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Review

Complement inhibitors: a resurgent concept in anti-inflammatory therapeutics

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Abstract

In addition to its essential role in immune defense, the complement system contributes to tissue damage in many clinical conditions. Thus, there is a pressing need to develop therapeutically effective complement inhibitors to prevent these adverse effects. This concept, though old, received little scientific attention until recently. Data from animal models of diseases that have been produced using complement-deficient, knockout, and transgenic animals, as well as data demonstrating that complement proteins are produced in many important tissue sites (including the brain) have attracted the interest of many basic research scientists and applied scientists from the biotechnology field and larger pharmaceutical firms. This resurgence of interest has generated a wealth of new information in the field of complement inhibition. In this article, we comprehensively review up-to-date information in the field of complement inhibitors.

Keywords: Complement inhibitors; Anti-inflammatory therapeutics; Immune defense

1. Introduction

The complement system, which emerged about 600–700 million years ago, constitutes an important part of the innate immune system that is designed to eliminate ''harmful'' substances from the body. This elimination is accomplished in many different ways: (1) the complement system tags pathogens with its components, promoting the engulfment of the foreign cells by phagocytes, and it causes direct lysis of certain pathogens as a result of membrane attack complex (MAC) formation; (2) it produces local inflammatory responses against pathogens by generating anaphylatoxic peptides; (3) it prevents immune precipitation (lattice formation) and helps solubilize and clear immune complexes from the circulation; (4) the complement system also instructs the adaptive immune response to select appropriate antigens for a humoral response; and (5) it helps the body to eliminate self-reactive B cells.

If the complement system is intended to perform all of these wide-ranging functions, it must not discriminate between the self and the non-self. Consistent with this notion, complement proteins that are involved in the activation process do not make such discriminations; they have the potential to destroy

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any cells to which they bind, including those of the host. As a means of preventing this destructive activity, the complement system is tightly regulated (Fig. 1) by a family of structurally and functionally related proteins termed regulators of complement activation (RCA). The RCA family includes both plasma proteins (factor H and C4 binding protein [C4BP]) and membrane proteins (primarily complement receptor type 1, decay-accelerating factor [DAF] and membrane cofactor protein [MCP]). In addition to RCA proteins, control is also achieved through the activity of C1 inhibitor (C1-Inh), carboxypeptidase N, and CD59.

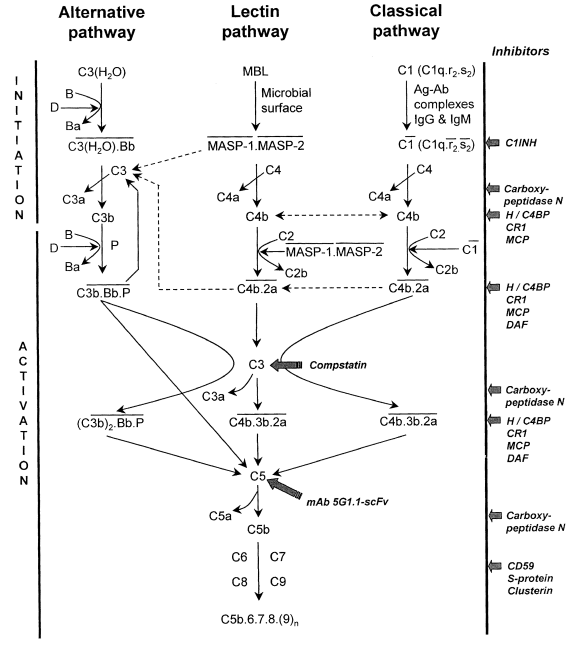

Fig. 1. Activation pathways of the complement system. The system is activated by three different pathways: The classical pathway is activated by antigen–antibody complexes, and the alternative and lectin pathways are activated by microbial surfaces. Activation of these pathways results in the generation of C3a, C4a, and C5a anaphylatoxins and the membrane attack complex $(C5b,6,7,8,(9)_n)$. The complement system is regulated by soluble (C1 INH, factor H, C4BP, carboxypeptidase N, S-protein, clusterin) and membrane-bound (CR1, DAF, MCP, CD59) proteins. A bar over a symbol indicates that the component is in its activated state. Compstatin and monoclonal antibody h5G1.1-scFv act on C3 and C5, respectively. Dotted arrows represent the recruitment of one pathway by another.

Table 1
Pathologic conditions associated with complement activation

Alzheimer's disease (Rogers et al., 1992)
Allotransplantation (Pruitt and Bollinger, 1991)
Asthma (Regal et al., 1993)
ARDS (Robbins et al., 1987)
Arthus reaction (Szalai et al., 2000)
Bullos pemphigoid (Liu et al., 1995)
Burn injuries (Gallinaro et al., 1992)
Crohn's disease (Ahrenstedt et al., 1990)
EAE (Davoust et al., 1999)
EAN (Vriesendorp et al., 1995)
Forssman shock (Higgins et al., 1997)
Glomerulonephritis (Couser et al., 1985)
Haemolytic anemia (Schreiber and Frank, 1972)
Hemodialysis (Amadori et al., 1983)
Hereditary angioedema (Gadek et al., 1980)
Ischemia/reperfusion injuries (Kilgore et al., 1994; Weiser et al., 1996)
IC-induced vasculitis (Cochrane, 1984)
Multiple system organ failure (Heideman and Hugli, 1984)
Multiple sclerosis (Williams et al., 1994)
Myasthenia gravis (Piddlesden et al., 1996)
Post-CBP inflammation (Pekna et al., 1993)
Psoriasis (Rosenberg et al., 1990)
Rheumatoid arthritis (Wang et al., 1995)
Septic shock (Hack et al., 1992)
SLE (Buyon et al., 1992)
Stroke (Huang et al., 1999)
Vascular leak syndrome (Hack et al., 1994)
Xenotransplantation (Dalmasso, 1992)

ARDS, adult respiratory distress syndrome; EAE, experimental allergic encephalomyelitis; EAN, experimental allergic neuritis; IC, immune complex; CPB, cardiopulmonary bypass; SLE, systemic lupus erythematosus.

Given that the complement proteins that participate in the activation process do not discriminate between self and non-self, it is not unexpected that unregulated activation of complement leads to host cell damage. Although complement activation per se is not an etiological factor in any known disease, its inappropriate activation is a cause of tissue injury in many disease states (Table 1). A wealth of data obtained from animal models of diseases generated using complement-deficient (Larsen et al., 1981; Brauer et al., 1993), knockout (Sheerin et al., 1997; Quigg et al., 1998; Williams et al., 1999) and transgenic animals (Alexander et al., 1999; Davoust et al., 1999) have clearly demonstrated that complement activation plays an essential role in the pathogenesis of many diseases (reviewed in Ward et al., 2000).

Table 2
List of complement inhibitors that are under development

Inhibitor	Target protein/protease
Proteins	
C1-Inh	C1
sCR1	C3b, C4b, C3bBb, $C3b_2Bb$, C4b2a, C4b3b2a
sDAF	C3bBb, $C3b_2Bb$, C4b2a, C4b3b2a
sMCP	C3b, C4b
sMCP-DAF	C3b, C4b, C3bBb, $C3b_2Bb$, C4b2a, C4b3b2a
sCD59	C5b-8
DAF-CD59	C3bBb, $C3b_2Bb$, C4b2a, C4b3b2a, C5b-8
C5a mutants	C5aR
Anti-C5	C5
Anti-C3	C3
Anti-C3a	C3a
Anti-C5a	C5a
Small molecules	
N MeFKPdChaWdR	C5aR
F-(OpdChaWR)	C5aR
Compstatin	C3
RNA aptamer	C5
BCX-1470	Factor D
FUT-175	C1s, Factor D, C3bBb, $C3b_2Bb$, C4b2a, C4b3b2a
K-76	C5
Thioester inhibitors	C3, C4

Thus, there is a clear need for specific complement inhibitors. As yet, there are no inhibitors of complement activation available in the clinic; however, several inhibitors have been identified, and some of them are currently in phase I or phase II clinical trials (Table 2). In this review, we discuss the mechanism of complement-mediated tissue injury and identify the target proteins for drug development. We also focus on the various approaches that are being used for therapeutically targeting complement proteins. Therapeutic targeting of complement receptors is beyond the scope of this review; an excellent review on this subject is available elsewhere (Ross et al., 1999).

2. What mediates tissue injury?

Activation of complement (Fig. 1) by the classical, alternative or lectin pathways leads to the formation of C3 convertase (C4b,2a or C3b,Bb) which cleaves C3 into an anaphylatoxic peptide C3a and an opsonic fragment C3b. Covalent attachment of C3b to target cells undergoing complement attack initiates the formation of C5 convertase ($C3b,Bb/C3b_2,Bb$ or C4b,2a,3b), which cleaves C5, releasing C5a peptide. C5b, the major cleavage product of C5, initiates the formation of the MAC composed of C5b-9. The tissue injury that results from complement activation is directly mediated by the MAC, and indirectly by the generation of anaphylatoxic peptides C3a and C5a. As described below, these peptides induce damage through their effect on neutrophils, eosinophils, and mast cells (Wetsel, 1995; Ember et al., 1998).

C5a is a potent chemoattractant for neutrophils, eosinophils, basophils, monocytes/macrophages, and microglial cells (Ember et al., 1998). Studies have demonstrated that upon stimulation with C5a, neutrophils produce a wide range of inflammatory mediators, including serine elastase, peroxidase, glucuronidase, and lactoferrin (Goldstein, 1984; Goldstein and Weissmann, 1974; Smedly et al., 1986). Stimulation of eosinophils results in release of peroxidase, major basic protein, eosinophil-derived neurotoxin, and eosinophil cationic protein (Wetsel, 1995). C5a also triggers the generation of toxic oxygen-derived free radicals from neutrophils (Ehrengruber et al., 1994) and enhances expression of β2 integrins on both neutrophils and eosinophils (Kishimoto et al., 1989; Neeley et al., 1993; Lundahl and Hed, 1994). In addition to these properties, C5a is capable of inducing the production of inflammatory cytokines such as IL-1, IL-6, and IL-8 from unprimed as well as lipopolysaccharide-primed monocytes (Okusawa et al., 1987; Scholz et al., 1990; Ember et al., 1994).

Although C3a is not a chemoattractant for neutrophils, it mediates migration of eosinophils (Daffern et al., 1995) and mast cells (Nilsson et al., 1996). It acts as an activator of mast cells leading to mediator release. Like C5a, C3a has the ability to increase granule release and the expression of β2 integrins on eosinophils (Takafuji et al., 1994; Daffern et al., 1995). The relative activity of C3a for these responses, however, is at least 10-fold lower than that of C5a (Wetsel, 1995). This lower activity does not undermine the importance of C3a as a

proinflammatory molecule, since its concentration in plasma can exceed that of C5a by as much as 20-fold (Wagner and Hugli, 1984).

Although the formation of MAC on host cells may result in cell lysis, most host cells are resistant to lysis by the MAC. Recent studies have indicated that the proinflammatory activity of the MAC is due to induction of cell activation. Sublytic assembly of MAC on host cells induces expression of P-selectin, E-selectin, and intercellular adhesion molecules (Kilgore et al., 1995; Tedesco et al., 1997). The complex is also capable of enhancing the expression of tissue factor and the production of chemokines such as IL-8 and monocyte chemoattractant protein-1 (Kilgore et al., 1997).

3. Which proteins should be targeted?

It has been recognized for some time that activation of one of the complement pathways (classical or alternative, or lectin) leads to the recruitment of another (Fig. 1). For example, activation of the classical pathway results in activation of the alternative pathway (Meri and Pangburn, 1990). Similarly, activation of the lectin pathway supports the activation of the alternative pathway (Reid and Turner, 1994; Matsushita, 1996). Thus, in most clinical conditions, multiple pathways are activated, and it is difficult to discern which pathway initiated the activation. These results suggest the usefulness of a complement inhibitor that blocks all three pathways. The three pathways converge at the C3 activation step; therefore, blocking this step would result in total shutoff of the complement cascade, including generation of C3a and C5a and MAC formation. In fact, most physiological regulators of complement (e.g., factor H, CR1, DAF and MCP) act on C3b to inhibit complement activation. Another school of thought suggests that the pathways should be inhibited at the C5 activation step. The rationale for selecting C5 as a target protein is that it would block the generation of C5a and MAC, while leaving the initial complement components intact for opsonization and immune complex clearance.

In principle, which complement protein(s) should be targeted for the drug development would depend on the pathological condition of interest. For example, if damage is initiated by only one pathway, then inhibition of initial steps of activation of that pathway (e.g., by targeting C1, C2, factor D, factor B, or MASP) would result in suppression of the injury. Similarly, if it is known that damage is caused by C3a, C5a, or C5b-9, then their selective inhibition would alleviate the damage. Such selective inhibition of complement would allow partial functioning of the system, which would be desirable. Thus, inhibition of the classical pathway would not affect the infection-fighting capability of the alternative pathway, and inhibition of the alternative pathway, anaphylatoxin activity, and, C5b-9 would leave the classical pathway intact for immune-complex processing.

4. Complement inhibitors

The concept of developing complement inhibitors for therapeutic benefit is not new. Hereditary angioedema (HAE), an autosomal dominant disorder, was found to be associated with C1-Inh deficiency by Donaldson and Evans (1963). In the early 1970s, this disorder was treated with ε-aminocaproic acid (Frank et al., 1972), an antifibrinolytic agent that also inhibits complement; later, infusion of C1-Inh purified from plasma was used. As the list of diseases associated with complement activation continued to grow, interest in designing complement inhibitors also began to increase among complementologists. With the advent of molecular biology techniques came the first generation of inhibitors that were designed after natural complement inhibitors. Originally developed by Weisman et al. (1990), this concept was the first major step in the development of complement inhibitors. Since then, several recombinant complement inhibitors have been developed (see below), which show promise in various experimental diseases. In addition, monoclonal antibodies (mAbs) against complement components have been developed as inhibitors. Recent studies have focused on a second generation of smaller molecular-weight derivatives, with a goal of developing cost-effective therapeutics with more desirable pharmacologic properties.

4.1. Natural proteins

C1-Inh is the only plasma-derived protein that has been thoroughly studied as an in vivo complement

inhibitor. It is a member of the serine proteinase inhibitor (serpin) family that inhibits activated C1s and C1r (components of C1), in addition to factor XIIa, kallikrein, and factor XIa of the contact system. C1-Inh is a suicide inhibitor that inhibits proteinases by binding to their active sites through the reactive center (Sim et al., 1979). These stable complexes are then cleared from the circulation by binding to specific receptors on hepatocytes (Perlmutter et al., 1990). In addition to its successful use in replacement therapy in patients with HAE (Gadek et al., 1980; Bergamaschini et al., 1983; Sim and Grant, 1990; Waytes et al., 1996), C1-Inh has also showed promise in other diseases such as sepsis (Hack et al., 1992, 1994), vascular leak syndrome (Nurnberger et al., 1992; Ogilvie et al., 1994; Hack et al., 1994), and acute myocardial infarction (Buerke et al., 1995; Horstick et al., 1997). C1-Inh, like other members of serpin family, suffers from a drawback of its susceptibility to inactivation by neutrophil elastase. To overcome this problem, C1-Inh mutants have been developed that are resistant to elastase (Eldering et al., 1993); however, the therapeutic efficacy of these mutants has not yet been established.

The plasma proteins Factor H and C4BP are two important members of the RCA family. These proteins inhibit complement activation by causing dissociation of the subunits of the C3 and C5 convertases and by supporting the proteolytic inactivation of the subunits by factor I (Lambris et al., 1998). Though effective against spontaneous activation, these proteins are poor inhibitors of induced activation and are therefore considered as unlikely to be useful for therapeutic purposes (Kalli et al., 1994).

4.2. Recombinant proteins

The first recombinant complement inhibitor made was soluble CR1 (sCR1), which lacks the transmembrane region and the cytoplasmic tail of the parent molecule. The choice of CR1 over other RCA proteins was obvious: It was known to inhibit C3 convertase as well as C5 convertase and to serve as a cofactor for the inactivation of C3b and C4b. Most importantly, the concentrations of soluble CR1 (sCR1) required to inhibit the classical pathway-mediated lysis of sensitized erythrocytes and zymosan-induced activation of the alternative pathway were over 100-fold lower than serum concentrations of factor H and C4BP (Weisman et al., 1990).

sCR1 has been shown to protect against tissue injury in several animal models of acute and chronic inflammatory conditions such as, the Arthus reaction (Yeh et al., 1991), immune complex-induced alveolitis (Mulligan et al., 1992), lung injury (Rabinovici et al., 1992), trauma (Kaczorowski et al., 1995), ischemia/reperfusion (Weisman et al., 1990; Hill et al., 1992), myasthenia gravis (Piddlesden et al., 1996), glomerulonephritis (Couser et al., 1985), multiple sclerosis (Piddlesden et al., 1994) and asthma (Regal et al., 1993). In addition, it has shown a protective effect against tissue injury resulting from bioincompatibility situations such as those encountered during dialysis and cardiopulmonary bypass (Larsson et al., 1997), allotransplantation (Pruitt and Bollinger, 1991), and xenotransplantation (Pruitt et al., 1991). In spite of being an effective complement inhibitor, sCR1 suffers from a relatively short half-life in vivo ($t_{1/2}\,\beta = 8$ h in humans) (Makrides, 1998). This problem appears to have been solved by modifying the culture conditions; the modified preparations have a $t_{1/2}\,\beta$ of approximately 30 h in humans (Dellinger et al., 1996). sCR1 has shown encouraging results in Phase II trials in ARDS patients and patients with end-stage pulmonary disease undergoing lung transplant surgery.

Recently, efforts have been made to modify CR1 for selective inhibition of the alternative pathway. sCR1(desLHR-A), a mutant CR1 lacking the LHR-A region (a region that interacts with C4b), has been produced and used along with C1-Inh for dissecting the relative contributions of the classical and alternative pathways in a model of ischemia and reperfusion injury (Murohara et al., 1995); the study concluded that both pathways contribute to reperfusion injury (Murohara et al., 1995). More recently, in an effort to make sCR1 a bifunctional inhibitor, it has been covalently modified by sialyl Lewis x (sLe^x) glycosylation (Rittershaus et al., 1999). This sCR1sLe^x contains at least 10 sLe^x tetrasaccharide moieties per molecule (Rittershaus et al., 1999). This molecule possesses the ability to simultaneously inhibit both complement activation and selectin-mediated adhesion. When tested in a model of stroke, sCR1sLe^x was more potent than sCR1 in minimizing the volume of infarcted cerebral tissue and reduc-

ing the neuronal deficit (Huang et al., 1999). In another study, decorated sCR1 showed an enhanced ability to bind to activated vascular endothelium and enhanced anti-inflammatory effects (Mulligan et al., 1999).

Two other RCA proteins, DAF and MCP, intrinsically protect host tissue from complement-mediated damage by down-regulating complement activation on the cells on which they are expressed (reviewed in Kinoshita et al., 1985; Liszewski and Atkinson, 1992; Nicholson-Weller and Wang, 1994). DAF is an integral membrane glycoprotein that is anchored to the cell membrane via a covalent linkage with a glycosyl-phosphatidyl inositol (GPI) anchor. It is distinguished from other complement regulatory proteins in that it accelerates the decay of convertases but does not serve as a cofactor for factor I (Kinoshita et al., 1985; Nicholson-Weller and Wang, 1994). MCP, a type I transmembrane protein, serves as a cofactor for factor I to cleave and inactivate C3b and C4b, but does not accelerate the decay of convertases (Liszewski and Atkinson, 1992). Both inhibitors have been expressed as soluble recombinant proteins (sDAF and sMCP) by deleting the transmembrane and cytoplasmic regions of MCP (Christiansen et al., 1996) and the C-terminal amino acids of DAF that are required for GPI linkage (Moran et al., 1992). The two expressed proteins inhibited complement activation in vitro as well as in vivo (Moran et al., 1992; Christiansen et al., 1996) in the reverse passive Arthus reaction model. A comparison of the inhibitory activities of sDAF and sMCP with that of sCR1 showed that sCR1 was a more effective inhibitor than either sDAF or sMCP (Christiansen et al., 1996). Since DAF showed only decay-accelerating activity and MCP possessed only factor I cofactor activity, it seemed reasonable to expect that a hybrid molecule would show both these activities and would be a better inhibitor. Indeed, when an MCP-DAF hybrid, complement activation blocker-2 (CAB-2), was produced, it did exhibit both inhibitory activities (Higgins et al., 1997). Furthermore, CAB-2 showed inhibitory potential in vivo, by blocking the Arthus reaction and Forssman shock in guinea pigs (Higgins et al., 1997).

CD59, another GPI-anchored membrane protein, protects host cells from MAC-mediated damage. By tightly binding to C5b-8 complex, it prevents incorporation of C9 molecules into the membrane that is necessary for pore formation (Davies, 1996; Morgan, 1999). A soluble form of CD59 (sCD59) has been produced and shown to inhibit complement in vitro (Sugita et al., 1994). The molecule contains a single N-linked carbohydrate moiety, removal of which led to a 7-fold increase in the molecule's activity (Suzuki et al., 1996). Although sCD59 has not been tested in animal models of disease, it can serve as an excellent tool for defining the contribution of MAC-mediated damage in various disease models.

Efforts have also been made to construct DAF-CD59 hybrid molecules, with goal of blocking complement at the C3/C5 convertase level as well as at the MAC level. Two different constructs were made: CD (NH_2-CD59-DAF-GPI) and DC (NH_2-DAF-CD59-GPI) (Fodor et al., 1995). Analysis of their function indicated that the CD hybrid functioned only as a decay-accelerating molecule, whereas the DC hybrid retained the activity of DAF as well as CD59 (Fodor et al., 1995). The DC hybrid may serve as a molecule of choice for preventing hyperacute rejection of xenogeneic organs.

4.3. Antibodies

As described above, inhibition of complement at the C5 activation step would lead to inhibition of C5a generation and MAC formation, making C5 an attractive target protein. Since no natural complement inhibitors of C5 have been identified, development of mAbs against C5 became the obvious alternative because antibodies are known to recognize their targets with high specificity and affinity, and they have a relatively long half-life. Moreover, with the recent advent of genetically engineered antibodies, large quantities of specific antibody can be produced for therapeutic use with little difficulty. Although they are an attractive choice, mAbs do suffer from several limitations, such as the problem of immunogenicity and the requirement for administration by intravenous perfusion. The first limitation can be minimized to a great extent by ''humanization'' of antibodies (Fishwild et al., 1996; Bruggemann and Taussig, 1997); however, it cannot be totally avoided (Sandborn and Hanauer, 1999).

Several laboratories have developed anti-C5 mAbs that block complement activation (Frei et al., 1987; Wurzner et al., 1991; Wang et al., 1996a; Rollins et

al., 1998). The anti-human C5 mAb aC5-12 has been shown to block C5 cleavage by C5 convertases and the formation of C5b-9 (Wang et al., 1996a). Epitope mapping has localized the binding site of the antibody to the β-chain of C5 between residues Tyr^{334} and Lys^{418}. Deletion of 27 residues from either the N- or the C-terminal end of this 85-amino acid region resulted in a loss of aC5-12 binding, indicating that residues near each end are needed to form a tertiary epitope recognized by the antibody. The anti-human C5 mAb N19/8 (Wurzner et al., 1991), when tested in an in vitro extracorporeal blood circuit, inhibited C5a and soluble C5b-9 generation and CD11b up-regulation, and reduced the formation of leukocyte-platelet aggregates and eliminated P-selectin-positive platelets (Rinder et al., 1995).

The generation of anti-C5 mAb BB5.1 (Frei et al., 1987) was instrumental in the development of anti-C5 inhibitors. Early studies using this antibody have clearly established the pathological role of C5a and the MAC in various disease models. Anti-C5 mAbs have been tested in a mouse model of immune complex nephritis (Wang et al., 1996b) and collagen-induced arthritis (Wang et al., 1995), in a rat model of myocardial ischemia and reperfusion (Vakeva et al., 1998), and in cardiopulmonary bypass patients (Rollins et al., 1998). In the mouse models, anti-C5 therapy resulted in significant improvement in the course of glomerulonephritis (Wang et al., 1996b), prevented the onset of collagen-induced arthritis, and ameliorated the established disease (Wang et al., 1995). In the rat model, it reduced the ischemia/reperfusion-induced tissue injury (Vakeva et al., 1998). A single-chain (scFv) antibody constructed from anti-human C5 mAb N19-8, has been shown to prevent C5b-9 deposition in mouse hearts perfused with human plasma (Evans et al., 1995). Recently, another scFv antibody (h5G1.1-scFv) has been tested in cardiopulmonary bypass patients (Fitch et al., 1999). A dose of 2 mg/kg of this antibody inhibited $> 50\%$ of the total complement activity for about 14 h. Serum from treated patients showed no sC5b-9 generation and a significant reduction in leukocyte activation, as judged by CD11b up-regulation. Most importantly, these patients showed a significant reduction in cardiopulmonary bypass-induced myocardial damage, cognitive deficits, and blood loss (Fitch et al., 1999).

In addition to C5, mAbs are also being developed against C3 (Kemp et al., 1994), C3a (Burger et al., 1988; Nilsson et al., 1988; Elsner et al., 1994) and C5a (Ames et al., 1994; Amsterdam et al., 1995; Hopken et al., 1996; Park et al., 1999).

4.4. Small-molecule inhibitors

Small molecular-weight inhibitors offer several advantages over large therapeutic proteins, in that they are cost-effective, have better tissue penetration, and can be developed for oral use. Such considerations are of prime importance when the drug must be administered over a long period of time, such as during management of autoimmune disorders. Many small synthetic compounds have been identified in the past and have been reviewed extensively by others (Johnson, 1977; Reynard, 1980; Asghar, 1984; Makrides, 1998). In this section, we discuss only selected well-characterized compounds.

4.4.1. Anaphylatoxin receptor antagonists

Development of antagonists of the C5a receptor (C5aR) is a relatively old concept. Initial studies provided important information on the structure–function aspects of the ligand–receptor interaction, but the peptide analogs that were generated acted, at best, as partial antagonists (Hensens et al., 1991; Lanza et al., 1992; Mollison et al., 1992). In subsequent work, one of the hexapeptide analogs (*N*MeFKPdCHaFdR, where *N*ME is *N*-methyl phenylalanine, and Cha is cyclohexylalanine) that showed partial agonist activity was chosen for further study. This study demonstrated that increasing the aromaticity at position five led to a progressive loss in agonist activity, with little difference in binding affinity (Konteatis et al., 1994). Most importantly, this work led to the identification of the first full antagonist of C5aR, *N*MeFKPdCHaWdR (Konteatis et al., 1994). Surprisingly, this acyclic molecule possessed a well-defined conformation in solution (Wong et al., 1998). The nuclear magnetic resonance (NMR) structure of this antagonist was then used to design a cyclic peptide F-(OpdChaWR) that was able to inhibit myeloperoxidase secretion from human polymorphonuclear leukocytes and the human umbilical artery contraction induced by C5a (Paczkowski et al., 1999). The peptide also showed

inhibition of C5a-mediated neutropenia in rats (Short et al., 1999). Apart from small peptide antagonists, C5aR antagonists devoid of agonist activity were also developed by site-directed mutagenesis of the C-terminus of C5a (Pellas et al., 1998), and by screening C5a phage-display libraries in which the C-terminus of des-Arg^{74}-hC5a was mutated (Heller et al., 1999). The C5aR antagonists identified using both these approaches were shown to be effective in vivo (Pellas et al., 1998; Heller et al., 1999).

The gene encoding the C3a receptor has recently been cloned (Ames et al., 1996; Crass et al., 1996; Roglic et al., 1996), and the structural features that are important for ligand binding are currently being studied. These studies may facilitate the development of a much-needed C3a antagonist. No complete antagonist for the C3a receptor has been reported to date, although partial antagonists have been described (Kretzschmar et al., 1992; Pohl et al., 1993).

4.4.2. Compstatin

In our laboratory, we have also focused our attention on the development of small molecular-weight inhibitors of C3. In our initial efforts, we chose to use combinatorial peptide libraries to identify C3-interactive peptides, with the goal of identifying C3-binding peptides that would functionally mimic other C3-regulating proteins. This approach led to the identification of a novel 13-residue cyclic peptide (Sahu et al., 1996), later named Compstatin. Unlike natural inhibitors of complement that act on C3b, Compstatin binds to native C3 and inhibits its cleavage by C3 convertase. Most importantly, this inhibition is not caused by sterically hindered access to the C3a/C3b cleavage site (Sahu et al., 1996). The peptide displays exquisite specificity towards human and monkey C3 but does not inhibit rat, mouse, guinea pig, rabbit or swine complement (Sahu et al., 1998). Thus far, Compstatin has been tested in three different clinically relevant models: (1) hyperacute rejection in discordant kidney xenotransplantation has been studied ex vivo in a porcine-to-human perfusion model. In this model, Compstatin significantly prolonged the survival of the kidneys (Fiane et al., 1999a,b). (2) Its effect has also been tested in models of extracorporeal circulation (Nilsson et al., 1998), where it effectively inhibited the generation of C3a and sC5b-9 and the binding of C3/C3 fragments to a polymer surface. As a result of the inhibition of complement activation, the activation of polymorphonuclear leukocytes (as assessed by the expression of CD11b) and the binding of these cells ($CD16^+$) to the polymer surface were almost completely lost (Nilsson et al., 1998). (3) Most recently, Compstatin has been tested in vivo in primates to examine its effect on complement activation induced by a heparin–protamine complex; here it effectively inhibited complement activation (Soulika et. al., unpublished observation).

Structure-based rational design of peptidomimetics and crafting of small-molecule inhibitors requires knowledge of the complete three-dimensional (3D) structure of the peptide inhibitor and the target protein. We have achieved the first step towards this direction by determining the 3D structure of a major conformer of Compstatin in solution by two-dimensional (2D) NMR (Morikis et al., 1998). Although the peptide in its current form is effective in vivo, the structural information obtained for Compstatin is being used for the rational design of a small-molecule inhibitor that can be administered orally.

4.4.3. RNA aptamer inhibitor

Combinatorial chemistry seems to be the key to the development of complement inhibitors, since the lack of availability of the 3D structures of most complement proteases effectively limits the rational design of active site-based inhibitors. The SELEX combinatorial chemistry technique was recently used to develop a pool of $> 10^{14}$ unique RNA sequences. This technique utilizes a random DNA sequence flanked by 5′ and 3′ fixed-sequence primer regions as a template. The template is amplified by PCR to yield dsDNA, which is then transcribed into single-stranded RNA to generate the pool. The RNA molecules developed by this technique were screened against partially trypsinized C5 in the hope of developing aptamers specific to neo-epitopes that are exposed during complement activation (Biesecker et al., 1999). Cloning and sequencing of the bound RNA pool led to the identification of 28 clones, seven of which showed sequence homology. These aptamers bound C5 with K_d values of 20–30 nM, and they inhibited C5 cleavage. Further development of one of the aptamers was obtained in an inhibitor with a K_d value of 2–5 nM. This aptamer inhibited

human complement-mediated lysis of antibody-coated sheep cells. Currently, these inhibitors and other aptamers developed against rat C5 are being evaluated in in vitro and in vivo models (Biesecker et al., 1999).

4.4.4. BCX-1470

Factor D is one of the eight serine proteases of the complement system. It catalyzes the cleavage of factor B bound either to C3(H_2O) or C3b and initiates and amplifies the alternative pathway. It is also the first complement protease whose 3D structure was determined by X-ray crystallography (Narayana et al., 1994). Later, in order to design a potent factor D inhibitor, the same group determined the crystal structure of factor D in complex with its inhibitors diisopropyl fluorophosphate, isatoic anhydride, and 3,4 dichloroisocoumarin (Jing et al., 1998). Information obtained from these studies helped them to develop BCX-1470, which inhibits factor D in the nanomolar range and is approximately 180 times more potent than FUT-175 (presented at XVII Int. Complement Workshop, Rhodes, Greece). BCX-1470, however, is not specific for factor D; it also inhibits C1s, thrombin, factor Xa, and trypsin. In recent in vivo tests it inhibited the development of reverse passive Arthus reaction-induced edema in rats (Szalai et al., 2000). BCX-1470 has also been successfully tested in phase I clinical trials in healthy subjects, in which its safety and pharmacokinetic profile were evaluated.

4.4.5. FUT-175 (Nafamostat)

FUT-175 is a broad-spectrum synthetic serine protease inhibitor that has been shown to be an inhibitor of C1s, factor D, and C3/C5 convertases (Inagi et al., 1991). This inhibitor has been successfully tested in several animal models. It was effective in myocardial ischemia/reperfusion (Homeister and Lucchesi, 1994), acute experimental pancreatitis (Araida et al., 1995), and discordant xenotransplantation (Kobayashi et al., 1996). In addition, when administered to glomerulonephritis patients with hypocomplementemia, it improved serum complement levels and reduced proteinuria (Fujita et al., 1993). Its effect on cerebral vasospasm after subarachnoid hemorrhage has also been investigated. Patients treated with FUT-175 showed decreased incidence of cerebral infarction resulting from vasospasm (Yanamoto et al., 1992). Since the inhibitor is not specific for complement proteases, it is difficult to discern whether these effects were due to complement inhibition.

4.4.6. K-76 monocarboxylic acid

K76 monocarboxylic acid is a fungal metabolite derived from *Stachybotrys complementi* (Miyazaki et al., 1980). In vitro, it inhibits both the classical and alternative pathways of complement. Although it primarily inhibits the complement pathway at the C5 level (Hong et al., 1979), it has also been shown to inhibit factor I activity (Hong et al., 1980). K76 has been tested in several experimental models of complement activation: It reduced complement-mediated leukocyte accumulation in the subcutaneous air pouch of rats (Konno and Tsurufuji, 1983); decreased proteinuria in the early stage of BSA nephritis, with a 50% reduction in serum C5 (Iida et al., 1987); and prevented complement-mediated injuries in a localized acid aspiration model (Yamada et al., 1997). Recently, it has been tested in various xenotransplantation models, but it failed to prolong the survival of xenografts (Kobayashi et al., 1996; Tanaka et al., 1996; Blum et al., 1998).

4.4.7. Thioester inhibitors

Complement components C3 and C4 have the ability to attach covalently to the amino and hydroxyl groups of activating surfaces. This property is attributed to the intramolecular thioester bond present in these molecules. Targeting this thioester with compounds containing amino or hydroxyl groups results in inhibition of complement activation because these groups prevent attachment of C3 and C4 molecules to the target surface; moreover, the reacted species are susceptible to proteolytic inactivation by factor I in the presence of appropriate cofactors. This inactivation can be accomplished either by targeting the thioester of the native molecules (Levine and Dodds, 1990) or by attacking the activated thioester of nascent C3b and C4b (Isenman and Young, 1984; Law et al., 1984). Recent studies have examined in detail the reactivity of the activated thioester of metastable C3b with synthetic compounds (Sahu and Pangburn, 1995, 1996; Sahu et al., 1994, 1999). Several off-the–shelf compounds and

drugs were found to be up to 20,000 times more reactive than the natural targets such as carbohydrates. These studies have revealed that the nucleophilic character of the hydroxyl group, as well as other neighboring structural features, affects the reactivity with this thioester. The results suggest that additional improvements in reactivity are possible.

5. Possible risks associated with anti-complement therapeutics

The exact risk associated with complement inhibition is not known, primarily because of a paucity of sufficient data. It is known, however, that complement deficiencies are associated with increased susceptibility to infection (Wetsel and Colten, 1990), increased susceptibility to endotoxin as a result of impaired clearance (Fischer et al., 1997), and with autoimmunity such as that seen in systemic lupus erythematosus and glomerulonephritis (Wetsel and Colten, 1990). Thus, in principle, these effects may be perceived as risks. However, such complications would be likely to arise only when total complement inhibition was necessary to achieve therapeutic benefit. Studies have shown that even 60% inhibition of complement is sufficient to provide therapeutic benefit in collagen-induced arthritis (Wang et al., 1995). If this situation holds true for other clinical conditions, then the risks described above can be perceived, at the most, as theoretical.

6. Perspective

Studies of complement inhibitors, in particular sCR1 and anti-C5 mAb, have provided convincing evidence that complement inhibition cannot only prevent disease progression but can also ameliorate established disease. Both sCR1 and anti-human C5 mAb (5G1.1, 5G1.1-scFv) are currently in clinical trials in various diseases and have shown encouraging results. Undoubtedly, their use as anti-complement therapeutics will reduce the clinical morbidity in several diseases. That being said, it cannot be denied that recombinant protein therapies are not cost-effective (Grindley and Ogden, 1995), and in the long term, complement inhibitors will have to be developed as ''pills''. Currently, many laboratories including our own are engaged in designing small-molecule complement inhibitors with desirable pharmacologic properties. It is our conviction that these endeavors will lead to the development of much-needed small-molecule inhibitors of complement.

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