ANTIBODIES DIRECTED AGAINST AMYLOID-BETA PEPTIDE AND METHODS USING SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of provisional patent applications U.S. Serial No. 60/676,093, filed April 29, 2005, and U.S. Serial No. 60/704,818, filed August 1, 2005, all of which are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

[0002] The invention concerns antibodies to amyloid-beta peptide. The invention further concerns use of such antibodies in the treatment and/or prevention of diseases, such as Alzheimer's disease.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0003] Not applicable.

BACKGROUND OF THE INVENTION

[0004] Alzheimer's disease (AD) is a degenerative brain disorder characterized clinically by progressive memory deficits, confusion, gradual physical deterioration and, ultimately, death. Approximately 15 million people worldwide are affected by Alzheimer's disease, and the number is expected to increase dramatically as lifespan increases. Histologically, the disease is characterized by neuritic plaques, found primarily in the association cortex, limbic system and basal ganglia. The major constituent of these plaques is amyloid beta peptide (Aβ), which is the cleavage product of beta amyloid precursor protein (βAPP or APP). APP is a type I transmembrane glycoprotein that contains a large ectopic N-terminal domain, a transmembrane domain, and a small cytoplasmic C-terminal tail. Alternative splicing of the transcript of the single APP gene on chromosome 21 results in several isoforms that differ in the number of amino acids.

[0005] Aβ appears to have a central role in the neuropathology of Alzheimer's disease. Familial forms of the disease have been linked to mutations in APP and the presenilin genes

(Tanzi et al., 1996, Neurobiol. Dis. 3:159-168; Hardy, 1996, Ann. Med. 28:255-258). Diseased-linked mutations in these genes result in increased production of the 42-amino acid form of Aβ, the predominant form found in amyloid plaques. Moreover, immunization of transgenic mice that overexpress a disease-linked mutant form of APP with human Aβ reduces plaque burden and associated pathologies (Schenk et al., 1999, Nature 400:173-177; WO 99/27944), and peripheral administration of antibodies directed against Aβ also reduces plaque burden in the brain (Bard et al., 2000, Nature Medicine 6(8):916-919; WO 2004/032868; WO 00/72880).

[0006] It has been reported that Fc-mediated phagocytosis by microglial cells and/or macrophages is important to the process of plaque clearance in vivo. Bard et al., *Proc. Natl. Acad. Sci. USA* 100, 2023-2028 (2003). However, it has also been reported that non-Fc-mediated mechanisms are involved in clearance of amyloid-β in vivo by immunotherapy. Bacskai et al., *J. Neurosci.* 22:7873-7878 (2002); Das et al., *J. Neurosci.* 23:8532-8538 (2003).

[0007] Antibody therapy therefore provides a promising approach to the treatment and prevention of Alzheimer's disease. However, human clinical trials with a vaccine including Aβ1-42 were suspended due to meningoencephalititis in a subset of patients. Orgogozo et al., *Neruology* 61:7-8 (2003); Ferrer et al., *Brain Pathol.* 14:11-20 (2004). It has been reported that passive immunization with an N-terminal specific anti-Aβ antibody results in a significant reduction of mainly diffuse amyloid, but induces an increase of cerebral microhemorrhage frequency in transgenic mice that exhibit the age-related development of amyloid plaques and neurodegeneration as well as cerebral amyloid angiopathy (CAA) similar to that observed in the human AD brain. Pfeifer et al., *Science* 298:1379 (2002). It has been suggested that exacerbation of cerebral amyloid angiopathy (CAA)-associated microhemorrhage in APP transgenic mice by passive immunization with antibody directed to beta-amyloid is dependent on antibody recognition of deposited forms of amyloid beta peptide. Racke et al., *J. Neurosci.* 25:629-636 (2005). Passive immunization with antibodies against a peptide component of an amyloid deposit, which antibodies are devoid of Fc regions, has been suggested in order to decrease the risk of inflammation. WO 03/086310. There remains a need for antibodies and other immunotherapeutic agents directed against Aβ having improved efficacy and safety profile, and which are suitable for use with human patients.

[0008] Throughout this application various publications (including patents and patent applications) are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference.

BRIEF SUMMARY OF THE INVENTION

[0009] The invention disclosed herein concerns antibodies and polypeptides that bind to C-terminus of Aβ peptide. In one aspect, the invention provides an antibody or a polypeptide that binds to $A\beta_{1-40}$, $A\beta_{1-42}$, and $A\beta_{1-43}$, wherein the antibody or the polypeptide binds to $A\beta_{1-40}$ with higher affinity than its binding to $A\beta_{1-42}$, and $A\beta_{1-43}$, and wherein the antibody or the polypeptide binds to an epitope on $A\beta_{1-40}$ that includes amino acids 25-34 and 40. In some embodiments, the antibody binds to $A\beta_{1-40}$ with at least about 40-fold higher affinity than its binding to $A\beta_{1-42}$ and/or $A\beta_{1-43}$. In some embodiments, the antibody is not antibody 2294.

[0010] In another aspect, the invention provides an antibody 6G (interchangeably termed "6G"). The amino acid sequences of the heavy chain and light chain variable regions of 6G are shown in Figure 1. The complementarity determining region (CDR) portions of antibody 6G (including Chothia and Kabat CDRs) are also shown in Figure 1.

[0011] In another aspect, the invention also provides antibody variants of 6G with amino acid sequences depicted in Table 3.

[0012] In another aspect, the invention provides an antibody comprising a fragment or a region of the antibody 6G or its variants shown in Table 3. In one embodiment, the fragment is a light chain of the antibody 6G. In another embodiment, the fragment is a heavy chain of the antibody 6G. In yet another embodiment, the fragment contains one or more variable regions from a light chain and/or a heavy chain of the antibody 6G. In yet another embodiment, the fragment contains one or more variable regions from a light chain and/or a heavy chain shown in Figure 1. In yet another embodiment, the fragment contains one or more CDRs from a light chain and/or a heavy chain of the antibody 6G.

[0013] In another aspect, the invention provides polypeptides (which may or may not be an antibody) comprising any one or more of the following: a) one or more CDR(s) of antibody 6G or its variants shown in Table 3; b) CDR H3 from the heavy chain of antibody 6G or its variants shown in Table 3; c) CDR L3 from the light chain of antibody 6G or its variants shown

in Table 3; d) three CDRs from the light chain of antibody 6G or its variants shown in Table 3; e) three CDRs from the heavy chain of antibody 6G or its variants shown in Table 3; f) three CDRs from the light chain and three CDRs from the heavy chain of antibody 6G or its variants shown in Table 3. The invention further provides polypeptides (which may or may not be an antibody) comprising any one or more of the following: a) one or more (one, two , three, four, five, or six) CDR(s) derived from antibody 6G or its variants shown in Table 3; b) a CDR derived from CDR H3 from the heavy chain of antibody 6G; and/or c) a CDR derived from CDR L3 from the light chain of antibody 6G. In some embodiments, the CDR is a CDR shown in Figure 1. In some embodiments, the one or more CDRs derived from antibody 6G or its variants shown in Table 3 are at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to at least one, at least two, at least three, at least four, at least five, or at least six CDRs of 6G or its variants.

[0014] In some embodiments, the CDR is a Kabat CDR. In other embodiments, the CDR is a Chothia CDR. In other embodiments, the CDR is a combination of a Kabat and a Chothia CDR (also termed "combined CDR" or "extended CDR"). In other words, for any given embodiment containing more than one CDR, the CDRs may be any of Kabat, Chothia, and/or combined.

[0015] In some embodiments, the antibody of the invention is a human antibody. In other embodiments, the antibody of the invention is a humanized antibody. In some embodiments, the antibody is monoclonal. In some embodiments, the antibody (or polypeptide) is isolated. In some embodiments, the antibody (or polypeptide) is substantially pure.

[0016] The heavy chain constant region of the antibodies may be from any types of constant region, such as IgG, IgM, IgD, IgA, and IgE; and any isotypes, such as IgG1, IgG2, IgG3, and IgG4.

[0017] In some embodiments, the antibody or the polypeptide described herein has impaired effector function. In some embodiments, the antibody or the polypeptide comprises a heavy chain constant region having impaired effector function, wherein the heavy chain constant region comprises a Fc region. In some embodiments, the N-glycosylation in the Fc region is

removed. In some embodiments, the Fc region comprises a mutation within the N-glycosylation recognition sequence, whereby the Fc region of the antibody or polypeptide is not N-glycosylated. In some embodiments, the Fc region is PEGylated. In some embodiments, the heavy chain constant region of the antibody or the polypeptide is a human heavy chain IgG2a constant region containing the following mutations: A330P331 to S330S331 (amino acid numbering with reference to the wildtype IgG2a sequence). In some embodiments, the antibody or the polypeptide comprises a constant region of IgG4 comprising the following mutations: E233F234L235 to P233V234A235. These amino acid positions are based on Kabat numbering.

[0018] In another aspect, the invention provides a polynucleotide (which may be isolated) comprising a polynucleotide encoding a fragment or a region of the antibody 6G or its variants shown in Table 3. In one embodiment, the fragment is a light chain of the antibody 6G. In another embodiment, the fragment is a heavy chain of the antibody 6G. In yet another embodiment, the fragment contains one or more variable regions from a light chain and/or a heavy chain of the antibody 6G. In yet another embodiment, the fragment contains one or more (i.e., one, two, three, four, five, six) complementarity determining regions (CDRs) from a light chain and/or a heavy chain of the antibody 6G.

[0019] In another aspect, the invention is a polynucleotide (which may be isolated) comprising a polynucleotide that encodes for antibody 6G or its variants shown in Table 3. In some embodiments, the polynucleotide comprises either or both of the polynucleotides shown in SEQ ID NO:9 and SEQ ID NO:10.

[0020] In another aspect, the invention provides polynucleotides encoding any of the antibodies (including antibody fragments) or polypeptides described herein.

[0021] In another aspect, the invention provides vectors (including expression and cloning vectors) and host cells comprising any of the polynucleotide disclosed herein.

[0022] In another aspect, the invention is a host cell comprising a polynucleotide encoding any of the antibodies described herein.

[0023] In another aspect, the invention is a complex of $A\beta_{1-40}$ bound by antibody 6G or its variants shown in Table 3.

[0024] In another aspect, the invention is a complex of $A\beta_{1-40}$ bound by any of the antibodies or polypeptides described herein.

[0025] In another aspect, the invention is a pharmaceutical composition comprising an effective amount of any of the antibodies, polypeptides, or polynucleotides described herein, and a pharmaceutically acceptable excipient. In some embodiments, the antibodies or the polypeptides comprise one or more CDRs of antibody 6G.

[0026] In another aspect, the invention is a method of generating antibody 6G comprising culturing a host cell or progeny thereof under conditions that allow production of antibody 6G, wherein the host cell comprises an expression vector that encodes for antibody 6G; and, in some embodiments, purifying the antibody 6G. In some embodiments, the expression vector comprises one or both of the polynucleotide sequences shown in SEQ ID NO:9 and SEQ ID NO:10.

[0027] In another aspect, the invention provides methods of generating any of the antibodies or polypeptides described herein by expressing one or more polynucleotides encoding the antibody (which may be separately expressed as a single light or heavy chain, or both a light and a heavy chain are expressed from one vector) or the polypeptide in a suitable cell, generally followed by recovering and/or isolating the antibody or polypeptides of interest.

[0028] The invention also provides a method for preventing, treating, inhibiting, or delaying the development of Alzheimer's disease and other diseases associated with altered Aβ or βAPP expression, or accumulation of Aβ peptide, such as Down's syndrome, Parkinson's disease, multi-infarct dementia, mild cognitive impairment, cerebral amyloid angiopathy, depression, Creutzfeldt-Jakob disease, dementia with Lewy body, and AIDS. The method comprises administering an effective dosage a pharmaceutical composition comprising an antibody, a polypeptide, or a polynucleotide of the invention to a subject.

[0029] The invention also provides a method of delaying development of a symptom associated with Alzheimer's disease or other diseases related to accumulation of Aβ peptide in a subject comprising administering an effective dosage of a pharmaceutical composition comprising an antibody, a polypeptide, or a polynucleotide of the invention to the subject.

[0030] The invention also provides a method of suppressing formation of amyloid plaques and/or amyloid accumulation in a subject comprising administering an effective dosage of a pharmaceutical composition comprising an antibody, a polypeptide, or a polynucleotide of the invention to the subject. In some embodiments, the amyloid plaques are in the brain (brain tissue) of

the subject. In some embodiments, the amyloid plaques are in the cerebral vasculature. In other embodiments, the amyloid accumulation is in the circulatory system.

[0031] The invention also provides a method of reducing amyloid plaques and/or amyloid accumulation in a subject comprising administering an effective dosage of a pharmaceutical composition comprising an antibody, a polypeptide, or a polynucleotide of the invention to the subject. In some embodiments, the amyloid plaques are in the brain (brain tissue) of the subject. In some embodiments, the amyloid plaques are in the cerebral vasculature. In other embodiments, the amyloid accumulation is in the circulatory system.

[0032] The invention also provides a method of removing or clearing amyloid plaques and/or amyloid accumulation in a subject comprising administering an effective dosage of a pharmaceutical composition comprising an antibody, a polypeptide, or a polynucleotide of the invention to the subject. In some embodiments, the amyloid plaques are in the brain (brain tissue) of the subject. In some embodiments, the amyloid plaques are in the cerebral vasculature. In other embodiments, the amyloid accumulation is in the circulatory system.

[0033] Additionally, the invention provides a method for inhibiting the accumulation of Aβ peptide in a tissue comprising contacting the tissue with an antibody or a polypeptide of the invention.

[0034] The invention also provides a method of reducing Aβ peptide (such as soluble, oligomeric and deposited form) in a subject comprising administrating to the subject an effective amount of an antibody, a polypeptide, or a polynucleotide of the invention. In some embodiments, the accumulation of Aβ peptide is inhibited and/or reduced in the brain. In some embodiments, the toxic effects of Aβ peptide are inhibited and/or reduced. Thus, the method of the invention can be used to treat any disease in which accumulation of Aβ peptide is present or suspected, such as Alzheimer's disease, Down's syndrome, Parkinson's disease, multi-infarct dementia, mild cognitive impairment, cerebral amyloid angiopathy, depression, Creutzfeldt-Jakob disease, or dementia with Lewy body.

[0035] The invention also provides methods of improving cognition or reversing cognitive decline associated with diseases associated with amyloid deposit of Aβ in a subject, such as Alzheimer's disease, comprising administering an effective dosage of a pharmaceutical

composition comprising an antibody, a polypeptide, or a polynucleotide of the invention to the subject.

[0036] Any antibodies, polypeptides, or polynucleotides described herein may be used for the methods of the invention. In some embodiments, the antibody is antibody 6G.

[0037] Antibodies and polypeptides of the invention can further be used in the detection, diagnosis and monitoring of Alzheimer's disease and other diseases associated with altered Aβ or βAPP expression, such as Down's syndrome, and AIDS. The method comprises contacting a specimen of a patient suspected of having altered Aβ or βAPP expression with an antibody of the invention and determining whether the level of Aβ or βAPP differs from that of a control or comparison specimen. In some embodiments, serum level of Aβ is measured before and after administration of an anti-Aβ antibody; and any increase of serum level of Aβ is assessed.

[0038] Administration of any antibody or polypeptide of the invention may be by any means known in the art, including: intravenously, subcutaneously, via inhalation, intraarterially, intramuscularly, intracardially, intraventricularly, parenteral, intrathecally, and intraperitoneally. Administration may be systemic, *e.g.* intravenously, or localized. This also generally applies to polypeptides and polynucleotides of the invention.

[0039] In another aspect, the invention provides kits and compositions comprising any one or more of the compositions described herein. These kits, generally in suitable packaging and provided with appropriate instructions, are useful for any of the methods described herein.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

[0040] Figure 1 shows the amino acid sequence of the heavy chain variable region (SEQ ID NO:1) and light chain variable region (SEQ ID NO:2) of the 6G antibody. The Kabat CDRs are in bold text, and the Chothia CDRs are underlined. The amino acid residues for the heavy chain and light chain variable region are numbered sequentially.

[0041] Figure 2 shows epitope mapping of antibody 6G by ELISA. Aβ peptides (1-16, 1-28, 17-40, 17-42, 22-35, 28-40, 28-42, 1-38, 1-40, 1-42, 1-43, and 33-40) were immobilized on ELISA plates. Monoclonal antibody 6G (20 nM) was incubated for 1 h with various immobilized peptides. Antibody 6G bound to immobilized Aβ peptides was measured using goat anti-human kappa HRP conjugated secondary antibody.

[0042] Figure 3 shows epitope mapping of antibody 6G by ELISA. Various Aβ peptides (assigned SEQ ID NO:18-29 from top to bottom sequences) were immobilized on ELISA plates. Antibody 6G was incubated for 1 h with various immobilized peptides. Antibody 6G bound to immobilized Aβ peptides was measured using goat anti-human kappa HRP conjugated secondary antibody. "NB" refers to no binding detected.

[0043] Figure 4 is a schematic graph showing epitope that antibody 6G binds on Aβ. Relative positions of Aβ in amyloid precursor protein (APP) and portion of APP in cell membrane are shown. "CT99" refers to C-terminal 99 amino acids of APP. The amino acid sequence shown is assigned SEQ ID NO:30.

[0044] Figure 5 is a photograph showing immunostaining of APP expression cells with monoclonal antibody directed to $A\beta_{1-16}$ (m2324) and antibody 6G. The top panels show cells under fluorescence microscope after the cells were incubated with m2324 or 6G (each 5 ug/ml) and binding was detected by secondary Cy3-conjugated goat anti-mouse or anti-human antibody. The bottom panels show cells observed under microscope.

[0045] Figure 6 shows epitope mapping of antibody 2294 and 6G by ELISA. Various Aβ peptides (assigned SEQ ID NO:18-26, 31, and 27-29 from top to bottom sequences) were immobilized on ELISA plates. Antibodies were incubated for 1 hour with various immobilized peptides. Antibody 6G bound to immobilized Aβ peptides were measured using goat anti-human kappa HRP conjugated secondary antibody. Antibody 2294 bound to immobilized Aβ peptides were measured using goat anti-mouse that binds to both heavy and light chain and is HRP conjugated secondary antibody. "NB" refers to no binding detected. The numbers in the columns under "2294" and "6G" represent absorbance at 450 nm.

DETAILED DESCRIPTION OF THE INVENTION

[0046] The invention disclosed herein provides antibodies and polypeptides that bind to C-terminus of Aβ. The invention also provides polynucleotides encoding these antibodies and/or polypeptides. The invention also provides methods of making and using these antibodies and polypeptides.

[0047] The invention also provides methods for treating or preventing diseases associated with β-amyloid deposit in an individual, such as Alzheimer's disease, Down's syndrome, multi-

infarct dementia, mild cognitive impairment, cerebral amyloid angiopathy, depression, Creutzfeldt-Jakob disease, and dementia with Lewy body in a subject by administering to the subject an effective amount of a pharmaceutical composition comprising an antibody, a polypeptide, or a polynucleotide encoding the antibody or the polypeptide described herein.

General Techniques

[0048] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, Molecular Cloning: A Laboratory Manual, second edition (Sambrook et al., 1989) Cold Spring Harbor Press; Oligonucleotide Synthesis (M.J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J.E. Cellis, ed., 1998) Academic Press; Animal Cell Culture (R.I. Freshney, ed., 1987); Introduction to Cell and Tissue Culture (J.P. Mather and P.E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., 1993-1998) J. Wiley and Sons; Methods in Enzymology (Academic Press, Inc.); Handbook of Experimental Immunology (D.M. Weir and C.C. Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (J.M. Miller and M.P. Calos, eds., 1987); Current Protocols in Molecular Biology (F.M. Ausubel et al., eds., 1987); PCR: The Polymerase Chain Reaction, (Mullis et al., eds., 1994); Current Protocols in Immunology (J.E. Coligan et al., eds., 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C.A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: a practical approach (D. Catty., ed., IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using antibodies: a laboratory manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti and J.D. Capra, eds., Harwood Academic Publishers, 1995).

Definitions

[0049] An "antibody" is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one

antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term encompasses not only intact polyclonal or monoclonal antibodies, but also fragments thereof (such as Fab, Fab', $F(ab')_2$, Fv), single chain (ScFv), mutants thereof, fusion proteins comprising an antibody portion, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site. An antibody includes an antibody of any class, such as IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant domain of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0050] As used herein, "monoclonal antibody" 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 antigenic site. Furthermore, in contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler and Milstein, 1975, Nature, 256:495, or may be made by recombinant DNA methods such as described in U.S. Pat. No. 4,816,567. The monoclonal antibodies may also be isolated from phage libraries generated using the techniques described in McCafferty et al., 1990, Nature, 348:552-554, for example.

[0051] As used herein, "humanized" antibodies refer to forms of non-human (*e.g.* murine) antibodies that are specific chimeric immunoglobulins, immunoglobulin chains, or fragments

thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, the humanized antibody may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences, but are included to further refine and optimize antibody performance. 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 optimally also will comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin. Antibodies may have Fc regions modified as described in WO 99/58572. Other forms of humanized antibodies have one or more CDRs (one, two, three, four, five, six) which are altered with respect to the original antibody, which are also termed one or more CDRs "derived from" one or more CDRs from the original antibody.

[0052] As used herein, "human antibody" means an antibody having an amino acid sequence corresponding to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies known in the art or disclosed herein. This definition of a human antibody includes antibodies comprising at least one human heavy chain polypeptide or at least one human light chain polypeptide. One such example is an antibody comprising murine light chain and human heavy chain polypeptides. Human antibodies can be produced using various techniques known in the art. In one embodiment, the human antibody is selected from a phage library, where that phage library expresses human antibodies (Vaughan et al., 1996, Nature Biotechnology, 14:309-314; Sheets et al., 1998, PNAS, (USA) 95:6157-6162; Hoogenboom and Winter, 1991, J. Mol. Biol., 227:381; Marks et al., 1991, J. Mol. Biol., 222:581). Human antibodies can also be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been

partially or completely inactivated. This approach is described in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016. Alternatively, the human antibody may be prepared by immortalizing human B lymphocytes that produce an antibody directed against a target antigen (such B lymphocytes may be recovered from an individual or may have been immunized *in vitro)*. See, e.g., Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985); Boerner et al., 1991, J. Immunol., 147 (1):86-95; and U.S. Patent No. 5,750,373.

[0053] As used herein, the terms "6G" and "antibody 6G" are used interchangeably to refer to an antibody having the heavy chain amino acid sequence shown in SEQ ID NO:11 and the light chain amino acid sequence shown in SEQ ID NO:12. The amino acid sequence of the heavy chain and light chain variable regions are shown in Figure 1. The CDR portions of antibody 6G (including Chothia and Kabat CDRs) are diagrammatically depicted in Figure 1. The polynucleotides encoding the heavy and light chain are shown in SEQ ID NO:13 and SEQ ID NO:14. The characterization of 6G is described in the Examples.

[0054] The terms "polypeptide", "oligopeptide", "peptide" and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art. It is understood that, because the polypeptides of this invention are based upon an antibody, the polypeptides can occur as single chains or associated chains.

[0055] "Polynucleotide," or "nucleic acid," as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present,

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

[0056] A "variable region" of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. The variable regions of the heavy and light chain each consist of four framework regions (FR) connected by three complementarity determining regions (CDRs) also known as hypervariable regions. The CDRs in each chain are held together in close proximity by the FRs and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies. There are at least two techniques for determining CDRs: (1) an approach based on cross-species sequence variability (i.e., Kabat et al. Sequences of Proteins of Immunological Interest, (5th ed., 1991, National Institutes of Health, Bethesda MD)); and (2) an approach based on crystallographic studies of antigen-antibody complexes (Al-lazikani et al (1997) J. Molec. Biol. 273:927-948)). As used herein, a CDR may refer to CDRs defined by either approach or by a combination of both approaches.

[0057] A "constant region" of an antibody refers to the constant region of the antibody light chain or the constant region of the antibody heavy chain, either alone or in combination.

[0058] An epitope that "preferentially binds" or "specifically binds" (used interchangeably herein) to an antibody or a polypeptide is a term well understood in the art, and methods to determine such specific or preferential binding are also well known in the art. A molecule is said to exhibit "specific binding" or "preferential binding" if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. An antibody "specifically binds" or "preferentially binds" to a target if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, an antibody that specifically or preferentially binds to an $A\beta_{1-40}$ epitope is an antibody that binds this epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other $A\beta_{1-40}$ epitopes or non-$A\beta_{1-40}$ epitopes. It is also understood by reading this definition that, for example, an antibody (or moiety or epitope) that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, "specific binding" or "preferential binding" does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding.

[0059] As used herein, "substantially pure" refers to material which is at least 50% pure (*i.e.*, free from contaminants), more preferably at least 90 % pure, more preferably at least 95% pure, more preferably at least 98% pure, more preferably at least 99% pure.

[0060] A "host cell" includes an individual cell or cell culture that can be or has been a recipient for vector(s) for incorporation of polynucleotide inserts. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected *in vivo* with a polynucleotide(s) of this invention.

[0061] The term "Fc region" is used to define a C-terminal region of an immunoglobulin heavy chain. The "Fc region" may be a native sequence Fc region or a variant Fc region. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The numbering of the residues in the Fc region is that of the EU index as in Kabat. Kabat et al., Sequences of Proteins of Imunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991. The Fc region of an immunoglobulin generally comprises two constant domains, CH2 and CH3.

[0062] As used herein, "Fc receptor" and "FcR" describe a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. FcRs are reviewed in Ravetch and Kinet, 1991, Ann. Rev. Immunol., 9:457-92; Capel et al., 1994, Immunomethods, 4:25-34; and de Haas et al., 1995, J. Lab. Clin. Med., 126:330-41. "FcR" also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., 1976, J. Immunol., 117:587; and Kim et al., 1994, J. Immunol., 24:249).

[0063] "Complement dependent cytotoxicity" and "CDC" refer to the lysing of a target in the presence of complement. The complement activation pathway is initiated by the binding of

the first component of the complement system (C1q) to a molecule (*e.g.* an antibody) complexed with a cognate antigen. To assess complement activation, a CDC assay, *e.g.* as described in Gazzano-Santoro *et al.*, J. Immunol. Methods, 202:163 (1996), may be performed.

[0064] A "functional Fc region" possesses at least one effector function of a native sequence Fc region. Exemplary "effector functions" include C1q binding; complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down-regulation of cell surface receptors (e.g. B cell receptor; BCR), etc. Such effector functions generally require the Fc region to be combined with a binding domain (e.g. an antibody variable domain) and can be assessed using various assays known in the art for evaluating such antibody effector functions.

[0065] A "native sequence Fc region" comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. A "variant Fc region" comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification, yet retains at least one effector function of the native sequence Fc region. Preferably, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, e.g. from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein will preferably possess at least about 80% sequence identity with a native sequence Fc region and/or with an Fc region of a parent polypeptide, and most preferably at least about 90% sequence identity therewith, more preferably at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% sequence identity therewith.

[0066] As used herein "antibody-dependent cell-mediated cytotoxicity" and "ADCC" refer to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FcRs) (e.g. natural killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cell. ADCC activity of a molecule of interest can be assessed using an *in vitro* ADCC assay, such as that described in U.S. Patent No. 5,500,362 or 5,821,337. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and NK cells. Alternatively, or additionally, ADCC activity of the

molecule of interest may be assessed *in vivo*, e.g., in a animal model such as that disclosed in Clynes et al., 1998, PNAS (USA), 95:652-656.

[0067] As used herein, an "effective dosage" or "effective amount" drug, compound, or pharmaceutical composition is an amount sufficient to effect beneficial or desired results. For prophylactic use, beneficial or desired results includes results such as eliminating or reducing the risk, lessening the severity, or delaying the outset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results includes clinical results such as inhibiting, suppressing or reducing the formation of amyloid plaques, reducing, removing, clearing amyloid plaques, improving cognition, reversing or slowing cognitive decline, sequestering or increasing soluble Aβ peptide circulating in biological fluids, decreasing one or more symptoms resulting from the disease (biochemical, histological and/or behavioral), including its complications and intermediate pathological phenotypes presenting during development of the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing effect of another medication, delaying the progression of the disease, and/or prolonging survival of patients. An effective dosage can be administered in one or more administrations. For purposes of this invention, an effective dosage of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective dosage of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective dosage" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

[0068] As used herein, "treatment" or "treating" is an approach for obtaining beneficial or desired results including clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: inhibiting, suppressing or reducing the formation of amyloid plaques, reducing, removing, or clearing amyloid plaques, improving cognition, reversing or slowing cognitive decline, sequestering

soluble Aβ peptide circulating in biological fluids, reducing Aβ peptide (including soluble, oligomeric and deposited) in a tissue (such as brain), inhibiting, slowing and/or reducing accumulation of Aβ peptide in the brain, inhibiting, slowing and/or reducing toxic effects of Aβ peptide in a tissue (such as brain), decreasing symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, delaying the progression of the disease, and/or prolonging survival of patients.

[0069] As used herein, "delaying" development of Alzheimer's disease means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease. This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. A method that "delays" development of Alzheimer's disease is a method that reduces probability of disease development in a given time frame and/or reduces extent of the disease in a given time frame, when compared to not using the method. Such comparisons are typically based on clinical studies, using a statistically significant number of subjects.

[0070] "Development" of Alzheimer's disease means the onset and/or progression of Alzheimer's disease within an individual. Alzheimer's disease development can be detectable using standard clinical techniques as described herein. However, development also refers to disease progression that may be initially undetectable. For purposes of this invention, progression refers to the biological course of the disease state, in this case, as determined by a standard neurological examination, or patient interview or may be determined by more specialized testing. A variety of these diagnostic tests include, but not limited to, neuroimaging, detecting alterations of levels of specific proteins in the serum or cerebrospinal fluid (e.g., amyloid peptides and Tau), computerized tomography (CT), and magnetic resonance imaging (MRI). "Development" includes occurrence, recurrence, and onset. As used herein "onset" or "occurrence" of Alzheimer's disease includes initial onset and and/or recurrence.

[0071] As used herein, administration "in conjunction" includes simultaneous administration and/or administration at different times. Administration in conjunction also encompasses administration as a co-formulation or administration as separate compositions. As

used herein, administration in conjunction is meant to encompass any circumstance wherein an anti-Aβ antibody and another agent are administered to an individual, which can occur simultaneously and/or separately. As further discussed herein, it is understood that an anti-Aβ antibody and the other agent can be administered at different dosing frequencies or intervals. For example, an anti-Aβ antibody can be administered weekly, while the other agent can be administered less frequently. It is understood that the anti-Aβ antibody and the other agent can be administered using the same route of administration or different routes of administration.

[0072] A "biological sample" encompasses a variety of sample types obtained from an individual and can be used in a diagnostic or monitoring assay. The definition encompasses blood and other liquid samples of biological origin, solid tissue samples such as a biopsy specimen or tissue cultures or cells derived therefrom, and the progeny thereof. The definition also includes samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components, such as proteins or polynucleotides, or embedding in a semi-solid or solid matrix for sectioning purposes. The term "biological sample" encompasses a clinical sample, and also includes cells in culture, cell supernatants, cell lysates, serum, plasma, biological fluid, and tissue samples.

[0073] A "subject" (alternatively referred to as an "individual") is a mammal, more preferably a human. Mammals also include, but are not limited to, farm animals (such as cows), sport animals, pets (such as cats, dogs, horses), primates, mice and rats.

[0074] As used herein, "vector" means a construct, which is capable of delivering, and preferably expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, plasmid, cosmid or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, DNA or RNA expression vectors encapsulated in liposomes, and certain eukaryotic cells, such as producer cells.

[0075] As used herein, "expression control sequence" means a nucleic acid sequence that directs transcription of a nucleic acid. An expression control sequence can be a promoter, such as a constitutive or an inducible promoter, or an enhancer. The expression control sequence is operably linked to the nucleic acid sequence to be transcribed.

[0076] As used herein, "pharmaceutically acceptable carrier" includes any material which, when combined with an active ingredient, allows the ingredient to retain biological activity and is non-reactive with the subject's immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Preferred diluents for aerosol or parenteral administration are phosphate buffered saline or normal (0.9%) saline. Compositions comprising such carriers are formulated by well known conventional methods (see, for example, *Remington's Pharmaceutical Sciences*, 18th edition, A. Gennaro, ed., Mack Publishing Co., Easton, PA, 1990; and *Remington, The Science and Practice of Pharmacy* 20th Ed. Mack Publishing, 2000).

[0077] The term "k_{on}", as used herein, is intended to refer to the on rate constant for association of an antibody to an antigen.

[0078] The term "k_{off}", as used herein, is intended to refer to the off rate constant for dissociation of an antibody from the antibody/antigen complex.

[0079] The term "K_D", as used herein, is intended to refer to the equilibrium dissociation constant of an antibody-antigen interaction.

Compositions and Methods of Making the Compositions

Anti-β-amyloid antibodies and polypeptides

[0080] The present invention provides an antibody that binds to the C-terminus of Aβ peptide. The invention provides an antibody or a polypeptide that binds to $A\beta_{1-40}$, $A\beta_{1-42}$, and $A\beta_{1-43}$. In some embodiments, the antibody or the polypeptide binds to $A\beta_{1-40}$ with higher affinity than its binding to $A\beta_{1-42}$, and $A\beta_{1-43}$. In some embodiments, the antibody binds to $A\beta_{1-36}$, $A\beta_{1-37}$, $A\beta_{1-38}$, and $A\beta_{1-39}$. In some embodiments, the antibody binds to $A\beta_{22-35}$. In some embodiments, the antibody binds to $A\beta_{28-40}$. In some embodiments, the antibody or the polypeptide binds to an epitope on $A\beta_{1-40}$ that includes amino acids 25-34 and 40.

[0081] This invention also provides compositions, including pharmaceutical compositions, comprising any of the antibodies or polypeptides described herein (such as antibody 6G and its variants shown in Table 3 or polypeptide derived from antibody 6G and its variants shown in Table 3); or polynucleotides described herein. As used herein, compositions

comprise one or more antibodies or polypeptides (which may or may not be an antibody) that bind to C-terminus of $A\beta_{1-40}$, and/or one or more polynucleotides comprising sequences encoding one or more antibodies or polypeptides that bind to C-terminus of $A\beta_{1-40}$. These compositions may further comprise suitable excipients, such as pharmaceutically acceptable excipients including buffers, which are well known in the art.

[0082] The antibodies and polypeptides of the invention are characterized by any (one or more) of the following characteristics: (a) binds to $A\beta_{1-40}$, $A\beta_{1-42}$, and $A\beta_{1-43}$; (b) binds to $A\beta_{1-40}$, $A\beta_{1-42}$, and $A\beta_{1-43}$ with higher affinity binding to $A\beta_{1-40}$ than to $A\beta_{1-42}$ and $A\beta_{1-43}$; (c) binds to an epitope on $A\beta_{1-40}$ that includes amino acids 25-34 and 40; (d) binds to $A\beta_{1-36}$, $A\beta_{1-37}$, $A\beta_{1-38}$, and $A\beta_{1-39}$, but with lower affinity as compared to its binding to $A\beta_{1-40}$; (e) binds to $A\beta_{22-37}$ with a K_D of less than about 1 μM; (f) binds to $A\beta_{22-35}$; (g) binds to $A\beta_{28-40}$; (h) does not bind to APP expressed in a cell; (i) suppresses formation of amyloid plaques in a subject; (j) reduces amyloid plaques in a subject; (k) treats, prevents, ameliorates one or more symptoms of Alzheimer's disease or other Aβ accumulation associated diseases (e.g., Down's syndrome, Parkinson's disease, multi-infarct dementia, mild cognitive impairment, cerebral amyloid angiopathy, depression, Creutzfeldt-Jakob disease, dementia with Lewy body) ; (l) improves cognitive function. The antibodies and polypeptides of the invention may also have impaired effector function described herein. Antibodies and polypeptides having impaired effector function may exhibit a desirable safety profile in contrast to other reported anti-Aβ antibodies. For example, the compositions of the invention may not cause significant or unacceptable levels of any one or more of: bleeding in the brain vasculature (cerebral hemorrhage); meningoencephalitis (including changing magnetic resonance scan); elevated white blood count in cerebral spinal fluid; central nervous system inflammation.

[0083] Accordingly, the invention provides any of the following, or compositions (including pharmaceutical compositions) comprising any of the following: (a) antibody 6G or its variants shown in Table 3; (b) a fragment or a region of antibody 6G or its variants shown in Table 3; (c) a light chain of antibody 6G or its variants shown in Table 3; (d) a heavy chain of antibody 6G or its variants shown in Table 3; (e) one or more variable region(s) from a light chain and/or a heavy chain of antibody 6G or its variants shown in Table 3; (f) one or more CDR(s) (one, two, three, four, five or six CDRs) of antibody 6G or its variants shown in

Table 3; (g) CDR H3 from the heavy chain of antibody 6G; (h) CDR L3 from the light chain of antibody 6G or its variants shown in Table 3; (i) three CDRs from the light chain of antibody 6G or its variants shown in Table 3; (j) three CDRs from the heavy chain of antibody 6G or its variants shown in Table 3; (k) three CDRs from the light chain and three CDRs from the heavy chain, of antibody 6G or its variants shown in Table 3; and (l) an antibody comprising any one of (b) through (k). The invention also provides polypeptides comprising any one or more of the above.

[0084] The CDR portions of antibody 6G (including Chothia and Kabat CDRs) are diagrammatically depicted in Figure 1. Determination of CDR regions is well within the skill of the art. It is understood that in some embodiments, CDRs can be a combination of the Kabat and Chothia CDR (also termed "combined CDRs" or "extended CDRs"). In some embodiments, the CDRs are the Kabat CDRs. In other embodiments, the CDRs are the Chothia CDRs. In other words, in embodiments with more than one CDR, the CDRs may be any of Kabat, Chothia, combination CDRs, or combinations thereof.

[0085] In some embodiments, the invention provides a polypeptide (which may or may not be an antibody) which comprises at least one CDR, at least two, at least three, or at least four, at least five, or all six CDRs that are substantially identical to at least one CDR, at least two, at least three, at least four, at least five or all six CDRs of 6G or its variants shown in Table 3. Other embodiments include antibodies which have at least two, three, four, five, or six CDR(s) that are substantially identical to at least two, three, four, five or six CDRs of 6G or derived from 6G. In some embodiments, the at least one, two, three, four, five, or six CDR(s) are at least about 85%, 86%, 87%, 88%, 89%, 90%, 95%, 96%, 97%, 98%, or 99% identical to at least one, two, three, four, five or six CDRs of 6G or its variants shown in Table 3. It is understood that, for purposes of this invention, binding specificity and/or overall activity is generally retained, although the extent of activity may vary compared to 6G or its variants shown in Table 3 (may be greater or lesser).

[0086] The invention also provides a polypeptide (which may or may not be an antibody) which comprises an amino acid sequence of 6G or its variants shown in Table 3 that has any of the following: at least 5 contiguous amino acids, at least 8 contiguous amino acids, at least about 10 contiguous amino acids, at least about 15 contiguous amino acids, at least

about 20 contiguous amino acids, at least about 25 contiguous amino acids, at least about 30 contiguous amino acids of a sequence of 6G or its variants shown in Table 3, wherein at least 3 of the amino acids are from a variable region of 6G (Figure 1) or its variants shown in Table 3. In one embodiment, the variable region is from a light chain of 6G. In another embodiment, the variable region is from a heavy chain of 6G. An exemplary polypeptide has contiguous amino acid (lengths described above) from both the heavy and light chain variable regions of 6G. In another embodiment, the 5 (or more) contiguous amino acids are from a complementarity determining region (CDR) of 6G shown in Figure 1. In some embodiments, the contiguous amino acids are from a variable region of 6G.

[0087] The binding affinities of the antibodies and polypeptides of the invention may vary, and need not be (but can be) a particular value or range, as the exemplary embodiments described below. The binding affinity (K_D) of the antibodies and polypeptides of the invention to $A\beta_{1\text{-}40}$ can be about 0.10 to about 0.80 nM, about 0.15 to about 0.75 nM and about 0.18 to about 0.72 nM. In some embodiments, the binding affinity is about 2 pM, about 5 pM, about 10 pM, about 15 pM, about 20 pM, about 40 pM, or greater than about 40 pM. In one embodiment, the binding affinity is between about 2 pM and 22 pM. In other embodiments, the binding affinity is less than about 10 nM, about 5 nM, about 4 nM, about 3 nM, about 2 nM, about 1 nM, about 900 pM, about 800 pM, about 700 pM, about 600 pM, about 500 pM, about 400 pM, about 300 pM, about 200 pM, about 150 pM, about 100 pM, about 90 pM, about 80 pM, about 70 pM, about 60 pM, about 50 pM, about 40 pM, about 30 pM, about 10 pM. In some embodiment, the binding affinity is about 10 nM. In other embodiments, the binding affinity is less than about 10 nM, less than about 50 nM, less than about 100 nM, less than about 150 nM, less than about 200 nM, less than about 250 nM, less than about 500 nM, or less than about 1000 nM. In other embodiments, the binding affinity is less than about 5 nM. In other embodiments, the binding affinity is less than about 1 nM. In other embodiments, the binding affinity is about 0.1 nM or about 0.07 nM. In other embodiments, the binding affinity is less than about 0.1 nM or less than about 0.07 nM. In other embodiments, the binding affinity is from any of about 10 nM, about 5 nM, about 1 nM, about 900 pM, about 800 pM, about 700 pM, about 600 pM, about 500 pM, about 400 pM, about 300 pM, about 200 pM, about 150 pM, about 100 pM, about 90 pM, about 80 pM,

about 70 pM, about 60 pM, about 50 pM, about 40 pM, about 30 pM, about 10 pM to any of about 2 pM, about 5 pM, about 10 pM, about 15 pM, about 20 pM, or about 40 pM. In some embodiments, the binding affinity is any of about 10 nM, about 5 nM, about 1 nM, about 900 pM, about 800 pM, bout 700 pM, about 600 pM, about 500 pM, about 400 pM, about 300 pM, about 200 pM, about 150 pM, about 100 pM, about 90 pM, about 80 pM, about 70 pM, about 60 pM, about 50 pM, about 40 pM, about 30 pM, about 10 pM. In still other embodiments, the binding affinity is about 2 pM, about 5 pM, about 10 pM, about 15 pM, about 20 pM, about 40 pM, or greater than about 40 pM.

[0088] The antibodies and polypeptides of the invention may also bind to any one or more of $A\beta_{1-36}$, $A\beta_{1-37}$, $A\beta_{1-38}$, $A\beta_{1-39}$, $A\beta_{1-42}$, and $A\beta_{1-43}$, but the binding affinity to any one or more of these peptides is less than their binding affinities to $A\beta_{1-40}$. In some embodiments, the K_D of the antibodies or polypeptides to any one or more of $A\beta_{1-36}$, $A\beta_{1-37}$, $A\beta_{1-38}$, $A\beta_{1-39}$, $A\beta_{1-42}$, and $A\beta_{1-43}$ is at least about 5-fold, at least about 10-fold, at least about 20-fold, at least about 30-fold, at least about 40-fold, at least about 50-fold, at least about 80-fold, at least about 100-fold, at least about 150-fold, at least about 200-fold, or at least about 250-fold of the K_D to $A\beta_{1-40}$.

[0089] The invention also provides methods of making any of these antibodies or polypeptides. The antibodies of this invention can be made by procedures known in the art. For example, the antibody may be generated by immunizing a mammal with an Aβ peptide (such as Aβ25-40 as the immunogen). The polypeptides can be produced by proteolytic or other degradation of the antibodies, by recombinant methods (i.e., single or fusion polypeptides) as described above or by chemical synthesis. Polypeptides of the antibodies, especially shorter polypeptides up to about 50 amino acids, are conveniently made by chemical synthesis. Methods of chemical synthesis are known in the art and are commercially available. For example, an antibody could be produced by an automated polypeptide synthesizer employing the solid phase method. See also, U.S. Patent Nos. 5,807,715; 4,816,567; and 6,331,415.

[0090] In another alternative, the antibodies can be made recombinantly using procedures that are well known in the art. In one embodiment, a polynucleotide comprises a sequence encoding the heavy chain and/or the light chain variable regions of antibody 6G

shown in SEQ ID NO:9 and SEQ ID NO:10. In another embodiment, the polynucleotide comprising the nucleotide sequence shown in SEQ ID NO:9 and SEQ ID NO:10 are cloned into one or more vectors for expression or propagation. The sequence encoding the antibody of interest may be maintained in a vector in a host cell and the host cell can then be expanded and frozen for future use. Vectors (including expression vectors) and host cells are further described herein.

[0091] The invention also encompasses single chain variable region fragments ("scFv") of antibodies of this invention, such as 6G. Single chain variable region fragments are made by linking light and/or heavy chain variable regions by using a short linking peptide. Bird et al. (1988) Science 242:423-426. An example of a linking peptide is $(GGGGS)_3$, which bridges approximately 3.5 nm between the carboxy terminus of one variable region and the amino terminus of the other variable region. Linkers of other sequences have been designed and used. Bird et al. (1988). Linkers can in turn be modified for additional functions, such as attachment of drugs or attachment to solid supports. The single chain variants can be produced either recombinantly or synthetically. For synthetic production of scFv, an automated synthesizer can be used. For recombinant production of scFv, a suitable plasmid containing polynucleotide that encodes the scFv can be introduced into a suitable host cell, either eukaryotic, such as yeast, plant, insect or mammalian cells, or prokaryotic, such as E. coli. Polynucleotides encoding the scFv of interest can be made by routine manipulations such as ligation of polynucleotides. The resultant scFv can be isolated using standard protein purification techniques known in the art.

[0092] Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see e.g., Holliger, P., et al. (1993) Proc. Natl. Acad Sci. USA 90:6444-6448; Poljak, R. J., et al. (1994) Structure 2:1121-1123).

[0093] For example, bispecific antibodies, monoclonal antibodies that have binding specificities for at least two different antigens, can be prepared using the antibodies disclosed

herein. Methods for making bispecific antibodies are known in the art (see, e.g., Suresh et al., 1986, Methods in Enzymology 121:210). Traditionally, the recombinant production of bispecific antibodies was based on the coexpression of two immunoglobulin heavy chain-light chain pairs, with the two heavy chains having different specificities (Millstein and Cuello, 1983, Nature 305, 537-539).

[0094] According to one approach to making bispecific antibodies, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2 and CH3 regions. It is preferred to have the first heavy chain constant region (CH1), containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are cotransfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

[0095] In one approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. This asymmetric structure, with an immunoglobulin light chain in only one half of the bispecific molecule, facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations. This approach is described in PCT Publication No. WO 94/04690, published March 3, 1994.

[0096] Heteroconjugate antibodies, comprising two covalently joined antibodies, are also within the scope of the invention. Such antibodies have been used to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (PCT application publication Nos. WO 91/00360 and WO 92/200373; EP 03089).

Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents and techniques are well known in the art, and are described in U.S. Patent No. 4,676,980.

[0097] Chimeric or hybrid antibodies also may be prepared *in vitro* using known methods of synthetic protein chemistry, including those involving cross-linking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

[0098] Humanized antibody comprising one or more CDRs of antibody 6G or one or more CDRs derived from antibody 6G can be made using any methods known in the art. For example, four general steps may be used to humanize a monoclonal antibody. These are: (1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy variable domains (2) designing the humanized antibody, i.e., deciding which antibody framework region to use during the humanizing process (3) the actual humanizing methodologies/techniques and (4) the transfection and expression of the humanized antibody. *See*, for example, U.S. Patent Nos. 4,816,567; 5,807,715; 5,866,692; 6,331,415; 5,530,101; 5,693,761; 5,693,762; 5,585,089; 6,180,370; 5,225,539; 6,548,640.

[0099] In the recombinant humanized antibodies, the Fcγ portion can be modified to avoid interaction with Fcγ receptor and the complement immune system. This type of modification was designed by Dr. Mike Clark from the Department of Pathology at Cambridge University, and techniques for preparation of such antibodies are described in WO 99/58572, published November 18, 1999.

[0100] For example, the constant region may be engineered to more resemble human constant regions to avoid immune response if the antibody is used in clinical trials and treatments in humans. See, for example, U.S. Patent Nos. 5,997,867 and 5,866,692.

[0101] The invention encompasses modifications to antibody 6G, including functionally equivalent antibodies which do not significantly affect their properties and variants which have enhanced or decreased activity and/or affinity. For example, amino acid sequence of antibody 6G may be mutated to obtain an antibody with the desired binding affinity to $A\beta_{1-40}$ peptide. Modification of polypeptides is routine practice in the art and need

not be described in detail herein. Modification of polypeptides is exemplified in the Examples. Examples of modified polypeptides include polypeptides with conservative substitutions of amino acid residues, one or more deletions or additions of amino acids which do not significantly deleteriously change the functional activity, or use of chemical analogs.

[0102] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue or the antibody fused to an epitope tag. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody of an enzyme or a polypeptide which increases the serum half-life of the antibody.

[0103] Substitution variants have at least one amino acid residue in the antibody molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table 1 under the heading of "conservative substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" in Table 1, or as further described below in reference to amino acid classes, may be introduced and the products screened.

Table 1: Amino Acid Substitutions

Original Residue	Conservative Substitutions	Exemplary Substitutions
Ala (A)	Val	Val; Leu; Ile
Arg (R)	Lys	Lys; Gln; Asn
Asn (N)	Gln	Gln; His; Asp, Lys; Arg
Asp (D)	Glu	Glu; Asn
Cys (C)	Ser	Ser; Ala
Gln (Q)	Asn	Asn; Glu
Glu (E)	Asp	Asp; Gln
Gly (G)	Ala	Ala
His (H)	Arg	Asn; Gln; Lys; Arg

Ile (I)	Leu	Leu; Val; Met; Ala; Phe; Norleucine
Leu (L)	Ile	Norleucine; Ile; Val; Met; Ala; Phe
Lys (K)	Arg	Arg; Gln; Asn
Met (M)	Leu	Leu; Phe; Ile
Phe (F)	Tyr	Leu; Val; Ile; Ala; Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr	Tyr; Phe
Tyr (Y)	Phe	Trp; Phe; Thr; Ser
Val (V)	Leu	Ile; Leu; Met; Phe; Ala; Norleucine

[0104] Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

(1) Non-polar: Norleucine, Met, Ala, Val, Leu, Ile;

(2) Polar without charge: Cys, Ser, Thr, Asn, Gln;

(3) Acidic (negatively charged): Asp, Glu;

(4) Basic (positively charged): Lys, Arg;

(5) Residues that influence chain orientation: Gly, Pro; and

(6) Aromatic: Trp, Tyr, Phe, His.

[0105] Non-conservative substitutions are made by exchanging a member of one of these classes for another class.

[0106] Any cysteine residue not involved in maintaining the proper conformation of the antibody also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant cross-linking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability, particularly where the antibody is an antibody fragment such as an Fv fragment.

[0107] Amino acid modifications can range from changing or modifying one or more amino acids to complete redesign of a region, such as the variable region. Changes in the variable region can alter binding affinity and/or specificity. In some embodiments, no more than one to five conservative amino acid substitutions are made within a CDR domain. In other embodiments, no more than one to three conservative amino acid substitutions are made within a CDR domain. In still other embodiments, the CDR domain is CDRH3 and/or CDR L3.

[0108] Modifications also include glycosylated and nonglycosylated polypeptides, as well as polypeptides with other post-translational modifications, such as, for example, glycosylation with different sugars, acetylation, and phosphorylation. Antibodies are glycosylated at conserved positions in their constant regions (Jefferis and Lund, 1997, Chem. Immunol. 65:111-128; Wright and Morrison, 1997, TibTECH 15:26-32). The oligosaccharide side chains of the immunoglobulins affect the protein's function (Boyd et al., 1996, Mol. Immunol. 32:1311-1318; Wittwe and Howard, 1990, Biochem. 29:4175-4180) and the intramolecular interaction between portions of the glycoprotein, which can affect the conformation and presented three-dimensional surface of the glycoprotein (Hefferis and Lund, supra; Wyss and Wagner, 1996, Current Opin. Biotech. 7:409-416). Oligosaccharides may also serve to target a given glycoprotein to certain molecules based upon specific recognition structures. Glycosylation of antibodies has also been reported to affect antibody-dependent cellular cytotoxicity (ADCC). In particular, CHO cells with tetracycline-regulated expression of β(1,4)-N-acetylglucosaminyltransferase III (GnTIII), a glycosyltransferase catalyzing formation of bisecting GlcNAc, was reported to have improved ADCC activity (Umana et al., 1999, Mature Biotech. 17:176-180).

[0109] Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine, asparagine-X-threonine, and asparagine-X-cysteine, where X is any amino acid except proline, are the recognition sequences for enzymatic

attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[0110] Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

[0111] The glycosylation pattern of antibodies may also be altered without altering the underlying nucleotide sequence. Glycosylation largely depends on the host cell used to express the antibody. Since the cell type used for expression of recombinant glycoproteins, e.g. antibodies, as potential therapeutics is rarely the native cell, variations in the glycosylation pattern of the antibodies can be expected (see, e.g. Hse et al., 1997, J. Biol. Chem. 272:9062-9070).

[0112] In addition to the choice of host cells, factors that affect glycosylation during recombinant production of antibodies include growth mode, media formulation, culture density, oxygenation, pH, purification schemes and the like. Various methods have been proposed to alter the glycosylation pattern achieved in a particular host organism including introducing or overexpressing certain enzymes involved in oligosaccharide production (U. S. Patent Nos. 5,047,335; 5,510,261 and 5.278,299). Glycosylation, or certain types of glycosylation, can be enzymatically removed from the glycoprotein, for example using endoglycosidase H (Endo H), N-glycosidase F as described in Example 1, endoglycosidase F1, endoglycosidase F2, endoglycosidase F3. In addition, the recombinant host cell can be genetically engineered to be defective in processing certain types of polysaccharides. These and similar techniques are well known in the art.

[0113] Other methods of modification include using coupling techniques known in the art, including, but not limited to, enzymatic means, oxidative substitution and chelation. Modifications can be used, for example, for attachment of labels for immunoassay. Modified 6G

polypeptides are made using established procedures in the art and can be screened using standard assays known in the art, some of which are described below and in the Examples.

[0114] Other antibody modifications include antibodies that have been modified as described in PCT Publication No. WO 99/58572, published November 18, 1999. These antibodies comprise, in addition to a binding domain directed at the target molecule, an effector domain having an amino acid sequence substantially homologous to all or part of a constant domain of a human immunoglobulin heavy chain. These antibodies are capable of binding the target molecule without triggering significant complement dependent lysis, or cell-mediated destruction of the target. In some embodiments, the effector domain is capable of specifically binding FcRn and/or FcγRIIb. These are typically based on chimeric domains derived from two or more human immunoglobulin heavy chain C_H2 domains. Antibodies modified in this manner are particularly suitable for use in chronic antibody therapy, to avoid inflammatory and other adverse reactions to conventional antibody therapy.

[0115] The invention includes affinity matured embodiments. For example, affinity matured antibodies can be produced by procedures known in the art (Marks et al., 1992, Bio/Technology, 10:779-783; Barbas et al., 1994, Proc Nat. Acad. Sci, USA 91:3809-3813; Schier et al., 1995, Gene, 169:147-155; Yelton et al., 1995, J. Immunol., 155:1994-2004; Jackson et al., 1995, J. Immunol., 154(7):3310-9; Hawkins et al, 1992, J. Mol. Biol., 226:889-896; and WO2004/058184).

[0116] The following methods may be used for adjusting the affinity of an antibody and for characterizing a CDR. One way of characterizing a CDR of an antibody and/or altering (such as improving) the binding affinity of a polypeptide, such as an antibody, termed "library scanning mutagenesis". Generally, library scanning mutagenesis works as follows. One or more amino acid positions in the CDR are replaced with two or more (such as 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) amino acids using art recognized methods. This generates small libraries of clones (in some embodiments, one for every amino acid position that is analyzed), each with a complexity of two or more members (if two or more amino acids are substituted at every position). Generally, the library also includes a clone comprising the native (unsubstituted) amino acid. A small number of clones, e.g., about 20-80 clones (depending on the complexity of the library), from each library are screened for binding affinity to the target polypeptide (or other

binding target), and candidates with increased, the same, decreased or no binding are identified. Methods for determining binding affinity are well-known in the art. Binding affinity may be determined using BIAcore surface plasmon resonance analysis, which detects differences in binding affinity of about 2-fold or greater. BIAcore is particularly useful when the starting antibody already binds with a relatively high affinity, for example a K_D of about 10 nM or lower. Screening using BIAcore surface plasmon resonance is described in the Examples, herein.

[0117] Binding affinity may be determined using Kinexa Biocensor, scintillation proximity assays, ELISA, ORIGEN immunoassay (IGEN), fluorescence quenching, fluorescence transfer, and/or yeast display. Binding affinity may also be screened using a suitable bioassay.

[0118] In some embodiments, every amino acid position in a CDR is replaced (in some embodiments, one at a time) with all 20 natural amino acids using art recognized mutagenesis methods (some of which are described herein). This generates small libraries of clones (in some embodiments, one for every amino acid position that is analyzed), each with a complexity of 20 members (if all 20 amino acids are substituted at every position).

[0119] In some embodiments, the library to be screened comprises substitutions in two or more positions, which may be in the same CDR or in two or more CDRs. Thus, the library may comprise substitutions in two or more positions in one CDR. The library may comprise substitution in two or more positions in two or more CDRs. The library may comprise substitution in 3, 4, 5, or more positions, said positions found in two, three, four, five or six CDRs. The substitution may be prepared using low redundancy codons. See, e.g., Table 2 of Balint et al. , (1993) Gene 137(1):109-18).

[0120] The CDR may be CDRH3 and/or CDRL3. The CDR may be one or more of CDRL1, CDRL2, CDRL3, CDRH1, CDRH2, and/or CDRH3. The CDR may be a Kabat CDR, a Chothia CDR, or an extended CDR.

[0121] Candidates with improved binding may be sequenced, thereby identifying a CDR substitution mutant which results in improved affinity (also termed an "improved" substitution). Candidates that bind may also be sequenced, thereby identifying a CDR substitution which retains binding.

[0122] Multiple rounds of screening may be conducted. For example, candidates (each comprising an amino acid substitution at one or more position of one or more CDR) with

improved binding are also useful for the design of a second library containing at least the original and substituted amino acid at each improved CDR position (i.e., amino acid position in the CDR at which a substitution mutant showed improved binding). Preparation, and screening or selection of this library is discussed further below.

[0123] Library scanning mutagenesis also provides a means for characterizing a CDR, in so far as the frequency of clones with improved binding, the same binding, decreased binding or no binding also provide information relating to the importance of each amino acid position for the stability of the antibody-antigen complex. For example, if a position of the CDR retains binding when changed to all 20 amino acids, that position is identified as a position that is unlikely to be required for antigen binding. Conversely, if a position of CDR retains binding in only a small percentage of substitutions, that position is identified as a position that is important to CDR function. Thus, the library scanning mutagenesis methods generate information regarding positions in the CDRs that can be changed to many different amino acid (including all 20 amino acids), and positions in the CDRs which cannot be changed or which can only be changed to a few amino acids.

[0124] Candidates with improved affinity may be combined in a second library, which includes the improved amino acid, the original amino acid at that position, and may further include additional substitutions at that position, depending on the complexity of the library that is desired, or permitted using the desired screening or selection method. In addition, if desired, adjacent amino acid position can be randomized to at least two or more amino acids. Randomization of adjacent amino acids may permit additional conformational flexibility in the mutant CDR, which may in turn, permit or facilitate the introduction of a larger number of improving mutations. The library may also comprise substitution at positions that did not show improved affinity in the first round of screening.

[0125] The second library is screened or selected for library members with improved and/or altered binding affinity using any method known in the art, including screening using BIAcore surface plasmon resonance analysis, and selection using any method known in the art for selection, including phage display, yeast display, and ribosome display.

[0126] The invention also encompasses fusion proteins comprising one or more fragments or regions from the antibodies (such as 6G) or polypeptides of this invention. In one

embodiment, a fusion polypeptide is provided that comprises at least 10 contiguous amino acids of the variable light chain region shown in SEQ ID NO:2 (Figure 1) and/or at least 10 amino acids of the variable heavy chain region shown in SEQ ID NO:1 (Figure 1). In other embodiments, a fusion polypeptide is provided that comprises at least about 10, at least about 15, at least about 20, at least about 25, or at least about 30 contiguous amino acids of the variable light chain region shown in SEQ ID NO:2 (Figure 1) and/or at least about 10, at least about 15, at least about 20, at least about 25, or at least about 30 contiguous amino acids of the variable heavy chain region shown in SEQ ID NO:1 (Figure 1). In another embodiment, the fusion polypeptide comprises a light chain variable region and/or a heavy chain variable region of 6G, as shown in SEQ ID NO:2 and SEQ ID NO:1 of Figure 1. In another embodiment, the fusion polypeptide comprises one or more CDR(s) of 6G. In still other embodiments, the fusion polypeptide comprises CDR H3 and/or CDR L3 of antibody 6G. For purposes of this invention, an 6G fusion protein contains one or more 6G antibodies and another amino acid sequence to which it is not attached in the native molecule, for example, a heterologous sequence or a homologous sequence from another region. Exemplary heterologous sequences include, but are not limited to a "tag" such as a FLAG tag or a 6His tag. Tags are well known in the art.

[0127] A 6G fusion polypeptide can be created by methods known in the art, for example, synthetically or recombinantly. Typically, the 6G fusion proteins of this invention are made by preparing an expressing a polynucleotide encoding them using recombinant methods described herein, although they may also be prepared by other means known in the art, including, for example, chemical synthesis.

[0128] This invention also provides compositions comprising 6G antibodies or polypeptides conjugated (for example, linked) to an agent that facilitate coupling to a solid support (such as biotin or avidin). For simplicity, reference will be made generally to 6G or antibodies with the understanding that these methods apply to any of the $A\beta_{1-40}$ binding embodiments described herein. Conjugation generally refers to linking these components as described herein. The linking (which is generally fixing these components in proximate association at least for administration) can be achieved in any number of ways. For example, a direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or

sulfhydryl group, on one may be capable of reacting with a carbonyl-containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (e.g., a halide) on the other.

[0129] An antibody or polypeptide of this invention may be linked to a labeling agent (alternatively termed "label") such as a fluorescent molecule, a radioactive molecule or any others labels known in the art. Labels are known in the art which generally provide (either directly or indirectly) a signal.

[0130] The invention also provides compositions (including pharmaceutical compositions) and kits comprising antibody 6G, and, as this disclosure makes clear, any or all of the antibodies and/or polypeptides described herein.

Anti-Aβ antibodies and polypeptides having impaired effector function

[0131] The antibodies or polypeptides (including pharmaceutical compositions comprising the antibodies or polypeptides) described herein may have impaired effector function. As used herein, an antibody or a polypeptide having an "impaired effector function" (used interchangeably with "immunologically inert" or "partially immunologically inert") refers to antibodies or polypeptides that do not have any effector function or have reduced activity or activities of effector function (compared to antibody or polypeptide having an unmodified or a naturally occurring constant region), e.g., having no activity or reduced activity in any one or more of the following: a) triggering complement mediated lysis; b) stimulating antibody-dependent cell mediated cytotoxicity (ADCC); and c) activating microglia. The effector function activity may be reduced by about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, and 100%. In some embodiments, the antibody binds to a beta-amyloid peptide without triggering significant complement dependent lysis, or cell mediated destruction of the target. For example, the Fc receptor binding site on the constant region may be modified or mutated to remove or reduce binding affinity to certain Fc receptors, such as FcγRI, FcγRII, and/or FcγRIII. For simplicity, reference will be made to antibodies with the understanding that embodiments also apply to polypeptides. EU numbering system (Kabat et al., Sequences of Proteins of Immunological Interest; 5th ed. Public Health Service, National Institutes of Healthy, Bethesda, Md., 1991) is used to indicate which amino acid residue(s) of the constant region (e.g.,

of an IgG antibody) are altered or mutated. The numbering may be used for a specific type of antibody (e.g., IgG1) or a species (e.g., human) with the understanding that similar changes can be made across types of antibodies and species.

[0132] In some embodiments, the antibody that specifically binds to the an Aβ peptide comprises a heavy chain constant region having impaired effector function. The heavy chain constant region may have naturally occurring sequence or is a variant. In some embodiments, the amino acid sequence of a naturally occurring heavy chain constant region is mutated, e.g., by amino acid substitution, insertion and/or deletion, whereby the effector function of the constant region is impaired. In some embodiments, the N-glycosylation of the Fc region of a heavy chain constant region may also be changed, e.g., may be removed completely or partially, whereby the effector function of the constant region is impaired.

[0133] In some embodiments, the effector function is impaired by removing N-glycosylation of the Fc region (e.g., in the CH 2 domain of IgG) of the anti-Aβ peptide. In some embodiments, N-glycosylation of the Fc region is removed by mutating the glycosylated amino acid residue or flanking residues that are part of the glycosylation recognition sequence in the constant region. The tripeptide sequences asparagine-X-serine (N-X-S), asparagine-X-threonine (N-X-T) and asparagine-X-cysteine (N-X-C), where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain for N-glycosylation. Mutating any of the amino acid in the tripeptide sequences in the constant region yields an aglycosylated IgG. For example, N-glycosylation site N297 of human IgG1 and IgG3 may be mutated to A, D, Q, K, or H. See, Tao et al., *J. Immunology* 143: 2595-2601 (1989); and Jefferis et al., *Immunological Reviews* 163:59-76 (1998). It has been reported that human IgG1 and IgG3 with substitution of Asn-297 with Gln, His, or Lys do not bind to the human FcγRI and do not activate complement with C1q binding ability completely lost for IgG1 and dramatically decreased for IgG3. In some embodiments, the amino acid N in the tripeptide sequences is mutated to any one of amino acid A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, Y. In some embodiments, the amino acid N in the tripeptide sequences is mutated to a conservative substitution. In some embodiments, the amino acid X in the tripeptide sequences is mutated to proline. In some embodiments, the amino acid S in the tripeptide sequences is mutated to A, D, E, F, G, H, I, K, L, M, N, P, Q, R, V, W, Y. In some embodiments, the amino

acid T in the tripeptide sequences is mutated to A, D, E, F, G, H, I, K, L, M, N, P, Q, R, V, W, Y. In some embodiments, the amino acid C in the tripeptide sequences is mutated to A, D, E, F, G, H, I, K, L, M, N, P, Q, R, V, W, Y. In some embodiments, the amino acid following the tripeptide is mutated to P. In some embodiments, the N-glycosylation in the constant region is removed enzymatically (such as N-glycosidase F as described in Example 1, endoglycosidase F1, endoglycosidase F2, endoglycosidase F3, and englycosidase H). Removing N-glycosylation may also be achieved by producing the antibody in a cell line having deficiency for N-glycosylation. Wright et al., J Immunol. 160(7):3393-402 (1998).

[0134] In some embodiments, amino acid residue interacting with oligosaccharide attached to the N-glycosylation site of the constant region is mutated to reduce binding affinity to FcγRI. For example, F241, V264, D265 of human IgG3 may be mutated. See, Lund et al., *J. Immunology* 157:4963-4969 (1996).

[0135] In some embodiments, the effector function is impaired by modifying regions such as 233-236, 297, and/or 327-331 of human IgG as described in PCT WO 99/58572 and Armour et al., *Molecular Immunology* 40: 585-593 (2003); Reddy et al., *J. Immunology* 164:1925-1933 (2000). Antibodies described in PCT WO 99/58572 and Armour et al. comprise, in addition to a binding domain directed at the target molecule, an effector domain having an amino acid sequence substantially homologous to all or part of a constant region of a human immunoglobulin heavy chain. These antibodies are capable of binding the target molecule without triggering significant complement dependent lysis, or cell-mediated destruction of the target. In some embodiments, the effector domain has a reduced affinity for FcγRI, FcγRIIa, and FcγRIII. In some embodiments, the effector domain is capable of specifically binding FcRn and/or FcγRIIb. These are typically based on chimeric domains derived from two or more human immunoglobulin heavy chain C_H2 domains. Antibodies modified in this manner are particularly suitable for use in chronic antibody therapy, to avoid inflammatory and other adverse reactions to conventional antibody therapy. In some embodiments, the heavy chain constant region of the antibody is a human heavy chain IgG1 with any of the following mutations: 1) A327A330P331 to G327S330S331; 2) E233L234L235G236 to P233V234A235 with G236 deleted; 3) E233L234L235 to P233V234A235; 4) E233L234L235G236A327A330P331 to P233V234A235G327S330S331 with G236 deleted; 5) E233L234L235A327A330P331 to

P233V234A235G327S330S331; and 6) N297 to A297 or any other amino acid except N. These mutations may combined, for example, any of 1) - 5) may be combined with 6). In some embodiments, the heavy chain constant region of the antibody is a human heavy chain IgG2 with the following mutations: A330P331 to S330S331; N297 to Q297; and N297G327A330P331 to Q297G327S330S331. In some embodiments, the heavy chain constant region of the antibody is a human heavy chain IgG4 with any of the following mutations: E233F234L235G236 to P233V234A235 with G236 deleted; E233F234L235 to P233V234A235; P228L235 to S228E235; N297 to Q297; and E233F234L235G236N297 to P233V234A235G236Q297.

[0136] The constant region of the antibodies may also be modified to impair complement activation. For example, complement activation of IgG antibodies following binding of the C1 component of complement may be reduced by mutating amino acid residues in the constant region in a C1 binding motif (e.g., C1q binding motif). It has been reported that Ala mutation for each of D270, K322, P329, P331 of human IgG1 significantly reduced the ability of the antibody to bind to C1q and activating complement. For murine IgG2b, C1q binding motif constitutes residues E318, K320, and K322. Idusogie et al., *J. Immunology* 164:4178-4184 (2000); Duncan et al., Nature 322: 738-740 (1988).

[0137] Clq binding motif E318, K320, and K322 identified for murine IgG2b is believed to be common for other antibody isotypes. Duncan et al., Nature 322: 738-740 (1988). C1q binding activity for IgG2b can be abolished by replacing any one of the three specified residues with a residue having an inappropriate functionality on its side chain. It is not necessary to replace the ionic residues only with Ala to abolish Clq binding. It is also possible to use other alkyl-substituted non-ionic residues, such as Gly, Ile, Leu, or Val, or such aromatic non-polar residues as Phe, Tyr, Trp and Pro in place of any one of the three residues in order to abolish Clq binding. In addition, it is also be possible to use such polar non-ionic residues as Ser, Thr, Cys, and Met in place of residues 320 and 322, but not 318, in order to abolish Clq binding activity.

[0138] The invention also provides antibodies having impaired effector function wherein the antibody has a modified hinge region. Binding affinity of human IgG for its Fc receptors can be modulated by modifying the hinge region. Canfield et al., *J. Exp. Med.* 173:1483-1491 (1991); Hezareh et al., *J. Virol.* 75:12161-12168 (2001); Redpath et al., *Human Immunology* 59:720-727 (1998). Specific amino acid residues may be mutated or deleted. The modified

hinge region may comprise a complete hinge region derived from an antibody of different antibody class or subclass from that of the CH1 domain. For example, the constant domain (CH1) of a class IgG antibody can be attached to a hinge region of a class IgG4 antibody. Alternatively, the new hinge region may comprise part of a natural hinge or a repeating unit in which each unit in the repeat is derived from a natural hinge region. In some embodiments, the natural hinge region is altered by converting one or more cysteine residues into a neutral residue, such as alanine, or by converting suitably placed residues into cysteine residues. U.S. Pat. No.5,677,425. Such alterations are carried out using art recognized protein chemistry and, preferably, genetic engineering techniques and as described herein.

[0139] Polypeptides that specifically binds to an Aβ peptide and fused to a heavy chain constant region having impaired effector function may also be used for the methods described herein. In some embodiments, the polypeptide comprises a sequence derived from antibody 6G or its variants shown in Table 3. In some embodiments, the polypeptide is derived from a single domain antibody that binds to an Aβ peptide. Single domain antibody can be generated using methods known in the art. Omidfar et al., *Tumour Biol.* 25:296-305 (2004); Herring et al., *Trends in Biotechnology* 21:484-489 (2003).

[0140] In some embodiments, the antibody or polypeptide is a $F(ab')_2$ fragment. In some embodiments, the antibody or polypeptide is a Fab fragment. In some embodiments, the antibody or polypeptide is a single chain antibody scFv. In some embodiments, the antibody or polypeptide is a PEGylated $F(ab')_2$ fragment. In some embodiments, the antibody or polypeptide is a PEGylated Fab fragment. In some embodiments, the antibody or polypeptide is a PEGylated single chain antibody scFv.

[0141] Other methods to make antibodies having impaired effector function known in the art may also be used.

[0142] Antibodies and polypeptides with modified constant region can be tested in one or more assays to evaluate level of effector function reduction in biological activity compared to the starting antibody. For example, the ability of the antibody or polypeptide with an altered Fc region to bind complement or Fc receptors (for example, Fc receptors on microglia), or altered hinge region can be assessed using the assays disclosed herein as well as any art recognized assay. PCT WO 99/58572; Armour et al., *Molecular Immunology* 40: 585-593 (2003); Reddy et

al., J. Immunology 164:1925-1933 (2000); Song et al., *Infection and Immunity* 70:5177-5184 (2002).

[0143] Competition assays can be used to determine whether two antibodies bind the same epitope by recognizing identical or sterically overlapping epitopes or one antibody competitively inhibits binding of another antibody to the antigen. These assays are known in the art. Typically, antigen is immobilized on a multi-well plate and the ability of unlabeled antibodies to block the binding of labeled antibodies is measured. Common labels for such competition assays are radioactive labels or enzyme labels.

[0144] Antibodies and polypeptides that specifically bind to Aβ can be screened for efficacy in removing amyloid deposit and other beneficial effects, such as improving cognition. For example, antibodies or polypeptides may be administered to an animal having Alzheimer's pathology. Various animal models for Alzheimer's disease are known in the art. Following administration, level of compact and diffuse amyloid plaques, behavior analysis for cognition, and microglia activation and microhemorrhage may tested using methods known in the art and described in detail in Example 2. PCT WO 2004/032868; Wilcock et al., *J. Neurosci.* 23:3745-3751 (2003); Wilcock et al., *J. Neuroinflammation* 1:24 (2004).

Polynucleotides, vectors and host cells

[0145] The invention also provides isolated polynucleotides encoding the antibodies and polypeptides of the invention (including an antibody comprising the polypeptide sequences of the light chain and heavy chain variable regions shown in Figure 1), and vectors and host cells comprising the polynucleotide.

[0146] Accordingly, the invention provides polynucleotides (or compositions, including pharmaceutical compositions), comprising polynucleotides encoding any of the following: (a) antibody 6G or its variants shown in Table 3; (b) a fragment or a region of antibody 6G or its variants shown in Table 3; (c) a light chain of antibody 6G or its variants shown in Table 3; (d) a heavy chain of antibody 6G or its variants shown in Table 3; (e) one or more variable region(s) from a light chain and/or a heavy chain of antibody 6G or its variants shown in Table 3; (f) one or more CDR(s) (one, two, three, four, five or six CDRs) of antibody 6G or its variants shown in Table 3; (g) CDR H3 from the heavy chain of antibody 6G; (h) CDR L3 from the light chain of

antibody 6G or its variants shown in Table 3; (i) three CDRs from the light chain of antibody 6G or its variants shown in Table 3; (j) three CDRs from the heavy chain of antibody 6G or its variants shown in Table 3; (k) three CDRs from the light chain and three CDRs from the heavy chain, of antibody 6G or its variants shown in Table 3; and (l) an antibody comprising any one of (b) through (k). In some embodiments, the polynucleotide comprises either or both of the polynucleotide(s) shown in SEQ ID NO:9 and SEQ ID NO:10.

[0147] In another aspect, the invention provides polynucleotides encoding any of the antibodies (including antibody fragments) and polypeptides described herein, such as antibodies and polypeptides having impaired effector function. Polynucleotides can be made by procedures known in the art.

[0148] In another aspect, the invention provides compositions (such as a pharmaceutical compositions) comprising any of the polynucleotides of the invention. In some embodiments, the composition comprises an expression vector comprising a polynucleotide encoding the 6G antibody as described herein. In other embodiment, the composition comprises an expression vector comprising a polynucleotide encoding any of the antibodies or polypeptides described herein. In still other embodiments, the composition comprises either or both of the polynucleotides shown in SEQ ID NO:9 and SEQ ID NO:10. Expression vectors, and administration of polynucleotide compositions are further described herein.

[0149] In another aspect, the invention provides a method of making any of the polynucleotides described herein.

[0150] Polynucleotides complementary to any such sequences are also encompassed by the present invention. Polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

[0151] Polynucleotides may comprise a native sequence (i.e., an endogenous sequence that encodes an antibody or a portion thereof) or may comprise a variant of such a sequence. Polynucleotide variants contain one or more substitutions, additions, deletions and/or insertions

such that the immunoreactivity of the encoded polypeptide is not diminished, relative to a native immunoreactive molecule. The effect on the immunoreactivity of the encoded polypeptide may generally be assessed as described herein. Variants preferably exhibit at least about 70% identity, more preferably at least about 80% identity and most preferably at least about 90% identity to a polynucleotide sequence that encodes a native antibody or a portion thereof.

[0152] Two polynucleotide or polypeptide sequences are said to be "identical" if the sequence of nucleotides or amino acids in the two sequences is the same when aligned for maximum correspondence as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

[0153] Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins - Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J., 1990, Unified Approach to Alignment and Phylogenes pp. 626-645 Methods in Enzymology vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M., 1989, CABIOS 5:151-153; Myers, E.W. and Muller W., 1988, CABIOS 4:11-17; Robinson, E.D., 1971, Comb. Theor. 11:105; Santou, N., Nes, M., 1987, Mol. Biol. Evol. 4:406-425; Sneath, P.H.A. and Sokal, R.R., 1973, Numerical Taxonomy the Principles and Practice of Numerical Taxonomy, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J., 1983, Proc. Natl. Acad. Sci. USA 80:726-730.

[0154] Preferably, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (i.e. gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent,

as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (i.e. the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

[0155] Variants may also, or alternatively, be substantially homologous to a native gene, or a portion or complement thereof. Such polynucleotide variants are capable of hybridizing under moderately stringent conditions to a naturally occurring DNA sequence encoding a native antibody (or a complementary sequence).

[0156] Suitable "moderately stringent conditions" include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0. 1 % SDS.

[0157] As used herein, "highly stringent conditions" or "high stringency conditions" are those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 μg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

[0158] It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide

sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present invention. Further, alleles of the genes comprising the polynucleotide sequences provided herein are within the scope of the present invention. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides. The resulting mRNA and protein may, but need not, have an altered structure or function. Alleles may be identified using standard techniques (such as hybridization, amplification and/or database sequence comparison).

[0159] The polynucleotides of this invention can be obtained using chemical synthesis, recombinant methods, or PCR. Methods of chemical polynucleotide synthesis are well known in the art and need not be described in detail herein. One of skill in the art can use the sequences provided herein and a commercial DNA synthesizer to produce a desired DNA sequence.

[0160] For preparing polynucleotides using recombinant methods, a polynucleotide comprising a desired sequence can be inserted into a suitable vector, and the vector in turn can be introduced into a suitable host cell for replication and amplification, as further discussed herein. Polynucleotides may be inserted into host cells by any means known in the art. Cells are transformed by introducing an exogenous polynucleotide by direct uptake, endocytosis, transfection, F-mating or electroporation. Once introduced, the exogenous polynucleotide can be maintained within the cell as a non-integrated vector (such as a plasmid) or integrated into the host cell genome. The polynucleotide so amplified can be isolated from the host cell by methods well known within the art. See, e.g., Sambrook et al. (1989).

[0161] Alternatively, PCR allows reproduction of DNA sequences. PCR technology is well known in the art and is described in U.S. Patent Nos. 4,683,195, 4,800,159, 4,754,065 and 4,683,202, as well as PCR: The Polymerase Chain Reaction, Mullis et al. eds., Birkauswer Press, Boston (1994).

[0162] RNA can be obtained by using the isolated DNA in an appropriate vector and inserting it into a suitable host cell. When the cell replicates and the DNA is transcribed into RNA, the RNA can then be isolated using methods well known to those of skill in the art, as set forth in Sambrook et al., (1989), for example.

[0163] Suitable cloning vectors may be constructed according to standard techniques, or may be selected from a large number of cloning vectors available in the art. While the cloning

vector selected may vary according to the host cell intended to be used, useful cloning vectors will generally have the ability to self-replicate, may possess a single target for a particular restriction endonuclease, and/or may carry genes for a marker that can be used in selecting clones containing the vector. Suitable examples include plasmids and bacterial viruses, e.g., pUC18, pUC19, Bluescript (e.g., pBS SK+) and its derivatives, mp18, mp19, pBR322, pMB9, ColE1, pCR1, RP4, phage DNAs, and shuttle vectors such as pSA3 and pAT28. These and many other cloning vectors are available from commercial vendors such as BioRad, Strategene, and Invitrogen.

[0164] Expression vectors generally are replicable polynucleotide constructs that contain a polynucleotide according to the invention. It is implied that an expression vector must be replicable in the host cells either as episomes or as an integral part of the chromosomal DNA. Suitable expression vectors include but are not limited to plasmids, viral vectors, including adenoviruses, adeno-associated viruses, retroviruses, cosmids, and expression vector(s) disclosed in PCT Publication No. WO 87/04462. Vector components may generally include, but are not limited to, one or more of the following: a signal sequence; an origin of replication; one or more marker genes; suitable transcriptional controlling elements (such as promoters, enhancers and terminator). For expression (i.e., translation), one or more translational controlling elements are also usually required, such as ribosome binding sites, translation initiation sites, and stop codons.

[0165] The vectors containing the polynucleotides of interest can be introduced into the host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (*e.g.*, where the vector is an infectious agent such as vaccinia virus). The choice of introducing vectors or polynucleotides will often depend on features of the host cell.

[0166] The invention also provides host cells comprising any of the polynucleotides described herein. Any host cells capable of over-expressing heterologous DNAs can be used for the purpose of isolating the genes encoding the antibody, polypeptide or protein of interest. Non-limiting examples of mammalian host cells include but not limited to COS, HeLa, and CHO cells. *See also* PCT Publication No. WO 87/04462. Suitable non-mammalian host cells include prokaryotes (such as *E. coli* or *B. subtillis*) and yeast (such as *S. cerevisae*, *S. pombe*; or *K.*

lactis). Preferably, the host cells express the cDNAs at a level of about 5 fold higher, more preferably 10 fold higher, even more preferably 20 fold higher than that of the corresponding endogenous antibody or protein of interest, if present, in the host cells. Screening the host cells for a specific binding to $A\beta_{1-40}$ is effected by an immunoassay or FACS. A cell overexpressing the antibody or protein of interest can be identified.

Diagnostic uses of 6G derived antibodies and anti-Aβ antibodies having impaired effector function

[0167] Antibody 6G which binds to C-terminus of Aβ may be used to identify or detect the presence or absence of Aβ. For simplicity, reference will be made generally to 6G or antibodies with the understanding that these methods apply to any of Aβ binding embodiments (such as polypeptides) described herein. Detection generally involves contacting a biological sample with an antibody described herein that binds to Aβ and the formation of a complex between Aβ and an antibody (*e.g.*, 6G) which binds specifically to Aβ. The formation of such a complex can be *in vitro* or *in vivo*. The term "detection" as used herein includes qualitative and/or quantitative detection (measuring levels) with or without reference to a control.

[0168] Any of a variety of known methods can be used for detection, including, but not limited to, immunoassay, using antibody that binds the polypeptide, e.g. by enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and the like; and functional assay for the encoded polypeptide, e.g. binding activity or enzymatic assay. In some embodiments, the antibody is detectably labeled. Other embodiments are known in the art and described herein.

[0169] Antibodies and polypeptides of the invention can be used in the detection, diagnosis and monitoring of a disease, condition, or disorder associated with altered or aberrant Aβ or βAPP expression, such as Alzheimer's disease and Down's syndrome. Thus, in some embodiments, the invention provides methods comprises contacting a specimen (sample) of an individual suspected of having altered or aberrant Aβ expression with an antibody or polypeptide of the invention and determining whether the level of Aβ differs from that of a control or comparison specimen. In other embodiments, the invention provides methods comprises contacting a specimen (sample) of an individual and determining level of Aβ expression.

[0170] For diagnostic applications, the antibody may be labeled with a detectable moiety including but not limited to radioisotopes, fluorescent labels, and various enzyme-substrate labels. Methods of conjugating labels to an antibody are known in the art. In other embodiment of the invention, antibodies of the invention need not be labeled, and the presence thereof can be detected using a labeled antibody which binds to the antibodies of the invention.

[0171] 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).

[0172] The antibodies may also be used for in vivo diagnostic assays, such as in vivo imaging. Generally, the antibody is labeled with a radionuclide (such as ^{111}In, ^{99}Tc, ^{14}C, ^{131}I, ^{125}I, or ^{3}H) so that the cells or tissue of interest can be localized using immunoscintiography.

[0173] The antibody may also be used as staining reagent in pathology, following techniques well known in the art.

[0174] Anti-Aβ antibodies having impaired effector function may be used for measuring brain amyloid burden for diagnosis of subject at risk of or diagnosed with AD, and assessing progress of any treatment and disease stage. It has been reported that peripheral administration of a monoclonal anti-Aβ antibody results in a rapid increase in plasma Aβ and the magnitude of this increase is highly correlated with amyloid burden in the hippocampus and cortex. DeMattos et al., *Science* 295:2264-2267 (2002). In some embodiments, an anti-Aβ antibody having impaired effector function is administered to a subject, and level of Aβ in the plasma is measured, whereby an increase in plasma Aβ indicates presence and/or level of brain amyloid burden in the subject. These methods may be used to monitor effectiveness of the treatment and disease stage and to determine future dosing and frequency. Antibodies having impaired effector function may have a better safety profile and provide advantage for these diagnostic uses.

Methods of using anti-Aβ antibody for therapeutic purposes

[0175] The antibodies (including polypeptides), polynucleotides, and pharmaceutical compositions described herein can be used in methods for treating, preventing and inhibiting the development of Alzheimer's disease and other diseases associated with altered Aβ or βAPP

expression, or accumulation or deposit of Aβ peptide (collectively termed "Aβ-associated diseases"), such as Down's syndrome, Parkinson's disease, multi-infarct dementia, mild cognitive impairment, cerebral amyloid angiopathy, vascular disorder caused by deposit of Aβ peptide in blood vessels (such as stroke and HCHWA-D), depression, Creutzfeldt-Jakob disease, and dementia with Lewy body. Such methods comprise administering the antibodies, polypeptides, or polynucleotides, or a pharmaceutical composition to the subject. In prophylactic applications, pharmaceutical compositions or medicaments are administered to a patient susceptible to, or otherwise at risk of, Alzheimer's disease (or other Aβ-associated disease) in an amount sufficient to eliminate or reduce the risk, lessen the severity, or delay the outset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. In therapeutic applications, compositions or medicaments are administered to a patient suspected of, or already suffering from such a disease in amount sufficient to cure, or at least partially arrest, the symptoms of the disease (biochemical, histological and/or behavioral), including its complications and intermediate pathological phenotypes in development of the disease.

[0176] The invention also provides a method of delaying development of a symptom associated with Alzheimer's disease (or other Aβ-associated disease) in a subject comprising administering an effective dosage of a pharmaceutical composition comprising an antibody, a polypeptide, or a polynucleotide described herein to the subject. Symptoms associated with Alzheimer disease includes, but not limited to, abnormalities of memory, problem solving, language, calculation, visuospatial perception, judgment, and behavior.

[0177] This invention also provides methods of inhibiting or suppressing the formation of amyloid plaques and/or Aβ accumulation in a subject comprising administering an effective dose of a pharmaceutical composition comprising an antibody, a polypeptide, or a polynucleotide described herein described herein to the subject. In some embodiments, the amyloid plaques are in the brain of the subject. In some embodiments, the amyloid plaques are in the cerebral vasculature of the subject. In other embodiments, the Aβ accumulation is in the circulatory system of the subject.

[0178] This invention also provides methods of reducing amyloid plaques and/or reducing or slowing Aβ accumulation in a subject comprising administering an effective dose of a

pharmaceutical composition comprising an antibody, a polypeptide, or a polynucleotide described herein to the subject. In some embodiments, the amyloid plaques are in the brain of the subject. In some embodiments, the amyloid plaques are in the cerebral vasculature of the subject. In other embodiments, the Aβ accumulation is in the circulatory system of the subject.

[0179] This invention also provides methods of removing or clearing amyloid plaques and/or Aβ accumulation in a subject comprising administering an effective dose of a pharmaceutical composition comprising an antibody, a polypeptide, or a polynucleotide described herein to the subject. In some embodiments, the amyloid plaques are in the brain of the subject. In some embodiments, the amyloid plaques are in the cerebral vasculature of the subject. In other embodiments, the Aβ accumulation is in the circulatory system of the subject.

[0180] This invention also provides methods of reducing Aβ peptide in a tissue (such as brain), inhibiting and/or reducing accumulation of Aβ peptide in a tissue (such as brain), and inhibiting and/or reducing toxic effects of Aβ peptide in a tissue (such as brain) in a subject comprising administering an effective dose of a pharmaceutical composition comprising an antibody, a polypeptide, or a polynucleotide described herein to the subject. Aβ polypeptide may be in soluble, oligomeric, or deposited form. Oligomeric form of Aβ may be composed of 2-50 Aβ polypeptides, which can be a mixture of full length 1-40 and 1-42 peptides and/or any truncated version of the these peptides.

[0181] The invention also provides methods of improving cognition or reversing cognitive decline associated with diseases associated with amyloid deposit of Aβ in a subject, such as Alzheimer's disease, comprising administering an effective dosage of a pharmaceutical composition comprising an antibody, a polypeptide, or a polynucleotide described herein to the subject.

[0182] The methods described herein (including prophylaxis or therapy) can be accomplished by a single direct injection at a single time point or multiple time points to a single or multiple sites. Administration can also be nearly simultaneous to multiple sites. Frequency of administration may be determined and adjusted over the course of therapy, and is base on accomplishing desired results. In some cases, sustained continuous release formulations of antibodies (including polypeptides), polynucleotides, and pharmaceutical compositions of the

invention may be appropriate. Various formulations and devices for achieving sustained release are known in the art.

[0183] Patients, subjects, or individuals include mammals, such as human, bovine, equine, canine, feline, porcine, and ovine animals. The subject is preferably a human, and may or may not be afflicted with disease or presently show symptoms. In the case of Alzheimer's disease, virtually anyone is at risk of suffering from Alzheimer's disease if he or she lives long enough. Therefore, the present methods can be administered prophylactically to the general population without the need for any assessment of the risk of the subject patient. The present methods are useful for individuals who do have a known genetic risk of Alzheimer's disease. Such individuals include those having relatives who have experienced this disease, and those whose risk is determined by analysis of genetic or biochemical markers. Genetic markers of risk toward Alzheimer's disease include mutations in the APP gene, particularly mutations at position 717 and positions 670 and 671 referred to as the Hardy and Swedish mutations respectively (see Hardy (1997) Trends Neurosci. 20:154-9). Other markers of risk are mutations in the presenilin genes, PS1 and PS2, and ApoE4, family history of AD, hypercholesterolemia or atherosclerosis. Individuals presently suffering from Alzheimer's disease can be recognized from characteristic dementia, as well as the presence of risk factors described above. In addition, a number of diagnostic tests are available for identifying individuals who have AD. These include measurement of CSF tau and Aβ42 levels. Elevated tau and decreased Aβ42 levels signify the presence of AD. Individuals suffering from Alzheimer's disease can also be diagnosed by ADRDA (Alzheimer's Disease and Related Disorders Association) criteria. In asymptomatic patients, treatment can begin at any age (e.g., 10, 20, 30). Usually, however, it is not necessary to begin treatment until a patient reaches 40, 50, 60 or 70. Treatment typically entails multiple dosages over a period of time. Treatment can be monitored by various ways known in the art over time. In the case of potential Down's syndrome patients, treatment can begin antenatally by administering therapeutic agent to the mother or shortly after birth.

[0184] The pharmaceutical composition that can be used in the above methods include, any of the antibodies, polypeptides, and/or polynucleotides described herein. In some embodiments, antibody is antibody 6G or its variants shown in Table 3. In some embodiments,

the antibody is an antibody that specifically binds to an Aβ peptide and comprises a constant region having impaired effector function.

Administration and Dosage

[0185] The antibody is preferably administered to the mammal in a carrier; preferably a pharmaceutically-acceptable carrier. Suitable carriers and their formulations are described in *Remington's Pharmaceutical Sciences*, 18th edition, A. Gennaro, ed., Mack Publishing Co., Easton, PA, 1990; and *Remington, The Science and Practice of Pharmacy* 20th Ed. Mack Publishing, 2000. Typically, an appropriate amount of a pharmaceutically-acceptable salt is used in the formulation to render the formulation isotonic. Examples of the carrier include saline, Ringer's solution and dextrose solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7 to about 7.5. Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, liposomes or microparticles. It will be apparent to those persons skilled in the art that certain carriers may be more preferable depending upon, for instance, the route of administration and concentration of antibody being administered.

[0186] The antibody can be administered to the mammal by injection (e.g., systemic, intravenous, intraperitoneal, subcutaneous, intramuscular, intraportal, intracerebral, intracerebralventricular, and intranasal), or by other methods, such as infusion, which ensure its delivery to the bloodstream in an effective form. The antibody may also be administered by isolated perfusion techniques, such as isolated tissue perfusion, to exert local therapeutic effects. Intravenous injection is preferred.

[0187] Effective dosages and schedules for administering the antibody may be determined empirically, and making such determinations is within the skill in the art. Those skilled in the art will understand that the dosage of antibody that must be administered will vary depending on, for example, the mammal that will receive the antibody, the route of administration, the particular type of antibody used and other drugs being administered to the mammal. Guidance in selecting appropriate doses for antibody is found in the literature on therapeutic uses of antibodies, e.g., *Handbook of Monoclonal Antibodies*, Ferrone et al., eds., Noges Publications, Park Ridge, N.J., 1985, ch. 22 and pp. 303-357; Smith et al., *Antibodies in Human Diagnosis and Therapy*, Haber et al., eds., Raven Press, New York, 1977, pp. 365-389. A typical daily dosage of the antibody used

alone might range from about 1 μg/kg to up to 100 mg/kg of body weight or more per day, depending on the factors mentioned above. Generally, any of the following doses may be used: a dose of at least about 50 mg/kg body weight; at least about 10 mg/kg body weight; at least about 3 mg/kg body weight; at least about 1 mg/kg body weight; at least about 750 μg/kg body weight; at least about 500 μg/kg body weight; at least about 250 ug/kg body weight; at least about 100 μg /kg body weight; at least about 50 μg /kg body weight; at least about 10 ug /kg body weight; at least about 1 μg/kg body weight, or more, is administered. Antibodies may be administered at lower doses or less frequent at the beginning of the treatment to avoid potential side effect, such as temporary cerebral amyloid angiopathy (CAA).

[0188] In some embodiments, more than one antibody may be present. Such compositions may contain at least one, at least two, at least three, at least four, at least five different antibodies (including polypeptides) of the invention.

[0189] The antibody may also be administered to the mammal in combination with effective amounts of one or more other therapeutic agents. The antibody may be administered sequentially or concurrently with the one or more other therapeutic agents. The amounts of antibody and therapeutic agent depend, for example, on what type of drugs are used, the pathological condition being treated, and the scheduling and routes of administration but would generally be less than if each were used individually.

[0190] Following administration of antibody to the mammal, the mammal's physiological condition can be monitored in various ways well known to the skilled practitioner.

[0191] The above principles of administration and dosage can be adapted for polypeptides described herein.

[0192] A polynucleotide encoding an antibody or a polypeptide described herein may also be used for delivery and expression of the antibody or the polypeptide in a desired cell. It is apparent that an expression vector can be used to direct expression of the antibody. The expression vector can be administered systemically, intraperitoneally, intravenously, intramuscularly, subcutaneously, intrathecally, intraventricularly, orally, enterally, parenterally, intranasally, dermally, or by inhalation. For example, administration of expression vectors includes local or systemic administration, including injection, oral administration, particle gun or catheterized administration, and topical administration. One skilled in the art is familiar with

administration of expression vectors to obtain expression of an exogenous protein in vivo. *See*, e.g., U.S. Patent Nos. 6,436,908; 6,413,942; and 6,376,471.

[0193] Targeted delivery of therapeutic compositions comprising a polynucleotide encoding an antibody of the invention can also be used. Receptor-mediated DNA delivery techniques are described in, for example, Findeis *et al., Trends Biotechnol.* (1993) 11:202; Chiou *et al., Gene Therapeutics: Methods And Applications Of Direct Gene Transfer* (J.A. Wolff, ed.) (1994); Wu *et al., J. Biol. Chem.* (1988) 263:621; Wu *et al., J. Biol. Chem.* (1994) 269:542; Zenke *et al., Proc. Natl. Acad. Sci. (USA)* (1990) 87:3655; Wu *et al., J. Biol. Chem.* (1991) 266:338. Therapeutic compositions containing a polynucleotide are administered in a range of about 100 ng to about 200 mg of DNA for local administration in a gene therapy protocol. Concentration ranges of about 500 ng to about 50 mg, about 1 μg to about 2 mg, about 5 μg to about 500 μg, and about 20 μg to about 100 μg of DNA can also be used during a gene therapy protocol. The therapeutic polynucleotides and polypeptides of the present invention can be delivered using gene delivery vehicles. The gene delivery vehicle can be of viral or non-viral origin (*see* generally, Jolly, *Cancer Gene Therapy* (1994) 1:51; Kimura, *Human Gene Therapy* (1994) 5:845; Connelly, *Human Gene Therapy* (1995) 1:185; and Kaplitt, *Nature Genetics* (1994) 6:148). Expression of such coding sequences can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence can be either constitutive or regulated.

[0194] Viral-based vectors for delivery of a desired polynucleotide and expression in a desired cell are well known in the art. Exemplary viral-based vehicles include, but are not limited to, recombinant retroviruses (*see*, e.g., PCT Publication Nos. WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; WO 93/11230; WO 93/10218; WO 91/02805; U.S. Patent Nos. 5, 219,740; 4,777,127; GB Patent No. 2,200,651; and EP 0 345 242), alphavirus-based vectors (e.g., Sindbis virus vectors, Semliki forest virus (ATCC VR-67; ATCC VR-1247), Ross River virus (ATCC VR-373; ATCC VR-1246) and Venezuelan equine encephalitis virus (ATCC VR-923; ATCC VR-1250; ATCC VR 1249; ATCC VR-532)), and adeno-associated virus (AAV) vectors (*see*, e.g., PCT Publication Nos. WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655). Administration of DNA linked to killed adenovirus as described in Curiel, *Hum. Gene Ther.* (1992) 3:147 can also be employed.

[0195] Non-viral delivery vehicles and methods can also be employed, including, but not limited to, polycationic condensed DNA linked or unlinked to killed adenovirus alone (*see*, e.g., Curiel, *Hum. Gene Ther.* (1992) 3:147); ligand-linked DNA(*see*, e.g., Wu, *J. Biol. Chem.* (1989) 264:16985); eukaryotic cell delivery vehicles cells (*see*, e.g., U.S. Patent No. 5,814,482; PCT Publication Nos. WO 95/07994; WO 96/17072; WO 95/30763; and WO 97/42338) and nucleic charge neutralization or fusion with cell membranes. Naked DNA can also be employed. Exemplary naked DNA introduction methods are described in PCT Publication No. WO 90/11092 and U.S. Patent No. 5,580,859. Liposomes that can act as gene delivery vehicles are described in U.S. Patent No. 5,422,120; PCT Publication Nos. WO 95/13796; WO 94/23697; WO 91/14445; and EP 0 524 968. Additional approaches are described in Philip, *Mol. Cell Biol.* (1994) 14:2411, and in Woffendin, *Proc. Natl. Acad. Sci.* (1994) 91:1581.

Kits

[0196] The invention also provides articles of manufacture and kits containing materials useful for treating pathological conditions such as Alzheimer's disease or other Aβ-associated diseases (such as Down's syndrome, Parkinson's disease, multi-infarct dementia, mild cognitive impairment, cerebral amyloid angiopathy, vascular disorder caused by deposit of Aβ peptide in blood vessels (such as stroke and HCHWA-D)), or detecting or purifying Aβ or βAPP. The article of manufacture comprises a container with a label. Suitable containers include, for example, bottles, vials, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition having an active agent which is effective for treating pathological conditions or for detecting or purifying Aβ or βAPP. The active agent in the composition is an antibody and preferably, comprises monoclonal antibodies specific for Aβ or βAPP. In some embodiments, the active agent comprises antibody 6G or any antibodies or polypeptides derived from antibody 6G. In some embodiments, the active agent comprises an anti-Aβ antibody or polypeptide described herein having impaired effector function. In some embodiments, the anti-Aβ antibody or polypeptide comprises a heavy chain constant region, wherein the constant region has impaired effector function. The label on the container indicates that the composition is used for treating pathological conditions such as Alzheimer's

disease or detecting or purifying Aβ or βAPP, and may also indicate directions for either *in vivo* or *in vitro* use, such as those described above.

[0197] The invention also provides kits comprising any of the antibodies (such as 6G), polypeptides, polynucleotides described herein. In some embodiments, the kit of the invention comprises the container described above. In other embodiments, the kit of the invention comprises the container described above and a second container comprising a buffer. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for performing any methods described herein (such as methods for treating Alzheimer's disease, and methods for inhibiting or reducing accumulation of Aβ peptide in the brain). In kits to be used for detecting or purifying Aβ or βAPP, the antibody is typically labeled with a detectable marker, such as, for example, a radioisotope, fluorescent compound, bioluminescent compound, a chemiluminescent compound, metal chelator or enzyme.

[0198] In some embodiments, the invention provides compositions (described herein) for use in any of the methods described herein, whether in the context of use as a medicament and/or use for manufacture of a medicament.

[0199] The following examples are provided to illustrate, but not to limit, the invention.

EXAMPLES

Example 1. Binding affinity determination of antibody 6G and its variants

A. General methods

[0200] The following general methods were used in this example and other examples.

Expression vector used in clone characterization

[0201] Expression of the Fab fragment of the antibodies was under control of an IPTG inducible lacZ promotor similar to that described in Barbas (2001) *Phage display: a laboratory manual*, Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press pg 2.10. Vector pComb3X), however, modifications included addition and expression of the following additional domains: the human Kappa light chain constant domain and the CHI constant domain of IgG2a human immunoglobulin, Ig gamma-2 chain C region, protein accession number P01859; Immunoglobulin kappa light chain (homosapiens), protein accession number CAA09181.

Small scale Fab preparation

[0202] Small scale expression of Fabs in 96 wells plates was carried out as follows. Starting from *E. coli* transformed with a Fab library, colonies were picked to inoculate both a master plate (agar LB + Ampicillin (50 μg/ml) + 2% Glucose) and a working plate (2 ml/well, 96 well/plate containing 1.5 mL of LB + Ampicillin (50 μg/ml) + 2% Glucose). Both plates were grown at 30°C for 8-12 hours. The master plate was stored at 4°C and the cells from the working plate were pelleted at 5000 rpm and resuspended with 1 mL of LB+Ampicillin (50 μg/ml)+ 1 mM IPTG to induce expression of Fabs. Cells were harvested by centrifugation after 5 h expression time at 30°C, then resuspended in 500 μL of buffer HBS-EP (100 mM HEPES buffer pH 7.4, 150 mM NaCl, 0.005% P20). Lysis of HBS-EP resuspended cells was attained by one cycle of freezing (-80°C) then thawing at 37°C. Cell lysates were centrifuged at 5000 rpm for 30 min to separate cell debris from supernatants containing Fabs. The supernatants were then injected into the BIAcore plasmon resonance apparatus to obtain affinity information for each Fab. Clones expressing Fabs were rescued from the master plate to sequence the DNA and for large scale Fab production and detailed characterization as described below.

Large Scale Fab preparation

[0203] To obtain detailed kinetic parameters, Fabs were expressed and purified from large cultures. Erlenmeyer flasks containing 200 mL of LB+Ampicillin (50 μg/ml) + 2% Glucose were inoculated with 5 mL of over night culture from a selected Fab-expressing *E. coli* clone. Clones were incubated at 30°C until an OD_{550nm} of 1.0 was attained and then induced by replacing the media for 200 ml, of LB+Ampicillin (50 μg/ml) + 1 mM IPTG. After 5h expression time at 30°C, cells were pelleted by centrifugation, then resuspended in 10 mL PBS (pH 8). Lysis of the cells was obtained by two cycles of freeze/thaw (at -80°C and 37°C, respectively). Supernatant of the cell lysates were loaded onto Ni-NTA superflow sepharose (Qiagen, Valencia. CA) columns equilibrated with PBS, pH 8, then washed with 5 column volumes of PBS, pH 8. Individual Fabs eluted in different fractions with PBS (pH 8) + 300 mM Imidazol. Fractions containing Fabs were pooled and dialized in PBS, then quantified by ELISA prior to affinity characterization.

Full antibody preparation

[0204] For expression of full antibodies, heavy and light chain variable regions were cloned in mammalian expression vectors and transfected using lipofectamine into HEK 293 cells for transient expression. Antibodies were purified using protein A using standard methods.

[0205] Vector pDb.6G.hFc2a is an expression vector comprising the heavy chain of the 6G antibody, and is suitable for transient or stable expression of the heavy chain. Vector pDb.6G.hFc2a has nucleotide sequences corresponding to the following regions: the murine cytomegalovirus promoter region (nucleotides 1-612); a synthetic intron (nucleotides 619-1507); the DHFR coding region (nucleotides 707-1267); human growth hormone signal peptide (nucleotides 1525-1602); heavy chain variable region of 6G; human heavy chain IgG2a constant region containing the following mutations: A330P331 to S330S331 (amino acid numbering with reference to the wildtype IgG2a sequence; see Eur. J. Immunol. (1999) 29:2613-2624); SV40 late polyadenylation signal; SV40 enhancer region; phage f1 region and beta lactamase (AmpR) coding region.

[0206] Vector pEb.6G.hK is an expression vector comprising the light chain of the 6G antibody, and is suitable for transient expression of the light chain. Vector pEb.6G.hK has nucleotide sequences corresponding to the following regions: the murine cytomegalovirus promoter region (nucleotides 1-612); human EF-1 intron (nucleotides 619-1142); human growth hormone signal peptide (nucleotides 1173-1150); antibody 6G light chain variable region; human kappa chain constant region; SV40 late polyadenylation signal; SV40 enhancer region; phage f1 region and beta lactamase (AmpR) coding region.

Biacore Assay

[0207] Affinities of 6G monoclonal antibody were determined using the BIAcore3000™ surface plasmon resonance (SPR) system (BIAcore, INC, Piscaway NJ). One way of determining the affinity was immobilizing of 6G on CM5 chip and measuring binding kinetics of $A\beta_{1-40}$ peptide or other Aβ peptides to the antibody. CM5 chips were activated with N-ethyl-N'-(3-dimethylaminopropyl)-carbodiinide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Antibody 6G or its variants was diluted into 10 mM sodium acetate pH 4.0 or 5.0 and injected over the activated chip at a concentration of 0.005 mg/mL. Using variable flow time across the individual chip channels, a range of antibody density was achieved: 1000-2000 or 2000-3000 response units (RU). The chip was blocked with

ethanolamine. Regeneration studies showed that a solution containing 2 volumes of PIERCE elution buffer and 1 volumes of 4 M NaCl effectively removed the bound Aβ peptide while keeping the activity of 6G on the chip for over 200 injections. HBS-EP buffer (0.01M HEPES, pH 7.4, 0.15 NaCl, 3 mM EDTA, 0.005% Surfactant P20) was used as running buffer for all the BIAcore assays. Serial dilutions (0.1-10x estimated K_D) of purified $A\beta_{1-40}$ synthetic peptide or other Aβ peptide samples were injected for 1 min at 100 μL/min and dissociation times of 10 min were allowed. Kinetic association rates (k_{on}) and dissociation rates (k_{off}) were obtained simultaneously by fitting the data to a 1:1 Langmuir binding model (Karlsson, R. Roos, H. Fagerstam, L. Petersson, B. (1994). Methods Enzymology 6. 99-110) using the BIAevaluation program. Equilibrium dissociation constant (K_D) values were calculated as k_{off}/k_{on}.

[0208] Alternatively, affinity was determined by immobilizing $A\beta_{1-40}$ peptide or other Aβ peptides on SA chip and measuring binding kinetics of 6G Fab and Fab of 6G variants to the immobilized Aβ peptide. Affinities of 6G Fab fragment and its variants Fab fragments were determined by Surface Plasmon Resonance (SPR) system (BIAcore 3000™, BIAcore, Inc., Piscaway, NJ). SA chips (streptavidin) were used according to the supplier's instructions. Biotinylated Aβ peptide was diluted into HBS-EP (100 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% P20) and injected over the chip at a concentration of 0.005 mg/mL. Using variable flow time across the individual chip channels, two ranges of antigen density were achieved: 100-400 response units (RU) for detailed kinetic studies and 500-2000 RU for concentration studies and screening. Regeneration studies showed that 100 mM phosphoric acid (may also be followed by a solution containing 2 volumes of 50 mM NaOH and 1 volume of 70% ethanol) effectively removed the bound Fab while keeping the activity of Aβ peptide on the chip for over 200 injections. HBS-EP buffer was used as running buffer for all the BIAcore assays. Serial dilutions (0.1-10x estimated KD) of purified Fab samples were injected for 2 min at 100 μL/min and dissociation times of 10 min were allowed. The concentrations of the Fab proteins were determined by ELISA and/or SDS-PAGE electrophoresis using a standard Fab of known concentration (determined by amino acid analysis). Kinetic association rates (k_{on}) and dissociation rates (k_{off}) were obtained simultaneously by fitting the data to a 1:1 Langmuir binding model (Karlsson, R. Roos, H. Fagerstam, L. Petersson, B. (1994). Methods Enzymology

6, 99-110) using the BIAevaluation program. Equilibrium dissociation constant (K_D) values were calculated as k_{off}/k_{on}.

ELISA ASSAY

[0209] ELISA was used for measuring binding of antibody 6G and variants to nonbiotinylated Aβ peptides. NUNC maxisorp plates were coated with 2.5 ug/ml of Aβ peptides in PBS pH 7.4 for more than 1 hour at 4°C. Plates were blocked with 1% BSA in PBS buffer pH 7.4. Primary antibody (from cell supernatants, serum containing anti-Aβ antibody, or purified full antibody or Fabs at desired dilution) was incubated with the immobilized Aβ peptides for 1 h at room temperature. After washing, the plates were incubated with secondary antibody, a HRP conjugated goat anti-human kappa chain antibody (MP Biomedicals, 55233) at 1:5000 dilution. After washing, bound secondary antibody was measured by adding TMB substrate (KPL, 50-76-02, 50-65-02). The HRP reaction was stopped by adding 1M phosphoric acid and absorbance at 450 nm was measured.

[0210] ELISA was used for measuring binding of antibody 6G and variants to biotinylated Aβ peptides. NUNC maxisorp plates were coated with 6 ug/ml of streptavidin (Pierce, 21122) in PBS pH 7.4 for more than 1 h at 4°C. Plates were blocked with 1% BSA in PBS buffer pH 7.4. After washing, biotinylated Aβ peptides in PBS pH 7.4 were incubated 1 hour at room temperature. Primary antibody (from cell supernatants, serum containing anti-Aβ antibody, or purified full antibody or Fabs at desired dilution) was incubated with the immobilized Aβ peptides for 1 h at room temperature. After washing, plates were incubated with secondary antibody, a HRP conjugated goat anti-human kappa chain antibody (MP Biomedicals, 55233) at 1:5000 dilution. After washing, bound secondary antibody was measured by adding TMB substrate (KPL, 50-76-02, 50-65-02). HRP reaction was stopped by adding 1M phosphoric acid and absorbance at 450 nm was measured.

B. Binding affinity of antibody 6G and variants to $A\beta_{1-40}$, $A\beta_{1-42}$, and other Aβ peptides

[0211] The amino acid sequences of the heavy chain and light chain variable regions of antibody 6G is shown in Figure 1. The binding affinity of 6G antibody to $A\beta_{1-40}$, $A\beta_{1-42}$, and $A\beta_{22-37}$ determined using Biacore described above is shown in Table 2 below.

Table 2. Binding affinity of antibody 6G Fab fragment

	k_{on} (1/Ms)	k_{off} (1/s)	K_D (nM)
Biotinylated $A\beta_{1-40}$ immobilized on streptavidin chip, 6G Fab flowed onto it	3.0×10^5	7.0×10^{-4}	2
Biotinylated $A\beta_{1-42}$ immobilized on streptavidin chip, 6G Fab flowed onto it	1.8×10^4	1.6×10^{-3}	80
Biotinylated $A\beta_{22-37}$ immobilized on streptavidin chip, 6G Fab flowed onto it	3.6×10^5	3.9×10^{-3}	11

[0212] The amino acid sequence of the variants of 6G is shown in Table 3 below. All amino acid substitutions of the variants shown in Table 3 are described relative to the sequence of 6G. The relative binding of 6G variants are also shown in Table 3. Binding was determined by ELISA described above with nonbiotinylated $A\beta_{1-40}$ or $A\beta_{1-42}$ immobilized on the surface of an ELISA plate.

Table 3. Amino acid sequences and binding data for antibody 6G variants.

6G Heavy chain mutant variants binding data by ELISA							A450 ELISA	
clone number	mutations						$A\beta_{1-40}$	$A\beta_{1-42}$
6G	F99	D100	N101	Y102	D103	R104	2.55	0.95
1A	Y						1.60	0.26
1B	M						0.37	0.22
1G	L						0.51	0.21
2G	P						0.30	0.50
3E	C						0.26	0.40
4G		S					1.41	0.30
5D		N					1.52	0.39
6A		T					0.86	0.31
7B			S				0.44	0.27
7D			C				0.23	0.31
8H			H				0.21	0.19
9E			R				0.22	0.26
10A				F			1.85	0.34
10E				L			0.41	0.24
10G				I			0.63	0.22
11D				M			0.29	0.24
2F					P		1.89	0.38

3A					A		1.16	0.28
3B					R		1.43	0.43
3C					G		2.30	0.76
4A						G	2.17	0.40
4B						F	2.48	0.71
4D						Q	2.45	1.00
6F						S	2.28	0.62

6G Light chain mutant variants binding data by ELISA										A450 ELISA	
Clone number	mutations									$A\beta_{1-40}$	$A\beta_{1-42}$
6G	Q93	Q94	S95	K96	E97	F98	P99	W100	S101	2.49	0.61
2H	K									0.07	0.13
3A	P									0.08	0.13
4F	S									2.00	0.30
5B		G								0.09	0.14
7E		R								0.09	0.18
7F		K								0.12	0.19
10E			L							0.08	0.12
1A				N						2.02	0.32
1C				F						0.05	0.05
4A				A						2.09	0.28
4G				F						1.07	0.28
5H					R					2.60	0.85
6C					G					0.05	0.05
6D					T					2.41	1.34
6E					P					0.12	0.20
8G					V					2.60	0.90

Example 2: Characterization of epitope on Aβ peptide that antibody 6G binds

[0213] To determine the epitope on Aβ peptide that is recognized by antibody 6G, ELISA binding analysis was used. Various Aβ peptides (Global Peptide Services, CO) was immobilized on a ELISA plate. The binding of 6G full antibody (at 20 nM) to the immobilized Aβ was determined by ELISA as described above. Amino acid sequences of $A\beta_{1-40}$, $A\beta_{1-42}$, and $A\beta_{1-43}$ are shown in Table 5 below. As shown in Figure 2, antibody 6G binds to Aβ peptides 17-40, 17-42, 22-35, 28-40, 1-38, 1-40, 1-42, 1-43, and 28-42; but binding to 28-42 is much weaker than the other Aβ peptides. Antibody 6G did not bind to Aβ peptide 1-16, 1-28 and 33-40. Thus, antibody 6G binds to the C-terminus of various truncated Aβ peptide, for example, 22-35, 1-38, 1-40, 1-42, and 1-43.

[0214] Table 4 below shows binding affinity comparison of 6G to $A\beta_{1-40}$ to other Aβ peptide as measured by k_{off} (1/s) using Biacore assay. Antibody 6G binds to $A\beta_{1-40}$ with highest affinity as compared to other peptides, with significantly lower affinity to truncated $A\beta_{1-40}$ (such 1-36, 1-37, 1-38, and 1-39), $A\beta_{1-42}$ and $A\beta_{1-43}$. This indicates that the side chain or backbone of amino acid 40 (Valine) of Aβ is involved in binding of 6G to $A\beta_{1-40}$; and binding is significantly reduced (for example from about 10 to about 50-250 fold loss of affinity) in absence of this amino acid. Binding with lower affinity to carboxy-terminal amidated $A\beta_{1-40}$ indicates that binding of 6G to $A\beta_{1-40}$ involves but is not dependent on the free C-terminus of $A\beta_{1-40}$. Lower affinity binding to $A\beta_{1-42}$ and $A\beta_{1-43}$ may be due to conformational differences between monomer form of $A\beta_{1-40}$ and $A\beta_{1-42}$ or $A\beta_{1-43}$. It has been shown that monomer of $A\beta_{1-42}$ has a conformation different from $A\beta_{1-40}$ monomer in solution. See, the monomer structure coordinate for $A\beta_{1-42}$ shown in Protein Data Bank (pdb files) with accession no. 1IYT; and the monomer structure coordinate for $A\beta_{1-40}$ shown in Protein Data Bank (pdb files) with accession nos. 1BA6 and 1BA4.

Table 4.

Aβ peptide fragment	k_{off} (1/s)	K_{off} Aβ peptide/k_{off} $A\beta_{1-40}$ (fold loss of affinity)
1-28	-	
1-43	Very low binding	
22-35	0.0285	215.9
1-36	0.0205	155.3
1-37	0.0149	112.8
1-38	$9.3x10^{-3}$	70.4
1-39	$7.92x10^{-3}$	60.0
17-42	0.0465	352.2
1-42	$1.9x10^{-3}$	14.4
28-42	$3.37x10^{-3}$	25.5
28-40-NH2#	$3.62x10^{-3}$	27.4
28-40	$6.4x10^{-4}$	4.8
17-40	$2.15x10^{-4}$	1.6
1-40	$1.32x10^{-4}$	1

Peptide flowed as analyte onto a CM5 chip with 6G monoclonal antibody (ligand) immobilized by amine chemistry
#peptide with carboxy-terminal amidated

[0215] Epitope mapping of antibody 6G was performed by ELISA assay. Biotinylated 15-mer or 10-mer of various Aβ peptides (these peptides have glycine added to the C-terminal end) were immobilized on streptavidin coated plates. Antibody 6G (from 2.5 ug/ml to 10 ug/ml) was incubated with the immobilized peptides and binding was measured as described above. As shown in Figure 3, antibody 6G binds to Aβ peptides with amino acids 20-34, 21-35, 22-36, 23-37, 24-38, 25-39 and 25-34 with a glycine at the C-terminus; but does not bind to Aβ peptides with amino acids 19-33, 26-40, 27-41, 24-33, and 26-35 having a glycine at the C-terminus of these peptides. This suggests that the epitope of antibody 6G binds includes amino acids from 25 to 34.

[0216] Based on data shown above, the epitope that antibody 6G binds seems to include amino acids 25-34 and 40. Figure 4 is a schematic graph showing epitope of antibody 6G.

Table 5. Amino acid sequences of β-amyloid peptides

1-40 (WT)	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGL MVGGVV (SEQ ID NO:15)
1-42 (WT)	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGL MVGGVVIA (SEQ ID NO:16)
1-43 (WT)	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGL MVGGVVIAT (SEQ ID NO:17)

B. Antibody 6G does not bind to APP

[0217] To determine whether 6G binds to amyloid precursor proteins (APP), binding of 6G to cells transfected with wildtype APP was determined. HEK293 cells were transfected with a cDNA encoding wild type human amyloid precursor protein. Forty eight hours after the transfection, cells were incubated on ice for 45 minutes with monoclonal antibodies anti-$A\beta_{1-16}$, (m2324) or 6G (5 ug/ml in DMEM with 10% FCS). The cells were then washed three times in PBS for 5 minutes, fixed with 4% PFA. The cells were washed three times again in PBS, and antibody binding was detected with secondary Cy3-conjugated goat anti-mouse antibody (dilution of 1:500) from Jackson Immunoresearch under fluorescence microscope.

[0218] As shown in Figure 5, anti-$A\beta_{1-16}$ antibody, which recognize N-terminal epitopes in Aβ, showed significant binding to APP precursor proteins expressed on cells. In contrast, 6G did not bind to APP expressing cells.

Example 3. Characterization of epitope on Aβ peptide that antibody 2294 binds

[0219] Antibody 2294 is a murine antibody raised by immunizing a mouse with $A\beta_{1-40}$. This antibody is described in US 2004/0146512 and WO 04/032868.

[0220] Binding affinity for antibody 2294 to $A\beta_{1-40}$, $A\beta_{1-42}$, or $A\beta_{22-37}$ was measured using Biacore as described above. Table 6 below shows the affinity of antibody 2294 Fab fragment to various Aβ peptides.

Table 6. Binding affinity of antibody 2294 Fab fragment

	k_{on} (1/Ms)	k_{off} (1/s)	K_D (nM)
Biotinylated $A\beta_{1-40}$ immobilized on streptavidin chip, 2294 Fab flowed onto it	6.6×10^4	3.95×10^{-4}	6
Biotinylated $A\beta_{1-42}$ immobilized on streptavidin chip, 2294 Fab flowed onto it	1.1×10^4	4.87×10^{-3}	400
Biotinylated $A\beta_{22-37}$ immobilized on streptavidin chip, 2294 Fab flowed onto it	5×10^3	0.049	10,000

[0221] Epitope mapping of antibody 2294 was performed by ELISA assay. Biotinylated 15-mer or 10-mer of various Aβ peptides (these peptides have glycine added to the C-terminal end) were immobilized on streptavidin coated plates. NUNC maxisorp plates were coated with 6 ug/ml of streptavidin (Pierce, 21122) in PBS pH 7.4 for more than 1 h at 4°C. Plates were blocked with 1% BSA in PBS buffer pH 7.4. After washing, biotinylated Aβ peptides in PBS pH 7.4 were incubated 1 hour at room temperature. Antibody 2294 (from 2.5 ug/ml to 10 ug/ml) was incubated with the immobilized Aβ peptides for 1 h at room temperature. After washing, plates were incubated with secondary antibody, a HRP conjugated goat anti-human kappa chain antibody (MP Biomedicals, 55233) at 1:5000 dilution. After washing, bound secondary antibody was measured by adding TMB substrate (KPL, 50-76-02, 50-65-02). HRP reaction was stopped by adding 1M phosphoric acid and absorbance at 450 nm was measured. As shown in Figure 6, antibody 2294 binds to Aβ peptides with amino acids 20-34, 21-35, 22-36, 23-37, 24-38, 25-39, 26-40, and 25-34 with a glycine at the C-terminus; but does not bind to Aβ peptides with amino acids 19-33, 27-41, 24-33, and 27-35 having a glycine at the C-terminus of these peptides. This suggests that the epitope of antibody 2294 binds includes amino acids from 26 to 34.

[0222] To further determine the epitope on Aβ peptide that is recognized by antibody 2294, ELISA binding analysis was used. Various Aβ peptides (Global Peptide Services, CO) was immobilized on a ELISA plate. The binding of 2294 full antibody (at 20 nM) to the immobilized Aβ was determined by ELISA as described above. Antibody 2294 binds to Aβ peptides 17-40, 17-42, 28-40, 1-38, 1-40, 1-42, and 1-43. Antibody 2294 did not bind to Aβ peptide 1-16, 1-28, 28-42, 22-35, and 33-40. Thus, antibody 2294 binds to the C-terminus of various truncated Aβ peptide, for example, 1-38, 1-40, 1-42, and 1-43.

[0223] Table 7 below shows binding comparison of 2294 to $A\beta_{1-40}$ to other Aβ peptide as measured by Biacore assay. Antibody 2294 (full antibody) has the strongest binding to $A\beta_{1-40}$ as compared to other peptides, with significantly lower binding to truncated $A\beta_{1-40}$ (such as 1-36, 1-37, 1-38, and 1-39), $A\beta_{1-42}$ and $A\beta_{1-43}$. This indicates that the side chain or backbone of amino acid 40 (Valine) of Aβ is involved in binding of 2294 to $A\beta_{1-40}$; and binding is significantly reduced in absence of this amino acid.

Table 7.

Aβ peptide fragment	Binding
1-28	-
1-43	-
22-35	-
1-36	+
1-37	+
1-38	++
1-39	++
17-42	+++
1-42	+++
17-40	++++
1-40	++++

"-" indicates no binding; "+" indicates very low binding; "++" indicates medium binding; "+++" indicates strong binding; and "++++" indicates very strong binding.

[0224] Based on data shown above, the epitope that antibody 2294 binds seems to include amino acids 26-34 and 40. Antibody 2294 binds to an epitope very similar to antibody 6G as shown in Figure 6. However, binding of antibody 6G is less dependent on amino acid 40 than antibody 2294.

[0225] Antibody binding competition experiments between 2294, 6G, 2H6, and 2289 using Biacore assay were performed. Antibody 2H6 is an antibody that binds to $A\beta_{33-40}$ and is described in U.S. provisional application 60/653,197, filed February 14, 2005. Antibody 2289 is an antibody that binds to Aβ16-28 and is described in U.S. Pub. No. 2004/0146512 and PCT WO 04.032868. Competition experiments were performed using Biacore assay. Antibody 2294, 6G, 2H6, and 2289 were immobilized on different channels of a CM5 chip. CM5 chip channels were activated with N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Antibody 2294, 6G, 2H6, and 2289 were each diluted into 10 mM sodium acetate pH 4.0 and injected over an activated chip at a concentration of 0.005 mg/mL. Each channel was blocked with ethanolamine. $A\beta_{1-40}$ peptide (150 uM) was flowed onto the chip for 2 min. Then antibody 2294 (to be tested for competition of binding) at 0.6 uM was flowed onto the chip for 1 min. HBS-EP buffer (0.01M HEPES, pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% Surfactant P20) was used as running buffer for all the BIAcore assays. After measuring binding of $A\beta_{1-40}$, all channels of the chip were regenerated by washing twice with a mixture of Pierce elution buffer (Product No. 21004, Pierce Biotechnology, Rockford, IL) and 4 M NaCl (2:1) for 6 sec. Competition binding was then performed for antibody 6G, 2H6, and then antibody 2289. Competition between 2294 and 6G and between 2294 and 2H6 for binding to $A\beta_{1-40}$ was observed, but no competition was observed between 2294 and 2289 or between 6G and 2289. Observations of competition between the antibody immobilized and the same antibody flowed onto the chip served as the positive control. Data indicate that antibody 2294 competes with 2H6 and 6G for binding to $A\beta_{1-40}$.

Example 4. Binding affinity of antibody 2294 Fc regions to murine Fcγ receptors

[0226] Binding affinity of the antibody Fc regions to Fcγ receptors were measured using BIAcore as described above. Briefly, purified murine Fcγ receptors (from R&D Systems) were immobilized on BIAcore CM5 chip by amine chemistry. Serial dilutions of monoclonal antibodies (ranging from 2 nM to the maximum concentration as indicated in Tables 8) were injected. HBS-EP (0.01M HEPES, pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% Surfactant P20) as running and sample buffer. Binding data were analyzed using 1:1

langmuir interaction model for high affinity interactions, or steady state affinity model for low affinity interactions.

[0227] Table 8 below shows the binding affinity of antibody 2294 as measured by K_D (nM) to murine FcγRI, FcγRIIb, and FcγRIII. Deglycosylated antibodies have a constant region with removed N-glycosylation. As shown in Table 8, deglycosylated 2294 had reduced affinity to all murine Fcγ receptors tested as compared to each corresponding antibody without removed N-glycosylation.

Table 8. Binding affinity of antibodies to murine Fcγ receptors as measured by K_D (nM)

Antibody	FcγRI	FcγRIIb	FcγRIII	Isotype	Maximum antibody concentration tested for binding to Fcγ receptors (nM)
2294	1,200	13,000	19,000	Murine IgG2b	18,000
Deglycosylated 2294	8,600	NB	NB	Deglycosylated murine IgG2b	22,000

NB: no significant binding when antibody was used at the maximum concentration tested.

Example 5. Effect of antibody 2294 and deglycosylated antibody 2294 in reducing Aβ deposit and cognition in animal model of Alzheimer's disease

[0228] Deglycosylated antibody 2294 was prepared as by incubating purified antibody 2294 at 37°C for 7 days with peptide-N-glycosidase F (Prozyme, 0.05 U per mg of antibody) in 20 mM Tris-HCl pH 8.0. Completeness of deglycosylation was verified by MALDI-TOF-MS and protein gel electrophoresis. Deglycosylated antibodies were purified by Protein A chromatography and endotoxin was removed by Q-Sepharose. The binding affinity to $A\beta_{1-40}$ of the deglycosylated 2294 was tested using Biacore assay described above, and the binding affinity of the deglycosylated 2294 to $A\beta_{1-40}$ was found to be identical to the intact antibody 2294.

[0229] Antibody 2294 and deglycosylated 2294 were tested in transgenic mice APP Tg2576 for their effect on reversal of cognitive deficits, histological symptoms, and

microhemorrhage. Antibody administration, histological and behavioral analysis are performed as described below.

[0230] *Administration of antibodies.* Transgenic mice over-expressing the "Swedish" mutant amyloid precursor protein (APP Tg2576 with K670N/M671; Hsiao et al., Science 274:99-102 (1996)) were used for the experiments. The Alzheimer's-like phenotype present in these mice has been well-characterized. Holcomb et al., Nat. Med. 4:97-100 (1998); Holcomb et al., Behav. Gen. 29:177-185 (1999); and McGowan E, Neurobiol. Dis. 6:231-244 (1999). For the sixteen weeks treatment study, APP-transgenic mice, aged 20 months, were assigned to one of the four groups. The first group received weekly intraperitoneal anti-Aβ antibody 2294 injections for a period of 16 weeks ($n = 4$). The second group received weekly intraperitoneal deglycosylated anti-Aβ antibody 2294 injections for a period of 16 weeks ($n = 5$). The third group received weekly intraperitoneal anti-AMN antibody (2906; mouse-monoclonal anti-*Drosophila* amnesiac protein IgG1) injections for a period of 16 weeks ($n = 6$). Non-transgenic littermates were treated for 16 weeks with either anti-AMN antibody ($n = 4$) or 2294 ($n = 2$).

[0231] *Behavioral analysis.* Following 16 weeks of antibody treatment, the mice from the study are subjected to a two-day radial-arm water-maze paradigm as described previously. Wilcock et al., *J. Neuroinflammation* 1:24 (2004). The apparatus is a 6-arm maze as described previously. Gordon et al., *Neurobiol. Aging* 22:377–385 (2001). On day one, 15 trials are run in three blocks of 5. A cohort of 4 mice are run sequentially for each block (i.e., each of 4 mice get trial one, then the same mice get trial two, etc.). After each 5-trial block, a second cohort of mice is run permitting an extended rest period before mice are exposed to the second block of 5 trials. The goal arm is different for each mouse in a cohort to minimize odor cues. The start arm is varied for each trial, with the goal arm remaining constant for a given individual for both days. For the first 11 trials, the platform is alternately visible then hidden (hidden for the last 4 trials). On day two, the mice are run in exactly the same manner as day one except that the platform is hidden for all trials. The number of errors (incorrect arm entries) is measured in a one-minute time frame. Mice failing to make an arm choice in 20 seconds are assigned one error, but no mice in this study has to be assigned an error in this manner. Due to the numbers of mice in the study, the tester are unaware of treatment group identity of each mouse. Since the dependent

measures in the radial-arm water-maze task are quantitative, not evaluative, the potential for tester bias is reduced. In order to minimize the influence of individual trial variability, each mouse's errors for 3 consecutive trials are averaged producing 5 data points for each day, which are analyzed statistically by ANOVA using StatView (SAS Institute Inc., NC).

[0232] *Histological analysis.* On the day of sacrifice, mice are weighed, overdosed with 100 mg/kg Nembutal (Abbott laboratories, North Chicago, IL), and then intracardially perfused with 25 mL of 0.9% sodium chloride. Brains are rapidly removed, and the left half of the brain is immersion fixed for 24 h in freshly prepared 4% paraformaldehyde in 100 mM KPO_4 (pH 7.2) for histopathology. The hemi-brains are then incubated for 24 h in 10%, 20% and 30% sucrose sequentially for cyroprotection. Horizontal sections of 25 μ thickness are collected using a sliding microtome and stored at 4°C in Dulbecco's phosphate-buffered saline with sodium azide (pH 7.2) to prevent microbial growth. A series of 8 equally spaced tissue sections 600 μ apart are randomly selected spanning the entire brain and stained using free-floating immunohistochemistry for total Aβ (rabbit polyclonal anti-pan Aβ; Biosource, Camarillo, CA, 1:10,000) as previously described. Gordon et al., *Exp. Neurol.* 173:183–195 (2002); Wilcock et al., J. Neurosci. 24:6144–6151 (2004). A second series of tissue sections 600 μm apart are stained using 0.2% Congo red in NaCl-saturated 80% ethanol. Another set of sections are also mounted and stained for hemosiderin using 2% potassium ferrocyanide in 2% hydrochloric acid for 15 min, followed by a counterstain in a 1% neutral red solution for 10 min. Quantification of Congo red staining and Aβ immunohistochemistry is performed using the Image-Pro Plus (Media Cybernetics, Silver Spring, MD) to analyze the percent area occupied by positive stain. One region of the frontal cortex and three regions of the hippocampus are analyzed (to ensure that there is no regional bias in the hippocampal values). The initial analysis of Congo red is performed to give a total value. A second analysis is performed after manually editing out all of the parenchymal amyloid deposits to yield a percent area restricted to vascular Congo red staining. To estimate the parenchymal area of Congo red, the vascular amyloid values are subtracted from the total percentage. For the hemosiderin stain the numbers of Prussian blue-positive sites are counted on all sections and the average number of sites per section calculated. Qualitative differences between animals are observed at the sections at a low magnification. Eight equally spaced sections are examined and the number of positive profiles is determined and

averaged to a per-section value. To assess possible treatment-related differences, the values for each treatment group are analyzed by one-way ANOVA followed by Fisher's LSD means comparisons.

[0233] *Measurement of serum level of Aβ peptide using ELISA.* Serum collected one day after the last dosing of antibodies is diluted and incubated in 96-well microtiter plates (MaxiSorp; Nunc, Rosklide, Denmark), which are precoated with antibody 6E10 (anti-beta amyloid antibody that binds to $A\beta_{1-17}$; Signet, Dedham, MA) at 5 ug/ml in PBS buffer, pH 7.4. The secondary antibody is biotinylated 4G8 (anti-beta amyloid antibody that binds to $A\beta_{17-24}$; Signet) at a 1:5000 dilution. Detection is done using a streptavidin-horseradish peroxidase conjugate (Amersham Biosciences), followed by TMB substrate (KPL, Gaithersburg, MD). $A\beta_{1-40}$ (American Peptide) scaling from 6–400 pM are used for standard curves.

[0234] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application. All publications, patents and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent or patent application were specifically and individually indicated to be so incorporated by reference.

Deposit of biological material

[0235] The following materials have been deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, USA (ATCC):

Material	Antibody No.	ATCC Accession No.	Date of Deposit
pDb.6G.hFc2a	6G heavy chain	PTA-6786	June 15, 2005
pEb.6G.hK	6G light chain	PTA-6787	June 15, 2005

[0236] Vector pEb.6G.hK is a polynucleotide encoding the 6G light chain variable region and the light chain kappa constant region; and vector pDb.6G.hFc2a is a polynucleotide encoding

the 6G heavy chain variable region and the heavy chain IgG2a constant region containing the following mutations: A330P331 to S330S331 (amino acid numbering is based on Kabat numbering with reference to the wildtype IgG2a sequence; *see Eur. J. Immunol.* (1999) 29:2613-2624).

[0237] These deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Rinat Neuroscience Corp. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC Section 122 and the Commissioner's rules pursuant thereto (including 37 CFR Section 1.14 with particular reference to 886 OG 638).

[0238] The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

Antibody sequences

6G heavy chain variable region amino acid sequence (SEQ ID NO: 1)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYAIHWVRQ
APGQGLEWMGFTSPYSGVSNYNQKFKGRVTMTRDTSTST
VYMELSSLRSEDTAVYYCARFDNYDRGYVRDYWGQGTLV
TVS

6G light chain variable region amino acid sequence (SEQ ID NO:2)

DIVMTQSPDSLAVSLGERATINCRASESVDNDRISFLNW
YQQKPGQPPKLLIYAATKQGTGVPDRFSGSGSGTDFTLT
ISSLQAEDVAVYYCQQSKEFPWSFGGGTKVEIKRTV

6G CDR H1 (extended CDR) (SEQ ID NO: 3)
GYTFTTYAIH

6G CDR H2 (extended CDR) (SEQ ID NO: 4)
FTSPYSGVSNYNQKFKG

6G CDR H3 (extended CDR) (SEQ ID NO: 5)
FDNYDRGYVRDY

6G CDR L1 (extended CDR) (SEQ ID NO: 6)
RASESVDNDRISFLN

6G CDR L2 (extended CDR) (SEQ ID NO: 7)
AATKQGT

6G CDR L3 (extended CDR) (SEQ ID NO: 8)
QQSKEFPWS

6G heavy chain variable region nucleotide sequence (SEQ ID NO:9)
CAGGTGCAACTGGTGCAATCCGGTGCCGAGGTGAAAAAGCCAGGCGCCTCCGTGA
AAGTGTCCTGCAAAGCCTCCGGTTACACCTTTACCACCTATGCCATCCATTGGGTG
CGCCAGGCCCCAGGCCAGGGTCTGGAGTGGATGGGCTTTACTTCCCCCTACTCCG
GGGTGTCGAATTACAATCAGAAGTTCAAAGGCCGCGTCACCATGACCCGCGACAC
CTCCACCTCCACAGTGTATATGGAGCTGTCCTCTCTGCGCTCCGAAGACACCGCCG
TGTATTACTGTGCCCGCTTCGACAATTACGATCGCGGCTATGTGCGTGACTATTGG
GGCCAGGGCACCCTGGTCACCGTCTCC

6G light chain variable region nucleotide sequence (SEQ ID NO:10)
GACATCGTGATGACCCAGTCCCCAGACTCCCTGGCCGTGTCCCTGGGCGAGCGCG
CCACCATCAACTGCCGCGCCAGCGAATCCGTGGATAACGATCGTATTTCCTTTCT

GAACTGGTACCAGCAGAAACCAGGCCAGCCTCCTAAGCTGCTCATTTACGCCGC
CACCAAACAGGGTACCGGCGTGCCTGACCGCTTCTCCGGCAGCGGTTCCGGCAC
CGATTTCACTCTGACCATCTCCTCCCTGCAGGCCGAAGATGTGGCAGTGTATTAC
TGTCAGCAGTCCAAAGAGTTTCCCTGGTCCTTTGGCGGTGGCACCAAGGTGGAGA
TCAAACGCACTGTG

6G heavy chain full antibody amino acid sequence (including modified IgG2a as described herein) (SEQ ID NO:11)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYAIHWVRQAPGQGLEWMGFTSPYSG
VSNYNQKFKGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARFDNYDRGYVRDYW
GQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVE
CPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPSSIEKTISKTKG
QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPML
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

6G light chain full antibody amino acid sequence (SEQ ID NO:12)

~~DIVMTQSPDSLAVSLGERATINCRASESVDNDRISFLNWYQQKPGQPPKLLIYAATK~~
QGTGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQSKEFPWSFGGGTKVEIKRTV
AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

6G heavy chain full antibody nucleotide sequence (including modified IgG2a as described herein) (SEQ ID NO:13)

CAGGTGCAACTGGTGCAATCCGGTGCCGAGGTGAAAAAGCCAGGCGCCTCCGTG
AAAGTGTCCTGCAAAGCCTCCGGTTACACCTTTACCACCTATGCCATCCATTGGGT
GCGCCAGGCCCCAGGCCAGGGTCTGGAGTGGATGGGCTTTACTTCCCCCTACTCC
GGGGTGTCGAATTACAATCAGAAGTTCAAAGGCCGCGTCACCATGACCCGCGAC
ACCTCCACCTCCACAGTGTATATGGAGCTGTCCTCTCTGCGCTCCGAAGACACCG
CCGTGTATTACTGTGCCCGCTTCGACAATTACGATCGCGGCTATGTGCGTGACTAT
TGGGGCCAGGGCACCCTGGTCACCGTCTCCTCAGCCTCCACCAAGGGCCCATCTG
TCTTCCCACTGGCCCCATGCTCCCGCAGCACCTCCGAGAGCACAGCCGCCCTGGG

CTGCCTGGTCAAGGACTACTTCCCAGAACCTGTGACCGTGTCCTGGAACTCTGGC
GCTCTGACCAGCGGCGTGCACACCTTCCCAGCTGTCCTGCAGTCCTCAGGTCTCTA
CTCCCTCAGCAGCGTGGTGACCGTGCCATCCAGCAACTTCGGCACCCAGACCTAC
ACCTGCAACGTAGATCACAAGCCAAGCAACACCAAGGTCGACAAGACCGTGGAG
AGAAAGTGTTGTGTGGAGTGTCCACCTTGTCCAGCCCCTCCAGTGGCCGGACCAT
CCGTGTTCCTGTTCCCTCCAAAGCCAAAGGACACCCTGATGATCTCCAGAACCCC
AGAGGTGACCTGTGTGGTGGTGGACGTGTCCCACGAGGACCCAGAGGTGCAGTTC
AACTGGTATGTGGACGGAGTGGAGGTGCACAACGCCAAGACCAAGCCAAGAGAG
GAGCAGTTCAACTCCACCTTCAGAGTGGTGAGCGTGCTGACCGTGGTGCACCAGG
ACTGGCTGAACGGAAAGGAGTATAAGTGTAAGGTGTCCAACAAGGGACTGCCAT
CCAGCATCGAGAAGACCATCTCCAAGACCAAGGGACAGCCAAGAGAGCCACAGG
TGTATACCCTGCCCCCATCCAGAGAGGAGATGACCAAGAACCAGGTGTCCCTGAC
CTGTCTGGTGAAGGGATTCTATCCATCCGACATCGCCGTGGAGTGGGAGTCCAAC
GGACAGCCAGAGAACAACTATAAGACCACCCCTCCAATGCTGGACTCCGACGGA
TCCTTCTTCCTGTATTCCAAGCTGACCGTGGACAAGTCCAGATGGCAGCAGGGAA
ACGTGTTCTCTTGTTCCGTGATGCACGAGGCCCTGCACAACCACTATACCCAGAA
GAGCCTGTCCCTGTCTCCAGGAAAG

6G light chain full antibody nucleotide sequence (SEQ ID NO:14)

GACATCGTGATGACCCAGTCCCCAGACTCCCTGGCCGTGTCCCTGGGCGAGCGCG
CCACCATCAACTGCCGCGCCAGCGAATCCGTGGATAACGATCGTATTTCCTTTCTG
AACTGGTACCAGCAGAAACCAGGCCAGCCTCCTAAGCTGCTCATTTACGCCGCCA
CCAAACAGGGTACCGGCGTGCCTGACCGCTTCTCCGGCAGCGGTTCCGGCACCGA
TTTCACTCTGACCATCTCCTCCCTGCAGGCCGAAGATGTGGCAGTGTATTACTGTC
AGCAGTCCAAAGAGTTTCCCTGGTCCTTTGGCGGTGGCACCAAGGTGGAGATCAA
ACGCACTGTGGCTGCACCATCTGTCTTCATCTTCCCTCCATCTGATGAGCAGTTGA
AATCCGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCACGCGAGGCC
AAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCCGGTAACTCCCAGGAGAGT
GTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACC
CTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCAT
CAGGGCCTGAGTTCTCCAGTCACAAAGAGCTTCAACCGCGGTGAGTGC

CLAIMS

1. A monoclonal antibody that specifically binds to an Aβ peptide, wherein said antibody binds to an epitope on $A\beta_{1-40}$ that includes amino acids 25-34 and 40, wherein said antibody binds to $A\beta_{1-40}$ with higher affinity than its binding to $A\beta_{1-42}$ and $A\beta_{1-43}$, wherein said antibody binds to $A\beta_{22-37}$ with a K_D of less than 1 μM, and wherein said antibody comprises a heavy chain variable region comprising three CDRs at least 85% identical to the three CDRs from the heavy chain variable region shown in SEQ ID NO:1, and a light chain variable region comprising three CDRs at least 85% identical to the three CDRs from the light chain variable region shown in SEQ ID NO:2.

2. The antibody of claim 1, wherein said antibody comprises a heavy chain variable region comprising three CDRs from the heavy chain variable region shown in SEQ ID NO:1, and a light chain variable region comprising three CDRs from the light chain variable region shown in SEQ ID NO:2.

3. The antibody of claim 2, wherein said antibody comprises a heavy chain variable region comprising the three CDRs shown in SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5.

4. The antibody of claim 2 or 3, wherein said antibody comprises a light chain variable region comprising the three CDRs shown in SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8.

5. The antibody of claim 4, wherein said heavy chain variable region comprises the amino acid sequence shown in SEQ ID NO:1,

6. The antibody of claim 4 or 5, wherein said light chain variable region comprises the amino acid sequence shown in SEQ ID NO:2.

7. The antibody of claim 6, wherein said heavy chain amino acid sequence is shown in SEQ ID NO:11, and the light chain amino acid sequence is shown in SEQ ID NO:12.

8. The antibody of claim 1, wherein the antibody binds to $A\beta_{1-40}$ with at least about 40-

fold higher affinity than its binding to $A\beta_{1-42}$ and $A\beta_{1-43}$.

9. The antibody of claim 1 or 8, wherein the Fab fragment of the antibody binds $A\beta_{1-40}$ with an affinity of about 10 nM or less.

10. The antibody of claim 9, wherein the Fab fragment of the antibody binds $A\beta_{1-40}$ with an affinity of about 5 nM or less.

11. The antibody of any one of claims 1 to 10, wherein the isotype of the antibody is selected from the group consisting of IgG1, IgG2, IgG3, and IgG4.

12. The antibody of any one of claims 1 to 11, wherein the antibody comprises a heavy chain constant region comprising a Fc region, wherein the heavy chain constant region has impaired effector function.

13. The antibody of claim 12, wherein N-glycosylation in the Fc region is removed.

14. The antibody of claim 12, wherein the heavy chain constant region of the antibody is a human IgG2a constant region comprising amino acid mutations A330P331 to S330S331, wherein the amino acid position is based on Kabat numbering with reference to human wildtype IgG2a sequence.

15. The antibody of claim 12, wherein the heavy chain constant region of the antibody is a human IgG4 constant region comprising amino acid mutations from E233F234L235 to P233V234A235, wherein the amino acid position is based on Kabat numbering with reference to human wildtype IgG4 sequence.

16. The antibody of any one of claims 1 to 15, wherein the antibody is a human antibody.

17. The antibody of any one of claims 1 to 15, wherein the antibody is a humanized antibody.

18. A fragment of a monoclonal antibody of any one claim 2 to 7, wherein said fragment has or retains the binding specificity of said monoclonal antibody.

19. The fragment of claim 18, wherein the fragment is a Fab, a Fab', a $F(ab')_2$, or a Fv.

20. A polynucleotide comprising a nucleotide sequence encoding a variable region of an antibody heavy chain of SEQ ID: 1 or an antibody light chain of SEQ ID: 2.

21. A vector comprising the polynucleotide of claim 20.

22. The vector of claim 21, wherein said vector is pDb.6G.hFc2a with a deposit number of ATCC No. PTA-6786 or wherein said vector is pEb.6G.hK with a deposit number of ATCC No. PTA-6787.

23. A host cell comprising one or more of the polynucleotides of claim 20.

24. A method of producing an antibody, comprising culturing the host cell of claim 23 containing one or more of the polynucleotides of claim 20 under conditions such that the antibody is produced; and isolating the antibody from the host cell or culture.

25. A pharmaceutical composition comprising an effective amount of the antibody of any of claims 1-17 or of a fragment of any one of claims 18 or 19, and a pharmaceutically acceptable excipient.

26. Use of the pharmaceutical composition of claim 25 for the manufacture of a medicament for preventing, treating, inhibiting or delaying development of a disease associated with altered Aβ or βAPP expression or accumulation of Aβ peptide.

27. An antibody of any one of claims 1-17 or a fragment of any one of claims 18 or 19 for use in preventing, treating, inhibiting or delaying development of a disease associated with altered Aβ or βAPP expression or accumulation of Aβ peptide.

28. The use of claim 26 or the antibody or fragment of claim 27 for the treatment of Alzheimer's disease or delaying development of a symptom associated with Alzheimer's disease in a subject.

29. The use of claim 26 or the antibody or fragment of claim 27, wherein the disease is multi-infarct dementia, mild cognitive impairment, cerebral amyloid angiopathy, Down's syndrome, Parkinson's disease, Creutzfeld-Jakob, dementia with Lewy body, or AIDS.

30. Use of the pharmaceutical composition of claim 25 for the manufacture of a medicament for the suppression of formation of or reduction of amyloid plaques in a subject.

31. Use of the pharmaceutical composition of claim 25 for the manufacture of a medicament for improving cognition or reversing cognitive decline associated with amyloid deposit of Aβ in a subject.

32. A monoclonal antibody comprising a heavy chain variable region comprising the three CDRs shown in SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO:5; and a light chain variable region comprising the three CDRs shown in SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8.

33. A monoclonal antibody comprising a heavy chain amino acid sequence as shown in SEQ ID NO:11 and a light chain amino acid sequence as shown in SEQ ID NO:12.