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Therapeutic Inhibition of the Complement System

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I. Introduction

Activation of the complement system plays a key role in normal inflammatory response to injury but may cause substantial injury when activated inappropriately. The cytolytic properties of serum were first described more than a century ago (Bordet, 1896), but there is still no therapeutic compound available on the market for complement inhibition. This is about to change as several companies and academic investigators are actively engaged in the development of complement therapeutics (Morgan, 1995a; Pascual and French, 1995). The molecular cloning and biochemical dissection of the many components of the complement pathway during the last 2 decades has led to a detailed understanding of the mechanisms of complement activation in inflammation. This, in turn, has allowed for the potential for drug development based on the genetic engineering of receptors and other components of the complement pathway. Coupled with the ability to express human transgenes in animal organs, these developments hold promise for the therapeutic management of complement-mediated injury in certain diseases.

Although complement activation is probably not the primary etiology of many diseases, the damage to tissues in certain conditions is clearly complement-mediated. Indeed, the inappropriate activation of complement is at the core of a long list of disease pathologies (Morgan, 1994) that affect the immune, renal, cardiovascular, neurological, as well as other, systems in the body (table 1). Examination of the evidence for the involvement of

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TABLE 1
Disorders associated with complement activation

Disorder	References
Acute	
Adult respiratory distress syndrome	Zilow et al., 1992; Rinaldo and Christman 1990; Langlois et al., 1989; Meade et al., 1994
Ischemia-reperfusion injury:	
Myocardial infarct	Hill and Ward, 1971; Earis et al., 1985; Rubin et al., 1989; Fox, 1990; Entman et al., 1991; Kilgore et al., 1994; Homeister and Lucchesi, 1994
Skeletal muscle	Rubin et al., 1989; Weiser et al., 1996
Lung inflammation	Ward, 1996, 1997; Eppinger et al., 1997
Hyperacute rejection (transplantation)	Bach et al., 1995; Baldwin et al., 1995; Sanfilippo, 1996; White, 1996; Lawson and Platt, 1996
Sepsis	Hack et al., 1989; Gardinali et al., 1992
Cardiopulmonary bypass	Kirklin et al., 1983; Homeister et al., 1992
Burns, wound healing	Ward and Till, 1990; Oldham et al., 1988; Davis et al., 1987; Ljunghusen et al., 1996
Asthma	Regal et al., 1993; Regal and Fraser, 1996
Restenosis	Niculescu et al., 1987
Multiple organ dysfunction syndrome	Miller et al., 1996
Trauma, hemorrhagic shock	Gallinaro et al., 1992; Kaczorowski et al., 1995
Guillain-Barré syndrome	Hartung et al., 1987; Sanders et al., 1986; Koski et al., 1987; Koski, 1990
Chronic	
Paroxysmal nocturnal hemoglobinuria	Yomtovian et al., 1993; Shichishima, 1995; Rosse, 1997
Glomerulonephritis	Couser, 1993; Couser et al., 1985, 1995; Spitzer et al., 1969
Systemic lupus erythematosus	Belmont et al., 1986; Hopkins et al., 1988; Negoro et al., 1989; Gatenby, 1991
Rheumatoid arthritis	Kemp et al., 1992; Satsuma et al., 1993; Abbink et al., 1992
Infertility	D'Cruz et al., 1990, 1991; Anderson et al., 1993
Alzheimer's disease	Johnson et al., 1992; Rogers et al., 1992; Pasinetti, 1996; Eikelenboom et al., 1994; Velazquez et al., 1997; Jiang et al., 1994; McGeer et al., 1997; Chen et al., 1996; Morgan et al., 1997
Organ rejection (transplantation)	Platt, 1996; Baldwin et al., 1995; Marsh and Ryan, 1997; Dalmasso, 1997
Myasthenia gravis	Lennon et al., 1978; Piddlesden et al., 1996
Multiple sclerosis	Piddlesden et al., 1994
Biomaterials incompatibility	
Platelet storage	Gyongyossy-Issa et al., 1994
Hemodialysis	Cheung et al., 1994; Himmelfarb et al., 1995; Mollnes, 1997
Cardiopulmonary bypass equipment	Craddock et al., 1977; Haslam et al., 1980; Gillinov et al., 1993; Mollnes, 1997; te Velthuis et al., 1996

complement in these conditions is beyond the scope of this review. References are provided in table 1 for further reading, and several excellent reviews have covered clinical complementology (Ross and Densen, 1984; Frank, 1987; Morgan, 1990, 1994, 1995b; Morgan et al., 1997; Homeister and Lucchesi, 1994; Kalli et al., 1994; Asghar, 1995; Baldwin et al., 1995; Mossakowska and Smith, 1997). In addition, only a brief overview of the complement system is provided here as this area has been covered extensively (Liszewski et al., 1996; Ross, 1986; Rother and Till, 1988; Fearon and Wong, 1983; Reid, 1986; Müller-Eberhard, 1988; Dalmasso, 1986; Baldwin et al., 1995; Frank, 1994; Morgan and Meri, 1994). The main objective in this study is to review the published literature on the use of inhibitors for the therapeutic abrogation of pathological complement activation. A section is also included on the use of bispecific antibodies in human disease. This latter approach does not attempt to inhibit complement, but rather uses components of the complement system to facilitate the clearance of blood-borne pathogens from the circulation.

II. The Complement System and Its Regulation

The complement system consists of more than 30 serum and cellular proteins, including positive and negative regulators, linked in two biochemical cascades, the classical and alternative pathways (fig. 1). The activation of complement encompasses a series of initiation, amplification, and lytic steps and their discrete reactions (Parker, 1992; Liszewski et al., 1996). The system is regulated at multiple levels temporally as well as spatially. This regulation facilitates recognition of self from foreign tissue (Farries and Atkinson, 1987) and, therefore, allows for control over the potent tissue-damaging capabilities of complement activation. It has been recognized that some of the endogenous complement regulatory proteins might serve as potential therapeutic agents in blocking inappropriate activation of complement in human disease. Soluble and membrane-bound variants of complement regulators have been produced and shown to be effective in blocking complement activation in vitro as well as in animal models of complement-mediated pathologies (Homeister and Lucchesi, 1994).

A. The Classical Pathway

The classical pathway is usually initiated when a complex of antigen and IgM or IgG antibody binds to the first component of complement C1. Activation of this step of complement is regulated by the C1 inhibitor that binds to C1r and C1s and dissociates them from C1q (Liszewski et al., 1996). Activated C1 cleaves both C4 and C2 to generate C4a and C4b, as well as C2a and C2b. The C4b and C2a fragments combine to form the C3 convertase, which, in turn, cleaves the third component of complement, C3, to form C3a and C3b. The

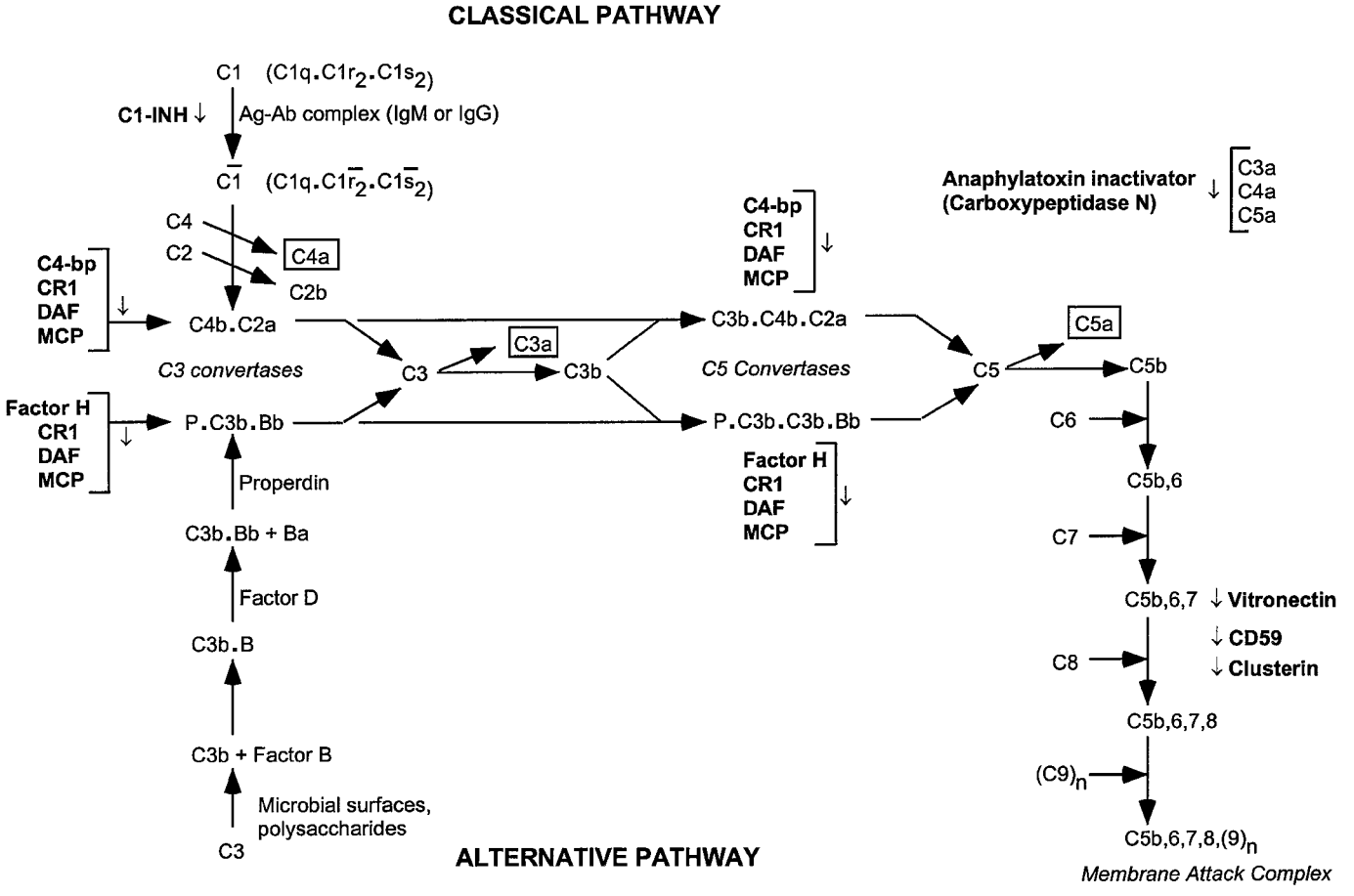

FIG. 1. The complement system and its regulators. The classical pathway is activated by complexes of antigen and IgM or IgG antibody classes. The alternative pathway is activated by microbial surfaces and complex polysaccharides, e.g., yeast cell walls, endotoxins, viral particles. In both the classical and alternative pathways C3 is converted into C3b by the C3 convertases, whereas in the classical pathway C5 is converted into C5b by the C5 convertases. The three anaphylatoxins, C3a, C4a and C5a are released during the various enzymatic reactions of the cascade. The membrane attack complex is formed by the sequential binding of C5b to C6, C7, C8 and C9. Both pathways are subject to fine regulation by soluble (C1 inhibitor, C4bp, factor H, vitronectin, clusterin) as well as membrane-bound (CR1, DAF, MCP, CD59) proteins. The anaphylatoxins are inactivated by carboxypeptidase N.

binding of C3b to the C3 convertase yields the C5 convertase, which cleaves C5 into C5a and C5b, the latter becoming part of the membrane attack complex (MAC)[b]. It must be noted that activators other than antibodies are capable of initiating the classical pathway. For example, in the absence of antibody, β-amyloid activates complement in the brain by binding to the collagen-like domain of the C1q A chain (Rogers et al., 1992; Jiang et al., 1994; Velazquez et al., 1997; Webster et al., 1997; Cadman and Puttfarcken, 1997). These observations have therapeutic implications for Alzheimer's disease (Barnum, 1995; Pasinetti, 1996; Chen et al., 1996).

The three peptides released during these steps, C3a, C4a, and C5a, are known as anaphylatoxins (Hugli and Müller-Eberhard, 1978), and they differ in their relative potencies. C5a is the most potent anaphylatoxin, followed by C3a, which, in turn, is 10- to 100-fold more active than C4a (Cui et al., 1994; Hugli and Müller-Eberhard, 1978; Liszewski et al., 1996). The anaphylatoxins mediate multiple reactions in the acute inflammatory response, including smooth muscle contraction, changes in vascular permeability, histamine release from mast cells, neutrophil chemotaxis, platelet activation and aggregation (Morgan, 1986; Hugli, 1989; Gerard and Gerard, 1994), as well as up-regulation of adhesion molecules that can also play key roles in neutrophil recruitment (Foreman et al., 1994; Mulligan et al., 1996, 1997; Schmid et al., 1997a). Recently, C3a and C5a have been shown to be potent chemotactic factors for human

[b] Abbreviations: AHB, antisense homology boxes; C1qR, C1q receptor; C3aR, C3a receptor; C5aR, C5a receptor; CAB-2, complement activation blocker-2; cDNA, complementary deoxyribonucleic acid; CR2, complement receptor type 2; CVF, cobra venom factor; DAF-CD59, decay accelerating factor-CD59; GPI, glycosyl phosphatidyl inositol; HAR, hyperacute rejection; HEL, hen egg lysozyme; IC, immune complexes; Ig, immunoglobulins; LHR, long homologous repeat; mAb, monoclonal antibody; MAC, membrane attack complex; MCP-DAF, membrane cofactor protein-decay accelerating factor; P, properdin; RCA, regulators of complement activation; scFv, single chain Fv; SCR, short consensus repeat; sCR1, soluble complement receptor type 1; sCR1[desLHR-A], soluble complement receptor type 1 lacking long homologous repeat-A; sCR1-SLe^x, soluble complement receptor type 1-sialyl Lewis^x; sDAF, soluble decay accelerating factor; sMCP, soluble membrane cofactor protein.

mast cells (Hartmann et al., 1997). The anaphylatoxins are rapidly inactivated by carboxypeptidase N, which cleaves the carboxyl terminal arginyl residue from each anaphylatoxin, thus converting them into their des-Arg forms (Bokisch et al., 1969; Bokisch and Müller-Eberhard, 1970; Chenoweth, 1986). A C5a-inactivating enzyme isolated from human peritoneal fluid has been described (Ayesh et al., 1995).

The C3 and C5 convertases of the classical pathway (fig. 1) are controlled by members of the Regulators of Complement Activation (RCA) family (Rey-Campos et al., 1987; Carroll et al., 1988; Campbell et al., 1988; Hourcade et al., 1989; Morgan and Meri, 1994) (fig. 2; table 2). This protein family includes the membrane-bound regulators complement receptor type 1 (CR1; C3b/C4b receptor; CD35), complement receptor type 2 (CR2; CD21; Epstein-Barr virus receptor), membrane cofactor protein (MCP; CD46; measles virus receptor), decay-accelerating factor (DAF; CD55), and the serum proteins factor H and C4b-binding protein (C4bp).

B. The Alternative Pathway

This arm of the complement system is triggered by microbial surfaces and a variety of complex polysaccha-

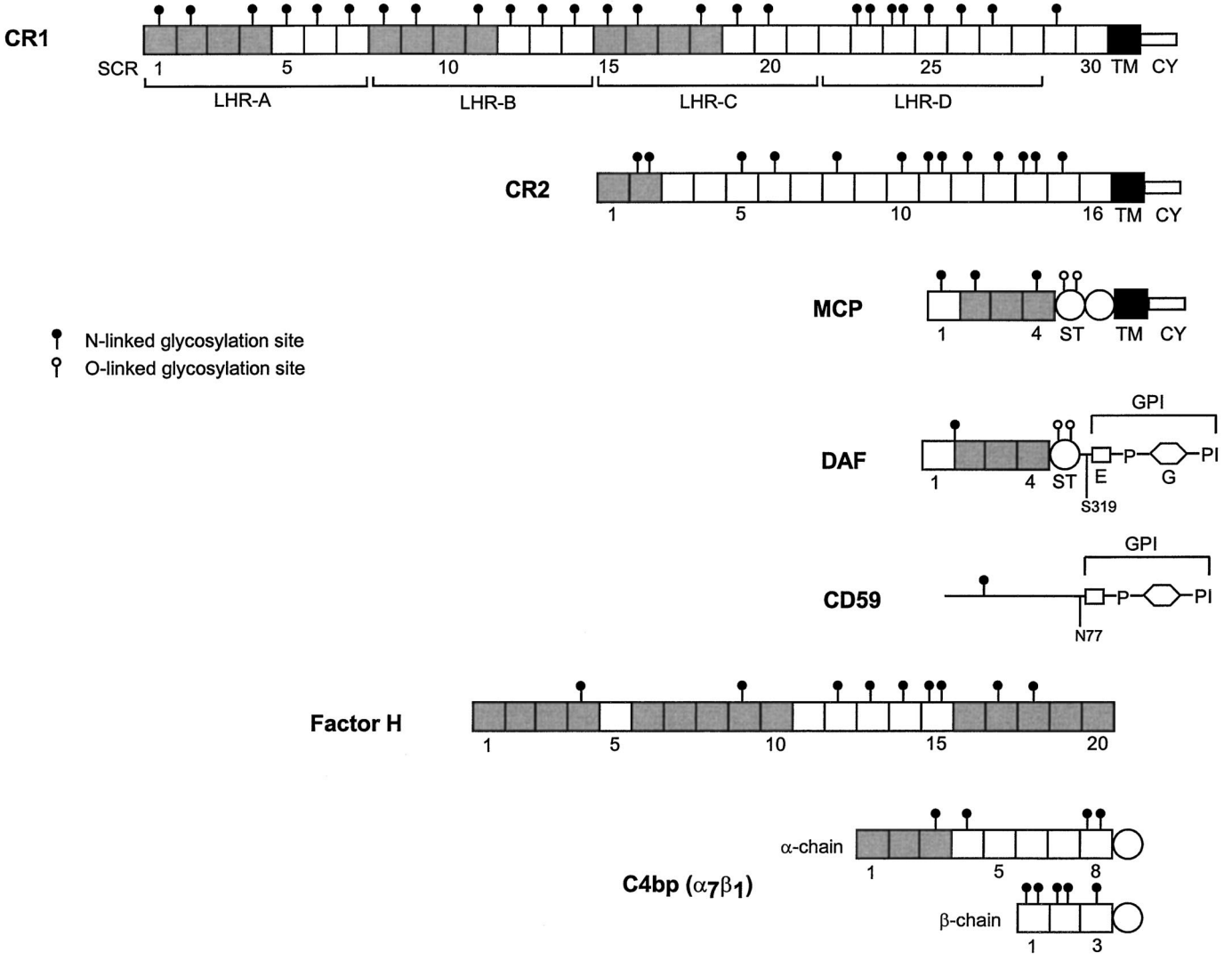

FIG. 2. Schematic representation of the structure of the six members of the RCA family and CD59. Only the common isoforms are shown here. SCRs in each protein are represented by square blocks, and transmembrane regions are shown in solid black. In CR1, groups of seven SCRs are further subdivided into four LHRs (Ahearn and Fearon, 1989). The number of *N*-linked glycosylation sites is shown based on the cDNA sequence of CR1 (Klickstein et al., 1988), CR2 (Moore et al., 1987; Weis et al., 1988), MCP (Liszewski et al., 1991), DAF (Caras et al., 1987; Medof et al., 1987), CD59 (Sugita et al., 1989; Davies et al., 1989), factor H (Ripoche et al., 1988), C4bp α-chain (Chung et al., 1985) and C4bp β-chain (Hillarp and Dahlbäck, 1990). The ligand-binding active sites are stippled in the appropriate SCRs for CR1 (Klickstein et al., 1988; Kalli et al., 1991; Makrides et al., 1992), CR2 (Fearon and Carter, 1995), MCP (Adams et al., 1991), DAF (Coyne et al., 1992; Kuttner-Kondo et al., 1996), factor H (Gordon et al., 1995; Sharma and Pangburn, 1996) and C4bp (Ogata et al., 1993; Härdig et al., 1997). In factor H, only the first C3b-binding site (SCR 1–4) exhibits factor I cofactor activity (Sharma and Pangburn, 1996). Factor H also contains two heparin-binding sites, one near SCR 13 and another in SCR 6–10 (Sharma and Pangburn, 1996) or SCR 7 (Blackmore et al., 1996). The amino acid residues in the active site of CD59 have been identified (Zhou et al., 1996; Yu et al., 1997). CY, cytoplasmic domain; GPI, glycosyl phosphatidyl inositol membrane anchor (E, ethanolamine; G, glycan; PI, phosphatidyl inositol); LHR, long homologous repeat; SCR, short consensus repeat; ST, serine/threonine-enriched domain capable of extensive *O*-linked glycosylation; TM, transmembrane region.

TABLE 2
Regulatory proteins of the complement pathway

Protein	Location	Ligand	Function/activity	References
C1-inhibitor	Plasma	C1r, C1s	Dissociates C1; regulates the contact (kinin-forming pathway); a serpin (Serine Protease Inhibitor)	Davis, 1988; Davis et al., 1993
Factor I	Plasma	C4b, C3b	Cleaves and inactivates C4b and C3b using CR1, MCP, C4bp, or factor H as cofactors	Goldberger et al., 1987; Catterall et al., 1987; Vyse et al., 1996
Factor H	Plasma	C3b	Accelerates decay of C3 convertases in alternative pathway; dissociates B and Bb from C3b; cofactor for cleavage of C3b by factor I	Whaley and Ruddy, 1976; Weiler et al., 1976; Ripoche et al., 1988
C4bp	Plasma	C4b (C3b)	Accelerates decay of C3 convertases in classical pathway; dissociates C2 and C2a from C4b; cofactor for C4b cleavage by factor I	Chung et al., 1985; Gigli et al., 1979
CR1 (CD35)	Membrane	C4b, C3b, iC3b	Accelerates decay of C3 and C5 convertases in classical and alternative pathways; dissociates C2 and C2a from C4b; dissociates B and Bb from C3b; cofactor for cleavage of C4b and C3b by factor I	Ahearn and Fearon, 1989; Klickstein et al., 1988; Krych et al., 1991
CR2 (CD21)	Membrane	iC3b, C3dg, C3d	B cell receptor for complexes having bound C3 fragments; cofactor for cleavage of iC3b by factor I; Epstein-Barr virus receptor	Ahearn and Fearon, 1989; Fearon and Carter, 1995
MCP (CD46)	Membrane	C3b (C4b)	Blocks formation of C3 convertases in classical and alternative pathways; cofactor for cleavage of C3b and C4b by factor I; receptor for measles virus and Streptococcus pyogenes	Seya et al., 1986; Cho et al., 1991; Naniche et al., 1993; Dorig et al., 1993; Okada et al., 1995
DAF (CD55)	Membrane	C4b, C3bA	Accelerates decay of C3 convertases in classical and alternative pathways; dissociates C2 and C2a from C4b; dissociates B and Bb from C3b	Fujita et al., 1987; Nicholson-Weller et al., 1982; Nicholson-Weller and Wang, 1994; Kuttner-Kondo et al., 1996
CD59	Membrane	C7, C8	Blocks formation of MAC on host cells	Davies et al., 1989; Sugita et al., 1993; Rollins et al., 1991
Vitronectin (S-protein)	Plasma	C5b-7	Blocks formation of fluid-phase MAC	Podack and Müller-Eberhard, 1979; Jenne and Stanley, 1985; Suzuki et al., 1985; Hayman et al., 1983; Tschopp et al., 1988; Johnson et al., 1994; Preissner, 1991; Sheehan et al., 1995
Clusterin (SP-40,40)	Plasma	C5b-9	Blocks formation of fluid-phase MAC	Kirszbaum et al., 1989; Tschopp et al., 1993; Rosenberg and Silkensen, 1995; McDonald and Nelsestuen, 1997
Anaphylatoxin inhibitor	Plasma	C5a, C4a, C3a	Cleaves terminal arginine residue and inactivates anaphylatoxins; carboxypeptidase N	Chenoweth, 1986
Properdin	Plasma	C3bBb	Binds to and stabilizes C3 convertase in alternative pathway	Farries et al., 1988; Fearon and Austen, 1975
Nephritic factors	Plasma	C3bBb, C4bC2a	Bind to and stabilize the C3 convertases in classical and alternative pathways resulting in chronic C3 cleavage	Spitzer et al., 1969; Daha et al., 1977; Hiramatsu and Tsokos, 1988

rides. C3b, formed by the spontaneous low-level cleavage of C3, can bind to nucleophilic targets on cell surfaces and form a complex with factor B that is subsequently cleaved by factor D (fig. 1). The resulting C3 convertase is stabilized by the binding of properdin (P) that increases the half-life of this convertase (Fearon and Austen, 1975). Cleavage of C3 and binding of an additional C3b to the C3 convertase give rise to the C5 convertase of the alternative pathway (fig. 1). Subsequent reactions are common to both pathways and lead to the formation of the MAC. The C3 and C5 convertases of the alternative pathway are controlled by CR1, DAF, MCP, and by factor H. These regulators differ in their mode of action, i.e., their decay-accelerating activity (ability to dissociate convertases) and ability to serve as required cofactors in the degradation of C3b or C4b by factor I (tables 2 and 3). In addition, CR2 may have a minor role in regulating complement activation (Fearon and Carter, 1995).

C. The Membrane Attack Complex

The C5 convertases in both the classical and alternative pathways cleave C5 to produce C5a and C5b. Thereafter, C5b sequentially binds to C6, C7, and C8 to form C5b-8 that catalyzes the polymerization of C9 to form the MAC (Tschopp et al., 1982). This structure inserts into target membranes and causes cell lysis (Hu et al., 1981; Podack et al., 1982). However, deposition of small amounts of the MAC on cell membranes of nucleated cells may mediate a range of cellular processes without causing cell death (Morgan, 1992; Nicholson-Weller and Halperin, 1993; Benzaquen et al., 1994).

TABLE 3
Functions of proteins of the RCA family

Protein	Decay-accelerating activity		Factor I cofactor activity		Substrate
	Classical	Alternative	C4b	C3b	
CR1	+	+	+	+	C3b/C4b
CR2	−	−	−	−[a]	iC3b/C3dg
DAF	+	+	−	−	C3b/C4b
MCP	−	−	+	+	C3b/C4b
C4-bp	+	−	+	−	C4b
Factor H	−	+	−	+	C3b

[a] CR2 possesses factor I cofactor activity for iC3b.

Three different molecules are known to be involved in the control of the MAC formation. Vitronectin controls fluid-phase MAC by binding to the C5b-7 complex, preventing its insertion into membranes (Podack et al., 1977). Similarly, clusterin (SP-40,40; cytolysis inhibitor; sulfated glycoprotein 2; apolipoprotein J) (Liszewski et al., 1996) blocks fluid-phase MAC by binding to the C5b-7 complex (Jenne and Tschopp, 1989; Choi et al., 1989; Murphy et al., 1989). CD59 blocks MAC formation by binding to C8 and C9, and inhibiting the incorporation and subsequent polymerization of C9 (Rollins et al., 1991). An additional protein, homologous restriction factor (Zalman, 1992), may be involved in MAC regulation, but it has been suggested that the functional activity reported for homologous restriction factor might possibly be due to a contamination by CD59 aggregates during purification (Liszewski et al., 1996).

III. Modified Native Complement Components That Block Complement Activation

A. Soluble Complement Receptor Type 1

The primary structure of the human CR1 (CD35) has been derived from its complementary deoxyribonucleic acid (cDNA) sequence (Klickstein et al., 1987, 1988; Hourcade et al., 1988). The mature protein of the most common allotype of CR1 contains 1998 amino acid residues: an extracellular domain of 1930 residues, a transmembrane region of 25 residues, and a cytoplasmic domain of 43 residues. The entire extracellular domain is composed of 30 repeating units (fig. 2) referred to as short consensus repeats (SCRs) or complement control protein repeats (CCPRs), each consisting of 60 to 70 amino acid residues. Within each SCR a loop structure is maintained by disulfide linkages between the conserved cysteines-1 and -3, and -2 and -4 (Ahearn and Fearon, 1989). The SCR motif, first shown in β_2-glycoprotein I (Lozier et al., 1984), or a variation thereof, is found in other complement proteins as well as in a large number of noncomplement proteins (Reid and Day, 1989). In CR1, groups of seven SCRs have been organized into four long homologous repeats (LHRs), so that only the SCRs 29 and 30 are not part of a LHR (fig. 2). CR1 has 25 Asn-X-Ser(Thr) sequence motifs (Klickstein et al., 1988) that confer potential *N*-linked glycosylation (Winzler, 1973) (fig. 2). The ligand-binding active sites of CR1 (fig. 2) were originally identified by Klickstein et al. (1988) who demonstrated a C4b-binding site within LHR-A and C3b-binding sites within both LHR-B and LHR-C. These observations were subsequently confirmed by site-directed mutagenesis studies (Krych et al., 1991, 1994). Optimal binding affinities equivalent to those of native CR1 were later demonstrated to reside within SCRs 8–11 and 15–18 for C3b (Kalli et al., 1991; Makrides et al., 1992) and in SCRs 1–4 for C4b (Reilly et al., 1994). CR1 has extrinsic activity (Medof et al., 1982), i.e., it inactivates convertases assembled on external membranes, and it also exhibits intrinsic activity (Kinoshita et al., 1986; Makrides et al., 1992), i.e., it inactivates convertases formed on the same membrane on which it is expressed. Among the members of the RCA family (table 3), CR1 is the only one that possesses decay-accelerating activity for both C3 and C5 convertases in both the classical and alternative pathways, as well as factor I cofactor activity for the degradation of both C3b and C4b (Fearon, 1991). Recent data indicate that C1q binds specifically to human CR1 (Klickstein et al., 1997). Thus, CR1 recognizes all three complement opsonins, namely C3b, C4b, and C1q.

A soluble version of recombinant human CR1 (sCR1) lacking the transmembrane and cytoplasmic domains was produced and shown to retain all the known functions of the native CR1 (Weisman et al., 1990a,b). Initial studies centered on the use of sCR1 in animal models of ischemia/reperfusion injury. Although thrombolytic agents have been used effectively in ischemic myocardium to induce reperfusion, blood reflow into ischemic tissue may induce necrosis because of complement activation, neutrophil accumulation in the microvasculature, and consequent damage to the endothelium (Homeister and Lucchesi, 1994). Administration of sCR1 in a rat model of ischemia/reperfusion injury reduced myocardial infarct size by 44% assessed at 7 days postdosage and minimized the accumulation of neutrophils within the infarcted area, probably because of a decreased generation of the anaphylatoxin C5a (Weisman et al., 1990a,b). In addition, sCR1 attenuated the deposition of the C5b-9 MAC. This was the first demonstration that a recombinant soluble form of a member of the RCA family might provide a potential therapeutic agent in inflammation. The cardioprotective role of sCR1 in animal models of ischemia/reperfusion injury has been confirmed (Shandelya et al., 1993; Smith et al., 1993; Homeister et al., 1993). Similarly, sCR1 reduced tissue injury in ischemia/reperfusion of mouse skeletal muscle (Pemberton et al., 1993), rat intestine (Hill et al., 1992), rat liver (Chávez-Cartaya et al., 1995), and remote organs after lower torso ischemia in the rat (Lindsay et al., 1992). In addition to its efficacy in models of ischemia/reperfusion, sCR1 has been shown to reduce complement-mediated tissue injury in animal models with a wide range of human acute and chronic inflammatory diseases. These include dermal vascular reactions (Yeh

et al., 1991; Mulligan et al., 1992b), lung injury (Rabinovici et al., 1992; Mulligan et al., 1992a,b), trauma (Kaczorowski et al., 1995), myasthenia gravis (Piddlesden et al., 1996), glomerulonephritis (Couser et al., 1995), multiple sclerosis (Piddlesden et al., 1994), allergic reactions (Lima et al., 1997), and asthma (Regal et al., 1993). In addition, sCR1 protects against vascular injury and cellular infiltration in allografts (Pratt et al., 1996a,b) and attenuates hyperacute rejection in xenografts (Baldwin et al., 1995; Ryan, 1995; Levin et al., 1996) (see Section VII.).

Pharmacokinetic studies of earlier preparations of sCR1 showed that the β-phase half-life ($t_{1/2}\beta$) was approximately 1.7 h in rats, and 8 h in humans (J. Levin, unpublished data). A longer circulating half-life might permit bolus-dosage administration, allow lower dosages of a drug to achieve comparable therapeutic effects and reduce the cost per therapeutic dosage. One experimental approach used to extend the circulating half-life of sCR1 utilized the albumin-binding terminus "BA" or "BABA" of Streptococcal protein G as a fusion partner with sCR1 (Makrides et al., 1996). The resulting sCR1-BA fusion construct exhibited a significantly longer half-life (297 min) than sCR1 (103 min) in rats (Makrides et al., 1996). Fearon and colleagues chose the Ig molecule as a fusion partner with SCRs 8–11, the C3b-binding site of CR1. The $(CR1)_2$-$F(ab')_2$ chimera was as effective as sCR1 in binding to C3b dimers, promoting cleavage of C3b by factor I and inhibiting activation of the alternative pathway (Kalli et al., 1991). A potential application of this finding is the fusion of CR1 active units to full-length IgG to create chimeras with a longer half-life than sCR1 because of the long plasma half-life of the Fc moiety (Capon et al., 1989). An additional advantage of an Ig fusion partner is the potential for targeting complement inhibition to specific tissues (Kalli et al., 1994). Thus, a monoclonal antibody (mAb) specific for antigen localized in the area of complement activation could be used to construct a CR1/Ig molecule that might act as a local rather than a systemic complement blocker.

However, none of the above molecular constructions is likely to be therapeutically useful because of the potential immunogenicity of the fusion partners. The genetic engineering or "humanization" of antibodies (Co and Queen, 1991; Rapley, 1995; Morrison and Shin, 1995) might minimize immunogenic reactions but not completely eliminate anti-idiotypic effects. Most important, the problems associated with the short half-life of sCR1 appear to have been solved. Thus, a subsequent preparation of sCR1 obtained using modified culture conditions showed a $t_{1/2}\beta$ of approximately 30 h in humans (Dellinger et al., 1995, 1996). The reason for the longer half-life of sCR1 is unknown but may be related to a potentially altered glycosylation pattern resulting from the culture conditions.

It has been suggested that the effects of complement on the endothelium are mediated primarily by the MAC because the human vascular endothelium is apparently devoid of receptors for anaphylatoxins (conference discussion cited in Morgan, 1995a). Although the expression of the C5a receptor (C5aR) was thought to be limited to leukocytes, the molecular cloning of the human C5aR demonstrated its expression on nonmyeloid cells, including the vascular endothelium (Haviland et al., 1995; Wetsel, 1995). Similarly, the human C3a receptor (C3aR) has recently been cloned by three groups (Ames et al., 1996; Roglic et al., 1996; Crass et al., 1996). The C3aR, originally thought to be an orphan receptor (Roglic et al., 1996), was shown to be expressed in endothelial cells (Roglic et al., 1996). It is now clear that complement activation products have many diverse effects on endothelial cells, and, in fact, the endothelium may be a major target of the complement system (Ward, 1996).

The ability of sCR1 to block activation of both the classical as well as the alternative pathways has been thought (Evans et al., 1995) to potentially reduce its therapeutic value because it inhibits generation of C3b, a C3 opsonic product that is critical for antibacterial defenses (Ross and Densen, 1984). The possibility that a global inhibitor of complement activation might compromise antibacterial defenses was recognized by Becker (1972) who concluded that "this risk might not be unacceptably high." To date, there is no credible evidence that sCR1 compromises bacterial defenses in animal models of inflammation. More importantly, two phase I clinical trials of sCR1 in patients with myocardial infarct or burn-induced adult respiratory distress syndrome revealed no safety issues in this regard, including rates of bacterial infection (J. Levin, personal communication). The adult respiratory distress syndrome trial is of particular relevance in this context because people who are severely burned may die of bacterial sepsis. In this environment, sCR1 had no effect on systemic bacterial infections (J. Levin, personal communication).

B. Soluble Complement-Receptor Type 1 Lacking Long Homologous Repeat-A

A mutant version of sCR1 lacking LHR-A sCR1[desLHR-A] was constructed with the objective of generating a selective inhibitor of the alternative pathway (Scesney et al., 1996). The rationale for this is based on the fact that C4b is a component of the classical pathway exclusively, whereas C3b functions in both classical and alternative pathways (fig. 1). Thus, removal of the C4b-binding LHR-A from sCR1 would be expected to abrogate the ability of sCR1[desLHR-A] to accelerate the decay of the C3 and C5 convertases in the classical pathway (fig. 1). Indeed, sCR1[desLHR-A] was shown to be quantitatively equivalent to sCR1 in its ability to inhibit the alternative pathway in vitro (Scesney et al., 1996). On the other hand, sCR1[desLHR-

A] was less effective than sCR1 in blocking activation of the classical pathway in vitro. Both sCR1[desLHR-A] and sCR1 exhibited equal capacities to serve as cofactor in the degradation of fluid-phase C3b by factor I (Scesney et al., 1996). These results are consistent with the observations of Kalli et al. (1991) who constructed a chimera between SCR 8–11 of CR1 and an antibody $F(ab')_2$ fragment, thus allowing the bivalent presentation of SCR 8–11 (C3b-binding site). In that case, the chimera $(CR1)_2$-$F(ab')_2$ and sCR1 were shown to be equivalent in their capacity to inhibit the alternative pathway of complement activation, although the chimera, which lacks the C4b-binding site found in LHR-A, was considerably less effective at inhibiting the classical pathway (Kalli et al., 1991).

The availability of sCR1[desLHR-A] facilitated examination of the relative contributions of the classical and alternative pathways in a model of discordant xenotransplantation in which an isolated perfused heart from a rabbit is exposed to human plasma that serves as a complement source (Homeister et al., 1992). The interaction of rabbit heart tissue with plasma activates complement, leading to the production of anaphylatoxins and the generation of C5b-9 membrane attack complex. Both sCR1 and sCR1[desLHR-A] had a cardioprotective effect in the rabbit heart perfused with human plasma (Gralinski et al., 1996). Complement activation was also shown to attenuate endothelium-dependent relaxation in rabbit tissue (Lennon et al., 1996). This attenuation was dependent on the formation of C5b-9 via the classical and alternative pathways, as demonstrated through the use of human serum depleted in factor B, C2, or C8. The use of sCR1 and sCR1[desLHR-A] decreased the loss of endothelium-dependent relaxation in rabbit thoracic aortic rings (Lennon et al., 1996). Murohara et al. (Murohara et al., 1995a) examined the relative contribution of the classical and alternative pathways in a rat model of ischemia and reperfusion injury using either C1 esterase inhibitor (see Section III.J), a classical pathway inhibitor, or sCR1[desLHR-A]. These authors concluded that both the classical and alternative pathways contribute to reperfusion injury in myocardial ischemia by a neutrophil-dependent mechanism. Selective inhibition of the classical pathway appeared to be slightly more effective in limiting tissue injury than the selective inhibition of the alternative pathway in this model (Murohara et al., 1995a).

C. Soluble Complement Receptor Type 1-Sialyl Lewis^x

This compound is designed to simultaneously inhibit both complement activation and neutrophil recruitment at sites of inflammation (C. Rittershaus, personal communication). The rationale behind the development of this complement inhibitor is based on the current understanding of the interaction between complement and selectins in inflammation (Lefer et al., 1994a; Lefer, 1995; Mulligan et al., 1996) and the demonstration that C5a up-regulates P-selectin (Foreman et al., 1994; Mulligan et al., 1997). The migration of leukocytes to sites of inflammation is a complex and highly regulated process that is orchestrated by chemoattractants and a large number of adhesion molecules that are involved in cell-cell and cell-matrix interactions. These adhesion molecules are members of the four families of receptors, the selectins, the integrins, the Ig superfamily, and the cadherins (Zimmerman et al., 1992; Pardi et al., 1992; Mackay and Imhof, 1993; Albelda et al., 1994; Springer, 1994; Malik and Lo, 1996; Butcher and Picker, 1996). The selectins, L-, P-, and E-selectins, participate in the initial "rolling" adhesions, bringing the circulating leukocytes into close proximity with chemoattractants released from endothelial cells of the vessel wall. Chemoattractants bind to G protein-coupled receptors on leukocytes, signaling the activation of integrins that, together with members of the Ig superfamily effect the arrest and subsequent migration of leukocytes into the tissue (Springer, 1994). Although this model of neutrophil extravasation suggests that rolling is a necessary precursor to subsequent adhesive events, experimental evidence indicates the possibility of simultaneous, rather than sequential activity of the various adhesion molecules of the inflammatory cascade (Doerschuk et al., 1993; Hogg and Doerschuk, 1995; Ward, 1995; Lowe and Ward, 1997).

Selectin function, unlike that of most other adhesion molecules, appears to be restricted to interactions between leukocytes and the vascular endothelium (Tedder et al., 1995). The selectins bind carbohydrate ligands containing fucose, including SLe^x (Neu5Acα2–3Galβ1–4(Fucα1–3)GlcNAc-) (Phillips et al., 1990; Polley et al., 1991; Foxall et al., 1992; Rosen and Bertozzi, 1994; Bertozzi, 1995; McEver et al., 1995). Other proteins, including PSGL-1, CD34, and GlyCAM-1 have been identified as high-affinity ligands for selectins (Lasky, 1995; Kansas, 1996). There is a diversity of opinions as to the identities of the physiologically relevant ligands for selectins (Varki, 1994, 1997; Kansas, 1996). Nevertheless, the observation that SLe^x can inhibit neutrophil adhesion mediated by both E- and P-selectins (Phillips et al., 1990; Lasky, 1992) led to vigorous efforts to develop compounds for the therapeutic disruption of the selectin-SLe^x interaction in inflammation. Such antagonists include SLe^x and its analogs (Mulligan et al., 1993a; Rao et al., 1994; Bertozzi et al., 1995; Flynn et al., 1996; Lefer et al., 1994b; Buerke et al., 1994; Murohara et al., 1995c; Maaheimo et al., 1995; Lin et al., 1996; Tojo et al., 1996; Zhang et al., 1996), antibodies against SLe^x (Dinh et al., 1996; Seko et al., 1996) or against P-selectin (Lefer et al., 1996; Doerschuk et al., 1996), peptides (Briggs et al., 1995; Geng et al., 1992; Heavner et al., 1993; Briggs et al., 1996; Martens et al., 1995; Norman et al., 1996), oligonucleotides (Murohara et al., 1996; Hicke et al., 1996; O'Connell et al., 1996), fucoidin (Kubes et al., 1995), inositol polyanions (Cecconi et al.,

1994), sulfatides (Mulligan et al., 1995), heparin-derived oligosaccharides (Nelson et al., 1993), sulfated neoglycopolymers (Manning et al., 1997), a hydroxamic acid-based peptide inhibitor of matrix metalloproteases (Walcheck et al., 1996), and chimeric proteins (Mulligan et al., 1993b; Fujise et al., 1997). Recently, the 3′-sulfated Lewis^a pentasaccharide was demonstrated to prevent ischemia-reperfusion lung injury in a rat model (Reignier et al., 1997). The 3′-sulfated Lewis^a has been shown to be a more potent ligand for E- and L-selectins as compared with SLe^x (Green et al., 1995; Yuen et al., 1994). The biological effects of many of these compounds in selectin-dependent animal models of inflammation have been critically reviewed (Lowe and Ward, 1997).

The protective effects of SLe^x synthetic analogues have been demonstrated in several models of inflammation, including feline (Buerke et al., 1994) and canine (Lefer et al., 1994b; Flynn et al., 1996) models of myocardial ischemia/reperfusion, as well as in a rat model of lung injury (Mulligan et al., 1993a). However, the use of the SLe^x analogue CY-1503 did not reduce myocardial infarct or neutrophil accumulation in dogs subjected to ischemia/reperfusion injury (Gill et al., 1996). These conflicting results may in part be explained by the dosing regimes employed by the different investigators and the relatively short half-life of the SLe^x analogue. Of key importance is the high IC_{50} (0.5 to 1.0 mM) of the monovalent SLe^x tetrasaccharide in inhibiting E- and P-selectin-dependent adhesion of leukocytes, as determined in static adhesion assays (Jacob et al., 1995). SLe^x multivalency appears to enhance its binding to L-selectin (Maaheimo et al., 1995). Thus, a synthetic SLe^x analog was tested as an inhibitor of L-selectin-mediated lymphocyte-endothelium interactions in rejecting rat kidney transplant. Although the nonfucosylated *O*-glycosidic oligosaccharide did not possess any inhibitory activity, the monovalent SLe^x molecule prevented the binding significantly, and the divalent SLe^x saccharide was the most potent inhibitor (Maaheimo et al., 1995).

Complement activation and, in particular, generation of C5a attract and stimulate neutrophils, causing their sequestration within capillaries. Activated neutrophils produce toxic oxygen metabolites that damage endothelial cells (Mulligan et al., 1996, 1997). C5a is necessary for up-regulation of vascular P-selectin after systemic activation of complement (Foreman et al., 1994; Mulligan et al., 1997; Ward, 1996). To control the damaging effects of both complement and neutrophil activation during inflammation, sCR1 was produced in a mammalian cell line capable of SLe^x glycosylation (Picard et al., 1996; Sen et al., 1966; Bertino et al., 1996). It was shown that sCR1 purified from conditioned media possessed SLe^x moieties on the *N*-linked oligosaccharides. sCR1 potentially has 25 *N*-glycosylation sites (Klickstein et al., 1988) and, although not every Asn-X-Ser(Thr) sequon is an efficient oligosaccharide acceptor (Kasturi et al., 1997), it is expected that sCR1-SLe^x would be extensively decorated with SLe^x moieties. Thus, in addition to blocking complement activation, the potential multivalent interactions between sCR1-SLe^x and its selectin counterligands might render this molecule particularly effective at inhibiting neutrophil activation and recruitment to sites of inflammation on the endothelial surface. It is important to determine the half-life of sCR1-SLe^x and, especially, whether it localizes to sites of inflammation. Notably, the basic SCR structure of CR1 occurs in selectins, and this might allow relatively easy structural modifications and a "cassette" approach to the molecular construction of hybrid molecules. For example, constructs possessing the ability to home to areas of inflamed endothelium might be readily combined with elements effecting multivalent complement inhibition. Spacing of the active segments along the construct could be varied for optimal interaction with complement elements while still retaining the affinity of the selectins for targeted endothelium.

D. Complement Receptor Type 2

The molecular cloning of the human CR2 (Moore et al., 1987; Weis et al., 1988) facilitated its structural and functional characterization (Ahearn and Fearon, 1989; Fearon and Carter, 1995; Carroll and Fischer, 1997). CR2 (CD21; Epstein-Barr virus receptor) is present on follicular dendritic cells, mature B cells, and a subpopulation of T cells, and it binds the C3 breakdown fragments, C3dg and C3d. CR2 has relatively weak cofactor activity for the factor I-mediated breakdown of iC3b to C3dg and C3c (Mitomo et al., 1987), and it probably plays a minor role in complement regulation. CR2 has B cell-stimulating functions, as it associates with CD19, a B cell surface molecule that activates B cells, and participates in T cell-dependent B cell responses (Fearon and Carter, 1995; Carroll and Fischer, 1997). Fearon and colleagues provided direct evidence that attachment of C3d to antigen significantly enhances humoral responses, a process that is mediated by CR2 (Dempsey et al., 1996). The immune-augmenting function of C3d was demonstrated by the fusion of murine C3d to hen egg lysozyme (HEL). Thus, HEL bearing three copies of C3d was ten thousand-fold more immunogenic than HEL alone, suggesting that such manipulations might allow for development of effective strategies for vaccination without the need for adjuvant (Dempsey et al., 1996).

E. Soluble Decay Accelerating Factor

Decay accelerating factor (DAF) (CD55) is composed of four SCRs plus a serine/threonine-enriched domain that is capable of extensive *O*-linked glycosylation (fig. 2) (Nicholson-Weller and Wang, 1994). DAF is attached to cell membranes by a glycosyl phosphatidyl inositol (GPI) anchor (Davitz et al., 1986; Medof et al., 1986) and, through its ability to bind C4b and C3b, it acts by dissociating the C3 and C5 convertases in both the classical and alternative pathways (fig. 1). Unlike CR1, which

possesses both extrinsic (Medof et al., 1982) and intrinsic activity (Kinoshita et al., 1986; Makrides et al., 1992), DAF functions only intrinsically by inactivating convertases assembled on the same cell membrane on which it is expressed and not those convertases formed on external membranes (Medof et al., 1984). Soluble versions of DAF (sDAF) have been shown to inhibit complement activation in vitro (Christiansen et al., 1996; Moran et al., 1992) as well as in the reversed passive Arthus reaction in guinea pigs (Moran et al., 1992) (table 4).

The clinical usefulness of a complement blocker depends on several requirements (Kalli et al., 1994). These include the ability to inhibit the C5 convertases of both classical and alternative pathways, a high affinity for the C3b and C4b components of the convertases, the irreversible inactivation of the convertases, and the ability to recycle in order to block multiple convertases (Kalli et al., 1994). The modest inhibitory activity of sDAF (Christiansen et al., 1996; Moran et al., 1992; Kalli et al., 1994) and its lack of factor I cofactor activity limit its therapeutic potential as a complement blocker.

F. Soluble Membrane Cofactor Protein

Membrane cofactor protein (CD46; measles virus receptor) (fig. 2) has factor I cofactor activity but no decay-accelerating activity. It acts jointly with DAF, which has decay-accelerating activity but no cofactor activity (table 3) to block C3b/C4b deposition on cell membranes (Liszewski et al., 1991). MCP is an intrinsic regulator of complement activation, i.e., it protects cells on which it is expressed, but it does not protect neighboring cells (Oglesby et al., 1992). It is expressed primarily as four isoforms, termed BC1, BC2, C1, and C2, that are formed by alternative splicing of a single gene and that differ in the domains for *O*-glycosylation and cytoplasmic regions (reviewed in Liszewski et al., 1996). The BC isoforms have been shown to cleave cell-bound C4b more efficiently than the C isoforms and to provide enhanced cytoprotection against the classical pathway (Liszewski and Atkinson, 1996). A recombinant sMCP was shown to inhibit immune complex-mediated inflammation in the reverse passive Arthus reaction model in rats (Christiansen et al., 1996). As in the case of sDAF, the single activity of sMCP limits its potential as an effective therapeutic reagent. However, sMCP may prove to be a valuable reagent in combination with other complement inhibitors (see Section III.I.).

G. Soluble CD59

CD59, also known by several other names (Liszewski et al., 1996), is a single-chain glycoprotein that is GPI-anchored to cell membranes (Holguin et al., 1989; Davies et al., 1989; Davies and Lachmann, 1993). The carbohydrate moiety at the single *N*-glycosylation site is not required for complement inhibition (Suzuki et al., 1996; Rushmere et al., 1997). CD59 functions as an inhibitor of the formation of the MAC on cells by binding to C8 and C9, thereby blocking the addition of polymerized C9 molecules (Meri et al., 1990; Rollins et al., 1991). sCD59 has been shown to possess complement inhibitory activity in vitro (Sugita et al., 1994). However, the potential usefulness of sCD59 as a therapeutic complement blocker is limited by its lack of certain functional properties (as discussed in Section III.E.) (Kalli et al., 1994). Although the inhibition of MAC assembly would be of benefit in inflammation, the late stage in the complement cascade at which CD59 acts (fig. 1), leaves the generation of anaphylatoxins and their pathological sequelae unaffected.

H. Decay Accelerating Factor-CD59 Hybrid

The molecular fusion of different complement regulatory proteins has been used to create chimeric molecules endowed with novel functions. Fodor and colleagues (Fodor et al., 1995) constructed two such chimeric complement inhibitors for cell surface expression using a GPI anchor: CD (NH2-CD59-DAF-GPI) and DC (NH2-DAF-CD59-GPI). The rationale behind this work was to create a single protein that blocks C3 and C5 convertase activity as well as the assembly of the MAC. Of the two molecules, CD retained DAF function, but did not inhibit C5b-9 assembly. The DC chimera, however, exhibited both DAF and CD59 activity. The reason for the differential function of the two molecules was thought to be the different orientation of the protein domains. Thus, in the CD molecule, the CD59 moiety occupies a membrane-distal position where it cannot interact with the C5b-8 and C5b-9 complex, although in the DC molecule the membrane-proximal position of the CD59 domain facilitates the interaction between CD59 and the MAC (Fodor et al., 1995). The DC chimera may have utility in the production of transgenic organs (see also Section VII.) for the inhibition of hyperacute rejection in xenotransplantation (Kennedy et al., 1994; Fodor et al., 1994; McCurry et al., 1995a,b; Miyagawa et al., 1995; Heckl-Östreicher et al., 1996; Kroshus et al., 1996b; Diamond et al., 1996; Byrne et al., 1997).

I. Membrane Cofactor Protein-Decay Accelerating Factor Hybrid

The molecular fusion of membrane cofactor protein (MCP) and decay acclerating factor (DAF) brings together the complementary activities of these two regulatory molecules to create a single protein that has both factor I cofactor activity and decay-accelerating activity. A membrane-bound chimeric MCP-DAF was expressed in CHO cells, and its activity was compared with that of transfectants expressing MCP or DAF or MCP plus DAF (Iwata et al., 1994). The proteins differed in their ability to block C3 deposition on sensitized CHO cells through activation of the classical pathway, in the order of MCP + DAF > DAF > MCP-DAF > MCP. C3 deposition via the alternative pathway was blocked in the order MCP-

TABLE 4
Protein inhibitors of complement activation

Protein	Identity	Site/mode of action	Disease indication/model	References
TP10 (sCR1)	Soluble CR1	C3/C5 convertases; classical/alternative	ARDS (clinical); lung allotransplantation (clinical); xenotransplantation	Weisman et al., 1990a,b; Dellinger et al., 1995, 1996; Levin et al., 1996; Marsh and Ryan, 1997
sCR1-SLe^x	sCR1 glycosylated with SLe^x	C3/C5 convertases; classical/alternative; selectin-mediated	Pre-clinical	Picard et al., 1996; Bertino et al., 1996; Marsh and Ryan, 1997
sCR1[desLHR-A]	sCR1 minus LHR-A	C3/C5 convertases alternative	In vitro Discordant xenotransplantation Endothelial dysfunction Ischemia reperfusion injury	Scesney et al., 1996 Gralinski et al., 1996 Lennon et al., 1996 Murohara et al., 1995a
sCR1[desLHR-A]-SLe^x	sCR1 minus LHR-A glycosylated with SLe^x	C3/C5 convertases; alternative; selectin-mediated	In vitro	Marsh, 1997[a]
sCD59	Soluble CD59	MAC assembly	In vitro	Sugita et al., 1994; Suzuki et al., 1996
sDAF	Soluble DAF	C3/C5 convertases Classical/alternative	Reverse passive Arthus reaction In vitro	Moran et al., 1992 Christiansen et al., 1996
sMCP	Soluble MCP	Factor I cofactor activity	Reverse passive Arthus reaction	Christiansen et al., 1996
C1-INH	C1 esterase inhibitor	C1 inactivation; classical	Reperfusion injury Xenograft hyperacute rejection	Horstick et al., 1997; Buerke et al., 1995; Murohara et al., 1995b Dalmasso and Platt, 1993, 1994
CAB-2	Soluble chimeric MCP-DAF	C3/C5 convertases Classical/alternative	Arthus reaction, Forssman shock In vitro	Higgins et al., 1997 Iwata et al., 1994
CD	Membrane-bound chimeric NH_2-CD59-DAF-GPI	C3/C5 convertases Classical/alternative	In vitro	Fodor et al., 1995
DC	Membrane-bound chimeric NH_2-DAF-CD59-GPI	C3/C5 convertases Classical/alternative MAC assembly	In vitro	Fodor et al., 1995
N19-8 mAb	Anti-human C5 murine monoclonal antibody	C5, MAC assembly	Cardiopulmonary bypass Ex vivo model	Würzner et al., 1991; Rinder et al., 1995
N19-8 scFv	Anti-human C5 murine single chain Fv	C5, MAC assembly	Myocardial reperfusion injury	Evans et al., 1995
5G1.1-SC	Anti-human C5 humanized scFv	C5, MAC assembly	Cardiopulmonary bypass	Thomas et al., 1996
C1qR	66-kDa C1qR, detergent-solubilized	Classical	In vitro	van den Berg et al., 1995
C5aR antagonists	C5a oligopeptide analogs	C5aR	In vitro; dermal inflammation; C5a-induced neutropenia	van Oostrum et al., 1996
Factor J	Glycoprotein	Classical/alternative	In vitro	López-Trascasa et al., 1989; González-Rubio et al., 1994

[a] Marsh H Combined complement inhibition and selectin binding by sCR1sLe^x and sCR1[desLHR-A]sLe^x. Paper presented at: "New Therapeutic Targets Based on Control of Complement System," June 9–11, 1997; Boston, MA.

DAF > MCP + DAF > DAF > MCP (Iwata et al., 1994). Thus, in this in vitro system, the hybrid surface-bound protein appeared to have greater potency at blocking alternative rather than classical pathway activation. Similar studies were performed in vitro in stably transfected swine endothelial cells exposed to human complement (Miyagawa et al., 1994). In this model of xenograft hyperacute rejection, mediated mainly by the classical pathway, the surface-expressed MCP-DAF hybrid inhibited cell lysis more effectively than MCP alone, and apparently as effectively as DAF. Differences in lysis, however, were rather small, and the quantitative differences in the levels of surface expression of the molecules make it difficult to draw firm conclusions regarding their relative effectiveness (Iwata et al., 1994; Miyagawa et al., 1994). Nevertheless, these studies demonstrate the dual functionality and complement inhibitory activity of the MCP-DAF hybrid.

A soluble version of chimeric MCP-DAF, referred to as complement activation blocker-2 (CAB-2), possessed factor I cofactor activity and decay-accelerating activity, and inactivated both classical and alternative C3 and C5 convertases in vitro as measured by assays of inhibition of cytotoxicity and anaphylatoxin generation (Higgins et al., 1997). CAB-2 had inhibitory activity against cell-bound convertases that was greater than that of either sMCP or sDAF or both factors combined. This hybrid was shown to inhibit complement activation in vivo, in the reversed passive Arthus reaction and in the direct passive Arthus reaction, as well as in the Forssman shock model in guinea pigs. The $t_{1/2}\beta$ of CAB-2 in rats was 8 h (Higgins et al., 1997), which is suitable for human therapy. It is possible that the half-life of CAB-2 may be longer in humans than in rats, as has been the case for sCR1 (see Section III.A.). One potential limitation of CAB-2 as a therapeutic is its potential immunogenicity. The molecular fusion of two otherwise natural proteins is likely to create novel epitopes, which might trigger an immune response. In this case, CAB-2 might be useful in acute indications, depending on the severity of the anti-CAB-2 response.

J. C1 Inhibitor

C1 inhibitor, a member of the "serpin" family of serine protease inhibitors, is a heavily glycosylated plasma protein that prevents fluid-phase C1 activation (reviewed in Davis, 1988; Davis et al., 1993). C1 inhibitor regulates the classical pathway of complement activation (fig. 1) by blocking the active site of C1r and C1s and dissociating them from C1q (Ziccardi and Cooper, 1979). Studies of the role of complement activation in myocardial ischemia and reperfusion injury (reviewed in Homeister and Lucchesi, 1994; Makrides and Ryan, 1997) have used C1 inhibitor in feline (Buerke et al., 1995), rat (Murohara et al., 1995a), and pig (Horstick et al., 1997) models. All these studies have demonstrated that blocking the classical pathway of complement activation by C1 inhibitor is an effective means of protecting ischemic myocardial tissue from reperfusion injury.

K. C1q Receptor

Several types of human C1q receptors (C1qR) have been described. These include the ubiquitously distributed 60- to 67-kDa receptor, referred to as cC1qR because it binds the collagen-like domain of C1q (Peerschke et al., 1993; Malhotra et al., 1993). This C1qR variant was shown to be calreticulin (Malhotra et al., 1993; Stuart et al., 1996); a 126-kDa receptor that modulates monocyte phagocytosis, designated $C1qR_p$ (Guan et al., 1991, 1994; Nepomuceno et al., 1997); and a 28- to 33-kDa protein isolated and cloned from Raji cells, termed gC1qR because it interacts preferentially with the globular domains of C1q (Ghebrehiwet et al., 1994; Peerschke et al., 1996). A recent study showed that CR1 also acts as a receptor for C1q (Klickstein et al., 1997). Experimental evidence supports the hypothesis that gC1qR is not a membrane-bound molecule, but rather a secreted soluble protein with affinity for the globular regions of C1q (van den Berg et al., 1997). Thus, it may act as a fluid-phase regulator of complement activation. van den Berg et al. (1997) did not detect surface expression of gC1qR but were able to demonstrate strong intracellular staining for this protein, as well as its presence in human and rat sera and in supernatants of cultured HUVEC. Furthermore, other data are consistent with the molecular properties of gC1qR. Thus, the cDNA sequence (Ghebrehiwet et al., 1994) encodes a protein that lacks a membrane-spanning domain (Fasman and Gilbert, 1990) or a consensus sequence for GPI-anchoring (Medof et al., 1996). It is possible, however, that under certain conditions gC1qR may be surface-expressed at low levels, or it may bind to cell membranes as a complex with other fluid-phase molecules (van den Berg et al., 1997). The ability of C1qR (66 kDa) to inhibit the classical pathway of complement has been demonstrated in vitro. Membrane-associated C1qR as well as detergent-solubilized C1qR, purified from polymorphonuclear leukocytes and endothelial cells, blocked complement-mediated lysis of C1q-sensitized erythrocytes (van den Berg et al., 1995).

The mechanisms by which the different types of C1qR regulate complement activation in vivo and the physiological significance of the putative fluid-phase C1qR (van den Berg et al., 1995, 1997) remain unclear. However, the studies cited here, and the demonstration that C1q is required for immune complexes to stimulate endothelial cells to express adhesion molecules (Lozada et al., 1995), suggest a potential therapeutic use in preventing vascular injury.

IV. Complement-Inhibitory Antibodies

A. Anti-C5 Monoclonal Antibody

Inhibition of C5 activation using high-affinity (K_d < 100 pM) anti-C5 monoclonal antibodies (mAbs) repre-

sents another therapeutic approach for blocking complement activation (Matis and Rollins, 1995; Rinder et al., 1995). This strategy is aimed at inhibiting the formation of C5a and C5b-9 via both the classical and alternative pathways (fig. 1), without affecting the generation of C3b, a C3 opsonic product that is critical for antibacterial defenses (Ross and Densen, 1984). This is scientifically sound, although, as discussed above (section III.A.), the on-going clinical trials using sCR1 have produced no evidence to date that blockade of the C3 and C5 convertases in both the classical and alternative pathways compromises bacterial defenses. Another suggested advantage (Wang et al., 1995) of using monoclonal antibodies to block C5 activation is the prevention of the direct cleavage and activation of C5 by oxygen radicals (Vogt et al., 1989) or by enzymes released from injured tissue (Wetsel and Kolb, 1982, 1983) during inflammation.

The efficacy of a mAb specific for murine C5 was demonstrated in the treatment of collagen-induced arthritis, an animal model for human rheumatoid arthritis. It was shown that the systemic administration of the anti-C5 mAb in mice blocked complement activation, prevented the onset of arthritis in immunized animals, and ameliorated established disease (Wang et al., 1995). The same anti-C5 mAb was tested in mice that develop an autoimmune disorder similar to human systemic lupus erythematosus. Continuous treatment with the antibody resulted in significant reduction in glomerulonephritis and in increased survival (Wang et al., 1996).

The anti-human C5 mAb N19/8 (Würzner et al., 1991) that does not inhibit formation of C3a was tested in an in vitro model of extracorporeal blood flow that activates complement, platelets, and neutrophils (Rinder et al., 1995). This mAb inhibited the generation of C5a and soluble C5b-9 and blocked serum complement hemolytic activity, without affecting the production of C3a. In addition, the anti-C5 mAb inhibited neutrophil CD11b upregulation, abolished the increase in P selectin-positive platelets, and reduced formation of leukocyte-platelet aggregates (Rinder et al., 1995). Thus, it appears that C5a and C5b-9, but not C3a contribute to platelet and neutrophil activation during extracorporeal procedures. Although the N19/8 mAb could be used in human therapy, it is recognized that chronic application of monoclonal antibodies would elicit human anti-mouse antibody responses (Waldmann, 1991; Khazaeli et al., 1994). The "humanization" of antibodies (Co and Queen, 1991; Rapley, 1995; Morrison and Shin, 1995) should minimize immunogenic reactions, although it might be difficult to completely eliminate anti-idiotypic effects. Recent advances in transgenic animal technology now make it possible to produce completely human monoclonal antibodies that are devoid of mouse or other nonhuman sequences (Fishwild et al., 1996; Brüggemann and Neuberger, 1996; Brüggemann and Taussig, 1997; Jakobovitz, 1995; Sherman-Gold, 1997).

B. Anti-C5 Single Chain Fv

A recombinant single chain (scFv) antibody, constructed from the variable region of the N19/8 mAb, was shown to inhibit human C5b-9-mediated hemolysis of chicken erythrocytes and to partially inhibit C5a generation (Evans et al., 1995). The ability of this scFv to protect against complement-mediated myocardial injury was demonstrated in isolated mouse hearts perfused with 6% human plasma. Pharmacokinetic analysis in rhesus monkeys revealed a $t_{1/2}\alpha$ of 28 minutes and a $t_{1/2}\beta$ of 17 h (Evans et al., 1995). Humanized anti-C5 antibody and scFv have been produced (Thomas et al., 1996).

V. Synthetic Inhibitors of Complement Activation

Compared with conventional drugs, recombinant proteins for therapy remain attractive to date, for reasons having to do with both the biological properties of proteins and the economics of drug development (Buckel, 1996). The time required to develop protein drugs is shorter than that for conventional drugs and, although a therapeutic protein has a 40% probability of becoming a marketable drug, this figure is approximately 10% for a new chemical entity, partly because of the lower toxicity of proteins compared with chemical compounds (Buckel, 1996). However, the high cost of therapeutic proteins is increasingly becoming a problem (Grindley and Ogden, 1995). The emergence of structure-based drug design for the development of small synthetic molecules for therapy holds promise, in spite of formidable technical challenges (Verlinde and Hol, 1994; Hruby, 1997).

The existing plethora of synthetic blockers of complement prompted Becker in 1972 to note that "a comprehensive review of all compounds found to inhibit complement would turn into a catalogue of a chemical supply house." Twenty five years later, this task becomes even more daunting. Several excellent reviews on the use of synthetic complement inhibitors (table 5) for therapeutic, as well as for other uses, have been published (Becker, 1972; Patrick and Johnson, 1980; Asghar, 1984; Fujii and Aoyama, 1984; Hagmann and Sindelar, 1992). The objective here is to present a brief and selective summary of the findings using synthetic molecules for the therapeutic inhibition of complement.

A. Peptides and Analogs

The anaphylatoxins exert their multiple biological functions (Gerard and Gerard, 1994; Mulligan et al., 1997; Hartmann et al., 1997) by binding to their respective receptors (Wetsel, 1995). C5a, the most potent anaphylatoxin, is a 74-amino acid polypeptide, the sequence of which (Fernandez and Hugli, 1978) has been used to synthesize peptide analogs to downregulate the transducing functions of the C5aR, a member of the G protein-coupled receptor superfamily (Gerard and Gerard, 1991;

TABLE 5
Synthetic inhibitors of complement activation

Name	Compound identity	MW	Structural template/source	Site of action	IC_{50} (μM)	References
	Peptide analogues and derivatives		C5a	C5aR antagonist	2	van Oostrum et al., 1996
	Peptide		C5a, C-terminal octapeptides	C5aR antagonist		Kawai et al., 1991; Kawai et al., 1992
	Peptide		C5a, His^{67}-modified C-terminal octapeptide analogues	C5aR antagonist		Or et al., 1992
	Peptide		C5a	C5aR antagonist		Zhang et al., 1997
C089	Peptide with aromatic substitutions		C5a hexapeptide	C5aR antagonist	0.070	Konteatis et al., 1994
PR226	Peptide	2157	C5aR	C5aR: antagonist at >500 nM agonist at <2.0 nM		Baranyi et al., 1996
	Peptide		C3a C-terminus	C3aR antagonist		Kretzschmar et al., 1992
	Peptide	1430	C3b-based phage display screening	C3	Classical: 63; alternative: 12	Sahu et al., 1996
	Peptide		CH_2 domain of human IgG			Boackle et al., 1979; Lukas et al., 1981
	Peptide		C1q			Reid et al., 1977
	Peptides containing Phe/Tyr			C3 convertase		Kossorotow et al., 1977
	Peptide		Factor B-related hexapeptides	Factor D, alternative		Lesavre et al., 1982
CBP2	Peptide		C1q B chain helical region	C1q		Fryer et al., 1997
	Peptide		C3	C3	25	Ogata and Low, 1997
DFP	Diisopropyl fluorophosphate (model compound)			factor D		Cole et al., 1997; Fearon et al., 1974
BCX-1470				factor D	0.096	Kilpatrick, 1997[a]
	K-76 analogs		K-76 (see table 6)		Classical: 1,600; alternative: 2,500	Kaufman et al., 1995a,b
TKIXc	K-76 derivative		K-76 (see table 6)		Classical: 160; alternative: 1,360	Sindelar et al., 1996
K-76 COOH	K-76 derivative		K-76 (see table 6)		C5	Hong et al., 1979; Miyazaki et al., 1984; Tanaka et al., 1996
FUT-175	Nafamstat mesilate			Classical, alternative		Fujii and Hitomi, 1981; Hitomi and Fujii, 1982; Ikari et al., 1983; Aoyama et al., 1984; Kreil et al., 1989; Issekutz et al., 1990; Homeister et al., 1992; Inose et al., 1997
PS-oligo	Oligodeoxyribonucleotide containing phosphorothioate backbone linkages			Classical, alternative		Shaw et al., 1997
	Other compounds					Fujii and Aoyama, 1984; Asghar, 1984

[a] Kilpatrick, JM Development of small molecule inhibitors of factor D. Paper presented at: "New Therapeutic Targets Based on Control of the Complement System," June 9–11, 1997; Boston, MA.

Boulay et al., 1991). A series of hexapeptide analogs of the form NMePhe-Lys-Pro-dCha-X-dArg has been synthesized (Mollison et al., 1992; Konteatis et al., 1994) and tested for C5aR antagonism. The peptide C089 (IC_{50} 70 nM) containing Trp at position X lacked agonist properties and inhibited C5a-induced degranulation and GTPase activity, a measure of G protein activation (Konteatis et al., 1994). The in vivo functionality of this peptide has not been reported. Other C5a peptide derivatives having no anaphylatoxin or agonist activity have been described (van Oostrum et al., 1996) and shown to be active in reducing inflammation in animal models. The elucidation of the tertiary structure of a peptide antagonist of C5aR (Zhang et al., 1997) should provide information for the future structure-based design of C5aR antagonists.

A different experimental approach to the design of peptide antagonists of C5aR is based on the molecular recognition theory proposed by Blalock (reviewed in Blalock, 1990; Trospha et al., 1992; Blalock, 1995). This theory is based on the concept of "complementary" or "antisense" peptide and proposes that peptides encoded in the same reading frame on opposite strands of deoxyribonucleic acid (DNA) can bind to each other on the basis of their complementary hydropathy (Blalock, 1995). Furthermore, the theory suggests that receptors and their cognate ligands may have evolved from complementary regions of the same nucleotide sequence (see discussion in Baranyi et al., 1996). Amphiphilic peptides consisting of 8–15 residues and their corresponding antisense peptides have been identified within proteins and termed antisense homology boxes (AHB) (Baranyi et al., 1995). These regions may represent important structural elements that somehow influence the function of their respective proteins. A peptide derived from an AHB of the human endothelin A receptor inhibited endothelin in a smooth muscle relaxation assay and blocked endotoxin-induced shock in rats (Baranyi et al., 1995). Similarly, computer analysis of human C5a and the C5aR revealed several AHBs, and peptides derived from the AHBs acted as agonists or antagonists of C5aR function, depending on their concentration (Baranyi et al., 1996). It is possible that the ability to locate AHBs in proteins may provide an efficient means to identify peptides with biological activity. Other peptides that inhibit specific components of the complement system are summarized in table 5.

B. Organic Molecules

The crystal structure of factor D has been elucidated in a series of studies designed to produce an inhibitor for the therapeutic modulation of the alternative pathway (Narayana et al., 1994; Kim et al., 1995; Cole et al., 1997). This strategy is based on the rationale that factor D is the limiting enzyme in the alternative pathway and is positioned early in the biochemical cascade. The ability of diisopropyl fluorophosphate to completely inactivate factor D (Fearon et al., 1974) has been exploited in crystallographic studies to compare the active sites between factor D and the diisopropyl fluorophosphate-inhibited factor D (Cole et al., 1997) with the objective of designing small molecule inhibitors. This work resulted in the synthesis of a factor D inhibitor (BCX-1470, IC_{50} 96 nM, see table 5).

The fungal metabolite K76 (see table 6) has been modified to yield complement inhibitors of modest IC_{50} values (Kaufman et al., 1995a,b). TKIXc, a K76 derivative, inhibited both the classical and the alternative pathways (see table 5). Other synthetic inhibitors of complement activation are listed in table 5.

VI. Naturally Occurring Compounds That Block Complement Activation

There is voluminous literature on naturally-occurring complement inhibitors isolated from animal and plant tissues (table 6). Some of these compounds may serve as leads to new chemical structures, although others have not yet been purified to homogeneity. Heparin and its related glycosaminoglycan compounds and derivatives have been actively pursued as complement inhibitors. Heparin is a sulfated copolymer of uronic acid and glucosamine (Jaques, 1979a,b). Its protein core is removed during commercial processing to yield glycosaminoglycan heparin. The anticomplement activity of heparin was first demonstrated in 1929 (Ecker and Gross, 1929), and its mechanism of action has been extensively studied (Weiler et al., 1978, 1992; Linhardt et al., 1988; Maillet et al., 1983). Heparin blocks the interaction between C1q and complement activators and inhibits the assembly of C3 convertases in the classical and alternative pathways. In addition, it may potentiate C1 inhibitor-mediated inactivation of C1s, a mechanism shared by heparin and related glycosaminoglycans (Wuillemin et al., 1997; Kirschfink et al., 1997). A highly sulfated, low-molecular weight heparin derivative has been shown to prevent complement-mediated myocardial injury in the perfused rabbit heart (Gralinski et al., 1997). Heparin-coated extracorporeal circuits inhibit complement activation during cardiac surgery (te Velthuis et al., 1996). For more information on naturally-occurring complement-inhibitory compounds, refer to the references in table 6.

VII. Complement Inhibition in Xenotransplantation

Xenotransplantation, the ability to engraft organs across the species barrier, would theoretically meet the demand for organ transplantation that has doubled since 1988 and is growing by 15% per annum, requiring approximately 150,000 people worldwide to wait for donor organs (Nainggolan, 1996). It is estimated that by the year 2010 the xenotransplantation market could be worth $6 billion (Nainggolan, 1996). In recent years there has been remarkable progress in prolonging sur-

TABLE 6
Naturally occurring compounds that inhibit complement activation

Compound	Identity	MW	Source	Site of action/model	IC_{50} (μM)	References
Extract	Polyphenolic flavonoid		Plant pollen			Berrens et al., 1997
Glycyrrhizin	β-glycyrrhetinic acid steroid-like		Glycyrrhiza glabra roots	C2, classical, in vitro	35	Kroes et al., 1997
GR-2II	Polysaccharide		Glycyrrhiza uralensis roots	In vitro		Zhao et al., 1991
	Polysaccharide	11,000	Malva sylvestris leaves	In vitro		Gonda et al., 1990
AGIIb-1, AR-2IIa	Polysaccharide		Angelica acutiloba roots	In vitro		Kiyohara et al., 1989a,b; Yamada et al., 1987
Rosmarinic acid	Ester		Rosmarinus officinalis Melissa officinalis	C3 convertase, classical, in vitro	5–10	Englberger et al., 1988
Extract	Polyanionic carbohydrate		Ephedra sinica	C2, C9, classical, alternative, in vitro		Ling et al., 1995
Extract			Eugenia malaccensis	Classical inhibition, alternative activation, in vitro		Locher et al., 1995
Extract	Alkaloid		Berberis vulgaris roots	In vitro, in vivo (DTH reaction)		Ivanovska and Philipov, 1996
Extract			Fraxinus			Ivanovska et al., 1996
Extract	Polysaccharide		Panax ginseng roots, leaves	In vitro		Gao et al., 1989
BR-5-I	Polysaccharide	18,500	Bupleurum falcatum, roots	In vitro		Yamada et al., 1988
Extract			Pokeweed	In vitro		Gancevici and Popescu, 1987a; Popescu et al., 1988
Extract			Spices			Gancevici and Popescu, 1987b
Extract	Proanthocyanidin Ca^{++}-binding polymer		Jatropha multifida latex	Classical, in vitro		Kosasi et al., 1989
Fucan	Sulfated polysaccharide	16,000–22,000	Ascophyllum nodosum brown seaweed	C1, C4, factor B, classical, alternative		Blondin et al., 1994; Charreau et al., 1997
CI	Glycoprotein		Aspergillus fumigatus	Alternative, in vitro		Washburn et al., 1990
Complestatin	Peptide-like related to glycopeptide antibiotics	1325	Streptomyces lavendulae	Classical, alternative, in vitro, in vivo		Kaneko et al., 1980, 1989; Seto et al., 1989; Momota et al., 1991
K76	Sesquiterpene	402	Stachybotrys complementi	Classical, alternative, in vitro, in vivo		Miyazaki et al., 1980; Kitano et al., 1992; Miyagawa et al., 1993; Kobayashi et al., 1996
Decorin	Dermatan sulfate proteoglycan	100,000	Extracellular matrix (cartilage, bone, skin, cornea)	C1q, in vitro		Krumdieck et al., 1992
Heparin	Glycosaminoglycan		Mast cells, basophils	C3 convertases, classical, alternative, in vitro, in vivo		Weiler et al., 1978, 1992; Linhardt et al., 1988; Maillet et al., 1983; te Velthuis et al., 1996
LU 51198	Sulfated heparin fraction			Myocardial injury, ex vivo perfused heart		Gralinski et al., 1997
Dextran sulfate	Glycosaminoglycan	5000		Potentiates C1 inhibitor		Wuillemin et al., 1997
GCRF[a]	Chondroitin sulphate B proteoglycan		Glomerular epithelial cells	PC3bBb, factor B?		Quigg, 1992
CSPG	Chondroitin sulphate proteoglycan		B cell lines	C1q		Kirschfink et al., 1997
Extract	Phenolic		Propolis (bee product)	C3, classical, alternative, in vitro		Georgieva et al., 1997
C4 inactivator	Serum protein		Ginglymostoma cirratum Nurse shark	C4, in vitro, in vivo, Forssman shock, Arthus reaction		Jensen, 1969
L-156,602	Cyclic hexadepsipeptide	822–839	Streptomyces sp. MA6348	C5aR antagonist, in vitro, in vivo		Hensens et al., 1991; Tsuji et al., 1992a,b
CVF[b]	Glycoprotein	144,000	Cobra Naja naja	C3, in vitro, in vivo		Cochrane et al., 1970; Schwartz and Naff, 1971; Müller-Eberhard and Fjellström, 1971; Kourounakis et al., 1973; Jungi and McGregor, 1979
M5	Fibrinolytic proteinase	25,000	Crotalus molossus molossus			Chen and Rael, 1997

[a] Glomerular complement regulatory factor.
[b] Cobra venom factor; causes extensive complement activation, resulting in complement-depletion (see section VII).

vival of xenogeneic organs in animal models of xenotransplantation, and there is optimism in the scientific community about overcoming the various immunological barriers to xenotransplantation (Auchincloss, 1997). However, there are serious obstacles to be overcome, and, occasionally, we are reminded of the severe hurdles evolution has set for xenotransplantation (Hammer, 1997). In an excellent review of comparative physiology, biochemistry, and anatomy in the context of xenotransplantation, Hammer (1997) concludes that "In the pig-to-primate model, little convincing organ function has been achieved. . . . Today's approaches are not convincing." The field of xenotransplantation has been reviewed often and in great detail. The recent volume edited by Cooper et al. (1997) provides an excellent single source of information on this topic. The objective here is to summarize briefly the main areas of research activity as they pertain to complement inhibition in xenotransplantation.

Organ transplantation between widely disparate species is termed "discordant" as opposed to "concordant" transplantation between closely related species (Calne, 1970). The hallmark of discordant xenotransplantation is the rapid and destructive rejection of the xenograft, a process referred to as hyperacute rejection (HAR) (table 7). Activation of the complement system, after recognition of the discordant organ by xenoreactive antibodies, plays a crucial role in HAR (Baldwin et al., 1995; Sanfilippo, 1996; Dalmasso, 1997). It is generally accepted that the relative importance of the classical versus the alternative pathway in HAR depends on the species combination studied. For example, the complete elimination of natural antibodies from rats had little effect on their ability to reject guinea pig hearts hyperacutely (Pruitt et al., 1993; Soares et al., 1994). On the other hand, blocking or absorption of natural antibodies in primates is an effective method of preventing HAR of porcine hearts. Several other studies using different species combinations have shown that the alternative pathway of complement is activated in HAR (Miyagawa et al., 1988; Wang et al., 1992; Forty et al., 1992; Hengster et al., 1996). A recent study examined whether the alternative and classical pathways can be activated independently in HAR: human plasma was depleted of both C1q and factor D and then reconstituted with purified C1q or factor D to restore the classical and alternative pathways, respectively (Romanella et al., 1997). The modified plasmas were tested in an ex vivo isolated mouse heart perfusion model, and it was demonstrated that, in the mouse-to-human species combination, both the classical and alternative pathways are independently activated (Romanella et al., 1997).

Two main approaches have been used to prevent HAR (table 8). One method attempts to block the interactions between native xenoreactive antibodies and the xenograft endothelium. This strategy is aimed at the major xenoantigen responsible for HAR, the α-galactosyl epitope (Rother and Squinto, 1996; Oriol and Cooper, 1997). The other approach aims to block complement activation using soluble complement inhibitors or transgenic technology. Cobra venom factor (CVF) has been shown to deplete complement and prolong graft survival (Leventhal et al., 1994). However, CVF achieves its effect by activating complement and generating the anaphylatoxins C3a and C5a, which may cause endothelial damage (Till et al., 1982; Schmid et al., 1997b). In addition, the immunogenicity of CVF limits its usefulness. C1 inhibitor (C1-Inh), in combination with heparin, blocks HAR mediated by the classical pathway (Dalmasso and Platt, 1993). sCR1 has been shown to effectively delay HAR in a variety of xenotransplantation models (reviewed in Baldwin et al., 1995; Sanfilippo, 1996; Ryan, 1995; Levin et al., 1996; Marsh and Ryan, 1997).

An alternative strategy for the suppression of HAR uses transgenic technology for the production of animals expressing molecules of the human RCA family (Cozzi

TABLE 7
Temporal stages of discordant xenograft rejection

Hyperacute rejection	Acute/delayed rejection	Chronic rejection
Deposition of recipient XNA on xenograft endothelium	Factors involved in hyperacute rejection	Cell-mediated
Complement activation: C3a/C5a	Activated endothelium	Mechanisms unclear
Upregulation of adhesion molecules	Disordered thromboregulation	
Leukocyte recruitment	Upregulation of TF on activated ECs and monocytes	
Activation of ECs (prothrombotic surface):	Secretion of cytokines by activated NK cells and monocytes:	
Breakdown of EC barrier to plasma proteins and blood cells	IFN-γ, IL-1β, IL-6, IL-7, IL-8, IL-12, TNF-α, MCP-1	
Loss of TM, AT-III: ↑ thrombin	Cytokine-mediated recruitment of leukocytes to the graft	
Loss of TFPI	Inflammation and thrombosis	
Loss of ADPase: ↑ ADP, platelet aggregation and thrombi	Organ rejection within days	
Loss of heparan sulfate which tethers SOD		
Platelet adherence and activation		
Release of inflammatory mediators: histamine, PAF, thrombin, leukotrienes		
Fibrin deposition, thrombosis		
Organ rejection within minutes to hours		

Abbreviations: AT-III, anti-thrombin III; EC, endothelial cell; IFN, interferon; IL, interleukin; MCP, monocyte chemoattractant protein; NK, natural killer; PAF, platelet-activating factor; SOD, superoxide dismutase; TF, tissue factor; TFPI, tissue factor pathway inhibitor; TM, thrombomodulin; TNF, tumor necrosis factor; XNA, xenoreactive antibodies. After Auchincloss, 1997; Bach, et al., 1995, 1996; Parker et al., 1996; Platt, 1996; Saadi et al., 1996; Takahashi et al., 1997

TABLE 8
Methods of inhibiting hyperacute rejection in xenotransplantation

Method	Rationale/comments	References
Inhibition of interactions between XNA and xenograft endothelium		
Immunoadsorption through columns containing Galα(1–3)Gal linkages	Depletion of XNA; temporary suppression of XNA titers	Good et al., 1992; Pascher et al., 1997; Liu et al., 1997
Intravenous infusion of α-galactosyl carbohydrates.	Saturation of XNA sites prior to transplantation; temporary suppression of XNA titers	Ye et al., 1994
Expression of α(1,2)-FT (EC 2.4.1.69) in transgenic pig cells.	Reduced surface expression of Ga1α(1–3)Ga1; reduced binding of human anti-pig XNA	Sandrin et al., 1995; Koike et al., 1996, 1997; Sharma et al., 1996
Adenovirus-mediated gene transfer of antisense ribozyme to α(1–3)-FT (EC 2.4.1.51).	Ribozyme in xenogeneic organ inhibits expression of Ga1α(1–3)Ga1 epitope	Hayashi et al., 1997
Expression of GnT-III in transgenic animals.	Suppression of Ga1α(1–3)Ga1 transferase	Tanemura et al., 1997a,b
Neutralization of anti-αGa1 antibodies with monoclonal anti-idiotypic antibodies	Neutralization of the cytotoxic activity of human anti-αGa1 antibodies	Koren et al., 1997
Deletion of α1,3-Galactosyltransferase gene in xenograft (gene knockout)	Currently not feasible because pig embryonic stem cell technology has not yet been developed; possibility of horizontal transmission of retroviruses?	Sandrin et al., 1997; Rother et al., 1995; Rother and Squinto, 1996
Down-regulation of integrin GpIIIa	The porcine integrin GpIIIa expresses XNA-reactive carbohydrate epitopes; down-regulation of GpIIIa alone does not significantly alter xenograft rejection	Kearns-Jonker et al., 1997
Infusion of galactosyl peptide mimetics	Peptides that mimic the α(1–3)Ga1 determinant for blocking or absorbing XNA.	Vaughan et al., 1996; Kooyman et al., 1996
Infusion of synthetic sulfated oligosaccharides	Inhibition of endothelial activation by blocking release of heparan sulfate	Deng et al., 1996
Depletion of xenoreactive IgM natural antibody	Suppression of HAR	Kroshus et al., 1996a
Direct inhibition of complement activation		
Systemic anti-complement agents: sCR1, C1 inhibitor, cobra venom factor, FUT-175, K76 (see section III; tables 4–6).	Inhibition of both classical and alternative pathways, or selective inhibition of classical pathway. FUT-175, K76 are relatively ineffective at suppressing HAR; dual-pathway inhibitors in long-term therapy carry the theoretical risk of bacterial infections	Levin et al., 1996; Baldwin et al., 1995; Marsh and Ryan, 1997; Candinas et al., 1996; Dalmasso, 1997; Pruitt et al., 1997; Leventhal et al., 1993, 1994; Dalmasso and Platt, 1993, 1994; Miyagawa et al., 1993; Tanaka et al., 1996
Infusion of large doses of IgG	IgG around target cells competes for binding of C3b and C4b	Basta et al., 1991; Latremouille et al., 1994; Magee et al., 1995; Gautreau et al., 1995
Antibodies directed against complement components	Anti-C5 and anti-C8 antibodies	Rollins et al., 1995; Kroshus et al., 1995; Thomas et al., 1996
Xenogeneic organs expressing membrane-bound human RCA proteins.	Expression of human RCA proteins in xenogeneic vascular endothelial cells would efficiently inhibit activation of human complement, as RCA proteins are species-specific; localization of recipient RCA proteins in graft would not impair systemic complement activation; transgenic animals expressing human RCA proteins which serve as microbial receptors may be susceptible to human pathogens, and may require appropriate vaccination	Dalmasso et al., 1991; Cozzi and White, 1995; Dalmasso, 1997; Platt and Logan, 1997; Squinto and Fodor, 1997; Hancock, 1997
	Requires additional immunosuppression, e.g., cyclosporin A, steroids, and cyclophosphamide	Navia, 1996; Morris, 1996; Brazelton and Morris, 1996
	Possibility of xenosis or xenozoonosis	Chapman and Fishman, 1997; Allan, 1997; Murphy, 1996; Stoye, 1997; Stoye and Coffin, 1995; Chapman et al., 1995; Patience et al., 1997; Bach et al., 1998; Kennedy and Sewell, 1998
	Complex biochemical differences between recipient and xenogeneic organs: need to "outwit evolution", a daunting task	Hammer, 1997

Abbreviations: FT, fucosyltransferase; GnT-III, β-D-mannoside β-1,4-N-acetylglucosaminyl transferase III; RCA, regulators of complement activation; XNA, xenoreactive antibodies.

and White, 1995; Platt and Logan, 1997; Squinto and Fodor, 1997; Hancock, 1997). This is a promising area with its own limitations (table 8). Disturbingly, the recent demonstration that pig cell lines harbor endogenous retroviruses that could infect human cells in vitro (Patience et al., 1997) raises the possibility that pig

retroviruses might infect human cells in xenotransplantation (Chapman and Fishman, 1997; Allan, 1997; Murphy, 1996; Stoye, 1997; Stoye and Coffin, 1995; Chapman et al., 1995; Patience et al., 1997; Bach et al., 1998).

Clearly, there has been significant progress in our understanding of the mechanisms of HAR, and it is reasonable to anticipate that this principal immunological barrier to xenotransplantation, as well as delayed rejection, will be overcome in the near future (Auchincloss, 1997). However, formidable obstacles to overcome chronic rejection of xenografts remain (Hammer, 1997). Evidence indicates that chronic rejection may be mediated by several complement-independent mechanisms, including the activation of endothelium by IgM xenoreactive antibodies (Platt et al., 1991; Blakely et al., 1994), activation of macrophages or T cells (Blakely et al., 1994; Chen et al., 1992; Fryer et al., 1994, 1995), activation of natural killer cells (Inverardi et al., 1992; Arakawa et al., 1994), and antibody-dependent cellular cytotoxicity (Schaapherder et al., 1994; Dennert, 1974; Lin et al., 1997).

VIII. Bispecific Antibodies for Immune Complex Removal

The potential role of erythrocytes in the body's defense against bacteria and viruses was recognized by Nelson (1953, 1955) who demonstrated in vitro the binding of microorganisms to the erythrocyte surface in the presence of antibody and complement and showed that the immobilization of C3b-opsonized microbes on erythrocytes led to increased phagocytosis of the adherent pathogens by leukocytes. Erythrocyte-associated CR1 (Fearon, 1979) in primates plays a key role in the elimination of antibody/antigen immune complexes (IC) by binding C3b/C4b-opsonized IC in the circulation. The IC are then removed from erythrocytes by macrophages for subsequent clearance in the liver and spleen (Schifferli et al., 1986; Ahearn and Fearon, 1989). The erythrocytes are returned to the circulation without lysis. Nelson's original observations indicated that the erythrocyte-CR1 system could possibly be manipulated for the removal of pathogens in human disease. Thus, Taylor and colleagues (Taylor et al., 1991) speculated that if targeted antigens could be bound to erythrocytes via CR1 in the absence of complement, then it might be possible to use erythrocytes to treat a variety of infectious diseases associated with blood-born pathogens. This concept was systematically studied using bispecific, cross-linked monoclonal antibodies (heteropolymers) with specificity for both targeted antigen and the human CR1 (Taylor et al., 1991) (fig. 3A).

The potential value of bispecific antibodies in this therapeutic approach rests on their ability to facilitate antigen clearance in vivo without destruction of erythrocytes. In studies designed to answer this question, the injection into monkeys of sensitized erythrocytes (containing ^{125}I-labeled Ag attached to ^{51}Cr-labeled monkey

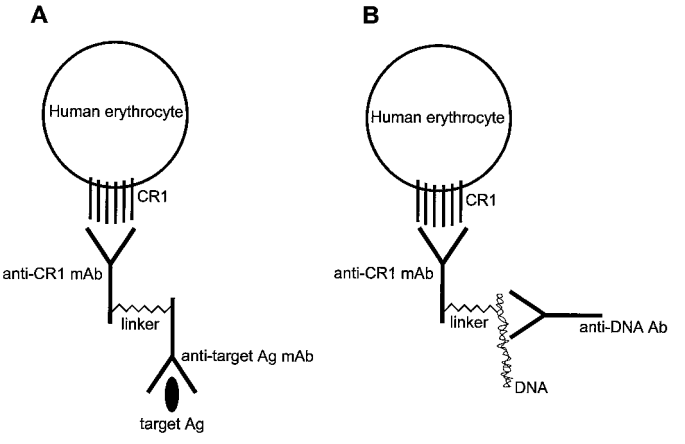

FIG. 3. Schematic representation of the concept of bispecific monoclonal antibody therapy for the clearance of pathogens from the circulation. A, bispecific antibody targeted to a soluble circulating antigen. B, bispecific antibody targeted to autoantibody, in this case anti-DNA autoantibody. After Taylor and Ferguson (1995), with permission from the author and publisher.

erythrocytes) led to rapid clearance from the circulation of several different antigens with no sequestration, lysis, or clearance of erythrocytes (Taylor et al., 1992; Reist et al., 1993). Thus, large amounts of IgG can be bound via CR1 to human or monkey erythrocytes without any phagocytic uptake by mononuclear cells. In contrast, erythrocytes that bind comparable levels of IgG at sites other than CR1, are rapidly phagocytosed (Reinagel et al., 1997). The primary organs for uptake of the IC were the liver and spleen (Reist et al., 1994). Similar studies in experimental monkey models demonstrated the feasibility of using bispecific antibodies to clear prototype viruses (Taylor et al., 1997a,b) and autoantibodies (Ferguson et al., 1995a,b; Taylor and Ferguson, 1995) from the circulation (fig. 3B), and, once again, the cleared substrates were phagocytosed and destroyed in the liver (Taylor et al., 1997a). In vitro studies using bispecific antibodies suggest that a modification of this approach may be used to clear bacterial pathogens from cystic fibrosis patients (McCormick et al., 1997). Mouse monoclonal antibodies are inherently immunogenic in humans, but this problem could be minimized through antibody engineering, "humanization" methods, or transgenic technology for production of completely human antibodies.

In contrast to the above approach that avoids complement activation, bispecific antibodies have also been engineered to recruit complement effector functions (Kontermann et al., 1997; Holliger et al., 1997). In this case, human antibody fragments directed against human C1q were isolated from a phage display library and coupled to lysozyme-specific antibody fragments, creating bispecific antibodies (diabodies). These were able to recruit C1q, effecting the lysis of lysozyme-coated sheep erythrocytes (Kontermann et al., 1997). Other diabody constructions were directed against the target antigen as well as against serum Ig and were shown to recruit

complement and promote cytotoxicity toward colon carcinoma cells in conjunction with $CD8^+$ T cells (Holliger et al., 1997). Such bispecific antibodies may have therapeutic utility in situations requiring complement activation.

IX. Summary

The use of powerful methodologies in molecular biology, biochemistry, and physiology in the last 2 decades has led to impressive progress in our understanding of the mechanisms of complement activation and its role as either a protective or a pathogenic factor in human disease. With respect to disease pathogenesis, the complexity of the complement cascade provides opportunities for several different therapeutic targets within the complement pathways. More than a century after complement was first described, we are about to witness in the near future the availability of a variety of complement inhibitors for specific therapies. Progress in the area of xenotransplantation has been substantial, but formidable obstacles remain to selective inhibition of the factors that block successful clinical xenotransplantation. Bispecific antibodies, designed to enhance rather than inhibit existing complement pathways, hold strong promise for the clearance of viral and bacterial pathogens from the circulation.

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