

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
9 November 2006 (09.11.2006)

PCT

(10) International Publication Number
WO 2006/118959 A2

(51) International Patent Classification: Not classified

(21) International Application Number:
PCT/US2006/016071

(22) International Filing Date: 28 April 2006 (28.04.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/676,093 29 April 2005 (29.04.2005) US
60/704,818 1 August 2005 (01.08.2005) US

(71) Applicant (for all designated States except US): **RINAT NEUROSCIENCE CORP.** [US/US]; 230 E. Grand Ave, South San Francisco, CA 94080 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **ROSENTHAL, Arnon** [US/US]; 150 Normandy Lane, Woodside, CA 94062 (US). **PONS, Jaume** [ES/US]; 3311 La Mesa Drive #3, San Carlos, CA 94070 (US). **HO, Wei-Hsien** [US/US]; 153 California Avenue, F211, Palo Alto, CA 94306 (US).

(74) Agents: **ZHOU, Jie** et al.; **MORRISON & FOERSTER LLP**, 755 Page Mill Road, Palo Alto, CA 94304-1018 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ANTIBODIES DIRECTED AGAINST AMYLOID-BETA PEPTIDE AND METHODS USING SAME

(57) Abstract: Antibodies directed to the C-terminal side of β -amyloid peptide and methods of using these antibodies for diagnosing and treatment of Alzheimer's disease and A β peptide associated diseases are described.

WO 2006/118959 A2

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 (A β), which is the cleavage product of beta amyloid precursor protein (β APP 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] A β 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). Disease-linked mutations in these genes result in increased production of the 42-amino acid form of A β , the predominant form found in amyloid plaques. Moreover, immunization of transgenic mice that overexpress a disease-linked mutant form of APP with human A β reduces plaque burden and associated pathologies (Schenk et al., 1999, *Nature* 400:173-177; WO 99/27944), and peripheral administration of antibodies directed against A β 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 A β 1-42 were suspended due to meningoencephalitis in a subset of patients. Orgogozo et al., *Neurology* 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-A β 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 A β 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 A β peptide. In one aspect, the invention provides an antibody or a polypeptide that binds to A β ₁₋₄₀, A β ₁₋₄₂, and A β ₁₋₄₃, wherein the antibody or the polypeptide binds to A β ₁₋₄₀ with higher affinity than its binding to A β ₁₋₄₂, and A β ₁₋₄₃, and wherein the antibody or the polypeptide binds to an epitope on A β ₁₋₄₀ that includes amino acids 25-34 and 40. In some embodiments, the antibody binds to A β ₁₋₄₀ with at least about 40-fold higher affinity than its binding to A β ₁₋₄₂ and/or A β 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 β ₁₋₄₀ bound by antibody 6G or its variants shown in Table 3.

[0024] In another aspect, the invention is a complex of A β 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 A β or β APP expression, or accumulation of A β 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 A β 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 A β 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 A β peptide (such as soluble, oligomeric and deposited form) in a subject comprising administering to the subject an effective amount of an antibody, a polypeptide, or a polynucleotide of the invention. In some embodiments, the accumulation of A β peptide is inhibited and/or reduced in the brain. In some embodiments, the toxic effects of A β peptide are inhibited and/or reduced. Thus, the method of the invention can be used to treat any disease in which accumulation of A β 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 A β 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 A β or β APP expression, such as Down's syndrome, and AIDS. The method comprises contacting a specimen of a patient suspected of having altered A β or β APP expression with an antibody of the invention and determining whether the level of A β or β APP differs from that of a control or comparison specimen. In some embodiments, serum level of A β is measured before and after administration of an anti-A β antibody; and any increase of serum level of A β 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. A β 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 A β peptides was measured using goat anti-human kappa HRP conjugated secondary antibody.

[0042] Figure 3 shows epitope mapping of antibody 6G by ELISA. Various A β 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 A β 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 A β . Relative positions of A β 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 β 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 A β 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 A β peptides were measured using goat anti-human kappa HRP conjugated secondary antibody. Antibody 2294 bound to immobilized A β 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 A β . 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, phosphoramidates, 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, poly-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 β 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 β ₁₋₄₀ epitopes or non-A β 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 Immunological 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 A β 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 A β peptide circulating in biological fluids, reducing A β peptide (including soluble, oligomeric and deposited) in a tissue (such as brain), inhibiting, slowing and/or reducing accumulation of A β peptide in the brain, inhibiting, slowing and/or reducing toxic effects of A β 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-A β 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-A β antibody and the other agent can be administered at different dosing frequencies or intervals. For example, an anti-A β antibody can be administered weekly, while the other agent can be administered less frequently. It is understood that the anti-A β 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 A β peptide. The invention provides an antibody or a polypeptide that binds to A β_{1-40} , A β_{1-42} , and A β_{1-43} . In some embodiments, the antibody or the polypeptide binds to A β_{1-40} with higher affinity than its binding to A β_{1-42} , and A β_{1-43} . In some embodiments, the antibody binds to A β_{1-36} , A β_{1-37} , A β_{1-38} , and A β_{1-39} . In some embodiments, the antibody binds to A β_{22-35} . In some embodiments, the antibody binds to A β_{28-40} . In some embodiments, the antibody or the polypeptide binds to an epitope on A β_{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 β ₁₋₄₀, and/or one or more polynucleotides comprising sequences encoding one or more antibodies or polypeptides that bind to C-terminus of A β 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 β ₁₋₄₀, A β ₁₋₄₂, and A β ₁₋₄₃; (b) binds to A β ₁₋₄₀, A β ₁₋₄₂, and A β ₁₋₄₃ with higher affinity binding to A β ₁₋₄₀ than to A β ₁₋₄₂ and A β ₁₋₄₃; (c) binds to an epitope on A β ₁₋₄₀ that includes amino acids 25-34 and 40; (d) binds to A β ₁₋₃₆, A β ₁₋₃₇, A β ₁₋₃₈, and A β ₁₋₃₉, but with lower affinity as compared to its binding to A β ₁₋₄₀; (e) binds to A β ₂₂₋₃₇ with a K_D of less than about 1 μ M; (f) binds to A β ₂₂₋₃₅; (g) binds to A β 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 A β 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-A β 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 β_{1-40} 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, 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 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 $A\beta$ peptide (such as $A\beta_{25-40}$ 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 Fc γ portion can be modified to avoid interaction with Fc γ 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 β 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 $\beta(1,4)$ -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 Fc γ Rn and/or Fc γ RIIb. 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 β 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-A β 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 Fc γ RI, Fc γ RII, and/or Fc γ RIII. 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 Health, 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 A β 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-A β 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 Fc γ RI 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 endoglycosidase 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 be 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 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 A β 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 A β 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 A β 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 be 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 Phylogenies 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., Birkhäuser 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. subtilis*) and yeast (such as *S. cerevisiae*, *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 β 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-A β antibodies having impaired effector function

[0167] Antibody 6G which binds to C-terminus of A β may be used to identify or detect the presence or absence of A β . For simplicity, reference will be made generally to 6G or antibodies with the understanding that these methods apply to any of A β binding embodiments (such as polypeptides) described herein. Detection generally involves contacting a biological sample with an antibody described herein that binds to A β and the formation of a complex between A β and an antibody (*e.g.*, 6G) which binds specifically to A β . 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 A β or β APP 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 A β expression with an antibody or polypeptide of the invention and determining whether the level of A β 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 A β 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 ^3H) so that the cells or tissue of interest can be localized using immunoscintigraphy.

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

[0174] Anti-A β 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-A β antibody results in a rapid increase in plasma A β 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-A β antibody having impaired effector function is administered to a subject, and level of A β in the plasma is measured, whereby an increase in plasma A β 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-A β 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 A β or βAPP

expression, or accumulation or deposit of A β peptide (collectively termed "A β -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 A β 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 A β -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 A β -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 A β 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 A β accumulation is in the circulatory system of the subject.

[0178] This invention also provides methods of reducing amyloid plaques and/or reducing or slowing A β 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 A β accumulation is in the circulatory system of the subject.

[0179] This invention also provides methods of removing or clearing amyloid plaques and/or A β 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 A β accumulation is in the circulatory system of the subject.

[0180] This invention also provides methods of reducing A β peptide in a tissue (such as brain), inhibiting and/or reducing accumulation of A β peptide in a tissue (such as brain), and inhibiting and/or reducing toxic effects of A β 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. A β polypeptide may be in soluble, oligomeric, or deposited form. Oligomeric form of A β may be composed of 2-50 A β 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 A β 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 A β 42 levels. Elevated tau and decreased A β 42 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 A β 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., Noyes 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 µg/kg body weight; at least about 100 µg/kg body weight; at least about 50 µg/kg body weight; at least about 10 µg/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 A β -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 A β peptide in blood vessels (such as stroke and HCHWA-D)), or detecting or purifying A β or β APP. 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 A β or β APP. The active agent in the composition is an antibody and preferably, comprises monoclonal antibodies specific for A β or β APP. 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-A β antibody or polypeptide described herein having impaired effector function. In some embodiments, the anti-A β 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 A β or β APP, 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 A β peptide in the brain). In kits to be used for detecting or purifying A β or β APP, 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 dialyzed 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 β ₁₋₄₀ peptide or other A β peptides to the antibody. CM5 chips were activated with N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide 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 volume of 4 M NaCl effectively removed the bound A β 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 β_{1-40} synthetic peptide or other A β peptide samples were injected for 1 min at 100 μ L/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 β_{1-40} peptide or other A β peptides on SA chip and measuring binding kinetics of 6G Fab and Fab of 6G variants to the immobilized A β peptide. Affinities of 6G Fab fragment and its variants Fab fragments were determined by Surface Plasmon Resonance (SPR) system (BIAcore 3000TM, BIAcore, Inc., Piscaway, NJ). SA chips (streptavidin) were used according to the supplier's instructions. Biotinylated A β 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 A β 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 K_D) of purified Fab samples were injected for 2 min at 100 μ L/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 A β peptides. NUNC maxisorp plates were coated with 2.5 ug/ml of A β 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-A β antibody, or purified full antibody or Fabs at desired dilution) was incubated with the immobilized A β 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 A β 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 A β peptides in PBS pH 7.4 were incubated 1 hour at room temperature. Primary antibody (from cell supernatants, serum containing anti-A β antibody, or purified full antibody or Fabs at desired dilution) was incubated with the immobilized A β 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 β_{1-40} , A β_{1-42} , and other A β 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 β_{1-40} , A β_{1-42} , and A β_{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 β_{1-40} immobilized on streptavidin chip, 6G Fab flowed onto it	3.0×10^5	7.0×10^{-4}	2
Biotinylated A β_{1-42} immobilized on streptavidin chip, 6G Fab flowed onto it	1.8×10^4	1.6×10^{-3}	80
Biotinylated A β_{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 β_{1-40} or A β_{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 β_{1-40}	A β_{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 β_{1-40}	A β_{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 A β peptide that antibody 6G binds

[0213] To determine the epitope on A β peptide that is recognized by antibody 6G, ELISA binding analysis was used. Various A β peptides (Global Peptide Services, CO) was immobilized on a ELISA plate. The binding of 6G full antibody (at 20 nM) to the immobilized A β was determined by ELISA as described above. Amino acid sequences of A β_{1-40} , A β_{1-42} , and A β_{1-43} are shown in Table 5 below. As shown in Figure 2, antibody 6G binds to A β 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 A β peptides. Antibody 6G did not bind to A β peptide 1-16, 1-28 and 33-40. Thus, antibody 6G binds to the C-terminus of various truncated A β 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 β ₁₋₄₀ to other A β peptide as measured by k_{off} (1/s) using Biacore assay. Antibody 6G binds to A β ₁₋₄₀ with highest affinity as compared to other peptides, with significantly lower affinity to truncated A β ₁₋₄₀ (such 1-36, 1-37, 1-38, and 1-39), A β ₁₋₄₂ and A β ₁₋₄₃. This indicates that the side chain or backbone of amino acid 40 (Valine) of A β is involved in binding of 6G to A β 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 β ₁₋₄₀ indicates that binding of 6G to A β ₁₋₄₀ involves but is not dependent on the free C-terminus of A β ₁₋₄₀. Lower affinity binding to A β ₁₋₄₂ and A β ₁₋₄₃ may be due to conformational differences between monomer form of A β ₁₋₄₀ and A β ₁₋₄₂ or A β ₁₋₄₃. It has been shown that monomer of A β ₁₋₄₂ has a conformation different from A β ₁₋₄₀ monomer in solution. See, the monomer structure coordinate for A β ₁₋₄₂ shown in Protein Data Bank (pdb files) with accession no. 1IYT; and the monomer structure coordinate for A β ₁₋₄₀ shown in Protein Data Bank (pdb files) with accession nos. 1BA6 and 1BA4.

Table 4.

A β peptide fragment	k_{off} (1/s)	K_{off} A β peptide/ k_{off} A β 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	9.3×10^{-3}	70.4
1-39	7.92×10^{-3}	60.0
17-42	0.0465	352.2
1-42	1.9×10^{-3}	14.4
28-42	3.37×10^{-3}	25.5
28-40-NH2#	3.62×10^{-3}	27.4
28-40	6.4×10^{-4}	4.8
17-40	2.15×10^{-4}	1.6
1-40	1.32×10^{-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 A β 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 A β 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 A β 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)	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV (SEQ ID NO:15)
1-42 (WT)	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA (SEQ ID NO:16)
1-43 (WT)	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIAT (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 β_{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 β_{1-16} antibody, which recognize N-terminal epitopes in A β , 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 A β peptide that antibody 2294 binds

[0219] Antibody 2294 is a murine antibody raised by immunizing a mouse with A β ₁₋₄₀. This antibody is described in US 2004/0146512 and WO 04/032868.

[0220] Binding affinity for antibody 2294 to A β ₁₋₄₀, A β ₁₋₄₂, or A β ₂₂₋₃₇ was measured using Biacore as described above. Table 6 below shows the affinity of antibody 2294 Fab fragment to various A β peptides.

Table 6. Binding affinity of antibody 2294 Fab fragment

	k_{on} (1/Ms)	k_{off} (1/s)	K_D (nM)
Biotinylated A β ₁₋₄₀ immobilized on streptavidin chip, 2294 Fab flowed onto it	6.6×10^4	3.95×10^{-4}	6
Biotinylated A β ₁₋₄₂ immobilized on streptavidin chip, 2294 Fab flowed onto it	1.1×10^4	4.87×10^{-3}	400
Biotinylated A β 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 A β 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 A β 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 A β 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 A β 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 A β 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 A β peptide that is recognized by antibody 2294, ELISA binding analysis was used. Various A β peptides (Global Peptide Services, CO) was immobilized on a ELISA plate. The binding of 2294 full antibody (at 20 nM) to the immobilized A β was determined by ELISA as described above. Antibody 2294 binds to A β peptides 17-40, 17-42, 28-40, 1-38, 1-40, 1-42, and 1-43. Antibody 2294 did not bind to A β peptide 1-16, 1-28, 28-42, 22-35, and 33-40. Thus, antibody 2294 binds to the C-terminus of various truncated A β peptide, for example, 1-38, 1-40, 1-42, and 1-43.

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

Table 7.

A β 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 β 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 A β ₁₆₋₂₈ 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 β ₁₋₄₀ peptide (150 μ M) was flowed onto the chip for 2 min. Then antibody 2294 (to be tested for competition of binding) at 0.6 μ M 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 β 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 β 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 β ₁₋₄₀.

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

[0226] Binding affinity of the antibody Fc regions to Fc γ receptors were measured using Biacore as described above. Briefly, purified murine Fc γ 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 Fc γ RI, Fc γ RIIb, and Fc γ RIII. Deglycosylated antibodies have a constant region with removed N-glycosylation. As shown in Table 8, deglycosylated 2294 had reduced affinity to all murine Fc γ receptors tested as compared to each corresponding antibody without removed N-glycosylation.

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

Antibody	Fc γ RI	Fc γ RIIb	Fc γ RIII	Isotype	Maximum antibody concentration tested for binding to Fc γ 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 A β 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 β_{1-40} of the deglycosylated 2294 was tested using Biacore assay described above, and the binding affinity of the deglycosylated 2294 to A β_{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-A β antibody 2294 injections for a period of 16 weeks ($n = 4$). The second group received weekly intraperitoneal deglycosylated anti-A β 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 KPO₄ (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 A β (rabbit polyclonal anti-pan A β ; 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 μ m 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 A β 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 A β peptide using ELISA.* Serum collected one day after the last dosing of antibodies is diluted and incubated in 96-well microtiter plates (MaxiSorp; Nunc, Roskilde, Denmark), which are precoated with antibody 6E10 (anti-beta amyloid antibody that binds to A β 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 β 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 β 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):

<i>Material</i>	<u>Antibody No.</u>	<u>ATCC Accession No.</u>	<u>Date of Deposit</u>
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
VYMELSSLRSEDTAVYYCARFDNYDRGYVRDYGQGTLLV
TVS

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

DIVMTQSPDSLAVSLGERATINCRASESVDNDRISFLNW
YQQKPGQPPKLLIYAATKQGTGVPDRFSGSGSGTDFTLT
ISSLQAEDVAVYYCQQSKEFPWSFGGGGTKVEIKRTV

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
AAGTGTCTTGCAAAGCCTCCGGTTACACCTTTACCACCTATGCCATCCATTGGGTG
CGCCAGGCCCCAGGCCAGGGTCTGGAGTGGATGGGCTTTACTTCCCCCTACTCCG
GGGTGTCGAATTACAATCAGAAGTTCAAAGGCCGCGTCACCATGACCCGCGACAC
CTCCACCTCCACAGTGTATATGGAGCTGTCCTCTCTGCGCTCCGAAGACACCGCCG
TGTATTACTGTGCCCCGCTTCGACAATTACGATCGCGGCTATGTGCGTGACTATTGG
GGCCAGGGCACCCCTGGTCACCGTCTCC

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

GACATCGTGATGACCCAGTCCCCAGACTCCCTGGCCGTGTCCTGGGCGAGCGCG
CCACCATCAACTGCCGCGCCAGCGAATCCGTGGATAACGATCGTATTTCTTTCT

GAAGTGGTACCAGCAGAAACCAGGCCAGCCTCCTAAGCTGCTCATTTACGCCGC
 CACCAAACAGGGTACCGGCGTGCTGACCGCTTCTCCGGCAGCGGTTCCGGCAC
 CGATTTCACTCTGACCATCTCCTCCCTGCAGGCCGAAGATGTGGCAGTGTATTAC
 TGTCAGCAGTCCAAAGAGTTTCCCTGGTCCTTTGGCGGTGGCACCAAGGTGGAGA
 TCAAACGCACTGTG

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

QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYAIHWVRQAPGQGLEWMGFTSPYSG
 VSNNYQKFKGRVTMTRDTSTSTVYMESSLRSEDTAVYYCARFDNYDRGYVRDYW
 GQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTS
 GVHTFPAVLQSSGLYSLSSVTVPSNFGTQTYTCNVDHKPSNTKVDKTVRKCCVE
 CPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEV
 HNAKTKPREEQFNSTFRVSVLTVVHQDWLNGKEYKCKVSNKGLPSSIEKTISKTKG
 QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPML
 DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

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

DIVMTQSPDSLAVSLGERATINCRASESDNDNISFLNWFYQQKPGQPPKLLIYAATK
 QGTGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQSKEFPWSFGGGTKVEIKRTV
 AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
 SKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

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

CAGGTGCAACTGGTGCAATCCGGTGCCGAGGTGAAAAAGCCAGGCGCCTCCGTG
 AAAGTGTCTTGCAAAGCCTCCGGTTACACCTTTACCACCTATGCCATCCATTGGGT
 GCGCCAGGCCCCAGGCCAGGGTCTGGAGTGGATGGGCTTTACTTCCCCCTACTCC
 GGGGTGTCGAATTACAATCAGAAGTTCAAAGGCCGCGTCACCATGACCCGCGAC
 ACCTCCACCTCCACAGTGTATATGGAGCTGTCCTCTCTGCGCTCCGAAGACACCG
 CCGTGTATTACTGTGCCCGCTTCGACAATTACGATCGCGGCTATGTGCGTGACTAT
 TGGGGCCAGGGCACCTGGTCACCGTCTCCTCAGCCTCCACCAAGGGCCCCTCTG
 TCTTCCCACTGGCCCCATGCTCCCGCAGCACCTCCGAGAGCACAGCCGCCCTGGG

CTGCCTGGTCAAGGACTACTTCCCAGAACCTGTGACCGTGTCTGGAACCTCTGGC
GCTCTGACCAGCGGCGTGCACACCTTCCCAGCTGTCTGCAGTCCTCAGGTCTCTA
CTCCCTCAGCAGCGTGGTGACCGTGCCATCCAGCAACTTCGGCACCCAGACCTAC
ACCTGCAACGTAGATCACAAAGCCAAGCAACACCAAGGTCGACAAGACCGTGGAG
AGAAAGTGTGTGTGGAGTGTCCACCTTGTCCAGCCCCTCCAGTGGCCGGACCAT
CCGTGTTCTGTTCCTCCAAAGCCAAGGACACCCTGATGATCTCCAGAACCCC
AGAGGTGACCTGTGTGGTGGTGGACGTGTCCACGAGGACCCAGAGGTGCAGTTC
AACTGGTATGTGGACGGAGTGGAGGTGCACAACGCCAAGACCAAGCCAAGAGAG
GAGCAGTTCAACTCCACCTTCAGAGTGGTGAGCGTGCTGACCGTGGTGCACCAGG
ACTGGCTGAACGGAAAGGAGTATAAGTGTAAGGTGTCCAACAAGGGACTGCCAT
CCAGCATCGAGAAGACCATCTCCAAGACCAAGGGACAGCCAAGAGAGCCACAGG
TGTATACCCTGCCCCCATCCAGAGAGGAGATGACCAAGAACCAGGTGTCCCTGAC
CTGTCTGGTGAAGGGATTCTATCCATCCGACATCGCCGTGGAGTGGGAGTCCAAC
GGACAGCCAGAGAACAACCTATAAGACCACCCCTCCAATGCTGGACTCCGACGGA
TCCTTCTTCCTGTATTCCAAGCTGACCGTGGACAAGTCCAGATGGCAGCAGGGAA
ACGTGTTCTCTTGTTCCTGATGCACGAGGCCCTGCACAACCACTATACCCAGAA
GAGCCTGTCCCTGTCTCCAGGAAAG

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

GACATCGTGATGACCCAGTCCCCAGACTCCCTGGCCGTGTCCCTGGGCGAGCGCG
CCACCATCAACTGCCGCGCCAGCGAATCCGTGGATAACGATCGTATTTCTTTCTG
AACTGGTACCAGCAGAAACCAGGCCAGCCTCCTAAGCTGCTCATTTACGCCGCCA
CCAAACAGGGTACCGGCGTGCCTGACCGCTTCTCCGGCAGCGGTTCCGGCACCGA
TTTCACTCTGACCATCTCCTCCCTGCAGGCCGAAGATGTGGCAGTGTATTACTGTC
AGCAGTCCAAAGAGTTTTCCCTGGTCTTTTGGCGGTGGCACCAAGGTGGAGATCAA
ACGCACTGTGGCTGCACCATCTGTCTTCATCTTCCCTCCATCTGATGAGCAGTTGA
AATCCGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCACGCGAGGCC
AAAGTACAGTGGAAAGGTGGATAACGCCCTCCAATCCGGTAACTCCCAGGAGAGT
GTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACC
CTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCAT
CAGGGCCTGAGTTCTCCAGTCACAAAGAGCTTCAACCGCGGTGAGTGC

CLAIMS

The claimed invention is:

1. A monoclonal antibody that specifically binds to an A β peptide, wherein the antibody binds to an epitope on A β ₁₋₄₀ that includes amino acids 25-34 and 40, wherein the antibody binds to A β ₁₋₄₀ with higher affinity than its binding to A β ₁₋₄₂ and A β ₁₋₄₃, and wherein the antibody is not antibody 2294.
2. The antibody of claim 1, wherein the antibody binds to A β ₁₋₄₀ with at least about 40-fold higher affinity than its binding to A β ₁₋₄₂ or A β ₁₋₄₃.
3. The antibody of claim 1, wherein the Fab fragment of the antibody binds A β ₁₋₄₀ with an affinity of about 5 nM or less.
4. The antibody of claim 1, wherein the isotype of the antibody is selected from the group consisting of IgG1, IgG2, IgG3, and IgG4.
5. The antibody of claim 1, wherein the antibody comprises a heavy chain constant region comprising a Fc region, wherein the heavy chain constant region has impaired effector function.
6. The antibody of claim 5, wherein N-glycosylation in the Fc region is removed.
7. The antibody of claim 5, 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.
8. The antibody of claim 5, 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.

9. The antibody of claim 1, wherein the antibody is a human antibody.

10. The antibody of claim 1, wherein the antibody is a humanized antibody.

11. The antibody of claim 1, wherein the antibody comprises a heavy chain variable region comprising three CDRs from heavy chain variable region of an antibody shown in Table 3, and a light chain variable region comprising three CDRs from light chain variable region of an antibody shown in Table 3.

12. The antibody of claim 1, wherein the antibody comprises a heavy chain variable region comprising three CDRs from antibody 6G heavy chain variable region shown in SEQ ID NO:1, and a light chain variable region comprising three CDRs from antibody 6G light variable region shown in SEQ ID NO:2.

13. The antibody of claim 1, wherein the 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, 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.

14. The antibody of claim 1, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence shown in SEQ ID NO:1, and a light chain variable region comprising the amino acid sequence shown in SEQ ID NO:2.

15. The antibody of claim 1, wherein the antibody comprises 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.

16. An antibody heavy chain comprising three CDRs from heavy chain of an antibody shown in Table 3.

17. The antibody heavy chain of claim 16, wherein the three CDRs are from antibody 6G heavy chain variable region shown in SEQ ID NO:1.

18. The antibody heavy chain of claim 16, wherein the heavy chain comprises the amino acid sequence shown in SEQ ID NO:1.

19. An antibody light chain comprising three CDRs from light chain of an antibody shown in Table 3.

20. The antibody light chain of claim 19, wherein the three CDRs are from antibody 6G light chain variable region shown in SEQ ID NO:2.

21. The antibody light chain of claim 19, wherein the light chain comprises the amino acid sequence shown in SEQ ID NO:2.

22. A fragment of antibody 6G, wherein the fragment has or retains the binding specificity of antibody 6G.

23. The fragment of claim 22, wherein the fragment is a Fab, a Fab', a F(ab')₂, or a Fv.

24. A pharmaceutical composition comprising the antibody of any one of claims 1-15 and a pharmaceutically acceptable carrier.

25. A polynucleotide comprising a nucleotide sequence encoding the antibody of any one of claims 1-15.

26. The polynucleotide of claim 25, wherein the polynucleotide comprises the nucleotide sequence of SEQ ID NOS:13 and 14.

27. A polynucleotide comprising a nucleotide sequence encoding the heavy chain of any one of claims 16-18.

28. A polynucleotide comprising a nucleotide sequence encoding the light chain of any one of claims 19-21.

29. A polynucleotide comprising a nucleotide sequence encoding the fragment of claims 22 or 23.

30. A vector comprising the polynucleotide of claim 25.

31. A vector comprising the polynucleotide of claim 27.

32. The vector of claim 31, wherein the vector is pDb.6G.hFc2a with a deposit number of ATCC No. PTA-6786.

33. A vector comprising the polynucleotide of claim 28.

34. The vector of claim 33, wherein the vector is pEb.6G.hK with a deposit number of ATCC No. PTA-6787.

35. A vector comprising the polynucleotide of claim 29.

36. A host cell comprising the polynucleotide of claim 25.

37. A host cell comprising the polynucleotide of claim 27.

38. A host cell comprising the polynucleotide of claim 28.

39. A host cell comprising the polynucleotide of claim 29.

40. A method of producing an antibody, comprising culturing the host cell of claim 36 under conditions such that the antibody is produced; and isolating the antibody from the host cell or culture.

41. A method for treating Alzheimer's disease in a subject comprising administering to the subject an effective amount of the pharmaceutical composition of claim 24.

42. A method of suppressing formation of amyloid plaques in a subject comprising administering to the subject an effective amount of the pharmaceutical composition of claim 24.

43. A method of reducing amyloid plaques in a subject comprising administering to the subject an effective amount of the pharmaceutical composition of claim 24.

44. A method of delaying development of a symptom associated with Alzheimer's disease in a subject comprising administering to the subject an effective amount of the pharmaceutical composition of claim 24.

Figure 1**BOLD=Kabat CDR**Underline=Chothia CDR**6G Heavy Chain variable domain**

H1
1 5 10 15 20 25 30 35
QVQLVQSGAEVKKPGASVKVSCKASGYTFT**TYAI**HWVRQ

H2
40 45 50 55 60 65 70 75
APGQGLEWMG**FTSPYSGVSNYNQKFKGR**VTMTRDTSTST

H3
80 85 90 95 100 105 110 115
VYMEISSLRSEDTAVYYCAR**FDNYDRGYVRDY**WGQGTLV

120
TVS

6G Light Chain variable domain

L1
1 5 10 15 20 25 30 35
DIVMTQSPDSLAVSLGERATINCR**RASESVDNDRIS**FLNW

L2
40 45 50 55 60 65 70 75
YQQKPGQPPKLLIY**AATKQGT**GVDPDRFSGSGSGTDFTLT

L3
80 85 90 95 100 105 110 114
ISSLQAEDVAVYYC**QQSKEFPWS**FGGGTKVEIKRTV

Figure 2

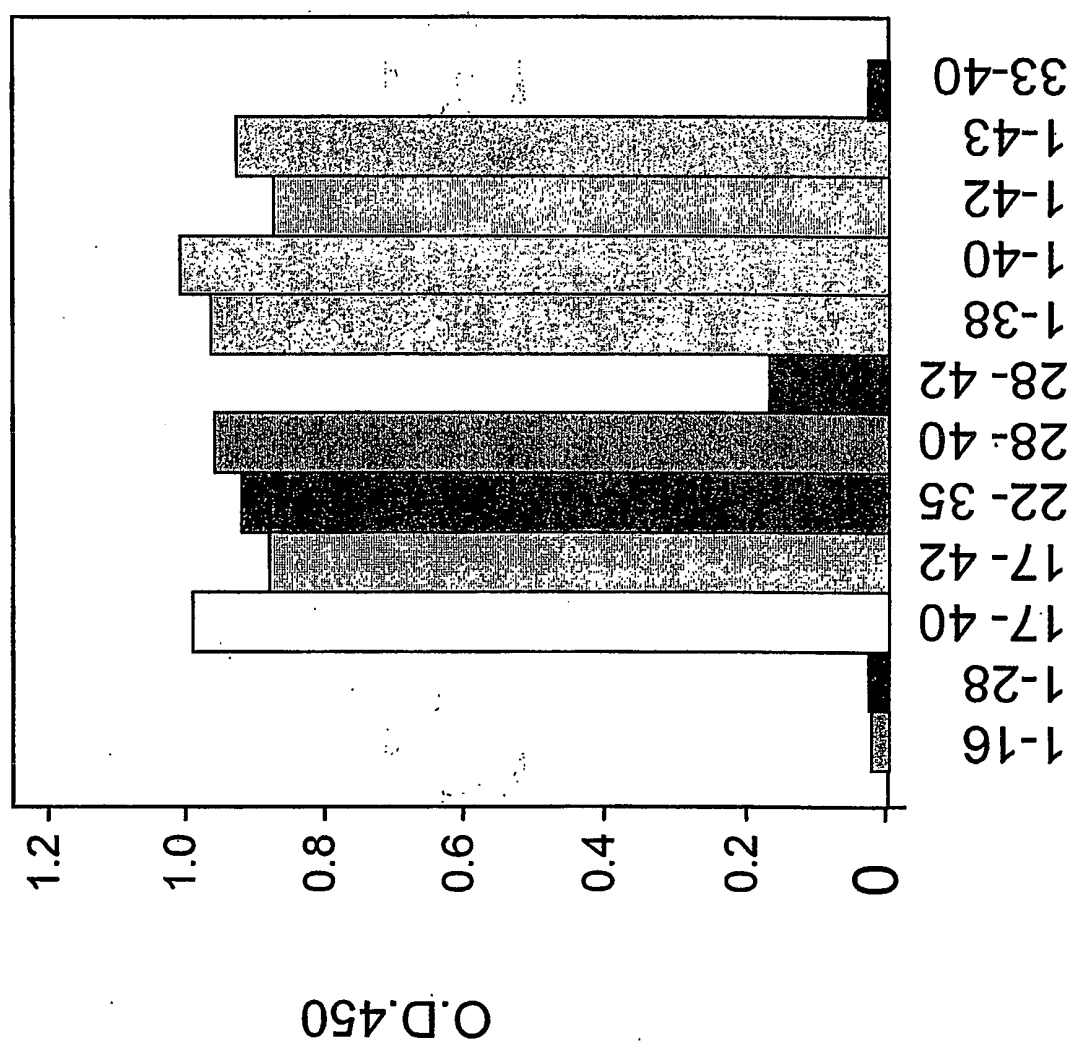


Figure 3

Epitope mapping of antibody 6G by ELISA peptide scanning

A450 ELISA15-mer Peptides

FFAEDVGSNKGAIIGG	NB
FAEDVGSNKGAIIGLG	>3
AEDVGSNKGAIIGLMG	>3
EDVGSNKGAIIGLMVG	>3
DVGSNKGAIIGLMVGG	2.3
VGSNKGAIIGLMVGGG	2.1
GSNKGAIIGLMVGGVG	1.5
SNKGAIIGLMVGGWVG	NB
NKGAIIGLMVGGWVIG	NB

10-mer Peptides
(min. epitope tested)

VGSNKGAIIGG	NB
GSNKGAIIGLG	2
SNKGAIIGLMG	NB

Figure 4

Schematic representation of antibody 6G epitope

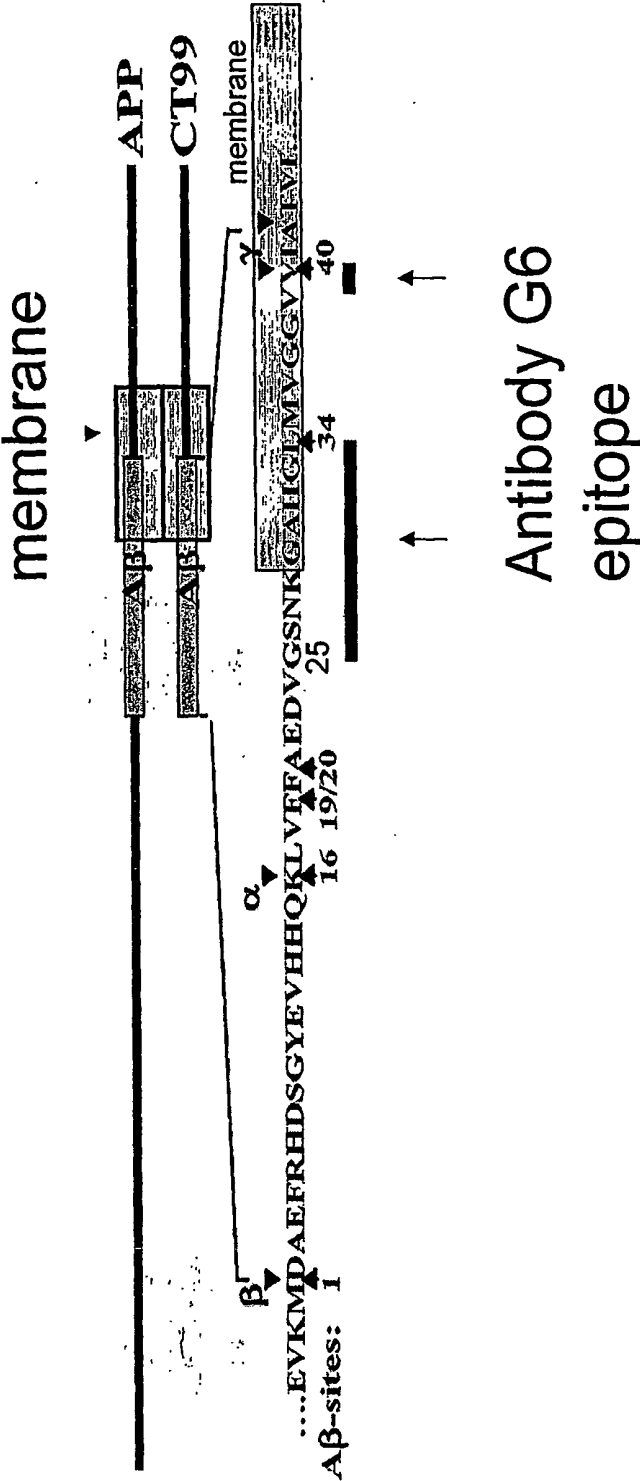
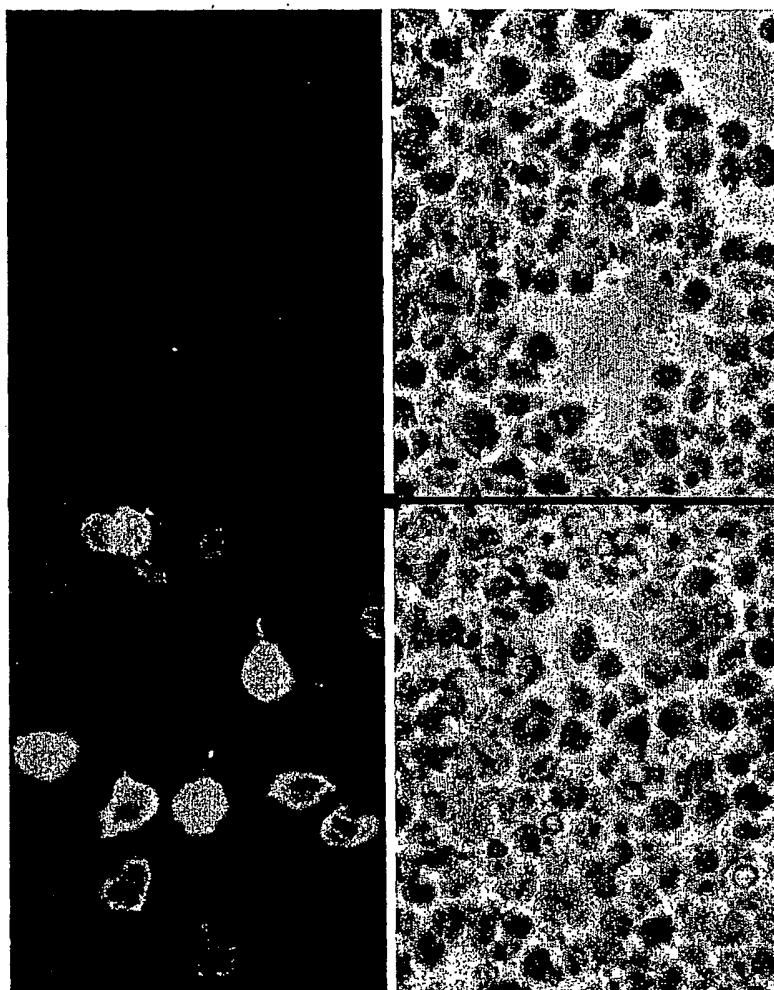


Figure 5m2324
(1-16)

6G



Epitope mapping:		
15 MER PEPTIDE	2294	6G
19 FFAEDVGSNKGAIIGG	NB	NB
20 FAEDVGSNKGAIIGLG	>3	>3
21 AEDVGSNKGAIIGLMG	2.9	>3
22 EDVGSNKGAIIGLMVG	>3	>3
23 DVGSNKGAIIGLMVGG	>3	2.3
24 VGSNKGAIIGLMVGGG	>3	2.1
25 GSNKGAIIGLMVGGVG	>3	1.5
26 SNKGAIIGLMVGGVVG	>3	NB.
27 NKGAIIGLMVGGVVIG	NB	NB
28 KGAIIGLMVGGVVIAG	NB	NB
10 MER PEPTIDES BINDING DATA: MINIMUM EPITOPE TESTED		
VGSNKGAIIGG	2294	6G
GSNKGAIIGLG	NB	NB
SNKGAIIGLMG	2	>3
	NB	NB

Figure 6

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
9 November 2006 (09.11.2006)

PCT

(10) International Publication Number
WO 2006/118959 A3

(51) International Patent Classification:
C07K 16/18 (2006.01) A61P 25/00 (2006.01)

(21) International Application Number:
PCT/US2006/016071

(22) International Filing Date: 28 April 2006 (28.04.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/676,093 29 April 2005 (29.04.2005) US
60/704,818 1 August 2005 (01.08.2005) US

(71) Applicant (for all designated States except US): **RINAT NEUROSCIENCE CORP.** [US/US]; 230 E. Grand Ave, South San Francisco, CA 94080 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **ROSENTHAL, Arnon** [US/US]; 150 Normandy Lane, Woodside, CA 94062 (US). **PONS, Jaume** [ES/US]; 3311 La Mesa Drive #3, San Carlos, CA 94070 (US). **HO, Wei-Hsien** [US/US]; 153 California Avenue, F211, Palo Alto, CA 94306 (US).

(74) Agents: **ZHOU, Jie** et al.; **MORRISON & FOERSTER LLP**, 755 Page Mill Road, Palo Alto, CA 94304-1018 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:
10 May 2007

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ANTIBODIES DIRECTED AGAINST AMYLOID-BETA PEPTIDE AND METHODS USING SAME

(57) Abstract: Antibodies directed to the C-terminal side of β -amyloid peptide and methods of using these antibodies for diagnosing and treatment of Alzheimer's disease and $A\beta$ peptide associated diseases are described.



WO 2006/118959 A3

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2006/016071

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K16/18 A61P25/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2004/146512 A1 (ROSENTHAL ARNON ET AL) 29 July 2004 (2004-07-29) paragraph [0003] paragraph [0007] paragraph [0015] paragraph [0018] - paragraph [0024] paragraph [0048] - paragraph [0052]	1-44
X	WO 03/077858 A (NEURALAB LIMITED; WYETH; BASI, GURIO; SALDANHA, JOSE) 25 September 2003 (2003-09-25) page 2, line 29 - page 3, line 20 page 8, line 8 - line 19 page 14, line 14 - page 16, line 19 page 42, line 20 - page 47, line 10 examples claim 102	1-44

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

23 February 2007

Date of mailing of the international search report

06/03/2007

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Rankin, Robert

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2006/016071

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99/58572 A (CAMBRIDGE UNIVERSITY TECHNICAL SERVICES LIMITED; ARMOUR, KATHRYN, LESL) 18 November 1999 (1999-11-18) page 4, line 26 - page 5, line 9 page 6, line 13 - page 7, line 13 claims; example 1	1-44
A	WO 03/086310 A (RAMOT AT TEL AVIV UNIVERSITY LTD; SOLOMON, BEKA; MCINNIS, PATRICIA) 23 October 2003 (2003-10-23) cited in the application paragraph [0001] paragraph [0007] paragraph [0009] - paragraph [0012]	1-44

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2006/016071

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 41-44 are directed to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the compound.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2006/016071

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2004146512 A1	29-07-2004	NONE	
WO 03077858 A	25-09-2003	AU 2003214152 A1 BR PI0308143 A CA 2478049 A1 CN 1703426 A EP 1554311 A2 HR 20040950 A2 IS 7499 A JP 2006503549 T KR 20050071368 A MX PA04008740 A	29-09-2003 23-01-2007 25-09-2003 30-11-2005 20-07-2005 31-08-2005 11-10-2004 02-02-2006 07-07-2005 13-07-2005
WO 9958572 A	18-11-1999	AU 752185 B2 AU 3837399 A BR 9910281 A CA 2326501 A1 CN 1308637 A EE 200000643 A EP 1075496 A1 HK 1039624 A1 HU 0101915 A2 JP 2002514406 T NO 20005612 A NZ 507694 A PL 343931 A1 RU 2226196 C2 TR 200003292 T2 ZA 200005870 A	12-09-2002 29-11-1999 02-01-2001 18-11-1999 15-08-2001 15-04-2002 14-02-2001 29-09-2006 28-09-2001 21-05-2002 29-12-2000 25-07-2003 10-09-2001 27-03-2004 21-03-2001 25-10-2002
WO 03086310 A	23-10-2003	AU 2003226356 A1	27-10-2003

[19] 中华人民共和国国家知识产权局

[51] Int. Cl.

C07K 16/18 (2006.01)

A61P 25/00 (2006.01)



[12] 发明专利申请公布说明书

[21] 申请号 200680018904.0

[43] 公开日 2008 年 6 月 4 日

[11] 公开号 CN 101193914A

[22] 申请日 2006.4.28

[21] 申请号 200680018904.0

[30] 优先权

[32] 2005. 4. 29 [33] US [31] 60/676,093

[32] 2005. 8. 1 [33] US [31] 60/704,818

[86] 国际申请 PCT/US2006/016071 2006.4.28

[87] 国际公布 WO2006/118959 英 2006.11.9

[85] 进入国家阶段日期 2007.11.29

[71] 申请人 瑞纳神经科学公司

地址 美国加利福尼亚

[72] 发明人 A·罗森塔尔 J·彭斯 何维贤

[74] 专利代理机构 中国国际贸易促进委员会专利商
标事务所

代理人 罗菊华

(Heo70828)

权利要求书 5 页 说明书 72 页 附图 6 页

[54] 发明名称

抗 β - 淀粉样肽抗体及其使用方法

[57] 摘要

本发明描述抗 β - 淀粉样肽的 C 端侧的抗体以及使用这些抗体诊断和治疗阿尔茨海默氏病和 $A\beta$ 肽相关疾病的方法。

1. 能够特异地与 A β 肽结合的单克隆抗体, 其中所述抗体可以结合 A β_{1-40} 上包括氨基酸 25-34 和 40 的表位, 其中所述抗体结合 A β_{1-40} 的亲合力比其结合 A β_{1-42} 及 A β_{1-43} 的高, 并且其中所述抗体不是抗体 2294。

2. 权利要求 1 的抗体, 其中所述抗体结合 A β_{1-40} 的亲合力是其结合 A β_{1-42} 或 A β_{1-43} 的亲和力的至少大约 40 倍。

3. 权利要求 1 的抗体, 其中所述抗体的 Fab 片段以大约 5nM 或更低的亲合力结合 A β_{1-40} 。

4. 权利要求 1 的抗体, 其中所述抗体的同种型选自: IgG1, IgG2, IgG3, 和 IgG4。

5. 权利要求 1 的抗体, 其中所述抗体包含含有 Fc 区的重链恒定区, 其中所述重链恒定区具有受损的效应子功能。

6. 权利要求 5 的抗体, 其中 Fc 区中的 N-糖基化被去除。

7. 权利要求 5 的抗体, 其中所述抗体的重链恒定区是含有氨基酸突变 A330P331 至 S330S331 的人 IgG2a 恒定区, 其中所述氨基酸位置参照人野生型 IgG2a 序列以 Kabat 编号为基础。

8. 权利要求 5 的抗体, 其中所述抗体的重链恒定区是含有氨基酸突变 E233F234L235 至 P233V234A235 的人 IgG4 恒定区, 其中所述氨基酸位置参照人野生型 IgG4 序列以 Kabat 编号为基础。

9. 权利要求 1 的抗体，其中所述抗体是人抗体。

10. 权利要求 1 的抗体，其中所述抗体是人源化抗体。

11. 权利要求 1 的抗体，其中所述抗体包含含有来自表 3 所示抗体的重链可变区的 3 个 CDR 的重链可变区、以及含有来自表 3 所示抗体的轻链可变区的 3 个 CDR 的轻链可变区。

12. 权利要求 1 的抗体，其中所述抗体包含含有 SEQ ID NO: 1 所示抗体 6G 重链可变区的 3 个 CDR 的重链可变区、以及含有 SEQ ID NO: 2 所示抗体 6G 轻链可变区的 3 个 CDR 的轻链可变区。

13. 权利要求 1 的抗体，其中所述抗体包含含有 SEQ ID NO: 3、SEQ ID NO: 4 和 SEQ ID NO: 5 所示 3 个 CDR 的重链可变区，以及含有 SEQ ID NO: 6、SEQ ID NO: 7 和 SEQ ID NO: 8 所示 3 个 CDR 的轻链可变区。

14. 权利要求 1 的抗体，其中所述抗体包含含有 SEQ ID NO: 1 所示氨基酸序列的重链可变区，以及含有 SEQ ID NO: 2 所示氨基酸序列的轻链可变区。

15. 权利要求 1 的抗体，其中所述抗体包含 SEQ ID NO: 11 所示重链氨基酸序列和 SEQ ID NO: 12 所示轻链氨基酸序列。

16. 抗体重链，其含有来自表 3 所示抗体的重链的 3 个 CDR。

17. 权利要求 16 的抗体重链，其中所述 3 个 CDR 来自 SEQ ID NO: 1 所示抗体 6G 重链可变区。

18. 权利要求 16 的抗体重链, 其中所述重链包含 SEQ ID NO: 1 所示氨基酸序列。

19. 抗体轻链, 其含有来自表 3 所示抗体的轻链的 3 个 CDR。

20. 权利要求 19 的抗体轻链, 其中所述 3 个 CDR 来自 SEQ ID NO: 2 所示抗体 6G 轻链可变区。

21. 权利要求 19 的抗体轻链, 其中所述轻链包含 SEQ ID NO: 2 所示氨基酸序列。

22. 抗体 6G 的片段, 其中所述片段具有或者保留抗体 6G 的结合特异性。

23. 权利要求 22 的片段, 其中所述片段是 Fab、Fab'、F(ab')₂ 或 Fv。

24. 包含权利要求 1-15 之任一项的抗体和药物可接受载体的药物组合物。

25. 包含编码权利要求 1-15 之任一项的抗体的核苷酸序列的多核苷酸。

26. 权利要求 25 的多核苷酸, 其中所述多核苷酸包含 SEQ ID NO: 13 和 14 的核苷酸序列。

27. 包含编码权利要求 16-18 之任一项的重链的核苷酸序列的多核苷酸。

28. 包含编码权利要求 19-21 之任一项的轻链的核苷酸序列的多核苷酸。

29. 包含编码权利要求 22 或 23 的片段的核苷酸序列的多核苷酸。

30. 包含权利要求 25 的多核苷酸的载体。

31. 包含权利要求 27 的多核苷酸的载体。

32. 权利要求 31 的载体，其中所述载体是具有保藏号 ATCC No. PTA-6786 的 pDb. 6G. hFc2a。

33. 包含权利要求 28 的多核苷酸的载体。

34. 权利要求 33 的载体，其中所述载体是具有保藏号 ATCC No. PTA-6787 的 pEb. 6G. hK。

35. 包含权利要求 29 的多核苷酸的载体。

36. 包含权利要求 25 的多核苷酸的宿主细胞。

37. 包含权利要求 27 的多核苷酸的宿主细胞。

38. 包含权利要求 28 的多核苷酸的宿主细胞。

39. 包含权利要求 29 的多核苷酸的宿主细胞。

40. 制备抗体的方法，包括在使得所述抗体产生的条件下培养

权利要求 36 的宿主细胞；和自所述宿主细胞或培养物中分离所述抗体。

41. 在个体中治疗阿尔茨海默氏病的方法，包括将有效量的权利要求 24 的药物组合物施用给所述个体。

42. 在个体中抑制淀粉样蛋白斑形成的方法，包括将有效量的权利要求 24 的药物组合物施用给所述个体。

43. 在个体中减少淀粉样蛋白斑的方法，包括将有效量的权利要求 24 的药物组合物施用给所述个体。

44. 在个体中延迟阿尔茨海默氏病相关症状的发展的方法，包括将有效量的权利要求 24 的药物组合物施用给所述个体。

抗 β -淀粉样肽抗体及其使用方法

相关申请的交叉引用

本申请要求 2005 年 4 月 29 日提交的美国临时专利申请系列号 60/676,093 以及 2005 年 8 月 1 日提交的美国临时专利申请系列号 60/704,818 的优先权,这两份临时专利申请完整地并入此处作为参考。

技术领域

本发明涉及抗 β -淀粉样肽抗体。本发明还涉及此类抗体在治疗和/或预防疾病,例如阿尔茨海默氏病中的用途。

关于联邦政府资助的研究或开发的声明

不适用。

背景技术

阿尔茨海默氏病(AD)是退行性脑病变,其临床特征在于进行性的记忆缺损、混乱、逐渐身体恶化,以及最终死亡。全球大约一千五百万人患有阿尔茨海默氏病,预期该数目将随着人类寿命的延长而急剧地增加。组织学上,该疾病的特征在于主要出现在联络皮层、边缘系统和基底神经节中的神经炎性斑块(neuritic plaque)。这些斑块的主要成分是 β -淀粉样肽(A β),该肽是 β 淀粉样前体蛋白(β APP 或 APP)的切割产物。APP 是 I 型跨膜糖蛋白,含有一个大的异位 N 端域、一个跨膜域和一个小的胞质 C 端尾。染色体 21 上的 APP 单基因的转录物的可变拼接导致具有不同氨基酸数目的几种同种型。

A β 似乎在阿尔茨海默氏病的神经病理学上具有主要作用。该疾病的家族形式已经与 APP 及早老素(presenilin)基因中的突变联系起来(Tanzi 等, 1996, Neurobiol. Dis. 3: 159-168; Hardy, 1996,

Ann. Med. 28: 255-258)。这些基因中的疾病相关突变导致 A β 的 42 氨基酸形式——淀粉样蛋白斑中发现的主要形式——的产生增加。而且，用人 A β 免疫过表达 APP 的疾病相关突变形式的转基因小鼠，减轻了斑块负担及相关病理 (Schenk 等, 1999, Nature, 400: 173-177; WO 99/27944)，并且外周施用抗 A β 抗体也降低脑中的斑块负担 (Bard 等, 2000, Nature Medicine 6(8): 916-919; WO 2004/032868; WO 00/72880)。

已经有报道，Fc 介导的小胶质细胞和/或巨噬细胞的吞噬作用对于体内斑块的清除过程是重要的。Bard 等, Proc. Natl. Acad. Sci. USA 100: 2023-2028 (2003)。然而，也有报道，在通过免疫疗法体内清除 β 淀粉样肽中涉及非 Fc 介导的机制。Bacskai 等, J. Neurosci. 22: 7873-7878 (2002); Das 等, J. Neurosci. 23: 8532-8538 (2003)。

因此，抗体疗法提供了一种有希望的治疗和预防阿尔茨海默氏病的方法。然而，使用包括 A β 1-42 的疫苗进行的人类临床试验由于一些患者中的脑膜脑炎而被暂停。Orgogozo 等, Neurology 61: 7-8 (2003); Ferrer 等 Brain Pathol. 14: 11-20 (2004)。已经报道，在表现出与人 AD 脑中观察到的相似的年龄相关性淀粉样蛋白斑发生以及神经变性和脑淀粉样血管病 (CAA) 的转基因小鼠中，使用 N 端特异的抗 A β 抗体实施被动免疫，导致大部分弥散的淀粉样蛋白的显著减少，但诱导脑微出血频率增加。Pfeifer 等, Science 298: 1379 (2002)。已经提示，在 APP 转基因小鼠中，由抗 β 淀粉样肽抗体的被动免疫引起的脑淀粉样血管病 (CAA) 相关性微出血的加剧，依赖于抗体对 β 淀粉样肽的沉积形式的识别。Racke 等, J. Neurosci. 25: 629-636 (2005)。为了减少发生炎症的风险，已经提议用缺乏 Fc 区的抗淀粉样蛋白沉积物的肽成分的抗体被动免疫。WO 03/086310。仍然需要具有改善的效果和安全谱并且适用于人类患者的、针对 A β 的抗体和其它免疫治疗剂。

在本申请中引用了各种出版物(包括专利和专利申请)。这些出

版物的公开内容特此完整地并入作为参考。

发明概述

在此公开的本发明涉及与 A β 肽 C 端结合的抗体和多肽。一方面，本发明提供与 A β ₁₋₄₀、A β ₁₋₄₂ 和 A β ₁₋₄₃ 结合的抗体或多肽，其中所述抗体或多肽以比其结合 A β ₁₋₄₂ 和 A β ₁₋₄₃ 的亲合力更高的亲合力与 A β ₁₋₄₀ 结合，而且其中所述抗体或多肽与 A β ₁₋₄₀ 上包括第 25-34 和 40 位氨基酸的表位结合。一些实施方案中，抗体与 A β ₁₋₄₀ 结合的亲合力是其与 A β ₁₋₄₂ 和/或 A β ₁₋₄₃ 结合的亲合力的至少大约 40 倍。一些实施方案中，所述抗体不是抗体 2294。

另一方面，本发明提供抗体 6G（可以与术语“6G”互换）。6G 的重链和轻链可变区的氨基酸序列显示在图 1 中。抗体 6G 的互补决定区 (CDR) 部分 (包括 Chothia 和 Kabat CDR) 也显示在图 1 中。

另一方面，本发明还提供具有表 3 所示氨基酸序列的 6G 的抗体变体。

另一方面，本发明提供包含抗体 6G 或表 3 所示的其变体的片段或区域的抗体。一个实施方案中，所述片段是抗体 6G 的轻链。另一实施方案中，所述片段是抗体 6G 的重链。再一实施方案中，所述片段包含来自抗体 6G 轻链和/或重链的一个或多个可变区。再一实施方案中，所述片段包含来自图 1 所示轻链和/或重链的一个或多个可变区。再一实施方案中，所述片段包含来自抗体 6G 轻链和/或重链的一个或多个 CDR。

另一方面，本发明提供包含以下项目中的任意一个或多个的多肽 (其可以是或者可以不是抗体)：a) 抗体 6G 或其显示在表 3 中的变体的一个或多个 CDR；b) 来自抗体 6G 或其显示在表 3 中的变体的重链的 CDR H3；c) 来自抗体 6G 或其显示在表 3 中的变体的轻链的 CDR L3；d) 来自抗体 6G 或其显示在表 3 中的变体的轻链的 3 个 CDR；e) 来自抗体 6G 或其显示在表 3 中的变体的重链的 3 个 CDR；f) 来自抗体 6G 或其显示在表 3 中的变体的轻链的 3 个 CDR 以及来自抗体

6G 或其显示在表 3 中的变体的重链的 3 个 CDR。本发明还提供包含以下项目中的任意一个或多个的多肽(其可以是或者可以不是抗体): a) 衍生自抗体 6G 或其显示在表 3 中的变体的一个或多个(一个、两个、三个、四个、五个或六个)CDR; b) 衍生自抗体 6G 重链 CDR H3 的 CDR; 和/或 c) 衍生自抗体 6G 轻链 CDR L3 的 CDR。一些实施方案中, 所述 CDR 是图 1 中所示的 CDR。一些实施方案中, 所述衍生自抗体 6G 或其显示在表 3 中的变体的一个或多个 CDR 与 6G 或其变体的至少一个、至少两个、至少三个、至少四个、至少五个或至少六个 CDR 有至少大约 85%、至少大约 86%、至少大约 87%、至少大约 88%、至少大约 89%、至少大约 90%、至少大约 91%、至少大约 92%、至少大约 93%、至少大约 94%、至少大约 95%、至少大约 96%、至少大约 97%、至少大约 98% 或至少大约 99% 的同一性。

一些实施方案中, 所述 CDR 是 Kabat CDR。一个实施方案中, 所述 CDR 是 Chothia CDR。其它实施方案中, 所述 CDR 是 Kabat 及 Chothia CDR 的组合(也称作“组合 CDR”或“扩展 CDR”)。换言之, 对于包含一个以上 CDR 的任何给定的实施方案, 这些 CDR 可以是选自 Kabat、Chothia 和/或组合 CDR 的任何 CDR。

一些实施方案中, 本发明抗体是人抗体。其它实施方案中, 本发明抗体是人源化抗体。一些实施方案中, 抗体是单克隆的。一些实施方案中, 抗体(或多肽)是分离的。一些实施方案中, 抗体(或多肽)是实质上纯的。

抗体重链恒定区可以来自任何类型的恒定区, 例如 IgG、IgM、IgD、IgA 和 IgE; 以及任何同种型, 例如, IgG1、IgG2、IgG3 和 IgG4。

一些实施方案中, 本文所述抗体或多肽具有被受损的效应子功能。一些实施方案中, 抗体或多肽包含具有受损的效应子功能的重链恒定区, 其中所述重链恒定区包含 Fc 区。一些实施方案中, Fc 区中的 N-糖基化被去除。一些实施方案中, Fc 区在 N-糖基化识别序列中包含突变, 由此该抗体或多肽的 Fc 区不发生 N-糖基化。一些实施方案中, Fc 区被 PEG 化。一些实施方案中, 抗体或多肽的重链恒定区

是含有以下突变的人重链 IgG2a 恒定区：A330P331 至 S330S331（氨基酸编号参照野生型 IgG2 序列进行）。一些实施方案中，抗体或多肽包含含有如下突变的 IgG4 恒定区：E233F234L235 至 P233V234A235。这些氨基酸位置以 Kabat 编号方式为基础。

另一方面，本发明提供包含编码抗体 6G 或其显示在表 3 中的变体的片段或区域的多核苷酸的多核苷酸（其可以是分离的）。一个实施方案中，所述片段是抗体 6G 的轻链。另一实施方案中，所述片段是抗体 6G 的重链。再一实施方案中，所述片段含有来自抗体 6G 的轻链和/或重链的一个或多个可变区。再一实施方案中，所述片段包含来自抗体 6G 的轻链和/或重链的一个或多个（即，一个、两个、三个、四个、五个、六个）互补决定区（CDR）。

另一方面，本发明是包含编码抗体 6G 或其在表 3 中所示的变体的多核苷酸的多核苷酸（其可以是分离的）。一些实施方案中，所述多核苷酸包含 SEQ ID NO: 9 和 SEQ ID NO: 10 中所示多核苷酸之一或两者。

另一方面，本发明提供编码本文所述任何抗体（包括抗体片段）或多肽的多核苷酸。

另一方面，本发明提供包含本文所述任何多核苷酸的载体（包括表达和克隆载体）和宿主细胞。

另一方面，本发明是包含编码本文所述任何抗体的多核苷酸的宿主细胞。

另一方面，本发明是 $A\beta_{1-40}$ 与抗体 6G 或其在表 3 中显示的变体结合的复合物。

另一方面，本发明是 $A\beta_{1-40}$ 与本文所述任何抗体或多肽结合的复合物。

另一方面，本发明是包含有效量的任何本文所述抗体、多肽或多核苷酸以及药物可接受赋形剂的药物组合物。一些实施方案中，所述抗体或多肽包含抗体 6G 的一个或多个 CDR。

另一方面，本发明是制备抗体 6G 的方法，包括：在允许抗体 6G

产生的条件下培养宿主细胞或其后代,其中所述宿主细胞包含编码抗体 6G 的表达载体;和,在一些实施方案中,纯化该抗体 6G。一些实施方案中,表达载体包含 SEQ ID NO: 9 和 SEQ ID NO: 10 中所示多核苷酸序列之一或两者。

另一方面,本发明提供通过如下方式制备本文所述任何抗体或多肽的方法:在适宜细胞中表达编码所述抗体(其可以分开表达为单独的轻链或重链,或者从一个载体既表达轻链也表达重链)或多肽的一种或多种多核苷酸,一般地随后回收和/或分离目的抗体或多肽。

本发明也提供预防、治疗、抑制或延迟阿尔茨海默氏病及与改变的 A β 或 β APP 表达或者 A β 肽的累积有关的其它疾病发展的方法,其中所述其它疾病有例如 Down 氏综合症、帕金森病、多发性脑梗塞痴呆、轻度认知缺损、脑淀粉样血管病、抑郁、Creutzfeldt-Jakob 病、伴有 Lewy 小体的痴呆和 AIDS。该方法包括给个体施用有效剂量的包含本发明抗体、多肽或多核苷酸的药物组合物。

本发明还提供在个体中延迟和阿尔茨海默氏病或与 A β 肽累积有关的其它疾病相关的症状发展的方法,包括给个体施用有效剂量的包含本发明抗体、多肽或多核苷酸的药物组合物。

本发明还提供抑制个体中淀粉样蛋白斑和/或淀粉样蛋白累积的形成的方法,包括给个体施用有效剂量的包含本发明抗体、多肽或多核苷酸的药物组合物。一些实施方案中,淀粉样蛋白斑存在于个体的脑(脑组织)中。一些实施方案中,淀粉样蛋白斑存在于脑血管结构中。其它实施方案中,淀粉样蛋白累积出现在循环系统中。

本发明还提供在个体中减少淀粉样蛋白斑和/或淀粉样蛋白累积的方法,包括给个体施用有效剂量的包含本发明抗体、多肽或多核苷酸的药物组合物。一些实施方案中,淀粉样蛋白斑存在于个体的脑(脑组织)中。一些实施方案中,淀粉样蛋白斑存在于脑血管结构中。其它实施方案中,淀粉样蛋白累积出现在循环系统中。

本发明还提供在个体中除去或清除淀粉样蛋白斑和/或淀粉样蛋白累积的方法,包括给个体施用有效剂量的包含本发明抗体、多肽

或多核苷酸的药物组合物。一些实施方案中，淀粉样蛋白斑存在于个体的脑(脑组织)中。一些实施方案中，淀粉样蛋白斑存在于脑血管结构中。其它实施方案中，淀粉样蛋白累积出现在循环系统中。

此外，本发明还提供抑制 A β 肽在组织中累积的方法，包括使所述组织与本发明抗体或多肽接触。

本发明也提供在个体中减少 A β 肽(例如，可溶性的、寡聚体的和沉积的形式)的方法，包括给个体施用有效量的本发明抗体、多肽或多核苷酸。一些实施方案中，抑制和/或降低脑中 A β 肽的累积。一些实施方案中，抑制和/或降低 A β 肽的毒性作用。因此，本发明方法可以用于治疗其中存在或怀疑存在 A β 肽累积的任何疾病，例如，阿尔茨海默氏病、Down 氏综合症、帕金森病、多发性脑梗塞痴呆、轻度认知缺损、脑淀粉样血管病、抑郁、Creutzfeldt-Jakob 病或伴有 Lewy 小体的痴呆。

本发明还提供在个体中改善认知功能或逆转与 A β 淀粉样蛋白沉积相关疾病(如，阿尔茨海默氏病)有关的认知衰退的方法，包括给个体施用有效剂量的包含本发明抗体、多肽或多核苷酸的药物组合物。

本文所述任何抗体、多肽或多核苷酸均可以用于本发明的方法。一些实施方案中，所述抗体是抗体 6G。

本发明抗体和多肽还可以用于检测、诊断和监测阿尔茨海默氏病及与改变的 A β 或 β APP 表达有关的其它疾病，例如，Down 氏综合症和 AIDS。该方法包括使怀疑具有改变的 A β 或 β APP 表达的患者样本与本发明抗体接触，以及确定 A β 或 β APP 的水平是否不同于对照或比较样本的水平。一些实施方案中，在给予抗 A β 抗体之前和之后测量血清 A β 水平；并且评估血清 A β 水平的任何增加。

本发明任何抗体或多肽的施用都可以通过本领域已知的任何方式进行，包括：静脉内、皮下、通过吸入、动脉内、肌肉内、心内、心室内、胃肠外、鞘内、以及腹膜内。施用可以是全身性的，例如，静脉内，或局部的。一般地，这也适用于本发明的多肽和多核苷酸。

另一方面, 本发明提供包含本文所述任何一种或多种组合物的药盒及组合物。这些药盒通常在适宜的包装中并提供了适当的说明, 可以用于本文所述任何方法中。

附图简述

图 1 显示抗体 6G 的重链可变区氨基酸序列 (SEQ ID NO: 1) 和轻链可变区氨基酸序列 (SEQ ID NO: 2)。Kabat CDR 以粗体显示, Chothia CDR 以下划线显示。重链和轻链可变区的氨基酸残基顺序地编号。

图 2 显示通过 ELISA 对抗体 6G 进行的表位作图。A β 肽 (1-16、1-28、17-40、17-42、22-35、28-40、28-42、1-38、1-40、1-42、1-43、和 33-40) 固定于 ELISA 板上。单克隆抗体 6G (20 nM) 与各种固定的肽温育 1 小时。使用 HRP 缀合的山羊抗人 κ 二抗测量与固定的 A β 肽结合的抗体 6G。

图 3 显示通过 ELISA 对抗体 6G 进行的表位作图。将各种 A β 肽 (序列从上到下被指定为 SEQ ID NO: 18-29) 固定于 ELISA 板上。单克隆抗体 6G 与各种固定的肽温育 1 小时。使用 HRP 缀合的山羊抗人 κ 二抗测量与固定的 A β 肽结合的抗体 6G。“NB” 指未检测到结合。

图 4 是显示 A β 上与抗体 6G 结合的表位的示意图。显示了 A β 在淀粉样前体蛋白 (APP) 及 APP 的细胞膜部分中的相对位置。“CT99” 指 APP 的 C 端 99 个氨基酸。所示氨基酸序列被指定为 SEQ ID NO: 30。

图 5 是显示抗 A β ₁₋₁₆ 的单克隆抗体 (m2324) 和抗体 6G 对 APP 表达细胞进行免疫染色的照片。顶图显示在细胞与 m2324 或 6G (各 5 μ g/ml) 温育以及使用 Cy3 缀合的山羊抗小鼠或抗人二抗检测结合后荧光显微镜下观察到的细胞。底图显示显微镜下观察到的细胞。

图 6 显示通过 ELISA 对抗体 2294 和 6G 进行的表位作图。将各种 A β 肽 (序列从上到下为 SEQ ID NO: 18-26、31 及 27-29) 固定于 ELISA 板上。抗体与各种固定的肽温育 1 小时。使用 HRP 缀合的山羊抗人 κ 二抗测量与固定的 A β 肽结合的抗体 6G。使用山羊抗小鼠抗体测量与固定的 A β 肽结合的抗体 2294, 其中所述山羊抗小鼠抗体能

够既结合重链也结合轻链，是缀合了 HRP 的二抗。“NB”指未检测到结合。在“2294”和“6G”下的列中的数字表示 450nm 的吸收值。

发明详述

此处公开的本发明提供与 A β 的 C 端结合的抗体和多肽。本发明还提供编码这些抗体和/或多肽的多核苷酸。本发明还提供制备和使用这些抗体和多肽的方法。

本发明还提供通过给个体施用有效量的包含本文所述抗体、多肽或编码本文所述抗体或多肽的多核苷酸的药物组合物，治疗或预防个体中与 β -淀粉样蛋白沉积有关的疾病的方法，所述疾病有例如，阿尔茨海默氏病、Down 氏综合症、多发性脑梗塞痴呆、轻度认知缺损、脑淀粉样血管病、抑郁、Creutzfeldt-Jakob 病及伴有 Lewy 小体的痴呆。

一般技术

除非另有说明，否则本发明的实施将利用分子生物学(包括重组技术)、微生物学、细胞生物学、生物化学和免疫学的常规技术，这些技术属于本领域技术的范围。这些技术在文献中有全面的说明，例如，Molecular Cloning: A Laboratory Manual, 第二版(Sambrook 等, 1989) Cold Spring Harbor Press; Oligonucleotide Synthesis (M. J. Gait 编, 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J. E. Cellis, 编, 1998) Academic Press; Animal Cell Culture (R. I. Freshney, 编, 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, 和 D. G. Newell 编, 1993-1998) J. Wiley and Sons; Methods in Enzymology (Academic Press, Inc.); Handbook of Experimental Immunology (D. M. Weir 和 C. C. Blackwell, 编); Gene Transfer Vectors

For Mammalian Cells (J.M. Miller 和 M. P. Calos 编, 1987); Current Protocols in Molecular Biology (F.M. Ausubel 等, 编, 1987); PCR: The Polymerase Chain Reaction (Mullis 等编, 1994); Current Protocols in Immunology (J.E. Coligan 等, 编, 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C.A. Janeway 和 P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: A practical approach (D. Catty. 编, IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd 和 C. Dean 编, Oxford University Press, 2000); Using antibodies: a laboratory manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti 和 J. D. Capra 编, Harwood Academic Publishers, 1995)。

定义

“抗体”是能够通过至少一个抗原识别位点特异结合靶(例如糖、多核苷酸、脂质、多肽等)的免疫球蛋白分子,其中所述抗原识别位点位于该免疫球蛋白分子的可变区中。本文中,该术语不仅包括完整的多克隆或单克隆抗体,也包括其片段(例如 Fab, Fab', F(ab')₂, Fv)、单链(ScFv)、其突变体、包含抗体部分的融合蛋白、以及包含抗原识别位点的该免疫球蛋白分子的任何其它经修饰的构型(configuration)。本发明抗体包括任何类型的抗体,例如 IgG, IgA 或 IgM (或其亚型),该抗体不必是任何特定类型的。根据抗体重链恒定区的氨基酸序列,免疫球蛋白可以划分为不同的类型。有五大类的免疫球蛋白: IgA, IgD, IgE, IgG 和 IgM,其中几种可以进一步细分成亚型(同种型),例如, IgG1, IgG2, IgG3, IgG4, IgA1 和 IgA2。对应于不同类型的免疫球蛋白的重链恒定区被分别称作 α 、 δ 、 ϵ 、 γ 和 μ 。不同类型的免疫球蛋白的亚基结构和三维构型是熟知的。

本文中,“单克隆抗体”指从基本上均质的抗体群体(即,构

成该群体的各个抗体,除了可能微量存在的可能的天然突变外,都是相同的)获得的抗体。单克隆抗体是高度特异的,指向单个抗原位点。而且,与典型地包括针对不同决定簇(表位)的不同抗体的多克隆抗体制备物不同,每个单克隆抗体针对抗原上的单个决定簇。修饰词“单克隆”指的是抗体获自基本上均质的抗体群的该特征,而不能理解为需要通过任何特定的方法来产生该抗体。例如,根据本发明使用的单克隆抗体可以通过 Kohler 和 Milstein, 1975, Nature 256:495 最先描述的杂交瘤方法制备,或者可以通过诸如美国专利 4,816,567 描述的重组 DNA 方法制备。单克隆抗体也可以从使用例如 McCafferty 等, 1990, Nature, 348: 552-554 中描述的技术制备的噬菌体文库分离。

本文中,“人源化”抗体指含有最少的源于非人免疫球蛋白的序列的非人(例如鼠)抗体形式,该抗体形式是特异的嵌合免疫球蛋白、免疫球蛋白链、或其片段(例如 Fv, Fab, Fab', F(ab')₂ 或抗体的其它抗原结合亚序列)。人源化抗体主要是人免疫球蛋白(受体抗体),在该蛋白中来自该受体的互补决定区(CDR)的残基被来自具有期望特异性、亲和性和能力的、非人物种(供体抗体)例如小鼠、大鼠或兔的 CDR 的残基替换。一些情况下,人免疫球蛋白的 Fv 构架区(FR)残基被相应的非人残基替换。而且,人源化抗体可以包含既不存在于受体抗体也不存在于引入的 CDR 或构架序列中但被包括在人源化抗体中以进一步改善和优化抗体的性能的残基。一般地,人源化抗体包含至少一个,以及典型地两个,可变区的基本上所有部分,其中全部或基本上全部的 CDR 区对应于非人免疫球蛋白的 CDR 区,而全部或基本上全部的 FR 区是具有人免疫球蛋白共有序列的那些 FR 区。最佳地,人源化抗体还将包含免疫球蛋白(典型地,人免疫球蛋白)的恒定区或域(Fc)的至少一部分。抗体可以具有按照 WO 99/58572 所述修饰的 Fc 区。其它人源化抗体形式具有相对于原始抗体发生了改变的一个或多个 CDR(一个、两个、三个、四个、五个、六个),这些 CDR 也被称作“衍生自”原始抗体的一个或多个 CDR 的一个或多个

CDR。

本文中，“人抗体”指具有与人产生的抗体的氨基酸序列相对应的氨基酸序列的抗体，和/或使用本领域已知的或本文所述的制备人抗体的任何技术制备的抗体。人抗体的该定义包括含有至少一条人重链多肽或至少一条人轻链多肽的抗体。一个这样的实例是包含鼠轻链和人重链多肽的抗体。人抗体可以使用本领域已知的各种技术制备。一个实施方案中，从噬菌体文库选择人抗体，其中该噬菌体文库表达人抗体 (Vaughan 等 1996, *Nature Biotechnology*, 14: 309-314; Sheets 等, 1998, *PNAS*, (USA) 95: 6157-6162; Hoogenboom 和 Winter, 1991, *J. Mol. Biol.* 227: 381; Marks 等, 1991, *J. Mol. Biol.* 222: 581)。人抗体也可以通过将人免疫球蛋白基因座引入转基因动物，例如，内源性免疫球蛋白基因已经部分或完全被失活的小鼠，而制备。该方法描述在美国专利 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425 和 5,661,016 中。或者，人抗体可以通过使产生抗靶抗原的抗体的人 B 淋巴细胞(该 B 淋巴细胞可以从个体回收或者可以已经在体外免疫)永生而制备。见例如，Cole 等, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p.77 (1985); Boerner 等, 1991, *J. Immunol.* 147(1) 86-95; 和美国专利 5,750,373。

本文中，术语“6G”和“抗体 6G”可互换使用，指具有 SEQ ID NO: 11 所示重链氨基酸序列和 SEQ ID NO: 12 所示轻链氨基酸序列的抗体。图 1 中显示了重链和轻链可变区的氨基酸序列。图 1 中图解显示了抗体 6G 的 CDR 部分(包括 Chothia 和 Kabat CDR)。编码重链和轻链的多核苷酸显示在 SEQ ID NO: 13 和 SEQ ID NO: 14 中。6G 的特征在实施例中描述。

术语“多肽”、“寡肽”、“肽”和“蛋白质”在本文中可互换使用，指任何长度的氨基酸聚合物。该聚合物可以是直链的或分支的，它可以包含修饰的氨基酸，并且可以被非氨基酸中断。该术语也包括已经天然地或通过干预的方式被修饰的氨基酸聚合物;所述修饰

有例如，二硫键形成、糖基化、脂质化、乙酰化、磷酸化、或任何其它操作或修饰，例如与标记成分缀合。该定义也包括例如，含有一个或多个氨基酸类似物(包括例如，非天然的氨基酸等)以及本领域已知的其它修饰的多肽。可以理解，由于本发明多肽是以抗体为基础的，故该多肽可以是单链形式或连接在一起的多条链的形式。

“多核苷酸”或“核酸”在本文中可互换使用，指任何长度的核苷酸聚合物，包括DNA和RNA。核苷酸可以是脱氧核糖核苷酸、核糖核苷酸、修饰的核苷酸或碱基，和/或它们的类似物，或者是可以通过DNA或RNA聚合酶掺入聚合物中的任何底物。多核苷酸可以包含修饰的核苷酸，例如，甲基化的核苷酸及它们的类似物。如果存在修饰的话，对核苷酸结构的修饰可以在聚合物组装之前或之后发生。核苷酸序列可以被非核苷酸成分中断。多核苷酸可以在聚合之后被进一步修饰，例如，与标记成分缀合。其它类型的修饰包括，例如，“带帽”、将一个或多个天然存在的核苷酸替换成类似物、核苷酸间的修饰，例如，不带电荷的键接(例如，甲基膦酸酯、磷酸三酯、phosphoamidates、cabamates等)、带电荷的键接(例如，硫代磷酸酯、二硫代磷酸酯等)、含有吊挂部分例如蛋白质(例如，核酸酶、毒素、抗体、信号肽、聚-L-赖氨酸等)、具有嵌入剂(例如，吡啶、补骨脂素等)、含有螯合剂(例如，金属、放射性金属、硼、氧化金属等)、含有烷化剂、具有修饰的键接(例如， α 异头核酸等)，以及这些多核苷酸的未修饰形式。而且，任何通常存在于糖中的羟基基团均可以被置换为例如膦酸基团、磷酸基团，被标准保护基保护、或被活化以预备与另外核苷酸的另外连接、或者可以缀合至固相支持物上。5'和3'端OH可以被磷酸化或者替代为胺或具有1至20个碳原子的有机封端基团部分。其它羟基也可以被衍生化为标准的保护基。多核苷酸也可以含有本领域通常已知的核糖或脱氧核糖的糖的类似形式，包括例如，2'-O-甲基-、2'-O-烯丙基、2'-氟-或2'-叠氮-核糖、碳环糖类似物、 α -异头糖、差向异构糖例如阿拉伯糖、木糖类或来苏糖类、吡喃糖类、呋喃糖类、景天庚酮糖类、无环类似物及脱碱基核

苷类似物例如甲基核苷。可以将一个或多个磷酸二酯键替换成备选的连接基团。这些备选连接基团包括,但不限于,其中磷酸酯被 $P(O)S$ (“硫代酯”)、 $P(S)S$ (“二硫代酯”)、 $(O)NR_2$ (“酰胺化物”)、 $P(O)R$ 、 $P(O)OR'$ 、 CO 或 CH_2 (“formacetal”) 替代的实施方案,其中每一个 R 或 R' 均独立地是 H 或是取代的或未取代的烷基(1-20C) (任选地含有醚(-O-)键)、芳基、烯基、环烷基、环烯基或 araldyl。多核苷酸中并不是所有的连接都需要相同。前面的描述适用于本文中提及的所有多核苷酸,包括 RNA 和 DNA。

抗体“可变区”指单独的抗体轻链可变区或抗体重链可变区或者两者的组合。重链和轻链的可变区均各自由通过三个互补决定区(CDR,也称作超变区)连接在一起的4个构架区(FR)组成。每条链中的CDR通过FR而紧靠在一起,并与来自另一链的CDR一起促成抗体的抗原结合位点的形成。存在至少两种确定CDR的技术:(1)基于交叉物种序列变异性的方法(即,Kabat等, *Sequences of Proteins of Immunological Interest* (第5版,1991, National Institutes of Health, Bethesda, MD)); 和(2)基于抗原抗体复合物的晶体学研究(Al-lazikani等(1997), *J. Molec. Biol.* 273: 927-948))。本文中,CDR可以指通过其中一种方法或者通过这两种方法组合确定的CDR。

抗体“恒定区”指单独的抗体轻链恒定区或抗体重链恒定区或者两者的组合。

与抗体或多肽“优先地结合”或“特异地结合”(本文中可互换使用)的“表位”是本领域熟知的术语,确定此特异结合或优先结合的方法也是本领域熟知的。如果分子与特定细胞或物质比其与其它细胞或物质能以更高的频率、更快的速度、更长的持续时间和/或更大的亲和力反应或结合,则称该分子表现出“特异的结合”或“优先的结合”。如果抗体与靶的结合比其与其它物质的结合具有更高的亲和性、亲和力、更为容易和/或持续时间更长,则该抗体“特异地结合”或“优先地结合”靶。例如,特异地或优先地与某 $A\beta_{1-40}$ 表位结

合的抗体是这样的抗体，该抗体与该表位的结合比它与其它 $A\beta_{1-40}$ 表位或非 $A\beta_{1-40}$ 表位的结合具有更高的亲和性、亲和力、更为容易和/或持续时间更长。通过阅读该定义也可以理解，例如，特异地或优先地与第一靶结合的抗体(或部分或表位)可以或者不可以特异地或优先地与第二靶结合。如此，“特异的结合”或“优先的结合”不要求(尽管它可以包括)排他式的结合。一般地，但不是必需的，提及到结合意味着优先的结合。

本文中，“基本上纯的”指至少 50% 纯(即，无杂质)、更优选地至少 90% 纯、更优选地至少 95% 纯、更优选地至少 98% 纯，更优先地至少 99% 纯的物质。

“宿主细胞”包括单个细胞或细胞培养物，其可以是或者已经是用于掺入多核苷酸插入物的载体的受体。宿主细胞包括单个宿主细胞的后代，该后代由于自然的、偶发的或有意的突变而可以不必与原始亲本细胞完全相同(在形态学上或在基因组 DNA 互补性上)。宿主细胞包括在体内被本发明多核苷酸转染的细胞。

术语“Fc 区”用于定义免疫球蛋白重链的 C 端区域。“Fc 区”可以是天然序列的 Fc 区或者变异的 Fc 区。尽管免疫球蛋白重链 Fc 区的界限可以变化，但人 IgG 重链 Fc 区通常被定义为自 Cys226 或 Pro230 位置的氨基酸残基延伸至其羧基末端。Fc 区中残基的编号为如 Kabat 等，Sequence of Proteins of Immunological Interest, 第 5 版, Public Health Service, National Institute of Health, Bethesda, Md., 1991 中 EU 索引(EU index)的编号。免疫球蛋白 Fc 区一般包含两个恒定区，CH2 和 CH3。

本文中，“Fc 区”和“FcR”描述与抗体 Fc 区结合的受体。优选的 FcR 是天然序列的人 FcR。而且，优选的 FcR 是与 IgG 抗体结合(γ 受体)的 FcR，其包括 Fc γ R1、Fc γ RII 和 Fc γ RIII 亚型受体，包括这些受体的等位变体和可变拼接形式。Fc γ RII 受体包括 Fc γ RIIA (“激活性受体”)和 Fc γ RIIB (“抑制性受体”)，两者具有相似氨基酸序列，它们的主要差异在于它们的胞质域。关于 FcR

的综述见 Ravetch 和 Kinet, 1991, *Ann. Rev. Immunol.* 9: 457-92; Capel 等, 1994, *Immunomethods*, 4: 25-34; 和 de Haas 等, 1995, *J. Lab. Clin. Med.* 126: 330-41。"FcR" 也包括新生儿受体 FcRn, 该受体负责将母亲的 IgG 转移给胎儿 (Guyer 等, 1976, *J. Immunol.* 117: 587; 和 Kim 等, 1994, *J. Immunol.* 24: 249)。

"补体依赖性细胞毒性" 和 "CDC" 指靶在补体存在下发生的裂解。补体系统的第一成分 (C1q) 与和关连抗原形成复合物的分子 (例如抗体) 结合, 将导致补体活化途径的起始。为了评价补体活化, 可以实施 CDC 试验, 例如 Gazzano-Santoro 等, *J. Immunol. Methods*, 202: 163 (1996) 中描述的 CDC 试验。

"功能性 Fc 区" 具有天然序列的 Fc 区的至少一种效应子功能。示例性 "效应子功能" 包括 C1q 结合; 补体依赖性细胞毒性 (CDC); Fc 受体结合; 抗体依赖性细胞介导的细胞毒性 (ADCC); 吞噬作用; 细胞表面受体 (例如, B 细胞受体; BCR) 的下调, 等。该效应子功能一般需要 Fc 区与结合域 (例如, 抗体可变区结构域) 组合, 并且可以使用本领域已知用于评价此类抗体效应子功能的各种试验来进行评价。

"天然序列的 Fc 区" 包含与自然界中存在的 Fc 区的氨基酸序列相同的氨基酸序列。"变异的 Fc 区" 包含由于至少一个氨基酸修饰而不同于天然序列的 Fc 区的氨基酸序列但保留了该天然序列的 Fc 区的至少一种效应子功能的氨基酸序列。优选地, 变异的 Fc 区与天然序列的 Fc 区或者亲本多肽的 Fc 区相比具有至少一个氨基酸替代, 例如, 在天然序列的 Fc 区中或在亲本多肽的 Fc 区中大约 1 个至大约 10 个氨基酸替代, 优选地大约 1 个至大约 5 个氨基酸替代。本文中, 变异的 Fc 区优选地与天然序列的 Fc 区和/或与亲本多肽的 Fc 区具有至少大约 80% 序列同一性, 最优选地至少大约 90% 序列同一性, 更优选地至少大约 95%、至少大约 96%、至少大约 97%、至少大约 98%、至少大约 99% 序列同一性。

本文中, "抗体依赖性细胞介导的细胞毒性" 和 "ADCC" 指细胞介导的反应, 其中表达 Fc 受体 (FcR) 的非特异性细胞毒性细胞 (例如,

自然杀伤(NK)细胞、嗜中性粒细胞和巨噬细胞)识别靶细胞上结合的抗体并随后导致靶细胞的裂解。目的分子的ADCC活性可以使用体外ADCC试验,例如,美国专利5,500,362或5,821,337中所述的试验来评价。对于该试验有用的效应细胞包括外周血单核细胞(PBMC)和NK细胞。备选地,或者此外,目的分子的ADCC活性可以在体内,例如,在动物模型,例如 Clynes 等 1998 PNAS (USA) 95: 652-656 中公开的动物模型中评价。

本文中,药物、化合物或药物组合物的“有效剂量”或“有效量”是可以有效地实现有益或期望结果的量。对于预防性应用,有益的结果包括诸如消除或降低疾病的风险、减轻疾病的严重性或延迟疾病的发作,其中所述疾病包括疾病的生物化学的、组织学的和/或行为上的症状、其并发症以及疾病发展过程中表现出的中间病理学表型。对于治疗性应用,有益的结果包括临床结果,例如,抑制、阻碍或降低淀粉样蛋白斑的形成,减少、除去、清除淀粉样蛋白斑,改善认知、逆转或减慢认知衰退、隔离或增加生物学液体中循环的可溶性A β 肽、减少因该疾病导致的一种或多种症状(生物化学的、组织学的和/或行为上的),包括其并发症和疾病发展过程中出现的中间病理学表型,增加该疾病患者的生活质量,降低其它药物治疗该疾病时所需的剂量,增强其它药物的效果,延迟疾病进展,和/或延长患者的存活时间。有效剂量可以分一次或多次施用。对于本发明的目的,药物、化合物或药物组合物的有效剂量是足以直接地或间接地实现预防性或治疗性治疗的量。在临床上可以理解,药物、化合物或药物组合物的有效剂量可以通过或者可以不通过与其它药物、化合物或药物组合物联合而实现。因此,可以在施用一种或多种治疗剂的情况下认定“有效剂量”,而且如果单个药剂在与一个或多个其它药剂联合时达到了或者可以达到期望的结果,则该单个药剂可被认为是以有效量给予的。

本文中,“治疗”或“疗法”是用于获得有益的或期望的结果(包括临床结果)的方法。为了本发明目的,有益的或期望的临床结果包

括，但不限于，以下之一种或多种：抑制、阻碍或降低淀粉样蛋白斑的形成，减少、除去或清除淀粉样蛋白斑，改善认知、逆转或减慢认知衰退、隔离在生物学液体中循环的可溶性 A β 肽、减少组织（例如脑）中的 A β 肽（包括，可溶性的、寡聚体的和沉积的），抑制、减慢和/或降低脑中 A β 肽的累积，抑制、减缓和/或降低 A β 肽在组织（例如脑）中的毒性作用、减少因该疾病导致的症状，增加该疾病患者的生活质量，降低其它药物治疗该疾病时所需的剂量，延迟疾病进展，和/或延长患者的存活时间。

本文中，“延迟”阿尔茨海默氏病的发展意味着推迟、阻碍、减缓、阻止、平抑和/或延缓该疾病的发展。根据该疾病的病史和/或待治疗的个体，此延迟可以具有不同的时间长度。正如本领域技术人员明了的，充分的或显著的延迟可以，实际上，包括预防，即，个体不出现该疾病。“延迟”阿尔茨海默氏病的发展的方法是，与未使用该方法相比，在给定的时间框架内降低疾病发展的可能性和/或在给定的时间框架内降低疾病的程度的方法。该比较典型地使用统计学显著数量的个体基于临床研究进行。

阿尔茨海默氏病的“发展”意味着阿尔茨海默氏病在个体内发作和/或进展，可以使用本文所述的标准临床技术检测阿尔茨海默氏病的发展。然而，发展也指最初可能检测不到的疾病进展。为了本发明的目的，进展指疾病状态的生物学过程，在此情况下，可以通过标准神经学检查、或患者访问来确定，或者可以通过更为专业的测试来确定。各种此类诊断试验包括，但不限于，神经成像、检测血清或脑脊液中特定蛋白质（例如，淀粉样肽和 Tau）的水平变化、计算机化的 X 线断层摄影术 (CT)、和核磁共振成像 (MRI)。“发展”包括发生、复发和发作。本文中，阿尔茨海默氏病的“发作”或“发生”包括最初的发作和/或复发。

本文中，“联合”施用包括同时施用和/或在不同时间施用。联合施用也包括以共制剂的形式施用或者以分开的组合物的形式施用。本文中，联合施用意味着包括其中将抗 A β 抗体和另一药剂施用给个体

的任何情况，其中抗 A β 抗体和另一药剂的施用可以同时和/或分开进行。如本文中进一步讨论的，应当理解抗 A β 抗体和所述另一药剂可以以不同的给药频率或间隔施用。例如，可以每周施用抗 A β 抗体，而所述另一药剂可以以较低的频率施用。可以理解，抗 A β 抗体和所述另一药剂可以使用相同的施用途径或不同的施用途径施用。

“生物学样品”包括各种从个体获得的样品类型，其可以用于诊断或监测试验中。该定义包括血液和生物学来源的其它液体样品、固体组织样品例如生物活检标本或组织培养物或来源于它们的细胞及其后代。该定义也包括在获得之后以任何方式被操作过的样品，所述操作例如用试剂处理、增溶或富集某些成分如蛋白质或多核苷酸、或包埋在半固体或固体基质中以便用于切片目的。术语“生物学样品”包括临床样品，也包括培养的细胞、细胞上清液、细胞裂解物、血清、血浆、生物学液体和组织样品。

“对象”（或者称作“个体”）是哺乳动物、更优选地人。哺乳动物也包括，但不限于，农畜（例如奶牛）、运动动物、宠物（例如猫、狗、马）、灵长类动物、小鼠和大鼠。

本文中，“载体”指能够向宿主细胞递送，优选地在宿主细胞中表达，一个或多个目的基因或序列的构建体。载体的例子包括，但不限于，病毒载体、裸 DNA 或 RNA 表达载体、质粒、粘粒或噬菌体载体、与阳离子缩合剂结合的 DNA 或 RNA 表达载体、包在脂质体中的 DNA 或 RNA 表达载体、以及某些真核细胞例如生产细胞。

本文中，“表达控制序列”指指导核酸表达的核酸序列。表达控制序列可以是启动子，例如组成型或诱导型启动子，或增强子。表达控制序列与待转录的核酸序列可操作地连接。

本文中，“药物可接受载体”包括当与活性成分组合时能够允许所述成分保持生物学活性并且其与个体的免疫系统不发生反应的任何物质。实例包括，但不限于，任何标准的药物载体，例如，磷酸缓冲盐水溶液、水、乳剂如油/水乳剂、和各种类型的润湿剂。对于气雾剂或肠胃外施用，优选的稀释剂是磷酸缓冲盐水或生理盐水（0.9%）。包

含此类载体的组合物可以通过熟知的常规方法配制(见例如, Remington's Pharmaceutical Sciences, 第18版, A. Gennaro, 编, Mack Publishing Co., Easton, P.A., 1990; 和 Remington, The Science and Practice of Pharmacy 第20版, Mack Publishing, 2000)。

术语“ k_{on} ”在本文中旨在指抗体与抗原结合的结合速率常数(on rate constant)。

术语“ k_{off} ”在本文中旨在指抗体自抗体/抗原复合物解离的解离速率常数(off rate constant)。

术语“ K_D ”在本文中旨在指抗体抗原相互作用的平衡解离常数。

组合物和制备组合物的方法

抗 β 淀粉样蛋白抗体和多肽

本发明提供与 $A\beta$ 肽的C端结合的抗体。本发明提供与 $A\beta_{1-40}$ 、 $A\beta_{1-42}$ 和 $A\beta_{1-43}$ 结合的抗体或多肽。一些实施方案中,所述抗体或多肽以比其结合 $A\beta_{1-42}$ 和 $A\beta_{1-43}$ 时更高的亲和性结合 $A\beta_{1-40}$ 。一些实施方案中,所述抗体与 $A\beta_{1-36}$ 、 $A\beta_{1-37}$ 、 $A\beta_{1-38}$ 和 $A\beta_{1-39}$ 结合。一些实施方案中,所述抗体与 $A\beta_{22-35}$ 结合。一些实施方案中,所述抗体与 $A\beta_{28-40}$ 结合。一些实施方案中,所述抗体或多肽与 $A\beta_{1-40}$ 上包括氨基酸25-34及40的表位结合。

本发明还提供包含本文所述任何抗体或多肽(例如,抗体6G和其显示在表3中的变体或者来源于抗体6G和其显示在表3中的变体的多肽);或本文所述多核苷酸的组合物,包括药物组合物。本文中,组合物包含一种或多种与 $A\beta_{1-40}$ 的C端结合的抗体或多肽(其可以是或可以不是抗体)和/或一种或多种包含编码一种或多种与 $A\beta_{1-40}$ 的C端结合的抗体或多肽的序列的多核苷酸。这些组合物还可以包含适宜的赋形剂,例如,本领域熟知的药物可接受赋形剂,包括缓冲液。

本发明抗体和多肽的特征在于以下特征之任何(一个或多个):(a)与 $A\beta_{1-40}$ 、 $A\beta_{1-42}$ 和 $A\beta_{1-43}$ 结合;(b)与 $A\beta_{1-40}$ 、 $A\beta_{1-42}$ 和 $A\beta_{1-43}$ 结合,

其中与 $A\beta_{1-40}$ 结合的亲和性高于与 $A\beta_{1-42}$ 和 $A\beta_{1-43}$ 结合的亲和性; (c) 与 $A\beta_{1-40}$ 上包括氨基酸 25-34 及 40 的表位结合; (d) 与 $A\beta_{1-36}$ 、 $A\beta_{1-37}$ 、 $A\beta_{1-38}$ 和 $A\beta_{1-39}$ 结合, 但是亲和性低于其结合 $A\beta_{1-36}$ 的亲和性; (e) 以小于大约 $1\mu M$ 的 K_D 与 $A\beta_{22-37}$ 结合; (f) 与 $A\beta_{22-35}$ 结合; (g) 与 $A\beta_{28-40}$ 结合; (h) 不结合细胞中表达的 APP; (i) 抑制个体中淀粉样蛋白斑的形成; (j) 减少个体中的淀粉样蛋白斑; (k) 治疗、预防、改善阿尔茨海默氏病或其它 $A\beta$ 累积相关疾病(例如, Down 氏综合症、帕金森病、多发性脑梗塞痴呆、轻度认知缺损、脑淀粉样血管病、抑郁、Creutzfeldt-Jakob 病、伴有 Lewy 小体的痴呆)的一个或多个症状; (l) 改善认知功能。本发明抗体和多肽也可以具有本文所述的受损的效应子功能。与报道的其它抗 $A\beta$ 抗体不同, 具有受损的效应子功能的抗体和多肽可以表现出期望的完全谱。例如, 本发明组合物可以不造成显著或不可接受水平的任一种或多种下列情况: 脑血管系统中的出血(脑出血); 脑膜脑炎(包括改变的核磁共振扫描); 脑脊液中升高的白细胞计数; 中枢神经系统炎症。

因此, 本发明提供任何以下物质, 或包含任何以下物质的组合物(包括药物组合物): (a) 抗体 6G 或其显示在表 3 中的变体; (b) 抗体 6G 或其显示在表 3 中的变体的片段或区域; (c) 抗体 6G 或其显示在表 3 中的变体的轻链; (d) 抗体 6G 或其显示在表 3 中的变体的重链; (e) 来自抗体 6G 或其显示在表 3 中的变体的轻链和/或重链的一个或多个可变区; (f) 抗体 6G 或其显示在表 3 中的变体的一个或多个 CDR(1, 2, 3, 4, 5, 或 6 个 CDR); (g) 来自抗体 6G 重链的 CDR H3; (h) 来自抗体 6G 或其显示在表 3 中的变体的轻链的 CDR L3; (i) 来自抗体 6G 或其显示在表 3 中的变体的轻链的 3 个 CDR; (j) 来自抗体 6G 或其显示在表 3 中的变体的重链的 3 个 CDR; (k) 来自抗体 6G 或其显示在表 3 中的变体的轻链的 3 个 CDR 以及来自抗体 6G 或其显示在表 3 中的变体的重链的 3 个 CDR; 和 (l) 包含 (b) 至 (k) 中的任意一个的抗体。本发明还提供包含以上项目任意一个或多个的多肽。

抗体 6G 的 CDR 部分(包括 Chothia 和 Kabat CDR)图解显示在图 1

中。CDR 区的确定是本领域技术人员熟知的。可以理解, 在一些实施方案中, CDR 可以是 Kabat 和 Chothia CDR 的组合(也称作“组合 CDR”或“扩展 CDR”)。一些实施方案中, CDR 是 Kabat CDR。其它实施方案中, CDR 是 Chothia CDR。换言之, 在具有一个以上 CDR 的实施方案中, 所述 CDR 可以是 Kabat、Chothia、组合 CDR 或其组合中的任意一种。

一些实施方案中, 本发明提供多肽(可以是或可以不是抗体), 该多肽包含与 6G 或其显示在表 3 中的变体的至少一个 CDR、至少两个、至少三个、至少四个、至少五个或全部六个 CDR 基本上相同的至少一个 CDR、至少两个、至少三个、或至少四个、至少五个或全部六个 CDR。其它实施方案包括具有与 6G 的或衍生自 6G 的至少两个、三个、四个、五个或六个 CDR 基本上相同的至少两个、三个、四个、五个或六个 CDR 的抗体。一些实施方案中, 所述至少一个、两个、三个、四个、五个、或六个 CDR 与 6G 或其显示在表 3 中的变体的至少一个、两个、三个、四个、五个或六个 CDR 有至少大约 85%、86%、87%、88%、89%、90%、95%、96%、97%、98% 或 99% 的同一性。可以理解, 为了本发明的目的, 结合特异性和/或总的活性一般地得到保持, 但是与 6G 或其显示在表 3 中的变体相比活性程度可以不同(可以较高或较低)。

本发明还提供包含 6G 或其显示在表 3 中的变体的氨基酸序列的多肽(可以是或可以不是抗体), 其中所述氨基酸序列具有以下之任一: 抗体 6G 或其显示在表 3 中的变体的序列中的至少 5 个连续氨基酸、至少 8 个连续氨基酸、至少大约 10 个连续氨基酸、至少大约 15 个连续氨基酸、至少大约 20 个连续氨基酸、至少大约 25 个连续氨基酸、至少大约 30 个连续氨基酸, 其中这些氨基酸中的至少 3 个来自 6G(图 1)或其显示在表 3 中的变体的可变区。一个实施方案中, 所述可变区来自 6G 的轻链。另一实施方案中, 所述可变区来自 6G 的重链。一个示例性多肽具有来自 6G 的重链可变区和轻链可变区的连续氨基酸(长度如上述)。另一实施方案中, 所述 5 个(或更多个)连续氨基酸来自图 1 所示 6G 的互补决定区(CDR)。一些实施方案中, 所述连续氨基酸来自

6G 的可变区。

本发明抗体和多肽的结合亲和性可以变化，不必(但可以)是特定的值或范围，如下述示例性实施方案。本发明抗体和多肽与 $A\beta_{1-40}$ 的结合亲和性(K_D)可以是大约 0.10 至大约 0.80 nM、大约 0.15 至大约 0.75 nM、和大约 0.18 至大约 0.72 nM。一些实施方案中，所述结合亲和性是大约 2pM、大约 5pM、大约 10pM、大约 15pM、大约 20pM、大约 40pM、或大于大约 40pM。一个实施方案中，结合亲和性是大约 2pM 至大约 22pM。其它实施方案中，结合亲和性小于大约 10nM、大约 5nM、大约 4nM、大约 3nM、大约 2nM、大约 1nM、大约 900pM、大约 800pM、大约 700pM、大约 600pM、大约 500pM、大约 400pM、大约 300pM、大约 200pM、大约 150pM、大约 100pM、大约 90pM、大约 80pM、大约 70pM、大约 60pM、大约 50pM、大约 40pM、大约 30pM、大约 10pM。一些实施方案中，结合亲和性为大约 10nM。其它实施方案中，结合亲和性小于大约 10nM、小于大约 50nM、小于大约 100nM、小于大约 150nM、小于大约 200nM、小于大约 250nM、小于大约 500nM、或小于大约 1000nM。其它实施方案中，结合亲和性小于大约 5nM。其它实施方案中，结合亲和性小于大约 1nM。其它实施方案中，结合亲和性为大约 0.1nM 或大约 0.07nM。其它实施方案中，结合亲和性小于大约 0.1nM 或小于大约 0.07nM。其它实施方案中，结合亲和性为从大约 10nM、大约 5nM、大约 1nM、大约 900pM、大约 800pM、大约 700pM、大约 600pM、大约 500pM、大约 400pM、大约 300pM、大约 200pM、大约 150pM、大约 100pM、大约 90pM、大约 80pM、大约 70pM、大约 60pM、大约 50pM、大约 40pM、大约 30pM、大约 10pM 中的任意一个至大约 2pM、大约 5pM、大约 10pM、大约 15pM、大约 20pM、或大约 40pM 中的任意一个。一些实施方案中，结合亲和性是大约 10nM、大约 5nM、大约 1nM、大约 900pM、大约 800pM、大约 700pM、大约 600pM、大约 500pM、大约 400pM、大约 300pM、大约 200pM、大约 150pM、大约 100pM、大约 90pM、大约 80pM、大约 70pM、大约 60pM、大约 50pM、大约 40pM、大约 30pM、大约 10pM 中的任意一个。再其它实施方案中，结合亲和性为大约 2pM、大约 5pM、大约 10pM、

大约 15pM、大约 20pM、大约 40pM 或大于大约 40pM。

本发明抗体和多肽还可以与 $A\beta_{1-36}$ 、 $A\beta_{1-37}$ 、 $A\beta_{1-38}$ 、 $A\beta_{1-39}$ 、 $A\beta_{1-42}$ 和 $A\beta_{1-43}$ 中的任意一个或多个结合，但是与这些肽中的任意一个或多个的结合亲和性小于它们与 $A\beta_{1-40}$ 的结合亲和性。一些实施方案中，所述抗体或多肽与 $A\beta_{1-36}$ 、 $A\beta_{1-37}$ 、 $A\beta_{1-38}$ 、 $A\beta_{1-39}$ 、 $A\beta_{1-42}$ 和 $A\beta_{1-43}$ 中的任意一个或多个的 K_D 是其与 $A\beta_{1-40}$ 的 K_D 的至少大约 5 倍、至少大约 10 倍、至少大约 20 倍、至少大约 30 倍、至少大约 40 倍、至少大约 50 倍、至少大约 80 倍、至少大约 100 倍、至少大约 150 倍、至少大约 200 倍或至少大约 250 倍。

本发明还提供制备这些抗体或多肽中的任何抗体或多肽的方法。本发明抗体可以通过本领域已知的方法制备。例如，可以通过用 $A\beta$ 肽（例如， $A\beta_{25-40}$ 作为免疫原）免疫哺乳动物，制备所述抗体。可以通过蛋白水解或其它的方式降解抗体，或通过如上述的重组方法（即，单个的或融合的多肽）或通过化学合成，而制备所述多肽。所述抗体的多肽，尤其是不超过大约 50 个氨基酸的较短多肽，可以通过化学合成方便地制备。化学合成方法是本领域已知的，并且是可以通过商业途径获得的。例如，可以通过自动多肽合成仪，使用固相方法，制备抗体。也参见美国专利 5,807,715；4,816,567；和 6,331,415。

在另一备选方案中，抗体可以使用本领域熟知的方法重组制备。一个实施方案中，多核苷酸包含 SEQ ID NO: 9 和 SEQ ID NO: 10 中所示编码抗体 6G 的重链和/或轻链可变区的序列。另一实施方案中，包含 SEQ ID NO: 9 和 SEQ ID NO: 10 中所示核苷酸序列的多核苷酸被克隆至一个或多个用于表达或扩增的载体中。编码目的抗体的序列在宿主细胞中可以维持在载体中，然后可以扩增宿主细胞并将之冷冻以备用。对载体（包括表达载体）和宿主细胞，本文将进一步作出描述。

本发明还包括本发明抗体，例如 6G，的单链可变区片段（“scFv”）。单链可变区片段可以通过使用短的连接肽将轻链和/或重链可变区连接在一起而制备。Bird 等(1988) Science 242: 423-426。连接肽的一个实例是 (GGGGS)₃，其在一个可变区的羧基端和另一可变区的氨基

端之间形成了大约 3.5nm 的桥。具有其它序列的接头也已有设计和应用。Bird 等(1988)。接头反过来可以被修饰以具有额外的功能,例如附着药物或附着固相支持物。所述单链变体可以通过重组方式或合成方式制备。对于 scFv 的合成制备,可以使用自动合成仪。对于 scFv 的重组制备,可以将含有编码该 scFv 的多核苷酸的适宜质粒导入适宜的宿主细胞,它可以是真核细胞如酵母、植物、昆虫或哺乳动物细胞,或者原核细胞例如大肠杆菌(*E. coli*)。编码目的 scFv 的多核苷酸可以通过常规操作例如多核苷酸的连接而产生。所得 scFv 可以使用本领域已知的标准蛋白质纯化技术分离。

本发明也包括其它形式的单链抗体,例如 diabody。Diabody 是二价双特异性抗体,其中 VH 和 VL 域表达在单一一条多肽链上,但是通过使用短至不允许同一条链上的这两个域发生配对的接头,从而迫使这些域与另一条链上的互补域配对而产生两个抗原结合位点(见例如, Holliger, P. 等(1993) *Proc. Natl. Acad. Sci. USA* 90: 6444-6448; Poljak, R. J. 等(1994) *Structure* 2: 1121-1123)。

例如,双特异性抗体,对至少两种不同抗原具有结合特异性的单克隆抗体可以使用本文公开的抗体制备。制备双特异性抗体的方法是本领域已知的(见例如, Suresh 等, 1986, *Methods in Enzymology* 121: 210)。传统上,双特异性抗体的重组制备以共表达两对免疫球蛋白重链-轻链为基础,其中所述两条重链具有不同的特异性(Millstein 和 Cuello, 1983, *Nature*, 305: 537-539)。

根据制备双特异性抗体的一个方法,将具有所述期望结合特异性的抗体可变域(抗体-抗原结合位点)与免疫球蛋白恒定域序列融合。该融合物优选具有包含至少一部分铰链, CH2 和 CH3 区的免疫球蛋白重链恒定域。优选地,在至少一个融合物中存在含有结合轻链所必需的位点的第一重链恒定区(CH1)。将编码这些免疫球蛋白重链融合物以及,如果期望的话,免疫球蛋白轻链的 DNA 插入分开的表达载体中,并共转染至适宜的宿主生物。在所述构建体中所用的这三条多肽链具有不等比率可以提供最佳产率的实施方案中,这使得可以极为灵活地

调整这三个多肽片段的相互比例。然而，当至少两条多肽链以等比率表达可以导致高产率时或者当所述比率不具有特别意义时，将两条或全部三条多肽链的编码序列插入一个表达载体中是可能的。

一个方法中，双特异性抗体由位于一条臂中的具有第一结合特异性的杂合免疫球蛋白重链和位于另一臂中的杂合免疫球蛋白重链-轻链对(提供第二结合特异性)组成。该不对称结构——免疫球蛋白轻链仅存在于该双特异性分子的一半中——有利于将期望的双特异性化合物与不想要的免疫球蛋白链组合分开。该方法描述在1994年3月3日公布的PCT公布号WO 94/04690中。

包含两个共价连接的抗体的杂缀合抗体(heteroconjugate antibody)也在本发明范围内。此类抗体已经用于使免疫系统细胞靶向不想要的细胞(美国专利号4,676,980)和用于治疗HIV感染(PCT申请公开号WO 91/00360和WO 92/200373; EP 03089)。杂缀合抗体可以使用任何方便的交联方法制备。适宜的交联剂和技术是本领域熟知的，描述在美国专利号4,676,980中。

嵌合或杂合抗体也可以使用合成蛋白质化学的已知方法，包括涉及交联剂的那些方法，在体外制备。例如，可以使用二硫化物交换反应或者通过形成硫醚键，构建免疫毒素。适用于此目的的试剂的例子包括亚氨基硫醇酯和甲基-4-巯基丁酰亚胺酸酯(butyrimidate)。

包含抗体6G的一个或多个CDR或者衍生自抗体6G的一个或多个CDR的人源化抗体可以使用本领域已知的任何方法制备。例如，可以使用4个一般步骤对单克隆抗体实施人源化。这些步骤是：(1)确定起始抗体轻链和重链可变域的核苷酸序列和预测的氨基酸序列；(2)设计人源化抗体，即，决定在此人源化过程中使用何种抗体构架区；(3)实际的人源化方法/技术；和(4)转染和表达该人源化抗体。见例如，美国专利号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。

在重组人源化抗体中，可以修饰Fc γ 部分以避免与Fc γ 受体及补

体免疫系统发生相互作用。剑桥大学病理学系的 Mike Clark 博士设计了该类型的修饰,并且在 1999 年 11 月 18 日公开的 W0 99/58572 中描述制备此类抗体的技术。

例如,如果抗体用于临床试验和人类治疗中,则可以对恒定区进行工程化改造以便更类似于人类恒定区从而避免免疫反应。见例如美国专利号 5,997,867 和 5,866,692。

本发明包括抗体 6G 的修饰形式,包括不显著影响其性质的功能等价抗体和具有增强的或减弱的活性和/或亲和性的变体。例如,可以突变抗体 6G 的氨基酸序列以获得具有期望的 AB_{1-40} 肽结合亲和性的抗体。多肽的修饰是本领域的常规操作,无需在此详细描述。在实施例中举例说明了对多肽的修饰。修饰的多肽的实例包括具有保守的氨基酸残基替代、一个或多个不显著有害地改变功能活性的氨基酸缺失或添加、或应用化学类似物的多肽。

氨基酸序列插入包括长度从 1 个残基至含有 100 个或多个残基的多肽的氨基和/或羧基端融合,以及序列内的单个或多个氨基酸残基插入。末端插入的实例包括具有 N 末端甲硫氨酰残基的抗体或与表位标签融合的抗体。抗体分子的其它插入变体包括抗体 N 或 C 端与酶或增加抗体血清半衰期的多肽的融合。

替代变体在抗体分子中去除至少一个氨基酸残基并在该位置插入不同的残基。对于替代诱变而言最有意义的位点包括超变区,但是 FR 改变也在考虑之列。保守替代显示表 1 中“保守替代”标题之下。如果此类替代导致生物学活性改变,则可以引入表 1 中命名为“示例性替代”的或者以下参考氨基酸类别进一步描述的更实质性改变,并筛选产物。

表 1: 氨基酸替代

原始残基	保守替代	示例性替代
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; 正亮氨酸
Leu (L)	Ile	正亮氨酸; 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; 正亮氨酸

抗体生物学性质的实质性修饰可以通过选择对于维持(a)替代区域中多肽骨架的结构, 例如, 片层或螺旋构象; (b) 靶位点处分子的电荷或疏水性; 或(c) 侧链体积具有显著不同的影响的替代而实现。天然残基基于通常的侧链性质可以划分为以下几组:

- (1) 非极性: 正亮氨酸、Met、Ala、Val、Leu、Ile;
- (2) 极性不带电荷: Cys、Ser、Thr、Asn、Gln;
- (3) 酸性(带负电荷): Asp、Glu;
- (4) 碱性(带正电荷): Lys、Arg;
- (5) 影响侧链取向的残基: Gly、Pro; 和
- (6) 芳族的: Trp、Tyr、Phe、His。

非保守性替代可以通过将属于这些类型中的一种类型的成员变换

为另一种类型来进行。

任何不参与维持抗体正常构象的半胱氨酸残基也可以被替代，一般地使用丝氨酸替代，以改善该分子的氧化稳定性和防止异常的交联。反过来，可以给抗体添加半胱氨酸键，尤其是当所述抗体是抗体片段如 Fv 片段时，以便提高其稳定性。

氨基酸修饰的范围可以从改变或修饰一个或多个氨基酸到完全地重现设计一个区域，例如可变区。可变区中的变化可以改变结合亲和性和/或特异性。在一些实施方案中，在 CDR 域中进行不超过 1 个至 5 个保守氨基酸替代。在其它实施方案中，在 CDR 域中进行不超过 1 个至 3 个保守氨基酸替代。在再其它实施方案中，所述 CDR 域是 CDRH3 和/或 CDR L3。

修饰也包括糖基化的和非糖基化的多肽，以及具有其它翻译后修饰的多肽，例如，具有不同糖的糖基化、乙酰化和磷酸化。抗体在其恒定区的保守位置发生糖基化 (Jefferis 和 Lund, 1997, Chem. Immunol. 65: 111-128; Wright 和 Morrison, 1997, TibTECH 15: 26-32)。免疫球蛋白的寡糖侧链影响该蛋白的功能 (Boyd 等, 1996, Mol. Immunol. 32: 1311-1318; Wittwe 和 Howard, 1990, Biochem. 29: 4175-4180) 以及该糖蛋白的不同部分之间的分子内相互作用——这可以影响该糖蛋白的构象和所呈现出的三维表面 (Hefferis 和 Lund, 上述引文; Wyss 和 Wagner, 1996, Current Opin. Biotech. 7: 409-416)。寡糖也可以用于使给定的糖蛋白基于特异的识别结构而靶向某些分子。抗体的糖基化也已经被报道可以影响抗体依赖的细胞介导的细胞毒性 (ADCC)。具体地，据报道，在四环素的调节下表达 β (1, 4)-N-乙酰基葡萄糖酰氨基转移酶 III (GnTIII)——一种催化分叉性 GlcNAc 形成的糖基转移酶——的 CHO 细胞具有改善的 ADCC 活性 (Umana 等, 1999, Mature Biotech. 17: 176-180)。

抗体的糖基化典型地是 N-连接的或 O-连接的。N-连接的糖基化指糖部分与天冬酰胺残基的侧链连接。三肽序列，天冬酰胺-X-丝氨酸、天冬酰胺-X-苏氨酸和天冬酰胺-X-半胱氨酸 (其中 X 是除了脯氨酸之

外的任何氨基酸), 是使糖部分与天冬酰胺侧链发生酶促连接的识别序列。因此, 当多肽中存在这些三肽序列之任一个时将产生潜在的糖基化位点。O-连接的糖基化指糖 N-乙酰基半乳糖胺、半乳糖或木糖之一与羟基氨基酸的连接, 其中所述羟基氨基酸最通常是丝氨酸或苏氨酸, 但是也可以使用 5-羟基脯氨酸或 5-羟基赖氨酸。

向抗体添加糖基化位点可以通过改变氨基酸序列使其包含上述三肽序列之一或多个(对 N-连接的糖基化位点)而方便地实现。该改变也可以通过向原始抗体序列中添加或替代入一个或多个丝氨酸或苏氨酸残基而实现(对 O-连接的糖基化位点)。

也可以改变抗体的糖基化模式而不改变基础的核苷酸序列。糖基化在相当大程度上取决于用于表达抗体的宿主细胞。由于用于表达重组糖蛋白, 例如抗体, 作为潜在治疗剂的细胞类型很少是原初的细胞, 故可以预期抗体糖基化模式的变化(见例如, Hse 等, 1997, J. Biol. Chem. 272: 9062-9070)。

除了宿主细胞的选择外, 在抗体重组生产过程中影响糖基化的因素还包括生长模式、培养基的配制、培养密度、充氧、pH、纯化方案等。已经提出各种方法来改变在特定宿主生物体中获得的糖基化模式, 包括引入或过表达参与寡糖产生的某些酶(美国专利号 5, 047, 335; 5, 510, 261 和 5, 278, 299)。糖基化, 或某些类型的糖基化, 可以通过酶学方法, 例如, 使用内切糖苷酶 H(Endo H)、N-糖苷酶 F(见实施例 1 的描述)、内切糖苷酶 F1、内切糖苷酶 F2、内切糖苷酶 F3, 从糖蛋白上除去。此外, 可以遗传工程改造重组宿主细胞以便在某些类型的多糖的加工上出现缺陷。这些以及类似技术是本领域熟知的。

其它修饰方法包括使用本领域已知的偶联技术, 包括, 但不限于, 酶学方法、氧化取代和螯合。可以通过修饰来例如连接标记物用于免疫分析试验。可以使用本领域成熟的程序制备修饰的 6G 多肽, 并且可以使用本领域已知的标准分析试验(其中一些在下面和实施例中进行了描述)筛选该多肽。

其它抗体修饰包括按照 1999 年 11 月 18 日公布的 PCT 公布号 WO

99/58572 所述修饰的抗体。这些抗体，除了包含指向靶分子的结合域外，还包含具有与人免疫球蛋白重链恒定域之全部或部分基本上同源的氨基酸序列的效应子域。这些抗体能够结合靶分子而不触发显著的补体依赖性裂解、或者细胞介导的靶破坏。一些实施方案中，所述效应子域能够特异地结合 FcRn 和/或 Fc γ RIIb。这些典型地以衍生自两个或更多个人免疫球蛋白重链 C_H2 域的嵌合域为基础。以此方式修饰的抗体特别适合用于长期抗体治疗中，以避免针对常规抗体治疗出现的炎性和其它不良反应。

本发明包括亲和力饱和的实施方案。例如，亲和力饱和的抗体可以通过本领域已知的程序制备 (Marks 等, 1992, Bio/Technology, 10: 779-783; Barbas 等, 1994, Proc Nat. Acad. Sci, USA 91: 3809-3813; Schier 等, 1995, Gene, 169: 147-155; Yelton 等, 1995, J. Immunol. 155: 1994-2004; Jackson 等, 1995, J. Immunol. 154(7): 3310-9; Hawkins 等, 1992, J. Mol. Biol., 226: 889-896; 和 WO 2004/058184)。

以下方法可以用于调整抗体的亲和力以及用于表征 CDR。表征抗体 CDR 和/或改变 (例如提高) 多肽如抗体的结合亲和力的一种方法称作“文库扫描诱变”。一般地，文库扫描诱变按如下方式进行。使用本领域已知的方法，用两个或更多个 (例如，3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19 或 20 个) 氨基酸替换 CDR 中的一个或多个氨基酸位置。这产生小的克隆文库 (在一些实施方案中，针对每一个分析的氨基酸位置有 1 个文库)，每一个文库具有两个或更多个成员的复杂度 (如果在每一个位置替换两个或更多个氨基酸)。一般地，该文库还包括含有原始 (未被替代的) 氨基酸的克隆。每个文库取少量克隆，例如，大约 20-80 个克隆 (取决于文库的复杂度)，基于与靶多肽 (或其它结合剂) 的结合亲和力，筛选这些克隆，并鉴定具有增加的、相同的、降低的结合或无结合的候选物。确定结合亲和力的方法是本领域熟知的。可以使用检测大约 2 倍或更多的结合亲和力差异的 BIAcore 表面等离子共振分析来测定结合亲和力。当起始抗

体已经以相对高的亲和力，例如大约 10nM 或更低的 K_D ，发生结合时，BIAcore 是特别有用的。本文实施例中描述了使用 BIAcore 表面等离子共振进行的筛选。

可以使用 Kinexa Biocensor、亲近闪烁检测法 (Scintillation Proximity Assay)、ELISA、ORIGEN 免疫分析 (IGEN)、荧光淬灭、荧光转移、和/或酵母展示，测定结合亲和力。也可以使用适宜的生物分析试验，筛选结合亲和力。

一些实施方案中，使用本领域认可的诱变方法 (本文中描述了其中一些方法)，在 CDR 的每一个氨基酸位置 (一些实施方案中，每次一个位置) 实施所有 20 种天然氨基酸的替换。这产生小的克隆文库 (一些实施方案中，每一个分析的氨基酸位置一个文库)，每一个文库具有 20 个成员的复杂度 (如果每一个位置发生全部的 20 种氨基酸替代)。

一些实施方案中，待筛选的文库包含发生在两个或更多个位置的替代，所述位置可以位于相同 CDR 中或位于两个或更多个 CDR 中。因此，该文库可以包含发生在一个 CDR 中的两个或更多个位置的替代。该文库可以包含发生在两个或更多个 CDR 中的两个或更多个位置的替代。该文库可以包含发生在 3 个、4 个、5 个或更多个位置的替代，所述位置存在于两个、三个、四个、五个或六个 CDR 中。所述替代可以使用低冗余密码子制备。见例如 Balint 等 (1993) Gene 137(1): 109-18 之表 2。

所述 CDR 可以是 CDRH3 和/或 CDRL3。所述 CDR 可以是 CDRL1、CDRL2、CDRL3、CDRH1、CDRH2 和/或 CDRH3 中的一个或多个。所述 CDR 可以是 Kabat CDR、Chothia CDR 或扩展 CDR。

可以对具有改良的结合的候选物进行测序，由此鉴定导致改善的亲合力的 CDR 替代突变体 (也称作“改善的”替代)。也可以对发生结合的候选物进行测序，由此鉴定保持结合的 CDR 替代。

可以实施多轮筛选。例如，具有改善的结合的候选物 (每一个包含位于一个或多个 CDR 的一个或多个位置的氨基酸替代) 也可以用于设计在每一个改善的 CDR 位置 (即，显示出改良的结合的替代突变体所在

的 CDR 氨基酸位置) 包含至少原始的和替代的氨基酸的第二文库。以下进一步讨论该文库的制备、和筛选或选择。

文库扫描诱变也为表征 CDR 提供了一种手段, 具有改良的结合、相同的结合、降低的结合或无结合的克隆的频率也提供了有关每个氨基酸位置对于抗体抗原复合物的稳定性而言所具有的重要性的信息。例如, 如果 CDR 的一个位置在变化成所有 20 种氨基酸时仍保持结合, 则该位置可以被鉴定为不可能是抗原结合所必需的位置。相反地, 如果 CDR 的一个位置仅在一小部分替代中保持结合, 则该位置被鉴定为是对 CDR 功能具有重要性的位置。因此, 文库扫描诱变方法可以产生关于 CDR 中可以被变化成许多不同氨基酸(包括全部 20 种氨基酸) 的位置以及 CDR 中不能被改变或仅能够被改变成少数几种氨基酸的位置的信息。

可以将具有改良的亲合力的候选物合并的第二文库中, 该文库包括在该位置的改良的氨基酸、原始氨基酸, 并且还可以包括在该位置的其它替代, 这取决于所期望的或者使用期望的筛选或选择方法所能允许的文库复杂度。此外, 如果期望的话, 可以随机地赋予邻近的氨基酸位置至少两种或更多种氨基酸。邻近氨基酸的随机化可以允许在突变 CDR 中具有额外的构象灵活性, 这反过来可以允许或有利于引入更大数量的改良突变。该文库也可以包含在第一轮筛选中未显示出改良的亲合力的位置的替代。

使用任何本领域已知方法, 包括利用 BIAcore 表面等离子共振分析进行筛选, 以及利用本领域已知用于选择的任何选择方法, 包括噬菌体展示、酵母展示和核糖体展示, 进行选择, 从第二文库筛选或选择具有改良的和/或改变的结合亲和力的文库成员。

本发明还包括包含来自本发明抗体(如 6G)或多肽的一个或多个片段或区域的融合蛋白。一个实施方案中, 提供包含 SEQ ID NO: 2 所示轻链可变区(图 1)的至少 10 个连续氨基酸和/或 SEQ ID NO: 1 所示重链可变区(图 1)的至少 10 个氨基酸的融合多肽。其它实施方案中, 提供包含 SEQ ID NO: 2 所示轻链可变区(图 1)的至少大约 10 个、至

少大约 15 个、至少大约 20 个、至少大约 25 个、或至少大约 30 个连续氨基酸和/或 SEQ ID NO: 1 所示重链可变区(图 1)的至少大约 10 个、至少大约 15 个、至少大约 20 个、至少大约 25 个、或至少大约 30 个连续氨基酸的融合多肽。在另一实施方案中,融合多肽包含如图 1 之 SEQ ID NO: 2 和 SEQ ID NO: 1 所示的 6G 轻链可变区和/或重链可变区。在另一实施方案中,融合多肽包含 6G 的一个或多个 CDR。在再一实施方案中,融合多肽包含抗体 6G 的 CDR H3 和/或 CDR L3。为了本发明的目的,6G 融合蛋白含有一个或多个 6G 抗体和在天然分子中并不与 6G 连接的另一氨基酸序列,例如,异源序列或来自另一区域的同源序列。异源序列的实例包括但不限于“标签”,例如 FLAG 标签或 6His 标签。标签是本领域熟知的。

6G 融合多肽可以通过本领域已知的方法,例如,合成或重组方法,制备。典型地,本发明 6G 融合蛋白通过使用本文所述重组方法制备并表达编码它们的多核苷酸而产生,但是也可以通过本领域已知的其它方式,包括例如化学合成来产生。

本发明也提供包含与利于和固相支持物偶联的试剂(例如生物素或抗生物素蛋白)缀合(例如,连接)的 6G 抗体或多肽的组合物。为了简便,一般提及 6G 或抗体,但应明了这些方法适用于本文描述的任何 $A\beta_{1-40}$ 结合实施方案。缀合一般指按本文所述将这些成分连接起来。连接(其一般是指至少为了施用的目的而固定这些成分使它们处于紧密联系中)可以通过多种方式实现。例如,当试剂和抗体各具有能够与对方反应的取代基时,试剂和抗体可以直接反应。例如,位于其中一个分子上的亲核基团,例如,氨基或巯基,可能能够与位于另一分子上的含羰基基团,例如,酐或酰基卤,或者含有良好离去基团(例如卤素)的烷基基团反应。

本发明抗体或多肽可以与标记试剂(或者称作“标记物”)例如荧光分子、放射性分子或本领域已知的任何其它标记物连接。标记物是本领域已知的,一般提供(直接地或者间接地)信号。

本发明还提供包含抗体 6G,以及,正如本公开所解释的,本文所

述任何或所有的抗体和/或多肽的组合物(包括药物组合物)和药盒。

具有受损的效应子功能的抗 A β 抗体和多肽

本文所述抗体或多肽(包括包含该抗体或多肽的药物组合物)可以具有受损的效应子功能。在本文中,具有“受损的效应子功能”(可以与“免疫学惰性的”或“部分免疫学惰性的”互换使用)的抗体或多肽指不具有任何效应子功能或者具有一种或多种降低的效应子功能活性(与具有未修饰的或者天然存在的恒定区的抗体或多肽相比)的抗体或多肽,例如不具有或具有降低的如下活性之任一种或多种:a) 触发补体介导的裂解;b) 刺激抗体依赖性细胞介导的细胞毒性(ADCC);和 c) 激活小胶质细胞。该效应子功能活性可以降低大约 10%、20%、30%、40%、50%、60%、70%、80%、90%、95%、99% 或 100%。一些实施方案中,抗体与 β -淀粉样肽结合而不触发显著的补体依赖性靶裂解或者细胞介导的靶破坏。例如,恒定区上的 Fc 受体结合位点可以被修饰或突变以去除或降低与某些 Fc 受体,例如 Fc γ RI、Fc γ RII 和/或 Fc γ RIII 的结合亲和力。为了简便,提及抗体时应理解所述实施方案也适用于多肽。EU 编号系统(Kabat 等, Sequences of Proteins of Immunological Interest; 第 5 版, Public Health Service, National Institutes of Health, Bethesda, Md., 1991)被用于指示恒定区(例如, IgG 抗体的恒定区)中哪个(或哪些)氨基酸残基被改变或突变。该编号可以用于特定类型的抗体(例如, IgG1)或物种(例如人),但应理解可以跨抗体和物种类型进行类似变化。

一些实施方案中,特异地结合 A β 肽的抗体包含具有受损的效应子功能的重链恒定区。该重链恒定区可以具有天然序列或者是变体。一些实施方案中,突变天然重链恒定区的氨基酸序列,例如,通过氨基酸替代、插入和/或缺失进行突变,由此损害该恒定区的效应子功能。一些实施方案中,也可以改变,例如,可以完全或部分地除去,重链恒定区 Fc 区的 N-糖基化,由此损害该恒定区的效应子功能。

一些实施方案中,通过去除抗 A β 肽 Fc 区(例如, IgG 的 CH2 域中的)的 N-糖基化,损害效应子功能。一些实施方案中,通过突变被糖基化的氨基酸残基或者构成恒定区糖基化识别序列一部分的侧翼残基,去除 Fc 区的 N-糖基化。三肽序列,天冬酰胺-X-丝氨酸(N-X-S)、天冬酰胺-X-苏氨酸(N-X-T)和天冬酰胺-X-半胱氨酸(N-X-C)(其中 X 是除脯氨酸之外的任何氨基酸),是糖部分与天冬酰胺侧链发生酶促连接形成 N-糖基化的识别序列。对恒定区中此三肽序列中任何氨基酸进行突变,产生无糖基化的 IgG。例如,可以将人 IgG1 和 IgG3 的 N-糖基化位点 N297 突变为 A、D、Q、K 或 H。见, Tao 等, *J. Immunology*, 143: 2595-2601 (1989); 和 Jefferis 等, *Immunological Reviews* 163: 59-76 (1998)。已经报道,具有 Asn-297 至 Gln、His 或 Lys 的替代的人 IgG1 和 IgG3 不与人 Fc γ RI 结合,并且不激活补体,其中对于 IgG1 而言 C1q 结合能力完全丧失,对于 IgG3 而言该能力急剧降低。一些实施方案中,三肽序列中的氨基酸 N 被突变为以下任一种氨基酸: A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, Y。在一些实施方案中,三肽序列中的氨基酸 N 被突变为保守替代。一些实施方案中,三肽序列中的氨基酸 X 被突变为脯氨酸。一些实施方案中,三肽序列中的氨基酸 S 被突变为 A, D, E, F, G, H, I, K, L, M, N, P, Q, R, V, W, Y。一些实施方案中,三肽序列中的氨基酸 T 被突变为 A, D, E, F, G, H, I, K, L, M, N, P, Q, R, V, W, Y。一些实施方案中,三肽序列中的氨基酸 C 被突变为 A, D, E, F, G, H, I, K, L, M, N, P, Q, R, V, W, Y。一些实施方案中,位于该三肽之后的氨基酸被突变为 P。一些实施方案中,恒定区的 N-糖基化通过酶学方式去除(例如,如实施例 1 中所示的 N-糖苷酶 F、内切糖苷酶 F1、内切糖苷酶 F2、内切糖苷酶 F3 和内切糖苷酶 H)。也可以通过在具有 N-糖基化作用缺陷的细胞系中生产抗体,而去除 N-糖基化。Wright 等, *J Immunol.* 160(7): 3393-402 (1998)。

一些实施方案中,对与附着于恒定区 N-糖基化位点的寡糖发生相互作用的氨基酸残基进行突变,以降低和 Fc γ RI 的结合亲和力。例如,

可以突变人 IgG3 的 F241、V264、D265。见, Lund 等, J. Immunology 157: 4963-4969 (1996)。

一些实施方案中, 通过修饰区域例如人 IgG 的 233-236, 297, 和 / 或 327-331 (参见 PCT WO 99/58572 和 Armour 等, Molecular Immunology 40: 585-593 (2003); Reddy 等, J. Immunology 164: 1925-1933 (2000)), 损害效应子功能。PCT WO 99/58572 和 Armour 等描述的抗体除了包含指向靶分子的结合域外还包含具有与人免疫球蛋白重链恒定区之全部或部分基本上同源的氨基酸序列的效应子域。这些抗体能够结合靶分子同时不触发显著的补体依赖性靶裂解或细胞介导的靶破坏。一些实施方案中, 所述效应子域对 Fc γ RI、Fc γ RIIa 和 / 或 Fc γ RIII 具有降低的亲合力。一些实施方案中, 所述效应子域能够特异地结合 FcRn 和 / 或 Fc γ RIIb。这些典型地以衍生自两个或更多个人免疫球蛋白重链 C_H2 域的嵌合域为基础。以此方式修饰的抗体尤其适用于长期抗体治疗, 以避免常规抗体治疗时出现的炎症和其它不良反应。一些实施方案中, 抗体重链恒定区是具有任何如下突变的人重链 IgG1: 1) A327A330P331 至 G327S330S331; 2) E233L234L235G236 至 P233V234A235 且 G236 缺失; 3) E233L234L235 至 P233V234A235; 4) E233L234L235G236A327 A330P331 至 P233V234A235G327S330S331 且 G236 缺失; 5) E233L234L235A327A330P331 至 P233V234A235G327S330S331; 和 6) N297 至 A297 或除 N 之外的任何其它氨基酸。这些突变可以组合, 例如, 1) 至 5) 之任一可以和 6) 组合。一些实施方案中, 所述抗体重链恒定区是具有如下突变的人重链 IgG2: A330P331 至 S330S331; N297 至 Q297; 和 N297G327A330P331 至 Q297G327S330S331。一些实施方案中, 所述抗体重链恒定区是具有以下任何突变的人重链 IgG4: E233F234L235G236 至 P233V234A235 且 G236 缺失; E233F234L235 至 P233V234A235; P228L235 至 S228E235; N297 至 Q297; 和 E233F234L235G236N297 至 P233V234A235G236Q297。

也可以修饰所述抗体恒定区以破坏补体的活化。例如, IgG 抗体

在结合补体的 C1 成分后的补体激活作用可以通过突变恒定区中 C1 结合基序(例如, C1q 结合基序)内的氨基酸残基而降低。已经报道, 针对人 IgG1 的 D270、K322、P329 和 P331 之每一个的 Ala 突变均显著地降低该抗体结合 C1q 和激活补体的能力。对于鼠 IgG2b, C1q 结合基序由残基 E318、K320 和 K322 组成。Idusogie 等, *J. Immunology* 164: 4178-4184 (2000); Duncan 等, *Nature* 322: 738-740(1988)。

据认为, 针对鼠 IgG2b 所鉴定的 C1q 结合基序 E318、K320 和 K322 是其它抗体同种型所共有的。Duncan 等, *Nature* 322: 738-740 (1988)。可以通过将所述三个特定残基中的任意一个残基置换为在其侧链上具有不适宜的功能性的残基, 而废除 IgG2b 的 C1q 结合活性。不一定只有用 Ala 替代离子残基才能废除 C1q 的结合。也可以使用其它烷基取代的非离子残基, 例如, Gly, Ile, Leu 或 Val, 或者芳族非极性残基如 Phe, Tyr, Trp 和 Pro 替代所述三个残基中的任意一个以废除 C1q 的结合。此外, 也可以使用极性非离子残基例如 Ser, Thr, Cys 和 Met 替代残基 320 和 322 而非残基 318, 以废除 C1q 结合活性。

本发明还提供具有受损的效应子功能的抗体, 其中所述抗体具有修饰的铰链区。人 IgG 对其 Fc 受体的结合亲和力可以通过修饰铰链区来调整。Canfield 等, *J. Exp. Med.* 173: 1483-1491 (1991); Hezareh 等, *J. Virol.* 75: 12161-12168 (2001); Redpath 等, *Human Immunology* 59: 720-727 (1998)。可以突变或缺失特定的氨基酸残基。修饰的铰链区可以包含从与 CH1 域的抗体类型或亚类不同的抗体类型或亚类的抗体衍生的完整铰链区。例如, IgG 类抗体的恒定区(CH1)可以与 IgG4 类抗体的铰链区连接。或者, 该新的铰链区可以包含部分的天然铰链或者重复单元, 其中所述重复中的每一个单元都衍生自天然铰链区。一些实施方案中, 通过将一个或多个半胱氨酸残基转换成中性残基, 例如, 丙氨酸, 或者通过将位于适宜位置的残基转换成半胱氨酸残基, 而改变天然铰链区。美国专利号 5,677,425。这些改变可以使用本领域认可的蛋白化学以及优选地, 遗传工程技术, 按照本文所述进行。

特异地与 A β 肽结合并与具有受损的效应子功能的重链恒定区融合的多肽也可以用于本文所述的方法中。一些实施方案中, 所述多肽包含衍生自抗体 6G 或其显示在表 3 中的变体的序列。一些实施方案中, 所述多肽衍生自与 A β 肽结合的单域抗体。单域抗体可以使用本领域已知的方法制备。Omidfar 等, *Tumour Biol.* 25: 296-305 (2004); Herring 等, *Trends in Biotechnology* 21: 484-489 (2003)。

一些实施方案中, 所述抗体或多肽是 F(ab')₂ 片段。一些实施方案中, 所述抗体或多肽是 Fab 片段。一些实施方案中, 所述抗体或多肽是单链抗体 scFv。一些实施方案中, 所述抗体或多肽是 PEG 化的 F(ab')₂ 片段。一些实施方案中, 所述抗体或多肽是 PEG 化的 Fab 片段。一些实施方案中, 所述抗体或多肽是 PEG 化的单链抗体 scFv。

也可以使用本领域已知的制备具有受损的效应子功能的抗体的其它方法。

可以在一或多个试验中检测具有修饰的恒定区的抗体或多肽, 以评价相对于起始抗体而言效应子功能的生物活性降低水平。例如, 可以使用本文所公开的试验以及任何本领域认可的试验, 评价具有改变的 Fc 区的抗体或多肽与补体或 Fc 受体(例如, 小胶质细胞上的 Fc 受体)结合的能力或具有改变的铰链区的抗体或多肽的能力。PCT WO 99/58572; Armour 等, *Molecular Immunology* 40: 585-593 (2003); Reddy 等, *J. Immunology* 164:1925-1933 (2000); Song 等, *Infection and Immunity* 70:5177-5184 (2002)。

可以使用竞争试验确定是否是两个抗体通过识别相同的或空间上重叠的表位而与相同的表位结合还是一个抗体竞争地抑制另一抗体与抗原的结合。这些试验是本领域已知的。典型地, 将抗原固定在多孔板上并测量未标记的抗体阻断标记抗体的结合的能力。对此此类竞争试验, 常用的标记物是放射性标记物或酶标记物。

可以基于清除淀粉样蛋白沉积物的效力和其它有益效果, 例如改善的认知能力, 筛选与 A β 特异地结合的抗体和多肽。例如, 可以将抗体或多肽施用给具有阿尔茨海默氏病的病理情况的动物。本领域已

知各种阿尔茨海默氏病动物模式。在施用之后,可以使用本领域已知的和实施例2中详细描述的方法,检测致密的和弥散的淀粉样蛋白斑的水平、认知的行为分析、以及小胶质细胞的活化和微出血。PCT WO 2004/032868; Wilcock 等, J. Neurosci. 23: 3745-3751 (2003); Wilcock 等, J. Neuroinflammation 1:24 (2004)。

多核苷酸、载体和宿主细胞

本发明还提供编码本发明抗体和多肽(包括包含图1所示轻链和重链可变区的多肽序列的抗体)的分离的多核苷酸、以及包含该多核苷酸的载体和宿主细胞。

因此,本发明提供包含编码如下之任一项的多核苷酸的多核苷酸(或者组合物,包括药物组合物): a) 抗体6G或其显示在表3中的变体; b) 抗体6G或其显示在表3中的变体的片段或区域; c) 抗体6G或其显示在表3中的变体的轻链; d) 抗体6G或其显示在表3中的变体的重链; e) 来自抗体6G或其显示在表3中的变体的轻链和/或重链的一个或多个可变区; f) 抗体6G或其显示在表3中的变体的一个或多个CDR(一个、两个、三个、四个、五个或六个CDR); g) 来自抗体6G重链的CDR H3; h) 来自抗体6G或其显示在表3中的变体的轻链的CDR L3; i) 来自抗体6G或其显示在表3中的变体的轻链的3个CDR; j) 来自抗体6G或其显示在表3中的变体的重链的3个CDR; k) 来自抗体6G或其显示在表3中的变体的轻链的3个CDR以及来自抗体6G或其显示在表3中的变体的重链的3个CDR; 和 l) 包含(b)至(k)至任一项的抗体。一些实施方案中,所述多核苷酸包含SEQ ID NO: 9和SEQ ID NO: 10中所示多核苷酸之一或两者。

另一方面,本发明提供编码本文所述任何抗体(包括抗体片段)和多肽,例如,具有受损的效应子功能的抗体和多肽的多核苷酸。多核苷酸可以通过本领域已知的方法制备。

另一方面,本发明提供包含本发明任何多核苷酸的组合物(例如药物组合物)。一些实施方案中,所述组合物包含含有编码本文所述抗体

6G的多核苷酸的表达载体。其它实施方案中,所述组合物包含含有编码本文所述任何抗体或多肽的多核苷酸的表达载体。再其它实施方案中,所述组合物包含 SEQ ID NO: 9 和 SEQ ID NO: 10 中所示多核苷酸之一或两者。表达载体和多核苷酸组合物的施用将在本文中作进一步描述。

另一方面,本发明提供制备本文所述任何多核苷酸的方法。

与任何此类序列互补的多核苷酸也包括在本发明中。多核苷酸可以是单链的(编码链或反义链)或者双链的,并且可以是 DNA(基因组 DNA、cDNA 或合成的 DNA)或 RNA 分子。RNA 分子包括含有内含子并以一对一的方式对应于 DNA 分子的 HnRNA 分子,以及不含内含子的 mRNA 分子。其它的编码或非编码序列可以,但不必然,存在于本发明的多核苷酸中。多核苷酸可以,但不必然,与其它分子和/或支持材料相连。

多核苷酸可以包含天然序列(即,编码抗体或其部分的内源序列)或者可以包含该序列的变体。多核苷酸变体含有一个或多个替代、添加、缺失和/或插入以致所编码的多肽的免疫反应性相对于天然免疫反应性分子而言不减低。对所编码的多肽的免疫反应性的影响一般可以按照本文所述进行评估。变体优选地表现出与编码天然抗体或其部分的多核苷酸序列具有至少大约 70% 同一性,更优选地至少大约 80% 同一性,最优选地至少大约 90% 同一性。

如果两个多核苷酸或多肽序列在按照下述进行比对以获得最大对应性的情况下两个序列中的核苷酸或氨基酸序列相同,则这两个多核苷酸或多肽序列被称作是“同一的”。两个序列之间的比较典型地通过比较窗中比较序列以鉴定和比较局部区域的序列相似性来进行。本文中,“比较窗”指具有至少大约 20 个连续位置、通常 30 至大约 75, 40 至大约 50 个连续位置的区段,在该区段中序列可以在实施最佳比对之后与具有相同数目的连续位置的参考序列进行比较。

为了比较的目的实施的序列最佳比对可以使用 Lasergene 生物信息软件组(DNASTAR, Inc., Madison, WI)中的 Megalign 程序以及默认参数进行。该程序体现了以下参考文献中描述的几种比对方案:

Dayhoff, M. O. (1978) 蛋白质的进化改变模型 - 用于检测远源关系的矩阵 (A model of evolutionary change in proteins - Matrices for detecting distant relationships.) Dayhoff, M. O. (编) 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 Phylogenesis pp. 626-645 Methods in Enzymology vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D. G. 和 Sharp, P. M., 1989, CABIOS 5:151-153; Myers, E. W. 和 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. 和 Sokal, R. R., 1973, Numerical Taxonomy the Principles and Practice of Numerical Taxonomy, Freeman Press, San Francisco, CA; Wilbur, W. J. 和 Lipman, D. J., 1983, Proc. Natl. Acad. Sci. USA 80:726-730.

优选地,通过在至少 20 个位置的比较窗中比较两个最佳比对的序列,确定“序列同一性百分数”,其中为了获得所述两个序列的最佳比对,比较窗中的多核苷酸或多肽序列部分可以相对于参考序列(不含添加或缺失)而言包含 20%或更少、通常 5%至 15%或 10%至 12%的添加或缺失(即,缺口)。该百分数通过如下方式计算:确定两个序列中出现相同核酸碱基或氨基酸残基的位置的数目以产生匹配位置的数目,然后用该匹配位置的数目除以参考序列中位置的总数(即,窗的大小),并将结果乘以 100 以产生序列同一性百分数。

变体也或者备选地可以与天然基因或其部分或互补物基本上同源。此类多核苷酸变体能够在中等严紧条件下与编码天然抗体的天然 DNA 序列(或互补序列)杂交。

适宜的“中等严紧条件”包括在 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0)的溶液中预洗涤;在 50°C-65°C, 5 X SSC 中杂交过夜;之后在 65°C 分别用含有 0.1 % SDS 的 2X, 0.5X 和 0.2X SSC 各洗涤两次

20 分钟。

本文中，“高严谨条件”或“高严谨性条件”是指利用以下条件的那些：(1)使用低离子强度和高温进行洗涤，例如，0.015 M 氯化钠/0.0015 M 柠檬酸钠/0.1%十二烷基硫酸钠于 50℃；(2)杂交过程中使用变性剂，例如甲酰胺，例如，50% (v/v) 甲酰胺与 0.1%牛血清白蛋白/0.1% Ficoll/0.1% 聚乙烯吡咯烷酮/50mM 磷酸钠缓冲液 pH 6.5 和 750 mM 氯化钠，75 mM 柠檬酸钠于 42℃；或者(3)使用 50%甲酰胺，5 x SSC (0.75 M NaCl, 0.075 M 柠檬酸钠)，50 mM 磷酸钠 (pH 6.8)，0.1%焦磷酸钠，5 x Denhardt 氏溶液，超声的鲑鱼精子 DNA (50 $\mu\text{g}/\text{ml}$)，0.1% SDS, 和 10%硫酸葡聚糖于 42℃，并在 0.2 x SSC (氯化钠/柠檬酸钠)中 42℃洗涤和在 50%甲酰胺中 55℃洗涤，之后用含有 EDTA 的 0.1 x SSC 在 55℃下进行高严谨性洗涤。本领域技术人员将明了如何按照需要调整温度、离子强度等以适应诸如探针长度等因素。

本领域技术人员将明了，由于遗传密码的简并性，存在许多编码本文所述多肽的核苷酸序列。其中一些多核苷酸与任何天然基因的核苷酸序列具有极小的同源性。然而，由于密码子使用差异而不同的多核苷酸是本发明所特别考虑。而且，包含此处提供的多核苷酸序列的基因的等位基因也在本发明范围内。等位基因是由于一个或多个核苷酸突变，例如缺失、添加和/或替代而被改变的内源性基因。所产生的 mRNA 和蛋白质可以，但不必，具有改变的结构或功能。等位基因可以使用标准技术(例如，杂交、扩增和/或数据库序列比较)鉴定。

本发明多核苷酸可以使用化学合成、重组方法、或 PCR 获得。化学多核苷酸合成方法是本领域熟知的，无需在此作详细的描述。本领域技术人员可以使用本文提供的序列和商业 DNA 合成仪产生期望的 DNA 序列。

对于使用重组方法制备多核苷酸，可以将含有期望序列的多核苷酸插入适宜载体中，接着将该载体导入适宜的宿主细胞以便复制和扩增，这将在本文中作进一步讨论。可以利用本领域已知的任何方式，将多核苷酸插入宿主细胞中。可以通过直接摄取、胞吞作用、转染、

F-交配或电穿孔引入外源多核苷酸，来转化细胞。一旦引入后，外源多核苷酸可以以非整合型载体(例如质粒)的形式维持在细胞中或者可以整合至宿主细胞基因组中。如此扩增的多核苷酸可以通过本领域熟知的方法从宿主细胞中分离出来。见例如，Sambrook 等(1989)。

备选地，PCR 允许 DNA 序列进行复制。PCR 技术是本领域熟知的，描述在美国专利号 4,683,195、4,800,159、4,754,065 和 4,683,202 以及 PCR: The Polymerase Chain Reaction, Mullis et al. eds., Birkanswer Press, Boston (1994) 中。

可以通过在适宜的载体中使用分离的 DNA 并将其插入适宜的宿主细胞，获得 RNA。当所述细胞复制和所述 DNA 转录为 RNA 时，可以使用本领域熟知的方法(见例如 Sambrook 等(1989))分离该 RNA。

适宜的克隆载体可以使用标准技术构建，或者可以从本领域可以获得的大量克隆载体中选择。尽管所选的克隆载体可以因意在使用的宿主细胞而变化，但一般地有用的克隆载体将具有自我复制能力，可以具有用于特定限制性内切核酸酶的单一靶，和/或可以带有标记基因，其中所述标记可以用于选择含有所述载体的克隆。适宜的实例包括质粒和细菌病毒，例如，pUC18, pUC19, Bluescript (如 pBS SK+) 及其衍生物，mp18, mp19, pBR322, pMB9, ColE1, pCR1, RP4, 噬菌体 DNA, 和穿梭载体如 pSA3 和 pAT28。这些和许多其它的克隆载体可以从供应商如 BioRad、Strategene 和 Invitrogen 获得。

表达载体一般是含有本发明多核苷酸的可复制的多核苷酸构建体。这意味着表达载体必须以附加体形式或者作为整合至染色体 DNA 中的部分能够在宿主细胞中复制。适宜的表达载体包括但不限于质粒、病毒载体，包括腺病毒、腺相关病毒、逆转录病毒、粘粒和 PCT 公布号 WO 87/04462 中公开的表达载体。载体成分一般可以包括但不限于以下一种或多种：信号序列；复制起点；一个或多个标记基因；适宜的转录控制元件(例如，启动子、增强子和终止子)。为了表达(即，翻译)，通常也需要一个或多个翻译控制元件，例如，核糖体结合位点、翻译起始位点和终止密码子。

含有目的多核苷酸的载体可以通过多种适宜方式中的任何一种，包括电穿孔，使用氯化钙、氯化铷、磷酸钙、DEAE-葡聚糖或其它物质的转染；微粒轰击；脂转染；和感染（例如，当载体是感染剂如痘苗病毒时）。对载体或多核苷酸引入的选择常常取决于宿主细胞的特性。

本发明也提供包含本文所述任何多核苷酸的宿主细胞。能够过表达异源 DNA 的任何宿主细胞都可以用于分离编码目的抗体、多肽或蛋白质的基因的目的。哺乳动物宿主细胞的非限制性例子包括但不限于 COS、HeLa 和 CHO 细胞。也参见 PCT 公布号 WO 87/04462。适宜的非哺乳动物宿主细胞包括原核细胞（例如大肠杆菌 (*E. coli*) 或枯草芽孢杆菌 (*B. subtilis*)) 和酵母（例如，酿酒酵母 (*S. cerevisiae*)、粟酒裂殖酵母 (*S. pombe*)；或乳酸克鲁维酵母 (*K. lactis*))。优选地，宿主细胞以比宿主细胞中相应的目的内源抗体或蛋白质（如果存在的话）的水平高大约 5 倍、更优选地 10 倍、甚至更优选地 20 倍的水平表达所述 cDNA。通过免疫分析试验或 FACS，基于与 $A\beta_{1-40}$ 的特异结合，筛选宿主细胞。可以鉴定过表达目的抗体或多肽的细胞。

6G 衍生抗体和具有受损的效应子功能的抗 $A\beta$ 抗体的诊断用途

可以使用与 $A\beta$ 的 C 端结合的抗体 6G 鉴定或检测 $A\beta$ 存在与否。为了简便，一般地提及 6G 或抗体，但应理解这些方法适用于本文所述的任何 $A\beta$ 结合实施方案（如多肽）。检测一般涉及本文所述和 $A\beta$ 结合的抗体与生物学样品的接触以及 $A\beta$ 和可以特异地结合 $A\beta$ 的抗体（例如，6G）之间复合物的形成。此复合物可以在体外或体内形成。术语“检测”在本文中包括在参考或不参考对照的情况下的定性和/或定量（测量水平）检测。

多种已知的方法均可以用于检测，包括，但不限于，使用可以结合所述多肽的抗体的免疫分析试验，例如，酶联免疫吸附试验 (ELISA)、放射免疫分析试验 (RIA) 等；和用于所编码的多肽的功能分析试验，例如，结合活性或酶分析试验。一些实施方案中，所述抗体被可检测地标记。其它实施方案是本领域已知的并在本文中描述。

本发明抗体和多肽可以用于检测、诊断和监测与改变的或异常的 A β 或 β APP 表达有关的疾病、状况或病症,例如,阿尔茨海默氏病和 Down 氏综合症。因此,一些实施方案中,本发明提供方法,所述方法包括使被怀疑具有改变的或异常的 A β 表达的个体的样本(样品)与本发明抗体或多肽接触,和确定此 A β 水平是否不同于对照或比较样本的 A β 水平。其它实施方案中,本发明提供方法,所述方法包括接触个体样本(样品)和确定 A β 表达水平。

对于诊断应用,可以用可检测的部分,包括但不限于放射性同位素、荧光标记物和各种酶-底物标记物,来标记抗体。用于将标记物和抗体缀合的方法是本领域已知的。本发明其它实施方案中,本发明抗体无需标记,其存在可以通过使用能够与本发明抗体结合的标记抗体来检测。

本发明抗体可以用于任何已知的分析试验方法,例如,竞争结合试验、直接和间接的三明治分析试验和免疫沉淀分析试验。Zola, Monoclonal Antibodies: A Manual of Techniques, pp.147-158 (CRC Press, Inc. 1987)。

也可以将抗体用于体内诊断试验,例如体内成像。一般地,用放射性核素(例如, ^{111}In , ^{99}Tc , ^{14}C , ^{131}I , ^{125}I , 或 ^3H) 标记抗体以便可以使用免疫闪烁术(immunoscintigraphy)定位目的细胞或组织。

也可以利用本领域熟知的技术,将抗体作为染色剂用于病理学中。

具有受损的效应子功能的抗 A β 抗体可以用于测量脑淀粉样蛋白负担,以诊断有罹患 AD 危险的个体或者已经诊断为患有 AD 的个体,和评价任何治疗的进展和疾病阶段。已经报道,外周施用单克隆抗 A β 抗体导致血浆 A β 快速增加,并且该增加的幅度与海马和皮层中的淀粉样蛋白负担高度相关。DeMattos 等, Science 295: 2264-2267 (2002)。一些实施方案中,将具有受损的效应子功能的抗 A β 抗体施用给个体,并测量血浆中 A β 水平,由此血浆 A β 的增加将指示个体中脑淀粉样蛋白负担的存在和/或水平。这些方法可以用于监测治疗的有效性和疾病阶段以及用于确定未来的给药剂量和频率。具有受损的效应子

功能的抗体可以具有更好的安全谱并为这些诊断应用提供优势。

将抗 A β 抗体用于治疗目的的方法

本文所述抗体(包括多肽)、多核苷酸和药物组合物可以用于治疗、预防和抑制阿尔茨海默氏病及与改变的 A β 或 β APP 表达或 A β 肽的累积或沉积相关的其它疾病(总称为“A β 相关疾病”),例如,Down 氏综合症、帕金森病、多发性脑梗塞痴呆、轻度认知缺损、脑淀粉样血管病、由血管中 A β 肽的沉积引起的血管病症(例如中风和 HCHWA-D)、抑郁、Creutzfeldt-Jakob 病、伴有 Lewy 小体的痴呆等的方法中。该方法包括给个体施用所述抗体、多肽、或多核苷酸、或药物组合物。在预防性应用中,将药物组合物或药物施用给阿尔茨海默氏病的易感患者或有罹患阿尔茨海默氏病(或其它 A β 相关疾病)的危险的患者,施用量为足以消除或降低所述危险、减轻疾病严重程度、或延迟疾病发作的量,其中所述疾病包括疾病的生物化学的、组织学的和/或行为的症状、其并发症和疾病发展过程出现的中间病理学表型。在治疗性应用中,将组合物或药物施用给此类疾病的疑似患者或者已经患有此类疾病的患者,施用量将足以治愈或者至少部分地抑制该疾病的症状(生物化学的、组织学的和/或行为的),包括其并发症和疾病发展过程中存在的中间病理学表型。

本发明还提供在个体中延迟阿尔茨海默氏病(或其它 A β 相关疾病)的有关症状发展的方法,包括给个体施用有效剂量的包含本文所述抗体、多肽或多核苷酸的药物组合物。与阿尔茨海默氏病有关的症状包括但不限于记忆、问题解决、语言、计算、视觉空间感知、判断和行为的异常。

本发明还提供在个体中抑制或减低淀粉样蛋白斑和/或 A β 累积的形成的方法,包括给个体施用有效剂量的包含本文所述抗体、多肽或多核苷酸的药物组合物。一些实施方案中,淀粉样蛋白斑存在于个体的脑中。一些实施方案中,淀粉样蛋白斑存在于个体的脑血管系统中。其它实施方案中,A β 累积存在于个体的循环系统中。

本发明还提供在个体中减少淀粉样蛋白斑和/或减少或减缓 A β 累积的方法, 包括给个体施用有效剂量的包含本文所述抗体、多肽或多核苷酸的药物组合物。一些实施方案中, 淀粉样蛋白斑存在于个体的脑中。一些实施方案中, 淀粉样蛋白斑存在于个体的脑血管系统中。其它实施方案中, A β 累积存在于个体的循环系统中。

本发明还提供在个体中去除或清除淀粉样蛋白斑和/或 A β 累积的方法, 包括给个体施用有效剂量的包含本文所述抗体、多肽或多核苷酸的药物组合物。一些实施方案中, 淀粉样蛋白斑存在于个体的脑中。一些实施方案中, 淀粉样蛋白斑存在于个体的脑血管系统中。其它实施方案中, A β 累积存在于个体的循环系统中。

本发明还提供在个体中减少组织(例如脑)中 A β 肽、抑制和/或降低组织(例如脑)中 A β 肽的累积、和抑制和/或降低组织(例如脑)中 A β 肽的毒性作用的方法, 包括给个体施用有效剂量的包含本文所述抗体、多肽或多核苷酸的药物组合物。A β 多肽可以是可溶性的、寡聚体的或沉积的形式。寡聚体形式的 A β 可以由 2 至 50 个 A β 多肽组成, 其可以是全长 1-40 和 1-42 肽和/或这些肽的任何截短形式的混合物。

本发明还提供在个体中改善认知或逆转与 A β 淀粉样蛋白沉积相关疾病(例如阿尔茨海默氏病)有关的认知降低的方法, 包括给个体施用有效剂量的包含本文所述抗体、多肽或多核苷酸的药物组合物。

此处所述方法(包括预防或治疗)可以通过在一个时间点或多个时间点单次直接注射至单个或多个位点来进行。也可以几乎同时施用于多个位点。施用频率可以在治疗过程中进行确定和调整, 并且以所要达到的期望结果为基础。一些情况下, 本发明抗体(包括多肽)、多核苷酸和药物组合物的持久的连续释放制剂可能是合适的。本领域已知多种制剂和装置可以用于实现持久释放。

患者、对象或个体包括哺乳动物、例如, 人、牛、马、狗、猫、猪和羊动物。对象优选是人, 其可以患有或未患有疾病或者可以目前表现出或不表现出症状。就阿尔茨海默氏病而言, 实际上任何人如果他或她活得够长则都有罹患阿尔茨海默氏病的危险。因此, 本发明方

法可以预防性地施用给一般群体而无需对患者个体的危险性作任何评价。本发明方法对于确实具有已知的阿尔茨海默氏病遗传危险的个体是有用的。此类个体包括有罹患该病的亲属的个体、和通过对遗传或生物化学标志分析被确定存在危险的个体。对于阿尔茨海默氏病，患病危险性的遗传标志包括 APP 基因中的突变，尤其是分别称作 Hardy 和 Swedish 突变的 717 位和 670 及 671 位的突变(见 Hardy (1997) Trends Neurosci. 20: 154-9)。危险性的其它标志是早老素基因 PS1 和 PS2、及 ApoE4 中的突变、AD 家族史、高胆固醇血症或动脉粥样硬化。当前患有阿尔茨海默氏病的个体可以通过特征性痴呆以及上述危险因素的存在而识别。此外，可以获得多种诊断试验用于鉴定患有 AD 的个体。这些试验包括测量 CSF tau 和 A β 42 的水平。升高的 tau 和降低的 A β 42 水平意味着存在 AD。也可以通过 ADRDA (阿尔茨海默氏病及相关病症协会) 的标准，诊断患有阿尔茨海默氏病的个体。在无症状患者中，可以在任何年龄(例如，10、20、30 岁)开始治疗。然而，通常，在患者达到 40、50、60、或 70 岁前无需开始治疗。治疗典型地需要在一段时间内多次给药。可以利用本领域已知的多种方法，随着时间推移对治疗实施监测。在潜在的 Down 氏综合症患者的情况下，治疗可以通过将治疗剂施用给母亲在出生前开始或者在出生后不久开始。

可以用于上述方法的药物组合物包括本文所述任何抗体、多肽和/或多核苷酸。一些实施方案中，抗体是抗体 6G 或其显示在表 3 中的变体。一些实施方案中，抗体是能够特异地结合 A β 肽并包含具有受损的效应子功能的恒定区的抗体。

施用和剂量

抗体优选地在载体，优选地药物可接受载体中施用给哺乳动物。适宜的载体和其配制描述在 Remington's Pharmaceutical Sciences, 18 版, A. Gennaro, 编, Mack Publishing Co., Easton, PA, 1990; 和 Remington, The Science and Practice of Pharmacy 20 版. Mack Publishing, 2000。典型地，在制剂中施用合适量的药物可接受盐以

使所述制剂等渗。该载体的实例包括盐水、Ringer 氏溶液和葡萄糖溶液。该溶液的 pH 优选为大约 5 至大约 8，更优选大约 7 至大约 7.5。其它载体包括持久释放制品，例如，包含抗体的固体疏水性聚合物的半透性基质，该基质是成形的物体的形式，例如，膜、脂质体或微粒。本领域技术人员明了，根据例如施用途径和所施用的抗体浓度，某些载体可能更为优选。

抗体可以通过注射(例如，全身、静脉、腹膜内、皮下、肌内、门静脉内、脑内、脑室内和鼻内)或者通过保证将抗体以有效形式递送至血流的其它方法，例如输注，施用给哺乳动物。也可以通过隔离灌注技术，例如，隔离组织灌注，施用抗体以实行局部治疗作用。优选静脉内注射。

施用抗体的有效剂量和进度表可以通过经验决定，而且如何作出此类决定属于本领域技术的范畴。本领域技术人员明了，取决于例如接受抗体的哺乳动物、施用途径、所用的抗体特定类型和正在施用给所述哺乳动物的其它药物，必须施用的抗体剂量将发生变化。在关于抗体治疗应用的文献中可以找到如何选择适当剂量的抗体的指导，所述文献有例如，Handbook of Monoclonal Antibodies, Ferrone 等编，Noges Publications, Park Ridge, N.J., 1985, ch. 22 and pp. 303-357; Smith 等，Antibodies in Human Diagnosis and Therapy, Haber 等编，Raven Press, New York, 1977, pp. 365-389。单独使用的抗体的典型日剂量，根据上述因素，可以是每天大约 $1\mu\text{g}/\text{kg}$ 体重至不超过 $100\text{mg}/\text{kg}$ 体重或更多。一般地，可以使用以下任何剂量：施用至少大约 $50\text{mg}/\text{kg}$ 体重；至少大约 $10\text{mg}/\text{kg}$ 体重；至少大约 $3\text{mg}/\text{kg}$ 体重；至少大约 $1\text{mg}/\text{kg}$ 体重；至少大约 $750\mu\text{g}/\text{kg}$ 体重；至少大约 $500\mu\text{g}/\text{kg}$ 体重；至少大约 $250\mu\text{g}/\text{kg}$ 体重；至少大约 $100\mu\text{g}/\text{kg}$ 体重；至少大约 $50\mu\text{g}/\text{kg}$ 体重；至少大约 $10\mu\text{g}/\text{kg}$ 体重；至少大约 $1\mu\text{g}/\text{kg}$ 体重；或更大剂量。在治疗开始时可以以较低的剂量或较低频率施用抗体以避免潜在的副作用，例如，暂时性淀粉样脑血管病(CAA)。

一些实施方案中，可以存在一种以上抗体。此类组合物可以含有至少一种、至少两种、至少三种、至少四种、至少五种不同的本发明抗体(包括多肽)。

抗体也可以与有效量的一种或多种其它治疗剂联合施用给哺乳动物。所述抗体与所述一种或多种治疗剂可以相继地或同时地施用。抗体和治疗剂的量取决于例如所用的药物类型、正在治疗的病理学情况、和施用的进度和途径，但是通常该量少于每一种单独使用时的量。

在将抗体施用给哺乳动物后，可以以本领域技术人员熟知的各种方法监测该哺乳动物的生理学状况。

以上的施用和剂量原则可以根据本文所述的多肽进行适应性修改。

编码本文所述抗体或多肽的多核苷酸也可以用于将所述抗体或多肽递送至期望的细胞以及在该细胞中表达。明显地，可以使用表达载体指导抗体表达。表达载体可以全身地、腹膜内、静脉内、肌内、皮下、鞘内、心室内、经口、肠、胃肠外、鼻内、经皮、或通过吸入施用。例如，表达载体的施用包括局部或全身施用，包括注射、口服、基因枪或插导管施用和表面施用。本领域技术人员对于施用表达载体以获得外源蛋白在体内的表达是熟悉的。见例如，美国专利号 6,436,908; 6,413,942; 和 6,376,471。

也可以定向递送包含编码本发明抗体的多核苷酸的治疗性组合物。受体介导的 DNA 递送技术描述在例如，Findeis 等，Trends Biotechnol. (1993) 11:202; Chiou 等，Gene Therapeutics: Methods And Applications Of Direct Gene Transfer (J.A. Wolff, ed.) (1994); Wu 等，J. Biol. Chem. (1988) 263:621; Wu 等，J. Biol. Chem. (1994) 269:542; Zenke 等，Proc. Natl. Acad. Sci. (USA) (1990) 87:3655; Wu 等，J. Biol. Chem. (1991) 266:338。在基因治疗方案中含有多核苷酸的治疗性组合物以大约 100ng 至大约 200mg DNA 局部施用。基因治疗方案过程中也可以使用大约 500 ng 至大约 50 mg，大约 1 μ g 至大约 2 mg，大约 5 μ g 至大约 500 μ g，以及大约

20 μg 至大约 100 μg 的 DNA 的浓度范围。本发明的治疗性多核苷酸和多肽可以使用基因递送载体递送。基因递送载体可以是病毒和非病毒来源的(一般, 见, Jolly, Cancer Gene Therapy (1994) 1:51; Kimura, Human Gene Therapy (1994) 5:845; Connelly, Human Gene Therapy (1995) 1:185; 和 Kaplitt, Nature Genetics (1994) 6:148)。这些编码序列的表达可以使用内源性哺乳动物启动子或异源启动子诱导。编码序列的表达可以是组成型的或是受到调节的。

用于递送期望多核苷酸和在期望细胞中实施表达的基于病毒的载体是本领域熟知的。示例性基于病毒的载体包括, 但不限于, 重组逆转录病毒(见例如, PCT 公布号 WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; WO 93/11230; WO 93/10218; WO 91/02805; 美国专利号 5, 219, 740; 4, 777, 127; GB 专利号 2, 200, 651; 和 EP 0 345 242), 基于甲病毒的载体(例如, 辛德毕斯病毒载体, Semliki 森林病毒(ATCC VR-67; ATCC VR-1247), 罗斯河病毒(Ross river virus)(ATCC VR-373; ATCC VR-1246)和委内瑞拉马脑炎病毒(Venezuelan equine encephalitis virus)(ATCC VR-923; ATCC VR-1250; ATCC VR 1249; ATCC VR-532)), 以及腺相关病毒(AAV)载体(见例如 PCT 公布号 WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 和 WO 95/00655)。也可以如 Curiel, Hum. Gene Ther. (1992) 3:147 中所述施用与杀死的腺病毒连接的 DNA。

也可以使用非病毒递送载体和方法, 包括但不限于, 单独的与杀死的腺病毒连接的或未连接的聚阳离子缩合的 DNA(见例如, Curiel, Hum. Gene Ther. (1992) 3:147); 配体连接的 DNA(见例如 Wu, J. Biol. Chem. (1989) 264:16985); 真核细胞递送载体细胞(见例如, 美国专利号 5, 814, 482; PCT 公布号 WO 95/07994; WO 96/17072; WO 95/30763; 和 WO 97/42338)以及核酸电荷中和(nucleic charge neutralization)或与细胞膜的融合。也可以使用裸 DNA。引入裸 DNA 的方法的例子描述在 PCT 公布号 WO 90/11092 和美国专利号 5, 580, 859 中。可以充当基因递送载体的脂质体描述在美国专利号

5,422,120; PCT 公布号 WO 95/13796; WO 94/23697; WO 91/14445; 和 EP 0 524 968 中。Philip, Mol. Cell Biol. (1994) 14:2411, 和 Woffendin, Proc. Natl. Acad. Sci. (1994) 91:1581 中描述了其它方法。

药盒

本发明还提供含有用于治疗诸如阿尔茨海默氏病或其它 A β 相关疾病(例如 Down 氏综合症、帕金森病、多发性脑梗塞痴呆、轻度认知缺损、脑淀粉样血管病、因 A β 肽在血管中的沉积引起的血管病症(例如中风和 HCHWA-D))等病理情况或者用于检测或纯化 A β 或 β APP 的物质的产品和药盒。所述产品包含带有标签的容器。适宜的容器包括例如瓶、小瓶和试管。所述容器可以利用各种材料例如玻璃或塑料形成。所述容器容纳具有可以有效地治疗病理学情况或检测或纯化 A β 或 β APP 的活性剂的组合物。组合物中的活性剂是抗体,优选地包含对 A β 或 β APP 特异的单克隆抗体。一些实施方案中,活性剂包含抗体 6G 或衍生自抗体 6G 的任何抗体或多肽。一些实施方案中,活性剂包含本文所述具有受损的效应子功能的抗 A β 抗体或多肽。一些实施方案中,所述抗 A β 抗体或多肽包含重链恒定区,其中所述恒定区具有受损的效应子功能。容器上的标签指示所述组合物用于治疗诸如阿尔茨海默氏病等病理情况或者用于检测或纯化 A β 或 β APP,并且还可以指示用于体内或体外应用,例如上述那些应用的用法。

本发明还提供包含本文所述任何抗体(例如 6G)、多肽、多核苷酸的药盒。一些实施方案中,本发明药盒包含上述容器。其它实施方案中,本发明药盒包含上述容器以及含有缓冲液的第二容器。其还可以包括从商业和用户角度看可能期望的其它物质,包括其它缓冲液、稀释剂、滤器、针、注射器以及带有实施本文所述任何方法(例如,用于治疗阿尔茨海默氏病的方法、和用于抑制或减低脑中 A β 肽累积的方法)的说明书的包裹插页。在用于检测或纯化 A β 或 β APP 的药盒中,抗体典型地用可检测标记物,例如,放射性同位素、荧光化合物、生物发光化合物、化学发光化合物、金属螯合剂或酶标记。

一些实施方案中,本发明提供无论是在用作药物和/或用于制备药物的情况下用于本文所述任何方法的组合物(见本文描述)。

提供下列实施例用于举例说明而非限制本发明。

实施例

实施例 1: 抗体 6G 及其变体的结合亲和力测定

A. 一般方法

在该实施例和其它实施例中使用了以下一般方法。

用于克隆表征的表达载体

类似于 Barbas (2001) Phage display: a laboratory manual, Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press pg 2.10. Vector pComb3X) 中所述, 在 IPTG 诱导型 lacZ 启动子控制下表达抗体 Fab 片段, 但改变包括添加和表达下列额外结构域: 人 κ 轻链恒定域和 IgG2a 人免疫球蛋白 CHI 恒定域, Ig γ 2 链 C 区, 蛋白登录号 P01859; 免疫球蛋白 κ 轻链(智人(homosapiens)), 蛋白登录号 C1A09181。

小规模 Fab 制备

按下述在 96 孔板中小规模表达 Fab。从转化了 Fab 文库的大肠杆菌开始, 挑取菌落接种母板(琼脂 LB+氨苄青霉素(50 μ g/ml) + 2%葡萄糖)和工作板(2ml/孔, 96 孔/板, 含有 1.5mL LB+氨苄青霉素(50 μ g/ml) + 2%葡萄糖)。两板均在 30 $^{\circ}$ C 生长 8 至 12 小时。母板 4 $^{\circ}$ C 保存, 来自工作板的细胞以 5000 rpm 沉淀并用 1mL LB+氨苄青霉素(50 μ g/ml) + 1mM IPTG 重悬以诱导 Fab 表达。30 $^{\circ}$ C 5 小时的表达时间后, 离心收获细胞, 然后重悬于 500 μ L 缓冲液 HBS-EP (100mM HEPES 缓冲液 pH 7.4, 150mM NaCl, 0.005% P20)。通过一轮冻(-80 $^{\circ}$ C)融(37 $^{\circ}$ C), 裂解 HBS-EP 重悬的细胞。细胞裂解物以 5000 rpm 离心 30 分钟以分离细胞碎片和含有 Fab 的上清液。然后将上清液注射入 BIAcore 等离子体共振仪以获得每种 Fab 的亲合力信息。从母板营救表达 Fab 的克隆对该 DNA 进行测序和大规模的 Fab 生产和详细表征, 见下述。

大规模 Fab 制备

为了获得详细的动力学参数, 从大培养物表达和纯化 Fab。用来自所选择的表达 Fab 的大肠杆菌克隆的 5ml 过夜培养物, 接种含有 200ml LB+氨苄青霉素($50\mu\text{g/ml}$) + 2%葡萄糖的三角瓶。30℃温育克隆直到获得 1.0 的 $\text{OD}_{550\text{nm}}$, 然后通过将该培养基置换成 200ml LB+氨苄青霉素($50\mu\text{g/ml}$) + 1mM IPTG 进行诱导。30℃ 5h 表达时间后, 离心沉淀细胞, 然后重悬于 10ml PBS (pH 8) 中。通过两轮冻融 (分别于 -80℃ 和 37℃) 循环裂解细胞。将细胞裂解物的上清液加载于 PBS (pH 8) 平衡的 Ni-NTA Superflow 琼脂糖 (Qiagen, Valencia, CA) 柱上, 然后用 5 倍柱体积的 PBS (pH 8) 洗涤。利用 PBS (pH 8) + 300mM 咪唑将各个 Fab 洗脱在不同级分中。汇合含有 Fab 的级分, 并在 PBS 中透析, 然后通过 ELISA 定量之后进行亲和力表征。

全抗体制备

为了表达全抗体, 将重链和轻链可变区克隆在哺乳动物表达载体中, 使用 lipofectamine 将其转染至 HEK293 细胞中以便实现瞬时表达。使用标准方法, 利用蛋白 A 纯化抗体。

载体 pDb. 6G. hFc2a 是含有抗体 6G 重链的表达载体, 其适于瞬时和稳定表达该重链。载体 pDb. 6G. hFc2a 具有相应于以下区域的核苷酸序列: 鼠巨细胞病毒启动子区 (核苷酸 1-612); 合成的内含子 (核苷酸 619-1507); DHFR 编码区 (核苷酸 707-1267); 人生长激素信号肽 (核苷酸 1525-1602); 6G 重链可变区; 含有以下突变的人重链 IgG2a 恒定区: A330P331 至 S330S331 (氨基酸编号参照野生型 IgG2a 序列进行; 见 Eur. J. Immunol. (1999) 29: 2613-2624); SV40 晚期聚腺苷酸化信号; SV40 增强子区域; 噬菌体 f1 区域和 β 内酰胺酶 (AmpR) 编码区。

载体 pEb. 6G. hK 是包含 6G 抗体轻链的表达载体, 其适于瞬时表达该轻链。载体 pEb. 6G. hK 具有相应于以下区域的核苷酸序列: 鼠巨细胞病毒启动子区域 (核苷酸 1-612); 人 EF-1 内含子 (核苷酸 619-1142); 人生长激素信号肽 (核苷酸 1173-1150); 抗体 6G 轻链可变区; 人 κ 链恒定区; SV40 晚期聚腺苷酸化信号; SV40 增强子区域; 噬菌体 f1 区

域和 β 内酰胺酶 (AmpR) 编码区。

BIACore 试验

使用 BIAcore3000™ 表面等离子体共振 (SPR) 系统 (BIAcore, INC, Piscaway NJ), 测定 6G 单克隆抗体的亲和力。测定亲和力的一个方法是将 6G 固定在 CM5 芯片上并测量 $A\beta_{1-40}$ 肽或其它 $A\beta$ 肽与该抗体的结合动力学。CM5 芯片用 N-乙基-N'-(3-二甲基氨基丙基)-碳二亚胺盐酸盐 (EDC) 和 N-羟基琥珀酰亚胺 (NHS) 根据供应商的说明书活化。将抗体 6G 或其变体稀释在 10mM 乙酸钠 pH 4.0 或 pH 5.0 中, 并以 0.005mg/mL 的浓度注射在活化的芯片上。使用不同的流动时间通过各芯片通道, 获得一系列抗体密度: 1000-2000 或 2000-3000 响应单位 (RU)。用乙醇胺封闭芯片。再生研究显示, 含有 2 体积 PIERCE 洗脱缓冲液和 1 体积 4M NaCl 的溶液有效地除去结合的 $A\beta$ 肽同时保持芯片上 6G 的活性长达 200 次以上注射。HBS-EP 缓冲液 (0.01M HEPES, pH 7.4, 0.15 NaCl, 3 mM EDTA, 0.005% 表面活性剂 P20) 用作所有 BIAcore 试验的运行缓冲液。将纯化的 $A\beta_{1-40}$ 合成肽或其它 $A\beta$ 肽样品的系列稀释物 ($0.1-10 \times$ 估计的 K_D) 以 100 μ L/min 注射 1 分钟, 允许 10 分钟的解离时间。通过用 BIA 评价程序以该数据模拟 1:1 Langmuir 结合模型 (Karlsson, R. Roos, H. Fagerstam, L. Petersson, B. (1994). Methods Enzymology 6. 99-110), 同时获得动力学结合速率 (k_{on}) 和解离速率 (k_{off})。以 k_{on}/k_{off} 计算平衡解离常数 (K_D) 值。

备选地, 通过将 $A\beta_{1-40}$ 肽或其它 $A\beta$ 肽固定在 SA 芯片上并测量 6G 的 Fab 和 6G 变体的 Fab 与固定的 $A\beta$ 肽的结合动力学, 由此确定亲和力。6G Fab 片段及其变体的 Fab 片段的亲和力通过表面等离子体共振 (SPR) 系统 (BIAcore 3000™, BIAcore, Inc., Piscaway NJ) 确定。SA 芯片 (链霉亲和素) 根据供应商说明书使用。将生物化的 $A\beta$ 肽稀释在 HBS-EP (100mM HEPES, pH 7.4, 150mM NaCl, 3 mM EDTA, 0.005% P20) 中并以 0.005mg/mL 浓度注射在芯片上。使用不同的流动时间通过各芯片通道, 获得两组抗原密度: 100-400 响应单位 (RU) 用于详细的动力学研究和 500-2000 RU 用于浓度研究和筛选。再生研究显示, 100mM

磷酸(也可以尾随含有 2 体积 50mM NaOH 和 1 体积 70% 乙醇的溶液)有效地除去结合的 Fab 同时保持芯片上 A β 肽的活性长达 200 次以上注射。HBS-EP 缓冲液用作所有 BIAcore 试验的运行缓冲液。纯化的 Fab 样品的系列稀释物(0.1-10 $\times$ 估计的 KD)以 100 μ L/min 注射 2 分钟,允许 10 分钟的解离时间。通过 ELISA 和/或 SDS-PAGE 电泳,使用已知浓度(通过氨基酸分析确定)的标准 Fab,确定所述 Fab 蛋白的浓度。通过用 BIA 评价程序通过以数据模拟 1:1 Langmuir 结合模型(Karlsson, R. Roos, H. Fagerstam, L. Petersson, B. (1994). Methods Enzymology 6. 99-110),同时获得动力学结合速率(k_{on})和解离速率(k_{off})。以 k_{on}/k_{off} 计算平衡解离常数(K_D)值。

ELISA 试验

使用 ELISA 测量抗体 6G 和变体与未生物素化的 A β 肽的结合。NUNC maxisorp 板用 PBS pH 7.4 中的 2.5 μ g/ml A β 肽 4 $^{\circ}$ C 包被 1 小时以上。用 PBS 缓冲液 pH 7.4 中的 1%BSA 封闭板子。一抗(来自细胞上清液,含有抗 A β 抗体的血清、或期望稀释度的纯化的全抗体或 Fab)与固定的 A β 肽室温孵育 1 小时。洗涤后,板子与 1:5000 稀释的二抗(HRP 缀合的山羊抗人 κ 链抗体,MP Biomedicals, 55233)一起孵育。洗涤后,通过添加 TMB 底物(KPL, 50-76-02, 50-65-02)测量结合的二抗。通过添加 1M 磷酸终止 HRP 反应并测量 450nm 的吸光度。

使用 ELISA 测量抗体 6G 和变体与生物素化的 A β 肽的结合。NUNC maxisorp 板用 6 μ g/ml 链霉亲和素(Pierce, 21122)在 PBS pH 7.4 中 4 $^{\circ}$ C 包被 1 小时以上。用 1%BSA 在 PBS 缓冲液 pH7.4 中封闭板子。洗涤后,在 PBS pH 7.4 中室温温育生物素化的 A β 肽 1 小时。一抗(来自细胞上清液、含有抗 A β 抗体的血清、或期望稀释度的纯化的全抗体或 Fab)与固定的 A β 肽一起室温温育小时。洗涤后,板子与 1:5000 稀释的二抗(HRP 缀合的山羊抗人 κ 链抗体,MP Biomedicals, 55233)一起孵育。洗涤后,通过添加 TMB 底物(KPL, 50-76-02, 50-65-02)测量结合的二抗。通过添加 1M 磷酸终止 HRP 反应并测量 450nm 的吸光度。

B. 抗体 6G 和变体与 $A\beta_{1-40}$ 、 $A\beta_{1-42}$ 和其它 $A\beta$ 肽的结合亲和力

图 1 显示抗体 6G 的重链和轻链可变区的氨基酸序列。使用以上描述的 Biacore 测定的 6G 抗体与 $A\beta_{1-40}$ 、 $A\beta_{1-42}$ 和 $A\beta_{22-37}$ 的结合亲和力显示在下表 2 中。

表 2. 抗体 6G Fab 片段的结合亲和力

	k_{on} (1/Ms)	k_{off} (1/s)	K_D (nM)
生物素化的 $A\beta_{1-40}$ 固定在链霉亲和素芯片上, 6G Fab 在其上流过	3.0×10^5	7.0×10^{-4}	2
生物素化的 $A\beta_{1-42}$ 固定在链霉亲和素芯片上, 6G Fab 在其上流过	1.8×10^4	1.6×10^{-3}	80
生物素化的 $A\beta_{22-37}$ 固定在链霉亲和素芯片上, 6G Fab 在其上流过	3.6×10^5	3.9×10^{-3}	11

6G 变体的氨基酸序列显示在下表 3 中。表 3 中所示变体的所有氨基酸替代是相对于 6G 序列进行描述的。6G 变体的相对结合也显示在表 3 中。按照上述 ELISA 使用固定在 ELISA 板表面上的未生物素化的 $A\beta_{1-40}$ 或 $A\beta_{1-42}$ 测定结合。

表 3. 抗体 6G 变体的氨基酸序列和结合数据

通过 ELISA 获得的 6G 重链突变变体的结合数据							A450 ELISA	
克隆编号	突变						$A\beta_{1-40}$	$A\beta_{1-42}$
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

通过 ELISA 获得的 6G 轻链突变变体的结合数据										A450 ELISA	
克隆编号	突变									$A\beta_{1-40}$	$A\beta_{1-42}$
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

实施例 2. 表征 A β 肽上与抗体 6G 结合的表位

为了确定 A β 肽上抗体 6G 所识别的表位, 使用 ELISA 结合分析。将各种 A β 肽 (Global Peptide Services, CO) 固定在 ELISA 板上。通过上述 ELISA 测定 6G 全抗体 (20nM) 与固定的 A β 的结合。下表 5 显示 A β_{1-40} 、A β_{1-42} 和 A β_{1-43} 的氨基酸序列。如图 2 所示, 抗体 6G 与 A β 肽 17-40、17-42、22-35、28-40、1-38、1-40、1-42、1-43 和 28-42 结合; 但与 28-42 的结合比其它 A β 肽弱得多。抗体 6G 不结合 A β 肽 1-16、1-28 和 33-40。因此, 抗体 6G 与各种截短的 A β 肽, 例如, 22-35、1-38、1-40、1-42 和 1-43 的 C 端结合。

下表 4 显示使用 Biacore 试验通过 k_{off} (1/s) 测定的 A β_{1-40} 与其它 A β 肽和 6G 的结合亲和力的比较。与其它肽相比, 抗体 6G 与 A β_{1-40} 具有最高的结合亲和力, 与截短的 A β_{1-40} (例如, 1-36、1-37、1-38 和 1-39)、A β_{1-42} 和 A β_{1-43} 具有显著较低的亲和力。这说明, A β 的氨基酸 40 (缬氨酸) 的侧链或骨架参与 6G 和 A β_{1-40} 的结合; 而且, 在缺少该氨基酸的情况下结合显著地降低 (例如, 大约 10 至大约 50-250 倍的亲和力降低)。与羧基末端酰胺化的 A β_{1-40} 以较低的亲和力结合, 这说明 6G 与 A β_{1-40} 的结合涉及但不依赖于 A β_{1-40} 的游离 C 端。与 A β_{1-42} 和 A β_{1-43} 的较低亲和力结合可能是由于 A β_{1-40} 与 A β_{1-42} 或 A β_{1-43} 的单体形式之间的构象差异所致的。已经证实, 溶液中 A β_{1-42} 单体具有与 A β_{1-40} 单体不同的构象。见, 蛋白质数据库 Protein Data Bank (pbd 档案) 中登录号 1IYT 下所示 A β_{1-42} 的单体结构坐标; 和蛋白质数据库 (pbd 档案) 中登录号 1BA6 和 1BA4 下所示 A β_{1-40} 的单体结构坐标。

表 4

A β 肽片段	k_{off} (1/s)	K_{off} A β 肽/ k_{off} A β ₁₋₄₀ (亲和力降低的倍数)
1-28	-	
1-43	非常低的结合	
22-35	0.0285	215.9
1-36	0.0205	155.3
1-37	0.0149	112.8
1-38	9.3×10^{-3}	70.4
1-39	7.92×10^{-3}	60.0
17-42	0.0465	352.2
1-42	1.9×10^{-3}	14.4
28-42	3.37×10^{-3}	25.5
28-40-NH2#	3.62×10^{-3}	27.4
28-40	6.4×10^{-4}	4.8
17-40	2.15×10^{-4}	1.6
1-40	1.32×10^{-4}	1

肽作为被分析物在具有通过胺化学固定的 6G 单克隆抗体(配体)的 CM5 芯片上流动

#羧基端酰胺化的肽

通过 ELISA 试验进行抗体 6G 的表位作图。将生物素化的 15-mer 或 10-mer 各种 A β 肽(这些肽在 C 末端添加了甘氨酸)固定在链霉亲和素包被的板子上。抗体 6G (2.5 μ g/ml 至 10 μ g/ml) 与固定的肽一起孵育并如上所述测定结合。如图 3 所示, 抗体 6G 与具有氨基酸 20-34、21-35、22-36、23-37、24-38、25-39 和 25-34 且 C 端具有甘氨酸的 A β 肽结合; 但不与具有氨基酸 19-33、26-40、27-41、24-33 和 26-35 且在肽 C 端具有甘氨酸的 A β 肽结合。这提示, 抗体 6G 的表位包括从 25 至 34 位的氨基酸。

基于以上所示数据, 抗体 6G 所结合的表位看来包括氨基酸 25-34 和 40。图 4 是显示抗体 6G 的表位的示意图。

表 5. β 淀粉样肽的氨基酸序列

1-40 (WT)	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV (SEQ ID NO:15)
1-42 (WT)	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA (SEQ ID NO:16)
1-43 (WT)	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIAT (SEQ ID NO:17)

B. 抗体 6G 不结合 APP

为了确定 6G 是否结合淀粉样前体蛋白 (APP)，测定 6G 与转染了野生型 APP 的细胞的结合。用编码野生型人淀粉样前体蛋白的 cDNA 转染 HEK293 细胞。转染后 48 小时，细胞在冰上与单克隆抗体抗 $A\beta_{1-16}$ (m2324) 或 6G ($5\mu\text{g/ml}$ ，在具有 10% FCS 的 DMEM 中) 一起孵育 45 分钟。然后在 PBS 中洗涤细胞 3 次 5 分钟，用 4% PFA 固定。再次在 PBS 中洗涤细胞 3 次，用来自 Jackson ImmunoResearch 的二抗 Cy3 缀合的山羊抗小鼠抗体 (1:500 稀释) 在荧光显微镜下检测抗体的结合。

如图 5 所示，识别 $A\beta$ N 端表位的抗 $A\beta_{1-16}$ 抗体显示出与细胞上表达的 APP 前体蛋白有显著的结合。相反，6G 不结合 APP 表达细胞。

实施例 3. 表征 $A\beta$ 肽上抗体 2294 所结合的表位

抗体 2294 是通过以 $A\beta_{1-40}$ 免疫小鼠产生的鼠源抗体。该抗体描述在 US 2004/0146512 和 WO 04/032868 中。

抗体 2294 与 $A\beta_{1-40}$ 、 $A\beta_{1-42}$ 或 $A\beta_{22-37}$ 的结合亲和力使用上述 Biacore 进行测量。下表 6 显示抗体 2294 Fab 片段与各种 $A\beta$ 肽的亲和力。

表 6. 抗体 2294 Fab 片段的结合亲和力

	k_{on} (1/Ms)	k_{off} (1/s)	K_D (nM)
生物素化的 $A\beta_{1-40}$ 固定在链霉亲和素芯片上，2294 Fab 在其上流过	6.6×10^4	3.95×10^{-4}	6
生物素化的 $A\beta_{1-42}$ 固定在链霉亲和素芯片上，2294 Fab 在其上流过	1.1×10^4	4.87×10^{-3}	400
生物素化的 $A\beta_{22-37}$ 固定在链霉亲和素芯片上，2294 Fab 在其上流过	5×10^3	0.049	10,000

通过 ELISA 试验对抗体 2294 进行表位作图。将生物素化的 15-mer 或 10-mer 各种 A β 肽(这些肽在 C 末端添加了甘氨酸)固定在链霉亲和素包被的板子上。用 6 μ g/ml 链霉亲和素(Pierce, 21122)在 PBS pH7.4 中 4 $^{\circ}$ C 包被 NUNC maxisorp 板 1 小时以上。用 1%BSA 在 PBS 缓冲液 pH 7.4 中封闭板子。洗涤后,在 PBS pH 7.4 中室温温育生物素化的 A β 肽 1 小时。抗体 2294 (2.5 μ g/ml 至 10 μ g/ml)与固定的 A β 肽一起室温温育 1 小时。洗涤后,板子与 1:5000 稀释的二抗(HRP 缀合的山羊抗人 κ 链抗体, MP Biomedicals, 55233)一起孵育。洗涤后,通过添加 TMB 底物(KPL, 50-76-02, 50-65-02)测量结合的二抗。通过添加 1M 磷酸终止 HRP 反应并测量 450nm 的吸光度。如图 6 所示,抗体 2294 与具有氨基酸 20-34、21-35、22-36、23-37、24-38、25-39、26-40 和 25-34 且 C 端具有甘氨酸的 A β 肽结合;但不与具有氨基酸 19-33、27-41、24-33 和 27-35 且在肽 C 端具有甘氨酸的 A β 肽结合。这提示,抗体 2294 的表位包括从 26 至 34 位的氨基酸。

为了进一步确定 A β 肽上抗体 2294 所识别的表位,使用 ELISA 结合分析。将各种 A β 肽(Global Peptide Services, CO)固定在 ELISA 板上。2294 全抗体 (20nM)与固定的 A β 的结合通过上述 ELISA 进行测定。抗体 2294 与 A β 肽 17-40、17-42、28-40、1-38、1-40、1-42 和 1-43 结合。抗体 2294 不结合 A β 肽 1-16、1-28、28-42、22-35 和 33-40。因此,抗体 2294 与各种截短的 A β 肽,例如,1-38、1-40、1-42 和 1-43 的 C 端结合。

下表 7 显示通过 Biacore 试验测定的 A β ₁₋₄₀ 与其它 A β 肽和 2294 结合的比较。与其它肽相比,抗体 2294(全抗体)与 A β ₁₋₄₀ 具有最强的结合,与截短的 A β ₁₋₄₀ (例如,1-36、1-37、1-38 和 1-39)、A β ₁₋₄₂ 和 A β ₁₋₄₃ 具有显著较低的结合。这说明,A β 的氨基酸 40(缬氨酸)的侧链或骨架参与 2294 和 A β ₁₋₄₀ 的结合;而且,在缺乏该氨基酸的情况下结合显著地降低。

表 7

β 肽片段	结合
1-28	-
1-43	-
22-35	-
1-36	+
1-37	+
1-38	++
1-39	++
17-42	+++
1-42	+++
17-40	++++
1-40	++++

“-”表示无结合；“+”表示非常低的结合；“++”表示中等程度的结合；“+++”表示强结合；以及“++++”表示非常强的结合。

基于以上数据，抗体 2294 所结合的表位看来包括氨基酸 26-34 和 40。如图 6 中所示，抗体 2294 所结合的表位与抗体 6G 所结合的表位非常相似。然而，抗体 6G 的结合比抗体 2294 较少地依赖于氨基酸 40。

使用 Biacore 试验在 2294、6G、2H6 和 2289 之间进行抗体结合竞争实验。抗体 2H6 是与 $A\beta_{33-40}$ 结合的抗体，其描述在 2005 年 2 月 14 日提交的美国临时专利申请 60/653,197 中。抗体 2289 是与 $A\beta_{16-28}$ 结合的抗体，描述在美国公开号 2004/0146512 和 PCT WO 04/032868 中。使用 Biacore 试验进行竞争实验。抗体 2294、6G、2H6 和 2289 固定在 CM5 芯片的不同通道内。CM5 芯片通道用 N-乙基-N'-(3-二甲基氨基丙基)-碳二亚胺盐酸盐 (EDC) 和 N-羟基琥珀酰亚胺 (NHS) 根据供应商的说明书活化。抗体 2294、6G、2H6 和 2289 分别稀释在 10mM 乙酸钠 pH 4.0 中，并以 0.005mg/mL 的浓度注射在活化的芯片上。用乙醇胺封闭各通道。 $A\beta_{1-40}$ 肽 (150 μ M) 在芯片上流动 2 分钟。然后，0.6 μ M 的抗体 2294 (待检测对结合的竞争作用) 在芯片上流动 1 分钟。HBS-EP 缓冲液 (0.01M HEPES, pH 7.4, 0.15 NaCl, 3 mM EDTA, 0.005% 表面活性剂 P20) 用作所有这些 BIACore 试验的运

行缓冲液。在测量 $A\beta_{1-40}$ 的结合后,芯片上所有的通道通过用 Pierce 洗脱缓冲液(产品号 21004, Pierce Biotechnology, Rockford, IL)和 4M NaCl (2:1)的混合物洗涤 2 次 6 秒以再生。然后对抗体 6G、2H6、以及之后对抗体 2289 实施竞争结合。观察到 2294 和 6G 之间以及 2294 和 2H6 之间存在对 $A\beta_{1-40}$ 的竞争结合,但是在 2294 和 2289 之间以及 6G 和 2289 之间未观察到竞争。通过对固定的抗体和在芯片上流动的相同抗体之间的竞争的观察,作为阳性对照。数据说明,抗体 2294 与 2H6 及 6G 竞争地与 $A\beta_{1-40}$ 结合。

实施例 4. 抗体 2294 Fc 区与鼠 Fc γ 受体的结合亲和力

使用上述 BIAcore 试验测量该抗体 Fc 区与 Fc γ 受体的结合亲和力。简要地,通过胺化学将纯化的鼠 Fc γ 受体(来自 R&D Systems)固定在 BIAcore CM5 芯片上。注射单克隆抗体的系列稀释物(从 2nM 至表 8 中所示最大浓度)。HBS-EP (0.01M HEPES, pH 7.4, 0.15M NaCl, 3mM EDTA, 0.005%表面活性剂 P20)用作运行缓冲液和样品缓冲液。使用用于高亲和力相互作用的 1:1 Langmuir 相互作用模型或者用于低亲和力相互作用的稳态亲和力模型,分析结合数据。

下表 8 显示通过 K_D (nM)测量的抗体 2294 对鼠 Fc γ RI、Fc γ RIIb 和 Fc γ RIII 的结合亲和力。脱糖基化的抗体具有除去了 N-糖基化的恒定区。如表 8 中所示,脱糖基化的 2294 与未除去 N-糖基化的相应抗体相比,对所有被测的鼠 Fc γ 受体具有降低的亲和力。

表 8. 通过 K_D (nM)测量的抗体对鼠 Fc γ 受体的结合亲和力

抗体	Fc γ RI	Fc γ RIIb	Fc γ RIII	同种型	测定的用于结合 Fc γ 受体的最大抗体浓度 (nM)
2294	1,200	13,000	19,000	鼠 IgG2b	18,000
脱糖基化的 2294	8,600	NB	NB	脱糖基化的鼠 IgG2b	22,000

NB: 当以测定的最大浓度使用抗体时没有显著的结合。

实施例 5. 抗体 2294 和脱糖基化的抗体 2294 在阿尔茨海默氏病

动物模型中对减少 A β 沉积和认知的作用

通过将纯化的抗体 2294 在 37℃ 下与肽-N-糖苷酶 F (Prozyme, 每 mg 抗体 0.05U) 在 20mM Tris-HCl pH 8.0 中温育 7 天, 制备脱糖基化的抗体 2294。通过 MALDI-TOF-MS 和蛋白质凝胶电泳, 验证脱糖基化的完全度。利用蛋白 A 层析纯化脱糖基化的抗体, 并通过 Q-Sepharose 除去内毒素。使用上述 BIAcore 试验, 测定脱糖基化的 2294 与 A β ₁₋₄₀ 的结合亲和力, 发现脱糖基化的 2294 对 A β ₁₋₄₀ 的结合亲和力和完整抗体 2294 的相同。

在转基因小鼠 APP Tg2576 中检查抗体 2294 和脱糖基化的 2294 对逆转认知缺陷、组织学症状及微出血 (microhemorrhage) 的作用。按下述进行抗体的施用、组织学和行为分析。

抗体施用。过表达 “Swedish” 突变淀粉样前体蛋白的转基因小鼠 (具有 K670N/M671 的 APP Tg2576; Hsiao et al., Science 274: 99-102 (1996)) 用于该实验。这些小鼠中出现的阿尔茨海默氏病样表型已经得以充分的表征。Holcomb 等, Nat. Med. 4: 97-100 (1998); Holcomb 等, Behav. Gen. 29: 177-185 (1999); 和 McGowan E, Neurobiol. Dis. 6: 231-244 (1999)。对于该 16 周的处理研究, 将 20 月龄的 APP 转基因小鼠分为 4 组。第一组每周接受腹膜内抗 A β 抗体 2294 注射, 持续 16 周 (n=4)。第二组每周接受腹膜内脱糖基化抗 A β 抗体 2294 注射, 持续 16 周 (n=5)。第三组每周接受腹膜内抗 AMN 抗体 (2906; 小鼠单克隆抗果蝇 (*Drosophila*) amnesiac 蛋白 IgG1) 注射, 持续 16 周 (n=6)。非转基因的同窝出生仔畜用抗 AMN 抗体 (n=4) 或 2294 (n=2) 处理 16 周。

行为分析。抗体处理 16 周后, 按以前的描述使来自该研究的小鼠接受两天放射状臂形水迷宫训练。Wilcock 等, J. Neuroinflammation 1: 24 (2004)。该装置为早有描述的 6 臂迷宫。Gordon 等, Neurobiol. Aging 22: 377-385 (2001)。第 1 天, 15 次试验分三批各 5 次试验进行。对于每一批一组 4 只小鼠相继地进行试验 (即, 4 只小鼠中的每一只都进行试验 1, 然后相同的小鼠进行试验

2, 等等)。每个 5 次试验批次之后, 试验第二组小鼠, 使得在小鼠进行第二个批次 5 次试验之前有延长的休息期。目标臂对于一组中的每一只小鼠都是不同的以使气味线索最小化。起始臂对于每次试验是变化的, 而目标臂对于给定的个体两天保持恒定。对于前 11 次试验, 平台交替地可见然后隐藏(对于最后 4 次试验, 平台隐藏)。在第 2 天, 除了平台在所有的试验中都隐藏外, 按照以第 1 天完全相同的方式训练小鼠。在 1 分钟的时间框中测量错误(不正确的臂进入)次数。在 20 秒内未能对臂作出选择的小鼠被定为一次错误, 但是在本研究中没有小鼠以此方式不得被定为错误。由于本研究中小鼠的数量, 试验者不会知道每只小鼠的处理组身份。由于放射状臂形水迷宫任务中的依赖性测量是定量的而非估计性的, 故降低了试验者偏见的可能性。为了使各单次试验的变异的影响最小化, 将 3 次连续试验中每只小鼠的错误取平均值, 每天得到 5 个数据点, 使用 StatView (SAS Institute Inc., NC) 通过 ANOVA 统计学分析这些数据点。

组织学分析。在处死当天, 将小鼠称重, 以 100mg/kg 过量给药 Nembutal (Abbott 实验室, North Chicago, IL), 然后用 25ml 0.9% 氯化钠进行心脏内灌流。快速取出脑, 将左半脑在 100mM KPO_4 (pH 7.2) 中的新鲜制备的 4% 多聚甲醛中浸没固定 24 小时用于组织病理学。为了获得超低温保护, 然后将该半脑相继地在 10%、20% 和 30% 蔗糖中孵育 24 小时。使用滑动切片机收集 25 μ 厚的水平切片, 4℃ 储存在具有叠氮化钠 (pH 7.2) 的 Dulbecco 氏磷酸盐缓冲盐水中以防止微生物生长。随机挑选一系列相隔 600 μ 的 8 个等间隔组织切片跨整个脑并按照早有的描述使用自由漂浮 (free-floating) 免疫组织化学染色总 A β (兔多克隆抗总 A β ; Biosource, Camarillo, CA, 1:10,000)。Gordon 等, *Exp. Neurol.* 173:183-195 (2002); Wilcock 等, *J. Neurosci.* 24:6144-6151 (2004)。使用 0.2% Congo 红在 NaCl 饱和的 80% 乙醇中染色相隔 600 μ m 的第二个系列的组织切片。此外, 还封固另一组切片, 并使用 2% 亚铁氰化钾在 2% 盐酸中染色血铁黄素 15 分钟, 之后在 1% 中性红溶液中复染 10 分钟。使用 Image-Pro

Plus (Media Cybernetics, Silver Spring, MD) 定量 Congo 红染色和 A β 免疫组织化学, 以分析阳性染色所占的面积百分数。分析额皮质的一个区域和海马的三个区域 (以确保在海马值中不存在区域偏差)。最初分析 Congo 红给出总值。在手动编辑删除全部实质淀粉样沉淀后进行第二次分析以得到局限于血管 Congo 红染色的面积百分数。为了估计 Congo 红的实质面积, 从总百分数中扣除血管淀粉样变的值。对于血铁黄素染色, 在所有切片上计数普鲁士兰阳性位点的数目, 并计算每切片的位点平均数目。在低放大倍率下在切片上定性地观察动物之间的差异。检查 8 个等间隔的切片, 确定阳性谱的数目, 并平均为每切片的值。为了评估可能的处理相关差异, 通过单因素 ANOVA 以及之后通过 Fisher 氏 LSD 平均数比较, 分析每个处理组的值。

使用 ELISA 测量血清 A β 肽水平。在最后一次抗体给药后 1 天收集血清, 将其稀释, 并在用 5 μ g/ml 抗体 6E10 (与 A β ₁₋₁₇ 结合的抗 β 淀粉样蛋白抗体; Signet, Dedham, MA) 在 PBS 缓冲液 pH 7.4 中预先包被的 96 孔微滴定板 (MaxiSorp; Nunc, Roskilde, Denmark) 中温育。二抗为 1:5000 稀释的生物素化的 4G8 (与 A β ₁₇₋₂₄ 结合的抗 β 淀粉样蛋白抗体; Signet)。使用链霉亲和素-辣根过氧化物酶缀合物 (Amersham Biosciences), 之后用 TMB 底物 (KPL, Gaithersburg, MD) 进行检测。使用从 6 至 400pM 按比例增加的 A β ₁₋₄₀ (American Peptide) 制作标准曲线。

应当理解, 本文描述的实施例和实施方案仅用于举例说明的目的, 在其基础上的各种修饰或改变是本领域技术人员可以想到的, 并将包括在本申请的精神和范围内。为了所有的目的特此将本文引用的所有出版物、专利和专利申请完整地并入作为参考, 这等同于专门单独地指出每一份单独的出版物、专利或专利申请如此并入作为参考一样。

生物材料保藏

以下材料保藏在美国典型培养物保藏中心, 10801 University Boulevard, Manassas, Virginia 20110-2209, USA (ATCC):

材料	抗体编号	ATCC 保藏号	保藏日
pDb. 6G. hFc2a	6G 重链	PTA-6786	2005 年 6 月 15 日
pEb. 6G. hK	6G 轻链	PTA-6787	2005 年 6 月 15 日

载体 pEb. 6G. hK 是编码 6G 轻链可变区和轻链 κ 恒定区的多核苷酸; 载体 pDb. 6G. hFc2a 是编码 6G 重链可变区和含有突变 A330P331 至 S330S331 (氨基酸编号以 Kabat 编号为基础, 参考野生型 IgG2a 序列进行; 见 Eur. J. Immunol. (1999) 29: 2613-2624) 的重链 IgG2a 恒定区的多核苷酸。

这些保藏是根据国际承认用于专利程序的微生物保藏布达佩斯条约和其实施细则(布达佩斯条约)的规定进行的。这保证该保藏物的活培养物自该保藏日起维持 30 年。该保藏物可以根据布达佩斯条约通过 ATCC 获得, 并且其服从 Rinat Neuroscience 公司和 ATCC 之间的协议, 这就保证在相关美国专利颁发后或者在任何美国或外国专利申请公布给公众后(以先到者为准), 公众可以持久而无限制地获得该保藏物的培养物后代, 并且保证被美国专利商标局长根据 35 USC Section 122 和据此的局长令(包括 37 CFR Section 1.14 并特别参考 886 OG 638)确定为有资格的人可以获得该后代。

本发明申请的受让人同意如果保藏材料的培养物在适宜条件下培养时死亡或丢失或损坏, 在接到通知后将立即以相同的另一份材料替换该保藏材料。该保藏材料的可获得性并不能理解为违反任何政府权利机构根据其专利法授予的权利而许可实施本发明。

抗体序列

6G 重链可变区氨基酸序列 (SEQ ID NO: 1)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYAIHWVRQ
APGQGLEWMGFTSPYSGVSNYNQKFKGRVTMTRDTSTST
VYMELSSLRSEDVAVYYCARFDNYDRGYVRDYWGQGTLV
TVS

6G 轻链可变区氨基酸序列 (SEQ ID NO: 2)

DIVMTQSPDSLAVSLGERATINCRASESDNDNRISFLNW
YQQKPGQPPKLLIYAATKQGTGVPDRFSGSGSGTDFTLT
ISLQAEDVAVYYCQQSKEFPWSFGGGTKVEIKRTV

6G CDR H1 (扩展 CDR) (SEQ ID NO: 3)

GYTFTTYAIH

6G CDR H2 (扩展 CDR) (SEQ ID NO: 4)

FTSPYSGVSNYNQKFKG

6G CDR H3 (扩展 CDR) (SEQ ID NO: 5)

FDNYDRGYVRDY

6G CDR L1 (扩展 CDR) (SEQ ID NO: 6)

RASESDNDNRISFLN

6G CDR L2 (扩展 CDR) (SEQ ID NO: 7)

AATKQGT

6G CDR L3 (扩展 CDR) (SEQ ID NO: 8)

QQSKEFPWS

6G 重链可变区核苷酸序列 (SEQ ID NO: 9)

CAGGTGCAACTGGTGCAATCCGGTGCCGAGGTGAAAAAGCCAGGCGCCTCCGTGA
AAGTGTCCTGCAAAGCCTCCGGTTACACCTTTACCACCTATGCCATCCATTGGGTG
CGCCAGGCCCCAGGCCAGGGTCTGGAGTGGATGGGCTTTACTTCCCCCTACTCCG
GGGTGTCGAATTACAATCAGAAAGTTCAAAGGCCGCGTCACCATGACCCGCGACAC

CTCCACCTCCACAGTGTATATGGAGCTGTCCTCTCTGCGCTCCGAAGACACCGCCG
TGTATTACTGTGCCCCTTCGACAATTACGATCGCGGCTATGTGCGTGACTATTGG
GGCCAGGGCACCCTGGTCACCGTCTCC

6G 轻链可变区核苷酸序列 (SEQ ID NO: 10)

GACATCGTGATGACCCAGTCCCCAGACTCCCTGGCCGTGTCCCTGGGCGAGCGCG
CCACCATCAACTGCCGCGCCAGCGAATCCGTGGATAACGATCGTATTTCTTTCT
GAACTGGTACCAGCAGAAACCAGGCCAGCCTCCTAAGCTGCTCATTACGCCGC
CACCAAACAGGGTACCGGCGTGCCTGACCGCTTCTCCGGCAGCGGTTCCGGCAC
CGATTTCACTCTGACCATCTCCTCCCTGCAGGCCGAAGATGTGGCAGTGTATTAC
TGTCAGCAGTCCAAAGAGTTTCCCTGGTCCTTTGGCGGTGGCACCAAGGTGGAGA
TCAAACGCACTGTG

6G 重链全抗体氨基酸序列 (包括本文中描述的经修饰的 IgG2a) (SEQ ID NO: 11)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYAIHWVRQAPGQGLEWMGFTSPYSG
VSNYNQKFKGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARFDNYDRGYVRDYW
GQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVTVPSNFGTQTYTCNVDHKPSNTKVDKTVRKCCEVE
CPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTFRVVSFLTIVVHQDWLNGKEYKCKVSNKGLPSSIEKTISKTKG
QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPML
DSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSLSPGK

6G 轻链全抗体氨基酸序列 (SEQ ID NO: 12)

DIVMTQSPDSLAVSEGERATINCRASESDNDNRISFLNWFYQQKPGQPPKLLIYAATK
QGTGVPDRFSGSGSGTDFTLTISLQAEDVAVYYCQQSKEFPWSFGGGTKVEIKRTV
AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

6G 重链全抗体核苷酸序列 (包括本文中描述的经修饰的 IgG2a) (SEQ ID NO: 13)

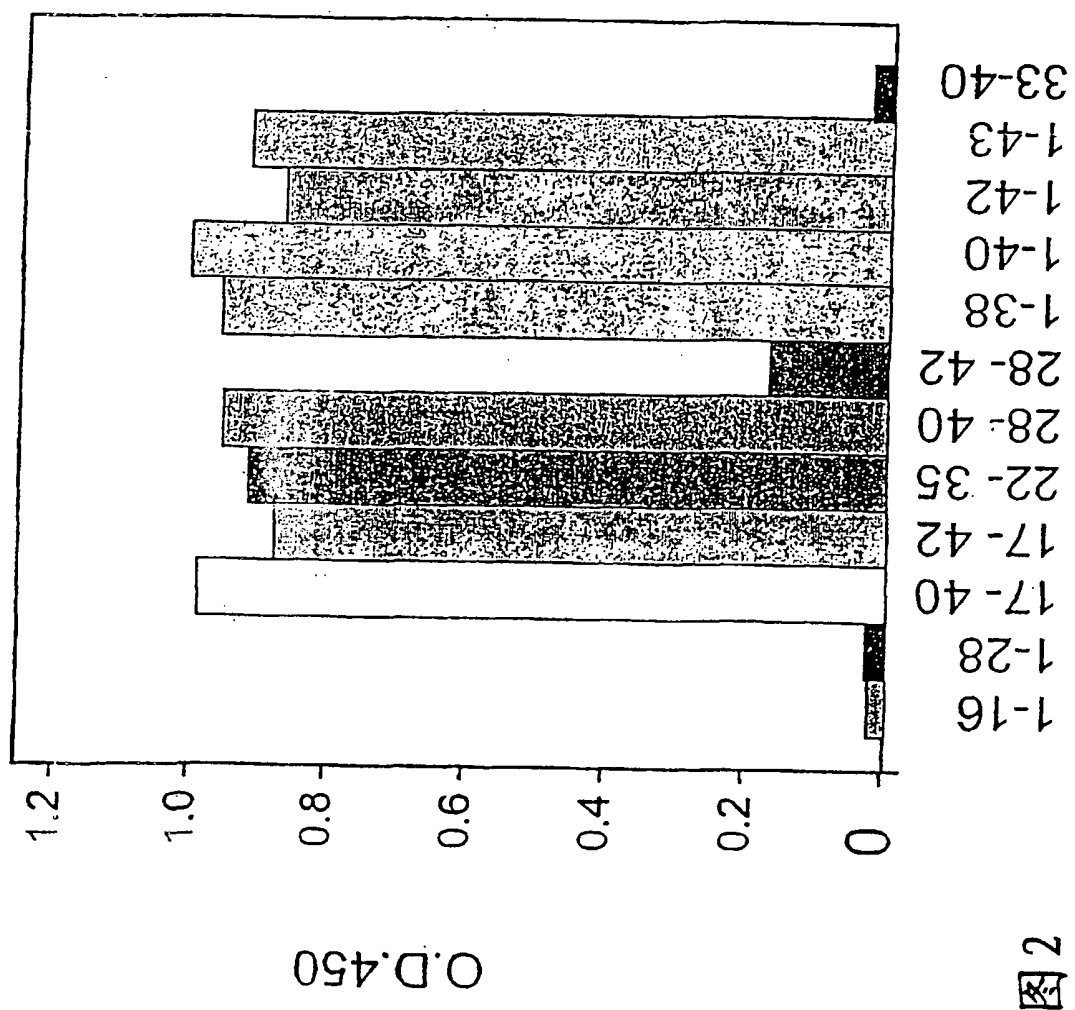
CAGGTGCAACTGGTGCAATCCGGTGCCGAGGTGAAAAAGCCAGGCGCCTCCGTG
AAAGTGTCCTGCAAAGCCTCCGGTTACACCTTTACCACCTATGCCATCCATTGGGT
GCGCCAGGCCCCAGGCCAGGGTCTGGAGTGGATGGGCTTTACTTCCCCCTACTCC
GGGGTGTCGAATTACAATCAGAAGTTCAAAGGCCGCGTCACCATGACCCGCGAC
ACCTCCACCTCCACAGTGTATATGGAGCTGTCCTCTCTGCGCTCCGAAGACACCG
CCGTGTATTACTGTGCCCCTTCGACAATTACGATCGCGGCTATGTGCGTGACTAT
TGGGGCCAGGGCACCCTGGTCACCGTCTCCTCAGCCTCCACCAAGGGCCCATCTG
TCTTCCCACTGGCCCCATGCTCCCGCAGCACCTCCGAGAGCACAGCCGCCCTGGG

CTGCCTGGTCAAGGACTACTTCCCAGAACCTGTGACCGTGTCCCTGGAACCTCTGGC
GCTCTGACCAGCGGCGTGCACACCTTCCCAGCTGTCTGCAGTCCTCAGGTCTCTA
CTCCCTCAGCAGCGTGGTGACCGTGCCATCCAGCAACTTCGGCACCCAGACCTAC
ACCTGCAACGATAGATCACAAGCCAAGCAACACCAAGGTCGACAAGACCGTGGAG
AGAAAGTGTTGTGTGGAGTGTCCACCTTGTCCAGCCCCTCCAGTGGCCGGACCAT
CCGTGTTCCCTGTTCCCTCCAAAGCCAAAGGACACCCTGATGATCTCCAGAACCCC
AGAGGTGACCTGTGTGGTGGTGGACGTGTCCCACGAGGACCCAGAGGTGCAGTTC
AACTGGTATGTGGACGGAGTGGAGGTGCACAACGCCAAGACCAAGCCAAGAGAG
GAGCAGTTCAACTCCACCTTCAGAGTGGTGAGCGTGCTGACCGTGGTGCACCAGG
ACTGGCTGAACGGAAAGGAGTATAAGTGTAAGGTGTCCAACAAGGGACTGCCAT
CCAGCATCGAGAAGACCATCTCCAAGACCAAGGGACAGCCAAGAGAGCCACAGG
TGTATACCCTGCCCCCATCCAGAGAGGAGATGACCAAGAACCAGGTGTCCCTGAC
CTGTCTGGTGAAGGGATTCTATCCATCCGACATCGCCGTGGAGTGGGAGTCCAAC
GGACAGCCAGAGAACAACTATAAGACCACCCCTCCAATGCTGGACTCCGACGGA
TCCTTCTTCCTGTATTCCAAGCTGACCGTGGACAAGTCCAGATGGCAGCAGGGAA
ACGTGTTCTCTTGTTCCTGATGCACGAGGCCCTGCACAACCACTATACCCAGAA
GAGCCTGTCCCTGTCTCCAGGAAAG

6G 轻链全抗体核苷酸序列 (SEQ ID NO: 14)

GACATCGTGATGACCCAGTCCCCAGACTCCCTGGCCGTGTCCCTGGGCGAGCGCG
CCACCATCAACTGCCGCGCCAGCGAATCCGTGGATAACGATCGTATTTCTTTCTG
AACTGGTACCAGCAGAAACCAGGCCAGCCTCCTAAGCTGCTCATTTACGCCGCCA
CCAAACAGGGTACCGGCGTGCCTGACCGCTTCTCCGGCAGCGGTTCCGGCACCGA
TTTCACTCTGACCATCTCCTCCCTGCAGGCCGAAGATGTGGCAGTGTATTACTGTC
AGCAGTCCAAAGAGTTTCCCTGGTCCCTTGGCGGTGGCACCAAGGTGGAGATCAA
ACGCACTGTGGCTGCACCATCTGTCTTCATCTTCCCTCCATCTGATGAGCAGTTGA
AATCCGGAACCTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCACGCGAGGCC
AAAGTACAGTGGAAAGGTGGATAACGCCCTCCAATCCGGTAACTCCCAGGAGAGT
GTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACC
CTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCAT
CAGGGCCTGAGTTCTCCAGTCACAAAGAGCTTCAACCGCGGTGAGTGC

下划线=Chothia CDR



通过ELISA肽扫描对抗体6G的表位作图

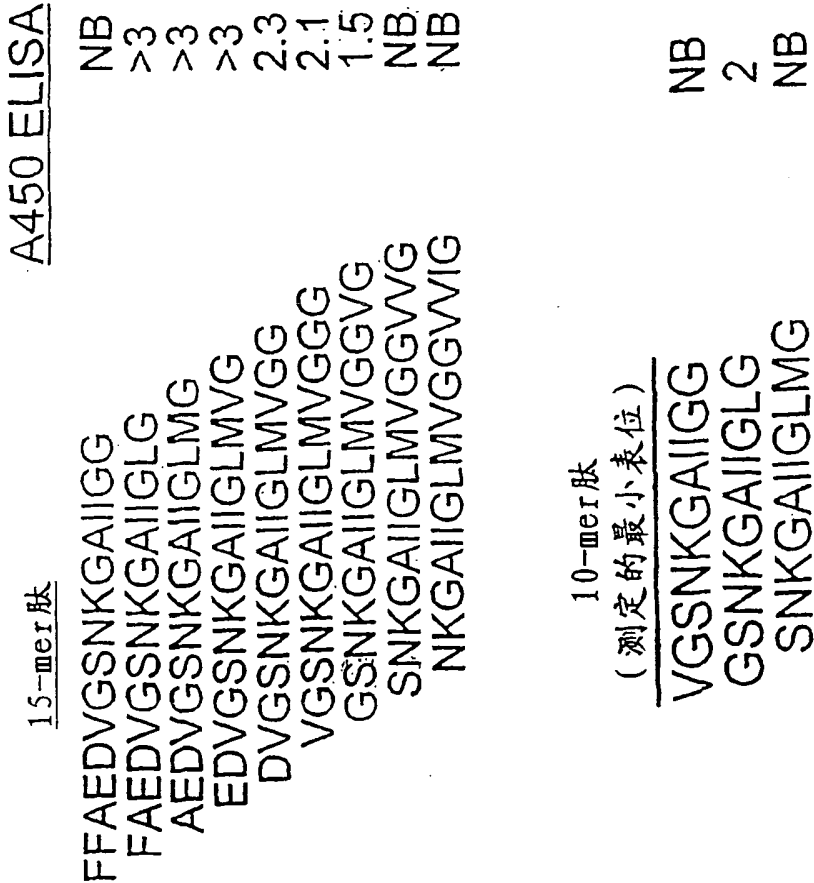


图3

抗体G6表位的示意图

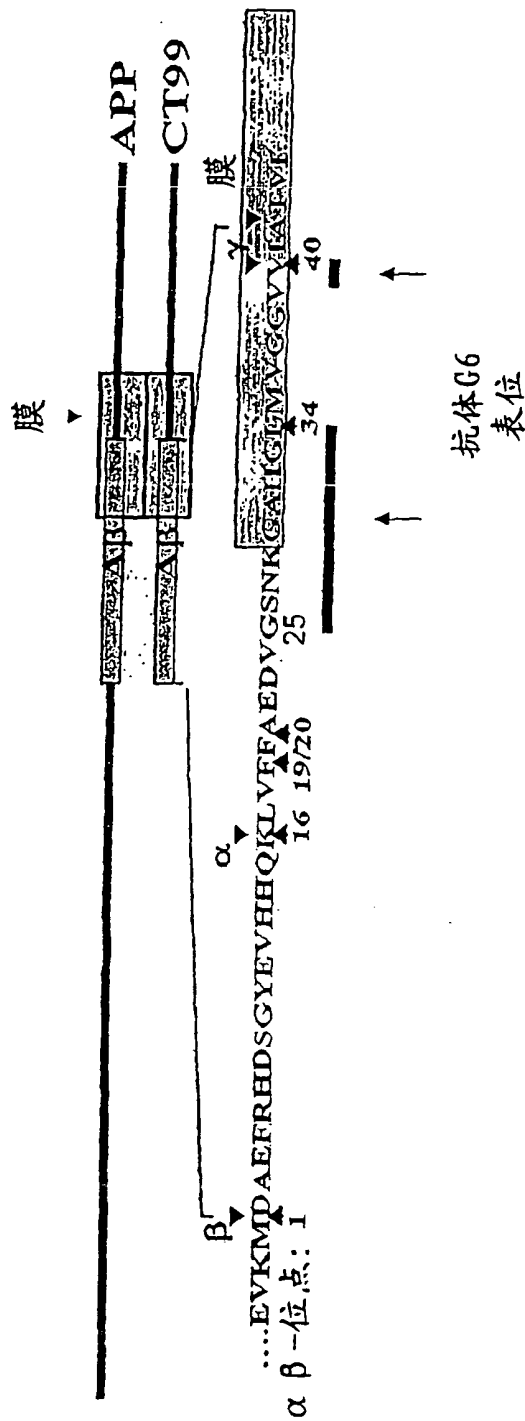


图4

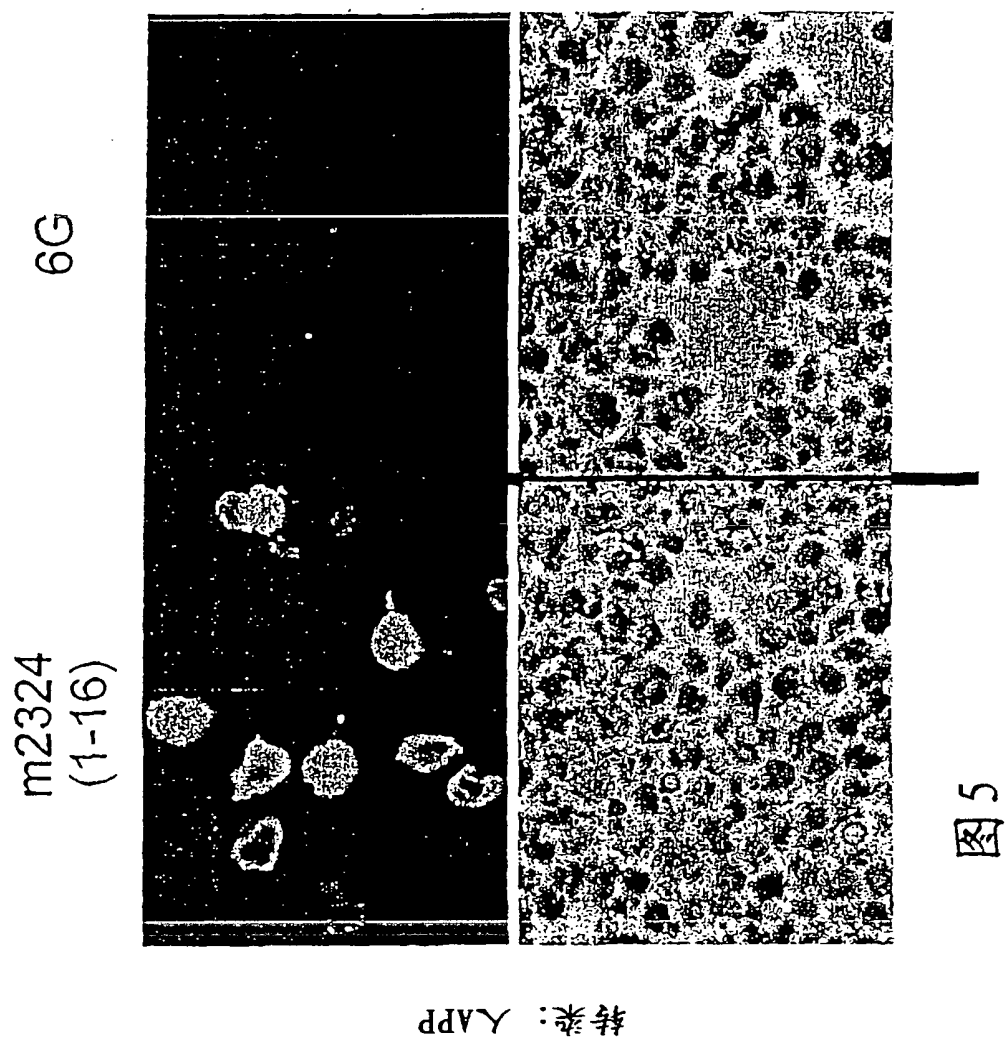


图5

表位作图:		
15 MER肽		
19	FFAEDVGSNKGAIIGG	6G
20	FAEDVGSNKGAIIGLG	NB
21	AEDVGSNKGAIIGLMG	>3
22	EDVGSNKGAIIGLMVG	>3
23	DVGSNKGAIIGLMVGG	>3
24	VGSNKGAIIGLMVGGG	2.3
25	GSNKGAIIGLMVGGVG	2.1
26	SNKGAIIGLMVGGVVG	1.5
27	NKGAIIGLMVGGVVIG	NB
28	KGAIIGLMVGGVVIAG	NB
2294		
	NB	
	>3	
	2.9	
	>3	
	>3	
	>3	
	>3	
	>3	
	NB	
	NB	
10 MER肽结合数据: 测定的最小表位		
	VGSNKGAIIGG	6G
	GSNKGAIIGLG	NB
	SNKGAIIGLMG	>3
2294		
	NB	
	2	
	NB	

图6

(57) **Abstract:** Antibodies directed to the C-terminal side of β -amyloid peptide and methods of using these antibodies for diagnosing and treatment of Alzheimer's disease and $A\beta$ peptide associated diseases are described.

24C070928