Published in final edited form as:

Immunobiology. 2012 November ; 217(11): 1057–1066. doi:10.1016/j.imbio.2012.07.016.

Manipulating the mediator: modulation of the alternative complement pathway C3 convertase in health, disease and therapy¹

Daniel Ricklin

Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia

Abstract

The complement network is increasingly recognized as an important triage system that is able to differentiate between healthy host cells, microbial intruders, cellular debris and immune complexes, and tailor its actions accordingly. At the center of this triage mechanism is the alternative pathway C3 convertase (C3bBb), a potent enzymatic protein complex capable of rapidly converting the inert yet abundant component C3 into powerful effector fragments (C3a and C3b), thereby amplifying the initial response on unprotected surfaces and inducing a variety of effector functions. A fascinating molecular mechanism of convertase assembly and intrinsic regulation, as well as the interplay with a panel of cell surface-bound and soluble inhibitors are essential for directing complement attack to intruders and protecting healthy host cells. While efficiently keeping immune surveillance and homeostasis on track, the reliance on an intricate cascade of interaction and conversion steps also renders the C3 convertase vulnerable to derail. On the one hand, tissue damage, accumulation of debris, or polymorphisms in complement genes may unfavorably shift the balance between activation and regulation, thereby contributing to a variety of clinical conditions. On the other hand, pathogens developed powerful evasion strategies to avoid complement attack by targeting the convertase. Finally, we increasingly challenge our bodies with foreign materials such as biomaterial implants or drug delivery vehicles that may induce adverse effects that are at least partially caused by complement activation and amplification via the alternative pathway. The involvement of the C3 convertase in a range of pathological conditions put this complex into the spotlight of complement-targeted drug discovery efforts. Fortunately, the physiological regulation and microbial evasion approaches provide a rich source of inspiration for the development of powerful treatment options. This review provides insight into the current knowledge about the molecular mechanisms that drive C3 convertase activity, reveals common and divergent strategies of convertase inhibition employed by host and pathogens, and how this inhibitory arsenal can be tapped for developing therapeutic options to treat complement-related diseases.

¹This review article is part of the Young Investigator Award for Research in Complement that will be presented at the XXIV International Complement Workshop 2012

© 2012 Elsevier GmbH. All rights reserved.

Corresponding author: Dr. Daniel Ricklin, 401 Stellar Chance, 422 Curie Blvd., Philadelphia, PA 19104, USA. Phone: +1-215-746-5760, ricklin@upenn.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

complement; complement-targeted therapeutics; convertase; immune evasion; innate immunity; regulators of complement activation

Introduction

Although often depicted as a ‘first line of defense’, recent years have impressively demonstrated that complement is more than just a defender against microbial intruders but rather a tightly integrated surveillance and triage system (Ricklin et al. 2010). Under normal conditions, complement may probe healthy cells by low levels of opsonization through the tick-over mechanism but such activity is instantly impaired by regulators of complement activation (RCA). Apoptotic and diseased cells, as well as cellular debris, may induce a more directed and potent yet carefully restricted response mediated by pattern recognition proteins (PRPs), which marks them for elimination without causing danger signaling or inflammation. Invading microorganisms, however, will ideally face the full power of complement activity, including recognition, opsonization, amplification, recruitment and activation of immune cells, pro-inflammatory signaling, phagocytosis and, on susceptible surfaces, formation of lytic complexes (Fig. 1A). A close interplay and crosstalk with other contact, defense, and housekeeping systems ranging from innate and adaptive immunity to coagulation and cytokine networks is essential in all these processes (Le Frc and Kemper 2009; Oikonomopoulou et al. 2012; Ricklin et al. 2010). In order to execute such tailored responses to different cells and surfaces, complement relies on an intricate network of PRPs, protein components, enzymes, regulators and cell-surface-receptors. Whereas a complement response may be triggered by a variety of different damage/danger and pathogen-associated patterns (DAMPs and PAMPs, respectively) and mediated through at least three initiation pathways (i.e., classical, lectin and alternative pathway), it is often the alternative pathway (AP) C3 convertase that defines the fate of the probed cell. Studies on model systems have shown that some 80% of the observed complement response may be derived from AP convertase-mediated amplification even if initially induced by the classical pathway (CP) (Harboe et al. 2004). This is even more important in clinical situations, where any imbalance between convertase activity and regulation may contribute to a number of inflammatory, age-related, infectious, (auto)immune or ischemia/reperfusion (I/R) diseases (Fig. 1B; also see below). Recent molecular studies of this powerful complex and its interactions with various host and pathogen proteins have provided unprecedented insight into the role of the convertase in health and disease, its susceptibility to immune evasion approaches and its potential as a target for therapeutic intervention in complement-associated diseases. Intriguingly, many of the concepts related to manipulating the convertase are shared between physiological regulators, bacterial or viral evasion proteins, and complement-targeted therapeutics currently in development. The following sections provide a brief overview of these concepts and their implications for research and therapy.

Zooming to the molecular level: a fascinating picture of intrinsic convertase control

A host of biochemical studies early identified the AP C3 convertase as central element of complement activation and amplification (Fearon et al. 1973; Muller-Eberhard 1988; Muller-Eberhard and Gotze 1972; Schreiber et al. 1978). Once initial C3b is deposited by any route, this key opsonin forms AP C3 convertase complexes (i.e., C3bBb) via a tired interaction with the serine proteases factor B (FB) and factor D (FD); C3bBb cleaves native C3 and releases the anaphylatoxin fragment C3a while enabling covalent deposition of additional C3b on the target surface. In the presence of RCA on host cells, the convertase is

quickly destabilized and C3b is degraded into fragments (i.e., iC3b, C3c, C3dg) that are unable of generating new convertases. In the absence of such regulators, however, the succession of C3b deposition and convertase assembly rapidly amplifies the response, thereby decorating the target cells with large numbers of opsonins that facilitate phagocytosis. This increasing C3b density also favors the formation of C5 convertases, which produce the potent anaphylatoxin C5a and trigger the formation of terminal complement complexes (TCC; also known as membrane attack complex). While the individual players and basic mechanisms of convertase function have been described for some time, it was only the recent wealth of insight provided by structural studies and advanced biophysical and biochemical experiments that completed the fascinating picture of how complement is able to control the power of the convertase on a molecular level (Fig. 2A&B) (Gros et al. 2008; Ricklin et al. 2010).

Complement faces a delicate challenge as it needs to be able to quickly probe and react to surfaces with which it comes into contact, yet at the same time needs to contain unnecessary amplification and prevent attack of self-cells. One key element in this task is the high plasma concentration of C3 (~1 mg/ml) that, like a sentinel on patrol, is non-reactive in its native form but quickly gains powerful functionality upon activation. The molecular base of this central mechanism was solved with publications of the crystal structures of native C3 (Janssen et al. 2005), consisting of two chains organized in 13 domains, and activated C3b (Janssen et al. 2006; Wiesmann et al. 2006). Most importantly, these structures revealed that after enzymatic removal of the ANA domain (i.e., C3a), the residual domains of the alpha chains undergo dramatic conformational rearrangements that not only expose and break the reactive thioester moiety, but also uncover a number of novel ligand binding sites that allow for interaction of C3b with activators, regulators, and receptors. Among those sites is the binding area of FB, a plasma protein divided into two segments (Ba/Bb, latter of which contains the serine protease [SP] domain). Intriguingly, formation of the pro-convertase (C3bB) upon binding of FB to C3b induces conformational changes of the SP domain during which FB changes from a compact ("closed") to an "open" form that exposes a new binding site for FD (Forneris et al. 2010). FD does not readily interact via its catalytic center but rather via a distant exosite; this binding induces the dynamic removal of a self-inhibitory loop in FD, which subsequently enables binding and cleavage of a scissile loop in FB that leads to the release of Ba (Forneris et al. 2010). The residual Bb fragment again rearranges and remains bound to the C-terminal C345C domain of C3b, thereby forming the final transient AP C3 convertase complex (C3bBb) (Rooijakkers et al. 2009). Though structural details still have to be resolved, current molecular evidence suggests that native C3 interacts with the convertase via a homodimerization site on C3 and convertase-bound C3b (involving MG4 and MG5). While docking studies confirmed that such dimerization brings the ANA domain of C3 close to the SP domain of C3bBb, the scissile loop of ANA and the catalytic center of SP is still too far distant to allow for direct cleavage (Rooijakkers et al. 2009); it is likely, therefore, that movements in the rather flexible C345C domain of C3b may bring Bb closer to C3 to mediate the cleavage. This activation hypothesis has been further supported by the co-crystal structure between C5 and cobra venom factor (CVF), which both share homology with C3/C3b; the study revealed an interaction interface that resembles the proposed convertase:C3 complex and even provided clues about potential structural changes that would facilitate substrate cleavage (Laursen et al. 2011). Finally, two clinically observed mutations in C3 featuring either a deletion of two residues in MG7 (C3 923ΔDG) or a point mutation in MG4 (C3 M351T) were recently described on the molecular level; both affected areas are supposed to be involved in the convertase:C3 contact regions and resulted in C3 species that were unable to be cleaved by the AP C3 convertase (Martinez-Barricarte et al. 2010; Sfyroera et al. 2010). Upon activation, the conformational changes in C3b described above position the TED domain with its freshly activated thioester moiety close to the cell surface (Fig. 2A). This is of utmost importance,

since the reactive acyl group is only able to attack its nucleophilic targets (mostly hydroxyl- and amino-groups on cell surface molecules) with a half-life of less than a second before it gets hydrolyzed; this mechanism prevents circulation of reactive C3b and ensures that only the target cell is opsonized. An equally clever control mechanisms further provides selectivity at the level of the proteases. Although both C3 and FB are present in rather high concentration in plasma, and despite FB appears to possess an active catalytic center in solution, the Ba segment only allows binding to C3b but not C3, and the C3b part of the convertase acts as an essential bridge to mediate the interaction between enzyme and substrate. The convertase has limited stability (half-life of some 90 s) and, after release of Ba, Bb cannot rebind to C3b, thereby requiring the continuous presence of convertase building blocks to keep amplification going. Finally, FD is a highly active protease but intrinsically impaired by its self-inhibitory loop in the unbound form. In summary, it is that unique interplay between differentially active proteases, bridging molecules, conformational rearrangements, and kinetic limitations that ensures quick yet highly specific activation of the convertase.

Yet the convertase cannot simply rely on such intrinsic control steps but is tightly regulated on self-surfaces by members of the RCA family of regulators (Fig. 2B). They are all composed of 4–30 complement control-protein domains (CCP) and are either anchored to the host cell surface or available in circulation. On membranes, CD55 (decay accelerating factor; DAF) prevents formation of the AP C3 convertase and decreases its stability (decay accelerating activity; DAA), whereas CD46 (membrane cofactor protein; MCP) mediates the degradation of C3b by factor I (FI), which results in iC3b that cannot form new convertases (cofactor activity; CA). Further, membrane-bound CD35 (complement receptor 1; CR1) contains three regulatory areas and exerts potent DAA and CA towards both AP and CP/LP convertases; it is the only RCA that allows further degradation of iC3b to C3dg and C3c. Finally, the abundant soluble regulator factor H (FH) shows DAA/CA to inhibit the AP C3 convertase; in addition, FH contains CPP domains that recognize distinct pattern on self surfaces (mostly sialic acid and glycosaminoglycans [GAG]). The co-crystal structure of the regulatory N-terminal four CCP domains (i.e., FH1-4) with C3b revealed important insight into the characteristics of these activities (Wu et al. 2009). Surprisingly, FH1-4 forms a large interaction interface that stretches across a whole flank of C3b. Superimposition of C3bBb of the convertase structure with C3b/FH1-4 revealed that the two ligands occupy a partially overlapping binding site, thereby suggesting that the DAA is reached via competitive replacement of Bb (similarly, FH would also prevent initial binding of FB via the Ba segment) (Wu et al. 2009). In the case of CA, the binding of FH to C3b appears of form a shared binding platform for FI that positions the protease in proximity of the CUB domain of C3b to allow for the necessary two cleavages to produce iC3b (Fig. 2B) (Roversi et al. 2011; Wu et al. 2009). While it is expected that all RCA share these principal mechanisms, more structural insight is needed to decipher what makes one a better DAA regular and what favors CA.

Small bugs are big on convertase inhibition

Given its central role in complement amplification on microbial surface, it is not surprising that the AP C3 convertase is a major target of innate immune evasion strategies by several human pathogens (Blom et al. 2009; Jongerius et al. 2009; Lambris et al. 2008). One of the most common approaches employed by many bacteria and certain fungi is RCA recruitment (Fig. 2C); lacking self-surface pattern like GAG, these pathogens express specialized proteins that bind soluble regulators like C4b-binding protein (C4BP; CP/LP regulation), FH (AP regulation) or vitronectin (terminal pathway regulation). Prominent members of this class of pathogens include *Neisseria*, *Streptococcus*, *Borrelia*, *Candida* and *Aspergillus* species (Blom et al. 2009; Serruto et al. 2010). In the case of *N. meningitidis*, the surface-

exposed lipoprotein fHbp (GNA1870) appears to mimic the polyanion patches on host surfaces that are recognized by CCP domains 6–7 of FH and was shown to strongly bind human FH but not FH from non-human primates or lower species (Schneider et al. 2006; Schneider et al. 2009; Serruto et al. 2010). While orthopox viruses also count on the regulatory power of RCA, they follow a distinct approach by expressing soluble or membrane-anchored RCA homologs with strong DAA and/or CA properties (Fig. 2C); among those, the smallpox inhibitor of complement enzymes (SPICE) and the vaccinia virus complement control protein (VCP) are the most thoroughly studied viral RCA mimics (Mullick et al. 2003). Although they do not share homology to RCA, certain glycoproteins from herpes viruses (i.e., HSV gC-1) have been shown to exert DAA and prevent binding of the convertase-stabilizing protein properdin (Mullick et al. 2003).

Staphylococcus aureus, on the other hand, seems to primarily focus on a different strategy to tame the action of the convertase (Lambris et al. 2008; Serruto et al. 2010), even though molecules that either enhance the binding of FH to C3b (e.g., via Sbi and Efb) or attract FH to the surface (i.e., via SdrE) have recently been described (Chen et al. 2010; Haupt et al. 2008; Sharp et al. 2012). Instead, *S. aureus* at least two families of proteins that directly bind and act at the C3 level. Intriguingly, both the extracellular fibrinogen-binding protein (Efb; and its homolog Ehp) and the staphylococcal complement inhibitor family (SCIN-A, SCIN-B, SCIN-C) share a highly similar structural motif, bind to C3 fragments and block convertase activity, but follow completely distinct inhibitor mechanisms (Garcia et al. 2012a; Hammel et al. 2007a; Hammel et al. 2007b; Jongerius et al. 2007). Efb combines a fibrinogen-binding region with a C-terminal domain (Efb-C) that binds to the TED of C3, C3b, iC3b, and C3dg (Hammel et al. 2007b; Haspel et al. 2008). In the case of C3b, the Efb binding site is not readily accessible in the conformer found in all available C3b crystal structures. Using a combination of different structural and biophysical methods, we could recently show that binding of Efb-C induces and/or stabilizes a conformational state of C3b, where the TED domain is dislocated from its primary position (Chen et al. 2010). This conformational distortion propagates along the CUB domain into the “shoulder region” of C3b (MG7/8), thereby affecting several key ligand binding sites. Most notably, Efb-C strongly impaired the binding of FB to C3b and, consequently, formation of the AP C3 convertase. Its acting as a conformational wedge that influences activities on distant areas of the target protein therefore made Efb the first known allosteric complement inhibitor (Fig. 2D) (Chen et al. 2010). In comparison with Efb, the SCIN proteins binds to two distinct regions of C3b with a primary site at domain MG8 (Garcia et al. 2010; Garcia et al. 2012b; Ricklin et al. 2009; Rooijakkers et al. 2009). Rather than preventing convertase formation, as in the case of Efb, SCIN actually stabilizes the C3bBb complex and thereby traps it in an inactive state. When mixed with C3b, FB, and FD rapidly forms mixed convertase dimers (i.e., C3b₂Bb₂SCIN₂; Fig. 2E) that are highly stable and enabled crystallization of a C3 convertase complex (Rooijakkers et al. 2009). Surface plasmon resonance (SPR)-based studies indicated that the C3 substrate can still bind to the SCIN-stabilized convertase but cannot be cleaved and deposited, thereby preventing opsonization and participation in the amplification loop (Ricklin et al. 2009). It is likely that SCIN, by forming trimeric complexes with C3b and Bb, prevents domain movements in the convertase that would bring Bb into contact with the scissile loop of C3. Notably, SCIN also renders the convertase virtually inert towards decay acceleration via FH, thereby keeping it in the trapped state (Ricklin et al. 2009). Besides inhibiting the convertase, both Efb and SCIN have been associated with other complement-targeted activities: The binding of Efb and Ehp to C3dg has been shown to prevent the interaction of this opsonin with complement receptor 2 (CD21) and impairs co-stimulation of the B cell receptor on B lymphocytes (Ricklin et al. 2008). SCIN-induced convertase dimerization, on the other hand, blocks essential sites in C3b that are involved in the signaling via CD35 and the complement receptor of the immunoglobulin superfamily (CRIg) (Jongerius et al. 2010). Yet previous and recent studies

indicate that the impressive convertase-directed arsenal of *S. aureus* may not be restricted to the Efb and SCIN families: for example, the staphylococcal metalloprotease aureolysin was shown to cleave native C3 into C3b, which is subsequently degraded by host factors (Laarman et al. 2011); in addition, clumping factor A (CfIA) binds to FI and mediates the degradation of C3b, staphylokinase was shown to induce plasminogen to remove opsonic C3b, and the immunoglobulin-binding protein Sbi also appears to exert inhibitory or even depleting actions via binding to C3 (Laarman et al. 2010; Lambris et al. 2008; Smith et al. 2011). Importantly, the molecular study of this exceptionally diverse panel of potent complement inhibitors not only provided insight into complement evasion by *S. aureus* but also taught important lessons about the structure and function of the convertase and may well contribute to the development of complement-targeted therapeutics.

Convertase out of bounds: key to complement-related diseases

As a centerpiece of the complement-mediated triage and surveillance system described above (Fig. 1A), the AP C3 convertase and its regulatory minders are also vulnerable spots that may tip the entire system off balance. Indeed, dysregulation and erroneous activation/amplification of the AP are increasingly recognized as important contributors to a variety of clinical conditions (Fig. 1B) ranging from the autoimmune to the degenerative and inflammatory spectrum (Holers 2008; Ricklin and Lambris 2007). Mutations and polymorphisms in the genes of C3, FB, FH and other RCA (and to a lesser degree FD) have been associated with disease states of the eye (e.g., age-related macular degeneration [AMD]), the kidneys (e.g., dense deposit disease), the brain (e.g., CD35 in Alzheimer's disease) or other bodily systems (e.g., atypical hemolytic uremic syndrome [aHUS] that includes hemolytic anemia, thrombocytopenia and renal impairment among its symptoms) (Rodriguez de Cordoba et al. 2011; Veerhuis et al. 2011). In many of these cases, initial triggers (such as accumulating debris or plaque) set a vicious cycle of recognition and amplification in motion that cannot be adequately controlled and ultimately lead to danger signaling and inflammatory events (Fig. 1B). In the case of AMD, for example, polymorphisms of FH that lower its interaction with either C3b or self/disease surface patches have been associated with a higher risk, whereas a polymorphic form of FB that decreases the Ba-mediated interaction with C3b was found to have a partially protective effect (Rodriguez de Cordoba et al. 2011); very often, the observed activity/affinity changes are moderate but highly significant in diseases that develop over years. Dysfunctions and deficiencies in individual components may induce similar conditions and may also contribute to increased susceptibility to certain types of infection (Botto et al. 2009; Reis et al. 2006), especially since many human pathogens already have acquired mechanisms of evading considerable parts of complement-mediated defense (see above; Fig. 1B). Importantly, similar molecular consequences may lead to distinct clinical manifestations; for example, while one of the two C3 mutations that impair cleavage by the AP C3 convertase (C3 393ΔDG; see above) is associated with a unique dense deposit disease pedigree, the other (C3 M351T) does not show similar association but was rather causing symptoms like rashes or episodes of certain infections (Martinez-Barricarte et al. 2010; Sfyroera et al. 2010). A special example is paroxysmal nocturnal hemoglobinuria (PNH), where genetic defects do not alter the protein sequence directly but prevent the attachment of the GPI linker, thereby rendering cells devoid of the regulators CD55 and CD59; PNH erythrocytes thereby become highly susceptible to opsonization and amplification via the AP and, ultimately, TCC-mediated lysis (Risitano et al. 2011). In addition, PNH also affects complement regulation on platelets and other blood cells, and the higher rate of complement-mediated platelet activation or release of tissue factor may contribute to the high risk of thrombosis observed in PNH patients (Markiewski et al. 2007; Oikonomopoulou et al. 2012). Overwhelming infection (in the case of sepsis) but also other acute triggers like trauma, burns, or ischemia/reperfusion injury (e.g., in the case of myocardial infarction) can

induce tissue damage, thereby strongly activating complement and its amplification loop, in combination with other defense and downstream inflammatory pathways that often fuel each other to arrive at severe clinical consequences (Castellheim et al. 2009; Ioannou et al. 2011; Markiewski et al. 2008; Neher et al. 2011; Ward and Gao 2009). Finally, while modern medicine enabled fascinating applications in the transplantation of organs and cells as well as the use of biomaterials in extracorporeal circuits (e.g., hemodialysis), implants (e.g., meshes for hernia repair) or drug delivery systems (e.g., nanoparticles, liposomes, or pumps), the involved surfaces are commonly recognized as foreign and/or are insufficiently protected by regulators, therefore leading to AP-dominated complement attack and adverse reactions that affect the function of the transplant, implant, or vehicle and the quality-of-life of the patient (Fig. 1B) (Ekdahl et al. 2011; Nilsson et al. 2010; Sacks and Zhou 2012). Given that AP activation and amplification are driving forces, or at least critically involved, in the wide range of clinical conditions described above, pharmacologic control of the convertase activity is considered a highly important and promising strategy for taming complement-related diseases (Ricklin and Lambris 2007; Ricklin and Lambris 2012).

Although complement-related therapeutics have meanwhile been entering the market and can be used for clinical application, none of the available drugs, i.e. purified C1 inhibitor (e.g., Cinryze, ViroPharma) and the C5 antibody Eculizumab (Soliris, Alexion), are acting at the level of the AP C3 convertase. The necessity of such options can perhaps best be exemplified with the case of PNH, where Eculizumab effectively prevents intravascular lysis via unprotected formation of TCC yet does not interrupt amplification, thereby leaving heavily opsonized erythrocytes that show increased susceptibility for extravascular lysis (Risitano et al. 2011). In many clinical conditions that are driven by AP-mediated amplification of complement responses, convertase-directed therapeutics are therefore highly desired.

Taming the beast: therapeutic modulation of convertase activity

When not hampered by polymorphisms, members of the RCA family of regulators represent highly powerful inhibitors, as they have been evolutionary designed to control the activity of the C3 convertase. It is not surprising, therefore, that considerable efforts in complement-related drug discovery have been invested in utilizing this power in a therapeutic manner (Ricklin and Lambris 2007; Ricklin and Lambris 2012). Initial strategies primarily focused on soluble recombinant versions of the membrane-bound RCAs CD35, CD46, and CD55. Examples include a fusion protein that linked the decay accelerating domains of CD55 with the cofactor area of CD46 (CAB-2, MLN2222; Xoma/Millennium) and soluble CD35 (sCR1, TP10; Avant). TP10 had shown promising effects in preclinical models and clinical trials, mostly for taming inflammatory reactions during transplantation and cardiopulmonary bypass surgery (Li et al. 2006), yet development had slowed due to inconclusive results during a phase II trial (gender-specific effects) and company mergers (Lazar et al. 2007). Currently, sCR1 is developed for the treatment of renal diseases by Celldex (CDX-1135). In order to reduce the size and increase the cell surface selectivity of this powerful regulator, a targeted form of a truncated CD35 fragment attached to a lipopeptide membrane anchor has been developed (APT070, Mirococept) (Smith 2002), which has recently demonstrated high promise in prolonging the lifetime of kidney transplants after perfusion with this drug (Sacks and Zhou 2012). In view of the many disease associations of FH, the AP-specific inhibitory potency of this regulator has moved into the spotlight of RCA-based therapies. The reconstitution of quantitative of functional FH deficiencies in aHUS by plasma therapy has been described (Lapeyraque et al. 2008; Licht et al. 2005), and the use of plasma-purified or recombinant FH has been proposed as a viable alternative that could be used in diseases like AMD or aHUS (Gehrs et al. 2010; Schmidt et al. 2011). In many situations, however, novel concepts based on engineered, targeted FH constructs may be advantageous concerning

activity, specificity and production cost. The most prominent member of this category is TT30 (ALXN1102, Alexion), a fusion protein between the five N-terminal CCP domains of FH (including the regulatory CCP1-4) and the N-terminus of complement receptor 2 (CR2 CCP1-4). The binding of the CR2 part to surface patches of iC3b and C3dg preferentially directs TT30 to sites of ongoing complement activation/amplification and thereby targets the inhibitory activity of FH to diseased cells and tissue (Fridkis-Hareli et al. 2011). TT30 and related constructs have successfully been evaluated in various animal models related to AMD and other diseases and have demonstrated high potency in suppressing both C3b deposition and lysis in PNH in humans (Risitano et al. 2012); it is currently in a phase I trial for PNH. More recently, our group has developed another FH construct (called Mini-FH (Schmidt et al. 2012)) that uniquely links the regulatory N-terminus (CCP1-4) to the surface-recognizing C-terminus (CCP19-20) via a short peptide linker based on novel structural insight about the interaction of these areas with C3b (Kajander et al. 2011; Morgan et al. 2011; Wu et al. 2009). Similar to TT30, mini-FH maintains full regulatory activity of FH and exerts targeting to sites of ongoing activation via binding of CCP19-20 to C3b, iC3b, and C3d. Importantly, mini-FH also binds to self-surface pattern such as GAGs and may even be targeted to certain disease-related surface modifications, as for example to oxidative stress-induced malondialdehyde adducts that have recently been associated with complement-mediated disease contributions in AMD (Schmidt et al. 2012; Weismann et al. 2011). Mini-FH showed high activity in models of PNH and in blood of PNH patients, and may prove useful in a variety of complement-mediated disease models (Schmidt et al. 2012).

Yet convertase inhibition is not restricted to RCA-derived therapeutics. Although the use of serine protease inhibitors for blocking the key activation steps mediated by FB and FD have been actively pursued, pharmacokinetic and specificity issues proved to be rather challenging (Qu et al. 2009; Ricklin and Lambris 2007; Ricklin and Lambris 2012). Most of the non-RCA-based drug candidates rather focus on inhibiting the various protein-protein interactions (PPI) that are involved in the assembly, activation and substrate cleavage of the AP C3 convertase. For example, a humanized antibody fragment against FB (TA106, mAb 1379) had been developed by Taligen (now Alexion); this antibody binds to the CCP3 domain of the Ba segment of FB and prevents the initial interaction with C3b (Thurman et al. 2005). Similarly, a Fab fragment against an exosite on FD that blocks the binding of FD to the open form of the C3bB pro-convertase is under clinical development by Genentech (FCFD4514S, mAb 166–32) for treating AMD (Katschke et al. 2012; Yehoshua et al. 2011). In addition, various C3b-directed antibodies with therapeutic potential have been described; they usually prevent the binding of FB to form the pro-convertase or the interaction of native C3 with the assembled convertase. Examples include mAb S77 that binds to the shoulder region of C3b (MG7) and blocks FB binding (Katschke et al. 2009), and mAb 3E7 that interacts with an non-disclosed epitope on C3b/iC3b and competes with the binding of FB and FH (DiLillo et al. 2006; Lindorfer et al. 2010). Finally, Genentech also described the use of a soluble, affinity-maturated form of the complement receptor of the immunoglobulin superfamily (CRIg), which primarily binds to the MG core of the beta-chain of C3b (and iC3b/C3c) and was found to inhibit both the AP C3 and C5 convertases (Li et al. 2009; Wiesmann et al. 2006). While engineered proteins and, especially, therapeutic antibodies represent the most successful class of PPI inhibitors due to their high affinity and large potential for steric hindrance, they are also costly to produce and usually need to be injected. Though more challenging to develop, smaller molecules such as peptides hold high promise as PPI inhibitors as they potentially offer key advantages concerning cost, and in some cases, administration routes and immunogenicity (Bray 2003; Vlieghe et al. 2010; Wells and McClendon 2007). In the case of convertase-directed therapeutics, the peptidic inhibitor is currently the only compound that appears to fit this category. Compstatin is a cyclic peptide of 13 amino acid residues that binds to both native C3 and C3b (as well as to C3(H₂O), iC3b and C3c) and potently inhibits the activation of C3 by both AP and CP C3 convertases

(Ricklin and Lambris 2008). Structural studies revealed a binding site at the MG4/5 domains and indicated the importance of this area for the interaction of C3 with the AP C3 convertase (Janssen et al. 2007; Rooijakkers et al. 2009); it is very likely, therefore, that compstatin acts as a PPI inhibitor of this interaction. Intriguingly, however, recent experiments based on SPR and hydrogen-deuterium exchange mass spectrometry indicated that compstatin analogs also induce allosteric effects in C3 and C3b (Chen et al., in preparation). In the past decade, optimization efforts produced a large panel of compstatin analogs with largely improved efficacy, physicochemical properties and pharmacokinetic profiles (Knerr et al. 2011; Magotti et al. 2009; Qu et al. 2011; Qu et al. 2012; Ricklin and Lambris 2008). The most recent analogs feature subnanomolar binding affinities for C3b and surprisingly favorable plasma elimination half-life values of up to 12 hours in non-human primates, making them interesting candidates for systemic applications (Qu et al. 2012). Compstatin analogs have been successfully used in a variety of disease models ranging from AMD and transplantation to sepsis and hemodialysis-induced thromboinflammatory complications (Chi et al. 2010; Kourtzelis et al. 2010; Ricklin and Lambris 2008; Silasi-Mansat et al. 2010); one analog is currently in clinical evaluation of the local treatment of AMD (Ricklin and Lambris 2012; Yehoshua et al. 2011). Interestingly, the three C3b-directed PPI inhibitors for which a co-crystal structure has been reported, i.e., compstatin, sCRIg and the Fab fragment of S77 (Janssen et al. 2007; Katschke et al. 2009; Wiesmann et al. 2006), bind to the same flank of C3b, thereby further supporting the importance of this area for convertase-mediated C3 activation (Rooijakkers et al. 2009)(Fig. 3C).

As illustrated above, the principle of preventing convertase activity directly on the surface via active recruiting of RCAs is likewise being exploited by the host (via recognition of self-surface pattern) and many human pathogens (via expression of RCA-binding proteins). This evolutionary successful principle can also be utilized in a therapeutic context to shields foreign cells and organs (e.g., during allo- and xeno-transplantation) or artificial materials (e.g., biomaterials implants, extracorporeal circuits or drug delivery vehicles) from complement attack and downstream inflammatory events (Ekdahl et al. 2011; Nilsson et al. 2010). Indeed, coating of biomaterials with the GAG heparin was previously been shown to have regulatory effects on both complement and coagulation pathways, though it is not clear how much of these have been mediated by FH recruitment. In addition, FH had been directly immobilized on model biomaterials and efficiently prevented complement activation after contact with blood. Finally, the principle of using bacterial protein coatings to recruit RCA to biomaterial surfaces has been demonstrated by immobilization of streptococcal M-protein that subsequently bound C4b-binding protein and attenuated CP activation (Ekdahl et al. 2011). However, all these approaches suffer from the use of comparatively large molecules that are often difficult and costly to coat. Phage-display libraries were therefore screened for small peptides capable of binding to FH, which identified a promising candidate (peptide 5C6) that captures FH without negatively influencing its regulatory or binding affinities; on model materials, 5C6 efficiently recruited FH from plasma and largely prevented biomaterial-induced complement activation (Wu et al. 2011). Importantly, using membrane-targeted anchoring strategies, 5C6-mediated FH binding also protected clinically relevant targets such as erythrocytes or porcine aortic endothelial cells from complement attack; in combination with immobilized apyrase, such mixed coating produced an effective autoregulatory surface to prevent thromboinflammation (Nilsson et al. 2012).

Alongside RCA and receptor domains such as CRIg, microbial complement evasion proteins could also serve as promising templates for the design of complement-targeted therapeutics (Laarman et al. 2010; Lambris et al. 2008). The orthopox virus-derived RCA mimic VCP has been evaluated in disease models such as in renal I/R injury (Ghebremariam et al. 2010; Jha and Kotwal 2003), and recombinant versions of the C5a receptor antagonist CHIPS from *S. aureus* are in clinical development for treating myocardial infarction (ADC-1004;

Alligator Biosciences) (Gustafsson et al. 2009; van der Pals et al. 2010). Potential issues concerning immunogenicity, plasma elimination half-life, and species specificity certainly need to be considered for clinical development, but may be sufficiently resolved by truncation, mutagenesis, conjugation (e.g., using PEGylation) or other measures. Furthermore, the potent and intriguing mechanisms of convertase inhibition by Efb and SCIN identified vulnerable points in the convertase and potential hot spots for the discovery of novel complement inhibitors based on peptides or small molecules. After all, millennia of coevolution have rendered many pathogens into efficient anti-immune/inflammatory “drug development centers” from which we have a lot to learn.

Conclusion and Outlook

The AP C3 convertase undeniably plays an important role in immune surveillance, homeostasis, and defense and it often takes little to tip the balance between physiological and pathophysiological effects. Convertase activity is therefore not only tightly controlled by the host, but the importance of modulating this driving force of complement response has also been recognized by human pathogens as well as in the development for complement related drugs. Very often, the mechanisms and strategies of convertase modulation are heavily intertwined as the utilization of RCA in regulation, evasion, and therapy impressively illustrates. Yet there are also unique concepts that may serve as inspiration for future therapeutic approaches. In any case, the study of molecular activation and inhibition mechanisms was greatly enabled by advances in structural and biophysical/biochemical methods and led to a tremendous gain of knowledge about how the convertase works and what may trigger disease. Nonetheless, there are still essential gaps in our understanding of the convertases and associated processes. For example, the described mechanism of convertase induction and/or stabilization by properdin has not yet been resolved on the structural level, the hypothesis about how C3 binds to the assembled convertase still awaits confirmation, and additional structures of RCA bound to C3b or C4b may reveal important key aspects of convertase regulation. Moreover, little is still known about the C3 convertases of the classical/lectin and tick-over pathways, and about what exact mechanisms propagate the shift from C3 to C5 convertases. Various structural studies indicated that C3b possesses a higher degree of conformational flexibility than available (co-)crystal structures of this protein suggest (Chen et al. 2010; Nishida et al. 2006; Schuster et al. 2008); however, the functional impact of this dynamic flexibility and whether it can be employed for the development of allosteric convertase inhibitors is not yet clear. Finally, the identification of novel microbial evasion proteins and therapeutic inhibitors is likely to provide additional insight into mechanistic aspects of the convertase, reveal new targeting spots and potential therapeutic concepts. Our molecular picture of the AP C3 convertase and its many modulators becomes increasingly detailed and colorful but still needs work before completion. Knowing complement, it seems almost certain that one or the other fascinating surprise awaits us on that journey...

Acknowledgments

This work was supported by National Institutes of Health grants AI030040, AI068730, GM062134, EY020633 and AI097805.

Abbreviations

AMD	age-related macular degeneration
AP	alternative pathway
CA	cofactor activity

CCP	complement control protein domain
CP	classical pathway
CR1	complement receptor 1
CUB	complement C1r/C1s, Uegf, Bmp1 domain
DAA	decay acceleration activity
DAF	decay accelerating factor
Efb	extracellular fibrinogen-binding protein
FB	factor B
FH	factor H
FI	factor I
GAG	glycosaminoglycan
MCP	membrane cofactor protein
PNH	paroxysmal nocturnal hemoglobinuria
PPI	protein-protein interaction
RCA	regulator of complement activation
SCIN	staphylococcal complement inhibitor
SPICE	smallpox inhibitor of complement enzymes
SPR	surface plasmon resonance
TCC	terminal complement complex
TED	thioester-containing domain
VCP	vaccinia virus complement control protein

References

- Blom AM, Hallstrom T, Riesbeck K. Complement evasion strategies of pathogens-acquisition of inhibitors and beyond. *Mol Immunol.* 2009; 46:2808–2817. [PubMed: 19477524]
- Botto M, Kirschfink M, Macor P, Pickering MC, Wurzner R, Tedesco F. Complement in human diseases: Lessons from complement deficiencies. *Mol Immunol.* 2009; 46:2774–2783. [PubMed: 19481265]
- Bray BL. Large-scale manufacture of peptide therapeutics by chemical synthesis. *Nat Rev Drug Discov.* 2003; 2:587–593. [PubMed: 12815383]
- Castellheim A, Brekke OL, Espevik T, Harboe M, Mollnes TE. Innate immune responses to danger signals in systemic inflammatory response syndrome and sepsis. *Scand J Immunol.* 2009; 69:479–491. [PubMed: 19439008]
- Chen H, Ricklin D, Hammel M, Garcia BL, McWhorter WJ, Sfyroera G, Wu YQ, Tzekou A, Li S, Geisbrecht BV, Woods VL Jr, Lambris JD. Allosteric inhibition of complement function by a staphylococcal immune evasion protein. *Proc Natl Acad Sci U S A.* 2010; 107:17621–17626. [PubMed: 20876141]
- Chi ZL, Yoshida T, Lambris JD, Iwata T. Suppression of drusen formation by compstatin, a peptide inhibitor of complement C3 activation, on cynomolgus monkey with early-onset macular degeneration. *Adv Exp Med Biol.* 2010; 703:127–135. [PubMed: 20711711]
- DiLillo DJ, Pawluczko AW, Peng W, Kennedy AD, Beum PV, Lindorfer MA, Taylor RP. Selective and efficient inhibition of the alternative pathway of complement by a mAb that recognizes C3b/iC3b. *Mol Immunol.* 2006; 43:1010–1019. [PubMed: 15961157]

- Ekdahl KN, Lambris JD, Elwing H, Ricklin D, Nilsson PH, Teramura Y, Nicholls IA, Nilsson B. Innate immunity activation on biomaterial surfaces: a mechanistic model and coping strategies. *Adv Drug Deliv Rev.* 2011; 63:1042–1050. [PubMed: 21771620]
- Fearon DT, Austen KF, Ruddy S. Formation of a hemolytically active cellular intermediate by the interaction between properdin factors B and D and the activated third component of complement. *J Exp Med.* 1973; 138:1305–1313. [PubMed: 4202731]
- Forneris F, Ricklin D, Wu J, Tzekou A, Wallace RS, Lambris JD, Gros P. Structures of C3b in complex with factors B and D give insight into complement convertase formation. *Science.* 2010; 330:1816–1820. [PubMed: 21205667]
- Fridkis-Hareli M, Storek M, Mazsaroff I, Risitano AM, Lundberg AS, Horvath CJ, Holers VM. Design and development of TT30, a novel C3d-targeted C3/C5 convertase inhibitor for treatment of human complement alternative pathway-mediated diseases. *Blood.* 2011; 118:4705–4713. [PubMed: 21860027]
- Garcia BL, Ramyar KX, Ricklin D, Lambris JD, Geisbrecht BV. Advances in understanding the structure, function, and mechanism of the SCIN and Efb families of Staphylococcal immune evasion proteins. *Adv Exp Med Biol.* 2012a; 946:113–133. [PubMed: 21948365]
- Garcia BL, Ramyar KX, Tzekou A, Ricklin D, McWhorter WJ, Lambris JD, Geisbrecht BV. Molecular basis for complement recognition and inhibition determined by crystallographic studies of the staphylococcal complement inhibitor (SCIN) bound to C3c and C3b. *J Mol Biol.* 2010; 402:17–29. [PubMed: 20654625]
- Garcia BL, Summers BJ, Lin Z, Ramyar KX, Ricklin D, Kamath DV, Fu ZQ, Lambris JD, Geisbrecht BV. Diversity in the C3b [corrected] contact residues and tertiary structures of the staphylococcal complement inhibitor (SCIN) protein family. *J Biol Chem.* 2012b; 287:628–640. [PubMed: 22086928]
- Gehrs KM, Jackson JR, Brown EN, Allikmets R, Hageman GS. Complement, age-related macular degeneration and a vision of the future. *Arch Ophthalmol.* 2010; 128:349–358. [PubMed: 20212207]
- Ghebremariam YT, Engelbrecht G, Tyler M, Lotz Z, Govender D, Kotwal GJ, Kahn D. Vaccinia virus complement control protein (VCP) improves kidney structure and function following ischemia/reperfusion injury in rats. *J Surg Res.* 2010; 159:747–754. [PubMed: 19691975]
- Gros P, Milder FJ, Janssen BJ. Complement driven by conformational changes. *Nat Rev Immunol.* 2008; 8:48–58. [PubMed: 18064050]
- Gustafsson E, Forsberg C, Haraldsson K, Lindman S, Ljung L, Furebring C. Purification of truncated and mutated Chemotaxis Inhibitory Protein of Staphylococcus aureus--an anti-inflammatory protein. *Protein Expr Purif.* 2009; 63:95–101. [PubMed: 18950716]
- Hammel M, Sfyroera G, Pyrpassopoulos S, Ricklin D, Ramyar KX, Pop M, Jin Z, Lambris JD, Geisbrecht BV. Characterization of Ehp, a secreted complement inhibitory protein from Staphylococcus aureus. *J Biol Chem.* 2007a; 282:30051–30061. [PubMed: 17699522]
- Hammel M, Sfyroera G, Ricklin D, Magotti P, Lambris JD, Geisbrecht BV. A structural basis for complement inhibition by Staphylococcus aureus. *Nat Immunol.* 2007b; 8:430–437. [PubMed: 17351618]
- Harboe M, Ulvund G, Vien L, Fung M, Mollnes TE. The quantitative role of alternative pathway amplification in classical pathway induced terminal complement activation. *Clin Exp Immunol.* 2004; 138:439–446. [PubMed: 15544620]
- Haspel N, Ricklin D, Geisbrecht BV, Kavraki LE, Lambris JD. Electrostatic contributions drive the interaction between Staphylococcus aureus protein Efb-C and its complement target C3d. *Protein Sci.* 2008; 17:1894–1906. [PubMed: 18687868]
- Haupt K, Reuter M, van den Elsen J, Burman J, Halbach S, Richter J, Skerka C, Zipfel PF. The Staphylococcus aureus protein Sbi acts as a complement inhibitor and forms a tripartite complex with host complement Factor H and C3b. *PLoS Pathog.* 2008; 4:e1000250. [PubMed: 19112495]
- Holers VM. The spectrum of complement alternative pathway-mediated diseases. *Immunol Rev.* 2008; 223:300–316. [PubMed: 18613844]
- Ioannou A, Dalle Lucca J, Tsokos GC. Immunopathogenesis of ischemia/reperfusion-associated tissue damage. *Clin Immunol.* 2011; 141:3–14. [PubMed: 21839685]

- Janssen BJ, Christodoulidou A, McCarthy A, Lambris JD, Gros P. Structure of C3b reveals conformational changes that underlie complement activity. *Nature*. 2006; 444:213–216. [PubMed: 17051160]
- Janssen BJ, Halff EF, Lambris JD, Gros P. Structure of compstatin in complex with complement component C3c reveals a new mechanism of complement inhibition. *J Biol Chem*. 2007; 282:29241–29247. [PubMed: 17684013]
- Janssen BJ, Huizinga EG, Raaijmakers HC, Roos A, Daha MR, Nilsson-Ekdahl K, Nilsson B, Gros P. Structures of complement component C3 provide insights into the function and evolution of immunity. *Nature*. 2005; 437:505–511. [PubMed: 16177781]
- Jha P, Kotwal GJ. Vaccinia complement control protein: multi-functional protein and a potential wonder drug. *J Biosci*. 2003; 28:265–271. [PubMed: 12734405]
- Jongerijs I, Kohl J, Pandey MK, Ruyken M, van Kessel KP, van Strijp JA, Rooijackers SH. Staphylococcal complement evasion by various convertase-blocking molecules. *J Exp Med*. 2007; 204:2461–2471. [PubMed: 17893203]
- Jongerijs I, Puister M, Wu J, Ruyken M, van Strijp JA, Rooijackers SH. Staphylococcal complement inhibitor modulates phagocyte responses by dimerization of convertases. *J Immunol*. 2010; 184:420–425. [PubMed: 19949103]
- Jongerijs I, Ram S, Rooijackers S. Bacterial complement escape. *Adv Exp Med Biol*. 2009; 666:32–48. [PubMed: 20054973]
- Kajander T, Lehtinen MJ, Hyvarinen S, Bhattacharjee A, Leung E, Isenman DE, Meri S, Goldman A, Jokiranta TS. Dual interaction of factor H with C3d and glycosaminoglycans in host-nonhost discrimination by complement. *Proc Natl Acad Sci U S A*. 2011; 108:2897–2902. [PubMed: 21285368]
- Katschke KJ Jr, Stawicki S, Yin J, Steffek M, Xi H, Sturgeon L, Hass PE, Loyet KM, Deforge L, Wu Y, van Lookeren Campagne M, Wiesmann C. Structural and functional analysis of a C3b-specific antibody that selectively inhibits the alternative pathway of complement. *J Biol Chem*. 2009; 284:10473–10479. [PubMed: 19196712]
- Katschke KJ Jr, Wu P, Ganesan R, Kelley RF, Mathieu MA, Hass PE, Murray J, Kirchhofer D, Wiesmann C, van Lookeren Campagne M. Inhibiting alternative pathway complement activation by targeting the factor D exosite. *J Biol Chem*. 2012; 287:12886–12892. [PubMed: 22362762]
- Knerr PJ, Tzekou A, Ricklin D, Qu H, Chen H, van der Donk WA, Lambris JD. Synthesis and activity of thioether-containing analogues of the complement inhibitor compstatin. *ACS Chem Biol*. 2011; 6:753–760. [PubMed: 21520911]
- Kourtzelis I, Markiewski MM, Doumas M, Rafail S, Kambas K, Mitroulis I, Panagoutsos S, Passadakis P, Vargemezis V, Magotti P, Qu H, Mollnes TE, Ritis K, Lambris JD. Complement anaphylatoxin C5a contributes to hemodialysis-associated thrombosis. *Blood*. 2010; 116:631–639. [PubMed: 20424189]
- Laarman A, Milder F, van Strijp J, Rooijackers S. Complement inhibition by gram-positive pathogens: molecular mechanisms and therapeutic implications. *J Mol Med (Berl)*. 2010; 88:115–120. [PubMed: 20062962]
- Laarman AJ, Ruyken M, Malone CL, van Strijp JA, Horswill AR, Rooijackers SH. *Staphylococcus aureus* metalloprotease aureolysin cleaves complement C3 to mediate immune evasion. *J Immunol*. 2011; 186:6445–6453. [PubMed: 21502375]
- Lambris JD, Ricklin D, Geisbrecht BV. Complement evasion by human pathogens. *Nat Rev Microbiol*. 2008; 6:132–142. [PubMed: 18197169]
- Lapeyraque AL, Wagner E, Phan V, Clermont MJ, Merouani A, Fremeaux-Bacchi V, Goodship TH, Robitaille P. Efficacy of plasma therapy in atypical hemolytic uremic syndrome with complement factor H mutations. *Pediatr Nephrol*. 2008; 23:1363–1366. [PubMed: 18425537]
- Laursen NS, Andersen KR, Braren I, Spillner E, Sottrup-Jensen L, Andersen GR. Substrate recognition by complement convertases revealed in the C5-cobra venom factor complex. *EMBO J*. 2011; 30:606–616. [PubMed: 21217642]
- Lazar HL, Keilani T, Fitzgerald CA, Shapira OM, Hunter CT, Shemin RJ, Marsh HC Jr, Ryan US, Group TPCSS. Beneficial effects of complement inhibition with soluble complement receptor 1

- (TP10) during cardiac surgery: is there a gender difference? *Circulation*. 2007; 116:I83–88. [PubMed: 17846331]
- Le Friec G, Kemper C. Complement: coming full circle. *Arch Immunol Ther Exp (Warsz)*. 2009; 57:393–407. [PubMed: 19866344]
- Li B, Xi H, Diehl L, Lee WP, Sturgeon L, Chinn J, Deforge L, Kelley RF, Wiesmann C, van Lookeren Campagne M, Sidhu SS. Improving therapeutic efficacy of a complement receptor by structure-based affinity maturation. *J Biol Chem*. 2009; 284:35605–35611. [PubMed: 19833734]
- Li JS, Jagers J, Anderson PA. The use of TP10, soluble complement receptor 1, in cardiopulmonary bypass. *Expert Rev Cardiovasc Ther*. 2006; 4:649–654. [PubMed: 17081086]
- Licht C, Weyersberg A, Heinen S, Stapenhorst L, Devenge J, Beck B, Waldherr R, Kirschfink M, Zipfel PF, Hoppe B. Successful plasma therapy for atypical hemolytic uremic syndrome caused by factor H deficiency owing to a novel mutation in the complement cofactor protein domain 15. *Am J Kidney Dis*. 2005; 45:415–421. [PubMed: 15685522]
- Lindorfer MA, Pawluczakowycz AW, Peek EM, Hickman K, Taylor RP, Parker CJ. A novel approach to preventing the hemolysis of paroxysmal nocturnal hemoglobinuria: both complement-mediated cytolysis and C3 deposition are blocked by a monoclonal antibody specific for the alternative pathway of complement. *Blood*. 2010; 115:2283–2291. [PubMed: 20068220]
- Magotti P, Ricklin D, Qu H, Wu YQ, Kaznessis YN, Lambris JD. Structure-kinetic relationship analysis of the therapeutic complement inhibitor compstatin. *J Mol Recognit*. 2009; 22:495–505. [PubMed: 19658192]
- Markiewski MM, DeAngelis RA, Lambris JD. Complexity of complement activation in sepsis. *J Cell Mol Med*. 2008; 12:2245–2254. [PubMed: 18798865]
- Markiewski MM, Nilsson B, Ekdahl KN, Mollnes TE, Lambris JD. Complement and coagulation: strangers or partners in crime? *Trends Immunol*. 2007; 28:184–192. [PubMed: 17336159]
- Martinez-Barricarte R, Heurich M, Valdes-Canedo F, Vazquez-Martul E, Torreira E, Montes T, Tortajada A, Pinto S, Lopez-Trascasa M, Morgan BP, Llorca O, Harris CL, Rodriguez de Cordoba S. Human C3 mutation reveals a mechanism of dense deposit disease pathogenesis and provides insights into complement activation and regulation. *J Clin Invest*. 2010; 120:3702–3712. [PubMed: 20852386]
- Morgan HP, Schmidt CQ, Guariento M, Blaum BS, Gillespie D, Herbert AP, Kavanagh D, Mertens HD, Svergun DI, Johansson CM, Uhrin D, Barlow PN, Hannan JP. Structural basis for engagement by complement factor H of C3b on a self surface. *Nat Struct Mol Biol*. 2011; 18:463–470. [PubMed: 21317894]
- Muller-Eberhard HJ. Molecular organization and function of the complement system. *Annu Rev Biochem*. 1988; 57:321–347. [PubMed: 3052276]
- Muller-Eberhard HJ, Gotze O. C3 proactivator convertase and its mode of action. *J Exp Med*. 1972; 135:1003–1008. [PubMed: 4111773]
- Mullick J, Kadam A, Sahu A. Herpes and pox viral complement control proteins: ‘the mask of self’. *Trends Immunol*. 2003; 24:500–507. [PubMed: 12967674]
- Neher MD, Weckbach S, Flierl MA, Huber-Lang MS, Stahel PF. Molecular mechanisms of inflammation and tissue injury after major trauma-is complement the “bad guy”. *J Biomed Sci*. 2011; 18:90. [PubMed: 22129197]
- Nilsson B, Korsgren O, Lambris JD, Ekdahl KN. Can cells and biomaterials in therapeutic medicine be shielded from innate immune recognition? *Trends Immunol*. 2010; 31:32–38. [PubMed: 19836998]
- Nilsson PH, Ekdahl KN, Magnusson PU, Hilborn J, Qu H, Iwata H, Ricklin D, Hong J, Lambris JD, Nilsson B, Teramura Y. Autoregulation of thromboinflammation on biomaterials and cells by a novel therapeutic coating technique. *Nat Methods*. 2012 submitted.
- Nishida N, Walz T, Springer TA. Structural transitions of complement component C3 and its activation products. *Proc Natl Acad Sci U S A*. 2006; 103:19737–19742. [PubMed: 17172439]
- Oikonomopoulou K, Ricklin D, Ward PA, Lambris JD. Interactions between coagulation and complement--their role in inflammation. *Semin Immunopathol*. 2012; 34:151–165. [PubMed: 21811895]

- Qu H, Magotti P, Ricklin D, Wu EL, Kourtzelis I, Wu YQ, Kaznessis YN, Lambris JD. Novel analogues of the therapeutic complement inhibitor compstatin with significantly improved affinity and potency. *Mol Immunol*. 2011; 48:481–489. [PubMed: 21067811]
- Qu H, Ricklin D, Bai H, Chen H, Reis ES, Maciejewski M, Tzekou A, DeAngelis RA, Resuello RRG, Lupu F, Barlow PN, Lambris JD. New analogs of the clinical complement inhibitor compstatin with subnanomolar affinity and enhanced pharmacokinetic properties. *Immunobiology*. 2012 in press.
- Qu H, Ricklin D, Lambris JD. Recent developments in low molecular weight complement inhibitors. *Mol Immunol*. 2009; 47:185–195. [PubMed: 19800693]
- Reis ES, Falcao DA, Isaac L. Clinical aspects and molecular basis of primary deficiencies of complement component C3 and its regulatory proteins factor I and factor H. *Scand J Immunol*. 2006; 63:155–168. [PubMed: 16499568]
- Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. *Nat Immunol*. 2010; 11:785–797. [PubMed: 20720586]
- Ricklin D, Lambris JD. Complement-targeted therapeutics. *Nat Biotechnol*. 2007; 25:1265–1275. [PubMed: 17989689]
- Ricklin D, Lambris JD. Compstatin: a complement inhibitor on its way to clinical application. *Adv Exp Med Biol*. 2008; 632:273–292. [PubMed: 19025129]
- Ricklin D, Lambris JD. Progress and trends in complement therapeutics. *Adv Exp Med Biol*. 2012; 1007/978–1–4614–4118–2_1
- Ricklin D, Ricklin-Lichtsteiner SK, Markiewski MM, Geisbrecht BV, Lambris JD. Cutting edge: members of the *Staphylococcus aureus* extracellular fibrinogen-binding protein family inhibit the interaction of C3d with complement receptor 2. *J Immunol*. 2008; 181:7463–7467. [PubMed: 19017934]
- Ricklin D, Tzekou A, Garcia BL, Hammel M, McWhorter WJ, Sfyroera G, Wu YQ, Holers VM, Herbert AP, Barlow PN, Geisbrecht BV, Lambris JD. A molecular insight into complement evasion by the staphylococcal complement inhibitor protein family. *J Immunol*. 2009; 183:2565–2574. [PubMed: 19625656]
- Risitano AM, Notaro R, Pascariello C, Sica M, Del Vecchio L, Horvath CJ, Fridkis-Hareli M, Selleri C, Lindorfer MA, Taylor RP, Luzzatto L, Holers VM. The complement receptor 2/factor H fusion protein TT30 protects paroxysmal nocturnal hemoglobinuria erythrocytes from complement-mediated hemolysis and C3 fragment. *Blood*. 2012; 119:6307–6316. [PubMed: 22577173]
- Risitano AM, Perna F, Selleri C. Achievements and limitations of complement inhibition by eculizumab in paroxysmal nocturnal hemoglobinuria: the role of complement component 3. *Mini Rev Med Chem*. 2011; 11:528–535. [PubMed: 21561403]
- Rodriguez de Cordoba S, Harris CL, Morgan BP, Llorca O. Lessons from functional and structural analyses of disease-associated genetic variants in the complement alternative pathway. *Biochim Biophys Acta*. 2011; 1812:12–22. [PubMed: 20837143]
- Rooijackers SH, Wu J, Ruyken M, van Domselaar R, Planken KL, Tzekou A, Ricklin D, Lambris JD, Janssen BJ, van Strijp JA, Gros P. Structural and functional implications of the alternative complement pathway C3 convertase stabilized by a staphylococcal inhibitor. *Nat Immunol*. 2009; 10:721–727. [PubMed: 19503103]
- Roversi P, Johnson S, Caesar JJ, McLean F, Leath KJ, Tsiftoglou SA, Morgan BP, Harris CL, Sim RB, Lea SM. Structural basis for complement factor I control and its disease-associated sequence polymorphisms. *Proc Natl Acad Sci U S A*. 2011; 108:12839–12844. [PubMed: 21768352]
- Sacks SH, Zhou W. The role of complement in the early immune response to transplantation. *Nat Rev Immunol*. 2012; 12:431–442. [PubMed: 22627861]
- Schmidt CQ, Bai H, Lin Z, Risitano AM, Barlow PN, Ricklin D, Lambris JD. Rational engineering of a novel complement regulator affords triple targeting of cells and controls innate immunity in disease condition. 2012 in preparation.
- Schmidt CQ, Slingsby FC, Richards A, Barlow PN. Production of biologically active complement factor H in therapeutically useful quantities. *Protein Expr Purif*. 2011; 76:254–263. [PubMed: 21146613]

- Schneider MC, Exley RM, Chan H, Feavers I, Kang YH, Sim RB, Tang CM. Functional significance of factor H binding to *Neisseria meningitidis*. *J Immunol*. 2006; 176:7566–7575. [PubMed: 16751403]
- Schneider MC, Prosser BE, Caesar JJ, Kugelberg E, Li S, Zhang Q, Quoraishi S, Lovett JE, Deane JE, Sim RB, Roversi P, Johnson S, Tang CM, Lea SM. *Neisseria meningitidis* recruits factor H using protein mimicry of host carbohydrates. *Nature*. 2009; 458:890–893. [PubMed: 19225461]
- Schreiber RD, Pangburn MK, Lesavre PH, Muller-Eberhard HJ. Initiation of the alternative pathway of complement: recognition of activators by bound C3b and assembly of the entire pathway from six isolated proteins. *Proc Natl Acad Sci U S A*. 1978; 75:3948–3952. [PubMed: 279011]
- Schuster MC, Ricklin D, Papp K, Molnar KS, Coales SJ, Hamuro Y, Sfyroera G, Chen H, Winters MS, Lambris JD. Dynamic structural changes during complement C3 activation analyzed by hydrogen/deuterium exchange mass spectrometry. *Mol Immunol*. 2008; 45:3142–3151. [PubMed: 18456336]
- Serruto D, Rappuoli R, Scarselli M, Gros P, van Strijp JA. Molecular mechanisms of complement evasion: learning from staphylococci and meningococci. *Nat Rev Microbiol*. 2010; 8:393–399. [PubMed: 20467445]
- Sfyroera G, Ricklin D, Chen H, Nilsson-Ekdahl K, Nilsson B, Wu EL, Kaznessis YN, Lambris JD. Impaired ability of complement activation via the alternative pathway C3 convertase caused by a single-point mutation in the beta chain of C3. *Mol Immunol*. 2010; 47:2289–2289.
- Sharp JA, Echague CG, Hair PS, Ward MD, Nyalwidhe JO, Geoghegan JA, Foster TJ, Cunnion KM. *Staphylococcus aureus* surface protein SdrE binds complement regulator factor H as an immune evasion tactic. *PLoS One*. 2012; 7:e38407. [PubMed: 22675461]
- Silasi-Mansat R, Zhu H, Popescu NI, Peer G, Sfyroera G, Magotti P, Ivanciu L, Lupu C, Mollnes TE, Taylor FB, Kinasewitz G, Lambris JD, Lupu F. Complement inhibition decreases the procoagulant response and confers organ protection in a baboon model of *Escherichia coli* sepsis. *Blood*. 2010; 116:1002–1010. [PubMed: 20466856]
- Smith EJ, Visai L, Kerrigan SW, Speziale P, Foster TJ. The Sbi protein is a multifunctional immune evasion factor of *Staphylococcus aureus*. *Infect Immun*. 2011; 79:3801–3809. [PubMed: 21708997]
- Smith RA. Targeting anticomplement agents. *Biochem Soc Trans*. 2002; 30:1037–1041. [PubMed: 12440967]
- Thurman JM, Kraus DM, Girardi G, Hourcade D, Kang HJ, Royer PA, Mitchell LM, Giclas PC, Salmon J, Gilkeson G, Holers VM. A novel inhibitor of the alternative complement pathway prevents antiphospholipid antibody-induced pregnancy loss in mice. *Mol Immunol*. 2005; 42:87–97. [PubMed: 15488947]
- van der Pals J, Koul S, Andersson P, Gotberg M, Ubachs JF, Kanski M, Arheden H, Olivecrona GK, Larsson B, Erlinge D. Treatment with the C5a receptor antagonist ADC-1004 reduces myocardial infarction in a porcine ischemia-reperfusion model. *BMC Cardiovasc Disord*. 2010; 10:45. [PubMed: 20875134]
- Veerhuis R, Nielsen HM, Tenner AJ. Complement in the brain. *Mol Immunol*. 2011; 48:1592–1603. [PubMed: 21546088]
- Vlieghe P, Lisowski V, Martinez J, Khrestchatsky M. Synthetic therapeutic peptides: science and market. *Drug Discov Today*. 2010; 15:40–56. [PubMed: 19879957]
- Ward PA, Gao H. Sepsis, complement and the dysregulated inflammatory response. *J Cell Mol Med*. 2009; 13:4154–4160. [PubMed: 19725914]
- Weismann D, Hartvigsen K, Lauer N, Bennett KL, Scholl HP, Charbel Issa P, Cano M, Brandstatter H, Tsimikas S, Skerka C, Superti-Furga G, Handa JT, Zipfel PF, Witztum JL, Binder CJ. Complement factor H binds malondialdehyde epitopes and protects from oxidative stress. *Nature*. 2011; 478:76–81. [PubMed: 21979047]
- Wells JA, McClendon CL. Reaching for high-hanging fruit in drug discovery at protein-protein interfaces. *Nature*. 2007; 450:1001–1009. [PubMed: 18075579]
- Wiesmann C, Katschke KJ, Yin J, Helmy KY, Steffek M, Fairbrother WJ, McCallum SA, Embuscado L, DeForge L, Hass PE, van Lookeren Campagne M. Structure of C3b in complex with CRIg

- gives insights into regulation of complement activation. *Nature*. 2006; 444:217–220. [PubMed: 17051150]
- Wu J, Wu YQ, Ricklin D, Janssen BJ, Lambris JD, Gros P. Structure of complement fragment C3b-factor H and implications for host protection by complement regulators. *Nat Immunol*. 2009; 10:728–733. [PubMed: 19503104]
- Wu YQ, Qu H, Sfyroera G, Tzekou A, Kay BK, Nilsson B, Nilsson Ekdahl K, Ricklin D, Lambris JD. Protection of nonself surfaces from complement attack by factor H-binding peptides: implications for therapeutic medicine. *J Immunol*. 2011; 186:4269–4277. [PubMed: 21339361]
- Yehoshua Z, Rosenfeld PJ, Albini TA. Current Clinical Trials in Dry AMD and the Definition of Appropriate Clinical Outcome Measures. *Semin Ophthalmol*. 2011; 26:167–180. [PubMed: 21609230]

Figure 1.

Complement-mediated surveillance and triage in health and disease. (A) Under normal conditions, complement tailors its activity towards the potential threat via interplay of pattern recognition, amplification loop and regulators. While any complement response is contained on healthy human cells, restricted opsonization allows for clearance of cellular debris and immune complexes; finally, a more forceful response against microbial intruders leads to elimination, killing and danger signaling. (B)

Figure 2.

Molecular mechanisms of activation, amplification, regulation, and evasion of the AP C3 convertase. (A) Convertase-mediated activation of C3 on unprotected surfaces. After the initial deposition of C3b by various routes, FB binds to the opsonin to form a pro-convertase that converts from a closed to an open form and allows binding of FD. Bound FD gains the ability to cleave FB and releases the Ba fragment, thereby creating the final AP C3 convertase (C3bBb). C3 appears to bind the convertase and gets cleaved by the Bb protease. Conformational changes expose the thioester and bring it close to the target surface to allow covalent deposition. The C3a/ANA domain is depicted as a pale blue polygon in the cartoon of C3. (B) Convertase regulation by RCA on self-surfaces. Decay acceleration activity (DAA) is mediated by binding of CD55, CD59 or FH to the convertase and competitive replacement of Bb. In the case of cofactor activity (CA), the regulators CD35, CD46 and FH form a binding platform for factor I (FI) and mediate the cleavage of C3b to iC3b (or C3dg). In the RCA cartoons, red and pink circles reflect regulatory and recognition domains, respectively. (C–E) Convertase-targeted evasion strategies by human pathogens. While most pathogens either attract RCA to their surface or express RCA mimics (C), *Staphylococcus aureus* secretes at least two direct inhibitors that either allosterically impair formation of the

convertase (Efb; D) or trap it in a state where it is unable to cleave C3 or participate in any other downstream events (SCIN; E).

\$watermark-text

\$watermark-text

\$watermark-text

Figure 3.

Therapeutic modulation of the AP C3 convertase activity. (A) Soluble and targeted RCA constructs for convertase inhibition via decay acceleration (DAA) and cofactor activity (CA). Grey arrows signify targeting to sites of ongoing complement activation (iC3b, C3dg) and/or self-pattern (GAG); red, pink, and cyan circles represent regulatory, recognition, and receptor domains, respectively. (B) Protein-protein interaction inhibitors based on therapeutic antibodies, proteins and peptides. Though only labeled on C3, compstatin and its analogs bind to both C3 and C3b. (C) Theoretical structural model of native C3 binding to C3bBb (as suggested in (Roijakkers et al. 2009); combined from PDB structures 2WIN and 2A73) and superimposition of the co-crystal structures of the three C3b-binding inhibitors compstatin (PDB 2QKI), CRIg (PDB 2ICF) and S77 (PDB 3G6J).