Published in final edited form as:

Immunobiology. 2016 June ; 221(6): 740–746. doi:10.1016/j.imbio.2015.06.012.

Therapeutic control of complement activation at the level of the central component C3

Daniel Ricklin* and John D. Lambris

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

Abstract

The increasing recognition of the complement system's association with diseases of the inflammatory spectrum and with biomaterial and transplant-related complications has generated growing interest in the therapeutic modulation of this innate immune cascade. As a central functional hub that largely drives the activation, amplification, and effector generation of the complement response, the plasma protein C3 has long been recognized as an attractive target. While pharmacological modulation of C3 activation may offer a powerful opportunity to interfere with or even prevent complement-driven pathologies, the development of C3 inhibitors has often been accompanied by concerns regarding the safety and feasibility of this approach. Although no C3-targeted inhibitors have thus far been approved for clinical use, several promising concepts and candidates have emerged in recent years. At the same time, experiences from preclinical development and clinical trials are slowly providing a more detailed picture of therapeutic complement inhibition at the level of C3. This review highlights the current therapeutic strategies to control C3 activation and discusses the possibilities and challenges on the road to bringing C3-targeted therapeutics to the clinic.

Keywords

Complement; C3; Therapeutics; Convertase; Alternative Pathway

From guardian to “bull in a china shop”

The complement system is typically viewed as a “first responder” that protects us from microbial intruders and prevents accumulation of harmful debris (Ricklin et al., 2010). However, the defining ability of complement to instantaneously recognize non-self and altered surfaces also presents one of its biggest vulnerabilities, which may turn this defense system into a burden, or even a threat (Ricklin et al., 2010; Ricklin and Lambris, 2013). For

*Corresponding author: Daniel Ricklin, Ph.D., Department of Pathology and Laboratory Medicine, University of Pennsylvania, 401 Stellar Chance, 422 Curie Blvd., Philadelphia, PA 19104, Phone: 215-746-5760, ; Email: ricklin@upenn.edu.

Disclosure

D.R. and J.D.L. are co-inventors of patents and/or patent applications describing complement inhibitors and their clinical use. J.D.L. is the founder of Amyndas Pharmaceuticals.

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.

example, exposure of our bodies to implants, extracorporeal circuits, or drug delivery vehicles can induce an adverse complement response by the foreign material (Ekdahl et al., 2011). Complement-mediated inflammation is also a major complication in transplantation medicine, contributing to rejection or limited graft function (Sacks and Zhou, 2012). Although our cells are normally protected from complement attack, any type of tissue damage resulting from trauma, infection, or other insult can potentially trigger the complement cascade via damage-associated molecular patterns. Even worse, complement effector molecules can cause additional tissue damage and create an inflammatory milieu that fuels a vicious cycle in which complement suddenly acts like a bull in a china shop. Deficiencies and polymorphisms in complement components can further exacerbate such inflammatory reactions or contribute to autoimmune diseases. Finally, even small functional changes in the balance of complement activation and regulation can contribute to age-related diseases (Ricklin and Lambris, 2013).

This strong disease involvement, often at the early stages of pathophysiological processes, has rendered complement an attractive target for pharmacological intervention (Ricklin and Lambris, 2013). Although the arsenal of complement-targeted drugs has remained limited thus far, clinical experience with C1 inhibitor (e.g., Cinryze, Shire) and an anti-C5 antibody eculizumab (Soliris, Alexion) have provided important insights into the efficacy and versatility of this approach. However, it has also become evident that not all pathologies will benefit equally from the available options, and a broadening of targets and inhibitors needs to be achieved. Among the many potential targets within the cascade, the central complement component C3 is of particular interest because it is often the driving force behind complement-mediated damage. Although the development of C3-targeted inhibitors has faced challenges and is often accompanied by discussions about safety and feasibility, enormous progress has been achieved in recent years. The following paragraphs briefly discuss the important concepts, chances, and challenges of the modulation of C3 activation and highlight approaches currently in clinical development.

Aiming for the center: the promise of therapeutic C3 inhibition

The central position of C3 in the complement activation process renders this plasma protein a highly attractive target for therapeutic complement inhibition (Fig. 1A) (Ricklin, 2012; Ricklin and Lambris, 2013). Initiation of the complement response by any activation route, i.e., the classical, lectin, and/or alternative pathway (CP, LP and AP, respectively), leads to the formation of C3 convertases on the surface of the recognized cell or material. Despite their distinct composition, all convertases bind and activate C3 to generate the opsonic fragment C3b and the pro-inflammatory anaphylatoxin C3a. In the immediate proximity of the site of activation, C3b is covalently deposited on the surface and can bind complement factor B (FB) to form a pro-convertase (C3bB). Factor D (FD) transforms this complex into the final AP C3 convertase (C3bBB), which can in turn activate additional C3 (Fig. 1B). This amplification of C3 activation via the AP often fuels the overall complement response, independent of the initial trigger (Harboe et al., 2009; Harboe et al., 2004). Increasing C3b deposition is a prerequisite for the generation of C5 convertases that cleave C5 into the inflammatory mediator C5a and into C5b, which initiates the formation of membrane attack complexes (MAC) that can induce lysis or cell damage (Fig. 1A).

In order to protect self-cells from complement attack, this detrimental C3 activation cycle is carefully controlled by the C3b breakdown cycle, which destabilizes the convertase and degrades C3b into fragments that can no longer participate in convertase amplification (Fig. 1B) (Lachmann, 2009; Ricklin, 2012; Ricklin et al., 2010). It should be noted, however, that the resulting fragments (iC3b, C3dg) still play important roles in mediating phagocytosis and/or stimulating adaptive immune responses.

In most complement-mediated pathologies, the balance between C3 activation and C3b breakdown directly determines the progression of adverse responses and controls the generation of almost all effector molecules. As compared to inhibition at the level of C1 or C5, which leave C3b opsonization and amplification via the AP intact, therapeutic inhibition at the level of C3 promises a more comprehensive control of complement activation. Although it is expected that the broad inhibitory effect of C3-targeted drugs would render them effective in almost all complement-mediated conditions, there are several indications that are particularly interesting and some that are challenging to manage with the existing options in the clinic. Among the most suitable conditions are pathologies that are largely driven by aberrant C3 activation, such as dense deposit disease (DDD) or C3 glomerulonephritis, as well as conditions with complex and broad complement involvement, such as age-related macular degeneration (AMD) (Ricklin and Lambris, 2013). Even in diseases with an established efficacy of the standard treatment, such as paroxysmal nocturnal hemoglobinuria (PNH), C3-targeted approaches may provide benefits for affected patients (Mastellos et al., 2014). While the advantages of distinct therapeutic strategies need to be evaluated for each individual disease, the availability of a diverse palette of complement inhibitors is important for the clinic in any case.

Therapeutic concepts to control C3 activation

Over the past few years, a series of creative and promising therapeutic concepts have emerged that aim to control the activation of C3 at different levels (Fig. 1C). Although initial C3 activation can also be prevented by compounds acting on the CP and LP (Ricklin and Lambris, 2013), thereby controlling the CP/LP convertase, this review primarily focuses on approaches acting on C3 itself, on its fragments, or on the AP C3 convertase.

Inhibition of native C3

Despite being the centerpiece of the complement activation machinery, native C3 has proven to be a difficult target for pharmacological targeting. In contrast to C5, in which an antibody binding to the native state (i.e., eculizumab) has proved highly successful, therapeutic antibodies against C3 typically bind to its activated form, C3b, and mainly control convertase formation (see below).

The compstatin family of peptidic C3 inhibitors is the rare exception among the therapeutics that act on native C3 and prevent its activation. Compstatin was discovered as a cyclic peptide of 13 amino acids that blocks convertase-mediated activation of C3 by all pathways (Sahu et al., 1996). Thereby, compstatin also inhibits the amplification of the response and prevents downstream formation of complement effectors. Structural studies have revealed that compstatin binds to a shallow pocket in the β -chain of C3 and C3b, which appears to be

critical for the binding of C3 to the convertase (Janssen et al., 2007; Mastellos et al., 2015; Rooijakkers et al., 2009). It should be noted that compstatin and its analogs neither prevent direct cleavage of C3 by proteases such as thrombin nor the “activation” of C3 into C3(H₂O) via spontaneous hydrolysis; also, deposition of C4b via the CP/LP remains intact (Mastellos et al., 2015; Ricklin and Lambris, 2008). The optimization of compstatin led to analogs with largely improved efficacy, stability, and pharmacodynamic properties.

Despite their species specificity for human and non-human primate (NHP) C3, compstatin derivatives have been evaluated in a broad spectrum of disease models (Mastellos et al., 2015; Ricklin and Lambris, 2008). An early compstatin analog built the basis for the clinical candidate POT-4 (Potentia Pharmaceuticals), which has been developed for the treatment of AMD. After successful phase 1 studies indicating efficacy after intravitreal injection (ClinicalTrials.gov NCT00473928), phase 2 clinical trials did not corroborate efficacy; however, much lower dose ranges were used in these studies (ClinicalTrials.gov NCT01157065; ClinicalTrials.gov NCT01603043). Apellis Pharmaceuticals acquired the technology from Potentia and is developing this compstatin analog (as APL-1) and a long-acting PEGylated derivative (APL-2) for various indications, including AMD, chronic obstructive pulmonary disease (COPD) and PNH (ClinicalTrials.gov NCT02264639). At the same time, next-generation compstatin analogs have been licensed to Amyndas Pharmaceuticals, which is developing AMY-101 for ABO-incompatible kidney transplantation and PNH. AMY-101 is based on the compstatin analog Cp40, which showed a 6,000-fold increased target-binding affinity over original compstatin as well as pharmacokinetic profiles suitable for systemic administration (Qu et al., 2013). Cp40 showed promising results in preclinical studies of PNH, periodontal disease, hemodialysis-induced inflammation, and C3 glomerulopathies (Maekawa et al., 2014; Reis et al., 2014; Risitano et al., 2014; Zhang et al., 2015). Amyndas is expected to start clinical trials with AMY-101 in ABO-incompatible kidney transplantation and PNH in 2015/2016.

An alternative strategy to interfering at the level of native C3 is to remove it from the equation via selective depletion. This concept stems from the discovery of cobra venom factor (CVF), a structural homolog of C3b/C3c found in the venom of certain cobra species, which forms exceptionally stable convertases that consume circulating C3. Although this depletion results in the generation of effector molecules (C3a, C3b) in solution, adverse inflammatory reactions appear low in the variants that are not also acting as a C5 convertase to generate C5a (Vogel et al., 2014). CVF has shown efficacy in various disease models, including transplant rejection in NHP, and a humanized form of CVF had been developed by InCode (Vogel et al., 2014). However, no plans for clinical development of CVF-based therapeutics have been announced. The concept of selective C3 depletion has recently been taken up by Catalyst Biosciences, which specializes in engineered human proteases (Craik et al., 2011). Although no details have been disclosed, Catalyst’s anti-C3 protease technology appears to be capable of rapidly inactivating C3 in vivo. Catalyst lists AMD and ischemia-reperfusion injury as indications, with one lead candidate (CB 2782) entering preclinical development for delayed graft function after kidney transplantation.

Control of convertase formation

The formation of the AP C3 convertase relies critically on the coordinated interaction of C3b, FB and FD (Forneris et al., 2010). Therapeutic blockage of any of these components can therefore be effective in controlling AP-mediated complement activation and amplification. Indeed, several antibodies have been identified that tightly bind to C3b and impair convertase formation. For example, the phage-derived antibody S77 (Genentech) has been shown to bind to an epitope in the MG7 domain of C3b (but not C3) that also harbors part of the FB binding site, therefore preventing assembly of the C3bB pro-convertase (Katschke et al., 2009). S77 efficiently inhibited AP-mediated hemolysis in human serum but not in samples from other tested species, including several NHP (Katschke et al., 2009). The murine antibody 3E7 and its chimeric-deimmunized version H17 are another prominent example of blockage at the level of C3b (DiLillo et al., 2006). Like S77, these monoclonal antibodies (mAbs) block the interaction with FB, which was recently explained by studies showing that mAb H17 binds to the MG6/7 region of C3b (DiLillo et al., 2006; Paixao-Cavalcante et al., 2014). These antibodies have shown promising results in in vitro models of PNH and DDD; an interesting finding in the latter case was that mAb H17 can bind to preformed C3 convertases stabilized by C3 nephritic factors, indicating that the inhibitory activity in this model is comes partially from preventing C3 from binding to the convertase (Paixao-Cavalcante et al., 2014). No clinical development programs appear to have been announced for these C3b antibodies. In the case of FB, a therapeutic antibody for the treatment of asthma and other inflammatory diseases has been developed by Taligen (TA-106); however, this antibody has not been evaluated in clinical trials since the acquisition of the program by Alexion.

Occupying a bottleneck function in the AP as a result of its essential role in the formation of the active convertase and its comparatively low plasma concentration (~2 µg/ml), FD moved early on into the spotlight of complement-targeted drug discovery. BCX-1470 (BioCryst), a small-molecule FD inhibitor, has been reported, but has been shown to also act potently on C1s of the CP. Achillion Pharmaceuticals recently provided the first public insight into their development program for small-molecule FD inhibitors (Morgan et al., 2014; Wiles et al., 2014). One of their candidate drugs binds to FD with subnanomolar affinity, blocks access to the catalytic site, and potently inhibits AP-mediated complement activation (Morgan et al., 2014; Wiles et al., 2014). Importantly, this FD inhibitor shows oral bioavailability, and inhibitory plasma levels can be achieved with an oral dose of 100 mg/kg every 12 hours in cynomolgus monkeys (Morgan et al., 2014; Wiles et al., 2014). In a preclinical model of PNH, compound A inhibited hemolysis with an IC₅₀ of 25 nM; it also prevented the deposition of C3 activation fragments on rabbit erythrocytes (Morgan et al., 2014). Achillion is expected to announce a lead candidate and initiate clinical development of their FD inhibitors in 2015.

Rather than interfering with the catalytic site of FD, the humanized antibody fragment lampalizumab (Genentech) binds to the exosite of FD that mediates the essential interaction with C3b-bound FB to form the AP C3 convertase (Forneris et al., 2010; Katschke et al., 2012). Genentech is clinically developing lampalizumab as an intravitreal therapy for dry AMD. In phase 2 trials (ClinicalTrials.gov NCT01229215), lampalizumab reduced the

progression of geographic atrophy by 20% in the treatment group and by 44% in a subpopulation that tested positive for factor I polymorphism (Holz et al., 2013). Based on these promising results, Genentech is currently recruiting patients for phase 3 trials (ClinicalTrials.gov NCT02247479; ClinicalTrials.gov NCT02247531).

Another approach to influencing the assembly and stability of the AP C3 convertases is the blockage of properdin (Leshner et al., 2013). This oligomeric glycoprotein has long been known to stabilize the C3bBb complex and extend its activity, but it has also been connected with inducing AP activation by binding to cell surfaces and recruiting C3b. The involvement of properdin in AP-mediated pathologies appears to be complex, and rodent studies have revealed distinct roles in models of arthritis, tissue injury, and C3 glomerulopathy, among others (sometimes in concert with the impairment of other regulators) (Leshner et al., 2013; Leshner et al., 2013; Ruseva et al., 2013). Therapeutic inhibition of properdin has had beneficial effects in hemolysis, arthritis and ischemia-reperfusion injury models (Kimura et al., 2010; Miwa et al., 2013; Pauly et al., 2014), and anti-properdin antibodies are in development.

Supporting the breakdown cycle

On human cells, regulators of complement activation (RCA) control the formation and activity of C3 convertases, either by destabilizing the complexes or enabling the FI-mediated degradation of C3b and iC3b. Whereas CD35, CD46, and CD55 are membrane-bound, factor H (FH) controls AP activation in solution and also supports its regulation on cells by recognizing self-surface patterns such as glycosaminoglycans. In many diseases, a loss of RCA expression, binding, or activity (e.g., because of polymorphisms) drives complement-mediated complications (Ricklin and Lambris, 2013). Topping off physiological regulators with purified or recombinant forms has therefore been considered a promising concept for controlling excessive complement activity.

As the most versatile RCA, controlling both the CP/LP and AP convertases and being the only regulator mediating opsonin degradation down to C3dg, CD35 (complement receptor 1; CR1) was selected early on as a clinical candidate. Originally developed as TP10 and evaluated in cardiopulmonary bypass surgery settings (Lazar et al., 2007; Li et al., 2006), the extracellular part of CR1 (CDX-1135; Celldex) has more recently been evaluated in a phase 1 safety/efficacy trial for DDD (ClinicalTrials.gov NCT01791686). One caveat with this approach is the large size of the protein (240 kDa), which likely adversely affects production and cost. The same is true for purified or recombinant FH (150 kDa), which has been considered as a therapeutic but not yet evaluated in clinical trials. Facilitated by the modular architecture of the RCA, smaller engineered regulators have therefore gained traction in recent years.

In most cases, the regulatory domains of FH have been combined with a targeting entity to direct the regulators to sites of ongoing complement activation (see specialized reviews for more information) (Holers et al., 2013; Ricklin and Lambris, 2013). In model systems, this targeting approach has been shown to result in an increased level of selectivity (Atkinson et al., 2005). In the case of TT30 (Alexion), targeting is achieved by including domains of complement receptor 2 (CR2; CD21) that recognize iC3b and C3dg, which typically

accumulate on host cells under complement attack (Fridkis-Hareli et al., 2011). TT30 has shown promise in models of PNH, controlling both intravascular hemolysis and opsonization (Risitano et al., 2012). Phase 1 trials were initiated by Alexion but not completed (ClinicalTrials.gov NCT01335165), and no plans for clinical development have been announced. Mini-FH follows a similar route but utilizes the C-terminal self cell-recognition domains of FH itself to target the inhibitor. Despite a 70% reduction of the size when compared to full-length FH, the mini-FH form showed a higher efficacy than that of FH in a PNH model with patient-derived PNH erythrocytes (Schmidt et al., 2013).

RCA constructs have also been directly tethered to surfaces in order to protect them from complement attack. Mirococept, containing three regulatory domains of CR1 and a membrane-binding entity, has been used to perfuse kidney transplants and has shown positive effects on allograft function in clinical pilot studies (Sacks et al., 2013); phase 2b trials are currently initiated (Medical Research Council). An alternative approach is to recruit FH from the circulation to a material or cell surface. For example, the peptide 5C6 is able to specifically bind FH and protect coated surfaces from complement attack (Wu et al., 2011). In cell-based models of transplantation, 5C6 coating has been shown to reduce complement activation and the generation of anaphylatoxins (Nilsson et al., 2013).

An interesting alternative to using regulators for enhancing the C3b breakdown cycle is treatment with exogenous factor I (FI), which also affects the degradation of C3 opsonins. Although not being cell-targeted or acting on convertase stability, recombinant FI may offer some specific advantages of its own (Lachmann, 2009; Lay et al., 2014). Like FD in the C3 activation process, FI has a bottleneck function in the breakdown cycle because of its rather low plasma concentration (~35 µg/ml). In addition, and in contrast to FH-based regulators, FI also enhances the CR1-mediated degradation of opsonins to the C3dg stage, thereby avoiding the biological activities of iC3b that are mediated by the integrin receptors CR3 and CR4 (Lachmann, 2009). In a recent in vitro study, recombinant FI (GlaxoSmithKline) showed high activity in sera from individuals carrying combinations of disease-related polymorphisms in C3, FB, and FH, thereby supporting a potential use for this factor in AMD or other age-related inflammatory diseases (Lay et al., 2014). It will be important to evaluate the effects of FI therapy, and the associated generation of large amounts of complement fragments, using in vivo models.

Chances and challenges on the way to the clinic

Despite the apparent advantages of C3-targeted inhibition in various indications, and the steady progress being made in developing new clinical candidates, therapeutic complement inhibition at the level of C3 remains controversial. Skeptical voices primarily raise concerns about the technical feasibility of the approach or potential adverse consequences of blocking a central step of a host defense system. However, as delineated below, these concerns are not always supported by actual clinical experience and need to be viewed in the appropriate context.

On the technical side, the high plasma concentrations and/or rapid metabolic turnover of several components in the C3 activation cycle, particularly C3 (Alper and Rosen, 1967), may

impose demands on inhibitor doses and administration frequencies. However, these potential limitations largely depend on the exact target, inhibitor, and type of treatment (e.g., local versus systemic) and may be addressed by carefully selecting the site and route of administration. For example, the anti-FD antibody lampalizumab was shown to have only transient activity after systemic (intravenous) administration, but it efficiently blocked local complement activation in the retinal tissue for several days after intravitreal injection (Loyet et al., 2014). In the case of the compstatin family, the tight binding of recent analogs such as Cp40/AMY-101 to the abundant plasma protein C3 contributes to an exceptionally long terminal half-life, exceeding that of typical peptide drugs (Mastellos et al., 2015). By employing the depot effect of subcutaneous administration, doses as low as 1 mg/kg every 12 hours were shown to maintain target-saturating Cp40 levels in NHP (Risitano et al., 2014). In addition to a modulation of the pharmacokinetic properties by means of PEGylation or albumin-binding entities (Huang et al., 2014; Risitano et al., 2014), even an adjustment of the dose and formulation may extend the injection frequency to a single daily dose (Lambris and Ricklin, unpublished observations), thereby rendering therapeutic options such as patient self-administration in PNH or DDD increasingly realistic. Finally, it is not yet clear whether C3 inhibition needs to be complete or continuous in all indications. In a NHP model of hemodialysis, for example, a single bolus injection of compstatin Cp40 was sufficient to suppress filter-induced complement activation during the entire HD session (Reis et al., 2014). These and other examples, such as the oral administration of Achillion's FD inhibitor mentioned above (Wiles et al., 2014), show that treatment at the C3 level may have its technical challenges but can certainly be accomplished successfully.

In contrast to these technical aspects, the question about the safety of C3-targeted complement inhibition is more difficult to assess because of the lack of sufficient clinical experience. Discussions for and against such an approach therefore remain largely hypothetical. Arguments emphasizing the potential dangers of therapeutically inhibiting C3 activation are mainly based on the clinical observation of patients with primary C3 deficiencies. Most of these patients show increased susceptibility to certain pyogenic infections (Botto et al., 2009; Grumach and Kirschfink, 2014; Reis et al., 2006), which may be explained by the reduction in opsonization and the loss of MAC-mediated lysis. However, this susceptibility to recurrent infection is strongest in the early stages of life and typically subsides once patients reach adulthood, indicating that the contribution of complement to antimicrobial defense becomes less prominent after the maturation of the immune system (Botto et al., 2009). It is also not clear to what extent microbial killing relies on MAC formation; recent studies using the C5 inhibitor OmCI revealed that even for Gram-negative 'serum-sensitive' bacteria, there are other factors in serum besides complement that mediate bacterial killing (Berends et al., 2015). Moreover, because C3 plays important roles in shaping immunity during development (Botto et al., 2009), it is difficult to judge to what extent an altered "immune baseline" in C3-deficient individuals would influence clinical consequences. Most importantly, however, C3-targeted therapy is reversible and can be interrupted in the case of adverse events such as infection. In this context, the high synthetic rate of C3 may prove advantageous for quickly restoring protective levels of active C3.

Despite these considerations, the potential risk of infection needs to be minimized and controlled during the clinical development and evaluation of C3 inhibitors. In addition to

meningococcal vaccines, which are already successfully used as standard prophylaxis during anti-C5 therapy (Hillmen et al., 2013), vaccinations against pneumococci and *Haemophilus influenzae* may be considered. Inclusion of a reserve antibiotic might further strengthen antimicrobial control. The mode of treatment will also largely influence the risk assessment, with different considerations needing to be made for systemic and local applications. A striking example in this context is the effect of C3 inhibition in periodontitis, which is considered an infectious disease; in a recent NHP study, local administration of compstatin Cp40 was able to reduce inflammation and bone loss in the teeth by shifting microbial dysbiosis (Maekawa et al., 2014).

Although immune complex-mediated inflammation or even “auto-immune diseases” are occasionally mentioned as potential risks of C3-targeted therapy, these associations have to be reassessed very critically. While C3 is indeed involved in the clearance of immune complexes, clinical signs of immune complex diseases have so far only been described in very few cases among C3-deficient patients and it is likely that other factors may determine the incidence (Botto et al., 2009). Importantly, and in contrast to a lack in CP components such as C4 and C1q, patients deficient in C3 deficiency are not commonly predisposed to autoimmune diseases. In fact, recent insight from cell-based studies in mice suggest that C3 deficiency may even protect from autoimmunity by affecting both the trafficking of apoptotic cargo in dendritic cells and the shaping of optimal T cell responses (Baudino et al., 2014; Scott and Botto, 2015).

As with every therapy, risks and adverse effects need to be carefully evaluated and taken into consideration when weighing the benefits of individual indications. At the same time, the potential adverse effects and technical limitations increasingly appear manageable, thereby bringing C3-targeted therapies closer to the clinic. The clinical trials planned for various compounds acting at the level of C3 will be essential for finally gaining real clinical experience and directing discussions about possibilities and challenges. Owing to the promise this strategy holds for many indications, the progress and creativity in the development of C3-targeted drugs is exciting to observe and fuels the hope that the arsenal of therapeutics to treat complement-related disorders will continue to grow.

Acknowledgments

We thank Deborah McClellan for her excellent editorial assistance. This work was supported by National Institutes of Health grants AI068730, AI030040, EY020633, and AI097805, the National Science Foundation grant 1423304, a pilot grant from the Penn-CHOP Blood Center for Patient Care and Discovery, and by funding by the European Community's Seventh Framework Programme under grant agreement number 602699 (DIREKT).

References

- Alper CA, Rosen FS. Studies of the in vivo behavior of human C'3 in normal subjects and patients. *J Clin Invest.* 1967; 46:2021. [PubMed: 6074005]
- Atkinson C, Song H, Lu B, Qiao F, Burns TA, Holers VM, Tsokos GC, Tomlinson S. Targeted complement inhibition by C3d recognition ameliorates tissue injury without apparent increase in susceptibility to infection. *J Clin Invest.* 2005; 115:2444. [PubMed: 16127466]
- Bajic G, Yatime L, Klos A, Andersen GR. Human C3a and C3a desArg anaphylatoxins have conserved structures, in contrast to C5a and C5a desArg. *Protein Sci.* 2013; 22:204. [PubMed: 23184394]

- Baudino L, Sardini A, Ruseva MM, Fossati-Jimack L, Cook HT, Scott D, Simpson E, Botto M. C3 opsonization regulates endocytic handling of apoptotic cells resulting in enhanced T-cell responses to cargo-derived antigens. *Proc Natl Acad Sci USA*. 2014; 111:1503. [PubMed: 24474777]
- Berends ET, Mohan S, Mielliet WR, Ruyken M, Rooijackers SH. Contribution of the complement Membrane Attack Complex to the bactericidal activity of human serum. *Mol Immunol*. 2015; 65:328. [PubMed: 25725315]
- 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. [PubMed: 19481265]
- Craik CS, Page MJ, Madison EL. Proteases as therapeutics. *Biochem J*. 2011; 435:1. [PubMed: 21406063]
- 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. [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. [PubMed: 21771620]
- 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. [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. [PubMed: 21860027]
- Grumach AS, Kirschfink M. Are complement deficiencies really rare? Overview on prevalence, clinical importance and modern diagnostic approach. *Mol Immunol*. 2014; 61:110. [PubMed: 25037634]
- Harboe M, Garred P, Karlstrom E, Lindstad JK, Stahl GL, Mollnes TE. The down-stream effects of mannan-induced lectin complement pathway activation depend quantitatively on alternative pathway amplification. *Mol Immunol*. 2009; 47:373. [PubMed: 19800125]
- 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. [PubMed: 15544620]
- Hillmen P, Muus P, Roth A, Elebute MO, Risitano AM, Schrezenmeier H, Szer J, Browne P, Maciejewski JP, Schubert J, Urbano-Ispizua A, de Castro C, Socie G, Brodsky RA. Long-term safety and efficacy of sustained eculizumab treatment in patients with paroxysmal nocturnal haemoglobinuria. *Br J Haematol*. 2013; 162:62. [PubMed: 23617322]
- Holers VM, Rohrer B, Tomlinson S. CR2-mediated targeting of complement inhibitors: bench-to bedside using a novel strategy for site-specific complement modulation. *Adv Exp Med Biol*. 2013; 735:137. [PubMed: 23402024]
- Holz FG, Li ZR, Beres T, Le K, Strauss E. The MAHALO Phase II Study: Safety, Tolerability and Evidence of Activity of FCFD4514S (Anti-factor D) in Patients with Geographic Atrophy (GA) Secondary to Age-Related Macular Degeneration (AMD). *Ophthalmologica*. 2013; 230:12.
- Huang Y, Reis ES, Knerr PJ, van der Donk WA, Ricklin D, Lambris JD. Conjugation to albumin-binding molecule tags as a strategy to improve both efficacy and pharmacokinetic properties of the complement inhibitor compstatin. *Chem Med Chem*. 2014; 9:2223. [PubMed: 25056114]
- Janssen BJ, Christodoulidou A, McCarthy A, Lambris JD, Gros P. Structure of C3b reveals conformational changes that underlie complement activity. *Nature*. 2006; 444:213. [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. [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. [PubMed: 16177781]
- 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. [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. [PubMed: 22362762]
- Kimura Y, Zhou L, Miwa T, Song WC. Genetic and therapeutic targeting of properdin in mice prevents complement-mediated tissue injury. *J Clin Invest*. 2010; 120:3545. [PubMed: 20941861]
- Lachmann PJ. The amplification loop of the complement pathways. *Adv Immunol*. 2009; 104:115. [PubMed: 20457117]
- Lay E, Nutland S, Smith JE, Hiles I, Smith RA, Seilly DJ, Buchberger A, Schwaebler W, Lachmann PJ. Complotype affects the extent of down-regulation by Factor I of the C3b feedback cycle in-vitro. *Clin Exp Immunol*. 2014; in press. doi: 10.1111/cei.12437
- 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; 116:183. [PubMed: 17846331]
- Leshner AM, Nilsson B, Song WC. Properdin in complement activation and tissue injury. *Mol Immunol*. 2013; 56:191. [PubMed: 23816404]
- Leshner AM, Zhou L, Kimura Y, Sato S, Gullipalli D, Herbert AP, Barlow PN, Eberhardt HU, Skerka C, Zipfel PF, Hamano T, Miwa T, Tung KS, Song WC. Combination of factor H mutation and properdin deficiency causes severe C3 glomerulonephritis. *J Am Soc Nephrol*. 2013; 24:53. [PubMed: 23204401]
- Li JS, Jaggars J, Anderson PA. The use of TP10, soluble complement receptor 1, in cardiopulmonary bypass. *Expert Rev Cardiovasc Ther*. 2006; 4:649. [PubMed: 17081086]
- Loyet KM, Good J, Davanecze T, Sturgeon L, Wang X, Yang J, Le KN, Wong M, Hass PE, Campagne M, Haughney PC, Morimoto A, Damico-Beyer LA, DeForge LE. Complement inhibition in cynomolgus monkeys by anti-factor d antigen-binding fragment for the treatment of an advanced form of dry age-related macular degeneration. *J Pharmacol Exp Ther*. 2014; 351:527. [PubMed: 25232192]
- Maekawa T, Abe T, Hajishengallis E, Hosur KB, DeAngelis RA, Ricklin D, Lambris JD, Hajishengallis G. Genetic and intervention studies implicating complement C3 as a major target for the treatment of periodontitis. *J Immunol*. 2014; 192:6020. [PubMed: 24808362]
- Mastellos DC, Ricklin D, Yancopoulou D, Risitano A, Lambris JD. Complement in paroxysmal nocturnal hemoglobinuria: exploiting our current knowledge to improve the treatment landscape. *Expert Rev Hematol*. 2014; 7:583. [PubMed: 25213458]
- Mastellos DC, Yancopoulou D, Kokkinos P, Huber-Lang M, Hajishengallis G, Biglarnia AR, Lupu F, Nilsson B, Risitano AM, Ricklin D, Lambris JD. Compstatin: a C3-targeted complement inhibitor reaching its prime for bedside intervention. *Eur J Clin Invest*. 2015; 45:423. [PubMed: 25678219]
- Medical Research Council. A Double Blind Randomized Controlled Investigation into the efficacy of Mirococept (APT070) for preventing ischaemia-reperfusion injury in the kidney allograft (EMPIRIKAL). UK Clinical Research Network Study Portfolio. [Internet; cited 2015 Feb 17]. Available from: <http://public.ukcrn.org.uk/search/StudyDetail.aspx?StudyID=16181> UKCRN Identifier: 16181
- Milder FJ, Gomes L, Schouten A, Janssen BJ, Huizinga EG, Romijn RA, Hemrika W, Roos A, Daha MR, Gros P. Factor B structure provides insights into activation of the central protease of the complement system. *Nat Struct Mol Biol*. 2007; 14:224. [PubMed: 17310251]
- Miwa T, Sato S, Gullipalli D, Nangaku M, Song WC. Blocking properdin, the alternative pathway, and anaphylatoxin receptors ameliorates renal ischemia-reperfusion injury in decay-accelerating factor and CD59 double-knockout mice. *J Immunol*. 2013; 190:3552. [PubMed: 23427256]

- Morgan, BP.; Thanassi, J.; Podos, S.; Yang, G.; Phadke, A.; Gadachanda, V.; Pais, G.; Hashimoto, A.; Wang, Q.; Chen, D.; Wang, X.; Agarwal, A.; Deshpande, M.; Huang, Y.; Wiles, J.; Huang, M. Novel small-molecule inhibitors targeting complement factor D for therapy of paroxysmal nocturnal hemoglobinuria. 56th ASH Annual Meeting and Exposition; San Francisco, CA. 2014.
- Nagar B, Jones RG, Diefenbach RJ, Isenman DE, Rini JM. X-ray crystal structure of C3d: a C3 fragment and ligand for complement receptor 2. *Science*. 1998; 280:1277. [PubMed: 9596584]
- Nilsson PH, Ekdahl KN, Magnusson PU, Qu H, Iwata H, Ricklin D, Hong J, Lambris JD, Nilsson B, Teramura Y. Autoregulation of thromboinflammation on biomaterial surfaces by a multicomponent therapeutic coating. *Biomaterials*. 2013; 34:985. [PubMed: 23137394]
- Paixao-Cavalcante D, Torreira E, Lindorfer MA, Rodriguez de Cordoba S, Morgan BP, Taylor RP, Llorca O, Harris CL. A humanized antibody that regulates the alternative pathway convertase: potential for therapy of renal disease associated with nephritic factors. *J Immunol*. 2014; 192:4844. [PubMed: 24729617]
- Pauly D, Nagel BM, Reinders J, Killian T, Wulf M, Ackermann S, Ehrenstein B, Zipfel PF, Skerka C, Weber BH. A novel antibody against human properdin inhibits the alternative complement system and specifically detects properdin from blood samples. *PLoS One*. 2014; 9:e96371. [PubMed: 24797388]
- Qu H, Ricklin D, Bai H, Chen H, Reis ES, Maciejewski M, Tzekou A, DeAngelis RA, Resuello RR, Lupu F, Barlow PN, Lambris JD. New analogs of the clinical complement inhibitor compstatin with subnanomolar affinity and enhanced pharmacokinetic properties. *Immunobiology*. 2013; 218:496. [PubMed: 22795972]
- Reis ES, DeAngelis RA, Chen H, Resuello RR, Ricklin D, Lambris JD. Therapeutic C3 inhibitor Cp40 abrogates complement activation induced by modern hemodialysis filters. *Immunobiology*. 2014; 220:476. [PubMed: 25468722]
- 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.
- Ricklin D. Manipulating the mediator: modulation of the alternative complement pathway C3 convertase in health, disease and therapy. *Immunobiology*. 2012; 217:1057. [PubMed: 22964231]
- Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. *Nat Immunol*. 2010; 11:785. [PubMed: 20720586]
- Ricklin D, Lambris JD. Compstatin: a complement inhibitor on its way to clinical application. *Adv Exp Med Biol*. 2008; 632:273. [PubMed: 19025129]
- Ricklin D, Lambris JD. Complement in immune and inflammatory disorders: pathophysiological mechanisms. *J Immunol*. 2013; 190:3831. [PubMed: 23564577]
- Ricklin D, Lambris JD. Complement in immune and inflammatory disorders: therapeutic interventions. *J Immunol*. 2013; 190:3839. [PubMed: 23564578]
- Ricklin D, Lambris JD. Progress and trends in complement therapeutics. *Adv Exp Med Biol*. 2013; 735:1.
- 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. [PubMed: 22577173]
- Risitano AM, Ricklin D, Huang Y, Reis ES, Chen H, Ricci P, Lin Z, Pascariello C, Raia M, Sica M, Del Vecchio L, Pane F, Lupu F, Notaro R, Resuello RR, DeAngelis RA, Lambris JD. Peptide inhibitors of C3 activation as a novel strategy of complement inhibition for the treatment of paroxysmal nocturnal hemoglobinuria. *Blood*. 2014; 123:2094. [PubMed: 24497537]
- 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. [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 USA*. 2011; 108:12839. [PubMed: 21768352]

- Ruseva MM, Vernon KA, Leshner AM, Schwaebler WJ, Ali YM, Botto M, Cook T, Song W, Stover CM, Pickering MC. Loss of properdin exacerbates C3 glomerulopathy resulting from factor H deficiency. *J Am Soc Nephrol*. 2013; 24:43. [PubMed: 23184055]
- Sacks S, Kargli J, Farrar CA, Asgari E, Schwaebler W, Zhou W, Smith RA. Targeting complement at the time of transplantation. *Adv Exp Med Biol*. 2013; 735:247. [PubMed: 23402032]
- Sacks SH, Zhou W. The role of complement in the early immune response to transplantation. *Nat Rev Immunol*. 2012; 12:431. [PubMed: 22627861]
- Sahu A, Kay BK, Lambris JD. Inhibition of human complement by a C3-binding peptide isolated from a phage-displayed random peptide library. *J Immunol*. 1996; 157:884. [PubMed: 8752942]
- Schmidt CQ, Bai H, Lin Z, Risitano AM, Barlow PN, Ricklin D, Lambris JD. Rational engineering of a minimized immune inhibitor with unique triple-targeting properties. *J Immunol*. 2013; 190:5712. [PubMed: 23616575]
- Scott D, Botto M. The paradoxical roles of C1q and C3 in autoimmunity. *Immunobiology*. 2015; in press. doi: 10.1016/j.imbio.2015.05.001
- Vogel CW, Finnegan PW, Fritzinger DC. Humanized cobra venom factor: structure, activity, and therapeutic efficacy in preclinical disease models. *Mol Immunol*. 2014; 61:191. [PubMed: 25062833]
- Wiles, J.; Podos, S.; Thanassi, J.; Phadke, A.; Gadhachanda, V.; Pais, G.; Hashimoto, A.; Wang, Q.; Chen, D.; Wang, X.; Agarwal, A.; Rivera, J.; Elliot, M.; Marlor, C.; Zhang, J.; Fabrycki, J.; Patel, D.; Deshpande, M.; Huang, M.; Huang, Y. Preclinical evaluation of orally bioavailable small-molecule inhibitors of complement factor D as a potential treatment for paroxysmal nocturnal hemoglobinuria. 56th ASH Annual Meeting and Exposition; San Francisco, CA. 2014.
- 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. [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. [PubMed: 21339361]
- Zhang Y, Shao D, Ricklin D, Hilkin BM, Nester CM, Lambris JD, Smith RJ. Compstatin analog Cp40 inhibits complement dysregulation in vitro in C3 glomerulopathy. *Immunobiology*. 2015; 220:993. [PubMed: 25982307]

Figure 1. Mechanisms of complement activation and points of therapeutic inhibition at the level of C3

(A) The complement response as a driver of clinical conditions. On a diseased cell surface, damage-associated molecular patterns (DAMPs) and/or (auto)antibodies may trigger complement activation via binding of pattern recognition receptors of the classical (CP) and lectin pathways (LP) or through probing via the tick-over mechanisms of the alternative pathway (AP). The formation of convertases leads to the cleavage of C3 and deposition of C3b, which forms additional convertases and amplifies the response via the AP. C3b deposition also enables the formation of C5 convertases, thereby leading to the generation of membrane attack complexes (MAC) and the inflammatory effector C5a, which can exacerbate tissue damage and fuel an additional complement response. This process is counteracted by regulators of complement activation (RCA) that act on the C3 convertase

and mediate the degradation of C3b by factor I (FI), generating opsonin fragments with distinct biological activities. **(B)** A molecular view of C3 activation and its regulation. Initial deposition of C3b by any route allows the binding of factor B (FB) and formation of the AP C3 convertase after cleavage by factor D (FD). C3 likely binds to the assembled convertase through a dimerization site, thereby enabling the removal of C3a and the generation of additional C3b that can participate in convertase formation. Binding of RCAs to C3bBb leads to accelerated decay of the convertase by competitive removal of Bb and provides a platform for the binding of FI to mediate the cleavage of C3b to iC3b and C3dg. **(C)** The various concepts to therapeutically control activation of C3. Whereas antibodies against C3b or FB prevent the formation of the pro-convertase (1, 2), mAb- or small molecule-based inhibitors of FD block the conversion to the active convertase (3, 4). C3 inhibitors of the compstatin family protect native C3 by preventing its binding to and cleavage by C3 convertases (5). C3 may also be depleted via cleavage by cobra venom factor or anti-C3 proteases (6, 7). The breakdown of the convertase and C3b can be therapeutically enhanced by adding recombinant FI (8) or by providing purified, recombinant and/or engineered RCA (9). Surfaces may also be coated by entities that recruit factor H (FH) from the circulation (10) or by tethered RCA constructs (11). In panels B and C, the structural figures have been prepared using the crystal structures of C3 (PDB 2A73) (Janssen et al., 2005), C3a (PDB 4HW5) (Bajic et al., 2013), C3b (PDB 2I07) (Janssen et al., 2006), C3d (PDB 1C3D) (Nagar et al., 1998), FB (PDB 2OK5) (Milder et al., 2007), FI (PDB 2XRC) (Roversi et al., 2011), C3bBD (PDB 2XWB) (Forneris et al., 2010), C3b₂Bb₂SCIN₂ (PDB 2WIN) (Rooijakkers et al., 2009), and C3b/FH1-4 (PDB 2WII) (Wu et al., 2009). The complex between C3b, FH1-4, and FI was prepared according to published instructions (Roversi et al., 2011). The hypothetical structure of full-length FH in panel C was composed from multiple copies of FH1-4 derived from the C3b/FH1-4 complex.