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(54) **ANTIBODY SPECIFIC BINDING TO A BETA OLIGOMER AND THE USE**

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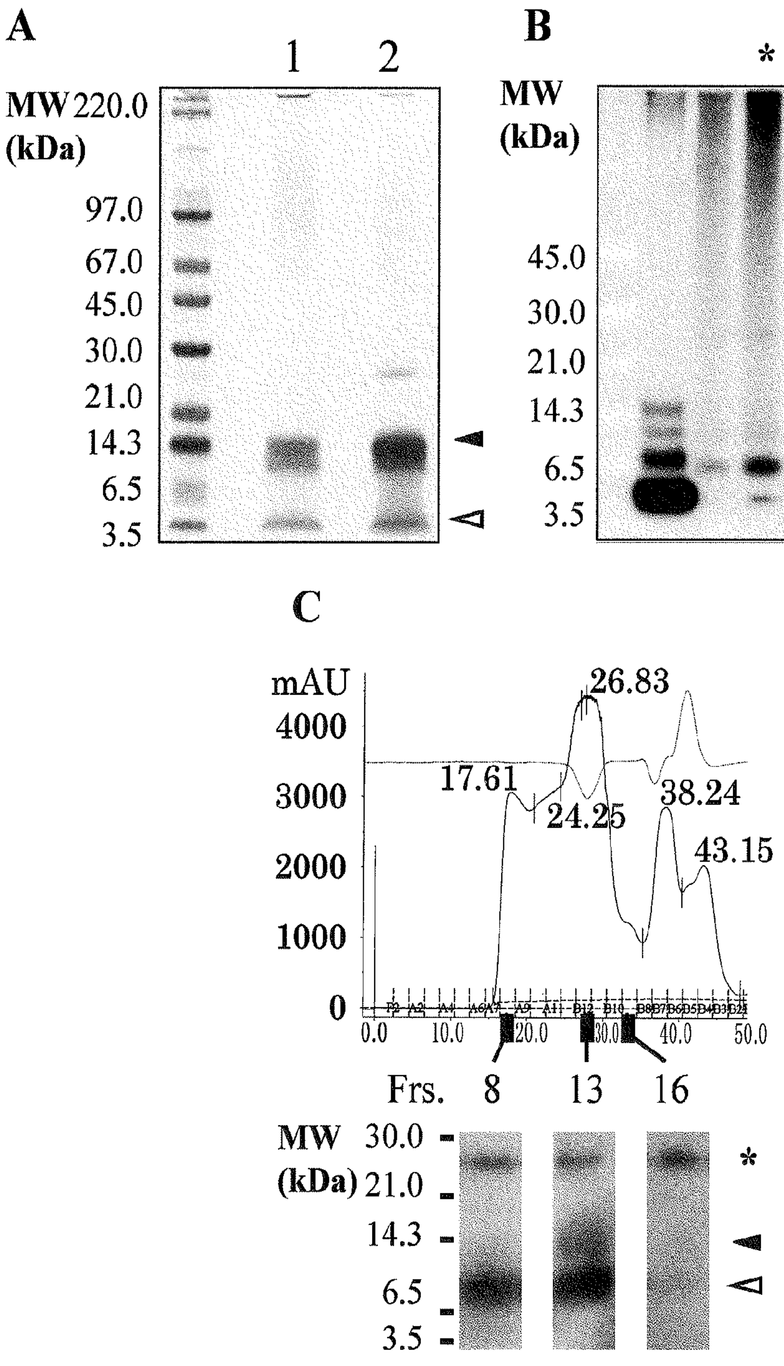
(57) **ABSTRACT**

The present inventors successfully produced monoclonal antibodies that are specific to only soluble Aβ oligomers, but do not recognize soluble Aβ monomers, which are physiological molecules. It was demonstrated that the antibodies are useful as diagnostic/therapeutic monoclonal antibodies for Alzheimer's disease.



IP	1A9
IB	4G8

FIG. 1

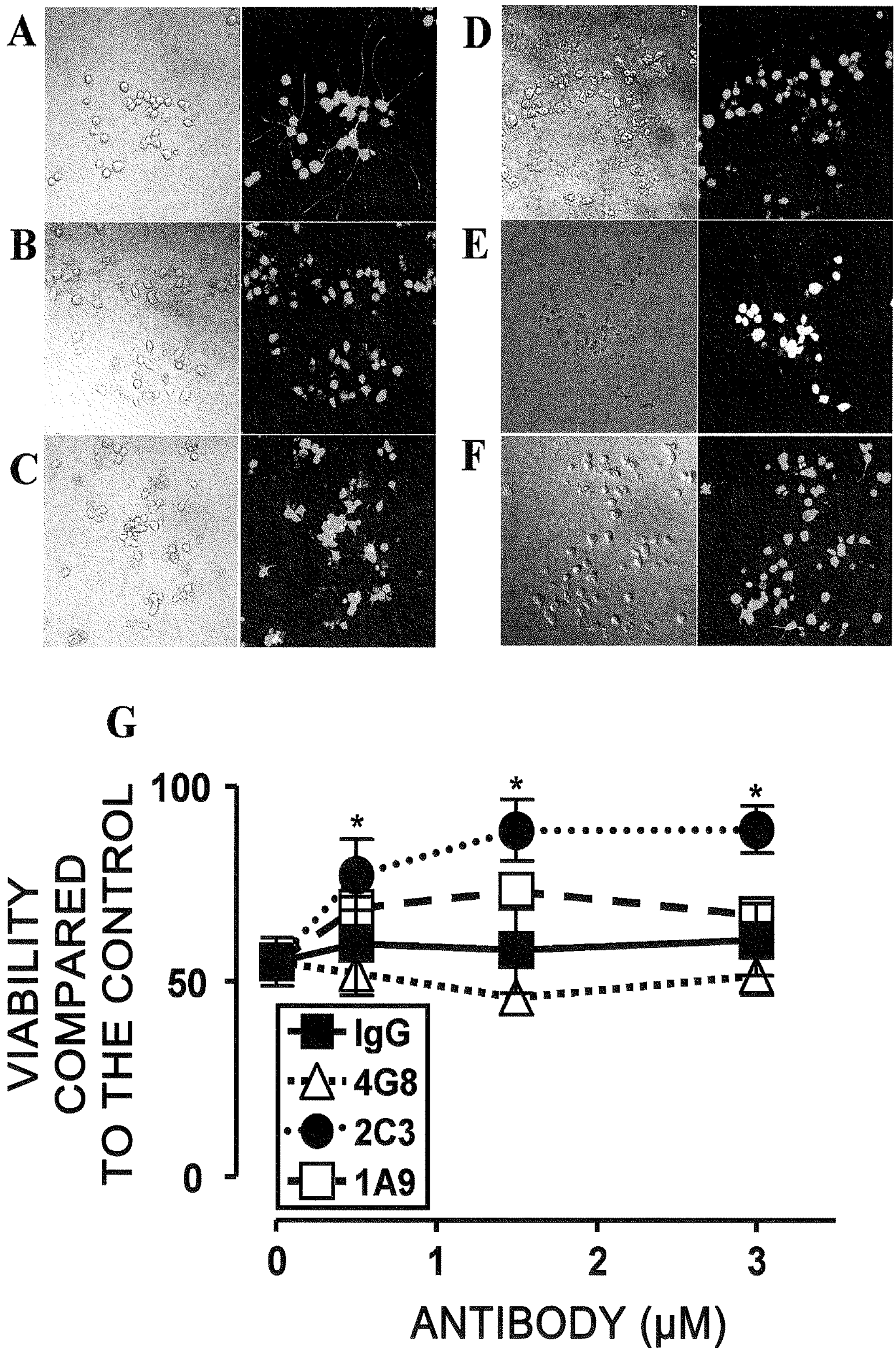

A
B
C
D
E
F
G
VIABILITY COMPARED TO THE CONTROL
100
50
0
*
IgG
4G8
2C3
1A9
0
1
2
3
ANTIBODY (μM)

FIG. 2

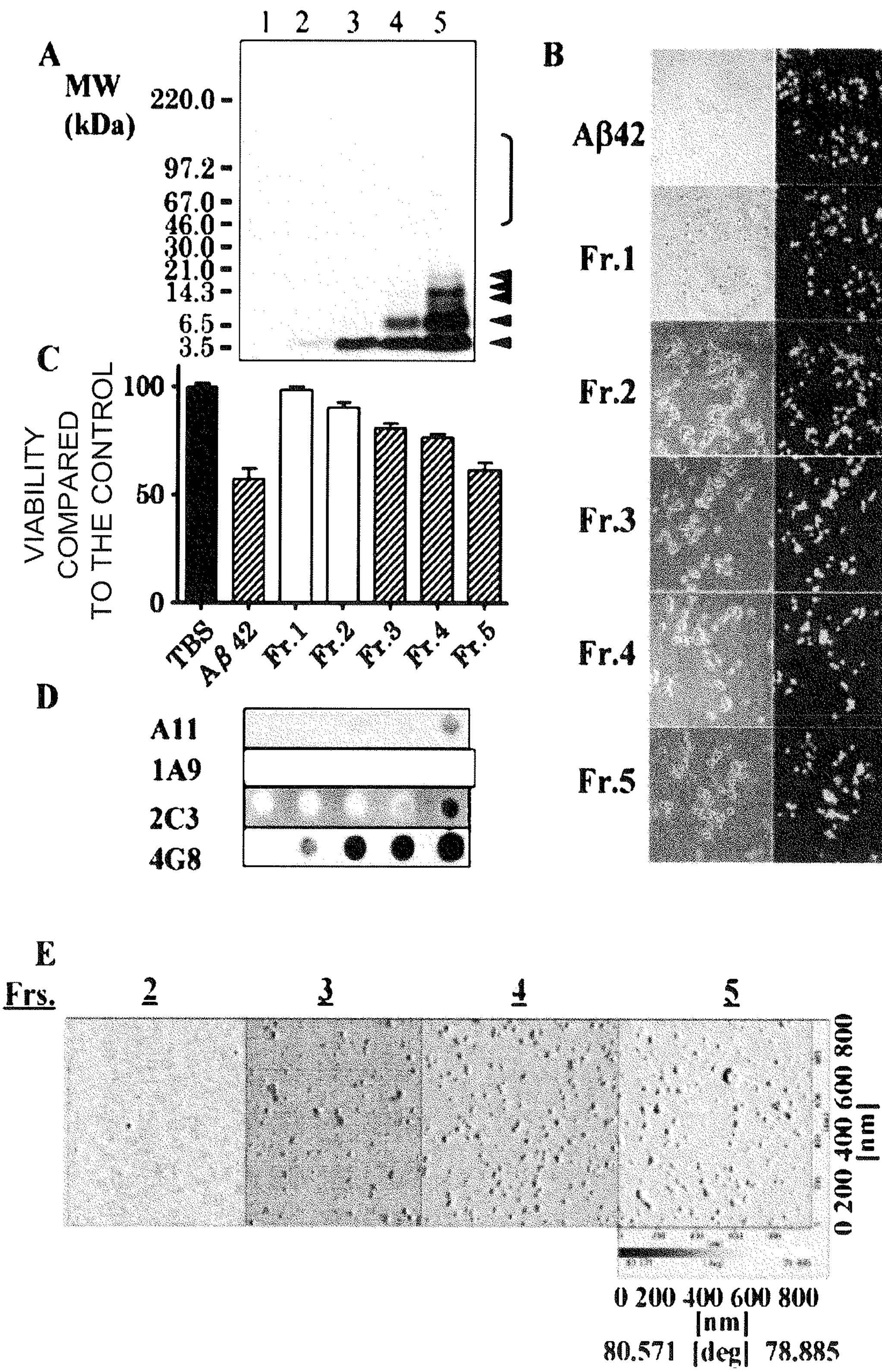

A
MW (kDa)
1 2 3 4 5
220.0
97.2
67.0
46.0
30.0
21.0
14.3
6.5
3.5
B
Aβ42
Fr.1
Fr.2
Fr.3
Fr.4
Fr.5
C
VIABILITY COMPARED TO THE CONTROL
100
50
0
TBS
Aβ42
Fr.1
Fr.2
Fr.3
Fr.4
Fr.5
D
A11
1A9
2C3
4G8
E
Frs.
2
3
4
5
0 200 400 600 800 [nm]
0 200 400 600 800 [nm]
80.571 [deg] 78.885

FIG. 3

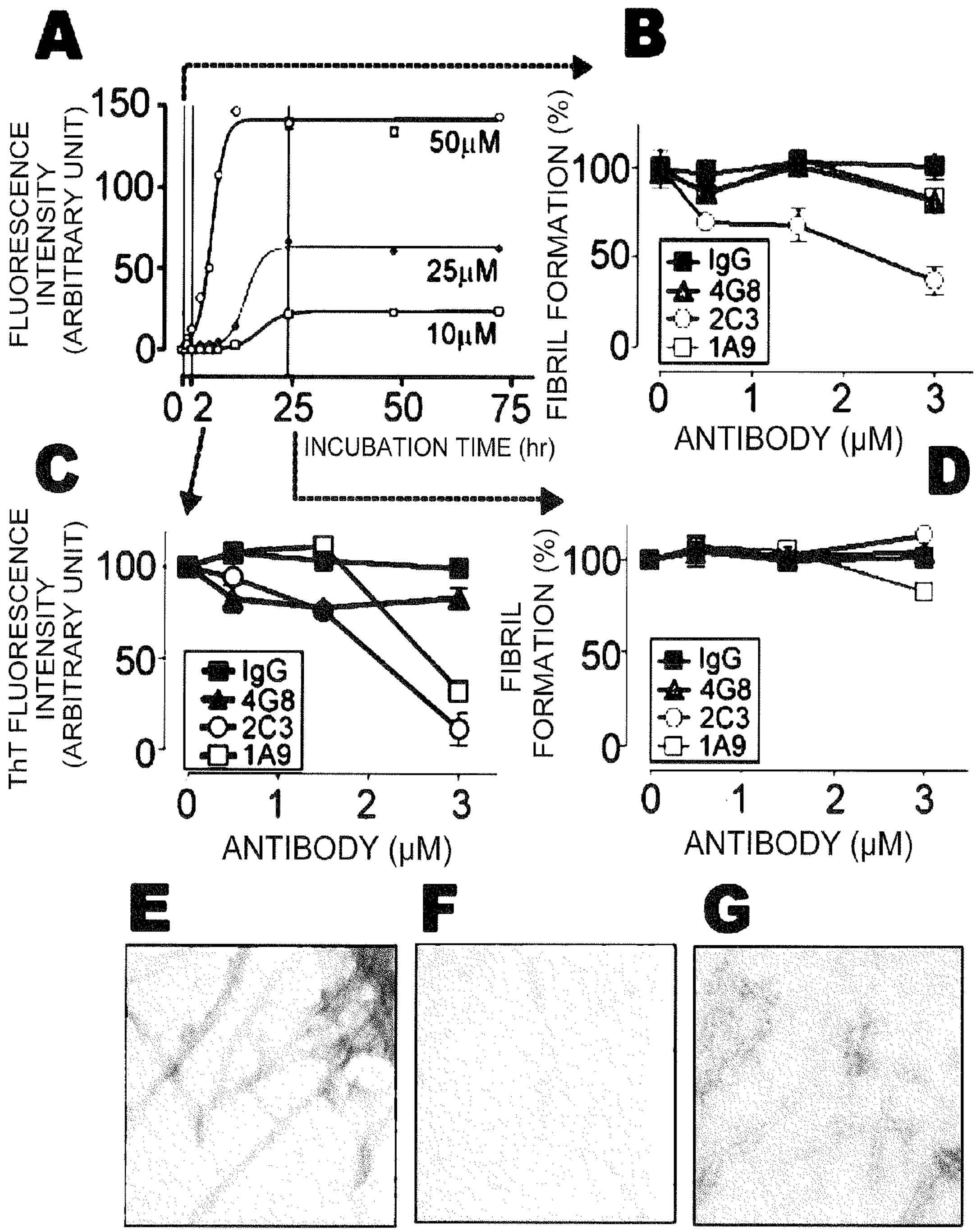

A
B
C
D
E
F
G
FLUORESCENCE INTENSITY (ARBITRARY UNIT)
150
100
50
0
50µM
25µM
10µM
0 2 25 50 75
INCUBATION TIME (hr)
FIBRIL FORMATION (%)
100
50
0
IgG
4G8
2C3
1A9
0 1 2 3
ANTIBODY (µM)
ThT FLUORESCENCE INTENSITY (ARBITRARY UNIT)
100
50
0
IgG
4G8
2C3
1A9
0 1 2 3
ANTIBODY (µM)
FIBRIL FORMATION (%)
100
50
0
IgG
4G8
2C3
1A9
0 1 2 3
ANTIBODY (µM)

FIG. 4

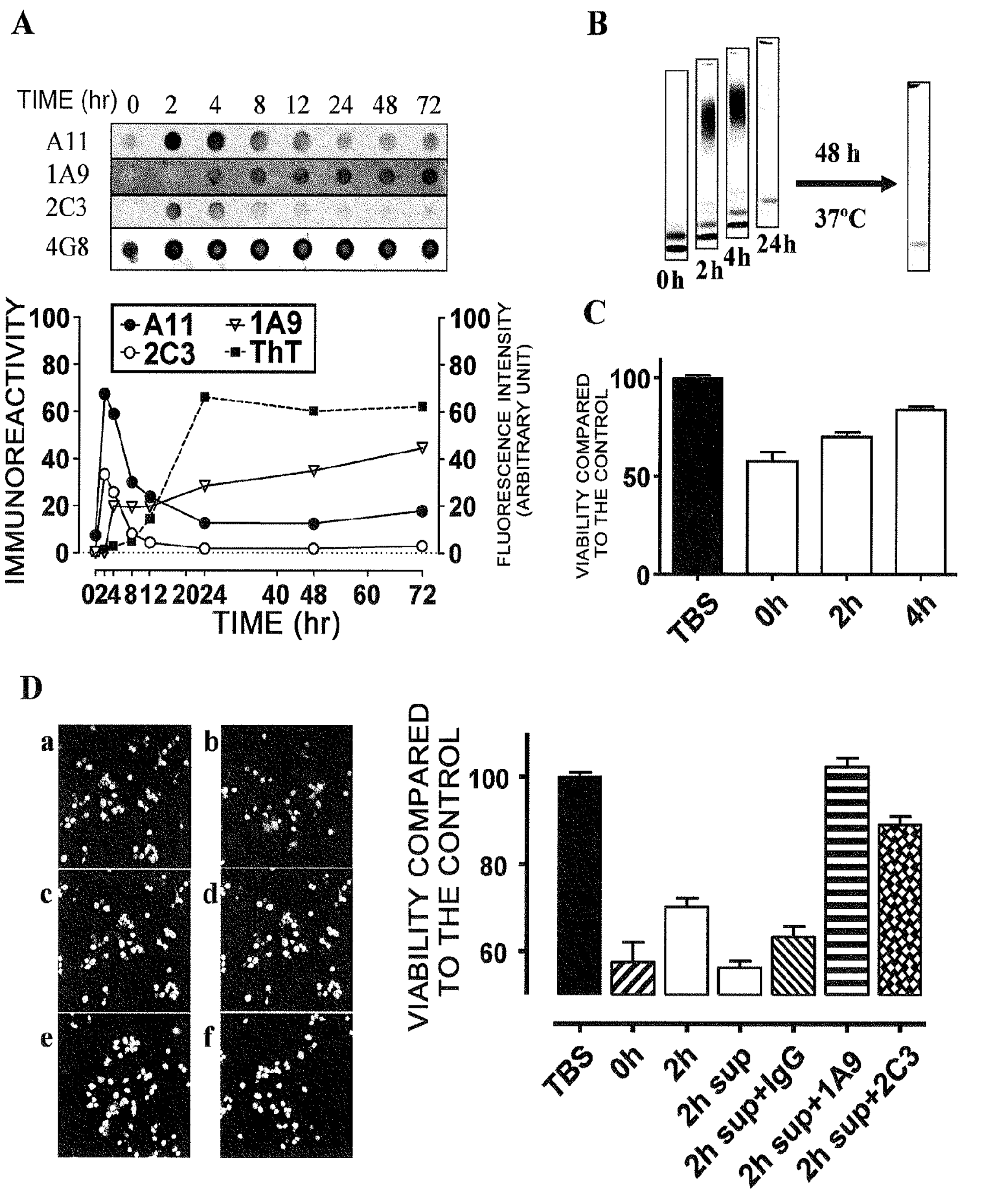

A
TIME (hr) 0 2 4 8 12 24 48 72
A11
1A9
2C3
4G8
IMMUNOREACTIVITY
FLUORESCENCE INTENSITY (ARBITRARY UNIT)
A11
1A9
2C3
ThT
TIME (hr)
B
48 h
37°C
0h
2h
4h
24h
C
VIABILITY COMPARED TO THE CONTROL
TBS
0h
2h
4h
D
a
b
c
d
e
f
VIABILITY COMPARED TO THE CONTROL
TBS
0h
2h
2h sup
2h sup+IgG
2h sup+1A9
2h sup+2C3

FIG. 5

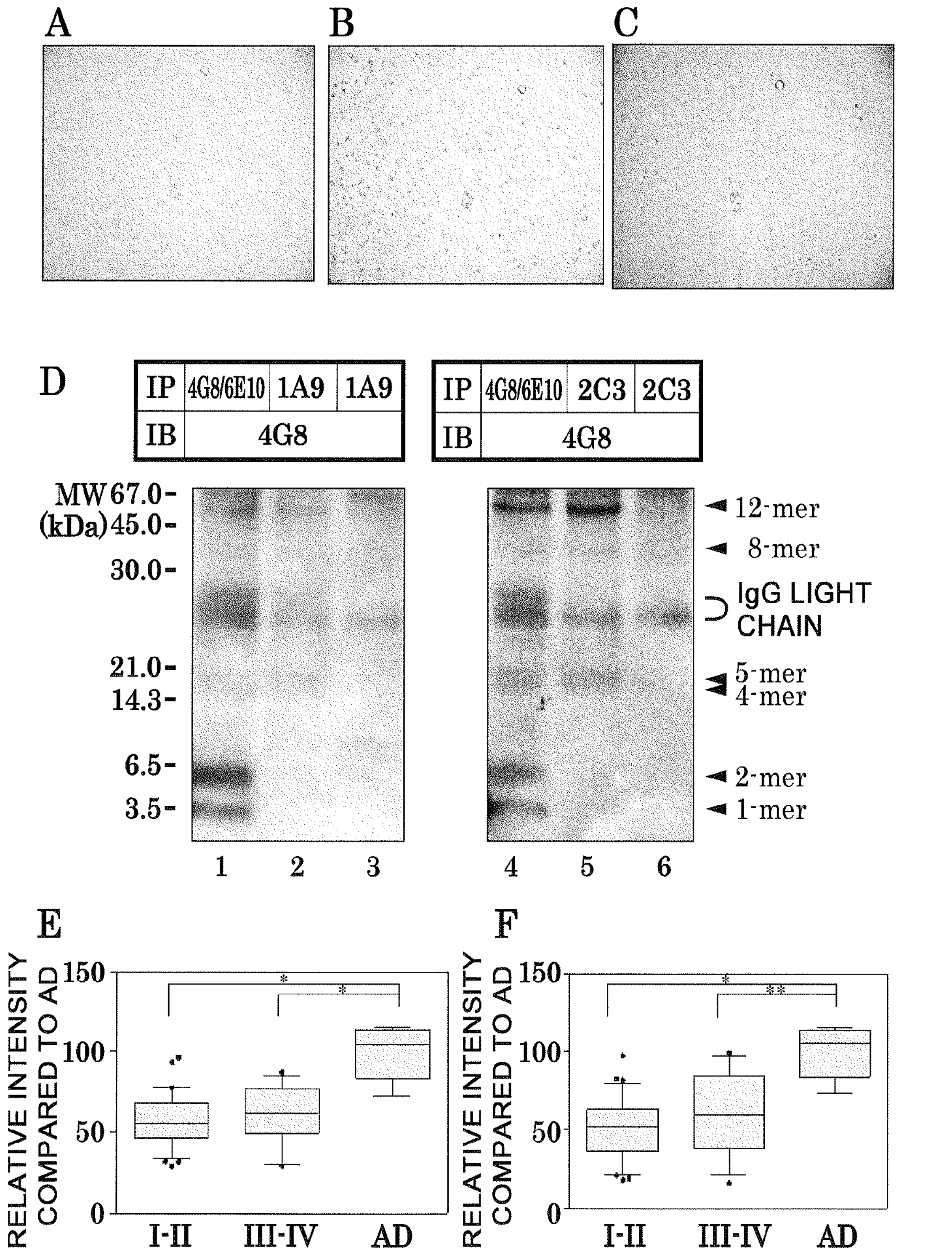

A
B
C
D
IP 4G8/6E10 1A9 1A9
IB 4G8
IP 4G8/6E10 2C3 2C3
IB 4G8
MW (kDa)
67.0
45.0
30.0
21.0
14.3
6.5
3.5
12-mer
8-mer
IgG LIGHT CHAIN
5-mer
4-mer
2-mer
1-mer
1 2 3 4 5 6
E
F
RELATIVE INTENSITY COMPARED TO AD
150
100
50
0
I-II
III-IV
AD
*
**

FIG. 6

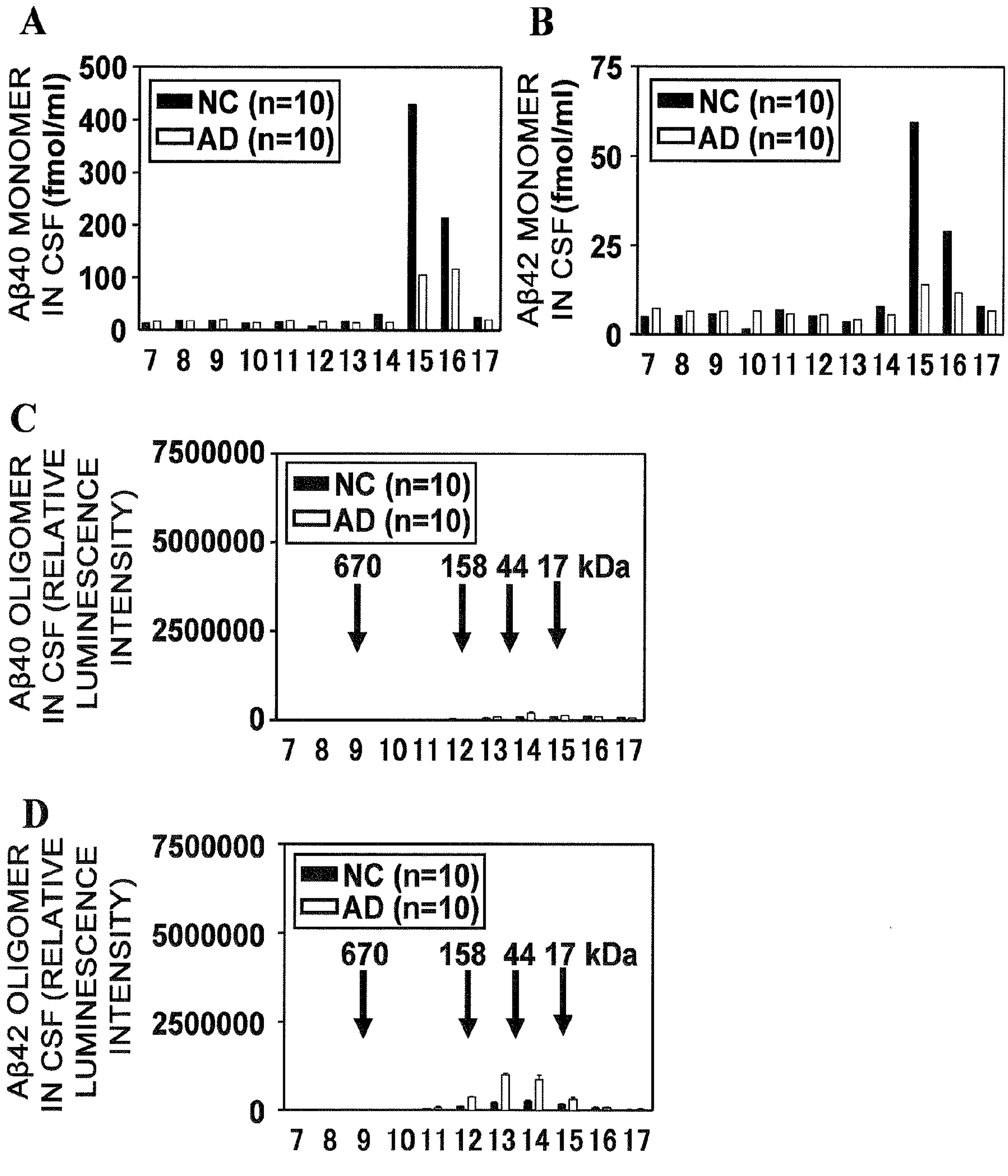

A
Aβ40 MONOMER IN CSF (fmol/ml)
500
400
300
200
100
0
NC (n=10)
AD (n=10)
7 8 9 10 11 12 13 14 15 16 17
B
Aβ42 MONOMER IN CSF (fmol/ml)
75
50
25
0
NC (n=10)
AD (n=10)
7 8 9 10 11 12 13 14 15 16 17
C
Aβ40 OLIGOMER IN CSF (RELATIVE LUMINESCENCE INTENSITY)
7500000
5000000
2500000
0
NC (n=10)
AD (n=10)
670 158 44 17 kDa
7 8 9 10 11 12 13 14 15 16 17
D
Aβ42 OLIGOMER IN CSF (RELATIVE LUMINESCENCE INTENSITY)
7500000
5000000
2500000
0
NC (n=10)
AD (n=10)
670 158 44 17 kDa
7 8 9 10 11 12 13 14 15 16 17

FIG. 7-1

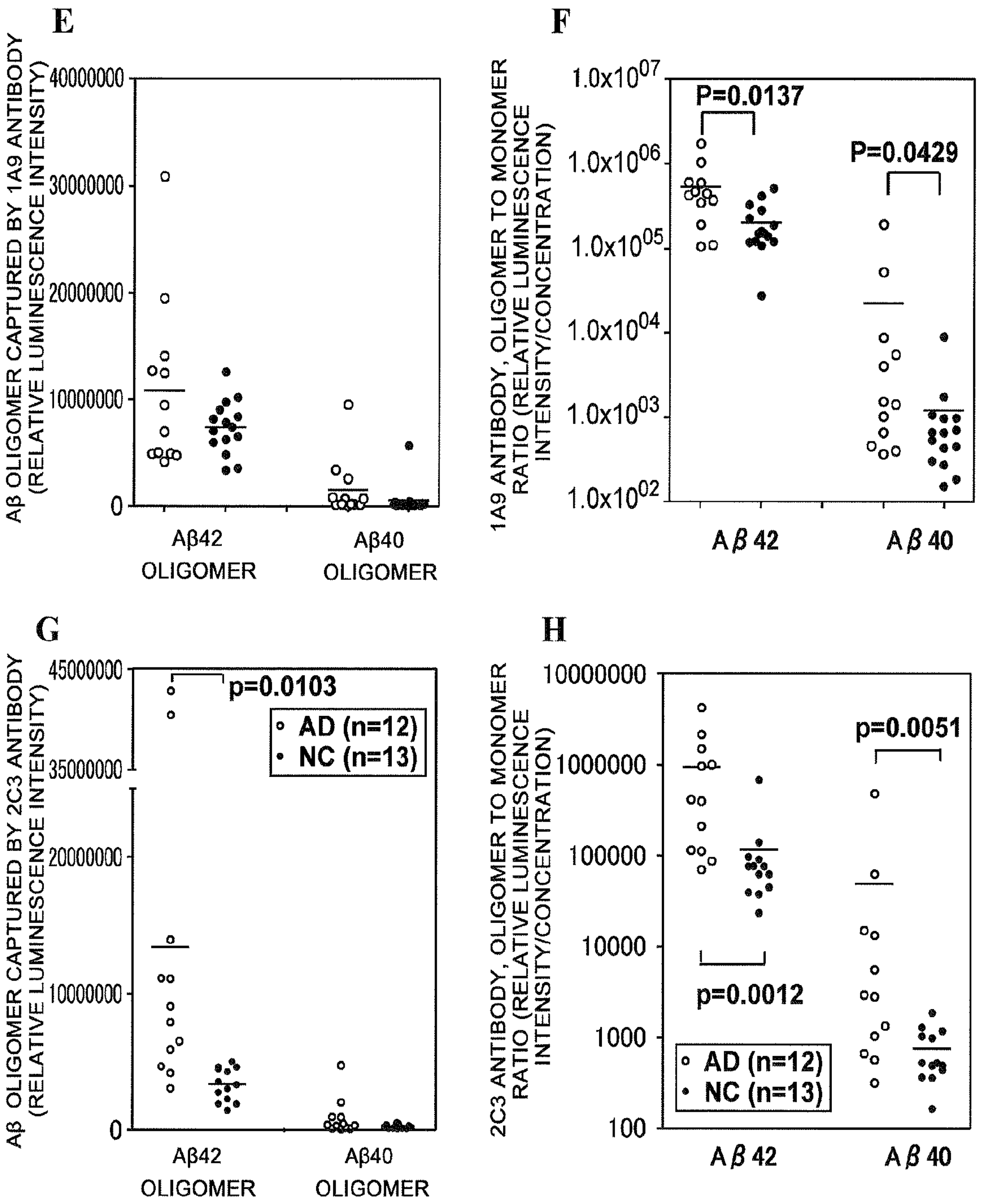

E
Aβ OLIGOMER CAPTURED BY 1A9 ANTIBODY (RELATIVE LUMINESCENCE INTENSITY)
40000000
30000000
20000000
10000000
0
Aβ42 OLIGOMER
Aβ40 OLIGOMER
F
1A9 ANTIBODY, OLIGOMER TO MONOMER RATIO (RELATIVE LUMINESCENCE INTENSITY/CONCENTRATION)
1.0x10^07
1.0x10^06
1.0x10^05
1.0x10^04
1.0x10^03
1.0x10^02
P=0.0137
P=0.0429
Aβ 42
Aβ 40
G
Aβ OLIGOMER CAPTURED BY 2C3 ANTIBODY (RELATIVE LUMINESCENCE INTENSITY)
45000000
35000000
20000000
10000000
0
p=0.0103
AD (n=12)
NC (n=13)
Aβ42 OLIGOMER
Aβ40 OLIGOMER
H
2C3 ANTIBODY, OLIGOMER TO MONOMER RATIO (RELATIVE LUMINESCENCE INTENSITY/CONCENTRATION)
10000000
1000000
100000
10000
1000
100
p=0.0051
p=0.0012
AD (n=12)
NC (n=13)
Aβ 42
Aβ 40

FIG. 7-2

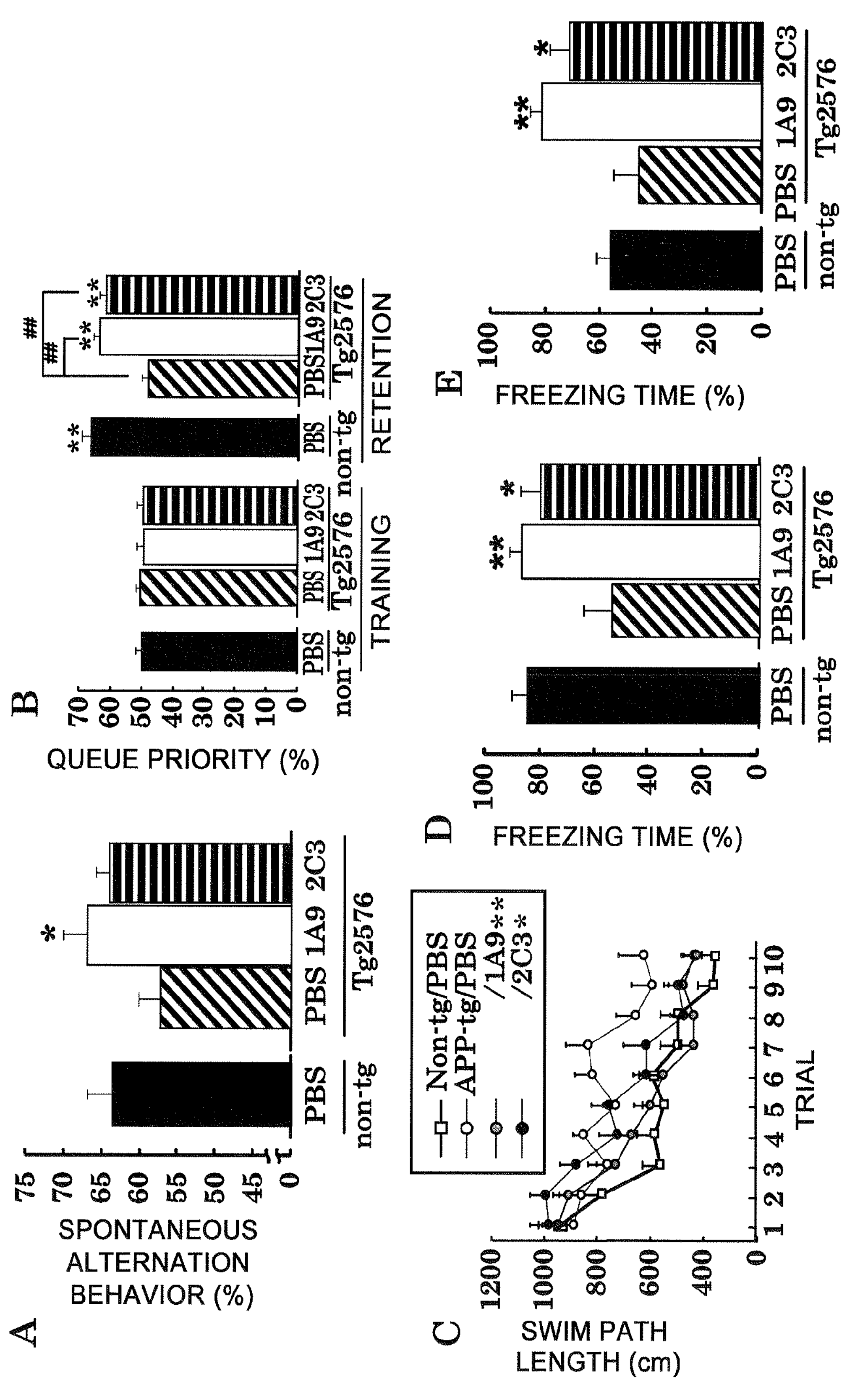

A
SPONTANEOUS ALTERNATION BEHAVIOR (%)
PBS
non-tg
PBS 1A9 2C3
Tg2576
B
QUEUE PRIORITY (%)
TRAINING
RETENTION
##
C
SWIM PATH LENGTH (cm)
TRIAL
Non-tg/PBS
APP-tg/PBS
/1A9**
/2C3*
D
FREEZING TIME (%)
E
FREEZING TIME (%)

FIG. 8

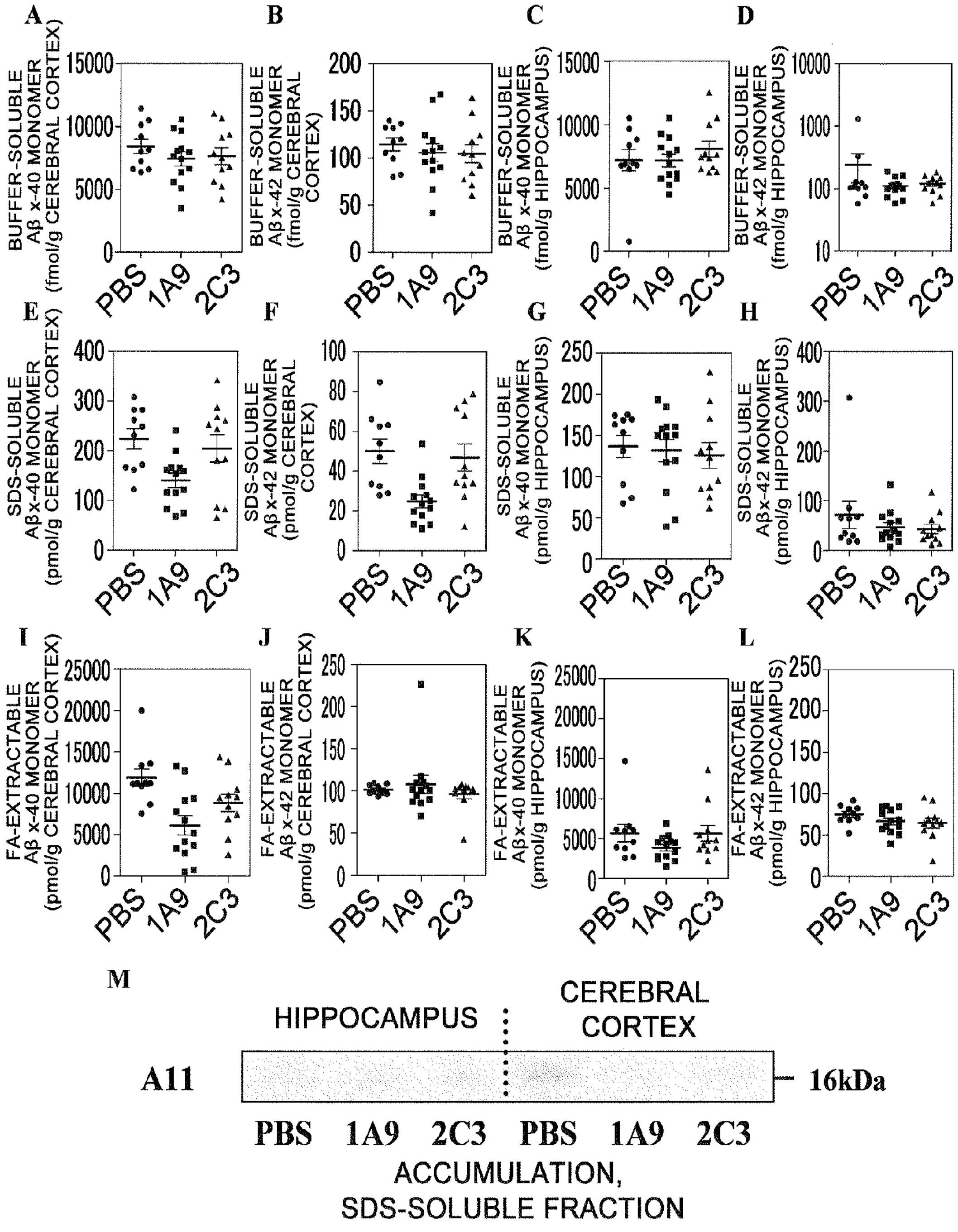

A
BUFFER-SOLUBLE Aβ x-40 MONOMER (fmol/g CEREBRAL CORTEX)
B
BUFFER-SOLUBLE Aβ x-42 MONOMER (fmol/g CEREBRAL CORTEX)
C
BUFFER-SOLUBLE Aβ x-40 MONOMER (fmol/g HIPPOCAMPUS)
D
BUFFER-SOLUBLE Aβ x-42 MONOMER (fmol/g HIPPOCAMPUS)
E
SDS-SOLUBLE Aβx-40 MONOMER (pmol/g CEREBRAL CORTEX)
F
SDS-SOLUBLE Aβ x-42 MONOMER (pmol/g CEREBRAL CORTEX)
G
SDS-SOLUBLE Aβ x-40 MONOMER (pmol/g HIPPOCAMPUS)
H
SDS-SOLUBLE Aβ x-42 MONOMER (pmol/g HIPPOCAMPUS)
I
FA-EXTRACTABLE Aβ x-40 MONOMER (pmol/g CEREBRAL CORTEX)
J
FA-EXTRACTABLE Aβ x-42 MONOMER (pmol/g CEREBRAL CORTEX)
K
FA-EXTRACTABLE Aβx-40 MONOMER (pmol/g HIPPOCAMPUS)
L
FA-EXTRACTABLE Aβ x-42 MONOMER (pmol/g HIPPOCAMPUS)
PBS
1A9
2C3
M
HIPPOCAMPUS
CEREBRAL CORTEX
A11
16kDa
PBS 1A9 2C3 PBS 1A9 2C3
ACCUMULATION, SDS-SOLUBLE FRACTION

FIG. 9

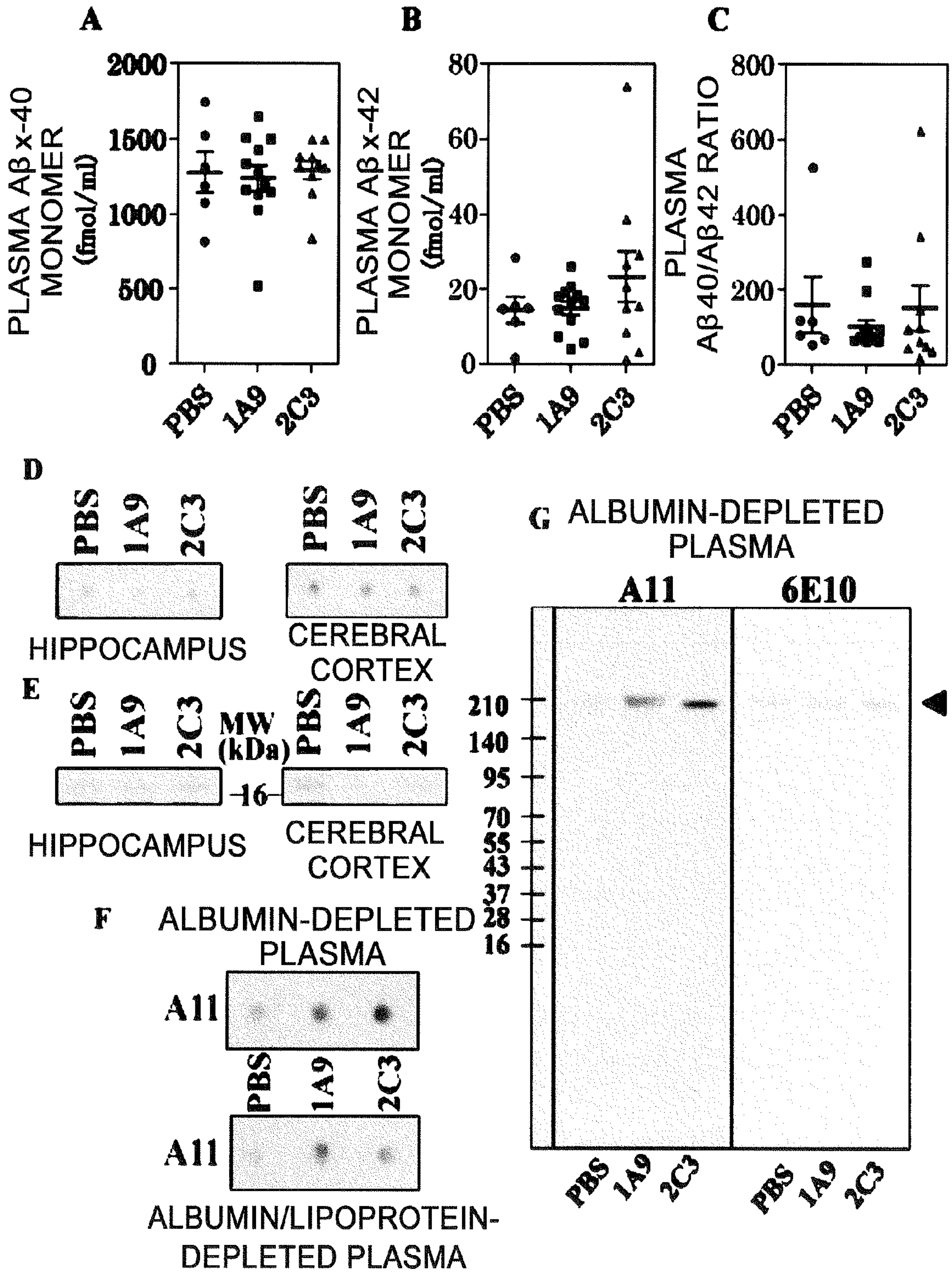

A
B
C
PLASMA Aβ x-40 MONOMER (fmol/ml)
PLASMA Aβ x-42 MONOMER (fmol/ml)
PLASMA Aβ40/Aβ42 RATIO
2000
1500
1000
500
0
80
60
40
20
0
800
600
400
200
0
PBS
1A9
2C3
D
HIPPOCAMPUS
CEREBRAL CORTEX
E
MW (kDa)
16
F
ALBUMIN-DEPLETED PLASMA
A11
ALBUMIN/LIPOPROTEIN-DEPLETED PLASMA
G
ALBUMIN-DEPLETED PLASMA
A11
6E10
210
140
95
70
55
43
37
28
16

FIG. 10

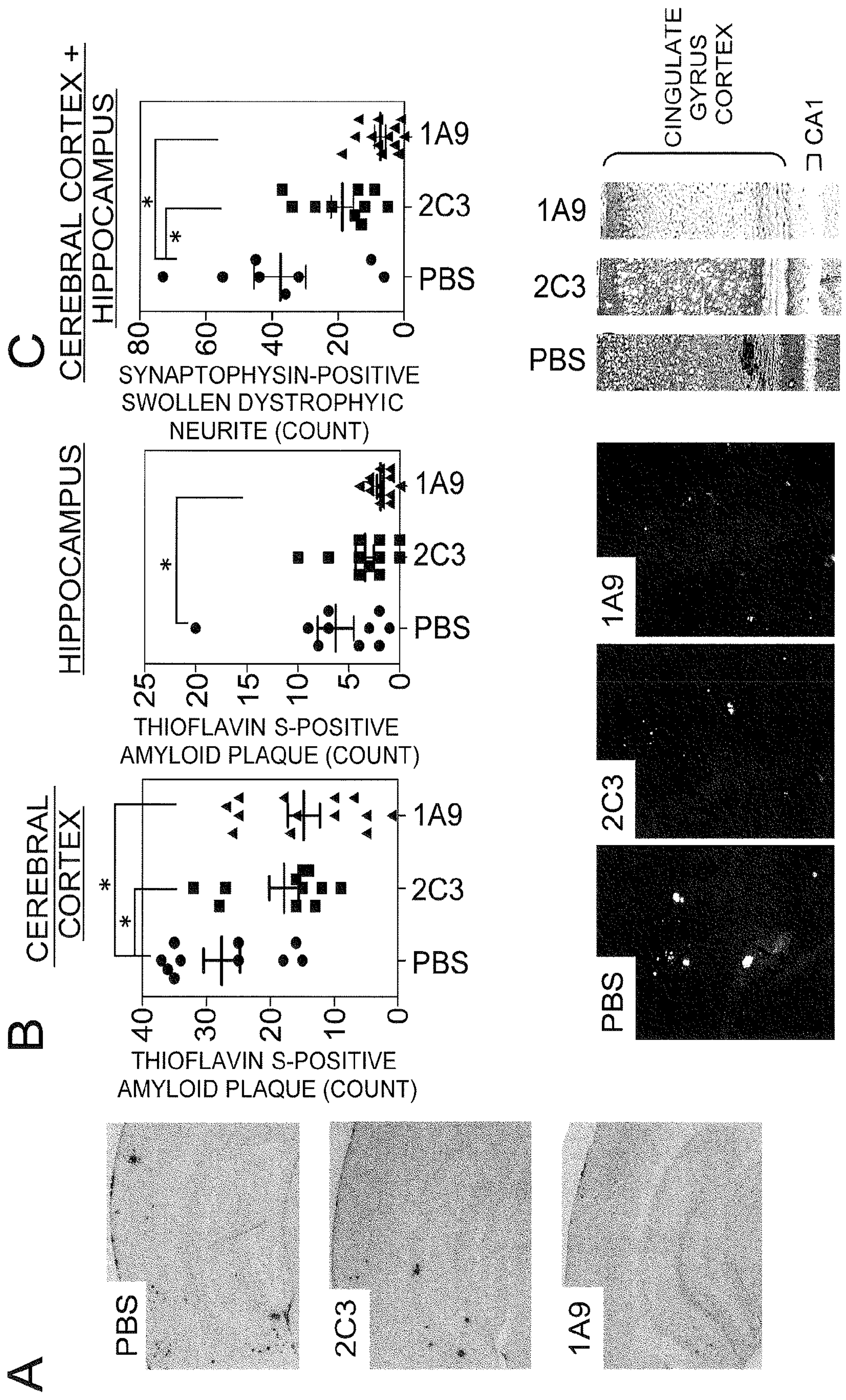

A
PBS
2C3
1A9
B
CEREBRAL CORTEX
THIOFLAVIN S-POSITIVE AMYLOID PLAQUE (COUNT)
40
30
20
10
0
PBS
2C3
1A9
HIPPOCAMPUS
THIOFLAVIN S-POSITIVE AMYLOID PLAQUE (COUNT)
25
20
15
10
5
0
PBS
2C3
1A9
C
CEREBRAL CORTEX + HIPPOCAMPUS
SYNAPTOPHYSIN-POSITIVE SWOLLEN DYSTROPHYIC NEURITE (COUNT)
80
60
40
20
0
PBS
2C3
1A9
CINGULATE GYRUS CORTEX
CA1

FIG. 11

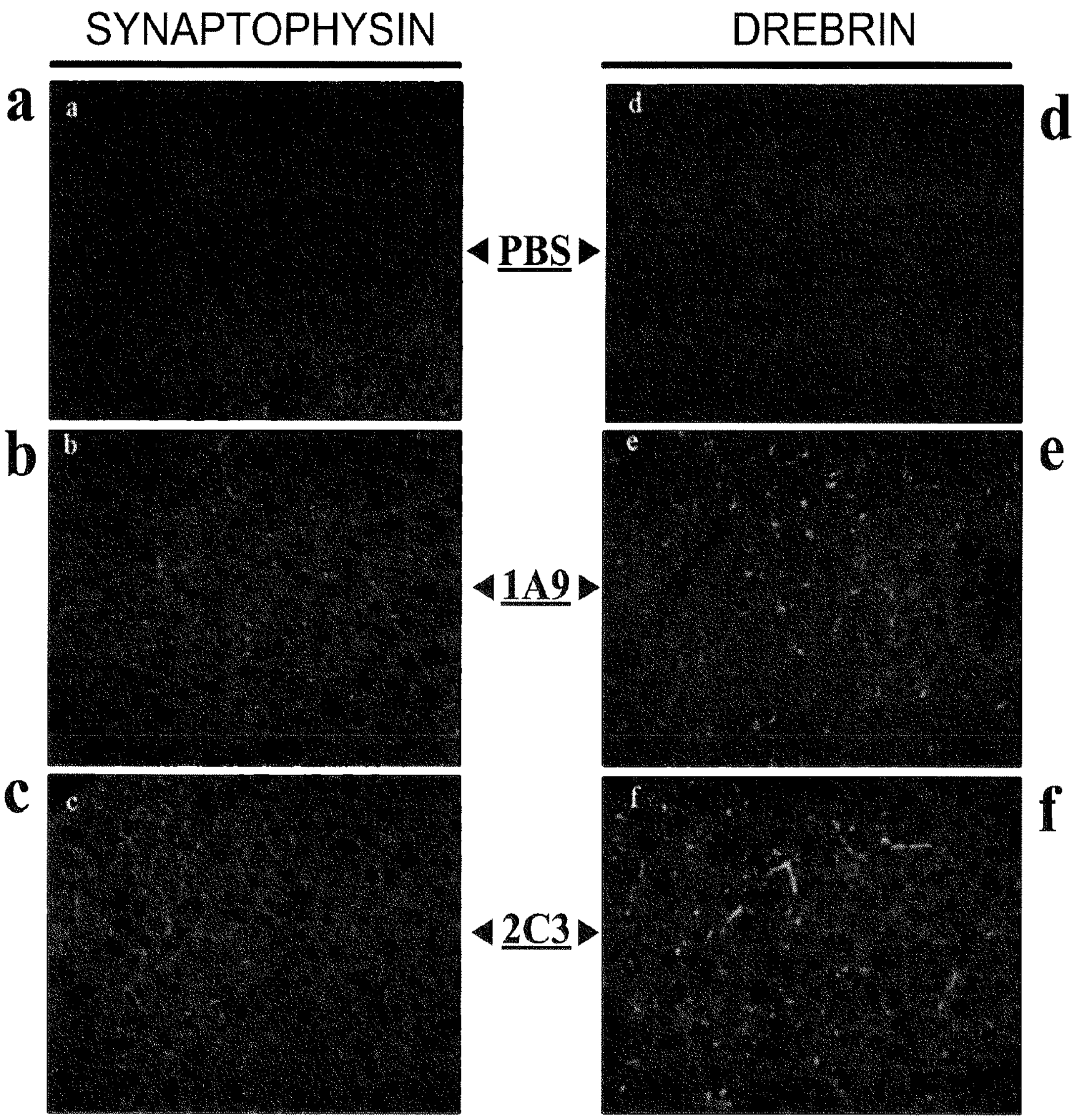


FIG. 12

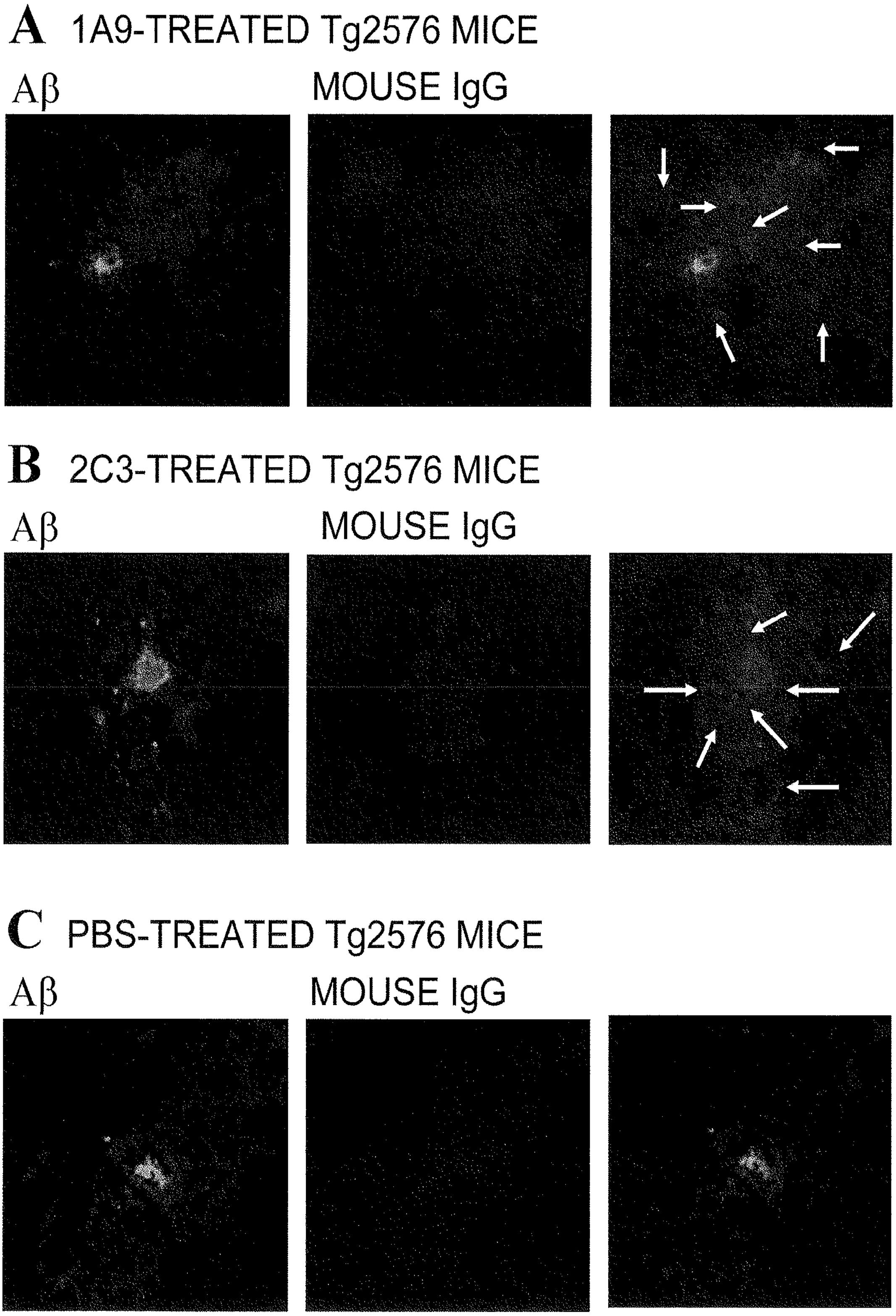

A 1A9-TREATED Tg2576 MICE
Aβ
MOUSE IgG
B 2C3-TREATED Tg2576 MICE
Aβ
MOUSE IgG
C PBS-TREATED Tg2576 MICE
Aβ
MOUSE IgG

FIG. 13

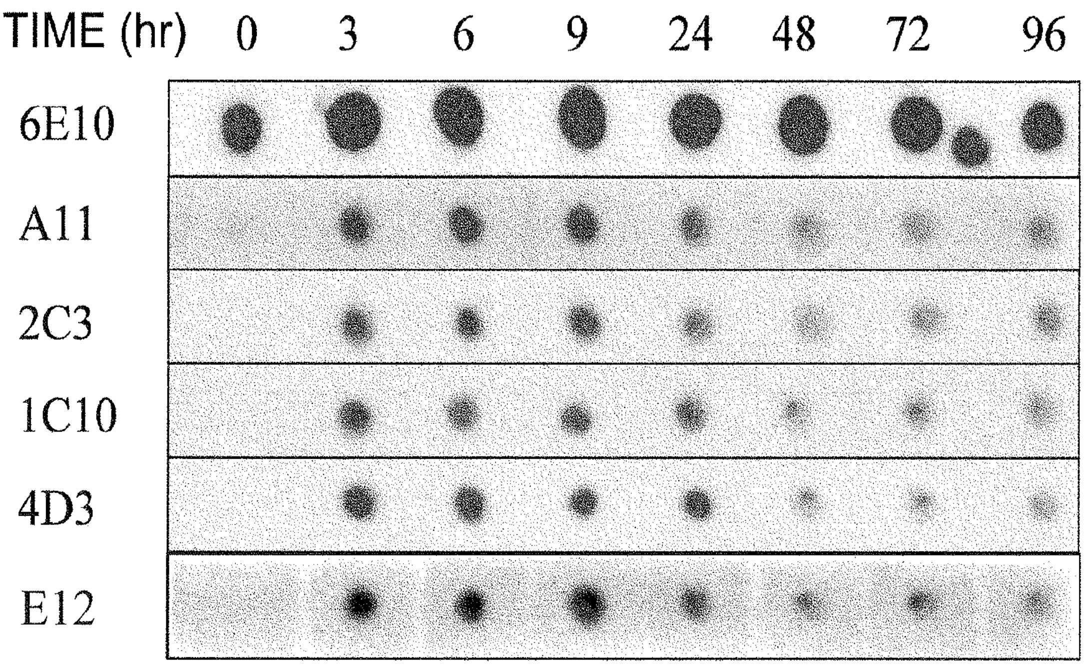


FIG. 14

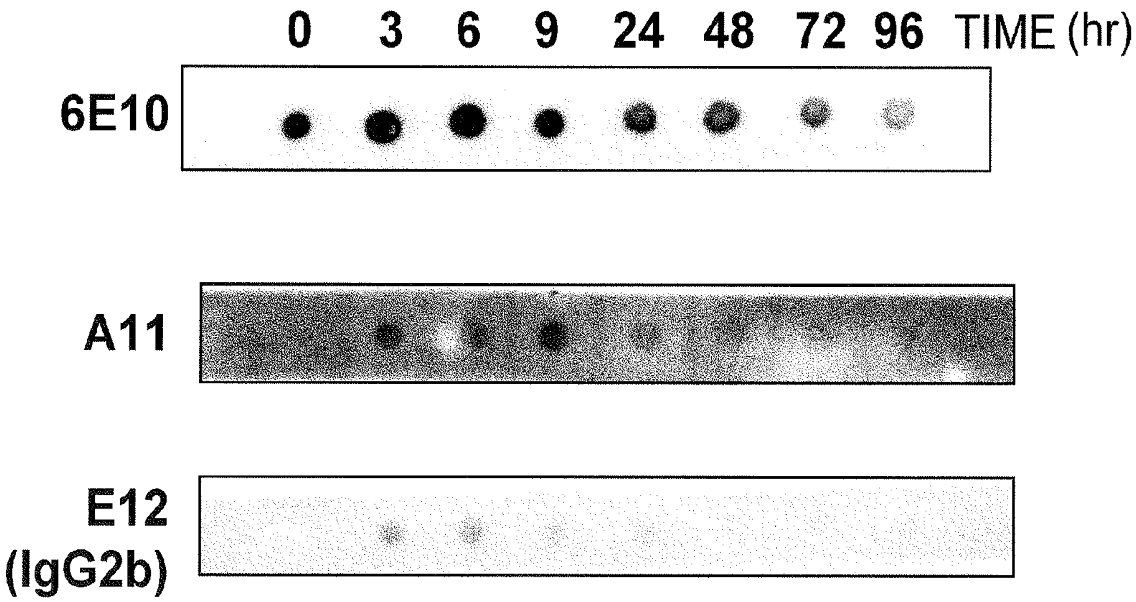


FIG. 15

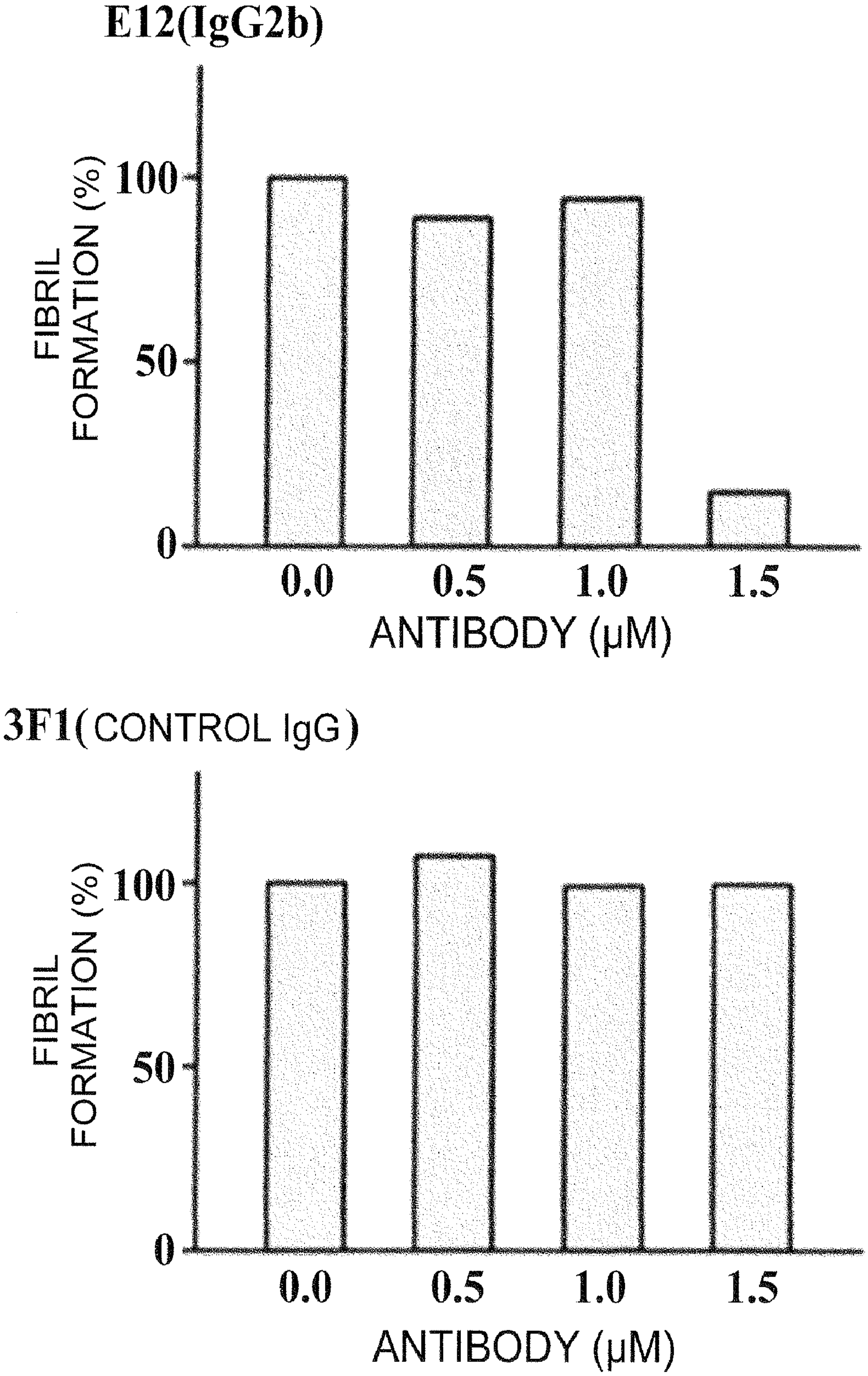

E12(IgG2b)
FIBRIL FORMATION (%)
100
50
0
0.0
0.5
1.0
1.5
ANTIBODY (μM)
3F1(CONTROL IgG)
FIBRIL FORMATION (%)
100
50
0
0.0
0.5
1.0
1.5
ANTIBODY (μM)

FIG. 16

ANTIBODY SPECIFIC BINDING TO A BETA OLIGOMER AND THE USE

PRIORITY

[0001] The present application is a continuation of U.S. application Ser. No. 12/533,294, filed Jul. 31, 2009, now pending. U.S. application Ser. No. 12/533,294 is a nonprovisional of provisional U.S. Application No. 61/085,540, filed Aug. 1, 2008, now abandoned. The present application is also a continuation-in-part of International Application No. PCT/JP2008/068848, filed Oct. 17, 2008, now pending. International Application No. PCT/JP2008/068848 claims priority to Japanese Patent Application No. 2007-272015, filed Oct. 19, 2007, now abandoned, and claims benefit to provisional U.S. Application No. 61/085,540, filed Aug. 1, 2008, now abandoned. The entire contents of these applications are incorporated by reference herein.

FIELD OF THE INVENTION

[0002] The present invention relates to antibodies that specifically bind to Aβ oligomers and uses thereof.

BACKGROUND OF THE INVENTION

[0003] Various evidence has shown that deterioration of memory arises from synaptic dysfunction triggered by soluble Aβ oligomers (see Klein W L, Trends Neurosci. 24: 219-224, 2001; and Selkoe D J, Science 298: 789-791, 2002). Excessive accumulation and deposition of Aβ oligomers may be the trigger for a series of pathological cascades that lead to Alzheimer's disease (AD). Therefore, therapeutic intervention targeting Aβ oligomers may be effective for blocking these cascades. However, findings on core molecules of this amyloid cascade hypothesis which are responsible for neurodegeneration, particularly on neurodegeneration mediated by Aβ oligomers, originate from in vitro experiments (see Hass C et al.: Nature Review 8: 101-12, 2007). This neurodegeneration has not been proven directly in vivo. The greatest defect of previously reported in vivo experiments is that they failed to demonstrate synaptic toxicity of endogenous Aβ oligomers due to the lack of conformation-specific molecular tools (see Lee E B, et al.: J. Biol. Chem. 281: 4292-4299, 2006). There has been known no technique capable of proving the toxicity within the human brain, an aspect which is difficult to demonstrate even in Alzheimer's disease mouse models. Thus, the in vivo neurotoxicity of endogenous Aβ has been often disregarded. It has been unknown why NFT formation and loss of nerve cells precede senile plaque formation in the human entorhinal cortex, and how Aβ oligomers are involved in this mechanism.

SUMMARY OF THE INVENTION

[0004] The present invention was achieved in view of the above circumstances. An objective of the present invention is to provide antibodies that bind specifically to Aβ oligomers, and uses thereof. More specifically, the present invention provides antibodies that bind specifically to Aβ oligomers, methods for detecting Aβ oligomers using the antibodies, methods for diagnosing Alzheimer's disease using the antibodies, and pharmaceutical agents comprising the antibodies.

[0005] The present inventors produced monoclonal antibodies that are specific to only soluble amyloid β (Aβ) oligomers and do not recognize soluble Aβ monomers which are physiological molecules, and confirmed that the antibodies have the following:

(1) anti-neurotoxic activity;
(2) activity to suppress Aβ amyloid fibril formation;
(3) specificity to recognize only Aβ oligomers;
(4) ability to capture Aβ oligomers in AD brain; and
(5) ability to prevent the development of Alzheimer's disease-like phenotypes (memory impairment, brain Aβ accumulation) in APPswe transgenic mice (Tg2576).

[0006] Using an ultrafiltration/molecular sieve method, among the antibodies produced, monoclonal 1A9 and 2C3 were determined to specifically recognize oligomers of 30 kDa or more, mainly 100 kDa or more, but not monomers of approximately 4.5 kDa. The two antibodies were confirmed to have neurotoxicity-neutralizing activity by evaluating the neutralizing effect against Aβ 1-42-induced neurotoxicity in PC12 cells differentiated into nerve cells. Thioflavin T assay and electron microscopy showed that the antibodies have activity to suppress Aβ amyloid fibril formation. The ability of 1A9 and 2C3 to capture Aβ oligomers in AD brain was confirmed by immunoprecipitation using the antibodies in the presence of SDS-stable 4-, 5-, 8-, and 12-mers. Furthermore, to determine the in vivo neurotoxicity in the human brain, the amount of polymers recognized by the antibodies was evaluated in the human entorhinal cortex mostly at Braak NFT Stages I to III. By particularly focusing on the 12-mer, which has been reported to have neurotoxicity in animal studies, it was confirmed that the polymer accumulation precedes the occurrence of cognitive impairment, and is increased with the progression of Braak NFT stage. This result shows for the first time that the 12-mer, which is specifically recognized by the antibodies, is a conformational assembly that causes in vivo neurotoxicity in the human brain. The present inventors also discovered that the oligomeric conformational structure recognized by the antibodies is present in cerebrospinal fluid (CSF), and is increased in AD patients. The present inventors used 1A9 or 2C3 in passive immunotherapy by intravenous injection as with other neurological disorders. It was confirmed that Tg2576 mice are protected from memory impairment, senile plaque formation, synaptic dysfunction, and Aβ accumulation by subchronic passive immunotherapy, without harmful side-effects. The results obtained by the present inventors demonstrated for the first time that monoclonal 1A9 and 2C3 are promising candidates for therapeutic antibodies for preventing Alzheimer's disease-like phenotypes in Tg2576 mice, which are expected to show their effect by conventional peripheral intravenous administration, and thus there is no need to consider brain transfer.

[0007] The present inventors also confirmed that passive immunotherapy using the 1A9 and 2C3 antibodies suppresses senile plaque amyloid formation and swollen dystrophic neurite formation. Furthermore, the present inventors discovered that a fraction of the 1A9 and 2C3 antibodies administered into the blood transfers into the brain.

[0008] As described above, the present inventors disclose herein that monoclonal 1A9 and 2C3, which are antibodies that specifically bind to Aβ oligomers, fulfill all of the diagnostic/therapeutic antibody criteria, and are promising candidates for therapeutic antibodies for diagnosing/preventing Alzheimer's disease.

[0009] The present inventors succeeded in obtaining the E12, 1C10, and 4D3 antibodies that, similarly to the 1A9 and 2C3 antibodies, do not recognize Aβ monomers but bind specifically to Aβ oligomers, and that, among them, the E12 antibody was found to have activity to suppress Aβ amyloid fibril formation.

[0010] Accordingly, the present inventors disclose that the above-mentioned E12, 1C10, and 4D3 antibodies are also promising candidates for therapeutic antibodies for diagnosing/preventing Alzheimer's disease.

[0011] More specifically, the present invention provides the following:

[1] an antibody that binds to an Aβ oligomer, wherein the antibody does not bind to an Aβ monomer;

[2] an antibody binding to an Aβ oligomer that binds to an antibody comprising an H chain having the amino acid sequence of SEQ ID NO: 1 and an L chain having the amino acid sequence of SEQ ID NO: 3;

[3] an antibody binding to an Aβ oligomer that binds to an antibody comprising an H chain having the amino acid sequence of SEQ ID NO: 21 and an L chain having the amino acid sequence of SEQ ID NO: 23;

[4] an antibody binding to an Aβ oligomer that binds to an antibody comprising an H chain having the amino acid sequence of SEQ ID NO: 41 and an L chain having the amino acid sequence of SEQ ID NO: 43;

[5] an antibody of any one of (1) to (20) below:

(1) an antibody that comprises an H chain having the amino acid sequence of SEQ ID NO: 9 as CDR1, the amino acid sequence of SEQ ID NO: 11 as CDR2, and the amino acid sequence of SEQ ID NO: 13 as CDR3;

(2) an antibody that comprises an L chain having the amino acid sequence of SEQ ID NO: 15 as CDR1, the amino acid sequence of SEQ ID NO: 17 as CDR2, and the amino acid sequence of SEQ ID NO: 19 as CDR3;

(3) an antibody that comprises the H chain of (1) and the L chain of (2);

(4) an antibody that comprises an H chain having the amino acid sequence of SEQ ID NO: 5 as VH;

(5) an antibody that comprises an L chain having the amino acid sequence of SEQ ID NO: 7 as VL;

(6) an antibody that comprises the H chain of (4) and the L chain of (5);

(7) an antibody that comprises an H chain having the amino acid sequence of SEQ ID NO: 29 as CDR1, the amino acid sequence of SEQ ID NO: 31 as CDR2, and the amino acid sequence of SEQ ID NO: 33 as CDR3;

(8) an antibody that comprises an L chain having the amino acid sequence of SEQ ID NO: 35 as CDR1, the amino acid sequence of SEQ ID NO: 37 as CDR2, and the amino acid sequence of SEQ ID NO: 39 as CDR3;

(9) an antibody that comprises the H chain of (7) and the L chain of (8);

(10) an antibody that comprises an H chain having the amino acid sequence of SEQ ID NO: 25 as VH;

(11) an antibody that comprises an L chain having the amino acid sequence of SEQ ID NO: 27 as VL;

(12) an antibody that comprises the H chain of (10) and the L chain of (11);

(13) an antibody that comprises an H chain having the amino acid sequence of SEQ ID NO: 49 as CDR1, the amino acid sequence of SEQ ID NO: 51 as CDR2, and the amino acid sequence of SEQ ID NO: 53 as CDR3;

(14) an antibody that comprises an L chain having the amino acid sequence of SEQ ID NO: 55 as CDR1, the amino acid sequence of SEQ ID NO: 57 as CDR2, and the amino acid sequence of SEQ ID NO: 59 as CDR3;

(15) an antibody that comprises the H chain of (13) and the L chain of (14);

(16) an antibody that comprises an H chain having the amino acid sequence of SEQ ID NO: 45 as VH;

(17) an antibody that comprises an L chain having the amino acid sequence of SEQ ID NO: 47 as VL;

(18) an antibody that comprises the H chain of (16) and the L chain of (17);

(19) an antibody that comprises one or more amino acid substitutions, deletions, additions, and/or insertions in the antibody of any one of (1) to (18), which has equivalent activity to the antibody of any one of (1) to (18); and

(20) an antibody that binds to the epitope bound by the antibody of any one of (1) to (18);

[6] the antibody of [5], wherein the antibody is a chimeric antibody or a humanized antibody;

[7] a composition comprising the antibody of any one of [1] to [6] and a pharmaceutically acceptable carrier;

[8] an agent against cognitive impairment, which comprises the antibody of any one of [1] to [6] or the composition of [7] as an active ingredient;

[9] a therapeutic agent for Alzheimer's disease, which comprises the antibody of any one of [1] to [6] or the composition of [7] as an active ingredient;

[10] an agent for suppressing the progression of Alzheimer's disease, which comprises the antibody of any one of [1] to [6] or the composition of [7] as an active ingredient;

[11] an agent for suppressing senile plaque formation, which comprises the antibody of any one of [1] to [6] or the composition of [7] as an active ingredient;

[12] an agent for suppressing Aβ accumulation, which comprises the antibody of any one of [1] to [6] or the composition of [7] as an active ingredient;

[13] an anti-neurotoxic agent, which comprises the antibody of any one of [1] to [6] or the composition of [7] as an active ingredient;

[14] an agent for inhibiting Aβ amyloid fibril formation, which comprises the antibody of any one of [1] to [6] or the composition of [7] as an active ingredient;

[15] an agent against synaptic toxicity, which comprises the antibody of any one of [1] to [6] or the composition of [7] as an active ingredient;

[16] a method for preventing and/or treating cognitive impairment, which comprises the step of administering the antibody of any one of [1] to [6] or the composition of [7] as an active ingredient;

[17] a method for preventing and/or treating Alzheimer's disease, which comprises the step of administering the antibody of any one of [1] to [6] or the composition of [7] as an active ingredient;

[18] a method for suppressing the progression of Alzheimer's disease, which comprises the step of administering the antibody of any one of [1] to [6] or the composition of [7] as an active ingredient;

[19] a method for suppressing senile plaque formation, which comprises the step of administering the antibody of any one of [1] to [6] or the composition of [7] as an active ingredient;

[20] a method for suppressing Aβ accumulation, which comprises the step of administering the antibody of any one of [1] to [6] or the composition of [7] as an active ingredient;

[21] a method for neutralizing neurotoxicity, which comprises the step of administering the antibody of any one of [1] to [6] or the composition of [7] as an active ingredient;

[22] a method for inhibiting Aβ amyloid fibril formation, which comprises the step of administering the antibody of any one of [1] to [6] or the composition of [7] as an active ingredient;
[23] a method for neutralizing synaptic toxicity, which comprises the step of administering the antibody of any one of [1] to [6] or the composition of [7] as an active ingredient;
[24] a method for detecting an Aβ oligomer, which comprises the step of detecting an Aβ oligomer contained in a sample collected from a subject using the antibody of any one of [1] to [6];
[25] a method of diagnosing whether or not a subject suffers from or is at a risk of developing Alzheimer's disease, which comprises using the antibody of any one of [1] to [6] to detect an Aβ oligomer in a sample collected from a subject;
[26] a method of diagnosing whether or not a subject suffers from or is at a risk of developing Alzheimer's disease, which comprises the steps of:
(a) contacting a sample collected from a subject with the antibody of any one of [1] to [6]; and
(b) measuring the amount of Aβ oligomer in the sample,
wherein the subject is determined to suffer from or be at a risk of developing Alzheimer's disease, when the amount measured in step (b) is higher than that of a healthy individual;
[27] a method of diagnosing whether or not a subject suffers from or is at a risk of developing Alzheimer's disease, which comprises the steps of:
(a) contacting a sample collected from a subject with the antibody of any one of [1] to [6] and an antibody that binds to an Aβ monomer; and
(b) measuring the ratio of Aβ oligomer to Aβ monomer in the sample,
wherein the subject is determined to suffer from or be at a risk of developing Alzheimer's disease, when the ratio measured in step (b) is higher than that of a healthy individual;
[28] the method of any one of [24] to [27], wherein the sample is blood or cerebrospinal fluid;
[29] a pharmaceutical agent for use in the method of any one of [24] to [27]; and
[30] a kit for use in the method of any one of [24] to [27].

[0012] Furthermore, the present invention provides the following:
[31] use of the antibody of any one of [1] to [6] or the composition of [7] in the production of an agent against cognitive impairment;
[32] use of the antibody of any one of [1] to [6] or the composition of [7] in the production of a therapeutic agent for Alzheimer's disease;
[33] use of the antibody of any one of [1] to [6] or the composition of [7] in the production of an agent for suppressing the progression of Alzheimer's disease;
[34] use of the antibody of any one of [1] to [6] or the composition of [7] in the production of an agent for suppressing senile plaque formation;
[35] use of the antibody of any one of [1] to [6] or the composition of [7] in the production of an agent for suppressing Aβ accumulation;
[36] use of the antibody of any one of [1] to [6] or the composition of [7] in the production of an agent for neutralizing (suppressing) neurotoxicity;
[37] use of the antibody of any one of [1] to [6] or the composition of [7] in the production of an agent for inhibiting Aβ amyloid fibril formation;
[38] use of the antibody of any one of [1] to [6] or the composition of [7] in the production of an agent for neutralizing (suppressing) synaptic toxicity;
[39] the antibody of any one of [1] to [6] or the composition of [7] for use in preventing and/or treating cognitive impairment;
[40] the antibody of any one of [1] to [6] or the composition of [7] for use in preventing and/or treating Alzheimer's disease;
[41] the antibody of any one of [1] to [6] or the composition of [7] for use in suppressing the progression of Alzheimer's disease;
[42] the antibody of any one of [1] to [6] or the composition of [7] for use in suppressing senile plaque formation;
[43] the antibody of any one of [1] to [6] or the composition of [7] for use in suppressing Aβ accumulation;
[44] the antibody of any one of [1] to [6] or the composition of [7] for use in neutralizing (suppressing) neurotoxicity;
[45] the antibody of any one of [1] to [6] or the composition of [7] for use in inhibiting Aβ amyloid fibril formation; and
[46] the antibody of any one of [1] to [6] or the composition of [7] for use in neutralizing (suppressing) synaptic toxicity

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] FIG. **1** presents photographs and a graph showing the results of production and characteristic determination of oligomer-specific antibodies. A: Electrophoresis of immunogens. The Aβ 1-42 tetramer (red arrowhead) which is free of contamination of the Aβ 1-42 monomer (outlined arrowhead) was isolated using SDS-PAGE. Lane 1: Aβ 1-42 dissolved in 10 mM phosphate buffer; and Lane 2: Aβ 1-42 dissolved in distilled deionized water. B: Aβ amyloid, which is insoluble in a buffer but can be extracted using formic acid from the brain of Alzheimer's disease patients, was immunoprecipitated using the supernatant of a positive hybridoma cell culture, and the immune complex was selectively separated using protein-G agarose (Amersham). Nine clones were tested; lane 2 (red asterisk) is 1A9 and lane 6 (red double asterisk) is 2C3. C: Elution profile of SEC of a conditioned medium. Among the 24 SEC-collected fractions, fractions 8, 13, and 16 were subjected to 1A9 immunoprecipitation. Aβ immunoreactivity was detected using 4G8. The red arrowhead indicates the trimer and the outlined arrowhead indicates the dimer. Asterisk (*) indicates the anti-mouse IgG light chain.

[0014] FIG. **2** presents photographs and a graph showing the antitoxic activity of 1A9 and 2C3. A to F: Representative images of NGF-treated PC12 (PC12N) cells, which were exposed to seed-free Aβ 1-42 at 37° C. for 48 hours in the presence or absence of the antibodies (left half of each panel). Representative calcein AM/PI staining where live cells were stained green and dead cells were stained red (right half of each panel). G: The viability of cells exposed to seed-free Aβ 1-42 (25 μM) with the following antibodies: non-specific IgG2b (filled square); 4G8 (open triangle); 1A9 (open square); and 2C3 (filled circle).

[0015] FIG. **3** presents photographs and a graph showing the size and morphological characteristics of the toxic Aβ assemblies targeted by 1A9 and 2C3. A: The 540,000×g supernatant of Aβ 1-42 (25 μM) was subjected to a continuous molecular sieving process using ultrafiltration membranes having a molecular weight cutoff value of 3, 10, 30, and 100 kDa (Microcon). The four types of filtrates thus fractioned were named as follows: fraction 1 (<3 kDa), fraction 2 (3 to 10 kDa), fraction 3 (10 to 30 kDa), fraction 4 (30 to 100 kDa); and fraction 5 (>100 kDa) which was finally retained. The presence of Aβ 1-42 in each of the above-mentioned fractions was detected by 4G8 immunoblotting. B: Representative images of NGF-treated PC12 (PC12N) cells treated with the five fractions at 37° C. for 48 hours. The toxicity of each fraction was evaluated as described above for FIG. **2**. C: The viability of cells treated with the 540,000×g supernatant of Aβ 1-42 and the five fractions (fractions 1 to 5). Similar results were obtained from two independent experiments. The values are presented in percentage (mean±SD) with respect to the control. D: Dot blot analysis of the five fractions (fractions 1 to 5). The blots were reacted with A11, 1A9, 2C3, and 4G8. E: AFM images of the five fractions. In fraction 5 (Fr. 5) that had the strongest toxicity, ring-shaped and bead-shaped structures were observed in addition to granular molecules.

[0016] FIG. **4** presents photographs and graphs showing the activity of 1A9 and 2C3 to suppress Aβ amyloid fibril formation. A: Amyloid fibril formation of Aβ 1-42 at various concentrations (10 μM (open square), 25 μM (filled diamond), and 50 μM (open circle)) was monitored by ThT assay at 37° C. for up to 72 hours. B: Coexisting antibody dose-dependent inhibition of amyloid fibril formation of Aβ 1-42 was observed for 2C3 (open circle). In contrast, the 1A9 (open square), 4G8 (filled triangle), and non-specific IgG (filled square) antibodies did not inhibit fibril-forming assembly of seed-free Aβ 1-42 (ThT-negative 540,000×g supernatant). C: Coexisting antibody dose-dependent inhibition of fibril-forming assembly of Aβ 1-42 was observed for 2C3 (open circle), and nearly complete inhibition was observed also for 1A9 (open square) at 3 μM. D: None of the test antibodies added after a 24-hour pre-incubation for Aβ 1-42 amyloid fibril formation could dissolve nor disassemble the Aβ 1-42 amyloid fibrils. E to G: EM images of Aβ 1-42 in the absence (Panel E) and presence of 2C3 (Panel F) and 1A9 (Panel G).

[0017] FIG. **5** presents photographs and graphs on toxicity-related Aβ 1-42 oligomers. A: Dot blot assay (upper half of Panel A): Aβ 1-42 monomers (25 μM) were incubated for a specified time (0 to 72 hours) at 37° C., and immobilized onto a nitrocellulose membrane, and subjected to dot blot assay that uses A11, 1A9, 2C3, or 4G8. The emergence of immunoreactivity-positive structures for each antibody was tested. Immunoreactivity intensity analysis (lower half of Panel A): The results of dot blot assay were analyzed semiquantitatively using the Multi Gauge v 3.0 software (Fuji Film, Tokyo). To correlate the oligomer formation and amyloid fibril formation, the ThT fluorescence value (the right Y axis) was overlaid on the same time axis. B: The Aβ 1-42 assembly after 0-, 2-, 4-, and 24-hour incubation at 37° C., and the change in Aβ 1-42 assembly after further 48-hour incubation. The Aβ 1-42 assembly was detected by 4G8 immunoblotting. C: The toxic activity of the above-mentioned various Aβ 1-42 assemblies. The viability of nerve cells was determined by the LIVE/DEAD assay as described for FIG. **2**. D: The anti-neurotoxic activity of 1A9 and 2C3 was evaluated using various Aβ assemblies (the Aβ 1-42 assemblies formed at 37° C. for 0 and 2 hours ("0 h" and "2 h"); and the ThT-positive supernatant collected after ultracentrifugation at 540,000×g for two hours ("2 h sup")). Representative images of PC12N cells exposed to various Aβ 1-42 assemblies in the absence or presence of the antibodies are shown in the left half of Panel D (a: "0 h"; b: "2 h"; c: "2 h sup"; d: "2 h sup" +IgG2b; e: "2 h sup"+1A9; f: "2 h sup"+2C3). The viability of cells exposed to various Aβ 1-42 assemblies in the absence or presence of the antibodies is presented in percentage (mean±SD) with respect to the control, and this is shown in the right half of Panel D. Compared to the "0 h" Aβ 1-42 assembly, the "2 h" Aβ 1-42 assembly lowered the neurotoxicity. "2 h sup" recovered the neurotoxicity to a degree similar to that of the "0 h" Aβ 1-42 assembly. Non-specific IgG2b could not block the neurotoxicity induction of the "2 h sup" Aβ 1-42 assembly. Monoclonal 1A9 completely inhibited the "2 h sup"-induced neurotoxicity, while the ability of 2C3 to inhibit the toxicity was slightly inferior. In the experiments using the two monoclonal antibodies (mAbs), the antitoxic activity of the mAbs was observed at a mAb:Aβ mole ratio of 1:<25 to 50. This suggests that structurally different 1A9- and 2C3-recognized oligomeric assemblies exist at a relatively low concentration.

[0018] FIG. **6** presents photographs and graphs showing that soluble 1A9- and 2C3-recognized oligomers exist in the human brain. Antibodies against Aβ oligomers can detect senile plaques and vascular amyloids in AD brain only after pretreatment with Protease K. A: 1A9 staining; B: 2C3 staining; and C: A11 staining. D: 4G8 immunoblotting of 1A9- or 2C3-immunoprecipitated Aβ in buffer-soluble AD brain (lanes 1, 2, 4, and 5) and healthy control brain (lanes 3 and 6). Representative results for 1A9 and 2C3 are shown in the left and right half of the panel, respectively. E and F: Semiquantitative analysis (with actin control) of soluble 1A9-immunoreactive 12-mer (Panel E) and soluble 2C3-immunoreactive 12-mer (Panel F) in the human entorhinal cortex obtained from 50 autopsy cases of a healthy elderly population (Braak NFT Stage I or II: n=35; Braak NFT Stage III or IV: n=13; and Braak NFT Stage>IV, AD cases: n=2).

[0019] FIG. **7-1** present graphs showing that soluble 1A9- and 2C3-recognized oligomers exist in human CSF. Pooled whole cerebrospinal fluid (CSF) (AD=10 and NC=10) (Panels A and B) and pooled lipoprotein-depleted CSF (AD=10, and NC=10) (Panels C and D) were subjected to size exclusion chromatography (SEC). In Panels A and B, the collected fractions were analyzed for the distribution of Aβ 40 and Aβ 42 monomers by BNT77-BA27 and BNT77-BC05 ELISAs. Panels C and D show the presence of Aβ 40 and Aβ 42 oligomers captured by 1A9/2C3 mixed antibodies.

[0020] FIG. **7-2** is the continuation of FIG. **7-1**. The amount of 1A9-recognized oligomeric assembly (1A9-BC05 and 1A9-BA27 ELISAs) and the amount of 2C3-recognized assembly (2C3-BC05 and 2C3-BA27 ELISAs) were measured for 12 AD cases (open circle) and 13 NC cases (filled circle) (Panels E and G). The oligomer/monomer ratio is shown in Panels F (1A9) and H (2C3).

[0021] FIG. **8** presents graphs showing that the onset of memory impairment in Tg2576 mice can be prevented by passive immunization treatment. 13-month-old Tg2576 mice were divided into the following three groups to perform learning/behavior tests: PBS-administered group: n=10; 1A9-administered group: n=13; and 2C3-administered group: n=11. All of the measured values were indicated as mean±SE. (A) Y-maze test. Spontaneous alteration behavior was monitored in each group during an eight-minute session of the Y-maze task. The results of one-way ANOVA were as follows: $F(1, 52)=3.09$, $p<0.05$; * $p<0.05$ in the comparison with PBS-administered Tg2576 mice. (B) Novel object recognition test. The retention session was performed 24 hours after training. The exploratory preference in a ten-minute session in the novel object recognition test was determined in each group. The results of two-way ANOVA were as follows: training/

retention, F(1, 64)=31.53, p<0.01; animal group, F(2, 64)~7.49, p<0.01; repeated training/retention by the animal group, F(2, 64)=10.12, p<0.01; ** p <0.01 in the comparison with the corresponding untrained mice, ## p<0.01 in the comparison with PBS-administered Tg2576 mice. (C) The swimming path length during a 60-second session of water maze test was measured for each group. The results of two-way ANOVA were as follows: trial, F(9, 320)=20.46, p<0.01; animal group, F(2, 320)=12.59, p<0.01; repeated trial by the animal group, F(18, 320)=1.78, p<0.05; p<0.05, ** p<0.01 in the comparison with PBS-administered Tg2576 mice. Fear-conditioned learning test: Context-dependent (D) and clue-dependent freezing times (E) were determined. The results of two-way ANOVA were as follows: context-dependent test, F(2, 32)=5.94, p<0.01; clue-dependent test, F(2, 32)=7.33, p<0.01; * p<0.05 and ** p<0.01 in the comparison with PBS-administered Tg2576 mice.

[0022] FIG. **9** presents graphs and a photograph showing that the brain Aβ accumulation in Tg2576 can be prevented by passive immunotherapy. The hippocampus and cerebral cortex of three groups of 13-month-old Tg2576 mice (PBS-administered group, n=10; 1A9-administered group, n=13; and 2C3-administered group, n=11) were extracted in three continuous steps to prepare the buffer-soluble, SDS-soluble, and formic acid (FA)-extractable fractions. Each of the fractions was subjected to Aβ-specific ELISAs (WAKO kit: BNT77-BA27 for Aβ x-40; BNT77-BC05 for Aβ x-42). The accumulation of Aβ 40 (SDS and FA) and Aβ 42 (SDS) was found to be significantly suppressed only in the 1A9-treated group. The accumulation-suppressing effect for the A11-positive oligomer (4-mer) was confirmed in the SDS-soluble cerebral cortex fractions from the two antibody-treated groups.

[0023] FIG. **10** presents photographs and graphs on Aβ oligomers in the plasma and brain of Tg2576. A and B: As a result of ELISA analysis, no significant difference in the amount of Aβ x-40 and Aβ x-42 in the plasma was observed between the PBS-administered group and the immunotherapy group. C: Similarly, no difference in the Aβ 40/Aβ 42 ratio was observed among the three groups tested. D: As a result of dot blot analysis using pooled brain homogenates, no difference in the amount of physiological saline-soluble A11-positive oligomer was observed among the three groups tested. Hippocampus (left panel) and cerebral cortex (right panel). PBS-administered group, n=10; 1A9-administered group, n=13; and 2C3-administered group, n=11. E: According to immunoblot analysis using the anti-oligomer A11 antibody, the immunoreactivity of the Aβ tetramer in the SDS-extracted cerebral cortex fraction (right panel) was decreased in the 1A9- and 2C3-administered groups compared to the PBS-administered group. On the other hand, this was not observed in the hippocampus (left panel). F: Blood (albumin-depleted plasma, upper part of Panel F; albumin/lipoprotein-depleted plasma, lower part of Panel F) was pooled from each of the groups, and subjected to A11 dot blot analysis. As a result, the A11 immunoreactivity was found to be increased in the 1A9- and 2C3-administered groups compared to the PBS-administered group (Panel F). The proportion of the lipoprotein-bound form of 2C3-recognized oligomers was higher than that of 1A9-recognized oligomers (lower part of Panel F). Furthermore, the A11 immunoblotting also showed positive signals at approximately 200 kDa, and the immunoreactivity was clearly increased in the 1A9- and 2C3-administered groups compared to the PBS-administered group (Panel G). From these results, it is conceivable that the therapeutic effect selective only to target Aβ oligomer molecules was obtained in the antibody-administered groups without affecting physiological molecules.

[0024] FIG. **11** presents photographs and graphs showing that senile plaque amyloid formation (A: Aβ-specific antibody staining; and B: thioflavin-S-positive analysis) and swollen dystrophic neurite formation (C: synaptophysin-positive analysis) were suppressed in the Tg2576 mouse brain by passive immunization treatment.

[0025] FIG. **12** presents photographs showing the suppression of synaptic degeneration by passive immunization treatment with 1A9 and 2C3. Immunostaining of synaptophysin (left panels) and drebrin (right panels) in presynaptic and postsynaptic dot-like peripheral cells. Top: PBS administration; middle: 1A9 administration; and bottom: 2C3 administration.

[0026] FIG. **13** presents photographs showing the brain transfer of the antibodies by passive immunization treatment. The distribution of administered antibodies in the Tg2576 mouse brain is shown. Staining with anti-Aβ antibodies (left panels) and IgG (center panels). 1A9 administration (A), 2C3 administration (B), and PBS administration (C).

[0027] FIG. **14** presents photographs showing, by dot blot analysis, that the monoclonal antibodies 2C3, E12, 1C10 (isotype: IgM), and 4D3 are specific to oligomers (3-96 h), but do not recognize monomers (0 h).

[0028] FIG. **15** presents photographs showing, by dot blot analysis, that the E12 antibody whose class has been changed from IgM to IgG2b by genetic engineering, does not recognize Aβ monomers (0 hr), but is specific to Aβ oligomers (3-96 hr).

[0029] FIG. **16** presents graphs showing the suppressing activity of the E12 antibody whose class has been changed from IgM to IgG2b by genetic engineering against Aβ amyloid fibril formation. The antibodies were added at three different concentrations to a Aβ 1-42 solution (12.5 μM). After incubation at 37° C. for 24 hours, the level of Aβ amyloid fibril formation was measured by the ThT fluorescence intensity method. The horizontal axis indicates the amount of antibody added, and the vertical axis shows the level of amyloid fibril formation by the antibody addition that is relative to the level of amyloid fibril formation without antibody addition as the standard.

DETAILED DESCRIPTION OF THE INVENTION

[0030] The present invention will be described more specifically below.

[0031] As described above, the present inventors succeeded in obtaining antibodies that bind specifically to Aβ oligomers but not to Aβ monomers. That is, the present invention provides antibodies that bind to Aβ oligomers but not to Aβ monomers. The antibodies are preferably isolated or purified.

[0032] The terms "isolated" and "purified" used for substances (antibodies and such) of the present invention indicate that the substances do not substantially include at least one other substance that may be contained in the natural source. Therefore, "isolated antibodies" and "purified antibodies" refer to antibodies that do not substantially include cell materials such as hydrocarbons, lipids, or other contaminant proteins from the cell or tissue source from which the antibodies (proteins) are derived. When the antibodies are chemically synthesized, the terms refer to antibodies that do not substantially include chemical precursor substances or other chemical substances. In a preferred embodiment, the antibodies of the present invention are isolated or purified.

[0033] "Antibodies" refers to glycoproteins that have the same structural characteristics. Antibodies show binding specificity towards specific antigens. Herein, "antigens" refers to proteins that have the ability to bind to the corresponding antibodies, and induce antigen-antibody reactions in vivo.

[0034] Aβ proteins, which are the major constituents of amyloids, are peptides consisting of 40 to 42 amino acids, and are known to be produced from precursor proteins called amyloid precursor proteins (APPs) by the action of proteases. Besides amyloid fibrils collected in ultracentrifuged sediment fractions, the amyloid molecules produced from APPs include oligomeric non-fibrous assemblies in addition to soluble monomers. "Aβ oligomers" of the present invention refer to non-fibrous assemblies. The "Aβ oligomers" of the present invention include, for example, Aβ40 (Aβ 1-40) oligomers and Aβ42 (Aβ 1-42) oligomers. For example, "Aβ42 oligomers" of the present invention are molecules showing a molecular weight of 45 to 160 kDa in SDS-PAGE, and 22.5 to 1,035 kDa in Blue Native PAGE. Using molecular sieves, the molecules are collected mainly in the >100 kDa retention solution. When observed under an atomic force microscope, the molecules show mixed morphologies of granular, bead-shaped, and ring-shaped molecules having a height of 1.5 to 3.1 nm. By the gel filtration method, the molecules can be eluted in the void volume fraction 8 with a molecular weight of 680 kDa or more, and in fraction 15 with a molecular weight of 17 to 44 kDa.

[0035] There is no limitation on the origin and form of the antibodies used in the present invention as long as they bind to Aβ oligomers but not to Aβ monomers.

[0036] "Antibodies" of the present invention include both monoclonal and polyclonal antibodies. The antibodies of the present invention also include any type of antibodies such as non-human animal antibodies, humanized antibodies, chimeric antibodies, human antibodies, the later-described minibodies, amino acid sequence-modified antibodies, modified antibodies conjugated to other molecules (for example, polymers such as polyethylene glycol), and sugar chain-modified antibodies.

[0037] Herein, the term "monoclonal antibodies" refers to antibodies that are obtained from a substantially homogeneous population of antibodies. That is, the individual antibodies constituting the population are identical with the exception of possible natural mutants that may be present in a trace amount. Monoclonal antibodies are highly specific and recognize a single antigenic site. Each of the monoclonal antibodies recognizes a single determinant of the antigen, in contrast to conventional (polyclonal) antibody preparations that typically contain different antibodies against different antigenic determinants (epitopes).

[0038] In addition to the above-mentioned specificity, monoclonal antibodies have the advantage that they can be synthesized from a hybridoma culture that is not contaminated with other immunoglobulins. Therefore, "monoclonal" indicates the characteristics of antibodies that can be obtained from a substantially homogeneous antibody population. This term does not indicate the requirement for any specific method for antibody production.

[0039] Basically, monoclonal antibodies can be produced by using known techniques. For example, they may be produced by the hybridoma method first described by Kohler and Milstein (Nature 256: 495-7, 1975), or by the recombinant DNA method (Cabilly et al., Proc. Natl. Acad. Sci. USA 81:3273-7, 1984), but the methods are not limited thereto. For example, when using the hybridoma method, an Aβ oligomer (for example, the Aβ tetramer described in the Examples) is used as a sensitizing antigen, and immunization is carried out according to a conventional immunization method. The obtained immune cells are fused with known parent cells by a conventional cell fusion method, and monoclonal antibody-producing cells can be screened and isolated using a conventional screening method.

[0040] The monoclonal antibodies of the present invention can be produced as follows. First, synthetic Aβ 1-42 (Peptide Institute, Inc., Osaka) is dissolved in distilled deionized water or a 10 mM phosphate buffer solution, and this is incubated at 37° C. for 18 hours. Then, the peptides are separated by 4-12% SDS-PAGE, and visualized by CBB staining, and the portion of the Aβ 1-42 tetramer alone which is not contaminated with the Aβ 1-42 monomer is cut out. Next, BALB/c mice are immunized at their foot pad with 2.5 μg of the Aβ 1-42 tetramer emulsified using complete Freund's adjuvant. Subsequently, booster immunizations are carried out six times. Hybridomas are produced from the inguinal lymph node by fusion with Sp2/O-Ag14 cells using Polyethylene Glycol 1500.

[0041] In the present invention, the animals immunized with sensitizing antigens are not particularly limited, but are preferably selected considering the compatibility with parent cells used for cell fusion. Generally, rodents, lagomorphs, or primates are used. Rodents include, for example, mice, rats, and hamsters. Lagomorphs include, for example, rabbits. Primates include, for example, Catarrhini (old-world) monkeys such as *Macaca fascicularis, Macaca mulatta*, hamadryas, and chimpanzees.

[0042] Animals are immunized with sensitizing antigens according to known methods. For example, as a standard method, immunization is performed by intraperitoneal or subcutaneous injection of a sensitizing antigen into mammals.

[0043] An example of the parent cells fused with the aforementioned immunocytes is the Sp2/O-Ag14 cell, which will be described below in the Examples. However, various other known cell lines can be used.

[0044] Cell fusion between the aforementioned immunocyte and a myeloma cell can be carried out basically according to known methods including the method by Kohler and Milstein (Kohler G. and Milstein C., Methods Enzymol. (1981) 73, 3-46).

[0045] Hybridomas obtained in this manner are selected by culturing them in a conventional selection culture medium such as a HAT culture medium, which contains hypoxanthine, aminopterin, and thymidine. Culturing in the above-mentioned HAT culture medium is generally continued for several days to several weeks for an adequate time for killing cells other than the desired hybridomas (non-fused cells). Next, a conventional limiting dilution method is performed for screening and singly-cloning of a hybridoma that produces the desired antibody.

[0046] Thereafter, the obtained hybridoma is transplanted into the abdominal cavity of a mouse, and ascitic fluid containing the desired monoclonal antibodies is extracted. For example, the antibodies can be purified from the ascitic fluid by conventional protein separation and/or purification methods such as a selected combination of column chromatography including, but not limited to, affinity chromatography, filtration, ultrafiltration, salt precipitation, dialysis, SDS polyacrylamide gel electrophoresis, and isoelectric focusing (Antibodies: A Laboratory manual, Harlow and David, Lane (edit.), Cold Spring Harbor Laboratory, 1988).

[0047] Protein A columns and Protein G columns can be used for affinity columns. Examples of the Protein A columns used include Hyper D, POROS, and Sepharose F.F. (Pharmacia).

[0048] Chromatography (excluding affinity chromatography) includes ion exchange chromatography, hydrophobic chromatography, gel filtration, reverse-phase chromatography, and adsorption chromatography ("Strategies for Protein Purification and Characterization: A Laboratory Course Manual", Daniel R Marshak et al., Cold Spring Harbor Laboratory Press, 1996). When chromatography is carried out, liquid-phase chromatography methods such as HPLC and FPLC can be used.

[0049] Monoclonal antibody-producing hybridomas prepared in this manner can be subcultured in a conventional culture medium, and they can be stored for a long time in liquid nitrogen.

[0050] Any mammal can be immunized using an immunogen for antibody production. However, when preparing monoclonal antibodies by producing hybridomas, the compatibility with parent cells used in cell fusion for hybridoma production is preferably considered.

[0051] Generally, rodents, lagomorphs, or primates are used for the immunization. Rodents include, for example, mice, rats, and hamsters. Lagomorphs include, for example, rabbits. Primates include, for example, Catarrhini (old-world) monkeys such as *Macaca fascicularis, Macaca mulatta*, hamadryas, and chimpanzees.

[0052] The use of transgenic animals that have a human antibody gene repertoire is known in the art (Ishida I, et al., Cloning and Stem Cells 4: 91-102, 2002). As with other animals, to obtain human monoclonal antibodies, the transgenic animals are immunized, then antibody-producing cells are collected from the animals and fused with myeloma cells to produce hybridomas, and anti-protein human antibodies can be prepared from these hybridomas (see International Publication Nos. WO92/03918, WO94/02602, WO94/25585, WO96/33735, and WO96/34096).

[0053] Alternatively, lymphocytes immortalized with oncogenes may be used for monoclonal antibody production. For example, human lymphocytes infected with EB virus or such is immunized in vitro with immunogens. Next, the immunized lymphocytes are fused with human-derived myeloma cells (U266, etc) capable of unlimited division, and thus hybridomas that produce the desired human antibodies are obtained (Japanese Patent Application Kokai Publication No. (JP-A) S63-17688 (unexamined, published Japanese patent application)).

[0054] Once monoclonal antibodies can be obtained by any of the aforementioned methods, the antibodies may also be prepared using genetic engineering methods (see, for example, Borrebaeck C A K and Larrick J W, Therapeutic Monoclonal Antibodies, MacMillan Publishers, UK, 1990). For example, recombinant antibodies may be prepared by cloning DNAs that encode the desired antibodies from antigen-producing cells such as hybridomas or immunized lymphocytes that produce the antibodies, then inserting the cloned DNAs into appropriate vectors, and transfecting the vectors into suitable host cells. Such recombinant antibodies are also included in the present invention.

[0055] Examples of the monoclonal antibodies of the present invention include the 1A9 monoclonal antibody, 2C3 monoclonal antibody, E12 monoclonal antibody, 1C10 monoclonal antibody, and 4D3 monoclonal antibody. Preferably, the monoclonal antibodies include the antibodies of (i) to (iii) below:

(i) an antibody that comprises an H chain (heavy chain) having the amino acid sequence of SEQ ID NO: 1 and an L chain (light chain) having the amino acid sequence of SEQ ID NO: 3;

(ii) an antibody that comprises an H chain (heavy chain) having the amino acid sequence of SEQ ID NO: 21 and an L chain (light chain) having the amino acid sequence of SEQ ID NO: 23;

(iii) an antibody that comprises an H chain (heavy chain) having the amino acid sequence of SEQ ID NO: 41 and an L chain (light chain) having the amino acid sequence of SEQ ID NO: 43.

[0056] In an embodiment, the antibodies of the present invention include minibodies. A minibody contains an antibody fragment lacking a portion of a whole antibody, and is not particularly limited as long as it has the ability to bind to an antigen. Examples of antibody fragments include Fab, Fab', F(ab')2, and Fv. Examples of minibodies include Fab, Fab', F(ab')2, Fv, scFv (single chain Fv), diabody, and sc(Fv)2 (single chain (Fv)2).

[0057] To obtain polyclonal antibodies against the proteins of the present invention, blood is removed from a mammal sensitized with an antigen after the serum level of the desired antibody is confirmed to be increased. Serum is separated from blood by a known method. When a polyclonal antibody is used, serum containing the polyclonal antibody may be utilized. Alternatively, if necessary, a fraction containing the polyclonal antibody may be isolated from serum and then used. For example, immunoglobulin G or M can be prepared by obtaining a fraction that specifically recognizes a protein of the present invention using an affinity column coupled with the protein, and then purifying this fraction using a Protein A or Protein G column.

[0058] In the present invention, the antibody that binds to an Aβ oligomer is an antibody binding to an Aβ oligomer that binds 1A9, 2C3, E12, 1C10, or 4D3. Preferably, the antibody is any one of the antibodies of (A) to (C) below:

(A) an antibody binding to an Aβ oligomer that binds to an antibody comprising an H (heavy) chain having the amino acid sequence of SEQ ID NO: 1 and an L (light) chain having the amino acid sequence of SEQ ID NO: 3;

(B) an antibody binding to an Aβ oligomer that binds to an antibody comprising an H chain having the amino acid sequence of SEQ ID NO: 21 and an L chain having the amino acid sequence of SEQ ID NO: 23;

(C) an antibody binding to an Aβ oligomer that binds to an antibody comprising an H chain having the amino acid sequence of SEQ ID NO: 41 and an L chain having the amino acid sequence of SEQ ID NO: 43.

[0059] Furthermore, the present invention provides Aβ oligomers to which the antibodies of the present invention bind. Preferably, the antibodies include, for example, the 1A9 monoclonal antibody, 2C3 monoclonal antibody, E12 monoclonal antibody, 1C10 monoclonal antibody, and 4D3 monoclonal antibody. Such Aβ oligomers can be used as antigens for preparing antibodies, or vaccines.

[0060] In a preferred embodiment, the antibodies of the present invention include, for example, the antibody of any one of (1) to (20) below:

(1) an antibody that comprises an H chain having the amino acid sequence of SEQ ID NO: 9 as CDR1, the amino acid sequence of SEQ ID NO: 11 as CDR2, and the amino acid sequence of SEQ ID NO: 13 as CDR3;

(2) an antibody that comprises an L chain having the amino acid sequence of SEQ ID NO: 15 as CDR1, the amino acid sequence of SEQ ID NO: 17 as CDR2, and the amino acid sequence of SEQ ID NO: 19 as CDR3;

(3) an antibody that comprises the H chain of (1) and the L chain of (2);

(4) an antibody that comprises an H chain having the amino acid sequence of SEQ ID NO: 5 as VH;

(5) an antibody that comprises an L chain having the amino acid sequence of SEQ ID NO: 7 as VL;

(6) an antibody that comprises the H chain of (4) and the L chain of (5);

(7) an antibody that comprises an H chain having the amino acid sequence of SEQ ID NO: 29 as CDR1, the amino acid sequence of SEQ ID NO: 31 as CDR2, and the amino acid sequence of SEQ ID NO: 33 as CDR3;

(8) an antibody that comprises an L chain having the amino acid sequence of SEQ ID NO: 35 as CDR1, the amino acid sequence of SEQ ID NO: 37 as CDR2, and the amino acid sequence of SEQ ID NO: 39 as CDR3;

(9) an antibody that comprises the H chain of (7) and the L chain of (8);

(10) an antibody that comprises an H chain having the amino acid sequence of SEQ ID NO: 25 as VH;

(11) an antibody that comprises an L chain having the amino acid sequence of SEQ ID NO: 27 as VL;

(12) an antibody that comprises the H chain of (10) and the L chain of (11);

(13) an antibody that comprises an H chain having the amino acid sequence of SEQ ID NO: 49 as CDR1, the amino acid sequence of SEQ ID NO: 51 as CDR2, and the amino acid sequence of SEQ ID NO: 53 as CDR3;

(14) an antibody that comprises an L chain having the amino acid sequence of SEQ ID NO: 55 as CDR1, the amino acid sequence of SEQ ID NO: 57 as CDR2, and the amino acid sequence of SEQ ID NO: 59 as CDR3;

(15) an antibody that comprises the H chain of (13) and the L chain of (14);

(16) an antibody that comprises an H chain having the amino acid sequence of SEQ ID NO: 45 as VH;

(17) an antibody that comprises an L chain having the amino acid sequence of SEQ ID NO: 47 as VL;

(18) an antibody that comprises the H chain of (16) and the L chain of (17);

(19) an antibody that comprises one or more amino acid substitutions, deletions, additions, and/or insertions in the antibody of any one of (1) to (18), which has equivalent activity as the antibody of any one of (1) to (18); and

(20) an antibody that binds to the epitope bound by the antibody of any one of (1) to (18).

[0061] An example of the VH in the above-mentioned "H chain having the amino acid sequence of SEQ ID NO: 9 (sequence of the E12 antibody H-chain CDR1) as CDR1, the amino acid sequence of SEQ ID NO: 11 (sequence of the E12 antibody H-chain CDR2) as CDR2, and the amino acid sequence of SEQ ID NO: 13 (sequence of the E12 antibody H-chain CDR3) as CDR3" of (1) is a VH having the amino acid sequence of SEQ ID NO: 5 (sequence of the E12 antibody VH).

[0062] An example of the VL in the above-mentioned "L chain having the amino acid sequence of SEQ ID NO: 15 (sequence of the E12 antibody L-chain CDR1) as CDR1, the amino acid sequence of SEQ ID NO: 17 (sequence of the E12 antibody L-chain CDR2) as CDR2, and the amino acid sequence of SEQ ID NO: 19 (sequence of the E12 antibody L-chain CDR3) as CDR3" of (2) is a VL having the amino acid sequence of SEQ ID NO: 7 (sequence of the E12 antibody VL).

[0063] An example of the VH in the above-mentioned "H chain having the amino acid sequence of SEQ ID NO: 29 (sequence of the 1C10 antibody H-chain CDR1) as CDR1, the amino acid sequence of SEQ ID NO: 31 (sequence of the 1C10 antibody H-chain CDR2) as CDR2, and the amino acid sequence of SEQ ID NO: 33 (sequence of the 1C10 antibody H-chain CDR3) as CDR3" of (7) is a VH having the amino acid sequence of SEQ ID NO: 25 (sequence of the 1C10 antibody VH).

[0064] An example of the VL in the above-mentioned "L chain having the amino acid sequence of SEQ ID NO: 35 (sequence of the 1C10 antibody L-chain CDR1) as CDR1, the amino acid sequence of SEQ ID NO: 37 (sequence of the 1C10 antibody L-chain CDR2) as CDR2, and the amino acid sequence of SEQ ID NO: 39 (sequence of the 1C10 antibody L-chain CDR3) as CDR3" of (8) is a VL having the amino acid sequence of SEQ ID NO: 27 (sequence of the 1C10 antibody VL).

[0065] An example of the VH in the above-mentioned "H chain having the amino acid sequence of SEQ ID NO: 49 (sequence of the 4D3 antibody H-chain CDR1) as CDR1, the amino acid sequence of SEQ ID NO: 51 (sequence of the 4D3 antibody H-chain CDR2) as CDR2, and the amino acid sequence of SEQ ID NO: 53 (sequence of the 4D3 antibody H-chain CDR3) as CDR3" of (13) is a VH having the amino acid sequence of SEQ ID NO: 45 (sequence of the 4D3 antibody VH).

[0066] An example of the VL in the above-mentioned "L chain having the amino acid sequence of SEQ ID NO: 55 (sequence of the 4D3 antibody L-chain CDR1) as CDR1, the amino acid sequence of SEQ ID NO: 57 (sequence of the 4D3 antibody L-chain CDR2) as CDR2, and the amino acid sequence of SEQ ID NO: 59 (sequence of the 4D3 antibody L-chain CDR3) as CDR3" of (14) is a VL having the amino acid sequence of SEQ ID NO: 47 (sequence of the 4D3 antibody VL).

[0067] For the E12 antibody of the present invention, the amino acid sequence and the nucleotide sequence of the full-length H chain are shown in SEQ ID NO: 1 and SEQ ID NO: 2, respectively; the amino acid sequence and the nucleotide sequence of the full-length L chain are shown in SEQ ID NO: 3 and SEQ ID NO: 4, respectively; the amino acid sequence and the nucleotide sequence of the H-chain variable region (VH) are shown in SEQ ID NO: 5 and SEQ ID NO: 6, respectively; the amino acid sequence and the nucleotide sequence of the L-chain variable region (VL) are shown in SEQ ID NO: 7 and SEQ ID NO: 8, respectively; the amino acid sequence and the nucleotide sequence of the H-chain CDR1 are shown in SEQ ID NO: 9 and SEQ ID NO: 10, respectively; the amino acid sequence and the nucleotide sequence of the H-chain CDR2 are shown in SEQ ID NO: 11 and SEQ ID NO: 12, respectively; the amino acid sequence and the nucleotide sequence of the H-chain CDR3 are shown in SEQ ID NO: 13 and SEQ ID NO: 14, respectively; the amino acid sequence and the nucleotide sequence of the L-chain CDR1 are shown in SEQ ID NO: 15 and SEQ ID NO: 16, respectively; the amino acid sequence and the nucleotide sequence of the L-chain CDR2 are shown in SEQ ID NO: 17 and SEQ ID NO: 18, respectively; and the amino acid sequence and the nucleotide sequence of the L-chain CDR3 are shown in SEQ ID NO: 19 and SEQ ID NO: 20, respectively.

[0068] For the 1C10 antibody of the present invention, the amino acid sequence and the nucleotide sequence of the full-length H chain are shown in SEQ ID NO: 21 and SEQ ID NO: 22, respectively; the amino acid sequence and the nucleotide sequence of the full-length L chain are shown in SEQ ID NO: 23 and SEQ ID NO: 24, respectively; the amino acid sequence and the nucleotide sequence of the H-chain variable region (VH) are shown in SEQ ID NO: 25 and SEQ ID NO: 26, respectively; the amino acid sequence and the nucleotide sequence of the L-chain variable region (VL) are shown in SEQ ID NO: 27 and SEQ ID NO: 28, respectively; the amino acid sequence and the nucleotide sequence of the H-chain CDR1 are shown in SEQ ID NO: 29 and SEQ ID NO: 30, respectively; the amino acid sequence and the nucleotide sequence of the H-chain CDR2 are shown in SEQ ID NO: 31 and SEQ ID NO: 32, respectively; the amino acid sequence and the nucleotide sequence of the H-chain CDR3 are shown in SEQ ID NO: 33 and SEQ ID NO: 34, respectively; the amino acid sequence and the nucleotide sequence of the L-chain CDR1 are shown in SEQ ID NO: 35 and SEQ ID NO: 36, respectively; the amino acid sequence and the nucleotide sequence of the L-chain CDR2 are shown in SEQ ID NO: 37 and SEQ ID NO: 38, respectively; and the amino acid sequence and the nucleotide sequence of the L-chain CDR3 are shown in SEQ ID NO: 39 and SEQ ID NO: 40, respectively.

[0069] For the 4D3 antibody of the present invention, the amino acid sequence and the nucleotide sequence of the full-length H chain are shown in SEQ ID NO: 41 and SEQ ID NO: 42, respectively; the amino acid sequence and the nucleotide sequence of the full-length L chain are shown in SEQ ID NO: 43 and SEQ ID NO: 44, respectively; the amino acid sequence and the nucleotide sequence of the H-chain variable region (VH) are shown in SEQ ID NO: 45 and SEQ ID NO: 46, respectively; the amino acid sequence and the nucleotide sequence of the L-chain variable region (VL) are shown in SEQ ID NO: 47 and SEQ ID NO: 48, respectively; the amino acid sequence and the nucleotide sequence of the H-chain CDR1 are shown in SEQ ID NO: 49 and SEQ ID NO: 50, respectively; the amino acid sequence and the nucleotide sequence of the H-chain CDR2 are shown in SEQ ID NO: 51 and SEQ ID NO: 52, respectively; the amino acid sequence and the nucleotide sequence of the H-chain CDR3 are shown in SEQ ID NO: 53 and SEQ ID NO: 54, respectively; the amino acid sequence and the nucleotide sequence of the L-chain CDR1 are shown in SEQ ID NO: 55 and SEQ ID NO: 56, respectively; the amino acid sequence and the nucleotide sequence of the L-chain CDR2 are shown in SEQ ID NO: 57 and SEQ ID NO: 58, respectively; and the amino acid sequence and the nucleotide sequence of the L-chain CDR3 are shown in SEQ ID NO: 59 and SEQ ID NO: 60, respectively.

[0070] The above-mentioned antibodies of (1) to (20) include not only monovalent antibodies but also multivalent antibodies with two or more valencies. The multivalent antibodies of the present invention include multivalent antibodies whose antigen binding sites are all the same and multivalent antibodies whose antigen binding sites are partially or completely different.

[0071] In a preferred embodiment, the above-mentioned antibody of (19) is an antibody with no modified CDRs. For example, the "antibody that comprises one or more amino acid substitutions, deletions, additions, and/or insertions in the antibody of (1), which has equivalent activity as the antibody of (1)" of the above-mentioned antibody of (19) is preferably "an antibody that has equivalent activity as the antibody of (1), and comprises one or more amino acid substitutions, deletions, additions, and/or insertions in the antibody of (1), and comprises an H chain having the amino acid sequence of SEQ ID NO: 9 as CDR1, the amino acid sequence of SEQ ID NO: 11 as CDR2, and the amino acid sequence of SEQ ID NO: 13 as CDR3". Another preferred antibody of the above-mentioned antibody of (19) can be expressed in a similar manner.

[0072] Herein, "equivalent activity" means that the antibody of interest has biological or biochemical activity similar to that of an antibody of the present invention. Examples of the "activity" of the present invention include the activity to bind specifically to Aβ oligomers but not to Aβ monomers, anti-neurotoxic activity, activity to suppress Aβ amyloid fibril formation, anti-synaptic toxicity activity, and anti-memory impairment activity.

[0073] Methods for preparing a polypeptide having activity equivalent to that of a certain polypeptide that are well known to those skilled in the art include methods for introducing mutations into a polypeptide. For example, one skilled in the art can prepare an antibody having activity equivalent to that of an antibody of the present invention by introducing appropriate mutations into the antibody using site-directed mutagenesis (Hashimoto-Gotoh, T. et al. (1995) Gene 152, 271-275; Zoller, M J, and Smith, M. (1983) Methods Enzymol. 100, 468-500; Kramer, W. et al. (1984) Nucleic Acids Res. 12, 9441-9456; Kramer W, and Fritz H J (1987) Methods. Enzymol. 154, 350-367; Kunkel, T A (1985) Proc. Natl. Acad. Sci. USA. 82, 488-492; Kunkel (1988) Methods Enzymol. 85, 2763-2766) and such. Amino acid mutations may also occur naturally. The antibodies of the present invention also include an antibody that comprises an amino acid sequence with one or more amino acid mutations in the amino acid sequence of an antibody of the present invention, and which has activity equivalent to that of the antibody of the present invention. The number of mutated amino acids in such mutants may be generally 50 amino acids or less, preferably 30 amino acids or less, and more preferably ten amino acids or less (for example, five amino acids or less).

[0074] Amino acid residues are preferably mutated into other amino acids that conserve the properties of the amino acid side chains. For example, amino acids are categorized as follows depending on the side chain properties: hydrophobic amino acids (A, I, L, M, F, P, W, Y, and V), hydrophilic amino acids (R, D, N, C, E, Q, G, H, K, S, and T), amino acids with aliphatic side chains (G, A, V, L, I, and P), amino acids with hydroxyl-containing side chains (S, T, and Y), amino acids with sulfur atom-containing side chains (C and M), amino acids with carboxylic acid- and amide-containing side chains (D, N, E, and Q), amino acids with basic side chains (R, K, and H), and amino acids with aromatic ring-containing side chains (H, F, Y, and W) (amino acids are represented by one-letter codes in parentheses).

[0075] A polypeptide having an amino acid sequence, in which one or more amino acid residues are modified (deleted, added, and/or substituted with other amino acids) in a certain amino acid sequence, is known to retain its original biological activity (function).

[0076] In addition to the above-mentioned modifications, the antibodies of the present invention may be conjugated to other substances as long as the activity is maintained. Examples of the substances include peptides, lipids, sugars and sugar chains, acetyl groups, and natural and synthetic polymers. These modifications may be performed to confer additional functions to the antibodies, or to stabilize the antibodies.

[0077] Antibodies in which several amino acid residues have been added to the amino acid sequence of an antibody of the present invention include fusion proteins containing the antibody. In the fusion proteins, the antibody is fused with another peptide or protein. Methods for producing a fusion protein can be carried out by ligating a polynucleotide encoding an antibody of the present invention in frame with a polynucleotide encoding another peptide or polypeptide, and inserting this into an expression vector, and expressing the fusion construct in a host. Techniques known to those skilled in the art can be used for this purpose. The peptides or polypeptides fused with an antibody of the present invention include, for example, known peptides such as FLAG (Hopp, T. P. et al., BioTechnology (1988) 6, 1204-1210), 6× His consisting of six histidine (His) residues, 10× His, Influenza hemagglutinin (HA), human c-myc fragments, VSV-GP fragments, p18HIV fragments, T7-tag, HSV-tag, E-tag, SV40T antigen fragments, lck tag, α-tubulin fragments, B-tag, and Protein C fragments; glutathione-S-transferase (GST); immunoglobulin constant regions; β-galactosidase; and maltose-binding protein (MBP), etc. Commercially available polynucleotides encoding these peptides or polypeptides can be fused with polynucleotides encoding the antibodies of the present invention, and the fusion polypeptides can be produced by expressing the fusion polynucleotides thus prepared.

[0078] The antibodies of the present invention may differ in the amino acid sequence, molecular weight, presence or absence of sugar chains, structure and such, depending on the cell or host producing the antibodies or the purification method. However, as long as the obtained antibody has an activity equivalent to an antibody of the present invention, it is included in the present invention.

[0079] Antibodies that bind to an epitope to which an antibody of any one of (1) to (18) above binds can be obtained by methods known to those skilled in the art. For example, the antibodies can be obtained by (i) determining the epitope bound by the antibody of any one of (1) to (18) using a conventional method, and producing the antibodies using a polypeptide comprising an amino acid sequence included in the epitope as an immunogen; or (ii) determining the epitopes of antibodies produced by a conventional method, and selecting antibodies whose epitope is the same as that of the antibody of any one of (1) to (18).

[0080] The above-mentioned antibodies of (1) to (20) also include any type of antibodies such as the above-described minibodies, antibodies with modified amino acid sequences such as humanized antibodies and chimeric antibodies, non-human animal antibodies, human antibodies, modified antibodies conjugated to other molecules (for example, polymers such as polyethylene glycol), and sugar chain-modified antibodies.

[0081] In a preferred embodiment, the antibodies of the present invention are modified antibodies such as chimeric antibodies and humanized antibodies. Examples of preferred antibodies include (i) a chimeric antibody whose variable region is derived from the E12 antibody, 1C10 antibody, or 4D3 antibody, and whose constant region is derived from a human immunoglobulin; and (ii) a humanized antibody whose CDR is derived from the E12 antibody, 1C10 antibody, or 4D3 antibody, and whose FR is derived from a human immunoglobulin, and whose constant region is derived from a human immunoglobulin. These modified antibodies can be produced using known methods.

[0082] Since the antigenicity of a chimeric antibody or a humanized antibody in the human body is reduced, such an antibody is useful for administration to humans for therapeutic purposes or such.

[0083] Chimeric antibodies are produced by combining sequences derived from different animals. Examples of chimeric antibodies include antibodies comprising the heavy-chain and light-chain variable regions of a mouse antibody and the heavy-chain and light-chain constant regions of a human antibody. The production of chimeric antibodies can be carried out using known methods (see, for example, Jones et al., Nature 321:522-5, 1986; Riechmann et al., Nature 332:323-7, 1988; and Presta, Curr. Opin. Struct. Biol. 2:593-6, 1992). For example, first, genes encoding the variable regions or CDRs of the antibody of interest are prepared from the RNAs of antibody-producing cells by polymerase chain reaction (PCR) or such (see, for example, Larrick et al., "Methods: a Companion to Methods in Enzymology", Vol. 2: 106, 1991; Courtenay-Luck, "Genetic Manipulation of Monoclonal Antibodies" in Monoclonal Antibodies: Production, Engineering and Clinical Application; Ritter et al. (eds.), page 166, Cambridge University Press, 1995, and Ward et al., "Genetic Manipulation and Expression of Antibodies" in Monoclonal Antibodies: Principles and Applications; and Birch et al. (eds.), page 137, Wiley-Liss, Inc., 1995). The prepared genes encoding the variable regions are linked to genes encoding the constant regions or framework regions. The genes encoding the constant regions or framework regions may be determined in a manner similar to that for the CDR-encoding genes, or alternatively, they can be prepared based on the sequence information of known antibodies. DNA sequences encoding chimeric products and CDR-grafted products may be synthesized completely or partially using oligonucleotide synthesis techniques. For example, the oligonucleotide synthesis described by Jones et al. (Nature 321:522-5, 1986) may be performed. Furthermore, in some cases, site-directed mutagenesis and polymerase chain reaction techniques may be appropriately used. Techniques for oligonucleotide-specific mutagenesis of known variable regions described by Verhoeyen et al. (Science 239: 1534-6, 1988) and Riechmann et al. (Nature 332: 323-7, 1988) may be used for modifying the variable region sequences, for example, to enhance the binding ability of chimeric antibodies. Furthermore, if necessary, enzymatic fill-in of gapped oligonucleotides using T4 DNA polymerase may be performed, for example, as described by Queen et al. (Proc. Natl. Acad. Sci. USA 86: 10029-33, 1989; and WO 90/07861).

[0084] For example, CDR-grafting techniques are known in the art ("Immunoglobulin genes", Academic Press (London), pp 260-74, 1989; and Michael A et al., Proc. Natl. Acad. Sci. USA 91: 969-73, 1994). Using the techniques, the CDRs of a certain antibody are replaced with the CDRs of another antibody. Through such replacement, the binding specificity of the former antibody is changed to that of the latter antibody. Among such chimeric antibodies, those in which the framework amino acids are derived from a human antibody are called "humanized antibodies (CDR-grafted antibodies)". When using antibodies to treat humans, human antibodies or humanized antibodies are preferably utilized.

[0085] Generally, chimeric antibodies comprise the variable regions of a non-human mammal-derived antibody and the constant regions derived from a human antibody. On the other hand, humanized antibodies comprise the complementarity-determining regions (CDR) of a non-human mammal-derived antibody and the framework regions and constant regions derived from a human antibody.

[0086] After producing the chimeric antibodies or humanized antibodies, amino acids in the variable regions (for example, FRs) or the constant regions may be substituted with other amino acids.

[0087] The origin of the variable regions of the chimeric antibodies or the CDRs of the humanized antibodies is not particularly limited.

[0088] Human antibody-derived C-regions are used for the C-regions of the chimeric antibodies and humanized antibodies. For example, $C\gamma1$, $C\gamma2$, $C\gamma3$, $C\gamma4$, $C\gamma\mu$, $C\delta$, $C\alpha1$, $C\alpha2$, and $C\epsilon$ can be used for the H-chain C-regions, and $C\kappa$ and $C\lambda$ can be used for the L-chain C-regions. Their sequences are known. Furthermore, the human antibody C regions can be modified to improve the stability of the antibodies or their production.

[0089] The binding activity of the antibodies of the present invention to the antigens (Aβ oligomers) can be measured using, for example, an absorbance measurement method, an enzyme-linked immunosorbent assay (ELISA) method, an enzyme immunoassay (EIA) method, a radioimmunoassay (RIA) method, and/or a fluoroimmunoassay method. In ELISA, an antibody is immobilized on a plate, and an antigen for the antibody is added to the plate, then a sample containing the desired antibody, such as the culture supernatant of antibody-producing cells or a purified antibody is added. Next, a secondary antibody which recognizes the primary antibody and is tagged with an enzyme such as alkaline phosphatase is added to the plate, and this is preincubated, After washing, an enzyme substrate such as p-nitrophenyl phosphate is added to the plate, and the absorbance is measured to evaluate the antigen-binding ability of the sample of interest. The evaluation can be performed using BIAcore (Pharmacia).

[0090] Furthermore, the present invention provides compositions comprising the above-mentioned antibody of the present invention and a pharmaceutically acceptable carrier.

[0091] As described below, the present invention strongly suggests that monoclonal 1A9 and 2C3 antibody are promising candidates for therapeutic antibodies for preventing Alzheimer-like phenotypes. Memory deterioration has been shown to be related to synaptic dysfunction caused by soluble Aβ oligomers (Klein W L, 2001, Trends Neurosci; and Selkoe D J, 2002, Science). Excessive accumulation and deposition of Aβ oligomers may trigger the complicated downstream cascades that cause Alzheimer's disease. If this is the case, therapeutic intervention using a composition comprising an antibody of the present invention and a pharmaceutically acceptable carrier could be effective for blocking the pathologic cascades, and thus this could enable the treatment of Alzheimer's disease.

[0092] The "treatment" of the present invention does not necessarily have complete therapeutic or preventive effects against organs or tissues exhibiting symptoms of disorders or diseases, but may have partial effects.

[0093] "Treatment of Alzheimer's disease" in the present invention means amelioration of at least one symptom that may be caused by Alzheimer's disease, and examples include amelioration or suppression of cognitive impairment, amelioration or suppression of senile plaque formation, amelioration or suppression of synaptic dysfunction, and reduction or suppression of Aβ accumulation in brain tissues, blood, or such. Herein, "cognitive impairment" includes, for example, memory impairment including long term/short term memory impairment, object recognition memory impairment, spatial memory impairment, and associative and emotional memory impairment.

[0094] The present invention provides pharmaceutical compositions or pharmaceutical agents which comprise as an active ingredient the above-described composition comprising an antibody of the present invention and a pharmaceutically acceptable carrier.

[0095] In the present invention, the phrase "comprising as an active ingredient the above-described composition comprising an antibody of the present invention and a pharmaceutically acceptable carrier" means comprising the above-described composition comprising an antibody of the present invention and a pharmaceutically acceptable carrier as a major ingredient, but does not limit its content rate.

[0096] Examples of the above-mentioned pharmaceutical compositions include agents against cognitive impairment, Alzheimer's disease therapeutic agents, agents for suppressing the progression of Alzheimer's disease, agents for suppressing senile plaque formation, agents for suppressing Aβ accumulation, anti-neurotoxic agents (agents for neutralizing neurotoxicity), agents for inhibiting Aβ amyloid fibril formation, and anti-synaptic toxicity agents (agents for neutralizing synaptic toxicity).

[0097] The above-mentioned pharmaceutical composition of the present invention can be expressed, for example, as "methods for suppressing Alzheimer's disease" which comprise the step of administering to a subject (individual) the above-described composition comprising an effective amount of an antibody of the present invention and a pharmaceutically acceptable carrier. In other embodiments, examples include methods for suppressing cognitive impairment, methods for suppressing the progression of Alzheimer's disease, methods for suppressing senile plaque formation, methods for suppressing Aβ accumulation, methods for neutralizing (suppressing) neurotoxic activity, methods for inhibiting Aβ amyloid fibril formation, and methods for neutralizing (suppressing) synaptic toxicity. In further embodiments, examples include methods for preventing and/or treating cognitive impairment, and methods for preventing and/or treating Alzheimer's disease.

[0098] The present invention also provides use of a composition comprising the above-described antibody of the present invention and a pharmaceutically acceptable carrier in the production of the above-mentioned pharmaceutical composition.

[0099] Furthermore, the present invention relates to the following compositions.

[0100] A composition comprising the above-described antibody of the present invention and a pharmaceutically acceptable carrier for use in preventing and/or treating cognitive impairment.

[0101] A composition comprising the above-described antibody of the present invention and a pharmaceutically acceptable carrier for use in preventing and/or treating Alzheimer's disease.

[0102] A composition comprising the above-described antibody of the present invention and a pharmaceutically acceptable carrier for use in suppressing the progression of Alzheimer's disease.

[0103] A composition comprising the above-described antibody of the present invention and a pharmaceutically acceptable carrier for use in suppressing senile plaque formation.

[0104] A composition comprising the above-described antibody of the present invention and a pharmaceutically acceptable carrier for use in suppressing Aβ accumulation.

[0105] A composition comprising the above-described antibody of the present invention and a pharmaceutically acceptable carrier for use in neutralizing (suppressing) neurotoxic activity.

[0106] A composition comprising the above-described antibody of the present invention and a pharmaceutically acceptable carrier for use in inhibiting Aβ amyloid fibril formation.

[0107] A composition comprising the above-described antibody of the present invention and a pharmaceutically acceptable carrier for use in neutralizing (suppressing) synaptic toxicity

[0108] The above-mentioned pharmaceutical agents of the present invention can be administered to humans or other animals. In the present invention, non-human animals to which the pharmaceutical agents are administered include mice, rats, guinea pigs, rabbits, chickens, cats, dogs, sheep, pigs, cattle, monkeys, baboons, and chimpanzees. These animals preferably exhibit at least one symptom selected from, for example, cognitive impairment, senile plaque formation, synaptic dysfunction, Aβ accumulation in brain tissues or blood, etc.

[0109] Antibodies contained in the pharmaceutical compositions of the present invention are not particularly limited as long as they are included in the above-mentioned antibodies of the present invention, and examples include the antibodies described herein.

[0110] When using the above-mentioned antibodies of the present invention for pharmaceutical compositions, they may be formulated by methods known to those skilled in the art. For example, as necessary, they can be prepared in the form of injectable sterile solutions or suspensions using water or another pharmaceutically acceptable liquid, and can be administered parenterally. For example, the antibodies to be included in the pharmaceutical compositions can be combined with acceptable carriers or media, specifically, sterile water, physiological saline, vegetable oils, emulsifiers, suspensions, surfactants, stabilizers, flavoring agents, excipients, solvents, preservatives, binders, or such, and mixed into a unit dose form required for generally accepted pharmaceutical practice. The phrase "pharmaceutically acceptable" indicates that the substance is inactive, and contains conventional substances used as diluents or vehicles for pharmaceuticals. Suitable excipients and their formulations are described, for example, in Remington's Pharmaceutical Sciences, 16^{th} ed. (1980) Mack Publishing Co., ed. Oslo et al.

[0111] Physiological saline and other isotonic solutions containing glucose or adjuvants (for example, D-sorbitol, D-mannose, D-mannitol, and sodium chloride) can be used as aqueous solutions for injection. They can be used together with appropriate solubilizers such as alcohols, more specifically, ethanol and polyalcohols (propylene glycol, polyethylene glycol, and such), and non-ionic surfactants (Polysorbate 80™, HCO-50, and such).

[0112] Sesame oil or soybean oil can be used as an oleaginous liquid, and benzyl benzoate or benzyl alcohol can be used in combination as a solubilizer. Buffers (for example, phosphate buffer and sodium acetate buffer), soothing agents (for example, procaine hydrochloride), stabilizers (for example, benzyl alcohol and phenol), and antioxidants can be used for the formulations. Prepared injection solutions can be filled into appropriate ampules.

[0113] The administration is preferably parenteral administration, and specific examples include administration by injection, transnasal administration, transpulmonary administration, and transdermal administration. Examples of administration by injection include systemic and local administration by intravenous injection, intramuscular injection, intraperitoneal injection, subcutaneous injection, and such.

[0114] The pharmaceutical compositions contain a pharmaceutically effective amount of the active component (the above-mentioned antibody of the present invention). "Pharmaceutically effective amount (of a compound)" refers to an amount sufficient for treating and/or preventing disorders in which the antigens for the above-mentioned antibodies of the present invention play an important role. For example, "a pharmaceutically acceptable amount" may be an amount required for reducing Aβ accumulation, neutralizing Aβ-induced toxicity, reducing Aβ fibril formation, or such, thereby treating or preventing conditions caused by Alzheimer's disease, when the compound is administered to individuals (patients). The reduction or neutralization may be, for example, a reduction or neutralization of at least approximately 5%, 10%, 20%, 30%, 40%, 50%, 75%, 80%, 90%, 95%, 99%, or 100%.

[0115] Assessment for determining such a pharmaceutically effective amount of the above-mentioned antibodies of the present invention may be carried out using a standard clinical protocol including histopathological diagnosis.

[0116] A suitable administration method may be selected depending on the age and symptoms of the patient. The dosage of an antibody-containing pharmaceutical composition may be selected, for example, within the range of 0.0001 mg to 1000 mg per kilogram body weight for each administration. Alternatively, for example, the dosage for each patient may be selected within the range of 0.001 to 100,000 mg/body; however, the dosage is not necessarily limited to these ranges. Although the dosage and administration methods vary depending on the patient's body weight, age, symptoms, and such, one skilled in the art can appropriately select them. In the later-described animal experiments, the dosage was selected based on the high-dose intravenous immunoglobulin therapy (400 mg/kg) covered by health insurance for humans.

[0117] Furthermore, the present invention provides methods for detecting Aβ oligomers (examples include Aβ40 (Aβ 1-40) and Aβ42 (Aβ 1-42) oligomers) in samples. Examples of "samples" of the present invention include samples collected from subjects. Specifically, the present methods include the step of detecting Aβ oligomers contained in a sample collected from a subject using an antibody of the present invention. Aβ oligomers in a sample can be detected using, for example, sandwich solid-phase enzyme immunoassay methods that use chemiluminescence (chemiluminescence ELISA), immunoprecipitation methods that use the obtained antibodies, immunoblotting, flow cytometry, mass spectrometry, and immunohistochemical analysis.

[0118] When Aβ oligomers are detected in a sample collected from a subject by the above-mentioned measurement methods, the subject is a possible Alzheimer's disease patient. For example, when the amount of Aβ oligomers in a sample collected from a subject is compared with that from a healthy individual, and if the amount of Aβ oligomers is greater in the subject than in the healthy individual, the subject is determined to suffer from or be at a risk of developing Alzheimer's disease. Whether or not a subject suffers from or is at a risk of developing Alzheimer's disease is diagnosed usually by physicians (including individuals under instructions from physicians; same herein below). Data on the amount of Aβ oligomers in samples collected from a subject and a healthy individual, which are obtained by the present methods of diagnosis, will be useful for diagnosis by physicians. Therefore, the present methods of diagnosis can be expressed as methods of collecting and presenting data useful for diagnosis by physicians.

[0119] Specifically, the present invention provides methods for diagnosing whether or not a subject suffers from or is at a risk of developing Alzheimer's disease, wherein the methods comprise detecting Aβ oligomers in a sample collected from the subject using an antibody of the present invention.

[0120] Furthermore, the present invention provides methods of diagnosing whether or not a subject suffers from or is at a risk of developing Alzheimer's disease, which comprise the steps of:

(a) contacting a sample collected from a subject with an antibody of the present invention and an antibody that binds to an Aβ monomer; and

(b) measuring the ratio of Aβ oligomer to Aβ monomer in the sample,

wherein the subject is determined to suffer from or be at a risk of developing Alzheimer's disease, if the ratio measured in step (b) is higher than that of a healthy individual.

[0121] First, in the present methods, a sample collected from a subject is contacted with an antibody of the present invention and an antibody that binds to an Aβ monomer. Herein, "contact" may be carried out, for example, by adding each of the above-mentioned antibodies to a sample collected from a subject, which is placed in a test tube. In this case, the antibody is added suitably in the form of a solution, a solid obtained by freeze-drying, or such. When adding the antibody as an aqueous solution, the solution may purely contain the antibody alone, or may contain, for example, surfactants, excipients, coloring agents, flavors, preservatives, stabilizers, buffers, suspending agents, tonicity agents, binding agents, disintegrants, lubricants, fluidity promoters, or corrigents. The concentration at which the antibody is added is not particularly limited. For example, as with human immunoglobulin formulations, 500-mg, 1000-mg, and 2500-mg freeze-dried formulations and such may be suitably used.

[0122] Next, the ratio of Aβ oligomer to Aβ monomer (herein, this is also referred to as "O/M index") in the aforementioned sample is measured. To measure this ratio, the following method is suitably used. For example, as described below in the Examples, the measurement can be carried out using a method of comparing the oligomer and monomer ELISA values obtained from the same sample.

[0123] Then, this ratio is compared with the ratio for a healthy individual. When the ratio is higher in the subject than in the healthy individual, the subject is determined to suffer from or be at a risk of developing Alzheimer's disease.

[0124] The methods of diagnosis of the present invention can be performed both in vitro and in vivo, but they are preferably performed in vitro.

[0125] Preferably, the "sample" of the present invention is not particularly limited as long as it is a tissue derived from a subject. Examples include the brain (brain parenchyma, and such), organs, and body fluids (blood, cerebrospinal fluid, and such) of a subject. In the present invention, the sample is preferably blood (more preferably, plasma) or cerebrospinal fluid.

[0126] Furthermore, the present invention provides pharmaceutical agents for use in the above-mentioned methods of measuring Aβ oligomers in a sample, or methods of diagnosing whether or not a subject suffers from or is at a risk of developing Alzheimer's disease.

[0127] In the present invention, the pharmaceutical compositions comprising an antibody may be included in products and kits containing materials useful for treating pathological conditions of a subject. The products may comprise any labeled container for a compound. Suitable containers include bottles, vials, and test tubes. The containers may be formed from a variety of materials such as glass and plastic. The label on the container surface should indicate that the composition is used to treat or prevent one or more conditions of the disease. The label may also indicate descriptions for administration, and such.

[0128] In addition to the above-mentioned container, a kit containing a pharmaceutical composition comprising an antibody may optionally include a second container that stores a pharmaceutically acceptable diluent. The kit may further include other materials desirable from a commercial and user's standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with descriptions for use.

[0129] If necessary, the pharmaceutical compositions may be provided in a pack or dispenser device that may contain one or more unit dosage forms comprising an active ingredient. The pack may comprise metal or plastic foil, and, for example, it is a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

[0130] In the above-mentioned pharmaceutical agents and kits, besides the antibody of the present invention that is an active ingredient, sterile water, physiological saline, vegetable oils, surfactants, lipids, solubilizing agents, buffers, protein stabilizers (BSA, gelatin, etc.), preservatives, blocking solutions, reaction solutions, reaction quenching solutions, reagents for treating samples, and such, may be mixed as necessary.

[0131] The antibodies provided by the present invention are expected to greatly contribute to the establishment of preventive/therapeutic methods selective to molecules responsible for evoking pathological conditions of Alzheimer's disease, and the establishment of early diagnostic markers for Alzheimer's disease. The present inventors obtained evidence showing that, even in antibody therapy targeting pathological conditions in the brain, peripheral intravenous administration is sufficient and there is no need to consider brain transfer. Thus, the present invention is expected to rapidly accelerate the progress of antibody drugs for Alzheimer's disease.

[0132] All prior art references cited herein are incorporated by reference into this description.

EXAMPLES

[0133] Hereinbelow, the present invention is specifically described with reference to the Examples, but it is not to be construed as being limited thereto.

Methods

Preparation of Monoclonal 1A9, 2C3, E12, 1C10, and 4D3

[0134] Synthetic Aβ 1-42 (Peptide Institute, Inc., Osaka) was dissolved in distilled water or 10 mM phosphate buffer, and incubated at 37° C. for 18 hours. Then, the peptides were separated by SDS-PAGE (4-12% NuPAGE Tris-Glycine gel), and after visualization by CBB staining, just the Aβ 1-42 tetramer was excised without contamination of the Aβ 1-42 monomer.

[0135] Next, BALB/c mice were immunized at their foot pad with 2.5 μg of the Aβ 1-42 tetramer emulsified using complete Freund's adjuvant. Subsequently, booster immunizations were carried out six times. Hybridomas were produced using the inguinal lymph node by fusion with Sp2/O-Ag14 cells using Polyethylene Glycol 1500.

Antibody Isotyping

[0136] Isotyping of purified immunoglobulins was carried out using a Serotec (Oxford, UK) mouse monoclonal antibody isotyping kit. The isotype of 1A9 and 2C3 was IgG2b.

Preparation of Antigens (4F7, 4H5, 5A5, 5A9, 6E4, and 6H4)

[0137] A fluorescent dye, 6-carboxytetramethylrhodamine (6-TAMRA) (SIGMA) was chemically linked to the N terminus of a synthetic Aβ 1-40 peptide (Peptide Institute, Inc.) to produce a modified Aβ. An oligomer-rich sample (Aβ1-40 oligomer) was prepared by copolymerizing the modified Aβ and synthetic Aβ 1-40 peptide. It is preferable to adjust the conditions so that the fluorescence intensity determined by ThT assay, which is described below, is one-fourth or less the fluorescence intensity in the absence of modified Aβ. More specifically, it is preferred that 100 μM each of the modified Aβ and synthetic Aβ 1-40 peptide are mixed, and polymerized for 20 hours.

Preparation of Antibody-Producing Hybridomas

[0138] Balb/c mice were immunized by injecting the antigen prepared by the method described above into their foot pads. Then, booster immunization was carried out six times. Hybridomas were prepared from inguinal lymph nodes by fusion with Sp2/O-Ag14 cells using Polyethylene Glycol 1500.

Antibody Isotyping

[0139] Isotyping of purified immunoglobulins was carried out using a Serotec (Oxford, UK) mouse monoclonal antibody isotyping kit.

Dot Blot Analysis (Primary Screening)

[0140] The initial screening was carried out by dot blot analysis using a nitrocellulose membrane onto which 2.5 μl of Aβ 1-42 (2.5 μg/dot) pre-incubated for 18 hours was immobilized. Non-specific binding sites on the membrane were blocked with a phosphate buffer containing 5% low-fat milk, 1% BSA, and 0.05% Tween-20, and then the membrane was incubated with a culture supernatant. Aβ oligomer-binding antibodies in the culture supernatant were detected by horseradish peroxidase-labeled goat anti-mouse $F(ab')_2$ (1:3000; Amersham), and visualized using an enhanced chemiluminescence (ECL) kit and LAS3000 mini (Fujitsu, Tokyo, Japan). Among 400 clones, 16 clones positive in the dot blotting, including 1A9 and 2C3, were subjected to the secondary screening described below.

Immunoprecipitation and Immunoblot Analysis (Secondary Screening)

[0141] Immunoprecipitation experiments (Ghiso J, et al., Biochem J, 1993) were conducted using an Aβ oligomer-rich amyloid fraction (Matsubara E, et al., Neurobiol Aging, 2004) for the secondary screening to assess whether the 16 clones selected in the primary screening can capture Aβ oligomers in AD brain. A buffer-insoluble, formic acid-soluble fraction prepared from AD brain was incubated with a culture supernatant and Protein G-Sepharose. The immunoprecipitated Aβ oligomers were separated using an NuPAGE 4-12% Bis-Tris-Glycine gel, and transferred onto a nitrocellulose membrane or Immobilon P (Millipore) using 10 mM 3-cyclohexylamino-1-propane sulfonic acid (pH 11) containing 10% methanol at 400 mA for one hour. Non-specific binding sites on the membrane were blocked with a phosphate buffer containing 5% low-fat milk, 1% BSA, and 0.05% Tween-20 at room temperature for three hours. The immunoprecipitated Aβ oligomers were detected by immunoblotting using the 4G8 (1:1000) or 6E10 (1:1000) monoclonal antibody as described above. Two clones, 1A9 and 2C3, were selected from the 16 clones as candidates for therapeutic antibodies for Alzheimer's disease.

Antibodies

[0142] The 6E10 and 4G8 monoclonal antibodies (Covance Immuno-Technologies, Dedham, Mass.) recognize the epitopes at amino acid positions 1-16 and 17-24 of the human Aβ sequence, respectively. Polyclonal A11 which specifically recognizes Aβ oligomers was purchased from Biosource (Camarillo, Calif.). Alex Fluor(AF)488- or 594-conjugated goat anti-mouse IgG and Alex Fluor(AF)488-conjugated goat anti-rat IgG were purchased from Molecular Probes (Eugene, Oreg.). Anti-mouse IgG2b was purchased from Sigma (St. Louis, Mo.). An anti-synaptophysin antibody was purchased from Santa Cruz (Santa Cruz, Calif.), and an anti-drebrin antibody was purchased from MBL (Nagoya, Japan).

Size Exclusion Chromatography (SEC)

[0143] SEC was carried out to assess 1A9 and 2C3 for their size specificity. According to a previously reported method (Matsubara E., et al., Neurobiol Aging, 25: 833-841, 2004), one can selectively separate Aβ monomers and Aβ oligomers, or lipoprotein-bound Aβ and lipoprotein-free Aβ. The present inventors concentrated the culture supernatant from APP/PS1-overexpessing HEK293 cells about ten-fold using a Microcon 3 kDa molecular weight cut-off filter (Millipore Corp.). Then, this concentrate was fractionated into 28 one-milliliter fractions using a Superose 12 size exclusion column

(1 cm×30 cm; Pharmacia Biotech., Uppsala, Sweden; flow rate of 0.5 ml/min) pre-equilibrated with a phosphate buffer. Half of each fraction was subjected to immunoprecipitation using 1A9 or 2C3. Aβ contained in the resulting immunoprecipitates was detected by immunoblotting using 4G8.

[0144] Cerebrospinal fluid (CSF) pooled from ten cases of Alzheimer's disease patients or age-matched healthy individuals, and lipoprotein-depleted CSF from the pools were also fractionated under the same conditions as described above. Aβ in the collected fractions was detected by ELISA analysis. To detect the lipid, the total cholesterol was enzymatically quantified using a standard kit (Wako, Osaka, Japan). Under the experimental conditions of the present inventors, the CSF lipoproteins were eluted at fractions 7 to 14, while fractions 15 to 28 contained cholesterol-free proteins.

Preparation of Seed-Free Aβ Solution

[0145] Synthetic Aβ 1-42 was dissolved at 250 μM in 0.02% ammonia water. Then, in order to prepare a seed-free Aβ solution, insoluble peptides, which may function as a seed, were precipitated by ultracentrifugation using an Optima TL ultracentrifuge (Beckman, USA) at 540,000×g for three hours. The resulting supernatant was collected, aliquoted, and stored at −80° C. until use. Samples were prepared by thawing the Aβ stock solutions immediately before use, and diluting them ten-fold with Tris-buffered saline (TBS; 150 mM NaCl and 10 mM Tris-HCl (pH 7.4)). The resulting 25 μM solutions were used in the experiments described below. Synthetic Aβ 1-40 (HCL form; Peptide Institute, Inc., Osaka) was prepared at 50 μM.

Aβ Incubation and ThT Assay (Yamamoto N, et al., J Biol Chem, 282: 2646-2655, 2007)

[0146] An Aβ solution (25 μM) was incubated in the presence of a predetermined concentration of an antibody at 37° C. for two or 24 hours. The ThT fluorescence intensity of the incubation mixture was determined using a fluorescence spectrophotometer (RF-5300PC; Shimadzu Co., Kyoto, Japan). The optimal fluorescence intensity was determined for Aβ amyloid fibrils at excitation and emission wavelengths of 446 and 490 nm, respectively, using 1.0 ml of a reaction mixture containing 5 μM ThT and 50 mM glycine-NaOH (pH 8.5). The fluorescence intensity was determined immediately after preparation of the mixture.

[0147] Furthermore, the activity of the E12 antibody to suppress Aβ amyloid fibril formation was assessed by the following procedure. An Aβ 1-42 solution diluted to 12.5 μM with cell culture medium was incubated in the presence or absence of the E12 antibody at 37° C. for 24 hours. The amount of formed amyloid fibrils was determined by the above-described ThT fluorescence intensity assay method.

Aβ-Induced Neurotoxicity Assay (Yamamoto N, et al., J Biol Chem, 282: 2646-2655, 2007)

[0148] Rat pheochromocytoma PC12 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen, Carlsbad, Calif.) containing 10% heat-inactivated horse serum (Invitrogen) and 5% fetal bovine serum (FBS) (Invitrogen). In order to induce the differentiation into nerve cells, PC12 cells were plated at a density of 20,000 cells/cm^2 in culture dishes coated with poly-L-lysine (10 mg/ml), and cultured for six days in DMEM supplemented with 100 ng/ml nerve growth factor (NGF; Alomone Labs, Jerusalem, Israel) (PC12N). PC12N was exposed to 25 μM seed-free Aβ 1-42 or pre-incubated Aβ 1-42 in the presence or absence of the 1A9 or 2C3 antibody at 4° C. for 48 hours. The neurotoxicity induced by Aβ 1-42 was assessed by Live/Dead dual-color fluorescence assay according to the supplier's instructions (Molecular Probes, Eugene, Oreg.).

[0149] Furthermore, the activity of the E12 antibody to neutralize Aβ-induced neurotoxicity was assessed by the method described below. First, human neuroblastoma cells (SH-SY5Y) were cultured for 24 hours in DMEM containing 10% FBS, at a density of 150,000 cells/well in 24-well plates. Then, the medium was replaced with serum-free culture medium containing Aβ 1-42 (12.5 μM) in the presence or absence of the E12 antibody, and the cells were cultured for another 24 hours. To determine the cytotoxicity induced by Aβ 1-42, the level of dead cell-derived LDH released into the medium was measured by a CytoTox96 kit (Promega).

Ultrafiltration and Molecular Sieve

[0150] In order to determine the size-dependent characteristics of neurotoxic Aβ oligomers, the four types of filtrates (<3 kDa, 3 to 10 kDa, 10 to 30 kDa, 30 to 100 kDa) and the retention solution (>100 kDa) were prepared from a 25 μM Aβ oligomer solution by sequential ultrafiltration using Microcon 3 kDa, 10 kDa, 30 kDa, and 100 kDa cut-off membranes. Each of the fractions was subjected to the Aβ-induced neurotoxicity assay described above. PC12N was exposed to each fraction to identify the toxic fraction as described above. The distribution of the three-dimensional structures recognized by A11, 1A9, 2C3, and 4G8 was also identified by the dot blot analysis described above. The morphological characterization of the neurotoxic oligomers was performed by examining each fraction using an atomic force microscope.

Electron Microscopy (EM) and Atomic Force Microscopy (AFM)

[0151] Samples were diluted with distilled water and sprayed over carbon-coated grids to conduct electron microscopy. The grids were negatively-stained with 1% phosphotungstic acid and observed under a Hitachi H-7000 electron microscope (Tokyo, Japan) with an acceleration voltage of 77 kV. AFM assessment was carried out as recently reported. Drops of the samples were placed onto freshly cleaved mica. The mica was allowed to stand for 30 minutes and then washed with water, and the liquid samples were analyzed using Nanoscope IIIa (Digital Instruments, Santa Barbara, Calif., USA) set to the tapping mode (Tero, R, et al., Langmuir 20, 7526-7531, 2004). The cantilever used was OMCL-TR400PSA (Olympus, Japan).

Subject Tissues and Extraction

[0152] The present study was conducted based on autopsy cases (n=50; 26 male and 24 female cases) from the Tokyo Metropolitan Brain Bank for Aging Research of the Tokyo Metropolitan Institute of Gerontology (Itabashi, Tokyo, Japan). This research project was approved by the institutional ethical committees of the Faculty of Medicine, the University of Tokyo; the Tokyo Metropolitan Geriatric Hospital of the Tokyo Metropolitan Institute of Gerontology; and the National Center of Geriatrics and Gerontology. Details of subjects and sample collection have been reported (Katsuno T, Neurology, 64: 687-692, 2005). However, that study analyzed insoluble brain fractions, whereas in this research project (Katsuno T, Neurology, 64: 687-692, 2005), the present inventors analyzed soluble brain fractions, which remain uncharacterized in previous studies. Frozen tissue samples (the anterior portion of entorhinal cortex) were homogenized in nine volumes of Tris-buffered saline (TS) containing a protease inhibitor cocktail. The homogenates were ultracentrifuged at 265,000×g for 20 minutes. One-third aliquots (0.5 ml) of the resulting supernatants were subjected to immunoblot analysis.

Immunohistochemistry

[0153] The left brain hemispheres of Tg2576 mice were sliced into 30-μm-thick sagittal sections using a cryotome (RM 2145; Leica, Wetzlar, Germany), and stained with thioflavin S as previously described (Wyss-Coray et al., 2001). The formation of swollen dystrophic neurites was observed using an anti-synaptophysin antibody (Chemicon, Temecula, Calif.). The number of thioflavin S-positive plaques and synaptophysin-positive swollen dystrophic neurites was counted by observing four or five sections from the left brain hemisphere of each mouse at 40-fold magnification. To observe Aβ deposition, serial sections briefly pre-treated with formic acid or Protease K were stained using an Aβ immunostaining kit (Sigma, St. Louis, Mo.), and immunopositive signals were visualized using an ABC elite kit (Vector Laboratories). Images of the cerebral cortex and hippocampus were recorded using a digital camera connected with a microscope, and analyzed using a simple PCI software (Compix Imaging System, Lake Oswego, Oreg.). The brain translocation of antibodies was observed using a confocal laser microscope (Carl Zeiss LSM510). The number of thioflavin S-positive plaques and synaptophysin-positive swollen dystrophic neurites was determined in a double blind manner.

Passive Immunotherapy and Behavioral Analysis

[0154] Three-month-old female non-transgenic (non-Tg) mice, and Tg2576 mice having and overexpressing the Swedish-type mutant human APP gene with dual mutations (K670N and M671 L) derived from familial AD were purchased from Taconics (Germantown, NY, USA). These mice were reared until 13 months old in the animal facility of the present inventors. To determine whether the Alzheimer-like phenotype is prevented by passive immunotherapy, 1A9 or 2C3 (0.4 mg/kg/week), or PBS was administered into the caudal vein of four-month-old Tg2576, and the administration was continued until 13 months. The memory function was assessed at month 13 as previously described (Mouri A, FASEB J, 21: 2135-2148, 2007), based on the following four behavioral paradigms:
(1) Y-maze test for short-term memory;
(2) novel object recognition test;
(3) Morris water maze test; and
(4) contextual fear conditioning test.
Three days after the behavioral tests, the mice were sacrificed for biochemical and histological assessments. The experimental results were analyzed by one-way ANOVA and two-way ANOVA. Post-hoc analysis was carried out using Fisher test.

Separation and Removal of Lipoprotein

[0155] CSF was collected from 12 AD patients and 13 NC individuals. Then, lipoproteins were removed from 600 μl each of the CSF by preparative continuous density gradient ultracentrifugation according to a protocol reported previously (Matsubara E, et al., Ann Neurol, 45: 537-541, 1999). The density of CSF was adjusted to 1.25 g/ml with KBr. The CSF was ultracentrifuged at 100,000 rpm and 16° C. for eight hours using a Hitachi RP100AT centrifuge. Lipoproteins floating at a density of 1.25 g/ml and lipoprotein-depleted CSF (LPD-CSF) were subjected to ultrafiltration using a 3 kDa cut-off membrane (Microcon 3; Arnicon, Inc), and then frozen and stored, or stored at 4° C., until use.

[0156] Lipoproteins were also removed by affinity chromatography using PHML-LIPOSORB (Calbiochem, La Jolla, Calif.). Each sample (plasma or brain) and PHML-LIPOSORB (Calbiochem, La Jolla, Calif.) were combined at a ratio of 1.5:1, and mixed for 60 seconds. Then, the mixture was centrifuged at 3,000 rpm for ten minutes. The resulting supernatants (lipoprotein-free samples) were subjected to ELISA using 6E10 for the oligomers. The lipoprotein-bound samples were eluted from PHML-LIPOSORB using 20 mM sodium deoxycholate. The removal of specific lipoproteins was confirmed by agarose electrophoresis using 1% gel (Beckmann), followed by staining with FAST-RED 7B (Wako, Osaka, Japan).

Quantification of Human Aβ

[0157] Whole plasma and LPDP Aβ species were specifically quantified by sandwich ELISA as previously described (Matsubara E, et al., Ann Neurol, 537-541, 1999; Matsubara E, et al., Neurobiol Aging, 25: 833-841, 2004). To analyze brain Aβ species, soluble Aβ species in 100 μl of buffer were directly subjected to ELISA, while insoluble Aβ samples extracted with 70% formic acid were neutralized with 1 M Tris-HCl (pH 8.0) and diluted 1,000-fold prior to ELISA. The values obtained by the assay were normalized using the brain wet weight, and ultimately presented in pmol/g. Normalization among plates was done by including the three standard plasma samples in all three plates.

Aβ Oligomer-Specific ELISA

[0158] Chemiluminescence-based sandwich solid-phase enzyme immunoassay (chemiluminescent ELISA) was used to specifically detect oligomeric Aβ but not monomeric Aβ. Microplates were coated with monoclonal 1A9 (IgG2b isotype) or 2C3 (IgG2b isotype), or a mixture of 1A9 and 2C3. 100 μl of a sample (brain or cerebrospinal fluid) was added and incubated continuously for 24 hours at 4° C. Then, horseradish peroxidase-conjugated BA27 Fab' fragment (anti-Aβ 1-40 specific to Aβ 40; Wako pure chemical, Osaka, Japan) or horseradish peroxidase-conjugated BC05 Fab' fragment (anti-Aβ 35-43 specific to Aβ 42; Wako pure chemical, Osaka, Japan) was added and incubated at 4° C. for 24 hours. The chemiluminescence generated using SuperSignal ELISA Pico Chemiluminescent Substrate (Pierce, Rockford, Ill., USA) was quantified by a Veritas Microplate Luminometer (Promega).

[0159] To assess the in vivo efficacy of the peripheral administration of monoclonal 1A9 and 2C3, plasma and organ samples were collected from administered mice and analyzed for Aβ oligomers by ELISA using HRP-labeled 6E10 (Senetek PLC, Napa, Calif., USA) specific to the human oligomers. High-sensitivity detection was achieved by using the above-described chemiluminescent system. To avoid interference by lipoprotein-bound Aβ monomers, the present inventors pre-treated plasma and organ samples using PHML-LIPOSORB in the same way as described above. The resulting lipoprotein-depleted samples were used for the assay.

Example 1

Preparation of Aβ Oligomer-Specific Monoclonal Antibodies (1A9 and 2C3)

[0160] Aβ oligomers and monomers co-exist in a solution. Thus, it is essential to remove Aβ monomers for preparation of antigens to produce Aβ oligomer-specific antibodies. As shown in FIG. **1**A, the present inventors succeeded in isolating SDS-stable Aβ tetramers without contamination of Aβ monomers by SDS-PAGE. After in vivo immunization with the isolated Aβ tetramers, positive hybridoma clones were selected by two-step screening using dot blot analysis followed by immunoprecipitation. Among 400 clones subjected to dot blot analysis, 16 clones were determined to be positive (positivity rate=4%). To assess the specificity of the isolated positive clones to the oligomers, a phosphate buffer-insoluble and formic acid (FA)-soluble amyloid fraction derived from AD brain (Matsubara E et al., Neurobiol Aging, 25: 833-841, 2004) was analyzed by immunoprecipitation using the cell culture supernatants of the positive hybridomas (FIG. **1**B). The Aβ dimer, a smaller amount of the trimer, and a high-molecular-weight smear characteristic to aggregated Aβ molecular species were detected by immunoblot analysis using anti-Aβ monoclonal 4G8. A very small amount of Aβ monomers dissociated in the presence of SDS was also detected. To further confirm the existence of three-dimensional structures recognized by native 1A9 and 2C3 (i.e., oligomers), the present inventors detected the oligomers in conditioned medium (CM) of human embryonic kidney (HEK) 293 cells transfected with mutant PS1 cDNA (Nakaya Y et al., J Biol Chem, 280: 19070-19077, 2005). The present inventors fractionated HEK293 CM by SEC, and then identified the oligomers. As reported previously (Matsubara E et al., Neurobiol Aging, 25: 833-841, 2004; Yamamoto N, et al., J Biol Chem, 282: 2646-2655, 2007), this method can effectively separate the oligomers (fractions 8 to 13) from monomers (fractions 14 to 20). When immunoprecipitated with monoclonal 1A9, SDS-stable Aβ dimers secreted into CM were precipitated in fraction 8 (>680 kDa); SDS-stable Aβ dimers and trimers were precipitated in fraction 13 (17 to 44 kDa); and a very small amount of the dimers was precipitated in fraction 16 (FIG. **1**C). Similar results were obtained when immunoprecipitation was carried out using 2C3 (data not shown). These data demonstrate that monoclonal 1A9 and 2C3 are exactly specific to Aβ oligomers but do not recognize Aβ monomers.

Example 2

The Anti-Neurotoxic Activity of Monoclonal 1A9 and 2C3

[0161] To assess whether monoclonal 1A9 and 2C3 can prevent Aβ-induced neurotoxicity, NOF-differentiated PC12 cells (PC12N) were incubated with 25 μM seed-free Aβ 1-42 (ThT-negative 540,000×g supernatant) in the presence or absence of the monoclonal antibodies (mAbs) at 37° C. for 48 hours. The viability of nerve cells was determined by LIVE/DEAD assay (FIG. **2**). Nerve cell death was detected at a significantly high level (50%) in the presence of Aβ 1-42 (FIGS. **2**B and **2**G), as compared to the control assay (FIG. **2**A). Non-specific IgG2b (FIGS. **2**C and **2**G) could not inhibit the Aβ 1-42-induced neurotoxicity under the same conditions. The commercially available Aβ-specific monoclonal antibody 4G8 (IgG2b isotype; FIGS. **2**D and **2**G) had a tendency to enhance the toxicity. Monoclonal 2C3 (IgG2b isotype; FIGS. **2**F and **2**G) neutralized the neurotoxicity of Aβ 1-42 almost completely in a concentration-dependent manner. Thus, the de novo-formed neurotoxic three-dimensional structure recognized by 2C3 was speculated to take an oligomer form. Meanwhile, the anti-neurotoxic activity of 1A9 (IgG2b isotype; FIGS. **2**E and **2**G) falls between the anti-neurotoxic activity of 2C3 and non-specific IgG2b. This suggests that the three-dimensional structure recognized by 1A9 is structurally different from the 2C3-recognized oligomers.

Example 3

[0162] Currently, the determination of the precise size and conformation of neurotoxic Aβ 1-42 oligomers is one of the most urgent issues and which is subjected to intense competition. The present inventors succeeded in isolating soluble neurotoxic Aβ 1-42 molecular species and fractionating the species into the following five fractions by ultrafiltration and molecular sieving (UC/MS) (FIG. **3**A):
fraction 1, filtrate of <3 kDa (lane 1);
fraction 2, filtrate of 3 to 10 kDa (lane 2);
fraction 3, filtrate of 10 to 30 kDa (lane 3);
fraction 4, filtrate of 30 to 100 kDa (lane 4); and
fraction 5, retention solution of >100 kDa (lane 5).

[0163] The immunoblot analysis using monoclonal 4G8 (FIG. **3**A) revealed that:
fraction 1 does not contain Aβ (lane 1);
fraction 2 contains Aβ monomers (lane 2);
fraction 3 contains Aβ monomers and a small amount of Aβ dimers (lane 3);
fraction 4 contains Aβ monomers to pentamers (lane 4); and
fraction 5 contains Aβ monomers to pentamers, and molecules of 45 to 160 kDa (lane 5).

[0164] These data suggest that 2% SDS depolymerizes high-molecular-weight (HMW) Aβ oligomers into Aβ monomers and low-molecular-weight (LMW) Aβ oligomers. To assess the size distribution of toxic Aβ1-42, the present inventors measured the biological activity of each fraction incubated with PC12N at 37° C. for 48 hours. As shown in FIGS. **3**B and **3**C, it was demonstrated that: fraction 1 was non-toxic, and fraction 2 had a very weak toxicity, suggesting that Aβ monomers and dimers are unlikely to be toxic. Fractions 3 to 5 were significantly toxic (one-way ANOVA; $p<0.0001$), suggesting that the size of neurotoxic oligomers theoretically corresponds to the size of trimers or higher-molecular-weight polymers. The dot blot analysis using the oligomer-specific A11 antibody demonstrated that the above-mentioned three neurotoxic fractions (3 to 5) were positive for A11, supporting the evidence that the neurotoxic molecules are oligomeric (FIG. **3**D). The 2C3-recognized oligomers were detected in fractions 4 and 5 (FIG. **3**D). Thus, 2C3 was demonstrated to actually react with neurotoxic Aβ oligomers (>30 kDa). Furthermore, the majority of the 2C3-recognized oligomers was detected in fraction 5 (>100 kDa) that was the most toxic, and thus the 2C3-recognized oligomers having a molecular weight over 100 kDa were considered to show a strong neurotoxicity (FIG. **3**D). Meanwhile, only an extremely small amount of the 1A9-recognized oligomers was distributed in fraction 5 that was the most toxic. This is consistent with the result that the neutralization of neurotoxicity by 1A9 was insufficient (FIGS. **2**E and **2**G). By contrast, monoclonal 4G8 having no anti-neurotoxic activity detected the Aβ species distributed in all of the fractions (FIG. **3**D). This suggests the possibility that non-toxic and toxic oligomers of the same size co-exist.

[0165] To further assess the toxicity-structure correlation, each fraction was subjected to atomic force microscopy (AFM). The presence of globular particle morphology consistent with the fraction size was detected in the three neurotoxic fractions. FIG. **3**E shows the atomic force microscopic images of non-toxic fraction 2 (Fr. 2), toxic fractions 3 (Fr. 3) and 4 (Fr. 4), and the most toxic fraction 5 (Fr. 5). The formation of many granular polymer molecules was clearly observed in the toxic fractions. In particular, fraction 5 was revealed to contain heterogeneous toxic molecules including bead-shaped and ring-shaped molecules in addition to various large and small granular molecules.

Example 4

The Activity of 1A9 and 2C3 to Suppress Aβ Amyloid Fibril Formation

[0166] Next, the present inventors assessed the activity of 1A9 and 2C3 to suppress Aβ amyloid fibril formation. The formation of Aβ 1-42 amyloid fibrils (at 0, 10, 25, and 50 μM) was assessed by measuring the ThT fluorescence for 72 hours at 37° C. Under the conditions used by the present inventors, seed-free Aβ 1-42 (ThT-negative supernatant fraction obtained by ultracentrifugation at 540,000×g) was polymerized into amyloid fibrils by nucleation-dependent polymerization (FIG. **4**A). To assess the activity of 1A9 and 2C3 to suppress Aβ amyloid fibril formation, the present inventors incubated 25 μM seed-free Aβ 1-42 in the presence or absence of the antibodies at 37° C. for 48 hours. As shown in FIG. **4**B, the ThT fluorescence intensity was altered in a 2C3 concentration-dependent manner, while none of monoclonal 1A9 and 4G8 and non-specific IgG2b altered the florescence intensity. Meanwhile, when Aβ was polymerized by incubation for two hours, 1A9, as well as 2C3, showed the activity to almost completely suppress the fibril formation (FIG. **4**C). Since the activity to suppress Aβ amyloid fibril formation was detected even when the molar ratio of 2C3 to Aβ was low, 2C3 was inferred to have the activity to inhibit the polymerization nucleus formed de novo or the seed function at an early stage of Aβ 1-42 amyloid fibril formation. Similar results were obtained by morphological observation. As shown in FIG. **4**E (Aβ 42 alone) and FIG. **4**F (Aβ 42+2C3, 25:3), electron microscopy (EM) demonstrated that the formation of Aβ amyloid fibrils was partially inhibited in the presence of monoclonal 2C3, while only a weak inhibitory effect was produced in the presence of 1A9 (FIG. **4**G). Meanwhile, none of the test antibodies exhibited the effect of lysing or depolymerizing Aβ 1-42 amyloid fibrils that were formed by incubation for 24 hours (FIG. **4**D).

Example 5

Toxicity-Related Oligomers Targeted by 1A9 and 2C3

[0167] To elucidate the structural and kinetic connection between the Aβ 1-42 oligomerization and amyloid fibril formation, the polymerization time course was analyzed by dot blotting using A11, 1A9, 2C3, and 4G8. As shown in FIG. **5**A, the majority of A11 antibody-reactive oligomers was formed during the lag time phase of polymerization (0 to 8 hours), and the ThT fluorescence intensity was relatively weak. During the next fibril extension phase (8 to 24 hours), the level of A11-immunoreactive oligomers reached a plateau, and then was constant (about 20% of the peak level) until 72 hours (plateau phase). It has been demonstrated that, since the anti-oligomer A11 antibody does not recognize amyloid fibrils, the Aβ oligomer formation can be specifically observed using the antibody (Kayed R, et al., Science 300, 486-489, 2003). Hence, the present results suggest that the Aβ oligomer formation precedes amyloid fibril formation, and there is an oligomerization state that does not directly enter the amyloid fibril formation pathway. The 2C3-recognized oligomers were kinetically similar to the A11-recognized oligomers, but not the 1A9-recognized oligomers. The 1A9-recognized oligomers were detected only after four hours, and then the immunoreactivity to 1A9 increased twofold over time. This suggests that the 1A9-recognized oligomers are slowly formed. Meanwhile, it was revealed that the 2C3-recognized oligomers are transiently increased during the lag time phase (0 to 8 hours), and then exist at a very low level (less than 5%) in a oligomerized state from 8 to 72 hours. The above-described data obtained by the present inventors suggest the possibility that the A11-, 1A9-, and 2C3-recognized oligomers have structurally and immunologically different conformations or stability, and the 2C3-recognized oligomers are relatively unstable as compared to the 1A9-recognized oligomers.

[0168] To characterize the de novo toxic polymerization state, PC12N were exposed at 37° C. for 48 hours to seed-free Aβ 1-42 (0 hour), or Aβ 1-42 pre-incubated for two, four, or 24 hours (FIG. **5**B), and the neurotoxic activity was assayed. As shown in FIG. **5**B, the immunoblotting analysis using 4G8 revealed that the monomers, dimers, and timers exist even at the 0 hour time point. The pre-incubation of two or four hours resulted in a high-molecular-weight (HMW) smear pattern of 45 to 160 kDa, in addition to the monomers to pentamers. At the time point of 24 hours, the HMW smear was dramatically reduced, and there were two types of major components: a high-molecular-weight species that could not enter the gel and thus remained in the well, and a small amount of the monomers. The HMW smear disappeared after further incubation at 37° C. for 48 hours. As shown in FIGS. **3**A and **5**C, the molecular sieve experiment revealed that seed-free Aβ 1-42 is converted into molecular species of 100 kDa or more, and exhibits the strongest toxicity. By SDS-PAGE, it was demonstrated that the toxic molecules include molecular species showing a high-molecular-weight (HMW) smear pattern of 45 to 160 kDa, in addition to the monomer to pentamer species, and the toxic polymers can be easily depolymerized into low-molecular-weight species in the presence of SDS. However, when seed-free Aβ 1-42 was pre-incubated for two and four hours, the neurotoxic activity of de novo-formed Aβ oligomers was reduced by about 12.5% and 26%, respectively (FIG. **5**C). This result suggests that the level of de novo-formed Aβ oligomers in the early period of Aβ polymerization is a determining factor for neurotoxicity, and that the formation reaches a peak in the period of zero to two hours, and then the level of formed Aβ oligomers reduces over time. Alternatively, there is a possibility that nuclei for the de novo polymerization of Aβ amyloid fibrils, or amyloid fibrils themselves have the neurotoxicity-neutralizing activity. The present inventors incubated Aβ 1-42 for two hours, and then removed insoluble Aβ polymerization nuclei and amyloid fibrils by ultracentrifugation for three hours at 540,000×g. The supernatant and pellet obtained by ultracentrifugation at 540,000×g exhibited similar levels of thioflavin T signals, suggesting that the 540,000×g supernatant contains soluble ThT-positive Aβ polymers (The ThT binding indicates structural changes to form a β sheet-rich structure, but not fibril formation). The neurotoxicity was restored and enhanced when PC12N was exposed to the soluble polymers (FIG. **5**D). This suggests that insoluble Aβ 1-42 itself has the anti-toxic activity. Under the conditions described above, monoclonal 1A9 completely neutralized the neurotoxicity induced by soluble Aβ oligomers enriched in β sheet structures, and this neutralizing activity was greater than that of 2C3. Meanwhile, non-specific IgG2b has no effect on the viability of cultured PC12N. Accordingly, it is speculated that neurotoxic 1A9-recognized polymers are basically soluble toxic oligomers that have been slightly stabilized due to some structural change, while neurotoxic 2C3-recognized polymers are basically short-lived oligomeric intermediates that are very unstable due to drastic structural changes during the early stage of polymerization process.

Example 6

Monoclonal 1A9 and 2C3 Recognize Aβ Oligomers in the Brain Parenchyma

[0169] The present inventors demonstrated the specificity and biological activity of 1A9 and 2C3. Furthermore, the inventors detected 1A9 and 2C3 polymers in the brain by immunohistochemistry. The present inventors performed conventional immunohistochemistry methods to enhance immune reaction by formaldehyde fixation, and formic acid, SDS, or microwave treatment of brain sections. The two antibodies exhibited no immunoreactivity to AD brain by any one of the enhancement methods. Thus, the present inventors pre-treated the sections with Protease K, which is known to improve immunostaining (Wrzolek M A, et al., Am J Pathol, 141: 343-355, 1992). As a result, many senile plaques were stained with 1A9 (FIG. **6**A), 2C3 (FIG. **6**B), and A11 (FIG. **6**C). Together with the finding from the in vitro experiments by the present inventors that Aβ amyloid fibrils neutralize the Aβ oligomer-induced neurotoxicity, the result described above suggests that a senile plaque serves as a defensive reservoir to isolate and store Aβ oligomers, and thus the interior of the reservoir is hardly accessible for antibodies. Indeed, immunoprecipitation using 1A9 and 2C3 demonstrated that amyloid fractions composed of senile plaques contain Aβ oligomers recognized by the two antibodies. Thus, the hypothesis of the present inventors was proven to be consistent with the in vivo finding (see FIG. **1**B).

[0170] To further assess the existence of "soluble" 1A9- and 2C3-recognized polymers in the brain, the present inventors carried out immunoprecipitation experiments using the two antibodies. Brain homogenates were prepared using Tris-buffered saline (TBS) to avoid chemical modification during the extraction of soluble oligomers. The oligomers having a molecular weight of 4mer, 5mer, 8mer, and 12mer were immunoprecipitated with 1A9 from TBS samples of the cerebral cortex from AD brain (FIG. **6**D, lane 2), while the level of the oligomers in the control healthy brain was below the detection limit (lane 3). While the intensity of 4mer, 5mer, and 8mer was comparable between 1A9 (lane 2) and the monoclonal 4G8/6E10 mixture (lane 1), 1A9 appeared to recover a larger amount of 12mer than 4G8/6E10. The immunoprecipitation with 2C3 showed a comparable result (FIG. **6**, lanes 4 to 6). Next, the present inventors identified the molecules responsible for the in vivo neurotoxicity in the human entorhinal cortex. It is well known that neurofibrillary tangle (NFT) and nerve cell loss precede the formation of senile plaques in lesions in general elderly populations. The present inventors hypothesized that the lack of functional reservoirs such as senile plaques for 1A9- and 2C3-recognized polymers is harmful for entorhinal cortex neurons, and is a possible cause of memory disturbance. The level of 12mer in the buffer-soluble fractions of previously reported 50 autopsy cases was determined by immunoblotting using monoclonal 1A9 and 2C3. The 50 cases include two AD cases, 35 cases at Braak NFT stages I to II, and 13 cases at NFT stages III to IV (Katsuno et al., Neurology, 64: 687-692, 2005). As shown in FIGS. **6**E (1A9) and **6**F (2C3), the immunological activity of 1A9- or 2C3-immunoreactive 12mer relative to actin was significantly higher in AD patients as compared to the healthy control group (Braak NFT stages I to II) and mild cognitive impairment group (Braak stages III to IV). Interestingly, the 12mer was accumulated in the entorhinal cortex of the healthy control group (Braak NFT stages I to II) and mild cognitive impairment group (Braak stages III to IV) at a level of about 40% and 60% (the level of AD cases is 100%), respectively (FIGS. **6**E and **6**F). This result indicates that the accumulation of 12mer precedes the onset of cognitive impairment, and is increased as the Braak NFT stage advances, suggesting that the 1A9- and 2C3-imunoreactive 12mer are polymers responsible for the in vivo neurotoxicity.

Example 7

Monoclonal 1A9 and 2C3 Recognize Aβ Oligomers in the Cerebrospinal Fluid

[0171] The Aβ polymers (soluble 1A9- and 2C3-imuunoreactive 12mer) responsible for the in vivo neurotoxicity were found in the brain parenchyma. Thus, the present inventors speculated that CSF also contains the polymers. To verify this, the present inventors fractionated pooled CSFs from ten AD patients and ten age-matched healthy individuals as a control by SEC, and assayed the fractions by Aβ oligomer-specific sandwich ELISA using monoclonal BC05 or BA27 in the capturing and detection systems. The BC05/BC05 oligomer ELISA detected soluble Aβ 1-42 in fraction 13, while the BA27/BA27 ELISA detected soluble Aβ 1-40 in fractions 7 to 14 (data not shown). However, in each ELISA, the absorbance (O.D. at 450 nm) was low for sensitive detection of a small amount of Aβ oligomers in CSF. The detection of Aβ monomers in the same fractions by BNT77 ELISA showed that lipoprotein-bound Aβ monomers (fractions 7 to 14) and lipoprotein-free Aβ monomers (fractions 15 to 17) coexist with Aβ oligomers in the fractions (FIGS. **7**-**1**A and **7**-**1**B) (Matsubara E, et al., Neurobiol Aging, 25: 833-841, 2004). The level of lipoprotein-bound Aβ monomer in AD was comparable to that of the healthy control, while the level of lipoprotein-free Aβ40 monomer (FIG. **7**-**1**A) and A1342 monomer (FIG. **7**-**1**B) in AD was lower as compared to the age-matched healthy control. The present inventors also found that lipoprotein-bound Aβ monomers, in addition to the oligomers, can be detected when ELISA is designed to use HRP-labeled BC05 or BA27 as a capture antibody. This problem remained unnoticed in the prior art document (Lee EB, et al., J Biol Chem, 281: 4292-4299, 2006), which describes assay methods (for example, 6E10/6E10 ELISA) that are similar to the methods described herein. Since the oligomers and lipoprotein-bound Aβ monomers are eluted at a comparable retention time in SEC, it is impossible to distinguish them by oligomer ELISA using the same antibody in capturing and detection. Thus, it was revealed that CSF containing lipoproteins is unsuitable for a test sample when Aβ oligomers are analyzed using Aβ oligomer-nonselective antibodies.

[0172] To overcome the weaknesses of the prior art methods, the present inventors improved the detection antibodies and samples used in ELISA. Lipoproteins were pre-depleted from CSF, and the resulting lipoprotein-depleted CSF (LPD-CSF) was used as an assay sample. Aβ oligomer-specific 1A9 and 2C3 were used as detection antibodies for ELISA. Furthermore, chemiluminescence ELISA was developed to enhance the sensitivity. Pooled LPD-CSF (FIGS. **7-1**C to D) was fractionated by SEC, and each fraction was analyzed for Aβ oligomer distribution by luminescence ELISA using 1A9 or 2C3 as a detection antibody. As shown in FIGS. **7-1**C to D, Aβ oligomers were detected in SEC fractions 12 to 15 (relatively large Aβ with a molecular weight ranging within 18 to 108 kDa, which corresponds to the size of 4mer to 24mer). The level of 1A9- and 2C3-recognized oligomers was elevated in all of the AD patient-derived fractions in which the oligomers were detectable. To assess the usefulness of the Aβ oligomers as therapeutic markers, the level of Aβ oligomers in LPD-CSF from AD patients was compared to that from the age-matched healthy control, although a limited number of cases were analyzed. As shown in FIG. **7-2**G, 2C3-recognized oligomers composed of Aβ x-42 were significantly increased in the AD patient group as compared to the normal control group (nonparametric analysis; p=0.0103). By contrast, for 2C3-recognized oligomers composed of Aβ x-42, there was no significant difference between the two groups. Meanwhile, the level of 1A9-recognized oligomers composed of Aβ x-42 was higher in AD than in the control, although the difference was not statistically significant. For 1A9-recognized oligomers composed of Aβ x-40, there was no significant difference between the two groups (FIG. **7-2**E). The structural change from Aβ monomer to oligomer occurs in the earliest period of the process of Aβ polymerization. The ratio between Aβ oligomer and monomer (O/M index) can be used as a clinical indicator reflecting the pathological conditions of AD. As shown in FIGS. **7-2**F and 7-2H, the O/M indices for Aβ42 and Aβ40 were significantly increased in the AD patient group as compared to the healthy control group (1A9, P=0.0137 for Aβ42 and P=0.0429 for Aβ40; 2C3, P=0.0012 for Aβ42 and P=0.0051 for Aβ40). The results described above show that the 1A9- and 2C3-positive three-dimensional structures are present as Aβ oligomers in LPD-CSF, and increased in AD patients. In addition, the results obtained by the present inventors demonstrated that the structural conversion of lipoprotein-free soluble Aβ to the oligomeric intermediates occurs in CSF of AD patients, and the oligomers can be detected as useful biological markers for diagnosis of sporadic AD.

Example 8

Passive Immunotherapy Using Monoclonal 1A9 and 2C3 Prevents the Onset of Memory Disturbance in Tg2576

[0173] To assess the in vivo preventive/therapeutic effect of passive immunotherapy based on the administration of 1A9 (n=13) or 2C3 (n=11), the present inventors administered 1A9 or 2C3 (0.4 mg/kg/week), or PBS to Tg2576 mice via the caudal vein during the 4 to 13 month period. The memory function was assessed at 13 months old in terms of the following four types of learning/behavioral paradigms:

(1) short-term memory in Y-maze test (FIG. **8**A);

(2) object recognition memory in novel object recognition test (FIG. **8**B);

(3) spacial memory in water maze test (FIG. **8**C); and

(4) associative emotional memory in contextual fear conditioning test (FIG. **8**D).

[0174] As compared to 1A9- and 2C3-administered Tg2576 mice, PBS-administered Tg2576 mice showed significant learning and behavioral impairments (FIG. **8**A to **8**D). Unlike the memory function of PBS-administered Tg2576 mice (n=10), the memory function of 1A9 and 2C3-administered Tg2576 mice was indistinguishable from that of age-matched non-administered wild type cohort mice, which was previously determined. Therefore, 1A9 and 2C3-administered Tg2576 mice were shown to retain both short- and long-term memory, which were impaired in the PBS administration group. That is, the present inventors obtained evidence supporting the view that the onset of memory disturbance, in particular AD, can be prevented by conducting passive immunotherapy targeting Aβ oligomers before the onset. Furthermore, the result described above presents the first in vivo evidence that directly indicates that Aβ oligomers are responsible for the onset of memory disturbance.

Example 9

Monoclonal 1A9 Prevents Aβ Accumulation in the Brain of Tg2576

[0175] Tg2576 mice administered with PBS (n=10) and Tg2576 mice treated with passive immunotherapy during the 4 to 13 month period (1A9 administration group, n=13; 2C3 administration group, n=11) were dissected after the learning/behavioral experiments. The amount of Aβ accumulated in the brain (cerebral cortex vs. hippocampus) was determined in the following three fractions (150 mg/extract) prepared by serial extraction: soluble fraction in Tris buffer containing protease inhibitors; 2% SDS-soluble amyloid fraction; and 2% SDS-insoluble and 70% formic acid-soluble amyloid fraction. It is considered that non-accumulative, physiological Aβ molecules are contained in the Tris buffer fraction, while 2% SDS-soluble Aβ includes Aβ in diffuse senile plaques before amyloid fibril formation, immunocytochemically undetectable Aβ, and conformationally altered, accumulative soluble oligomeric Aβ. Aβ was selectively quantified by Aβ40 and Aβ42 end-specific ELISA (BNT77/BA27 specific for Aβ40, BNT77/BC05 specific for Aβ42, WAKO kit). There was no marked difference among the three groups in the Aβ concentration in the Tris buffer fraction where the major components were non-accumulative, physiological Aβ molecules (FIGS. **9**A and **9**C, Aβ x-40; FIGS. **9**B and **9**D, Aβ x-42). Regarding soluble Aβ accumulated in the brain (SDS fraction), a significant suppressive effect on the accumulation of Aβ x-40 and Aβ x-42 in the cerebral cortex was observed only in the 1A9 administration group (FIG. **9**E, Aβ x-40; FIG. **9**F, Aβ x-42). No accumulation-suppressive effect was observed in the hippocampus (FIG. **9**G, Aβ x-40; FIG. **9**H, Aβ x-42). Meanwhile, regarding insoluble Aβ accumulated in the brain (FA fraction), a significant suppressive effect on the accumulation of Aβ x-40 in the cerebral cortex was observed only in the 1A9 administration group (FIG. **9**I, Aβ x-**40**; FIG. **9**J, Aβ x-42). No accumulation-suppressive effect was observed in the hippocampus (FIG. **9**K, Aβ x-40; FIG. **9**L, Aβ x-42). The A11 immunoblot analysis of the SDS-soluble fractions showed a suppressive effect on the accumulation of A11-positive oligomer (4mer) in the cerebral cortex in the two antibody treatment groups (FIG. **9**M).

Example 10

Plasma Aβ Oligomers Are Increased by Passive Immunotherapy with 1A9 and 2C3

[0176] There was no significant difference in the plasma Aβ concentration among the following three groups: Tg2576 mice administered with PBS (n=10), and Tg2576 mice treated with passive immunotherapy during the 4 to 13 month period (1A9 administration group, n=13; 2C3 administration group, n=11) (FIG. **10**A, Aβ x-40; FIG. **10**B, Aβ x-42). There was also no significant difference in the Aβ40/42 ratio (FIG. **10**C).

[0177] In order to elucidate the mechanism underlying the preventive effect of passive immunotherapy with 1A9 and 2C3 (IVIg) against the AD-like phenotype in Tg2576 mice, the present inventors assessed the level of physiological saline-soluble and -insoluble Aβ oligomers in pooled brain homogenates, and the level of Aβ oligomers in the peripheral blood and plasma. There was no difference in the amount of physiological saline-soluble Aβ oligomers in the pooled brain homogenates among the treatment groups (FIG. **10**D). Meanwhile, the amount of insoluble Aβ oligomers was shown to be reduced in the 1A9 and 2C3 treatment groups (FIG. **10**E). Furthermore, pooled plasma from each group (albumin-depleted plasma, upper part of Panel F; albumin/lipoprotein-depleted plasma, lower part of Panel F) was assayed for Aβ oligomers by A11 dot blotting. The result shows that the oligomers were present in the plasma from PBS-administered Tg2576 mice (FIG. **10**F). A11-positive oligomers in plasma were clearly increased in the passive immunotherapy groups as compared to the PBS administration group (FIG. **10**F). The proportion of 2C3-recognized oligomers in a lipoprotein-bound form was greater than that of 1A9-recognized oligomers (lower part of Panel F). Furthermore, plasma Aβ oligomers were detected by A11 immunoprecipitation. The result shows that the oligomers of about 200 kDa were increased in Tg2576 mice treated with passive immunotherapy as compared to the PBS administration group (FIG. **10**G). The increase in plasma Aβ oligomers in the passive immunotherapy groups can be considered to directly reflect enhanced cerebral clearance. Thus, the present inventors obtained evidence that direct target molecules for intravenous passive immunotherapy are also present in blood in addition to brain, and that oligomer-selective cerebral clearance can be enhanced through peripheral sites of action. That is, the present inventors showed the clinical usefulness of the intravenous passive immunotherapy.

Example 11

Formation of Senile Amyloid Plaques and Swollen Dystrophic Neurites Can Be Suppressed by Passive Immunotherapy Using 1A9 and 2C3

[0178] Immunohistochemical Aβ deposition was suppressed in the passive immunotherapy groups (FIG. **11**A). The formation of thioflavin S-positive senile amyloid plaques was significantly suppressed in both the cerebral cortex and hippocampus (FIG. **11**B, upper part), and the reduction was also clearly demonstrated by histochemistry (FIG. **11**B, lower part). The formation of synaptophysin-positive swollen dystrophic neurites was also significantly suppressed in the passive immunotherapy groups (FIG. **11**C).

Example 12

Immunostaining Analysis Using Anti-Synaptophysin and Anti-Drebrin Antibodies

[0179] 1A9 and 2C3 suppressed the presynaptic and postsynaptic degeneration in the cerebral neocortex (FIG. **12**).

Example 13

The Antibodies Translocate to the Brain

[0180] The existence/localization of deposited Aβ and cerebral mouse IgG was assessed using a confocal laser microscope. The result shows that mouse IgG is localized almost independently of deposited Aβ within the areas containing diffuse senile plaques. Mouse IgG was observed only in the passive immunotherapy groups (1A9 (FIG. **13**A) and 2C3 (FIG. **13**B)), but not in the PBS administration group (FIG. **13**C). Thus, a fraction of the antibodies administered into the blood was considered to translocate to the brain. This result shows that the preventive effect on memory disturbance was produced not only through the direct neutralization of the toxicity of soluble Aβ polymers by the antibodies translocated to the brain, but also through the clearance of soluble Aβ polymers in the form of a complex with the antibodies into the blood. Thus, the therapeutic effect was considered to be based on multiple action mechanisms.

Example 14

The Antibodies (E12, 1C10, and 4D3) that Do Not Recognize Other Aβ Monomers but Are Oligomer-Specific

[0181] 1C10 and 4D3 are the two strongly positive cells from the 24 positive-cell wells among the 386 wells containing hybridomas obtained by reimmunization of mice. 50 μM seed-free Aβ 1-40 (monomer) was prepared from Synthetic Aβ 1-40 (HCl form) which readily forms a beta sheet structure from a random coil structure, and this was incubated at 37° C. for 0 to 96 hours to produce oligomers, and the oligomer specificity of the antibodies was examined by dot blotting. It was confirmed that, as with 2C3 described above, E12, 1C10, and 4D3 (all IgM isotype) do not react with monomers but are oligomer specific (FIG. **14**).

Example 15

Immuno-Dot Blot Analysis of the E12 Antibody Whose Class Was Changed to IgG2b

[0182] Immuno-dot blot analysis was performed on the E12 antibody whose isotype was changed to IgG2b by a conventional method. As a result, the E12 antibody (IgG2b isotype) was found to be an antibody that does not recognize Aβ monomers (0 hr), but binds specifically to Aβ oligomers

(FIG. **15**). Furthermore, the characteristics of the antibody were confirmed not to be altered as a result of the isotype change.

Example 16

The Aβ Amyloid Fibril Formation-Suppressing Activity of the E12 Antibody Whose Class Has Been Changed to IgG2b

[0183] To assess whether the E12 antibody (IgG2b isotype) has an activity of suppressing Aβ amyloid fibril formation, Aβ amyloid fibril formation was detected by the above-described ThT fluorescence intensity assay method in a solution (medium) whose composition was the same as that used in the Aβ-induced neurotoxicity experiment. As a result, the Aβ amyloid fibril formation was observed to be suppressed in the presence of 1.5 μM of the E12 antibody (FIG. **16**).

Discussion

[0184] The data obtained by the present inventors show that monoclonal 1A9 and 2C3 specifically recognize the "neurotoxic epitope" and "polymerization epitope" of soluble Aβ polymers that are responsible for the toxic activity and neurofibril formation activity. Since monoclonal 1A9 and 2C3 do not react with soluble Aβ monomers, which are physiological molecules, it can be concluded that a three-dimensional structure having the epitope that is recognized by 1A9 or 2C3 is specific to soluble oligomeric polymers. The experiments using ultrafiltration and molecular sieve revealed that the size of 1A9- and 2C3-immunoreactive oligomers is greater than 100 kDa (>20mer). The result of morphological observation by AFM demonstrated that the toxic polymers are morphologically heterogeneous (granular, bead-shaped, and ring-shaped).

[0185] To demonstrate that the toxic polymers are actually bioactive molecules that exhibit in vivo synaptic toxicity, the present inventors commenced treatment of young Tg2576 mice before the onset of memory disturbance with anti-Aβ oligomer passive immunotherapy targeting 1A9- and 2C3-recognized toxic polymers. For the first time, the present inventors presented evidence supporting that age-dependent memory deterioration that naturally develops in Tg2576 mice can be prevented by passive immunotherapy using anti-Aβ oligomer-specific antibodies (1A9 and 2C3). Herein, short-term memory disturbance assessed by the Y-maze test is similar to the Aβ accumulation-associated memory disturbance observed in mild cognitive impairment (MCI) and early AD. The Y-maze test showed excellent and almost normal results in Tg2576 mice administered with 1A9 and 2C3, respectively. When assessed by the novel object recognition task, Morris water maze, and contextual fear conditioning task, the long-teen memory was maintained nearly normal by the anti-Aβ oligomer antibodies.

[0186] A selective increase in A11-positive oligomers in blood was observed in the antibody-treated mouse groups as compared to the PBS treatment group, which is consistent with the ability of the antibodies to prevent the onset of memory disturbance (the memory maintenance ability). The 1A9 antibody treatment also exhibited the effect of suppressing cerebral Aβ accumulation. The 2C3 antibody treatment demonstrated a higher blood level of A11-positive oligomers as compared to the 1 A9 antibody treatment. However, the cerebral Aβ accumulation-suppressing effect of the 2C3 antibody treatment was unclear. Accordingly, 1A9-recognized oligomers were considered to have greater contribution to the cerebral Aβ accumulation than 2C3-recognized oligomers. The involvement of the polymers in cerebral Aβ accumulation can be explained based on the following assumption: neurotoxic 1A9 polymers are soluble toxic oligomers that are somewhat conformationally, while neurotoxic 2C3 polymers are very unstable, short-lived oligomeric intermediates that appear at an early stage of the polymerization process, the conformation of which is easily changed.

[0187] The present inventors disclose herein the in vivo preventive effect of anti-oligomer antibodies on Alzheimer's disease, and this is the first evidence that directly demonstrates that toxic Aβ oligomers formed in vivo can inhibit the functions of nerve cells, thereby inducing the symptoms of Alzheimer's disease.

[0188] The data obtained by the present inventors is also the first evidence supporting the view that Aβ exhibits in vivo neurotoxicity in the human brain. It is well known that the human entorhinal cortex is an area that is easily affected with AD. In this area, NFT formation and nerve cell loss precede the formation of senile plaques. Thus, the entorhinal cortex is an exceptional area to which the commonly accepted amyloid cascade hypothesis cannot be applied. However, this inconsistency has been neglected and remained unstudied for a long time.

[0189] The present inventors proposed and examined the hypothesis that previously unidentifiable, invisible Aβ oligomers are harmful for nerve cells in the entorhinal cortex and cause memory disturbance. To examine this hypothesis, the present inventors performed semi-quantitative analysis of 1A9- and 2C3-immunoreactive 12mer in the entorhinal cortex of elderly individuals who were mostly at Braak NFT stages I to III. The 1A9- and 2C3-immunoreactive 12mer were already present in the entorhinal cortex of healthy individuals at Braak NFT stages I to II, and increased with the advancement of Braak NFT stage. The 12mer was found to be significantly increased in AD. Thus, the appearance of 1A9- and 2C3-immunoreactive 12mer was demonstrated to precede the onset of cognitive impairment in the human brain. On the other hand, by biochemical and immunohistochemical techniques, it was demonstrated that senile plaques contain 1A9- and 2C3-immunoreactive Aβ oligomers. In addition, insolubilized amyloid fibrils themselves were revealed to have an activity of neutralizing the neurotoxicity. These findings suggest that, under conditions where Aβ oligomers are present without senile plaque formation, Aβ oligomers exert in vivo toxicity and thus can be a cause of memory disturbance.

[0190] As described above, the data of the present inventors show for the first time evidence that directly demonstrates in vivo the memory disturbance resulting from synaptic dysfunction caused by endogenous Aβ oligomers. Although active immunotherapy (Janus D, 2000, Nature; Morgan D, 2000, Nature) and passive immunotherapy (Bard F, 2222, Nat med; DeMattos R B, PNAS, 2001) have been used previously, the mechanism by which learning disability and memory disturbance can be prevented has remained a matter of conjecture. One widely proposed possibility is that the antibodies reach the brain through the blood-brain barrier and directly neutralize in vivo soluble Aβ oligomers that cause memory impairment. The second possibility, the "sink theory", is that the antibodies act peripherally to deplete the peripheral blood Aβ pool and thus activate Aβ clearance from the brain. DeMattos et al. have reported that a peripherally administered anti-Aβ antibody rapidly transports not only cerebral Aβ monomers but also Aβ dimers into plasma, and also cerebral Aβ into CSF (DeMattos R B et al., PNAS, 98; 8850-8855, 2001). The present inventors also revealed that Aβ oligomers are present in human CSF and increased in AD patients. Thus, the present inventors demonstrated that the Aβ oligomers can be used as diagnostic markers for AD. Furthermore, the present inventors presented the first evidence supporting the view that Aβ oligomers are present in the plasma of Tg2576 mice, and, in passive immunotherapy by which Aβ oligomers are specifically captured and neutralized through intravenous injection, intracerebral antibody delivery is not required and the clearance of Aβ oligomers from the brain to blood can be enhanced at the peripheral sites of action, i.e., blood vessels. In addition, the present inventors presented the first evidence that passive immunotherapy can suppress senile amyloid plaque formation, and indirectly suppress nerve cell damage (swollen dystrophic neurite formation) through senile amyloid plaque suppression. These results confirm that the Aβ oligomer is the molecular basis for the onset of Alzheimer's disease, and selective control using oligomer-specific antibodies enables the control of Alzheimer's disease from a prophylactic viewpoint, in addition to a therapeutic viewpoint. Furthermore, a fraction of the administered antibodies was proven to translocate into the brain. This suggests that the effect of suppressing memory disturbance is exerted by a combination of multiple actions such as direct neutralization of soluble Aβ oligomers in the brain, transport of antibody-Aβ oligomer immune complexes into blood by the neonatal Fc receptor (Deane R, 2005, J Neurosci), and the "sink" action described above.

[0191] The establishment of accurate pre-onset diagnosis to identify cases at a high risk of developing AD is essential to design preventive/therapeutic strategies. The significant increase in the CSF O/M ratio in AD, which is reported herein, is expected to be one of the leading candidates for pre-onset diagnostic markers.

INDUSTRIAL APPLICABILITY

[0192] The antibodies provided by the present invention can be used, for example, in intravenous injection-based preventive passive immunotherapy for Alzheimer's disease, and as biological markers for pre-onset diagnosis, disease monitoring, drug efficacy monitoring/assessment for the disease, and such.

[0193] Furthermore, the antibodies of the present invention are expected to greatly contribute to the establishment of preventive/therapeutic methods for Alzheimer's disease that are selective to molecules responsible for evoking the pathological conditions of the disease, and the establishment of early diagnostic markers. The present inventors obtained evidence supporting that antibody therapies, even when they target intracerebral pathological conditions, can be satisfactorily achieved by peripheral intravenous administration, without the need to consider intracerebral transfer of the antibodies. In addition, the present inventors obtained evidence demonstrating that a fraction of administered antibodies translocates to the brain and produces a direct effect even in peripheral intravenous administration therapy, again without the need to consider intracerebral transfer of the antibodies. Thus, the present invention is expected to rapidly accelerate the progress of antibody therapeutics for Alzheimer's disease.

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<213> ORGANISM: Mus musculus

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

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

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

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

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

Ala Arg His Arg Glu Val Arg Pro Trp Ala Tyr Trp Gly Gln Gly Thr
            100                 105                 110

Leu Val Thr Val Ser Ala Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro
        115                 120                 125
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Leu Ala Pro Gly Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu Gly
    130                 135                 140

Cys Leu Val Lys Gly Tyr Phe Pro Glu Ser Val Thr Val Thr Trp Asn
145                 150                 155                 160

Ser Gly Ser Leu Ser Ser Ser Val His Thr Phe Pro Ala Leu Leu Gln
                165                 170                 175

Ser Gly Leu Tyr Thr Met Ser Ser Ser Val Thr Val Pro Ser Ser Thr
            180                 185                 190

Trp Pro Ser Gln Thr Val Thr Cys Ser Val Ala His Pro Ala Ser Ser
        195                 200                 205

Thr Thr Val Asp Lys Lys Leu Glu Pro Ser Gly Pro Ile Ser Thr Ile
    210                 215                 220

Asn Pro Cys Pro Pro Cys Lys Glu Cys His Lys Cys Pro Ala Pro Asn
225                 230                 235                 240

Leu Glu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Asn Ile Lys Asp
                245                 250                 255

Val Leu Met Ile Ser Leu Thr Pro Lys Val Thr Cys Val Val Val Asp
            260                 265                 270

Val Ser Glu Asp Asp Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn
        275                 280                 285

Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn
    290                 295                 300

Ser Thr Ile Arg Val Val Ser Thr Leu Pro Ile Gln His Gln Asp Trp
305                 310                 315                 320

Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro
                325                 330                 335

Ser Pro Ile Glu Arg Thr Ile Ser Lys Ile Lys Gly Leu Val Arg Ala
            340                 345                 350

Pro Gln Val Tyr Ile Leu Pro Pro Pro Ala Glu Gln Leu Ser Arg Lys
        355                 360                 365

Asp Val Ser Leu Thr Cys Leu Val Val Gly Phe Asn Pro Gly Asp Ile
    370                 375                 380

Ser Val Glu Trp Thr Ser Asn Gly His Thr Glu Glu Asn Tyr Lys Asp
385                 390                 395                 400

Thr Ala Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe Ile Tyr Ser Lys
                405                 410                 415

Leu Asn Met Lys Thr Ser Lys Trp Glu Lys Thr Asp Ser Phe Ser Cys
            420                 425                 430

Asn Val Arg His Glu Gly Leu Lys Asn Tyr Tyr Leu Lys Lys Thr Ile
        435                 440                 445

Ser Arg Ser Pro Gly Lys
    450
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<210> SEQ ID NO 2
<211> LENGTH: 1365
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 2

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tcctgtgcag cctctggatt cactttcagt agctatggca tgtcttgggt tcgccagact     120
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ccagacaaga ggctggagtg ggtcgcaacc attagtagtg gtggtagtta cacctactat    180

ccagacagtg tgaaggggcg attcaccatc tccagagaca atgccaagaa caccctgtac    240

ctgcaaatga gcagtctgaa gtctgaggac acagccatgt attactgtgc aagacatagg    300

gaggtacgcc cctgggctta ctggggccaa gggactctgg tcactgtctc tgcagccaaa    360

acaacacccc catcagtcta tccactggcc cctgggtgtg gagatacaac tggttcctcc    420

gtgactctgg gatgcctggt caagggctac ttccctgagt cagtgactgt gacttggaac    480

tctggatccc tgtccagcag tgtgcacacc ttcccagctc tcctgcagtc tggactctac    540

actatgagca gctcagtgac tgtcccctcc agcacctggc caagtcagac cgtcacctgc    600

agcgttgctc acccagccag cagcaccacg gtggacaaaa aacttgagcc cagcgggccc    660

atttcaacaa tcaacccctg tcctccatgc aaggagtgtc acaaatgccc agctcctaac    720

ctcgagggtg gaccatccgt cttcatcttc cctccaaata tcaaggatgt actcatgatc    780

tccctgacac ccaaggtcac gtgtgtggtg gtggatgtga gcgaggatga cccagacgtc    840

cagatcagct ggtttgtgaa caacgtggaa gtacacacag ctcagacaca aacccataga    900

gaggattaca acagtactat ccgggtggtc agcaccctcc ccatccagca ccaggactgg    960

atgagtggca aggagttcaa atgcaaggtc aacaacaaag acctcccatc acccatcgag   1020

agaaccatct caaaaattaa agggctagtc agagctccac aagtatacat cttgccgcca   1080

ccagcagagc agttgtccag gaaagatgtc agtctcactt gcctggtcgt gggcttcaac   1140

cctggagaca tcagtgtgga gtggaccagc aatgggcata cagaggagaa ctacaaggac   1200

accgcaccag tcctggactc tgacggttct tacttcatat atagcaagct caatatgaaa   1260

acaagcaagt gggagaaaac agattccttc tcatgcaacg tgagacacga gggtctgaaa   1320

aattactacc tgaagaagac catctcccgg tctccgggta aatga                   1365
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<210> SEQ ID NO 3
<211> LENGTH: 214
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 3

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Asp Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Val Gly
1               5                   10                  15

Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Gly Thr Asn
            20                  25                  30

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

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

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

Glu Asp Leu Ala Glu Tyr Phe Cys Gln Gln Tyr Asn Ser Tyr Pro Tyr
                85                  90                  95

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

Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly
        115                 120                 125

Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Lys Asp Ile
    130                 135                 140
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-continued

Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly Val Leu
145 150 155 160

Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr Ser Met Ser
165 170 175

Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His Asn Ser Tyr
180 185 190

Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro Ile Val Lys Ser
195 200 205

Phe Asn Arg Asn Glu Cys
210

<210> SEQ ID NO 4
<211> LENGTH: 645
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 4

gacattgtga tgacccagtc tcaaaaattc atgtccacat cagtaggaga cagggtcagc 60

gtcacctgca aggccagtca gaatgtgggt actaatgtag cctggtatca acagaaacca 120

gggcaatctc ctaaagcact gatttactcg gcatcctacc ggtacagtgg agtccctgat 180

cgcttcacag gcagtggatc tgggacagat ttcactctca ccatcagcaa tgtgcagtct 240

gaagacttgg cagagtattt ctgtcagcaa tataacagct atccgtacac gttcggaggg 300

gggaccaagc tggaaataaa acgggctgat gctgcaccaa ctgtatccat cttcccacca 360

tccagtgagc agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac 420

cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa tggcgtcctg 480

aacagttgga ctgatcagga cagcaaagac agcacctaca gcatgagcag caccctcacg 540

ttgaccaagg acgagtatga acgacataac agctatacct gtgaggccac tcacaagaca 600

tcaacttcac ccattgtcaa gagcttcaac aggaatgagt gttag 645

<210> SEQ ID NO 5
<211> LENGTH: 118
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 5

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

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

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

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

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

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

Ala Arg His Arg Glu Val Arg Pro Trp Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ala

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115

<210> SEQ ID NO 6
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<212> TYPE: DNA
<213> ORGANISM: Mus musculus

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tcctgtgcag cctctggatt cactttcagt agctatggca tgtcttgggt tcgccagact 120

ccagacaaga ggctggagtg ggtcgcaacc attagtagtg gtggtagtta cacctactat 180

ccagacagtg tgaaggggcg attcaccatc tccagagaca atgccaagaa caccctgtac 240

ctgcaaatga gcagtctgaa gtctgaggac acagccatgt attactgtgc aagacatagg 300

gaggtacgcc cctgggctta ctggggccaa gggactctgg tcactgtctc tgca 354

<210> SEQ ID NO 7
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 7

Asp Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Val Gly
1 5 10 15

Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Gly Thr Asn
20 25 30

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

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

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

Glu Asp Leu Ala Glu Tyr Phe Cys Gln Gln Tyr Asn Ser Tyr Pro Tyr
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> SEQ ID NO 8
<211> LENGTH: 321
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 8

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gtcacctgca aggccagtca gaatgtgggt actaatgtag cctggtatca acagaaacca 120

gggcaatctc ctaaagcact gatttactcg gcatcctacc ggtacagtgg agtccctgat 180

cgcttcacag gcagtggatc tgggacagat ttcactctca ccatcagcaa tgtgcagtct 240

gaagacttgg cagagtattt ctgtcagcaa tataacagct atccgtacac gttcggaggg 300

gggaccaagc tggaaataaa a 321

<210> SEQ ID NO 9
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

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<400> SEQUENCE: 9

Ser Tyr Gly Met Ser
1 5

<210> SEQ ID NO 10
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 10

agctatggca tgtct 15

<210> SEQ ID NO 11
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 11

Thr Ile Ser Ser Gly Gly Ser Tyr Thr Tyr
1 5 10

<210> SEQ ID NO 12
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 12

accattagta gtggtggtag ttacacctac 30

<210> SEQ ID NO 13
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 13

His Arg Glu Val Arg Pro Trp Ala Tyr
1 5

<210> SEQ ID NO 14
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 14

catagggagg tacgcccctg ggcttac 27

<210> SEQ ID NO 15
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 15

Lys Ala Ser Gln Asn Val Gly Thr Asn Val Ala
1 5 10

<210> SEQ ID NO 16
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 16

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aaggccagtc agaatgtggg tactaatgta gcc                          33


<210> SEQ ID NO 17
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 17

Ser Ala Ser Tyr Arg Tyr Ser
1               5


<210> SEQ ID NO 18
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 18

tcggcatcct accggtacag t                                       21


<210> SEQ ID NO 19
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 19

Gln Gln Tyr Asn Ser Tyr Pro Tyr Thr
1               5


<210> SEQ ID NO 20
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 20

cagcaatata acagctatcc gtacacg                                 27


<210> SEQ ID NO 21
<211> LENGTH: 461
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 21

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

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

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

Met Gly Tyr Lys Arg Tyr Asp Gly Ser Asn Asn Tyr Asn Pro Ser Leu
    50                  55                  60

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

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

Ala Arg Glu Ala Tyr Tyr Tyr Arg Tyr Asp Gly Gly Leu Tyr Tyr Phe
            100                 105                 110

Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr
        115                 120                 125
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Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Cys Gly Asp Thr Thr
    130                 135                 140

Gly Ser Ser Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu
145                 150                 155                 160

Ser Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Ser Val His
                165                 170                 175

Thr Phe Pro Ala Leu Leu Gln Ser Gly Leu Tyr Thr Met Ser Ser Ser
            180                 185                 190

Val Thr Val Pro Ser Ser Thr Trp Pro Ser Gln Thr Val Thr Cys Ser
        195                 200                 205

Val Ala His Pro Ala Ser Ser Thr Thr Val Asp Lys Lys Leu Glu Pro
    210                 215                 220

Ser Gly Pro Ile Ser Thr Ile Asn Pro Cys Pro Pro Cys Lys Glu Cys
225                 230                 235                 240

His Lys Cys Pro Ala Pro Asn Leu Glu Gly Gly Pro Ser Val Phe Ile
                245                 250                 255

Phe Pro Pro Asn Ile Lys Asp Val Leu Met Ile Ser Leu Thr Pro Lys
            260                 265                 270

Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln
        275                 280                 285

Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln
    290                 295                 300

Thr His Arg Glu Asp Tyr Asn Ser Thr Ile Arg Val Val Ser Thr Leu
305                 310                 315                 320

Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu Phe Lys Cys Lys
                325                 330                 335

Val Asn Asn Lys Asp Leu Pro Ser Pro Ile Glu Arg Thr Ile Ser Lys
            340                 345                 350

Ile Lys Gly Leu Val Arg Ala Pro Gln Val Tyr Ile Leu Pro Pro Pro
        355                 360                 365

Ala Glu Gln Leu Ser Arg Lys Asp Val Ser Leu Thr Cys Leu Val Val
    370                 375                 380

Gly Phe Asn Pro Gly Asp Ile Ser Val Glu Trp Thr Ser Asn Gly His
385                 390                 395                 400

Thr Glu Glu Asn Tyr Lys Asp Thr Ala Pro Val Leu Asp Ser Asp Gly
                405                 410                 415

Ser Tyr Phe Ile Tyr Ser Lys Leu Asn Met Lys Thr Ser Lys Trp Glu
            420                 425                 430

Lys Thr Asp Ser Phe Ser Cys Asn Val Arg His Glu Gly Leu Lys Asn
        435                 440                 445

Tyr Tyr Leu Lys Lys Thr Ile Ser Arg Ser Pro Gly Lys
    450                 455                 460
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<210> SEQ ID NO 22
<211> LENGTH: 1386
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 22

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acctgctctg tcactggcta ctccatcacc agtggttatt actggaactg gatccggcag     120
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tttccaggaa acaaactgga atggatgggc tacaaaaggt acgacggtag caataactac    180

aacccatctc tcaaaaatcg aatctccatc actcgtgaca catctaagaa ccagtttttc    240

ctgaagttga attctgtgac tactgaggac acagctacat attactgtgc aagagaggcc    300

tactactata ggtacgacgg gggcctatac tactttgact actggggcca aggcaccact    360

ctcacagtct cctcagccaa aacaacaccc ccatcagtct atccactggc ccctgggtgt    420

ggagatacaa ctggttcctc cgtgactctg ggatgcctgg tcaagggcta cttccctgag    480

tcagtgactg tgacttggaa ctctggatcc ctgtccagca gtgtgcacac cttcccagct    540

ctcctgcagt ctggactcta cactatgagc agctcagtga ctgtcccctc cagcacctgg    600

ccaagtcaga ccgtcacctg cagcgttgct cacccagcca gcagcaccac ggtggacaaa    660

aaacttgagc ccagcgggcc catttcaaca atcaacccct gtcctccatg caaggagtgt    720

cacaaatgcc cagctcctaa cctcgagggt ggaccatccg tcttcatctt ccctccaaat    780

atcaaggatg tactcatgat ctccctgaca cccaaggtca cgtgtgtggt ggtggatgtg    840

agcgaggatg acccagacgt ccagatcagc tggtttgtga acaacgtgga agtacacaca    900

gctcagacac aaacccatag agaggattac aacagtacta tccgggtggt cagcaccctc    960

cccatccagc accaggactg gatgagtggc aaggagttca aatgcaaggt caacaacaaa   1020

gacctcccat cacccatcga gagaaccatc tcaaaaatta aagggctagt cagagctcca   1080

caagtataca tcttgccgcc accagcagag cagttgtcca ggaaagatgt cagtctcact   1140

tgcctggtcg tgggcttcaa ccctggagac atcagtgtgg agtggaccag caatgggcat   1200

acagaggaga actacaagga caccgcacca gtcctggact ctgacggttc ttacttcata   1260

tatagcaagc tcaatatgaa aacaagcaag tgggagaaaa cagattcctt ctcatgcaac   1320

gtgagacacg agggtctgaa aaattactac ctgaagaaga ccatctcccg gtctccgggt   1380

aaatga                                                              1386
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<210> SEQ ID NO 23
<211> LENGTH: 214
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 23

Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly
1               5                   10                  15

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

Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile
        35                  40                  45

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

Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu His
65                  70                  75                  80

Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Tyr
                85                  90                  95

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

Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly
        115                 120                 125
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Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Lys Asp Ile
    130                 135                 140

Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly Val Leu
145                 150                 155                 160

Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr Ser Met Ser
                165                 170                 175

Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His Asn Ser Tyr
            180                 185                 190

Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro Ile Val Lys Ser
        195                 200                 205

Phe Asn Arg Asn Glu Cys
    210


<210> SEQ ID NO 24
<211> LENGTH: 645
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 24

gatatccaga tgacacagac tacatcctcc ctgtctgcct ctctgggaga cagagtcacc      60

atcagttgca gggcaagtca ggacattagc aattatttaa actggtatca gcagaaacca     120

gatggaactg ttaaactcct gatctacttc acatcaagat tacactcagg agtcccatca     180

aggttcagtg gcagtgggtc tggaacagat tattctctca ccattagcaa cctggagcat     240

gaagatattg ccacttactt ttgccaacag ggtaatacgc ttccgtacac gttcggaggg     300

gggaccaagc tggaaataaa acgggctgat gctgcaccaa ctgtatccat cttcccacca     360

tccagtgagc agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac     420

cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa tggcgtcctg     480

aacagttgga ctgatcagga cagcaaagac agcacctaca gcatgagcag taccctcacg     540

ttgaccaagg acgagtatga acgacataac agctatacct gtgaggccac tcacaagaca     600

tcaacttcac ccattgtcaa gagcttcaac aggaatgagt gttag                     645


<210> SEQ ID NO 25
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 25

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

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

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

Met Gly Tyr Lys Arg Tyr Asp Gly Ser Asn Asn Tyr Asn Pro Ser Leu
    50                  55                  60

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

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

Ala Arg Glu Ala Tyr Tyr Tyr Arg Tyr Asp Gly Gly Leu Tyr Tyr Phe
            100                 105                 110
```

-continued

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Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
        115                 120                 125


<210> SEQ ID NO 26
<211> LENGTH: 375
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 26

gatgtacagc ttcaggagtc aggacctggc ctcgtgaaac cttctcagtc tctgtctctc     60

acctgctctg tcactggcta ctccatcacc agtggttatt actggaactg gatccggcag    120

tttccaggaa acaaactgga atggatgggc tacaaaaggt acgacggtag caataactac    180

aacccatctc tcaaaaatcg aatctccatc actcgtgaca catctaagaa ccagtttttc    240

ctgaagttga attctgtgac tactgaggac acagctacat attactgtgc aagagaggcc    300

tactactata ggtacgacgg gggcctatac tactttgact actggggcca aggcaccact    360

ctcacagtct cctca                                                     375


<210> SEQ ID NO 27
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 27

Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly
1               5                   10                  15

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

Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile
        35                  40                  45

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

Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu His
65                  70                  75                  80

Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Tyr
                85                  90                  95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105


<210> SEQ ID NO 28
<211> LENGTH: 321
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 28

gatatccaga tgacacagac tacatcctcc ctgtctgcct ctctgggaga cagagtcacc     60

atcagttgca gggcaagtca ggacattagc aattatttaa actggtatca gcagaaacca    120

gatggaactg ttaaactcct gatctacttc acatcaagat tacactcagg agtcccatca    180

aggttcagtg gcagtgggtc tggaacagat tattctctca ccattagcaa cctggagcat    240

gaagatattg ccacttactt ttgccaacag ggtaatacgc ttccgtacac gttcggaggg    300

gggaccaagc tggaaataaa a                                              321
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<210> SEQ ID NO 29
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 29

Ser Gly Tyr Tyr Trp Asn
1   5

<210> SEQ ID NO 30
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 30

agtggttatt actggaac   18

<210> SEQ ID NO 31
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 31

Tyr Lys Arg Tyr Asp Gly Ser Asn Asn
1   5

<210> SEQ ID NO 32
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 32

tacaaaaggt acgacggtag caataac   27

<210> SEQ ID NO 33
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 33

Glu Ala Tyr Tyr Tyr Arg Tyr Asp Gly Gly Leu Tyr Tyr Phe Asp Tyr
1   5   10   15

<210> SEQ ID NO 34
<211> LENGTH: 48
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 34

gaggcctact actataggta cgacgggggc ctatactact ttgactac   48

<210> SEQ ID NO 35
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 35

Arg Ala Ser Gln Asp Ile Ser Asn Tyr Leu Asn
1   5   10

<210> SEQ ID NO 36
<211> LENGTH: 33

-continued

<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 36

agggcaagtc aggacattag caattattta aac 33

<210> SEQ ID NO 37
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 37

Phe Thr Ser Arg Leu His Ser
1 5

<210> SEQ ID NO 38
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 38

ttcacatcaa gattacactc a 21

<210> SEQ ID NO 39
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 39

Gln Gln Gly Asn Thr Leu Pro Tyr Thr
1 5

<210> SEQ ID NO 40
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 40

caacagggta atacgcttcc gtacacg 27

<210> SEQ ID NO 41
<211> LENGTH: 455
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 41

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

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

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

Ala Glu Ile Arg Leu Lys Ser Asn Asn Tyr Ala Thr His Tyr Ala Glu
50 55 60

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

Val Tyr Leu Gln Met Asn Asn Leu Arg Ala Glu Asp Thr Gly Ile Tyr
85 90 95

Tyr Cys Thr Arg Glu Leu Arg Tyr Ala Met Asp Tyr Trp Gly Gln Gly

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            100                 105                 110

Thr Ser Val Thr Val Ser Ser Ala Lys Thr Thr Pro Pro Ser Val Tyr
        115                 120                 125

Pro Leu Ala Pro Gly Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu
    130                 135                 140

Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Ser Val Thr Val Thr Trp
145                 150                 155                 160

Asn Ser Gly Ser Leu Ser Ser Ser Val His Thr Phe Pro Ala Leu Leu
                165                 170                 175

Gln Ser Gly Leu Tyr Thr Met Ser Ser Ser Val Thr Val Pro Ser Ser
            180                 185                 190

Thr Trp Pro Ser Gln Thr Val Thr Cys Ser Val Ala His Pro Ala Ser
        195                 200                 205

Ser Thr Thr Val Asp Lys Lys Leu Glu Pro Ser Gly Pro Ile Ser Thr
    210                 215                 220

Ile Asn Pro Cys Pro Pro Cys Lys Glu Cys His Lys Cys Pro Ala Pro
225                 230                 235                 240

Asn Leu Glu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Asn Ile Lys
                245                 250                 255

Asp Val Leu Met Ile Ser Leu Thr Pro Lys Val Thr Cys Val Val Val
            260                 265                 270

Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile Ser Trp Phe Val Asn
        275                 280                 285

Asn Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg Glu Asp Tyr
    290                 295                 300

Asn Ser Thr Ile Arg Val Val Ser Thr Leu Pro Ile Gln His Gln Asp
305                 310                 315                 320

Trp Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn Lys Asp Leu
                325                 330                 335

Pro Ser Pro Ile Glu Arg Thr Ile Ser Lys Ile Lys Gly Leu Val Arg
            340                 345                 350

Ala Pro Gln Val Tyr Ile Leu Pro Pro Pro Ala Glu Gln Leu Ser Arg
        355                 360                 365

Lys Asp Val Ser Leu Thr Cys Leu Val Val Gly Phe Asn Pro Gly Asp
    370                 375                 380

Ile Ser Val Glu Trp Thr Ser Asn Gly His Thr Glu Glu Asn Tyr Lys
385                 390                 395                 400

Asp Thr Ala Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe Ile Tyr Ser
                405                 410                 415

Lys Leu Asn Met Lys Thr Ser Lys Trp Glu Lys Thr Asp Ser Phe Ser
            420                 425                 430

Cys Asn Val Arg His Glu Gly Leu Lys Asn Tyr Tyr Leu Lys Lys Thr
        435                 440                 445

Ile Ser Arg Ser Pro Gly Lys
    450                 455
```

<210> SEQ ID NO 42
<211> LENGTH: 1368
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 42

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gaagtgaagc ttgaggagtc tggaggaggc ttggtgcgac ctggaggatc catgaaactc     60
tcctgtgttg cctctggatt cactttcagt aactactgga tgaactgggt ccgccagtct    120
ccagagaagg ggctcgagtg ggttgctgaa attagattga aatctaataa ttatgcaaca    180
cattatgcgg agtctgtgaa agggaggttc accatctcaa gggatgattc caaaagtagt    240
gtctacctgc aaatgaacaa cttaagagct gaagacactg gcatttatta ctgtaccagg    300
gaactacggt atgctatgga ctactggggt caaggaacct cagtcaccgt ctcctcagcc    360
aaaacaacac ccccatcagt ctatccactg gcccctgggt gtggagatac aactggttcc    420
tccgtgactc tgggatgcct ggtcaagggc tacttccctg agtcagtgac tgtgacttgg    480
aactctggat ccctgtccag cagtgtgcac accttcccag ctctcctgca gtctggactc    540
tacactatga gcagctcagt gactgtcccc tccagcacct ggccaagtca gaccgtcacc    600
tgcagcgttg ctcacccagc cagcagcacc acggtggaca aaaaacttga gcccagcggg    660
cccatttcaa caatcaaccc ctgtcctcca tgcaaggagt gtcacaaatg cccagctcct    720
aacctcgagg gtggaccatc cgtcttcatc ttccctccaa atatcaagga tgtactcatg    780
atctccctga cacccaaggt cacgtgtgtg gtggtggatg tgagcgagga tgacccagac    840
gtccagatca gctggtttgt gaacaacgtg gaagtacaca cagctcagac acaaacccat    900
agagaggatt acaacagtac tatccgggtg gtcagcaccc tccccatcca gcaccaggac    960
tggatgagtg gcaaggagtt caaatgcaag gtcaacaaca aagacctccc atcacccatc   1020
gagagaacca tctcaaaaat taaagggcta gtcagagctc cacaagtata catcttgccg   1080
ccaccagcag agcagttgtc caggaaagat gtcagtctca cttgcctggt cgtgggcttc   1140
aaccctggag acatcagtgt ggagtggacc agcaatgggc atacagagga gaactacaag   1200
gacaccgcac cagtcctgga ctctgacggt tcttacttca tatatagcaa gctcaatatg   1260
aaaacaagca agtgggagaa aacagattcc ttctcatgca acgtgagaca cgagggtctg   1320
aaaaattact acctgaagaa gaccatctcc cggtctccgg gtaaatga                1368
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<210> SEQ ID NO 43
<211> LENGTH: 214
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 43

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

Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Gly Asn Ile His Asn Tyr
            20                  25                  30

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

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

Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Asn Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Gly Ser Tyr Tyr Cys Gln His Phe Trp Ser Thr Pro Arg
                85                  90                  95

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

Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly
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        115                 120                 125

Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Lys Asp Ile
    130                 135                 140

Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly Val Leu
145                 150                 155                 160

Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr Ser Met Ser
                165                 170                 175

Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His Asn Ser Tyr
            180                 185                 190

Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro Ile Val Lys Ser
        195                 200                 205

Phe Asn Arg Asn Glu Cys
    210


<210> SEQ ID NO 44
<211> LENGTH: 645
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 44

gacatccaga tgactcagtc tccagcctcc ctatctgcat ctgtgggaga aactgtcacc      60

atcacatgtc gagcaagtgg gaatattcac aattatttag catggtatca gcagaaacag     120

ggaaaatctc ctcagctcct ggtctataat gcaaaaacct tagcagatgg tgtgccatca     180

aggttcagtg gcagtggatc aggaacacaa tattctctca agatcaacag cctgcagcct     240

gaagattttg ggagttatta ctgtcaacat ttttggagta ctcctcggac gttcggtgga     300

ggcaccaagc tggaaatcaa acgggctgat gctgcaccaa ctgtatccat cttcccacca     360

tccagtgagc agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac     420

cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa tggcgtcctg     480

aacagttgga ctgatcagga cagcaaagac agcacctaca gcatgagcag taccctcacg     540

ttgaccaagg acgagtatga acgacataac agctatacct gtgaggccac tcacaagaca     600

tcaacttcac ccattgtcaa gagcttcaac aggaatgagt gttag                     645


<210> SEQ ID NO 45
<211> LENGTH: 119
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 45

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

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

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

Ala Glu Ile Arg Leu Lys Ser Asn Asn Tyr Ala Thr His Tyr Ala Glu
    50                  55                  60

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

Val Tyr Leu Gln Met Asn Asn Leu Arg Ala Glu Asp Thr Gly Ile Tyr
                85                  90                  95
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Tyr Cys Thr Arg Glu Leu Arg Tyr Ala Met Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Ser Val Thr Val Ser Ser
        115


<210> SEQ ID NO 46
<211> LENGTH: 357
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 46

gaagtgaagc ttgaggagtc tggaggaggc ttggtgcgac ctggaggatc catgaaactc     60

tcctgtgttg cctctggatt cactttcagt aactactgga tgaactgggt ccgccagtct    120

ccagagaagg ggctcgagtg ggttgctgaa attagattga aatctaataa ttatgcaaca    180

cattatgcgg agtctgtgaa agggaggttc accatctcaa gggatgattc caaaagtagt    240

gtctacctgc aaatgaacaa cttaagagct gaagacactg gcatttatta ctgtaccagg    300

gaactacggt atgctatgga ctactggggt caaggaacct cagtcaccgt ctcctca       357


<210> SEQ ID NO 47
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 47

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

Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Gly Asn Ile His Asn Tyr
            20                  25                  30

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

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

Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Asn Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Gly Ser Tyr Tyr Cys Gln His Phe Trp Ser Thr Pro Arg
                85                  90                  95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105


<210> SEQ ID NO 48
<211> LENGTH: 321
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 48

gacatccaga tgactcagtc tccagcctcc ctatctgcat ctgtgggaga aactgtcacc     60

atcacatgtc gagcaagtgg gaatattcac aattatttag catggtatca gcagaaacag    120

ggaaaatctc ctcagctcct ggtctataat gcaaaaacct tagcagatgg tgtgccatca    180

aggttcagtg gcagtggatc aggaacacaa tattctctca agatcaacag cctgcagcct    240

gaagattttg ggagttatta ctgtcaacat ttttggagta ctcctcggac gttcggtgga    300

ggcaccaagc tggaaatcaa a                                              321
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-continued

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<210> SEQ ID NO 49
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 49

Asn Tyr Trp Met Asn
1               5


<210> SEQ ID NO 50
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 50

aactactgga tgaac                                                      15


<210> SEQ ID NO 51
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 51

Glu Ile Arg Leu Lys Ser Asn Asn Tyr Ala Thr His
1               5                   10


<210> SEQ ID NO 52
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 52

gaaattagat tgaaatctaa taattatgca acacat                               36


<210> SEQ ID NO 53
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 53

Glu Leu Arg Tyr Ala Met Asp Tyr
1               5


<210> SEQ ID NO 54
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 54

gaactacggt atgctatgga ctac                                            24


<210> SEQ ID NO 55
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 55

Arg Ala Ser Gly Asn Ile His Asn Tyr Leu Ala
1               5                   10


<210> SEQ ID NO 56
<211> LENGTH: 33
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-continued

```
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 56

cgagcaagtg ggaatattca caattattta gca                              33


<210> SEQ ID NO 57
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 57

Asn Ala Lys Thr Leu Ala Asp
1               5


<210> SEQ ID NO 58
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 58

aatgcaaaaa ccttagcaga t                                           21


<210> SEQ ID NO 59
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 59

Gln His Phe Trp Ser Thr Pro Arg Thr
1               5


<210> SEQ ID NO 60
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 60

caacattttt ggagtactcc tcggacg                                     27
```

1. An antibody that binds to an Aβ oligomer, wherein the antibody does not bind to an Aβ monomer.

2. An antibody binding to an Aβ oligomer that binds to an antibody comprising an H chain having the amino acid sequence of SEQ ID NO: 1 and an L chain having the amino acid sequence of SEQ ID NO: 3.

3. An antibody binding to an Aβ oligomer that binds to an antibody comprising an H chain having the amino acid sequence of SEQ ID NO: 21 and an L chain having the amino acid sequence of SEQ ID NO: 23.

4. An antibody binding to an Aβ oligomer that binds to an antibody comprising an H chain having the amino acid sequence of SEQ ID NO: 41 and an L chain having the amino acid sequence of SEQ ID NO: 43.

5. An antibody of any one of (1) to (20) below:

(1) an antibody that comprises an H chain having the amino acid sequence of SEQ ID NO: 9 as CDR1, the amino acid sequence of SEQ ID NO: 11 as CDR2, and the amino acid sequence of SEQ ID NO: 13 as CDR3;

(2) an antibody that comprises an L chain having the amino acid sequence of SEQ ID NO: 15 as CDR1, the amino acid sequence of SEQ ID NO: 17 as CDR2, and the amino acid sequence of SEQ ID NO: 19 as CDR3;

(3) an antibody that comprises the H chain of (1) and the L chain of (2);

(4) an antibody that comprises an H chain having the amino acid sequence of SEQ ID NO: 5 as VH;

(5) an antibody that comprises an L chain having the amino acid sequence of SEQ ID NO: 7 as VL;

(6) an antibody that comprises the H chain of (4) and the L chain of (5);

(7) an antibody that comprises an H chain having the amino acid sequence of SEQ ID NO: 29 as CDR1, the amino acid sequence of SEQ ID NO: 31 as CDR2, and the amino acid sequence of SEQ ID NO: 33 as CDR3;

(8) an antibody that comprises an L chain having the amino acid sequence of SEQ ID NO: 35 as CDR1, the amino acid sequence of SEQ ID NO: 37 as CDR2, and the amino acid sequence of SEQ ID NO: 39 as CDR3;

(9) an antibody that comprises the H chain of (7) and the L chain of (8);

(10) an antibody that comprises an H chain having the amino acid sequence of SEQ ID NO: 25 as VH;
(11) an antibody that comprises an L chain having the amino acid sequence of SEQ ID NO: 27 as VL;
(12) an antibody that comprises the H chain of (10) and the L chain of (11);
(13) an antibody that comprises an H chain having the amino acid sequence of SEQ ID NO: 49 as CDR1, the amino acid sequence of SEQ ID NO: 51 as CDR2, and the amino acid sequence of SEQ ID NO: 53 as CDR3;
(14) an antibody that comprises an L chain having the amino acid sequence of SEQ ID NO: 55 as CDR1, the amino acid sequence of SEQ ID NO: 57 as CDR2, and the amino acid sequence of SEQ ID NO: 59 as CDR3;
(15) an antibody that comprises the H chain of (13) and the L chain of (14);
(16) an antibody that comprises an H chain having the amino acid sequence of SEQ ID NO: 45 as VH;
(17) an antibody that comprises an L chain having the amino acid sequence of SEQ ID NO: 47 as VL;
(18) an antibody that comprises the H chain of (16) and the L chain of (17);
(19) an antibody that comprises one or more amino acid substitutions, deletions, additions, and/or insertions in the antibody of any one of (1) to (18), which has equivalent activity to the antibody of any one of (1) to (18); and
(20) an antibody that binds to the epitope bound by the antibody of any one of (1) to (18).

6. The antibody of claim **5**, wherein the antibody is a chimeric antibody or a humanized antibody.

7. A composition comprising the antibody of any one of claims **1** to **5** and a pharmaceutically acceptable carrier.

8. A method for preventing and/or treating cognitive impairment, which comprises administering the antibody of any one of claims **1** to **5** as an active ingredient.

9. A method for preventing and/or treating Alzheimer's disease, which comprises administering the antibody of any one of claims **1** to **5** as an active ingredient.

10. A method for suppressing the progression of Alzheimer's disease, which comprises administering the antibody of any one of claims **1** to **5** as an active ingredient.

11. A method for suppressing senile plaque formation, which comprises administering the antibody of any one of claims **1** to **5** as an active ingredient.

12. A method for suppressing Aβ accumulation, which comprises administering the antibody of any one of claims **1** to **5** as an active ingredient.

13. A method for neutralizing neurotoxicity, which comprises administering the antibody of any one of claims **1** to **5** as an active ingredient.

14. A method for inhibiting Aβ amyloid fibril formation, which comprises administering the antibody of any one of claims **1** to **5** as an active ingredient.

15. A method for neutralizing synaptic toxicity, which comprises administering the antibody of any one of claims **1** to **5** as an active ingredient.

16. A method for detecting an Aβ oligomer, which comprises detecting an Aβ oligomer contained in a sample collected from a subject using the antibody of any one of claims **1** to **5**.

17. The method of claim **16**, wherein the sample is blood or cerebrospinal fluid.

18. A method of diagnosing whether or not a subject suffers from or is at a risk of developing Alzheimer's disease, which comprises using the antibody of any one of claims **1** to **5** to detect an Aβ oligomer in a sample collected from a subject.

19. A method of diagnosing whether or not a subject suffers from or is at a risk of developing Alzheimer's disease, which comprises:
(a) contacting a sample collected from a subject with the antibody of any one of claims **1** to **5**; and
(b) measuring the amount of Aβ oligomer in the sample,
wherein the subject is determined to suffer from or be at a risk of developing Alzheimer's disease, when the amount measured in (b) is higher than that of a healthy individual.

20. A method of diagnosing whether or not a subject suffers from or is at a risk of developing Alzheimer's disease, which comprises:
(a) contacting a sample collected from a subject with the antibody of any one of claims **1** to **5** and an antibody that binds to an Aβ monomer; and
(b) measuring the ratio of Aβ oligomer to Aβ monomer in the sample,
wherein the subject is determined to suffer from or be at a risk of developing Alzheimer's disease, when the ratio measured in (b) is higher than that of a healthy individual.

21. The method of claim **18**, wherein the sample is blood or cerebrospinal fluid.

* * * * *

专利名称(译)	抗体特异性结合β寡聚体及其用途		
公开(公告)号	US20100260783A1	公开(公告)日	2010-10-14
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[标]申请(专利权)人(译)	NAT癌症CENT		
申请(专利权)人(译)	IMMUNAS PHARMA , INC. 国家中心的老年医学和老年病学		
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优先权	2007272015 2007-10-19 JP 61/085540 2008-08-01 US		
外部链接	Espacenet USPTO		

摘要(译)

本发明人成功地制备了仅对可溶性Aβ寡聚体特异的单克隆抗体，但不识别作为生理分子的可溶性Aβ单体。已证明该抗体可用作阿尔茨海默病的诊断/治疗性单克隆抗体。

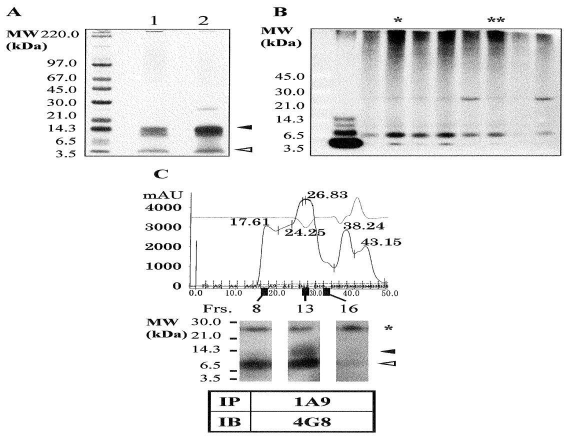

FIG. 1