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(54) **HUMANIZED ANTIBODIES THAT RECOGNIZE BETA AMYLOID PEPTIDE**

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(58) **Field of Classification Search** ..... None
See application file for complete search history.

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(57) **ABSTRACT**

The invention provides improved agents and methods for treatment of diseases associated with amyloid deposits of Aβ in the brain of a patient. Preferred agents include antibodies, e.g., humanized antibodies.

22 Claims, 6 Drawing Sheets

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Figure 1

Effect of CENTRAL Aβ mAbs 2B1, 1C2 & 15C11 on Contextual Memory in Tg2576 Mice

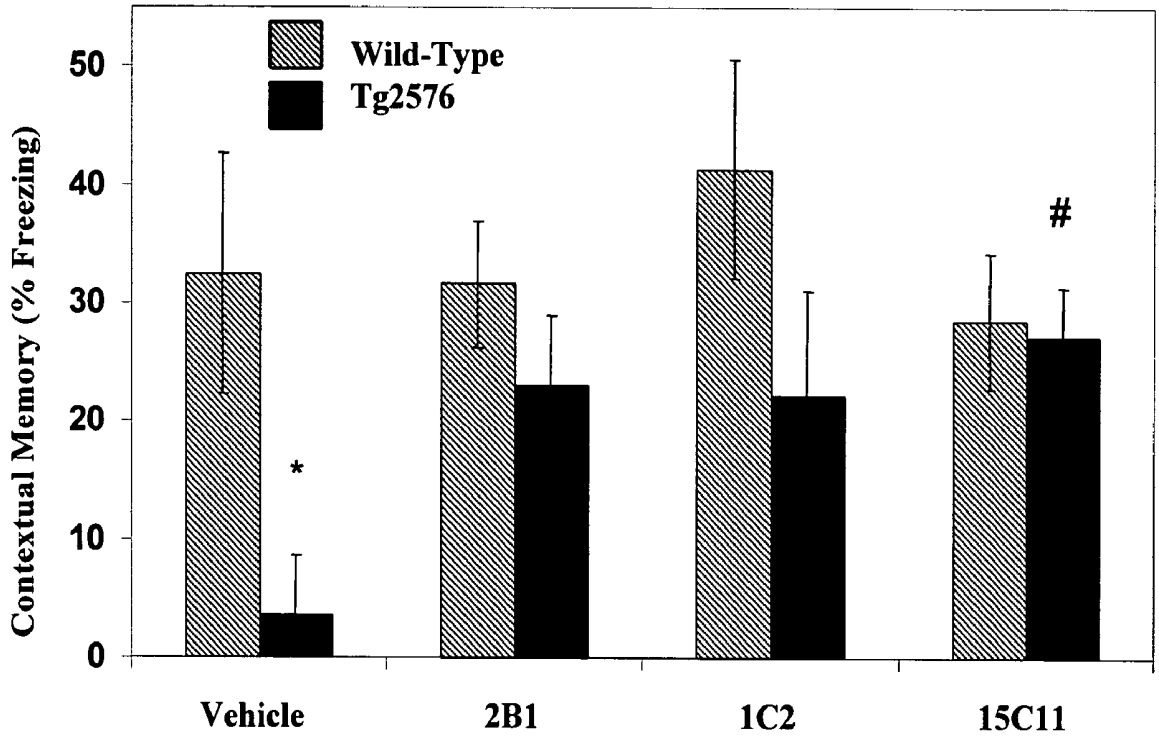

Figure 2

Effect of low dose 15C11 on Contextual Memory in Tg2576 Mouse

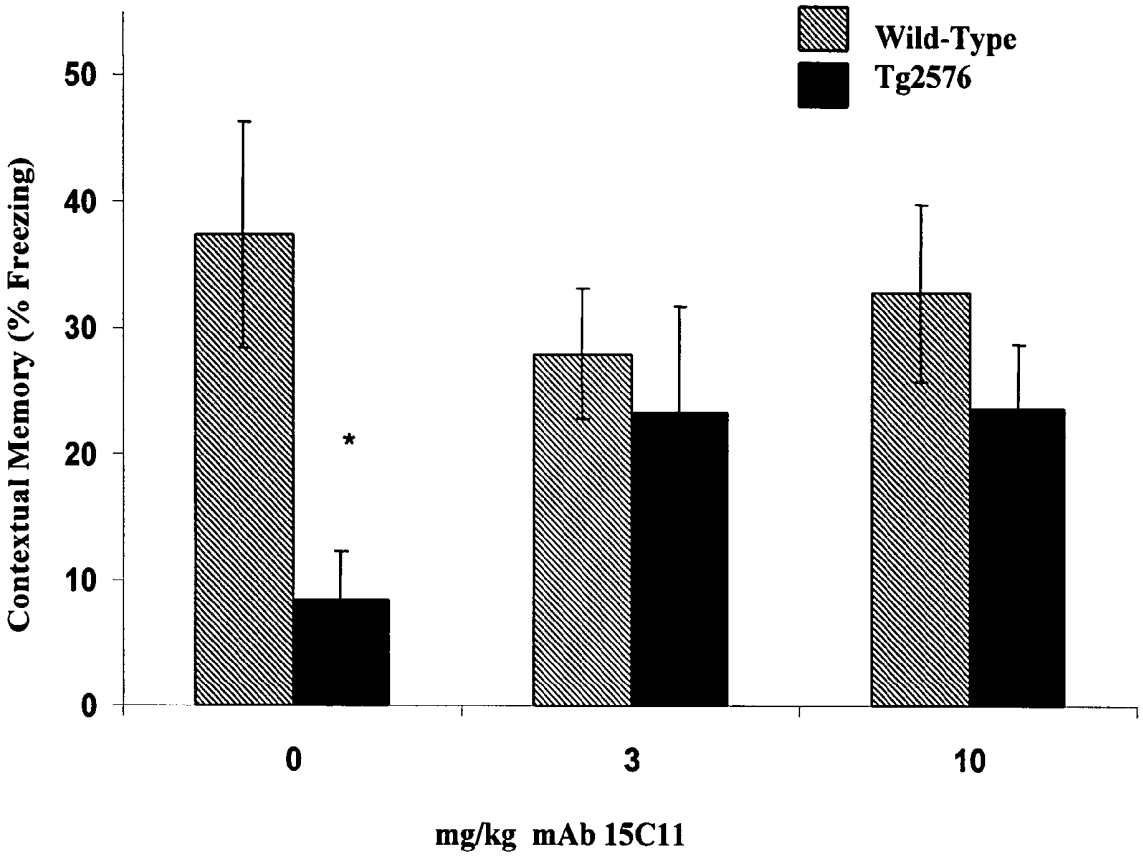

Figure 3

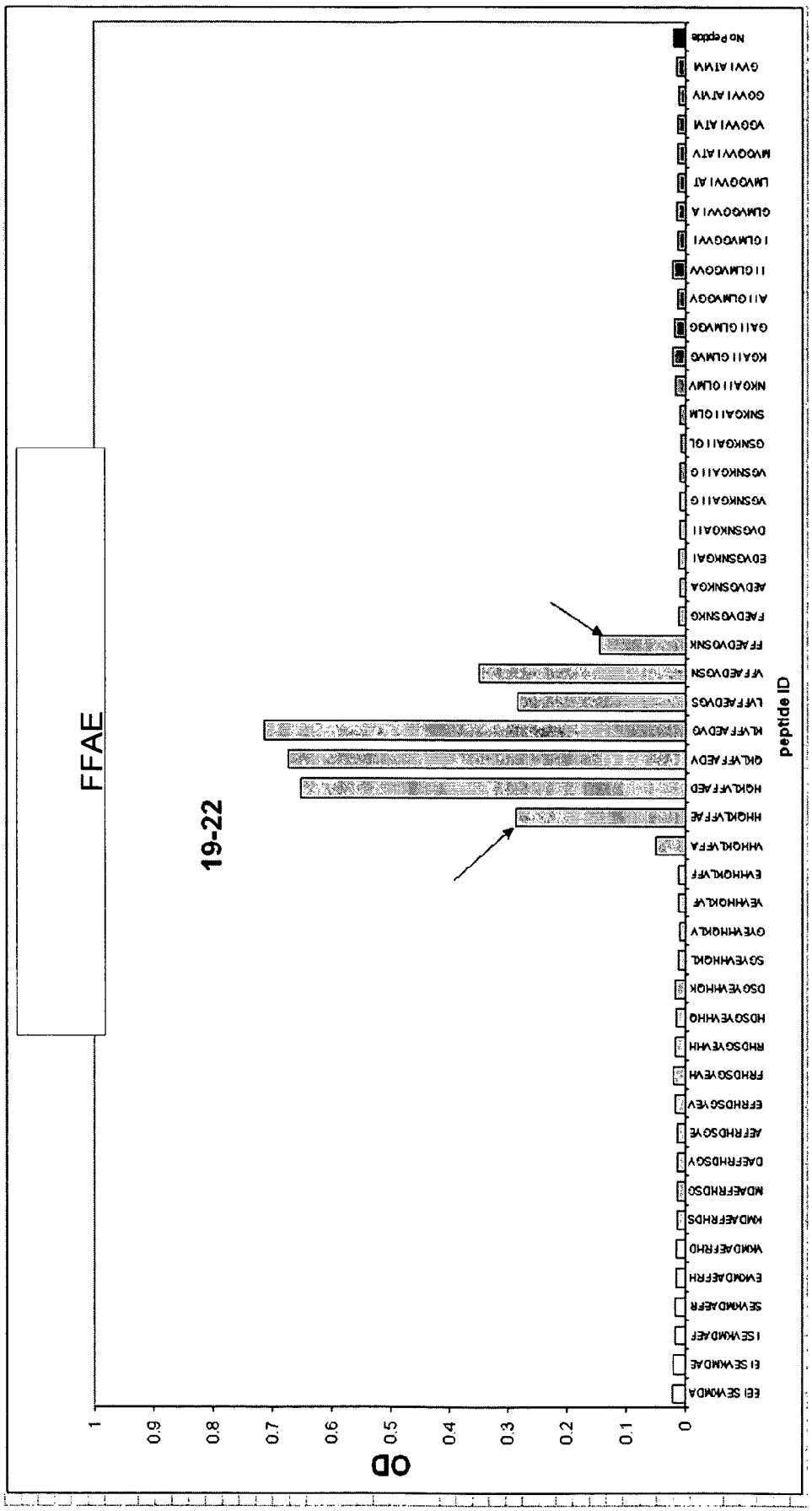
FFAE
19-22
OD
peptide ID
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
No Peptide
GVVIATVM
GGVVIATVV
VGGVVIATM
MVGGVVIATV
LMVGGVVIAT
GLMVGGVVIA
IGLMVGGVVI
IIGLMVGGVV
AIIGLMVGGV
GAIIGLMVGG
KGAIIGLMVG
NKGAIIGLMV
SNKGAIIGLM
QSNKGAIIGL
VQSNKGAIIG
VGSNKGAIIG
DVGSNKGAII
EDVGSNKGAI
AEDVGSNKGA
FAEDVGSNKG
FFAEDVGSNK
VFFAEDVGSN
LVFFAEDVGS
KLVFFAEDVG
QKLVFFAEDV
HQKLVFFAED
HHQKLVFFAE
VHHQKLVFFA
EVHHQKLVFF
VEVHHQKLVF
GVEVHHQKLV
SGVEVHHQKL
DSGVEVHHQK
HDSGVEVHHQ
RHDSGVEVHH
FRHDSGVEVH
EFRHDSGVEV
AEFRHDSGVE
DAEFRHDSGV
MDAEFRHDSG
KMDAEFRHDS
VKMDAEFRHD
EVKMDAEFRH
SEVKMDAEFR
ISEVKMDAEF
EISEVKMDAE
EEISEVKMDA

Figure 4

```
                                                    10                  20
15C11 VH    m n f g l s l i f l v l v l k g - v l c E V K
9G8   VH    m n f g l s l i f l v l v l k g - v l c E V K
266   VH    m n f g l s l i f l v l v l k g - v l c E V K
6H9   VH    - - - - - - - - f l l l i v p a y v l s Q V I

15C11 VH    L V E S G G G L V Q P G G S L K L S C A A S G
9G8   VH    L V E S G G G L V Q P G G S L K L S C A A S G
266   VH    L V E S G G G L V Q P G G S L K L S C A V S G
6H9   VH    L K E S G P G I L Q P S Q T L S L T C S I S G

                        30                          40

15C11 VH    F T F S R Y S - - M S W V R Q T P E K R L E L
9G8   VH    F T F S D Y T - - M S W V R Q T P E K R L E L
266   VH    F T F S R Y S - - M S W V R Q T P E K R L E L
6H9   VH    F S I S T S G S G V S W I R Q T S G K G L E W

                     50   52A                 60

15C11 VH    V A K I S N S G D N T Y Y P D T L K G R F T I
9G8   VH    V A E I S N T G G S T Y Y P D T V K G R F T I
266   VH    V A Q I N S V G N S T Y Y P D T V K G R F T I
6H9   VH    L A H I Y W N G N F R Y N P - S I K S R L T I

                70                  80     82A 82B 82C

15C11 VH    S R D N A Q N T L Y L Q M S S L K S E D T A M
9G8   VH    S R D N A K N T L Y L Q M S S L K S E D T A M
266   VH    S R D N A E Y T L S L Q M S G L R S D D T A T
6H9   VH    S K D T S N N Q V F L K I I S V D T T D T A T

                90                          100

15C11 VH    Y Y C A - S G - - - - - - - D Y W G Q G T T L
9G8   VH    Y Y C A - S G - - - - - - - D Y W G Q G T T L
266   VH    Y Y C A - S G - - - - - - - D Y W G Q G T T L
6H9   VH    Y Y C A L R G S N K E E V F D Y W G Q G T F L

                110

15C11 VH    T V S S
9G8   VH    T V S S
266   VH    T V S S
6H9   VH    T V S S
```

Figure 5

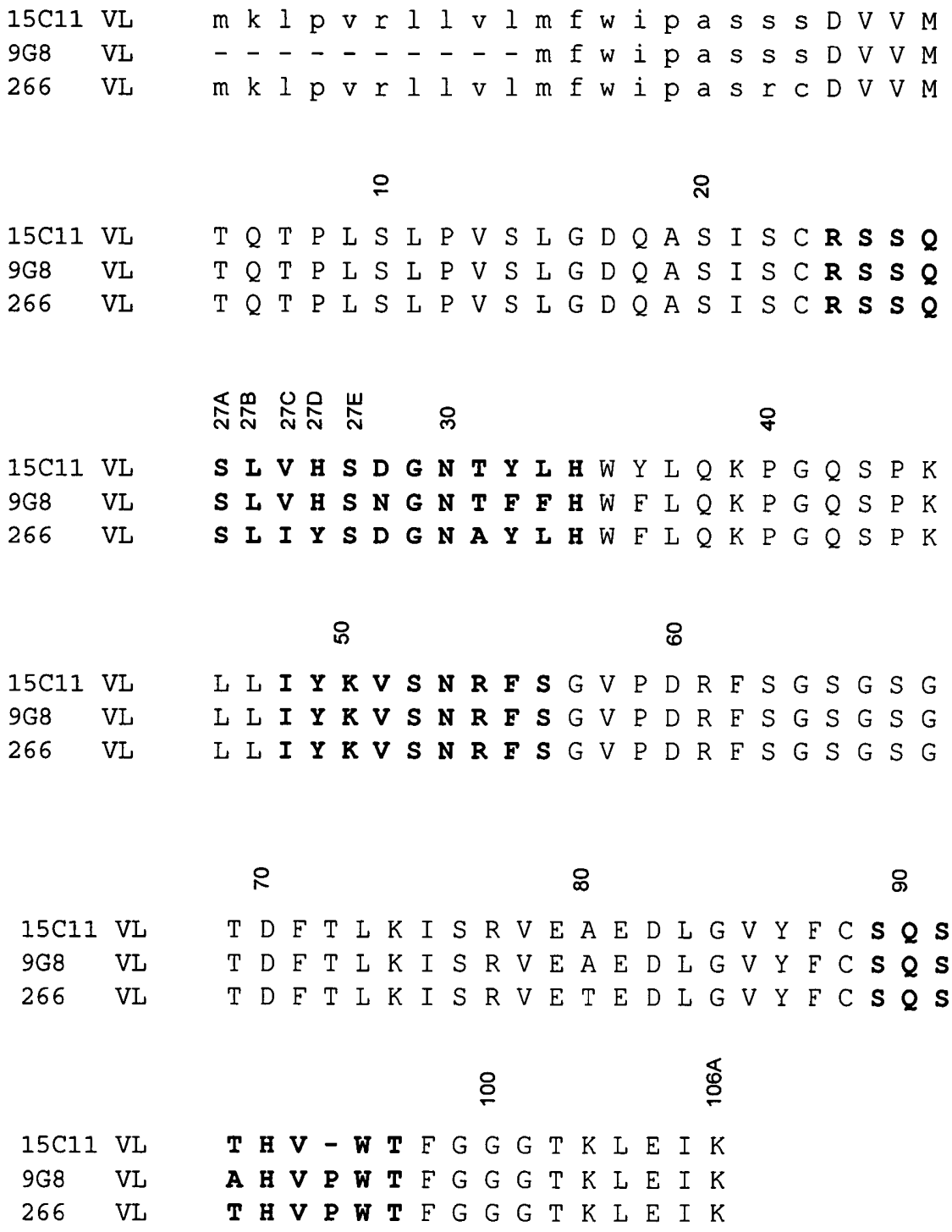
15C11 VL m k l p v r l l v l m f w i p a s s s D V V M
9G8 VL - - - - - - - - - - - m f w i p a s s s D V V M
266 VL m k l p v r l l v l m f w i p a s r c D V V M
10
20
15C11 VL T Q T P L S L P V S L G D Q A S I S C R S S Q
9G8 VL T Q T P L S L P V S L G D Q A S I S C R S S Q
266 VL T Q T P L S L P V S L G D Q A S I S C R S S Q
27A 27B 27C 27D 27E
30
40
15C11 VL S L V H S D G N T Y L H W Y L Q K P G Q S P K
9G8 VL S L V H S N G N T F F H W F L Q K P G Q S P K
266 VL S L I Y S D G N A Y L H W F L Q K P G Q S P K
50
60
15C11 VL L L I Y K V S N R F S G V P D R F S G S G S G
9G8 VL L L I Y K V S N R F S G V P D R F S G S G S G
266 VL L L I Y K V S N R F S G V P D R F S G S G S G
70
80
90
15C11 VL T D F T L K I S R V E A E D L G V Y F C S Q S
9G8 VL T D F T L K I S R V E A E D L G V Y F C S Q S
266 VL T D F T L K I S R V E T E D L G V Y F C S Q S
100
106A
15C11 VL T H V - W T F G G G T K L E I K
9G8 VL A H V P W T F G G G T K L E I K
266 VL T H V P W T F G G G T K L E I K

FIGURE 6

Antibody:	3D6	6C6	12A11	12B4	3A3	266	9G8	15C11	6H9
Aβ Epitope:	1-5	3-7	3-7	3-7	3-7	16-24	16-21	19-22	19-22
CFC :	+	(+/-)	+	-	ND	+	ND	+	-

HUMANIZED ANTIBODIES THAT RECOGNIZE BETA AMYLOID PEPTIDE

RELATED APPLICATIONS

This application claims the benefit of provisional patent application U.S. Ser. No. 60/636684, filed Dec. 15, 2004, entitled "Humanized Antibodies That Recognize Beta Amyloid Peptide," the entire content of which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

Alzheimer's disease (AD) is a progressive disease resulting in senile dementia. See generally Selkoe, *TINS* 16:403 (1993); Hardy et al., WO 92/13069; Selkoe, *J. Neuropathol. Exp. Neurol.* 53:438 (1994); Duff et al., *Nature* 373:476 (1995); Games et al., *Nature* 373:523 (1995). Broadly speaking, the disease falls into two categories: late onset, which occurs in old age (65+ years) and early onset, which develops well before the senile period, i.e., between 35 and 60 years. In both types of disease, the pathology is the same but the abnormalities tend to be more severe and widespread in cases beginning at an earlier age. The disease is characterized by at least two types of lesions in the brain, neurofibrillary tangles and senile plaques. Neurofibrillary tangles are intracellular deposits of microtubule associated tau protein consisting of two filaments twisted about each other in pairs. Senile plaques (i.e., amyloid plaques) are areas of disorganized neuropil up to 150 βm across with extracellular amyloid deposits at the center which are visible by microscopic analysis of sections of brain tissue. The accumulation of amyloid plaques within the brain is also associated with Down's syndrome and other cognitive disorders.

The principal constituent of the plaques is a peptide termed Aβ or β-amyloid peptide. Aβ peptide is a 4-kDa internal fragment of 39-43 amino acid residues of a larger transmembrane glycoprotein protein termed amyloid precursor protein (APP). As a result of proteolytic processing of APP by different secretase enzymes, Aβ is primarily found in both a short form, 40 amino acids in length, and a long form, ranging from 42-43 amino acids in length. Part of the hydrophobic transmembrane domain of APP is found at the carboxy end of Aβ, and may account for the ability of Aβ to aggregate into plaques, particularly in the case of the long form. Accumulation of amyloid plaques in the brain eventually leads to neuronal cell death. The physical symptoms associated with this type of neural deterioration characterize Alzheimer's disease.

Several mutations within the APP protein have been correlated with the presence of Alzheimer's disease. See, e.g., Goate et al., *Nature* 349:704) (1991) (valine^{717} to isoleucine); Chartier Harlan et al. *Nature* 353:844 (1991)) (valine^{717} to glycine); Murrell et al., *Science* 254:97 (1991) (valine^{717} to phenylalanine); Mullan et al., *Nature Genet.* 1:345 (1992) (a double mutation changing lysine^{595}-methionine^{596} to asparagine^{595}-leucine^{596}). Such mutations are thought to cause Alzheimer's disease by increased or altered processing of APP to Aβ, particularly processing of APP to increased amounts of the long form of Aβ (i.e., Aβ1-42 and Aβ1-43). Mutations in other genes, such as the presenilin genes, PS1 and PS2, are thought indirectly to affect processing of APP to generate increased amounts of long form Aβ (see Hardy, *TINS* 20: 154 (1997)).

Mouse models have been used successfully to determine the significance of amyloid plaques in Alzheimer's (Games et al., supra, Johnson-Wood et al., *Proc. Natl. Acad. Sci. USA* 94:1550 (1997)). In particular, when PDAPP transgenic mice, (which express a mutant form of human APP and develop Alzheimer's disease at a young age), are injected with the long form of Aβ, they display both a decrease in the progression of Alzheimer's and an increase in antibody titers to the Aβ peptide (Schenk et al., *Nature* 400, 173 (1999)). The observations discussed above indicate that Aβ, particularly in its long form, is a causative element in Alzheimer's disease.

The Aβ peptide can exist in solution and can be detected in CNS (e.g., CSF) and plasma. Under certain conditions, soluble Aβ is transformed into fibrillary, toxic, β-sheet forms found in neuritic plaques and cerebral blood vessels of patients with AD. Treatments involving immunization with monoclonal antibodies against Aβ have been investigated. Both active and passive immunization have been tested in mouse models of AD. Active immunization resulted in some reduction in plaque load in the brain, but only when administered nasally. Passive immunization of PDAPP transgenic mice has also been investigated (Bard, et al. (2000) *Nat. Med.* 6:916-19). Antibodies recognizing the amino-terminal and central domains of Aβ were found to stimulate phagocytosis of Aβ deposits, whereas antibodies against domains near the carboxy-terminal domain were not.

The mechanism of clearance of Aβ after passive or active immunization is under continued investigation. Two mechanisms are proposed for effective clearance, i.e., central degradation and peripheral degradation. The central degradation mechanism relies on antibodies being able to cross the blood-brain barrier, bind to plaques, and induce clearance of pre-existing plaques. Clearance has been shown to be promoted through an Fc-receptor-mediated phagocytosis (Bard, et al. (2000) *Nat. Med.* 6:916-19). The peripheral degradation mechanism of Aβ clearance relies on a disruption of the dynamic equilibrium of Aβ between brain, CSF, and plasma upon administration of antibody, leading to transport of Aβ from one compartment to another. Centrally derived Aβ is transported into the CSF and the plasma where it is degraded. Recent studies have suggested that soluble and unbound Aβ are involved in the memory impairment associated with AD, even without reduction in amyloid deposition in the brain. Further studies are needed to determine the action and/or interplay of these pathways for Aβ clearance (Dodel, et al. (2003) *The Lancet Vol.* 2:215)

Accordingly, there exists the need for new therapies and reagents for the treatment of Alzheimer's disease, in particular, therapies and reagents capable of effecting a therapeutic benefit at physiologic (e.g., non-toxic) doses. Successful approaches to the prevention and/or treatment of AD include interventions aimed at preventing Aβ accumulation and/or accelerating Aβ clearance, e.g., from Aβ plaques.

SUMMARY OF THE INVENTION

The present invention features new immunological reagents, in particular, therapeutic antibody reagents for the prevention and treatment of amyloidogenic disease (e.g., Alzheimer's disease) as well as relevant behavioral deficits associated with such disease. The invention is based, at least in part, on the identification and characterization of monoclonal antibodies, e.g., 15C11 and 9G8, that specifically bind to Aβ. Antibodies that bind to Aβ oligomers improve cognition in mammals with amyloidogenic disorders. In one embodiment, the invention pertains to antibodies that are capable of rapidly improving cognition in a patient as demonstrated in animal models predictive of human efficacy.

Structural and functional analysis of these antibodies leads to the design of various humanized antibodies for prophylactic and/or therapeutic use. In particular, the invention features

humanization of the variable regions of these antibodies and, accordingly, provides for humanized immunoglobulin or antibody chains, intact humanized immunoglobulins or antibodies, and functional immunoglobulin or antibody fragments, in particular, CDRs or antigen binding fragments, of the featured antibodies.

Polypeptides comprising the complementarity determining regions (CDRs) of the featured monoclonal antibodies are also disclosed, as are polynucleotides encoding the antibodies or polypeptides derived therefrom and vectors and host cells comprising said polypeptides.

Methods for treating amyloidogenic diseases or disorders (e.g., Alzheimer's disease) and/or symptoms associated with such diseases or disorders are disclosed, as are pharmaceutical compositions and kits for use in such applications.

Also featured are methods of identifying residues within the featured monoclonal antibodies which are important for proper immunologic function and for identifying residues which are amenable to substitution in the design of humanized antibodies having improved binding affinities and/or reduced immunogenicity, when used as therapeutic reagents.

Also featured are antibodies (e.g., humanized antibodies) having altered effector functions, and therapeutic uses thereof.

Accordingly, in one aspect, the invention pertains to a humanized immunoglobulin light chain comprising (i) variable region complementarity determining regions (CDRs) from the 15C11 immunoglobulin light chain variable region sequence set forth as SEQ ID NO:2, and (ii) a variable framework region from a human acceptor immunoglobulin light chain sequence, optionally having at least one framework residue substituted with the corresponding amino acid residue from the mouse 15C11 light chain variable region sequence, wherein the framework residue is selected from the group consisting of: (a) a residue that non-covalently binds antigen directly; (b) a residue adjacent to a CDR; (c) a CDR-interacting residue; and (d) a residue participating in the VL-VH interface.

In another aspect, the invention pertains to a humanized immunoglobulin heavy chain comprising (i) variable region complementarity determining regions (CDRs) from the 15C11 immunoglobulin heavy chain variable region sequence set forth as SEQ ID NO:4, and (ii) a variable framework region from a human acceptor immunoglobulin heavy chain, optionally having at least one framework residue substituted with the corresponding amino acid residue from the mouse 15C11 heavy chain variable region sequence, wherein the framework residue is selected from the group consisting of: (a) a residue that non-covalently binds antigen directly; (b) a residue adjacent to a CDR; (c) a CDR-interacting residue; and (d) a residue participating in the VL-VH interface.

In one embodiment, a CDR-interacting residue is identified by modeling the 15C11 light chain based on the solved structure of a murine immunoglobulin light chain that shares at least 70% sequence identity with the 15C11 light chain.

In another embodiment, a CDR-interacting residue is identified by modeling the 15C11 light chain based on the solved structure of a murine immunoglobulin light chain that shares at least 80% sequence identity with the 15C11 light chain.

In one embodiment, a CDR-interacting residue is identified by modeling the 15C11 light chain based on the solved structure of a murine immunoglobulin light chain that shares at least 90% sequence identity with the 15C11 light chain.

In another embodiment, a CDR-interacting residue is identified by modeling the 15C11 heavy chain based on the solved structure of a murine immunoglobulin heavy chain that shares at least 70% sequence identity with the 15C11 heavy chain.

In yet another embodiment, a CDR-interacting residue is identified by modeling the 15C11 heavy chain based on the solved structure of a murine immunoglobulin heavy chain that shares at least 80% sequence identity with the 15C11 heavy chain.

In another embodiment, a CDR-interacting residue is identified by modeling the 15C11 heavy chain based on the solved structure of a murine immunoglobulin heavy chain that shares at least 90% sequence identity with the 15C11 heavy chain.

In another aspect, the invention pertains to a humanized immunoglobulin light chain comprising (i) variable region complementarity determining regions (CDRs) from the 15C11 immunoglobulin light chain variable region sequence set forth as SEQ ID NO:2, and (ii) a variable framework region from a human acceptor immunoglobulin light chain sequence, optionally having at least one framework residue substituted with the corresponding amino acid residue from the mouse 15C11 light chain variable region sequence, wherein the framework residue is a residue capable of affecting light chain variable region conformation or function as identified by analysis of a three-dimensional model of the 15C11 immunoglobulin light chain variable region.

In another aspect, the invention pertains to a humanized immunoglobulin heavy chain comprising (i) variable region complementarity determining regions (CDRs) from the 15C11 immunoglobulin heavy chain variable region sequence set forth as SEQ ID NO:4, and (ii) a variable framework region from a human acceptor immunoglobulin heavy chain, optionally having at least one framework residue substituted with the corresponding amino acid residue from the mouse 15C11 heavy chain variable region sequence, wherein the framework residue is a residue capable of affecting heavy chain variable region conformation or function as identified by analysis of a three-dimensional model of the 15C11 immunoglobulin heavy chain variable region.

In another embodiment, the framework residue is selected from the group consisting of a residue capable of interacting with antigen, a residue proximal to the antigen binding site, a residue capable of interacting with a CDR, a residue adjacent to a CDR, a residue within 6 Å of a CDR residue, a canonical residue, a vernier zone residue, an interchain packing residue, a rare residue, and a glycoslyation site residue on the surface of the structural model.

In yet another embodiment, the framework residue is selected from the group consisting of a residue capable of interacting with antigen, a residue proximal to the antigen binding site, a residue capable of interacting with a CDR, a residue adjacent to a CDR, a residue within 6 Å of a CDR residue, a canonical residue, a vernier zone residue, an interchain packing residue, an unusual residue, and a glycoslyation site residue on the surface of the structural model.

In still another embodiment, the framework residue is substituted at a position selected from the group consisting of position 2, 4, 35, 64, and 71 of the light chain as numbered according to Kabat. In another embodiment, the framework residue is substituted at a position selected from the group consisting of position 26-30, 71, 93, 94, and 103 of the heavy chain as numbered according to Kabat.

In another embodiment, the framework residue is identified by modeling the 15C11 light chain based on the solved structure of a murine immunoglobulin light chain that shares at least 70% sequence identity with the 15C11 light chain.

In still another embodiment, the framework residue is identified by modeling the 15C11 light chain based on the solved structure of a murine immunoglobulin light chain that shares at least 80% sequence identity with the 15C11 light chain.

In another embodiment, the framework residue is identified by modeling the 15C11 light chain based on the solved structure of a murine immunoglobulin light chain that shares at least 90% sequence identity with the 15C11 light chain.

In yet another embodiment, the framework residue is identified by modeling the 15C11 heavy chain based on the solved structure of a murine immunoglobulin heavy chain that shares at least 70% sequence identity with the 15C11 heavy chain.

In another embodiment, the framework residue is identified by modeling the 15C11 heavy chain based on the solved structure of a murine immunoglobulin heavy chain that shares at least 80% sequence identity with the 15C11 heavy chain.

In another embodiment, the framework residue is identified by modeling the 15C11 heavy chain based on the solved structure of a murine immunoglobulin heavy chain that shares at least 90% sequence identity with the 15C11 heavy chain.

In some embodiments, an immunoglobulin of the invention comprises one or more amino acid alterations in the hinge region, for example, at EU positions 234, 235, 236 and 237. In a particular embodiment, an immunoglobulin according to the invention is a humanized antibody including amino acid alterations at positions 234 and 237 of the hinge region (i.e., L234A and G237A).

In further embodiments, immunoglobulins of the invention comprise pegylated antibody fragments, e.g., Fabs and Fab's. In yet other embodiments, immunoglobulins of the invention comprise an aglycosylated constant region. In an exemplary embodiment, an immunoglobulin includes an amino acid substitution of an asparagine at position 297 to an alanine, thereby preventing glycosylation of the immunoglobulin.

In some embodiments, a humanized immunoglobulin of the invention comprises complementarity determining regions (CDRs) of the 9G8 antibody produced by the cell line having ATCC Accession Number PTA-7201. In other embodiments, a humanized immunoglobulin is a humanized version of the monoclonal antibody 9G8 produced by the cell line having ATCC Accession Number PTA-7201. The cell line designated 9G8 producing the antibody 9G8 has the American Type Culture Collection (ATCC) accession number PTA-7201, having been deposited on Nov. 1, 2005 under the terms of the Budapest Treaty. The address of the American Type Culture Collection is 10801 University Boulevard, Manassas, Va. 20010-2209.

Also featured herein are methods of increasing expression of immunoglobulins by deleting one or more introns in a gene which encodes the heavy chain of the immunoglobulin.

Additionally, this invention relates to methods of treatment, as described herein, using one or more immunoglobulins of the invention.

In one embodiment, an immunoglobulin or antigen binding fragment specifically binds to beta amyloid peptide (Aβ) with a binding affinity of at least 10^{-7} M. In yet another embodiment, immunoglobulin or antigen binding fragment specifically binds to beta amyloid peptide (Aβ) with a binding affinity of at least 10^{-8} M. In another embodiment, an immunoglobulin or antigen binding fragment specifically binds to beta amyloid peptide (Aβ) with a binding affinity of at least 10^{-9} M.

In one embodiment, an immunoglobulin or antigen binding fragment of the invention comprises a heavy chain isotype γ1.

In another embodiment, an immunoglobulin or antigen binding fragment of the invention binds to soluble beta amyloid peptide (Aβ).

In one embodiment, an immunoglobulin or antigen binding fragment binds to oligomeric beta amyloid peptide (Aβ).

In one embodiment, an immunoglobulin or antigen binding fragment captures beta amyloid peptide (Aβ).

In another embodiment, an immunoglobulin or antigen binding fragment of the invention crosses the blood-brain barrier in a patient.

In another embodiment, an immunoglobulin or antigen binding fragment of the invention reduces beta amyloid peptide (Aβ) plaque burden in a patient.

In another aspect, the invention is directed to a humanized immunoglobulin comprising a humanized heavy chain and a humanized light chain, wherein (a) the humanized light chain comprises three complementarity determining regions (CDR1, CDR2 and CDR3) having amino acid sequences from the corresponding complementarily determining regions of the mouse 15C11 immunoglobulin light chain variable domain designated SEQ ID NO:2, and a variable region framework from a human light chain variable region framework sequence optionally having at least one framework residue selected from the group consisting of a canonical residue, a vernier residue, a packing residue and a rare residue, occupied by the same amino acid residue present in the equivalent position of the mouse 15C11 immunoglobulin light chain variable region framework; and (b) the humanized heavy chain comprises three complementarity determining regions (CDR1, CDR2 and CDR3) having amino acid sequences from the corresponding complementarity determining regions of the mouse 15C11 immunoglobulin heavy chain variable domain designated SEQ ID NO:4, and a variable region framework from a human heavy chain variable region framework sequence optionally having at least one framework residue selected from a second group consisting of a canonical residue, a vernier residue, a packing residue and a rare residue, occupied by the same amino acid residue present in the equivalent position of the mouse 15C11 immunoglobulin heavy chain variable region framework; wherein the humanized immunoglobulin specifically binds to beta amyloid peptide ("Aβ") with a binding affinity of at least 10^{-7} M.

In one embodiment, the human light chain variable region framework is from a kappa light chain variable region.

In another embodiment, the human heavy chain variable region framework is from an IgG1 heavy chain variable region.

In another embodiment, the human heavy chain variable region framework is from an IgG4 heavy chain variable region.

In one embodiment, the light chain variable region framework is from a human immunoglobulin light chain having at least 70% sequence identity with light chain sequence of the 15C11 immunoglobulin.

In one embodiment, the heavy chain variable region framework is from a human immunoglobulin heavy chain having at least 70% sequence identity with heavy chain sequence of the 15C11 immunoglobulin.

In one embodiment, the invention pertains to a humanized light chain comprising complementarity determining regions that are identical to the corresponding complementarity determining regions of the mouse 15C11 heavy chain, and a humanized heavy chain comprising complementarity determining regions that are identical to the corresponding complementarity determining regions of the mouse 15C11 heavy chain.

In one embodiment, the invention pertains to a humanized immunoglobulin comprising the complementarity determining regions (CDR1, CDR2 and CDR3) of the 15C11 variable light chain sequence set forth as SEQ ID NO:2.

In another embodiment, the invention pertains to a humanized immunoglobulin comprising the complementarity deter-

mining regions (CDR1, CDR2 and CDR3) of the 15C11 variable heavy chain sequence set forth as SEQ ID NO:4.

In still another embodiment, the invention pertains to a humanized immunoglobulin, or antigen-binding fragment thereof, which specifically binds to beta amyloid peptide (Aβ), comprising a variable region comprising complementarity determining regions (CDRs) corresponding to CDRs from the mouse 15C11 antibody.

In yet another embodiment, the invention pertains to a chimeric immunoglobulin comprising variable region sequence substantially as set forth in SEQ ID NO:2 or SEQ ID NO:4, and constant region sequences from a human immunoglobulin.

In one embodiment, the invention pertains to a method of preventing or treating an amyloidogenic disease in a patient, comprising administering to the patient an effective dosage of the humanized immunoglobulin described herein.

In another embodiment, method of preventing or treating Alzheimer's disease in a patient, comprising administering to the patient an effective dosage of the humanized immunoglobulin described herein.

In one embodiment, the effective dosage of humanized immunoglobulin is 1 mg/kg body weight. In another embodiment, the effective dosage of humanized immunoglobulin is 10 mg/kg body weight. In still another embodiment, the effective dosage of humanized immunoglobulin is 30 mg/kg body weight.

In another embodiment, the invention pertains to a pharmaceutical composition comprising an immunoglobulin molecule described herein and a pharmaceutical carrier.

In another aspect, the invention pertains to an isolated polypeptide comprising a fragment of SEQ ID NO:2, wherein said fragment is selected from the group consisting of amino acids 24-39 of SEQ ID NO:2, amino acids 55-61 of SEQ ID NO:2 and amino acids 94-101 of SEQ ID NO:2.

In another aspect, the invention pertains to an isolated polypeptide comprising amino acids 24-39 of SEQ ID NO:2, amino acids 55-61 of SEQ ID NO:2 and amino acids 94-101 of SEQ ID NO:2.

In another aspect, the invention pertains to an isolated polypeptide comprising a fragment of SEQ ID NO:4, wherein said fragment is selected from the group consisting of amino acids 26-35 of SEQ ID NO:4, amino acids 50-66 of SEQ ID NO:4 and amino acids 99-101 of SEQ ID NO:4.

In still another aspect, the invention pertains to an isolated polypeptide comprising amino acids 26-35 of SEQ ID NO:4, amino acids 50-66 of SEQ ID NO:4 and amino acids 99-101 of SEQ ID NO:4.

In another aspect, the invention pertains to an isolated polypeptide comprising amino acids 1-111 of SEQ ID NO:2.

In another aspect, the invention pertains to an isolated polypeptide comprising amino acids 1-112 of SEQ ID NO:4.

In still another aspect, the invention pertains to an isolated polypeptide having at least 85% identity to amino acids 1-111 of SEQ ID NO: 2.

In another aspect, the invention pertains to an isolated polypeptide having at least 85% identity to amino acids 1-112 of SEQ ID NO: 4.

In another embodiment, the invention pertains to an isolated polypeptide having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, or at least 90% identity to amino acids 1-131 of SEQ ID NO: 4. In one embodiment, the polypeptide has at least 90% or more identity.

In one aspect, the invention pertains to a variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, wherein said variant comprises at least one conservative amino acid substitution, and wherein the variant retains the ability to specifically bind beta amyloid peptide (Aβ) with a binding affinity of at least 10^7 M^{-1}.

In another aspect, the invention pertains to a variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:4, wherein said variant comprises at least one conservative amino acid substitution, and wherein the variant retains the ability to direct specific binding to beta amyloid peptide (Aβ) with a binding affinity of at least 10^7 M^{-1}.

In one embodiment, the invention pertains to an isolated nucleic acid molecule encoding a light chain described herein. In another embodiment, the invention pertains to an isolated nucleic acid molecule encoding a heavy chain described herein.

In one embodiment, the invention pertains to an isolated nucleic acid molecule encoding the polypeptide of the invention. In one embodiment, the invention pertains to an isolated nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1 or 3.

In another embodiment, the invention pertains to a vector comprising a nucleic acid molecule of the invention. In another embodiment, the invention pertains to a host cell comprising a nucleic acid molecule of the invention.

In another embodiment, the invention pertains to a transgenic animal expressing a polypeptide encoded by a nucleic acid molecule of the invention.

In one embodiment, the polypeptide is expressed in the milk of said animal.

In another embodiment, the invention pertains to a method of producing an antibody, or fragment thereof, comprising culturing the host cell comprising a nucleic acid molecule of the invention under conditions such that the antibody or fragment is produced and isolating said antibody from the host cell or culture.

In another embodiment, the invention pertains to a method of producing an antibody, or fragment thereof, comprising a fragment of SEQ ID NO:2 selected from the group consisting of amino acids 24-39 of SEQ ID NO:2, amino acids 55-61 of SEQ ID NO:2 and amino acids 94-101 of SEQ ID NO:2, said method comprising culturing a host cell comprising a nucleic acid molecule that encodes said antibody, or fragment thereof, under conditions such that the antibody or fragment is produced, and isolating said antibody from the host cell or culture.

In one embodiment, the invention pertains to a method of producing an antibody, or fragment thereof, comprising a fragment of SEQ ID NO:4 selected from the group consisting of amino acids 26-35 of SEQ ID NO:4, amino acids 50-66 of SEQ ID NO:4 and amino acids 99-101 of SEQ ID NO:4, said method comprising culturing a host cell comprising a nucleic acid molecule that encodes said antibody, or fragment thereof, under conditions such that the antibody or fragment is produced, and isolating said antibody from the host cell or culture.

In one embodiment, the invention pertains to a method for identifying residues amenable to substitution in a humanized 15C11 immunoglobulin variable framework region, comprising modeling the three-dimensional structure of the 15C11 variable region based on a solved immunoglobulin structure and analyzing said model for residues capable of affecting 15C11 immunoglobulin variable region conformation or function, such that residues amenable to substitution are identified.

In one aspect, the invention pertains to a variable region sequence set forth as SEQ ID NO:2 or SEQ ID NO:4, or any portion thereof, in producing a three-dimensional image of a 15C11 immunoglobulin, 15C11 immunoglobulin chain, or domain thereof.

In another aspect, the invention pertains to a method of imaging amyloid deposits in the brain of a patient comprising administering to the patient an agent that specifically binds to Aβ, and detecting the antibody bound to Aβ. In one embodiment, the agent is an antibody comprising a light chain variable sequence as set forth in SEQ ID NO:2 and a heavy chain variable region sequence as set forth in SEQ ID NO:4, or an antigen-binding fragment of said antibody. In one embodiment, the antigen-binding fragment is a Fab fragment.

In another embodiment, the invention pertains to a kit for imaging including instructions for use.

The invention further pertains to a method of treating an amyloidogenic disease comprising administering to a patient having said amyloidogenic disease, a nucleic acid molecule that encodes an immunoglobulin light chain comprising the CDRs of the amino acid sequence of SEQ ID NO:2 and a nucleic acid molecule that encodes an immunoglobulin heavy chain that comprises the CDRs of the amino acid sequence of SEQ ID NO:4, under conditions such that said immunoglobulin chains are expressed, such that a beneficial therapeutic response in said patient is generated.

In another aspect, the invention pertains to an antibody selected from the group consisting of: a 15C11 antibody, a humanized 15C11 antibody, and a chimeric 15C11 antibody, or a fragment thereof. In still another aspect, the invention pertains to a cell line producing such an antibody.

In one embodiment, the invention pertains to an antibody selected from the group consisting of: a 9G8 antibody, a humanized 9G8 antibody, and a chimeric 9G8 antibody, or a fragment thereof. In still another aspect, the invention pertains to a cell line producing an antibody of the invention.

In one aspect, the invention pertains to a method for effecting rapid improvement in cognition in a patient, comprising administering to the patient an effective dose of an antibody of the invention such that the rapid improvement in cognition is achieved.

In one embodiment, the patient has or is at risk for an Aβ-related disease or disorder. In another embodiment, the patient has or is at risk for an amyloidogenic disease or disorder. In another embodiment, the patient has or is at risk for Alzheimer's disease.

In one embodiment, the patient is human.

In one embodiment, the effective dose of an antibody of the invention is from about 100 μg/kg to 100 mg/kg body weight of the patient. In another embodiment, the effective dose of an antibody of the invention is from about 300 μg/kg to 30 mg/kg body weight of the patient. In another embodiment, the effective dose of an antibody of the invention is from about 1 mg/kg to 10 mg/kg body weight of the patient.

In one embodiment, rapid improvement in cognition is achieved within 12 hours after administration of the antibody. In another embodiment, rapid improvement in cognition is achieved within 24 hours after administration of the antibody. In still another embodiment, rapid improvement in cognition is achieved within 36 hours after administration of the antibody. In yet another embodiment, rapid improvement in cognition is achieved within 48 hours after administration of the antibody.

In one embodiment, the antibody agent is a 15C11 antibody or antigen-binding fragment thereof.

The invention still further pertains to a polypeptide comprising a light chain variable region that binds Aβ, wherein the light chain variable region comprises a first, a second, and a third complementarity determining region (CDR), wherein

the first CDR comprises an amino acid sequence selected from the group consisting of: amino acids 24-39 of SEQ ID NO:8, amino acids 24-39 of SEQ ID NO:2, and amino acids 24-39 of SEQ ID NO:9; the second CDR comprises amino acids 55-61 of SEQ ID NO:2; and the third CDR comprises an amino acid sequence selected from the group consisting of: amino acids 94-102 of SEQ ID NO:8, amino acids 94-101 of SEQ ID NO:2, and amino acids 94-102 of SEQ ID NO:9; provided that where the first CDR comprises amino acids 24-39 of SEQ ID NO:9, the third CDR does not comprise amino acids 94-102 of SEQ ID NO:9 and that where the third CDR comprises amino acids 94-102 of SEQ ID NO:9, the first CDR does not comprise amino acids 24-39 of SEQ ID NO:9.

In one embodiment, where a first CDR comprises amino acids 24-39 of SEQ ID NO:8, a third CDR comprises amino acids 94-102 of SEQ ID NO:8.

In another embodiment, where the first CDR comprises amino acids 24-39 of SEQ ID NO:8, the third CDR comprises amino acids 94-101 of SEQ ID NO:2.

In another embodiment, where the first CDR comprises amino acids 24-39 of SEQ ID NO:8, the third CDR comprises amino acids 94-102 of SEQ ID NO:9.

In one embodiment, where the first CDR comprises amino acids 24-39 of SEQ ID NO:2, the third CDR comprises amino acids 94-101 of SEQ ID NO:2.

In one embodiment, the first CDR comprises amino acids 24-39 of SEQ ID NO:2, the third CDR comprises amino acids 94-102 of SEQ ID NO:8.

In one embodiment, where the first CDR comprises amino acids 24-39 of SEQ ID NO:2, the third CDR comprises amino acids 94-102 of SEQ ID NO:9.

In one embodiment, where the first CDR comprises amino acids 24-39 of SEQ ID NO:9, the third CDR comprises amino acids 94-102 of SEQ ID NO:8.

In one embodiment, where the first CDR comprises amino acids 24-39 of SEQ ID NO:9, and the third CDR comprises amino acids 94-101 of SEQ ID NO:2.

In another aspect, the invention pertains to a polypeptide comprising a heavy chain variable region that binds Aβ, wherein the heavy chain variable region comprises a first, a second, and a third, complementarity determining region (CDR), wherein

the first CDR comprises an amino acid sequence selected from the group consisting of: amino acids 26-35 of SEQ ID NO:5 and amino acids 26-35 of SEQ ID NO:4;

the second CDR comprises an amino acid sequence selected from the group consisting of: amino acids 50-66 of SEQ ID NO:4, amino acids 50-66 of SEQ ID NO:5, and amino acids 50-66 of SEQ ID NO:6; and the third CDR comprises amino acids 99-101 of SEQ ID NO:4; provided that where the first CDR comprises amino acids 26-35 of SEQ ID NO:4, the second CDR does not comprise amino acids 50-66 of SEQ ID NO:6 and that where the second CDR comprises amino acids 50-66 of SEQ ID NO:6, the first CDR does not comprise amino acids 26-35 of SEQ ID NO:4.

In one embodiment, where the first CDR comprises amino acids 26-35 of SEQ ID NO:5, the second CDR comprises amino acids 50-66 of SEQ ID NO:5.

In one embodiment, where the first CDR comprises amino acids 26-35 of SEQ ID NO:5, the second CDR comprises amino acids 50-66 of SEQ ID NO:4.

In one embodiment, where the first CDR comprises amino acids 26-35 of SEQ ID NO:5, the second CDR comprises amino acids 50-66 of SEQ ID NO:6.

In another embodiment, where the first CDR comprises amino acids 26-35 of SEQ ID NO:4, and the second CDR comprises amino acids 50-66 of SEQ ID NO:4.

In one embodiment, where the first CDR comprises amino acids 26-35 of SEQ ID NO:4, the second CDR comprises amino acids 50-66 of SEQ ID NO:5.

In another aspect, the invention pertains to a polypeptide comprising a light chain variable region that binds Aβ, wherein the light chain variable region comprises a first, a second, and a third complementarity determining region (CDR), wherein the first CDR comprises the amino acid sequence shown in SEQ ID NO:12; the second CDR comprises amino acids 55-61 of SEQ ID NO:2; and the third CDR comprises the amino acid sequence shown in SEQ ID NO:13; provided that where the first CDR comprises amino acids 24-39 of SEQ ID NO:9, the third CDR does not comprise amino acids 94-102 of SEQ ID NO:9 and that where the third CDR comprises amino acids 94-102 of SEQ ID NO:9, the first CDR does not comprise amino acids 24-39 of SEQ ID NO:9.

In still another aspect, the invention pertains to a polypeptide comprising a heavy chain variable region that binds Aβ, wherein the heavy chain variable region comprises a first, a second, and a third, complementarity determining region (CDR), wherein the first CDR comprises the amino acid sequence shown in SEQ ID NO:10; the second CDR comprises the amino acid sequence shown in SEQ ID NO:11; and the third CDR comprises amino acids 99-101 of SEQ ID NO:4; provided that where the first CDR comprises amino acids 26-35 of SEQ ID NO:4, the second CDR does not comprise amino acids 50-66 of SEQ ID NO:6 and that where the second CDR comprises amino acids 50-66 of SEQ ID NO:6, the first CDR does not comprise amino acids 26-35 of SEQ ID NO:4.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a graph depicting the effect of 15C11, 2B1 and 1C2 (30 mg/kg) on contextual memory in Tg2576 mice, as determined by contextual fear conditioning assays. The memory deficit reversal was full and significant in Tg2576 mice administered 15C11. The asterisk (*) indicates significant difference from wild-type and the number sign (#) indicates significant difference from vehicle treated heterozygotes.

FIG. **2** is a graph depicting the effect of low dose (3 mg/kg and 10 mg/kg) 15C11 on contextual memory in Tg2576 mouse as determined by contextual fear conditioning assays. The data shows a trend towards no impairment as dosage of 15C11 is increased. Improvement in contextual memory is significant for Tg2576 mice which received 30 mg/kg of 15C11. The asterisk (*) indicates significant difference from wild-type.

FIG. **3** depicts the results of an epitope map assay for 15C11. Results indicate that 15C11 recognizes residues 19-22 (FFAE (SEQ ID NO: 34)) of Aβ. Peptide ID's disclosed as SEQ ID NOS 49-80 and 80-94, respectively, in order of appearance.

FIG. **4** is an alignment of the heavy chain variable domains of 15C11 (SEQ ID NO: 4), 9G8 (SEQ ID NO: 5), 266 (SEQ ID NO: 6) and 6H9 (SEQ ID NO: 7) anti-Aβ antibodies. Kabat numbering of the amino acids for 15C11 is shown above the sequence. The leader sequence is shown in lower case and the CDRs are bolded.

FIG. **5** is an alignment of the light chain variable domains of 15C11 (SEQ ID NO: 2), 9G8 (SEQ ID NO: 8) and 266 (SEQ ID NO: 9) anti-Aβ antibodies. Kabat numbering of the amino acids for 15C11 is shown above the sequence. The leader sequence is shown in lower case and the CDRs are bolded.

FIG. **6** depicts a Western blot of immunoprecipitates of peroxynitrite treated oligomeric $A\beta_{1-42}$ preparation precipitated with various Aβ antibodies (3D6, 6C6, 12A11, 12B4, 3A3, 266, 9G8, 15C11, and 6H9) and imaged with 3D6. The approximate positions of $A\beta_{1-42}$ monomer, dimer, trimer and tetramer bands are indicated on the left-hand side of the figure. Indicated below each Aβ antibody is the Aβ epitope recognized by the antibody and CFC assay results for the antibody, a "+" notation indicates an observation of increased cognition upon treatment with the antibody, a "−" notation indicates an observation of no change in cognition upon treatment with the antibody, a "+/−" notation indicates an observation of a trend of increased cognition upon treatment with the antibody but the observed trend was not statistically significant enough to be indicated as an observation of increased cognition, and "ND" notation indicates no CFC assay data compared for this antibody.

DETAILED DESCRIPTION OF THE INVENTION

The present invention features novel immunological reagents and methods for preventing or treating Alzheimer's disease or other amyloidogenic diseases using such reagents. The invention is based, at least in part, on the characterization of a monoclonal immunoglobulins, e.g., 15C11 and 9G8, effective at binding beta amyloid protein (Aβ, e.g., Aβ oligomers) and improving cognition in a patient, (e.g., in a subject having an amyloidogenic disease or disorder).

The invention is further based on the determination and structural characterization of the primary and secondary structure of the variable light and heavy chains of the 15C11 and 9G8 immunoglobulins, e.g., the identification of residues important for antigen binding and/or activity.

Immunoglobulins are featured which include a variable light and/or variable heavy chain of the 15C11 or 9G8 monoclonal immunoglobulins described herein. Preferred immunoglobulins, e.g., therapeutic immunoglobulins, are featured which include a humanized variable light and/or humanized variable heavy chain. Preferred variable light and/or variable heavy chains include at least one complementarity determining region (CDR) from the 15C11 or 9G8 immunoglobulin (e.g., donor immunoglobulin) and variable framework regions derived from or substantially derived from a human acceptor immunoglobulin. The phrase "substantially from a human acceptor immunoglobulin" means that the majority or key framework residues are from the human acceptor sequence, allowing however, for substitution of residues at certain positions with residues selected to improve or do not diminish activity of the humanized immunoglobulin (e.g., alter activity such that it more closely mimics the activity of the donor immunoglobulin) or selected to decrease the immunogenicity of the humanized immunoglobulin.

In one embodiment, the invention features an immunoglobulin light or heavy chain that includes at least one 15C11 variable region complementarity determining region (CDR) (e.g., includes one, two or three CDRs (i.e., CDRL1, CDRL2, or CDRL3) from the light chain variable region sequence set forth as SEQ ID NO:2 and/or includes one, two or three CDRs (i.e., CDRH1, CDRH2, and CDRH3) from the heavy chain variable region sequence set forth as SEQ ID NO:4) and includes a variable framework region derived from or substantially derived from a human acceptor immunoglobulin light or heavy chain sequence.

In one embodiment, the invention features an immunoglobulin light or heavy chain that includes at least one 15C11 variable region complementarity determining region (CDR) (i.e., includes one, two or three CDRs from the light chain

variable region sequence set forth as SEQ ID NO:2 and/or includes one, two or three CDRs from the heavy chain variable region sequence set forth as SEQ ID NO:4), and includes a variable framework region substantially from a human acceptor immunoglobulin light or heavy chain sequence.

In one embodiment, the invention features an immunoglobulin light or heavy chain that includes at least one 9G8 variable region complementarity determining region (CDR) (e.g., includes one, two or three CDRs (i.e., CDRL1, CDRL2, or CDRL3) from the light chain variable region sequence set forth as SEQ ID NO:8 and/or includes one, two or three CDRs (i.e., CDRH1, CDRH2, and CDRH3) from the heavy chain variable region sequence set forth as SEQ ID NO:5) and includes a variable framework region derived from or substantially derived from a human acceptor immunoglobulin light or heavy chain sequence.

In one embodiment, the invention features an immunoglobulin light or heavy chain that includes at least one 9G8 variable region complementarity determining region (CDR) (i.e., includes one, two or three CDRs from the light chain variable region sequence set forth as SEQ ID NO:8 and/or includes one, two or three CDRs from the heavy chain variable region sequence set forth as SEQ ID NO:5), and includes a variable framework region substantially from a human acceptor immunoglobulin light or heavy chain sequence.

In one embodiment, an immunoglobulin light or heavy chain of the invention, an antibody of the invention, or fragment thereof is monoclonal. In another embodiment, an immunoglobulin light or heavy chain of the invention, an antibody of the invention, or fragment thereof is chimeric. In another embodiment, an immunoglobulin light or heavy chain of the invention, an antibody of the invention, or fragment thereof is humanized.

In one embodiment, at least one amino acid residue of the framework region derived substantially from a human Ig heavy or light chain sequence in a humanized antibody, immunoglobulin heavy chain or fragment thereof is backmutated to (i.e., substituted with) a corresponding amino acid residue from the mouse antibody sequence (e.g., 15C11 or 9G8 light or heavy chain variable region sequence), wherein said backmutation does not substantially affect the ability of the chain to direct Aβ binding.

In another embodiment, the invention features a humanized immunoglobulin light or heavy chain that includes at least one 15C11 variable region complementarity determining region (CDR) (e.g., includes one, two or three CDRs from the light chain variable region sequence set forth as SEQ ID NO:2 and/or includes one, two or three CDRs from the heavy chain variable region sequence set forth as SEQ ID NO:4), and includes a variable framework region substantially from a human acceptor immunoglobulin light or heavy chain sequence.

In another embodiment, the invention features a humanized immunoglobulin light or heavy chain that includes at least one 9G8 variable region complementarity determining region (CDR) (e.g., includes one, two or three CDRs from the light chain variable region sequence set forth as SEQ ID NO:8 and/or includes one, two or three CDRs from the heavy chain variable region sequence set forth as SEQ ID NO:5), and includes a variable framework region substantially from a human acceptor immunoglobulin light or heavy chain sequence.

As disclosed herein, CDRs from central epitope monoclonal antibodies which are effective in preventing or treating an amyloidogenic disease in a patient are highly conserved. For example, as shown in FIG. **4**, CDRH1 regions of the 15C11, 9G8, and 266 antibodies are conserved, while that of the 6H9 antibody is more divergent in sequence. The same is true for the CDRH2 and CDRH3 for these antibodies. With respect to the light chain variable region, as is shown in FIG. **5**, the CDRL1 regions of the 15C11, 9G8, and 266 antibodies are all conserved. Similarly, the CDRL2 and CDRL3 regions for these antibodies are also conserved. Accordingly, various combinations of CDR sequences from these antibodies can be included in a composite antibody or antigen binding fragment thereof which comprises CDRs from at least two different antibodies (e.g., at least one CDR from a first antibody and at least one CDR from a second antibody).

For example, in one embodiment, the invention features a polypeptide comprising a light chain variable region where the light chain variable region comprises a first, a second, and a third complementarity determining region (CDR), where the first CDR comprises an amino acid sequence selected from the group consisting of: CDRL1 of the 9G8 antibody, CDRL1 of the 15C11 antibody, and CDRL1 of the 266 antibody; the second CDR comprises CDRL2 of the 15C11 antibody; and the third CDR comprises an amino acid sequence selected from the group consisting of: CDRL3 of the 9G8 antibody, amino CDRL3 of the 15C11 antibody, and CDRL3 of the 266 antibody; provided that where the first CDR comprises CDRL1 of the 266 antibody, the third CDR does not comprise CDRL3 of the 266 antibody and that where the third CDR comprises CDRL3 of the 266 antibody, the first CDR does not comprise CDRL1 of the 266 antibody.

In one embodiment, a polypeptide of the invention comprises CDRL1 of the 9G8 antibody and a CDRL3 of the 9G8 antibody. In one embodiment, a polypeptide of the invention comprises CDRL1 of the 9G8 antibody and a CDRL3 of the 15C11 antibody.

In one embodiment, a polypeptide of the invention comprises CDRL1 of the 15C11 antibody and a CDRL3 of the 9G8 antibody. In one embodiment, a polypeptide of the invention comprises CDRL1 of the 15C11 antibody and a CDRL3 of the 15C11 antibody. In one embodiment, a polypeptide of the invention comprises CDRL1 of the 15C11 antibody and a CDRL3 of the 9G8 antibody. In one embodiment, a polypeptide of the invention comprises CDRL1 of the 15C11 antibody and a CDRL3 of the 266 antibody.

In one embodiment, a polypeptide of the invention comprises CDRL1 of the 266 antibody and a CDRL3 of the 9G8 antibody. In one embodiment, a polypeptide of the invention comprises CDRL1 of the 266 antibody and a CDRL3 of the 15C11 antibody.

In another embodiment, the invention features a polypeptide comprising a heavy chain variable region where the heavy chain variable region comprises a first, a second, and a third, complementarity determining region (CDR), where the first CDR comprises an amino acid sequence selected from the group consisting of: CDRH1 from the 9G8 antibody and CDRH1 from the 15C11 antibody; the second CDR comprises an amino acid sequence selected from the group consisting of: CDRH2 from the 15C11 antibody, CDRH2 from the 9G8 antibody, and CDRH2 from the 266 antibody; and the third CDR comprises CDRH3 from the 15C11 antibody; provided that where the first CDR comprises CDRH1 from the 15C11 antibody, the second CDR does not comprise CDRH2 from the 266 antibody and that where the second CDR comprises CDRH2 from the 266 antibody, the first CDR does not comprise CDRH1 from the 15C11 antibody.

In one embodiment, a polypeptide of the invention comprises CDRH1 of the 9G8 antibody and a CDRH2 of the 9G8 antibody. In one embodiment, a polypeptide of the invention comprises CDRH1 of the 9G8 antibody and a CDRH2 of the

15C11 antibody. In one embodiment, a polypeptide of the invention comprises CDRH1 of the 9G8 antibody and a CDRH2 of the 266 antibody.

In one embodiment, a polypeptide of the invention comprises CDRH1 of the 15C11 antibody and a CDRH2 of the 15C11 antibody. In one embodiment, a polypeptide of the invention comprises CDRH1 of the 15C11 antibody and a CDRH2 of the 9G8 antibody.

In another embodiment, the invention pertains to a polypeptide comprising a light chain variable region where the light chain variable region comprises a first, a second, and a third complementarity determining region (CDR), wherein the first CDR comprises the amino acid sequence R S S Q S L X (where X is V or I) X (where X is H or Y) S X (where X is D or N) G N X (where X is T or A) X (where X is Y or F) X (where X is L or F) H; the second CDR comprises CDRL2 of the 15C1 antibody; and the third CDR comprises the amino acid sequence S Q S X (where X is T or A) H V X (where X is absent or is P) W T; provided that where the first CDR comprises CDRL1 of the 266 antibody, the third CDR does not comprise CDRL3 of the 266 antibody and that where the third CDR comprises CDRL3 of the 266 antibody, the first CDR does not comprise CDRL1 of the 266 antibody.

In another embodiment, the invention pertains to a polypeptide comprising a light chain variable region where the light chain variable region comprises a first, a second, and a third complementarity determining region (CDR), wherein the first CDR comprises the amino acid sequence R S S Q S L X (where X is V or I) X (where X is H or Y) S X (where X is D or N) G N X (where X is T or A) X (where X is Y or F) X (where X is L or F) H (SEQ ID NO: 12); the second CDR comprises CDRL2 of the 15C11 antibody; and the third CDR comprises the amino acid sequence S Q S X (where X is T or A) H V X (where X is absent or is P) W T (SEQ ID NO: 13); provided that where the first CDR comprises CDRL1 of the 266 antibody, the third CDR does not comprise CDRL3 of the 266 antibody and that where the third CDR comprises CDRL3 of the 266 antibody, the first CDR does not comprise CDRL1 of the 266 antibody.

In another embodiment, the invention pertains to a polypeptide comprising a heavy chain variable region where the heavy chain variable region comprises a first, a second, and a third, complementarity determining region (CDR), where the first CDR comprises the amino acid sequence G F T F S X (where X is R or D) Y X (where X is S or T) M S (SEQ ID NO: 10); the second CDR comprises the amino acid sequence X (where X is any amino acid) I X (where X is S or N) X (where X is N or S) X (where X is any amino acid) G X (where X is any amino acid) X (where X is N or S) T Y Y P D T X (where X is L or V) K G (SEQ ID NO: 11); and the third CDR comprises CDRH3 of the 15C11 antibody; provided that where the first CDR comprises CDRH1 of the 15C11 antibody, the second CDR does not comprise CDRH2 of the 266 antibody and that where the second CDR comprises CDRH2 of the 266 antibody, the first CDR does not comprise CDRH1 of the 15C11 antibody.

The 15C11 VL amino acid sequence is shown in SEQ ID NO:2 and the VH amino acid sequence is shown in SEQ ID NO:4. The 9G8 VL amino acid sequence is shown in SEQ ID NO:8 and the VH amino acid sequence is shown in SEQ ID NO:5. The 266 VL amino acid sequence is shown in SEQ ID NO:9 and the VH amino acid sequence is shown in SEQ ID NO:6.

It will be understood that the polypeptides comprising the light and heavy chain variable regions described herein can be combined to form antibody molecules or fragments thereof. For example, in one embodiment, a 15C11 variable region light chain can be combined with a variable region heavy chain from a 9G8 or 266 antibody or one of the composite variable region heavy chains described herein (e.g., comprising a first CDR comprising amino acids 26-35 of SEQ ID NO:5, a second CDR comprising amino acids 50-66 of SEQ ID NO:5, and a third CDR comprising amino acids 99-101 of SEQ ID NO:4; a first CDR comprising amino acids 26-35 of SEQ ID NO:5, a second CDR comprising amino acids 50-66 of SEQ ID NO:4, and a third CDR comprising amino acids 99-101 of SEQ ID NO:4; a first CDR comprising amino acids 26-35 of SEQ ID NO:5, and the second CDR comprising amino acids 50-66 of SEQ ID NO:6, and a third CDR comprising amino acids 99-101 of SEQ ID NO:4; a first CDR comprising amino acids 26-35 of SEQ ID NO:4, and the second CDR comprises amino acids 50-66 of SEQ ID NO:4, and a third CDR comprising amino acids 99-101 of SEQ ID NO:4; a first CDR comprising amino acids 26-35 of SEQ ID NO:4, a second CDR comprises amino acids 50-66 of SEQ ID NO:5, and a third CDR comprising amino acids 99-101 of SEQ ID NO:4). Similarly, a 9G8 variable region light chain can be combined with a variable region heavy chain from a 15C11 or 266 antibody or one of the composite variable region heavy chains described herein. Likewise, a 266 variable region light chain can be combined with a variable region heavy chain from a 9G8 or 15C11 antibody or one of the composite variable region heavy chains described herein

In another embodiment, a 15C11 variable region heavy chain can be combined with a variable region light chain from a 9G8 or 266 antibody or one of the composite variable region light chains described herein (e.g., comprising a first CDR comprising amino acids 24-39 of SEQ ID NO:8, a second CDR comprising amino acids 55-61 of SEQ ID NO:2, and a third CDR comprising amino acids 94-102 of SEQ ID NO:8; a first CDR comprising amino acids 24-39 of SEQ ID NO:8, a second CDR comprising amino acids 55-61 of SEQ ID NO:2, and a third CDR comprising amino acids 94-101 of SEQ ID NO:2; a first CDR comprising amino acids 24-39 of SEQ ID NO:8, a second CDR comprising amino acids 55-61 of SEQ ID NO:2, and a third CDR comprising amino acids 94-102 of SEQ ID NO:9; comprising a first CDR comprising amino acids 24-39 of SEQ ID NO:2, a second CDR comprising amino acids 55-61 of SEQ ID NO:2, and a third CDR comprising amino acids 94-101 of SEQ ID NO:2; a first CDR comprising amino acids 24-39 of SEQ ID NO:2, a second CDR comprising amino acids 55-61 of SEQ ID NO:2, and a third CDR comprising amino acids 94-102 of SEQ ID NO:8; a first CDR comprising amino acids 24-39 of SEQ ID NO:2, a second CDR comprising amino acids 55-61 of SEQ ID NO:2, and a third CDR comprising amino acids 94-102 of SEQ ID NO:9; a first CDR comprising amino acids 24-39 of SEQ ID NO:9, a second CDR comprising amino acids 55-61 of SEQ ID NO:2, and a third CDR comprising amino acids 94-102 of SEQ ID NO:8; a first CDR comprising amino acids 24-39 of SEQ ID NO:9, a second CDR comprising amino acids 55-61 of SEQ ID NO:2, and a third CDR comprising amino acids 94-101 of SEQ ID NO:2). Similarly, a 9G8 variable region heavy chain can be combined with a variable region light chain from a 15C11 or 266 antibody or one of the composite variable region light chains described herein. Likewise, a 266 variable region heavy chain can be combined with a variable region light chain from a 9G8 or 15C11 antibody or one of the composite variable region heavy chains described herein.

In one embodiment, in making a humanized antibody of the invention at least one framework residue is substituted with the corresponding amino acid residue from the mouse light or heavy chain variable region sequence (e.g., 15C11 or

9G8 sequence), where the framework residue for substitution is selected from the group consisting of (a) a residue that non-covalently binds antigen directly; (b) a residue adjacent to a CDR; (c) a CDR-interacting residue (e.g., identified by modeling the light or heavy chain on the solved structure of a homologous known immunoglobulin chain); and (d) a residue participating in the VL-VH interface.

In another embodiment, the invention features a humanized immunoglobulin light or heavy chain that includes variable region CDRs and variable framework regions from a human acceptor immunoglobulin light or heavy chain sequence, provided that at least one framework residue is substituted with the corresponding amino acid residue from the mouse light or heavy chain variable region sequence, where the framework residue is a residue capable of affecting light chain variable region conformation or function as identified by analysis of a three-dimensional model of the variable region, for example a residue capable of interacting with antigen, a residue proximal to the antigen binding site, a residue capable of interacting with a CDR, a residue adjacent to a CDR, a residue within 6 Å of a CDR residue, a canonical residue, a vernier zone residue, an interchain packing residue, an unusual residue, or a glycoslyation site residue on the surface of the structural model.

In another embodiment, the invention features, in addition to the substitutions described above, a substitution of at least one rare human framework residue. For example, a rare residue can be substituted with an amino acid residue which is common for human variable chain sequences at that position. Alternatively, a rare residue can be substituted with a corresponding amino acid residue from a homologous germline variable chain sequence.

In another embodiment, the invention features a humanized immunoglobulin that includes a light chain and a heavy chain, as described above, or an antigen-binding fragment or CDR of said immunoglobulin. In an exemplary embodiment, the humanized immunoglobulin binds (e.g., specifically binds) to beta amyloid peptide (Aβ) with a binding affinity of at least 10^7 M^{-1}, 10^8 M^{-1}, or 10^9 M^{-1}. In another embodiment, the immunoglobulin or antigen binding fragment includes a heavy chain having isotype γ1. In another embodiment, the immunoglobulin or antigen binding fragment binds (e.g., specifically binds) to either or both soluble beta amyloid peptide (Aβ) and aggregated Aβ. In another embodiment, the immunoglobulin or antigen binding fragment captures soluble Aβ (e.g., soluble Aβ1-42) circulating in the blood or present in the central nervous system (CNS) of a patient, thus preventing the accumulation of Aβ in the CNS and/or promoting the removal of Aβ from the CNS. Capture of soluble Aβ can lead to the rapid improvement of cognition in patient. In another embodiment, the invention features chimeric immunoglobulins that include at least one 15C11 or 9G8 or 266 CDR or at least one 15C11 or 9G8 or 266 or composite variable region (e.g., the variable region sequences set forth as SEQ ID NO:2 or 8 or SEQ ID NO:4 or 5). In yet another embodiment, the immunoglobulin, or antigen-binding fragment thereof, further includes at least one constant region from IgG1. In yet another embodiment, the immunoglobulin, or antigen-binding fragment thereof, further includes at least one constant region from IgG4.

The immunoglobulins described herein are particularly suited for use in therapeutic methods aimed at preventing or treating amyloidogenic diseases and/or the symptoms and/or behavioral deficits associated with amyloidogenic diseases or disorders. In one embodiment, the invention features a method of preventing or treating an amyloidogenic disease (e.g., Alzheimer's disease) that involves administering to the patient an effective dosage of a humanized immunoglobulin as described herein. In another embodiment, the invention features pharmaceutical compositions that include a humanized immunoglobulin as described herein and a pharmaceutical carrier. Also featured are isolated nucleic acid molecules, vectors and host cells for producing the immunoglobulins or immunoglobulin fragments or chains described herein, as well as methods for producing said immunoglobulins, immunoglobulin fragments or immunoglobulin chains

The present invention further features a method for identifying 15C11 or 9G8 amino acid residues amenable to substitution when producing a humanized immunoglobulin. For example, a method for identifying variable framework region residues amenable to substitution involves modeling the three-dimensional structure of a variable region on a solved homologous immunoglobulin structure and analyzing said model for residues capable of affecting immunoglobulin variable region conformation or function, such that residues amenable to substitution are identified. The invention further features use of the variable region sequence set forth as SEQ ID NO:2, 4, 8, or 5 or any portion thereof (or composite variable regions or portions thereof), in producing a three-dimensional image of a immunoglobulin, immunoglobulin chain, or domain thereof.

The present invention further features immunoglobulins having altered effector function, such as the ability to bind effector molecules, for example, complement or a receptor on an effector cell. In particular, the immunoglobulin of the invention has an altered constant region, e.g., Fc region, wherein at least one amino acid residue in the Fc region has been replaced with a different residue or side chain. In one embodiment, the modified immunoglobulin is of the IgG class, comprises at least one amino acid residue replacement in the Fc region such that the immunoglobulin has an altered effector function, e.g., as compared with an unmodified immunoglobulin. In particular embodiments, the immunoglobulin of the invention has an altered effector function such that it is less immunogenic (e.g., does not provoke undesired effector cell activity, lysis, or complement binding), has improved amyloid clearance properties, and/or has a desirable half-life.

The immunoglobulins of the present invention are capable of rapidly improving cognition in a patient. In one embodiment, the immunoglobulins of the invention are capable of capturing soluble Aβ (e.g., soluble Aβ1-42) circulating in the blood or present in the CNS, thereby preventing accumulation and/or promoting removal of soluble Aβ from the blood and/or CNS. This activity, e.g., rapid improvement in cognition, is demonstrated in in vivo assays wherein the immunoglobulin, e.g., 15C11, is administered in an animal model of Alzheimer's disease, e.g., a Tg2576 mouse, and the animal is tested for contextual fear conditioning. Significant improvement in contextual memory is seen in mice which were administered 15C11 over a relatively short period of time, suggesting that Aβ plaque clearance may not be necessary for efficacy. Accordingly, the immunoglobulins described herein, e.g., 15C11, may be administered to a patient, e.g., a subject suffering from Alzheimer's disease, to rapidly improve impairment status. In one embodiment, a single dose of antibody is administered to the patient, e.g., about 30 mg/kg.

Prior to further describing the invention, it may be helpful to an understanding thereof to set forth definitions of certain terms to be used hereinafter.

The term "Aβ-related disease or disorder" as used herein refers to a disease or disorder associated with, or character-

ized by, the development or presence of an Aβ peptide. In one embodiment, the Aβ-related disease or disorder is associated with or characterized by the presence of soluble Aβ. In another embodiment, the Aβ-related disease or disorder is associated with or characterized by the presence of insoluble Aβ. In another embodiment, the Aβ-related disease or disorder is associated with or characterized by the presence of a neuroactive Aβ species (NAβ). In another embodiment, the Aβ-related disease or disorder is also an amyloidogenic disorder. In another embodiment, the Aβ-related disease or disorder is characterized by an Aβ-related cognitive deficit or disorder, for example, an Aβ-related dementia disorder. Exemplary Aβ-related diseases or disorders include Alzheimer's disease (AD), Down's syndrome, cerebral amyloid angiopathy, certain vascular dementias, and mild cognitive impairment (MCI).

The terms "β-amyloid protein", "β-amyloid peptide", "β-amyloid", "Aβ" and "Aβ peptide" are used interchangeably herein. Aβ peptide (e.g., Aβ39, Aβ40, Aβ41, Aβ42 and Aβ43) is a ~4-kDa internal fragment of 39-43 amino acids of the larger transmembrane glycoprotein termed Amyloid Percursor Protein (APP). Multiple isoforms of APP exist, for example APP^{695}, APP^{751}, and APP^{770}. Amino acids within APP are assigned numbers according to the sequence of the APP^{770} isoform (see e.g., GenBank Accession No. P05067). Examples of specific isotypes of APP which are currently known to exist in humans are the 695 amino acid polypeptide described by Kang et al. (1987) *Nature* 325:733-736 which is designated as the "normal" APP; the 751 amino acid polypeptide described by Ponte et al. (1988) *Nature* 331:525-527 (1988) and Tanzi et al. (1988) *Nature* 331:528-530; and the 770-amino acid polypeptide described by Kitaguchi et al. (1988) *Nature* 331:530-532. As a result of proteolytic processing of APP by different secretase enzymes in vivo or in situ, Aβ is found in both a "short form", 40 amino acids in length, and a "long form", ranging from 42-43 amino acids in length. The short form, $Aβ_{40}$, consists of residues 672-711 of APP. The long form, e.g., $Aβ_{42}$ or $Aβ_{43}$, consists of residues 672-713 or 672-714, respectively. Part of the hydrophobic domain of APP is found at the carboxy end of Aβ, and may account for the ability of Aβ to aggregate, particularly in the case of the long form. Aβ peptide can be found in, or purified from, the body fluids of humans and other mammals, e.g. cerebrospinal fluid, including both normal individuals and individuals suffering from amyloidogenic disorders.

The terms "β-amyloid protein", "β-amyloid peptide", "β-amyloid", "Aβ" and "Aβ peptide" include peptides resulting from secretase cleavage of APP and synthetic peptides having the same or essentially the same sequence as the cleavage products. AP peptides of the invention can be derived from a variety of sources, for example, tissues, cell lines, or body fluids (e.g. sera or cerebrospinal fluid). For example, an Aβ can be derived from APP-expressing cells such as Chinese hamster ovary (CHO) cells stably transfected with $APP_{717V\rightarrow F}$, as described, for example, in Walsh et al., (2002), *Nature,* 416, pp 535-539. An Aβ preparation can be derived from tissue sources using methods previously described (see, e.g., Johnson-Wood et al., (1997), *Proc. Natl. Acad. Sci. USA* 94:1550). Alternatively, Aβ peptides can be synthesized using methods which are well known to those in the art. See, for example, Fields et al., Synthetic Peptides: A User's Guide, ed. Grant, W.H. Freeman & Co., New York, N.Y., 1992, p 77). Hence, peptides can be synthesized using the automated Merrifield techniques of solid phase synthesis with the α-amino group protected by either t-Boc or F-moc chemistry using side chain protected amino acids on, for example, an Applied Biosystems Peptide Synthesizer Model 430A or 431. Longer peptide antigens can be synthesized using well known recombinant DNA techniques. For example, a polynucleotide encoding the peptide or fusion peptide can be synthesized or molecularly cloned and inserted in a suitable expression vector for the transfection and heterologous expression by a suitable host cell. Aβ peptide also refers to related Aβ sequences that results from mutations in the Aβ region of the normal gene.

The term "soluble Aβ" or "dissociated Aβ" refers to non-aggregating or disaggregated Aβ polypeptide, including monomeric soluble as well as oligomeric soluble Aβ polypeptide (e.g., soluble Aβ dimers, trimers, and the like). Soluble Aβ can be found in vivo in biological fluids such as cerebrospinal fluid and/or serum. Soluble Aβ can also be prepared in vitro, e.g., by solubilizing Aβ peptide in appropriate solvents and/or solutions. For example, soluble Aβ can be prepared by dissolving lyophilized peptide in alcohol, e.g., HFIP followed by dilution into cold aqueous solution. Alternatively, soluble Aβ can be prepared by dissolving lyophilized peptide in neat DMSO with sonication. The resulting solution can be centrifuged (e.g., at 14,000×g, 4° C., 10 minutes) to remove any insoluble particulates.

The term "insoluble Aβ" or "aggregated Aβ" refers to aggregated Aβ polypeptide, for example, Aβ held together by noncovalent bonds and which can occur in the fibrillary, toxic, β-sheet form of Aβ peptide that is found in neuritic plaques and cerebral blood vessels of patients with AD. Aβ (e.g., Aβ42) is believed to aggregate, at least in part, due to the presence of hydrophobic residues at the C-terminus of the peptide (part of the transmembrane domain of APP).

As used herein, the phrase "neuroactive AD species" refers to an Aβ species (e.g., an Aβ peptide or form of Aβ peptide) that effects at least one activity or physical characteristic of a neuronal cell. Neuroactive Aβ species effect, for example, the function, biological activity, viability, morphology and/or architecture of a neuronal cell. The effect on neuronal cells can be cellular, for example, effecting the long-term-potentiation (LPT) of a neuronal cell or viability of a neuronal cell (neurotoxicity). Alternatively, the effect can be on an in vivo neuronal system, for example, effecting a behavioral outcome in an appropriate animal test (e.g., a cognitive test). The term "neutralize" as used herein means to make neutral, counteract or make ineffective an activity or effect.

As used herein, the term "neurodegenerative disease" refers broadly to disorders or diseases associated with or characterized by degeneration of neurons and/or nervous tissues, e.g. an amyloidogenic disease.

The term "amyloidogenic disease" or "amyloidogenic disorder" includes any disease associated with (or caused by) the formation or deposition of insoluble amyloid fibrils. Exemplary amyloidogenic diseases include, but are not limited to systemic amyloidosis, Alzheimer's disease (AD), cerebral amyloid angiopathy (CAA), mature onset diabetes, Parkinson's disease, Huntington's disease, fronto-temporal dementia, and the prion-related transmissible spongiform encephalopathies (kuru and Creutzfeldt-Jacob disease in humans and scrapie and BSE in sheep and cattle, respectively). Different amyloidogenic diseases are defined or characterized by the nature of the polypeptide component of the fibrils deposited. For example, in subjects or patients having Alzheimer's disease, β-amyloid protein (e.g., wild-type, variant, or truncated β-amyloid protein) is the principal polypeptide component of the amyloid deposit. Accordingly, Alzheimer's disease is an example of a "disease characterized by deposits of Aβ" or a "disease associated with deposits of Aβ", e.g., in the brain of a subject or patient. Other diseases characterized by deposits of Aβ can include uncharacterized diseases where amy-

loidogenic deposits are found in one or more regions of the brain associated with learning and/or memory, e.g., the hippocampus, amygdala, subiculum, cingulated cortex, prefrontal cortex, perirhinal cortex, sensory cortex, and medial temporal lobe.

The term "cognition" refers to cognitive mental processes performed by a patient including, but not limited to, learning or memory (e.g., short-term or long term learning or memory), knowledge, awareness, attention and concentration, judgement, visual recognition, abstract thinking, executive functions, language, visual-spatial (i.e., visuo-spatial orientation) skills, visual recognition, balance/agility and sensorimotor acitivity. Exemplary cognitive processes include learning and memory.

The terms "cognitive disorder", "cognitive deficit", or "cognitive impairment" are used interchangeably herein and refer to a deficiency or impairment in one or more cognitive mental processes of a patient. Cognitive deficits may have a number of origins: a functional mechanism (anxiety, depression), physiological aging (age-associated memory impairment), brain injury, psychiatric disorders (e.g. schizophrenia), drugs, infections, toxicants, or anatomical lesions. Exemplary cognitive deficits include deficiency or impairment in learning or memory (e.g., in short-term or long term learning and/or memory loss of intellectual abilities, judgment, language, motor skills, and/or abstract thinking).

As used herein, the term "Aβ-related cognitive disorder" (or "deficit" or "impairment") refers to a cognitive disorder associated with, or characterized by, the development or presence of an Aβ peptide. In one embodiment, the Aβ-related disease or disorder is associated with or characterized by the presence of soluble Aβ. In another embodiment, the Aβ-related disease or disorder is associated with or characterized by the presence of insoluble Aβ. In another embodiment, the Aβ-related disease or disorder is associated with or characterized by the presence of a neuroactive Aβ species (NAβ).

The term "dementia disorder", as used herein, refers to a disorder characterized by dementia (i.e., general deterioration or progressive decline of cognitive abilities or dementia-like symptoms). Dementia disorders are often associated with, or caused by, one or more aberrant processes in the brain or central nervous system (e.g. neurodegeneration). Dementia disorders commonly progress from mild through severe stages and interfere with the ability of a patient to function independently in everyday life. Dementia may be classified as cortical or subcortical depending on the area of the brain affected. Dementia disorders do not include disorders characterized by a loss of consciousness (as in delirium) or depression, or other functional mental disorders (pseudodementia). Dementia disorders include the irreversible dementias such Alzheimer's disease, vascular dementia, Lewy body dementia, Jakob-Creutzfeldt disease, Pick's disease, progressive supranuclear palsy, Frontal lobe dementia, idiopathic basal ganglia calcification, Huntington disease, multiple sclerosis, and Parkinson's disease, as well as reversible dementias due to trauma (posttraumatic encephalopathy), intracranial tumors (primary or metastatic), subdural hematomas, metabolic and endocrinologic conditions (hypo- and hyperthyroidism, Wilson's disease, uremic encephalopathy, dialysis dementia, anoxic and post-anoxic dementia, and chronic electrolyte disturbances), deficiency states (Vitamin B12 deficiency and pellagra (vitamin B6)), infections (AIDS, syphilitic meningoencephalitis, limbic encephalitis, progressive multifocal leukoencephalopathy, fungal infections, tuberculosis), and chronic exposure to alcohol, aluminum, heavy metals (arsenic, lead, mercury, manganese), or prescription drugs (anticholinergics, sedatives, barbiturates, etc.).

As used herein, the term "Aβ-related dementia disorder" refers to a dementia disorder associated with, or characterized by, the development or presence of an Aβ peptide.

As used herein, the phrase "improvement in cognition" refers to an enhancement or increase in a cognitive skill or function. Likewise, the phrase "improving cognition" refers to the enhancing or increasing of a cognitive skill or function. An improvement in cognition is relative, for example, to cognition in the patient before a treatment according to the instant invention. Preferably, the improvement in cognition trends towards that of a normal subject or towards a standard or expected level.

The term "rapid", as used, for example, in the phrase "rapid improvement in cognition" (or "rapidly improving cognition") means taking a relatively or comparatively short time or occurring within a comparatively short time interval; i.e., that an effect (e.g., improvement) is accomplished, observed or achieved comparatively quickly, in terms of clinical relevance.

An exemplary "rapid improvement in cognition" is accomplished, observed or achieved within one day (i.e., within 24 hours). A "rapid improvement in cognition" may be accomplished, observed or achieved in less than one day (i.e., less than 24 hours), for example, within 23, 22, 21, 20, 29, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 hour(s). A "rapid improvement in cognition" may alternatively be accomplished, observed or achieved in more than one day but preferably within one month, for example, within 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3 or 2 days. Exemplary time intervals for accomplishing, observing or achieving a rapid improvement in cognition are within weeks, e.g., within three weeks, within two weeks or within one week or within, for example, 120 hours, 96 hours, 72 hours, 48 hours, 24 hours, 18 hours, 12 hours and/or 6 hours.

The term "prolonged", as used, for example, in the phrase "prolonged improvement in cognition" means occurring over a comparatively or relatively longer time interval than a suitable control; i.e., that a desired effect (e.g., improvement) occurs or is observed to be sustained without interruption for an extended or protracted time period, in terms of clinical relevance.

An exemplary "prolonged improvement in cognition" is accomplished, observed or achieved for at least one week. A "prolonged improvement in cognition" may be accomplished, observed or achieved for more than one day (i.e., more than 24 hours), for example, for more than 36 hours, 48 hours (i.e., 2 days), 72 hours (i.e., 3 days), 96 hours (i.e., 4 days) 108 hours (i.e., 5 days) or 132 hours (i.e., 6 days). A "prolonged improvement in cognition" may alternatively be accomplished, observed or achieved for more than one week, e.g., for 8, 9, 10, 11, 12, 13, or 14 days (i.e., two weeks), three weeks, four weeks, five weeks, six weeks, or more. Exemplary time intervals over which a prolonged improvement in cognition is accomplished, observed or achieved include 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 days.

The term "modulation" as used herein refers to both upregulation, i.e. stimulation, and downregulation, i.e. suppression, of a response.

The term "treatment" as used herein, is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a

disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease or the predisposition toward disease.

The term "effective dose" or "effective dosage" is defined as an amount sufficient to achieve or at least partially achieve the desired effect. The term "therapeutically effective dose" is defined as an amount sufficient to cure or at least partially arrest the disease and its complications in a patient already suffering from the disease. Amounts effective for this use will depend upon the severity of the disease, the patient's general physiology. e.g., the patient's body mass, age, gender, the route of administration, and other factors well known to physicians and/or pharmacologists. Effective doses may be expressed, for example, as the total mass of antibody (e.g., in grams, milligrams or micrograms) or as a ratio of mass of antibody to body mass (e.g., as grams per kilogram (g/kg), milligrams per kilogram (mg/kg), or micrograms per kilogram (μg/kg). An effective dose of antibody used in the present methods will range, for example, between 1 μg/kg and 500 mg/kg. An exemplary range for effective doses of antibodies used in the methods of the present invention is between 0.1 mg/kg and 100 mg/kg. Exemplary effective doses include, but are not limited to, 10 μg/kg, 30 μg/kg, 100 μg/kg, 300 μg/kg, 1 mg/kg, 30 mg/kg and 100 mg/kg.

As used herein, the term "administering" refers to the act of introducing a pharmaceutical agent into a patient's body. An exemplary route of administration in the parenteral route, e.g., subcutaneous, intravenous or intraperitoneal administration.

The terms "patient" includes human and other mammalian subjects that receive either prophylactic or therapeutic treatment with one or more agents (e.g., immunotherapeutic agents) of the invention. Exemplary patients receive either prophylactic or therapeutic treatment with the immunotherapeutic agents of the invention.

The term "animal model" or "model animal", as used herein, includes a member of a mammalian species such as rodents, non-human primates, sheep, dogs, and cows that exhibit features or characteristics of a certain system of disease or disorder, e.g., a human system, disease or disorder. Exemplary non-human animals selected from the rodent family include rabbits, guinea pigs, rats and mice, most preferably mice. An "animal model" of, or "model animal" having, a dementia disorder exhibits, for example, prominent cognitive deficits associated with a dementia-related disorder (e.g., AD). Preferably the model animal exhibits a progressive worsening of the cognitive deficit with increasing age, such that the disease progression in the model animal parallels the disease progression in a patient suffering from the dementia disorder.

The term "immunological agent" or "immunological reagent" refers to an agent that comprises or consists of one or more immunoglobulins, antibodies, antibody fragments or antibody chains, as defined herein, or combinations thereof. The term "immunological agent" also includes nucleic acids encoding immunoglobulins, antibodies; antibody fragments, or antibody chains. Such nucleic acids can be DNA or RNA. A nucleic acid encoding an immunoglobulin is typically linked to regulatory elements, such as a promoter and enhancer, that allow expression of the nucleic acid in an appropriate cell or tissue.

The term "immunotherapeutic agent" refers to an agent that comprises or consists of one or more immunoglobulins, antibodies, antibody fragments or antibody chains, as defined herein, or combinations thereof, for therapeutic use. The term "immunotherapeutic agent" also includes nucleic acids encoding immunoglobulins, antibodies, antibody fragments, or antibody chains, for therapeutic use. Such nucleic acids can be DNA or RNA. A nucleic acid encoding an immunoglobulin is typically linked to regulatory elements, such as a promoter and enhancer, that allow expression of the nucleic acid in an intended target cell or tissue of a subject or patient.

The term "immunoglobulin" or "antibody" (used interchangeably herein) refers to a protein having a basic four-polypeptide chain structure consisting of two heavy and two light chains, said chains being stabilized, for example, by interchain disulfide bonds, which has the ability to specifically bind antigen. It is intended that the term "antibody" encompass any Ig class or any Ig subclass (e.g. the IgG1, IgG2, IgG3, and IgG4 subclassess of IgG) obtained from any source (e.g., in exemplary embodiments, humans and non-human primates, and in additional embodiments, rodents, lagomorphs, caprines, bovines, equines, ovines, etc.).

The term "Ig class" or "immunoglobulin class", as used herein, refers to the five classes of immunoglobulin that have been identified in humans and higher mammals, IgG, IgM, IgA, IgD, and IgE. The term "Ig subclass" refers to the two subclasses of IgM (H and L), three subclasses of IgA (IgA1, IgA2, and secretory IgA), and four subclasses of IgG (IgG_1, IgG_2, IgG_3, and IgG_4) that have been identified in humans and higher mammals.

The term "IgG subclass" refers to the four subclasses of immunoglobulin class IgG—IgG_1, IgG_2, IgG_3, and IgG_4 that have been identified in humans and higher mammals by the γ heavy chains of the immunoglobulins, γ_1-γ_4, respectively.

The term "single-chain immunoglobulin" or "single-chain antibody" (used interchangeably herein) refers to a protein having a two-polypeptide chain structure consisting of a heavy and a light chain, said chains being stabilized, for example, by interchain peptide linkers, which has the ability to specifically bind antigen. The term "domain" refers to a globular region of a heavy or light chain polypeptide comprising peptide loops (e.g., comprising 3 to 4 peptide loops) stabilized, for example, by β-pleated sheet and/or intrachain disulfide bond. Domains are further referred to herein as "constant" or "variable", based on the relative lack of sequence variation within the domains of various class members in the case of a "constant" domain, or the significant variation within the domains of various class members in the case of a "variable" domain. Antibody or polypeptide "domains" are often referred to interchangeably in the art as antibody or polypeptide "regions". The "constant" domains of an antibody light chain are referred to interchangeably as "light chain constant regions", "light chain constant domains", "CL" regions or "CL" domains. The "constant" domains of an antibody heavy chain are referred to interchangeably as "heavy chain constant regions", "heavy chain constant domains", "CH" regions or "CH" domains). The "variable" domains of an antibody light chain are referred to interchangeably as "light chain variable regions", "light chain variable domains", "VL" regions or "VL" domains). The "variable" domains of an antibody heavy chain are referred to interchangeably as "heavy chain constant regions", "heavy chain constant domains", "VH" regions or "VH" domains).

The term "region" can also refer to a part or portion of an antibody chain or antibody chain domain (e.g., a part or portion of a heavy or light chain or a part or portion of a constant or variable domain, as defined herein), as well as more discrete parts or portions of said chains or domains. For example, light and heavy chains or light and heavy chain variable domains include "complementarity determining regions" or "CDRs" interspersed among "framework regions" or "FRs", as defined herein.

Immunoglobulins or antibodies can exist in monomeric or polymeric form, for example, IgM antibodies which exist in pentameric form and/or IgA antibodies which exist in monomeric, dimeric or multimeric form. The term "fragment" refers to a part or portion of an antibody or antibody chain comprising fewer amino acid residues than an intact or complete antibody or antibody chain. Fragments can be obtained via chemical or enzymatic treatment of an intact or complete antibody or antibody chain. Fragments can also be obtained by recombinant means. Exemplary fragments include Fab, Fab', F(ab')2, Fabc and/or Fv fragments. The term "antigen-binding fragment" refers to a polypeptide fragment of an immunoglobulin or antibody that binds antigen or competes with intact antibody (i.e., with the intact antibody from which they were derived) for antigen binding (i.e., specific binding).

The term "conformation" refers to the tertiary structure of a protein or polypeptide (e.g., an antibody, antibody chain, domain or region thereof). For example, the phrase "light (or heavy) chain conformation" refers to the tertiary structure of a light (or heavy) chain variable region, and the phrase "antibody conformation" or "antibody fragment conformation" refers to the tertiary structure of an antibody or fragment thereof.

"Specific binding" of an antibody means that the antibody exhibits appreciable affinity for a particular antigen or epitope and, generally, does not exhibit significant crossreactivity. In exemplary embodiments, the antibody exhibits no crossreactivity (e.g., does not crossreact with non-Aβ peptides or with remote epitopes on Aβ. "Appreciable" or preferred binding includes binding with an affinity of at least 10^6, 10^7, 10^8, 10^9 M^{-1}, or 10^{10} M^{-1}. Affinities greater than 10^7 M^{-1}, preferably greater than 10^8 M^{-1} are more preferred. Values intermediate of those set forth herein are also intended to be within the scope of the present invention and a preferred binding affinity can be indicated as a range of affinities, for example, 10^6 to 10^{10} M^{-1}, preferably 10^7 to 10^{10} M^{-1}, more preferably 10^8 to 10^{10} M^{-1}. An antibody that "does not exhibit significant crossreactivity" is one that will not appreciably bind to an undesirable entity (e.g., an undesirable proteinaceous entity). For example, an antibody that specifically binds to Aβ will appreciably bind Aβ but will not significantly react with non-Aβ proteins or peptides (e.g., non-Aβ proteins or peptides included in plaques). An antibody specific for a particular epitope will, for example, not significantly crossreact with remote epitopes on the same protein or peptide. Specific binding can be determined according to any art-recognized means for determining such binding. Preferably, specific binding is determined according to Scatchard analysis and/or competitive binding assays.

As used herein, the term "affinity" refers to the strength of the binding of a single antigen-combining site with an antigenic determinant. Affinity depends on the closeness of stereochemical fit between antibody combining sites and antigen determinants, on the size of the area of contact between them, on the distribution of charged and hydrophobic groups, etc. Antibody affinity can be measured by equilibrium dialysis or by the kinetic BIACORE™ method. The BIACORE™ method relies on the phenomenon of surface plasmon resonance (SPR), which occurs when surface plasmon waves are excited at a metal/liquid interface. Light is directed at, and reflected from, the side of the surface not in contact with sample, and SPR causes a reduction in the reflected light intensity at a specific combination of angle and wavelength. Bimolecular binding events cause changes in the refractive index at the surface layer, which are detected as changes in the SPR signal.

The dissociation constant, KD, and the association constant, KA, are quantitative measures of affinity. At equilibrium, free antigen (Ag) and free antibody (Ab) are in equilibrium with antigen-antibody complex (Ag-Ab), and the rate constants, ka and kd, quantitate the rates of the individual reactions:

$$Ag + Ab \underset{kd}{\overset{ka}{\rightleftharpoons}} Ag - Ab$$

At equilibrium, ka [Ab][Ag]=kd [Ag-Ab]. The dissociation constant, KD, is given by: KD=kd/ka=[Ag][Ab]/[Ag-Ab]. KD has units of concentration, most typically M, mM, μM, nM, pM, etc. When comparing antibody affinities expressed as KD, having greater affinity for Aβ is indicated by a lower value. The association constant, KA, is given by: KA=KA/KD=[Ag-Ab]/[Ag][Ab]. KA has units of inverse concentration, most typically M^{-1}, mM^{-1}, μM^{-1}, nM^{-1}, pM^{-1}, etc. As used herein, the term "avidity" refers to the strength of the antigen-antibody bond after formation of reversible complexes.

Binding fragments are produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact immunoglobulins. Binding fragments include Fab, Fab', $F(ab')_2$, Fabc, Fv, single chains, and single-chain antibodies. Other than "bispecific" or "bifunctional" immunoglobulins or antibodies, an immunoglobulin or antibody is understood to have each of its binding sites identical. A "bispecific" or "bifunctional antibody" is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, e.g., Songsivilai & Lachmann, *Clin. Exp. Immunol.* 79:315-321 (1990); Kostelny et al., *J. Immunol.* 148, 1547-1553 (1992).

As used herein, the term "monoclonal antibody" refers to an antibody derived from a clona population of antibody-producing cells (e.g., B lymphocytes or B cells) which is homogeneous in structure and antigen specificity. The term "polyclonal antibody" refers to a plurality of antibodies originating from different clonal populations of antibody-producing cells which are heterogeneous in their structure and epitope specificity but which recognize a common antigen. Monoclonal and polyclonal antibodies may exist within bodily fluids, as crude preparations, or may be purified, as described herein.

The term "humanized immunoglobulin" or "humanized antibody" refers to an immunoglobulin or antibody that includes at least one humanized immunoglobulin or antibody chain (i.e., at least one humanized light or heavy chain). The term "humanized immunoglobulin chain" or "humanized antibody chain" (i.e., a "humanized immunoglobulin light chain" or "humanized immunoglobulin heavy chain") refers to an immunoglobulin or antibody chain (i.e., a light or heavy chain, respectively) having a variable region that includes a variable framework region substantially from a human immunoglobulin or antibody and complementarity determining regions (CDRs) (e.g., at least one CDR, preferably two CDRs, more preferably three CDRs) substantially from a non-human immunoglobulin or antibody, and further includes constant regions (e.g., at least one constant region or portion thereof, in the case of a light chain, and preferably three constant regions in the case of a heavy chain). The term "humanized variable region" (e.g., "humanized light chain variable region" or "humanized heavy chain variable region")

refers to a variable region that includes a variable framework region substantially from a human immunoglobulin or antibody and complementarity determining regions (CDRs) substantially from a non-human immunoglobulin or antibody. See, Queen et al., *Proc. Natl. Acad. Sci. USA* 86:10029-10033 (1989), U.S. Pat. Nos. 5,530,101, 5,585,089, 5,693,761, 5,693,762, Selick et al., WO 90/07861, and Winter, U.S. Pat. No. 5,225,539 (incorporated by reference in their entirety for all purposes).

A "humanized immunoglobulin" or "humanized antibody" of the invention can be made using any of the methods described herein or those that are well known in the art.

The phrase "substantially from a human immunoglobulin or antibody" or "substantially human" means that, when aligned to a human immunoglobulin or antibody amino sequence for comparison purposes, the region shares at least 80-90%, 90-95%, or 95-99% identity (i.e., local sequence identity) with the human framework or constant region sequence, allowing, for example, for conservative substitutions, consensus sequence substitutions, germline substitutions, backmutations, and the like. The introduction of conservative substitutions, consensus sequence substitutions, germline substitutions, backmutations, and the like, is often referred to as "optimization" of a humanized antibody or chain. The phrase "substantially from a non-human immunoglobulin or antibody" or "substantially non-human" means having an immunoglobulin or antibody sequence at least 80-95%, preferably at least 90-95%, more preferably, 96%, 97%, 98%, or 99% identical to that of a non-human organism, e.g., a non-human mammal.

Accordingly, all regions or residues of a humanized immunoglobulin or antibody, or of a humanized immunoglobulin or antibody chain, except possibly the CDRs, are substantially identical to the corresponding regions or residues of one or more native human immunoglobulin sequences. The term "corresponding region" or "corresponding residue" refers to a region or residue on a second amino acid or nucleotide sequence which occupies the same (i.e., equivalent) position as a region or residue on a first amino acid or nucleotide sequence, when the first and second sequences are optimally aligned for comparison purposes.

The term "significant identity" means that two polypeptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 60-70% sequence identity, more preferably at least 70-80% sequence identity, more preferably at least 80-90% identity, even more preferably at least 90-95% identity, and even more preferably at least 95% sequence identity or more (e.g., 99% sequence identity or more). The term "substantial identity" means that two polypeptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80-90% sequence identity, preferably at least 90-95% sequence identity, and more preferably at least 95% sequence identity or more (e.g., 99% sequence identity or more). For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection (see generally Ausubel et al., Current Protocols in Molecular Biology). One example of algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al., *J. Mol. Biol.* 215:403 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (publicly accessible through the National Institutes of Health NCBI internet server). Typically, default program parameters can be used to perform the sequence comparison, although customized parameters can also be used. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)).

Preferably, residue positions which are not identical differ by conservative amino acid substitutions. For purposes of classifying amino acids substitutions as conservative or non-conservative, amino acids are grouped as follows: Group I (hydrophobic sidechains): leu, met, ala, val, leu, ile; Group II (neutral hydrophilic side chains): cys, ser, thr; Group III (acidic side chains): asp, glu; Group IV (basic side chains): asn, gln, his, lys, arg; Group V (residues influencing chain orientation): gly, pro; and Group VI (aromatic side chains): trp, tyr, phe. Conservative substitutions involve substitutions between amino acids in the same class. Non-conservative substitutions constitute exchanging a member of one of these classes for a member of another class.

Preferably, humanized immunoglobulins or antibodies bind antigen with an affinity that is within a factor of three, four, or five of that of the corresponding non-humanized antibody. For example, if the nonhumanized antibody has a binding affinity of 10^9 M^{-1}, humanized antibodies will have a binding affinity of at least 3×10^9 M^{-1}, 4×10^9 M^{-1} or 5×10^9 M^{-1}. When describing the binding properties of an immunoglobulin or antibody chain, the chain can be described based on its ability to "direct antigen (e.g., Aβ) binding". A chain is said to "direct antigen binding" when it confers upon an intact immunoglobulin or antibody (or antigen binding fragment thereof) a specific binding property or binding affinity. A mutation (e.g., a backmutation) is said to substantially affect the ability of a heavy or light chain to direct antigen binding if it affects (e.g., decreases) the binding affinity of an intact immunoglobulin or antibody (or antigen binding fragment thereof) comprising said chain by at least an order of magnitude compared to that of the antibody (or antigen binding fragment thereof) comprising an equivalent chain lacking said mutation. A mutation "does not substantially affect (e.g., decrease) the ability of a chain to direct antigen binding" if it affects (e.g., decreases) the binding affinity of an intact immunoglobulin or antibody (or antigen binding fragment thereof) comprising said chain by only a factor of two, three, or four of that of the antibody (or antigen binding fragment thereof) comprising an equivalent chain lacking said mutation.

The term "chimeric immunoglobulin" or antibody refers to an immunoglobulin or antibody whose variable regions derive from a first species and whose constant regions derive from a second species. Chimeric immunoglobulins or antibodies can be constructed, for example by genetic engineering, from immunoglobulin gene segments belonging to different species. The terms "humanized immunoglobulin" or

"humanized antibody" are not intended to encompass chimeric immunoglobulins or antibodies, as defined infra. Although humanized immunoglobulins or antibodies are chimeric in their construction (i.e., comprise regions from more than one species of protein), they include additional features (i.e., variable regions comprising donor CDR residues and acceptor framework residues) not found in chimeric immunoglobulins or antibodies, as defined herein.

An "antigen" is an entity (e.g., a proteinaceous entity or peptide) to which an immunoglobulin or antibody (or antigen-binding fragment thereof) specifically binds.

The term "epitope" or "antigenic determinant" refers to a site on an antigen to which an immunoglobulin or antibody (or antigen binding fragment thereof) specifically binds. Epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, e.g., *Epitope Mapping Protocols in Methods in Molecular Biology*, Vol. 66, G. E. Morris, Ed. (1996).

Antibodies that recognize the same epitope can be identified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen, i.e., a competitive binding assay. Competitive binding is determined in an assay in which the immunoglobulin under test inhibits specific binding of a reference antibody to a common antigen, such as Aβ. Numerous types of competitive binding assays are known, for example: solid phase direct or indirect radioimmunoassay (RIA), solid phase direct or indirect enzyme immunoassay (EIA), sandwich competition assay (see Stahli et al., *Methods in Enzymology* 9:242 (1983)); solid phase direct biotin-avidin EIA (see Kirkland et al., *J. Immunol.* 137:3614 (1986)); solid phase direct labeled assay, solid phase direct labeled sandwich assay (see Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press (1988)); solid phase direct label RIA using I-125 label (see Morel et al., *Mol. Immunol.* 25(1):7 (1988)); solid phase direct biotin-avidin EIA (Cheung et al., *Virology* 176:546 (1990)); and direct labeled RIA. (Moldenhauer et al., *Scand. J. Immunol.* 32:77 (1990)). Typically, such an assay involves the use of purified antigen bound to a solid surface or cells bearing either of these, an unlabeled test immunoglobulin and a labeled reference immunoglobulin. Competitive inhibition is measured by determining the amount of label bound to the solid surface or cells in the presence of the test immunoglobulin. Usually the test immunoglobulin is present in excess. Usually, when a competing antibody is present in excess, it will inhibit specific binding of a reference antibody to a common antigen by at least 50-55%, 55-60%, 60-65%, 65-70% 70-75% or more.

An epitope is also recognized by immunologic cells, for example, B cells and/or T cells. Cellular recognition of an epitope can be determined by in vitro assays that measure antigen-dependent proliferation, as determined by ^{3}H-thymidine incorporation, by cytokine secretion, by antibody secretion, or by antigen-dependent killing (cytotoxic T lymphocyte assay).

Exemplary epitopes or antigenic determinants to which an antibody of the invention binds can be found within the human amyloid precursor protein (APP), but are preferably found within the Aβ peptide of APP. Exemplary epitopes or antigenic determinants within Aβ, as described herein, are located within the N-terminus, central region, or C-terminus of Aβ.

An "N-terminal epitope", is an epitope or antigenic determinant comprising residues located within the N-terminus of Aβ peptide. Exemplary N-terminal epitopes include residues within amino acids 1-10 of Aβ, preferably from residues 1-3, 1-4, 1-5, 1-6, 1-7, 2-6, 3-6, or 3-7 of Aβ42. Other exemplary N-terminal epitopes start at residues 1-3 and end at residues 7-11 of Aβ. Additional exemplary N-terminal epitopes include residues 2-4, 5, 6, 7 or 8 of Aβ, residues 3-5, 6, 7, 8 or 9 of Aβ, or residues 4-7, 8, 9 or 10 of Aβ42.

"Central epitopes" are epitopes or antigenic determinants comprising residues located within the central or mid-portion of the Aβ peptide. Exemplary central epitopes include residues within amino acids 10-18, 16-21, 16-22, 16-23, 16-24, 18-21, 19-21, 19-22, 19-23, or 19-24 of Aβ.

"C-terminal epitopes" are epitopes or antigenic determinants comprising residues located within the central or mid-portion of the Aβ peptide. Additional exemplary epitopes or antigenic determinants include residues 33-40 or 33-42 of Aβ. Such epitopes can be referred to as "C-terminal epitopes".

When an antibody is said to bind to an epitope within specified residues, such as within Aβ 13-28, what is meant is that the antibody specifically binds to a polypeptide containing the specified residues (i.e., Aβ 13-28, inclusive, in this example). Such an antibody does not necessarily contact every residue within Aβ 13-28. Nor does substitution or deletion of every individual amino acid residue within Aβ 13-28 necessarily significantly affect binding affinity.

The terms "Aβ antibody" and "anti-Aβ" are used interchangeably herein to refer to an antibody that binds to one or more epitopes or antigenic determinants within Aβ protein. Exemplary Aβ antibodies include N-terminal Aβ antibodies, central Aβ antibodies, and C-terminal Aβ antibodies. As used herein, the term "N-terminal Aβ antibody" shall refer to an Aβ antibody that recognizes at least one N-terminal epitope or antigenic determinant. As used herein, the term "central Aβ antibody" shall refer to an Aβ antibody that recognizes at least one central epitope or antigenic determinant. As used herein, the term "C-terminal Aβ antibody" shall refer to an Aβ antibody that recognizes at least one C-terminal epitope or antigenic determinant.

As used herein, the term "antigen binding site" refers to a site that specifically binds (immunoreacts with) an antigen (e.g., a cell surface or soluble antigen). Antibodies of the invention preferably comprise at least two antigen binding sites. An antigen binding site commonly includes immunoglobulin heavy chain and light chain CDRs and the binding site formed by these CDRs determines the specificity of the antibody. An "antigen binding region" or "antigen binding domain" is a region or domain (e.g., an antibody region or domain that includes an antibody binding site as defined herein).

An "immunogenic agent" or "immunogen" is capable of inducing an immunological response against itself on administration to a mammal, optionally in conjunction with an adjuvant.

As used herein, the term "immunotherapy" refers to a treatment, for example, a therapeutic or prophylactic treatment, of a disease or disorder intended to and/or producing an immune response (e.g., an active or passive immune response).

The term "adjuvant" refers to a compound that when administered in conjunction with an antigen augments the

immune response to the antigen, but when administered alone does not generate an immune response to the antigen. Adjuvants can augment an immune response by several mechanisms including lymphocyte recruitment, stimulation of B and/or T cells, and stimulation of macrophages.

As used herein, the term "kit" is used in reference to a combination of reagents and other materials which facilitate sample analysis. In some embodiments, the immunoassay kit of the present invention includes a suitable antigen, binding agent comprising a detectable moiety, and detection reagents. A system for amplifying the signal produced by detectable moieties may or may not also be included in the kit. Furthermore, in other embodiments, the kit includes, but is not limited to, components such as apparatus for sample collection, sample tubes, holders, trays, racks, dishes, plates, instructions to the kit user, solutions or other chemical reagents, and samples to be used for standardization, normalization, and/or control samples.

Various methodologies of the instant invention include a step that involves comparing a value, level, feature, characteristic, property, etc. to a "suitable control", referred to interchangeably herein as an "appropriate control". A "suitable control" or "appropriate control" is any control or standard familiar to one of ordinary skill in the art useful for comparison purposes. In one embodiment, a "suitable control" or "appropriate control" is a value, level, feature, characteristic, property, etc. determined prior to performing a methodology of the invention, as described herein. In another embodiment, a "suitable control" or "appropriate control" is a value, level, feature, characteristic, property, etc. determined in a patient, e.g., a control or normal subject exhibiting, for example, normal traits. In yet another embodiment, a "suitable control" or "appropriate control" is a predefined value, level, feature, characteristic, property, etc.

"Capturing soluble Aβ" refers to binding of soluble Aβ which is present in the plasma, e.g., as part of protein complexes or in the central nervous system, by an immunoglobulin, thereby preventing accumulation of Aβ and/or promoting removal of Aβ from the CNS.

The term "Fc immunoglobulin variant" or "Fc antibody variant" includes immunoglobulins or antibodies (e.g., humanized immunoglobulins, chimeric immunoglobulins, single chain antibodies, antibody fragments, etc.) having an altered Fc region. Fc regions can be altered, for example, such that the immunoglobulin has an altered effector function. In some embodiments, the Fc region includes one or more amino acid alterations in the hinge region, for example, at EU positions 234, 235, 236 and/or 237. Antibodies including hinge region mutations at one or more of amino acid positions 234, 235, 236 and/or 237, can be made, as described in, for example, U.S. Pat. Nos. 5,624,821, and 5,648,260, incorporated by reference herein.

The term "effector function" refers to an activity that resides in the Fc region of an antibody (e.g., an IgG antibody) and includes, for example, the ability of the antibody to bind effector molecules such as complement and/or Fc receptors, which can control several immune functions of the antibody such as effector cell activity, lysis, complement-mediated activity, antibody clearance, and antibody half-life.

The term "effector molecule" refers to a molecule that is capable of binding to the Fc region of an antibody (e.g., an IgG antibody) including, but not limited to, a complement protein or a Fc receptor.

The term "effector cell" refers to a cell capable of binding to the Fc portion of an antibody (e.g., an IgG antibody) typically via an Fc receptor expressed on the surface of the effector cell including, but not limited to, lymphocytes, e.g., antigen presenting cells and T cells.

The term "Fc region" refers to a C-terminal region of an IgG antibody, in particular, the C-terminal region of the heavy chain(s) of said IgG antibody. Although the boundaries of the Fc region of an IgG heavy chain can vary slightly, a Fc region is typically defined as spanning from about amino acid residue Cys226 to the carboxyl-terminus of a human IgG heavy chain(s).

The term "aglycosylated" antibody refers to an antibody lacking one or more carbohydrates by virtue of a chemical or enzymatic process, mutation of one or more glycosylation sites, expression in bacteria, etc. An aglycosylated antibody may be a deglycosylated antibody, that is an antibody for which the Fc carbohydrate has been removed, for example, chemically or enzymatically. Alternatively, the aglycosylated antibody may be a nonglycosylated or unglycosylated antibody, that is an antibody that was expressed without Fc carbohydrate, for example by mutation of one or more residues that encode the glycosylation pattern or by expression in an organism that does not attach carbohydrates to proteins, for example bacteria.

"Kabat numbering" unless otherwise stated, is as taught in Kabat et al. (Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)), expressly incorporated herein by reference. "EU numbering" unless otherwise stated, is also taught in Kabat et al. (Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)) and, for example, refers to the numbering of the residues in heavy chain antibody sequences using the EU index as described therein. This numbering system is based on the sequence of the Eu antibody described in Edelman et al., 63(1):78-85 (1969).

The term "Fc receptor" or "FcR" refers to a receptor that binds to the Fc region of an antibody. Typical Fc receptors which bind to an Fc region of an antibody (e.g., an IgG antibody) include, but are not limited to, receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. Other Fc receptors include the neonatal Fc receptors (FcRn) which regulate antibody half-life. Fc receptors are reviewed in Ravetch and Kinet, Annu. Rev. Immunol 9:457-92 (1991); Capel et al., Immunomethods 4:25-34 (1994); and de Haas et al., J. Lab. Clin. Med. 126:330-41 (1995).

I. Immunological and Therapeutic Reagents

Immunological and therapeutic reagents of the invention comprise or consist of immunoglobulins or antibodies, or functional or antigen binding fragments thereof, as defined herein. The basic antibody structural unit is known to comprise a tetramer of subunits. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function.

Light chains are classified as either kappa or lambda and are about 230 residues in length. Heavy chains are classified as gamma (γ), mu (μ), alpha (α), delta (δ), or epsilon (ϵ), are about 450-600 residues in length, and define the antibody's isotype as IgG, IgM, IgA, IgD and IgE, respectively. Both heavy and light chains are folded into domains. The term "domain" refers to a globular region of a protein, for example, an immunoglobulin or antibody. Immunoglobulin or antibody domains include, for example, three or four peptide

loops stabilized by β-pleated sheet and an interchain disulfide bond. Intact light chains have, for example, two domains (V_L and C_L) and intact heavy chains have, for example, four or five domains (V_H, C_H1, C_H2, and C_H3).

Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. (See generally, *Fundamental Immunology* (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989), Ch. 7, incorporated by reference in its entirety for all purposes).

The variable regions of each light/heavy chain pair form the antibody binding site. Thus, an intact antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are the same. The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. Naturally-occurring chains or recombinantly produced chains can be expressed with a leader sequence which is removed during cellular processing to produce a mature chain. Mature chains can also be recombinantly produced having a non-naturally occurring leader sequence, for example, to enhance secretion or alter the processing of a particular chain of interest.

The CDRs of the two mature chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. "FR4" also is referred to in the art as the D/J region of the variable heavy chain and the J region of the variable light chain. The assignment of amino acids to each domain is in accordance with the definitions of Kabat, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987 and 1991). An alternative structural definition has been proposed by Chothia et al., *J. Mol. Biol.* 196:901 (1987); *Nature* 342:878 (1989); and *J. Mol. Biol.* 186:651 (1989) (hereinafter collectively referred to as "Chothia et al." and incorporated by reference in their entirety for all purposes).

A. Aβ Antibodies

Therapeutic agents of the invention include antibodies that specifically bind to Aβ or to other components of the amyloid plaque. Preferred antibodies are monoclonal antibodies. Some such antibodies bind specifically to the aggregated form of Aβ without binding to the soluble form. Some bind specifically to the soluble form without binding to the aggregated form. Some bind to both aggregated and soluble forms. Some antibodies bind Aβ in plaques. Some antibodies can cross the blood-brain barrier. Some antibodies can reduce amyloid burden in a patient. Some antibodies can reduce neuritic dystrophy in a patient. Some antibodies can maintain synaptic architecture (e.g., synaptophysin). Antibodies used in therapeutic methods can have an intact constant region or at least sufficient of the constant region to interact with an Fc receptor. Some antibodies are efficacious at stimulating Fc-mediated phagocytosis of Aβ in plaques. Exemplary human isotype include IgG1 and IgG4. Human IgG1 is the equivalent of murine IgG2a and human IgG4 is the equivalent of murine IgG1. Thus, the latter are suitable for testing in vivo efficacy in animal (e.g., mouse) models of Alzheimer's. Bispecific Fab fragments can also be used, in which one arm of the antibody has specificity for Aβ, and the other for an Fc receptor. In exemplary embodiments, antibodies bind to Aβ with a binding affinity greater than (or equal to) about 10^6 M^{-1}, 10^7 M^{-1}, 10^8 M^-, 10^9 M^{-1}, or 10^{10} M^{-1} (including affinities intermediate of these values). In other exemplary embodiments, antibodies bind to Aβ with a binding

Preferred antibodies also include those antibodies which are capable of capturing soluble Aβ, e.g., in the bloodstream or CNS of a patient. Preferred antibodies are capable of rapidly improving cognition in a patient, e.g., via capture of soluble Aβ.

Monoclonal antibodies bind to a specific epitope within Aβ that can be a conformational or nonconformational epitope. Prophylactic and therapeutic efficacy of antibodies can be tested in an animal model, e.g., using the transgenic animal model procedures described in the Examples. Preferred monoclonal antibodies bind to an epitope within residues 13-28 of Aβ (with the first N terminal residue of natural Aβ designated 1), more preferably to an epitope within residues 19-22 of Aβ. In some methods, multiple monoclonal antibodies having binding specificities to different epitopes are used, for example, an antibody specific for an epitope within residues 19-22 of Aβ can be co-administered with an antibody specific for an epitope outside of residues 19-22 of Aβ. Such antibodies can be administered sequentially or simultaneously. Antibodies to amyloid components other than Aβ can also be used in combination with the instant reagents, (e.g., administered or co-administered).

Epitope specificity of an antibody can be determined, for example, by forming a phage display library in which different members of the library display different subsequences of Aβ. The phage display library is then screened for members specifically bind to an antibody under test. A family of sequences is selected and isolated. Typically, such a family contains a common core sequence, and varying lengths of flanking sequences in different members. The shortest core sequence showing specific binding to the antibody defines the epitope bound by the antibody. Antibodies can also be tested for epitope specificity in a competition assay with an antibody whose epitope specificity has already been determined. For example, antibodies that compete with the 15C11 antibody for binding to Aβ bind to the same or similar epitope as 15C11, i.e., within residues Aβ 19-22. Screening antibodies for epitope specificity is a useful predictor of therapeutic efficacy. For example, an antibody determined to bind to an epitope within residues 13-28 (e.g., to Aβ 19-22) of Aβ is likely to be effective in preventing and treating Alzheimer's disease according to the methodologies of the present invention.

Antibodies that specifically bind to a preferred segment of Aβ without binding to other regions of Aβ have a number of advantages relative to monoclonal antibodies binding to other regions or polyclonal sera to intact Aβ. First, for equal mass dosages, dosages of antibodies that specifically bind to preferred segments contain a higher molar dosage of antibodies effective in clearing amyloid plaques. Second, antibodies specifically binding to preferred segments can induce a clearing response against amyloid deposits without inducing a clearing response against intact APP polypeptide, thereby reducing the potential side effects.

1. Production of Nonhuman Antibodies

The present invention features non-human antibodies, for example, antibodies having specificity for the preferred Aβ epitopes of the invention. Such antibodies can be used in formulating various therapeutic compositions of the invention or, preferably, provide complementarity determining regions for the production of humanized or chimeric antibodies (described in detail below). The production of non-human monoclonal antibodies, e.g., murine, guinea pig, primate, rabbit or rat, can be accomplished by, for example, immunizing the animal with Aβ. A longer polypeptide comprising Aβ or an immunogenic fragment of Aβ or anti-idiotypic antibodies to an antibody to Aβ can also be used. (See Harlow &

Lane, supra, incorporated by reference for all purposes). Such an immunogen can be obtained from a natural source, by peptide synthesis or by recombinant expression. Optionally, the immunogen can be administered fused or otherwise complexed with a carrier protein, as described below. Optionally, the immunogen can be administered with an adjuvant. The term "adjuvant" refers to a compound that when administered in conjunction with an antigen augments the immune response to the antigen, but when administered alone does not generate an immune response to the antigen. Adjuvants can augment an immune response by several mechanisms including lymphocyte recruitment, stimulation of B and/or T cells, and stimulation of macrophages. Several types of adjuvant can be used as described below. Complete Freund's adjuvant followed by incomplete adjuvant is preferred for immunization of laboratory animals.

Rabbits or guinea pigs are typically used for making polyclonal antibodies. Exemplary preparation of polyclonal antibodies, e.g., for passive protection, can be performed as follows. 125 non-transgenic mice are immunized with 100 μg Aβ1-42, plus CFA/IFA adjuvant, and euthanized at 4-5 months. Blood is collected from immunized mice. IgG is separated from other blood components. Antibody specific for the immunogen may be partially purified by affinity chromatography. An average of about 0.5-1 mg of immunogen-specific antibody is obtained per mouse, giving a total of 60-120 mg.

Mice are typically used for making monoclonal antibodies. Monoclonals can be prepared against a fragment by injecting the fragment or longer form of Aβ into a mouse, preparing hybridomas and screening the hybridomas for an antibody that specifically binds to Aβ. Optionally, antibodies are screened for binding to a specific region or desired fragment of Aβ without binding to other nonoverlapping fragments of Aβ. The latter screening can be accomplished by determining binding of an antibody to a collection of deletion mutants of an Aβ peptide and determining which deletion mutants bind to the antibody. Binding can be assessed, for example, by Western blot or ELISA. The smallest fragment to show specific binding to the antibody defines the epitope of the antibody. Alternatively, epitope specificity can be determined by a competition assay is which a test and reference antibody compete for binding to Aβ. If the test and reference antibodies compete, then they bind to the same epitope or epitopes sufficiently proximal such that binding of one antibody interferes with binding of the other. Exemplary isotypes for such antibodies are mouse isotype IgG2a or IgG1, or equivalent isotypes in other species. Mouse isotype IgG2a is the equivalent of human isotype IgG1. Mouse isotype IgG1 is the equivalent of human isotype IgG4.

2. Chimeric and Humanized Antibodies

The present invention also features chimeric and/or humanized antibodies (i.e., chimeric and/or humanized immunoglobulins) specific for beta amyloid peptide. Chimeric and/or humanized antibodies have the same or similar binding specificity and affinity as a mouse or other nonhuman antibody that provides the starting material for construction of a chimeric or humanized antibody. A preferred antibody for humanization is the mouse 15C11 antibody described herein. The mouse 9G8 antibody is a further exemplary antibody for humanization, as described herein.

a. Production of Chimeric Antibodies

The term "chimeric antibody" refers to an antibody whose light and heavy chain genes have been constructed, typically by genetic engineering, from immunoglobulin gene segments belonging to different species. For example, the variable (V) segments of the genes from a mouse monoclonal antibody may be joined to human constant (C) segments, such as IgG1 and IgG4. Human isotypes IgG1 and IgG4 are exemplary. A typical chimeric antibody is thus a hybrid protein consisting of the V or antigen-binding domain from a mouse antibody and the C or effector domain from a human antibody.

b. Production of Humanized Antibodies

The term "humanized antibody" refers to an antibody comprising at least one chain comprising variable region framework residues substantially from a human antibody chain (referred to as the acceptor immunoglobulin or antibody) and at least one complementarity determining region substantially from a mouse antibody, (referred to as the donor immunoglobulin or antibody). See, Queen et al., *Proc. Natl. Acad. Sci. USA* 86:10029-10033 (1989), U.S. Pat. Nos. 5,530,101, 5,585,089, 5,693,761, 5,693,762, Selick et al., WO 90/07861, and Winter, U.S. Pat. No. 5,225,539 (incorporated by reference in their entirety for all purposes). The constant region(s), if present, are also substantially or entirely from a human immunoglobulin.

The substitution of mouse CDRs into a human variable domain framework is most likely to result in retention of their correct spatial orientation if the human variable domain framework adopts the same or similar conformation to the mouse variable framework from which the CDRs originated. This is achieved by obtaining the human variable domains from human antibodies whose framework sequences exhibit a high degree of sequence identity with the murine variable framework domains from which the CDRs were derived. The heavy and light chain variable framework regions can be derived from the same or different human antibody sequences. The human antibody sequences can be the sequences of naturally occurring human antibodies or can be consensus sequences of several human antibodies. See Kettleborough et al., *Protein Engineering* 4:773 (1991); Kolbinger et al., *Protein Engineering* 6:971 (1993) and Carter et al., WO 92/22653.

Having identified the complementarity determining regions of the murine donor immunoglobulin and appropriate human acceptor immunoglobulins, the next step is to determine which, if any, residues from these components should be substituted to optimize the properties of the resulting humanized antibody. In general, substitution of human amino acid residues with murine should be minimized, because introduction of murine residues increases the risk of the antibody eliciting a human-anti-mouse-antibody (HAMA) response in humans. Art-recognized methods of determining immune response can be performed to monitor a HAMA response in a particular patient or during clinical trials. Patients treated with humanized antibodies can be given an immunogenicity assessment at the beginning and throughout the administration of said therapy. The HAMA response is measured, for example, by detecting antibodies to the humanized therapeutic reagent, in serum samples from the patient using a method known to one in the art, including surface plasmon resonance technology (BIACORE) and/or solid-phase ELISA analysis.

Certain amino acid residues from the human variable region framework residues are selected for substitution based on their possible influence on CDR conformation and/or binding to antigen. The unnatural juxtaposition of murine CDR regions with human variable framework region can result in unnatural conformational restraints, which, unless corrected by substitution of certain amino acid residues, may lead to loss of binding affinity.

In one embodiment, the selection of amino acid residues for substitution is determined, in part, by computer modeling. Computer hardware and software are described herein for producing three-dimensional images of immunoglobulin

molecules. In general, molecular models are produced starting from solved structures for immunoglobulin chains or domains thereof. The chains to be modeled are compared for amino acid sequence similarity with chains or domains of solved three-dimensional structures, and the chains or domains showing the greatest sequence similarity is/are selected as starting points for construction of the molecular model. Chains or domains sharing at least 50% sequence identity are selected for modeling, and preferably those sharing at least 60%, 70%, 80%, 90% sequence identity or more are selected for modeling. The solved starting structures are modified to allow for differences between the actual amino acids in the immunoglobulin chains or domains being modeled, and those in the starting structure. The modified structures are then assembled into a composite immunoglobulin. Finally, the model is refined by energy minimization and by verifying that all atoms are within appropriate distances from one another and that bond lengths and angles are within chemically acceptable limits.

The selection of amino acid residues for substitution can also be determined, in part, by examination of the characteristics of the amino acids at particular locations, or empirical observation of the effects of substitution or mutagenesis of particular amino acids. For example, when an amino acid differs between a murine variable region framework residue and a selected human variable region framework residue, the human framework amino acid should usually be substituted by the equivalent framework amino acid from the mouse antibody when it is reasonably expected that the amino acid:

(1) noncovalently binds antigen directly,

(2) is adjacent to a CDR region,

(3) otherwise interacts with a CDR region (e.g., is within about 3-6 Å of a CDR region as determined by computer modeling), or

(4) participates in the VL-VH interface.

Residues which "noncovalently bind antigen directly" include amino acids in positions in framework regions which are have a good probability of directly interacting with amino acids on the antigen according to established chemical forces, for example, by hydrogen bonding, Van der Waals forces, hydrophobic interactions, and the like.

CDR and framework regions are as defined by Kabat et al. or Chothia et al., supra. When framework residues, as defined by Kabat et al., supra, constitute structural loop residues as defined by Chothia et al., supra, the amino acids present in the mouse antibody may be selected for substitution into the humanized antibody.

Residues which are "adjacent to a CDR region" include amino acid residues in positions immediately adjacent to one or more of the CDRs in the primary sequence of the humanized immunoglobulin chain, for example, in positions immediately adjacent to a CDR as defined by Kabat, or a CDR as defined by Chothia (See e.g., Chothia and Lesk J M B 196: 901 (1987)). These amino acids are particularly likely to interact with the amino acids in the CDRs and, if chosen from the acceptor, to distort the donor CDRs and reduce affinity. Moreover, the adjacent amino acids may interact directly with the antigen (Amit et al., Science, 233:747 (1986), which is incorporated herein by reference) and selecting these amino acids from the donor may be desirable to keep all the antigen contacts that provide affinity in the original antibody.

Residues that "otherwise interact with a CDR region" include those that are determined by secondary structural analysis to be in a spatial orientation sufficient to affect a CDR region. In one embodiment, residues that "otherwise interact with a CDR region" are identified by analyzing a three-dimensional model of the donor immunoglobulin (e.g., a computer-generated model). A three-dimensional model, typically of the original donor antibody, shows that certain amino acids outside of the CDRs are close to the CDRs and have a good probability of interacting with amino acids in the CDRs by hydrogen bonding, Van der Waals forces, hydrophobic interactions, etc. At those amino acid positions, the donor immunoglobulin amino acid rather than the acceptor immunoglobulin amino acid may be selected. Amino acids according to this criterion will generally have a side chain atom within about 3 angstrom units (Å) of some atom in the CDRs and must contain an atom that could interact with the CDR atoms according to established chemical forces, such as those listed above.

In the case of atoms that may form a hydrogen bond, the 3 Å is measured between their nuclei, but for atoms that do not form a bond, the 3 Å is measured between their Van der Waals surfaces. Hence, in the latter case, the nuclei must be within about 6 Å (3 Å plus the sum of the Van der Waals radii) for the atoms to be considered capable of interacting. In many cases the nuclei will be from 4 or 5 to 6 Å apart. In determining whether an amino acid can interact with the CDRs, it is preferred not to consider the last 8 amino acids of heavy chain CDR 2 as part of the CDRs, because from the viewpoint of structure, these 8 amino acids behave more as part of the framework.

Amino acids that are capable of interacting with amino acids in the CDRs, may be identified in yet another way. The solvent accessible surface area of each framework amino acid is calculated in two ways: (1) in the intact antibody, and (2) in a hypothetical molecule consisting of the antibody with its CDRs removed. A significant difference between these numbers of about 10 square angstroms or more shows that access of the framework amino acid to solvent is at least partly blocked by the CDRs, and therefore that the amino acid is making contact with the CDRs. Solvent accessible surface area of an amino acid may be calculated based on a three-dimensional model of an antibody, using algorithms known in the art (e.g., Connolly, J. Appl. Cryst. 16:548 (1983) and Lee and Richards, J. Mol. Biol. 55:379 (1971), both of which are incorporated herein by reference). Framework amino acids may also occasionally interact with the CDRs indirectly, by affecting the conformation of another framework amino acid that in turn contacts the CDRs.

The amino acids at several positions in the framework are known to be important for determining CDR confirmation (e.g., capable of interacting with the CDRs) in many antibodies (Chothia and Lesk, supra, Chothia et al., supra and Tramontano et al., J. Mol. Biol. 215:175 (1990), all of which are incorporated herein by reference). These authors identified conserved framework residues important for CDR conformation by analysis of the structures of several known antibodies. The antibodies analyzed fell into a limited number of structural or "canonical" classes based on the conformation of the CDRs. Conserved framework residues within members of a canonical class are referred to as "canonical" residues. Canonical residues include residues 2, 25, 29, 30, 33, 48, 64, 71, 90, 94 and 95 of the light chain and residues 24, 26, 29, 34, 54, 55, 71 and 94 of the heavy chain. Additional residues (e.g., CDR structure-determining residues) can be identified according to the methodology of Martin and Thorton (1996) *J. Mol. Biol.* 263:800. Notably, the amino acids at positions 2, 48, 64 and 71 of the light chain and 26-30, 71 and 94 of the heavy chain (numbering according to Kabat) are known to be capable of interacting with the CDRs in many antibodies. The amino acids at positions 35 in the light chain and 93 and 103 in the heavy chain are also likely to interact with the CDRs. Additional residues which may effect conformation of the

CDRs can be identified according to the methodology of Foote and Winter (1992) *J. Mol. Biol.* 224:487. Such residues are termed "vernier" residues and are those residues in the framework region closely underlying (i.e., forming a "platform" under) the CDRs. At all these numbered positions, choice of the donor amino acid rather than the acceptor amino acid (when they differ) to be in the humanized immunoglobulin is preferred. On the other hand, certain residues capable of interacting with the CDR region, such as the first 5 amino acids of the light chain, may sometimes be chosen from the acceptor immunoglobulin without loss of affinity in the humanized immunoglobulin.

Residues which "participate in the VL-VH interface" or "packing residues" include those residues at the interface between VL and VH as defined, for example, by Novotny and Haber, Proc. Natl. Acad. Sci. USA, 82:4592-66 (1985) or Chothia et al, supra. Generally, rare packing residues should be retained in the humanized antibody if they differ from those in the human frameworks.

In general, one or more of the amino acids fulfilling the above criteria can be substituted. In some embodiments, all or most of the amino acids fulfilling the above criteria are substituted. Occasionally, there is some ambiguity about whether a particular amino acid meets the above criteria, and alternative variant immunoglobulins are produced, one of which has that particular substitution, the other of which does not. Alternative variant immunoglobulins so produced can be tested in any of the assays described herein for the desired activity, and the preferred immunoglobulin selected.

Usually the CDR regions in humanized antibodies are substantially identical, and more usually, identical to the corresponding CDR regions of the donor antibody. However, in certain embodiments, it may be desirable to modify one or more CDR regions to modify the antigen binding specificity of the antibody and/or reduce the immunogenicity of the antibody. Typically, one or more residues of a CDR are altered to modify binding to achieve a more favored on-rate of binding, a more favored off-rate of binding, or both, such that an idealized binding constant is achieved. Using this strategy, an antibody having ultra high binding affinity of, for example, 10^{10} M^{-1} or more, can be achieved. Briefly, the donor CDR sequence is referred to as a base sequence from which one or more residues are then altered. Affinity maturation techniques, as described herein, can be used to alter the CDR region(s) followed by screening of the resultant binding molecules for the desired change in binding. The method may also be used to alter the donor CDR, typically a mouse CDR, to be less immunogenic such that a potential human anti-mouse antibody (HAMA) response is minimized or avoided. Accordingly, as CDR(s) are altered, changes in binding affinity as well as immunogenicity can be monitored and scored such that an antibody optimized for the best combined binding and low immunogenicity are achieved (see, e.g., U.S. Pat. No. 6,656,467 and U.S. Pat. Pub. US20020164326A1).

In another approach, the CDR regions of the antibody are analyzed to determine the contributions of each individual CDR to antibody binding and/or immunogenicity by systemically replacing each of the donor CDRs with a human counterpart. The resultant panel of humanized antibodies is then scored for antigen affinity and potential immunogenicity of each CDR. In this way, the two clinically important properties of a candidate binding molecule, i.e., antigen binding and low immunogenicity, are determined. If patient sera against a corresponding murine or CDR-grafted (humanized) form of the antibody is available, then the entire panel of antibodies representing the systematic human CDR exchanges can be screened to determine the patients anti-idiotypic response against each donor CDR (for technical details, see, e.g., Iwashi et al., *Mol. Immunol.* 36:1079-91 (1999). Such an approach allows for identifying essential donor CDR regions from non-essential donor CDRs. Nonessential donor CDR regions may then be exchanged with a human counterpart CDR. Where an essential CDR region cannot be exchanged without unacceptable loss of function, identification of the specificity-determining residues (SDRs) of the CDR is performed by, for example, site-directed mutagenesis. In this way, the CDR can then be reengineered to retain only the SDRs and be human and/or minimally immunogenic at the remaining amino acid positions throughout the CDR. Such an approach, where only a portion of the donor CDR is grafted, is also referred to as abbreviated CDR-grafting (for technical details on the foregoing techniques, see, e.g., Tamura et al., *J. of Immunology* 164(3):1432-41. (2000); Gonzales et al., *Mol. Immunol* 40:337-349 (2003); Kashmiri et al., *Crit. Rev. Oncol. Hematol.* 38:3-16 (2001); and De Pascalis et al., *J. of Immunology* 169(6):3076-84. (2002).

Moreover, it is sometimes possible to make one or more conservative amino acid substitutions of CDR residues without appreciably affecting the binding affinity of the resulting humanized immunoglobulin. By conservative substitutions are intended combinations such as gly, ala; val, ile, leu; asp, glu; asn, gin; ser, thr; lys, arg; and phe, tyr.

Additional candidates for substitution are acceptor human framework amino acids that are "rare" for a human immunoglobulin at that position. These amino acids can be substituted with amino acids from the equivalent position of the mouse donor antibody or from the equivalent positions of more typical human immunoglobulins. For example, substitution may be desirable when the amino acid in a human framework region of the acceptor immunoglobulin is rare for that position and the corresponding amino acid in the donor immunoglobulin is common for that position in human immunoglobulin sequences; or when the amino acid in the acceptor immunoglobulin is rare for that position and the corresponding amino acid in the donor immunoglobulin is also rare, relative to other human sequences. Whether a residue is rare for acceptor human framework sequences should also be considered when selecting residues for backmutation based on contribution to CDR conformation. For example, if backmutation results in substitution of a residue that is rare for acceptor human framework sequences, a humanized antibody may be tested with and without for activity. If the backmutation is not necessary for activity, it may be eliminated to reduce immunogenicity concerns. For example, backmutation at the following residues may introduce a residue that is rare in acceptor human framework sequences; vl=V2(2.0%), L3 (0.4%), T7 (1.8%), Q18 (0.2%), L83 (1.2%), I85 (2.9%), A100 (0.3%) and L106 (1.1%); and vh=T3 (2.0%), K5 (1.8%), I11 (0.2%), S23 (1.5%), F24 (1.5%), S41 (2.3%), K71 (2.4%), R75 (1.4%), I82 (1.4%), D83 (2.2%) and L109 (0.8%). These criteria help ensure that an atypical amino acid in the human framework does not disrupt the antibody structure. Moreover, by replacing a rare human acceptor amino acid with an amino acid from the donor antibody that happens to be typical for human antibodies, the humanized antibody may be made less immunogenic.

The term "rare", as used herein, indicates an amino acid occurring at that position in less than about 20%, preferably less than about 10%, more preferably less than about 5%, even more preferably less than about 3%, even more preferably less than about 2% and even more preferably less than about 1% of sequences in a representative sample of sequences, and the term "common", as used herein, indicates an amino acid occurring in more than about 25% but usually

more than about 50% of sequences in a representative sample. For example, when deciding whether an amino acid in a human acceptor sequence is "rare" or "common", it will often be preferable to consider only human variable region sequences and when deciding whether a mouse amino acid is "rare" or "common", only mouse variable region sequences. Moreover, all human light and heavy chain variable region sequences are respectively grouped into "subgroups" of sequences that are especially homologous to each other and have the same amino acids at certain critical positions (Kabat et al., supra). When deciding whether an amino acid in a human acceptor sequence is "rare" or "common" among human sequences, it will often be preferable to consider only those human sequences in the same subgroup as the acceptor sequence.

Additional candidates for substitution are acceptor human framework amino acids that would be identified as part of a CDR region under the alternative definition proposed by Chothia et al., supra. Additional candidates for substitution are acceptor human framework amino acids that would be identified as part of a CDR region under the AbM and/or contact definitions.

Additional candidates for substitution are acceptor framework residues that correspond to a rare donor framework residue. Rare donor framework residues are those that are rare (as defined herein) for murine antibodies at that position. For murine antibodies, the subgroup can be determined according to Kabat and residue positions identified which differ from the consensus. These donor specific differences may point to somatic mutations in the murine sequence which enhance activity. Rare residues that are predicted to affect binding (e.g., packing canonical and/or vernier residues) are retained, whereas residues predicted to be unimportant for binding can be substituted.

Additional candidates for substitution are non-germline residues occurring in an acceptor framework region. For example, when an acceptor antibody chain (i.e., a human antibody chain sharing significant sequence identity with the donor antibody chain) is aligned to a germline antibody chain (likewise sharing significant sequence identity with the donor chain), residues not matching between acceptor chain framework and the germline chain framework can be substituted with corresponding residues from the germline sequence.

Other than the specific amino acid substitutions discussed above, the framework regions of humanized immunoglobulins are usually substantially identical, and more usually, identical to the framework regions of the human antibodies from which they were derived. Of course, many of the amino acids in the framework region make little or no direct contribution to the specificity or affinity of an antibody. Thus, many individual conservative substitutions of framework residues can be tolerated without appreciable change of the specificity or affinity of the resulting humanized immunoglobulin. Thus, in one embodiment the variable framework region of the humanized immunoglobulin shares at least 85% sequence identity to a human variable framework region sequence or consensus of such sequences. In another embodiment, the variable framework region of the humanized immunoglobulin shares at least 90%, preferably 95%, more preferably 96%, 97%, 98% or 99% sequence identity to a human variable framework region sequence or consensus of such sequences. In general, however, such substitutions are undesirable.

The humanized antibodies preferably exhibit a specific binding affinity for antigen of at least 10^7 M^{-1}, 10^8 M^{-1}, 10^9 M^{-1} or 10^{10} M^{-1}. Usually the upper limit of binding affinity of the humanized antibodies for antigen is within a factor of three, four or five of that of the donor immunoglobulin. Often the lower limit of binding affinity is also within a factor of three, four or five of that of donor immunoglobulin. Alternatively, the binding affinity can be compared to that of a humanized antibody having no substitutions (e.g., an antibody having donor CDRs and acceptor FRs, but no FR substitutions). In such instances, the binding of the optimized antibody (with substitutions) is preferably at least two- to three-fold greater, or three- to four-fold greater, than that of the unsubstituted antibody. For making comparisons, activity of the various antibodies can be determined, for example, by BIACORE (i.e., surface plasmon resonance using unlabelled reagents) or competitive binding assays.

In one embodiment, humanized antibodies of the invention include a variable region framework sequence selected from human antibody genes (e.g., germline antibody gene segments) which include one or more canonical CDR structure types that are identical or similar to the canonical CDR structure types for the corresponding non-human antibody (e.g., murine) which is humanized. See, U.S. Pat. No. 6,881,557 and Tan et al., *Journal of Immunol* 169:1119-1125 (2002) (incorporated by reference in their entirety for all purposes).

Also featured are humanized antibodies comprising a framework region having a consensus amino acid sequence, for example, as described in U.S. Pat. No. 6,300,064, incorporated by reference herein in its entirety for all purposes. The following table lists various consensus sequences that can be used as framework regions in the humanized antibodies described herein. Therefore, any one of the consensus sequences shown below can be used as in combination with one or more CDRs described herein, thereby resulting in a humanized immunoglobulin or humanized antibody of the invention.

Consensus Sequences for light chain framework regions	Amino Acid Sequence
Kappa chain	DIQMTQSPSSLSASVGDRVTITCRASQGISS YLAWYQQKPGKAPKLLIYAASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQHYT TPPTFGQGTKVEIKRT (SEQ ID NO:14)
Kappa chain	DIVMTQSPLSLPVTPGEPASISCRSSQSLLH SNGYNYLDWYLQKPGQSPQLLIYLGSNRASG VPDRFSGSGSGTDFTLKISRVEAEDVGVYYC QQHYTTPPTFGQGTKVEIKRT (SEQ ID NO:15)
Kappa chain	DIVLTQSPATLSLSPGERATLSCRASQSVSS SYLAWYQQKPGQAPRLLIYGASSRATGVPAR FSGSGSGTDFTLTISSLEPEDFAVYYCQQHY TTPPTFGQGTKVEIKRT (SEQ ID NO:16)
Kappa chain	DIVMTQSPDSLAVSLGERATINCRSSQSVLY SSNNKNYLAWYQQKPGQPPKLLIYWASTRES GVPDRFSGSGSGTDFTLTISSLQAEDVAVYY CQQHYTTPPTFGQGTKVEIKRT (SEQ ID NO:17)
Lambda chain	QSVLTQPPSVSGAPGQRVTISCSGSSSNIGS NYVSWYQQLPGTAPKLLIYDNNQRPSGVPDR FSGSKSGTSASLAITGLQSEDEADYYCQQHY TTPPVFGGGTKLTVLG (SEQ ID NO:18)
Lambda chain	QSALTQPASVSGSPGQSITISCTGTSSDVGG YNYVSWYQQHPGKAPKLMIYDVSNRPSGVSN RFSGSKSGNTASLTISGLQAEDEADYYCQQH YTTPPVFGGGTKLTVLG (SEQ ID NO:19)

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Lambda chain	SYELTQPPSVSVAPGQTARISCSGDALGDKY ASWYQQKPGQAPVLVIYDDSDRPSGIPERFS GSNSGNTATLTISGTQAEDEADYYCQQHYTT PPVFGGGTKLTVLG (SEQ ID NO:20)

Consensus Sequences for Heavy chain framework regions	Amino Acid Sequence
Heavy chain	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSS YAISWVRQAPGQGLEWMGGIIPIFGTANYAQ KFQGRVTITADESTSTAYMELSSLRSEDTAV YYCARWGGDGFYAMDYWGQGTLVTVSS (SEQ ID NO:21)
Heavy chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTS YYMHWVRQAPGQGLEWMGWINPNSGGTNYAQ KFQGRVTMTRDTSISTAYMELSSLRSEDTAV YYCARWGGDGFYAMDYWGQGTLVTVSS (SEQ ID NO:22)
Heavy chain	QVQLKESGPALVKPTQTLTLTCTFSGFSLST SGVGVGWIRQPPGKALEWLALIDWDDDKYYS TSLKTRLTISKDTSKNQVVLTMTNMDPVDTA TYYCARWGGDGFYAMDYWGQGTLVTVSS (SEQ ID NO:23)
Heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGFTFSS YAMSWVRQAPGKGLEWVSAISGSGGSTYYAD SVKGRFTISRDNSKNTLYLQMNSLRAEDTAV YYCARWGGDGFYAMDYWGQGTLVTVSS (SEQ ID NO:24)
Heavy chain	QVQLQESGPGLVKPSETLSLTCTVSGGSISS YYWSWIRQPPGKGLEWIGYIYYSGSTNYNPS LKSRVTISVDTSKNQFSLKLSSVTAADTAVY YCARWGGDGFYAMDYWGQGTLVTVSS (SEQ ID NO:25)
Heavy chain	EVQLVQSGAEVKKPGESLKISCKGSGYSFTS YWIGWVRQMPGKGLEWMGIIYPGDSDTRYSP SFQGQVTISADKSISTAYLQWSSLKASDTAM YYCARWGGDGFYAMDYWGQGTLVTVSS (SEQ ID NO:26)
Heavy chain	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSS NSAAWNWIRQSPGRGLEWLGRTYYRSKWYND YAVSVKSRITINPDTSKNQFSLQLNSVTPED TAVYYCARWGGDGFYAMDYWGQGTLVTVSS (SEQ ID NO:27)

Yet another strategy that can be used to produce the humanized antibodies of the invention is to select the closest human germline sequence as the framework which receives the CDRs from a murine antibody to be humanized. See, Mercken et al., US 2005/0129695 (incorporated by reference in their entirety for all purposes). Germline sequences originate from un-rearranged immunoglobulin genes and therefore do not present somatic hypermtuation that is potentially immunogenic. This approach is based on the search for the closest human germline sequence. In particular, variable domains from germline sequences that exhibit a high degree of sequence identity with the murine VL and VH framework regions can be identified using the V-Base and/or IMGT databases (publicly accessible through the Medical Research Council Center for Protein Engineering internet server and the European Bioinformatics Institute internet server, respectively). The murine CDRs are then grafted on to the chosen human germline variable region acceptor sequences.

Additionally, framework residues can be analyzed using any of the techniques as described above to determine which, if any, residues should be substituted to optimize the properties of the resulting humanized antibody. For example, computer modeling can be used to identify residues which have a good probability of directly or indirectly influencing antigen binding.

The following human heavy chain germline framework acceptor sequences can be used.

VH3a

Xaa_1-Xaa_{30}-Xaa_{31}-Xaa_{42}-Xaa_{43}-Xaa_{56}-Xaa_{57}-Xaa_{75}-
FR1 CDR1 FR2 CDR2

Xaa_{76}-Xaa_{107}-Xaa_{108}-Xaa_{132}-Xaa_{133}-Xaa_{155}
FR3 CDR3 FR4

Xaa_1=(−),E,Q,V,D,P,A,G,R,C,K,L,S; Xaa_2=V,(−),E,M,A,K, L,R,P,Q; Xaa_3=Q,(−),H,K,L,R,E,N,V,P,D,I; Xaa_4=L,(−),V, M,Q,P,R,I; Xaa_5=(−),V,L,Q,E,R,S,A,M; Xaa_6=E,(−),Q,D,G, V,K,R; Xaa_7=S,(−),T,P,F,R,A; Xaa_8=G,(−),R,A,W; Xaa_9=G, (−),E,R; Xaa_{10}=G,(−),D,A,V,S,N,H,T; Xaa_{11}=L,(−),V,F,S, W,G; Xaa_{12}=V,(−),I,A,L,F,G,S; Xaa_{13}=Q,(−),K,R,H,T,P,E, L,N; Xaa_{14}=P,(−),V,S,T,A,H; Xaa_{15}=G,(−),D,E,W,R,L; Xaa_{16}=G,(−),R,K,T,E,W,M,V,N; Xaa_{17}=S,(−),P,A,F,C,Y; Xaa_{18}=L,(−)M,V,R,Q,P; Xaa_{19}=R,(−),K,T,G,I,S; Xaa_{20}=L, (−),V,I,P,F; Xaa_{21}=S,(−),C,A,F,T; Xaa_{22}=C,(−),R,S,Q,T; Xaa_{23}=A,(−),V,T,S,E,G,I,P,L,C; Xaa_{24}=A,(−),V,T,G,S,L,P, K,M,D; Xaa_{25}=S,A,P,T,F,Y,C,L,R; Xaa_{26}=G,(−),R,E,D,A,K, W,P,S; Xaa_{27}=F,(−), V,L,I,S,Y,D; Xaa_{28}=T,(−),S,I,N,P,A,R, G,M,E,H,V; Xaa_{29}=F,(−),V,L,S,I,A,C,Y; Xaa_{30}=S,(−),D, R,N,T,G,K,I,Y,L,E,H

Xaa_{31}-Xaa_{40}=CDR1 residues 1-10; Xaa_{41}-Xaa_{42}=CDR1 residues 11 to 12, if present

Xaa_{43}=W,(−),G,L,R,F; Xaa_{44}=V,I,(−),A,G,P,E,F,S,C; Xaa_{45}=R,(−),L,H; Xaa_{46}=Q,(−),R,K,H,S,L,G,I,F, D,C; Xaa_{47}=A,T,V,(−),P; Xaa_{48}=P,T,(−),S,A,L,Q; Xaa_{49}=G,(−),E, R,A,C,D,W,K; Xaa_{50}=K,R,E,Q,M, T,(−),N,A,G; Xaa_{51}=G, R,A,(−),E,S; Xaa_{52}=L,P,(−),Q,S,V; Xaa_{53}=E,V,D,Q,(−),A, G,M,T,K,Y; Xaa_{54}=W,Y,C,R,(−),L,S; Xaa_{55}=V,L,I,A,M,S, (−),E,G; Xaa_{56}=S,A,T,P,G,L,(−),V

Xaa_{57}-Xaa_{62}=CDR2 residues 1-6; Xaa_{63}-Xaa_{75}=CDR2 residues 7 to 19, if present

Xaa_{76}=R,Q,H,G; Xaa_{77}=F,L,S,V,C,Y; Xaa_{78}=T,I,S,V,A,F, G,(−),D; Xaa_{79}=I,V,L,T,M,A,N,S,F; Xaa_{80}=S,F,A,T,Y,G,C, W,L; Xaa_{81}=R,G,S,K,I; Xaa_{82}=D,E,N,G,H,S,V,Y; Xaa_{83}=N, D,K,S,T,I,R,Q,G,C,E,V; Xaa_{84}=S,A,T,V,G,D,P,F,L,Y,N,R,I; Xaa_{85}=K,R,N,E,Q,M,T,H,L,D,S,(−); Xaa_{86}=N,S,K,D,T,H, R,Y,Q; Xaa_{87}=T,S,L,M,A,I,P,R,K,Y,F,V; Xaa_{88}=L,V,M,I,A, F,P,Q,W; Xaa_{89}=Y,F,S,H,C,D,N,L,T; Xaa_{90}=L,V, M,F,I,(−); Xaa_{91}=Q,E,H,L,R,N,D,K,T,V; Xaa_{92}=M,I,L,V,T,K; Xaa_{93}=N,S,D,K,T,G,H,R,Y,I; Xaa_{94}=S,N,G,T,R,I,A,F,V; Xaa_{95}=L,V,M,R,P,Q; Xaa_{96}=R,T,G,K,S,N,(−),E,I,M; Xaa_{97}=A,V,D,T,P,G,S,L,E,H, I,(−),N; Xaa_{98}=E,D,G,A,(−), V,K,N,Q; Xaa_{99}=D,E,N,G,(−),Q; Xaa_{100}=T,M,S,A,(−),R,K, P; Xaa_{101}=A,G, S,T,(−),P,V,D,C,L; Xaa_{102}=V,L,I,M,A,F,(−), T,E,G,Q,K,S,D,R,W,Y; Xaa_{103}=Y,F,H,(−),S; Xaa_{104}=Y,F,H, S,L,(−),N,T,W; Xaa_{105}=C,(−),W,Y,S,R,F; Xaa_{106}=A,V,T,G, S,L,(−),P,K,E,I,R,C,Y; Xaa_{107}=R,K,S,(−),T,N, G,A,I,Q,E,L, P,C,V,H,D,F

Xaa_{108}-Xaa_{110}=CDR3 residues 1-3; Xaa_{111}-Xaa_{32}=CDR3 residues 4 to 25, if present

Xaa_{133}=W,(−),Y,L,F,G,V,S,R,P,D,C,N,T,A,Q,M,E,H,I; Xaa_{134}=G,(−),A,V,D,S,W,Y,T,P,H,F,R,E,L, Q,C,M,I,K; Xaa_{135}=(−),Q,K,R,P,G,S,D,T,Y,W,L,H,E,V,F,A,M,I; Xaa_{136}=(−),G,Q,R,S,K,D,P,Y,M,A, V,F,W,N,H,E,T,L,I,C;

Xaa_{137}=(−),Q,S,L,A,F,G,I,P,V,M,R,K,T,N,W,Y; Xaa_{138}=(−), G,T,Y,A,D,R,S,F, V,W,L,Q,N,P,I,E,C,H; Xaa_{139}=(−),T,L,M, Y,S,A,P,V,G,I,W,D,N,R,F,H,Q,E,K,C; Xaa_{140}=(−), L,V,T,M, Q, G,W,S,R,D,Y,A,F,I,P,K,H,E,C; Xaa_{141}=(−),V,T,G,D,S,L, I,A,P,W,R,F,Q,H,Y,N,K,C,E; Xaa_{142}=(−),T,V, P,I,S,A,Y,G,L, Q,W,D,K,M,E,F,C,N,H,R; Xaa_{143}=(−),V,S,L,W,G,F,T,Q,R, A,P,I,Y,D,M; Xaa_{144}=(−),S,V, G,T,P,D,W,L,F,M,A,Q,H,R,E, I; Xaa_{145}=(−),S,G,T,A,V,D,R,L,I,K,Q,N,P,H,M,E,W,Y; Xaa_{146}=(−),G,A,S, V,W,L,P,Y,Q,R,T,D,M,I; Xaa_{147}=(−),S,T, A,P,H,W,G,R,F,L,Y,E,V,M,C; Xaa_{148}=(−),T,S,A,K,P,R,Q,L, M, V,D,F,Y; Xaa_{149}=(−),K,S,G,A,V,P,R,L,W,I,T,D,Y; Xaa_{150}=(−),A,P,G,T,S,H,V,F,W; Xaa_{151}=(−),P,S,H,T, V,R,K, I,L,N,M; Xaa_{152}=(−),S,T,V,L,R,D,F,K,N,P,G,W; Xaa_{153}=V, L,F,S,D,A,P,Y,G,T; Xaa_{154}=F,P,S, Y,G,H,Q; Xaa_{155}=P,L,S, H,R

VH3b (SEQ ID NO: 35)

Xaa_1-Xaa_{30}-Xaa_{31}-Xaa_{42}-Xaa_{43}-Xaa_{56}-Xaa_{57}-Xaa_{75}-
FR1 CDR1 FR2 CDR2

Xaa_{76}-Xaa_{107}-Xaa_{108}-Xaa_{132}-Xaa_{133}-Xaa_{155}
FR3 CDR3 FR4

Xaa_1=E,(−),Q; Xaa_2=V,(−),A,E; Xaa_3=Q,(−),H,R; Xaa_4=L, (−),Q; Xaa_5=V,(−),Q,L; Xaa_6=E,(−),Q,D; Xaa_7=S,(−); Xaa_8=G,(−),A,R; Xaa_9=G,(−); Xaa_{10}=G,(−),A,D,S,N; Xaa_{11}=L,(−),S,V; Xaa_{12}=V,(−),I,A; Xaa_{13}=K,(−),Q,E,H; Xaa_{14}=P,(−),V,R; Xaa_{15}=G,(−),E,D,R; Xaa_{16}=G,(−),R,E; Xaa_{17}=S,(−),P; Xaa_{18}=L,(−); Xaa_{19}=R,(−),K,T; Xaa_{20}=L, (−),V,F; Xaa_{21}=S,(−),T,A; Xaa_{22}=C,(−); Xaa_{23}=A,(−)V,E,T, G; Xaa_{24}=A,(−),V,T,D,P,G; Xaa_{25}=S,(−),A,L; Xaa_{26}=G,(−), R,N,W; Xaa_{27}=F,(−),I,L; Xaa_{28}=T,(−),I,S,A,P,L,N; Xaa_{29}=F, (−),C,L; Xaa_{30}=S,(−),N,T,G,P,Q,R

Xaa_{31}-Xaa_{40}=CDR1 residues 1-10; Xaa_{41}-Xaa_{42}=CDR1 residues 11 to 12, if present

Xaa_{43}=W,(−); Xaa_{44}=V,(−),A,L; Xaa_{45}=R,(−),H; Xaa_{46}=Q, (−),H,L; Xaa_{47}=A,(−),P,V,T,G,L,S; Xaa_{48}=P,S,(−),Q; Xaa_{49}=G,(−),R,E; Xaa_{50}=K,R,(−); Xaa_{51}=G,E,(−); Xaa_{52}=L,V,(−); Xaa_{53}=E,Q,(−); Xaa_{54}=W,L,(−); Xaa_{55}=V,L, I,(−); Xaa_{56}=G,A,(−)

Xaa_{57}-Xaa_{62}=CDR2 residues 1-6; Xaa_{63}-Xaa_{75}=CDR2 residues 7 to 19, if present

Xaa_{76}=R,S,T,G,K; Xaa_{77}=F,L,V,I; Xaa_{78}=T,I,S,V; Xaa_{79}=I, V,F,C,M; Xaa_{80}=S,A; Xaa_{81}=R,S; Xaa_{82}=D,E,H,N,V; Xaa_{83}=D,E,S; Xaa_{84}=S,P; Xaa_{85}=K,Q,E,R,N,T; Xaa_{86}=N,S, D,K,Q,T; Xaa_{87}=T,S,M,A,I; Xaa_{88}=L,A,V,M; Xaa_{89}=Y,W,F, S,D,C; Xaa_{90}=L; Xaa_{91}=Q,E,R,T,H,K; Xaa_{92}=M,I,L; Xaa_{93}=N,S,I, T,D,K; Xaa_{94}=S,R,N,G,I; Xaa_{95}=L,V; Xaa_{96}=K,E,R,Q,T,G,S; Xaa_{97}=T,I,S,A,D,V,P,L,N; Xaa_{98}=E, D,G; Xaa_{99}=D; Xaa_{100}=T,L; Xaa_{101}=A,G,T; Xaa_{102}=V,I,L, M,E,F; Xaa_{103}=Y,H; Xaa_{104}=Y,S,F,C; Xaa_{105}=C, A,R; Xaa_{106}=T,I,A,G,V,N,P,L,S,C,K,Q,F; Xaa_{107}=T,R,(−),S,G,A, V,I,K,P,W

Xaa_{108}-Xaa_{109}=CDR3 residues 1-3; Xaa_{111}-Xaa_{132}=CDR3 residues 4 to 25, if present

Xaa_{133}=W,(−),L,G,T,V,Y,P,K,D,I,E; Xaa_{134}=G,(−),V,T,N,D, R,S,Q,E; Xaa_{135}=Q,(−),K,T,R,P,H,N,E,F, L,S,M; Xaa_{136}=G, (−),V,D,N,P,L; Xaa_{137}=T,(−),S,W,Y,V,N,H,A,F,G; Xaa_{138}= (−),L,T,F,M,S,W,R,H; Xaa_{139}=V,(−),D,G,F,R,A; Xaa_{140}T, (−),S,L,P,R,G,W,Y; Xaa_{141}=V,(−),W,S,P,R,A,T,F,G; Xaa_{142}=(−), S,G,T,E,K; Xaa_{143}=(−),S,P,R,A,L,G; Xaa_{144}= (−),A,G,P,V; Xaa_{145}=(−),S,T; Xaa_{146}=(−),T,A,V,L,P,W; Xaa_{147}=(−),K,S,G,T,V; Xaa_{148}=(−),G,A,L,S,T; Xaa_{149}=(−), P,H,S,V; Xaa_{150}=(−),S,T,D,K; Xaa_{151}=(−),V,L; Xaa_{152}=(−), F; Xaa_{153}=(−),P; Xaa_{154}=(−),L; Xaa_{155}=(−),A,G

The following human light chain germline framework acceptor sequences can be used.

κ2 (κ Configuration Disclosed as SEQ ID NO: 36; λ Configuration Disclosed as SEQ ID NO: 37)

Xaa_1-Xaa_{23}-Xaa_{24}-Xaa_{35}-Xaa_{36}-Xaa_{50}-Xaa_{51}-Xaa_{69}-
FR1 CDR1 FR2 CDR2

Xaa_{70}-Xaa_{101}-Xaa_{102}-Xaa_{126}-Xaa_{127}-Xaa_{136}
FR3 CDR3 FR4

Xaa_1=(−),D,E,A,Q; Xaa_2=(−),I,V,L,E,A,T; Xaa_3=V,(−),L,E, M; Xaa_4=M,(−),L,V; Xaa_5=T,(−); Xaa_6=Q,(−); Xaa_7=(−),S, T,N; Xaa_8=P,(−); Xaa_9=L,(−),P,D,S,I,F; Xaa_{10}=S,(−),D; Xaa_{11}=L,(−),S; Xaa_{12}=P,(−),S,T,A; Xaa_{13}=V,(−); Xaa_{14}=T, (−),S,N,I; Xaa_{15}=P,L,(−); Xaa_{16}=G,(−); Xaa_{17}=Q,E,(−),D; Xaa_{18}=P,(−),Q,S,R; Xaa_{19}=A,(−); Xaa_{20}=S,(−); Xaa_{21}=I,(−), L,M,F,V; Xaa_{22}=S,(−); Xaa_{23}=C,(−),F

Xaa_{24}-Xaa_{33}=CDR1 residues 1-10; Xaa_{34}-Xaa_{35}=CDR1 residues 11 to 12, if present

Xaa_{36}=W,C,R; Xaa_{37}=Y,F,L,H; Xaa_{38}=L,Q,V,P,R,H; Xaa_{39}=Q,E; Xaa_{40}=K,R,T; Xaa_{41}=P,A; Xaa_{42}=G; Xaa_{43}=Q, R,H,E,K,(−); Xaa_{44}=S,P,T,(−); Xaa_{45}=P,L,(−); Xaa_{46}=Q,R, K,H,E,W,L; Xaa_{47}=L,R,V,I; Xaa_{48}=L,V; Xaa_{49}=I,L,F,V; Xaa_{50}=Y,S,F,H,(−)

Xaa_{51}-Xaa_{56}=CDR2 residues 1-6; Xaa_{57}-Xaa_{69}=CDR2 residues 7 to 19, if present

Xaa_{70}=G; Xaa_{71}=V; Xaa_{72}=P,S; Xaa_{73}=D,H,N; Xaa_{74}=R,S, T,K; Xaa_{75}=F,I,L; Xaa_{76}=S,T; Xaa_{77}=G,D,A; Xaa_{78}=S,G,T; Xaa_{79}=G,(−); Xaa_{80}=S,A; Xaa_{81}=G,(−),D; Xaa_{82}=T,A,S; Xaa_{83}=D,A,E,B,S,H; Xaa_{84}=F,V; Xaa_{85}=T; Xaa_{86}=L; Xaa_{87}=K,E,R,T,A,I,L,M,N; Xaa_{88}=I,V; Xaa_{89}=S,N; Xaa_{90}=R,W,K,S; Xaa_{91}=V,M,A; Xaa_{92}=E,G,Q,K; Xaa_{93}=A, P,T,V; Xaa_{94}=E,D,A,G,Q; Xaa_{95}=D,B; Xaa_{96}=V,A,L,D,E,I; Xaa_{97}=G,A; Xaa_{98}=V,I,L,T; Xaa_{99}=Y,H; Xaa_{100}=Y,F; Xaa_{101}=C,(−)

Xaa_{102}-Xaa_{104}=CDR3 residues 1-3; Xaa_{105}-Xaa_{126}=CDR3 residues 4 to 25, if present

κ: Xaa_{127}=F; Xaa_{128}=G; Xaa_{129}=Q,P,G; Xaa_{130}=G; Xaa_{131}=T; Xaa_{132}=K,R; Xaa_{133}=V,L; Xaa_{134}=E,D; Xaa_{135}=I; Xaa_{136}=K

λ: Xaa_{127}=F; Xaa_{128}=G; Xaa_{129}=G,T; Xaa_{130}=G; Xaa_{131}=T; Xaa_{132}=K,Q; Xaa_{133}=V,L; Xaa_{134}=T; Xaa_{135}=V; Xaa_{136}=L

The following table lists exemplary CDRs of the antibodies of the invention which can be grafted onto human germline acceptor framework sequences.

Antibody	CDRL1	CDRL2	CDRL3
15C11	RSSQSLVHSDGNTYLH (SEQ ID NO: 38)	KVSNRFS (SEQ ID NO: 39)	SQSTHVWT (SEQ ID NO: 40)
9G8	RSSQSLVHSNGNTFFH (SEQ ID NO: 41)	KVSNRFS (SEQ ID NO: 39)	SQSAHVPWT (SEQ ID NO: 42)

-continued

Antibody	CDRH1	CDRH2	CDRH3
15C11	GFTFSRYSMS (SEQ ID NO: 43)	KISNSGDNTYYPDTLKG (SEQ ID NO: 44)	GDY
9G8	GFTFSDYTMS (SEQ ID NO: 45)	EISNTGGSTYYPDTVKG (SEQ ID NO: 46)	GDY

In additional embodiments, framework residues can be analyzed using any of the techniques as described above to determine which, if any, residues should be substituted to optimize the properties of the resulting humanized antibody. For example, computer modeling can be used to identify residues which have a good probability of directly or indirectly influencing antigen binding.

Additional exemplary humanization techniques that can be used for humanizing the immunoglobulins of the invention are described in, for example, Presta et al., J. Immunol., 151: 2623-2632 (1993); Carter et al., Proc. Natl. Acad. Sci. USA., 89: 4285-4289 (1992); Couto et al., Cancer Res., 55: 5973s-77s (1995); O'Conner et al., Protein Eng., 11: 321-328 (1998); and Antibody Engineering-Methods and Protocols by Lo, Vol. 248 (2004).

c. Production of Humanized 15C11 Antibodies

A preferred embodiment of the present invention features a humanized antibody to the central portion of Aβ, in particular, for use in the therapeutic and/or diagnostic methodologies described herein. A particularly preferred starting material for production of humanized antibodies is 15C11. 15C11 is specific for the central portion of Aβ, e.g., the portion between the N-terminus and the C-terminus (e.g., within Aβ 13-28), and has been shown to (1) specifically bind Aβ1-42 (e.g., Aβ oligomer), (2) capture soluble Aβ, and (3) improve cognition in a patient. The in vivo efficacy of the 15C11 antibody is described in Example I. The cloning and sequencing of cDNA encoding the 15C11 antibody heavy and light chain variable regions is described in Example III.

Suitable human acceptor antibody sequences are identified by computer comparisons of the amino acid sequences of the mouse variable regions with the sequences of known human antibodies. The comparison is performed separately for heavy and light chains but the principles are similar for each. In particular, variable domains from human antibodies whose framework sequences exhibit a high degree of sequence identity with the murine VL and VH framework regions were identified by query of the Kabat Database using NCBI BLAST (publicly accessible through the National Institutes of Health NCBI internet server) with the respective murine framework sequences. In one embodiment, acceptor sequences sharing greater that 50% sequence identity with murine donor sequences are selected. Preferably, acceptor antibody sequences sharing 60%, 70%, 80%, 90% or more are selected.

In one embodiment, the choice of the acceptor framework is from the same human subgroup as that which corresponds to the murine V region, has no unusual framework residues, and in which the CDRs belong to the same Chothia canonical structure groups. For example, CDR L1 of 15C11 belongs to Chothia-equivalent class 4, CDR L2 belongs to class 1, CDRL3 is similar to class 3, CDR H1 is similar to class 1, and CDR H2 is similar to class 3.

A computer comparison of 15C11 heavy and light chains can be used to identify human heavy and light chains having a great degree of sequence identity. Light and heavy human framework regions can be derived from such human antibodies, or from consensus sequences of such antibodies.

Residues are next selected for substitution, as follows. When an amino acid differs between a 15C11 variable framework region and an equivalent human variable framework region, the human framework amino acid should usually be substituted by the equivalent mouse amino acid if it is reasonably expected that the amino acid:

(1) noncovalently binds antigen directly,

(2) is adjacent to a CDR region, is part of a CDR region under the alternative definition proposed by Chothia et al., supra, or otherwise interacts with a CDR region (e.g., is within about 3A of a CDR region), or

(3) participates in the VL-VH interface.

Computer modeling of the 15C11 antibody heavy and light chain variable regions is performed as follows. Briefly, a three-dimensional model is generated based on the closest solved murine antibody structures for the heavy and light chains. The model is further refined by a series of energy minimization steps to relieve unfavorable atomic contacts and optimize electrostatic and van der Walls interactions.

Three-dimensional structural information for the antibodies described herein is publicly available, for example, from the Research Collaboratory for Structural Bioinformatics' Protein Data Bank (PDB). The PDB is freely accessible via the World Wide Web internet and is described by Berman et al. (2000) *Nucleic Acids Research,* 28:235. Computer modeling allows for the identification of CDR-interacting residues. The computer model of the structure of 15C11 can in turn serve as a starting point for predicting the three-dimensional structure of an antibody containing the 15C11 complementarity determining regions substituted in human framework structures. Additional models can be constructed representing the structure as further amino acid substitutions are introduced.

In general, substitution of one, most or all of the amino acids fulfilling the above criteria is desirable. Accordingly, the humanized antibodies of the present invention will usually contain a substitution of a human light chain framework residue with a corresponding 15C11 residue in at least 1, 2, 3 or more of the chosen positions. The humanized antibodies also usually contain a substitution of a human heavy chain framework residue with a corresponding 15C11 residue in at least 1, 2, 3 or more of the chosen positions.

Occasionally, however, there is some ambiguity about whether a particular amino acid meets the above criteria, and alternative variant immunoglobulins are produced, one of which has that particular substitution, the other of which does not. In instances where substitution with a murine residue would introduce a residue that is rare in human immunoglobulins at a particular position, it may be desirable to test the antibody for activity with or without the particular substitution. If activity (e.g., binding affinity and/or binding specificity) is about the same with or without the substitution, the antibody without substitution may be preferred, as it would be expected to elicit less of a HAMA response, as described herein.

Other candidates for substitution are acceptor human framework amino acids that are unusual for a human immunoglobulin at that position. These amino acids can be substituted with amino acids from the equivalent position of more typical human immunoglobulins. Alternatively, amino acids from equivalent positions in the mouse 15C11 can be introduced into the human framework regions when such amino acids are typical of human immunoglobulin at the equivalent positions.

Other candidates for substitution are non-germline residues occurring in a framework region. By performing a computer comparison of 15C11 with known germline sequences, germline sequences with the greatest degree of sequence identity to the heavy or light chain can be identified. Alignment of the framework region and the germline sequence will reveal which residues may be selected for substitution with corresponding germline residues. Residues not matching between a selected light chain acceptor framework and one of these germline sequences could be selected for substitution with the corresponding germline residue.

Rare mouse residues are identified by comparing the donor VL and/or VH sequences with the sequences of other members of the subgroup to which the donor VL and/or VH sequences belong (according to Kabat) and identifying the residue positions which differ from the consensus. These donor specific differences may point to somatic mutations which enhance activity. Unusual or rare residues close to the binding site may possibly contact the antigen, making it desirable to retain the mouse residue. However, if the unusual mouse residue is not important for binding, use of the corresponding acceptor residue is preferred as the mouse residue may create immunogenic neoepitopes in the humanized antibody. In the situation where an unusual residue in the donor sequence is actually a common residue in the corresponding acceptor sequence, the preferred residue is clearly the acceptor residue.

Kabat ID sequences referenced herein are publicly available, for example, from the Northwestern University Biomedical Engineering Department's Kabat Database of Sequences of Proteins of Immunological Interest. Three-dimensional structural information for antibodies described herein is publicly available, for example, from the Research Collaboratory for Structural Bioinformatics' Protein Data Bank (PDB). The PDB is freely accessible via the World Wide Web internet and is described by Berman et al. (2000) *Nucleic Acids Research, p*235-242. Germline gene sequences referenced herein are publicly available, for example, from the National Center for Biotechnology Information (NCBI) database of sequences in collections of Igh, Ig kappa and Ig lambda germline V genes (as a division of the National Library of Medicine (NLM) at the National Institutes of Health (N1H)). Homology searching of the NCBI "Ig Germline Genes" database is provided by IgG BLAST™.

In a preferred embodiment, a humanized antibody of the present invention contains (i) a light chain comprising a variable domain comprising murine 15C11 VL CDRs and a human acceptor framework, the framework having zero, one, or more residues substituted with the corresponding 15C11 residue and (ii) a heavy chain comprising 15C11 VH CDRs and a human acceptor framework, the framework having at least one, preferably two, three, four, five, six, seven, eight, nine or more residues substituted with the corresponding 15C11 residue, and, optionally, at least one, preferably two or three residues substituted with a corresponding human germline residue.

In another preferred embodiment, a humanized antibody of the present invention has structural features, as described herein, and further has at least one (preferably two, three, four or all) of the following activities: (1) binds soluble Aβ; (2) binds aggregated Aβ1-42 (e.g., as determined by ELISA); (3) captures soluble Aβ; (4) binds Aβ in plaques (e.g., staining of AD and/or PDAPP plaques); (5) binds Aβ with an affinity no less than two to three fold lower than chimeric 15C11 (e.g., 15C11 having murine variable region sequences and human constant region sequences); (6) mediates phagocytosis of Aβ (e.g., in an ex vivo phagocytosis assay, as described herein); and (7) crosses the blood-brain barrier (e.g., demonstrates short-term brain localization, for example, in a PDAPP animal model, as described herein).

In another preferred embodiment, a humanized antibody of the present invention has structural features, as described herein, such that it binds Aβ in a manner or with an affinity sufficient to elicit at least one of the following in vivo effects: (1) reduce Aβ plaque burden; (2) prevent plaque formation; (3) reduce levels of soluble Aβ; (4) reduce the neuritic pathology associated with an amyloidogenic disorder; (5) lessen or ameliorate at least one physiological symptom associated with an amyloidogenic disorder; and/or (6) improve cognitive function, e.g., rapidly improve cognition without crossing the blood-brain barrier.

In another preferred embodiment, a humanized antibody of the present invention has structural features as described herein, and specifically binds to an epitope within residues 13-28 of Aβ, e.g., comprising residues 19-22 of Aβ).

The activities described above can be determined utilizing any one of a variety of assays described herein or in the art (e.g., binding assays, phagocytosis assays, etc.). Activities can be assayed either in vivo (e.g., using labeled assay components and/or imaging techniques) or in vitro (e.g., using samples or specimens derived from a patient). Activities can be assayed either directly or indirectly. In certain preferred embodiments, neurological endpoints (e.g., amyloid burden, neuritic burden, etc.) are assayed. Such endpoints can be assayed in living subjects (e.g., in animal models of Alzheimer's disease or in human subjects, for example, undergoing immunotherapy) using non-invasive detection methodologies. Alternatively, such endpoints can be assayed in subjects post mortem. Assaying such endpoints in animal models and/or in human subjects post mortem is useful in assessing the effectiveness of various agents (e.g., humanized antibodies) to be utilized in similar immunotherapeutic applications. In other preferred embodiments, behavioral or neurological parameters can be assessed as indicators of the above neuropathological activities or endpoints.

3. Production of Variable Regions

Having conceptually selected the CDR and framework components of humanized immunoglobulins, a variety of methods are available for producing such immunoglobulins. In general, one or more of the murine complementarity determining regions (CDR) of the heavy and/or light chain of the antibody can be humanized, for example, placed in the context of one or more human framework regions, using primer-based polymerase chain reaction (PCR). Briefly, primers are designed which are capable of annealing to target murine CDR region(s) which also contain sequence which overlaps and can anneal with a human framework region. Accordingly, under appropriate conditions, the primers can amplify a murine CDR from a murine antibody template nucleic acid and add to the amplified template a portion of a human framework sequence. Similarly, primers can be designed which are capable of annealing to a target human framework region(s) where a PCR reaction using these primers results in an amplified human framework region(s). When each amplification product is then denatured, combined, and annealed to the other product, the murine CDR region, having overlapping human framework sequence with the amplified human framework sequence, can be genetically linked. Accordingly, in one or more such reactions, one or more murine CDR regions can be genetically linked to intervening human framework regions.

In some embodiments, the primers may also comprise desirable restriction enzyme recognition sequences to facilitate the genetic engineering of the resultant PCR amplified

sequences into a larger genetic segment, for example, a variable light or heavy chain segment, heavy chain, or vector. In addition, the primers used to amplify either the murine CDR regions or human framework regions may have desirable mismatches such that a different codon is introduced into the murine CDR or human framework region. Typical mismatches introduce alterations in the human framework regions that preserve or improve the structural orientation of the murine CDR and thus its binding affinity, as described herein.

It should be understood that the foregoing approach can be used to introduce one, two, or all three murine CDR regions into the context of intervening human framework regions. Methods for amplifying and linking different sequences using primer-based PCR are described in, for example, Sambrook, Fritsch and Maniatis, *Molecular Cloning: Cold Spring Harbor Laboratory Press* (1989); *DNA Cloning*, Vols. 1 and 2, (D. N. Glover, Ed. 1985); *PCR Handbook Current Protocols in Nucleic Acid Chemistry*, Beaucage, Ed. John Wiley & Sons (1999) (Editor); *Current Protocols in Molecular Biology*, eds. Ausubel et al., John Wiley & Sons (1992).

Because of the degeneracy of the code, a variety of nucleic acid sequences will encode each immunoglobulin amino acid sequence. The desired nucleic acid sequences can be produced by de novo solid-phase DNA synthesis or by PCR mutagenesis of an earlier prepared variant of the desired polynucleotide. Oligonucleotide-mediated mutagenesis is a preferred method for preparing substitution, deletion and insertion variants of target polypeptide DNA. See Adelman et al., DNA 2:183 (1983). Briefly, the target polypeptide DNA is altered by hybridizing an oligonucleotide encoding the desired mutation to a single-stranded DNA template. After hybridization, a DNA polymerase is used to synthesize an entire second complementary strand of the template that incorporates the oligonucleotide primer, and encodes the selected alteration in the target polypeptide DNA.

4. Selection of Constant Regions

The variable segments of antibodies produced as described supra (e.g., the heavy and light chain variable regions of chimeric or humanized antibodies) are typically linked to at least a portion of an immunoglobulin constant region (Fc region), usually that of a human immunoglobulin. Human constant region DNA sequences can be isolated in accordance with well known procedures from a variety of human cells, but preferably immortalized B cells (see Kabat et al., supra, and Liu et al., WO87/02671) (each of which is incorporated by reference in its entirety for all purposes). Ordinarily, the antibody will contain both light chain and heavy chain constant regions. The heavy chain constant region usually includes CH1, hinge, CH2, CH3, and CH4 regions. The antibodies described herein include antibodies having all types of constant regions, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG1, IgG2, IgG3 and IgG4. When it is desired that the antibody (e.g., humanized antibody) exhibit cytotoxic activity, the constant domain is usually a complement fixing constant domain and the class is typically IgG1. When it is desired that the antibody (e.g., humanized antibody) exhibit reduced cytotoxic activity, the constant domain the class is typically IgG4. Exemplary human isotypes include IgG1 and IgG4. Light chain constant regions can be lambda or kappa. The humanized antibody may comprise sequences from more than one class or isotype. Antibodies can be expressed as tetramers containing two light and two heavy chains, as separate heavy chains, light chains, as Fab, Fab'F(ab')$_2$, and Fv, or as single chain antibodies in which heavy and light chain variable domains are linked through a spacer.

In one embodiment, a humanized antibody of the invention includes the 15C11 VH region linked to an IgG1 constant region. In another embodiment, a humanized antibody of the invention includes the 15C11 VH region linked to an IgG4 constant region.

In one embodiment, a humanized antibody of the invention includes a humanized 15C11 VH region linked to an IgG1 constant region, encoded by the nucleotide sequence set forth in SEQ ID NO:28. In another embodiment, a humanized antibody of the invention includes a humanized 15C11 region linked to an IgG1 constant region, as shown below in SEQ ID NO: 29. In another embodiment, a humanized antibody of the invention includes a humanized 15C11 region linked to an IgG1 constant region, as shown below in SEQ ID NO:32.

In one embodiment, a humanized antibody of the invention includes a humanized 15C11 VL region linked to an IgG1 constant region, encoded by the nucleotide sequence set forth in SEQ ID NO:30. In another embodiment, a humanized antibody of the invention includes a humanized 15C11 VH region linked to an IgG1 constant region, as shown below in SEQ ID NO: 31.

In another embodiment, a humanized antibody of the invention includes a humanized 15C11 VH region linked to an IgG4 constant region, as shown below in SEQ ID NO: 33.

In some embodiments, humanized antibodies described herein are modified to enhance their antigen dependent cellular cytotoxicity (ADCC) activity using techniques, such as, for example, those described in U.S. Pat. No. 6,946,292, the entire contents of which are incorporated by reference herein. ADCC activity of antibodies is generally thought to require the binding of the Fc region of an antibody to an antibody receptor existing on the surface of an effector cell, such as, for example, a killer cell, a natural killer cell and an activated macrophage. By altering fucosylation (e.g., reducing or eliminating) of the carbohydrate structure of a humanized antibody (i.e., in the Fe region), the ADCC activity of the antibody can be enhanced in vitro by, for example, 10-fold, or 20-fold, or 30-fold, or 40-fold, or 50-fold, or 100-fold, relative to an unmodified humanized antibody. Because of increased ADCC activity, such modified antibodies can be used at lower dosages than their unmodified counterparts and generally have fewer or reduced side effects in patients.

In some embodiments, aglycosyl versions of humanized antibodies are featured, wherein such antibodies include an aglycosylated constant region. Oligosaccharide at Asn-297 is a characteristic feature of normal human IgG antibodies (See, Kabat et al., 1987, Sequence of Proteins of Immunological Interest, U.S. Department of Health Human Services Publication). Each of the two heavy chains in IgG molecules have a single branched chain carbohydrate group which is linked to the amide group of the asparagine residue, for example, at position 297. Substitution of, for example, asparagine with alanine prevents the glycosylation of the antibody, as described in, for example, U.S. Pat. No. 6,706,265, incorporated by reference herein. In a particular embodiment, the amino acid residue Asn at position 297 is mutated to alanine.

5. Expression of Recombinant Antibodies

Chimeric and humanized antibodies are typically produced by recombinant expression. Nucleic acids encoding light and heavy chain variable regions, optionally linked to constant regions, are inserted into expression vectors. The light and heavy chains can be cloned in the same or different expression vectors. The DNA segments encoding immunoglobulin chains are operably linked to control sequences in the expression vector(s) that ensure the expression of immunoglobulin polypeptides. Expression control sequences include, but are not limited to, promoters (e.g., naturally-

associated or heterologous promoters), signal sequences, enhancer elements, and transcription termination sequences. Preferably, the expression control sequences are eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells (e.g., COS or CHO cells). Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and the collection and purification of the crossreacting antibodies.

These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors contain selection markers (e.g., ampicillin-resistance, hygromycin-resistance, tetracycline resistance, kanamycin resistance or neomycin resistance) to permit detection of those cells transformed with the desired DNA sequences (see, e.g., Itakura et al., U.S. Pat. No. 4,704,362).

E. coli is one prokaryotic host particularly useful for cloning the polynucleotides (e.g., DNA sequences) of the present invention. Other microbial hosts suitable for use include bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as *Salmonella, Serratia*, and various *Pseudomonas* species. In these prokaryotic hosts, one can also make expression vectors, which will typically contain expression control sequences compatible with the host cell (e.g., an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence, and have ribosome binding site sequences and the like, for initiating and completing transcription and translation.

Other microbes, such as yeast, are also useful for expression. *Saccharomyces* is a preferred yeast host, with suitable vectors having expression control sequences (e.g., promoters), an origin of replication, termination sequences and the like as desired. Typical promoters include 3-phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase, isocytochrome C, and enzymes responsible for maltose and galactose utilization.

In addition to microorganisms, mammalian tissue cell culture may also be used to express and produce the polypeptides of the present invention (e.g., polynucleotides encoding immunoglobulins or fragments thereof). See Winnacker, From Genes to Clones, VCH Publishers, N.Y., N.Y. (1987). Eukaryotic cells are actually preferred, because a number of suitable host cell lines capable of secreting heterologous proteins (e.g., intact immunoglobulins) have been developed in the art, and include CHO cell lines, various COS cell lines, HeLa cells, preferably, myeloma cell lines, or transformed B-cells or hybridomas. Preferably, the cells are nonhuman. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, and an enhancer (Queen et al., *Immunol. Rev.* 89:49 (1986)), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences.

Preferred expression control sequences are promoters derived from immunoglobulin genes, SV40, adenovirus, bovine papilloma virus, cytomegalovirus and the like. See Co et al., *J. Immunol.* 148:1149 (1992).

Alternatively, antibody-coding sequences can be incorporated in transgenes for introduction into the genome of a transgenic animal and subsequent expression in the milk of the transgenic animal (see, e.g., Deboer et al., U.S. Pat. No. 5,741,957, Rosen, U.S. Pat. No. 5,304,489, and Meade et al., U.S. Pat. No. 5,849,992). Suitable transgenes include coding sequences for light and/or heavy chains in operable linkage with a promoter and enhancer from a mammary gland specific gene, such as casein or beta lactoglobulin.

The vectors containing the polynucleotide sequences of interest (e.g., the heavy and light chain encoding sequences and expression control sequences) can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment, electroporation, lipofection, biolistics or viral-based transfection may be used for other cellular hosts. (See generally Sambrook et al., *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Press, 2nd ed., 1989) (incorporated by reference in its entirety for all purposes). Other methods used to transform mammalian cells include the use of polybrene, protoplast fusion, liposomes, electroporation, and microinjection (see generally, Sambrook et al., supra). For production of transgenic animals, transgenes can be microinjected into fertilized oocytes, or can be incorporated into the genome of embryonic stem cells, and the nuclei of such cells transferred into enucleated oocytes.

When heavy and light chains are cloned on separate expression vectors, the vectors are co-transfected to obtain expression and assembly of intact immunoglobulins. Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin forms of the present invention can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, HPLC purification, gel electrophoresis and the like (see generally Scopes, Protein Purification (Springer-Verlag, N.Y., (1982)). Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses.

6. Antibody Fragments

Also contemplated within the scope of the instant invention are antibody fragments. In one embodiment, fragments of non-human, and/or chimeric antibodies are provided. In another embodiment, fragments of humanized antibodies are provided. Typically, these fragments exhibit specific binding to antigen with an affinity of at least 10^7, and more typically 10^8 or $10^9 M^{-1}$. Humanized antibody fragments include separate heavy chains, light chains, Fab, Fab', F(ab')2, Fabc, and Fv. Fragments are produced by recombinant DNA techniques, or by enzymatic or chemical separation of intact immunoglobulins.

In some embodiments, the generally short half-life of antibody fragments (e.g., Fabs or Fab's) is extended by pegylation. This is generally achieved by fusion to polyethylene glycol (PEG), as described by, for example, Leong, et al. *Cytokine* 16, 106-119 (2001). Pegylation has the added advantage of eliminating Fc receptor mediated function, where desired, and/or reducing immunogenicity. In exemplary embodiments, 2-20 kDa PEG molecules are covalently attached, for example, to an antibody heavy chain hinge region via a K-linker-C (See, e.g., Choy et al., Rheumatol. 41:1133-1137 (2002)).

7. Epitope Mapping

Epitope mapping can be performed to determine which antigenic determinant or epitope of Aβ is recognized by the antibody. In one embodiment, epitope mapping is performed according to Replacement NET (rNET) analysis. The rNET epitope map assay provides information about the contribution of individual residues within the epitope to the overall binding activity of the antibody. rNET analysis uses synthe-

sized systematic single substituted peptide analogs. Binding of an antibody being tested is determined against native peptide (native antigen) and against 19 alternative "single substituted" peptides, each peptide being substituted at a first position with one of 19 non-native amino acids for that position. A profile is generated reflecting the effect of substitution at that position with the various non-native residues. Profiles are likewise generated at successive positions along the antigenic peptide. The combined profile, or epitope map, (reflecting substitution at each position with all 19 non-native residues) can then be compared to a map similarly generated for a second antibody. Substantially similar or identical maps indicate that antibodies being compared have the same or similar epitope specificity.

8. Testing Antibodies for Therapeutic Efficacy (e.g., Plaque Clearing Activity) in Animal Models

Groups of 7-9 month old PDAPP mice each are injected with 0.5 mg in PBS of polyclonal anti-Aβ or specific anti-Aβ monoclonal, humanized, or chimeric antibodies. All antibody preparations are purified to have low endotoxin levels. Monoclonals can be prepared against a fragment by injecting the fragment or longer form of Aβ into a mouse, preparing hybridomas and screening the hybridomas for an antibody that specifically binds to a desired fragment of Aβ without binding to other nonoverlapping fragments of Aβ. Humanized and/or chimeric antibodies are prepared as described herein.

Mice are injected intraperitoneally as needed over a 4 month period to maintain a circulating antibody concentration measured by ELISA titer of greater than 1/1000 defined by ELISA to Aβ42 or other immunogen. Titers are monitored and mice are euthanized at the end of 6 months of injections. Histochemistry, Aβ levels and toxicology are performed post mortem. Ten mice are used per group.

9. Testing Antibodies for Binding to Soluble Oligomeric Aβ

The invention also provides methods of testing the ability of an antibody to bind to soluble, oligomeric Aβ in a biochemical assay. The biochemical assay is based, at least in part, on a comparison of the binding of an antibody to one or more forms of soluble, oligomeric Aβ (e.g., Aβ dimers, Aβ trimers, Aβ tetramers, Aβ pentamers, and the like) as compared to the binding of the antibody to monomeric Aβ. This comparison can be used to determine a relative binding of the antibody to soluble, oligomeric Aβ as compared to monomeric Aβ. In various embodiments, this relative binding is compared to a corresponding relative binding of a control reagent to one or more soluble oligomeric Aβ species versus monomeric Aβ. In other aspects, the affinity of an antibody for one or more oligomeric Aβ species is compared to the antibody's affinity for monomeric Aβ in the Aβ preparation. It has been discovered that a strong correlation exists between an Aβ antibody's ability to preferentially bind soluble, oligomeric Aβ species and the ability of the antibody to rapidly improve cognition as assessed by a CFC assay in an approporate model animal, as described in detail infra. An antibody's ability to improve cognition in the CFC assay is further believed to be a strong indicator or predictor of the antibody's ultimate human therapeutic efficacy (in particular, efficacy in rapidly improving cognition in a patient). Accordingly, a comparison of Aβ antibody binding preferences and/or affinities leads to the identification of certain antibodies as candidates for use in the therapeutic methods of the invention, in particular, for use in method for effecting rapid improvement in cognition in a patient.

Candidate antibodies exhibit a preferential or greater binding to one or more soluble oligomeric Aβ species as compared to monomeric Aβ. Antibodies that preferentially bind to, for example, Aβ dimers, trimers and tetramers as compared to monomeric Aβ are preferred candidates for use in methods for effecting rapid improvement in cognition in a patient. For example, candidate antibodies exhibiting a two-fold, three-fold, four-fold, five-fold, ten-fold, twenty-fold or more greater binding to soluble oligomeric Aβ species as compared to monomeric Aβ are selected for use in the threapeutic methods.

The binding of an antibody to one or more soluble, oligomeric Aβ species or to monomeric Aβ can be determined qualitatively, quantitatively, or combination of both. In general, any technique capable of distinguishing oligomeric Aβ species from monomeric Aβ in an Aβ preparation comprising the species can be used. In exemplary embodiments, one or more of immunoprecipitation, electrophoretic separation, and chromatographic separation (e.g., liquid chromatography), can be used to distinguish oligomeric Aβ species from monomeric Aβ in an Aβ preparation comprising the species.

In preferred embodiments, the binding of the antibody to one or more soluble, oligomeric Aβ species or to monomeric Aβ is determined by immunoprecipitating the Aβ species from the preparation. The immunoprecipitate is then subjected to an electrophoretic separation (e.g., SDS-PAGE) to distinguish oligomeric species from monomeric Aβ in the precipitate. The amount of oligomeric Aβ species and monomeric Aβ present in the electrophoretic bands can be visualized, for example, by immunoblotting of the electrophoretic gel or by direct quantitation of the respective species in the bands of the electrophoretic gel. The amount of precipitate for an Aβ species can be determined, for example, from the intensity of the corresponding electrophoretic bands, immunoblot bands, or a combination of both. The intensity determination can be qualitative, quantitative, or a combination of both.

Assessment of band intensity can be performed, for example, using appropriate film exposures which can be scanned and the density of bands determined with software, for example, AlphaEase™ software (AlphaInnotech™). Assessment of band intensity can be performed, for example, using any of a number of labels incorporated into the antibody, an imaging reagent (e.g., an antibody used in an immunoblot), or both. Suitable labels include, but are not limited to, fluorescent labels, radioactive labels, paramagnetic labels, or combinations thereof.

In various embodiments, the amount of one or more oligomeric Aβ species and/or monomeric Aβ which bind to an antibody can be assessed using mass spectrometry, for example, on the Aβ preparation itself a suitable time after it has been contacted with the antibody, or on monomeric Aβ and/or one or more soluble, oligomeric Aβ species bound to the antibody which have been extracted from the Aβ preparation.

In certain aspects, the affinity of an antibody for one or more oligomeric Aβ species is compared to the antibody's affinity for monomeric Aβ to identify the antibody as a candidate for use in the therapeutic methods of the invention, in particular, for use in method for effecting rapid improvement in cognition in a patient. The affinity of the test antibody (e.g., an Aβ antibody) for oligomeric Aβ as compared to monomeric Aβ can be compared to the binding affinities of a control reagent. Labels can be used to assess the affinity of an antibody for monomeric Aβ, oligomeric Aβ, or both. In various embodiments, a primary reagent with affinity for Aβ is unlabelled and a secondary labeling agent is used to bind to the primary reagent. Suitable labels include, but are not limited to, fluorescent labels, paramagnetic labels, radioactive labels, and combinations thereof.

10. Screening Antibodies for Clearing Activity

The invention also provides methods of screening an antibody for activity in clearing an amyloid deposit or any other antigen, or associated biological entity, for which clearing activity is desired. To screen for activity against an amyloid deposit, a tissue sample from a brain of a patient with Alzheimer's disease or an animal model having characteristic Alzheimer's pathology is contacted with phagocytic cells bearing an Fc receptor, such as microglial cells, and the antibody under test in a medium in vitro. The phagocytic cells can be a primary culture or a cell line, and can be of murine (e.g., BV-2 or C8-B4 cells) or human origin (e.g., THP-1 cells). In some methods, the components are combined on a microscope slide to facilitate microscopic monitoring. In some methods, multiple reactions are performed in parallel in the wells of a microtiter dish. In such a format, a separate miniature microscope slide can be mounted in the separate wells, or a nonmicroscopic detection format, such as ELISA detection of Aβ can be used. Preferably, a series of measurements is made of the amount of amyloid deposit in the in vitro reaction mixture, starting from a baseline value before the reaction has proceeded, and one or more test values during the reaction. The antigen can be detected by staining, for example, with a fluorescently labeled antibody to Aβ or other component of amyloid plaques. The antibody used for staining may or may not be the same as the antibody being tested for clearing activity. A reduction relative to baseline during the reaction of the amyloid deposits indicates that the antibody under test has clearing activity. Such antibodies are likely to be useful in preventing or treating Alzheimer's and other amyloidogenic diseases. Particularly useful antibodies for preventing or treating Alzheimer's and other amyloidogenic diseases include those capable of clearing both compact and diffuse amyloid plaques, for example, the 15C11 or 9G8 antibody of the instant invention, or chimeric or humanized versions thereof.

Analogous methods can be used to screen antibodies for activity in clearing other types of biological entities. The assay can be used to detect clearing activity against virtually any kind of biological entity. Typically, the biological entity has some role in human or animal disease. The biological entity can be provided as a tissue sample or in isolated form. If provided as a tissue sample, the tissue sample is preferably unfixed to allow ready access to components of the tissue sample and to avoid perturbing the conformation of the components incidental to fixing. Examples of tissue samples that can be tested in this assay include cancerous tissue, precancerous tissue, tissue containing benign growths such as warts or moles, tissue infected with pathogenic microorganisms, tissue infiltrated with inflammatory cells, tissue bearing pathological matrices between cells (e.g., fibrinous pericarditis), tissue bearing aberrant antigens, and scar tissue. Examples of isolated biological entities that can be used include Aβ, viral antigens or viruses, proteoglycans, antigens of other pathogenic microorganisms, tumor antigens, and adhesion molecules. Such antigens can be obtained from natural sources, recombinant expression or chemical synthesis, among other means. The tissue sample or isolated biological entity is contacted with phagocytic cells bearing Fc receptors, such as monocytes or microglial cells, and an antibody to be tested in a medium. The antibody can be directed to the biological entity under test or to an antigen associated with the entity. In the latter situation, the object is to test whether the biological entity is phagocytosed with the antigen. Usually, although not necessarily, the antibody and biological entity (sometimes with an associated antigen), are contacted with each other before adding the phagocytic cells. The concentration of the biological entity and/or the associated antigen remaining in the medium, if present, is then monitored. A reduction in the amount or concentration of antigen or the associated biological entity in the medium indicates the antibody has a clearing response against the antigen and/or associated biological entity in conjunction with the phagocytic cells.

11. Testing Antibodies for a Rapid or Prolonged Improvement in Cognition in a CFC Assay

In various aspects, an antibody of the invention can be tested for the ability to improve cognition in an appropriate animal model. For example, the ability of an antibody to improve cognition in an animal model for AD, as assessed via a contextual fear conditioning (CFC) assay, can be used to select the antibody as a candidate for use in the therapeutic methods of the invention, in particular, in methods for effecting rapid improvement in cognition in a patient.

Contextual fear conditioning is a common form of learning that is exceptionally reliable and rapidly acquired in most animals, for example, mammals. Test animals learn to fear a previously neutral stimulus because of its association with an aversive experience and/or environmental cue(s). (see, e.g., Fanselow, *Anim. Learn. Behav.* 18:264-270 (1990); Wehner et al., *Nature Genet.* 17:331-334. (1997); Caldarone et al., *Nature Genet.* 17:335-337 (1997)).

Contextual fear conditioning is especially useful for determining cognitive function or dysfunction, e.g., as a result of disease or a disorder, such as a neurodegenerative disease or disorder, an Aβ-related disease or disorder, an amyloidogenic disease or disorder, the presence of an unfavorable genetic alteration affective cognitive function (e.g., genetic mutation, gene disruption, or undesired genotype), and/or the efficacy of an agent, e.g., an antibody agent, on cognitive ability. Accordingly, the CFC assay provides a method for independently testing and/or validating the therapeutic effect of agents for preventing or treating a cognitive disease or disorder, and in particular, a disease or disorder affecting one or more regions of the brains, e.g., the hippocampus, subiculum, cingulated cortex, prefrontal cortex, perirhinal cortex, sensory cortex, and medial temporal lobe.

Typically, the CFC assay is performed using standard animal chambers and the employment of conditioning training comprising a mild shock (e.g., 0.35 m foot shock) paired with an auditory (e.g., a period of 85 db white noise), olfactory (e.g., almond or lemon extract), touch (e.g., floor cage texture), and/or visual cue (light flash). Alternatively, conditioning training comprises administration of the shock absent a paired cue (i.e., shock associated with context). The response to the aversive experience (shock) is typically one of freezing (absence of movement except for respiration) but may also include eye blink, or change in the nictitating membrane reflex, depending on the test animal selected. The aversive response is usually characterized on the first day of testing to determine a baseline for unconditioned fear with aversive response results on subsequent test days (e.g., freezing in the same context but in the absence of the aversive stimulus and/or freezing in presence of the cue but in the absence of the aversive experience) being characterized as contextually conditioned fear. For improved reliability, test animals are typically tested separately by independent technicians and scored over time. Additional experimental design details can be found in the art, for example, in Crawley, J N, *What's Wrong with my Mouse; Behavioral Phenotyping of Transgenic and Knockout Mice*, Wiley-Liss, NY (2000).

Exemplary test animals (e.g., model animals) include mammals (e.g. rodents or non-human primates) that exhibit prominent symptoms or pathology that is characteristic of an

amyloidogenic disorder such as Alzheimer's. Model animals may be created by selective inbreeding for a desired or they may genetically engineered using transgenic techniques that are well-known in the art, such that a targeted genetic alteration (e.g. a genetic mutation, gene disruption) in a gene that is associated with the dementia disorder, leading to aberrant expression or function of the targeted gene. For example, several transgenic mouse strains are available that overexpress APP and develop amyloid plaque pathology and/or develop cognitive deficits that are characteristic of Alzheimer's disease (see for example, Games et al., supra, Johnson-Wood et al., *Proc. Natl. Acad. Sci. USA* 94:1550 (1997); Masliah E and Rockenstein E. (2000) *J Neural Transm Suppl.;* 59:175-83).

Alternatively, the model animal can be created using chemical compounds (e.g. neurotoxins, anaesthetics) or surgical techniques (e.g. stereotactic ablation, axotomization, transection, aspiration) that ablate or otherwise interfere with the normal function of an anatomical brain region (e.g. hippocampus, amygdala, perirhinal cortex, medial septal nucleus, locus coeruleus, mammalary bodies) or specific neurons (e.g. serotonergic, cholinergic, or dopaminergic neurons) that are associated with characteristic symptoms or pathology of the amyloidogenic disorder. In certain preferred embodiments, the animal model exhibits a prominent cognitive deficit associated with learning or memory in addition to the neurodegenerative pathology that associated with a amyloidogenic disorder. More preferably, the cognitive deficit progressively worsens with increasing age, such that the disease progression in the model animal parallels the disease progression in a subject suffering from the amyloidogenic disorder.

Conditional fear conditioning and other in vivo assays to test the functionality of the antibodies described herein may be performed using wild-type mice or mice having a certain genetic alteration leading to impaired memory or mouse models of neurodegenerative disease, e.g., Alzheimer's disease, including mouse models which display elevated levels of soluble Aβ in the cerebrospinal fluid (CSF) or plasma. For example, animal models for Alzheimer's disease include transgenic mice that overexpress the "Swedish" mutation of human amyloid precursor protein (hAPPswe; Tg2576) which show age-dependent memory deficits and plaques (Hsiao et al. (1996) *Science* 274:99-102). The in vivo functionality of the antibodies described herein can also be tested using PDAPP transgenic mice, which express a mutant form of human APP (APP^{V71F}) and develop Alzheimer's disease at a young age (Bard, et al. (2000) *Nature Medicine* 6:916-919; Masliah E, et al. (1996) *J Neurosci.* 15; 16(18):5795-811). Other mouse models for Alzheimer's disease include the PSAPP mouse, a doubly transgenic mouse (PSAPP) overexpressing mutant APP and PS1 transgenes, described in Holcomb, et al. (1998) *Nature Medicine* 4:97-110, and the PS-1 mutant mouse, described in Duff, et al. (1996) *Nature* 383, 710-713. Other genetically altered transgenic models of Alzheimer's disease are described in Masliah E and Rockenstein E. (2000) *J Neural Transm Suppl.* 59:175-83.

12. Chimeric/Humanized Antibodies Having Altered Effector Function

For the above-described antibodies of the invention comprising a constant region (Fc region), it may also be desirable to alter the effector function of the molecule. Generally, the effector function of an antibody resides in the constant or Fc region of the molecule which can mediate binding to various effector molecules, e.g., complement proteins or Fc receptors. The binding of complement to the Fc region is important, for example, in the opsonization and lysis of cell pathogens and the activation of inflammatory responses. The binding of antibody to Fc receptors, for example, on the surface of effector cells can trigger a number of important and diverse biological responses including, for example, engulfment and destruction of antibody-coated pathogens or particles, clearance of immune complexes, lysis of antibody-coated target cells by killer cells (i.e., antibody-dependent cell-mediated cytotoxicity, or ADCC), release of inflammatory mediators, placental transfer of antibodies, and control of immunoglobulin production.

Accordingly, depending on a particular therapeutic or diagnostic application, the above-mentioned immune functions, or only selected immune functions, may be desirable. By altering the Fc region of the antibody, various aspects of the effector function of the molecule, including enhancing or suppressing various reactions of the immune system, with beneficial effects in diagnosis and therapy, are achieved.

The antibodies of the invention can be produced which react only with certain types of Fc receptors, for example, the antibodies of the invention can be modified to bind to only certain Fc receptors, or if desired, lack Fc receptor binding entirely, by deletion or alteration of the Fc receptor binding site located in the Fc region of the antibody. Other desirable alterations of the Fc region of an antibody of the invention are cataloged below. Typically the EU numbering system (ie. as in the EU index of Kabat et al., supra) is used to indicate which amino acid residue(s) of the Fc region (e.g., of an IgG antibody) are altered (e.g., by amino acid substitution) in order to achieve a desired change in effector function. The numbering system is also employed to compare antibodies across species such that a desired effector function observed in, for example, a mouse antibody, can then be systematically engineered into a human, humanized, or chimeric antibody of the invention.

For example, it has been observed that antibodies (e.g., IgG antibodies) can be grouped into those found to exhibit tight, intermediate, or weak binding to an Fc receptor (e.g., an Fc receptor on human monocytes (FcγRI)). By comparison of the amino-acid sequences in these different affinity groups, a monocyte-binding site in the hinge-link region (Leu234-Ser239 according to EU numbering system) has been identified. Moreover, the human FcγRI receptor binds human IgG1 and mouse IgG2a as a monomer, but the binding of mouse IgG2b is 100-fold weaker. A comparison of the sequence of these proteins in the hinge-link region shows that the sequence from EU numbering positions 234 to 238, i.e., Leu-Leu-Gly-Gly-Pro (SEQ ID NO: 47) in the strong binders becomes Leu-Glu-Gly-Gly-Pro (SEQ ID NO:48) in mouse gamma 2b, i.e., weak binders. Accordingly, a corresponding change in a human antibody hinge sequence can be made if reduced FcγI receptor binding is desired. It is understood that other alterations can be made to achieve the same or similar results. For example, the affinity of FcγRI binding can be altered by replacing the specified residue with a residue having an inappropriate functional group on its sidechain, or by introducing a charged functional group (e.g., Glu or Asp) or for example an aromatic non-polar residue (e.g., Phe, Tyr, or Trp).

These changes can be equally applied to the murine, human, and rat systems given the sequence homology between the different immunoglobulins. It has been shown that for human IgG3, which binds to the human FcγRI receptor, changing Leu at EU position 235 to Glu destroys the interaction of the mutant for the receptor. The binding site for this receptor can thus be switched on or switched off by making the appropriate mutation.

Mutations on adjacent or close sites in the hinge link region (e.g., replacing residues at EU positions 234, 236 or 237 by Ala) indicate that alterations in residues 234, 235, 236, and 237 at least affect affinity for the FcγRI receptor. Accordingly, the antibodies of the invention can also have an altered Fc region with altered binding affinity for FcγRI as compared with the unmodified antibody. Such an antibody conveniently has a modification at EU amino acid positions 234, 235, 236, or 237.

In some embodiments, an antibody of the invention is a humanized antibody including amino acid alterations at one or more EU positions 234, 235, 236 and 237. In a particular embodiment of the invention, a humanized antibody includes amino acid alterations at EU positions 234 and 237 of the hinge link region derived from IgG1 (i.e., L234A and G237A).

Affinity for other Fc receptors can be altered by a similar approach, for controlling the immune response in different ways.

As a further example, the lytic properties of IgG antibodies following binding of the Cl component of complement can be altered.

The first component of the complement system, Cl, comprises three proteins known as Clq, Clr and Cls which bind tightly together. It has been shown that Clq is responsible for binding of the three protein complex to an antibody.

Accordingly, the Clq binding activity of an antibody can be altered by providing an antibody with an altered CH 2 domain in which at least one of the amino acid residues at EU amino acid positions 318, 320, and 322 of the heavy chain has been changed to a residue having a different side chain. Other suitable alterations for altering, e.g., reducing or abolishing specific C1q-binding to an antibody include changing any one of residues at EU positions 318 (Glu), 320 (Lys) and 322 (Lys), to Ala.

Moreover, by making mutations at these residues, it has been shown that Clq binding is retained as long as residue 318 has a hydrogen-bonding side chain and residues 320 and 322 both have a positively charged side chain.

Clq binding activity can be abolished by replacing any one of the three specified residues with a residue having an inappropriate functionality on its side chain. It is not necessary to replace the ionic residues only with Ala to abolish Clq binding. It is also possible to use other alkyl-substituted non-ionic residues, such as Gly, Ile, Leu, or Val, or such aromatic non-polar residues as Phe, Tyr, Trp and Pro in place of any one of the three residues in order to abolish Clq binding. In addition, it is also be possible to use such polar non-ionic residues as Ser, Thr, Cys, and Met in place of residues 320 and 322, but not 318, in order to abolish Clq binding activity.

It is also noted that the side chains on ionic or non-ionic polar residues will be able to form hydrogen bonds in a similar manner to the bonds formed by the Glu residue. Therefore, replacement of the 318 (Glu) residue by a polar residue may modify but not abolish Clq binding activity.

It is also known that replacing residue 297 (Asn) with Ala results in removal of lytic activity while only slightly reducing (about three fold weaker) affinity for Clq. This alteration destroys the glycosylation site and the presence of carbohydrate that is required for complement activation. Any other substitution at this site will also destroy the glycosylation site.

The invention also provides an antibody having an altered effector function wherein the antibody has a modified hinge region. 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 one example, 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. Such alterations are carried out using art recognized protein chemistry and, preferably, genetic engineering techniques, as described herein.

In one embodiment of the invention, the number of cysteine residues in the hinge region of the antibody is reduced, for example, to one cysteine residue. This modification has the advantage of facilitating the assembly of the antibody, for example, bispecific antibody molecules and antibody molecules wherein the Fc portion has been replaced by an effector or reporter molecule, since it is only necessary to form a single disulfide bond. This modification also provides a specific target for attaching the hinge region either to another hinge region or to an effector or reporter molecule, either directly or indirectly, for example, by chemical means.

Conversely, the number of cysteine residues in the hinge region of the antibody is increased, for example, at least one more than the number of normally occurring cysteine residues. Increasing the number of cysteine residues can be used to stabilize the interactions between adjacent hinges. Another advantage of this modification is that it facilitates the use of cysteine thiol groups for attaching effector or reporter molecules to the altered antibody, for example, a radiolabel.

Accordingly, the invention provides for an exchange of hinge regions between antibody classes, in particular, IgG classes, and/or an increase or decrease in the number of cysteine residues in the hinge region in order to achieve an altered effector function (see for example U.S. Pat. No. 5,677,425 which is expressly incorporated herein). A determination of altered antibody effector function is made using the assays described herein or other art recognized techniques.

Importantly, the resultant antibody can be subjected to one or more assays to evaluate any change in biological activity compared to the starting antibody. For example, the ability of the antibody with an altered Fc region to bind complement or Fc receptors can be assessed using the assays disclosed herein as well as any art recognized assay.

Production of the antibodies of the invention is carried out by any suitable technique including techniques described herein as well as techniques known to those skilled in the art. For example an appropriate protein sequence, e.g. forming part of or all of a relevant constant domain, e.g., Fc region, i.e., CH2, and/or CH3 domain(s), of an antibody, and include appropriately altered residue(s) can be synthesized and then chemically joined into the appropriate place in an antibody molecule.

Preferably, genetic engineering techniques are used for producing an altered antibody. Preferred techniques include, for example, preparing suitable primers for use in polymerase chain reaction (PCR) such that a DNA sequence which encodes at least part of an IgG heavy chain, e.g., an Fc or constant region (e.g., CH2, and/or CH3) is altered, at one or more residues. The segment can then be operably linked to the remaining portion of the antibody, e.g., the variable region of the antibody and required regulatory elements for expression in a cell.

The present invention also includes vectors used to transform the cell line, vectors used in producing the transforming vectors, cell lines transformed with the transforming vectors, cell lines transformed with preparative vectors, and methods for their production.

Preferably, the cell line which is transformed to produce the antibody with an altered Fc region (i.e., of altered effector function) is an immortalized mammalian cell line (e.g., CHO cell).

Although the cell line used to produce the antibody with an altered Fc region is preferably a mammalian cell line, any other suitable cell line, such as a bacterial cell line or a yeast cell line, may alternatively be used.

B. Nucleic Acid Molecules Encoding Immunologic and Therapeutic Agents

Immune responses against amyloid deposits can also be induced by administration of nucleic acids encoding antibodies and their component chains used for passive immunization. Such nucleic acids can be DNA or RNA. A nucleic acid segment encoding an immunogen is typically linked to regulatory elements, such as a promoter and enhancer, that allow expression of the DNA segment in the intended target cells of a patient. For expression in blood cells, as is desirable for induction of an immune response, exemplary promoter and enhancer elements include those from light or heavy chain immunoglobulin genes and/or the CMV major intermediate early promoter and enhancer (Stinski, U.S. Pat. Nos. 5,168,062 and 5,385,839). The linked regulatory elements and coding sequences are often cloned into a vector. For administration of double-chain antibodies, the two chains can be cloned in the same or separate vectors.

A number of viral vector systems are available including retroviral systems (see, e.g., Lawrie and Tumin, *Cur. Opin. Genet. Develop.* 3:102-109 (1993)); adenoviral vectors (see, e.g., Bett et al., *J. Virol.* 67:5911 (1993)); adeno-associated virus vectors (see, e.g., Zhou et al., *J. Exp. Med.* 179:1867 (1994)), viral vectors from the pox family including vaccinia virus and the avian pox viruses, viral vectors from the alpha virus genus such as those derived from Sindbis and Semliki Forest Viruses (see, e.g., Dubensky et al., *J. Virol.* 70:508 (1996)), Venezuelan equine encephalitis virus (see Johnston et al., U.S. Pat. No. 5,643,576) and rhabdoviruses, such as vesicular stomatitis virus (see Rose, U.S. Pat. No. 6,168,943) and papillomaviruses (Ohe et al., *Human Gene Therapy* 6:325 (1995); Woo et al., WO 94/12629 and Xiao & Brandsma, *Nucleic Acids. Res.* 24, 2630-2622 (1996)).

DNA encoding an antibody of the invention, e.g., 15C11, or a vector containing the same, can be packaged into liposomes. Suitable lipids and related analogs are described by Eppstein et al., U.S. Pat. No. 5,208,036, Felgner et al., U.S. Pat. No. 5,264,618, Rose, U.S. Pat. No. 5,279,833, and Epand et al., U.S. Pat. No. 5,283,185. Vectors and DNA encoding an immunogen can also be adsorbed to or associated with particulate carriers, examples of which include polymethyl methacrylate polymers and polylactides and poly (lactide-co-glycolides), see, e.g., McGee et al., *J. Micro Encap.* (1996).

Gene therapy vectors or naked polypeptides (e.g., DNA) can be delivered in vivo by administration to an individual patient, typically by systemic administration (e.g., intravenous, intraperitoneal, nasal, gastric, intradermal, intramuscular, subdermal, or intracranial infusion) or topical application (see e.g., Anderson et al., U.S. Pat. No. 5,399,346). The term "naked polynucleotide" refers to a polynucleotide not delivered in association with a transfection facilitating agent. Naked polynucleotides are sometimes cloned in a plasmid vector. Such vectors can further include facilitating agents such as bupivacaine (Weiner et al., U.S. Pat. No. 5,593,972). DNA can also be administered using a gene gun. See Xiao & Brandsma, supra. The DNA encoding an immunogen is precipitated onto the surface of microscopic metal beads. The microprojectiles are accelerated with a shock wave or expanding helium gas, and penetrate tissues to a depth of several cell layers. For example, The Accel™ Gene Delivery Device manufactured by Agricetus, Inc. Middleton Wis. is suitable. Alternatively, naked DNA can pass through skin into the blood stream simply by spotting the DNA onto skin with chemical or mechanical irritation (see Howell et al., WO 95/05853).

In a further variation, vectors encoding immunogens can be delivered to cells ex vivo, such as cells explanted from an individual patient (e.g., lymphocytes, bone marrow aspirates, tissue biopsy) or universal donor hematopoietic stem cells, followed by reimplantation of the cells into a patient, usually after selection for cells which have incorporated the vector.

II. Prophylactic and Therapeutic Methods

The present invention is directed inter alia to treatment of Alzheimer's and other amyloidogenic diseases by administration of therapeutic immunological reagents (e.g., humanized immunoglobulins) to specific epitopes within Aβ to a patient under conditions that generate a beneficial therapeutic response in a patient (e.g., capture of soluble Aβ, reduction of plaque burden, inhibition of plaque formation, reduction of neuritic dystrophy, improving cognitive function, e.g., rapidly improving cognition, and/or reversing, treating or preventing cognitive decline) in the patient, for example, for the prevention or treatment of an amyloidogenic disease. The invention is also directed to use of the disclosed immunological reagents (e.g., humanized immunoglobulins) in the manufacture of a medicament for the treatment or prevention of an amyloidogenic disease.

In one aspect, the invention provides methods of preventing or treating a disease associated with amyloid deposits of Aβ in the brain of a patient. Such diseases include Alzheimer's disease, Down's syndrome and cognitive impairment. The latter can occur with or without other characteristics of an amyloidogenic disease. Some methods of the invention comprise administering an effective dosage of an antibody that specifically binds to a component of an amyloid deposit to the patient. Such methods are particularly useful for preventing or treating Alzheimer's disease in human patients. Exemplary methods comprise administering an effective dosage of an antibody that binds to Aβ. Preferred methods comprise administering an effective dosage of an antibody that specifically binds to an epitope within residues 13-28 of Aβ, for example, antibodies that specifically bind to an epitope within residues 14-27 of Aβ, antibodies that specifically bind to an epitope within residues 15-26 of Aβ, antibodies that specifically bind to an epitope within residues 16-25 of Aβ, antibodies that specifically bind to an epitope within residues 17-24 of Aβ, antibodies that specifically bind to an epitope within residues 18-23 of Aβ, or antibodies that specifically bind to an epitope within residues 19-22 of Aβ. In yet another aspect, the invention features administering antibodies that bind to an epitope comprising a free N-terminal residue of Aβ. In yet another aspect, the invention features administering antibodies that specifically bind to Aβ peptide without binding to full-length amyloid precursor protein (APP). In yet another aspect, the isotype of the antibody is human IgG1. In yet another aspect, the isotype of the antibody is human IgG4. In yet another embodiment, the invention features administering antibodies that bind to and/or capture soluble Aβ.

In yet another aspect, the isotype of the antibody is IgG4. In another aspect, an antibody of the invention is engineered to have an isotype having reduced effector function (e.g., reduced Fc-mediated phagocytosis, reduced ability to opsonize plaques etc.). In a particular embodiment, an antibody of the invention is a humanized 15C11 antibody having an IgG4 isotype.

In yet another aspect, the invention features administering antibodies that bind to an amyloid deposit in the patient and induce a clearing response against the amyloid deposit. For example, such a clearing response can be effected by Fc receptor mediated phagocytosis. Such a clearing response can be engineered into an antibody, for example, by including an Fc receptor binding domain (e.g., an IgG2a constant region).

Therapeutic agents of the invention are typically substantially pure from undesired contaminant. This means that an agent is typically at least about 50% w/w (weight/weight) pure, as well as being substantially free from interfering proteins and contaminants. Sometimes the agents are at least about 80% w/w and, more preferably at least 90 or about 95% w/w pure. However, using conventional protein purification techniques, homogeneous peptides of at least 99% w/w pure can be obtained.

The methods can be used on both asymptomatic patients and those currently showing symptoms of disease. The antibodies used in such methods can be human, humanized, chimeric or nonhuman antibodies, or fragments thereof (e.g., antigen binding fragments) and can be monoclonal or polyclonal, as described herein. In yet another aspect, the invention features administering antibodies prepared from a human immunized with Aβ peptide, which human can be the patient to be treated with antibody.

In another aspect, the invention features administering an antibody with a pharmaceutical carrier as a pharmaceutical composition. Alternatively, the antibody can be administered to a patient by administering a polynucleotide encoding at least one antibody chain. The polynucleotide is expressed to produce the antibody chain in the patient. Optionally, the polynucleotide encodes heavy and light chains of the antibody. The polynucleotide is expressed to produce the heavy and light chains in the patient. In exemplary embodiments, the patient is monitored for level of administered antibody in the blood of the patient.

The invention thus fulfills a longstanding need for therapeutic regimes for preventing or ameliorating the neuropathology and, in some patients, the cognitive impairment associated with Alzheimer's disease.

A. Rapid Improvement in Cognition

The present invention provides methods for effecting rapid improvement in cognition in a patient having or at risk for an suffering from an Aβ-related disease or disorder or amyloidogenic disease or disorder (e.g., AD). In preferred aspects, the methods feature administering an effective dose of an antibody agent such that rapid improvement in cognition is achieved. In exemplary aspects of the invention, improvement in one or more cognitive deficits in the patient (e.g., procedural learning and/or memory, deficits) is achieved. The cognitive deficit can be an impairment in explicit memory (also known as "declarative" or "working" memory), which is defined as the ability to store and retrieve specific information that is available to consciousness and which can therefore be expressed by language (e.g. the ability to remember a specific fact or event). Alternatively, the cognitive deficit can be an impairment in procedural memory (also known as "implicit" or "contextual" memory), which is defined as the ability to acquire, retain, and retrieve general information or knowledge that is not available to consciousness and which requires the learning of skills, associations, habits, or complex reflexes to be expressed, e.g. the ability to remember how to execute a specific task. Individuals suffering from procedural memory deficits are much more impaired in their ability to function normally. As such, treatments which are effective in improving deficits in procedural memory are highly desirable and advantageous.

B. Patients Amenable to Treatment

Patients amenable to treatment include individuals at risk of an Aβ-related disease or disorder or amyloidogenic disease or disorder but not showing symptoms, as well as patients presently showing 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 especially useful for individuals who are at risk for AD, e.g., those who exhibit risk factors of AD. The main risk factor for AD is increased age. As the population ages, the frequency of AD continues to increase. Current estimates indicate that up to 10% of the population over the age of 65 and up to 50% of the population over the age of 85 have AD.

Although rare, certain individuals can be identified at an early age as being genetically predisposed to developing AD. Individuals carrying the heritable form of AD, known as "familial AD" or "early-onset AD", can be identified from a well documented family history of AD, of the analysis of a gene that is known to confer AD when mutated, for example the APP or presenilin gene. Well characterized APP mutations include the "Hardy" mutations at codons 716 and 717 of APP770 (e.g., valine^{717} to isoleucine (Goate et al., (1991), *Nature* 349:704); valine^{717} to glycine (Chartier et al. (1991) *Nature* 353:844; Murrell et al. (1991), *Science* 254:97); valine^{717} to phenylalanine (Mullan et al. (1992), *Nature Genet.* 1:345-7)), the "Swedish" mutations at codon 670 and 671 of APP770, and the "Flemish" mutation at codon 692 of APP770. Such mutations are thought to cause Alzheimer's disease by increased or altered processing of APP to Aβ, particularly processing of APP to increased amounts of the long form of Aβ (i.e., Aβ1-42 and Aβ 1-43). Mutations in other genes, such as the presenilin genes, PS1 and PS2, are thought indirectly to affect processing of APP to generate increased amounts of long form Aβ (see Hardy, *TINS* 20: 154 (1997); Kowalska et al., (2004), *Polish J. Pharmacol.,* 56: 171-8). In addition to AD, mutations at amino acid 692 or 693 of the 770-amino acid isoform of APP has been implicated in cerebral amyloidogenic disorder called Hereditary Cerebral Hemorrhage with Amyloidosis of the Dutch-type (HCHWA-D).

More commonly, AD is not inherited by a patient but develops due to the complex interplay of a variety of genetic factors. These individuals are said to have "sporadic AD" (also known as "late-onset AD"), a form which is much more difficult to diagnose. Nonetheless, the patient population can be screened for the presence of susceptibility alleles or traits that do not cause AD but are known to segregate with AD at a higher frequency than in the general population, e.g., the ϵ2, ϵ3, and ϵ4 alleles of apolipoprotein E (Corder et. al. (1993), *Science,* 261: 921-923). In particular, patients lacking the ϵ4 allele, preferably in addition to some other marker for AD, may be identified as "at risk" for AD. For example, patients lacking the ϵ4 allele who have relatives who have AD or who suffer from hypercholesterolemia or atherosclerosis may be identified as "at risk" for AD. Another potential biomarker is the combined assessment of cerebral spinal fluid (CSF) Aβ42 and tau levels. Low Aβ42 and high tau levels have a predictive value in identifying patients at risk for AD.

Other indicators of patients at risk for AD include in vivo dynamic neuropathological data, for example, in vivo detection of brain beta amyloid, patterns of brain activation, etc. Such data can be obtained using, for example, three-dimensional magnetic resonance imaging (MRI), positron emission

tomography (PET) scan and single-photon emission computed tomography (SPECT). Indicators of patients having probable AD include, but are not limited to, patients (1) having dementia, (2) of an age of 40-90 years old, (3) cognitive deficits, e.g., in two or more cognitive domains, (4) progression of deficits for more than six months, (5) consciousness undisturbed, and/or (6) absence of other reasonable diagnoses.

Individuals suffering either sporadic or familial forms of AD are usually, however, diagnosed following presentation of one or more characteristic symptoms of AD. Common symptoms of AD include cognitive deficits that affect the performance of routine skills or tasks, problems with language, disorientation to time or place, poor or decreased judgement, impairments in abstract thought, loss of motor control, mood or behaviour alteration, personality change, or loss of initiative. The number deficits or the degree of the cognitive deficit displayed by the patient usually reflects the extent to which the disease has progressed. For example, the patient may exhibit only a mild cognitive impairment, such that the patient exhibits problems with memory (e.g. contextual memory) but is otherwise able to function well.

The present methods are also useful for individuals who have an Aβ-related cognitive deficit, e.g. Aβ-related dementia. In particular, the present methods are especially useful for individuals who have a cognitive deficit or aberrancy caused by or attributed to the presence of soluble oligomeric Aβ in the central nervous system (CNS), for example, in the brain or CSF. Cognitive deficits caused by or associated with Aβ also include those caused by or associated with: (1) the development of β-amyloid plaques in the brain; (2) abnormal rates of Aβ synthesis, processing, degradation or clearance; (3) the formation or activity of soluble oligomeric Aβ species (e.g., in the brain); and/or (4) the formation of abnormal forms of Aβ. It is not necessary that an actual causative link be established between an Aβ abnormality and cognitive deficit in a particular patient, however, some the link should be indicated, for example, by one of the above-described markers of AD to distinguish patients suffering from non-Aβ related cognitive deficits who would not be expected to benefit from treatment with an Aβ immunotherapeutic agent.

Several tests have been developed to assess cognitive skills or performance in human subjects, for example, subjects at risk for or having symptoms or pathology of dementia disorders (e.g., AD). Cognitive deficits can be identified by impaired performance of these tests, and many treatments have been proposed based on their ability to improve performance in these tests. Although some tasks have evaluated behaviors or motor function of subjects, most tasks have been designed to test learning or memory.

Cognition in humans may be assessed using a wide variety of tests including, but not limited to, the following tests. The ADAS-Cog (Alzheimer Disease Assessment Scale-Cognitive) is 11-part test that takes 30 minutes to complete. The ADAS-Cog is a preferred brief exam for the study of language and memory skills. See Rosen et al. (1984) *Am J Psychiatry.* 141(11):1356-64; Ihl et al. (2000) *Neuropsychobiol.* 41(2): 102-7; and Weyer et al. (1997) *Int Psychogeriatr.* 9(2):123-38.

The Blessed Test is another quick (~10 minute) test of cognition which assesses activities of daily living and memory, concentration and orientation. See Blessed et al. (1968) *Br J Psychiatry* 114(512):797-811.

The Cambridge Neuropsychological Test Automated Battery (CANTAB) is used for the assessment of cognitive deficits in humans with neurodegenerative diseases or brain damage. It consists of thirteen interrelated computerized tests of memory, attention, and executive function, and is administered via a touch sensitive screen from a personal computer. The tests are language and largely culture free, and have shown to be highly sensitive in the early detection and routine screening of Alzheimer's disease. See Swainson et al. (2001) *Dement Geriatr Cogn Disord.;* 12:265-280; and Fray and Robbins (1996) *Neurotoxicol Teratol.* 18(4):499-504. Robbins et al. (1994) *Dementia* 5(5):266-81.

The Consortium to Establish a Registry for Alzheimer's Disease (CERAD) Clinical and Neuropsychological Tests include a verbal fluency test, Boston Naming Test, Mini Mental State Exam (MMSE), ten-item word recall, constructional praxis, and delayed recall of praxis items. The test typically takes 20-30 minutes and is convenient and effective at assessing and tracking cognitive decline. See Morris et al. (1988) *Psychopharmacol Bull.* 24(4):641-52; Morris et al. (1989) *Neurology* 39(9):1159-65; and Welsh et al. (1991) *Arch Neurol.* 48(3):278-81.

The Mini Mental State Exam (MMSE) developed in 1975 by Folestein et al, is a brief test of mental status and cognition function. It does not measure other mental phenomena and is therefore not a substitute for a full mental status examination. It is useful in screening for dementia and its scoring system is helpful in following progress over time. The Mini-Mental State Examination MMSE is widely used, with norms adjusted for age and education. It can be used to screen for cognitive impairment, to estimate the severity of cognitive impairment at a given point in time, to follow the course of cognitive changes in an individual over time, and to document an individual's response to treatment. Cognitive assessment of subjects may require formal neuropsychologic testing, with follow-up testing separated by nine months or more (in humans). See Folstein et al. (1975) *J Psychiatr Res.* 12:196-198; Cockrell and Folstein (1988) *Psychopharm Bull.* 24(4): 689-692; and Crum et al. (1993) *J. Am. Med. Association* 18:2386-2391.

The Seven-Minute Screen is a screening tool to help identify patients who should be evaluated for Alzheimer's disease. The screening tool is highly sensitive to the early signs of AD, using a series of questions to assess different types of intellectual functionality. The test consists of 4 sets of questions that focus on orientation, memory, visuospatial skills and expressive language. It can distinguish between cognitive changes due to the normal aging process and cognitive deficits due to dementia. See Solomon and Pendlebury (1998) *Fam Med.* 30(4):265-71, Solomon et al. (1998) *Arch Neurol.* 55(3):349-55.

Individuals presently suffering from Alzheimer's disease can be recognized from characteristic dementia, as well as the presence of risk factors described above. In addition, a number of diagnostic tests are available for identifying individuals who have AD. These include measurement of CSF tau and Aβ42 levels. Elevated tau and decreased Aβ42 levels signify the presence of AD. Individuals suffering from Alzheimer's disease can also be diagnosed by ADRDA criteria as discussed in the Examples section.

C. Treatment Regimes and Dosages

In prophylactic applications, pharmaceutical compositions or medicaments are administered to a patient susceptible to, or otherwise at risk of, Alzheimer's disease in an amount sufficient to eliminate or reduce the risk, lessen the severity, or delay the outset of the disease, including biochemical, histologic 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 an amount sufficient to cure, or at least partially arrest, the symptoms of the disease (biochemical, histologic and/or behavioral), including its complications and intermediate pathological phenotypes in development of the disease.

In some methods, administration of agent reduces or eliminates myocognitive impairment in patients that have not yet developed characteristic Alzheimer's pathology. An amount adequate to accomplish therapeutic or prophylactic treatment is defined as a therapeutically- or prophylactically-effective dose. In both prophylactic and therapeutic regimes, agents are usually administered in several dosages until a sufficient immune response has been achieved. The term "immune response" or "immunological response" includes the development of a humoral (antibody mediated) and/or a cellular (mediated by antigen-specific T cells or their secretion products) response directed against an antigen in a recipient subject. Such a response can be an active response, i.e., induced by administration of immunogen, or a passive response, i.e., induced by administration of immunoglobulin or antibody or primed T-cells. Typically, the immune response is monitored and repeated dosages are given if the immune response starts to wane.

Effective doses of the compositions of the present invention, for the treatment of the above described conditions vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. Usually, the patient is a human but non-human mammals including transgenic mammals can also be treated. Treatment dosages need to be titrated to optimize safety and efficacy.

For passive immunization with an antibody, the dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg (e.g., 0.02 mg/kg, 0.25 mg/kg, 0.5 mg/kg, 0.75 mg/kg, 1 mg/kg, 2 mg/kg, etc.), of the host body weight. For example dosages can be 1 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg, preferably at least 1 mg/kg. In another example, dosages can be 0.5 mg/kg body weight or 15 mg/kg body weight or within the range of 0.5-15 mg/kg, preferably at least 1 mg/kg. In another example, dosages can be 0.5 mg/kg body weight or 20 mg/kg body weight or within the range of 0.5-20 mg/kg, preferably at least 1 mg/kg. In another example, dosages can be 0.5 mg/kg body weight or 30 mg/kg body weight or within the range of 0.5-30 mg/kg, preferably at least 1 mg/kg. In a preferred example, dosages can be about 30 kg/mg. In a particularly preferred example, the 15C11 antibody is administered intraperitoneally at a dose range from approximately 0.3 mg/kg to approximately 30 mg/kg.

Doses intermediate in the above ranges are also intended to be within the scope of the invention. Subjects can be administered such doses daily, on alternative days, weekly or according to any other schedule determined by empirical analysis. An exemplary treatment involves administration in multiple dosages over a prolonged period, for example, of at least six months. Additional exemplary treatment regimes involve administration once per every two weeks or once a month or once every 3 to 6 months. Exemplary dosage schedules include 1-10 mg/kg or 15 mg/kg on consecutive days, 30 mg/kg on alternate days or 60 mg/kg weekly. In some methods, two or more monoclonal antibodies with different binding specificities are administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated.

Antibody is usually administered on multiple occasions. Intervals between single dosages can be weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of antibody to Aβ in the patient. In some methods, dosage is adjusted to achieve a plasma antibody concentration of 1-1000 μg/ml and in some methods 25-300 μg/ml. Alternatively, antibody can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the patient. In general, humanized antibodies show the longest half-life, followed by chimeric antibodies and nonhuman antibodies.

The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, compositions containing the present antibodies or a cocktail thereof are administered to a patient not already in the disease state to enhance the patient's resistance. Such an amount is defined to be a "prophylactic effective dose." In this use, the precise amounts again depend upon the patient's state of health and general immunity, but generally range from 0.1 to 25 mg per dose, especially 0.5 to 2.5 mg per dose. A relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives.

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 involves multiple dosages over a period of time. Treatment can be monitored by assaying antibody levels over time. If the response falls, a booster dosage is indicated. In the case of potential Down's syndrome patients, treatment can begin antenatally by administering therapeutic agent to the mother or shortly after birth.

In therapeutic applications, a relatively high dosage (e.g., from about 1 to 200 mg of antibody per dose, with dosages of from 5 to 25 mg being more commonly used) at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patent can be administered a prophylactic regime.

Doses for nucleic acids encoding antibodies range from about 10 ng to 1 g, 100 ng to 100 mg, 1 μg to 10 mg, or 30-300 μg DNA per patient. Doses for infectious viral vectors vary from 10-100, or more, virions per dose.

Therapeutic agents can be administered by parenteral, topical, intravenous, oral, subcutaneous, intraarterial, intracranial, intraperitoneal, intranasal or intramuscular means for prophylactic and/or therapeutic treatment. The most typical route of administration of an immunogenic agent is subcutaneous although other routes can be equally effective. The next most common route is intramuscular injection. This type of injection is most typically performed in the arm or leg muscles. In some methods, agents are injected directly into a particular tissue where deposits have accumulated, for example intracranial injection. Intramuscular injection or intravenous infusion are preferred for administration of antibody. In some methods, particular therapeutic antibodies are injected directly into the cranium. In some methods, antibodies are administered as a sustained release composition or device, such as a MEDIPAD™ device.

Agents of the invention can optionally be administered in combination with other agents that are at least partly effective in treatment of amyloidogenic disease. In certain embodiments, a humanized antibody of the invention (e.g., humanized 15C11 or 9G8) is administered in combination with a second immunogenic or immunologic agent. For example, a humanized 15C11 or 9G8 antibody of the invention can be

administered in combination with another humanized antibody to Aβ. In other embodiments, a humanized antibody is administered to a patient who has received or is receiving an Aβ vaccine. In the case of Alzheimer's and Down's syndrome, in which amyloid deposits occur in the brain, agents of the invention can also be administered in conjunction with other agents that increase passage of the agents of the invention across the blood-brain barrier. Agents of the invention can also be administered in combination with other agents that enhance access of the therapeutic agent to a target cell or tissue, for example, liposomes and the like. Coadministering such agents can decrease the dosage of a therapeutic agent (e.g., therapeutic antibody or antibody chain) needed to achieve a desired effect.

D. Pharmaceutical Compositions

Agents of the invention are often administered as pharmaceutical compositions comprising an active therapeutic agent, i.e., and a variety of other pharmaceutically acceptable components. See *Remington's Pharmaceutical Science* (15th ed., Mack Publishing Company, Easton, Pa. (1980)). The preferred form depends on the intended mode of administration and therapeutic application. The compositions can also include, depending on the formulation desired, pharmaceutically-acceptable, non-toxic carriers or diluents, which are defined as vehicles commonly used to formulate pharmaceutical compositions for animal or human administration. The diluent is selected so as not to affect the biological activity of the combination. Examples of such diluents are distilled water, physiological phosphate-buffered saline, Ringer's solutions, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation may also include other carriers, adjuvants, or nontoxic, nontherapeutic, nonimmunogenic stabilizers and the like.

Pharmaceutical compositions can also include large, slowly metabolized macromolecules such as proteins, polysaccharides such as chitosan, polylactic acids, polyglycolic acids and copolymers (such as latex functionalized Sepharose™, agarose, cellulose, and the like), polymeric amino acids, amino acid copolymers, and lipid aggregates (such as oil droplets or liposomes). Additionally, these carriers can function as immunostimulating agents (i.e., adjuvants).

For parenteral administration, agents of the invention can be administered as injectable dosages of a solution or suspension of the substance in a physiologically acceptable diluent with a pharmaceutical carrier that can be a sterile liquid such as water oils, saline, glycerol, or ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, surfactants, pH buffering substances and the like can be present in compositions. Other components of pharmaceutical compositions are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, and mineral oil. In general, glycols such as propylene glycol or polyethylene glycol are preferred liquid carriers, particularly for injectable solutions. Antibodies can be administered in the form of a depot injection or implant preparation, which can be formulated in such a manner as to permit a sustained release of the active ingredient. An exemplary composition comprises monoclonal antibody at 5 mg/mL, formulated in aqueous buffer consisting of 50 mM L-histidine, 150 mM NaCl, adjusted to pH 6.0 with HCl.

Typically, compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared. The preparation also can be emulsified or encapsulated in liposomes or micro particles such as polylactide, polyglycolide, or copolymer for enhanced adjuvant effect, as discussed above (see Langer, *Science* 249: 1527 (1990) and Hanes, *Advanced Drug Delivery Reviews* 28:97 (1997)). The agents of this invention can be administered in the form of a depot injection or implant preparation, which can be formulated in such a manner as to permit a sustained or pulsatile release of the active ingredient.

Additional formulations suitable for other modes of administration include oral, intranasal, and pulmonary formulations, suppositories, and transdermal applications. For suppositories, binders and carriers include, for example, polyalkylene glycols or triglycerides; such suppositories can be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1%-2%. Oral formulations include excipients, such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, and magnesium carbonate. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10%-95% of active ingredient, preferably 25%-70%.

Topical application can result in transdermal or intradermal delivery. Topical administration can be facilitated by co-administration of the agent with cholera toxin or detoxified derivatives or subunits thereof or other similar bacterial toxins (See Glenn et al., *Nature* 391, 851 (1998)). Co-administration can be achieved by using the components as a mixture or as linked molecules obtained by chemical crosslinking or expression as a fusion protein.

Alternatively, transdermal delivery can be achieved using a skin patch or using transferosomes (Paul et al., *Eur. J. Immunol.* 25:3521 (1995); Cevc et al., *Biochem. Biophys. Acta* 1368:201-15 (1998)).

E. Monitoring the Course of Treatment

The invention provides methods of monitoring treatment in a patient suffering from or susceptible to Alzheimer's, i.e., for monitoring a course of treatment being administered to a patient. The methods can be used to monitor both therapeutic treatment on symptomatic patients and prophylactic treatment on asymptomatic patients. In particular, the methods are useful for monitoring passive immunization (e.g., measuring level of administered antibody).

Some methods involve determining a baseline value, for example, of an antibody level or profile in a patient, before administering a dosage of agent, and comparing this with a value for the profile or level after treatment. A significant increase (i.e., greater than the typical margin of experimental error in repeat measurements of the same sample, expressed as one standard deviation from the mean of such measurements) in value of the level or profile signals a positive treatment outcome (i.e., that administration of the agent has achieved a desired response). If the value for immune response does not change significantly, or decreases, a negative treatment outcome is indicated.

In other methods, a control value (i.e., a mean and standard deviation) of level or profile is determined for a control population. Typically the individuals in the control population have not received prior treatment. Measured values of the level or profile in a patient after administering a therapeutic agent are then compared with the control value. A significant increase relative to the control value (e.g., greater than one standard deviation from the mean) signals a positive or sufficient treatment outcome. A lack of significant increase or a decrease signals a negative or insufficient treatment outcome. Administration of agent is generally continued while the level is increasing relative to the control value. As before, attainment of a plateau relative to control values is an indicator that the administration of treatment can be discontinued or reduced in dosage and/or frequency.

In other methods, a control value of the level or profile (e.g., a mean and standard deviation) is determined from a control population of individuals who have undergone treatment with a therapeutic agent and whose levels or profiles have plateaued in response to treatment. Measured values of levels or profiles in a patient are compared with the control value. If the measured level in a patient is not significantly different (e.g., more than one standard deviation) from the control value, treatment can be discontinued. If the level in a patient is significantly below the control value, continued administration of agent is warranted. If the level in the patient persists below the control value, then a change in treatment may be indicated.

In other methods, a patient who is not presently receiving treatment but has undergone a previous course of treatment is monitored for antibody levels or profiles to determine whether a resumption of treatment is required. The measured level or profile in the patient can be compared with a value previously achieved in the patient after a previous course of treatment. A significant decrease relative to the previous measurement (i.e., greater than a typical margin of error in repeat measurements of the same sample) is an indication that treatment can be resumed. Alternatively, the value measured in a patient can be compared with a control value (mean plus standard deviation) determined in a population of patients after undergoing a course of treatment. Alternatively, the measured value in a patient can be compared with a control value in populations of prophylactically treated patients who remain free of symptoms of disease, or populations of therapeutically treated patients who show amelioration of disease characteristics. In all of these cases, a significant decrease relative to the control level (i.e., more than a standard deviation) is an indicator that treatment should be resumed in a patient.

The tissue sample for analysis is typically blood, plasma, serum, mucous fluid or cerebrospinal fluid from the patient. The sample is analyzed, for example, for levels or profiles of antibodies to Aβ peptide, e.g., levels or profiles of humanized antibodies. ELISA methods of detecting antibodies specific to Aβ are described in the Examples section. In some methods, the level or profile of an administered antibody is determined using a clearing assay, for example, in an in vitro phagocytosis assay, as described herein. In such methods, a tissue sample from a patient being tested is contacted with amyloid deposits (e.g., from a PDAPP mouse) and phagocytic cells bearing Fc receptors. Subsequent clearing of the amyloid deposit is then monitored. The existence and extent of clearing response provides an indication of the existence and level of antibodies effective to clear Aβ in the tissue sample of the patient under test.

The antibody profile following passive immunization typically shows an immediate peak in antibody concentration followed by an exponential decay. Without a further dosage, the decay approaches pretreatment levels within a period of days to months depending on the half-life of the antibody administered.

In some methods, a baseline measurement of antibody to Aβ in the patient is made before administration, a second measurement is made soon thereafter to determine the peak antibody level, and one or more further measurements are made at intervals to monitor decay of antibody levels. When the level of antibody has declined to baseline or a predetermined percentage of the peak less baseline (e.g., 50%, 25% or 10%), administration of a further dosage of antibody is administered. In some methods, peak or subsequent measured levels less background are compared with reference levels previously determined to constitute a beneficial prophylactic or therapeutic treatment regime in other patients. If the measured antibody level is significantly less than a reference level (e.g., less than the mean minus one standard deviation of the reference value in population of patients benefiting from treatment) administration of an additional dosage of antibody is indicated.

Additional methods include monitoring, over the course of treatment, any art-recognized physiologic symptom (e.g., physical or mental symptom) routinely relied on by researchers or physicians to diagnose or monitor amyloidogenic diseases (e.g., Alzheimer's disease). For example, one can monitor cognitive impairment. The latter is a symptom of Alzheimer's disease and Down's syndrome but can also occur without other characteristics of either of these diseases. For example, cognitive impairment can be monitored by determining a patient's score on the Mini-Mental State Exam in accordance with convention throughout the course of treatment.

F. Kits

The invention further provides kits for performing the monitoring methods described above. Typically, such kits contain an agent that specifically binds to antibodies to Aβ. The kit can also include a label. For detection of antibodies to Aβ, the label is typically in the form of labeled anti-idiotypic antibodies. For detection of antibodies, the agent can be supplied prebound to a solid phase, such as to the wells of a microtiter dish. Kits also typically contain labeling providing directions for use of the kit. The labeling may also include a chart or other correspondence regime correlating levels of measured label with levels of antibodies to Aβ. The term labeling refers to any written or recorded material that is attached to, or otherwise accompanies a kit at any time during its manufacture, transport, sale or use. For example, the term labeling encompasses advertising leaflets and brochures, packaging materials, instructions, audio or videocassettes, computer discs, as well as writing imprinted directly on kits.

The invention also provides diagnostic kits, for example, research, detection and/or diagnostic kits (e.g., for performing in vivo imaging). Such kits typically contain an antibody for binding to an epitope of Aβ, wherein the epitope is accessible to the antibody (e.g., the epitope is accessible in plaque-associated Aβ or amyloid deposits), e.g., residues 13-28. Preferably, the antibody is labeled or a secondary labeling reagent is included in the kit. Preferably, the kit is labeled with instructions for performing the intended application, for example, for performing an in vivo imaging assay. Exemplary antibodies are those described herein.

F. In Vivo Imaging

The invention provides methods of in vivo imaging amyloid deposits in a patient. Such methods are useful to diagnose or confirm diagnosis of Alzheimer's disease, or susceptibility thereto. For example, the methods can be used on a patient presenting with symptoms of dementia. If the patient has abnormal amyloid deposits, then the patient is likely suffering from Alzheimer's disease. The methods can also be used on asymptomatic patients. Presence of abnormal deposits of amyloid indicates susceptibility to future symptomatic disease. The methods are also useful for monitoring disease progression and/or response to treatment in patients who have been previously diagnosed with Alzheimer's disease.

The methods work by administering a reagent, such as antibody that binds to Aβ, to the patient and then detecting the agent after it has bound. Preferred antibodies bind to Aβ deposits in a patient without binding to full length APP polypeptide. Certain antibodies bind without inducing a substantial clearing response. Other antibodies bind and induce a clearing response to Aβ. However, the clearing response can

be avoided by using antibody fragments lacking a full-length constant region, such as Fabs. In some methods, the same antibody can serve as both a treatment and diagnostic reagent.

Diagnostic reagents can be administered by intravenous injection into the body of the patient, or directly into the brain by intracranial injection or by drilling a hole through the skull. The dosage of reagent should be within the same ranges as for treatment methods. Typically, the reagent is labeled, although in some methods, the primary reagent with affinity for Aβ is unlabelled and a secondary labeling agent is used to bind to the primary reagent. The choice of label depends on the means of detection. For example, a fluorescent label is suitable for optical detection. Use of paramagnetic labels is suitable for tomographic detection without surgical intervention. Radioactive labels can also be detected using PET or SPECT.

Diagnosis is performed by comparing the number, size, and/or intensity of labeled loci, to corresponding baseline values. The base line values can represent the mean levels in a population of undiseased individuals. Baseline values can also represent previous levels determined in the same patient. For example, baseline values can be determined in a patient before beginning treatment, and measured values thereafter compared with the baseline values. A decrease in values relative to baseline signals a positive response to treatment.

The present invention will be more fully described by the following non-limiting examples.

EXAMPLES

The following Sequence identifiers are used throughout the Examples section to refer to immunoglobulin chain variable region nucleotide and amino acid sequences.

Antibody	VL nucleotide sequence	VL amino acid sequence	VH nucleotide sequence	VH amino acid sequence
15C11	SEQ ID NO: 1 (coding)	SEQ ID NO: 2	SEQ ID NO: 3 (coding)	SEQ ID NO: 4
9G8		SEQ ID NO: 8		SEQ ID NO: 5
266		SEQ ID NO: 9		SEQ ID NO: 6

As used throughout the specification, an antibody or immunoglobulin sequence comprising a VL and/or VH sequence as set forth in any one of SEQ ID NOs: 1-9 can comprise (or encode) either the full sequence or can comprise the mature sequence (i.e., mature peptide without the signal or leader peptide).

Example I

In Vivo Efficacy of Mouse 15C11 Antibody

Mouse Antibody 15C11 Improves Cognition In Vivo.

To determine the in vivo efficacy of 15C11, antibodies (including 15C11) were administered to wild type and Tg2576 mice at 3 mg/kg, 10 mg/kg, and 30 mg/kg. Mice were assayed for contextual fear conditioning as described herein.

Tg2576 mice which were administered 30 mg/kg of 15C11 displayed full and significant memory deficit reversal (see FIG. **1**). Furthermore, a trend towards no impairment was found in animals receiving 3 mg/kg and 10/mg/kg (see FIG. **2**) (p value=0.1246 at 3 mg/kg of 15C11; 0.1156 at 10 mg/kg of 15C11; 0.0274 at 30 mg/kg of 15C11).

Mice displaying memory deficit reversal did so within a short time period. Without being bound by the following, this rapid improvement in cognition in mice administered 15C11 suggest a mechanism of action of 15C11 that involves the capture of soluble Aβ in the blood and the subsequent removal of Aβ from the CNS into the plasma.

Example II

Capture Ability of Mouse 15C11

The ability of various antibodies (including 15C11) to capture soluble Aβ was assayed as follows. Various concentrations of antibody (up to 10 μg/ml) were incubated with 50,000 CPM of ^{125}I-Aβ 1-42 (or ^{125}I-Aβ 1-40). The concentration of antibody sufficient to bind 25% of the radioactive counts was determined in a capture radioimmunoassay. Certain antibodies did not bind 25% of the counts at the highest concentration tested (i.e., 10 μg/ml). For such antibodies, the percentage of counts bound at 10 μg/ml was determined. At 3 μg/ml, 15C11 bound 25% of the radioactive counts (i.e., ^{125}I-Aβ). This capture was significant as compared to other monoclonal antibodies raised against central Aβ fragments (e.g., Aβ 13-28 or Aβ 17-28). The range of concentrations necessary to capture 25% of the labeled Aβ for such antibodies is from about 0.1 μg/ml to 10 μg/ml with some antibodies capturing less than 25% labeled Aβ (e.g., 10-20%) when assayed at 10 μg/ml.

The ability of 15C11 was also tested for its ability to bind soluble Aβ oligomers, e.g., dimers, trimers, tetramers, etc. (and Aβ monomers). Aβ 1-42 peptide (synthetic, purified) was solubilized in hexafluoroisopropanol (HFIP), dried under a vacuum, resuspended in DMSO and diluted into cold F12 culture media. Oligomers were formed in the presence of peroxynitrate cross-linking agent. The oligomeric reagent was immunoprecipitated. Immunoprecipitates were visualized following SDS-PAGE using 3D6 antibody as a detecting agent. 15C11 exhibited preferential affinity for oligomeric Aβ species as compared to monomeric Aβ. This preferential binding correlates with efficacy in the CFC animal model described above and is predictive of therapeutic efficacy of the antibody (e.g., effecting rapid improvement in cognition) in vivo.

Example III

Cloning and Sequencing of the Mouse 15C11 Variable Regions

Cloning and Sequence Analysis of 15C11 VL. The light chain variable VL region of 15C11 was cloned in an analogous manner as the VH region. The nucleotide sequence (coding, SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) derived from two independent cDNA clones encoding the presumed 15C11 VL domain, are set forth in Table 1 and Table 2, respectively.

TABLE 1

Mouse 15C11 VL DNA sequence
ATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTC (SEQ ID NO: 1)
TGGATTCCTGCTTCCAGCAGTGATGTTGTGATGACC
CAAACTCCACTCTCCCTGCCTGTCAGTCTTGGAGAT
CAAGCCTCCATCTCTTGCAGATCTAGTCAGAGCCTT
GTACACAGTGATGGAAACACCTATTTACATTGGTAC
CTGCAGAAGCCAGGCCAGTCTCCAAAACTCCTGATC
TACAAAGTTTCCAACCGATTTTCTGGGGTCCCAGAC

TABLE 1-continued

Mouse 15C11 VL DNA sequence

```
AGGTTCAGTGGCAGTGGATCAGGGACAGATTTCACA
CTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGA
GTTTATTTCTGCTCTCAAAGTACACATGTGTGGACG
TTCGGTGGAGGCACCAAGCTGGAAATCAAA
```

TABLE 2

Mouse 15C11 VL amino acid sequence

```
mklpvrllvlmfwipasssDVVMTQTPLSLPVSLGD (SEQ ID NO: 2)
QASISCrssqslvhsdgntylhWYLQKPGQSPKLLi
ykvsnrfsGVPDRFSGSGSGTDFTLKISRVEAEDLG
VYFCsqsthvwtFGGGTKLEIK
```

* Leader peptide and CDRs in lower case.

Cloning and Sequence Analysis of 15C11 VH.

The VH and VL regions of 15C11 from hybridoma cells were cloned by RT-PCR and 5' RACE using mRNA from hybridoma cells and standard cloning methodology. The nucleotide sequence (coding, SEQ ID NO:3) and deduced amino acid sequence (SEQ ID NO:4) derived from independent cDNA clones encoding the presumed 15C11 VH domain, are set forth in Table 3 and Table 4 respectively.

TABLE 3

Mouse 15C11 VH DNA sequence.

```
ATGAATTTCGGGCTCAGCTTGATTTTCCTT       (SEQ ID NO: 3)
GTCCTTGTTTTAAAAGGTGTCCTGTGTGAA
GTGAAGCTGGTGGAGTCTGGGGGAGGTTTA
GTGCAGCCTGGAGGGTCCCTGAAACTCTCC
TGTGCAGCCTCTGGATTTACTTTCAGTAGA
TATAGTATGTCTTGGGTTCGCCAGACTCCA
GAGAAGAGGCTGGAGTTGGTCGCAAAAATT
AGTAATAGTGGTGATAACACCTACTATCCA
GACACTTTAAAGGGCCGATTCACCATCTCC
AGAGACAATGCCCAGAACACCCTGTACCTG
CAAATGAGCAGTCTGAAGTCTGAGGACACG
GCCATGTATTACTGTGCAAGCGGGGACTAC
TGGGGCCAAGGCACCACTCTCACAGTCTCC
TCA
```

TABLE 4

Mouse 15C11 VH amino acid sequence

```
mnfglsliflvlvlkgvlcEVKLVESGGGLVQPGGS (SEQ ID NO: 4)
LKLSCAASgftfsrysmsWVRQTPEKRLELVAkisn
sgdntyypdtlkgRFTISRDNAQNTLYLQMSSLKSE
DTAMYYCASgdyWGQGTTLTVSS
```

* Leader peptide and CDRs in lower case.

The 15C11 VL and VH sequences meet the criteria for functional V regions in so far as they contain a contiguous ORF from the initiator methionine to the C-region, and share conserved residues characteristic of immunoglobulin V region genes. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4.

An epitope map assay was performed which identified residues 19-22 of Aβ as the epitope for 15C11 (see FIG. **3**).

A cell line producing the 15C11 antibody having the ATCC Accession No. PTA-7270 was deposited on Dec. 12, 2005, under the terms of the Budapest Treaty. The cell line designated 15C11 producing the antibody 15C11 has the American Type Culture Collection (ATCC) accession number PTA-7270, having been deposited on Dec. 13, 2005 under the terms of the Budapest Treaty. The address of the American Type Culture Collection is 10801 University Boulevard, Manassas, VA 20110-2209.

Example IV

In Vitro Efficacy of a Various Aβ Antibodies: Binding Soluble, Oligomeric Aβ

In this Example, the Aβ preparation was derived from synthetic Aβ oligimers substantially as follows:

(1) lyophilized $A\beta_{1-42}$ peptide was dissolved to 1 mM in 100% hexafluoroisopropanol (HFIP) (mixed then incubated at room temperature for 1 hour) and separated into aliquots in microcentrifuge tubes (each tube containing 0.5 mg of $A\beta_{1-42}$ peptide);

(2) the HFIP was removed by evaporation followed by lyophilization to remove residual HFIP;

(3) the resultant Aβ peptide film/residue was stored, desiccated, at −20° C.;

(4) the Aβ peptide residue was resuspended in DMSO to a final concentration of 5 mM of peptide then added to ice cold Ham's F-12 (phenol red free) culture media to bring the peptide to a final concentration of 100 μM;

(5) the peptide was incubated at 4° C. for 24 h to produce synthetic Aβ oligomers at an approximately 100 μM concentration; and

(6) the synthetic Aβ oligomers were treated with peroxynitrite.

Portions of the AD preparation were then each contacted with a test immunological reagent, in this case antibodies, and the Aβ monomers and one or more Aβ oligomers which bound to the test immunological reagent were extracted from the Aβ preparation by immunoprecipitation. The various immunoprecipitates were separated by gel electrophoresis and immunoblotted with the 3D6 antibody substantially as follows. Immunoprecipitate samples of FIG. **6** were diluted in sample buffer and separated by SDS-PAGE on a 16% Tricine gel. The protein was transferred to nitrocellulose membranes, the membranes boiled in PBS, and then blocked overnight at 4° C. in a solution of TBS/Tween/5% Carnation dry milk. The membranes were then incubated with 3D6, a mouse monoclonal Aβ antibody to residues 1-5. For detection, the membranes were incubated with anti-mouse Ig-HRP, developed using ECL Plus, and visualized using film. Molecular mass was estimated by SeeBlue™ Plus2 molecular weight markers.

FIG. **6** depicts the results of contacting samples of the above $A\beta_{1-42}$ preparation with various Aβ antibodies to determine the binding to Aβ monomers, dimers, trimers, tetramers, pentamers, etc. in the Aβ preparation. FIG. **6** depicts Western blots (imaged with 15C11) of immunoprecipitates of a peroxynitrite treated oligomeric $A\beta_{1-42}$ preparation contacted with various Aβ antibodies. The approximate positions of $A\beta_{1-42}$ monomer, dimer, trimer and tetramer bands are indicated on the left-hand side of each figure. Indicated below each Aβ antibody is the Aβ epitope recognized by the antibody and CFC assay results for the antibody. A "+" notation indicates an observation of increased cognition upon treatment with the antibody, a "−" notation indicates an observation of no change in cognition upon treatment with the antibody, a "+/−" notation indicates an observation of a trend of increased cognition upon treatment with the antibody but which is not statistically significant enough to be indicated as

an observation of increased cognition, and "ND" notation indicates no CFC assay data available or compared for this antibody.

In FIG. **6**, an increased binding of an Aβ antibody for Aβ dimers or higher ordered oligomers in the Aβ preparation, relative to the binding of the Aβ antibody for Aβ monomers in the Aβ preparation, predicts that the Aβ antibody has therapeutic efficacy for the treatment of Alzheimer's disease. Notably, Aβ antibodies 3D6, 15C11, 10D5, 12A11 and 266 exhibited preferential binding for oligomeric Aβ species as compared to monomeric Aβ with 12A11 exhibiting the most significant preferential binding to oligomeric Aβ. Accordingly, these antibodies are predicted to have therapeutic efficacy in the treatment cognitive deficits, e.g., those associated with AD.

Example V

Prevention and Treatment of Human Patients

A single-dose phase I trial is performed to determine safety in humans. A therapeutic agent is administered in increasing dosages to different patients starting from about 0.01 the level of presumed efficacy, and increasing by a factor of three until a level of about 10 times the effective mouse dosage is reached.

A phase II trial is performed to determine therapeutic efficacy. Patients with early to mid Alzheimer's Disease defined using Alzheimer's disease and Related Disorders Association (ADRDA) criteria for probable AD are selected. Suitable patients score in the 12-26 range on the Mini-Mental State Exam (MMSE). Other selection criteria are whether patients are likely to survive for the duration of the study and lack complicating issues such as use of concomitant medications that may interfere. Baseline evaluations of patient function are made using classic psychometric measures, such as the MMSE, and the ADAS, which is a comprehensive scale for evaluating patients with Alzheimer's Disease status and function. These psychometric scales provide a measure of progression of the Alzheimer's condition. Suitable qualitative life scales can also be used to monitor treatment. Disease progression can also be monitored by MRI. Blood profiles of patients can also be monitored including assays of immunogen-specific antibodies and T-cells responses.

Following baseline measurements, patients begin receiving treatment. They are randomized and treated with either therapeutic agent or placebo in a blinded fashion. Patients are monitored at least every six months. Efficacy is determined by a significant reduction in progression of a treatment group relative to a placebo group.

A second phase II trial is performed to evaluate conversion of patients from non-Alzheimer's Disease early memory loss, sometimes referred to as age-associated memory impairment (AAMI) or mild cognitive impairment (MCI), to probable Alzheimer's disease as defined as by ADRDA criteria. Patients with high risk for conversion to Alzheimer's Disease are selected from a non-clinical population by screening reference populations for early signs of memory loss or other difficulties associated with pre-Alzheimer's symptomatology, a family history of Alzheimer's Disease, genetic risk factors, age, sex, and other features found to predict high-risk for Alzheimer's Disease. Baseline scores on suitable metrics including the MMSE and the ADAS together with other metrics designed to evaluate a more normal population are collected. These patient populations are divided into suitable groups with placebo comparison against dosing alternatives with the agent. These patient populations are followed at intervals of about six months, and the endpoint for each patient is whether or not he or she converts to probable Alzheimer's Disease as defined by ADRDA criteria at the end of the observation.

Although the foregoing invention has been described in detail for purposes of clarity of understanding, it will be obvious that certain modifications may be practiced within the scope of the appended claims. All publications and patent documents cited herein, as well as text appearing in the figures and sequence listing, are hereby incorporated by reference in their entirety for all purposes to the same extent as if each were so individually denoted.

From the foregoing it will be apparent that the invention provides for a number of uses. For example, the invention provides for the use of any of the antibodies to Aβ described above in the treatment, prophylaxis or diagnosis of amyloidogenic disease, or in the manufacture of a medicament or diagnostic composition for use in the same.

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tcc agc agt gat gtt gtg atg acc caa act cca ctc tcc ctg cct gtc       96
Ser Ser Ser Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val
```

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```
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agt ctt gga gat caa gcc tcc atc tct tgc aga tct agt cag agc ctt      144
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     15                  20                  25

gta cac agt gat gga aac acc tat tta cat tgg tac ctg cag aag cca      192
Val His Ser Asp Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro
 30                  35                  40                  45

ggc cag tct cca aaa ctc ctg atc tac aaa gtt tcc aac cga ttt tct      240
Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser
                 50                  55                  60

ggg gtc cca gac agg ttc agt ggc agt gga tca ggg aca gat ttc aca      288
Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
             65                  70                  75

ctc aag atc agc aga gtg gag gct gag gat ctg gga gtt tat ttc tgc      336
Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys
         80                  85                  90

tct caa agt aca cat gtg tgg acg ttc ggt gga ggc acc aag ctg gaa      384
Ser Gln Ser Thr His Val Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu
     95                  100                 105

atc aaa                                                             390
Ile Lys
110
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             1               5                  10

Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu
    15                  20                  25

Val His Ser Asp Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro
30                  35                  40                  45

Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser
                50                  55                  60

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
            65                  70                  75

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys
        80                  85                  90

Ser Gln Ser Thr His Val Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu
    95                  100                 105

Ile Lys
110
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                -15                 -10                 -5

gtc ctg tgt gaa gtg aag ctg gtg gag tct ggg gga ggt tta gtg cag       96
Val Leu Cys Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Gln
            1               5                  10

cct gga ggg tcc ctg aaa ctc tcc tgt gca gcc tct gga ttt act ttc      144
Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
     15                  20                  25

agt aga tat agt atg tct tgg gtt cgc cag act cca gag aag agg ctg      192
Ser Arg Tyr Ser Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu
 30                  35                  40                  45

gag ttg gtc gca aaa att agt aat agt ggt gat aac acc tac tat cca      240
Glu Leu Val Ala Lys Ile Ser Asn Ser Gly Asp Asn Thr Tyr Tyr Pro
                50                  55                  60

gac act tta aag ggc cga ttc acc atc tcc aga gac aat gcc cag aac      288
Asp Thr Leu Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Gln Asn
            65                  70                  75

acc ctg tac ctg caa atg agc agt ctg aag tct gag gac acg gcc atg      336
Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met
         80                  85                  90

tat tac tgt gca agc ggg gac tac tgg ggc caa ggc acc act ctc aca      384
Tyr Tyr Cys Ala Ser Gly Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr
     95                  100                 105

gtc tcc tca                                                          393
Val Ser Ser
110
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Val Leu Cys Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Gln
            1               5                  10

Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
    15                  20                  25

Ser Arg Tyr Ser Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu
30                  35                  40                  45

Glu Leu Val Ala Lys Ile Ser Asn Ser Gly Asp Asn Thr Tyr Tyr Pro
                50                  55                  60

Asp Thr Leu Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Gln Asn
            65                  70                  75

Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met
        80                  85                  90

Tyr Tyr Cys Ala Ser Gly Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr
    95                  100                 105

Val Ser Ser
110
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Val Leu Cys Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Gln
1 5 10

Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
15 20 25

Ser Asp Tyr Thr Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu
30 35 40 45

Glu Leu Val Ala Glu Ile Ser Asn Thr Gly Gly Ser Thr Tyr Tyr Pro
50 55 60

Asp Thr Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
65 70 75

Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met
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Tyr Tyr Cys Ala Ser Gly Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr
95 100 105

Val Ser Ser
110

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Val Leu Cys Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Gln
1 5 10

Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
15 20 25

Ser Arg Tyr Ser Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu
30 35 40 45

Glu Leu Val Ala Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr Pro
50 55 60

Asp Thr Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Glu Tyr
65 70 75

Thr Leu Ser Leu Gln Met Ser Gly Leu Arg Ser Asp Asp Thr Ala Thr
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95 100 105

Val Ser Ser
110

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Lys Glu Ser Gly Pro Gly Ile Leu Gln Pro Ser Gln Thr Leu Ser Leu
5 10 15 20

Thr Cys Ser Ile Ser Gly Phe Ser Ile Ser Thr Ser Gly Ser Gly Val
25 30 35

Ser Trp Ile Arg Gln Thr Ser Gly Lys Gly Leu Glu Trp Leu Ala His
40 45 50

Ile Tyr Trp Asn Gly Asn Phe Arg Tyr Asn Pro Ser Ile Lys Ser Arg
55 60 65

Leu Thr Ile Ser Lys Asp Thr Ser Asn Asn Gln Val Phe Leu Lys Ile
70 75 80

Ile Ser Val Asp Thr Thr Asp Thr Ala Thr Tyr Tyr Cys Ala Leu Arg
85 90 95 100

Gly Ser Asn Lys Glu Glu Val Phe Asp Tyr Trp Gly Gln Gly Thr Phe
105 110 115

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Pro Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys
10 15 20

Arg Ser Ser Gln Ser Leu Val His Ser Asn Gly Asn Thr Phe Phe His
25 30 35

Trp Phe Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys
40 45 50 55

Val Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly
60 65 70

Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp
75 80 85

Leu Gly Val Tyr Phe Cys Ser Gln Ser Ala His Val Pro Trp Thr Phe
90 95 100

Gly Gly Gly Thr Lys Leu Glu Ile Lys
105 110

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Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu
    15                  20                  25

Ile Tyr Ser Asp Gly Asn Ala Tyr Leu His Trp Phe Leu Gln Lys Pro
30                  35                  40                  45

Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser
                50                  55                  60

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
            65                  70                  75

Leu Lys Ile Ser Arg Val Glu Thr Glu Asp Leu Gly Val Tyr Phe Cys
        80                  85                  90

Ser Gln Ser Thr His Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu
    95                  100                 105

Glu Ile Lys
110


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<223> OTHER INFORMATION: Xaa = Arg or Asp
<220> FEATURE:
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<222> LOCATION: 8
<223> OTHER INFORMATION: Xaa =  Ser or Thr

<400> SEQUENCE: 10

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 1               5                  10


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<223> OTHER INFORMATION: Xaa = Any Amino Acid
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<223> OTHER INFORMATION: Xaa = Ser or Asn
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<222> LOCATION: 15
<223> OTHER INFORMATION: Xaa = Leu or Val

<400> SEQUENCE: 11

Xaa Ile Xaa Xaa Xaa Gly Xaa Xaa Thr Tyr Tyr Pro Asp Thr Xaa Lys
 1               5                  10                  15

Gly


<210> SEQ ID NO 12
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<223> OTHER INFORMATION: Xaa = Asp or Asn
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<223> OTHER INFORMATION: Xaa = Leu or Phe

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 1   5   10   15

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<223> OTHER INFORMATION: Xaa = Thr or Ala
<220> FEATURE:
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<222> LOCATION: 7
<223> OTHER INFORMATION: Xaa = Pro, may or may not be present

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 1   5

<210> SEQ ID NO 14
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct, consensus sequence for
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<400> SEQUENCE: 14

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 1   5   10   15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Tyr
            20   25   30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35   40   45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50   55   60

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

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
                85   90   95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr
            100   105

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<212> TYPE: PRT
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<400> SEQUENCE: 15

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1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Gln Gln His
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Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr

<210> SEQ ID NO 16
<211> LENGTH: 110
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct, consensus sequence for kappa chain

<400> SEQUENCE: 16

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Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Val Pro Ala Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu
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Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro
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Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr
100 105 110

<210> SEQ ID NO 17
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct, consensus sequence for kappa chain

<400> SEQUENCE: 17

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1 5 10 15

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Glu Arg Ala Thr Ile Asn Cys Arg Ser Ser Gln Ser Val Leu Tyr Ser
            20                  25                  30

Ser Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
        35                  40                  45

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
    50                  55                  60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65                  70                  75                  80

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

His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
            100                 105                 110

Lys Arg Thr
        115


<210> SEQ ID NO 18
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Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
 1               5                  10                  15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
            20                  25                  30

Tyr Val Ser Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35                  40                  45

Ile Tyr Asp Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50                  55                  60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu Gln
65                  70                  75                  80

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

Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100                 105


<210> SEQ ID NO 19
<211> LENGTH: 110
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct, consensus sequence for
      lambda chain

<400> SEQUENCE: 19

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

Ser Ile Thr Ile Ser Cys Thr Gly Thr Ser Ser Asp Val Gly Gly Tyr
            20                  25                  30

Asn Tyr Val Ser Trp Tyr Gln Gln His Pro Gly Lys Ala Pro Lys Leu
        35                  40                  45

Met Ile Tyr Asp Val Ser Asn Arg Pro Ser Gly Val Ser Asn Arg Phe
    50                  55                  60

Ser Gly Ser Lys Ser Gly Asn Thr Ala Ser Leu Thr Ile Ser Gly Leu
65                  70                  75                  80
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Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Gln His Tyr Thr Thr
                85                  90                  95

Pro Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100                 105                 110


<210> SEQ ID NO 20
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct, consensus sequence for
      lambda chain

<400> SEQUENCE: 20

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

Thr Ala Arg Ile Ser Cys Ser Gly Asp Ala Leu Gly Asp Lys Tyr Ala
            20                  25                  30

Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
        35                  40                  45

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

Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Gly Thr Gln Ala Glu
65                  70                  75                  80

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

Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100                 105


<210> SEQ ID NO 21
<211> LENGTH: 120
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct, consensus sequence for
      heavy chain framework region

<400> SEQUENCE: 21

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

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

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

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
    50                  55                  60

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

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
            100                 105                 110

 Gly Thr Leu Val Thr Val Ser Ser
        115                 120


<210> SEQ ID NO 22
<211> LENGTH: 120
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct, consensus sequence for heavy chain framework region

<400> SEQUENCE: 22

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

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

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

Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> SEQ ID NO 23
<211> LENGTH: 121
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct, consensus sequence for heavy chain framework region

<400> SEQUENCE: 23

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

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30

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

Trp Leu Ala Leu Ile Asp Trp Asp Asp Asp Lys Tyr Tyr Ser Thr Ser
50 55 60

Leu Lys Thr Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> SEQ ID NO 24
<211> LENGTH: 120
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct, consensus sequence for heavy chain framework region

<400> SEQUENCE: 24

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

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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

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

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

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

Ala Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser
        115                 120


<210> SEQ ID NO 25
<211> LENGTH: 119
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct, consensus sequence for
      heavy chain framework region

<400> SEQUENCE: 25

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

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

Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Tyr Tyr Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
    50                  55                  60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65                  70                  75                  80

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

Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Leu Val Thr Val Ser Ser
        115


<210> SEQ ID NO 26
<211> LENGTH: 120
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct, consensus sequence for
      heavy chain framework region

<400> SEQUENCE: 26

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

Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Ser Tyr
            20                  25                  30

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

Gly Ile Ile Tyr Pro Gly Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe
    50                  55                  60
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Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr
65 70 75 80

Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> SEQ ID NO 27
<211> LENGTH: 123
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct, consensus sequence for heavy chain framework region

<400> SEQUENCE: 27

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

Thr Leu Ser Leu Thr Cys Ala Ile Ser Gly Asp Ser Val Ser Ser Asn
20 25 30

Ser Ala Ala Trp Asn Trp Ile Arg Gln Ser Pro Gly Arg Gly Leu Glu
35 40 45

Trp Leu Gly Arg Thr Tyr Tyr Arg Ser Lys Trp Tyr Asn Asp Tyr Ala
50 55 60

Val Ser Val Lys Ser Arg Ile Thr Ile Asn Pro Asp Thr Ser Lys Asn
65 70 75 80

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

Tyr Tyr Cys Ala Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> SEQ ID NO 28
<211> LENGTH: 993
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct, IgG 1 Heavy chain constant region DNA (codons only)

<400> SEQUENCE: 28

gcctccacca agggcccatc ggtcttcccc ctggcaccct cctccaagag cacctctggg 60

ggcacagcgg ccctgggctg cctggtcaag gactacttcc ccgaaccggt gacggtgtcg 120

tggaactcag gcgccctgac cagcggcgtg cacaccttcc cggctgtcct acagtcctca 180

ggactctact ccctcagcag cgtggtgacc gtgccctcca gcagcttggg cacccagacc 240

tacatctgca acgtgaatca caagcccagc aacaccaagg tggacaagag agttgagccc 300

aaatcttgtg acaaaactca cacatgccca ccgtgcccag cacctgaact cctgggggga 360

ccgtcagtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggaccccct 420

gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg 480

tacgtggacg gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac 540

agcacgtacc gtgtggtcag cgtcctcacc gtcctgcacc aggactggct gaatggcaag 600

gagtacaagt gcaaggtctc caacaaagcc ctcccagccc ccatcgagaa aaccatctcc 660

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aaagccaaag ggcagccccg agaaccacag gtgtacaccc tgcccccatc ccgggaggag    720

atgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc    780

gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg    840

ctggactccg acggctcctt cttcctctat agcaagctca ccgtggacaa gagcaggtgg    900

cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg    960

cagaagagcc tctccctgtc cccgggtaaa tga                                 993


<210> SEQ ID NO 29
<211> LENGTH: 330
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct, IgG1 Heavy chain
      constant region protein

<400> SEQUENCE: 29

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
 1               5                  10                  15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65                  70                  75                  80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            100                 105                 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        115                 120                 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    130                 135                 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145                 150                 155                 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                165                 170                 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            180                 185                 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        195                 200                 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    210                 215                 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
225                 230                 235                 240

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                245                 250                 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            260                 265                 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
        275                 280                 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
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    290                 295                 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305                 310                 315                 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                325                 330


<210> SEQ ID NO 30
<211> LENGTH: 324
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct, kappa light chain
      constant region DNA

<400> SEQUENCE: 30

cgaactgtgg ctgcaccatc tgtcttcatc ttcccgccat ctgatgagca gttgaaatct     60

ggaactgcct ctgttgtgtg cctgctgaat aacttctatc ccagagaggc caaagtacag    120

tggaaggtgg ataacgccct ccaatcgggt aactcccagg agagtgtcac agagcaggac    180

agcaaggaca gcacctacag cctcagcagc accctgacgc tgagcaaagc agactacgag    240

aaacacaaag tctacgcctg cgaagtcacc catcagggcc tgagctcgcc cgtcacaaag    300

agcttcaaca ggggagagtg ttag                                           324


<210> SEQ ID NO 31
<211> LENGTH: 106
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct, kappa light chain
      constant region protein

<400> SEQUENCE: 31

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
 1               5                  10                  15

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

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
        35                  40                  45

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
    50                  55                  60

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
65                  70                  75                  80

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
                85                  90                  95

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
            100                 105


<210> SEQ ID NO 32
<211> LENGTH: 330
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: IgG1 heavy chain constant region

<400> SEQUENCE: 32

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1               5                  10                  15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30
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Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65                  70                  75                  80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            100                 105                 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        115                 120                 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    130                 135                 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145                 150                 155                 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                165                 170                 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            180                 185                 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        195                 200                 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    210                 215                 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
225                 230                 235                 240

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                245                 250                 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            260                 265                 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
        275                 280                 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
    290                 295                 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305                 310                 315                 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                325                 330


<210> SEQ ID NO 33
<211> LENGTH: 327
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: IgG4 heavy chain constant region

<400> SEQUENCE: 33

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1               5                   10                  15

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

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60
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Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr
65                  70                  75                  80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro
            100                 105                 110

Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
        115                 120                 125

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
    130                 135                 140

Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp
145                 150                 155                 160

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe
                165                 170                 175

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
            180                 185                 190

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
        195                 200                 205

Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
    210                 215                 220

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
225                 230                 235                 240

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
                245                 250                 255

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
            260                 265                 270

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
        275                 280                 285

Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
    290                 295                 300

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
305                 310                 315                 320

Leu Ser Leu Ser Leu Gly Lys
                325
```

We claim:

1. A humanized immunoglobulin which specifically binds beta amyloid peptide (Aβ), or antigen-binding fragment thereof, the humanized immunoglobulin comprising:

(i) a light chain comprising three complementarity determining regions (CDRs) from the 15C11 immunoglobulin light chain variable region sequence set forth as SEQ ID NO:2, and a variable framework region from a human acceptor immunoglobulin light chain; and

(ii) a heavy chain comprising three complementarity determining regions (CDRs) from the 15C11 immunoglobulin heavy chain variable region sequence set forth as SEQ ID NO:4, and variable framework region from a human acceptor immunoglobulin heavy chain, provided that at least one framework residue in the light or heavy chain is substituted with the corresponding amino acid residue from the mouse 15C11 light or heavy chain variable region sequence, wherein the framework residue is selected from the group consisting of:

(a) a residue that non-covalently binds antigen directly;

(b) a residue adjacent to a CDR;

(c) a CDR-interacting residue; and

(d) a residue participating in the VL-VH interface.

2. A humanized immunoglobulin which specifically binds beta amyloid peptide (Aβ), or antigen-binding fragment thereof, the humanized immunoglobulin comprising:

(i) a light chain comprising three complementarity determining regions (CDRs) from the 15C11 immunoglobulin light chain variable region sequence set forth as SEQ ID NO:2, and a variable framework region from a human acceptor immunoglobulin light chain; and

(ii) a heavy chain comprising three complementarity determining regions (CDRs) from the 15C11 immunoglobulin heavy chain variable region sequence set forth as SEQ ID NO:4, and a variable framework region from a human acceptor immunoglobulin heavy chain, provided that at least one framework residue in both the light and heavy chain is substituted with the corresponding amino acid residue from the mouse 15C11 light or heavy chain variable region sequence, wherein the framework residue is selected from the group consisting of:

(a) a residue that non-covalently binds antigen directly;

(b) a residue adjacent to a CDR;

(c) a CDR-interacting residue; and

(d) a residue participating in the VL-VH interface.

3. A humanized immunoglobulin which specifically binds beta amyloid peptide (Aβ), or antigen-binding fragment thereof, the humanized immunoglobulin comprising:

(i) a light chain comprising three complementarity determining regions (CDRs) from the 15C11 immunoglobulin light chain variable region sequence set forth as SEQ ID NO:2, and a variable framework region from a human acceptor immunoglobulin light chain, and

(ii) a heavy chain comprising three complementarity determining regions (CDRs) from the 15C11 immunoglobulin heavy chain variable region sequence set forth as SEQ ID NO:4, and a variable framework region from a human acceptor immunoglobulin heavy chain, provided that at least one framework residue in the light or heavy chain is substituted with the corresponding amino acid residue from the mouse 15C11 light chain or heavy variable region sequence, wherein the framework residue is a residue capable of affecting the light or heavy chain variable region conformation or function as identified by analysis of a three-dimensional model of the variable region.

4. A humanized immunoglobulin which specifically binds beta amyloid peptide (Aβ), or antigen-binding fragment thereof, the humanized immunoglobulin comprising:

(i) a light chain comprising three complementarity determining regions (CDRs) from the 15C11 immunoglobulin light chain variable region sequence set forth as SEQ ID NO:2, and a variable framework region from a human acceptor immunoglobulin light chain, and

(ii) a heavy chain comprising three complementarity determining regions (CDRs) from the 15C11 immunoglobulin heavy chain variable region sequence set forth as SEQ ID NO:4, and a variable framework region from a human acceptor immunoglobulin heavy chain, provided that at least one framework residue in the light and heavy chain is substituted with the corresponding amino acid residue from the mouse 15C11 light or heavy chain variable region sequence, wherein the framework residue is a residue capable of affecting heavy chain variable region conformation or function as identified by analysis of a three-dimensional model of the variable region.

5. The humanized immunoglobulin or antigen-binding fragment of claim **3** or **4**, wherein the framework residue in the light chain is selected from the group consisting of a residue capable of interacting with antigen, a residue proximal to the antigen binding site, a residue capable of interacting with a CDR, a residue adjacent to a CDR, a residue within 6 Å of a CDR residue, a canonical residue, a vernier zone residue, an interchain packing residue, a rare residue, and a glycoslyation site residue on the surface of the three-dimensional model.

6. The humanized immunoglobulin or antigen-binding fragment of claim **3** or **4**, wherein the framework residue in the heavy chain is selected from the group consisting of a residue capable of interacting with antigen, a residue proximal to the antigen binding site, a residue capable of interacting with a CDR, a residue adjacent to a CDR, a residue within 6 Å of a CDR residue, a canonical residue, a vernier zone residue, an interchain packing residue, a rare residue, and a glycoslyation site residue on the surface of the three-dimensional model.

7. The humanized immunoglobulin or antigen-binding fragment of claim **3** or **4**, wherein the framework residue in the light chain is substituted at a position selected from the group consisting of position 2, 4, 35, 64, and 71 of the light chain as numbered according to Kabat.

8. The humanized immunoglobulin or antigen-binding fragment of claim **3** or **4**, wherein the framework residue in the heavy chain is substituted at a position selected from the group consisting of position 26-30, 71, 93, 94, and 103 of the heavy chain as numbered according to Kabat.

9. A humanized immunoglobulin which specifically binds beta amyloid peptide (Aβ), or antigen-binding fragment thereof, the human immunoglobulin comprising:

(a) a light chain comprising three complementarity determining regions (CDR1, CDR2 and CDR3) from the monoclonal antibody 15C11 light chain variable region sequence set forth as SEQ ID NO:2, and a variable framework region from a human acceptor immunoglobulin light chain provided that at least one framework residue in the light chain is substituted with the corresponding amino acid residue from the 15C11 light chain variable region sequence, wherein the framework residue is selected from the group consisting of a canonical residue, a vernier residue, a packing residue and a rare residue; and

(b) a heavy chain comprising three complementarity determining regions (CDR1, CDR2 and CDR3) from the monoclonal antibody 15C11 immunoglobulin heavy chain variable region sequence set forth as SEQ ID NO:4, and a variable framework region from a human acceptor immunoglobulin heavy chain provided that at least one framework residue in the heavy chain is substituted with the corresponding amino acid residue from the 15C11 heavy chain variable region sequence, wherein the framework residue is selected from a second group consisting of a canonical residue, a vernier residue, a packing residue and a rare residue.

10. A humanized immunoglobulin which specifically binds beta amyloid peptide (Aβ), or antigen-binding fragment thereof, the human immunoglobulin comprising a light chain and a heavy chain, the light chain comprising the complementarity determining regions (CDR1, CDR2 and CDR3) of the 15C11 light chain variable region sequence set forth as SEQ ID NO:2, and the heavy chain comprising the complementarity determining regions (CDR1, CDR2, and CDR3) of the 15C11 heavy chain variable region sequence set forth as SEQ ID NO:4.

11. The humanized immunoglobulin or antigen binding fragment of any one of claims **1**, **2**, **3**, **4**, **9**, and **10**, which specifically binds to beta amyloid peptide (Aβ) with a binding affinity of at least 10^7 M^{-1}.

12. The humanized immunoglobulin or antigen binding fragment of any one of claims **1**, **2**, **3**, **4**, **9**, and **10**, which specifically binds to beta amyloid peptide (Aβ) with a binding affinity of at least 10^8 M^{-1}.

13. The humanized immunoglobulin or antigen binding fragment of any one of claims **1**, **2**, **3**, **4**, **9**, and **10**, which specifically binds to beta amyloid peptide (Aβ) with a binding affinity of at least 10^9 M^{-1}.

14. The humanized immunoglobulin or antigen binding fragment of any one of claims **1**, **2**, **3**, **4**, **9**, and **10**, wherein the heavy chain isotype is γ1.

15. The humanized immunoglobulin or antigen binding fragment of any one of claims **1**, **2**, **3**, **4**, **9**, and **10**, wherein the heavy chain isotype is γ4.

16. The humanized immunoglobulin or antigen binding fragment of any one of claims **1**, **2**, **3**, **4**, **9**, and **10**, which binds to soluble beta amyloid peptide (Aγ).

17. The humanized immunoglobulin or antigen binding fragment of any one of claims **1**, **2**, **3**, **4**, **9**, and **10**, which binds to oligomeric beta amyloid peptide (Aβ).

18. The humanized immunoglobulin or antigen binding fragment of any one of claims **1**, **2**, **3**, **4**, **9**, and **10**, which captures beta amyloid peptide (Aβ).

19. The humanized immunoglobulin or antigen binding fragment of any one of claims **1**, **2**, **3**, **4**, **9**, and **10**, which crosses the blood-brain barrier in a patient.

20. The humanized immunoglobulin or antigen binding fragment of any one of claims **1**, **2**, **3**, **4**, **9**, and **10**, which reduces beta amyloid peptide (Aβ) plaque burden in a patient.

21. A chimeric immunoglobulin, which specifically binds beta amyloid peptide (Aβ) with a binding affinity of at least $10^7 M^{-1}$, comprising the light chain variable region sequence as set forth in amino acid residues 1-111 of SEQ ID NO:2 and the heavy chain variable region sequence set forth in amino acid residues 1-112 of SEQ ID NO:4, and comprising constant region sequences from a human immunoglobulin.

22. A pharmaceutical composition comprising the humanized immunoglobulin of any one of claims **1**, **2**, **3** and **4** and a pharmaceutical carrier.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,625,560B2
APPLICATION NO. : 11/304986
DATED : December 1, 2009
INVENTOR(S) : Basi et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the claims:

Claim 16, Column 114, Line 67, delete "γ", insert --β--

Signed and Sealed this

Twenty-fifth Day of May, 2010

David J. Kappos

David J. Kappos
Director of the United States Patent and Trademark Office

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,625,560 B2
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INVENTOR(S) : Basi et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the title page:

(73) Assignee: Insert --Wyeth, Five Giralda Farms, Madison, NJ 07940--.

[*] Delete the phrase "by 128 days" and insert --by 449 days--.

Signed and Sealed this

Thirty-first Day of August, 2010

David J. Kappos

David J. Kappos
Director of the United States Patent and Trademark Office

专利名称(译)	识别β淀粉样肽的人源化抗体		
公开(公告)号	US7625560	公开(公告)日	2009-12-01
申请号	US11/304986	申请日	2005-12-15
[标]申请(专利权)人(译)	巴斯GURIQ JACOBSEN插孔输入S		
申请(专利权)人(译)	巴斯GURIQ JACOBSEN插孔输入S		
当前申请(专利权)人(译)	JANSSEN阿尔茨海默免疫治疗		
[标]发明人	BASI GURIQ JACOBSEN JACK STEVEN		
发明人	BASI, GURIQ JACOBSEN, JACK STEVEN		
IPC分类号	C07K16/46 A61K39/395 C07K16/28 G01N33/53		
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代理机构(译)	TOWNSEND和TOWNSEND和机组LLP		
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摘要(译)

本发明提供了用于治疗与患者脑中Aβ的淀粉样沉积物相关的疾病的改进的药剂和方法。优选的试剂包括抗体，例如人源化抗体。