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Systemic vaccination with anti-oligomeric monoclonal antibodies improves cognitive function by reducing A β deposition and tau pathology in 3xTg-AD mice

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Abstract

Alzheimer's disease (AD) is a devastating disorder that is clinically characterized by a comprehensive cognitive decline. Accumulation of the amyloid-beta (A β) peptide plays a pivotal role in the pathogenesis of AD. In AD, the conversion of A β from a physiological soluble monomeric form into insoluble fibrillar conformation is an important event. The most toxic form of A β is oligomers, which is the intermediate step during the conversion of monomeric form to fibrillar form. There are at least two types of oligomers: oligomers that are immunologically related to fibrils and those that are not. In transgenic AD animal models, both active and passive anti-A β immunotherapies improve cognitive function and clear the parenchymal accumulation of amyloid plaques in the brain. In this report we studied effect of immunotherapy of two sequence-independent non-fibrillar oligomer specific monoclonal antibodies on the cognitive function, amyloid load and tau pathology in 3xTg-AD mice. Anti-oligomeric monoclonal antibodies significantly reduce the amyloid load and improve the cognition. The clearance of amyloid load was significantly correlated with reduced tau hyperphosphorylation and improvement in cognition. These results demonstrate that systemic immunotherapy using oligomer-specific monoclonal antibodies effectively attenuates behavioral and pathological impairments in 3xTg-AD mice. These findings demonstrate the potential of using oligomer specific monoclonal antibodies as a therapeutic approach to prevent and treat Alzheimer's disease.

Keywords

abeta oligomers; Alzheimer's disease; amyloid plaques; monoclonal antibodies; tau pathology; vaccination

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Accumulation of the amyloid- β (A β) peptide plays a pivotal role in the pathogenesis of Alzheimer disease (AD) (Golde *et al.* 2000; Hardy and Selkoe 2002; and Lee *et al.* 2007). In animal models of AD, both active and passive anti-A β immunotherapies improve cognitive functions and clear the parenchymal accumulation of amyloid plaques in the brain (Schenk *et al.* 1999; Bard *et al.* 2000; Janus *et al.* 2000; Morgan *et al.* 2000; DeMattos *et al.* 2001; Dodart *et al.* 2002; Frazer *et al.* 2008). Accordingly, immunization against A β has offered a promising approach toward the therapeutic management of AD (Schenk 2002; Vasilevko and Cribbs 2006; Brody and Holtzman 2008). Promising pre-clinical findings (Schenk *et al.* 1999) led to clinical trials with AN1792, a synthetic A β 42 vaccine, but further development was halted when 6% of immunized patients developed meningoencephalitis. Direct administration of anti-A β antibodies is believed to represent a safer alternative that minimizes the risk of a proinflammatory T cell response (Monsonogo *et al.* 2003) and permitting dosage control. Recent pre-clinical studies illustrate that passive immunization with antibodies that target amyloid plaques also increase cerebral amyloid angiopathy (CAA) and CAA-associated microhemorrhage in transgenic mouse models of AD (Pfeifer *et al.* 2002 and Wilcock *et al.* 2004, 2006; Racke *et al.* 2005). The mechanisms proposed for the effects of anti-A β immunotherapy on amyloid accumulation remain elusive and generally rely on systemic regimens that yield high titers of anti-A β antibodies in the peripheral circulation (Vasilevko and Cribbs 2006 and Levites *et al.* 2006). High antibody titers bind to substantial amounts of A β in the bloodstream, which is believed to shift the overall A β equilibrium and create a 'peripheral sink' that facilitates an efflux of A β from the brain (DeMattos *et al.* 2001 and Lemere *et al.* 2003). Furthermore, high doses of antibody in the periphery are required because of the low-level penetration of antibody across the blood-brain barrier to effectively engage the local (i.e., central) mechanisms for clearing the cerebral amyloid (Bard *et al.* 2000; Levites *et al.* 2006 and Banks *et al.* 2002). Interestingly, a single intracerebroventricular injection of anti-A β antibodies is able to prevent the A β -induced impairment of synaptic plasticity in the hippocampus (Oddo *et al.* 2003), and also transiently reverse the memory deficit in a transgenic AD mouse model (Oddo *et al.* 2004). It is also reported that passive immunization reduces oligomers, which directly induce GSK3b(β) activation and tau phosphorylation (Ma *et al.* 2006).

As antibodies that target plaque amyloid deposits are associated with CAA and microhemorrhage, we examined antibodies that are specific for pre-fibrillar oligomers that do not bind to plaques. In addition to polyclonal oligomeric specific antibody (A11) (Kayed *et al.* 2003), we isolated a number of different rabbit monoclonal antibodies (Mabs) including 204 and 205 that are specific for pre-fibrillar oligomers (Kayed *et al.* 2010). To gain further insight into the actions of anti-A β antibodies we tested the short time immunotherapeutic effects of anti-oligomeric antibodies in the aged 3xTg-AD mouse model. Our results not only show improvements in the cognitive function but also significantly reduced amyloid deposits and hyperphosphorylated tau.

Material and methods

All animal procedures were performed in accordance with animal protocols approved by the Institutional Animal Care and Use Committee at the University of California, Irvine (UCI). The 3xTg-AD have been described previously (Oddo *et al.* 2003). Briefly, these mice harbor

a knock-in mutation of presenilin 1 (PS1_{M146V}), the Swedish double mutation of amyloid precursor protein (APP_{KM670/671NL}), and a frontotemporal dementia mutation in tau (tau_{p301L}) on a 129/C57BL/6 background. These mice were a kind gift from Dr. Frank Laferla (University of California Irvine, USA). We used 32 female 3xTg-AD mice for our experiment. 3xTg-AD mice are widely used strain that exhibit both intracellular and extracellular A β deposits, tau pathology and cognitive deficits.

Vaccination

The detailed purification of these antibodies has already been reported previously (Kayed *et al.* 2010). These rabbit monoclonal antibodies were produced under a contract with Epitomics, Incorporated, Burlingame, CA. New Zealand white rabbits were immunized subcutaneously with A β 40-colloidal gold oligomer mimics at a 0.25 mg A β dose bit and boosted seven times with the same amount at 3 weeks intervals. The titer and specificity of the immune response was determined by ELISA and dot blot analysis as previously described using serum collected after the third and sixth boosts (Kayed *et al.* 2003) The mice were distributed equally into four groups with eight mice in each group. Oligomer specific antibodies were injected (intraperitoneal 300 μ g/150 μ L). One group received the polyclonal oligomer-specific antibody, A11 (Kayed *et al.* 2003), whereas the other two groups received monoclonal oligomer-specific antibodies 204 and 205 (Kayed 2010). The control group was vaccinated with pre-immune rabbit IgGs (Jackson Immuno-Research Lab, West Grove, PA, USA). The antibodies were administered weekly intraperitoneally for 5 weeks in 13-month-old 3xTg-AD mice. After 5 weeks, mice went through a battery of behavioral test and were killed at 15 months.

Morris water maze

The Morris water maze test was used to assess memory related to the hippocampal function as previously described (Billings *et al.* 2005). After 5 weeks of antibody administration morris water maze test was performed. For each trial, the mouse was placed into the tank at one of four designated start points in a pseudorandom order. Mice were given four trials a day until they reach the given criterion (< 20 s). The 3xTg-AD mice were trained for 6 days to meet the criterion. After 24 h of retention of spatial memory was assessed. In the probe trial consisted of a 60 s free swim in the pool without the platform. The parameters measured during the probe trial included: (i) initial latency to cross the platform location, (ii) number of platform location crosses, and (iii) time spent in the quadrant opposite to the one containing the platform during training.

Passive inhibitory avoidance

Amygdala dependent tasks were evaluated using the passive inhibitory avoidance (Decker *et al.* 1990). This was measured using a Gemini Avoidance System instrument (San Diego Instruments, San Diego, CA, USA). During the trial period, the mice were placed inside the lighted compartment and latency was recorded (time to enter in the dark compartment). On complete entry into the dark compartment, the animal received a mildly aversive foot shock (0.15 mA, 1 s). After 24 h the mouse was again placed back in the lighted compartment and the step-through latency before re-entering the dark side was measured for a period up to

180 s. The retention trial was interrupted if the animal took more than 180 s to cross into the dark compartment.

Tissue collection and immunohistochemistry

After mice were anesthetized with pentobarbital (150 mg/kg, IP), blood was collected by cardiac puncture, and mice were perfused transcardially with cold phosphate-buffered saline (PBS). Brain tissues were fixed overnight with 4% paraformaldehyde in PBS, pH 7.4 at 4°C and stored in PBS/0.02% sodium azide (NaN₃) at 4°C until use. Fixed brain tissues were sectioned (40 µm) with a vibratome. Coronal sections were collected in PBS (containing 0.02% sodium azide) and stored at 4°C prior to staining. To stain for Aβ plaques, sections were immersed in 70% formic acid for 5 min. Endogenous peroxidase in tissue was blocked by treating with 3% H₂O₂ in PBS for 10 min at 25°C. Nonspecific background staining was blocked by 1 h incubation in 2% bovine serum albumin, 0.3% Triton X-100 (TX) at 25°C. Tissues were incubated with primary antibodies (6E10, AT8, and CD45) overnight at 4°C, rinsed three times with PBS, 0.1% TX, followed by biotinylated secondary antibodies (anti-rabbit, anti-mouse, and anti-rat), detection with an ABC peroxidase kit, and visualization with a 3,3'-diaminobenzidine substrate kit (Vector, Burlingame, CA, USA). CD45 (1 : 3000; Serotec, Raleigh, NC, USA) staining was done as described previously (Gordon *et al.* 2002). Control experiments with primary or secondary antibody omitted resulted in no staining.

Image quantification

Image quantification was done as described in our previous publication (Rasool *et al.* 2012). Briefly immunostaining was observed under a Zeiss Axiovert- 200 inverted microscope (Carl Zeiss, Thornwood, NY, USA) and images were acquired with a Zeiss AxioCam high-resolution digital color camera (1300 × 1030 pixel) using Axiovision 4.1 or 4.6 software. The same software (Carl Zeiss) was used to analyze the digital images. Percent of immunopositive area (% Field Area) (immunopositive area/total image area × 100) was determined for all the markers studied by averaging images of the hippocampus and subiculum area from 4 to 5 sections per animal. Digital images were obtained using the same settings and the segmentation parameters constant within a range per given marker and experiment. The mean value of the % Field Area for each marker in each animal was averaged per genotype group with the number of animals per group indicated in Figure legends.

Enzyme-linked immunosorbent assay for soluble and insoluble Aβ

We used the same procedure for detecting soluble and insoluble form of Aβ (Rasool *et al.* 2012). Briefly soluble and insoluble Aβ fractions were isolated from whole brain homogenates using a four step extraction protocol (Kawarabayashi *et al.* 2001). Frozen hemibrains were sequentially extracted. At each step, sonication in an appropriate buffer was followed by centrifugation at 100 000 g for 1 h at 4°C. The supernatant was then removed, and the pellet was sonicated in the next solution used in the sequential extraction process. For four-step extraction, sonication of the frozen brain (150 mg/mL wet weight) began in Tris-buffered saline (20 mM Tris and 137 mM NaCl, pH 7.6), which contained protease inhibitors (Protease inhibitor cocktail from Sigma St. Louis USA). The next three

sequential extraction steps used 1% Triton X-100 in Tris-buffered saline with protease inhibitors, 2% sodium dodecyl sulfate in ddH₂O with the same protease inhibitors, and 70% formic acid in ddH₂O. To measure the soluble and insoluble form of A β , insoluble fractions were diluted to 1 : 20 in a neutralization buffer (1 mol/L Tris base, 0.5 mol/L NaH₂PO₄) before loading on MaxiSorp immunoplates (Nunc, Rochester, NY, USA), whereas soluble fractions were loaded directly. The immunoplates were coated with Mab 20.1 antibody (kind gift from Dr. David Cribbs, University of California, Irvine) at a concentration of 25 μ g/mL in coating buffer (0.1 M/L Na₂CO₃, pH 9.6) and blocked with 3% bovine serum albumin. Standard solutions for both A β 1-40 and A β 1-42 were made in the antigen capture buffer (20 mmol/L NaH₂PO₄, 2 mmol/EDTA, 0.4M NaCl, 0.05% 3-[(3- cholamidopropyl) dimethylammonio]propanesulfonate, and 1% bovine serum albumin, pH 7.0) and loaded onto ELISA plates in duplicate. Samples were then loaded (also in duplicate) and incubated overnight at 4°C. Plates were then washed and probed with either horseradish peroxidase conjugated anti-A β 1-40(C49) or anti-A β 1-42(D32) overnight at 4°C. The chromogen was 3,3 – 5,5 tetramethylbenzidine and the reaction was stopped by 30% phosphoric acid. The plates were read at 450 nm using a plate reader (Molecular Dynamics, Sunnyvale, CA, USA). Readings were then normalized to protein concentrations of the samples.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). For comparison between antigen vaccinated groups and control vaccinated group, one-way ANOVA followed by Dunnet post-test was performed. Probability values less than 0.05 were accepted as statistically significant.

Results

Vaccination improves cognitive performance

We first sought to determine whether aged mice harboring an extensive plaque and tangle burden would show improved learning and memory following passive immunization. At the end of the treatment period, we evaluated the behavioral phenotype of the 3xTg-AD mice using two different behavioral paradigms, the Morris water maze and the passive inhibitory avoidance Fig. 1a, shows the time line of the passive immunization trial. 13-month-old 3xTg-AD mice were immunized with A11, 204, and 205 antibody at 1 week intervals for 5 weeks. We evaluated the reference memory after 5 weeks of vaccination in a Morris water maze test. All of the mice vaccinated with anti-oligomer antibodies demonstrated a significant improvement ($p < 0.001$) in the acquisition and retention of the Morris Water Maze test as compared to control IgG vaccinated mice (Fig. 1b). At 24 h probe trials, mice vaccinated with A11, 204, and 205 showed significant improvement ($***p < 0.001$) in retention memory as evident by their improved latency to cross the platform location and the number of platform crosses during the 24 h tests as compared with controls (Fig. 1c). Contextual learning and memory was evaluated using the passive avoidance task. In retention trials, the step-through latency of antibody-vaccinated mice significantly increased ($**p < 0.01$) as compared to that of the control rabbit IgG-vaccinated group (Fig. 1d). These results indicate that vaccination with oligomer specific antibodies showed significant improvement in behavior as compared with control vaccinated mice.

Vaccination with oligomer antibodies reduces A β plaque accumulation in 3xTg-AD mice

We next determined the effect of passive immunization on plaque burden in the aged mice. Total amyloid deposits in the 3xTg-AD mice after 5 weeks of vaccination were analyzed by immunohistochemistry using 6E10. We compared the effectiveness of the immunization on amyloid deposition by quantifying the amount of anti-A β (6E10) immunoreactive material. Fig. 2a, shows representative photomicrographs from the hippocampus of control and vaccinated animals. Image analysis of sections from multiple animals demonstrated that A β deposits in A11, 204, and 205 vaccinated mice were decreased significantly as compared with control vaccinated mice. Fig. 2b, shows that all the immunized groups displayed a significant reduction in A β plaque load in hippocampus as compared to the respective controls ($***p < 0.001$, $**p < 0.01$). These results indicate that administration of oligomer specific antibodies lower the amount of plaque deposits, even in mice with extensive plaque and tangle neuropathology.

Vaccination with oligomeric antibodies reduces both soluble and insoluble A β 40 and A β 42 levels

3xTg-AD mice increasingly accumulate A β as they age with fibrillar amyloid plaques starting to develop from 12 months of age (Oddo *et al.* 2006a,b). It has been previously reported that the initial cognitive deficits in these mice are caused by intraneuronal A β levels and that cognitive deficits increase with increasing brain pathology. To evaluate whether vaccination with oligomer-specific antibodies not only reduce plaque load but also affect cerebral total A β levels, brain homogenates were processed using four step extraction which distinguishes between soluble and insoluble A β levels. Subsequently, A β 40 and 42 levels were quantified using a high-sensitivity sandwich ELISA as described in Materials and Methods. Consistent with a decrease in amyloid plaques immunostaining, we observed a significant reduction in brain soluble and insoluble A β 40 and A β 42 levels (Fig. 3) in all three of the oligomer-specific antibody vaccinated groups.

Vaccination with antibodies reduces tau pathology

The formation of tau tangles is believed to be dependent on A β deposition (Vossel *et al.* 2010). Therefore, we assessed whether administration of anti-oligomer antibodies could reduce the tau pathology. The hyperphosphorylated micro-tubule-associated protein tau is the major component of the paired helical filament of Alzheimer's disease, we also examined tau pathology using AT8, an antibody that selectively recognizes tau that is phosphorylated at serine 202 and threonine 205. As hyperphosphorylation of tau is a critical step in the pathway leading to neurofibrillary pathology, we determined whether the A β -mediated clearance of tau was dependent on its phosphorylation state. At 14 months of age, homozygous 3xTgAD mice show extensive tau hyperphosphorylation in CA1 pyramidal neurons and were immunopositive for tau antibodies. To investigate the effect of oligomer specific antibodies on hyperphosphorylated, we stained the brain section with AT8 antibody. Fig. 4a, shows the representative photomicrographs from the hippocampus of control and vaccinated mice stained with AT8 antibody. After 5 weeks of vaccination we observed statistically significant reduction in hyperphosphorylated tau aggregates as compared with control IgG vaccinated mice (Fig. 4b, $**p < 0.01$). We also investigated the effect of

antibodies on total tau using HT7Ab. Immunoreactivity with HT7 antibody displays a reduction in antibody vaccinated mice (Fig. 5). Moreover, we cannot exclude the possibility of the reduction in HT7 staining in the 3xTg-AD-treated mice is also due to the decrease in A β , as it has been previously showed the lowering A β reduces HT7-positive, non-phosphorylated tau (Oddo *et al.* 2004).

Vaccination reduces microglial activation

One of the main characteristics accompanying the accumulation of A β plaques in both human AD brains and in transgenic mouse models of AD is an enhanced neuroinflammatory response characterized by activation of microglia. It has been previously reported that immunization with A β reduces the microglial activation (Wilcock *et al.* 2003). We assessed the effect of vaccination by anti-oligomeric antibodies on microglial reactivity as measured by CD45, which is a protein-tyrosine phosphatase that is normally moderately expressed on microglia around amyloid deposits in aged APP transgenic mice and is a widely used marker for microglial activation. Fig. 6a, shows representative photomicrographs from the cortex including white matter of control and vaccinated animals. Image analysis of sections from multiple animals demonstrated that CD45 immunore-activity in A11, 204, and 205 vaccinated mice were decreased significantly as compared with control vaccinated mice. After 5 weeks of vaccination, we observed a statistically significant decrease in % age of CD45 immunostaining relative to the respective control vaccinated mice (Fig. 6b, *** $p < 0.001$).

Discussion

Passive immunization has been shown to improve cognition by reducing amyloid plaque levels (Chen *et al.* 2000; Morgan *et al.* 2000). Passive immunotherapy in APP transgenic mice has been shown to be applicable to human clinical trials (Bard *et al.* 2000; DeMattos *et al.* 2001; Dodart *et al.* 2002) and so are other treatments that reduce A β burden (Blennow *et al.* 2006). Diffuse A β plaques in mouse models such as Tg2576, PDAPP and 3xTg-AD were efficiently reduced by most N-terminal anti-A β antibodies (Bard *et al.* 2000; Levites *et al.* 2006; Oddo *et al.* 2006b). It has also been reported that thioflavin-S positive plaques are cleared by certain anti-A β antibodies of the IgG2a isotype (Bussiere *et al.* 2004). In transgenic models with highly insoluble dense-cored plaques, like tg-ArcSwe mice (Philipson *et al.* 2009), the effects of anti-A β immunotherapy were subtle and purely preventive (Levites *et al.* 2006; Tucker *et al.* 2008) with only diffuse non-fibrillar A β deposits being cleared (Levites *et al.* 2006). In such studies, induced transgene driven intracerebral injections of A β antibodies lacked efficiency as only nascent A β production was blocked (Tucker *et al.* 2008). A β protofibril levels were lowered while levels of insoluble A β were unaffected as detected using protofibril-selective antibody mAb158 (Lord A *et al.* 2009). In contrast, our data indicate that the formation of amyloid deposits can be reversed with passive immunization using pre-fibrillar oligomer-specific antibodies. These antibodies are specific for oligomers that are immunologically distinct from fibrils and do not bind plaques (Kayed *et al.* 2010). We observed a significant reduction in both soluble and insoluble levels of A β 40 and 42. This reduction may be affected by the opsonization of antibody-bound oligomeric A β 42 or 40 by microglia or infiltrated macrophages. It has been

previously reported that intracranial injection of anti-A β in 19-month-old Tg2576 mice showed a dramatic reduction in cerebral amyloid by 24 h after a single dose, which was maintained up to 7 days post-injection (Wilcock *et al.* 2003)

Current evidence suggests that hyperphosphorylation and aggregation of tau protein is an important pathway driving neurodegeneration in AD. Tau is a microtubule-binding protein involved in regulation of neuronal microtubule assembly and stabilization (Caceres and Kosik 1990; Avila *et al.* 1994; Esmaeli-Azad *et al.* 1994; Harada *et al.* 1994). Aggregates of A β are candidate triggers of tau hyperphosphorylation, aggregation and the subsequent degeneration of affected neurons (Greenberg *et al.* 1992; Iqbal *et al.* 1994; Sigurdsson *et al.* 1997; Lovestone and Reynolds 1997; Spillantini and Goedert 1998; Lee and Trojanowski 1999). In 3xTg-AD mice, only the early, but not the late forms of phosphorylated human tau were removed by a single injection of anti-A β antibody into the brain (Oddo *et al.* 2004).

Clearance of tau is critically dependent on its phosphorylation state. At late-stages, hyperphosphorylated aggregates appear to be unaffected by anti-A β antibody treatment. Notably, the injection of the anti-oligomeric A β antibody A11, leads to the reduction of tau pathology and A β pathology in 3x Tg-AD mice and strongly suggest that A β oligomers may represent a link between A β and tau pathology (Oddo *et al.* 2006a,b). It has been previously reported that passive immunization reduces A β oligomeric levels, which has been shown to directly induce GSK3 β activation and tau phosphorylation (Ma *et al.* 2006). Anti-A β active immunization has been shown to decrease tau hyperphosphorylation in two other mouse models that sequentially develop amyloid plaques and neuronal aggregates of hyperphosphorylated native murine tau, thus adding further evidence for its potential beneficial effect on tau pathology (Wilcock *et al.* 2009). These data are consistent with our results showing that passive immunization with anti-oligomer antibodies efficiently clears hyperphosphorylated tau. It has been shown that A β fibrils accelerate the formation of abnormally phosphorylated neurofibrillary tangles in a tau transgenic mouse (Gotz *et al.* 2001), and that A β could induce tau phosphorylation and toxicity in cultured septal cholinergic neurons (Zheng *et al.* 2002). More recently, it has been shown that A β oligomers cause abnormal tau phosphorylation and morphology changes of spines by missorting of endogenous tau into dendrites (Zempel *et al.* 2010). Naturally occurring A β dimers isolated from AD brains are sufficient to induce AD-type tau phosphorylation and, consequently, neuritic dystrophy (Jin *et al.* 2011). For synaptic plasticity, it has been found that the absence of tau protein inhibits the impairment of LTP and neurotoxicity caused by A β (Shipton *et al.* 2011) and targeting tau by immunotherapy prevents cognitive decline in a novel tangle mouse model (Asuni *et al.* 2007; Boutajangout *et al.* 2010).

CD45 is a protein-tyrosine phosphatase that is elevated with microglial activation. Loss of microglia activation after intracranial antibody administration (Wilcock *et al.* 2003) and active immunization (Wilcock *et al.* 2001) has been previously reported. In our study, we also observed a significant reduction in CD45 levels as compared with control IgG vaccinated mice. This suggests that the reduced microglial activation could possibly be attributable to the clearance of most amyloid plaques. It is also conceivable that the microglia could be undergoing apoptosis attributable to the robust activation, as described

previously by Liu *et al.* (2001), when microglia are over-activated by lipopolysaccharide. An alternative explanation could be tolerance of the microglia to antibody-opsonized A β .

Our findings demonstrated that conformation-dependent antibodies efficiently clear amyloid plaque, tau pathology and improve cognition, which highlight that these oligomer specific antibodies have a therapeutic potential to prevent and treat Alzheimer's disease. Because these antibodies do not target plaques and other fibrillar amyloid deposits, they may have a more favorable safety profile than antibodies that increase CAA and the risk of hemorrhage.

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Abbreviations used

A β	amyloid-beta
AD	Alzheimer's disease
APP	amyloid precursor protein
CAA	cerebral amyloid angiopathy
PBS	phosphate-buffered saline

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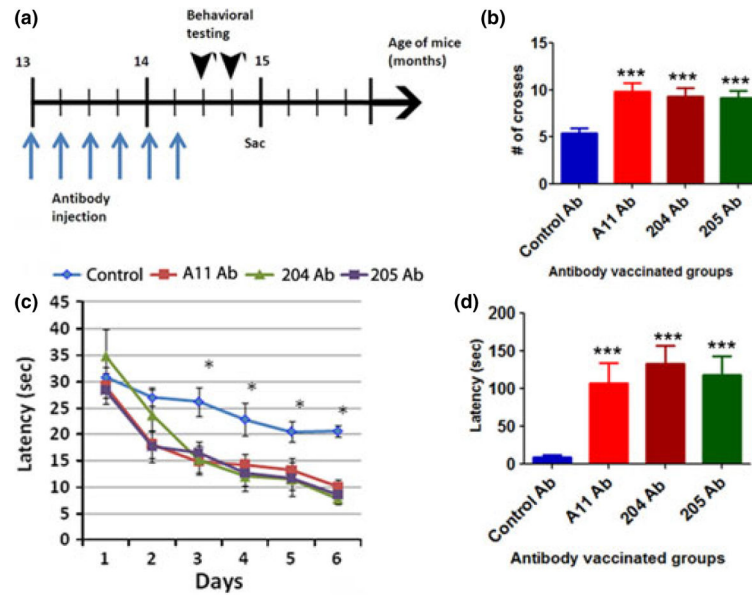


Fig. 1. Vaccination with antibodies improves spatial cognitive deficits in 3xTg-Alzheimer's disease (AD) mice. Schematic representation of the passive immunization paradigm. The *arrows* represent the antibody treatment and the *arrowheads* represents the behavior testing (a). After 5 weeks of passive vaccination with oligomeric antibodies, 3xTg-AD mice were evaluated using a spatial reference paradigm and compared with control treated mice vaccinated with colloidal gold and adjuvant. (b) Mice were trained on the spatial reference version of the Morris water maze task after treatment. All the vaccinated mice showed significant improvement ($*p < 0.05$) as compared with control (b). (c) In probe trial during 24 h testing, all vaccinated 3xTg-AD mice also display significantly more number of crosses around to platform than rabbit IgG control vaccinated mice (ANOVA, $***p < 0.001$). Vaccination prevents contextual fear memory deficits in a mainly amygdala-dependent task. (d) Mice were tested for retention of memory for fear-associated environments 24 h after training. Mice were taken out after 180 s if they did not cross over. At 24 h all the oligomeric antibody vaccinated mice showed significant improvement ($***p < 0.001$).

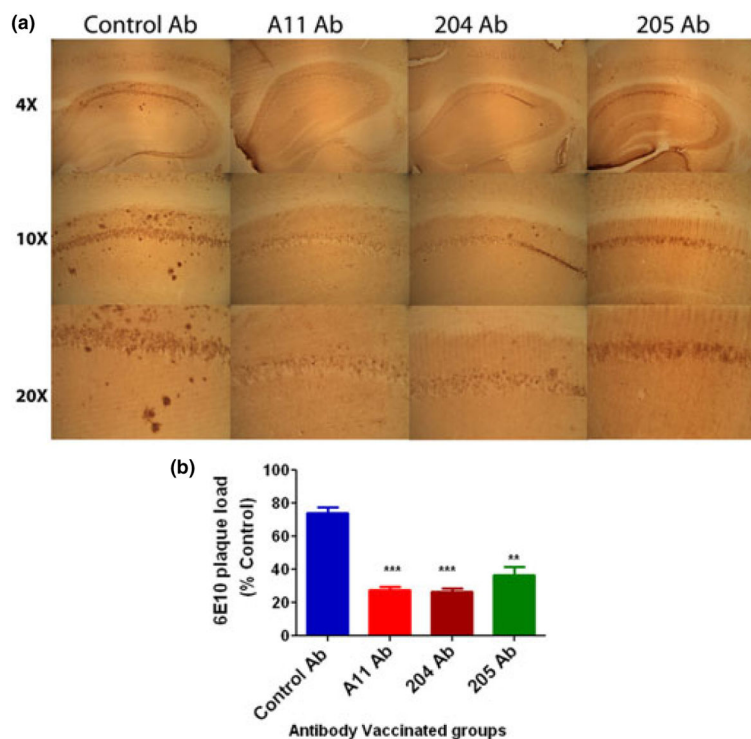


Fig. 2. Passive vaccination decreases total amyloid deposits. (a) Representative photomicrographs of sections from brains (hippocampus) of 3xTg-Alzheimer's disease mice after 5 weeks of vaccination (Control antibody, A11 antibody, 204 antibody, and 205 antibody vaccinated). Immunostaining was done with 6E10 (which reacts with the human amyloid β peptide). Scale bar: 100 μ m. Image analysis (% Field area) of A β (6E10 antibody) immunoreactivity in hippocampus. Mean of each animal is the average of 4 sections (except untreated control which is 1 section per animal) assessing 4–8 images per section (most to all of the area of the section was analyzed). Bars represent group mean \pm SEM of *n* mice per group: Control antibody vaccinated *n* = 8, A11 antibody vaccinated *n* = 8, 204 antibody vaccinated *n* = 8 and 205 antibody vaccinated *n* = 8, ****p* < 0.001, ***p* < 0.01 by ANOVA (b).

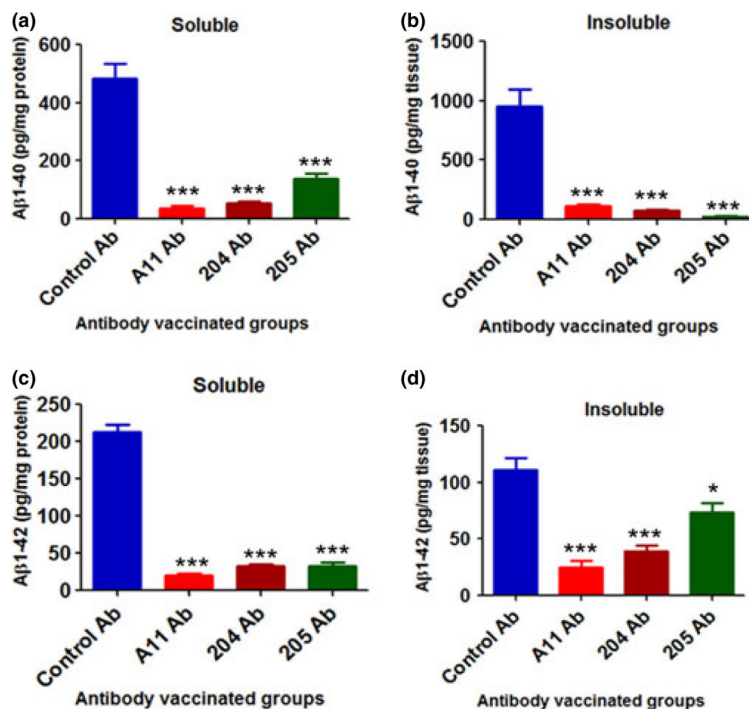


Fig. 3. Vaccination with oligomeric antibodies strongly reduces levels of soluble and insoluble Aβ40 and Aβ42 in brain. Following 5 weeks of passive vaccination, levels of soluble and insoluble Aβ40 (a, b) & Aβ42 (c, d) on homogenates of the whole brain hemisphere of 3xTg-Alzheimer's disease mice were determined using ELISA and reported in pg of Aβ/mg of protein in case of soluble and pg of Aβ/mg of wet tissue weight (insoluble). Oligomer antibody treatment was associated with significantly lower levels of both soluble and insoluble levels of Aβ40 and Aβ42. Error bars represent standard errors of the mean ($n = 8$). *** $p < 0.001$ for soluble and insoluble level of Aβ40 and *** $p < 0.001$ for soluble Aβ42 and *** $p < 0.001$ for insoluble levels of Aβ42 in A11 antibody vaccinated mice and 204 antibody vaccinated mice, * $p < 0.05$ for 205 antibody vaccinated mice by one-way ANOVA test.

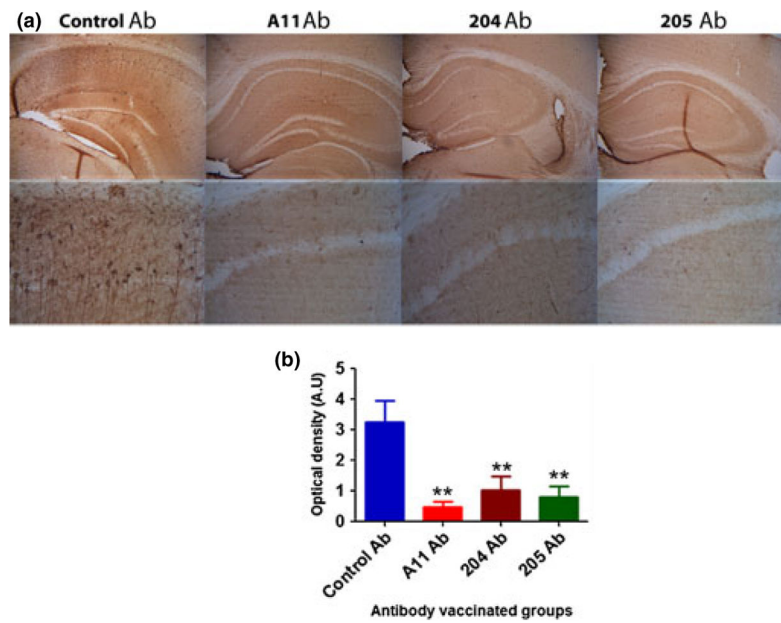


Fig. 4. Passive vaccination with oligomer antibodies led to a reduction in total tau levels: (a) Representative photomicrographs of sections from brains (hippocampus) of 3xTg-Alzheimer's disease mice after 5 weeks of vaccination (Control antibody, A11 antibody, 204 antibody, and 205 antibody vaccinated). Immunostaining was done with HT7 (which recognizes the total tau). Immunohistochemical analysis of oligomer antibody vaccinated mice showed a prominent reduction of total tau in the hippocampus (scale bar = 100 μ m) as compared with control antibody vaccinated mice (b). The reduction was statistically significant $**p < 0.01$.

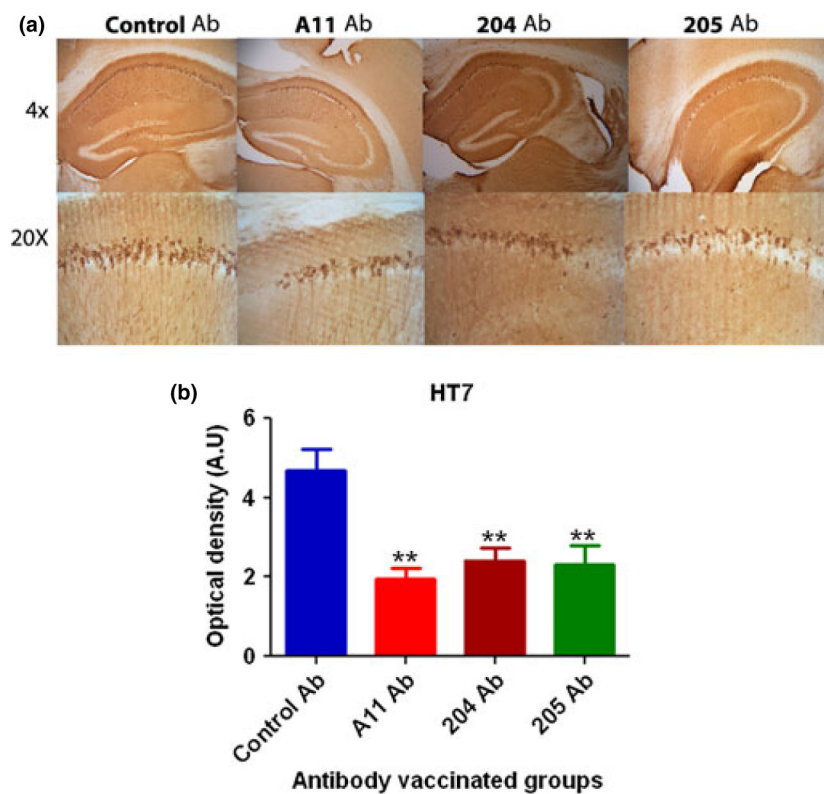


Fig. 5. Antibodies against oligomeric A β decrease hyperphosphorylated tau using AT8 antibody. (a) Representative photomicrographs of sections from brains (hippocampus) of 3xTg-AD Alzheimer's disease mice after 5 weeks of vaccination (Control antibody, A11 antibody, 204 antibody, and 205 antibody vaccinated). Brain sections were subjected to immunostaining using anti-phosphorylated tau (Ser202) (AT8 antibody). Immunohistochemical analysis of oligomer antibody vaccinated mice showed a prominent reduction of hyperphosphorylated tau in the hippocampus (scale bar = 100 μ m) as compared with control antibody vaccinated mice. The reduction was statistically significant $**p < 0.01$ (b).

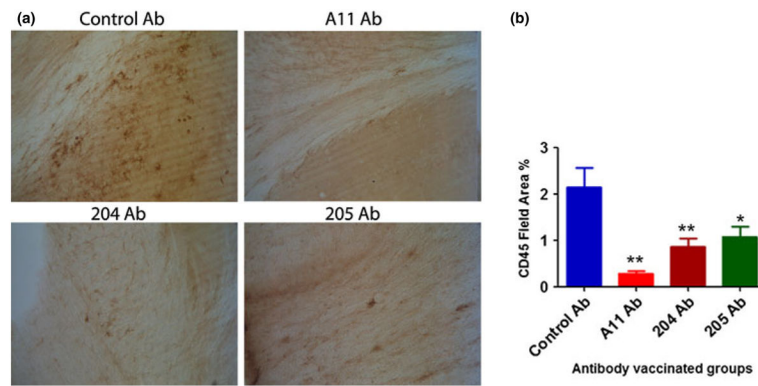


Fig. 6. Vaccination with oligomeric antibodies decreases activated microglia. Brain sections were subjected to immunostaining using microglial marker (CD45 antibody). Representative photomicrographs of CD45 (brown) staining in the cortex and white matter of brain sections from 3xTg-Alzheimer's disease mice (a). Mean of each animal is the average of two sections in which most to all the area of study was analyzed (4–8 images per section) (b) Bars represent mean \pm SD of $n = 8$ mice per group. ** $p < 0.01$ for A11 antibody and 204 antibody vaccinated mice, * $p < 0.05$ for 205 antibody vaccinated mice.