receptor-mediated over-activation of microglia and to avoid complement activation. MABT binds with high affinity to multiple forms of Aβ1-42 and Aβ1-40, protected against Aβ1-42 oligomer-induced cytotoxicity, mediated uptake of neurotoxic Aβ by microglia both *in vitro* and *in vivo*. When compared to a human IgG1 wild-type subclass containing the same antigen-binding variable domains and with equal binding avidity to Aβ, MABT showed reduced activation of the stress-activated p38 mitogen-activated protein kinase (p38MAPK) in microglia, and induced less release of pro-inflammatory mediators.

[0014] The present invention is also based in part on the unexpected role of p38MAP kinase activation in microglia cells for anti-Aβ antibody-mediated neuroprotection in AD. p38MAP kinase activity is generally thought to be pro-inflammatory and thus would have been thought to contribute to the pathogenic inflammatory state of amyloidosis, such as AD. Surprisingly, however, intermediate p38MAP kinase activation in microglia cells contributes to anti-Aβ antibody-mediated neuroprotection without generation of a pathogenic inflammatory state.

[0015] Provided herein are methods and compositions for the safe treatment and/or prevention of an amyloidosis, including but not limited to Alzheimer's Disease. Amyloidosis includes, but is not limited to, neurological disorders such as Alzheimer's Disease (AD), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type), the Guam Parkinson-Dementia complex, as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis, Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), Adult Onset Diabetes, senile cardiac amyloidosis, endocrine tumors, and others, including ocular disorders such as macular degeneration, cortical visual deficits, glaucoma, optic nerve drusen, optic neuropathy, optic neuritis, cataract, ocular amyloidosis and lattice dystrophy.

[0015a] Thus, in one aspect, there is provided a method for testing the safety of an anti-beta amyloid antibody, wherein the method comprises incubating microglia cells with beta amyloid in the presence of the anti-beta amyloid antibody; and measuring p38 MAP kinase activation in microglia cells in the presence of the anti-beta amyloid antibody; and identifying the anti-beta amyloid antibody as safe if the anti-beta amyloid antibody induces an intermediate level of p38 MAP kinase activation in the microglia cells, wherein the intermediate level of p38 MAP kinase activation is a level higher than a level of activation of p38 MAP kinase in the

presence of beta amyloid without the anti-beta amyloid antibody, but lower than a level of activation of p38 MAP kinase in the presence of beta amyloid and an IgG1 antibody that specifically binds beta amyloid, and wherein the IgG1 antibody comprises a human IgG1 constant region.

[0015b] In another aspect, there is provided a method for monitoring the course of treatment of amyloidosis in a patient being treated with an anti-beta amyloid antibody, wherein the method comprises a. measuring the degree of p38 MAP kinase activation in microglia cells obtained from the patient; and adjusting the course of treatment if the degree of p38 MAP kinase activation is different from an intermediate level of p38 MAP kinase activation in the microglia cells, wherein the intermediate level of p38 MAP kinase activation is a level higher than a level of activation of p38 MAP kinase in the absence of the course of treatment, but lower than a level of activation of p38 MAP kinase in the presence of beta amyloid and an IgG1 antibody that specifically binds beta amyloid, and wherein the IgG1 antibody comprises a human IgG1 constant region.

[0016] In particular, provided herein are anti-Aβ antibodies with effector regions that have been selected or modified to trigger an intermediate activation of p38MAP kinase in microglia cells. Further provided herein are methods for the treatment and prevention of an amyloidosis, including but not limited to Alzheimer's Disease, wherein the dose and/or administration regimen are selected such that p38MAP kinase is activated at an intermediate level in microglia cells. In specific ~~embodiments~~examples, a safe and functional anti-Aβ antibody has the effector region of an IgG4 antibody. In certain more specific examples~~embodiments~~, a safe and functional anti-Aβ antibody

has the CH2 region of an IgG4 antibody. In certain specific examplesembodiments, an intermediate level of p38 MAP kinase activation is a level above the level of p38 MAP kinase activation by toxic beta-amyloid oligomers alone but less than the level of p38 MAP kinase activation by an IgG1 anti-Aβ antibody in conjunction with the toxic beta-amyloid oligomers. In certain examplesembodiments, the effector region of the anti-Aβ antibody is modified such that its effector function is reduced. In certain examplesembodiments the modification can be any genetic alteration resulting in an amino acid substitution and/or a deletion.

[0017] Further provided are methods for improving the safety of an anti-Aβ antibody. In one exampleembodiment, method is provided for improving the safety of a non-IgG4 anti-Aβ antibody comprising replacing the constant region of said non-IgG4 anti-Aβ antibody with a constant region derived from an IgG4 antibody. In another exampleembodiment, a method is provided for improving the safety of a non-IgG4 anti-Aβ antibody comprising replacing the constant region of said non-IgG4 antibody with a constant region derived from an non IgG1 antibody. In a specific exampleembodiment, the method for improving the safety of a non-IgG4 anti-beta-amyloid antibody is for improving the safety of an IgG1 anti-Aβ antibody.

[0018] Further provided herein is a cell culture-based assay system to test anti-Aβ antibodies for their safety and functionality to treat and/or prevent an amyloidosis, including, but not limited to Alzheimer's Disease. In certain embodimentsexamples, microglia cells are incubated with toxic beta-amyloid oligomers and the test anti-Aβ antibody. Anti-Aβ antibody-mediated uptake of beta amyloid into the microglia cells demonstrates functionality of the antibody, *e.g.*, in mediating the clearance of beta amyloid. Anti-Aβ antibody-mediated p38 MAP kinase activation at intermediate levels in microglia cells indicates both the antibody is functional and safe. In certain examplesembodiments, an intermediate level of p38 MAP kinase activation is a level above the level of p38 MAP kinase activation by toxic beta-amyloid oligomers alone but less than level of p38 MAP kinase activation by an IgG1 anti-Aβ antibody having the level of effector function of the wild type (i.e., unmodified IgG1 constant region). This cell culture-based assay system can be used to test anti-Aβ antibodies for their ability to protect neurons from the neurotoxic effects of Aβ. Further, this cell culture-based assay system can be used to test anti-Aβ antibodies for their ability to trigger p38 MAP kinase activation at intermediate levels.

[0019] In certain examplesembodiments, the cell culture-based assay system further includes neurons. The survival rate of the neurons upon co-incubation with toxic beta-amyloid oligomers,

the test anti-Aβ antibody, and microglia cells demonstrates the functionality of the test anti-Aβ antibody.

[0020] In certain examples~~embodiments~~, the cell culture-based assay system is a primary cortical cell culture. The primary cortical cell culture is incubated with toxic beta-amyloid oligomers and the test anti-Aβ antibody. Anti-Aβ antibody-mediated uptake of beta amyloid into the microglia cells indicates functionality of the antibody, *e.g.*, in mediating the clearance of beta amyloid. Anti-Aβ antibody-mediated p38 MAP kinase activation at intermediate levels in microglia cells demonstrates both the functionality and safety of the antibody.

[0021] Further provided herein are safe and functional antibodies for the treatment and/or prevention of an amyloidosis, including, but not limited to Alzheimer's Disease. In certain examples~~embodiments~~, the variable region of a non-IgG4 humanized antibody which binds to Aβ is combined with the constant region of a human IgG4 antibody. In other examples~~embodiments~~, the variable region of an IgG1 humanized antibody which binds to Aβ is combined with the constant region of a human IgG4 antibody. In certain examples~~embodiments~~, the CH2 domain of a non-IgG4 humanized antibody which binds to Aβ is replaced with the CH2 domain of a human IgG4 antibody. In other examples~~embodiments~~, the CH2 domain of an IgG1 humanized antibody which binds to Aβ is replaced with the CH2 domain of a human IgG4 antibody. In certain examples~~embodiments~~, the constant region of the constant region is derived from an IgG1 antiby wherein the constant region of the IgG1 antibody is modified such that the modified constant region has a reduced or eliminated effector function.

[0022] In other examples~~embodiments~~, methods are provided for the treatment and/or prevention of an amyloidosis, including but not limited to Alzheimer's Disease, wherein an IgG1 anti-Aβ antibody is administered in combination with an anti-inflammatory agent such that p38 MAP kinase is activated at an intermediate level in microglia cells. In certain specific examples~~embodiments~~, intermediate levels of p38 MAP kinase activation are levels above the levels of p38 MAP kinase activation by toxic beta-amyloid oligomers alone but less than levels of p38 MAP kinase activation by an IgG1 anti-Aβ antibody having normal levels of effector function in the presence of oligomers alone without the anti-inflammatory agent.

[0023]

antibody (right). Quantitative measures of fluorescence are shown for 30 min treatment and 18 h treatment (B). Mean results (±SEM) of two experiments are shown. Aβ1-42 tagged with HyLite Fluor-488 verified that MABT inhibited binding of Aβ1-42 to neurites. Cortical cultures from P1 rats were treated as described for (A), except that Aβ1-42 tagged with HyLite Fluor-488 was used (C). Aβ1-42-labeled samples are shown in the upper panel and DAPI-stained samples are shown in the lower panel. One representative experiment is shown. Aβ1-42 fluorescence was quantified, with the mean (±SD) of the two independent experiments shown (D). Intracellular accumulation of Aβ1-42 was assayed by performing an ELISA for Aβ1-42 on trypsin-cleared cells as described in Methods (EC). Mean results (±SEM) of three experiments are shown. Upon treatment with MABT, Aβ1 42 oligomers appeared to be taken up by cells resembling microglia, as shown in (F). DAPI was used to label the cell nuclei; and an anti-Aβ antibody was used to label beta-amyloid.

[0065] Figure 4 provides graphs and confocal images corresponding to the experiments described in Example 6.5. Aβ1-42 oligomers were taken up via an FcR-mediated mechanism into microglia upon MABT treatment. Confocal imaging was used to show that Aβ1-42 oligomers complexed to MABT are taken up into microglia. DAPI was used to label the cell nuclei to show the microglia itself; and an anti-Aβ antibody was used to label Aβ1-42 oligomers (A). An apical-to-distal slice was obtained from a three-dimensional image rendered from Z-stacks. Confocal imaging was performed on mixed cortical cells labeled for Aβ (periphery) and stained with DAPI (center) as described in the Example Section under Materials and Methods. Microglia were identified and scanned for the maximum fluorescence intensity through a series of confocal stacks representing intracellular locations (B), and the total area of fluorescent signal above a minimum threshold was quantified (C). Each mark on the graph represents the total area of Aβ stained within a single cell. A minimum of 20 cells were analyzed for each treatment condition. Data were compared using one-way ANOVA followed by Tukey post-hoc multiple comparison. Means (±SEM) are shown. Microglia (Iba1+) was verified as the cell-type taking up Aβ1-42 complexed to MABT (D). Iba1 was used as a marker for microglia, HyLite Fluor-488-labeled Aβ1-42 was used to show Aβ1-42 A, and DAPI was used to show cell nuclei. Collocalization of Iba1 and Aβ1-42 is shown. Aβ1-42 oligomer internalization by different IgG monoclonal antibodies correlates with FcγR-binding. Differential binding to FcγRIIIa-V158 was verified in a binding assay (E). An anti-Aβ monoclonal antibody requires FcγR-binding for full

but effector functions such as cytotoxicity in the presence of effector cells, are reduced relative to the unmodified constant region.

5.3.2 Variable Regions

[00110] In certain embodiments, the variable regions of an antibody known to bind specifically to beta amyloid and / or its pathological form(s) can be used for the generation of a safe and functional antibody. In certain more specific embodiments, the variable regions of a humanized antibody known to bind specifically to beta amyloid and / or its pathological form(s) can be used for the generation of a safe and functional antibody. In certain embodiments, the variable regions are obtained from a non-IgG4 humanized antibody. In a specific embodiment, the variable regions are obtained from a non-IgG4 humanized antibody and an IgG4 constant region is used as constant region.

[00111] CDRs of antibodies known to bind to an amyloid protein of interest can be used for the generation of a safe and functional antibody useful in the methods described herein. In certain embodiments, CDRs of an antibody known to bind specifically to beta amyloid and/or its pathological form(s) can be used for the generation of such a safe and functional antibody.

[00112] Illustrative antibodies whose CDRs or variable regions can be used include, but are not limited to those set forth in Table 2.

Table 2: Illustrative anti-beta amyloid antibodies

Antibody	**Reference / Source**
mACI-01-Ab7 C2	WO 2007/068412 published June 21, 2007
mACI-01-Ab7 C2	WO 2008/060364 published May 22, 2008
mACI-02-Ab6	WO 2007/068412 published June 21, 2007
mACI-11-Ab9	WO 2007/068412 published June 21, 2007
mACI-12-Ab11	WO 2007/068412 published June 21, 2007
mACI-24-Ab4	WO 2007/068412 published June 21, 2007
ACI-24-Ab-3	WO 2008/156621 published December 24, 2008
ACI-11-Ab-9	WO 2008/156621 published December 24, 2008
ACI-12-Ab-11	WO 2008/156621 published December 24, 2008
20C2	WO 2007/050359 published May 3, 2007
8F5	WO 2007/064972 published June 7, 2007
8C5	WO 2007/064972 published June 7, 2007
6E10	Pirttilä *et al.* 1994, J Neurol Sci 127:90-95
4G8	Pirttilä *et al.* 1994, J Neurol Sci 127:90-95
MS-Roche#3 and MS-Roche#3 derived antibodies	WO 03/070760 published August 28, 2003