Humanized Anti-Factor D Antibodies And Uses Thereof

Background of the Invention

[0001] The complement system plays a central role in the clearance of immune complexes and the immune response to infectious agents, foreign antigens, virus-infected cells and tumor cells. However, complement is also involved in pathological inflammation and in autoimmune diseases. Therefore, inhibition of excessive or uncontrolled activation of the complement cascade could provide clinical benefit to patients with such diseases and conditions.

[0002] The complement system encompasses two distinct activation pathways, designated the classical and the alternative pathways (V.M. Holers, In *Clinical Immunology: Principles and Practice*, ed. R.R. Rich, Mosby Press; 1996, 363-391). The classical pathway is a calcium/magnesium-dependent cascade which is normally activated by the formation of antigen-antibody complexes. The alternative pathway is a magnesium-dependent cascade which is activated by deposition and activation of C3 on certain susceptible surfaces (*e.g.* cell wall polysaccharides of yeast and bacteria, and certain biopolymer materials). Activation of the complement pathway generates biologically active fragments of complement proteins, *e.g.* C3a, C4a and C5a anaphylatoxins and C5b-9 membrane attack complexes (MAC), which mediate inflammatory activities involving leukocyte chemotaxis, activation of macrophages, neutrophils, platelets, mast cells and endothelial cells, vascular permeability, cytolysis, and tissue injury.

[0003] Factor D is a highly specific serine protease essential for activation of the alternative complement pathway. It cleaves factor B bound to C3b, generating the C3b/Bb enzyme which is the active component of the alternative pathway C3/C5 convertases. Factor D may be a suitable target for inhibition, since its plasma concentration in humans is very low (1.8 μg/ml), and it has been shown to be the limiting enzyme for activation of the alternative complement pathway (P.H. Lesavre and H.J. Müller-Eberhard. *J. Exp. Med.*, 1978; 148: 1498-1510; J.E. Volanakis et al., *New Eng. J. Med.*, 1985; 312: 395-401).

[0004] The down-regulation of complement activation has been demonstrated to be effective in treating several disease indications in animal models and in *ex vivo* studies, *e.g.* systemic lupus erythematosus and glomerulonephritis (Y. Wang et al., *Proc. Natl. Acad. Sci.*; 1996, 93: 8563-8568),

rheumatoid arthritis (Y. Wang et al., *Proc. Natl. Acad. Sci.*, 1995; 92: 8955-8959), cardiopulmonary bypass and hemodialysis (C.S. Rinder, *J. Clin. Invest.*, 1995; 96: 1564-1572), hypercute rejection in organ transplantation (T.J. Kroshus et al., *Transplantation*, 1995; 60: 1194-1202), myocardial infarction (J. W. Homeister et al., *J. Immunol.*, 1993; 150: 1055-1064; H.F. Weisman et al., *Science*, 1990; 249: 146-151), reperfusion injury (E.A. Amsterdam et al., *Am. J. Physiol.*, 1995; 268: H448-H457), and adult respiratory distress syndrome (R. Rabinovici et al., *J. Immunol.*, 1992; 149: 1744-1750). In addition, other inflammatory conditions and autoimmune/immune complex diseases are also closely associated with complement activation (V.M. Holers, *ibid.*, B.P. Morgan. *Eur. J. Clin. Invest.*, 1994: 24: 219-228), including thermal injury, severe asthma, anaphylactic shock, bowel inflammation, urticaria, angioedema, vasculitis, multiple sclerosis, myasthenia gravis, membranoproliferative glomerulonephritis, and Sjögren's syndrome.

[0005] There is a need for antibody therapeutics in the field of complement-mediated disorders and the humanized antibodies of the present invention provide high affinity antibodies useful to meet this need.

Summary of the Invention

[0006] The present disclosure relates generally to antibodies comprising the heavy and light chain variable domain sequences of murine antibody 166-32, which is an antibody capable of inhibiting biological activities associated with Factor D. For example, at a concentration of 18 μg/ml (equivalent to about 1.5 times the molar concentration of human factor D in the blood; molar ratio of anti-Factor D antibody to Factor D of about 1.5:1), significant inhibition of the alternative complement activity by the antibody can be observed (see, e.g., US Patent No. 6,956,107)

[0007] The present invention also relates to humanized antibodies of murine MAb 166-32. The invention includes the amino acid sequences of the variable heavy and light chain of the antibodies and their corresponding nucleic acid sequences. Another embodiment of the invention includes the CDR sequences of these antibodies.

[0007a] According to a first aspect, there is provided a variable domain of a humanized antibody comprising a heavy chain variable domain amino acid sequence selected from the group consisting of: SEQ ID NO: 6; SEQ ID NO: 8; SEQ ID NO: 10; and SEQ ID NO: 12.

[0007b] According to a second aspect, there is provided a variable domain of a humanized antibody comprising a light chain variable domain amino acid sequence selected from the group consisting of SEQ ID NO: 5; SEQ ID NO: 7; SEQ ID NO: 9 and SEQ ID NO: 11.

[0007c] According to a third aspect, there is provided a humanized anti-Factor D antibody comprising the variable domain sequence of SEQ ID NO: 5 and the variable domain sequence in SEQ ID NO: 6.

[0007d] According to a fourth aspect, there is provided a humanized anti-Factor D antibody comprising the variable domain sequence of SEQ ID NO: 7 and the variable domain sequence in SEQ ID NO: 8.

[0007e] According to a fifth aspect, there is provided a humanized anti-Factor D antibody comprising the variable domain sequence of SEQ ID NO: 9 and the variable domain sequence in SEQ ID NO: 10.

[0007f] According to a sixth aspect, there is provided a humanized anti-Factor D antibody comprising the variable domain sequence of SEQ ID NO: 11 and the variable domain sequence in SEQ ID NO: 12.

[0007g] According to a seventh aspect, there is provided a humanized anti-Factor D antibody having a variable light chain comprising a CDR-L1 having the sequence of SEQ ID NO: 16; a CDR-L2 having the sequence of SEQ ID NO: 17, 21, or 24, and a CDR-L3 having the sequence of SEQ ID NO: 18, 19 or 22.

[0007h] According to an eighth aspect, there is provided a humanized anti-Factor D antibody having a variable heavy chain comprising a CDR-H1 having the sequence of SEQ ID NO: 13, 23 or 25; a CDR-H2 having the sequence of SEQ ID NO: 14; and a CDR-H3 having the sequence of SEQ ID NO: 15 or 20.

[0007i] According to a ninth aspect, there is provided a polypeptide comprising the following amino acid sequence: $QX_1QLVQSGX_2E$ LKKPGASVKV SCKASGYTFT SYGMNWVX_3QA PGQGLEWMGW INTYTGETTYADDFKGRFVF SLDTSVSTAY LQISSLKAED TAX_4YYCX_5REG GVNNWGQGTL VTVSS (SEQ ID NO: 27), wherein X_1 is I or V; X_2 is P or S; X_3 is K or R; X_4 is T or V; and X_5 is E or A.

[0007j] According to a tenth aspect, there is provided a polypeptide comprising the following amino acid sequence: DIQX_6TQSPSSLSX_7SVGDRVTITCITSTDIDDDMNWYQQKPGKX_8PKLLIX_9DGNT LRPGVPSRFSX_{10}SGSGX_{11}DFTLTISSLQPEDVATYYCLQSDSLPYTFGQ GTKLEIK (SEQ ID NO: 26),

wherein X_6 is V or M; X_7 is M or A; X_8 is P or V; X_9 is S or Y; X_{10} is S or G and X_{11} is A or T.

[0007k] According to an eleventh aspect, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 6, 8, 10, or 12.

[0007l] According to a twelfth aspect, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 5, 7, 9, or 11.

[0007m] According to a thirteenth aspect, there is provided a humanized antibody comprising the polypeptide as defined above.

[0007n] According to a fourteenth aspect, there is provided an antibody fragment as defined above.

[0007o] According to a fifteenth aspect, there is provided an isolated nucleic acid encoding an antibody as defined above.

[0007p] According to a sixteenth aspect, there is provided an isolated nucleic acid encoding the polypeptide as defined above.

[0007q] According to a seventeenth aspect, there is provided a vector comprising the nucleic acid as defined above.

[0007r] According to an eighteenth aspect, there is provided a cell line comprising the vector as defined above.

[0007s] According to a nineteenth aspect, there is provided a composition comprising the antibody as defined above.

[0007t] According to a twentieth aspect, there is provided the use of the composition as defined above in the manufacture of a medicament for treating complement mediated disorders.

[0007u] According to a twenty first aspect, there is provided a variable domain of as defined above wherein amino acid at position 104 of SEQ ID NO: 7 is a valine or a leucine.

[0007v] According to a twenty second aspect, there is provided the method of making a humanized anti-Factor D antibody or fragment thereof as defined above.

[0007w] According to a twenty third aspect, there is provided an antibody which competes with murine antibody 166-32.

[0007x] According to a twenty fourth aspect, there is provided an antibody which competes with humanized anti-Factor D antibody clone #56, #111, #250 or #416 and/or an antibody comprising variable domain or HVR sequences of humanized anti-Factor D antibody clone #56, #111, #250 or #416.

[0007y] According to a twenty fifth aspect, there is provided an antibody which binds to the same epitope as murine antibody 166-32.

[0007z] According to a twenty sixth aspect, there is provided an antibody which binds to the same epitope as humanized anti-Factor D antibody clone #56, #111, #250 or #416 and/or an antibody comprising variable domain or HVR sequences of humanized anti- Factor D antibody clone #56, #111, #250 or #416.

[0007aa] According to a twenty seventh aspect, there is provided a humanized antibody comprising a heavy chain variable domain amino acid sequence selected from the group consisting of: SEQ ID NO: 6; SEQ ID NO: 8; SEQ ID NO: 10; and SEQ ID NO: 12.

[0007bb] According to a twenty eighth aspect, there is provided a humanized antibody comprising a light chain variable domain amino acid sequence selected from the group consisting of: SEQ ID NO: 5; SEQ ID NO: 7; SEQ ID NO: 9; and SEQ ID NO: 11.

[0007cc] According to a twenty ninth aspect, there is provided a humanized antibody comprising the polypeptide as defined above.

[0007dd] According to a thirtieth aspect, there is provided a humanized anti-Factor D antibody having a variable light chain comprising a CDR-L1 having the sequence of SEQ ID NO: 16; a CDR-L2 having the sequence of SEQ ID NO: 21 or 24, and a CDR-L3 having the sequence of SEQ ID NO: 18, 19 or 22.

[0007ee] According to a thirty first aspect, there is provided a humanized anti-Factor D antibody having a variable light chain comprising a CDR-L1 having the sequence of SEQ ID NO: 16; a CDR-L2 having the sequence of SEQ ID NO: 17, 21 or 24, and a CDR-L3 having the sequence of SEQ ID NO: 19 or 22.

[0007ff] According to a thirty second aspect, there is provided a humanized anti-Factor D antibody having a variable heavy chain comprising a CDR-H1 having the sequence of SEQ ID NO: 23 or 25; a CDR-H2 having the sequence of SEQ ID NO: 14; and a CDR-H3 having the sequence of SEQ ID NO: 15 or 20.

[0007gg] According to a thirty third aspect, there is provided a humanized anti-Factor D antibody having a variable heavy chain comprising a CDR-H1 having the sequence of SEQ ID NO: 13, 23 or 25; a CDR-H2 having the sequence of SEQ ID NO: 14; and a CDR-H3 having the sequence of SEQ ID NO: 20.

[0007hh] According to a thirty fourth aspect, there is provided a humanized anti-Factor D antibody having (i) a variable light chain comprising a CDR-L1 having the sequence of SEQ ID NO: 16; a CDR-L2 having the sequence of SEQ ID NO: 21 or 24, and a CDR-L3 having the sequence of SEQ ID NO: 18, 19 or 22; and (ii) a variable heavy chain comprising a CDR-H1 having the sequence of SEQ ID NO: 13, 23 or 25; a CDR-H2 having the sequence of SEQ ID NO: 14; and a CDR-H3 having the sequence of SEQ ID NO: 15 or 20.

[0007ii] According to a thirty fifth aspect, there is provided a humanized anti-Factor D antibody having (i) a variable light chain comprising a CDR-L1 having the sequence of SEQ ID NO: 16; a CDR-L2 having the sequence of SEQ ID NO: 17, 21 or 24, and a CDR-L3 having the sequence of SEQ ID NO: 19 or 22; and (ii) a variable heavy chain comprising a CDR-H1 having the sequence of SEQ ID NO: 13, 23 or 25; a CDR-H2 having the sequence of SEQ ID NO: 14; and a CDR-H3 having the sequence of SEQ ID NO: 15 or 20.

[0007jj] According to a thirty sixth aspect, there is provided a humanized anti-Factor D antibody having (i) a variable light chain comprising a CDR-L1 having the sequence of SEQ ID NO: 16; a CDR-L2 having the sequence of SEQ ID NO: 17, 21 or 24, and a CDR-L3 having the sequence of SEQ ID NO: 18, 19 or 22; and (ii) a variable heavy chain comprising a CDR-H1 having the sequence of SEQ ID NO: 23 or 25; a CDR-H2 having the sequence of SEQ ID NO: 14; and a CDR-H3 having the sequence of SEQ ID NO: 15 or 20.

[0007kk] According to a thirty seventh aspect, there is provided a humanized anti-Factor D antibody having (i) a variable light chain comprising a CDR-L1 having the sequence of SEQ ID NO: 16; a CDR-L2 having the sequence of SEQ ID NO: 17, 21 or 24, and a CDR-L3 having the sequence of SEQ ID NO: 18, 19 or 22; and (ii) a variable heavy chain comprising a CDR-H1 having the sequence of SEQ ID NO: 13, 23 or 25; a CDR-H2 having the sequence of SEQ ID NO: 14; and a CDR-H3 having the sequence of SEQ ID NO: 20.

[0007ll] According to a thirty eighth aspect, there is provided a variable domain of a humanized antibody comprising a heavy chain variable domain amino acid sequence of SEQ ID NO: 8.

[0007mm] According to a thirty ninth aspect, there is provided a variable domain of a humanized antibody comprising a light chain variable domain amino acid sequence of SEQ ID NO: 7.

[0007nn] According to a fortieth aspect, there is provided humanized antibody comprising a heavy chain variable domain amino acid sequence of SEQ ID NO: 8.

[0007oo] According to a forty first aspect, there is provided a humanized antibody comprising a light chain variable domain amino acid sequence of SEQ ID NO: 7.

[0007pp] According to a forty second aspect, there is provided a humanized anti-Factor D antibody having a variable light chain comprising a CDR-L1 having the sequence of SEQ ID NO: 16; a CDR-L2 having the sequence of SEQ ID NO: 17, and a CDR-L3 having the sequence of SEQ ID NO: 19.

[0007qq] According to a forty third aspect, there is provided a humanized anti-Factor D antibody having a variable heavy chain comprising a CDR-H1 having the sequence of SEQ ID NO: 13; a CDR-H2 having the sequence of SEQ ID NO: 14; and a CDR-H3 having the sequence of SEQ ID NO: 20.

[0007rr] According to a forty fourth aspect, there is provided a humanized anti-Factor D antibody having a variable light chain comprising a CDR-L1 having the sequence of SEQ ID NO: 16; a CDR-L2 having the sequence of SEQ ID NO: 17, and a CDR-L3 having the sequence of SEQ ID NO: 19; and a variable heavy chain comprising a CDR-H1 having the sequence of SEQ ID NO: 13; a CDR-H2 having the sequence of SEQ ID NO: 14; and a CDR-H3 having the sequence of SEQ ID NO: 20.

[0007ss] According to a forty fifth aspect, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 8.

[0007tt] According to forty sixth aspect, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 7.

[0008] Another embodiment of the present disclosure includes compositions comprising an antibody of the invention. In another embodiment, the invention

provides cell lines and vectors harboring the antibody sequences of the present invention. In one embodiment, the invention includes method of making and using antibodies and compositions of the invention.

[0009] Another embodiment of the present invention is the use of these humanized antibodies for the preparation of a medicament or composition for the treatment of disorders associated with excessive or uncontrolled complement activation. They include complement activation during cardiopulmonary bypass operations; complement activation due to ischemia-reperfusion following acute myocardial infarction, aneurysm, stroke, hemorrhagic shock, crush injury, multiple organ failure, hypobolemic shock, intestinal ischemia or other events causing ischemia. Complement activation has also been shown to be associated with inflammatory conditions such as severe burns, endotoxemia, septic shock, adult respiratory distress syndrome, hemodialysis; anaphylactic shock, severe asthma, angioedema, Crohn's disease, sickle cell anemia, poststreptococcal glomerulonephritis and pancreatitis. The disorder may be the result of an adverse drug reaction, drug allergy, IL-2 induced vascular leakage syndrome or radiographic contrast media allergy. It also includes autoimmune disease such as systemic lupus erythematosus, myasthenia gravis, rheumatoid arthritis, Alzheimer's disease and multiple sclerosis. Complement activation is also associated with transplant rejection.Complement activation is also associated with ocular diseases such as age-related macular degeneration, diabetic retinopathy.

Brief Description of the Figures

[00010] Figures 1A and 1B depict the amino acid sequence of the Murine MAb 166-32 Variable Heavy Chain (Figure 1A) and the Variable Light Chain (Figure 1B).

[00011] Figures 2A and 2B depict nucleic acid sequence of the Murine MAb 166-32 Variable Heavy Chain (Figure 2A) and the Variable Light Chain (Figure 2B).

[00012] Figure 3 depicts the comparison of the light chains of the murine MAb 166-32.

[00013] Figure 4 depicts the comparison of the heavy chains of the murine MAb 166-32.

[00014] Figure 5 depicts the amino acid sequences of the Variable Heavy Chain and the Variable Light Chain for each humanized antibody clone #56, #111, # 250, and #416.

[00015] Figure 6 depicts the hemolytic assay results for humanized antibody Fab clone #56, #111, # 250, and #416.

[00016] Figure 7 depicts the inhibition of the alternative complement activity by humanized antibody Fab clones #56, #111, # 250, and #416.

[00017] Figure 8A-B (variable heavy (VH) consensus frameworks) and Figure 9A-B (variable light (VL) consensus frameworks) depict exemplary acceptor human consensus framework sequences that can be used in practicing the instant invention with sequence identifiers as follows: (Figure 8A-B) human VH subgroup I consensus framework minus Kabat CDRs (SEQ ID NO: 28), human VH subgroup I consensus framework minus extended hypervariable regions (SEQ ID NOs: 29-31), human VH subgroup II consensus framework minus Kabat CDRs (SEQ ID NO: 32), human VH subgroup II consensus framework minus extended hypervariable regions (SEQ ID NOs: 33-35), human VH subgroup III consensus framework minus Kabat CDRs (SEQ ID NO: 36), human VH subgroup III consensus framework minus extended hypervariable regions (SEQ ID NOs: 37-39), human VH subgroup VII consensus framework minus Kabat CDRs (SEQ ID NO: 55), human VH subgroup VII consensus framework minus extended hypervariable regions (SEQ ID NOs: 56-58), human VH acceptor framework minus Kabat CDRs (SEQ ID NO: 40), human VH acceptor framework minus extended hypervariable regions (SEQ ID NOs: 41-42), human VH acceptor 2 framework minus Kabat CDRs (SEQ ID NO: 43) and human VH acceptor 2 framework minus extended hypervariable regions (SEQ ID NOs: 44-46) and (Figure 9A-B) human VL kappa subgroup I consensus framework (SEQ ID NO: 47), human VL kappa subgroup consensus framework (SEQ ID NO: 48), human kappa subgroup III consensus framework (SEQ ID NO: 49) and human kappa subgroup IV consensus framework (SEQ ID NO: 50).

Detailed Description of the Invention

Definitions

[00018] Terms used throughout this application are to be construed with ordinary and typical meaning to those of ordinary skill in the art. However,

Applicants desire that the following terms be given the particular definition as defined below.

[00019] The phrase "substantially identical" with respect to an antibody chain polypeptide sequence may be construed as an antibody chain exhibiting at least 70%, or 80%, or 90% or 95% sequence identity to the reference polypeptide sequence. The term with respect to a nucleic acid sequence may be construed as a sequence of nucleotides exhibiting at least about 85%, or 90%, or 95% or 97% sequence identity to the reference nucleic acid sequence.

[00020] The term "identity" or "homology" shall be construed to mean the percentage of amino acid residues in the candidate sequence that are identical with the residue of a corresponding sequence to which it is compared, after aligning the sequences and introducing gaps, if necessary to achieve the maximum percent identity for the entire sequence, and not considering any conservative substitutions as part of the sequence identity. Neither N- or C-terminal extensions nor insertions shall be construed as reducing identity or homology. Methods and computer programs for the alignment are well known in the art. Sequence identity may be measured using sequence analysis software.

[00021] The term "antibody" is used in the broadest sense, and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, and multispecific antibodies (e.g., bispecific antibodies). Antibodies (Abs) and immunoglobulins (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific target, immunoglobulins include both antibodies and other antibody-like molecules which lack target specificity. Native antibodies and immunoglobulins are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. 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.

[00022] As used herein, "anti-human Factor D antibody" means an antibody which specifically binds to human Factor D in such a manner so as to inhibit or substantially reduce complement activation.

[00023] The term "variable" in the context of variable domain of antibodies, refers to the fact that certain portions of the variable domains differ extensively in

sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular target. However, the variability is not evenly distributed through the variable domains of antibodies. It is concentrated in three segments called complementarity determining regions (CDRs) also known as 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 (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely a adopting a β-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the target binding site of antibodies (see Kabat et al.). As used herein, numbering of immunoglobulin amino acid residues is done according to the immunoglobulin amino acid residue numbering system of Kabat et al., (Sequences of Proteins of Immunological Interest, National Institute of Health, Bethesda, Md. 1987), unless otherwise indicated.

[00024] The term "hypervariable region", "HVR", or "HV", when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six hypervariable regions; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). A number of hypervariable region delineations are in use and are encompassed herein. The Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat *et al., Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). Chothia refers instead to the location of the structural loops (Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)). The AbM hypervariable regions represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The "contact" hypervariable regions are based on an analysis of the available complex crystal structures. The residues from each of these hypervariable regions 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 (Kabat Numbering)	H31-H35B	H26-H35B	H26-H32	H30-H35B
H1 (Chothia Numbering)	H31-H35	H26-H35	H26-H32	H30-H35
H2	H50-H65	H50-H58	H53-H55	H47-H58
H3	H95-H102	H95-H102	H96-H101	H93-H101

[00025] Hypervariable regions may comprise "extended hypervariable regions" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 (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 definitions.

[00026] "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues or CDR residues herein defined.

[00027] The term "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., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991). 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 CDR 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.

[00028] 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; hinge region in constant domain of heavy chain is approximately residues 216-230 (EU numbering) of the heavy chain). 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 Provisional Application No. 60/640,323, Figures for EU numbering).

[00029] The term "antibody fragment" refers to a portion of a full-length antibody, generally the target binding or variable region. Examples of antibody fragments include Fab, Fab', $F(ab')_2$ and Fv fragments. The phrase "functional fragment or analog" of an antibody is a compound having qualitative biological activity in common with a full-length antibody. For example, a functional fragment or analog of an anti-human Factor D antibody is one which can bind to Factor D in such a manner so as to prevent or substantially reduce the complement activation. As used herein, "functional fragment" with respect to antibodies, refers to Fv, F(ab) and $F(ab')_2$ fragments. An "Fv" fragment is the minimum antibody fragment which contains a complete target recognition and binding site. This region consists of a dimer of one heavy and one light chain variable domain in a tight, non-covalent association (V_H -V_L dimer). It is in this configuration that the three CDRs of each variable domain interact to define an target binding site on the surface of the V_H -V_L dimer. Collectively, the six CDRs confer target binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an target) has the ability to recognize and bind target. "Single-chain Fv" or "sFv" antibody fragments comprise the V_H and V_L domains of an antibody, wherein these domains are present in a single

polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for target binding.

[00030] The Fab fragment contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. F(ab') fragments are produced by cleavage of the disulfide bond at the hinge cysteines of the $F(ab')_2$ pepsin digestion product. Additional chemical couplings of antibody fragments are known to those of ordinary skill in the art.

[00031] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single targetic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the target. In addition to their specificity, monoclonal antibodies are advantageous in that they may be 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 for use with the present invention may be isolated from phage antibody libraries using the well known techniques. The parent monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler and Milstein, Nature 256, 495 (1975), or may be made by recombinant methods.

[00032] "Humanized" forms of non-human (e.g. murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other target-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. In general, the humanized antibody will comprise substantially all of at least one, and typically

two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody may also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin template chosen.

[00033] Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[00034] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies can in some instances be important to reduce antigenicity and/or HAMA response (human anti-mouse antibody) when the antibody is intended for human therapeutic use. Reduction or elimination of a HAMA response is generally a significant aspect of clinical development of suitable therapeutic agents. See, e.g., Khaxzaeli et al., J. Natl. Cancer Inst. (1988), 80:937; Jaffers et al., Transplantation (1986), 41:572; Shawler et al., J. Immunol. (1985), 135:1530; Sears et al., J. Biol. Response Mod. (1984), 3:138; Miller et al., Blood (1983), 62:988; Hakimi et al., J. Immunol. (1991), 147:1352; Reichmann et al., Nature (1988), 332:323; Junghans et al., Cancer Res. (1990), 50:1495. As described herein, the invention provides antibodies that are humanized such that HAMA response is reduced or eliminated. Variants of these antibodies can further be obtained using routine methods known in the art, some of which are further described below. According to the so-called "best-fit" method,

the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable domain sequences. The human V domain sequence which is closest to that of the rodent is identified and the human framework region (FR) within it accepted for the humanized antibody (Sims et al., J. Immunol. 151:2296 (1993); Chothia et al., J. Mol. Biol., 196:901 (1987)). Another method uses a particular framework region derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter et al., Proc. Natl. Acad. Sci. USA, 89:4285 (1992); Presta et al., J. Immunol. 151:2623 (1993)).

[00035] For example, an amino acid sequence from an antibody as described herein can serve as a starting (parent) sequence for diversification of the framework and/or hypervariable sequence(s). A selected framework sequence to which a starting hypervariable sequence is linked is referred to herein as an acceptor human framework. While the acceptor human frameworks may be from, or derived from, a human immunoglobulin (the VL and/or VH regions thereof), the acceptor human frameworks may be from, or derived from, a human consensus framework sequence as such frameworks have been demonstrated to have minimal, or no, immunogenicity in human patients. An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a VL or VH framework derived from a human immunoglobulin framework, or from a human consensus framework. An acceptor human framework "derived from" a human immunoglobulin framework or human consensus framework may comprise the same amino acid sequence thereof, or may contain pre-existing amino acid sequence changes. Where pre-existing amino acid changes are present, preferably no more than 5 and preferably 4 or less, or 3 or less, pre-existing amino acid changes are present. In one embodiment, the VH acceptor human framework is identical in sequence to the VH human immunoglobulin framework sequence or human consensus framework sequence. In one embodiment, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence. A "human consensus framework" is a framework which represents the most commonly occurring amino acid residue 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.* In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat *et al.* In one embodiment, for the VH, the subgroup is subgroup III as in Kabat *et al.*

[00036] Where the acceptor is derived from a human immunoglobulin, one may optionally select a human framework sequence that is selected based on its homology to the donor framework sequence by aligning the donor framework sequence with various human framework sequences in a collection of human framework sequences, and select the most homologous framework sequence as the acceptor. The acceptor human framework may be from or derived from human antibody germline sequences available in the public databases.

[00037] In one embodiment, human consensus frameworks herein are from, or derived from, VH subgroup VII and/or VL kappa subgroup I consensus framework sequences.

[00038] In one embodiment, the human framework template used for generation of an anti-Factor D antibody may comprise framework sequences from a template comprising a combination of VI-4.1b+ (VH7 family) and JH4d for VH chain (Figure 3) and/or a combination of DPK4 (VκI family) and JK2 for VL chain (Figure 4).

[00039] Thus, the VH acceptor human framework may comprise one, two, three or all of the following framework sequences: FR1 comprising $QX_1QLVQSGX_2ELKKPGASVKVSCKAS$ (amino acids 1-25 of SEQ ID NO: 27), wherein X_1 is I or V, X_2 is P or S; FR2 comprising $WVX_3QAPGQGLE$ (amino acids 36-46 of SEQ ID NO: 27), wherein X_3 is K or R; FR3 comprising $RFVFSLDTSVSTAYLQISSLKAEDTAX_4YYCX_5R$ (amino acids 67-98 of SEQ ID NO: 27), wherein X_4 is T or V, X_5 is E or A; FR4 comprising WGQGTLVTVSS (amino acids 105-115 of SEQ ID NO: 8 or amino acids 105-115 of SEQ ID NO: 27)

[00040] Examples of VH consensus frameworks include:

human VH subgroup I consensus framework minus Kabat CDRs (SEQ ID NO: 28);

human VH subgroup I consensus framework minus extended hypervariable regions (SEQ ID NOs: 29-31);

human VH subgroup II consensus framework minus Kabat CDRs (SEQ ID NO: 32);
human VH subgroup II consensus framework minus extended hypervariable regions (SEQ ID NOs: 33-35);
human VH subgroup III consensus framework minus Kabat CDRs (SEQ ID NO: 36);
human VH subgroup III consensus framework minus extended hypervariable regions (SEQ ID NO: 37-39);
human VH subgroup VII consensus framework minus Kabat CDRs (SEQ ID NO: 55);
human VH subgroup VII consensus framework minus extended hypervariable regions (SEQ ID NO: 56-58);
human VH acceptor framework minus Kabat CDRs (SEQ ID NO: 40);
human VH acceptor framework minus extended hypervariable regions (SEQ ID NOs: 41-42);
human VH acceptor 2 framework minus Kabat CDRs (SEQ ID NO: 43); or
human VH acceptor 2 framework minus extended hypervariable regions (SEQ ID NOs: 44-45).

[00041] In one embodiment, the VH acceptor human framework comprises one, two, three or all of the following framework sequences:
FR1 comprising QVQLVQSGPELKKPGASVKVSCKAS (amino acids 1-25 of SEQ ID NO: 8),
FR2 comprising WVRQAPGQGLE (amino acids 36-46 of SEQ ID NO: 8),
FR3 comprising RFVFSLDTSVSTAYLQISSLKAEDTAVYYCER (amino acids 67-98 of SEQ ID NO: 8),
RFVFSLDTSVSTAYLQISSLKAEDTAVYYCE (amino acids 67-97 of SEQ ID NO: 8),
RFVFSLDTSVSTAYLQISSLKAEDTAVYYC (amino acids 67-96 of SEQ ID NO: 8),
RFVFSLDTSVSTAYLQISSLKAEDTAVYYCS (SEQ ID NO: 51), or
RFVFSLDTSVSTAYLQISSLKAEDTAVYYCSR (SEQ ID NO: 52)
FR4 comprising WGQGTLVTVSS (amino acids 105-115 of SEQ ID NO: 8 or amino acids 105-115 of SEQ ID NO: 27).

[00042] The VL acceptor human framework may comprise one, two, three or all of the following framework sequences:

FR1 comprising DIQX$_6$TQSPSSLSX$_7$SVGDRVTITC (amino acids 1-23 of SEQ ID NO: 26), wherein X$_6$ is V or M, X$_7$ is M or A;
FR2 comprising WYQQKPGKX$_8$PKLLIX$_9$ (amino acids 35-49 of SEQ ID NO: 26), wherein X$_8$ is P or V, X$_9$ is S or Y;
FR3 comprising GVPSRFSX$_{10}$SGSGX$_{11}$DFTLTISSLQPEDVATYYC (amino acids 57-88 of SEQ ID NO: 26), wherein X$_{10}$ is S or G, X$_{11}$ is A or T;
FR4 comprising FGQGTKX$_{12}$EIK (SEQ ID NO: 54), wherein X$_{12}$ is V or L.

[00043] Examples of VL consensus frameworks include:
human VL kappa subgroup I consensus framework (SEQ ID NO: 47);
human VL kappa subgroup II consensus framework (SEQ ID NO: 48);
human VL kappa subgroup III consensus framework (SEQ ID NO: 49); or
human VL kappa subgroup IV consensus framework (SEQ ID NO: 50)

[00044] In one embodiment, the VL acceptor human framework may comprise one, two, three or all of the following framework sequences:
FR1 comprising DIQVTQSPSSLSASVGDRVTITC (amino acids 1-23 of SEQ ID NO: 7),
FR2 comprising WYQQKPGKVPKLLIS (amino acids 35-49 of SEQ ID NO: 7),
FR3 comprising GVPSRFSGSGSGTDFTLTISSLQPEDVATYYC (amino acids 57-88 of SEQ ID NO: 7),
FR4 comprising FGQGTKLEIK (amino acids 98-107 of SEQ ID NO: 7), or FGQGTKVEIK (SEQ ID NO: 53).

[00045] While the acceptor may be identical in sequence to the human framework sequence selected, whether that be from a human immunoglobulin or a human consensus framework, the present invention contemplates that the acceptor sequence may comprise pre-existing amino acid substitutions relative to the human immunoglobulin sequence or human consensus framework sequence. These pre-existing substitutions are preferably minimal; usually four, three, two or one amino acid differences only relative to the human immunoglobulin sequence or consensus framework sequence.

[00046] Hypervariable region residues of the non-human antibody are incorporated into the VL and/or VH acceptor human frameworks. For example, one may incorporate residues corresponding to the Kabat CDR residues, the Chothia hypervariable loop residues, the Abm residues, and/or contact residues. Optionally, the extended hypervariable region residues as follows are incorporated: 24-34 (L1), 50-56 (L2) and 89-97 (L3), 26-35 (H1), 50-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3).

[00047] In one aspect, the invention provides an antibody comprising at least one, two, three, four, five or six HVRs selected from (a) an HVR-H1 comprising the amino acid sequence selected from SEQ ID NO: 13 and SEQ ID NO: 25; (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14; (c) an HVR-H3 comprising the amino acid sequence selected from SEQ ID NO: 15 and SEQ ID NO: 20; (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 16; (e) an HVR-L2 comprising the amino acid sequence selected from SEQ ID NO: 17, SEQ ID NO; 21 and SEQ ID NO: 23; and (f) an HVR-L3 comprising the amino acid sequence selected from SEQ ID NO: 18, SEQ ID NO: 22 and SEQ ID NO: 24.

[00048] In one aspect, the invention provides an anti-Factor D antibody comprising at least one, two, three, four, five or six HVRs selected from (a) an HVR-H1 comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to an amino acid sequence selected from SEQ ID NO: 13 and SEQ ID NO: 25; (b) an HVR-H2 comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to an amino acid sequence of SEQ ID NO: 14; (c) an HVR-H3 comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to an amino acid sequence selected from SEQ ID NO: 15 and SEQ ID NO: 20; (d) an HVR-L1 comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to an amino acid sequence of SEQ ID NO: 16; (e) an HVR-L2 comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to an amino acid sequence selected from SEQ ID NO: 17, SEQ ID NO; 21 and SEQ ID NO: 23; and (f) an HVR-L3 comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or

99% sequence identity to an amino acid sequence selected from SEQ ID NO: 18, SEQ ID NO: 22 and SEQ ID NO: 24. In some embodiments, an HVR having an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity contains substitutions, insertions, or deletions relative to the reference sequence, but an antibody comprising that amino acid sequence retains the ability to bind to Factor D. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted, or deleted in the reference sequence selected from the group consisting of SEQ ID NO: 13, SEQ ID NO: 25, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 20, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO; 21, SEQ ID NO: 23, SEQ ID NO: 18, SEQ ID NO: 22 and SEQ ID NO: 24. In some embodiments, the invention provides an antibody comprising at least one, two, three, four, five or six HVRs selected from (a) an HVR-H1 comprising the amino acid sequence selected from SEQ ID NO: 13 and SEQ ID NO: 25; (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14; (c) an HVR-H3 comprising the amino acid sequence selected from SEQ ID NO: 15 and SEQ ID NO: 20; (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 16; (e) an HVR-L2 comprising the amino acid sequence selected from SEQ ID NO: 17, SEQ ID NO; 21 and SEQ ID NO: 23; and (f) an HVR-L3 comprising the amino acid sequence selected from SEQ ID NO: 18, SEQ ID NO: 22 and SEQ ID NO: 24.

[00049] In one aspect, the invention provides an antibody comprising a heavy chain variable domain selected from SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12. In one aspect, the invention provides an antibody comprising a light chain variable domain selected from SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and SEQ ID NO: 11. In one aspect, the invention provides an antibody comprising a heavy chain variable domain comprising SEQ ID NO: 6. In one aspect, the invention provides an antibody comprising a light chain variable domain comprising SEQ ID NO: 5. In one aspect, the invention provides an antibody comprising a heavy chain variable domain comprising SEQ ID NO: 6 and a light chain variable domain comprising SEQ ID NO: 5. In one aspect, the invention provides an antibody comprising a heavy chain variable domain comprising SEQ ID NO: 8. In one aspect, the invention provides an antibody comprising a light chain variable domain comprising SEQ ID NO: 7. In one aspect, the invention provides an antibody comprising a heavy chain variable

domain comprising SEQ ID NO: 8 and a light chain variable domain comprising SEQ ID NO: 7. In one aspect, the invention provides an antibody comprising a heavy chain variable domain comprising SEQ ID NO: 10. In one aspect, the invention provides an antibody comprising a light chain variable domain comprising SEQ ID NO: 9. In one aspect, the invention provides an antibody comprising a heavy chain variable domain comprising SEQ ID NO: 10 and a light chain variable domain comprising SEQ ID NO: 9. In one aspect, the invention provides an antibody comprising a heavy chain variable domain comprising SEQ ID NO: 12. In one aspect, the invention provides an antibody comprising a light chain variable domain comprising SEQ ID NO: 11. In one aspect, the invention provides an antibody comprising a heavy chain variable domain comprising SEQ ID NO: 12 and a light chain variable domain comprising SEQ ID NO: 11.

[00050] In one aspect, the invention provides an anti-Factor D antibody comprising a heavy chain variable domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 8, 10 and 12. In some embodiments, an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity contains substitutions, insertions, or deletions relative to the reference sequence, but an antibody comprising that amino acid sequence retains the ability to bind to Factor D. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted, or deleted in a sequence selected from the group consisting of SEQ ID NO: 6, 8, 10 or 12. In some embodiments, the substitutions, insertions or deletions occur in regions outside the HVRs (i.e., in the FRs). In some embodiments, an anti-Factor D antibody comprises a heavy chain variable domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 8, 10 or 12.

[00051] In some embodiments, the invention provides an anti-Factor D antibody comprising a light chain variable domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 5, 7, 9 and 11. In some embodiments, an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity contains substitutions, insertions, or deletions relative to

the reference sequence, but an antibody comprising that amino acid sequence retains the ability to bind to Factor D. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted, or deleted in a sequence selected from the group consisting of SEQ ID NO: 5, 7, 9 and 11. In some embodiments, the substitutions, insertions or deletions occur in regions outside the HVRs (i.e., in the FRs). In some embodiments, an anti-Factor D antibody comprises a light chain variable domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 5, 7, 9 and 11.

[00052] An anti-Factor D antibody may comprise any suitable framework variable domain sequence, provided that the antibody retains the ability to bind Factor D. For example, in some embodiments, anti-Factor D antibodies of the invention comprise a heavy chain variable domain framework sequence that is a combination of VI.4.1 b+ and JH4d (See Figure 3). In some embodiments, anti-Factor D antibodies of the invention comprise a human subgroup VII heavy chain framework consensus sequence. In some embodiments, anti-Factor D antibodies of the invention comprise a heavy chain variable domain framework sequence comprising FR1 comprising amino acids 1-25 of SEQ ID NO: 8, FR2 comprising amino acids 36-46 of SEQ ID NO: 8, FR3 comprising amino acids 67-98 of SEQ ID NO: 8 and FR4 comprising amino acids 105-115 of SEQ ID NO: 8 In one embodiment of these antibodies, the heavy chain variable domain sequence comprises substitution(s) at position 40 and/or 88 (Kabat numbering). In one embodiment of these antibodies, position 40 is cysteine (C) or alanine (A) and/or position 88 is cysteine (C) or alanine (A). In some embodiments, anti-Factor D antibodies of the invention comprise a light chain variable domain framework sequence that is a combination of DPK4 and JK2 (See Figure 4). In some embodiments, anti-Factor D antibodies of the invention comprise a human kappa I (κI) light chain framework consensus sequence. In some embodiments, anti-Factor D antibodies of the invention comprise a light chain variable domain framework sequence comprising FR1 comprising amino acids 1-23 of SEQ ID NO: 7, FR2 comprising amino acids 35-49 of SEQ ID NO: 7, FR3 comprising amino acids 57-88 of SEQ ID NO: 7 and FR4 comprising amino acids 98-107 of SEQ ID NO: 7. In one embodiment of these antibodies, the light chain variable framework sequence comprises one or more substitution(s) at position 15, 43 and/or 104

(Kabat numbering). In one embodiment of these antibodies, position 15 is cysteine (C) or valine (V), position 43 is cysteine (C) or alanine (A) and/or position 104 is valine (V) or leucine (L).

[00053] Further, an anti-Factor D antibody may comprise any suitable constant domain sequence, provided that the antibody retains the ability to bind Factor D. For example, in some embodiments, anti-Factor D antibodies of the invention comprise at least a portion of a heavy chain constant domain. In one embodiment, anti-Factor D antibodies of the invention comprise a heavy chain constant domain of either one or a combination of an α, δ, ε, γ, or μ heavy chain. Depending on the amino acid sequence of the constant domain of their heavy chains (C_H), 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 α, δ, ε, γ, and μ, respectively. The γ and α classes are further divided into subclasses on the basis of relatively minor differences in C_H sequence and function, e.g., humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. In one embodiment, anti-Factor D antibodies of the invention comprise a heavy chain constant domain comprising substitutions at amino acid positions that results in a desired effect on effector function (e.g.. binding affinity). In one embodiment, anti-Factor D antibodies of the invention comprise a heavy chain constant domain comprising substitutions at amino acid positions that do not result in an effect on effector function (e.g.. binding affinity). In one embodiment, anti-Factor D antibodies of the invention comprise a heavy chain constant domain of the IgG type (e.g. IgG1, IgG2, IgG3 or IgG4) and further comprise a substitution at position 114 (Kabat numbering; equivalent to 118 in EU numbering), 168 (Kabat numbering; equivalent to 172 in EU numbering), 172 (Kabat numbering; equivalent to 176 in EU numbering) and/or 228 (EU numbering). In one embodiment, anti-Factor D antibodies of the invention comprise a heavy chain constant domain of the IgG (e.g. IgG1, IgG2, IgG3 or IgG4) type and further comprise a substitution at position 114 wherein position 114 is a cysteine (C) or alanine (A), position 168 is cysteine (C) or alanine (A), position 172 is a cysteine (C) or alanine (A) and/or position 228 is a proline (P), arginine (R) or serine (S).

[00054] Further, for example, in some embodiments, anti-Factor D antibodies of the invention comprise at least a portion of a light chain constant

domain. In one embodiment, anti-Factor D antibodies of the invention comprise a light chain constant domain of either one or a combination of a kappa or a lambda light chain, as the light chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. In one embodiment, anti-Factor D antibodies of the invention comprise a light chain constant domain comprising substitutions at amino acid positions that results in a desired effect on effector function (e.g.. binding affinity). In one embodiment, anti-Factor D antibodies of the invention comprise a light chain constant domain comprising substitutions at amino acid positions that do not result in an effect on effector function (e.g., binding affinity). In one embodiment, anti-Factor D antibodies of the invention comprise a light chain constant domain of the kappa type and further comprise a substitution at position 110, 144, 146 and/or 168 (Kabat numbering). In one embodiment, anti-Factor D antibodies of the invention comprise a light chain constant domain of the kappa type and further comprise a substitution at position 110 wherein 110 is a cysteine (C) or valine (V), at position 144 wherein 144 is a cysteine (C) or alanine (A), at position 146 wherein 146 is a isoleucine (I) or valine (V) and/or at position 168 wherein 168 is a cysteine (C) or serine (S).

[00055] In one aspect, the invention provides antibodies that compete with murine antibody 166-32 and/or humanized anti-Factor D antibody clone #56, #111, #250 or #416, and/or an antibody comprising variable domain or HVR sequences of humanized anti-Factor D antibody clone #56, #111, #250 or #416. Antibodies that bind to the same epitope as murine antibody 166-32 and/or humanized anti-Factor D antibody clone #56, #111, #250 or #416, and/or an antibody comprising variable domain or HVR sequences of humanized anti-Factor D antibody clone #56, #111, #250 or #416 are also provided.

[00056] In one embodiment, the invention provides an anti-Factor D antibody wherein the monovalent affinity of the antibody to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is lower, for example at least 1-fold or 2-fold lower than the monovalent affinity of a chimeric antibody (e.g. affinity of the cihmeric antibody as a Fab fragment to Factor D), comprising, consisting or consisting essentially of a light chain variable domain of SEQ ID NO: 2 and heavy chain variable domain of SEQ ID NO: 1.

[00057] In one embodiment, the invention provides an anti-Factor D antibody wherein the bivalent affinity of the antibody to Factor D (e.g., affinity of the antibody as an IgG to Factor D) is lower, for example at least 1-fold or 2-fold lower than the bivalent affinity of a chimeric antibody (e.g. affinity of the cihmeric antibody as an IgG to Factor D), comprising, consisting or consisting essentially of a light chain variable domain of SEQ ID NO: 2 and heavy chain variable domain of SEQ ID NO: 1.

[00058] In another embodiment, the invention provides an anti-Factor D antibody wherein the monovalent affinity of the antibody to FactorD (e.g., affinity of the antibody as a Fab fragment to Factor D) is greater, for example at least 1-fold or 2-fold greater than the monovalent affinity of a chimeric antibody (e.g. affinity of the chimeric antibody as a Fab fragment to Factor D), comprising, consisting or consisting essentially of a light chain variable domain of SEQ ID NO: 2 and heavy chain variable domain of SEQ ID NO: 1.

[00059] In another embodiment, the invention provides an anti-Factor D antibody wherein the bivalent affinity of the antibody to FactorD (e.g., affinity of the antibody as an IgG to Factor D) is greater, for example at least 1-fold or 2-fold greater than the bivalent affinity of a chimeric antibody (e.g. affinity of the chimeric antibody as an IgG to Factor D), comprising, consisting or consisting essentially of a light chain variable domain of SEQ ID NO: 2 and heavy chain variable domain of SEQ ID NO: 1.

[00060] In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is 1.0 nM ($1.0x10^{-9}$ M) or better. In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is 0.5 nM ($0.5x10^{-9}$ M) or better. In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is 1.0 pM ($1.0x10^{-12}$ M) or better. In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is 0.5 pM ($0.5x10^{-12}$ M) or better.

[00061] In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as an IgG to Factor D) is 1.0 nM ($1.0x10^{-9}$ M) or better. In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its its bivalent form to Factor D (e.g., affinity of the antibody as an IgG to Factor D) is 0.5 nM ($0.5x10^{-9}$ M) or better. In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its its bivalent form to Factor D (e.g., affinity of the antibody as an IgG to Factor D) is 1.0 pM ($1.0x10^{-12}$ M) or better. In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its its bivalent form to Factor D (e.g., affinity of the antibody as an IgG to Factor D) is 0.5 pM ($0.5x10^{-12}$ M) or better.

[00062] In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is between 0.5 mM ($0.5x10^{-6}$ M) and 0.5 pM ($0.5x10^{-12}$ M). In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is between 15 nM ($15x10^{-9}$ M) and 0.1 nM ($0.1x10^{-9}$ M). In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is between 5.5 nM ($5.5x10^{-9}$ M) and 1 nM ($1x10^{-9}$ M). In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is between 0.5 pM ($0.5x10^{-12}$ M) and 2 pM ($2x10^{-12}$ M).

[00063] In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as an IgG to Factor D) is between 0.5 mM ($0.5x10^{-6}$ M) and 0.5 pM ($0.5x10^{-12}$ M). In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as an IgG to Factor D) is between 10 nM ($10x10^{-9}$ M) and 0.05 nM ($0.05x10^{-9}$ M). In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its bivalent form to

Factor D (e.g., affinity of the antibody as an IgG to Factor D) is between 5.5 nM ($5.5x10^{-9}$ M) and 1 nM ($1x10^{-9}$ M). In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as an IgG to Factor D) is between 0.5 pM ($0.5x10^{-12}$ M) and 2 pM ($2x10^{-12}$ M).

[00064] In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is about 0.37 nM ($3.7x10^{-10}$ M). In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is about 0.33 nM ($3.3x10^{-10}$ M). In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is about 0.51 nM ($5.1x10^{-10}$ M). In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is about 2.7 nM ($2.7x10^{-9}$ M). In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is about 1.14 nM ($1.14x10^{-9}$ M). In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is about 1.4 pM ($1.4x10^{-12}$ M). In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as a IgG to Factor D) is about 1.1 pM ($1.1x10^{-12}$ M). In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is about 0.19 nM ($0.19x10^{-9}$ M). In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as a IgG to Factor D) is about 0.08 nM ($0.08x10^{-9}$ M). In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to

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Factor D) is about 12.3 nM ($12.3x10^{-9}$ M). In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as a IgG to Factor D) is about 9.0 nM ($9.0x10^{-9}$ M).

[00065] In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is about 1.4 pM ($1.4x10^{-12}$ M) +/- 0.5. In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as an IgG to Factor D) is about 1.1 pM ($1.1x10^{-12}$ M) +/- 0.6. In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is about 0.19 nM ($0.19x10^{-9}$ M) +/- .01. In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as a IgG to Factor D) is about 0.08 nM ($0.08x10^{-9}$ M) +/- 0.01. In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is about 12.3 nM ($12.3x10^{-9}$ M) +/- 2. In another embodiment, the invention provides an anti-Factor D antibody wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as a IgG to Factor D) is about 9.0 nM ($9.0x10^{-9}$ M) +/- 1.

[00066] In another embodiment, an anti-Factor D antibody may have an affinity in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) of about 3.7 nM ($3.7x10^{-9}$ M) +/- 2. In another embodiment, an anti-Factor D antibody may have an affinity in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) of about 3.3 nM ($3.3x10^{-9}$ M) +/- 2. In another embodiment, an anti-Factor D antibody may have an affinity in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) of about 5.1 nM ($5.1x10^{-9}$ M) +/- 2. In another embodiment, an anti-Factor D antibody may have an affinity in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) of about 2.7 nM ($2.7x10^{-9}$ M) +/- 2. In another embodiment, an anti-Factor D antibody may have an affinity in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab

fragment to Factor D) of about 1.4 nM ($1.4x10^{-9}$ M) +/- 2. In another embodiment, an anti-Factor D antibody may have an affinity in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) of about 1.4 pM ($1.4x10^{-12}$ M) +/- 2. In another embodiment, an anti-Factor D antibody may have an affinity in its bivalent form to Factor D (e.g., affinity of the antibody as a IgG to Factor D) of about 1.1 pM ($1.1x10^{-12}$ M) +/- 2. In another embodiment, an anti-Factor D antibody may have an affinity in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is about 0.19 nM ($0.19x10^{-9}$ M) +/- 2. In another embodiment, an anti-Factor D antibody may have an affinity in its bivalent form to Factor D (e.g., affinity of the antibody as a IgG to Factor D) is about 0.08 nM ($0.08x10^{-9}$ M) +/- 2. In another embodiment, an anti-Factor D antibody may have an affinity in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is about 12.3 nM ($12.3x10^{-9}$ M) +/- 2. In another embodiment, an anti-Factor D antibody may have an affinity in its bivalent form to Factor D (e.g., affinity of the antibody as a IgG to Factor D) is about 9.0 nM ($9.0x10^{-9}$ M) +/- 2.

[00067] As is well-established in the art, binding affinity of a ligand to its receptor can be determined using any of a variety of assays, and expressed in terms of a variety of quantitative values. Accordingly, in one embodiment, the binding affinity is expressed as Kd values and reflects intrinsic binding affinity (e.g., with minimized avidity effects). Generally and preferably, binding affinity is measured *in vitro*, whether in a cell-free or cell-associated setting. As described in greater detail herein, fold difference in binding affinity can be quantified in terms of the ratio of the monovalent binding affinity value of a humanized antibody (e.g., in Fab form) and the monovalent binding affinity value of a reference/comparator antibody (e.g., in Fab form) (e.g., a murine antibody having donor hypervariable region sequences), wherein the binding affinity values are determined under similar assay conditions. Thus, in one embodiment, the fold difference in binding affinity is determined as the ratio of the Kd values of the humanized antibody in Fab form and said reference/comparator Fab antibody. For example, in one embodiment, if an antibody of the invention (A) has an affinity that is "3-fold lower" than the affinity of a reference antibody (M), then if the Kd value for A is 3x, the Kd value of M would be 1x, and the ratio of Kd of A to Kd of M would be 3:1. Conversely, in one embodiment, if an antibody of the invention (C) has an affinity

that is "3-fold greater" than the affinity of a reference antibody (R), then if the Kd value for C is 1x, the Kd value of R would be 3x, and the ratio of Kd of C to Kd of R would be 1:3. Any of a number of assays known in the art, including those described herein, can be used to obtain binding affinity measurements, including, for example, Biacore, radioimmunoassay (RIA) and ELISA.

[00068] Further, Kd values for an antibody of the invention may vary depending on conditions of the particular assay used. For example, in one embodiment, binding affinity measurements may be obtained in an assay wherein the Fab or antibody is immobilized and binding of the ligand, i.e. Factor D, is measured or alternatively, the ligand, i.e. Factor D, for the Fab or antibody is immobilized and binding of the Fab or antibody is measured. In one embodiment, the binding affinity measurements may be obtained in an assay wherein the regeneration conditions may comprise (1) 10mM glycein or 4M $MgCl_2$ at pH 1.5, and (2) pH between pH of 1.0 and pH of 7.5, including pH of 1.5, pH of 5.0, pH of 6.0 and pH of 7.2. In one embodiment, the binding affinity measurements may be obtained in an assay wherein the binding conditions may comprise (1) PBS or HEPES-buffered saline and (2) Tween-20, i.e. 0.1% Tween-20. In one embodiment, the binding affinity measurements may be obtained in an assay wherein the source of the ligand, i.e Factor D, may be from commercially available sources. In one embodiment, binding affinity measurements may be obtained in an assay wherein (1) the Fab or antibody is immobilized and binding of the ligand, i.e. Factor D is measured, (2) the regeneration conditions comprise 4M $MgCl_2$ at pH 7.2 and (3) the binding conditions comprise HEPES-buffered saline, pH 7.2 containing 0.1% Tween-20. In one embodiment, binding affinity measurements may be obtained in an assay wherein (1) the ligand, i.e. Factor D, is immobilized and binding of the Fab or antibody is measured, (2) the regeneration conditions comprise 10mM glycine at pH 1.5 and (3) the binding conditions comprise PBS buffer.

[00069] The terms "cell", "cell line" and "cell culture" include progeny. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Variant progeny that have the same function or biological property, as screened for in the originally transformed cell, are included. The "host cells" used in the present invention generally are prokaryotic or eukaryotic hosts.

[00070] The term "vector" means a DNA construct containing a DNA sequence which is operably linked to a suitable control sequence capable of effecting the expression of the DNA in a suitable host. Such control sequences include a promoter to effect transcription, an optional operator sequence to control such transcription, a sequence encoding suitable mRNA ribosome binding sites, and sequences which control the termination of transcription and translation. The vector may be a plasmid, a phage particle, or simply a potential genomic insert. Once transformed into a suitable host, the vector may replicate and function independently of the host genome, or may in some instances, integrate into the genome itself. In the present specification, "plasmid" and "vector" are sometimes used interchangeably, as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of vectors which serve equivalent function as and which are, or become, known in the art.

[00071] The word "label" when used herein refers to a detectable compound or composition which can be conjugated directly or indirectly to a molecule or protein, e.g., an antibody. The label may itself be detectable (e.g., radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

[00072] As used herein, "solid phase" means a non-aqueous matrix to which the antibody of the present invention can adhere. Example of solid phases encompassed herein include those formed partially or entirely of glass (e.g. controlled pore glass), polysaccharides (e.g., agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (e.g. an affinity chromatography column).

GENERATION OF ANTIBODIES

SELECTION AND TRANSFORMATION OF HOST CELLS

[00073] Suitable host cells for cloning or expressing the DNA in the vectors herein are prokaryotic, yeast, or higher eukaryotic cells. Suitable prokaryotes for this purpose include both Gram-negative and Gram-positive organisms, for example, Enterobacteria such as *E. coli, Enterobacter, Erwinia, Klebsiella, Proteus, Salmonella, Serratia,* and *Shigella*, as well as Bacilli, Pseudomonas, and Streptomyces. One preferred *E. coli* cloning host is E. coli 294 (ATCC 31,446),

although other strains such as E. coli B, E. coli X1776 (ATCC 31,537), and E. coli W3110 (ATCC 27,325) are suitable. These examples are illustrative rather than limiting.

[00074] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors. *Saccharomyces cerevisiae* is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe*; Kluyveromyces; Candida; Trichoderma; *Neurospora crassa*; and filamentous fungi such as e.g., Neurospora, Penicillium, Tolypocladium, and Aspergillus hosts, such as *A. nidulans* and *A. niger.*

[00075] Suitable host cells for the expression of glycosylated antibodies are derived from multicellular organisms. In principal, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. Examples of invertebrate cells include plant and insect cells, Luckow et al., Bio/Technology 6, 47-55 (1988); Miller et al., Genetic Engineering, Setlow et al. eds. Vol. 8, pp. 277-279 (Plenam publishing 1986); Mseda et al., Nature 315, 592-594 (1985). Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as Spodoptera frugiperda (caterpillar), Aedes (mosquito), Drosophila melanogaster (fruitfly), and Bombyx mori have been identified. A variety of viral strains for transfection are publicly available, e.g., the L-1 variant of Autographa californica NPV and the Bm-5 strain of Bombyx mori NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of Spodoptera frugiperda cells. Moreover, plant cells cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco and also be utilized as hosts.

[00076] Vertebrate cells, and propagation of vertebrate cells, in culture (tissue culture) has become a routine procedure. See Tissue Culture, Academic Press, Kruse and Patterson, eds. (1973). Examples of useful mammalian host cell lines are monkey kidney; human embryonic kidney line; baby hamster kidney cells; Chinese hamster ovary cells/-DHFR (CHO, Urlaub et al., Proc. Natl. Acad. Sci. USA 77: 4216 (1980)); mouse sertoli cells; human cervical carcinoma cells (HELA); canine kidney cells; human lung cells; human liver cells; mouse mammary tumor; and NS0 cells.

[00077] Host cells are transformed with the above-described vectors for antibody production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

[00078] The host cells used to produce the antibody variant of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium (MEM, Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium (DMEM, Sigma) are suitable for culturing host cells. In addition, any of the media described in Ham et al., Meth. Enzymol. 58: 44 (1979), Barnes et al., Anal. Biochem. 102: 255 (1980), U.S. Pat. Nos. 4,767,704; 4,657,866; 4,560,655; 5,122,469; 5,712,163; or 6,048,728 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as X-chlorides, where X is sodium, calcium, magnesium; and phosphates), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCIN.TM. drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

ANTIBODY PURIFICATION

[00079] When using recombinant techniques, the antibody can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the antibody variant is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, may be removed, for example, by centrifugation or ultrafiltration. Carter et al., Bio/Technology 10: 163-167 (1992) describe a procedure for isolating antibodies which are secreted to the periplasmic space of E. coli. Briefly, cell paste is thawed in the presence of sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonylfluoride (PMSF) over about 30 minutes. Cell debris can be removed by centrifugation. Where the antibody variant is secreted into the medium, supernatants from such expression systems are

generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

[00080] The antibody composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel elecrophoresis, dialysis, and affinity chromatography, with affinity chromatography being the preferred purification technique. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody variant. Protein A can be used to purify antibodies that are based on human IgG1, IgG2 or IgG4 heavy chains (Lindmark et al., J. Immunol Meth. 62: 1-13 (1983)). Protein G is recommended for all mouse isotypes and for human IgG3 (Guss et al., EMBO J. 5: 1567-1575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody variant comprises a CH3 domain, the Bakerbond ABXTM resin (J. T. Baker, Phillipsburg, N.J.) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody variant to be recovered.

[00081] Following any preliminary purification step(s), the mixture comprising the antibody variant of interest and contaminants may be subjected to low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, preferably performed at low salt concentrations (e.g., from about 0-0.25M salt).

PHARMACEUTICAL FORMULATIONS

[00082] Therapeutic formulations of the polypeptide or antibody may be prepared for storage as lyophilized formulations or aqueous solutions by mixing the polypeptide having the desired degree of purity with optional

"pharmaceutically-acceptable" carriers, excipients or stabilizers typically employed in the art (all of which are termed "excipients"). For example, buffering agents, stabilizing agents, preservatives, isotonifiers, non-ionic detergents, antioxidants and other miscellaneous additives. (See Remington's Pharmaceutical Sciences, 16th edition, A. Osol, Ed. (1980)). Such additives must be nontoxic to the recipients at the dosages and concentrations employed.

[00083] Buffering agents help to maintain the pH in the range which approximates physiological conditions. They are preferably present at concentration ranging from about 2 mM to about 50 mM. Suitable buffering agents for use with the present invention include both organic and inorganic acids and salts thereof such as citrate buffers (e.g., monosodium citrate-disodium citrate mixture, citric acid-trisodium citrate mixture, citric acid-monosodium citrate mixture, etc.), succinate buffers (e.g., succinic acid-monosodium succinate mixture, succinic acid-sodium hydroxide mixture, succinic acid-disodium succinate mixture, etc.), tartrate buffers (e.g., tartaric acid-sodium tartrate mixture, tartaric acid-potassium tartrate mixture, tartaric acid-sodium hydroxide mixture, etc.), fumarate buffers (e.g., fumaric acid-monosodium fumarate mixture, etc.), fumarate buffers (e.g., fumaric acid-monosodium fumarate mixture, fumaric acid-disodium fumarate mixture, monosodium fumarate-disodium fumarate mixture, etc.), gluconate buffers (e.g., gluconic acid-sodium glyconate mixture, gluconic acid-sodium hydroxide mixture, gluconic acid-potassium glyuconate mixture, etc.), oxalate buffer (e.g., oxalic acid-sodium oxalate mixture, oxalic acid-sodium hydroxide mixture, oxalic acid-potassium oxalate mixture, etc.), lactate buffers (e.g., lactic acid-sodium lactate mixture, lactic acid-sodium hydroxide mixture, lactic acid-potassium lactate mixture, etc.) and acetate buffers (e.g., acetic acid-sodium acetate mixture, acetic acid-sodium hydroxide mixture, etc.). Additionally, there may be mentioned phosphate buffers, histidine buffers and trimethylamine salts such as Tris.

[00084] Preservatives may be added to retard microbial growth, and may be added in amounts ranging from 0.2%-1% (w/v). Suitable preservatives for use with the present invention include phenol, benzyl alcohol, meta-cresol, methyl paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalconium halides (e.g., chloride, bromide, iodide), hexamethonium chloride,

alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, and 3-pentanol.

[00085] Isotonicifiers sometimes known as "stabilizers" may be added to ensure isotonicity of liquid compositions of the present invention and include polhydric sugar alcohols, preferably trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol.

[00086] Stabilizers refer to a broad category of excipients which can range in function from a bulking agent to an additive which solubilizes the therapeutic agent or helps to prevent denaturation or adherence to the container wall. Typical stabilizers can be polyhydric sugar alcohols (enumerated above); amino acids such as arginine, lysine, glycine, glutamine, asparagine, histidine, alanine, ornithine, L-leucine, 2-phenylalanine, glutamic acid, threonine, etc., organic sugars or sugar alcohols, such as lactose, trehalose, stachyose, mannitol, sorbitol, xylitol, ribitol, myoinisitol, galactitol, glycerol and the like, including cyclitols such as inositol; polyethylene glycol; amino acid polymers; sulfur containing reducing agents, such as urea, glutathione, thioctic acid, sodium thioglycolate, thioglycerol, .alpha.-monothioglycerol and sodium thio sulfate; low molecular weight polypeptides (i.e. <10 residues); proteins such as human serum albumin, bovine serum albumin, gelatin or immunoglobulins; hydrophylic polymers, such as polyvinylpyrrolidone monosaccharides, such as xylose, mannose, fructose, glucose; disaccharides such as lactose, maltose, sucrose and trisaccacharides such as raffinose; polysaccharides such as dextran. Stabilizers may be present in the range from 0.1 to 10,000 weights per part of weight active protein.

[00087] Non-ionic surfactants or detergents (also known as "wetting agents") may be added to help solubilize the therapeutic agent as well as to protect the therapeutic protein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stressed without causing denaturation of the protein. Suitable non-ionic surfactants include polysorbates (20, 80, etc.), polyoxamers (184, 188 etc.), Pluronic.RTM. polyols, polyoxyethylene sorbitan monoethers (Tween.RTM.-20, Tween.RTM.-80, etc.). Non-ionic surfactants may be present in a range of about 0.05 mg/ml to about 1.0 mg/ml, preferably about 0.07 mg/ml to about 0.2 mg/ml.

[00088] Additional miscellaneous excipients include bulking agents, (e.g. starch), chelating agents (e.g. EDTA), antioxidants (e.g., ascorbic acid, methionine, vitamin E), and cosolvents. The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desireable to further provide an immunosuppressive agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended. The active ingredients may also be entrapped in microcapsule prepared, for example, by coascervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsule and poly-(methylmethacylate) microcapsule, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microphores, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington 's Pharmaceutical Sciences, 16th edition, A. Osal, Ed. (1980).

[00089] The formulations to be used for in vivo administration must be sterile. This is readily accomplished, for example, by filtration through sterile filtration membranes. Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the antibody variant, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D- (-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37° C resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be

intermolecular S--S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

[00090] The amount of therapeutic polypeptide, antibody or fragment thereof which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. Where possible, it is desirable to determine the dose-response curve and the pharmaceutical compositions of the invention first in vitro, and then in useful animal model systems prior to testing in humans.

[00091] In a preferred embodiment, an aqueous solution of therapeutic polypeptide, antibody or fragment thereof is administered by subcutaneous injection. Each dose may range from about 0.5 μg to about 50 μg per kilogram of body weight, or more preferably, from about 3 μg to about 30 μg per kilogram body weight.

[00092] The dosing schedule for subcutaneous administration may vary form once a month to daily depending on a number of clinical factors, including the type of disease, severity of disease, and the subject's sensitivity to the therapeutic agent.

USES FOR THE HUMANIZED ANTIBODY

[00093] The humanized antibodies of the present invention are useful in diagnostic assays, e.g., for detecting expression of a target of interest in specific cells, tissues, or serum. For diagnostic applications, the antibody variant typically will be labeled with a detectable moiety. Numerous labels are available. Techniques for quantifying a change in fluorescence are described above. The chemiluminescent substrate becomes electronically excited by a chemical reaction and may then emit light which can be measured (using a chemiluminometer, for example) or donates energy to a fluorescent acceptor. Examples of enzymatic labels include luciferases (e.g., firefly luciferase and bacterial luciferase; U.S. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase such as horseradish peroxidase (HRPO), alkaline phosphatase, .beta.-galactosidase, glucoamylase, lysozyme, saccharide oxidases (e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (such

as uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like. Techniques for conjugating enzymes to antibodies are described in O'Sullivan et al., Methods for the Preparation of Enzyme-Antibody Conjugates for Use in Enzyme Immunoassay, in Methods in Enzym. (Ed. J. Langone & H. Van Vunakis), Academic press, New York, 73: 147-166 (1981).

[00094] Sometimes, the label is indirectly conjugated with the antibody variant. The skilled artisan will be aware of various techniques for achieving this. For example, the antibody variant can be conjugated with biotin and any of the three broad categories of labels mentioned above can be conjugated with avidin, or vice versa. Biotin binds selectively to avidin and thus, the label can be conjugated with the antibody variant in this indirect manner. Alternatively, to achieve indirect conjugation of the label with the antibody variant, the antibody variant is conjugated with a small hapten (e.g. digloxin) and one of the different types of labels mentioned above is conjugated with an anti-hapten antibody variant (e.g. anti-digloxin antibody). Thus, indirect conjugation of the label with the antibody variant can be achieved.

[00095] In another embodiment of the invention, the antibody variant need not be labeled, and the presence thereof can be detected using a labeled antibody which binds to the antibody variant.

[00096] The antibodies of the present invention may be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, Monoclonal Antibodies: A Manual of Techniques, pp. 147-158 (CRC Press, Inc. 1987).

[00097] Competitive binding assays rely on the ability of a labeled standard to compete with the test sample for binding with a limited amount of antibody variant. The amount of target in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies generally are insolubilized before or after the competition. As a result, the standard and test sample that are bound to the antibodies may conveniently be separated from the standard and test sample which remain unbound.

[00098] Sandwich assays involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, or the protein to be detected. In a sandwich assay, the test sample to be analyzed is bound by a first

antibody which is immobilized on a solid support, and thereafter a second antibody binds to the test sample, thus forming an insoluble three-part complex. See e.g., U.S. Pat. No. 4,376,110. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assay). For example, one type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme.

[00099] For immunohistochemistry, the tumor sample may be fresh or frozen or may be embedded in paraffin and fixed with a preservative such as formalin, for example.

[000100] The antibodies may also be used for in vivo diagnostic assays. Generally, the antibody variant is labeled with a radionucleotide (such as .sup.111 In, .sup.99 Tc, .sup.14 C, .sup.131 I, .sup.3 H, .sup.32 P or .sup.35 S) so that the tumor can be localized using immunoscintiography. For example, a high affinity anti-IgE antibody of the present invention may be used to detect the amount of IgE present in, e.g., the lungs of an asthmatic patient.

[000101] The antibody of the present invention can be provided in a kit, i.e., packaged combination of reagents in predetermined amounts with instructions for performing the diagnostic assay. Where the antibody variant is labeled with an enzyme, the kit may include substrates and cofactors required by the enzyme (e.g., a substrate precursor which provides the detectable chromophore or fluorophore). In addition, other additives may be included such as stabilizers, buffers (e.g., a block buffer or lysis buffer) and the like. The relative amounts of the various reagents may be varied widely to provide for concentrations in solution of the reagents which substantially optimize the sensitivity of the assay. Particularly, the reagents may be provided as dry powders, usually lyophilized, including excipients which on dissolution will provide a reagent solution having the appropriate concentration.

IN VIVO USES FOR THE ANTIBODY

[000102] It is contemplated that the antibodies of the present invention may be used to treat a mammal. In one embodiment, the antibody is administered to a nonhuman mammal for the purposes of obtaining preclinical data, for example. Exemplary nonhuman mammals to be treated include nonhuman primates, dogs, cats, rodents and other mammals in which preclinical studies are performed.

Such mammals may be established animal models for a disease to be treated with the antibody or may be used to study toxicity of the antibody of interest. In each of these embodiments, dose escalation studies may be performed on the mammal.

[000103] The antibody or polypeptide is administered by any suitable means, including parenteral, subcutaneous, intraperitoneal, intrapulmonary, and intranasal, and, if desired for local immunosuppressive treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. In addition, the antibody variant is suitably administered by pulse infusion, particularly with declining doses of the antibody variant. Preferably the dosing is given by injections, most preferably intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic.

[000104] For the prevention or treatment of disease, the appropriate dosage of the antibody or polypeptide will depend on the type of disease to be treated, the severity and course of the disease, whether the antibody variant is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician.

[000105] Depending on the type and severity of the disease, about 0.1 mg/kg to 150 mg/kg (e.g., 0.1-20 mg/kg) of antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 mg/kg to 100 mg/kg or more, depending on the factors mentioned above. 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. An exemplary dosing regimen is disclosed in WO 94/04188.

[000106] The antibody compositions may be formulated, dosed and administered in a manner consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of

administration, the scheduling of administration, and other factors known to medical practitioners. The "therapeutically effective amount" of the antibody to be administered will be governed by such considerations, and is the minimum amount necessary to prevent, ameliorate, or treat a disease or disorder. The antibody need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as used hereinbefore or about from 1 to 99% of the heretofore employed dosages.

[000107] The antibodies of the present invention which recognize Factor D as their target may be used to treat complement-mediated disorders. These disorders are associated with excessive or uncontrolled complement activation. They include: Complement activation during cardiopulmonary bypass operations; complement activation due to ischemia-reperfusion following acute myocardial infarction, aneurysm, stroke, hemorrhagic shock, crush injury, multiple organ failure, hypobolemic shock and intestinal ischemia. These disorders can also include disease or condition is an inflammatory condition such as severe burns, endotoxemia, septic shock, adult respiratory distress syndrome, hemodialysis; anaphylactic shock, severe asthma, angioedema, Crohn's disease, sickle cell anemia, poststreptococcal glomerulonephritis and pancreatitis. The disorder may be the result of an adverse drug reaction, drug allergy, IL-2 induced vascular leakage syndrome or radiographic contrast media allergy. It also includes autoimmune disease such as systemic lupus erythematosus, myasthenia gravis, rheumatoid arthritis, Alzheimer's disease and multiple sclerosis. Complement activation is also associated with transplant rejection. Recently there has been a strong correlation shown between complement activation and ocular diseases such as age-related macular degeneration, diabetic retinopathy.

EXAMPLES

[000108] The following examples are offered by way of illustration and not by way of limitation.

Example 1: Humanization of Factor D Murine MAb 166-32

[000109] The sequences of the heavy chain variable region (V_H) and the light chain variable region (V_L) of murine mAb 166-32 were compared with human

antibody germline sequences available in the public databases. Several criteria were used when deciding on a template as described in step 1 above, including overall length, similar CDR position within the framework, overall homology, size of the CDR, etc. All of these criteria taken together provided a result for choosing the optimal human template as shown in the sequence alignment between 166-32 MAb heavy and light chain sequences and the respective human template sequences depicted in Figures 3 and 4.

[000110] In this case, more than one human framework template was used to design this antibody. The human template chosen for the V_H chain was a combination of VI-4.1b+ (7-04.1 locus)(access# X62110)(VH7 family) and JH4d (See Figure 3). The human template chosen for the V_L chain was a combination of DPK4 (VK I family) combined with JK2 (See Figure 4).

[000111] Once the template was chosen, a Fab library was constructed by DNA synthesis and overlapping PCR. The library was composed of synthesized MAb 166-32 CDRs synthesized with the respective chosen human templates. The overlapping nucleotides encoding partial V_H and V_L sequences were synthesized in the range of about 63 to about 76 nucleotides with 18 to 21 nucleotide overlaps. Vectors expressing a library of humanized Fabs against Factor D antigen were constructed, and transformed into *E. coli* DH10B then plated on XL-1B bacterial lawn.

[000112] Library quality was evaluated for the size (the number of independent clones) and the diversity (the distribution of the mutations). The individual clones with double insertion of both light and heavy chain was about 14 out of 20 sequenced. Framework wobble mutations were evenly distributed.

[000113] PCR amplification of V_L and V_H gene was performed using a biotinylated forward primer containing the specific sequence to the framework region FR1 and an overhanging sequence annealed to the end of leader sequence (GeneIII) and a reverse primer from the conserved constant region (Cκ or CH1) under standard PCR conditions. The PCR product was purified by agarose gel electrophoresis, or by commercial PCR purification kit to remove unincorporated biotinylated primers and non-specific PCR.

EXAMPLE 2: Library Screening

[000114] Capture Filter Lift was used for primary screening. The actual screening sized is more than 3 times larger than the theoretic library size. The

candidates were further screened by single-point ELISA assay. The best binders were further confirmed by direct antigen titration using Factor D based on Fab concentration

[000115] *Capture lift screening*

[000116] Capture Filter Lift Assay was used for primary screening for the binding of Fab to Factor D. High titer phage were plated and incubated at 37^{0}C till use (about 6-8 hr). Goat anti-human kappa was diluted to 10ug/ml in 10 ml PBST; Nitrocellulose filters for lifting plaques were prepared according to standard plaque lifting procedures and then immersed in10ml blocking buffer for 2 hrs on a shaker. The filters were rinsed 3x with PBST. The filters were applied to a plaque lawn and incubated at RT for approximately 15-24 hours. The filters were then removed from the plates and rinsed with TBST 3x.

[000117] Factor D (50ug/ml) was diluted in PBST to 0.1ug/ml and 4ml per filter was added. The filters were incubated in the solution for 2hr on a shaker at RT followed by rinsing 3x, each time 5min. Diluted 166-222-HRP (1:10, 000 with PBST) was added at a volume of 4 ml per filter and the filters were incubated for 1hr on a shaker. The filters were rinsed 4x. The filters were dried and then immersed in TMB substrate followed by immersion in water to stop the reaction. Positive clones were identified.

EXAMPLE 3: Single-Point ELISA screening

[000118] Single-Point ELISA assay was used for the secondary screening. Immulon II plates were coated with goat anti-human Fab (1:12,000, 50ul/well) over night at RT. The next day the plates were washed 4x with a plate washer. Blocking buffer was added at a volume of100ul per well and plates incubated for 1hr at RT. The plates were then washed 4x.

[000119] Each Fab to be screened was added at a volume of 50ul per well (either from 15ml periplasmic preparation or supernatant) and incubated 1 hr at RT. Plates were washed 4x followed by the addition of 50ul/well biotinylated factor D at 0.01ug/ml. Plates were incubated for 1hr at RT and then washed 4x. StreptAvidin-HRP was added (1:10,000 in PBST) and incubated for 1hr at RT. Plates were washed 5x and then developed by adding TMB substrate at 50ul/well. Stop buffer was added at a volume of 50ul when it is well-developed (10-45 min) and the plates were read at 450nm.

Example 4: Sequencing of Humanized anti-Factor D clones

[000120] Sixteen humanized clones with good binding affinity for human Factor D were sequenced (see Table 1). Among these, position 2 (100% human) and 49 (100% mouse) in the light chain, and position 93 (100% mouse) in the heavy chain are highly conserved indicating that they are important in maintaining antibody binding ability.

Table 1. Amino acid sequence analysis of humanized clones from the humanization library

VK	2	4	13	43	49	64	69	VH	2	9	38	93	97
Mouse	T	V	M	P	S	S	A		I	P	K	T	E
Human	I	M	A	V	Y	G	T		V	S	R	V	A
7	I	M	M	V	S	S	T		I	P	K	V	E
30	I	V	A	V	S	S	A		V	P	K	T	E
45	I	V	M	V	S	G	A		I	S	R	V	E
46	I	V	M	V	S	S	T		I	S	R	V	E
47	I	V	A	V	S	S	T		I	S	R	V	E
48	I	V	M	V	S	S	T		V	P	R	V	E
50	I	V	M	V	S	G	A		V	P	R	T	E
51	I	M	M	V	S	G	T		I	S	K	T	E
56	**I**	**V**	**A**	**V**	**S**	**G**	**T**		**V**	**P**	**K**	**T**	**E**
57	I	M	M	V	S	S	A		V	S	R	V	E
58	I	V	M	P	S	G	A		V	P	R	V	E
59	I	V	A	P	S	S	T		V	P	K	V	E
60	I	V	M	P	S	G	T		V	P	R	V	E
63	I	V	M	V	S	S	T		V	S	R	T	E
74	I	V	M	V	S	S	T		I	S	R	V	E

[000121] Clone #56 was evaluated by BIAcore analysis and hemolytic inhibition assay. BIAcore analysis showed that clone #56 has a similar affinity to human Factor D as chimeric 166-32 Fab (see Table 4). Hemolytic inhibition assay showed that clone #56 is somewhat more potent than chimeric 166-32 Fab (see Figure 6). Clone # 56 contains two murine residues in the framework of light

chain and four murine residues in the heavy chain. (see Table 1). Based on these results, further optimization was carried out.

Table 2. Amino acid sequence analysis of optimized antibodies from the humanization/CDR3s optimization library

VK Positions	2	4	13	43	49	64	69	CDR-L3	92	93	97	
166-32	T	V	M	P	S	S	A		D	N	T	
Human Template	I	M	A	V	Y	G	T					
104	I	V	M	P	S	S	T		D	S	T	
109	I	V	A	V	S	G	A		M	N	T	
111	I	V	A	V	S	G	T		D	S	T	
112	I	V	A	V	S	G	T		D	S	T	
114	I	V	A	V	S	G	T		D	C	T	
121	I	V	A	V	S	S	A		D	N	T	
125	I	V	A	P	S	S	T		D	N	T	
130	I	V	A	V	S	S	T		D	N	S	

VH Positions	2	9	38	93	97	CDR-H3	98	99	100
166-32	I	P	K	T	E		V	D	N
Human Template	V	S	R	V	A				
104	V	S	R	V	E		V	D	T
109	V	S	R	V	E		V	N	N
111	V	S	R	V	E		V	N	N
112	V	S	R	V	E		V	N	N
114	V	S	K	V	E		V	N	N
121	I	S	R	V	E		V	N	T
125	V	S	R	V	E		P	D	N
130	V	S	R	V	E		V	D	H

[000122] Clone #111 and #114 were characterized by BIAcore analysis (see Table 4). Clone #104, #111, #114 and #130 were also characterized by hemolytic inhibition assay (see Figure 6). These clones have higher affinities than chimeric 166-32, and are more potent than chimeric Fab in inhibiting the

alternative pathway as shown by hemolytic inhibition assay (Figure 7). Clone #111 contains the same two murine residues in the light chain (position 4 and 49) as clone # 56. It also contains the conserved murine residue in the heavy chain position 97 as found in clone #56. There is one beneficial mutation in both light and heavy chain CDR3 in clone #111. From two independent libraries screened (humanization library, and humanization/CDR3s optimization library), we found that the best clones have similar consensus residues.

[000123] To further optimize the affinity of clone #111, an antibody library was constructed by introducing single mutations into the CDR-H1 and CDR-L2 simultaneously. In brief, site-directed mutagenesis approach was used to construct such libraries by annealing oligonucleotides encoding single mutations to the template of clone #111. A total of 24 clones with very high affinity to human Factor D were sequenced. Among those 24 clones, several redundant beneficial mutations were identified. Clones #250, #315, #345 and #416 were selected for BIAcore analysis (see Table 4). BIAcore data showed that these clones have higher affinity to human Factor D than initial clone #111. Clone #250, #315, #348 and #416 were also tested in the hemolytic inhibition assay (see Figure 6) and inhibition of the alternative pathway (Figure 7).

Example 5: AP Hemolysis Assay

[000124] Biological function of the humanized clones was determined using hemolytic inhibition assay and BIAcore analysis (See Example 6 below). Hemolytic assay was performed according to the following procedure. 20 ul of 1:20 diluted rabbit red blood cells (RRBC) (0.5 ml + 9.5 ml GVB/Mg-EGTA buffer) in 20 ml Saline (0.9% NaCl) at approximately 1 : $2x10^4$ dilution were counted by Coulter Counter. The cell concentration was then adjusted to about 2-5 $x10^4$ cells/ml. Each plate received about $500x10^6$ / plate RRBC or about 1 ml RRBC/plate ($500x10^6$ / 2-5 $x10^4$).

[000125] Cells were diluted in 6 ml GVB/Mg-EGTA buffer/plate, mixed and washed 3 times by spinning at 1360 rpm x 4 min at 4 °C. The RRBC pellet is suspended in 3 ml GVB/Mg-EGTA buffer /plate and kept on ice.

[000126] Human serum from –80°C freezer was thawed just prior to use. The serum was diluted to a concentration of 20 % serum in GVB/Mg-EGTA buffer, 5 ml/plate (final is 10 %) and kept on ice.

Table 3

	1	2	3	S	SB
GVB/Mg-EGTA:	50 ul	50 ul	50 ul	50 ul	50 ul
mAb: 50 ul mix	50 ul	50 ul	50 ul	---	---
20% Hu serum:	50 ul	50 ul	50 ul	50 ul	50 ul
	Shake 30 sec at 5-6°, then keep at RT for 7 min.				
Rabbit RBC:	30ul	30 ul	30 ul		30 ul

[000127] Samples were shaken for 30 sec at 5-6°C and then for 40 minutes at 37 °C. Samples were cooled to 5-6°C while shaking and then centrifuged at 2,000 rpm x 3 min at 4 °C. Approximately 80 ul supernatant was transferred to a flat-bottom 96 well plate and the OD value at 590 nm was read using a standard plate reader. The percent inhibition was calculated as follows: % Inhibition = {[(S-SB) – (U-SB)] / (S-SB)} x 100%. (U = sample 1, 2 or 3 (columns 1, 2 or 3 of Table 3, respectively).

Example 6: Kinetic Analysis of anti-Human Factor D Fab by BiaCore

[000128] *Immobilization*- Human factor D (Advanced Research Inc, 0.1 mg/ml) was directly immobilized onto the CM5 chip (BiaCore) using amine-coupling method. The procedure is briefly described as following: (1) Constant flow (PBS) is at 5 µl/min. (2) Injection of 35 µl EDC/NHS (1:1). (3) Injection of 35 µl of human factor D in acetate buffer, pH 4.5. (4) Block the activated group by injection of 35 µl etholamine. (5) Clean-up the surface by 5 µl 10 mM Glycine pH 1.5. The ligand (human factor D) immobilization level is about 1,000 RU. Test run using α-human factor D (huDi, 40 µl, 31.5 µg/ml) yielded a relative response around 900 RU.

[000129] *Kinetic analysis*- All anti-human factor D Fabs were diluted in PBS buffer. Each sample was prepared in a series of concentrations: 12.5 nM, 25 nM, 50 nM, 75nM, 100 nM, 125 nM, and 150 nM, with 40 µl injection pulse at high acquisition rate. Regeneration was accomplished by applying 5 µl pulse of 10 mM Glycine at pH 1.5. Kinetic parameters were obtained by fitting Fabs binding traces to 1:1 binding model under pseudo-first order kinetic using BIAvaluation version

3.0. The results are presented in Table 2 below. All data are obtained by global fitting routine.

Table 4 BIAcore Results

Fab Clone	ka (M-1s-1)	kd (s-1)	Kd (M)
Anti-factor D Fab 315	$7.1x10^{5}$	$2.7x10^{-4}$	$3.7x10^{-10}$
Anti-factor D Fab 416	$8.2x10^{5}$	$1.8x10^{-4}$	$3.3x10^{-10}$
Anti-factor D Fab 345	$6.8x10^{5}$	$3.5x10^{-4}$	$5.1x10^{-10}$
Anti-factor D Fab 250	$5.7x10^{5}$	$1.9x10^{-4}$	$3.3x10^{-10}$
Anti-factor D Fab 56	$3.6x10^{5}$	$9.8x10^{-4}$	$2.7x10^{-9}$
chimeric	$4.4x10^{5}$	$1.2x10^{-3}$	$2.7x10^{-9}$
Anti-factor D Fab 111	$3.3x10^{5}$	$3.7x10^{-4}$	$1.14x10^{-9}$

[000130] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

We Claim:

1. A variable domain of a humanized antibody comprising a heavy chain variable domain amino acid sequence selected from the group consisting of: SEQ ID NO: 6; SEQ ID NO: 8; SEQ ID NO: 10; and SEQ ID NO: 12.

2. A variable domain of a humanized antibody comprising a light chain variable domain amino acid sequence selected from the group consisting of SEQ ID NO: 5; SEQ ID NO: 7; SEQ ID NO: 9 and SEQ ID NO: 11.

3. A humanized anti-Factor D antibody comprising the variable domain sequence of SEQ ID NO: 5 and the variable domain sequence in SEQ ID NO: 6.

4. A humanized anti-Factor D antibody comprising the variable domain sequence of SEQ ID NO: 7 and the variable domain sequence in SEQ ID NO: 8.

5. A humanized anti-Factor D antibody comprising the variable domain sequence of SEQ ID NO: 9 and the variable domain sequence in SEQ ID NO: 10.

6. A humanized anti-Factor D antibody comprising the variable domain sequence of SEQ ID NO: 11 and the variable domain sequence in SEQ ID NO: 12.

7. A humanized anti-Factor D antibody having a variable light chain comprising a CDR-L1 having the sequence of SEQ ID NO: 16; a CDR-L2 having

the sequence of SEQ ID NO: 17, 21, or 24, and a CDR-L3 having the sequence of SEQ ID NO: 18, 19 or 22.

8. A humanized anti-Factor D antibody having a variable heavy chain comprising a CDR-H1 having the sequence of SEQ ID NO: 13, 23 or 25; a CDR-H2 having the sequence of SEQ ID NO: 14; and a CDR-H3 having the sequence of SEQ ID NO: 15 or 20.

9. A polypeptide comprising the following amino acid sequence: $QX_1QLVQSGX_2E$ LKKPGASVKV SCKASGYTFT SYGMNWVX_3QA PGQGLEWMGW INTYTGETTYADDFKGRFVF SLDTSVSTAY LQISSLKAED TAX_4YYCX_5REG GVNNWGQGTL VTVSS (SEQ ID NO: 27), wherein X_1 is I or V; X_2 is P or S; X_3 is K or R; X_4 is T or V; and X_5 is E or A.

10. A polypeptide comprising the following amino acid sequence: DIQX_6TQSPSSLSX_7SVGDRVTITCITSTDIDDDMNWYQQKPGKX_8PKLLIX_9DGNT LRPGVPSRFSX_{10}SGSGX_{11}DFTLTISSLQPEDVATYYCLQSDSLPYTFGQ GTKLEIK (SEQ ID NO: 26), wherein X_6 is V or M; X_7 is M or A; X_8 is P or V; X_9 is S or Y; X_{10} is S or G and X_{11} is A or T.

11. A polypeptide comprising the amino acid sequence of SEQ ID NO: 6, 8, 10, or 12.

12. A polypeptide comprising the amino acid sequence of SEQ ID NO: 5, 7, 9, or 11.

13. A humanized antibody comprising the polypeptide of Claim 9.

14. The humanized antibody of Claim 13 further comprising the polypeptide of Claim 10 or 25.

15. An antibody fragment of any one of Claims 1-8, 13, 14, 23, 24 or 34 to 52.

16. An isolated nucleic acid encoding an antibody of any one of Claims 1-8, 13, 14, 23, 24 or 34 to 52.

17. An isolated nucleic acid encoding the polypeptide of any one of Claims 9 to 12, 25, 26, 53 or 54.

18. A vector comprising the nucleic acid of Claim 16 or 17.

19. A cell line comprising the vector of Claim 18.

20. A composition comprising the antibody of any one of Claims 1-8, 13, 14, 23, 24 or 34 to 52.

21. Use of the composition of Claim 20 in the manufacture of a medicament for treating complement mediated disorders.

22. The use according to Claim 21, wherein the disorder is an ocular disease such as age-related macular degeneration or diabetic retinopathy.

23. A variable domain of Claim 2 or 47 wherein amino acid at position 104 of SEQ ID NO: 7 is a valine or a leucine.

24. A humanized anti-Factor D antibody of Claim 4 comprising the variable domain sequence of SEQ ID NO: 7 wherein the amino acid at position 104 of SEQ ID NO: 7 is a valine or a leucine and the variable domain sequence of SEQ ID NO: 8.

25. A polypeptide of Claim 10 wherein the amino acid at position 104 of SEQ ID NO: 26 is a valine or a leucine.

26. A polypeptide of Claim 12 or 54 wherein the amino acid at position 104 of SEQ ID NO: 7 is a valine or a leucine.

27. The method of making a humanized anti-Factor D antibody or fragment thereof of any of claims 1-8, 13, 14, 23, 24 or 34 to 52.

28. An antibody which competes with murine antibody 166-32.

29. An antibody which competes with humanized anti-Factor D antibody clone #56, #111, #250 or #416 and/or an antibody comprising variable domain or HVR sequences of humanized anti-Factor D antibody clone #56, #111, #250 or #416.

30. An antibody which binds to the same epitope as murine antibody 166-32.

31. An antibody which binds to the same epitope as humanized anti-Factor D antibody clone #56, #111, #250 or #416 and/or an antibody comprising variable domain or HVR sequences of humanized anti- Factor D antibody clone #56, #111, #250 or #416.

32. The method of claim 27 wherein said method comprising expressing the isolated nucleic acid of claim 16 or 17 in a cell.

33. The method of claim 32 wherein said method further comprising purifying the antibody or fragment thereof.

34. A humanized antibody comprising a heavy chain variable domain amino acid sequence selected from the group consisting of: SEQ ID NO: 6; SEQ ID NO: 8; SEQ ID NO: 10; and SEQ ID NO: 12.

35. A humanized antibody comprising a light chain variable domain amino acid sequence selected from the group consisting of: SEQ ID NO: 5; SEQ ID NO: 7; SEQ ID NO: 9; and SEQ ID NO: 11.

36. The humanized antibody of Claim 35 or 49 comprising the light chain variable domain amino acid sequence of SEQ ID NO:7, wherein the amino acid at position 104 of SEQ ID NO:7 is a valine or a leucine.

37. A humanized antibody comprising the polypeptide of Claim 10 or 25.

38. A humanized anti-Factor D antibody having a variable light chain comprising a CDR-L1 having the sequence of SEQ ID NO: 16; a CDR-L2 having the sequence of SEQ ID NO: 21 or 24, and a CDR-L3 having the sequence of SEQ ID NO: 18, 19 or 22.

39. A humanized anti-Factor D antibody having a variable light chain comprising a CDR-L1 having the sequence of SEQ ID NO: 16; a CDR-L2 having the sequence of SEQ ID NO: 17, 21 or 24, and a CDR-L3 having the sequence of SEQ ID NO: 19 or 22.

40. A humanized anti-Factor D antibody having a variable heavy chain comprising a CDR-H1 having the sequence of SEQ ID NO: 23 or 25; a CDR-H2 having the sequence of SEQ ID NO: 14; and a CDR-H3 having the sequence of SEQ ID NO: 15 or 20.

41. A humanized anti-Factor D antibody having a variable heavy chain comprising a CDR-H1 having the sequence of SEQ ID NO: 13, 23 or 25; a CDR-H2 having the sequence of SEQ ID NO: 14; and a CDR-H3 having the sequence of SEQ ID NO: 20.

42. A humanized anti-Factor D antibody having (i) a variable light chain comprising a CDR-L1 having the sequence of SEQ ID NO: 16; a CDR-L2 having the sequence of SEQ ID NO: 21 or 24, and a CDR-L3 having the sequence of SEQ ID NO: 18, 19 or 22; and (ii) a variable heavy chain comprising a CDR-H1 having the sequence of SEQ ID NO: 13, 23 or 25; a CDR-H2 having the sequence of SEQ ID NO: 14; and a CDR-H3 having the sequence of SEQ ID NO: 15 or 20.

43. A humanized anti-Factor D antibody having (i) a variable light chain comprising a CDR-L1 having the sequence of SEQ ID NO: 16; a CDR-L2 having the sequence of SEQ ID NO: 17, 21 or 24, and a CDR-L3 having the sequence of SEQ ID NO: 19 or 22; and (ii) a variable heavy chain comprising a CDR-H1 having the sequence of SEQ ID NO: 13, 23 or 25; a CDR-H2 having the sequence of SEQ ID NO: 14; and a CDR-H3 having the sequence of SEQ ID NO: 15 or 20.

44. A humanized anti-Factor D antibody having (i) a variable light chain comprising a CDR-L1 having the sequence of SEQ ID NO: 16; a CDR-L2 having the sequence of SEQ ID NO: 17, 21 or 24, and a CDR-L3 having the sequence of SEQ ID NO: 18, 19 or 22; and (ii) a variable heavy chain comprising a CDR-H1 having the sequence of SEQ ID NO: 23 or 25; a CDR-H2 having the sequence of SEQ ID NO: 14; and a CDR-H3 having the sequence of SEQ ID NO: 15 or 20.

45. A humanized anti-Factor D antibody having (i) a variable light chain comprising a CDR-L1 having the sequence of SEQ ID NO: 16; a CDR-L2 having the sequence of SEQ ID NO: 17, 21 or 24, and a CDR-L3 having the sequence of SEQ ID NO: 18, 19 or 22; and (ii) a variable heavy chain comprising a CDR-H1 having the sequence of SEQ ID NO: 13, 23 or 25; a CDR-H2 having the sequence of SEQ ID NO: 14; and a CDR-H3 having the sequence of SEQ ID NO: 20.

46. A variable domain of a humanized antibody comprising a heavy chain variable domain amino acid sequence of SEQ ID NO: 8.

47. A variable domain of a humanized antibody comprising a light chain variable domain amino acid sequence of SEQ ID NO: 7.

48. A humanized antibody comprising a heavy chain variable domain amino acid sequence of SEQ ID NO: 8.

49. A humanized antibody comprising a light chain variable domain amino acid sequence of SEQ ID NO: 7.

50. A humanized anti-Factor D antibody having a variable light chain comprising a CDR-L1 having the sequence of SEQ ID NO: 16; a CDR-L2 having the sequence of SEQ ID NO: 17, and a CDR-L3 having the sequence of SEQ ID NO: 19.

51. A humanized anti-Factor D antibody having a variable heavy chain comprising a CDR-H1 having the sequence of SEQ ID NO: 13; a CDR-H2 having the sequence of SEQ ID NO: 14; and a CDR-H3 having the sequence of SEQ ID NO: 20.

52. A humanized anti-Factor D antibody having a variable light chain comprising a CDR-L1 having the sequence of SEQ ID NO: 16; a CDR-L2 having the sequence of SEQ ID NO: 17, and a CDR-L3 having the sequence of SEQ ID NO: 19; and a variable heavy chain comprising a CDR-H1 having the sequence of SEQ ID NO: 13; a CDR-H2 having the sequence of SEQ ID NO: 14; and a CDR-H3 having the sequence of SEQ ID NO: 20.

53. A polypeptide comprising the amino acid sequence of SEQ ID NO: 8.

54. A polypeptide comprising the amino acid sequence of SEQ ID NO: 7.

SEQUENCE LISTING

<110> Genentech, Inc.
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<120> Humanized Anti-Factor D Antibodies and Uses Thereof

<130> P4028R1 WO

<150> US 60/856,505
<151> 2006-11-02

<160> 58

<170> PatentIn version 3.4

<210> 1
<211> 115
<212> PRT
<213> ARTIFICIAL

<220>
<223> VARIABLE HEAVY CHAIN DOMAIN OF MAB 166-32

<400> 1

Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
1 5 10 15

Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr
65 70 75 80

Leu Glu Ile Asn Asn Leu Lys Asn Glu Asp Met Ala Thr Tyr Phe Cys
85 90 95

Glu Arg Glu Gly Gly Val Asp Asn Trp Gly Gln Gly Thr Thr Leu Thr
100 105 110

Val Ser Ser
115

<210> 2
<211> 107
<212> PRT
<213> ARTIFICIAL

<220>
<223> VARIABLE LIGHT CHAIN DOMAIN OF MAB 166-32

<400> 2

Glu Thr Thr Val Thr Gln Ser Pro Ala Ser Leu Ser Met Ala Ile Gly
1 5 10 15

Glu Lys Val Thr Ile Arg Cys Ile Thr Ser Thr Asp Ile Asp Asp Asp
20 25 30

Met Asn Trp Tyr Gln Gln Lys Pro Gly Glu Pro Pro Lys Leu Leu Ile
35 40 45

Ser Gly Gly Asn Thr Leu Arg Pro Gly Val Pro Ser Arg Phe Ser Ser
50 55 60

Ser Gly Tyr Gly Ala Asp Phe Val Phe Thr Ile Asp Asn Met Leu Ser
65 70 75 80

Glu Asp Val Ala Asp Tyr Tyr Cys Leu Gln Ser Asp Asn Leu Pro Tyr
85 90 95

Thr Phe Gly Gly Gly Thr Arg Leu Glu Ile Lys
100 105

<210> 3
<211> 345
<212> DNA
<213> ARTIFICIAL

<220>
<223> VARIABLE HEAVY CHAIN DOMAIN OF MAB 166-32

<400> 3
cagatccagt tggtgcagtc tggacctgag ctgaagaagc ctggagagac agtcaagatc 60

tcctgcaagg cttctgggta taccttcaca aactatggaa tgaactgggt gaagcaggct 120

ccaggaaagg gtttaaagtg gatgggctgg ataaacacct acactggaga gacaacatat 180

gctgatgact tcaagggacg gtttgtcttc tctttggaaa cctctgccag cactgcctat 240

ttggagatca acaacctcaa aaatgaggac atggctacat atttctgtga aagagagggg 300

ggggttgaca actggggcca aggcaccact ctcacagtct cctca 345

<210> 4
<211> 321
<212> DNA
<213> ARTIFICIAL

<220>
<223> VARIABLE LIGHT CHAIN DOMAIN OF MAB 166-32

<400> 4
gaaacaactg tgacccagtc tcctgcatcc ctgtccatgg ctataggaga aaaagtcacc 60

atcagatgca taaccagcac tgatattgat gatgatatga actggtacca gcagaagcca 120

ggggaacctc ctaagctcct tatttcagga ggcaatactc ttcgtcctgg agtcccatcc 180

cgattctcca gcagtggcta tggtgcagat tttgttttta caattgacaa catgctctca 240

gaagatgttg cagattacta ctgtttgcaa agtgataact tgccgtacac gttcggaggg 300

gggaccaggc tggaaataaa a 321

<210> 5
<211> 107
<212> PRT
<213> ARTIFICIAL

<220>
<223> VARIABLE LIGHT CHAIN DOMAIN OF HUMANIZED CLONE #56

<400> 5

Asp Ile Gln Val Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Arg
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ile Thr Ser Thr Asp Ile Asp Asp Asp
20 25 30

Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Leu Ile
35 40 45

Ser Gly Gly Asn Thr Leu Arg Pro Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Val Ala Thr Tyr Tyr Cys Leu Gln Ser Asp Asn Leu Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 6
<211> 115
<212> PRT
<213> ARTIFICIAL

<220>
<223> VARIABLE HEAVY CHAIN DOMAIN OF HUMANIZED CLONE #56

<400> 6

Gln Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Lys Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Thr Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asp Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 7
<211> 107
<212> PRT
<213> ARTIFICIAL

<220>
<223> VARIABLE LIGHT CHAIN DOMAIN OF HUMANIZED CLONE #111

<400> 7

Asp Ile Gln Val Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ile Thr Ser Thr Asp Ile Asp Asp Asp
20 25 30

Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Leu Ile
35 40 45

Ser Gly Gly Asn Thr Leu Arg Pro Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Val Ala Thr Tyr Tyr Cys Leu Gln Ser Asp Ser Leu Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 8
<211> 115
<212> PRT
<213> ARTIFICIAL

<220>
<223> VARIABLE HEAVY CHAIN DOMAIN OF HUMANIZED CLONE # 111

<400> 8

Gln Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 9
<211> 107
<212> PRT
<213> ARTIFICIAL

<220>
<223> VARIABLE LIGHT CHAIN DOMAIN OF HUMANIZED CLONE #250

<400> 9

Asp Ile Gln Val Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ile Thr Ser Thr Asp Ile Asp Asp Asp
20 25 30

Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Leu Ile
35 40 45

Ser His Gly Asn Thr Leu Arg Pro Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Val Ala Thr Tyr Tyr Cys Leu Gln Ser Asp Ser Leu Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 10
<211> 115
<212> PRT
<213> ARTIFICIAL

<220>
<223> VARIABLE HEAVY CHAIN DOMAIN OF HUMANIZED CLONE #250

<400> 10

Gln Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Leu Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 11
<211> 107
<212> PRT
<213> ARTIFICIAL

<220>
<223> VARIABLE LIGHT CHAIN DOMAIN OF HUMANIZED CLONE #416

<400> 11

Asp Ile Gln Val Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ile Thr Ser Thr Asp Ile Asp Asp Asp
20 25 30

Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Leu Ile
35 40 45

Ser Asp Gly Asn Thr Leu Arg Pro Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Val Ala Thr Tyr Tyr Cys Leu Gln Ser Asp Ser Leu Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 12
<211> 115
<212> PRT
<213> ARTIFICIAL

<220>
<223> VARIABLE HEAVY CHAIN DOMAIN OF HUMANIZED CLONE #416

<400> 12

Gln Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 13
<211> 10
<212> PRT
<213> ARTIFICIAL

<220>
<223> CDR-H1 OF MAB 166-32

<400> 13

Gly Tyr Thr Phe Thr Asn Tyr Gly Met Asn
1 5 10

<210> 14
<211> 19
<212> PRT
<213> ARTIFICIAL

<220>
<223> CDR-H2 OF MAB 166-32

<400> 14

Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp
1 5 10 15

Asp Phe Lys

<210> 15
<211> 6
<212> PRT
<213> ARTIFICIAL

<220>
<223> CDR-H3 OF MAB 166-32

<400> 15

Glu Gly Gly Val Asp Asn
1 5

<210> 16
<211> 11
<212> PRT
<213> ARTIFICIAL

<220>
<223> CDR-L1 OF MAB 166-32

<400> 16

Ile Thr Ser Thr Asp Ile Asp Asp Asp Met Asn
1 5 10

<210> 17
<211> 7
<212> PRT
<213> ARTIFICIAL

<220>
<223> CDR-L2 OF MAB 166-32

<400> 17

Gly Gly Asn Thr Leu Arg Pro
1 5

<210> 18
<211> 9
<212> PRT
<213> ARTIFICIAL

<220>
<223> CDR-L3 OF MAB 166-32

<400> 18

Leu Gln Ser Asp Asn Leu Pro Tyr Thr
1 5

<210> 19
<211> 9
<212> PRT
<213> ARTIFICIAL

<220>
<223> CDR-L3 OF HUMANIZED CLONE #111

<400> 19

Leu Gln Ser Asp Ser Leu Pro Tyr Thr
1 5

<210> 20
<211> 6
<212> PRT
<213> ARTIFICIAL

<220>
<223> CDR-H3 OF HUMANIZED CLONE #111

<400> 20

Glu Gly Gly Val Asn Asn
1 5

<210> 21
<211> 7
<212> PRT
<213> ARTIFICIAL

<220>
<223> CDR-L2 OF HUMANIZED CLONE #250

<400> 21

His Gly Asn Thr Leu Arg Pro
1 5

<210> 22
<211> 9
<212> PRT
<213> ARTIFICIAL

<220>
<223> CDR-L3 OF HUMANIZED CLONE #250

<400> 22

Leu Gln Ser Asp Ser Leu Pro Tyr Thr
1 5

<210> 23
<211> 10

<212> PRT
<213> ARTIFICIAL

<220>
<223> CDR-H1 OF HUMANIZED CLONE #250

<400> 23

Gly Tyr Thr Phe Thr Asn Tyr Gly Leu Asn
1 5 10

<210> 24
<211> 7
<212> PRT
<213> ARTIFICIAL

<220>
<223> CDR-L2 OF HUMANIZED CLONE #416

<400> 24

Asp Gly Asn Thr Leu Arg Pro
1 5

<210> 25
<211> 10
<212> PRT
<213> ARTIFICIAL

<220>
<223> CDR-H1 OF HUMANIZED CLONE #416

<400> 25

Gly Tyr Thr Phe Thr Ser Tyr Gly Met Asn
1 5 10

<210> 26
<211> 107
<212> PRT
<213> ARTIFICIAL

<220>
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<220>
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<222> (4)..(4)
<223> Xaa can be any naturally occurring amino acid

<220>
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<222> (13)..(13)
<223> Xaa can be any naturally occurring amino acid

<220>
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<222> (43)..(43)
<223> Xaa can be any naturally occurring amino acid

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<223> Xaa can be any naturally occurring amino acid

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<222> (64)..(64)
<223> Xaa can be any naturally occurring amino acid

<220>
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<222> (69)..(69)
<223> Xaa can be any naturally occurring amino acid

<400> 26

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Asp Ile Gln Xaa Thr Gln Ser Pro Ser Ser Leu Ser Xaa Ser Val Gly
1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Ile Thr Ser Thr Asp Ile Asp Asp Asp
            20                  25                  30

Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Xaa Pro Lys Leu Leu Ile
        35                  40                  45

Xaa Asp Gly Asn Thr Leu Arg Pro Gly Val Pro Ser Arg Phe Ser Xaa
    50                  55                  60

Ser Gly Ser Gly Xaa Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Val Ala Thr Tyr Tyr Cys Leu Gln Ser Asp Ser Leu Pro Tyr
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
            100                 105
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<210> 27
<211> 115
<212> PRT
<213> ARTIFICIAL

<220>
<223> VARIABLE HEAVY CHAIN COMPOSITE OF HUMANIZED CLONES

<220>
<221> misc_feature
<222> (2)..(2)
<223> Xaa can be any naturally occurring amino acid

<220>
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<220>
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<222> (38)..(38)
<223> Xaa can be any naturally occurring amino acid

<220>
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<222> (93)..(93)

<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (97)..(97)
<223> Xaa can be any naturally occurring amino acid

<400> 27

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Gln Xaa Gln Leu Val Gln Ser Gly Xaa Glu Leu Lys Lys Pro Gly Ala
1               5                  10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30

Gly Met Asn Trp Val Xaa Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
    50                  55                  60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Xaa Tyr Tyr Cys
                85                  90                  95

Xaa Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
            100                 105                 110

Val Ser Ser
        115
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<210> 28
<211> 87
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 28

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Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                  10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Trp Val
            20                  25                  30

Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Arg Val Thr Ile
        35                  40                  45

Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu
    50                  55                  60

Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Trp Gly Gln Gly
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65 70 75 80

Thr Leu Val Thr Val Ser Ser
85

<210> 29
<211> 81
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 29

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Trp Val Arg Gln Ala Pro Gly
20 25 30

Gln Gly Leu Glu Trp Met Arg Val Thr Ile Thr Ala Asp Thr Ser Thr
35 40 45

Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala
50 55 60

Val Tyr Tyr Cys Ala Arg Trp Gly Gln Gly Thr Leu Val Thr Val Ser
65 70 75 80

Ser

<210> 30
<211> 80
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 30

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Trp Val Arg Gln Ala Pro Gly
20 25 30

Gln Gly Leu Glu Trp Met Arg Val Thr Ile Thr Ala Asp Thr Ser Thr
35 40 45

Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala
50 55 60

Val Tyr Tyr Cys Ala Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

65 70 75 80

<210> 31
<211> 79
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 31

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Trp Val Arg Gln Ala Pro Gly
20 25 30

Gln Gly Leu Glu Trp Met Arg Val Thr Ile Thr Ala Asp Thr Ser Thr
35 40 45

Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala
50 55 60

Val Tyr Tyr Cys Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
65 70 75

<210> 32
<211> 87
<212> PRT
<213> ARTIFICIAL

<220>
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<400> 32

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Val Ser Trp Ile
20 25 30

Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile Gly Arg Val Thr Ile
35 40 45

Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu Lys Leu Ser Ser Val
50 55 60

Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg Trp Gly Gln Gly
65 70 75 80

Thr Leu Val Thr Val Ser Ser
85

<210> 33

<211> 81
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 33

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Trp Ile Arg Gln Pro Pro Gly
20 25 30

Lys Gly Leu Glu Trp Ile Arg Val Thr Ile Ser Val Asp Thr Ser Lys
35 40 45

Asn Gln Phe Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala
50 55 60

Val Tyr Tyr Cys Ala Arg Trp Gly Gln Gly Thr Leu Val Thr Val Ser
65 70 75 80

Ser

<210> 34
<211> 80
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 34

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Trp Ile Arg Gln Pro Pro Gly
20 25 30

Lys Gly Leu Glu Trp Ile Arg Val Thr Ile Ser Val Asp Thr Ser Lys
35 40 45

Asn Gln Phe Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala
50 55 60

Val Tyr Tyr Cys Ala Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
65 70 75 80

<210> 35
<211> 79
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 35

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Trp Ile Arg Gln Pro Pro Gly
20 25 30

Lys Gly Leu Glu Trp Ile Arg Val Thr Ile Ser Val Asp Thr Ser Lys
35 40 45

Asn Gln Phe Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala
50 55 60

Val Tyr Tyr Cys Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
65 70 75

<210> 36
<211> 87
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 36

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Trp Val
20 25 30

Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Arg Phe Thr Ile
35 40 45

Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu
50 55 60

Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Trp Gly Gln Gly
65 70 75 80

Thr Leu Val Thr Val Ser Ser
85

<210> 37
<211> 81
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 37

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Trp Val Arg Gln Ala Pro Gly
            20                  25                  30

Lys Gly Leu Glu Trp Val Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys
        35                  40                  45

Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
    50                  55                  60

Val Tyr Tyr Cys Ala Arg Trp Gly Gln Gly Thr Leu Val Thr Val Ser
65                  70                  75                  80

Ser
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<210> 38
<211> 80
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 38

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Trp Val Arg Gln Ala Pro Gly
            20                  25                  30

Lys Gly Leu Glu Trp Val Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys
        35                  40                  45

Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
    50                  55                  60

Val Tyr Tyr Cys Ala Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
65                  70                  75                  80
```

<210> 39
<211> 79
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 39

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15
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Ser Leu Arg Leu Ser Cys Ala Ala Ser Trp Val Arg Gln Ala Pro Gly
20 25 30

Lys Gly Leu Glu Trp Val Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys
35 40 45

Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
50 55 60

Val Tyr Tyr Cys Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
65 70 75

<210> 40
<211> 87
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 40

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Trp Val
20 25 30

Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Arg Phe Thr Ile
35 40 45

Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu
50 55 60

Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gln Gly
65 70 75 80

Thr Leu Val Thr Val Ser Ser
85

<210> 41
<211> 81
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 41

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Trp Val Arg Gln Ala Pro Gly
20 25 30

Lys Gly Leu Glu Trp Val Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys
35 40 45

Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
50 55 60

Val Tyr Tyr Cys Ser Arg Trp Gly Gln Gly Thr Leu Val Thr Val Ser
65 70 75 80

Ser

<210> 42
<211> 80
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 42

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Trp Val Arg Gln Ala Pro Gly
20 25 30

Lys Gly Leu Glu Trp Val Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys
35 40 45

Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
50 55 60

Val Tyr Tyr Cys Ser Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
65 70 75 80

<210> 43
<211> 87
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 43

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Trp Val
20 25 30

Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Arg Phe Thr Ile
35 40 45

```
Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu
     50                  55                  60

Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Trp Gly Gln Gly
65                  70                  75                  80

Thr Leu Val Thr Val Ser Ser
                85
```

<210> 44
<211> 81
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 44

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Trp Val Arg Gln Ala Pro Gly
            20                  25                  30

Lys Gly Leu Glu Trp Val Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys
        35                  40                  45

Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
    50                  55                  60

Val Tyr Tyr Cys Ala Arg Trp Gly Gln Gly Thr Leu Val Thr Val Ser
65                  70                  75                  80

Ser
```

<210> 45
<211> 80
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 45

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Trp Val Arg Gln Ala Pro Gly
            20                  25                  30

Lys Gly Leu Glu Trp Val Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys
        35                  40                  45
```

Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
50 55 60

Val Tyr Tyr Cys Ala Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
65 70 75 80

<210> 46
<211> 79
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 46

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Trp Val Arg Gln Ala Pro Gly
20 25 30

Lys Gly Leu Glu Trp Val Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys
35 40 45

Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
50 55 60

Val Tyr Tyr Cys Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
65 70 75

<210> 47
<211> 80
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 47

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Trp Tyr Gln Gln Lys Pro Gly Lys Ala
20 25 30

Pro Lys Leu Leu Ile Tyr Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
35 40 45

Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp
50 55 60

Phe Ala Thr Tyr Tyr Cys Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
65 70 75 80

<210> 48
<211> 80
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 48

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Trp Tyr Leu Gln Lys Pro Gly Gln Ser
20 25 30

Pro Gln Leu Leu Ile Tyr Gly Val Pro Asp Arg Phe Ser Gly Ser Gly
35 40 45

Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp
50 55 60

Val Gly Val Tyr Tyr Cys Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
65 70 75 80

<210> 49
<211> 80
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 49

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Trp Tyr Gln Gln Lys Pro Gly Gln Ala
20 25 30

Pro Arg Leu Leu Ile Tyr Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly
35 40 45

Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu Asp
50 55 60

Phe Ala Val Tyr Tyr Cys Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
65 70 75 80

<210> 50
<211> 80
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 50

```
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1               5                  10                  15

Glu Arg Ala Thr Ile Asn Cys Trp Tyr Gln Gln Lys Pro Gly Gln Pro
            20                  25                  30

Pro Lys Leu Leu Ile Tyr Gly Val Pro Asp Arg Phe Ser Gly Ser Gly
        35                  40                  45

Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp
    50                  55                  60

Val Ala Val Tyr Tyr Cys Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
65                  70                  75                  80
```

<210> 51
<211> 31
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 51

```
Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr Leu Gln
1               5                  10                  15

Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ser
            20                  25                  30
```

<210> 52
<211> 32
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 52

```
Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr Leu Gln
1               5                  10                  15

Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ser Arg
            20                  25                  30
```

<210> 53
<211> 10
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 53

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
1 5 10

<210> 54
<211> 10
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<220>
<221> misc_feature
<222> (7)..(7)
<223> Xaa can be any naturally occurring amino acid

<400> 54

Phe Gly Gln Gly Thr Lys Xaa Glu Ile Lys
1 5 10

<210> 55
<211> 87
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 55

Gln Val Gln Leu Val Gln Ser Gly Ser Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Trp Val
20 25 30

Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Arg Phe Val Phe
35 40 45

Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr Leu Gln Ile Ser Ser Leu
50 55 60

Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Trp Gly Gln Gly
65 70 75 80

Thr Ser Leu Thr Val Ser Ser
85

<210> 56
<211> 81
<212> PRT
<213> artificial

<220>
<223> Sequence is synthesized

<400> 56

Gln Val Gln Leu Val Gln Ser Gly Ser Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Trp Val Arg Gln Ala Pro Gly
20 25 30

Gln Gly Leu Glu Trp Met Arg Phe Val Phe Ser Leu Asp Thr Ser Val
35 40 45

Ser Thr Ala Tyr Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala
50 55 60

Val Tyr Tyr Cys Ala Arg Trp Gly Gln Gly Thr Ser Leu Thr Val Ser
65 70 75 80

Ser

<210> 57
<211> 80
<212> PRT
<213> artificial

<220>
<223> Sequence is synthesized

<400> 57

Gln Val Gln Leu Val Gln Ser Gly Ser Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Trp Val Arg Gln Ala Pro Gly
20 25 30

Gln Gly Leu Glu Trp Met Arg Phe Val Phe Ser Leu Asp Thr Ser Val
35 40 45

Ser Thr Ala Tyr Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala
50 55 60

Val Tyr Tyr Cys Ala Trp Gly Gln Gly Thr Ser Leu Thr Val Ser Ser
65 70 75 80

<210> 58
<211> 79
<212> PRT
<213> ARTIFICIAL

<220>
<223> Sequence is synthesized

<400> 58

Gln Val Gln Leu Val Gln Ser Gly Ser Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

```
Ser Val Lys Val Ser Cys Lys Ala Ser Trp Val Arg Gln Ala Pro Gly
            20                  25                  30

Gln Gly Leu Glu Trp Met Arg Phe Val Phe Ser Leu Asp Thr Ser Val
        35                  40                  45

Ser Thr Ala Tyr Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala
    50                  55                  60

Val Tyr Tyr Cys Trp Gly Gln Gly Thr Ser Leu Thr Val Ser Ser
65                  70                  75
```