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(71) Applicant *(for all designated States except US)*: **BIOTHERAPIX MOLECULAR MEDICINES S.L.U.** [ES/ES]; Parque Tecnológico de Madrid, Marconi, 1, E-28760 Tres Cantos - Madrid (ES).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **TORÁN GARCÍA, José Luis** [ES/ES]; Parque Tecnológico de Madrid, Marconi, 1, E-28760 Tres Cantos - Madrid (ES). **LAÍN DE LERA, María Teresa** [ES/ES]; Parque Tecnológico de Madrid, Marconi, 1, E-28760 Tres Cantos - Madrid (ES).

(74) Agent: **ARIAS SANZ, Juan**; ABG PATENTES, S.L., Orense, 68, E-28020 Madrid (ES).

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(54) **Title:** HUMAN ANTIBODIES WITH BETA-AMYLOID PEPTIDE-BINDING CAPACITY AND THEIR APPLICATIONS

(57) **Abstract:** The present invention relates to isolated specific binding members, particularly antibodies or fragments, derivatives or variants therof that recognize and bind amyloid beta 1-42 protein. Methods of diagnosing, prognosing or treating diseases or disorders in which elevated levels of amyloid beta protein, including amyloid beta 1-42 are prevalent, in particular Alzheimer's disease, are disclosed. The present invention also relates to pharmaceutical compositions comprising such binding members, antibodies and mimics therof and methods of screening for novel binding members, which may or may not be antibodies.

HUMAN ANTIBODIES WITH BETA-AMYLOID PEPTIDE-BINDING CAPACITY AND THEIR APPLICATIONS

FIELD OF THE INVENTION

[0001] The present invention relates to human antibodies, fragments thereof or derivatives, with beta-amyloid 1-42 peptide-binding capacity, obtaining said antibodies and their use as therapeutic and/or diagnostic agents for Alzheimer's disease.

BACKGROUND OF THE INVENTION

[0002] Alzheimer's disease is the most frequent cause of dementia among the elderly. It is a neurodegenerative disease essentially characterized by the progressive accumulation of beta-amyloid peptide in the areas of the brain involved in cognitive functions, such as memory and language. Alzheimer's disease generally affects older people over the age of 65, although it may also occur in younger people. According to data published by the World Health Organization (WHO), Alzheimer's disease currently affects 5% of men and 6% of women over the age of 60 worldwide. Close to 400,000 people are currently affected in Spain, over 8 million people in Europe, and due to the accelerated aging of the population, the number of Alzheimer's patients is expected to double in the year 2020 and triple in the year 2050 (Selmes van den Bril, 2003).

[0003] A number of studies indicate that the APP (Amyloid Precursor Protein), the normal function of which is unknown, plays an essential role in the onset of the disease. The presence of amyloid plaques caused by the anomalous processing of the APP protein constitutes a central event in the pathogenesis of Alzheimer's disease. This protein is sequentially cleaved by means of the action of alpha, beta and gamma-secretases. In a pathological process, APP is anomalously processed, resulting in insoluble peptides, with 40-42 amino acids (beta-amyloid peptide or beta-peptides), which tend to be aggregated as fibrils and are deposited as plaques in neuronal cells. The accumulation of said plaques results in cell death of several types of neuronal cells with the subsequent cerebral deterioration. Nevertheless, beta-amyloid peptide production from APP protein processing is not the only factor influencing the existence of a beta-peptide deposit. There is evidence that factors involved in catabolism and elimination, as well as in the aggregation of the beta-amyloid peptide, are also critical (Dodel et al., 2002).

[0004] Despite having made great progress in recent years in terms of knowledge of the etiology, genetics and physiopathology of Alzheimer's disease, the therapies existing today are palliative therapies. The absence of drugs which act by preventing or significantly delaying the development of Alzheimer's disease means that new alternative therapies and new neuroprotective active ingredients that allow reducing the deposit of beta-peptides and the inflammatory response, contributing to reducing progression of the disease, are required.

[0005] The immune system may play an important role in the development of Alzheimer's disease. Active immunotherapy studies conducted in transgenic mice capable of reproducing human Alzheimer's disease (Janus et al., 2000; Morgan et al., 2000; Schenk et al., 1999) indicate that after immunization with beta-amyloid peptide, said mice produce specific antibodies capable of disintegrating and reducing the amyloid plaques caused by the beta-peptide accumulation, as well as preventing the development of new plaques and the onset of associated symptoms. The reduction of the beta-amyloid peptide deposit observed in said *in vivo* assays was also accompanied by cognitive changes in the animal.

[0006] The results obtained in the mouse model prompted in 2001 the start of a clinical trial on active immunotherapy by vaccination in humans with beta-amyloid 1-42 peptide. After concluding phase I of the clinical trial without having observed adverse reactions, clinical phase II had to be suspended at the end of 2002 because 6% of the individuals vaccinated with beta-amyloid peptide developed reactive encephalitis (Orgogozo et al., 2003; Munch and Robinson, 2002; Schenk, 2002). Post-mortem studies performed on those individuals who exhibited an inflammatory adverse response showed, in addition to said encephalitic changes, a reduction in the number of amyloid plaques as well as signs of T cell infiltration in the central nervous system. The cellular immune response induced by the administration of beta-amyloid peptide is considered to be one of the factors involved in the occurrence of the observed adverse effects (Morgan and Gitter, 2004).

[0007] Despite having to suspend the clinical trial, the subjects participating in phase II of the clinical trial continued to be monitored (Hock et al., 2002). Most of the vaccinated individuals had developed anti beta-amyloid peptide antibodies that recognized amyloid plaques. It was observed that there was an inversely proportional relationship between the serum anti beta-amyloid antibodies in vaccinated subjects and the loss of intellectual capacity in said individuals (Hock et al., 2003), suggesting the protective role of anti beta-amyloid antibodies compared to the progression of Alzheimer's disease. An absence of correlation between serum anti beta-amyloid antibody levels present and the onset of meningoencephalitis was also detected (Orgogozo et al., 2003).

[0008] An alternative therapy to active immunization with beta-amyloid peptide for the treatment and/or prevention of Alzheimer's disease is the passive administration of specific anti beta-amyloid antibodies. Passive immunotherapy allows customizing the antibody dose and replacing one antibody with another one aimed at a different epitope, as well as to select the isotype of the antibody to be administered. Another advantage of the administration of specific antibodies compared to active immunization is that its effectiveness does not depend on there being an immune response in the recipient, which is particularly interesting in the case of elderly individuals since patients of this age group usually have a variable immune response. On the other hand, passive immunotherapy allows suspending or reducing the antibody dose administered in the event of the occurrence of adverse reactions (Morgan and Gitter, 2004).

[0009] Several passive immunotherapy studies have been conducted in transgenic mice which reproduce human Alzheimer's disease (DeMattos et al., 2001, Bard et al., 2000, Frenkel et al. 2000). Said studies show how there is a reduction of the number of amyloid plaques after the passive administration of antibodies with beta amyloid peptide-binding capacity, as well as a reduction in progression of the disease. Said results suggest that the T cell mediated immune response is not required in eliminating beta-amyloid deposits, which generates an expectation on the therapeutic potential offered by developing specific anti beta-amyloid peptide antibodies. Therefore, the administration of monoclonal antibodies against the beta-amyloid peptide would produce positive effects similar to those obtained by means of active immunization. It would in turn prevent the occurrence of undesirable adverse effects, such as reactive encephalitis, in the absence of a T cell mediated immune response.

[0010] The positive effects exerted by anti beta-amyloid antibodies observed in the *in vivo* passive immunotherapy assays conducted in transgenic mice prompted different studies for the purpose of investigating the presence of auto-antibodies against the beta-amyloid peptide in non-immunized healthy humans and their possible protective role compared to Alzheimer's disease or other diseases in which there is a beta-amyloid peptide deposit. However said studies generated contradictory results.

[0011] On one hand, there are articles providing data which support a possible protective role of anti beta-amyloid antibodies. In one of them (Du et al., 2001), the presence of antibodies (IgG) specifically recognizing beta-amyloid peptide in non-immunized humans, both in serum and in cerebral spinal fluid was identified. The antibody titer in cerebral spinal fluid of patients with Alzheimer's was significantly lower than that detected in control individuals. In a later study (Weksler et al., 2002) it was also observed that the serum anti beta-amyloid IgG titer was reduced in elderly Alzheimer patients with respect to healthy elderly individuals (> 65 years of age) who did not exhibit a loss of intellectual capacity. Both studies provide evidence in favor of the potential protective role of the specific humoral immune response compared to the beta-amyloid peptide in Alzheimer's disease. Nevertheless, the reduced anti beta antibody levels observed in said studies could be the consequence of an increase in the beta-peptide concentration in the central nervous system. Therefore, binding of the beta-peptides to the specific antibodies would result in an apparent reduction of the anti beta-amyloid peptide antibody concentration (Weksler and Goodhardt, 2002).

[0012] On the other hand, studies have been published which contradict the hypothesis of the possible protective role of serum auto-anti beta-amyloid antibodies present in non-immunized elderly individuals. Hyman et al. (Hyman et al, 2001) found no significant differences upon comparing the total serum anti beta-amyloid immunoglobulin levels in Alzheimer's patients with the levels detected in control individuals. In another recent study (Baril et al., 2004) the levels of antibodies specific against serum beta-amyloid peptide in elderly Alzheimer's patients were compared with the control individuals of the same age range (cases: 66-86 years of age, control:

63-82 years of age). The individuals of both groups showed low anti beta-amyloid antibody titers with variable levels. In said study, an analysis of the serum concentration of anti beta-amyloid 1-42 peptide IgG in different age groups (children: 1-10 years of age, young subjects: 20-48 years of age and elderly subjects: case and control subjects of the study) was performed. The results of said analysis show that there are no statistically significant differences in the levels of immunoglobulin aimed at the beta-amyloid 1-42 peptide between the different age groups.

[0013] It is currently accepted that beta-peptide deposition in the central nervous system takes place progressively with age and in Alzheimer's disease. Beta-amyloid peptide deposition and plaque formation begin 10-20 years before the onset of any symptom indicative of a loss of cognitive capacity suggesting the presence of dementia (Monsonego et al., 2003; Weksler and Goodhardt, 2002). Starting from this premise, healthy elderly individuals, selected for not having any altered intellectual capacity, who have taken part as control individuals in the studies mentioned, would probably have beta-amyloid peptide deposits and would probably develop Alzheimer's disease in the future. Therefore, the fact that some elderly individuals do not exhibit symptoms of dementia does not exclude those elderly individuals from developing the disease in the future. In a cohort study performed in healthy volunteers (Kawas et al., 2000), the evolution of the participating patients was monitored biennally for the purpose of determining the incidence rate of Alzheimer's disease for different age ranges. In that study, it was observed that the incidence rate increases with age from 0.08% per year for the age range between 60 and 65 years of age, to 6.48% per year in the group of individuals over the age of 85, considering the data for men and women combined, estimating that the incidence rate is doubled in about 4.4 years.

[0014] All publications, patent applications, patents and other reference material mentioned herein are incorporated by reference in their entirety. In addition, the materials, methods and examples are only illustrative and are not intended to be limiting. The citation of references herein shall not be construed as an admission that such is prior art to the present invention.

SUMMARY OF THE INVENTION

[0015] The present invention relates to specific binding members that recognize amyloid beta 1-42, more particularly, to antibodies, to functional fragments thereof or derivatives with beta-amyloid 1-42 peptide-binding capacity and to obtaining them by means of genetic engineering techniques from the genetic repertoire of immunoglobulins isolated from healthy elderly, and particularly, nonagenarian human donors. It particularly relates to antibodies, to functional fragments thereof or derivatives with beta-amyloid 1-42 peptide-binding capacity, to the nucleic acid sequences encoding for said antibodies, as well as to the uses and pharmaceutical compositions comprising these binding members and antibodies and fragments, derivatives or variants thereof.

[0016] The present Inventors proceeded to search for and identify healthy elderly individuals between the ages of 60 to 100, and particularly over the age of 90 who show full cognitive capacity and good health in the absence of symptoms of Alzheimer's disease or another neuropathy and who had never received any type of medication for the prevention or treatment of neurodegenerative diseases, i.e. a restricted group of individuals who had surpassed the ages considered as critical for the onset of Alzheimer's disease were chosen as candidates for obtaining anti beta-amyloid 1-42 peptide antibodies. Therefore, a group of healthy nonagenarian elderly subjects was selected in whom, in the absence of immunization, the presence of serum anti beta-amyloid 1-42 peptide antibodies was analyzed by means of performing specific immunoassays, detecting the existence of antibodies against the beta-amyloid 1-42 peptide.

[0017] Surprisingly, two of the antibodies of the invention have numerous somatic mutations in their nucleotide sequence in comparison to the corresponding genomic genes (germline), indicating the existence of antigenic maturation processes against the beta-amyloid 1-42 peptide. These facts indicate a competent immune response against the beta peptide and reflect the existence of natural anti beta amyloid antibodies, which do not exhibit harmful effects in healthy elderly patients, with a potential therapeutic application in Alzheimer's disease

[0018] Thus, the present invention relates to human anti beta-amyloid 1-42 peptide recombinant monoclonal antibodies and to obtaining them by means of the isolation, cloning and expression of immunoglobulin genes isolated from a representative portion of the genetic repertoire of immunoglobulins from healthy elderly, and particularly, nonagenarian human donors selectively chosen for having full cognitive capacity and for not exhibiting symptoms of Alzheimer's or another neuropathy.

[0019] Antibodies per se are widely known in the state of the art. Antibodies structurally consist of two different chains called heavy chain (HC) and light chain (LC) proteins which are bound by means of disulfide bridges. Both have a variable region (V) and a constant region (C). Variable regions are involved in antigen recognition, whereas the HC constant region determines the isotype of the antibody and the functional properties thereof. When comparing the sequence similarity of the different domains of an antibody molecule, it has been observed that most domains belonging to antibodies of the same subclass have the same sequence. Said domains are called constant (C) domains. CL is the light chain constant domain and the heavy chain constant domains are CH_1, CH_2 and CH_3. However, one domain of each one of the chains is variable (VH and VL). Kabat et al. (Kabat et al., 1978) observed that variability is concentrated in 3 regions of the two variable domains. Said hypervariable domains or CDRs (Complementary-Determining Regions) interact in a three-dimensional manner to give rise to the antigen binding site, which antigen contains six CDR regions, three VH domain regions and three VL domain regions. Different authors have observed that heavy chain CDR3 (HCDR3) is the region having a greater degree of variability and a predominant participation in antigen-antibody interaction.

[0020] The genes encoding HC and LC chains are located in different chromosomes, forming multigenic families in each case. The genes encoding the HC and LC variable and constant regions are separated in the genome; therefore somatic recombination thereof is first required for their expression (Tonegawa, 1983). The heavy chain variable region is encoded by three gene segments: Variability (V), Diversity (D) and Joining (J), whereas the light chain variable region consists of two gene segments: V and J. Each one of these segments consists of a variable number of genes that are grouped into families according to their degree of homology (Williams and Winter, 1993; Zachau, 1993). The segments of the heavy and light chain constant region are close to the J segments and are incorporated to the V(D)J complex by means of RNA splicing (Early et al. 1980). The existence of multiple copies of each one of the gene segments forming the HC and LC, as well as the different combination possibilities among them, contribute to generating the diversity of the antibody repertoire.

[0021] The somatic recombination of the V(D)J segments occurs during the B cell maturation process. Both the addition of nucleotides N by the terminal deoxynucleotidyl transferase enzyme (TdT) and deletions caused by exonucleases or the insertion of palindromic elements generated during the resolution of the recombinant products, contribute to the variability of the complementary-determining region (HCDR3). Said region is directly involved in antigenic recognition and contributes to the diversity of the immunoglobulin repertoire. The antibody repertoire is subsequently diversified during the antigenic response of the mature lymphocyte by means of somatic mutation. This process involves introducing isolated mutations in the genes of both the HC and LC chain variable regions generating intraclonal variables. The most frequent mutations are transitions, which entail substitutions in the amino acid sequence, resulting in the onset of new variants of the original immunoglobulin. An antigen-directed selective process then begins, which gradually enriches B cell populations with higher affinity immunoglobulins (and perhaps better kinetics as well). This phenomenon is what is known as affinity maturation. In selected clones, analyses of the somatic mutations of immunoglobulin variable chains show that the changes generally tend to be concentrated in the complementary-determinant regions (Foote and Milstein, 1991).

[0022] Within the context of the present invention, the term genetic repertoire of immunoglobulins is understood to be the group of gene segments of immunoglobulins which, once rearranged, encode for antibody synthesis.

[0023] Accordingly, a first aspect of the invention relates to a process of obtaining binding members, in particular, antibodies, fragments thereof or derivatives with beta-amyloid 1-42 peptide-binding capacity, by means of genetic engineering techniques from the genetic repertoire of immunoglobulins isolated from healthy elderly human donors. In the context of the present invention, the term elderly includes donors of 60 years of age or older, for example 70 years of age or older, 80 years of age or older or 90 years of age or older. Preferably the donor

subjects of the invention are nonagenarian donors of 90 years of age or older, for example between about 90 to about 100 years of age.

[0024] Murine antibodies are unsuitable as clinical candidate therapeutics since mouse antibodies are not immunogenic in humans and usually trigger an immune response generating human anti-mouse antibody (HAMA). Said immunoglobulins recognize therapeutic antibodies and will prevent them from reaching their target, and they furthermore trigger a type III hypersensitivity reaction (George and Urch, 2000). The antibodies of the present invention are recombinant antibodies of human origin, minimizing the possibilities that the immune system of the individual recipient rejects them.

[0025] A preferred aspect of the invention relates to the cloning and expression of the genetic repertoire of immunoglobulins isolated from healthy elderly, and particularly, nonagenarian human donors selectively chosen for having full cognitive capacity and for not exhibiting symptoms of Alzheimer's or another neuropathy, and in whose serum the presence of anti beta-amyloid 1-42 peptide antibodies was detected.

[0026] There are several known strategies for producing monoclonal antibodies. Hybridoma technology (Kennet, McKearn and Bechtol, 1980) meant a revolution for immunology investigation, allowing antibody production resulting from the synthesis activity of a single B cell clone. Recent advances in genetic engineering have greatly facilitated the production, identification and conjugation of recombinant antibody fragments. An even more preferred aspect of the invention relates to the construction of a library containing the genetic repertoire of immunoglobulins isolated from healthy nonagenarian human donors selectively chosen for having full cognitive capacity and for not exhibiting symptoms of Alzheimer's or another neuropathy, in whose serum the presence of anti beta-amyloid 1-42 peptide antibodies was detected. Said antibody gene library must preferably be widely representative of the genetic repertoire of immunoglobulins and can be obtained, for example, by means of PCR amplification of B cell cDNA. Preferably, the selection method of this library must have an efficacy that is comparable to that of the immune system, which can be achieved by displaying the produced antibodies on the surface of microorganisms (Little et al., 1994). Some examples of microorganisms which can be used are: filamentous phages such as M13 (McCafferty et al., 1990), bacteria such as E. coli (Fuchs et al., 1991) or yeasts (Boder and Wittrup, 1997). In the surface displaying strategy there is a physical link between the antigen binding capacity and the gene encoding for said antibody. The antibody displayed on the cell surface or on the viral capsid surface is encoded in the genetic material of the selected clone, such that affinity for the antigen allows the isolatation of the microorganism carrying the gene of the antibody of interest. Within the context of the present invention, any antibody with beta-amyloid 1-42 peptide-binding capacity is understood to be an antibody of interest. Another known strategy for displaying the antigen of the antibodies of the library is Ribosome Display (Hanes J and Pluckthun A., 1997), in which DNA is transcribed into mRNA, which is purified and translated in vitro. The in vitro

translation of mRNA is designed such that the dissociation of the mRNA-ribosome-peptide complex is prevented. Therefore, said complexes will be used for selecting, by antigen affinity, those clones encoding the antibody of interest.

[0027] The antigenic selection process consists of identifying those clones having affinity for the antigen and subsequently enriching the library in said clones by means of amplification in E. coli or another suitable microorganism. Said selection (bio-panning) can be done by means of different strategies that include but are not limited to: selection by means of the use of antigens immobilized by methods such as ELISA, immunotubes, affinity columns or BIAcore (surface plasmon reasonance) sensor chips; selection by means of the use of antigens in solution, such as for example: biotinylated antigens, and selection by markers expressed on the cell surface, both in monolayers of adhesive cells and in cells in suspension. It is generally necessary to perform several selection cycles in the antigen selection process. After the antigen selection from the constructed libraries, it is normal to obtain the clones of antibodies specific against the selecting molecules. Evaluation of their antigenic specificity can, for example, be carried out by means of enzyme immunoassay.

[0028] A second aspect of the present invention relates to an antibody, a functional fragment thereof or derivative with beta-amyloid 1-42 peptide-binding capacity.

[0029] A particular aspect of the invention relates to an antibody, functional fragment thereof or derivative with beta-amyloid 1-42 peptide-binding capacity obtained by means of genetic engineering techniques from the genetic repertoire of immunoglobulins isolated from healthy elderly, and particularly, nonagenarian human donors.

[0030] Within the context of the present invention, those antibody fragments that can be obtained by means of recombinant DNA technology and preserve antigen binding activity are understood to be functional antibody fragments. Included as functional antibody fragments are fragments of an IgG antibody molecule, such as Fab, Fab' and $F(ab')_2$. Fv fragments and derivatives thereof such as scFv (single-chain Fv) and dsFv (disulfide-stabilized Fv), which have been modified to increase stability of the recombinant Fv fragment are also included. The binding of two molecules other than scFv will give rise to bispecific scFvs. Other functional fragments that are very similar to bispecific scFvs are those called diabodies. Diabodies are antibody fragments consisting of two associated chains, in which each one of the chains comprises a light or heavy chain variable domain or bound to a linker. Changes in the length of said linker direct the formation of diabodies, triabodies or tetrabodies. Likewise, other functional fragments based on scFv fragments, such as minibodies, which are bivalent antibody fragments formed by the binding of two molecules of the fusion peptide scFv-CH_3, have also been described. Fd-like fragments (Chamow and Ashkenazy, 1999), domain antibodies (Holt et al., 2003) and other antibody fragments obtained by genetic engineering which by themselves are able to interact with the other antigen, are also understood as functional antibody fragments.

[0031] Within the context of the present invention, any molecule comprising an antibody or a functional fragment thereof in its structure is understood as a derivative, for example: immunoconjugates, understanding as such molecules formed by an antibody or a functional antibody fragment and an effector molecule.

[0032] A particular embodiment of the present invention relates to an antibody, a functional fragment thereof or derivative with beta-amyloid 1-42 peptide-binding capacity, comprising one or more of the sequences selected from the group consisting of: the amino acid sequence defined in SEQ ID NO: 2, which corresponds to the heavy chain of the antibody AD38 (Figure 8), the amino acid sequence defined in SEQ ID NO: 4, which corresponds to the light chain of AD38 (Figure 9), or sequences that are homologous to these.

[0033] Antibody AD38 of the present invention is defined in nucleotide sequence SEQ ID NO: 9, whose corresponding amino acid sequence is defined in SEQ ID NO: 10, and said antibody is referred to hereinafter as AD38.

[0034] Within the context of the present invention, those amino acid sequences showing a degree of similarity of at least 80% with the amino acid sequences of the antibodies of the present invention, preferably those having a degree of similarity of at least 85%, more preferably those having a degree of similarity of at least 90% with the amino acid sequences of the antibodies of the present invention, and even more preferably those amino acid sequences having a degree of similarity of at least 95%, are understood as homologous sequences. Sequence analysis algorithms are known in the state of the art, such as BLAST for example, disclosed in Altschul et al., 1990.

[0035] In a further particular embodiment, the antibody, functional fragment thereof or derivative with beta-amyloid 1-42 peptide-binding capacity, comprises at least one heavy chain and one light chain, in which the heavy chain comprises the amino acid sequence defined in SEQ ID NO: 2 and the light chain comprises the amino acid sequence defined in SEQ ID NO: 4.

[0036] In a yet further particular embodiment, reference is made to a functional antibody fragment with beta-amyloid 1-42 peptide-binding capacity comprising at least one heavy chain and one light chain, in which the heavy chain consists of the amino acid sequence defined in SEQ ID NO: 2 and the light chain consists of the amino acid sequence defined in SEQ ID NO: 4, such as the single chain (scFv) recombinant antibody AD38 (SEQ ID NO: 10) for example.

[0037] Another particular embodiment of the present invention relates to an antibody, functional fragment thereof or derivative with beta-amyloid 1-42 peptide-binding capacity comprising one or more of the sequences selected from the group consisting of: the amino acid sequence defined in SEQ ID NO: 6 which corresponds to the heavy chain of antibody AD64 (Figure 10); the amino acid sequence defined in SEQ ID NO: 8 which corresponds to the light chain of AD64 (Figure 11) or sequences that are homologous to these.

[0038] Antibody AD64 of the present invention is defined in nucleotide sequence SEQ ID NO: 11, the corresponding amino acid sequence of which is defined in SEQ ID NO: 12, and said antibody will be referred to hereinafter as AD64.

[0039] In a particular embodiment, the antibody, functional fragment thereof or derivative with beta-amyloid 1-42 peptide-binding capacity, comprises at least one heavy chain and one light chain, in which the heavy chain comprises sequence SEQ ID NO: 6, and the light chain comprises the sequence SEQ ID NO: 8.

[0040] In another particular embodiment, reference is made to a functional antibody fragment with beta-amyloid 1-42 peptide-binding capacity comprising at least one heavy chain and one light chain, in which the heavy chain consists of the amino acid sequence defined in SEQ ID NO: 6, and the light chain consists of the amino acid sequence defined in SEQ ID NO: 8, such as the single chain (scFv) recombinant antibody AD64 (SEQ ID NO: 12), for example.

[0041] Another particular embodiment relates to an antibody, functional fragment thereof or derivative with beta-amyloid 1-42 peptide-binding capacity, obtained by means of affinity maturation strategies from the antibodies of the present invention. Said affinity maturation strategies include but are not limited to: chain shuffling (Marks et al., 1992), introduction of directed mutations (Balint and Larrick, 1993) or random mutation strategies.

[0042] Within the context of the present invention, a recombinant polypeptide in which the sequences of the light and heavy chain variable domains (VL and VH) of an antibody have been linked by means of an artificial sequence are understood as scFv fragment. Therefore, for example the C-terminal end of the VH sequence is linked with the N-terminal end of the VL sequence by means of a binding peptide. The arrangement of the variable domains will affect the characteristics of the scFv. The scFv fragments have a series of advantages with respect to intact antibodies and to other functional antibody fragments: scFv fragments lack the Fc region, and therefore unwanted binding of the antibody to cells having Fc binding, such as cells of the reticular endothelial system, as well as complement fixation, is prevented. The absence of the Fc region implies an advantage in certain applications, such as directed immunotherapy and molecular imaging.

[0043] Due to their low molecular weight, scFv fragments have a fast biodistribution, accessing tissues more easily than intact antibodies and other functional fragments.Plasma elimination of scFv fragments is faster than that observed in Fab fragments and in intact antibodies. Due to the higher scFv elimination rate, the immunocomplexes formed by scFv conjugated with active ingredients or radionucleotides show lower toxicity. Due to their smaller size, the retention of scFv fragment antigen-antibody complexes in the renal glomerulus is less than that observed in Fab fragments and in intact antibodies.scFv fragments are highly versatile functional fragments since they are easily modified by means of genetic engineering. It is possible to generate other functional fragments from one scFv fragment.

[0044] Another particular embodiment relates to an antibody, antibody fragment or derivative comprising part of any of the amino acid sequences defined in sequences SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 or combinations of all or part of the sequence thereof, which maintains beta-amyloid 1-42 peptide-binding capacity.

[0045] In another particular embodiment, the heavy chain variable domain CDR1, CDR2 or CDR3 of antibody AD38 defined in Figure 8, the light chain variable domain CDR1, CDR2 or CDR3 of antibody AD38 defined in Figure 9, the heavy chain variable domain CDR1, CDR2 or CDR3 of antibody AD64 defined in Figure 10, and the light chain variable domain CDR1, CDR2 or CDR3 of antibody AD64 defined in Figure 11, are understood as part of any of the amino acid sequences defined in sequences SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8.

[0046] A third aspect of the invention relates to a nucleic acid molecule encoding the amino acid sequence of the light chain or heavy chain variable domain of an antibody with beta-amyloid 1-42 peptide-binding capacity of the present invention, and to variants of said nucleic acid molecule.

[0047] Within the context of the present invention, substantially similar sequences are understood as variants of a nucleic acid molecule. Substantially similar sequences include nucleotide sequences which, due to the degeneration of the genetic code, encode the amino acid sequence of the light chain or heavy chain variable domain of an antibody of the present invention. The amino acids are encoded by triplets consisting of three nucleotides, also called codons. Most amino acids are encoded by more than one triplet. This is called genetic code degeneration. Therefore, one or more triplets may be replaced by other triplets, but said nucleotide sequence will continue encoding for the same amino acid.

[0048] Substantially similar sequences also include nucleotide sequences encoding variants of the antibodies or antibody fragments of the invention. These nucleotide sequences homologous to a particular nucleotide sequence of the present invention will have at least a 40%, 50%, 60%, 70% identity with said sequence, preferably at least a 75%, 80%, 85%, 90% identity with said sequence, and even more preferably, it will have a sequence similarity of 95%, 96%, 97%, 98%, 99% or greater.

[0049] A further aspect of the invention relates to a nucleic acid molecule comprising any of the nucleotide sequences shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7, active fragments thereof, or any combination thereof.

[0050] A fourth aspect of the invention relates to a vector comprising a nucleic acid molecule according to the present invention. Said vector includes but is not limited to: viral plasmids, cosmids and vectors. Said vectors preferably contain one or more regulatory regions, such as promoters, termination and secretion signal sequences allowing the nucleic acid molecules of the present invention to be expressed as a protein. Even more particularly, said vector is a viral

vector that can be used to infect cells *in vitro* or *in vivo*. Said vector can be used in gene therapy.

[0051] A fifth aspect of the invention relates to a host cell containing at least one vector comprising a nucleic acid molecule of the present invention. Said host cells are cells that are known by any person skilled in the art, and they include but are not limited to: mammalian cells, yeast cells and prokaryotic cells such as *Escherichia coli*. Other unicellular host cells may be selected from the group consisting of Pseudomonas, Bacillus, Streptomyces, CHO, YB/20, NSO, SP2/0, R1.1, B-W, L-M, COS 1, COS 7, BSC1, BSC40, and BMT10 cells, plant cells, insect cells, and human cells in tissue culture.

[0052] In one embodiment, the unicellular host is transformed with:

(A) the DNA sequence of SEQ ID NO:1;

(B) the DNA sequence of SEQ ID NO:3;

(C) the DNA sequence of SEQ ID NO:1 and the DNA sequence of SEQ ID NO:3;

(D) a DNA sequence that hybridizes to any of the foregoing DNA sequences under standard hybridization conditions;

(E) a DNA sequence that codes on expression for an amino acid sequence encoded by any of the foregoing DNA sequences; or

(F) a fragment of any one of the foregoing DNA sequences which encodes a specific binding member which

(i) recognizes an epitope on amyloid beta 1-42, wherein said binding member is obtained from healthy elderly subjects having full cognitive capacity and who exhibit no symptoms of Alzheimer's disease or other neuropathies, for example where the subjects are 60 years of age or older, for example 70 years of age or older, 80 years of age or older or 90 years of age or older;

(ii) is an antibody having amyloid beta 1-42 binding capacity, and wherein the antibody comprises an amino acid sequence of SEQ ID NOs: 2, or 4, or an amino acid sequence that is substantially homologous to SEQ ID NOs: 2 or 4, and functional fragments, derivatives or variants thereof;

(iii) is an antibody, functional fragment or derivative thereof with beta-amyloid 1-42 peptide-binding capacity comprising one or more of the amino acid sequences selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4 and sequences substantially homologous to either one of SEQ ID NO: 2 or SEQ ID NO: 4;

(iv) is an antibody, functional fragment thereof or derivative with beta-amyloid 1-42 peptide-binding capacity, according to claim 4, comprising at least one heavy chain and

one light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 2 and the light chain comprises the amino acid sequence of SEQ ID NO: 4;

(v) is an antibody, functional fragment or derivative thereof with beta-amyloid 1-42 peptide-binding capacity comprising one or more of the amino acid sequences selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 8 and sequences substantially homologous to either one of SEQ ID NO: 6 or SEQ ID NO: 8;

or,

(vi) is an antibody, functional fragment thereof or derivative with beta-amyloid 1-42 peptide-binding capacity, according to the claim 6, comprising at least one heavy chain and one light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 6 and the light chain comprises the amino acid sequence of SEQ ID NO: 8,

wherein said DNA sequence is operatively linked to an expression control sequence.

[0053] In another embodiment, the unicellular host is transformed with:

(A) the DNA sequence of SEQ ID NO:5;

(B) the DNA sequence of SEQ ID NO:7;

(C) the DNA sequence of SEQ ID NO:5 and the DNA sequence of SEQ ID NO:7;

(D) a DNA sequence that hybridizes to any of the foregoing DNA sequences under standard hybridization conditions;

(E) a DNA sequence that codes on expression for an amino acid sequence encoded by any of the foregoing DNA sequences; or

(F) a fragment of any one of the foregoing DNA sequences which encodes a specific binding member which

(i) recognizes an epitope on amyloid beta 1-42, wherein said binding member is obtained from healthy elderly subjects having full cognitive capacity and who exhibit no symptoms of Alzheimer's disease or other neuropathies, for example where the subjects are 60 years of age or older, for example 70 years of age or older, 80 years of age or older or 90 years of age or older;

(ii) is an antibody having amyloid beta 1-42 binding capacity, and wherein the antibody comprises an amino acid sequence of SEQ ID NOs: 2, or 4, or an amino acid sequence that is substantially homologous to SEQ ID NOs: 2 or 4, and functional fragments, derivatives or variants thereof;

(iii) is an antibody, functional fragment or derivative thereof with beta-amyloid 1-42 peptide-binding capacity comprising one or more of the amino acid sequences selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4 and sequences substantially homologous to either one of SEQ ID NO: 2 or SEQ ID NO: 4;

(iv) is an antibody, functional fragment thereof or derivative with beta-amyloid 1-42 peptide-binding capacity, according to claim 4, comprising at least one heavy chain and one light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 2 and the light chain comprises the amino acid sequence of SEQ ID NO: 4;

(v) is an antibody, functional fragment or derivative thereof with beta-amyloid 1-42 peptide-binding capacity comprising one or more of the amino acid sequences selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 8 and sequences substantially homologous to either one of SEQ ID NO: 6 or SEQ ID NO: 8;

or,

(vi) is an antibody, functional fragment thereof or derivative with beta-amyloid 1-42 peptide-binding capacity, according to the claim 6, comprising at least one heavy chain and one light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 6 and the light chain comprises the amino acid sequence of SEQ ID NO: 8,

wherein said DNA sequence is operatively linked to an expression control sequence.

[0054] A sixth aspect of the invention relates to the use of an antibody, a fragment thereof including active and immunogenic fragments thereof, or derivatives thereof, for preparing a pharmaceutical composition. In a related aspect the invention also provides a binding member which :

(i) recognizes an epitope on amyloid beta 1-42, wherein said binding member is obtained from healthy elderly subjects having full cognitive capacity and who exhibit no symptoms of Alzheimer's disease or other neuropathies, for example where the subjects are 60 years of age or older, for example 70 years of age or older, 80 years of age or older or 90 years of age or older;

(ii) is an antibody having amyloid beta 1-42 binding capacity, and wherein the antibody comprises an amino acid sequence of SEQ ID NOs: 2, or 4, or an amino acid sequence that is substantially homologous to SEQ ID NOs: 2 or 4, and functional fragments, derivatives or variants thereof;

(iii) is an antibody, functional fragment or derivative thereof with beta-amyloid 1-42 peptide-binding capacity comprising one or more of the amino acid sequences selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4 and sequences substantially homologous to either one of SEQ ID NO: 2 or SEQ ID NO: 4;

(iv) is an antibody, functional fragment thereof or derivative with beta-amyloid 1-42 peptide-binding capacity, according to claim 4, comprising at least one heavy chain and one light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 2 and the light chain comprises the amino acid sequence of SEQ ID NO: 4;

(v) is an antibody, functional fragment or derivative thereof with beta-amyloid 1-42 peptide-binding capacity comprising one or more of the amino acid sequences selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 8 and sequences substantially homologous to either one of SEQ ID NO: 6 or SEQ ID NO: 8;

or

(vi) is an antibody, functional fragment thereof or derivative with beta-amyloid 1-42 peptide-binding capacity, according to the claim 6, comprising at least one heavy chain and one light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 6 and the light chain comprises the amino acid sequence of SEQ ID NO: 8.

for use as a medicament. Also provided is a pharmaceutical composition which comprises a binding member as described above and a pharmaceutical acceptable excipient. Further provided is the use of a binding member as described above for the manufacture of a medicament for the treatment or prevention of Alzheimer's disease, or for the preparation of a pharmaceutical composition for its use in the treatment or prevention of Alzheimer's disease.

[0055] A further particular aspect of the invention relates to a pharmaceutical composition containing an isolated specific binding member which recognizes amyloid beta 1-42, and in particular, an antibody, a fragment thereof including active and immunogenic fragments thereof, or derivatives thereof.

[0056] A yet further particular aspect of the invention relates to a pharmaceutical composition containing an antibody, a fragment thereof including active and immunogenic fragments thereof, or derivatives thereof, for use in treating or preventing Alzheimer's disease.

[0057] In one particular embodiment, the composition comprises a binding member, such as an antibody comprising one or more of the amino acid sequences set forth in SEQ ID NOs: 2, 4, 6, or 8 or amino acid sequences having substantial homology to one or more of the sequences set forth in SEQ ID NOs: 2, 4, 6 or 8.

[0058] In another particular embodiment, the binding member is the antibody aforesaid, and particularly, a scFv.

[0059] In another particular embodiment, the pharmaceutical composition further comprises an additional agent useful for treating Alzheimer's disease, selected from the group consisting of small organic molecules, other amyloid beta antibodies, and combinations thereof. The invention contemplates and includes therefore, compositions comprising at least one binding member of the invention, in combination with one or more other agents as aforesaid.

[0060] A seventh aspect of the invention relates to the use of an antibody, fragment thereof or derivative of the present invention for detecting the beta-amyloid 1-42 peptide *in vitro*. The use of antibodies as affinity reagents is of particular interest. Specific monoclonal antibody production will be obvious for any person skilled in the art. The use of specific antibodies in *in vitro* immunodetection techniques is also widely known. Said techniques include but are not limited to: ELISA, Western Blot, immunofluorescence, immunohistochemistry or flow cytometry.

[0061] An eighth aspect of the invention relates to the use of an antibody, fragments thereof or derivatives thereof, for detecting the beta-amyloid 1-42 peptide *in vivo*. Further provided by the invention is the use of an antibody, fragments thereof or derivatives thereof in the preparation of a composition for the *in vivo* detection of the beta-amyloid 1-42 peptide. The *in vivo* detection of the beta-amyloid 1-42 peptide can be carried out by means of molecular imaging techniques. Labeled monoclonal antibodies constitute specific detectable contrasts according to the presence of binding to their ligand (Weissleder, 2002). Molecular imaging techniques allow conducting in vivo specific detection studies. In animal experiments, it allows studying the evolution of a pathology or the effect of a drug on the animal without necessarily having to sacrifice it. In human beings, it allows the pharmacological study or study of the pathology in different tissues in a non-invasive and virtually painless manner. The use of a labeled antibody which specifically binds to the beta-amyloid 1-42 peptide would allow very early detection (before the onset of clinical symptoms characteristic of Alzheimer's disease) of the presence of beta-amyloid 1-42 peptide deposits, as well as the study of the evolution of the disease over time and of treatment efficacy.

[0062] A ninth aspect of the invention relates to the use of an antibody, a fragment thereof including active and immunogenic fragments thereof, or derivatives thereof for identifying a compound that is able to compete with said antibody, fragment or derivative for binding to the beta-amyloid 1-42 peptide.

[0063] In a preferred aspect of the invention, said compound able to compete with the antibody, fragment or derivative of the present invention is a peptide or a peptoid. Within the context of the present invention, a compound that is analogous to a peptide in which the peptide bonds have been replaced by peptide-like bonds, such as: Carba [psi] (CH_2-CH_2), Depsi [psi] (CO-OR), Hydroxyethylene [psi] (CHOH-CH_2), Ketomethylene [psi] (CH-CH_2), Methylene-oxy CH_2-O-,

Reduced CH_2-NH, Thiomethylene CH_2-S-, Thiopeptide CS-NH, N-modified -NRCO-, is understood as a peptoid.

[0064] In an even more preferred aspect of the invention, said compound able to compete with the antibody, fragment or derivative of the present invention is a mimotope. Within the context of the present invention, a peptide molecule the structure of which imitates that of the epitope recognized by the antibodies, fragments or derivatives of the present invention, is understood as a mimotope. The amino acid sequence of said mimotope does not necessarily have to present sequence similarity with the epitope; nevertheless, the mimotope will adopt a conformation that imitates that of the epitope (Steward et al., 1995).

[0065] In an especially preferred aspect of the invention, said compound able to compete with the antibody, fragment or derivative of the present invention is a synthetic peptide based on the sequence of the heavy chain CDR3 region of the antibodies of the present invention.

[0066] A tenth aspect of the invention provides a process of obtaining antibodies or functional fragments thereof with beta-amyloid 1-42 peptide-binding capacity from the genetic repertoire of immunoglobulins isolated from healthy donors, comprising the steps of:

A) purifying B cells from, for example, a blood sample or a bone marrow sample, said sample being obtained from a subject fulfilling the following criteria;
   (i) being 60 years of age or older;
   (ii) having full cognitive capacity and good health;
   (iii) having no symptoms of Alzheimer's disease or another neuropathy;
   (iv) not having previously received any medication for the prevention or treatment of neurodegenerative diseases; and
   (v) possessing detectable anti beta-amyloid 1-42 peptide antibodies in the blood serum;
B) obtaining the immunoglobulin gene repertoire for said anti beta-amyloid 1-42 peptide antibodies from said B cells;
C) using said repertoire to express said antibodies.

[0067] The method for obtaining the immunoglobulin gene repertoire for the anti beta-amyloid 1-42 peptide antibodies from the B cells in step B) can be carried out by the following steps:

(i) obtaining mRNA from the said B cells;
(ii) obtaining cDNA from the mRNA of step (i); and;
(iii) using a primer extension reaction to amplify from said cDNA the fragments corresponding to the heavy chains (HC) and the kappa light chains (LC) of said anti beta-amyloid 1-42 peptide antibodies.

In a preferred embodiment the B cells are peripheral blood B cells obtained from a blood sample.

[0068] An eleventh aspect of the invention provides a method of preparing a specific binding member capable of binding an epitope on amyloid beta 1-42, which method comprises:

a) providing a starting repertoire of nucleic acids encoding a VH domain which lacks a CDR3 encoding region;

b) combining said repertoire with a donor nucleic acid encoding an amino acid sequence substantially as set out in SEQ ID NOs: 2, 4, 6 or 8 such that said donor nucleic acid is inserted into the missing CDR3 region, so as to provide a product repertoire of nucleic acids encoding a VH domain;

c) expressing the nucleic acids of said product repertoire; and

d) selecting a specific binding member which recognizes amyloid beta 1-42; and

e) recovering said binding member or the nucleic acid encoding it.

[0069] A twelfth aspect of the invention provides a method of treating or preventing the progression of Alzheimer's disease in a subject, or for ameliorating the symptoms associated with Alzheimer's disease, or for diagnosing or screening a subject for the presence of Alzheimer's disease or for determining a subject's risk for developing Alzheimer's disease, which method comprises administering to said subject an effective amount of a specific binding member or antibody or fragment, derivative or variant thereof, and pharmaceuticals containing the binding member.

[0070] Other objects and advantages will become apparent to those skilled in the art from a review of the following description which proceeds with reference to the following illustrative drawings.

BRIEF DESCRIPTION OF THE FIGURES

[0071] Figures 1A-1D show the results obtained in the ELISA assays carried out using the beta-amyloid 1-42 peptide at a concentration of 5 µg/ml as a coating and anti IgG+IgM+IgA-HRP as a secondary antibody to detect the existence of serum anti beta-amyloid 1-42 peptide antibodies in donors Vj2, Vj3, Vj4 and Vj5. The serial dilutions of assayed serum are shown on the X-axis and optical density at 450 nm is shown on the Y-axis.

[0072] Figures 2A and 2B show the results obtained in the ELISA assays using the beta-amyloid 1-42 peptide at a concentration of (1 µg/ml) as a coating and anti IgG (H+L)-HRP as a secondary antibody, compared to the different dilutions of serums Vj2 and Vj5. The serial dilutions of assayed serum are shown on the X-axis and optical density at 492 nm is shown on the Y-axis. Figure 2A shows the titration curve of the serum from donor Vj2, and Figure 2B shows the titration curve of the serum from donor Vj5.

[0073] Figures 3A and 3B show the photograph of a 2% agarose gel electrophoresis of the PCR products obtained in the first amplification of the genetic human immunoglobulin repertoire for the construction of an antibody fragment (Fab) library. Figure 3A shows the gamma-1 heavy chains and Figure 3b shows the kappa chains. Figure 3C shows the photograph of a 2% agarose gel electrophoresis of the PCR products obtained in the second amplification using the purified PCR products from the first amplification as a mold.

[0074] Figures 4A and 4B show the photograph of a 2% agarose gel electrophoresis of the PCR products obtained in the first amplification of the genetic human immunoglobulin repertoire for the construction of a single chain fragment (scFv) library. Figure 4A shows the kappa chains and Figure 4B shows the gamma heavy chains. Figure 4C shows the photograph of a 2% agarose gel electrophoresis of the PCR products obtained in the second amplification, carried out in triplicate, using the purified PCR products from the first amplification as a mold.

[0075] Figure 5 shows the HCDR3 sequences of several clones chosen randomly from the antibody fragment (Fab) library and from the single chain antibody fragment (scFv) library, which have been sequenced for the purpose of checking the diversity of the obtained libraries.

[0076] Figure 6 shows the degree of enrichment obtained in the antigen selection step of the single chain fragment library against the beta-amyloid 1-42 peptide immobilized in solid phase (ELISA). The graph represents the progress of each selection cycle as the percentage of antibodies eluted in each round (CFU eluted/ml); final enrichment degree (x 3.9) was determined considering the negative control (c-).

[0077] Figures 7A and 7B show the binding profile of the recombinant antibodies AD38 and AD64, respectively, by means of specific immunoassaying (ELISA) against the beta-amyloid 1-42 peptide.

[0078] Figure 8 shows the nucleotide sequence of the heavy chain of recombinant antibody AD38 and its corresponding amino acid sequence, pointing out the hypervariable regions or CDRs.

[0079] Figure 9 shows the nucleotide sequence of the light chain of recombinant antibody AD38 and its corresponding amino acid sequence, pointing out the hypervariable regions or CDRs.

[0080] Figure 10 shows the nucleotide sequence of the heavy chain of recombinant antibody AD64 and its corresponding amino acid sequence, pointing out the hypervariable regions or CDRs.

[0081] Figure 11 shows the nucleotide sequence of the light chain of recombinant antibody AD64 and its corresponding amino acid sequence, pointing out the hypervariable regions or CDRs.

DETAILED DESCRIPTION OF THE INVENTION

[0082] Before the present methods and treatment methodology are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for purposes of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only in the appended claims.

[0083] As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, references to "the method" includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

[0084] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference in their entirety.

[0085] In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook et al, "Molecular Cloning: A Laboratory Manual" (1989); "Current Protocols in Molecular Biology" Volumes I-III [Ausubel, R. M., ed. (1994)]; "Cell Biology: A Laboratory Handbook" Volumes I-III [J. E. Celis, ed. (1994))]; "Current Protocols in Immunology" Volumes I-III [Coligan, J. E., ed. (1994)]; "Oligonucleotide Synthesis" (M.J. Gait ed. 1984); "Nucleic Acid Hybridization" [B.D. Hames & S.J. Higgins eds. (1985)]; "Transcription And Translation" [B.D. PHames & S.J. Higgins, eds. (1984)]; "Animal Cell Culture" [R.I. Freshney, ed. (1986)]; "Immobilized Cells And Enzymes" [IRL Press, (1986)]; B. Perbal, "A Practical Guide To Molecular Cloning" (1984).

Definitions

[0086] The terms used herein have the meanings recognized and known to those of skill in the art, however, for convenience and completeness, particular terms and their meanings are set forth below.

[0087] An "isolated" or "purified" polypeptide or protein, e.g., an "isolated antibody," refers to a protein, polypeptide or antibody that is purified to a state beyond that in which it exists in nature. For example, the "isolated" or "purified" polypeptide or protein, e.g., an "isolated antibody," refers to a protein that is separated from at least one component of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or separated from at least one component of the chemical precursors or other chemicals when chemically synthesized. For example, an isolated protein can be substantially free of other proteins, other cellular material, or chemical precursors. In some embodiments, the preparation of antibody protein having less than about 50% of non-antibody protein (also referred to herein as a "contaminating protein"), or of chemical precursors, is considered to be "substantially free." In other embodiments, 40%, 30%, 20%, 10% and more preferably 5% (by dry weight), of non-antibody protein, or of chemical precursors is considered to be substantially free. When the

antibody protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, e.g., the culture medium represents less than about 30%, 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume or mass of the protein preparation. Proteins or polypeptides referred to herein as "recombinant" are proteins or polypeptides produced by the expression of recombinant nucleic acids. Proteins can be purified by standard methods (including, e.g., ion exchange and affinity chromatography) to provide preparations in which a particular protein is at least 5, 10, 20, 25, 50, 75, 80, 90, 95, 98, 99% pure relative to other proteins or relative to other biologically active components.

[0088] The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human. Preferably, as used herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

[0089] The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water or aqueous solution saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin.

[0090] The phrase "therapeutically effective amount" is used herein to mean an amount sufficient to prevent, and preferably reduce by at least about 25 percent, more preferably by at least 50 percent, most preferably by at least 90 percent, a clinically significant change in a feature of pathology such as for example, elevated blood pressure, fever, or white cell count, as may attend its presence and activity. As related to the present invention, the term may also mean an amount sufficient to ameliorate or reverse one or more symptoms associated with Alzheimer's disease. In particular, a "therapeutically effective amount" of the treatment may result in amelioration, reduction or elimination of at least one of the following symptoms: memory impairment, persistent sadness or anxiety, feelings of emptiness, hopelessness, pessimism, guilt, worthlessness, helplessness, a loss of interest or pleasure in hobbies and activities that were once enjoyed, decreased energy, or fatigue, difficulty concentrating, remembering, or making decisions, insomnia, early-morning awakening, or oversleeping, appetite and/or weight loss or overeating and weight gain, thoughts of death or suicide and suicide attempts, restlessness, irritability, and persistent physical symptoms that do not respond to treatment, such as headaches, digestive disorders, and chronic pain.

[0091] "Treatment" or "treating" refers to therapy, prevention and prophylaxis and particularly refers to the administration of medicine or the performance of medical procedures with respect to a patient, for either prophylaxis (prevention) or to cure or reduce the extent of or likelihood of occurrence of the infirmity or malady or condition or event in the instance where the patient is afflicted.

[0092] "Subject" or "patient" refers to a mammal, preferably a human, in need of treatment for a condition, disorder or disease.

[0093] An "antibody" is any immunoglobulin, including antibodies and fragments or derivatives thereof, that binds a specific epitope. Such an antibody that binds a specific epitope is said to be "immunospecific". The term encompasses "polyclonal", "monoclonal", and "chimeric" antibodies, the last mentioned described in further detail in U.S. Patent Nos. 4,816,397 and 4,816,567. Commonly used carriers that are chemically coupled to peptides include bovine or chicken serum albumin, thyroglobulin, and other carriers known to those skilled in the art. The coupled peptide is then used to immunize the animal (e.g, a mouse, rat or rabbit). The "chimeric antibody" refers to a molecule in which different portions are derived from different animal species, such as those having a human immunoglobulin constant region and a variable region derived from a murine mAb. (See, e.g., Cabilly et al., U.S. Patent No. 4,816,567; and Boss et al., U.S. Patent No. 4,816,397.). The antibody may be a human or a humanized antibody. The antibody may be a single chain antibody. (See, e.g., Curiel et al., U.S. Patent No. 5,910,486 and U.S. Patent No. 6,028,059). The antibody may be prepared in, but not limited to, mice, rats, rabbits, goats, sheep, swine, dogs, cats, or horses. More particularly, the present invention provides for the isolation of antibodies from healthy elderly human, who do not exhibit any of the symptoms associated with Alzheimer's disease, such as, for example, loss of memory or irritability. As used herein, the term "single-chain antibody" refers to a polypeptide comprising a V_H region and a V_L region in polypeptide linkage, generally linked via a spacer peptide (e.g., [Gly-Gly-Gly-Gly-Ser] $_x$), and which may comprise additional amino acid sequences at the amino- and/or carboxy- termini. For example, a single-chain antibody may comprise a tether segment for linking to the encoding polynucleotide. As an example, a scFv (single chain fragment variable) is a single-chain antibody. Single-chain antibodies are generally proteins consisting of one or more polypeptide segments of at least 10 contiguous amino acids substantially encoded by genes of the immunoglobulin superfamily (e.g., see The Immunoglobulin Gene Superfamily, A. F. Williams and A. N. Barclay, in Immunoglobulin Genes, T. Honjo, F. W. Alt, and T. H. Rabbitts, eds., (1989) Academic Press: San Diego, Calif., pp.361-387, most frequently encoded by a rodent, non-human primate, avian, porcine, bovine, ovine, goat, or human heavy chain or light chain gene sequence. A functional single-chain antibody generally contains a sufficient portion of an immunoglobulin superfamily gene product so as to retain the property of binding to a specific target molecule, typically a receptor or antigen (epitope). For a review of scFv see Pluckthun in The Pharmacology of Monoclonal Antibodies,

vol. 113, Rosenburg and Moore eds. Springer-Verlag, N.Y., pp. 269-315 (1994). One particularly advantageous approach has been the use of so-called single-chain fragment variable (scFv) libraries (Marks et al. (1992) Biotechnology 10: 779; Winter G and Milstein C (1991) Nature 349: 293; Clackson et al. (1991) op.cit.; Marks et al. (1991) J. Mol. Biol. 222: 581; Chaudhary et al. (1990) Proc. Natl. Acad. Sci. (USA) 87: 1066; Chiswell et al. (1992) TIBTECH 10: 80; McCafferty et al. (1990) op.cit.; and Huston et al. (1988) Proc. Natl. Acad. Sci. (USA) 85: 5879). Various embodiments of scFv libraries displayed on bacteriophage coat proteins have been described.

[0094] Antibodies are naturally "bivalent antibodies", however, the term also includes diabodies. The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (V_M) connected to a light chain variable domain (V_L) in the same polypeptide chain (V_H-V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, (Holliger, P., et al. (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448; Poljak, R. J., et al. (I 994) Structure 2:1121-1123); EP 404,097; WO 93/11161). "Multivalent" antibodies include two or more binding domains which may all be of the same specificity or may have multiple specificities.

An "antibody combining site" is that structural portion of an antibody molecule comprised of heavy and light chain variable and hypervariable regions that specifically binds antigen.

[0095] The phrase "antibody molecule" in its various grammatical forms as used herein contemplates both an intact immunoglobulin molecule and an immunologically active portion of an immunoglobulin molecule.

[0100] Exemplary antibody molecules are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and those portions of an immunoglobulin molecule that contains the paratope, including those portions known in the art as Fab, Fab', $F(ab')_2$ and F(v), which portions are preferred for use in the therapeutic methods described herein.

[0101] Fab and $F(ab')_2$ portions of antibody molecules are prepared by the proteolytic reaction of papain and pepsin, respectively, on substantially intact antibody molecules by methods that are well-known. See for example, U.S. Patent No. 4,342,566 to Theofilopolous et al. Fab' antibody molecule portions are also well-known and are produced from $F(ab')_2$ portions followed by reduction of the disulfide bonds linking the two heavy chain portions as with mercaptoethanol, and followed by alkylation of the resulting protein mercaptan with a reagent such as iodoacetamide. An antibody containing intact antibody molecules is preferred herein.

[0102] The phrase "monoclonal antibody" in its various grammatical forms refers to an antibody having only one species of antibody combining site capable of immunoreacting with a particular antigen. A monoclonal antibody thus typically displays a single binding affinity for any antigen with which it immunoreacts. A monoclonal antibody may therefore contain an antibody molecule

having a plurality of antibody combining sites, each immunospecific for a different antigen; e.g., a bispecific (chimeric) monoclonal antibody.

[0103] "Screening" or "Diagnosis" refers to diagnosis, prognosis, monitoring, characterizing, selecting patients, including participants in clinical trials, and identifying patients at risk for or having a particular disorder or clinical event or those most likely to respond to a particular therapeutic treatment, or for assessing or monitoring a patient's response to a particular therapeutic treatment.

[0104] "Amyloid" describes various types of protein aggregations that share specific traits when examined microscopically. Amyloid is typically identified by a change in the fluorescence intensity of planar aromatic dyes such as Thioflavin T or Congo Red. This is generally attributed to the environmental change as these dyes intercolate between beta-strands. The amyloid fold is characterized by a cross-beta sheet quaternary structure, that is, a monomeric unit contributes a beta strand to a beta sheet which spans across more than one molecule. While amyloid is usually identified using fluorescent dyes, stain polarimetry, circular dichroism, or FTIR (all indirect measurements), the "gold-standard" test to see if a structure is amyloid is by placing a sample in an X-ray diffraction beam; there are two characteristic scattering bands produced at 4 and 10 angstroms each, corresponding to the interstrand distances in the beta sheet structure. The amyloid protein disclosed in the present application refers to amyloid beta, as described below.

[0105] "Amyloid beta", "Abeta", "Aß", "beta-amyloid" or "amyloid beta peptide" is a physiological product normally released from the amyloid beta protein precursor (βAPP or APP) through β and γ secretase cleavage and consists of two 40 and 42 amino acid peptides, usually abbreviated as Aβ40 or amyloid beta 1-40 and Aβ42 or amyloid beat 1-42, respectively (Selkoe, D. (2002), J. Clin. Invest. 110:1375-1381. The 42 amino acid amyloid beta peptide (Aß42) is more hydrophobic & "sticky" (and hence aggregates more readily) than the 40 amino acid amyloid beta peptide (Aß40), and as such may play a greater role in the pathogenesis of Alzheimer's disease, due to its increased tendency to form insoluble fibrils and increased neurotoxicity. Thus, under certain circumstances, as in Alzheimer's disease (AD), brain levels of these peptides increase dramatically, which can lead to the oligomerization of the peptides and eventually to the formation of insoluble fibrillar aggregates, which deposit in senile plaques. The term "amyloid beta40" may be used interchangeably with "Abeta40", Aβ40 and Abeta 1-40, and "amyloid beta42" is used interchangeably with "Abeta42", Aβ42 and Abeta 1-42. The sequences of these Abeta peptides and their relationship to the APP precursor are illustrated in FIG. 1 of Hardy et al., TINS 20, 155-158 (1997).

[0106] "Fragment" refers to either a protein or polypeptide comprising an amino acid sequence of at least 4 amino acid residues (preferably, at least 10 amino acid residues, at least 15 amino acid residues, at least 20 amino acid residues, at least 25 amino acid residues, at least 40 amino acid residues, at least 50 amino acid residues, at least 60 amino residues, at least 70

amino acid residues, at least 80 amino acid residues, at least 90 amino acid residues, at least 100 amino acid residues, at least 125 amino acid residues, or at least 150 amino acid residues) of the amino acid sequence of a parent protein or polypeptide, or a nucleic acid comprising a nucleotide sequence of at least 10 base pairs (preferably at least 20 base pairs, at least 30 base pairs, at least 40 base pairs, at least 50 base pairs, at least 50 base pairs, at least 100 base pairs, at least 200 base pairs) of the nucleotide sequence of the parent nucleic acid. Any given fragment may or may not possess a functional activity of the parent nucleic acid or protein or polypeptide.

[0107] Examples of binding fragments encompassed within the term "antigen-binding fragment" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al. (1989) Nature 341:544-46), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Camelid antibodies, and camelized antibodies can also be used. Such antibodies, e.g., can include CDRs from just one of the variable domains described herein. Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see, e.g., Bird et al. (1988) Science 242:423-26; Huston et al. (1988) Proc. Natl. Acad. Sci. U.S.A. 85:5879-83). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding fragment" of an antibody. These antibody fragments are obtained using conventional techniques known to those skilled in the art, and the fragments are evaluated for function in the same manner as are intact antibodies.

[0108] "Combination therapy" refers to the use of the agents of the present invention with other active agents or treatment modalities, in the manner of the present invention for treatment of Alzheimer's disease (AD). These other agents or treatments may include drugs such as antidepressants including those that are standardly used to treat other various depressive disorders. The agents of the invention may also be combined with corticosteroids, non-steroidal anti-inflammatory compounds, or other agents useful in treating inflammation or alleviating pain. The combined use of the agents of the present invention with these other therapies or treatment modalities may be concurrent, or given sequentially, that is, the two treatments may be divided up such that the antibody or fragment thereof of the present invention may be given prior to or after the other therapy or treatment modality.

[0109] "Slow release formulation" refers to a formulation designed to release a therapeutically effective amount of a drug or other active agent such as a polypeptide or a synthetic compound over an extended period of time, with the result being a reduction in the number of treatments

necessary to achieve the desired therapeutic effect. In the matter of the present invention, a slow release formulation would decrease the number of treatments necessary to achieve the desired effect in terms of amelioration of, reduction in, or reversal of at least one symptom of AD.

[0110] As used herein, "probe" refers to a labeled oligonucleotide primer, which forms a duplex structure with a sequence in the target nucleic acid, due to complementarity of at least one sequence in the probe with a sequence in the target region.

[0111] A "variant" (v) of polynucleotides or polypeptides, as the term is used herein, are polynucleotides or polypeptides that are different from a reference polynucleotide or polypeptide, respectively. Variant polynucleotides are generally limited so that the nucleotide sequence of the reference and the variant are closely related overall and, in many regions, identical. Changes in the nucleotide sequence of the variant may be silent. That is, they may not alter the amino acid sequence encoded by the polynucleotide. Where alterations are limited to silent changes of this type a variant will encode a polypeptide with the same amino acid sequence as the reference. Alternatively, changes in the nucleotide sequence of the variant may alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Such nucleotide changes may result in amino acid substitutions, additions, deletions, fusions, and truncations in the polypeptide encoded by the reference sequence. Variant polypeptides are generally limited so that the sequences of the reference and the variant are that are closely similar overall and, in many regions, identical. For example, a variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions, fusions, and truncations, which may be present or absent in any combination. Such variants can differ in their amino acid composition (e.g. as a result of allelic or natural variation in the amino acid sequence, e.g. as a result of alternative mRNA or pre-mRNA processing, e.g. alternative splicing or limited proteolysis) and in addition, or in the alternative, may arise from differential post-translational modification (e.g., glycosylation, acylation, phosphorylation, isoprenylation, lipidation).

[0112] "Derivative" refers to either a protein or polypeptide that comprises an amino acid sequence of a parent protein or polypeptide that has been altered by the introduction of amino acid residue substitutions, deletions or additions, or a nucleic acid or nucleotide that has been modified by either introduction of nucleotide substitutions or deletions, additions or mutations. The derivative nucleic acid, nucleotide, protein or polypeptide possesses a similar or identical function as the parent polypeptide.

[0113] A "homologue" refers to polypeptides having the same or conserved residues at a corresponding position in their primary, secondary or tertiary structure. The term also extends to two or more nucleotide sequences encoding the homologous polypeptides.

[0114] The term "standard hybridization conditions" refers to salt and temperature conditions substantially equivalent to 5 x SSC and 65°C for both hybridization and wash. However, one

skilled in the art will appreciate that such "standard hybridization conditions" are dependent on particular conditions including the concentration of sodium and magnesium in the buffer, nucleotide sequence length and concentration, percent mismatch, percent formamide, and the like. Also important in the determination of "standard hybridization conditions" is whether the two sequences hybridizing are RNA-RNA, DNA-DNA or RNA-DNA. Such standard hybridization conditions are easily determined by one skilled in the art according to well known formulae, wherein hybridization is typically 10-20°C below the predicted or determined T_m with washes of higher stringency, if desired.

[0115] Procedures using such conditions of high stringency are as follows. Prehybridization of filters containing DNA is carried out for 15 minutes to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 µg/ml denatured salmon sperm DNA. Filters are hybridized for 48 h at 65°C in prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 X 10^6 cpm of ^{32}P-labeled probe. Washing of filters is done at 37°C for 1 h in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1X SSC at 50°C for 45 min before autoradiography. Other conditions of high stringency that may be used are well known in the art.

[0116] "AD38" refers to an antibody to amyloid beta 1-42 designated as Colección Española de Cultivos Tipo (CECT) accession number 7167. The amino acid sequence for AD38 is found in SEQ ID NO: 10. The nucleic acid encoding AD38 can be found in SEQ ID NO: 9. The heavy chain of AD38, which is a functional antibody fragment that retains beta amyloid 1-42 binding capacity, has the amino acid sequence set forth in SEQ ID NO: 2. The nucleic acid encoding the heavy chain of AD38 is set forth in SEQ ID NO: 1. The light chain of AD38, which is a functional antibody fragment that retains beta amyloid 1-42 binding capacity, has the amino acid sequence set forth in SEQ ID NO: 4. The nucleic acid encoding the light chain of AD38 is set forth in SEQ ID NO: 3.

[0117] "AD64" refers to an antibody to amyloid beta 1-42 designated as CECT accession number 7168. The amino acid sequence for AD64 is found in SEQ ID NO: 12. The nucleic acid encoding AD64 can be found in SEQ ID NO: 11. The heavy chain of AD64, which is a functional antibody fragment that retains beta amyloid 1-42 binding capacity, has the amino acid sequence set forth in SEQ ID NO: 6. The nucleic acid encoding the heavy chain of AD64 is set forth in SEQ ID NO: 5. The light chain of AD64, which is a functional antibody fragment that retains beta amyloid 1-42 binding capacity, has the amino acid sequence set forth in SEQ ID NO: 8. The nucleic acid encoding the light chain of AD38 is set forth in SEQ ID NO: 7.

[0118] The term "nucleic acid molecule", as used herein, is intended to include DNA molecules and RNA molecules. A nucleic acid molecule may be single-stranded or double-stranded, but preferably is double-stranded DNA.

[0119] The term "vector," as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genorne. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

[0120] "Operably linked" when describing the relationship between two polynucleotide sequences, means that they are functionally linked to each other. For example, a promoter is operably linked to a coding sequence if it controls the transcription of the sequence. As a regulatory sequence commonly used promoter elements as well as enhancers may be used. Generally, such expression regulation sequences are derived from genes that are expressed primarily in the tissue or cell type chosen. Preferably, the genes from which these expression regulation sequences are obtained are expressed substantially only in the tissue or cell type chosen, although secondary expression in other tissue and/or cell types is acceptable if expression of the recombinant DNA in the transgene in such tissue or cell type is not detrimental to the transgenic animal.

[0121] "Complementary" is understood in its recognized meaning as identifying a nucleotide in one sequence that hybridizes (anneals) to a nucleotide in another sequence according to the rule A→T, U and C→G (and vice versa) and thus "matches" its partner for purposes of this definition. Enzymatic transcription has measurable and well known error rates (depending on the specific enzyme used), thus within the limits of transcriptional accuracy using the modes described herein, in that a skilled practitioner would understand that fidelity of enzymatic complementary strand synthesis is not absolute and that the amplicon need not be completely matched in every nucleotide to the target or template RNA.

[0122] A "reporter gene" refers to a gene whose phenotypic expression is easy to monitor and is used to study promoter activity in different tissues or developmental stages. Recombinant DNA constructs are made in which the reporter gene is attached to a promoter region of particular interest and the construct transfected into a cell or organism. As used herein, a

"reporter"gene is used interchangeably with the term "marker gene" and is a nucleic acid that is readily detectable and/or encodes a gene product that is readily detectable such as green fluorescent protein (as described in U.S. Patent No. 5,625,048 issued April 29, 1997, and WO 97/26333, published July 24, 1997, the disclosures of each are hereby incorporated by reference herein in their entireties), or red fluorescent protein, or yellow fluorescent protein, or wheat germ agglutinin (WGA) or a WGA-type molecule or luciferase.

[0123] The primers herein are selected to be "substantially" complementary to different strands of a particular target DNA sequence. This means that the primers must be sufficiently complementary to selectively hybridize with their respective strands. Therefore, the primer sequence need not reflect the exact sequence of the template. For example, a non-complementary nucleotide fragment may be attached to the 5' end of the primer, with the remainder of the primer sequence being complementary to the strand. Alternatively, non-complementary bases or longer sequences can be interspersed into the primer, provided that the primer sequence has sufficient complementarity with the sequence of the strand to selectively hybridize therewith and thereby form the template for the synthesis of the extension product.

[0124] Two DNA sequences are "substantially homologous" or have "substantial homology" when at least about 75% (preferably at least about 80%, and most preferably at least about 90 or 95%) of the nucleotides match over the defined length of the DNA sequences. Sequences that are substantially homologous can be identified by comparing the sequences using standard software available in sequence data banks, or in a Southern hybridization experiment under, for example, stringent conditions as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Maniatis et al., supra; DNA Cloning, Vols. I & II, supra; Nucleic Acid Hybridization, supra.

[0125] Two amino acid sequences are "substantially homologous" or show "substantial homology" when at least about 70% of the amino acid residues (preferably at least about 80%, and most preferably at least about 90 or 95%) are identical, or represent conservative substitutions.

[0126] The term "CDR" refers to the complementarity determining region or hypervariable region amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region or CDRs of the human IgG subtype of antibody comprise amino acid residues from residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain as described by Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)] and/or those residues from a hypervariable loop (i.e., residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain as described by

[Chothia et al., J. Mol. Biol. 196: 901-917 (1987)]. Framework or FR residues are those variable domain residues other than and bracketing the hypervariable regions.

[0127] The term "specific binding" refers to antibody binding to a predetermined antigen. Typically, the antibody binds with a dissociation constant (K_D) of 10^{-7} M or less, and binds to the predetermined antigen with a K_D that is at least twofold less than its K_D for binding to a non-specific antigen (e.g., BSA, casein, or any other specified polypeptide) other than the predetermined antigen. The phrases "an antibody recognizing an antigen" and "an antibody specific for an antigen" are used interchangeably herein with the term "an antibody which binds specifically to an antigen". As used herein "highly specific" binding means that the relative K_D of the antibody for the specific target epitope is at least 10-fold less than the K_D for binding that antibody to other ligands.

[0128] The affinity or avidity of an antibody for an antigen can be determined experimentally using any suitable method. (See, for example, Berzofsky, et al., "Antibody-Antigen Interactions," In Fundamental Immunology, Paul, W. E., Ed., Raven Press: New York, N.Y. (1984); Kuby, Janis Immunology, W. H. Freeman and Company: New York, N.Y. (1992); and methods described herein). The measured affinity of a particular antibody-antigen interaction can vary if measured under different conditions (e.g., salt concentration, pH). Thus, measurements of affinity and other antigen-binding parameters (e.g., K.sub.D, IC50) are preferably made with standardized solutions of antibody and antigen, and a standardized buffer, such as the buffer described herein.

[0129] A "binding member" as used in the context of the present invention relates primarily to antibodies, and fragments thereof, but may also refer to other non-antibody molecules that bind to the amyloid beta 1-42 protein.

[0130] A DNA "coding sequence" is a double-stranded DNA sequence which is transcribed and translated into a polypeptide in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

[0131] Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, polyadenylation signals, terminators, and the like, that provide for the expression of a coding sequence in a host cell.

[0132] A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum

number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Prokaryotic promoters contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences.

[0133] An "expression control sequence" is a DNA sequence that controls and regulates the transcription and translation of another DNA sequence. A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then translated into the protein encoded by the coding sequence.

[0134] A "signal sequence" can be included before the coding sequence. This sequence encodes a signal peptide, N-terminal to the polypeptide, that communicates to the host cell to direct the polypeptide to the cell surface or secrete the polypeptide into the media, and this signal peptide is clipped off by the host cell before the protein leaves the cell. Signal sequences can be found associated with a variety of proteins native to prokaryotes and eukaryotes.

[0135] The term "oligonucleotide," as used herein in referring to the probe of the present invention, is defined as a molecule comprised of two or more ribonucleotides, preferably more than three. Its exact size will depend upon many factors which, in turn, depend upon the ultimate function and use of the oligonucleotide.

[0136] A "small molecule" refers to a composition that has a molecular weight of less than 3 kilodaltons (kDa), and preferably less than 1.5 kilodaltons, and more preferably less than about 1 kilodalton. Small molecules may be nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, lipids or other organic (carbon-containing) or inorganic molecules. As those skilled in the art will appreciate, based on the present description, extensive libraries of chemical and/or biological mixtures, often fungal, bacterial, or algal extracts, may be screened with any of the assays of the invention to identify compounds that modulate a bioactivity. A "small organic molecule" is an organic compound (or organic compound complexed with an inorganic compound (e.g., metal)) that has a molecular weight of less than 3 kilodaltons, and preferably less than 1.5 kilodaltons, and more preferably less than about 1 kDa.

[0137] Blood-brain barrier: As used herein, the term "blood-brain barrier" or "BBB" refers to that obstacle to biological transport of drugs, ions, peptides, proteins and toxins that is formed by the membrane properties, structure and tight junctions of brain capillary endothelial cells.

[0138] By "scaffold" is meant a protein used to select or design a protein framework with specific and favorable properties, such as binding. When designing proteins from the scaffold, amino acid residues that are important for the framework's favorable properties are retained, while others residues may be varied. Such a scaffold has less than 50% of the amino acid residues that vary between protein derivatives having different properties and greater than or

equal to 50% of the residues that are constant between such derivatives. Most commonly, these constant residues confer the same overall three-dimensional fold to all the variant domains, regardless of their properties.

[0139] "Diseases characterized by amyloid deposits" include Alzheimer's disease (AD), both late and early onset. In both diseases, the amyloid deposit comprises a peptide termed Aβ, which accumulates in the brain of affected individuals. Examples of some other diseases characterized by amyloid deposits are SAA amyloidosis, hereditary Icelandic syndrome, multiple myeloma, and spongiform encephalopathies, including mad cow disease, Creutzfeldt Jakob disease, sheep scrapie, and mink spongiform encephalopathy (see Weissmann et al., Curr. Opin. Neurobiol. 7, 695-700 (1997); Smits et al., Veterinary Quarterly 19, 101-105 (1997); Nathanson et al., Am. J. Epidemiol. 145, 959-969 (1997)). The peptides forming the aggregates in these diseases are serum amyloid A, cystantin C, IgG kappa light chain respectively for the first three, and prion protein for the others.

[0140] "Diagnosis" or "screening" refers to diagnosis, prognosis, monitoring, characterizing, selecting patients, including participants in clinical trials, and identifying patients at risk for or having a particular disorder or clinical event or those most likely to respond to a particular therapeutic treatment, or for assessing or monitoring a patient's response to a particular therapeutic treatment.

[0141] The term "affinity maturation strategies" is described by Gonzales-Fernandez et al., "Low antigen dose favors selection of somatic mutants with hallmarks of antibody affinity maturation," Immunology, 93:149-153 (1998).

General Description

[0142] Amyloid beta 1-42 binding agents, e.g., anti-amyloid beta 1-42 antibodies and antigen-binding fragments thereof, pharmaceutical compositions thereof, nucleic acids encoding the aforesaid antibodies, as well as vectors and host cells containing the aforesaid nucleic acid sequences, are disclosed. More particularly, the present invention relates to the isolation and identification of anti-amyloid beta 1-42 antibodies from healthy elderly individuals who show full cognitive capacity and good health in the absence of symptoms of Alzheimer's disease or another neuropathy and who had never received any type of medication for the prevention or treatment of neurodegenerative diseases. More particularly, these patients were a restricted group of individuals who had surpassed the ages considered as critical for the onset of Alzheimer's disease and were chosen as candidates for obtaining anti beta-amyloid 1-42 peptide antibodies. Therefore, a group of healthy nonagenarian elderly subjects was selected in whom, in the absence of immunization, the presence of serum anti beta-amyloid 1-42 peptide antibodies was analyzed by means of performing specific immunoassays, detecting the existence of antibodies against the beta-amyloid 1-42 peptide.

[0143] More particularly, two of the antibodies of the invention have numerous somatic mutations in their nucleotide sequence in comparison to the corresponding genomic genes (germline), indicating the existence of antigenic maturation processes against the beta-amyloid 1-42 peptide. These facts indicate a competent immune response against the beta peptide and reflect the existence of natural anti beta amyloid antibodies, which do not exhibit harmful effects in healthy elderly patients, with a potential therapeutic application in Alzheimer's disease

[0144] Accordingly, the present invention also relates to human anti beta-amyloid 1-42 peptide recombinant and/or monoclonal antibodies and to obtaining them by means of the isolation, cloning and expression of immunoglobulin genes isolated from a representative portion of the genetic repertoire of immunoglobulins from healthy elderly, and particularly, nonagenarian human donors selectively chosen for having full cognitive capacity and for not exhibiting symptoms of Alzheimer's or another neuropathy.

[0145] Another aspect of the invention provides a method of treating and/or preventing the progression of Alzheimer's disease or the symptoms associated with Alzheimer's disease, such as, but not limited to, memory loss and irritability, comprising administering a therapeutically effective amount of an antibody to amyloid beta 1-42, which may reduce intracellular and/or extracellular amyloid beta levels in the brain. In addition, the invention provides a method of treating or preventing the progression of Alzheimer's disease or the symptoms associated with Alzheimer's disease, such as, but not limited to, memory loss and irritability, comprising administering an agent that prevents or interferes with amyloid beta-induced neurotoxicity.

[0146] Methods of obtaining the aforesaid antibodies, as well as methods for modulating one or more amyloid beta 1-42-associated activities using antibodies that bind to amyloid beta, e.g., human amyloid beta 1-42, are also disclosed. Anti-amyloid beta 1-42 antibodies can be used to mitigate amyloid beta 1-42-mediated disorders, e.g., Alzheimer's disease. Anti-amyloid beta 1-42 antibodies can be used alone or in combination with other therapies used to treat the same or another related disease, such as amyloidosis and other neurodegeneratve disorders for which amyloid beta is thought to play a role.

Anti-Human Amyloid beta 1-42 Antibodies

[0147] Antibodies that are capable of binding to, neutralizing and/or inhibiting one or more amyloid beta 1-42 -associated activities, are useful for treating amyloid beta 1-42 -mediated diseases, such as Alzheimer's disease.

[0148] In one embodiment, the anti-amyloid beta 1-42 antibodies disclosed herein are isolated or purified.

[0149] The antibodies of the invention may be used for treating and/or preventing the progression of Alzheimer's disease or to decrease the symptoms associated with Alzheimer's disease, such as for example, memory loss, irritability or anxiety. While not wishing to be bound by theory, the anti-amyloid beta 1-42 antibodies may be administered in a therapeutically

effective amount to reduce intracellular and/or extracellular amyloid beta levels in the brain or may interfere with amyloid beta-induced neurotoxicity.

[0150] In one particular embodiment, the anti-amyloid-beta 1-42 antibodies of the invention are human antibodies. In another particular embodiment, the antibodies are polyclonal or monoclonal antibodies. In yet another particular embodiment, the antibodies are single chain antibodies and fragments thereof.

[0151] Conservative substitutions may be contemplated in the antibodies of the invention. These typically include the substitution of one amino acid for another with similar characteristics, e.g., substitutions within the following groups: valine, glycine; glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; phenylalanine, and tyrosine.

[0152] Antibodies, also known as immunoglobulins, are typically tetrameric glycosylated proteins composed of two light (L) chains of approximately 25 kDa each and two heavy (H) chains of approximately 50 kDa each. Two types of light chain, termed lambda and kappa, may be found in antibodies. Depending on the amino acid sequence of the constant domain of heavy chains, immunoglobulins can be assigned to five major classes: A, D, E, G, and M, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. Each light chain includes an N-terminal variable (V) domain (VL) and a constant (C) domain (CL). Each heavy chain includes an N-terminal V domain (VH), three or four C domains (CHs), and a hinge region. The CH domain most proximal to VH is designated as CHI. The VH and VL domains consist of four regions of relatively conserved sequences called framework regions (FR1, FR2, FR3, and FR4), which form a scaffold for three regions of hypervariable sequences (complementarity determining regions, CDRs). The CDRs contain most of the residues responsible for specific interactions of the antibody with the antigen. CDRs are referred to as CDR1, CDR2, and CDR3. Accordingly, CDR constituents on the heavy chain are referred to as H1, H2, and H3, while CDR constituents on the light chain are referred to as L1, L2, and L3. CDR3 is typically the greatest source of molecular diversity within the antibody-binding site. H3, for example, can be as short as two amino acid residues or greater than 26 amino acids.

[0153] The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known in the art. For a review of the antibody structure, see Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, eds. Harlow et al., 1988. One of skill in the art will recognize that each subunit structure, e.g., a CH, VH, CL, VL, CDR, FR structure, comprises active fragments, e.g., the portion of the VH, VL, or CDR subunit the binds to the antigen, i.e., the antigen-binding fragment, or, e.g., the portion of the CH subunit that binds to and/or activates, e.g., an Fc receptor and/or complement. The CDRs typically refer to the Kabat CDRs, as described in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services (1991), eds. Kabat et al. Another standard for characterizing the antigen binding site is to refer to the hypervariable loops as described by

Chothia. See, e.g., Chothia, D. et al. (1992) J. Mol. Biol. 227:799-817; and Tomlinson et al. (1995) EMBO J. 14:4628-4638. Still another standard is the AbM definition used by Oxford Molecular's AbM antibody modeling software. See, generally, e.g., Protein Sequence and Structure Analysis of Antibody Variable Domains. In: Antibody Engineering Lab Manual (Ed.: Duebel, S. and Kontermann, R., Springer-Verlag, Heidelberg). Embodiments described with respect to Kabat CDRs can alternatively be implemented using similar described relationships with respect to Chothia hypervariable loops or to the AbM-defined loops.

[0154] Antibody diversity, in a natural system, is created by the use of multiple germline genes encoding variable regions and a variety of somatic events. The somatic events include recombination of variable gene segments with diversity (D) and joining (J) gene segments to make a complete VH region, and the recombination of variable and joining gene segments to make a complete VL region. The recombination process itself can be imprecise, resulting in the loss or addition of amino acids at the V(D)J junctions. These mechanisms of diversity occur in the developing B cell prior to antigen exposure. After antigenic stimulation, the expressed antibody genes in B cells undergo somatic mutation. Based on the estimated number of germline gene segments, the random recombination of these segments, and random VH-VL pairing, up to 1.6×10^7 different antibodies could be produced (Fundamental Immunology, 3rd ed. (1993), ed. Paul, Raven Press, New York, N.Y.). When other processes that contribute to antibody diversity (such as somatic mutation) are taken into account, it is thought that upwards of 10^{10} different antibodies could be generated (Immunoglobulin Genes, 2nd ed. (1995), eds. Jonio et al., Academic Press, San Diego, Calif.). Because of the many processes involved in generating antibody diversity, it is unlikely that independently derived monoclonal antibodies with the same antigen specificity will have identical amino acid sequences.

[0155] Thus, this disclosure provides, inter alia, antibodies, and antigen-binding fragments thereof, that bind to amyloid beta 1-42. The antigen-binding fragments, e.g., structures containing a CDR, will generally be an antibody heavy or light chain sequence, or an active fragment thereof, in which the CDR is placed at a location corresponding to the CDR of naturally occurring VH and VL. The structures and locations of immunoglobulin variable domains may be determined as described in Sequences of Proteins of Immunological Interest, U.S. Department of Health and Human Services (1991), eds. Kabat et al.

[0156] Another aspect of the invention provides for methods of treating and/or preventing the progression of Alzheimer's disease or decreasing one or more symptoms associated with Alzheimer's disease, comprising administering a therapeutically effective amount of an antibody that binds to amyloid beta 1-42. In so doing, one may reduce brain amyloid beta levels. Such reduction of brain amyloid beta levels may be the result of treatment with an antibody, which either prevents the production or generation of amyloid beta (such production or generation occurring through the cleavage of beta amyloid precursor protein or APP), or which prevents the aggregation of amyloid beta fibrils and deposition in cerebral parenchymal tissues and blood

vessels, or by preventing the formation of the oligomeric forms of amyloid beta, amyloid beta 1-40 or amyloid beta 1-42, or which increases the degradation of amyloid beta, or which increases the clearance of amyloid beta from the brain, or which facilitates the peripheral metabolism and clearance of amyloid beta. In another aspect the method of treating or preventing Alzheimer's disease comprises administering an agent, such as an antibody that might prevent or interfere with amyloid beta induced neurotoxicity. In one particular embodiment, the amyloid beta is amyloid beta 1-42 or fragments thereof. In another particular embodiment of the present invention, it is envisioned that amyloid beta, including amyloid beta 1-42 and oligomeric forms may be damaging (eg. neurotoxic) even if not deposited. That is, the present invention contemplates that amyloid beta 1-42 may be neurotoxic and can lead to neuronal damage or alterations in neurotransmitter levels or perturbations in neurotransmission through interference with signaling systems, the result of which is Alzheimer's disease and the symptoms associated with Alzheimer's disease.

[0157] Antibody molecules (including antigen-binding fragments) disclosed herein, i.e., antibody molecules that bind to amyloid beta 1-42, include, but are not limited to, human single chain (svFv) recombinant antibodies designated as AD38 (SEQ ID NO: 10) (CECT accession number 7167) and AD64, (SEQ ID NO: 12) (CECT accession number 7168), and variants or derivatives thereof. These antibody molecules and their fragments thereof may be useful in preventing or treating the progression of Alzheimer's disease and one or more symptoms associated with Alzheimer's disease.

[0158] The amino acid sequence of the heavy chain of AD38 (CECT accession number 7167) is set forth in SEQ ID NO: 2, whereas the light chain amino acid sequence of AD38 is shown in SEQ ID NO: 4. The nucleic acid sequence encoding a single chain (scFv) recombinant antibody designated as AD38 (CECT accession number 7167) is set forth in SEQ ID NO: 9. The nucleic acid sequences encoding the heavy or light chain fragments from the antibody designated as AD38 (CECT accession number 7167) are set forth in SEQ ID NOs: 1 and 3, respectively.

[0159] The amino acid sequences of the heavy chain of AD64 (CECT accession number 7168) is set forth in SEQ ID NO: 6 and the light chain amino acid sequence of AD64 is set forth in SEQ ID NO: 8. The nucleic acid sequence encoding a single chain (scFv) recombinant antibody designated as AD64 (CECT accession number 7168) is set forth in SEQ ID NO: 11. The nucleic acid sequences encoding the heavy or light chain fragments from the antibody designated as AD64 (CECT accession number 7168) are set forth in SEQ ID NOs: 5 and 7, respectively.

[0160] In a further particular embodiment, the heavy chain variable domain CDR1, CDR2 or CDR3 of antibody AD38 as defined in Figure 8, the light chain variable domain CDR1, CDR2 or CDR3 of antibody AD38 as defined in Figure 9, the heavy chain variable domain CDR1, CDR2 or CDR3 of antibody AD64 as defined in Figure 10, and the light chain variable domain CDR1,

CDR2 or CDR3 of antibody AD64 as defined in Figure 11, are understood as part of any of the amino acid sequences defined in sequences SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8. It is to be understood that the CDR regions of any of the above-noted antibodies may be genetically engineered into any other protein or antibody molecule or scaffold protein, as described herein, for increasing their stability, e.g. their half-life, or for targeting to the brain or for decreasing their immunogenicity if necessary.

[0161] The CDRs contain most of the residues responsible for specific interactions with the antigen, and are contained within the VH and VL domains, i.e., the heavy chain variable region and the light chain variable region, respectively. Exemplary antibodies include at least one CDR comprising an amino acid sequence selected from the amino acid sequences set forth in SEQ ID NOs: 2, 4, 6, and 8, or selected residues, particularly amyloid beta 1-42 contact residues from such CDRs. Such antibodies may also bind to amyloid beta 1-42 and, e.g., interfere with the formation of a functional amyloid beta 1-42 signaling complex. An antibody may include one or more CDRs of the VL chain or one or more CDRs of the VH chain.

[0162] Also described above, an antigen-binding fragment may be an Fv fragment, which includes VH and VL domains. Thus, an Fv fragment of AD38 or AD64 antibodies may constitute an antibody described herein. It may bind to amyloid beta 1-42 and interfere with the formation of amyloid beta 1-42 or interfere with the neurotoxic properties of amyloid beta 1-42. Other fragments include the Fv fragment, e.g., scFv fragments, Fab fragments, and F(ab')2 fragments of the amyloid beta 1-42 antibodies described herein or of an antibody that includes one or more CDRs having an amino acid sequence selected from the amino acid sequences set forth in SEQ ID NOs: 2, 4, 6, or 8.

[0163] Other such antibody molecules may be produced by methods known to those skilled in the art. For example, the sequences described herein may be used to isolate the antigenic portion of the beta amyloid protein for which the antibodies are specific and afterwards monoclonal antibodies specific for that region of the beta amyloid protein or amyloid beta 1-42 may be produced by generation of hybridomas in accordance with known methods. Hybridomas formed in this manner are then screened using standard methods, such as enzyme-linked immunosorbent assay (ELISA) and surface plasmon resonance (Biacore.TM.) analysis, to identify one or more hybridomas that produce an antibody that specifically binds with amyloid beta 1-42, and neutralizes or inhibits one or more of the amyloid beta 1-42 activities. Recombinant amyloid beta 1-42, naturally occurring amyloid beta 1-42 (i.e., the processed mature form of amyloid beta 1-42), any variants thereof, and antigenic peptide fragments of amyloid beta 1-42 may be used as the immunogen.

[0164] According to the invention, techniques described for the production of single chain antibodies (U.S. Patent Nos. 5,476,786 and 5,132,405 to Huston; U.S. Patent 4,946,778) can be adapted to produce other e.g., amyloid beta-specific single chain antibodies. An additional embodiment of the invention utilizes the techniques described for the construction of Fab

expression libraries (Huse et al., Science, 246:1275-1281 (1989)) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity for amyloid beta 1-42, or its derivatives, or analogs.

[0165] An antigenic peptide fragment of amyloid beta 1-42 can comprise at least 7 continuous amino acid residues and encompasses an epitope such that an antibody raised against the peptide forms a specific immune complex with amyloid beta 1-42. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, even more preferably at least 30 amino acid residues and most preferably at least 40 amino acid residues.

[0166] As an alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal antibody may be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with amyloid beta 1-42, including variants and/or portions thereof, to thereby isolate immunoglobulin library members that bind to amyloid beta 1-42. Techniques and commercially available kits for generating and screening phage display libraries are known to those skilled in the art. Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display include those described in U.S. Pat. No. 5,658,727, U.S. Pat. No. 5,667,988, and U.S. Pat. No. 5,885,793.

[0167] Polyclonal sera and antibodies described herein may be produced by immunizing a suitable subject with amyloid beta 1-42, its variants and/or portions thereof. The antibody titer in the immunized subject may be monitored over time by standard techniques, such as an Enzyme Linked immunosorbent Assay (ELISA), using immobilized amyloid beta 1-42 or other marker proteins (e.g., FLAG). Antibodies may be isolated from an animal or culture media. A variety of methods can be used to purify antibodies including well-known techniques, such as use of protein A chromatography to obtain an IgG fraction.

[0168] Certain embodiments comprise the VH and/or VL domain of the Fv fragment of the anti-amyloid beta 1-42 antibody. Fragments of antibodies e.g., Fab, F(ab')2, Fd, and dAb fragments, may be produced by cleavage of the antibodies or by recombinant engineering. For example, immunologically active Fab and F(ab')2 fragments may be generated by treating the antibodies with an enzyme such as pepsin.

[0169] Further embodiments comprise one or more complementarity determining regions (CDRs) of any of these VH and VL domains.

[0170] The VH and VL domains described herein, in certain embodiments, can be germlined, i.e., the framework regions (FRs) of these domains may be changed using conventional molecular biology techniques to match human germline genes or the consensus amino acid sequences of human germline gene products, at one or more positions (e.g., at least 70, 80, 85, 90, 95, 97, 98, or 99% of framework positions). In other embodiments, the framework sequences remain diverged from the germline.

[0171] Human germline sequences, for example, are disclosed in Tomlinson, I. A. et al. (1992) J. Mol. Biol. 227:776-798; Cook, G. P. et al. (1995) Immunol. Today Vol. 16 (5): 237-242; Chothia, D. et al. (1992) J. Mol. Biol. 227:799-817; and Tomlinson et al. (1995) EMBO J. 14:4628-4638. The V BASE directory provides a comprehensive directory of human immunoglobulin variable region sequences (compiled by Tomlinson, I. A. et al. MRC Centre for Protein Engineering, Cambridge, UK). These sequences can be used as a source of human sequence, e.g., for framework regions and CDRs. Consensus human framework regions can also be used, e.g., as described in U.S. Pat. No. 6,300,064.

[0172] Additionally, chimeric, humanized, and single-chain antibodies described herein, comprising both human and nonhuman portions, may be produced using standard recombinant DNA techniques. Humanized antibodies may also be produced using transgenic mice that express human heavy and light chain genes, but are incapable of expressing the endogenous mouse immunoglobulin heavy and light chain genes.

[0173] Additionally, the antibodies described herein also include those that bind to amyloid beta 1-42, interfere with the formation or function of amyloid beta 1-42 signaling, and have mutations in the constant regions of the heavy and light chains. It is sometimes desirable to mutate and inactivate certain fragments of the constant region. For example, mutations in the heavy constant region can be made to produce antibodies with reduced binding to the Fc receptor (FcR) and/or complement; such mutations are well known in the art.

[0174] Exemplary antibodies can include variants of the sequences provided that retain antigen-binding ability. Such variants may be derived from the provided sequences using techniques well known in the art. Amino acid substitutions, deletions, or additions, can be made in either the FRs or in the CDRs. While changes in the framework regions are usually designed to improve stability and reduce immunogenicity of the antibody, changes in the CDRs are usually designed to increase affinity of the antibody for its target. Such affinity-increasing changes are typically determined empirically by altering the CDR region and testing the antibody. Such alterations can be made according to the methods described in Antibody Engineering, 2nd. ed. (1995), ed. Borrebaeck, Oxford University Press.

[0175] An exemplary method for making a VH domain, which is an amino acid sequence variant of a VH domain, comprises a step of adding, deleting, substituting or inserting one or more amino acids in the amino acid sequence of the VH domain, optionally combining the VH domain with one or more VL domains, and testing the VH domain or VH/VL combination or combinations for specific binding to amyloid beta 1-42, and (preferably) testing the ability of such antigen-binding domain to modulate one or more amyloid beta 1-42 associated activities or signaling. An analogous method may be employed in which one or more sequence variants of a VL domain disclosed herein are combined with one or more VH domains.

[0176] A further aspect of the invention provides a method of preparing an antigen-binding fragment that specifically binds to amyloid beta 1-42. The method comprises: (a) providing a

starting repertoire of nucleic acids encoding a VH domain that either includes a CDR3 to be replaced or lacks a CDR3 encoding region; (b) combining the repertoire with a donor nucleic acid encoding a donor CDR, such that the donor nucleic acid is inserted into the CDR3 region in the repertoire so as to provide a product repertoire of nucleic acids encoding a VH domain; (c) expressing the nucleic acids of the product repertoire; (d) selecting a specific antibody or antigen-binding fragment specific for amyloid beta 1-42; and (e) recovering the specific antibody or antigen-binding fragment or nucleic acid encoding it.

[0177] Moreover, the present invention provides for a process of obtaining antibodies or functional fragments thereof with beta-amyloid 1-42 peptide-binding capacity from the genetic repertoire of immunoglobulins isolated from healthy elderly, and particularly, nonagenarian donors, the process comprising the steps of:

A) purifying B cells from a blood sample or from a bone marrow sample, said sample being obtained from a subject fulfilling the following criteria;
   (i) being 60 years of age or older;
   (ii) having full cognitive capacity and good health;
   (iii) having no symptoms of Alzheimer's disease or another neuropathy;
   (iv) not having previously received any medication for the prevention or treatment of neurodegenerative diseases; and
   (v) possessing detectable anti beta-amyloid 1-42 peptide antibodies in the blood serum;
B) obtaining the immunoglobulin gene repertoire for said anti beta-amyloid 1-42 peptide antibodies from said B cells;
C) using said repertoire to express said antibodies.

[0178] In a particular embodiment, the method for obtaining the immunoglobulin gene repertoire for the anti beta-amyloid 1-42 peptide antibodies from the B cells in step B) is carried out by the following steps:

(i) obtaining mRNA from the said B cells;
(ii) obtaining cDNA from the mRNA of step (i); and;
(iii) using a primer extension reaction to amplify from said cDNA the fragments corresponding to the heavy chains (HC) and the kappa light chains (LC) of said anti beta-amyloid 1-42 peptide antibodies.

In a preferred embodiment, the B cells are peripheral blood B cells obtained from a blood sample.

Other methodologies for obtaining the immunoglobulin gene repertoire from immune cells, for example peripheral blood B cells, are known in the art .

[0179] In another embodiment, an analogous method may be employed in which a VL CDR3 (i.e., L3) described herein is combined with a repertoire of nucleic acids encoding a VL domain, which either includes a CDR3 to be replaced or lacks a CDR3-encoding region. A coding

sequence of a CDR may be introduced into a repertoire of variable domains lacking a CDR (e.g., CDR3), using recombinant DNA technology. For example, Marks et al. (Bio/Technology (1992) 10:779-83) describes methods of producing repertoires of antibody variable domains in which consensus primers directed at or adjacent to the 5' end of the variable domain area are used in conjunction with consensus primers to the third framework region of human VH genes to provide a repertoire of VH variable domains lacking a CDR3. The repertoire may be combined with a CDR3 of a particular antibody. Using analogous techniques, the CDR3-derived sequences may be shuffled with repertoires of VH or VL domains lacking a CDR3, and the shuffled complete VH or VL domains combined with a cognate VL or VH domain to provide specific antigen-binding fragments. The repertoire may then be displayed in a suitable host system such as the phage display system of WO 92/01047, so that suitable antigen-binding fragments can be selected. Analogous shuffling or combinatorial techniques are also disclosed by Stemmer (Nature (1994) 370:389-91). A further alternative is to generate altered VH or VL regions using random mutagenesis of one or more selected VH and/or VL genes to generate mutations within the entire variable domain. See, e.g., Gram et al. Proc. Nat. Acad. Sci. U.S.A. (1992) 89:3576-80.

[0180] Another method that may be used is to direct mutagenesis to CDR regions of VH or VL genes. Such techniques are disclosed by, e.g., Barbas et al. (Proc. Nat. Acad. Sci. U.S.A. (1994) 91:3809-13) and Schier et al. (J. Mol. Biol. (1996) 263:551-67). Similarly, one or more, or all three CDRs may be grafted into a repertoire of VH or VL domains, or even some other scaffold (such as a fibronectin domain). The resulting protein is evaluated for ability to bind to amyloid beta 1-42.

[0181] In one particular embodiment, a binding protein that binds to a target is modified, e.g., by mutagenesis, to provide a pool of modified binding proteins. The modified binding proteins are then evaluated to identify one or more altered binding proteins which have altered functional properties (e.g., improved binding, improved stability, lengthened stability in vivo). In one implementation, display library technology is used to select or screen the pool of modified binding proteins. Higher affinity binding proteins are then identified from the second library, e.g., by using higher stringency or more competitive binding and washing conditions. Other screening techniques can also be used.

[0182] In some embodiments, the mutagenesis is targeted to regions known or likely to be at the binding interface. If, for example, the identified binding proteins are antibodies, then mutagenesis can be directed to the CDR regions of the heavy or light chains as described herein. Further, mutagenesis can be directed to framework regions near or adjacent to the CDRs, e.g., framework regions, particular within 10, 5, or 3 amino acids of a CDR junction. In the case of antibodies, mutagenesis can also be limited to one or a few of the CDRs, e.g., to make step-wise improvements.

[0183] In another embodiment, mutagenesis is used to make an antibody more similar to one or more germline sequences. One exemplary germlining method can include: identifying one or more germline sequences that are similar (e.g., most similar in a particular database) to the sequence of the isolated antibody. Then mutations (at the amino acid level) can be made in the isolated antibody, either incrementally, in combination, or both. For example, a nucleic acid library that includes sequences encoding some or all possible germline mutations is made. The mutated antibodies are then evaluated, e.g., to identify an antibody that has one or more additional germline residues relative to the isolated antibody and that is still useful (e.g., has a functional activity). In one embodiment, as many germline residues are introduced into an isolated antibody as possible.

[0184] In yet another embodiment, mutagenesis is used to substitute or insert one or more germline residues into a CDR region. For example, the germline CDR residue can be from a germline sequence that is similar (e.g., most similar) to the variable region being modified. After mutagenesis, activity (e.g., binding or other functional activity) of the antibody can be evaluated to determine if the germline residue or residues are tolerated. Similar mutagenesis can be performed in the framework regions.

[0185] Selecting a germline sequence can be performed in different ways. For example, a germline sequence can be selected if it meets a predetermined criteria for selectivity or similarity, e.g., at least a certain percentage identity, e.g., at least 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 99.5% identity. The selection can be performed using at least 2, 3, 5, or 10 germline sequences. In the case of CDR1 and CDR2, identifying a similar germline sequence can include selecting one such sequence. In the case of CDR3, identifying a similar germline sequence can include selecting one such sequence, but may including using two germline sequences that separately contribute to the amino-terminal portion and the carboxy-terminal portion. In other implementations more than one or two germline sequences are used, e.g., to form a consensus sequence.

[0186] The invention also provides methods of mutating antibodies to optimize their affinity, selectivity, binding strength or other desirable property. A mutant antibody refers to an amino acid sequence variant of an antibody. In general, one or more of the amino acid residues in the mutant antibody is different from what is present in the reference antibody. Such mutant antibodies necessarily have less than 100% sequence identity or similarity with the reference amino acid sequence. In general, mutant antibodies have at least 75% amino acid sequence identity or similarity with the amino acid sequence of either the heavy or light chain variable domain of the reference antibody. Preferably, mutant antibodies have at least 80%, more preferably at least 85%, even more preferably at least 90%, and most preferably at least 95% amino acid sequence identity or similarity with the amino acid sequence of either the heavy or light chain variable domain of the reference antibody. One method of mutating antibodies involves "affinity maturation" using phage display.

[0187] "Affinity maturation" using phage display refers to a process described in Lowman et al., Biochemistry 30(45): 10832-10838 (1991), see also Hawkins et al., J. Mol Biol. 254: 889-896 (1992). While not strictly limited to the following description, this process can be described briefly as involving mutation of several antibody hypervariable regions in a number of different sites with the goal of generating all possible amino acid substitutions at each site. The antibody mutants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusion proteins. Fusions are generally made to the gene III product of M13. The phage expressing the various mutants can be cycled through several rounds of selection for the trait of interest, e.g. binding affinity or selectivity. The mutants of interest are isolated and sequenced. Such methods are described in more detail in U.S. Pat. No. 5,750,373, U.S. Pat. No. 6,290,957 and Cunningham, B. C. et al., EMBO J. 13(11), 2508-2515 (1994).

[0188] Any cloning procedure used by one of skill in the art can be employed to make the expression vectors used in such affinity maturation/phage display procedures. For example, one of skill in the art can readily employ known cloning procedures to fuse a nucleic acid encoding an antibody hypervariable region to a nucleic acid encoding a phage coat protein. See, e.g., Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y., 1989; Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y., 2001.

[0189] In another embodiment, an antibody or fragment thereof has CDR sequences that differ only insubstantially from those of the antibodies described herein. Insubstantial differences include minor amino acid changes, such as substitutions of 1 or 2 out of any of typically 5-7 amino acids in the sequence of a CDR, e.g., a Chothia or Kabat CDR. Typically an amino acid is substituted by a related amino acid having similar charge, hydrophobic, or stereochemical characteristics. Such substitutions would be within the ordinary skills of an artisan. Unlike in CDRs, more substantial changes in structure framework regions (FRs) can be made without adversely affecting the binding properties of an antibody. Changes to FRs include, but are not limited to, humanizing a nonhuman-derived framework or engineering certain framework residues that are important for antigen contact or for stabilizing the binding site, e.g., changing the class or subclass of the constant region, changing specific amino acid residues which might alter an effector function such as Fc receptor binding (Lund et al. (1991) J. Immunol. 147:2657-62; Morgan et al. (1995) Immunology 86:319-24), or changing the species from which the constant region is derived. Antibodies may have mutations in the CH2 region of the heavy chain that reduce or alter effector function, e.g., Fc receptor binding and complement activation. For example, antibodies may have mutations such as those described in U.S. Pat. Nos. 5,624,821 and 5,648,260. Antibodies may also have mutations that stabilize the disulfide bond between the two heavy chains of an immunoglobulin, such as mutations in the hinge region of IgG4, as disclosed in the art (e.g., Angal et al. (1993) Mol. Immunol. 30:105-08).

[0190] The amyloid beta 1-42 binding proteins can be in the form of intact antibodies, fragments of antibodies, e.g., Fab, F(ab')2, Fd, dAb, and scFv fragments, and intact antibodies and fragments that have been mutated either in their constant and/or variable region (e.g., mutations to produce chimeric, partially humanized, or fully humanized antibodies, as well as to produce antibodies with a desired trait, e.g., enhanced amyloid beta 1-42 binding and/or reduced FcR binding).

[0191] In some embodiments, a substantial portion of an immunoglobulin variable domain can comprise at least one of the CDR regions and, optionally, their intervening framework regions from the variable regions as set out herein. The portion will also include at least about 50, 60, 70, 80, 85, 87, 88, 90, 92, 94, 95, 96, 97, 98% of either or both of FR1 and FR4. For example, the portion which may be contiguous or non-contiguous may include the C-terminal 50% of FR1 and the N-terminal 50% of FR4. Additional residues at the N-terminal or C-terminal end of the substantial part of the variable domain may be those not normally associated with naturally occurring variable domain regions. For example, construction of specific antigen-binding fragments made by recombinant DNA techniques may result in the introduction of N- or C-terminal residues encoded by linkers introduced to facilitate cloning or other manipulation steps. Other manipulation steps include the introduction of linkers to join variable domains described herein to further protein sequences, including immunoglobulin heavy chains, other variable domains (e.g., in the production of diabodies) or protein labels as discussed in more detail below.

[0192] The invention also encompasses binding fragments containing a single variable domain derived from either VH or VL domain sequences, especially VH domains. In the case of either of the single-chain specific binding domains, these domains may be used to screen for complementary domains capable of forming a two-domain specific antigen-binding domain capable of binding amyloid beta 1-42. This may be achieved by phage display screening methods using the so-called hierarchical dual combinatorial approach (as disclosed in, e.g., WO 92/01047) in which an individual colony containing either an H or L chain clone is used to infect a complete library of clones encoding the other chain (L or H) and the resulting two-chain specific antigen-binding domain is selected in accordance with phage display techniques such as those described in that reference. This technique is also disclosed in Marks et al., supra. Antibodies can be conjugated by chemical methods with radionuclides, drugs, macromolecules, or other agents, or can be made as fusion proteins comprising one or more CDRs described herein.

[0193] An antibody fusion protein contains a VH-VL pair where one of these chains (usually VH) and another protein are synthesized as a single polypeptide chain. These types of products differ from antibodies in that they generally have an additional functional element: e.g., the active moiety of a small molecule or the principal molecular structural feature of the conjugated or fused macromolecule.

[0194] In addition to the changes to the amino acid sequence noted above, the antibodies can be glycosylated, pegylated, or linked to albumin or a nonproteinaceous polymer. For instance, the presently disclosed antibodies may be linked to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Pat. Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192; or 4,179,337. The antibodies are chemically modified by covalent conjugation to a polymer to increase their circulating half-life, for example. Exemplary polymers, and methods to attach them to peptides, are also shown in U.S. Pat. Nos. 4,766,106; 4,179,337; 4,495,285; and 4,609,546.

[0195] In other embodiments, the antibody may be modified to have an altered glycosylation pattern (e.g., altered from the original or native glycosylation pattern). As used in this context, "altered" means having one or more carbohydrate moieties deleted, and/or having one or more glycosylation sites added to the original antibody. Addition of glycosylation sites to the presently disclosed antibodies may be accomplished by altering the amino acid sequence to contain glycosylation site consensus sequences; such techniques are well known in the art. Another means of increasing the number of carbohydrate moieties on the antibodies is by chemical or enzymatic coupling of glycosides to the amino acid residues of the antibody. These methods are described in, e.g., WO 87/05330, and Aplin and Wriston ((1981) CRC Crit. Rev. Biochem. 22:259-306). Removal of any carbohydrate moieties present on the antibodies may be accomplished chemically or enzymatically as described in the art (Hakimuddin et al. (1987) Arch. Biochem. Biophys. 259:52; Edge et al. (1981) Anal. Biochem. 118:131; and Thotakura et al. (1987) Meth. Enzymol. 138:350). See, e.g., U.S. Pat. No. 5,869,046 for a modification that increases in vivo half life by providing a salvage receptor binding epitope.

[0196] In addition, combinatorial libraries of antibodies have been generated in bacteriophage lambda expression systems which may be screened as bacteriophage plaques or as colonies of lysogens (Huse et al. (1989) Science 246: 1275; Caton and Koprowski (1990) Proc. Natl. Acad. Sci. (U.S.A.) 87: 6450; Mullinax et al (1990) Proc. Natl. Acad. Sci. (U.S.A.) 87: 8095; Persson et al. (1991) Proc. Natl. Acad. Sci. (U.S.A.) 88: 2432). Various embodiments of bacteriophage antibody display libraries and lambda phage expression libraries have been described (Kang et al. (1991) Proc. Natl. Acad. Sci. (U.S.A.) 88: 4363; Clackson et al. (1991) Nature 352: 624; McCafferty et al. (1990) Nature 348: 552; Burton et al. (1991) Proc. Natl. Acad. Sci. (U.S.A.) 88: 10134; Hoogenboom et al. (1991) Nucleic Acids Res. 19: 4133; Chang et al. (1991) J. Immunol. 147: 3610; Breitling et al. (1991) Gene 104: 147; Marks et al. (1991) J. Mol. Biol. 222: 581; Barbas et al. (1992) Proc. Natl. Acad. Sci. (U.S.A.) 89: 4457; Hawkins and Winter (1992) J. Immunol. 22: 867; Marks et al. (1992) Biotechnology 10: 779; Marks et al. (1992) J. Biol. Chem. 267: 16007; Lowman et al (1991) Biochemistry 30: 10832; Lerner et al. (1992) Science 258: 1313, incorporated herein by reference). Typically, a bacteriophage antibody display library is screened with an antigen (e.g., polypeptide, carbohydrate, glycoprotein, nucleic acid) that is

immobilized (e.g., by covalent linkage to a chromatography resin to enrich for reactive phage by affinity chromatography) and/or labeled (e.g., to screen plaque or colony lifts).

[0197] One particularly advantageous approach has been the use of so-called single-chain fragment variable (scFv) libraries (Marks et al. (1992) Biotechnology 10: 779; Winter G and Milstein C (1991) Nature 349: 293; Clackson et al. (1991) op.cit.; Marks et al. (1991) J. Mol. Biol. 222: 581; Chaudhary et al. (1990) Proc. Natl. Acad. Sci. (USA) 87: 1066; Chiswell et al. (1992) TIBTECH 10: 80; McCafferty et al. (1990) op.cit.; and Huston et al. (1988) Proc. Natl. Acad. Sci. (USA) 85: 5879). Various embodiments of scFv libraries displayed on bacteriophage coat proteins have been described.

[0198] Beginning in 1988, single-chain analogues of Fv fragments and their fusion proteins have been reliably generated by antibody engineering methods. The first step generally involves obtaining the genes encoding V_H and V_L regions with desired binding properties; these V genes may be isolated from a specific hybridoma cell line, selected from a combinatorial V-gene library, or made by V gene synthesis. The single-chain Fv is formed by connecting the component V genes with an oligonucleotide that encodes an appropriately designed linker peptide, such as $(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_3$ or equivalent linker peptide(s). The linker bridges the C-terminus of the first V region and N-terminus of the second, ordered as either V_H -linker-V_L or V_L -linker-V_H. In principle, the scFv binding site can faithfully replicate both the affinity and specificity of its parent antibody combining site.

[0199] Thus, scFv fragments are comprised of V_H and V_L regions linked into a single polypeptide chain by a flexible linker peptide. After the scFv genes are assembled, they are cloned into a phagemid and expressed at the tip of the M13 phage (or similar filamentous bacteriophage) as fusion proteins with the bacteriophage pIII (gene 3) coat protein. Enriching for phage expressing an antibody of interest is accomplished by panning the recombinant phage displaying a population scFv for binding to a predetermined epitope (e.g., target antigen, receptor).

[0200] The linked polynucleotide of a library member provides the basis for replication of the library member after a screening or selection procedure, and also provides the basis for the determination, by nucleotide sequencing, of the identity of the displayed peptide sequence or V_H and V_L amino acid sequence. The displayed peptide(s) or single-chain antibody (e.g., scFv) and/or its V_H and V_L regions or their CDRs can be cloned and expressed in a suitable expression system. Often polynucleotides encoding the isolated V_H and V_L regions will be ligated to polynucleotides encoding constant regions (C_H and C_L) to form polynucleotides encoding complete antibodies (e.g., chimeric or fully-human), antibody fragments, and the like. Often polynucleotides encoding the isolated CDRs will be grafted into polynucleotides encoding a suitable variable region framework (and optionally constant regions) to form polynucleotides encoding complete antibodies (e.g., humanized or fully-human), antibody fragments, and the like. Antibodies can be used to isolate preparative quantities of the antigen by immunoaffinity

chromatography. Various other uses of such antibodies are to diagnose and/or stage disease (e.g., neoplasia), and for therapeutic application to treat disease, such as for example: neoplasia, HIV infections and the like.

[0201] Various methods have been reported for increasing the combinatorial diversity of a scFv library to broaden the repertoire of binding species. The use of PCR (polymerase chain reaction) has permitted the variable regions to be rapidly cloned either from a specific hybridoma source or as a gene library from non-immunized cells, affording combinatorial diversity in the assortment of V_H and V_L cassettes which can be combined. Furthermore, the V_H and V_L cassettes can themselves be diversified, such as by random, pseudorandom, or directed mutagenesis. Typically, V_H and V_L cassettes are diversified in or near the complementarity-determining regions (CDRs), often the third CDR, CDR3. Enzymatic inverse PCR mutagenesis has been shown to be a simple and reliable method for constructing relatively large libraries of scFv site-directed mutants (Stemmer et al. (1993) Biotechniques 14: 256), as has error-prone PCR and chemical mutagenesis (Deng et al. (1994) J. Biol. Chem. 269: 9533). Riechmann et al. [Biochemistry 32: 8848; (1993)] showed semirational design of an antibody scFv fragment using site-directed randomization by degenerate oligonucleotide PCR and subsequent phage display of the resultant scFv mutants.

[0202] Selection of antibodies specific for amyloid beta 1-42 is based on binding affinity to amyloid beta 1-42 and may be determined by various well-known immunoassays including, enzyme-linked immunosorbent, chemiluminescent, immunofluorescent, immunohistochemical, radioimmunoassay, and immunoprecipitation assays and the like which may be performed in vitro, in vivo or in situ. The standard techniques known in the art for immunoassays are described in "Methods in Immunodiagnosis", 2nd Edition, Rose and Bigazzi, eds. John Wiley & Sons, 1980; Campbell et al., "Methods and Immunology", W.A. Benjamin, Inc., 1964; and Oellerich, M. (1984) J. Clin. Chem. Clin. Biochem. **22**:895-904.

[0203] Antibodies described herein may also be tagged with a detectable or functional label. Detectable labels include radiolabels such as ^{131}I or ^{99}Tc, which may be attached to antibodies described herein using conventional chemistry known in the art. Labels also include enzyme labels such as horseradish peroxidase or alkaline phosphatase. Labels further include chemical moieties such as biotin, which may be detected via binding to a specific cognate detectable moiety, e.g., labeled avidin.

[0204] The binding characteristics of an antibody disclosed herein may be measured by any suitable methods, including the following methods: Biacore analysis, Enzyme Linked Immunosorbent Assay (ELISA), x-ray crystallography, sequence analysis and scanning mutagenesis as described in the Examples below, and other methods that are well known in the art.

[0205] The antibodies of the present invention may be formulated, e.g., as a pharmaceutical composition.

[0206] The anti-amyloid beta 1-42 antibodies disclosed herein are also useful for isolating, purifying, and/or detecting amyloid beta 1-42 in supernatant, cellular lysate, or on the cell surface or in tissue. Antibodies disclosed herein can be used diagnostically to monitor amyloid beta 1-42 protein levels as part of a clinical testing procedure. The present disclosure also provides novel isolated and purified polynucleotides and polypeptides related to novel antibodies directed against human amyloid beta 1-42. The genes, polynucleotides, proteins, and polypeptides disclosed herein include, but are not limited to, a human antibody to amyloid beta 1-42 and fragments, variants or derivatives thereof.

Anti-Amyloid Beta 1-42 Antibody Polynucleotides and Polypeptides

[0207] The disclosure provides purified and isolated polynucleotides encoding the anti-amyloid beta 1-42 antibodies and fragments thereof. The isolated nucleic acids of the present invention encoding the anti amyloid beta 1-42 antibodies can be made using (a) recombinant methods, (b) synthetic techniques, (c) purification techniques, or combinations thereof, as well-known in the art. DNA encoding the monoclonal antibodies is readily isolated and sequenced using methods known in the art (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). Where a hybridoma is produced, such cells can serve as a source of such DNA. Alternatively, using display techniques such as phage or ribosomal display libraries, the selection of the binder and the nucleic acid is simplified. After phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described below. Techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in International publication No. WO 92/22324; Mullinax et al., 1992, BioTechniques 12(6):864-869; Sawai et al., 1995, AJR134:26-34; and Better et al., 1988, Science 240:1041-1043 (said references entirely incorporated by reference).

[0208] Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as E. coli cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce antibody protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra et al., Curr. Opinion in Immunol., 5:256-262 (1993) and Pluckthun, Immunol. Revs. 130:151-188 (1992).

[0209] Known methods of DNA or RNA amplification include, but are not limited to, polymerase chain reaction (PCR) and related amplification processes. PCR and other *in vitro* amplification methods can also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of the desired mRNA in samples, for nucleic acid sequencing, or for other purposes. Examples of

techniques sufficient to direct persons of skill through in vitro amplification methods are found in Berger, supra, Sambrook, supra, and Mullis, et al., U.S. Pat. No. 4,683,202 (1987); and Innis, et al., PCR Protocols A Guide to Methods and Applications, Eds., Academic Press Inc., San Diego, Calif. (1990). Commercially available kits for genomic PCR amplification are known in the art. See, e.g., Advantage-GC Genomic PCR Kit (Clontech). Additionally, e.g., the T4 gene 32 protein (Boehringer Mannheim) can be used to improve yield of long PCR products.

[0210] The isolated nucleic acid compositions of this invention, such as RNA, cDNA, genomic DNA, or any combination thereof, can be obtained from biological sources using any number of cloning methodologies known to those of skill in the art. In some embodiments, oligonucleotide probes that selectively hybridize, under stringent conditions, to the polynucleotides of the present invention are used to identify the desired sequence in a cDNA or genomic DNA library. The isolation of RNA, and construction of cDNA and genomic libraries, is well known to those of ordinary skill in the art. (See, e.g., Sambrook, supra).

[0211] The nucleic acid molecules of the invention are nucleic acids encoding antibodies or antibody portions (e.g., VH, VL, CDR3) that bind to amyloid beta 1-42. "Isolated nucleic acid molecules" of the invention is intended to refer to a nucleic acid molecule in which the nucleotide sequences encoding the antibody or antibody portion are free of other nucleotide sequences encoding antibodies or antibody portions that bind antigens other than amyloid beta 1-42.

[0212] In one particular embodiment, mutations can be introduced randomly along all or part of an anti-amyloid beta 1-42 antibody coding sequence, such as by saturation mutagenesis or by recombination, and the resulting modified anti-amyloid beta 1-42 antibodies can be screened for binding activity.

[0213] In a more particular embodiment, the nucleic acid sequence encoding a single chain (scFv) recombinant antibody designated as AD38 (CECT accession number 7167) is set forth in SEQ ID NO: 9. The nucleic acid sequences encoding the heavy or light chain fragments from the antibody designated as AD38 (CECT accession number 7167) are set forth in SEQ ID NOs: 1 and 3, respectively.

[0214] In yet another particular embodiment, the nucleic acid sequence encoding a single chain (scFv) recombinant antibody designated as AD64 (CECT accession number 7168) is set forth in SEQ ID NO: 11. The nucleic acid sequences encoding the heavy or light chain fragments from the antibody designated as AD64 (CECT accession number 7168) are set forth in SEQ ID NOs: 5 and 7, respectively.

[0215] The polynucleotides can also include polynucleotides that hybridize under stringent conditions to any of the sequences set forth in SEQ ID NOs: 1, 3, 5, 7, 9 and 11, or complements thereof, and/or that encode polypeptides that retain substantial biological activity (i.e., active fragments) of the variable regions encoded by these sequences. The polynucleotides can also include continuous portions of the any of the sequences set forth in SEQ ID NOs: 1, 3, 5, 7, 9 and 11, comprising at least 21 consecutive nucleotides.

[0216] The invention also provides for nucleotide sequences that are substantially homologous to the sequences encoding the antibodies to amyloid beta 1-42, as described herein. The percent identity between two nucleotide sequences can be determined using the GAP program in the GCG software package (available at http://www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. The percent identity between two nucleotide or amino acid sequences can also determined using the algorithm of E. Meyers and W. Miller (Comput. AppL. Biosci., 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM 1 20 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (J. Mol. Biol. 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blossum 62 matrix or a PAM2 5 0 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (1990) J. Mol. Biol. 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score=100, wordlength=12 to obtain nucleotide sequences homologous to the nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to the protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) Nucleic Acids Res. 25(17):3389. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov.

[0217] In a further particular embodiment, the heavy chain variable domain CDR1, CDR2 or CDR3 of antibody AD38 as defined in Figure 8, the light chain variable domain CDR1, CDR2 or CDR3 of antibody AD38 as defined in Figure 9, the heavy chain variable domain CDR1, CDR2 or CDR3 of antibody AD64 as defined in Figure 10, and the light chain variable domain CDR1, CDR2 or CDR3 of antibody AD64 as defined in Figure 11, are understood as part of any of the amino acid sequences defined in sequences SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8. It is to be understood that the CDR regions of any of the above-noted antibodies may be genetically engineered into any other protein or antibody molecule or scaffold protein, as described herein, for increasing their stability, e.g. their half-life, or for targeting to the brain or for decreasing their immunogenicity if necessary.

[0218] Polypeptides disclosed herein also include continuous portions of any of the sequences set forth in SEQ ID NOs: 2, 4, 6, 8, 10 and 12 comprising at least 4 consecutive amino acids and that retain substantial biological or binding activity (i.e., active fragments) of these sequences.

Preferably, polypeptides of the present application include continuous portions of any of the sequences set forth in SEQ ID NOs: 2, 4, 6, 8, 10 and 12 comprising 5-7 amino acids. More preferred polypeptides of the present application include any continuous portion of the any of sequences set forth in SEQ ID NOs: 2, 4, 6, 8, 10 and 12 that retains substantial biological or binding activity of the antibodies designated as AD38 and AD64. Polynucleotides disclosed herein also include, in addition to those polynucleotides described above, polynucleotides that encode any of the amino acid sequences set forth in SEQ ID NOs: 2, 4, 6, 8, 10 and 12, or a continuous portion thereof, and that differ from the polynucleotides described in SEQ ID NOs: 1, 3, 5, 7, 9 and 11 only due to the well-known degeneracy of the genetic code.

[0219] The isolated polynucleotides disclosed herein may be used as hybridization probes and primers to identify and isolate nucleic acids having sequences identical to or similar to those encoding the disclosed polynucleotides. Hybridization methods for identifying and isolating nucleic acids include polymerase chain reaction (PCR), Southern hybridization, in situ hybridization and Northern hybridization, and are well known to those skilled in the art.

[0220] Hybridization reactions can be performed under conditions of different stringency. The stringency of a hybridization reaction includes the difficulty with which any two nucleic acid molecules will hybridize to one another. Preferably, each hybridizing polynucleotide hybridizes to its corresponding polynucleotide under reduced stringency conditions, more preferably stringent conditions, and most preferably highly stringent conditions. The term "standard hybridization conditions" refers to salt and temperature conditions substantially equivalent to 5 x SSC and 65°C for both hybridization and wash. However, one skilled in the art will appreciate that such "standard hybridization conditions" are dependent on particular conditions including the concentration of sodium and magnesium in the buffer, nucleotide sequence length and concentration, percent mismatch, percent formamide, and the like. Also important in the determination of "standard hybridization conditions" is whether the two sequences hybridizing are RNA-RNA, DNA-DNA or RNA-DNA. Such standard hybridization conditions are easily determined by one skilled in the art according to well known formulae, wherein hybridization is typically 10-20°C below the predicted or determined T_m with washes of higher stringency, if desired.

[0221] Procedures using such conditions of high stringency are as follows. Prehybridization of filters containing DNA is carried out for 15 minutes to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 µg/ml denatured salmon sperm DNA. Filters are hybridized for 48 h at 65°C in prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 X 10^6 cpm of ^{32}P-labeled probe. Washing of filters is done at 37°C for 1 h in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1X SSC at 50°C for 45 min before autoradiography. Other conditions of high stringency that may be used are well known in the art.

[0222] The isolated polynucleotides disclosed herein may be used as hybridization probes and primers to identify and isolate DNA having sequences encoding allelic variants of the disclosed polynucleotides. Allelic variants are naturally occurring alternative forms of the disclosed polynucleotides that encode polypeptides that are identical to or have significant similarity to the polypeptides encoded by the disclosed polynucleotides. Preferably, allelic variants have at least 90% sequence identity (more preferably, at least 95% identity; most preferably, at least 99% identity) with the disclosed polynucleotides.

[0223] The isolated polynucleotides disclosed herein may also be used as hybridization probes and primers to identify and isolate DNAs having sequences encoding polypeptides homologous to the disclosed polynucleotides. These homologs are polynucleotides and polypeptides isolated from a different species than that of the disclosed polypeptides and polynucleotides, or within the same species, but with significant sequence similarity to the disclosed polynucleotides and polypeptides. Preferably, polynucleotide homologs have at least 50%, 70%, 75%, 80%, 85%, 87%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence with the disclosed polynucleotides

[0224] The isolated polynucleotides disclosed herein may also be used as hybridization probes and primers to identify cells and tissues that express the antibodies and the conditions under which they are expressed.

[0225] The isolated polynucleotides disclosed herein may be operably linked to an expression control sequence for recombinant production of the polypeptides described herein. A polynucleotide can be operably linked to a nucleotide sequence encoding a constant region, e.g., a constant region of one of the various antibody isotypes. For example, a polynucleotide that encodes a light chain disclosed herein (e.g., any one of those set forth in SEQ ID NOs: 4 or 8) may be operably linked to a nucleotide sequence that encodes the constant region (or derivatives thereof) of either a kappa light chain or lambda light chain, such that the expression of the linked nucleotides will result in a full kappa or lambda light chain with a variable region that specifically binds to amyloid beta 1-42 and interferes with the formation of a functional amyloid beta 1-42 signaling complex, and neutralizes or inhibits the formation of amyloid beta 1-42 or one or more amyloid beta 1-42 associated activities. Similarly, a polynucleotide that encodes a heavy chain region disclosed herein (e.g., any of those set forth in SEQ ID NOs:2 or 6) may be operably linked to a nucleotide sequence that encodes the constant region of a heavy chain isotype (or derivatives thereof), e.g., IgM, IgD, IgE, IgG and IgA. General methods of expressing recombinant proteins are well known in the art. Such recombinant proteins may be expressed in soluble form for use in treatment of disorders resulting from amyloid beta mediated signaling (e.g., Alzheimer's disease).

[0226] The recombinant expression vectors disclosed herein may carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into

which the vector has been introduced (see, e.g., U.S. Pat. Nos. 4,399,216, 4,634,665 and 5,179,017). For example, typically the selectable marker gene confers resistance to a drug, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr$^-$ host cells with methotrexate selection/amplification) and the neo gene (for G418 selection).

[0227] A number of cell lines are suitable host cells for recombinant expression. Mammalian host cell lines include, for example, COS cells, CHO cells, 293T cells, A431 cells, 3T3 cells, CV-1 cells, HeLa cells, L cells, BHK21 cells, HL-60 cells, U937 cells, HaK cells, Jurkat cells, as well as cell strains derived from in vitro culture of primary tissue and primary explants.

[0228] Alternatively, it may be possible to recombinantly produce polypeptides in lower eukaryotes such as yeast (e.g., Saccharomyces, Pichia, Kluyveromyces strains, and Candida strains) or in prokaryotes (e.g., *Escherichia coli*, *Bacillus subtilis*, and *Salmonella typhimurium*). Polypeptides made in yeast or bacteria can be modified, e.g., glycosylation of appropriate sites

[0229] Polypeptides can also be produced in animal cells, e.g., insect or mammalian cells. For example, a sequence encoding the polypeptide can be inserted into an insect expression vector, such as a baculovirus vector, and used in an insect cell expression system (e.g., the MAXBAC.RTM. kit, Invitrogen, Carlsbad, Calif.).

[0230] The polypeptides disclosed herein may then be purified from culture medium or cell extracts using known purification processes, such as gel filtration and ion exchange chromatography. Purification may also include affinity chromatography with agents known to bind the polypeptides disclosed herein.

[0231] Alternatively, the polypeptides disclosed herein may also be recombinantly expressed in a form that facilitates purification. For example, the polypeptides may be expressed as fusions with proteins such as maltose-binding protein (MBP), glutathione-S-transferase (GST), or thioredoxin (TRX) or as fusions to hexa-histidine, penta-histidine, or small epitope tags, e.g., the FLAG epitope.

[0232] The polypeptides disclosed herein also encompass molecules that are structurally different from the disclosed polypeptides (e.g., which have a slightly altered sequence), but which have substantially the same biochemical properties as the disclosed polypeptides (e.g., are changed only in functionally nonessential amino acid residues). Such molecules include naturally occurring allelic variants and deliberately engineered variants containing alterations, substitutions, replacements, insertions, or deletions. Techniques for such alterations, substitutions, replacements, insertions, or deletions are well known to those skilled in the art.

Other Specific Amyloid Beta 1-42 Binding Members or Agents

[0233] Also provided are binding members or agents, other than binding agents that are antibodies and fragments thereof, that bind to amyloid beta 1-42, particularly binding agents that compete with the antibodies AD38 and AD64 and other antibodies described herein for binding

to amyloid beta 1-42. For example, the binding agents can bind to the same epitope or an overlapping epitope as AD38 and AD64 on amyloid beta 1-42. The binding agents preferably inhibit formation of a neurotoxic form of amyloid beta 1-42 or neutralize amyloid beta 1-42 activity. Such binding agents can be used in the methods described herein, e.g., the methods of treating and preventing disorders, in particular Alzheimer's disease. All embodiments described herein can be adapted for use with amyloid beta 1-42 binding agents.

[0234] Binding agents can be identified by a number of means, including modifying a variable domain described herein or grafting one or more CDRs of a variable domain described herein onto another scaffold. Binding agents can also be identified from diverse libraries, e.g., by screening. One method for screening protein libraries uses phage display. Particular regions of a protein are varied and proteins that interact with amyloid beta 1-42 are identified, e.g., by retention on a solid support or by other physical association. To identify particular binding agents that bind to the same epitope or an overlapping epitope as AD38 or AD64 on amyloid beta 1-42, binding agents can be eluted by adding AD38 or AD64 (or related antibody), or binding agents can be evaluated in competition experiments with AD38 or AD64 (or related antibody). It is also possible to deplete the library of agents that bind to other epitopes by contacting the library to a complex that contains amyloid beta 1-42 and antibody AD38 or AD64 (or related antibody). The depleted library can then be contacted to amyloid beta 1-42 to obtain a binding agent that binds to amyloid beta 1-42 but not to amyloid beta 1-42 when it is bound by AD38 or AD64. It is also possible to use peptides from amyloid beta 1-42 that contain the AD38 or AD64 epitope as a target.

[0235] Phage display is described, for example, in U.S. Pat. No. 5,223,409; Smith (1985) Science 228:1315-1317; WO 92/18619; WO 91/17271; WO 92/20791; WO 92/15679; WO 93/01288; WO 92/01047; WO 92/09690; WO 90/02809; WO 94/05781; Fuchs et al. (1991) Bio/Technology 9:1370-1372; Hay et al. (1992) Hum Antibod Hybridomas 3:81-85; Huse et al. (1989) Science 246:1275-1281; Griffiths et al. (1993) EMBO J. 12:725-734; Hawkins et al. (1992) J Mol Biol 226:889-896; Clackson et al. (1991) Nature 352:624-628; Gram et al. (1992) PNAS 89:3576-3580; Garrard et al. (1991) Bio/Technology 9:1373-1377; Rebar et al. (1996) Methods Enzymol. 267:129-49; and Barbas et al. (1991) PNAS 88:7978-7982. Yeast surface display is described, e.g., in Boder and Wittrup (1997) Nat. Biotechnol. 15:553-557. Another form of display is ribosome display. See, e.g., Mattheakis et al. (1994) Proc. Natl. Acad. Sci. USA 91:9022 and Hanes et al. (2000) Nat Biotechnol. 18:1287-92; Hanes et al. (2000) Methods Enzymol. 328:404-30. and Schaffitzel et al. (1999) J Immunol Methods. 231(1-2):119-35.

[0236] Binding agents that bind to amyloid beta 1-42 can have structural features of one scaffold proteins, e.g., a folded domain. An exemplary scaffold domain, based on an antibody, is a "minibody" scaffold has been designed by deleting three beta strands from a heavy chain variable domain of a monoclonal antibody (Tramontano et al., 1994, J. Mol. Recognit. 7:9; and Martin et al., 1994, The EMBO Journal 13, pp. 5303-5309). This domain includes 61 residues

and can be used to present two hypervariable loops, e.g., one or more hypervariable loops of a variable domain described herein or a variant described herein. In another approach, the binding agent includes a scaffold domain that is a V-like domain (Coia et al. WO 99/45110). V-like domains refer to a domain that has similar structural features to the variable heavy (VH) or variable light (VL) domains of antibodies. Another scaffold domain is derived from tendamistatin, a 74 residue, six-strand beta sheet sandwich held together by two disulfide bonds (McConnell and Hoess, 1995, J. Mol. Biol. 250:460). This parent protein includes three loops. The loops can be modified (e.g., using CDRs or hypervariable loops described herein) or varied, e.g., to select domains that bind to amyloid beta 1-42. WO 00/60070 describes a β-sandwich structure derived from the naturally occurring extracellular domain of CTLA-4 is used as a scaffold.

[0237] Still another scaffold domain for an amyloid beta 1-42 binding agent is a domain based on the fibronectin type III domain or related fibronectin-like proteins. The overall fold of the fibronectin type III (Fn3) domain is closely related to that of the smallest functional antibody fragment, the variable region of the antibody heavy chain. Fn3 is a β-sandwich similar to that of the antibody VH domain, except that Fn3 has seven β-strands instead of nine. There are three loops at the end of Fn3; the positions of BC, DE and FG loops approximately correspond to those of CDR1, 2 and 3 of the VH domain of an antibody. Fn3 is advantageous because it does not have disulfide bonds. Therefore, Fn3 is stable under reducing conditions, unlike antibodies and their fragments (see WO 98/56915; WO 01/64942; WO 00/34784). An Fn3 domain can be modified (e.g., using CDRs or hypervariable loops described herein) or varied, e.g., to select domains that bind to amyloid beta 1-42.

[0238] Still other exemplary scaffold domains include: T-cell receptors; MHC proteins; extracellular domains (e.g., fibronectin Type III repeats, EGF repeats); protease inhibitors (e.g., Kunitz domains, ecotin, BPTI, and so forth); TPR repeats; trifoil structures; zinc finger domains; DNA-binding proteins; particularly monomeric DNA binding proteins; RNA binding proteins; enzymes, e.g., proteases (particularly inactivated proteases), RNase; chaperones, e.g., thioredoxin, and heat shock proteins; and intracellular signaling domains (such as SH2 and SH3 domains). U.S. 20040009530 describes examples of some alternative scaffolds.

[0239] Examples of small scaffold domains include: Kunitz domains (58 amino acids, 3 disulfide bonds), Cucurbida maxima trypsin inhibitor domains (31 amino acids, 3 disulfide bonds), domains related to guanylin (14 amino acids, 2 disulfide bonds), domains related to heat-stable enterotoxin IA from gram negative bacteria (18 amino acids, 3 disulfide bonds), EGF domains (50 amino acids, 3 disulfide bonds), kringle domains (60 amino acids, 3 disulfide bonds), fungal carbohydrate-binding domains (35 amino acids, 2 disulfide bonds), endothelin domains (18 amino acids, 2 disulfide bonds), and Streptococcal G IgG-binding domain (35 amino acids, no disulfide bonds). Examples of small intracellular scaffold domains include SH2, SH3, and EVH domains. Generally, any modular domain, intracellular or extracellular, can be used.

[0240] Exemplary criteria for evaluating a scaffold domain can include: (1) amino acid sequence, (2) sequences of several homologous domains, (3) 3-dimensional structure, and/or (4) stability data over a range of pH, temperature, salinity, organic solvent, oxidant concentration. In one embodiment, the scaffold domain is a small, stable protein domains, e.g., a protein of less than 100, 70, 50, 40 or 30 amino acids. The domain may include one or more disulfide bonds or may chelate a metal, e.g., zinc.

[0241] Still other binding agents are based on peptides, e.g., proteins with an amino acid sequence that are less than 30, 25, 24, 20, 18, 15 or 12 amino acids. Peptides can be incorporated in a larger protein, but typically a region that can independently bind to amyloid beta 1-42, e.g., to an epitope described herein. Peptides can be identified by phage display as described previously.

[0242] An amyloid beta 1-42 binding agent may include non-peptide linkages and other chemical modification. For example, part or all of the binding agent may be synthesized as a peptidomimetic, e.g., a peptoid (see, e.g., Simon et al. (1992) Proc. Natl. Acad. Sci. USA 89:9367-71 and Horwell (1995) Trends Biotechnol. 13:132-4). A binding agent may include one or more (e.g., all) non-hydrolyzable bonds. Many non-hydrolyzable peptide bonds are known in the art, along with procedures for synthesis of peptides containing such bonds. Exemplary non-hydrolyzable bonds include --[CH_2NH]-- reduced amide peptide bonds, --[$COCH_2$]-- ketomethylene peptide bonds, --[CH(CN)NH]-- (cyanomethylene)amino peptide bonds, --[$CH_2CH(OH)$]-- hydroxyethylene peptide bonds, --[CH_2O]-- peptide bonds, and --[CH_2S]-- thiomethylene peptide bonds (see e.g., U.S. Pat. No. 6,172,043).

[0243] While the present invention contemplates the identification and development of novel antibodies through variegation of codons corresponding to the hypervariable region of an antibody's variable domain, another aspect of the invention provides for the development of binding proteins which are not antibodies or even variable domains of antibodies.

[0244] In order to obtain a novel binding protein against a chosen target, such as in the instance of the present invention, amyloid beta 1-42, DNA molecules, each encoding a protein comprising one of a family of similar potential binding domains and a structural signal calling for the display of the protein on the outer surface of a chosen bacterial cell, bacterial spore or phage (genetic package) are introduced into a genetic package. The protein is expressed and the potential binding domain is displayed on the outer surface of the package. The cells or viruses bearing the binding domains which recognize the target molecule are then isolated and amplified. The successful binding domains are then characterized. One or more of these successful binding domains is then used as a model for the design of a new family of potential binding domains, and the process is repeated until a novel binding domain having a desired affinity for the target molecule is obtained.

[0245] Most larger proteins fold into distinguishable globules called domains. Protein domains have been defined various ways, but all definitions fall into one of three classes: a) those that

define a domain in terms of 3D atomic coordinates, b) those that define a domain as an isolable, stable fragment of a larger protein, and c) those that define a domain based on protein sequence homology plus a method from class a) or b). Frequently, different methods of defining domains applied to a single protein yield identical or very similar domain boundaries. The diversity of definitions for domains stems from the many ways that protein domains are perceived to be important, including the concept of domains in predicting the boundaries of stable fragments, and the relationship of domains to protein folding, function, stability, and evolution.

[0246] When a domain of a protein is primarily responsible for the protein's ability to specifically bind a chosen target, it is referred to herein as a "binding domain" (BD). A preliminary operation is to engineer the appearance of a stable protein domain, denoted as an "initial potential binding domain" (IPBD), on the surface of a genetic package.

[0247] For the purposes of this invention, the term "potential binding protein" refers to a protein encoded by one species of DNA molecule in a population of variegated DNA wherein the region of variation appears in one or more subsequences encoding one or more segments of the polypeptide having the potential of serving as a binding domain for the target substance. This is exemplified in U.S. Patent No. 6,979,538.

Pharmaceutical Compositions

[0248] Another aspect of the invention provides for pharmaceutical compositions for the treatment of Alzheimer's disease. The pharmaceutical composition comprises an agent, in the manner of the present invention, an anti-amyloid beta 1-42 antibody or fragment, derivative, or variant thereof, and a pharmaceutically acceptable carrier. While not wishing to be bound by theory, such an agent (antibody) may function in one or more ways such as:

a) preventing the production or generation of amyloid beta through cleavage of APP, or

b) preventing aggregation of amyloid beta fibrils and deposition in cerebral parenchymal tissues and blood vessels, or

c) preventing the formation of oligomeric forms of Abeta, Abeta 1-42, or

d) increasing the degradation of amyloid beta, or

e) increasing the clearance of amyloid beta from the brain, or

f) facilitating the metabolism of amyloid beta.

[0249] The pharmaceutical compositions may be formulated for oral delivery or parenteral delivery. Furthermore, the pharmaceutical compositions are formulated for delivery by a route selected from the group consisting of intravenous, intramuscular, oral, subcutaneous, intrathecal, intracranial and intraventricular. In a particular embodiment, the amyloid beta antibody in the composition binds to amyloid beta 1-42.

[0250] The compositions for parenteral administration will commonly comprise a solution of any of the agents described above, including an antibody or fragment thereof or a protein scaffold of

the invention or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be employed, e.g., water, buffered water, 0.4% saline, 0.3% glycine, and the like. These solutions are sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well-known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the agent of the invention in such pharmaceutical compositions may vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight, and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

[0251] Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 ml sterile buffered water, and a therapeutically effective amount of the antibody of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain 250 ml of sterile Ringer's solution, and a therapeutically effective amount of the antibody of the invention. Actual methods for preparing parenterally administrable compositions are well-known or will be apparent to those skilled in the art, and are described in more detail in, e.g., Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, PA. The therapeutically effective amount of the antibody can be determined by one skilled in the art. The age and weight of the patient, the stage of the disease, the mode of administration, the actual formulation and the possible coadministration of other therapeutic agents must all be taken into account when determining the optimal therapeutically effective amount of the antibody to be administered.

[0252] The agent (antibody) of the invention may be lyophilized for storage and reconstituted in a suitable carrier prior to use.

[0253] The pharmaceutical composition of the invention may be administered for prophylactic and/or therapeutic treatments. In therapeutic application, compositions are administered to a subject already suffering from Alzheimer's disease, in an amount sufficient to cure or at least partially arrest the disorder and its complications, or to stop the progression of the disease and one or more of its symptoms, for example, memory loss or anxiety or irritability. In prophylactic applications, compositions containing the present agents are administered to a subject not already in a disease state but one that may be predisposed to Alzheimer's disease to enhance the subject's resistance to such disorder. A "subject at risk for developing Alzheimer's disease" as described herein, refers to a subject who is predisposed to development of AD as described in the present application, whereby such risk factors are known to those skilled in the art.

[0254] It will be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of an agent of the invention will be determined by the nature and extent of the depressive disorder being treated, the form, route and site of administration, and the particular animal being treated, and that such optimums may be determined by conventional

techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, i.e., the number of doses of an agent of the invention given per day for a defined number of days, may be ascertained by those skilled in the art using conventional course of treatment determination tests.

[0255] In practicing an exemplary method of treatment or use described herein, a therapeutically effective amount of antibody that binds to amyloid beta 1-42 can be administered to a subject, e.g., mammal (e.g., a human). An antibody may be administered in accordance with the methods described either alone or in combination with other therapies such as treatments employing cytokines, lymphokines hematopoietic factors, one or more other antibodies that may or may not bind amyloid beta or amyloid beta 1-42, or anti-inflammatory agents. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with an antibody described herein. When coadministered with one or more agents, the antibody may be administered either simultaneously with the second agent, or separately, e.g., sequentially. If administered separately, e.g., sequentially, the attending physician will decide on the appropriate sequence of administering the antibody in combination with other agents.

[0256] In one embodiment, the pharmaceutical composition includes the anti-amyloid beta 1-42 antibody as the sole biologic (e.g., the sole protein component) or as the sole biologically active ingredient. For example, the composition can include less than 25, 20, 15, 10, 5, 3, 2, 1, 0.4, or 0.1% other protein components on a w/w basis.

[0257] The pharmaceutical compositions may be in the form of a liposome in which the anti-amyloid beta 1-42 antibody is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids that exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers while in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Pat. Nos. 4,235,871; 4,501,728; 4,837,028; and 4,737,323, all of which are incorporated herein by reference.

[0258] The phrase "therapeutically effective amount" is used herein to mean an amount sufficient to prevent, and preferably reduce by at least about 25 percent, more preferably by at least 50 percent, most preferably by at least 90 percent, a clinically significant change in a feature of pathology such as for example, elevated blood pressure, fever, or white cell count, as may attend its presence and activity. As related to the present invention, the term may also mean an amount sufficient to ameliorate or reverse one or more symptoms associated with Alzheimer's disease. In particular, a "therapeutically effective amount" of the treatment may result in amelioration, reduction or elimination of at least one of the following symptoms: memory impairment, persistent sadness or anxiety, feelings of emptiness, hopelessness, pessimism,

guilt, worthlessness, helplessness, a loss of interest or pleasure in hobbies and activities that were once enjoyed, decreased energy, or fatigue, difficulty concentrating, remembering, or making decisions, insomnia, early-morning awakening, or oversleeping, appetite and/or weight loss or overeating and weight gain, thoughts of death or suicide and suicide attempts, restlessness, irritability, and persistent physical symptoms that do not respond to treatment, such as headaches, digestive disorders, and chronic pain. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

Combination Therapy

[0259] It is envisioned that the antibodies of the present invention may be administered alone as a single composition or may be administered in combination with one or more other agents useful for treating Alzheimer's disease.

[0260] For example, amyloid beta formation could be inhibited by peptides homologous to Abeta (position 17-21) which have a similar degree of hydrophobicity, but also have a very low propensity to adopt a beta-sheet conformation due to the presence of proline residues (anti-beta-sheet peptides or beta-sheet inhibitors) (Soto C,' Kindy MS, Baumann M, Frangione B.Biochem, (1996), Biophys Res Commun., Sep 24;226(3):672-80). An 11-residue peptide with these characteristics binds to Abeta, inhibits Abeta fibril formation and partially disaggregates preformed fibrils in vitro. Shorter anti-beta-sheet peptides and analogs containing D-amino acids are also able to inhibit Abeta fibrillogenesis. The latter are more resistant to proteolytic degradation and may serve as a starting point to design more efficient peptides derivatives to inhibit amyloidogenesis in vivo.

[0261] Alternatively, it has been determined that N-methyl amino acid containing congeners of the hydrophobic "core domain" of Abeta inhibit the fibrillogenesis of full-length Abeta. These peptides also disassemble preformed fibrils of full-length Abeta. A key feature of the inhibitor peptides is that they contain N-methyl amino acids in alternating positions of the sequence. The most potent of these inhibitors, termed Abeta 16-22m, has the sequence NH(2)-K(Me or L)V(Me or F)F(Me or A)E-CONH(2) (SEQ ID NO:13). These inhibitors appear to act by binding to growth sites of Abeta nuclei and/or fibrils and preventing the propagation of the network of hydrogen bonds that is essential for the formation of an extended beta-sheet fibril. (Gordon DJ, Sciarretta KL, Meredith SC., (2001), Biochemistry. Jul 27;40(28):8237-45). Furthermore, it has been shown that single N-methyl amino acid-containing peptides related to the central hydrophobic region β_{16-20} (Lys-Leu-Val-Phe-Phe (SEQ ID NO:14) of the β-amyloid protein are able to reduce the cytotoxicity of natural β_{1-42} in PC12 cell cultures. N-methyl phenylalanine analogs yield statistically significant increments in cell viability (Student's t-test < 0.01%) and are nontoxic in

the same assay (M. Cruz, J.M. Tusell, D. Grillo-Bosch, F. Albericio, J. Serratosa, F. Rabanal and E. Giralt, (2004), The Journal of Peptide Research, 63(3): 324).

[0262] In addition, it has been shown that phenserine, a third generation acetylcholinesterase inhibitor (AChE-inhibitor), has the ability to reduce both beta amyloid precursor protein (APP) and beta amyloid peptide (amyloid-beta) formation in the brain (see http://www.axonyx.com/pipeline/index.html).

[0263] Other investigators have reported that injection of animals with low molecular weight heparins (enoxaprin and dalteparin) demonstrated a reduction in amyloid beta deposition. Moreover, these compounds were capable of arresting the progression of amyloid beta deposits and amyloid beta peptide fibril formation by impeding the structural changes necessary for fibril formation. Not only was amyloid beta progression retarded, but these molecules enhanced the clearance of established amyloid beta fibrils (Zhu, H. et al. (2001), Mol. Med. 7(8):517-522).

[0264] Furthermore, it has been shown that monoclonal antibodies are capable of inhibiting in vitro fibrillar aggregation of the Alzheimer beta-amyloid peptide (Solomon B, et al., (1996), Proc Natl Acad Sci U S A, 93(1):452-5). In one study, two monoclonal antibodies (mAbs), AMY-33 and 6F/3D, were raised against beta-amyloid fragments spanning amino acid residues 1-28 and 8-17 of the beta-amyloid peptide chain, respectively. In vitro aggregation of beta-amyloid peptide was induced by incubation for 3 hours at 37° C and monitored by ELISA, negative staining electron microscopy, and fluorimetric studies. The investigators found that the monoclonal antibodies prevented the aggregation of beta-amyloid peptide. Moreover, the inhibitory effect appeared to be related to the localization of the antibody-binding sites and the nature of the aggregating agents (Solomon B, et al., (1996), Proc Natl Acad Sci U S A, 93(1):452-5).

[0265] It may also be possible to prevent the effects of amyloid beta by administering an agent that effects a regulatory element necessary for its expression. Alternatively, the effects of amyloid beta in AD may be addressed by taking into account the effects of abeta in the inflammatory process. For example, studies by Suo Z. et al suggest that beta-amyloid (Abeta)-induced inflammatory reactions may partially drive the pathogenesis of Alzheimer's disease (AD) and in cerebral amyloid angiopathy (CAA). To evaluate the roles of Abeta in the inflammatory processes in vascular tissues, they tested the ability of Abeta to trigger inflammatory responses in cultured human vascular cells. They found that stimulation with Abeta dose-dependently increased the expression of CD40, and secretion of interferon-((IFN-() and interleukin-1ß (IL-1ß) in endothelial cells. Abeta also induced expression of IFN-(receptor (IFN-(R) both in endothelial and smooth muscle cells. Characterization of the Abeta-induced inflammatory responses in the vascular cells showed that the ligation of CD40 further increased cytokine production and/or the expression of IFN-(R. Moreover, IL-1 ß and IFN-(synergistically increased the Abeta-induced expression of CD40 and IFN-(R. They also found that Abeta induced expression of adhesion molecules, and that cytokine production and interaction of CD40-CD40 ligand (CD40L) further increased the Abeta-induced expression of adhesion molecules in these

same cells. These results suggest that Abeta can function as an inflammatory stimulator to activate vascular cells and induces an auto-amplified inflammatory molecular cascade, through interactions among adhesion molecules, CD40-CD40L and cytokines. Additionally, Abeta 1-42, the more pathologic form of Abeta, induces much stronger effects in endothelial cells than in smooth muscle cells, while the reverse is true for Abeta
1-40. Collectively, these findings support the hypothesis that the Abeta-induced inflammatory responses in vascular cells may play a significant role in the pathogenesis of CAA and AD (Suo et al., (1998), Brain Res, 807(1-2): 110-7).

[0266] Studies have also focused on a means of enhancing the degradation and clearance of Abeta. Studies by Qiu et al. have shown increased degradation of amyloid beta protein by a metalloprotease secreted by microglia. Such a metalloprotease was identified in a microglial cell line, BV-2 (Qiu, WQ et al. (1997), J. Biol. Chem. 272(10):6641-6). Substrate analysis revealed that the enzyme responsible for the degradation of $Abeta_{1-40}$ and $Abeta_{1-42}$ was a non-matrix metalloprotease. Thus, it may be possible to develop novel therapeutics that act to enhance the activity of this enzyme or to identify other enzymes that act in a similar manner to degrade the amyloid beta proteins, or alternatively, to develop a means of directing specific enzymes with such amyloid beta degrading activity to the site where needed.

[0267] Another enzyme, neprilysin, has been shown to be a major Abeta degrading enzyme in vivo. Studies by Hama, et al. investigated whether or not manipulation of neprilysin activity in the brain would be an effective strategy for regulating Abeta levels. Neprilysin was expressed in primary cortical neurons using a Sindbis viral vector and the effect on Abeta metabolism was examined. The investigators showed that the corresponding recombinant protein, expressed in the cell bodies and processes, exhibited thiorphan-sensitive endopeptidase activity, whereas a mutant neprilysin with an amino acid substitution in the active site did not show any such activity. Expression of the wild-type neprilysin, but not the mutant, resulted in a dose-dependent decrease in both the Abeta 40 and 42 levels in the culture media (Hama E. et al. (2001), Journal of Biochemistry, Vol 130, Issue 6: 721-726). Moreover, neprilysin expression also resulted in reducing cell-associated Abeta, which could be more neurotoxic than extracellular Abeta. These results indicate that the manipulation of neprilysin activity in neurons, the major source of Abeta in the brain, would be a relevant strategy for controlling the Abeta levels and thus the Abeta-associated pathology in brain tissues. In the matter of the present invention, such a strategy could be used to treat AD associated with elevated levels of Abeta peptides.

[0268] Studies have also shown that endothelin converting enzyme (ECE) has been shown to play a fundamental role in amyloid metabolic pathways. A published study (Journal of Biological Chemistry: 276(27):24540-8, 2001) reported that both ECE-1a and ECE-1b activation reduced the intracellular generation of Abeta40 and Abeta42, and that ECE-1a may also be able to degrade preexisting protein. The development of strategies able to stimulate or over-express

ECE could offer a novel therapy of treating AD characterized by elevated levels of Abeta 1-40 and Abeta 1-42.

[0269] Furthermore, it has been shown that a modest increase in astroglial TGF-beta 1 production in aged transgenic mice expressing the human beta-amyloid precursor protein (hAPP) results in a three-fold reduction in the number of parenchymal amyloid plaques, a 50% reduction in the overall Abeta load in the hippocampus and neocortex, and a decrease in the number of dystrophic neuritis (Wyss-Coray T, et al. (2001) Nat Med. 7(5):612-8). These investigators found that in mice expressing hAPP and TGF-ß1, Abeta accumulated substantially in cerebral blood vessels, but not in parenchymal plaques. Furthermore, in human cases of AD, Abeta immunoreactivity associated with parenchymal plaques was inversely correlated with Abeta in blood vessels and cortical TGF-ß1 mRNA levels. The reduction of parenchymal plaques in hAPP/TGF-beta1 mice was associated with a strong activation of microglia and an increase in inflammatory mediators. Recombinant TGF-ß1 stimulated Abeta clearance in microglial cell cultures. These results demonstrate that TGF-ß1 is an important modifier of amyloid beta deposition in vivo and indicate that TGF-ß1 might promote microglial processes that inhibit the accumulation of Abeta in the brain parenchyma. Accordingly, agents that modulate TGF-ß activity may prove useful to treat AD by virtue of their effect on Abeta deposition and/or clearance.

[0270] In studies related to the role of amyloid beta in Alzheimer's disease, several groups are exploring the possibility that Abeta-based vaccines can help the brain in Alzheimer's patients by increasing its clearance. Work by Lemere et al. demonstrated that vaccinating monkeys with synthetic amyloid beta peptide enhanced the clearance of amyloid beta protein from the brain and into the blood. In this study, five aged Caribbean vervet monkeys were given eight injections of the amyloid beta peptide over a period of nine-months. Five control monkeys did not receive the treatment. The investigators found that all of the immunized monkeys made antibodies to amyloid beta peptide. These antibodies were found in blood and, in lower amounts, in cerebrospinal fluid (CSF). More importantly, the investigators found that between 22 and 42 days after immunization, levels of amyloid beta protein dropped in the CSF and increased in the blood and no plaques were found in the immunized animals. These findings suggest that the antibodies bound to amyloid beta protein and increased its slow release from the CSF to the blood for clearance (http://www.ahaf.org/whatsnew/AD_Hope_Nov_2003.htm). One possible drawback to this approach is the finding that foreign beta-amyloid could have unwanted consequences, such as contributing to the death of brain cells or triggering harmful immune system responses. One possible concern is the induction of an inflammatory response in the brain. Thus, it may be necessary to administer other anti-inflammatory compounds at the time of vaccination. The present invention provides for such combination therapies.

[0271] One alternative is to find nontoxic molecules that will boost the clearance of beta-amyloid without inducing an inflammatory response. Agadjanyan et al. have found several

molecules that mimic beta-amyloid (termed "mimotopes") in mice, and they are now looking for corresponding mimotopes in humans. Future studies by this group will concentrate on identification of beta-amyloid mimotopes in the blood from patients with Alzheimer's disease. These molecules will then be tested in a mouse model of Alzheimer's disease to determine whether vaccination with human mimotopes can reduce mouse brain levels of beta-amyloid and reverse memory deficits, without side effects (see http://www.alz.org/Research/Funded/2003/03USA_AgadjanyanIIRG.asp).

[0272] Any of the above agents, whether they are small organic molecules, enzymes, peptides or antibodies (other than those disclosed herein), which prevent the production or generation of amyloid beta through cleavage of APP, or aggregation of amyloid beta fibrils, or promote the degradation and/or clearance of amyloid beta peptides, may be useful for treatment of AD when combined with the antibodies of the invention.

Administration of the Pharmaceutical Compositions

[0273] According to the present invention, a therapeutic composition, e.g., an antibody that binds to amyloid beta 1-42, and which may act as an inhibitor of amyloid beta production or generation through cleavage of amyloid precursor protein (APP), or as an inhibitor of amyloid beta aggregation into fibrils, or as an enhancer of amyloid beta clearance from the brain, or as an enhancer of amyloid beta degradation, or as an agent that interferes with the neurotoxic effects of amyloid beta, and a pharmaceutically acceptable carrier of the invention, or an amyloid beta 1-42 binding agent or a protein or antibody prepared in a scaffold to enhance the half-life of the molecule, may be introduced orally or parenterally, e.g. intramuscularly, intravenously, subcutaneously, transmucosally, or nasally. Alternatively, administration is by intracranial, intrathecal or intraventricular administration. In addition, the therapeutic composition can be placed (e.g., injected) into the bloodstream after coupling the agent to a carrier that will allow the agent-carrier complex to cross the blood-brain barrier. Such methods are known to those skilled in the art.

[0274] In a preferred aspect, the agent of the present invention can cross cellular or nuclear membranes, which would allow for intravenous or oral administration. Strategies are available for such crossing, including but not limited to, increasing the hydrophobic nature of a molecule; introducing the molecule as a conjugate to a carrier, such as a ligand to a specific receptor, targeted to a receptor; and the like.

[0275] The present invention also provides for the use of the antibody molecules and fragments thereof as "targeting molecules". A "targeting molecule" as used herein shall mean a molecule which, when administered in vivo, localizes to desired location(s). In various embodiments, the targeting molecule can be a peptide or protein, an antibody or fragment thereof, a lectin, a carbohydrate, or a steroid. In one embodiment, the targeting molecule is a peptide ligand of a receptor on the target cell. In a more particular embodiment, the targeting molecule is an

antibody, such as those described herein as AD38 (SEQ ID NO: 10), AD64 (SEQ ID NO: 12) and the amino acid sequences described in SEQ ID NOS: 2, 4, 6 and 8. Preferably, the targeting molecule is an antibody that binds to amyloid beta, preferably amyloid beta 1-42. The antibody may be useful for targeting other proteins or small organic molecules to the site of amyloid beta deposition in the brain.

[0276] Accordingly, in one embodiment, the use of agents that increase the transfer of molecules through the blood brain barrier is contemplated, such as that described in US 20060039859. One other strategy for drug delivery through the blood brain barrier (BBB) entails disruption of the BBB, either by osmotic means such as mannitol or leukotrienes, or biochemically by the use of vasoactive substances such as bradykinin. A BBB disrupting agent can be co-administered with the antibody therapeutic or imaging compositions of the invention when the compositions are administered by intravascular injection. Other strategies to go through the BBB may entail the use of endogenous transport systems, including carrier-mediated transporters such as glucose and amino acid carriers, receptor-mediated transcytosis for insulin or transferrin, and active efflux transporters such as p-glycoprotein. Active transport moieties may also be conjugated to the therapeutic or imaging compounds for use in the invention to facilitate transport across the epithelial wall of the blood vessel. Alternatively, drug delivery behind the BBB is by intrathecal delivery of therapeutics or imaging agents directly to the cranium, as through an Ommaya reservoir.

[0277] In one embodiment, to facilitate crosslinking, the antibody can be reduced to two heavy and light chain heterodimers, or the $F(ab')_2$ fragment can be reduced, and crosslinked to the agent via a reduced sulfhydryl.

[0278] In another embodiment, the therapeutic compound can be delivered in a vesicle, in particular a liposome (see Langer, (1990) Science, 249:1527-1533; Treat et al., (1989) in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss: New York, pp. 353-365; Lopez-Berestein, ibid., pp. 317-327; see generally ibid.).

[0279] In yet another embodiment, the therapeutic compound can be delivered in a controlled release system. For example, the agent may be administered using intravenous infusion, an implantable osmotic pump, a transdermal patch, liposomes, or other modes of administration. In one embodiment, a pump may be used (see Langer (1990) supra; Sefton, (1987) CRC Crit. Ref. Biomed. Eng., 14:201; Buchwald et al., (1980) Surgery, 88:507; Saudek et al., (1989) N. Engl. J. Med., 321:574). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Press: Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley: New York (1984); Ranger and Peppas, (1983) J. Macromol. Sci. Rev. Macromol. Chem., 23:61; see also Levy et al., (1985) Science, 228:190; During et al., (1989) Ann. Neurol., 25:351; Howard et al., (1989) J. Neurosurg., 71:105). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring

only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

[0280] When a therapeutically effective amount of an antibody that binds to amyloid beta 1-42 is administered orally, the binding agent will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, a pharmaceutical composition may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% binding agent, and preferably from about 25 to 90% binding agent. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of the binding agent, and preferably from about 1 to 50% the binding agent.

[0281] When a therapeutically effective amount of an antibody that binds to amyloid beta 1-42 is administered by intravenous, cutaneous, or subcutaneous injection, the binding agent will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to binding agent an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. A pharmaceutical composition may also contain stabilizers, preservatives, buffers, antioxidants, or other additive known to those of skill in the art.

[0282] The amount of an antibody (or other amyloid beta 1-42 binding agent) in the pharmaceutical composition can depend upon the nature and severity of the condition being treated, and on the nature of prior treatments that the patient has undergone. Ultimately, the attending physician will decide the amount of antibody with which to treat each individual patient. Initially, the attending physician will administer low doses of antibody and observe the patient's response. Larger doses of antibody may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not generally increased further. For example, doses in the range of 0.1-50 mg/kg, 0.5-50 mg/kg, 1-100 mg/kg, 0.5-25 mg/kg, 0.1-15 mg/kg, or 1-8 mg/kg of body weight can be administered. The physician may track the effectiveness of the pharmaceutical composition by monitoring the progression of the disease or the stabilization of the condition. For example, in the case of Alzheimer's disease, the physician may assess the overall mental health of the patient as related to signs of irritability or anxiety or may assess the effect of the composition on memory impairment. The pharmaceutical

composition can be administered to normal patients or patients who do not show symptoms, e.g., in a prophylactic mode.

[0283] A composition that includes an amyloid beta 1-42 antibody or fragment thereof can be formulated for inhalation or other mode of pulmonary delivery. Accordingly, the compounds described herein can be administered by inhalation to pulmonary tissue. The term "pulmonary tissue" as used herein refers to any tissue of the respiratory tract and includes both the upper and lower respiratory tract, except where otherwise indicated. An amyloid beta 1-42 antibody or fragment thereof can be administered in combination with one or more of the existing modalities for treating pulmonary diseases.

[0284] In one example, the compound is formulated for a nebulizer. In one embodiment, the compound can be stored in a lyophilized form (e.g., at room temperature) and reconstituted in solution prior to inhalation.

[0285] It is also possible to formulate the compound for inhalation using a medical device, e.g., an inhaler (see, e.g., U.S. Pat. No. 6,102,035 (a powder inhaler) and U.S. Pat. No. 6,012,454 (a dry powder inhaler). The inhaler can include separate compartments for the active compound at a pH suitable for storage and another compartment for a neutralizing buffer, and a mechanism for combining the compound with a neutralizing buffer immediately prior to atomization. In one embodiment, the inhaler is a metered dose inhaler.

[0286] The three common systems used to deliver drugs locally to the pulmonary air passages include dry powder inhalers (DPIs), metered dose inhalers (MDIs) and nebulizers. MDIs, used in the most popular method of inhalation administration, may be used to deliver medicaments in a solubilized form or as a dispersion. Typically MDIs comprise a Freon or other relatively high vapor pressure propellant that forces aerosolized medication into the respiratory tract upon activation of the device. Unlike MDIs, DPIs generally rely entirely on the inspiratory efforts of the patient to introduce a medicament in a dry powder form to the lungs. Nebulizers form a medicament aerosol to be inhaled by imparting energy to a liquid solution. Direct pulmonary delivery of drugs during liquid ventilation or pulmonary lavage using a fluorochemical medium has also been explored. These and other methods can be used to deliver an amyloid beta 1-42 antibody or fragment thereof. In one embodiment, the amyloid beta 1-42 antibody or fragment thereof is associated with a polymer, e.g., a polymer that stabilizes or increases half-life of the compound.

[0287] For example, for administration by inhalation, an amyloid beta 1-42 antibody or fragment thereof is delivered in the form of an aerosol spray from a pressured container or dispenser that contains a suitable propellant or a nebulizer. The compound may be in the form of a dry particle or a liquid. Particles that include the compound can be prepared, e.g., by spray drying, by drying an aqueous solution of the amyloid beta 1-42 antibody or fragment thereof with a charge neutralizing agent and then creating particles from the dried powder, or by drying an aqueous solution in an organic modifier and then creating particles from the dried powder.

[0288] The compound may be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide, or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges for use in an inhaler or insufflator may be formulated containing a powder mix of an amyloid beta 1-42 antibody or fragment thereof and a suitable powder base such as lactose or starch, if the particle is a formulated particle. In addition to the formulated or unformulated compound, other materials such as 100% DPPC or other surfactants can be mixed with the amyloid beta 1-42 42 antibody or fragment thereof to promote the delivery and dispersion of formulated or unformulated compound. Methods of preparing dry particles are described, for example, in PCT Publication WO 02/32406.

[0289] An amyloid beta 1-42 antibody or fragment thereof can be formulated for aerosol delivery, e.g., as dry aerosol particles, such that when administered it can be rapidly absorbed and can produce a rapid local or systemic therapeutic result. Administration can be tailored to provide detectable activity within 2 minutes, 5 minutes, 1 hour, or 3 hours of administration. In some embodiments, the peak activity can be achieved even more quickly, e.g., within 30 minutes or even within 10 minutes. An amyloid beta 1-42 antibody or fragment thereof can be formulated for longer biological half-life (e.g., by association with a polymer such as PEG) and can be used as an alternative to other modes of administration, e.g., such that the compound enters the circulation from the lungs and is distributed to other organs or to a particular target organ.

[0290] In one embodiment, the amyloid beta 1-42 antibody or fragment thereof is delivered in an amount such that at least 5% of the mass of the polypeptide is delivered to the lower respiratory tract or the deep lung. Deep lung has an extremely rich capillary network. The respiratory membrane separating the capillary lumen from the alveolar air space is very thin and extremely permeable. In addition, the liquid layer lining the alveolar surface is rich in lung surfactants. In other embodiments, at least 2%, 3%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, or 80% of the composition of an amyloid beta 1-42 antibody or fragment thereof is delivered to the lower respiratory tract or to the deep lung. Delivery to either or both of these tissues results in efficient absorption of the compound and high bioavailability. In one embodiment, the compound is provided in a metered dose using, e.g., an inhaler or nebulizer. For example, the compound is delivered in a dosage unit form of at least about 0.02, 0.1, 0.5, 1, 1.5, 2, 5, 10, 20, 40, or 50 mg/puff or more. The percent bioavailability can be calculated as follows:

Percent Bioavailability = (AUC noninvasive/AUC i.v. or s.c.) X (dose i.v. or s.c./dose noninvasive) X 100.

[0291] Although not necessary, delivery enhancers such as surfactants can be used to further enhance pulmonary delivery. A "surfactant" as used herein refers to a compound having hydrophilic and lipophilic moieties that promote absorption of a drug by interacting with an interface between two immiscible phases. Surfactants are useful with dry particles for several reasons, e.g., reduction of particle agglomeration, reduction of macrophage phagocytosis, etc. When coupled with lung surfactant, a more efficient absorption of the compound can be achieved because surfactants, such as DPPC, will greatly facilitate diffusion of the compound. Surfactants are well known in the art and include, but are not limited to, phosphoglycerides, e.g., phosphatidylcholines, L-α-phosphatidylcholine dipalmitoyl (DPPC) and diphosphatidyl glycerol (DPPG); hexadecanol; fatty acids; polyethylene glycol (PEG); polyoxyethylene-9-; auryl ether; palmitic acid; oleic acid; sorbitan trioleate (SPAN.TM. 85); glycocholate; surfactin; poloxomer; sorbitan fatty acid ester; sorbitan trioleate; tyloxapol; and phospholipids.

Stabilization and Retention

[0292] In one embodiment, an amyloid beta 1-42 antibody or fragment thereof is physically associated with a moiety that improves its stabilization and/or retention in circulation, e.g., in blood, serum, lymph, or other tissues, e.g., by at least 1.5, 2, 5, 10, or 50 fold. For example, an amyloid beta 1-42 antibody or fragment thereof can be associated with a polymer, e.g., a substantially nonantigenic polymer, such as polyalkylene oxides or polyethylene oxides. Suitable polymers will vary substantially by weight. Polymers having molecular number average weights ranging from about 200 to about 35,000 (or about 1,000 to about 15,000, or about 2,000 to about 12,500) can be used. For example, an amyloid beta 1-42 antibody or fragment thereof can be conjugated to a water-soluble polymer, e.g., hydrophilic polyvinyl polymers, e.g. polyvinylalcohol and polyvinylpyrrolidone. A nonlimiting list of such polymers includes polyalkylene oxide homopolymers such as polyethylene glycol (PEG) or polypropylene glycols, polyoxyethylenated polyols, copolymers thereof.and block copolymers thereof, provided that the water solubility of the block copolymers is maintained.

[0293] The molecular weight of the polymer can range up to about 500,000 Da, and preferably is at least about 20,000 Da, or at least about 30,000 Da, or at least about 40,000 Da. The molecular weight chosen can depend upon the effective size of the conjugate to be achieved, the nature (e.g., structure, such as linear or branched) of the polymer, and the degree of derivatization. A covalent bond can be used to attach an amyloid beta 1-42 antibody or fragment thereof to a polymer, for example, cross linking to the N-terminal amino group of the antibody and epsilon amino groups found on lysine residues of the antibody, as well as other amino, imino, carboxyl, sulfhydryl, hydroxyl or other hydrophilic groups. Functionalized PEG polymers that can be attached to an amyloid beta 1-42 antibody or fragment thereof are available, e.g., from Shearwater Polymers, Inc. (Huntsville, Ala.). The reaction conditions for coupling PEG and other polymers may vary depending on the amyloid beta 1-42 antibody or fragment thereof, the

desired degree of PEGylation, and the polymer utilized. Some factors involved in the choice of PEG derivatives include: the desired point of attachment (such as lysine or cysteine R-groups), hydrolytic stability and reactivity of the derivatives, stability, toxicity and antigenicity of the linkage, suitability for analysis, etc. Specific instructions for the use of any particular derivative are available from the manufacturer.

[0294] The conjugates of an amyloid beta 1-42 antibody or fragment thereof and a polymer can be separated from the unreacted starting materials, e.g., by gel filtration or ion exchange chromatography, e.g., HPLC. Heterologous species of the conjugates are purified from one another in the same fashion. Resolution of different species (e.g. containing one or two PEG residues) is also possible due to the difference in the ionic properties of the unreacted amino acids (see, e.g., WO 96/34015).

Therapeutic and Prophylactic Uses of Anti- amyloid beta 1-42 Antibodies

[0295] In yet another aspect, this disclosure features a method for neutralizing and/or inhibiting one or more disease-associated activities of amyloid beta 1-42 in vivo by administering an antibody that binds to amyloid beta 1-42, e.g., an antibody described herein in an amount sufficient to bind to and inhibit its activity and perhaps neutralize the neurotoxicity associated with beta amyloid 1-42. Such antibodies can also be administered to subjects having Alzheimer's disease or who are at risk of developing Alzheimer's disease or a related condition such as amyloidosis or other conditions in which the presence or accumulation of amyloid beta 1-42 may play a role in acquiring a disease or increasing the rate of progression of the disease.

Blood-Brain Barrier

[0296] The antibodies of the present invention, are contemplated for use in treating Alzheimer's disease. As such, the antibodies of the invention that exert their physiological effect *in vivo* in the brain may be more useful if they gain access to cells in the brain. Non-limiting examples of brain cells are neurons, glial cells (astrocytes, oligodendrocytes, microglia), cerebrovascular cells (muscle cells, endothelial cells), and cells that comprise the meninges. The blood brain barrier ("BBB") typically restricts access to brain cells by acting as a physical and functional blockade that separates the brain parenchyma from the systemic circulation (see, e.g., Pardridge, et al., J. Neurovirol. 5(6), 556-69 (1999); Rubin, et al., Rev. Neurosci. 22, 11-28 (1999)). Circulating molecules are normally able to gain access to brain cells via one of two processes: lipid-mediated transport through the BBB by free diffusion, or active (or catalyzed) transport.

[0297] The agents of the invention may be formulated to improve distribution *in vivo*, for example as powdered or liquid tablet or solution for oral administration or as a nasal spray, nose drops, a gel or ointment, through a tube or catheter, by syringe, by packtail, by pledget, or by submucosal infusion. For example, the blood-brain barrier (BBB) excludes many highly

hydrophilic agents. To ensure that the more hydrophilic therapeutic agents of the invention cross the BBB, they may be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, e.g., U.S. Pat. Nos. 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs ("targeting moieties" or "targeting groups" or "transporting vectors"), thus providing targeted drug delivery (see, e.g., V.V. Ranade J. Clin. Pharmacol. 29, 685 (1989)). Likewise, the agents may be linked to targeting groups that facilitate penetration of the blood brain barrier.

[0298] To facilitate transport of agents of the invention across the BBB, they may be coupled to a BBB transport vector (for review of BBB transport vectors and mechanisms, see, Bickel, et al., Adv. Drug Delivery Reviews 46, 247-79 (2001)). Exemplary transport vectors include cationized albumin or the OX26 monoclonal antibody to the transferrin receptor; these proteins undergo absorptive-mediated and receptor-mediated transcytosis through the BBB, respectively. Natural cell metabolites that may be used as targeting groups, include putrescine, spermidine, spermine, or DHA. Other exemplary targeting moieties include folate or biotin (see, e.g., U.S. Pat. No. 5,416,016); mannosides (Umezawa, et al., Biochem. Biophys. Res. Commun. 153, 1038 (1988)); antibodies (P.G. Bloeman, et al., FEBS Lett. 357, 140 (1995); M. Owais, et al., Antimicrob. Agents Chemother. 39, 180 (1995)); surfactant protein A receptor (Briscoe, et al., Am. J. Physiol. 1233, 134 (1995)); gp120 (Schreier, et al., J. Biol. Chem. 269, 9090 (1994)); see also, K. Keinanen and M.L. Laukkanen, FEBS Lett. 346, 123 (1994); J.J. Killion and I.J. Fidler, Immunomethods 4, 273 (1994).

[0299] Examples of other BBB transport vectors that target receptor-mediated transport systems into the brain include factors such as insulin, insulin-like growth factors ("IGF-I," and "IGF-II"), angiotensin II, atrial and brain natriuretic peptide ("ANP," and "BNP"), interleukin I ("IL-1") and transferrin. Monoclonal antibodies to the receptors that bind these factors may also be used as BBB transport vectors. BBB transport vectors targeting mechanisms for absorptive-mediated transcytosis include cationic moieties such as cationized LDL, albumin or horseradish peroxidase coupled with polylysine, cationized albumin or cationized immunoglobulins. Small basic oligopeptides such as the dynorphin analogue E-2078 and the ACTH analogue ebiratide may also cross the brain via absorptive-mediated transcytosis and are potential transport vectors.

[0300] Other examples of BBB transport vectors include hexose moieties, e.g., glucose and monocarboxylic acids, e.g., lactic acid and neutral amino acids, e.g., phenylalanine and amines, e.g., choline and basic amino acids, e.g., arginine, nucleosides, e.g., adenosine and purine bases, e.g., adenine, and thyroid hormone, e.g., triiodothyridine. Antibodies to the extracellular domain of nutrient transporters may also be used as transport vectors. Other possible vectors include angiotensin II and ANP, which may be involved in regulating BBB permeability.

[0301] In some cases, the bond linking the therapeutic agent to the transport vector may be cleaved following transport into the brain in order to liberate the biologically active agent.

Exemplary linkers include disulfide bonds, ester-based linkages, thioether linkages, amide bonds, acid-labile linkages, and Schiff base linkages. Avidin/biotin linkers, in which avidin is covalently coupled to the BBB drug transport vector, may also be used. Avidin itself may be a drug transport vector.

[0302] Transcytosis, including receptor-mediated transport of compositions across the blood brain barrier, may also be suitable for the agents of the invention. Transferrin receptor-mediated delivery is disclosed in U.S. Pat. Nos. 5,672,683; 5,383,988; 5,527,527; 5,977,307; and 6,015,555. Transferrin-mediated transport is also known. P.M. Friden, et al., Pharmacol. Exp. Ther. 278, 1491-98 (1996); H.J. Lee, J. Pharmacol. Exp. Ther. 292, 1048-52 (2000). EGF receptor-mediated delivery is disclosed in Y. Deguchi, et al., Bioconjug. Chem. 10, 32-37 (1999), and transcytosis is described in A. Cerletti, et al., J. Drug Target. 8, 435-46 (2000). Insulin fragments have also been used as carriers for delivery across the blood brain barrier. M. Fukuta, et al., Pharm. Res. 11. 1681-88 (1994). Delivery of agents via a conjugate of neutral avidin and cationized human albumin has also been described. Y.S. Kang, et al., Pharm. Res. 1, 1257-64 (1994).

[0303] Other modifications for enhancing penetration of the antibodies of the invention across the blood brain barrier may be accomplished using methods and derivatives known in the art. For example, U.S. Pat. No. 6,024,977 discloses covalent polar lipid conjugates for targeting to the brain and central nervous system. U.S. Pat. No. 5,017,566 discloses cyclodextrin derivatives comprising inclusion complexes of lipoidal forms of dihydropyridine redox targeting moieties. U.S. Pat. No. 5,023,252 discloses the use of pharmaceutical compositions comprising a neurologically active drug and a compound for facilitating transport of the drug across the blood-brain barrier including a macrocyclic ester, diester, amide, diamide, amidine, diamidine, thioester, dithioester, thioamide, ketone or lactone. U.S. Pat. No. 5,024,998 discloses parenteral solutions of aqueous-insoluble drugs with cyclodextrin derivatives. U.S. Pat. No. 5,039,794 discloses the use of a metastatic tumor-derived egress factor for facilitating the transport of compounds across the blood-brain barrier. U.S. Pat. No. 5,112,863 discloses the use of N-acyl amino acid derivatives as antipsychotic drugs for delivery across the blood-brain barrier. U.S. Pat. No. 5,124,146 discloses a method for delivery of therapeutic agents across the blood-brain barrier at sites of increase permeability associated with brain lesions. U.S. Pat. No. 5,153,179 discloses acylated glycerol and derivatives for use in a medicament for improved penetration of cell membranes. U.S. Pat. No. 5,177,064 discloses the use of lipoidal phosphonate derivatives of nucleoside antiviral agents for delivery across the blood-brain barrier. U.S. Pat. No. 5,254,342 discloses receptor-mediated transcytosis of the blood-brain barrier using the transferrin receptor in combination with pharmaceutical compounds that enhance or accelerate this process. U.S. Pat. No. 5,258,402 discloses treatment of epilepsy with imidate derivatives of anticonvulsive sulfamate. U.S. Pat. No. 5,270,312 discloses substituted piperazines as central nervous system agents. U.S. Pat. No. 5,284,876 discloses fatty acid conjugates of dopamine drugs. U.S. Pat.

No. 5,389,623 discloses the use of lipid dihydropyridine derivatives of anti-inflammatory steroids or steroid sex hormones for delivery across the blood-brain barrier. U.S. Pat. No. 5,405,834 discloses prodrug derivatives of thyrotropin releasing hormone. U.S. Pat. No. 5,413,996 discloses acyloxyalkyl phosphonate conjugates of neurologically-active drugs for anionic sequestration of such drugs in brain tissue. U.S. Pat. No. 5,434,137 discloses methods for the selective opening of abnormal brain tissue capillaries using bradykinin infused into the carotid artery. U.S. Pat. No. 5,442,043 discloses a peptide conjugate between a peptide having a biological activity and incapable of crossing the blood-brain barrier and a peptide which exhibits no biological activity and is capable of passing the blood-brain barrier by receptor-mediated endocytosis. U.S. Pat. No. 5,466,683 discloses water soluble analogues of an anticonvulsant for the treatment of epilepsy. U.S. Pat. No. 5,525,727 discloses compositions for differential uptake and retention in brain tissue comprising a conjugate of a narcotic analgesic and agonists and antagonists thereof with a lipid form of dihydropyridine that forms a redox salt upon uptake across the blood-brain barrier that prevents partitioning back to the systemic circulation.

[0304] Still further examples of modifications that enhance penetration of the blood brain barrier are described in International (PCT) Application Publication No. WO 85/02342, which discloses a drug composition comprising a glycerolipid or derivative thereof. PCT Publication No. WO 089/11299 discloses a chemical conjugate of an antibody with an enzyme which is delivered specifically to a brain lesion site for activating a separately-administered neurologically-active prodrug. PCT Publication No. WO 91/04014 discloses methods for delivering therapeutic and diagnostic agents across the blood-brain barrier by encapsulating the drugs in liposomes targeted to brain tissue using transport-specific receptor ligands or antibodies. PCT Publication No. WO 91/04745 discloses transport across the blood-brain barrier using cell adhesion molecules and fragments thereof to increase the permeability of tight junctions in vascular endothelium. PCT Publication No. WO 91/14438 discloses the use of a modified, chimeric monoclonal antibody for facilitating transport of substances across the blood-brain barrier. PCT Publication No. WO 94/01131 discloses lipidized proteins, including antibodies. PCT Publication No. WO 94/03424 discloses the use of amino acid derivatives as drug conjugates for facilitating transport across the blood-brain barrier. PCT Publication No. WO 94/06450 discloses conjugates of neurologically-active drugs with a dihydropyridine-type redox targeting moiety and comprising an amino acid linkage and an aliphatic residue. PCT Publication No. WO 94/02178 discloses antibody-targeted liposomes for delivery across the blood-brain barrier. PCT Publication No. WO 95/07092 discloses the use of drug-growth factor conjugates for delivering drugs across the blood-brain barrier. PCT Publication No. WO 96/00537 discloses polymeric microspheres as injectable drug-delivery vehicles for delivering bioactive agents to sites within the central nervous system. PCT Publication No. WO 96/04001 discloses omega-3-fatty acid conjugates of neurologically-active drugs for brain tissue delivery. PCT Publication No. WO 96/22303 discloses fatty acid and glycerolipid conjugates of neurologically-active drugs for brain

tissue delivery.

[0305] Methods for Diagnosing, Prognosing and Monitoring Amyloid Beta Associated Disorders

Non-Invasive Alzheimer's Disease (AD) Diagnostics

[0306] Certain non-invasive AD diagnostic probes are known, and hold some promise for enabling in vivo evaluation of the presence and/or extent of brain amyloid. Known non-invasive AD diagnostic probes include: a) Congo Red derivatized small organic molecules (Dezutter et al., 1999b; Klunk et al., 2002; Skovronsky et al., 2000); b) anti-Abeta. monoclonal antibodies that bind specific amino acid residues of Abeta 1-42/43 (Majocha et al., 1992; Walker et al., 1994); c) Abeta 1-40 peptide derivatized with putrescine for increased permeability across the BBB, with appended chelation cores holding gadolinium (Gd-DTPA) or monocrystalline iron oxide nanoparticles (MION) (see, e.g. Weissleder et al., 2000); and d) iodine-123/125 and carbon-11 labeled thioflavin-based organic compounds that have been developed for in vivo labeling of A.beta. plaques (Klunk et al., 2001; Kung et al., 2002).

[0307] However, these known imaging agents bear significant limitations. The Congo Red derivatized compounds are neutral, small molecular weight compounds which can permeate the blood-brain barrier, and can provide localization of an Abeta targeted probe, but do not provide quantification capabilities.

[0308] Thus, known non-invasive imaging tools as they apply to *in vivo* diagnosis of AD are currently quite limited for a variety of reasons. A clear need remains for tools and methods enabling premortem diagnosis of AD, elucidation of the pathogenesis of AD, and efficient monitoring of patients undergoing anti-amyloid therapeutic treatment. In particular, a need exists for non-invasive imaging techniques for visualizing AD-associated changes in the brain.

[0309] The proteins that bind to amyloid beta 1-42, e.g., antibodies, described herein have *in vitro* and *in vivo* diagnostic utilities for Alzheimer's disease. An exemplary method includes: (i) administering the amyloid beta 1-42 antibody to a subject; and (ii) detecting the amyloid beta 1-42 antibody in the subject. The detecting can include determining the location of the amyloid beta 1-42 antibody in the subject. Another exemplary method includes contacting an amyloid beta 1-42 antibody to a sample, e.g., a sample from a subject.

[0310] In another aspect, the present invention provides a diagnostic method for detecting the presence of amyloid beta 1-42, in vitro (e.g., a biological sample, such as tissue, biopsy) or in vivo (e.g., in vivo imaging in a subject). The method includes: (i) contacting a sample with amyloid beta 1-42 antibody; and (ii) detecting formation of a complex between the amyloid beta 1-42 antibody and the sample. The method can also include contacting a reference sample (e.g., a control sample) with the ligand, and determining the extent of formation of the complex between the ligand and the sample relative to the same for the reference sample. A change, e.g., a statistically significant change, in the formation of the complex in the sample or subject

relative to the control sample or subject can be indicative of the presence of amyloid beta 1-42 in the sample.

[0311] The amyloid beta 1-42 antibody can be directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound protein. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, and radioactive materials.

[0312] Complex formation between the amyloid beta 1-42 antibody and amyloid beta 1-42 can be detected by measuring or visualizing either the ligand bound to the amyloid beta 1-42 or unbound ligand. Conventional detection assays can be used, e.g., an enzyme-linked immunosorbent assays (ELISA), a radioimmunoassay (RIA) or tissue immunohistochemistry. Further to labeling the amyloid beta 1-42 antibody, the presence of amyloid beta 1-42 can be assayed in a sample by a competition immunoassay utilizing standards labeled with a detectable substance and an unlabeled amyloid beta 1-42 antibody.

[0313] The foregoing antibodies can be used in methods known in the art relating to the localization and activity of the amyloid beta 1-42 polypeptide, e.g., for Western blotting, imaging amyloid beta in situ, measuring levels thereof in appropriate physiological samples, etc. using any of the detection techniques mentioned herein or known in the art. The standard techniques known in the art for immunoassays are described in "Methods in Immunodiagnosis", 2nd Edition, Rose and Bigazzi, eds. John Wiley & Sons, 1980; Campbell et al., "Methods and Immunology",,W.A. Benjamin, Inc., 1964; and Oellerich, M. (1984) J. Clin. Chem. Clin. Biochem. 22:895-904.

[0314] One aspect of the invention provides a method of using an antibody against amyloid beta, for example amyloid beta 1-42, to diagnose Alzheimer's disease (AD) in a subject. As amyloid beta levels correlate with the presence of AD in a subject, amyloid beta 1-42 serves as a general biomarker for AD, and may be predictive of the future onset of such disease. Alternatively, it may also serve as a marker for monitoring efficacy of therapy for such disorder, as described herein. Thus, the antibody compositions and methods provided herein are particularly deemed useful for the diagnosis of AD. In a particular embodiment, AD is the result of build-up of amyloid beta in the brains of individuals suffering from such disease. More particularly, the amyloid beta is amyloid beta 1-42.

[0315] The diagnostic method of the invention provides contacting a biological sample such as a biopsy sample, tissue, cell or fluid (e.g., whole blood, plasma, serum, urine, or CSF) isolated from a subject with an antibody which binds amyloid beta (Ghiso et al. FEBS Letters 408 (1997) pages 105-108). The antibody is allowed to bind to the antigen to form an antibody-antigen complex. The conditions and time required to form the antibody-antigen complex may vary and are dependent on the biological sample being tested and the method of detection being used. Once non-specific interactions are removed by, for example, washing the sample, the antibody-antigen complex is detected using any one of the immunoassays described above as well a

number of well-known immunoassays used to detect and/or quantitate antigens (see, for example, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1988) 555-612). Such well-known immunoassays include antibody capture assays, antigen capture assays, and two-antibody sandwich assays. In an antibody capture assay, the antigen is attached to solid support, and labeled antibody is allowed to bind. After washing, the assay is quantitated by measuring the amount of antibody retained on the solid support. In an antigen capture assay, the antibody is attached to a solid support, and labeled antigen is allowed to bind. The unbound proteins are removed by washing, and the assay is quantitated by measuring the amount of antigen that is bound. In a two-antibody sandwich assay, one antibody is bound to a solid support, and the antigen is allowed to bind to this first antibody. The assay is quantitated by measuring the amount of a labeled second antibody that binds to the antigen.

[0316] These immunoassays typically rely on labeled antigens, antibodies, or secondary reagents for detection. These proteins may be labeled with radioactive compounds, enzymes, biotin, or fluorochromes. Of these, radioactive labeling may be used for almost all types of assays. Enzyme-conjugated labels are particularly useful when radioactivity must be avoided or when quick results are needed. Biotin-coupled reagents usually are detected with labeled streptavidin. Streptavidin binds tightly and quickly to biotin and may be labeled with radioisotopes or enzymes. Fluorochromes, although requiring expensive equipment for their use, provide a very sensitive method of detection. Those of ordinary skill in the art will know of other suitable labels which may be employed in accordance with the present invention. The binding of these labels to antibodies or fragments thereof may be accomplished using standard techniques such as those described by Kennedy, et al. ((1976) Clin. Chim. Acta 70:1-31), and Schuurs, et al. ((1977) Clin. Chim Acta 81:1-40).

[0317] In accordance with the diagnostic method of the invention, the presence or absence of the antibody-antigen complex is correlated with the presence or absence in the biological sample of the antigen, or a peptide fragment thereof. A biological sample containing elevated levels of said antigen (amyloid beta 1-42) is indicative of AD in a subject from which the biological sample was obtained. Accordingly, the diagnostic method of the invention may be used as part of a routine screen in subjects suspected of having AD or for subjects who may be predisposed to having AD. Moreover, the diagnostic method of the invention may be used alone or in combination with other well-known diagnostic methods to confirm the presence of AD.

[0318] The diagnostic method of the invention further provides that an antibody of the invention may be used to monitor the levels of amyloid beta antigen in patient samples at various intervals of drug treatment to identify whether and to which degree the drug treatment is effective in reducing or inhibiting the symptoms associated with such disorder, such reduction being an indication that the therapy may ultimately result in amelioration and/or cure of the disorder. Furthermore, antigen levels may be monitored using an antibody of the invention in studies evaluating efficacy of drug candidates in model systems and in clinical trials. The antigens

provide for surrogate biomarkers in biological fluids to non-invasively assess the global status of AD. For example, using an antibody of this invention, antigen levels may be monitored in biological samples of individuals treated with known or unknown therapeutic agents or toxins. This may be accomplished with cell lines in vitro or in model systems and clinical trials. Persistently increased total levels of amyloid beta antigen in biological samples during or immediately after treatment with a drug candidate indicates that the drug candidate has little or no effect on AD. Likewise, the reduction in total levels of amyloid beta antigen indicates that the drug candidate is effective in reducing or inhibiting the symptoms of AD. Furthermore, the continued reduction of amyloid beta in the subject may ultimately result in full remission of the individual suffering from such disorder. This may provide valuable information at all stages of pre-clinical drug development, clinical drug trials as well as subsequent monitoring of patients undergoing drug treatment.

[0319] The present invention also provides methods for monitoring the progression of AD by detecting the upregulation of amyloid beta 1-42. Monitoring methods involve determining the test amounts of amyloid beta 1-42in biological samples taken from a subject at a first and second time, and comparing the amounts. A change in amount of amyloid beta 1-42 between the first and second time indicates a change in the course of AD, with a decrease in amount indicating stabilization of AD progression, and an increase in amount indicating progression of AD. Such monitoring assays are also useful for evaluating the efficacy of a particular therapeutic intervention (e.g., disease attenuation and/or reversal) in patients being treated for AD.

[0320] Fluorophore- and chromophore-labeled protein ligands can be prepared. Since antibodies and other proteins absorb light having wavelengths up to about 310 nm, the fluorescent moieties should be selected to have substantial absorption at wavelengths above 310 nm, and preferably above 400 nm. A variety of suitable fluorescers and chromophores are described by Stryer (Science (1968) 162:526) and by Brand et al. (Annual Rev. Biochem. (1972) 41:843 868). The protein ligands can be labeled with fluorescent chromophore groups by conventional procedures such as those disclosed in U.S. Pat. Nos. 3,940,475, 4,289,747, and 4,376,110. One group of fluorescers having a number of the desirable properties described above is the xanthene dyes, which include the fluoresceins and rhodamines. Another group of fluorescent compounds are the naphthylamines. Once labeled with a fluorophore or chromophore, the protein ligand can be used to detect the presence or localization of the amyloid beta 1-42 in a sample, e.g., using fluorescent microscopy (such as confocal or deconvolution microscopy).

[0321] Immunohistochemistry can be performed using the protein ligands described herein. For example, in the case of an antibody, the antibody can be synthesized with a label (such as a purification or epitope tag), or can be detectably labeled, e.g., by conjugating a label or label-binding group. For example, a chelator can be attached to the antibody. The antibody is then contacted to a histological preparation, e.g., a fixed section of tissue that is on a microscope

slide. After an incubation for binding, the preparation is washed to remove unbound antibody. The preparation is then analyzed, e.g., using microscopy, to identify if the antibody bound to the preparation.

[0322] The antibody (or other polypeptide or peptide) can be unlabeled at the time of binding. After binding and washing, the antibody is labeled in order to render it detectable.

[0323] The amyloid beta 1-42 antibody can also be immobilized on a protein array. The protein array can be used as a diagnostic tool, e.g., to screen medical samples (such as isolated cells, blood, sera, biopsies, and the like). The protein array can also include other ligands, e.g., that bind to amyloid beta 1-42 or to other target molecules.

[0324] Methods of producing polypeptide arrays are described, e.g., in De Wildt et al. (2000) Nat. Biotechnol. 18:989-994; Lueking et al. (1999) Anal. Biochem. 270:103-111; Ge (2000) Nucleic Acids Res. 28, e3, I-VII; MacBeath and Schreiber (2000) Science 289:1760-1763; WO 01/40803 and WO 99/51773A1. Polypeptides for the array can be spotted at high speed, e.g., using commercially available robotic apparati, e.g., from Genetic MicroSystems or BioRobotics. The array substrate can be, for example, nitrocellulose, plastic, glass, e.g., surface-modified glass. The array can also include a porous matrix, e.g., acrylamide, agarose, or another polymer.

[0325] For example, the array can be an array of antibodies, e.g., as described in De Wildt, supra. Cells that produce the protein ligands can be grown on a filter in an arrayed format. Polypeptide production is induced, and the expressed polypeptides are immobilized to the filter at the location of the cell.

[0326] A protein array can be contacted with a labeled target to determine the extent of binding of the target to each immobilized polypeptide from the diversity strand library. If the target is unlabeled, a sandwich method can be used, e.g., using a labeled probed, to detect binding of the unlabeled target.

[0327] Information about the extent of binding at each address of the array can be stored as a profile, e.g., in a computer database. The protein array can be produced in replicates and used to compare binding profiles, e.g., of a target and a non-target. Thus, protein arrays can be used to identify individual members of the diversity strand library that have desired binding properties with respect to one or more molecules.

[0328] The amyloid beta 1-42 antibody can be used to label cells, e.g., cells in a sample (e.g., a patient sample). The ligand is also attached (or attachable) to a fluorescent compound. The cells can then be sorted using fluorescent activated cell sorted (e.g., using a sorter available from Becton Dickinson Immunocytometry Systems, San Jose Calif.; see also U.S. Pat. Nos. 5,627,037; 5,030,002; and 5,137,809). As cells pass through the sorter, a laser beam excites the fluorescent compound while a detector counts cells that pass through and determines whether a fluorescent compound is attached to the cell by detecting fluorescence. The amount of label bound to each cell can be quantified and analyzed to characterize the sample.

[0329] The sorter can also deflect the cell and separate cells bound by the ligand from those cells not bound by the ligand. The separated cells can be cultured and/or characterized.

[0330] In still another embodiment, the invention provides a method for detecting the presence of a amyloid beta 1-42 within a subject *in vivo*. The method includes (i) administering to a subject (e.g., a patient having an amyloid beta 1-42 associated disorder such as AD) an anti-amyloid beta 1-42 antibody, conjugated to a detectable marker; (ii) exposing the subject to a means for detecting the detectable marker. For example, the subject is imaged, e.g., by NMR or other tomographic means.

[0331] Examples of labels useful for diagnostic imaging include radiolabels such as ^{131}I, ^{111}In, ^{123}I, ^{99m}Tc, ^{32}P, ^{33}P, ^{125}I, ^{3}H, ^{14}C, and ^{188}Rh, fluorescent labels such as fluorescein and rhodamine, nuclear magnetic resonance active labels, positron emitting isotopes detectable by a positron emission tomography ("PET") scanner, chemiluminescers such as luciferin, and enzymatic markers such as peroxidase or phosphatase. Short range radiation emitters, such as isotopes detectable by short range detector probes can also be employed. The protein ligand can be labeled with such reagents using known techniques. For example, see Wensel and Meares (1983) Radioimmunoimaging and Radioimmunotherapy, Elsevier, N.Y. for techniques relating to the radiolabeling of antibodies and Colcher et al. (1986) Meth. Enzymol. 121: 802 816.

[0332] A radiolabeled ligand can also be used for in vitro diagnostic tests. The specific activity of a isotopically-labeled ligand depends upon the half life, the isotopic purity of the radioactive label, and how the label is incorporated into the antibody.

[0333] Procedures for labeling polypeptides with the radioactive isotopes (such as ^{14}C, ^{3}H, ^{35}S, ^{99m}Tc, ^{125}I, ^{32}P, ^{33}P, and ^{131}I) are generally known. See, e.g., U.S. Pat. No. 4,302,438; Goding, J. W. (Monoclonal antibodies: principles and practice: production and application of monoclonal antibodies in cell biology, biochemistry, and immunology 2nd ed. London; Orlando: Academic Press, 1986. pp 124 126) and the references cited therein; and A. R. Bradwell et al., "Developments in Antibody Imaging", Monoclonal Antibodies for Cancer Detection and Therapy, R. W. Baldwin et al., (eds.), pp 65 85 (Academic Press 1985).

[0334] Amyloid beta 1-42 antibodies described herein can be conjugated to Magnetic Resonance Imaging (MRI) contrast agents. Some MRI techniques are summarized in EP-A-0 502 814. These methods may be used in conjunction with contrast agents. Examples of such contrast agents include a number of magnetic agents paramagnetic agents (which primarily alter T1) and ferromagnetic or superparamagnetic (which primarily alter T2 response). Chelates (e.g., EDTA, DTPA and NTA chelates) can be used to attach (and reduce toxicity) of some paramagnetic substances (e.g., Fe^{3+}, Mn^{2+}, Gd^{3+}). Other agents can be in the form of particles, e.g., less than 10 mm to about 10 nm in diameter and having ferromagnetic, antiferromagnetic, or superparamagnetic properties.

[0335] The amyloid beta 1-42 antibodies can also be labeled with an indicating group containing the NMR active ^{19}F atom, or a plurality of such atoms as described by Pykett ((1982) Scientific American 246:78-88) to locate and image amyloid beta 1-42 distribution.

Cellular Transfection and Gene Therapy

[0336] The present invention encompasses the use of nucleic acids encoding the antibodies of the invention for transfection of cells *in vitro* and *in vivo*. These nucleic acids can be inserted into any of a number of well-known vectors for transfection of target cells and organisms. The nucleic acids are transfected into cells ex vivo and in vivo, through the interaction of the vector and the target cell. The compositions are administered to a subject in an amount sufficient to elicit a therapeutic response.

[0337] In another aspect, the invention provides a method of treating a target site, i.e., a target cell or tissue, in a human or other animal comprising transfecting a cell with a nucleic acid encoding an antibody of the invention, wherein the nucleic acid comprises an inducible promoter operably linked to the nucleic acid encoding the fusion polypeptide. For gene therapy procedures in the treatment or prevention of human disease, see for example, Van Brunt (1998) Biotechnology 6:1149-1154.

[0338] Non-limiting examples of techniques which can be used to introduce an expression vector encoding the antibodies into a host cell include: Adenovirus-Polylysine DNA Complexes: Naked DNA can be introduced into cells by complexing the DNA to a cation, such as polylysine, which is then coupled to the exterior of an adenovirus virion (e.g., through an antibody bridge, wherein the antibody is specific for the adenovirus molecule and the polylysine is covalently coupled to the antibody) (see Curiel et al. (1992) Human Gene Therapy 3:147-154). Entry of the DNA into cells exploits the viral entry function, including natural disruption of endosomes to allow release of the DNA intracellularly. A particularly advantageous feature of this approach is the flexibility in the size and design of heterologous DNA that can be transferred to cells.

[0339] Receptor-Mediated DNA Uptake: Naked DNA can also be introduced into cells by complexing the DNA to a cation, such as polylysine, which is coupled to a ligand for a cell-surface receptor (see for example Wu et al. (1988) J. Biol. Chem. 263:14621; Wilson et al. (1992) J Biol. Chem. 267:963-967; and U.S. Pat. No. 5,166,320). Binding of the DNA-ligand complex to the receptor facilitates uptake of the DNA by receptor-mediated endocytosis. Receptors to which a DNA-ligand complex have targeted include the transferrin receptor and the asialoglycoprotein receptor. Additionally, a DNA-ligand complex can be linked to adenovirus capsids which naturally disrupt endosomes, thereby promoting release of the DNA material into the cytoplasm and avoiding degradation of the complex by intracellular lysosomes (see for example Curiel et al. (1991) Proc. Natl. Acad. Sci. USA 88:8850; and Cotten et al. (1992) Proc. Natl. Acad. Sci. USA 89:6094-6098; Wagner et al. (1992) Proc. Natl. Acad. Sci. USA 89:6099-6103). Receptor-mediated DNA uptake can be used to introduce DNA into cells either in vitro or

in vivo and, additionally, has the added feature that DNA can be selectively targeted to a particular cell type by use of a ligand which binds to a receptor selectively expressed on a target cell of interest.

[0340] Liposome-Mediated transfection ("lipofection"): Naked DNA can be introduced into cells by mixing the DNA with a liposome suspension containing cationic lipids. The DNA/liposome complex is then incubated with cells. Liposome mediated transfection can be used to stably (or transiently) transfect cells in culture in vitro. Protocols can be found in Current Protocols in Molecular Biology, Ausubel, F. M. et al. (eds.) Greene Publishing Associates, (1989), Section 9.4 and other standard laboratory manuals. Additionally, gene delivery in vivo has been accomplished using liposomes. See for example Nicolau et al. (1987) Meth. Enz. 149:157-176; Wang and Huang (1987) Proc. Natl. Acad. Sci. USA 84:7851-7855; Brigham et al. (1989) Am. J Med. Sci. 298:278; and Gould-Fogerite et al. (1989) Gene 84:429-438.

[0341] Direct Injection: Naked DNA can be introduced into cells by directly injecting the DNA into the cells. For an in vitro culture of cells, DNA can be introduced by microinjection, although this not practical for large numbers of cells. Direct injection has also been used to introduce naked DNA into cells in vivo (see e.g., Acsadi et al. (1991) Nature 332:815-818; Wolff et al. (1990) Science 247:1465-1468). A delivery apparatus (e.g., a "gene gun") for injecting DNA into cells in vivo can be used. Such an apparatus is commercially available (e.g., from BioRad).

[0342] Retroviral Mediated Gene Transfer: Defective retroviruses are well characterized for use in gene transfer for gene therapy purposes (for a review see Miller, A. D. (1990) Blood 76:271). A recombinant retrovirus can be constructed having a nucleic acid encoding a gene of interest (e.g., an antibody homologue) inserted into the retroviral genome. Additionally, portions of the retroviral genome can be removed to render the retrovirus replication defective. The replication defective retrovirus is then packaged into virions which can be used to infect a target cell through the use of a helper virus by standard techniques. Protocols for producing recombinant retroviruses and for infecting cells *in vitro* or *in vivo* with such viruses can be found in Current Protocols in Molecular Biology, Ausubel, F. M. et al. (eds.) Greene Publishing Associates, (1989), Sections 9.10-9.14 and other standard laboratory manuals. Examples of suitable retroviruses include pLJ, pZIP, pWE and pEM which are well known to those skilled in the art.

[0343] Retroviruses have been used to introduce a variety of genes into many different cell types, including epithelial cells, endothelial cells, lymphocytes, myoblasts, hepatocytes, bone marrow cells, in vitro and/or in vivo (see for example Eglitis, et al. (1985) Science 230:1395-1398; Danos and Mulligan (1988) Proc. Natl. Acad. Sci. USA 85:6460-6464; Wilson et al. (1988) Proc. Natl. Acad. Sci. USA 85:3014-3018; Armentano et al. (1990) Proc. Natl. Acad. Sci. USA 87:6141-6145; Huber et al. (1991) Proc. Natl. Acad. Sci. USA 88:8039-8043; Ferry et al. (1991) Proc. Natl. Acad. Sci. USA 88:8377-8381; Chowdhury et al. (1991) Science 254:1802-1805; van Beusechem et al. (1992) Proc. Natl. Acad. Sci. USA 89:7640-7644; Kay et al. (1992) Human Gene Therapy 3:641-647; Dai et al. (1992) Proc. Natl. Acad. Sci. USA 89:10892-10895; Hwu et

al. (1993) J Immunol. 150:4104-4115; U.S. Pat. No. 4,868,116; U.S. Pat. No. 4,980,286; and PCT Publication Nos. WO 89/07136; WO 89/02468; WO 89/05345; and WO 92/07573). While any retrovirus may be utilized, the lentivirus approach allows for delivery to a broad variety of cellular targets, both ex vivo (cell lines, primary cells including stem cells, fertilized oocytes, and blastocysts) and in vivo (e.g., brain, lung, liver). The lentivirus vector-mediated delivery of siRNAs allows for the controllable suppression of cellular genes both with a high degree of efficacy and without significant leakiness.

[0344] Adenoviral Mediated Gene Transfer: The genome of an adenovirus can be manipulated such that it encodes and expresses a gene product of interest (e.g., an antibody homologue) but is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. See for example Berkner et al. (1988) BioTechniques 6:616; Rosenfeld et al. (1991) Science 252:431-434; and Rosenfeld et al. (1992) Cell 68:143-155. Suitable adenoviral vectors derived from the adenovirus strain Ad type 5 dl324 or other strains of adenovirus (e.g., Ad2, Ad3, Ad7 etc.) are well known to those skilled in the art. Recombinant adenoviruses are advantageous in that they do not require dividing cells to be effective gene delivery vehicles and can be used to infect a wide variety of cell types, including airway epithelium (Rosenfeld et al. (1992) cited supra), endothelial cells (Lemarchand et al. (1992) Proc. Natl. Acad. Sci. USA 89:6482-6486), hepatocytes (Herz and Gerard (1993) Proc. Natl. Acad. Sci. USA 90:2812-2816) and muscle cells (Quantin et al. (1992) Proc. Natl. Acad. Sci. USA 89:2581-2584). Additionally, introduced adenoviral DNA (and foreign DNA contained therein) is not integrated into the genome of a host cell but remains episomal, thereby avoiding potential problems that can occur as a result of insertional mutagenesis in situations where introduced DNA becomes integrated into the host genome (e.g., retroviral DNA). Moreover, the carrying capacity of the adenoviral genome for foreign DNA is large (up to 8 kilobases) relative to many other gene delivery vectors (Berkner et al. cited supra; Haj-Ahmand and Graham (1986) J Virol. 57:267). Most replication-defective adenoviral vectors currently in use are deleted for all or parts of the viral E1 and E3 genes but retain as much as 80% of the adenoviral genetic material.

[0345] Adeno-Associated Viral Mediated Gene Transfer: Adeno-associated virus (AAV) is a naturally occurring defective virus that requires another virus, such as an adenovirus or a herpes virus, as a helper virus for efficient replication and a productive life cycle. (For a review see Muzyczka et al. Curr. Topics in Micro. and Immunol. (1992) 158:97-129). It is also one of the few viruses that may integrate its DNA into non-dividing cells, and exhibits a high frequency of stable integration (see for example Flotte et al. (1992) Am. J Respir. Cell. Mol. Biol. 7:349-356; Samulski et al. (1989) J Virol. 63:3822-3828; and McLaughlin et al. (1989) J Virol. 62:1963-1973). Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate. Space for exogenous DNA is limited to about 4.5 kb. An AAV vector such as that described in Tratschin et al. (1985) Mol. Cell. Biol. 5:3251-3260 can be used to introduce DNA into cells. A variety of nucleic acids have been introduced into different cell types using AAV vectors (see for

example Hermonat et al. (1984) Proc. Natl. Acad. Sci. USA 81:6466-6470; Tratschin et al. (1985) Mol. Cell. Biol. 4:2072-2081; Wondisford et al. (1988) Mol. Endocrinol. 2:32-39; Tratschin et al. (1984) J Virol. 51:611-619; and Flotte et al. (1993) J Biol. Chem. 268:3781-3790).

[0346] The efficacy of a particular expression vector system and method of introducing nucleic acid into a cell can be assessed by standard approaches routinely used in the art. For example, DNA introduced into a cell can be detected by a filter hybridization technique (e.g., Southern blotting) and RNA produced by transcription of the introduced DNA can be detected, for example, by Northern blotting, RNase protection or reverse transcriptase-polymerase chain reaction (RT-PCR). Expression of the introduced gene product (e.g., the antibody homologue) in the cell can be detected by an appropriate assay for detecting proteins, for example by immunohistochemistry.

[0347] As will be appreciated by those skilled in the art, the choice of expression vector system will depend, at least in part, on the host cell targeted for introduction of the nucleic acid. For example, nucleic acid encoding a fusion polypeptide is preferably introduced into muscle cells.

[0348] The functional outcome of expression of the antibodies can be assessed by suitable assays that monitor the expression and/or function of the target protein, including standard immunohistochemistry or immunoelectron microscopy techniques.

Kits

[0349] Also within the scope described herein are kits comprising the protein ligand that binds to amyloid beta 1-42 and instructions for diagnostic use, e.g., the use of the amyloid beta 1-42 antibody (e.g., antibody or antigen-binding fragment thereof, or other polypeptide or peptide) to detect amyloid beta 1-42, *in vitro*, e.g., in a sample, e.g., a biopsy or cells from a patient having an amyloid beta 1-42associated disorder, or in vivo, e.g., by imaging a subject. The kit can further contain a least one additional reagent, such as a label or additional diagnostic agent. For *in vivo* use the ligand can be formulated as a pharmaceutical composition.

[0350] The foregoing antibodies can be used in methods known in the art relating to the localization and activity of the amyloid beta, e.g., for Western blotting, imaging amyloid beta in situ, measuring levels thereof in appropriate physiological samples, etc. using any of the detection techniques mentioned herein or known in the art. The standard techniques known in the art for immunoassays are described in "Methods in Immunodiagnosis", 2nd Edition, Rose and Bigazzi, eds. John Wiley & Sons, 1980; Campbell et al., "Methods and Immunology", W.A. Benjamin, Inc., 1964; and Oellerich, M. (1984) J. Clin. Chem. Clin. Biochem. 22:895-904.

[0351] An amyloid beta 1-42 antibody or fragment thereof, can be provided in a kit, e.g., as a component of a kit. For example, the kit includes (a) amyloid beta 1-42 antibody or fragment thereof, e.g., a composition that includes an amyloid beta 1-42 antibody or fragment thereof, and, optionally (b) informational material. The informational material can be descriptive, instructional, marketing, or other material that relates to the methods described herein and/or

the use of an amyloid beta 1-42 antagonist, e.g., anti- amyloid beta 1-42 antibody or fragment thereof, for the methods described herein.

[0352] The informational material of the kits is not limited in its form. In one embodiment, the informational material can include information about production of the compound, molecular weight of the compound, concentration, date of expiration, batch, or production site information, and so forth. In one embodiment, the informational material relates to using the ligand to treat, prevent, diagnose, prognose, or monitor a disorder described herein.

[0353] In one embodiment, the informational material can include instructions to administer an amyloid beta 1-42 antagonist, e.g., anti- amyloid beta 1-42 antibody or fragment thereof, in a suitable manner to perform the methods described herein, e.g., in a suitable dose, dosage form, or mode of administration (e.g., a dose, dosage form, or mode of administration described herein). In another embodiment, the informational material can include instructions to administer an amyloid beta 1-42 antagonist, e.g., anti- amyloid beta 1-42 antibody or fragment thereof, to a suitable subject, e.g., a human, e.g., a human having, or at risk for, Alzheimer's disease, or an amyloid beta 1-42 mediated disorder.

[0354] For example, the material can include instructions to administer an amyloid beta 1-42 antagonist, e.g., amyloid beta 1-42 antibody or fragment thereof, to a patient, a patient with or at risk for AD.

[0355] The kit can include one or more containers for the composition containing an amyloid beta 1-42 antagonist, e.g., anti- amyloid beta 1-42 antibody or fragment thereof. In some embodiments, the kit contains separate containers, dividers or compartments for the composition and informational material. For example, the composition can be contained in a bottle, vial, or syringe, and the informational material can be contained in a plastic sleeve or packet. In other embodiments, the separate elements of the kit are contained within a single, undivided container. For example, the composition is contained in a bottle, vial, or syringe that has attached thereto the informational material in the form of a label. In some embodiments, the kit includes a plurality (e.g., a pack) of individual containers, each containing one or more unit dosage forms (e.g., a dosage form described herein) of an amyloid beta 1-42 antagonist, e.g., anti- amyloid beta 1-42 antibody or fragment thereof. For example, the kit includes a plurality of syringes, ampules, foil packets, atomizers, or inhalation devices, each containing a single unit dose of an amyloid beta 1-42 antagonist, e.g., anti- amyloid beta 1-42 antibody or fragment thereof, or multiple unit doses.

[0356] The kit optionally includes a device suitable for administration of the composition, e.g., a syringe, inhalant, pipette, forceps, measured spoon, dropper (e.g., eye dropper), swab (e.g., a cotton swab or wooden swab), or any such delivery device. In a preferred embodiment, the device is an implantable device that dispenses metered doses of the ligand.

Screening Assays

[0357] A still further aspect of the invention relates to screening assays to identify agents which bind amyloid beta e.g. a specific binding member thereof, particularly antibodies or mimics thereof. While not wishing to be bound by theory, these agents may inhibit the production or generation of Abeta by cleavage of APP or the aggregation of amyloid beta fibrils or to enhance the degradation or clearance of Abeta. The invention encompasses both *in vivo* and *in vitro* assays to screen small molecules, compounds, recombinant proteins, peptides, enzymes, nucleic acids, antibodies, etc. which exhibit any of the above activities.

[0358] In a particular embodiment, the binding of the agent is determined through the use of competitive binding assays. The competitor is an antibody of the invention known to bind to amyloid beta protein, including amyloid beta 1-42, or fragments thereof. Competitive screening assays may be done by combining the amyloid beta protein and an antibody of the invention in a first sample. A second sample comprises a test agent, amyloid beta and an antibody of the invention. The binding of the antibody is determined for both samples, and a change, or difference in binding between the two samples indicates the presence of a test agent capable of binding to amyloid beta and potentially modulating its conformational structure and/or activity. That is, if the binding of the antibody is different in the second sample relative to the first sample, the test agent is capable of binding to amyloid beta protein. Similar designs that utilize antibodies of this invention for the identification of non-antibody compounds that bind to amyloid beta are obvious to those skilled in the art.

[0359] One variation provides that the agent is labeled. Either the agent, or the competitor, or both, is added first to amyloid beta protein for a time sufficient to allow binding. Incubations may be performed at any temperature which facilitates optimal activity, typically between 4°C and 40°C. Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high-throughput screening. Typically between 0.1 and 1 hour will be sufficient. Excess reagent is generally removed or washed away. The second component is then added, and the presence or absence of the labeled component is followed, to indicate binding.

[0360] It is preferred that the competitor is added first, followed by the test agent. Displacement of the competing antibody of this invention is an indication that the test agent is binding to amyloid beta protein and thus is capable of binding to, and potentially modulating, the conformational structure and/or activity of amyloid beta protein. In this reaction either component may be labeled. Thus, for example, if the competitor is labeled, the presence of label in the wash solution indicates displacement by the agent. Alternatively, if the test agent is labeled, the presence of the label on the support indicates displacement.

[0361] Alternatively, the test agent is added first, with incubation and washing, followed by the competitor. The absence of binding by the competitor may indicate the test agent is bound to amyloid beta protein with a higher affinity. Thus, if the agent is labeled, the presence of the label on the support, coupled with a lack of competitor binding, may indicate the test agent is capable of binding to amyloid beta protein.

[0362] The agents encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 100 and less than about 3,500 daltons. Agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Agents may also be found among biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof.

[0363] Agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds.

[0364] Alternatively, the antibodies of this invention may be used for the design and synthesis of either peptide or non-peptide compounds (mimetics) that specifically bind to amyloid beta.

[0365] The assays provided use amyloid beta protein, amyloid beta 1-42, or fragments of any of these molecules. In addition, the assays described herein may use either isolated amyloid beta, amyloid beta 1-42, or fragments thereof or cells expressing these molecules or animal models that express these molecules.

[0366] A variety of other reagents may be included in the screening assays. These include reagents like salts, neutral proteins, e.g. albumin, detergents, etc. which may be used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Also, reagents that otherwise improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, and the like may be used. The mixture of components may be added in any order that provides for the requisite binding.

[0367] The methods of the invention are used to identify compounds that bind amyloid beta and which may inhibit the production or generation of Abeta through cleavage of APP, such as a gamma or beta secretase inhibitor, or the aggregation of amyloid beta fibrils, or which enhance the degradation or clearance of Abeta, and are therefore useful in the treatment of disorders, diseases or conditions attributed to elevated levels of amyloid beta, in particular, amyloid 1-42 or fragments thereof.

[0368] In the same manner that a small organic molecule or enzyme or mimotope having the desired activity and characteristics noted above, *e.g.* a molecule that blocks the production or generation of Abeta, or blocks the aggregation of amyloid beta, or enhances its degradation or clearance, may be administered to treat AD, so may an antibody specific for amyloid beta, Abeta 1-40, Abeta 1-42 or fragments thereof be administered to treat AD. Accordingly, a further aspect of the invention provides methods for treating AD by administering to a subject with such disorder an antibody which binds to and therefore blocks the functionally significant region(s) of amyloid beta, Abeta 1-42 or fragments thereof. As one of skill in the art may appreciate, the pharmaceutical compositions comprising the antibody and a pharmaceutically acceptable

carrier, as well as the route of administration of such pharmaceutical compositions, would be similar to those provided above for the other agents of this invention. The optimal quantity and spacing of individual dosages of an agent of the invention will be determined by the nature of the agent, the nature and extent of the disorder being treated, the form, route and site of administration, and the particular animal being treated. Such optimums may be determined by conventional techniques of monitoring clinical depression.

EXAMPLES

[0369] The following Examples and Figures are provided as illustrative examples and not for the purpose of limiting the present invention.

EXAMPLE 1.- Obtaining genetic immunoglobulin repertoires from peripheral blood of healthy elderly donors

Choice of suitable donors for constructing combinatorial human antibody libraries

[0370] A group of 4 healthy elderly individuals with ages comprised between 90-102 years was selected for obtaining human antibody or recombinant antibody fragment libraries. Said non-immunized healthy donors (Vj2, Vj3, Vj4 and Vj5) were chosen as optimal candidates for obtaining antibodies against the beta-amyloid 1-42 peptide for having full cognitive capacity and good health with no symptoms of Alzheimer's or another neuropathy without having received any medication for the prevention or treatment of neurodegenerative diseases.

Determination of specific antibodies in the serum of the chosen donors

[0371] Detection of natural antibodies in the serum of the donors was examined by means of a specific immunodetection assay that allows detecting reactivity against the beta-amyloid 1-42 peptide.

[0372] A specific enzymatic immunodetection assay (ELISA) was carried out to identify natural anti beta-amyloid 1-42 peptide antibodies in human serum, which included optimization of the working dilutions of commercial antibodies as well as the adjustment of the detection of antibodies against the beta-amyloid 1-42 peptide. A commercial mouse anti beta-amyloid 1-42 antibody (Pharmingen) was evaluated by means of plate immunoassay, using human beta-amyloid 1-42 peptide (Pharmingen) at 5 µg/ml as a coating. The working concentration of the secondary antibody to be used was then determined. To that end, several assays were conducted using the secondary antibodies: anti-hFab-HRP (Pierce), anti IgG+IgM+IgA-HRP (Pierce); anti IgG (H+L)-HRP (Pierce), anti IgM (Pharmingen), anti MIg1-biothin (Pharmingen). It was concluded that only two of the tested secondary antibodies present suitable characteristics for the inventors' purpose: anti IgG+IgM+IgA-HRP (Pierce) and anti IgG (H+L) HRP (Pierce) antibody.

[0373] Once the working concentrations of the commercial anti-beta 1-42 antibody and of the secondary antibody were optimized, the existence of anti beta-amyloid 1-42 peptide antibodies was then detected in the donors' serums. To that end, an ELISA using the beta-amyloid 1-42 peptide (Pharmingen) at a concentration of 5 µg/ml as the coating was performed and several serum dilutions were incubated. The secondary antibody used was anti IgG+IgM+IgA-HRP (Pierce). The results are shown in Figures 1A-1D. In view of these results, reactivity against the beta-amyloid 1-42 peptide (1 µg/ml) of the Vj2 and Vj5 serums was checked by means of dilutions. Anti IgG (H+L)-HRP antibody (Pierce) was used as the secondary antibody. In Figures 2A and 2B, it can be observed how these two serums titrate against the beta-amyloid 1-42 peptide, the serum from donor Vj2 exhibiting the greatest titer of anti beta-amyloid antibodies. Based on the results obtained in the antibody detection assays and on the clinical history, donor Vj2 was chosen as the optimal candidate for isolating the genetic repertoire of immunoglobulins and the construction of libraries for selecting antibodies against Alzheimer's disease.

Obtaining the immunoglobulin repertoire

Purification of peripheral blood B cells and obtaining the RNA mold

[0374] Peripheral blood lymphocytes (between 0.1 and 2.10^8 cells) of the selected donor (Vj2) were obtained by means of Ficoll-Hypaque density gradient centrifugation. The genetic material (mRNA) was extracted using commercial product Tri-Reagent following the manufacturer's protocol. Integrity and purity of the extracted material was analyzed by means of agarose/high resolution formaldehyde gels and analytically using the bio-sizing method for its quantification. After checking the integrity of each sample, the resulting material was considered satisfactory in terms of quality and integrity and was stored at -80 °C for its subsequent processing into aliquots suitable for being used as a mold for amplification of all the genetic families of the immunoglobulin genes.

[0375] The obtained samples were used for constructing the following libraries:

- Antibody fragment (Fab) library
- Single chain antibody fragment (scFv) library

Obtaining the immunoglobulin gene repertoire by means of PCR

[0376] Single chain complementary DNA (cDNA) was synthesized from mRNA by means of reverse transcriptase reaction, using deoxythimine octomers (oligo-dt) (Pharmacia) as primers. The material was aliquoted and used as a mold to amplify the fragments corresponding to the heavy chains (HC) of the IgG1 immunoglobulins and the kappa light chains (LC) by PCR. PCR amplification was done using standard protocols and in an independent manner. The different IgG1 fragments were obtained independently, using various single chain oligonucleotides, representative of all families of variable genes (VH) as primers, together with the Cγ1 specific primer of the first gamma-1 chain constant domain (CH1). The kappa light chains (LC) were

amplified in a similar manner, using primers representative of each VK family, together with kappa constant region (CK) specific primers. In addition to containing sequences specific for the amplification of variable or constant genes, all the primers include specific restriction targets not present in the genes for the purpose of facilitating their cloning. The pairing temperature (Tp) used for each pair of primers was optimized in prior experiments for the purpose of achieving amplification of about 95% of the genes of each VH and VK family.

[0377] The PCR products (HC or LC) for each amplified family were isolated from purified, quantified and proportionally combined 2% agarose gels in order to obtain the final repertoire (Figures 3A and 3B). This material was used as a mold in a second PCR amplification using external oligonucleotides for the purpose of extending the products, facilitating the subsequent digestion with restriction enzymes and increasing their cloning efficiency. The results are illustrated in Figure 3C. Once amplified, the products of each repertoire (HC-G1 and LC-kappa) were independently purified. The VH repertoires were digested with restriction enzymes Xho I and SpeI and were purified from agarose gels by Quiackick. The LC-kappa repertoire was digested with Sac I and XbaI and purified in the same manner.

[0378] Additionally, the same starting material was used to obtain a single chain antibody fragment (scFv) repertoire by means of using specific primers. The approach used allows obtaining from the same donor two antibody libraries: on in Fab (antibody fragments) format and another one in scFv fragment. Said single chain fragments consist of VH segments fused by means of a flexible linker (G_4S_2) to the light chain variable region. All amplifications were previously optimized until obtaining a yield of 90-100%. The results are illustrated in Figures 4A and 4B. Once the amplifications were carried out, the positive bands representative of each family were extracted and purified from preparative gels, and the material was subsequently fused by means of PCR overlap for its combined amplification using for that purpose primers RSC-F and RSC-B (Figure 4C). The obtained bands were finally digested and purified.

EXAMPLE 2.- <u>Construction of high-complexity combinatorial antibody libraries</u>

<u>Fab fragment library</u>

[0379] The Fab fragment library was constructed by means of cloning and assembly, using the gamma-1 repertoires combined with all the kappa light chains in Fab format, using the phagemid vector pComb3 XX. This vector allows cloning the CLs at restriction sites SacI-Xba I and the HCs at restriction sites XhoI–SpeI. The libraries were constructed sequentially. First the corresponding light chain repertoire was cloned and then the heavy chain repertoire was cloned.

[0380] Preparation of vector pComb3 XX SacI/XbaI was optimized in pilot experiments until obtaining a high CL ligation level (>$1x10^8$ transforming colonies/µg of DNA) and a low religation efficiency (<1%) in the absence of inserts, before cloning the kappa LC repertoire. Then, all the ligation product was used to transform highly competent E. coli bacteria (Efficiency > 5.10^9

transforming colonies/µg of DNA) by means of electroporation. After bacterial growth, the entire bacterial culture was centrifuged at low speed and duly treated to extract the phagemid DNA. The DNA was purified, quantified and subjected to digestion with restriction enzymes XhoI and SpeI. The resulting vector was quantified and used for cloning the HC-IgG1 repertoires. After precipitation, the ligation products were transformed independently by means of electroporation in E. coli and the final assembly was carried out by means of infection with M13 helper phage to construct a kappa-IgG1 repertoire antibody expression library of $1x\ 10^7$ antibodies.

Single chain fragment (scFv) library

[0381] The single chain antibody fragment (scFv) library of the same donor (Vj2) was constructed by means of cloning single fragments of the variable regions of the immunoglobulins corresponding to the Gamma and Kappa repertoires overlapped by means of a flexible linker G_4S_2. After enzymatic digestion, the repertoires were cloned into vector pComB3XX. The obtained size was 5×10^6 independent clones.

Checking the diversity of antibody libraries by HCDR3 segment sequencing

[0382] The genetic material of 15-20 antibody-producing clones was extracted from each library. Random sequences of the HCDR3 segment indicated that the libraries consist of a wide diversity, both in the size of HC CDR3 (6-21 amino acids long), and in their sequence. As an example, Figure 5 shows the HCDR3 sequences of several clones chosen randomly from the antibody fragment (Fab) library and from the single chain antibody fragment (scFv) library.

[0383] Human antibody libraries were obtained including two formats (Fab and scFv). Said libraries represent a broad collection of human antibodies and constitute a highly useful tool in the search for specific antibodies directed against the beta-amyloid 1-42 peptide.

EXAMPLE 3.- Antigen selection from the single chain fragment library

[0384] The antigen selection against the beta-amyloid 1-42 peptide of the single chain fragment (scFv) library was carried out by means of an immunoassay against the beta-amyloid 1-42 peptide immobilized in solid phase (ELISA), using BSA as a negative control (c-). In each cycle, the scFv fragment library was amplified up to 10^{13} clones/ml and selected in the previously determined conditions, increasing the astringency of the washings in each selection round. The peptide concentrations to be used in the selection against the peptide amyloid 1-42 were previously optimized in solid phase. A significant degree of selection was obtained in the antigen selection process, as illustrated in Figure 6.

EXAMPLE 4.- Identification of high-affinity specific target human antibodies

[0385] After antigen selection from the single chain fragment library, the identification of antibody clones specific against the beta amyloid peptide was carried out by means of transfection of the genetic material of the antibodies obtained in bacteria suitable for the expression of soluble scFv molecules.

[0386] In the antigenic selection process, clones were chosen randomly from the total number of clones obtained in the last selection cycle and were arranged in master culture dishes. Antibody production in bacterial clones was optimized by means of pilot assays to obtain maximum expression. After determining the optimal conditions 235 clones chosen randomly from the master were analyzed. Each bacterial clone was grown in optimal culture conditions for antibody production and these were finally extracted from the supernatant for their characterization.

EXAMPLE 5.- Characterization of the obtained human antibodies against the beta-amyloid 1-42 peptide

[0387] After the identification of specific human antibodies, the genetic, structural and functional characterization of said antibodies were performed.

[0388] The antibodies obtained from the selection of the single chain antibody fragment library were analyzed by means of immunoassay specific against the beta-amyloid 1-42 peptide and BSA. A total of 5 recombinant antibodies showing specific reactivity against the beta-amyloid 1-42 peptide were obtained and the remaining antibodies showed reactivity against beta amyloid 1-42 and BSA. Two of said recombinant antibodies, AD38 and AD64, showed better beta-amyloid 1-42 peptide-binding profiles. These antibodies were purified to mid-scale (0.5-1 µg/ml) and evaluated at different concentrations in immunoassay against peptide 1-42. The obtained results are summarized in Figures 7A and 7B, in which it is seen that both antibodies specifically recognize the target binding in a dose-dependent manner.

[0389] Genetic characterization was carried out by means of the automatic determination of the nucleotide sequences. Reading and edition was conducted using the SeqEd program (Applied Biosystems) and subsequent analysis was conducted using GCG (University of Wisconsin Genetic Computer Group-Madison, WI) and Analize (MacMolly ®Tetra Sof Gen GmbH (version 3.10)). Identity of the VH, DH, JH and VK, JK genes was determined by using FASTA (GenBank), Blast and IgBlast (NCBI) together with MacMolly (Aling and Complign) and DNAPLOT (Version 2.0.1) locally applied to the human antibody database VBASE (Tomlinson et al., MRC Centre for Protein Engineering, UK) (Tomlinson, 1992). The index of replacement and silent (R:S) mutations observed in the HC and LC, after comparison with the corresponding genomic genes (germline), was calculated manually. The theoretical or inherent R:S indexes or each germinal gene were calculated individually for each codon and region using the InhSusCalc v1.0 program.

[0390] Once the nucleotide sequences of the scFv antibody fragments of AD38 (SEQ ID NO: 9) and AD64 (SEQ ID NO: 11) were known, said recombinant antibodies were used as the only antibodies. Their heavy chains are derived from the VHIII gene family and the light chains are derived from the VKIII and VKII gene families. Table 1 summarizes the genetic derivation and sequences of the CDR3 (Complementary-Determining Region-3) segments of recombinant antibodies AD38 and AD64; and Table 2 shows the R:S indexes. Numerous somatic mutations in comparison to their corresponding genomic genes (germline) were observed in both antibodies, indicating the existence of antigen maturation processes against the beta-amyloid 1-42 peptide.

[0391] Both the functional analysis and the genetic characteristics found after sequencing indicate that antibodies AD38 and AD64 are specific, exhibit antigenic maturation characteristics and reflect part of a competent immune response against the beta amyloid peptide. This makes them and antibodies derived from their structures excellent candidates as diagnostic, therapeutic or imaging agents.

EXAMPLE 6

Proposed Treatment of Subjects

[0392] A single-dose phase I trial can be performed to determine safety of the anti-amyloid beta 1-42 antibody. A therapeutic agent can be administered in increasing dosages to different patients starting from about 0.01mg per patient to a level of presumed efficacy, and increasing by a factor of three until a level of about 10 times the proposed effective dosage is reached.

[0393] A phase II trial can be performed to determine therapeutic efficacy of the anti-amyloid beta 1-42 antibody. Patients with early to mid Alzheimer's Disease defined using Alzheimer's disease and Related Disorders Association (ADRDA) criteria for probable AD will be selected. Suitable patients score in the 12-26 range on the Mini-Mental State Exam (MMSE). Other selection criteria are that patients are likely to survive the duration of the study and lack complicating issues such as use of concomitant medications that may interfere. Baseline evaluations of patient function can be made using classic psychometric measures, such as the MMSE, and the ADAS, which is a comprehensive scale for evaluating patients with Alzheimer's Disease status and function. These psychometric scales provide a measure of progression of the Alzheimer's condition. Suitable qualitative life scales can also be used to monitor treatment. Disease progression can also be monitored by MRI. Blood profiles of patients can also be monitored including assays of immunogen-specific antibodies and T-cells responses.

[0394] Following baseline measures, patients can begin receiving treatment. They will be randomized and treated with either therapeutic agent or placebo in a blinded fashion. Patients will be monitored at least every six months. Efficacy will be determined by a significant reduction in progression of a treatment group relative to a placebo group.

[0395] A second phase II trial can be performed to evaluate conversion of patients from non-Alzheimer's Disease early memory loss, sometimes referred to as age-associated memory impairment (AAMI), to probable Alzheimer's disease as defined as by ADRDA criteria. Patients with high risk for conversion to Alzheimer's Disease may be selected from a non-clinical population by screening reference populations for early signs of memory loss or other difficulties associated with pre-Alzheimer's symptomatology, a family history of Alzheimer's Disease, genetic risk factors, age, sex, and other features found to predict high-risk for Alzheimer's Disease. Baseline scores on suitable metrics including the MMSE and the ADAS together with other metrics designed to evaluate a more normal population will be collected. These patient populations will be divided into suitable groups with placebo comparison against dosing alternatives with the agent. These patient populations will be followed at intervals of about six months, and the endpoint for each patient is whether or not he or she converts to probable Alzheimer's Disease as defined by ADRDA criteria at the end of the observation.

[0396] **Table 1**

Antibody	**VH germline**	**DH germline**	**JH germline**	**HCDR3**
AD38	COS-6/DA-8	D6-6/DN4	JH4a	ELRGWSIEY
AD64	DP-38/9-1	D2	JH3a	DDYDF
Antibody	**Vk germline**		**JL germline**	**LCDR3**
AD38	Vg/38K		JK5	QHRRDWPPGAT
AD64	DPK15/A19		JK4	MQALHNPLT

[0397] **Table 2**

Heavy chain (HC)							
Antibody	**FR1**	**CDR1**	**FR2**	**CDR2**	**FR3**	**FRs R:S**	**CDRs R:S**
AD38	5:2	1:0	2:1	8:4	8:3	15:6	9:4
AD64	6:3	1:1	0:0	10:2	7:6	13:9	11:3
Light chain (LC)							
Antibody	**FR1**	**CDR1**	**FR2**	**CDR2**	**FR3**	**FRs R:S**	**CDRs R:S**
AD38	5:2	4:0	3:2	3:0	4:1	12:5	7:0
AD64	4:0	3:0	1:1	2:1	2:1	7:2	5:1

[0398] Biological Material Deposit

An *Escherichia coli* XL 1-B culture containing the AD38 encoding plasmid vector has been deposited pursuant to, and in satisfaction of, the requirements of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure with the Spanish Type Culture Collection (CECT) (Colección Española de Cultivos Tipo), Burjassot, Valencia (Spain), on March 28, 2006, under the access number CECT 7167.

Also, an *Escherichia coli* XL 1-B culture containing the AD64 encoding plasmid vector has been deposited pursuant to, and in satisfaction of, the requirements of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure with the Spanish Type Culture Collection (CECT) (Colección Española de Cultivos Tipo), Burjassot, Valencia (Spain), on March 28, 2006, under the access number CECT 7168.

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WHAT IS CLAIMED IS:

1. An isolated specific binding member which recognizes an epitope on amyloid beta 1-42, wherein said binding member is obtained from healthy elderly subjects having full cognitive capacity and who exhibit no symptoms of Alzheimer's disease or other neuropathies.

2. The isolated specific binding member of claim 1, wherein the subjects from which the binding member is obtained range in age from 60 years of age and above, 70 years of age and above, 80 years of age and above or 90 years of age and above.

3. An isolated specific binding member of claim 1, wherein the binding member is an antibody having amyloid beta 1-42 binding capacity, and wherein the antibody comprises an amino acid sequence of SEQ ID NOs: 2, or 4, or an amino acid sequence that is substantially homologous to SEQ ID NOs: 2 or 4, and functional fragments, derivatives or variants thereof.

4. An antibody, functional fragment or derivative thereof with beta-amyloid 1-42 peptide-binding capacity comprising one or more of the amino acid sequences selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4 and sequences substantially homologous to either one of SEQ ID NO: 2 or SEQ ID NO: 4.

5. An antibody, functional fragment thereof or derivative with beta-amyloid 1-42 peptide-binding capacity, according to claim 4, comprising at least one heavy chain and one light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 2 and the light chain comprises the amino acid sequence of SEQ ID NO: 4.

6. An antibody, functional fragment or derivative thereof with beta-amyloid 1-42 peptide-binding capacity comprising one or more of the amino acid sequences selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 8 and sequences substantially homologous to either one of SEQ ID NO: 6 or SEQ ID NO: 8.

7. An antibody, functional fragment thereof or derivative with beta-amyloid 1-42 peptide-binding capacity, according to the claim 6, comprising at least one heavy chain and one light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 6 and the light chain comprises the amino acid sequence of SEQ ID NO: 8.

8. An isolated specific binding member which recognizes amyloid beta 1-42, wherein said binding member is obtained by means of affinity maturation strategies from the antibodies of any one of claims 1-7.

9. A specific binding member or functional antibody fragment or derivative thereof according to any of claims 1 to 8, wherein said fragment is a scFv.

10. A specific binding member, or antibody, functional fragment thereof or derivative thereof, of any one of claims 1-8, which is detectably labeled.

11. The specific binding member, or antibody, functional fragment thereof or derivative thereof, of claim 10, wherein the detectable label is selected from an enzyme, a radioisotope, a fluorophore and a heavy metal.

12. A specific binding member, or antibody, functional fragment thereof or derivative thereof, of any one of claims 1-9 which is attached to a drug.

13. A specific binding member, or antibody, functional fragment thereof or derivative thereof, comprising a functional or binding portion of any one of the sequences set forth in claims 3 to 7 or combinations thereof with beta-amyloid 1-42 peptide-binding capacity.

14. An isolated nucleic acid molecule selected from the group consisting of:

a. a nucleic acid molecule encoding a specific binding member, or antibody, functional fragment thereof or derivative thereof,, according to any one of claims 1 to 7;

b. a nucleic acid molecule the nucleotide sequence of which differs from the sequences defined in (a) due to degeneration of the genetic code; and

c. a nucleic acid molecule substantially homologous to the sequences defined in (a).

15. The isolated nucleic acid of claim 14 encoding a binding member which is an antibody molecule or a fragment, derivative or variant thereof, which binds amyloid beta 1-42.

16. The isolated nucleic acid molecule of claim 15, comprising any one of the nucleotide sequences set forth in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7, or combinations thereof.

17. A vector comprising a nucleic acid molecule according to any of claims 14,15 or 16.

18. A host cell containing a vector according to claim 17.

19. A unicellular host transformed with:

(A) the DNA sequence of SEQ ID NO:1;

(B) the DNA sequence of SEQ ID NO:3;

(C) the DNA sequence of SEQ ID NO:1 and the DNA sequence of SEQ ID NO:3;

(D) a DNA sequence that hybridizes to any of the foregoing DNA sequences under standard hybridization conditions;

(E) a DNA sequence that codes on expression for an amino acid sequence encoded by any of the foregoing DNA sequences; or

(F) a fragment of any one of the foregoing DNA sequences which encodes a specific binding member, or antibody, functional fragment thereof or derivative thereof, as defined in any one of claims 1 to 7,

wherein said DNA sequence is operatively linked to an expression control sequence.

20. A unicellular host transformed with:

(A) the DNA sequence of SEQ ID NO:5;

(B) the DNA sequence of SEQ ID NO:7;

(C) the DNA sequence of SEQ ID NO:5 and the DNA sequence of SEQ ID NO:7;

(D) a DNA sequence that hybridizes to any of the foregoing DNA sequences under standard hybridization conditions;

(E) a DNA sequence that codes on expression for an amino acid sequence encoded by any of the foregoing DNA sequences; or

(F) a fragment of any one of the foregoing DNA sequences which encodes a specific binding member, or antibody, functional fragment thereof or derivative thereof, as defined in any one of claims 1 to 7,

wherein said DNA sequence is operatively linked to an expression control sequence.

21. The unicellular host of either one of claims 19 or 20 wherein the unicellular host is selected from the group consisting of E. coli, Pseudomonas, Bacillus, Streptomyces, yeasts, CHO, YB/20, NSO, SP2/0, R1.1, B-W, L-M, COS 1, COS 7, BSC1, BSC40, and BMT10 cells, plant cells, insect cells, and human cells in tissue culture.

22. The use of a specific binding member, or antibody, fragment thereof or derivative thereof according to any of claims 1 to 8, 9, 12 or 13 for preparing a pharmaceutical composition for the treatment of Alzheimer's disease.

23. A pharmaceutical composition comprising a specific binding member, or antibody, fragment thereof or derivative thereof according to any of claims 1 to 8, 9, 12 or 13, or an isolated nucleic acid molecule of claims 14-16, for use in the treatment of Alzheimer's disease.

24. A pharmaceutical composition according to claim 22, for use in treating or preventing the progression of Alzheimer's disease, or for the amelioration of symptoms associated with Alzheimer's disease, or for diagnosing or screening a subject for the presence of Alzheimer's disease or for determining a subject's risk for developing Alzheimer's disease, said composition comprising a binding member that recognizes amyloid beta 1-42 and a pharmaceutically acceptable carrier.

25. The use of a specific binding member, or antibody, fragment thereof or derivative thereof according to any of claims 1 to 8, 9, 12 or 13, for the manufacture of a medicament for the treatment of Alzheimer's disease.

26. The pharmaceutical composition of claim 22 or the use of claim 25, wherein the binding member is an antibody comprising one or more of the amino acid sequences set forth in SEQ ID NOs: 2, 4, 6, or 8 or amino acid sequences having substantial homology to one or more of the sequences set forth in SEQ ID NOs: 2, 4, 6 or 8.

27. The pharmaceutical composition of claim 24 or the use of claim 25, wherein the binding member is a scFv.

28. The pharmaceutical composition of claim 24 or the use of claim 25, wherein said composition or medicament further comprises an additional agent useful for treating Alzheimer's disease, selected from the group consisting of small organic molecules, other amyloid beta antibodies, and combinations thereof.

29. A process of obtaining antibodies or functional fragments thereof with beta-amyloid 1-42 peptide-binding capacity from the genetic repertoire of immunoglobulins isolated from healthy donors, comprising the steps of:

A) purifying B cells from a sample, said sample being obtained from a subject fulfilling the following criteria;
   (i) being 60 years of age or older;
   (ii) having full cognitive capacity and good health;
   (iii) having no symptoms of Alzheimer's disease or another neuropathy;
   (iv) not having previously received any medication for the prevention or treatment of neurodegenerative diseases; and
   (v) possessing detectable anti beta-amyloid 1-42 peptide antibodies in the blood serum;
B) obtaining the immunoglobulin gene repertoire for said anti beta-amyloid 1-42 peptide antibodies from said B cells;
C) using said repertoire to express said antibodies.

30. The process of claim 29, wherin step B) comprises the steps of:
   (i) obtaining mRNA from the said B cells;
   (ii) obtaining cDNA from the mRNA of step (d); and
   (iii) using a primer extension reaction to amplify from said cDNA the fragments corresponding to the heavy chains (HC) and the kappa light chains (LC) of said anti beta-amyloid 1-42 peptide antibodies.

31. An antibody, functional fragment thereof or derivative with beta-amyloid 1-42 peptide-binding capacity obtained by a process according to claims 29 and 30.

32. The binding member or antibody, fragment or derivative thereof, according to any of claims 1 to 8, 9, 12 or 13 for use as a medicament.

33. A pharmaceutical composition comprising a binding member or an antibody, fragment or derivative thereof, according to any of claims 1 to 8, 9, 12 or 13 and a pharmaceutically acceptable excipient.

34. The use of a binding member or an antibody, fragment or derivative thereof, according to any of claims 1 to 8, 9, 12 or 13 for the *in vitro* detection of the beta-amyloid 1-42 peptide.

35. The use of a binding member or an antibody, fragment or derivative thereof, according to any of claims 1 to 8, 9, 12 or 13 for the *in vivo* detection of the beta-amyloid 1-42 peptide.

36. The use of a binding member or an antibody, fragment or derivative thereof, according to any of claims 1 to 8, 9, 12 or 13 for the preparation of a composition for the *in vivo* detection of the beta-amyloid 1-42 peptide.

37. The use of a binding member or an antibody, fragment or derivative thereof, according to any of claims 1 to 8, 9, 12 or 13, for the identification of a compound able to compete with said antibody, fragment or derivative.

38. A specific binding member or antibody or fragment, derivative or variant thereof according to any one of claims 1 to 8, 9, 12 or 13 for use in a method of treatment or diagnosis of the human or animal body.

39. A method of preparing a specific binding member capable of binding an epitope on amyloid beta 1-42, which method comprises:

a) providing a starting repertoire of nucleic acids encoding a VH domain which lacks a CDR3 encoding region;

b) combining said repertoire with a donor nucleic acid encoding an amino acid sequence substantially as set out in SEQ ID NOs: 2, 4, 6 or 8 such that said donor nucleic acid is inserted into the missing CDR3 region, so as to provide a product repertoire of nucleic acids encoding a VH domain;

c) expressing the nucleic acids of said product repertoire; and

d) selecting a specific binding member which recognizes amyloid beta 1-42; and

e) recovering said binding member or the nucleic acid encoding it.

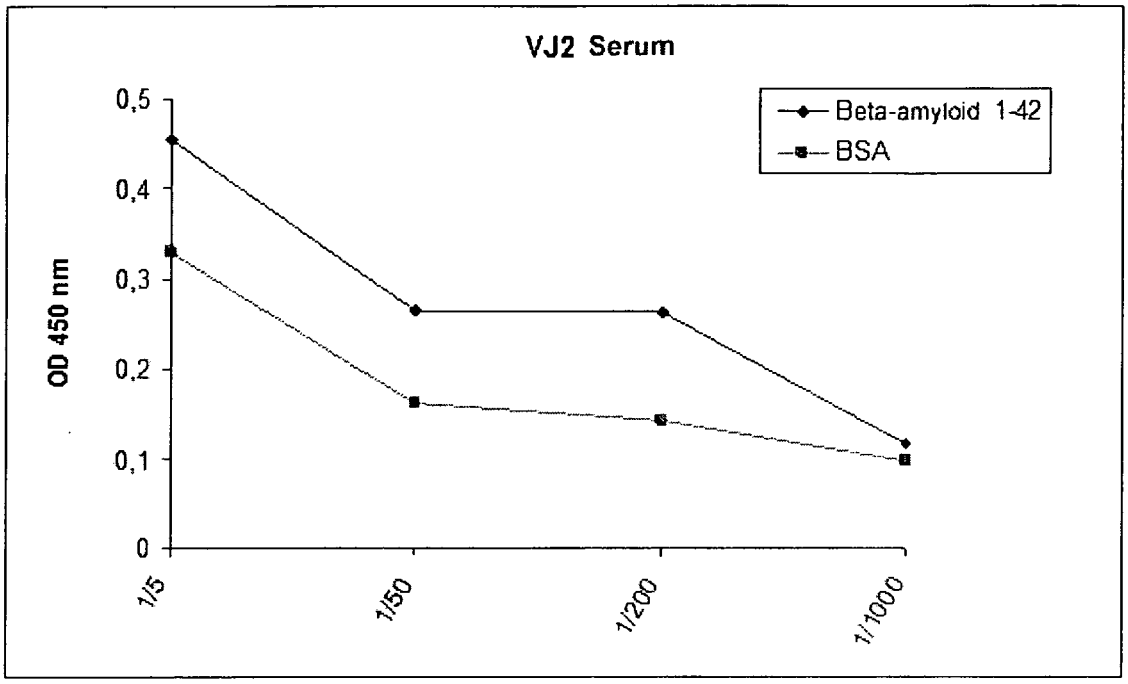
40. A method of treating or preventing the progression of Alzheimer's disease in a subject, or for ameliorating the symptoms associated with Alzheimer's disease, or for diagnosing or screening a subject for the presence of Alzheimer's disease or for determining a subject's risk for developing Alzheimer's disease, which method comprises administering to said subject an effective amount of a specific binding member or antibody or fragment, derivative or variant thereof, as defined in any one of claims 1 to 8, 9, 12 or 13.

41. A method of treating or preventing the progression of Alzheimer's disease in a subject, or for ameliorating the symptoms associated with Alzheimer's disease, or for diagnosing or screening a subject for the presence of Alzheimer's disease or for determining a subject's risk for developing Alzheimer's

disease, which method comprises administering to said subject an effective amount of the pharmaceutical composition of any one of claims 23, 24 or 26 to 28.

42. A kit for the diagnosis of Alzheimer's disease, said kit comprising a specific binding member or antibody of any one of claims 1 to 8, 9, 12 or 13, optionally with reagents and/or instructions for use.

FIG. 1A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Beta-amyloid 1-42
BSA
OD 450 nm
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0,3
0,2
0,1
0
1/5
1/50
1/200
1/1000

FIG. 1B

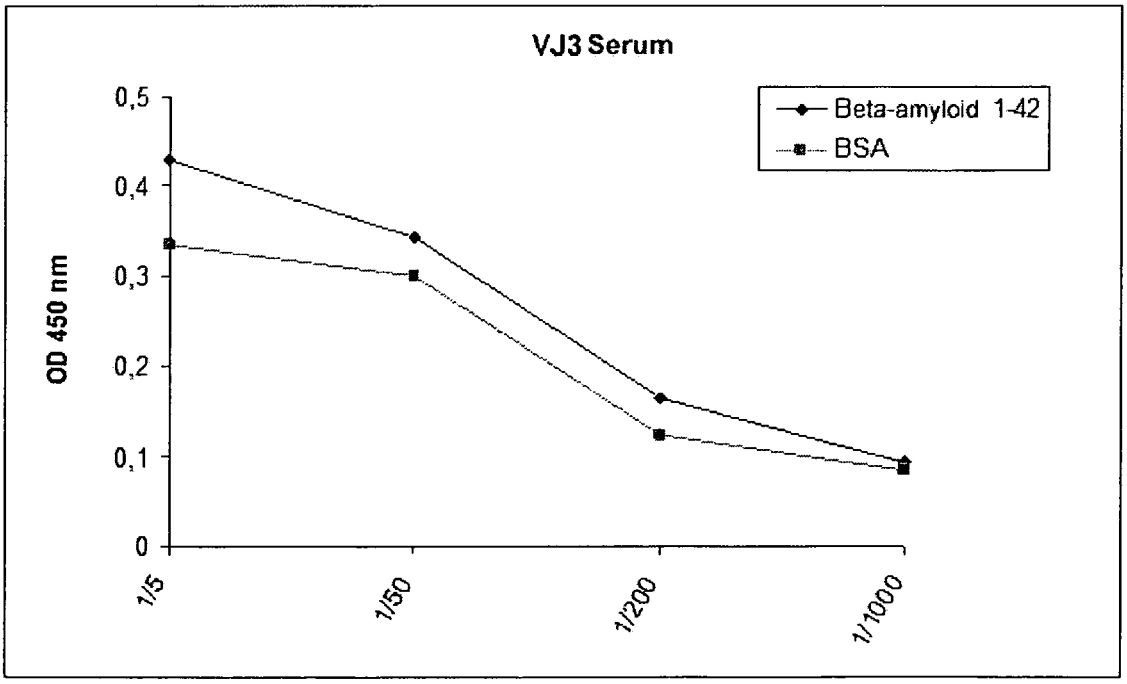

VJ3 Serum
Beta-amyloid 1-42
BSA
OD 450 nm
0,5
0,4
0,3
0,2
0,1
0
1/5
1/50
1/200
1/1000

FIG. 1C

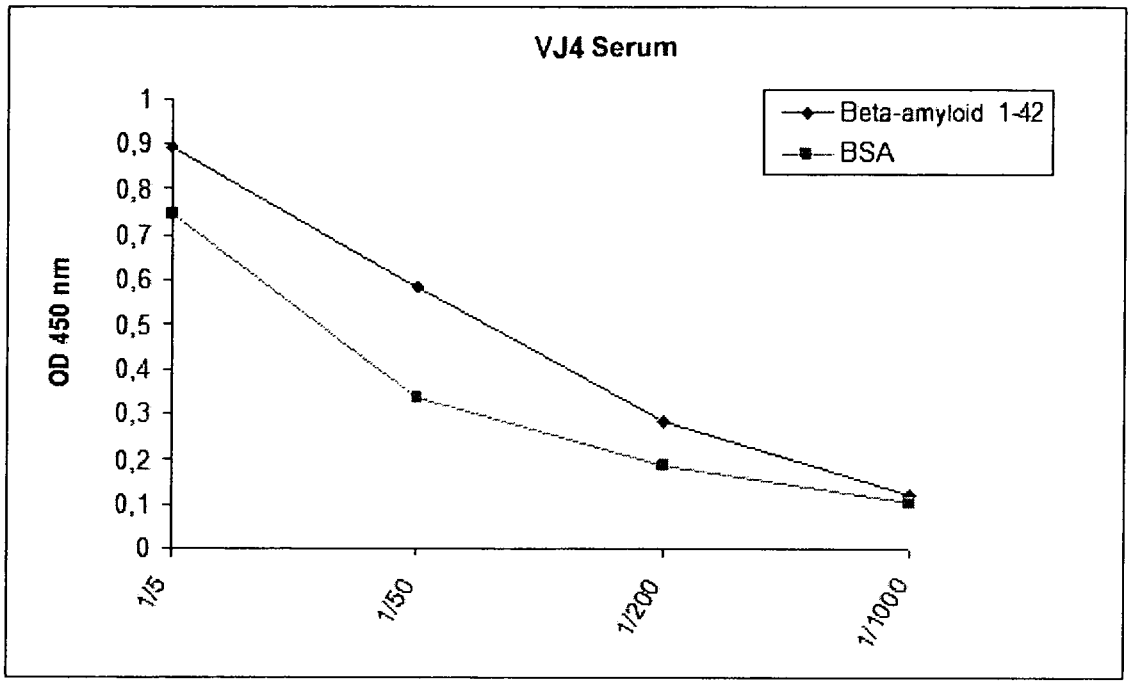
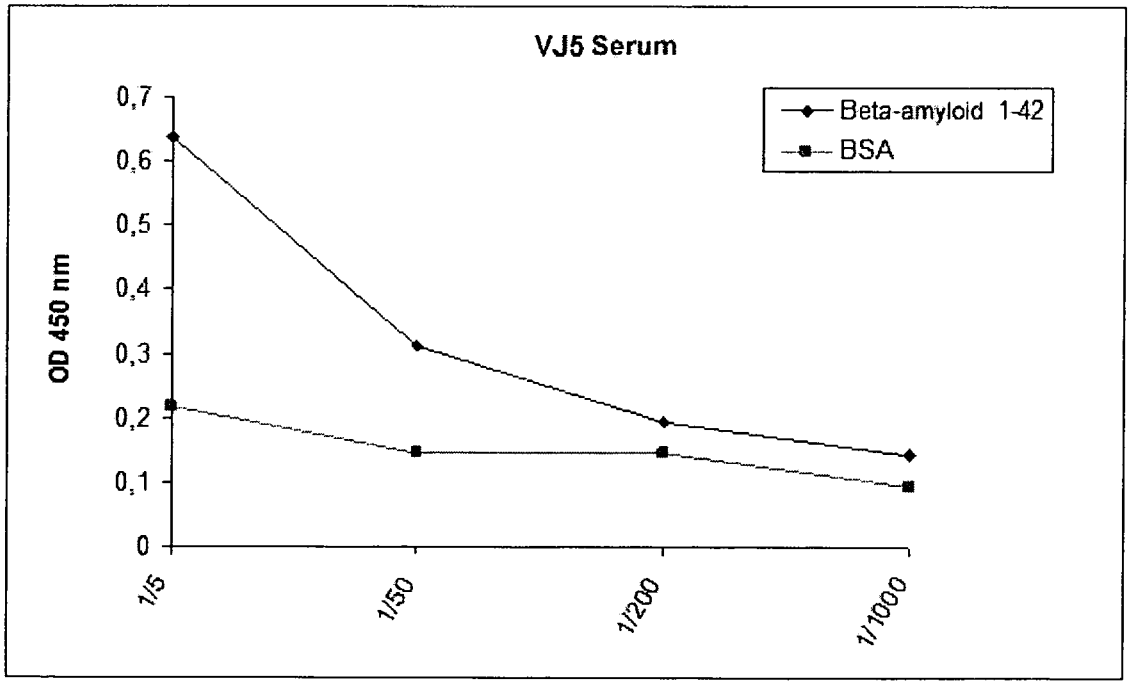

VJ4 Serum
Beta-amyloid 1-42
BSA
OD 450 nm
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
1/5
1/50
1/200
1/1000

FIG. 1D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Beta-amyloid 1-42
BSA
OD 450 nm
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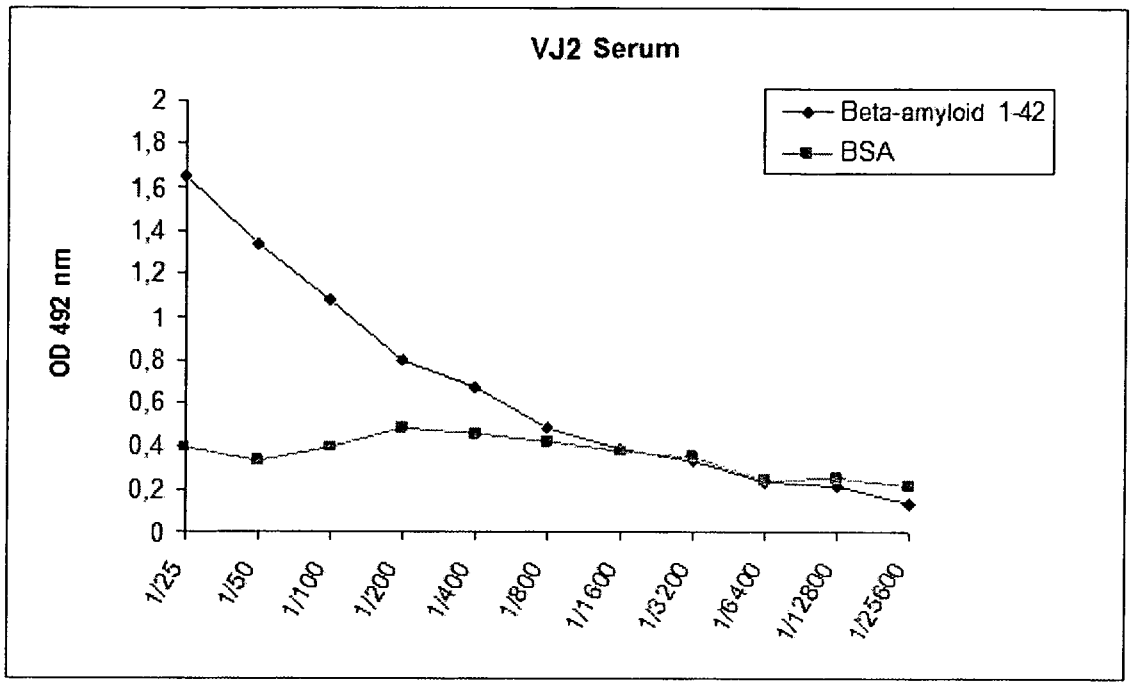
0,4
0,3
0,2
0,1
0
1/5
1/50
1/200
1/1000

FIG. 2A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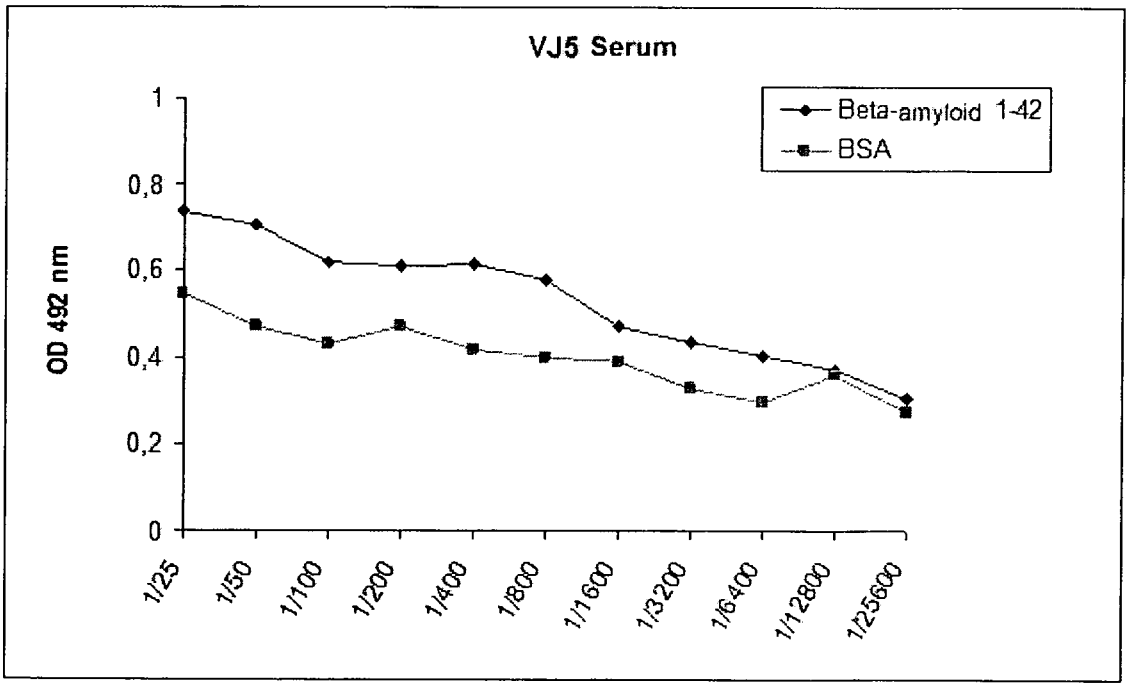
Beta-amyloid 1-42
BSA
OD 492 nm
2
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
1/25
1/50
1/100
1/200
1/400
1/800
1/1600
1/3200
1/6400
1/12800
1/25600

FIG. 2B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Beta-amyloid 1-42
BSA
OD 492 nm
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0,6
0,4
0,2
0
1/25
1/50
1/100
1/200
1/400
1/800
1/1600
1/3200
1/6400
1/12800
1/25600

FIG. 3A

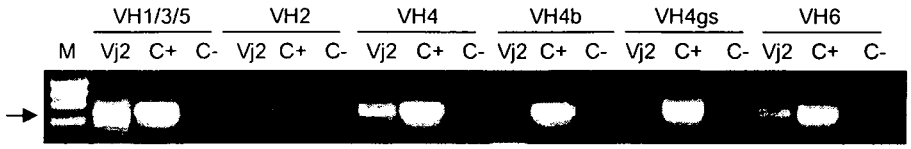


FIG. 3B

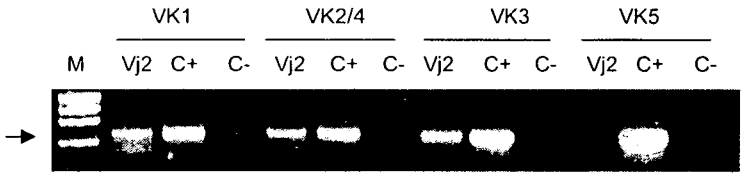


FIG. 3C

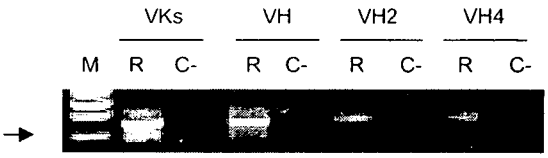


FIG. 4A

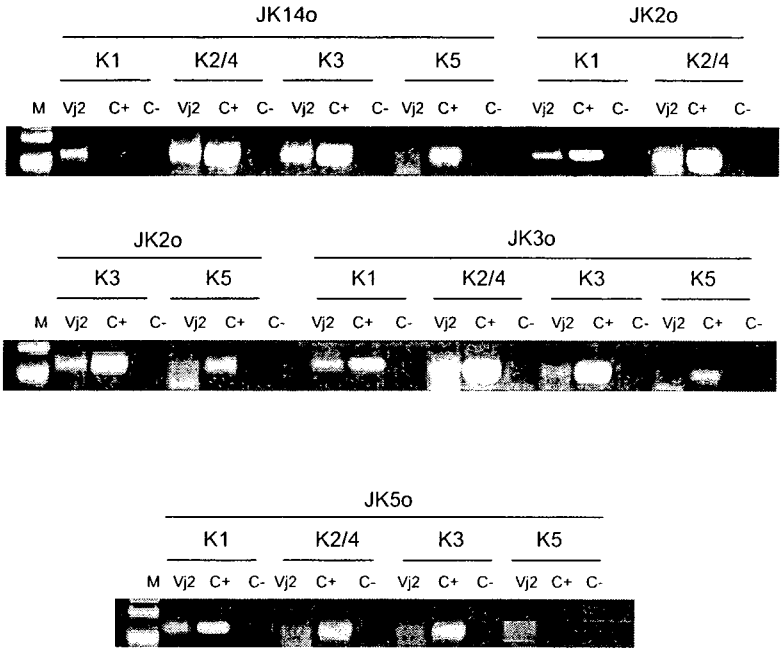


FIG. 4B

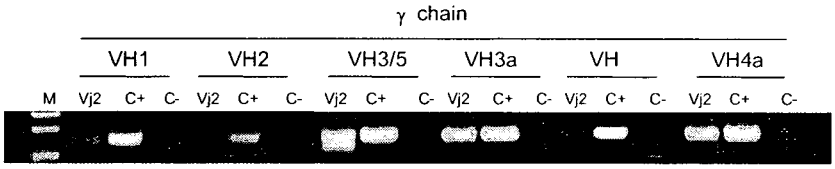


FIG. 4C

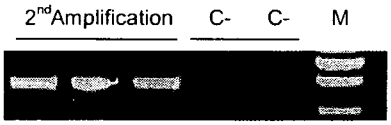


FIG. 5

YYF	/ CLQHDSFPRT(10)	/ FGQG	CAR	/ LSEYYSRAHGMDV(13)	/ WGQG
YYC	/ QQYGSSLT(8)	/ FGGG	CAR	/ DRGLGWLRFPL(10)	/ WGQG
YYC	/ QQSYSYPLT(9)	/ FGQG	CAR	/ DGYDFWSGYRMDV(13)	/ WGQG
YYC	/ QQRNNWPLT(9)	/ FGGG	CAR	/ GGTGEDWYFDL(11)	/ WGQG
YYC	/ QQYNSYSWT(9)	/ FGQG	CAR	/ DGAPLDYKNSGYYMDV(16)	/ WGEG
YYC	/ QQYGTSWT(8)	/ FGQG	CAT	/ DPLTVPTGPLTTGTMET(17)	/ WGEG
YYC	/ QQIYSSPPEST(11)	/ FGQG	CAR	/ LRYLKEGFDY(10)	/ WGQG
YYC	/ QQRSNWPPSLT(11)	/ FGGG	CAT	/ GDVFDV(6)	/ WGQG
YYC	/ QQYGSSEWT(9)	/ FGQG	CNR	/ DPPPSIGVESPAIGDDY(17)	/ WGQG
YYC	/ QQRNSWPWT(9)	/ FGQG	CAR	/ ESPTAGDYSEDY(12)	/ WCQD
			CAR	/ HQQLYYYYIDV(11)	/ WGKG
			CAL	/ TSGWYPRSASEI(12)	/ WDGQ
			CAR	/ VSRYDYKSSLKY(12)	/ WGQG
			CAR	/ DPYGANGPSNDT(12)	/ WGAG

FIG. 6

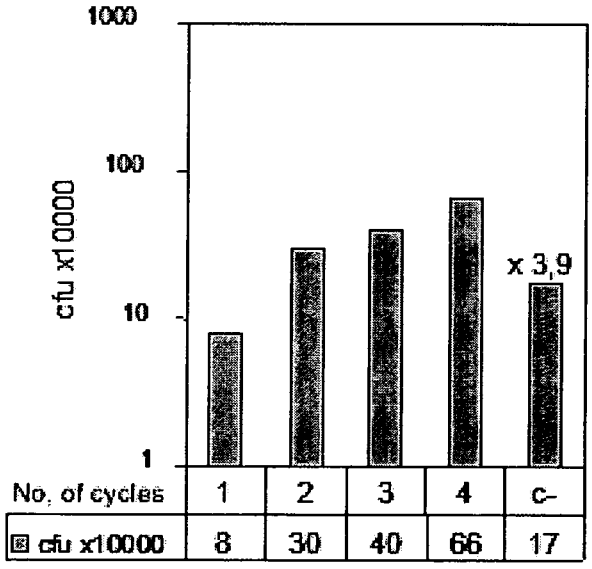
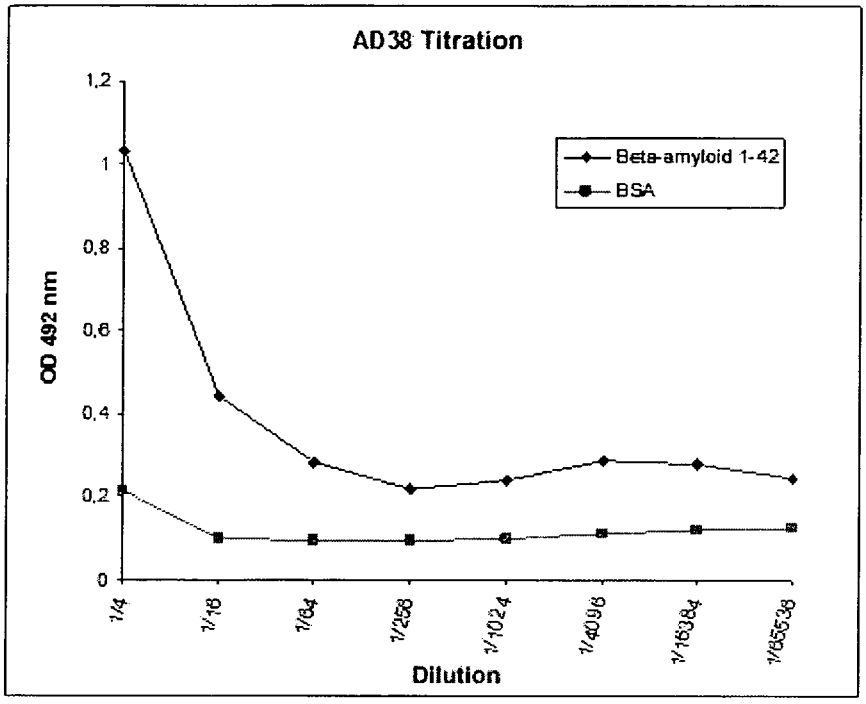


FIG. 7A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OD 492 nm
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1
0,8
0,6
0,4
0,2
0
Beta-amyloid 1-42
BSA
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1/16
1/64
1/256
1/1024
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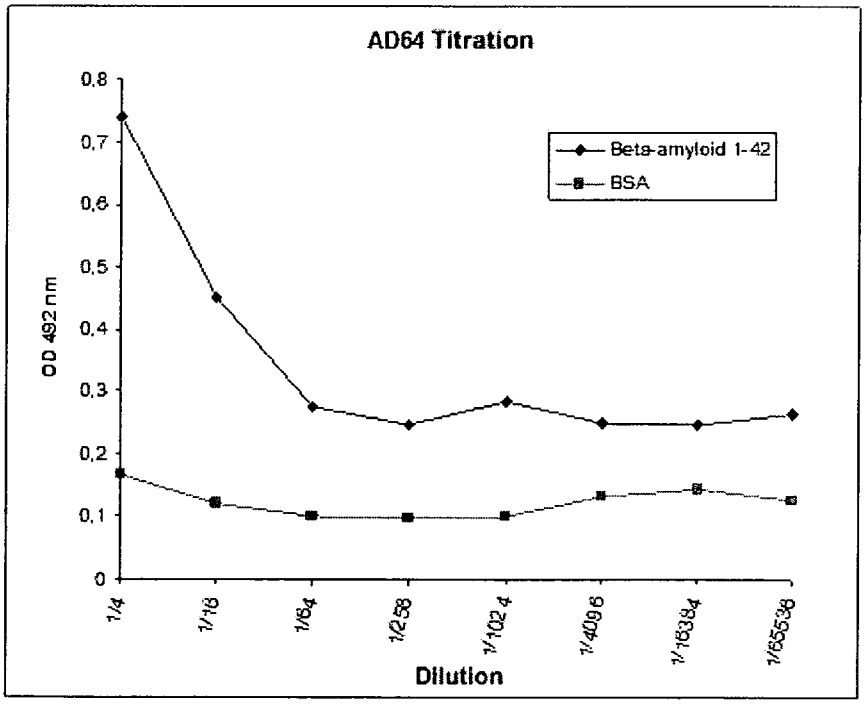
Dilution

FIG. 7B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0,6
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0
Beta-amyloid 1-42
BSA
1/4
1/16
1/64
1/256
1/1024
1/4096
1/16384
1/65536
Dilution

FIG. 8

HEAVY CHAIN AD38 (HC38)

```
                GAG GTG CAG CTG TTG GAG TCT GGG GGA GGC CTA GTT CAG CCG GGG GGG TCC CTG AGA CTC TCC
HC/38            E   V   Q   L   L   E   S   G   G   G   L   V   Q   P   G   G   S   L   R   L   S

                                          _______CDR1_______
                TGT ACA GCC TCT GGA TTC GTC TTC AGT GAC TAC TGG ATG CAC TGG GTC CGC CAA GCT CCA GGG
HC/38            C   T   A   S   G   F   V   F   S   D   Y   W   M   H   W   V   R   Q   A   P   G

                                                                        CDR2
                AAG GGG CTG GTT TGG ATT TCA CGT CTT AAG AGT GAT GGA AGT AGT AGA AGT TAT GCG GAC TTT
HC/38            K   G   L   V   W   I   S   R   L   K   S   D   G   S   S   R   S   Y   A   D   F

                GTG AAG GGC CGA TTC ACC GTC TCC AGA GAC AAC GTC AAG AAT ACA CTG TTT TTG CAA ATC AAC
HC/38            V   K   G   R   F   T   V   S   R   D   N   V   K   N   T   L   F   L   Q   I   N

                                                                    _____CDR3_____
                AGT CTG AGA GCC GAG GAC ACG GCT GTC TAT TAC TGT GCA AGA GAG TTA CGT GGC TGG TCC ATT
HC/38            S   L   R   A   E   D   T   A   V   Y   Y   C   A   R   E   L   R   G   W   S   I

                GAA TAT TGG GGC CAC GGA ACT CAG GTC ACC GTC TCC TCA GCT TCC ACC AAG
                 E   Y   W   G   H   G   T   Q   V   T   V   S   S   A   S   T   K
```

FIG. 9

LIGHT CHAIN AD38 (LC38)

```
                GAG CTC GTG TTG ACG CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGA GAA AGA GCC ACC CTC
LC/38            E   L   V   L   T   Q   S   P   A   T   L   S   L   S   P   G   E   R   A   T   L

                                    ____________________CDR1____________________
                TTC TGC AGG GCC AGT CAG GAT ATT AGC ACC TCC TTA GCC TGG TAT CAA CAA AAA CCT GGC CAG
LC/38            F   C   R   A   S   Q   D   I   S   T   S   L   A   W   Y   Q   Q   K   P   G   Q

                                                ___________CDR2___________
                GTT CCC AGG CTC CTC ATC TAT GGC ACA TCC TAT AGG GCC ACT GGC ATC TCA GCC AGG TTC AGT
LC/38            V   P   R   L   L   I   Y   G   T   S   Y   R   A   T   G   I   S   A   R   F   S

                GGC CGT GGG TCT GGG ACA GAC TTC ACT CTC ACC ATC AGC AGC CTA GAG CCT GAA GAC TTT GCA
LC/38            G   R   G   S   G   T   D   F   T   L   T   I   S   S   L   E   P   E   D   F   A

                                    ____________________CDR3____________________
                TTG TAT TAC TGT CAA CAC CGG AGG GAC TGG CCT CCG GGG GCC ACC TTC GGC CAA GGG ACA CGA
LC/38            L   Y   Y   C   Q   H   R   R   D   W   P   P   G   A   T   F   G   Q   G   T   R

                CTG GAG ATT AAA
                 L   E   I   K
```

FIG. 10

HEAVY CHAIN AD64 (HC64)

HC/64

GAG GTG CAG CTG GTG CAG TCT GGG GGA GGC TTG GTA AAG CCG GGG GGG TCC CTC AGA CTC TCC
E V Q L V Q S G G G L V K P G G S L R L S

CDR1

TGT GTC GGC TCT GGA TTC ACT TTC ACT AAT GCC TGG ATG AGC TGG GTC CGC CAG GCT CCA GGG
C V G S G F T F T N A W M S W V R Q A P G

CDR2

AAG GGG CTG GAG TGG GTT GGC CGT ATG AAA AGC AAG ACT TAT GGC TGG ACA ACA GAA TTT GCT
K G L E W V G R M K S K T Y G W T T E F A

ACA GCC GTG GAA GGC AGA TTC ACC ATG TCA AGA GAT GAT TCA AAA AAC ACA CTC TAT TTG CAA
T A V E G R F T M S R D D S K N T L Y L Q

CDR3

ATG AAC AGC CTG AAA ACC GAG GAC ACA GCC GTG TAT TAT TGC TCC ACA GAT GAC TAT GAT TTT
M N S L K T E D T A V Y Y C S T D D Y D F

TGG GGC CAA GGG ACA ATG GTC ACC GTC TCT TCA GCT TCC ACC AAG
W G Q G T M V T V S S A S T K

FIG. 11

LIGHT CHAIN AD64 (LC AD64)

```
LC/64   GAG CTC GTG ATG ACT CAG TCT CCA GTC TCC CTG GCC GTC ACC CCT GGA GAG CCG GCC TCC ATC
         E   L   V   M   T   Q   S   P   V   S   L   A   V   T   P   G   E   P   A   S   I

                                    _____CDR1_____
        TCC TGC AGG TCT AGT CAG AGC CTC CTG TTT AGT AAT GGA TAC AAC TAT TTG GAT TGG TAC CTT
LC/64    S   C   R   S   S   Q   S   L   L   F   S   N   G   F   H   Y   L   D   W   Y   L

                                            _____CDR2_____
        CAG AAG CCA GGG CAG TCT CCA CAG CTC CTG ATC TAT TTG GGT TCC AGT CGG GCC TCC GGG GTC
LC/64    Q   K   P   G   Q   S   P   Q   L   L   I   Y   L   G   S   S   R   A   S   G   V

        CCT GAC AGG TTC AGT GGC AGT GGA TCA GGC TCA GAT TTC ACA CTG AAA ATC AGC AGA GTG GAG
LC/64    P   D   R   F   S   G   S   G   S   G   S   D   F   T   L   K   I   S   R   V   E

                                        _____CDR3_____
        GCT GAG GAT GTT GGG GTT TAT TAC TGC ATG CAA GCT CTA CAT AAT CCG CTC ACT TTC GGC GGA
LC/64    A   E   D   V   G   V   Y   Y   C   M   Q   A   L   H   N   P   L   T   F   G   G

        GGG ACC AAG CTG GAG ATC AAA
         G   T   K   L   E   I   K
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List of Sequences

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<150> ES P200500753
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<222> (1)..(366)
<223> Recombinant antibody AD38 heavy chain variable domain

<400> 1

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gag gtg cag ctg ttg gag tct ggg gga ggc cta gtt cag ccg ggg ggg       48
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

tcc ctg aga ctc tcc tgt aca gcc tct gga ttc gtc ttc agt gac tac       96
Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Val Phe Ser Asp Tyr
            20                  25                  30

tgg atg cac tgg gtc cgc caa gct cca ggg aag ggg ctg gtt tgg att      144
Trp Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Val Trp Ile
        35                  40                  45

tca cgt ctt aag agt gat gga agt agt aga agt tat gcg gac ttt gtg      192
Ser Arg Leu Lys Ser Asp Gly Ser Ser Arg Ser Tyr Ala Asp Phe Val
    50                  55                  60

aag ggc cga ttc acc gtc tcc aga gac aac gtc aag aat aca ctg ttt      240
Lys Gly Arg Phe Thr Val Ser Arg Asp Asn Val Lys Asn Thr Leu Phe
65                  70                  75                  80

ttg caa atc aac agt ctg aga gcc gag gac acg gct gtc tat tac tgt      288
Leu Gln Ile Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

gca aga gag tta cgt ggc tgg tcc att gaa tat tgg ggc cac gga act      336
Ala Arg Glu Leu Arg Gly Trp Ser Ile Glu Tyr Trp Gly His Gly Thr
            100                 105                 110

cag gtc acc gtc tcc tca gct tcc acc aag                             366
Gln Val Thr Val Ser Ser Ala Ser Thr Lys
        115                 120
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<210> 2
<211> 122
<212> PRT
<213> Homo sapiens

<400> 2

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

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

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

Ser Arg Leu Lys Ser Asp Gly Ser Ser Arg Ser Tyr Ala Asp Phe Val
50 55 60

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

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

Ala Arg Glu Leu Arg Gly Trp Ser Ile Glu Tyr Trp Gly His Gly Thr
100 105 110

Gln Val Thr Val Ser Ser Ala Ser Thr Lys
115 120

<210> 3
<211> 327
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(327)
<223> Recombinant antibody AD38 light chain variable domain

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

gaa aga gcc acc ctc ttc tgc agg gcc agt cag gat att agc acc tcc 96
Glu Arg Ala Thr Leu Phe Cys Arg Ala Ser Gln Asp Ile Ser Thr Ser
20 25 30

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tta gcc tgg tat caa caa aaa cct ggc cag gtt ccc agg ctc ctc atc      144
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Val Pro Arg Leu Leu Ile
        35                  40                  45

tat ggc aca tcc tat agg gcc act ggc atc tca gcc agg ttc agt ggc      192
Tyr Gly Thr Ser Tyr Arg Ala Thr Gly Ile Ser Ala Arg Phe Ser Gly
    50                  55                  60

cgt ggg tct ggg aca gac ttc act ctc acc atc agc agc cta gag cct      240
Arg Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80

gaa gac ttt gca ttg tat tac tgt caa cac cgg agg gac tgg cct ccg      288
Glu Asp Phe Ala Leu Tyr Tyr Cys Gln His Arg Arg Asp Trp Pro Pro
                85                  90                  95

ggg gcc acc ttc ggc caa ggg aca cga ctg gag att aaa                 327
Gly Ala Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys
            100                 105
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<210> 4
<211> 109
<212> PRT
<213> Homo sapiens

<400> 4

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

Glu Arg Ala Thr Leu Phe Cys Arg Ala Ser Gln Asp Ile Ser Thr Ser
            20                  25                  30

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

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

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

Glu Asp Phe Ala Leu Tyr Tyr Cys Gln His Arg Arg Asp Trp Pro Pro
                85                  90                  95

Gly Ala Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys
            100                 105
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<210> 5
<211> 360
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(360)
<223> Recombinant antibody AD64 heavy chain variable domain

<400> 5

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

tcc ctc aga ctc tcc tgt gtc ggc tct gga ttc act ttc act aat gcc        96
Ser Leu Arg Leu Ser Cys Val Gly Ser Gly Phe Thr Phe Thr Asn Ala
            20                  25                  30

tgg atg agc tgg gtc cgc cag gct cca ggg aag ggg ctg gag tgg gtt       144
Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

ggc cgt atg aaa agc aag act tat ggc tgg aca aca gaa ttt gct aca       192
Gly Arg Met Lys Ser Lys Thr Tyr Gly Trp Thr Thr Glu Phe Ala Thr
    50                  55                  60

gcc gtg gaa ggc aga ttc acc atg tca aga gat gat tca aaa aac aca       240
Ala Val Glu Gly Arg Phe Thr Met Ser Arg Asp Asp Ser Lys Asn Thr
65                  70                  75                  80

ctc tat ttg caa atg aac agc ctg aaa acc gag gac aca gcc gtg tat       288
Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
                85                  90                  95

tat tgc tcc aca gat gac tat gat ttt tgg ggc caa ggg aca atg gtc       336
Tyr Cys Ser Thr Asp Asp Tyr Asp Phe Trp Gly Gln Gly Thr Met Val
            100                 105                 110

acc gtc tct tca gct tcc acc aag                                       360
Thr Val Ser Ser Ala Ser Thr Lys
        115                 120
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<210> 6
<211> 120
<212> PRT
<213> Homo sapiens

<400> 6

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

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

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

Gly Arg Met Lys Ser Lys Thr Tyr Gly Trp Thr Thr Glu Phe Ala Thr
    50                  55                  60
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Ala Val Glu Gly Arg Phe Thr Met Ser Arg Asp Asp Ser Lys Asn Thr
65                  70                  75                  80

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

Tyr Cys Ser Thr Asp Asp Tyr Asp Phe Trp Gly Gln Gly Thr Met Val
            100                 105                 110

Thr Val Ser Ser Ala Ser Thr Lys
        115                 120
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<210> 7
<211> 336
<212> DNA
<213> Homo sapiens

<220>
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<222> (1)..(336)
<223> Recombinant antibody AD64 light chain variable domain

<400> 7

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

gag ccg gcc tcc atc tcc tgc agg tct agt cag agc ctc ctg ttt agt       96
Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu Phe Ser
            20                  25                  30

aat gga ttc cac tat ttg gat tgg tac ctt cag aag cca ggg cag tct      144
Asn Gly Phe His Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

cca cag ctc ctg atc tat ttg ggt tcc agt cgg gcc tcc ggg gtc cct      192
Pro Gln Leu Leu Ile Tyr Leu Gly Ser Ser Arg Ala Ser Gly Val Pro
    50                  55                  60

gac agg ttc agt ggc agt gga tca ggc tca gat ttc aca ctg aaa atc      240
Asp Arg Phe Ser Gly Ser Gly Ser Gly Ser Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

agc aga gtg gag gct gag gat gtt ggg gtt tat tac tgc atg caa gct      288
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
                85                  90                  95

cta cat aat ccg ctc act ttc ggc gga ggg acc aag ctg gag atc aaa      336
Leu His Asn Pro Leu Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110
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<210> 8
<211> 112
<212> PRT

<213> Homo sapiens

<400> 8

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

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

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

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

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

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

Leu His Asn Pro Leu Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 9
<211> 714
<212> DNA
<213> Homo sapiens

<220>
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<222> (1)..(714)
<223> Single chain recombinant antibody AD38

<400> 9

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1 5 10 15

gaa aga gcc acc ctc ttc tgc agg gcc agt cag gat att agc acc tcc 96
Glu Arg Ala Thr Leu Phe Cys Arg Ala Ser Gln Asp Ile Ser Thr Ser
20 25 30

tta gcc tgg tat caa caa aaa cct ggc cag gtt ccc agg ctc ctc atc 144
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Val Pro Arg Leu Leu Ile
35 40 45

tat ggc aca tcc tat agg gcc act ggc atc tca gcc agg ttc agt ggc 192
Tyr Gly Thr Ser Tyr Arg Ala Thr Gly Ile Ser Ala Arg Phe Ser Gly
50 55 60

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cgt ggg tct ggg aca gac ttc act ctc acc atc agc agc cta gag cct      240
Arg Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80

gaa gac ttt gca ttg tat tac tgt caa cac cgg agg gac tgg cct ccg      288
Glu Asp Phe Ala Leu Tyr Tyr Cys Gln His Arg Arg Asp Trp Pro Pro
                85                  90                  95

ggg gcc acc ttc ggc caa ggg aca cga ctg gag att aaa ggt ggt tcc      336
Gly Ala Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Gly Gly Ser
            100                 105                 110

tct aga tct tcc gag gtg cag ctg ttg gag tct ggg gga ggc cta gtt      384
Ser Arg Ser Ser Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val
        115                 120                 125

cag ccg ggg ggg tcc ctg aga ctc tcc tgt aca gcc tct gga ttc gtc      432
Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Val
    130                 135                 140

ttc agt gac tac tgg atg cac tgg gtc cgc caa gct cca ggg aag ggg      480
Phe Ser Asp Tyr Trp Met His Trp Val Arg Gln Ala Pro Gly Lys Gly
145                 150                 155                 160

ctg gtt tgg att tca cgt ctt aag agt gat gga agt agt aga agt tat      528
Leu Val Trp Ile Ser Arg Leu Lys Ser Asp Gly Ser Ser Arg Ser Tyr
                165                 170                 175

gcg gac ttt gtg aag ggc cga ttc acc gtc tcc aga gac aac gtc aag      576
Ala Asp Phe Val Lys Gly Arg Phe Thr Val Ser Arg Asp Asn Val Lys
            180                 185                 190

aat aca ctg ttt ttg caa atc aac agt ctg aga gcc gag gac acg gct      624
Asn Thr Leu Phe Leu Gln Ile Asn Ser Leu Arg Ala Glu Asp Thr Ala
        195                 200                 205

gtc tat tac tgt gca aga gag tta cgt ggc tgg tcc att gaa tat tgg      672
Val Tyr Tyr Cys Ala Arg Glu Leu Arg Gly Trp Ser Ile Glu Tyr Trp
    210                 215                 220

ggc cac gga act cag gtc acc gtc tcc tca gct tcc acc aag              714
Gly His Gly Thr Gln Val Thr Val Ser Ser Ala Ser Thr Lys
225                 230                 235
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<210> 10
<211> 238
<212> PRT
<213> Homo sapiens

<400> 10

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            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Val Pro Arg Leu Leu Ile
        35                  40                  45
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Tyr Gly Thr Ser Tyr Arg Ala Thr Gly Ile Ser Ala Arg Phe Ser Gly
    50                  55                  60

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

Glu Asp Phe Ala Leu Tyr Tyr Cys Gln His Arg Arg Asp Trp Pro Pro
                85                  90                  95

Gly Ala Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Gly Gly Ser
            100                 105                 110

Ser Arg Ser Ser Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val
        115                 120                 125

Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Val
    130                 135                 140

Phe Ser Asp Tyr Trp Met His Trp Val Arg Gln Ala Pro Gly Lys Gly
145                 150                 155                 160

Leu Val Trp Ile Ser Arg Leu Lys Ser Asp Gly Ser Ser Arg Ser Tyr
                165                 170                 175

Ala Asp Phe Val Lys Gly Arg Phe Thr Val Ser Arg Asp Asn Val Lys
            180                 185                 190

Asn Thr Leu Phe Leu Gln Ile Asn Ser Leu Arg Ala Glu Asp Thr Ala
        195                 200                 205

Val Tyr Tyr Cys Ala Arg Glu Leu Arg Gly Trp Ser Ile Glu Tyr Trp
    210                 215                 220

Gly His Gly Thr Gln Val Thr Val Ser Ser Ala Ser Thr Lys
225                 230                 235
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<210> 11
<211> 717
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(717)
<223> Single chain recombinant antibody AD64

<400> 11

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

gag ccg gcc tcc atc tcc tgc agg tct agt cag agc ctc ctg ttt agt       96
Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu Phe Ser
            20                  25                  30

aat gga ttc cac tat ttg gat tgg tac ctt cag aag cca ggg cag tct      144
Asn Gly Phe His Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

cca cag ctc ctg atc tat ttg ggt tcc agt cgg gcc tcc ggg gtc cct      192
Pro Gln Leu Leu Ile Tyr Leu Gly Ser Ser Arg Ala Ser Gly Val Pro
    50                  55                  60

gac agg ttc agt ggc agt gga tca ggc tca gat ttc aca ctg aaa atc      240
Asp Arg Phe Ser Gly Ser Gly Ser Gly Ser Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

agc aga gtg gag gct gag gat gtt ggg gtt tat tac tgc atg caa gct      288
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
                85                  90                  95

cta cat aat ccg ctc act ttc ggc gga ggg acc aag ctg gag atc aaa      336
Leu His Asn Pro Leu Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110

ggt ggt tcc tct aga tct tcc gag gtg cag ctg gtg cag tct ggg gga      384
Gly Gly Ser Ser Arg Ser Ser Glu Val Gln Leu Val Gln Ser Gly Gly
        115                 120                 125

ggc ttg gta aag ccg ggg ggg tcc ctc aga ctc tcc tgt gtc ggc tct      432
Gly Leu Val Lys Pro Gly Gly Ser Leu Arg Leu Ser Cys Val Gly Ser
    130                 135                 140

gga ttc act ttc act aat gcc tgg atg agc tgg gtc cgc cag gct cca      480
Gly Phe Thr Phe Thr Asn Ala Trp Met Ser Trp Val Arg Gln Ala Pro
145                 150                 155                 160

ggg aag ggg ctg gag tgg gtt ggc cgt atg aaa agc aag act tat ggc      528
Gly Lys Gly Leu Glu Trp Val Gly Arg Met Lys Ser Lys Thr Tyr Gly
                165                 170                 175

tgg aca aca gaa ttt gct aca gcc gtg gaa ggc aga ttc acc atg tca      576
Trp Thr Thr Glu Phe Ala Thr Ala Val Glu Gly Arg Phe Thr Met Ser
            180                 185                 190

aga gat gat tca aaa aac aca ctc tat ttg caa atg aac agc ctg aaa      624
Arg Asp Asp Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Lys
        195                 200                 205

acc gag gac aca gcc gtg tat tat tgc tcc aca gat gac tat gat ttt      672
Thr Glu Asp Thr Ala Val Tyr Tyr Cys Ser Thr Asp Asp Tyr Asp Phe
    210                 215                 220

tgg ggc caa ggg aca atg gtc acc gtc tct tca gct tcc acc aag          717
Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys
225                 230                 235
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<210> 12
<211> 239
<212> PRT
<213> Homo sapiens

<400> 12

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

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

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

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

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

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

Leu His Asn Pro Leu Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

Gly Gly Ser Ser Arg Ser Ser Glu Val Gln Leu Val Gln Ser Gly Gly
115 120 125

Gly Leu Val Lys Pro Gly Gly Ser Leu Arg Leu Ser Cys Val Gly Ser
130 135 140

Gly Phe Thr Phe Thr Asn Ala Trp Met Ser Trp Val Arg Gln Ala Pro
145 150 155 160

Gly Lys Gly Leu Glu Trp Val Gly Arg Met Lys Ser Lys Thr Tyr Gly
165 170 175

Trp Thr Thr Glu Phe Ala Thr Ala Val Glu Gly Arg Phe Thr Met Ser
180 185 190

Arg Asp Asp Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Lys
195 200 205

Thr Glu Asp Thr Ala Val Tyr Tyr Cys Ser Thr Asp Asp Tyr Asp Phe
210 215 220

Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys
225 230 235

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/003363

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395 G01N33/53 A61P25/28

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, Sequence Search, PAJ, WPI Data, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 172 378 A (DODEL, RICHARD, DR; DU, YANSHENG, DR) 16 January 2002 (2002-01-16) the whole document	1,2, 8-12, 22-25, 31-38, 40-42
X	CAI JIONG ET AL: "Cloning and expression of human single-chain Fv antibody against amyloid beta peptide involved in Alzheimer's disease" ZHONGGUO YIXUE KEXUEYUAN XUEBAO - ACTA ACADEMIAE MEDICINAE SINICAE, BEIJING, CN, vol. 25, no. 5, October 2003 (2003-10), pages 557-562, XP002994528 ISSN: 1000-503X abstract; figure 4	1,2,8,9, 31

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 June 2006	17/07/2006

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Bernhardt, W

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/003363

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/028511 A (CENTOCOR, INC; MERCKEN, MARC; BENSON, JACQUELINE, M) 31 March 2005 (2005-03-31) pages 2-3; claims 1-38; examples 1,2,4	1,2, 8-12, 22-25, 31-38, 40-42
X	WO 2004/108895 A (NEURALAB LIMITED; WYETH; BASI, GURIQ; SALDANHA, JOSE, W; BARD, FREDERI) 16 December 2004 (2004-12-16) page 4; claims 43,69-75; figure 5a; sequence 8	6,13-15, 17-26, 31-33, 38,40-42
X	GEYLIS V ET AL: "P4-388 Human monoclonal antibodies against amyloid-beta (Abeta) engendered by EBV-immortalized lymphocytes from healthy adults" NEUROBIOLOGY OF AGING, TARRYTOWN, NY, US, vol. 25, July 2004 (2004-07), page S585, XP004626560 ISSN: 0197-4580 abstract	1-8, 13-16, 29-32,39
X,P	GEYLIS ET AL: "Immunotherapy of Alzheimer's disease (AD): From murine models to anti-amyloid beta (Abeta) human monoclonal antibodies" AUTOIMMUNITY REVIEWS, ELSEVIER, AMSTERDAM, NL, vol. 5, no. 1, 1 August 2005 (2005-08-01), pages 33-39, XP005203566 ISSN: 1568-9972 the whole document	1-42
A	KANG J ET AL: "THE PRECURSOR OF ALZHEIMER'S DISEASE AMYLOID A4 PROTEIN RESEMBLES A CELL- SURFACE RECEPTOR" NATURE, NATURE PUBLISHING GROUP, LONDON, GB, vol. 325, 19 February 1987 (1987-02-19), pages 733-736, XP000196840 ISSN: 0028-0836 abstract; figure 1	1-42

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	& DATABASE UniProt [Online] 13 August 1987 (1987-08-13), "Amyloid beta A4 protein precursor (APP) (ABPP) (Alzheimer disease amyloid protein) (Cerebral vascular amyloid peptide) (CVAP) (Protease nexin-II) (PN-II) (APPI) (PreA4) [Contains: Soluble APP-alpha (S-APP-alpha); Soluble APP-beta (S-APP-beta); C99; Beta-amyloid protein 42 (Beta-APP42); Beta-amyloid" retrieved from EBI accession no. UNIPROT:P05067 Database accession no. P05067	
A	LITTLE M ET AL: "Of mice and men: hybridoma and recombinant antibodies" IMMUNOLOGY TODAY, ELSEVIER PUBLICATIONS, CAMBRIDGE, GB, vol. 21, no. 8, 1 August 2000 (2000-08-01), pages 364-370, XP004215163 ISSN: 0167-5699 abstract; figure 3	1-42

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Although claims 40 and 41 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

In addition, although claim 35 is directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box II.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy
Rule 39.1(iv) PCT - Diagnostic method practised on the human or animal body

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2006/003363

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

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

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2006/003363

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专利名称(译)	具有β-淀粉样肽结合能力的人抗体及其应用		
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[标]发明人	TORAN GARCIA JOSE LUIS LAIN DE LERA MARIA TERESA		
发明人	TORÁN GARCÍA, JOSÉ LUIS LAÍN DE LERA, MARÍA TERESA		
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优先权	2005000753 2005-04-01 ES		
外部链接	Espacenet		

摘要(译)

本发明涉及分离的特异性结合成员，特别是识别和结合淀粉状蛋白β1-42蛋白的抗体或片段，衍生物或变体。公开了诊断，预后或治疗其中淀粉样蛋白β蛋白（包括淀粉状蛋白β1-42）普遍存在的疾病或病症，特别是阿尔茨海默氏病的方法。本发明还涉及包含此类结合成员，抗体和模拟物的药物组合物以及筛选新结合成员的方法，所述新结合成员可以是或可以不是抗体。