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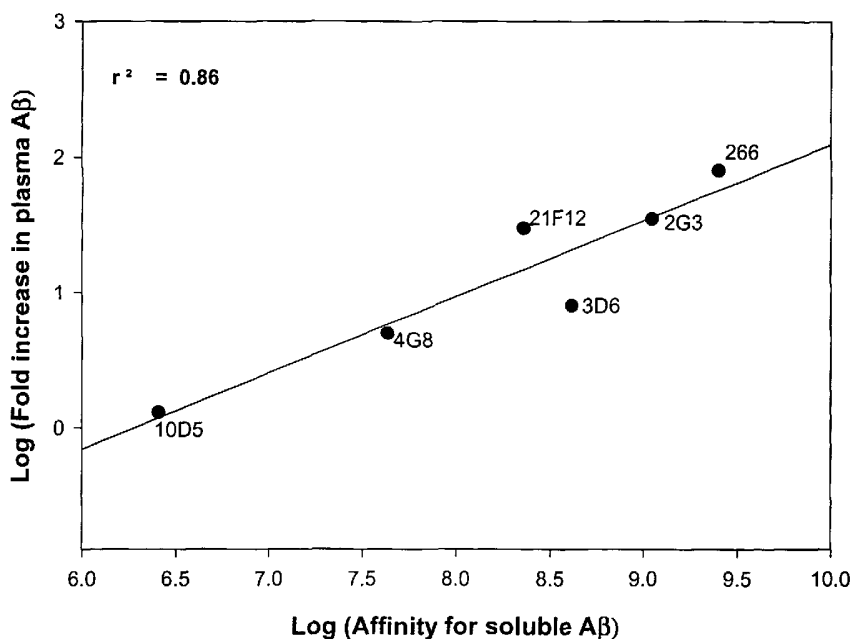
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(54) Title: USE OF ANTIBODIES HAVING HIGH AFFINITY FOR SOLUBLE A β TO TREAT CONDITIONS AND DISEASES RELATED TO A β



(57) Abstract: This invention provides a method for treating a subject having a condition or disease related to the A β peptide comprising administering to the subject an antibody that recognizes A β , wherein the antibody has an affinity for soluble A β higher than 10^{-9} M.



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-1-

**USE OF ANTIBODIES HAVING HIGH AFFINITY FOR SOLUBLE A β TO
TREAT CONDITIONS AND DISEASES RELATED TO A β**

This application claims the priority of U.S. Provisional Application 60/313,576, filed August 17, 2001, and U.S. Provisional Application 60/383,851, filed May 28, 2002, both of which are expressly incorporated by reference.

This invention is in the field of medicine. More particularly, this invention is in the field of treatment of conditions and diseases related to the A β peptide, such as Alzheimer's disease, Down's syndrome, cerebral amyloid angiopathy, mild cognitive impairment, and the like.

Chronic administration (several months) of antibodies that recognize certain N-terminal epitopes within A β (e.g., polyclonal antibodies raised against aggregated A β 1-42 and monoclonal antibodies 3D6 and 10D5) reduced amyloid plaque in the brains of PDAPP mice [Bard, *et al. Nature Med.* 6:916-919 (2000); Schenk, *et al.* WO00/72880, December 7, 2000]. Schenk found that efficacy in reducing plaque was not related to antibody affinity for aggregated A β , despite stating a preference for antibodies with affinity for A β greater than 10^6 M⁻¹, 10^7 M⁻¹, 10^8 M⁻¹, 10^9 M⁻¹, or 10^{10} M⁻¹. These affinities must be in terms of affinity for aggregated A β because: 1) the affinities for aggregated A β are the only affinities specifically mentioned in the disclosure for specific antibodies; 2) the disclosure reported active immunization that relied on aggregated A β for vaccinations; 3) the disclosure reported that A β plaque reduction following passive immunization with polyclonal antibodies raised against aggregated A β or with other anti-A β antibodies requires adherence of the antibodies to aggregated A β in plaques followed by cell mediated plaque clearance.

Several studies have shown that administration of aggregated A β improves performance in various tests of memory and cognition in animal models of Alzheimer's disease after chronic administration (several weeks to several months) [Weiner, *et al.*, *Ann. Neurol.* 48:567-579 (2000); Janus, *et al.*, *Nature* 408:979-982 (2000); Morgan, *et al.*, *Nature* 408:982-985 (2000)]. However, these investigators used aggregated A β , and so presumably obtained polyclonal antibodies against aggregated A β . Such treatment,

-2-

apparently somewhat effective to improve cognition, is very prolonged and inefficient. Furthermore, the use of forms of A β as antigens may be hazardous and produce immunological responses that are difficult to control and quite variable between subjects.

DeMattos, *et al.* [*Proc. Nat'l Acad. Sci. (USA)* 98:8850-8855 (2001)] identified an A β -antibody complex after administration of antibodies 266 [Seubert, *et al.*, *Nature* 359:325 – 327 (1992)] or 4G8 [Shoji, *et al.*, *Science* 258:126-129 (1992)], antibodies that recognize epitopes between amino acids 13 and 28 of A β . No complexes were observed when antibodies that recognize other portions of A β were administered, even though some had affinities for soluble A β similar to that of 4G8. It was concluded that antibodies recognizing epitopes in the 13-28 region of A β would cause formation of the complex and sequester A β in the plasma. The affinity of the antibody for A β was not considered an important factor in sequestration of A β in plasma. DeMattos also showed that administration of 266 causes a rapid, massive efflux of A β from the CNS to the plasma within hours after administration.

We have now found that the rate of A β flux in the first 24 hours after administration of antibodies is related to the affinity of the antibody for soluble A β , and that antibodies with higher affinity for soluble A β cause a more rapid efflux of soluble A β . Most surprisingly, rapid recovery of cognitive performance was seen within 24 hours of administering certain antibodies. The relative magnitude of recovery was related to the rate of efflux of soluble A β within the first 24 hours, and to the affinity of the antibody for soluble A β .

The invention proceeds from the first recognition of an important feature of anti-A β antibodies that was previously unrecognized and could not have been deduced from the prior art, which in fact taught that the important features of effective anti-A β antibodies were either the particular epitope recognized (e.g., N-terminal) or the affinity of the antibody for aggregated forms of A β . The newly recognized, important feature of anti-A β antibodies is their affinity for soluble forms of A β . An antibody having a higher affinity for soluble A β will be more effective in treating conditions or diseases related to the A β peptide.

-3-

SUMMARY OF THE INVENTION

Therefore, this invention provides a method for treating a subject having a condition or disease related to the A β peptide comprising administering to the subject an antibody that recognizes A β , wherein the antibody has greater affinity for soluble A β than 10⁻⁹ M. More particularly, the invention is a method for treating cognitive symptoms of a condition or disease associated with A β in a subject, comprising administering to the subject an effective amount of an anti-A β antibody that has greater affinity for soluble A β than antibody 266 has. More particularly, the affinity is with respect to A β 1-40 or A β 1-42.

The invention includes a method for reducing disease progression in a subject having a condition or disease associated with A β , comprising administering to the subject an effective amount of an anti-A β antibody that has greater affinity for soluble A β than 10⁻⁹ M. More particularly, the invention is a method for reducing disease progression in a subject having a condition or disease associated with A β , comprising administering to the subject an effective amount of an anti-A β antibody that has greater affinity for soluble A β than antibody 266 has. More particularly, the affinity is with respect to A β 1-40 or A β 1-42.

The invention also includes a method for treating cognitive symptoms of a condition or disease associated with A β in a subject, comprising administering to the subject an effective amount of an anti-A β antibody that has affinity for soluble A β 1-40 or A β 1-42 higher than 10⁻⁹ M, more particularly, higher than the affinity of antibody 266.

Also included is the use of an anti-A β antibody that has affinity for soluble A β 1-40 or A β 1-42 higher than 10⁻⁹ M (i.e., higher than the affinity of antibody 266) for preparing medicaments for reducing disease progression or treating cognitive symptoms of a condition or disease associated with A β . More particularly, the invention is the use of an anti-A β antibody having higher affinity for soluble A β than antibody 266 has to prepare a medicament for treating cognitive symptoms or reducing disease progression in a subject having a condition or disease associated with A β .

BRIEF DESCRIPTION OF THE DRAWINGS

- Figure 1. Object recognition memory performance 24 hours after administration of m266 anti-A β antibody. The recognition index is the percentage of time spent exploring a novel object during trial 2 (test trial). Both saline- and control IgG-treated tg mice performed at chance levels (recognition index = 50%), whereas m266-treated tg mice and WT mice significantly performed above chance (t-test analysis). Values are means \pm SEM; ** means $p < 0.0001$ vs. saline- and IgG-treated tg groups; ## means $p < 0.0001$ vs. wild type (WT) mice.
- Figure 2. Plasma A β 40 and A β 42 levels 24 hours after administration of m266. Plasma levels correlated with object recognition memory performance. (A) Plasma levels of both peptides are markedly increased in APP^{V717F} tg mice acutely administered m266, compared to saline or control IgG-treated tg mice. Values are means \pm SEM; (B) Bivariate scattergrams showing highly significant correlation between plasma levels of A β and the object recognition memory performance.
- Figure 3. Apparatus used for holeboard spatial learning assay.
- Figure 4. Acute A β antibody treatment improved reference memory in APPV717F mice.
- Figure 5. Acute A β antibody treatment decreased total errors in APPV717F mice.
- Figure 6. Correlation between Log (A β flux) and Log (affinity of various anti-A β antibodies for soluble A β).
- Figure 7. Lack of correlation between Log (A β flux) and Log (affinity of various anti-A β antibodies for insoluble A β).
- Figure 8. Object recognition memory performance 24 hours after administration of 266 or 3D6 anti-A β antibody. (* means $p < 0.05$ vs. saline or IgG, *** means $p < 0.001$ vs. saline or IgG).
- Figure 9. Correlation between Log (A β flux) and Log (affinity of various anti-A β antibodies for soluble A β using altered BIAcore method).

DETAILED DESCRIPTION OF THE INVENTION

By “cognition” is meant short-term memory, long-term memory, abstraction, judgment, language, praxis, visuospatial skills, behavior or personality. Cognition may be assessed in non-human subjects using any of a wide array of tests [Weiner, *et al.*, *Ann. Neurol.* 48:567-579 (2000); Janus, *et al.*, *Nature* 408:979-982. (2000); Morgan, *et al.*, *Nature* 408:982-985 (2000); Dodart, *et al.*, *Neuroreport.* 8:1173-1178 (1997)], and the methods described herein. Cognition in humans may be assessed using any of the tests mentioned below, plus tests such as the Alzheimer’s Disease Assessment Scale – Cognitive subscale (ADAS-Cog) component [Rosen, *et al.*, *Am J Psychiatry* 141: 1356–1364 (1984)]. The ADAS-Cog is a 70-point test that briefly assesses memory, language capabilities, and other cognitive functions in patients with dementia.

By “subject” is meant a mammal, preferably a human. A subject will benefit from the present invention if the subject has a cognitive deficiency or aberration caused by or related to the presence of toxic forms and/or concentrations of soluble A β in the subject’s brain. Even though the nature or concentration of A β in a subject’s brain may not be known with certainty, the administration of well-known tests of cognition in subjects who are suspected or known to suffer from an A β -related disease will suffice to identify many subjects who will benefit from the present methods. For other subjects, a combination of clinical assessment, subject history, and perhaps laboratory or other diagnostic assessments may be needed to identify subjects likely to benefit from the present invention.

A mental status examination of cognitive domains such as language, memory, visuospatial function, executive function (ability to manipulate previously acquired information, multitasking, abstraction, judgment, calculation, etc.), personality, and mood will aid in identifying subjects most likely to benefit from the present invention. Subjects meeting the criteria for a diagnosis of probable AD (i.e., dementia, 40-90 years old, cognitive deficits in two or more cognitive domains, progression of deficits for more than six months, consciousness undisturbed, and absence of other reasonable diagnoses) will benefit from the present invention. Likewise, subjects with Down’s syndrome will benefit from the present invention.

The Mini-Mental State Examination (MMSE) is widely used, with norms adjusted for age and education [Folstein *et al.*, *J. Psych. Res.* 12:196-198 (1975); Anthony, *et al.*,

-6-

Psychological Med. 12: 397-408 (1982); Cockrell, *et al.*, *Psychopharmacology* 24: 689-692 (1988); Crum, *et al.*, *J. Am. Med. Assoc.* 18:2386-2391 (1993)]. The MMSE is a brief, quantitative measure of cognitive status in adults. 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). The "cognitive symptoms" treated by the present invention are cognitive deficits known to be associated with conditions and diseases related to A β as discussed herein.

Laboratory assessment or structural imaging studies may identify reversible causes of cognitive impairment, which are not likely to respond to the present invention, and to identify focal lesions, significant white matter disease implicating vascular dementia, significant temporal atrophy. In AD, functional studies such as positron emission tomography (PET) or single-photon emission computed tomography (SPECT) typically show abnormalities most marked in the parietal and temporal lobes bilaterally. These studies are particularly useful in differentiating early AD from normal aging or frontotemporal degeneration. They are not required to identify subjects who are likely to benefit from the present invention. The combination of medial temporal atrophy determined by structural imaging and parietal impairment determined with functional imaging is, however, a useful biomarker of AD. MRI can be used to exclude subjects with significant cerebrovascular disease.

ApoE genotyping is not useful in isolation, but may increase the specificity of the diagnosis when patients do not have the E4 allele if the diagnosis is in question. Another potential biomarker is the combined assessment of cerebral spinal fluid (CSF) A β 42 and tau concentrations. A low A β 42 and high tau concentration have a high predictive value (90%) and negative predictive value (95%) based on a clinical diagnosis of probable AD.

By "condition or disease related to A β " is meant conditions and diseases that are associated with: 1) the development of β -amyloid plaques in the brain, 2) the synthesis of abnormal forms of A β , 3) the formation of particularly toxic forms of A β , or 4) abnormal rates of synthesis, degradation, or clearance of A β . Conditions and diseases such as clinical and pre-clinical Alzheimer's disease, Down's syndrome, cerebral amyloid

-7-

angiopathy, certain vascular dementias, and mild cognitive impairment are known or suspected of having relationship to A β . "Disease progression" refers to worsening of signs or symptoms of the condition or disease with time.

Alzheimer's disease, discussed above, is the most prevalent disease related to A β (60-80% of dementias). Definite diagnosis of AD is only possible presently with a post-mortem examination. But, a diagnosis of probable AD correlates highly with AD pathology. Vascular dementia (VaD), dementia with Lewy bodies (DLB), and frontotemporal dementia (FTD) together probably account for 15% to 20% of dementias, with other disorders (e.g., hydrocephalus; vitamin B12 deficiency) accounting for about 5%. Of these, only certain vascular dementias are suspected of having a significant A β component.

A state of increased risk or early manifestation of cognitive problems that often progresses to AD is termed mild cognitive impairment (MCI). MCI is a clinical entity characterized by memory loss, without significant dysfunction in other cognitive domains and without impairment in activities of daily living (ADL) function. Early diagnosis and treatment of MCI, including with the use of the present invention, is important. Currently the best predictor of preclinical AD is a diagnosis of MCI, because 30-50% of subjects with MCI develop AD within 3-5 years. One structural correlate of MCI that may be predictive for which subjects will develop AD is the volume of the hippocampus. Subjects with MCI have smaller hippocampi than age-equivalent controls and appear to experience atrophy of the structure at a more rapid pace.

By "administering" is meant the act of introducing a pharmaceutical agent into the subject's body. The parenteral route is the preferred route of administering the antibodies in the methods of the present invention. Preferred parenteral routes include subcutaneous, intravenous, and intraperitoneal.

By "effective dose" is meant an amount of antibody, which when administered to the subject, will cause improvement in cognition. The amount of antibody in an effective dose can be readily determined by a skilled physician or clinical pharmacologist, taking into account the subject's body mass, age, gender, severity of the A β -related condition or disease, affinity of the antibody for soluble A β , route of administration, and similar factors well known to physicians and pharmacologists. Effective doses may be expressed, for example, as the total mass of antibody (e.g., in grams, milligrams or micrograms) or as

-8-

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 ($\mu\text{g/kg}$). An effective dose of antibody in the present methods will range between 1 $\mu\text{g/kg}$ and 100 mg/kg. A more preferred range for effective dose in the present invention is between 1 $\mu\text{g/kg}$ and 30 mg/kg. Yet
5 more preferred ranges are between 1 $\mu\text{g/kg}$ and 10 mg/kg, 1 $\mu\text{g/kg}$ and 10 mg/kg, between 1 $\mu\text{g/kg}$ and 1 mg/kg, between 1 $\mu\text{g/kg}$ and 0.3 mg/kg, and between 1 $\mu\text{g/kg}$ and 0.1 mg/kg.

“A β peptide” and “A β ” refer to a peptide that is derived from amyloid precursor protein (“APP” – Alzheimer's disease amyloid A4 protein [Precursor]) by proteolytic
10 cleavage. Full-length A β peptides are from 39 to 43 amino acids long in humans, for example. Full length A β peptide may undergo further cleavage *in vivo* to produce A β fragments that are shorter at the N-terminus, at the C-terminus, or both, by one to several amino acids. Soluble full-length A β peptide or fragments thereof may be used as antigens to raise antibodies that bind soluble A β peptide with high specificity and affinity. For
15 example, among the many A β peptide fragments used for this purpose, the A β 13 – 28 fragment (conjugated via m-maleimidobenzoyl-N-hydroxysuccinimide ester to an anti-CD3 antibody) was used to raise antibody 266 [Seubert, *et al.*, *Nature* 359:325 – 327 (1992)]. The use of antibody 266 for selective measurement of soluble A β is well known [Schenk, *et al.*, U.S. Patents 5,593,846, 5,766,846, 5,872,672, and 6,284,221 B1].
20 Assessment of binding to “soluble A β ” is carried out with A β in an unaggregated form, predominantly monomeric form, as described hereinbelow.

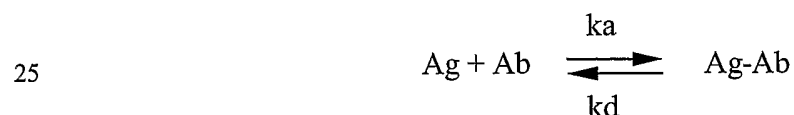
The expression “anti-A β antibody” means an antibody that binds to soluble A β . The antibody preferably binds with high affinity to soluble A β . Affinity higher than that of antibody 266 is preferred. Affinity higher than 10^{-9} M is preferred. Affinity higher
25 than 10^{-10} M is more preferred. Affinity higher than 10^{-11} M is yet more preferred. Affinity higher than 10^{-12} M is highly preferred. The term “A β ” in this context includes the 39, 40, 41, 42, and 43 amino acid peptides derived from the APP protein *in vivo* by proteolysis, and any fragments of those peptides, such as N-terminally shortened peptides derived from those peptides (e.g., denoted by, for example, x-42, where x = 1, 2, 3, etc.),
30 C-terminally shortened peptides derived from 1-39, 40, 41, and 42 peptides, and peptides shortened at both termini. The expression “A β 40” is used to denote peptides that bind to antibodies that bind only at an A β C-terminus that ends at position 40. The expression

-9-

"A β 42" denotes peptides that bind to antibodies that bind only at an A β C-terminus that ends at position 42.

By "affinity" is meant the strength of the binding of a single antigen-combining site with an antigenic determinant. It is a measure of the binding strength between antibody and a simple hapten or antigen determinant. It depends on the closeness of stereochemical fit between antibody combining sites and antigen determinants, on the size of the area of contact between them, and on the distribution of charged and hydrophobic groups. It includes the concept of "avidity," which refers to the strength of the antigen-antibody bond after formation of reversible complexes. The most direct way of measuring antibody affinity is by the well known method of equilibrium dialysis. Methods requiring less time or materials than equilibrium dialysis are known, for example, the method of Griswold, *et al. Immunology Letters* 9:15-18 (1985) and the kinetic BIAcore method described herein. 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, K_D , and the association constant, K_A , 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, k_a and k_d , quantitate the rates of the individual reactions:



At equilibrium, $k_a [\text{Ab}][\text{Ag}] = k_d [\text{Ag-Ab}]$. The dissociation constant, K_D , is given by: $K_D = k_d/k_a = [\text{Ag}][\text{Ab}]/[\text{Ag-Ab}]$. K_D has units of concentration, most typically M, mM, μM , nM, pM, etc. When comparing antibody affinities expressed as K_D , having greater affinity for A β is indicated by a lower value. The association constant, K_A , is given by: $K_A = k_a/k_d = [\text{Ag-Ab}]/[\text{Ag}][\text{Ab}]$. K_A has units of inverse concentration, most typically M^{-1} , mM^{-1} , μM^{-1} , nM^{-1} , pM^{-1} , or the like. When comparing

-10-

antibody affinities expressed as KA , having greater affinity for $A\beta$ is indicated by a higher value. "Affinity for soluble" $A\beta$ is measured as described herein using samples of $A\beta$, typically $A\beta$ 1-40 or $A\beta$ 1-42, that are reasonably free of aggregated forms of $A\beta$. For antibodies having high affinity for soluble $A\beta$, particular care must be taken when using the BIAcore technology, as described hereinbelow.

As used herein, the word "treat" includes therapeutic treatment, where a condition to be treated is already known to be present, and prophylaxis - *i.e.*, prevention of, or amelioration of, the possible future onset of a condition. The term "treating" includes prophylaxis (preventing), amelioration (reducing or reversing), or elimination of a sign, symptom, condition, disease, or disorder.

By "antibody" is meant a whole antibody, including without limitation an animal-derived antibody (e.g., murine), chimeric, humanized, human sequence, recombinant, transgenic, grafted and single chain antibody, and the like, and any fusion proteins, conjugates, fragments, or derivatives thereof. An antibody comprises protein resembling an antibody in the broadest sense in that the protein comprises a binding site for an antigen, which binding site is comprised of three pairs of complementarity determining regions. Antibody includes a whole immunoglobulin molecule, a monoclonal antibody, a chimeric antibody, a humanized antibody, a human antibody, or an immunologically effective fragment of any of these. An antibody fragment, or simply fragment, means an Fv, a disulfide linked Fv, scFv, Fab, Fab', or F(ab')₂ fragment, which terms are well known in the art. In some contexts herein, fragments will be mentioned specifically for emphasis. Nevertheless, it will be understood that regardless of whether fragments are specified, the term "antibody" includes such fragments as well as single-chain forms. As long as a protein retains the ability specifically to bind its intended target, it is included within the term "antibody." Also included within the definition "antibody" are single chain forms. Preferably, but not necessarily, the antibodies useful in the invention are produced recombinantly. Antibodies may or may not be glycosylated, though glycosylated antibodies are preferred under some circumstances, such as when prolonged residence in the body is desirable, or when minimum risk of developing neutralizing antibodies. Antibodies, except perhaps for certain types in which cross-linking between chains is accomplished by peptide or other chemical chains, are properly cross-linked via disulfide bonds.

-11-

The basic antibody structural unit is known to comprise a tetramer. 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 kappa and lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, and define the antibody's isotype as IgG, IgM, IgA, IgD and IgE, respectively. IgG isotypes are preferred. Of the IgG subclasses, IgG1 and IgG4 are preferred.

The variable regions of each light/heavy chain pair form the antibody binding site. Thus, an intact non-fragment antibody and certain fragments (e.g., an F(ab')₂ fragment) has two binding sites, whereas, most fragments have only one binding site per molecule. 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. The CDRs from the two 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. The assignment of amino acids to each domain is in accordance with well known conventions [Kabat, *et al.*, "Sequences of Proteins of Immunological Interest" National Institutes of Health, Bethesda, Md., 1987 and 1991; Chothia, *et al.*, *J. Mol. Biol.* 196:901-917 (1987); Chothia, *et al.*, *Nature* 342:878-883 (1989)].

By "humanized antibody" is meant an antibody that is composed partially or fully of amino acid sequences derived from a human antibody germline by altering the sequence of an antibody having non-human complementarity determining regions (CDR). Humanized antibodies are also referred to as CDR-grafted or reshaped antibodies. A humanized immunoglobulin does not encompass a chimeric antibody having a mouse variable region and a human constant region. However, the variable region of the antibody and even the CDR are humanized by techniques that are by now well known in the art. The framework regions of the variable regions are substituted by the corresponding human framework regions leaving the non-human CDR substantially intact. As mentioned above, it is sufficient for use in the methods of the invention, to

-12-

employ an immunologically specific fragment of the antibody, including fragments representing single chain forms.

Although the mechanics of producing an engineered, humanized mAb using the techniques of molecular biology are relatively straightforward, simple grafting of
5 xenogenic (usually rodent) complementarity-determining regions (CDRs) into human frameworks does not always reconstitute the binding affinity and specificity of the original mAb. The design of the engineered mAb is the critical step in reproducing the function of the original molecule. This design includes various choices: the extents of the CDRs, the human frameworks to use and the substitution of residues from the rodent
10 mAb into the human framework regions (backmutations). The positions of these backmutations have been identified principally by sequence/structural analysis or by analysis of homology models of the variable regions' 3D structure. Recently, phage libraries have been used to vary the amino acids at chosen positions. Similarly, many approaches have been used to choose the most appropriate human frameworks in which to
15 graft the rodent CDRs. Variable regions with high amino acid sequence identity to the rodent variable regions (homology matching or best-fit), consensus or germline sequences, or fragments of the framework sequences within each light or heavy chain variable region from several different human mAbs may be used. Alternatively the surface rodent residues may be replaced with the most common residues found in human
20 mAbs ("resurfacing" or "veneering").

The design of humanized immunoglobulins starting from a non-human antibody that has properties found to be critical in the present invention may be carried out as follows. As to the human framework region, a framework or variable region amino acid sequence of a CDR-providing non-human immunoglobulin is compared with
25 corresponding sequences in a human immunoglobulin variable region sequence collection, and a sequence having a high percentage of identical amino acids is selected. When an amino acid falls under the following category, the framework amino acid of a human immunoglobulin to be used (acceptor immunoglobulin) is replaced by a framework amino acid from a CDR-providing non-human immunoglobulin (donor
30 immunoglobulin):

(a) the amino acid in the human framework region of the acceptor immunoglobulin is unusual for human immunoglobulin at that position, whereas the

-13-

corresponding amino acid in the donor immunoglobulin is typical for human immunoglobulin at that position;

(b) the position of the amino acid is immediately adjacent to one of the CDRs; or

(c) any side chain atom of a framework amino acid is within about 5-6 angstroms
5 (center-to-center) of any atom of a CDR amino acid in a three dimensional immunoglobulin model [Queen, *et al.*, *Proc. Nat'l. Acad. Sci. (USA)* 86:10029-10033 (1989); Co, *et al.*, *Proc. Nat'l. Acad. Sci. (USA)* 88:2869 (1991)]. When each of the amino acid in the human framework region of the acceptor immunoglobulin and a corresponding amino acid in the donor immunoglobulin is unusual for human
10 immunoglobulin at that position, such an amino acid is replaced by an amino acid typical for human immunoglobulin at that position.

Human antibodies may be readily obtained using known methods, such as, from human immune donors, from phage libraries, and from transgenic animals such as mice. Antibodies may be rescued from immune human donors using either EBV transformation
15 of B-cells or by PCR cloning and phage display. Synthetic phage libraries may be created that use randomized combinations of synthetic human antibody V-regions. By selection on antigen, so called 'fully human antibodies' can be made, in which it is assumed that the V-regions are very human-like in nature. Transgenic mice can be created that have a repertoire of human immunoglobulin germline gene segments. These mice, when
20 immunized with soluble A β , make human antibodies directed against soluble A β .

Preparation of high affinity humanized or human antibodies for use in the present invention may be carried out by methods well known in the art, including preparing monoclonal antibodies using well known techniques and screening for high affinity antibodies, or by first identifying a monoclonal antibody having reasonably high affinity
25 and then improving the affinity using well known methods such as those described, for example, in: US Patent Nos. 5,976,562, 5,824,514, 5,817,483, 5,814,476, 5,763,192, 5,723,323; WO97/29131; Thomas, *et al.*, *J. Biol. Chem.* 277:2059-2064 (2002); Shreder, *Methods* 20:372-379 (2000); Boder, *et al.*, *Proc. Nat'l Acad. Sci. (USA)* 97:10701-10705 (2000); Chen, *et al.*, *J. Mol. Biol.* 293:865-881 (1999); Wu, *et al.*, *Proc. Nat'l. Acad. Sci. (USA)* 95:6037-6042 (1998); Hoogenbloom, *Trends Biotechnol.* 15:62-70 (1997); Rader, *et al.*, *Curr. Opin. Biotechnol.*, 8:503-508 (1997); Crameri, *et al.*, *Nature Medicine* 2:100-
30 102 (1996); Crameri, *et al.*, *Nature Medicine* 2:100-103 (1996); Schier, *et al.*, *J. Mol.*

-14-

Biol. 255:28-43 (1996); Yang, *et al.*, *J. Mol. Biol.* 254:392-403 (1995); Yang, *et al.*, *J. Mol. Biol.* 254:392-403 (1995); Yelton, *et al.*, *J. Immunol.* 155:1994-2004 (1995); Stemmer, *Proc. Nat'l. Acad. Sci. (USA)* 91:10747-10751 (1994); Stemmer, *Nature* 370:389-391 (1994); Huse, *et al.*, *Internat'l Rev. Immunol.* 10:129-137 (1993); the
5 portion of each of which having to do with preparation of high affinity antibodies is incorporated herein by reference.

The antibodies used in the present invention will most advantageously be expressed in recombinant hosts and purified using well known techniques [Page, *et al.*, *Bio/Technol.* 9, 64-68 (1991); Carroll, *et al.*, *Mol. Immunol.* 29, 821-827 (1992); Coloma,
10 *et al.*, *J. Immunol. Meth.* 152, 89-104 (1992); Bebbington, *et al.*, *Bio/Technol.* 10, 169-175 (1992); Deyev, *et al.*, *FEBS Lett.* 330, 111-113 (1993); Bender, *et al.*, *Hum. Antibodies Hybridomas* 4, 74-79 (1993); Norderhaug, *et al.*, *J. Immunol. Meth.* 204, 77-87 (1997); Poul, *et al.*, *Eur. J. Immunol.* 25, 2005-2009 (1995), each of which is incorporated herein by reference].

15 A preferred antibody for use in the present invention is an antibody that binds to the same epitope on A β that 266 binds or any antibody that competitively inhibits the binding of 266 and human or mouse A β . The skilled reader will know how to determine, using well known methods, whether any particular antibody competitively inhibits the binding of 266 and human A β . For example, a competitive ELISA method could be used.
20 Wells of a 96-well ELISA plate (e.g., Nunc-Immuno plate, Cat # 439454, NalgeNunc) are coated with A β peptide (1-40 is particularly convenient, but other lengths could be used also), optionally conjugated to a larger protein such as albumin. After washing the wells, they are blocked as appropriate, and then rinsed and dried appropriately. A mixture of biotinylated 266 antibody (e.g., biotinylated humanized 266, having as light chain the
25 amino acid sequence of SEQ ID NO:11 and as heavy chain the amino acid sequence of SEQ ID NO:12) at 0.3 μ g/ml final concentration, for example, and a competitor antibody (starting at 750 μ g/ml final concentration and serial 3-fold dilutions) are added in a final volume of 100 μ l per well. No-competitor and background controls are run. The ELISA plate is incubated at an appropriate temperature for an appropriate length of time, and then
30 the wells are washed. After washing the wells, HRP-conjugated streptavidin (Cat # 21124, Pierce), or equivalent, is added to each well (e.g., 100 μ l of 1 μ g/ml). The plate is incubated at room temperature for an appropriate length of time, e.g., 30 min, and then is

-15-

thoroughly washed. For color development, 100 μ l/well of ABTS Peroxidase Substrate (Kirkegaard & Perry Laboratories), or equivalent, is added. Color development is stopped and absorbance is read (e.g., at 415 nm). The absorbances are plotted against the log of the competitor concentration, curves are fitted to the data points (e.g., using Prism or equivalent) and the IC₅₀ determined using methods well known in the art. An antibody having an IC₅₀ greater than 100-fold less than of that of 266 is considered to competitively inhibit the binding of 266 to A β . The affinity of an antibody for soluble A β can be determined using methods well known in the art or described herein.

Antibody 266 has the following amino acid sequences as CDRs:

10

light chain CDR1:

```

1           5           10           15
Arg Ser Ser Gln Ser Leu Ile Tyr Ser Asp Gly Asn Ala Tyr Leu His
(SEQ ID NO:1)

```

15

light chain CDR2:

```

1           5
Lys Val Ser Asn Arg Phe Ser (SEQ ID NO:2)

```

20

light chain CDR3:

```

1           5
Ser Gln Ser Thr His Val Pro Trp Thr (SEQ ID NO:3)

```

heavy chain CDR1:

25

```

1           5
Arg Tyr Ser Met Ser (SEQ ID NO:4)

```

heavy chain CDR2:

30

```

1           5           10           15
Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr Pro Asp Thr Val Lys Gly
(SEQ ID NO:5)

```

and, heavy chain CDR3:

35

```

1
Gly Asp Tyr (SEQ ID NO:6) .

```

-16-

In humanized versions of 266, human framework regions may optionally have substitutions of one to several residues from mouse 266 for the purpose of maintaining the strength or specificity of the binding of humanized antibody 266 [WO01/62801]. A preferred light chain variable region of a humanized 266 antibody has the following amino acid sequence:

```

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

10          20          25          30
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Xaa Tyr Ser

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

15          50          55          60
Pro Xaa Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro

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

          85          90          95
Ser Arg Val Glu Ala Glu Asp Xaa Gly Val Tyr Tyr Cys Ser Gln Ser

25          100          105          110
Thr His Val Pro Trp Thr Phe Gly Xaa Gly Thr Xaa Xaa Glu Ile Lys

```

Arg (SEQ ID NO:7)

30

wherein:

- Xaa at position 2 is Val or Ile;
- Xaa at position 7 is Ser or Thr;
- Xaa at position 14 is Thr or Ser;
- 35 Xaa at position 15 is Leu or Pro;
- Xaa at position 30 is Ile or Val;
- Xaa at position 50 is Arg, Gln, or Lys;
- Xaa at position 88 is Val or Leu;

-17-

Xaa at position 105 is Gln or Gly;

Xaa at position 108 is Lys or Arg; and

Xaa at position 109 is Val or Leu.

5 A preferred heavy chain variable region of a humanized 266 antibody has the following amino acid sequence:

```

1           5           10           15
Xaa Val Gln Leu Val Glu Xaa Gly Gly Gly Leu Val Gln Pro Gly Gly
10
           20           25           30
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr
           35           40           45
15 Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Xaa Leu Val
           50           55           60
Ala Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr Pro Asp Xaa Val
20 65           70           75           80
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Xaa Xaa Asn Thr Leu Tyr
           85           90           95
Leu Gln Met Asn Ser Leu Arg Ala Xaa Asp Thr Ala Val Tyr Tyr Cys
25
           100          105          110
Ala Ser Gly Asp Tyr Trp Gly Gln Gly Thr Xaa Val Thr Val Ser Ser

```

(SEQ ID NO:8)

30

wherein:

Xaa at position 1 is Glu or Gln;

Xaa at position 7 is Ser or Leu;

Xaa at position 46 is Glu, Val, Asp, or Ser;

35 Xaa at position 63 is Thr or Ser;

Xaa at position 75 is Ala, Ser, Val, or Thr;

Xaa at position 76 is Lys or Arg;

-18-

Xaa at position 89 is Glu or Asp; and

Xaa at position 107 is Leu or Thr.

A particularly preferred light chain variable region of a humanized 266 antibody
 5 has the following amino acid sequence:

```

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

10          20          25          30
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Ile Tyr Ser

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

15          50          55          60
Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro

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

          85          90          95
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Ser

25          100         105         110
Thr His Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys

Arg          (SEQ ID NO:9) .

30

```

A particularly preferred heavy chain variable region of a humanized 266 antibody
 has the following amino acid sequence:

```

35   1           5           10           15
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly

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

```

-19-

```

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

5          50          55          60
Ala Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr Pro Asp Thr Val

          65          70          75          80
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
10
          85          90          95
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys

          100          105          110
15 Ala Ser Gly Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

```

(SEQ ID NO:10).

20 A preferred light chain for a humanized 266 antibody has the amino acid sequence:

```

          1          5          10          15
Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu
25
          20          25          30
Gly Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Ile

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

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

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

          80          85          90
Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val
40

```

-20-

```

          95              100              105
Tyr Tyr Cys Ser Gln Ser Thr His Val Pro Trp Thr Phe Gly Gln

          110              115              120
5  Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val

          125              130              135
Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala

10          140              145              150
Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys

          155              160              165
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln

15          170              175              180
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu

          185              190              195
20 Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys

          200              205              210
Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val

25          215
Thr Lys Ser Phe Asn Arg Gly Glu Cys (SEQ ID NO:11).

```

30 A preferred heavy chain for a humanized 266 antibody has the amino acid sequence:

```

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

35          20              25              30
Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser

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

```

-21-

		50		55		60										
		Glu	Leu	Val	Ala	Gln	Ile	Asn	Ser	Val	Gly	Asn	Ser	Thr	Tyr	Tyr
		65		70		75										
5		Pro	Asp	Thr	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala
		80		85		90										
		Lys	Asn	Thr	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp
10		95		100		105										
		Thr	Ala	Val	Tyr	Tyr	Cys	Ala	Ser	Gly	Asp	Tyr	Trp	Gly	Gln	Gly
		110		115		120										
		Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val
15		125		130		135										
		Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala
		140		145		150										
20		Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr
		155		160		165										
		Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe
25		170		175		180										
		Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val
		185		190		195										
		Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys
30		200		205		210										
		Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val
		215		220		225										
35		Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro
		230		235		240										
		Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro
40		245		250		255										
		Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr

-22-

	260	265	270
	Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe		
	275	280	285
5	Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys		
	290	295	300
	Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val		
10	305	310	315
	Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys		
	320	325	330
	Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr		
15	335	340	345
	Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr		
	350	355	360
20	Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu		
	365	370	375
	Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu		
25	380	385	390
	Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		
	395	400	405
	Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu		
30	410	415	420
	Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys		
	425	430	435
35	Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser		
	440		
	Leu Ser Leu Ser Pro Gly Lys	(SEQ ID NO:12).	

40 A preferred antibody for use in the present invention is an analog of 266, in which an N-glycosylation site within CDR2 of the heavy chain (SEQ ID NO:5) is engineered so

as not to be glycosylated. Such an analog has higher affinity for A β than 266 does, and comprises a light chain and a heavy chain, wherein the light chain comprises the three light chain complementarity determining regions (CDRs) from mouse monoclonal antibody 266 (SEQ ID NO:1-3), and wherein the heavy chain comprises heavy chain CDR1 and CDR3 from mouse monoclonal antibody 266 (SEQ ID NO: 4 and 6, respectively), and a heavy chain CDR2 having the sequence given by SEQ ID NO:13:

10

Xaa at position 7 is any amino acid, provided that if Xaa at position 8 is neither
15 Asp nor Pro and Xaa at position 9 is Ser or Thr, then Xaa at position 7 is not Asn;

15

20

20

25

30

-24-

and Tyr, provided that if Xaa at position 7 is Asn and Xaa at position 9 is Ser or Thr, then Xaa at position 8 is Asp or Pro; and

Xaa at position 9 of SEQ ID NO:13 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr, provided that if Xaa at position 7 is Asn and Xaa at position 8 is neither Asp nor Pro, then Xaa at position 9 is neither Ser nor Thr.

Another description of the preferred group is: antibodies or fragments thereof having as light chain CDR1-CDR3 the sequences SEQ ID NO:1-3, respectively, as heavy chain CDR1 and CDR3 the sequences SEQ ID NO:4 and 6, respectively, and wherein the sequence of heavy chain CDR2 is selected from the group consisting of:

```

1)      SEQ ID NO:14
          1              5              10              15
Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val Lys
15
Gly      (SEQ ID NO:14)

```

wherein:

Xaa at position 7 of SEQ ID NO:14 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr;

Xaa at position 8 of SEQ ID NO:14 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr; and

25 Xaa at position 9 of SEQ ID NO:14 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr;

```

2)      SEQ ID NO:15
30      1              5              10              15
      Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val Lys

      Gly  (SEQ ID NO:15)

```

-25-

wherein:

Xaa at position 7 of SEQ ID NO:15 is Asn;

Xaa at position 8 of SEQ ID NO:15 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr; and

Xaa at position 9 of SEQ ID NO:15 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Val, Trp, and Tyr; and

3) SEQ ID NO:16

1	5	10	15
Gln	Ile Asn Ser Val	Gly Xaa Xaa Xaa	Tyr Tyr Pro Asp Thr Val Lys

Gly (SEQ ID NO:16)

wherein:

Xaa at position 7 of SEQ ID NO:16 is Asn;

Xaa at position 8 of SEQ ID NO:16 is selected from the group consisting of Asp and Pro; and

Xaa at position 9 of SEQ ID NO:16 is selected from the group consisting of Ser and Thr.

Preferred sequences for CDR2 of the heavy chain include those in which only a single amino acid is changed, those in which only two amino acids are changed, or all three are changed. It is preferred to replace Asn at position 7, or to replace Thr at position 9, or to replace both. Conservative substitutions at one, two, or all three positions are preferred. The most preferred species are those in which Asn at position 7 is replaced with Ser or Thr.

Preferred deglycosylated 266 antibodies for use in the present invention are those in which in CDR2 of the heavy chain (i.e., within SEQ ID NO:13, as described above):

Xaa at position 7 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser, and Thr, provided that if Xaa at position 9 is Ser or Thr, then Xaa at position 7 is not Asn;

-26-

Xaa at position 8 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser, and Thr; and

Xaa at position 9 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser, and Thr, provided that if Xaa at position 7 is Asn, then Xaa at position 9 is neither
5 Ser nor Thr.

An alternate description of preferred declycoglycosylated 266 antibodies is: antibodies or fragments thereof having as light chain CDR1-CDR3 the sequences SEQ ID NO:1-3, respectively, as heavy chain CDR1 and CDR3 the sequences SEQ ID NO:4 and 6, respectively, and wherein the sequence of heavy chain CDR2 is selected from the group
10 consisting of:

1) SEQ ID NO:17

1 5 10 15
Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val Lys
15
Gly (SEQ ID NO:17)

wherein:

Xaa at position 7 of SEQ ID NO:17 is selected from the group consisting of Ala,
20 Gly, His, Gln, Ser, and Thr;

Xaa at position 8 of SEQ ID NO:17 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser, and Thr; and

Xaa at position 9 of SEQ ID NO:17 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser, and Thr; and
25

2) SEQ ID NO:18

1 5 10 15
Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val Lys
30
Gly (SEQ ID NO:18)

wherein:

Xaa at position 7 of SEQ ID NO:18 is Asn;

-27-

Xaa at position 8 of SEQ ID NO:18 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser, and Thr; and

Xaa at position 9 of SEQ ID NO:18 is selected from the group consisting of Ala, Gly, His, Asn, and Gln.

5

A preferred humanized antibody for use in the present invention has the light chain variable region of SEQ ID NO:7 and a heavy chain variable region of SEQ ID NO:19:

```

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

              20              25              30
      Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
15
              35              40              45
      Arg Tyr Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu

              50              55              60
20      Xaa Leu Val Ala Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr

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

25              80              85              90
      Xaa Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Xaa Asp

              95              100              105
      Thr Ala Val Tyr Tyr Cys Ala Ser Gly Asp Tyr Trp Gly Gln Gly
30
              110
      Thr Xaa Val Thr Val Ser Ser (SEQ ID NO:19)

```

wherein:

- 35 Xaa at position 1 is Glu or Gln;
 Xaa at position 7 is Ser or Leu;
 Xaa at position 46 is Glu, Val, Asp, or Ser;

-28-

Xaa at position 56 is any amino acid, provided that if Xaa at position 57 is neither Asp nor Pro and Xaa at position 59 is Ser or Thr, then Xaa at position 56 is not Asn;

Xaa at position 57 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 58 is Ser or Thr, then Xaa at position 57 is Asp or Pro; and

5 Xaa at position 58 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 57 is neither Asp nor Pro, then Xaa at position 58 is neither Ser nor Thr

Xaa at position 63 is Thr or Ser;

Xaa at position 75 is Ala, Ser, Val, or Thr;

10 Xaa at position 76 is Lys or Arg;

Xaa at position 89 is Glu or Asp; and

Xaa at position 107 is Leu or Thr.

15 A preferred humanized antibody for use in the present invention has the light chain variable region of SEQ ID NO:9 and a heavy chain variable region of SEQ ID NO:20:

1	5	10	15
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly			
20	20	25	30
Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser			
25	35	40	45
Arg Tyr Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu			
	50	55	60
Glu Leu Val Ala Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr			
30	65	70	75
Pro Asp Thr Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala			
	80	85	90
Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp			
35	95	100	105
Thr Ala Val Tyr Tyr Cys Ala Ser Gly Asp Tyr Trp Gly Gln Gly			

-29-

110

Thr Leu Val Thr Val Ser Ser

(SEQ ID NO:20) .

5 wherein:

Xaa at position 56 is any amino acid, provided that if Xaa at position 57 is neither Asp nor Pro and Xaa at position 59 is Ser or Thr, then Xaa at position 56 is not Asn;

Xaa at position 57 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 58 is Ser or Thr, then Xaa at position 57 is Asp or Pro; and

10 Xaa at position 58 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 57 is neither Asp nor Pro, then Xaa at position 58 is neither Ser nor Thr.

A preferred humanized antibody for use in the present invention has the light chain variable region of SEQ ID NO:11 and a heavy chain given by SEQ ID NO:21:

15

1	5	10	15
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly			
	20	25	30
20 Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser			
	35	40	45
Arg Tyr Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu			
25	50	55	60
Glu Leu Val Ala Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr			
	65	70	75
Pro Asp Thr Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala			
30	80	85	90
Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp			
	95	100	105
35 Thr Ala Val Tyr Tyr Cys Ala Ser Gly Asp Tyr Trp Gly Gln Gly			
	110	115	120
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val			

-30-

		125		130		135
	Phe	Pro	Leu	Ala	Pro	Ser
		Ser	Ser	Lys	Ser	Thr
				Ser	Thr	Ser
				Gly	Gly	Thr
						Ala
5		140		145		150
	Ala	Leu	Gly	Cys	Leu	Val
		Lys	Asp	Tyr	Phe	Pro
				Glu	Pro	Val
						Thr
		155		160		165
	Val	Ser	Trp	Asn	Ser	Gly
		Ala	Leu	Thr	Ser	Gly
				Val	His	Thr
						Phe
10		170		175		180
	Pro	Ala	Val	Leu	Gln	Ser
		Ser	Ser	Gly	Leu	Tyr
				Ser	Leu	Ser
						Val
		185		190		195
	Val	Thr	Val	Pro	Ser	Ser
		Ser	Ser	Ser	Leu	Gly
				Thr	Gln	Thr
				Tyr	Ile	Cys
15		200		205		210
	Asn	Val	Asn	His	Lys	Pro
		Ser	Asn	Thr	Lys	Val
				Asp	Lys	Lys
						Val
		215		220		225
	Glu	Pro	Lys	Ser	Cys	Asp
		Lys	Thr	His	Thr	Cys
				Pro	Pro	Cys
						Pro
		230		235		240
	Ala	Pro	Glu	Leu	Leu	Gly
		Gly	Gly	Pro	Ser	Val
				Phe	Leu	Phe
						Pro
						Pro
25		245		250		255
	Lys	Pro	Lys	Asp	Thr	Leu
		Met	Ile	Ser	Arg	Thr
				Pro	Glu	Val
						Thr
		260		265		270
	Cys	Val	Val	Val	Asp	Val
		Ser	His	Glu	Asp	Pro
				Glu	Val	Lys
						Phe
30		275		280		285
	Asn	Trp	Tyr	Val	Asp	Gly
		Val	Glu	Val	His	Asn
				Ala	Lys	Thr
						Lys
		290		295		300
	Pro	Arg	Glu	Glu	Gln	Tyr
		Asn	Ser	Thr	Tyr	Arg
				Val	Val	Ser
						Val
		305		310		315
	Leu	Thr	Val	Leu	His	Gln
		Asp	Trp	Leu	Asn	Gly
				Lys	Glu	Tyr
						Lys
40		320		325		330
	Cys	Lys	Val	Ser	Asn	Lys
		Ala	Leu	Pro	Ala	Pro
			Ile	Glu	Lys	Thr

-31-

335 340 345
 Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
 5 350 355 360
 Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
 365 370 375
 Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
 10 380 385 390
 Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
 395 400 405
 15 Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
 410 415 420
 Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
 20 425 430 435
 Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
 440
 Leu Ser Leu Ser Pro Gly Lys (SEQ ID NO:21)
 25

wherein:

Xaa at position 56 is any amino acid, provided that if Xaa at position 57 is neither Asp nor Pro and Xaa at position 59 is Ser or Thr, then Xaa at position 56 is not Asn;

30 Xaa at position 57 is any amino acid, provided that if Xaa at position 56 is Asn
 and Xaa at position 58 is Ser or Thr, then Xaa at position 57 is Asp or Pro; and

Xaa at position 58 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 57 is neither Asp nor Pro, then Xaa at position 58 is neither Ser nor Thr.

Preferred deglycosylated 266 antibodies having the heavy variable region
 35 according to SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21 are those wherein:

Xaa at position 56 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser, and Thr, provided that if Xaa at position 58 is Ser or Thr, then Xaa at position 56 is not Asn;

-32-

Xaa at position 57 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser, and Thr; and

Xaa at position 58 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser, and Thr, provided that if Xaa at position 56 is Asn, then Xaa at position 58 is
5 neither Ser nor Thr.

Preferred sequences for CDR2 (positions 56, 57, and 58) of the heavy chain SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21 include those in which only a single amino acid is changed, those in which only two amino acids are changed, or all three are changed. It is preferred to replace Asn at position 56. It is preferred to replace Thr at
10 position 58 with an amino acid other than Ser. It is preferred to not destroy the N-glycosylation site in the CDR2 of the 266 heavy chain by replacing Ser at position 57 with Pro or Asp. Conservative substitutions at one, two, or all three positions are preferred. The most preferred species are those in which Asn at position 56 is replaced with Ser or Thr. Particularly preferred antibodies are those in which Ser or Thr is at position 56, Ser
15 is at position 57, and Thr is at position 58 of SEQ ID NO:19, SEQ ID NO:20, or SEQ ID NO:21.

The most preferred species are antibodies comprising a light chain of SEQ ID NO:11 and a heavy chain of SEQ ID NO:21, wherein in SEQ ID NO:21, Xaa at position 56 is Ser, Xaa at position 57 is Ser, and Xaa at position 58 is Thr ("N56S"), or wherein in
20 SEQ ID NO:21, Xaa at position 56 is Thr, Xaa at position 57 is Ser, and Xaa at position 58 is Thr ("N56T").

The antibodies (including immunologically reactive fragments) are administered to a subject as identified above using standard parenteral, peripheral administration techniques, by intravenous, intraperitoneal, subcutaneous, pulmonary, transdermal,
25 intramuscular, intranasal, buccal, sublingual, or suppository administration. The preferred routes of administration are intravenous, subcutaneous, and intraperitoneal.

The preparation of an acceptable pharmaceutical preparation of the antibodies used in the present invention, including its strength, excipients, pH, isotonicity, presentation, dosage form, and the like, is well known to the skilled person.
30 Pharmaceutical compositions for use in the present invention should be appropriate for the selected mode of administration, and pharmaceutically acceptable excipients such as, buffers, surfactants, preservatives, solubilizing agents, isotonicity agents, stabilizing

-33-

agents and the like are used as appropriate. Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton PA, latest edition, incorporated herein by reference, provides a compendium of formulation techniques as are generally known to practitioners.

Pharmaceutical preparations for use in the present invention should be sterile or at least
5 nearly so, and if necessary preserved or rendered bacteriostatic.

The following examples are intended to illustrate, not limit, the invention. The examples describe experiments conducted in murine systems, thus the use of murine monoclonal antibodies is satisfactory. However, in the treatment methods of the invention intended for human use, humanized or fully human antibodies are preferred.

10

EXAMPLES

Example 1

Rapid Improvement in Cognition after Administration of Anti-A β Antibody 266

15 APPV717F transgenic mice (PDAPP mice, eleven month old) were used [Games et al., *Nature* 373:523-527 (1995)]. The mice were handled daily 5 days before the behavioral testing. All animals had free access to food and water. They were housed at a room temperature of $23 \pm 1^\circ\text{C}$ and with a light-dark cycle of 12:12 h with lights on at 6:00 a.m. Behavioral experiments were conducted during the light period, between 8:00 a.m.
20 and 2 p.m.

The object recognition task is based on the spontaneous tendency of rodents to explore a novel object more often than a familiar one [Ennaceur et Delacour, *Behavioral Brain Research*. 31:47-59 (1988); Dodart et al., *Neuroreport*. 8:1173-1178 (1997)]. This task was performed in a black PlexiglasTM open field (50 X 50 X 40 cm). The objects to
25 be discriminated were a marble (1.5 cm diameter) and a plastic dice (1.8 cm edge). After each trial, the objects were handled with disposable gloves and immersed in alcohol to eliminate olfactory cues. Before experiments, several male mice were placed in the open field in order to condition the testing environment. On the first day of testing, mice were submitted to a familiarization session by placing them in the empty open field for 30 min
30 and the distance traveled (cm) was recorded by at 5-minute intervals using a computer-assisted video tracking system (San Diego Instrument, CA). On the following day, mice were submitted to two 10 min trials with a 3 hour inter-trial delay. During trial 1, mice

-34-

were allowed to explore the open field in the presence of object A (marble or dice). The distance traveled (cm) and the time spent exploring the object (nose pointing toward the object at a distance ≤ 1 cm) were recorded with the video tracking system and by hand, respectively. For trial 2, mice were allowed to explore the open field in the presence of two objects: the familiar object ("object A") and a novel object ("object B") (e.g., a marble and a die).

A recognition index calculated for each animal was expressed by the ratio $(t_B \times 100)/(t_A + t_B)$ where t_A and t_B are the time spent on object A and object B respectively. An observer blind to the treatment status of the animals recorded the object exploration time.

Murine anti-A β antibody 266 ["m266", Seubert, *et al.*, *Nature* 359:325-327 (1992)] and a purified mouse IgG1 isotype control (Pharmingen) were diluted from stock solutions in PBS prior to administration. Animals were injected (500 μ L, i.p.) with m266 (n=8, 1 mg/mL) control IgG solution (n=8, 1 mg/mL) or PBS (n=7) 3 hours before the familiarization session, which corresponds to 24 hours before trial 1. An additional control group of wild-type mice (WT) animals was tested in parallel in the object recognition task (n=6).

After completion of trial 2, blood and CSF fluids were sampled, and brains were processed using a 3-step extraction procedure. The first step consisted of homogenizing samples in cold PBS and complex of proteinase inhibitors (CompleteTM, Boehringer-Mannheim, IN) followed by centrifugation at 10,000 rpm for 10 min at 4°C, the supernatant was considered as the PBS "soluble" pool. The second step consisted of re-suspension of the pellet in RIPA (50mM Tris, 150mM NaCl, 0.5% DOC, 1% NP40, 0.1% SDS and CompleteTM, pH 8.0) followed by centrifugation at 10,000 rpm for 10 min at 4°C, the supernatant was designated the "detergent soluble" pool. Finally, the third step consisted of re-suspension of the pellet in 5M Guanidine-HCl, rocking the tubes for 2 hours at room temperature, followed by centrifugation at 10,000 rpm for 10 min at 4°C. This step produced the "insoluble" pool. A β 40 and A β 42 were quantified in each pool using an ELISA [Bales, *et al.*, *Proc. Natl. Acad. Sci. USA*. 96:15233-15238 (1999)].

Briefly, for the ELISA, the monoclonal antibodies 2G3 and 21F12 were used to capture A β peptides terminating at residues 40 and 42 respectively [Johnson-Wood, *et al.*,

-35-

1997]. Biotinylated 3D6, which recognizes the A β 1-5 region, was used as the reporter antibody.

Proteins of plasma and CSF samples were separated by electrophoresis under non-denaturing conditions utilizing a 4-20% TBE gel (Criterion gel, Bio Rad, CA) and
5 transferred in CAPS buffer (CAPS 10 mM, 0.01% SDS, 1% Methanol, pH 11) onto PVDF membrane. After a 1-hour block in SuperBlock blocking buffer (Pierce, IL), the membrane was probed with biotinylated 3D6 (0.045 mg/ml), thereafter reacted with StreptAvidin (1:200000) and visualized utilizing SuperSignal West Femto (Pierce, IL).

To compare behavioral data as well as the A β levels between groups, one-way or
10 two-way analyses of variance (ANOVA) and correlation analyses were performed using the Statview5 software (SAS Institute Inc., NC).

As shown in Fig. 1, the object recognition index differed significantly between treatment groups (group effect: $F_{3,25} = 25.085$, $p < 0.0001$). Performance of m266-treated tg mice was significantly higher than performance of saline-treated tg mice and control
15 IgG-treated tg mice ($p < 0.0001$) and comparable to WT mice. The object recognition index did not differ between saline- and control IgG-treated tg mice. Moreover, a within-group t-test analysis confirmed that m266- (t-value = 9.526, $p < 0.0001$) treated tg mice and WT mice (t-value = 9.581, $p < 0.0002$) performed above chance levels (50%) whereas saline- and control IgG-treated tg mice did not (t-values = 0.081 and 1.157 respectively,
20 NS). A group effect analysis also confirmed that there were no differences between groups in total object exploration time during trial 1 ($F_{3,25} = 0.555$, NS) or trial 2 ($F_{3,25} = 0.679$, NS). The distance traveled also did not differ between groups during the familiarization session (group x block interaction: $F_{15,125} = 1.455$, NS; group effect: $F_{3,25} = 1.200$, NS), during trial 1 (group effect: $F_{3,25} = 1.326$, NS) or during trial 2 (group effect:
25 $F_{3,23} = 1.334$, NS).

Following administration of m266, as detected by ELISA (Fig.2a), the levels of both A β 40 and A β 42 peptides were significantly increased in plasma samples when compared to saline- and IgG-treated mice ($p < 0.0001$). The increase for A β 40, when compared to saline-treated mice was approximately 150-fold and the increase for A β 42,
30 was approximately 70-fold. Neither A β 40 nor A β 42 levels differed between saline- and control IgG-treated mice. Remarkably, the plasma levels of both A β peptides following

-36-

m266 treatment were highly correlated with object recognition memory performance ($r = 0.774$, $p < 0.0001$ for A β 40; $r = 0.781$, $p < 0.0001$ for A β 42) (Fig.2b).

In CSF samples, levels of A β 40 significantly differed between groups ($F_{2,19} = 4.798$, $p < 0.05$), m266-treated animals showing increased levels of this peptide compared to saline- or control IgG-treated animals ($p < 0.05$, levels of A β 40 in ng per ml of protein: saline, 7.79 ± 1.73 ; control IgG, 8.72 ± 2.9 ; m266, 16.57 ± 3.25). No difference in CSF levels of A β 42 was observed between groups ($F_{2,19} = 3.006$, NS).

In the cerebral cortex, a significant difference between groups was observed only for A β 40 in the PBS-soluble pool ($F_{2,20} = 7.785$, $p < 0.01$); m266-treated mice showing increased levels of soluble A β peptide. No significant differences in levels of A β were found between groups in the hippocampal extracts (data not shown). Interestingly, object recognition performance was also significantly correlated with levels of A β 40 in CSF and in the cortical PBS-soluble pool ($r = 0.491$, $p < 0.05$ and $r = 0.605$, $p < 0.01$).

Administration of 360 μ g of 3D6 per animal 24 hours prior to the habituation session in 11-12 month old APP^{V717F} tg mice improved OR performance in only 2 of 8 mice tested (Figure 8) ($p < 0.05$). This improvement was much less significant than that obtained using antibody 266 that has a significantly greater affinity for soluble A β than 3D6, and a significantly greater ability to cause flux of A β from brain than does 3D6.

These results demonstrate that administration of an antibody having a very high affinity for soluble A β (but a very low affinity for insoluble A β) rapidly and efficiently reverses object recognition memory impairments in APPV717F transgenic mice. This rapid improvement in cognitive function is accompanied by significant and marked increases in plasma, CSF and cortical soluble levels of A β , but not by any measurable changes in the brain insoluble pool of A β .

25

Example 2

Rapid Effect of Administration of Anti-A β Antibodies on Cognition Correlated with Affinity for Soluble A β

The anti-A β murine antibodies 21F12 (recognizing A β 42, but not A β 40), 2G3 (recognizing A β 40, but not A β 42), 4G8 (binding A β between 13 and 28), 10D5

30

-37-

(recognizing 1-16), and 3D6 (binding 1-5) are administered to transgenic PDAPP mice as described above.

The performance of the mice administered these antibodies is then determined in the object recognition test as described above. Performance will correlate positively with the affinity of the antibody of soluble A β , that is, the higher the affinity of an antibody for soluble A β , the generally higher will be the performance in tests of cognition within a short time after administering the antibody.

Antibody m266 causes more significant flux of A β into the plasma and faster, more complete recovery of object recognition than does an antibody such as 3D6, which has an affinity for soluble A β that is about 1,000-fold less than that of m266. Antibodies having higher binding affinity for soluble A β will cause more pronounced flux and significantly faster and better improvement in cognitive function.

Example 3

Spatial Learning in APPV717F Mice Following a Single anti-A β Antibody Treatment

The subjects were female APPV717F and wild-type mice approximately 11 months old. Each mouse was administered 355 μ g of murine 266 antibody or vehicle (PBS) administered 24 hours prior to start of testing (i.p.) Mice were tested in a holeboard spatial learning assay for four consecutive days (Figure 3). Four holes were baited with access to a single food pellet and the remaining holes were baited beneath a screen without access. Mice were food-deprived each night before testing the next morning. Mice were tested for four, 180-second trials per day. Testing occurred for four consecutive days.

A single dose of A β antibody (266) significantly enhanced cognitive functioning of 11 month-old APPV717F mice compared to vehicle-treated transgenic mice (Figure 4).

A significant decrease in total errors was noted on Day 4 for vehicle-treated WT mice while the number of errors was similar across Days 3 and 4 for antibody-treated wild type mice (Figure 5).

Example 4

-38-

Effect of administration of various antibodies on plasma and cortical soluble A β concentrations after 24 hours

Transgenic (+/-) mice (4 months of age) were administered 355 μ g of each antibody (intraperitoneal). Samples were obtained 24 hours later.

5

Table 1. Concentration of soluble A β 42 in cortex (μ g/g) 24 hours after administration of various anti-A β antibodies.

	Mean	S.E.M.
Control (5)	0.058	0.007
266 (4)	0.169	0.047
3D6 (5)	0.091	0.007
10D5 (5)	0.065	0.004

10 Table 2. Concentration (ng/mL) of A β in plasma samples 24 hours after administering various anti-A β antibodies.

	A β 40		A β 42	
	Mean	S.E.M.	Mean	S.E.M.
Control (4)	0.054	0.0045	0.064	0.004
266 (4)	5.0	0.13	9.2	0.055
10D5 (5)	0.19	0.022	0.20	0.002
Control (4)	0.50	0.02	0.13	0.005
3D6 (5)	3.0	0.35	1.18	0.15

Antibody 3D6 administration caused an increase in plasma A β 40 as well as A β 42 (6-fold, and 8-fold, respectively). Plasma A β 40 and A β 42 levels were increased by 10D5 administration as well (approx. 3-4 fold). Antibody 266 administration caused a very significant increase in both A β 40 and A β 42 (93-fold and 144-fold, respectively).

Soluble A β 40 from cortical tissue was significantly increased by 266 only. Administration of 3D6 or 10D5 was without effect on soluble A β 40 levels in brain.

20 In another study of the rapid effects of administration of antibodies, 355 μ g of each of 266, 3D6, and 4G8 was administered ip to hemizygous PDAPP transgenic mice (3 months old). Samples were obtained 24 hours later.

-39-

Table 3. Concentration (ng/mL) of A β 42 in plasma samples 24 hours after administering various anti-A β antibodies.

A β 42		
	Mean	S.E.M.
Control (7)	0.048	0.0018
266 (5)	3.8	0.30
4G8 (5)	0.23	0.035
Control (7)	0.088	0.0035
3D6 (5)	0.72	0.15

5 In another study of the rapid effects of administration of antibodies, 355 μ g of each of anti-A β antibodies 2G3 (recognizing the C-terminus of A β 40 but not A β 42), 10D5 (recognizing the N-terminus of A β), and 21F12 (recognizing the C-terminus of A β 42, but not A β 40) was administered intraperitoneally. Samples were obtained 24 hours later. The only significant difference in plasma A β 42 levels was in the 10D5 group.

10

Table 4. Concentration (ng/mL) of A β 42 in plasma samples 24 hours after administering various anti-A β antibodies.

A β 42		
	Mean	S.E.M.
Control (5)	0.065	0.0055
2G3 (5)	2.34	0.091
10D5 (5)	0.087	0.0044
Control (5)	0.058	0.0029
21F12 (5)	1.80	0.036

15 Table 5. Concentration (μ g/g) of soluble A β 40 and A β 42 in cortex 24 hours after administering various anti-A β antibodies.

	A β 40		A β 42	
	Mean	S.E.M.	Mean	S.E.M.
Control (5)	27	3	7.0	0.61
2G3 (5)	48	5	4.0	0.23
10D5 (5)	62	9	5.2	0.49
21F12 (5)	91	14	5.4	0.16

-40-

There were no significant differences in insoluble A β in any group. Administration of 21F12 and 10D5 resulted in a statistically significant increase in soluble A β 40 levels in the brain. Soluble levels of A β 42 were significantly decreased following treatment with all 3 antibodies.

5 In yet another study, administration of 360 μ g of 266, 3D6, or 10D5 antibody per animal (5 animals per group, saline control) raised average plasma A β 1-40 approximately 334-, 92-, and 14-fold, respectively, and raised average plasma A β 1-42 approximately 168-, 32-, and 19-fold, respectively. Clearly the relative magnitudes of these responses are directly related to the relative affinities of the antibodies toward
10 soluble A β .

Taken together, these studies of the effects of administration of anti-A β antibodies after 24 hours shows a strong relationship between the levels of plasma A β and the affinity of the antibody for soluble A β 40 and A β 42. Also, an antibody that causes a significantly higher flux of A β (i.e., 266 compared with 3D6) after 24 hours also causes a
15 significantly higher recovery of cognition in the same period. Therefore, we believe that improvement in cognition will be faster and/or greater in magnitude when an antibody having a higher affinity for soluble A β is administered.

Example 5

20 Cognition after administration of antibodies having a range of affinities for soluble A β

Antibodies having affinities for soluble A β between about 1 nM and about 1 pM are obtained or prepared as described herein. The antibodies are administered to transgenic mice as described above in Example 1. Antibodies having higher affinity for soluble A β will generally cause greater flux of A β within a short time after administration
25 and also more greatly effect rapid improvement in cognition.

Example 6

Binding affinity for soluble A β

30 Antibody affinity for soluble A β is determined using a BIAcore biosensor 2000 and data analyzed with BIAevaluation (v. 3.1) software. A capture antibody (rabbit anti-

-41-

mouse Ig or anti-human Ig) is coupled via free amine groups to carboxyl groups on flow cell 2 of a biosensor chip (CM5) using N-ethyl-N-dimethylaminopropyl carbodiimide and N-hydroxysuccinimide (EDC/NHS). A non-specific rabbit IgG is coupled to flow cell 1 as a background control. Test antibodies are captured to yield 300 resonance units (RU).

- 5 Soluble A β 1-40 or 1-42 (Biosource International, Inc.) is then flowed over the chip at decreasing concentrations (1000 to 0.1 times KD). To regenerate the chip, bound anti-A β antibody is eluted from the chip using a wash with glycine-HCl (pH 2). A control injection containing no amyloid-beta serves as a control for baseline subtraction. Sensorgrams demonstrating association and dissociation phases are analyzed to determine
- 10 kd and ka. Using this method, the affinity of the following antibodies was determined for 1-42 and/or 1-40, and they are presented in Table 1. Two affinities were found for antibody 10D5.

Table 6. Affinity (nM) of various antibodies for soluble A β 1-40 and/or soluble A β 1-42.

15

	Soluble A β 1-40	Soluble A β 1-42
3D6	2.4	2.4
10D5	390	0.57/4,950
266	0.004	0.004
266 N56S*	-	0.0025
266 N56T†	-	0.0019
4G8	23	24
21F12	-	4.4
2G3	0.9	-

* deglycosylated 266 analog, wherein Asn at position 56 of the heavy chain variable region is replaced with Ser

† deglycosylated 266 analog, wherein Asn at position 56 of the heavy chain variable

20 region is replaced with Thr

In Figure 6 is plotted log of flux vs. log affinity for soluble A β . Flux is defined as the fold increase in plasma A β (40 or 42) 24 hours after administration of antibody, as described in Example 4 above. Affinity is given in Table 2 above. Because 10D5

-42-

apparently had two affinities for soluble A β 42 that varied in the extreme, only data on A β 40 were used for 10D5. A distinct relationship between affinity for soluble A β and flux is evident.

In Figure 7 is plotted flux data against affinity for aggregated A β , using data taken from Bard, *et al.*, *Nat. Med.* 6:916-919 (2000). Antibody 266 was reported not to bind to aggregated A β , and so no data are plotted for it. There is clearly no relationship between affinity for aggregated A β and flux.

As demonstrated herein, the affinity of anti-A β antibodies for soluble, not aggregated, A β is positively correlated with flux of A β from the brain into the plasma within 24 hours after administering the antibody. Furthermore, the rate of flux of A β is also related to acute improvement in cognitive performance as demonstrated in Example 1 and Figure 2. Antibodies having higher affinity for soluble A β will cause more pronounced flux and will more quickly and more significantly effect improvement in cognitive function in conditions and diseases involving A β . Furthermore, chronic administration of antibodies having high affinity for soluble forms of A β will more effectively cause flux of A β from brain and sequester A β forms away from brain tissues which are adversely affected in conditions and diseases related to A β . We conclusively demonstrate here that high affinity for soluble, not insoluble or aggregated, forms of A β is an important feature of anti-A β antibodies for treating cognitive impairment in A β related conditions and diseases. We also believe that high affinity for soluble, not insoluble or aggregated, forms of A β is an important feature of anti-A β antibodies for treating cognitive impairment in A β related conditions and diseases.

Example 7

Alternate procedure for determining binding affinity for antibodies having high affinity for soluble A β

The procedure is very similar to that used above in Example 6, with certain modifications as described below. Protein A or protein A/G is immobilized via amine coupling to flow cells 1 and 2 of a B1 or CM5 sensor chip (BIAcore). The test antibody is then captured in flow cell 2 at desired levels (usually a 10 – 60 second injection of antibody) and 5 minutes is allowed for the antibody to stabilize. An aliquot of frozen A β

-43-

1-40 solution is thawed and then diluted to make up the highest concentration (e.g., 200 nM), which is then serially diluted (1:2 dilutions) to the lowest concentration (e.g., 6.25 nM). Each concentration is injected over the surface for 5 minutes at a flow rate of 50 $\mu\text{L}/\text{min}$. To obtain an accurate measurement of off-rate for high affinity antibodies, the dissociation of A β 1-40 is followed for 5 hours. A β 1-40 and antibody are then eluted from both flow cells with a 40 second injection of glycine (pH 1.5). The signal is allowed to stabilize for 2 minutes before the next cycle. The data from flow cell 1 is subtracted from flow cell 2 to account for any bulk shifts due to buffer differences or non-specific binding to the sensor chip or protein A. The various concentrations are injected randomly and each concentration is run in duplicate. Two 0 nM runs are used to control for any dissociation of antibody from the protein A or A/G capture surface. The data is analyzed using the Biaevaluation software. A 1:1 model with mass transfer and a local Rmax is used as the best fit for the data.

Using this alternate method, the affinity of humanized 266 antibody for soluble A β 1-40 was found to be $0.4 \text{ M}^{-9} (\pm 0.2 \text{ M}^{-9})$. Using this value of affinity, together with data for other antibodies (above), the log of flux vs. log affinity for soluble A β 1-40 was plotted (Figure 8). The very distinct relationship between affinity for soluble A β and flux continues to be evident.

Soluble A β 1-40 stocks and diluted samples are prepared as follows. Solid A β 1-40 (Biosource International, Camarillo California USA 93012) is dissolved to 1 mg/mL (about 230 μM) in water, and the solution is immediately aliquoted into 20 – 50 μL portions and then frozen (-70°C). Alkaline conditions can be used to dissolve A β , as described by Fezoui, *et al.*, *Amyloid: Int'l J. Exp. Clin. Invest.* 7:166-178 (2000). An alternate method for preparing aggregate-free soluble stock A β solutions is that of Zagorski, *et al.* [*Meth. Enzymol.* 309:189-204 (1999)]. This procedure involves, in sequence, dissolving the peptide in trifluoroacetic acid (TFA), evaporating the TFA, redissolving in hexafluoroisopropanol (HFIP), removing HFIP, and dissolving in water. Aliquoting and freezing may be performed either before or after removing HFIP. Stock A β solutions can be checked for aggregates by methods well known in the art, for example, light scattering [Tomski, *et al.*, *Arch. Biochem. Biophys.* 294:630-638 (1992)], thioflavin T binding [LaVine, *Meth. Enzymol.* 309:274-285 (1999)], or Congo red binding [Klunk *et al.*, *Anal. Biochem.* 266:66 (1999)]. Immediately before use, a stock aliquot is

-44-

thawed and diluted to the highest concentration to be used (typically, about a thousand-fold dilution to about 200 nM). This 1000-fold diluted sample is expected to contain soluble A β that is predominantly monomeric. The great tendency of A β to self-associate means that even when carefully prepared as described, samples of A β may contain small proportions of A β dimer especially, and perhaps even some higher order association states. Stock aliquots are not refrozen after they have been thawed. Stock aliquots are not used after the day on which they are thawed, nor are diluted samples used after the day on which they are prepared.

Example 8

Effect of administration of various humanized antibodies on plasma A β concentrations in male cynomolgus monkeys after 24 hours

The animals (2 to 4 kg, young adult to adult, 2 animals per group) were given a bolus intravenous administration of antibody (1 mg/kg; humanized 266 - expressed in a host cell transformed to express SEQ ID NO:11 and SEQ ID NO:12; N56S - expressed in a host cell transformed to express SEQ ID NO:11 and SEQ ID NO:21; or humanized 3D6 - US 60/287,539, filed 2001 April 30). Animals were housed individually in stainless steel cages. Environmental controls were set to maintain 18 to 29 degrees Centigrade, a relative humidity of 30% to 70%, and a 12-hour light/12-hour dark cycle. They received certified primate diet one or two times daily. Water was provided *ad libitum*. The animals were selected for study based on body weights, clinical observations, clinical pathology data, and other data as appropriate. Plasma samples (pre-dose and 24 hours post-dose) were collected in EDTA and frozen until analysis.

Plasma concentrations of immunoreactive A β ₁₋₄₀ or A β ₁₋₄₂ were determined using ELISA assays. Immunoreactive A β ₁₋₄₀ was captured on the ELISA plate using mouse monoclonal antibody 2G3 or A β ₁₋₄₂ was captured using mouse monoclonal antibody 21F12. The bound complex was detected using biotinylated-3D6 antibody, followed by addition of streptavidin-HRP. Color development was performed using TMB as a substrate. Optical density values were read at 450/630 nm, and raw data was analyzed with a 5-parameter logistic algorithm using STATLIA software (Brendan Scientific). Serum concentrations of immunoreactive A β ₁₋₄₀ were estimated using calibrators ranging from 16 to 1000 pg/ml prepared in heat-treated, charcoal stripped human serum. Based

-45-

on recovery of control samples and the back-fit of calibrators, the lower and upper limits of quantitation in this assay are estimated to be 50 and 1000 pg/mL, respectively. Results for A β 1-40 are shown below in Table 7. Flux could not be calculated for A β 1-42 because pre-dose concentrations were below the detection limit.

5

Table 7. Mean concentrations of immunoreactive antibodies and A β 1-40 in cynomolgus monkeys (two animals per group).

	humanized 266	N56S	humanized 3D6
[A β 1-40] _{Pre-dose} (ng/mL)	0.6	1.4	0.6
[A β 1-40] _{24 hour} (ng/mL)	109	124	12.2
Difference (ng/mL)	108	123	11.6
Flux (-fold increase)	181	87.6	19.3

The increase in plasma A β 1-40 24 hours after administration of humanized 266 and N56S was approximately the same, and much greater than the increase after administration of humanized 3D6. These data, obtained in normal monkeys (i.e., not having any known defect that affects A β metabolism or any known A β -related conditions or diseases), are consistent with the mouse studies described herein – that is, that the affinity of anti-A β antibodies for soluble, not aggregated, A β is positively correlated with flux of A β from the brain into the plasma within 24 hours after administering the antibody. N56S has a much faster turn-over than humanized 266 or 3D6 (5.5- and 8.6-times as much 266 and 3D6 in the plasma as N56S at 24 hours, though the doses were the same). Thus, the A β 1-40 levels at 24 hours for N56S are higher than those for humanized 266 when normalized to the concentration of antibody present, as would be expected on the basis of the present invention, because N56S has a higher affinity for soluble A β than humanized 266. Differences in flux caused by 266 and 3D6 cannot be attributed to differences in the pharmacokinetics for the two antibodies either in these monkeys or in the mice used in other experiments described herein.

-46-

We claim:

1. A method for treating cognitive symptoms of a condition or disease associated with A β in a subject, comprising administering to the subject an effective amount of an anti-A β antibody that has greater affinity for soluble A β than 10^{-9} M.
- 5 2. A method for reducing disease progression in a subject having a condition or disease associated with A β , comprising administering to the subject an effective amount of an anti-A β antibody that has greater affinity for soluble A β than 10^{-9} M.
- 10 3. A method for treating cognitive symptoms of a condition or disease associated with A β in a subject, comprising administering to the subject an effective amount of an anti-A β antibody that has affinity (KD) for soluble A β 1-40 or A β 1-42 higher than 10^{-9} M.
- 15 4. A method for reducing disease progression in a subject having a condition or disease associated with A β , comprising administering to the subject an effective amount of an anti-A β antibody that has affinity (KD) for soluble A β 1-40 or A β 1-42 higher than 10^{-9} M.
- 20 5. A method for treating cognitive symptoms of a condition or disease associated with A β in a subject, comprising administering to the subject an effective amount of an anti-A β antibody that has greater affinity for soluble A β than antibody 266 has.
- 25 6. A method for reducing disease progression in a subject having a condition or disease associated with A β , comprising administering to the subject an effective amount of an anti-A β antibody that has greater affinity for soluble A β than antibody 266 has.
7. The method any one of Claims 1-6, wherein the anti-A β antibody has greater affinity for soluble A β than 10^{-10} M.
8. The method of Claim 7, wherein the anti-A β antibody has greater affinity for soluble A β than 10^{-11} M.
9. The method of Claim 8, wherein the anti-A β antibody has greater affinity for soluble A β than 10^{-12} M.

-47-

10. The method of any one of Claims 1 – 9, wherein the affinity of the anti-A β antibody is measured with respect to soluble A β 1-40 or A β 1-42.
11. The method of any one of Claims 1 – 10, wherein the subject is human and the anti-A β antibody is a human or humanized antibody.
- 5 12. The method of any one of Claims 1 – 11, wherein the anti-A β antibody recognizes the same epitope that antibody 266 recognizes or competes with antibody 266 for binding to soluble A β .
13. The method of any one of Claims 1 – 12, wherein the condition or disease is Alzheimer's disease.
- 10 14. The method of any one of Claims 1 – 12, wherein the condition or disease is Down's syndrome.
15. The method of any one of Claims 1 – 12, wherein the condition or disease is cerebral amyloid angiopathy.
16. The method of any one of Claims 1 – 12, wherein the condition or disease
15 is vascular dementia.
17. The method of any one of Claims 1 – 12, wherein the condition or disease is mild cognitive impairment.
18. The use of an anti-A β antibody having affinity (KD) for soluble A β 1-40 or A β 1-42 higher than 10^{-9} M to prepare a medicament for treating cognitive symptoms of a
20 condition or disease associated with A β .
19. The use of an anti-A β antibody having affinity (KD) for soluble A β 1-40 or A β 1-42 higher than 10^{-9} M to prepare a medicament for reducing disease progression in a subject having a condition or disease associated with A β .
20. The use of an anti-A β antibody having greater affinity for soluble A β than
25 antibody 266 has to prepare a medicament for treating cognitive symptoms in a subject having a condition or disease associated with A β .
21. The use of an anti-A β antibody having greater affinity for soluble A β than antibody 266 has to prepare a medicament for reducing disease progression in a subject having a condition or disease associated with A β .

-48-

22. The use of an anti-A β antibody to prepare a medicament for use in a method of any one of Claims 1 –17.

FIG. 1.

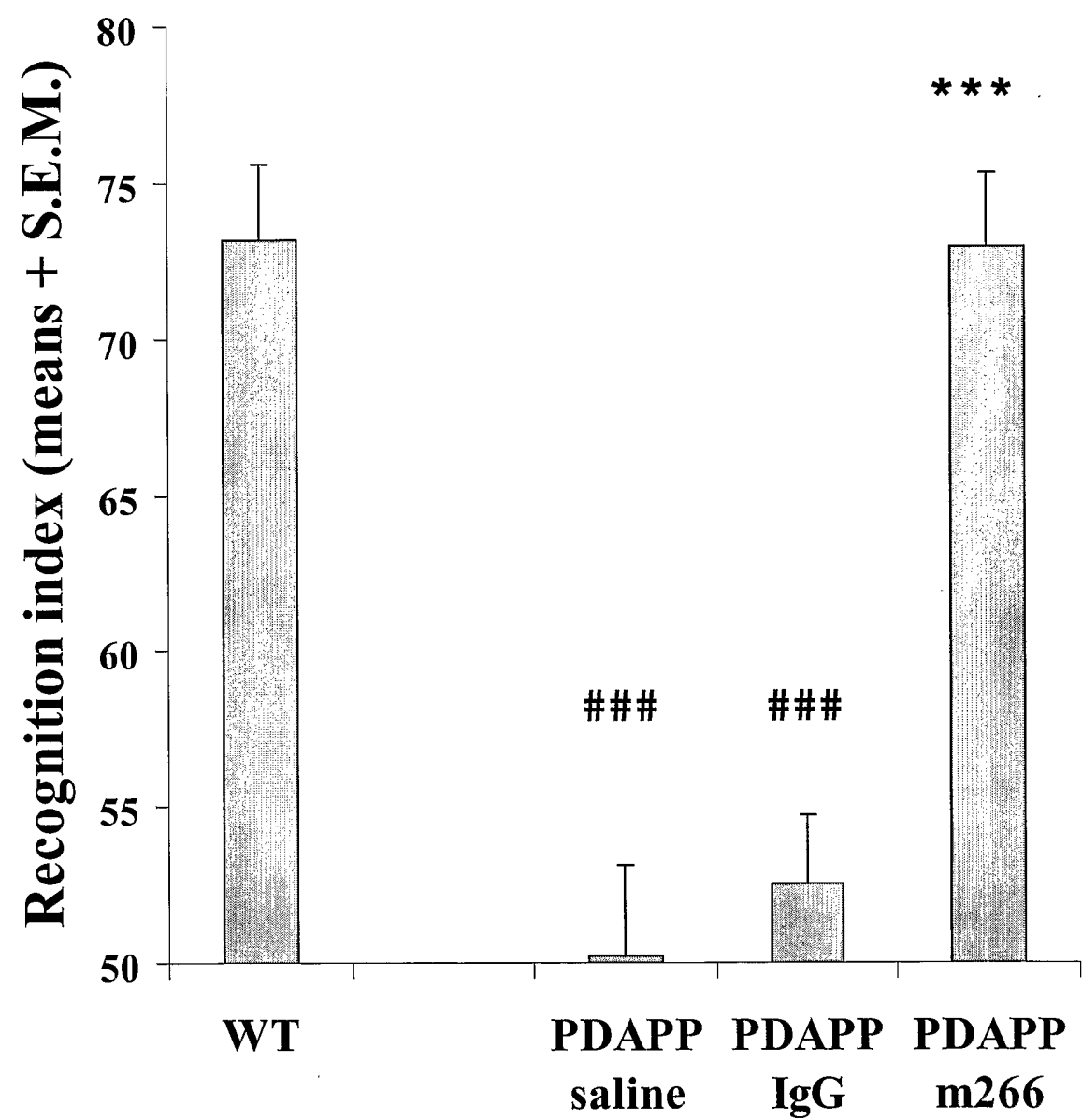
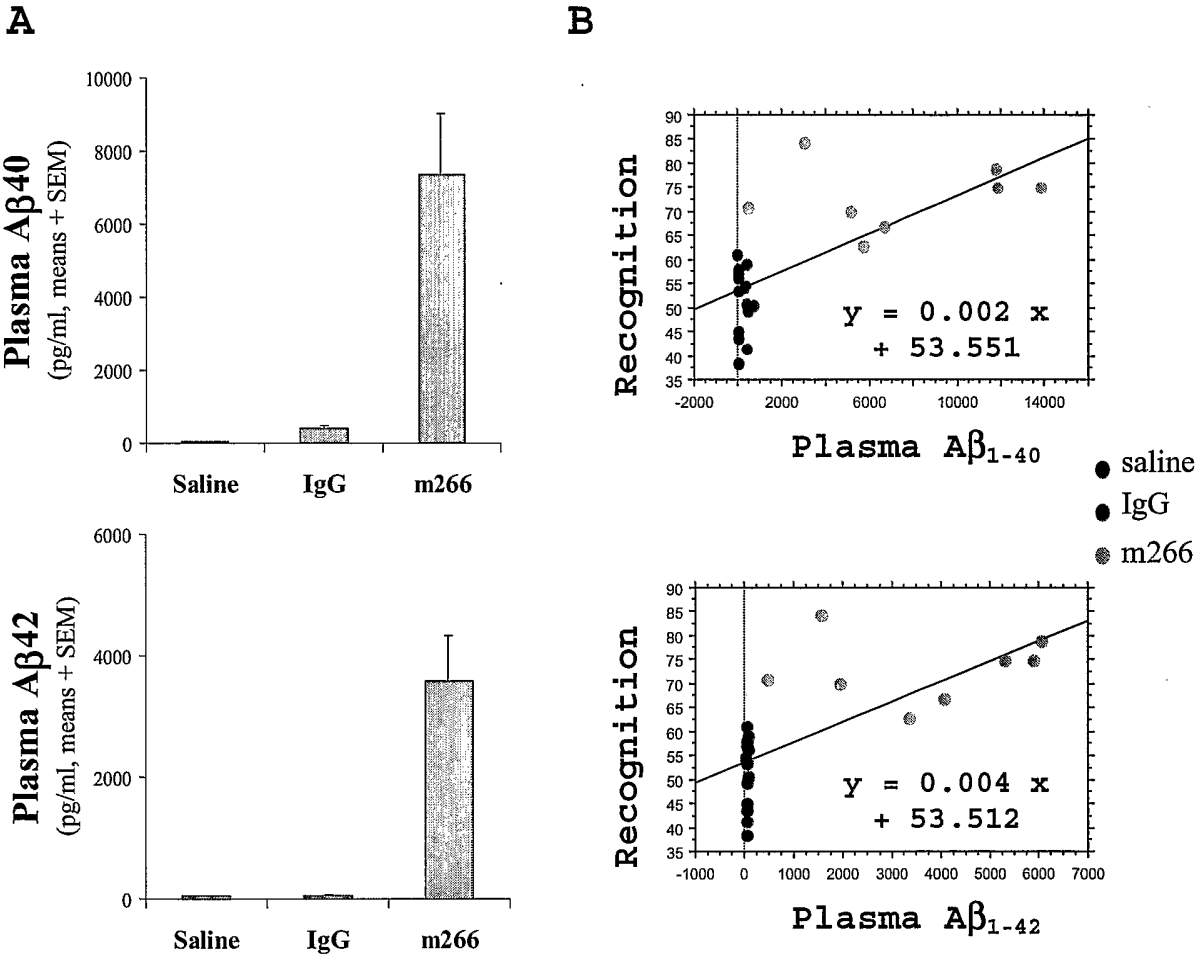
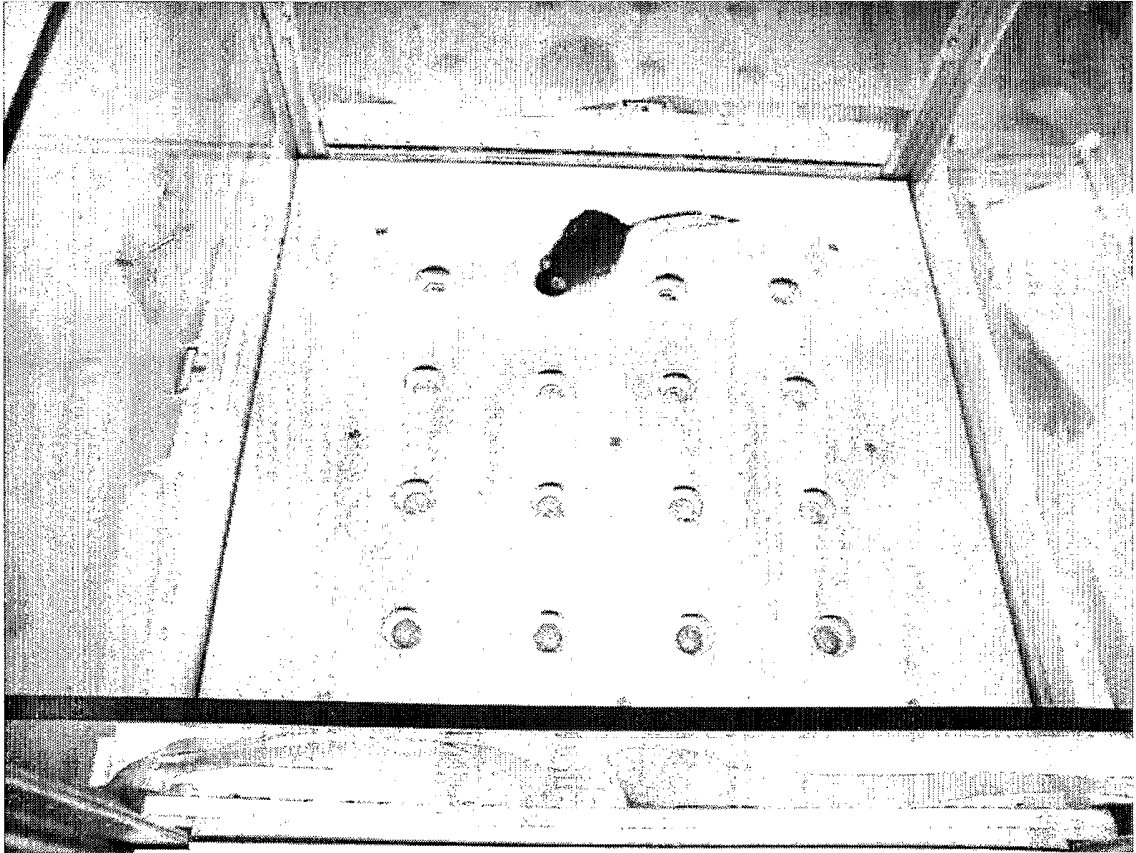


FIG. 2.



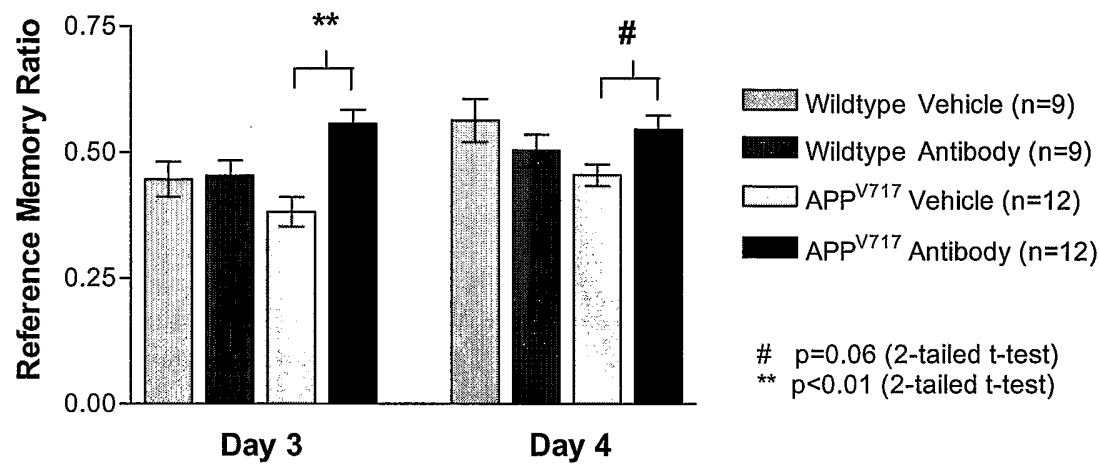
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FIG. 3.



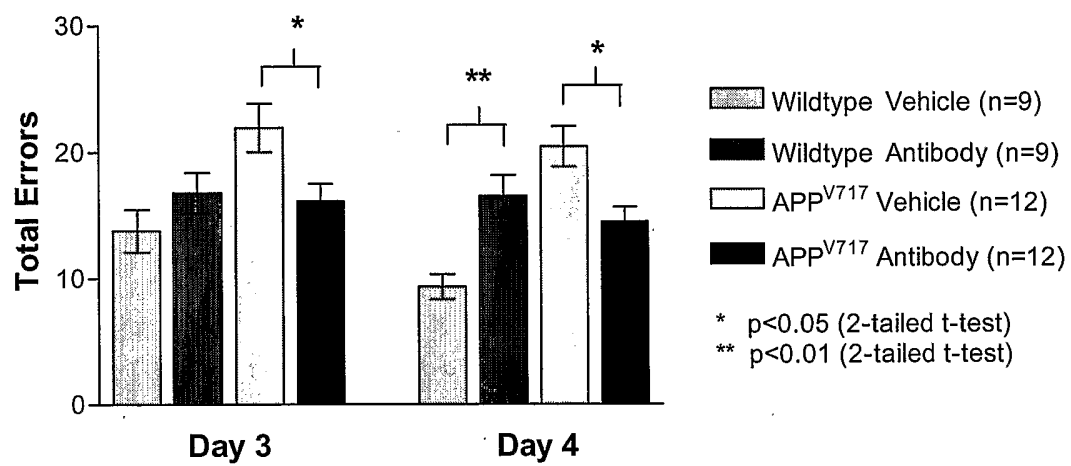
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FIG. 4.



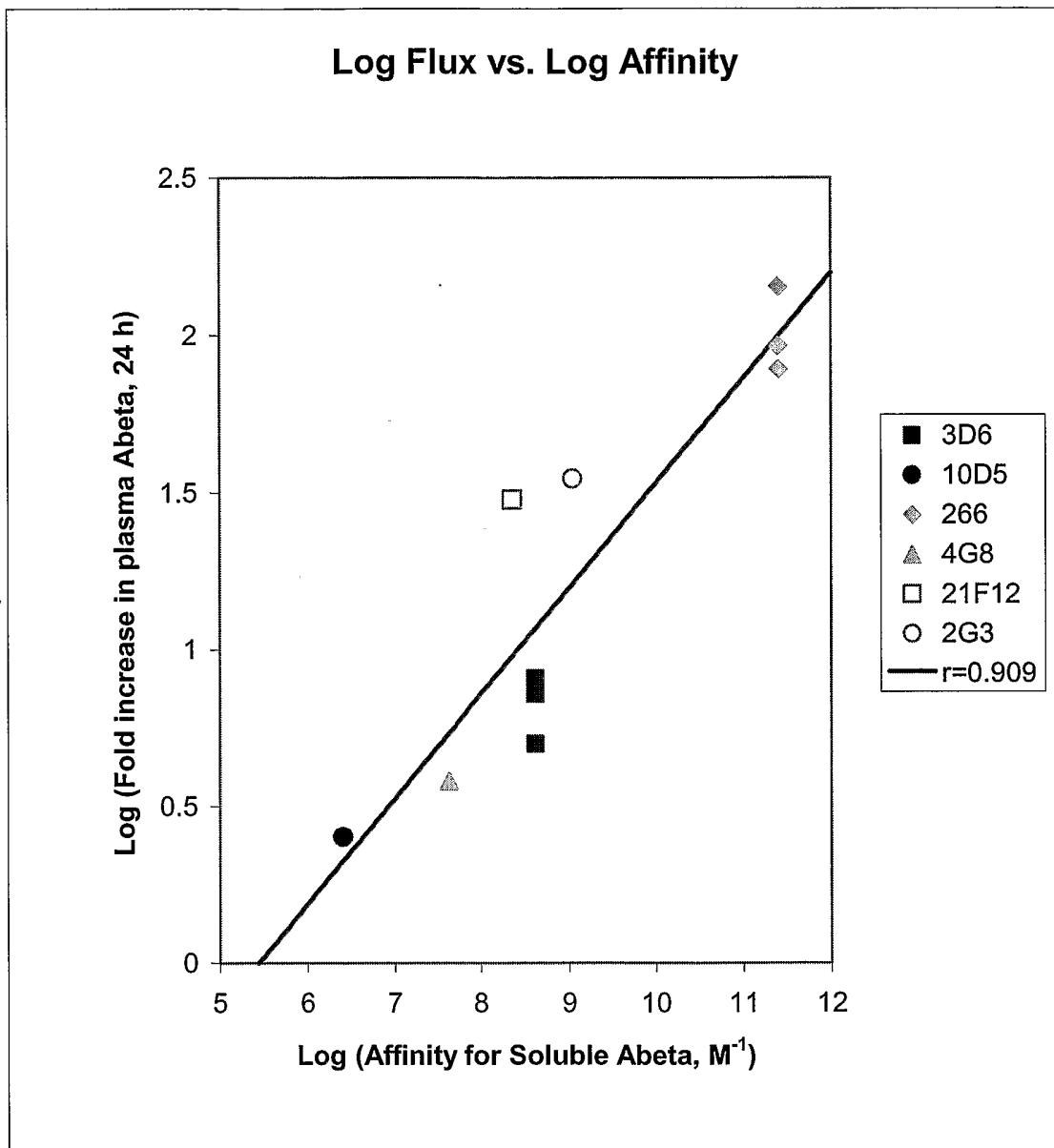
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FIG. 5.



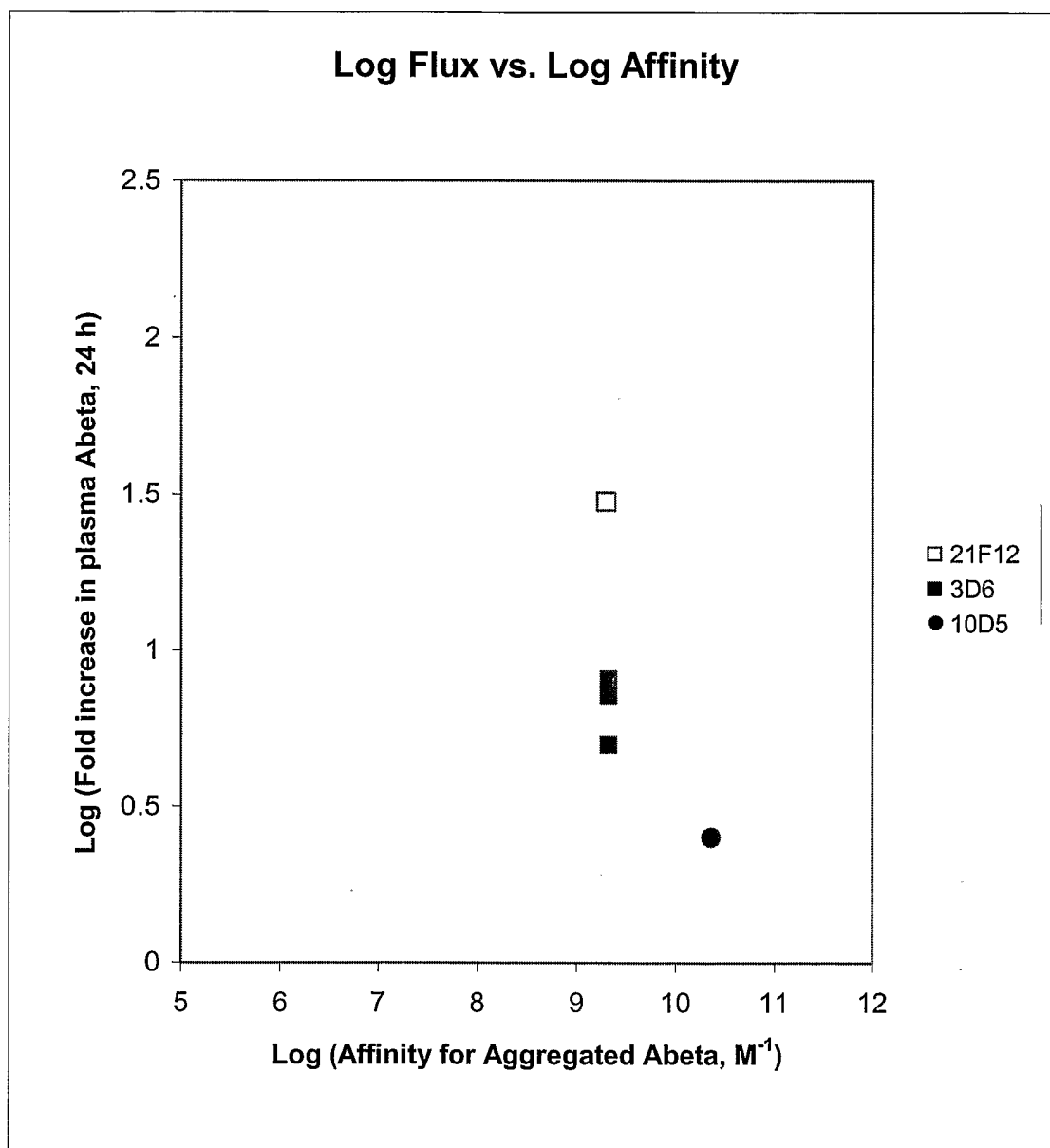
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FIG. 6.



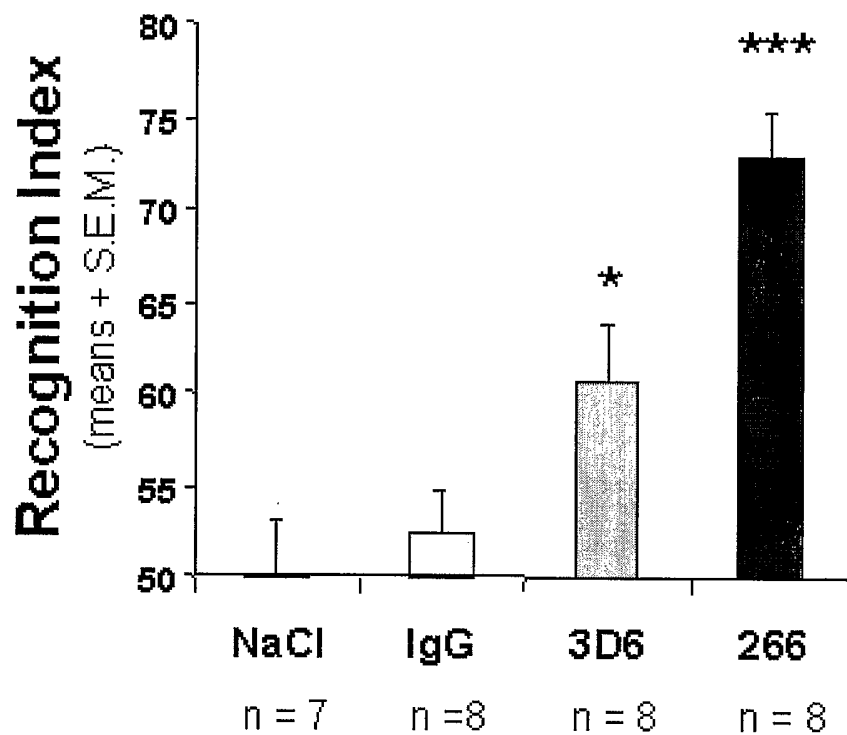
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FIG. 7.



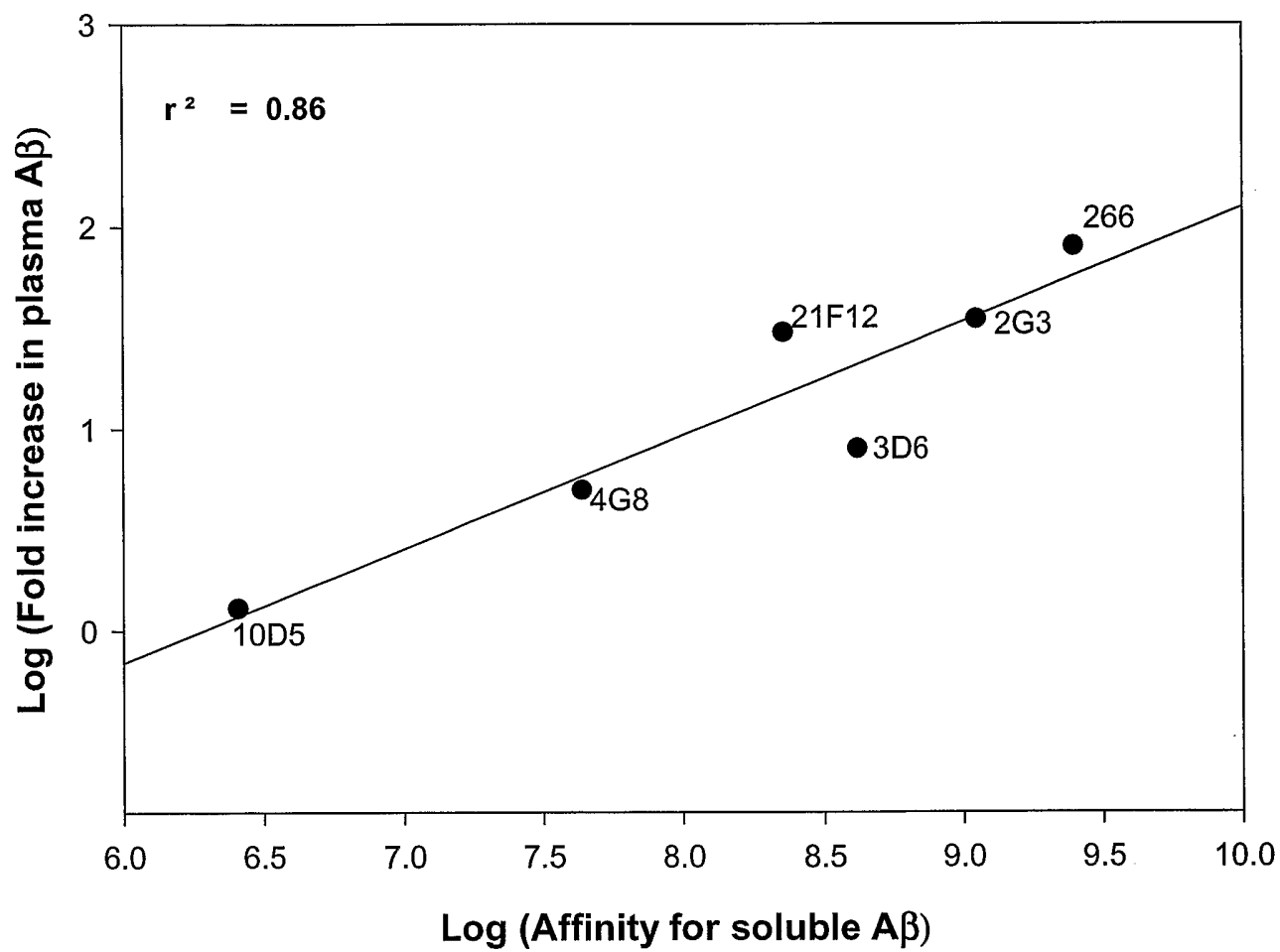
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FIG. 8.



9/9

FIG. 9.



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Gly

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Asp	Gly	Asn	Ala
	35	Tyr	Leu
His	Trp	Phe	Leu
	40	Gln	Lys
Pro	Gly	Gln	Ser
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Pro	Xaa	Leu	Leu
	50	Ile	Tyr
Lys	Val	Ser	Asn
	55	Arg	Phe
Ser	Ser	Gly	Val
	60		
Asp	Arg	Phe	Ser
	65	Gly	Ser
Gly	Ser	Gly	Thr
	70	Asp	Phe
Thr	Leu	Lys	Ile
	75		80
Ser	Arg	Val	Glu
	85	Ala	Glu
Asp	Xaa	Gly	Val
	90	Tyr	Tyr
Cys	Ser	Gln	Ser
	95		
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Xaa	Glu	Ile	Lys
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Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Xaa Leu Val
 35 40 45

Ala Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr Pro Asp Xaa Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Xaa Xaa Asn Thr Leu Tyr
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 20 25 30

Asp Gly Asn Ala Tyr Leu His Trp Phe Leu Gln Lys Pro Gly Gln Ser

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Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe 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 Ser Gln Ser	85	90	95	
Thr His Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys	100	105	110	

Arg

$\langle 210 \rangle$ 10

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$\langle 222 \rangle$ (1) . . (112)

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20 25 30

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

Ala Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr Pro Asp Thr Val
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Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

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 20 25 30

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

Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe 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 Ser Gln Ser
 85 90 95

Thr His Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
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Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
 115 120 125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe

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Ser	Gly	Asn	Ser	Gln 165	Glu	Ser	Val	Thr	Glu 170	Gln	Asp	Ser	Lys	Asp 175	Ser
Thr	Tyr	Ser	Leu 180	Ser	Ser	Thr	Leu	Thr 185	Leu	Ser	Lys	Ala	Asp 190	Tyr	Glu
Lys	His	Lys 195	Val	Tyr	Ala	Cys	Glu 200	Val	Thr	His	Gln	Gly 205	Leu	Ser	Ser
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 Ala Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr Pro Asp Thr Val
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 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
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Leu Gln Met Asn Ser₈₅ Leu Arg Ala Glu Asp₉₀ Thr Ala Val Tyr Tyr Cys
 Ala Ser Gly Asp₁₀₀ Tyr Trp Gly Gln Gly₁₀₅ Thr Leu Val Thr Val₁₁₀ Ser Ser
 Ala Ser Thr₁₁₅ Lys Gly Pro Ser Val₁₂₀ Phe Pro Leu Ala Pro₁₂₅ Ser Ser Lys
 Ser Thr₁₃₀ Ser Gly Gly Thr Ala₁₃₅ Ala Leu Gly Cys Leu Val₁₄₀ Lys Asp Tyr
 Phe Pro Glu Pro Val Thr₁₅₀ Val Ser Trp Asn Ser₁₅₅ Gly Ala Leu Thr Ser₁₆₀
 Gly Val His Thr Phe₁₆₅ Pro Ala Val Leu Gln₁₇₀ Ser Ser Gly Leu Tyr Ser₁₇₅
 Leu Ser Ser Val₁₈₀ Val Thr Val Pro Ser₁₈₅ Ser Ser Leu Gly Thr₁₉₀ Gln Thr
 Tyr Ile Cys₁₉₅ Asn Val Asn His Lys₂₀₀ Pro Ser Asn Thr Lys₂₀₅ Val Asp Lys
 Lys Val₂₁₀ Glu Pro Lys Ser Cys₂₁₅ Asp Lys Thr His Thr₂₂₀ Cys Pro Pro Cys
 Pro Ala Pro Glu Leu Leu₂₃₀ Gly Gly Pro Ser Val₂₃₅ Phe Leu Phe Pro Pro₂₄₀
 Lys Pro Lys Asp Thr₂₄₅ Leu Met Ile Ser Arg₂₅₀ Thr Pro Glu Val Thr₂₅₅ Cys
 Val Val Val Asp₂₆₀ Val Ser His Glu Asp₂₆₅ Pro Glu Val Lys Phe₂₇₀ Asn Trp
 Tyr Val Asp₂₇₅ Gly Val Glu Val His₂₈₀ Asn Ala Lys Thr Lys₂₈₅ Pro Arg Glu
 Glu Gln Tyr Asn Ser Thr Tyr₂₉₅ Arg Val Val Ser Val₃₀₀ Leu Thr Val Leu
 His Gln Asp Trp Leu Asn₃₁₀ Gly Lys Glu Tyr Lys₃₁₅ Cys Lys Val Ser Asn₃₂₀
 Lys Ala Leu Pro Ala₃₂₅ Pro Ile Glu Lys Thr₃₃₀ Ile Ser Lys Ala Lys₃₃₅ Gly

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Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
 355 360 365

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
 370 375 380

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
 385 390 395 400

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
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Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
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<222> (8)..(8)

<223> Xaa is at position 8 is any amino acid, provided that if Xaa at position 7 is Asn and Xaa at position 9 is Ser or Thr, then Xaa at position 8 is Asp or Pro

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<222> (9)..(9)

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<222> (1)..(17)

<223> heavy chain CDR2

<400> 13

Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val Lys
 1 5 10 15

Gly

<210> 14

<211> 17

<212> PRT

<213> Mus sp.

<220>

<221> MISC_FEATURE

<222> (7)..(7)

<223> Xaa at position 7 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr

<220>

<221> MISC_FEATURE

<222> (1)..(17)

<223> heavy chain CDR2

X-15047.ST25.txt

<220>

<221> MISC_FEATURE

<222> (8)..(8)

<223> Xaa at position 8 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr

<220>

<221> MISC_FEATURE

<222> (9)..(9)

<223> Xaa at position 9 is selected from the group consisting Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp and Tyr

<400> 14

Gln	Ile	Asn	Ser	Val	Gly	Xaa	Xaa	Xaa	Tyr	Tyr	Pro	Asp	Thr	Val	Lys
1				5					10					15	

Gly

<210> 15

<211> 17

<212> PRT

<213> Mus sp.

<220>

<221> MISC_FEATURE

<222> (7)..(7)

<223> Xaa at position 7 is Asn

<220>

<221> MISC_FEATURE

<222> (8)..(8)

<223> Xaa at position 8 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Val, Trp, and Tyr

X-15047.ST25.txt

<220>

<221> MISC_FEATURE

<222> (9)..(9)

<223> Xaa at position 9 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Val, Trp and Tyr

<400> 15

Gln	Ile	Asn	Ser	Val	Gly	Xaa	Xaa	Xaa	Tyr	Tyr	Pro	Asp	Thr	Val	Lys
1				5					10					15	

Gly

<210> 16

<211> 17

<212> PRT

<213> Mus sp.

<220>

<221> MISC_FEATURE

<222> (7)..(7)

<223> Xaa at position 7 is Asn

<220>

<221> MISC_FEATURE

<222> (8)..(8)

<223> Xaa at position 8 is selected from the group consisting of Asp and Pro

<220>

<221> MISC_FEATURE

<222> (9)..(9)

<223> Xaa at position 9 is selected from the group consisting of Ser and Thr

X-15047.ST25.txt

<400> 16

Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val Lys
1 5 10 15

Gly

<210> 17

<211> 17

<212> PRT

<213> Mus sp.

<220>

<221> MISC_FEATURE

<222> (7)..(7)

<223> Xaa at position 7 is selected from the group consisting of Ala, Gly, His, Gln, Ser, and Thr

<220>

<221> MISC_FEATURE

<222> (1)..(17)

<223> heavy chain CDR2

<220>

<221> MISC_FEATURE

<222> (8)..(8)

<223> Xaa at position 8 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser and Thr

<220>

<221> MISC_FEATURE

<222> (9)..(9)

<223> Xaa at position 9 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser, and Thr

X-15047.ST25.txt

<400> 17

Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val Lys
 1 5 10 15

Gly

<210> 18

<211> 17

<212> PRT

<213> Mus sp.

<220>

<221> MISC_FEATURE

<222> (7)..(7)

<223> Xaa at position 7 is Asn

<220>

<221> MISC_FEATURE

<222> (8)..(8)

<223> Xaa at position 8 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser, and Thr

<220>

<221> MISC_FEATURE

<222> (9)..(9)

<223> Xaa at position 9 is selected from the group consisting of Ala, Gly, His, Asn and Gln

<400> 18

Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val Lys
 1 5 10 15

Gly

X-15047.ST25.txt

<210> 19

<211> 112

<212> PRT

<213> humanized antibody

<220>

<221> MISC_FEATURE

<222> (1)..(1)

<223> Xaa at position 1 is Glu or Gln

<220>

<221> MISC_FEATURE

<222> (1)..(112)

<223> humanized antibody heavy chain variable

<220>

<221> MISC_FEATURE

<222> (7)..(7)

<223> Xaa at position 7 is Ser or Leu

<220>

<221> MISC_FEATURE

<222> (46)..(46)

<223> Xaa at position 46 is Glu, Val, Asp, or Ser

<220>

<221> MISC_FEATURE

<222> (56)..(56)

<223> Xaa at position 56 is any amino acid, provided that if Xaa at position 57 is neither Asp nor Pro and Xaa at position 59 is Ser or Thr, then Xaa at position 56 is not Asn

<220>

X-15047.ST25.txt

<221> MISC_FEATURE

<222> (57)..(57)

<223> Xaa at position 57 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 58 is Ser or Thr, then Xaa at position 57 is Asp or Pro

<220>

<221> MISC_FEATURE

<222> (58)..(58)

<223> Xaa at position 58 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 57 is neither Asp nor Pro, then Xaa at position 58 is neither Ser nor Thr

<220>

<221> MISC_FEATURE

<222> (63)..(63)

<223> Xaa at position 63 is Thr or Ser

<220>

<221> MISC_FEATURE

<222> (75)..(75)

<223> Xaa at position 75 is Ala, Ser, Val, or Thr

<220>

<221> MISC_FEATURE

<222> (76)..(76)

<223> Xaa at position 76 is Lys or Arg

<220>

<221> MISC_FEATURE

<222> (89)..(89)

<223> Xaa at position 89 is Glu or Asp

<220>

X-15047.ST25.txt

<221> MISC_FEATURE

<222> (107)..(107)

<223> Xaa at position 107 is Leu or Thr

<400> 19

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

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

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

Ala Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Xaa Val
 50 55 60

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

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

Ala Ser Gly Asp Tyr Trp Gly Gln Gly Thr Xaa Val Thr Val Ser Ser
 100 105 110

<210> 20

<211> 112

<212> PRT

<213> humanized antibody

<220>

<221> MISC_FEATURE

<222> (56)..(56)

<223> Xaa at position 56 is any amino acid, provided that if Xaa at position 57 is neither Asp nor Pro and Xaa at position 59 is Ser or Thr, then Xaa at position 56 is not Asn

<220>

<221> MISC_FEATURE

X-15047.ST25.txt

<222> (1)..(112)

<223> heavy chain variable

<220>

<221> MISC_FEATURE

<222> (57)..(57)

<223> xaa at position 57 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 58 is Ser or Thr, then Xaa at position 57 is Asp or Pro

<220>

<221> MISC_FEATURE

<222> (58)..(58)

<223> Xaa at position 58 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 57 is neither Asp nor Pro then Xaa at position 58 is neither Ser nor Thr

<400> 20

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

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

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

Ala Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala 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 Ser Gly Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
100 105 110

<210> 21

<211> 442

X-15047.ST25.txt

<212> PRT

<213> humanized antibody

<220>

<221> MISC_FEATURE

<222> (56)..(56)

<223> Xaa at position 56 is any amino acid provided that if Xaa at position 57 is neither Asp nor Pro and Xaa at position 59 is Ser or Thr, then Xaa at position 56 is not Asn

<220>

<221> MISC_FEATURE

<222> (1)..(442)

<223> heavy chain antibody

<220>

<221> MISC_FEATURE

<222> (57)..(57)

<223> Xaa at position 57 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 58 is Ser or Thr, then Xaa at position 57 is Asp or Pro

<220>

<221> MISC_FEATURE

<222> (58)..(58)

<223> Xaa at position 58 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 57 is neither Asp nor Pro, then Xaa at position 58 is neither Ser nor Thr

<400> 21

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

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

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

X-15047.ST25.txt

Ala Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala 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 Ser Gly Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 100 105 110
 Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
 115 120 125
 Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
 130 135 140
 Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
 145 150 155 160
 Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
 165 170 175
 Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
 180 185 190
 Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
 195 200 205
 Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
 210 215 220
 Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
 225 230 235 240
 Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
 245 250 255
 Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
 260 265 270
 Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
 275 280 285
 Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
 290 295 300

X-15047.ST25.txt

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
 305 310 315 320

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
 325 330 335

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
 340 345 350

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
 355 360 365

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
 370 375 380

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
 385 390 395 400

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
 405 410 415

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
 420 425 430

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 435 440