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Review Article

Complement factor 5a as a therapeutic target

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Abstract

The complement system is an innate immune defense mechanism that protects the host from infection and injury. Complement activation results in the formation of anaphylatoxins, including the biologically active protein C5a. This anaphylatoxin is a potent chemotactic agent for immune and inflammatory cells and induces cell activation. In situations of excessive or uncontrolled complement activation, the overproduction of C5a can cause deleterious effects to the host, and this process is implicated in the pathogenesis of numerous immunoinflammatory disease states, including rheumatoid arthritis, psoriasis, inflammatory bowel disease, ischemia-reperfusion injuries and others. The presence of C5a in a wide variety of conditions has prompted many groups to examine the potential of inhibiting this complement activation product, with the aim of controlling these diseases and reducing the pathologic process. However, to date there is no clinically available specific C5a inhibitor and development of this new drug class is still in a relatively early stage, although limited phase I and phase II human clinical trials have been undertaken in the last few years with selected agents. In this review, examination of the current evidence supporting a specific role of C5a in selected disease states and an overview of potential therapeutic C5a inhibitors will enable the critical evaluation of the potential for C5a as a therapeutic target.

Introduction

The complement system

The complement system consists of over 30 components that play an essential role in host defense responses to infection and injury. This complement cascade can be initiated via either the classical pathway, which is initiated by immune complex formation, or the antibody-independent mannan-binding lectin (MBL) pathway or the alternate pathway, initiated by contact with foreign cell surface components (1). All three pathways converge at C3 and terminate in formation of the membrane attack complex (MAC; C5b-9), which is involved in bacterial cell lysis (1).

In addition, complement cascade activation results in formation of a group of biologically active peptides (74-77 amino acids) termed the anaphylatoxins, C5a, C4a and C3a. The anaphylatoxins are released from the alpha chains of complement components C5, C4 and C3, respectively, by convertase-mediated proteolytic cleavage of an arginyl-X bond in each parent peptide (1). C3a and C5a are produced upon complement activation via all three pathways and C4a is produced upon activation of the classical and MBL pathways (2). All three anaphylatoxins are highly cationic, stabilized by three conserved disulfide linkages and have very similar tertiary structures composed of an alpha-helical bundle core and carboxy-terminal (*C*-terminal) region. While the role of C4a remains ill-defined, C5a and C3a are known to be involved in the modulation of immune responses and inflammatory processes (2).

C5a and the C5a receptor

C5a is a glycoprotein composed of 74 amino acids folded into a four alpha-helical bundle core (residues 1-63) of approximately anti-parallel topology, stabilized by three disulfide bonds (between Cys^{21}-Cys^{47}, Cys^{22}-Cys^{54} and Cys^{34}-Cys^{55}) (3). Six *C*-terminal residues of C5a assume an elongated 1.5 turn helix (4). C5a and its degradation product C5adesArg, which is produced through carboxypeptidase-*N*-mediated cleavage of the C5a C-terminal arginine residue, reportedly interact with two cell surface receptors, the C5a receptor (C5aR) and a recently discovered second receptor, termed C5L2.

The human C5aR is a 350-residue, seven transmembrane-spanning, G-protein-coupled receptor which is responsible for the defined activities of C5a in biological processes. A two-site binding model has been devised to describe the interaction between C5a and the C5aR. The first "recognition" binding site is hypothesized to involve an interaction between the acidic Asp residues of the amino-terminal (*N*-terminal) of the C5aR and residues of the cationic, helical core of C5a, namely Lys^{19}, Lys^{20} and Arg^{46} from the *N*-terminal and loop 3 of C5a. The second "activation" binding site lies within the pore formed by the transmembrane domains of the receptor, including Asp^{82}, Glu^{199} and Arg^{206}, and interacts with the *C*-terminal residues of C5a, which is responsible for C5aR activation (5).

The newly defined C5L2 receptor is composed of 337 amino acids, contains seven transmembrane domains and reportedly can form high affinity interactions with both C5a and C5adesArg (6). The C5L2 receptor is co-expressed with the C5a receptor on many cells, including polymorphonuclear neutrophils, the expression of which has been shown to be upregulated in an immunoinflammatory response (7). However, as a result of an Arg to Lys mutation in the DRY sequence (in the third transmembrane region) of C5L2, this receptor is unable to couple to G proteins (8). The C5L2 receptor may serve to balance C5a-induced responses given that anti-C5L2 antibody administration to septic mice induced an increase in IL-6 when compared with untreated animals (7). Moreover, an enhanced *in vitro* chemotactic response of $C5L2^{-/-}$ murine bone marrow cells to C5a, coupled with amplified neutrophil influx in response to C5a, further support an antiinflammatory role for C5L2 (9). In some situations, the C5L2 receptor may act as a nonsignaling decoy receptor, binding surplus C5a and limiting the proinflammatory actions of C5a at the receptor (8). Alternatively, C5L2 may interact with the C5aR intracellularly through complex formation, or it may signal through a non-G-protein-coupled pathway. The definitive biological role of C5L2 and the mechanism through which C5a/C5adesArg interacts with C5L2 remain to be fully illuminated.

The proinflammatory and immunoregulatory activities of C5a have been extensively characterized (see 2 for review). C5a induces smooth muscle contraction via the release of secondary mediators and chemotaxis of inflammatory cells by a direct action (2). Chemotaxis of these cells is accompanied by cell adhesion and diapedesis mediated by C5a-induced transient upregulation of CD11/CD18 integrins on inflammatory cells and P-selectin molecules on endothelial cells (1). Furthermore, C5a is responsible for induction of bactericidal lysosomal enzymes and reactive oxygen species from PMNs and proinflammatory and immunomodulatory cytokines from monocytes and macrophages (2). C5a-induced immune enhancement is mediated through chemotaxis of antigen-presenting cells, augmentation of antigen- and mitogen-induced antibody production, antigen- and alloantigen-mediated T-cell proliferation and major histocompatibility complex restricted T-cell proliferation, as well as C5a-induced release of IL-1 from macrophages (10). Moreover, *in vivo*, C5a causes numerous effects, and infusion to rats induces acute neutropenia, systemic hypotension and elevation of cytokines including TNF-α and IL-6 (11, 12). These varied proinflammatory effects of C5a are suggestive of a key role in inflammation and inflammatory diseases.

C5a and disease

As described above, the generation of C5a in the body produces a powerful immune and inflammatory response when it binds to the C5aR. These innate responses have evolved to remove and destroy invading organisms and foreign material, and ultimately protect the host from infection. However, the powerful actions of C5a can have deleterious effects on the host, particularly in situations where complement is inappropriately or excessively activated or under-regulated (13). In these situations, the abnormal and chronic production of C5a, which

Table I: Diseases in which C5a is believed to be involved in the pathogenesis.

Disorder/Disease	Ref.
Acute brain trauma	97
Acute respiratory distress syndrome	101
Atherosclerosis	102
Burn injuries	103
Cardiopulmonary bypass	104
Glomerulonephritis	105
Hemorrhagic shock	93
Inflammatory bowel disease	51
Ischemia-reperfusion injuries	53
Liver fibrosis	94
Macular degeneration	106
Neurodegenerative diseases	98, 107
Pseudoallergic reactions	108
Psoriasis	32, 109
Recurrent pregnancy loss	96
Rheumatoid arthritis	21
Sepsis	91
Systemic lupus erythematosus	95

is thought to contribute to the pathogenesis of numerous disease states, can lead to devastating effects to the body. A list of diseases where C5a is thought to contribute in the pathogenesis is shown in Table I. This list is by no means comprehensive, and the precise role of C5a in these diseases is constantly being refined as more studies are conducted. The evidence for C5a contributing to a few of these diseases is described in more detail below.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a debilitating, chronic autoimmune condition that affects approximately 1-2% of the population (14). The disease is primarily characterized by a severe and destructive immunoinflammatory response in the small peripheral joints, resulting in severe joint swelling, pain and ultimately joint deformity (15). The precise etiology of RA remains unknown, but genetic and environmental factors influence susceptibility to the disease by affecting the reactivity of the immune system (14).

An overview of the general pathogenesis of RA is as follows: immune complexes form following the binding of an unknown antigen to immunoglobulin rheumatic factors (IgM or IgG) deposited in the joint. This initiates the inflammatory response through local activation of the classical complement cascade and subsequent recruitment and activation of immune and inflammatory cells. These activated cells then release a host of chemical mediators that lead to the clinical manifestations of RA (15).

Given that complement activation products are elevated, both in the circulation and the joint, in patients with RA (16, 17), the production of C5a is inevitable. Indeed, C5a has been detected in the synovial fluids and plasma of RA patients (18). A role for C5a in RA has also been shown through the demonstration of elevated levels of C5aR on synoviocytes isolated from RA patients (19).

The effect of specifically inhibiting C5a and/or C5aRs has been demonstrated in several animal models of RA with relative success. Using C5aR knockout mice, Grant and colleagues demonstrated that the C5aR is essential to produce pathology in a model of collagen-induced arthritis (20). C5aR-deficient mice had reduced paw swelling, inflammatory cell infiltration, inflammatory cytokines and histopathology compared to the C5aR-sufficient animals (20). Interestingly, in the same study, the authors found no effect from the deletion of C3aRs, indicating that C3a is not involved in the pathology of arthritis in this model (20). Our group has also shown the deleterious effects of C5a in a model of experimental arthritis by using a specific, potent C5aR antagonist developed in our laboratories, PMX53 (AcF-[OP(D-Cha)WR], Promics Ltd.). This cyclic peptide antagonist was utilized in a model of antigen-induced arthritis in rats. Rats treated with the antagonist had reduced joint swelling and associated gait disturbances, inflammatory cell influx, inflammatory cytokines and histopathology (21). The results were similar to the results of Grant and co-workers except that C5aR antagonism did not provide a complete inhibition of arthritis, as it did in the C5aR-deficient mice (20). Another study conducted using the linear C5aR antagonist MeFKP(D-Cha)WR failed to demonstrate an effective inhibition of disease progression in an acute MAC-dependent anti-CD59 antibody-induced arthritis model (22). Several reasons for the apparent lack of C5a involvement in this model include the impaired clearance of MAC rather than activation of the complement cascade as the instigator of pathology, the failure of pathology to be affected by complement depletion, and the resolution of the lesion within 3 days (23). In a model of collagen-induced arthritis performed in cynomologus monkeys, animals treated with the C5a antagonist W-54011 (Mitsubishi Pharma Corp.) for 15 days had a significant reduction in joint swelling and radiographic scores (24). Another recent study conducted in collagen-induced arthritic mice vaccinated with the anti-C5/C5a immunotherapy MBP-C5a (Resistentia Pharmaceuticals AB) has also demonstrated the positive effects of inhibiting C5a (25).

Based on the weight of evidence indicating a deleterious role for C5a in the pathology of RA, several clinical studies have been performed to date to determine the effect of blocking C5a in patients with RA. Neurogen Corp. has conducted a phase IIa clinical trial with its non-peptide C5aR antagonist (NGD-2000-1) in 49 patients with mild to moderate RA (26). In this trial, patients were dosed orally twice daily for 14 days with either a placebo or various doses of NGD-2000-1. The trial failed to meet the primary endpoint of a change in C-reactive protein levels, but significant reductions were seen in the highest dose group (100 mg b.i.d.) in several of the secondary endpoints, including ACR20 scores (20% improvement in swollen and tender joint numbers and 3/5 assessments of disease) (26). Promics Ltd. has also conducted a phase Ib/IIa clinical trial in patients with mild to moderate RA using its cyclic peptide C5aR antagonist PMX53 (27). This trial involved 21 patients, all of whom were concurrently treated with methotrexate, dosed orally once daily for 28 days with either a placebo or 8 mg/kg PMX53 (28). The trial's primary endpoint of safety and tolerability was successfully completed, and there were trends towards efficacy in several disease parameters (27). However, there were no clear indications of significant reductions in disease parameters (28). The trials conducted by Neurogen Corp. and Promics Ltd. involved a relatively small number of patients, and were run over a limited time period (14 and 28 days, respectively). Given this, it is not entirely surprising that there were no major reductions in disease pathology.

Alexion Pharmaceuticals has developed a humanized antibody to the complement factor 5 (C5) which prevents the production of C5a, C5b and the terminal MAC (29). A subcutaneous formulation of this antibody (eculizumab) was tested in a more extensive phase IIb clinical trial in RA patients, involving 350 patients over 6 months (29). This trial demonstrated that there was a significant improvement in patients' ACR20 scores and a significant

reduction in the erythrocyte sedimentation rate (ESR) at 6 months (29). Although the therapeutic effects of eculizumab cannot be differentiated between inhibition of C5a or the MAC, the results from this trial suggest that inhibiting C5a could be an effective therapy in RA. At present, however, assessment of the potential therapeutic effects of drugs selectively blocking C5a in RA requires more extensive and longer term clinical trials to be conducted using specific C5a inhibitors.

Psoriasis

Psoriasis is a chronic and recurrent inflammatory disease in which silvery scales or plaques appear on the skin that are red and inflamed underneath, resulting in considerable discomfort and pain (30). Complement activation and neutrophil and other inflammatory cell infiltration participate in many of the inflammatory events seen in psoriasis (30, 31). Several studies have demonstrated that C5a and its degradation product, C5adesArg, are highly elevated in scale extracts from patients with psoriasis (32, 33). However, the circulating levels of C5a in psoriasis patients are not yet fully defined (34, 35). Intradermal injection of C5a also induces an inflammatory cutaneous response, with associated neutrophil infiltration, edema and erythema (36, 37), indicating that C5a is a proinflammatory agent in human dermis.

Although there are no particularly good animal models of psoriasis, models of contact senstitivity, delayed-type hypersensitivity and other models of dermal inflammation share some of the pathological features of psoriasis, and can be used to explore the pathogenesis of the disease (38). In models of contact sensitivity, C5a has been shown to play a major role in the development of the disease through the use of C5aR knockout mice and C5a neutralizing antibodies (39). C5a has also been shown to be important in the mediation of delayed-type hypersensitivity reactions through the use of the C5aR antagonist L-156602 (40); however, this compound may act through other mechanisms (41). The selective C5aR antagonist PMX53 is able to reduce or block markers of dermal immunoinflammation in the rat dermal Arthus reaction (42) and a mouse model of contact hypersensitivity (43). PMX53 as a topical formulation has also been examined for efficacy in a small, uncontrolled, open-label clinical trial in patients with mild psoriasis. This trial involved 10 patients with active psoriasis, and results indicated that 90% of patients showed an improvement in their psoriatic lesion score (LPSI) following application of the compound to specific psoriatic lesions (44). Taken together, these results suggest a potential clinical role for C5a in inflammatory skin conditions such as psoriasis.

Inflammatory bowel disease

Ulcerative colitis and Crohn's disease are chronic inflammatory bowel disorders that fall under the banner of inflammatory bowel disease (IBD). IBD is characterized by spontaneously occurring, chronic relapsing bowel inflammation of unknown origin (45). The pathogenesis of IBD is thought to involve either a destructive autoimmune response directed toward endogenous antigens in the bowel of patients, an overactive immune and inflammatory response to the bowel following an infection with a pathogenic organism, or a deregulated or abnormal immune response to commensal bacteria (45, 46).

The role of C5a and the complement system in IBD has not been extensively studied. The complement system has been suggested to be activated in patients with IBD and plays a role in the disease pathogenesis (47, 48), but evidence for this has been relatively limited. Activated complement products are found at the luminal face of surface epithelial cells, as well as in the muscularis mucosa and submucosal blood vessels in IBD patients (49, 50). However, the contribution of these complement activation products in creating bowel damage is still unclear.

Our laboratory has provided the first evidence of a direct role for C5a in a model of experimentally induced colitis in rats. Administration of PMX53 reduced mortality and significantly improved macroscopic scores, colon edema, colon myeloperoxidase levels, reduced concentrations of TNF-α in the colon and serum, and increased food intake in diseased rats (51). These results support the view that C5a may play a deleterious role in IBD. Clinical studies are now required to ascertain whether inhibiting C5a may provide a therapeutic effect in patients with IBD.

Ischemia-reperfusion injuries

The re-establishment of blood flow to previously ischemic organs and tissues leads to ischemia-reperfusion (I/R) injuries. These injuries are characterized by local inflammation leading to tissue damage, as well as systemic complications as a result of the "reperfusion syndrome" (52). Reperfusion of the ischemic tissue, although necessary for its ultimate survival, triggers the release of numerous factors that exacerbate the injury. Neutrophil infiltration and proinflammatory cytokine production are key events in the pathogenesis of these diseases, and the involvement of the complement system in this process has long been recognized as an important mediator of tissue injury following I/R (53). Complement is activated in the ischemic tissue, resulting in subsequent generation of C5a, which may promote local and remote tissue injury (52). I/R injuries can occur in virtually all sites within the body. Following is a brief discussion of the evidence for C5a as a pathogenic factor in I/R injuries to the liver, kidney, gut, heart, skeletal muscle and brain.

In the case of I/R injury to the liver, the role of C5a is not yet completely understood. The serum complement system is activated and C5a generated following liver transplantation and subsequent reperfusion in humans (54). Our group has recently investigated the protective effects of administering PMX53 in an animal model of hepatic I/R (55). In this study, we found that blocking C5aRs was effective at reducing numerous parameters of liver injury following hepatic I/R (55). The inhibition was

not complete, however, particularly for the histopathological picture, indicating that C5a inhibition alone is not sufficient to completely abrogate hepatic I/R injury. It remains to be seen whether blocking C5a in a clinical situation of hepatic I/R will provide a therapeutic effect. The role of C5a in the liver is complex, for it has also been shown that C5a is a critical factor involved in regeneration of the liver following partial hepatectomy (56). This nuancing of the role of C5a in the liver may require judicious use of complement-modulating agents in particular clinical situations.

The complement system as a whole has been shown to be involved in the pathogenesis of renal I/R through several animal models (57, 58), although not all models have strongly implicated complement (59). Zhou and colleagues argued that C5a did not play a role in renal I/R injuries in their studies using C6-deficient mice, which were additionally treated with anti-C5 antibodies (57). They found that blocking the formation of C5a with this antibody provided no additional therapeutic effects in the C6-deficient mice, with the conclusion that C5a production was therefore not causing tissue injury (57). Studies by our group, however, using PMX53 have shown that blocking the activity of C5a ameliorates numerous parameters of tissue injury in a model of renal I/R in rats (60). A separate group has also shown that specific inhibition of C5a using a C5aR antagonist selected from phage libraries (ΔpIII-A8) is able to reduce the loss of renal function following renal I/R in mice, independent of neutrophils (61). Despite these studies using specific C5a inhibitors, debate remains regarding the role of C5a in inducing injury in renal I/R and the relative roles of the other complement factors (57). It would appear that for this condition, C5a alone is not the principal complement activation product critical for renal I/R pathology. Clinical studies with complement inhibitors, which would further elucidate the role of C5a, have yet to be performed for this I/R condition. It could be hypothesized that species and organ differences may account for these conflicting reports on complement and C5a in renal I/R, and definitive studies will require administration of complement inhibitors in human clinical trials.

Intestinal I/R injury can result in severe systemic complications, and the role of complement in these pathologies has been well documented (62). Complement activation and generation of plasma C5a is seen in canines with bowel I/R (63). Numerous studies have also shown the importance of C5a in various animal models of intestinal I/R. Studies by Heller and co-workers showed that the administration of the proteinaceous C5aR antagonist, ΔpIII-A8, reduced local and remote tissue injury caused by intestinal I/R in mice (64). Work by Austen and colleagues, however, suggested that the MAC is more important in inducing tissue injury following intestinal I/R in mice (65), although specific C5a inhibitors were not used in this study. Our group examined the protective effects of PMX53 in a rat model of intestinal I/R. We found that blockade of C5aRs provided a significant protection against both local and remote injury parameters in this model (66). In a separate study, Fleming and colleagues showed a similar protective effect in a mouse model of mesenteric I/R using the closely related antagonist, PMX57 (F-[OP(D-Cha)WR]) (67). The results of these studies using selective C5aR inhibitors strongly indicate a specific role for C5a in inducing tissue injury following intestinal I/R. Although the inhibition of C5a alone does not appear to provide complete inhibition of all parameters in intestinal I/R, clinical studies using one of these or other C5a inhibitors are warranted based on these studies.

Complement overactivation in cardiac I/R has long been proposed as a detrimental mediator of tissue injury. This has been shown in several clinical studies where complement activation has been measured (68, 69) and in numerous animal models of myocardial infarction where complement inhibitors have provided protection (70, 71). Indeed, analysis of human postmortem material has shown that following an infarct essentially all of the complement components are expressed inside the myocardial muscle cells (72). Specifically, C5a has been proposed to be a major mediator of pathology in cardiac I/R. Several animal models have also indicated the involvement of C5a. The recombinant human C5a antagonist, CGS-32359, reduced infarct size after coronary artery occlusion in a porcine model of surgical revascularization (73). Anti-C5a antibodies have also been used to successfully reduce tissue injury following myocardial I/R in pigs (74). Experiments by Tanhehco and colleagues, however, indicate that low concentrations of C5a may be protective against myocardial I/R in rabbits (75). C5a activity in the lymph is elevated following coronary artery I/R in canines (76); however, in human clinical studies C5a either does not appear to be elevated or is minimally elevated following myocardial ischemia (69, 77). Recently, Alexion Corp. released results from a phase III clinical trial in coronary artery bypass graft (CABG) surgery patients using the C5 antibody, pexelizumab (78). The trial, involving approximately 4,250 patients, examined the ability of pexelizumab to reduce heart attack and death following CABG surgery, with or without concomitant valve surgery (78). The results showed that during the 30 days following surgery, pexelizumab treatment did not significantly reduce the incidence of nonfatal myocardial infarction or death (78). Given that pexelizumab inhibits both C5a and the MAC, the trial results suggest that inhibiting C5a may not be a valid therapeutic target for this condition. However, until trials are conducted using specific C5a inhibitors, the role of C5a in myocardial I/R remains inconclusive.

Complement activation is seen in humans with ischemic lower extremities, with studies showing an increase in serum levels of C3a and C5a (79). The role of C5a specifically in causing limb I/R injuries has been demonstrated by Bless and co-workers using an anti-C5a antibody. This group showed a reduction in lung vascular permeability, myeloperoxidase content and bronchoalveolar lavage levels of cytokine-induced neutrophil chemoattractant in anti-C5a antibody-treated rats,

demonstrating a specific role for C5a in this model (80). However, evidence by Kyriakides and colleagues suggested that the MAC is more important in inducing injury, and that the role of C5a is relatively minor (81). Our group has recently shown that specific blockade of C5aRs using PMX53 was able to reduce numerous parameters of local and remote injury following hindlimb I/R in rats (82). This study confirms the deleterious role of C5a in induced tissue injury, at least in this model of I/R injury. Once again, clinical studies with specific C5a inhibitors such as PMX53 will need to be conducted to determine the precise role of C5a in limb I/R.

Complement factors are also upregulated in human stroke patients (83). Animal models of cerebral ischemia in which complement activation is inhibited have also shown the importance of the complement system in disease pathology (84). Elevation in C5aR expression is observed in the brain following middle cerebral artery occlusion in rodents (85, 86), suggesting the involvement of C5a in the pathological process. To date, however, no one has investigated the effects of specific and selective C5a inhibitors in cerebral I/R, and this certainly is an area requiring further investigation. Interestingly, C5a has also been proposed as a potential neuroprotective agent in certain neurological situations (87), so the potential for a therapeutic effect by inhibiting C5a in situations of cerebral I/R has yet to be determined.

C5a as a therapeutic target

Given the multitude of diseases associated with C5a and its receptor (see above), it is little wonder that over the years numerous studies and development programs have been aimed at inhibiting the function of C5a (see 88 for review). Targeting C5a and/or its receptor has been an elusive task, and as yet, there is still no clinically available C5a inhibitor and will not be for some years to come.

Neurogen Corp. has developed a specific nonpeptide, orally active C5aR antagonist, NGD-2000-1, which has completed two phase IIa clinical trials. The compound is a substituted tetrahydroisoquinoline of undisclosed structure. Clinical trials were conducted in patients suffering from asthma and RA, and preliminary results demonstrated some efficacy in RA patients but not in asthma patients (89). In earlier phase I clinical studies, it was discovered that NGD-2000-1 inhibited cytochrome P450 3A4, potentially limiting its use in patients taking other drugs that are metabolized by this enzyme (26). Therefore, Neurogen Corp. has suspended further clinical development of NGD-2000-1 but has indicated it will continue profiling additional C5a antagonist compounds for future development (26).

The most advanced small-molecule inhibitor of C5a reported to date is PMX53 (Fig.1A), being developed by Promics Ltd. This cyclic peptide drug is a selective antagonist for human C5aRs with an *in vitro* potency of 2-6 nM (90). Surprisingly, the compound is quite active following oral administration and displays demonstrable oral bioavailability, despite its peptidic nature (42), which is

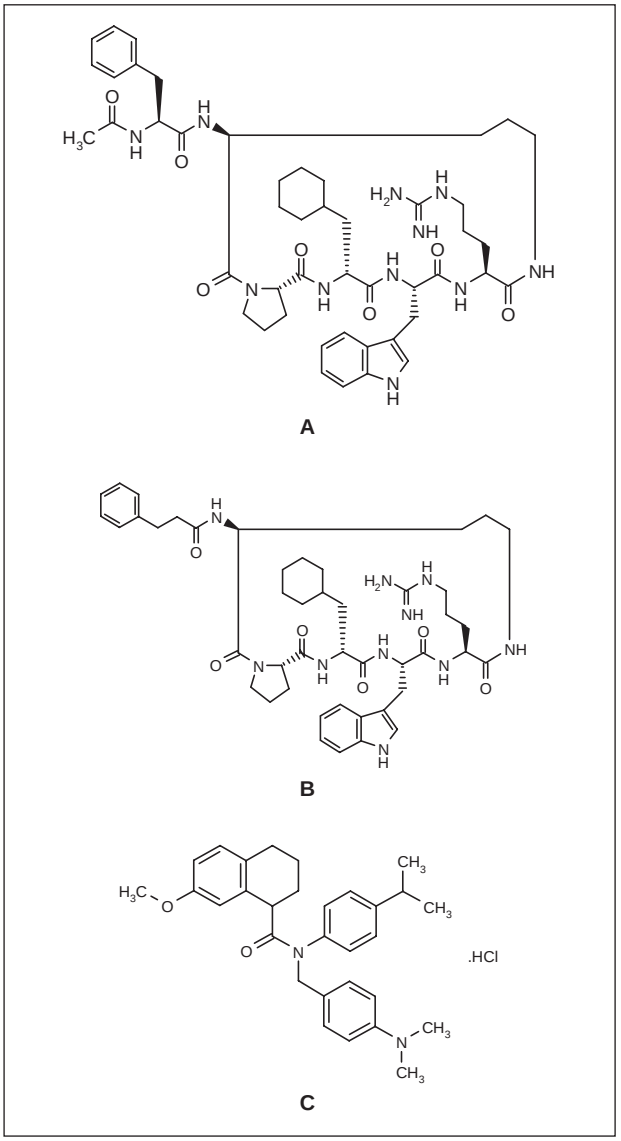

Fig.1. Structures of small-molecule cyclic peptide C5aR antagonists (A) PMX53 and (B) PMX205 (Promics Ltd.) and the nonpeptide C5aR antagonist (C) W-54011 (Mitsubishi Corp.).

likely due to the cyclic nature of the compound which renders it stable to peptidase and acidic breakdown (90). This compound has been used for several years by numerous investigators as a pharmacological tool to delineate the specific role of C5a in various immunoinflammatory situations. Specifically, the compound is effective against models of arthritis; ischemia-reperfusion injuries of the gut, kidney, liver and limb; inflammatory bowel disease; sepsis; ruptured abdominal aortic aneurysm; immune complex-mediated reactions of the peritoneum and dermis; liver fibrosis; experimental lupus nephritis; antiphospholipid Ab-induced fetal injury; and traumatic brain cryoinjury (21, 42, 51, 55, 60, 66, 67, 82, 91-97), and is currently under investigation in numerous other disease models (98). PMX53 has undergone three clinical trials to date, a phase Ia safety and tolerability

trial, a phase Ib/IIa trial in RA patients, and a phase Ib trial in psoriasis patients (www.promics.com), with successful primary outcomes in all three trials. Promics Ltd. is currently continuing development with PMX53 and other potentially more potent analogues such as PMX205 (hydrocinnamate-[OP(D-Cha)WR]; Fig. 1B) (90).

There are numerous other specific inhibitors of C5a reported which are in clinical development by pharmaceutical companies and scientific laboratories; however, to date none of these have progressed beyond phase Ia clinical trials. One important factor in progressing a drug to clinical trials is the development of a compound which has the appropriate physical characteristics for further drug development, and ideally, for oral administration. These include factors such as low molecular weight and economical cost of production, appropriate pharmacokinetic properties for oral drug delivery and elimination from the body, and lack of toxicity (99). Unfortunately, many of the early-stage C5a inhibitors reported by various groups do not display many of these favorable characteristics and, as such, are unlikely to progress further and enter clinical trials. A possible exception to this, is the small-molecule, nonpeptide competitive antagonist of the C5aR, *N*-[4-dimethylaminophenyl)methyl]-*N*-(4-isopropylphenyl)-7-methoxy-1,2,3,4-tetrahydronaphthalene-1-carboxamide hydrochloride (W-54011; Fig. 1C) developed by Mitsubishi Corp. (100). This compound displays oral activity and is efficacious in animal models of inflammatory disease but has yet to enter clinical trials and is now available as a commercial laboratory reagent (24).

Alexion Corp. has also developed advanced inhibitors of C5 (C5 antibodies, pexelizumab and eculizumab) which have successfully completed numerous clinical trials in diseases such as paroxysmal nocturnal hemoglobinuria (phase III) and rheumatoid arthritis (phase IIb). These antibodies inhibit the formation of C5a, C5b and the membrane attack complex and, as such, their therapeutic effects in these trials cannot be differentiated among these complement factors. However, it is likely that some of the protective effects seen with the C5 antibodies in the clinical trials are due, in part, to inhibition of C5a.

Conclusions

The complement activation product C5a is produced by any of the three pathways of complement activation. The local production of C5a, in situations of injury or infection, leads to the infiltration and activation of immune and inflammatory cells, inducing the “innate” immune response and the “classic” inflammatory response. In normal and controlled situations, this process aids in the destruction of foreign matter and organisms, and promotes the healing process. However, increasing evidence suggests that C5a is produced in situations of human disease, and is responsible for the exacerbation and propagation of many disease states such as rheumatoid arthritis, psoriasis, inflammatory bowel disease, and ischemia-reperfusion injuries. Many animal models of these disease states have shown that inhibition of C5a can reduce the severity of the disease in these animals, suggesting that inhibiting C5a in human inflammatory diseases could be a viable therapeutic option. To date, the C5aR antagonist PMX53 (Promics Ltd.) is the most advanced specific, small-molecule C5a inhibitor and has demonstrated safety in early clinical trials, with some indications of efficacy in rheumatoid arthritis and psoriasis patients. Although the development of C5a inhibitors is still in the early stages, the weight of emerging experimental and clinical evidence indicates that specific inhibitors of C5a function could provide substantial therapeutic benefits in the future to patients with inflammatory diseases. Certainly, this represents an entirely new class of antiinflammatory drug and, as such, only controlled clinical trials conducted over the next few decades with optimized agents will illuminate their broad therapeutic potential in humans, as well as in veterinary medicine.

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