Synthetic small-molecule complement inhibitors
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During the past few years, several large molecular-weight compounds with complement-inhibitory activities have entered clinical trials for a wide variety of acute and chronic inflammatory conditions. Various small synthetic compounds that offer several advantages over the larger complement inhibitors are also being discovered at a rapid pace. In this review, the focus will be on three of these small molecules, a C3-binding peptide, compstatin; a synthetic peptide antagonist of the C3a anaphylatoxin receptor, D353; and a non-peptidergic antagonist of the C3a anaphylatoxin receptor, SB-290157. In recent years, compstatin has undergone a series of optimizations that have led to more active and stable analogs, while D353 and SB-290157 have been more extensively tested in animal models of various human inflammatory diseases. These compounds have been shown to be effective and display little or no toxicity, and as such may be promising new candidates for further therapeutic development.

Keywords Complement, inflammation, inhibitors, peptides, synthetic

Introduction
Complement is an important component of the innate immune system and protects the host from pathogenic organisms and chemical and physical insults through a variety of mechanisms involving cell lysis, opsonization, immune clearance and inflammation (Figure 1) [1]. Complement activation is tightly regulated by a family of structurally related proteins known as regulators of complement activation (RCA) that are either soluble (eg, factor H and C4 binding protein) or surface-bound (eg, decay-accelerating factor (DAF), membrane-co-factor protein (MCP) and complement receptor type 1 (CR1)) [1,2]. Despite the presence of these regulatory molecules, uncontrolled complement activation has occurred in response to various iatrogenic situations (eg, transplantation, reperfusion and cardiopulmonary bypass surgery), resulting in serious tissue damage and hypersensitivity reactions [1]. Excessive complement activation has also been associated with over 25 immune-inflammatory conditions, such as rheumatoid arthritis, asthma, psoriasis, hemolytic anemia, multiple sclerosis, lupus erythematosus and glomerulonephritis, in which complement can affect the occurrence as well as the exacerbation of the disease [2,3].

Complement plays an important role in inflammation and interacts with the cytokine and eicosanoid networks at various levels [4-8]. However, despite the relevance of complement to human disease, complement inhibitors are not yet available in the clinic. The slow development of these compounds may be related to the difficulty with which complement activation can be regulated. In addition, until recently, none of the generated experimental compounds were considered safe or effective enough to be suitable candidates for therapeutic development [9].

Complement as a therapeutic target
During the past ten years, several novel compounds targeting complement have entered clinical trials. These include, but are not limited to, soluble versions of CRI, such as TP-10 (Avant Immunotherapeutics Inc), TP-20 and microcept (APT-670, Adprotech Ltd), a soluble chimeric molecule of DAF and MCP termed MLN-2222 (previously known as CAB-2, Xoma Ltd/Millennium Pharmaceuticals Inc), humanized antibodies against complement factor (C)5, (eg, pexelizumab; Alexion Pharmaceuticals Inc/The Procter & Gamble Co, and eculizumab; Alexion Pharmaceuticals Inc). These compounds are currently being tested in phase I, II or III clinical trials in the US and/or the UK for rheumatoid arthritis, cardiopulmonary bypass, myocardial infarction, systemic lupus erythematosus, asthma, psoriasis, reperfusion injury, nephritis, paroxysmal nocturnal hemoglobinuria, dermatomyositis or pemphigoid.

It seems likely that a complement inhibitor will be on the market within the next few years, an event that will markedly affect inflammatory and complement research. In this review we will discuss some of the progress that has recently been made in the discovery and development of several small synthetic complement inhibitors, which have several advantages over the larger compounds with regard to their possible routes of administration, cost-effectiveness and pharmacokinetics. We will focus on three of the currently most promising compounds, a C3-binding peptide, compstatin; a C5a receptor antagonist, D353 (University of Queensland, Australia); and a C3aR antagonist, SB-290157 (GlaxoSmithKline plc). Two small synthetic molecules, both C5a receptor antagonists, have recently entered clinical trials in Australia (PMX-53, Promics Pty Ltd) for psoriasis and asthma (topical and oral formulations, respectively) and in the US for asthma (NGD-2000-I, Neurogen Corp). Therefore, it will not be surprising if small synthetic compounds become a new generation of complement inhibitors.
Comstatin: An inhibitor of C3 activation

Comstatin, a 13-residue cyclic peptide with a molecular weight of 1551 Da, was initially isolated from a phage-displayed random peptide library in 1996 [10]. It blocks complement activation by binding to the C3c portion of C3 and preventing its cleavage into C3a and C3b. Interestingly, this binding, which is fully reversible, does not sterically hinder access to the C3a/C3b cleavage site by C3 convertase. Therefore, it was suggested that the lack of C3 cleavage may, in part, be due to an inhibition of the formation or stabilization of the convertase (C3bBb) [10]. Comstatin's three-dimensional structure in solution was determined in 1998 and revealed the presence of a type I β-turn in the region Gln^2-Asp^3-Trp^4-Gly^5 and a hydrophobic cluster at the linked termini [11,12]. A combination of nuclear magnetic resonance (NMR) and deletion/replacement studies, identified the structural characteristics and residues that are most critical for compstatin's activity [11-14]. These include: (i) the disulfide bridge between Cys^2 and Cys^9, (ii) an 11-membered peptide located between the disulfide bond, (iii) a type I β-turn formed by residues 5 to 8, (iv) Val at position 3, and (v) the hydrophobic cluster at the linked termini (formed by Ile^1-Cys^2-Val^3-Val^4 and Cys^5-Thr^6). Further details on the structural aspects of compstatin can be found in reference [15].

The side chains of several of the residues are important for the conformational stability of compstatin, as well as for compstatin-C3-binding interactions [13]. For example, the side chain of Trp^4, which caps the β-turn, may be involved in C3 binding via electrostatic interactions with aromatic or cation partners on C3 (Figure 2). Surface plasmon resonance (SPR) analyses have demonstrated that compstatin binds to C3, C3b and C3c, but not to the C3d(g) fragment [14].

Interestingly, the binding of compstatin to C3 is species specific [10]. Saha et al [16] recently demonstrated that reconstitution of C3-deficient mouse serum with human C3 protects rabbit erythrocytes from complement-mediated lysis, thus demonstrating that the lack of compstatin efficacy in mouse models is solely due to the C3 species of origin. More detailed studies on the binding kinetics showed that compstatin exhibits exclusive specificity for primate C3, and does not bind to C3 from pig, rabbit, guinea pig, rat or mouse, or to human C4 or C5, two structural homologs of human C3. The binding interactions between compstatin and non-human primate C3 molecules were similar to those of human C3. The exact reason for the lack of species cross-reactivity of compstatin is still under intense investigation.

Because of the specificity of compstatin for primate C3, studies on the activity of the peptide are limited to clinically relevant in vitro models using human blood and in vivo studies using non-human primates. Table 1 lists the models in which compstatin's inhibitory activity has been tested. Of special interest are the recent studies by Lappégaard
and co-workers [8,17], in which the effects of compstatin on polymer-induced blood cell activation have been investigated in an in vitro model of extracorporeal circulation. In these studies, compstatin displayed an efficacy similar to that of anti-factor D and anti-C5 antibodies in inhibiting the formation of the membrane-attack complex (MAC or C5b-9) and reducing expression of the neutrophil activation marker CD11b. However, only compstatin and anti-factor D were able to inhibit the formation of the alternative-pathway C3 convertase [17]. Both compstatin and the C5a receptor (C5aR or CD88) antagonist 3D53 reduced leukotriene B₄ (LTB₄) production in this model, thus indicating that C5a-C5aR interactions play a role in the production of this eicosanoid [8]. The studies in Table 1 demonstrate the efficacy of compstatin in preventing complement activation and associated inflammatory responses in a variety of experimental settings. Thus far, no cytotoxicity or deleterious effects on hemodynamic and cardiovascular parameters, including the clotting cascade, have been observed at any of the doses tested [18,19]. These findings bode well for compstatin's potential therapeutic applications, but more stringent pharmacological testing needs to be conducted.

**Novel analogs of compstatin displaying improved activity**

Since its discovery, compstatin has undergone a series of optimizations that have led to various novel analogs with greatly improved activities (Table 2). The design of these novel analogs was based on structural analyses using NMR, alanine scans, phage display screenings and computational approaches. These strategies have been recently reviewed by Morikis et al [20•] and will therefore not be addressed in great detail in this review.

The first analog to display improved activity (a 3-fold increase over the original compstatin) was generated by acetylation of the N-terminus of the peptide, which resulted in the neutralization of the N-terminal charge and an enhanced stability [14,21]. Other alterations included replacing the bulky His at position 9 with the simpler Ala, which introduced more conformational freedom immediately outside the β-turn (Ac-H9A). A second phage display screen against C3, in which residues known to be important for compstatin’s activity were kept fixed, yielded a more active analog in which three residues were substituted (Ac-I1L/H9W/T13G). A study has determined the structures of Ac-I1L/H9W/T13G and Ac-compstatin using NMR [21]; both structures were similar to that of the original compstatin. The binding kinetics to C3 were similar for Ac-I1L/H9W/T13G and Ac-H9A, but differed from those of Ac-compstatin. Based on structural and kinetic data, results of this study suggest that the improved activity of the two analogs is the result of the compensatory effects of flexibility outside the β-turn, combined with tryptophan ring stacking in the presence of two tryptophans.
<table>
<thead>
<tr>
<th>Model</th>
<th>Clinical relevance</th>
<th>Setting</th>
<th>Inhibitor and dose</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte lysis (alternative pathway)</td>
<td>Complement activation</td>
<td>In vitro</td>
<td>Comstatin 12 μM</td>
<td>50% inhibition of hemolysis</td>
<td>[10,16]</td>
</tr>
<tr>
<td>Porcine-to-human kidney perfusion</td>
<td>Hyper-acute rejection in discordant kidney xenotransplantation</td>
<td>Ex vivo</td>
<td>Ac-comstatin 1 57 μM (perfusion)</td>
<td>Graft survival was prolonged. C3, fibrin and C5b-9 deposition on kidney tissue was reduced</td>
<td>[58]</td>
</tr>
<tr>
<td>Human serum-induced porcine endothelial cell activation</td>
<td>Xenotransplantation</td>
<td>In vitro</td>
<td>Ac-comstatin 10 to 240 μM</td>
<td>The upregulation of E-selectin on porcine endothelial cells was significantly reduced</td>
<td>[59]</td>
</tr>
<tr>
<td>Tubing loops</td>
<td>Extracorporeal circulation during cardiopulmonary bypass surgery</td>
<td>In vitro</td>
<td>Comstatin 250 μM, Ac-comstatin 100 μM, 3 and 16 μM, 250 μM, 100 μM, 12 mg/kg and 0.2 mg/min/kg (bolus followed by infusion)</td>
<td>Generation of C3a and sc5b-9 in fluid phase and deposition of C3/C3 fragments on polymer surface were inhibited. Monocyte and PMN activation (CR3 upregulation) and the binding of CD16+ cells to the polymer surface were prevented without affecting blood cell counts. Granulocyte-platelet formation was attenuated. The synthesis of LTB4 in whole blood was inhibited</td>
<td>[60] [17] [8]</td>
</tr>
<tr>
<td>Heparin-protease complex-induced complement activation</td>
<td>Cardiopulmonary bypass surgery (baboons)</td>
<td>In vivo</td>
<td>Ac-comstatin 12 mg/kg and 0.2 mg/min/kg (bolus followed by infusion)</td>
<td>The heparin-protease complex-induced rise in activated C3 fragments in blood was completely prevented. Blood cell counts and the coagulation pathway were not affected</td>
<td>[19]</td>
</tr>
<tr>
<td>Escherichia coli-induced complement activation in whole blood</td>
<td>Sepsis</td>
<td>In vitro</td>
<td>Ac-comstatin 50 μM</td>
<td>C3bc, C5a and sc5b-9 formation were inhibited. Oxidative burst of granulocytes and monocytes was reduced by at least 70%. IL-8 production was partially reduced</td>
<td>[45]</td>
</tr>
<tr>
<td>Polymer-bead assay</td>
<td>Implanted biomaterials</td>
<td>In vitro</td>
<td>Comstatin 116 μM</td>
<td>C5a generation in polymer-exposed human blood was inhibited. Polymer-induced CR3 upregulation by human neutrophils was prevented</td>
<td>[61]</td>
</tr>
<tr>
<td>CRP-substrate-induced complement activation</td>
<td>CRP-mediated inflammatory conditions</td>
<td>In vitro</td>
<td>Ac-comstatin 30 μM</td>
<td>C5b-9 formation and the deposition of complement fragments by CRP or IgG-activated cell lines were blocked</td>
<td>[62]</td>
</tr>
<tr>
<td>Polystyrene-induced complement activation</td>
<td>Biomaterials</td>
<td>In vitro</td>
<td>Ac-V4W/H9A 7 μM</td>
<td>The binding of C3 fragments to polystyrene was significantly reduced</td>
<td>[63]</td>
</tr>
</tbody>
</table>

1Ac-comstatin is a complement analog in which the N terminus is acetylated. 2Ac-V4W/H9A is a potent analog of complement in which Val is replaced with Trp and His with Ala. CR3 complement receptor 3 (CD11b/CD18 or Mac-1), CRP collagen-related peptide, IL-8 interleukin-8, LTB4 leukotriene B₄, PMN polymorphonuclear cells.

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Table 2. Some of the complement analogs obtained in recent years displaying increased inhibitor activity.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sequence</th>
<th>RA</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comstatin</td>
<td>B[CVQWDWGHHRCT]-NH₂</td>
<td>1</td>
<td>[10]</td>
</tr>
<tr>
<td>Ac-comstatin</td>
<td>Ac-[CVQWDWGHHRCT]-NH₂</td>
<td>3</td>
<td>[21]</td>
</tr>
<tr>
<td>Ac-H9A</td>
<td>Ac-[CVQWDWGARCT]-NH₂</td>
<td>4</td>
<td>[13,21]</td>
</tr>
<tr>
<td>Ac-11L/H9W/T13G</td>
<td>Ac-[CVQWDWGHHRCT]-NH₂</td>
<td>4</td>
<td>[21]</td>
</tr>
<tr>
<td>Ac-V4Y/H9A-NH₂</td>
<td>Ac-[CVQWDWGARCT]-NH₂</td>
<td>14</td>
<td>[22]</td>
</tr>
<tr>
<td>Ac-V4W-NH₂</td>
<td>Ac-[CVQWDWGHHRCT]-NH₂</td>
<td>24</td>
<td>A</td>
</tr>
<tr>
<td>Ac-V4W/H9A-NH₂</td>
<td>Ac-[CVQWDWGARCT]-NH₂</td>
<td>45</td>
<td>A</td>
</tr>
<tr>
<td>Ac-V4(2NaN)H9A-NH₂</td>
<td>Ac-[CVQWDWGARCT]-NH₂</td>
<td>99</td>
<td>A</td>
</tr>
</tbody>
</table>

The greatest improvements in compstatin's activity were obtained with computational combinatorial optimization [22,1]. This approach led to the design of various analogs displaying dramatically enhanced activity (Table 2). The positions most amenable to optimization were Val′ and His′, which flank both ends of the β-turn structure. An analog in which Val′ was replaced with Tyr, and His′ with Alα, was 14-fold more active than compstatin (Table 2) [22,2]. A further recent improvement involved the replacement of Val′ with Trp, another aromatic ring-containing residue, which resulted in a 45-fold higher activity [B Mallik, M Katragadda, L Spruce, C Carafides, GC Tsokos, D Morikis, JD Lambris, unpublished data]. Moreover, when this replacement approach was recently combined with a rational design strategy involving the incorporation of non-natural amino acids, analogs were obtained that displayed up to 99-fold higher inhibitory activity than that of the parent molecule [B Mallik, M Katragadda, L Spruce, C Carafides, GC Tsokos, D Morikis, JD Lambris, unpublished data].

Compstatin, like other small peptides, is very flexible in solution and can assume multiple interconverting conformations. Recent studies using the original NMR structures and molecular dynamics simulations have identified five of these interconverting conformers in compstatin [23,1]. Together with non-natural amino acid replacement experiments, the structures of these additional conformers will be used for further optimization studies to design analogs displaying even higher complement inhibitory activities.

**Antagonists of the anaphylatoxin receptors**

The anaphylatoxins C3a and C5a are small (approximately 10 kDa) cleavage fragments of C3 and C5, respectively, that are generated during complement activation (Figure 1). They are powerful mediators of inflammation and interact with their specific receptors, the C3a receptor (C3aR) and C5aR, with nanomolar affinities [24,25]. Both receptors are members of the G protein-coupled seven transmembrane receptor superfamily and are expressed by a large number of myeloid and non-myeloid cells.

C5a has been linked to a multitude of inflammatory diseases, such as rheumatoid arthritis, sepsis, asthma, adult respiratory distress syndrome and psoriasis, and has been a target for therapeutic intervention for almost two decades [26]. Several C5aR antagonists have been developed over the years, but only a few have proven useful in vivo studies. These include the polypeptide C5a analog C5aRAM and its dimer C5aRAD [27], a C5a mutant [28], a non-peptidic antagonist [29] and the cyclic peptide AcFlopDCaWR known as 3D53 [30]. 3D53 will be focused on in this review, since it currently shows the most promise for further development as a therapeutic agent.

C3a has also contributed to the pathogenesis of various conditions. Due to its effects on eosinophils and mast cells, C3a has been implicated in the pathogenesis of asthma and allergy [31]. However, it may also contribute to the development of sepsis and adult respiratory distress syndrome [24,32,33]. It has been less of a target for therapeutic intervention than C5a, perhaps because of its lower overall activity and the relatively late identification of the C3aR [34]. Over the past 12 years, several compounds displaying antagonist activity have been reported. These include a class of cyclized peptides [35], diiminosodiolne analogs [36], and a small non-peptidic compound N-[2,2-diphenylethoxy]acetyl]-L-arginine, also known as SB-290157 [37]. Only the diiminosodiolne and SB-290157 have been tested in vivo.

**3D53: An antagonist of the C5aR (CD88)**

Binding of C5a to its receptor involves three binding sites [38], however, only one, the eight-residue C-terminus domain of C5a, is responsible for C5aR activation [38,39]. Consequently, this region has been the basis for the development of potential C5aR agonists and antagonists. Structure-activity studies have yielded numerous receptor agonists, a partial agonist/antagonist, and the full antagonist NMe-Phe-Lys-Pro-D-Cha-Trp-D-Arg-CO2H or MeFkPdChaWR [39]. Similar to other hexapeptides, this peptide adopted a conformation in solution as determined by 1H-NMR spectroscopy [40,41]. In a recent study by March and colleagues [42,1], this conformation was shared by other C5aR antagonists as well as agonists (including the C-terminus of C5a) and may be a crucial determinant of receptor binding [42,1]. In an attempt to limit conformational flexibility and to increase the stability of the turn motif of the peptide, a cyclic derivative was produced [41]. This new compound, F[KPdChaWR], produced via backbone-to-side chain cyclization, was further optimized to yield the highly potent Ac-Phe-[orn-Pro-D-Cha-Trp-Arg] or AcFopDCaWR, which displayed a receptor affinity of approximately 300 nM and an antagonist potency of 20 nM (Figure 2) [30]. This cyclic peptide was later named 3D53 [43] and is one of the most potant C5aR antagonists available.

The antagonistic properties of 3D53 have been determined in a variety of in vitro assays, including inhibition assays of myeloperoxidase release from human polymorphonuclear cells (PMNs), C5a-mediated chemotaxis of human PMNs, cytokine release by human monocytes, C5a-induced human umbilical artery contractions and Escherichia coli-induced oxidative burst and phagocytosis [4,30,44,45]. In addition, 3D53 was highly receptor selective and did not interfere with the effects of C3a, interleukin (IL)-8, LT-B4, platelet-activating factor, or formyl-Met-Leu-Phe (fMLP) on PMN enzyme release [4]. In recent years, 3D53 and its non-acetylated variant, which is only slightly less active than 3D53 [30], have also been used in numerous short- and long-term animal models of inflammatory disease (Table 3). These studies have demonstrated that 3D53 is highly effective in reducing the pathogenesis of various immune-inflammatory diseases. This effectiveness combined with versatility in terms of route of administration, makes it an attractive drug candidate.

Pharmacokinetic analyses have shown that 3D53 appears in the plasma within 5 min of oral administration (3 mg/kg) to rats, with peak blood levels of approximately 0.3 μM being reached within 20 min. The plasma elimination half-life was approximately 70 min in this case [44]. Topical administration did not result in elevated plasma drug levels, suggesting that its mode of action is local only. Although the
Table 3. Animal models of inflammatory conditions in which the C5aR antagonist 3D53 and analogs have been tested.

<table>
<thead>
<tr>
<th>Model</th>
<th>Clinical relevance</th>
<th>Species</th>
<th>Inhibitor and dose</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>C5a- and endotoxin-induced neuroinflammia</td>
<td>Sepsis</td>
<td>Rat</td>
<td>F[OPtChaWR] 0.3 to 3 mg/kg iv</td>
<td>Inhibition of C5a- and LPS-induced neuroinflammia</td>
<td>[64, 65]</td>
</tr>
<tr>
<td>Dermal Arthus reaction</td>
<td>Dermal inflammation</td>
<td>Rat</td>
<td>AcF[OPtChaWR] 1 mg/kg iv, 1 to 10 mg/kg po or 0.4 mg/site topically</td>
<td>Inhibition of immune complex-mediated dermal vascular leakage and systemic cytokine production</td>
<td>[65, 44]</td>
</tr>
<tr>
<td>Peritoneal Arthus reaction</td>
<td>Peritoneal inflammation</td>
<td>Rat</td>
<td>AcF[OPtChaWR] 1 to 10 mg/kg po</td>
<td>Inhibition of immune complex-induced vascular leakage, PMN infiltration, TNFα and IL-6 production</td>
<td>[66]</td>
</tr>
<tr>
<td>Immune complex-induced monoarticular arthritis</td>
<td>Immune-mediated monoarticular arthritis</td>
<td>Rat</td>
<td>AcF[OPtChaWR] 1 and 3 mg/kg po</td>
<td>Significant reduction in knee swelling, gait disturbance, lavaged joint cell numbers, histopathology and cytokine levels</td>
<td>[67]</td>
</tr>
<tr>
<td>Intestinal I/R injury</td>
<td>Intestinal I/R injury</td>
<td>Rat</td>
<td>AcF[OPtChaWR] 1 mg/kg iv or 10 mg/kg po</td>
<td>Inhibition of neuropenia, intestinal edema, plasma levels of TNFα, haptoglobin and AST and a reduction in mucosal layer damage</td>
<td>[68]</td>
</tr>
<tr>
<td>Intestinal I/R injury</td>
<td>Intestinal I/R injury</td>
<td>Mouse</td>
<td>AcF[OPtChaWR] 1 or 25 μg/animal iv</td>
<td>Limited local injury and prevented lung injury, Reduction of PMN infiltration and I/R-induced PMN oxygen radical production and adhesion molecule expression</td>
<td>[69]</td>
</tr>
<tr>
<td>Hepatic I/R injury</td>
<td>Hepatic I/R injury</td>
<td>Rat</td>
<td>AcF[OPtChaWR] 1 mg/kg iv or 10 mg/kg po and sc</td>
<td>Reduction in hepatic I/R-induced mortality and an attenuation of the rise in liver enzymes, TNFα, MPO activity and PMN infiltration, and liver histopathology</td>
<td>[70]</td>
</tr>
<tr>
<td>Renal I/R injury</td>
<td>Renal I/R injury</td>
<td>Rat</td>
<td>AcF[OPtChaWR] 1 mg/g iv or 10 mg/kg po</td>
<td>Inhibition of I/R-induced hematuria, vascular leakage, tissue TNFα and MPO levels, and serum AST and creatine levels</td>
<td>[71]</td>
</tr>
<tr>
<td>Acute limb I/R injury</td>
<td>Acute limb I/R injury</td>
<td>Rat</td>
<td>AcF[OPtChaWR] 1 mg/g iv or 10 mg/kg po</td>
<td>Reduction of a multitude of disease markers (e.g., serum creatine, TNFβ, AST, BUN, PMN) of local and distant tissue injury</td>
<td>[72]</td>
</tr>
<tr>
<td>Cecal ligation/puncture</td>
<td>Sepsis</td>
<td>Mouse</td>
<td>F[OPtChaWR] 1 and 3 mg/kg iv</td>
<td>Improved survival rates</td>
<td>[47]</td>
</tr>
<tr>
<td>Immune complex-induced lung injury</td>
<td>Inflammatory lung injury</td>
<td>Mouse</td>
<td>F[OPtChaWR] 1 and 3 mg/kg iv</td>
<td>Reduction in pulmonary permeability</td>
<td>[47]</td>
</tr>
<tr>
<td>Induced hemorrhagic shock followed by I/R</td>
<td>Ruptured abdominal aortic aneurysm</td>
<td>Rat</td>
<td>AcF[OPtChaWR] 2 mg/kg iv</td>
<td>Reduction in pulmonary and intestinal permeability and pulmonary MPO activity. Resulted in a better maintenance of blood pressure</td>
<td>[73]</td>
</tr>
<tr>
<td>TNBS-induced colitis</td>
<td>Ulcerative colitis</td>
<td>Rat</td>
<td>AcF[OPtChaWR] 10 mg/kg po followed by 0.3 mg/kg/day sc</td>
<td>Reduction in colon edema, mortality, colon MPO and TNFα levels, and improved macroscopic scores. Greater food intake and weight gains</td>
<td>[74]</td>
</tr>
<tr>
<td>Influenza virus challenge</td>
<td>Influenza</td>
<td>Mouse</td>
<td>AcF[OPtChaWR] 1 mg/kg ip every other day</td>
<td>Reduced frequency and absolute numbers of flu-specific CD8+ T-cells and attenuated antiviral cytolytic activity in the lungs</td>
<td>[75]</td>
</tr>
<tr>
<td>Hepatectomy-induced liver injury</td>
<td>Liver regeneration after partial hepatectomy</td>
<td>Mouse</td>
<td>AcF[OPtChaWR] 1 mg/kg ip</td>
<td>Inhibited hepatocyte DNA synthesis and abrogated liver regeneration. Significantly elevated mortality by blocking the actions of C5a, which is a critical mediator of hepatocyte differentiation</td>
<td>[76]</td>
</tr>
<tr>
<td>Antiphospholipid Ig-induced fetal loss</td>
<td>Antiphospholipid syndrome</td>
<td>Mouse</td>
<td>AcF[OPtChaWR] 50 μg/mouse ip</td>
<td>Prevented pregnancy complications and fetal loss</td>
<td>[77]</td>
</tr>
<tr>
<td>Antigen-induced airway response</td>
<td>Asthma</td>
<td>Rat</td>
<td>NMeFKPotChaWr 1 mg/kg iv</td>
<td>Reduction in bronchial eosinophil and neutrophil infiltration, and the expression of cytokines of lung tissue</td>
<td>[78]</td>
</tr>
<tr>
<td>Antibody-induced thrombotic glomerulonephritis</td>
<td>Thrombotic glomerulonephritis</td>
<td>Rat</td>
<td>NMeFKPotChaWr 1 mg/kg iv</td>
<td>Inhibition of leukocyte accumulation and thrombus formation in glomeruli</td>
<td>[79]</td>
</tr>
<tr>
<td>Endotoxin-induced lethal shock</td>
<td>Sepsis</td>
<td>Rat</td>
<td>NMeFKPotChaWr 3 mg/kg iv</td>
<td>Prevented the lethal reaction in rats that had been pretreated with LPS</td>
<td>[80]</td>
</tr>
</tbody>
</table>

AST aspartate aminotransferase, BUN blood urea nitrogen, Ig antibody, IL-6 interleukin 6, iv intraperitoneally, I/R ischemia reperfusion, iv intravenously, LPS lipoplysaccharide, MPO myeloperoxidase, PMN polymorphonuclear cell, po orally, sc subcutaneously, TNBS trinitrobenzene sulfonic acid, TNF tumor necrosis factor.
affinity of 3D53 and its non-acetylated variant were reported to be lower in mice than in rats or humans [46], further studies have shown that the non-acetylated version of 3D53 binds to isolated mouse neutrophils with a \( K_d \) value of 30 nM (mouse 3a binds with a \( K_d \) value of 0.3 nM) and inhibited mouse 3a-induced chemotaxis with an \( IC_{50} \) value of 0.5 nM [47]. Clearance of this antagonist after iv administration to mice, showed a multiphasic profile, with an early rapid distribution phase (< 3 h) followed by a slower clearance phase (24 to 72 h).

The linear antagonist MeFkPdChaWr bound human PMNs and umbilical artery membranes with different affinities [48]. A similar tissue selectivity had previously been reported for C5a agonists [49,50]. The reason for this selectivity is still unclear, as only one C5aR isotype has been identified, but it may be related to tissue-specific differences in the coupling of the C5aR to various G proteins or to the differential expression of another C5a receptor, C5L2. Although the biological role of C5L2 is still enigmatic [51,52], it appears to be differentially expressed on different cell types and may affect the bioavailability of C5a and participate in some C5a-mediated activities [53••]. A recent study by Otto and co-workers [53••] demonstrated little or no affinity of 3D53 for C5L2. However, these authors have generated a novel C5a mutant that exhibits affinity for both CDB8 and C5L2 [53••]. The use of this peptide may shed some light on the involvement of C5L2 in inflammatory processes.

**Novel analogs of 3D53 displaying improved affinity but not activity**

Structure-activity relationships of 60 novel cyclic 3D53 analogs have recently been evaluated by March and colleagues [42••], and residues critical for receptor affinity as well as antagonist activity have been identified. This study revealed that the Phe, Pro, d-Cha and Arg residues are important for receptor affinity, but Trp alone seems to dictate the agonistic or antagonistic behavior of the compound. The acetyl group, on the other hand, could be widely varied without any loss of activity. Based on the solution structures of several of these compounds, the authors suggested that the side chains of the individual residues are important for the ligand fit to the receptor, rather than contributing to the peptide’s macrocyclic shape. Competition studies between the several agonists and antagonists have indicated that the binding sites for these peptides on intact human PMNs are similar or overlapping [42••]. This study also described 20 additional compounds with receptor affinities of < 1 \( \mu M \), six of which exhibited a higher affinity for the C5aR compared with 3D53, but none of them displayed improved antagonist activity. However, these compounds may serve as powerful probes to increase our understanding of C5a-C5aR interactions.

**SB-290157: A synthetic antagonist of the C3a receptor**

SB-290157 was identified from high-throughput screening and further chemically optimized as a selective C3aR antagonist by Ames and co-workers at SmithKline Beecham, now GlaxoSmithKline plc, in 2001 (Figure 2) [37]. Using C3aR transfected cells and a competitive C3a binding assay, this compound bound to the C3aR with a relative affinity of 200 nM. It was evaluated for inhibitory activity using assays of C3a-induced Ca\(^{2+}\) mobilization, mast cell chemotaxis, ATP release from guinea pig platelets and spasmodogenic activity of rat caudal artery, and was effective in concentrations ranging from 30 nM to 5 \( \mu M \).

SB-290157 is a selective antagonist, as it did not affect Ca\(^{2+}\) mobilization by other compounds, such as C5a, FMLP or LTb4 [37]. Interestingly, this inhibitor was a potent antagonist for the human, mouse, rat and guinea pig C3aRs, despite the fact that the C3aR sequences of these species display low overall identity (60 to 65%).

This C3aR antagonist was first tested in vivo using a guinea pig lipopolysaccharide-induced airway neutrophilia model, in which it reduced neutrophil accumulation, probably via indirect mechanisms, at a dose of 30 mg/kg ip. In a rat model of adjuvant-induced arthritis, it reduced paw edema at a dose of 30 mg/kg ip twice daily [37]. In addition, it was recently used in a mouse study addressing the role of C3a and the C3aR in hematopoietic stem cell mobilization, and accelerated and enhanced granulocyte colony-stimulating factor (G-CSF)-induced stem cell mobilization in a dose-dependent manner (0.1 to 0.5 \( \mu g/\text{mouse} \) ip) [54•]. SB-290157 was ineffective at enhancing the effects of G-CSF in C3- and C3aR-deficient mice, thus demonstrating its selectivity in blocking C3a-C3aR interactions. Moreover, this study assessed the effects of SB-290157 on hematopoietic stem cell proliferation, survival and several intracellular signaling mechanisms, and demonstrated no toxic side effects of the compound.

Recently, Taylor and co-workers tested this inhibitor in a rat model of intestinal ischemia/reperfusion [55••] and compared its anti-inflammatory effects with that of the C5aR antagonist 3D53. When administered intravenously 45 min prior to reperfusion, 0.3 and 1.0 mg/kg of SB-290157 significantly reduced intestinal edema, while 1 mg/kg also attenuated the ischemia/reperfusion-mediated rise in serum amino alanine transferase levels and ameliorated mucosal damage. These authors found no additive beneficial effects of a combined SB-290157 and 3D53 treatment on any of the parameters tested.

This group also provided additional information on the pharmacology and pharmacokinetics of SB-290157 in rats. Intravenous administration of 1.0 mg/kg of SB-290157 led to a rapid distribution phase with a half-life of 7.6 min, followed by a slower clearance phase with a half-life of 195 min. Surprisingly, infusion of the inhibitor 2 h prior to ischemia/reperfusion, which was an effective time course in blocking the actions of C3a, did not provide protection against ischemia/reperfusion injury. This observation together with the fact that SB-290157 induced rapid neutropenia combined with hypertension, effects that have also been observed with C3a agonists, led the authors to suggest that SB-290157 may not be receptor selective and could exert some of its effects via other yet unidentified receptors.
Clearly, further studies are needed to elucidate the pharmacology of SB-290157, as well as its affinity for other receptors. An additional complication, however, is that receptors other than the C3aR may mediate some of the actions of C3a, since not all biological activity of C3a and its desarginated derivative can be explained based on the existence of one receptor only [6,56]. C3a already interacts with at least one other receptor, the IgE receptor FcεRI, on mucosal mast cells [57].

Conclusion
Significant progress has been made in recent years in understanding the role of complement in inflammatory processes that contribute to the pathogenesis of a variety of human conditions and diseases. The small-molecule synthetic inhibitors described in this review have contributed significantly to this increased knowledge and have paved the way for further development of this class of inhibitors for potential therapeutic purposes. The fact that complement is an important regulator of cytokine and eicosanoid release in various inflammatory settings, suggests that complement inhibitors may be used as an alternative to, or in combination with, anticytokine or anti-eicosanoid therapies. The recently discovered, highly potent analogs of compstatin are currently being tested in a non-human primate model and may yield a suitable candidate for the treatment of various acute inflammatory conditions requiring complete blockade of the activities of complement. Antagonists of the C5aR and, perhaps also the C3aR, may find application in the treatment of more chronic conditions, since their actions target only one specific interaction (C5a-C5aR or C3a-C3aR) and they leave other complement-effector mechanisms that are important for immune protection intact (eg, opsonizing fragments and MAC deposition). The fact that at least one of these compounds, the C5aR antagonist, is orally and topically active underscores the potential therapeutic value of this compound.

Although peptides are not always popular compounds for therapeutic development, the cyclic compounds described in this review have been stable in various animal models. A recent study describing an inexpensive production method for medium- to large-scale synthesis of cyclic peptides, like the C5aR antagonist, may be applicable to the mass production of these compounds for clinical testing [43]. In addition, optimization of small-molecule inhibitors is an ongoing process and these compounds can potentially serve as a template for the development of non-peptidic complement inhibitors.

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References

In this paper, various complement inhibitors, including compstatin, are used to identify the complement components and the inflammatory processes involved in bio-incompatibility.


This is a comprehensive mini-review of the various optimization strategies used over the years to generate compstatin analogs with improved activity.


• This paper describes a two-stage computational protein design method for the selection, ranking and validation of stability and specificity of sequences leading to improved complement analogs.


• This paper reports the dynamic character of complement with the identification of interconverting conformations, which will form the basis for further optimization studies.


25. Wetsel RA, Kildsgaard J, Haviland DL: Complement anaphylatoxins (C3a, C4a, C5a) and their receptors (C3AR, C5aR/CDB8) as therapeutic targets in inflammation. In: Therapeutic Interventions in the Complement System, Lamberis JD, Holers MV (Eds), Humana Press, Totowa, NJ, USA (2000):113-153.


• Using structure-activity relationships and computer modeling comparisons, the authors of this paper identified the residues and structural characteristics important for 3D25-C5aR binding interactions and antagonist activities.


• This paper stresses the enzymic role of the second C5a receptor, C5L2.


- Using site-directed mutagenesis, these authors identified residue 69 of a C5a mutant to be important for C5aR antagonism. An analog of this compound displaying dual affinity for the C5aR (CD88) and C5L2 is also described.


- This study is the first to demonstrate the efficacy of the C3AR antagonist SB-290157 in blocking a recently described novel activity of the C3a anaphylatoxin.


- In this study the C3aR and C5aR antagonists are used together for the first time to dissect the involvement of C3aR and C5aR in ischaemia/reperfusion injury in rats. Some novel pharmacological and pharmacokinetic information on SB-290157 is also provided.


