Chapter 4

Complement Inhibitors Targeting C3, C4, and C5

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Introduction

Activation of the complement system (Fig. 1) is the key to the development of normal inflammatory responses against foreign pathogens. During the course of this activation process a number of biological events are initiated, including generation of small peptides that induce local inflammatory responses, tagging of foreign pathogens with complement components that aid engulfment by phagocytes, and direct lysis of certain pathogens as a result of membrane attack complex (MAC) formation. Thus, complement deficiencies are often associated with a diminished ability to clear circulating immune complexes or to fight infection.

Because complement plays a pivotal role in inflammation, it is not unexpected that its unregulated activation leads to host cell damage. Although complement activation *per se* is not an etiological factor in any known disease, its inappropriate activation has been a cause of tissue injury in many disease states. The tissue injury that results from complement activation is directly mediated by the MAC, C5b–C9, and indirectly by the generation of anaphylatoxic peptides

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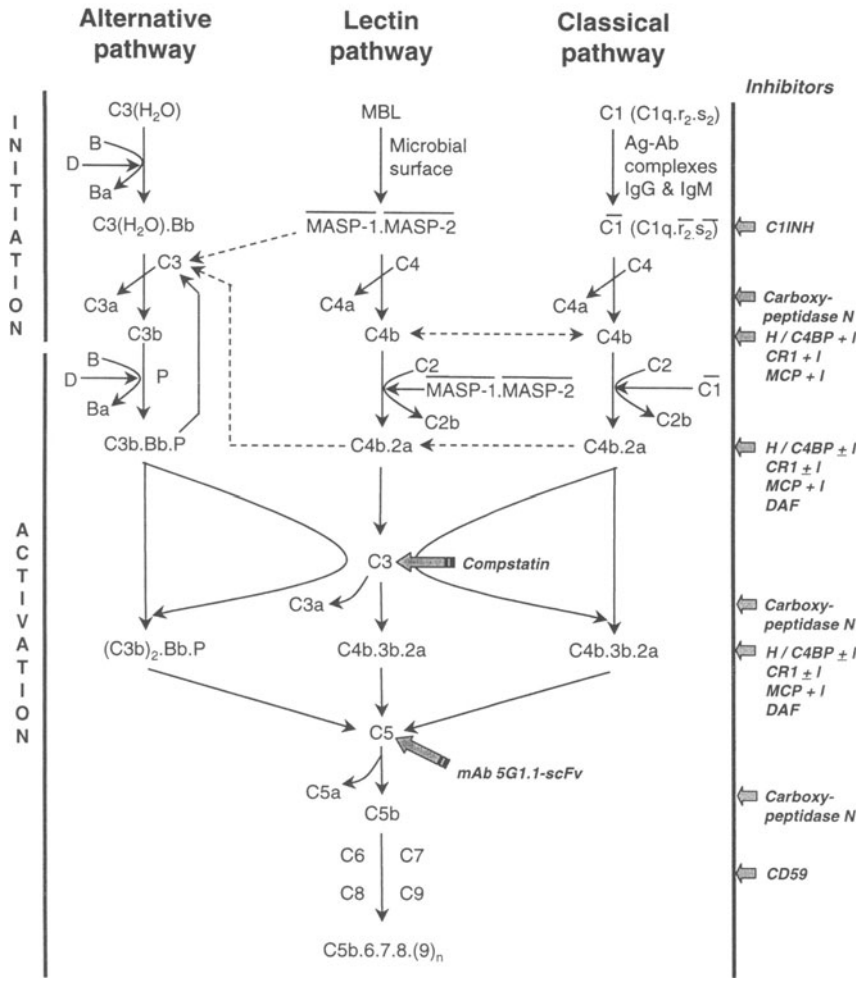

Fig. 1. Activation pathways of the complement system. The system is activated by three different pathways. The classical pathway is activated by antigen–antibody complexes, and the alternative and lectin pathways are activated by microbial surfaces. Activation of these pathways results in the generation 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) and membrane-bound (CR1, DAF, MCP, CD59) proteins. Compstatin and monoclonal antibody h5G1.1-scFv act on C3 and C5, respectively. Dotted arrows represent the recruitment of one pathway by an other.

C3a, C4a, and C5a. Complement-mediated tissue injury has been reported in a variety of disease states, including but not limited to autoimmune diseases *(1)*, adult respiratory distress syndrome

(ARDS) *(2)*, Alzheimer's disease *(3,4)*, stroke *(5)*, heart attack *(6)*, burn injuries *(7)*, and reperfusion injuries *(8,9)*. Complement-mediated tissue injury has also been found to occur as a consequence of bioincompatibility situations such as those encountered during dialysis and cardiopulmonary bypass *(10,11)*. In certain situations (e.g., organ transplantation across a species barrier) complement activation has been shown to play a central pathophysiological role in hyperacute rejection, in which grafts are rejected within minutes of transplantation *(12,13)*.

These problems associated with complement-mediated tissue injury underscore the 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. The complement proteins C3, C4, and C5 are involved in key steps of complement pathways, and activation of these components results in the generation of anaphylatoxins and leads to formation of the MAC (Fig. 1); thus, these proteins are considered ideal targets for drug development *(14)*. As discussed later, many physiological regulators of complement target these proteins. In this chapter we provide a brief overview on the structure and function of C3, C4, and C5 (for details *see [15]*) and focus on the various approaches being used to therapeutically target these molecules. A special emphasis is given to the recently developed C3-specific peptide inhibitor named Compstatin.

Structure, Function, and Regulation of C3, C4, and C5

The complement components C3, C4, and C5 are structural homologs, and interactions among them are a prerequisite for activation of the complement system. A comparison of their deduced amino acid sequences and their interactions with other complement proteins suggest that they are more closely related to each other than to any other known proteins. It is believed that these proteins evolved from a common ancestor although such an ancestor molecule has not yet been characterized. Structurally these proteins are similar in size (~200 kDa), subunit structure (α–β in C3 and C5 and α–β–γ in C4), order of chains during synthesis, arginine linker, presence of an internal thioester bond (not in C5), and probably even the disulfide linkages. All three proteins are proteolytically cleaved during

complement activation to produce anaphylatoxins (C3a, C4a, and C5a), all of which are inactivated by carboxypeptidase N; both C3 and C4 are inactivated by factor I in the presence of different cofactors to generate essentially similar cleavage products.

Complement Component C3

Complement component C3 plays a central role in the classical, alternative, and lectin pathways of complement activation (Fig. 1). The primary structure of human C3, deduced from the cDNA sequence *(16)*, consists of 1663 amino acids, including a 22-amino-acid signal peptide. It is the most abundant plasma complement component (1.2 mg/mL) composed of an α-chain (115 kDa) and a β-chain (75 kDa) linked by a single disulfide linkage. A complete disulfide linkage pattern has been determined for C3 *(17,18)*: four linkages were found in C3a, and a single bridge was identified in the β-chain as well as in the C3d portion of the α-chain. The N- and C-terminal regions of the α-chain were found to be connected to each other through a disulfide linkage. It is particularly interesting that the six linkages are clustered together in the 46-kDa C-terminal peptide of the α-chain. Three dimensional (3-D) structural information is available for the C3a *(17)* and C3d *(19)* fragments of this molecule, but the entire 3-D structure of the C3 is still unavailable, in part because of its size. Carbohydrate analysis has revealed that human C3 possesses two N-linked carbohydrate moieties, positioned at residues 63 of the β-chain ($Man_5GlcNac_2$ + $Man_6GlcNac_2$) and 917 of the α-chain ($Man_8GlcNac_2$ + $Man_9GlcNac_2$). Together these moieties account for 1.5% of the molecular weight of C3 *(20,21)*.

Native C3 contains an intramolecular thioester bond; this type of bond is present in only two other plasma proteins, complement component C4 and α_2-macroglobulin. The thioester bond allows C3 to bind covalently to target molecules on activating surfaces *(22)* through ester or amide linkages *(23)*. Binding studies have indicated that C3 shows a preference for the hydroxylated targets. The thioester bond is formed through an intramolecular transacylation reaction between the thiol group of the cysteine and the γ-amide group of the glutamine within the C3 sequence Gly-Cys^{988}-Gly-Glu-Gln^{991}-Asn *(24)*. In native C3, the thioester bond appears to be protected within a hydrophobic pocket and is exposed in the C3b fragment upon cleavage of C3 by C3 convertases. Thus, the transiently exposed thioester

bond (half-life ~100 μs) can then participate in a transacylation reaction with nucleophilic groups present on cell surfaces or with complex carbohydrates or immune complexes *(23,25,26)*. Until recently, the attachment of C3b to various activators (such as fungi, bacteria, and viruses) was considered as a nonspecific reaction. However, recent studies have clearly demonstrated that C3b displays a high degree of specificity in reacting with targets such as carbohydrates on different microorganisms and with proteins such as C3b, C4b, and IgG *(25–28)*, and they have further indicated that this specificity can be an important factor in complement activation *(25)*. In human IgG_1 and C4b, Thr^{144} and Ser^{1213}, respectively, have been identified as the major, if not the only, sites with which C3b reacts *(26,27)*. These findings demonstrate the selection of a single residue out of several hundred potential hydroxyl and amino-group-containing targets on these two proteins. Thus, the previous supposition that metastable C3b reacts randomly or nonspecifically is not correct. Covalent attachment of C3b to surface structures is necessary to initiate the formation of the MAC, phagocytosis of foreign particles, and enhancement of effector cell–target cell contact.

Proteolytic cleavage of the C3 molecule between Arg^{726} and Ser^{727} by the classical/lectin (C4b2a) or alternative (C3bBb) pathway C3 convertases results in the generation of a small anaphylatoxic peptide, C3a (9 kDa), and a large fragment, C3b (185 kDa), which becomes covalently attached to the activating surface. Once C3 is cleaved to C3b, it expresses binding sites for many complement components, including factor B, properdin (P), C5, factors H and I, C4 binding protein (C4BP), complement receptors 1 (CR1) and 2 (CR2), and the membrane cofactor protein (MCP). Which particular protein binds to C3b determines the fate of the ensuing activation process. Binding of factor B in the presence of factor D leads to amplification of the alternative pathway and initiation of the MAC (C5b–9) (Fig. 1), and binding of factor H, C4BP, CR1, or MCP in the presence of factor I shuts off the activation process (Fig. 2). Whether amplification or inactivation occurs depends on the nature of the surface to which C3b becomes attached.

Complement Component C4

The fourth component of complement, C4, is an important constituent of the classical and lectin pathways. It exists in two isoforms

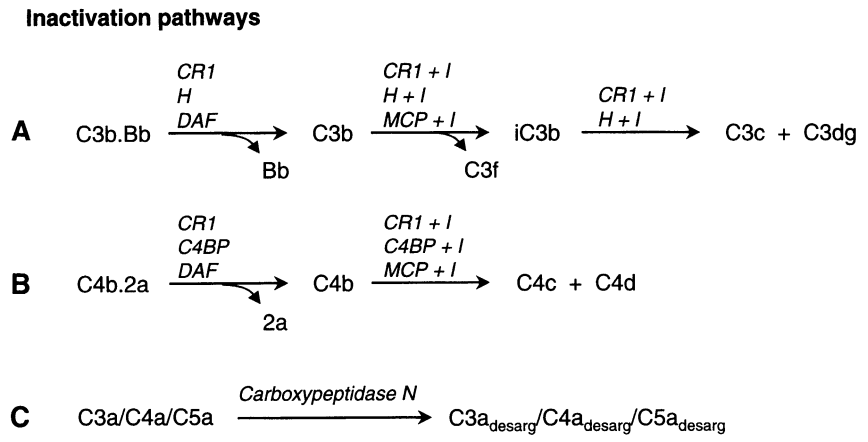

Fig. 2. Regulation of C3, C4, and C5. C3 convertases (C3bBb and C4b2a) generated during the activation of the complement system are dissociated into their subunits by the members of the RCA (CR1, factor H, DAF, and C4BP). After dissociation, C3b and C4b molecules are proteolytically inactivated by factor I in the presence of the appropriate cofactor.

(C4A and C4B) that are the transcription products of two separate genes. C4 is a plasma glycoprotein (average concentration in human serum, 0.64 mg/mL) of 200 kDa of which 7% is carbohydrate. It contains three polypeptide chains: α (95 kDa), β (75 kDa), and γ (33 kDa). Interchain disulfide analysis has shown that the N-terminus of the α-chain is linked by a disulfide bond to both the β- and the γ-chains, whereas the C-terminus of the α-chain is linked only to the γ-chain *(31)*; intrachain disulfide linkages have not been studied. The primary structure of C4 has been deduced from a cDNA sequence encoded by a 5.5-kb mature mRNA and contains 1722 amino acids *(32)*. Analogous to C3 and C5, C4 is synthesized as a single polypeptide chain (pro-C4), which is glycosylated *(33)* before being processed by a plasmin-like protease *(34)*, organized, sulfated *(35)*, and secreted as a multichain protein. The order of the three subunits in the pro-C4 molecule is β-α-γ *(32)*. After secretion, the protein is further processed in the plasma by the removal of a small C-terminal fragment of the α-chain by metalloprotease *(36)*.

Like C3 and α_2-macroglobulin, C4 also contains an intramolecular thioester bond between the sulfhydryl group of Cys^{991} and the carbonyl group of Gln^{994}. It is believed that this bond formation

is entirely spontaneous and does not involve any enzymes or high-energy metabolites *(37)*. During activation of the classical pathway, proteolytic cleavage of native C4 by C1s produces an anaphylatoxic peptide C4a (8.8 kDa) and an opsonic fragment C4b (191 kDa) that contains a short-lived, highly reactive thioester bond. Similar cleavage products of C4 are observed when the lectin pathway is activated and C4 is cleaved by activated mannan-binding lectin-associated serine protease 2 (MASP-2) *(38)*. The activated thioester of C4b reacts with hydroxyl or amino groups of the receptive surface to form ester or amide bonds, which are required for subsequent steps in complement activation. The two isotypes of C4 differ in their reactivity; C4A shows strong reactivity with amino groups, while C4B displays a preference for hydroxyl groups *(39,40)*.

Cleavages of C4 to C4b by either C1s *(30,41)* or by MASP-2 *(42)* (between residues 737 and 738) produces conformational changes in the molecule and exposes binding sites for many proteins. The newly formed C4b encompasses binding sites for many complement proteins, including C2, C3, C5, C4BP, CR1, and MCP. Binding of C2, C3, or C5 to C4b leads to the activation of the pathway, while that of C4BP, CR1, and MCP inactivates the pathway. Binding of C2 to C4b in the presence of magnesium ions makes the C4b susceptible to cleavage by C1s or MASP-2 and, in turn, results in the formation of C3 convertase, C4b2a . This complex then cleaves C3, and the cleaved molecules that are in close proximity to C4b bind covalently to C4b to form the C5 convertase, C4b2a3b. In contrast to the binding of C2 and C3, the binding of C4BP, CR1, and MCP to C4b results in cleavage of C4b by factor I into the functionally inactive species C4c and C4d (Fig. 2; *[45–48]*). The factor I-mediated cleavages have been reported to occur at residues Arg^{937}-Thr^{938} and Arg^{1318}-Asn^{1319} of the α'-chain of C4b (numbering according to *[32]*).

Complement Component C5

C5 is a plasma glycoprotein of 190 kDa, of which 3% is carbohydrate *(49,50)*. It is present at a concentration of 0.075 mg/mL in human serum. The molecule is composed of an α- (115 kDa) and a β-chain (75 kDa) which are linked together by at least one disulfide bond *(51,52)*. A disulfide linkage analysis has not yet been done for this molecule. C5 is synthesized as a pro-C5 molecule of 1676 amino

acid residues, including an 18-amino-acid signal peptide and an arginine-rich linker sequence (RPRR) located at the β–α junction. During secretion, the signal peptide and the linker sequence are removed to generate the native two-chain structure. Fine-structural information is not available for C5; however, it has been crystallized and the crystals diffracted at 6 Å *(53)*. Although these data were collected at low resolution, this degree of resolution should allow us to discern the overall structure and domain arrangement of the molecule. Electron microscopy, neutron and X-ray scattering, and physical chemical studies have indicated that C5 exhibits an asymmetrical two-domain shape, with a sedimentation coefficient of 7.9 S *(50,54)*.

Proteolytic cleavage of C5 to C5b occurs during the activation of all three complement pathways (Fig. 1). Activation of these pathways results in the formation of classical/lectin (C4b2a3b) or alternative (C3b3bBb) pathway C5 convertases, which cleave C5 at Arg^{74}-Leu^{75} to generate anaphylatoxic peptide C5a (11.2 kDa) and C5b. On a molar basis C5a is 100 times more potent than C3a in eliciting responses. In addition, C5a also acts directly on neutrophils and monocytes: It is a potent chemoattractant, enhancing the ability of neutrophil and monocytes their adhere to vessel walls, and it also enhances the expression of CR1 and CR3 and their ability to engulf particles. C5a is inactivated by the plasma enzyme carboxypeptidase N, which removes the C-terminal arginine from C5a to form the C5a des Arg derivative *(55)*. On a molar basis, human C5a des Arg exhibits only 1% as much anaphylactic activity *(56)* and 1.0% as much chemotactic activity for polymorphonuclear leukocytes (PMNs) as does the undigested C5a *(57,58)*. C5b, on the other hand, participates in the formation of the C5b–9 MAC. Unlike C3b and C4b, C5b is not inactivated by factor I.

Complement Inhibitors Specific to C3, C4, and C5

Human Complement Proteins and Chimeras

Complement proteins that are involved in the activation process do not have the ability to discriminate between self and nonself, and thus they have the potential to destroy any cell to which they bind. As a means of preventing this destructive activity, human cells are protected 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 CR1, decay-accelerating factor (DAF), and MCP (Table 1). The inhibition mediated by these proteins is achieved through the dissociation of the subunits of C3 and C5 convertases and/or through the proteolytic inactivation of the subunits by factor I (Fig. 2). In addition, control is also achieved through the activity of carboxypeptidase N, which inactivates C3a, C4a, and C5a (for details *see* Chapter 5); C1INH, which prevents C1 activation (*see* Chapter 2); and CD59, which prevents MAC formation (*see* Chapter 8) (Fig. 1).

At present some of these human complement-regulating proteins are being developed for therapeutic applications. A soluble form of recombinant CR1 (sCR1) has been generated by deleting the transmembrane region and the cytoplasmic tail. This recombinant protein retains all the function of native CR1 and has been tested in many animal models *(1,59)*. sCR1 is currently in phase II trials for use in ARDS patients. Other C3-, C4-, and C5-interacting proteins that are being developed for therapeutic use include the soluble forms of DAF and MCP and chimeras such as DAF–CD59 and MCP–DAF (for details *see* Chapter 6).

Proteins of Foreign Origin

Activation of the complement system can lead to destruction of pathogens. Successful pathogens have developed strategies that allow them to circumvent these defenses and evade complement-mediated destruction (Table 1; for reviews *see [60–62]*). Of relevance to this chapter are viral proteins that mimic human complement control proteins. These proteins are of no direct therapeutic value because of the obvious problem of immunogenicity. However, identification of structural determinants that are important for their function may allow the construction of human–virus chimeras with little or no immunogenicity.

The first known and best-studied example of a viral homolog of human complement control proteins is the vaccinia virus complement control protein (VCP) *(63)*. VCP is one of the two major proteins secreted by vaccinia virus-infected cells. This protein received scientific attention when it was discovered that an attenuated mutant of vaccinia virus does not secrete this protein. Sequence analysis

Table 1
Complement Inhibitors Targeting C3, C4, and C5

Inhibitor	Specificity	Molecular mass	Location/source	Key features	Refs.
Cell surface proteins					
CR1 (CD35)	C3b, C4b iC3b, C3c	4 allotypes 190 kDa 220 250 280	Erythrocytes,eosinophils, monocytes, macrophages, neutrophils, B and some T lymphocytes, glomerular podocytes, follicular dendritic cells, mast cells, polymorphonuclear cells	Member of RCA, accelerates decay of CP and AP C3 convertases, cofactor for factor I, helps processing immune complexes, involved in phagocytosis. A soluble form (sCR1) is used as complement inhibitor.	*(59,144–146)*
DAF (CD55)	C3bBb C4b2a	75 kDa	Erythrocytes, all leukocytes, platelets	Accelerates decay of CP and AP C3 convertases A soluble form (sDAF) is used as complement inhibitor.	*(147–149)*
MCP (CD46)	C3b, iC3b C4b	45–70 kDa	Neutrophils, monocytes, platelets, reticulocytes, most lymphocytes, granulocytes, endothelial cells, epithelial cells, mesenchymal cells	Member of RCA, cofactor for factor I, does not accelerate decay of C3 convertases. A soluble form (sMCP) is used as complement inhibitor.	*(48,149,150)*
Soluble proteins					
Factor H	C3b, iC3b C3c, C3d	150 kDa	Plasma	Accelerates the decay of AP C3 convertase, cofactor for factor I	*(151–153)*
Factor I	C3b, iC3b	88 kDa	Plasma	C4b/C3b inactivator	*(154,155)*
C4BP	C4b, C3b	460-540 kDa 70 kDa α chain 45 kDa β chain	Plasma	Cofactor for factor I, accelerates the decay of CP C3 convertase	*(156,157)*
Factor J	C3bBb, C1	65 kDa	Plasma	Inhibits generation and accelerates decay of AP C3 convertase, inhibits C1	*(158–160)*
Undefined	C3	26 kDa	Bronchoalveolar lavage	Inhibits C3 activation	*(161)*
Chimeric human proteins					
CAB-2	C3/C5 convertase (MCP-DAF)		Soluble expressed protein	Accelerates decay of CP and AP C3/C5 convertase Possesses factor I cofactor activity	*(162,163)*
CD (CD59-DAF)	C3/C5 convertase MAC assembly		Membrane-bound expressed protein	Accelerates decay of CP and AP C3/C5 convertase Inhibits MAC assembly	*(164)*

DC (DAF-CD59)	C3/C5 convertase MAC assembly		Membrane-bound expressed protein	Accelerates decay of CP and AP C3/C5 convertase Inhibits MAC assembly	*(164)*
Proteins of foreign origin					
VCP	C3b,C4b	27 kDa	Secreted protein	Vaccinia virus complement control protein homolog Accelerates the decay of CP and AP convertases factor I cofactor for C3b and C4b, Inhibits CP and AP	*(64–67)*
IMP	C3b?,C4b?		Secreted protein	Cowpox virus complement control protein homolog Modulates in vivo complement-mediated inflammatory responses.	*(165)*
HVS CCPH	C3b?,C4b?	65–75 kDa	Virion surface (membrane form)	Herpesvirus saimeri complement control protein homolog	*(70,71)*
		47–53 kDa	Secreted protein (soluble form)	Protects cell from complement-mediated damage	
HHV8 CCPH	C3b?, C4b?	—	Virion surface (membrane form)	Kaposis sarcoma associated herpesvirus complement	*(72)*
			Secreted protein (soluble form)	Control protein homolog. Functions not characterized.	
SPICE	C3b?, C4b?	35 kDa	Secreted protein	Secreted by Variola major. Inhibits human complement	*(166)*
γHV68	C3b?, C4b?	—	Virion surface (membrane form)	γ Herpesvirus 68 complement control protein homolog.	*(73)*
			Secreted protein (soluble form)	Functions not characterized.	
Antibodies					
N19-8 mAb	C5	160 kDa	Hybridoma	Murine mAb against human C5. Inhibits generation of C5a and C5b-9	*(77,79)*
N19-8 scFv	C5	Murine scFv	Recombinant	Murine single chain Fv Ab against human C5. Inhibits C5a and C5b-9 generation.	*(83)*
5G1.1	C5	Humanized scFv	Recombinant	Humanized single chain Fv Ab against C5. Inhibits C5a and C5b-9 generation.	*(78)*
aC5-12	C5	160 kDa	Hybridoma	Murine mAb against human C5. Binds to C5 β-chain between Tyr^{334}-Lys^{418}. Inhibits C5a and C5b-9 generation, but not C5 binding to C3b	*(76)*

(continued)

Table 1 *(continued)*

Inhibitor	Specificity	Molecular mass	Location/source	Key features	Refs.
Small-molecule inhibitors					
Synthetic peptides					
Compstatin	C3	1551 Da		Inhibits CP and AP by inhibiting C3 cleavage.	*(99)*
Tyr peptides	C3			Inhibit complement by covalent attachment to C3 thioester.	*(112)*
Unnamed	C3aR			C3aR antagonist	*(167)*
Unnamed	C3 convertase			Peptides containing Phe/Tyr	*(167a)*
Unnamed	C5aR			C5aR antagonist	*(168)*
Unnamed	C5aR	8477 Da		C5aR antagonist	*(169)*
CO89	C5aR			C5aR antagonist	*(170)*
PR226	C5aR	2463 Da		C5aR antagonist at >500 nM, agonist at 0.04 nM	*(171)*
Other synthetic compounds					
K-76 analogs	C5			Inhibit CP and AP	*(172–174)*
FUT-175	C3/C5 convertase			Inhibits C3/C5 convertase activity	*(175–178)*
Hydroxylated compounds	C3			Inhibit CP and AP by covalent attachment to the thioester of C3	*(100)*
Naturally occurring compounds					
Rosmarinic acid	C3, C49		Meliss officinalis, Rosmarinus officinalis	Inhibits CP and AP. Inactivates C3 by covalent attachment to the thioester.	*(129,130)*
Fucan	C4, C1, B	16,000–22,000	Ascophyllum nodosum	Inhibits CP and AP.	*(179,180)*
Heparin	C3bBb, C1INH		Mast cells, basophils	Inhibits C3 convertase, potentiates activity of C1INH and factor H, Interfere with binding of C1s to C4 and C2 and C2 to C4b.	*(114,120)* *(117,121–123)*
	C1, C4, C2, H				
K76	C5		*Stachybotrys complementi*	Inhibits CP and AP	*(135,137–139)*
GCRF	C3bBb		Glomerular epithelial cells	Inhibits C3bBbP and factor B?	*(181)*
Phenolic extract	C3		Propolis (bee product)	Inhibits CP and AP	*(182)*
C4 inactivator	C4		Nurse shark serum	Inhibits C4	*(183)*
L-156,602	822-839		*Streptomyces* sp. MA6348	C5aR antagonist	*(184–186)*

CP, classical pathway; AP, alternative pathway; RAC, regulators of complement activation.

revealed that it is structurally related to the members of the RCA family *(63)*. VCP, which is encoded by the C3L open reading frame (ORF) of the vaccinia genome, is a 27-kDa protein that is composed entirely of four tandemly repeating domains called short consensus repeats (SCRs) or complement control protein repeats (CCPs) *(64)*.

VCP apparently protects the infected cells and released virions from attack by host complement. Evidence for this theory has come from experiments in which VCP was shown to abrogate complement-mediated, antibody-dependent neutralization of vaccinia virions *(65)*. In addition, studies with recombinant vaccinia viruses that do not express VCP have clearly shown that these viruses are attenuated in vivo *(65)*. A culture medium containing secreted VCP has been shown to inhibit complement-mediated lysis of sheep erythrocytes, to bind to C3b and C4b, and to accelerate the decay of the classical as well as the alternative pathway C3 convertases.

To understand the detailed mechanisms by which VCP inactivates complement, our laboratory has generated a recombinant form of VCP *(67)*. A comparison of its effect on complement to that of human factor H and sCR1 revealed that the recombinant VCP was less effective than CR1 in inhibiting the classical and alternative pathways of complement and less effective than factor H in inhibiting the alternative pathway. However, it is noteworthy that on a molar basis this VCP was four times more effective than factor H in inhibiting the classical pathway, possibly owing to its dual action on C3 and C4. The study also demonstrated that VCP supports the factor I-mediated cleavages of C3b and C4b. It is known that CR1 and factor H support the factor I-mediated cleavage of C3b between $Arg^{1281}$and Ser^{1282} (site 1), $Arg^{1298}$and Ser^{1299} (site 2), and $Arg^{932}$and Glu^{933} (site 3) *(15)*. Analysis of VCP-supported cleavages of C3b showed that it primarily served as a cofactor for the first site, leading to generation of C3b through a single cleavage ($iC3b_1$) . The factor I cofactor activity of VCP for C4b was similar to that of CR1. Purification and functional analysis of the VCP-generated $iC3b_1$ showed that it was unable to interact with factor B to form the alternative pathway C3 convertase (C3b,Bb) *(67)*. These results demonstrate that the interaction of VCP with C3 is different from all the other factor I cofactors characterized to date.

Proteins that have four SCRs and are also homologous to complement control proteins have been found in various members of

the poxvirus family including smallpox virus *(68,69)*, herpesvirus saimiri *(70,71)*, Kaposi's sarcoma-associated herpesvirus (HHV-8) *(72)*, and murine γ-herpesvirus 68 *(73)*.

Antibodies

Because antibodies recognize their targets with high specificity and high affinity and have a relatively long half-life, they are attractive candidates for use as therapeutic agents. With the recent advent of genetically engineered antibodies, it is no longer difficult to produce large quantities of antibody for therapeutic use. Limitations to this strategy include the problem of immunogenicity and the requirement for administration by intravenous perfusion. Production of completely human monoclonal antibodies in transgenic animals can overcome the first limitation .

Several laboratories have developed anti-C5 monoclonal antibodies to block complement activation . The anti-human C5 monoclonal antibody (mAb) aC5–12 has been shown to block both C5 cleavage by C5 convertases and formation of C5b-9 *(76)*. Epitope mapping has localized the antibody binding site 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 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 *(77)*, when tested in an in vitro extracorporeal blood circuit, inhibited C5a and soluble C5b–9 generation and CD11b upregulation; it also reduced the formation leukocyte–platelet aggregates and eliminated P selectin-positive platelets *(79)*.

The in vivo efficacy of anti-C5 mAbs has been tested in mouse models of immune complex nephritis *(80)* and collagen-induced arthritis *(81)*, in a rat model of myocardial ischemia and reperfusion *(82)*, and in cardiopulmonary bypass patients *(78)*. In mice, anti-C5 therapy resulted in a significant slowing of the course of glomerulonephritis *(80)*, prevented the onset of collagen-induced arthritis, and ameliorated the established disease *(81)*. In the rat model it reduced ischemia/reperfusion-induced tissue injury *(82)*. A single-chain (scFv) antibody has been constructed from anti-human C5 mAb N19-8; this antibody prevented C5b–9 deposition in mouse hearts perfused with human plasma *(83)*. Recently, another scFv antibody (h5G1.1–scFv) has been tested in cardiopulmonary bypass patients

(78). In these trials a dose of 2 mg/kg produced a >50% inhibition of complement activity for about 12 h. The patients' sera showed no sC5b–9 generation and a significant reduction in leukocyte activation, as judged by CD11b upregulation. Most importantly, these patients showed a significant reduction in CBP-induced myocardial damage.

Small-Molecule Inhibitors

Use of therapeutic proteins such as complement control proteins is the first step toward the management of complement-mediated injuries. The emphasis has now moved toward the generation of small-molecule inhibitors that are cost-effective and have more desirable pharmacokinetic properties. Such considerations are of prime importance when drugs will need to be administered over a long period of time, such as during transplantation of xenografts or management of autoimmune disorders. A large number of small synthetic compounds and antiinflammatory drugs have been identified in the past and have been reviewed extensively by others *(85–88)*. Many of theses antiinflammatory drugs *(84)* inhibit complement activation; however, the doses required to bring any significant inhibition were much higher than the blood level of drugs achieved after therapeutic exposure. Therefore in this section we have reviewed only selected well-characterized compounds that have recently been developed.

Compstatin and Other Peptide Inhibitors

Most of the work in developing peptide inhibitors of complement has focused on the production of peptide antagonists of anaphylatoxins C3a and C5a. A large body of literature is available in this area, which is elegantly summarized in Chapter 5 of this volume. In our laboratory, we have instead focused our attention on the use of combinatorial peptide libraries to identify C3-interactive peptides. This approach has led to the identification of a novel C3-binding peptide inhibitor, Compstatin, which shows significant activity in both in vitro and ex vivo models.

The rational for selecting C3 as a target protein for identifying peptide inhibitors of complement is as follows: It has been recognized for some time that activation of one of the complement pathways (classical or alternative, or lectin) leads to recruitment of an

other (Fig. 1). For example, activation of the classical pathway results in activation of the alternative pathway *(89)*. Similarly, activation of the lectin pathway supports the activation of the alternative pathway *(90,91)*. Thus, in most clinical conditions, multiple pathways are activated. 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, for example, factor H, CR1, DAF, and MCP, act on C3b to inhibit complement activation.

Within the last decade random peptide libraries have become a rich source of structural diversity *(92)*. They have proved to be a useful tool for identifying small-molecule drug lead *(93)*, the peptide epitopes of monoclonal antibodies *(94)*, and mimetics of ligands for various proteins *(95–98)*. To identify C3-interactive peptides we have screened a phage-displayed random peptide library against C3b. We hypothesized that (a) binding of the peptide to C3b might affect the interaction of C3b with other complement proteins and (b) the peptide could functionally mimic other C3-binding proteins that regulate complement and in turn produce complement inhibition. This approach indeed resulted in the isolation of a 27-mer C3-binding peptide, which inactivated complement at a concentration approximately twice that of human C3 in normal human serum *(99)*.

To identify the minimal region of the 27-mer peptide (peptide I, Table 2) that is required for interaction with C3, two overlapping peptides were synthesized, and their activity was measured in the classical and alternative pathway-mediated hemolytic assays (these are standard assays for measuring complement inhibition *[100]*). These two analogs were a cyclic 13-mer N-terminal peptide (peptide IV, Table 2) and a linear 17-mer C-terminal peptide (peptide III, Table 2). The N-terminal cyclic peptide (peptide IV) retained the functional activity of the parent peptide and inhibited the classical as well as the alternative pathway at concentrations similar to those of peptide I; no inhibitory activity was detected for peptide III. The N-terminal cyclic peptide (peptide IV) was later named Compstatin. We made the interesting discovery that reduction and alkylation of Compstatin destroyed its inhibitory activity (Table 2), an observation that strongly suggests that oxidation of cysteines is important in

Table 2
Inhibition of Human Complement by Compstatin (Peptide IV) and Its Analogs

Peptide	Amino acid sequence[a]	Mass spectral analyses		Inhibition of complement activity[b] IC_{50} (μM)
		Expected	Observed	
Peptide I	I*CVVQDWGHHRC*TAGHMANLTSHASAI	2913	2919	19
Peptide II	ICVVQDWGHHRCTAGHMANLTSHASAI	3021	3018	>300
Peptide III	RATAGHMANLTSHASAI	1709	1708	>300
Peptide IV (Compstatin)	I*CVVQDWGHHRC*T	1552	1551	12
Peptide V	ICVVQDWGHHRCT	1660	1664	>600
Peptide VI	*CVVQDWGHHRC*	1340	1339	33
Peptide VII	*CAVQDWGHHRC*	1311	1309	1200
Peptide VIII	*CVAQDWGHHRC*	1311	1309	67
Peptide IX	*CVVADWGHHRC*	1282	1281	910
Peptide X	*CVVQAWGHHRC*	1296	1297	257
Peptide XI	*CVVQDAGHHRC*	1224	1223	182
Peptide XII	*CVVQDWAHHRC*	1353	1352	>1200
Peptide XIII	*CVVQDWGAHRC*	1273	1272	15
Peptide XIV	*CVVQDWGHARC*	1273	1272	74
Peptide XV	*CVVQDWGHHAC*	1254	1255	70

[a]Asterisks denotes oxidized cysteines.
[b]Alternative pathway complement activity was measured by Er lysis assay *(100)*.

maintaining the stable and preferred structure of the peptide *(99)*. Compstatin contains two flanking amino acid residues outside the constrained region. To further reduce the size of the molecule, we deleted these two residues (peptide VI, Table 2). This change resulted in a 2.8-fold reduction in the activity of Compstatin, indicating the importance of these residues in enhancing the inhibitory activity of Compstatin *(99)*. A series of experiments were then performed to unravel the mechanism by which Compstatin inhibits complement. The data we obtained indicated that the peptide reversibly binds to native C3 to inhibit its activation, and this inhibition is not due to sterically hindered access to the C3a/C3b cleavage site *(99)*.

Identification of the contribution of each residue to maintaining the biological activity of Compstatin was determined by systematic replacement by alanine of each residue of the 11-membered ring, except for the two cysteines (peptides VII-XV, Table 2) *(101)*. Replacement of Val^4, His^9, His^{10}, or Arg^{11} did not significantly affect the functional activity, suggesting that these residues do not contribute significantly to the interaction with C3. However, replacement of Val^3, Gln^5, Asp^6, or Trp^7 reduced the activity of the peptide from 6- to 36-fold, as compared to peptide VI (Table 2). These residues are clustered together in the N-terminal half of the peptide. Replacement of Gly^8 with Ala dramatically reduced the activity of the peptide by more than 100-fold, suggesting that the side chain of the Ala may sterically hinder the binding of Compstatin to C3 or may alter the local structure of Compstatin, thus prohibiting binding to C3. From these results it is evident that the side chains of Val^3, Gln^5, Asp^6, and Trp^7 are essential for binding and biological activity of Compstatin.

Structure-based rational design of peptidomimetics and crafting of small molecule inhibitors requires knowledge of the complete 3-D structure of the peptide inhibitor and the target protein. The first step in this direction has been achieved by determining the 3-D structure of a major conformer of Compstatin in solution, using 2-D nuclear magnetic resonance (NMR) restraints and two different computational methods. The first set of structure calculations of Compstatin was performed using a hybrid distance geometry/restraint simulated annealing and refinement (hDG/SA) method and the full set of NMR restraints *(101)*. The second set of structure calculations of Compstatin was performed using a deterministic global optimization method and a subset of the available NMR restraints

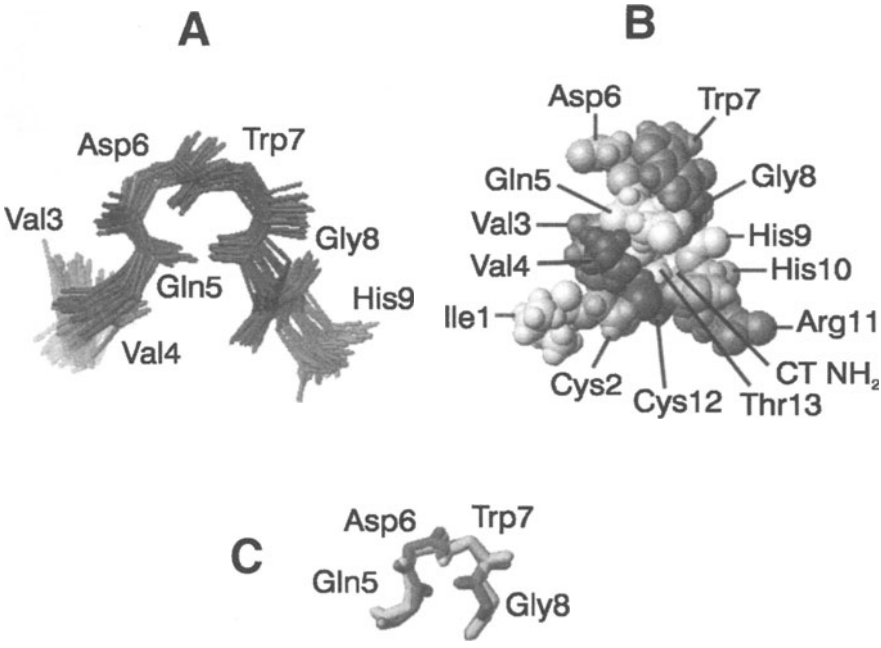

Fig. 3. **(A)** A backbone representation ($N, C^{\alpha}, C, H^{N}, O^{C}$) of the ensemble of the 21 structures of Compstatin calculated using the hDG/SA method (code 1a1p of the Brookhaven Protein Data Bank). Only the best defined region between residues 3 and 9 is shown. Residues Gln^{5}-Asp^{6}-Trp^{7}-Gly^{8} form a type I β-turn. In this representation, residues closer to the viewer appear darker than residues farther from the viewer to give an indication of the depth of field and the relative topology. **(B)** A space-filling model of the lowest energy structure of Compstatin calculated using the hDG/SA method. Four shades of gray are used to represent different amino acids as follows: white for Ile^{1}, Gln^{5}, His^{9}, Thr^{13}; light gray for Cys^{2}, Asp^{6}, His^{10}, C-terminus NH_2 blocking group; gray for Val^{3}, Trp^{7}, Arg^{11}; black for Val^{4}, Gly^{8}, Cys^{12}. **(C)** A superposition of the type I β-turn segment of the lowest energy structure of Compstatin calculated using the hDG/SA method (*in black*) and the structure of Compstatin calculated using the deterministic global optimization method (*in gray*).

(101a). Both calculations yielded similar results in the best defined region of Compstatin, which is the region that contains most of the NMR restraints. Figure 3A shows the backbone of residues 3–11 of the ensemble of the 21 structures of Compstatin calculated using the hDG/SA method. The backbone forms a type I β-turn comprising residues Gln^{5}-Asp^{6}-Trp^{7}-Gly^{8}. Figure 3B shows a space-filling

model of the lowest energy structure of Compstatin. The side-chain characteristics of the structure of Compstatin, as indicated in Fig. 3B, are (a) hydrophobic clustering of Thr^{13}, $Cys1^{2}$, Cys^{2}, Ile^{1}, Val^{3}, Val^{4} and Trp^{7} that might have a direct effect on the structural stability of Compstatin; (b) capping of the β-turn by Trp^{7} and no side-chain contact of Gln^{5} and Asp^{6}, which are oriented in opposite directions; (c) side-chain contact of Asp^{6} with His^{9} and Trp^{7}; (d) disorder of His^{10} and Arg^{11}, which precludes electrostatic interactions with Asp^{6}; (e) reduced solvent accessibility of the β-turn end residues (as shown by a solvent accessibility calculation), Gln^{5} and Gly^{8}, probably due to the effect of capping of the turn by the rings of Trp^{7}; and (f) the presence of a disulfide bond between Cys^{2} and Cys^{12} that determines the opening of the cyclic peptide and might also have a direct impact on the formation of the β-turn. Figure 3C presents a comparison of the type I β-turn region of the lowest energy structure of Compstatin calculated with the hDG/SA method and the structure of Compstatin calculated with the deterministic global optimization method. The structures are in excellent agreement in this region.

The alanine scanning experiments described in the preceeding indicated that residues Val^{3}, Gln^{5}, Asp^{6}, Trp^{7}, and Gly^{8} are critical for maintaining the inhibitory activity of Compstatin. The structural basis, however, was not apparent from the alanine scan experiments. It is clear from the solution structure of Compstatin that four of five residues (Gln^{5}-Gly^{8}) are involved in the formation of the type I β-turn, and the fifth residue (Val^{3}) is a critical residue in the formation of the hydrophobic cluster of Compstatin. The two end-residues of the type I β-turn (Gln^{5} and Gly^{8}), which are somehow buried (as shown by a solvent accessibility calculation) *(101)*, are more essential for Compstatin activity. Replacement of Gly^{8} with Ala caused a dramatic total loss of the inhibitory activity of Compstatin (Table 2). Analysis of turn structures in a number of proteins has indicated that Gly is the most favorable residue at the fourth position of type I β-turns because it releases steric hindrance and stabilizes the turn structure *(101)*. However, the role of Val^{3} in peptide binding is not obvious. As discussed previously, a hydrophobic clustering of side chains of Val^{3} with Val^{4}, Trp^{7}, Cys^{2}, Cys^{12}, Ile^{1}, and Thr^{13} is present in Compstatin. These hydrophobic interactions might be important in stabilizing the β-turn. The same residues that are involved in side-chain and backbone interactions that stabilize the β-turn are also

important for the functional activity of Compstatin. It is possible that disruption of the turn structure and/or the hydrophobic cluster can cause a loss of structural stability and affect the inhibitory activity of Compstatin. The question that Compstatin undergoes structural reorientation upon binding to C3 is still open and comparison between the structures of the free and bound Compstatin could yield significant insight into the C3–Compstatin recognition process.

Recent studies have determined the species specificity and analyzed the biotransformation of Compstatin *(103)*. The peptide specifically inhibited human and monkey complement but failed to inhibit rat, mouse, guinea pig, rabbit, or swine complement. This exquisite species specificity was further confirmed by inhibiting the hemolytic activity of C3-depleted mouse serum that had been reconstituted by adding human C3. Compstatin inhibited the hemolytic activity of the reconstituted serum at concentrations similar to those seen for human serum. In vitro biotransformation of Compstatin was also studied in human blood. A major pathway of biotransformation was the removal of Ile^1, which could be blocked by *N*-acetylation of the peptide. A recently developed acetylated Compstatin is very stable against proteolytic cleavage and has an in vitro half-life of 24 h in human blood.

The most important question is, Does Compstatin show clinical potential? Up until now it has been tested in three different clinically relevant models. Hyperacute rejection in discordant kidney xenotransplantation has been studied ex vivo using a porcine-to-human perfusion model . In this model Compstatin significantly prolonged the survival of the kidneys *(104)*. Its effect has also been tested in models for extracorporeal circulation *(104,105)*, 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 PMNs (assessed by the expression of CD11b) and the binding of these cells ($CD16^+$) to the polymer surface were almost completely lost. Most recently it has been tested in vivo in primates to examine its effect on complement activation induced by heparin–protamine complex. It effectively inhibited the generation of C3b/c fragments in this model (A. Soulika, M. Khan, A. Sahu, T. Hattori, F. W. Bowen, B. A. Richardson, L. H. Edmonds, and J. D. Lambris, *unpublished observations*). These properties make Compstatin a useful complement inhibitor.

Thioester Inhibitors

When they have been proteolytically cleaved by their respective convertases, 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 *(107,108)* or by attacking the activated thioester of nascent C3b and C4b *(39,40,109)*. Interaction of nucleophilic compounds with the thioester of native complement components was first discussed in 1926 *(110)*. It was found that ammonia, at a pH >8, when it is present as NH_3, is an effective inhibitor of C4. Since then many nucleophilic compounds have been reported to interact with the thioester of native C3 and C4 *(24,107,111)*.

Recent studies have examined in detail the reactivity of the activated thioester of metastable C3b with synthetic compounds *(25,26,100,112)*. 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. 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 *(100)*. These studies, however, did not made any attempt to incorporate additional specificity features into the model compounds and their results suggest that additional improvements in reactivity are possible. Some neuroactive compounds such as L-DOPA, epinephrine, and norepinephrine were also identified as inhibitiors. These findings are especially interesting in light of the involvement of complement in Alzheimer's disease and other neurological diseases *(3,4)*. Whether the complement-inhibiting properties of these compounds contribute in part to their efficacy is unknown at present.

RNA Aptamer Inhibitors

Combinatorial chemistry seems to be the key to the development of complement inhibitors, because the complete 3-D structures of most complement proteases are still unavailable, limiting the

rational design of active-site-based inhibitors. Recently, the SELEX combinatorial chemistry technique was used to develop a pool of $>10^{14}$ unique RNA sequences *(113)*. These molecules were screened against partially trypsinized C5 in the hope of developing aptamers specific to neo-epitopes that are exposed during complement activation . Cloning and sequencing of the bound RNA pool led to the identification of 28 clones, 7 of which showed sequence homology. These aptamers bound C5 with a K_d of 20–30 n*M*, and all of them inhibited C5 cleavage. One of the aptamers was further developed, yielding an aptamer with a K_d of 2–5 n*M* and the ability to inhibit human complement-mediated lysis of antibody-coated sheep erythrocytes. These aptamers and others developed against rat C5 are being evaluated in in vitro and in vivo models .

Naturally Occurring Compounds

A diverse array of compounds that affect complement has been isolated from plants, insects, small organisms, and particular cell types. In only a few cases has their specificity been defined. Here we review the literature that deals with compounds known to interact with C3, C4, and C5. A detailed list of other compounds is provided elsewhere *(87)*.

A large body of literature is available regarding the interaction of heparin with the complement system, a phenomenon that has been recognized since 1929 *(114)*. This area of research is still very active, and heparin is being pursued as a complement inhibitor *(115,116)*. Heparin is known to modulate the classical and alternative pathways of complement *(117,120)*, and it has been shown to interact with multiple complement components, thereby inhibiting various steps of the complement cascade. Heparin is known to bind to C4 but not to C3 or C5 *(121)*. It interferes with the binding of C4 to C1s and C2 *(117,122,123)*. Apart from these in vitro studies, it has also been shown to inhibit complement activation in vivo *(120)*. Most direct evidence of its usefulness as complement inhibitor comes from its effect on complement activation during the use of biomaterials. For example, it is well documented that the use of heparin-coated bypass circuits during cardiopulmonary bypass reduces complement activation *(124–127)*. Studies of the structural requirements for inhibition have shown that *N*- and *O*-sulfation is necessary for the biological effects of heparin on complement *(128)*.

Rosmarinic acid, isolated from *Rosmarinus officinalis* and *Melissa officinalis*, is a natural herbal antiinflammatory agent with anticomplement activity. This compound has been shown to inhibit the classical as well as alternative pathways of complement *(129,130)*. In vivo it is effective in inhibiting cobra venom factor induced paw edema *(129,131)*, immune complex mediated passive cutaneous anaphylaxis *(129)*, and complement-dependent stimulation of prostacyclin synthesis *(132)*. Early studies on the mechanism of complement inhibition by this compound suggested that it inhibited C3 and C5 convertases *(129,133)*. A recent careful analysis, however, has shown that its action on complement is primarily related to its reaction with the activated thioester of metastable C3 *(130)*. At present it is not known whether rosmarinic acid also reacts with the thioester of C4, but the structure suggests that such a reaction is possible, and its overall effect on complement may be due to its effect on C3 as well as C4.

K76 monocarboxalic acid is a fungal metabolite that is derived from *Stachybotrys complementi (134)* and is known to inhibit the classical and alternative pathways of complement. This anticomplement agent inhibits the complement pathway at the C5 step *(135)*; it also inhibits factor I activity *(136)*. When tested in several experimental models of complement activation, it was found to reduce complement-mediated leukocyte accumulation in the subcutaneous air pouch of rats *(137)*, to decrease proteinuria in the early stage of bovine serum albumin (BSA) nephritis (with a 50% reduction in the level of serum C5) *(138)*, and to prevent complement-mediated injuries in a localized acid-aspiration model *(139)*. This agent was also tested in several xenotransplantation models, but it failed to prolong the survival of xenografts *(140–142)*.

Perspective

As discussed above, complement inhibitors ranging from large molecular weight proteins to small synthetic compounds have been identified. A soluble form of complement receptor type 1 and antihuman C5 mAb (h5G1.1–scFv) have shown promise and are currently in clinical trials. Recombinant protein therapy, although an attractive solution, has not proved cost-effective *(143)*. It cannot be denied that in the long term, complement inhibitors will have to be developed as "pills." The design of such therapeutically effective

complement inhibitors can be expedited with the aid of structural information concerning the lead compound and the target protein. Recently, using a combinatorial phage-displayed peptide library, we have identified a 13-mer cyclic peptide inhibitor named Compstatin *(99)*. We have determined the 3-D structure of free Compstatin in solution *(101,101a)* and are using this information to generate analogs with preferred pharmacological properties. Experiments are also underway to localize the binding site of Compstatin on C3 and determine the solution structure of the C3–Compstatin complex. Information obtained from these studies should not only provide important insight into the as yet unidentified "hot spot" in C3 and the mechanism of Compstatin binding to C3 but should also provide useful information to assist molecular docking computer programs to screen databases of commercially available compounds for molecules that are complementary to the bound structure of Compstatin. It is our conviction that such endeavors will lead to a much needed complement inhibitor.

References

1. Kalli, K. R., Hsu, P., and Fearon, D. T. (1994) Therapeutic uses of recombinant complement protein inhibitors. *Springer Semin. Immunopathol.* **15,** 417–431.
2. Robbins, R. A., Russ, W. D., Rasmussen, J. K., and Clayton, M. M. (1987) Activation of the complement system in the adult respiratory distress syndrome. *Am. Rev. Respir. Dis.* **135,** 651–658.
3. Bradt, B. M., Kolb, W. P., and Cooper, N. R. (1998) Complement-dependent proinflammatory properties of the Alzheimer's disease beta-peptide. *J. Exp. Med.* **188,** 431–438.
4. Rogers, J., Cooper, N. R., Webster, S., Schultz, J., Mcgeer, P. L., Styren, S. D., Civin, W. H., Brachova, L., Bradt, B., Ward, P., and Lieberburg, I. (1992) Complement activation by beta-amyloid in Alzheimer-disease. *Proc. Natl. Acad. Sci. USA* **89,** 10,016–10,020.
5. Vasthare, U. S., Rosenwasser, R. H., Barone, F. C., and Tuma, R. F. (1993) Involvement of the complement system in cerebral ischemic and reperfusion injury. *FASEB J.* **7,** A424.
6. Kilgore, K. S., Friedrichs, G. S., Homeister, J. W., and Lucchesi, B. R. (1994) The complement system in myocardial ischaemia/reperfusion injury. *Cardiovasc. Res.* **28,** 437–444.
7. Gallinaro, R., Cheadle, W. G., Applegate, K., and Polk, H. C., Jr. (1992) The role of the complement system in trauma and infection. *Surg. Gynecol. Obstet.* **174,** 435–440.
8. Beranek, J. T. (1997) Terminal complement-complex in myocardial reperfusion injury. *Cardiovasc. Res.* **33,** 495–496.

9. Weiser, M. R., Williams, J. P., Moore, F. D., Kobzik, L., Ma, M. H., Hechtman, H. B., and Carroll, M. C. (1996) Reperfusion injury of ischemic skeletal muscle is mediated by natural antibody and complement. *J. Exp. Med.* **183,** 2343–2348.
10. Johnson, R. J. (1991) Complement activation by biomaterials. *Prog. Clin. Biol. Res.* **337,** 507–512.
11. Pekna, M., Nilsson, L., Nilsson Ekdahl, K., Nilsson, U. R., and Nilsson, B. (1993) Evidence for iC3 generation during cardiopulmonary bypass as the result of blood–gas interaction. *Clin. Exp. Immunol.* **91,** 404–409.
12. Baldwin, W. M., Pruitt, S. K., Brauer, R. B., Daha, M. R., and Sanfilippo, F. (1995) Complement in organ-transplantation-contributions to inflammation, injury, and rejection. *Transplantation* **59,** 797–808.
13. Dalmasso, A. P. (1992) The complement-system in xenotransplantation. *Immunopharmacology* **24** , 149–160.
14. Persidis, A. (1998) Complement inhibitors. *Nature Biotech.* **16,** 882–883.
15. Lambris, J. D., Sahu, A., and Wetsel, R. (1998) The chemistry and biology of C**3,** C**4,** and C5, in *The Human Complement System in Health and Disease* (Volanakis, J. E. and Frank, M., eds.), Marcel Dekker, New York, pp. 83–118.
16. De Bruijn, M. H. L. and Fey, G. H. (1985) Human complement component C3: cDNA coding sequence and derived primary structure. *Proc. Natl. Acad. Sci. USA* **82,** 708–712.
17. Huber, R., Scholze, H., Paques, E. P., and Deisenhofer, J. (1980) Crystal structure analysis and molecular model of human C3a anaphylatoxin. *Hoppe Seylers Z. Physiol. Chemie.* **361,** 1389–1399.
18. Dolmer, K. and Sottrupjensen, L. (1993) Disulfide bridges in human complement component C3b. *FEBS Lett.* **315,** 85–90.
19. Nagar, B., Jones, R. G., Diefenbach, R. J., Isenman, D. E., and Rini, J. M. (1998) X-ray crystal structure of C3d: a C3 fragment and ligand for complement receptor 2. *Science* **280,** 1277–1281.
20. Hase, S., Kikuchi, N., Ikenaka, T., and Inoue, K. (1985) Structures of sugar chains of the third component of human complement. *J. Biochem. (Tokyo)* **98,** 863–874.
21. Hirani, S., Lambris, J. D., and Muller-Eberhard, H. J. (1986) Structural analysis of the asparagine-linked oligosaccharides of human complement component C3. *Biochem. J.* **233,** 613–616.
22. Müller-Eberhard, H. J., Dalmasso, A. P., and Calcott, M. A. (1966) The reaction mechanism of β1c-Globulin (C'3) in immune hemolysis. *J. Exp. Med.* **123,** 33–54.
23. Law, S. K. A. and Dodds, A. W. (1997) The internal thioester and the covalent binding properties of the complement proteins C3 and C4. *Prot. Sci.* **6,** 263–274.
24. Tack, B. F., Harrison, R. A., Janatova, J., Thomas, M. L., and Prahl, J. W. (1980) Evidence for presence of an internal thiolester bond in third component of human complement. *Proc. Natl. Acad. Sci. USA* **77,** 5764–5768.
25. Sahu, A., Kozel, T. R., and Pangburn, M. K. (1994) Specificity of the thioester-containing reactive site of human C3 and its significance to complement activation. *Biochem. J* **302,** 429–436.

26. Sahu, A. and Pangburn, M. K. (1994) Covalent attachment of human complement C3 to IgG: Identification of the amino acid residue involved in ester linkage formation. *J. Biol. Chem.* **269,** 28,997–29,002.
27. Kim, Y. U., Carroll, M. C., Isenman, D. E., Nonaka, M., Pramoonjago, P., Takeda, J., Inoue, K., and Kinoshita, T. (1992) Covalent binding of C3b to C4b within the classical complement pathway C5 convertase: determination of amino acid residues involved in ester linkage formation. *J. Biol. Chem.* **267,** 4171–4176.
28. Kinoshita, T., Takata, Y., Kozono, H., Takeda, J., Hong, K., and Inoue, K. (1988) C5 convertase of the alternative complement pathway: covalent linkage between two C3b molecules within the trimolecular complex enzyme. *J. Immunol.* **141,** 3895–3901.
29. Gigli, I., von Zabern, I., and Porter, R. R. (1977) The isolation and structure of C**4,** the fourth component of human complement. *Biochem. J.* **165,** 439–446.
30. Schreiber, R. D. and Muller-Eberhard, H. J. (1974) Fourth component of human complement: Description of a three chain structure. *J. Exp. Med.* **140,** 1324–1335.
31. Seya, T., Nagasawa, S., and Atkinson, J. P. (1986) Location of the interchain disulfide bonds of the fourth component of human complement (C4): evidence based on the liberation of fragments secondary to thiol-disulfide interchange reactions. *J. Immunol.* **136,** 4152–4156.
32. Belt, K. T., Carroll, M. C., and Porter, R. R. (1984) The structural basis of the multiple forms of human complement component C4. *Cell* **36,** 907–914.
33. Chan, A. C. and Atkinson, J. P. (1985) Oligosaccharide structure of human C4. *J. Immunol.* **134,** 1790–1798.
34. Goldberger, G. and Colten, H. R. (1980) Precursor complement protein (pro-C4) is converted in vitro to native C4 by plasmin. *Nature* **286,** 514–516.
35. Karp, D. R. (1983) Post-translational modification of the fourth component of complement. Sulfation of the alpha chain. *J. Biol. Chem.* **258,** 12,745–12,748.
36. Chan, A. C., Mitchell, K. R., Munns, T. W., Karp, D. R., and Atkinson, J. P. (1983) Identification and partial characterization of the secreted form of the fourth component of human complement. Evidence that it is different from major plasma form. *Proc. Natl. Acad. Sci. USA* **80,** 268–272.
37. Pangburn, M. K. (1992) Spontaneous thioester bond formation in alpha 2-macroglobulin, C3 and C4. *FEBS Lett.* **308,** 280–282.
38. Matsushita, M. and Fujita, T. (1992) Activation of the classical complement pathway by mannose-binding protein in association with a novel C1s-like serine protease. *J. Exp. Med.* **176,** 1497–1502.
39. Isenman, D. E. and Young, J. R. (1984) The molecular basis for the difference in immune hemolysis activity of the Chido and Rodgers isotypes of human complement component C4. *J. Immunol.* **132,** 3019–3027.
40. Law, S. K. A., Dodds, A. W., and Porter, R. R. (1984) A comparison of the properties of two classes, C4A and C4B, of the human complement component C4. *EMBO J.* **3,** 1819–1823.
41. Bolotin, C., Morris, S., Tack, B., and Prahl, J. (1977) Purification and structural analysis of the fourth component of human complement. *Biochemistry* **16,** 2008–2015.

42. Matsushita, M., Takahashi, M., Thiel, S., Jensenius, J. C., and Fujita, T. (1998) Distinct proteolytic activities of MASP-1 and MASP-2. *Mol. Immunol.* **35,** 349.
43. Kerr, M. A. (1980) The human complement system: assembly of the classical pathway C3 convertase. *Biochem. J.* **189,** 173–181.
44. Muller-Eberhard, H. J., Polley, M. J., and Calcott, R. M. (1967) Formation and functional significance of a molecular complex derived from the second and the fourth component of human complement. *J. Exp. Med.* **125,** 359–380.
45. Press, E. M. and Gagnon, J. (1981) Human complement component C4: structural studies on the fragments derived from C4b by cleavage with C3b inactivator. *Biochem. J.* **199,** 351–357.
46. von Zabern, I., Bloom, E. L., Chu, V., and Gigli, I. (1982) The fourth component of human complement treated with amines or chaotropes or frozen-thawed (C4b-Like C4): interaction with C4 binding protein and cleavage by C3b/C4b inactivator. *J. Immunol.* **128,** 1433–1438.
47. Kinoshita, T., Medof, M. E., Hong, K., and Nussenzweig, V. (1986) Membrane-bound C4b interacts endogenously with complement receptor CR1 of human red cells. *J. Exp. Med.* **164,** 1377–1388.
48. Seya, T., Turner, J. R., and Atkinson, J. P. (1986) Purification and characterization of a membrane protein (gp45–70) that is a cofactor for cleavage of C3b and C4b. *J. Exp. Med.* **163,** 837–855.
49. Tomana, M., Niemann, M., Garner, C., and Volanakis, J. E. (1985) Carbohydrate composition of the second, third and fifth components and factors B and D of human complement. *Mol. Immunol.* **22,** 107–111.
50. DiScipio, R. G., Smith, C. A., Müller-Eberhard, H. J., and Hugli, T. E. (1983) The activation of human complement component C5 by a fluid phase C5 convertase. *J. Biol. Chem.* **258,** 10,629–10,636.
51. Ooi, Y. M. and Colten, H. R. (1979) Biosynthesis and post-synthetic modification of a precursor (pro-C5) of the fifth component of mouse complement (C5). *J. Immunol.* **123,** 2494–2498.
52. Ooi, Y. M., Harris, D. E., Edelson, P. J., and Colten, H. R. (1980) Post-translational control of complement (C5) production by resident and stimulated mouse macrophages. *J. Immunol.* **124,** 2077–2081.
53. DiScipio, R. G. and Stura, E. A. (1996) Crystallization of human complement component C5. *Mol. Immunol.* **33,** 43–43 (Abstr).
54. Perkins, S. J., Smith, K. F., Nealis, A. S., Lachmann, P. J., and Harrison, R. A. (1990) Structural homologies of component C5 of human complement with components C3 and C4 by neutron scattering. *Biochemistry* **29,** 1175–1180.
55. Goetzl, E. J. and Austen, K. F. (1974) Stimulation of neutrophil leucocyte aerobic glucose metabolism by purified chemotactic factors. *J. Clin. Invest.* **53,** 591–599.
56. Gerard, C. and Hugli, T. E. (1981) Identification of classical anaphylatoxin as the des-Arg form of the C5a molecule: evidence of a modulator role for the oligosaccharide unit in human des-Arg74-C5a. *Proc. Natl. Acad. Sci. USA* **78,** 1833–1837.

57. Chenoweth, D. E. and Hugli, T. E. (1980) Human C5a and C5a analogs as probes for the neutrophil C5a receptor. *Mol. Immunol.* **17,** 151–161.
58. Webster, R. O., Hong, S. R., Johnston, R. B., Jr., and Henson, P. M. (1980) Biological effects of the human complement fragments C5a and C5ades Arg on neutrophil function. *Immunopharmacology* **2,** 201–219.
59. Weisman, H. F., Bartow, T., Leppo, M. K., Marsh, H. C. Jr., Carson, G. R., Concino, M. F., Boyle, M. P., Roux, K. H., Weisfeldt, M. L., and Fearon, D. T. (1990) Soluble human complement receptor type 1: in vivo inhibitor of complement suppressing post-ischemic myocardial inflammation and necrosis. *Science* **249,** 146–151.
60. Lachmann, P. J. and Davies, A. (1997) Complement and immunity to viruses. *Immunol. Rev.* **159,** 69–77.
61. Cooper, N. R. (1991) Complement evasion strategies of microorganisms. *Immunol. Today* **12,** 327–331.
62. Sahu, A., Sunyer, J. O., Moore, W. T., Sarrias, M. R., Soulika, A. M., and Lambris, J. D. (1998) Structure, functions, and evolution of the third complement component and viral molecular mimicry. *Immunol. Res.* **17,** 109–121.
63. Kotwal, G. J., and Moss, B. (1988) Vaccinia virus encodes a secretory polypeptide structurally related to complement control proteins. *Nature* **335,** 176–178.
64. Kotwal, G. J., Isaacs, S. N., Mckenzie, R., Frank, M. M., and Moss, B. (1990) Inhibition of the complement cascade by the major secretory protein of vaccinia virus. *Science* **250,** 827–830.
65. Isaacs, S. N., Kotwal, G. J., and Moss, B. (1992) Vaccinia virus complement-control protein prevents antibody-dependent complement-enhanced neutralization of infectivity and contributes to virulence. *Proc. Natl. Acad. Sci. USA* **89,** 628–632.
66. Mckenzie, R., Kotwal, G. J., Moss, B., Hammer, C. H., and Frank, M. M. (1992) Regulation of complement activity by vaccinia virus complement-control protein. *J. Infect. Dis.* **166,** 1245–1250.
67. Sahu, A., Isaacs, S. N., Soulika, A. M., and Lambris, J. D. (1998) Interaction of vaccinia virus complement control protein with human complement proteins: factor I-mediated degradation of C3b to $iC3b_1$ inactivates the alternative complement pathway. *J. Immunol.* **160,** 5596–5604.
68. Massung, R. F., Esposito, J. J., Liu, L. I., Qi, J., Utterback, T. R., Knight, J. C., Aubin, L., Yuran, T. E., Parsons, J. M., Loparev, V. N., Selivanov, N. A., Cavallaro, K. F., Kerlavage, A. R., Mahy, B. W. J., and Venter, J. C. (1993) Potential virulence determinants in terminal regions of variola smallpox virus genome. *Nature* **366,** 748–751.
69. Shchelkunov, S. N., Blinov, V. M., Totmenin, A. V., Marennikova, S. S., Kolykhalov, A. A., Frolov, I. V., Chizhikov, V. E., Gutorov, V. V., Gashnikov, P. V., Belanov, E. F., Belavin, P. A., Resenchuk, S. M., Shelikhina, E. M., Netesov, S. V., Andzhaparidze, O. G., and Sandakhchiev, L. S. (1992) Structural-functional organization of the smallpox virus genome. 1. cloning of viral-DNA HINDIII and XHOI fragments and sequencing of HINDIII fragment-M, fragment-L, and fragment-I. *Mol. Biol.* **26,** 731–744.

70. Albrecht, J. C. and Fleckenstein, B. (1992) New member of the multigene family of complement control proteins in herpesvirus saimiri. *J. Virol.* **66,** 3937–3940.
71. Fodor, W. L., Rollins, S. A., Biancocaron, S., Rother, R. P., Guilmette, E. R., Burton, W. V., Albrecht, J. C., Fleckenstein, B., and Squinto, S. P. (1995) The complement control protein homolog of herpesvirus saimiri regulates serum complement by inhibiting C3 convertase activity. *J. Virol.* **69,** 3889–3892.
72. Russo, J. J., Bohenzky, R. A., Chien, M. C., Chen, J., Yan, M., Maddalena, D., Parry, J. P., Peruzzi, D., Edelman, I. S., Chang, Y. A., and Moore, P. S. (1996) Nucleotide sequence of the Kaposi sarcoma-associated herpesvirus (HHV8). *Proc. Natl. Acad. Sci. USA* **93,** 14,862–14,867.
73. Virgin, H. W., Latreille, P., Wamsley, P., Hallsworth, K., Weck, K. E., DalCanto, A. J., and Speck, S. H. (1997) Complete sequence and genomic analysis of murine gammaherpesvirus 68. *J. Virol.* **71,** 5894–5904.
74. Bruggemann, M. and Taussig, M. J. (1997) Production of human antibody repertoires in transgenic mice. *Curr. Opin. Biotechnol.* **8,** 455–458.
75. Fishwild, D. M., ODonnell, S. L., Bengoechea, T., Hudson, D. V., Harding, F., Bernhard, S. L., Jones, D., Kay, R. M., Higgins, K. M., Schramm, S. R., and Lonberg, N. (1996) High-avidity human IgG kappa monoclonal antibodies from a novel strain of minilocus transgenic mice. *Nat. Biotech.* **14,** 845–851.
76. Wang, X., Sahu, A., Pangburn, M. K., and Wetsel, R. A. (1996) Inhibition of C5 cleavage but not C5 binding by a monoclonal antibody that recognizes an 85 aamino acid region of C5 β-chain. *Mol. Immunol.* **33,** 56 (Abstr).
77. Wurzner, R., Schulze, M., Happe, L., Franzke, A., Bieber, F. A., Oppermann, M., and Gotze, O. (1991) Inhibition of terminal complement complex-formation and cell-lysis by monoclonal-antibodies. *Compl. Inflam.* **8,** 328–340.
78. Rollins, S. A., Fitch, J. C. K., Shernan, S., Rinder, C. S., Rinder, H. M., Smith, B. R., Collard, C. D., Stahl, G. L., Alford, B. L., Li, L., and Matis, L. A. (1998) Anti-C5 single chain antibody therapy blocks complement and leukocyte activation and reduces myocardial tissue damage in CPB patients. *Mol. Immunol.* **35,** 397.
79. Rinder, C. S., Rinder, H. M., Smith, B. R., Fitch, J. C. K., Smith, M. J., Tracey, J. B., Matis, L. A., Squinto, S. P., and Rollins, S. A. (1995) Blockade of C5a and C5b–9 generation inhibits leukocyte and platelet activation during extracorporeal-circulation. *J. Clin. Invest.* **96,** 1564–1572.
80. Wang, Y., Hu, Q. L., Madri, J. A., Rollins, S. A., Chodera, A., and Matis, L. A. (1996) Amelioration of lupus-like autoimmune disease in NZB/WF1 mice after treatment with a blocking monoclonal antibody specific for complement component C5 *Proc. Natl. Acad. Sci. USA* **93,** 8563–8568.
81. Wang, Y., Rollins, S. A., Madri, J. A., and Matis, L. A. (1995) Anti-C5 monoclonal antibody therapy prevents collagen-induced arthritis and ameliorates established disease. *Proc. Natl. Acad. Sci. USA* **92,** 8955–8959.

82. Vakeva, A. P., Agah, A., Rollins, S. A., Matis, L. A., Li, L., and Stahl, G. L. (1998) Myocardial infarction and apoptosis after myocardial ischemia and reperfusion—Role of the terminal complement components and inhibition by anti-C5 therapy. *Circulation* **97,** 2259–2267.
83. Evans, M. J., Rollins, S. A., Wolff, D. W., Rother, R. P., Norin, A. J., Therrien, D. M., Grijalva, G. A., Mueller, J. P., Nye, S. H., Squinto, S. P., and Wilkins, J. A. (1995) In vitro and in vivo inhibition of complement activity by a single-chain Fv fragment recognizing human C5. *Mol. Immunol.* **32,** 1183–1195.
84. Sahu, A., Saha, K., Kashyap, A., and Chakrabarty, A. K. (1988) Interaction of anti-leprosy drugs with the rat serum complement system. *Immunopharmacol.* **15,** 143–150.
85. Reynard, A. M. (1980) The regulation of complement activity by pharmacologic agents. *J. Immunopharmacology* **2,** 1–47.
86. Johnson, B. J. (1977) Complement: a host defense mechanism ready for pharmacological manipulation? *J. Pharmaceut. Sci.* **66,** 1367–1377.
87. Makrides, S. C. (1998) Therapeutic inhibition of the complement system. *Pharmacol. Rev.* **50,** 59–87.
88. Asghar, S. S. (1984) Pharmacological manipulation of complement system. *Pharmacol. Rev.* **36,** 223–244.
89. Meri, S. and Pangburn, M. K. (1990) A mechanism of activation of the alternative complement pathway by the classical pathway—protection of C3b from inactivation by covalent attachment to C4b. *Eur. J. Immunol.* **20,** 2555–2561.
90. Reid, K. B. M. and Turner, M. W. (1994) Mammalian lectins in activation and clearance mechanisms involving the complement system. *Springer Semin. Immunopathol.* **15,** 307–326.
91. Matsushita, M. (1996) The lectin pathway of the complement system. *Microbiol. Immunol.* **40,** 887–893.
92. Terrett, N. K., Gardner, M., Gordon, D. W., Kobylecki, R. J., and Steele, J. (1995) Combinatorial synthesis—the design of compound libraries and their application to drug discovery. *Tetrahedron* **51,** 8135–8173.
93. Kay, B. K., Kurakin, A. V., and Hyde-DeRuyscher, R. (1998) From peptides to drugs via phage display. *Drug Discovery Today* **3,** 370–378.
94. Scott, J. K. and Smith, G. P. (1990) Searching for peptide ligands with an epitope library. *Science* **249,** 386–390.
95. Sparks, A. B., Quilliam, L. A., Thorn, J. M., Der, C. J., and Kay, B. K. (1994) Identification and characterization of Src SH3 ligands from phage-displayed random peptide libraries. *J. Biol. Chem.* **269,** 23,853–23,856.
96. Blond-Elguindi, S., Cwirla, S. E., Dower, W. J., Lipshutz, R. J., Sprang, S. R., Sambrook, J. F., and Gething, M.-J. H. (1993) Affinity panning of a library of peptides dispalyed on bacteriophages reveals the binding specificity of BiP. *Cell* **75,** 717–728.
97. Dedman, J. R., Kaetzel, M. A., Chan, H. C., Nelson, D. J., and Jamieson, G. A., Jr. (1993) Selection of targeted biological modifiers fron a bacteriophage library of random peptides: The identification of novel calmodulin regulatory peptides. *J Biol. Chem.* **268,** 23,025–23,030.

98. Devlin, J. J., Panganiban, L. C., and Devlin, P. E. (1990) Random peptide libraries: a source of specific protein binding molecules. *Science* **245,** 404–406.
99. Sahu, A., Kay, B. K., and Lambris, J. D. (1996) Inhibition of human complement by a C3-binding peptide isolated from a phage displayed random peptide library. *J. Immunol.* **157,** 884–891.
100. Sahu, A. and Pangburn, M. K. (1996) Investigation of mechanism-based inhibitors of complement targeting the activated thioester of human C3. *Biochem. Pharmacol.* **51,** 797–804.
101. Morikis, D., Assa-Munt, N., Sahu, A., and Lambris, J. D. (1998) Solution structure of Compstatin, a potent complement inhibitor. *Protein Sci.* **7,** 619–627.
101a. Klepeis, J. L., Floudas, C. A., Morikis, D., and Lambris, J. D. (1999) Predicting peptide structures using NMR data and deterministic global optimization. *J. Comput. Chem.* **20,** 1354–1370.
102. Wilmot, C. M. and Thornton, J. M. (1988) Analysis and prediction of the different types of beta-turn in proteins. *J. Mol. Biol.* **203,** 221–232.
103. Sahu, A., Morikis, D., Soulika, A. M., Spruce, L., Moore, W. T., and Lambris, J. D. (1998) Species specificity, structural functional analysis and biotransformation studies on Compstatin, a potent complement inhibitor. *Mol. Immunol.* **35,** 371–371.
104. Fiane, A. E., Mollnes, T. E., Videm, V. Hovig, T., Høgåsen, K., Mellbye, O. J., Spruce, L. Moore, W. T. Sahu, A., and Lambris, J. D. (1999) Compstatin, a peptide inhibitor of C**3,** prolongs survival of ex vivo perfused pig xenografts. *Xenotransplantation* **6,** 52–65.
105. Fiane, A. E., Mollnes, T. E., Videm, V., Hovig, T., Hogasen, K., Mellbye, O. J., Spruce, L., Moore, W. T., Sahu, A., and Lambris, J. D. (1999) Prolongation of ex-vivo-perfused pig xenograft survival by the complement inhibitor Compstatin. *Transplant. Proc.* **31,** 934–935.
106. Nilsson, B., Larsson, R., Hong, J., Elgue, G., Ekdahl, K. N., Sahu, A., and Lambris, J. D. (1998) Compstatin inhibits complement and cellular activation in whole blood in two models of extracorporeal circulation. *Blood* **92,** 1661–1667.
107. Levine, R. P. and Dodds, A. W. (1990) The thiolester bond of C3. *Curr. Top. Microbiol. Immunol.* **153,** 73–82.
108. Law, S. K., Lichtenberg, N. A., and Levine, R. P. (1980) Covalent binding and hemolytic activity of complement proteins. *Proc. Natl. Acad. Sci. USA* **77,** 7194–7198.
109. Law, S. A., Minich, T. M., and Levine, R. P. (1981) Binding reaction between the third human complement protein and small molecules. *Biochemistry* **20,** 7457–7463.
110. Gordon, J., Whitehead, H., and Wormall, A. (1926) The action of ammonia on complement. The fourth component. *Biochem. J.* 2**0,** 1028–1035.
111. Pangburn, M. K. and Müller-Eberhard, H. J. (1980) Relation of a putative thioester bond in C3 to activation of the alternative pathway and the binding of C3b to biological targets of complement. *J. Exp. Med.* **152,** 1102–1114.

112. Sahu, A. and Pangburn, M. K. (1995) Tyrosine is a potential site for covalent attachment of activated commplement component C3. *Mol. Immunol.* **32,** 711–716.
113. Biesecker, G., Dihel, L., Enney, K., and Bendele, R. (1998) Derivation of RNA aptamer inhibitors of human C5. *Mol. Immunol.* **35,** 334.
114. Ecker, E. E. and Gross, P. (1929) Anticomplementary power of heparin. *J. Infect. Dis.* **44,** 250–253.
115. Wan, S., LeClerc, J. L., and Vincent, J. L. (1997) Inflammatory response to cardiopulmonary bypass—mechanisms involved and possible therapeutic strategies. *Chest* **112,** 676–692.
116. Marsters, S. A., Ayres, T. M., Skubatch, M., Gray, C. L., Rothe, M., and Ashkenazi, A. (1997) Herpesvirus entry mediator, a member of the tumor necrosis factor receptor (TNFR) family, interacts with members of the TNFR-associated factor family and activates the transcription factors NF-kappa B and AP-1. *J. Biol. Chem.* **272,** 14,029–14,032.
117. Raepple E., Hill H. U., and Loos M. (1976) Mode of interaction of different polyanions with the first (C1), the second (C2) and the fourth (C4) component of complement—I. effect on fluid phase C1 and on C1 bound to EA or to EAC4. *Immunochemistry* **13,** 251–255.
118. Weiler J. M., Yurt R. W., Fearon D. T., and Austen, K. F. (1978) Modulation of the formation of the amplification convertase of complement, C3b, Bb, by native and commercial heparin. *J. Exp. Med.* **147,** 409–421.
119. Weiler, J. M. and Linhardt, R. J. (1989) Comparison of the activity of polyanion and polycations on the classical and alternative pathways of complement. *Immunopharmacology* **17,** 65–72.
120. Weiler, J. M., Edens, R. E., Linhardt, R. J., and Kapelanski, D. P. (1992) Heparin and modified heparin inhibit complement activation in vivo. *J. Immunol.* **148,** 3210–3215.
121. Sahu, A. and Pangburn, M. K. (1993) Identification of multiple sites of interaction between heparin and the complement system. *Mol. Immunol.* **30,** 679–684.
122. Loos, M., Volanakis, J. E., and Stroud, R. M. (1976) Mode of interaction of different polyanions with the first (C1), the second (C2) and the fourth (C4) component of complement II: effect of polyanions on the binding of C2 to EAC4b. *Immunochemistry* **13,** 257–261.
123. Loos, M., Volanakis, J. E., and Stroud, R. M. (1976) Mode of interaction of different polyanions with the first (C1), the second (C2) and the fourth (C4) component of complement III: inhibition of C4 and C2 binding site(s) on C1s by polyanions. *Immunochemistry* **13,** 789–791.
124. Fosse, E., Moen, O., Johnson, E., Semb, G., Brockmeier, V., Mollnes, T. E., Fagerhol, M. K., and Venge, P. (1994) Reduced complement and granulocyte activation with heparin- coated cardiopulmonary bypass. *Ann. Thorac. Surg.* **58,** 472–477.
125. Svennevig, J. L., Geiran, O. R., Karlsen, H., Pedersen, T., Mollnes, T. E., Kongsgard, U., and Froysaker, T. (1993) Complement activation during extracorporeal circulation—in vitro comparison of Duraflo-II heparin-coated and uncoated oxygenator circuits. *J. Thorac. Cardiovasc. Surg.* **106,** 466–472.

126. Nilsson, U. R., Larm, O., Nilsson, B., Storm, K. E., Elwing, H., and Ekdahl, K. N. (1993) Modification of the complement binding properties of polystyrene—effects of end-point heparin attachment. *Scand. J. Immunol.* **37,** 349–354.
127. Pekna, M., Hagman, L., Halden, E., Nilsson, U. R., Nilsson, B., and Thelin, S. (1994) Complement activation during cardiopulmonary bypass: effects of immobilized heparin. *Ann. Thorac. Surg.* **58,** 421–424
128. Kazatchkine, M. D., Fearon, D. T., Metcalfe, D. D., Rosenberg, R. D., and Austen, K. F. (1981) Structural determinants of the capacity of heparin to inhibit the formation of the amplification C3 convertase. *J. Clin. Invest.* **67,** 223–228.
129. Englberger, W., Hadding, U., Etschenberg, E., Graf, E., Leyck, S., Winkelmann, J., and Parnham, M. J. (1988) Rosmarinic acid- a new inhibitor of complement C3 convertase with anti-inflammatory activity. *Int. J. Immunopharmacol.* **10,** 729–737.
130. Sahu, A., Rawal, N., and Pangburn, M. K. (1999) Inhibition of complement by covalent attachment of rosmarinic acid to activated C3b. *Biochem. Pharmacol.* **57,** 1439–1446.
131. Leyck, E., Etschenberg, E., Hadding, U., and Winkelmann, J. (1983) A new model of acute inflammation: cobra venom factor induced paw oedema. *Agents Actions* **13,** 437–438.
132. Rampart, M., Beetens, J. R., Bult, H., Herman, A. G., Parnham, M. J., and Winkelmann, J. (1986) Complement-dependent stimulation of prostacyclin biosynthesis: inhibition by rosmarinic acid. *Biochem. Pharmacol.* **35,** 1397–1400.
133. Peake, P. W., Pussell, B. A., Martyn, P., Timmermans, V., and Charlesworth, J. A. (1991) The inhibitory effect of rosmarinic acid on complement involves the C5 convertase. *Int. J. Immunopharmacol.* **13,** 853–857.
134. Miyazaki, W., Tomaoka, H., Shinohara, M., Kaise, H., Izawa, T., Nakano, Y., Kinoshita, T., Hong, K., and Inoue, K. (1980) A complement inhibitor produced by *Stachybotrys complementi*, nov. sp. K-7**6,** a new species of fungi imperfecti. *Microbiol. Immunol.* **24,** 1091–1108.
135. Hong, K., Kinoshita, T., Miyazaki, W., Izawa, T., and Inoue, K. (1979) An anticomplementary agent, K–76 monocarboxylic acid: its site and mechanism of inhibition of the complement activation cascade. *J. Immunol.* **122,** 2418–1223.
136. Hong, K., Kinoshita, T., Kitajima, H., and Inoue, K. (1980) Inhibitory effect of K-76 monocarboxylic acid, an anticomplementary agent, on the C3b inactivator system. *J. Immunol.* **127,** 104–108.
137. Konno, S. and Tsurufuji, S. (1983) Induction of zymosan-air-pouch inflammation in rats and its characterization with reference to the effects of anticomplementary and anti-inflammatory agents. *Br. J. Pharmacol.* **80,** 269–277.
138. Iida, H., Izumino, K., Asaka, M., Takata, M., Mizumura, Y., and Sasayama, S. (1987) Effect of anticomplementary agent, K-76 monocarboxylic acid, on experimental immune complex glomerulonephritis in rats. *Clin. Expt. Immunol.* **67,** 130–134.

139. Yamada, H., Kudoh, I., Nishizawa, H., Kaneko, K., Miyazaki, H., Ohara, M., and Okumura, F. (1997) Complement partially mediates acid aspiration-induced remote organ injury in the rat. *Acta Anaesthesiol. Scand.* **41,** 713–718
140. Tanaka, M., Murase, N., Ye, Q., Miyazaki, W., Nomoto, M., Miyazawa, H., Manez, R., Toyama, Y., Demetris, A. J., Todo, S., and Starzl, T. E. (1996) Effect of anticomplement agent K76 COOH on hamster-to-rat and guinea pig-to-rat heart xenotransplantation. *Transplantation* **62,** 681–688.
141. Blum, M. G., Collins, B. J., Chang, A. C., Zhang, J. P., Knaus, S. A., and Pierson, R. N. (1998) Complement inhibition by FUT–175 and K76-COOH in a pig-to-human lung xenotransplant model. *Xenotransplantation* **5,** 35–43.
142. Kobayashi, T., Neethling, F. A., Taniguchi, S., Ye, Y., Niekrasz, M., Koren, E., Hancock, W. W., Takagi, H., and Cooper, D. K. C. (1996) Investigation of the anti-complement agents, FUT-175 and K76COOH, in discordant xenotransplantation. *Xenotransplantation* **3,** 237–245.
143. Grindley, J. N. and Ogden, J. E. (1995) Forecasting the future for protein drugs. *Scrip. Mag.* November, 53–56.
144. Ahearn, J. M. and Fearon D. T. (1989) Structure and function of the complement receptors, CR1 (CD35), and CR2 (CD21). *Adv. Immunol.* **46,** 183–219.
145. Dellinger, R. P., Zimmerman, J. L., Straube, R. C., Metzler, M. H., Wall, M., Brown, B. K., Levin, J. L., Toth, C. A., and Ryan, U. S. (1996) Results of phase I trial of soluble complement receptor type 1 (TP10) in acute lung injury (ALI). *Crit. Care Med.* **24** (Suppl. 2), A29.
146. Ryan, U. S. (1995) Complement inhibitory therapeutics and xenotransplantation. *Nat . Med.* **1,** 967–968.
147. Medof, M. E., Kinoshita, T., and Nussenzweig,V. (1984) Inhibition of complement activation on the surface of cells after incorporation of decay-acceleration factor (DAF) into their membranes. *J. Exp. Med.* **160,** 1558–1578.
148. Fujita, T., Inoue, T., Ogawa, K., Iida, K., and Tamura, N. (1987) The mechanism of action of decay-accelerating factor (DAF): DAF inhibits the assembly of C3 convertases by dissociating C2a and Bb. *J. Exp. Med.* **166,** 1221–1228.
149. Christiansen, D., Milland, J., Thorley, B. R., Mckenzie, I. F. C., and Loveland, B. E. (1996) A functional analysis of recombinant soluble CD46 in vivo and a comparison with recombinant soluble forms of CD55 and CD35 in vitro. *Eur. J. Immunol.* **26,** 578–585.
150. Oglesby, T. J., Allen, C. J., Liszewski, M. K., White, D. J. G., and Atkinson, J. P. (1992) Membrane cofactor protein (CD46) protects cells from complement-mediated attack by an intrinsic mechanism. *J. Exp. Med.* **175,** 1547–1551
151. Whaley, K., and Ruddy, S. (1976) Modulation of C3b hemolytic activity by a plasma protein distinct from C3b inactivator. *Science* **193,** 1011–1013.
152. Weiler J. M., Daha, M. R., Austen, K. F., and Fearon D. T. (1976) Control of the amplification convertase of complement by the plasma protein beta1H. *Proc. Natl. Acad. Sci. USA* **73,** 3268–3272.

153. Pangburn, M. K., Schreiber, R. D., and Müller-Eberhard, H. J. (1977) Human complement C3b inactivator: isolation, characterization, and demonstration of an absolute requirement for the serum protein β1H for cleavage of C3b and C4b in solution. *J. Exp. Med.* **146,** 257–270.
154. Goldberger, G., Bruns, G. A., Rits, M., Edge, M. D., and Kwiatkowski, D. J. (1987) Huamn complement factor I: analysis of cDNA-derived primary structure and assignment of its gene to chromosome 4. *J. Biol. Chem.* **262,** 10,065–10,071.
155. Chamberlain, D., Ullman, C. G., and Perkins, S. J. (1998) Possible arrangement of the five domains in human complement factor I as determined by a combination of X-ray and neutron scattering and homology modeling. *Biochemistry* **37,** 13918–13929.
156. Chung, L. P., Bentley, D. R., and Reid, K. B. M. (1985) Molecular cloning and characterization of the cDNA coding for C4b-binding protein, a regulatory protein of theclassical pathway of the human complement system. *Biochem. J.* **230,** 133–141.
157. Gigli, I., Fujita, T., and Nussenzweig, V. (1979) Modulation of the classical pathway C3 convertase by plasma proteins C4 binding protein and C3b inactivator. *Proc. Natl. Acad. Sci. USA* **76,** 6596–6600.
158. Lopez Trascasa, M., Bing, D. H., Rivard, M., and Nicholson-Weller, A. (1989) Factor J—isolation and characterization of a new polypeptide inhibitor of complement C1. *J. Biol. Chem.* **264,** 16,214–16,221.
159. Gonzalez Rubio, C., Jimenez Clavero, M. A., Fontan, G., and Lopez Trascasa, M. (1994) The inhibitory effect of factor J on the alternative complement pathway. *J. Biol. Chem.* **269,** 26,017–26,024.
160. Jimenezclavero, M. A., Gonzalezrubio, C., Larrucea, S., Gamallo, C., Fontan, G., and Lopeztrascasa, M. (1995) Cell-surface molecules related to factor J in human lymphoid cells and cell-lines. *J. Immunol.* **155,** 2143–2150.
161. Giclas, P. C., King, T. E., Baker, S. L., Russo, J., and Henson, P. M. (1987) Complement activity in normal rabbit bronchoalveolar fluid description of an inhibitor of C3 activation. *Am. Rev. Respir. Dis.* **135,** 403–411.
162. Iwata, K., Seya, T., Ariga, H., and Nagasawa, S. (1994) Expression of a hybrid complement regulatory protein, membrane cofactor protein-decay accelerating factor on chinese hamster ovary—Comparison of its regulatory effect with those of decay accelerating factor and membrane cofactor protein. *J. Immunol.* **152,** 3436–3444.
163. Higgins, P. J., Ko, J. L., Lobell, R., Sardonini, C., Alessi, M. K., and Yeh, C. G. (1997) A soluble chimeric complement inhibitory protein that possesses both decay-accelerating and factor I cofactor activities. *J. Immunol.* **158,** 2872–2881.
164. Fodor, W. L., Rollins, S. A., Guilmette, E. R., Setter, E., and Squinto, S. P. (1995) A novel bifunctional chimeric complement inhibitor that regulates C3 convertase and formation of the membrane attack complex. *J. Immunol.* **155,** 4135–4138.
165. Miller, C. G., Shchelkunov, S. N., and Kotwal, G. J. (1997) The cowpox virus-encoded homolog of the vaccinia virus complement control protein is an inflammation modulatory protein. *Virology* **229,** 126–133.

166. Rosengard, A. M. and Ahearn, J. M. (1998) Creation and functional characterization of spice, the small pox inhibitor of complement enzymes. *Mol. Immunol.* **35,** 397.
Kretzschmar, T., Pohl, M., Casaretto, M., Przewosny, M., Bautsch, W., Klos, A., Saunders, D., and Kohl, J. (1992) Synthetic peptides as antagonists of the anaphylotoxin C3a. *Eur. J. Biochem.* **210,** 185–191.

167a. Kossorotow, A., Optiz, W., Etschenberg, E., and Hadding, U. (1977) Studies on C3 convertase: inhibition of C5 convertase formation by peptides containing aromatic amino acids. *Biochem. J.* **167,** 377–382.

168. Pellas, T. C., Boyar, W., van Oostrum, J., Wasvary, J., Fryer, L. R., Pastor, G., Sills, M., Braunwalder, A., Yarwood, D. R., Kramer, R., Kimble, E., Hadala, J., Haston, W., Moreira-Ludewig, R., Uziel-Fusi, S., Peters, P., Bill, K., and Wennogle, L. P. (1998) Novel C5a receptor antagonists regulate neutrophil functions in vitro and in vivo. *J. Immunol.* **160,** 5616–5621.

169. Zhang, X. L., Boyar, W., Galakatos, N., and Gonnella, N. C. (1997) Solution structure of a unique C5a semi-synthetic antagonist: Implications in receptor binding. *Prot. Sci.* **6,** 65–72.

170. Konteatis, Z. D., Siciliano, S. J., Vanriper, G., Molineaux, C. J., Pandya, S., Fischer, P., Rosen, H., Mumford, R. A., and Springer, M. S. (1994) Development of C5a receptor antagonists—differential loss of functional responses. *J. Immunol.* **153,** 4200–4205.

171. Baranyi, L., Campbell, W., and Okada, H. (1996) Antisense homology boxes in C5a receptor and C5a anaphylatoxin—a new method for identification of potentially active peptides. *J. Immunol.* **157,** 4591–4601.

172. Kaufman, T. S., Srivastava, R. P., Sindelar, R. D., Scesney, S. M., and Marsh, H. C. (1995) Design, synthesis, and evaluation of A/C/D-ring analogs of the fungal metabolite K-76 as potential complement inhibitors. *J. Med. Chem.* **38,** 1437–1445.

173. Kaufman, T. S., Srivastava, R. P. S., Sindelar, R. D., Scesney, S. M., and Marsh, H. C. (1995) Design, synthesis, and evaluation of A/C/D-ring analogs of the fungal metabolite K-76 as potential complement inhibitors—a potential probe for the absolute stereochemistry at position. *Bioorg. Med. Chem. Lett.* **5,** 501–506.

174. Sindelar, R. D., Srivastava, R. P., Bartyzel, P., Assefa, H., Walker, L. A., Zhu, X., Marsh, H. C., and Scesney, S. M. (1997) The design, synthesis and evaluation of potential human complement inhibitors based on a natural product model. *Abstr. Am. Chem. Soc.* **214**(Pt 1), U93–U93.

175. Fujii, S. and Hitomi, Y. (1981) New synthetic inhibitors of C1r, C1 esterase, thrombin, plasmin, kallikren and trypsin. *Biochim. Biophys. Acta* **661,** 342–345.

176. Ikari, N., Sakai, Y., Hitomi, Y., and Fujii, S. (1983) New synthetic inhibitor to the alternative complement pathway. *Immunology* **49,** 685–691.

177. Homeister, J. W., Satoh, P., and Lucchesi, B. R. (1992) Effects of complement activation in the isolated heart—role of the terminal complement components. *Circ. Res.* **71,** 303–319.

178. Inose, K., Ono, K., Tsutida, A., Onai, M., Komai, M., Uchara, K., Yano, S., and Naruse, T. (1997) Active inhibitory effect of nafamostat mesylate against the elevation of plasma myeloperoxidase during hemodialysis. *Nephron* **75,** 420–425.
179. Blondin, C., Fischer, E., Boissonvidal, C., Kazatchkine, M. D., and Jozefonvicz, J. (1994) Inhibition of complement activation by natural sulfated polysaccharides (fucans) from brown seaweed. *Mol. Immunol.* **31,** 247–253.
180. Charreau, B., Blondin, C., Boisson-Vidal, C., Soulillou, J. P., and Anegon, I. (1997) Efficiency of fucans in protecting porcine endothelial cells against complement activation and lysis by human serum. *Transplant. Proc.* **29,** 889–890.
181. Quigg, R. J. (1992) Inhibition of the alternative pathway of complement by glomerular chondroitin sulphate proteoglycan. *Immunology* **76,** 373–377
182. Georgieva, P., Ivanovska, N., Bankova, V., and Popov, S. (1997) Anticomplement activity of lysine complexes of propolis phenolic constituents and their synthetic analogs. *Zeitsch. Naturforsch. C- A J. Biosci.* **52,** 60–64.
183. Jansen, J. A. (1969) A specific inactivator of mammalian C'4 isolated from nurse shark (*Ginglymostroma cirratum*) serum. *J. Exp. Med.* **130,** 217–241.
184. Hensens, O. D., Borris, R. P., Koupal, L. R., Caldwell, C. G., Currie, S. A., Haidri, A. A., Homnick, C. F., Honeycutt, S. S., Lindnmayer, S. M., Schwartz, C. D., Weissberger, B. A., Woodruff, H. B., Zink, D. L., Zitano, L., Fieldhouse, J. M., Rollins, T., Springer, M. S., and Springer, J. P. (1991) L–15**6,**60**2,** a C5a antagonist with a novel cyclic hexadepsipeptide structure from *streptomyces*-Sp MA6348—fermentation, isolation and structure determination *J. Antibiotics* **44,** 249–254.
185. Tsuji, R. F., Magae, J., Nagai, K., and Yamasaki, M. (1992) Effects of L-15**6,**60**2,** a C5a receptor antagonist, on experimental models of inflammation. *Biosci. Biotechnol. Biochem.* **56,** 2034–2036.
186. Tsuji, R. F., Uramoto, M., Koshino, H., Tsuji, N. M., Magae, J., Nagai, K., and Yamasaki, M. (1992) Preferential suppression of delayed-type hypersensitivity by L–15**6,**60**2,** a C5a receptor antagonist. *Biosci. Biotechnol. Biochem.* **56,** 1686–1689.