Mucosal immunotherapy in an Alzheimer mouse model by recombinant Sendai virus vector carrying Aβ1–43/IL-10 cDNA

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1. Introduction

Alzheimer’s disease (AD) is characterized by progressive loss of cognitive functions related to amyloid β (Aβ) deposits in the central nervous system. Based on the amyloid cascade hypothesis [1], many reports have indicated the efficacy of immunotherapy for AD [2]. This strategy was originated by the finding that active immunization with Aβ peptide plus adjuvant showed effective clearance and prevention of amyloid deposits in PDAPP mice [3].

Although the phase Ila trial of AN1792, a mixture of synthetic Aβ1–42 peptide and adjuvant QS21 was halted because of meningoencephalitis as the side effect [4], pathological reports have indicated the effective removal of senile plaques in vaccinated patients [5–7]. AN1792 also ameliorates cognitive functions of AD patients [8–10], although another report showed no clinical benefit in spite of significant clearance of senile plaque amyloid [11]. Since administration of some anti-Aβ antibodies has also shown the therapeutic efficacy in animals [12,13], some clinical trials of passive immunization are under investigation. However, repeated injections of monoclonal anti-Aβ antibody are required, which may produce anti-idiotypic and neutralizing antibodies. Increases of micro-hemorrhage and vasogenic edema have also been reported after systemic administration of anti-Aβ antibodies into APP-tg mice and humans [14–16]. Furthermore, passive immunization is not useful for prophylaxis for diseases with insidious onset such as AD. Thus, active immunization, if it is safe and easy, still has an advantage. One of the active immunizations is intramuscular injection of DNA encoding Aβ [17,18]. However, repeated injections are required, and it may require a strategy of suppressing T helper 1 (Th1) immune responses.

The mucosal immune system representing Peyer’s patch and nasopharyngeal associated lymphoid tissue (NALT) has distinct functions such as predominant humoral immune responses and efficient immune induction via mucosal tissue. To induce mucosal immune responses nasal administration of Aβ peptide and adjuvant has been successful in mice [19,20]. However, use of adjuvant induces T cell infiltration in the brain. Administration of viral vectors carrying cDNA encoding genes of targeting antigens can stimulate mucosal immune system without adjuvant [21].

Now, we have developed a new nasal vaccine for AD by using the recombinant Sendai virus (SeV) vector. We found an excellent effect of the vaccine in APP-tg mice (Tg2576) pathologically and functionally without inducing brain inflammation.
2. Materials and methods

2.1. Ethics

This work was conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). All experiments were performed in accordance with Guidelines for Animal Experiments of the NCCG/NILS animal experimentation committee and of Nagoya University School of Medicine. The procedures involving animals and their care conformed to the international guidelines set out in “Principles of Laboratory Animal Care” (NIH publication no. 85–23, revised 1985).

2.2. Mice

Tg2576 mice [22] expressing the Swedish mutation of APP (AβPPK670N, M671L) at high levels under control of the hamster prion protein (PrP) promoter were obtained from Taconic Co. (USA). Animals were kept in a specific-pathogen-free condition and fed ad libitum.

2.3. Recombinant Sendai virus vector

We developed recombinant SeV vector carrying human Aβ1–43 cDNA and mouse interleukin-10 (mIL-10) cDNA (rSeV-ΔΔ). Recombinant SeV vector carrying LacZ cDNA (rSeV-LacZ) was used as control. The experiment was approved by the recombinant DNA experiment safety committee in the institutions.

In order to make the vaccine, we utilized F gene-deleted non-transmissible SeV [23] further bearing temperature-sensitive mutations in M (G69E, T116A, and A183S), HN (A262T, G264R, and K461G) [24], and L (N1197S, K1795E) identified in SeV strains capable of persistent infection in vitro [25]. Thus generated and named SeV/ΔΔ vector was used to construct the SeVΔΔ/Aβ1–43/ΔΔ-mIL10 vector carrying Aβ1–43 gene with APP secretion signal [21] and mIL10 according to the method described previously with a little modification [23,24,26] (Fig. 1). In brief, Aβ1–43 gene was amplified with a pair of NotI-tagged primers that contained SeV-specific transcriptional regulatory signal sequences, 5'-ATTGCGGCCGCGCAAGGGTTCACGATGCGCCGGTTCGCACTGGTCTTGG-3' and 5'-ATTGCGGCCGCGATGAACTTCTACCTTGAATGCGATAGCACACACCCGGACCATGAGTCC-3'. The amplified fragment was introduced into the NotI site of the parental pSeVΔΔ′/ΔΔ, cDNA of SeV of SeV/ΔΔ, to generate pSeVΔΔ′/Aβ43/ΔΔ, cDNA of mL10 (accession no. NP_034678) that was amplified with a pair of NotI-tagged primers, 5'-ACGTGCAGCCGCAAGGGTTCACGATGCGCCGGTTCGCACTGGTCTTGG-3' and 5'-ATGCGGCCGCGATGAACTTCTACCTTGAATGCGATAGCACACACCCGGACCATGAGTCC-3', which were subcloned into the F gene-deleted site of the LitmusSalINHeflfrag/ΔΔ vector (Sall and Nhel digested fragment containing M and HN genes from pSeVΔΔ′/ΔΔ in LITMUS38 (NEB) [27]). The Sall and Nhel digested fragment of pSeVΔΔ′Aβ1–43/ΔΔ was substituted with the corresponding fragment of the mL10 gene-introduced LitmusSalINHeflfrag/ΔΔ. The cDNA of SeVΔΔ′LacZ/ΔΔ (pSeVΔΔ′lacZ/ΔΔ) was constructed in similar manner using an amplified fragment of LacZ [26]. pSeVΔΔ′Aβ1–43/ΔΔ-mIL10 or pSeVΔΔ′LacZ/ΔΔ was transfected into 293T cells with T7-expressing plasmid. The T7-driven recombinant SeVΔΔ′Aβ1–43/ΔΔ-mIL10 and SeVΔΔ′LacZ/ΔΔ RNA genomes were encapsulated by NP, P, and L proteins, which were derived from their respective co-transfected plasmids. The recovered SeV vectors were propagated using F protein-expressing packaging cell line [23]. The virus titers were determined using infectivity and were expressed in cell infectious units (CUI). The SeV vectors were stored at −80 °C until use.

2.4. Vaccination with rSeV-Aβ or rSeV-LacZ

rSeV was diluted with PBS to give 5 × 10^6 CFIU/head in a final volume of 0.02 ml and was administered once nasally or intramuscularly (left quadriceps) to 12-month-old Tg2576 mice for analysis of cognitive functions and body weight, or to 24-month-old Tg2576 mice for evaluation of amyloid burdens and Aβ contents in the brain. Control Tg2576 mice received SeV-LacZ and were analyzed in the same way.

2.5. Pathological study

Tg2576 mice received the vaccine nasally or intramuscularly at the age of 24 months and were sacrificed 8 weeks after by CO2 asphyxiation. Their brains were removed and cut in half sagittally.

2.6. Serum antibodies against Aβ42

Anti-human Aβ antibody titers in the serum of nasally or intramuscularly vaccinated mice with rSeV-Aβ or rSeV-LacZ (n = 4 each) were quantified by a sandwich ELISA. Microtiter ELISA plates were coated overnight at 4 °C with 2 μg/ml of synthetic human Aβ1–42 in 0.1 M NaHCO3, pH 8.3, washed twice with washing buffer, blocked with 1% BSA and 2% normal goat serum in PBS for 2 h at room temperature (RT), washed twice and incubated with mouse serum samples diluted 1:500 in blocking buffer for 2 h at RT while shaking, washed × 4 and incubated horseradish peroxidase-conjugated goat-anti-mouse IgG for 2 h at RT, washed × 4 and analyzed colorimetrically after incubation with the chromogen substrate 3,3′,5,5′-tetramethylbenzidine (Kirkegaard & Perry Laboratories, Gaithersburg) at RT.

2.7. Aβ measurements in brain tissue by ELISA

Using highly specific antibodies and a sensitive sandwich ELISA, we quantified insoluble Aβ40 and Aβ42 in brain homogenates extracted with TBS, 2% SDS and 70% formic acid according to the method described [28]. The frozen brain tissue obtained from rSeV-Aβ-treated and rSeV-LacZ–treated Tg2576 mice (n = 4 each) was homogenized in 1 ml of TBS/complete protease inhibitor and 20 μg/ml peptatin A (Roche, Indianapolis), then centrifuged at 100,000 × g for 1 h at 4 °C. The TBS supernatants were stored at −80 °C and the pellets were homogenized in 1 ml of 2% SDS/TBS with protease inhibitor (Roche), then centrifuged at 100,000 × g for 1 h at 25 °C following 15 min incubation at 37 °C. The pellet was washed once, then extracted further with 1 ml of 70% formic acid, and centrifuged at 100,000 × g for 1 h. The 70% formic acid extracts were neutralized with 1 ml Tris–HCl, pH 8.0 at dilution of 1:20. For quantification of Aβ in the insoluble fractions, we used β-amyloid ELISA kit (Wako, Japan). The supernatant was diluted with standard dilution buffer at 1:2000 for Aβ40 or 1:400 for Aβ42 and measured according to the manufacturer’s instructions. The obtained values were corrected with the wet weight of each brain hemisphere samples and expressed as pmol/g brain.
2.8. Western blot analysis for detection of Aβ oligomers in soluble fraction

For analysis of Aβ oligomers in the SDS soluble fractions, 5 μl of the supernatant referring to the sample preparation in ELISA was electrophoresed on 15/25% gradient SDS-PAGE gel (Daichi, Japan) and transferred onto 0.2 μm nitrocellulose membrane at 200 mA for 1 h. Filters were blocked with 5% non-fat milk in a 20 mM Tris–HCl, pH 7.4 containing 150 mM NaCl and 0.05% Tween 20 (TBS-T). After washing the membranes in TBS-T, monoclonal anti-Aβ antibody 6E10 (Senetek, Napa, CA) was used to probe the blots. Bound antibody was visualized using horseradish peroxidase-conjugated anti-mouse IgG (at 1:10,000) and ECL+ detection (Amersham Pharmacia Biotech, Arlington Heights, IL).

2.9. Immunohistochemistry

Cryosections were fixed for 15 min with 70% formic acid for Aβ staining or 4% paraformaldehyde in 0.1 M phosphate buffer and rinsed with PBS–Triton before incubation in 0.3% H2O2 in methanol for 30 min. Sections were incubated at RT for 2 h with antibody as indicated below. Sections were washed with PBS–Triton before incubation with secondary goat anti-mouse or anti-rabbit antibodies for 2 h. After PBS–Triton washes, sections were stained by the avidin–biotin HRP/DAB method. For immunofluorescent labeling, the fluorochrome-conjugated immunoreagents were applied at a concentration of 20 μg/ml PBS containing 1% BSA and 2% normal goat serum. Aβ plaque-containing sections were stained with polyclonal rabbit anti-Aβ antibody (Senetek, Napa, CA). The following primary antibodies were used at 1:50: CD3e, CD4, CD86, CD19 and CD11b (BD Biosciences Pharmingen, San Jose, CA), Cy3-tagged anti-mouse GFAP (Sigma, Saint Louis, MS; 1:400), and Iba-1 for microglia (kind gift from Dr. U. Imai, NCNP, Tokyo).

Quantitative analysis of Aβ burden was performed as described previously [21] in three different brain regions, the hippocampus, the frontal cortex, and the parietal association cortex of rSeV-LacZ-treated and rSeV-Aβ-treated Tg2576 mice (n = 4 each). The Aβ burden was defined as the percentage of a brain region covered by Aβ-immunoreactive deposits. Images were projected from an Olympus Vanox microscope onto a computer screen through a 3CCD Fujix digital camera and analyzed with an image analysis system (Mac Scope, Mitani Corporation).

2.10. Behavioral analysis

2.10.1. Experimental design

Cognitive dysfunctions are directly correlated with Aβ oligomers in Tg2576 mice, which start at around 6 months old and are stable until 14 months old [29]. Thus, we first evaluated cognitive functions in both non-tg (n = 18) and Tg2576 mice (n = 24) at the age of 12 months. After the behavioral test, mice were divided into two groups to be treated with rSeV-LacZ or rSeV-Aβ. There is no difference between the two groups in behavioral scores at the age of 12 months. To evaluate the effect of vaccine treatment, each group (rSeV-LacZ-treated non-tg mice, n = 9; rSeV-Aβ-treated non-tg mice, n = 9; rSeV-LacZ-treated Tg2576 mice, n = 10; rSeV-Aβ-treated Tg2576 mice, n = 14) was subjected to behavioral tests at the age of 15 months. All tests were done according to the methods described previously [30].

2.10.2. Novel-object recognition test

24 h after 10 min-training session following 3 day-habitation, each mouse was placed back into the same box in which one of the familiar objects used during training was replaced with a novel one. The animals were then allowed to explore freely for 10 min and the time spent exploring each object was recorded. The exploratory preference (%), a ratio of the amount of time spent exploring any one of the two objects (training session) or the novel object (retention session) over the total time spent exploring both objects was used to measure cognitive function.

2.10.3. Spontaneous alternation in a Y-maze test

Each mouse was placed at the center of the apparatus and allowed to move freely through the maze during an 8-min session, and the series of arm entries was recorded visually. Alternation was defined as successive entry into the three arms on overlapping triplet sets. The % alternation was calculated as the ratio of actual alternations to the possible alternations (defined as the number of arm entries minus two) multiplied by 100.

2.10.4. Morris water maze test

The Morris water maze test was conducted in a circular pool (1.2 m in diameter) with a hidden platform (7 cm in diameter) filled with water at a temperature of 22 ± 1 °C. The mice were given two trials (one block) for 10 consecutive days during which the platform was left in the same position. The time and distance taken to reach to the escape platform (escape latency and distance moved) was determined in each trial by using the Etho Vision system (Brainscience Co. Ltd., Osaka, Japan). Three hours after the last training trial, the platform was removed, and mice were allowed for 60 s to search the removed platform.

2.10.5. Cued and contextual fear conditioning tests

For measuring basal levels of freezing response (preconditioning phase), mice were individually placed in a neutral cage for 1 min, and then in the conditioning cage for 2 min. For conditioning, mice were placed in the cage, and an 80 dB tone was delivered for 15 s. During the last 5 s of the tone stimulus, a foot shock of 0.6 mA was delivered as an unconditioned stimulus through a shock generator (Brainscience Idea Co. Ltd.). This procedure was repeated four times with 15-s intervals. Cued and contextual tests were carried out 1 day after fear conditioning. For the cued test, the freezing response was measured in the neutral cage for 1 min in the presence of a continuous-tone stimulus identical to the conditioned stimulus. For the contextual test, mice were placed in the conditioning cage, and the freezing response was measured for 2 min in the absence of the conditioned stimulus.

2.10.6. Statistic analysis

All results were expressed as the mean ± S.E.M. for each group. The difference among groups was analyzed with a one-way, two-way, or repeated ANOVA, followed by the Student–Newman–Keuls multiple range-test. The Student’s t-test was used to compare two sets of data.

3. Results

3.1. Antibody response in the SeV-Aβ-treated APP-tg mice

IgG antibodies to Aβ were detected in the serum of nasally treated Tg2576 mice with rSeV-Aβ at 4 weeks and less amount at 8 weeks after vaccination (Fig. 2a). However, intramuscularly treated mice showed poor antibody response (not shown). The immune sera from nasally vaccinated mice stained the senile plaque amyloid in the tissue.

3.2. Reduction of Aβ burden after nasal vaccine with rSeV-Aβ in Tg2576 mice

Nasal vaccination with rSeV-Aβ resulted in marked reduction of Aβ burden in the frontal cortex, parietal association cortex and hippocampus compared to the control (Fig. 2b and c). Thioflavin...
S-positive senile plaques were also significantly reduced in vaccinated mice. However, intramuscular injection of rSeV-Aβ had little effects on Aβ clearance (Fig. 2d and e). Quantitative analyses showed a marked reduction of Aβ deposition in nasally vaccinated mice compared to the control (Fig. 2f), but intramuscular injection showed no difference in Aβ clearance (Fig. 2g).

To investigate the expression of Aβ43 in the olfactory bulb and brain stem through trafficking of rSeV via the olfactory or trigeminal nerves, we stained the brain tissue with anti-Aβ43 antibody. Although Tg2576 mice expressed very little endogenous Aβ43, we could not find any Aβ43 deposits after the nasal administration of rSeV-Aβ (data not shown).

### 3.3. Aβ contents in brain homogenates and Western blot analysis of Aβ*56 oligomer

Soluble/insoluble Aβ40 and Aβ42 in brain homogenate fractions extracted with TBS or 2% SDS and 70% formic acid were quantified using the sandwich ELISA.

Nasal vaccination of rSeV-Aβ significantly reduced the contents of soluble and insoluble Aβ40 and Aβ42 compared to the control (Aβ40 in TBS, p = 0.04; 2% SDS, p = 0.027; formic acid, p = 0.001. Aβ42 in TBS, p = 0.008; 2% SDS, p = 0.01; formic acid, p = 0.045.) (Fig. 3A), but again intramuscular injection of rSeV-Aβ was ineffective (Aβ40 in TBS, p = 0.3; 2% SDS, p = 0.45; formic acid, p = 0.41. Aβ42 in TBS, p = 0.15; 2% SDS, p = 0.27; formic acid, p = 0.48) (Fig. 3B).

The trimeric, tetrameric, nonameric and dodecameric (Aβ*56) Aβ oligomers in soluble fraction of Tg2576 mice were detected by using Western blotting. Nasal vaccination with rSeV-Aβ in Tg2576 mice resulted in a marked reduction in the contents of Aβ*56 (dodecamer) but not in soluble sAPPα (Fig. 3C).

### 3.4. Absence of brain inflammation and synaptic restoration after nasal vaccination with rSeV-Aβ

HE staining of the brain sections of the vaccinated mice showed no lymphocyte infiltrations in the leptomeninges or brain parenchyma, and immunohistochemistry did not reveal any lymphocyte infiltrations, including CD3+, CD4+ and CD8+ T cells and CD19+ B cells (data not shown).

In the hippocampus of the control APP-tg mice, there were many Iba-1+ and CD11b+ microglia cells surrounding the senile plaques (Fig. 4A), while nasally vaccinated mice with rSeV-Aβ showed the uniform distribution of Iba-1+ CD11b+ microglia (Fig. 4B). GFAP positive cells were less frequent in mice nasally vaccinated with rSeV-Aβ.

Synaptophysin immunoreactivity was shrunken and disrupted in control mice with rSeV-LacZ. The nasally vaccinated mice with rSeV-Aβ showed the amelioration of abnormal change in synaptic densities and distribution patterns (Fig. 4C and d).
3.5. Effect of rSeV-Aβ on the body weight in Tg2576 mice

We examined the changes of body weight in Tg2576 mice treated with SeV-Aβ nasally at the age of 12 months. The body weight measured at the age of 15 months was 28.2 ± 1.4 g for rSeV-LacZ-vaccinated non-tg mice, 26.3 ± 1.1 g for rSeV-Aβ-vaccinated non-tg mice, 23.8 ± 0.9 g for rSeV-LacZ-vaccinated Tg2576 mice, and 22.6 ± 0.7 g for rSeV-Aβ-vaccinated Tg2576 mice. Results with the two-way ANOVA were significantly different in genotype ($F(1,38) = 17.08, p < 0.01$) but not vaccination ($F(1,38) = 2.24, p = 0.14$) nor interaction of genotype with vaccination ($F(1,38) = 0.16, p = 0.74$).

3.6. Effect of nasal vaccination with rSeV-Aβ on the impaired learning and memory

3.6.1. Object recognition in a novel-object recognition test

During the training session, there were no significant differences in exploratory preference between the two objects and total exploratory time among the groups (data not shown), suggesting that all groups of mice have the same levels of motivation, curiosity, and interest in exploring novel objects. For the retention session at age 12 months, the level of exploratory preference for the novel object in Tg2576 mice was significantly decreased compared to that in non-tg mice (supplemental Fig. 1).

At age 15 months, the rSeV-LacZ-vaccinated Tg2576 mice also showed a significant reduction in the exploratory preference for the novel object compared with rSeV-LacZ-vaccinated non-tg mice, however rSeV-Aβ vaccination improved the impairment of recognition memory in Tg2576 mice significantly (supplemental Fig. 1).

3.6.2. Spontaneous alternation in a Y-maze test

There was no significant difference in the number of arm entries among the groups (data not shown), suggesting that all mice have the same levels of motivation, curiosity, and motor function. At age 12 months, Tg2576 mice showed significantly reduced spontaneous alternation behavior in a Y-maze test compared with non-tg mice (Fig. 5a). At age 15 months, the rSeV-LacZ-vaccinated Tg2576
mice also showed a significant reduction in spontaneous alternation behavior compared with rSeV-LacZ-vaccinated non-tg mice, however rSeV-AB\text{\textregistered} vaccination improved alternation behavior in Tg2576 mice significantly (Fig. 5b).

3.6.3. Associative learning in the cued and contextual fear conditioning tests

In the preconditioning phase, the mice hardly showed any freezing response. There were no differences in basal levels of freezing response between the groups (data not shown). In the contextual learning test, the rSeV-LacZ- and rSeV-AB\text{\textregistered}-vaccinated non-tg mice showed a marked contextual freezing response 24 h after fear conditioning. There was no difference in freezing response between the two groups of non-tg mice. However, the rSeV-LacZ-vaccinated Tg2576 mice exhibited significantly less freezing response in the contextual tests, indicating an impairment of associative learning, while the rSeV-AB\text{\textregistered}-vaccinated Tg2576 mice were indistinguishable from the rSeV-LacZ-vaccinated non-tg mice (Fig. 5c).

In the cued learning test, there was no difference in the cued freezing response 24 h after fear conditioning among the groups. No alterations of nociceptive response were found in any of the mutant mice: there was no difference in the minimal current required to elicit flinching or jumping among the mice (Fig. 5d).

3.6.4. Reference memory in the Morris water maze test

At the age 12 months, Tg2576 mice took significantly longer time and distance to reach the platform than non-tg mice, indicating an impairment of reference memory (Fig. 6A and B). When the transfer test was carried out following the tenth training trial, Tg2576 mice showed a significant decrease in the time spent in the trained quadrant compared to non-tg mice (Fig. 6C). At age 15 months, rSeV-AB\text{\textregistered} vaccination improved all these parameters in Tg2576 mice significantly (Fig. 6D–F). The decreased ability of the rSeV-LacZ-vaccinated Tg2576 mice did not reflect a loss of swimming ability and motivation because swimming speed and distance in the transfer test were similar to those in other mice (data not shown).

4. Discussion

There are numerous approaches in active immunization therapies for AD [31]. An interesting approach to avoid autoimmune encephalitis is to avoid use of autoantigen AB\text{\textregistered}. Nasal administration of glatiramer acetate (GA) and adjuvant [32] or subcutaneous administration of GA alone [33] is reported safe and effective in Alzheimer model mice. GA is a synthetic random polymer composed of alanine, lysine, tyrosine and glutamic acid, which is now used for treatment of multiple sclerosis (MS). It has been speculated that GA activates regulatory T cells against myelin antigen-reactive auto-aggressive T cells, which in turn activate microglia, resulting in increased phagocytosis of amyloid. However, such non-specific clearance may not last for long. Further, GA must be injected everyday in MS.

Our nasal vaccine seems to be safe, easy, non-invasive and long lasting. Long term expression of recombinant protein in the mucosal epithelial cells and antigen presentation to the mucosal immune system have many advantages such as less frequent administrations and induction of continuous specific antibody production. In addition, majority of administered DNA is spontaneously eliminated in accordance with epithelial cell renewal.

SeV belonging to the Genus Respirovirus, infects and multiplies its genome copy in most mammalian cells, and expresses high levels of the transgene. Its replication is independent of nuclear functions and does not have a DNA phase during its life cycle, so it does not need to be concerned about the transformation of cells by integration of vector materials into the host chromosomes. These properties are very promising devices for gene therapy of new age (Cytoplastic Gene Therapy) because of its genotoxicity-free nature. Further, it is non-pathogenic for humans.
Since Sendai virus is a murine parainfluenza virus (PIV) with certain homologies to human PIV, it was tested as xenotropic vaccine in African Green monkeys and humans without any significant adverse reactions [34,35]. Recombinant SeV vector carrying human PIV was also tested in rats [36,37]. Further, recombinant SeV vaccine for human immunodeficiency virus (HIV) infection is going to be tested in humans (http://www.dnavec.co.jp/en/index.html). Thus, safety of Sendai virus vector is gradually established.

We inserted mouse IL-10 cDNA to construct rSeV-AB with the aim of helping antibodies productions and suppressing Th1 type T cell activations. Nasal administration of rSeV-AB without IL-10 had less effect to remove Aβ deposits (data not shown).

Recently, soluble Aβ oligomers, but not fibrils nor monomers, have been considered responsible for cognitive dysfunction prior to the formation of Aβ plaques [22], and Aβ3-56, a 56-kDa soluble Aβ dodecamer was found responsible in Tg2576 mice [29]. Our nasal vaccine efficiently reduced not only senile plaque amyloid but also the contents of Aβ3-56 oligomer without changing sAPPα and improved cognitive dysfunction in water maze, Y-maze and contextual fear test which could evaluate hippocampus-related cognition. Thus, our vaccine, if applicable, can be given at the stage of mild cognitive impairment or earlier.

Aβ is released from presynaptic sites and deposited in extracellular plaques [38], and APP and synaptophysin are co-localized at the growth cones of developing neurons in culture [39]. These reports have indicated that Aβ deposition plays an important role in degeneration of presynaptic structures. In addition, it is reported that Aβ oligomers directly disrupt synaptic structures [40]. In our study, synaptophysin staining showed amelioration of presynaptic degeneration following our nasal vaccine at 24 months old, suggesting prevention of synaptic degeneration or repair of synaptic structures after removal of Aβ deposits including Aβ oligomers.

Fig. 5. Effect of nasal vaccination with rSeV-AB on Y-maze test, and cued and contextual fear conditioning tests. (a) Percent alternation during an 8-min session in the Y-maze tested in 12-month-old mice. Values indicate the mean ± S.E.M. *p < 0.05 vs non-transgenic mice (Student’s t-test). (b) Y-maze test done 3 months after vaccination. Values indicate the mean ± S.E.M. Results with the two-way ANOVA were: genotype; F[1,38] = 7.17, p < 0.05, vaccination; F[1,38] = 2.40, p = 0.13, interaction of genotype with vaccination; F[1,38] = 4.53, p < 0.05, *p < 0.01 vs rSeV-LacZ-vaccinated non-Tg mice. *p < 0.05 vs rSeV-LacZ-vaccinated Tg2576 mice. N, non-Tg mice; TG, Tg2576 mice; C, rSeV-LacZ-treated mice; V, rSeV-AB-treated mice. (c and d) Effect of oral vaccination with rSeV-AB/mil-10 on associative learning in a conditioned-fear learning test. The retention session was carried out 24 h after the training. Vaccine was given at 12 months and context-dependent (c) and tone-dependent (d) freezing time was measured at 15 months after the birth. Values indicate the mean ± S.E.M. Results with the two-way ANOVA were: context-dependent test: genotype; F[1,38] = 1.06, p = 0.31, vaccination; F[1,38] = 1.17, p = 0.29, interaction of genotype with vaccination; F[1,38] = 4.33, p < 0.05, cued-dependent test: genotype; F[1,38] = 1.25, p = 0.27, vaccination; F[1,38] = 0.78, p = 0.38, interaction of genotype with vaccination; F[1,38] = 1.44, p = 0.24. *p < 0.05 vs rSeV-LacZ-vaccinated non-transgenic mice. *p < 0.05 vs rSeV-LacZ-vaccinated Tg2576 mice. C, rSeV-LacZ vaccination; V, rSeV-AB/mil-10 vaccination; TG, Tg2576 mice; N, non-transgenic mice.
**Fig. 6.** Effect of nasal vaccination with rSeV-Aβ on reference memory in Morris water maze test. Morris water maze test was done at the age of 12 months (A–C) and was repeated 3 months after vaccination (D–F). All values indicate the mean and S.E.M. (A) Escape latency (Esc. Lat.) measured during a 60-s session in the water maze test. Results with the repeated ANOVA were: trial: F(9,360) = 9.24, p < 0.01, animal group: F(1,40) = 5.98, p < 0.05, interaction of trial with animal group: F(9,369) = 0.86, p = 0.56, **p < 0.01 vs non-tg mice. (B) Distance moved (Dist. Mov.) measured during a 60-s session in the water maze test. Results with the repeated ANOVA were: trial: F(9,360) = 18.27, p < 0.01, animal group: F(1,40) = 24.97, p < 0.01, interaction of trial with animal group: F(9,360) = 1.92, p < 0.05, **p < 0.01 vs non-transgenic mice. (C) Spatial memory during the transfer test after the training. Percent search time of a platform location during a 60-s session in the water maze test was measured. *p < 0.05 vs non-tg mice (Student’s t-test). (D) Same test as (A). Results with the repeated ANOVA were: trial: F(9,360) = 18.27, p < 0.01, animal group: F(3,38) = 3.56, p < 0.05, interaction of trial with animal group: F(27,342) = 0.83, p = 0.71, **p < 0.01 vs rSeV-LacZ-vaccinated non-tg mice. **p < 0.01 vs rSeV-LacZ-vaccinated Tg2576 mice. (E) Same test as (B). Results with the repeated ANOVA were: trial: F(9,360) = 16.16, p < 0.01, animal group: F(3,38) = 9.45, p < 0.01, interaction of trial with animal group: F(27,342) = 1.00, p = 0.46, **p < 0.01 vs rSeV-LacZ-vaccinated non-tg mice. **p < 0.01 vs rSeV-LacZ-vaccinated Tg2576 mice. (F) Same test as (C). Results with the two-way ANOVA were: genotype: F(1,38) = 6.56, p < 0.05, vaccination: F(1,38) = 0.48, p = 0.49, interaction of genotype with vaccination: F(1,38) = 1.37, p = 0.25, *p < 0.05 vs rSeV-LacZ-vaccinated, non-transgenic mice.
Our next plan is to see whether Tg2576 mice show improvement of cognitive functions by eliminating senile plaque amyloid even at 24 months old.

5. Conclusion

In conclusion, a new vaccine using Sendai virus vector with Ab3 and IL-10 cDNA was developed. A nasal administration of this vaccine reduced amyloid burden including Ab3 oligomers significantly in AD mice and improved cognitive functions without causing side effects such as brain inflammation. This vaccine can be used to treat and prevent Alzheimer disease.

Acknowledgements

Authors are grateful to Dr. Y. Noda at Meijo University, Dr. T. Nagai at Nagoya University, and Dr. M. Inoue at DNAVEC Co. for their kind help in this study. This study was supported in part by a grant (NIBIO 05–27) and by Health and Labor Sci. Res. Grant, Regulator Sci. Pharmaceut. Med. Devices from the Ministry of Health, Labor and Welfare, Japan; Acad. Front. Project for Private Univ. (2007–2011) from the Ministry of Education, Culture, Sports, Science and Technology of Japan; Internat. Res. Project, The Meijo Asian Res. Center; Grant-in-aid for Explor. Res.; Grant-in-Aid for Scientific Res. (B); Grant-in-Aid on Priority Areas, and Grant from INSERM-JSPS Joint Res. Project, JSPS.

Appendix A. Supplementary data


References