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DESCRIPTION

Description

BACKGROUND

1. Field

[0001] The present invention is defined by the claims and relates to humanized antiC1q antibodies as defined by the claims.

2. Description of Related Art

[0002] Excessive complement activation has been associated with a range of disease conditions, including numerous inflammatory and autoimmune diseases. More recently, the complement system has also been shown to contribute to neurodegenerative disease pathology. Specifically, complement factors, such as C1q, were shown to be expressed in neuronal synapses and to mark these synapses for elimination. *See, e.g.*, U.S. Patent Publication Nos. 2012/0195880 and 2012/328601. While selective synapse loss is an essential aspect of normal brain development ("synaptic pruning"), excessive synapse loss, especially in a mature or aging brain, results in neurodegeneration and cognitive decline. Elevated synaptic complement expression was found to contribute to synaptic loss in normal aging and in neurodegenerative disease progression. Conversely, lowering neuronal complement expression was found to be neuroprotective. Based on these findings, neutralizing the activity of complement factors such as C1q is regarded as a promising therapeutic strategy to prevent synapse loss and to slow neurodegenerative disease progression as well as cognitive decline in normal aging.

[0003] Neurodegenerative diseases involving synapse loss and considered to be amenable to treatments aiming at the neutralization of complement factors such as C1q include Alzheimer's disease, amyotrophic lateral sclerosis, multiple sclerosis, glaucoma, myotonic dystrophy, Down syndrome, Parkinson's disease, Huntington's disease, and the like.

[0004] Only a limited number of complement neutralizing antibodies are known to date *(see, e.g.,* Klos A. et al., MolImmunol. 2009, 46(14), 2753-2766; Carroll S. & Georgiou G., Immunobiology 2013, 218(8), 1041-1048; Tuzun et al., J. Neuroimmunol. 2007, 182, 167-176; Nelson et al., J. Clin. Invest. 2006, 116:2892-2900; Heinz et al., J. Immunol. 1984, 133, 400-404; Jiang et al., J. Immunol. 1991, 146, 2324-2330; Trinder et al., Scand. J. Immunol. 1999, 50, 635-641; Hwang et al., Mol. Immunol. 2008, 45, 2570-2580). WO2005/002513 discloses neutralizing anti-C1q antibody P1 H10. WO2014/169076 and Tuzun et al., Current Topics in Complement II, 632, 254-261 disclose neutralizing anti-C1q antibody 4A4B11. Only the C5 neutralizing antibody Eculizumab, an inhibitor of the terminal complement activation pathway, has obtained regulatory approval to date; Eculizumab is marketed for the treatment of paroxysmal nocturnal hemoglobinuria (PNH; Hillmen et al., N Engl J Med. 2006, 355(12):1233-43).

[0005] Thus, there is a need to develop further antibodies that specifically bind to and neutralize biological

activities of complement factors such as C1q.

BRIEF SUMMARY

[0006] The present invention is defined by the claims and relates to a humanized anti-C1q antibody , or an antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof comprises: a) a heavy chain variable domain comprising an amino acid sequence of SEQ ID NO: 1 and a light chain variable domain comprising an amino acid sequence of SEQ ID NO: 5; b) a heavy chain variable domain comprising an amino acid sequence of SEQ ID NO: 3 and a light chain variable domain comprising an amino acid sequence of SEQ ID NO: 8; c) a heavy chain variable domain comprising an amino acid sequence of SEQ ID NO: 3 and a light chain variable domain comprising an amino acid sequence of SEQ ID NO: 7; or d) a heavy chain variable domain comprising an amino acid sequence of SEQ ID NO: 4 and a light chain variable domain comprising an amino acid sequence of SEQ ID NO: 7..

[0007] In certain aspects of the invention, the antibody or antigen-binding fragment comprises a human IgG4 heavy chain constant region.

[0008] In some embodiments of the invention the human IgG4 heavy chain constant region comprises the amino acid sequence of SEQ ID NO: 37.

[0009] In certain aspects of the invention, the human IgG4 heavy chain constant region comprises a Fc region and the Fc region comprises an amino acid substitution at position 248 and/or position 241 according to Kabat numbering convention.

[0010] In certain aspects of the invention, the amino acid substitution at position 248 is a leucine to glutamate amino acid substitution.

[0011] In certain aspects, the amino acid substitution at position 241 is a serine to proline amino acid substitution. In certain aspects, the antigen-binding fragment of the invention is a Fab, F(ab')2 or Fab' fragment.

[0012] In certain aspects, the present invention provides an isolated polynucleotide comprising a nucleic acid sequence encoding the antibody or antigen-binding fragment of the invention.

[0013] In certain aspects, the present invention provides an isolated host cell comprising the nucleic acid sequence of the invention.

[0014] In certain aspects, the present invention provides a pharmaceutical composition comprising the antibody or antigen-binding fragment of the invention and a pharmaceutically acceptable carrier.

[0015] In certain aspects, the present invention provides the antibody or antigen-binding fragment of the invention for use in medicine.

[0016] In certain aspects of the invention, the use in medicine is for treating or preventing a disease associated with complement activation, wherein the disease is a neurodegenerative disorder, inflammatory disease, autoimmune disease or metabolic disorder.

[0017] In some embodiments of the invention the disease is a neurodegenerative disorder selected from Alzheimer's disease, amyotrophic lateral sclerosis, multiple sclerosis, glaucoma, myotonic dystrophy,

Guillain-Barre' syndrome (GBS), Myastenia Gravis, Bullous Pemphigoid, spinal muscular atrophy, Down syndrome, Parkinson's disease, and Huntington's disease.

[0018] In some other embodiments of the invention the disease is an inflammatory disease, autoimmune disease or metabolic disorder selected from diabetes, vitiligo, Hashimoto's thyroiditis, Addison's disease, Coeliac disease, Crohn's disease, pernicious anaemia, myasthenia gravis, obesity, autoimmune hemolytic anemias, paraneoplastic syndromes, hypocomplementemic urticarial vasculitis (HUV), polymyalgia rheumatic, Wegener's Granulomatosis, rheumatoid arthritis (RA), acute respiratory distress syndrome (ARDS), remote tissue injury after ischemia and reperfusion, complement activation during cardiopulmonary bypass surgery, dermatomyositis, pemphigus, lupus nephritis and resultant glomerulonephritis and vasculitis, cardiopulmonary bypass, cardioplegia-induced coronary endothelial dysfunction, type II membranoproliferative glomerulonephritis, IgA nephropathy, acute renal failure, cryoglobulemia, antiphospholipid syndrome, Chronic open-angle glaucoma, acute closed angle glaucoma, macular degenerative diseases, age-related macular degeneration (AMD), (AMD-wet), Geographic atrophy, choroidal neovascularization (CNV), uveitis, diabetic retinopathy, ischemia-related retinopathy, endophthalmitis, intraocular neovascular disease, diabetic macular edema, pathological myopia, von Hippel-Lindau disease, histoplasmosis of the eye, Neuromyelitis Optica (NMO), Central Retinal Vein Occlusion (CRVO), corneal neovascularization, retinal neovascularization, Leber's hereditary optic neuropathy, optic neuritis, Behcet's retinopathy, ischemic optic neuropathy, retinal vasculitis, ANCA vasculitis, Purtscher retinopathy, Sjogren's dry eye disease, dry AMD, sarcoidosis, temporal arteritis, polyarteritis nodosa, multiple sclerosis, allo-transplantation, hyperacute rejection, hemodialysis, chronic occlusive pulmonary distress syndrome (COPD), asthma, and aspiration pneumonia.

DESCRIPTION OF THE FIGURES

[0019]

FIG. 1 depicts plasmid maps for light chain and heavy chain expression vectors. **FIG. 1A** depicts a plasmid map for light chain expression vector pANTVk. **FIG. 1B** depicts a plasmid map for and heavy chain expression vector pANTVhG4 (S241P L248E). Both VH and Vκ vectors contain genomic DNA fragments incorporating introns and polyA sequences. Expression of both chains is driven by a CMV promoter and selection (on the heavy chain vector) is via a DHFR mini gene.

FIG. 2A depicts an alignment of amino acid sequence of the heavy chain variable region (VH) of the M1 antibody and the amino acid sequences of the humanized VH variants VH1-VH2. **FIG. 2B** depicts an alignment of amino acid sequence of the heavy chain variable region (VH) of the M1 antibody and the amino acid sequences of the humanized VH variants VH3-VH4. **FIG. 2C** depicts an alignment of amino acid sequence of the kappa light chain variable region (Vκ) of the M1 antibody and the amino acid sequences of the humanized Vκ variants Vκ1-Vκ2. **FIG. 2D** depicts an alignment of amino acid sequence of the kappa light chain variable region (Vκ) of the M1 antibody and the amino acid sequences of the humanized Vκ variants Vκ3-Vκ4.

FIG. 3 depicts a Coomassie Blue-stained SDS-PAGE gel of protein A-purified antibodies. 2µg of each sample was loaded on a NuPage 4-12% Bis-Tris gel and run at 200V for 35min. Size marker is pre-stained protein standard Fermentas PageRuler Plus.

FIG. 4 depicts competition ELISA assays for human C1q. A dilution series of purified humanized anti-Clq antibodies were competed against a fixed concentration of biotinylated monoclonal antibody M1 for binding to human C1q. Bound biotinylated M1 antibody was detected using streptavidin-peroxidase conjugate and TMB substrate. **FIG. 4A** depicts the results with the humanized antibodies VH1/Vκ1, VH1/Vκ2, and VH1/Vκ3.

FIG. 4B depicts the results with the humanized antibodies VH1/Vκ4, VH2/Vκ1, VH2/Vκ2, VH2/Vκ3, and VH2/Vκ4. **FIG. 4C** depicts the results with the humanized antibodiesVH3/Vκ1, VH3/Vκ2, VH3/Vκ3, and VH3/Vκ4. **FIG. 4D** depicts the results with the humanized antibodies VH4/Vκ1, VH4/Vκ2, VH4/Vκ3, and VH4/Vκ4.

FIG. 5 depicts competition ELISA assays for mouse C1q. A dilution series of purified humanized anti-Clq antibodies were competed against a fixed concentration of the chimeric M1 antibody for binding to mouse C1q. Bound biotinylated chimeric M1 was detected using streptavidin-peroxidase conjugate and TMB substrate.

FIG. 6 depicts a Coomassie Blue-stained SDS-PAGE gel of gel filtration-purified antibodies. 1 µg of each sample was loaded on a NuPage 4-12% Bis-Tris gel and run at 200V for 35min. Size marker (M) is pre-stained protein standard Fermentas PageRuler Plus. Lane 1 depicts Fab VH3/Vκ3 reduced; lane 2 depicts Fab VH3/Vκ3 non-reduced; lane 3 depicts IgG V VH3/Vκ3 reduced; and lane 4 depicts IgG VH3/Vκ3 non-reduced.

FIG. 7 illustrates the C1q-neutralizing activities of anti-C1q antibodies in human, and rat CH50 hemolytic assays in a dose-response format. **FIG. 7A** illustrates results from a human CH50 hemolytic assay. **FIG. 7B** illustrates results from a rat CH50 hemolytic assay. "ANN-005" corresponds to the monoclonal antibody M1, "3E2" corresponds to a chimeric M1 antibody, "2B12" corresponds to antibody VH1/Vκ1, "5H7" corresponds to antibody VH3/Nκ3, "3F1" corresponds to antibody VH3/Vκ4, and "1D3" corresponds to antibody VH4/Vκ3.

FIG. 8 depicts the time course of serum 5H7 levels in monkeys for single IV dose at 15 and 100 mg/Kg.

FIG. 9 illustrates the time course of serum C1q levels in monkeys for single IV dose at 15 and 100 mg/Kg. **FIG 9A** depicts the time course of serum C1q levels in monkeys using the JL1-M1 assay. FIG. **9B** depicts the time course of serum C1q levels in monkeys using the JL1-JL1 assay.

FIG 10 shows sustained reduction of serum hemolysis in monkeys for single IV dose at 15 and 100 mg/Kg.

DETAILED DESCRIPTION

General Techniques

[0020] The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook et al., Molecular Cloning: A Laboratory Manual 3d edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Current Protocols in Molecular Biology (F.M. Ausubel, et al. eds., (2003)); the series Methods in Enzymology (Academic Press, Inc.): PCR 2: A Practical Approach (M.J. MacPherson, B.D. Hames and G.R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) Antibodies, A Laboratory Manual, and Animal Cell Culture (R.I. Freshney, ed. (1987)); Oligonucleotide Synthesis (M.J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J.E. Cellis, ed., 1998) Academic Press; Animal Cell Culture (R.I. Freshney), ed., 1987); Introduction to Cell and Tissue Culture (J.P. Mather and P.E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., 1993-8) J. Wiley and Sons; Handbook of Experimental Immunology (D.M. Weir and C.C. Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (J.M. Miller and M.P. Calos, eds., 1987); PCR: The Polymerase Chain Reaction, (Mullis et al., eds., 1994); Current Protocols in Immunology (J.E. Coligan et al., eds., 1991); Short Protocols in

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Definitions

[0021] As used herein, the term "*preventing*" includes providing prophylaxis with respect to occurrence or recurrence of a particular disease, disorder, or condition in an individual. An individual may be predisposed to, susceptible to a particular disease, disorder, or condition, or at risk of developing such a disease, disorder, or condition, but has not yet been diagnosed with the disease, disorder, or condition.

[0022] As used herein, an individual "*at risk*" of developing a particular disease, disorder, or condition may or may not have detectable disease or symptoms of disease, and may or may not have displayed detectable disease or symptoms of disease prior to the treatment methods described herein. "At risk" denotes that an individual has one or more risk factors, which are measurable parameters that correlate with development of a particular disease, disorder, or condition, as known in the art. An individual having one or more of these risk factors has a higher probability of developing a particular disease, disorder, or condition than an individual without one or more of these risk factors.

[0023] As used herein, the term "*treatment*" refers to clinical intervention designed to alter the natural course of the individual being treated during the course of clinical pathology. Desirable effects of treatment include decreasing the rate of progression, ameliorating or palliating the pathological state, and remission or improved prognosis of a particular disease, disorder, or condition. An individual is successfully "treated", for example, if one or more symptoms associated with a particular disease, disorder, or condition are mitigated or eliminated.

[0024] An "*effective amount*" refers to at least an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. An effective amount can be provided in one or more administrations.

[0025] A "*therapeutically effective amount*" is at least the minimum concentration required to effect a measurable improvement of a particular disease, disorder, or condition. A therapeutically effective amount herein may vary according to factors such as the disease state, age, sex, and weight of the patient, and the ability of the anti-Clq antibody to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the anti-Clq antibody are outweighed by the therapeutically beneficial effects.

[0026] *"Chronic"* administration refers to administration of the medicament(s) in a continuous as opposed to acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "*Intermittent*" administration refers to treatment that is not consecutively done without interruption, but rather is cyclic in nature.

[0027] As used herein, administration "*in conjunction*" with another compound or composition includes simultaneous administration and/or administration at different times. Administration in conjunction also encompasses administration as a co-formulation or administration as separate compositions, including at different dosing frequencies or intervals, and using the same route of administration or different routes of

administration.

[0028] An "*individual*" for purposes of treatment, prevention, or reduction of risk refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sport, or pet animals, such as dogs, horses, rabbits, cattle, pigs, hamsters, gerbils, mice, ferrets, rats, cats, and the like. In some implementations, the individual is human.

[0029] As used herein, "*autoantibody*" means any antibody that recognizes a host antigen.

[0030] The term "*immunoglobulin*" (Ig) is used interchangeably with *"antibody"* herein. The term "antibody" herein is used in the broadest sense and specifically covers monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity.

[0031] The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. The pairing of a V_H and V_L together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, *see, e.g.,* Basic and Clinical Immunology, 8th Ed., Daniel P. Stites, Abba I. Terr and Tristram G. Parslow (eds.), Appleton & Lange, Norwalk, CT, 1994, page 71 and Chapter 6.

[0032] The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa ("κ") and lambda ("λ"), based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (CH), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, having heavy chains designated alpha ("α"), delta ("δ"), epsilon ("ε"), gamma ("γ") and mu ("μ"), respectively. The y and α classes are further divided into subclasses (isotypes) on the basis of relatively minor differences in the CH sequence and function, *e.g.*, humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The subunit structures and three dimensional configurations of different classes of immunoglobulins are well known and described generally in, for example, Abbas et al., Cellular and Molecular Immunology, 4th ed. (W.B. Saunders Co., 2000).

[0033] "*Native antibodies*" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

[0034] An "*isolated*" antibody, such as an anti-Clq antibody of the present disclosure, is one that has been identified, separated and/or recovered from a component of its production environment (e.g., naturally or recombinantly). In some implementations, the isolated polypeptide is free of association with all other contaminant components from its production environment. Contaminant components from its production environment, such as those resulting from recombinant transfected cells, are materials that would typically interfere with research, diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In some implementations, the polypeptide will be purified: (1) to greater than 95% by weight of antibody as determined by, for example, the Lowry method, and in some implementations, to greater than 99% by weight; (2) to a degree sufficient to

obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or silver stain. Isolated antibody includes the antibody *in situ* within recombinant T-cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, an isolated polypeptide or antibody will be prepared by at least one purification step.

[0035] The "*variable region*" or "*variable domain*" of an antibody, such as an anti-Clq antibody of the present disclosure, refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domains of the heavy chain and light chain may be referred to as "V_H" and "V_L", respectively. These domains are generally the most variable parts of the antibody (relative to other antibodies of the same class) and contain the antigen binding sites.

[0036] The term "*variable*" refers to the fact that certain segments of the variable domains differ extensively in sequence among antibodies, such as anti-Clq antibodies of the present disclosure. The V domain mediates antigen binding and defines the specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the entire span of the variable domains. Instead, it is concentrated in three segments called hypervariable regions (HVRs) both in the light-chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three HVRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The HVRs in each chain are held together in close proximity by the FR regions and, with the HVRs from the other chain, contribute to the formation of the antigen binding site of antibodies *(see* Kabat et al., Sequences of Immunological Interest, Fifth Edition, National Institute of Health, Bethesda, MD (1991)). The constant domains are not involved directly in the binding of antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent-cellular toxicity.

[0037] The term "*monoclonal antibody*" as used herein refers to an antibody, such as an anti- C1q antibody of the present disclosure, obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-translation modifications (e.g., isomerizations, amidations) that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. In contrast to polyclonal antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present disclosure may be made by a variety of techniques, including, for example, the hybridoma method *(e.g.,* Kohler and Milstein., Nature, 256:495-97 (1975); Hongo et al., Hybridoma, 14 (3):253-260 (1995), Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2d ed. 1988); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (see, *e.g.,* U.S. Patent No. 4,816,567), phage-display technologies (*see, e.g.,* Clackson et al., Nature, 352:624-628 (1991); Marks et al., J. Mol. Biol. 222:581-597 (1992); Sidhu et al., J. Mol. Biol. 338(2): 299-310 (2004); Lee et al., J. Mol. Biol. 340(5):1073-1093 (2004); Fellouse, Proc. Nat'l Acad. Sci. USA 101(34):12467-472 (2004); and Lee et al., J. Immunol. Methods 284(1-2):119-132 (2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, *e.g.,* WO 1998/24893; WO 1996/34096; WO 1996/33735; WO 1991/10741; Jakobovits et al., Proc. Nat'l Acad. Sci. USA 90:2551 (1993); Jakobovits et al., Nature 362:255-

258 (1993); Bruggemann et al., Year in Immunol. 7:33 (1993); U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016; Marks et al., Bio/Technology 10:779-783 (1992); Lonberg et al., Nature 368:856-859 (1994); Morrison, Nature 368:812-813 (1994); Fishwild et al., Nature Biotechnol. 14:845-851 (1996); Neuberger, Nature Biotechnol. 14:826 (1996); and Lonberg and Huszar, Intern. Rev. Immunol. 13:65-93 (1995).

[0038] The terms "*full-length antibody," "intact antibody*" or *"whole antibody"* are used interchangeably to refer to an antibody, such as and anti-Clq antibody of the present disclosure, in its substantially intact form, as opposed to an antibody fragment. Specifically whole antibodies include those with heavy and light chains including an Fc region. The constant domains may be native sequence constant domains (e.g., human native sequence constant domains) or amino acid sequence variants thereof. In some cases, the intact antibody may have one or more effector functions.

[0039] An *"antibody fragment*" comprises a portion of an intact antibody, the antigen binding and/or the variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', $F(ab')_2$ and Fv fragments; diabodies; linear antibodies *(see* U.S. Patent 5,641,870, Example 2; Zapata et al., Protein Eng. 8(10):1057-1062 (1995)); single-chain antibody molecules and multispecific antibodies formed from antibody fragments.

[0040] Papain digestion of antibodies, such as anti-Clq antibodies of the present disclosure, produces two identical antigen-binding fragments or regions, called *"Fab"* fragments or regions, and a residual "*Fc*" fragment or region, a designation reflecting the ability to crystallize readily. The Fab fragment or region consists of an entire L chain along with the variable region domain of the H chain (V_H), and the first constant domain of one heavy chain (C_H1). Each Fab fragment or region is monovalent with respect to antigen binding, *i.e.,* it has a single antigen-binding site. Pepsin treatment of an antibody yields a single large $F(ab')_2$ fragment or region which roughly corresponds to two disulfide linked Fab fragments having different antigen-binding activity and is still capable of cross-linking antigen. Fab' fragments or regions differ from Fab fragments by having a few additional residues at the carboxy terminus of the C_H1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0041] The Fc fragment or region comprises the carboxy-terminal portions of both H chains held together by disulfides. The effector functions of antibodies are determined by sequences in the Fc region, the region which is also recognized by Fc receptors (FcR) found on certain types of cells.

[0042] *"Fv"* is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three HVRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0043] *"Single-chain Fv"* also abbreviated as *"sFv"* or *"scFv"* are antibody fragments that comprise the VH and VL antibody domains connected into a single polypeptide chain. In some implementations, the sFv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for antigen binding. For a review of the sFv, *see* Pluckthun in The Pharmacology

of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[0044] *"Functional fragments"* of antibodies, such as anti-Clq antibodies of the present disclosure, comprise a portion of an intact antibody, generally including the antigen binding or variable region of the intact antibody or the Fc region of an antibody which retains or has modified FcR binding capability. Examples of antibody fragments include linear antibody, single-chain antibody molecules and multispecific antibodies formed from antibody fragments.

[0045] The term *"diabodies"* refers to small antibody fragments prepared by constructing sFv fragments (see preceding paragraph) with short linkers (about 5-10) residues) between the V_H and V_L domains such that inter-chain but not intra-chain pairing of the V domains is achieved, thereby resulting in a bivalent fragment, *i.e.,* a fragment having two antigen-binding sites. Bispecific diabodies are heterodimers of two "crossover" sFv fragments in which the V_H and V_L domains of the two antibodies are present on different polypeptide chains. Diabodies are described in greater detail in, for example, EP 404,097; WO 93/11161; Hollinger et al., Proc. Nat'l Acad. Sci. USA 90:6444-48 (1993).

[0046] As used herein, a "chimeric antibody" refers to an antibody (immunoglobulin), such as an anti-Clq antibody of the present disclosure, in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is(are) identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison et al., Proc. Nat'l Acad. Sci. USA, 81:6851-55 (1984)). Chimeric antibodies of interest herein include PRIMATIZED® antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, *e.g.*, immunizing macaque monkeys with an antigen of interest. As used herein, "humanized antibody" is used a subset of "chimeric antibodies."

[0047] *"Humanized"* forms of non-human *(e.g.,* murine) antibodies, such as anti-Clq antibodies of the present disclosure, are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one implementation, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from an HVR of the recipient are replaced by residues from an HVR of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired specificity, affinity, and/or capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance, such as binding affinity. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin sequence, and all or substantially all of the FR regions are those of a human immunoglobulin sequence, although the FR regions may include one or more individual FR residue substitutions that improve antibody performance, such as binding affinity, isomerization, immunogenicity, and the like. The number of these amino acid substitutions in the FR is typically no more than 6 in the H chain, and in the L chain, no more than 3. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, *see, e.g.,* Jones et al., Nature 321:522-525 (1986); Riechmann et al., Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992). *See also,* for example, Vaswani and Hamilton, Ann. Allergy, Asthma & Immunol. 1:105-115 (1998); Harris, Biochem. Soc. Transactions 23:1035-1038 (1995); Hurle and Gross, Curr. Op. Biotech. 5:428-433 (1994); and U.S. Patent Nos. 6,982,321 and 7,087,409.

[0048] A *"human antibody"* is one that possesses an amino-acid sequence corresponding to that of an antibody, such as an anti-Clq antibody of the present disclosure, produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985); Boerner et al., J. Immunol., 147(1):86-95 (1991). *See also* van Dijk and van de Winkel, Curr. Opin. Pharmacol. 5:368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, *e.g.,* immunized xenomice *(see, e.g.,* U.S. Patent Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). *See also,* for example, Li et al., Proc. Nat'l Acad. Sci. USA, 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

[0049] The term "*hypervariable region," "HVR,"* or "*HV,*" when used herein refers to the regions of an antibody-variable domain, such as that of an anti-Clq antibody of the present disclosure, that are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). In native antibodies, H3 and L3 display the most diversity of the six HVRs, and H3 in particular is believed to play a unique role in conferring fine specificity to antibodies. *See, e.g.,* Xu et al., Immunity 13:37-45 (2000); Johnson and Wu in Methods in Molecular Biology 248:1-25 (Lo, ed., Human Press, Totowa, NJ, 2003)). Indeed, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain. See, *e.g.*, Hamers-Casterman et al., Nature 363:446-448 (1993) and Sheriff et al., Nature Struct. Biol. 3:733-736 (1996).

[0050] A number of HVR delineations are in use and are encompassed herein. The HVRs that are Kabat complementarity-determining regions (CDRs) are based on sequence variability and are the most commonly used (Kabat et al., *supra*). Chothia refers instead to the location of the structural loops (Chothia and Lesk J. Mol. Biol. 196:901-917 (1987)). The AbM HVRs represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody-modeling software. The "contact" HVRs are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below.

Loop	Kabat	AbM	Chothia	Contact
L1	L24-L34	L24-L34	L26-L32	L30-L36
L2	L50-L56	L50-L56	L50-L52	L46-L55
L3	L89-L97	L89-L97	L91-L96	L89-L96
H1	H31-H35B	H26-H35B	H26-H32	H30-H35B (Kabat numbering)
H1	H31-H35	H26-H35	H26-H32	H30-H35 (Chothia numbering)
H2	H50-H65	H50-H58	H53-H55	H47-H58
H3	H95-H102	H95-H102	H96-H101	H93-H101

[0051] HVRs may comprise "extended HVRs" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2), and 89-97 or 89-96 (L3) in the VL, and 26-35 (H1), 50-65 or 49-65 (H2), and 93-102, 94-102, or 95-102 (H3) in the VH. The variable-domain residues are numbered according to Kabat et al., *supra,* for each of these extended-HVR definitions.

[0052] "*Framework*" or *"FR"* residues are those variable-domain residues other than the HVR residues as herein defined.

[0053] The phrase *"variable-domain residue-numbering as in Kabat"* or *"amino-acid-position numbering as in Kabat*," and variations thereof, refers to the numbering system used for heavy-chain variable domains or light-chain variable domains of the compilation of antibodies in Kabat et al., *supra.* Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy-chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues *(e.g.,* residues 82a, 82b, and 82c, *etc.* according to Kabat) after heavy-chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

[0054] The Kabat numbering system is generally used when referring to a residue in the variable domain (approximately residues 1-107 of the light chain and residues 1-113 of the heavy chain) *(e.g.,* Kabat et al., Sequences of Immunological Interest. 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The "EU numbering system" or "EU index" is generally used when referring to a residue in an immunoglobulin heavy chain constant region *(e.g.,* the EU index reported in Kabat *et al.*, *supra*). The "EU index as in Kabat" refers to the residue numbering of the human IgG1 EU antibody. Unless stated otherwise herein, references to residue numbers in the variable domain of antibodies means residue numbering by the Kabat numbering system. Unless stated otherwise herein, references to residue numbers in the constant domain of antibodies means residue numbering by the EU numbering system (*e.g., see* United States Patent Publication No. 2010-280227).

[0055] An "*acceptor human framework"* as used herein is a framework comprising the amino acid sequence of a VL or VH framework derived from a human immunoglobulin framework or a human consensus framework. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain pre-existing amino acid sequence changes. In some implementations, the number of pre-existing amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some implementations, where pre-existing amino acid changes are present in a VH, those changes occur at only three, two, or one of positions 71H, 73H and 78H; for instance, the amino acid residues at those positions may by 71A, 73T and/or 78A. In one implementation, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

[0056] A *"human consensus framework*" is a framework that represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991). Examples include for the VL, the subgroup may be subgroup kappa I, kappa II, kappa III or kappa IV as in Kabat *et al.*, *supra.* Additionally, for the VH, the subgroup may be subgroup I, subgroup II, or subgroup III as in Kabat *et al.*, *supra.*

[0057] An "*amino-acid modification*" at a specified position, *e.g.,* of an anti-Clq antibody of the present disclosure, refers to the substitution or deletion of the specified residue, or the insertion of at least one amino acid residue adjacent the specified residue. Insertion "adjacent" to a specified residue means insertion within one to two residues thereof. The insertion may be N-terminal or C-terminal to the specified residue. In some implementations, the amino acid modification herein is a substitution.

[0058] An "*affinity-matured*" antibody, such as an anti- C1q antibody of the present disclosure, is one with one or more alterations in one or more HVRs thereof that result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody that does not possess those alteration(s). In one implementation, an affinity-matured antibody has nanomolar or even picomolar affinities for the target antigen. Affinity-matured antibodies are produced by procedures known in the art. For example, Marks et al., Bio/Technology 10:779-783 (1992) describes affinity maturation by VH- and VL-domain shuffling. Random mutagenesis of HVR and/or framework residues is described by, for example: Barbas et al. Proc Nat. Acad. Sci. USA 91:3809-3813 (1994); Schier et al. Gene 169:147-155 (1995); Yelton et al. J. Immunol. 155:1994-2004 (1995); Jackson et al., J. Immunol. 154(7):3310-9 (1995); and Hawkins et al, J. Mol. Biol. 226:889-896 (1992).

[0059] As use herein, the term *"specifically recognizes"* or "*specifically binds"* refers to measurable and reproducible interactions such as attraction or binding between a target and an antibody, such as an anti-C1q antibody of the present disclosure, that is determinative of the presence of the target in the presence of a heterogeneous population of molecules including biological molecules. For example, an antibody, such as an anti-C1q antibody of the present disclosure, that specifically or preferentially binds to a target or an epitope is an antibody that binds this target or epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other targets or other epitopes of the target. It is also understood by reading this definition that, for example, an antibody (or a moiety) that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, *"specific binding"* or "*preferential binding"* does not necessarily require (although it can include) exclusive binding. An antibody that specifically binds to a target may have an association constant of at least about 10^{3} M^{-1} or 10^{4} M^{-1}, sometimes about 10^{5} M^{-1} or 10^{6} M^{-1}, in other instances about 10^{6} M^{-1} or 10^{7} M^{-1}, about 10^{8} M^{-1} to 10^{9} M^{-1}, or about 10^{10} M^{-1} to 10^{11} M^{-1} or higher. A variety of immunoassay formats can be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. *See, e.g.,* Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Publications, New York, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

[0060] As used herein, an "*interaction*" between a complement protein, such as complement factor C1q, and a second protein encompasses, without limitation, protein-protein interaction, a physical interaction, a chemical interaction, binding, covalent binding, and ionic binding. As used herein, an antibody "inhibits interaction" between two proteins when the antibody disrupts, reduces, or completely eliminates an interaction between the two proteins. An antibody of the present disclosure, or fragment thereof, "inhibits interaction" between two proteins when the antibody or fragment thereof binds to one of the two proteins.

[0061] A *"blocking"* antibody, an "*antagonist*" antibody, an "*inhibitory*" antibody, or a *"neutralizing"* antibody is an antibody, such as an anti-Clq antibody of the present disclosure that inhibits or reduces one or more biological activities of the antigen it binds, such as interactions with one or more proteins. In some implementations, blocking antibodies, antagonist antibodies, inhibitory antibodies, or *"neutralizing"* antibodies substantially or completely inhibit one or more biological activities or interactions of the antigen.

[0062] Antibody "*effector functions*" refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody, and vary with the antibody isotype.

[0063] The term *"Fc region"* herein is used to define a C-terminal region of an immunoglobulin heavy chain, including native-sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an

immunoglobulin heavy chain might vary, the human IgG heavy-chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a composition of intact antibodies may comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations having a mixture of antibodies with and without the K447 residue. Suitable native-sequence Fc regions for use in the antibodies of the present disclosure include human IgG1, IgG2, IgG3 and IgG4.

[0064] A *"native sequence Fc region"* comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. Native sequence human Fc regions include a native sequence human IgG1 Fc region (non-A and A allotypes); native sequence human IgG2 Fc region; native sequence human IgG3 Fc region; and native sequence human IgG4 Fc region as well as naturally occurring variants thereof.

[0065] A "*variant Fc region"* comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification. In some implementations, the variant Fc region differs in one or more amino acid substitution(s). In some implementations, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, e.g. from about one to about ten amino acid substitutions, and, in some implementations, from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein will, in some implementations, possess at least about 80% homology with a native sequence Fc region and/or with an Fc region of a parent polypeptide, and, in some implementations, at least about 90% homology therewith, and, in some implementations, at least about 95% homology therewith.

[0066] *"Fc receptor"* or *"FcR"* describes a receptor that binds to the Fc region of an antibody. In some implementations, the FcR is a native sequence human FcR. Moreover, in some implementations, a FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors, FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif ("ITAM") in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif ("ITIM") in its cytoplasmic domain *(see, e.g.,* M. Daëron, Annu. Rev. Immunol. 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, Annu. Rev. Immunol. 9:457-92 (1991); Capel et al., Immunomethods 4:25-34 (1994); and de Haas et al., J. Lab. Clin. Med. 126: 330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. FcRs can also increase the serum half-life of antibodies.

[0067] Binding to FcRn *in vivo* and serum half-life of human FcRn high-affinity binding polypeptides can be assayed, e.g., in transgenic mice or transfected human cell lines expressing human FcRn, or in primates to which the polypeptides having a variant Fc region are administered. WO 2004/42072 (Presta) describes antibody variants with improved or diminished binding to FcRs. *See also, e.g.,* Shields et al., J. Biol. Chem. 9(2):6591-6604 (2001).

[0068] The term "k_{on}", as used herein, is intended to refer to the rate constant for association of an antibody to an antigen.

[0069] The term "k_{off}", as used herein, is intended to refer to the rate constant for dissociation of an antibody

from the antibody/antigen complex.

[0070] The term "K_D", as used herein, is intended to refer to the equilibrium dissociation constant of an antibody-antigen interaction.

[0071] As used herein, *"percent* (%) *amino acid sequence identity"* and *"homology"* with respect to a peptide, polypeptide or antibody sequence refers to the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid 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 or MEGALIGN™ (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms known in the art needed to achieve maximal alignment over the full length of the sequences being compared.

[0072] An "*isolated*" molecule or cell is a molecule or a cell that is identified and separated from at least one contaminant molecule or cell with which it is ordinarily associated in the environment in which it was produced. In some implementations, the isolated molecule or cell is free of association with all components associated with the production environment. The isolated molecule or cell is in a form other than in the form or setting in which it is found in nature. Isolated molecules therefore are distinguished from molecules existing naturally in cells; isolated cells are distinguished from cells existing naturally in tissues, organs, or individuals. In some implementations, the isolated molecule is an anti-Clq antibody of the present disclosure. In other implementations, the isolated cell is a host cell or hybridoma cell producing an anti-Clq antibody of the present disclosure.

[0073] An "*isolated*" nucleic acid molecule encoding an antibody, such as an anti-Clq antibody of the present disclosure, is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the environment in which it was produced. In some implementations, the isolated nucleic acid is free of association with all components associated with the production environment. The isolated nucleic acid molecules encoding the polypeptides and antibodies herein is in a form other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from nucleic acid encoding the polypeptides and antibodies herein existing naturally in cells.

[0074] The term *"vector,"* as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid," which refers to a circular double stranded DNA into which additional DNA segments may be ligated. Another type of vector is a phage vector. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors," or simply, "expression vectors." In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector.

[0075] *"Polynucleotide,"* or *"nucleic acid,"* as used interchangeably herein, refers to polymers of nucleotides

of any length, and includes DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase or by a synthetic reaction. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may comprise modification(s) made after synthesis, such as conjugation to a label. Other types of modifications include, for example, "caps," substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, *etc.)* and with charged linkages (e.g., phosphorothioates, phosphorodithioates, *etc.),* those containing pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, ply-L-lysine, *etc.),* those with intercalators *(e.g.,* acridine, psoralen, *etc.),* those containing chelators *(e.g.,* metals, radioactive metals, boron, oxidative metals, *etc.),* those containing alkylators, those with modified linkages *(e.g.,* alpha anomeric nucleic acids, *etc.),* as well as unmodified forms of the polynucleotides(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid or semi-solid supports. The 5' and 3' terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2'-O-methyl-, 2'-O-allyl-, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, α-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs, and basic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, implementations wherein phosphate is replaced by P(O)S ("thioate"), P(S)S ("dithioate"), $(O)NR_2$ ("amidate"), P(O)R, P(O)OR', CO, or CH_2 ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-O-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

[0076] A *"host cell"* includes an individual cell or cell culture that can be or has been a recipient for vector(s) for incorporation of polynucleotide inserts. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected *in vivo* with a polynucleotide(s) of this disclosure.

[0077] *"Carriers"* as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers that are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

[0078] The term *"about"* as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to *"about"* a value or parameter herein includes

(and describes) implementations that are directed to that value or parameter *per se.*

[0079] As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly indicates otherwise. For example, reference to an "antibody" is a reference to from one to many antibodies, such as molar amounts, and includes equivalents thereof known to those skilled in the art, and so forth.

[0080] It is understood that aspects and implementations of the present disclosure described herein include "comprising," "consisting," and "consisting essentially of" aspects and implementations.

Overview

[0081] The present disclosure provides humanized anti-Clq antibodies and uses thereof. The humanized anti-Clq antibodies of the present disclosure specifically bind a C1q protein of this disclosure. In some implementations, the humanized anti-Clq antibodies are C1q neutralizing antibodies. In some implementations, the humanized antiC1q antibodies of this disclosure may bind to C1 complex.

[0082] In certain aspects, the present disclosure provides a humanized antibody that specifically binds to a C1q protein, wherein the antibody comprises a heavy chain variable region and a human heavy chain constant region, wherein the heavy chain variable region comprises an Fab region and the heavy chain constant region comprises an Fc region, wherein the Fab region specifically binds to the C1q protein, and wherein the Fc region is incapable of binding the C1q protein.

[0083] In certain aspects, the present disclosure provides a humanized anti-Clq antibody, or an antigen-binding fragment thereof, the antibody comprising a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence selected from SEQ ID NOs: 1-4, or an amino acid sequence with at least about 90% homology to the amino acid sequence selected from SEQ ID NOs: 1-4.

[0084] In certain aspects, the present disclosure provides a humanized anti-Clq antibody, or an antigen-binding fragment thereof, the antibody comprising a heavy chain variable domain and a light chain variable domain, wherein the light chain variable domain comprises an amino acid sequence selected from SEQ ID NOs: 5-8, or an amino acid sequence with at least about 90% homology to the amino acid sequence selected from SEQ ID NOs: 5-8.

[0085] In certain aspects, the present disclosure provides a humanized anti-Clq antibody, or an antigen-binding fragment thereof, the antibody comprising: a heavy chain variable domain comprising an amino acid sequence selected from SEQ ID NOs: 1-4, or an amino acid sequence with at least about 90% homology to the amino acid sequence selected from SEQ ID NOs: 1-4; and/or a light chain variable domain comprising an amino acid sequence selected from SEQ ID NOs: 5-8, or an amino acid sequence with at least about 90% homology to the amino acid sequence selected from SEQ ID NOs: 5-8.

[0086] In some implementations, the humanized anti-Clq antibodies of the present disclosure neutralize a biological activity of C1q. Uses for humanized anti-Clq antibodies include, without limitation, the detection of complement factor C1q, *e.g.,* in individuals having a neurodegenerative disorder associated with complement factor 1 (CF1)-dependent pathological synapse loss. Additional non-limiting uses include the inhibition of the classical pathway of complement activation, *e.g.*, in cases where the classical complement pathway is activated by autoantibodies. Further non-limiting uses for humanized anti-Clq antibodies include the diagnosis and treatment of disorders that are associated with elevated expression of complement

factors, such as C1q, or associated with the activation of the complement pathway. Such disorders may include, without limitation, autoimmune disorders, inflammatory disorders, and neurodegenerative disorders, including neurodegenerative disorders associated with synapse loss.

[0087] In another aspect, the present disclosure provides an isolated nucleic acid molecule encoding an antibody of the present disclosure.

[0088] The present disclosure also provides isolated host cells containing a nucleic acid molecule that encodes an antibody of this disclosure. Additionally, pharmaceutical compositions are provided containing anti-Clq antibodies, such as humanized C1q neutralizing antibodies of this disclosure, in combination with pharmaceutically acceptable carriers. The present disclosure also provides a kit containing a humanized anti-Clq antibody for use in any of the methods described herein.

[0089] The present disclosure further provides methods of using the humanized antiC1q antibodies of the present disclosure (e.g., humanized C1q neutralizing antibodies of this disclosure) to treat or prevent a neurodegenerative disease or autoimmune disease in an individual in need of such treatment, to detect synapses in an individual having a neurodegenerative disease or autoimmune disease, and to detect synapses in a biological sample. The present disclosure also provides kits containing humanized anti-Clq antibodies of the present disclosure (e.g., humanized C1q neutralizing antibodies of this disclosure).

Complement Proteins

[0090] The antibodies of this disclosure specifically recognize complement factor C1q and/or C1q in the C1 complex of the classical complement activation pathway. The recognized complement factor may be derived, without limitation, from any organism having a complement system, including any mammalian organism such as human, mouse, rat, rabbit, monkey, dog, cat, cow, horse, camel, sheep, goat, or pig.

[0091] As used herein *"C1 complex"* refers to a protein complex that may include, without limitation, one C1q protein, two C1r proteins, and two C1s proteins (*e.g.*, $C1qr^2s^2$).

[0092] As used herein *"complement factor Clq"* refers to both wild type sequences and naturally occurring variant sequences.

[0093] A non-limiting example of a complement factor C1q recognized by antibodies of this disclosure is human C1q, including the three polypeptide chains A, B, and C:

C1q, chain A (homo sapiens), Accession No. Protein Data Base: NP_057075.1; GenBank No.: NM_015991:
>gi|7705753|ref]NP_057075.1| complement C1q subcomponent subunit A precursor [Homo sapiens]
MEGPRGWLVLCVLAISLASMVTEDLCRAPDGKKGEAGRPGRRGRPGLKGEQGEP
GAPGIRTGIQGLKGDQGEPGPSGNPGKVGYPGPSGPLGARGIPGIKGTKGSPGNIKD
QPRPAFSAIRRNPPMGGNVVIFDTVITNQEEPYQNHSGRFVCTVPGYYYFTFQVLSQ
WEICLSIVSSSRGQVRRSLGFCDTTNKGLFQVVSGGMVLQLQQGDQVWVEKDPKK
GHIYQGSEADSVFSGFLIFPSA (SEQ ID NO:9).

Clq, chain B (homo sapiens), Accession No. Protein Data Base: NP_000482.3; GenBank No.: NM_000491.3:
>gi|87298828|ref]NP_000482.3| complement C1q subcomponent subunit B precursor [Homo sapiens]
MMMKIPWGSIPVLMLLLLLGLIDISQAQLSCTGPPAIPGIPGIPGTPGPDGQPGTPGIK
GEKGLPGLAGDHGEFGEKGDPGIPGNPGKVGPKGPMGPKGGPGAPGAPGPKGESG
[illegible]

DYKATQKIAFSATRTINVPLRRDQTIRFDHVITNMNNNYEPRSGKFTCKVPGLYYFT
YHASSRGNLCVNLMRGRERAQKVVTFCDYAYNTFQVTTGGMVLKLEQGENVFLQ
ATDKNSLLGMEGANSIFSGFLLFPDMEA (SEQ ID NO:10).

C1q, chain C (homo sapiens), Accession No. Protein Data Base: NP_001107573.1; GenBank No.: NM_001114101.1:
>gi|166235903|ref]NP_001107573.1| complement C1q subcomponent subunit C precursor [Homo sapiens]
MDVGPSSLPHLGLKLLLLLLLLPLRGQANTGCYGIPGMPGLPGAPGKDGYDGLPGP
KGEPGIPAIPGIRGPKGQKGEPGLPGHPGKNGPMGPPGMPGVPGPMGIPGEPGEEGR
YKQKFQSVFTVTRQTHQPPAPNSLIRFNAVLTNPQGDYDTSTGKFTCKVPGLYYFV
YHASHTANLCVLLYRSGVKVVTFCGHTSKTNQVNSGGVLLRLQVGEEVWLAVND
YYDMVGIQGSDSVFSGFLLFPD (SEQ ID NO:11).

[0094] Accordingly, a humanized anti-Clq antibody of the present disclosure may bind to polypeptide chain A, polypeptide chain B, and/or polypeptide chain C of a C1q protein. In some implementations, a humanized anti-C1q antibody of the present disclosure binds to polypeptide chain A, polypeptide chain B, and/or polypeptide chain C of human C1q or a homolog thereof, such as mouse, rat, rabbit, monkey, dog, cat, cow, horse, camel, sheep, goat, or pig C1q.

Humanized anti-Clq Antibodies

[0095] Humanized antibodies of the present disclosure specifically bind to a complement factor C1q and/or C1q protein in the C1 complex of the classical complement pathway. In some implementations, the humanized anti-Clq antibodies specifically bind to human C1q. In some implementations, the humanized anti-Clq antibodies specifically bind to human and mouse C1q. In some implementations, the humanized anti-Clq antibodies specifically bind to rat C1q. In some implementations, the humanized anti-Clq antibodies specifically bind to human C1q, mouse C1q, and rat C1q.

[0096] In some implementations, humanized anti-Clq antibodies of the present disclosure include a heavy chain variable region that contains an Fab region and a heavy chain constant regions that contains an Fc region, where the Fab region specifically binds to a C1q protein of the present disclosure, but the Fc region is incapable of binding the C1q protein. In some implementations, the Fc region is from a human IgG1, IgG2, IgG3, or IgG4 isotype. In some implementations, the Fc region is incapable of inducing complement activity and/or incapable of inducing antibody-dependent cellular cytotoxicity (ADCC). In some implementations, the Fc region comprises one or more modifications, including, without limitation, amino acid substitutions. In certain implementations, the Fc region of humanized anti-Clq antibodies of the present disclosure comprises an amino acid substitution at position 248 according to Kabat numbering convention or a position corresponding to position 248 according to Kabat numbering convention, and/or at position 241 according to Kabat numbering convention or a position corresponding to position 241 according to Kabat numbering convention. In some implementations, the amino acid substitution at position 248 or a position corresponding to position 248 inhibits the Fc region from interacting with an Fc receptor. In some implementations, the amino acid substitution at position 248 or a position corresponding to position 248 is a leucine to glutamate amino acid substitution. In some implementations, the amino acid substitution at position 241 or a position corresponding to position 241 prevents arm switching in the antibody. In some implementations, the amino acid substitution at position 241 or a position corresponding to position 241 is a serine to proline amino acid substitution. In certain implementations, the Fc region of humanized anti-Clq antibodies of the present disclosure comprises the amino acid sequence of SEQ ID NO: 37, or an amino acid sequence with at least

about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 37.

[0097] In some implementations, the humanized anti-Clq antibodies of the present disclosure neutralize a biological activity of complement factor C1q. In some implementations, the antibodies inhibit the interaction between complement factor C1q and other complement factors, such as C1r or C1s or between C1q and an antibody, such as an autoantibody. As disclosed herein, an autoantibody of the present disclosure includes, without limitation, an antibody that recognizes a host antigen and activates the classical pathway of complement activation. In the first step of this activation process complement factor C1q binds to the autoantibody-autoantigen-immune complex. In some implementations, the antibodies inhibit the interaction between complement factor C1q and a non-complement factor. A non-complement factor may include phosphatidylserine, pentraxin-3, C-reactive protein (CRP), globular C1q receptor (gC1qR), complement receptor 1 (CR1), β-amyloid, and calreticulin. In some implementations, the antibodies inhibit the classical complement activation pathway. In certain implementations, the antibodies further inhibit the alternative pathway. In some implementations, the antibodies inhibit autoantibody- and complement-dependent cytotoxicity (CDC). In some implementations, the antibodies inhibit complement-dependent cell-mediated cytotoxicity (CDCC). In some implementations, the antibodies inhibit B-cell antibody production, dendritic cell maturation, T-cell proliferation, cytokine production, or microglia activation. In some implementations, the antibodies inhibit the Arthus reaction. In some implementations, the antibodies inhibit phagocytosis of synapses or nerve endings. In some implementations, the antibodies inhibit the activation of complement receptor 3 (CR3/C3) expressing cells.

[0098] The functional properties of the antibodies of the present disclosure, such as dissociation constants for antigens, inhibition of protein-protein interactions (e.g., C1q-autoantibody interactions), inhibition of autoantibody-dependent and complement-dependent cytotoxicity (CDC), inhibition of complement-dependent cell-mediated cytotoxicity (CDCC), or lesion formation, may, without limitation, be measured in *in vitro, ex vivo,* or *in vivo* experiments.

[0099] The dissociation constants (K_D) of the humanized anti-Clq antibodies for C1q may be less than 125 nM, less than 120 nM, less than 115 nM, less than 110 nM, less than 100 nM, less than 90 nM, less than 80 nM, less than 70 nM, less than 60 nM, less than 50 nM, less than 40 nM, less than 30 nM, less than 20 nM, less than 10 nM, less than 9 nM, less than 8 nM, less than 7 nM, less than 6 nM, less than 5 nM, less than 4 nM, less than 3 nM, less than 2 nM, less than 1 nM, less than 0.5 nM, less than 0.1 nM, less than 0.05 nM, less than 0.01 nM, or less than 0.005 nM. In some implementations, dissociation constants range from less than about 125 nM to less than about 5 pM.

[0100] In some implementations, dissociation constants of humanized anti-Clq antibodies of the present disclosure are less than about 10 pM to less than about 5 pM for human C1q. In some implementations, dissociation constants are less than about 10 pM, less than about 9.9 pm, less than about 9.8 pM, less than about 9.7 pM, less than about 9.6 pM, less than about 9.5 pM, less than about 9.4 pM, less than about 9.3 pM, less than about 9.2 pM, less than about 9.1 pM, less than about 9 pM, less than about 8.9 pm, less than about 8.8 pM, less than about 8.7 pM, less than about 8.6 pM, less than about 8.5 pM, less than about 8.4 pM, less than about 8.3 pM, less than about 8.2 pM, less than about 8.1 pM, less than about 8 pM, less than about 7.9 pm, less than about 7.8 pM, less than about 7.7 pM, less than about 7.6 pM, less than about 7.5 pM, less than about 7.4 pM, less than about 7.3 pM, less than about 7.2 pM, less than about 7.1 pM, less than about 7 pM, less than about 6.9 pm, less than about 6.8 pM, less than about 6.7 pM, less than about 6.6 pM, less than about 6.5 pM, less than about 6.4 pM, less than about 6.3 pM, less than about 6.2 pM, less than about 6.1 pM, less than about 6 pM, less than about 5.9 pm, less than about 5.8 pM, less than about 5.7 pM, less than about 5.6 pM, less than about 5.5 pM, less than about 5.4 pM, less than about 5.3 pM, less than about 5.2 pM, less than about 5.1 pM, or less than about 5 pM, for human C1q.

[0101] In some implementations, dissociation constants of humanized anti-Clq antibodies of the present disclosure are less than 125 nM, less than 120 nM, less than 115 nM, less than 110 nM, less than 100 nM, less than 90 nM, less than 80 nM, less than 70 nM, less than 60 nM, less than 50 nM, less than 40 nM, less than 30 nM, less than 20 nM, less than 10 nM, less than 9 nM, less than 8 nM, less than 7 nM, less than 6 nM, less than 5 nM, less than 4 nM, less than 3 nM, less than 2 nM, less than 1 nM, less than 0.5 nM, less than 0.1 nM, or less than 0.05 nM for mouse C1q.

[0102] Antibody dissociation constants for antigens other than C1q may be least 5-fold, at least 10-fold, at least 100-fold, at least 1,000-fold, at least 10,000-fold, or at least 100,000-fold higher that the dissociation constants for C1q. For example, the dissociation constant of a humanized anti-Clq antibody of the present disclosure may be at least 1,000-fold higher for C1s than for C1q. Dissociation constants may be determined through any analytical technique, including any biochemical or biophysical technique such as ELISA, surface plasmon resonance (SPR), bio-layer interferometry *(see, e.g.,* Octet System by ForteBio), isothermal titration calorimetry (ITC), differential scanning calorimetry (DSC), circular dichroism (CD), stopped-flow analysis, and colorimetric or fluorescent protein melting analyses. Dissociation constants (K_D) of the anti-Clq antibodies for C1q may be determined, *e.g.*, using full-length antibodies or antibody fragments, such as Fab fragments.

[0103] One exemplary way of determining binding affinity of humanized antibodies to C1q is by measuring binding affinity of monofunctional Fab fragments of the antibody. To obtain monofunctional Fab fragments, an antibody (for example, IgG) can be cleaved with papain or expressed recombinantly. The affinity of an Fab fragment of an antibody can be determined by surface plasmon resonance (Biacore3000™ surface plasmon resonance (SPR) system, Biacore.TM., INC, Piscataway N.J.) equipped with pre-immobilized streptavidin sensor chips (SA) using HBS-EP running buffer (0.01M HEPES, pH 7.4, 0.15 NaCl, 3 mM EDTA, 0.005% v/v Surfactant P20). Biotinylated human C1q (or any other C1q) can be diluted into HBS-EP buffer to a concentration of less than 0.5 µg/mL and injected across the individual chip channels using variable contact times, to achieve two ranges of antigen density, either 50-200 response units (RU) for detailed kinetic studies or 800-1,000 RU for screening assays. Regeneration studies have shown that 25 mM NaOH in 25% v/v ethanol effectively removes the bound Fab while keeping the activity of C1q on the chip for over 200 injections. Typically, serial dilutions (spanning concentrations of 0.1-10.times. estimated K_D) of purified Fab samples are injected for 1 min at 100 µL/minute and dissociation times of up to 2 hours are allowed. The concentrations of the Fab proteins are determined by ELISA and/or SDS-PAGE electrophoresis using a Fab of known concentration (as determined by amino acid analysis) as a standard. Kinetic association rates (k_{on}) and dissociation rates (k_{off}) are obtained simultaneously by fitting the data globally to a 1:1 Langmuir binding model (Karlsson, R. Roos, H. Fagerstam, L. Petersson, B. (1994). Methods Enzymology 6. 99-110) using the BIAevaluation program. Equilibrium dissociation constant (K_D) values are calculated as k_{off}/k_{on}. This protocol is suitable for use in determining binding affinity of an antibody to any C1q, including human C1q, C1q of another mammal (such as mouse C1q, rat C1q, primate C1q), as well as different forms of C1q. Binding affinity of an antibody is generally measured at 25°C, but can also be measured at 37°C.

[0104] The humanized antibodies of the present disclosure may bind to C1q antigens derived from any organism having a complement system, including any mammalian organism such as human, mouse, rat, rabbit, monkey, dog, cat, cow, horse, camel, sheep, goat, or pig. In some implementations, the anti-Clq antibodies bind specifically to epitopes on human C1q. In some implementations, the anti-Clq antibodies specifically bind to epitopes on both human and mouse C1q. In some implementations, the anti-Clq antibodies specifically bind to epitopes on human, mouse, and rat C1q.

[0105] In some implementations, provided herein is a humanized anti-Clq antibody that binds to an epitope of C1q that is the same as or overlaps with the C1q epitope bound by another antibody of this disclosure. In

certain implementations, provided herein is a humanized anti-Clq antibody that binds to an epitope of C1q that is the same as or overlaps with the C1q epitope bound by anti-Clq antibody M1 produced by the hybridoma cell line with ATCC Accession Number PTA-120399. In some implementations, the humanized anti-Clq antibody competes with another antibody of this disclosure for binding to C1q. In certain implementations, the anti-Clq antibody competes with anti-Clq antibody M1 produced by the hybridoma cell line with ATCC Accession Number PTA-120399 or antiC1q binding fragments thereof.

[0106] Methods that may be used to determine which C1q epitope of a humanized antiC1q antibody binds to, or whether two antibodies bind to the same or an overlapping epitope, may include, without limitation, X-ray crystallography, NMR spectroscopy, Alanine-Scanning Mutagenesis, the screening of peptide libraries that include C1q-derived peptides with overlapping C1q sequences, and competition assays. Competition assays are especially useful to determine whether two antibodies bind the same epitope by recognizing identical or sterically overlapping epitopes or whether one antibody competitively inhibits binding of another antibody to the antigen. These assays are known in the art. Typically, an antigen or antigen expressing cells are immobilized on a multi-well plate and the ability of unlabeled antibodies to block the binding of labeled antibodies is measured. Common labels for such competition assays are radioactive labels or enzyme labels.

[0107] Competitive antibodies encompassed herein are humanized antibodies that inhibit (i.e., prevent or interfere with in comparison to a control) or reduce the binding of any anti-Clq antibody of this disclosure (such as M1 or an antigen-binding fragment of M1) to C1q by at least 50%, 60%, 70%, 80%, 90% and 95% at 1 μM or less. For example, the concentration competing antibody in the competition assay may be at or below the K_D of antibody M1 or an antigen-binding fragment of M1. Competition between binding members may be readily assayed *in vitro* for example using ELISA and/or by monitoring the interaction of the antibodies with C1q in solution. The exact means for conducting the analysis is not critical. C1q may be immobilized to a 96-well plate or may be placed in a homogenous solution. In specific implementations, the ability of unlabeled candidate antibody(ies) to block the binding of the labeled anti-Clq antibody, e.g. M1, can be measured using radioactive, enzyme or other labels. In the reverse assay, the ability of unlabeled antibodies to interfere with the interaction of a labeled anti-Clq antibody with C1q wherein said labeled anti-Clq antibody, e.g., M1, and C1q are already bound is determined. The readout is through measurement of bound label. C1q and the candidate antibody(ies) may be added in any order or at the same time.

[0108] In some implementations, the humanized anti-Clq antibody inhibits the interaction between C1q and an autoantibody.

[0109] In some implementations, a humanized anti-Clq antibody of the present disclosure binds essentially the same C1q epitope as antibody M1 produced by the hybridoma cell line with ATCC Accession Number PTA-120399 or anti-Clq binding fragments thereof.

[0110] In some implementations, the humanized anti-Clq antibody is an antibody, or an antigen-binding fragment thereof, comprising a heavy chain variable domain comprising an amino acid sequence selected from SEQ ID NOs: 1-4, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence selected from SEQ ID NOs: 1-4. In some implementations, the humanized anti-Clq antibody is an antibody, or an antigen-binding fragment thereof, comprising a light chain variable domain comprising an amino acid sequence selected from SEQ ID NOs: 5-8, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence selected from SEQ ID NOs: 5-8. In some implementations, the humanized anti-Clq antibody is an antibody, or an antigen-binding fragment thereof, comprising a heavy chain variable domain comprising an amino acid sequence selected from SEQ ID NOs: 1-4, or an amino acid

sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence selected from SEQ ID NOs: 1-4; and/or a light chain variable domain comprising an amino acid sequence selected from SEQ ID NOs: 5-8, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence selected from SEQ ID NOs: 5-8.

[0111] In some implementations, the humanized anti-Clq antibody, or an antigen-binding fragment thereof, comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 1; and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 5, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 5. In some implementations, the humanized anti-Clq antibody, or an antigen-binding fragment thereof, comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 1; and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 6. In some implementations, the humanized anti-Clq antibody, or an antigen-binding fragment thereof, comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 1; and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 7, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 7. In some implementations, the humanized anti-Clq antibody, or an antigen-binding fragment thereof, comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 1; and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 8, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 8.

[0112] In some implementations, the humanized anti-Clq antibody, or an antigen-binding fragment thereof, comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 2, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 2; and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 5, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 5. In some implementations, the humanized anti-Clq antibody, or an antigen-binding fragment thereof, comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 2, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 2; and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 6. In some implementations, the humanized anti-Clq antibody, or an antigen-binding fragment thereof, comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 2, or an amino acid sequence with at least about 70%, at least about 75%, at least

about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 2; and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 7, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 7. In some implementations, the humanized anti-Clq antibody, or an antigen-binding fragment thereof, comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 2, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 2; and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 8, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 8.

[0113] In some implementations, the humanized anti-Clq antibody, or an antigen-binding fragment thereof, comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 3, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 3; and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 5, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 5. In some implementations, the humanized anti-Clq antibody, or an antigen-binding fragment thereof, comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 3, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 3; and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 6. In some implementations, the humanized anti-Clq antibody, or an antigen-binding fragment thereof, comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 3, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 3; and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 7, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 7. In some implementations, the humanized anti-Clq antibody, or an antigen-binding fragment thereof, comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 3, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 3; and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 8, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 8.

[0114] In some implementations, the humanized anti-Clq antibody, or an antigen-binding fragment thereof, comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 4, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 4; and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 5, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 5. In some implementations, the humanized anti-Clq antibody, or an antigen-binding fragment thereof, comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 4, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about

95% homology to the amino acid sequence of SEQ ID NO: 4; and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 6. In some implementations, the humanized anti-Clq antibody, or an antigen-binding fragment thereof, comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 4, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 4; and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 7, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 7. In some implementations, the humanized anti-Clq antibody, or an antigen-binding fragment thereof, comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 4, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 4; and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 8, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95%homology to the amino acid sequence of SEQ ID NO: 8.

[0115] In some implementations, humanized anti-Clq antibodies of the present disclosure may comprise at least one HVR selected from HVR-L1, HVR-L2, and HVR-L3 of the light chain variable domains of monoclonal antibody M1 produced by the hybridoma cell line having ATCC Accession Number PTA-120399, or progeny thereof. In some implementations, humanized anti-Clq antibodies of the present disclosure may comprise at least one HVR selected from HVR-H1, HVR-H2, and HVR-H3 of the heavy chain variable domains of monoclonal antibody M1 produced by the hybridoma cell line having ATCC Accession Number PTA-120399, or progeny thereof. In some implementations, humanized anti-Clq antibodies of the present disclosure may comprise at least one HVR selected from HVR-L1, HVR-L2, and HVR-L3 of the light chain variable domains and at least one HVR selected from HVR-H1, HVR-H2, and HVR-H3 of the heavy chain variable domains of monoclonal antibody M1 produced by the hybridoma cell line having ATCC Accession Number PTA-120399, or progeny thereof.

[0116] In some implementations, humanized anti-Clq antibodies of the present disclosure may bind to a C1q protein and binds to one or more amino acids of the C1q protein within amino acid residues selected from (a) amino acid residues 196-226 of SEQ ID NO: 9 (SEQ ID NO: 12), or amino acid residues of a C1q protein chain A (C1qA) corresponding to amino acid residues 196-226

(GLFQVVSGGMVLQLQQGDQVWVEKDPKKGHI) of SEQ ID NO: 9 (SEQ ID NO: 12); (b) amino acid residues 196-221 of SEQ ID NO: 9 (SEQ ID NO: 13), or amino acid residues of a ClqA corresponding to amino acid residues 196-221

(GLFQVVSGGMVLQLQQGDQVWVEKDP) of SEQ ID. NO: 9 (SEQ ID NO:13); (c) amino acid residues 202-221 of SEQ ID NO: 9 (SEQ ID NO:14), or amino acid residues of a ClqA corresponding to amino acid residues 202-221

(SGGMVLQLQQGDQVWVEKDP) of SEQ ID NO: 9 (SEQ ID NO:14); (d) amino acid residues 202-219 of SEQ ID NO: 9 (SEQ ID NO:15), or amino acid residues of a ClqA corresponding to amino acid residues 202-219 (SGGMVLQLQQGDQVWVEK) of SEQ ID NO: 9 (SEQ ID NO:15); and (e) amino acid residues Lys 219 and/or Ser 202 of SEQ ID NO: 9, or amino acid residues of a ClqA corresponding Lys 219 and/or Ser 202 of SEQ ID NO: 9.

[0117] In some implementations, the humanized anti-Clq antibodies may further bind to one or more amino

acids of the C1q protein within amino acid residues selected from: (a) amino acid residues 218-240 of SEQ ID NO: 11 (SEQ ID NO:16) or amino acid residues of a C1q protein chain C (ClqC) corresponding to amino acid residues 218-240 (WLAVNDYYDMVGI QGSDSVFSGF) of SEQ ID NO: 11 (SEQ ID NO:16); (b) amino acid residues 225-240 of SEQ ID NO: 11 (SEQ ID NO:17) or amino acid residues of a ClqC corresponding to amino acid residues 225-240 (YDMVGI QGSDSVFSGF) of SEQ ID NO: 11 (SEQ ID NO:17); (c) amino acid residues 225-232 of SEQ ID NO: 11 (SEQ ID NO:18) or amino acid residues of a ClqC corresponding to amino acid residues 225-232 (YDMVGIQG) of SEQ ID NO: 11 (SEQ ID NO:18); (d) amino acid residue Tyr 225 of SEQ ID NO: 11 or an amino acid residue of a ClqC corresponding to amino acid residue Tyr 225 of SEQ ID NO: 11; (e) amino acid residues 174-196 of SEQ ID NO: 11 (SEQ ID NO:19) or amino acid residues of a ClqC corresponding to amino acid residues 174-196 (HTANLCVLLYRSGVKVVTFCGHT) of SEQ ID NO: 11 (SEQ ID NO:19); (f) amino acid residues 184-192 of SEQ ID NO: 11 (SEQ ID NO:20) or amino acid residues of a ClqC corresponding to amino acid residues 184-192 (RSGVKVVTF) of SEQ ID NO: 11 (SEQ ID NO:20); (g) amino acid residues 185-187 of SEQ ID NO: 11 or amino acid residues of a ClqC corresponding to amino acid residues 185-187 (SGV) of SEQ ID NO: 11; (h) amino acid residue Ser 185 of SEQ ID NO: 11 or an amino acid residue of a ClqC corresponding to amino acid residue Ser 185 of SEQ ID NO: 11.

[0118] In certain implementations, humanized anti-Clq antibodies of the present disclosure may bind to amino acid residues Lys 219 and Ser 202 of the human ClqA as shown in SEQ ID NO: 9 or amino acids of a human ClqA corresponding to Lys 219 and Ser 202 as shown in SEQ ID NO: 9, and amino acid residue Tyr 225 of the human ClqC as shown in SEQ ID NO: 11 or an amino acid residue of a human ClqC corresponding to Tyr 225 as shown in SEQ ID NO: 11. In certain implementations, the anti-Clq antibody binds to amino acid residue Lys 219 of the human ClqA as shown in SEQ ID NO: 9 or an amino acid residue of a human ClqA corresponding to Lys 219 as shown in SEQ ID NO: 9, and amino acid residue Ser 185 of the human ClqC as shown in SEQ ID NO: 11 or an amino acid residue of a human C1qC corresponding to Ser 185 as shown in SEQ ID NO: 11.

[0119] In some implementations, humanized anti-Clq antibodies of the present disclosure may bind to a C1q protein and binds to one or more amino acids of the C1q protein within amino acid residues selected from: (a) amino acid residues 218-240 of SEQ ID NO: 11 (SEQ ID NO:16) or amino acid residues of a C1qC corresponding to amino acid residues 218-240 (WLAVNDYYDMVGI QGSDSVFSGF) of SEQ ID NO: 11 (SEQ ID NO:16); (b) amino acid residues 225-240 of SEQ ID NO: 11 (SEQ ID NO:17) or amino acid residues of a ClqC corresponding to amino acid residues 225-240 (YDMVGI QGSDSVFSGF) of SEQ ID NO: 11 (SEQ ID NO:17); (c) amino acid residues 225-232 of SEQ ID NO: 11 (SEQ ID NO:18) or amino acid residues of a ClqC corresponding to amino acid residues 225-232 (YDMVGIQG) of SEQ ID NO: 11 (SEQ ID NO:18); (d) amino acid residue Tyr 225 of SEQ ID NO: 11 or an amino acid residue of a ClqC corresponding to amino acid residue Tyr 225 of SEQ ID NO: 11; (e) amino acid residues 174-196 of SEQ ID NO: 11 (SEQ ID NO:19) or amino acid residues of a ClqC corresponding to amino acid residues 174-196 (HTANLCVLLYRSGVKWTFCGHT) of SEQ ID NO: 11 (SEQ ID NO:19); (f) amino acid residues 184-192 of SEQ ID NO: 11 (SEQ ID NO:20) or amino acid residues of a ClqC corresponding to amino acid residues 184-192 (RSGVKWTF) of SEQ ID NO: 11 (SEQ ID NO:20); (g) amino acid residues 185-187 of SEQ ID NO: 11 or amino acid residues of a ClqC corresponding to amino acid residues 185-187 (SGV) of SEQ ID NO: 11; (h) amino acid residue Ser 185 of SEQ ID NO: 11 or an amino acid residue of a ClqC corresponding to amino acid residue Ser 185 of SEQ ID NO: 11.

[0120] In some implementations, a humanized anti-Clq antibody of the present disclosure inhibits the interaction between C1q and C1s. In some implementations, the humanized anti-Clq antibody inhibits the interaction between C1q and C1r. In some implementations the humanized anti-Clq antibody inhibits the interaction between C1q and C1s and between C1q and C1r. In some implementations, the humanized anti-Clq antibody inhibits the interaction between C1q and another antibody, such as an autoantibody. In some implementations, the humanized anti-Clq antibody inhibits the respective interactions, at a stoichiometry of

less than 2.5:1; 2.0:1; 1.5:1; or 1.0:1. In some implementations, the humanized C1q antibody inhibits an interaction, such as the C1q-C1s interaction, at approximately equimolar concentrations of C1q and the anti-Clq antibody. In other implementations, the anti-C1q antibody binds to C1q with a stoichiometry of less than 20:1; less than 19.5:1; less than 19: 1; less than 18.5:1; less than 18:1; less than 17.5:1; less than 17:1; less than 16.5:1; less than 16:1; less than 15.5:1; less than 15:1; less than 14.5:1; less than 14:1; less than 13.5:1; less than 13:1; less than 12.5:1; less than 12:1; less than 11.5:1; less than 11:1; less than 10.5:1; less than 10:1; less than 9.5:1; less than 9:1; less than 8.5:1; less than 8:1; less than 7.5:1; less than 7:1; less than 6.5:1; less than 6:1; less than 5.5:1; less than 5:1; less than 4.5:1; less than 4:1; less than 3.5:1; less than 3:1; less than 2.5:1; less than 2.0:1; less than 1.5:1; or less than 1.0:1. In certain implementations, the humanized anti-Clq antibody binds C1q with a binding stoichiometry that ranges from 20:1 to 1.0:1 or less than1.0:1. In certain implementations, the humanized anti-Clq antibody binds C1q with a binding stoichiometry that ranges from 6:1 to 1.0:1 or less than1.0:1. In certain implementations, the humanized anti-Clq antibody binds C1q with a binding stoichiometry that ranges from 2.5:1 to 1.0:1 or less than1.0:1. In some implementations, an anti-Clq antibody of the present disclosure having a binding stoichiometry for C1q of 1.0:1 yeilds approximately 50% inhibition of C1F hemolysis, as deptermined for example by CH50 assays of the present disclosure. In some implementations, the humanized anti-Clq antibody inhibits the interaction between C1q and C1r, or between C1q and C1s, or between C1q and both C1r and C1s. In some implementations, the humanized anti-Clq antibody inhibits the interaction between C1q and C1r, between C1q and C1s, and/or between C1q and both C1r and C1s. In some implementations, the humanized anti-Clq antibody binds to the C1q A-chain. In other implementations, the humanized anti-Clq antibody binds to the C1q B-chain. In other implementations, the humanized anti-Clq antibody binds to the C1q C-chain. In some implementations, the humanized anti-Clq antibody binds to the C1q A-chain, the C1q B-chain and/or the C1q C-chain. In some implementations, the humanized anti-Clq antibody binds to the globular domain of the C1q A-chain, B-chain, and/or C-chain. In other implementations, the humanized anti-Clq antibody binds to the collagen-like domain of the C1q A-chain, the C1q B-chain, and/or the C1q C-chain.

[0121] Where humanized antibodies of this disclosure inhibit the interaction between two or more complement factors, such as the interaction of C1q and C1s, or the interaction between C1q and C1r, the interaction occurring in the presence of the antibody may be reduced by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% relative to a control wherein the antibodies of this disclosure are absent. In certain implementations, the interaction occurring in the presence of the humanized antibody is reduced by an amount that ranges from at least 30% to at least 99% relative to a control wherein the humanized antibodies of this disclosure are absent.

[0122] In some implementations, humanized anti-Clq antibodies of the present disclosure inhibit C4-cleavage by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99%, or by an amount that ranges from at least 30% to at least 99%, relative to a control wherein the antibodies of this disclosure are absent. Methods for measuring C4-cleavage are well known in the art. The EC_{50} values for antibodies of this disclosure with respect C4-cleavage may be less than 3 µg/ml; 2.5 µg/ml; 2.0 µg/ml; 1.5 µg/ml; 1.0 µg/ml; 0.5 µg/ml; 0.25 µg/ml; 0.1 µg/ml; 0.05 µg/ml. In some implementations, the antibodies of this disclosure inhibit C4-cleavage at approximately equimolar concentrations of C1q and the respective anti-Clq antibody.

[0123] In some implementations, humanized anti-Clq antibodies of the present disclosure inhibit autoantibody-dependent and complement-dependent cytotoxicity (CDC) by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99%, or by an amount that ranges from at least 30% to at least 99%, relative to a control wherein the antibodies of this disclosure are absent. The EC_{50} values for antibodies of this disclosure with respect to inhibition of autoantibody-dependent and complement-dependent cytotoxicity may be less than 3 µg/ml; 2.5 µg/ml; 2.0

µg/ml; 1.5 µg/ml; 1.0 µg/ml; 0.5 µg/ml; 0.25 µg/ml; 0.1 µg/ml; 0.05 µg/ml.

[0124] In some implementations, humanized anti-Clq antibodies of the present disclosure inhibit complement-dependent cell-mediated cytotoxicity (CDCC) by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99%, or by an amount that ranges from at least 30% to at least 99%, relative to a control wherein the antibodies of this disclosure are absent. Methods for measuring CDCC are well known in the art. The EC_{50} values for antibodies of this disclosure with respect CDCC inhibition may be less than 3 µg/ml; 2.5 µg/ml; 2.0 µg/ml; 1.5 µg/ml; 1.0 µg/ml; 0.5 µg/ml; 0.25 µg/ml; 0.1 µg/ml; 0.05 µg/ml. In some implementations, the antibodies of this disclosure inhibit CDCC but not antibody-dependent cellular cytotoxicity (ADCC).

[0125] In some implementations, humanized anti-Clq antibodies of the present disclosure inhibit C1F hemolysis (also referred to as CH50 hemolysis) by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99%, or by an amount that ranges from at least 30% to at least 99%,relative to a control wherein the antibodies of this disclosure are absent or wherein control antibodies are used that do not bind to a complement factor or another antibody such as an autoantibody *(see, e.g.,* Examples section below). Methods for measuring C1F hemolysis are well known in the art *(see, e.g.,* Examples section below). The EC_{50} values for humanized antibodies of this disclosure with respect to C1F hemolysis may be less than 3 µg/ml; 2.5 µg/ml; 2.0 µg/ml; 1.5 µg/ml; 1.0 µg/ml; 0.5 µg/ml; 0.25 µg/ml; 0.1 µg/ml; 0.05 µg/ml. In some implementations, humanized anti-Clq antibodies of this disclosure neutralize at least 50% of C1F hemolysis at a dose of less than 200 ng/ml, less than 100 ng/ml, less than 50 ng/ml, or less than 20 ng/ml. In some implementations, humanized antibodies of this disclosure neutralize C1F hemolysis at approximately equimolar concentrations of C1q and the anti-Clq antibody. In some implementations, humanized anti-Clq antibodies of this disclosure neutralize hemolysis in a human C1F hemolysis assay. In some implementations, humanized anti-Clq antibodies of this disclosure neutralize hemolysis in a human and rat C1F hemolysis assay *(see, e.g.,* Examples section below).

[0126] In some implementations, the alternative pathway may amplify CDC initiated by C1q binding and subsequent C1s activation; in at least some of these implementations, the antibodies of this disclosure inhibit the alternative pathway by at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99%, or by an amount that ranges from at least 30% to at least 99%, relative to a control wherein the antibodies of this disclosure were absent.

[0127] In some implementations, humanized anti-Clq antibodies of the present disclosure prevent synaptic loss in a cellular *in vitro* model or an *in vivo* model of synaptic loss, such as an *in vivo* mouse model. *In vivo* mouse models may include Tg2576, a mouse amyloid precursor protein (APP) transgenic model of Alzheimer's disease, R6/2 NT-CAG150, a transgenic model for Huntington's disease, or SMAΔ7, a mouse model for Spinal Muscular Atrophy, or DBA/2J, a genetic mouse model of glaucoma. In general, any neurodegenerative disease model may be used that displays synapse loss.

[0128] Methods for measuring synaptic loss *in vitro* or *in vivo* are well known in the art. *In vitro* lesion formation may be reduced by at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%, or by an amount that ranges from at least 30% to at least 95%, relative to a control experiment in which antibodies of this disclosure are absent. The EC_{50} values for antibodies of this disclosure with respect to the prevention of *in vitro* lesion formation may be less than 3 µg/ml; 2.5 µg/ml; 2.0 µg/ml; 1.5 µg/ml; 1.0 µg/ml; 0.5 µg/ml; 0.25 µg/ml; 0.1 µg/ml; 0.05 µg/ml. *In vivo* synaptic loss may be reduced by at least 5%, at least 10%, at least 15%, at least 20%, at least 35%, at least 40%, or at least 50%, or by an amount that ranges from at least 5% to at least 50%, relative to a control experiment in which antibodies of this disclosure are absent.

[0129] In some implementations, humanized anti-Clq antibodies of the present disclosure prevent lesion formation in an *ex vivo* spinal cord slice model of NMO or in an *in vivo* mouse model of NMO. Methods for measuring lesion formation *ex vivo* or *in vivo* are well known in the art. *Ex vivo* lesion formation may be reduced at least by a relative score of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, or 4.0. The EC_{50} values for antibodies of this disclosure with respect to the prevention of *ex vivo* lesion formation may be less than 3µg/ml; less than 2.5 µg/ml; less than 2.0 µg/ml; less than 1.5 µg/ml; less than 1.0 µg/ml; less than 0.5 µg/ml; less than 0.25 µg/ml; less than 0.1 µg/ml; or less than 0.05 µg/ml. *In vivo* lesion formation may be reduced by at least 5%, at least 10%, at least 15%, at least 20%, at least 35%, at least 40%, or at least 50%, or by an amount that ranges from at least 5% to at least 50%, in terms of loss of staining (% of area). Staining may be assessed, without limitation, by AQP4 staining, GFAP staining, or MBP staining.

[0130] The present disclosure provides humanized anti-Clq antibodies. The humanized antibodies of the present disclosure may have one or more of the following characteristics. The antibodies of this disclosure may be polyclonal antibodies, monoclonal antibodies, chimeric antibodies, human antibodies, antibody fragments, bispecific and polyspecific antibodies, multivalent antibodies, or heteroconjugate antibodies. Antibody fragments of this disclosure may be functional fragments that bind the same epitope as any of the humanized anti-Clq antibodies of this disclosure. In some implementations, the antibody fragments of this disclosure specifically bind to and neutralize a biological activity of C1q. In some implementations, the antibody fragments are miniaturized versions of the humanized anti-Clq antibodies or antibody fragments of this disclosure that have the same epitope of the corresponding full-length antibody, but have much smaller molecule weight. Such miniaturized anti-Clq antibody fragments may have better brain penetration ability and a shorter half-life, which is advantageous for imaging and diagnostic utilities (*see e.g.,* Lütje S et al., Bioconjug Chem. 2014 Feb 19;25(2):335-41; Tavaré R et al., Proc Natl Acad Sci USA. 2014 Jan 21;111(3):1108-13; and Wiehr S et al., Prostate. 2014 May;74(7):743-55). Accordingly, in some implementations, humanized anti-Clq antibody fragments of this disclosure have better brain penetration as compared to their corresponding full-length antibodies and/or have a shorter half-life as compared to their corresponding full-length antibodies. In some implementations, humanized anti-Clq antibodies of the present disclosure are bispecific antibodies recognizing a first antigen and a second antigen. In some implementations, the first antigen is a C1q antigen. In some implementations, the second antigen is an antigen facilitating transport across the blood-brain-barrier, including without limitation, transferrin receptor (TR), insulin receptor (HIR), insulin-like growth factor receptor (IGFR), low-density lipoprotein receptor related proteins 1 and 2 (LPR-1 and 2), diphtheria toxin receptor, CRM197, a llama single domain antibody, TMEM 30(A), a protein transduction domain, TAT, Syn-B, penetratin, a poly-arginine peptide, an angiopep peptide, and ANG1005.

[0131] Humanized anti-Clq antibodies of the present disclosure may further contain engineered effector functions, amino acid sequence modifications or other antibody modifications known in the art; e.g., the constant region of the anti-Clq antibodies described herein may be modified to impair complement activation. For example, and without wishing to be bound by theory, unlike the Fc region of human IgG1, IgG2, and IgG3, the Fc region of human IgG4 does not bind to C1q. Accordingly, in some implementations, humanized anti-Clq antibodies of this disclosure may further comprise the Fc region of human IgG4. In some implementations, humanized anti-Clq antibodies of this disclosure comprise one or more amino acid substitutions within the Fc region that, for example, prevent arm switching and/or reduces or otherwise inhibits the ability of Fc region from interacting with Fc receptors expressed on cells *(see e.g.,* Angal S et al., Mol Immunol. 1993 Jan;30(1):105-8; and Morgan A et al., Immunology 1995 86 319-324). In some implementations humanized anti-Clq antibodies of this disclosure comprise an Fc region that comprises an amino acid substitution at position 241 or 248 according to Kabat numbering convention. In some implementations, the Fc region comprises a serine to proline amino acid substitution at position 241 that prevent arm switching. In some implementations, the Fc region comprises a serine to proline amino acid substitution at position 241 according to Kabat numbering convention. In some implementations, the Fc

region comprises a leucine to glutamate amino acid substitution at position 248 that reduces or otherwise inhibits the ability of Fc region from interacting with an Fc receptor. In some implementations, the Fc region comprises a leucine to glutamate amino acid substitution at position 248 according to Kabat numbering convention. In some implementations humanized anti-Clq antibodies of this disclosure comprise an Fc region comprising the amino acid sequence of SEQ ID NO: 37.

[0132] Additional humanized anti-C1q antibodies, e.g., humanized antibodies that specifically bind to a C1q protein of the present disclosure, may be identified, screened, and/or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

Antibody preparation

[0133] Anti-Clq antibodies of the present disclosure may be produced using any methods described herein or known in the art. Monoclonal antibodies (e.g., humanized antibodies) of the of the present disclosure can be produced using a variety of known techniques, such as the standard somatic cell hybridization technique described by Kohler and Milstein, Nature 256: 495 (1975). Although somatic cell hybridization procedures are preferred, in principle, other techniques for producing monoclonal antibodies also can be employed, e.g., viral or oncogenic transformation of B lymphocytes and phage display technique using libraries of human antibody genes.

[0134] One method for generating hybridomas which produce monoclonal antibodies of the present disclosure is the murine system. Hybridoma production in the mouse is well-known in the art, including immunization protocols and techniques for isolating and fusing immunized splenocytes.

[0135] Polyclonal antibodies can be prepared by immunizing a suitable subject with a polypeptide immunogen. The polypeptide antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized polypeptide. If desired, the antibody directed against the antigen can be isolated from the mammal (e.g., from the blood) and further purified by well-known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, e.g., when the antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) Nature 256:495-497) (see also Brown et al. (1981) J. Immunol. 127:539-46; Brown et al. (1980) J. Biol. Chem. 255:4980-83; Yeh et al. (1976) Proc. Natl. Acad. Sci. 76:2927-31; and Yeh et al. (1982) Int. J. Cancer 29:269-75), the more recent human B cell hybridoma technique (Kozbor et al. (1983) Immunol. Today 4:72), the EBV-hybridoma technique (Cole et al. (1985) Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well-known (see generally Kenneth, R. H. in Monoclonal Antibodies: A New Dimension In Biological Analyses, Plenum Publishing Corp., New York, New York (1980); Lerner, E. A. (1981) Yale J. Biol. Med. 54:387-402; Gefter, M. L. et al. (1977) Somatic Cell Genet. 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with an immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds to the polypeptide antigen, preferably specifically.

[0136] Any of the many well-known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-PD-1, PD-L1, or PD-L2 monoclonal antibody (see, *e.g.,* Galfre, G. et al. (1977) Nature 266:55052; Getter *et al.* (1977) *supra;* Lerner (1981) *supra;* Kenneth (1980) *supra).* Moreover, the ordinary skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line *(e.g.,* a myeloma cell line) is derived from the same

mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present disclosure with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, *e.g.*, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from the American Type Culture Collection (ATCC), Rockville, Md. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the present disclosure are detected by screening the hybridoma culture supernatants for antibodies that bind a given polypeptide, *e.g.*, using a standard ELISA assay.

[0137] As an alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal specific for a desired polypeptide (e.g., Clq) can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with the appropriate polypeptide to thereby isolate immunoglobulin library members that bind the polypeptide. Kits for generating and screening phage display libraries are commercially available *(e.g.,* the Pharmacia *Recombinant Phage Antibody System,* Catalog No. 27-9400-01; and the Stratagene *SurfZAP*™ *Phage Display Kit,* Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening an antibody display library can be found in, for example, Ladner et al. U.S. Patent No. 5,223,409; Kang et al. International Publication No. WO 92/18619; Dower et al. International Publication No. WO 91/17271; Winter et al. International Publication WO 92/20791; Markland et al. International Publication No. WO 92/15679; Breitling et al. International Publication WO 93/01288; McCafferty et al. International Publication No. WO 92/01047; Garrard et al. International Publication No. WO 92/09690; Ladner et al. International Publication No. WO 90/02809; Fuchs et al. (1991) Biotechnology (NY) 9:1369-1372; Hay et al. (1992) Hum. Antibod. Hybridomas 3:81-85; Huse et al. (1989) Science 246:1275-1281; Griffiths et al. (1993) EMBO J. 12:725-734; Hawkins et al. (1992) J. Mol. Biol. 226:889-896; Clarkson et al. (1991) Nature 352:624-628; Gram et al. (1992) Proc. Natl. Acad. Sci. USA 89:3576-3580; Garrard et al. (1991) Biotechnology (NY) 9:1373-1377; Hoogenboom et al. (1991) Nucleic Acids Res. 19:4133-4137; Barbas et al. (1991) Proc. Natl. Acad. Sci. USA 88:7978-7982; and McCafferty et al. (1990) Nature 348:552-554.

[0138] Additionally, recombinant anti-Clq antibodies, such as humanized and chimeric monoclonal antibodies, which can be made using standard recombinant DNA techniques, can be generated. Such humanized and chimeric monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson et al. International Patent Publication PCT/US86/02269; Akira et al. European Patent Application 184,187; Taniguchi, M. European Patent Application 171,496; Morrison et al. European Patent Application 173,494; Neuberger et al. PCT Application WO 86/01533; Cabilly et al. U.S. Patent No. 4,816,567; Cabilly et al. European Patent Application 125,023; Better et al. (1988) Science 240:1041-1043; Liu et al. (1987) Proc. Natl. Acad. Sci. USA 84:3439-3443; Liu et al. (1987) J. Immunol. 139:3521-3526; Sun et al. (1987) Proc. Natl. Acad. Sci. 84:214-218; Nishimura et al. (1987) Cancer Res. 47:999-1005; Wood et al. (1985) Nature 314:446-449; and Shaw et al. (1988) J. Natl. Cancer Inst. 80:1553-1559); Morrison, S. L. (1985) Science 229:1202-1207; Oi et al. (1986) Biotechniques 4:214; Winter U.S. Patent 5,225,539; Jones et al. (1986) Nature 321:552-525; Verhoeyan et al. (1988) Science 239:1534; and Beidler et al. (1988) J. Immunol. 141:4053-4060.

[0139] In addition, humanized antibodies can be made according to standard protocols such as those disclosed in US patent 5,565,332. In another implementation, antibody chains or specific binding pair members can be produced by recombination between vectors comprising nucleic acid molecules encoding a fusion of a polypeptide chain of a specific binding pair member and a component of a replicable generic

display package and vectors containing nucleic acid molecules encoding a second polypeptide chain of a single binding pair member using techniques known in the art, *e.g.,* as described in US patents 5,565,332, 5,871,907, or 5,733,743. The use of intracellular antibodies to inhibit protein function in a cell is also known in the art (see *e.g.,* Carlson, J. R. (1988) Mol. Cell. Biol. 8:2638-2646; Biocca, S. et al. (1990) EMBO J. 9:101-108; Werge, T. M. et al. (1990)FEBSLett. 274:193-198; Carlson, J. R. (1993) Proc. Natl. Acad. Sci. USA 90:7427-7428; Marasco, W. A. et al. (1993) Proc. Natl. Acad. Sci. USA 90:7889-7893 ; Biocca, S. et al. (1994) Biotechnology (NY) 12:396-399; Chen, S-Y. et al. (1994) Hum. Gene Ther. 5:595-601; Duan, L et al. (1994) Proc. Natl. Acad. Sci. USA 91:5075-5079; Chen, S-Y. et al. (1994) Proc. Natl. Acad. Sci. USA 91:5932-5936; Beerli, R. R. et al. (1994) J. Biol. Chem. 269:23931-23936; Beerli, R. R. et al. (1994) Biochem. Biophys. Res. Commun. 204:666-672; Mhashilkar, A. M. et al. (1995) EMBO J. 14:1542-1551; Richardson, J. H. et al. (1995) Proc. Natl. Acad. Sci. USA 92:3137-3141 ; PCT Publication No. WO 94/02610 by Marasco et al.; and PCT Publication No. WO 95/03832 by Duan et al.).

[0140] In another implementation, human monoclonal anti-Clq antibodies can be generated using transgenic or transchromosomal mice carrying parts of the human immune system rather than the mouse system. In one implementation, transgenic mice, referred to herein as "HuMAb mice" which contain a human immunoglobulin gene miniloci that encodes unrearranged human heavy (μ and γ) and κ light chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous μ and κ chain loci (Lonberg, N. et al. (1994) Nature 368(6474): 856 859). Accordingly, the mice exhibit reduced expression of mouse IgM or κ, and in response to immunization, the introduced human heavy and light chain transgenes undergo class switching and somatic mutation to generate high affinity human IgGκ monoclonal antibodies (Lonberg, N. *et al.* (1994), supra; reviewed in Lonberg, N. (1994) Handbook of Experimental Pharmacology 113:49 101; Lonberg, N. and Huszar, D. (1995) Intern. Rev. Immunol. Vol. 13: 65 93, and Harding, F. and Lonberg, N. (1995) Ann. N. Y Acad. Sci 764:536 546). The preparation of HuMAb mice is described in Taylor, L. et al. (1992) Nucleic Acids Research 20:6287 6295; Chen, J. et al. (1993) International Immunology 5: 647 656; Tuaillon et al. (1993) Proc. Natl. Acad. Sci USA 90:3720 3724 ; Choi et al. (1993) Nature Genetics 4:117 123; Chen, J. et al. (1993) EMBO J. 12: 821 830; Tuaillon et al. (1994) J. Immunol. 152:2912 2920; Lonberg et al., (1994) Nature 368(6474): 856 859; Lonberg, N. (1994) Handbook of Experimental Pharmacology 113:49 101; Taylor, L. et al. (1994) International Immunology 6: 579 591; Lonberg, N. and Huszar, D. (1995) Intern. Rev. Immunol. Vol. 13: 65 93; Harding, F. and Lonberg, N. (1995) Ann. N.Y. Acad. Sci 764:536 546; Fishwild, D. et al. (1996) Nature Biotechnology 14: 845 851. See further, U.S. Pat. Nos. 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,789,650; 5,877,397; 5,661,016; 5,814,318; 5,874,299; and 5,770,429; all to Lonberg and Kay, and GenPharm International; U.S. Pat. No. 5,545,807 to Surani et al.*;* International Publication Nos. WO 98/24884, published on Jun. 11, 1998; WO 94/25585, published Nov. 10, 1994; WO 93/1227, published Jun. 24, 1993; WO 92/22645, published Dec. 23, 1992; WO 92/03918, published Mar. 19, 1992.

[0141] Yet another aspect of the present disclosure relates to anti-C1q antibodies that are obtainable by a process comprising, immunizing an animal with an immunogenic C1q polypeptide, respectively, or an immunogenic portion thereof; and then isolating from the animal antibodies that specifically bind to the polypeptide.

[0142] In still another aspect of the present disclosure, partial or known antibody sequences can be used to generate and/or express new antibodies. Antibodies interact with target antigens predominantly through amino acid residues that are located in the six heavy and light chain complementarity determining regions (CDRs). For this reason, the amino acid sequences within CDRs are more diverse between individual antibodies than sequences outside of CDRs. Because CDR sequences are responsible for most antibody-antigen interactions, it is possible to express recombinant antibodies that mimic the properties of specific naturally occurring antibodies by constructing expression vectors that include CDR sequences from the specific naturally occurring antibody grafted onto framework sequences from a different antibody with

different properties (see, *e.g.,* Riechmann, L. et al., 1998, Nature 332:323 327; Jones, P. et al., 1986, Nature 321:522 525; and Queen, C. et al., 1989, Proc. Natl. Acad. See. U.S.A. 86:10029 10033). Such framework sequences can be obtained from public DNA databases that include germline or non-germline antibody gene sequences. These germline sequences will differ from mature antibody gene sequences because they will not include completely assembled variable genes, which are formed by V(D)J joining during B cell maturation. Germline gene sequences will also differ from the sequences of a high affinity secondary repertoire antibody at individual evenly across the variable region. For example, somatic mutations are relatively infrequent in the amino-terminal portion of framework region. For example, somatic mutations are relatively infrequent in the amino terminal portion of framework region 1 and in the carboxy-terminal portion of framework region 4. Furthermore, many somatic mutations do not significantly alter the binding properties of the antibody. For this reason, it is not necessary to obtain the entire DNA sequence of a particular antibody in order to recreate an intact recombinant antibody having binding properties similar to those of the original antibody (see PCT/US99/05535 filed on Mar. 12, 1999). Partial heavy and light chain sequence spanning the CDR regions is typically sufficient for this purpose. The partial sequence is used to determine which germline and/or non-germline variable and joining gene segments contributed to the recombined antibody variable genes. The germline and/or non-germline sequence is then used to fill in missing portions of the variable regions. Heavy and light chain leader sequences are cleaved during protein maturation and do not contribute to the properties of the final antibody. To add missing sequences, cloned cDNA sequences can be combined with synthetic oligonucleotides by ligation or PCR amplification. Alternatively, the entire variable region can be synthesized as a set of short, overlapping, oligonucleotides and combined by PCR amplification to create an entirely synthetic variable region clone. This process has certain advantages such as elimination or inclusion or particular restriction sites, or optimization of particular codons. The process can also be used to screen libraries of particular immunoglobulin encoding sequences in one species (*e.g.,* human) to design cognate immunoglobulin encoding sequences from known antibody sequence in another species (*e.g.,* mouse) (see, for example, the Examples section below).

[0143] The nucleotide sequences of heavy and light chain transcripts from a hybridoma may be used to design an overlapping set of synthetic oligonucleotides to create synthetic V sequences with identical amino acid coding capacities as the natural sequences. The synthetic heavy and kappa chain sequences can differ from the natural sequences in three ways: strings of repeated nucleotide bases are interrupted to facilitate oligonucleotide synthesis and PCR amplification; optimal translation initiation sites are incorporated according to Kozak's rules (Kozak, 1991, J. Biol. Chem. 266L19867019870); and, HindIII sites are engineered upstream of the translation initiation sites.

[0144] For both the heavy and light chain variable regions, the optimized coding, and corresponding non-coding, strand sequences are broken down into 30-50 nucleotide approximately the midpoint of the corresponding non-coding oligonucleotide. Thus, for each chain, the oligonucleotides can be assembled into overlapping double stranded sets that span segments of 150-400 nucleotides. The pools are then used as templates to produce PCR amplification products of 150-400 nucleotides. Typically, a single variable region oligonucleotide set will be broken down into two pools which are separately amplified to generate two overlapping PCR products. These overlapping products are then combined by PCR amplification to form the complete variable region. It may also be desirable to include an overlapping fragment of the heavy or light chain constant region in the PCR amplification to generate fragments that can easily be cloned into the expression vector constructs.

[0145] The reconstructed heavy and light chain variable regions are then combined with cloned promoter, leader sequence, translation initiation, leader sequence, constant region, 3' untranslated, polyadenylation, and transcription termination, sequences to form expression vector constructs. The heavy and light chain expression constructs can be combined into a single vector, co-transfected, serially transfected, or separately transfected into host cells which are then fused to form a host cell expressing both chains.

[0146] Plasmids for this use are known in the art and include the plasmids provided in the Examples section below. Fully human and chimeric antibodies of the present disclosure also include IgG1, IgG2, IgG3, IgG4, IgE, IgA, IgM, and IgD antibodies, and variants and mutants thereof. Similar plasmids can be constructed for expression of other heavy chain isotypes, or for expression of antibodies comprising lambda light chains.

[0147] Thus, in one aspect of the present disclosure, the structural features of known, non-human or human antibodies (*e.g.,* a mouse anti-human anti-C1q antibody, such as the monoclonal antibody M1 produced by the hybridoma cell line having ATCC Accession Number PTA-120399) are used to create structurally related human anti-human C1q antibodies that retain at least one functional property of the antibodies of the present disclosure, such as binding to a C1q protein. Another functional property includes inhibiting binding of the monoclonal antibody M1 to C1q in a competition ELISA assay. In some implementations, the structurally related anti-human C1q antibodies have a comparable binding affinity to the antigen as compared to the monoclonal antibody M1 as measured by the IC_{50} value as described in the Examples section below. In some implementations, the structurally related anti-human C1q antibodies have a higher affinity to the antigen as compared to the monoclonal antibody M1 as measured by the IC50 value as described in the Examples section below. In addition, one or more CDR or variable regions of an anti-C1q antibody (*e.g.,* monoclonal antibody M1 produced by the hybridoma cell line having ATCC Accession Number PTA-120399) can be combined recombinantly with known human framework regions and CDRs to create additional, recombinantly-engineered, human anti-C1q antibodies of the present disclosure.

[0148] Since it is well-known in the art that antibody heavy and light chain CDR3 domains play a particularly important role in the binding specificity/affinity of an antibody for an antigen, the recombinant antibodies of the present disclosure prepared as set forth above may, in some implementations, comprise the heavy and light chain CDR3s of variable regions of the monoclonal antibody M1 produced by the hybridoma cell line having ATCC Accession Number PTA-120399. In some implementations, the antibodies further can comprise the CDR2s of variable regions of the monoclonal antibody M1. In some implementations, the antibodies further can comprise the CDR1s of variable regions of the monoclonal antibody M1. In some implementations, the antibodies can further comprise any combinations of the CDRs.

[0149] In some implementations, the CDR1, 2, and/or 3 regions of the engineered antibodies described above may comprise the exact amino acid sequence(s) as those of variable regions of the monoclonal antibody M1 produced by the hybridoma cell line having ATCC Accession Number PTA-120399. However, the ordinarily skilled artisan will appreciate that some deviation from the exact CDR sequences may be possible while still retaining the ability of the antibody to bind C1q effectively (*e.g.,* conservative sequence modifications). Accordingly, in another implementation, the engineered antibody may be composed of one or more CDRs that are, for example, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identical to one or more CDRs of the monoclonal antibody M1.

Antibody fragments

[0150] In certain implementations there are advantages to using anti-C1q antibody fragments, rather than whole anti-C1q antibodies. Smaller fragment sizes allow for rapid clearance.

[0151] Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (*see, e.g.,* Morimoto et al., J. Biochem. Biophys. Method. 24:107-117 (1992); and Brennan et al., Science 229:81 (1985)). However, these fragments can now be produced directly by recombinant host-cells, for example, using nucleic acids encoding anti-C1q antibodies of the present disclosure. Fab, Fv and scFv antibody fragments can all be expressed in and secreted from *E. coli,* thus allowing the straightforward production of large amounts of

these fragments. A anti-C1q antibody fragments can also be isolated from the antibody phage libraries as discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab')_2$ fragments (Carter et al., Bio/Technology 10:163-167 (1992)). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host-cell culture. Production of Fab and $F(ab')_2$ antibody fragments with increased *in vivo* half-lives are described in U.S. Patent No. 5,869,046. In other implementations, the antibody of choice is a single chain Fv fragment (scFv). See WO 93/16185; U.S. Patent No. 5,571,894 and U.S. Patent No. 5,587,458. The anti-C1q, anti-C1r, or anti-C1q antibody fragment may also be a "linear antibody," *e.g.*, as described in U.S. Patent 5,641,870. Such linear antibody fragments may be monospecific or bispecific.

Bispecific and polyspecific antibodies

[0152] In some implementations, antibodies of the present disclosure encompass bispecific antibodies and polyspecific antibodies.

[0153] Bispecific antibodies (BsAbs) are antibodies that have binding specificities for at least two different epitopes, including those on the same or another protein (*e.g.,* one or more C1q proteins of the present disclosure). Alternatively, one part of a BsAb can be armed to bind to the target C1q antigen, and another can be combined with an arm that binds to a second protein. Such antibodies can be derived from full length antibodies or antibody fragments (*e.g.,* $F(ab')_2$ bispecific antibodies).

[0154] Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy-chain/light chain pairs, where the two chains have different specificities. Millstein et al., Nature, 305:537-539 (1983). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829 and in Traunecker et al., EMBO J., 10:3655-3659 (1991).

[0155] According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion may be with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, C_H2, and C_H3 regions. In some implementations, the first heavy-chain constant region (C_H1) containing the site necessary for light chain binding is present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides great flexibility in adjusting the mutual proportions of the three polypeptide fragments in implementations when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

[0156] In some implementations of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only half of the

bispecific molecules provides an easy way of separation. This approach is disclosed in WO 94/04690. For further details of generating bispecific antibodies, see, for example, Suresh et al., Methods in Enzymology 121: 210 (1986).

[0157] According to another approach described in WO 96/27011 or U.S. Patent No. 5,731,168, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant-cell culture. The interface may comprise at least a part of the C_H3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g., tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chains(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (*e.g.,* alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

[0158] Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan et al., Science 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate $F(ab')_2$ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

[0159] Fab' fragments may be directly recovered from *E. coli* and chemically coupled to form bispecific antibodies. Shalaby et al., J. Exp. Med. 175: 217-225 (1992) describes the production of fully humanized bispecific antibody $F(ab')_2$ molecules. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T-cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

[0160] Various techniques for making and isolating bivalent antibody fragments directly from recombinant-cell culture have also been described. For example, bivalent heterodimers have been produced using leucine zippers. Kostelny et al., J. Immunol., 148(5): 1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. The "diabody" technology described by Hollinger et al., Proc. Nat'l Acad. Sci. USA, 90: 6444-6448 (1993) has provided an alternative mechanism for making bispecific/bivalent antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific/bivalent antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. *See* Gruber et al., J. Immunol., 152:5368 (1994).

[0161] Antibodies with more than two valencies are also contemplated. For example, trispecific antibodies can be prepared. Tutt et al., J. Immunol. 147:60 (1991).

[0162] Exemplary bispecific antibodies may bind to two different antigens. In some implementations a bispecific antibody binds to a first antigen, C1q, and a second antigen facilitating transport across the blood-brain barrier. Numerous antigens are known in the art that facilitate transport across the blood-brain barrier

(*see, e.g.,* Gabathuler R., Approaches to transport therapeutic drugs across the blood-brain barrier to treat brain diseases, Neurobiol. Dis. 37 (2010) 48-57). Such second antigens include, without limitation, transferrin receptor (TR), insulin receptor (HIR), Insulin-like growth factor receptor (IGFR), low-density lipoprotein receptor related proteins 1 and 2 (LPR-1 and 2), diphtheria toxin receptor, including CRM197 (a non-toxic mutant of diphtheria toxin), llama single domain antibodies such as TMEM 30(A) (Flippase), protein transduction domains such as TAT, Syn-B, or penetratin, poly-arginine or generally positively charged peptides, and Angiopep peptides such as ANG1005 (*see, e.g.,* Gabathuler, 2010).

Multivalent antibodies

[0163] In some implementations, antibodies of the present disclosure encompass multivalent antibodies. A multivalent antibody may be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind. The anti-C1q antibodies of the present disclosure or antibody fragments thereof can be multivalent antibodies (which are other than of the IgM class) with three or more antigen binding sites (*e.g.,* tetravalent antibodies), which can be readily produced by recombinant expression of nucleic acid encoding the polypeptide chains of the antibody. The multivalent antibody can comprise a dimerization domain and three or more antigen binding sites. In some implementations, the dimerization domain comprises an Fc region or a hinge region. In this scenario, the antibody will comprise an Fc region and three or more antigen binding sites amino-terminal to the Fc region. In some implementations, the multivalent antibody herein contains three to about eight, and in some implementations four, antigen binding sites. The multivalent antibody contains at least one polypeptide chain (and in some implementations two polypeptide chains), wherein the polypeptide chain or chains comprise two or more variable domains. For instance, the polypeptide chain or chains may comprise VD1-(X1)n-VD2-(X2)n-Fc, wherein VD1 is a first variable domain, VD2 is a second variable domain, Fc is one polypeptide chain of an Fc region, X1 and X2 represent an amino acid or polypeptide, and n is 0 or 1. Similarly, the polypeptide chain or chains may comprise V_H-C_H1-flexible linker-V_H-C_H1-Fc region chain; or V_H-C_H1-V_H-C_H1-Fc region chain. The multivalent antibody herein may further comprise at least two (and in some implementations four) light chain variable domain polypeptides. The multivalent antibody herein may, for instance, comprise from about two to about eight light chain variable domain polypeptides. The light chain variable domain polypeptides contemplated here comprise a light chain variable domain and, optionally, further comprise a CL domain.

Heteroconjugate antibodies

[0164] Heteroconjugate antibodies are also within the scope of the present disclosure. Heteroconjugate antibodies are composed of two covalently joined antibodies (*e.g.,* antiC1q antibodies of the present disclosure or antibody fragments thereof). For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells, U.S. Patent No. 4,676,980, and have been used to treat HIV infection. International Publication Nos. WO 91/00360, WO 92/200373 and EP 0308936. It is contemplated that the antibodies may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980. Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Patent No. 4,676,980, along with a number of cross-linking techniques.

Effector function engineering

[0165] In some implementations, it may be desirable to modify a humanized anti-C1q antibody of the present disclosure to modify effector function and/or to increase serum half-life of the antibody. For example, the Fc receptor binding site on the constant region may be modified or mutated to remove or reduce binding affinity to certain Fc receptors, such as FcγRI, FcγRII, and/or FcγRIII. In some implementations, the effector function is impaired by removing N-glycosylation of the Fc region (*e.g.,* in the CH 2 domain of IgG) of the antibody. In some implementations, the effector function is impaired by modifying regions such as 233-236, 297, and/or 327-331 of human IgG as described in PCT WO 99/58572 and Armour et al., Molecular Immunology 40: 585-593 (2003); Reddy et al., J. Immunology 164:1925-1933 (2000).

[0166] The constant region of the anti-complement antibodies described herein may also be modified to impair complement activation. For example, complement activation of IgG antibodies following binding of the C1 component of complement may be reduced by mutating amino acid residues in the constant region in a C1 binding motif (*e.g.,* C1q binding motif). It has been reported that Ala mutation for each of D270, K322, P329, P331 of human IgG1 significantly reduced the ability of the antibody to bind to C1q and activating complement. For murine IgG2b, C1q binding motif constitutes residues E318, K320, and K322. Idusogie et al. (2000) J. Immunology 164:4178-4184; Duncan et al. (1988) Nature 322: 738-740. As the C1s binding motif E318, K320, and K322 identified for murine IgG2b is believed to be common for other antibody isotypes (Duncan et al. (1988) Nature 322:738-740), C1q binding activity for IgG2b can be abolished by replacing any one of the three specified residues with a residue having an inappropriate functionality on its side chain. It is not necessary to replace the ionic residues only with Ala to abolish C1q binding. It is also possible to use other alkyl-substituted non-ionic residues, such as Gly, Ile, Leu, or Val, or such aromatic non-polar residues as Phe, Tyr, Trp, and Pro in place of any one of the three residues in order to abolish C1q binding. In addition, it is also possible to use such polar non-ionic residues as Ser, Thr, Cys, and Met in place of residues 320 and 322, but not 318, in order to abolish C1s binding activity. In addition, removal of carbohydrate modifications of the Fc region necessary for complement binding can prevent complement activation Glycosylation of a conserved asparagine (Asn-297) on the CH2 domain of IgG heavy chains is essential for antibody effector functions (Jefferis et al. (1998) Immunol Rev 163:59-76). Modification of the Fc glycan alters IgG conformation and reduces the Fc affinity for binding of complement protein C1q and effector cell receptor FcR (Alhorn et al. (2008) PLos ONE 2008;3:e1413). Complete removal of the Fc glycan abolishes CDC and ADCC. Deglycosylation can be performed using glycosidase enzymes for example Endoglycosidase S (EndoS), a 108kDa enzyme encoded by the gene endoS of Streptococcus pyogenes that selectively digests asparagine-linked glycans on the heavy chain of all IgG subclasses, without action on other immunoglobulin classes or other glycoproteins (Collin et al. (2001) EMBO J 2001;20:3046-3055).

[0167] To increase the serum half-life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in U.S. Patent 5,739,277, for example. As used herein, the term *"salvage receptor binding epitope"* refers to an epitope of the Fc region of an IgG molecule (*e.g.,* IgG_1, IgG_2, IgG_3, or IgG_4) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule.

Other amino acid sequence modifications

[0168] Amino acid sequence modifications of humanized anti-C1q antibodies of the present disclosure, or antibody fragments thereof, are also contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibodies or antibody fragments. Amino acid sequence

variants of the antibodies or antibody fragments are prepared by introducing appropriate nucleotide changes into the nucleic acid encoding the antibodies or antibody fragments, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics (*i.e.,* the ability to bind or physically interact with a C1q protein of the present disclosure). The amino acid changes also may alter post-translational processes of the antibody, such as changing the number or position of glycosylation sites.

[0169] A useful method for identification of certain residues or regions of the anti-C1q antibody that are preferred locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells in Science, 244:1081-1085 (1989). Here, a residue or group of target residues are identified (*e.g.,* charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with the target antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to analyze the performance of a mutation at a given site, alanine scanning or random mutagenesis is conducted at the target codon or region and the expressed antibody variants are screened for the desired activity.

[0170] Amino acid sequence insertions include amino- ("N") and/or carboxy- ("C") terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue or the antibody fused to a cytotoxic polypeptide. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme or a polypeptide which increases the serum half-life of the antibody.

[0171] Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the antibody molecule replaced by a different residue. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in the Table A below under the heading of "preferred substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" in Table A, or as further described below in reference to amino acid classes, may be introduced and the products screened.

TABLE A: Amino Acid Substitutions

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	val; leu; ile	val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; asp, lys; arg	gln
Asp (D)	glu; asn	glu
Cys (C)	ser; ala	ser
Gln (Q)	asn; glu	asn
Glu (E)	asp; gln	asp
Gly (G)	ala	ala
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe; norleucine	leu
Leu (L)	norleucine; ile; val; met; ala; phe	ile

Original Residue	Exemplary Substitutions	Preferred Substitutions
Lys (K)	arg; gln; asn	arg
Met (M)	leu; phe; ile	leu
Phe (F)	leu; val; ile; ala; tyr	tyr
Pro (P)	ala	ala
Ser (S)	thr	thr
Thr (T)	ser	ser
Trp (W)	tyr; phe	tyr
Tyr (Y)	trp; phe; thr; ser	phe
Val (V)	ile; leu; met; phe; ala; norleucine	leu

[0172] Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

1. (1) hydrophobic: norleucine, met, ala, val, leu, ile;
2. (2) neutral hydrophilic: cys, ser, thr;
3. (3) acidic: asp, glu;
4. (4) basic: asn, gln, his, lys, arg;
5. (5) residues that influence chain orientation: gly, pro; and
6. (6) aromatic: trp, tyr, phe.

[0173] Non-conservative substitutions entail exchanging a member of one of these classes for another class.

[0174] Any cysteine residue not involved in maintaining the proper conformation of the antibody also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability (particularly where the antibody is an antibody fragment, such as an Fv fragment).

[0175] In some implementations, the substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.* a humanized or human antiC1q antibody). Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sites (*e.g.,* 6-7 sites) are mutated to generate all possible amino substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (*e.g.,* binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and the antigen (*e.g.,* a C1q protein of the present disclosure). Such contact residues and neighboring residues are

candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

[0176] Another type of amino acid variant of the antibody alters the original glycosylation pattern of the antibody. By altering is meant deleting one or more carbohydrate moieties found in the antibody, and/or adding one or more glycosylation sites that are not present in the antibody.

[0177] Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[0178] Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

[0179] Nucleic acid molecules encoding amino acid sequence variants of the anti-IgE antibody are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the antibodies (*e.g.,* anti-C1q antibody of the present disclosure) or antibody fragments.

Other antibody modifications

[0180] In some implementations, humanized anti-C1q antibodies of the present disclosure, or antibody fragments thereof, may be further modified to contain additional non-proteinaceous moieties that are known in the art and readily available. In some implementations, the moieties suitable for derivatization of the antibody are water-soluble polymers. Non-limiting examples of water-soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, polypropylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.,* glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer is attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, *etc.* Such techniques and other suitable formulations are disclosed in Remington: The Science and Practice of Pharmacy, 20th Ed., Alfonso Gennaro, Ed., Philadelphia College of Pharmacy and Science (2000).

Nucleic acids, vectors, and host cells

[0181] Humanized anti-C1q antibodies of the present disclosure may be produced using recombinant methods and compositions, *e.g.*, as described in U.S. Patent No. 4,816,567. In some implementations, isolated nucleic acids having a nucleotide sequence encoding any of the anti-C1q antibodies of the present disclosure are provided. Such nucleic acids may encode an amino acid sequence containing the VL and/or an amino acid sequence containing the VH of the anti-C1q antibody (*e.g.,* the light and/or heavy chains of the antibody). In some implementations, one or more vectors (*e.g.,* expression vectors) containing such nucleic acids are provided. In some implementations, a host cell containing such nucleic acid is also provided. In some implementations, the host cell contains (*e.g.,* has been transduced with): (1) a vector containing a nucleic acid that encodes an amino acid sequence containing the VL of the antibody and an amino acid sequence containing the VH of the antibody, or (2) a first vector containing a nucleic acid that encodes an amino acid sequence containing the VL of the antibody and a second vector containing a nucleic acid that encodes an amino acid sequence containing the VH of the antibody. In some implementations, the host cell is eukaryotic, *e.g.*, a Chinese Hamster Ovary (CHO) cell or lymphoid cell (*e.g.,* Y0, NS0, Sp20 cell).

[0182] Methods of making an anti-C1q antibody of the present disclosure are provided. In some implementations, the method includes culturing a host cell of the present disclosure containing a nucleic acid encoding the anti-C1q antibody, under conditions suitable for expression of the antibody. In some implementations, the antibody is subsequently recovered from the host cell (or host cell culture medium).

[0183] For recombinant production of a humanized anti-C1q antibody of the present disclosure, a nucleic acid encoding the anti-C1q antibody is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (*e.g.,* by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

[0184] Suitable vectors containing a nucleic acid sequence encoding any of the antiC1q antibodies of the present disclosure, or fragments thereof polypeptides (including antibodies) described herein include, without limitation, cloning vectors and expression vectors. Suitable cloning vectors can be constructed according to standard techniques, or may be selected from a large number of cloning vectors available in the art. While the cloning vector selected may vary according to the host cell intended to be used, useful cloning vectors generally have the ability to self-replicate, may possess a single target for a particular restriction endonuclease, and/or may carry genes for a marker that can be used in selecting clones containing the vector. Suitable examples include plasmids and bacterial viruses, *e.g.,* pUC18, pUC19, Bluescript (*e.g.,* pBS SK+) and its derivatives, mpl8, mpl9, pBR322, pMB9, ColE1, pCR1, RP4, phage DNAs, and shuttle vectors such as pSA3 and pAT28. These and many other cloning vectors are available from commercial vendors such as BioRad, Strategene, and Invitrogen.

[0185] Expression vectors generally are replicable polynucleotide constructs that contain a nucleic acid of the present disclosure. The expression vector may replicable in the host cells either as episomes or as an integral part of the chromosomal DNA. Suitable expression vectors include but are not limited to plasmids, viral vectors, including adenoviruses, adeno-associated viruses, retroviruses, cosmids, and expression vector(s) disclosed in PCT Publication No. WO 87/04462. Vector components may generally include, but are not limited to, one or more of the following: a signal sequence; an origin of replication; one or more marker genes; suitable transcriptional controlling elements (such as promoters, enhancers and terminator). For expression (*i.e.,* translation), one or more translational controlling elements are also usually required, such as ribosome binding sites, translation initiation sites, and stop codons.

[0186] The vectors containing the nucleic acids of interest can be introduced into the host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (*e.g.,* where the vector is an infectious agent such as vaccinia virus). The choice of introducing vectors or polynucleotides will often depend on features of the host cell. In some implementations, the vector contains a nucleic acid containing one or more amino acid sequences encoding an anti-C1q antibody of the present disclosure.

[0187] Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells. For example, anti-C1q antibodies of the present disclosure may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria (*e.g.,* U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523; and Charlton, Methods in Molecular Biology, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments *in E. coli.*). After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

[0188] In addition to prokaryotes, eukaryotic microorganisms, such as filamentous fungi or yeast, are also suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern (*e.g.,* Gerngross, Nat. Biotech. 22:1409-1414 (2004); and Li et al., Nat. Biotech. 24:210-215 (2006)).

[0189] Suitable host cells for the expression of glycosylated antibody can also be derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells. Plant cell cultures can also be utilized as hosts (*e.g.,* U.S. Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429, describing PLANTIBODIES™ technology for producing antibodies in transgenic plants.).

[0190] Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, *e.g.,* in Graham et al., J. Gen Virol. 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, *e.g.,* in Mather, Biol. Reprod. 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, *e.g.,* in Mather et al., Annals N.Y. Acad. Sci. 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR-CHO cells (Urlaub et al., Proc. Natl. Acad. Sci. USA 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, *e.g.*, Yazaki and Wu, Methods in Molecular Biology, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).

Pharmaceutical compositions

[0191] Humanized anti-C1q antibodies of the present disclosure can be incorporated into a variety of formulations for therapeutic use (*e.g.,* by administration) or in the manufacture of a medicament (*e.g.,* for treating or preventing a neurodegenerative disease or autoimmune disease) by combining the antibodies

with appropriate pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms. Examples of such formulations include, without limitation, tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants, gels, microspheres, and aerosols. Pharmaceutical compositions can include, depending on the formulation desired, pharmaceutically-acceptable, non-toxic carriers of diluents, which are vehicles commonly used to formulate pharmaceutical compositions for animal or human administration. The diluent is selected so as not to affect the biological activity of the combination. Examples of such diluents include, without limitation, distilled water, buffered water, physiological saline, PBS, Ringer's solution, dextrose solution, and Hank's solution. A pharmaceutical composition or formulation of the present disclosure can further include other carriers, adjuvants, or non-toxic, nontherapeutic, nonimmunogenic stabilizers, excipients and the like. The compositions can also include additional substances to approximate physiological conditions, such as pH adjusting and buffering agents, toxicity adjusting agents, wetting agents and detergents.

[0192] A pharmaceutical composition of the present disclosure can also include any of a variety of stabilizing agents, such as an antioxidant for example. When the pharmaceutical composition includes a polypeptide, the polypeptide can be complexed with various well-known compounds that enhance the *in vivo* stability of the polypeptide, or otherwise enhance its pharmacological properties (*e.g.,* increase the half-life of the polypeptide, reduce its toxicity, and enhance solubility or uptake). Examples of such modifications or complexing agents include, without limitation, sulfate, gluconate, citrate and phosphate. The polypeptides of a composition can also be complexed with molecules that enhance their *in vivo* attributes. Such molecules include, without limitation, carbohydrates, polyamines, amino acids, other peptides, ions (*e.g.,* sodium, potassium, calcium, magnesium, manganese), and lipids.

[0193] Further examples of formulations that are suitable for various types of administration can be found in Remington's Pharmaceutical Sciences, Mace Publishing Company, Philadelphia, PA, 17th ed. (1985). For a brief review of methods for drug delivery, see, Langer, Science 249:1527-1533 (1990).

[0194] For oral administration, the active ingredient can be administered in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, and suspensions. The active component(s) can be encapsulated in gelatin capsules together with inactive ingredients and powdered carriers, such as glucose, lactose, sucrose, mannitol, starch, cellulose or cellulose derivatives, magnesium stearate, stearic acid, sodium saccharin, talcum, magnesium carbonate. Examples of additional inactive ingredients that may be added to provide desirable color, taste, stability, buffering capacity, dispersion or other known desirable features are red iron oxide, silica gel, sodium lauryl sulfate, titanium dioxide, and edible white ink. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric-coated for selective disintegration in the gastrointestinal tract. Liquid dosage forms for oral administration can contain coloring and flavoring to increase patient acceptance.

[0195] Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives.

[0196] The components used to formulate the pharmaceutical compositions are preferably of high purity and are substantially free of potentially harmful contaminants (*e.g.,* at least National Food (NF) grade, generally at least analytical grade, and more typically at least pharmaceutical grade). Moreover, compositions

intended for *in vivo* use are usually sterile. To the extent that a given compound must be synthesized prior to use, the resulting product is typically substantially free of any potentially toxic agents, particularly any endotoxins, which may be present during the synthesis or purification process. Compositions for parental administration are also sterile, substantially isotonic and made under GMP conditions.

[0197] Formulations may be optimized for retention and stabilization in the brain or central nervous system. When the agent is administered into the cranial compartment, it is desirable for the agent to be retained in the compartment, and not to diffuse or otherwise cross the blood brain barrier. Stabilization techniques include cross-linking, multimerizing, or linking to groups such as polyethylene glycol, polyacrylamide, neutral protein carriers, *etc.* in order to achieve an increase in molecular weight.

[0198] Other strategies for increasing retention include the entrapment of the antibody, such as a humanized anti-C1q antibody of the present disclosure, in a biodegradable or bioerodible implant. The rate of release of the therapeutically active agent is controlled by the rate of transport through the polymeric matrix, and the biodegradation of the implant. The transport of drug through the polymer barrier will also be affected by compound solubility, polymer hydrophilicity, extent of polymer cross-linking, expansion of the polymer upon water absorption so as to make the polymer barrier more permeable to the drug, geometry of the implant, and the like. The implants are of dimensions commensurate with the size and shape of the region selected as the site of implantation. Implants may be particles, sheets, patches, plaques, fibers, microcapsules and the like and may be of any size or shape compatible with the selected site of insertion.

[0199] The implants may be monolithic, *i.e.* having the active agent homogenously distributed through the polymeric matrix, or encapsulated, where a reservoir of active agent is encapsulated by the polymeric matrix. The selection of the polymeric composition to be employed will vary with the site of administration, the desired period of treatment, patient tolerance, the nature of the disease to be treated and the like. Characteristics of the polymers will include biodegradability at the site of implantation, compatibility with the agent of interest, ease of encapsulation, a half-life in the physiological environment.

[0200] Biodegradable polymeric compositions which may be employed may be organic esters or ethers, which when degraded result in physiologically acceptable degradation products, including the monomers. Anhydrides, amides, orthoesters or the like, by themselves or in combination with other monomers, may find use. The polymers will be condensation polymers. The polymers may be cross-linked or non-cross-linked. Of particular interest are polymers of hydroxyaliphatic carboxylic acids, either homo- or copolymers, and polysaccharides. Included among the polyesters of interest are polymers of D-lactic acid, L-lactic acid, racemic lactic acid, glycolic acid, polycaprolactone, and combinations thereof. By employing the L-lactate or D-lactate, a slowly biodegrading polymer is achieved, while degradation is substantially enhanced with the racemate. Copolymers of glycolic and lactic acid are of particular interest, where the rate of biodegradation is controlled by the ratio of glycolic to lactic acid. The most rapidly degraded copolymer has roughly equal amounts of glycolic and lactic acid, where either homopolymer is more resistant to degradation. The ratio of glycolic acid to lactic acid will also affect the brittleness of in the implant, where a more flexible implant is desirable for larger geometries. Among the polysaccharides of interest are calcium alginate, and functionalized celluloses, particularly carboxymethylcellulose esters characterized by being water insoluble, a molecular weight of about 5 kD to 500 kD, *etc.* Biodegradable hydrogels may also be employed in the implants of the present disclosure. Hydrogels are typically a copolymer material, characterized by the ability to imbibe a liquid. Exemplary biodegradable hydrogels which may be employed are described in Heller in: Hydrogels in Medicine and Pharmacy, N. A. Peppes ed., Vol. III, CRC Press, Boca Raton, Fla., 1987, pp 137-149.

Pharmaceutical dosages

[0201] Pharmaceutical compositions of the present disclosure containing a humanized anti-C1q antibody of the present disclosure may be used (*e.g.,* administered to an individual in need of treatment with an anti-C1q antibody, such as a human individual) in accordance with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, intracranial, intraspinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes.

[0202] Dosages and desired drug concentration of pharmaceutical compositions of the present disclosure may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary artisan. Animal experiments provide reliable guidance for the determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles described in Mordenti, J. and Chappell, W. "The Use of Interspecies Scaling in Toxicokinetics," In Toxicokinetics and New Drug Development, Yacobi et al., Eds, Pergamon Press, New York 1989, pp.42-46.

[0203] For *in vivo* administration of any of the humanized anti-C1q antibodies of the present disclosure, normal dosage amounts may vary from about 10 ng/kg up to about 100 mg/kg of an individual's body weight or more per day, depending upon the route of administration. In some implementations, the dose amount is about 1 mg/kg/day to 10 mg/kg/day. For repeated administrations over several days or longer, depending on the severity of the disease, disorder, or condition to be treated, the treatment is sustained until a desired suppression of symptoms is achieved.

[0204] An exemplary dosing regimen may include administering an initial dose of a humanized anti-C1q antibody, of about 2 mg/kg, followed by a weekly maintenance dose of about 1 mg/kg every other week. Other dosage regimens may be useful, depending on the pattern of pharmacokinetic decay that the physician wishes to achieve. For example, dosing an individual from one to twenty-one times a week is contemplated herein. In certain implementations, dosing ranging from about 3 µg/kg to about 2 mg/kg (such as about 3 µg/kg, about 10 µg/kg, about 30 µg/kg, about 100 µg/kg, about 300 µg/kg, about 1 mg/kg, or about 2 mg/kg) may be used. In certain implementations, dosing frequency is three times per day, twice per day, once per day, once every other day, once weekly, once every two weeks, once every four weeks, once every five weeks, once every six weeks, once every seven weeks, once every eight weeks, once every nine weeks, once every ten weeks, or once monthly, once every two months, once every three months, or longer. Progress of the therapy is easily monitored by conventional techniques and assays. The dosing regimen, including the humanized anti-C1q antibody administered, can vary over time independently of the dose used.

[0205] Dosages for a particular humanized anti-C1q antibody may be determined empirically in individuals who have been given one or more administrations of the humanized anti-C1q antibody. Individuals are given incremental doses of a humanized anti-C1q antibody. To assess efficacy of a humanized anti-C1q antibody, any clinical symptom of a neurodegenerative disorder, inflammatory disorder, or autoimmune disorder can be monitored.

[0206] Administration of a humanized anti-C1q antibody of the present disclosure can be continuous or intermittent, depending, for example, on the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of a humanized anti-C1q antibody may be essentially continuous over a preselected period of time or may be in a series of spaced doses.

[0207] Guidance regarding particular dosages and methods of delivery is provided in the literature; see, for example, U.S. Patent Nos. 4,657,760; 5,206,344; or 5,225,212. It is within the scope of the present

disclosure that different formulations will be effective for different treatments and different disorders, and that administration intended to treat a specific organ or tissue may necessitate delivery in a manner different from that to another organ or tissue. Moreover, dosages may be administered by one or more separate administrations, or by continuous infusion. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

Therapeutic uses

[0208] The present disclosure provides humanized anti-C1q antibodies, and antigen-binding fragments thereof, which can bind to and neutralize a biologic activity of C1q. These humanized anti-C1q antibodies are useful for preventing, reducing risk, or treating a range of diseases associated with complement activation, including, without limitation, neurodegenerative disorders, inflammatory disorders, and autoimmune disorders. Accordingly, as disclosed herein, humanized anti-C1q antibodies of the present disclosure may be used for treating, preventing, or reducing risk of a disease associated with complement activation, including, without limitation, neurodegenerative disorders, inflammatory disorders, and autoimmune disorders, in an individual. In some implementations, the individual has such a disease. In some implementations, the individual is a human.

[0209] Neurodegenerative disorders that may be treated with humanized anti-C1q antibodies of this disclosure include disorders associated with loss of nerve connections or synapses, including CF1-dependent synapse loss. Such disorders may include, without limitation, Alzheimer's disease, amyotrophic lateral sclerosis, multiple sclerosis, glaucoma, myotonic dystrophy, Guillain-Barre' syndrome (GBS), Myasthenia Gravis, Bullous Pemphigoid, spinal muscular atrophy, Down syndrome, Parkinson's disease, and Huntington's disease. In some neurodegenerative disorders, synapse loss is dependent on the complement receptor 3 (CR3)/C3 or complement receptor CR1. In some neurodegenerative disorders, synapse loss is associated with pathological activity-dependent synaptic pruning. In some disorders, synapses are phagocytosed by microglia. Accordingly, the humanized anti-C1q antibodies of the present disclosure may be used to treat, prevent, or improve one or more symptoms of a neurodegenerative disorder of the present disclosure. In some implementations, the present disclosure provides methods of treating, preventing, or improving one or more symptoms in individuals having a neurodegenerative disorder of the present disclosure by administering a humanized antiC1q antibody of the present disclosure to, for example, inhibit the interaction between C1q and an autoantibody, the interaction of C1q and C1r, and/or the interaction of C1q and C1s.

[0210] Inflammatory or autoimmune diseases that may be treated with humanized antiC1q antibodies of this disclosure include, without limitation, rheumatoid arthritis (RA), acute respiratory distress syndrome (ARDS), remote tissue injury after ischemia and reperfusion, complement activation during cardiopulmonary bypass surgery, dermatomyositis, pemphigus, lupus nephritis and resultant glomerulonephritis and vasculitis, cardiopulmonary bypass, cardioplegia-induced coronary endothelial dysfunction, type II membranoproliferative glomerulonephritis, IgA nephropathy, acute renal failure, cryoglobulemia, antiphospholipid syndrome, Chronic open-angle glaucoma, acute closed angle glaucoma, macular degenerative diseases, age-related macular degeneration (AMD), (AMD-wet), Geographic atrophy, choroidal neovascularization (CNV), uveitis, diabetic retinopathy, ischemia-related retinopathy, endophthalmitis, intraocular neovascular disease, diabetic macular edema, pathological myopia, von Hippel-Lindau disease, histoplasmosis of the eye, Neuromyelitis Optica (NMO), Central Retinal Vein Occlusion (CRVO), corneal neovascularization, retinal neovascularization, Leber's hereditary optic neuropathy, optic neuritis, Behcet's retinopathy, ischemic optic neuropathy, retinal vasculitis, ANCA vasculitis, Purtscher retinopathy, Sjogren's

dry eye disease, dry AMD, sarcoidosis, temporal arteritis, polyarteritis nodosa, multiple sclerosis, as well as allo-transplantation, hyperacute rejection, hemodialysis, chronic occlusive pulmonary distress syndrome (COPD), asthma, and aspiration pneumonia. In some implementations, autoimmune disease may further include, without limitation, Guillain-Barré syndrome, myasthenia gravis, Diabetes mellitus type 1, Hashimoto's thyroiditis, Addison's disease, Coeliac disease, Crohn's disease, pernicious anaemia, Pemphigus vulgaris, vitiligo, autoimmune hemolytic anemias, paraneoplastic syndromes, a vasculitis disease, hypocomplementemic urticarial vasculitis (HUV), polymyalgia rheumatica, temporal arteritis, and Wegener's granulomatosis.

[0211] In autoimmune diseases, such as Neuromyelitis Optica (NMO), autoantibodies activate the complement system. In NMO patients, the classical complement pathway is triggered by the binding of an autoantibody, such as an AQP4-targeted autoantibody, to its autoantigen, AQP4. AQP4 thereby activates the classical pathway of complement activation. In the first step of this activation process complement factor C1q binds to the autoantibody-autoantigen-immune complex. Autoantibodies may include naturally occurring antibodies, such as serum antibodies from NMO patients (commonly referred to as NMO-IgG) or monoclonal antibodies, such as rAb-53.

[0212] Accordingly, the humanized anti-C1q antibodies of the present disclosure may be used to treat, prevent, or improve one or more symptoms of an inflammatory or autoimmune disease of the present disclosure. In some implementations, the present disclosure provides methods of treating, preventing, or improving one or more symptoms in individuals having an inflammatory or autoimmune disease of the present disclosure by administering a humanized anti-C1q antibody of the present disclosure to, for example, inhibit the interaction between C1q and an autoantibody, the interaction of C1q and C1r, and/or the interaction of C1q and C1s.

[0213] Metabolic diseases that may be treated with humanized anti-C1q antibodies include, without limitation, diabetes, such as type II diabetes, and obesity. *In vitro* and *in vivo* models of metabolic disorders that can be used for the testing of humanized anti-C1q antibodies are well known in the art. Accordingly, the humanized anti-C1q antibodies of the present disclosure may be used to treat, prevent, or improve one or more symptoms of a metabolic disease of the present disclosure. In some implementations, the present disclosure provides methods of treating, preventing, or improving one or more symptoms in individuals having metabolic disease of the present disclosure by administering a humanized anti-C1q antibody of the present disclosure to, for example, inhibit the interaction between C1q and an autoantibody, such as an anti-ganglioside autoantibody, the interaction of C1q and C1r, and/or the interaction of C1q and C1s.

Combination Treatments

[0214] The antibodies of the present disclosure may be used, without limitation, in combination with any additional treatment for neurodegenerative disorders, inflammatory disorders, and/or autoimmune disorders.

[0215] In some implementations, a humanized anti-C1q antibody of this disclosure is administered in therapeutically effective amounts in combination with a second anti-complement factor antibody (*e.g.,* a neutralizing anti-complement factor antibody), such as an anti-C1s or anti-C1r antibody, or a second anti-C1q antibody. In some implementations, a humanized anti-C1q antibody of this disclosure is administered in therapeutically effective amounts with a second and a third neutralizing anti-complement factor antibody, such as a second anti-C1q antibody, an anti-C1s antibody, and/or an anti-C1r antibody.

[0216] In some implementations, the humanized anti-C1q antibodies of this disclosure are administered in combination with an inhibitor of antibody-dependent cellular cytotoxicity (ADCC). ADCC inhibitors may

include, without limitation, soluble NK cell inhibitory receptors such as the killer cell Ig-like receptors (KIRs), which recognize HLA-A, HLA-B, or HLA-C and C-type lectin CD94/NKG2A heterodimers, which recognize HLA-E (*see, e.g.,* López-Botet M., T. Bellón, M. Llano, F. Navarro, P. Garcia & M. de Miguel. (2000), Paired inhibitory and triggering NK cell receptors for HLA class I molecules. Hum. Immunol. 61: 7-17; Lanier L.L. (1998) Follow the leader: NK cell receptors for classical and nonclassical MHC class I. Cell 92: 705-707.), and cadmium (*see, e.g.,* Immunopharmacology 1990; Volume 20, Pages 73-8).

[0217] In some implementations, the humanized anti-C1q antibodies of this disclosure are administered in combination with an inhibitor of the alternative pathway of complement activation. Such inhibitors may include, without limitation, factor B blocking antibodies, factor D blocking antibodies, soluble, membrane-bound, tagged or fusion-protein forms of CD59, DAF, CR1, CR2, Crry or Comstatin-like peptides that block the cleavage of C3, non-peptide C3aR antagonists such as SB 290157, Cobra venom factor or non-specific complement inhibitors such as nafamostat mesilate (FUTHAN; FUT-175), aprotinin, K-76 monocarboxylic acid (MX-1) and heparin (*see, e.g.,* T.E. Mollnes & M. Kirschfink, Molecular Immunology 43 (2006) 107-121). In some implementations, the humanized anti-C1q antibodies of this disclosure are administered in combination with an inhibitor of the interaction between the autoantibody and its autoantigen. Such inhibitors may include purified soluble forms of the autoantigen, or antigen mimetics such as peptide or RNA-derived mimotopes, including mimotopes of the AQP4 antigen. Alternatively, such inhibitors may include blocking agents that recognize the autoantigen and prevent binding of the autoantibody without triggering the classical complement pathway. Such blocking agents may include, *e.g.*, autoantigen-binding RNA aptamers or antibodies lacking functional C1q binding sites in their Fc domains (*e.g.,* Fab fragments or antibody otherwise engineered not to bind C1q).

Diagnostic uses

[0218] The humanized anti-C1q antibodies of the present disclosure, or functional fragments thereof, also have diagnostic utility. This disclosure therefore provides methods of using the antibodies of this disclosure, or functional fragments thereof, for diagnostic purposes, such as the detection of C1q in an individual or in tissue samples derived from an individual. In some implementations, the individual is a human. In some implementations, the individual is a human patient suffering from a neurodegenerative disorder or an inflammatory, or autoimmune disease. In some implementations, the humanized anti-C1q antibodies of this disclosure are used to detect synapses and synapse loss. For example, synapse loss may be measured in an individual suffering from a neurodegenerative disorder such as Alzheimer's disease or glaucoma.

[0219] In some implementations, the diagnostic methods involve the steps of administering a humanized anti-C1q antibody of this disclosure, or functional fragment thereof, to an individual and detecting the antibody bound to a synapse of the individual. Antibody-binding to synapses may be quantified, for example, by non-invasive techniques such as positron emission tomography (PET), X-ray computed tomography, single-photon emission computed tomography (SPECT), computed tomography (CT), and computed axial tomography (CAT).

[0220] In some implementations, the diagnostic methods involve detecting synapses in a biological sample, such as a biopsy specimen, a tissue, or a cell. A humanized anti-C1q antibody, or functional fragment thereof, is contacted with the biological sample and synapse-bound antibody is detected. The detection method may involve quantification of the synapse-bound antibody. Antibody detection in biological samples may occur with any method known in the art, including immunofluorescence microscopy, immunocytochemistry, immunohistochemistry, ELISA, FACS analysis, immunoprecipitation, or micro-positron emission tomography. In certain implementations, the antibody is radiolabeled, for example with ^{18}F and subsequently detected utilizing micro-positron emission tomography analysis.

[0221] The quantification of synapse-bound antibodies provides a relative measure for the number of synapses present in the individual. Typically, synapses are quantified repeatedly over a period of time. The exact periodicity of synapse quantification depends on many factors, including the nature of the neurodegenerative disease, the stage of disease progression, treatment modalities and many other factors. Repeat measurements commonly reveal progressive synapse loss in individuals having a neurodegenerative disorder. Alternatively, relative synapse counts may be compared in populations of diseased individuals and healthy control individuals at a single time point. In diseased individuals undergoing treatment, the treatment's efficacy can be assessed by comparing the rates of synapse loss in the treated individuals with the rates of synapse loss in a control group. Control group members have received either no treatment or a control treatment, such as a placebo control.

Kits

[0222] The present disclosure also provides kits containing a humanized anti-C1q antibody of the present disclosure, or a functional fragment thereof. Kits of the present disclosure include one or more containers comprising a purified humanized anti-C1q antibody of this disclosure. In some implementations, the kits further include instructions for use in accordance with the methods of this disclosure. In some implementations, these instructions comprise a description of administration of the humanized anti-C1q antibody to treat or diagnose a disease associated with complement activation including, without limitation a neurodegenerative disorder (*e.g.,* Alzheimer's disease), inflammatory disease, autoimmune disease, and/or metabolic disorder, according to any methods of this disclosure. In some implementations, the instructions comprise a description of how to detect C1q, for example in an individual, in a tissue sample, or in a cell. The kit may further comprise a description of selecting an individual suitable for treatment based on identifying whether that individual has the disease and the stage of the disease.

[0223] The instructions generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (*e.g.,* multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the present disclosure are typically written instructions on a label or package insert (*e.g.,* a paper sheet included in the kit), but machine-readable instructions (*e.g.,* instructions carried on a magnetic or optical storage disk) are also acceptable.

[0224] The label or package insert indicates that the composition is used for treating, *e.g.,* a neurodegenerative disease. Instructions may be provided for practicing any of the methods described herein.

[0225] The kits of this disclosure are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (*e.g.,* sealed Mylar or plastic bags), and the like. Also contemplated are packages for use in combination with a specific device, such as an inhaler, nasal administration device (*e.g.,* an atomizer) or an infusion device such as a minipump. A kit may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The container may also have a sterile access port (*e.g.,* the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an inhibitor of classical complement pathway. The container may further comprise a second pharmaceutically active agent.

[0226] Kits may optionally provide additional components such as buffers and interpretive information. Normally, the kit comprises a container and a label or package insert(s) on or associated with the container.

[0227] The present disclosure will be more fully understood by reference to the following Examples. They should not, however, be construed as limiting any aspect or scope of the present disclosure in any way.

EXAMPLES

Example 1: Production of Humanized Anti-C1q Antibodies

Introduction

[0228] This example describes the generation of fully humanized antibodies from the murine hybridoma M1 (expressing the mouse anti-human C1q antibody M1). Composite human antibody variable region genes were generated using synthetic oligonucleotides encoding combinations of selected human sequence segments. These were then cloned into vectors encoding human IgG4 (S241P L248E) heavy chain and human kappa light chain. Humanized antibodies were stably expressed in NS0 (mouse myeloma cell-line) cells, Protein A purified and tested for binding to human C1q using a competition ELISA assay against biotinylated murine M1 antibody. Selected antibodies were also tested for binding to mouse C1q using competition ELISA assay against biotinylated chimeric antibody.

Results

Sequencing of anti-human C1q V regions

[0229] RNA was extracted from the hybridoma cell pellet expressing M1 antibody using an RNAqueousR-4PCR kit (Ambion cat. no. AM1914). Initially, RT-PCR was performed using degenerate primer pools for murine signal sequences together with constant region primers for both of IgG and Igκ. Heavy chain V region RNA was amplified using a set of six degenerate primer pools (HA to HF) and light chain V region mRNA was amplified using a set of seven degenerate primer pools for the κ cluster (KA to KG).

[0230] For the VH region, amplification products of the expected size were found in IgG primer pools HB and HE. For the Vκ region, amplification products of the expected size were found in kappa primer pools KC, KE, and KG. The PCR products obtained from each of the successful amplifications were purified and cloned into a 'TA' cloning vector (pGEM-T Easy, Promega cat. no. A1360) and sequenced. A total of 14 VH and 24 Vκ clones were sequenced.

[0231] A single functional VH gene was identified in 14 clones from IgG pools HB and HE. A single functional Vκ gene sequence was identified from 9 clones from primer pool KC. The 3' coding sequence downstream of the variable region obtained from IgG primer pools was consistent with the antibody isotype being IgG.

[0232] The functional VH and Vκ gene sequences were identical to the hybridoma sequences with the exception of five amino acids at the beginning of the VH sequence and two amino acids at the beginning of the Vκ sequence. These differences were most likely due to the method of sequencing, and were a result of using primers that are degenerate to the signal sequence rather than primers that are degenerate to 5' end of V regions.

[0233] The amino acid sequence of the functional VH is:
QVQLQQPGAELVKPGASVKLSCKSS**GYHFTSYWMH**WVKQRPGQGLEWIGVIHP
NSGSINYNEKFESKATLTVDKSSSTAYMQLSSLTSEDSAVYYCAG**ERDSTEVLPM DY**WGQGTSVTVSS (SEQ ID NO: 21). The hyper variable regions (HVRs) of the VH are depicted in bolded and underlined text.

[0234] The amino acid sequence of the functional Vκ is:
DVQITQSPSYLAASPGETITINC**RASKSINKYLA**WYQEKPGKTNKLLIY**SGSTLQS**G
IPSRFSGSGSGTDFTLTISSLEPEDFAMYYC**QQHNEYPLT**FGAGTKLELK (SEQ ID NO: 22). The hyper variable regions (HVRs) of the Vκ are depicted in bolded and underlined text.

Construction of chimeric antibody

[0235] The VH and Vκ sequences of the murine M1 antibody were PCR amplified using primers that introduced flanking restriction enzyme sites for cloning into the IgG4 (S241P L248E) heavy and kappa chain expression vectors **(FIG. 1).** The *Bam*HI, *Hind*III and *Ssp*I restriction sites were removed from the Vκ sequence in order to clone the gene. The VH region was cloned using *Mlu*I and *Hind*III sites, and the Vκ region was cloned using *BssH*II and *BamH*I restriction sites. Both constructs were confirmed by sequencing.

Design of composite human variable region sequences

[0236] Structural models of the murine M1 antibody V regions were produced using Swiss PDB and analyzed in order to identify important "constraining" amino acids in the V regions that were likely to be essential for the binding properties of the antibody. Most residues contained within the HVRs (using both Kabat and Chothia definitions) together with a number of framework residues were considered to be important. The VH and Vκ sequences of M1 contain typical framework residues and the HVR 1, 2 and 3 motifs are comparable to many murine antibodies.

[0237] From the above analysis, it was considered that composite human sequences of M1 could be created with a wide latitude for alternative residues outside of the HVRs but with only a narrow menu of possible residues within the HVR sequences. Preliminary analysis indicated that corresponding sequence segments from several human antibodies could be combined to create HVRs similar or identical to those in the murine sequences. For regions outside of and flanking the HVRs, a wide selection of human sequence segments were identified as possible components of the novel humanized V regions.

CD4$^+$ T cell epitope avoidance

[0238] Based upon the structural analysis, a large preliminary set of sequence segments that could be used to create M1 humanized variants were selected and analyzed using iTope™ technology for *in silico* analysis of peptide binding to human MHC class II alleles (Perry, LCA et al. Drugs R D. 2008;9(6):385-96), and using the TCED™ T-cell epitope database of known antibody sequence-related T cell epitopes (Bryson, CJ et al. BioDrugs. 2010 Feb 1;24(1):1-8). Sequence segments that were identified as significant non-human germline binders to human MHC class II or that scored significant hits against the TCED™ were discarded. This resulted in a reduced set of segments, and combinations of these were again analyzed, as above, to ensure that the junctions between segments did not contain potential T cell epitopes. Selected sequence segments were assembled into complete V region sequences that were devoid of significant T cell epitopes.

Four heavy chain sequences (VH1-VH4) and four light chain sequences (Vκ1- Vκ4) were then chosen for gene synthesis, expression in mammalian cells, and testing for activity.

Sequences of humanized heavy chain and light variable domains

[0239] Using standard techniques, the amino acid and nucleic acid sequences encoding the heavy chain variable domain (VH) and kappa light chain variable domain (Vκ) variants were determined.

[0240] The amino acid sequence of heavy chain variable domain variant 1 (VH1) is: QVQLVQSGAELKKPGASVKVSCKSS**GYHFTSYWMH**WVKQAPGQGLEWIG**VIHP NSGSINYNEKFES**KATITVDKSTSTAYMOLSSLTSEDSAVYYCAG**ERDSTEVLPM DY**WGOGTSVTVSS (SEQ ID NO: 1). The hyper variable regions (HVRs) of VH1 are depicted in bolded and underlined text.

[0241] The amino acid sequence of heavy chain variable domain variant 2 (VH2) is: QVQLVQSGAELKKPGASVKVSCKSS**GYHFTSYWMH**WVKQAPGQGLEWIG**VIHP NSGSINYNEKFES**RATITVDKSTSTAYMELSSLRSEDTAVYYCAG**ERDSTEVLPM DY**WGQGTTVTVSS (SEQ ID NO: 2). The hyper variable regions (HVRs) of VH2 are depicted in bolded and underlined text.

[0242] The amino acid sequence of heavy chain variable domain variant 3 (VH3) is: QVQLVQSGAELKKPGASVKVSCKSS**GYHFTSYWMH**WVKQAPGQGLEWIG**VIHP NSGSINYNEKFES**RVTITVDKSTSTAYMELSSLRSEDTAVYYCAG**ERDSTEVLPM DY**WGQGTTVTVSS (SEQ ID NO: 3). The hyper variable regions (HVRs) of VH3 are depicted in bolded and underlined text.

[0243] The amino acid sequence of heavy chain variable domain variant 4 (VH4) is: QVQLVQSGAELKKPGASVKVSCKSS**GYHFTSYWMH**WVRQAPGQGLEWIG**VIHP NSGSINYNEKFES**RVTITVDKSTSTAYMELSSLRSEDTAVYYCAG**ERDSTEVLPM DY**WGQGTTVTVSS (SEQ ID NO: 4). The hyper variable regions (HVRs) of VH4 are depicted in bolded and underlined text.

[0244] In some embodiments, the HVR-H1 of any one of VH1, VH2, VH3, or VH4 has the sequence GYHFTSYWMH (SEQ ID NO: 23), the HVR-H2 of V any one of VH1, VH2, VH3, or VH4 has the sequence VIHPNSGSINYNEKFES (SEQ ID NO: 24), and the HVR-H3 of any one of VH1, VH2, VH3, or VH4 has the sequence ERDSTEVLPMDY (SEQ ID NO: 25).

[0245] The nucleic acid sequence encoding heavy chain variable domain variant 1 (VH1) is:
CAGGTGCAGCTGGTGCAGTCAGGGGCTGAGCTGAAGAAGCCTGGGGCTTCAGT
GAAGGTTTCCTGCAAGTCTTCTGGCTACCATTTCACCAGCTACTGGATGCACTG
GGTGAAGCAGGCCCCTGGACAAGGCCTTGAGTGGATTGGAGTGATTCATCCTA
ATAGTGGTAGTATTAACTACAATGAGAAGTTCGAGAGCAAGGCCACAATTACT
GTAGACAAATCCACCAGCACAGCCTACATGCAACTCAGCAGCCTGACATCTGA
GGACTCGGCGGTCTATTATTGTGCAGGAGAGAGAGATTCTACGGAGGTTCTCCC
TATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCA (SEQ ID NO: 26).

[0246] The nucleic acid sequence encoding heavy chain variable domain variant 2 (VH2) is:
CAGGTGCAGCTGGTGCAGTCAGGGGCTGAGCTGAAGAAGCCTGGGGCTTCAGT
GAAGGTTTCCTGCAAGTCTTCTGGCTACCATTTCACCAGCTACTGGATGCACTG
GGTGAAGCAGGCCCCTGGACAAGGCCTTGAGTGGATTGGAGTGATTCATCCTA
ATAGTGGTAGTATTAACTACAATGAGAAGTTCGAGAGCAGAGCCACAATTACT

GTAGACAAATCCACCAGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGA
GGACACGGCGGTCTATTATTGTGCAGGAGAGAGAGATTCTACGGAGGTTCTCC
CTATGGACTACTGGGGTCAAGGAACCACGGTCACCGTCTCCTCA (SEQ ID NO:
27).

[0247] The nucleic acid sequence encoding heavy chain variable domain variant 3 (VH3) is:
CAGGTGCAGCTGGTGCAGTCAGGGGCTGAGCTGAAGAAGCCTGGGGCTTCAGT
GAAGGTTTCCTGCAAGTCTTCTGGCTACCATTTCACCAGCTACTGGATGCACTG
GGTGAAGCAGGCCCCTGGACAAGGCCTTGAGTGGATTGGAGTGATTCATCCTA
ATAGTGGTAGTATTAACTACAATGAGAAGTTCGAGAGCAGAGTCACAATTACT
GTAGACAAATCCACCAGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGA
GGACACGGCGGTCTATTATTGTGCAGGAGAGAGAGATTCTACGGAGGTTCTCC
CTATGGACTACTGGGGTCAAGGAACCACGGTCACCGTCTCCTCAG (SEQ ID NO:
28).

[0248] The nucleic acid sequence encoding heavy chain variable domain variant 4 (VH4) is:
CAGGTGCAGCTGGTGCAGTCAGGGGCTGAGCTGAAGAAGCCTGGGGCTTCAGT
GAAGGTTTCCTGCAAGTCTTCTGGCTACCATTTCACCAGCTACTGGATGCACTG
GGTGCGACAGGCCCCTGGACAAGGCCTTGAGTGGATTGGAGTGATTCATCCTA
ATAGTGGTAGTATTAACTACAATGAGAAGTTCGAGAGCAGAGTCACAATTACT
GTAGACAAATCCACCAGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGA
GGACACGGCGGTCTATTATTGTGCAGGAGAGAGAGATTCTACGGAGGTTCTCC
CTATGGACTACTGGGGTCAAGGAACCACGGTCACCGTCTCCTCA (SEQ ID NO:
29).

[0249] The amino acid sequence of kappa light chain variable domain variant 1 (Vκ1) is:
DVOITOSPSYLAASLGERATINC**RASKSINKYLA**WYOOKPGKTNKLLIY**SGSTLOS**
GIPARFSGSGSGTDFTLTISSLEPEDFAMYYC**QQHNEYPLT**FGOGTKLEIK (SEQ ID NO: 5). The hyper variable regions (HVRs) of Vκ1 are depicted in bolded and underlined text.

[0250] The amino acid sequence of kappa light chain variable domain variant 2 (Vκ2) is:
DVOITOSPSSLSASLGERATINC**RASKSINKYLA**WYOOKPGKANKLLIY**SGSTLOS**
GIPARFSGSGSGTDFTLTISSLEPEDFAMYYC**QQHNEYPLT**FGOGTKLEIK (SEQ ID NO: 6). The hyper variable regions (HVRs) of Vκ2 are depicted in bolded and underlined text.

[0251] The amino acid sequence of kappa light chain variable domain variant 3 (Vκ3) is:
DVOITOSPSSLSASLGERATINC**RASKSINKYLA**WYOOKPGKAPKLLIY**SGSTLOS**
GIPARFSGSGSGTDFTLTISSLEPEDFAMYYC**QQHNEYPLT**FGOGTKLEIK (SEQ ID NO: 7). The hyper variable regions (HVRs) of Vκ3 are depicted in bolded and underlined text.

[0252] The amino acid sequence of kappa light chain variable domain variant 4 (Vκ4) is:
DIOLTOSPSSLSASLGERATINC**RASKSINKYLA**WYOOKPGKAPKLLIY**SGSTLQS**G
IPARFSGSGSGTDFTLTISSLEPEDFAMYYC**QQHNEYPLT**FGQGTKLEIK (SEQ ID NO: 8). The hyper variable regions (HVRs) of Vκ4 are depicted in bolded and underlined text.

[0253] In some embodiments, the HVR-L1 of any one of Vκ1, Vκ2, Vκ3, or Vκ4 has the sequence has the

sequence RASKSINKYLA (SEQ ID NO: 30), the HVR-L2 of any one of Vκ1, Vκ2, Vκ3, or Vκ4 has the sequence SGSTLQS (SEQ ID NO: 31), and the HVR-L3 of any one of Vκ1, Vκ2, Vκ3, or Vκ4 has the sequence QQHNEYPLT (SEQ ID NO: 32).

[0254] The nucleic acid sequence encoding kappa light chain variable domain variant 1 (Vκ1) is:
GATGTCCAGATCACACAGTCTCCATCTTATCTTGCTGCATCTCTCGGAGAAAGA
GCTACTATTAATTGCAGGGCAAGTAAGAGCATTAACAAATACTTAGCCTGGTAT
CAACAGAAACCTGGGAAAACTAATAAGCTCCTTATCTACTCTGGCTCCACTTTG
CAATCTGGAATTCCAGCAAGGTTCAGTGGCAGTGGATCTGGTACAGATTTCACT
CTCACCATCAGTAGCCTGGAGCCTGAAGATTTTGCAATGTATTACTGTCAACAA
CATAATGAATACCCGCTCACGTTCGGTCAGGGGACCAAGCTGGAGATCAAA
(SEQ ID NO: 33).

[0255] The nucleic acid sequence encoding kappa light chain variable domain variant 2 (Vκ2) is:
GATGTCCAGATCACACAGTCTCCATCTTCCCTTTCTGCATCTCTCGGAGAAAGA
GCTACTATTAATTGCAGGGCAAGTAAGAGCATTAACAAATACTTAGCCTGGTAT
CAACAGAAACCTGGGAAAGCTAATAAGCTCCTTATCTACTCTGGCTCCACTTTG
CAATCTGGAATTCCAGCAAGGTTCAGTGGCAGTGGATCTGGTACAGATTTCACT
CTCACCATCAGTAGCCTGGAGCCTGAAGATTTTGCAATGTATTACTGTCAACAA
CATAATGAATACCCGCTCACGTTCGGTCAGGGGACCAAGCTGGAGATCAAA
(SEQ ID NO: 34).

[0256] The nucleic acid sequence encoding kappa light chain variable domain variant 3 (Vκ3) is:
GATGTCCAGATCACACAGTCTCCATCTTCCCTTTCTGCATCTCTCGGAGAAAGA
GCTACTATTAATTGCAGGGCAAGTAAGAGCATTAACAAATACTTAGCCTGGTAT
CAACAGAAACCTGGGAAAGCTCCTAAGCTCCTTATCTACTCTGGCTCCACTTTG
CAATCTGGAATTCCAGCAAGGTTCAGTGGCAGTGGATCTGGTACAGATTTCACT
CTCACCATCAGTAGCCTGGAGCCTGAAGATTTTGCAATGTATTACTGTCAACAA
CATAATGAATACCCGCTCACGTTCGGTCAGGGGACCAAGCTGGAGATCAAA
(SEQ ID NO: 35).

[0257] The nucleic acid sequence encoding kappa light chain variable domain variant 4 (Vκ4) is:
GATATTCAGCTCACACAGTCTCCATCTTCCCTTTCTGCATCTCTCGGAGAAAGA
GCTACTATTAATTGCAGGGCAAGTAAGAGCATTAACAAATACTTAGCCTGGTAT
CAACAGAAACCTGGGAAAGCTCCTAAGCTCCTTATCTACTCTGGCTCCACTTTG
CAATCTGGAATTCCAGCAAGGTTCAGTGGCAGTGGATCTGGTACAGATTTCACT
CTCACCATCAGTAGCCTGGAGCCTGAAGATTTTGCAATGTATTACTGTCAACAA
CATAATGAATACCCGCTCACGTTCGGTCAGGGGACCAAGCTGGAGATCAAA
(SEQ ID NO: 36).

Sequences of human IgG4 (S241P L248E) heavy chain constant domain

[0258] Using standard techniques, the amino acid and nucleic acid sequences encoding the human IgG4

(S241P L248E) heavy chain constant domain *(i.e.,* CH1, CH2, CH3, and hinge region,) were determined.

[0259] The amino acid sequence of human IgG4 (S241P L248E) heavy chain constant domain is:
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCP**P**CPAPEF
EGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKT
KPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPRE
PQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO: 37). The S241P mutation and the L248E mutation are depicted in bolded and underlined text.

[0260] The amino acid sequence of the human IgG4 (S241P L248E) CH1 is:
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRV (SEQ ID NO: 38).

[0261] The amino acid sequence of the human IgG4 (S241P L248E) hinge region is: ESKYGPPCP**P**CP (SEQ ID NO: 39). The S241P mutation is depicted in bolded and underlined text.

[0262] The amino acid sequence of the human IgG4 (S241P L248E) CH2 is:
APEF**E**GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK (SEQ ID NO: 40). The L248E mutation is depicted in bolded and underlined text.

[0263] The amino acid sequence of the human IgG4 (S241P L248E) CH3 is:
GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ
ID NO: 41).

[0264] The nucleic acid sequence encoding human IgG4 (S241P L248E) heavy chain constant domain is:
GTAAGCTTTCTGGGGCAGGCCGGGCCTGACTTTGGCTGGGGGCAGGGAGGGGG
CTAAGGTGACGCAGGTGGCGCCAGCCAGGTGCACACCCAATGCCCATGAGCCC
AGACACTGGACCCTGCATGGACCATCGCGGATAGACAAGAACCGAGGGGCCTC
TGCGCCCTGGGCCCAGCTCTGTCCCACACCGCGGTCACATGGCACCACCTCTCT
TGCAGCTTCCACCAAGGGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAG
CACCTCCGAGAGCACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCG
AACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACC
TTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACC
GTGCCCTCCAGCAGCTTGGGCACGAAGACCTACACCTGCAATGTAGATCACAA
GCCCAGCAACACCAAGGTGGACAAGAGAGTTGGTGAGAGGCCAGCACAGGGA
GGGAGGGTGTCTGCTGGAAGCCAGGCTCAGCCCTCCTGCCTGGACGCACCCCG

GCTGTGCAGCCCCAGCCCAGGGCAGCAAGGCAGGCCCCATCTGTCTCCTCACCT
GGAGGCCTCTGACCACCCCACTCATGCTCAGGGAGAGGGTCTTCTGGATTTTTC
CACCAGGCTCCGGGCAGCCACAGGCTGGATGCCCCTACCCCAGGCCCTGCGCA
TACAGGGGCAGGTGCTGCGCTCAGACCTGCCAAGAGCCATATCCGGGAGGACC
CTGCCCCTGACCTAAGCCCACCCCAAAGGCCAAACTCTCCACTCCCTCAGCTCA
GACACCTTCTCTCCTCCCAGATCTGAGTAACTCCCAATCTTCTCTCTGCAGAGTC

CAAATATGGTCCCCCATGCCCACCATGCCCAGGTAAGCCAACCCAGGCCTCGC
CCTCCAGCTCAAGGCGGGACAGGTGCCCTAGAGTAGCCTGCATCCAGGGACAG
GCCCCAGCCGGGTGCTGACGCATCCACCTCCATCTCTTCCTCAGCACCTGAGTT
CGAGGGGGGACCATCAGTCTTCCTGTTCCCCCCAAAACCCAAGGACACTCTCAT
GATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGAGCCAGGAAG
ACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCC
AAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCGT
CCTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGG
TCTCCAACAAAGGCCTCCCGTCCTCCATCGAGAAAACCATCTCCAAAGCCAAA
GGTGGGACCCACGGGGTGCGAGGGCCACATGGACAGAGGTCAGCTCGGCCCAC
CCTCTGCCCTGGGAGTGACCGCTGTGCCAACCTCTGTCCCTACAGGGCAGCCCC
GAGAGCCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAGAAC
CAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTG
GAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGT
GCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAGGCTAACCGTGGACAAGAG
CAGGTGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCA
CAACCACTACACACAGAAGAGCCTCTCCCTGTCTCTGGGTAAA (SEQ ID NO:
42).

[0265] The nucleic acid sequence encoding the human IgG4 (S241P L248E) heavy chain CH1 is:
CTTCCACCAAGGGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCT
CCGAGAGCACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCG
GTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCC
GGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCC
CTCCAGCAGCTTGGGCACGAAGACCTACACCTGCAATGTAGATCACAAGCCCA
GCAACACCAAGGTGGACAAGAGAGTTG (SEQ ID NO: 43).

[0266] The nucleic acid sequence encoding the human IgG4 (S241P L248E) heavy chain hinge is:
AGTCCAAATATGGTCCCCCATGCCCACCATGCCCAG (SEQ ID NO: 44).

[0267] The nucleic acid sequence encoding the human IgG4 (S241P L248E) heavy chain CH2 is:
CACCTGAGTTCGAGGGGGGACCATCAGTCTTCCTGTTCCCCCCAAAACCCAAGG
ACACTCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGA
GCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTG
CATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGT
GGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACA
AGTGCAAGGTCTCCAACAAAGGCCTCCCGTCCTCCATCGAGAAAACCATCTCCA
AAGCCAAAG (SEQ ID NO: 45).

[0268] The nucleic acid sequence encoding the human IgG4 (S241P L248E) heavy chain CH3 is:
GGCAGCCCCGAGAGCCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATG
ACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGA
CATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC
ACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAGGCTAACC
GTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGATGCA

TGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCTGGGTAA
A (SEQ ID NO: 46).

[0269] FIG. 2 shows an alignment of the amino acid sequences of the heavy chain variable region (VH) of M1 and the amino acid sequences of the humanized VH variants VH1-VH4, and of the amino acid sequences of the kappa light chain variable region (Vκ) of M1 and the amino acid sequences of the humanized Vκ variants Vκ1-Vκ4.

Construction of composite human antibody variants

[0270] All variant VH and Vκ region genes for M1 were synthesized using a series of overlapping oligonucleotides that were annealed, ligated and PCR amplified to give full length synthetic V regions with any restriction sites removed. The assembled variants were then cloned directly into the pANT expression vector system for IgG4 (S241 L248E) heavy chain and kappa light chain **(FIG. 1).** The VH region was cloned using *Mlu*I and *Hind*III sites, and the Vκ region was cloned using *BssH*II and *BamH*I restriction sites. All constructs were confirmed by sequencing.

Expression and purification of antibodies

[0271] A total of 16 fully humanized antibodies were stably transfected into NS0 cells via electroporation. In addition, the chimeric antibody M1 along with two controls - chimeric VH (ChVH) with variant Vκ1 and variant VH1 with chimeric Vκ (ChVκ) - were included. Stable transfectants were selected using 200 nM methotrexate (Sigma Cat. No. M8407). Methotrexate-resistant colonies for each construct were tested for IgG expression levels using an IgG4 ELISA, and the best expressing lines were selected, expanded and frozen under liquid nitrogen. Successful transfection and stable clone selection was achieved for all of the 16 humanized M1 variants as well as chimeric M1, ChVH/Vκ1 and VH1/ChVκ antibodies. The identity of each cell line was confirmed by DNA sequencing of the variable domains from genomic DNA.

[0272] Antibodies were purified from cell culture supernatants on a Protein A sepharose column (GE Healthcare cat. co. 110034-93), buffer exchanged into PBS, pH 7.4 and quantified by OD_{280nm} using an extinction coefficient based on the predicted amino acid sequences. Chimeric and humanized variant IgGs were analyzed by reducing SDS-PAGE. Bands corresponding to the predicted sizes of the VH and Vκ chains were observed with no evidence of any aggregation, degradation or other unusual features **(FIG. 3).**

Competition ELISA against human C1q antigen

[0273] The binding of humanized M1 variants to human C1q was analyzed by competition ELISA. A three-fold dilution series of test antibody (5 µg/ml to 0.002 µg/ml) was premixed with a constant concentration of biotinylated mouse M1 antibody (0.02 µg/ml, final concentration) before incubating for 1 hour at 37°C with shaking on plates pre-coated with human C1q at 1.0µg/ml. The binding of mouse M1 antibody was detected with streptavidin-peroxidase conjugate (Sigma-Aldrich cat. no. S5512) and TMB (3,3',5,5'-tetramethylbenzidine) substrate (Thermo Scientific cat. no. 34029). The reaction was stopped with 1M HCl, absorbance read at 450 nm on a Dynex Technologies MRX TC II plate reader and the binding curves plotted.

[0274] FIG. 4 shows that all humanized M1 variants generated have similar binding profiles to the chimeric M1 antibody. The binding curves were used to calculate IC_{50} values (concentration of test antibody that inhibits labelled competitor binding by 50%) for each antibody normalized to the IC_{50} of chimeric M1 and antibody yields from NS0 transfectants were also compared (Table 1).

TABLE 1: Relative IC_{50} values for binding to human C1q and protein expression levels

Antibody	Relative IC_{50}	Expression levels (µg/ml)
Chimeric M1	1.00	12.6
ChV1/Vκ1	1.09	7.0
VH1/ChVκ	0.92	11.9
VH1/Vκ1	0.90	14.0
VH1/Vκ2	0.84	14.5
VH1/Vκ3	0.91	28.9
VH1/Vκ4	0.80	22.6
VH2/Vκ1	1.15	1.4
VH2/Vκ2	1.12	3.8
VH2/Vκ3	0.75	21.3
VH2/Vκ4	0.72	6.1
VH3/Vκ1	0.65	16.9
VH3/Vκ2	0.82	8.7
VH3/Vκ3	0.63	19.8
VH3/Vκ4	0.83	32.2
VH4/Vκ1	1.03	8.5
VH4/Vκ2	0.84	1.6
VH4/Vκ3	0.77	18.3
VH4/Vκ4	0.92	2.4

[0275] Table 1 shows calculated relative IC_{50} values for humanized M1 variants binding to human C1q and protein expression levels of corresponding NS0 cell line.

[0276] The normalized IC_{50} data for all variants tested were in the range of 0.63 to 1.15 indicating that the binding efficiencies of all of the fully humanized M1 antibodies to human C1q were comparable to that of chimeric M1. Furthermore, most humanized variants achieved an increase in expression level compared to the chimeric antibody.

Competition ELISA against mouse C1q antigen

[0277] The binding of humanized M1 variants to mouse C1q was analyzed by competition ELISA on four selected antibodies, VH1/Vκ1, VH3/Vκ3, VH3/Vκ4 and VH4/Vκ3. An irrelevant IgG4 (S241P L248E) antibody was also included as a binding control. A three-fold dilution series of test antibody (100 µg/ml to 0.046 µg/ml) was premixed with a constant concentration of biotinylated chimeric M1 antibody (0.03 µg/ml, final concentration) before incubating for 1 hour at 37°C with shaking on plates pre-coated with mouse C1q at 5.0µg/ml. The binding of chimeric M1 antibody was detected with streptavidin-peroxidase conjugate (Sigma-

Aldrich cat. no. S5512) and TMB substrate (Thermo Scientific cat. no. 34029). The reaction was stopped with 1M HCl, absorbance read at 450 nm on a Dynex Technologies MRX TC II plate reader and the binding curves plotted.

[0278] FIG. 5 shows that humanized M1 variants generated have similar binding profiles to the chimeric M1 antibody. The binding curves were used to calculate IC_{50} values for each antibody normalized to the IC_{50} of chimeric M1 (Table 2).

TABLE 2: Relative IC_{50} values for binding to mouse C1q

Antibody	Relative IC_{50}
Chimeric M1	1.00
VH1/Vκ1	1.62
VH3/Vκ3	1.50
VH3/Vκ4	1.91
VH4/Vκ3	1.84

[0279] Table 2 shows calculated relative IC_{50} values for humanized M1 variants binding to mouse C1q.

Conclusion

[0280] The V region genes from the murine antibody M1 were cloned into vectors to generate a chimeric antibody comprising the murine V regions combined with the human IgG4 (S241P L248E) heavy chain constant region and κ light chain constant regions. Additionally, a series of four humanized VH regions for IgG4 (S241P L248E) and four humanized Vκ regions were designed and constructed.

[0281] The chimeric antibody and the combinations of humanized V region genes (16 antibodies in total) were expressed in NS0 cells, purified and tested for binding to human C1q in a competition ELISA assay. The binding data (Table 1) were used to rank the humanized M1 variants in comparison with the chimeric M1 antibody. No significant differences in quality of the heavy and light chain bands were detected by SDS-PAGE.

Example 2: Kinetic Characterization of Humanized Anti-C1q Antibody VH3/Vκ3 Introduction

[0282] Immunological biosensors, for example Biacore™ surface plasmon resonance (SPR) instruments, that measure the binding and dissociation of antigen-antibody complexes in real time allow the elucidation of binding kinetics. The rate of dissociation and its subsequent optimization is especially important for biopharmaceutical antibody development.

[0283] The Biacore uses SPR to monitor the interaction between a surface bound molecule `ligand' and its binding partner in solution `analyte', in real time. SPR is an electron charge-density wave phenomenon, which arises at the surface of a metallic layer when light is reflected at the layer under conditions of total internal reflectance. The surface plasmons that are generated are sensitive to any changes in the refractive index of the medium on the opposite side of the metallic layer from the reflected light. Protein-protein interactions occurring at the surface affect the refractive index of the medium and can therefore be detected. Binding of molecules to the ligand modified sensor surface generates a response, which is proportional to

the bound mass allowing small changes in the amount of bound analyte to be detected (down to low picogram levels). The technique can be used to measure affinity constants (K_D) over the range 10^{-5} M to 10^{-12} M, association rate constants (k_a) between 10^3 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, and dissociation rate constants (k_d) between 10^{-1} $s^{-1}$and 10^{-6} s^{-1}.

[0284] The technique requires only small amounts of material and both of the interacting biomolecules can be used in a label-free form. Experimental design is important, however, as some features of the technology, and the fact that one of the proteins must be surfaced attached, can give rise to misleading results (Huber and Mueller, Curr Pharm Des. 2006;12(31):3999-4021; and Lakey and Raggett, Curr Opin Struct Biol., 1998. 8(1): p. 119-123).

[0285] This example describes the kinetic characterization of the interaction between the humanized anti-C1q antibody VH3/Vκ3 (both Fab fragment and full-length IgG) and the C1q protein utilizing the Biacore T200 surface plasmon resonance instrument for the high resolution.

Materials and Methods

Samples

[0286] The reagents used in this example are listed in Table 3.

TABLE 3: Samples

Sample	Volume at concentration (mg/ml)
IgG-VH3/Vκ3	400 µl at 1.2 mg/ml
Fab-VH3/Vκ3	450 µl at 0.3 mg/ml
Mouse C1q	4 × 50 µl at 1.0 mg/ml
Human C1q	10 × 50 µl at 1.0 mg/ml

[0287] Mouse and human C1q were stored at -80°C, except once defrosted they were stored at + 4°C. Fab and IgG VH3/Vκ3 were stored at +4°C. Once diluted, C1q solutions were kept on ice and used within 24 hours.

Equipment

[0288] A Biacore T200 instrument (SN: CN 12231) running Biacore T200 Evaluation Software V1.1 (Uppsala, Sweden) was used.

Materials

[0289] The following materials were obtained from Biacore as follows:

Biacore Preventative Maintenance Kit 2:	BR-1006-51, Lot No. 164110
Series S CM5 Sensor Chips:	BR-1006-68, Lot No. 10102398

Amine Coupling Kit:	BR-1000-50, Lot No. 2027942/41
10 mM Acetate pH 5.0:	BR-1003-51, Lot No. 21702813
HBS-EP Running buffer:	BR-1006-69, Lot No. 2027942/59

[0290] BSA was obtained from Sigma (A3294).

Procedures

[0291] All experiments were developed with Biacore `wizard' software. The following Biacore methods were used:

Immobilization

Kinetics/Affinity

Desorb and Sanitize

Results

VH3/Vκ3 Fab preparation

[0292] The Fab fragment of the anti-C1q humanized antibody VH3/Vκ3 was prepared using a Fab Micro Preparation Kit following the manufacturer's protocol. The starting concentration of the full-length IgG VH3/Vκ3 was 1.88 mg/ml. To obtain sufficient amount of the Fab fragments, 6 reactions of 225 µg each of full-length IgG were digested, pooled, and purified. The purified Fab and the full-length IgG were further purified by size exclusion chromatography using a Superdex 200 Increase 10/300 GL column (GE Healthcare Cat. No. 28-9909-44) with 1× PBS as running buffer. Samples were quantified by OD_{280nm} using an extinction coefficient ($EC_{(0.1\%)}$) based on the predicted amino acid sequence ($Ec_{(0.1\%)}$ = 1.45 for IgG VH3/Vκ3 and 1.4 for Fab VH3/Vκ3). Both samples were analyzed by non-reducing and reducing SDS-PAGE **(FIG. 6). FIG. 6** depicts a Coomassie Blue-stained SDS-PAGE gel of gel filtration-purified antibodies. 1 µg of each sample was loaded on a NuPage 4-12% Bis-Tris gel (Invitrogen Cat. No. NP0322BOX) and run at 200V for 35min. Size marker (M) is prestained protein standard Fermentas PageRuler Plus (Cat. No. SM1811). Lane 1 shows the reduced VH3/Vκ3 Fab; lane 2 shows the nonreduced VH3/Vκ3 Fab; lane 3 shows the reduced VH3/Vκ3 IgG; and lane 4 shows the nonreduced VH3/Vκ3 IgG.

Antigen preparation

[0293] The samples of mouse C1q (mClq) and human C1q (hC1q) antigens were stored at -80°C, and upon initial defrosting, multiple aliquots were prepared, re-frozen and stored at -80°C. Further dilutions of the analytes into HBS-EP (10 mM HEPES pH 7.4 and 150 mM NaCl, 3 mM EDTA and 0.05 % (v/v) P20) were performed for the kinetic runs.

Instrument preparation

[0294] Before running any samples, and during the study, a system check (Biacore Preventative Maintenance Kit 2) was performed. All the systems tested passed (Reagent pump, Refractometer, Injections, Noise, Mixing and Buffer Selector) indicating that the instrument was performing to criteria set by the manufacturer.

System preparation

[0295] Upon insertion of a CM5 chip the system was primed and then normalized with BIA normalizing solution (Biacore Preventative Maintenance Kit 2). All samples were run at 25°C with a sample rack incubated at 10°C. The chip was added to the system with HBS-EP used as the running buffer; prior to immobilization the chip surface was primed with two injections of 50 mM NaOH.

Immobilization conditions

[0296] Due to concerns about stability, two chips were prepared; one with hClq and mClq (Chip A11) as ligands and one with IgG and Fab (Chip A13) as ligands. Immobilization for m/h C1q was carried out at a protein concentration of 5 µg/ml in 10 mM acetate buffer pH 5.5, whereas immobilization for IgG and Fab was carried out at a protein concentration of 0.5-2 µg/ml in 10 mM acetate buffer pH 5.0, both on a CM5 Series S sensor chip (Biacore). The final response levels for Chips A11 and A13 used in the kinetic analysis are shown in Table 4.

TABLE 4: Final response level (RU)

	F_c1	F_c2	F_c3	F_c4
A11	Blank	hC1q 808.3	mC1q 801.3	mC1q 824.1
A13	Blank	Fab 10.4	IgG 12.8	IgG 51.9

[0297] Table 4 depicts the final immobilization levels achieved from Chips A11 and A13 for each flow cell (F_c).

[0298] For kinetic experiments the amount of immobilized/captured ligand needs to be limited to avoid mass transfer effects at the surface of the chip and the surface should ideally have an analyte maximum binding response (R_{max}) of 100-150 response uints (RUs). The amount of ligand to immobilize is therefore calculated using **Equation 1:**

$$Analyte\ Binding\ Capacity(RU) = \frac{analyte\ Mw}{ligand\ Mw} \times immobilised\ ligand(RU) \times Sm$$

[0299] An average MW of 410 kDa (literature and reagent manufacturers) for both mClq and hC1q, 150 kDa for IgG (estimated value for antibodies), and 50 kDa for the Fab (estimated) were employed. Aiming for 100 RU for R_{max} and the stoichiometry (Sm) as 1, an ideal target amount of C1q to immobilize would be ~ 820 RUs. Due to concerns associated with avidity for the Fab and IgG immobilized surfaces, the amount of immobilized ligand was kept as low as possible (limit of 10 RUs in Biacore immobilization software).

Non-specific binding (NSB) control

[0300] Non-specific binding can be due to either the analyte or analyte contaminants, interacting with the ligand (non-specific and difficult to detect), the capture protein, or the sensor chip surface. When analyzing the response of the blank F_c1 surface after a relatively high concentration (500 nM), 300 second injection of both mClq and hC1q, significant non-specific binding (NSB) was observed. An additional blocking step, a post-ligand immobilization of 50 µg/ml BSA (10 mM acetate pH 4.25) was therefore included (Moore et al., *MAbs.* 2010 Mar-Apr;2(2):181-9). The BSA blocking step was also repeated on the reference channel (Fc1); for both, immobilization levels were -8000 RU. No NSB was observed using Fab, or IgG at 500 nM on the CM5 surface, and at the concentration used in the kinetic runs, no NSB to the BSA blocked surface was seen.

Regeneration scouting

[0301] Where required, either a single injection or two sequential 30 second injections of
1 M NaCl / 50 mM NaOH were used to regenerate all surfaces. A 180 second wait step was introduced after the last regeneration injection to allow the surface to stabilize before starting the next binding cycle.

Surface performance

[0302] The performance of the surface was analyzed by repeated control injections of analyte at the start, interspersed and at the end of a kinetic run. Stable binding was typically observed throughout the kinetic run, highlighting the suitability of the system for kinetic analysis.

Mass transfer control

[0303] Mass transport limitation occurs when the rate of association contains a significant component associated with the rate of transport of the analyte to and from the chip surface. Where mass transfer is found to be significant, the resulting kinetic analysis could be inaccurate. Lowering the density of immobilized ligand, or increasing the flow rate, can reduce mass transport limitations. From previous experience of using low density surfaces and similar MW antigens a flow rate of 40 µL/min was selected for this study.

Linked reactions control

[0304] The linked reaction control experiment is used to assess the ligand-analyte interaction to check for deviations from a 1-to-1 binding model. The analyte is injected over the surface for different periods of time (contact times) and the dissociation rate is analyzed to determine if it varies with the contact time. If such a relationship is observed, it indicates that a second interaction event is taking place after the initial binding event that results in a stabilized complex at the surface.

[0305] Avidity associated with a single hClq binding two antibodies could be expected if the antibody was employed as the ligand. A linked reaction control was therefore performed to provide supporting evidence for more complex data analysis models.

Kinetic analysis

[0306] A 1:1 binding model was initially assumed for all ligand/complex interactions (see **Equation 2**). Due to ligand activity and drift in the baseline (BSA blocked surfaces) the parameter R_{max} was set to local as opposed to global analysis for selected kinetic analyses. If necessary, additional models, such as Heterogeneous Ligand (see **Equation 3**) and Bivalent Binding (see **Equation 4**), were also assessed for goodness of fit.

Equation 2: 1-to-1 binding:

$$A + B \underset{kd}{\overset{ka}{\rightleftharpoons}} AB$$

Equation 3: Heterogeneous ligand:

$$A + B1 \underset{kd1}{\overset{ka1}{\rightleftharpoons}} AB1 \qquad A + B2 \underset{kd2}{\overset{ka2}{\rightleftharpoons}} AB2$$

Equation 4: Bivalent (Avidity):

$$2B + A \underset{kd1}{\overset{ka1}{\rightleftharpoons}} BA \quad + \quad B \underset{kd2}{\overset{ka2}{\rightleftharpoons}} BBA$$

Antibody characterization

[0307] Both C1q complexes were immobilized based on concerns over avidity (*i.e.,* two immobilized antibodies binding C1q), and the very high levels of NSB observed using mC1q and to a lesser extent hC1q to the CM5 surface (charge mediated estimated C1q: pI 8-9). Single cycle kinetics (SCK) were used initially to derive estimated kinetic constants due to uncertainty over the stability of the C1q complex and the regeneration conditions. Full kinetic analysis was performed after SCK. The ligand stability control injections performed during the kinetic run indicated either the regeneration conditions used were inactivating the ligand, or the ligand itself was unstable at 25°C during the 48 hours required for kinetic analysis. The lower affinity displayed by mClq for Fab allowed kinetic analysis to be performed without surface regeneration.

[0308] Due to stability issues both Fab and IgG were used as ligands for kinetic analysis, with the amount of ligand minimized to avoid potential avidity.

[0309] Kinetic data was obtained at a flow rate of 40 µL/min to minimize any potential mass transfer effects. Two repeats of the blank (no antigen) and a single concentration of the analyte were programmed into the kinetic run in order to check the stability of both the surface and analyte over the kinetic cycles. For the initial kinetic runs 3.33-fold dilutions of analyte were run. For kinetic analysis and on subsequent runs a 2-fold dilution range was selected.

[0310] The association phase was monitored for 500 seconds to allow some of the higher concentrations of analyte to reach steady state. In order to observe a sufficient signal decrease (≥10 %) during the dissociation phase of the kinetic cycle, dissociation was measured for up to 10,800 seconds to allow sufficient dissociation to occur for accurate assessment of the kinetics. The signal from the reference channel F_c1 was subtracted from that of F_c2, F_c3, and F_c4.

[0311] The kinetic parameters for the interaction of mC1q and the Fab fragment of VH3/Vκ3 in various assay

formats are shown in Table 5. The K_D value for mClq when used as an analyte was 123 nM, and the K_D value for mClq when used as a ligand was 677 nM. The difference in the KD values reported could be due to the mode of interaction or the effect of chemically coupling a multi-subunit mClq to a surface, resulting in changes to the secondary or tertiary structure of the protein.

TABLE 5: Kinetic analysis with mouse C1q

Ligand	Analyte	k_a (1/Ms)	SE (k_a)	k_d (1/S)	SE (k_d)	R_{max} (RU)	SE (R_{max})	Chi^2 (RU^2)	K_D (nM)
mC1q	Fab VH3/Vκ3	6.23×10^3	1.4×10^2	4.6×10^{-3}	1.7×10^{-5}	37.0	0.6	0.8	747.9
		6.9×10^3	2.4×10^2	4.1×10^{-3}	1.0×10^{-4}	33.4	0.5	0.9	606.6
	Mean	**6.5×10^3**		**4.3×10^{-3}**		**35.2**			**677.3**
	Std	**5.0×10^2**		**3.1×10^{-4}**		**2.6**			**99.9**
Fab VH3/Vκ3	mC1q	4.5×10^5	2.6×10^4	5.6×10^{-2}	3.4×10^{-3}	88.6	0.6	1.9	125.6
		1.7×10^5	1.8×10^3	9.1×10^{-3}	5.4×10^{-5}	50.0	0.3	5.6	121.2
	Mean	**3.1×10^5**		**3.2×10^{-2}**		**69.3**			**123.4**
	Std	**2.0×10^5**		**3.3×10^{-2}**		**27.3**			**3.1**

[0312] Table 5 depicts the kinetic parameters for the 1-to-1 interactions of mClq and Fab as determined using the Biacore T200. The Chi^2 values show how well the association and dissociation data fit the proposed binding model. The lower the value the better the fit. The associated SE values for the rate constants represent the uncertainty associated with fitting the data to the model described, and do not represent the total uncertainty for the true kinetic values. The mean response data represents the average kinetic values and the associated SD from 2 independent analyses.

[0313] The kinetic parameters for both the IgG and Fab interactions with hC1q were in the low picomolar range (Table 6 for 1-to-1 model and Table 7 for the heterogeneous model). In order to avoid avidity, hClq was initially used as the ligand, however this limited the analysis to single cycle kinetics and the use of more complex models, for example, heterogeneous ligand binding models **(Equation 3),** that may represent the different forms of hC1q structure that were associated with chemical coupling of a multi-subunit protein to a surface. When hClq was used as the analyte both IgG and Fab were immobilized at the lowest concentrations possible to avoid avidity. The data was analyzed using a 1-to-1 model and a more complex bivalent analyte model **(Equation 4).** Complex model fitting did not significantly improve the fitting metrics and a linked reaction control did not show a time-dependent dissociation phase. The results indicate that, at the lower ligand density, the binding was predominately not due to avidity. Using hClq as the analyte, the KD value for the full-length VH3/Vκ3 IgG was 5.8 pM and the KD value for the VH3/Vκ3 Fab was 8.6. It should be noted that the dissociation rate was too slow to measure accurately with this technique. Longer dissociation times were limited by the stability of the system (BSA blocking layer) and the low levels of binding used to avoid avidity. These results correlate well with results obtained using hClq as a ligand.

TABLE 6: Kinetic analysis with human C1q

Ligand	Analyte	k_a (1/Ms)	SE (k_a)	k_d (1/S)	SE (k_d)	R_{max} (RU)	SE (R_{max})	Chi^2 (RU^2)	K_D (pM)
hC1q	Fab VH3/Vκ3	5.2×10^4	82	4.6×10^{-8}	1.3×10^{-7}	154.9	0.033	14.8	0.87
	IgG VH3/Vκ3	3.9×10^4	96	3.1×10^{-7}	5.3×10^{-8}	387.9	0.11	206	7.9

Ligand	Analyte	k_a (1/Ms)	SE (k_a)	k_d (1/S)	SE (k_d)	R_{max} (RU)	SE (R_{max})	Chi^2 (RU^2)	K_D (pM)
Fab VH3/Vκ3	hC1q	1.1×10^{6}	190	9.1×10^{-6}	1.4×10^{-8}	13.5	0.0015	0.0773	**8.6**
IgG VH3/Vκ3	hC1q	9.6×10^{5}	360	6.4×10^{-6}	2.9×10^{-8}	6.5	1.5×10^{-3}	0.076	6.7
		1.1×10^{6}	190	5.1×10^{-6}	1.4×10^{-8}	17.4	0.002	0.143	4.9
	Mean	**1.0×10^{6}**		**5.8×10^{-6}**					**5.8**
	Std	**6.2×10^{4}**		**9.1×10^{-7}**					**1.3**

[0314] Table 6 depicts the kinetic parameters for the 1-to-1 interactions of hClq with Fab, or IgG as determined using the Biacore T200. Data highlighted in red indicate poor fitting criteria, these data sets have therefore been analyzed using a more complex model (Table 7).

TABLE 7: Kinetic analysis for heterogeneous ligand interaction

Ligand	Analyte	k_a1 (1/Ms)	k_d1 (1/S)	k_a2 (1/Ms)	k_d2 (1/S)	$R_{max}1$(RU)	$R_{max}2$ (RU)	Chi^2 (RU^2)	K_D1 (nM)	K_D2 (nM)
hC1q	Fab VH3/Vκ3	1.3×10^{5}	2.3×10^{-5}	1.8×10^{4}	6.3×10^{-8}	80.3	78.7	0.1	**175.0**	**3.4**
	IgG VH3/Vκ3	2.0×10^{5}	4.6×10^{-6}	1.6×10^{4}	1.1×10^{-7}	152.6	247.1	2.41	**22.7**	**7.3**

[0315] Table 7 depicts the kinetic parameters for the heterogeneous ligand interactions of hClq and Fab, or IgG as determined using the Biacore T200. Data fit to a heterogeneous ligand model to represent the expected heterogeneity of immobilizing a multi-subunit protein on a surface.

Conclusion

[0316] The interaction of hC1q and mC1q with full-length IgG and the Fab fragment of VH3/Vκ3 5was analyzed using both species as ligands. Issues with the stability and chemical coupling of C1q complexes to the CMS dextran surface required the development of an IgG and Fab surface; the results indicate that the binding mode observed with this surface was mainly 1-to-1, *i.e.,* the kinetic profile did not show signs of avidity. Both the full-length IgG and Fab fragment of VH3/Vκ3 displayed tight binding in the low picomolar range for hC1q (5.8 and 8.6 pM, respectively), whereas a lower affinity was observed for mC1q binding to the Fab fragment of VH3/Vκ3 (123 nM).

Example 3: Humanized Anti-C1q Antibodies Inhibit Complement-Mediated Hemolysis

[0317] Humanized anti-Clq antibodies were tested in human and rodent hemolytic assays (CH50) for their ability to neutralize C1q and block its activation of the downstream complement cascade.

[0318] The humanized anti-Clq antibodies utilized in this example were produced as described in Example 1. The following humanized antibodies from Example 1 were utilized: the antibody VH1/Vκ1 (2B12), the antibody VH3/Vκ3 (5H7), the antibody VH3/Vκ4 (3F1), and the antibody VH4/Vκ3 (1D3). The mouse monoclonal antibody M1 (ANN-005) and the chimeric Mlantibody (3E2) were also utilized as controls.

[0319] CH50 assays were conducted essentially as described in Current Protocols in Immunology (1994) Supplement 9 Unit 13.1. In brief, 5 microliters (µl) of human serum (Cedarlane, Burlington, NC) or 0.625 µl of Wistar rat serum was diluted to 50 µl of GVB buffer (Cedarlane, Burlington, NC) and added to 50 µl of the humanized antibodies (1 µg) diluted in GVB buffer. The antibody: serum mixture was pre-incubated for 30 minutes on ice and then added to 100 µl of EA cells (2×10^8/ml) for both rat and human assays. The EA cells were generated exactly as specified in Current Protocols using Sheeps blood in Alsever's (Cedarlane Cat #CL2581) and hemolysin (Cedalane Cat #CL9000). The EA cells, serum and antibody mixture was incubated for 30 minutes at 37°C. and then placed on ice. Next 1.2 ml of 0.15 M NaCl was added to the mixture and the OD_{412} of the sample was read in a spectrophotometer to determine the amount of cell lysis. The percent inhibition of the test antibodies was determined relative to a control mouse IgG1 antibody (Abeam ab18447).

[0320] Four C1q-binding antibodies (2B12, 5H7, 3F1, and 1D3) were tested for their C1q neutralizing activity in the human CH50 hemolysis assay in a dose-response format (**FIG. 7A**). Each of the antibodies was tested at doses of 3.9 ng, 15.9 ng, 62.5 ng, and 260 ng, which correspond to an effective dosing range that results in the anti-Clq antibody binding to C1q with a stoichiometry that ranges from approximately 10:1 to approximately 1:1. The murine anti-C1q antibody M1 (ANN-005) and the chimeric M1 antibody (3E2) were used as references. The VH3/Vκ3 antibody (5H7) inhibited CH50 hemolysis in a dose-dependent manner to degree that was comparable to both the murine M1 antibody and the chimeric M1 antibody (**FIG. 7A**). Moreover, approximately 60 ng of the VH3/Vκ3 antibody (5H7), the VH4/Vκ3 antibody (1D3), and the VH1/Vκ1 antibody (2B12) was required to inhibit 50% of the hemolysis observed (**FIG. 7A**). Approximately 250 ng of the antibody VH3/Vκ4 (3F1) was required to inhibit approximately 95% of the hemolysis observed (**FIG. 7A**).

[0321] Four C1q-binding antibodies (2B12, 5H7, 3F1, and 1D3) were also tested for their C1q neutralizing activity in the rat CH50 assays (**FIG. 7B**). Each of the antibodies was tested at doses of 3.9 ng, 15.9 ng, 62.5 ng, and 260 ng, which correspond to an effective dosing range that results in the anti-Clq antibody binding to C1q with a stoichiometry that ranges from approximately 10:1 to approximately 1:1. Testing was conducted in dose-response formats. The murine anti-C1q antibody M1 (ANN-005) and the chimeric M1 antibody (3E2) were used as references. The VH1/Vκ1 antibody (2B12) inhibited CH50 hemolysis in a dose-dependent manner to degree that was comparable to both the murine M1 antibody and the chimeric M1 antibody (**FIG. 7B**). Moreover, approximately 60 ng of the VH1/Vκ1 antibody (2B12), the antibody VH3/Vκ4 (3F1), the VH3/Vκ3 antibody (5H7), and the VH4/Vκ3 antibody (1D3) was required to inhibit approximately 50% to approximately 80% of the hemolysis observed (**FIG. 7B**).

Example 4: Intravenous dosing study in monkey to evaluate pharmacokinetics of humanized anti-Clq antibody, pharmacodynamic effects on serum C1q levels and ex-vivo complement mediated hemolysis

[0322] Cynomolgus monkeys were dosed with humanized anti-Clq antibody VH3/Vκ3 (5H7) via single intravenous bolus injection (I.V.) at 15 and 100 mg/Kg dose (N=2 per dose, 1 male and 1 female monkey per dose).

[0323] Blood samples were collected at the following time points - Day 1: pre-study, 0.5, 2, 4, 8, 12, 24, 72, 96 and 120 hours post dose and on Days: 7, 9, 12, 15, 18 and 21. Blood samples were allowed to clot, serum was separated by centrifugation and then stored frozen at -80C until analysis.

[0324] Determination of serum levels of VH3/Vκ3 (5H7) from monkey samples: Serum anti-C1q antibody

levels were measured using a direct ELISA with hC1q used as the capture analyte, followed by detection of human 5H7 antibody. Black 96 well ELISA plates (Corning, Cat# 3925) were coated with human C1q (Complement Technology A099) at 2 µg/mL. After overnight incubation at 4C, plates were washed thrice with Dulbecco's phosphate buffered saline (DPBS) (Thermo Scientific 28372) and blocked overnight at 4°C with DPBS containing 3% BSA. Next day, blocking solution was removed and 5H7 standards or individual serum samples at a range of dilutions (2000 to 2000000-fold) were added to the plates at 50 µL per sample in assay buffer, DPBS containing 0.3% BSA and 0.1% tween (KPL Inc. 51-12-10). Samples were incubated at room temperature, shaking at 300 rpm for 1 hr. Then, 50 µL of goat anti-human FC antibody conjugated with alkaline phosphatase (Jackson Immuno research, 109-055-098) was added at a concentration of 0.5 µg/mL in assay buffer. After incubation for 1 hr at room temperature, plates were washed three times in DPBS containing 0.05% tween. Each wash was for a duration of 10 minutes with shaking at 300 rpm on a plate shaker. Plates were then tapped dry and developed using alkaline phosphatase substrate incubation for 20 minutes (Life Technologies, #T2214). Luminescence counts were read on a Perkin Elmer envision reader. Standard curves were fit using 4PL logistic fit and unknown signal counts were converted µg/mL concentration and plotted using Graphpad Prism.

[0325] Determination of serum C1q levels from monkey samples: Serum levels of C1q were determined using two distinct hC1q specific ELISA assays. In both ELISA assays, JL1, an antibody that binds to the collagen tail of C1q was used as the capture antibody (Abeam ab71940). In the first assay, the murine version of VH3/Vκ3 (5H7) or M1, which binds to the same site as 5H7, was used as detection antibody to isolate Free C1q levels. In the second assay, JL1 was used as the detection antibody to measure C1q which is both free and bound to ANX in the serum samples.

[0326] Black 96 well ELISA plates (Corning, Cat# 3925) were coated with JL1 at 1 µg/mL. After overnight incubation at 4C, plates were washed thrice with Dulbecco's phosphate buffered saline (DPBS) (Thermo Scientific 28372) and blocked overnight with DPBS containing 3% BSA at 4°C. Next day, blocking solution was removed and C1q standards or individual serum samples were run at dilutions in the range of 1000x to 10000x, in assay buffer DPBS with 0.3% BSA and 0.1% tween, at 50 µL per sample. Following incubation at room temperature for 1 hr, 50 uL of respective alkaline phosphatase conjugated antibodies M1 or JL1 were added, at a final concentration of 200-400 ng/mL in assay buffer. Samples were incubated overnight with shaking at 4C. Next day, plates were washed three times in DPBS containing 0.05% tween. Each wash was for a duration of 10 minutes with shaking at 300 rpm on a plate shaker. Plates were then tapped dry and developed using alkaline phosphatase substrate incubation for 20 minutes. Luminescence counts were read on a Perkin Elmer envision reader. Standard curves were fit using 4PL logistic fit and unknown signal counts were converted to concentration, dilution correction and then plotted using Graphpad Prism.

[0327] Determination of ex-vivo hemolysis activity in monkey serum samples: The hemolysis assays were similar to that in example 3 with the following modification. Monkey serum samples from the study were diluted 1:50 in GVB ++ buffer solution (Complement Technology Cat# B100) and mixed with an equal volume of antibody sensitized sheep red blood cells at 17 million cells/mL (Complement Technology Cat# B201). Samples were incubated for 1 hr at 37C. Control wells were set up to determine baseline (buffer only without any serum) and 100% hemolysis (using deionized water). Samples were then spun down and supernatants were transferred to clear ELISA plates and absorbance read at 415 nm. The absorbance for all samples were baseline subtracted and normalized to 100% hemolysis (deionized water). At the 1:50 dilution, serum samples showed 50-70% of hemolysis observed with water. % Hemolysis was plotted for each individual monkey following baseline normalization.

[0328] A dose dependent increase in serum 5H7 levels was observed following IV dosing with a maximal exposure of ~250 ug/mL at the 15 mg/Kg dose and ~ 2000 ug/mL at the 100 mg/Kg dose. Sustained serum levels of 5H7 were evident over the 20 days of sampling at the 100 mg/Kg dose, while serum 5H7 levels

declined to levels below limit of detection after 4 days at the 15 mg/Kg dose (**FIG. 8**). Serum C1q levels (JL1-M1 assay) were reduced > 90% over 5 days at the 15 mg/Kg dose and recovered back to baseline between 5-11 days after onset of dosing (**FIG. 9A**). In contrast, the 100 mg/Kg dose led to a sustained reduction of serum C1q levels up to 20 days after onset of dosing (**FIG. 9A**). A similar pattern of reduction and time course of serum C1q was observed with the JL1-JL1 assay (**FIG. 9B**). The observation of robust and sustained reduction in serum C1q in 2 independent ELISA assays, one with a detection antibody that binds to the same site on C1q as 5H7 and the other with detection antibody against an independent site on C1q, suggests that serum C1q levels are cleared following treatment with 5H7. Consistent with the reduction in serum C1q levels, a sustained reduction of ex-vivo hemolysis was observed at the 100 mg/Kg dose up to 20 days after onset of dosing (**FIG. 10**). At the 15 mg/Kg dose of ANX, hemolysis was reduced > 90% over 5 days and recovered back to baseline between 5-11 days after onset of dosing (**FIG. 10**).
These results demonstrate that the anti-C1q antibody VH3/Vκ3 (5H7) shows a robust pharmacokinetic exposure and time course along with sustained reduction of serum C1q levels and hemolysis in cynomolgus monkeys.

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Patentkrav

1. Humaniseret anti-C1q-antistof eller et antigenbindende fragment deraf, hvori antistoffet eller det antigenbindende fragment deraf omfatter:
    a) et variabelt tungkædedomæne, der omfatter en aminosyresekvens ifølge SEQ ID NO: 1 og et variabelt letkædedomæne, der omfatter en aminosyresekvens af ifølge SEQ ID NO: 5;
    b) et variabelt tungkædedomæne, der omfatter en aminosyresekvens ifølge SEQ ID NO: 3 og et variabelt letkædedomæne, der omfatter en aminosyresekvens ifølge SEQ ID NO: 8;
    c) et variabelt tungkædedomæne, der omfatter en aminosyresekvens ifølge SEQ ID NO: 3 og et variabelt letkædedomæne, der omfatter en aminosyresekvens ifølge SEQ ID NO: 7; eller
    d) et variabelt tungkædedomæne, der omfatter en aminosyresekvens ifølge SEQ ID NO: 4 og et variabelt letkædedomæne, der omfatter en aminosyresekvens ifølge SEQ ID NO: 7.
2. Antistof eller antigenbindende fragment ifølge krav 1, hvori antistoffet eller det antigenbindende fragment omfatter en human IgG4 konstant tungkæderegion.
3. Antistof eller antigenbindende fragment ifølge krav 2, hvori den humane IgG4 konstante tungkæderegion omfatter aminosyresekvensen ifølge SEQ ID NO: 37.
4. Antistof eller antigenbindende fragment ifølge krav 2, hvor den humane IgG4 konstante tungkæderegion omfatter en Fc-region, og hvori Fc-regionen omfatter en aminosyresubstitution i position 248 og/eller position 241 ifølge Kabat-nummereringskonventionen.
5. Antistof eller antigenbindende fragment ifølge krav 4, hvori aminosyresubstitutionen i position 248 er en leucin til glutamat aminosyresubstitution.
6. Antistof eller antigenbindende fragment ifølge krav 4, hvori aminosyresubstitutionen i position 241 er en serin til prolin aminosyresubstitution.
7. Antistof eller antigenbindende fragment ifølge et hvilket som helst af kravene 1-6, hvori det antigenbindende fragment er et Fab-, $F(ab')_2$- eller Fab'-fragment.
8. Isoleret polynukleotid, der omfatter en nukleinsyresekvens, der koder for antistoffet eller det antigenbindende fragment ifølge et hvilket som helst af kravene 1-7.
9. Isoleret værtscelle, der omfatter nukleinsyresekvensen ifølge krav 8.

10. Farmaceutisk sammensætning, der omfatter antistoffet eller det antigenbindende fragment ifølge et hvilket som helst af kravene 1-7 og en farmaceutisk acceptabel bærer.
11. Antistof eller antigenbindende fragment ifølge et af kravene 1 til 7 til anvendelse i medicin.
12. Antistof eller antigenbindende fragment til anvendelse ifølge krav 11, hvori anvendelsen i medicin er til behandling eller forebyggelse af en sygdom forbundet med komplementaktivering, hvori sygdommen er en neurodegenerativ lidelse, inflammatorisk sygdom, autoimmun sygdom eller metabolisk lidelse.
13. Antistof eller antigenbindende fragment til anvendelse ifølge krav 12, hvori sygdommen er en neurodegenerativ lidelse udvalgt fra Alzheimers sygdom, amyotrofisk lateral sklerose, multipel sklerose, glaukom, myotonisk dystrofi, Guillain-Barré syndrom (GBS), myasthenia gravis, bulløst pemphigoid, spinal muskelatrofi, Downs syndrom, Parkinsons sygdom og Huntingtons sygdom.
14. Antistof eller antigenbindende fragment til anvendelse ifølge krav 12, hvori sygdommen er en inflammatorisk sygdom, autoimmun sygdom eller metabolisk lidelse udvalgt fra diabetes, vitiligo, Hashimotos thyroiditis, Addisons sygdom, cøliaki, Crohns sygdom, perniciøs anæmi, myasthenia gravis, fedme, autoimmune hæmolytiske anæmier, paraneoplastiske syndromer, hypokomplementæmisk urticarial vaskulitis (HUV), polymyalgi reumatisk, Wegeners granulomatose, reumatoid artritis (RA), akut respiratorisk distress syndrom (ARDS), skader på fjerne væve efter iskæmi og reperfusion, komplementaktivering under kardiopulmonal bypass-operation, dermatomyositis, pemphigus, lupus nefritis og resulterende glomerulonefritis og vaskulitis, kardiopulmonal bypass, kardioplegi-induceret koronar endotelial dysfunktion, type II membranoproliferativ glomerulonefritis, IgA nefropati, akut nyresvigt, kryoglobulinæmi, antifosfolipidsyndrom, kronisk åbenvinklet glaukom, akut snævervinklet glaukom, makulære degenerative sygdomme, aldersrelateret makuladegeneration (AMD), (AMD-våd), geografisk atrofi, choroidal neovaskularisering (CNV), uveitis, diabetisk retinopati, iskæmirelateret retinopati, endophthalmitis, intraokulær neovaskulær sygdom, diabetisk makulaødem, patologisk myopi, von Hippel-Lindaus sygdom, histoplasmose i øjet, neuromyelitis optica (NMO), okklusion af den centrale retinavene (CRVO), neovaskularisering af cornea, neovaskularisering af retina, Lebers hereditære opticus neuropati, optisk neuritis, Behcets retinopati, iskæmisk optisk neuropati, retinavasculitis, ANCA-vasculitis, Purtschers retinopati, Sjøgrens tørre øjne, tør AMD, sarcoidose, temporal arteritis, polyarteritis nodosa, multipel sklerose, allotransplantation, hyperakut

afstødning, hæmodialyse, kronisk obstruktiv lungesygdom (KOL), astma og aspirationspneumoni.

DRAWINGS

Drawing

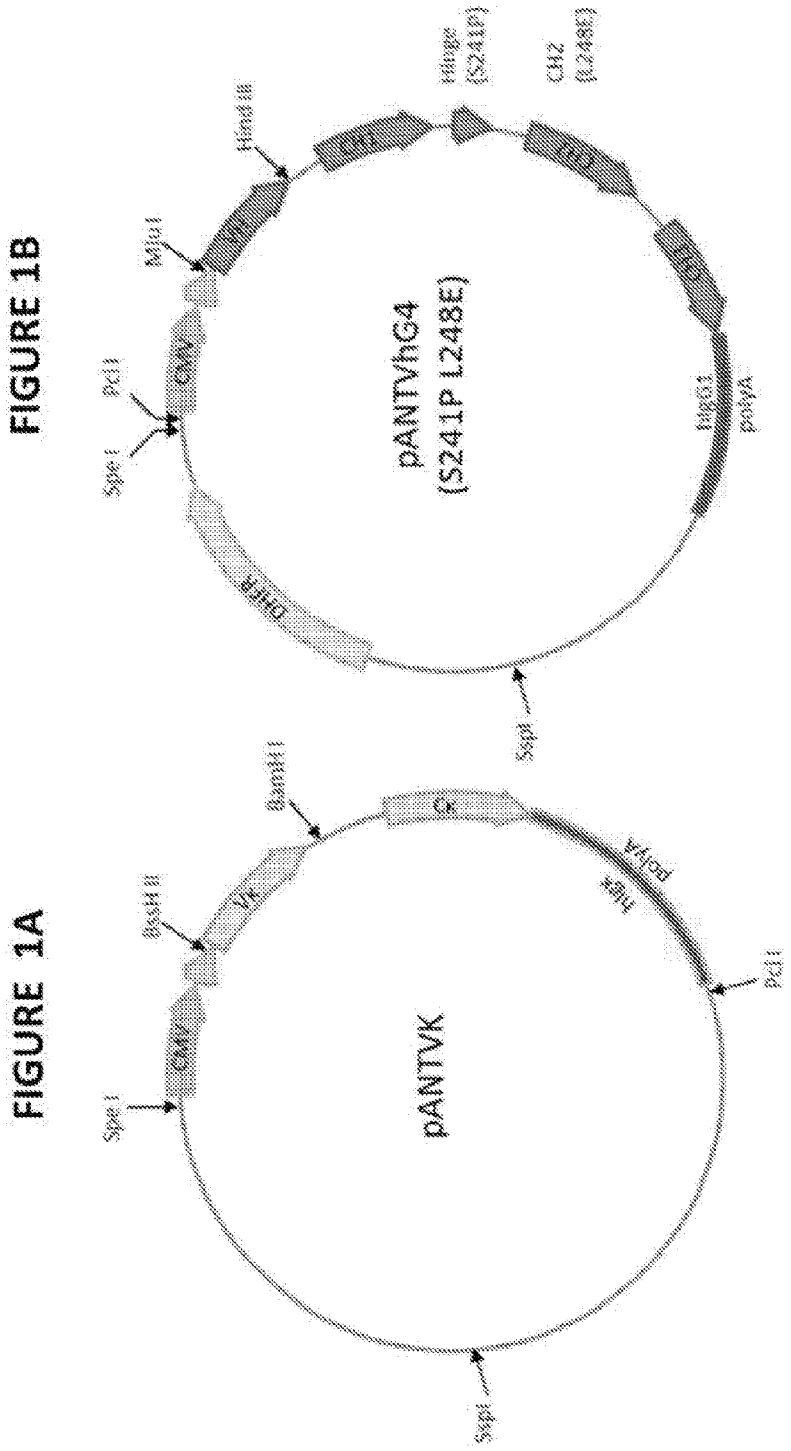
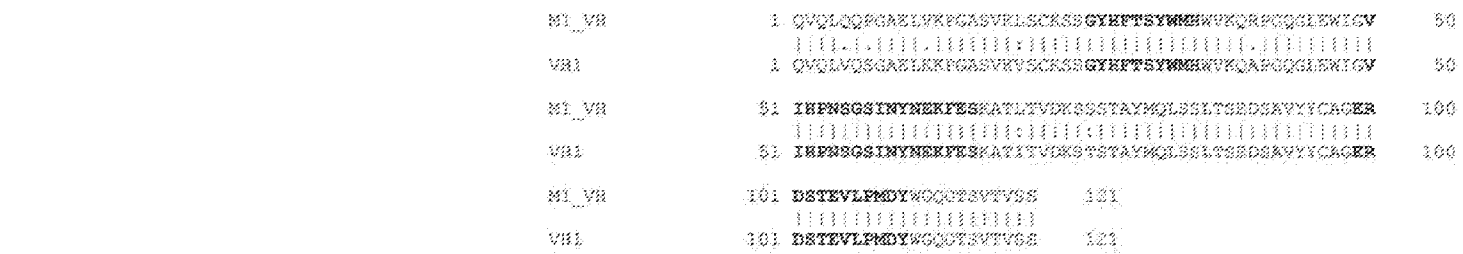


FIGURE 2A

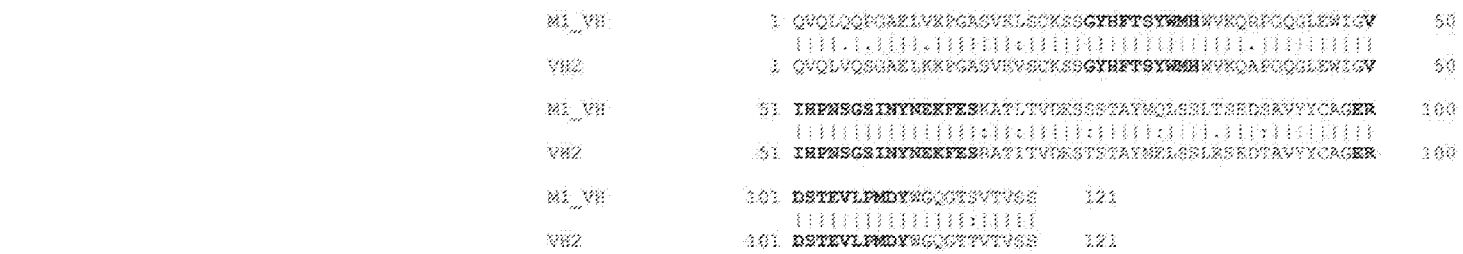
Alignment of amino acid sequence of the heavy chain variable region (VH) of M1 anti-C1q and the amino acid sequences of the humanized VH variants 1-2

VH variant 1:



M1_VH refers to the VH of the M1 antibody, and VH1 refers to VH variant 1. The three CDR sequences are depicted in **bold**.

VH variant 2:



M1_VH refers to the VH of the M1 antibody, and VH2 refers to VH variant 2. The three CDR sequences are depicted in **bold**.

FIGURE 2B

Alignment of amino acid sequence of the heavy chain variable region (VH) of M1 anti-C1q and the amino acid sequences of the humanized VH variants 3-4

VH variant 3:

```
M1_VH          1 QVQLQQPGAELVRPGASVKLSCKSSGYIFTSYWMHWVKQRPGQGLEWIGV   50
                 ||||.|.||||.|||||||:|||||||||||||||||||.|||||||||
VH3            1 QVQLVQSGAELKRPGASVKVSCKSSGYIFTSYWMHWVRQAPGQGLEWIGV   50

M1_VH         51 IHPNSGSINYNEKFESKATLTVDKSSSTAYMQLSSLTSEDSAVYYCAGER  100
                 ||||||||||||||||.|:||||:|||||:||||.|||:|||||||||||
VH3           51 IHPNSGSINYNEKFESRVTITVDKSTSTAYMELSSLRSEDTAVYYCAGER  100

M1_VH        101 DSTEVLPMDYWGQGTSVTVSS     121
                 |||||||||||||||:|||||
VH3          101 DSTEVLPMDYWGQGTTVTVSS     121
```

M1_VH refers to the VH of the M1 antibody, and VH3 refers to VH variant 3. The three CDR sequences are depicted in **bold.**

VH variant 4:

```
M1_VH          1 QVQLQQPGAELVRPGASVKLSCKSSGYIFTSYWMHWVKQRPGQGLEWIGV   50
                 ||||.|.||||.|||||||:|||||||||||||||||||.|||||||||
VH4            1 QVQLVQSGAELKRPGASVKVSCKSSGYIFTSYWMHWVRQAPGQGLEWIGV   50

M1_VH         51 IHPNSGSINYNEKFESKATLTVDKSSSTAYMQLSSLTSEDSAVYYCAGER  100
                 ||||||||||||||||.|:||||:|||||:||||.|||:|||||||||||
VH4           51 IHPNSGSINYNEKFESRVTITVDKSESTAYMELSSLRSEDTAVYYCAGER  100

M1_VH        101 DSTEVLPMDYWGQGTSVTVSS     121
                 |||||||||||||||:|||||
VH4          101 DSTEVLPMDYWGQGTTVTVSS     121
```

M1_VH refers to the VH of the M1 antibody, and VH4 refers to VH variant 4. The three CDR sequences are depicted in **bold.**

FIGURE 2C

Alignment of amino acid sequence of the kappa light chain variable region (Vκ) of M1 anti-C1q and the amino acid sequences of the humanized Vκ variants 1-2

Vκ variant 1:

```
M1_VK      1 DVQITQSPSYLAASPGETITINCRASKSINKYLAWYQEKPGKTNKLLIYS  50
             ||||||||||||||.||..||||||||||||||||:|||||||||||||
VK1        1 DVQITQSPSYLAASLGERATINCRASKSINKYLAWYQQKPGKTNKLLIYS  50

M1_VK     51 GSTLQSGIPSRFSGSGSGTDFTLTISSLEPEDFAMYYCQQHNEYPLTFGA 100
             ||||||||||:||||||||||||||||||||||||||||||||||||||||.
VK1       51 GSTLQSGIPARFSGSGSGTDFTLTISSLEPEDFAMYYCQQHNEYPLTFGQ 100

M1_VK    101 GTKLELK    107
             |||||||
VK1      101 GTKLEIK    107
```

M1_VK refers to the Vκ of the M1 antibody, and VK1 refers to Vκ variant 1. The three CDR sequences are depicted in **bold.**

Vκ variant 2

```
M1_VK      1 DVQITQSPSYLAASPGETITINCRASKSINKYLAWYQEKPGKTNKLLIYS  50
             ||||||||||.|:||.||..||||||||||||||||:||||.|||||||
VK2        1 DVQITQSPSSLSASVGDRVTINCRASKSINKYLAWYQQKPGKANKLLIYS  50

M1_VK     51 GSTLQSGIPSRFSGSGSGTDFTLTISSLEPEDFAMYYCQQHNEYPLTFGA 100
             ||||||||||:||||||||||||||||||||||||||||||||||||||||.
VK2       51 GSTLQSGIPARFSGSGSGTDFTLTISSLEPEDFAMYYCQQHNEYPLTFGQ 100

M1_VK    101 GTKLELK    107
             |||||||
VK2      101 GTKLEIK    107
```

M1_VK refers to the Vκ of the M1 antibody, and VK2 refers to Vκ variant 2. The three CDR sequences are depicted in **bold.**

FIGURE 2D

Alignment of amino acid sequence of the kappa light chain variable region (Vκ) of M1 anti-C1q and the amino acid sequences of the humanized Vκ variants 3-4

Vκ variant 3:

```
M1_VK      1 DVQITQSPSYLAASPGETITINCRASKSINKYLAWYQEKPGKTNKLLIYS  50
             :::::::::.:::.::..::::::::::::::::::::::::::..::::
VK3        1 DVQITQSPSSLSASLGERATINCRASKSINKYLAWYQQKPGKAPKLLIYS  50

M1_VK     51 GSTLQSGIPSRFSGSGSGTDFTLTISSLEPEDFAMYYCQQHNEYPLTFGA 100
             :::::::::::::::::::::::::::::::::::::::::::::::::.
VK3       51 GSTLQSGVPARFSGSGSGTEFTLTISSLQPEDFAMYYCQQHNEYPLTFGQ 100

M1_VK    101 GTKLELK 107
             :::::::
VK3      101 GTKLEIK 107
```

M1_VK refers to the Vκ of the M1 antibody, and VK3 refers to Vκ variant 3. The three CDR sequences are depicted in **bold.**

Vκ variant 4:

```
M1_VK      1 DVQITQSPSYLAASPGETITINCRASKSINKYLAWYQEKPGKTNKLLIYS  50
             :::::::::.::::.::..::::::::::::::::::::::::::..::::
VK4        1 DIQLTQSPSFLSASLGERATINCRASKSINKYLAWYQQKPGKAPKLLIYS  50

M1_VK     51 GSTLQSGIPSRFSGSGSGTDFTLTISSLEPEDFAMYYCQQHNEYPLTFGA 100
             :::::::::::::::::::::::::::::::::::::::::::::::::.
VK4       51 GSTLQSGVPARFSGSGSGTDFTLTISSLEPEDFAMYYCQQHNEYPLTFGQ 100

M1_VK    101 GTKLELK 107
             :::::::
VK4      101 GTKLEIK 107
```

M1_VK refers to the Vκ of the M1 antibody, and VK4 refers to Vκ variant 4. The three CDR sequences are depicted in **bold.**

FIGURE 3

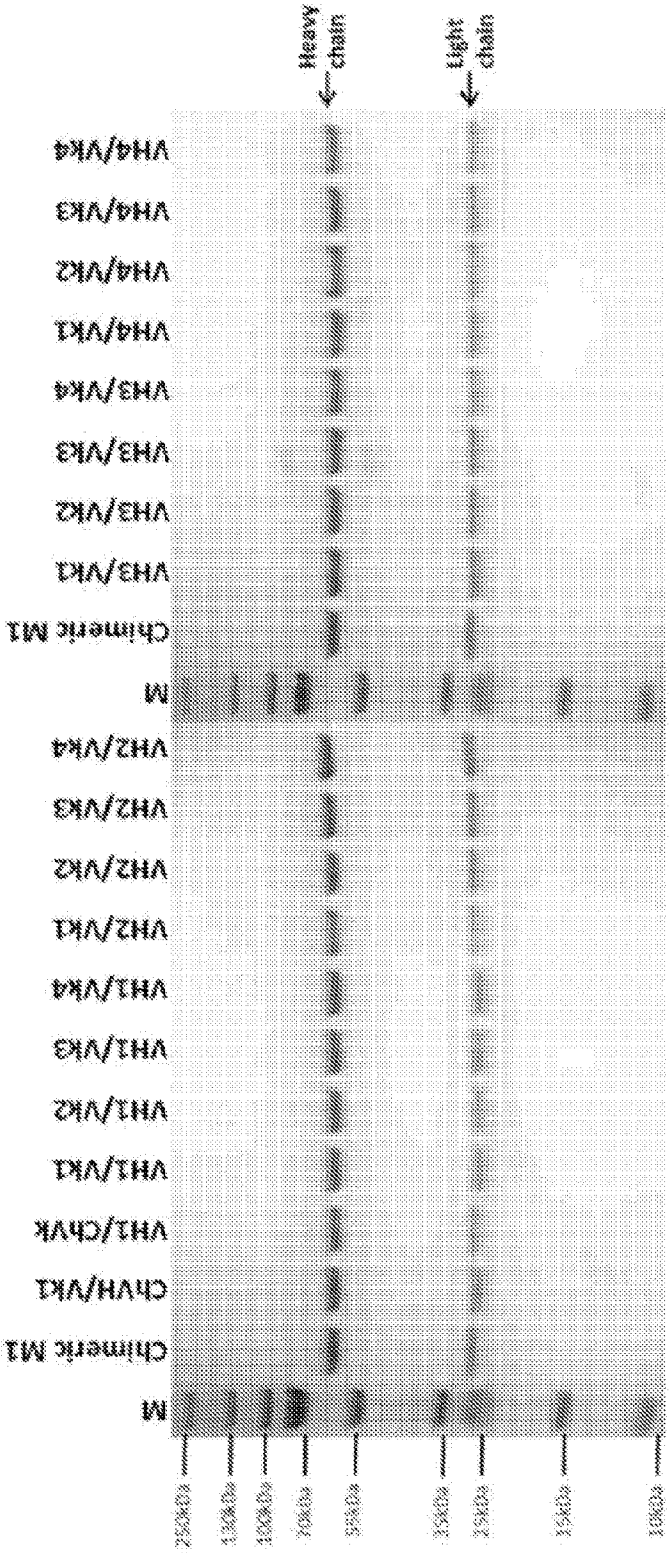

Heavy chain
Light chain
250KDa
130KDa
100KDa
70KDa
55KDa
35KDa
25KDa
15KDa
10KDa
M
Chimeric M1
ChVH/VK1
VH1/ChVK
VH1/VK1
VH1/VK2
VH1/VK3
VH1/VK4
VH2/VK1
VH2/VK2
VH2/VK3
VH2/VK4
M
Chimeric M1
VH3/VK1
VH3/VK2
VH3/VK3
VH3/VK4
VH4/VK1
VH4/VK2
VH4/VK3
VH4/VK4

FIGURE 4A

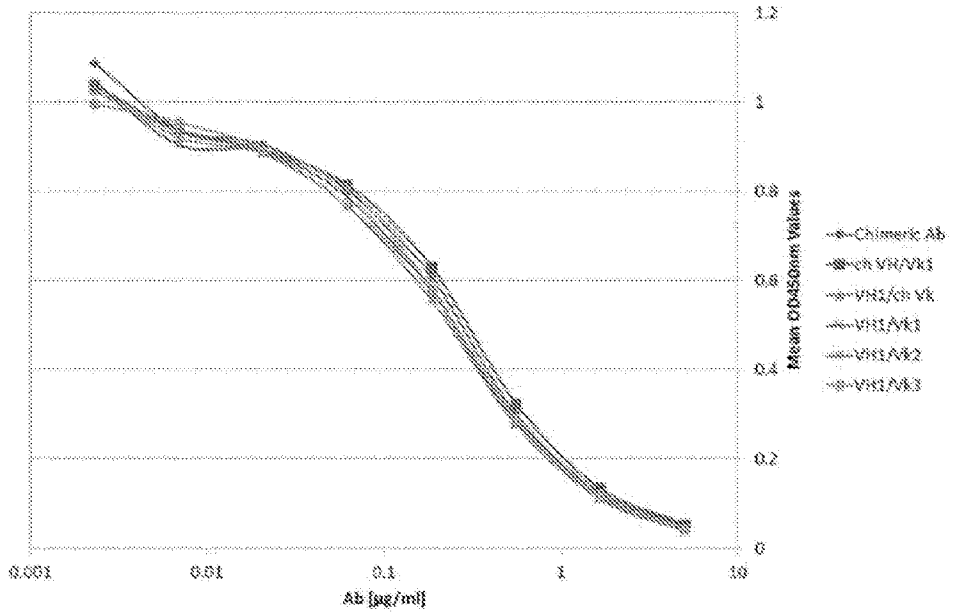


FIGURE 4B

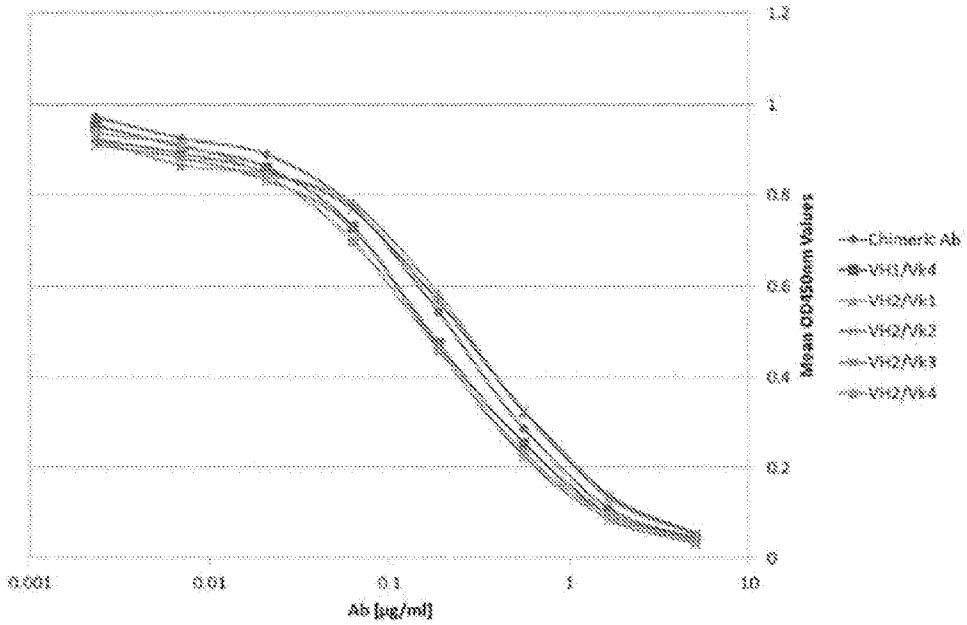


FIGURE 4C

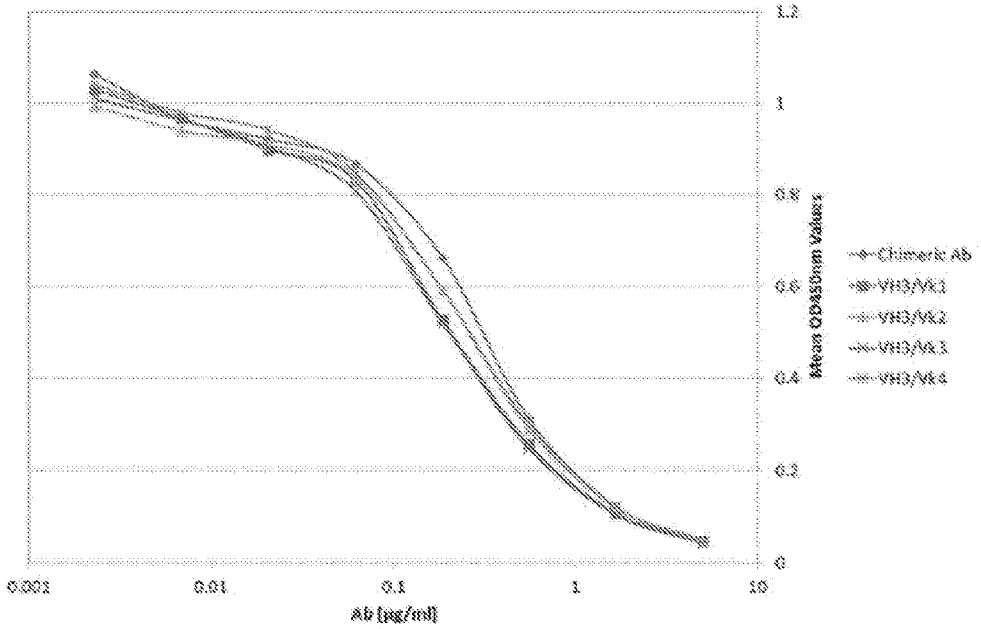


FIGURE 4D

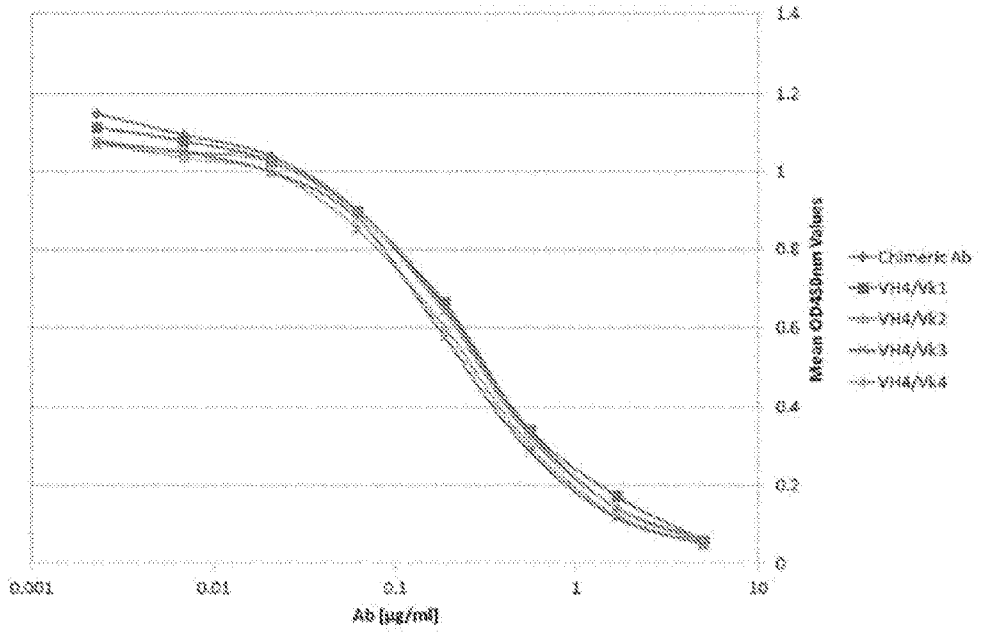


FIGURE 5

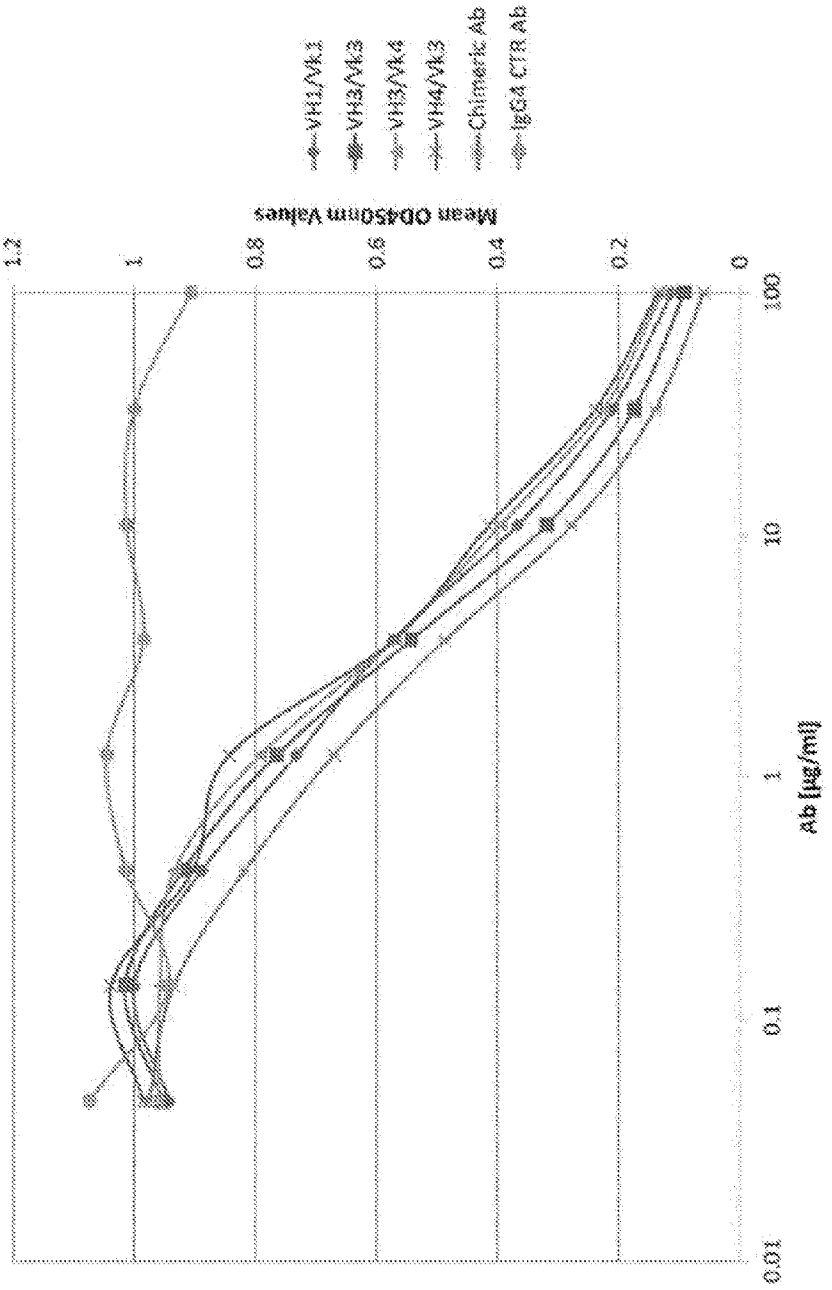

VH1/VL1
VH3/VL3
VH3/VL4
VH4/VL3
Chimeric Ab
IgG4 CTR Ab
Mean OD450nm Values
1.2
1
0.8
0.6
0.4
0.2
0
100
10
1
0.1
0.01
Ab [µg/ml]

FIGURE 6

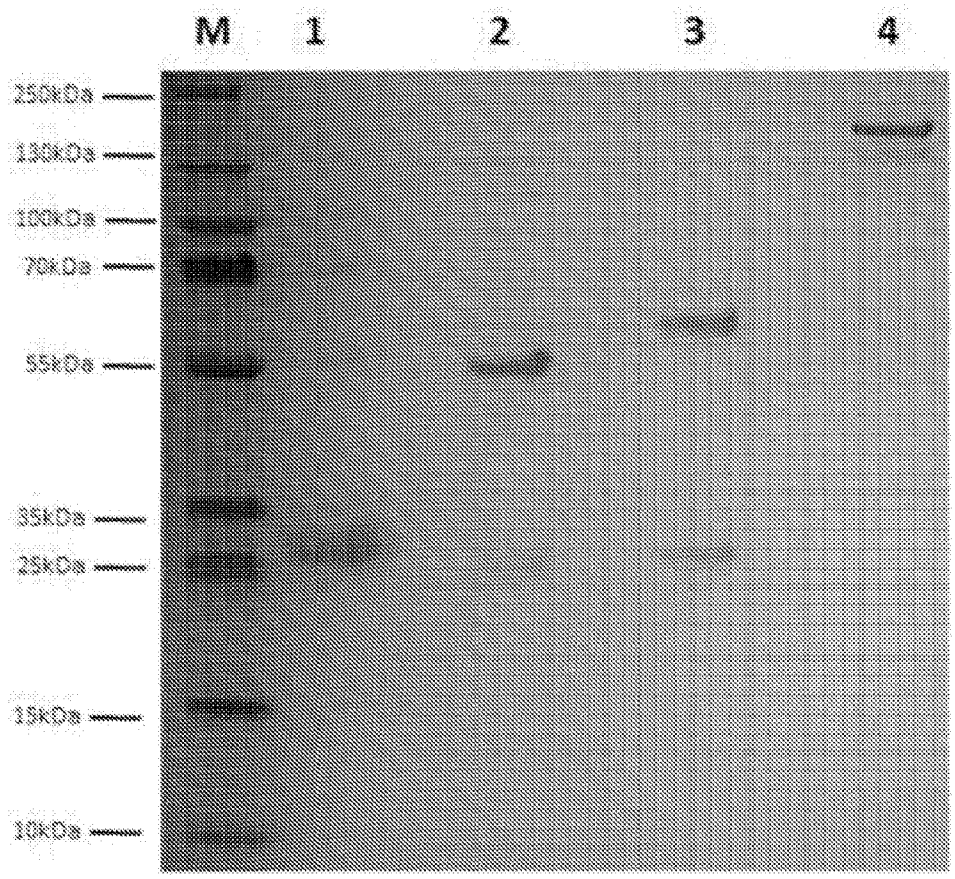

M
1
2
3
4
250kDa
130kDa
100kDa
70kDa
55kDa
35kDa
25kDa
15kDa
10kDa

FIGURE 7A

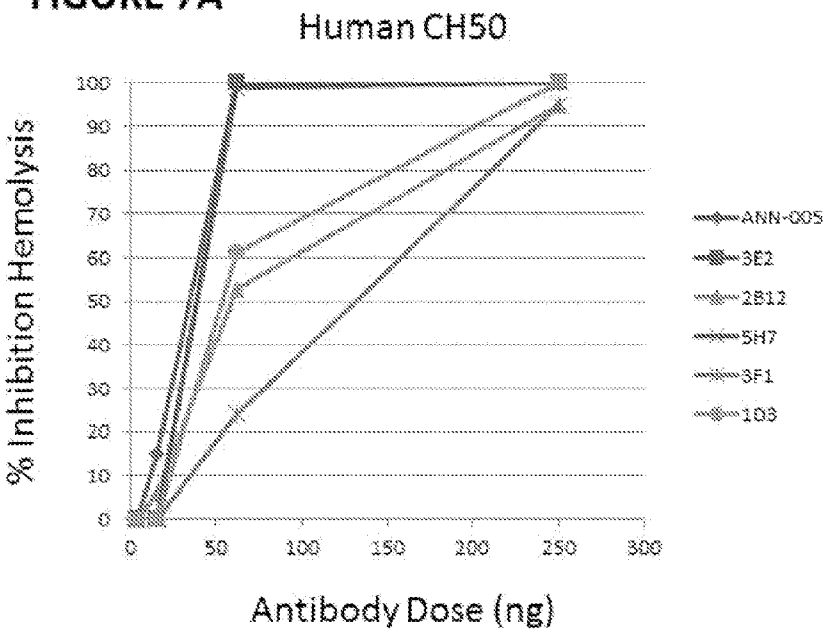


FIGURE 7B

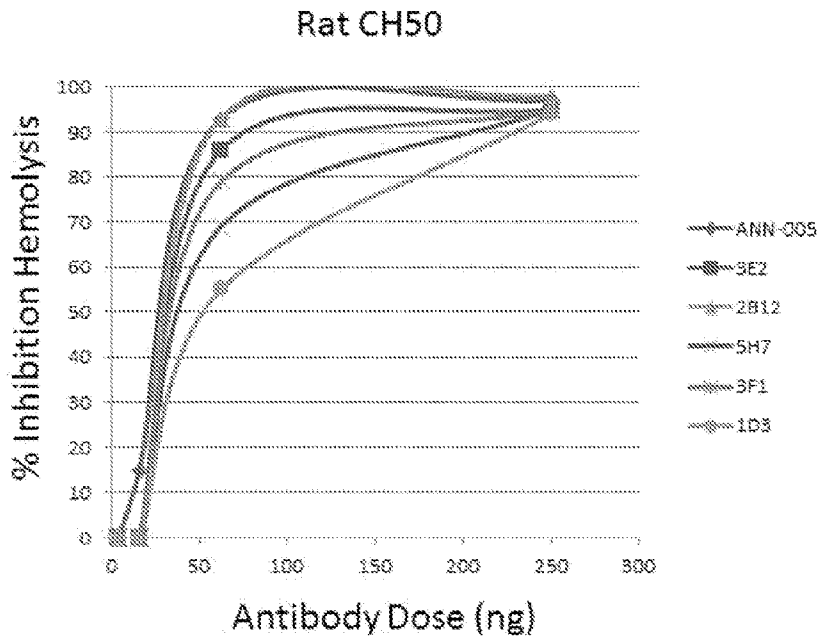


FIGURE 8

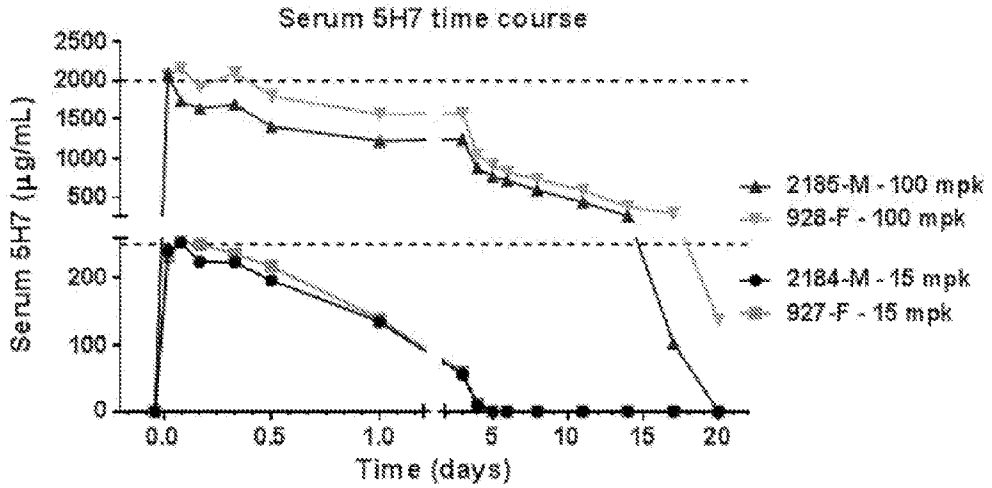

Serum 5H7 time course
Serum 5H7 (µg/mL)
2500
2000
1500
1000
500
200
100
0
0.0
0.5
1.0
5
10
15
20
Time (days)
2185-M - 100 mpk
928-F - 100 mpk
2184-M - 15 mpk
927-F - 15 mpk

FIGURE 9A

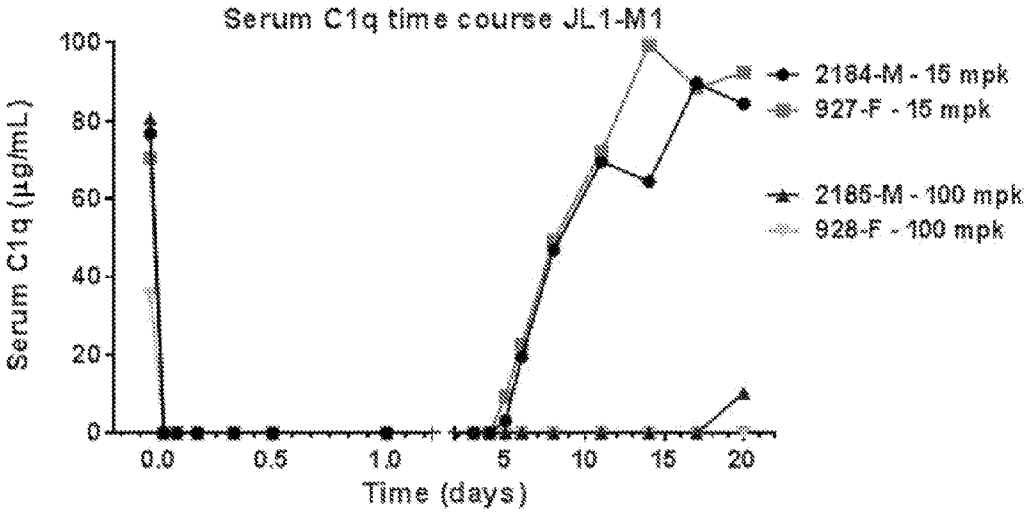


FIGURE 9B

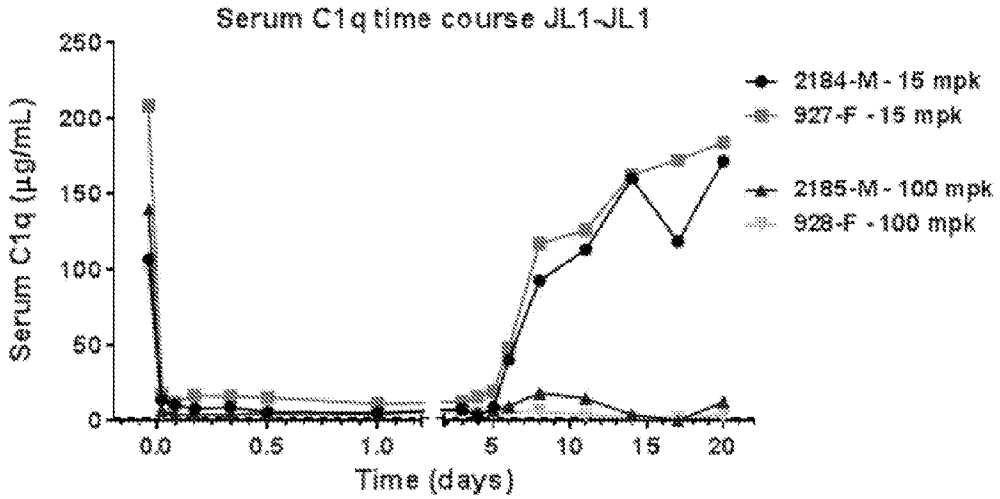
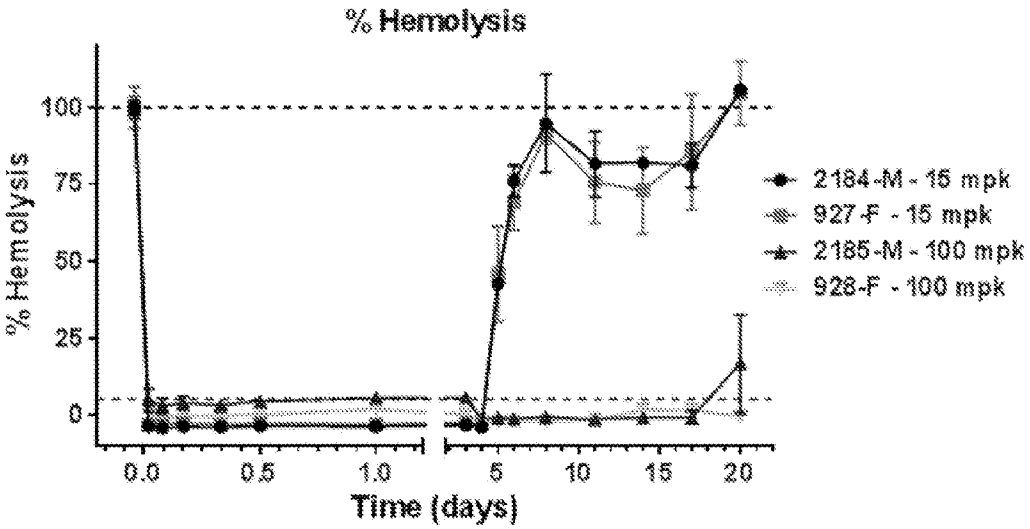


FIGURE 10


% Hemolysis
% Hemolysis
100
75
50
25
0
0.0
0.5
1.0
5
10
15
20
Time (days)
2184-M - 15 mpk
927-F - 15 mpk
2185-M - 100 mpk
928-F - 100 mpk