers), which are typically smaller that C1q (hexamers). It also explains why the pentameric and hexameric forms of human MBL (relatively minor components) are most efficient at activating C1rs tetramers (Lu et al., 1990): these oligomers most resemble C1q so are better able to bind and active C1rs.

A notable feature of C1, MBL-MASP and ficolin-MASP complexes is their Ca^{2+} dependence. Ca^{2+} mediates interactions between C1r and C1s, C1rs and C1q as well as between MBL and MASP and ficolin and MASP. The Ca^{2+} binding sites are conserved in each of the CUB domains as well as in the EGF domains of the protease subcomponents (Figure 1). Thus, each C1rs tetramer probably binds 12 Ca^{2+} and each MASP dimer 6 Ca^{2+}. The interfaces of the MASP homodimers and the two C1r-C1s heterodimers are created by a set of conserved contacts between the CUB1-EGF domains (Feinberg et al., 2003; Gregory et al., 2003; Gregory et al., 2004). Several residues, which lie in or near the Ca^{2+} site in the EGF domain, stabilise the dimer by interacting with the CUB1 domain of the adjacent protomer. The CUB domains co-ordinate the Ca^{2+} through a group of acidic residues found on surface exposed loops of the CUB domains. Mutation of these residues weakens binding to the collagenous domains of the respective recognition component implying they play direct roles in the protein-protein interaction (Teillet et al., 2008). However, mutations that disrupt Ca^{2+} binding probably also perturb protease binding indirectly, by disrupting the conformation of the surface loops. Thus the assignment of the roles of the residues in binding remains ambiguous.

C1 subcomponents associate spontaneously in solution and do so even when C1q is already attached to an activating surface to yield functional complexes (Gigli et al., 1976). This would not be possible without considerable conformational flexibility of the protease subcomponents. C1rs tetramers adopt bent but relatively extended structures in solution and in electron micrographs (Perkins & Nealis, 1989), so presumably are able to pass through gaps between C1q stalks, where they adopt a more constrained bound conformation. The dynamic nature of interactions of individual C1q globular heads with the activating surface probably helps to allow access of C1rs tetramers to immobilized

C1q. Such "breathing" of the globular heads would seem likely because each interaction is relatively weak and high-affinity target binding is instead achieved through multiple head-surface interactions.

Transmission of the activation signal from C1q to C1r

A critical facet of the complement cascade is that activation is only triggered when C1 complexes bind to an activating surface. There are several factors intrinsic to C1 that combine to prevent inappropriate activation: (1) the C1r polypeptides are located inside the complex (i.e. within the cone created by the C1q stalks) so are relatively inaccessible to other serum proteins and proteases; (2) they circulate as zymogens; and (3) reciprocal interactions between the SP domain of one C1r polypeptide and the CCP domains of its partner prevent enzyme-substrate interactions that could lead to autoactivation. Remarkably, each polypeptide must move by some 46 Å to allow activation (Budayova-Spano et al., 2002). C1q is the only subcomponent that contacts the activating surface directly, so upon binding it must undergoe considerable conformational changes to trigger autocatalysis of C1r. Two alternative mechanisms can be considered: "relaxed-to-strained", in which binding to the surface distorts C1q thereby driving conversion of the zymogen complex (relaxed) to the active conformation (strained); or "strained-to-relaxed", in which C1 converts from a strained zymogen to a relaxed active conformation. In the former mechanism, the energy for activation comes from binding to the activating surface, whereas in the latter, some of the energy gained from assembly of subcomponents primes the complex for activation. Although neither mechanism can be completely ruled out, the available solution and structural data favour a strained-to-relaxed mechanism (Figure 3). In particular, both subcomponents are more constrained in the complex compared to their unbound states. Thus, unliganded C1q is highly flexible in solution, with large differences in the angles between collagenous stalks, but becomes fixed upon binding to C1rs tetramers (Perkins, 1985). Severe constraints are also imposed upon C1r and C1s, which adopt more compact configurations on association with each other and upon binding to C1q (Strang et al., 1982). Importantly, a strained-to-relaxed mechanism would require relatively little energy input from target binding to trigger activation of the "primed" complex, hence providing an explanation for the low level of spontaneous C1 autoactivation observed *in vitro*, even in the absence of immune complexes or other activators (Ziccardi, 1982). It would also explain how activation is independent of the nature of the activating surface, thereby accounting for the diverse targets that trigger activation and the different ways in which the globular heads of C1q are believed to bind to these targets (Gaboriaud et al., 2003). On the other hand, a relaxed-to-strained model would seem much less likely, because different activating targets would somehow have to induce comparable, ordered conformational changes in C1q to trigger the steps necessary for activation. Such changes would seem particularly unlikely given the innate flexibility of the C1q stalks.

Autoactivation of C1r

We propose that the inter-protomer C1r CCP-SP interactions not only prevent autoactivation of the zymogen complex, but also induce strain into circulating C1 complexes (Figure 3). Compatible with this hypothesis, modelling studies have shown that zymogen C1rs tetramers cannot be docked onto C1q without distorting the subcomponents, such as through changes at the junction between the EGF and CUB-2 domains of C1r (which is likely to be flexible), the relative positions of C1r and C1s and consequently the relative positions of the C1q stalks (Phillips et al., 2009). For example, in the model of the zymogen C1 complex proposed by Bally *et al.* (Bally et al., 2009), the six binding sites on C1rs tetramers are arranged about a 2 x 3 rectangle when viewed from above, rather than a hexagon as in the active conformation (Phillips et al., 2009). The overall effect of these changes would be to pull some (or all) of the C1q stalks closer together, and thus distort the natural 6-fold symmetry of C1q observed in solution (Perkins, 1985). Complement activation would then be initiated by splaying apart of the C1q bouquets when they bind to a target, breaking the reciprocal interactions between C1r polypeptides and releasing the imparted strain. The SP domains would then naturally separate to allow mutual autocatalysis (Budayova-Spano et al., 2002). This mechanism would explain why complexes assembled from truncated forms of C1rs, lacking the CCP and SP domains, have properties compatible with the active (relaxed) rather than the zymogen (strained) conformation (Phillips et al., 2009).

A strained-to-relaxed activation mechanism shares a number of similarities with the "split-and-reassembly" model proposed by Gal and colleagues (Kardos et al., 2008). Here, binding of C1q heads to the target results in a change in the relative position of the C1q arms, which in turn shifts the equilibrium to the dissociated form of the C1r dimer. However, a major difference concerns the mode of separation and realignment of C1r. In the split-and-reassembly model, the polypeptides reassemble spontaneously into an enzyme-substrate complex. Whereas in the strained-to-relaxed model they would spring apart as the strain is released. In this respect, the motion resembles that proposed in earlier models (e.g. (Gaboriaud et al., 2007; Gaboriaud et al., 2004)), with the key innovation being that separation of polypeptides is driven by relaxation, rather than induced by distortion. Irrespective of the mode of separation, autocatalysis is probably facilitated by stabilizing intermolecular CCP2–SP interactions, and occurs via a substrate-induced mechanism (discussed in more detail in (Gal et al., 2009)), followed by cleavage of the remaining zymogen polypeptide by its active partner. Importantly, by the nature of the strained-to-relaxed mechanism, once activated, the C1r polypeptides would not tend to reassociate with each other as has been suggested in other models, but rather would be free to activate C1s.

Activation of C1s by C1r

The next step in C1 activation is the cleavage of C1s by activated C1r. In addition to blocking the latent proteolytic activity within the zymogen complex and facilitating autocatalysis through

intermolecular CCP-SP contacts, the CCP domains of C1r play an additional, more structural role by acting as spacers to separate the C1q binding domains (CUB1-EGF-CUB2) from the enzymatic SP domain. Together with inter-domain flexibility, most likely between CUB2 and CCP1 of both C1r and C1s, this would allow the required protein dynamics for C1r to contact the C1s polypeptide and activate it. Modelling reveals that there would be sufficient space available for this process to occur inside the complex, so that activated C1r is protected from C1-inhibitor until after C1s activation has occurred (Figure 4).

Cleavage of C4 and C2 by C1s

The junctions between the C1q-binding (CUB1-EGF-CUB2) and proteolytic (CCP1-CCP2-SP) portions C1r and C1s are located towards the outer perimeter of the complex in gaps between adjacent C1q stalks (Figures 3 and 4). Flexibility at these junctions would allow the proteolytic portions to swing out into solution through the gaps, placing the CCP1-CCP2-SP domains of C1s in a position to cleave the next components of pathway – C4 and C2. C4 cleavage results in two products: the peptide anaphylatoxin C4a and C4b which contains a reactive thioester that anchors C4b covalently to the target surface. Next, C2 binds C4b (it is unable to bind full-length C4) and is itself cleaved by C1s, yielding the C4b2a complex. Also known as the C3 convertase, this component proteolytically activates C3 to form C3a (another peptide analphylatoxin) and C3b, which joins C4b2a to make the C5 convertase that furthers the complement cascade. MASP-2, within the MBL-MASP complex, has also been shown to act in a similar manner to produce the C3 convertase (Chen & Wallis, 2004). Here, accessory binding sites for C4 on the CCP domains of the MASP play key roles in regulating C3 convertase formation. These additional interactions not only facilitate specific catalysis of C4 by increasing the affinity of enzyme-substrate complex (Gal et al., 2005), but also reduce the rate of dissociation of C4b from the MASP. As a consequence, the reaction of the C4b thioester with the cell surface occurs at least an order of magnitude faster than dissociation of the C4b-MASP-2 complex (Chen & Wallis, 2004). The overall effect is to ensure that C4b deposits onto the target surface before it dissociates from the MBL-MASP complex, so that it is close enough for the same MBL-MASP complex to rapidly activate C2 upon its binding to C4b.

A similar mechanism might regulate C3 convertase formation by the classical pathway, and kinetic data show that interactions with the CCP modules also facilitate cleavage of C4 by C1s (Rossi et al., 1998). However, the CCP modules of MASP-2 appear to contribute more towards recognition of C4 than for C1s (Rossi et al., 2005), so this mechanism might be less important in the classical pathway, perhaps reflecting the greater abundance of components in the serum. This conclusion is supported by studies comparing C3 convertase formation on different surfaces, which showed that almost every C4b molecule deposited via the lectin pathway was capable of forming a convertase, compared to only one in four C4b molecules deposited through classical pathway activation (Rawal et al., 2008). The

potential for increased amplification in the lectin pathway and the subsequent production of pro-inflammatory products, such as C3a, C4a, and C5a, provides a possible mechanism to explain why complement activation via the lectin pathway is a more prominent contributor to the pathology of inflammatory reactions.

Binding of C1 inhibitor

C1 inhibitor (C1-INH/SERPING1) has a number of different biological roles including regulation of vascular permeability and acting as an anti-inflammatory agent (Davis et al., 2008). It functions primarily by controlling the activities of range of blood-borne proteases including those of the contact system (factor XII, plasma kallikrein), coagulation (factor XI) and fibrinolysis (plasmin, tissue plasminogen activator) in addition to the complement proteases (C1r, C1s and MASPs) in their activated forms. Loss of this regulation by C1-INH through genetic or acquired deficiencies results in disease states such as angioedema (Cugno et al., 2009). In common with other members of the serpin family of protease inhibitors, C1-INH functions by acting as a suicide substrate. A reactive centre loop inserts into the active site and cleavage triggers a startling conformational change. This is perhaps best illustrated in inhibition of trypsin by α_1-antitrypsin, where once cleaved, the reactive centre loop and the covalently tethered protease move 71 Å to the other end of the serpin, 'crushing' the protease in the process (Huntington et al., 2000). This remarkable process, sometimes compared to the mechanism of a 'mouse-trap', is thermodynamically driven by the cleaved serpin being hyperstable compared to the uncleaved form (an extreme example of a strained-to-relaxed mechanism). C1-INH also displays this characteristic property; for example, melting temperatures of the uncleaved active and cleaved forms are ~60 °C and ~116 °C, respectively (Beinrohr et al., 2007). Thus, C1-INH probably inhibits C1r and C1s through similar distortion of the SP domains. Notably, post-C1r activation, the two C1rs hetero-dimers have already separated from each other and subsequently bind to C1q an order of magnitude weaker than the C1rs tetramer (zymogen form); so the C1 complex is already intrinsically less stable in this form (Phillips et al., 2009). Association with C1-INH (~100 kDa) presumably further disrupts and weakens their interactions with C1q by promoting dissociation and blocking reassociation through steric-electronic repulsion/clashes. Weakening, rather than total disruption of binding is suggested by the observation that C1-INH strips C1r and C1s from C1 more efficiently at higher salt concentrations (Sim et al., 1980). Nevertheless, under physiological conditions the protease and recognition subcomponents separate and much of the C1q remains bound to the activating surface. Another feature conserved between C1-INH-C1r/s and some other serpin-protease interactions is that heparin appears to potentiate the protein-protein interaction by accelerating the second-order association rate constant. For C1-INH binding to C1s, heparin shields unfavourable electrostatic clashes (Beinrohr et al., 2007). In the case of MASP-1, this shielding effect leads to anti-thrombin being a faster inhibitor, even than C1-INH (Dobo et al., 2009).

C1-INH provides a crucial regulatory function by limiting the lifetime of surface-bound activated C1 and thus regulating C3 convertase formation. Given the relatively compact nature of C1 and the appreciable size of C1-INH, it is likely that once C1 is bound to an activating surface, C1r and C1s are only targeted once their SP domains have moved outside of the C1q stalks. Protease-C1-INH complexes are released rapidly from C1-Ab-Ag complexes, so active C1 exists only transiently on the activating surface (Sim et al., 1980). Consequently, there would be competition between C4 cleavage and C1-INH-mediated inhibition. The C1q separated from C1rs but still attached to cell surfaces would be free to bind to receptors (such as gC1qR/collectin receptor/calreticulin) to further modulate immune function (Gasque, 2004). Another crucial function of C1-INH is to neutralise spontaneous C1 activation, which occurs at low-level in serum (Ziccardi, 1982). Such neutralisation is key to preventing activation in solution, which would lead to depletion of complement components in plasma. Based on their more exposed positions in unbound C1, C1-INH would be able to readily access the SP domains of C1r and C1s once activated. The efficiency of this neutralization is likely to be greatly enhanced by reversible interactions between C1 and C1-INH, as components circulate in plasma (Ziccardi, 1985).

Parallels between C1 complex and MBL-MASP formation

Given the similarities between the initiating complexes of the classical and lectin pathways and their abilities to cross-bind and cross-activate *in vitro*, it is likely that MBL-MASP and ficolin-MASP complexes undergo similar changes to C1 during activation. Post-activation, MASP-2 is largely functionally analogous to C1s, although intriguingly, their common catalytic specificities are achieved through different mechanisms (Harmat et al., 2004). Where the two complexes differ is that two activation steps (C1r and C1s) are required for C1 activation, but only one step (autoactivation of MASP-2) is required for MBL-MASP activation. MASP dimers have four separate binding sites for MBL stalks (Teillet et al., 2008): one for each stalk of a MBL tetramer. Nevertheless, MBL dimers are able to activate complement (although with reduce activity compared to trimers and tetramers), so only two of these sites must be occupied for activation to occur (Wallis & Drickamer, 1999). Unlike C1r, the CCP1-CCP2-SP fragments of each zymogen MASP polypeptide do not interact with each other significantly (Gal et al., 2005). Nevertheless, MASP binding might still distort the MBL/ficolin, leading to a similar strained-to-relaxed activation mechanism. Interestingly, in a recent study of MBL by atomic force microscopy, stalks were not symmetrically arranged in individual oligomers (e.g. 90° for a MBL tetramers), but rather with only 35–40° between neighbouring stalks. Consequently significant conformational changes would be required to bind readily to MASPs (Jensenius et al., 2009). It will be interesting to determine the solution properties of MBL and MBL-MASP complexes to investigate this phenomenon further.

Figure legends

Figure 1: Schematic representations of the individual components of the initiating complexes of lectin and classical complement pathways. *Top,* C1q, MBL and ficolins (not shown) resemble bouquets, comprising N-terminal collagen-like domains linked to C-terminal target-recognition domains (carbohydrate-recognition, antibody-binding and fibrinogen-like, respectively). *Middle*, domain organisations of C1rs heterotetramers and MASP homodimers. C1r-C1s and MASP-MASP interactions are conserved (*black boxes*), so the C1rs tetramer is effectively equivalent to two MASP dimers linked through the CCP-SP domains of C1r. *Bottom*, crystal structures of MASP-1 CUB1-EGF-CUB2 (green; PDB: 3DEM (Teillet et al., 2008)) and C1s CUB1-EGF (orange and purple; PDB: 1NZI, (Gregory et al., 2003)) fragments. Each EGF domain binds a Ca^{2+} ion (red sphere) aiding binding to the CUB1 domain of its partner. Additional Ca^{2+} ions (*red spheres*) are co-ordinated within the CUB domains and organize the loops that form the binding sites for the MBL/C1q stalks. Putative binding residues are coloured *red* and *yellow* for large and lesser effects on binding, respectively. *In vivo*, C1s does not form homodimers, but each chain instead binds to a C1r polypeptide through homologous interactions.

Figure 2: C1 and MBL-MASP complexes. Top (*top*) and side (*bottom*) views of C1 and MBL-MASP complexes derived from modelling and binding studies. Only the CUB1-EGF-CUB2 domains of C1r and C1s (*purple* and *orange*) and MASP (green) are shown. In both cases the CCP1-CCP2-SP domains are located inside the complex, on top of the CUB1-EGF-CUB2 domains. Binding sites are: C1q (blue), MBL (dark blue), C1rs (yellow) and MASP (orange). Notably, interactions of C1q with C1rs and MBL with MASP are analogous, with four binding sites on each MASP dimer and three on each C1r-C1s (CUB2 of C1s lacks a binding site), making a total of six sites on a C1rs heterotetramer.

Figure 3: Strained-to-relaxed model of C1 activation. Phase 1: Zymogen C1. The active sites of C1r are kept apart through the interactions of one SP domain with the CCP domains of its partner. Binding to C1q induces strain, pulling the C1q stalks closer together (as indicated by the arrows). Although C1r and C1s are zymogens, their SP domains are relatively exposed in circulating complexes, so are accessible to reversible binding by C1-INH (Ziccardi, 1985). However, irreversible binding and inhibition by C1-INH only occurs upon activation of C1r and/or C1s and serves to neutralise the low level of spontaneous activation that occurs in solution (Ziccardi, 1982). The CCP-SP domains of C1s are not shown for clarity. **Phase 2: target binding induced C1r autoactivation.** Multiple simultaneous interactions of the C1q heads with an activating surface (e.g., IgG bound to bacterial surfaces) enable the stalks to move apart again (see arrows), releasing the induced strain within the complex. The CCP-SP domains of C1r spring apart allowing autocatalysis of one polypeptide by its partner. *Side* and *top* views are shown. **Phase 3: C1s activation.** Flexibility at the inter-domain CUB2-CCP junctions allows the C1r SP domain to cleave C1s, converting it to the active form. **Phase 4: Activated C1.** The catalytic domains of C1s, and possibly C1r, move out into solution, where the former activate downstream substrates C4 and C2. Once C1 is bound to a surface, C1-INH can only access the SP domains of C1s and/or C1r after they have moved outside of the complex.

Figure 4: Conformational changes in C1 and MBL-MASP complexes. Side and top views of complexes during activation and fully activated. Autoactivation of C1r and MASPs occurs inside the complexes. Flexibility at the interdomain CUB2-CCP1 junctions would then allow the CCP-protease domains to move outside the cones created by the C1q/MBL stalks and into solution where they would be able to activate downstream substrates C4 and C4b2.

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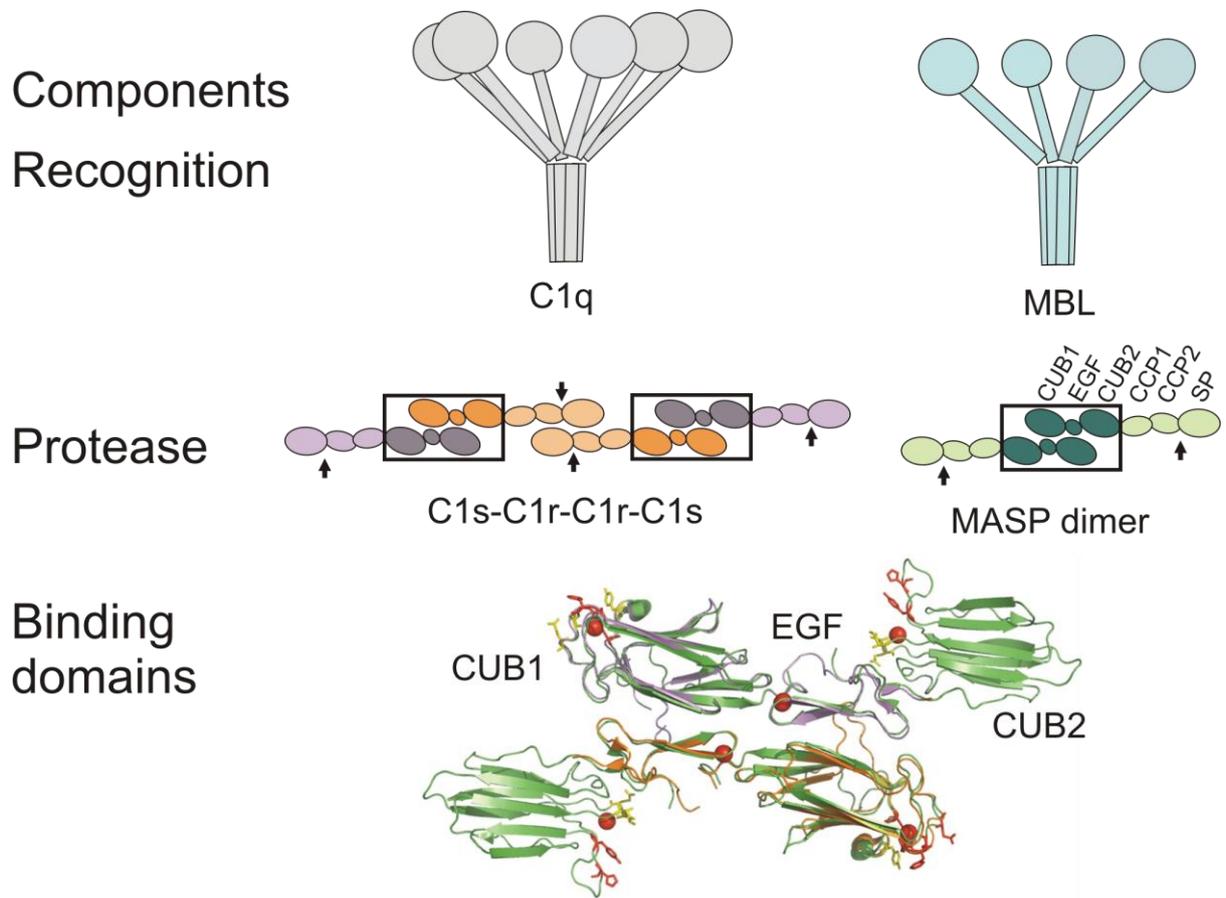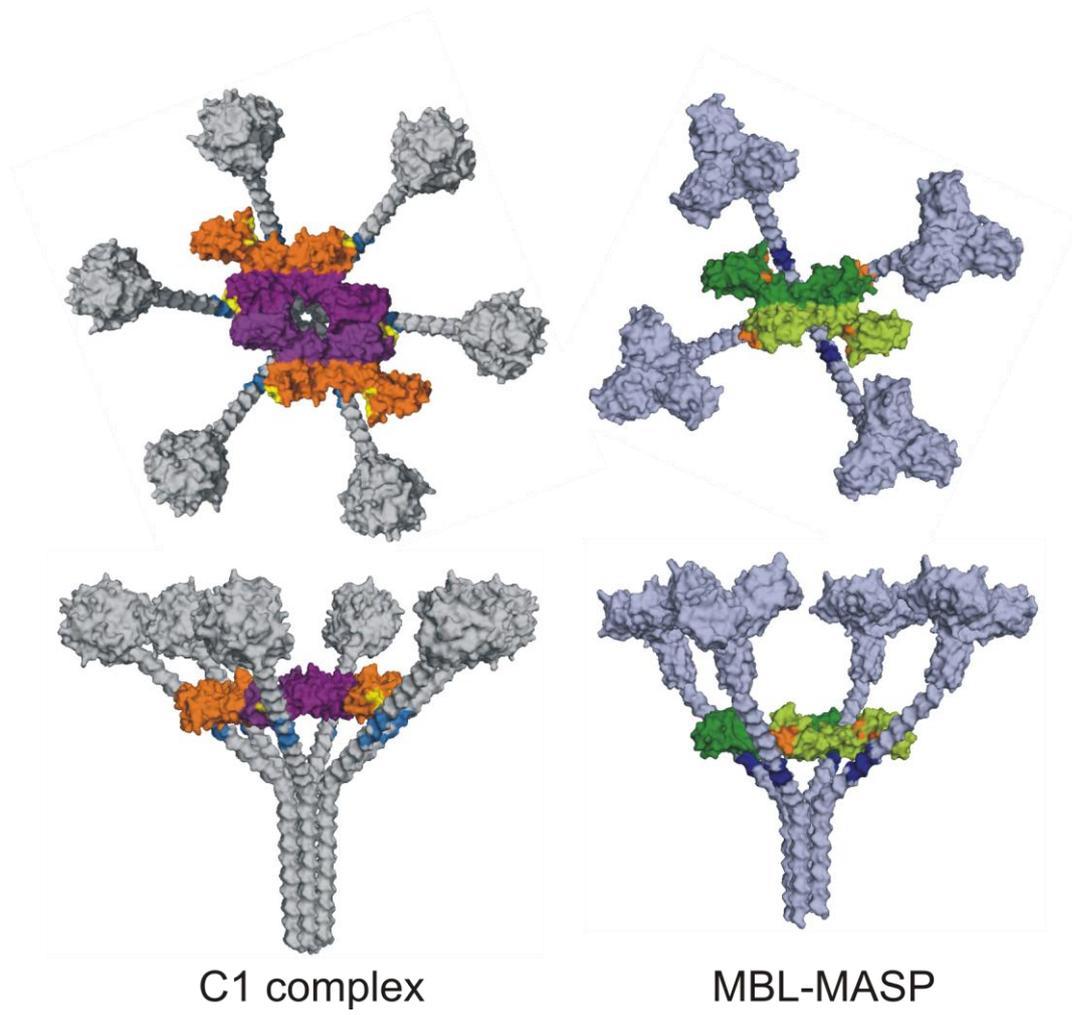
Figure 1

Components
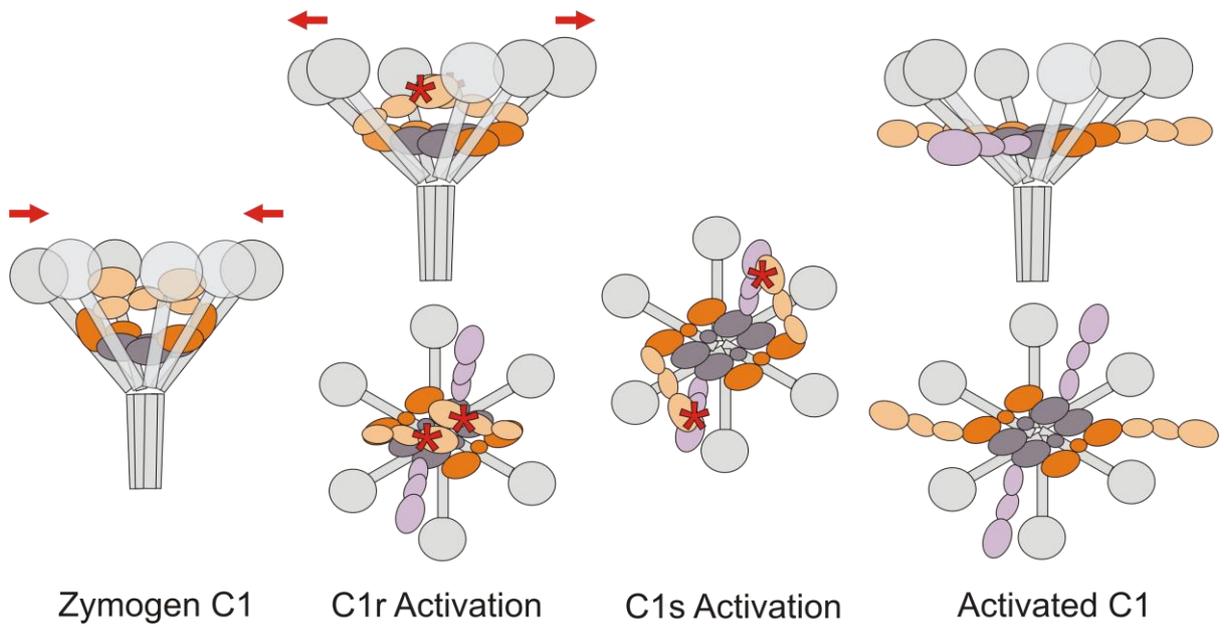Recognition

C1q

MBL

Protease

C1s-C1r-C1r-C1s

CUB1 EGF CUB2 CCP1 CCP2 SP

MASP dimer

Binding
domains

CUB1

EGF

CUB2

Figure 2

C1 complex   MBL-MASP

Figure 3

Zymogen C1   C1r Activation   C1s Activation   Activated C1

Figure 4

Activation

Fully activated

C1 complex   MBL-MASP