The complement system

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Abstract

The mammalian complement system plays an important role in the immune defense in blood and interstitial fluids. This set of ~30, mostly multi-domain, plasma proteins and cell-surface receptors enables the host to recognize and clear invading pathogens and altered host cells, while protecting healthy host cells and tissues. In addition to humoral immune defense, complement proteins are produced in the brain, where these proteins contribute to clearance processes such as synaptic pruning. Overall, the complement system may be considered as a broad surveillance mechanism to maintain healthy tissue in mammals. These lecture notes present structural data that provided insights into the underlying molecular mechanism that regulate complement activity. Examples of experimental results are taken primarily from the Gros lab. For an overview of the field the reader is referred to reviews published elsewhere.

Introduction

The complement system can be activated through three main routes: 1) the classical pathway, 2) the lectin-mediated pathway and 3) the alternative pathway of complement activation; see figure 1 (for reviews see e.g. Ricklin et al., 2010; Dunkelberger & Song, 2010). The alternative pathway may be considered as the evolutionary core of the complement system. It is activated non-specifically by a low level of hydrolysis of complement component C3, yielding C3(H₂O), which serves as a starting point for activation, a process referred to as “tick-over mechanism”. The other two pathways provide specificity to complement. The classical and lectin pathways recognize danger patterns present of surfaces of cells and non-cell materials. All three pathways initiate a proteolytic cascade that results in labeling (called opsonization) of the targeted surfaces by complement proteins C4b and C3b. Opsonized surfaces induces cellular immune response, such as phagocytosis by macrophages and stimulation of B cells. Moreover, in the terminal pathway of complement activation further proteolytic activity initiates formation of membrane-attack complexes (MAC), which form large 10-nm pores in the cell membranes leading to cell lysis.

The alternative pathway provides a positive-feedback amplification loop of complement activation leading to opsonization. The central complement protein C3 harbors a reactive thioester moiety. Upon cleavage of C3 into C3a and C3b, C3b covalently binds to target surfaces through its reactive thioester moiety. C3b binding is amplified by generation of C3 convertases on the targeted surface. To form C3 convertases (of the alternative pathway), pro-protease factor B (FB) binds to surface-bound C3b, yielding the pro-convertase C3bB. Subsequently, protease factor D (FD)
cleaves C3bB, which results in release of the pro-peptide fragment Ba yielding the C3bBb complex. The C3bBb complex is a C3 convertase that cleaves C3 into C3a and C3b, which amplifies C3b attachment to the targeted surface by producing C3b locally on the surface.

Figure 1, Schematic overview of the complement cascade: alternative pathway (AP), classical pathway (CP), lectin-binding pathway (LP), host protection and terminal pathway (TP). Red indicates target-surface associated and blue & bold covalent-bound proteins.

Hydrolysis of C3 into C3(H2O) yields a molecule that is functionally homologous to C3b. That is, together with FB and FD, C3(H2O) generates C3(H2O)Bb, which also functions as a C3 convertase. This convertase is considered responsible for the tick-over mechanism.

Formation of C3(H2O)Bb and C3bBb are indiscriminate of self and foreign surfaces. Therefore, the host requires complement regulators to protect healthy host cells and surfaces (Sjoberg et al., 2009). This is a critical step as witnessed by the many mutations in regulators causing dysfunctional regulation and hence disease. Typical examples of disease are: age-related macular degeneration (AMD), atypical hemolytic uremic syndrome (aHUS) and membranoproliferative glomerulonephritis type II (MPGN-II) or dense-deposit disease (DDD) (Ricklin et al., 2010; Anderson et al., 2010; Holers, 2008). Complement regulators consist of strings of complement-control-protein (CCP) domains. They are either expressed on host cell surfaces (e.g., the membrane cofactor protein CD46/MCP) or are present in plasma and bind host cells and matrix specifically, e.g. the soluble, abundant regulator factor H (FH).

The classical and lectin pathways contain multi-molecular initiation complexes, such as C1 and maltose-binding lectin (MBL) complexes, that recognize surface-danger signals specifically. As an example, the large C1 complex consists of a hexameric heterotrimeric C1q that binds either directly or indirectly, through e.g. antibody-antigen complexes, to surfaces. Serine proteases are associated with these recognition molecules, C1r and C1s with C1q and MBL-associated serine proteases 1 and 2 (MASP1 and MASP2). Activation of these pro-enzymes initiates the cascade of proteolytic reactions that is characteristic for complement activation. As shown in figure 1, activated C1 and MBL-MASP cleave C4 into C4a and C4b. Next, C2 binds C4b forming the pro-convertase C4b2, which is cleaved by C1 or MBL-MASP yielding C4b2a, which is the C3 convertase of the classical and lectin pathways. C4 and C2 are homologous to C3 and FB, thus these processes are related to convertase formation in the alternative pathway. One important difference, however, is that in the classical and lectin pathways C4 and C4b2 are cleaved by surface bound C1 or MBL-MASP complexes, whilst in the alternative pathway these complexes are cleaved by soluble FD, which allows for easy spreading and amplification of the opsonization process.
Activation of the terminal pathway of complement results in formation of membrane-attack-complexes (MAC) that form large (100-Å wide) pores in the target membrane (Muller-Eberhard, 1986). These complexes are formed when C5 is cleaved into C5b by the C5 convertase. The C5 convertase is formed by association of one (or possibly more) C3b molecule to the C3 convertase. This somehow shifts the substrate specificity from C3 to its homologue C5. C5b binds subsequently C6, C7, hetero-trimeric C8αβγ and multiple copies of C9. C6 to C9 are homologous proteins build-up from a central MAC-perforin (MACPF) domain and several N- and C-terminal regulatory domains. Together these multi-domain proteins change from soluble proteins into a pore-forming complex that perforates the membrane.

Antibody-mediated activation of the classical pathway

In collaboration with Roman Koning, Bram Koster (Leiden) and Genmab (Utrecht), we used cryo-EM tomography to reveal the arrangement of IgG antibodies on a liposomal surface that binds and activates C1 (Diebolder, Beurskens et al. 2014). Addition of anti-dinitrophenyl (DNP) polyclonal antibodies to DNP-coupled liposomes (Yamamoto et al., 1995) yielded patches of single layers of antibodies on the liposomal surfaces (figure 2, left side). C1 bound on top of the antibody patches (blue arrows in figure 2). Based on 200 tilt series collected on a Krios Titan at NeCEN, we class averaged ~100 complexes resulting in a density map at 6-nm resolution. This very-low resolution map was consistent with IgG molecules forming Fc platforms, similar to the Fc hexamers observed as ‘crystal contacts’ in 1hzh.pdb (Saphire et al., 2001). Specific mutations at the Fc-Fc interface enhanced antibody-mediated complement activation against tumor models in vitro. Modifying a monospecific (bivalent) and non-complement activating EGFR antibody into a monovalent antibody induced potent complement activation. This switch strongly supports the structural interpretation that complement activation by antibodies requires binding of one Fab arm to the target and one Fab "free", which allows formation oligomeric Fc-platforms by the IgG molecules.

![Figure 2](image_url)

Figure 2, Cryo-EM tomograms and averaged density of antibody-mediated complement activation via the classical pathway.

Activation states of the central component C3

Human C3 is a large (1,641 amino-acid residues) protein with a remarkable structural arrangement of 13 domains formed by two protein-chains β and α (Janssen et al.,
The internal domain homologies indicate that this type of protein molecule evolved from a core of eight homologous "macroglobulin" (MG) domains, marking the beginning of a generic host defense mechanism more than 1,300 million years ago; well before the emergence of antibodies. Five other domains are attached to these eight MG domains: a linker and an anaphylatoxin (ANA/C3a) domain are inserted into MG6, a CUB and thioester domain (TED/C3d) are located in between MG7 and MG8, and a netrin-like C345C domain is attached via an anchor region to the C-terminus of domain MG8. In native C3 the reactive thioester is protected in two ways. First, the thioester is sequestered from water to prevent hydrolysis and second, domain-domain interactions prevent transformation of the reactive thioester into a highly reactive thiolate and acyl-imidazole intermediate (Law & Dodds, 1997).

Proteolytic activation of C3 produces anaphylatoxin C3a and opsonin C3b. The major fragment C3b changes its conformation markedly compared to that of native C3 (Janssen et al., 2006; Wiesmann et al., 2006); see figure 3. The thioester region moves over 85 Å, becomes fully exposed and is activated into the acylimidazole intermediate for reaction with hydroxyls on the targeted surfaces. Moreover, the rearranged structure now exposes previously hidden binding sites for pro-protease FB and a variety of regulator proteins that function to protect healthy host cells and tissue.

![Figure 3, Structures of C3 and C3b.](image)

**Native pro-enzyme factor B**

FB is the pro-protease that after assembly and proteolysis provides the serine-protease activity of the central C3 convertase complex (C3bBb) responsible for amplification of the complement response.

The structure of the pro-protease FB (Milder et al., 2007) consists of three N-terminal CCP domains, a linker helix αL (which together with the CCP domains forms the pro-peptide segment Ba), a central Von Willebrand A-type (VWA) domain and C-terminal serine protease (SP) domain (the two latter forming together the protease segment Bb); see figure 4. The VWA domain is homologous to the regulatory "inserted" (I) domains of integrins (Springer, 2006). Similar to integrin I-domains, VWA has a metal-ion (Mg²⁺) dependent adhesion site (MIDAS) critical for ligand (C3b) binding and a C-terminal helix α7 that may be involved in determining ligand-binding affinity at the MIDAS and transmitting conformational changes upon ligand binding (Milder et al., 2006). In the pro-enzyme FB, however, we observe a novel arrangement of these elements in the VWA domain. Helix α7 is displaced from its canonical groove by helix αL of the pro-peptide and the MIDAS, which is critical for C3b binding, is disrupted. Furthermore, the P1 arginine residue of the scissile bond (cleaved by FD in activation of the pro-convertase C3bB) is located in a shallow cleft formed by the helices αL and α7 and forms salt bridges with both helices. These
arrangements suggest a "locked" state for native FB and that binding of C3b likely induces large conformational rearrangements of the helices αL and α7 that expose the scissile bond and make FB susceptible to cleavage by FD.

Figure 4, Structure of factor B.

### Convertase formation

The C3 convertase of the Alternative Pathway is formed in two steps. First, pro-protease FB binds in an Mg$^{2+}$-dependent manner to C3b yielding the pro-convertase C3bB. Second, protease FD cleaves C3bB releasing the pro-peptide fragment Ba resulting in the active C3 convertase, C3bBb.

We first studied the pro-convertase formed by FB and cobra-venom factor (CVF), which is a potent homologue of C3b (Janssen et al., 2009). The isolated structure of CVF (Krishnan et al., 2009) and the complex CVF-B showed that CVF is structurally homologous to C3b. FB binds CVF through two interfaces: one formed by the CCP domains of the pro-peptide segment Ba and one through the VWA domain of the protease segment Bb. The interactions with the Ba segment are apparently critical to "load" FB onto CVF or C3b, because the Bb fragment alone does not bind to CVF or C3b. The Bb segment interacts with CVF through its MIDAS present in the VWA domain, where the carboxy terminus of CVF chelates the Mg$^{2+}$ ion. Surprisingly, no overall domain rearrangement in FB is observed upon binding to CVF. Notably, the scissile bond remains occluded as in the structure of native FB. Negative-stain EM data indicated, however, conformational changes as FB binds to C3b (Torreira et al., 2009a). We hypothesized that CVF-B may represent a "loading" state whereas the EM data of C3bB represents an "activation" state of the pro-convertase, which can be cleaved by FD. Subsequently, this hypothesis was supported by EM data revealing the co-existence of a closed and open state of the pro-convertase (Torreira et al., 2009b).

A crystal structure of the C3bB complex revealed a large conformational change in FB (Forneris et al., 2010); see figure 5. Whereas the crystal structure of CVF-B correlated well with the EM data of the closed (loading) form (Torreira et al., 2009b), the structure of C3bB correlated well with the open (activation) form (Torreira et al., 2009a). The conformational change involved an unexpected, marked reorientation of the catalytic serine protease (SP) domain of FB. This rotation partially extends the VWA-SP linker and unwinds the C-terminal α7 helix of the VWA domain. In conjunction, helix αL that precedes the scissile bond in FB extends by two turns positioning hydrophobic residues in pockets that are vacated by the scissile loop. Together, these changes destabilize the binding of the P1 arginine (Arg234) to the αL and α7 helices, resulting in exposure of the whole scissile loop.
FD circulates in blood in an inactive conformation, in which the catalytic Ser-His-Asp triad is distorted and the P1-binding pocket is blocked by an arginine (Narayana et al., 1994). FD binds FB specifically in the open/activation state of C3bB. The interface formed between the "exosite" of FD and both the VWA and SP domains of FB explains the high affinity (Kd = 9 nM). Binding of FD to C3bB activates FD. Arg202, which blocked the P1-binding pocket, swings out and the self-inhibitory loop rearranges allowing the catalytic Ser-His-Asp triad to be restored (Forneris et al., 2010). What causes these conformational changes is not fully clear, though. Interaction between Glu230 of the scissile loop in FB and Arg202 of FD contributes to the substrate specificity and possibly provides a trigger for the conformational changes that activate FD. Finally, FD cleaves FB and liberates the Ba fragment. The proteolytic fragment Bb, formed by the VWA and SP domains, remains bound to C3b yielding the active C3bBb protease complex.

C3 convertase activity and specificity

C3 convertases are unstable complexes that dissociate irreversibly (with a half life time of ~90 seconds for C3bBb) and thus provide a local and brief burst of complement amplification.

The crystallographic studies of the labile C3bBb protease complex were facilitated by an immune evasion protein, called staphylococcal complement inhibitor (SCIN), which is secreted by S. aureus, that inhibits the C3 convertase, while stabilizing it (Rooijakkers et al. 2007; Rooijakkers et al., 2009). The crystal structure of the triple complex (C3b-Bb-SCIN) revealed a dimeric arrangement of convertases (C3bBb) stabilized by two bridging SCIN molecules (Rooijakkers et al., 2009; Garcia et al., 2010); see figure 6. Two C3b molecules form the center of the dimer. The protease fragment Bb is bound to the C-terminal C345C domain of C3b through its VWA domain, whereas the SP domain is oriented side-ways without making contacts to C3b. SCIN interacts with both C3b and Bb stabilizing the loose arrangement. The observed C3b:C3b contacts suggests a putative substrate:enzyme complex, consistent with earlier considerations based on inhibitor binding sites that block substrate binding (Janssen et al., 2007). Replacement of one C3b by native C3 positions the scissile bond of C3 in front of the catalytic site of the SP domain in Bb. A swing of the Bb fragment towards substrate would result in a productive orientation of the scissile loop in the active site, while the inhibitor SCIN prevents such a movement in the inhibited complex. Such a putative dimerization of the substrate C3
with C3b of the C3 convertase (C3bBb) explains the high specificity and activity of the central C3 convertases (Rooijakkers et al., 2009).

Host protection

Factor H (FH) is an abundant soluble regulator that protects tissues and cells with limited surface regulators. It consists of 20 CCP domains. The first 4 CCP domains are essential and sufficient for the functional activity, whereas the other domains CCP5-20 are involved in distinguishing self from foreign (Pangburn, 2000). We have determined the structure of FH domains 1-4 with its target C3b (Wu et al., 2009); see Figure 6. FH(1-4) binds C3b in an extended arrangement forming a 100-Å long binding site. Comparison of this structure with that of C3bBb shows that domains CCP1-2 of FH are directly involved in displacing Bb from C3b, which explains the "decay-accelerating activity" that breaks down C3 convertases and thereby stops the amplification and production of C3b. In a second mechanism, referred to as "cofactor activity", FH serves as a cofactor to bind the protease factor I (FI). FI cleaves C3b in the CUB domain yielding inactive iC3b that cannot bind FB to form convertases and thus blocks complement amplification. Mutational data (in particular those of the homologue from variola virus done by Sahu and co-workers) suggest a role for CCP2-3 in binding FI. Domains CCP2-3 lie adjacent to the CUB domain of C3b and, thus, provide an appropriate binding site for FI to cleave C3b. A recent crystal structure of FI reveals an arrangement of its five domains that would putatively be consistent with binding of FI in between CCP2-3 of FH and C345C of C3b with the catalytic SP domain oriented towards the scissile bonds in the CUB domain of C3b (Roversi et al., 2011).
Membrane-attack complex

The structure of the membrane-insertion (MACPF) domain of complement component C8α revealed a surprising structural resemblance to the bacterial cholesterol-dependent cytolysins (Hadders et al., 2007; Rosado et al., 2007). This suggests a common membrane perforation mechanism for MAC and perforin of the mammalian immune system and these bacterial pore forming proteins. Sodetz and co-workers resolved additional structures, C8α-MACPF-γ and that of the heterotrimer C8αβγ, revealing both the interactions with the N- and C-terminal ancillary domains and those within the α-β MAC dimer of C8 (Lovelace et al., 2011, Slade et al., 2008).

Next, we solved the structure of the C5b-C6 (C5b6) complex (Hadders et al., 2012; Aleshin et al., 2012b); see figure 8. This structure reveals that, when C5 is cleaved into C5b, large structural changes occur (Laursen et al., 2010). In part, these changes are similar to those of the C3 to C3b conversion. However, in the case of C5 to C5b the TED/C5d domain ends up in a position halfway the MG ring. This conformation of C5b is captured by C6. C6 consists of a core of domains similar to C8 (with an additional TSP domain at the N-terminus) and a C-terminal extension of two CCP domains and 2 FIMACs (Aleshin et al., 2012a). The core of C6 binds the bottom part of the MG ring of C5b. The C-terminal linker and CCP domains of C6 wrap around the TED of C5b. As a consequence the C6 MACPF domain with its pore-forming segments is positioned below C5b. In collaboration with the labs of Susan Lea (Oxford) and Oscar Llorca (Madrid) we placed the C5b6 crystal structure into the cryo-EM reconstruction map of the soluble MAC (sMAC or sC5b6-9) (Hadders et al., 2012). These data indicate that the arrangement of C5b-C6-C7-C8β-C8αγ-C9 yields an arc of the MAC proteins with a protrusion at the beginning formed by C5b. Below the arc large blobs of density indicate the likely presence of clusterin and vitronectin that enwrap the pore-forming segments, thereby providing protection to host cells to bystander damage.

Figure 8, Structure of the C5b6 complex (left) and MAC pore based on cryo-EM tomography (right).

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