Complement in Immune and Inflammatory Disorders: Therapeutic Interventions

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Complement in Immune and Inflammatory Disorders: Therapeutic Interventions

Daniel Ricklin and John D. Lambris

With the awareness that immune-inflammatory crosstalk is at the heart of many disorders, the desire for novel immunomodulatory strategies in the therapy of such diseases has grown dramatically. As a prime initiator and important modulator of immunological and inflammatory processes, the complement system has emerged as an attractive target for early and upstream intervention in inflammatory diseases and has moved into the spotlight of drug discovery. Although prevalent conditions such as age-related macular degeneration have attracted the most attention, the diverse array of complement-mediated pathologies, with distinct underlying mechanisms, demands a multifaceted arsenal of therapeutic strategies. Fortunately, efforts in recent years have not only introduced the first complement inhibitors to the clinic but also filled the pipelines with promising candidates. With a focus on immunomodulatory strategies, in this review we discuss complement-directed therapeutic concepts and highlight promising candidate molecules. The Journal of Immunology, 2013, 190: 3839–3847.

Many concepts of modern drug discovery have deep roots in immunology research, with Paul Ehrlich’s notions of the “magic bullet” and his “side-chain theory” being among the most prominent examples (1). The connection between these two disciplines has even intensified in recent years in view of biological drugs such as mAbs. Therapeutic immune modulation is increasingly recognized as a promising strategy for tackling inflammatory diseases, yet it is in need of more selective modulators that help restore the immune balance and resolve inflammation (2). Fueled by the discovery of new functional roles, immune cross-talk mechanisms, and a growing number of disease associations (see accompanying review in Ref. 3), complement has emerged as focal point of interest in immunomodulatory and anti-inflammatory strategies (4). The variety of intervention points and high number of extracellular targets within the complement cascade (Fig. 1), the availability of potent natural inhibitor templates, and a spike in structure/function insight have all contributed to rapid advances in the field of complement-related drug discovery. Although only two drugs with connection to complement are currently available in the clinic, ongoing research efforts have produced a plethora of innovative and diverse drug candidates that demonstrate great promise in many clinical conditions. In this review, we provide an overview of current therapeutic strategies and highlight drug candidates that are in preclinical or clinical development.

The therapeutic arsenal to tackle complement-related diseases

In view of the diversity and impact of complement-related disorders (3), potent complement inhibitors are desired, yet a “one-size-fits-all” solution for therapeutic management is improbable. Fortunately, complement offers many intervention points from pattern recognition molecules that detect pathogen- and damage-associated surface structures, proteases that drive the activation cascade, opsonins that mark target cells for elimination, to anaphylatoxins that attract immune cells and mediate inflammatory responses (Fig. 1). With purified or recombinant C1 inhibitor (C1-INH) concentrates (Table I) and the anti-C5 Ab eculizumab (Alexion Pharmaceuticals), the first complement-directed drugs have meanwhile entered the clinic. C1-INH is a host serine protease inhibitor that was first recognized for its ability to regulate the activity of the C1 complex, but it also acts at the level of mannose-binding lectin (MBL)–associated serine proteases (MASP), thereby preventing complement initiation via the classical and lectin pathways (CP and LP, respectively). In fact, the specificity of C1-INH is even broader and includes serine proteases of the kinin, coagulation, and fibrinolytic systems; additionally, it may exert anti-inflammatory effects through nonprotease-directed mechanisms (5). Whereas the primary indication of C1-INH preparations, that is, as a substitution therapy in hereditary angioedema, is likely mediated by the kinin rather than the complement system, the availability of a CP/LP inhibitor in the clinic enables exploring the drug in complement-related diseases. Indeed, C1-INH has shown promising effects in several disease models, in-

Abbreviations used in this article: aHUS, atypical hemolytic uremic syndrome; AMD, age-related macular degeneration; AP, alternative pathway; C1-INH, C1 inhibitor; CP, classical pathway; CR, complement receptor; CVF, cobra venom factor; FB, factor B; FD, factor D; FH, factor H; IPI, ischemia/reperfusion injury; LP, lectin pathway; MAC, membrane attack complex; MASP, mannose-binding lectin–associated serine protease; MBL, mannose-binding lectin; PNH, paroxysmal nocturnal hemoglobinuria; RCA, regulator of complement activation.
FIGURE 1. Simplified scheme of the complement cascade with currently exploited targets of complement-directed therapeutic intervention. Only the AP C5 convertase (C3bBb3b) is shown for reasons of simplicity; however C5 convertases (C4b2b3b) can also be formed with contribution from the CP/LP. The enzymatic fragment of C2 is referred to as C2b (or 2b as part of the CP/LP convertases); the same fragment is designated as C2a in some publications. AP, Alternative pathway; C3(H2O), hydrolyzed C3; C1-INH, C1 inhibitor; C5L2, C5a receptor-like 2; CP, classical pathway; CR, complement receptor; FB, factor B; Fcn, ficolins; FD, factor D; FH, factor H; FP, factor P (properdin); GPCR, G protein–coupled receptor; LP, lectin pathway; MAC, membrane attack complex; MASP, mannose-binding lectin–associated protease; MBL, mannose-binding lectin.

Including myocardial infarction (6), transplantation (7), and, recently, in a mouse model of type 1 diabetes (8); clinical trials have been initiated for the attenuation of thromboinflammatory responses in trauma (ClinicalTrials.gov identifier NCT01275976) and kidney transplantation (NCT01147302, NCT01134510). In contrast to C1-INH, eculizumab is complement-specific, as this mAb binds to C5 and prevents its activation by the convertase and, consequently, the generation of C5a and the membrane attack complex (MAC). Eculizumab was originally approved for the orphan disease paroxysmal nocturnal hemoglobinuria (PNH), in which it prevents intravascular lysis of insufficiently protected erythrocytes with high efficacy. Meanwhile, indications for eculizumab have been extended to include atypical hemolytic uremic syndrome (aHUS) where it was shown to improve clinical parameters such as renal function or microangiopathy (9). Despite its high cost, the availability of this potent terminal pathway inhibitor has allowed off-label use of the drug, perhaps most noticeably for the treatment of hemolytic uremic syndrome caused by Shiga toxin–producing Escherichia coli during a recent outbreak in Europe (10). Additionally, eculizumab has been tested in various disease models and is currently being evaluated in clinical trials ranging from age-related macular degeneration (AMD) to transplantation (Table I).

The pursuit of extending the arsenal of complement inhibitors for pathway or tissue-specific prevention of complement attack has produced various attractive candidates that cover a wide range of targets and applications. Traditionally, selective blockage of the CP has focused on its major activating serine protease C1s, yet attempts to arrive at small molecule inhibitors were hampered by poor specificity and pharmacokinetics (11). More recently, structure-guided discovery approaches and PEGylation were able to improve such shortcomings and may lead to clinical C1s inhibitors (12, 13). In addition to small molecule approaches, Ab-centered strategies to prevent pattern recognition by C1q have also shown promise; for example, a single-chain Ab fragment that binds to the globular heads of C1q (QuVHVL) was shown to block recognition of both IgG and C-reactive protein and to largely reduce CP activation by apoptotic cells (14).

Novel candidates have also emerged for LP-associated targets such as the MASP family. For example, Omeros runs a preclinical program that involves MASP-2–specific Abs (e.g., OMS721) with potential application in aHUS and other conditions ranging from ischemic diseases to AMD (15). Indeed, anti–MASP-2 mAbs have shown beneficial effects in mouse models of myocardial and gastrointestinal ischemia/reperfusion injury (IRI) (16). Additionally, phage-display library screening using an insect proteinase inhibitor as a tem-
plate produced peptides that are monospecific for MASP-1 and MASP-2, respectively, and inhibit the LP with nanomolar activity (17). Finally, the therapeutic potential of the endogenous LP regulator MBL/ficolin-associated protein-1, which displaces MASP proteins from the MBL complex, has been demonstrated in mouse models of myocardial infarction (18).

Owing to the role of the AP as a major amplifier of complement responses, contributing up to some 80% of activity even in the case of CP-mediated initiation (19), strategies that lead to comprehensive complement inhibition by targeting at the level of C3 have been actively pursued in recent years (20). Currently, the peptide compstatin and its analogs are the only inhibitors that act on native C3; by preventing activation of the AP, they can also mask the APC system and thereby reduce the levels of C3 convertase.

### Table 1. Complement therapeutics currently (or recently) listed in the pipelines of pharmaceutical companies

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Manufacturer</th>
<th>Complement Target</th>
<th>Compound Class</th>
<th>Phase/Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 inhibitor† (Cinryze, Berinert, Cetor, Ruconest)</td>
<td></td>
<td>C1s, Fc, other proteases</td>
<td>Peptide</td>
<td>P4 (HAE), P3 (trauma), P1/2 (TP)</td>
</tr>
<tr>
<td>Nafamostat† (FUT-175, Furhat)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OMS521</td>
<td>Genentech</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FCD4514S, TNX-234</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA106</td>
<td>Alexion Pharmaceuticals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bikaciomab, NM9308</td>
<td>Novelmed</td>
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<td></td>
<td></td>
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<tr>
<td>NM9401</td>
<td>Properdin</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CVF, HC3-1496</td>
<td>InCode</td>
<td></td>
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</tr>
<tr>
<td>Complatin®</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POT-4, AL-7898A</td>
<td>Potentia Pharmaceuticals/Alcon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cp40, AMY-101</td>
<td>Amyndas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP-1</td>
<td>Apellis Pharmaceuticals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sCR1, CDX-1135</td>
<td>Cellnex</td>
<td>C3-conv, C4b, C3b</td>
<td>Peptide</td>
<td>P4 (HAE), P3 (trauma), P1/2 (TP)</td>
</tr>
<tr>
<td>Miropoibio</td>
<td>(U.K. Medical Research Council)</td>
<td>C3-conv, C4b, C3b</td>
<td>Peptide</td>
<td>P1 (DDD)</td>
</tr>
<tr>
<td>ALXN1102/ALXN1103d (TT30)</td>
<td>Alexion Pharmaceuticals</td>
<td>C3-conv, C3b</td>
<td>Peptide</td>
<td>P1 (TP)</td>
</tr>
<tr>
<td>rFH</td>
<td>(Optherion, Taligent)</td>
<td>C3-conv, C3b</td>
<td>C3-conv, C3b</td>
<td>Peptide</td>
</tr>
<tr>
<td>mini-FH, AMY-201</td>
<td>Amyndas</td>
<td>C3-conv, C3b</td>
<td>Peptide</td>
<td>P1 (TP)</td>
</tr>
<tr>
<td>5C6, AMY-301</td>
<td>Amyndas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eculizumab (Soliris)</td>
<td>Alexion Pharmaceuticals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eculizumab (Soliris)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mubodina</td>
<td>Adienne Pharma and Biotech</td>
<td>C5</td>
<td>Ab (miniboody)</td>
<td>P2 (MDM)</td>
</tr>
<tr>
<td>Ergidina</td>
<td>Adienne Pharma and Biotech</td>
<td>C5</td>
<td>Ab (miniboody, targeted)</td>
<td>P1 (ADMD)</td>
</tr>
<tr>
<td>ARC1905</td>
<td>Ophthotech</td>
<td>C5</td>
<td></td>
<td></td>
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<td>LFG316</td>
<td>Novartis, MorphoSys</td>
<td>C5</td>
<td></td>
<td></td>
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<tr>
<td>MED17814</td>
<td>MedImmune, AstraZeneca</td>
<td>C5/C5a</td>
<td></td>
<td></td>
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<tr>
<td>NOX-D19</td>
<td>Noxon</td>
<td>C5a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFX-1, CaPC29</td>
<td>InfraRx</td>
<td>C5a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMX33, PMX205</td>
<td>Cephalon/Teva</td>
<td>C5aR</td>
<td>Peptidomimetic</td>
<td>N/A</td>
</tr>
<tr>
<td>CCX168</td>
<td>ChemoCentryx</td>
<td>C5aR</td>
<td>Small molecule</td>
<td>P2 (ANCA-vasculitis)</td>
</tr>
<tr>
<td>ADC-1004</td>
<td>Alligator Bioscience</td>
<td>C5aR</td>
<td>Protein</td>
<td>P1 (RA)</td>
</tr>
<tr>
<td>Anti-C5aR-151, NN8209</td>
<td>Novo Nordisk</td>
<td>C5aR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-C5aR-215, NN8210</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imprine PGG</td>
<td>Biothera</td>
<td>CR3, other targets</td>
<td>Soluble β-glucan</td>
<td>P1–P3 (cancer; add-on)</td>
</tr>
</tbody>
</table>

*Clinical development is indicated as follows: PC, preclinical; P1–P3, clinical trial phases 1–3; P4, marketed (phase 4). Preclinical indications extracted from company websites and other sources and may be subject to change. Clinical trial indicators (ClinicalTrials.gov) are indicated in the text.

†C1 inhibitor and Nafamostat also inhibit proteases outside the complement network; clinical effects may be partially or not complement-related.

‡POT-4 is developed by Potentia/Alcon for age-related macular degeneration. Apellis Pharmaceuticals develops POT-4 for other indications under the name APL-1. Amyndas develops advanced compstatin analogs.

§clinical trials with Miropoibio are performed by the U.K. Medical Research Council using a government-sponsored grant.

Recombinant H (preclinically indicated for age-related macular degeneration) had been developed by Ophtherion and Taligen before both companies left the market.

Cephalon has recently been acquired by Teva; no plans for the clinical development of PMX had been disclosed by Teva at the time of publication.

Target has not been officially disclosed for IFX-1.
of C3 by convertases, compstatin blocks C3b opsonization, amplification, and generation of effectors. Despite specificity for human/primate C3, compstatin analogs have been tested in models ranging from sepsis to hemodialysis-induced thromboinflammation (11, 21, 22). In a primate model of AMD, intravitreal compstatin suppressed or reversed drusen formation (23), and a compstatin analog (AL-78898A; Alcon) is in clinical development for the treatment of AMD. The same analog (termed APL-1) is developed by Apellis Pharmaceuticals for asthma and chronic obstructive pulmonary disease. Although peptidic drugs are often hampered by rapid plasma elimination, recent optimization efforts have produced compstatin analogs (e.g., AMY-101; Amyndas) that feature subnanomolar target affinities and plasma half-lives of up to 12 h (24), which is expected to facilitate systemic applications. In addition to compstatin, Abs against C3b, factor B (FB), and factor D (FD) have been disclosed. For example, mAb S77 (Genentech) was shown to prevent convertase formation by blocking the binding area of FB on C3b (25), and the anti-C3b mAb 3E7 demonstrated high efficacy in a model of PNH (26). Genentech also developed an mAb against FD (FCFD45145), which is being tested in clinical trials for dry AMD (NCT01229215) (27). A fragment of the anti-FB mAb 1379 (28) had been disclosed by Taligen Therapeutics (TA106), but no development plans have been revealed since its acquisition by Alexion Pharmaceuticals. More recently, properdin emerged as an attractive therapeutic target, and Abs against this modulator showed efficacy in models of arthritis and abdominal aortic aneurism (29, 30). Although not a classical “inhibitor,” the C3 homolog cobra venom factor (CVF) also targets C3 by forming long-lasting C3 convertases that rapidly deplete C3 stores (31). Therapeutic C3 depletion by CVF and its humanized form (HC3-1496; InCode) has shown efficacy in disease models, including AMD (32) and transplantation (33). Of note, HC3-1497 does not act as a C5 convertase (in contrast to some forms of native CVF), thereby alleviating toxicity concerns due to direct generation of massive C5a levels (31). Still, what effect instant solution activation of complement may have on diseased tissue and which indications may benefit most from depletion strategies will need to be further evaluated.

Whereas these concepts aim at preventing formation of the convertases, our body possesses formidable inhibitors, that is, the regulators of complement activation (RCA) family, to deal with those potent enzyme complexes and prevent AP amplification. Their potency, specificity, and modular architecture (consisting of 4–30 complement control protein domains) render RCA attractive templates for inhibitor development (20, 34). For example, soluble complement receptor (CR)1 (TP10; Avant Immunotherapeutics) has been evaluated for various diseases (35, 36) and the program has meanwhile been continued by Celldex Therapeutics (CDX-1135) with focus on renal pathologies; phase I clinical trials of CDX-1135 for the treatment of dense deposit disease have recently been initiated (NCT01791686). A truncated, membrane-targeted form of CR1 (APIT070/Miroceopt) (37) was recently shown to improve early transplant function in a pilot study of kidney transplantation, and is scheduled to enter formal clinical trials (38). Owing to its strong disease associations in AMD and kidney disorders (3), the direct use of recombinant factor H (FH) for therapeutic purposes has been considered but is not being actively pursued at this time. Hence, recent strategies focus on engineered protein therapeutics based on the regulatory domains of FH. Among those, TT30 (ALXN1102; Alexion Pharmaceuticals) is the most established candidate. TT30 combines the regulatory complement control protein domains 1–5 of FH with the N-terminal four domains of CR2 that bind to iC3b, C3dg, and C3d. In this manner, TT30 inhibits AP activity (by accelerating the decay of convertases and acting as a cofactor for the factor I–mediated degradation of C3b to prevent further C3/C5 convertase formation) on sites of ongoing complement activation that typically accumulate these downstream opsonins (39, 40). TT30 and its rodent homologs have shown promising results for AP-specific complement inhibition in disease models ranging from AMD to PNH (39, 41, 42), and TT30 is now being evaluated in phase I trials for PNH (NCT01335165). This targeting approach has been extended to combine the N-terminal regions of CR1 and CR2 (TT32), thereby producing an inhibitor with activities for both CP/LP and AP (by acting on C4b and C3b, respectively) that has been tested in an arthritis model (43). Based on insight into the structure of FH, a streamlined FH derivative that directly links the regulatory and targeting domains was recently disclosed (mini-FH, AMY-201; Amyndas) (44). Despite a size reduction by 70%, mini-FH preserved functional activities compared with FH and showed a unique targeting profile toward sites of ongoing activation (similar to TT30), self cells (via binding to glycosaminoglycans), and markers of oxidative stress–induced damage (i.e., recognition of malondialdehyde); in models of PNH, mini-FH showed activities exceeding those of FH and other inhibitors (44). Inspired by complement evasion strategies employed by human pathogens (45), an approach for protecting biomaterial surfaces has been described in which surfaces are coated with an FH-binding peptide (5C6, AMY-301; Amyndas) that recruits host FH and prevents AP amplification (46). Recently, this approach was expanded to transplantation-relevant cell surfaces and combined with apyrase immobilization to produce anti-thromboinflammatory coatings (47).

The therapeutic value of inhibiting at the level of C5, that is, simultaneously blocking the generation of C5a and MAC, remains a major focus of complement-targeted drug discovery (20, 48). For example, an mAb against mouse C5 (BB5.1) with antihemolytic activity was disclosed in 1987 (49) and subsequently used in disease models (50–52), spearheading a development that resulted in human-specific C5 Abs (53) and, eventually, the clinical availability of eculizumab (see above). A single-chain variable fragment of the Ab (pexelizumab; Alexion Pharmaceuticals) was evaluated for use in myocardial infarction and cardiopulmonary bypass surgery, among other models (54, 55), but it appears to be discontinued after those studies did not meet expectations (56). More recently, a fully human anti-C5 mAb (FG316, Novartis/MorphoSys) has entered phase II clinical trials for AMD, multifocal choroiditis, and panuveitis (NCT01624636, NCT01527500, NCT01526889, NCT01535950). Additionally, Adienne Pharma and Biotech is developing neutralizing minibodies against C5 in which a single-chain variable fragment is linked to the IgG hinge region. A rat homolog of this minibody showed promising results in models of posttransplant IRI (57), and the human minibody (Mubodina)
PMX205 has shown promising effects in a mouse model of compound demonstrated beneficial safety profiles, it did not psoriasis and rheumatoid arthritis (48, 68); although the used candidate and has shown promise in disease models PMX53 (originally developed by Promics) is the most widely representative, and several small molecule antagonists have indeed been developed over the years (11, 12). As a G protein–coupled receptor, the C5aR (C5aIP, AcPepA) have been tested in islet transplantation and sepsis (66, 67). As a target specific for the synovial microvascular endothelium to the minibody, the resulting molecule (MT07) resolved inflammation by reducing recruitment of polymorphonuclear cells, cytokine release, and tissue injury (58). C5-neutralizing entities have also been developed on the basis of aptamers (ARC1905; Ophthotech). Whereas a phase I trial for AMD in combination with the anti–vascular endothelial growth factor mAb Lucentis has been completed in 2011 with positive safety results (NCT00709527) (59), no details about the future development of this compound have been released. Finally, nature provides potent C5 inhibitors such as SSL7 from Staphylococcus aureus (60) or the tick-derived OmCI, the latter of which was shown to prevent terminal pathway activation and partially reduce inflammatory markers in an ex vivo human/porcine whole blood model of sepsis and completely prevented the development of experimental myasthenia gravis in rats (61, 62). As with all exogenous natural products, however, potential immunogenicity concerns need to be carefully considered and may render a direct use of these inhibitors challenging.

Instead of inhibiting the entire terminal pathway, the selective blockage of either C5a-mediated signaling or MAC formation is desired under certain clinical conditions (48). Although MAC-directed therapy is the road less traveled, it produced promising concepts for the treatment of PNH or AMD based on soluble or membrane-targeted forms of the regulator CD59 (63, 64). Given the strong proinflammatory and modulatory activities of C5a signaling, therapeutic intervention at the level of C5a or the C5aR (CD88) remains a focal area. Neutralizing Abs against C5a have demonstrated protective effects in experimental sepsis (65), and this promising therapeutic concept appears to apply to the use of mAb IFX-1 (CaCP29; InflaRx; target has not been officially disclosed) that has been evaluated in phase I trials (NCT01319903). Additionally, two strategies have been developed that exploit structurally complementary molecules as C5a inhibitors: whereas NOX-D19 (Noxxon Pharma) is based on Spiegelmer technology (i.e., biotable RNA aptamers), antisense peptides (C5aIP, AcPepA) have been tested in islet transplantation and sepsis (66, 67). As a G protein–coupled receptor, the C5aR represents a druggable target, and several small molecule antagonists have indeed been developed over the years (11, 48). Among those, the peptidomimetic C5aR antagonist PMX53 (originally developed by Promics) is the most widely used candidate and has shown promise in disease models ranging from sepsis, cancer, and IRI to inflammatory bowel disease, arthritis, and pregnancy-related complications. Clinical studies have been conducted with PMX53 for use in psoriasis and rheumatoid arthritis (48, 68); although the compound demonstrated beneficial safety profiles, it did not significantly reduce synovial inflammation in the arthritis study (69). A derivative of the compound with increased metabolic stability and ability to cross the blood–brain barrier (PMX205) has shown promising effects in a mouse model of Alzheimer’s disease (70) and has recently been tested in models of periodontitis and colitis (71, 72). The technology has undergone several acquisitions, and future plans for the clinical development of PMX53 or PMX205 have not been revealed. A structurally similar antagonist (JPE-1375) has been discontinued after operations of the developing company (JeriPharma) had been closed in 2009. The only small molecule C5aR antagonist under clinical development, to the best of our knowledge, remains CCX168 (ChemoCentryx), which is evaluated in phase II for anti-neutrophil cytoplasmic Ab–associated renal vasculitis (NCT01363388). Alongside synthetic antagonists, blockage of C5aR signaling can also be achieved by protein therapeutics. Novo Nordisk is developing two anti-C5aR Abs (Table I) and is conducting phase I trials for rheumatoid arthritis (NCT01223911, NCT01611688). A protein antagonist based on the immune evasion protein CHIPS from S. aureus (ADC-1004; Alligator Bioscience) had been tested in a myocardial IRI model, in which it reduced infarct size by 21% (73), but the company has announced a delay in further development of this compound. Compared with C5aR, therapeutic modulation of the C3aR has proven challenging and a C3aR antagonist candidate (SB 290157) showed partial agonism (74). Still, SB 290157 continues to be tested in experimental disease studies, primarily in rodents, and the compound has shown modulatory effect in models of intracerebral hemorrhage and metabolic dysfunction, among others (75, 76).

Translation into complement-centered immunomodulatory strategies

Ongoing research on underlying disease mechanisms using clinically relevant models will continue to be of high importance for validating the involvement of complement in a specific disease and for selecting the most promising indications. These studies will also guide target selection, which is a balancing act between comprehensive amelioration of complement-induced damage, maintenance of physiological complement activities, treatment cost, and other factors. The ideal level of intervention will be different for each disease and may even vary within groups of affected patients. As a major mediator of immune responses, C5a signaling can be an attractive target to tame inflammation without affecting opsonization or lytic functions of complement. In other diseases, it may be more important to disrupt the amplification cycle to avoid perpetual opsonization and immune cell recognition. Finally, in disorders with a known initiator, the upstream inhibition of a single pathway may be preferred. The complexity and intricate consequences of choosing an appropriate target level for therapeutic complement inhibition is nicely illustrated in the case of PNH. Because MAC-induced lysis is the hallmark symptom of this disease, inhibition needs to occur at or upstream of C5 cleavage, thereby excluding the C5a/C5aR axis. C5-directed therapy using eculizumab potently prevents intravascular lysis and presents a highly effective (yet costly) treatment option. However, studies have shown that some PNH patients under eculizumab show insufficient success and remain dependent on transfusion. In these patients, uncontrolled AP amplification on PNH erythrocytes in the absence of CD55 can lead to an accumulation of opsonized cells in circulation, which are recognized by immune cells and become subject to C3-dependent extravascular hemolysis (77). Upstream intervention at the level of C3 may therefore provide dosage.
a valuable alternative to ecuizumab, especially for patients with poor response to the treatment. The study of PNH patients treated with ecuizumab also established that the effects of complement-directed therapy reaches beyond prevention of lysis and may beneficially shape other clinical aspects, including lower risk of thrombosis and normalization of immune parameters (77, 78).

The clinical availability of complement inhibitors and a growing number of drug trials have also shed more light on the important question of safety. With long-term data for chronic ecuizumab treatment available, the results suggest good safety and tolerability for this C5-directed strategy (79, 80). Similarly, the various C1-INH preparations share a favorable safety/tolerability profile when used in the typical dosage regimen for the treatment and/or prophylaxis of hereditary angioedema. Finally, clinical trials conducted so far with inhibitors acting at various levels ranging from C3 to C5aR (e.g., SCR1, PMX53, compstatin, ARC1905; Table I) did not reveal toxicity or other major complications. Increased susceptibility to infection still remains the biggest concern in the context of complement inhibition. Indeed, although patients with a primary deficiency in proximal components may develop a higher risk for infection with certain pathogens such as Streptococcus pneumoniae, immune complex diseases, or systemic lupus erythematosus–like symptoms, complete lack of one of the terminal components primarily raises the susceptibility to neisserial infections (81, 82); patients receiving ecuizumab are therefore prophylactically vaccinated against Neisseria meningitidis. On a functional level, even downstream effectors such as C5a have been shown to critically contribute to the protection against certain pathogens such as S. aureus (83), thereby emphasizing the notion that no inhibition level is a priori to be considered “safe” or “unsafe.” Importantly, however, severe infections in patients with primary complement deficiencies are predominantly seen in childhood, with a significant improvement in adulthood; it has therefore been concluded that the antibacterial functions of complement become less critical once adaptive immunity is fully developed and high IgG titers are reached (84). Moreover, in the case of C3, even low residual plasma levels, as for example observed in patients with nephritic factors that deplete the C3 store, appear to confer a considerable level of protection. In this context, one open question is whether full complement inhibition can be achieved (e.g., due to rapid turnover of some components) or even needs to be achieved. In diseases based on a chronically imbalanced complement system (e.g., as a consequence of RCA polymorphisms), it may be enough to tip the balance into the normal range. Additionally, even C5 inhibition does not lead to complete complement impairment, as it does not interrupt opsonization with CP/LP components, and direct activation of C3 and C5 by nonconvertase proteases remains intact in most inhibitory regimens. Finally, safety considerations are also dependent on the timeframe and localization of inhibitor administration, with potential concerns being less pronounced when inhibitors are administered locally (e.g., intravitreal injection in AMD) and/or for a limited time frame (e.g., in sepsis). Although the final “verdict” on the safety of complement-targeted therapy is still out, and certainly needs to carefully be reassessed for each compound, the current picture is encouraging.

One of the emerging strategies that may further add to the selectivity and safety of complement-directed therapies is drug targeting. Because most complement activation and amplification occur on the surface of foreign or diseased cells, and given the importance of peripherally synthesized complement, a directed intervention at the site of major activity is considered preferable in many situations. Approaches that target specific surfaces via coating (e.g., 5C6), membrane anchoring (e.g., Mirococept), or cell/tissue-specific peptide tags (e.g., Ergidina) have shown great promise and will be interesting to follow through clinical development. The same is true for the concept of directing regulatory entities toward sites of ongoing AP activity (as marked by accumulating iC3b/C3dg) using FH- or CR2-derived recognition domains (e.g., in mini-FH or TT30). In the latter case, comparative studies impressively demonstrated the efficacy of the targeted compound in attenuating IRI-induced damage on affected tissue while retaining systemic complement activity (85).

Novel therapeutic concepts increasingly attempt to exploit the cross-talk between complement and systems of innate/adaptive immunity and coagulation during pathological events. For example, because complement and the TLR system show cooperative effects during confrontation with danger patterns, their combined attenuation was hypothesized to be advantageous (86). Indeed, synergistic beneficial effects of concerted inhibition of complement (using compstatin or anti-C5) and TLR (via anti-CD14) have been shown in whole blood models of sepsis (87) and might have great implications for systemic inflammatory response syndrome (86) and other inflammatory diseases such as periodontitis. Moreover, the newly discovered negative feedback loop in the C5aRa-FcyR cross-talk via dectin-1 and galactosylated IgG may influence the development of glycoengineered intravenous immunoglobulin (IVIG) preparations similar to those used as anti-inflammatory treatment in autoimmune disorders (88, 89). In some situations, such as during infection, the explicit stimulation of C5a mediation via C5aR agonists such as peptide EP67 may lead to a beneficial enhancement of immune responses (90); the same agonist has also been suggested as potential adjuvant owing to its ability to drive Th1-mediated immune responses (91). Even in cases of complement-inhibitory monotherapy, one should consider that associated physiological systems may be affected and aim for a comprehensive analysis. In the case of sepsis, for example, compstatin mediated by boboons 6 h after a sublethal dose of E. coli not only prevented complement activation but also influenced inflammatory and thrombotic parameters, leading to significant organ protection (22). Especially in complex disorders such as sepsis, there may be several routes (e.g., targeting complement, coagulation, or downstream inflammation) that lead to similar results, as they provide the body with sufficient free resources to cope with the remaining complications. In many aspects, tipping the balance from an excessive back to an effective complement response may be an essential and highly promising strategy for restoring immune homeostasis and resolving inflammation.

Conclusions

In coming back to the drug discovery concepts coined by Paul Ehrlich and other pioneers in immunology, it appears unlikely that there will be one “magic bullet” to treat all complement-related diseases. Rather, we may aim for a diverse armory...
of therapeutics and for carefully tailoring the treatment strategies for each disease. The commonalities within many complement-related pathologies indicate that once on the market for one disease, a new complement-targeted drug may also benefit patients with other disorders, as the continuous expansion of indications for both eculizumab and C1-INH have already demonstrated. In any case, the pipelines of academic research and biopharmaceutical companies look highly promising, as they are already filled with a diverse panel of intriguing candidates, rendering the goal of a complement therapeutic “toolbox” ever more likely. Finally, the increasing use of diverse and highly specific complement inhibitors for the dissection and exploration of disease mechanisms may not only reveal novel candidates with therapeutic potential but also help discover even more fascinating cross-talk mechanisms between complement and other branches of immunity.

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Disclosures

D.R. and J.D.L. are the inventors of patents and/or patent applications that describe the use of complement inhibitors for therapeutic purposes. J.D.L. is the founder of Amyndas Biotherapeutics and Amyndas Pharmaceuticals, which are developing complement inhibitors for clinical applications.

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4. Dempulos, G. A., T. Dudler, and W. J. Schwaeble, inventors; Omeros Corporation, the founder of Amyndas Biotherapeutics and Amyndas Pharmaceuticals, which have already demonstrated. In any case, the pipelines of academic research and biopharmaceutical companies look highly promising.


