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$A\beta_{42}$ gene vaccination reduces brain amyloid plaque burden in transgenic mice

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Abstract

Objective: To demonstrate that in APPswe/PS1DE9 transgenic mice, gene gun mediated $A\beta_{42}$ gene vaccination elicits a high titer of anti-$A\beta_{42}$ antibodies causal of a significant reduction of $A\beta_{42}$ deposition in brain.

Methods: Gene gun immunization is conducted with transgenic mice using the $A\beta_{42}$ gene in a bacterial plasmid with the pSP72-E3L-$A\beta_{42}$ construct. Enzyme-linked immunoabsorbent assays (ELISA) and Western blots are used to monitor anti-$A\beta_{42}$ antibody levels in serum and $A\beta_{42}$ levels in brain tissues. Enzyme-linked immunospot (ELISPOT) assays are used for detection of peripheral blood T cells to release γ-interferon. Immunofluorescence detection of $A\beta_{42}$ plaques and quantification of amyloid burden of brain tissue were measured and sections were analyzed with Image J (NIH) software.

Results: Gene gun vaccination with the $A\beta_{42}$ gene resulted in high titers of anti-$A\beta_{42}$ antibody production of the Th2-type. Levels of $A\beta_{42}$ in treated transgenic mouse brain were reduced by 60-77.5%. The Mann-Whitney U-test P = 0.0286.

Interpretation: We have developed a gene gun mediated $A\beta_{42}$ gene vaccination method that is efficient to break host $A\beta_{42}$ tolerance without using adjuvant and induces a Th2 immune response. $A\beta_{42}$ gene vaccination significantly reduces the $A\beta_{42}$ burden of the brain in treated APPswe/PS1DE9 transgenic mice with no overlap between treated and control mice.

Keywords

$A\beta_{42}$ gene vaccination; Brain amyloid plaque; Transgenic mice

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia and currently there is no cure or effective treatment to prevent its progression. The cause of AD has been associated with the accumulation, aggregation, and deposition of amyloid beta peptides (Ah) in cerebral cortex, hippocampus, and other subcortical structures [1,2]. Aβ is derived proteolytically from a glycosylated membrane protein, beta-amyloid precursor protein [3,4] (APP). APP is

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ubiquitously expressed but at higher levels in the central nervous system [5,6]. The derived Ah in brain is 39 to 43 amino acids in length but the major processed species are $A\beta_{40}$ and $A\beta_{42}$. The aggregated form of $A\beta_{42}$ has been identified to play a major role in the initiation of neuropathology of AD [7-9].

In recent years, reduction in the level of Aβ in brain has become a major therapeutic goal in prevention and treatment of AD. In transgenic mice carrying mutations causal of AD, reduction of the amyloid burden in brain and cognitive improvement have been achieved by active immunization of these mice with $A\beta_{42}$ peptide or by infusion of anti-Ah antibodies [10-14]. A clinical trial of patients with Alzheimer's disease was conducted with active $A\beta_{42}$ peptide immunization but the trial was halted due to the complications associated with a cell-mediated cytotoxic T cell mediated meningoencephalitis in 6% of immunized AD patients [15,16]. Neuropsychological testing of immunized patients showed some slowing of cognitive loss in a subset of patients with significant anti-Ah antibody production who subsequently had Ah plaque reduction and clearance on postmortem examination [17-21].

In the present paper, we demonstrate that in APPswe/PS1ΔE9 transgenic mice, gene gun mediated $A\beta_{42}$ gene vaccination efficiently elicits a high titer of anti-$A\beta_{42}$ antibodies and the $A\beta_{42}$ deposition in the brains of these mice is significantly reduced without a significant adverse cellular immune response. We believe gene vaccination can be an approach to reduce Aβ levels in the brain of patients with Alzheimer's disease.

2. Materials and methods

2.1. Mice

APP-PS1 co-injected transgenic mice (7-10weeks) carrying the human APP-Swedish and PS1DE9 mutations were purchased from Jackson Laboratory (Bar Harbor, ME) (Stock Number 004462). These animals develop numerous amyloid plaque deposits in the cerebral cortex and hippocampus by 6 months of age. The wild type female Balb/c mice (age 5-7week) were obtained from Harlan Inc. (Indianapolis, IN). The use of animals for this study was approved by the UT Southwestern Medical Center Animal Care and Use Committee.

2.2. DNA constructs

The open reading frame of the human $A\beta_{42}$ and $A\beta_{16}$ genes were chemically synthesized with optimal codons for mammalian cell expression and cloned into the genetic immunization vector as described previously [22]. The $A\beta_{42}$ and $A\beta_{16}$ genes were fused with the leader sequence of adenovirus E3 gene [23]. A lysosome targeting sequence was fused downstream of the $A\beta_{42}$ and $A\beta_{16}$ sequences [24]. For comparison, we also replaced the E3 leader with the human alpha-1 antitrypsin leader sequence, or insert between the leader and $A\beta_{42}$ or $A\beta_{16}$, a B-cell targeted CTA1-DD gene [25] (ADP-ribosylation domain of cholera toxin with an Ig-binding fragment D of Staphylococcus aureus protein A) or the rat cartilage oligomerization matrix protein (COMP) gene [26]. The artificial SP72 and CMV promoters were also compared [22, 26].

2.3. Gene gun immunization

Immunizations of mice with plasmid DNA encoding the $A\beta_{42}$ gene were performed on mouse ear skin using the Helios gene gun (Bio-Rad, Hercules, CA) as described [21]. Briefly, DNA-coated gold particles were prepared by binding DNA to the gold beads (1-2Am, Ferro Electronic Material Systems, South Plainfield, NJ) in 1Ag DNA/1mg gold beads in the presence of spermidine and calcium chloride. The DNA-gold was attached to the insides of the tubing and cut into 1.3cm long as a bullet. The DNA-coated gold particles were bombarded to both sides of the mouse ears using the gene gun with a helium gas of 400psi. The APPswe-

PS1ΔE9 mice ($N = 4$) were immunized with the $A\beta_{42}$ gene starting from the age of 3months with first three immunizations at 2-week intervals and then once per month for a total of 11 immunizations. The control group ($N = 4$) received either a non-Ah peptide vector (2) or $A\beta_{16}$ peptide vector (2) with the same immunization schedule. The blood was drawn from the tail vein and the serum sample was used to monitor the humoral immune response.

2.4. Immunoassay for detection of anti-Abβ_{42} antibodies in serum

Enzyme-linked immunoabsorbent assay (ELISA) and Western blot were used to monitor the humoral immune responses [22]. In brief, mouse blood was collected from tail vein and serum was used to detect Aβ peptide by ELISA with 96-microwell plate coated with GST-Ah proteins. For Western blot, the GST-Ah proteins in bacteria extract were separated by SDS-PAGE, blotted onto nitrocellulose, incubated with the sera at 1:2000 dilutions. Antibodies against Aβ were detected using peroxidase-conjugated affinity-purified rabbit antiserum against mouse Ig. To determine the specific isotypes, sera from mice were diluted 1:200 and tested with ELISA method as described above. To detect mouse IgG1, IgG2a, we used anti-mouse Ig-subclass-specific rabbit antibody (Pierce, Rockford, IL), followed by incubation with HRP-conjugated Donkey anti-rabbit IgG.

2.5. Enzyme-linked immunospot assay (ELISPOT)

The cell-mediated immune response was evaluated by enzyme-linked immunospot assays (ELISPOT assay) for detection of peripheral blood T cell to release interferon-gamma during in vitro re-stimulation with Aβ peptide [27]. Briefly, 96-well polyvinylidine diflouride (PVDF) plates (Millipore, Bedford, MA) were coated with antibody to IFNs gamma. Cells were cultured at 2×10^5 per well in 0.2ml of medium for re-stimulation with Aβ peptides. After 36h of incubation at 37 °C, the plates were washed, incubated with biotinylated anti-mouse IFN-gamma, and further with Streptavidin-AP conjugate. After three washes, spots were developed with one-step NBT/BCIP reagent. Spots were counted using a stereomicroscope.

2.6. Enzyme-linked immunosorbent assay for detection of Abβ_{42} peptide in brain tissues and in plasma

The frontal lobe of brain was homogenized in 10volumes of guanidine-tris buffer (5.0M guanidine HCl/50mM Tris-HCl, pH 8.0). The homogenates were mixed for 3 to 4h at RT and stored at 20°C until measured [28,29]. For ELISA assay, 96-well plates (Nunc) were coated with polyclonal antibody (50μl, 1:400 dilutions) that specifically recognize the C-terminal of $A\beta_{42}$ (Sigma) at 4°C overnight. The plate was further blocked by adding 100μl blocking buffer (PBS with 1% milk, 0.05% Tween 20) for 2h in RT. After washing with washing buffer (PBS containing 0.05% Tween 20), the brain extracts in guanidine buffer (4Ag protein perμl) or the plasma were diluted in 1:100 in blocking buffer and added to the well. The plate was incubated overnight in 4°C. After washing, 50μl of monoclonal anti-$A\beta_{1\text{-}17}$ antibody (Sigma, 1:1000 in blocking buffer) was added to the well to detect the bound $A\beta_{42}$. After 2-h incubation in RT, 50μl of peroxidase-labeled anti-mouse immunoglobulin antibody (dilution of 1:1000) was added to each well. Following 2-h incubation and washing, 50μl of substrate solution (TMB, Calbiochem, La Jolla, CA) was added and terminated after 20min RT incubation with 50μl 0.5M HCl. The different concentrations (1-2000ng/ml) of synthetic $A\beta_{42}$ peptide were used to establish the standard curve. The absorbance of the plates was read at 450nm with a spectrophotometer.

2.7. Immunofluorescence detection of Abβ_{42} plaques and quantification of amyloid burden

Portions of the brain from APPswe/PS1ΔE9 transgenic mice were dissected and put into a tube filled with PBS and the tube was snap frozen in liquid nitrogen. Part of the brain was also fixed with paraformaldehyde and embedded in paraffin. The frozen tissues were subsequently cut

into 8-Amsections with a cryotome, collected on glass slides, dried and fixed for 4h in 4% paraformaldehyde. After washing with PBS, the section was penetrated with 0.2% Triton X-100 for 10min and further washed and blocked with blocking buffer (1% BSA in PBS with 0.05% Tween 20) for 30min. After incubation with rabbit polyclonal anti-serum [30] (A1916, Sigma, St. Louis, MO) against $A\beta_{42}$(5Ag/ml, 2h at RT), the sections were washed three times for 5min each in PBS, again treated with blocking buffer (5min, RT) before reacting with the secondary antibody (Alexa488-labeled goat anti-rabbit IgG, (10Ag/ml, 1h, RT) (Invitrogen Co.; Carlsbad, CA). Finally, the preparations were washed three times in PBS and observed by confocal fluorescence microscopy. The amyloid plaque burden was analyzed with Image J (NIH) software. Five representative sections of each mouse brain were imaged and the areas and densities of the plaques were measured by the software. Sections from paraffin embedded tissue (8Am) were deparaffinized and immunolabeled as above. In addition, formic acid antigen retrieval was performed on sections of frontal cortex from one control and one treated mouse and stained as above.

2.8. Statistical analysis

Because the assumptions of the two independent samples t-test were violated (unequal group variances and non-normal distributions), quantitative measurement of $A\beta_{42}$ burden in frozen and paraffin-embedded sections for control and treatment groups were compared using the non-parametric Mann-Whitney U-test. Analyses were performed using SAS and statistical testing was conducted using $p < 0.05$.

3. Results

3.1. Cloning and immune response in Balb/c wild-type mice immunized with various constructs

The $A\beta_{42}$ or $A\beta_{16}$ genes were cloned into a genetic immunization plasmid vector under the control of an synthetic mammalian-cell specific promoter named SP72 and fused upstream with a leader signal of adenovirus E3 gene (E3L) (pSP72-E3L-$A\beta_{42}$ or $A\beta_{16}$) or alpha1 antitrypsin leader signal (ATL) (pSP72-ATL-$A\beta_{42}$). SP72 promoter was replaced with cytomegalovirus-immediate early enhancer/promoter (CMV) to create pCMV-ATL-$A\beta_{42}$, or insert COMP or CTA1-DD gene between ATL and $A\beta_{42}$ gene to create (pSP72-ATL-COM-$A\beta_{42}$, SP72-ATL-CTA1-$A\beta_{42}$). All the constructs have the same downstream endosome targeting sequence fused in frame with $A\beta_{42}$ or $A\beta_{16}$. The constructs were sequenced to confirm the insert and the correct open reading frame. We immunized initially the wild type Balb/c mice with these plasmid constructs by gene gun to compare the efficiency to incite the $A\beta_{42}$ immune response. After two gene gun immunizations to the mice ears (each immunization injecting 4Ag DNA in 4 bullets in both sides of the ears) at 2-week intervals, tail vein blood was taken 2weeks after the second immunization and ELISA was performed to measure the anti-Ah antibodies in sera. We identified that the pSP72-E3L-Ah42 construct showed the best antibody response. The fusion of the adjuvant sequence COM, or CTA1-DD did not enhance the humoral immune responses against Aβ peptide. SP72 promoter is slightly better than CMV and the E3 leader is better than the AAT leader sequence (Fig. 1A). The result prompted us to apply the SP72-E3L-$A\beta_{42}$ plasmid for gene immunization in APPswe/PS1ΔE9 transgenic mice without using any other immune adjuvant. The encoded protein of E3 leader, human $A\beta_{42}$ and endosomal targeting sequence are shown in Fig. 1B and the finally applied plasmid vector construct is shown in Fig. 1C.

3.2. Immune response in APPswe/PS1ΔE9 transgenic mice

On the basis of data obtained in wild-type Balb/c mice, we used the pSP72-E3L-$A\beta_{42}$ construct for gene immunization in APP/PS1 co-injected double transgenic mice APPswe/PS1ΔE9. These mice begin to develop amyloid plaques by the age of 6months. Four treated mice received

the human $A\beta_{42}$ gene vaccine at the age of 3months and four control mice received DNA plasmid without the Aβ peptide gene (2) or with the $A\beta_{16}$ gene (2). The humoral response was detectable by ELISA 2weeks after the third immunization and reached a peak level after six immunizations. The antibody titer against $A\beta_{1\text{-}16}$ was estimated at 1:10,000 and 1:4000 for $A\beta_{17\text{-}28}$ and $A\beta_{29\text{-}42}$ peptides in all four treated Tg mice. There are no detectable specific anti-Ah antibodies in the sera of vector only control Tg mice. Results of ELISA and Western blots (Fig. 2A and B) showed similar conclusions. Fig. 2A shows the antibody concentrations of the sera of $A\beta_{42}$ treated mice obtained 2weeks after the last immunization with about 1.2Ag/ml specific anti-$A\beta_{1\text{-}16}$ antibodies and 350ng/ml of anti-$A\beta_{17\text{-}28}$ and $A\beta_{29\text{-}42}$.

We also analyzed isotypes of anti-$A\beta_{42}$ antibodies in the sera of the $A\beta_{42}$ treated Tg mice. All treated Tg mice generated IgG1 antibodies, whereas the level of IgG2a was in an undetectable level (Fig. 2C). The production of IgG1 antibodies is an indirect measure of the relative contribution of Th2-type cytokines, whereas IgG2a antibodies reflect the contribution of Th1 cytokines to the immune response. Thus, our data indicate that gene gun mediated $A\beta_{42}$ gene vaccine gun induces a highly Th2-polarized response. Consistent with the Th2 response in antibody isotyping, these $A\beta_{42}$ treated mice also showed insignificant cellular immune responses as tested by ELISPOT stimulated with $A\beta_{1\text{-}42}$ and $A\beta_{9\text{-}18}$ synthetic peptide although a slightly higher number of interferon positive T cells was observed (Fig. 2D).

3.3. Abβ_{42} levels were reduced in brain but increased in plasma in vaccinated Tg mice as measured by ELISA

After 11 $A\beta_{42}$ gene vaccinations, the Aβ ELISA analysis was performed to measure the brain and blood $A\beta_{42}$ levels in 15-month-old Tg mice. The Aβ of forebrain tissue was extracted with 5M guanidine-tris buffer and subjected to sandwich ELISA to measure the specific $A\beta_{42}$. The median level of $A\beta_{42}$ in the cerebral cortex of the four control mice at 15months was 4500ng/g wet tissue. In contrast, $A\beta_{42}$ gene vaccinated animals had 66% less $A\beta_{42}$ at 15months (1500 ng/g) than the control vector immunized group ($p < 0.05$) (Fig. 3A). The heparinized plasma was directly subjected to $A\beta_{42}$ measurement without further extraction and showed a 33% increase of plasma $A\beta_{42}$ in the vaccinated mice (160ng/ml) than the four control mice (120ng/ml), though the differences were not statistically different (Fig. 3B).

3.4. Aβ plaque burden was attenuated as demonstrated by Abβ_{42} immunolabeling

To evaluate the Aβ burden in vaccinated Tg mouse brain, we performed fluorescence immunolabeling for $A\beta_{42}$ on sections from both snap frozen tissue and paraformaldehyde fixed and paraffin embedded tissue. All eight mice (treated N = 4, and control N = 4) were subjected to immunolabeling analysis for $A\beta_{42}$ with sigma antibody A1916. Preparations from all four control mice showed numerous large volume plaques in cortical and hippocampal regions. In contrast, while plaques remained in preparations from all four vaccinated mice, they were smaller and fewer in number. Representative photomicroimages of $A\beta_{42}$ labelled sections from control and treated mice are shown in frozen sections of frontal cortex (Fig. 4) and in paraformaldehyde fixed paraffin-embedded sections from hippocampus (Fig. 5). To quantitatively measure the brain $A\beta_{42}$ burden in these mice, 10 representative high-resolution pictures were taken with confocal microscopy from cortical and hippocampal regions of each mouse (5 in frozen sections and 5 in paraffin-embedded sections). The fluorescence plaque areas of each microimage were traced by Image J (NIH) software and the plaque areas and mean plaque intensity were registered by the software. The plaque intensity was defined as 0 in black and 255 in white and the fluorescence intensity was registered as a gray signal in between. The fluorescence plaque areas were registered in units of pixels, a small dot defined by the software and computer, in a given resolution. The density (total amount) was given by the measured plaque area in pixels multiplied by the average plaque intensity. With this method, we quantitatively evaluated the plaque burden in brain and calculated the reduction of $A\beta_{42}$ in

the treated mice compared to the untreated control mice. The measured values are given in Tables 1 and 2. Table 1 shows the quantitative result of frozen sections and Table 2 of paraffin-embedded sections. With frozen sectioning methods, the median plaque area of four control mice with five imaged areas being measured in each mouse was 177,100 pixels and 31,500 pixels in the four treated mice. The plaque area was reduced by 82.2% in the treated compared to the control mice. Similarly, the median density of four control mice (25.3 million pixels) was also significantly reduced in the treated mice (5.7 million pixels) with a Mann-Whitney P = 0.0286. There was a 77.5% reduction in plaque burden in treated mice as calculated by plaque density (total amount) (Table 1). We used the same method to measure the $A\beta_{42}$ burden reduction in the vaccinated mice brains in paraffin-embedded sections. The median plaque volume in four control mice was 201,600 pixels and 69,100 pixels in four vaccinated mice with a 65.7% reduction in plaque area in the treated mice. The median plaque density (total amount) of the control mice was 35.3 million pixels and 14.2 million pixels in the treated mice. The reduction of the plaque burden in the treated mice was 60% as calculated by the plaque density (total amount) with a Mann-Whitney P = 0.0286 (Table 2). In conclusion, with Image J software quantitation of $A\beta_{42}$ burden in a total of 80 representative confocal imaged areas (40 in control and 40 in treated mice), the reduction of $A\beta_{42}$ burden was 60-77.5% in the gene vaccinated group of mice compared to the control group of mice. Both tissue processing methods give the same conclusion and there is no overlap of the data between the control and treated groups both for frozen and paraffin-embedded brain for $A\beta_{42}$ levels. Sections from one control and one treated mouse which included formic antigen retrieval methodology were studied with Image J software. Formic acid antigen retrieval is commonly employed in the diagnostic evaluation of human AD brain tissue for Aβ levels. The median plaque density (total amount) of the vector only control mouse (C1) frontal cortex was 36.2 million pixels and the median plaque density (total amount) of the treated mouse (T2) frontal cortex was 21.6 million pixels, giving a 41% reduction in $A\beta_{42}$ immunolabelling. This additional evaluation is included to assure quantitation of all potential amyloid antigenicity and demonstration of significant reduction in $A\beta_{42}$ immunolabelling in the treated mouse brain. In addition, the immune response induced by gene vaccination to $A\beta_{42}$ does not produce any obvious signs of damage to the neurons in $A\beta_{42}$ gene-immunized animals. Histological examination of brain revealed no signs of immune-mediated complications (data not shown). The weight gain is similar in both groups with an average 40g body weight at 15months of age.

4. Discussion

APPswe/PS1ΔE9 double transgenic mice develop $A\beta_{42}$ plaques beginning at the age of 6months, much earlier than APPswe/PS1(A246E) (9months) and PDAPP (APPswe) Tg mice [30-32] (18months). We demonstrate, even in this highly Aβ expressing strain, significant attenuation of the $A\beta_{42}$ plaque formation in cortical and hippocampal brain regions in all four mice with $A\beta_{42}$ gene vaccination. To our knowledge, our findings represent the first report of gene vaccination that significantly reduced the $A\beta_{42}$ plaque burden in a co-injected double transgenic animal AD model, although we and others have reported the $A\beta_{42}$ gene vaccine can elicit an immune response to $A\beta_{42}$[22,33,34].

Gene vaccination using a plasmid vector has many advantages over peptide or protein vaccination including simple, low-cost preparation and purification [35-37]. Furthermore with gene vaccination, the immune response of the host can be easily manipulated to bias towards a Th1 or Th2 type reaction. It has been found that muscle injection of gene vaccines generally induces a Th1 predominant reaction. In contrast, gene gun-based gene vaccines can induce predominantly Th2 responses with certain antigens [38,39]. In our study, gene gun-administered $A\beta_{42}$ gene vaccination induces a Th2 response without a detectable Th1 type reaction and the cellular response to $A\beta_{42}$ is insignificant. This point may be a key advantage for gene vaccination, as it could avoid cell-mediated encephalitis caused by $A\beta_{42}$ peptide

immunization. Recent reports on clinical trials with gene vaccines are encouraging that this method can be effective in humans [40].

In the present study, we show that the construct SP72-E3-$A\beta_{42}$ elicits an excellent immune response. The SP72 promoter was rationally designed and it is a part of our effort to create a better immunization vector to improve the gene vaccination method. The E3 leader may help to target $A\beta_{42}$ peptide to the endoplasmic reticulum to bind MHC molecules for presentation. The $A\beta_{16}$ containing vector served as a positive control as it did not reduce $A\beta_{42}$ plaque density compared to vector only vaccinated mice. Antibody against the entire $A\beta_{42}$ peptide may be required for subsequent $A\beta_{42}$ targeting and reduction from brain.

In summary, we have developed a gene gun-mediated $A\beta_{42}$ gene vaccination method that is efficient to break host $A\beta_{42}$ tolerance without using adjuvant and induces a Th2 immune response that minimizes producing a T cell mediated meningoencephalitis. In the present studies, we demonstrate further that $A\beta_{42}$ gene vaccination can significantly reduce the $A\beta_{42}$ burden of the brain in all treated APPswe/PS1ΔE9 transgenic mice with no overlap between treated and control mice. Collectively, the results of this study support the position that $A\beta_{42}$ gene immunization should now be studied in a non-human primate to see if is safe and effective and then consider it in a clinical trial of patients with Alzheimer's disease [41].

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References

1. Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. Physiol Rev 2001;81:741–66. [PubMed: 11274343]
2. Rosenberg RN. The molecular and genetic basis of alzheimer's disease: the end of the beginning. Neurology 2000;54:2045–54. [PubMed: 10851361]
3. Haass C, Schlossmacher MG, Hung AY, Vigo-Pelfrey C, Mellon A, Ostaszewski BL, et al. Amyloid beta-peptide is produced by cultured cells during normal metabolism. Nature 1992;359:322–5. [PubMed: 1383826]
4. Shoji M, Golde TE, Ghiso J, Cheung TT, Estus S, Shaffer LM, et al. Production of the Alzheimer amyloid beta protein by normal proteolytic processing. Science 1992;258:126–9. [PubMed: 1439760]
5. Lahiri DK, Ge RW. Role of the APP promoter in Alzheimer's disease: cell type-specific expression of the h-amyloid precursor protein. Ann N Y Acad Sci 2004;1030:310–6. [PubMed: 15659812]
6. Fox NW, Johnstone EM, Ward KE, Schrementi J, Little SP. APP gene promoter constructs are preferentially expressed in the CNS and testis of transgenic mice. Biochem Biophys Res Commun 1997;240:759–62. [PubMed: 9398640]
7. Iwatsubo T, Odaka A, Suzuki N, Mizusawa H, Nukina N, Ihara Y. Visualization of A beta 42(43) and A beta 40 in senile plaques with end-specific A beta monoclonals: evidence that an initially deposited species is A beta 42(43). Neuron 1994;13:45–53. [PubMed: 8043280]
8. Iwatsubo T, Mann DM, Odaka A, Suzuki N, Ihara Y. Amyloid beta protein (A beta) deposition: a beta 42(43) precedes A beta 40 in Down syndrome. Ann Neurol 1995;37:294–9. [PubMed: 7695229]
9. Yankner BA. Mechanisms of neuronal degeneration in Alzheimer's disease. Neuron 1996;16:921–32. [PubMed: 8630250]
10. Selkoe DJ. Translating cell biology into therapeutic advances in Alzheimer's disease. Nature 1999;399:A23–31. [PubMed: 10392577]

11. Monsonego A, Weiner HL. Immunotherapeutic approaches to Alzheimer's disease. Science 2003;302:834–8. [PubMed: 14593170]
12. Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, et al. Immunization with amyloid —attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature 1999;400:173–7. [PubMed: 10408445]
13. Morgan D, Diamond DM, Gottschall PE, Ugen KE, Dickey C, Hardy J, et al. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. Nature 2000;408:982–5. [PubMed: 11140686]
14. DeMattos RB, Bales KR, Cummins DJ, Dodart JC, Paul SM, Holtzman DM. Free in PMC peripheral anti-A beta antibody alters CNS and plasma A beta clearance and decreases brain A beta burden in a mouse model of Alzheimer's disease. Proc Natl Acad Sci U S A 2001;98:8850–5. [PubMed: 11438712]
15. Schenk D. Amyloid-beta immunotherapy for Alzheimer's disease: the end of the beginning. Nat Rev Neurosci 2002;3:824–8. [PubMed: 12360327]
16. Rosenberg RN. Immunization with β-amyloid$_{(1-42)}$ protein for Alzheimer disease: genomics predicts the response. JAMA 2005;294:2352–3.
17. Kotilinek LA, Bacskai B, Westerman M, Kawarabayshi T, Younkin L, Hyman BT, et al. Reversible memory loss in a mouse transgenic model of Alzheimer's disease. J Neurosci 2002;22:6331–5. [PubMed: 12151510]
18. Hock C, Konietzko U, Streffer JR, Tracy J, Signorell A, Muller-Tillmanns B, et al. Antibodies against beta-amyloid slow cognitive decline in Alzheimer's disease. Neuron 2003;38:547–54. [PubMed: 12765607]
19. Sorbi S. Beta-amyloid immunisation in Alzheimer's disease. Neurol Sci 2005;26(Suppl 1):S5. [PubMed: 15883696]
20. Fox NC, Black RS, Gilman S, Rossor MN, Griffith SG, Jenkins L, et al. AN1792(QS-21)-201 Study Team. Effects of Abeta immunization (AN1792) on MRI measures of cerebral volume in Alzheimer disease. Neurology 2005;64:1563–72. [PubMed: 15883317]
21. Gilman S, Koller M, Black RS, Jenkins L, Griffith SG, Fox NC, et al. AN1792(QS-21)-201 Study Team. Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial. Neurology 2005;64:1553–62. [PubMed: 15883316]
22. Qu B, Rosenberg RN, Li L, Boyer PJ, Johnston SA. Gene vaccination to bias the immune response to amyloid-beta peptide as therapy for Alzheimer disease. Arch Neurol 2004;61:1859–64. [PubMed: 15596606]
23. Ciernik IF, Berzofsky JA, Carbone DP. Induction of cytotoxic T lymphocytes and antitumor immunity with DNA vaccines expressing single T cell epitopes. J Immunol 1996;156:2369–75. [PubMed: 8786293]
24. Marques ET Jr, Chikhlikar P, de Arruda LB, Leao IC, Lu Y, Wong J, et al. HIV-1 p55Gag encoded in the lysosome-associated membrane protein-1 as a DNA plasmid vaccine chimera is highly expressed, traffics to the major histocompatibility class II compartment, and elicits enhanced immune responses. J Biol Chem 2003;278:37926–36. [PubMed: 12824194]
25. Eriksson A, Lycke N. The CTA1-DD vaccine adjuvant binds to human B cells and potentiates their T cell stimulating ability. Vaccine 2003;22:185–93. [PubMed: 14615145]
26. Chambers RS, Johnston SA. High-level generation of polyclonal antibodies by genetic immunization. Nat Biotechnol 2003;9:1088–92. [PubMed: 12910245]
27. Kumar A, Weiss W, Tine JA, Hoffman SL, Rogers WO. ELISPOT assay for detection of peptide specific interferon-gamma secreting cells in rhesus macaques. J Immunol Methods 2001;247:49–60. [PubMed: 11150536]
28. Vanderstichele H, Van Kerschaver E, Hesse C, Davidsson P, Buyse MA, Andreasen N, et al. Amyloid. Standardization of measurement of beta-amyloid(1 - 42) in cerebrospinal fluid and plasma. Amyloid 2000;7:245–58. [PubMed: 11132093]
29. Johnson-Wood K, Lee M, Motter R, Hu K, Gordon G, Barbour R, et al. Amyloid precursor protein processing and A beta42 deposition in a transgenic mouse model of Alzheimer disease. Proc Natl Acad Sci U S A 1997;94:1550–5. [PubMed: 9037091]

30. Borchelt DR, Ratovitski T, van Lare J, Lee MK, Gonzales V, Jenkins NA, et al. Accelerated amyloid deposition in the brains of transgenic mice coexpressing mutant presenilin 1 and amyloid precursor proteins. Neuron 1997;19:939–45. [PubMed: 9354339]

31. Lamb BT, Siscodia SS, Lawler AM, Slunt HH, Kitt CA, Kearns WG, et al. Introduction and expression of the 400 kilobase precursor amyloid protein gene in transgenic mice. Nat Genet 1993;5:22–30. [PubMed: 8220418]

32. Wong PC, Cai H, Borchelt DR, Price DL. Genetically engineered mouse models of neurodegenerative diseases. Nat Neurosci 2002;5:633–9. [PubMed: 12085093]

33. Schiltz JG, Salzer U, Mohajeri MH, Franke D, Heinrich J, Pavlovic J, et al. Antibodies from a DNA peptide vaccination decrease the brain amyloid burden in a mouse model of Alzheimer's disease. J Mol Med 2004;82:706–14. [PubMed: 15241501]

34. Ghochikyan A, Vasilevko V, Petrushina I, Movsesyan N, Babikyan D, Tian W, et al. Generation and characterization of the humoral immune response to DNA immunization with a chimeric beta-amyloid-interleukin-4 minigene. Eur J Immunol 2003;33:3232–41. [PubMed: 14635031]

35. Tang DC, DeVit M, Johnston SA. Genetic immunization is a simple method for eliciting an immune response. Nature 1992;356:152–4. [PubMed: 1545867]

36. Barry MA, Lai WC, Johnston SA. Protection against mycoplasma infection using expression-library immunization. Nature 1995;377:632–5. [PubMed: 7566175]

37. Ulmer JB, Donnelly JJ, Parker SE, Rhodes GH, Felgner PL, Dwarki VJ, et al. Heterologous protection against influenza by injection of DNA encoding a viral protein. Science 1993;259:1745–9. [PubMed: 8456302]

38. Feltquate DM, Heaney S, Webster RG, Robinson HL. Different T helper cell types and antibody isotypes generated by saline and gene gun DNA immunization. J Immunol 1997;158:2278–84. [PubMed: 9036975]

39. Weiss R, Scheiblhofer S, Freund J, Ferreira F, Lirey I, Thalhamer J, et al. Gene gun bombardment with gold particles displays a particular Th2-promoting signal that over-rules the Th1-inducing effect of immunostimulatory CpG motifs in DNA. Vaccine 2002;20:3148–54. [PubMed: 12163266]

40. Drape, RJ.; Macklin, MD.; Barr, LJ.; Jones, S.; Haynes, JR.; Dearn, HJ. Epidermal DNA vaccine for influenza is immunogenic in humans. Vaccine. www.elsevier.com/locate/vaccinewww.sciencedirect.com.in presson line Science Direct at

41. Rosenberg RN. Translational research on the way to effective therapy for Alzheimer Disease. Arch Gen Psychiatry 2005;62:1186–92. [PubMed: 16275806]

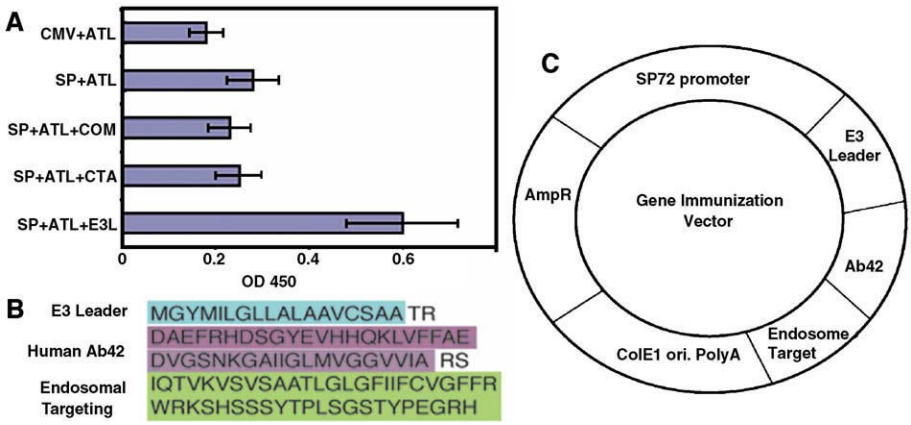

Fig. 1.

(A) Aβ peptide specific immune responses in BALB/c wild-type mice immunized with human $A\beta_{42}$ gene vaccine cloned in various vectors (N = 3). Serum was obtained 14days after a second vaccination at 2-week intervals with dilution of 1:200. The serum was tested by ELISA for $A\beta_{42}$ peptide and shows that mice vaccinated with pSP-E3L-$A\beta_{42}$ had the highest anti-$A\beta_{42}$ antibodies than the other constructs. (B) Peptide sequence encoded by the open reading frame of the gene immunization vector and (C) schematic representation of the construct. DNA sequence encoding adenovirus E3 leader, $A\beta_{42}$ or $A\beta_{16}$ peptides, and endosome targeting peptide were cloned in frame in EcoRI and XbaI restriction sites under the control of a synthetic SP72 mammalian cell-specific promoter.

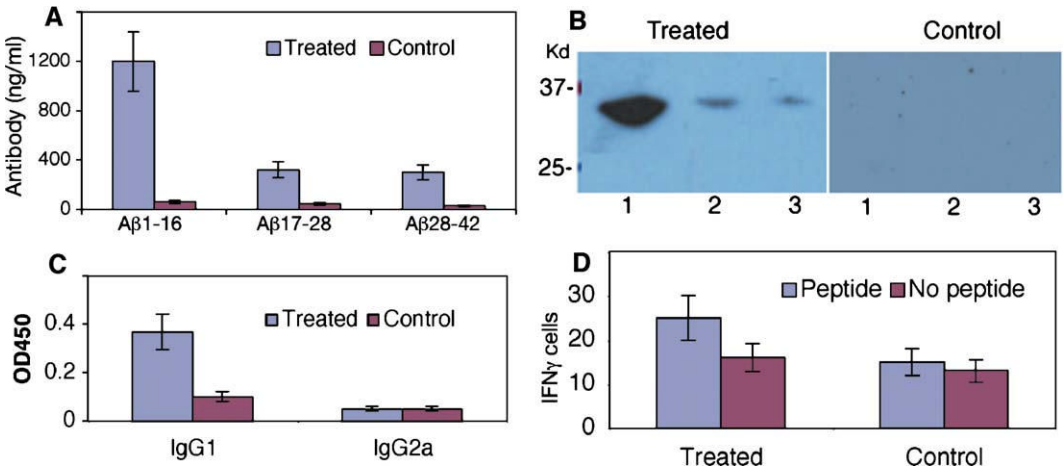

Fig. 2.

Human $A\beta_{42}$ specific immune responses in APPswe/PS1ΔE9 double transgenic mice immunized with human $A\beta_{42}$ gene vaccine. (A) Anti-Ah peptide antibody titer assayed by ELISA in Tg mice immunized with $A\beta_{42}$ for 6 times in 4months. The serum was obtained 2weeks after the 6th immunization and titers were tested against GST fused Aβ peptide$_{1\text{-}16,\ 17\text{ - }28,\ 29\text{ - }42}$. Higher titers against $A\beta_{1\text{-}16}$ was seen than the other epitopes (the bar value represent mean ± S.E.M. of four mice). (B) The same serum tested with Western blot shows a similar result, with a higher response against the $A\beta_{1\text{-}16}$ epitope. The sera from control mice are negative. Lane 1: $A\beta_{1\text{-}16}$, lane 2: $A\beta_{17\text{-}28}$, lane 3: $A\beta_{29\text{-}42}$ peptide fused to GST was loaded and probed with the serum in 1:2000 dilution. (C) Isotyping of anti-$A\beta_{42}$ antibodies after immunization of mice with p SP72-E3L-$A\beta_{42}$. The sera were diluted 1:200 and used for detection of IgG1, IgG2a subclasses of anti-$A\beta_{42}$ antibodies. All mice demonstrated an IgG1 (Th2) response without detectable IgG2a (Th1) response (mean ± S.E.M., N = 4). (D) ELISPOT assay shows that no significant cellular immune response was observed in human $A\beta_{42}$ gene vaccinated Tg mice. Peripheral blood T cells were pooled from the vaccinated and vector only control mice and the cells were cultured in quadruplicate (2×10^5 cells per well in 96-well plate format) in the presence of peptide or absence of peptide using a mixture of Aβ and $A\beta_{42}$ peptide $A\beta_{9\text{-}18}$ at 10μg/ml for 36h and further processed for detection of released interferon. Control mice (A - D) received vector only construct without any Aβ gene insert and induced no detectable specific anti-Ah antibodies in sera and no significant cellular immune response.

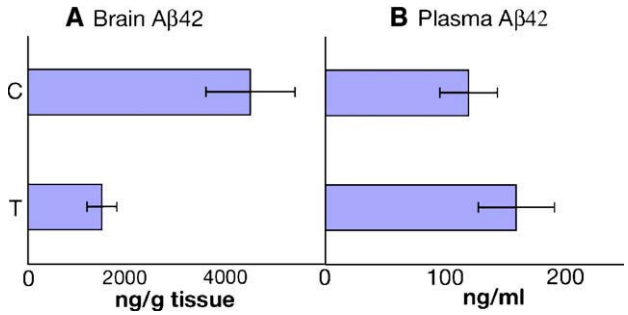

Fig. 3.

Levels of $A\beta_{42}$ in forebrain (A) and in plasma (B) of 15-month-old APPswe/PS1ΔE9 transgenic mice treated with the $A\beta_{42}$ gene vaccine (T) (4) and control (C) (2 with $A\beta_{16}$ construct and 2 vector only). Bars represent mean ± S.E.M. of four mice in both groups. (A) The forebrain was extracted with 5M guanidine-tris buffer. $A\beta_{42}$ was quantified by sandwich ELISA. There is about 70% reduction of total $A\beta_{42}$ in the forebrain of vaccinated mice compared to the control. (B) Plasma samples were diluted in 1:100 in blocking buffer and $A\beta_{42}$ levels were measured by sandwich ELISA.

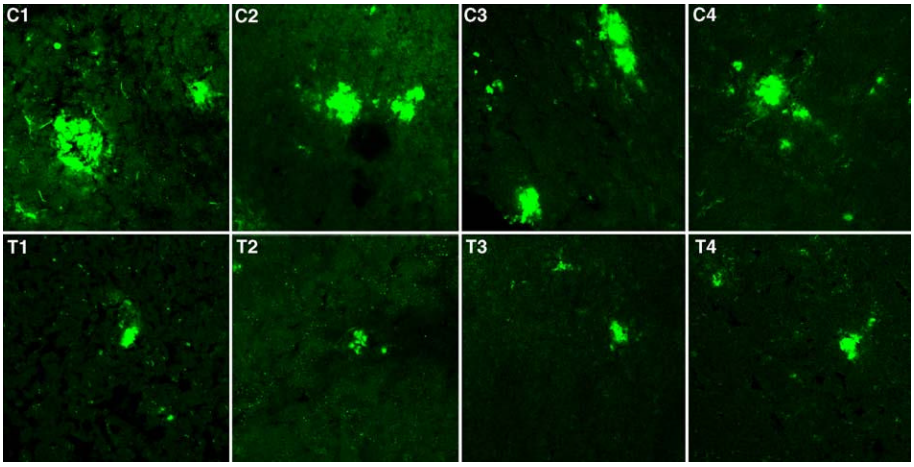

Fig. 4.

Reduction of Aβ deposition in frontal cortex of 15-month-old APPswe/PS1ΔE9 mice immunized with $A\beta_{42}$ gene vaccination 11 times. Cryostat sections of the frozen frontal cortex were labeled with anti-$A\beta_{42}$ antibody followed by a fluorescently labeled second antibody. Shown are representative images taken by confocal microscopy at × 40 in each mouse brain. The upper panel images were taken from four control mice (C1 and C2, vector only and C3 and C4, vector with $A\beta_{16}$) with large multiple plaques being seen scattered in all cortical areas. The lower panels of images were from four treated (vector with $A\beta_{42}$) (T1, T2, T3, T4) mice with much smaller and fewer plaques. There is no significant difference in plaque volume in vector only control mice or control mice with $A\beta_{16}$.

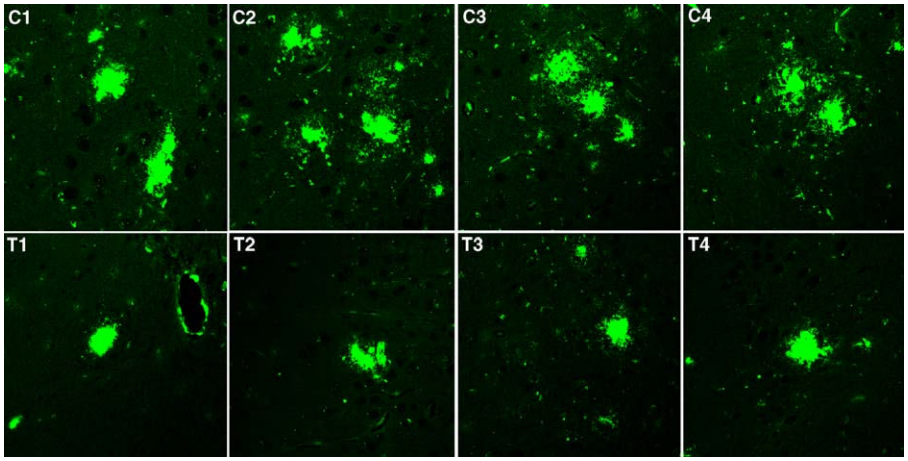

Fig. 5.

Reduction of Aβ deposition in hippocampus of 15-month-old APPswe/PS1ΔE9 mice immunized with $A\beta_{42}$ gene vaccination for 11 times. Paraffin-embedded sections of paraformaldehyde-fixed mice brain were labeled with anti-$A\beta_{42}$ antibody followed by a fluorescently labeled second antibody. Shown are representative images taken by confocal microscopy at × 40 in each mouse brain. The upper panel images were taken from four control mice (C1 and C2, vector only; C3 and C4, vector with $A\beta_{16}$) with large multiple plaques being seen scattered in brain areas. The lower panels of images were from four treated (vector with $A\beta_{42}$) (T1, T2, T3, T4) mice with smaller and fewer plaques. There is no significant difference in plaque volume in vector only control mice or control mice with $A\beta_{16}$.

Table 1

Quantitative measurement of Aβ42 burden in frozen sections of APPswe/PS1ΔE9 mouse frontal cortex after immunolabeling for Aβ42

Mice	Sum of plaque area (× 10^3)	Mean of plaque intensity	Plaque density (× 10^6)(total amount)
C1	221.7	169.7	37.6
C2	200.5	141.9	28.5
C3	153.7	144.5	22.2
C4	148.9	145.9	21.7
Median C1-4	177.1*	145.2	25.3*
T1	66.1	166.3	11.0
T2	26.0	86.1	2.2
T3	20.0	181.4	3.6
T4	37.0	207.7	7.7
Median T1-4	31.5* (17.8%)	173.8	5.7* (22.5%)

C1-4 are four control mice (C1 and 2, vector only and C3 and 4, vector with Aβ16) and T1-4 are Aβ42 vaccinated mice. The area represents the sum of 5 imaged areas measured by Image J in units of pixels. There is no significant difference in plaque volume in vector only control mice or control mice with vector containing Aβ16. The median plaque density (totalamount) is the result of the median plaque area multiplied by the median plaque intensity. The number in parenthesis represents the percentage of Aβ42 burden in treated compared to control mice.

Table 2

Quantitative measurement of Aβ42 burden in paraffin-embedded sections APPswe/PS1ΔE9 mouse hippocampus after Aβ42 immunolabeling

Mice	Sum of plaque area ($\times 10^3$)	Mean of plaque intensity	Plaque density ($\times 10^6$)(total amount)
C1	288.5	210.1	60.6
C2	179.7	185.2	33.3
C3	219.3	168.8	37.0
C4	183.8	182.3	33.5
Median C1-4	201.6*	183.7	35.3*
T1	57.0	223.8	12.8
T2	45.1	212.1	9.6
T3	81.1	193.3	15.7
T4	88.3	205.3	18.1
Median T1-4	69.1(34.3%)*	208.7	14.2 (40.2%)*

Same as in Table 1 but in paraffin-embedded sections.

* Mann-Whitney P =0.0286.