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- (54) **ANTIBODIES TO THE C3D FRAGMENT OF COMPLEMENT COMPONENT 3**
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(57) **ABSTRACT**

The present invention relates to methods and materials for modulating the complement alternative pathway (CAP), the complement classical pathway (CCP), the complement lectin/mannose pathway (CMP), or combinations thereof, as well as methods and materials for targeting diagnostic, prophylactic and therapeutic agents to localized areas of tissue within the body where they may more directly exert their effects upon the intended target cells or tissue, with reduced, associated systemic effects compared with administration of the same or similar agents in an untargeted, systemic manner. The methods and materials of the present invention may therefore allow for increased efficacy, lower threshold effective dosages and/or lower effective maintenance doses, and/or reduced associated undesired or adverse effects in terms of frequency or severity of occurrence, or both. The present invention also relates to methods and materials for modulating a host humoral immune response, especially reducing, inhibiting, or preventing a host humoral immune response.

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- Certificate of Deposit for ATCC Patent Deposit Designations PTA-11025, PTA-11026 and PTA-11027, Jun. 21, 2010.
- Certificate of Deposit for ATCC Patent Deposit Designations PTA-10998, PTA-10999 and PTA-11000, Jun. 8, 2010.

(56)

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Lemoli et al., "Immunological effects of omalizumab in chronic urticaria: a case report" *Journal Investigational Allergology Clinical Immunology*, (2010), vol. 20, pp. 252-254.

Wigler et al., "A Murine Leukemia Virus Mutant with a Temperature-Sensitive Defect in Membrane Glycoprotein Synthesis", *Cell*, (1979), vol. 16, pp. 77-88.

* cited by examiner

Figure 1

Cleavage of C3

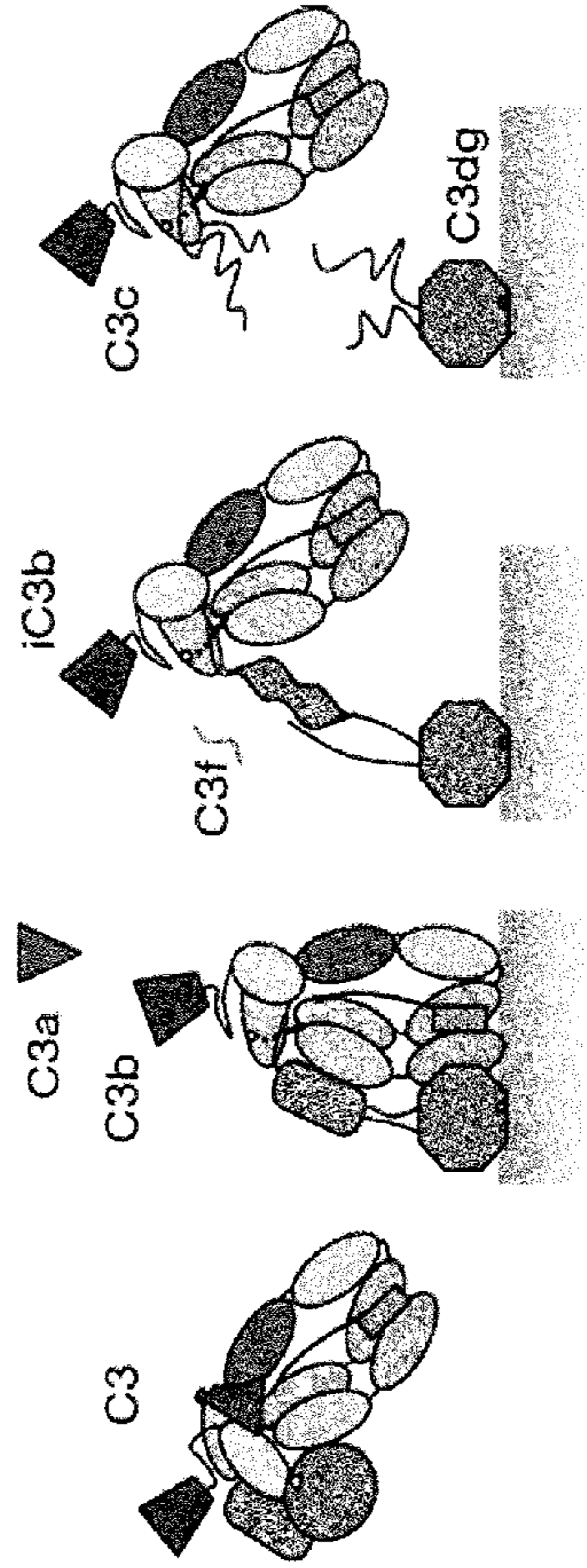
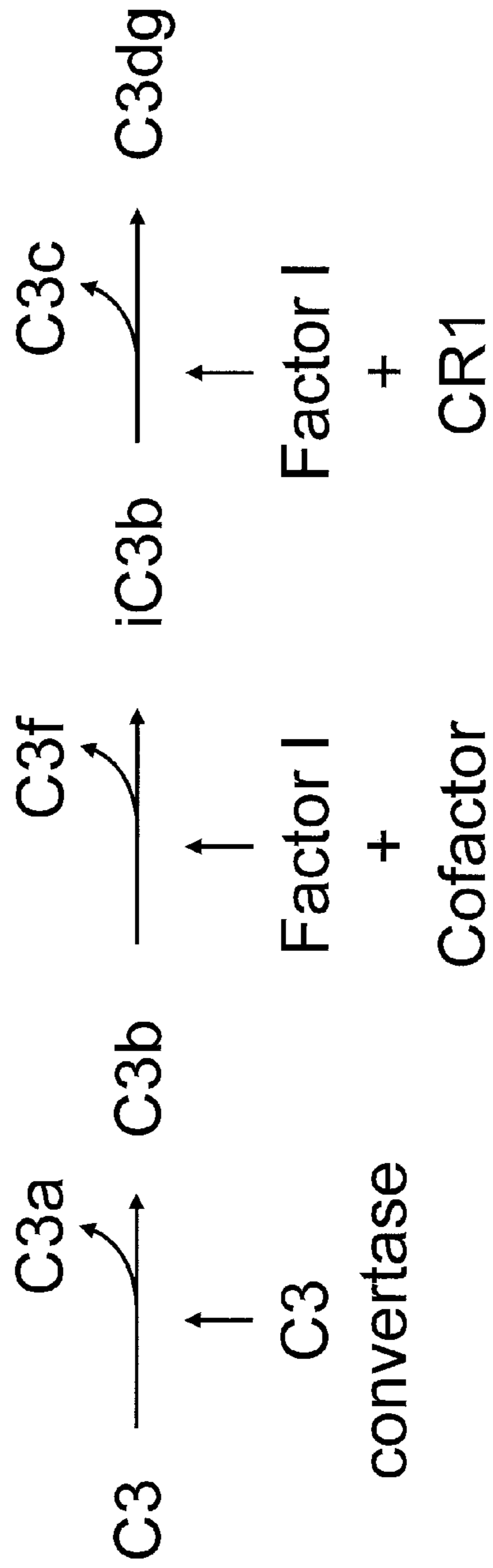
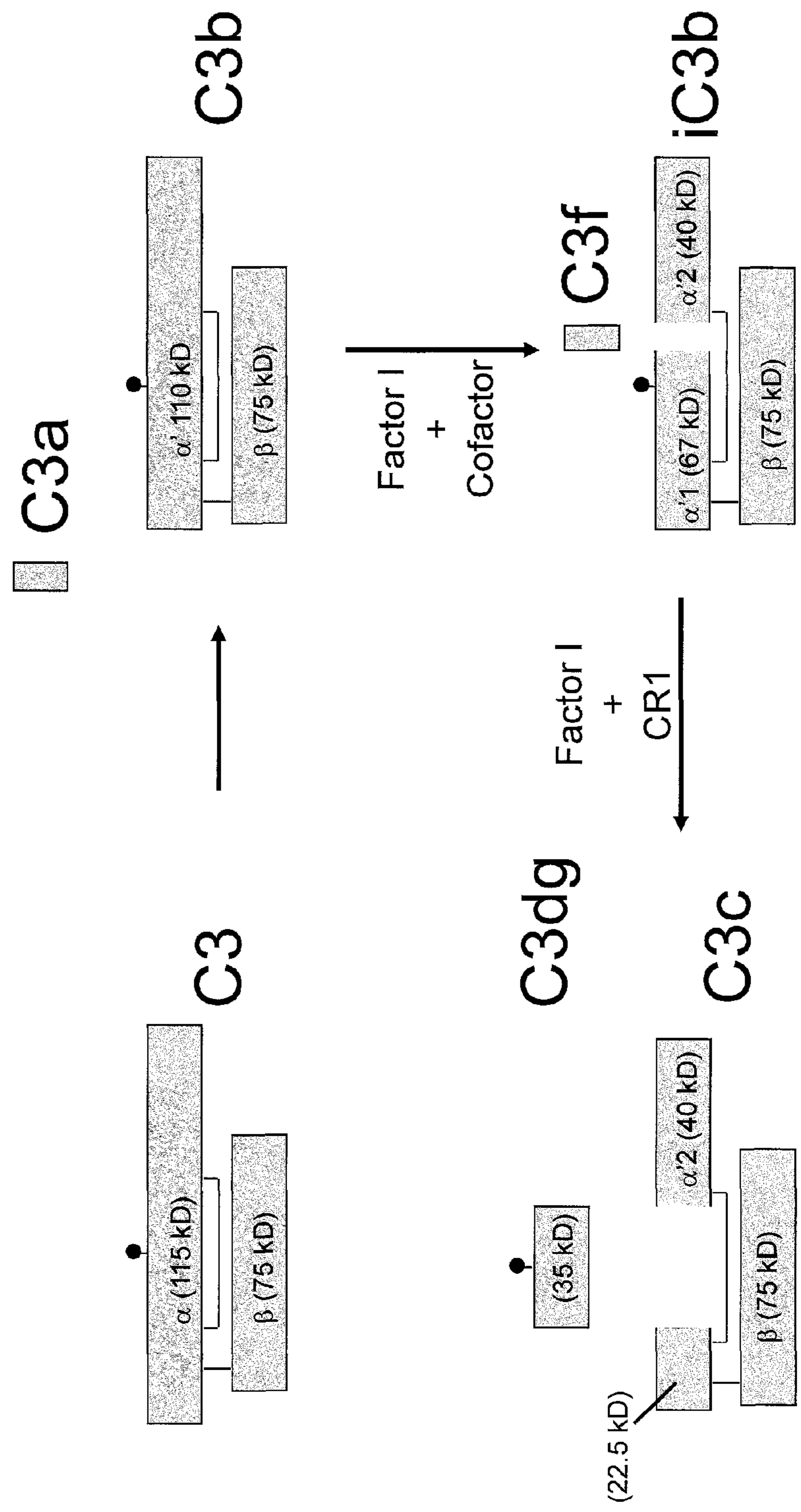


Figure 2

C3 Activation Fragments



Anticipate anti-C3d mAbs could bind to α (115 kD), α' (110 kD), α' 1 (67 kD), α' 2 (~35 kD), C3dg/C3d (~35 kD)

Figure 3

A Subset of mAbs (Group 1) Preferentially Bind C3d by Western Blot Analysis

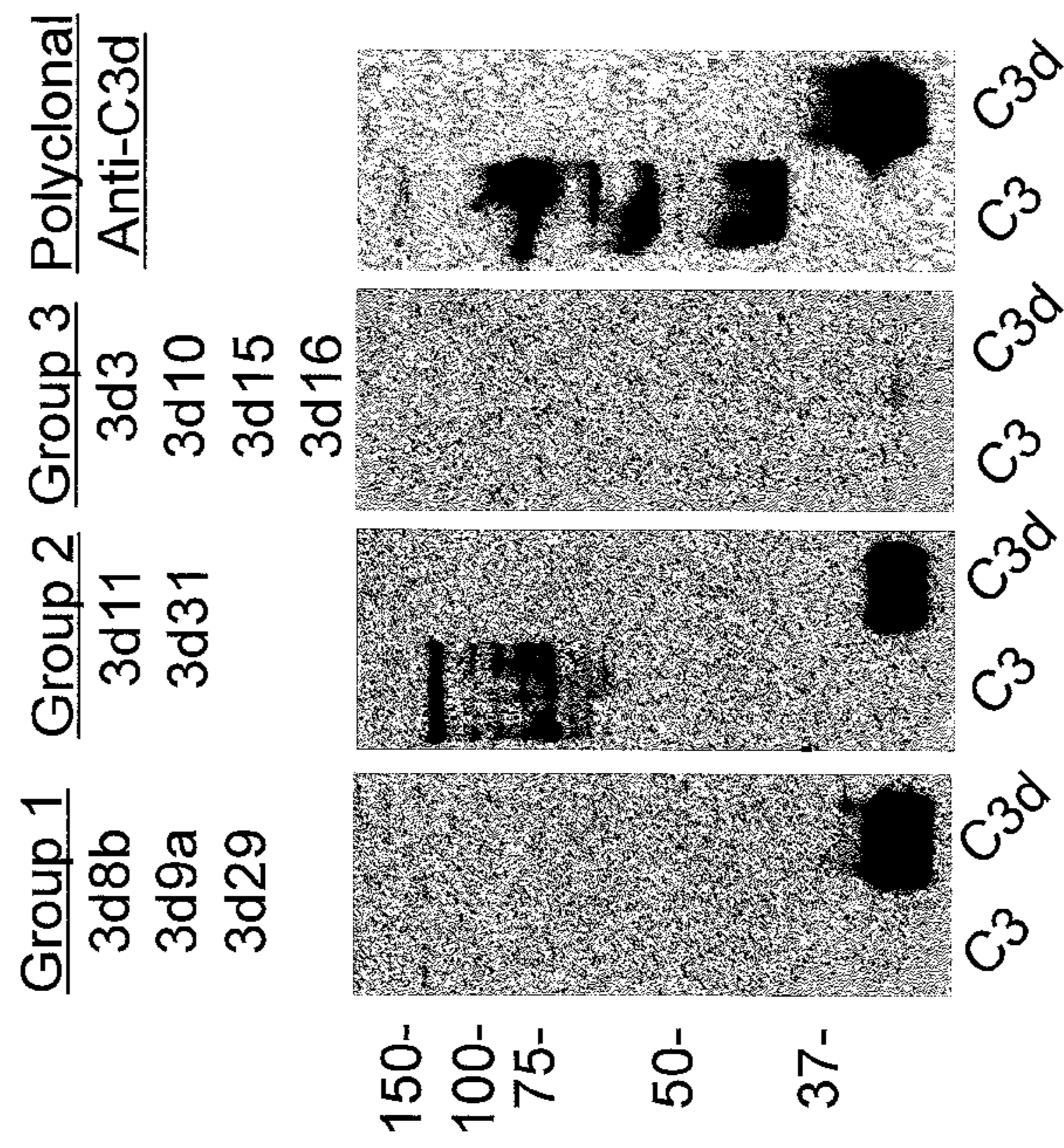


Figure 4

Binding to Native C3 Fragments - Opsonized Zymosan Particles Only By Group 1 Antibodies

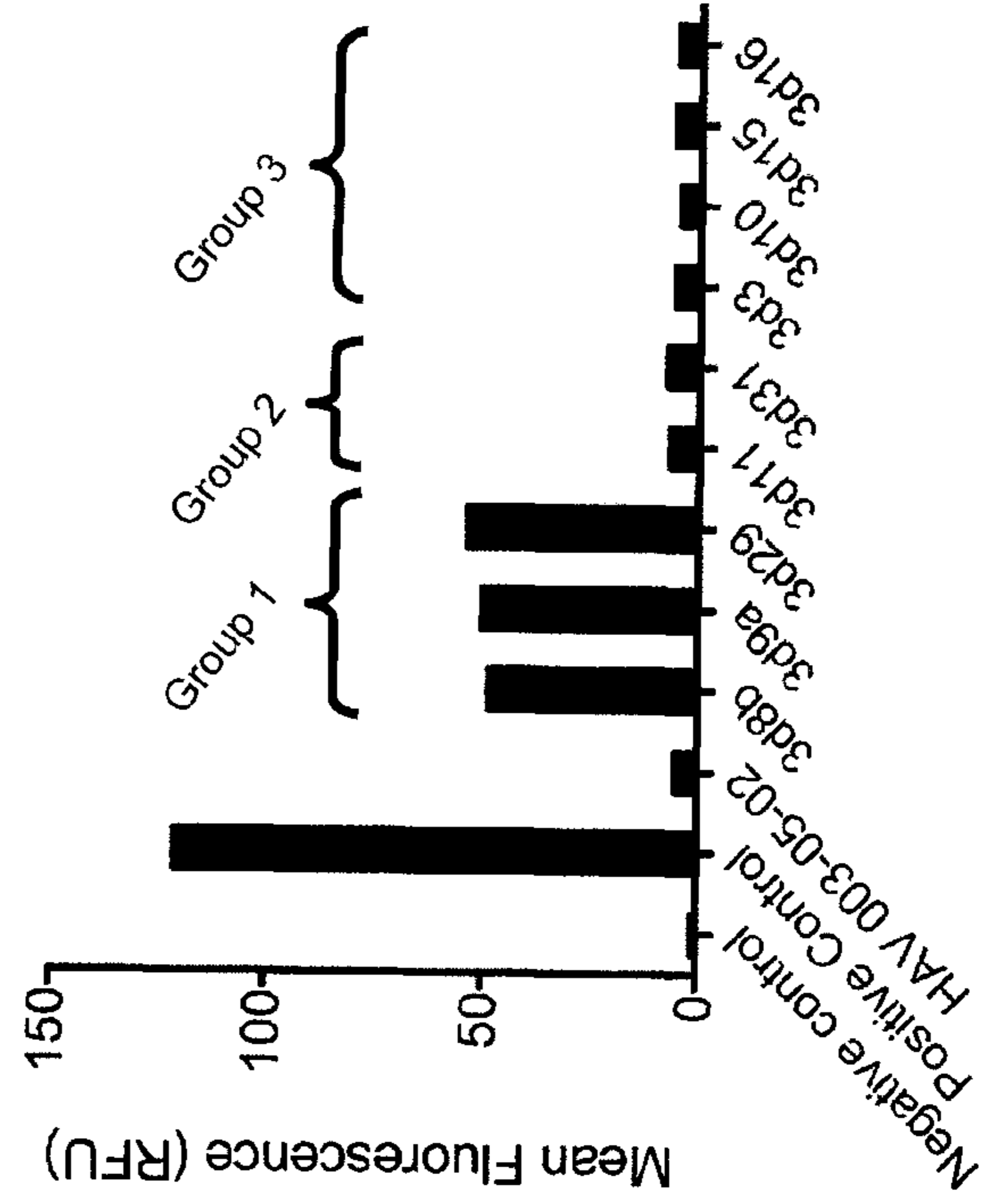


Figure 5 Immunoprecipitation by Group 1 Antibodies of Specific C3 Fragments from iC3b and C3dg in Zymosan-Activated Plasma

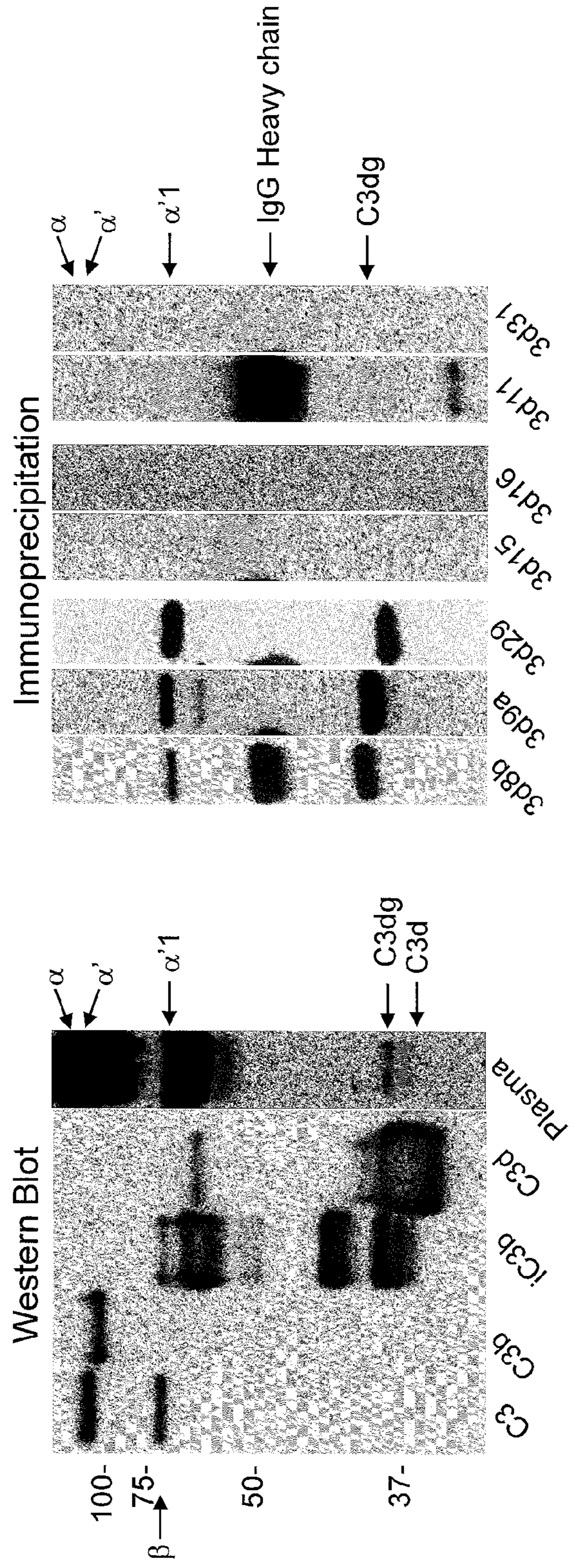


Figure 6

Biacore Analyses and Kd of Group 1 Monoclonal Antibodies

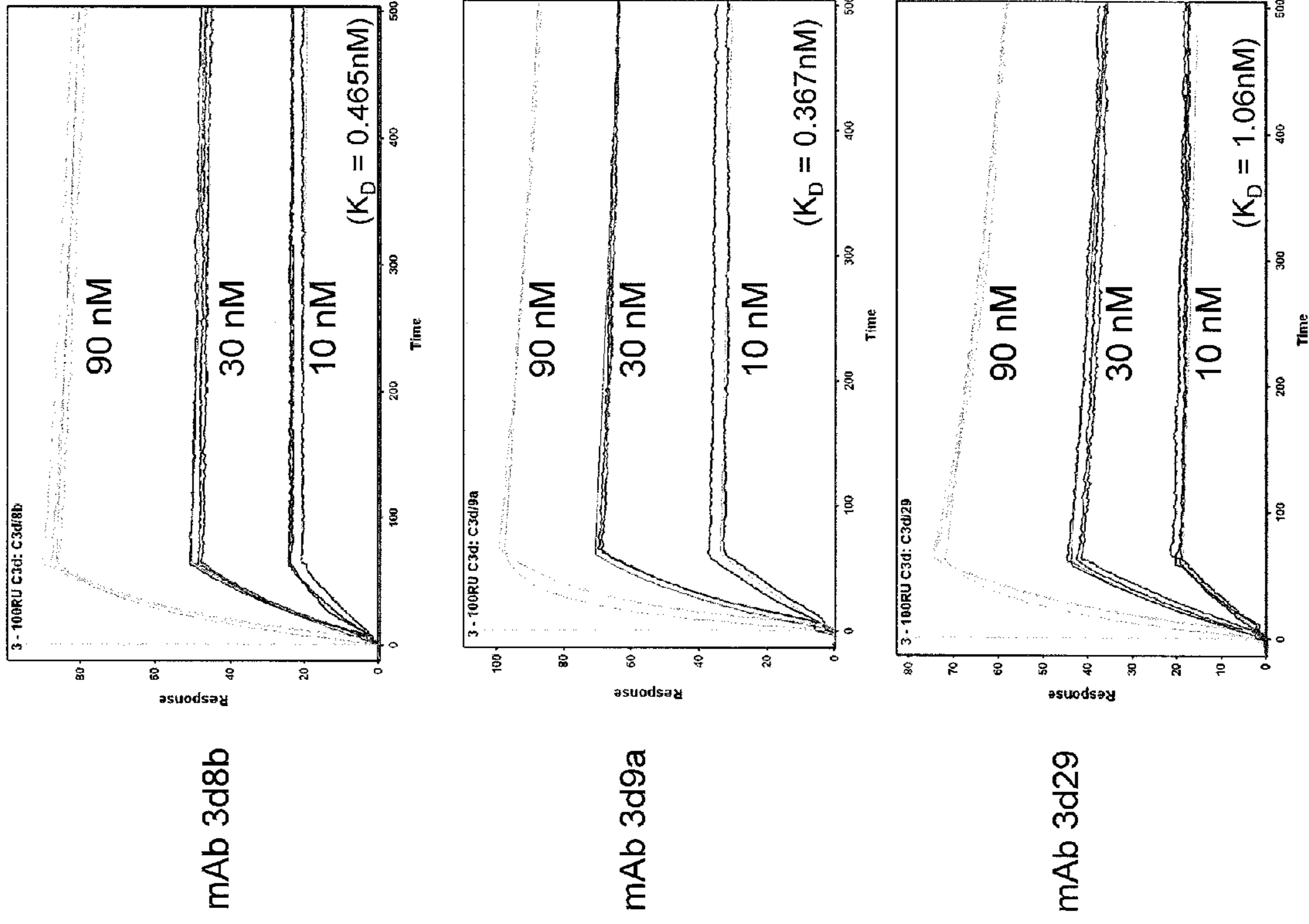


Figure 7

CR2 Competition assay results

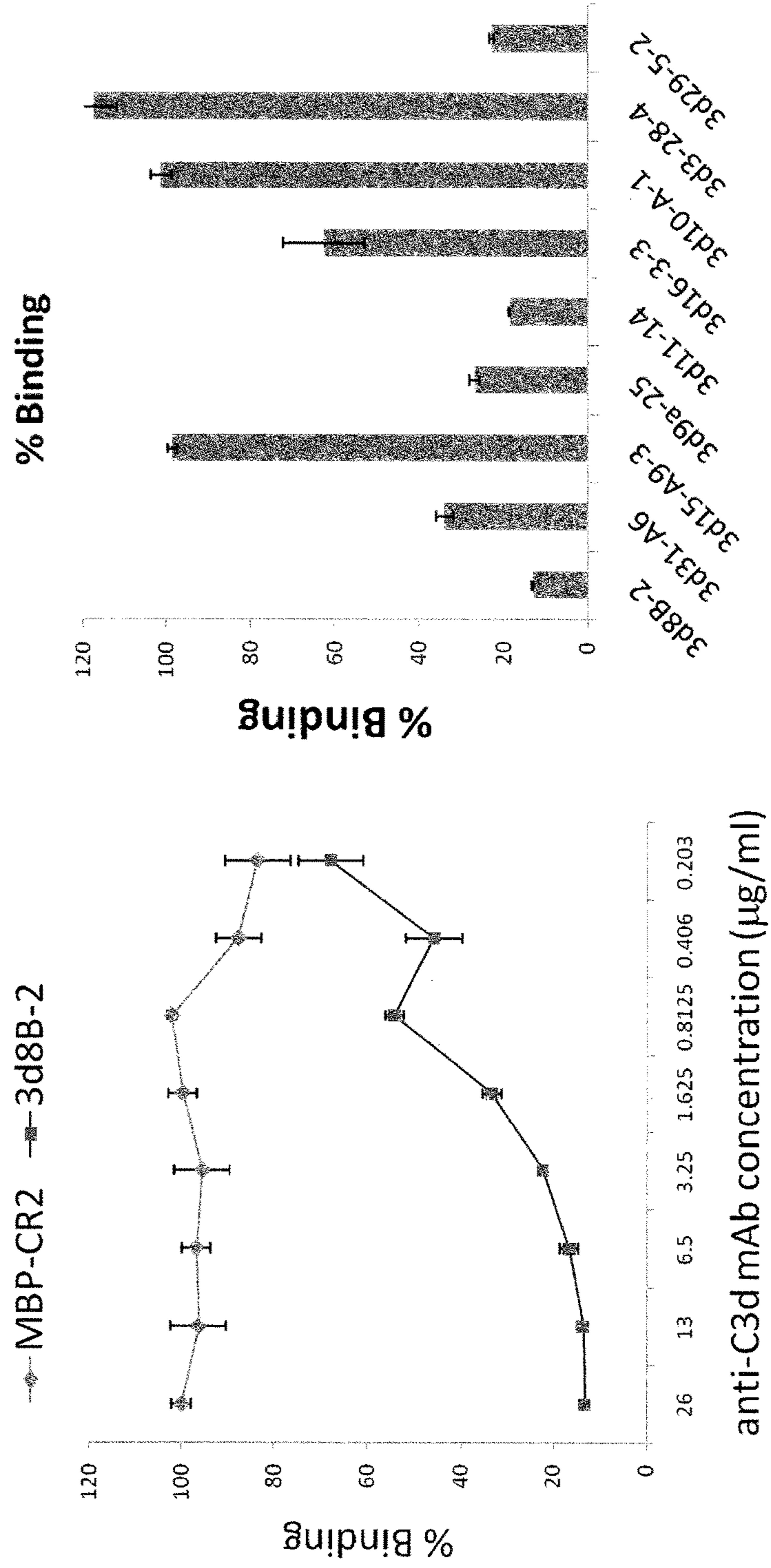


Figure 8

Summary of Key Characteristics of Test Monoclonal Antibodies

	Designation	Isotype	hC3d binding by ELISA	Inhibition of hC3d-hCR2 binding	Zymosan activated serum binding	Mouse C3d binding	Cyno C3d binding
1	3d3	IgG1	+			+	
2	3d10	IgG1	+			+	
3	3d11	IgG1	+	+		+	+
4	3d16	IgG1	+	+/-		+	+/-
5	3d9a	IgG2a/c	+	+	+	+	+
6	3d15	IgG2a/c	+			+	
7	3d29	IgG2a/c	+	+	+	+	+
8	3d31	IgG2a/c	+	+		+	+
9	3d8b	IgG2b	+	+	+	+	+

Figure 9

Only Group 1 Monoclonal Antibodies Target Tissue-Bound C3 Fragments Following In Vivo Injection

A. Glomerular Anti-C3
Fragment Staining



Glomerular C3:
iC3b, C3d

B. Glomerular Mouse IgG Staining

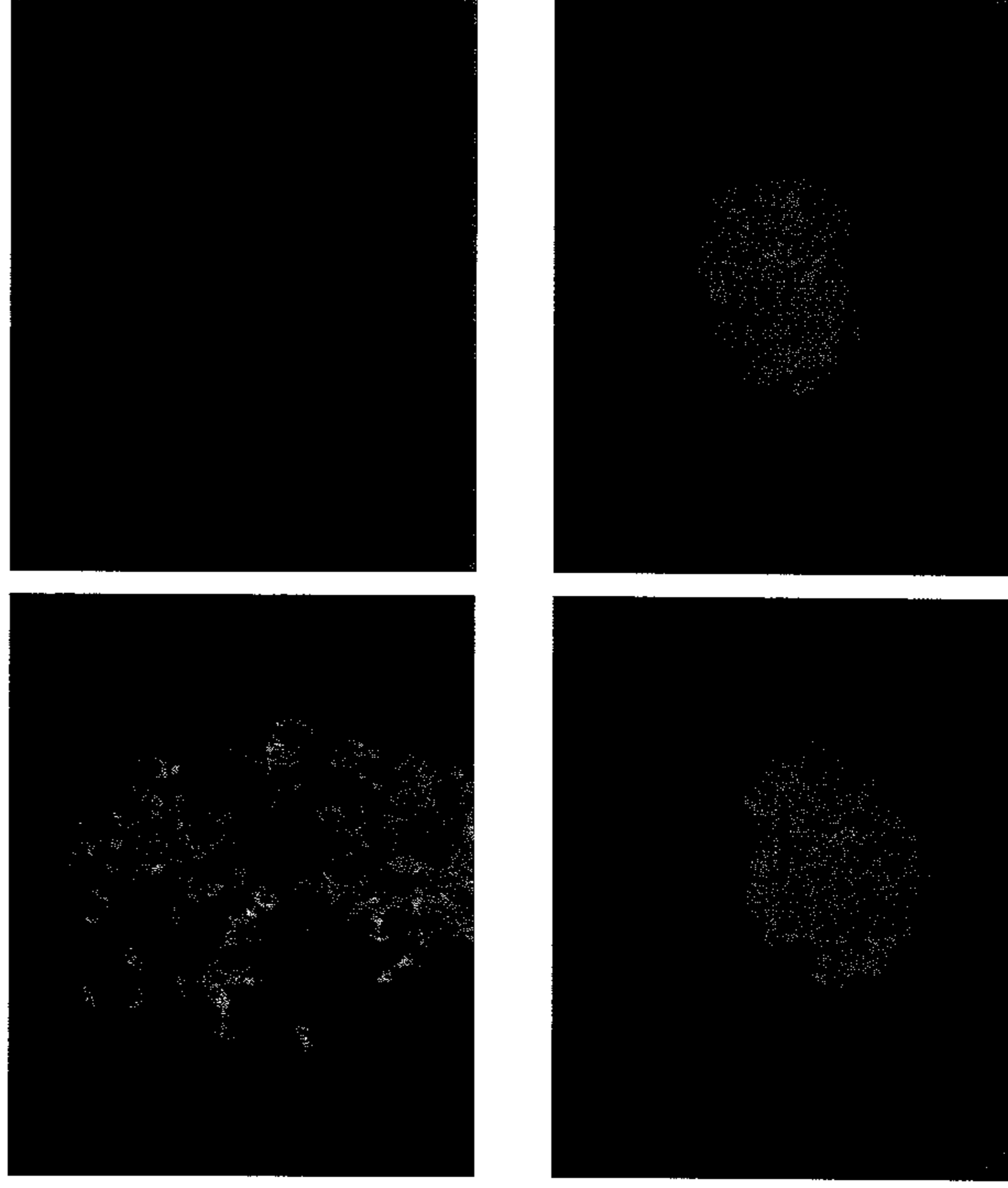


Figure 10

Group 1 Anti-C3d Monoclonal Antibodies Do Not Enhance Complement Activation

Rabbit erythrocyte lysis using 3.3% serum

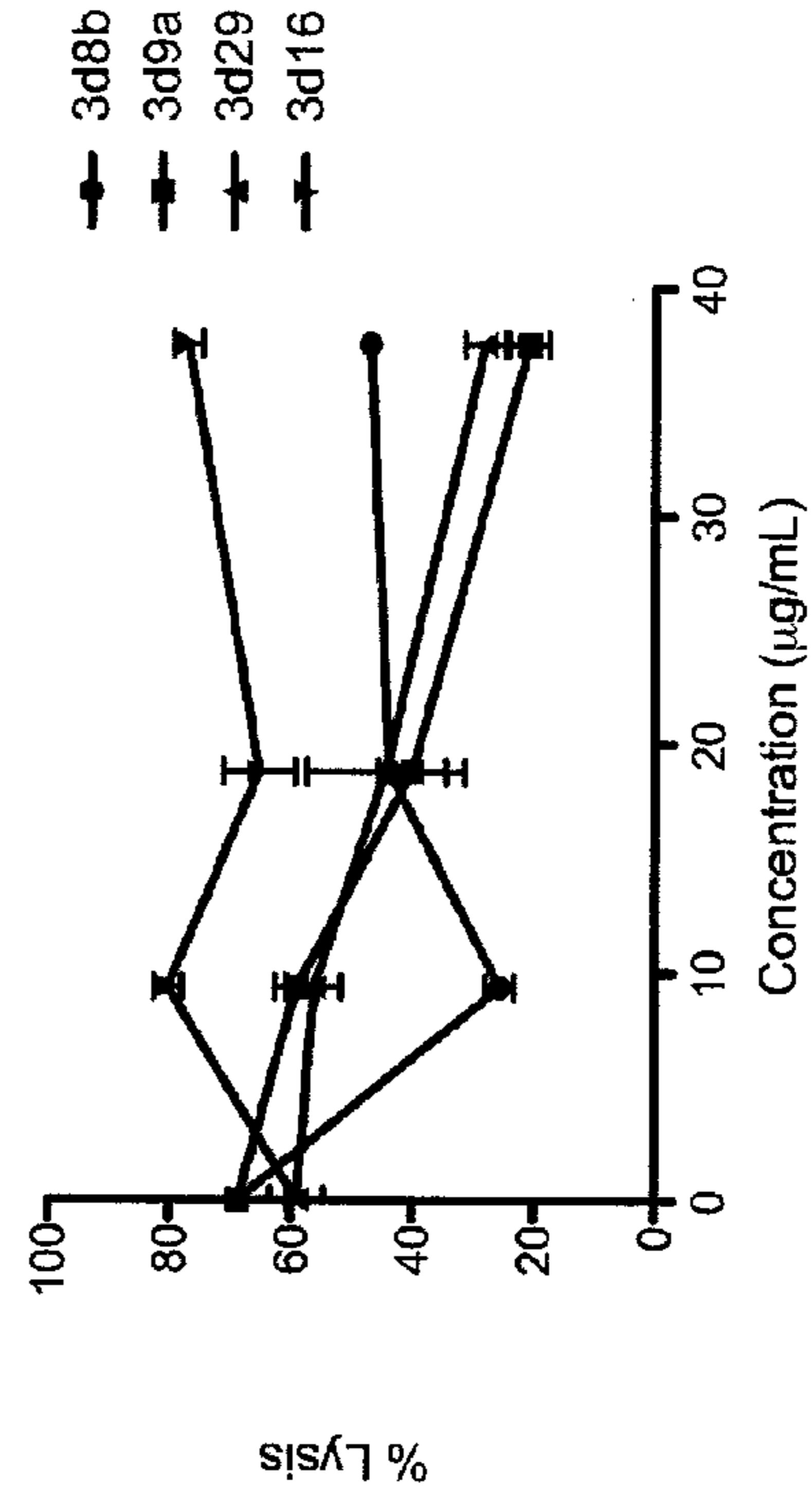


Figure 11

Protocol for Analysis of Effects of Anti-C3d Monoclonal Antibodies on Model Antigen Immune Response

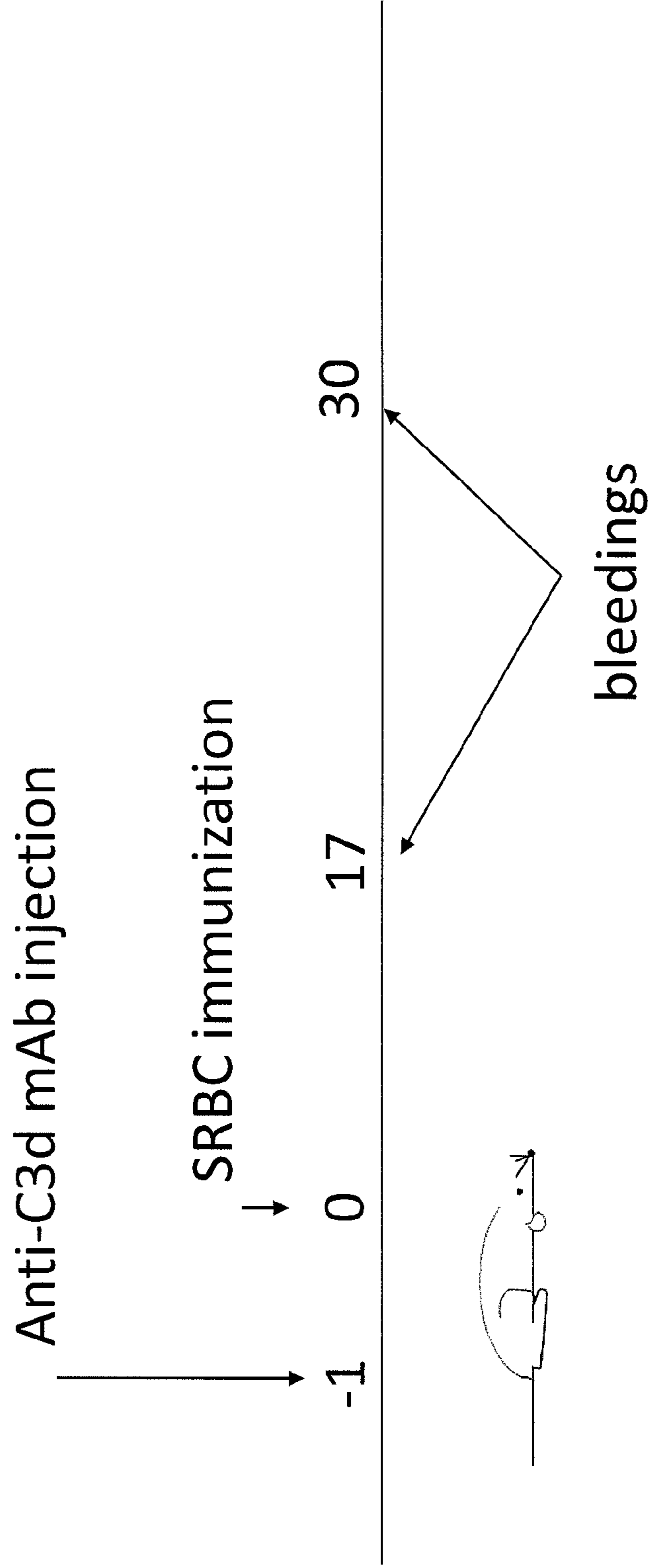
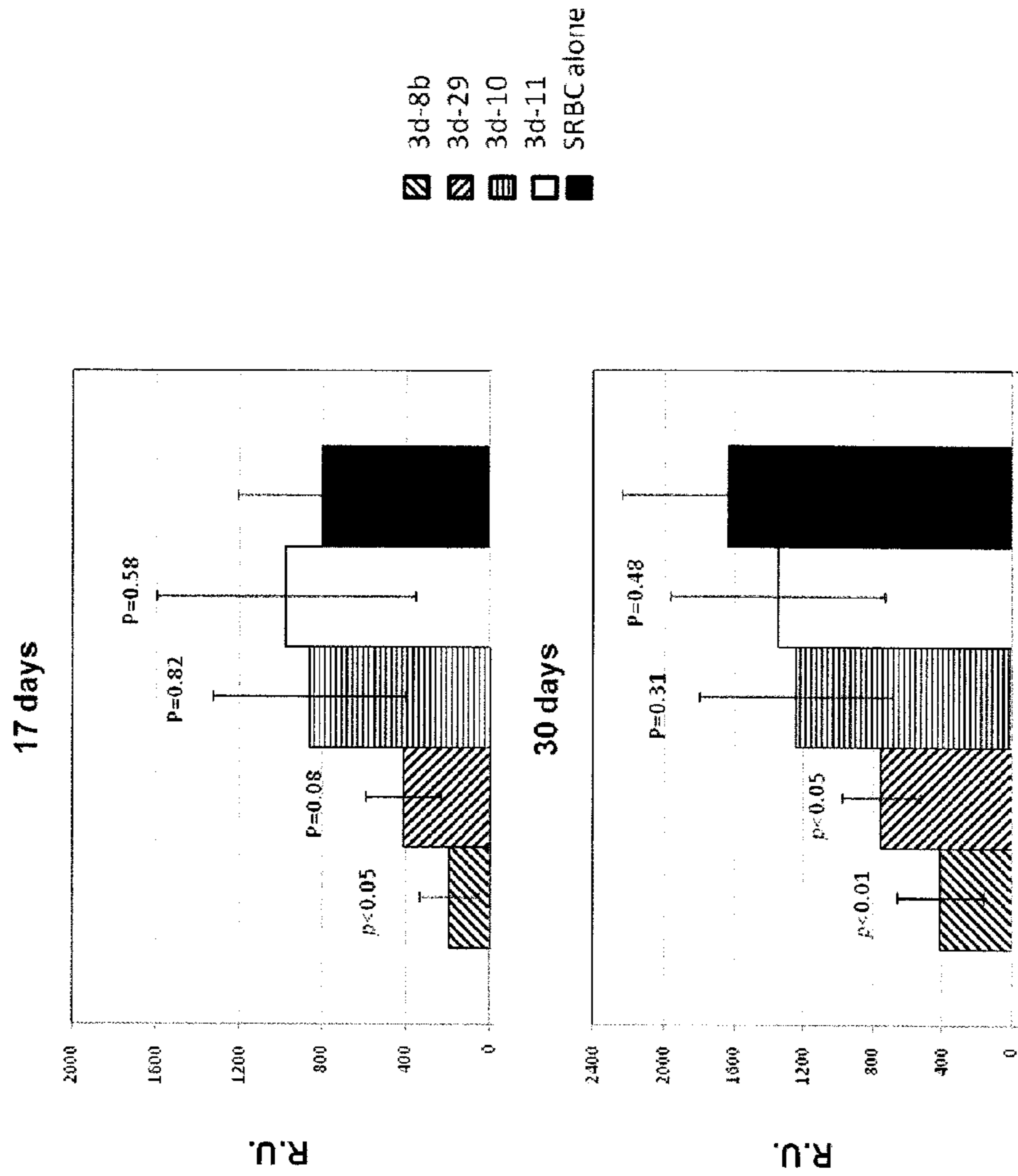


Figure 12 Inhibitory Effect of Group 1 Anti-C3d Monoclonal Antibodies on Model Sheep Red Blood Cell Antigen-Specific Immune Response



ANTIBODIES TO THE C3D FRAGMENT OF COMPLEMENT COMPONENT 3

Matter enclosed in heavy brackets [] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue; a claim printed with strikethrough indicates that the claim was canceled, disclaimed, or held invalid by a prior post-patent action or proceeding.

RELATED APPLICATION

This application is the U.S. National Stage filing under 35 U.S.C. §371 of International Application No. PCT/US2011/041517, filed Jun. 22, 2011, which claims the benefit of U.S. provisional patent application Ser. No. 61/357,499, filed on Jun. 22, 2010, the disclosure of which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to methods and materials for modulating the complement alternative pathway (CAP), the complement classical pathway (CCP), the complement lectin/mannose pathway (CMP), or combinations thereof, as well as methods and materials for targeting diagnostic, prophylactic and therapeutic agents to localized areas of tissue within the body where they may more directly exert their effects upon the intended target cells or tissue, with reduced, associated systemic effects compared with administration of the same or similar agents in an untargeted, systemic manner. The methods and materials of the present invention may therefore allow for increased efficacy, lower threshold effective dosages and/or lower effective maintenance doses, and/or reduced associated undesired or adverse effects in terms of frequency or severity of occurrence, or both. The present invention also relates to methods and materials for modulating a host humoral immune response, especially reducing, inhibiting, or preventing a host humoral immune response, e.g., to self antigens as occurs in patients with autoimmune disease.

BACKGROUND OF THE INVENTION

The complement system plays an important role in the pathology of many autoimmune, inflammatory and ischemic diseases. Inappropriate complement activation and its deposition on host cells can lead to complement-mediated lysis and/or injury of cells and target tissues, as well as tissue destruction due to the generation of powerful mediators of inflammation. Key to the activity of the complement system is the covalent attachment of processed protein fragments derived from a serum protein, complement C3, to tissue sites of complement activation. This unusual property is due to the presence of a thioester bond in C3 that, when cleaved during C3 activation, converts C3 to a form designated C3b which can then utilize ester or amide bonds to link to cell and tissue-attached molecules. Once C3b is covalently attached, it is rapidly processed to the iC3b, C3dg and C3d forms, each of which remain covalently attached to the target tissue site. This process results in the "marking" of the tissue as one in which an inflammatory injury or other complement-related process is underway.

Complement can be activated by any of three pathways: the classical, lectin and alternative pathways. The classical pathway is activated through the binding of the complement

system protein C1q to antigen-antibody complexes, pentraxins or apoptotic cells. The pentraxins include C-reactive protein and serum amyloid P component. The lectin pathway is initiated by binding of microbial carbohydrates to mannose-binding lectin or by the binding of ficolins to carbohydrates or acetylated molecules.

The alternative pathway is activated on surfaces of pathogens that have neutral or positive charge characteristics and do not express or contain complement inhibitors. This results from the process termed 'tickover' of C3 that occurs spontaneously, involving the interaction of conformationally altered C3 with factor B, and results in the fixation of active C3b on pathogens or other surfaces. The alternative pathway can also be initiated when certain antibodies block endogenous regulatory mechanisms, by IgA-containing immune complexes, or when expression of complement regulatory proteins is decreased. In addition, the alternative pathway is activated by a mechanism called the 'amplification loop' when C3b that is deposited onto targets via the classical or lectin pathway, or indeed through the tickover process itself, binds factor B. See Muller-Eberhard (1988) *Ann. Rev. Biochem.* 57:321. For example, Holers and colleagues have shown that the alternative pathway is amplified at sites of local injury when inflammatory cells are recruited following initial complement activation. Girardi et al., *J. Clin. Invest.* 2003, 112:1644. Dramatic complement amplification through the alternative pathway then occurs through a mechanism that involves either the additional generation of injured cells that fix complement, local synthesis of alternative pathway components, or more likely because infiltrating inflammatory cells that carry preformed C3 and properdin greatly increase activation specifically at that site.

Alternative pathway amplification is initiated when circulating factor B binds to activated C3b. This complex is then cleaved by circulating factor D to yield an enzymatically active C3 convertase complex, C3bBb. C3bBb cleaves additional C3 generating C3b, which drives inflammation and also further amplifies the activation process, generating a positive feedback loop. Factor H is a key regulator (inhibitor) of the alternative complement pathway activation and initiation mechanisms that competes with factor B for binding to conformationally altered C3 in the tickover mechanism and to C3b in the amplification loop. Binding of C3b to Factor H also leads to degradation of C3b by factor I to the inactive form iC3b (also designated C3bi), thus exerting a further check on complement activation. Factor H regulates complement in the fluid phase, circulating at a plasma concentration of approximately 500 µg/ml, but its binding to cells is a regulated phenomenon enhanced by the presence of a negatively charged surface as well as fixed C3b, iC3b, C3dg or C3d. Jozsi et al., *Histopathol.* (2004) 19:251-258.

Complement activation, C3 fragment fixation and complement-mediated inflammation are involved in the etiology and progression of numerous diseases. The down-regulation of complement activation has been shown to be effective in treating several diseases in animal models and in ex vivo studies, including, for example, systemic lupus erythematosus and glomerulonephritis (Y. Wang et al., *Proc. Nat'l Acad. Sci. USA* (1996) 93:8563-8568), rheumatoid arthritis (Y. Wang et al., *Proc. Nat'l Acad. Sci. USA* (1995) 92:8955-8959), cardiopulmonary bypass and hemodialysis (C. S. Rinder, *J. Clin. Invest.* (1995) 96:1564-1572), hyperacute rejection in organ transplantation (T. J. Kroshus et al., *Transplantation* (1995) 60:1194-1202), myocardial infarction (J. W. Homeister et al., *J. Immunol.* (1993) 150:1055-1064; H. F. Weisman et al., *Science* (1990) 249:146-151),

ischemia/reperfusion injury (E. A. Amsterdam et al., *Am. J. Physiol.* (1995) 268:H448-H457), antibody-mediated allograft rejection, for example, in the kidneys (J. B. Colvin, *J. Am. Soc. Nephrol.* (2007) 18(4):1046-56), and adult respiratory distress syndrome (R. Rabinovici et al., *J. Immunol.* (1992) 149:1744-1750). Moreover, other inflammatory conditions and autoimmune/immune complex diseases are also closely associated with complement activation (B. P. Morgan, *Eur. J. Clin. Invest.* (1994) 24:219-228), including, but not limited to, thermal injury, severe asthma, anaphylactic shock, bowel inflammation, urticaria, angioedema, vasculitis, multiple sclerosis, myasthenia gravis, myocarditis, membranoproliferative glomerulonephritis, atypical hemolytic uremic syndrome, Sjögren's syndrome, renal and pulmonary ischemia/reperfusion, and other organ-specific inflammatory disorders. It is currently uncertain whether complement activation is essential to the pathogenesis and injury of all diseases in which local tissue C3 activation and inflammatory injury occurs; nevertheless, C3 fragment fixation is almost universally found as an associated event.

The use of complement receptor 2 (CR2), or functional fragments thereof, to target complement modulators to tissues which exhibit or express C3, or fragments of C3 to which the CR2 is able to bind, including C3b, iC3b, C3d and C3dg, is described in US 2008/0267980 and US 2008/0221011, the disclosures of which are hereby incorporated herein by reference. Such CR2 molecules, and functional fragments thereof, can be used for targeting because the first two N-terminal short consensus repeat domains (SCRs) comprise an active binding site for the exposed C3d domain that is contained within iC3b and C3dg.

The disclosures of all publications, patents, patent applications and published patent applications referred to herein are hereby incorporated herein by reference in their entirety.

SUMMARY OF THE INVENTION

Because C3 fragments are expressed and exhibited on tissue and cells which have been subject to physical, chemical or other insult or injury, the present inventors hypothesized that modulation of complement may be effected using antibodies to C3 fragments, such as C3d. Moreover, by targeting a therapeutic or prophylactic agent to the sites of such insult or injury, more effective treatment or prevention of complement-related disorders may result. Such targeting can be accomplished, for example, by joining such therapeutic or prophylactic agents to a targeting moiety that is able to bind to C3 fragments, such as an antibody against C3d or C3dg. Using such means, elevated local concentrations of targeted therapeutic or prophylactic agents can be achieved at the site of such injury when the agent is administered systemically. Thus, therapeutic levels of agent may be achieved at the site of complement activation through systemic administration, using lower concentrations of and/or less frequent dosage regimens than would be necessary for an untargeted therapeutic. Because the agent is targeted to the tissue and cells where the agent may exert the greatest effect, the desired therapeutic or prophylactic concentrations and results may be achieved with reduced systemic side effects. Accordingly, the present disclosure provides materials and methods for targeting therapeutic and/or prophylactic agents to tissue and cells at the site of an injury or insult using targeting moieties that are capable of binding to C3 fragments, namely antibodies to C3d.

The inventors herefor have found that binding to the C3d fragment of complement provides for optimal modulation of the complement alternative pathway at sites of local comple-

ment activation in vivo. Thus, the inventors have identified novel methods and materials for the utilization of the unique "flag" or "target" provided by covalent decoration of inflamed tissues with the complement C3 fragments C3dg and/or C3d in order to provide novel methods and materials by which therapeutic or diagnostic agents can be directed preferentially to areas of such inflamed tissues.

Accordingly, in one aspect, the present disclosure provides an isolated antibody or antigen-binding fragment thereof specifically binding to complement protein C3d or C3dg. In some embodiments, the isolated antibody or antigen-binding fragment thereof disclosed herein specifically binds to mammalian C3d or C3dg, e.g., human C3d or C3dg.

In another aspect, the present disclosure provides an isolated anti-C3d/C3dg antibody or antigen-binding fragment thereof, wherein said antibody or antigen-binding fragment thereof has a binding affinity to C3d of 1.1 nM or better (in K_D value). (The term "C3d/C3dg" as used throughout this disclosure means either C3d and/or C3dg.) In some embodiments, the isolated anti-C3d/C3dg antibody or antigen-binding fragment thereof described in the present disclosure has a binding affinity to C3d of 0.5 nM or better (in K_D value). In some embodiments, the isolated antibody or antigen-binding fragment thereof disclosed herein binds to human C3d or C3dg with a K_D of 1.1 nM or better (i.e., with a lower K_D value). In some embodiments, such K_D to human C3d/C3dg is of 0.5 nM or better.

In another aspect, the present disclosure provides an isolated anti-C3d/C3dg antibody or antigen-binding fragment thereof, wherein said antibody or antigen-binding fragment thereof preferably binds to C3d, C3dg or iC3b compared to binding to complement proteins C3, C3a, C3b, C3c or C3f. Generally, the preference described herein refers to a preference to C3d/C3dg of a species over the C3, C3a, C3b, C3c or C3f protein of the same species, which includes, e.g., human, non-human mammals (e.g., cynomolgus monkey), mouse, or rat. Further, the preference described herein refers to either in vitro or in vivo or both in vitro and in vivo preferences, which can be determined by many well-known measurements, e.g., standard affinity determination techniques or competitive binding techniques, many of which are recited and/or described herein. In some embodiments, the isolated anti-C3d/C3dg antibody or antigen-binding fragment thereof described in the present disclosure does not bind to complement proteins C3, C3a, C3b, C3c or C3f of human or other mammalian species in vitro or in vivo. In some embodiments, the disclosure features an isolated anti-C3d/C3dg antibody or antigen-binding fragment thereof that binds to C3d or C3dg of human or other mammalian species with an affinity that is at least 2 (e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 300, 400, or 500, or more) -fold greater than its corresponding affinity for uncleaved, native C3 protein or other C3 fragments C3a, C3b, C3c or C3f from the same species. For example, an anti-C3d/C3dg antibody or antigen-binding fragment thereof described herein can, in some embodiments, bind to human C3d/C3dg with a K_D of 0.5 nM and to at least a subpopulation of uncleaved human C3 protein, or other human C3 fragments C3a, C3b, C3c or C3f, with a K_D that is at least 2-fold higher (e.g., at least 1 nM). In just another embodiment, the disclosure features an isolated anti-C3d/C3dg antibody or antigen-binding fragment thereof that binds to C3d/C3dg polypeptide from human or other species with a K_D that is less than 1.1 nM, or less than 0.5 nM, in the presence of a molar excess (e.g., a 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 300, 400, or 500-fold molar

excess) of uncleaved, native C3 or other C3 fragments C3a, C3b, C3c or C3f from the same species over C3d/C3dg.

In some embodiments, the present disclosure provides an isolated anti-C3d/C3dg antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof binds to deposited C3 fragments. In some embodiments, the isolated anti-C3d/C3dg antibody or antigen-binding fragment thereof described herein preferentially binds to deposited C3 fragments compared to binding to free or circulating or undeposited C3 or C3 fragments. The deposited C3 fragments described herein include, but are not limited to, C3d, C3dg, and iC3b. The free or circulating or undeposited C3 includes, but is not limited to, C3 and (C3H₂O). The free or undeposited C3 fragments include, but are not limited to, C3a, C3b, C3c and C3f. In some embodiments, the disclosure features an isolated anti-C3d/C3dg antibody or antigen-binding fragment thereof that binds to deposited C3 fragments, e.g., C3d or C3dg, from human or other species with an affinity that is at least 2 (e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 300, 400, or 500, or more) -fold greater than its corresponding affinity for free or undeposited C3 protein (e.g., C3 or (C3H₂O)) or other C3 fragments C3a, C3b, C3c or C3f from the same species.

In some embodiments, the present disclosure provides an isolated anti-C3d/C3dg antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof binds to C3d or C3dg proteins of at least two species (such antibodies can be referred to herein as species cross-reactive antibodies). In some embodiments, at least one of such species is a mammal. In some embodiments, the mammal described herein includes, but is not limited to, human, cynomolgus monkey, mouse, and rat. In some embodiments, the isolated antibody or antigen-binding fragment thereof described herein binds to both human and cynomolgus C3d or C3dg. In another embodiment, the isolated antibody or antigen-binding fragment thereof described herein binds to both human and rodent C3d or C3dg. In some embodiments, the isolated antibody or antigen-binding fragment thereof described herein binds to both human and mouse C3d or C3dg.

In some embodiments, the present disclosure provides an isolated anti-C3d/C3dg antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof competes with complement receptor 2 (CR2) for binding to C3d/C3dg. In some embodiments, the isolated antibody or antigen-binding fragment thereof described herein decreases, at a 1:1 molar concentration ratio, the binding of CR2 for C3d/C3dg in vitro or in vivo by any of about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%, inclusive, as well as any numerical value in between these percentages. In another embodiment, the isolated antibody or antigen-binding fragment thereof described herein decreases the binding of CR2 to C3d/C3dg in vitro or in vivo by any of about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%, inclusive, as well as any numerical value in between these percentages. In some embodiments, the competition with CR2 for binding to C3d/C3dg by the antibody or antigen-binding fragment thereof described herein leads to decreased or inhibited B cell activation. In some embodiments, such competition described herein leads to decreased, reduced or inhibited host humoral immune response. Thus, the present disclosure also provides an isolated anti-C3d/C3dg antibody or antigen-binding fragment thereof capable of decreasing, reducing or inhibiting host B cell activation. The present disclo-

sure further provides an isolated anti-C3d/C3dg antibody or antigen-binding fragment thereof capable of decreasing, reducing or inhibiting host humoral immune response.

In another aspect, the present disclosure provides an isolated anti-C3d antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof does not enhance complement activation. In some embodiments, a host being treated with the antibody or antigen-binding fragment thereof described herein has a complement activity level of about 10%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%, inclusive, as well as any numerical value in between these percentages, of the complement activity level in that host before being treated with the antibody or fragment.

In some embodiments, the present disclosure provides an isolated anti-C3d antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof does not compete with factor H binding to C3, C3(H₂O), C3b and C3dg.

In some embodiments, the present disclosure provides an isolated anti-C3d antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof does not activate C3 or stabilize complement alternative pathway (CAP) or complement classical pathway (CCP)C3/C5 convertases.

In some embodiments, the isolated anti-C3d antibody or antigen-binding fragment thereof described in the present disclosure includes, but is not limited to, a monoclonal antibody or antibody fragment, a diabody, a chimerized or chimeric antibody or antibody fragment, a humanized antibody or antibody fragment, a deimmunized human antibody or antibody fragment, a fully human antibody or antibody fragment, a bispecific antibody or antibody fragment, a monovalent antibody or antibody fragment, a single chain antibody, an Fv, an Fd, an Fab, an Fab', and an F(ab')₂. In some embodiments, the antibody described herein is a monoclonal antibody. In some embodiments, the antibody described herein is the mAb 3d8b, produced by hybridoma cell line 3d-8b/2 (ATCC Deposit PTA-10999). In some embodiments, the antibody described herein is mAb 3d9a, produced by hybridoma cell line 3d-9a/25 (ATCC Deposit PTA-10998). In some embodiments, the antibody described herein is mAb 3d29, produced by hybridoma cell line 3d-29/5/2 (ATCC Deposit PTA-11000). In some embodiments, the antibody or antigen-binding fragment thereof described in the present disclosure includes, but is not limited to, any engineered or recombinant antibody or antigen-binding fragment thereof originated from mAb 3d8b, 3d9a, 3d29, or other mAb described in this disclosure, which can be easily screened or produced by standard methods well known in the art, many of which are discussed in this disclosure. Generally, all these antibodies or fragments originating from mAbs in this disclosure may be designed, screened, produced and/or tested to modify, without being limiting, their binding affinity, avidity, or cross-species activity to the C3d/C3dg protein, selectivity over C3 or other C3 fragments, or their expression pattern and solubility, stability, half-life, cross-reactivity to other proteins/targets, or other inherent activities or characteristics of these antibodies or fragments, such as the effector activity.

In another aspect, the present disclosure provides a hybridoma cell selected from the group consisting of: 3d-8b/2 (ATCC Deposit PTA-10999), 3d-9a/25 (ATCC Deposit number: PTA-10998), 3d-29/5/2 (ATCC Deposit number: PTA-11000), 3d-11/14 (ATCC Deposit number: PTA-11011), 3d-31/A6/9 (ATCC Deposit number: PTA-

11027), 3d-3/28/4 (ATCC Deposit number: PTA-11025), 3d-15A9 (ATCC Deposit number: PTA-11012), 3d-10/14/1 (ATCC Deposit number: PTA-11010), and 3d-16/3/3 (ATCC Deposit number: PTA-11026).

In another aspect, the present disclosure provides an isolated antibody produced by the above-listed hybridoma cells. In yet another aspect, the disclosure features a humanized, primatized, or chimerized antibody comprising the set of six (6) CDRs of any of the antibodies produced by the above-listed hybridomas.

In another aspect, the present disclosure provides an isolated nucleic acid molecule encoding the antibody or antigen-binding fragment thereof described in this disclosure.

In another aspect, the present disclosure provides a vector containing the nucleic acid sequence of an isolated nucleic acid encoding the antibody or antigen-binding fragment thereof described in this disclosure. Such a vector includes, but is not limited to, a plasmid vector, a cosmid vector, a viral vector, a shuttle vector, or any vector well known in the art for expression in prokaryotic or eukaryotic cells.

In another aspect, the present disclosure provides a cell containing a vector containing the nucleic acid sequence of an isolated nucleic acid encoding the antibody or antigen-binding fragment thereof described in this disclosure. Such a cell includes, for example, a prokaryotic cell or a eukaryotic cell.

In another aspect, the present disclosure provides a pharmaceutical composition comprising any of the isolated antibodies or antigen-binding fragments thereof described in this disclosure. In some embodiments, the present disclosure provides a pharmaceutical composition comprising a nucleic acid encoding the antibody or antigen-binding fragment thereof described in this disclosure. In some embodiments, the present disclosure provides a pharmaceutical composition comprising a vector containing the nucleic acid sequence of an isolated nucleic acid encoding the antibody or antigen-binding fragment thereof described in this disclosure. In some embodiments, the present disclosure provides a pharmaceutical composition comprising a cell containing such vector described herein.

In another aspect, the present disclosure provides a pharmaceutical composition comprising any of the isolated antibodies or antigen-binding fragments thereof described in this disclosure and a therapeutically acceptable excipient. Suitable excipients are well known in the art and recited herein.

Such antibodies described herein may be useful as modulators of complement activity and, in particular, of complement alternative pathway (CAP), such that complement activation can be modulated, stabilized or reduced as part of a prophylactic or therapeutic regimen. In addition, such antibodies may find use as targeting moieties for the targeting of prophylactic or therapeutic agents, such as complement inhibitors, to areas of local complement activation, in order to modulate, stabilize or reduce complement activation. For example, such antibodies or targeted modulators of complement activity may be useful in controlling or lessening an inflammatory response, including localized inflammatory response or systemic inflammatory responses.

Modulation of the complement system represents a therapeutic modality for numerous pathologic conditions associated with complement activation. It has previously been postulated by the inventors herein that targeting complement inhibitors to the sites of complement activation and disease can facilitate the effectiveness of said complement inhibitors. The present inventors postulated that this is in part

because targeting to the sites of complement activation allows the complement inhibitors to act in a more focused area, achieving therapeutic results at the site or sites of complement activation, allowing lower systemic doses to be effective while avoiding or reducing adverse or undesired systemic effects.

The present inventors have found that targeting of prophylactic or therapeutic agents to particular epitopes present on the C3d fragment of complement is surprisingly effective in terms of localizing the prophylactic or therapeutic agents such that they can exert optimal effects on tissue or cells which are the site of complement activation. Thus, the present inventors have isolated antibodies which bind to the C3d fragment of complement and used them for the targeting of prophylactic and therapeutic agents.

Accordingly, in another aspect, the present disclosure provides a construct comprising: (a) a C3d binding portion; and (b) a complement modulator portion, wherein (a) and (b) are joined. In some embodiments, (a) is amino-terminal to (b). In some embodiments, (b) is amino-terminal to (a).

In some embodiments, the C3d binding portion comprises any of the anti-C3d/C3dg antibodies or antigen-binding fragments thereof described herein. In some embodiments, the complement modulatory portion is a compound, composition, or protein.

In yet another aspect, the disclosure provides a construct for modulating complement activation, comprising: (a) a C3d binding portion comprising said anti-C3d antibody or antigen-binding fragment thereof described herein; and (b) a complement modulatory portion which is a compound, composition, or protein.

In some embodiments, the construct disclosed herein modulates complement activity in the complement alternative pathway (CAP). In some embodiments, the construct disclosed herein is a fusion protein.

In some embodiments, said C3d binding portion and said complement modulator portion of the construct disclosed herein are joined directly without a linker. In some embodiments, such two portions are joined directly through a chemical bond. In other embodiments, such two portions are joined by a linker. Such linker may include, but is not limited to, a peptide. An exemplary peptide linker is, but is not limited to, (GlySer)_n, wherein n=1 (SEQ ID NO:12), 2 (SEQ ID NO:13), 3 (SEQ ID NO:14), 4 (SEQ ID NO:15), 5 (SEQ ID NO:16), 6 (SEQ ID NO:17), 7 (SEQ ID NO:18), or 8 (SEQ ID NO:19); (GlyGlyGlySer)_n, wherein n=1 (SEQ ID NO:20), 2 (SEQ ID NO:21), 3 (SEQ ID NO:22), or 4 (SEQ ID NO:23); (GlyGlyGlyGlySer)_n, wherein n=1 (SEQ ID NO:24), 2 (SEQ ID NO:25), 3 (SEQ ID NO:26), 4 (SEQ ID NO:27), 5 (SEQ ID NO:28), 6 (SEQ ID NO:29), 7 (SEQ ID NO:30), or 8 (SEQ ID NO:31); or (GlySerSerGly)_n, wherein n=1 (SEQ ID NO:32), 2 (SEQ ID NO:33), 3 (SEQ ID NO:34), or 4 (SEQ ID NO:35).

In some embodiments, said complement modulator portion comprises a complement inhibitor or biologically active fragment thereof. In some embodiments, such complement inhibitor is selected from the group consisting of: human membrane complement protein (MCP), human decay accelerating factor (DAF), mouse DAF, mouse complement receptor 1-related gene/protein y (Crry), human CD59, mouse CD59 isoform A, mouse CD59 isoform B, human complement receptor 1 (CR1), human factor H, and mouse factor H, and biologically active fragments thereof. Such biologically active fragment described herein may include, but is not limited to, SCRs 1-4 (amino acids 35-285 of SEQ ID NO:1) of human MCP, SCRs 1-4 plus the serine/threonine-rich domain (amino acids 35-326 of SEQ ID NO:1) of

human MCP, the extracellular domain (amino acids 35-343 of SEQ ID NO:1) of human MCP, SCRs 1-4 (amino acids 25-285 of SEQ ID NO:2) of human DAF, SCRs 1-4 plus the O-glycosylated serine/threonine-rich domain (amino acids 25-353 of SEQ ID NO:2) of human DAF, SCRs 1-4 (amino acids 35-286 of SEQ ID NO:3) of mouse DAF, SCRs 1-4 plus the O-glycosylated serine/threonine-rich domain (amino acids 35-362 of SEQ ID NO:3) of mouse DAF, SCRs 1-5 (amino acids 41-400 of SEQ NO:7) of Crry, the extracellular domain (amino acids 41-405 of SEQ ID NO:7) of Crry, the extracellular domain of human CD59 lacking its GPI anchor (amino acids 26-101 of SEQ ID NO:4), the extracellular domain of mouse CD59 isoform A lacking its GPI anchor (amino acids 24-95 of SEQ ID NO:5), the extracellular domain of mouse CD59 isoform B lacking its GPI anchor (amino acids 24-103 of SEQ ID NO:6), SCRs 1-3 (amino acids of 42-234 of SEQ ID NO:8) of human CR1, SCRs 1-4 (amino acids 42-295 of SEQ NO:8) of human CR1, SCRs 1-10 (amino acids 42-684 of SEQ ID NO:8) of human CR1, SCRs 8-10 (amino acids of 491-684 of SEQ ID NO:8) of human CR1, SCRs 8-11 (amino acids 491-745 of SEQ ID NO:8) of human CR1, SCRs 15-17 (amino acids of 941-1134 of SEQ ID NO:8) of human CR1, SCRs 15-18 (amino acids 941-1195 of SEQ ID NO:8) of human CR1, SCRs 22-28 (amino acids 1394-1842 of SEQ ID NO:8) of human CR1, SCRs 1-4 (amino acids 21-262 of SEQ ID NO:9) of human factor H, SCRs 1-5 (amino acids 21-320 of SEQ ID NO:9) of human factor H, SCRs 1-8 (amino acids 21-507 of SEQ ID NO:9) of human factor H, SCRs 1-18 (amino acids 21-1104 of SEQ ID NO:9) of human factor H, SCRs 1-4 (amino acids 19-264 of SEQ ID NO:10) of mouse factor H, SCRs 1-5 (amino acids 19-322 of SEQ ID NO:10) of mouse factor H, SCRs 1-8 (amino acids 19-507 of SEQ ID NO:10) of mouse factor H, and SCRs 1-18 (amino acids 19-1109 of SEQ ID NO:10) of mouse factor H.

In some embodiments, said complement modulator portion comprises a complement activator or biologically active fragment thereof. In some embodiments, such complement activator is selected from the group consisting of: human IgG1, human IgG1 Fc domain, human IgM, human IgM Fc domain, mouse IgG3, mouse IgG3 Pc domain, mouse IgM, mouse IgM Fc domain, and cobra venom factor (CVF).

In another aspect, the present disclosure provides an isolated nucleic acid molecule encoding the fusion construct described herein.

In another aspect, the present disclosure provides a vector containing the nucleic acid sequence of the fusion construct described in this disclosure. Such vector includes, but is not limited to, a plasmid vector, a cosmid vector, a viral vector, a shuttle vector, or any vector well known in the art for expression in prokaryotic or eukaryotic cells.

In another aspect, the present disclosure provides a cell containing a vector containing the nucleic acid sequence of an isolated nucleic acid encoding the fusion construct described in this disclosure. Such cell includes, for example, a prokaryotic cell or a eukaryotic cell.

In another aspect, the present disclosure provides a pharmaceutical composition comprising the fusion construct described in this disclosure. In some embodiments, the present disclosure provides a pharmaceutical composition comprising a nucleic acid encoding the fusion construct described in this disclosure. In some embodiments, the present disclosure provides a pharmaceutical composition comprising a vector containing the nucleic acid sequence of the fusion construct described in this disclosure. In some

embodiments, the present disclosure provides a pharmaceutical composition comprising a cell containing such vector described herein.

In another aspect, the present disclosure provides a pharmaceutical composition comprising the fusion construct described in this disclosure and further comprising a therapeutically acceptable excipient. Such excipient includes any excipient well known in the art.

In another aspect, the disclosure features: (a) a nucleic acid encoding any one of the antibodies, antigen-binding fragments, or constructs described herein; (b) a vector (e.g., an expression vector) comprising the nucleic acid; and (c) a cell (e.g., a bacterial, plant, fungal, insect, or mammalian cell) comprising the vector or expression vector.

In yet another aspect, the disclosure features a method for producing an antibody, an antigen-binding fragment of the antibody, or a construct described herein. The method includes culturing the aforementioned cell under conditions suitable to allow for expression of the antibody, fragment, or construct by the cell. The method can optionally include purifying the antibody, fragment, or construct from the cell or from the media in which the cell is cultured.

As described above, the disclosure also provides the results of experiments in which the inventors observed that an antagonist anti-C3d antibody (one that prevents the association between C3d and CR2) was capable of reducing antibody production in a mammal. While not bound by any particular theory or mechanism of action, the inventors believe that inhibition of the interaction between C3d or C3dg and CR2 on host B cells reduces B cell activation and/or activity. Accordingly, the disclosure provides antibodies (e.g., anti-C3d, anti-CR2, or anti-C3dg antibodies), antigen-binding fragments of the antibodies, and constructs that are useful for inhibiting B cell activation and/or activity, all of which can be used for, among other things, reducing a humoral immune response (e.g., in a mammal afflicted with an autoimmune disease).

For example, the aforementioned constructs contain a first portion that is capable of inhibiting B cell activation or activity and a second portion that is capable of inhibiting complement activity. The first portion can be, e.g., an antibody or an antigen-binding fragment thereof that binds to a natural ligand of CR2 such as C3d, C3dg, and iC3b. The antibody or antigen-binding fragment thereof can be any of the antibodies described herein. In some embodiments, the first portion comprises an antagonist anti-CR2 antibody (or antigen-binding fragment thereof) that inhibits the interaction between CR2 and C3d or C3dg. In some embodiments, the second portion can be, e.g., any of the complement inhibitor polypeptides (including variants and functional fragments) described herein.

In another aspect, the present disclosure provides a method of decreasing a humoral immune response in a subject comprising administering to said subject the isolated antibody or antigen-binding fragment thereof disclosed herein. In some embodiments, the isolated antibody or antigen-binding fragment thereof disclosed herein reduces B cell stimulation in said subject. In some embodiments, the isolated antibody or antigen-binding fragment thereof reduces B cell stimulation by its competition with CR2 for binding to C3d in said subject. In some embodiments, the subject is a mammal. In some embodiments, the mammal is a human.

In another aspect, the present disclosure provides a method of modulating complement activation in a subject, the method comprising administering to said subject an effective amount of a fusion construct disclosed herein.

In another aspect, the present disclosure provides a method of decreasing or inhibiting complement activation in a subject, which method comprises administering to said subject an effective amount of a fusion construct described herein.

In another aspect, the present disclosure provides a method of increasing complement activation in a subject. The method comprises administering to said subject an effective amount of a fusion construct disclosed herein.

In another aspect, the present disclosure provides a method of decreasing complement activation and humoral immune response simultaneously in a subject. The method includes administering to said subject the fusion construct described herein.

In another aspect, provided herein is a method of treating a subject having or suspected of having a disease or preventing a subject from developing a disease wherein said disease is selected from the group consisting of: tissue damage resulting from ischemia-reperfusion injury, an inflammatory disorder, transplant rejection, a pregnancy-related disease, an adverse drug reaction, and an autoimmune or immune complex disorder, said method comprising administering to said subject a therapeutically effective amount of the isolated antibody or antigen-binding fragment thereof described herein. In some embodiments, said tissue damage resulting from ischemia-reperfusion injury is associated with a disorder selected from the group consisting of: myocardial infarction, aneurysm, stroke, hemorrhagic shock, crush injury, multiple organ failure, hypovolemic shock, intestinal ischemia, spinal cord injury and traumatic brain injury. In some embodiments, said inflammatory disorder is selected from the group consisting of: burns, endotoxemia, septic shock, adult respiratory distress syndrome, cardiopulmonary bypass, hemodialysis, anaphylactic shock, asthma, angioedema, Crohn's disease, sickle cell anemia, poststreptococcal glomerulonephritis, membranous nephritis, and pancreatitis. In some embodiments, said transplant rejection is hyperacute xenograft rejection. In some embodiments, said pregnancy-related disease is selected from the group consisting of: recurrent fetal loss and pre-eclampsia. In some embodiments, said adverse drug reaction is selected from the group consisting of: drug allergy and IL-2 induced vascular leakage syndrome. In a further embodiment, said autoimmune or immune complex disorder is selected from the group consisting of: myasthenia gravis, Alzheimer's disease, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, lupus nephritis, insulin-dependent diabetes mellitus, acute disseminated encephalomyelitis, Addison's disease, antiphospholipid antibody syndrome, autoimmune hepatitis, Crohn's disease, Goodpasture's syndromes, Graves' disease, Guillain-Barré syndrome, Hashimoto's disease, idiopathic thrombocytopenic purpura, pemphigus, Sjögren's syndrome, Takayasu's arteritis, autoimmune glomerulonephritis, membranoproliferative glomerulonephritis type II, paroxysmal nocturnal hemoglobinuria, age-related macular degeneration, diabetic maculopathy, uveitis, retinal degeneration disorders, diabetic nephropathy, focal segmental glomerulosclerosis, ANCA associated vasculitis, hemolytic uremic syndrome, and atypical hemolytic uremic syndrome. In some embodiments, said autoimmune glomerulonephritis is selected from the group consisting of: immunoglobulin A nephropathy and membranoproliferative glomerulonephritis type I.

In one aspect, provided herein is a method of treating a subject having or suspected of having a disease or preventing a subject from developing a disease wherein said disease is selected from the group consisting of: cancer, a viral

infection, a bacterial infection, a parasitic infection, and a fungal infection, said method comprising administering to said subject any of the constructs described herein (e.g., a construct described herein that activates complement) in an amount effective to increase complement activation in said subject.

In some embodiments of any of the methods described herein, the subject is a mammal. In some embodiments of any of the methods described herein, the subject is a human.

In another aspect, the present disclosure provides methods of targeting complement regulatory proteins to areas within an individual, such as tissue and/or cells, to which complement C3 activation fragments are covalently attached. The methods comprise: (a) administering to the individual a composition comprising an effective amount of a targeted complement regulatory protein as a therapeutic agent. In some embodiments, the individual is a mammal. In some embodiments, the mammal is a human, a non-human primate (e.g., cynomolgus monkey), a mouse, or a rat. In some embodiments, the composition is administered by injection. In some embodiments, the injection is parenteral, intraocular, intravenous, subcutaneous, or intramuscular.

The targeting moiety can be, e.g., a monoclonal antibody, which specifically binds to a binding partner selected from the group consisting of C3d and C3dg; or fragments thereof which retain the ability to bind to their respective binding partner. In some embodiments, the monoclonal antibody will bind to a binding partner selected from the group consisting of C3d and/or C3dg, but will not bind to C3, C3a, C3b, C3c or C3f (e.g., as determined by immunoprecipitation from activated serum).

In some embodiments, the targeting moiety comprises a fusion protein comprising a C3d binding Fv-domain fused to an Fc-domain of human immunoglobulin isotype G₁ (IgG₁) or G₂ (IgG₂). In some embodiments, the fusion protein comprises the Fv-domain of an antibody selected from 3d9a, 3d29 and 3d8b, fused to the Fc-domain of human immunoglobulin isotype G₁ (IgG₁) or other IgGs or IgG_{2/4} grafts.

In any of the embodiments described herein, the complement-mediated inflammation may be associated with tissue damage resulting from ischemia-reperfusion injury, inflammatory disorders, transplant rejection, pregnancy-related diseases, adverse drug reactions, and autoimmune or immune complex disorders. In any of the embodiments described herein, the tissue damage resulting from ischemia-reperfusion injury may be associated with a disorder selected from the group consisting of myocardial infarction, aneurysm, stroke, hemorrhagic shock, crush injury, multiple organ failure, hypovolemic shock, intestinal ischemia, spinal cord injury and traumatic brain injury. In any of the embodiments described herein, the inflammatory disorder may be selected from the group consisting of burns, endotoxemia, septic shock, adult respiratory distress syndrome, cardiopulmonary bypass, hemodialysis, anaphylactic shock, asthma, angioedema, Crohn's disease, sickle cell anemia, poststreptococcal glomerulonephritis, membranous nephritis, and pancreatitis. In any of the embodiments described herein, the transplant rejection may be hyperacute xenograft rejection. In any of the embodiments described herein, the pregnancy-related disease may be selected from the group consisting of recurrent fetal loss and pre-eclampsia. In any of the embodiments described herein, the adverse drug reaction may be selected from the group consisting of drug allergy and IL-2 induced vascular leakage syndrome. In any of the embodiments described herein, the autoimmune or immune complex disorder may be selected from the group consisting of myasthenia gravis, Alzheimer's disease, multiple sclerosis,

rheumatoid arthritis, systemic lupus erythematosus, lupus nephritis, insulin-dependent diabetes mellitus, acute disseminated encephalomyelitis, Addison's disease, antiphospholipid antibody syndrome, autoimmune hepatitis, Crohn's disease, Goodpasture's syndrome, Graves' disease, Guillain-Barre syndrome, Hashimoto's disease, idiopathic thrombocytopenic purpura, pemphigus, Sjögren's syndrome, Takayasu's arteritis, autoimmune glomerulonephritis, membranoproliferative glomerulonephritis type II, paroxysmal nocturnal hemoglobinuria, age-related macular degeneration, diabetic maculopathy, uveitis, retinal degeneration disorders, diabetic nephropathy, focal segmental glomerulosclerosis, ANCA associated vasculitis, hemolytic uremic syndrome, and atypical hemolytic uremic syndrome. In any of the embodiments described herein, the autoimmune glomerulonephritis may be selected from the group consisting of immunoglobulin A nephropathy and membranoproliferative glomerulonephritis type I.

In another aspect, provided herein is the use of any of the compositions as described herein in connection with the methods as described herein, unless otherwise noted or as is clear from the specific context. Any of the compositions as described herein may also be used in the preparation of a medicament for use in the methods as described herein.

In another aspect, provided herein are articles of manufacture or kits containing pharmaceutical compositions comprising an effective amount of any of the targeted prophylactic or therapeutic agent moieties and instructions for their use in the methods described herein. Thus, in some embodiments, the article of manufacture comprises instructions for the use of pharmaceutical compositions comprising an effective amount of a fusion protein comprising a monoclonal antibody which binds to a binding partner selected from C3d and C3dg, joined to a prophylactic or therapeutic moiety. The pharmaceutical compositions may further comprise one or more pharmaceutically acceptable excipients formulated for administration to an individual as described herein. The kit may further comprise means for administration, such as a syringe, inhaler or other device useful for systemic administration.

In yet another aspect, the disclosure features an article of manufacture comprising: a container comprising a label; and a composition comprising any of the antibodies or antigen-binding fragments or constructs described herein, wherein the label indicates that the composition is to be administered to a human having, suspected of having, or at risk for developing, a complement-associated disorder. The article of manufacture can comprise one or more additional active agents.

In another aspect, the disclosure features a therapeutic kit comprising: (i) any of the antibodies or antigen-binding fragments thereof described herein and (ii) means for delivering the antibody or antigen-binding fragment to a human; or (ii) any of the constructs described herein and (iv) means for delivering the construct to a human. The means can be suitable for subcutaneous delivery of the construct, or the antibody or antigen-binding fragment thereof, to the human. The means can be suitable for intraocular delivery of the construct, or the antibody or antigen-binding fragment thereof, to the human. The means can be suitable for intraarticular delivery of the construct, or the antibody or antigen-binding fragment thereof, to the human.

In some embodiments, the means can be a syringe, e.g., a double-barreled syringe. In some embodiments, the means can be a trans-scleral patch or a contact lens comprising the construct or the antibody or antigen-binding fragment thereof.

In some embodiments, the means is suitable for intrapulmonary delivery of the construct, or the antibody or antigen-binding fragment thereof, to the human. For example, the means can be an inhaler or a nebulizer.

In some embodiments, the kits include at least one additional active agent for use in treating a complement-associated disorder in a human.

In yet another aspect, the disclosure features a pre-filled syringe comprising: (a) any of the antibodies or antigen-binding fragments thereof described herein or any of the constructs described herein. The construct, or the antibody or antigen-binding fragment thereof, can be formulated for intraocular, intravitreal, or intraarticular administration.

In some embodiments, the construct, or the antibody or antigen-binding fragment thereof, is formulated for intramuscular or subcutaneous administration.

In some embodiments, the syringe comprises at least one pharmaceutical unit dosage form of the construct, or the antibody or antigen-binding fragment thereof. In some embodiments, the syringe comprises between 0.05 mg to 10 mg of the construct, or the antibody or antigen-binding fragment thereof. In some embodiments, the syringe comprises between about 1 mg and 100 mg of the construct, or the antibody or antigen-binding fragment thereof. In some embodiments, each pharmaceutical unit dosage form has a volume of between 0.02 mL to 1 mL, inclusive. In some embodiments, the pharmaceutical unit dosage form has a volume of no more than 0.05 mL.

The active prophylactic or therapeutic moiety is preferably a complement regulatory moiety, and is preferably capable of exerting a localized effect on surrounding cells and tissue when the targeting moiety binds its binding partner in vivo. Because the prophylactic or therapeutic moiety is targeted to localized tissue where complement is activated, one or more of the undesired systemic effects of such prophylactic or therapeutic moiety may be reduced or eliminated.

The interactions between complement receptor type 2 (CR2) and the complement components C3d, C3dg and iC3b are essential for the initiation of a normal immune response. A crystal structure of the two N-terminal short consensus repeat (SCR1-2) domains of CR2 in complex with C3d has previously been elucidated. However, a number of biochemical and biophysical studies targeting both CR2 and C3d appear to be in conflict with available structural data. For example, additional published mutagenesis and heteronuclear NMR spectroscopy studies directed towards the C3d-binding site on CR2 have indicated that the CR2-C3d co-crystal structure complex may reflect an incomplete reflection of complex formation under solution conditions. With regards to the CR2-binding site on C3d, mutagenesis studies by Isenman and coworkers have implicated an electronegative concave pocket on C3d in the binding process. This surface is discrete from the CR2-C3d interface identified in the crystal structure of the complex. A new publication by Isenman and colleagues has reported a co-crystal structure of CR2 with C3d consistent with binding of CR2 within the concave surface of C3d. See van den Elsen et al. A Crystal Structure of the Complex Between Human Complement Receptor 2 and Its Ligand C3d. *Science* 332, 608 (2011).

As used herein, the term "antibody fragment," "antigen-binding fragment," or similar terms refer to fragment of an antibody that retains the ability to bind to an antigen (e.g., a complement component C3dg, C3d, or CR2), e.g., a single chain antibody, a single chain Fv fragment (scFv), an Fd fragment, an Fab fragment, an Fab' fragment, or an F(ab')₂.

fragment. An scFv fragment is a single polypeptide chain that includes both the heavy and light chain variable regions of the antibody from which the scFv is derived. In addition, diabodies (Poljak (1994) *Structure* 2(12):1121-1123; Hudson et al. (1999) *J. Immunol. Methods* 23(1-2):177-189, the disclosures of each of which are incorporated herein by reference in their entirety), minibodies, triabodies (Schoonooghe et al. (2009) *BMC Biotechnol* 9:70), and domain antibodies (also known as “heavy chain immunoglobulins” or camelids; Holt et al. (2003) *Trends Biotechnol* 21(11): 484-490), (the disclosures of each of which are incorporated herein by reference in their entirety) that bind to a complement component C3d or C3dg protein can be incorporated into the compositions, and used in the methods, described herein.

“Polypeptide,” “peptide,” and “protein” are used herein interchangeably and mean any peptide-linked chain of amino acids, regardless of length or post-translational modification. As noted below, the polypeptides described herein can be, e.g., wild-type proteins, biologically-active fragments of the wild-type proteins, or variants of the wild-type proteins or fragments. Variants, in accordance with the disclosure, can contain amino acid substitutions, deletions, or insertions. The substitutions can be conservative or non-conservative. Conservative substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine, and leucine; aspartic acid and glutamic acid; asparagine, glutamine, serine and threonine; lysine, histidine and arginine; and phenylalanine and tyrosine.

In some embodiments of any of the constructs described herein, the complement modulator portion is a complement modulator polypeptide (e.g., a complement inhibitor polypeptide) such as, e.g., MCP, DAF, Crry, CR1, CD59, or factor H. The polypeptides can be human polypeptides or polypeptides of a non-human species. For example, the complement modulator polypeptides can be from a non-human primate (e.g., orangutan, chimpanzee, macaque, gorilla, lemur, or gibbon), horse, cow, pig, sheep, goat, dog, cat, or rodent (e.g., mouse, rabbit, hamster, gerbil, Guinea pig, or rat).

Variant complement modulator polypeptides, in some embodiments, contain no more than 60 (e.g., no more than 59, 58, 57, 56, 55, 54, 53, 52, 51, 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, nine, eight, seven, six, five, four, three, two, or one) amino acid substitutions as compared to the corresponding wild-type sequence. The amino acid substitutions can be conservative, non-conservative, or a mixture of both. Variant polypeptides may, in some embodiments, contain one or more deletions or additions, or a combination of one or more deletions, additions, and substitutions. In some embodiments, the variant polypeptides contain no more than six (e.g., no more than five, four, three, two, or one) amino acid deletions, additions, or substitutions per 100 amino acids of the polypeptide. In embodiments where the complement modulator polypeptide comprises SCRs, variant polypeptides contain no amino acid substitutions, deletions, or additions in the complement modulator polypeptide SCRs.

In some embodiments, a variant complement modulator polypeptide comprises an amino acid sequence that is at least 70 (e.g., at least 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99) % identical to the corresponding wild-type amino acid sequence. For example, a variant functional fragment of human MCP containing all four SCRs of hMCP

can, in some embodiments, comprise an amino acid sequence that is at least 70 (e.g., at least 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99) % identical to amino acids 35 to 285 of SEQ ID NO:1.

Functional fragments of a complement modulator polypeptide or variant polypeptide described herein are shorter than the full-length polypeptides. Variant polypeptides, and functional fragments of wild-type proteins or variants, retain at least 50 (e.g., at least 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 or greater) % of a complement modulatory activity of the corresponding wild-type polypeptide. For example, a variant soluble CD59 polypeptide (e.g., one that has 90% or greater identity with amino acids 26 to 102 of SEQ ID NO:4, soluble human CD59) retains at least 50% of the complement modulatory activity (e.g., the ability to inhibit formation of the terminal complement complex) of the corresponding wild-type soluble human CD59 protein having amino acids 26 to 102 of SEQ ID NO:4.

In some embodiments, a variant complement modulator polypeptide, or functional fragment of the complement modulator polypeptide, has greater than 100% of the ability of the corresponding wild-type protein to modulate complement activity. Methods for detecting and/or quantifying complement activity are known in the art and described herein.

As used herein, percent (%) amino acid sequence identity is defined as the percentage of amino acids in a candidate sequence that are identical to the amino acids in a reference sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR) software. Appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared can be determined by known methods.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure pertains. In case of conflict, the present document, including definitions, will control. Exemplary methods and materials are described below, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the presently disclosed methods and compositions. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

Other features and advantages of the present disclosure, e.g., methods for treating or preventing a complement-associated disorder, will be apparent from the following description, the examples, and from the claims.

BRIEF DESCRIPTION OF DEPOSITED MATERIAL

Hybridoma line 3d-9a/25 was deposited on May 26, 2010 and has been designated as ATCC Patent Deposit PTA-10998.

Hybridoma line 3d-8b/2 was deposited on May 26, 2010 and has been designated as ATCC Patent Deposit PTA-10999.

Hybridoma line 3d-29/5/2 was deposited on May 26, 2010 and has been designated as ATCC Patent Deposit PTA-11000.

Hybridoma line 3d-10/14/1 was deposited on Jun. 2, 2010 and has been designated as ATCC Patent Deposit PTA-11010.

Hybridoma line 3d-11/14 was deposited on Jun. 2, 2010 and has been designated as ATCC Patent Deposit PTA-11011.

Hybridoma line 3d-15A9 was deposited on Jun. 2, 2010 and has been designated as ATCC Patent Deposit PTA-11012.

Hybridoma line 3d-3/28/4 was deposited on Jun. 9, 2010 and has been designated as ATCC Patent Deposit PTA-11025.

Hybridoma line 3d-16/3/3 was deposited on Jun. 9, 2010 and has been designated as ATCC Patent Deposit PTA-11026.

Hybridoma line 3d-31/A6/9 was deposited on Jun. 9, 2010 and has been designated as ATCC Patent Deposit PTA-11027.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 illustrates the cleavage of C3 which takes place under physiological conditions of complement activation at a local site. In the presence of a C3 convertase enzyme, C3 is cleaved into C3a and C3b, the latter of which becomes covalently attached to cells or tissues undergoing complement activation at that site. In the presence of Factor I and Cofactor, C3b is further rapidly cleaved into C3f and iC3b. In the presence of Factor I and CR1, iC3b is further cleaved into C3c, which is released, and C3dg, which remains attached to cells/tissues undergoing complement activation. C3dg is then converted to C3d by local action of proteases, which does not, however, change its receptor binding or cell/tissue attachment properties. In sum, relatively short-term markers of complement activity at the site include the presence of soluble C3a and tissue-bound C3b. Intermediate-term markers include iC3b, C3c and C3f. However, the most durable markers of complement activation are C3dg and C3d, which remain covalently attached to the cell/tissue for days to weeks. See Janssen et al., *Nature* 444:213 (2006) for a description of cleavage reactions.

FIG. 2 illustrates the molecular weights and organization of the cleavage fragments of C3. It is anticipated, but not necessary, that monoclonal antibodies generated to C3dg might bind by Western blot or ELISA to the α peptide of C3 (115 kD); the α' peptide of C3b (110 kD); the $\alpha'1$ peptide of iC3b (67 kD) or the ~35 kD fragment C3dg (or the slightly smaller C3d fragment). The preferred monoclonal antibodies of the present invention will bind only to the latter two, C3dg and C3d, or with preference to the latter two.

FIG. 3 illustrates the preferential binding to human C3d of monoclonal antibodies 3d8b, 3d9a and 3d29 in a Western blot analysis (Group 1). By comparison monoclonal antibodies 3d11 and 3d31 (Group 2) bind to both C3d and other C3 fragments in a Western blot analysis. Other anti-C3d antibodies (Group 3) do not clearly recognize fragments by Western blot analysis. As a positive control, a polyclonal anti-C3 antibody recognizes all bands.

FIG. 4 illustrates the binding of monoclonal antibodies to C3-opsonized zymosan particles derived from incubation of mouse serum with zymosan and generation of C3 fragments

iC3b and C3dg/C3d by using only endogenously available mechanisms. Notably, only Group 1 antibodies recognize C3 fragments bound to this target.

FIG. 5 illustrates the immunoprecipitation by Group 1 monoclonal antibodies only, but not by members of Group 2 or 3 antibodies, of the a1 and C3dg bands from zymosan-activated plasma. This result demonstrates that Group 1 antibodies recognize exposed epitopes on iC3b and C3dg but not C3 or C3b.

FIG. 6 illustrates the kinetics data revealing high affinity binding of clones 3d8b, 3d9a and 3d29 to C3d immobilized on a Biacore chip at increasing antibody concentrations. Lines representing injections of 90, 30 and 10 nM of antibody are shown. The data fit a simple 1:1 Langmuir binding model. K_{D} s of the individual monoclonal antibodies are included in the sensograms.

FIG. 7 illustrates the effect of anti-C3d monoclonal antibodies on the binding of human CR2-MBP to human C3d by ELISA. The top line (diamonds) illustrates one example of the binding in the absence, and bottom line (squares) the presence, of anti-C3d monoclonal antibody 3d8b. Summary histogram at right demonstrates percent binding of CR2 and C3d in the presence of the indicated monoclonal antibodies.

FIG. 8 is a summary of characteristics of the monoclonal antibodies. Note monoclonal antibodies of Group 1 (3d8b, 3d9a and 3d29) share functional effects.

FIGS. 9A and 9B illustrate the binding of monoclonal antibodies 3d8b, 3d9a and 3d29, but not other anti-C3d antibodies illustrated by 3d31, to tissue bound iC3b and C3dg C3 fragments following injection in factor H deficient (fH^{-/-}) mice of 0.5 mg of antibody (FIG. 9B). Note no glomerular IgG is present in the absence of anti-C3d monoclonal antibody injection in these mice. FIG. 9A is a positive control showing the presence of glomerular C3 fragments, which are primarily in the forms of iC3b and C3d, by staining with an anti-C3 fragment antibody.

FIG. 10 illustrates the effect of monoclonal antibodies against C3d on complement activation (AH50) and the lack of enhancement by Group 1 monoclonal antibodies. The lysis of cells in serum supplemented with 10, 20 and 40 μ g of each antibody is measured and compared with the lysis of cells in serum alone. The Y axis shows the percent change in lysis in serum with antibody compared with lysis in serum alone.

FIG. 11 illustrates the protocol utilized to assess the effect of anti-C3d monoclonal antibodies on the humoral immune response to a model complement-dependent antigen, sheep red blood cells.

FIG. 12 illustrates the effects of anti-C3d monoclonal antibodies on the humoral immune response, as assessed by antigen-specific IgG1 production at days 17 (top) and 30 (bottom). P values for individual monoclonal antibody effects on the antigen-specific immune response represent difference in IgG1 levels as compared to SRBC alone, and demonstrate inhibition by Group 1 monoclonal antibodies 3d8b and 3d29 as compared to other test antibodies.

DETAILED DESCRIPTION OF THE INVENTION

The present disclosure provides, among other things, molecules that bind to complement component proteins, e.g., antibodies such as anti-C3dg antibodies or anti-C3d antibodies. The molecules are useful for, e.g., inhibiting the activity and/or activation of a B cell.

The molecules are also useful for targeting a complement modulator to local sites of complement activation. Accord-

ingly, the present disclosure also provides fusion molecules/ constructs comprising a targeting moiety (e.g., an anti-C3d or anti-C3dg antibody or antigen-binding fragment thereof described herein) and an active prophylactic, therapeutic, or diagnostic moiety. In some embodiments, the targeting moiety comprises a molecule that binds to complement-related proteins (e.g., an antibody or antigen-binding fragment thereof that binds to C3d and/or C3dg). The therapeutic moiety can be, e.g., a complement modulator polypeptide such as, but not limited to, DAF, CD59, Crry, factor H, MCP, or CR1.

Furthermore, the present disclosure provides methods for using one or more of the complement component protein-binding molecules and/or the fusion molecules to treat or prevent a disorder in a subject. For example, as elaborated on herein, an anti-C3d antibody or antigen-binding fragment thereof described herein is useful for inhibiting a humoral immune response in a subject (e.g., a human afflicted with an autoimmune disease). Also featured are novel methods of generating monoclonal antibodies by immunizing complement C3 deficient mice with human C3d and performing the fusion and initial screening with minimal exposure to C3 contained within serum-based growth conditions and feeder cell populations that are used to support hybridoma growth. Following extensive *in vitro* and *in vivo* analyses, a subset of the mAbs was found to possess unique characteristics that in aggregate provide proof that these mAbs can be used to identify *in vivo* sites of complement activation and by this capability direct linked functional modules to these sites.

While in no way intended to be limiting, exemplary compositions (e.g., pharmaceutical compositions and formulations) and methods for using the compositions are elaborated on below.

Compositions

The compositions described herein contain molecules that bind to complement component proteins. For example, these molecules include a small molecule, a nucleic acid or nucleic acid analog, a peptide, a peptidomimetic, or a macromolecule that is not a nucleic acid or a peptide. In some embodiments, these molecules are proteins or protein fragments. In some embodiments, these molecules are antibodies or antibody fragments which retain their antigen-binding activity. In some embodiments, these molecules are anti-C3dg antibodies, anti-C3d antibodies, or antigen-binding fragments of any of the foregoing.

As used herein, the term “antibody” or “immunoglobulin” refers to proteins (including glycoproteins) of the immunoglobulin (Ig) superfamily of proteins. An antibody or immunoglobulin (Ig) molecule is tetrameric, comprising two identical light chain polypeptides and two identical heavy chain polypeptides. The two heavy chains are linked together by disulfide bonds, and each heavy chain is linked to a light chain by a disulfide bond. Each full-length Ig molecule contains at least two binding sites for a specific target or antigen.

The immune system produces several different classes of Ig molecules (isotypes), including IgA, IgD, IgE, IgG, and IgM, each distinguished by the particular class of heavy chain polypeptide present: alpha (α) found in IgA, delta (δ) found in IgD, epsilon (ϵ) found in IgE, gamma (γ) found in IgG, and mu (μ) found in IgM. There are at least five different γ heavy chain polypeptides (isotypes) found in IgG. In contrast, there are only two light chain polypeptide isotypes, referred to as kappa (κ) and lambda (λ) chains. The distinctive characteristics of antibody isotypes are defined by sequences of the constant domains of the heavy chain.

An IgG molecule comprises two light chains (either κ or λ form) and two heavy chains (γ form) bound together by disulfide bonds. The κ and λ forms of IgG light chain each contain a domain of relatively variable amino acid sequences, called the variable region (variously referred to as a “ V_L ,” “ V_κ ,” or “ V_λ -region”) and a domain of relatively conserved amino acid sequences, called the constant region (C_L -region). Similarly, each IgG heavy chain contains a variable region (V_H -region) and one or more conserved regions: a complete IgG heavy chain contains three constant domains (“ C_{H1} ,” “ C_{H2} ,” and “ C_{H3} -regions”) and a hinge region. Within each V_L - or V_H -region, hypervariable regions, also known as complementarity-determining regions (“CDR”), are interspersed between relatively conserved framework regions (“FR”). Generally, the variable region of a light or heavy chain polypeptide contains four FRs and three CDRs arranged in the following order along the polypeptide: NH_2 -FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-COOH. Together the CDRs and FRs determine the three-dimensional structure of the IgG binding site and thus, the specific target protein or antigen to which that IgG molecule binds. Each IgG molecule is dimeric, able to bind two antigen molecules. Cleavage of a dimeric IgG with the protease papain produces two identical antigen-binding fragments (“Fab”) and an “Fc” fragment or Fc domain, so named because it is readily crystallized.

As used throughout the present disclosure, the term “antibody” further refers to a whole or intact antibody (e.g., IgM, IgG, IgA, IgD, or IgE) molecule that is generated by any one of a variety of methods that are known in the art and described herein. The term “antibody” includes a polyclonal antibody, a monoclonal antibody, a chimerized or chimeric antibody, a humanized antibody, a deimmunized human antibody, and a fully human antibody. The antibody can be made in or derived from any of a variety of species, e.g., mammals such as humans, non-human primates (e.g., monkeys, baboons, or chimpanzees), horses, cattle, pigs, sheep, goats, dogs, cats, rabbits, guinea pigs, gerbils, hamsters, rats, and mice. The antibody can be a purified or a recombinant antibody.

Antibodies to C3 and C3 Fragments.

The compositions described herein can contain antibodies which bind to C3 or C3 fragments such as iC3b, C3d and C3dg, or an antigen-binding fragment thereof, such as the antibodies produced by the hybridoma cell lines deposited with the ATCC described herein. In some embodiments, an antibody described herein binds to C3d with greater specificity and broader species cross-reactivity than commercially available anti-human C3d antibodies. In some embodiments, the commercially available anti-C3d antibodies are designated by the Quidel catalog numbers A207 and A250, and are commercially available from the Quidel Corporation (Quidel Corp., San Diego and Santa Clara, Calif.). Antibodies that bind to C3 and to cleavage fragments C3b, iC3b and C3d, are known. For example, see U.S. Pat. No. 6,572,856, Taylor; Tosic et al., *J. Immunological Methods*, 120:241-249 (1989); Sokoloff et al., *Cancer Immunology and Immunotherapy*, 49:551-562 (2000); Mastellos et al., *Molecular Immunology*, 40:1213-1221 (2004); Dilillo et al., *Molecular Immunology*, 43:1010-1019 (2006); Campaigne, US 2009/0081211; Etemad-Gilbertson et al., U.S. patent application publication no. 2009/0175875; Aguado et al., *J. Clin. Invest.*, 76:1418-1426 (1985). The disclosures of these documents are incorporated herein by reference in their entirety. Such antibodies, and functional fragments thereof, may be useful in the present invention as the targeting moiety for directing therapeutic fragments to tissue

experiencing activated complement activity, and thus expressing C3 or its fragments.

However, the previously known antibodies to C3, C3b and C3d do not include all of the characteristics of the antibodies identified herein.

In some embodiments, the disclosure features an antibody, or antigen-binding fragment thereof, that binds to an epitope in the human C3d protein. In some embodiments, the disclosure features an antibody, or antigen-binding fragment thereof, that binds to an epitope in the human C3dg protein. For example, the anti-C3d or anti-C3dg antibody can bind to an epitope within, or overlapping with, an antigenic peptide fragment of a human complement component C3d protein, or to an epitope in the human complement component C3dg protein. In some embodiments, these anti-C3d or anti-C3dg antibodies are monoclonal antibodies or antibody fragments maintaining the antigen-binding activity. In some embodiments, these monoclonal antibodies include those produced by hybridoma cells 3d-8b/2 (ATCC Deposit number: PTA-10999), 3d-9a/25 (ATCC Deposit number: PTA-10998), 3d-29/5/2 (ATCC Deposit number: PTA-11000), 3d-11/14 (ATCC Deposit number: PTA-11011), 3d-31/A6/9 (ATCC Deposit number: PTA-11027), 3d3/28/4 (ATCC Deposit number: PTA-11025), 3d-15A9 (ATCC Deposit number: PTA-11012), 3d-10/14/1 (ATCC Deposit number: PTA-11010), and 3d-16/3/3 (ATCC Deposit number: PTA-11026). In some embodiments, the present disclosure provides antibodies, or antigen-binding fragments thereof, that bind to an epitope within, or overlapping with, an epitope recognized by any one of antibodies 3d8b, 3d9a, 3d29, 3d11, 3d31, 3d3, 3d15, 3d10, and 3d16. In some embodiments, these antibodies, or antigen-binding fragments thereof, which bind to an epitope within, or overlapping with, an epitope recognized by any one of antibodies 3d8b, 3d9a, 3d29, 3d11, 3d31, 3d3, 3d15, 3d10, and 3d16, do not compete with any one of antibodies 3d8b, 3d9a, 3d29, 3d11, 3d31, 3d3, 3d15, 3d10, and 3d16 for binding to C3d or C3dg. In some embodiments, these antibodies, or antigen-binding fragments thereof, which bind to an epitope within, or overlapping with, an epitope recognized by any one of antibodies 3d8b, 3d9a, 3d29, 3d11, 3d31, 3d3, 3d15, 3d10, and 3d16, compete with at least one of antibodies including 3d8b, 3d9a, 3d29, 3d11, 3d31, 3d3, 3d15, 3d10, and 3d16 for binding to C3d or C3dg. In some embodiments, these antibodies, or antigen-binding fragments thereof, which bind to an epitope within, or overlapping with, an epitope recognized by any one of antibodies 3d8b, 3d9a, 3d29, 3d11, 3d31, 3d3, 3d15, 3d10, and 3d16, inhibit at least one of antibodies 3d8b, 3d9a, 3d29, 3d11, 3d31, 3d3, 3d15, 3d10, and 3d16 for binding to C3d or C3dg.

As used herein, the term "epitope" refers to the site on a protein (e.g., a human complement component C3d or C3dg protein) that is bound by an antibody. "Overlapping epitopes" include at least one (e.g., two, three, four, five, or six) common amino acid residue(s).

As used herein, the terms "specific binding" or "specifically binds" refer to two molecules forming a complex (e.g., a complex between an antibody and a complement component C3d or C3dg protein) that is relatively stable under physiologic conditions. Typically, binding is considered specific when the association constant (K_a) is higher than 10^6 M^{-1} . Thus, an antibody can specifically bind to a C3d or C3dg protein with a K_a of at least (or greater than 10^6 (e.g., at least or greater than 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} , 10^{12} , 10^{13} , 10^{14} , or 10^{15} or higher) M^{-1}).

Methods for determining whether an antibody binds to a protein antigen and/or the affinity for an antibody to a protein antigen are known in the art. For example, the binding of an antibody to a protein antigen can be detected and/or quantified using a variety of techniques such as, but not limited to, Western blot, dot blot, surface plasmon resonance method (e.g., BIAcore system; Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.), or enzyme-linked immunosorbent assays (ELISA). See, e.g., Harlow and Lane (1988) "Antibodies: A Laboratory Manual" Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Benny K. C. Lo (2004) "Antibody Engineering: Methods and Protocols," Humana Press (ISBN: 1588290921); Borrebaek (1992) "Antibody Engineering, A Practical Guide," W.H. Freeman and Co., NY; Borrebaek (1995) "Antibody Engineering," 2nd Edition, Oxford University Press, NY, Oxford; Johne et al. (1993) J. Immunol. Meth. 160:191-198; Jonsson et al. (1993) Ann Biol. Clin. 51:19-26; and Jonsson et al. (1991) Biotechniques 11:620-627. See also, U.S. Pat. No. 6,355,245.

In some embodiments, the anti-C3d or anti-C3dg antibody, or antigen-binding fragment thereof, provided in the present disclosure can crossblock binding of another antibody or binding partner that binds to an epitope within, or overlapping with, a human complement component C3d or C3dg protein. In some embodiments, the anti-C3d or anti-C3dg antibody, or antigen-binding fragment thereof, can crossblock binding of an antibody that binds to an epitope within, or overlapping with, a peptide fragment of a human complement component C3d or C3dg protein.

As used herein, the term "crossblocking antibody" refers to an antibody, or antibody fragment thereof maintaining its antigen-binding activity, that lowers the amount of binding of anti-C3d or anti-C3dg antibody, or antibody fragment thereof maintaining its antigen-binding activity, to an epitope on a complement component C3d or C3dg protein relative to the amount of binding of the anti-C3d or anti-C3dg antibody, or antibody fragment thereof maintaining its antigen-binding activity, to the epitope in the absence of the crossblocking antibody, or antibody fragment thereof maintaining its antigen-binding activity. Suitable methods for determining whether a first antibody, or antibody fragment thereof, crossblocks binding of a second antibody, or antibody fragment thereof, to an epitope are known in the art. For example, crossblocking antibodies can be identified by comparing the binding of the 3d-9a/25 anti-C3d monoclonal antibody (produced by the hybridoma cell line ATCC designation PTA-11025) to C3d in the presence and absence of a test antibody. In such a case, decreased binding of the 3d-9a/25 antibody in the presence of the test antibody as compared to binding of the 3d-9a/25 antibody in the absence of the test antibody indicates that the test antibody is a crossblocking antibody.

Methods for identifying the epitope to which a particular antibody (e.g., an anti-C3d or anti-C3dg antibody) binds are also known in the art. For example, the binding epitope of an anti-C3d or anti-C3dg antibody can be identified by measuring the binding of the antibody to several (e.g., three, four, five, six, seven, eight, nine, 10, 15, 20, or 30 or more) overlapping peptide fragments of a complement component C3d or C3dg protein. Each of the different overlapping peptides is then bound to a unique address on a solid support, e.g., separate wells of a multi-well assay plate. Next, the anti-C3d or anti-C3dg antibody is interrogated by contacting it to each of the peptides in the assay plate for an amount of time and under conditions that allow for the antibody to bind to its epitope. Unbound anti-C3d or anti-C3dg antibody is

removed by washing each of the wells. Next, a detectably-labeled secondary antibody that binds to the anti-C3d or anti-C3dg antibody, if present in a well of the plate, is contacted to each of the wells, and unbound secondary antibody is removed by washing steps. The presence or amount of the detectable signal produced by the detectably-labeled secondary antibody in a well is an indication that the anti-C3d or anti-C3dg antibody binds to the particular peptide fragment associated with the well. For a similar method for identifying the epitope to which an anti-05 antibody binds, see, e.g., Harlow and Lane (supra), Benny K. C. Lo (supra), and U.S. Patent Application Publication No. 20060153836, the disclosure of which is incorporated by reference in its entirety. A particular epitope to which an antibody binds can also be identified using BIAcore chromatographic techniques (see, e.g., Pharmacia BIA technology Handbook, "Epitope Mapping," Section 6.3.2, (May 1994); and Johne et al. (1993) *J. Immunol. Methods* 160: 191-8).

In some embodiments, the present disclosure provides an anti-C3d or anti-C3dg antibody, or antigen-binding fragment thereof, which specifically binds to human C3d with a K_D value of 1.1×10^{-9} M or better. In some embodiments, such a K_D value is in the range from 1.1×10^{-9} M to 3.6×10^{-10} M. In some embodiments, the antibody or antigen-binding fragment thereof binds to human C3d with an affinity about $K_D = 1.06 \times 10^{-9}$ M. In some embodiments, the antibody is mAb 3d29. In some embodiments, the antibody or antigen-binding fragment thereof binds to human C3d with an affinity about $K_D = 4.65 \times 10^{-10}$ M. In some embodiments, the antibody is mAb 3d8b. In some embodiments, the antibody or antigen-binding fragment thereof binds to human C3d with an affinity about $K_D = 3.67 \times 10^{-10}$ M. In some embodiments, the antibody is mAb 3d9a.

Measurements to determine antibody affinity are standard and well known techniques. As an exemplary method to measure affinity, BIAcore analysis was used to quantify humanized antibodies' respective affinities for human C5a. See, e.g., Karlsson and Larsson (2004) *Methods Mol Biol* 248:389-415. Briefly, each of the humanized antibodies was screened with 3-4 concentrations of human C5a (antigen) using a capture technique. The antibodies were captured by an anti-Fc (human) directly immobilized on a CMS sensor chip with various concentrations in the range from 0.6 nM to 5.9 nM of human C5a passed over the sensor chip surface. The surface was regenerated with 20 mM HCl, 0.02% P20 after each cycle to remove bound antibody and antigen. The data were evaluated using Biacore BIAevaluation software using a 1:1 Langmuir Model Fit (Rmax:Global Fit; RI:Local Fit). Kinetics information such as (k_a : Association Rate constant), (k_d : Dissociation Rate constant) and K_D (Equilibrium Dissociation constant) was obtained from the fit. These and similar techniques are applicable to other antibodies such as those that bind to C3d or C3dg.

In some embodiments, the disclosure provides an antibody, or antigen-binding fragment thereof, which preferably binds to C3d or C3dg compared to binding to complement component proteins C3, C3a, C3b, C3c, or C3f. In some embodiments, the antibody is 3d8b, 3d9a, or 3d29. In some embodiments, an anti-C3d or anti-C3dg antibody described herein binds to C3d or C3dg but not to any one of complement component proteins C3, C3a, C3b, C3c, or C3f. In some embodiments, the present disclosure provides an antibody, or antigen-binding fragment thereof, which binds to C3d or C3dg at a comparable affinity as its binding to complement component proteins C3, C3a, C3b, C3c, or C3f. In some embodiments, the antibody is 3d11, or 3d31. In

some embodiments, the present disclosure provides an antibody, or antigen-binding fragment thereof, which binds weakly to C3d or C3dg but not to any one of complement component proteins C3, C3a, C3b, C3c, or C3f. In some embodiments, the antibody is 3d3, or 3d15.

Thus, in some embodiments, an antibody or antigen-binding fragment thereof binds to free C3d or C3dg (e.g., hC3d or hC3dg) with an affinity that is at least 10 (e.g., at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 225, 250, 275, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, or 10000)-fold greater than its corresponding affinity for uncleaved, native C3 protein.

In some embodiments, the disclosure provides an antibody, or antigen-binding fragment thereof, which preferably binds to deposited or opsonized C3 fragments, e.g., C3d or C3dg, compared to binding to free or circulating or undeposited C3 fragments. In some embodiments, such an antibody or antigen-binding fragment thereof only binds to deposited C3 fragments but not free C3 or C3 fragments. In other embodiments, the present disclosure comprises antibodies which bind to complement fragment C3d and are able to discriminate between tissue bound C3 fragments from circulating C3 (e.g., C3, C3b, or (C3H₂O)). In some embodiments, such antibodies include mAbs 3d9a, 3d29 and 3d8b. In some embodiments, antibodies of the invention bind to C3d with greater specificity than commercially available anti-C3d antibodies. In some embodiments, the commercially available anti-C3d antibodies are designated by the Quidel catalog numbers A207 and A250, and are commercially available from the Quidel Corporation (Quidel Corp., San Diego and Santa Clara, Calif.).

Each of the 3d9a, 3d29 and 3d8b antibodies was found to bind to kidney tissue sections exhibiting inflammation when injected into mice intravenously, and bind to C3-opsonized zymosan, which is known to express iC3b but not C3b. Thus, these antibodies are able to distinguish between tissue bound fragment C3d, and circulating native C3 and the fragment C3b.

In some embodiments, the disclosure comprises an antibody, or antigen-binding fragment thereof, that binds to C3d or C3dg from multiple species (species cross-reactive). In some embodiments, such an antibody or antigen-binding fragment thereof binds to C3d or C3dg from at least one species selected from human, non-human mammals (e.g., cynomolgus monkey or cynomolgus macaque, rhesus macaque, ape, baboon, chimpanzee, orangutan, or gorilla), rodents (e.g., mouse, rat, hamster, Guinea pig, gerbil, or rabbit), cattle, sheep, goat, donkey, pig, dog, cat, horse, and camel. In some embodiments, such an antibody or antigen-binding fragment thereof binds to cynomolgus macaque C3d or C3dg. In some embodiments, an antibody or antigen-binding fragment thereof described herein binds to C3d or C3dg from at least two species selected from the above list. In some embodiments, such an antibody or antigen-binding fragment thereof binds to both human and cynomolgus macaque C3d or C3dg. In some embodiments, such an antibody is 3d8b, 3d9a, 3d29, 3d11, 3d31, 3d3, 3d15, 3d10, or 3d16.

In some embodiments, the disclosure provides an anti-C3d or anti-C3dg antibody, or antigen-binding fragment thereof, which competes with CR2 for binding to C3d or C3dg. In some embodiments, such an antibody or antigen-binding fragment thereof reduces the ability of a CR2 protein to bind to human complement component C3d or C3dg by greater than 50 (e.g., greater than 55, 60, 65, 70, 75, 80, 85, 90, or 95 or more) %. In some embodiments, such an

antibody is mAb 3d11, 3d9a, 3d16, 3d29, 3d31 or 3d8b. In some embodiments, such an antibody or antigen-binding fragment thereof decreases CR2-C3d binding to at least 60%. In some embodiments, such an antibody or antigen-binding fragment thereof decreases CR2-C3d binding to at least 40%. In some embodiments, such an antibody or antigen-binding fragment thereof significantly inhibits or blocks CR2 binding to C3d. In some embodiments, such an antibody is 3d9a, 3d29 or 3d8b.

Immunoassays which can be used to analyze immunospecific binding and cross-reactivity of the antibodies include, but are not limited to, competitive and non-competitive assay systems using techniques such as Western blots, RIA, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, and protein A immunoassays. Such assays are routine and well known in the art.

Antibodies can also be assayed using any surface plasmon resonance (SPR)-based assays known in the art for characterizing the kinetic parameters of the interaction of the antibody with C3d or C3dg. Any SPR instrument commercially available including, but not limited to, Biacore Instruments (Biacore AB; Uppsala, Sweden); IAsys instruments (Affinity Sensors; Franklin, Mass.); IBIS system (Windsor Scientific Limited; Berks, UK), SPR-CELLIA systems (Nippon Laser and Electronics Lab; Hokkaido, Japan), and SPR Detector Spreeta (Texas Instruments; Dallas, Tex.) can be used in the methods described herein. See, e.g., Mullett et al. (2000) *Methods* 22: 77-91; Dong et al. (2002) *Reviews in Mol Biotech* 82: 303-323; Fivash et al. (1998) *Curr Opin Biotechnol* 9: 97-101; and Rich et al. (2000) *Curr Opin Biotechnol* 11: 54-61.

In some embodiments, the present disclosure provides an anti-C3d antibody or anti-C3dg antibody, or antigen-binding fragment thereof, which does not enhance complement activation. In some embodiments, such an antibody is mAb3d11, 3d9a, 3d16, 3d29, 3d31 or 3d8b.

While the disclosure is in no way limited by one particular theory or mechanism of action, the inventors assert that an anti-C3d or anti-C3dg antibody, or antigen-binding fragment thereof, disclosed herein is able to compete with CR2 on the membrane of host B cells for binding to C3d or C3dg or antigen-C3d/C3dg immune complexes, thereby reducing or inhibiting the downstream signaling through CR2, which is crucial for B cell activation and antibody production. As used herein, B cell activation or activity refers to any aspect of: (a) antigen-specific production or secretion of an antibody by a B cell or plasma cell; (b) proliferation of a B cell or plasma cell; (c) antigen processing by B cells; (d) antigen presentation by B cells to T cells; (e) upregulation of B cell surface markers; and/or (f) differentiation (e.g., antigen-driven) of a B cell into an antibody-producing plasma cell. Thus, in some embodiments, the disclosure features an anti-C3d or anti-C3dg antibody, or antigen-binding fragment thereof, which can decrease or inhibit host humoral immune response. In some embodiments, such an antibody or antigen-binding fragment thereof decreases or inhibits host antibody production. In some embodiments, such an antibody or antigen-binding fragment thereof reduces host immune response by greater than 20 (e.g., greater than 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, or 95 or more) %. In some embodiments, such an antibody or antigen-binding fragment thereof reduces host immune response by at least 50%. In some embodiments, such an antibody is mAb 3d11, 3d9a, 3d16, 3d29, 3d31 or 3d8b. In some

embodiments, such an antibody is 3d9a, 3d29, or 3d8b. Methods for determining whether an antibody or antigen-binding fragment thereof has reduced an immune response (e.g., an autoimmune response) in a mammal are well known in the art (e.g., mixed lymphocyte reaction (MLR)) and described in e.g., U.S. Pat. No. 7,576,182, and Lemoli et al. Immunological effects of omalizumab in chronic urticaria: a case report. *J Investig Allergol Clin Immunol.* 2010; 20(3): 252-4.

In some embodiments, the present disclosure also provides antibodies, or antigen-binding fragments thereof, which are variants of mouse monoclonal antibodies 3d8b, 3d9a, 3d29, 3d11, 3d31, 3d3, 3d15, 3d10, and 3d16 and maintain the C3d binding ability of these mouse antibodies. For example, the present disclosure provides an anti-C3d or anti-C3dg antibody, or antigen-binding fragments thereof, which is a polyclonal antibody, a monoclonal antibody or antibody fragment, a diabody, a chimerized or chimeric antibody or antibody fragment, a humanized antibody or antibody fragment, a deimmunized human antibody or antibody fragment, a fully human antibody or antibody fragment, a bispecific antibody or antibody fragment, a monovalent antibody or antibody fragment, a single chain antibody, an Fv, an Fab, an Fab', and an F(ab')₂. For example, the present disclosure provides chimerized, humanized, or single-chain versions of 3db8, 3d9a, 3d29, etc.

Methods for preparing a hybridoma cell line include immunizing Balb/c mice by injecting subcutaneously and/or intraperitoneally an immunogenic composition containing human C3d or C3dg protein (or an immunogenic fragment thereof) several times, e.g., four to six times, over several months, e.g., between two and four months. Spleen cells from the immunized mice are taken two to four days after the last injection and fused with cells of the myeloma cell line PAI in the presence of a fusion promoter, preferably polyethylene glycol. Preferably, the myeloma cells are fused with a three- to twenty-fold excess of spleen cells from the immunized mice in a solution containing about 30% to about 50% polyethylene glycol of a molecular weight around 4000. After the fusion, the cells are expanded in suitable culture media as described supra, supplemented with a selection medium, for example HAT medium, at regular intervals in order to prevent normal myeloma cells from overgrowing the desired hybridoma cells.

The antibodies and fragments thereof can be, in some embodiments, "chimeric." Chimeric antibodies and antigen-binding fragments thereof comprise portions from two or more different species (e.g., mouse and human). Chimeric antibodies can be produced with mouse variable regions of desired specificity spliced onto human constant domain gene segments (see, for example, U.S. Pat. No. 4,816,567). In this manner, non-human antibodies can be modified to make them more suitable for human clinical application (e.g., methods for treating or preventing a complement associated disorder in a human subject).

The monoclonal antibodies of the present disclosure include "humanized" forms of the non-human (e.g., mouse) antibodies. Humanized or CDR-grafted mAbs are particularly useful as therapeutic agents for humans because they are not cleared from the circulation as rapidly as mouse antibodies and do not typically provoke an adverse immune reaction. Methods of preparing humanized antibodies are generally well known in the art. For example, humanization can be essentially performed following the method of Winter and co-workers (see, e.g., Jones et al. (1986) *Nature* 321: 522-525; Riechmann et al. (1988) *Nature* 332:323-327; and

Verhoeyen et al. (1988) *Science* 239:1534-1536), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Also see, e.g., Staelens et al. (2006) *Mol Immunol* 43:1243-1257. In some embodiments, humanized forms of non-human (e.g., mouse) antibodies are human antibodies (recipient antibody) in which hypervariable (CDR) region residues of the recipient antibody are replaced by hypervariable region residues from a non-human species (donor antibody) such as a mouse, rat, rabbit, or non-human primate having the desired specificity, affinity, and binding capacity. In some instances, framework region residues of the human immunoglobulin are also replaced by corresponding non-human residues (so called "back mutations"). In addition, phage display libraries can be used to vary amino acids at chosen positions within the antibody sequence. The properties of a humanized antibody are also affected by the choice of the human framework. Furthermore, humanized and chimerized antibodies can be modified to comprise residues that are not found in the recipient antibody or in the donor antibody in order to further improve antibody properties, such as, for example, affinity or effector function.

Fully human antibodies are also provided in the disclosure. The term "human antibody" includes antibodies having variable and constant regions (if present) derived from human germline immunoglobulin sequences. Human antibodies can include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human antibody" does not include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences (i.e., humanized antibodies). Fully human or human antibodies may be derived from transgenic mice carrying human antibody genes (carrying the variable (V), diversity (D), joining (J), and constant (C) exons) or from human cells. For example, it is now possible to produce transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. (See, e.g., Jakobovits et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:2551; Jakobovits et al. (1993) *Nature* 362:255-258; Bruggemann et al. (1993) *Year in Immunol.* 7:33; and Duchosal et al. (1992) *Nature* 355:258.) Transgenic mice strains can be engineered to contain gene sequences from unrearranged human immunoglobulin genes. The human sequences may code for both the heavy and light chains of human antibodies and would function correctly in the mice, undergoing rearrangement to provide a wide antibody repertoire similar to that in humans. The transgenic mice can be immunized with the target protein (e.g., a complement component C3d or C3dg protein, fragments thereof, or cells expressing C3d or C3dg protein) to create a diverse array of specific antibodies and their encoding RNA. Nucleic acids encoding the antibody chain components of such antibodies may then be cloned from the animal into a display vector. Typically, separate populations of nucleic acids encoding heavy and light chain sequences are cloned, and the separate populations then recombined on insertion into the vector, such that any given copy of the vector receives a random combination of a heavy and a light chain. The vector is designed to express antibody chains so that they can be assembled and displayed on the outer surface of a display package containing the vector. For example, antibody chains can be expressed as fusion proteins with a phage coat protein

from the outer surface of the phage. Thereafter, display packages can be screened for display of antibodies binding to a target.

Thus, in some embodiments, the disclosure provides, e.g., humanized, deimmunized or primatized antibodies comprising one or more of the complementarity determining regions (CDRs) of the mouse monoclonal antibodies described herein, which retain the ability (e.g., at least 50, 60, 70, 80, 90, or 100%, or even greater than 100%) of the mouse monoclonal antibody counterpart to bind to its antigen (e.g., C3d or C3dg). For example, the disclosure features a humanized antibody comprising the set of six CDRs (e.g., heavy chain CDR1, heavy chain CDR2, heavy chain CDR3, light chain CDR1, light chain CDR2, and light chain CDR3) of any one of mouse monoclonal antibodies 3d8b, 3d9a, 3d29, 3d11, 3d31, 3d3, 3d15, 3d10, or 3d16 and human framework regions (with or without human constant or Fc regions).

The exact boundaries of CDRs and framework regions have been defined differently according to different methods and are well known to one of ordinary skill in the art of antibody engineering. In some embodiments, the positions of the CDRs or framework regions within a light or heavy chain variable domain can be as defined by Kabat et al. [(1991) "Sequences of Proteins of Immunological Interest." NIH Publication No. 91-3242, U.S. Department of Health and Human Services, Bethesda, Md.]. In such cases, the CDRs can be referred to as "Kabat CDRs" (e.g., "Kabat L_{CDR2}" or "Kabat H_{CDR1}") and the framework regions can be referred to as "Kabat framework regions," (e.g., "Kabat L_{FR1}" or "Kabat H_{FR3}"). In some embodiments, the positions of the CDRs or framework regions of a light or heavy chain variable region can be as defined by Chothia et al. (1989) *Nature* 342:877-883. Accordingly, these regions can be referred to as "Chothia CDRs" (e.g., "Chothia L_{CDR2}" or "Chothia H_{CDR3}") or "Chothia framework regions" (e.g., "Chothia L_{FR1}" or "Chothia L_{FR3}"), respectively. In some embodiments, the positions of the CDRs or framework regions of the light and heavy chain variable regions can be as defined by a Kabat-Chothia combined definition. In such embodiments, these regions can be referred to as "combined Kabat-Chothia CDRs" or "combined Kabat-Chothia framework regions," respectively. Thomas et al. [(1996) *Mol Immunol* 33(17/18):1389-1401] exemplifies the identification of CDRs and framework region boundaries according to Kabat and Chothia definitions.

In some embodiments, the positions of the CDRs and/or framework regions with a light or heavy chain variable domain can be as defined by Honnegger and Plückthun [(2001) *J Mol Biol* 309: 657-670].

In addition, human antibodies can be derived from phage-display libraries (Hoogenboom et al. (1991) *J. Mol. Biol.* 227:381; Marks et al. (1991) *J. Mol. Biol.*, 222:581-597; and Vaughan et al. (1996) *Nature Biotech* 14:309 (1996)). Synthetic phage libraries can be created which use randomized combinations of synthetic human antibody V-regions. By selection on antigen fully human antibodies can be made in which the V-regions are very human-like in nature. See, e.g., U.S. Pat. Nos. 6,794,132, 6,680,209, 4,634,666, and Ostberg et al. (1983), *Hybridoma* 2:361-367, the contents of each of which are incorporated herein by reference in their entirety.

For the generation of human antibodies, also see Mendez et al. (1998) *Nature Genetics* 15:146-156 and Green and Jakobovits (1998) *J. Exp. Med.* 188:483-495, the disclosures of which are hereby incorporated by reference in their entirety. Human antibodies are further discussed and delin-

eated in U.S. Pat. Nos. 5,939,598; 6,673,986; 6,114,598; 6,075,181; 6,162,963; 6,150,584; 6,713,610; and 6,657,103 as well as U.S. Patent Application Publication Nos. 2003-0229905 A1, 2004-0010810 A1, US 2004-0093622 A1, 2006-0040363 A1, 2005-0054055 A1, 2005-0076395 A1, and 2005-0287630 A1. See also International Publication Nos. WO 94/02602, WO 96/34096, and WO 98/24893, and European Patent No. EP 0 463 151 B1. The disclosures of each of the above-cited patents, applications, and references are hereby incorporated by reference in their entirety.

In an alternative approach, others, including GenPharm International, Inc., have utilized a "minilocus" approach. In the minilocus approach, an exogenous Ig locus is mimicked through the inclusion of pieces (individual genes) from the Ig locus. Thus, one or more VH genes, one or more DH genes, one or more JH genes, a mu constant region, and a second constant region (preferably a gamma constant region) are formed into a construct for insertion into an animal. This approach is described in, e.g., U.S. Pat. Nos. 5,545,807; 5,545,806; 5,625,825; 5,625,126; 5,633,425; 5,661,016; 5,770,429; 5,789,650; and 5,814,318; 5,591,669; 5,612,205; 5,721,367; 5,789,215; 5,643,763; 5,569,825; 5,877,397; 6,300,129; 5,874,299; 6,255,458; and 7,041,871, the disclosures of which are hereby incorporated by reference. See also European Patent No. 0 546 073 B1, International Patent Publication Nos. WO 92/03918, WO 92/22645, WO 92/22647, WO 92/22670, WO 93/12227, WO 94/00569, WO 94/25585, WO 96/14436, WO 97/13852, and WO 98/24884, the disclosures of each of which are hereby incorporated by reference in their entirety. See further Taylor et al. (1992) *Nucleic Acids Res.* 20: 6287; Chen et al. (1993) *Int. Immunol.* 5: 647; Tuaille et al. (1993) *Proc. Natl. Acad. Sci. USA* 90: 3720-4; Choi et al. (1993) *Nature Genetics* 4: 117; Lonberg et al. (1994) *Nature* 368: 856-859; Taylor et al. (1994) *International Immunology* 6: 579-591; Tuaille et al. (1995) *J. Immunol.* 154: 6453-65; Fishwild et al. (1996) *Nature Biotechnology* 14: 845; and Tuaille et al. (2000) *Eur. J. Immunol.* 10: 2998-3005, the disclosures of each of which are hereby incorporated by reference in their entirety.

In some embodiments, de-immunized antibodies or antigen-binding fragments thereof are provided. De-immunized antibodies or antigen-binding fragments thereof are antibodies that have been modified so as to render the antibody or antigen-binding fragment thereof non-immunogenic, or less immunogenic, to a given species (e.g., to a human). De-immunization can be achieved by modifying the antibody or antigen-binding fragment thereof utilizing any of a variety of techniques known to those skilled in the art (see, e.g., PCT Publication Nos. WO 04/108158 and WO 00/34317). For example, an antibody or antigen-binding fragment thereof may be de-immunized by identifying potential T cell epitopes and/or B cell epitopes within the amino acid sequence of the antibody or antigen-binding fragment thereof and removing one or more of the potential T cell epitopes and/or B cell epitopes from the antibody or antigen-binding fragment thereof, for example, using recombinant techniques. The modified antibody or antigen-binding fragment thereof may then optionally be produced and tested to identify antibodies or antigen-binding fragments thereof that have retained one or more desired biological activities, such as, for example, binding affinity, but have reduced immunogenicity. Methods for identifying potential T cell epitopes and/or B cell epitopes may be carried out using techniques known in the art, such as, for example, computational methods (see e.g., PCT Publication No. WO 02/069232), in vitro or in silico techniques, and biological assays or physical methods (such as, for example, determination of the

binding of peptides to MHC molecules, determination of the binding of peptide:MHC complexes to the T cell receptors from the species to receive the antibody or antigen-binding fragment thereof, testing of the protein or peptide parts thereof using transgenic animals with the MHC molecules of the species to receive the antibody or antigen-binding fragment thereof, or testing with transgenic animals reconstituted with immune system cells from the species to receive the antibody or antigen-binding fragment thereof, etc.). In various embodiments, the de-immunized anti-C3d or -C3dg antibodies described herein include de-immunized antigen-binding fragments, Fab, Fv, scFv, Fab' and F(ab')₂, monoclonal antibodies, murine antibodies, engineered antibodies (such as, for example, chimeric, single chain, CDR-grafted, humanized, fully human antibodies, and artificially selected antibodies), synthetic antibodies and semi-synthetic antibodies.

In some embodiments, the present disclosure also provides bispecific antibodies. Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for C3d or C3dg, the other one is for any other antigen.

Methods for making bispecific antibodies are within the purview of those skilled in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chain/light-chain pairs have different specificities (Milstein and Cuello (1983) *Nature* 305:537-539). Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion of the heavy chain variable region is preferably with an immunoglobulin heavy-chain constant domain, including at least part of the hinge, CH2, and CH3 regions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of illustrative currently known methods for generating bispecific antibodies see, e.g., Suresh et al. (1986) *Methods in Enzymology* 121:210; PCT Publication No. WO 96/27011; Brennan et al. (1985) *Science* 229:81; Shalaby et al., *J Exp Med* (1992) 175:217-225; Kostelny et al. (1992) *J Immunol* 148(5):1547-1553; Hollinger et al. (1993) *Proc Natl Acad Sci USA* 90:6444-6448; Gruber et al. (1994) *J Immunol* 152:5368; and Tutt et al. (1991) *J Immunol* 147:60. Bispecific antibodies also include cross-linked or heteroconjugate antibodies. Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Pat. No. 4,676,980, along with a number of cross-linking techniques.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. See, e.g., Kostelny et al. (1992) *J Immunol* 148(5):1547-1553. The leucine zipper peptides from the Fos and Jun proteins may be linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers may be reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al. (1993) *Proc Natl Acad Sci USA* 90:6444-6448 has provided an alternative mechanism for making bispecific antibody frag-

ments. The fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the VH and VL domains of one fragment are forced to pair with the complementary VL and VH domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (scFv) dimers has also been reported. See, e.g., Gruber et al. (1994) *J Immunol* 152:5368. Alternatively, the antibodies can be “linear antibodies” as described in, e.g., Zapata et al. (1995) *Protein Eng.* 8(10):1057-1062. Briefly, these antibodies comprise a pair of tandem Fd segments ($V_H-C_{H1}-V_H-C_{H1}$) which form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

Antibodies with more than two valencies (e.g., trispecific antibodies) are contemplated and described in, e.g., Tutt et al. (1991) *J Immunol* 147:60.

The disclosure also embraces variant forms of multi-specific antibodies such as the dual variable domain immunoglobulin (DVD-Ig) molecules described in Wu et al. (2007) *Nat Biotechnol* 25(11):1290-1297. The DVD-Ig molecules are designed such that two different light chain variable domains (VL) from two different parent antibodies are linked in tandem directly or via a short linker by recombinant DNA techniques, followed by the light chain constant domain. Similarly, the heavy chain comprises two different heavy chain variable domains (VH) linked in tandem, followed by the constant domain C_{H1} and Fc region. Methods for making DVD-Ig molecules from two parent antibodies are further described in, e.g., PCT Publication Nos. WO 08/024,188 and WO 07/024,715.

The disclosure also provides camelid or dromedary antibodies (e.g., antibodies derived from *Camelus bactrianus*, *Camelus dromaderius*, or *Lama paccos*). Such antibodies, unlike the typical two-chain (fragment) or four-chain (whole antibody) antibodies from most mammals, generally lack light chains. See U.S. Pat. No. 5,759,808; Stijlemans et al. (2004) *J Biol Chem* 279:1256-1261; Dumoulin et al. (2003) *Nature* 424:783-788; and Pleschberger et al. (2003) *Bioconjugate Chem* 14:440-448.

Engineered libraries of camelid antibodies and antibody fragments are commercially available, for example, from Ablynx (Ghent, Belgium). As with other antibodies of non-human origin, an amino acid sequence of a camelid antibody can be altered recombinantly to obtain a sequence that more closely resembles a human sequence, i.e., the nanobody can be “humanized” to thereby further reduce the potential immunogenicity of the antibody.

In some embodiments, the present disclosure also provides antibodies, or antigen-binding fragments thereof, which are mutants of mouse monoclonal antibodies 3d8b, 3d9a, 3d29, 3d11, 3d31, 3d3, 3d15, 3d10, and 3d16, or their variant antibodies, or antigen-binding fragments thereof, as described above and therein. Preferably, such a mutant antibody or antigen-binding fragments thereof maintain the C3d binding ability of the parent mouse mAbs. Such mutations and the methods to prepare these mutants are standard practices and well known in the art. In some embodiments, such a mutation introduces at least a single amino acid substitution, deletion, insertion, or other modification. In some embodiments, an antibody or antigen-binding fragment thereof described herein (e.g., mouse monoclonal antibodies 3d8b, 3d9a, 3d29, 3d11, 3d31, 3d3, 3d15, 3d10, or 3d16) comprises no more than 20 (e.g., no more than 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, nine, eight, seven, six, five,

four, three, two, or one) amino acid modifications (e.g., amino acid substitutions, deletions, or additions). In some embodiments, an antibody or antigen-binding fragment thereof described herein does not contain an amino acid modification in its CDRs. In some embodiments, an antibody or antigen-binding fragment thereof described herein does not contain an amino acid modification in the CDR3 of the heavy chain.

Twenty amino acids are commonly found in proteins. Those amino acids can be grouped into nine classes or groups based on the chemical properties of their side chains. Substitution of one amino acid residue for another within the same class or group is referred to herein as a “conservative” substitution. Conservative amino acid substitutions can frequently be made in a protein without significantly altering the conformation or function of the protein. Substitution of one amino acid residue for another from a different class or group is referred to herein as a “non-conservative” substitution. In contrast, non-conservative amino acid substitutions tend to disrupt conformation and function of a protein.

TABLE 1

Example of amino acid classification	
Small/Aliphatic residues:	Gly, Ala, Val, Leu, Ile
Cyclic Imino Acid:	Pro
Hydroxyl-containing Residues:	Ser, Thr
Acidic Residues:	Asp, Glu
Amide Residues:	Asn, Gln
Basic Residues:	Lys, Arg
Imidazole Residue:	His
Aromatic Residues:	Phe, Tyr, Trp
Sulfur-containing Residues:	Met, Cys

In some embodiments, the conservative amino acid substitution comprises substituting any of glycine (G), alanine (A), isoleucine (I), valine (V), and leucine (L) for any other of these aliphatic amino acids; serine (S) for threonine (T) and vice versa; aspartic acid (D) for glutamic acid (E) and vice versa; glutamine (Q) for asparagine (N) and vice versa; lysine (K) for arginine (R) and vice versa; phenylalanine (F), tyrosine (Y) and tryptophan (W) for any other of these aromatic amino acids; and methionine (M) for cysteine (C) and vice versa. Other substitutions can also be considered conservative, depending on the environment of the particular amino acid and its role in the three-dimensional structure of the protein. For example, glycine (G) and alanine (A) can frequently be interchangeable, as can alanine (A) and valine (V). Methionine (M), which is relatively hydrophobic, can frequently be interchanged with leucine and isoleucine, and sometimes with valine. Lysine (K) and arginine (R) are frequently interchangeable in locations in which the significant feature of the amino acid residue is its charge and the differing pKs of these two amino acid residues are not significant. Still other changes can be considered “conservative” in particular environments (see, e.g., *BIOCHEMISTRY* at pp. 13-15, 2nd ed. Lubert Stryer ed. (Stanford University); Henikoff et al., *Proc. Nat'l Acad. Sci. USA* (1992) 89:10915-10919; Lei et al., *J. Biol. Chem.* (1995) 270(20):11882-11886).

In some embodiments, the non-conservative amino acid substitution comprises substituting any of glycine (G), alanine (A), isoleucine (I), valine (V), and leucine (L) for any of serine (S), threonine (T), aspartic acid (D), glutamic acid (E), glutamine (Q), asparagine (N), lysine (K), arginine (R), phenylalanine (F), tyrosine (Y), tryptophan (W), methionine (M), cysteine (C), histidine (H), and proline (P). In some embodiments, the non-conservative amino acid substitution

comprises substituting any of serine (S) and threonine (T) for any of glycine (G), alanine (A), isoleucine (I), valine (V), leucine (L), aspartic acid (D), glutamic acid (E), glutamine (Q), asparagine (N), lysine (K), arginine (R), phenylalanine (F), tyrosine (Y), tryptophan (W), methionine (M), cysteine (C), histidine (H) and proline (P). In some embodiments, the non-conservative amino acid substitution comprises substituting any of aspartic acid (D) and glutamic acid (E) for any of glycine (G), alanine (A), isoleucine (I), valine (V), leucine (L), serine (S), threonine (T), glutamine (Q), asparagine (N), lysine (K), arginine (R), phenylalanine (F), tyrosine (Y), tryptophan (W), methionine (M), cysteine (C), histidine (H), and proline (P). In some embodiments, the non-conservative amino acid substitution comprises substituting any of glutamine (Q) and asparagine (N) for any of glycine (G), alanine (A), isoleucine (I), valine (V), leucine (L), serine (S), threonine (T), aspartic acid (D), glutamic acid (E), lysine (K), arginine (R), phenylalanine (F), tyrosine (Y), tryptophan (W), methionine (M), cysteine (C), histidine (H), and proline (P). In some embodiments, the non-conservative amino acid substitution comprises substituting any of lysine (K) and arginine (R) for any of glycine (G), alanine (A), isoleucine (I), valine (V), leucine (L), serine (S), threonine (T), aspartic acid (D), glutamic acid (E), glutamine (Q), asparagine (N), phenylalanine (F), tyrosine (Y), tryptophan (W), methionine (M), cysteine (C), histidine (H), and proline (P). In some embodiments, the non-conservative amino acid substitution comprises substituting any of phenylalanine (F), tyrosine (Y), and tryptophan (W) for any of glycine (G), alanine (A), isoleucine (I), valine (V), leucine (L), serine (S), threonine (T), aspartic acid (D), glutamic acid (E), glutamine (Q), asparagine (N), lysine (K), arginine (R), methionine (M), cysteine (C), histidine (H), and proline (P). In some embodiments, the non-conservative amino acid substitution comprises substituting any of methionine (M) and cysteine (C) for any of glycine (G), alanine (A), isoleucine (I), valine (V), leucine (L), serine (S), threonine (T), aspartic acid (D), glutamic acid (E), glutamine (Q), asparagine (N), lysine (K), arginine (R), phenylalanine (F), tyrosine (Y), tryptophan (W), histidine (H), and proline (P). In some embodiments, the non-conservative amino acid substitution comprises substituting histidine (H) for any of glycine (G), alanine (A), isoleucine (I), valine (V), leucine (L), serine (S), threonine (T), aspartic acid (D), glutamic acid (E), glutamine (Q), asparagine (N), lysine (K), arginine (R), phenylalanine (F), tyrosine (Y), tryptophan (W), methionine (M), cysteine (C), and proline (P). In some embodiments, the non-conservative amino acid substitution comprises substituting proline (P) for any of glycine (G), alanine (A), isoleucine (I), valine (V), leucine (L), serine (S), threonine (T), aspartic acid (D), glutamic acid (E), glutamine (Q), asparagine (N), lysine (K), arginine (R), phenylalanine (F), tyrosine (Y), tryptophan (W), methionine (M), cysteine (C), and histidine (H).

In some embodiments, the anti-C3d or anti-C3dg antibodies described herein comprise an altered or mutated sequence that leads to altered stability or half-life compared to parent antibodies. This includes, for example, an increased stability or half-life for higher affinity or longer clearance time in vitro or in vivo, or a decreased stability or half-life for lower affinity or quicker removal.

In some embodiments, the anti-C3d or anti-C3dg antibodies described herein comprise an altered heavy chain constant region that has reduced (or no) effector function relative to its corresponding unaltered constant region. That is, in some embodiments, an antibody described herein comprises an altered constant region that exhibits approxi-

mately 0 to 50% (e.g., less than 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1%) of the effector function of the corresponding unaltered (native) form of the constant region. Effector functions involving the constant region of the anti-C3d or anti-C3dg antibody may be modulated by altering properties of the constant or Fc region. Altered effector functions include, for example, a modulation in one or more of the following activities: antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), apoptosis, binding to one or more Fc-receptors, and pro-inflammatory responses. Modulation refers to an increase, decrease, or elimination of an effector function activity exhibited by a subject antibody containing an altered constant region as compared to the activity of the unaltered form of the constant region. In particular embodiments, modulation includes situations in which an activity is abolished or completely absent.

An altered constant region with altered FcR binding affinity and/or ADCC activity and/or altered CDC activity is a polypeptide which has an enhanced or diminished FcR binding activity and/or ADCC activity and/or CDC activity compared to the unaltered form of the constant region. An altered constant region which displays increased binding to an FcR binds at least one FcR with greater affinity than the unaltered polypeptide. An altered constant region which displays decreased binding to an FcR binds at least one FcR with lower affinity than the unaltered form of the constant region. Such variants which display decreased binding to an FcR may possess little or no appreciable binding to an FcR, e.g., 0 to 50% (e.g., less than 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1%) of the binding to the FcR as compared to the level of binding of a native sequence immunoglobulin constant or Fc region to the FcR. Similarly, an altered constant region that displays modulated ADCC and/or CDC activity may exhibit either increased or reduced ADCC and/or CDC activity compared to the unaltered constant region. For example, in some embodiments, the anti-C3d or -C3dg antibody comprising an altered constant region can exhibit approximately 0 to 50% (e.g., less than 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1%) of the ADCC and/or CDC activity of the unaltered form of the constant region. An anti-C3d or -C3dg antibody described herein comprising an altered constant region displaying reduced ADCC and/or CDC may exhibit reduced or no ADCC and/or CDC activity as exemplified herein.

In some embodiments, the altered constant region has at least one amino acid substitution, insertion, and/or deletion, compared to a native sequence constant region or to the unaltered constant region, e.g., from about one to about one hundred amino acid substitutions, insertions, and/or deletions in a native sequence constant region or in the constant region of the parent polypeptide. In some embodiments, the altered constant region herein will possess at least about 70% homology (similarity) or identity with the unaltered constant region and in some instances at least about 75% and in other instances at least about 80% homology or identity therewith, and in other embodiments at least about 85%, 90% or 95% homology or identity therewith. The altered constant region may also contain one or more amino acid deletions or insertions. Additionally, the altered constant region may contain one or more amino acid substitutions,

deletions, or insertions that result in altered post-translational modifications, including, for example, an altered glycosylation pattern (e.g., the addition of one or more sugar components, the loss of one or more sugar components, or a change in composition of one or more sugar components relative to the unaltered constant region).

Antibodies with altered or no effector functions may be generated by engineering or producing antibodies with variant constant, Fc, or heavy chain regions; recombinant DNA technology and/or cell culture and expression conditions may be used to produce antibodies with altered function and/or activity. For example, recombinant DNA technology may be used to engineer one or more amino acid substitutions, deletions, or insertions in regions (such as, for example, Fc or constant regions) that affect antibody function including effector functions. Alternatively, changes in post-translational modifications, such as, e.g., glycosylation patterns, may be achieved by manipulating the cell culture and expression conditions by which the antibody is produced. Suitable methods for introducing one or more substitutions, additions, or deletions into an Fc region of an antibody are well known in the art and include, e.g., standard DNA mutagenesis techniques as described in, e.g., Sambrook et al. (1989) "Molecular Cloning: A Laboratory Manual, 2nd Edition," Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Harlow and Lane (1988), supra; Borrebaek (1992), supra; Johne et al. (1993), supra; PCT publication no. WO 06/53301; and U.S. Pat. No. 7,704,497.

In some embodiments, an anti-C3d or anti-C3dg antibody described herein exhibits reduced or no effector function. In some embodiments, an anti-C3d or anti-C3dg antibody comprises a hybrid constant region, or a portion thereof, such as a G2/G4 hybrid constant region (see e.g., Burton et al. (1992) *Adv Immun* 51:1-18; Canfield et al. (1991) *J Exp Med* 173:1483-1491; and Mueller et al. (1997) *Mol Immunol* 34(6):441-452). See above.

In addition to using a G2/G4 construct as described above, an anti-C3d or anti-C3dg antibody described herein having reduced effector function may be produced by introducing other types of changes in the amino acid sequence of certain regions of the antibody. Such amino acid sequence changes include but are not limited to the Ala-Ala mutation described in, e.g., PCT Publication nos. WO 94/28027 and WO 98/47531; and Xu et al. (2000) *Cell Immunol* 200:16-26. Thus, in some embodiments, an anti-C3d or -C3dg antibody with one or more mutations within the constant region including the Ala-Ala mutation has reduced or no effector function. According to these embodiments, the constant region of the antibody can comprise a substitution to an alanine at position 234 or a mutation to an alanine at position 235. Additionally, the altered constant region may contain a double mutation: a mutation to an alanine at position 234 and a second mutation to an alanine at position 235. In some embodiments, an anti-C3d or anti-C3dg antibody comprises an IgG4 framework, wherein the Ala-Ala mutation would describe a mutation(s) from phenylalanine to alanine at position 234 and/or a mutation from leucine to alanine at position 235. In some embodiments, the anti-C3d or anti-C3dg antibody comprises an IgG1 framework, wherein the Ala-Ala mutation would describe a mutation(s) from leucine to alanine at position 234 and/or a mutation from leucine to alanine at position 235. An anti-C3d or anti-C3dg antibody may alternatively or additionally carry other mutations, including the point mutation K322A in the CH2 domain (Hezareh et al. (2001) *J Virol* 75:12161-12168). An antibody

with said mutation(s) in the constant region may furthermore be a blocking or non-blocking antibody.

Additional substitutions that, when introduced into a heavy chain constant region, result in decreased effector function are set forth in, e.g., Shields et al. (2001) *J Biol Chem* 276(9):6591-6604. See particularly Table 1 ("Binding of human IgG1 variants to human FcRn and FcγR") of Shields et al., the disclosure of which is incorporated herein by reference in its entirety. By screening a library of anti-IgE antibodies, each antibody of the library differing by one or more substitutions in the heavy chain constant region, for binding to a panel of Fc receptors (including FcRn, FcγRI, FcγRIIA, FcγRIIB, and FcγRIIA), the authors identified a number of substitutions that modulate specific Fc-Fc receptor interactions. For example, a variant IgG2a heavy chain constant region in which the CH2 domain contains a D265A substitution (heavy chain amino acid numbering according to Kabat et al. (supra)) results in a complete loss of interaction between the variant constant region and IgG Fc receptors FcγRIIB, FcγRIII, FcγRI, and FcγRIV. Shields et al. (2001) at page 6595, Table 1. See also Baudino et al. (2008) *J Immunol* 181:6664-6669 (supra).

Changes within the hinge region also affect effector functions. For example, deletion of the hinge region may reduce affinity for Fc receptors and may reduce complement activation (Klein et al. (1981) *Proc Natl Acad Sci USA* 78:524-528). The present disclosure therefore also relates to antibodies with alterations in the hinge region.

In some embodiments, an anti-C3d or anti-C3dg antibody may contain an altered constant region exhibiting enhanced or reduced complement dependent cytotoxicity (CDC). Modulated CDC activity may be achieved by introducing one or more amino acid substitutions, insertions, or deletions in an Fc region of the antibody. See, e.g., U.S. Pat. No. 6,194,551. Alternatively or additionally, cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved or reduced internalization capability and/or increased or decreased complement-mediated cell killing. See, e.g., Caron et al. (1992) *J Exp Med* 176:1191-1195 and Shopes (1992) *Immunol* 148:2918-2922; PCT publication nos. WO 99/51642 and WO 94/29351; Duncan and Winter (1988) *Nature* 322:738-40; and U.S. Pat. Nos. 5,648,260 and 5,624,821.

Another potential means of modulating effector function of antibodies includes changes in glycosylation, which is summarized in, e.g., Raju (2003) *BioProcess International* 1(4):44-53. According to Wright and Morrison, the microheterogeneity of human IgG oligosaccharides can affect biological functions such as CDC and ADCC, binding to various Fc receptors, and binding to C1q protein. (1997) *TIBTECH* 15:26-32. Glycosylation patterns of antibodies can differ depending on the producing cell and the cell culture conditions (Raju, supra). Such differences can lead to changes in both effector function and pharmacokinetics. See, e.g., Israel et al. (1996) *Immunology* 89(4):573-578; Newkirk et al. (1996) *Clin Exp Immunol* 106(2):259-264. Differences in effector function may be related to the IgG's ability to bind to the Fcγ receptors (FcγRs) on the effector cells. Shields et al. have shown that IgG, with alterations in amino acid sequence that have improved binding to FcγR, can exhibit up to 100% enhanced ADCC using human effector cells. (2001) *J Biol Chem* 276(9):6591-6604. While these alterations include changes in amino acids not found at the binding interface, both the nature of the sugar component as well as its structural pattern may also contribute to the

differences observed. In addition, the presence or absence of fucose in the oligosaccharide component of an IgG can improve binding and ADCC. See, e.g., Shields et al. (2002) *J Biol Chem* 277(30):26733-26740. An IgG that lacked a fucosylated carbohydrate linked to Asn297 exhibited normal receptor binding to the FcγRI receptor. In contrast, binding to the FcγRIIIA receptor was improved 50-fold and accompanied by enhanced ADCC, especially at lower antibody concentrations.

Shinkawa et al. demonstrated that an antibody to the human IL-5 receptor produced in a rat hybridoma showed more than 50% higher ADCC when compared to the antibody produced in Chinese hamster ovary cells (CHO) (Shinkawa et al. (2003) *J Biol Chem* 278(5):3466-73). Monosaccharide composition and oligosaccharide profiling showed that the rat hybridoma-produced IgG had a lower content of fucose than the CHO-produced protein. The authors concluded that the lack of fucosylation of an IgG1 has a critical role in enhancement of ADCC activity.

A different approach was taken by Umana et al. who changed the glycosylation pattern of chCE7, a chimeric IgG1 anti-neuroblastoma antibody. (1999) *Nat Biotechnol* 17(2):176-180). Using tetracycline, they regulated the activity of a glycosyltransferase enzyme (GnTIII) which bisects oligosaccharides that have been implicated in ADCC activity. The ADCC activity of the parent antibody was barely above background level. Measurement of ADCC activity of the chCE7 produced at different tetracycline levels showed an optimal range of GnTIII expression for maximal chCE7 in vitro ADCC activity. This activity correlated with the level of constant region-associated, bisected complex oligosaccharide. Newly optimized variants exhibited substantial ADCC activity. Similarly, Wright and Morrison produced antibodies in a CHO cell line deficient in glycosylation and showed that antibodies produced in this cell line were incapable of complement-mediated cytotoxicity. (1994) *J Exp Med* 180:1087-1096. Thus, as known alterations that affect effector function include modifications in the glycosylation pattern or a change in the number of glycosylated residues, the present disclosure relates to an anti-C3d or -C3dg antibody wherein glycosylation is altered to either enhance or decrease effector function(s) including ADCC and CDC. Altered glycosylation includes a decrease or increase in the number of glycosylated residues as well as a change in the pattern or location of glycosylated residues.

Still other approaches exist for altering the effector function of antibodies. For example, antibody-producing cells can be hypermutagenic, thereby generating antibodies with randomly altered polypeptide residues throughout an entire antibody molecule. See, e.g., PCT publication no. WO 05/011735. Hypermutagenic host cells include cells deficient in DNA mismatch repair. Antibodies produced in this manner may be less antigenic and/or have beneficial pharmacokinetic properties. Additionally, such antibodies may be selected for properties such as enhanced or decreased effector function(s). Additional details of molecular biology techniques useful for preparing an antibody or antigen-binding fragment thereof described herein are set forth below.

Fusion Molecules

The present disclosure also provides compositions containing a fusion molecule comprising a targeting moiety and an active prophylactic, therapeutic, or diagnostic moiety.

In some embodiments, the fusion molecules described herein are able to specifically deliver their active prophylactic, therapeutic, or diagnostic moiety or portion to a surface (e.g., cell surface, antigen surface, etc.) through the

specific binding of the targeting moiety or portion to a binding partner on such surface. The targeting moiety is preferably one which binds to a binding partner, which is a complement component protein and is preferably selected from the group consisting of C3dg, C3d, and CR2. Suitable targeting moieties can be small molecular compounds, peptides, proteins, or other moieties known in the art. Suitable targeting moieties include antibodies which bind a binding partner selected from the group consisting of C3dg and C3d; or fragments of such antibodies which retain the ability to bind to its respective binding partner. Suitable targeting moieties also include the antibodies of the present invention, or fragments thereof which retain the ability to bind to their respective binding partner. Examples of such targeting moieties or portions are discussed above.

In some embodiments, a fusion molecule is targeted for increased association to tissue that has been injured, damaged or has become inflamed by physical, chemical or other insult or injury. Targeting can be accomplished by tethering, fusing or otherwise associating an active agent to a targeting moiety. In some embodiments, the targeting moiety will bind to tissue-associated complement component 3 (C3) or one or more fragments of C3, including, but not limited to: C3b, iC3b, C3d and C3dg. In some embodiments, the targeting moieties include, for example, monoclonal antibodies to C3, C3b, iC3b, C3d, C3dg, or other fragments of C3, or fragments of such monoclonal antibodies that retain the ability to bind to their respective binding partner. In some embodiments, the targeting moieties can be monoclonal antibodies to iC3b, C3dg and C3d, as illustrated by the 3d9a, 3d29 and 3d8b antibodies. It is also possible to use non-complement regulatory fragments of Factor H that retain the ability to bind to one or more fragments of C3, including C3b, iC3b and C3d or other tissue-associated fragments of C3. These fragments of factor H potentially include fragments comprising SCR domains 5-8 and SCR domains 19-20.

In some embodiments, the fusion molecule described herein is designed to synergize two or more effects in a subject, preferably, a mammal. In some embodiments, the targeting moiety or portion, through its binding to its binding partner, has an in vitro and/or in vivo activity for stabilizing or modulating one target, activity, pathway, or mechanism which is different from the one stabilized or modulated by the active prophylactic, therapeutic, or diagnostic moiety or portion in the same fusion molecule. In some embodiments, the targeting moiety or portion may be fused to more than one active prophylactic, therapeutic, or diagnostic moiety or portion, each of which may have different activity for stabilizing or modulating different targets, activities, pathways, or mechanisms. In any case, the multiple functions of different moieties or portions of the fusion molecule described herein may lead to a synergy or better effect for this fusion molecule.

Active Prophylactic, Therapeutic, or Diagnostic Moiety

In some embodiments, the present disclosure provides an active prophylactic, therapeutic, or diagnostic moiety to be fused to the targeting moiety in the fusion molecule described herein. In some embodiments, an active prophylactic, therapeutic, or diagnostic moiety is targeted or presented to the near space of the binding partner recognized by the targeting moiety. In some embodiments, such targeting moiety is a binding molecule for a complement component protein, as discussed above. In some embodiments, such targeting moiety is an antibody to C3d or C3dg. In some embodiments, such active prophylactic, therapeutic, or diagnostic moiety is a complement modulator. While the disclo-

sure is in no way limited by one particular theory or mechanism of action, the inventors assert that through binding to the complement component protein, e.g., C3d or C3dg, by the targeting moiety, the active prophylactic, therapeutic, or diagnostic moiety, preferably, a complement modulator, is presented closely to the surface of the target, where it can function more effectively because of the increased local concentration of itself and other complement components modulated by it.

As used herein, the term "complement modulator" refers to a compound, composition, or protein that modulates (e.g., inhibits or activates) complement activity. A complement modulator can be a complement inhibitor or a complement activator. As used herein, the term "complement inhibitor" refers to any compound, composition, or protein that reduces or eliminates complement activity. The reduction in complement activity may be incremental (e.g., a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% reduction in activity) or complete. For example, in some embodiments, a complement inhibitor can inhibit complement activity by at least 10 (e.g., at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, or 95 or greater) % in a standard in vitro red blood cell hemolysis assay or an in vitro CH50eq assay. See, e.g., Kabat and Mayer (eds), "Experimental Immunochimistry, 2nd Edition," 135-240, Springfield, Ill., CC Thomas (1961), pages 135-139, or a conventional variation of that assay such as the chicken erythrocyte hemolysis method as described in, e.g., Hillmen et al. (2004) *N Engl J Med* 350(6):552.

The CH50eq assay is a method for measuring the total classical complement activity in serum. This test is a lytic assay, which uses antibody-sensitized erythrocytes as the activator of the classical complement pathway and various dilutions of the test serum to determine the amount required to give 50% lysis (CH50). The percent hemolysis can be determined, for example, using a spectrophotometer. The CH50eq assay provides an indirect measure of terminal complement complex (TCC) formation, since the TCC themselves are directly responsible for the hemolysis that is measured.

The assay is well known and commonly practiced by those of skill in the art. Briefly, to activate the classical complement pathway, undiluted serum samples (e.g., human serum samples) are added to microassay wells containing the antibody-sensitized erythrocytes to thereby generate TCC. Next, the activated sera are diluted in microassay wells, which are coated with a capture reagent (e.g., an antibody that binds to one or more components of the TCC). The TCC present in the activated samples bind to the monoclonal antibodies coating the surface of the microassay wells. The wells are washed and, to each well, is added a detection reagent that is detectably labeled and recognizes the bound TCC. The detectable label can be, e.g., a fluorescent label or an enzymatic label. The assay results are expressed in CH50 unit equivalents per milliliter (CH50 U Eq/mL).

Additional methods for detecting and/or measuring complement activity in vitro are set forth and exemplified in the working examples.

A complement inhibitor may be a soluble or membrane-bound protein such as, for example, membrane cofactor protein (MCP), decay accelerating factor (DAF/CD55), CD59, mouse complement receptor 1-related gene/protein y (Crry), human complement receptor 1 (CR1) or factor H, or an antibody specific for a component of a complement pathway such as, for example, eculizumab (an anti-05 antibody marketed under the trade name Soliris), pexeli-

zumab (the antigen-binding fragment of eculizumab), an anti-factor B antibody (such as the monoclonal antibody 1379 produced by ATCC Deposit No. PTA-6230), an anti-properdin antibody, an anti-factor D antibody, and the like (see below). Alternatively, a complement inhibitor may be a small molecule or a linear or cyclic peptide such as, for example, compstatin, N-acetylaspartylglutamic acid (NAAGA), and the like. As used herein the term "complement activator" refers to any compound, composition, or protein that increases or activates complement activity. The increase in complement activity may be incremental (e.g., a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% increase in activity). A complement activator may be a soluble or membrane-bound protein such as, for example, human Ig isotype G₁ (IgG₁), human Ig isotype M (IgM), mouse Ig isotype G₃ (IgG₃), and mouse IgM Fc, as well as cobra venom factor (CVF) and biologically-active fragments thereof, such as the Fc domain of Ig proteins, such as human IgG₁ Fc domain, human IgM Fc domain, mouse IgG₃ Fc domain, and mouse IgM Fc domain. Complement activators may also include, for example, hybrid CVF molecules comprising a CVF portion and a complement component 3 (C3) portion, such as those described in Fritzing et al., "Functional characterization of human C3/cobra venom factor hybrid proteins for therapeutic complement depletion," *Develop. Comp. Immunol.* 33(1):105-116 (2009). Those hybrids comprise proteins in which the 113 or 315 C-terminal residues of C3 were replaced with corresponding CVF sequences.

A number of endogenous soluble and membrane-bound proteins that inhibit complement have been identified. These complement inhibitor proteins include, but are not limited to, membrane cofactor protein (MCP), decay accelerating factor (DAF/CD55), CD59, mouse complement receptor 1-related gene/protein y (Crry), human complement receptor 1 (CR1) and factor H.

As used herein, the term "membrane cofactor protein," "MCP," or "CD46" refers to a widely distributed C3b/C4b-binding cell surface glycoprotein which inhibits complement activation on host cells and serves as a cofactor for the factor I-mediated cleavage of C3b and C4b, including homologs thereof. T. J. Oglesby et al., *J. Exp. Med.* (1992) 175:1547-1551. MCP belongs to a family known as the regulators of complement activation ("RCA"). Family members share certain structural features, comprising varying numbers of short consensus repeat (SCR) domains, which are typically between 60 and 70 amino acids in length. Beginning at its amino-terminus, MCP comprises four SCRs, a serine/threonine/proline-enriched region, an area of undefined function, a transmembrane hydrophobic domain, a cytoplasmic anchor and a cytoplasmic tail. It is understood that species and strain variations exist for the disclosed peptides, polypeptides, and proteins, and that human MCP or biologically active fragments thereof encompass all species and strain variations.

SEQ ID NO:1 represents the full-length human MCP amino acid sequence (see, e.g., UniProtKB/Swiss-Prot. Accession No. P15529). Amino acids 1-34 correspond to the signal peptide, amino acids 35-343 correspond to the extracellular domain, amino acids 344-366 correspond to the transmembrane domain, and amino acids 367-392 correspond to the cytoplasmic domain. In the extracellular domain, amino acids 35-96 correspond to SCR 1, amino acids 97-159 correspond to SCR 2, amino acids 160-225 correspond to SCR 3, amino acids 226-285 correspond to SCR 4, and amino acids 302-326 correspond to the serine/threonine-rich domain. It is understood that species and

strain variations exist for the disclosed peptides, polypeptides, and proteins, and that MCP or biologically active fragments thereof encompass all species and strain variations. As used herein, the term “biologically active” fragment of MCP refers to any soluble fragment lacking both the cytoplasmic domain and the transmembrane domain, including fragments comprising, consisting essentially of or consisting of 1, 2, 3, or 4 SCR domains, with or without the serine/threonine-rich domain, having some or all of the complement inhibitory activity of the full-length human MCP protein. In some embodiments, the complement inhibitor portion comprises full-length human MCP (amino acids 35-392 of SEQ ID NO:1), the extracellular domain of human MCP (amino acids 35-343 of SEQ ID NO:1), or SCRs 1-4 of human MCP (amino acids 35-285 of SEQ ID NO:1).

Decay accelerating factor, also referred to as CD55 (DAF/CD55) (SEQ ID NO:2 and SEQ ID NO:3), is an ~70 kiloDalton (kDa) membrane-bound glycoprotein which inhibits complement activation on host cells. Like several other complement regulatory proteins, DAF comprises several approximately 60 amino acid repeating motifs termed short consensus repeats (SCR).

As used herein, the term “decay accelerating factor,” “DAF,” or “CD55” refers to a seventy kilodalton (“kDa”) membrane glycoprotein comprising four short consensus repeat (SCR) domains followed by a heavily O-glycosylated serine/threonine-rich domain at the C-terminus that elevates the molecule from the membrane surface, followed by a glycosylphosphatidylinositol (“GPI”) anchor. DAF protects the cell surface from complement activation by dissociating membrane-bound C3 convertases that are required to cleave complement protein C3 and to amplify the complement cascade. DAF prevents assembly or accelerates decay of both the C3- and C5-convertases of the alternative and classical complement pathways.

SEQ ID NO:2 represents the full-length human DAF amino acid sequence (see, e.g., UniProtKB/Swiss-Prot. Accession No. P08173); SEQ ID NO:3 represents the full-length mouse DAF amino acid sequence (see, e.g., UniProtKB/Swiss-Prot. Accession No. Q61475). In the human DAF sequence, amino acids 1-34 correspond to the signal peptide, amino acids 35-353 appear in the mature protein, and amino acids 354-381 are removed from the polypeptide after translation. Within the mature protein, amino acids 35-96 correspond to SCR 1, amino acids 96-160 correspond to SCR 2, amino acids 161-222 correspond to SCR 3, amino acids 223-285 correspond to SCR 4, and amino acids 287-353 correspond to the O-glycosylated serine/threonine-rich domain. The GPI anchor is attached to human DAF at a serine at position 353. In the mouse DAF sequence, amino acids 1-34 correspond to the signal peptide, amino acids 35-362 appear in the mature protein, and amino acids 363-390 are removed from the polypeptide after translation. Within the mature protein, amino acids 35-96 correspond to SCR 1, amino acids 97-160 correspond to SCR 2, amino acids 161-222 correspond to SCR 3, amino acids 223-286 correspond to SCR 4, and amino acids 288-362 correspond to the O-glycosylated serine/threonine-rich domain. The GPI anchor is attached to mouse DAF at a serine at position 362. It is understood that species and strain variations exist for the disclosed peptides, polypeptides, and proteins, and that DAF or biologically active fragments thereof encompass all species and strain variations. As used herein, the term “biologically active” fragment of DAF refers to any fragment of DAF lacking a GPI anchor and/or the amino acid to which it is attached (i.e., Ser-353), including any fragments of the full-length DAF protein comprising, con-

sisting essentially of or consisting of 1, 2, 3, or 4 SCR domains, with or without the O-glycosylated serine/threonine-rich domain, having some or all the complement inhibitory activity of the full-length DAF protein.

As used herein, the term “CD59” refers to a membrane-bound 128 amino acid glycoprotein that potently inhibits the membrane attack complex (MAC) of complement. CD59 acts by binding to the C8 and/or C9 components of the MAC during assembly, ultimately preventing incorporation of the multiple copies of C9 required for complete formation of the osmolytic pore at the heart of the MAC. CD59 is both N- and O-glycosylated. The N-glycosylation comprises primarily bi- or tri-antennary structures with and without lactosamine and outer arm fucose residues, with variable sialylation present at some sites. Like DAF, CD59 is anchored in the cell membrane by a glycosylphosphatidylinositol (“GPI”) anchor, which is attached to an asparagine at amino acid 102. Soluble forms of CD59 (sCD59) have been produced, but they generally have low functional activity in vitro, particularly in the presence of serum, suggesting that unmodified sCD59 has little or no therapeutic efficacy. See, e.g., S. Meri et al., “Structural composition and functional characterization of soluble CD59: heterogeneity of the oligosaccharide and glycosylphosphoinositol (GPI) anchor revealed by laser-desorption mass spectrometric analysis,” *Biochem. J.*:923-935 (1996).

SEQ ID NO:4 represents the full-length human CD59 amino acid sequence (see, e.g., UniProtKB/Swiss-Prot. Accession No. P13987); SEQ ID NO:5 represents the full-length mouse CD59 sequence, isoform A (see, e.g., UniProtKB/Swiss-Prot. Accession No. 055186); SEQ ID NO:6 represents the full-length mouse CD59 sequence, isoform B (see, e.g., UniProtKB/Swiss-Prot. Accession No. P58019). In the human CD59 sequence, amino acids 1-25 of SEQ ID NO:4 correspond to the leader peptide, amino acids 26-102 of SEQ ID NO:4 correspond to the mature protein, and amino acids 103-128 of SEQ ID NO:4 are removed after translation. The GPI anchor is attached to CD59 at an asparagine at position 102 of SEQ ID NO:4. In isoform A of the mouse CD59 sequence, amino acids 1-23 of SEQ ID NO:5 correspond to the leader peptide, amino acids 24-96 of SEQ ID NO:5 correspond to the mature protein, and amino acids 97-123 of SEQ ID NO:5 are removed after translation. The GPI anchor is attached to CD59 at a serine at position 96 of SEQ ID NO:5. In isoform B of the mouse CD59 sequence, amino acids 1-23 of SEQ ID NO:6 correspond to the leader peptide, amino acids 24-104 of SEQ ID NO:6 correspond to the mature protein, and amino acids 105-129 of SEQ ID NO:6 are removed after translation. The GPI anchor is attached to CD59 at an asparagine at position 104 of SEQ ID NO:6. It is understood that species and strain variations exist for the disclosed peptides, polypeptides, and proteins, and that CD59 or biologically active fragments thereof encompass all species and strain variations.

As used herein, the term “biologically active” fragment of human CD59 refers to any fragment of human CD59 lacking a GPI anchor and/or the amino acid to which it is attached (i.e., Asn-102), including any fragments of the full-length human CD59 protein having some or all the complement inhibitory activity of the full-length CD59 protein; and the term “biologically active” fragment of mouse CD59 refers to any fragment of mouse CD59 isoform A or isoform B lacking a GPI anchor and/or the amino acid to which it is attached (i.e., Ser-96 of isoform A, or Asp-104 of isoform B), including any fragments of either full-length mouse CD59 protein isoform having some or all the complement inhibitory activity of the full-length CD59 protein.

As used herein, the term “mouse complement receptor 1-related gene/protein y” or “Crry” refers to a membrane-bound mouse glycoprotein that regulates complement activation, including homologs thereof. Crry regulates complement activation by serving as a cofactor for complement factor I, a serine protease which cleaves C3b and C4b deposited on host tissue. Crry also acts as a decay-accelerating factor, preventing the formation of C4b2a and C3bBb, the amplification convertases of the complement cascade.

SEQ ID NO:7 represents the full-length mouse Crry protein amino acid sequence. Amino acids 1-40 correspond to the leader peptide, amino acids 41-483 of SEQ ID NO:7 correspond to the mature protein, comprising amino acids 41-405 of SEQ ID NO:7, corresponding to the extracellular domain, amino acids 406-426 of SEQ ID NO:7, corresponding to the transmembrane domain, and amino acids 427-483 of SEQ ID NO:7, corresponding to the cytoplasmic domain. In the extracellular domain, amino acids 83-143 of SEQ ID NO:7 correspond to SCR 1, amino acids 144-205 of SEQ ID NO:7 correspond to SCR 2, amino acids 206-276 of SEQ ID NO:7 correspond to SCR 3, amino acids 277-338 of SEQ ID NO:7 correspond to SCR 4, and amino acids 339-400 of SEQ ID NO:7 correspond to SCR 5. It is understood that species and strain variations exist for the disclosed peptides, polypeptides, and proteins, and that mouse Crry protein or biologically active fragments thereof encompasses all species and strain variations. As used herein, the term “biologically active” fragment of mouse Crry protein refers to any soluble fragment of mouse Crry lacking the transmembrane domain and the cytoplasmic domain, including fragments comprising, consisting essentially of or consisting of 1, 2, 3, 4, or 5 SCR domains, including any fragments of the full-length mouse Crry protein having some or all the complement inhibitory activity of the full-length Crry protein.

As used herein, the term “complement receptor 1,” “CR1,” or “CD35” refers to a human gene encoding a protein of 2039 amino acids, with a predicted molecular weight of 220 kilodaltons (“kDa”), including homologs thereof. The gene is expressed principally on erythrocytes, monocytes, neutrophils, and B cells, but is also present on some T lymphocytes, mast cells, and glomerular podocytes. CR1 protein is typically expressed at between 100 and 1000 copies per cell. CR1 is the main system for processing and clearance of complement-opsonized immune complexes. CR1 negatively regulates the complement cascade, mediates immune adherence and phagocytosis, and inhibits both the classic and alternative complement pathways. The full-length CR1 protein comprises a 42 amino acid signal peptide, an extracellular domain of 1930 amino acids, a 25 amino acid transmembrane domain, and a 43 amino acid C-terminal cytoplasmic domain. The extracellular domain of CR1 has 25 potential N-glycosylation signal sequences, and comprises 30 short consensus (“SCR”) domains, also known as complement control protein (CCP) repeats, or sushi domains, each 60 to 70 amino acids long. The sequence homology between SCRs ranges between 60-99 percent. The 30 SCR domains are further grouped into four longer regions termed long homologous repeats (“LHRs”), each encoding approximately 45 kDa segments of the CR1 protein, designated LHR-A, -B, -C, and -D. The first three comprise seven SCR domains each, while LHR-D comprises 9 SCR domains. The active sites on the extracellular domain of CR1 protein include a C4b-binding site with lower affinity for C3b in SCRs 1-4 comprising amino acids 42-295, a C3b-binding site with lower affinity for C4b in SCRs 8-11 comprising amino acids 490-745, a C3b-binding

site with lower affinity for C4b in SCRs 15-18 comprising amino acids 940-1196, and a C1q-binding site in SCRs 22-28 comprising amino acids 1394-1842.

SEQ ID NO:8 represents the full-length human CR1 amino acid sequence (see, e.g., UniProtKB/Swiss-Prot. Accession No. P17927). Amino acids 1-41 correspond to the signal peptide, amino acids 42-2039 correspond to the mature protein, comprising amino acids 42-1971, corresponding to the extracellular domain, amino acids 1972-1996, corresponding to the transmembrane domain, and amino acids 1997-2039, corresponding to the cytoplasmic domain. In the extracellular domain, amino acids 42-101 correspond to SCR 1, 102-163 correspond to SCR2, amino acids 164-234 correspond to SCR3, amino acids 236-295 correspond to SCR4, amino acids 295-355 correspond to SCRS, amino acids 356-418 correspond to SCR6, amino acids 419-489 correspond to SCR7, amino acids 491-551 correspond to SCR8, amino acids 552-613 correspond to SCR9, amino acids 614-684 correspond to SCR10, amino acids 686-745 correspond to SCR11, amino acids 745-805 correspond to SCR12, amino acids 806-868 correspond to SCR13, amino acids 869-939 correspond to SCR14, amino acids 941-1001 correspond to SCR15, amino acids 1002-1063 correspond to SCR16, amino acids 1064-1134 correspond to SCR17, amino acids 1136-1195 correspond to SCR18, amino acids 1195-1255 correspond to SCR 19, amino acids 1256-1318 correspond to SCR 20, amino acids 1319-1389 correspond to SCR 21, amino acids 1394-1454 correspond to SCR 22, amino acids 1455-1516 correspond to SCR 23, amino acids 1517-1587 correspond to SCR 24, amino acids 1589-1648 correspond to SCR 25, amino acids 1648-1708 correspond to SCR 26, amino acids 1709-1771 correspond to SCR 27, amino acids 1772-1842 correspond to SCR 28, amino acids 1846-1906 correspond to SCR 29, amino acids 1907-1967 correspond to SCR 30. It is understood that species and strain variations exist for the disclosed peptides, polypeptides, and proteins, and that CR1 protein or biologically active fragments thereof encompass all species and strain variations. As used herein, the term “biologically active” fragment of CR1 protein refers to any soluble fragment of CR1 lacking the transmembrane domain and the cytoplasmic domain, including fragments comprising, consisting essentially of or consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 SCR domains, including any fragments of the full-length CR1 protein having some or all the complement inhibitory activity of the full-length CR1 protein.

As used herein, the term “complement factor H,” “factor H,” or “FH” refers to complement factor H, a single polypeptide chain plasma glycoprotein, including homologs thereof. The protein is composed of 20 conserved short consensus repeat (SCR) domains of approximately 60 amino acids, arranged in a continuous fashion like a string of beads, separated by short linker sequences of 2-6 amino acids each. Factor H binds to C3b, accelerates the decay of the alternative pathway C3-convertase (C3bBb), and acts as a cofactor for the proteolytic inactivation of C3b. In the presence of factor H, proteolysis by factor I results in the cleavage and inactivation of C3b. Factor H has at least three distinct binding domains for C3b, which are located within SCRs 1-4, SCRs 5-8, and SCRs 19-20. Each domain binds to a distinct region within the C3b protein: the N-terminal sites bind to native C3b; the second site, located in the middle region of factor H, binds to the C3c fragment and the site located within SCR19 and 20 binds to the C3d region. In addition, factor H also contains binding sites for heparin,

which are located within SCR 7, SCRs 5-12, and SCR 20 of factor H and overlap with those of the C3b binding sites. Structural and functional analyses have shown that the domains for the complement inhibitory activity of factor H are located within the first four N-terminal SCR domains.

SEQ ID NO:9 represents the full-length human factor H amino acid sequence (see, e.g., UniProtKB/Swiss-Prot. Accession No. P08603); SEQ ID NO:10 represents the full-length mouse factor H amino acid sequence (see, e.g., UniProtKB/Swiss-Prot. Accession No. P06909). In the human factor H sequence, amino acids 1-18 of SEQ ID NO:9 correspond to the signal peptide, and amino acids 19-1231 of SEQ ID NO:9 correspond to the mature protein. Within that protein, amino acids 21-80 of SEQ ID NO:9 correspond to SCR 1, amino acids 85-141 of SEQ ID NO:9 correspond to SCR 2, amino acids 146-205 of SEQ ID NO:9 correspond to SCR 3, amino acids 210-262 of SEQ ID NO:9 correspond to SCR 4, and amino acids 267-320 of SEQ ID NO:9 correspond to SCR 5. In the mouse factor H sequence, amino acids 1-18 of SEQ ID NO:10 correspond to the signal peptide, and amino acids 19-1234 of SEQ ID NO:10 correspond to the mature protein. Within that protein, amino acids 19-82 of SEQ ID NO:10 correspond to SCR 1, amino acids 83-143 of SEQ ID NO:10 correspond to SCR 2, amino acids 144-207 of SEQ ID NO:10 correspond to SCR 3, amino acids 208-264 of SEQ ID NO:10 correspond to SCR 4, and amino acids 265-322 of SEQ ID NO:10 correspond to SCR 5. It is understood that species and strain variations exist for the disclosed peptides, polypeptides, and proteins, and that factor H or biologically active fragments thereof encompass all species and strain variations.

As used herein, the term "biologically active" fragment of factor H refers to any portion of a factor H protein having some or all the complement inhibitory activity of the full-length factor H protein, and includes, but is not limited to, factor H fragments comprising SCRs 1-4, SCRs 1-5, SCRs 1-8, SCRs 1-18, SCRs 19-20, or any homolog of a naturally-occurring factor H or fragment thereof, as described in detail below. In some embodiments, the biologically active fragment of factor H has one or more of the following properties: (1) binding to C-reactive protein (CRP), (2) binding to C3b, (3) binding to heparin, (4) binding to sialic acid, (5) binding to endothelial cell surfaces, (6) binding to cellular integrin receptor, (7) binding to pathogens, (8) C3b co-factor activity, (9) C3b decay-acceleration activity, and (10) inhibiting the alternative complement pathway.

In some embodiments, the complement modulator portion of the construct comprises a complement inhibitor or biologically active fragment thereof. In some embodiments, the complement inhibitor is selected from the group consisting of human MCP, human DAF, mouse DAF, human CD59, mouse CD59 isoform A, mouse CD59 isoform B, mouse Crry protein, human CR1, human factor H, or mouse factor H, or a biologically active fragment thereof.

In some embodiments, the complement inhibitor portion of the construct comprises full-length human MCP (SEQ ID NO:1). In some embodiments, the complement inhibitor portion of the construct comprises a biologically active fragment of human MCP (SEQ ID NO:1). In some embodiments, the biologically active fragment of human MCP is selected from the group consisting of SCRs 1-4 (amino acids 35-285 of SEQ ID NO:1), SCRs 1-4 plus the serine/threonine-rich domain (amino acids 35-326 of SEQ ID NO:1), and the extracellular domain of MCP (amino acids 35-343 of SEQ ID NO:1).

In some embodiments, the complement inhibitor portion of the construct comprises full-length human DAF. In some

embodiments, the complement inhibitor portion of the construct comprises a biologically active fragment of human DAF (SEQ ID NO:2). In some embodiments, the biologically active fragment of human DAF is selected from the group consisting of SCRs 1-4 (amino acids 25-285 of SEQ ID NO:2) and SCRs 1-4 plus the O-glycosylated serine/threonine-rich domain (amino acids 25-353 of SEQ ID NO:2). In some embodiments, the complement inhibitor portion of the construct comprises full-length mouse DAF (SEQ ID NO:3). In some embodiments, the complement inhibitor portion of the construct comprises a biologically active fragment of mouse DAF. In some embodiments, the biologically active fragment of mouse DAF is selected from the group consisting of SCRs 1-4 (amino acids 35-286 of SEQ ID NO:3) and SCRs 1-4 plus the O-glycosylated serine/threonine-rich domain (amino acids 35-362 of SEQ ID NO:3).

In some embodiments, the complement inhibitor portion of the construct comprises full-length human CD59 (SEQ ID NO:4). In some embodiments, the complement inhibitor portion of the construct comprises a biologically active fragment of human CD59 (SEQ ID NO:4). In some embodiments, the biologically active fragment of human CD59 comprises the extracellular domain of human CD59 lacking its GPI anchor (amino acids 26-101 of SEQ ID NO:4). In some embodiments, the complement inhibitor portion of the construct comprises full-length mouse CD59, isoform A (SEQ ID NO:5). In some embodiments, the complement inhibitor portion of the construct comprises a biologically active fragment of mouse CD59, isoform A (SEQ ID NO:5). In some embodiments, the biologically active fragment of mouse CD59, isoform A comprises the extracellular domain of mouse CD59, isoform A lacking its GPI anchor (amino acids 24-95 of SEQ ID NO:5). In some embodiments, the complement inhibitor portion of the construct comprises full-length mouse CD59, isoform B (SEQ ID NO:6). In some embodiments, the complement inhibitor portion of the construct comprises a biologically active fragment of mouse CD59, isoform B (SEQ ID NO:6). In some embodiments, the biologically active fragment of mouse CD59, isoform B comprises the extracellular domain of mouse CD59, isoform B lacking its GPI anchor (amino acids 24-103 of SEQ ID NO:6).

In some embodiments, the complement inhibitor portion of the construct comprises full-length mouse Crry protein (SEQ ID NO:7). In some embodiments, the complement inhibitor portion of the construct comprises a biologically active fragment of mouse Crry protein (SEQ ID NO:7). In some embodiments, the biologically active fragment of mouse Crry protein is selected from the group consisting of SCRs 1-5 (amino acids 41-400 of SEQ ID NO:7) and the extracellular domain of mouse Crry protein (amino acids 41-405 of SEQ ID NO:7).

In some embodiments, the complement inhibitor portion of the construct comprises full-length human CR1 protein (SEQ ID NO:8). In some embodiments, the complement inhibitor portion of the construct comprises a biologically active fragment of human CR1 protein (SEQ ID NO:8). In some embodiments, the biologically active fragment of human CR1 protein is selected from the group consisting of SCRs 1-4 (amino acids 42-295 of SEQ ID NO:8), SCRs 1-10 (amino acids 42-684 of SEQ ID NO:8), SCRs 8-11 (amino acids 490-745 of SEQ ID NO:8), SCRs 15-18 (amino acids 940-1196 of SEQ ID NO:8), and SCRs 22-28 (amino acids 1394-1842 of SEQ ID NO:8).

In some embodiments, the complement inhibitor portion of the construct comprises full-length human (SEQ ID

NO:9) or mouse (SEQ ID NO:10) factor H. In some embodiments, the complement inhibitor portion of the construct comprises a biologically active fragment of human (SEQ ID NO:9) or mouse (SEQ ID NO:10) factor H. In some embodiments, the biologically active fragment of human factor H (SEQ ID NO:9) is selected from the group consisting of SCRs 1-4 (amino acids 21-262 of SEQ ID NO:9), SCRs 1-5 of factor H (amino acids 21-320 of SEQ ID NO:9), SCRs 1-8 of factor H (amino acids 21-507 of SEQ ID NO:9), and SCRs 1-18 of factor H (amino acids 21-1104 of SEQ ID NO:9). In some embodiments, the biologically active fragment of mouse factor H (SEQ ID NO:10) is selected from the group consisting of SCRs 1-4 (amino acids 19-264 of SEQ ID NO:10), SCRs 1-5 of factor H (amino acids 19-322 of SEQ ID NO:10), SCRs 1-8 of factor H (amino acids 19-507 of SEQ ID NO:10), and SCRs 1-18 of factor H (amino acids 19-1109 of SEQ ID NO:10). In some embodiments, the biologically active fragment of human (SEQ ID NO:9) or mouse (SEQ ID NO:10) factor H comprises (and in some embodiments consists of or consists essentially of) at least the first four N-terminal SCR domains of factor H, including for example, at least any of the first 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or more N-terminal SCR domains of factor H.

In some embodiments, the complement inhibitor portion is an antibody (or an antigen-binding fragment thereof) that binds to a complement component, e.g., a complement component selected from the group consisting of C1, C1q, C1s, C2, C2a, C3, C3a, C3b, C4, C4b, C5, C5a, C5b, C6, C7, C8, and C9. The complement polypeptides to which the antibodies or antigen-binding fragments thereof bind can be, in some embodiments, human polypeptides, e.g., human C1, C1q, C1s, C2, C2a, C3, C3a, C3b, C4, C4b, C5, C5a, C5b, C6, C7, C8, C9, factor B, factor D, or properdin polypeptides. The amino acid sequences for the foregoing complement proteins are well-known in the art as are methods for preparing the proteins or fragments thereof for use in preparing an antibody (or antigen-binding fragment thereof) specific for one or more of the complement proteins. Suitable methods are also described and exemplified herein.

Exemplary anti-complement protein antibodies, which are suitable for incorporation into the fusion proteins described herein and for subsequent use in any of the methods described herein, are also well known in the art. For example, antibodies that bind to complement component C5 and inhibit the cleavage of C5 into fragments C5a and C5b include, e.g., eculizumab (Soliris®; Alexion Pharmaceuticals, Inc., Cheshire, Conn.) and pexelizumab (Alexion Pharmaceuticals, Inc., Cheshire, Conn.). See, e.g., Kaplan (2002) *Curr Opin Investig Drugs* 3(7):1017-23; Hill (2005) *Clin Adv Hematol Oncol* 3(11):849-50; Rother et al. (2007) *Nature Biotechnol* 25(11):1256-1488; Whiss (2002) *Curr Opin Investig Drugs* 3(6):870-7; Patel et al. (2005) *Drugs Today (Barc)* 41(3):165-70; and Thomas et al. (1996) *Mol Immunol* 33(17-18):1389-401.

In some embodiments, the anti-05 antibody can bind to an epitope in the alpha chain of the human complement component C5 protein. Antibodies that bind to the alpha chain of C5 are described in, for example, PCT application publication no. WO 2010/136311 and U.S. Pat. No. 6,355,245.

In some embodiments, the anti-05 antibody can bind to an epitope in the beta chain of the human complement component C5 protein. Antibodies that bind to the C5 beta chain are described in, e.g., Moongkarndi et al. (1982) *Immunobiol* 162:397; Moongkarndi et al. (1983) *Immunobiol* 165:323; and Mollnes et al. (1988) *Scand J Immunol* 28:307-312.

Additional anti-05 antibodies, and antigen-binding fragments thereof, suitable for use in the fusion proteins described herein are described in, e.g., PCT application publication no. WO 2010/015608, the disclosure of which is incorporated herein by reference in its entirety.

Antibodies that bind to C3b and, for example, inhibit the C3b convertase are also well known in the art. For example, PCT application publication nos. WO 2010/136311, WO 2009/056631, and WO 2008/154251, the disclosures of each of which are incorporated herein by reference in their entirety. Antagonistic anti-C6 antibodies and anti-C7 antibodies have been described in, e.g., Brauer et al. (1996) *Transplantation* 61(4):588-594 and U.S. Pat. No. 5,679,345.

In some embodiments, the complement inhibitor portion is an anti-factor B antibody (such as the monoclonal antibody 1379 produced by ATCC Deposit No. PTA-6230). Anti-factor B antibodies are also described in, e.g., Ueda et al. (1987) *J Immunol* 138(4):1143-9; Tanhehco et al. (1999) *Transplant Proc* 31(5):2168-71; U.S. patent application publication nos. 20050260198 and 2008029911; and PCT publication no. WO 09/029,669.

In some embodiments, the complement inhibitor portion is an anti-factor D antibody, e.g., an antibody described in Pascual et al. (1990) *J Immunol Methods* 127:263-269; Sahu et al. (1993) *Mol Immunol* 30(7):679-684; Pascual et al. (1993) *Eur J Immunol* 23:1389-1392; Niemann et al. (1984) *J Immunol* 132(2):809-815; U.S. Pat. No. 7,439,331; or U.S. patent application publication no. 20080118506.

In some embodiments, the complement inhibitor portion is an anti-properdin antibody. Suitable anti-properdin antibodies are also well-known in the art and include, e.g., U.S. patent application publication nos. 20110014614 and PCT application publication no. WO2009110918.

In some embodiments, the complement inhibitor portion is an antibody (or antigen-binding fragment thereof) that specifically binds to a human complement component protein (e.g., human C5, C6, C7, C8, or C9). The terms "specific binding" or "specifically binds" refer to two molecules forming a complex (e.g., a complex between an antibody and C3b) that is relatively stable under physiologic conditions. Typically, binding is considered specific when the association constant (K_a) is higher than 10^6 M^{-1} . Thus, an antibody can specifically bind to a C5 protein with a K_a of at least (or greater than) 10^6 (e.g., at least or greater than 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} , 10^{12} , 10^{13} , 10^{14} , or 10^{15} or higher) M^{-1} . Examples of antibodies that specifically bind to a human complement component C5 protein are described in, e.g., U.S. Pat. No. 6,355,245 and PCT application publication no. WO 2010/015608.

Methods for determining whether an antibody binds to a protein antigen and/or the affinity for an antibody to a protein antigen are known in the art and described herein. For example, the binding of an antibody to a protein antigen can be detected and/or quantified using a variety of techniques such as, but not limited to, Western blot, dot blot, plasmon surface resonance method (e.g., BIAcore system; Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.), or enzyme-linked immunosorbent assay (ELISA) assays. See, e.g., Harlow and Lane (1988) "Antibodies: A Laboratory Manual" Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Benny K. C. Lo (2004) "Antibody Engineering: Methods and Protocols," Humana Press (ISBN: 1588290921); Borrebaek (1992) "Antibody Engineering, A Practical Guide," W.H. Freeman and Co., NY; Borrebaek (1995) "Antibody Engineering," 2nd Edition, Oxford University Press, NY, Oxford; Johne et al. (1993) *J Immunol Meth.* 160: 191-198; Jonsson et al. (1993) *Ann*

Biol Clin 51:19-26; and Jonsson et al. (1991) *Biotechniques* 11:620-627. See also, U.S. Pat. No. 6,355,245.

A number of endogenous soluble proteins that activate complement have also been identified. These complement activators include, but are not limited to, various immunoglobulin (Ig) proteins, including human Ig isotype G₁ (IgG₁), human Ig isotype M (IgM), mouse Ig isotype G₃ (IgG₃), and mouse IgM Fc, as well as cobra venom factor (CVF) and biologically-active fragments thereof. The complement activating activity of Ig proteins has been localized to the Fc domain. Therefore biologically-active fragments of complement-activating human and mouse Ig proteins include the Fc domain, such as human IgG₁ Fc domain, human IgM Fc domain, mouse IgG₃ Fc domain, and mouse IgM Fc domain.

As used herein, the terms "cobra venom factor," "CVF," and "C3b (Cobra)" refer to the non-toxic, complement-activating component of cobra venom. Like naturally occurring human C3b, CVF (SEQ ID NO:11) forms a complex, or convertase, with complement components Factor B and Factor D. This CVFBbD convertase is capable of activating C3 in a wide variety of species via the alternative complement pathway. CVFBbD convertase is Factor H-resistant and is therefore not blocked through the activity of Factor I or CR1 and can convert nearly 100% of the C3 to C3 fragments and C5 to C5 fragments. Levels of iC3b, C3a, C5b-9, C5a and the Factor B cleavage product Bb are all extremely high in CVF treated sera. The cloning and sequencing of CVF from the monocled cobra (*Naja kaouthia*) was reported in Fritzing, et al., "Molecular cloning and derived primary structure of cobra venom factor," *Proc. Nat'l Acad. Sci. USA* 91(26):12775-779 (1994); the sequence was deposited in the GenBank database under Accession Number U09969. Both the Fritzing et al. reference and the sequence deposited in GenBank under Accession Number U09969 are hereby incorporated herein by reference. The terms "cobra venom factor," "CVF," and "C3b (Cobra)" also refer to hybrid CVF molecules comprising a CVF portion and a complement component 3 (C3) portion, such as those described in Fritzing et al., "Functional characterization of human C3/cobra venom factor hybrid proteins for therapeutic complement depletion," *Develop. Comp. Immunol.* 33(1):105-116 (2009), which is incorporated herein by reference. Those hybrids comprise proteins in which the 113 or 315 C-terminal residues of C3 were replaced with corresponding CVF sequences. Both hybrids formed stable convertases that exhibited C3-cleaving activity, although at different rates. Neither convertase cleaved C5. Both convertases showed partial resistance to inactivation by factors H and I, allowing them to deplete complement in human serum.

In some embodiments, the complement modulator portion of the construct comprises a complement activator or biologically-active fragment thereof. In some embodiments, the complement activator portion of the construct comprises an Ig protein or biologically-active fragment thereof. In some embodiments, the Ig protein or biologically-active fragment thereof comprises human IgG₁, human IgG₁ Fc domain, human IgM, human IgM Fc domain, mouse IgG₃, mouse IgG₃ Fc domain, mouse IgM, and mouse IgM Fc domain. In some embodiments, the complement activator portion of the construct comprises a cobra venom factor (CVF) or biologically-active fragment thereof.

In any of the embodiments described herein, the construct comprising a C3d-binding antibody or a biologically active fragment thereof and a complement inhibitor portion comprising human CD59, mouse CD59 isoform A, mouse CD59

isoform B, mouse Crry protein, human factor H, mouse factor H, human CR1, human MCP, human DAF or mouse DAF or a biologically active fragment thereof also includes an amino acid linker sequence linking the anti-C3d or anti-C3dg portion and the complement inhibitor portion (e.g., human CD59, mouse CD59 isoform A, mouse CD59 isoform B, mouse Crry protein, human factor H, mouse factor H, human CR1, human MCP, human DAF or mouse DAF or a biologically active fragment thereof).

In any of the embodiments described herein, the construct comprising a C3d-binding antibody, or a biologically active fragment thereof and a complement activator portion comprising human IgG₁, human IgM, mouse IgG₃, mouse IgM, and CVF or a biologically-active fragment thereof also includes an amino acid linker sequence linking the anti-C3d or anti-C3dg portion and the complement activator portion (e.g., human IgG₁, human IgM, mouse IgG₃, mouse IgM, and CVF or a biologically active fragment thereof).

In some embodiments, the targeting portion of the fusion molecule is joined (e.g., directly or by way of a linker) to the amino-terminus of the complement modulator polypeptide (e.g., the complement inhibitor polypeptide). In some embodiments, the targeting portion of the fusion molecule is joined (e.g., directly or by way of a linker) to the carboxy-terminus of the complement modulator polypeptide (e.g., the complement inhibitor polypeptide). For example, the disclosure features an exemplary construct comprising a single chain scFv fragment of an anti-C3d antibody and a complement inhibitor polypeptide (such as soluble CD59, MCP, or DAF), in which the carboxy-terminal end of the complement inhibitor polypeptide (e.g., the soluble CD59 polypeptide) is joined, optionally by way of a linker (e.g., (GGGG)_n or any other linker described herein), to the amino-terminal end of the heavy or light chain polypeptide portion of the scFv.

In some embodiments, a fusion molecule described herein comprises more than one (e.g., two, three, four, five, six, or seven or more) complement modulators, e.g., more than one complement inhibitor polypeptide. The two or more complement modulators can be the same or different. For example, a fusion protein described herein can comprise, in some embodiments, two or more soluble CD59 portions (e.g., soluble human CD59 portions). In another example, a fusion protein described herein can contain two or more complement inhibitor polypeptide portions, wherein one is a soluble human CD59 and another is soluble human MCP. Thus, e.g., a fusion molecule described herein can comprise: (a) a targeting portion (e.g., an anti-C3d antibody, an anti-C3dg antibody, or an antigen-binding fragment of either of the foregoing); (b) a first complement inhibitor polypeptide (e.g., a soluble form of CD59, e.g., human CD59); and (c) a second complement inhibitor polypeptide (e.g., a soluble form of DAF, e.g., a soluble form of human DAF). The complement inhibitor polypeptides can be, e.g., any of those described herein including variants and biologically active fragments thereof.

In some embodiments, e.g., wherein the targeting portion is an antibody (or an antigen-binding fragment thereof), the light chain of the targeting portion comprises at least one complement modulator (e.g., a complement inhibitor polypeptide) and the heavy chain comprises at least one complement modulator. The two or more complement modulators (e.g., complement inhibitor polypeptides) can be the same or different. For example, in some embodiments, the fusion protein comprises a targeting portion comprising the Fab fragment of anti-C3d (or anti-C3dg) antibody, wherein: (i) the light chain of the Fab fragment comprises (at its C-terminal end) a complement inhibitor polypeptide such as

sDAF, sCD59, or any of the complement inhibitor polypeptides described herein and (ii) the heavy chain of the Fab fragment comprises (at its C-terminal end) the same or a different complement inhibitor polypeptide as in (i). Appropriate pairing of the two chains can be expected to occur as an inherent property of the Fab. The complement modulator (e.g., complement inhibitor) portion and the light chain or heavy chain of the Fab can be joined together directly or by way of a linker sequence (such as any of those described herein).

Immunomodulatory Agents

The disclosure also provides fusion molecules that are capable of inhibiting B cell activation or activity, by way of a first portion, and, by way of a second portion, inhibiting complement activity. The constructs are useful for, among other things, treating disorders such as, but not limited to, transplant rejection and autoimmune diseases (e.g., any of those described herein). As used herein, a compound (e.g., a small molecule or a polypeptide such as an antibody) which inhibits B cell activation or activity is one that inhibits any aspect of: (a) antigen-specific production or secretion of an antibody by a B cell or plasma cell; (b) proliferation of a B cell or plasma cell; (c) antigen processing by B cells; (d) antigen presentation by B cells to T cells; (e) upregulation of B cell cell surface markers; and/or (f) differentiation (e.g., antigen-driven) of a B cell into an antibody-producing plasma cell. For example, in some embodiments, the compound (in this case, the first portion of the construct) is one that inhibits one or more signaling or co-signaling events that are associated with the proliferation and/or differentiation of B cells into plasma cells. Exemplary compounds useful in the constructs described herein include, e.g., a molecule that binds to and inhibits the activity, expression, or surface presentation of a B cell receptor by a B cell. For example, the molecule can be an antibody or antigen-binding fragment thereof that binds to a B cell receptor and (a) inhibits its function, e.g., inhibits the interaction between B cell receptor and cognate receptors on T cells and/or (b) promotes the cellular internalization of the bound B cell receptor. In some embodiments, the compound is a molecule (e.g., an antibody or antigen-binding fragment thereof) that inhibits the interaction between a costimulatory molecule binding pair (e.g., a B cell-T cell costimulatory molecule binding pair), e.g., CD40 and CD40L. For example, the first portion can be an antagonist anti-CD40 antibody, an antagonist anti-CD40L antibody, or an antagonist antigen-binding fragment of either of the foregoing. Antagonist anti-CD40 antibodies, useful in conjunction with the constructs described herein, are well known in the art and include, e.g., HCD122 [Luqman et al. (2008) *Blood* 112(3):711-720]. See also the antagonist anti-CD40 antibodies disclosed in U.S. patent application publication no. 20090304706 and PCT application publication no. WO 07/129,895, the disclosures of each of which are incorporated herein by reference in their entirety. Suitable anti-CD40L antibodies are described in, e.g., PCT application publication no. WO 06/030220 and U.S. patent application publication no. 20030180292, the disclosures of which are incorporated herein in their entirety. Additional exemplary molecules that inhibit B cell activation or activity include, e.g., antagonists of CD19 such as an antagonist anti-CD19 antibody or antagonist CD19-binding fragments of such an antibody. Suitable antagonist anti-CD19 antibodies for use in the constructs described herein include, e.g., MDX-1342 (Bristol-Myers Squibb) Kardarelli et al. (2010) *Cancer Immunol Immunother* 59(2):257-2651.

While not bound by any particular theory or mechanism of action, the inventors believe that inhibition of the inter-

action between C3d or C3dg and CR2 on host B cells reduces B cell activation and/or activity, for example, by reducing antigen presentation by B cells to T cells and thus further inhibiting subsequent T cell activation. Thus, in some embodiments, the first portion, which inhibits B cell activation and/or activity, is, e.g., an antibody or an antigen-binding fragment thereof that: (i) binds to a natural ligand of CR2 such as C3d, C3dg, and iC3b and (ii) inhibits the interaction between CR2 and at least one of its natural ligands. The antibody or antigen-binding fragment thereof can be any of the antibodies described herein. The disclosure also embraces constructs comprising a first portion comprising an antagonist anti-CR2 antibody (or antigen-binding fragment thereof) that inhibits the interaction between CR2 and C3d or C3dg. Suitable anti-CR2 (CD21) antibodies are well known in the art and include, e.g., mAb OKB7 in Rao et al. OKB7, a monoclonal antibody that binds at or near the C3d binding site of human CR2. *Cellular Immunology* 1985; 93(2): 549-555. The amino acid sequence of human CR2 (NCBI accession no. NP_001006659) and mouse CR2 (NCBI accession no. NP_031784.1), for example, are well known in the art, as are methods for producing and isolating the protein (e.g., for use in immunizations associated with antibody production). See, e.g., Moore et al. (1987) *Proc Natl Acad Sci USA* 84:9194-9198; Prota et al. (2002) *Proc Natl Acad Sci USA* 99:10641-10646; and Weis et al. (1984) *Proc Natl Acad Sci USA* 81:881-885. Methods for making (e.g., immunizing mammals and isolating resultant antibodies) and testing such anti-CD21 antibodies are also known in the art and described herein. The second portion, which inhibits complement activity, present in the constructs described herein can be, e.g., any one of the complement inhibitor polypeptides (including variants and functional fragments) described herein.

Methods (e.g., competitive binding studies) for determining whether a molecule (e.g., an antibody or an antigen-binding fragment thereof such as an anti-CR2 antibody or an anti-C3d antibody) inhibits the interaction between two proteins (e.g., CR2 and a natural ligand of CR2 or CD40 and CD40L) are well-known in the art and described herein.

Linkers for Fusion Molecules

The present disclosure provides a fusion molecule described herein that may include two moieties or portions, e.g., the targeting moiety or portion and the active prophylactic, therapeutic, or diagnostic moiety or portion, which are directly fused together by a covalent bond or fused through a linker. Examples of linker sequences are known in the art, and include, for example, (Gly₄Ser), (Gly₄Ser)₂, (Gly₄Ser)₃, (Gly₃Ser)₄, (SerGly₄), (SerGly₄)₂, (SerGly₄)₃, and (SerGly₄)₄. Linking sequences can also comprise "natural" linking sequences found between different domains of complement factors. For example, VSVFPLE or EYFNKYSS, the linking sequence between the first two N-terminal short consensus repeat domains of human CR2, can be used. In some embodiments, the linking sequence between the fourth and the fifth N-terminal short consensus repeat domains of human CR2 (EEIF) is used.

Production Methods

The fusion proteins described herein can be produced using a variety of techniques known in the art of molecular biology and protein chemistry. For example, a nucleic acid encoding a fusion protein described herein can be inserted into an expression vector that contains transcriptional and translational regulatory sequences, which include, e.g., promoter sequences, ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, transcription terminator signals, polyadenylation

signals, and enhancer or activator sequences. The regulatory sequences include a promoter and transcriptional start and stop sequences. In addition, the expression vector can include more than one replication system such that it can be maintained in two different organisms, for example in mam-

malian or insect cells for expression and in a prokaryotic host for cloning and amplification.

Several possible vector systems are available for the expression of fusion proteins from nucleic acids in mammalian cells. One class of vectors relies upon the integration of the desired gene sequences into the host cell genome. Cells which have stably integrated DNA can be selected by simultaneously introducing drug resistance genes such as *E. coli* gpt (Mulligan and Berg (1981) Proc Natl Acad Sci USA 78:2072) or Tn5 neo (Southern and Berg (1982) Mol Appl Genet 1:327). The selectable marker gene can be either linked to the DNA gene sequences to be expressed, or introduced into the same cell by co-transfection (Wigler et al. (1979) Cell 16:77). A second class of vectors utilizes DNA elements which confer autonomously replicating capabilities to an extrachromosomal plasmid. These vectors can be derived from animal viruses, such as bovine papilloma-virus (Sarver et al. (1982) Proc Natl Acad Sci USA, 79:7147), polyoma virus (Deans et al. (1984) Proc Natl Acad Sci USA 81:1292), or SV40 virus (Lusky and Botchan (1981) Nature 293:79).

The expression vectors can be introduced into cells in a manner suitable for subsequent expression of the nucleic acid. The method of introduction is largely dictated by the targeted cell type, discussed below. Exemplary methods include CaPO₄ precipitation, liposome fusion, lipofectin, electroporation, viral infection, dextran-mediated transfection, polybrene-mediated transfection, protoplast fusion, and direct microinjection.

Appropriate host cells for the expression of the fusion proteins include yeast, bacteria, insect, plant, and, as described above, mammalian cells. Of interest are bacteria such as *E. coli*, fungi such as *Saccharomyces cerevisiae* and *Pichia pastoris*, insect cells such as SF9, mammalian cell lines (e.g., human cell lines), as well as primary cell lines (e.g., primary mammalian cells). In some embodiments, the fusion proteins can be expressed in Chinese hamster ovary (CHO) cells or in a suitable myeloma cell line such as (NSO). Suitable cell lines also include, for example, BHK-21 (baby hamster kidney) cells; 293 (human embryonic kidney) cells; HMEpC (Human Mammary Epithelial cells); 3T3 (mouse embryonic fibroblast) cells.

The targeting and active prophylactic or therapeutic moieties may optionally be directly joined to each other, or may optionally be joined via a linker moiety. Where the targeting and active moieties are directly joined, the hybrid vector is made where the DNA encoding the targeting and active moieties are themselves directly ligated to each other using known scientific methods. Where a linker moiety is used, the hybrid vector is made where the DNA encoding the targeting moiety is ligated to DNA encoding one end of the linker moiety; and the DNA encoding the active moiety is ligated to the other end of the linker moiety. Methods are known for performing such ligations in proper orientation. Such ligation may be performed either in series, or as a three way ligation. Examples of sequences which may serve as the linker sequence in the present invention include short peptides of about 2 to about 15 amino acids in length. Among the peptide sequences useful as linkers in the present invention are (Gly-Ser)_n, where n=1 (SEQ ID NO:12), 2 (SEQ ID NO:13) 3 (SEQ ID NO:14), 4 (SEQ ID NO:15), 5 (SEQ ID NO:16), 6 (SEQ ID NO:17), 7 (SEQ ID NO:18), or 8 (SEQ

ID NO:19); (GlyGlyGlySer)_n, where n=1 (SEQ ID NO:20), 2 (SEQ ID NO:21), 3 (SEQ ID NO:22), or 4 (SEQ ID NO:23); (GlySerSerGly)_n, where n=1 (SEQ ID NO:32), 2 (SEQ ID NO:33), 3 (SEQ ID NO:34), or 4 (SEQ ID NO:35).

Other examples of sequences useful as the linker sequence in the present invention include one or more short conserved region (SCR) domains from one or more of the following complement-related proteins: Factor H; complement receptor 1; complement receptor 2; Factor B; DAF; and others.

As will be recognized by the skilled artisan, many active moieties which may be used in the present invention occur in nature as secreted proteins in conjunction with a signal or leader peptide and/or as a pro-peptide which undergoes further intra- or extra-cellular processing. In such cases, the hybrid vectors of the present invention may include one or more DNA sequences encoding such signal or leader peptides and/or one or more DNA sequences encoding such pro-peptide sequence, depending upon whether such secretion and/or processing is desired. Alternatively, the hybrid vectors of the present disclosure may include DNA sequences encoding a different signal or leader peptide and/or pro-peptide sequence chosen to optimize the expression and localization of the fusion protein. In most cases, the signal peptide may be omitted, as the targeting moiety will supply sufficient information for targeting of the active moiety to the desired tissue and cells within the subject's body.

In some embodiments, a fusion protein described herein can be expressed in, and purified from, transgenic animals (e.g., transgenic mammals). For example, a fusion protein described herein can be produced in transgenic non-human mammals (e.g., rodents, sheep or goats) and isolated from milk as described in, e.g., Houdebine (2002) Curr Opin Biotechnol 13(6):625-629; van Kuik-Romeijn et al. (2000) Transgenic Res 9(2):155-159; and Pollock et al. (1999) J Immunol Methods 231(1-2):147-157. Additional methods for producing proteins in mammalian milk products are described in, e.g., U.S. patent application publication nos. 200600105347 and 20040006776 and U.S. Pat. No. 7,045, 676.

The fusion proteins described herein can be produced from cells by culturing a host cell transformed with the expression vector containing nucleic acid encoding the antibodies, under conditions, and for an amount of time, sufficient to allow expression of the proteins. Such conditions for protein expression will vary with the choice of the expression vector and the host cell, and will be easily ascertained by one skilled in the art through routine experimentation. For example, polypeptides expressed in *E. coli* can be refolded from inclusion bodies (see, e.g., Hou et al. (1998) Cytokine 10:319-30). Bacterial expression systems and methods for their use are well known in the art (see Current Protocols in Molecular Biology, Wiley & Sons, and Molecular Cloning—A Laboratory Manual—3rd Ed., Cold Spring Harbor Laboratory Press, New York (2001)). The choice of codons, suitable expression vectors and suitable host cells will vary depending on a number of factors, and may be easily optimized as needed. A fusion protein described herein can be expressed in mammalian cells or in other expression systems including but not limited to yeast, baculovirus, and in vitro expression systems (see, e.g., Kaszubska et al. (2000) Protein Expression and Purification 18:213-220).

Following expression, the fusion proteins can be isolated. The term "purified" or "isolated" as applied to any of the proteins described herein (e.g., a fusion protein described herein) refers to a polypeptide that has been separated or

purified from components (e.g., proteins or other naturally-occurring biological or organic molecules) which naturally accompany it, e.g., other proteins, lipids, and nucleic acid in a prokaryote expressing the proteins. Typically, a polypeptide is purified when it constitutes at least 60 (e.g., at least 65, 70, 75, 80, 85, 90, 92, 95, 97, or 99) %, by weight, of the total protein in a sample.

A fusion protein described herein can be isolated or purified in a variety of ways known to those skilled in the art depending on what other components are present in the sample. Standard purification methods include electrophoretic, molecular, immunological, and chromatographic techniques, including ion exchange, hydrophobic, affinity, and reverse-phase HPLC chromatography. For example, a fusion protein can be purified using a standard anti-fusion protein antibody affinity column. Ultrafiltration and diafiltration techniques, in conjunction with protein concentration, are also useful. See, e.g., Scopes (1994) "Protein Purification, 3rd edition," Springer-Verlag, New York City, N.Y. The degree of purification necessary will vary depending on the desired use. In some instances, no purification of the expressed polypeptide thereof will be necessary.

Methods for determining the yield or purity of a purified polypeptide are known in the art and include, e.g., Bradford assay, UV spectroscopy, Biuret protein assay, Lowry protein assay, amido black protein assay, high pressure liquid chromatography (HPLC), mass spectrometry (MS), and gel electrophoretic methods (e.g., using a protein stain such as Coomassie Blue or colloidal silver stain).

In some embodiments, a fusion protein described herein can be synthesized de novo in whole or in part, using chemical methods well known in the art. For example, the component amino acid sequences can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography followed by chemical linkage to form a desired polypeptide. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing.

Once expressed and/or purified, a fusion protein described herein can be assayed for any one of a number of desired properties using in vitro or in vivo assays such as any of those described herein. For example, a fusion protein described herein can be assayed for its ability to inhibit C5 convertase as described in, e.g., Heinen et al. (2009), supra.

In some embodiments, endotoxin can be removed from the fusion protein preparations. Methods for removing endotoxin from a protein sample are known in the art. For example, endotoxin can be removed from a protein sample using a variety of commercially available reagents including, without limitation, the ProteoSpin™ Endotoxin Removal Kits (Norgen Biotek Corporation), Detoxi-Gel Endotoxin Removal Gel (Thermo Scientific; Pierce Protein Research Products), MiraCLEAN® Endotoxin Removal Kit (Mirus), or Acrodisc™-Mustang® E membrane (Pall Corporation).

Methods for detecting and/or measuring the amount of endotoxin present in a sample (both before and after purification) are known in the art and commercial kits are available. For example, the concentration of endotoxin in a protein sample can be determined using the QCL-1000 Chromogenic kit (BioWhittaker), the limulus amoebocyte lysate (LAL)-based kits such as the Pyrotell®, Pyrotell®-T, Pyrochrome®, Chromo-LAL, and CSE kits available from the Associates of Cape Cod Incorporated.

Following expression and purification, the fusion proteins described herein can be modified. The modifications can be covalent or non-covalent modifications. Such modifications

can be introduced into the fusion proteins by, e.g., reacting targeted amino acid residues of the polypeptide with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues. Suitable sites for modification can be chosen using any of a variety of criteria including, e.g., structural analysis or amino acid sequence analysis of the fusion proteins described herein.

In some embodiments, a fusion protein described herein can be conjugated to a heterologous moiety. In embodiments where the heterologous moiety is a polypeptide, a fusion protein and a corresponding heterologous moiety described herein can be joined by way of fusion protein. The heterologous moiety can be, e.g., a heterologous polypeptide, a therapeutic agent (e.g., a toxin or a drug), or a detectable label such as, but not limited to, a radioactive label, an enzymatic label, a fluorescent label, or a luminescent label. Suitable heterologous polypeptides include, e.g., an antigenic tag (e.g., FLAG, polyhistidine, hemagglutinin (HA), glutathione-S-transferase (GST), or maltose-binding protein (MBP)) for use in purifying the antibodies. Heterologous polypeptides also include polypeptides that are useful as diagnostic or detectable markers, for example, luciferase, green fluorescent protein (GFP), or chloramphenicol acetyl transferase (CAT). Where the heterologous moiety is a polypeptide, the moiety can be incorporated into a fusion protein described herein, resulting in a fusion protein.

Conjugates

In some embodiments, the fusion molecules described herein are created by linkage of two independently produced polypeptide fragments, e.g., an antibody (e.g., a Fab fragment of an anti-C3d antibody) and a complement modulator polypeptide (e.g., a soluble form of CD59). Two proteins (e.g., a fusion protein described herein and a heterologous moiety or the two constituent parts of a fusion protein) can, in some embodiments, be chemically cross-linked using any of a number of known chemical cross linkers. Examples of such cross linkers are those which link two amino acid residues via a linkage that includes a "hindered" disulfide bond. In these linkages, a disulfide bond within the cross-linking unit is protected (by hindering groups on either side of the disulfide bond) from reduction by the action, for example, of reduced glutathione or the enzyme disulfide reductase. One suitable reagent, 4-succinimidylloxycarbonyl- α -methyl- α -(2-pyridyldithio)toluene (SMPT), forms such a linkage between two proteins utilizing a terminal lysine on one of the proteins and a terminal cysteine on the other. Heterobifunctional reagents that cross-link by a different coupling moiety on each protein can also be used. Other useful cross-linkers include, without limitation, reagents which link two amino groups (e.g., N-5-azido-2-nitrobenzoyloxysuccinimide), two sulfhydryl groups (e.g., 1,4-bis-maleimidobutane), an amino group and a sulfhydryl group (e.g., m-maleimidobenzoyl-N-hydroxysuccinimide ester), an amino group and a carboxyl group (e.g., 4-[p-azidosalicylamido]butylamine), and an amino group and a guanidinium group that is present in the side chain of arginine (e.g., p-azidophenyl glyoxal monohydrate).

In some embodiments, a fusion protein described herein can contain a heterologous moiety which is chemically linked to the fusion protein. For example, in some embodiments, a radioactive label can be directly conjugated to the amino acid backbone of the fusion protein (e.g., for use of the labeled fusion protein for in vivo imaging studies).

In some embodiments, the fusion proteins can be modified, e.g., with a moiety that improves the stabilization and/or retention of the antibodies in circulation, e.g., in blood, serum, or other tissues. For example, a fusion protein

described herein can be PEGylated as described in, e.g., Lee et al. (1999) *Bioconjug Chem* 10(6): 973-8; Kinstler et al. (2002) *Advanced Drug Deliveries Reviews* 54:477-485; and Roberts et al. (2002) *Advanced Drug Delivery Reviews* 54:459-476. The stabilization moiety can improve the stability, or retention of, the polypeptide by at least 1.5 (e.g., at least 2, 5, 10, 15, 20, 25, 30, 40, or 50 or more) fold.

In some embodiments, the fusion proteins described herein can be glycosylated. In some embodiments, a fusion protein described herein can be subjected to enzymatic or chemical treatment, or produced from a cell, such that the antibody has reduced or absent glycosylation. Methods for producing polypeptides with reduced glycosylation are known in the art and described in, e.g., U.S. Pat. No. 6,933,368; Wright et al. (1991) *EMBO J.* 10(10):2717-2723; and Co et al. (1993) *Mol Immunol* 30:1361.

Therapeutic Methods

The above-described constructs (e.g., fusion molecules) and antibodies (and antigen-binding fragments thereof) are useful in, inter alia, methods for treating or preventing a variety of complement-associated disorders in a subject. The compositions can be administered to a subject, e.g., a human subject, using a variety of methods that depend, in part, on the route of administration. The route can be, e.g., intravenous injection or infusion (IV), subcutaneous injection (SC), intraperitoneal (IP) injection, or intramuscular injection (IM).

Administration can be achieved by, e.g., local infusion, injection, or by means of an implant. The implant can be of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. The implant can be configured for sustained or periodic release of the composition to the subject. See, e.g., U.S. Patent Application Publication No. 20080241223; U.S. Pat. Nos. 5,501,856; 4,863,457; and 3,710,795; EP488401; and EP 430539, the disclosures of each of which are incorporated herein by reference in their entirety. An antibody or fusion protein described herein can be delivered to the subject by way of an implantable device based on, e.g., diffusive, erodible, or convective systems, e.g., osmotic pumps, biodegradable implants, electrodiffusion systems, electroosmosis systems, vapor pressure pumps, electrolytic pumps, effervescent pumps, piezoelectric pumps, erosion-based systems, or electromechanical systems.

In some embodiments, an antibody or fusion protein described herein is therapeutically delivered to a subject by way of local administration. As used herein, "local administration" or "local delivery," refers to delivery that does not rely upon transport of the composition or agent to its intended target tissue or site via the vascular system. For example, the composition may be delivered by injection or implantation of the composition or agent or by injection or implantation of a device containing the composition or agent. Following local administration in the vicinity of a target tissue or site, the composition or agent, or one or more components thereof, may diffuse to the intended target tissue or site.

In some embodiments, an antibody, antigen-binding fragment thereof, or fusion protein described herein can be locally administered to a joint (e.g., an articulated joint). For example, in embodiments where the complement-associated disorder is arthritis, the complement inhibitor can be administered directly to a joint (e.g., into a joint space) or in the vicinity of a joint. Examples of intraarticular joints to which a fusion protein described herein can be locally administered include, e.g., the hip, knee, elbow, wrist, sternoclavicular, temporomandibular, carpal, tarsal, ankle, and any other joint

subject to arthritic conditions. An antibody, antigen-binding fragment thereof, or fusion protein described herein can also be administered to bursa such as, e.g., acromial, bicipitoradial, cubitoradial, deltoid, infrapatellar, ischial, and any other bursa known in the art of medicine.

In some embodiments, an antibody, antigen-binding fragment thereof, or fusion protein described herein can be locally administered to the eye. As used herein, the term "eye" refers to any and all anatomical tissues and structures associated with an eye. The eye has a wall composed of three distinct layers: the outer sclera, the middle choroid layer, and the inner retina. The chamber behind the lens is filled with a gelatinous fluid referred to as the vitreous humor. At the back of the eye is the retina, which detects light. The cornea is an optically transparent tissue, which conveys images to the back of the eye. The cornea includes one pathway for the permeation of drugs into the eye. Other anatomical tissue structures associated with the eye include the lacrimal drainage system, which includes a secretory system, a distributive system and an excretory system. The secretory system comprises secretors that are stimulated by blinking and temperature change due to tear evaporation and reflex secretors that have an efferent parasympathetic nerve supply and secrete tears in response to physical or emotional stimulation. The distributive system includes the eyelids and the tear meniscus around the lid edges of an open eye, which spread tears over the ocular surface by blinking, thus reducing dry areas from developing.

In some embodiments, an antibody, antigen-binding fragment thereof, or fusion protein described herein is administered to the posterior chamber of the eye. In some embodiments, an antibody, antigen-binding fragment thereof, or fusion protein described herein is administered intravitreally. In some embodiments, an antibody, antigen-binding fragment thereof, or fusion protein described herein is administered trans-sclerally.

In some embodiments, e.g., in embodiments for treatment or prevention of a complement-associated pulmonary disorder such as COPD or asthma, an antibody, antigen-binding fragment thereof, or fusion protein described herein can be administered to a subject by way of the lung. Pulmonary drug delivery may be achieved by inhalation, and administration by inhalation herein may be oral and/or nasal. Examples of pharmaceutical devices for pulmonary delivery include metered dose inhalers, dry powder inhalers (DPIs), and nebulizers. For example, an antibody, antigen-binding fragment thereof, or fusion protein described herein can be administered to the lungs of a subject by way of a dry powder inhaler. These inhalers are propellant-free devices that deliver dispersible and stable dry powder formulations to the lungs. Dry powder inhalers are well known in the art of medicine and include, without limitation: the TurboHaler® (AstraZeneca; London, England) the AIR® inhaler (Alkermes®; Cambridge, Mass.); Rotahaler® (GlaxoSmithKline; London, England); and Eclipse™ (Sanofi-Aventis; Paris, France). See also, e.g., PCT Publication Nos. WO 04/026380, WO 04/024156, and WO 01/78693. DPI devices have been used for pulmonary administration of polypeptides such as insulin and growth hormone. In some embodiments, an antibody, antigen-binding fragment thereof, or fusion protein described herein can be intrapulmonarily administered by way of a metered dose inhaler. These inhalers rely on a propellant to deliver a discrete dose of a compound to the lungs. Examples of compounds administered by metered dose inhalers include, e.g., Astovent®

(Boehringer-Ingelheim; Ridgefield, Conn.) and Flovent® (GlaxoSmithKline). See also, e.g., U.S. Pat. Nos. 6,170,717; 5,447,150; and 6,095,141.

In some embodiments, an antibody, antigen-binding fragment thereof, or fusion protein described herein can be administered to the lungs of a subject by way of a nebulizer. Nebulizers use compressed air to deliver a compound as a liquefied aerosol or mist. A nebulizer can be, e.g., a jet nebulizer (e.g., air or liquid jet nebulizers) or an ultrasonic nebulizer. Additional devices and intrapulmonary administration methods are set forth in, e.g., U.S. Patent Application Publication Nos. 20050271660 and 20090110679, the disclosures of each of which are incorporated herein by reference in their entirety.

In some embodiments, an antibody, antigen-binding fragment thereof, or fusion protein described herein is present in unit dosage form, which can be particularly suitable for self-administration. A formulated product of the present disclosure can be included within a container, typically, for example, a vial, cartridge, prefilled syringe or disposable pen. A doser such as the doser device described in U.S. Pat. No. 6,302,855 may also be used, for example, with an injection system of the present disclosure.

An injection system of the present disclosure may employ a delivery pen as described in U.S. Pat. No. 5,308,341. Pen devices, most commonly used for self-delivery of insulin to patients with diabetes, are well known in the art. Such devices can comprise at least one injection needle (e.g., a 31 gauge needle of about 5 to 8 mm in length), are typically pre-filled with one or more therapeutic unit doses of a therapeutic solution, and are useful for rapidly delivering the solution to a subject with as little pain as possible.

One medication delivery pen includes a vial holder into which a vial of insulin or other medication may be received. The vial holder is an elongate generally tubular structure with proximal and distal ends. The distal end of the vial holder includes mounting means for engaging a double-ended needle cannula. The proximal end also includes mounting means for engaging a pen body which includes a driver and dose setting apparatus. A disposable medication (e.g., a high concentration solution of a fusion protein described herein) containing vial for use with the prior art vial holder includes a distal end having a pierceable elastomeric septum that can be pierced by one end of a double-ended needle cannula. The proximal end of this vial includes a stopper slidably disposed in fluid tight engagement with the cylindrical wall of the vial. This medication delivery pen is used by inserting the vial of medication into the vial holder. A pen body then is connected to the proximal end of the vial holder. The pen body includes a dose setting apparatus for designating a dose of medication to be delivered by the pen and a driving apparatus for urging the stopper of the vial distally for a distance corresponding to the selected dose. The user of the pen mounts a double-ended needle cannula to the distal end of the vial holder such that the proximal point of the needle cannula pierces the septum on the vial. The patient then selects a dose and operates the pen to urge the stopper distally to deliver the selected dose. The dose selecting apparatus returns to zero upon injection of the selected dose. The patient then removes and discards the needle cannula, and keeps the medication delivery pen in a convenient location for the next required medication administration. The medication in the vial will become exhausted after several such administrations of medication. The patient then separates the vial holder from the pen body. The empty vial may then be removed and discarded. A new vial can be inserted into the

vial holder, and the vial holder and pen body can be reassembled and used as explained above. Accordingly, a medication delivery pen generally has a drive mechanism for accurate dosing and ease of use.

A dosage mechanism such as a rotatable knob allows the user to accurately adjust the amount of medication that will be injected by the pen from a prepackaged vial of medication. To inject the dose of medication, the user inserts the needle under the skin and depresses the knob once as far as it will depress. The pen may be an entirely mechanical device or it may be combined with electronic circuitry to accurately set and/or indicate the dosage of medication that is injected into the user. See, e.g., U.S. Pat. No. 6,192,891.

In some embodiments, the needle of the pen device is disposable and the kits include one or more disposable replacement needles. Pen devices suitable for delivery of the any one of the presently featured antibodies, antigen-binding fragments thereof, or fusion proteins described herein are also described in, e.g., U.S. Pat. Nos. 6,277,099; 6,200,296; and 6,146,361, the disclosures of each of which are incorporated herein by reference in their entirety. A microneedle-based pen device is described in, e.g., U.S. Pat. No. 7,556,615, the disclosure of which is incorporated herein by reference in its entirety. See also the Precision Pen Injector (PPI) device, Molly™, manufactured by Scandinavian Health Ltd.

The present disclosure also presents controlled-release or extended-release formulations suitable for chronic and/or self-administration of a medication such as an antibody, antigen-binding fragment thereof, or fusion protein described herein. The various formulations can be administered to a patient in need of treatment with the medication as a bolus or by continuous infusion over a period of time.

In some embodiments, a high concentration solution of an antibody, antigen-binding fragment thereof, or fusion protein described herein is formulated for sustained-release, extended-release, timed-release, controlled-release, or continuous-release administration. In some embodiments, depot formulations are used to administer the fusion protein to the subject in need thereof. In this method, the antibody, antigen-binding fragment thereof, or fusion protein described herein is formulated with one or more carriers providing a gradual release of active agent over a period of a number of hours or days. Such formulations are often based upon a degrading matrix which gradually disperses in the body to release the active agent.

In some embodiments, an antibody, antigen-binding fragment thereof, or fusion protein described herein is administered by way of intrapulmonary administration to a subject in need thereof. For example, an antibody, antigen-binding fragment thereof, or fusion protein described herein can be delivered by way of a nebulizer or an inhaler to a subject (e.g., a human) afflicted with a complement-associated pulmonary disorder such as asthma or COPD.

A “subject,” as used herein, can be any mammal. For example, a subject can be a human, a non-human primate (e.g., orangutan, gorilla, macaque, baboon, or chimpanzee), a horse, a cow, a pig, a sheep, a goat, a dog, a cat, a rabbit, a guinea pig, a gerbil, a hamster, a rat, or a mouse. In some embodiments, the subject is an infant (e.g., a human infant).

As used herein, a subject “in need of prevention,” “in need of treatment,” or “in need thereof,” refers to one, who by the judgment of an appropriate medical practitioner (e.g., a doctor, a nurse, or a nurse practitioner in the case of humans; a veterinarian in the case of non-human mammals), would reasonably benefit from a given treatment (such as treatment

with a composition comprising an antibody, antigen-binding fragment thereof, or a fusion protein described herein).

The term "preventing" is art-recognized, and when used in relation to a condition, is well understood in the art, and includes administration of a composition which reduces the frequency of, or delays the onset of, symptoms of a medical condition in a subject relative to a subject which does not receive a fusion protein described herein. Thus, prevention of a complement-associated disorder such as asthma includes, for example, reducing the extent or frequency of coughing, wheezing, or chest pain in a population of patients receiving a prophylactic treatment relative to an untreated control population, and/or delaying the occurrence of coughing or wheezing in a treated population versus an untreated control population, e.g., by a statistically and/or clinically significant amount.

In some embodiments, the present disclosure provides a method of specifically targeting a moiety or portion of a fusion molecule to a pre-defined area or compartment in vivo, thus increasing the local concentration of such moiety or portion in such area or compartment but not in other areas or compartments or increasing the accessibility of such moiety or portion to at least one pre-defined molecule located in such area or compartment, by the specific interaction between a targeting moiety or portion of such fusion molecule and a target molecule located in such area or compartment. In some embodiments, the present disclosure provides a method of specifically targeting an active or therapeutic moiety or portion of a fusion molecule to a surface of complement activation by an antibody or antigen-binding fragment thereof which is fused to such active or therapeutic moiety or portion and is able to specifically bind to a complement component protein. In some embodiments, such complement component protein is C3d or C3dg.

In some embodiments, the present disclosure provides a method of reducing, inhibiting, or preventing host humoral immune response by using a composition comprising an anti-C3d/C3dg antibody, or antigen-binding fragment thereof, or a fusion molecule comprising such antibody or fragment, as described in this application. In some embodiments, the present disclosure provides a method of reducing, inhibiting, or preventing host B cell activation by using a composition comprising an anti-C3d/C3dg antibody, or antigen-binding fragment thereof, or a fusion molecule comprising such antibody or fragment, as described herein.

In some embodiments, the present disclosure provides a method of treating, alleviating, or preventing a disease characterized with upregulated humoral immune response, compared to the normal host immune response without the disease onset, in a subject by using a composition comprising an anti-C3d/C3dg antibody, or antigen-binding fragment thereof, or a fusion molecule comprising such antibody or fragment, as described in this application. In some embodiments, the present disclosure provides a method of treating, alleviating or preventing a disease characterized with upregulated B cell activation, compared to the normal B cell activation level without the disease onset, in a subject by using a composition comprising an anti-C3d/C3dg antibody, or antigen-binding fragment thereof, or a fusion molecule comprising such antibody or fragment, as described herein.

In some embodiments, the present disclosure provides a method of treating a subject having a disease or preventing a subject from developing a disease, wherein the subject has upregulated humoral immune response, compared to the normal immune response level in a subject without such disease, using a composition comprising an anti-C3d/C3dg antibody, or antigen-binding fragment thereof, or a fusion

molecule comprising such antibody or fragment, as described herein. In some embodiments, the present disclosure provides a method of treating a subject having a disease or preventing a subject from developing a disease, wherein the subject has upregulated B cell activation, compared to the normal B cell activation level in a subject without such disease, using a composition comprising an anti-C3d/C3dg antibody, or antigen-binding fragment thereof, or a fusion molecule comprising such antibody or fragment, as described herein.

In some embodiments, the present disclosure provides a method of modulating complement activity by using a composition comprising an anti-C3d/C3dg antibody, or antigen-binding fragment thereof, or a fusion molecule comprising such antibody or fragment, as described herein. In some embodiments, such fusion molecule comprises an anti-C3d/C3dg antibody, or antigen-binding fragment thereof, as the targeting moiety and a complement modulator as the active or therapeutic moiety.

In some embodiments, the present disclosure provides a method of increasing or activating complement activity by using a composition comprising an anti-C3d/C3dg antibody, or antigen-binding fragment thereof, or a fusion molecule comprising such antibody or fragment, as described herein.

In some embodiments, such fusion molecule comprises an anti-C3d/C3dg antibody, or antigen-binding fragment thereof, as the targeting moiety and a complement activator or agonist as the active or therapeutic moiety.

In some embodiments, the present disclosure provides a method of decreasing or inhibiting complement activity by using a composition comprising an anti-C3d/C3dg antibody, or antigen-binding fragment thereof, or a fusion molecule comprising such antibody or fragment, as described herein. In some embodiments, such fusion molecule comprises an anti-C3d/C3dg antibody, or antigen-binding fragment thereof, as the targeting moiety and a complement inhibitor or antagonist as the active or therapeutic moiety.

In some embodiments, the present disclosure provides a method of treating, alleviating, or preventing a disease characterized with abnormal complement activity, compared to the normal complement activity without the disease onset, in a subject by using a composition comprising an anti-C3d/C3dg antibody, or antigen-binding fragment thereof, or a fusion molecule comprising such antibody or fragment and a complement modulator, as described herein. In some embodiments, the present disclosure provides a method of treating, alleviating or preventing a disease characterized with increased complement activity, compared to the normal complement activity without the disease onset, in a subject by using a composition comprising an anti-C3d/C3dg antibody, or antigen-binding fragment thereof, or a fusion molecule comprising such antibody or fragment and a complement inhibitor or antagonist, as described herein. In some embodiments, the present disclosure provides a method of treating, alleviating or preventing a disease characterized with decreased complement activity, compared to the normal complement activity without the disease onset, in a subject by using a composition comprising an anti-C3d/C3dg antibody, or antigen-binding fragment thereof, or a fusion molecule comprising such antibody or fragment and a complement activator or agonist, as described herein.

In some embodiments, the present disclosure provides a method of treating a subject having a disease or preventing a subject from developing a disease, wherein the subject has abnormal complement activity, compared to the normal complement activity in a subject without such disease, using a composition comprising an anti-C3d/C3dg antibody, or

antigen-binding fragment thereof, or a fusion molecule comprising such antibody or fragment and a complement modulator, as described herein. In some embodiments, the present disclosure provides a method of treating a subject having a disease or preventing a subject from developing a disease, wherein the subject has increased complement activity, compared to the normal complement activity in a subject without such disease, using a composition comprising an anti-C3d/C3dg antibody, or antigen-binding fragment thereof, or a fusion molecule comprising such antibody or fragment and a complement inhibitor or antagonist, as described herein. In some embodiments, the present disclosure provides a method of treating a subject having a disease or preventing a subject from developing a disease, wherein the subject has decreased complement activity, compared to the normal complement activity in a subject without such disease, using a composition comprising an anti-C3d/C3dg antibody, or antigen-binding fragment thereof, or a fusion molecule comprising such antibody or fragment and a complement activator or agonist, as described herein.

In some embodiments, the present disclosure provides a method of synergizing the modulation of complement activity and at least one other *in vivo* function using a composition comprising a fusion molecule comprising an anti-C3d/C3dg antibody, or antigen-binding fragment thereof, and at least one another active or function moiety or portion, as described herein. In some embodiments, such fusion molecule comprises an anti-C3d/C3dg antibody, or antigen-binding fragment thereof, as the targeting moiety and a complement inhibitor or antagonist as the active or therapeutic moiety, leading to a synergy of inhibiting both complement activation and humoral immune response. In some embodiments, the present disclosure provides a method of synergizing the inhibition of complement activity and humoral immune response by using a composition comprising a fusion molecule comprising an anti-CR2 antibody, or antigen-binding fragment thereof, and a complement inhibitor or antagonist.

In some embodiments, the present disclosure provides a method of treating, alleviating, or preventing a disease characterized with both increased complement activity and humoral immune response, compared to the normal complement activity and immune response without the disease onset, in a subject by using a composition comprising a fusion molecule comprising an anti-C3d/C3dg antibody, or antigen-binding fragment thereof, and a complement inhibitor or antagonist. In other embodiments, an anti-CR2 antibody, or antigen-binding fragment thereof, is fused to such complement inhibitor or antagonist for the method.

In some embodiments, the present disclosure provides a method of treating a subject having a disease or preventing a subject from developing a disease, wherein the subject has both increased complement activity and humoral immune response, compared to the normal complement activity and immune response in a subject without the disease, using a composition comprising a fusion molecule comprising an anti-C3d/C3dg antibody, or antigen-binding fragment thereof, and a complement inhibitor or antagonist. In other embodiments, an anti-CR2 antibody, or antigen-binding fragment thereof, is fused to such complement inhibitor or antagonist for the method.

In some embodiments, the present disclosure provides a method of synergizing at least two modulator functions using a composition comprising a fusion molecule comprising an anti-C3d/C3dg antibody, or antigen-binding fragment thereof, and at least two active or functional moieties or portions, as described herein. In some embodiments, such

two active or functional moieties or portions are fused to the heavy and light chain of such antibody or fragment, respectively. Both the N-terminus and the C-terminus of the anti-C3d/C3dg antibody or antigen-binding fragment thereof can be used for the fusion to the active or functional moieties or portions in either direction. Generally the C-terminus of the antibody or antigen-binding fragment thereof is used for fusion since it may not disturb the antigen-binding activity. One example of the fusion molecule is an anti-C3d/C3dg antibody or Fab linked to both CD59 and DAF, each of which is on one of its heavy/light chains. Such a fusion protein will have an improved inhibitory function towards complement activation. Indications include, e.g., PNH patients. Another example is an anti-C3d/C3dg antibody or Fab fused to factor H (or equivalent) and to an anti-angiogenic peptide/protein (e.g. endostatin, angiostatin, and others reported in literature) for AMD patients.

As described above, the antibodies and biologically-active fragments or fusion molecules comprising such antibodies or fragments as the targeting moiety described herein can be used to treat a variety of complement-associated disorders such as, but not limited to: rheumatoid arthritis (RA); lupus nephritis; ischemia-reperfusion injury; atypical hemolytic uremic syndrome (aHUS); typical or infectious hemolytic uremic syndrome (tHUS); dense deposit disease (DDD); paroxysmal nocturnal hemoglobinuria (PNH); multiple sclerosis (MS); macular degeneration (e.g., age-related macular degeneration (AMD)); hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome; sepsis; dermatomyositis; diabetic retinopathy; thrombotic thrombocytopenic purpura (TTP); spontaneous fetal loss; Pauci-immune vasculitis; epidermolysis bullosa; recurrent fetal loss; multiple sclerosis (MS); and traumatic brain injury. See, e.g., Holers (2008) *Immunological Reviews* 223:300-316 and Holers and Thurman (2004) *Molecular Immunology* 41:147-152. In some embodiments, the complement-mediated disorder is a complement-mediated vascular disorder such as, but not limited to, a cardiovascular disorder, myocarditis, a cerebrovascular disorder, a peripheral (e.g., musculoskeletal) vascular disorder, a renovascular disorder, a mesenteric/enteric vascular disorder, revascularization to transplants and/or replants, vasculitis, Henoch-Schönlein purpura nephritis, systemic lupus erythematosus-associated vasculitis, vasculitis associated with rheumatoid arthritis, immune complex vasculitis, Takayasu's disease, capillary leak syndrome, dilated cardiomyopathy, diabetic angiopathy, thoracic-abdominal aortic aneurysm, Kawasaki's disease (arteritis), venous gas embolus (VGE), and restenosis following stent placement, rotational atherectomy, and percutaneous transluminal coronary angioplasty (PTCA). See, e.g., U.S. patent application publication no. 20070172483. In some embodiments, the complement-associated disorder is myasthenia gravis, cold-agglutinin disease (CAD), paroxysmal cold hemoglobinuria (PCH), dermatomyositis, scleroderma, warm autoimmune hemolytic anemia, Graves' disease, Hashimoto's thyroiditis, type I diabetes, psoriasis, pemphigus, autoimmune hemolytic anemia (AIHA), idiopathic thrombocytopenic purpura (ITP), Goodpasture's syndrome, antiphospholipid syndrome (APS), Degos disease, and catastrophic APS (CAPS).

In some embodiments, an anti-C3d/C3dg antibody or antigen-binding fragment thereof or a fusion molecule comprising such antibody or fragment thereof as the targeting moiety described herein, alone or in combination with a second anti-inflammatory agent, can be used to treat an inflammatory disorder such as, but not limited to, RA (above), inflammatory bowel disease, sepsis (above), septic

shock, acute lung injury, disseminated intravascular coagulation (DIC), or Crohn's disease. In some embodiments, the second anti-inflammatory agent can be one selected from the group consisting of NSAIDs, corticosteroids, methotrexate, hydroxychloroquine, anti-TNF agents such as etanercept and infliximab, a B cell depleting agent such as rituximab, an interleukin-1 antagonist, or a T cell costimulatory blocking agent such as abatacept.

In some embodiments, the complement-associated disorder is a complement-associated neurological disorder such as, but not limited to, amyotrophic lateral sclerosis (ALS), brain injury, Alzheimer's disease, and chronic inflammatory demyelinating neuropathy.

Complement-associated disorders also include complement-associated pulmonary disorders such as, but not limited to, asthma, bronchitis, a chronic obstructive pulmonary disease (COPD), an interstitial lung disease, α -1 anti-trypsin deficiency, emphysema, bronchiectasis, bronchiolitis obliterans, alveolitis, sarcoidosis, pulmonary fibrosis, and collagen vascular disorders.

In some embodiments, an anti-C3d/C3dg antibody or an antigen-binding fragment thereof described herein can be administered to a subject as a monotherapy. Alternatively, as described above, the antibody or fragment thereof can be administered to a subject as a combination therapy with another treatment, e.g., another treatment for a complement-associated disorder or a complement-associated inflammatory response. For example, the combination therapy can include administering to the subject (e.g., a human patient) one or more additional agents (e.g., anti-coagulants, anti-hypertensives, or anti-inflammatory drugs (e.g., steroids)) that provide a therapeutic benefit to a subject who has, or is at risk of developing, sepsis. In another example, the combination therapy can include administering to the subject one or more additional agents (e.g., an anti-IgE antibody, an anti-IL-4 antibody, an anti-IL-5 antibody, or an anti-histamine antibody) that provide therapeutic benefit to a subject who has, is at risk of developing, or is suspected of having a complement-associated pulmonary disorder such as COPD or asthma. In some embodiments, an anti-C3d/C3dg antibody and the one or more additional active agents are administered at the same time. In other embodiments, the anti-C3d/C3dg antibody is administered first in time and the one or more additional active agents are administered second in time. In some embodiments, the one or more additional active agents are administered first in time and the anti-C3d/C3dg antibody is administered second in time.

An anti-C3d/C3dg antibody or an antigen-binding fragment thereof described herein can replace or augment a previously or currently administered therapy. For example, upon treating with an anti-C3d/C3dg antibody or antigen-binding fragment thereof, administration of the one or more additional active agents can cease or diminish, e.g., be administered at lower levels. In some embodiments, administration of the previous therapy can be maintained. In some embodiments, a previous therapy will be maintained until the level of the anti-C3d/C3dg antibody reaches a level sufficient to provide a therapeutic effect. The two therapies can be administered in combination.

Monitoring a subject (e.g., a human patient) for an improvement in a complement-associated disorder (e.g., sepsis, severe burn, RA, lupus nephritis, Goodpasture's syndrome, or asthma), as defined herein, means evaluating the subject for a change in a disease parameter, e.g., an improvement in one or more symptoms of a given disorder. The symptoms of complement-associated disorders are well known in the art of medicine. In some embodiments, the

evaluation is performed at least one (1) hour, e.g., at least 2, 4, 6, 8, 12, 24, or 48 hours, or at least 1 day, 2 days, 4 days, 10 days, 13 days, 20 days or more, or at least 1 week, 2 weeks, 4 weeks, 10 weeks, 13 weeks, 20 weeks or more, after an administration. The subject can be evaluated in one or more of the following periods: prior to beginning of treatment; during the treatment; or after one or more elements of the treatment have been administered. Evaluation can include evaluating the need for further treatment, e.g., evaluating whether a dosage, frequency of administration, or duration of treatment should be altered. It can also include evaluating the need to add or drop a selected therapeutic modality, e.g., adding or dropping any of the treatments for a complement-associated disorder described herein.

15 Pharmaceutical Compositions

In another aspect, provided herein are pharmaceutical compositions comprising any of the constructs and/or fusion proteins described herein. Pharmaceutical compositions comprising any of the constructs and/or fusion proteins described herein are generally formulated as sterile, substantially isotonic pharmaceutical solutions in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food and Drug Administration. In some embodiments, the composition is free of pathogen. For injection, the pharmaceutical compositions can be in the form of liquid solutions, for example in physiologically compatible buffers such as Hank's Balanced Salt Solution, Phosphate-Buffered Saline or Ringer's solution. In addition, the pharmaceutical compositions provided herein can be in solid form and redissolved or resuspended immediately prior to use. Lyophilized compositions are also contemplated.

For oral administration, the pharmaceutical compositions can take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulfate). Liquid preparations for oral administration can take the form of, for example, solutions, syrups or suspensions, or they can be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations can also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

In some embodiments, the compositions are formulated in accordance with routine procedures as a pharmaceutical composition adapted for injection. In some embodiments, the pharmaceutical compositions provided herein are formulated for intravenous, intraperitoneal, or intraocular injection. Typically, compositions for injection are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water-free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the

composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The pharmaceutical compositions may further comprise additional ingredients, for example preservatives, buffers, tonicity agents, antioxidants and stabilizers, nonionic wetting or clarifying agents, viscosity-increasing agents, and the like.

Suitable preservatives for use in a solution include polyquaternium-1, benzalkonium chloride, thimerosal, chlorobutanol, methyl paraben, propyl paraben, phenylethyl alcohol, disodium-EDTA, sorbic acid, benzethonium chloride, and the like. Typically (but not necessarily) such preservatives are employed at a level of from 0.001% to 1.0% by weight.

Suitable buffers include boric acid, sodium and potassium bicarbonate, sodium and potassium borates, sodium and potassium carbonate, sodium acetate, sodium biphosphate and the like, in amounts sufficient to maintain the pH at between about pH 6 and pH 8, and preferably, between about pH 7 and pH 7.5.

Suitable tonicity agents include dextran 40, dextran 70, dextrose, glycerin, potassium chloride, propylene glycol, sodium chloride, and the like, such that the sodium chloride equivalent of the injectable solution is in the range 0.9 plus or minus 0.2%.

Suitable antioxidants and stabilizers include sodium bisulfite, sodium metabisulfite, sodium thiosulfite, thiourea and the like. Suitable wetting and clarifying agents include polysorbate 80, polysorbate 20, poloxamer 282 and tyloxapol. Suitable viscosity-increasing agents include dextran 40, dextran 70, gelatin, glycerin, hydroxyethylcellulose, hydroxymethylpropylcellulose, lanolin, methylcellulose, petrolatum, polyethylene glycol, polyvinyl alcohol, polyvinylpyrrolidone, carboxymethylcellulose and the like.

As described above, the antibodies, antigen-binding fragments thereof, or fusion proteins described herein can be formulated as relatively high concentrations in aqueous pharmaceutical solutions. For example, an antibody or a fusion protein described herein can be formulated in solution at a concentration of between about 10 mg/mL to 100 mg/mL (e.g., between about 9 mg/mL and 90 mg/mL; between about 9 mg/mL and 50 mg/mL; between about 10 mg/mL and 50 mg/mL; between about 15 mg/mL and 50 mg/mL; between about 15 mg/mL and 110 mg/mL; between about 15 mg/mL and 100 mg/mL; between about 20 mg/mL and 100 mg/mL; between about 20 mg/mL and 80 mg/mL; between about 25 mg/mL and 100 mg/mL; between about 25 mg/mL and 85 mg/mL; between about 20 mg/mL and 50 mg/mL; between about 25 mg/mL and 50 mg/mL; between about 30 mg/mL and 100 mg/mL; between about 30 mg/mL and 50 mg/mL; between about 40 mg/mL and 100 mg/mL; between about 50 mg/mL and 100 mg/mL; or between about 20 mg/mL and 50 mg/mL). In some embodiments, a fusion protein described herein can be formulated in an aqueous solution at a concentration of greater than 5 mg/mL and less than 50 mg/mL. Methods for formulating a protein in an aqueous solution are known in the art and are described in, e.g., U.S. Pat. No. 7,390,786; McNally and Hastedt (2007), "Protein Formulation and Delivery," Second Edition, Drugs and the Pharmaceutical Sciences, Volume 175, CRC Press; and Banga (1995), "Therapeutic peptides and proteins: formulation, processing, and delivery systems," CRC Press. In some embodiments, the aqueous solution has a neutral pH, e.g., a pH between, e.g., 6.5 and 8 (e.g., between and

inclusive of 7 and 8). In some embodiments, the aqueous solution has a pH of about 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, or 8.0. In some embodiments, the aqueous solution has a pH of greater than (or equal to) 6 (e.g., greater than or equal to 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, or 7.9), but less than pH 8.

In some embodiments, a therapeutic composition described herein (e.g., an antibody, an antigen-binding fragment thereof, or a fusion protein described herein) can be formulated with one or more additional active agents useful for treating or preventing a complement-associated disorder (e.g., an AP-associated disorder or a CP-associated disorder) in a subject. Additional agents for treating a complement-associated disorder in a subject will vary depending on the particular disorder being treated, but can include, without limitation, an antihypertensive (e.g., an angiotensin-converting enzyme inhibitor) [for use in treating, e.g., HELLP syndrome], an anticoagulant, a corticosteroid (e.g., prednisone), or an immunosuppressive agent (e.g., vincristine or cyclosporine A). Examples of anticoagulants include, e.g., warfarin (Coumadin), aspirin, heparin, phenindione, fondaparinux, idraparin, and thrombin inhibitors (e.g., argatroban, lepirudin, bivalirudin, or dabigatran). A fusion protein described herein can also be formulated with a fibrinolytic agent (e.g., ancrod, ϵ -aminocaproic acid, antiplasmin-a₁, prostacyclin, and defibrotide) for the treatment of a complement-associated disorder. In some embodiments, a fusion protein can be formulated with a lipid-lowering agent such as an inhibitor of hydroxymethylglutaryl CoA reductase. In some embodiments, a fusion protein can be formulated with, or for use with, an anti-CD20 agent such as rituximab (Rituxan™; Biogen Idec, Cambridge, Mass.). In some embodiments, e.g., for the treatment of RA, the fusion protein can be formulated with one or both of infliximab (Remicade®; Centocor, Inc.) and methotrexate (Rheumatrex®, Trexall®). In some embodiments, a fusion protein described herein can be formulated with a non-steroidal anti-inflammatory drug (NSAID). Many different NSAIDs are available, some over the counter including ibuprofen (Advil®, Motrin®, Nuprin®) and naproxen (Alleve®) and many others are available by prescription including meloxicam (Mobic®), etodolac (Lodine®), nabumetone (Relafen®), sulindac (Clinoril®), tolemin (Tolectin®), choline magnesium salicylate (Trilasate®), diclofenac (Cataflam®, Voltaren®, Arthrotec®), Diflusal (Dolobid®), indomethacin (Indocin®), Ketoprofen (Orudis®, Oruvail®), Oxaprozin (Daypro®), and piroxicam (Feldene®). In some embodiments a fusion protein can be formulated for use with an anti-hypertensive, an anti-seizure agent (e.g., magnesium sulfate), or an anti-thrombotic agent. Anti-hypertensives include, e.g., labetalol, hydralazine, nifedipine, calcium channel antagonists, nitroglycerin, or sodium nitroprussiate. (See, e.g., Mihu et al. (2007) J Gastrointest Liver Dis 16(4):419-424.) Anti-thrombotic agents include, e.g., heparin, antithrombin, prostacyclin, or low dose aspirin.

In some embodiments, a therapeutic composition described herein (e.g., an antibody, an antigen-binding fragment thereof, or a fusion protein described herein) can be formulated for administration (e.g., intrapulmonary administration) with at least one additional active agent for treating a pulmonary disorder. The at least one active agent can be, e.g., an anti-IgE antibody (e.g., omalizumab), an anti-IL-4 antibody or an anti-IL-5 antibody, an anti-IgE inhibitor (e.g., montelukast sodium), a sympathomimetic (e.g., albuterol), an antibiotic (e.g., tobramycin), a deoxyribonuclease (e.g.,

pulmozyme), an anticholinergic drug (e.g., ipratropium bromide), a corticosteroid (e.g., dexamethasone), a β -adreno-receptor agonist, a leukotriene inhibitor (e.g., zileuton), a 5-lipoxygenase inhibitor, a PDE inhibitor, a CD23 antago-
 5 nist, an IL-13 antagonist, a cytokine release inhibitor, a histamine H1 receptor antagonist, an anti-histamine, an anti-inflammatory agent (e.g., cromolyn sodium), or a his-
 tamine release inhibitor.

In some embodiments, a therapeutic composition described herein (e.g., an antibody, an antigen-binding frag-
 10 ment thereof, or a fusion protein described herein) can be formulated for administration with one or more additional therapeutic agents for use in treating a complement-associated disorder of the eye. Such additional therapeutic agents can be, e.g., bevacizumab or the Fab fragment of bevacizumab or ranibizumab, both sold by Roche Pharmaceuticals, Inc., and pegaptanib sodium (Mucogen®; Pfizer, Inc.). Such a kit can also, optionally, include instructions for adminis-
 15 tering the fusion protein to a subject.

In some embodiments, a therapeutic composition described herein (e.g., an antibody, an antigen-binding frag-
 20 ment thereof, or a fusion protein described herein) can be formulated for administration to a subject along with intravenous gamma globulin therapy (IVIG), plasmapheresis, plasma replacement, or plasma exchange. In some embodi-
 25 ments, a therapeutic composition described herein (e.g., an antibody, an antigen-binding fragment thereof, or a fusion protein described herein) can be formulated for use before, during, or after, a kidney transplant.

When a therapeutic composition described herein (e.g., an
 30 antibody, an antigen-binding fragment thereof, or a fusion protein described herein) is to be used in combination with a second active agent, the agents can be formulated separately or together. For example, the respective pharmaceu-
 tical compositions can be mixed, e.g., just prior to admin-
 35 istration, and administered together or can be administered separately, e.g., at the same or different times (see below).

As described above, a pharmaceutical composition can be formulated such that it includes a therapeutically effective amount of an antibody, an antigen-binding fragment thereof,
 40 or a fusion protein described herein. In some embodiments, a composition can be formulated to include a sub-therapeutic amount of a fusion protein and a sub-therapeutic amount of one or more additional active agents such that the components in total are therapeutically effective for treating
 45 or preventing a complement-associated disorder (e.g., an alternative complement pathway-associated complement disorder or a classical complement pathway-associated disorder) in a subject. Methods for determining a therapeutically effective dose of an agent such as a therapeutic fusion
 50 protein, antibody, or antigen-binding fragment thereof are known in the art and described herein.

Methods for Administration

The pharmaceutical compositions may be suitable for a variety of modes of administration described herein, includ-
 55 ing for example systemic or localized administration. The pharmaceutical compositions can be in the form of injectable solutions or in a form suitable for oral administration. The pharmaceutical compositions described herein can be packaged in single unit dosages or in multidosage forms. In
 60 some embodiments, the pharmaceutical compositions are suitable for administration to an individual, a vertebrate, a mammal, or a human by any route of administration described herein, including oral administration or intrave-
 nous injection.

The compositions described herein can be administered to an individual via any route, including, but not limited to,

intravenous (e.g., by infusion pumps), intraperitoneal, intraocular, intra-arterial, intrapulmonary, oral, inhalation, intravesicular, intramuscular, intra-tracheal, subcutaneous, intrathecal, transdermal, transpleural, topical, inhalational
 5 (e.g., as mists or sprays), mucosal (such as via nasal mucosa), subcutaneous, gastrointestinal, intraarticular, intra-cisternal, intraventricular, rectal (i.e., via suppository), vagi-
 nal (i.e., via pessary), intracranial, intraurethral, intrahepatic, and intratumoral. In some embodiments, the compositions
 10 are administered systemically (for example by intravenous injection). In some embodiments, the compositions are administered locally (for example by intraarterial or
 intraocular injection).

In some embodiments, the compositions are administered
 15 intravascularly, such as intravenously or intraarterially. In some embodiments (for example for the treatment of renal diseases), the compositions are administered directly into arteries (such as renal arteries). In some embodiments, the compositions are administered subcutaneously.

In some embodiments, the compositions are administered
 20 directly to the eye or the eye tissue. In some embodiments, the compositions are administered topically to the eye, for example, in eye drops. In some embodiments, the compo-
 25 sitions are administered by injection to the eye (intraocular injection) or to the tissues associated with the eye. The compositions can be administered, for example, by intraocu-
 lar injection, periocular injection, subretinal injection, intra-
 vitreal injection, trans-septal injection, subscleral injection, intrachoroidal injection, intracameral injection, subconjuncti-
 30 tival injection, sub-Tenon's injection, retrobulbar injection, peribulbar injection, or posterior juxtasclear delivery. These methods are known in the art. For example, for a description of exemplary periocular routes for retinal drug delivery, see Periocular routes for retinal drug delivery, Raghava et al.
 35 (2004), Expert Opin. Drug Deliv. 1(1):99-114. The compositions may be administered, for example, to the vitreous, aqueous humor, sclera, conjunctiva, the area between the sclera and conjunctiva, the retina choroids tissues, macula, or other area in or proximate to the eye of an individual.

The compositions can also be administered to the indi-
 40 vidual as an implant. Preferred implants are biocompatible and/or biodegradable sustained release formulations which gradually release the compounds over a period of time. Ocular implants for drug delivery are well-known in the art. See, e.g., U.S. Pat. Nos. 5,501,856, 5,476,511, and 6,331,
 45 313. The compositions can also be administered to the individual using iontophoresis, including, but are not limited to, the ionophoretic methods described in U.S. Pat. No. 4,454,151 and US 2003/0181531 and 2004/0058313.

Dosage

A suitable dose of an antibody, antigen-binding fragment thereof, or fusion protein described herein, which dose is capable of treating or preventing a complement-associated disorder in a subject, can depend on a variety of factors
 55 including, e.g., the age, sex, and weight of a subject to be treated and the particular inhibitor compound used. For example, a different dose of one fusion may be required to treat a subject with RA as compared to the dose of a different fusion required to treat the same subject. Other factors
 60 affecting the dose administered to the subject include, e.g., the type or severity of the complement-mediated disorder. For example, a subject having RA may require administration of a different dosage of a fusion protein described herein than a subject with AMD. Other factors can include, e.g.,
 65 other medical disorders concurrently or previously affecting the subject, the general health of the subject, the genetic disposition of the subject, diet, time of administration, rate

of excretion, drug combination, and any other additional therapeutics that are administered to the subject. It should also be understood that a specific dosage and treatment regimen for any particular subject will also depend upon the judgment of the treating medical practitioner (e.g., doctor or nurse).

An antibody, antigen-binding fragment thereof, or fusion protein described herein can be administered as a fixed dose, or in a milligram per kilogram (mg/kg) dose. In some embodiments, the dose can also be chosen to reduce or avoid production of antibodies or other host immune responses against one or more of the active fusion proteins in the composition. While in no way intended to be limiting, exemplary dosages of an antibody, such as a fusion protein described herein include, e.g., 1-1000 mg/kg, 1-100 mg/kg, 0.5-50 mg/kg, 0.1-100 mg/kg, 0.5-25 mg/kg, 1-20 mg/kg, and 1-10 mg/kg, 1-100 mg/kg, 0.5-50 mg/kg, 0.1-100 mg/kg, 0.5-25 mg/kg, 1-20 mg/kg, 0.100 mg/kg to 1 mg/kg, and 1-10 mg/kg. Exemplary dosages of an fusion protein described herein include, without limitation, 0.1 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 2.0 mg/kg, 4 mg/kg, and 8 mg/kg, 0.1 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 2.0 mg/kg, 4 mg/kg, 8 mg/kg, and 20 mg/kg. In some embodiments, the amount of composition administered to an individual is about 10 μ g to about 500 mg per dose, including for example any of about 10 μ g to about 50 μ g, about 50 μ g to about 100 μ g, about 100 μ g to about 200 μ g, about 200 μ g to about 300 μ g, about 300 μ g to about 500 μ g, about 500 μ g to about 1 mg, about 1 mg to about 10 mg, about 10 mg to about 50 mg, about 50 mg to about 100 mg, about 100 mg to about 200 mg, about 200 mg to about 300 mg, about 300 mg to about 400 mg, or about 400 mg to about 500 mg per dose.

A pharmaceutical composition can include a therapeutically effective amount of an antibody, antigen-binding fragment thereof, or fusion protein described herein. Such effective amounts can be readily determined by one of ordinary skill in the art based, in part, on the effect of the administered antibody, fragment, or fusion protein, or the combinatorial effect of the compound (e.g., antibody, fragment, or fusion protein) and one or more additional active agents, if more than one agent is used. A therapeutically effective amount of an antibody, antigen-binding fragment thereof, or fusion protein described herein can also vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the primary agent (and one or more additional active agents) to elicit a desired response in the individual, e.g., amelioration of at least one condition parameter, e.g., amelioration of at least one symptom of the complement-mediated disorder. For example, a therapeutically effective amount of an antibody, antigen-binding fragment thereof, or fusion protein described herein can inhibit (lessen the severity of or eliminate the occurrence of) and/or prevent a particular disorder, and/or any one of the symptoms of the particular disorder known in the art or described herein. A therapeutically effective amount is also one in which any toxic or detrimental effects of the composition are outweighed by the therapeutically beneficial effects.

Suitable human doses of any of the fusion proteins described herein can further be evaluated in, e.g., Phase I dose escalation studies. See, e.g., van Gorp et al. (2008) *Am J Transplantation* 8(8):1711-1718; Hanouska et al. (2007) *Clin Cancer Res* 13(2, part 1):523-531; and Hetherington et al. (2006) *Antimicrobial Agents and Chemotherapy* 50(10):3499-3500.

The terms "therapeutically effective amount" or "therapeutically effective dose," or similar terms used herein are

intended to mean an amount of an agent that will elicit the desired biological or medical response (e.g., an improvement in one or more symptoms of a complement-associated disorder). In some embodiments, a pharmaceutical composition described herein contains a therapeutically effective amount of at least one of said antibodies, antigen-binding fragments thereof, or fusion proteins described herein. In some embodiments, the composition contains any of the antibodies, antigen-binding fragments thereof, or fusion proteins described herein and one or more (e.g., two, three, four, five, six, seven, eight, nine, 10, or 11 or more) additional therapeutic agents such that the composition as a whole is therapeutically effective. For example, a composition can contain a fusion protein described herein and an immunosuppressive agent, wherein the fusion protein and agent are each at a concentration that when combined are therapeutically effective for treating or preventing a complement-associated disorder (e.g., a complement-associated inflammatory disorder such as COPD, asthma, sepsis, or RA) in a subject.

Toxicity and therapeutic efficacy of such compositions can be determined by known pharmaceutical procedures in cell cultures or experimental animals (e.g., animal models of any of the complement-mediated disorders described herein). These procedures can be used, e.g., for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. A fusion protein described herein that exhibits a high therapeutic index is preferred. While compositions that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue and to minimize potential damage to normal cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of the antibodies, antigen-binding fragments thereof, or fusion proteins described herein lies generally within a range of circulating concentrations of the fusion proteins that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For a fusion protein described herein, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (i.e., the concentration of the antibody which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography. In some embodiments, e.g., where local administration (e.g., to the eye or a joint) is desired, cell culture or animal modeling can be used to determine a dose required to achieve a therapeutically effective concentration within the local site.

In some embodiments, the methods can be performed in conjunction with other therapies for complement-associated disorders. For example, the composition can be administered to a subject at the same time, prior to, or after, plasmapheresis, IVIG therapy, or plasma exchange. See, e.g., Appel et al. (2005) *J Am Soc Nephrol* 16:1392-1404. In some embodiments, the composition can be administered to a subject at the same time, prior to, or after, a kidney transplant.

Compositions including an antibody, antigen-binding fragment thereof, or construct (e.g., fusion molecule) described herein may be administered in a single daily dose, or the total daily dose may be administered in divided dosages of two, three, or four times daily. The compositions can also be administered less frequently than daily, for example, six times a week, five times a week, four times a week, three times a week, twice a week, once a week, once every two weeks, once every three weeks, once a month, once every two months, once every three months, or once every six months. The compositions may also be administered in a sustained release formulation, such as in an implant which gradually releases the composition for use over a period of time, and which allows for the composition to be administered less frequently, such as once a month, once every 2-6 months, once every year, or even a single administration. The sustained release devices (such as pellets, nanoparticles, microparticles, nanospheres, microspheres, and the like) may be administered by injection or surgically implanted in various locations in the body.

Gene Therapy

The molecules can also be delivered by expression of the fusion protein *in vivo*, which is often referred to as "gene therapy." For example, cells may be engineered with a polynucleotide (DNA or RNA) encoding the fusion protein *ex vivo*, and the engineered cells are then provided to an individual to be treated with the fusion protein. Such methods are well-known in the art. For example, cells may be engineered by procedures known in the art by use of a retroviral particle containing RNA encoding the fusion protein of the present disclosure. Local delivery of the fusion proteins of the present disclosure using gene therapy may provide the therapeutic agent to a localized target area.

Methods of gene delivery are known in the art. These methods include, but are not limited to, 1) direct DNA transfer, see, e.g., Wolff et al. (1990) *Science* 247: 1465-1468; 2) Liposome-mediated DNA transfer, see, e.g., Caplen et al. (1995) *Nature Med.* 3:39-46; Crystal (1995) *Nature Med.* 1:15-17; Gao and Huang (1991) *Biochem. Biophys. Res. Comm* 179:280-285; 3) Retrovirus-mediated DNA transfer, see, e.g., Kay et al. (1993) *Science* 262:117-119; Anderson (1992) *Science* 256:808-813; and 4) DNA Virus-mediated DNA transfer. Such DNA viruses include adenoviruses (preferably Ad2 or Ad5 based vectors), herpes viruses (preferably herpes simplex virus based vectors), and parvoviruses (preferably "defective" or non-autonomous parvovirus based vectors, more preferably adeno-associated virus based vectors, most preferably AAV-2 based vectors). See, e.g., Ali et al. (1994) *Gene Therapy* 1:367-384; U.S. Pat. No. 4,797,368, incorporated herein by reference, and U.S. Pat. No. 5,139,941.

Retroviruses from which the retroviral plasmid vectors hereinabove mentioned may be derived include, but are not limited to, Moloney Mouse Leukemia Virus, spleen necrosis virus, retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, adenovirus, Myeloproliferative Sarcoma Virus, and mammary tumor virus. In some embodiments, the retroviral plasmid vector is derived from Moloney Mouse Leukemia Virus.

Adenoviruses have the advantage that they have a broad host range, can infect quiescent or terminally differentiated cells, such as neurons or hepatocytes, and appear essentially non-oncogenic. See, e.g., Ali et al. (1994), *supra*, p. 367. Adenoviruses do not appear to integrate into the host

genome. Because they exist extrachromosomally, the risk of insertional mutagenesis is greatly reduced. Ali et al. (1994), *supra*, p. 373.

Adeno-associated viruses exhibit similar advantages as adenoviral-based vectors. However, AAVs exhibit site-specific integration on human chromosome 19 (Ali et al. (1994), *supra*, p. 377).

The gene therapy vectors may include one or more promoters. In some embodiments, the vector has a promoter that drives expression in multiple cell types. In some embodiments, the vector has a promoter that drives expression in specific cell types (such as cells of retina or cells in the kidney). Suitable promoters which may be employed include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller et al. (1989) *Biotechniques* 7(9):980-990, or any other promoter (e.g., cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and β -actin promoters). Other viral promoters which may be employed include, but are not limited to, adenovirus promoters, thymidine kinase (TK) promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein.

The nucleic acid sequence encoding a fusion protein is preferably under the control of a suitable promoter. Suitable promoters which may be employed include, but are not limited to, adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoA1 promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs (including the modified retroviral LTRs hereinabove described); the β -actin promoter; and human growth hormone promoter.

Retroviral plasmid vectors can be employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected are described in Miller (1990) *Human Gene Therapy* 1:5-14. The vectors may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO_4 precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host. The producer cell line generates infectious retroviral vector particles which include the nucleic acid sequence(s) encoding the polypeptides. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the polypeptide. Eukaryotic cells which may be transduced include, but are not limited to, embryonic stem cells, embryonic carcinoma cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, myoblasts, keratinocytes, endothelial cells, and bronchial epithelial cells.

In some embodiments, the complement activation is inhibited by contacting a body fluid with a composition comprising a molecule *ex vivo* under conditions that permit the molecule to function to inhibit complement activation. Suitable body fluids include those that can be returned to the individual, such as blood, plasma, or lymph. Affinity adsorption apheresis is described generally in Nilsson et al. (1988) *Blood* 58(1):38-44; Christie et al. (1993) *Transfusion*

33:234-242; Richter et al. (1997) *ASAIO J.* 43(1):53-59; Suzuki et al. (1994) *Autoimmunity* 19: 105-112; U.S. Pat. No. 5,733,254; Richter et al. (1993) *Metabol. Clin. Exp.* 42:888-894; and Wallukat et al. (1996) *Int'l J. Card.* 54:1910195.

Accordingly, the disclosure includes methods of treating one or more diseases described herein in an individual comprising treating the individual's blood extracorporeally (i.e., outside the body or *ex vivo*) with a composition comprising a molecule under conditions that permit the molecule to function to inhibit complement activation, and returning the blood to the individual.

Unit Dosages, Articles of Manufacture, and Kits

Also provided are unit dosage forms of compositions, each dosage containing from about 0.01 mg to about 50 mg, including for example any of about 0.1 mg to about 50 mg, about 1 mg to about 50 mg, about 5 mg to about 40 mg, about 10 mg to about 20 mg, or about 15 mg of the molecule. In some embodiments, the unit dosage forms of molecule composition comprises about any of 0.01 mg-0.1 mg, 0.1 mg-0.2 mg, 0.2 mg-0.25 mg, 0.25 mg-0.3 mg, 0.3 mg-0.35 mg, 0.35 mg-0.4 mg, 0.4 mg-0.5 mg, 0.5 mg-1.0 mg, 1.0 mg-10 mg, 10 mg-20 mg, 20 mg-50 mg, 50 mg-80 mg, 80 mg-100 mg, 100 mg-150 mg, 150 mg-200 mg, 200 mg-250 mg, 250 mg-300 mg, 300 mg-400 mg, or 400 mg-500 mg molecule. In some embodiments, the unit dosage form comprises about 0.25 mg molecule. The term "unit dosage form" refers to a physically discrete unit suitable as unitary dosages for an individual, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical carrier, diluent, or excipient. These unit dosage forms can be stored in a suitable packaging in single or multiple unit dosages and may also be further sterilized and sealed.

Also provided are articles of manufacture comprising the compositions described herein in suitable packaging. Suitable packaging for compositions (such as ophthalmic compositions) described herein are known in the art, and include, for example, vials (such as sealed vials), vessels, ampules, bottles, jars, flexible packaging (e.g., sealed Mylar or plastic bags), and the like. These articles of manufacture may further be sterilized and/or sealed.

The present disclosure also provides kits comprising compositions (or unit dosage forms and/or articles of manufacture) described herein and may further comprise instruction(s) on methods of using the composition, such as uses described herein. The kits described herein may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for performing any methods described herein.

The compositions and formulations of the present disclosure are useful for the treatment of conditions associated with complement activation, preferably those which involve the complement alternative pathway, which is largely unaffected by terminal complement inhibitors [e.g., inhibitors of steps of the complement pathway subsequent to the activation of C3].

EXAMPLES

Anti-C3d Antibodies:

Recombinant human C3d was generated using the pGEX expression system in *E. coli* as has been described (Hannan,

J. P., et al. (2005) *J. Mol. Biol.* 346, 845-858). Briefly, ampicillin-resistant colonies were used to start overnight cultures that were expanded to 1 liter and grown at 37° C. until an A_{600} of 0.3 was achieved. Cultures were induced with 0.3 mM isopropyl- β -D-thiogalactoside at 30° C. overnight before harvesting by centrifugation. Harvested pellets were resuspended in glutathione S-transferase column buffer (50 mM Tris-HCl, pH 8.0, 250 mM NaCl, 1 mM EDTA) and lysed by sonication. Lysate was clarified by centrifugation and applied to a GStrap column (GE Biosciences). C3d was cleaved from the column by digesting with 50 units of thrombin overnight at 4° C. and subsequently purified by size exclusion chromatography. Purity of C3d was monitored via SDS-PAGE.

A C3^{-/-} mouse was immunized with 60 μ g of recombinant C3d emulsified in complete Freund's adjuvant. Two months later the mouse received a booster injection of 80 μ g of C3d in incomplete Freund's adjuvant. It received two more immunizations of 100 μ g of C3d in PBS at two-month intervals. The mouse was then sacrificed, and a single cell suspension of splenocytes was made. The splenocytes were fused with Sp2/0 mouse myeloma cell line.

The fused cells were grown in Hypoxanthine Aminopterin Thymidine (HAT)-DMEM medium containing 10% fetal calf serum for seven days. The cells were then changed to HAT medium that did not contain fetal calf serum and the medium was changed twice weekly. Peritoneal macrophages were obtained from C3^{-/-} mice by peritoneal lavage and were used as feeder cells for the cultures. Serum-free medium and feeder cells from C3^{-/-} mice were used in order to avoid exposure of the hybridomas to homologous C3d. The resulting clones were screened for anti-C3d antibodies using an anti-C3d ELISA protocol. Briefly, 100 μ l/well of 5 μ g/ml recombinant C3d was bound to the wells of a polyvinyl chloride 96-well plate (Falcon). Polyclonal supernatants of the hybridomas were incubated on the plates, and anti-C3d reactivity was detected using secondary antibodies to mouse IgM, mouse IgG, mouse IgG1, mouse IgG2a, and mouse IgG2b. Wells that displayed reactivity against C3d underwent further cloning by limiting dilution in serum-free medium, and the resulting clones were again screened for reactivity against recombinant C3d.

Cell cultures of hybridomas with anti-C3d reactivity were expanded. Serum containing and serum-free medium formulations were tested for each clone, and the formulation that yielded better growth characteristics for each clone was used. Antibody was then purified from the supernatants using protein G columns (GE Biosciences). Briefly, the supernatants were passed over the column. The column was washed with 20 mM sodium phosphate, pH 7. The bound antibody was then eluted with 0.1 M Glycine-HCl, pH 2.7. The eluent was neutralized in 1 M Tris-HCl, pH 9 and the buffer exchanged with PBS.

Binding Affinity of Anti-C3D Antibodies

The ability of the purified antibodies to bind several forms of C3d was tested. Direct binding of the purified antibodies to recombinant human C3d was tested as described above. In another protocol, a polyclonal rabbit anti-human C3d antibody was used to capture the recombinant human C3d to ELISA plates and binding of the hybridomas to the captured C3d was then tested. Commercially available human C3d (Complement Technology, Inc.) was also bound to ELISA plates and used as the target. In another ELISA-type assay, the antibodies were bound to the plates, and reactivity to C3d was detected using biotinylated recombinant human C3d generated as described above, and also using another construct for biotinylated, His-tagged C3d. To test the reac-

tivity of these antibodies to C3d of other species, murine and cynomolgus recombinant C3d was produced. The ability of the purified antibodies to bind murine and cynomolgus C3d was tested using protocols where the C3d was directly bound to the ELISA plate or where it was captured with the rabbit anti-human C3d antibody. The cross-species activity of 3d8b, 3d9a, 3d29, 3d11, and 3d31 is shown in FIG. 8.

The binding affinity of the anti-C3d/C3dg antibodies to C3d was further tested by BIAcore analysis. As shown in FIG. 6, 10, 30, and 90 nM of free antibodies were floated through bound C3d and their bindings were recorded respectively. mAb 3d8b binds to C3d with a $K_D=4.65 \times 10^{-10}$ M mAb 3d9a binds to C3d with a $K_D=3.67 \times 10^{-10}$ M. mAb 3d29 binds to C3d with a $K_D=1.06 \times 10^{-9}$ M.

Testing for Binding Discrimination

C3d and Intact C3 by Western Blot Analysis

One μ g of unreduced, purified intact C3 (Complement Technology, Inc.) or recombinant C3d was separated by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were incubated with purified anti-C3d antibodies, washed, and then incubated with HRP conjugated anti-mouse IgG. The antibodies demonstrated four patterns of reactivity: 1) they reacted weakly with intact C3 and very strongly with C3d (clones 3d8b, 3d9a, and 3d29), 2) they reacted strongly with both the intact C3 and the C3d (clones 3d11 and 3d31), or 3-4) they did not bind either protein (clones 3d10 and 3d16), or they reacted weakly with C3d (clones 3d3 and 3d15).

C3 Fragments on Zymosan Particles

To test whether the antibodies bind to surface-bound C3 fragments generated physiologically, zymosan particles were incubated with 10% mouse serum at 37° C. for 30 minutes in a reaction containing 5 mM $MgCl_2$ and 10 mM EGTA. The zymosan particles were washed and were then incubated with one μ g of the purified antibodies at 4° C. for an hour. The particles were washed, incubated with a FITC-conjugated antibody to mouse IgG, washed, and analyzed by flow cytometry. Binding of antibody to the particles was assessed by comparing fluorescence of the particles to negative controls (no primary antibody) and positive controls (a commercial polyclonal antibody to mouse C3). Clones that bound to the opsonized zymosan particles were 3d8b, 3d9a, and 3d29 (FIG. 4).

C3 Fragments on Tissue Sections.

Factor H deficient mice ($fH^{-/-}$ mice) are known to have heavy deposits of C3b/iC3b/C3d in the renal glomeruli (Paixao-Cavalcante, D., S. Hanson, M. Botto, H. T. Cook, and M. C. Pickering. 2009. Factor H facilitates the clearance of GBM bound iC3b by controlling C3 activation in fluid phase. *Mol Immunol* 46:1942-1950). To test the ability of the purified anti-C3d antibodies to bind to the tissue deposits of C3 fragments, 5 μ m sections of kidneys from $fH^{-/-}$ mice were fixed with acetone and incubated with approximately 5 ng of purified antibody. The tissue sections were washed and then incubate with a FITC-conjugated antibody to mouse IgG. The sections were examined using a fluorescent microscope, and glomeruli were examined in order to detect IgG bound to the glomerular capillary loops. Clones 3d8b, 3d9a, and 3d29 demonstrated IgG bound to the glomeruli in a pattern identical to the pattern of C3 fragment deposition. Targeting C3 Fragments In Vivo—Discrimination Between Tissue Bound Fragments and Circulating C3/C3b.

To test whether the purified anti-C3d antibodies could target to tissue bound C3d fragments in vivo, the antibodies were injected into $fH^{-/-}$ mice. $fH^{-/-}$ mice were chosen because they have discrete deposits of C3b/iC3b/C3d in the renal glomeruli, and a large proportion of the C3 in the

circulation is in the cleaved C3b form. Mice were injected via the tail-vein with 0.5 mg of purified antibody. As a control, another mouse was injected with 0.5 mg of a chimeric protein comprised of the iC3b/C3d binding region of human CR2 linked to the Fc region of mouse IgG1 (CR2-Fc). After 24 hours the mice were sacrificed and the kidneys were harvested. Five μ m sections of kidneys were cut, fixed with acetone, and incubated with a FITC-conjugated antibody to mouse IgG. The sections were examined using a fluorescent microscope, and glomeruli were examined in order to detect IgG that had bound to the glomerular capillary loops. Clones 3d8b, 3d9a, and 3d29 demonstrated IgG bound to the glomeruli in a pattern identical to the pattern of C3 fragment deposition (FIGS. 9A-B) and identical to the pattern of deposition seen with CR2-Fc.

Competition with CR2 Binding to C3d

To determine whether the purified antibodies compete with CR2 for binding to C3d, polyvinyl chloride plates were coated with 100 microliters of recombinant C3d at a concentration of 5 micrograms/ml in 1/3 PBS. The plates were washed three times in 1/3 PBS-Tween 20 (0.01%), and then blocked for 1 hour with PBS/1% BSA at 100 microliters per well at room temperature. The plates were again washed three times as above. Serial dilutions of the purified anti-C3d antibodies (concentrations ranging from 1.8-15 μ g/ml) were added to recombinant maltose binding protein (MBP)-CR2 SCRs 1-2 (10 micrograms/ml in PBS). This solution of anti-C3d+MBP-CR2 SCRs 1-2 was added to the plate and incubated for 1 hour at room temperature. The plates were washed three times in 1/3 PBS-Tween 20(0.01%). Following this 100 microliters per well of HRP-conjugated anti-MBP mAb (New England Biolabs) were added, and the plate was wrapped in foil and stored in darkness for 1 hour. The plates were then washed three times in 1/3 PBS-Tween 20(0.01%), following which 100 microliters of ABTS (prepared by dissolving 22 mg of azino-bis(3-ethylbenzothiazole sulfonic acid) diammonium salt in 100 ml of 50 mM sodium citrate, pH 4.0)+1/1000 35% hydrogen peroxide were added into each well. The plates were incubated in darkness for 10-20 minutes, and then read at 405 nm FIG. 7 illustrates exemplary data of the competition with CR2 for binding to C3d, in varied degrees, by the anti-C3d mAb clones 3d8b, 3d31, 3d9a, 3d11, and 3d29.

Effects on CR2 and FH Binding to C3d

For analysis of the binding of CR2 and factor H to C3d, Costar IIA/RIA plates are coated overnight at 4° C. with 50 microliters of wild-type or mutant C3d at a concentration of 5 micrograms/ml in 50 mM sodium bicarbonate buffer, pH 8.8. Then plates are washed three times in 100 microliters per well of PBS-Tween 20 (0.01%). Plates are then blocked for 1 hour with 100 microliters per well of PBS/1% BSA at room temperature, and washed three times as above. Serial dilutions of MBP-CR2 SCRs 1-2 are made up using a stock solution of 10 micrograms/ml (in PBS). Either monoclonal antibodies to C3d, or factor H (wild type or the factor H SCR19-20 or other fragments) are added to the solution of MBP-CR2 SCRs 1-2. This solution of MBP-CR2 SCRs 1-2 is added to the plate and incubated for 1 hour at room temperature. A stock of Horseradish peroxidase-conjugated anti-MBP monoclonal antibody (1/1000 dilution in PBS) (New England Biolabs: Anti-MBP Monoclonal Antibody HRP Conjugated, product number E8038L or E8038S) is prepared. The plates are washed three times in PBS-Tween 20(0.01%). Following this 50 microliters per well of HRP-conjugated anti-MBP mAb are added, and the plate is wrapped in foil and stored in darkness for 1 hour. The plates are then washed three times in PBS-Tween 20(0.01%),

following which 50 microliters of ABTS+1/1000 35% hydrogen peroxide are added into each well (add H₂O₂ immediately before developing color). The plates are incubated in darkness for 10-20 minutes, and then read at 405 mn.

Effects on CR1, CR3 Binding to iC3b/C3dg

For analysis of the effects of anti-C3d monoclonal antibodies on CR1 function, fresh sheep erythrocytes (SRBCs) are conjugated with purified C3b using standard protocols. The cells are then incubated with human polymorphonuclear cells expressing CR1. Inhibition and confirmation of CR1-dependent binding and rosette formation is measured using anti-CR1 monoclonal antibody, such as anti-CD35 mAb 3E11 (Edberg et al., Quantitative analyses of the binding of soluble complement-fixing antibody/dsDNA immune complexes to CR1 on human red blood cells. *J Immunol* (1987) 139:3739-3747). Inhibition of binding of C3 fragment coated cells to polymorphonuclear cells is assessed by pre-incubation of the C3b-coated erythrocytes with anti-C3d monoclonal antibodies for 60 minutes.

To measure CR3-dependent binding, fresh sheep erythrocytes (SRBCs) are sensitized with a predetermined sub-agglutinating amount of rabbit anti-SRBC IgM for 30 minutes at 37° C. in gelatin veronal buffer (GVB) (Advanced Research Technologies). After washing twice, C3b-opsonized SRBCs are prepared by incubating IgM-sensitized SRBCs with an equal volume of a 1:2 dilution of C6-deficient human serum in GVB⁺⁺ (120 minutes at 37° C.). Cells are washed twice and pellets are resuspended in GVB. Most of the C3 bound to erythrocytes after this treatment is in the form of iC3b or C3d degradation products (CR2 ligands). U937 cells are then added to 50 microliters of C3-fragment-opsonized SRBCs (2×10⁶ cells), and the mixture is minimally centrifuged and left at room temperature for 90 minutes. Cells are then examined by phase-contrast microscopy, and the number of U937 cells adherent to erythrocytes are determined. At least 100 erythrocytes are scored per sample, and the average number of U937 cells bound per erythrocyte is calculated. Triplicate determinations are made for each experiment performed. To increase CR3 levels, U937 cells are cultured for 3 days in the presence of 50 ng/ml PMA before harvest. Cells incubated with IgM-coated SRBCs alone or SRBCs incubated directly with C6-deficient human serum are used as controls.

Function Studies for Anti-C3d Monoclonal Antibodies Effects on (Non-Stabilization of) CAP.

To verify that the antibodies do not stabilize the CAP C3-convertase and amplify complement activation on surfaces, an AH50 assay was employed. To perform this assay, 5 ml of whole rabbit blood was washed in GVB-Mg⁺⁺-EGTA and resuspended in a volume of 20 ml. Fifty μl of the erythrocytes were then added to 15 μl of human serum. Purified antibody (0-150 μg/reaction) was added, and the volume was brought to 150 ml with GVB-Mg⁺⁺-EGTA. The mixture was incubated at 37° C. for 30 minutes with periodic shaking of the tubes. As a negative control, 10 mM EDTA was added to the reaction. As a positive control (full lysis), 100 μl of water was added to the erythrocytes. The mixture was incubated at 37° C. for 30 minutes with periodic shaking of the tubes. To stop the reaction, 1.2 ml of cold PBS was added to the tubes. The tubes were then centrifuged at 1000×g for five minutes and the supernatants were transferred to new tubes. The O.D. at 415 nm was read, and the percent lysis for each sample was determined relative to the negative and positive control values. One clone (3d16) showed higher lysis than serum alone (FIG. 10). Clones

3d8b, 3d9a, and 3d29 did not demonstrate increased lysis with increasing concentrations of the antibodies.

As an exemplary experiment to further demonstrate whether the mAbs described herein bind to the same, or overlapping, epitopes on C3d or C3dg as those for CR2 binding, mutations are introduced into the CR2 binding site on C3d using standard methods. These C3d mutants will be tested for binding to both CR2 and the mAbs described herein. Finding any mutant which binds to only CR2 but not any one of the anti-C3d/C3dg antibodies described herein, or vice versa, demonstrates that the mutated amino acid residue is crucial for binding for the antibody(es) but not CR2, or vice versa, and that CR2 and the antibody(es) bind to different epitopes. Similarly, different profiles of binding to these mutants among the anti-C3d/C3dg antibodies will help to determine different binding epitopes or crucial amino acid residue(s) for binding on C3d for these antibodies. The binding between C3d mutants and CR2 or the anti-C3d/C3dg antibodies can be tested by many standard methods well known in the art. One exemplary ELISA assay is illustrated herein. Following purification of C3d mutant proteins, ELISA plates are coated with wild-type or mutant C3d at a concentration of 5 micrograms/ml in 50 mM sodium bicarbonate buffer, pH 8.8. Plates are then washed three times in with PBS-Tween 20 (0.01%); 100 microliters per well. Plates are then blocked for 1 hour with PBS/1% BSA; 100 microliters per well at room temperature and washed three times as above. Serial dilutions of anti-C3d monoclonal antibodies are made up using a stock solution of 10 micrograms/ml (in PBS). Monoclonal antibodies are added to the plate and incubate for 1 hour at room temperature. A stock of Horseradish peroxidase-conjugated anti-mouse IgG antibody is prepared. The plates are washed three times in PBS-Tween 20(0.01%). Following this 50 microliters per well of HRP-conjugated anti-mouse antibody are added, and the plate is wrapped in foil and stored in darkness for 1 hour. The plates are then washed three times in PBS-Tween 20(0.01%), following which 50 microliters of ABTS+1/1000 35% hydrogen peroxide are added into each well (add H₂O₂ immediately before developing color). The plates are incubated in darkness for 10-20 minutes, and then read at 405 nm

Effects on Antigen Immune Response.

To assess the effect of anti-C3d monoclonal antibodies on host humoral immune responses, which is hypothesized based on the role of C3d in enhancing B cell responses, a model complement-dependent antigen, sheep red blood cells (SRBCs), was utilized to induce host immune response. In one exemplary experiment, as illustrated in FIG. 11, one day after the injection of anti-C3d monoclonal antibodies, mice were immunized with SRBCs as a foreign antigen. Blood samples were collected 17 and 30 days post immunization and analyzed for their antigen-specific IgG1 concentration. As shown in FIG. 12, SRBC induced a host humoral immune response, which led to an increase of SRBC-specific IgG1 concentration. Both mAb 3d8b and 3d29 of Group 1, with their inhibitory activity towards CR2-C3d binding, were able to decrease significantly the host IgG1 production, while a Group 3 C3d antibody, 3d10, did not decrease the IgG1 production, probably due to its inability to inhibit CR2-C3d interaction (FIG. 12, FIG. 7, and FIG. 8). Interestingly, a Group 2 C3d antibody, 3d11, which competed with CR2 for binding recombinant C3d (FIG. 7), did not decrease the SRBC-specific IgG1 production in mice (FIG. 12). However, this is consistent with its lack of ability to bind native C3d or iC3b fragments on opsonized zymosan particles (FIG. 4), which may suggest that 3d11 is not, at

least, a strong C3d binding partner under physiological settings of complement activation.

Preparation and Testing of Fusion Proteins of Anti-C3d Monoclonal Antibody with Complement Modulatory Moieties

The hybridoma cell lines producing the various anti-C3d monoclonal antibodies described herein have been deposited with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20108 U.S.A. as follows: (1) hybridoma cell line 3d-9a/25, deposited on May 26, 2010, and designated as ATCC Patent Deposit PTA-10998; (2) hybridoma cell line 3d-8b/2, deposited on May 26, 2010, and designated as ATCC Patent Deposit PTA-10999; (3) hybridoma cell line 3d-29/5/2, deposited on May 26, 2010, and designated as ATCC Patent Deposit PTA-11000; (4) hybridoma cell line 3d-10/14/1, deposited on Jun. 2, 2010, and designated as ATCC Patent Deposit PTA-11010; (5) hybridoma cell line 3d-11/14, deposited on Jun. 2, 2010, and designated as ATCC Patent Deposit PTA-11011; (6) hybridoma cell line 3d-15A9, deposited on Jun. 2, 2010, and designated as ATCC Patent Deposit PTA-11012; (7) hybridoma cell line 3d-3/28/4, deposited on Jun. 9, 2010, and designated as ATCC Patent Deposit PTA-11025; (8) hybridoma cell line 3d-16/3/3, deposited on Jun. 9, 2010, and designated as ATCC Patent Deposit PTA-11026; and (9) hybridoma cell line 3d-31/A6/9, deposited on Jun. 9, 2010, and designated as ATCC Patent Deposit PTA-11027. The complete nucleotide and amino acid sequences of the monoclonal antibodies produced by those hybridoma cell lines can easily be determined with standard methods such as, for example, the polymerase chain reaction (PCR) and automated sequencing.

Conversion of Anti-C3d Monoclonal Antibodies into Single-chain Variable Fragments (scFvs).

Single chain Fvs of the present disclosure preferably comprise V_H and V_L domains joined by a flexible peptide linker that prevents dissociation. The scFvs usually retain the specificity of the parental antibody and bind the target antigen in a monovalent manner. Conversion of antibodies to scFvs is a well-known process (see Nat. Biotechnol. 23:1126 (2005); Biomol Eng. 24:201 (2007); J Immunol Methods., 168:149 (1994); Arch Virol. 148:497 (2003)). ScFvs can be constructed by either de novo gene synthesis, overlap extension polymerase chain reaction (PCR) or sequential ligation of the individual heavy (V_H) and light (V_L) chain variable gene segments. Sequential cloning of the individual V_H and V_L genes into a vector containing a synthetic linker sequence (e.g., $(Gly_4Ser)_3$) can be performed as well. The sequence can be either V_H -linker- V_L or V_L -linker- V_H .

Attachment of Complement Modulator to scFvs Specific for C3d.

An ScFv generated according to the previous paragraph can be linked to the complement modulator protein, e.g. factor H SCR1-5 (SEQ ID NO: 5). A series of linkers can be used to identify the optimal distance between scFv and the complement modulator protein, ranging from no linker to linkers of 40 amino acids or more, and the sequence chosen for the linker can be, for example, $(GlyGlyGlyGlySer)_n$ where $n=0-8$. The construction of the scFv linked to the complement modulator can be done by de novo gene synthesis, overlap PCR, sequential cloning or ligation. The N-terminal moiety of the final construct can be either the complement modulator or the scFv.

In Vitro Inhibition of Alternative Pathway by Anti-C3d scFv-FH.

Human or mouse fusion proteins containing the anti-C3d scFv and the first five SCR domains of human or murine FH without linker (antiC3dFv-FH) or with linker (antiC3dFv-L-FH) are made by recombinant DNA cloning and gene expression methods. The sequence for FH to be used in one of the antiC3dFv-FH fusion proteins is provided in SEQ ID NO: 5 (human) or SEQ ID NO: 8 (murine). Note that, because the anti-C3d antibodies cross react with human and mouse C3d, the same antibody fragments can be used in in vitro assays and in vivo in both humans and mice. For use with human cells or in humans, the Factor H component of the fusion molecules is preferably of human origin. For use with murine cells or in mice, the Factor H component of the fusion molecules is preferably of murine origin.

A mouse antiC3dFv-FH fusion protein (designated as antiC3dFv-FHFH) containing the [antiC3dFv] and two tandemly linked FH portions (each containing the first five SCR domains of FH) is also made. The antiC3dFv portion and the first FH portion are linked by a linker sequence.

In vitro assays for activation of the alternative pathway are conducted as essentially described in Quigg et al., J. Immunol. 1998, 160(9):4553-60. Factor H (1H) or anti-C3d antibody are used as controls in the experiment. Specifically, 50 mg of zymosan beads in 10 ml of 0.15M NaCl are activated by boiling for 60 minutes, and washed twice in PBS. In each reaction mixture add: 1) 10 mM EGTA and 5 mM $MgCl_2$ (final concentration); 2) 1×10^7 beads; 3) 10 mM EDTA (negative control 1) or heat-inactivated serum (negative control 2) or increasing concentration of one of the antiC3dFab-FH fusion proteins or control proteins; 4) 10 μ l of serum; and 5) PBS to bring the total volume to 100 μ l. The mixtures are incubated at 37° C. for 20 minutes, and the reactions are stopped by addition of 10 mM EDTA (final concentration). The beads are washed twice with cold PBSB (PBS with 1% BSA), and incubated with FTIC-conjugated goat-anti-C3 antibody for one hour on ice. The sample is then washed twice in PBSB, resuspended with 1% paraformaldehyde and analyzed under flow cytometry.

Inhibition of complement activation by antiC3dFab-FH and antiC3dFab-FHFH, with and without the linker, is compared to that of FH alone; FH—FH; antiC3dFab alone and anti-C3d antibody alone. The effects of antiC3dFab-FH and antiC3dFab-FHFH proteins are measured relative to the independent function of each portion.

Treatment of Intestinal Ischemia and Reperfusion Injury by AntiC3dFab-FH.

This experiment is designed to illustrate the effects of treatment of intestinal ischemia and reperfusion injury in a mouse model.

Intestinal ischemia reperfusion injury.

For a detailed description of exemplary methods, see, e.g., Huang et al. "A novel targeted inhibitor of the alternative pathway of complement and its therapeutic application in ischemia/reperfusion injury." The Journal of Immunology (2008), 181:11:8068-76, the disclosure of which is hereby incorporated by reference in its entirety. Briefly, three adult male mice aged 8 weeks and weighing 20-25 g are anesthetized with 10 mg/kg ketamine and 6 mg/kg xylazine by i.p. injection. Animals are breathing spontaneously and body temperature is maintained using a heat mat for the entire experiment. A medial laparotomy is performed and the intestines are carefully moved allowing access to the superior mesenteric artery. The superior mesenteric artery is clamped using a microsurgical clamp (Fine Instruments, USA). Ischemia is confirmed by pallor of the small intestine. Sham treated mice undergo laparotomy without clamping of superior mesenteric artery. After 30 min ischemia, the arte-

rial clamp is removed, allowing reperfusion of the mesenteric vasculature. Animals are sutured using 6.0 ethicon suture and allowed to reperfuse for 2 hours. Then 0.1 mg or 0.05 mg antiC3dFab-FH, or control (PBS) are administered through i.v. 30 minutes post reperfusion and animals are sacrificed 90 minutes later following a total of 2 hours of reperfusion.

Histology.

Tissue samples for histological staining are taken from the intestine and either fixed in 10% formalin at 4° C. overnight and subsequently processed to paraffin, or frozen in liquid nitrogen for immunofluorescence analysis. Sections of intestine from each animal are stained with hematoxylin and eosin and scored for mucosal damage and villi height as previously described. See, e.g., Rehrig et al. Complement Inhibitor, Complement Receptor 1-Related Gene/Protein y-Ig Attenuates Intestinal Damage After the Onset of Mesenteric Ischemia/Reperfusion Injury in Mice. *J Immunol* (2001) 167:5921-5927. Briefly, a score of 0 is assigned to a normal villus; villi with tip distortion are scored as 1; villi lacking goblet cells and containing Gugenheims' spaces are scored as 2; villi with patchy disruption of the epithelial cells are scored as 3; villi exposed but intact lamina propria and epithelial cell sloughing are assigned as 4; villi in which lamina propria were exuding are scored as 5, and finally, villi displaying hemorrhage or denuded villi are scored as 6. All histological evaluations are carried out in a blinded fashion.

The effect of antiC3dFab-FH is measured relative to the control animal, which is expected to have normal levels of circulating endogenous factor H in excess of the amounts of antiC3dFab-FH to be administered.

Treatment of Renal Ischemia Reperfusion by Mouse antiC3dFab-FH

This example is designed to illustrate the effects of antiC3dFab-FH on renal ischemia reperfusion. Protocol for Induction of Ischemic Acute Renal Failure (ARF).

For a detailed description of exemplary methods, see, e.g., Thurman et al. Lack of a Functional Alternative Complement Pathway Ameliorates Ischemic Acute Renal Failure in Mice. *J Immunol* (2003) 170:1517-1523, the disclosure of which is hereby incorporated by reference in its entirety. Briefly, mice weighing 20-25 grams are anesthetized with 300 µl of 2,2,2-Tribromoethanol (Sigma-Aldrich) injected intra-peritoneally. After the mice are anesthetized, they are placed on a heating pad to maintain their body temperature during surgery. Laparotomies are then performed, and the renal pedicles are located and isolated by blunt dissection. The pedicles are clamped with surgical clips (Miltex Instrument Company, Inc.), and occlusion of blood flow is confirmed by visual inspection of the kidneys. The clamps are left in place for 24 minutes and then released. The time of ischemia is chosen to obtain a reversible model of ischemic ARF with a minimum of vascular thrombosis, and to avoid animal mortality. The kidneys are observed for approximately one minute to ensure blood re-flow. After 15 minutes of reperfusion the mice receive 0.25 mg of the mouse antiC3dFab-FH intra-peritoneally. Fascia and skin are sutured with 4-0 silk (United States Surgical). The mice are volume resuscitated with 0.5 ml of normal saline and kept in an incubator at 29° C. to maintain body temperature.

After 24 hours of reperfusion the mice are anesthetized, and blood is obtained by cardiac puncture. Laparotomy is performed and the kidneys are harvested. All animal studies are performed in compliance with standard humane laboratory practice.

Serum Urea Nitrogen Measurements.

Serum urea nitrogen is determined for each mouse using a Beckman Autoanalyzer (Beckman) The effect of antiC3dFab-FH on kidney function is measured using the serum urea nitrogen determination.

Renal Morphology.

After the kidneys are removed from the mice, sagittal sections are fixed in 4% paraformaldehyde. After being embedded in paraffin, four µm sections are cut and stained with periodic acid Schiff. The sections are evaluated by a renal pathologist in a blinded fashion. The cortex and outer stripe of the outer medulla are assessed for epithelial necrosis, loss of brush border, tubular dilatation and cast formation. At least ten fields (400×) are reviewed for each slide, and the percentage of tubules displaying these findings is determined. The kidney sections are scored as follows based on the percentage of affected tubules: 0, none; 1, <10%; 2, 11-25%; 3, 26-45%; 4, 46-75%; 5, >75%. The effects of antiC3dFab-FH are measured relative to the control animal. Immunofluorescence.

For immunofluorescence, sagittal sections of the kidneys are snap frozen in OCT compound (Sakura Finetek). Four µm sections are cut with a cryostat and stored at -70° C. The slides are later fixed with acetone and incubated with the FITC conjugated antibody to mouse C3 (Cappel). After hybridization with the antibody for one hour at room temperature, the slides are counterstained with hematoxylin (Vector Laboratories, Inc.). The effects of antiC3dFab-FH on the level of C3 deposited into kidneys of sham treated mice are measured relative to untreated control mice.

Treatment of Age-Related Macular Degeneration by antiC3dFab-FH.

For a detailed description of exemplary methods, see, e.g., Rohrer et al. Eliminating complement factor D reduces photoreceptor susceptibility to light-induced damage. *Investigative Ophthalmology & Visual Science* (2007) 48(11): 5282-9, the disclosure of which is hereby incorporated by reference in its entirety. Briefly, constant light exposed albino rats are used as animal models for age-related macular degeneration (dry AMD). Five to eight animals are injected intraocularly under anesthesia every other day with an antiC3dFab-FH fusion protein (1 µl of 4.3 mg/ml stock solution), starting with the first injection the day prior to the onset of continuous light exposure (days -1, 1, 3, 5, 7). One eye serves as the experimental, while the other eye serves as the PBS-injected control eye. Animals are tested with electroretinography (ERG) on day 8 and then euthanized for histology and PCR analysis. Number of rows of photoreceptors in eyes injected with antiC3dFab-FH is compared with those of the PBS control eyes.

The effects of anti-C3dFab-FH are measured using three parameters: functional activity (ERG) potentials, i.e., photoreceptor and retinal pigment epithelium (RPE) responses), histology and measures of inflammation (e.g., gene expression by RT-PCR and protein expression by immunohistochemistry).

Reduction in CNV Volume by AntiC3d-Murine FH.

For a detailed description of exemplary methods, see, e.g., Rohrer et al. "A targeted inhibitor of the alternative complement pathway reduces angiogenesis in a mouse model of age-related macular degeneration." *Invest Ophthalmol V is Sci.* (2009) 50:3056-3064, the disclosure of which is hereby incorporated by reference in its entirety. Briefly, for generation of CNV, 3-month-old mice are anesthetized using xylazine and ketamine (20 and 80 mg/kg, respectively) and pupils are dilated with a drop of phenylephrine HCl (2.5%) and atropine sulfate (1%). Argon laser photocoagulation (532 nm, 50 µm spot size, 0.05 s duration, 250 mW) is used

to generate four laser spots in each eye surrounding the optic nerve, using a handheld coverslip as a contact lens. A bubble formed at a laser spot indicates the rupture of Bruch's membrane. Nozaki et al., Proc. Natl. Acad. Sci. (2006) 103:2328-33.

The effects of antiC3dFab-FH are measured using four parameters: functional activity (ERG potentials, i.e., photoreceptor and RPE responses), histology, vascular integrity (choroidal flatmounts after fluorescein injections) and measures of inflammation (e.g., gene expression by RT-PCR and protein expression by immunohistochemistry).

For assessment of CNV lesions, CNV size is determined in flat-mount preparations of RPE/choroids stained with isolectin B (which binds to terminal beta-D-galactose residues on the surface of endothelial cells and selectively labels the mouse vasculature). Fluorescence measurements taken in 2 μ m sections using confocal microscopy are used for size determination. In short, a Z-stack of images through the CNV lesion is obtained, using the same laser intensity setting for all experiments. For each slice the overall fluorescence is determined and plotted against depth.

For electroretinography, animals are anesthetized using xylazine (20 mg/kg bodyweight) and ketamine (80 mg/kg bodyweight). Pupils are dilated with a drop of phenylephrine HCl (2.5%) and tropicamide (1%). Body temperature is stabilized via a DC-powered heating pad held at 37° C. The ERG setup used is previously described by Rohrer et al., J. Neurosci., 1999, 19(20): 8919-30 and is built according to Lyubarsky and Pugh Lyubarsky et al., J. Neurosci., 1996, 16:563-571. Stimulus light intensity was controlled using neutral density filters.

Stimulus Paradigms.

Animals are dark-adapted overnight and ERGs will be recorded. Rods are analyzed in response to single-flash stimuli of increasing light intensity. The single-flash responses are an average of at least 3 flashes with an inter-stimulus interval (ISI) of 15 s to 2 min (lowest intensity to highest, respectively). The different ISIs ensures that ERG amplitudes at a given intensity are identical between the first and the last flash.

Data Analysis.

For all ERG recordings, a-wave amplitude are measured from baseline to trough; b-wave amplitude are measured from a-wave trough or baseline to peak of b-wave, and implicit times are measured from onset of stimulus to a-wave trough or b-wave peak.

In one experiment, mice are treated with intravenous mouse antiC3dFabFH (250 μ g) at 30 minutes post laser burn, 48 hours post laser burn, or 6 hours post laser burn. After 6 days post laser burn, retinal function is assessed, and then mice are sacrificed for histology.

The effects of antiC3dFab-FH on a- and b-wave retinal responses in mice are measured relative to controls. Quantification of isolectin-b stained lesions is assessed for both antiC3dFab-FH and controls.

In a separate experiment, 1 μ g mouse antiC3dFab-FH is administered intraoptically immediately after laser burn, 48 hours post burn, or 96 hours post burn. Eyes are collected at day 6 for histology. Lesions are visualized by isolectin-b staining. The effects on lesion size of antiC3dFab-FH delivered directly to the eye are measured relative to controls. Delay of Onset of Antibody-Mediated Rejection in a Mouse Heterotopic Heart Transplant Model by Mouse AntiC3dFab-FH.

For a detailed description of exemplary methods, see, e.g., Atkinson et al. "Targeted Complement Inhibitors Protect against Posttransplant Cardiac Ischemia and Reperfusion

Injury and Reveal an Important Role for the Alternative Pathway of Complement Activation." J Immunol (2010) 185:7007-7013, the disclosure of which is hereby incorporated by reference by its entirety. Briefly in this experiment, hearts are heterotopically transplanted from C3H donor mice into Balb/c recipient mice. This strain combination promotes a TH2 immune phenotype which promotes acute vascular rejection, and is characterized by anti-graft antibody production and graft deposition of complement activation fragments.

Recipient mice are treated with 1) PBS, i.v., 2) a single 0.25 mg dose of mouse antiC3dFab-FH, i.v. 30 minutes post reperfusion, and 3) multiple doses of 0.25 mg mouse antiC3dFab-FH, i.v., starting 30 minutes post reperfusion and then every three days thereafter.

Hearts are harvested 24 hours post reperfusion for analysis. The effects of mouse antiC3dFab-FH on ischemia and reperfusion injury are assessed by histology, and measurements of C3, neutrophil infiltration, and inflammatory cytokines, compared to control animals. The effects of mouse antiC3dFab-FH on acute vascular rejection are also assessed by time of survival in mice treated with mouse antiC3dFab-FH when compared to controls.

Inhibition of Alternative Complement Pathway by Human AntiC3dFab-FH.

The protein sequence of the FH component present in human antiC3dFab-FH, also designated as antiC3dFH, and human antiC3dFab-FHFH, also designated as antiC3dFH2, are provided in SEQ ID NO: 9. The protein sequence of the FH present in murine antiC3dFH and murine antiC3dFH2, is shown in SEQ ID NO: 10.

Human antiC3dFH and human antiC3dFH2 are purified from transfected 293 cell supernatants by affinity chromatography using HB5-sepharose, which contains anti-FH antibody linked to CNBr-activated sepharose (Amersham Biosciences). Crude antiC3dFH or antiC3dFH2 supernatants are passed over the matrix, washed with PBS, and eluted in 0.1M glycine-HCl, pH 3.0. The eluted fraction is immediately neutralized by the addition of 1M Tris-Cl, pH 9.0, followed by exchange into PBS using centricon columns (Millipore). Three hundred nanograms of nonreduced, purified antiC3dFH and antiC3dFH2 are resolved on SDS-PAGE and visualized by Coomassie staining.

The effects of human antiC3dFH and human antiC3dFH2 on alternative pathway specific C3b deposition onto zymosan particles are assessed as follows. Briefly, Zymosan particles are incubated in PBS containing 5 mM Mg²⁺, 10 mM EGTA, 10% human serum, and increasing concentrations of antiC3dFH and antiC3dFH2 for 30 minutes at room temperature with a FITC conjugated goat anti-human C3 antibody. Zymosan is pelleted and washed, followed by FACS analysis. Endogenous FH levels can be measured and the effects of the targeted FH are compared with untargeted endogenous FH.

The effects of human antiC3dFH and human antiC3dFH2 on alternative pathway-mediated erythrocyte lysis are assessed as follows. Briefly, rabbit erythrocytes (1 \times 10⁸) are incubated with various concentrations of antiC3dFH or antiC3dFH2 in 1 \times GVB⁺⁺ (Boston BioProducts) and 17% human serum for 30 minutes at 37° C. The reaction is stopped with the addition of one tenth volume cold PBS followed by centrifugation to pellet unlysed erythrocytes. Hemolysis is quantified by measuring OD₄₁₅ nm. Endogenous FH levels can be measured and the effects of the targeted FH are compared with untargeted endogenous FH.

Inhibition of the Alternative Complement Pathway by antiC3d-muFH.

This example shows inhibition of the alternative complement pathway by mouse antiC3d-muFH using serum from mice deficient in the classical pathway.

ELISA assay with immune complexes of collagen-anti-collagen antibodies on the plates are used. C3 deposition/activation is measured by using anti-C3b antibody in the presence of serum from wild-type or from C4⁻/C4⁻ mice. Different amounts of full length mouse FH (2 µg/10 µl), the first four SCR domains of mouse CR2 (2 µg/10 µl), and antiC3d-muFH (2 µg/10 µl) are added to the serum.

To further demonstrate that the inhibition of C3b deposition observed with antiC3dFH is due to inhibition of the

alternative pathway, the effects of antiC3dFH on C3b deposition are studied in the absence of the classical pathway (C4⁻/C4⁻ mice). Calcium inhibits the lectin complement pathway.

In the foregoing specification and in the examples below, the invention has been described with specific embodiments thereof. However, it will be evident to those skilled in the art that various modifications and changes may be made thereto without departing from the broader scope of the invention.

All publications that are cited herein are hereby specifically incorporated herein by reference into the disclosure for the teachings for which they are cited.

SEQUENCE LISTING

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Lys  Lys  Gly  Tyr  Phe  Tyr  Ile  Pro  Pro  Leu  Ala  Thr  His  Thr  Ile  Cys
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Glu  Thr  Cys  Pro  Tyr  Ile  Arg  Asp  Pro  Leu  Asn  Gly  Gln  Ala  Val  Pro
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          165          170          175

Glu  Val  Glu  Val  Phe  Glu  Tyr  Leu  Asp  Ala  Val  Thr  Tyr  Ser  Cys  Asp
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Pro  Ala  Pro  Gly  Pro  Asp  Pro  Phe  Ser  Leu  Ile  Gly  Glu  Ser  Thr  Ile
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Tyr  Cys  Gly  Asp  Asn  Ser  Val  Trp  Ser  Arg  Ala  Ala  Pro  Glu  Cys  Lys
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          245          250          255

Asp  Lys  Gly  Phe  Tyr  Leu  Asp  Gly  Ser  Asp  Thr  Ile  Val  Cys  Asp  Ser
          260          265          270

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Phe Thr Met Ile Gly Glu His Ser Ile Tyr Cys Thr Val Asn Asn Asp
 260 265 270

Glu Gly Glu Trp Ser Gly Pro Pro Pro Glu Cys Arg Gly Lys Ser Leu
 275 280 285

Thr Ser Lys Val Pro Pro Thr Val Gln Lys Pro Thr Thr Val Asn Val
 290 295 300

Pro Thr Thr Glu Val Ser Pro Thr Ser Gln Lys Thr Thr Thr Lys Thr
 305 310 315 320

Thr Thr Pro Asn Ala Gln Ala Thr Arg Ser Thr Pro Val Ser Arg Thr
 325 330 335

Thr Lys His Phe His Glu Thr Thr Pro Asn Lys Gly Ser Gly Thr Thr
 340 345 350

Ser Gly Thr Thr Arg Leu Leu Ser Gly His Thr Cys Phe Thr Leu Thr
 355 360 365

Gly Leu Leu Gly Thr Leu Val Thr Met Gly Leu Leu Thr
 370 375 380

<210> SEQ ID NO 3
 <211> LENGTH: 390
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 3

Met Ile Arg Gly Arg Ala Pro Arg Thr Arg Pro Ser Pro Pro Pro Pro
 1 5 10 15

Leu Leu Pro Leu Leu Ser Leu Ser Leu Leu Leu Leu Ser Pro Thr Val
 20 25 30

Arg Gly Asp Cys Gly Pro Pro Pro Asp Ile Pro Asn Ala Arg Pro Ile
 35 40 45

Leu Gly Arg His Ser Lys Phe Ala Glu Gln Ser Lys Val Ala Tyr Ser
 50 55 60

Cys Asn Asn Gly Phe Lys Gln Val Pro Asp Lys Ser Asn Ile Val Val
 65 70 75 80

Cys Leu Glu Asn Gly Gln Trp Ser Ser His Glu Thr Phe Cys Glu Lys
 85 90 95

Ser Cys Val Ala Pro Glu Arg Leu Ser Phe Ala Ser Leu Lys Lys Glu
 100 105 110

Tyr Leu Asn Met Asn Phe Phe Pro Val Gly Thr Ile Val Glu Tyr Glu
 115 120 125

Cys Arg Pro Gly Phe Arg Lys Gln Pro Pro Leu Pro Gly Lys Ala Thr
 130 135 140

Cys Leu Glu Asp Leu Val Trp Ser Pro Val Ala Gln Phe Cys Lys Lys
 145 150 155 160

Lys Ser Cys Pro Asn Pro Lys Asp Leu Asp Asn Gly His Ile Asn Ile
 165 170 175

Pro Thr Gly Ile Leu Phe Gly Ser Glu Ile Asn Phe Ser Cys Asn Pro
 180 185 190

Gly Tyr Arg Leu Val Gly Val Ser Ser Thr Phe Cys Ser Val Thr Gly
 195 200 205

Asn Thr Val Asp Trp Asp Asp Glu Phe Pro Val Cys Thr Glu Ile His
 210 215 220

Cys Pro Glu Pro Pro Lys Ile Asn Asn Gly Ile Met Arg Gly Glu Ser
 225 230 235 240

Asp Ser Tyr Thr Tyr Ser Gln Val Val Thr Tyr Ser Cys Asp Lys Gly

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245				250				255							
Phe	Ile	Leu	Val	Gly	Asn	Ala	Ser	Ile	Tyr	Cys	Thr	Val	Ser	Lys	Ser
			260					265					270		
Asp	Val	Gly	Gln	Trp	Ser	Ser	Pro	Pro	Pro	Arg	Cys	Ile	Glu	Lys	Ser
		275					280					285			
Lys	Val	Pro	Thr	Lys	Lys	Pro	Thr	Ile	Asn	Val	Pro	Ser	Thr	Gly	Thr
	290					295					300				
Pro	Ser	Thr	Pro	Gln	Lys	Pro	Thr	Thr	Glu	Ser	Val	Pro	Asn	Pro	Gly
305					310					315					320
Asp	Gln	Pro	Thr	Pro	Gln	Lys	Pro	Ser	Thr	Val	Lys	Val	Ser	Ala	Thr
				325					330						335
Gln	His	Val	Pro	Val	Thr	Lys	Thr	Thr	Val	Arg	His	Pro	Ile	Arg	Thr
			340					345					350		
Ser	Thr	Asp	Lys	Gly	Glu	Pro	Asn	Thr	Gly	Gly	Asp	Arg	Tyr	Ile	Tyr
		355					360					365			
Gly	His	Thr	Cys	Leu	Ile	Thr	Leu	Thr	Val	Leu	His	Val	Met	Leu	Ser
		370				375					380				
Leu	Ile	Gly	Tyr	Leu	Thr										
385					390										

<210> SEQ ID NO 4
 <211> LENGTH: 128
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

Met	Gly	Ile	Gln	Gly	Gly	Ser	Val	Leu	Phe	Gly	Leu	Leu	Leu	Val	Leu
1				5					10					15	
Ala	Val	Phe	Cys	His	Ser	Gly	His	Ser	Leu	Gln	Cys	Tyr	Asn	Cys	Pro
			20					25					30		
Asn	Pro	Thr	Ala	Asp	Cys	Lys	Thr	Ala	Val	Asn	Cys	Ser	Ser	Asp	Phe
		35					40					45			
Asp	Ala	Cys	Leu	Ile	Thr	Lys	Ala	Gly	Leu	Gln	Val	Tyr	Asn	Lys	Cys
	50					55					60				
Trp	Lys	Phe	Glu	His	Cys	Asn	Phe	Asn	Asp	Val	Thr	Thr	Arg	Leu	Arg
65					70				75					80	
Glu	Asn	Glu	Leu	Thr	Tyr	Tyr	Cys	Cys	Lys	Lys	Asp	Leu	Cys	Asn	Phe
				85					90					95	
Asn	Glu	Gln	Leu	Glu	Asn	Gly	Gly	Thr	Ser	Leu	Ser	Glu	Lys	Thr	Val
			100					105					110		
Leu	Leu	Leu	Val	Thr	Pro	Phe	Leu	Ala	Ala	Ala	Trp	Ser	Leu	His	Pro
			115				120					125			

<210> SEQ ID NO 5
 <211> LENGTH: 123
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 5

Met	Arg	Ala	Gln	Arg	Gly	Leu	Ile	Leu	Leu	Leu	Leu	Leu	Leu	Ala	Val
1				5					10					15	
Phe	Cys	Ser	Thr	Ala	Val	Ser	Leu	Thr	Cys	Tyr	His	Cys	Phe	Gln	Pro
			20					25					30		
Val	Val	Ser	Ser	Cys	Asn	Met	Asn	Ser	Thr	Cys	Ser	Pro	Asp	Gln	Asp
		35					40					45			
Ser	Cys	Leu	Tyr	Ala	Val	Ala	Gly	Met	Gln	Val	Tyr	Gln	Arg	Cys	Trp

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Lys Gln Asp Ser Thr Trp Thr Ser Ala Glu Asp Lys Cys Ile Arg Lys
 130 135 140
 Gln Cys Lys Thr Pro Ser Asp Pro Glu Asn Gly Leu Val His Val His
 145 150 155 160
 Thr Gly Ile Gln Phe Gly Ser Arg Ile Asn Tyr Thr Cys Asn Gln Gly
 165 170 175
 Tyr Arg Leu Ile Gly Ser Ser Ser Ala Val Cys Val Ile Thr Asp Gln
 180 185 190
 Ser Val Asp Trp Asp Thr Glu Ala Pro Ile Cys Glu Trp Ile Pro Cys
 195 200 205
 Glu Ile Pro Pro Gly Ile Pro Asn Gly Asp Phe Phe Ser Ser Thr Arg
 210 215 220
 Glu Asp Phe His Tyr Gly Met Val Val Thr Tyr Arg Cys Asn Thr Asp
 225 230 235 240
 Ala Arg Gly Lys Ala Leu Phe Asn Leu Val Gly Glu Pro Ser Leu Tyr
 245 250 255
 Cys Thr Ser Asn Asp Gly Glu Ile Gly Val Trp Ser Gly Pro Pro Pro
 260 265 270
 Gln Cys Ile Glu Leu Asn Lys Cys Thr Pro Pro Tyr Val Glu Asn
 275 280 285
 Ala Val Met Leu Ser Glu Asn Arg Ser Leu Phe Ser Leu Arg Asp Ile
 290 295 300
 Val Glu Phe Arg Cys His Pro Gly Phe Ile Met Lys Gly Ala Ser Ser
 305 310 315 320
 Val His Cys Gln Ser Leu Asn Lys Trp Glu Pro Glu Leu Pro Ser Cys
 325 330 335
 Phe Lys Gly Val Ile Cys Arg Leu Pro Gln Glu Met Ser Gly Phe Gln
 340 345 350
 Lys Gly Leu Gly Met Lys Lys Glu Tyr Tyr Tyr Gly Glu Asn Val Thr
 355 360 365
 Leu Glu Cys Glu Asp Gly Tyr Thr Leu Glu Gly Ser Ser Gln Ser Gln
 370 375 380
 Cys Gln Ser Asp Gly Ser Trp Asn Pro Leu Leu Ala Lys Cys Val Ser
 385 390 395 400
 Arg Ser Ile Ser Gly Leu Ile Val Gly Ile Phe Ile Gly Ile Ile Val
 405 410 415
 Phe Ile Leu Val Ile Ile Val Phe Ile Trp Met Ile Leu Lys Tyr Lys
 420 425 430
 Lys Arg Asn Thr Thr Asp Glu Lys Tyr Lys Glu Val Gly Ile His Leu
 435 440 445
 Asn Tyr Lys Glu Asp Ser Cys Val Arg Leu Gln Ser Leu Leu Thr Ser
 450 455 460
 Gln Glu Asn Ser Ser Thr Thr Ser Pro Ala Arg Asn Ser Leu Thr Gln
 465 470 475 480
 Glu Val Ser

<210> SEQ ID NO 8
 <211> LENGTH: 2039
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

Met Gly Ala Ser Ser Pro Arg Ser Pro Glu Pro Val Gly Pro Pro Ala
 1 5 10 15

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Pro Gly Leu Pro Phe Cys Cys Gly Gly Ser Leu Leu Ala Val Val Val
 20 25 30
 Leu Leu Ala Leu Pro Val Ala Trp Gly Gln Cys Asn Ala Pro Glu Trp
 35 40 45
 Leu Pro Phe Ala Arg Pro Thr Asn Leu Thr Asp Glu Phe Glu Phe Pro
 50 55 60
 Ile Gly Thr Tyr Leu Asn Tyr Glu Cys Arg Pro Gly Tyr Ser Gly Arg
 65 70 75 80
 Pro Phe Ser Ile Ile Cys Leu Lys Asn Ser Val Trp Thr Gly Ala Lys
 85 90 95
 Asp Arg Cys Arg Arg Lys Ser Cys Arg Asn Pro Pro Asp Pro Val Asn
 100 105 110
 Gly Met Val His Val Ile Lys Gly Ile Gln Phe Gly Ser Gln Ile Lys
 115 120 125
 Tyr Ser Cys Thr Lys Gly Tyr Arg Leu Ile Gly Ser Ser Ser Ala Thr
 130 135 140
 Cys Ile Ile Ser Gly Asp Thr Val Ile Trp Asp Asn Glu Thr Pro Ile
 145 150 155 160
 Cys Asp Arg Ile Pro Cys Gly Leu Pro Pro Thr Ile Thr Asn Gly Asp
 165 170 175
 Phe Ile Ser Thr Asn Arg Glu Asn Phe His Tyr Gly Ser Val Val Thr
 180 185 190
 Tyr Arg Cys Asn Pro Gly Ser Gly Gly Arg Lys Val Phe Glu Leu Val
 195 200 205
 Gly Glu Pro Ser Ile Tyr Cys Thr Ser Asn Asp Asp Gln Val Gly Ile
 210 215 220
 Trp Ser Gly Pro Ala Pro Gln Cys Ile Ile Pro Asn Lys Cys Thr Pro
 225 230 235 240
 Pro Asn Val Glu Asn Gly Ile Leu Val Ser Asp Asn Arg Ser Leu Phe
 245 250 255
 Ser Leu Asn Glu Val Val Glu Phe Arg Cys Gln Pro Gly Phe Val Met
 260 265 270
 Lys Gly Pro Arg Arg Val Lys Cys Gln Ala Leu Asn Lys Trp Glu Pro
 275 280 285
 Glu Leu Pro Ser Cys Ser Arg Val Cys Gln Pro Pro Pro Asp Val Leu
 290 295 300
 His Ala Glu Arg Thr Gln Arg Asp Lys Asp Asn Phe Ser Pro Gly Gln
 305 310 315 320
 Glu Val Phe Tyr Ser Cys Glu Pro Gly Tyr Asp Leu Arg Gly Ala Ala
 325 330 335
 Ser Met Arg Cys Thr Pro Gln Gly Asp Trp Ser Pro Ala Ala Pro Thr
 340 345 350
 Cys Glu Val Lys Ser Cys Asp Asp Phe Met Gly Gln Leu Leu Asn Gly
 355 360 365
 Arg Val Leu Phe Pro Val Asn Leu Gln Leu Gly Ala Lys Val Asp Phe
 370 375 380
 Val Cys Asp Glu Gly Phe Gln Leu Lys Gly Ser Ser Ala Ser Tyr Cys
 385 390 395 400
 Val Leu Ala Gly Met Glu Ser Leu Trp Asn Ser Ser Val Pro Val Cys
 405 410 415
 Glu Gln Ile Phe Cys Pro Ser Pro Pro Val Ile Pro Asn Gly Arg His
 420 425 430

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Thr	Gly	Lys	Pro	Leu	Glu	Val	Phe	Pro	Phe	Gly	Lys	Ala	Val	Asn	Tyr
		435					440					445			
Thr	Cys	Asp	Pro	His	Pro	Asp	Arg	Gly	Thr	Ser	Phe	Asp	Leu	Ile	Gly
	450					455					460				
Glu	Ser	Thr	Ile	Arg	Cys	Thr	Ser	Asp	Pro	Gln	Gly	Asn	Gly	Val	Trp
465					470					475					480
Ser	Ser	Pro	Ala	Pro	Arg	Cys	Gly	Ile	Leu	Gly	His	Cys	Gln	Ala	Pro
				485					490						495
Asp	His	Phe	Leu	Phe	Ala	Lys	Leu	Lys	Thr	Gln	Thr	Asn	Ala	Ser	Asp
			500					505					510		
Phe	Pro	Ile	Gly	Thr	Ser	Leu	Lys	Tyr	Glu	Cys	Arg	Pro	Glu	Tyr	Tyr
		515					520					525			
Gly	Arg	Pro	Phe	Ser	Ile	Thr	Cys	Leu	Asp	Asn	Leu	Val	Trp	Ser	Ser
	530					535					540				
Pro	Lys	Asp	Val	Cys	Lys	Arg	Lys	Ser	Cys	Lys	Thr	Pro	Pro	Asp	Pro
545					550					555					560
Val	Asn	Gly	Met	Val	His	Val	Ile	Thr	Asp	Ile	Gln	Val	Gly	Ser	Arg
				565					570					575	
Ile	Asn	Tyr	Ser	Cys	Thr	Thr	Gly	His	Arg	Leu	Ile	Gly	His	Ser	Ser
			580					585					590		
Ala	Glu	Cys	Ile	Leu	Ser	Gly	Asn	Ala	Ala	His	Trp	Ser	Thr	Lys	Pro
		595					600					605			
Pro	Ile	Cys	Gln	Arg	Ile	Pro	Cys	Gly	Leu	Pro	Pro	Thr	Ile	Ala	Asn
	610					615					620				
Gly	Asp	Phe	Ile	Ser	Thr	Asn	Arg	Glu	Asn	Phe	His	Tyr	Gly	Ser	Val
625					630					635					640
Val	Thr	Tyr	Arg	Cys	Asn	Pro	Gly	Ser	Gly	Gly	Arg	Lys	Val	Phe	Glu
				645					650					655	
Leu	Val	Gly	Glu	Pro	Ser	Ile	Tyr	Cys	Thr	Ser	Asn	Asp	Asp	Gln	Val
			660					665					670		
Gly	Ile	Trp	Ser	Gly	Pro	Ala	Pro	Gln	Cys	Ile	Ile	Pro	Asn	Lys	Cys
		675					680						685		
Thr	Pro	Pro	Asn	Val	Glu	Asn	Gly	Ile	Leu	Val	Ser	Asp	Asn	Arg	Ser
	690					695					700				
Leu	Phe	Ser	Leu	Asn	Glu	Val	Val	Glu	Phe	Arg	Cys	Gln	Pro	Gly	Phe
705					710					715					720
Val	Met	Lys	Gly	Pro	Arg	Arg	Val	Lys	Cys	Gln	Ala	Leu	Asn	Lys	Trp
				725					730					735	
Glu	Pro	Glu	Leu	Pro	Ser	Cys	Ser	Arg	Val	Cys	Gln	Pro	Pro	Pro	Asp
			740					745					750		
Val	Leu	His	Ala	Glu	Arg	Thr	Gln	Arg	Asp	Lys	Asp	Asn	Phe	Ser	Pro
		755					760					765			
Gly	Gln	Glu	Val	Phe	Tyr	Ser	Cys	Glu	Pro	Gly	Tyr	Asp	Leu	Arg	Gly
	770					775					780				
Ala	Ala	Ser	Met	Arg	Cys	Thr	Pro	Gln	Gly	Asp	Trp	Ser	Pro	Ala	Ala
785					790					795					800
Pro	Thr	Cys	Glu	Val	Lys	Ser	Cys	Asp	Asp	Phe	Met	Gly	Gln	Leu	Leu
				805					810					815	
Asn	Gly	Arg	Val	Leu	Phe	Pro	Val	Asn	Leu	Gln	Leu	Gly	Ala	Lys	Val
			820					825					830		
Asp	Phe	Val	Cys	Asp	Glu	Gly	Phe	Gln	Leu	Lys	Gly	Ser	Ser	Ala	Ser
		835					840					845			
Tyr	Cys	Val	Leu	Ala	Gly	Met	Glu	Ser	Leu	Trp	Asn	Ser	Ser	Val	Pro

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850	855	860
Val Cys Glu Gln Ile Phe Cys Pro Ser Pro Pro Val Ile Pro Asn Gly 865 870 875 880		
Arg His Thr Gly Lys Pro Leu Glu Val Phe Pro Phe Gly Lys Ala Val 885 890 895		
Asn Tyr Thr Cys Asp Pro His Pro Asp Arg Gly Thr Ser Phe Asp Leu 900 905 910		
Ile Gly Glu Ser Thr Ile Arg Cys Thr Ser Asp Pro Gln Gly Asn Gly 915 920 925		
Val Trp Ser Ser Pro Ala Pro Arg Cys Gly Ile Leu Gly His Cys Gln 930 935 940		
Ala Pro Asp His Phe Leu Phe Ala Lys Leu Lys Thr Gln Thr Asn Ala 945 950 955 960		
Ser Asp Phe Pro Ile Gly Thr Ser Leu Lys Tyr Glu Cys Arg Pro Glu 965 970 975		
Tyr Tyr Gly Arg Pro Phe Ser Ile Thr Cys Leu Asp Asn Leu Val Trp 980 985 990		
Ser Ser Pro Lys Asp Val Cys Lys Arg Lys Ser Cys Lys Thr Pro Pro 995 1000 1005		
Asp Pro Val Asn Gly Met Val His Val Ile Thr Asp Ile Gln Val 1010 1015 1020		
Gly Ser Arg Ile Asn Tyr Ser Cys Thr Thr Gly His Arg Leu Ile 1025 1030 1035		
Gly His Ser Ser Ala Glu Cys Ile Leu Ser Gly Asn Thr Ala His 1040 1045 1050		
Trp Ser Thr Lys Pro Pro Ile Cys Gln Arg Ile Pro Cys Gly Leu 1055 1060 1065		
Pro Pro Thr Ile Ala Asn Gly Asp Phe Ile Ser Thr Asn Arg Glu 1070 1075 1080		
Asn Phe His Tyr Gly Ser Val Val Thr Tyr Arg Cys Asn Leu Gly 1085 1090 1095		
Ser Arg Gly Arg Lys Val Phe Glu Leu Val Gly Glu Pro Ser Ile 1100 1105 1110		
Tyr Cys Thr Ser Asn Asp Asp Gln Val Gly Ile Trp Ser Gly Pro 1115 1120 1125		
Ala Pro Gln Cys Ile Ile Pro Asn Lys Cys Thr Pro Pro Asn Val 1130 1135 1140		
Glu Asn Gly Ile Leu Val Ser Asp Asn Arg Ser Leu Phe Ser Leu 1145 1150 1155		
Asn Glu Val Val Glu Phe Arg Cys Gln Pro Gly Phe Val Met Lys 1160 1165 1170		
Gly Pro Arg Arg Val Lys Cys Gln Ala Leu Asn Lys Trp Glu Pro 1175 1180 1185		
Glu Leu Pro Ser Cys Ser Arg Val Cys Gln Pro Pro Pro Glu Ile 1190 1195 1200		
Leu His Gly Glu His Thr Pro Ser His Gln Asp Asn Phe Ser Pro 1205 1210 1215		
Gly Gln Glu Val Phe Tyr Ser Cys Glu Pro Gly Tyr Asp Leu Arg 1220 1225 1230		
Gly Ala Ala Ser Leu His Cys Thr Pro Gln Gly Asp Trp Ser Pro 1235 1240 1245		
Glu Ala Pro Arg Cys Ala Val Lys Ser Cys Asp Asp Phe Leu Gly 1250 1255 1260		

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Gln	Leu	Pro	His	Gly	Arg	Val	Leu	Phe	Pro	Leu	Asn	Leu	Gln	Leu
1265						1270					1275			
Gly	Ala	Lys	Val	Ser	Phe	Val	Cys	Asp	Glu	Gly	Phe	Arg	Leu	Lys
1280						1285					1290			
Gly	Ser	Ser	Val	Ser	His	Cys	Val	Leu	Val	Gly	Met	Arg	Ser	Leu
1295						1300					1305			
Trp	Asn	Asn	Ser	Val	Pro	Val	Cys	Glu	His	Ile	Phe	Cys	Pro	Asn
1310						1315					1320			
Pro	Pro	Ala	Ile	Leu	Asn	Gly	Arg	His	Thr	Gly	Thr	Pro	Ser	Gly
1325						1330					1335			
Asp	Ile	Pro	Tyr	Gly	Lys	Glu	Ile	Ser	Tyr	Thr	Cys	Asp	Pro	His
1340						1345					1350			
Pro	Asp	Arg	Gly	Met	Thr	Phe	Asn	Leu	Ile	Gly	Glu	Ser	Thr	Ile
1355						1360					1365			
Arg	Cys	Thr	Ser	Asp	Pro	His	Gly	Asn	Gly	Val	Trp	Ser	Ser	Pro
1370						1375					1380			
Ala	Pro	Arg	Cys	Glu	Leu	Ser	Val	Arg	Ala	Gly	His	Cys	Lys	Thr
1385						1390					1395			
Pro	Glu	Gln	Phe	Pro	Phe	Ala	Ser	Pro	Thr	Ile	Pro	Ile	Asn	Asp
1400						1405					1410			
Phe	Glu	Phe	Pro	Val	Gly	Thr	Ser	Leu	Asn	Tyr	Glu	Cys	Arg	Pro
1415						1420					1425			
Gly	Tyr	Phe	Gly	Lys	Met	Phe	Ser	Ile	Ser	Cys	Leu	Glu	Asn	Leu
1430						1435					1440			
Val	Trp	Ser	Ser	Val	Glu	Asp	Asn	Cys	Arg	Arg	Lys	Ser	Cys	Gly
1445						1450					1455			
Pro	Pro	Pro	Glu	Pro	Phe	Asn	Gly	Met	Val	His	Ile	Asn	Thr	Asp
1460						1465					1470			
Thr	Gln	Phe	Gly	Ser	Thr	Val	Asn	Tyr	Ser	Cys	Asn	Glu	Gly	Phe
1475						1480					1485			
Arg	Leu	Ile	Gly	Ser	Pro	Ser	Thr	Thr	Cys	Leu	Val	Ser	Gly	Asn
1490						1495					1500			
Asn	Val	Thr	Trp	Asp	Lys	Lys	Ala	Pro	Ile	Cys	Glu	Ile	Ile	Ser
1505						1510					1515			
Cys	Glu	Pro	Pro	Pro	Thr	Ile	Ser	Asn	Gly	Asp	Phe	Tyr	Ser	Asn
1520						1525					1530			
Asn	Arg	Thr	Ser	Phe	His	Asn	Gly	Thr	Val	Val	Thr	Tyr	Gln	Cys
1535						1540					1545			
His	Thr	Gly	Pro	Asp	Gly	Glu	Gln	Leu	Phe	Glu	Leu	Val	Gly	Glu
1550						1555					1560			
Arg	Ser	Ile	Tyr	Cys	Thr	Ser	Lys	Asp	Asp	Gln	Val	Gly	Val	Trp
1565						1570					1575			
Ser	Ser	Pro	Pro	Pro	Arg	Cys	Ile	Ser	Thr	Asn	Lys	Cys	Thr	Ala
1580						1585					1590			
Pro	Glu	Val	Glu	Asn	Ala	Ile	Arg	Val	Pro	Gly	Asn	Arg	Ser	Phe
1595						1600					1605			
Phe	Ser	Leu	Thr	Glu	Ile	Ile	Arg	Phe	Arg	Cys	Gln	Pro	Gly	Phe
1610						1615					1620			
Val	Met	Val	Gly	Ser	His	Thr	Val	Gln	Cys	Gln	Thr	Asn	Gly	Arg
1625						1630					1635			
Trp	Gly	Pro	Lys	Leu	Pro	His	Cys	Ser	Arg	Val	Cys	Gln	Pro	Pro
1640						1645					1650			

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<210> SEQ ID NO 9
<211> LENGTH: 1231
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 9

Met Arg Leu Leu Ala Lys Ile Ile Cys Leu Met Leu Trp Ala Ile Cys
1           5           10           15

Val Ala Glu Asp Cys Asn Glu Leu Pro Pro Arg Arg Asn Thr Glu Ile
20           25           30

Leu Thr Gly Ser Trp Ser Asp Gln Thr Tyr Pro Glu Gly Thr Gln Ala
35           40           45

Ile Tyr Lys Cys Arg Pro Gly Tyr Arg Ser Leu Gly Asn Val Ile Met
50           55           60

Val Cys Arg Lys Gly Glu Trp Val Ala Leu Asn Pro Leu Arg Lys Cys
65           70           75           80

Gln Lys Arg Pro Cys Gly His Pro Gly Asp Thr Pro Phe Gly Thr Phe
85           90           95

Thr Leu Thr Gly Gly Asn Val Phe Glu Tyr Gly Val Lys Ala Val Tyr
100          105          110

Thr Cys Asn Glu Gly Tyr Gln Leu Leu Gly Glu Ile Asn Tyr Arg Glu
115          120          125

Cys Asp Thr Asp Gly Trp Thr Asn Asp Ile Pro Ile Cys Glu Val Val
130          135          140

Lys Cys Leu Pro Val Thr Ala Pro Glu Asn Gly Lys Ile Val Ser Ser
145          150          155          160

Ala Met Glu Pro Asp Arg Glu Tyr His Phe Gly Gln Ala Val Arg Phe
165          170          175

Val Cys Asn Ser Gly Tyr Lys Ile Glu Gly Asp Glu Glu Met His Cys
180          185          190

Ser Asp Asp Gly Phe Trp Ser Lys Glu Lys Pro Lys Cys Val Glu Ile
195          200          205

Ser Cys Lys Ser Pro Asp Val Ile Asn Gly Ser Pro Ile Ser Gln Lys
210          215          220

Ile Ile Tyr Lys Glu Asn Glu Arg Phe Gln Tyr Lys Cys Asn Met Gly
225          230          235          240

Tyr Glu Tyr Ser Glu Arg Gly Asp Ala Val Cys Thr Glu Ser Gly Trp
245          250          255

Arg Pro Leu Pro Ser Cys Glu Glu Lys Ser Cys Asp Asn Pro Tyr Ile
260          265          270

Pro Asn Gly Asp Tyr Ser Pro Leu Arg Ile Lys His Arg Thr Gly Asp
275          280          285

Glu Ile Thr Tyr Gln Cys Arg Asn Gly Phe Tyr Pro Ala Thr Arg Gly
290          295          300

Asn Thr Ala Lys Cys Thr Ser Thr Gly Trp Ile Pro Ala Pro Arg Cys
305          310          315          320

Thr Leu Lys Pro Cys Asp Tyr Pro Asp Ile Lys His Gly Gly Leu Tyr
325          330          335

His Glu Asn Met Arg Arg Pro Tyr Phe Pro Val Ala Val Gly Lys Tyr
340          345          350

Tyr Ser Tyr Tyr Cys Asp Glu His Phe Glu Thr Pro Ser Gly Ser Tyr
355          360          365

Trp Asp His Ile His Cys Thr Gln Asp Gly Trp Ser Pro Ala Val Pro
370          375          380

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Cys	Leu	Arg	Lys	Cys	Tyr	Phe	Pro	Tyr	Leu	Glu	Asn	Gly	Tyr	Asn	Gln	385	390	395	400
Asn	Tyr	Gly	Arg	Lys	Phe	Val	Gln	Gly	Lys	Ser	Ile	Asp	Val	Ala	Cys	405	410	415	
His	Pro	Gly	Tyr	Ala	Leu	Pro	Lys	Ala	Gln	Thr	Thr	Val	Thr	Cys	Met	420	425	430	
Glu	Asn	Gly	Trp	Ser	Pro	Thr	Pro	Arg	Cys	Ile	Arg	Val	Lys	Thr	Cys	435	440	445	
Ser	Lys	Ser	Ser	Ile	Asp	Ile	Glu	Asn	Gly	Phe	Ile	Ser	Glu	Ser	Gln	450	455	460	
Tyr	Thr	Tyr	Ala	Leu	Lys	Glu	Lys	Ala	Lys	Tyr	Gln	Cys	Lys	Leu	Gly	465	470	475	480
Tyr	Val	Thr	Ala	Asp	Gly	Glu	Thr	Ser	Gly	Ser	Ile	Thr	Cys	Gly	Lys	485	490	495	
Asp	Gly	Trp	Ser	Ala	Gln	Pro	Thr	Cys	Ile	Lys	Ser	Cys	Asp	Ile	Pro	500	505	510	
Val	Phe	Met	Asn	Ala	Arg	Thr	Lys	Asn	Asp	Phe	Thr	Trp	Phe	Lys	Leu	515	520	525	
Asn	Asp	Thr	Leu	Asp	Tyr	Glu	Cys	His	Asp	Gly	Tyr	Glu	Ser	Asn	Thr	530	535	540	
Gly	Ser	Thr	Thr	Gly	Ser	Ile	Val	Cys	Gly	Tyr	Asn	Gly	Trp	Ser	Asp	545	550	555	560
Leu	Pro	Ile	Cys	Tyr	Glu	Arg	Glu	Cys	Glu	Leu	Pro	Lys	Ile	Asp	Val	565	570	575	
His	Leu	Val	Pro	Asp	Arg	Lys	Lys	Asp	Gln	Tyr	Lys	Val	Gly	Glu	Val	580	585	590	
Leu	Lys	Phe	Ser	Cys	Lys	Pro	Gly	Phe	Thr	Ile	Val	Gly	Pro	Asn	Ser	595	600	605	
Val	Gln	Cys	Tyr	His	Phe	Gly	Leu	Ser	Pro	Asp	Leu	Pro	Ile	Cys	Lys	610	615	620	
Glu	Gln	Val	Gln	Ser	Cys	Gly	Pro	Pro	Pro	Glu	Leu	Leu	Asn	Gly	Asn	625	630	635	640
Val	Lys	Glu	Lys	Thr	Lys	Glu	Glu	Tyr	Gly	His	Ser	Glu	Val	Val	Glu	645	650	655	
Tyr	Tyr	Cys	Asn	Pro	Arg	Phe	Leu	Met	Lys	Gly	Pro	Asn	Lys	Ile	Gln	660	665	670	
Cys	Val	Asp	Gly	Glu	Trp	Thr	Thr	Leu	Pro	Val	Cys	Ile	Val	Glu	Glu	675	680	685	
Ser	Thr	Cys	Gly	Asp	Ile	Pro	Glu	Leu	Glu	His	Gly	Trp	Ala	Gln	Leu	690	695	700	
Ser	Ser	Pro	Pro	Tyr	Tyr	Tyr	Gly	Asp	Ser	Val	Glu	Phe	Asn	Cys	Ser	705	710	715	720
Glu	Ser	Phe	Thr	Met	Ile	Gly	His	Arg	Ser	Ile	Thr	Cys	Ile	His	Gly	725	730	735	
Val	Trp	Thr	Gln	Leu	Pro	Gln	Cys	Val	Ala	Ile	Asp	Lys	Leu	Lys	Lys	740	745	750	
Cys	Lys	Ser	Ser	Asn	Leu	Ile	Ile	Leu	Glu	Glu	His	Leu	Lys	Asn	Lys	755	760	765	
Lys	Glu	Phe	Asp	His	Asn	Ser	Asn	Ile	Arg	Tyr	Arg	Cys	Arg	Gly	Lys	770	775	780	
Glu	Gly	Trp	Ile	His	Thr	Val	Cys	Ile	Asn	Gly	Arg	Trp	Asp	Pro	Glu	785	790	795	800
Val	Asn	Cys	Ser	Met	Ala	Gln	Ile	Gln	Leu	Cys	Pro	Pro	Pro	Pro	Gln				

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Trp Asp Gly Lys Leu Glu Tyr Pro Thr Cys Ala Lys Arg
 1220 1225 1230

<210> SEQ ID NO 10
 <211> LENGTH: 1234
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 10

Met Arg Leu Ser Ala Arg Ile Ile Trp Leu Ile Leu Trp Thr Val Cys
 1 5 10 15

Ala Ala Glu Asp Cys Lys Gly Pro Pro Pro Arg Glu Asn Ser Glu Ile
 20 25 30

Leu Ser Gly Ser Trp Ser Glu Gln Leu Tyr Pro Glu Gly Thr Gln Ala
 35 40 45

Thr Tyr Lys Cys Arg Pro Gly Tyr Arg Thr Leu Gly Thr Ile Val Lys
 50 55 60

Val Cys Lys Asn Gly Lys Trp Val Ala Ser Asn Pro Ser Arg Ile Cys
 65 70 75 80

Arg Lys Lys Pro Cys Gly His Pro Gly Asp Thr Pro Phe Gly Ser Phe
 85 90 95

Arg Leu Ala Val Gly Ser Gln Phe Glu Phe Gly Ala Lys Val Val Tyr
 100 105 110

Thr Cys Asp Asp Gly Tyr Gln Leu Leu Gly Glu Ile Asp Tyr Arg Glu
 115 120 125

Cys Gly Ala Asp Gly Trp Ile Asn Asp Ile Pro Leu Cys Glu Val Val
 130 135 140

Lys Cys Leu Pro Val Thr Glu Leu Glu Asn Gly Arg Ile Val Ser Gly
 145 150 155 160

Ala Ala Glu Thr Asp Gln Glu Tyr Tyr Phe Gly Gln Val Val Arg Phe
 165 170 175

Glu Cys Asn Ser Gly Phe Lys Ile Glu Gly His Lys Glu Ile His Cys
 180 185 190

Ser Glu Asn Gly Leu Trp Ser Asn Glu Lys Pro Arg Cys Val Glu Ile
 195 200 205

Leu Cys Thr Pro Pro Arg Val Glu Asn Gly Asp Gly Ile Asn Val Lys
 210 215 220

Pro Val Tyr Lys Glu Asn Glu Arg Tyr His Tyr Lys Cys Lys His Gly
 225 230 235 240

Tyr Val Pro Lys Glu Arg Gly Asp Ala Val Cys Thr Gly Ser Gly Trp
 245 250 255

Ser Ser Gln Pro Phe Cys Glu Glu Lys Arg Cys Ser Pro Pro Tyr Ile
 260 265 270

Leu Asn Gly Ile Tyr Thr Pro His Arg Ile Ile His Arg Ser Asp Asp
 275 280 285

Glu Ile Arg Tyr Glu Cys Asn Tyr Gly Phe Tyr Pro Val Thr Gly Ser
 290 295 300

Thr Val Ser Lys Cys Thr Pro Thr Gly Trp Ile Pro Val Pro Arg Cys
 305 310 315 320

Thr Leu Lys Pro Cys Glu Phe Pro Gln Phe Lys Tyr Gly Arg Leu Tyr
 325 330 335

Tyr Glu Glu Ser Leu Arg Pro Asn Phe Pro Val Ser Ile Gly Asn Lys
 340 345 350

Tyr Ser Tyr Lys Cys Asp Asn Gly Phe Ser Pro Pro Ser Gly Tyr Ser

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355					360					365					
Trp	Asp	Tyr	Leu	Arg	Cys	Thr	Ala	Gln	Gly	Trp	Glu	Pro	Glu	Val	Pro
370					375					380					
Cys	Val	Arg	Lys	Cys	Val	Phe	His	Tyr	Val	Glu	Asn	Gly	Asp	Ser	Ala
385				390						395					400
Tyr	Trp	Glu	Lys	Val	Tyr	Val	Gln	Gly	Gln	Ser	Leu	Lys	Val	Gln	Cys
			405						410					415	
Tyr	Asn	Gly	Tyr	Ser	Leu	Gln	Asn	Gly	Gln	Asp	Thr	Met	Thr	Cys	Thr
		420						425					430		
Glu	Asn	Gly	Trp	Ser	Pro	Pro	Pro	Lys	Cys	Ile	Arg	Ile	Lys	Thr	Cys
		435					440						445		
Ser	Ala	Ser	Asp	Ile	His	Ile	Asp	Asn	Gly	Phe	Leu	Ser	Glu	Ser	Ser
	450					455					460				
Ser	Ile	Tyr	Ala	Leu	Asn	Arg	Glu	Thr	Ser	Tyr	Arg	Cys	Lys	Gln	Gly
465				470						475					480
Tyr	Val	Thr	Asn	Thr	Gly	Glu	Ile	Ser	Gly	Ser	Ile	Thr	Cys	Leu	Gln
			485						490					495	
Asn	Gly	Trp	Ser	Pro	Gln	Pro	Ser	Cys	Ile	Lys	Ser	Cys	Asp	Met	Pro
		500						505					510		
Val	Phe	Glu	Asn	Ser	Ile	Thr	Lys	Asn	Thr	Arg	Thr	Trp	Phe	Lys	Leu
		515					520						525		
Asn	Asp	Lys	Leu	Asp	Tyr	Glu	Cys	Leu	Val	Gly	Phe	Glu	Asn	Glu	Tyr
	530					535					540				
Lys	His	Thr	Lys	Gly	Ser	Ile	Thr	Cys	Thr	Tyr	Tyr	Gly	Trp	Ser	Asp
545				550						555					560
Thr	Pro	Ser	Cys	Tyr	Glu	Arg	Glu	Cys	Ser	Val	Pro	Thr	Leu	Asp	Arg
			565						570					575	
Lys	Leu	Val	Val	Ser	Pro	Arg	Lys	Glu	Lys	Tyr	Arg	Val	Gly	Asp	Leu
			580					585					590		
Leu	Glu	Phe	Ser	Cys	His	Ser	Gly	His	Arg	Val	Gly	Pro	Asp	Ser	Val
	595						600					605			
Gln	Cys	Tyr	His	Phe	Gly	Trp	Ser	Pro	Gly	Phe	Pro	Thr	Cys	Lys	Gly
	610					615					620				
Gln	Val	Ala	Ser	Cys	Ala	Pro	Pro	Leu	Glu	Ile	Leu	Asn	Gly	Glu	Ile
625				630						635					640
Asn	Gly	Ala	Lys	Lys	Val	Glu	Tyr	Ser	His	Gly	Glu	Val	Val	Lys	Tyr
			645						650					655	
Asp	Cys	Lys	Pro	Arg	Phe	Leu	Leu	Lys	Gly	Pro	Asn	Lys	Ile	Gln	Cys
			660					665					670		
Val	Asp	Gly	Asn	Trp	Thr	Thr	Leu	Pro	Val	Cys	Ile	Glu	Glu	Glu	Arg
	675						680					685			
Thr	Cys	Gly	Asp	Ile	Pro	Glu	Leu	Glu	His	Gly	Ser	Ala	Lys	Cys	Ser
	690					695					700				
Val	Pro	Pro	Tyr	His	His	Gly	Asp	Ser	Val	Glu	Phe	Ile	Cys	Glu	Glu
705				710						715					720
Asn	Phe	Thr	Met	Ile	Gly	His	Gly	Ser	Val	Ser	Cys	Ile	Ser	Gly	Lys
			725						730					735	
Trp	Thr	Gln	Leu	Pro	Lys	Cys	Val	Ala	Thr	Asp	Gln	Leu	Glu	Lys	Cys
			740					745					750		
Arg	Val	Leu	Lys	Ser	Thr	Gly	Ile	Glu	Ala	Ile	Lys	Pro	Lys	Leu	Thr
	755						760					765			
Glu	Phe	Thr	His	Asn	Ser	Thr	Met	Asp	Tyr	Lys	Cys	Arg	Asp	Lys	Gln
	770					775					780				

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Glu Tyr Glu Arg Ser Ile Cys Ile Asn Gly Lys Trp Asp Pro Glu Pro
 785 790 795 800
 Asn Cys Thr Ser Lys Thr Ser Cys Pro Pro Pro Pro Gln Ile Pro Asn
 805 810 815
 Thr Gln Val Ile Glu Thr Thr Val Lys Tyr Leu Asp Gly Glu Lys Leu
 820 825 830
 Ser Val Leu Cys Gln Asp Asn Tyr Leu Thr Gln Asp Ser Glu Glu Met
 835 840 845
 Val Cys Lys Asp Gly Arg Trp Gln Ser Leu Pro Arg Cys Ile Glu Lys
 850 855 860
 Ile Pro Cys Ser Gln Pro Pro Thr Ile Glu His Gly Ser Ile Asn Leu
 865 870 875 880
 Pro Arg Ser Ser Glu Glu Arg Arg Asp Ser Ile Glu Ser Ser Ser His
 885 890 895
 Glu His Gly Thr Thr Phe Ser Tyr Val Cys Asp Asp Gly Phe Arg Ile
 900 905 910
 Pro Glu Glu Asn Arg Ile Thr Cys Tyr Met Gly Lys Trp Ser Thr Pro
 915 920 925
 Pro Arg Cys Val Gly Leu Pro Cys Gly Pro Pro Pro Ser Ile Pro Leu
 930 935 940
 Gly Thr Val Ser Leu Glu Leu Glu Ser Tyr Gln His Gly Glu Glu Val
 945 950 955 960
 Thr Tyr His Cys Ser Thr Gly Phe Gly Ile Asp Gly Pro Ala Phe Ile
 965 970 975
 Ile Cys Glu Gly Gly Lys Trp Ser Asp Pro Pro Lys Cys Ile Lys Thr
 980 985 990
 Asp Cys Asp Val Leu Pro Thr Val Lys Asn Ala Ile Ile Arg Gly Lys
 995 1000 1005
 Ser Lys Lys Ser Tyr Arg Thr Gly Glu Gln Val Thr Phe Arg Cys
 1010 1015 1020
 Gln Ser Pro Tyr Gln Met Asn Gly Ser Asp Thr Val Thr Cys Val
 1025 1030 1035
 Asn Ser Arg Trp Ile Gly Gln Pro Val Cys Lys Asp Asn Ser Cys
 1040 1045 1050
 Val Asp Pro Pro His Val Pro Asn Ala Thr Ile Val Thr Arg Thr
 1055 1060 1065
 Lys Asn Lys Tyr Leu His Gly Asp Arg Val Arg Tyr Glu Cys Asn
 1070 1075 1080
 Lys Pro Leu Glu Leu Phe Gly Gln Val Glu Val Met Cys Glu Asn
 1085 1090 1095
 Gly Ile Trp Thr Glu Lys Pro Lys Cys Arg Asp Ser Thr Gly Lys
 1100 1105 1110
 Cys Gly Pro Pro Pro Pro Ile Asp Asn Gly Asp Ile Thr Ser Leu
 1115 1120 1125
 Ser Leu Pro Val Tyr Glu Pro Leu Ser Ser Val Glu Tyr Gln Cys
 1130 1135 1140
 Gln Lys Tyr Tyr Leu Leu Lys Gly Lys Lys Thr Ile Thr Cys Thr
 1145 1150 1155
 Asn Gly Lys Trp Ser Glu Pro Pro Thr Cys Leu His Ala Cys Val
 1160 1165 1170
 Ile Pro Glu Asn Ile Met Glu Ser His Asn Ile Ile Leu Lys Trp
 1175 1180 1185

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Arg His Thr Glu Lys Ile Tyr Ser His Ser Gly Glu Asp Ile Glu
 1190 1195 1200
 Phe Gly Cys Lys Tyr Gly Tyr Tyr Lys Ala Arg Asp Ser Pro Pro
 1205 1210 1215
 Phe Arg Thr Lys Cys Ile Asn Gly Thr Ile Asn Tyr Pro Thr Cys
 1220 1225 1230
 Val

 <210> SEQ ID NO 11
 <211> LENGTH: 1642
 <212> TYPE: PRT
 <213> ORGANISM: Naja kaouthia

 <400> SEQUENCE: 11
 Met Glu Arg Met Ala Leu Tyr Leu Val Ala Ala Leu Leu Ile Gly Phe
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 Pro Gly Ser Ser His Gly Ala Leu Tyr Thr Leu Ile Thr Pro Ala Val
 20 25 30
 Leu Arg Thr Asp Thr Glu Glu Gln Ile Leu Val Glu Ala His Gly Asp
 35 40 45
 Ser Thr Pro Lys Gln Leu Asp Ile Phe Val His Asp Phe Pro Arg Lys
 50 55 60
 Gln Lys Thr Leu Phe Gln Thr Arg Val Asp Met Asn Pro Ala Gly Gly
 65 70 75 80
 Met Leu Val Thr Pro Thr Ile Glu Ile Pro Ala Lys Glu Val Ser Thr
 85 90 95
 Asp Ser Arg Gln Asn Gln Tyr Val Val Val Gln Val Thr Gly Pro Gln
 100 105 110
 Val Arg Leu Glu Lys Val Val Leu Leu Ser Tyr Gln Ser Ser Phe Leu
 115 120 125
 Phe Ile Gln Thr Asp Lys Gly Ile Tyr Thr Pro Gly Ser Pro Val Leu
 130 135 140
 Tyr Arg Val Phe Ser Met Asp His Asn Thr Ser Lys Met Asn Lys Thr
 145 150 155 160
 Val Ile Val Glu Phe Gln Thr Pro Glu Gly Ile Leu Val Ser Ser Asn
 165 170 175
 Ser Val Asp Leu Asn Phe Phe Trp Pro Tyr Asn Leu Pro Asp Leu Val
 180 185 190
 Ser Leu Gly Thr Trp Arg Ile Val Ala Lys Tyr Glu His Ser Pro Glu
 195 200 205
 Asn Tyr Thr Ala Tyr Phe Asp Val Arg Lys Tyr Val Leu Pro Ser Phe
 210 215 220
 Glu Val Arg Leu Gln Pro Ser Glu Lys Phe Phe Tyr Ile Asp Gly Asn
 225 230 235 240
 Glu Asn Phe His Val Ser Ile Thr Ala Arg Tyr Leu Tyr Gly Glu Glu
 245 250 255
 Val Glu Gly Val Ala Phe Val Leu Phe Gly Val Lys Ile Asp Asp Ala
 260 265 270
 Lys Lys Ser Ile Pro Asp Ser Leu Thr Arg Ile Pro Ile Ile Asp Gly
 275 280 285
 Asp Gly Lys Ala Thr Leu Lys Arg Asp Thr Phe Arg Ser Arg Phe Pro
 290 295 300
 Asn Leu Asn Glu Leu Val Gly His Thr Leu Tyr Ala Ser Val Thr Val
 305 310 315 320

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Met	Thr	Glu	Ser	Gly	Ser	Asp	Met	Val	Val	Thr	Glu	Gln	Ser	Gly	Ile	325	330	335	
His	Ile	Val	Ala	Ser	Pro	Tyr	Gln	Ile	His	Phe	Thr	Lys	Thr	Pro	Lys	340	345	350	
Tyr	Phe	Lys	Pro	Gly	Met	Pro	Tyr	Glu	Leu	Thr	Val	Tyr	Val	Thr	Asn	355	360	365	
Pro	Asp	Gly	Ser	Pro	Ala	Ala	His	Val	Pro	Val	Val	Ser	Glu	Ala	Phe	370	375	380	
His	Ser	Met	Gly	Thr	Thr	Leu	Ser	Asp	Gly	Thr	Ala	Lys	Leu	Ile	Leu	385	390	395	400
Asn	Ile	Pro	Leu	Asn	Ala	Gln	Ser	Leu	Pro	Ile	Thr	Val	Arg	Thr	Asn	405	410	415	
His	Gly	Asp	Leu	Pro	Arg	Glu	Arg	Gln	Ala	Thr	Lys	Ser	Met	Thr	Ala	420	425	430	
Ile	Ala	Tyr	Gln	Thr	Gln	Gly	Gly	Ser	Gly	Asn	Tyr	Leu	His	Val	Ala	435	440	445	
Ile	Thr	Ser	Thr	Glu	Ile	Lys	Pro	Gly	Asp	Asn	Leu	Pro	Val	Asn	Phe	450	455	460	
Asn	Val	Lys	Gly	Asn	Ala	Asn	Ser	Leu	Lys	Gln	Ile	Lys	Tyr	Phe	Thr	465	470	475	480
Tyr	Leu	Ile	Leu	Asn	Lys	Gly	Lys	Ile	Phe	Lys	Val	Gly	Arg	Gln	Pro	485	490	495	
Arg	Arg	Asp	Gly	Gln	Asn	Leu	Val	Thr	Met	Asn	Leu	His	Ile	Thr	Pro	500	505	510	
Asp	Leu	Ile	Pro	Ser	Phe	Arg	Phe	Val	Ala	Tyr	Tyr	Gln	Val	Gly	Asn	515	520	525	
Asn	Glu	Ile	Val	Ala	Asp	Ser	Val	Trp	Val	Asp	Val	Lys	Asp	Thr	Cys	530	535	540	
Met	Gly	Thr	Leu	Val	Val	Lys	Gly	Asp	Asn	Leu	Ile	Gln	Met	Pro	Gly	545	550	555	560
Ala	Ala	Met	Lys	Ile	Lys	Leu	Glu	Gly	Asp	Pro	Gly	Ala	Arg	Val	Gly	565	570	575	
Leu	Val	Ala	Val	Asp	Lys	Ala	Val	Tyr	Val	Leu	Asn	Asp	Lys	Tyr	Lys	580	585	590	
Ile	Ser	Gln	Ala	Lys	Ile	Trp	Asp	Thr	Ile	Glu	Lys	Ser	Asp	Phe	Gly	595	600	605	
Cys	Thr	Ala	Gly	Ser	Gly	Gln	Asn	Asn	Leu	Gly	Val	Phe	Glu	Asp	Ala	610	615	620	
Gly	Leu	Ala	Leu	Thr	Thr	Ser	Thr	Asn	Leu	Asn	Thr	Lys	Gln	Arg	Ser	625	630	635	640
Ala	Ala	Lys	Cys	Pro	Gln	Pro	Ala	Asn	Arg	Arg	Arg	Arg	Ser	Ser	Val	645	650	655	
Leu	Leu	Leu	Asp	Ser	Asn	Ala	Ser	Lys	Ala	Ala	Glu	Phe	Gln	Asp	Gln	660	665	670	
Asp	Leu	Arg	Lys	Cys	Cys	Glu	Asp	Val	Met	His	Glu	Asn	Pro	Met	Gly	675	680	685	
Tyr	Thr	Cys	Glu	Lys	Arg	Ala	Lys	Tyr	Ile	Gln	Glu	Gly	Asp	Ala	Cys	690	695	700	
Lys	Ala	Ala	Phe	Leu	Glu	Cys	Cys	Arg	Tyr	Ile	Lys	Gly	Val	Arg	Asp	705	710	715	720
Glu	Asn	Gln	Arg	Glu	Ser	Glu	Leu	Phe	Leu	Ala	Arg	Asp	Asp	Asn	Glu	725	730	735	
Asp	Gly	Phe	Ile	Ala	Asp	Ser	Asp	Ile	Ile	Ser	Arg	Ser	Asp	Phe	Pro				

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740					745					750					
Lys	Ser	Trp	Leu	Trp	Leu	Thr	Lys	Asp	Leu	Thr	Glu	Glu	Pro	Asn	Ser
		755					760					765			
Gln	Gly	Ile	Ser	Ser	Lys	Thr	Met	Ser	Phe	Tyr	Leu	Arg	Asp	Ser	Ile
		770					775					780			
Thr	Thr	Trp	Val	Val	Leu	Ala	Val	Ser	Phe	Thr	Pro	Thr	Lys	Gly	Ile
							790					795			800
Cys	Val	Ala	Glu	Pro	Tyr	Glu	Ile	Arg	Val	Met	Lys	Val	Phe	Phe	Ile
				805					810					815	
Asp	Leu	Gln	Met	Pro	Tyr	Ser	Val	Val	Lys	Asn	Glu	Gln	Val	Glu	Ile
			820						825					830	
Arg	Ala	Ile	Leu	His	Asn	Tyr	Val	Asn	Glu	Asp	Ile	Tyr	Val	Arg	Val
			835				840					845			
Glu	Leu	Leu	Tyr	Asn	Pro	Ala	Phe	Cys	Ser	Ala	Ser	Thr	Lys	Gly	Gln
							855					860			
Arg	Tyr	Arg	Gln	Gln	Phe	Pro	Ile	Lys	Ala	Leu	Ser	Ser	Arg	Ala	Val
							870					875			880
Pro	Phe	Val	Ile	Val	Pro	Leu	Glu	Gln	Gly	Leu	His	Asp	Val	Glu	Ile
				885					890					895	
Lys	Ala	Ser	Val	Gln	Glu	Ala	Leu	Trp	Ser	Asp	Gly	Val	Arg	Lys	Lys
			900					905					910		
Leu	Lys	Val	Val	Pro	Glu	Gly	Val	Gln	Lys	Ser	Ile	Val	Thr	Ile	Val
			915					920					925		
Lys	Leu	Asp	Pro	Arg	Ala	Lys	Gly	Val	Gly	Gly	Thr	Gln	Leu	Glu	Val
		930					935					940			
Ile	Lys	Ala	Arg	Lys	Leu	Asp	Asp	Arg	Val	Pro	Asp	Thr	Glu	Ile	Glu
				945			950					955			960
Thr	Lys	Ile	Ile	Ile	Gln	Gly	Asp	Pro	Val	Ala	Gln	Ile	Ile	Glu	Asn
				965				970						975	
Ser	Ile	Asp	Gly	Ser	Lys	Leu	Asn	His	Leu	Ile	Ile	Thr	Pro	Ser	Gly
			980					985					990		
Cys	Gly	Glu	Gln	Asn	Met	Ile	Arg	Met	Ala	Ala	Pro	Val	Ile	Ala	Thr
		995					1000					1005			
Tyr	Tyr	Leu	Asp	Thr	Thr	Glu	Gln	Trp	Glu	Thr	Leu	Gly	Ile	Asn	
		1010					1015					1020			
Arg	Arg	Thr	Glu	Ala	Val	Asn	Gln	Ile	Val	Thr	Gly	Tyr	Ala	Gln	
		1025					1030					1035			
Gln	Met	Val	Tyr	Lys	Lys	Ala	Asp	His	Ser	Tyr	Ala	Ala	Phe	Thr	
		1040					1045					1050			
Asn	Arg	Ala	Ser	Ser	Ser	Trp	Leu	Thr	Ala	Tyr	Val	Val	Lys	Val	
		1055					1060					1065			
Phe	Ala	Met	Ala	Ala	Lys	Met	Val	Ala	Gly	Ile	Ser	His	Glu	Ile	
		1070					1075					1080			
Ile	Cys	Gly	Gly	Val	Arg	Trp	Leu	Ile	Leu	Asn	Arg	Gln	Gln	Pro	
		1085					1090					1095			
Asp	Gly	Ala	Phe	Lys	Glu	Asn	Ala	Pro	Val	Leu	Ser	Gly	Thr	Met	
		1100					1105					1110			
Gln	Gly	Gly	Ile	Gln	Gly	Ala	Glu	Glu	Glu	Val	Tyr	Leu	Thr	Ala	
		1115					1120					1125			
Phe	Ile	Leu	Val	Ala	Leu	Leu	Glu	Ser	Lys	Thr	Ile	Cys	Asn	Asp	
		1130					1135					1140			
Tyr	Val	Asn	Ser	Leu	Asp	Ser	Ser	Ile	Lys	Lys	Ala	Thr	Asn	Tyr	
		1145					1150					1155			

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Leu	Leu	Lys	Lys	Tyr	Glu	Lys	Leu	Gln	Arg	Pro	Tyr	Thr	Thr	Ala
1160						1165					1170			
Leu	Thr	Ala	Tyr	Ala	Leu	Ala	Ala	Ala	Asp	Gln	Leu	Asn	Asp	Asp
1175						1180					1185			
Arg	Val	Leu	Met	Ala	Ala	Ser	Thr	Gly	Arg	Asp	His	Trp	Glu	Glu
1190						1195					1200			
Tyr	Asn	Ala	His	Thr	His	Asn	Ile	Glu	Gly	Thr	Ser	Tyr	Ala	Leu
1205						1210					1215			
Leu	Ala	Leu	Leu	Lys	Met	Lys	Lys	Phe	Asp	Gln	Thr	Gly	Pro	Ile
1220						1225					1230			
Val	Arg	Trp	Leu	Thr	Asp	Gln	Asn	Phe	Tyr	Gly	Glu	Thr	Tyr	Gly
1235						1240					1245			
Gln	Thr	Gln	Ala	Thr	Val	Met	Ala	Phe	Gln	Ala	Leu	Ala	Glu	Tyr
1250						1255					1260			
Glu	Ile	Gln	Met	Pro	Thr	His	Lys	Asp	Leu	Asn	Leu	Asp	Ile	Thr
1265						1270					1275			
Ile	Glu	Leu	Pro	Asp	Arg	Glu	Val	Pro	Ile	Arg	Tyr	Arg	Ile	Asn
1280						1285					1290			
Tyr	Glu	Asn	Ala	Leu	Leu	Ala	Arg	Thr	Val	Glu	Thr	Lys	Leu	Asn
1295						1300					1305			
Gln	Asp	Ile	Thr	Val	Thr	Ala	Ser	Gly	Asp	Gly	Lys	Ala	Thr	Met
1310						1315					1320			
Thr	Ile	Leu	Thr	Phe	Tyr	Asn	Ala	Gln	Leu	Gln	Glu	Lys	Ala	Asn
1325						1330					1335			
Val	Cys	Asn	Lys	Phe	His	Leu	Asn	Val	Ser	Val	Glu	Asn	Ile	His
1340						1345					1350			
Leu	Asn	Ala	Met	Gly	Ala	Lys	Gly	Ala	Leu	Met	Leu	Lys	Ile	Cys
1355						1360					1365			
Thr	Arg	Tyr	Leu	Gly	Glu	Val	Asp	Ser	Thr	Met	Thr	Ile	Ile	Asp
1370						1375					1380			
Ile	Ser	Met	Leu	Thr	Gly	Phe	Leu	Pro	Asp	Ala	Glu	Asp	Leu	Thr
1385						1390					1395			
Arg	Leu	Ser	Lys	Gly	Val	Asp	Arg	Tyr	Ile	Ser	Arg	Tyr	Glu	Val
1400						1405					1410			
Asp	Asn	Asn	Met	Ala	Gln	Lys	Val	Ala	Val	Ile	Ile	Tyr	Leu	Asn
1415						1420					1425			
Lys	Val	Ser	His	Ser	Glu	Asp	Glu	Cys	Leu	His	Phe	Lys	Ile	Leu
1430						1435					1440			
Lys	His	Phe	Glu	Val	Gly	Phe	Ile	Gln	Pro	Gly	Ser	Val	Lys	Val
1445						1450					1455			
Tyr	Ser	Tyr	Tyr	Asn	Leu	Asp	Glu	Lys	Cys	Thr	Lys	Phe	Tyr	His
1460						1465					1470			
Pro	Asp	Lys	Gly	Thr	Gly	Leu	Leu	Asn	Lys	Ile	Cys	Ile	Gly	Asn
1475						1480					1485			
Val	Cys	Arg	Cys	Ala	Gly	Glu	Thr	Cys	Ser	Ser	Leu	Asn	His	Gln
1490						1495					1500			
Glu	Arg	Ile	Asp	Val	Pro	Leu	Gln	Ile	Glu	Lys	Ala	Cys	Glu	Thr
1505						1510					1515			
Asn	Val	Asp	Tyr	Val	Tyr	Lys	Thr	Lys	Leu	Leu	Arg	Ile	Glu	Glu
1520						1525					1530			
Gln	Asp	Gly	Asn	Asp	Ile	Tyr	Val	Met	Asp	Val	Leu	Glu	Val	Ile
1535						1540					1545			

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Lys Gln Gly Thr Asp Glu Asn Pro Arg Ala Lys Thr His Gln Tyr
 1550 1555 1560

Ile Ser Gln Arg Lys Cys Gln Glu Ala Leu Asn Leu Lys Val Asn
 1565 1570 1575

Asp Asp Tyr Leu Ile Trp Gly Ser Arg Ser Asp Leu Leu Pro Thr
 1580 1585 1590

Lys Asp Lys Ile Ser Tyr Ile Ile Thr Lys Asn Thr Trp Ile Glu
 1595 1600 1605

Arg Trp Pro His Glu Asp Glu Cys Gln Glu Glu Glu Phe Gln Lys
 1610 1615 1620

Leu Cys Asp Asp Phe Ala Gln Phe Ser Tyr Thr Leu Thr Glu Phe
 1625 1630 1635

Gly Cys Pro Thr
 1640

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Gly Ser
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<210> SEQ ID NO 13
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 <223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 13

Gly Ser Gly Ser
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<210> SEQ ID NO 14
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 <212> TYPE: PRT
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 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Construct

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Gly Ser Gly Ser Gly Ser
 1 5

<210> SEQ ID NO 15
 <211> LENGTH: 8
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 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 15

Gly Ser Gly Ser Gly Ser Gly Ser
 1 5

<210> SEQ ID NO 16
 <211> LENGTH: 10
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Synthetic Construct

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Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser
 1 5 10

<210> SEQ ID NO 17

<211> LENGTH: 12

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 17

Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser
 1 5 10

<210> SEQ ID NO 18

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 18

Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser
 1 5 10

<210> SEQ ID NO 19

<211> LENGTH: 16

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 19

Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser
 1 5 10 15

<210> SEQ ID NO 20

<211> LENGTH: 4

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 20

Gly Gly Gly Ser
 1

<210> SEQ ID NO 21

<211> LENGTH: 8

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 21

Gly Gly Gly Ser Gly Gly Gly Ser
 1 5

<210> SEQ ID NO 22

<211> LENGTH: 12

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

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<400> SEQUENCE: 22

Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser
 1 5 10

<210> SEQ ID NO 23

<211> LENGTH: 16

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 23

Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser
 1 5 10 15

<210> SEQ ID NO 24

<211> LENGTH: 5

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

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<400> SEQUENCE: 24

Gly Gly Gly Gly Ser
 1 5

<210> SEQ ID NO 25

<211> LENGTH: 10

<212> TYPE: PRT

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Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 1 5 10

<210> SEQ ID NO 26

<211> LENGTH: 15

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<400> SEQUENCE: 26

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 1 5 10 15

<210> SEQ ID NO 27

<211> LENGTH: 20

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
 1 5 10 15

Gly Gly Gly Ser
 20

<210> SEQ ID NO 28

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Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
 1 5 10 15

Gly Gly Gly Ser Gly Gly Gly Gly Ser
 20 25

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Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
 1 5 10 15

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 20 25 30

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Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
 1 5 10 15

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
 20 25 30

Gly Gly Ser
 35

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Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
 1 5 10 15

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
 20 25 30

Gly Gly Ser Gly Gly Gly Gly Ser
 35 40

<210> SEQ ID NO 32
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<400> SEQUENCE: 32

Gly Ser Ser Gly
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<210> SEQ ID NO 33
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 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 33

Gly Ser Ser Gly Gly Ser Ser Gly
 1 5

<210> SEQ ID NO 34
 <211> LENGTH: 12
 <212> TYPE: PRT
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 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 34

Gly Ser Ser Gly Gly Ser Ser Gly Gly Ser Ser Gly
 1 5 10

<210> SEQ ID NO 35
 <211> LENGTH: 16
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
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<400> SEQUENCE: 35

Gly Ser Ser Gly Gly Ser Ser Gly Gly Ser Ser Gly Gly Ser Ser Gly
 1 5 10 15

What we claim is:

1. An isolated antibody or antigen-binding fragment thereof, comprising the complementarity determining regions of an antibody selected from the group consisting of:

- i) [3db8] 3d8b produced by hybridoma cell line 3d-8b/2 (ATCC Deposit PTA-10999);
- ii) 3d9a produced by hybridoma cell line 3d-9a/25 (ATCC Deposit PTA-10998);
- iii) 3d29 produced by hybridoma cell line 3d-29/5/2 (ATCC Deposit PTA-11000);
- iv) 3d11 produced by hybridoma cell line 3d-11/14 (ATCC Deposit PTA-10011);
- v) 3d31 produced by hybridoma cell line 3d-31/A6/9 (ATCC Deposit PTA-11027);
- vi) 3d3 produced by hybridoma cell line 3d-3/28/4 (ATCC Deposit PTA-11025);
- vii) 3d15 produced by hybridoma cell line 3d-15A9 (ATCC Deposit PTA-11012);
- viii) 3di0 produced by hybridoma cell line 3d-10/14/1 (ATCC Deposit PTA-11010); and
- ix) 3d16 produced by hybridoma cell line 3d-16/3/3 (ATCC Deposit PTA-11026);

wherein said isolated antibody or antigen-binding fragment thereof is capable of specifically binding to a mammalian complement component C3d protein.

2. The isolated antibody or antigen-binding fragment thereof of claim 1 or 19, wherein said antibody or antigen-binding fragment thereof is a humanized, primatized, or chimerized antibody or antigen-binding fragment thereof.

3. The isolated antibody or antigen-binding fragment thereof of claim 1 or 19, wherein said antibody or antigen-binding fragment thereof is selected from the group con-

35 sisting of: a monoclonal antibody or antibody fragment, a single chain antibody, an Fv, an Fab, an Fab', and an F(ab')₂.

4. The isolated antibody of claim 3, wherein said antibody is a monoclonal antibody.

5. The isolated antibody of claim 4, wherein said antibody is selected from the group consisting of:

- i) [3db8] 3d8b produced by hybridoma cell line 3d-8b/2 (ATCC Deposit PTA-10999);
- ii) 3d9a produced by hybridoma cell line 3d-9a/25 (ATCC Deposit PTA-10998);
- iii) 3d29 produced by hybridoma cell line 3d-29/5/2 (ATCC Deposit PTA-11000);
- iv) 3d11 produced by hybridoma cell line 3d-11/14 (ATCC Deposit PTA-10011);
- v) 3d31 produced by hybridoma cell line 3d-31/A6/9 (ATCC Deposit PTA-11027);
- vi) 3d3 produced by hybridoma cell line 3d-3/28/4 (ATCC Deposit PTA-11025);
- vii) 3d15 produced by hybridoma cell line 3d-15A9 (ATCC Deposit PTA-11012);
- viii) 3di0 produced by hybridoma cell line 3d-10/14/1 (ATCC Deposit PTA-11010); and
- ix) 3d16 produced by hybridoma cell line 3d-16/3/3 (ATCC Deposit PTA-11026).

6. A hybridoma cell selected from the group consisting of: 3d-8b/2 (ATCC Deposit PTA-10999), 3d-9a/25 (ATCC Deposit number: PTA-10998), 3d-29/5/2 (ATCC Deposit number: PTA-11000), 3d-11/14 (ATCC Deposit number: PTA-11011), 3d-31/A6/9 (ATCC Deposit number: PTA-11027), 3d-3/28/4 (ATCC Deposit number: PTA-11025), 3d-15A9 (ATCC Deposit number: PTA-11012), 3d-10/14/1 (ATCC Deposit number: PTA-11010), and 3d-16/3/3 (ATCC Deposit number: PTA-11026).

7. A pharmaceutical composition comprising the isolated antibody or antigen-binding fragment thereof of claim 1 or 19, and a pharmaceutically-acceptable excipient.

8. A therapeutic kit comprising: (i) the antibody or antigen-binding fragment thereof of claim 1 or 19, and (ii) means for delivering the antibody or antigen-binding fragment thereof to a human.

9. A pre-filled syringe comprising the antibody or antigen-binding fragment thereof of claim 1 or 19.

10. The isolated antibody or antigen-binding fragment thereof of claim 1 or 19, comprising the complementarity determining regions of the antibody [3db8] 3d8b produced by hybridoma cell line 3d-8b/2 (ATCC Deposit PTA-10999).

11. The isolated antibody or antigen-binding fragment thereof of claim 1 or 19, comprising the complementarity determining regions of the antibody 3d9a produced by hybridoma cell line 3d-9a/25 (ATCC Deposit PTA-10998).

12. The isolated antibody or antigen-binding fragment thereof of claim 1 or 19, comprising the complementarity determining regions of the antibody 3d29 produced by hybridoma cell line 3d-29/5/2 (ATCC Deposit PTA-11000).

13. The isolated antibody or antigen-binding fragment thereof of claim 1 or 19, comprising the complementarity determining regions of the antibody 3d11 produced by hybridoma cell line 3d-11/14 (ATCC Deposit PTA-10011).

14. The isolated antibody or antigen-binding fragment thereof of claim 1 or 19, comprising the complementarity determining regions of the antibody 3d31 produced by hybridoma cell line 3d-31/A6/9 (ATCC Deposit PTA-11027).

15. The isolated antibody or antigen-binding fragment thereof of claim 1 or 19, comprising the complementarity determining regions of the antibody 3d3 produced by hybridoma cell line 3d-3/28/4 (ATCC Deposit PTA-11025).

16. The isolated antibody or antigen-binding fragment thereof of claim 1 or 19, comprising the complementarity determining regions of the antibody 3d15 produced by hybridoma cell line 3d-15A9 (ATCC Deposit PTA-11012).

17. The isolated antibody or antigen-binding fragment thereof of claim 1 or 19, comprising the complementarity

determining regions of the antibody 3d10 produced by hybridoma cell line 3d-10/14/1 (ATCC Deposit PTA-11010).

18. The isolated antibody or antigen-binding fragment thereof of claim 1 or 19, comprising the complementarity determining regions of the antibody 3d16 produced by hybridoma cell line 3d-16/3/3 (ATCC Deposit PTA-11026).

19. An isolated antibody or antigen-binding fragment thereof, comprising all six of the complementarity determining region (CDR) of an antibody selected from the group consisting of:

i) 3d8b produced by hybridoma cell line 3d-8b/2 (ATCC Deposit PTA-10999);

ii) 3d9a produced by hybridoma cell line 3d-9a/25 (ATCC Deposit PTA-10998);

iii) 3d29 produced by hybridoma cell line 3d-29/5/2 (ATCC Deposit PTA-11000);

iv) 3d11 produced by hybridoma cell line 3d-11/14 (ATCC Deposit PTA-10011);

v) 3d31 produced by hybridoma cell line 3d-31/A6/9 (ATCC Deposit PTA-11027);

vi) 3d3 produced by hybridoma cell line 3d-3/28/4 (ATCC Deposit PTA-11025);

vii) 3d15 produced by hybridoma cell line 3d-15A9 (ATCC Deposit PTA-11012);

viii) 3d10 produced by hybridoma cell line 3d-10/14/1 (ATCC Deposit PTA-11010); and

ix) 3d16 produced by hybridoma cell line 3d-16/3/3 (ATCC Deposit PTA-11026);

wherein said isolated antibody or antigen-binding fragment thereof is capable of specifically binding to a mammalian complement component C3d protein, and wherein said isolated antibody or antigen-binding fragment thereof optionally further comprises a complement modulator conjugated to said antibody or antigen-binding fragment.

20. The isolated antibody or antigen-binding fragment thereof of claim 1 or 19, which comprises the heavy chain variable region (VH) of any one of the antibodies of i)-ix).

* * * * *