ANTIBODIES TO THE C3d FRAGMENT OF COMPLEMENT COMPONENT 3

RELATED APPLICATION

[0001] This application claims the benefit of U.S. provisional patent application serial no. 61/357,499, filed on 22 June 2010, ~~the disclosure of which is incorporated herein by reference in its entirety~~.

FIELD OF THE INVENTION

[0002] 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

[0003] 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

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.

[0007] 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.

[0008] 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.

[0008a] A murine monoclonal antibody raised against human C3d that possessed specificity for an antigenic determinant expressed by C3bi, C3dg and C3d, which is almost undetectable in C3 and C3b and completely absent from C3c is described in Tamerius J.D. et al, *The Journal of Immunology*, (1995), Vol. 135, No.3, pages 2015-2019. Ten monoclonal antibodies to C3d obtained by hybridization after the immunization of mice with purified human complement components are described in Dobbie D.et al, *Transfusion*, (1987), Vol. 27, No.6, pages 453-459.

~~[0009] 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

[0010] 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.

[0011] 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 complement 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.

[0012] 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.

[0013] In another aspect, the present disclosure provides an isolated anti-C3d/C3dg antibody

other C3 fragments C3a, C3b, C3c or C3f from the same species over C3d/C3dg.

[0015] 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 ($C3H_2O$). 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 ($C3H_2O$)) or other C3 fragments C3a, C3b, C3c or C3f from the same species.

[0016] 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.

[0017] 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 disclosure further provides an isolated anti-C3d/C3dg antibody or antigen-binding fragment thereof capable of decreasing, reducing or inhibiting host humoral immune response.

[0018] 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.

[0019] 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(H_2O), C3b and C3dg.

[0020] 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.

[0021] 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.

[0022] 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).

[0023] 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.

[0024] In another aspect, the present disclosure provides an isolated nucleic acid molecule encoding the antibody or antigen-binding fragment thereof described in this disclosure.

[0025] 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.

avoiding or reducing adverse or undesired systemic effects.

[0031] 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.

[0032] 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).

[0033] 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.

[0034] 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.

[0035] 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.

[0036] 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 to 8; $(GlyGlyGlySer)_n$, wherein n = 1 to 4; $(GlyGlyGlyGlySer)_n$, wherein n = 1 to 8; or $(GlySerSerGly)_n$, wherein n = 1 to 4.

[0037] 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 ID 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 ID 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.

[0038] 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 Fc domain, mouse IgM, mouse IgM Fc domain, and cobra venom factor (CVF).

[0039] In another aspect, the present disclosure provides an isolated nucleic acid molecule encoding the fusion construct described herein.

[0040] 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.

[0041] 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.

[0042] 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.

[0043] 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.

[0044] 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.

[0045] 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.

[0046] 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

Elsen et al. A Crystal Structure of the Complex Between Human Complement Receptor 2 and Its Ligand C3d. *Science* 332, 608 (2011).

[0072] 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.

[0073] "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.

[0074] 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).

[0075] 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,

[0079] 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.

[0080] 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.~~

[0081] 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

[0082] Hybridoma line 3d-9a/25 was deposited on May 26, 2010 and has been designated as ATCC Patent Deposit PTA-10998.

[0083] Hybridoma line 3d-8b/2 was deposited on May 26, 2010 and has been designated as ATCC Patent Deposit PTA-10999.

[0084] Hybridoma line 3d-29/5/2 was deposited on May 26, 2010 and has been designated as ATCC Patent Deposit PTA-11000.

[0085] Hybridoma line 3d-10/14/1 was deposited on June 2, 2010 and has been designated as ATCC Patent Deposit PTA-11010.

[0086] Hybridoma line 3d-11/14 was deposited on June 2, 2010 and has been designated as ATCC Patent Deposit PTA-11011.

[0087] Hybridoma line 3d-15A9 was deposited on June 2, 2010 and has been designated as

[0101] **Figure 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.

[0102] **Figure 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

[0103] 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.

[0104] The molecules are also useful for targeting a complement modulator to local sites of complement activation. Accordingly, 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.

[0105] 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

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.

[0113] However, the previously known antibodies to C3, C3b and C3d do not include all of the characteristics of the antibodies identified herein.

[0114] 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.

[0115] 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).

[0116] 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}.

[0117] 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. Patent No. 6,355,245.

[0118] 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.

[0119] 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/25anti-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.

[0120] 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-C5 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

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.

[0128] 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.

[0129] 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.

[0130] 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, Massachusetts); IBIS system (Windsor Scientific Limited; Berks, UK), SPR-CELLIA systems (Nippon Laser and Electronics Lab; Hokkaido, Japan), and SPR Detector Spreeta (Texas Instruments; Dallas, Texas) 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.

[0131] 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

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.

[0134] 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.

[0135] 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. Patent 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).

[0136] 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

[0140] 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].

[0141] 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. Patent 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.~~

[0142] 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 delineated in U.S. Patent 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.~~

[0143] 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. Patent 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; Tuaillon 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; Tuaillon et al. (1995) J. Immunol. 154: 6453-65; Fishwild et al. (1996) Nature Biotechnology 14: 845; and Tuaillon et al. (2000) Eur. J. Immunol. 10: 2998-3005, the disclosures of each of which are hereby incorporated by reference in their entirety.

[0144] 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

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.

[0162] 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.

[0163] 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 FcγRIIIA), 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

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

[0170] The present disclosure also provides compositions containing a fusion molecule comprising a targeting moiety and an active prophylactic, therapeutic, or diagnostic moiety.

[0171] 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.

[0172] 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.

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 Immunochemistry, 2nd Edition," 135-240, Springfield, IL, 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.

[0176] 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.

[0177] 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).

[0178] Additional methods for detecting and/or measuring complement activity *in vitro* are set forth and exemplified in the working examples.

[0179] 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-C5 antibody marketed under the trade name Soliris®), pexelizumab (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, and 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, and 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 G_1 (IgG_1), human Ig isotype M (IgM), mouse Ig isotype G_3 (IgG_3), 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 IgG_1 Fc domain, human IgM Fc domain, mouse IgG_3 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 Fritzinger 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.

[0180] 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.

[0181] 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.

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.

[0195] 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.

[0196] 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.

[0197] 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).

[0198] 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

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.

[0205] In some embodiments, the anti-C5 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. patent no. 6,355,245.

[0206] In some embodiments, the anti-C5 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.

[0207] Additional anti-C5 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.

[0208] 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. patent no. 5,679,345.

[0209] 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 09/029669.

[0210] 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. patent no. 7,439,331; or U.S. patent application publication no. 20080118506.

[0211] 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.

[0212] 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. patent no. 6,355,245 and PCT application publication no. WO 2010/015608.

[0213] 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. Patent No. 6,355,245.

[0214] 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 G_1 (IgG_1), human Ig isotype M (IgM), mouse Ig isotype G_3 (IgG_3), 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 IgG_1 Fc domain, human IgM Fc domain, mouse IgG_3 Fc domain, and mouse IgM Fc domain.

[0215] 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 Fritzinger, 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 Fritzinger 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 Fritzinger 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.

[0216] 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 IgG_1, human IgG_1 Fc domain, human IgM, human IgM Fc domain, mouse IgG_3, mouse IgG_3 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.

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.

[0221] 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

[0222] 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 07/129895~~, 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) [Cardarelli et al. (2010) *Cancer Immunol Immunother* 59(2):257-265].

[0223] 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, 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

mammalian milk products are described in, e.g., U.S. patent application publication nos. 200600105347 and 20040006776 and U.S. patent no. 7,045,676.

[0233] 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).

[0234] 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.

[0235] 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, New York. 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.

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.

[0242] 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

[0243] 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-succinimidyloxycarbonyl-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-

Publication No. 20080241223; U.S. Patent 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.

[0249] 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.

[0250] 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, temperomandibular, 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.

[0251] 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

[0254] 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~~.

[0255] 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. Patent No. 6,302,855 may also be used, for example, with an injection system of the present disclosure.

[0256] An injection system of the present disclosure may employ a delivery pen as described in U.S. Patent 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.

[0257] 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.

[0258] 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. Patent No. 6,192,891.

[0259] 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. patent 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. patent 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.

[0260] 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.

[0261] 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

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.

[0277] 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.

[0278] 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.

[0279] 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).

[0280] 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.

[0281] 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.

[0282] 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.

[0283] 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.

[0284] 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.

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.

[0288] 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.

[0289] The pharmaceutical compositions may further comprise additional ingredients, for example preservatives, buffers, tonicity agents, antioxidants and stabilizers, nonionic wetting or clarifying agents, and viscosity-increasing agents~~, and the like~~.

[0290] Suitable preservatives for use in a solution include polyquaternium-1, benzalkonium chloride, thimerosal, chlorobutanol, methyl paraben, propyl paraben, phenylethyl alcohol, disodium-EDTA, sorbic acid, and 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.

[0291] Suitable buffers include boric acid, sodium and potassium bicarbonate, sodium and potassium borates, sodium and potassium carbonate, sodium acetate, and 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.

[0292] Suitable tonicity agents include dextran 40, dextran 70, dextrose, glycerin, potassium chloride, propylene glycol, and 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%.

[0293] Suitable antioxidants and stabilizers include sodium bisulfite, sodium metabisulfite, sodium thiosulfite, and 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, and carboxymethylcellulose ~~and the like~~.

[0294] 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. Patent 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.

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 protein, antibody, or antigen-binding fragment thereof are known in the art and described herein.

Methods for Administration

[0301] The pharmaceutical compositions may be suitable for a variety of modes of administration described herein, including 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 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 intravenous injection.

[0302] 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 (e.g., as mists of sprays), mucosal (such as via nasal mucosa), subcutaneous, gastrointestinal, intraarticular, intracisternal, intraventricular, rectal (i.e., via suppository), vaginal (i.e., via pessary), intracranial, intraurethral, intrahepatic, and intratumoral. In some embodiments, the compositions are administered systemically (for example by intravenous injection). In some embodiments, the compositions are administered locally (for example by intraarterial or intraocular injection).

[0303] In some embodiments, the compositions are administered 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.

[0304] In some embodiments, the compositions are administered 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 compositions 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 intraocular injection, periocular injection, subretinal injection, intravitreal injection, trans-septal injection, subscleral injection, intrachoroidal injection, intracameral injection, subconjunctival injection, sub-Tenon's injection, retrobulbar injection, peribulbar injection, or posterior juxtascleral 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. (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.

[0305] The compositions can also be administered to the individual 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., US 5,501,856, 5,476,511, and 6,331,313. The compositions can also be administered to the individual using iontophoresis, including~~, but are not limited to,~~ the ionophoretic methods described in US 4,454,151 and US 2003/0181531 and 2004/0058313.

Dosage

[0306] 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 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 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., 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).

[0307] 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

achieve a circulating plasma concentration range that includes the IC_{50} (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.

[0313] 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.

[0314] 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, and microspheres, ~~and the like~~) may be administered by injection or surgically implanted in various locations in the body.

Gene Therapy

[0315] 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.

[0316] 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.

[0317] 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.

[0318] 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.

[0319] 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).

[0320] 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.

[0321] 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.

[0322] 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.

[0323] 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.

[0324] 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

[0325] 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.

[0326] 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, and flexible packaging (e.g., sealed Mylar or plastic bags), and the like. These articles of manufacture may further be sterilized and/or sealed.

[0327] 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)

[0351] 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 (fH) 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 µl of serum; and 5) PBS to bring the total volume to 100 µl. 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.

[0352] 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.

[0353] This experiment is designed to illustrate the effects of treatment of intestinal ischemia and reperfusion injury in a mouse model.

Intestinal ischemia reperfusion injury.

[0354] 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 arterial 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.

[0355] 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.

[0356] 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

[0357] This example is designed to illustrate the effects of antiC3dFab-FH on renal ischemia reperfusion.

Protocol for induction of ischemic acute renal failure (ARF).

[0358] 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

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.

[0363] 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.

[0364] 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.

[0365] 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 Vis 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.

[0366] The effects of antiC3dFab-FH are measured using four parameters: functional activity (ERG potentials, i.e., photoreceptor and RPE responses), histology, vascular integrity (choroidal

[0372] 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.

[0373] In a separate experiment, 1 μg 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.

[0374] 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.

[0375] 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.

[0376] 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.

[0377] 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.

[0394] SEQ ID NO:11 [amino acid sequence of CVF from *Naja kaouthia*]:

MERMALYLVAALLIGFPGSSHGALYTLITPAVLRTDTEEQILVEAHGDSTPKQLDIFVHD
FPRKQKTLFQTRVDMNPAGGMLVTPTIEIPAKEVSTDSRQNQYVVVQVTGPQVRLEKV
VLLSYQSSFLFIQTDKGIYTPGSPVLYRVFSMDHNTSKMNKTVIVEFQTPEGILVSSNSVD
LNFFWPYNLPDLVSLGTWRIVAKYEHSPENYTAYFDVRKYVLPSFEVRLQPSEKFFYID
GNENFHVSITARYLYGEEVEGVAFVLFGVKIDDAKKSIPDSLTRIPIIDGDGKATLKRDTF
RSRFPNLNELVGHTLYASVTVMTESGSDMVVTEQSGIHIVASPYQIHFTKTPKYFKPGMP
YELTVYVTNPDGSPAAHVPVVSEAFHSMGTTLSDGTAKLILNIPLNAQSLPITVRTNHGD
LPRERQATKSMTAIAYQTQGGSGNYLHVAITSTEIKPGDNLPVNFNVKGNANSLKQIKY
FTYLILNKGKIFKVGRQPRRDGQNLVTMNLHITPDLIPSFRFVAYYQVGNNEIVADSVW
VDVKDTCMGTLVVKGDNLIQMPGAAMKIKLEGDPGARVGLVAVDKAVYVLNDKYKIS
QAKIWDTIEKSDFGCTAGSGQNNLGVFEDAGLALTTSTNLNTKQRSAAKCPQPANRRR
RSSVLLLDSNASKAAEFQDQDLRKCCEDVMHENPMGYTCEKRAKYIQEGDACKAAFLE
CCRYIKGVRDENQRESELFLARDDNEDGFIADSDIISRSDFPKSWLWLTKDLTEEPNSQGI
SSKTMSFYLRDSITTWVVLAVSFTPTKGICVAEPYEIRVMKVFFIDLQMPYSVVKNEQVE
IRAILHNYVNEDIYVRVELLYNPAFCSASTKGQRYRQQFPIKALSSRAVPFVIVPLEQGLH
DVEIKASVQEALWSDGVRKKLKVVPEGVQKSIVTIVKLDPRAKGVGGTQLEVIKARKL
DDRVPDTEIETKIIIQGDPVAQIIENSIDGSKLNHLIITPSGCGEQNMIRMAAPVIATYYLDT
TEQWETLGINRRTEAVNQIVTGYAQQMVYKKADHSYAAFTNRASSSWLTAYVVKVFA
MAAKMVAGISHEIICGGVRWLILNRQQPDGAFKENAPVLSGTMQGGIQGAEEEVYLTAF
ILVALLESKTICNDYVNSLDSSIKKATNYLLKKYEKLQRPYTTALTAYALAAADQLNDD
RVLMAASTGRDHWEEYNAHTHNIEGTSYALLALLKMKKFDQTGPIVRWLTDQNFYGE
TYGQTQATVMAFQALAEYEIQMPTHKDLNLDITIELPDREVPIRYRINYENALLARTVET
KLNQDITVTASGDGKATMTILTFYNAQLQEKANVCNKFHLNVSVENIHLNAMGAKGAL
MLKICTRYLGEVDSTMTIIDISMLTGFLPDAEDLTRLSKGVDRYISRYEVDNNMAQKVA
VIIYLNKVSHSEDECLHFKILKHFEVGFIQPGSVKVYSYYNLDEKCTKFYHPDKGTGLLN
KICIGNVCRCAGETCSSLNHQERIDVPLQIEKACETNVDYVYKTKLLRIEEQDGNDIYVM
DVLEVIKQGTDENPRAKTHQYISQRKCQEALNLKVNDDYLIWGSRSDLLPTKDKISYIIT
KNTWIERWPHEDECQEEEFQKLCDDFAQFSYTLTEFGCPT

[0395] 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~~.

[0396] All publications that are cited herein are hereby specifically incorporated herein by reference into the disclosure for the teachings for which they are cited.

CLAIMS

1. An isolated antibody or antigen-binding fragment thereof specifically binding to a mammalian complement component C3d protein, wherein the antibody or antigen-binding fragment thereof is selected from the group consisting of:

a) an isolated anti-C3d 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;

b) an isolated anti-C3d antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof binds to C3d proteins of at least two species;

c) an isolated anti-C3d antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof competes with complement receptor 2 (CR2) for binding to C3d; and

d) an isolated anti-C3d antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof decreases host humoral immune response.

2. The isolated antibody or antigen-binding fragment thereof of claim 1(a), wherein said K_D is 0.5 nM or better; or

the isolated antibody or antigen-binding fragment thereof of claim 1, wherein said mammalian complement component C3d protein is a human complement component C3d protein; or

the isolated antibody or antigen-binding fragment thereof of claim 1, wherein said mammalian complement component C3d protein is a non-human primate complement component C3d protein selected from the group consisting of orangutan, chimpanzee, macaque, gorilla, lemur, or gibbon complement component C3d protein;

optionally wherein the isolated antibody or antigen-binding fragment is selected from the group consisting of: 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 $F(ab')_2$, optionally said antibody is selected from the group consisting of: i) 3d8b produced by hybridoma cell line 3d-8b/2 (ATCC Deposit number: PTA-10999); ii) 3d9a produced by hybridoma cell line 3d-9a/25 (ATCC Deposit number: PTA-10998); and iii) 3d29 produced by hybridoma cell line 3d-29/5/2 (ATCC Deposit number: PTA-11000).

3. 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).

4. An isolated antibody selected from the group consisting of: 3d8b, 3d9a, 3d29, 3d11, 3d31, 3d3, 3d15, 3d10, and 3d16.

5. An isolated nucleic acid molecule comprising a nucleotide sequence encoding the antibody or antigen-binding fragment thereof of any one of claims 1, 2, and 4.

6. An expression vector comprising the isolated nucleic acid molecule of claim 5.

7. A cell comprising the expression vector of claim 6.

8. A method for producing an antibody or antigen-binding fragment thereof, the method comprising culturing the cell of claim 7 under conditions suitable to allow expression of the antibody or antigen-binding fragment by the cell, optionally further comprising isolating the antibody or antigen-binding fragment thereof from the cell or the culture media in which the cell as cultured.

9. A pharmaceutical composition comprising the isolated antibody or antigen-binding fragment thereof of any one of claims 1, 2, and 4 and a pharmaceutically-acceptable excipient.

10. A construct comprising:

a) a C3d binding portion, optionally wherein said C3d binding portion comprises said anti-C3d antibody or antigen-binding fragment thereof of any one of claims 1, 2, and 4; and

b) a complement modulator portion, optionally wherein said complement modulator portion is a compound, composition, or protein;

wherein (a) and (b) are joined.

11. The construct of claim 10, wherein:

said construct modulates complement activity in the complement alternative pathway (CAP); or

said construct is a fusion protein; or

said C3d binding portion and said complement modulator portion are joined directly without a linker; or

said C3d binding portion and said complement modulator portion are joined by a linker, optionally wherein said linker is a peptide, optionally wherein said peptide is selected from the group consisting of: (GlySer)n, wherein n = 1 to 8; (GlyGlyGlySer)n, wherein n = 1 to 4; (GlyGlyGlyGlySer)n, wherein n = 1 to 8; and (GlySerSerGly)n, wherein n = 1 to 4.

12. The construct of claim 10, wherein said complement modulator portion comprises a complement inhibitor or biologically active fragment thereof, optionally wherein:

said complement inhibitor or biologically active fragment thereof is selected from the group consisting of: human membrane complement protein (MCP), human decay accelerating factor (DAF), human CD59, human complement receptor 1 (CR1), human factor H, Crry, and biologically active fragments or variants of any of the foregoing; or

said complement inhibitor is selected from the group consisting of: human membrane complement protein (MCP) (SEQ ID NO:1), human decay accelerating factor (DAF) (SEQ ID NO:2), mouse DAF (SEQ ID NO:3), mouse complement receptor 1-related gene/protein y (Crry) (SEQ ID NO:7), human CD59 (SEQ ID NO:4), mouse CD59 isoform A (SEQ ID NO:5), mouse CD59 isoform B (SEQ ID NO:6), human complement receptor 1 (CR1) (SEQ ID NO:8), human factor H (SEQ ID NO:9), and mouse factor H (SEQ ID NO:10), and biologically active fragments thereof, optionally wherein said biologically active fragment thereof is selected from the group consisting of: 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 ID 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 ID 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.

13. The construct of claim 10 or 11, wherein said complement modulator portion comprises a complement activator or biologically active fragment thereof, optionally wherein said complement activator or biologically active fragment thereof is selected from the group consisting of: human IgG1, human IgG1 Fc domain, human IgM, human IgM Fc domain, mouse IgG3, mouse IgG3 Fc domain, mouse IgM, mouse IgM Fc domain, and cobra venom factor (CVF).

14. An isolated nucleic acid molecule comprising a nucleotide sequence encoding the construct of claim 11, wherein said construct is a fusion protein.

15. An expression vector comprising the isolated nucleic acid molecule of claim 14.

16. A cell comprising the expression vector of claim 15.

17. A method for producing a construct, the method comprising culturing the cell of claim 16 under conditions suitable to allow expression of the construct by the cell, said method optionally further comprising isolating the construct from the cell or the culture media in which the cell was cultured.

18. The construct produced by the method of claim 17, said method further comprising isolating the construct from the cell or the culture media in which the cell was culture.

19. A pharmaceutical composition comprising the construct of any one of claims 10-13 and 18 and a pharmaceutically-acceptable excipient.

20. Use of:
a) the isolated antibody or antigen-binding fragment thereof of any one of claims 1, 2, and

4 in an amount effective to decrease a humoral immune response in a mammal, in the manufacture of a medicament for decreasing a humoral immune response in said mammal, optionally wherein the mammal is afflicted with an autoimmune disease or has received, or is likely to receive, a transplant; or

b) the construct of any one of claims 10-13 and 18 in an amount effective to decrease a humoral immune response in a mammal, in the manufacture of a medicament for decreasing a humoral immune response in said mammal, optionally wherein the mammal is afflicted with an autoimmune disease or has received, or is likely to receive, a transplant; or

c) the construct of any one of claims 10-13 and 18 in an amount effective to modulate complement activation in a mammal, in the manufacture of a medicament for modulating complement activation in said mammal; or

d) the construct of any one of claims 10-12 in an amount effective to decrease complement activation in a mammal, in the manufacture of a medicament for decreasing complement activation in said mammal; or

e) the construct of claim 13 in an amount effective to increase complement activation in a mammal, in the manufacture of a medicament for increasing complement activation in said mammal; or

f) the construct of any one of claims 10-12 in an amount effective to decrease complement activation and a humoral immune response in a mammal, in the manufacture of a medicament for decreasing complement activation and humoral immune response simultaneously in said mammal;

optionally wherein said mammal is a human.

21. Use of:

a) a therapeutically effective amount of the isolated antibody or antigen-binding fragment thereof of any one of claims 1, 2, and 4 or the construct of any one of claims 10-12, in the manufacture of a medicament for treating a mammal 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, optionally wherein

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; or

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; or

said transplant rejection is hyperacute xenograft rejection; or

said pregnancy-related disease is selected from the group consisting of: HELLP syndrome (*H*emolytic anemia, *E*levated *L*iver enzymes and *L*ow *P*latelet count), recurrent fetal loss, and pre-eclampsia; or

said adverse drug reaction is selected from the group consisting of: drug allergy and IL-2 induced vascular leakage syndrome; or

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, optionally wherein said autoimmune glomerulonephritis is selected from the group consisting of: immunoglobulin A nephropathy and membranoproliferative glomerulonephritis type I; or

b) the construct of claim 13 in an amount effective to increase complement activation in a mammal in the manufacture of a medicament for treating a mammal having or suspected of having a disease or preventing a mammal from developing a disease wherein said disease is selected from the group consisting of: a cancer, a viral infection, a bacterial infection, a parasitic infection, and a fungal infection;

optionally wherein said mammal is a human.

22. A therapeutic kit comprising:

(i) the antibody or antigen-binding fragment of any one of claims 1, 2, and 4 and (ii) means for delivering the antibody or antigen-binding fragment to a human; or

(iii) the construct of any one of claims 10-12 and (iv) means for delivering the construct to a human,

optionally wherein

the means is suitable for subcutaneous, intraocular, or intraarticular delivery of the construct, or the antibody or antigen-binding fragment thereof, to the human, optionally wherein the means is a syringe, optionally wherein the syringe is a double barreled syringe; or

the means is a trans-scleral path or a contact lens comprising the construct or the antibody or antigen-binding fragment thereof; or

the means is suitable for intrapulmonary delivery of the construct, or the antibody or antigen-binding fragment thereof, to the human, optionally wherein the means is an inhaler or a nebulizer;

optionally wherein said therapeutic kit further comprises at least one additional active agent for use in treating a complement-associated disorder in a human.

23. A pre-filled syringe comprising: (a) the antibody or antigen-binding fragment thereof of any one of claims 1, 2, and 4; or (b) the construct of any one of claims 10-12; optionally wherein:

the construct, or the antibody or antigen-binding fragment thereof, is formulated for intraocular, intravitreal, intraarticular, or subcutaneous administration, or

the syringe comprises at least one pharmaceutical unit dosage form of the construct, or the antibody or antigen-binding fragment thereof, optionally wherein:

each pharmaceutical unit dosage form has a volume of between 0.02 mL to 1 mL, inclusive, or

the pharmaceutical unit dosage form has a volume of no more than 0.05 mL; optionally wherein the syringe comprises between 0.05 mg to 10 mg, or between about 1 mg and 100 mg, of the construct, of the antibody or antigen-binding fragment thereof, optionally wherein the pharmaceutical unit dosage form has a volume of no more than 0.05 mL.

CLAIMS

1. An isolated antibody or antigen-binding fragment thereof specifically binding to a mammalian complement component C3d protein, wherein the antibody or antigen-binding fragment thereof is selected from the group consisting of:

a. an isolated anti-C3d 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;

b. an isolated anti-C3d antibody or antigen-binding fragment thereof, wherein said antibody or antigen-binding fragment thereof binds to C3d or C3dg with higher affinity than to complement proteins C3, C3a, C3b, C3c or C3f;

c. an isolated anti-C3d antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof binds to deposited C3 fragments;

d. an isolated anti-C3d antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof binds to C3d proteins of at least two species;

e. an isolated anti-C3d antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof competes with complement receptor 2 (CR2) for binding to C3d;

f. an isolated anti-C3d antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof does not enhance complement activation; and

g. an isolated anti-C3d antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof decreases host humoral immune response.

2. The isolated antibody or antigen-binding fragment thereof of claim 1(a), wherein said K_D is 0.5 nM or better; or

the isolated antibody or antigen-binding fragment thereof of claim 1(b), wherein said antibody or antigen-binding fragment thereof does not bind to complement proteins C3, C3a, C3b, C3c or C3f; or

the isolated antibody or antigen-binding fragment thereof of claim 1(c), wherein said antibody or antigen-binding fragment thereof binds to deposited C3d or C3dg with a higher affinity than it binds to circulating intact C3, C3b, or ($C3H_2O$); or

the isolated antibody or antigen-binding fragment thereof of claim 1, wherein said mammalian complement component C3d protein is a human complement component C3d protein; or

the isolated antibody or antigen-binding fragment thereof of claim 1, wherein said mammalian complement component C3d protein is a non-human primate complement component C3d protein selected from the group consisting of orangutan, chimpanzee, macaque, gorilla, lemur, or gibbon complement component C3d protein; or

the isolated antibody or antigen-binding fragment thereof of claim 1(f), wherein said antibody or antigen-binding fragment thereof reduces complement activation;

optionally wherein the antibody or antigen-binding fragment thereof is selected from the group consisting of: 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 $F(ab')_2$, optionally wherein said antibody is a monoclonal antibody, optionally wherein said antibody is selected from the group consisting of: i) 3db8 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), and iii) 3d29 produced by hybridoma cell line 3d-29/5/2 (ATCC Deposit PTA-11000).

3. 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).

4. An isolated antibody produced by the hybridoma of claim 3.

5. An isolated nucleic acid molecule comprising a nucleotide sequence encoding the antibody or antigen-binding fragment thereof of any one of claims 1, 2, and 4; or

an expression vector comprising said isolated nucleic acid molecule; or

a cell comprising said expression vector.

6. A method for producing an antibody or antigen-binding fragment thereof, the method comprising culturing the cell of claim 5 under conditions suitable to allow expression of the antibody or antigen-binding fragment by the cell, optionally further comprising isolating the antibody or antigen-binding fragment thereof from the cell or the culture media in which the cell was cultured.

7. The isolated antibody or antigen-binding fragment thereof produced by the method of claim 6, said method further comprising isolating the antibody or antigen-binding fragment thereof from the cell or the culture media in which the cell was cultured.

8. A pharmaceutical composition comprising the isolated antibody or antigen-binding fragment thereof of any one of claims 1, 2, 4, or 7 and a pharmaceutically-acceptable excipient.

9. A construct comprising:

a. a C3d binding portion, optionally wherein said C3d binding portion comprises said anti-C3d antibody or antigen-binding fragment thereof of any one of claims 1, 2, 4, or 7; and

b. a complement modulator portion, optionally wherein said complement modulator portion is a compound, composition, or protein;

wherein (a) and (b) are joined.

10. The construct of claim 9, wherein:

said construct modulates complement activity in the complement alternative pathway (CAP); or

said construct is a fusion protein; or

said C3d binding portion and said complement modulator portion are joined directly without a linker; or

said C3d binding portion and said complement modulator portion are joined by a linker, optionally wherein said linker is a peptide, optionally wherein said peptide is selected from the group consisting of: $(GlySer)_n$, wherein n = 1 to 8; $(GlyGlyGlySer)_n$, wherein n = 1 to 4; $(GlyGlyGlyGlySer)_n$, wherein n = 1 to 8; and $(GlySerSerGly)_n$, wherein n = 1 to 4.

11. The construct of claim 9, wherein said complement modulator portion comprises a complement inhibitor or biologically active fragment thereof, optionally wherein:

said complement inhibitor or biologically active fragment thereof is selected from the group consisting of: human membrane complement protein (MCP), human decay accelerating factor (DAF), human CD59, human complement receptor 1 (CR1), human factor H, Crry, and biologically active fragments or variants of any of the foregoing; or

said complement inhibitor is selected from the group consisting of: human membrane complement protein (MCP) (SEQ ID NO:1), human decay accelerating factor (DAF) (SEQ ID NO:2), mouse DAF (SEQ ID NO:3), mouse complement receptor 1-related gene/protein y (Crry) (SEQ ID NO:7), human CD59 (SEQ ID NO:4), mouse CD59 isoform A (SEQ ID NO:5), mouse CD59 isoform B (SEQ ID NO:6), human complement receptor 1 (CR1) (SEQ ID NO:8), human factor H (SEQ ID NO:9), and mouse factor H (SEQ ID NO:10), and biologically active fragments thereof, optionally wherein said biologically active fragment thereof is selected from the group consisting of: 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 ID 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 ID 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.

12. The construct of claim 9 or 10, wherein said complement modulator portion comprises a complement activator or biologically active fragment thereof, optionally wherein said complement activator or biologically active fragment thereof is selected from the group consisting of: human IgG1, human IgG1 Fc domain, human IgM, human IgM Fc domain, mouse IgG3, mouse IgG3 Fc domain, mouse IgM, mouse IgM Fc domain, and cobra venom factor (CVF).

13. An isolated nucleic acid molecule comprising a nucleotide sequence encoding the construct of claim10, wherein said construct is a fusion protein; or

an expression vector comprising said isolated nucleic acid molecule; or

a cell comprising said expression vector.

14. A method for producing a construct, the method comprising culturing the cell of claim 13 under conditions suitable to allow expression of the construct by the cell, said method optionally further comprising isolating the construct from the cell or the culture media in which the cell was cultured.

15. The construct produced by the method of claim 14, said method further comprising isolating the construct from the cell or the culture media in which the cell was cultured.

16. A pharmaceutical composition comprising the construct of any one of claims 9-12 or 15 and a pharmaceutically-acceptable excipient.

17. Use of:

a. the isolated antibody or antigen-binding fragment thereof of any one of claims 1, 2, 4, or 7 in an amount effective to decrease a humoral immune response in a mammal, in the manufacture of a medicament for decreasing a humoral immune response in said mammal, optionally wherein the mammal is afflicted with an autoimmune disease or has received, or is likely to receive, a transplant; or

b. the construct of any one of claims 9-12 or 15 in an amount effective to decrease a humoral immune response in a mammal, in the manufacture of a medicament for decreasing a humoral immune response in said mammal, optionally wherein the mammal is afflicted with an autoimmune disease or has received, or is likely to receive, a transplant; or

c. the construct of any one of claims 9-12 or 15 in an amount effective to modulate complement activation in a mammal, in the manufacture of a medicament for modulating complement activation in said mammal; or

d. the construct of any one of claims 9-11 in an amount effective to decrease complement activation in a mammal, in the manufacture of a medicament for decreasing complement activation in said mammal; or

e. the construct of claim 12 in an amount effective to increase complement activation in a mammal, in the manufacture of a medicament for increasing complement activation in said mammal; or

f. the construct of any one of claims 9-11 in an amount effective to decrease complement activation and a humoral immune response in a mammal, in the manufacture of a medicament for decreasing complement activation and humoral immune response simultaneously in said mammal;

optionally wherein said mammal is a human.

18. Use of:

a. a therapeutically effective amount of the isolated antibody or antigen-binding fragment thereof of any one of claims 1, 2, 4, or 7 or the construct of any one of claims 9-11, in the manufacture of a medicament for treating a mammal 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, optionally wherein:

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; or

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; or

said transplant rejection is hyperacute xenograft rejection; or

said pregnancy-related disease is selected from the group consisting of: HELLP syndrome (*H*emolytic anemia, *E*levated *L*iver enzymes and *L*ow *P*latelet count), recurrent fetal loss, and pre-eclampsia; or

said adverse drug reaction is selected from the group consisting of: drug allergy and IL-2 induced vascular leakage syndrome; or

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, optionally wherein said autoimmune glomerulonephritis is selected from the group consisting of: immunoglobulin A nephropathy and membranoproliferative glomerulonephritis type I; or

b. the construct of claim 12 in an amount effective to increase complement activation in a mammal in the manufacture of a medicament for treating a mammal having or suspected of having a disease or preventing a mammal from developing a disease wherein said disease is selected from the group consisting of: a cancer, a viral infection, a bacterial infection, a parasitic infection, and a fungal infection;

optionally wherein said mammal is a human.

19. A therapeutic kit comprising:

(i) the antibody or antigen-binding fragment of any one of claims 1, 2, 4, or 7 and

(ii) means for delivering the antibody or antigen-binding fragment to a human; or

(iii) the construct of any one of claims 9-11 and (iv) means for delivering the construct to a human,

optionally wherein:

the means is suitable for subcutaneous, intraocular, or intraarticular delivery of the construct, or the antibody or antigen-binding fragment thereof, to the human, optionally wherein the means is a syringe, optionally wherein the syringe is a double barreled syringe; or

the means is a trans-scleral patch or a contact lens comprising the construct or the antibody or antigen-binding fragment thereof; or

the means is suitable for intrapulmonary delivery of the construct, or the antibody or antigen-binding fragment thereof, to the human, optionally wherein the means is an inhaler or a nebulizer;

optionally wherein said therapeutic kit further comprises at least one additional active agent for use in treating a complement-associated disorder in a human.

20. A pre-filled syringe comprising: (a) the antibody or antigen-binding fragment thereof according to any one of claims 1, 2, 4, or 7; or (b) the construct of any one of claims 9-11; optionally wherein:

the construct, or the antibody or antigen-binding fragment thereof, is formulated for intraocular, intravitreal, intraarticular, intramuscular, or subcutaneous administration, or

the syringe comprises at least one pharmaceutical unit dosage form of the construct, or the antibody or antigen-binding fragment thereof, optionally wherein:

each pharmaceutical unit dosage form has a volume of between 0.02 mL to 1 mL, inclusive, or

the pharmaceutical unit dosage form has a volume of no more than 0.05 mL;

optionally wherein the syringe comprises between 0.05 mg to 10 mg, or between about 1 mg and 100 mg, of the construct, of the antibody or antigen-binding fragment thereof, optionally wherein the pharmaceutical unit dosage form has a volume of no more than 0.05 mL.

21. A construct comprising: (a) a first portion that inhibits one or both of the activity and activation of a B cell; and (b) a second portion comprising a complement inhibitor molecule, wherein (a) and (b) are joined, optionally wherein:

the first portion is an antibody or an antigen-binding fragment thereof that binds to a natural ligand of CR2 and inhibits the interaction between CR2 and the natural ligand of CR2, optionally wherein the natural ligand of CR2 is C3d or C3dg; or

the first portion is an antibody or an antigen-binding fragment thereof that binds to CR2 and inhibits the interaction between CR2 and at least one of its natural ligands,

optionally wherein the complement inhibitor molecule is a polypeptide, optionally wherein the complement inhibitor molecule comprises soluble human DAF, soluble human MCP, soluble human CD59, human factor H, soluble human CR1, soluble Crry, or a biologically-active fragment of any one of the foregoing.

22. A pharmaceutical composition comprising the construct of claim 21 and a pharmaceutically-acceptable excipient.