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Nasal inoculation of an adenovirus vector encoding 11 tandem repeats of A β 1-6 upregulates IL-10 expression and reduces amyloid load in a Mo/Hu APPswe PS1dE9 mouse model of Alzheimer disease

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Abstract

Background—One of the pathological hallmarks of Alzheimer disease (AD) is deposits of amyloid β -peptide (A β) in neuritic plaques and cerebral vessels. Immunization of AD mouse models with A β reduces A β deposits and improves memory and learning deficits. Because recent clinical trials of immunization with A β were halted due to brain inflammation that was presumably induced by a T cell-mediated autoimmune response, vaccination modalities that elicit predominantly humoral immune responses are currently being developed.

Methods—We have nasally immunized young AD mouse model with an adenovirus vector encoding 11 tandem repeats of A β 1-6 fused to the receptor-binding domain (Ia) of Pseudomonas exotoxin A (PEDI), AdPEDI-(A β 1-6)₁₁, in order to evaluate the efficacy of the vector in preventing A β deposits in the brain. We also have investigated immune responses of mice to AdPEDI-(A β 1-6)₁₁.

Results—Nasal immunization of an AD mouse model with AdPEDI-(A β 1-6)₁₁ elicited a predominant IgG1 response and reduced A β load in the brain. The plasma IL-10 level in the AD mouse model was upregulated after immunization and, upon the stimulation with PEDI-(A β 1-6)₁₁, marked IL-10 responses were found in splenic CD4⁺ T cells from C57BL/6 mice that had been immunized with AdPEDI-(A β 1-6)₁₁.

Conclusions—These results suggest that the induction of Th2-biased responses with AdPEDI-(A β 1-6)₁₁ in mice is mediated in part through the upregulation of IL-10, which inhibits activation of dendritic cells that dictate the induction of Th1 cells.

Keywords

DNA vaccine; amyloid; inflammation

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1. Introduction

Alzheimer disease (AD) is a neurodegenerative disorder characterized by the progressive loss of memory and cognitive functions. Cardinal pathological changes found in the brains of patients with AD are neurofibrillary tangles and deposits of aggregated A β in neuritic plaques and cerebral blood vessels. A β is derived from a larger transmembrane glycoprotein, amyloid β -protein precursor (APP). APP is processed through several proteolytic pathways. α -secretase cleaves the APP molecule at the cell surface between residues 16 and 17 of A β , releasing a soluble form of APP. This pathway is thought to be non-amyloidogenic since intact A β is not produced. Generation of A β requires cleavage of APP by β - and γ -secretase at the N-terminal and C-terminal ends of A β , respectively. A β found in the AD brain is heterogeneous at its carboxyl-terminus, resulting in peptides of 39–43 amino acids. Although the precise biological roles of APP are elusive, studies of over- and under-expression of APP suggest a number of important physiological functions [1,2]. Such functions include neurite outgrowth, synaptogenesis, neurogenesis [3], cell adhesion and migration, axonal transport [4], brain copper homeostasis [5], and brain and somatic development through Notch and Fe65 signaling pathway [6,7]. Because APP seems to have important roles in neuronal plasticity and synaptic functions, it has been hypothesized that alterations in physiological functions of APP may be the primary events leading to AD [8–10]. Furthermore, the crucial roles of A β and APP in the pathogenesis of AD are underscored by discoveries of missense mutations and duplications in the gene encoding APP in a small subset of familial AD [11]. Additionally, overexpression of the mutant forms of APP in transgenic mice produced AD-like pathologies including amyloid plaques in the brain and cognitive deficits [12,13]. These transgenic models of AD have been used to develop new therapeutic means as well as to investigate the pathogenesis.

Immunization of mouse models of AD with synthetic A β prevented or reduced A β deposits and improved their memory and learning deficits [14–16]. These impressive results prompted initiation of phase I/II clinical trials to evaluate the safety and tolerability of aggregated A β (AN1792) vaccination. The phase IIa clinical trial, however, was halted due to meningoencephalitis found in 6 % of AD patients subjected to the vaccine trial [17]. Clinical studies and autopsy results indicated aseptic T-lymphocyte meningo-encephalitis [17–19]. The reports of neuropathological analysis on the brains obtained at autopsy of AD patients who had a positive A β titer are striking: these brains had focal depletion of A β plaques and astrogliosis, but maintained neurofibrillary tangles [18,19]. These findings closely resemble the changes seen after immunotherapy in AD mouse models, indicating that immunotherapy is effective in removing A β plaques in human. Thus, A β immunotherapy is a promising therapeutic means and it is vitally important that a safe and effective vaccine be developed for treating AD patients. Peripheral administration of antibodies against A β also induced clearance of preexisting amyloid plaques in an AD mouse model [20], indicating that an active T cell-mediated immune response is unnecessary. Therefore, modalities which induce humoral (B cell) immune responses have become favorable or desirable for AD vaccines [21–25].

Genetic immunization is the approach for eliciting immune responses against specific proteins by expressing genes encoding the proteins in an animal's own cells. Endogenous expression of the antigens may play important roles, not only in the relatively greater efficacy of genetic vaccines, but also in induction of a different type of immune response compared with conventional vaccines [26,27]. Furthermore, it is possible to target a specific cellular compartment or specific cell type by manipulating genes encoding antigens. Thus, genetic immunization can be a safer modality for AD treatment. Indeed several groups reported induction of anti-inflammatory Th2 immune responses by DNA vaccines that encode A β [28,29]. Because A β 1-15 has been identified as a B cell epitope [30–32], and A β 6-28 and A β 15-42 contain a T cell epitope [21,30], we chose A β 1-6 as the antigen with the aim of developing a safe, effective vaccine and constructed an adenovirus vector encoding 11 tandem

repeats of A β 1-6, AdPEDI-(A β 1-6)₁₁ [33]. In this study, we have nasally immunized an AD mouse model with AdPEDI-(A β 1-6)₁₁ to evaluate the efficacy of the vector in preventing A β deposits in the brain and in improving behavioral deficits. We also have initiated the investigation of the mechanism by which nasal administration of AdPEDI-(A β 1-6)₁₁ in mice induces Th2 polarized immune responses.

2. Materials and Methods

2.1. E1/E3-defective adenovirus vectors

Construction and preparation of an adenovirus vector, AdPEDI-(A β 1-6)₁₁, were described previously [33]. AdPEDI-(A β 1-6)₁₁ carries cDNA for a fusion protein of the receptor-binding domain (Ia) of *Pseudomonas* exotoxin A (PEDI) and eleven-tandem repeats of A β 1-6 as immunogen. An adenovirus vector encoding murine granulocyte-macrophage colony stimulating factor (AdGM-CSF) [34] was used as control. The adenoviruses (serotype 5) were prepared as previously described [33].

2.2. Experimental animals and immunization protocol

A transgenic line of AD mouse model, Mo/Hu APPswe PS1dE9 [35] was obtained from Drs. David Borchelt and Joanna Jankowsky and maintained by crossing the transgenic males with B6C3F1 females. Mo/Hu APPswe PS1dE9 mice carry mouse APP with the double mutations (K670N and M671L) and human PS1 with a deletion of exon 9 found in familial AD patients. Mo/Hu APPswe PS1dE9 mice, however, develop amyloid deposits composed of human A β in the brain because the A β sequence in the mouse APP transgene has been humanized. Mo/Hu APPswe PS1dE9 mice develop amyloid deposits as early as 4 months of age and have numerous amyloid plaques by 8–9 months of age. Six Mo/Hu APPswe PS1dE9 mice at two months of age were used for vaccination with AdPEDI-(A β 1-6)₁₁. Another six Mo/Hu APPswe PS1dE9 mice were treated with AdGM-CSF ($n = 6$) as controls. C57BL/6 mice ($n = 4$) were treated with nasal administration of AdPEDI-(A β 1-6)₁₁ and the same number of untreated C57BL/6 mice were used as controls. Nasal inoculation was carried out by pipetting 20 μ l of adenovirus (1×10^8 PFU, Plaque Forming Unit) into one of the nostrils of an anesthetized mouse with an adenovirus vector followed by a booster every 3 weeks for 10 months for Mo/Hu APPswe PS1dE9 mice or every week for 4 weeks for C57BL/6 mice. Blood was collected through the tail 0 (preimmune), 6, 12 weeks for Mo/Hu APPswe PS1dE9 mice and 0 and 4 weeks for C57BL/6 mice after the initial immunization. All animal protocols used for this study were prospectively reviewed and approved by the Institutional Animal Care and Use Committees of the University of Alabama at Birmingham and the University of Illinois College of Medicine at Peoria.

2.3. ELISA for determination of serum titers

After incubating blood at 4°C for 3 h, the blood was centrifuged for 10 min at 10,000 g. The serum was collected and frozen at -80°C. Anti-A β immunoglobulin (IgM, IgG, IgG1, IgG2a and IgG2b) titers were determined by enzyme-linked immunosorbent assay (ELISA) as previously described [33]. Anti-A β antibody titers in the mouse sera were determined using serial dilutions of 6E10 (monoclonal anti-A β antibody) as the standard. Therefore, the concentrations (μ g/ml) of the serum titers presented here reflect the concentrations of 6E10 antibody, which produce the same ELISA readings, and may not accurately represent the absolute amounts. In the present study, the linear regression was found between 0.1 and 24 ng/ml of IgG. The minimum detectable anti-A β titer was 0.1 ng/ml which was determined by adding two standard deviations to the mean absorbance obtained when the zero standard was assayed 32 times. Comparison of treatment groups was performed by ANOVA with the post-hoc tests using the SigmaStat software (SPSS Science, Chicago, IL). $P < 0.05$ was considered statistically significant.

2.4. Cytokine-specific ELISA

Interferon- γ (IFN- γ), interleukin (IL)-2, IL-4, and IL-10 in mouse sera were determined by the BD OptEIA ELISA system (BD Biosciences, Pharmingen San Diego, CA, USA), according to the manufacturer's protocol. In brief, 96-well plates were pre-coated with an appropriate capture antibody (1:250) for overnight at 4°C. After rinsing three times with washing buffer (PBS with 0.05% Tween-20), the plates were incubated with 300 μ L of block solution (PBS with 10% FBS) at room temperature for 1 hour. The plates were incubated with diluted samples for overnight at 4°C. The plates were rinsed five times with washing buffer and incubated with an appropriate detection antibody for 1 h at room temperature. After washing, the plates were incubated with 3,3',5,5'-tetramethylbenzidine (TMB) for 30 min. The reaction was stopped by the addition of 0.18N H₂SO₄. Optic densities at 450 nm were determined using a Microplate Reader (Model 680XR, BioRad) within the linear regression. Each sample was measured in triplicates. The minimum detectable concentrations were 3.1 pg/ml for IL-2, 31.3 pg/ml for IFN- γ , 7.8 pg/ml for IL-4, and 31.3 pg/ml for IL-10.

2.5. Immunoreactivity of antisera to monomeric and oligomeric A β

Oligomeric A β was prepared as described by [36]. Synthetic A β 1-42 was dissolved in 1 mM hexafluoroisopropanol (Sigma) and then removed under vacuum in a Speed Vac (Savant, Holbrook, NY). The residual peptide was resuspended in dry dimethyl sulfoxide (Sigma) to a concentration of 5 mM. By adding phenol red free Ham's F-12 medium (Mediatech, Herndon, VA) to the resuspended peptide, the concentration was made to 100 μ M and the peptide was kept at 4°C for 24 hours. The samples were diluted in NuPage sample buffer (Invitrogen, Carlsbad, CA) and separated by 16.5% Tris-tricine SDS-PAGE. Western blotting was performed using induced anti-sera and an enhanced chemiluminescence system (Amersham, Arlington Heights, IL) as described previously [37].

2.6. Immunoreactivity of antisera to amyloid plaques in the brain

A 7-month-old-transgenic mouse (Mo/Hu APP^{swe} PS1^{dE9}) was terminated by intraperitoneal injection of sodium pentobarbital. The brain was fixed in 10% formaldehyde: 90% alcohol. Four mm sections were prepared for immunohistochemistry. To test immunoreactivity of sera from vaccinated mice with adenovirus vectors against amyloid plaques, the brain sections were stained with different dilutions of the sera by the avidin-biotin immunoperoxidase method using Vectastain ABC kit (Vector, Burlingame, CA). Endogenous peroxidase was eliminated by treatment with 3% H₂O₂ for 30 min. After washing with distilled water, the sections were blocked with 10% horse serum in 0.1 M Tris-saline (TBS) (pH 7.4) for 60 min at room temperature and incubated with diluted sera in 0.1 M TBS containing 10% horse serum for 16 hours at 4°C. The sections were rinsed in 0.1 M TBS containing 1% serum and incubated with biotinylated secondary antibody for 60 min at room temperature. After washing, the sections were incubated with Vectastain ABC reagent for 60 min at room temperature. Peroxidase activity was detected by treatment with 3,3'-diaminobenzidine. The sections were counterstained with hematoxylin.

2.7. Quantification of cerebral A β

Mice were terminated by lethal injection of sodium pentobarbital and the brain was quickly removed. After removing the cerebellum, the left hemisphere was processed for morphometric analysis as described previously [38]. In brief, three micron-tissue sections were immunostained with 6E10 antibody using Vectastain ABC reagent for detection of A β deposits. Ten micron brain sections were subjected to thioflavine-S staining for detection of A β fibrils. The amyloid burden was quantified by histomorphometry consisting of an Olympus BX61 automated microscope capable of both brightfield and fluorescence, an Olympus Fluoview system, and the Image Pro Plus v4 image analysis software (Media Cybernetics, Silver Spring,

MD) capable of color segmentation and automation via programmable macros. The entire hippocampus and neocortex in each slide were scanned. Approximately forty fields (1 mm² each, using a 10X objective and a 1X eyepiece lens) from 4 coronal brain sections, each separated by approximately consecutive 300 µm interval, from each mouse were analyzed with each staining method. Amyloid burden was expressed as a percentage of total area covered by Aβ immunoreactivity or thioflavine-S fluorescence. Data were expressed as mean ± standard error. Inter group differences were assessed by two-tailed Student's t-test. $P < 0.05$ was considered statistically significant.

The right hemisphere was homogenized in carbonate buffer (100 mM Na₂CO₃, 50 mM NaCl, pH 11.5) containing protease inhibitors [10 µg/ml aprotinin and 1mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF)]. Protein concentrations were determined by the Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA). An aliquot of homogenate was further homogenized with guanidine hydrochloride (final concentration, 5M) and then diluted with PBS at various concentrations for determination of Aβ concentration, which was done by using the Aβ1-42 and Aβ1-40 ELISA kits (Biosource International Camarillo, CA) according to the manufacturer's protocol.

2.8. H&E staining for general pathology and Iron stain (Prussian blue reaction) for cerebral hemorrhage

Hematoxylin and eosin (H&E) stained sections of brain collected from Mo/Hu APPswe PS1dE9 mice untreated ($n = 3$) and treated with AdGM-CSF ($n = 3$) or AdPEDI-(Aβ1-6)₁₁ ($n = 6$) were used for histologic safety evaluation. Five left hemisphere sections of brain including those from the cerebrum, hippocampus, and the cerebellum were presented on each slide and two slides from each mouse were examined. To study cerebral hemorrhage that may be associated with immunization, a series of 6 coronal brain sections (approximately 600 µm apart) from Mo/Hu APPswe PS1dE9 mice treated with AdPEDI-(Aβ1-6)₁₁ ($n = 4$) were stained with Sigma-Aldrich Iron Stain (Sigma-Aldrich, St. Louis, MO) according to the manufacturer's protocol. Brain sections from a Mo/Hu APPswe PS1dE9 mouse subjected to intracranial injection of lipopolysaccharide from *E. coli* 055:B5 (LPS) (Sigma, St. Louis, MO) were used as positive control.

2.9. Responses of splenic CD4⁺ T cell to PEDI-(Aβ1-6)₁₁ and cytokine-specific ELISA

CD4⁺ T cells were purified by the magnetic-activated cell sorter system (Miltenyl Biotec, Auburn, CA). Isolated cells were incubated in a nylon column (Polysciences, Warrington, PA) to remove B cells and macrophages. Enriched T cell fractions were then incubated with biotinylated anti-CD4 (GK 1.5) mAb, followed by streptavidin-conjugated microbeads, and passed through a magnetized column [39]. The purified T cell fractions were >97% CD4⁺ and were >99% viable. The purified CD4⁺ T cells (4×10^6 cells/ml) were cultured in the presence of T cell-depleted, irradiated (3000 rad) splenic antigen-presenting cells (APCs) with 5 % conditioned media that contained PEDI-(Aβ1-6)₁₁ or control medium. These APCs were derived from naïve mice and were placed in 24 well tissue culture plates (Corning Glass Works, Corning, NY) for 5 days at 37°C in a moist atmosphere of 5% CO₂ and 95% air. The condition medium containing PEDI-(Aβ1-6)₁₁ was prepared as follows: Medium was harvested from HEK293 cells transfected with pCA-PEDI-(Aβ1-6)₁₁ [33] and filtered through a Centricon (100 kDa molecular weight cut-off, Millipore). Medium from non-transfected HEK293 cells was used as a control. To assess T cell responses specific to PEDI-(Aβ1-6)₁₁, CD4⁺ T cells were cultured in the 5% conditioned medium for 3 days and T cell proliferation was quantified by CellTiter 96 non-radioactive cell proliferation assay (MTT assay, Promega, Madison, WI), according to the manufacturer's protocol. For determination of Th1 (IFN-γ and IL-2) and Th2 (IL-4 and IL-10) cytokines, supernatants were harvested after 5 days of incubation with 5%

conditioned or control medium, and were then subjected to cytokine-specific ELISA as described above.

3. RESULTS

3.1. AdPEDI-(A β 1-6)₁₁ elicits predominantly an IgG1 response

Serum titers against A β 1-42 were determined by ELISA using anti-mouse IgG antibody with anti-A β 6E10 antibody as a standard. Six weeks after immunization with AdPEDI-(A β 1-6)₁₁, anti-A β titers (14.3 ± 3.06 μ g/ml) were readily detectable in the sera in Mo/Hu APPswe PS1dE9 mice but those in the mice immunized with AdGM-CSF were less than the detectable anti-A β titer (0.1 ng/ml). The anti-A β antibody titers in the mice vaccinated with AdPEDI-(A β 1-6)₁₁ did not significantly increase thereafter in spite of the booster immunization (Figure 1A). Immunoglobulin isotype-specific anti-A β titers were quantified by ELISA (Figure 1B). The induced anti-A β antibodies are predominantly of the IgG1 isotype. The levels of IgM were negligible (<0.2 μ g/ml). In mice, the production of IgG1 is primarily induced by Th2-type cytokines, while IgG2a is produced through Th1-type cytokines. The IgG1/IgG2a ratio was approximately 6.1. Thus, these results indicate that nasal vaccination with AdPEDI-(A β 1-6)₁₁ induces a Th2-polarized immune responses in Mo/Hu APPswe PS1dE9 mice.

3.2. IL-10 is upregulated in Mo/Hu APPswe PS1dE9 mice immunized with AdPEDI-(A β 1-6)₁₁

Th1 cells are characterized by production of specific cytokines, IFN- γ and IL-2, while IL-4 and IL-10 are distinctive for Th2 cells. Six weeks after the initial vaccination, serum levels of cytokines were quantified by ELISA (Figure 2). A prominent induction of IL-10 (3.78 ± 0.95 ng/ml) was found in the Mo/Hu APPswe PS1dE9 mice immunized with AdPEDI-(A β 1-6)₁₁, whereas the levels of IL-10 were indiscernible (<3.1 pg/ml) in the sera from mice treated with AdGM-CSF ($P = 0.001$). The levels of IL-4 in the mice immunized with AdPEDI-(A β 1-6)₁₁ (3.57 ± 0.79 ng/ml) were greater than those with AdGM-CSF (1.72 ± 0.23 ng/ml) ($P = 0.038$). The mice immunized with AdPEDI-(A β 1-6)₁₁ had higher levels of serum IFN- γ (321 ± 33 ng/ml) compared with the mice treated with AdGM-CSF (113 ± 11 ng/ml) ($P = 0.039$). There were no differences in serum IL-2 levels between the mice immunized with AdPEDI-(A β 1-6)₁₁ (6.86 ± 1.58 ng/ml) and those treated with AdGM-CSF (4.36 ± 1.19 ng/ml) ($P = 0.17$). Thus, nasal immunization with AdPEDI-(A β 1-6)₁₁ mainly induced Th2 cytokines in Mo/Hu APPswe PS1dE9 mice.

3.3. Induced anti-A β antibodies are immunoreactive to A β oligomers as well as A β deposits in the brain

Immunoreactivity with monomeric and oligomeric A β for anti-sera induced by AdPEDI-(A β 1-6)₁₁ was determined by western blot analysis. When 6E10 antibody was used, A β monomer (4 kDa), trimer (12 kDa), and tetramer (16 kDa) were visualized as the major species (Figure 3A). All the anti-sera tested were also reactive to oligomeric as well as monomeric A β (Figure 3A). The observed bands by 6E10 antibody were not visualized by sera from mice immunized with AdGM-CSF (data not shown).

Brain sections from a 7-month old Mo/Hu APPswe PS1dE9 mouse were immunostained with 6-week sera from Mo/Hu APPswe PS1dE9 mice subjected to AdPEDI-(A β 1-6)₁₁ immunization. The 6-week sera reacted with amyloid plaques in the brain, whereas there was no plaque detected by 6-week sera from mice immunized with AdGM-CSF (Figure 3B and C). These findings demonstrate the specificity of anti-sera against amyloid deposits in the brain.

3.4. Effects of AdPEDI-(A β 1-6)₁₁ immunization on A β deposits

Diffuse and fibrillar A β deposits were detected by 6E10 antibody while fibrillar A β deposits (neuritic plaques) were stained by thioflavine-S. Representative results are shown in Figure 4. In the hippocampus, the A β loads (average percentage of area showing A β immunoreactivity by 6E10) were on average $0.687 \pm 0.081\%$ for AdPEDI-(A β 1-6)₁₁ treatment and $1.704 \pm 0.209\%$ for AdGM-CSF treatment ($P < 0.001$). The A β loads by 6E10 staining in the neocortex were on average $1.025 \pm 0.068\%$ for AdPEDI-(A β 1-6)₁₁ treatment and $2.189 \pm 0.189\%$ for AdGM-CSF treatment ($P < 0.001$). The A β loads showing thioflavine-S fluorescence in the hippocampus were $0.362 \pm 0.128\%$ for AdPEDI-(A β 1-6)₁₁ treatment and $0.716 \pm 0.011\%$ for AdGM-CSF treatment ($P = 0.03$). The A β loads by thioflavine-S fluorescence in the neocortex were $0.491 \pm 0.176\%$ for AdPEDI-(A β 1-6)₁₁ treatment and $1.11 \pm 0.099\%$ for AdGM-CSF treatment ($P = 0.04$). Thus, Mo/Hu APPswe PS1dE9 mice treated with AdPEDI-(A β 1-6)₁₁ had fewer diffuse and fibrillar A β deposits than those treated with AdGM-CSF.

3.5. Effects of AdPEDI-(A β 1-6)₁₁ immunization on cerebral A β load

The amount of total A β in the cerebrum was determined by A β 1-40 and A β 1-42 ELISA (Figure 5). The cerebral total A β 1-42 content in the mice treated with AdPEDI-(A β 1-6)₁₁ was on average $0.39 \pm 0.05 \mu\text{g/mg}$ protein and that with AdGM-CSF was $3.03 \pm 0.31 \mu\text{g/mg}$ protein ($P = 0.0009$). The cerebral total A β 1-40 content ($0.35 \pm 0.04 \mu\text{g/mg}$ protein) on average in the mice treated with AdPEDI-(A β 1-6)₁₁ was less than that with AdGM-CSF ($1.01 \pm 0.33 \mu\text{g/mg}$ protein) but the difference is not significant ($P = 0.07$). Thus, nasal immunization with AdPEDI-(A β 1-6)₁₁ reduced cerebral A β , particularly A β 1-42, in Mo/Hu APPswe PS1dE9 mice.

3.6. No noticeable inflammatory changes and hemorrhages associated with AdPEDI-(A β 1-6)₁₁ vaccination

Histopathological examination revealed no evidence of significant neuronal necrosis, demyelination, or generalized inflammation. A very few lymphocytes were occasionally found in the subependymal areas of the ventricles and in the choroids plexus in mice treated with AdGM-CSF ($n = 3$) and AdPEDI-(A β 1-6)₁₁ ($n = 6$), but not in mice untreated ($n = 3$). These changes, however, do not represent a generalized inflammation and do not appear to affect the functional activity of the brain. We further investigated possible cerebral hemorrhage in the experimental mice by iron stain (Prussian blue reaction). While hemorrhage was observed in a mouse subjected to intracranial injection of LPS, no cerebral hemorrhage was found in the mice treated with AdPEDI-(A β 1-6)₁₁ ($n = 4$) (data not shown).

3.7. Cytokine responses in C57BL/6 mice

As reported previously, nasal immunization with AdPEDI-(A β 1-6)₁₁ elicited anti-A β antibodies in C57BL/6 mice ($4.69 \pm 0.62 \mu\text{g/ml}$ serum), which were predominantly of the IgG1 isotype (IgG1/IgG2a ratio = 12.1 ± 3.3), indicating anti-inflammatory Th2 type [33]. We examined PEDI-(A β 1-6)₁₁-specific Th1 and Th2 cytokine responses in the mice treated with AdPEDI-(A β 1-6)₁₁ using splenic CD4⁺ T cells. Three days after stimulation with PEDI-(A β 1-6)₁₁-conditioned medium, splenic CD4⁺ T cell proliferative responses were determined by MTT assay. The stimulation index (SI) was 2.4: SI = (the 590 nm absorbance of the CD4⁺ T cell culture with 5% PEDI-(A β 1-6)₁₁-conditioned medium)/(the 590 nm absorbance of the CD4⁺ T cell culture with the control medium). The absorbance of the CD4⁺ T cell culture with 5% PEDI-(A β 1-6)₁₁-conditioned medium was significantly higher than that of the CD4⁺ T cell culture with 5%-conditioned control medium ($P = 0.002$). The levels of Th1 and Th2 cytokines were determined using the supernatants from splenic CD4⁺ T cell cultures that were stimulated with PEDI-(A β 1-6)₁₁-conditioned medium for 5 days (Figure 6). High levels of IL-10 responses ($4.82 \pm 0.36 \text{ ng/ml}$) upon the stimulation with PEDI-(A β 1-6)₁₁-conditioned

medium were found in the culture supernatants harvested from CD4⁺ T cells that were isolated from the C57BL/6 mice subjected to AdPEDI-(A β 1-6)₁₁ vaccination compared with those (0.36 $\pm$ 0.07 ng/ml) stimulated with control medium ($P < 0.01$). Although levels of IL-4 and IFN- γ responses in the culture of CD4⁺ T cells upon the stimulation with PEDI-(A β 1-6)₁₁, increased on average, the increases were not significant ($P > 0.05$). These results indicate that Th2-type cytokines, especially IL-10, mediate the immune responses elicited by nasal administration of AdPEDI-(A β 1-6)₁₁.

4. Discussion

Here, we show that nasal immunization of Mo/Hu APPswe PS1dE9 mice with AdPEDI-(A β 1-6)₁₁ successfully induced antibodies that were reactive with amyloid plaques as well as oligomeric A β . The induced anti-A β antibodies were predominantly of the IgG1 isotype, indicating induction of anti-inflammatory Th2 responses. This immunization modality was effective in reducing A β load in the brains of Mo/Hu APPswe PS1dE9 mice. Our preliminary data suggests that nasal administration of AdPEDI-(A β 1-6)₁₁ may be effective in improving learning and memory deficits in the AD model mice, also. The immune responses induced with AdPEDI-(A β 1-6)₁₁ were characterized by a marked upregulation of IL-10 expression, suggesting that innate IL-10 plays a crucial role in the induction of Th2 responses by AdPEDI-(A β 1-6)₁₁.

Nasal immunization of 2-month-old Mo/Hu APPswe PS1dE9 mice with AdPEDI-(A β 1-6)₁₁ for 10 months reduced total A β load (ELISA) and A β deposits (morphometric analysis) in the cerebrum by approximately 80% and 55%, respectively, compared with those in mice treated with AdGM-CSF. Amyloid deposits in the brains of Mo/Hu APPswe PS1dE9 mice become discernible at 4 months of age and subsequently increase significantly with age. Therefore, the effects of the adenovirus vector on amyloid deposition here should be considered to be prophylactic rather than therapeutic. Thus, amyloid deposition in the brain was not completely inhibited by this vaccination. Greater anti-A β titers and/or different epitopes of A β may be necessary to prevent A β accumulation entirely in the brain. As we and others previously demonstrated, it is feasible to increase anti-A β titers without shifting Th2-biased responses by use of Th2 adjuvants and cytokines such as Alum [40], monophosphoryl lipid A, cholera toxin B subunit, *E. coli* enterotoxin [41], IL-4 [28], and GM-CSF [42]. Alternatively, the complete prevention of amyloid deposits may be difficult without shifting Th2-biased responses because Bard et al. [43] reported that anti-A β IgG2a exhibited the highest degree of amyloid clearance among different IgG isotypes by passive immunization (intra peritoneal injection) of AD mouse models. While Chauhan and Siegel [44] reported that anti-A β IgG1 cleared cerebral A β more efficiently than IgG2a by intracerebroventricular injection, this discrepancy may be due to the differences in the injection routes and the former route seems to imitate active immunization better.

Adenoviruses have been extensively exploited as vaccine vectors for prevention of infectious diseases and treatment of cancer [45,46]. In such paradigms, Th1 or CD8 cytotoxic T cell mediated immune responses are thought to be essential for the destruction of cancer cells and microorganism-bearing cells. Indeed, the majority of reports on adenovirus vectors demonstrated remarkable Th1 responses after immunization with adenovirus vectored vaccines, particularly, in combination with DNA plasmid (DNA priming inoculation) [47–50]. Adenoviruses, however, activate dendritic cells without polarization toward a Th1 type [51]. In combination with Th2-type cytokines, adenovirus vectors can induce Th2 polarized responses [52]. IL-10 has been implicated in the induction of Th2 responses: IL-10 inhibits maturation of dendritic cells and the production of Th1 cytokines that direct the differentiation of naïve T cells into Th1 cells [53–55]. Consistent with this view, our results here suggest that

innate IL-10 plays a major role in driving the induction of Th2 cells after nasal immunization with AdPEDI-(A β 1-6)₁₁.

Upon stimulation with PEDI-(A β 1-6)₁₁, splenic CD4⁺ T cells from C57BL/6 mice that were nasally vaccinated with AdPEDI-(A β 1-6)₁₁, highly produced IL-10. Adjuvant, antigens, and delivery systems (such as adenovirus and recombinant salmonella) influence cytokine production by antigen-specific CD4⁺ T cells. For example, cholera toxin as adjuvant induces Th2-type cytokine responses regardless of its immunization routes (oral, nasal or systemic) [56–58]. In contrast, heat labile toxin as mucosal adjuvant elicits both Th1 and Th2 type cytokines [59]. When recombinant salmonella expressing fragment C of tetanus toxin was orally given to mice, high IFN- γ production was achieved [60]. In case of adenovirus vector, as we discussed above, Th1-type cytokine will be most likely induced. However, mucosal immunization with an adenovirus vector encoding β -galactosidase induced both Th1 and Th2-type cytokines in CD4⁺ T cells upon stimulation with adenovirus while IFN- γ and IL-6 were produced by CD4⁺ T cells upon stimulation with β -galactosidase [61]. In this study, plasma IL-10 was undetectable in mice vaccinated with AdGM-CSF. Therefore, it is most likely that AdPEDI-(A β 1-6)₁₁ induced IL-10 in our experimental animals rather than the mucosal immunization route.

It has been suggested that plasmid DNA coding for secreted antigens may induce Th2-polarized responses and IgG1 antibodies, whereas cytosolic or membrane anchored antigen induced IFN- γ -secreting cells and IgG2a antibodies [62,63]. Recently, Daly et al. (2005) [54] reported that plasmid DNA encoding secreted protein of HIV gp120 induced Th2-biased responses through the stimulation of innate IL-10. In order to achieve high levels of antigen secretion, we fused the signal sequence of immunoglobulin Kappa light chain to the N-terminus of PEDI-(A β 1-6)₁₁ [33,42]. Our observations also support the concept that DNA or vectored vaccines encoding secreted antigen induce Th2-biased responses.

The development of successful therapeutic vaccines against AD is thought to be dependent on the identification of immunization strategies that can induce potent A β -specific Th2 immune responses without eliciting adverse T cell-mediated autoimmune responses. The induction of innate anti-inflammatory cytokines, especially IL-10, is now recognized as an immune subversion strategy adopted by pathogens to suppress protective cellular immune responses [64]. Furthermore, IL-10 suppresses autoimmune encephalitis [65,66]. Our findings suggest that PEDI-(A β 1-6)₁₁ stimulates innate IL-10 production, inhibits activation of dendritic cells that induce Th1 cells, and promotes differentiation of Th2 cells.

A meningoencephalitis halted the clinical trial of AN1792. Transgenic mouse models of AD were not useful to predict such adverse effects associated with AN1792 [14–16]. It, however, may be possible to model the meningoencephalitis by addition of pertussis toxin or by passive immunization using a specific anti-A β antibody [67,68]. We occasionally observed a very few lymphocytes in the subependymal areas and choroids plexus in mice treated with AdGM-CSF and AdPEDI-(A β 1-6)₁₁, which are different from the pathological changes (focal lymphocytic and macrophage infiltration) reported in AD patients who developed aseptic meningoencephalitis after A β vaccine trial [17–19] and from the meningoencephalitis reported in mice [67,68]. Indeed, such minimal lymphocyte infiltration is reminiscent of changes found in the brain of an AD patient without encephalitis who was vaccinated with AN1792 [69]. Another possible side effect associated with A β vaccination is cerebral hemorrhages that were reported in one of AD patients subjected to the AN1792 clinical trial. Several groups [70–72] reported that passive immunization of anti-A β antibodies directed against N-terminal epitopes of A β and/or antibodies immunoreactive with A β deposits increased cerebral microhemorrhage in AD mouse models. This hemorrhage seems to be associated with passive immunization but not with active one [22]. We did not observe any hemorrhages in our experimental animals

vaccinated with AdPEDI-(A β 1-6)₁₁, also. It should be emphasized that none of the available AD models completely recapitulates the characteristics of human AD patients [73,74]. Thus, the safety of therapeutic means demonstrated in mice should not be translated directly into human and should be rigorously evaluated further.

In summary, the present study demonstrates that nasal administration of AdPEDI-(A β 1-6)₁₁ is an effective modality of priming therapeutic antibody and Th2-type responses in an AD mouse model. Because the differentiation of Th2 cells from naïve T cells is thought to be promoted by IL-4, it is possible to potentiate further the immune responses toward Th2-type by addition of IL-4 as an adjuvant.

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Reference List

1. Senechal Y, Larmet Y, Dev KK. Unraveling in vivo functions of amyloid precursor protein: insights from knockout and knockdown studies. *Neurodegener Dis* 2006;3:134–147. [PubMed: 16954700]
2. Zheng H, Koo EH. The amyloid precursor protein: beyond amyloid. *Mol Neurodegener* 2006;1:5. [PubMed: 16930452]
3. Caille I, Allinquant B, Dupont E, et al. Soluble form of amyloid precursor protein regulates proliferation of progenitors in the adult subventricular zone. *Development* 2004;131:2173–2181. [PubMed: 15073156]
4. Kamal A, menar-Queralt A, LeBlanc JF, et al. Kinesin-mediated axonal transport of a membrane compartment containing beta-secretase and presenilin-1 requires APP. *Nature* 2001;414:643–648. [PubMed: 11740561]
5. Inestrosa NC, Cerpa W, Varela-Nallar L. Copper brain homeostasis: role of amyloid precursor protein and prion protein. *IUBMB Life* 2005;57:645–650. [PubMed: 16203684]
6. Guenette S, Chang Y, Hiesberger T, et al. Essential roles for the FE65 amyloid precursor protein-interacting proteins in brain development. *EMBO J* 2006;25:420–431. [PubMed: 16407979]
7. Oh SY, Ellenstein A, Chen CD, et al. Amyloid precursor protein interacts with notch receptors. *J Neurosci Res* 2005;82:32–42. [PubMed: 16118793]
8. Selkoe DJ. Alzheimer's disease is a synaptic failure. *Science* 2002;298:789–791. [PubMed: 12399581]
9. Stokin GB, Lillo C, Falzone TL, et al. Axonopathy and transport deficits early in the pathogenesis of Alzheimer's disease. *Science* 2005;307:1282–1288. [PubMed: 15731448]
10. Tanzi RE. The synaptic Abeta hypothesis of Alzheimer disease. *Nat Neurosci* 2005;8:977–979. [PubMed: 16047022]
11. Hardy J. Amyloid double trouble. *Nat Genet* 2006;38:11–12. [PubMed: 16380721]
12. Games D, Adams D, Alessandrini R, et al. Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. *Nature* 1995;373:523–527. [PubMed: 7845465]
13. Hsiao K, Chapman P, Nilsen S, et al. Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science* 1996;274:99–102. [PubMed: 8810256]
14. Janus C, Pearson J, McLaurin J, et al. A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature* 2000;408:979–982. [PubMed: 11140685]
15. Morgan D, Diamond DM, Gottschall PE, et al. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* 2000;408:982–985. [PubMed: 11140686]
16. Schenk D, Barbour R, Dunn W, et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* 1999;400:173–177. [PubMed: 10408445]
17. Orgogozo JM, Gilman S, Dartigues JF, et al. Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization. *Neurology* 2003;61:46–54. [PubMed: 12847155]

18. Ferrer I, Boada RM, Sanchez Guerra ML, et al. Neuropathology and pathogenesis of encephalitis following amyloid-beta immunization in Alzheimer's disease. *Brain Pathol* 2004;14:11–20. [PubMed: 14997933]
19. Nicoll JA, Wilkinson D, Holmes C, et al. Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. *Nat Med* 2003;9:448–452. [PubMed: 12640446]
20. Bard F, Cannon C, Barbour R, et al. Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nat Med* 2000;6:916–919. [PubMed: 10932230]
21. Gelinas DS, DaSilva K, Fenili D, et al. Immunotherapy for Alzheimer's disease. *Proc Natl Acad Sci U S A* 2004;101:14657–14662. [PubMed: 15297619]
22. Goni F, Sigurdsson EM. New directions towards safer and effective vaccines for Alzheimer's disease. *Curr Opin Mol Ther* 2005;7:17–23. [PubMed: 15732525]
23. Monsonego A, Weiner HL. Immunotherapeutic approaches to Alzheimer's disease. *Science* 2003;302:834–838. [PubMed: 14593170]
24. Schenk D, Hagen M, Seubert P. Current progress in beta-amyloid immunotherapy. *Curr Opin Immunol* 2004;16:599–606. [PubMed: 15342006]
25. Wisniewski T, Frangione B. Immunological and anti-chaperone therapeutic approaches for Alzheimer disease. *Brain Pathol* 2005;15:72–77. [PubMed: 15779239]
26. Boyer JL, Kobinger G, Wilson JM, Crystal RG. Adenovirus-based genetic vaccines for biodefense. *Hum Gene Ther* 2005;16:157–168. [PubMed: 15761256]
27. Liu M, Acres B, Balloul JM, et al. Gene-based vaccines and immunotherapeutics. *Proc Natl Acad Sci U S A* 2004;101:14567–14571. [PubMed: 15333750]
28. Ghochikyan A, Vasilevko V, Petrushina I, 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;33:3232–3241. [PubMed: 14635031]
29. Qu B, Rosenberg RN, Li L, et al. Gene vaccination to bias the immune response to amyloid-beta peptide as therapy for Alzheimer disease. *Arch Neurol* 2004;61:1859–1864. [PubMed: 15596606]
30. Cribbs DH, Ghochikyan A, Vasilevko V, et al. Adjuvant-dependent modulation of Th1 and Th2 responses to immunization with beta-amyloid. *Int Immunol* 2003;15:505–514. [PubMed: 12663680]
31. McLaurin J, Cecal R, Kierstead ME, et al. Therapeutically effective antibodies against amyloid-beta peptide target amyloid-beta residues 4–10 and inhibit cytotoxicity and fibrillogenesis. *Nat Med* 2002;8:1263–1269. [PubMed: 12379850]
32. Town T, Tan J, Sansone N, et al. Characterization of murine immunoglobulin G antibodies against human amyloid-beta1-42. *Neurosci Lett* 2001;307:101–104. [PubMed: 11427310]
33. Kim HD, Maxwell JA, Kong FK, et al. Induction of anti-inflammatory immune response by an adenovirus vector encoding 11 tandem repeats of Abeta1-6: toward safer and effective vaccines against Alzheimer's disease. *Biochem Biophys Res Commun* 2005;336:84–92. [PubMed: 16126169]
34. Lee CT, Wu S, Ciernik IF, et al. Genetic immunotherapy of established tumors with adenovirus-murine granulocyte-macrophage colony-stimulating factor. *Hum Gene Ther* 1997;8:187–193. [PubMed: 9017422]
35. Jankowsky JL, Fadale DJ, Anderson J, et al. Mutant presenilins specifically elevate the levels of the 42 residue beta-amyloid peptide in vivo: evidence for augmentation of a 42-specific gamma secretase. *Hum Mol Genet* 2004;13:159–170. [PubMed: 14645205]
36. Dahlgren KN, Manelli AM, Stine WB Jr, et al. Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability. *J Biol Chem* 2002;277:32046–32053. [PubMed: 12058030]
37. Fukuchi K, Pham D, Hart M, et al. Amyloid-beta protein deposition in skeletal muscle of transgenic mice: possible model of inclusion body myopathy. *Am J Pathol* 1998;153:1687–1693. [PubMed: 9846958]
38. Li L, Cao D, Garber DW, et al. Association of aortic atherosclerosis with cerebral beta-amyloidosis and learning deficits in a mouse model of Alzheimer's disease. *Am J Pathol* 2003;163:2155–2164. [PubMed: 14633589]

39. Kataoka K, McGhee JR, Kobayashi R, et al. Nasal Flt3 ligand cDNA elicits CD11c+CD8+ dendritic cells for enhanced mucosal immunity. *J Immunol* 2004;172:3612–3619. [PubMed: 15004163]
40. Agadjanyan MG, Ghochikyan A, Petrushina I, et al. Prototype Alzheimer's disease vaccine using the immunodominant B cell epitope from beta-amyloid and promiscuous T cell epitope pan HLA DR-binding peptide. *J Immunol* 2005;174:1580–1586. [PubMed: 15661919]
41. Maier M, Seabrook TJ, Lemere CA. Modulation of the humoral and cellular immune response in Abeta immunotherapy by the adjuvants monophosphoryl lipid A (MPL), cholera toxin B subunit (CTB) and E.coli enterotoxin LT(R192G). *Vaccine* 2005;23:5149–5159. [PubMed: 16054274]
42. Kim HD, Kong FK, Cao Y, et al. Immunization of Alzheimer model mice with adenovirus vectors encoding amyloid beta-protein and GM-CSF reduces amyloid load in the brain. *Neurosci Lett* 2004;370:218–223. [PubMed: 15488326]
43. Bard F, Barbour R, Cannon C, et al. Epitope and isotype specificities of antibodies to beta-amyloid peptide for protection against Alzheimer's disease-like neuropathology. *Proc Natl Acad Sci U S A* 2003;100:2023–2028. [PubMed: 12566568]
44. Chauhan NB, Siegel GJ. Efficacy of anti-Abeta antibody isotypes used for intracerebroventricular immunization in TgCRND8. *Neurosci Lett* 2005;375:143–147. [PubMed: 15694248]
45. Santosuosso M, McCormick S, Xing Z. Adenoviral vectors for mucosal vaccination against infectious diseases. *Viral Immunol* 2005;18:283–291. [PubMed: 16035940]
46. Tatsis N, Ertl HC. Adenoviruses as vaccine vectors. *Mol Ther* 2004;10:616–629. [PubMed: 15451446]
47. Bruna-Romero O, Rocha CD, Tsuji M, Gazzinelli RT. Enhanced protective immunity against malaria by vaccination with a recombinant adenovirus encoding the circumsporozoite protein of Plasmodium lacking the GPI-anchoring motif. *Vaccine* 2004;22:3575–3584. [PubMed: 15315836]
48. Liu Y, Xia D, Li F, et al. Intratumoral administration of immature dendritic cells following the adenovirus vector encoding CD40 ligand elicits significant regression of established myeloma. *Cancer Gene Ther* 2005;12:122–132. [PubMed: 15565183]
49. Meng WS, Butterfield LH, Ribas A, et al. alpha-Fetoprotein-specific tumor immunity induced by plasmid prime-adenovirus boost genetic vaccination. *Cancer Res* 2001;61:8782–8786. [PubMed: 11751399]
50. Rollier C, Verschoor EJ, Paranhos-Baccala G, et al. Modulation of vaccine-induced immune responses to hepatitis C virus in rhesus macaques by altering priming before adenovirus boosting. *J Infect Dis* 2005;192:920–929. [PubMed: 16088843]
51. Rea D, Schagen FH, Hoebe RC, et al. Adenoviruses activate human dendritic cells without polarization toward a T-helper type 1-inducing subset. *J Virol* 1999;73:10245–10253. [PubMed: 10559341]
52. Xiang ZQ, Pasquini S, Ertl HC. Induction of genital immunity by DNA priming and intranasal booster immunization with a replication-defective adenoviral recombinant. *J Immunol* 1999;162:6716–6723. [PubMed: 10352290]
53. Corinti S, Albanesi C, la Sala A, et al. Regulatory activity of autocrine IL-10 on dendritic cell functions. *J Immunol* 2001;166:4312–4318. [PubMed: 11254683]
54. Daly LM, Johnson PA, Donnelly G, et al. Innate IL-10 promotes the induction of Th2 responses with plasmid DNA expressing HIV gp120. *Vaccine* 2005;23:963–974. [PubMed: 15603899]
55. Huang LY, Reis e Sousa, Itoh Y, et al. IL-12 induction by a TH1-inducing adjuvant in vivo: dendritic cell subsets and regulation by IL-10. *J Immunol* 2001;167:1423–1430. [PubMed: 11466361]
56. Xu-Amano J, Kiyono H, Jackson RJ, et al. Helper T cell subsets for immunoglobulin A responses: oral immunization with tetanus toxoid and cholera toxin as adjuvant selectively induces Th2 cells in mucosa associated tissues. *J Exp Med* 1993;178:1309–1320. [PubMed: 8376936]
57. Xu-Amano J, Jackson RJ, Fujihashi K, et al. Helper Th1 and Th2 cell responses following mucosal or systemic immunization with cholera toxin. *Vaccine* 1994;12:903–911. [PubMed: 7975832]
58. Yamamoto S, Kiyono H, Yamamoto M, et al. A nontoxic mutant of cholera toxin elicits Th2-type responses for enhanced mucosal immunity. *Proc Natl Acad Sci USA* 1997;94:5267–5272. [PubMed: 9144226]

59. Nakagawa I, Takahashi I, Kiyono H, et al. Oral immunization with the B subunit of the heat-labile enterotoxin of *Escherichia coli* induces early Th1 and late Th2 cytokine expression in Peyer's patches. *J Infect Dis* 1996;173:1428–1436. [PubMed: 8648216]
60. VanCott JL, Staats HF, Pascual DW, et al. Regulation of mucosal and systemic antibody responses by T helper cell subsets, macrophages, and derived cytokines following oral immunization with live recombinant *Salmonella*. *J Immunol* 1996;156:1504–1514. [PubMed: 8568254]
61. Van Ginkel FW, McGhee JR, Liu C, et al. Adenoviral gene delivery elicits distinct pulmonary-associated T helper cell responses to the vector and to its transgene. *J Immunol* 1997;159:685–693. [PubMed: 9218583]
62. Haddad D, Liljeqvist S, Stahl S, et al. Differential induction of immunoglobulin G subclasses by immunization with DNA vectors containing or lacking a signal sequence. *Immunol Lett* 1998;61:201–204. [PubMed: 9657276]
63. Lewis PJ, van Drunen Littel-van den Hurk, Babiuk LA. Altering the cellular location of an antigen expressed by a DNA-based vaccine modulates the immune response. *J Virol* 1999;73:10214–10223. [PubMed: 10559338]
64. McGuirk P, McCann C, Mills KH. Pathogen-specific T regulatory 1 cells induced in the respiratory tract by a bacterial molecule that stimulates interleukin 10 production by dendritic cells: a novel strategy for evasion of protective T helper type 1 responses by *Bordetella pertussis*. *J Exp Med* 2002;195:221–231. [PubMed: 11805149]
65. Frenkel D, Huang Z, Maron R, et al. Neuroprotection by IL-10-producing MOG CD4+ T cells following ischemic stroke. *J Neurol Sci* 2005;233:125–132. [PubMed: 15894335]
66. Gonnella PA, Waldner HP, Kodali D, Weiner HL. Induction of low dose oral tolerance in IL-10 deficient mice with experimental autoimmune encephalomyelitis. *J Autoimmun* 2004;23:193–200. [PubMed: 15501390]
67. Furlan R, Brambilla E, Sanvito F, et al. Vaccination with amyloid-beta peptide induces autoimmune encephalomyelitis in C57/BL6 mice. *Brain* 2003;126:285–291. [PubMed: 12538398]
68. Lee EB, Leng LZ, Lee VM, Trojanowski JQ. Meningoencephalitis associated with passive immunization of a transgenic murine model of Alzheimer's amyloidosis. *FEBS Lett* 2005;579:2564–2568. [PubMed: 15862291]
69. Masliah E, Hansen L, Adame A, et al. Abeta vaccination effects on plaque pathology in the absence of encephalitis in Alzheimer disease. *Neurology* 2005;64:129–131. [PubMed: 15642916]
70. Pfeifer M, Boncristiano S, Bondolfi L, et al. Cerebral hemorrhage after passive anti-Abeta immunotherapy. *Science* 2002;298:1379. [PubMed: 12434053]
71. Racke MM, Boone LI, Hepburn DL, et al. Exacerbation of cerebral amyloid angiopathy-associated microhemorrhage in amyloid precursor protein transgenic mice by immunotherapy is dependent on antibody recognition of deposited forms of amyloid beta. *J Neurosci* 2005;25:629–636. [PubMed: 15659599]
72. Wilcock DM, Rojiani A, Rosenthal A, et al. Passive immunotherapy against Aβeta in aged APP-transgenic mice reverses cognitive deficits and depletes parenchymal amyloid deposits in spite of increased vascular amyloid and microhemorrhage. *J Neuroinflammation* 2004;1:24. [PubMed: 15588287]
73. Codita A, Winblad B, Mohammed AH. Of mice and men: more neurobiology in dementia. *Curr Opin Psychiatry* 2006;19:555–563. [PubMed: 17012931]
74. Gandy S, Walker L. Toward modeling hemorrhagic and encephalitic complications of Alzheimer amyloid-beta vaccination in nonhuman primates. *Curr Opin Immunol* 2004;16:607–615. [PubMed: 15342007]

FIG. 1.

Induction of anti-A β antibodies in Mo/Hu APPswe PS1dE9 mice after vaccination with AdPEDI-(A β 1-6)₁₁. Anti-A β antibodies were indiscernible in mice treated with AdGM-CSF.

A: Serum titers for anti-A β antibodies were determined by ELISA using anti-mouse IgG antibody at the indicated time points; pre-serum (week 0), 6 weeks, and 12 weeks after the initial vaccination. **B:** Isotypes of anti-A β antibodies induced by AdPEDI-(A β 1-6)₁₁. Isotype specific titers for anti-A β antibodies were determined by ELISA, using anti mouse IgM, IgG1, IgG2a, and IgG2b antibodies 6 weeks after the initial vaccination. The error bars are S.E.M.

FIG. 2.

Levels of Th1 (IFN- γ and IL-2) and Th2 (IL-4 and IL-10) cytokines in Mo/Hu APPswe PS1dE9 mice vaccinated with AdPEDI-(A β 1-6)₁₁. Levels of serum cytokines (IL-2, IL-4, IL-10, and IFN- γ) in Mo/Hu APPswe PS1dE9 mice treated with AdPEDI-(A β 1-6)₁₁ or AdGM-CSF were determined by ELISA using cytokine specific antibodies. A remarkable induction of IL-10 is seen in Mo/Hu APPswe PS1dE9 mice treated with AdPEDI-(A β 1-6)₁₁. IL-10 in sera from the mice treated with AdGM-CSF are not detectable (less than 31 pg/ml).

FIG. 3.

Immunoreactivity of anti-sera from Mo/Hu APPswe PS1dE9 mice immunized with AdPEDI-(A β 1-6)₁₁. **A:** Immunoreactivity of anti-sera with oligomeric A β . Anti-serum, #5388, was obtained 12 weeks after immunization with AdPEDI-(A β 1-6)₁₁. A β oligomers were separated by 16.5% Tris-tricine SDS-PAGE and, after blotting to a PVDF membrane, were detected by anti-serum (#5388) or 6E10 antibody, using an enhanced chemiluminescence system. **B and C:** Immunoreactivity of anti-sera with amyloid plaques in the brain. Serial brain sections from a 7-month old Mo/Hu APPswe PS1dE9 mouse were stained with **(B)** #5426 serum drawn from a Mo/Hu APPswe PS1dE9 mouse 6 weeks after vaccination with AdPEDI-(A β 1-6)₁₁ and

(C) serum from a Mo/Hu APPswe PS1dE9 mouse treated with AdGM-CSF. The insets are high power magnifications of the indicated area by boxes. Scale bars indicate 500 μ m.

FIG. 4.

Nasal vaccination of Mo/Hu APPswe PS1dE9 mice with AdPEDI-(Aβ1-6)₁₁ reduces Aβ deposits in the brain. Ten months after the initial vaccination with AdPEDI-(Aβ1-6)₁₁ or AdGM-CSF, mice were terminated and the brains were subjected to immunohistochemical or histochemical analyses to evaluate the efficacy of the adenovirus vectors in reducing Aβ deposits in the hippocampus and neocortex. **A through D:** Diffuse and fibrillar Aβ deposits were detected with 6E10 antibody. **E through H:** fibrillar Aβ deposits (neuritic plaques) were stained with thioflavine-S. **A, C, E, and G:** AdPEDI-(Aβ1-6)₁₁-treated brain. **B, D, F, and H:** AdGM-CSF-treated brain. **A, B, E, and F:** Neocortex. **C, D, G, and H:** Hippocampus. Scale bars 200 μm (A through H).

FIG. 5.

Nasal vaccination of Mo/Hu APPswe PS1dE9 mice with AdPEDI-(Aβ1-6)₁₁ reduces cerebral Aβ. Ten months after vaccination with either AdPEDI-(Aβ1-6)₁₁ or AdGM-CSF, Mo/Hu APPswe PS1dE9 mice were terminated and cerebral protein was extracted by guanidine. Aβ40 and Aβ42 in the cerebrum were quantified by ELISA. The cerebral Aβ42 load in the mice vaccinated with AdPEDI-(Aβ1-6)₁₁ is reduced as compared with the mice treated with AdGM-CSF ($P = 0.0009$). The difference in the cerebral Aβ40 load is not significant between the two groups ($P = 0.2$). The values shown are the mean $\pm$ S.E.M. of 6 mice in each experimental group.

Vaccination: - - + +
 Induction
 [PEDI(Aβ1-6)₁₁] : - + - +

Vaccination: - - + +
 Induction
 [PEDI(Aβ1-6)₁₁] : - + - +

Vaccination: - - + +
 Induction
 [PEDI(Aβ1-6)₁₁] : - + - +

Vaccination: - - + +
 Induction
 [PEDI(Aβ1-6)₁₁] : - + - +

FIG. 6.

PEDI-(Aβ1-6)₁₁-induced CD4⁺ Th1- and Th2-type cytokine responses in C57BL/6 mice vaccinated with AdPEDI-(Aβ1-6)₁₁. Splenic CD4⁺ T cells from C57BL/6 mice vaccinated or unvaccinated with AdPEDI-(Aβ1-6)₁₁ were cultured in conditioned medium with or without PEDI-(Aβ1-6)₁₁ in the presence of T cell-depleted and irradiated splenic feeder cells. Culture supernatants were harvested after 5 days incubation and analyzed by the respective cytokine-specific ELISA. High levels of IL-10 responses upon the stimulation with PEDI-(Aβ1-6)₁₁ were found in the culture supernatants harvested from the C57BL/6 mice subjected to AdPEDI-(Aβ1-6)₁₁ vaccination compared with other groups ($P < 0.01$). The values shown are the mean $\pm$ S.E.M. of 4 mice.