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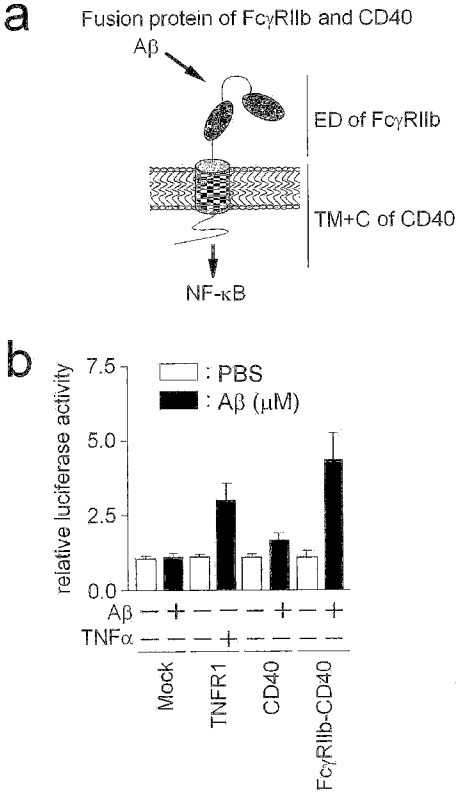
[Continued on next page]

(54) Title: COMPOSITIONS AND METHOD FOR THE DIAGNOSIS, PREVENTION AND TREAMENT OF ALZEIMER'S DISEASE

FIG. 8



(57) Abstract: The present disclosure is drawn to an agent for diagnosis, prophylaxis and treatment and a method for screening thereof. More particularly, an FcγRIIb inhibitor or antagonist selected from a group consisting of a soluble FcγRIIb, variants and extracellular domain proteins thereof, anti-FcγRIIb antibodies, FcγRIIb-specif ic peptides and FcγRIIb specific siRNAs and activity or expression inhibitors of intracellular kinases regulated by FcγRIIb can be used for diagnosis, prophylaxis and treatment of Alzheimer's disease by inhibiting signal transduction, cellular internalization, neuronal toxicity, apoptosis and loss of memory through inhibition of binding between FcγRIIb and Aβ.

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COMPOSITIONS AND METHOD FOR THE DIAGNOSIS, PREVENTION AND TREATMENT OF ALZHEIMER'S DISEASE

Field of the Invention

The present invention relates to methods of diagnosing, preventing and treating Alzheimer's disease based on the use of an inhibitor for the binding of amyloid-β to FcγRIIb, and a method of screening the inhibitor. More particularly, the present invention relates to methods of diagnosing, preventing and treating Alzheimer's disease using an inhibitor of the binding between amyloid-β and FcγRIIb, which is selected from the group consisting of an FcγRIIb protein or a variant thereof, an FcγRIIb extracellular domain, an anti-FcγRIIb antibody, a specific peptide and an FcγRIIb-specific siRNA, and a method of screening the inhibitor.

Background Art

About 50-70% of all people having dementia suffer from Alzheimer's disease (hereinafter, referred as "AD"), which is caused by the progressive degeneration of nerve cells in the brain, resulting in the loss of cognitive ability. AD is divided into two forms: familial AD, which has genetic links and runs in families, and sporadic AD, which develops in many people for no obvious reason. AD patients

typically have multiple cognitive deficiencies, which are manifested by memory impairment and psychological symptoms such as psychosomatic abnormalities, including increased anxiety and hypersensitivity.

Two pathological hallmarks are seen in the brains of patients who die of AD: senile plaques and neurofibrillary tangles. Senile plaques are extracellular accumulations of proteins and dead cells, and are primarily composed of amyloid-β (Aβ) peptides (Hardy, J. et al., *Nat Neurosci.* 1:355-358, 1998). The progressive loss of cognitive ability, which is the major pathological feature of AD patients, seems to be caused by the aberrant deposition of Aβ.

Aβ is produced from amyloid precursor protein (APP) through proteolytic cleavage. APP is cleaved by β-secretase (BACE) and γ-secretase, yielding Aβ (Craven, R., *Nat Rev. Neurosci*. 2: 533, 2001; David, H. S. *et al., Nat Rev. Neurosci.* 2: 595-598, 2001; Yankner, B. A., *Neuron* 16: 921-932, 1996; Selkoe, D. J., *Nature* 399: A23-A31, 1999).

Studies associated with AD to date resulted in the development of preventive and therapeutic agents for AD mainly using agents inhibiting Aβ production, such as secretase inhibitors, or inhibitors of neurotoxicity, such as antioxidants. Current medications for AD include

nicotinic receptor agonists, such as ABT-418; muscarinic receptor agonists, such as Xanomeline and YM-976; acetylcholine precursors, such as lecithin and acetyl-L-carnitine; metal chelators, such as desferrioxamine and clioquinol; beta-sheet breakers, such as iAβ5 and iAβ11; antioxidants, such as vitamin E, *Ginkgo biloba*, melatonin and idebenone; sAPP releasing agents, such as nicotine, acetylcholine and carbachol; β-secretase or γ-secretase inhibitors, such as OM99-1, OM99-2, OM99-3 and Z-VLL-CHO; non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen and indomethacin; hormones such as estrogen; vaccines, such as AN-1792; and cholesterol-lowering agents, such as simvastatin and atorvastatin. However, most medications are only marginally helpful in slightly relieving the pathological symptoms of AD or slowing AD progression, or are difficult to apply in practice due to their toxicity. Thus, there remains an urgent need for the development of stable and effective drugs for AD treatment.

Recent AD-associated studies have been focused on the identification of neurotoxic mechanisms of Aβ. Pro-apoptotic genes, such as prostate apoptosis response-4 (Par-4), tau protein kinase 1 (GSK-3β), Calsenilin/DREAM/KChIP3, and cell death-promoting gene 5 (DP5), are shown to be overexpressed or their activities

are increased in neuronal cells cultured in the presence of Aβ or neuronal cells from AD patients. The blocking of the functions of the proteins reduces Aβ-induced neuronal death (Guo, Q. *et al., Nat. Med.* **4**:597-562, 1998; Takashima, A. *et al., Proc. Natl. Acad. Sci. USA* **90**:7789-7793, 1993; Jo, D.G. *et al., FASEB J.* **15**:589-591, 2001; Imaizumi, K. *et al., J. Biol. Chem.* **274**:7975-7981, 1999). However, these reports are not sufficient to identify an intracellular signaling pathway for Aβ-induced neuronal toxicity so as to develop AD drugs for preventing Aβ-induced neuronal loss. To date, inhibitors of Aβ-induced neurotoxicity have not been found even *in vitro*.

An important step to define neurotoxic mechanisms of Aβ is to find a receptor for Aβ on neuronal cells. Many efforts have been made, but no specific receptor for Aβ has been identified yet. Several proteins interacting with Aβ, including receptors for advanced glycation end-product (RAGE) (Arancio, O. *et al., EMBO J.* 23:4096-4105, 2004) and amyloid-beta binding alcohol dehydrogenase (ABAD) (Takuma, K. *et al., FASEB J.* 19:597-598, 2005), were reported to be receptors for Aβ. However, such proteins have been shown to serve as cellular cofactors, rather than functioning to fundamentally modulate signal transduction in neuronal cells or neuronal toxicity. Thus, they are not likely to

be receptors for Aβ. This is because they were identified not using a knock-out method but through the observation that their overexpression increases signal transduction and neuronal toxicity.

On the other hand, Fcγ receptor IIb (hereinafter referred as "FcγRIIb"), expressed on immune cells, has been known to be a receptor having low binding affinity to immunoglobulin G. Individuals having a mutation in the FcγRIIb gene (FcγRIIb[I232T]), leading to abnormal immune responses, are susceptible to autoimmune diseases. Also, the FcγRIIb receptor has recently been known to play a regulatory role in arthritis (Nakamura, A. *et al.*, *Biomed. Pharmacother.* **58:** 292-298, 2004). However, the involvement of FcγRIIb in dementia and its potential as a therapeutic target for dementia have not been known.

The inventors of this application found for the first time that FcγRIIb serves as a receptor for Aβ as well as playing an immunoregulatory role. In particular, the present inventors found that FcγRIIb acts as a protein mediating Aβ neurotoxicity and serves as a receptor in an Aβ-initiated toxic signaling pathway, through which FcγRIIb binds Aβ as the first event of the toxic signaling in neuronal cells and transduces the cell death signal into the cells. The present inventors also found that FcγRIIb

enhances Aβ deposition, associated with memory impairment in AD, within neuronal cells. Based on these findings, the present inventors further found that an FcγRIIb protein or a variant thereof, an FcγRIIb extracellular domain, an anti-FcγRIIb antibody, an FcγRIIb-specific peptide and an FcγRIIb-specific siRNA suppress neuronal cell death and prevent memory loss in subjects, thereby leading to the present invention.

DISCLOSURE OF THE INVENTION

SUMMARY OF THE INVENTION

It is therefore an object of the present invention to provide an inhibitor for the binding of Aβ to FcγRIIb.

It is another object of the present invention to provide a method of screening an inhibitor for the binding of Aβ to FcγRIIb.

It is a further object of the present invention to provide a diagnostic method and a diagnostic kit for Alzheimer's disease.

It is yet another object of the present invention to provide a method of preventing and treating Alzheimer's disease.

In order to accomplish the above objects, the present

invention provides a method of preventing and treating Alzheimer's disease, by inhibiting binding of Aβ to FcγRIIb.

The present invention also provides an inhibitor for the binding of Aβ to FcγRIIb. The inhibitor includes an FcγRIIb protein or a variant thereof, an FcγRIIb extracellular domain, an anti-FcγRIIb antibody, an FcγRIIb-specific peptide, and an FcγRIIb-specific siRNA.

The present invention further provides a method of screening an agent inhibiting the interaction between Aβ and FcγRIIb. The screening method includes screening an agent inhibiting the activity of FcγRIIb, an agent suppressing the expression of FcγRIIb, an agent inhibiting the transduction of the toxic signal of Aβ into neuronal cells through FcγRIIb, and an agent inhibiting the interaction between Aβ and FcγRIIb. In addition, the ordinary skilled person may use computer software-based methods to identify the agent inhibiting the interaction between Aβ and FcγRIIb.

The present invention still further provides a method of diagnosing Alzheimer's disease comprising determining the expression level of FcγRIIb.

The present invention still further provides a kit for diagnosing Alzheimer's disease comprising reagents used for

determining the expression level of FcγRIIb.

The present invention still further provides a method of preventing and treating Alzheimer's disease based on the use of the method of inhibiting the interaction between Aβ and FcγRIIb.

DEFINITION OF TERMS

Hereinafter, we defines terms used in the present application.

A "peptide" means a molecule comprising two or more amino acids linked each other via peptide bond. The peptide may be chemically synthesized or prepared by common genetic engineering technologies.

An "siRNA" or "small interfering RNA" means an RNA molecule binding to a particular tagert mRNA and knock-out the mRNA, and may be a double stranded RNA molecule having a homologous sequence selected from the target mRNA and consisting of 17 to 25 consecutive nucleotide or may be a short hairpin RNA (hereinafter referred as "shRNA") comprising sequentially the homologous sequence, a loop and a complementary sequence of the homologous sequence thereby forming a stem-loop structure. The si RNA may be prepared by chemical synthesis of RNA oligonucleotides, in vitro transcription, cleavage of long dsRNA transcribed in vitro using RNase III or Dicer, expression through celluar transduction of plasmid or viral vector expressing siRNA or

cellular transduction of PCR-derived siRNA expression cassette.

A "variant" or "mutant" means a peptide having conserved amino acids for keeping the function thereof and whose one or more non-essential amino acids are substituted with different amino acids but reserving the original function.

An "inhibitor of interaction" means a substance inhibiting the interaction between proteins and includes compositions comprising a protein or a peptide inhibiting interaction between proteins or an antibody against the protein or the peptide or expression inhibitors.

An "expression inhibitor" means a material inhibiting transcription or translation of a particular gene. It includes compositions comprising molecules commonly used for inhibition of expression of the gene such as siRNA, shRNA, microRNA (hereinafter referred as "miRNA") and antisense oligonucleotide.

A "control group" means an experimental group treated with only buffer solution used for solving a tested compound or a compound known to have no effect on a target.

An "FcγRIIb chimeric protein" means a chimeric (or fusion) protein of FcγRIIb with an effector protein (a protein activating the expression, color development or color change of a specific protein or cellular signal transduction upon the interaction between FcγRIIb and Aβ).

An "Fcγ receptor IIb extracellular domain" means a portion of FcγRIIb which is exposed to extracellular space.

When recombinantly produced Fcγ receptor IIb extracellular domain is administrated to a subject, it can induce competitive inhibition with intrinsic Fcγ receptor IIbs.

An "antibody" means an immunoglobulin such as IgG, IgM and IgA or active fragment thereof such as Fab, $F(ab')_2$ and ScFv, which binds to a target antigen specifically. The antibody may be a polyclonal antibody prepared by immunizing a mammal such as a rabbit, a mouse, a rat or a goat through injecting a purified antigen along with an adjuvant; collecting blood from the mammal; and purifying antibodies using affinity column using protein A or G. In addition, the antibody may be a monoclonal antibody prepared by obtaining spleen cells from the immunized mammal; producing hybridoma with myeloma cells; and culturing the hybridoma under a selection medium. Further, the monoclonal antibody may be produced as a humanized antibody prepared by analyzing a gene encoding said monoclonal antibody; determining complementary determining region (hereinafter referred as "CDR"); reserving the CDR and replacing framework regions (hereinafter referred as "FRs") with human FRs. More preferably, a human antibody may be used by screening human antibody cDNA library using phage display technologies, etc.

DETAILED DESCRIPTION OF THE INVENTION

Hereinafter, the present invention will be described in detail.

I. The present invention provides a method of preventing and treating AD by inhibiting the binding of Aβ to FcγRIIb.

The present inventors identified a receptor for Aβ, which is the major pathological cause of AD, on neuronal cells, and, based on this finding, developed a method of preventing and treating AD by inhibiting the association between Aβ and FcγRIIb. The expression of FcγRIIb was found to increase in neuronal cells exposed to Aβ (FIG. 1). Then, a FcγRIIb wild type and a FcγRIIb variant were prepared and administered to neuronal cells along with Aβ. As a result, cells treated with the FcγRIIb variant were found to exhibit reduced cell death rates (FIG. 2). These results indicate that FcγRIIb mediates Aβ signaling.

The present inventors prepared siRNAs to suppress the transcription of FcγRIIb and RAGE, which is known to be a cell surface receptor for Aβ (FIG. 3a). The transcriptional suppression of RAGE expression did not result in any increase in cell survival, whereas all cells survived when the transcription of FcγRIIb was suppressed (FIG. 3b). These results indicate that FcγRIIb rather than

RAGE is the direct cell surface protein for Aβ signaling.

Then, an FcγRIIb extracellular domain was prepared, and neuronal cells exposed to Aβ were treated with this extracellular domain and examined for cell survival rates. The increased expression of FcγRIIb due to exposure to Aβ was suppressed (FIG. 4a), and the relative cell viability was increased to that of cells not exposed to Aβ (FIG. 4b). These results indicated that the FcγRIIb-mediated neuronal transduction of Aβ signaling was inhibited.

The *in vivo* distribution of Aβ and FcγRIIb was examined. Oligomeric Aβ and FcγRIIb were found to be co-localized in the brain tissue of Tg2576 mouse which is an animal model for AD, indicating that both of them are present in the same region (FIG. 5a). Also, Aβ was expressed together with FcγRIIb in brain specimens from AD patients, confirming that both of them are present in the same cells (FIG. 5b). The transcriptional suppression of RAGE expression did not result in a decrease in the intracellular accumulation of Aβ, whereas the transcriptional suppression of FcγRIIb expression markedly reduced intracellular Aβ accumulation (FIG. 6). These results indicate that FcγRIIb is involved in the intracellular accumulation of Aβ as well as in intracellular signal transduction of Aβ.

Based on the above results, the binding between Aβ and FcγRIIb was examined *in vitro*. The *in vitro* experiment revealed that Aβ binds to FcγRIIb (FIGs. 7 and 8).

In addition, the inventors of this application identified for the first time the structure of Aβ bound to FcγRIIb using a computer program. Researchers have made many efforts to determine the structure of Aβ, but failed to crystallize Aβ. Aβ is difficult to crystallize because it is present as amorphous aggregates, called amyloid plaques, in AD patients. However, recently, increasing evidence suggests that such Aβ aggregates are not directly involved in neuronal cell death, and that soluble oligomers of Aβ play a major role in neuronal toxicity. Recently, the three-dimensional structure of Aβ oligomers was determined by simulating the stable oligomerization of Aβ monomers using *in silico* methods (Urbanc, B. *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 101: 17345-17350, 2004). The present inventors predicted the FcγRIIb-bound structure of Aβ using the known three-dimensional structures of Aβ and FcγRIIb. This predicted structure showed that the structures of Aβ and FcγRIIb precisely fit together (FIG. 9). Aβ binds to an extracellular domain of FcγRIIb. Aβ is present in oligomeric forms, in which hydrophilic N-terminal regions are flexible and C-terminal regions

aggregate to form an oligomer. The present inventors found that extended N-terminal regions bind FcγRIIb to exert cytotoxicity using Insight II/Affinity program (Acelrys Co., USA).

When primary cultured neuronal cells from the cerebral cortex of rats were exposed to Aβ and treated with either IgG or IgA, Aβ neurotoxicity was blocked only in cultures treated with IgG, indicating that the Aβ binding site of FcγRIIb is identical to that for IgG. The co-crystal structure of Fcγ receptor IIIb, which has a structure similar to FcγRIIb, with IgG shows that a tryptophan pocket (Trp87 and Trp110) of FcγRIIIb interacts with a proline residue at 329 (Pro329) of IgG (Sondermann P. *et al.*, *Nature* 406(6793): 267-273, 2000). As well, a peptide containing a tryptophan pocket of FcγRIIb has been shown to inhibit the binding between IgG and FcγRIIb (Goldsmith, E. B. *et al.*, *Biochemistry*. 36(4): 952-959, 1997). Thus, the present inventors predicted the binding IgG and FcγRIIb also occurs in a tryptophan pocket (Trp92 and Trp115) and replaced two tryptophan residues (Trp92 and Trp115) of an FcγRIIb-CD40 chimeric protein with alanine. This replacement reduced NF-κB activation. The present inventors conducted an *in silico* simulation based on the above results. This simulation showed that a phenylalanine

residue at position 4 of Aβ makes a strong hydrophobic interaction with two tryptophan residues, Trp92 and Trp115, of FcγRIIb, an aspartate residue at position 7 of Aβ makes a strong hydrophilic interaction with two lysine residues, Lys116 and Lys118, of FcγRIIb, a glutamine residue at position 3 of Aβ makes a relatively weak interaction with Tyr165 of FcγRIIb, and Arg5 and His6 residues of Aβ rarely interact with residues of FcγRIIb.

Based on the structure of Aβ bound to FcγRIIb, the present inventors prepared peptides capable of inhibiting the binding between Aβ and FcγRIIb. The peptides were found to effectively inhibit the binding between Aβ and FcγRIIb (FIGs. 10 and 11).

The peptides were injected into the brain of mice, and the memory ability of mice was assessed. As a result, memory impairment, as seen in AD cases, was remarkably restored (FIG. 12). Also, the mice treated with the peptide inhibitors displayed no accumulation of the full length $A\beta_{1-42}$ peptide in the brain (FIG. 13).

II. The present invention provides interaction inhibitors inhibiting the interaction between Aβ and FcγRIIb.

The interaction inhibitors may be selected from the group consisting of an FcγRIIb protein or a variant

thereof, an FcγRIIb extracellular domain, an anti-FcγRIIb antibody, a peptide inhibiting the binding between Aβ and FcγRIIb, and an FcγRIIb expression inhibitor including an FcγRIIb-specific siRNA or an FcγRIIb-specific antisense nucleotide.

i) FcγRIIb protein or variant thereof

An FcγRIIb protein or a variant thereof competes with endogenous FcγRIIb in neuronal cells for Aβ binding to inhibit the binding of Aβ to endogenous FcγRIIb. Thus, a cell death signal of Aβ is not transduced into neuronal cells, thus preventing cell death.

The FcγRIIb protein has the nucleotide sequence of SEQ ID No. 35 and the amino acid sequence of SEQ ID No. 36, and variants thereof are also available. In a preferred embodiment, an FcγRIIb variant is prepared by replacing an isoleucine residue at 232 of human FcγRIIb with threonine, and has the amino acid sequence of SEQ ID No. 37. The variant is not specifically limited thereto, and any variant in which other residues of FcγRIIb are mutated and which is able to modulate the signal transduction mediated by FcγRIIb is available. In a preferred embodiment, when an FcγRIIb variant was prepared and introduced into neuronal cells, Aβ-induced neuronal cell death decreased

(FIG. 2).

ii) FcγRIIb extracellular domain

FcγRIIb is composed of an extracellular domain and an intracellular domain, and the extracellular domain binds to Aβ. Thus, the extracellular domain also competes with endogenous FcγRIIb in neuronal cells for Aβ binding to thus inhibit the binding of Aβ to endogenous FcγRIIb, thereby inhibiting neuronal cell death.

The FcγRIIb extracellular domain may be any one derived from humans, mice, rats, or the like. Preferred is a human-derived extracellular domain of FcγRIIb. Also, the FcγRIIb extracellular domain may be produced using a method known to those skilled in the art, for example, through cloning into *E. coli*, mass production and purification, or through gene introduction into animal cells or other eukaryotic cells (yeast or insect cells) and purification. In the practice of the present invention, the FcγRIIb extracellular domain is purified using a method described in Sondermann P. *et al.* (*EMBO J.*, 18(5): 1095-1103, 1999). The FcγRIIb extracellular domain was found to increase the relative viability of neuronal cells exposed to Aβ to the same level as cells not exposed to Aβ (FIG. 4).

iii) Anti-FcγRIIb antibody

An anti-FcγRIIb antibody, prepared using the entire region or extracellular domain of FcγRIIb as an antigen, competes with Aβ for FcγRIIb binding and thus inhibits the binding of Aβ to FcγRIIb in neuronal cells.

iv) Interaction inhibitory peptide

An interaction inhibitory peptides was prepared in order to inhibit the binding between Aβ and FcγRIIb. The peptide is designed based on an amino acid sequence predicted as a binding site of Aβ to FcγRIIb or vice versa (see FIG. 9), but is not limited thereto. Preferably, the peptide is a peptide or a mutant thereof, which consists of one to nine amino acids comprising phenylalanine at position 4 of SEQ ID No. 24, corresponding to the N-terminal region of Aβ. Also, preferably, the peptide is an amino acid, a peptide or a mutant thereof, which consists of one to nine amino acids, comprising tryptophan at position 5 of SEQ ID No. 32, spanning from position 107 to 114 of the amino acid sequence of FcγRIIb. In a preferred embodiment, peptides were designed to have sequences represented by SEQ ID Nos. 24 to 33 (peptides #1 to #10, respectively), but are not limited thereto. When the specific peptides were incubated with FcγRIIb-CD40 chimera

and Aβ, specific peptides #1, #4 and #9 effectively inhibited the binding between FcγRIIb-CD40 and Aβ (FIG. 10). When neuronal cells were treated with the peptides and Aβ, cells exhibited increased survival (FIG. 11). Also, the peptides were injected along with Aβ into the brains of mice, and mice were assessed for memory ability. Peptides #1 and #9 were found to restore Aβ-induced memory decline (FIG. 12). The immunohistochemical analysis of the brain of experimental animals showed that peptide #1 completely inhibited intracellular accumulation of Aβ in neuronal cells (FIG. 13).

v) FcγRIIb expression inhibitor

The term "FcγRIIb expression inhibitor" refers collectively to substances that specifically inhibit the transcriptional or translational expression of FcγRIIb, and may include siRNAs, antisense nucleotides and compounds.

FcγRIIb siRNAs are not limited to specific sequences, and any siRNA sequence capable of inhibiting the binding between Aβ and FcγRIIb by suppressing the expression of FcγRIIb may be used. In an embodiment, an FcγRIIb-specific siRNA consists of sense and antisense sequences, which are represented by SEQ ID Nos. 11 and 12. Sense and antisense sequences are suitably annealed and inserted into a pSuper-

neo vector (Oligoengine, USA). A siRNA expression vector useful in the present invention is not specifically limited, but is preferably prepared by introducing a nucleotide sequence corresponding to the siRNA into a commonly used siRNA expression vector, psiRNA (Invitrogen, USA), pRNA (GenScript, USA), psLentGene (USA), pSIREN (Clontech, USA), pU6shX (VectorCoreA, Korea), pSilencer (Ambion, USA), or pSuper-neo (Loigoengine, USA). The vector may be introduced into the nucleus of cells in the form of pure plasmid DNA or a complex with a transfection reagent or a target delivery substance, or in the form of a recombinant virus vector. Suitable viral vectors for use in the present invention include adenovirus, adeno-associated virus, and retrovirus including lentivirus. When the constructed vector was transfected into neuronal cells, it reduced Aβ-induced neuronal cell death (FIG. 3b) and effectively inhibited intracellular Aβ (FIG. 6).

Antisense nucleotides have been approved as drugs having potential for therapeutic application to various human diseases. According to the Watson-Crick base pairing rules, a nucleotide is annealed to (hybridized with) a complementary sequence of DNA, immature mRNA or mRNA to interrupt the transmission of genetic information. The specificity of antisense nucleotides to target sequences

makes them exceptionally multi-functional. An antisense-nucleotide is a long chain of monomer units and thus can be readily synthesized to correspond to a target RNA sequence. Many reports have recently demonstrated the usefulness of antisense nucleotides as a biochemical tool in the study of target proteins (Rothenberg *et al., J. Natl. Cancer Inst.*, 81: 1539-1544, 1999). Many advances have been recently made in the fields of oligonucleotide chemistry and the synthesis of nucleotides having improved cell adhesion, target binding affinity and resistance to nucleases, suggesting that antisense nucleotides may be used in novel therapeutic approaches. For example, an antisense oligonucleotide targeting cmyb has been used to completely eliminate myelogenous leukemia cells from the bone marrow of patients suffering from myelogenous leukemia (Gewirtz and Calabreta, U.S. Pat. No. 5,098,890). Antisense nucleotides are known to have *in vivo* therapeutic efficacy on cytomegalovirus retinitis. Antisense nucleotides to FcγRIIb are not limited to specific sequences, but any antisense nucleotides inhibiting the binding between Aβ and FcγRIIb by suppressing the expression of FcγRIIb may be used.

III. The present invention provides a pharmaceutical

composition for preventing or treating AD comprising the interaction inhibitor as an effective ingredient.

The present composition includes the effective ingredient in an amount of 0.0001 to 50 wt% based on the total weight of the composition.

In addition to the interaction inhibitor, the present composition may include one or more effective ingredients exhibiting functions that are the same as or similar to the interaction inhibitor.

The present composition may also include, in addition to the aforementioned effective ingredients, one or more pharmaceutically acceptable carriers for administration. The pharmaceutically acceptable carrier may include saline, sterile water, Ringer's solution, buffered saline, dextrose solution, maltodextrin solution, glycerol, ethanol, liposomes and mixtures of two or more thereof. If desired, the composition may further include other typical additives, such as antioxidants, buffers, and bacteriostatics. Also, diluents, dispersing agents, surfactants, binders and lubricants may be further added so as to be formulated into injectable formulations, such as solutions, suspensions and emulsions, pills, capsules, granules or tablets. The carrier may be conjugated to a target site-specific antibody or other ligands so as to act

specifically in the target site. Further, the composition may be desirably formulated according to each disease or ingredient using a proper method in the art or the method described in Remington's Pharmaceutical Science (updated version, Mack Publishing Company, Easton PA).

The pharmaceutical composition of the present invention, although not limited thereto, is administered orally or parenterally (e.g., intravenously, subcutaneously, intraperitoneally or locally). The dosage may vary depending on the patient's weight, age, gender, health state and diet, administration time, administration mode, excretion rate, and severity of illness. The daily dosage ranges from about 0.01 to 500 mg/kg, and preferably from 0.1 to 50 mg/kg. The daily dosage may be taken as a single dose or divided into several doses.

IV. The present invention provides a method of screening a substance inhibiting the binding between Aβ and FcγRIIb, FcγRIIb-mediated signal transduction, or the intracellular translocation of Aβ and FcγRIIb.

i) The present invention provides a method of screening an inhibitor of the interaction between Aβ and FcγRIIb.

The screening method includes the steps of 1) adding a compound to be tested before, after or during the binding

between all or part of FcγRIIb and all or part of Aβ; 2) measuring the binding degree between FcγRIIb and Aβ; and 3) determining whether the compound reduces the binding between Aβ and FcγRIIb in comparison with a control. At step 1), the entire FcγRIIb protein may have the sequence of SEQ ID No. 36, and the partial portion of FcγRIIb may be an FcγRIIb extracellular region which is represented by SEQ ID No. 38. The entire Aβ protein may have the sequence of SEQ ID No. 34, and the partial portion of Aβ may be an N-terminal region of Aβ. The screening may be carried out using various methods analyzing protein-protein interaction, which are known to those skilled in the art. Such methods for analyzing the association between proteins include yeast two-hybrid system (Parida *et al.*, *Tuberculosis*, 85: 347-355, 2005), immunoprecipitation (IP), BIAcore™, Fluorescence Energy Transfer (FRET), and GST-full down assay (Lee S. Y., *Biochem. Biophys. Res. Commun.*, 334: 1445-1451, 2005), but the present invention is not limited thereto, and any known methods for analyzing the association between proteins may be used.

ii) The present invention provides a method of screening an inhibitor of FcγRIIb.

The screening method includes the steps of 1)

contacting all or part of FcγRIIb with a compound to be tested; 2) measuring the binding degree of the compound to FcγRIIb; and 3) determining whether the compound has high binding affinity to FcγRIIb in comparison with a control. At step 1), the entire FcγRIIb protein may have the sequence of SEQ ID No. 36, and the partial portion of FcγRIIb may be an FcγRIIb extracellular region, which is represented by SEQ ID No. 38. The screening may be carried out using various methods analyzing protein-compound interaction, which are known to those skilled in the art. Such methods include MALDI-TOF, but the present invention is not limited thereto, and any known methods for analyzing the association between a protein and a compound may be used.

iii) The present invention provides a method of screening a substance inhibiting the expression of FcγRIIb.

The screening method includes the steps of 1) treating a brain cell culture with a compound to be tested; 2) measuring the expression level of FcγRIIb in the brain cell culture; and 3) determining whether the compound inhibits FcγRIIb expression in comparison with a control. At step 1), B103 cells or primary neuronal cells from the cerebral cortex may be used, but the present invention is not

limited thereto, and any known cell lines expressing FcγRIIb may be used. The FcγRIIb expression may be assessed using RT-PCR, an immunoassay, and the like, but the present invention is not limited thereto, and any known methods for measuring the amount of a transcript or a protein translated therefrom may be used.

iv) The present invention provides a method of screening a substance inhibiting the intracellular translocation of Aβ and FcγRIIb.

The screening method includes the steps of 1) treating a brain cell culture with Aβ and a compound to be tested; 2) detecting the intracellular level of Aβ in the brain cell culture; and 3) determining whether the compound inhibits the intracellular translocation of Aβ in comparison with a control. At step 1), B103 cells or primary neuronal cells from the cerebral cortex may be used, but the present invention is not limited thereto and any known cell lines expressing FcγRIIb may be used. The intracellular translocation of Aβ may be assessed using antibodies, compounds and peptides binding specifically to Aβ or FcγRIIb. Also, Aβ and FcγRIIb may be detected using a protein conjugated to a fluorescent, colorimetric or radioactive protein, compound or peptide. Thus, Aβ and

FcγRIIb may be detected using fluorescence detection, radioactive detection and colorimetric detection apparatuses.

v) The present invention provides a method of screening a substance inhibiting the interaction between Aβ and FcγRIIb using an FcγRIIb chimeric protein.

The screening method includes the steps of 1) treating a cell line expressing an FcγRIIb chimeric protein with Aβ and a compound to be tested; 2) measuring the activity of the FcγRIIb chimeric protein; and 3) determining whether the compound inhibits the activity of the chimeric protein in comparison with a control. At step 1), the FcγRIIb chimeric protein is a receptor, and any protein capable of measuring the force of interaction between Aβ and FcγRIIb, as determined through the expression, color development or color change thereof, or the cellular signal transduction mediated thereby, may be fused to FcγRIIb. The FcγRIIb chimeric protein may be created by linking FcγRIIb to a specific protein, of which the expression, color development, color change or cellular signal transduction is stimulated upon the interaction between FcγRIIb and Aβ. In a preferred embodiment, CD-40 which activates cellular signal transduction, was linked to FcγRIIb. In the present

invention, the transmembrane protein CD-40 mediating intracellular signal transduction was used, but a protein such as tyrosine receptor kinase (TRK), which contains both a transmembrane domain and a cytoplasmic domain, is preferred. However, the present invention is not limited thereto.

vi) The present invention provides a method of screening a substance inhibiting the interaction between Aβ and FcγRIIb using a software program.

The screening method includes the steps of 1) inputting information about the structure of a compound to be tested into a software program; and 2) determining whether the compound inhibits the binding between Aβ and FcγRIIb using the software program. The software program useful in the method may be selected from the group consisting of DOCK™, FlexX™, and Affinity™. The present inventors employed an Affinity Program (InsightII, Accelrys Inc). A compound inhibiting Aβ-FcγRIIb interaction may be determined based on 1) the protein structure of FcγRIIb, containing amino acids corresponding to glutamic acid at position 64, tryptophan at 132, tryptophan at 155, lysine at 156 and lysine at 158 of SEQ ID No. 36, and 2) the protein structure of Aβ, containing amino acids

corresponding to glutamic acid at position 3, phenylalanine at 4, histidine at 6 and aspartic acid at 7 of SEQ ID No. 34.

vii) The present invention provides a method for screening of an inhibitor of intracellular kinase acting downstream of FcγRIIb.

The present inventors confirmed that apoptosis occurred via activations of various intracellular kinases when oligomeric Aβ bound to FcγRIIb through a series of experiments using expression inhibitors and activity inhibitor of the kinases.

Therefore, it is possible to develop a therapeutic agent for treating AD by screening substances regulating activity or expression of said kinases.

The preferred intracellular kinase is Syk, Btk, Lyn, IP3K, JNK, GSK or IMPase, but not limited thereto.

The screening of an inhibitor inhibiting activity of the intracellular kinases may be performed by methods well-known in the art. Particularly, in a preferred embodiment the method is selected from a group consisting of:

i) Reacting an intracellular kinase, a substrate specific for the kinase and test compounds, and selecting a test compound reducing the extent of

phosphorylation of said kinase comparing to control which is not treated with the test compound;

ii) Reacting an intracellular kinase and test compounds, and selecting a test compound which is binding to the kinase; and

iii) Reacting an intracellular kinase, a substrate specific for the kinase and test compounds, and selecting a test compound inhibiting the interaction between the kinase and the substrate comparing to control which is not treated with the test compound.

The extent of phosphorylation of the kinase may be determined by various methods, for example, isotopes such as ^{32}P or ^{33}P, an agarose electrophoresis analysis in which the charge of a phosphorylated substrate changes by -2 or detection using antibodies specific for phosphorylated kinases may be used.

In the meantime, for screening of compounds binding to the intracellular kinases or inhibiting binding the kinase to the substrate, various well-known methods in the art such as protein or peptide microarray, western blot analysis, gel shift assay, yeast two-hybrid assay, surface plasmon resonance analysis, etc. may be used.

V. The present invention provides a method of diagnosing Alzheimer's disease by measuring the expression level of FcγRIIb.

The expression level of FcγRIIb may be detected using any known methods capable of measuring the expression level of the FcγRIIb protein. Examples of such methods include, but are not limited to, an immunoassay with an antibody binding specifically to FcγRIIb, and RT-PCR and Northern blotting with nucleic acid molecules capable of complementarily binding to the FcγRIIb gene.

VI. The present invention provides a kit for diagnosing Alzheimer's disease by measuring the expression level of FcγRIIb.

The diagnostic kit may include DNA, RNA and a protein, binding specifically to FcγRIIb, a buffer, a standard antibody, a secondary antibody labeled with an enzyme catalyzing a colorimetric reaction or a fluorescent substance, and a substance for color development. Also, when a compound binding specifically to FcγRIIb is used, this compound is used in a form in which it is conjugated to a fluorescent or colorimetric label, which may be visually detected.

The present invention also provides a method of diagnosing Alzheimer's disease using the diagnostic kit. The method includes the steps of 1) collecting a specimen from a subject; 2) reacting the specimen with a substance binding specifically to FcγRIIb and washing the specimen; and 3) measuring the amount of the specifically bound substance. At step 3), when an antibody specific to FcγRIIb is used, the antibody is allowed to react with a secondary antibody conjugated to a fluorescent substance, is washed, and is analyzed using a fluorescence microscope or scanner. When a compound specific to FcγRIIb is used, the bound compound may be quantified in a bound or separated state.

VII. The present invention provides a therapeutic agent for treating AD comprising an activity inhibitor or an expression inhibitor of intracellular kinase acting downstream of FcγRIIb.

In an embodiment, the intracellular kinase is preferably Syk, Btk, Lyn, IP3K, JNK, GSK or IMPase, but not limited thereto.

The activity inhibitor may be one known to the art, and a compound screened in the above method of vii, IV may be used.

In a more preferred embodiment, as the activity inhibitor, LY294002, SP600125, LiCl, L-690,330 or piceatannol, etc. may be used, but not limited thereto. As the expression inhibitor, antisense oligonucleotides, siRNA, miRNA or shRNA specific for the intracellular kinases may be used, but not limited thereto.

The therapeutic agent of the present invention, although not limited thereto, is administered orally or parenterally (e.g., intravenously, subcutaneously, intraperitoneally or locally). The dosage may vary depending on the patient's weight, age, gender, health state and diet, administration time, administration mode, excretion rate, and severity of illness. The daily dosage ranges from about 0.01 to 500 mg/kg, and preferably from 0.1 to 50 mg/kg. The daily dosage may be taken as a single dose or divided into several doses.

BREIF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the FcγRIIb expression, increased upon exposure to $A\beta_{1-42}$, which was detected using RT-PCR (a), Western blotting (b) and immunostaining (c):

Aβ: $A\beta_{1-42}$;

Bapta: Bapta-AM;

Calp.: Calpeptin;

Asc.: Ascorbic acid; and

Tuni: tunicamycin.

FIG. 2 shows neuronal cell death induced by overexpression of FcγRIIb and an FcγRIIb mutant.

FIG. 3a is a photograph showing results of western blot analyzing effect of siRNA against FcγRIIb and a siRNA against RAGE, and FIG. 3b is a graph comparing degree of cell death of various cell lines:

pSuper-Neo: mock vector;

siFcγRIIb #1: siRNA-expressing cell line #1;

siFcγRIIb #2: siRNA-expressing cell line #2;

siRAGE #1: siRNA-expressing cell line #1; and

siRAGE #2: siRNA-expressing cell line #2).

FIG. 4a is a photograph showing western blotting analyzing effect of treatment of FcγRIIb extracellular domain (ED), and FIG. 4b is a graph comparing a relative ratio of cell death treated with Aβ and/or FcγRIIb ED:

Extra-FcγRIIb: FcγRIIb-ED.

FIG. 5a and 5b are photographs showing the results of immunohistochemical analysis for FcγRIIb expression levels in the brains of Tg2576 mice, and AD patients, respectively.

FIG. 6 is a photograph showing the results of immunostaining for intracellular accumulation of $A\beta_{1-42}$ in

siFcγRIIb-transfected cells.

FIG. 7a is a photograph showing the results of an *in vitro* binding assay between FcγRIIb and $A\beta_{1-42}$ using gel shift assay, and FIG. 7b is a graph showing whether FcγRIIb binds to $A\beta_{1-40}$ and $A\beta_{1-42}$ using surface plasmon resonance analysis:

T: trimer;

D: dimmer; and

M: monomer.

FIG. 8a is a schematic diagram an FcγRIIb-CD40 chimeric protein, and FIG. 8b is a graph shwong the stimulation of NF-κB activation when $A\beta_{1-42}$ binds to FcγRIIb.

FIG. 9 is a computer simulation showing the predicted binding site structure of $A\beta_{1-42}$ and FcγRIIb.

FIG. 10a depicts the sequences of peptides antagonizing the binding between $A\beta_{1-42}$ and FcγRIIb and FIG. 10b is a graph showing degree of inhibition among the antagonistic peptides.

FIG. 11a and 11b are graphs showing the effects of the peptides inhibiting the binding between $A\beta_{1-42}$, FcγRIIb on Aβ-induced neurotoxicity in hippocampal neurons and cortical neurons, respectively.

FIG. 12 is a series of graphs showing the inhibitory

effects of the peptides inhibiting the binding between $A\beta_{1-42}$ and FcγRIIb on memory decline:

a and b: Y-maze test; and

c and d: passive avoidance test.

FIG. 13 is a series of photographs showing the results of immunostaining for intraneuronal accumulation of $A\beta_{1-42}$ in neurons treated with peptides inhibiting the binding between $A\beta_{1-42}$ and FcγRIIb.

FIG. 14a is a graph showing the results of experiments for investigating whether siRNAs against various intracellular kinases can inhibit apoptosis of neuronal cells, which is induced by overexpression of FcγRIIb, and FIG. 14b is a graph showing the result of experiments for investigating whether the siRNAs can inhibit apoptosis of neuronal cells, which is induced by interaction between $A\beta_{1-42}$ and FcγRIIb.

FIG. 15a is a graph showing the results of experiments for investigating whether inhibitors of various intracellular kinase can inhibit apoptosis of neuronal cells, which is induced by overexpression of FcγRIIb, and FIG. 15b is a graph showing the result of experiments for investigating whether the inhibitors can inhibit apoptosis of neuronal cells, which is induced by interaction between $A\beta_{1-42}$ and FcγRIIb.

EXAMPLES

A better understanding of the present invention may be obtained through the following examples which are set forth to illustrate, but are not to be construed as the limit of the present invention.

EXAMPLE 1: Gene expression profiling using DNA microarray

Neuronal cells were isolated from the cerebral cortex of 16 day-old rat embryos and cultured. The primary-cultured cortical neuronal cells were exposed to 5 μM of Aβ (500 μM in PBS; Sigma, USA) for 24 hrs. Total RNA was isolated from the cells using TRIZOL Reagent (GIBCO-BRL, USA) according to the manufacture's protocol. Gene expression was analyzed using DNA microarray filters (GF300, GF301, GF302, Invitrogen, USA) containing 17,000 rat cDNAs according to the manufacturer's instruction. Results obtained from three independent experiments were statistically analyzed using the Pathway3™ software program (Resgen™, Invitrogen, USA).

As a result, the expression of FcγRIIb exhibited a 2.740.5-fold increase compared to control DNA spots (consisting of total genomic DNA). Through this DNA microarray analysis, the increased expression of E2-

25K/Hip-2 and changes in the expression of other proteins of the ubiquitin/proteasome system were previously reported (Song *et al.*, *Molecular cell*, 12(3), 553-563, 2003).

EXAMPLE 2: Detection of changes in FcγRIIb expression upon exposure to Aβ

EXAMPLE 2-1: Reverse transcription polymerase chain reaction (RT-PCR)

The primary neuronal cells from the rat cerebral cortex were exposed to 5 μM of Aβ for 48 hrs. Cells were harvested, and total RNA for reverse transcription was isolated using TRIZOLR Reagent (Invitrogen, USA). cDNAs were synthesized through reverse transcription, which was carried out using 5 μg of total RNA and ImProm-II™ Reverse Transcriptase (Promega, USA) according to the manufacturer's protocol. RT-PCR was performed using the following primers: FcγRIIb-5'-*Eco*RI primer (5'-CGCGGAATTCGATGGACAGCAACAGGACT-3': SEQ ID No. 1), FcγRIIb-3'-*Kpn*I primer (5'-CGGGTACCATAATGTGGTTCTGGTAGTC-3': SEQ ID No. 2), FcγRI-RT-5' primer (5'-TTGGTGAACACAGTTCTCTATGTGAAAATAC-ACAGGCTGC-3': SEQ ID No. 3), FcγRI-RT-3' primer (5'-CTATCTTACAGTGGCTGTTACTTCTTCATACACGTCATCGCT-3': SEQ ID No. 4), FcγRIIa-RT-5' primer (5'-GCCGATTTCTGCCTAGTGATGTGCCTCCTGTTTGCA-GTGG-3': SEQ ID No. 5), and FcγRIIa-RT-3' primer (5'-TCATTTG-

TCCTGTGGAGCCTCTTTCCGACTGACAGGGATC-3': SEQ ID No. 6). β-actin was used as an internal control, and was amplified with the β-actin sense primer (5'-GCGTCCACCCGCGAG-3': SEQ ID No. 7) and the β-actin anti-sense primer (5'-TATAGCAGGGTCAAC-3': SEQ ID No. 8). PCR was carried out in a total volume of 50 μl using one-fifth of the reverse transcription reaction solution as a template. PCR conditions included denaturation at 95℃ for 5 min, and 10, 15, 20 or 25 cycles of denaturation at 95℃ for 60 sec, annealing at 56℃ for 60 sec and extension at 72℃ for 60 sec, followed by final extension at 72℃ for 7 min.

The exposure of B103 cells to Aβ resulted in a specific increase of FcγRIIb expression (FIG. 1a). These results were consistent with those of the DNA microarray analysis in Example 1.

EXAMPLE 2-2: Western blot analysis

Rat B103 neuronal cells were treated with a calcium chelator (BAPTA-AM, 5 μM; EGTA, 1 mM), a calpain protease inhibitor (calpeptin, 10 μM), and an antioxidant (ascorbic acid, 5 μM) for 2 hrs, and exposed to Aβ for 48 hrs. Then, cells were lysed with a sampling buffer (10% glycerol, 2% SDS, 62.5 mM Tris-HCl, 2% β-mercaptoethanol, pH 6.8). The cell lysates were separated on a 12% sodium dodecyl sulfate

polyacrylamide gel electrophoresis (SDS-PAGE) gel, and transferred onto a nitrocellulose membrane. Western blotting was performed with the primary antibody, monoclonal K9.631 (a gift from Dr. Hammerling, Memorial Sloa Kettering Cancer Center, NY) and goat anti-mouse IgG antibody-conjugated horseradish peroxidase as a secondary antibody (Santa Cruz Biotechnology, USA). A control was incubated with anti-α-tubulin antibody (T5168) (Sigma, USA) and the same HRP-conjugated secondary antibody.

The Western blotting showed that the Aβ-induced FcγRIIb expression was not suppressed upon treatment with the calcium chelators, calpain protease inhibitor and antioxidant (FIG. 1b). Thus, the Aβ-induced FcγRIIb expression seems to occur in a specific manner. Also, the FcγRIIb expression increased even upon the blocking of the action of calcium and active oxygen species, which mediate the toxic signaling of Aβ, indicating that Aβ acts upstream of Aβ signaling to induce FcγRIIb expression.

EXAMPLE 2-3: Immunostaining

As described in Example 2-1, B103 cells were exposed to PBS, tunicamycin (Tuni), which inhibits N-glycosylation as post-translational modification of proteins, and Aβ for 48 hrs. Cells were fixed, probed with the primary antibody

monoclonal K9.631 (Memorial Sloa Kettering Cancer Center, NY), and observed under a fluorescence microscope (Leica DMRBE, Germany). This test was carried out as described in Song *et al.*, *Molecular cell*, 12(3), 553-563, 2003.

When B103 cells were exposed to Aβ, the expression of FcγRIIb increased (FIG. 1c). These results were consistent with those of the DNA microarray analysis in Example 1.

EXAMPLE 3: Evaluation of cell death upon FcγRIIb overexpression and inhibition of Aβ neurotoxicity using FcγRIIb mutant

An FcγRIIb expression vector and an FcγRIIb mutant expression vector were constructed and transfected into rat neuronal B103 cells. The expression vectors were prepared as follows. The rat FcγRIIb gene was amplified by performing PCR using a rat brain cDNA library (Invitrogen, USA) as a template with a set of FcγRIIb-5'-*Eco*RI primer (5'-CGCGGAATTCGATGGACAGC-AACAGGACT-3': SEQ ID No. 1) and FcγRIIb-3'-*Kpn*I primer (5'-CGGGTACCATAATGTGGTTCTGGTAGTC-3': SEQ ID No. 2). PCR was carried out in a total volume of 100 μl using 20 pmol of each primer. PCR conditions included denaturation at 95℃ for 5 min, and 30 cycles of denaturation at 95℃ for 60 sec, annealing at 56℃ for 60 sec and extension at 72℃ for 60 sec, followed by final

extension at 72℃ for 7 min. The amplified rat FcγRIIb gene was inserted into pEGFP-N1 (Clonetech, USA), and the resulting vector was designated "pFcγRIIb". An FcγRIIb(I232T) mutant was prepared through PCR using FcγRIIb [I232T]-5' primer (5'-GCTGTCGCTGGAACTGTAGCTGCC-3': SEQ ID No. 9) and FcγRIIb [I232T]-3' primer (5'-GGCAGCTACA-GCAGTTCCAGCGACAGC-3': SEQ ID No. 10).

PCR was carried out in a total volume of 50 μl using 10 pmol of each primer. PCR conditions included denaturation at 95℃ for 5 min, and 30 cycles of denaturation at 95℃ for 5 min, annealing at 56℃ for 60 sec and extension at 72℃ for 10 sec, followed by final extension at 72℃ for 30 min. The amplified rat FcγRIIb[I232T] mutant gene was inserted into pEGFP-N1 (Clonetech, USA), and the resulting vector was designated "pFcγRIIb[I232T]". This vector was digested with *Dpn*I, and the excised mutant gene was subjected to DNA sequencing, which was performed by the COSMO Company (Korea). Then, B103 cells were transfected with 300 ng of pEGFP, 900 ng of pcDNA3 (void vector), 900 ng of pFcγRIIb, and 900 ng of pFcγRIIb[I232T] using lipofectamine (Invitrogen, USA) according to the manufacturer's instructions. Cells were then exposed to 5 μM of Aβ and phosphate buffered saline (PBS) for 48 hrs. Cell viability was estimated under a

fluorescence microscope based on the morphology of green fluorescent protein (GFP)-positive cells (expressing GFP through pEGFP introduction).

FcγRIIb-overexpressing B103 cells exhibited increased cell death, whereas neuronal cell death was inhibited in B103 cells transfected with the FcγRIIb mutant expression vector (FIG. 2). These results indicate that Aβ signaling occurs via FcγRIIb, and that an FcγRIIb mutant is useful to inhibit the toxic signaling of Aβ.

EXAMPLE 4: Construction of siRNAs specific to FcγIIb and RAGE

Small interfering RNAs (siRNAs) inhibiting the expression of FcγIIb and receptors for advanced glycation end-product (RAGE), which is known as a cell surface receptor of Aβ, were constructed, and their effects on cell death were compared to each other. A siRNA duplex was formed by hybridizing sense and antisense complementary RNA oligonucleotides, listed in the following Table 1, and was inserted into pSuper-neo (Oligoengine, USA). The siRNA expression vectors thus constructed were individually transfected into B103 cells using lipofectamine (Invitrogen, USA) according to the manufacturer's protocol. The resulting transfected cells were designated "pSuper-

neo", "psiFcγRIIb#1", "psiFcγRIIb#2", "psiRAGE#1", and "psiRAGE#2". Then, transfected cells were subjected to Western blot analysis. Western blotting was performed as described in Example 2-2 with anti-FcγRIIb antibody (primary antibody: K9.361; secondary antibody: goat anti-mouse IgG conjugated to horseradish peroxidase (Santa Cruz Biotechnology, USA)), anti-RAGE antibody (primary antibody: Sc8230 (Santa Cruz Biotechnology, USA); secondary antibody: donkey anti-goat IgG conjugated to horseradish peroxidase (Santa Cruz Biotechnology, USA)), and anti-α-tubulin antibody (primary antibody: T5168 (Sigma, USA); secondary antibody: goat anti-mouse IgG conjugated to horseradish peroxidase (Santa Cruz Biotechnology, USA)). As a result, siRNAs were found to completely suppress the expression of FcγRIIb and RAGE (FIG. 3a). Then, the transformed B103 cells were exposed to 5 μM of Aβ or PBS for 48 hrs, and their viability was evaluated. Cell survival was assessed through trypan blue exclusion, Hoechst staining (Sigma, USA), and Annexin V labeling (Promega, USA). The survival of effector cells and cells introduced with pEGFP (Clontech, USA) was determined by observing the morphology of GFP-positive cells under a fluorescence microscope (Leica DMRBE, Germany). Cells were determined to be dead when the cell morphology changed to a spherical shape and

the cell membrane was disrupted or destroyed.

Cell death was blocked in cells transfected with an siRNA against FcγRIIb. In contrast, cells transfected with an siRNA against RAGE, which is known to be a receptor of Aβ, exhibited low survival (FIG. 3b). That is, an siRNA against RAGE, which is known as a target for inhibiting Aβ signaling, was found to have a poor inhibitory effect on neuronal cell death, whereas the silencing of FcγRIIb expression was found to eliminate Aβ signaling.

TABLE 1

RNA oligonucleotides for siRNA construction

	Sequence	SEQ ID No.
siFcRb-5'-sense oligomer	5'-GATCCCCTCGGAGAGCCACTTATGCTTTCAAGAGAA-GCATAAGTGGCTCTCCGATTTTTGGAAA-3'	11
siFcRb-3'-antisense oligomer	5'-AGCTTTTCCAAAAATCGGAGAGCCACTTATGCTTCT-CTTGAAAGCATAAGTGGCTCTCCCGAGGAGTCGGG-3'	12
siRAGE-5'-sense oligomer	5'-GATCCCCGCTCCGGATGAAGAATCAGTTCAAGAGAC-TGATTCTTCATCCGGAGCTTTTTGGAAA-3'	13
siRAGE-3'-sense oligomer	5'-AGCTTTTCCAAAAAGCTCCGGATGAAGAATCAGTCT-CTTGAACTGATTCTTCATCCGGAGCGGAGTCGGG-3'	14

EXAMPLE 5: The effect of FcγRIIb extracellular domain (ED)

on neuronal cell death

An FcγRIIb extracellular domain (ED) was purified as described in Sondermann *et al.*, *EMBO J.*, 18:1095-1103, 1999. Neuronal B103 cells or primary-cultured neuronal cells were exposed to 5 μM of Aβ for 48 hrs. Then, cells were treated or not treated with 100 μg of the purified FcγRIIb ED, or treated or not treated with 100 μg of bovine serum albumin (BSA). B103 cells were subjected to Western blotting, which was performed with anti-FcγRIIb antibody as described in Example 2-2. The primary neuronal culture was evaluated for cell survival as described in Example 3.

Compared to BSA treatment, the FcγRIIb ED was found to completely inhibit Aβ signaling in Aβ-exposed cells (FIG. 4), indicating that the FcγRIIb ED is an extracellular receptor of Aβ. Thus, the FcγRIIb ED may have potential as a target for inhibiting the neurotoxic signaling initiated by Aβ.

EXAMPLE 6: Immunohistochemical assay

The transgenic mouse used in this test was Tg2576 (18 to 24 months old, female), which contained the human APP695 with the double mutation Lys670→Asn and Met671→Leu (K670N, M671L), which was found in a large Swedish family suffering from the early onset of Alzheimer's disease (Hsiao *et al.*,

Science, 274:99-102, 1996). The mouse was anesthetized with 7% chloral hydrate, and perfused transcardially with 4% phosphate-buffered paraformaldehyde (PFA; Sigma, USA). For neuropathological analysis, the brain was excised and immersed in PFA for 48 hrs. Then, the brain was cut into serial coronal sections on a freezing microtome. The sections were mounted on glass slides, dried, and fixed again with 4% PFA for 15 min. The sections were incubated in methanol, containing 3% H_2O_2 for 5 min, to remove endogenous peroxidase activity. Then, the brain sections were washed, immersed in 0.5% Triton X-100 for 30 min before being reacted with the primary antibody, and incubated with 1% bovine serum albumin for 1 hr.

Specimens from fifteen patients neuropathologically diagnosed as having AD (71 to 93 years of age; 83.83 years old on average; corpse dissection 2 to 16 hrs after death) were donated from McLean Hospital (Harvard Brain Tissue Resource Center, Belmont, Massachusetts) and Ohio state university (Columbus, Ohio). All tissues were confirmed through clinical records and neuropathological examinations.

Mouse brain sections and immunofluorescent-labeled brain sections from AD patients were observed under a fluorescence microscope (Leica DMRBE, Germany).

Subsequently, brain sections were stained with an alkaline Congo red solution (Sigma, USA). Tg2576 mouse samples were stained with anti-oligo-Aβ antibody (Biosource, USA), NeuN (Chemicon, USA), and anti-FcγRIIb antibody (K9.361, gift from Dr. Hammerling, Memorial Sloa Kettering Cancer Center, NY; or rabbit polyclonal Antibody, gift from Dr. Cambier, University of Colorado Health Sciences Center, CO). AD patient samples were stained with anti-Aβ monoclonal antibody (4G8:Signet, USA), anti-PHF-1 antibody (gift from Dr. Davis, Albert Einstein College of Medicine, NY).

Both amyloid plaques (asterisk) and FcγRIIb immunoreactivity (arrowheads) were detected in the brains of AD patients. Also, strong immunoreactivity was observed within neuronal cells (FIG. 5b). FcγRIIb was found to be strongly accumulated within neuronal cells and localized along with oligo-Aβ (FIG. 5). The strong increase of FcγRIIb in AD patients may be used in AD diagnosis. Also, these results demonstrate that FcγRIIb contributes to intraneuronal Aβ accumulation, indicating that FcγRIIb contributes to the intraneuronal accumulation of Aβ as well as the signaling ability of Aβ.

EXAMPLE 7: Evaluation of intracellular Aβ accumulation in B103 cells transfected with siRNA expression vectors

psiFcγRIIb #1 cells or psiRAGE #1 cells, prepared in Example 4, were exposed to 100 nM of Aβ or PBS for 12 hrs, and immunostained with anti-Aβ antibody (4G8; Signet, USA) according to the same method as in Example 2-3.

Intracellular Aβ accumulation was strongly inhibited in psiFcγRIIb cells but was maintained in psiRAGE cells, indicating that FcγRIIb mediates the intracellular accumulation of Aβ in neurons (FIG. 6). Thus, psiFcγRIIb of FcγRIIb mutants may be useful in inhibiting intraneuronal Aβ accumulation.

EXAMPLE 8: *In vitro* assay for the binding between FcγRII and $A\beta_{1-42}$

EXAMPLE 8-1: Gel shift assay

5 μM of Aβ was mixed with 20 μg of FcγRIIb-ED or 20 μg of BSA *in vitro*, and was incubated at 37℃ for 3 hrs. The reaction mixture was incubated with anti-FcγRIIb polyclonal antibody or anti-GST antibody for 2 hrs, and then with Protein G for 3 hrs (binding solution: 50 mM Tris-HCl, pH 7.4, 1 mM DTT, 0.5 mM EDTA, 0.01% Triton X-100, 0.5 mg/ml bovine serum albumin, 10%(v/v) glycerol, protease inhibitors cocktail, several concentrations of NP-40). The beads were washed three times and subjected to Western blotting to assess the association between FcγRII and $A\beta_{1-}$

$_{42}$. Western blotting was carried out with K9.361 antibody and anti-Aβ antibody (primary antibody: 71-5800 (Zymed, USA); secondary antibody: goat anti-rabbit IgG conjugated to horseradish peroxidase (Santa Cruz Biotechnology, USA).

Aβ was found to directly bind to FcγRIIb-ED (FIG. 7a). These results indicate that FcγRIIb is a receptor of Aβ and is thus useful in analysis for extracting inhibitors of the binding.

EXAMPLE 8-2: Surface Plasmon Resonance (SPR) analysis

In order to confirm the result of the above gel shift assay, the present inventors performed surface plasmon resonance assay.

Particularly, the inventors attached BSA and FcγRIIb-ED into CM5 chip (GE Healthcare, USA) and then determined binding activity with $A\beta_{1-42}$ using Biacore 3000 (GE Healthcare, USA) (FIG. 7b).

FcγRIIb-ED showed a distinct affinity with $A\beta_{1-42}$ comparing to BSA, and we confirmed strong affinity (Kd=2.3×10^{-7} M) even during the dissociation period (after 700 seconds). However, $A\beta_{1-40}$ showed weaker affinity than $A\beta_{1-42}$. This suggests that FcγRIIb binds to $A\beta_{1-42}$ preferentially.

EXAMPLE 9: Evaluation of the binding between FcγRIIb-CD40 chimera and Aβ

In order to investigate whether FcγRIIb binds to Aβ in a specific manner, an extracellular domain of FcγRIIb was genetically fused to CD40, consisting of a transmembrane domain and a cytoplasmic domain. The resulting FcγRIIb-CD40 fusion gene was expressed in NIH3T3 cells to increase NF-κB activity, a signal transducer of CD40, when the fusion protein binds to Aβ. The chimeric gene was constructed as follows: A rat FcγRIIb extracellular region and human CD40 transmembrane and cytoplasmic domains were amplified by performing PCR with FcγRIIb-ED-5'-*Nhe*I primer (5'-GCTAGCGCTATGGACAGCAACAGGACT-3': SEQ ID No. 15), FcγRIIb-ED-3'-*Hin*d III primer (5'-AAGCTTGGGAGGCAACGAACTGC-TGGATTT3': SEQ ID No. 16), CD40-TM+cyto-5' primer (5'-CCCAAGCTTGGGGCCCTGGTGGTGATCCCCATC-3': SEQ ID No. 17), and CD40-TM+cyto-3' primer (5'-CGGGTACCATTCACTGTCTCTCCTGCAC-3': SEQ ID No. 18). PCR products were inserted into a pEGFP-N1 vector according to the same method as in Example 3. Cells were transfected with the chimeric gene, CD40, TNFRI, pcDNA3 (mock) along with an NF-kB-luciferase gene, and were exposed to 5 μM of Aβ or 20 ng/ml of TNFα. NF-κB activity was assessed, as described in Woo *et al.* (*FEBS Lett.* 578: 239-244, 2004).

When cells were exposed to Aβ, CD40 did not stimulate NF-κB activity, but FcγRIIb-CD40 strongly stimulated NF-κB activity (FIG. 8). These results indicate that Aβ binds specifically to FcγRIIb to form a complex, which is capable of triggering signal transduction.

EXAMPLE 10: Prediction of the binding structure of FcγRIIb and Aβ

In recent years, it has become apparent that Aβ accumulated in an irregularly aggregated form or in a fibrillar form does not cause signal transduction of neurotoxicity, but soluble oligomers of five or six Aβ monomers initiate neurotoxic signaling and stimulate memory decline (Cleary, J.P. *et al. Nat. Neurosci.* **8**:79-84, 2005). The Aβ monomer is difficult to crystallize, and structure thereof is difficult to determine, due to its tendency to aggregate. For this reason, the structure of soluble oligomeric Aβ was predicted through computational analysis. A computational study revealed the assembly of Aβ monomers into a globular soluble oligomeric structure, in which N-terminal tails are exposed to the exterior and C-terminal hydrophobic regions aggregate to form an oligomer (Urbanc, B. *et al. Proc.Natl. Acad. Sci. U. S. A.* **101**:17345-17350, 2004). Also, the N-terminal structure of oligomers was

similar to that of monomeric Aβ. In this regard, the inventors of this application predicted that FcγRIIb binds the N-terminal region of Aβ, and identified first the binding site structures between FcγRIIb and Aβ using the N-terminal structure of Aβ, which was determined through a nuclear magnetic resonance (NMR) study of a computational prediction method (Affinity® program: InsightII, Accelrys Inc). The structures of the binding regions in FcγRIIb and Aβ were determined using a known crystal structure of FcγRIIb (Sondermann, P. *et al.*, *EMBO J.* **18**: 1095-1103, 1999). When a tryptophan residue critical for the binding of FcγRIIb to IgG was replaced with alanine, FcγRIIb showed remarkably reduced binding affinity to Aβ. Thus, the structure prediction was carried out by placing the N-terminal region of Aβ proximate into a tryptophan pocket of FcγRIIb.

In detail, *in silico* analysis was performed using the crystal structure of human FcγRIIb extracellular domain (PDB code: 2FCB, RCSB) and the NMR structure of $A\beta_{1-42}$ (PDB code: 1IYT, RCSB). Using Affinity program within InsightII (Accelrys, USA), the N-terminal region of $A\beta_{1-42}$ was docked with the IgG binding site of FcγRIIb. The binding site was defined as an 8 Å radius from Trp92 and Trp115 residues of human FcγRIIb (hFcγRIIb). $A\beta_{1-42}$ Phe4 was first

artificially located closed to Trp92 and Trp115 residues of hFcγRIIb, and the general binding procedure was then performed as follows. Molecular dynamic calculations for the binding between hFcγRIIb and Aβ were carried out using the CVFF force field. The initial structure was generated using a Monte Carlo minimization method, and simulated to generate actual non-bond contacts using a Cell Multipole method. Such simulated annealing started at 500 K, and the temperature was slowly cooled down to 300 K for stabilization through over 50 steps, followed by a final round of over 1000 steps of energy minimization for final structure calculation.

The structure calculations revealed that in a manner similar to that in which a proline residue of IgG is critical for FcγRIIb binding, the fourth residue phenylalanine of Aβ forms a strong hydrophobic bond with Trp92 and Trp115 of FcγRIIb. In contrast, the third residue glutamate of Aβ formed a relatively weak hydrophilic bond with Tyr165 of FcγRIIb. However, the fifth and sixth residues (Arg5, His6) of Aβ were not involved in FcγRIIb binding. Thus, the binding of Aβ to FcγRIIb was predicted to occur through the binding of a sequence stretch consisting of the third to seventh residues from the N-terminus of Aβ to a tryptophan pocket

and Tyr165 of FcγRIIb (FIG. 9). These results indicated that Aβ signaling can be inhibited by interrupting the binding thereof to FcγRIIb.

EXAMPLE 11: Interruption of the binding between FcγRIIb and Aβ

Based on the results of Example 10, a sequence spanning from the first to ninth residues from the N-terminus of Aβ, and a 95 to 101 sequence and a 107 to 114 sequence of mouse FcγRIIb, which are Aβ docking sites, were synthesized. Also, peptides, in which a residue involved in Aβ-cγRIIb binding in the above sequences was replaced with alanine, were synthesized. The peptides (wild type and mutant) have the sequences represented by SEQ ID Nos. 24 to 33 (FIG. 10a). The synthesized peptides were individually allowed to react with a mixture of FcγRIIb-CD40 and Aβ (5 μM) or PBS. Then, luciferase activity was measured.

The NF-κB activation, induced through the binding of Aβ to FcγRIIb-CD40, was strongly inhibited by peptides #1, #4 and #9, which corresponded to binding regions in Aβ and FcγRIIb. Mutant peptides #2, #3, #6, #7 and #10, having an alanine substitution for a residue responsible for Aβ-cγRIIb binding, exhibited a sharp decrease in inhibitory

effects on NF-κB activation (FIG. 10b). These results indicated that the binding structure of Aβ and FcγRIIb (See FIG. 9), determined in Example 9, is actually important in Aβ-FcγRIIb binding, and that the above peptides have the potential to inhibit Aβ signaling.

EXAMPLE 12: Inhibition of Aβ-induced neurotoxicity using the peptides inhibiting FcγRIIb-Aβ binding

Primary neuronal cells were treated with the peptides (15 μM each) prepared in Example 11 and Aβ (5 μM) for 48 hrs. Relative cell survival rates were then measured and compared with a control. Among rat primary neuronal cells, hippocampal neurons were treated with peptides #1 to #7, and rat cortical neurons were treated with peptides #8 to #10.

The binding inhibitory peptides were found to inhibit the neuronal toxicity induced by Aβ through its binding to FcγRIIb (FIG. 11). Thus, since the peptides are able to strongly inhibit the neurotoxic signaling of Aβ, they may be useful in the prevention and treatment of AD.

EXAMPLE 13: Memory test

Tg2576 mice were not used in this test because it takes a lot of time to breed the animals, and they are

expensive. Instead, normal mice were used in this memory test because the same AD symptoms as in Tg2576 mice were observed when Aβ was injected into the brains of normal mice. Normal BALB/c mice were injected intracerebroventricularly (i.c.v.) with Aβ (1.855 μg/5 μl, 410 pmole) alone or in combination with a specific peptide. After one day, a Y-maze test and a passive avoidance test were performed. Memory was assessed as described in Yan *et al.* (*Br. J. Pharmacol.*, 133:89-96, 2001).

When mice received i.c.v. injection of Aβ and a peptide, peptide #1 or #9, inhibiting FcγRIIb-Aβ binding, were found to strongly reduce Aβ-induced memory decline, whereas a mutant peptide #7 failed to reverse memory decline (FIG. 12). Thus, peptides #1 and #9 may be effective in the prevention and treatment of AD.

EXAMPLE 14: Evaluation of *in vivo* effects of the binding inhibitory peptides

Brain specimens were prepared from the mice of Example 13 according to the same method as in Example 6. Sections were immunostained with primary antibodies, anti-Aβ antibody (Biosource, USA) plus an antibody to a marker of neurons, anti-neuron specific enolase (NSE) antibody (Axxora, Swiss) or anti-neuron specific nuclear protein

(NeuN) antibody (Chemicon, USA), and with secondary antibodies, anti-mouse-FITC antibody (goat anti-mouse IgG conjugated to FITC (Santa Cruz Biotechnology, USA), anti-mouse-TRITC antibody (goat anti-mouse IgG conjugated to TRITC (Santa Cruz Biotechnology, USA), and anti-rabbit-goat-anti-mouse IgG conjugated to horseradish peroxidase (Santa Cruz Biotechnology, USA)).

Strong accumulation of intraneuronal Aβ was observed in mice treated with Aβ alone, but this phenomenon disappeared in mice treated with Aβ plus binding inhibitory peptide #1. In contrast, a strong intraneuronal Aβ staining was observed in mice treated with Aβ plus peptide #7, found not to have inhibitory capacity against Aβ-FcγRIIb binding (FIG. 13). These results indicate that the binding inhibitory peptide also effectively inhibits the binding between Aβ and FcγRIIb *in vivo*, and is thus useful as an effective therapeutic and preventive agent for AD.

EXAMPLE 15: Determination of intracellular signaling molecules of FcγRIIb

EXAMPLE 15-1: Analysis of change of apoptosis by knock-out of kinases through shRNAs

The present inventors analyzed degree of apoptosis after knocking out various kinases which are predicted to

act downstream of FcγRIIb using shRNAs against the kinase genes in order to investigate which molecules mediate intracellular signaling when oligomeric Aβs bind to FcγRIIb.

Particularly, the inventors transfected mouse hippocampal cell line HT22 with pFcγRIIb prepared in the Example 3 and recombinant vectors prepared by inserting oligonucleotides encoding shRNA sequence specific for kinases Syk, Btk, Lyn and Abl1 (SEQ ID NOs: 19 to 23, respectively, See Table 2) into pSM2c (Open Biosystems, USA). After incubating for 24 hours, we observed inhibitory effects of shRNA of those kinases on apoptosis induced by FcγRIIb. As a result, when Syk, Btk, Lyn and Abl1 kinase were knocked down, down-reglation of apoptosis are 46%, 43%, 16% and 15%, respectively and there is no such an effect in Abl2, a negative control (FIG. 14a).

Table 2

Oligonucleotides for siRNAs specific for various kinases

Name	Sequence	SEQ ID No.
siSyk	5'-TGCTGTTGACAGTGAGCGCCCTCATCAGGGAATATGTGAATA-GTGAAGCCACAGATGTATTCACATATTCCCTGATGAGGTTGCCTA-CTGCCTCGGA-3'	19
siBtk	5'-	20

	TGCTGTTGACAGTGAGCGAAGCTCGAAACTGTTTGGTAAAT-AGTGAAGCCACAGATGTATTTACCAAACAGTTTCGAGCTGTGCC-TACTGCCTCGGA-3'	
siLyn	5'-TGCTGTTGACAGTGAGCGAAGAGATCCAACGTCCAATAAAT-AGTGAAGCCACAGATGTATTTATTGGACGTTGGATCTCTCTGCCT-ACTGCCTCGGA-3'	21
siAbl1	5'-TGCTGTTGACAGTGAGCGCCCGGCCCTCCTTTGCTGAAATT-AGTGAAGCCACAGATGTAATTTCAGCAAAGGAGGGCCGGTTGCC-TACTGCCTCGGA-3'	22
siAbl2	5'-TGCTGTTGACAGTGAGCGACGGGACAAACCCTGTCCTTAAT-AGTGAAGCCACAGATGTATTAAGGACAGGGTTTGTCCCGGTGCC-TACTGCCTCGGA-3'	23

EXAMPLE 15-2: Analysis of apoptosis change by kinase inhibitors

The present inventors measured degree of apoptosis using selective inhibitors of various intracellular kinases in order to confirm whether the activities of the kinases resulted from binding between oligomeric Aβ and FcγRIIb.

Particularly, the inventors treated a mouse hippocampal cell line, HT22, with LY294002 (Sigma, USA) which is an inhibitor of PI3K, SP600125 (Sigma, USA) which is an inhibitor of JNK, LiCl (Sigma, USA) which is an inhibitor of SK3b/IMPase, L-690,330 Tocris, USA) which is an inhibitor of IMPase, PD98059 (Sigma, USA) which is an inhibitor of MEK, and SB203580 (Sigma, USA) which is an inhibitor of p38, respectively, in a dose of 5 μM (5 mM

only for LiCl). After 2 hours, the inventors transfected the cell line with pFcγRIIb prepared in the Example 3. As a result, experimental groups pre-treated with LY294002, SP600125, LiCl and L-690,330 showed the inhibition of apoptosis induced by FcγRIIb overexpression. However, there is no effect on experimental groups pre-treated with PD98059 and SB203580 (FIG. 15a).

In the meantime, the inventors treated the HT22 cells with the above kinase inhibitors and a Syk inhibitor, piceatannol (Sigma, USA) and 2 hours after the treatment the inventors treated the cells with 5 uM of $A\beta_{1-42}$, in order to confirm whether said kinase inhibitors inhibit apoptosis due to oligomeric Aβ. Observing under a microscope after 24 hours, the apoptosis of experimental groups pretreated with LY294002, SP600125, LiCl and L-690,330 and piceatnannol, respectively decreased, whereas no effect was seen in the experimental group pre-treated with PD98059 and SB203580, respectively (FIG. 15b).

These results mean that Syk, Btk, Lyn and Abl1 kinase are involved in cell signaling mediated by $A\beta_{1-42}$ and suggest that inhibitors of the kinases may be used for inhibiting loss of memory in Alzheimer's disease.

In accordance with the present invention, as described

above, an interaction inhibitor is provided for effectively inhibiting the binding of Aβ to FcγRIIb in neuronal cells and an animal model of Alzheimer's disease, thereby reducing Aβ-induced neurotoxicity and cell death therein. Thus, the present inhibitor is useful in the diagnosis, prevention and treatment of Alzheimer's disease.

Although the preferred embodiments of the present invention have been disclosed for illustrative purposes, those skilled in the art will appreciate that various modifications, additions and substitutions are possible, without departing from the scope and spirit of the invention as disclosed in the accompanying claims.

WHAT IS CLAIMED IS:

1. An interaction inhibitor inhibiting interaction between amyloid-β (Aβ) and Fcγ receptor IIb (FcγRIIb).

2. The interaction inhibitor according to claim 1, which is selected from the group consisting of an FcγRIIb protein or a variant thereof, an FcγRIIb extracellular domain, an anti-FcγRIIb antibody, a peptide inhibiting binding between Aβ and FcγRIIb, and an FcγRIIb-specific expression inhibitor.

3. The interaction inhibitor according to claim 2, wherein the FcγRIIb variant has an amino acid sequence represented by SEQ ID No. 37.

4. The interaction inhibitor according to claim 2, wherein the anti-FcγRIIb antibody includes an antibody recognizing an extracellular domain of FcγRIIb.

5. The interaction inhibitor according to claim 2, wherein the anti-FcγRIIb antibody is K.9.361.

6. The interaction inhibitor according to claim 2, wherein the FcγRIIb extracellular domain has an amino acid sequence represented by SEQ ID No. 38.

7. The interaction inhibitor according to claim 2, wherein the peptide is a peptide or a mutant thereof, which consists of one to nine amino acids comprising a third glutamic acid, a fourth phenylalanine, a sixth histidine or a seventh aspartic acid of SEQ ID No. 24.

8. The interaction inhibitor according to claim 7, wherein the peptide is selected from the group consisting of sequences of SEQ ID No. 24 to 31.

9. The interaction inhibitor according to claim 7, wherein the peptide has a sequence represented by SEQ ID No. 24.

10. The interaction inhibitor according to claim 2, wherein the peptide is a peptide or a mutant thereof, which consists of one to nine amino acids comprising a fifth tryptophan of SEQ ID No. 32.

11. The interaction inhibitor according to claim 10, wherein the peptide has a sequence represented by SEQ ID No. 33.

12. The interaction inhibitor according to claim 2, which is the FcγRIIb-specific expression inhibitor.

13. The interaction inhibitor according to claim 12, wherein the expression inhibitor is selected from the group consisting of an FcγRIIb-specific siRNA or an FcγRIIb-specific antisense oligonucleotide.

14. The interaction inhibitor according to claim 13, wherein the FcγRIIb-specific siRNA has a sequence represented by SEQ ID No. 11 or 12.

15. A recombinant expression vector comprising a nucleotide encoding the expression inhibitor of claim 13.

16. The recombinant expression vector according to claim 15, which is a plasmid vector or a viral vector.

17. A peptide having an amino acid sequence selected from the group consisting of SEQ ID Nos. 24 to 33.

18. A pharmaceutical composition for treating or preventing Alzheimer's disease, comprising any one selected from the group consisting of the interaction inhibitor of any one of claims 1 to 14, the recombinant expression vector of claim 15 or 16, and the peptide of claim 17 as an effective ingredient.

19. A method of screening a substance inhibiting binding between amyloid-β (Aβ) and Fcγ receptor IIb (FcγRIIb), FcγRIIb-mediated signal transduction, or intracellular translocation of Aβ and FcγRIIb.

20. The method of screening the substance inhibiting interaction between Aβ and FcγRIIb according to claim 19, comprising the steps of:

1) adding a compound to be tested before, after or during binding between all or part of FcγRIIb and all or part of Aβ;

2) measuring a binding degree between FcγRIIb and Aβ;

3) determining whether the compound reduces binding between Aβ and FcγRIIb in comparison with a control; and

4) determining whether the compound inhibits intracellular translocation of Aβ and FcγRIIb.

21. The method of screening the interaction inhibitor according to claim 19, wherein the part of FcγRIIb of step 1) is an FcγRIIb extracellular domain.

22. The method of screening the interaction inhibitor according to claim 21, wherein the part of FcγRIIb has a sequence represented by SEQ ID No. 38.

23. The method of screening the interaction inhibitor according to claim 20, wherein the part of Aβ of step 1) is an N-terminal region of Aβ.

24. The method of screening the interaction inhibitor according to claim 21, wherein the part of Aβ has a sequence represented by SEQ ID No. 31.

25. The method of screening the interaction inhibitor according to claim 23, wherein the part of Aβ consists of a third glutamic acid to a seventh aspartic acid of SEQ ID No. 34.

26. The method of screening an inhibitor of FcγRIIb according to claim 19, comprising the steps of:

1) contacting all or part of FcγRIIb with a compound to be tested;

2) measuring a binding degree of the compound to FcγRIIb; and

3) determining whether the compound has high binding affinity to FcγRIIb in comparison with a control.

27. The method of screening the FcγRIIb inhibitor according to claim 26, wherein the part of FcγRIIb of step 1) is an FcγRIIb extracellular domain.

28. The method of screening the FcγRIIb inhibitor according to claim 27, wherein the part of FcγRIIb has a sequence represented by SEQ ID No. 38.

29. The method of screening an FcγRIIb expression inhibitor according to claim 19, comprising the steps of:

1) treating a brain cell culture with a compound to be tested;

2) measuring an expression level of FcγRIIb in the brain cell culture; and

3) determining whether the compound inhibits FcγRIIb expression in comparison with a control.

30. The method of screening the FcγRIIb expression inhibitor according to claim 29, wherein the brain cell of step 1) is a rat B103 cell or a primary cortical cell.

31. The method of screening the substance inhibiting the intracellular translocation of Aβ and FcγRIIb according to claim 19, comprising the steps of:

1) treating a brain cell culture with Aβ and a compound to be tested;

2) detecting an intracellular level of Aβ in the brain cell culture; and

3) determining whether the compound inhibits the intracellular translocation of Aβ in comparison with a control.

32. The method of screening the substance inhibiting the intracellular translocation of Aβ and FcγRIIb according to claim 31, wherein the intracellular translocation of Aβ and FcγRIIb is assessed using an antibody, a compound and a peptide binding specifically to Aβ or FcγRIIb.

33. The method of screening the substance inhibiting the intracellular translocation of Aβ and FcγRIIb according to claim 31, wherein Aβ and FcγRIIb are detected using a protein conjugated to a fluorescent, colorimetric or radioactive protein, compound or peptide.

34. The method of screening the substance inhibiting the intracellular translocation of Aβ and FcγRIIb according to claim 31, wherein the intracellular translocation of Aβ or FcγRIIb is detected using fluorescence detection, radioactive detection and colorimetric detection apparatuses.

35. The method of screening a substance inhibiting interaction between Aβ and FcγRIIb according to claim 19, comprising the steps of:

1) treating a cell line expressing an FcγRIIb chimeric

protein with Aβ and a compound to be tested;

2) measuring activity of the FcγRIIb chimeric protein; and

3) determining whether the compound inhibits the activity of the chimeric protein in comparison with a control.

36. The method of screening the substance inhibiting the interaction between Aβ and FcγRIIb according to claim 35, wherein the FcγRIIb chimeric protein is a receptor protein that is capable of measuring a interaction force between Aβ and FcγRIIb as determined through expression, color development, color change or cellular signal transduction of a specific protein linked thereto.

37. The method of screening the substance inhibiting the interaction between Aβ and FcγRIIb according to claim 35, wherein the FcγRIIb chimeric protein is created by linking FcγRIIb to a specific protein, of which expression, color development, color change or cellular signal transduction is stimulated upon interaction between FcγRIIb and Aβ.

38. The method of screening the substance inhibiting the interaction between Aβ and FcγRIIb according to claim 35, wherein the FcγRIIb chimeric protein is created by fusing FcγRIIb with a protein mediating cellular signal transduction.

39. The method of screening the substance inhibiting the interaction between Aβ and FcγRIIb according to claim 38, wherein the FcγRIIb chimeric protein is created by fusing FcγRIIb with CD40.

40. The method of screening a substance inhibiting interaction between Aβ and FcγRIIb according to claim 19, comprising the steps of:

1) inputting information about a structure of a compound to be tested into a software program; and

2) determining whether the compound inhibits the interaction between Aβ and FcγRIIb by executing the software program.

41. The method of screening the substance inhibiting the interaction between Aβ and FcγRIIb according to claim 40, wherein the software program is an Affinity Program.

42. The method of screening the substance inhibiting the interaction between Aβ and FcγRIIb according to claim 40, wherein a compound inhibiting Aβ-FcγRIIb interaction is determined based on a protein structure of FcγRIIb, containing amino acids corresponding to glutamic acid at position 64, tryptophan at 132, tryptophan at 155, lysine at 156 and lysine at 158 of SEQ ID No. 36.

43. The method of screening the substance inhibiting the interaction between Aβ and FcγRIIb according to claim 40, wherein a compound inhibiting Aβ-FcγRIIb interaction is determined based on a protein structure of Aβ, containing amino acids corresponding to glutamic acid at position 3, phenylalanine at 4, histidine at 6 and aspartic acid at 7 of SEQ ID No. 31.

44. A method of inhibiting an interaction between amyloid-β and Fcγ receptor IIb, comprising treatment with the interaction inhibitor of claim 1.

45. A method of diagnosing Alzheimer's disease comprising measuring an expression level of Fcγ receptor IIb (FcγRIIb) in a specimen.

46. The method according to 45, wherein the expression level of FcγRIIb is assessed using an immunoassay with an antibody binding specifically to FcγRIIb, or using a method quantifying a transcript from an FcγRIIb gene.

47. A kit for diagnosing Alzheimer's disease comprising an antibody specific to Fcγ receptor IIb (FcγRIIb) or a nucleic acid molecule complementarily binding to an FcγRIIb gene.

48. A method of preventing and treating Alzheimer's disease comprising inhibiting interaction between amyloid-β and Fcγ receptor IIb in a subject.

49. The method according to claim 48, which comprises administering the interaction inhibitor of claim 1 to a subject in need thereof.

50. A method of preventing and treating Alzheimer's disease comprising inhibiting intracellular translocation of amyloid-β in a subject.

51. The method according to claim 50, which comprises administering the interaction inhibitor of claim 1 to an subject in need thereof.

52. The method according to claim 49, wherein the interaction inhibitor is administered to an individual orally, intraperitoneally, intravenously or intramuscularly, or is administered directly into a brain of the subject.

53. A therapeutic agent for preventing and treating Alzheimer's disease comprising an activity inhibitor or an expression inhibitor of intracellular kinases acting downstream

of FcγRIIb as an effective ingredient.

54. The therapeutic agent according to claim 53, wherein the kinase is Syk, Btk, Lyn, IP3K, IMPase, GSK or Abl1.

55. The therapeutic agent according to claim 53, the activity inhibitor is selected from a group consisting of LY294002, SP600125, LiCl, L-690,330 and piceatannol.

56. A method of preventing and treating of Alzheimer's disease comprising administrating an therapeutically effective amount of an activity inhibitor or an expression inhibitor of intracellular kinase acting downstream of FcγRIIb to a subject in need thereof.

57. A method of screening of a therapeutic agent for preventing and treating Alzheimer's disease comprising identifying a substance inhibiting intracellular kinase acting downstream of FcγRIIb.

58. The method according to claim 57, wherein the identifying a substance inhibiting intracellular kinase acting downstream of FcγRIIb is performed by a method selected from a group consisting of:

i) Reacting an intracellular kinase, a substrate

specific for the kinase and test compounds, and selecting a test compound reducing the extent of phosphorylation of said kinase comparing to control which is not treated with the test compound;

ii) Reacting an intracellular kinase and test compounds, and selecting a test compound which is binding to the kinase; and

iii) Reacting an intracellular kinase, a substrate specific for the kinase and test compounds, and selecting a test compound inhibiting the interaction between the kinase and the substrate comparing to control which is not treated with the test compound.

59. The method according to claim 58, wherein the step of i) is performed by analyzing using isotopes such as ^{32}P or ^{33}P, an agarose electrophoresis analysis in which the charge of a phosphorylated substrate changes by -2 or detection using antibodies specific for phosphorylated kinases.

60. The method according to claim 58, wherein the steps of ii) and iii) are performed by protein microarray, Western blot analysis, gel shift assay, yeast two hybrid assay, or surface plasmon resonance analysis (SPR).

DRAWINGS

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FIG. 1

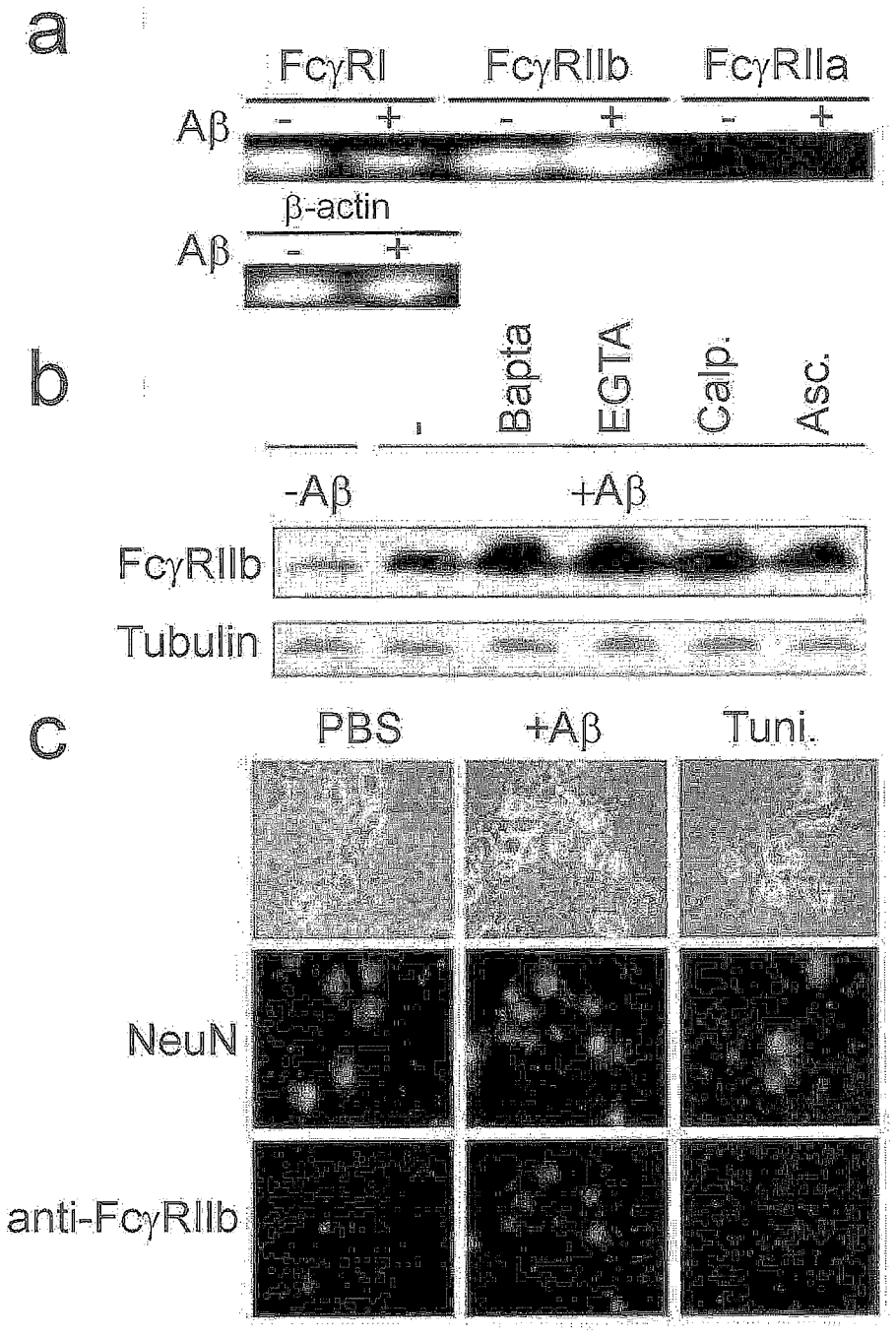


FIG. 2

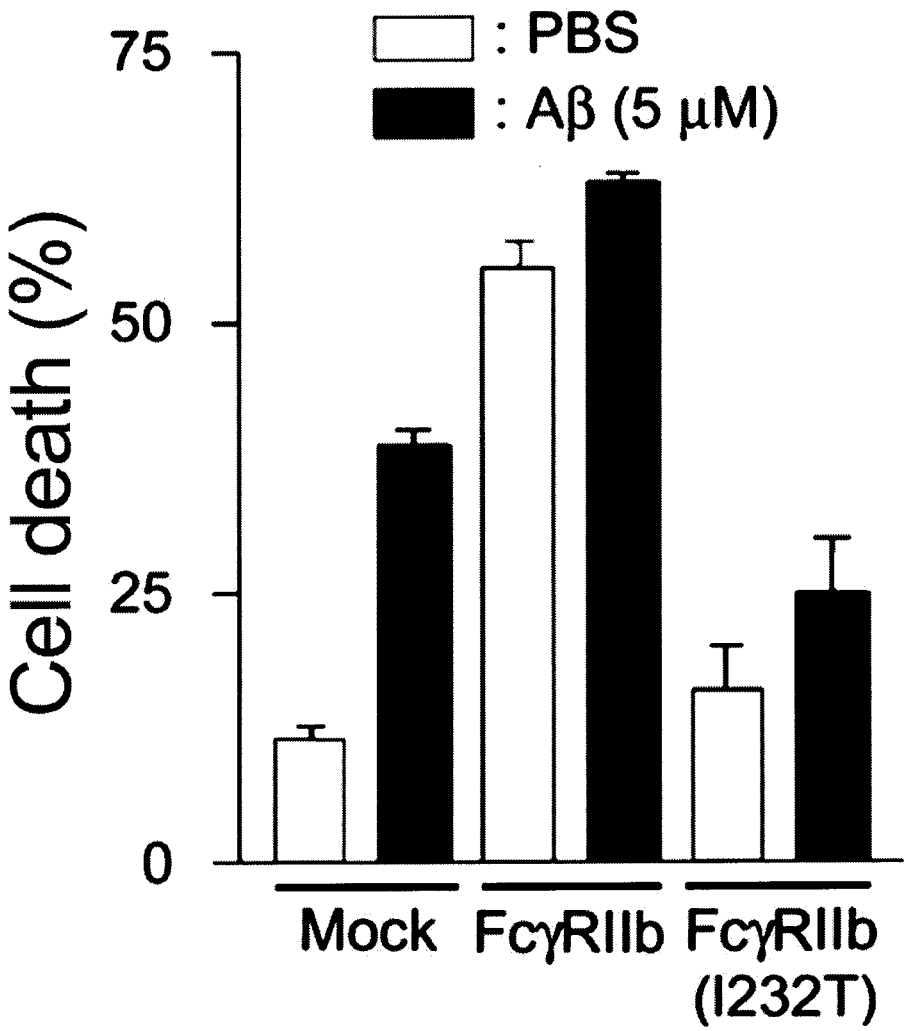


FIG. 3

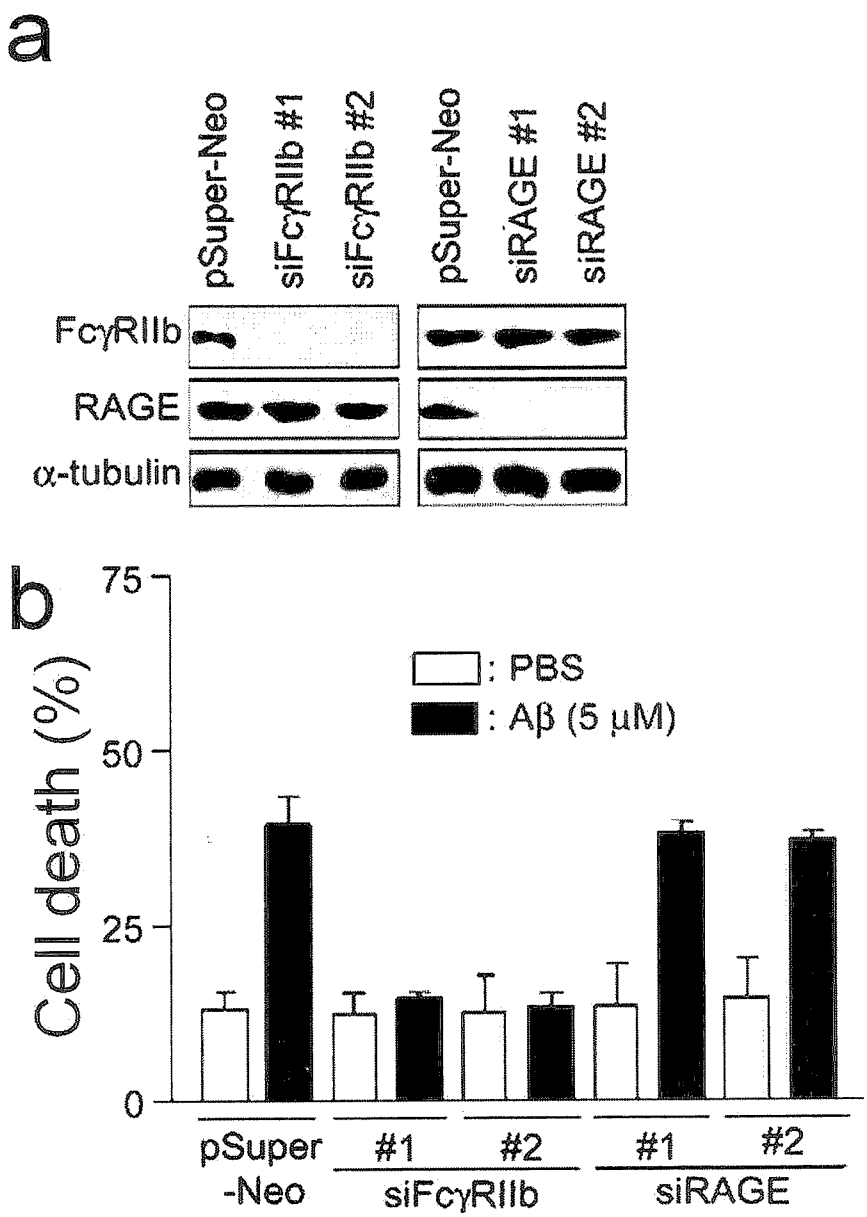

a
pSuper-Neo
siFcγRIIb #1
siFcγRIIb #2
pSuper-Neo
siRAGE #1
siRAGE #2
FcγRIIb
RAGE
α-tubulin
b
Cell death (%)
75
50
25
0
: PBS
: Aβ (5 μM)
pSuper -Neo
#1
#2
siFcγRIIb
#1
#2
siRAGE

FIG. 4

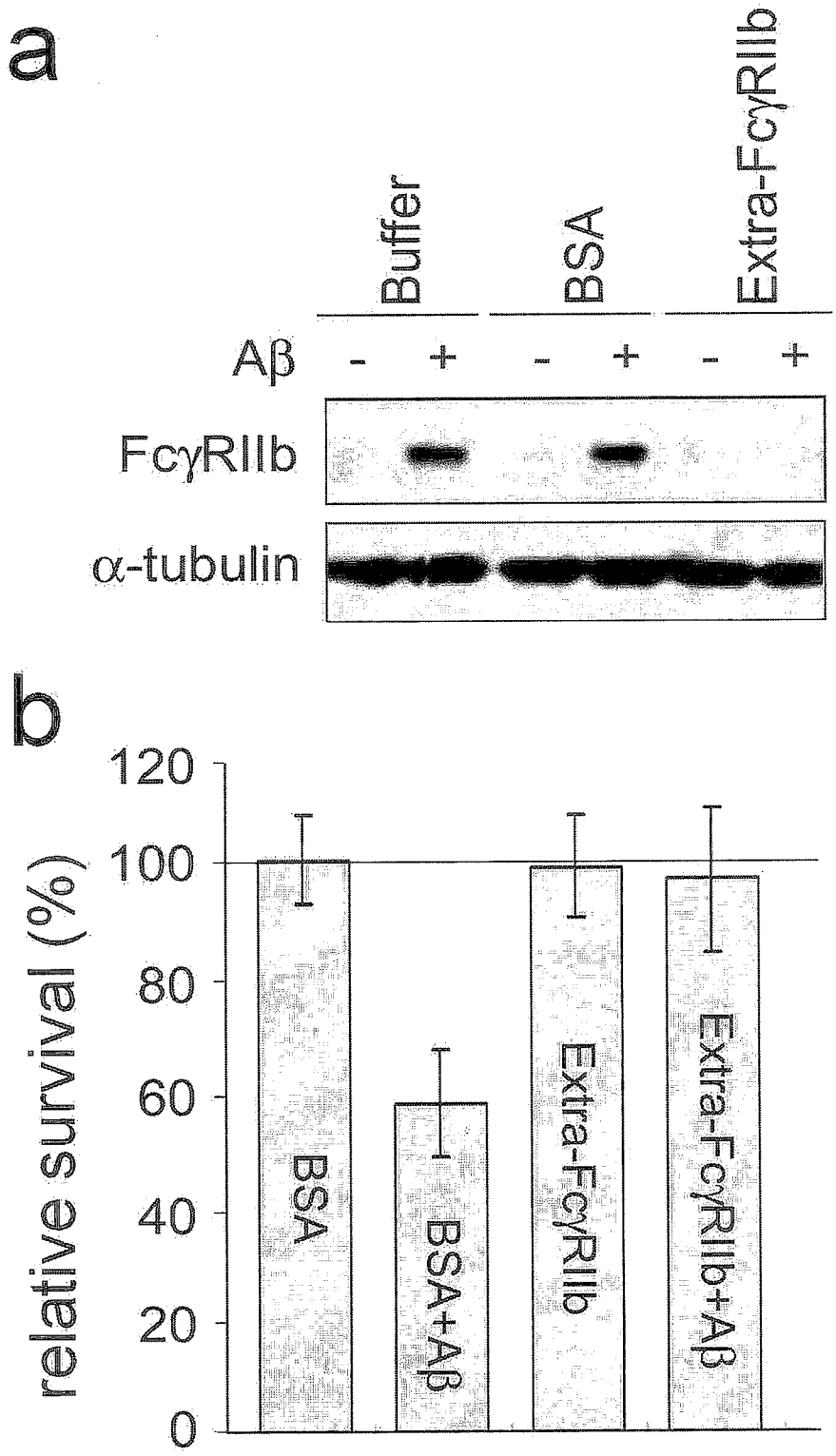

a
Buffer
BSA
Extra-FcγRIIb
Aβ - + - + - +
FcγRIIb
α-tubulin
b
relative survival (%)
120
100
80
60
40
20
0
BSA
BSA+Aβ
Extra-FcγRIIb
Extra-FcγRIIb+Aβ

FIG. 5

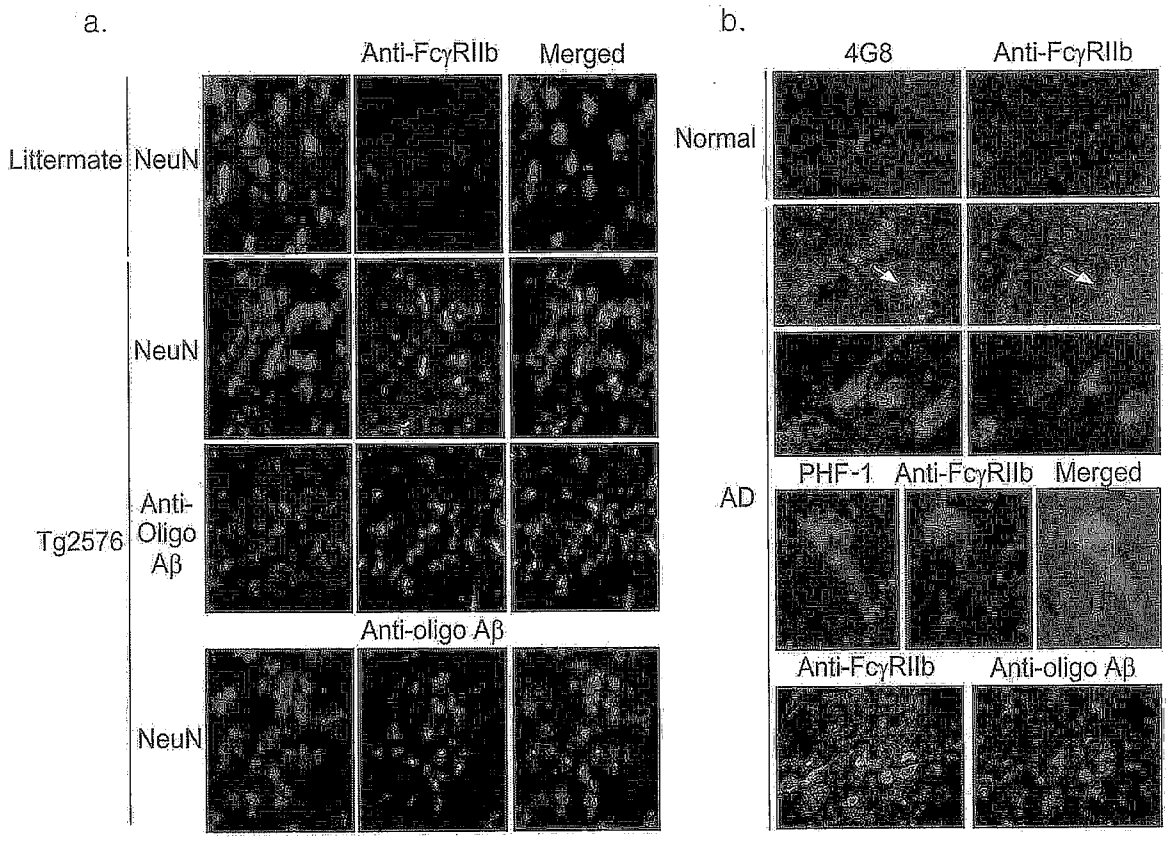

a.
Anti-FcγRIIb
Merged
Littermate
NeuN
NeuN
Tg2576
Anti-Oligo Aβ
Anti-oligo Aβ
NeuN
b.
4G8
Anti-FcγRIIb
Normal
PHF-1
Anti-FcγRIIb
Merged
AD
Anti-FcγRIIb
Anti-oligo Aβ

FIG. 6

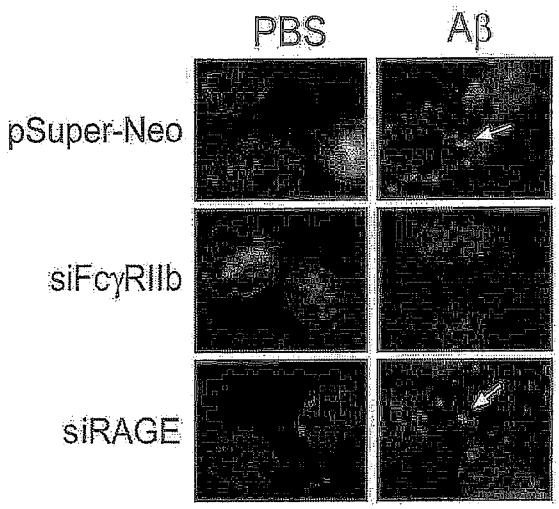
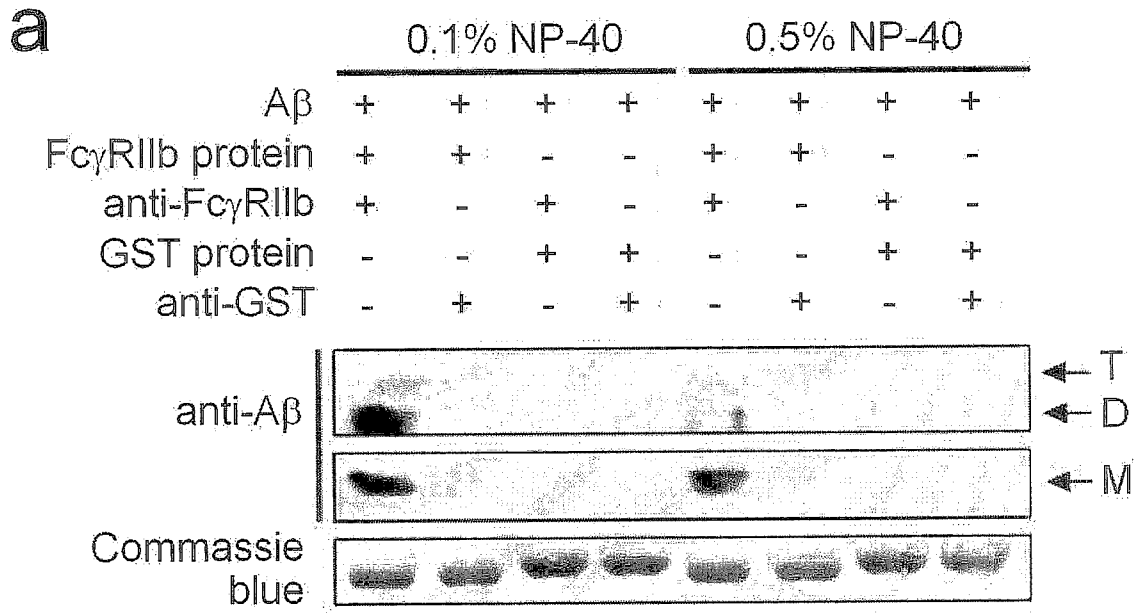

PBS
Aβ
pSuper-Neo
siFcγRIIb
siRAGE

FIG. 7


a
0.1% NP-40
0.5% NP-40
Aβ + + + + + + + +
FcγRIIb protein + + - - + + - -
anti-FcγRIIb + - + - + - + -
GST protein - - + + - - + +
anti-GST - + - + - + - +
anti-Aβ
T
D
M
Commassie blue

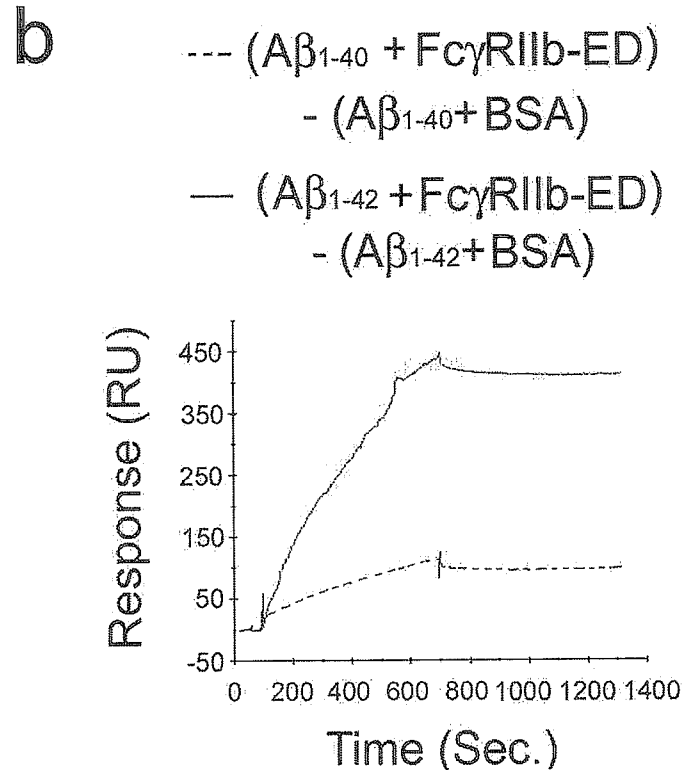

b
--- (Aβ1-40 + FcγRIIb-ED)
- (Aβ1-40+ BSA)
— (Aβ1-42 + FcγRIIb-ED)
- (Aβ1-42+ BSA)
Response (RU)
450
350
250
150
50
-50
0 200 400 600 800 1000 1200 1400
Time (Sec.)

FIG. 8

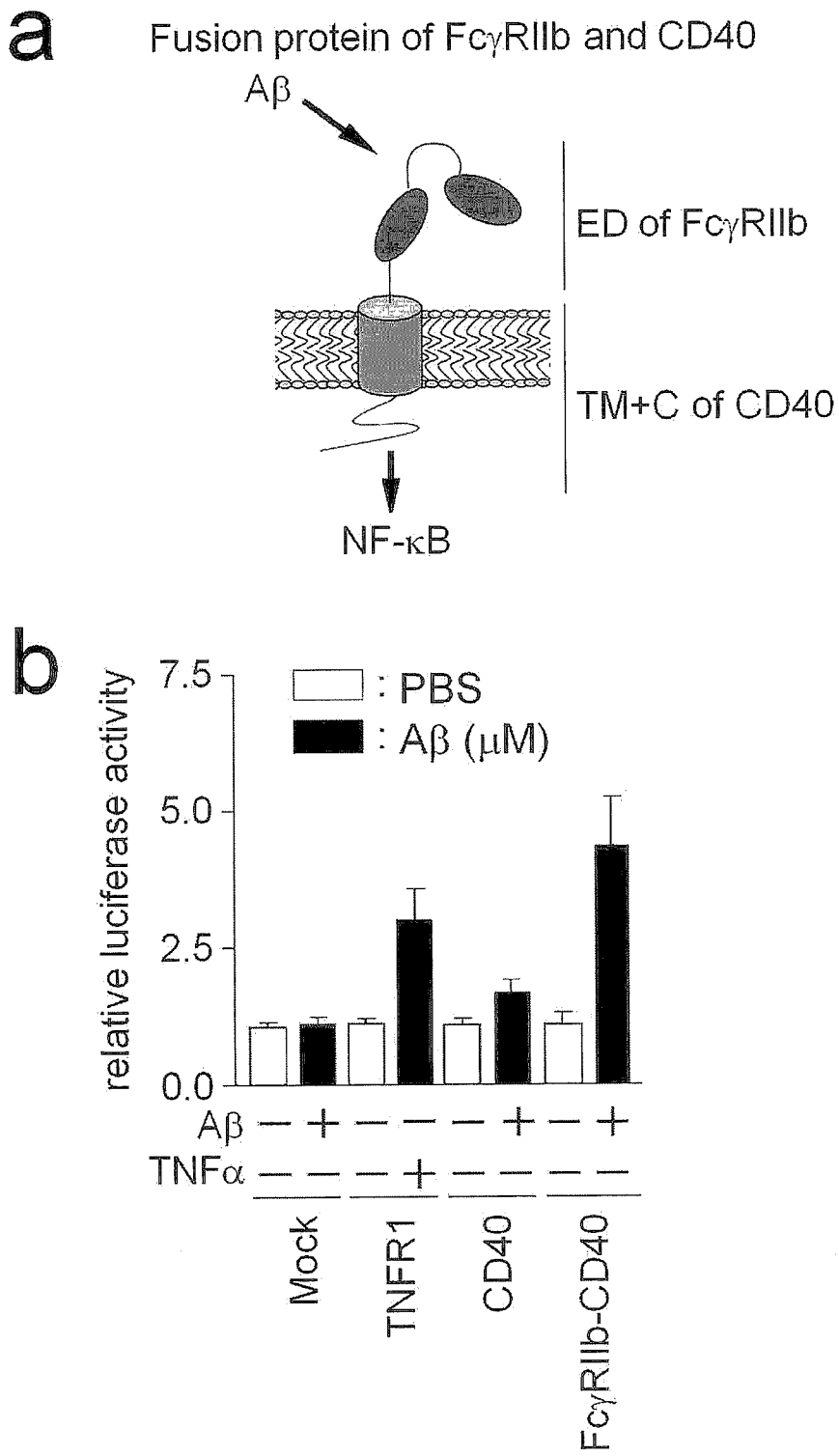

a
Fusion protein of FcγRIIb and CD40
Aβ
ED of FcγRIIb
TM+C of CD40
NF-κB
b
relative luciferase activity
7.5
5.0
2.5
0.0
: PBS
: Aβ (μM)
Aβ
TNFα
Mock
TNFR1
CD40
FcγRIIb-CD40

Fig. 9

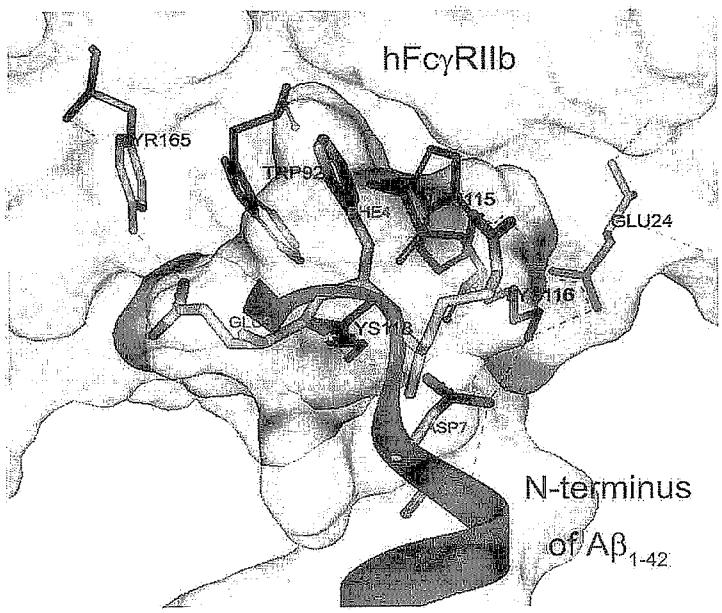


FIG. 10

a

#1: DAEFRHDSG	Aβ (1-9)	WT	SEQ ID NO. 24
#2: DAAFRHDSG		(EA)	SEQ ID NO. 25
#3: DAEARHDSG		(FA)	SEQ ID NO. 26
#4: DAEFAHDSG		(RA)	SEQ ID NO. 27
#5: DAEFRADSG		(HA)	SEQ ID NO. 28
#6: DAEFRHASG		(DA)	SEQ ID NO. 29
#7: DAEARHASG		(FDAA)	SEQ ID NO. 30
#8: QLVFLEG	mFcγRIIb	(95-101)	SEQ ID NO. 31
#9: RCHSWRNK		(107-114)	SEQ ID NO. 32
#10: RCHSARNK		(107-114)(WA)	SEQ ID NO. 33

b

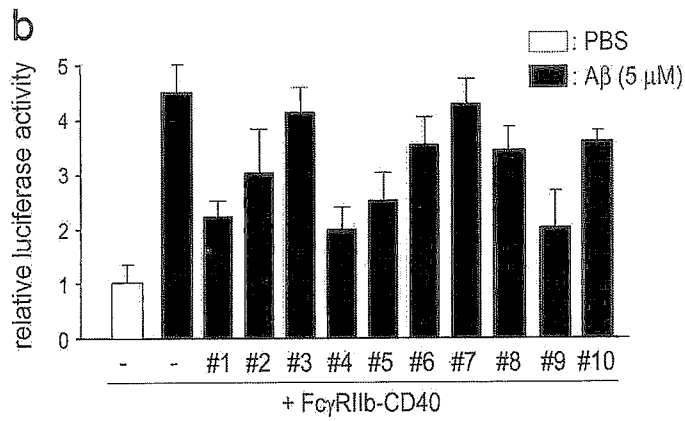


FIG. 11

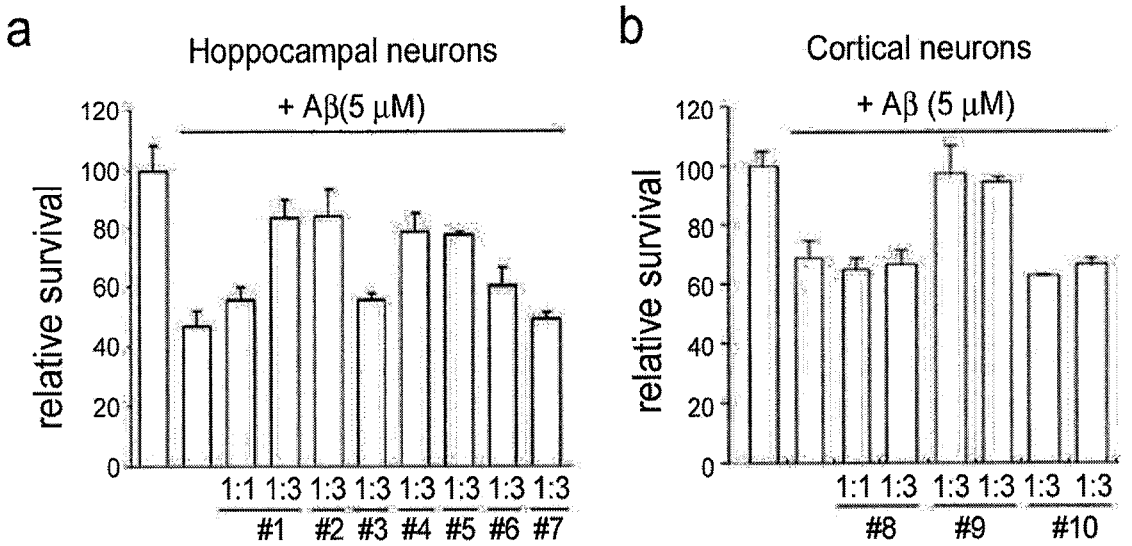

a
Hoppocampal neurons
+ Aβ(5 μM)
relative survival
120
100
80
60
40
20
0
1:1 1:3 1:3 1:3 1:3 1:3 1:3 1:3
#1 #2 #3 #4 #5 #6 #7
b
Cortical neurons
+ Aβ (5 μM)
relative survival
120
100
80
60
40
20
0
1:1 1:3 1:3 1:3 1:3 1:3
#8 #9 #10

FIG. 12

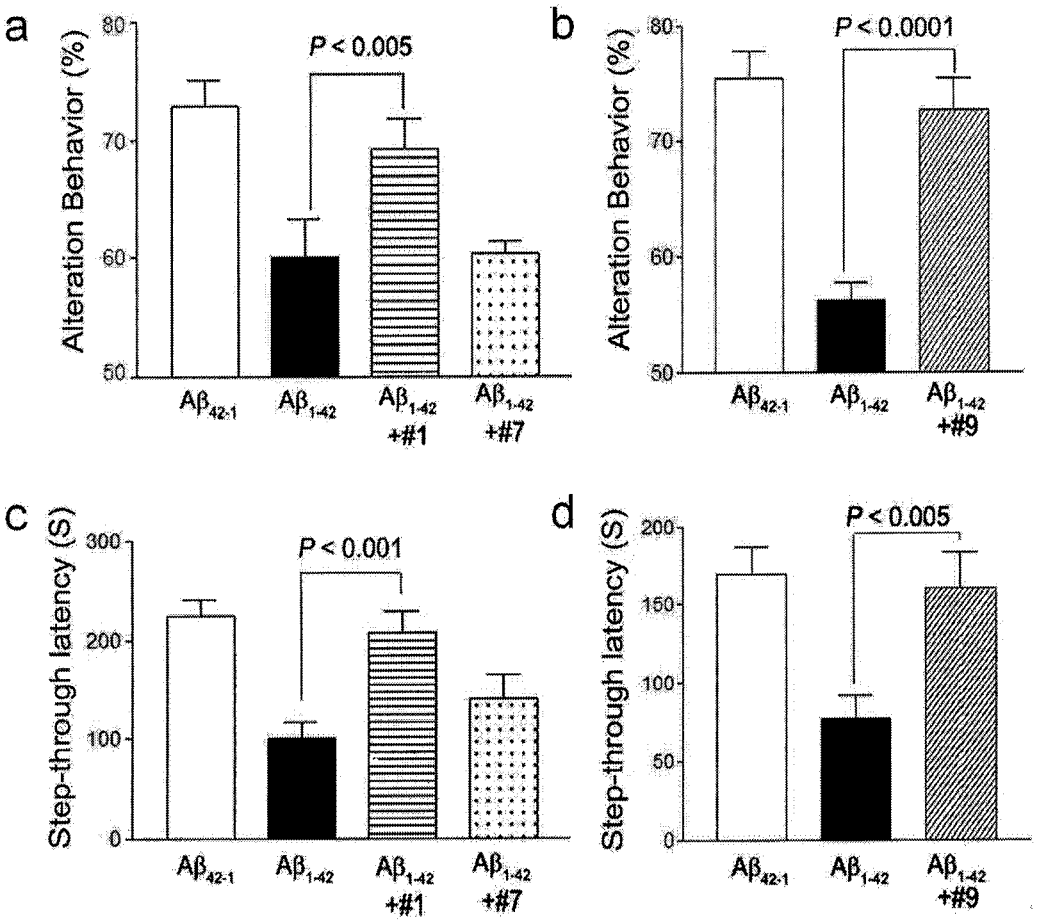

a
Alteration Behavior (%)
80
70
60
50
P < 0.005
Aβ42-1
Aβ1-42
Aβ1-42 +#1
Aβ1-42 +#7
b
Alteration Behavior (%)
80
70
60
50
P < 0.0001
Aβ42-1
Aβ1-42
Aβ1-42 +#9
c
Step-through latency (S)
300
200
100
0
P < 0.001
Aβ42-1
Aβ1-42
Aβ1-42 +#1
Aβ1-42 +#7
d
Step-through latency (S)
200
150
100
50
0
P < 0.005
Aβ42-1
Aβ1-42
Aβ1-42 +#9

FIG. 13

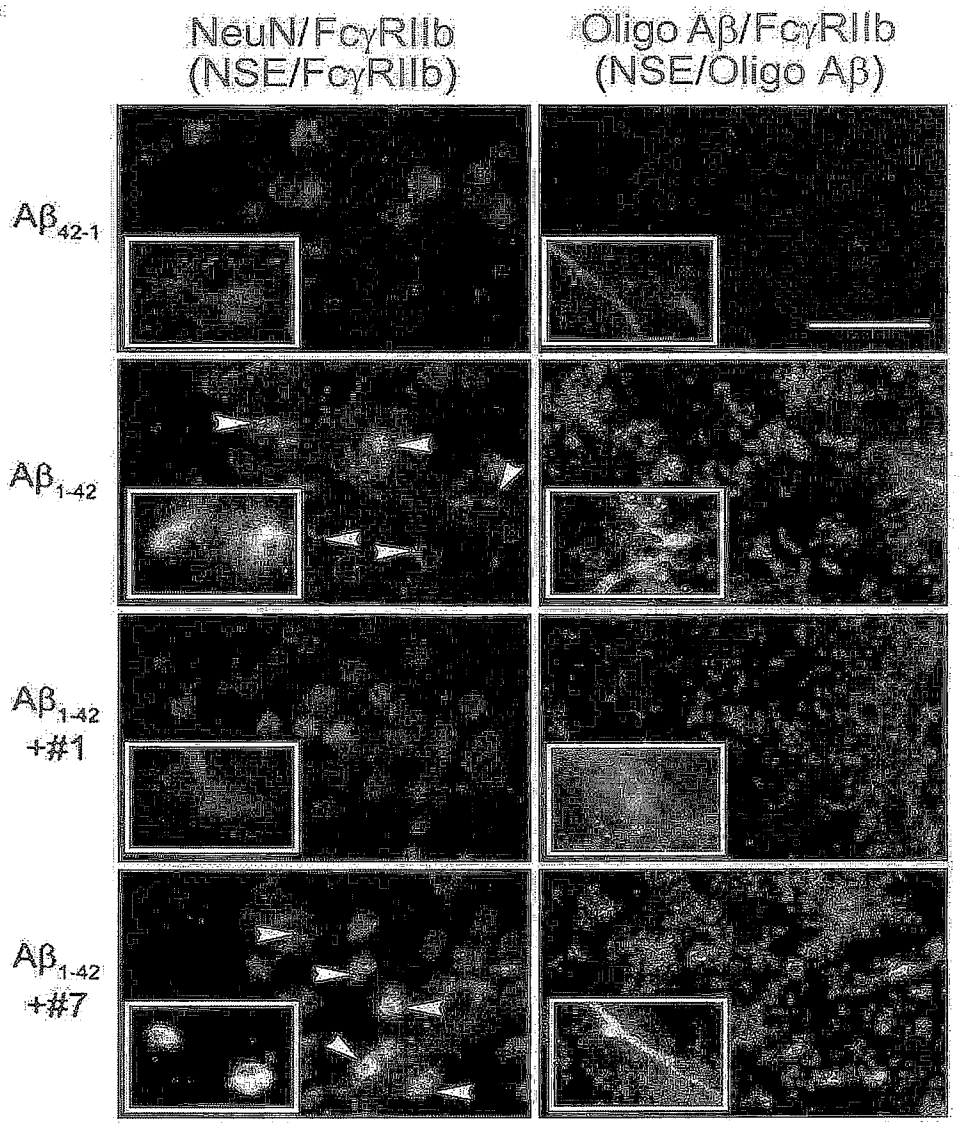

NeuN/FcγRIIb
(NSE/FcγRIIb)
Oligo Aβ/FcγRIIb
(NSE/Oligo Aβ)
Aβ42-1
Aβ1-42
Aβ1-42
+#1
Aβ1-42
+#7

FIG. 14

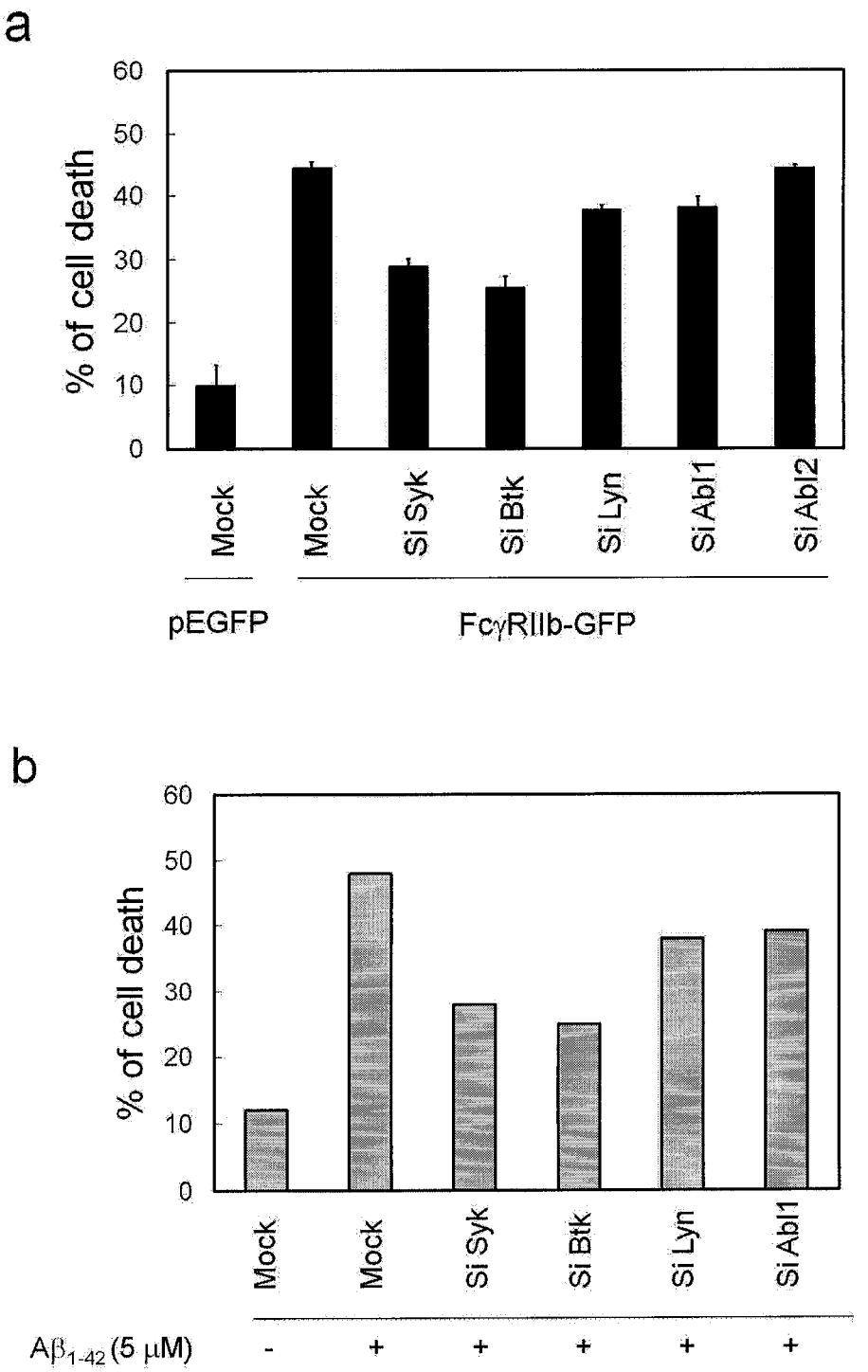
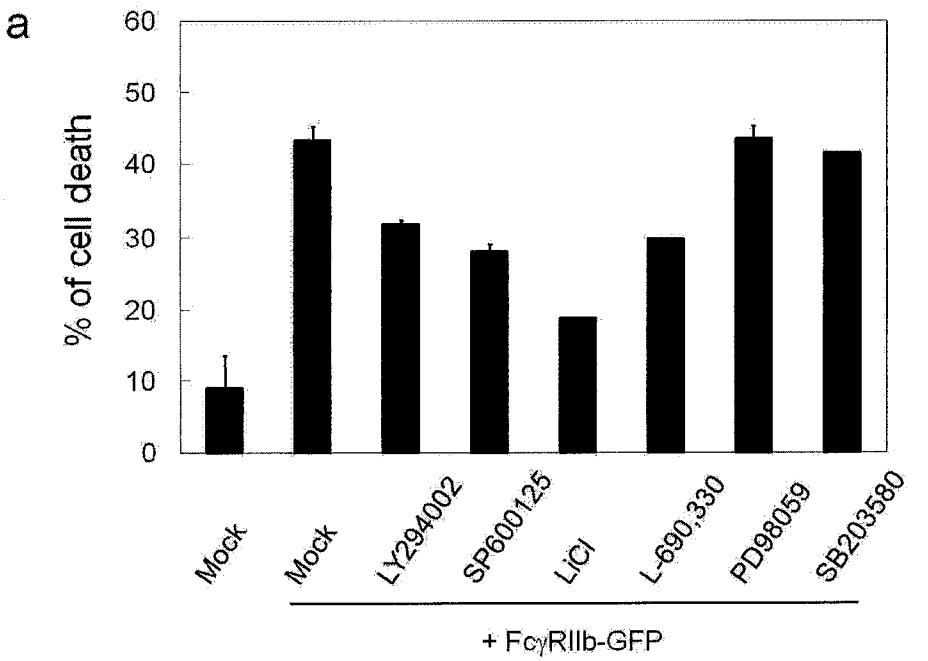
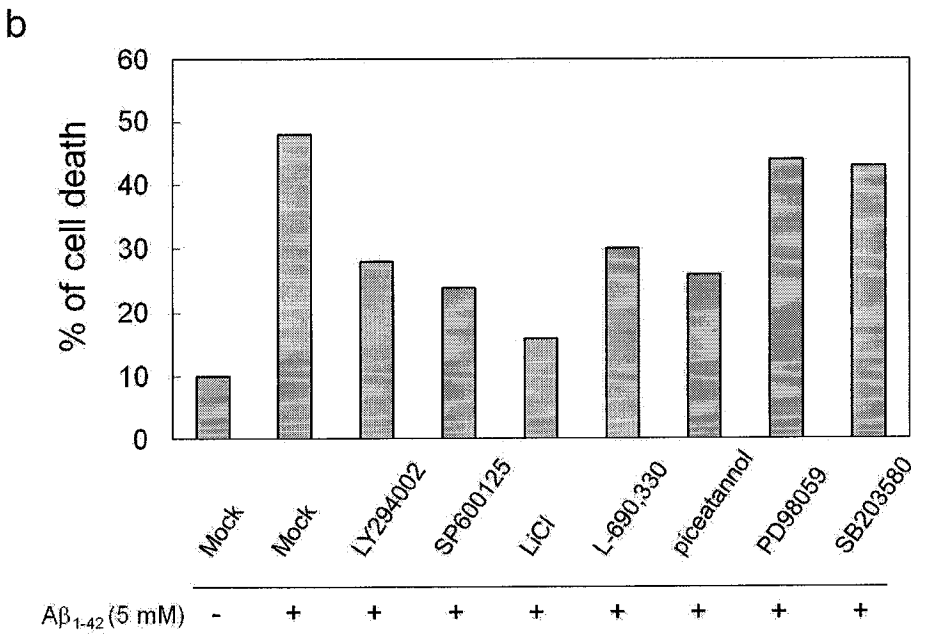

a
% of cell death
60
50
40
30
20
10
0
Mock
Mock
Si Syk
Si Btk
Si Lyn
Si Abl1
Si Abl2
pEGFP
FcγRIIb-GFP
b
% of cell death
60
50
40
30
20
10
0
Mock
Mock
Si Syk
Si Btk
Si Lyn
Si Abl1
Aβ1-42 (5 μM)
- + + + + +

FIG. 15


a
% of cell death
0
10
20
30
40
50
60
Mock
Mock
LY294002
SP600125
LiCl
L-690,330
PD98059
SB203580
+ FcγRIIb-GFP


b
% of cell death
0
10
20
30
40
50
60
Mock
Mock
LY294002
SP600125
LiCl
L-690,330
piceatannol
PD98059
SB203580
Aβ1-42 (5 mM) - + + + + + + + +

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2008/006570

A. CLASSIFICATION OF SUBJECT MATTER

A61K 38/16(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
e-KIPASS, PubMed(Keywords: Fc gamma receptor IIb, amyloid beta, Alzheimer's disease)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LUE, L. et al., "Modeling Alzheimer's disease immune therapy mechanisms: Interactions of human postmortem microglia with antibody-opsonized amyloid beta peptide", J. Neuros. Res., 2002, Vol. 70, pp. 599-610. See the abstract and fig. 7.	1-43, 53-55, 57-60
A	STEFANESCU, R. N. et al., "Inhibitory Fc gamma receptors: from gene to disease", J. Clin. Immunol., 2004, Vol. 24, No. 4, pp. 315-26. See the abstract and introduction.	1-43, 53-55, 57-60
A	NAKAMURA, K. et al., "CD3 and immunoglobulin G Fc receptor regulate cerebellar functions", Mol. Cell. Biol., July 2007, Vol. 27, No. 14, pp. 5128-34. See the abstract and fig. 2.	1-43, 53-55, 57-60

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 APRIL 2009 (07.04.2009)	**07 APRIL 2009 (07.04.2009)**

Name and mailing address of the ISA/KR	Authorized officer
Korean Intellectual Property Office Government Complex-Daejeon, 139 Seonsa-ro, Seo-gu, Daejeon 302-701, Republic of Korea	KIM, YUN-KYUNG
Facsimile No. 82-42-472-7140	Telephone No. 82-42-481-8406

Form PCT/ISA/210 (second sheet) (July 2008)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2008/006570

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of :

   a. type of material
   - [x] a sequence listing
   - [ ] table(s) related to the sequence listing

   b. format of material
   - [x] on paper
   - [x] in electronic form

   c. time of filing/furnishing
   - [x] contained in the international application as filed
   - [x] filed together with the international application in electronic form
   - [ ] furnished subsequently to this Authority for the purposes of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2008/006570

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 44-52, 56
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 44-52, 56 pertain to methods for treatment as well as diagnosis of the human or animal body by therapy and thus relate to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

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

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

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

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.