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Disease Modifying Strategies for the Treatment of Alzheimer's Disease: A Newer Concept of AD Vaccine

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Alzheimer's disease (AD) is characterized by progressive loss of cognitive function due to β amyloid deposits in the central nervous system[1]. Immunization of amyloid precursor protein (APP)-transgenic mice with synthetic Aβ in complete and subsequently incomplete Freund's adjuvant showed a marked reduction of amyloid burden in the brain[2]. Repetitive passive transfer of Aβ antibodies[3] was also effective for reducing the amyloid deposits. Although Aβ is not an infectious agent, this treatment is now widely accepted as "vaccination" from its analogy in mechanism. Since vaccinated mice showed an improvement of memory loss in mice[4, 5], clinical trials were performed in humans in the States and Europe. The phase II trial of AN-1792 vaccine composed of synthetic Aβ1-42 and adjuvant QS21 was halted because of complication, subacute meningoencephalitis which appeared in 6% of patients[6]. However autopsy cases with or without the complication suggested effective clearance of β amyloid by vaccination[7-9], and patients who produced antibodies against senile plaque amyloid showed better cognitive functions or less cognitive decline than those who did not produce such antibodies[10, 11]. Therefore, Aβ vaccination seems to be a promising way to delay the onset or to slow progression of AD, if the complication is minimized. We have developed an oral Aβ vaccine using the recombinant adeno-associated virus vector (AAV), which successfully reduced amyloid burden in Tg2576 APP transgenic mice without any complications[12].

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Production of AAV/Aβ vaccine

The AAV vector carrying human Aβ1-43 or Aβ1-21 was constructed using plasmid DNA pTRUF2 and the secreted form of Aβ was made by linking the APP signal sequence (SS) to the Aβ sequence (Fig. 1). Human embryonic kidney (HEK) 293 cells were co-transfected with SS-Aβ pTRUF2 and plasmid pXX2 and pXX6 as described[13].

To confirm the secretion of Aβ, we transfected HEK293 cells with the SS-Aβ1-43pTRUF2 expression vector. An immunoprecipitation and western blot method revealed Aβ monomer in the cell lysate and Aβ oligomers in the conditioned medium. When AAV/Aβ was given in mice, Aβ expression was observed primarily in the lamina propria of the upper part of the small intestine. There was no increase of Aβ1-43 or Aβ1-21 in the serum. Transduction of AAV was confirmed by PCR in intestinal cells, but not in the liver, spleen, heart, lung and kidney 4, 11 and 21 weeks after treatment, suggesting an absence of widespread infection of the virus vector.

Vaccination and tissue examinations

AAV/Aβ43 or AAV/Aβ21 was diluted with PBS, and 5×10^{11} genome in a final volume of 0.1 mL was administered once to Tg2576 mice (Taconic) using an orogastric tube in the treated group; control mice received once 0.1 mL of PBS. Since immunization with vector alone did not show any significant difference in our preliminary study, we used PBS as control. Mice were randomized to four groups; a group treated at age 15 weeks; a group treated at age 30 weeks; a group treated at age 45 weeks; and control groups treated with PBS at each age. Each group consisted of 4-6 mice. At

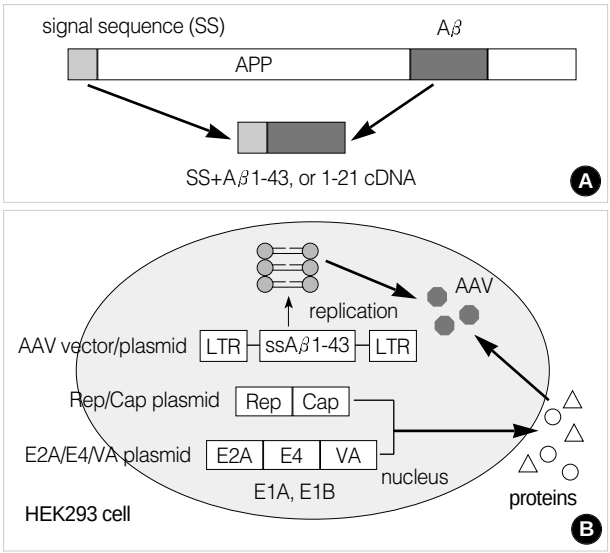

Fig. 1. cDNA construct of Aβ vaccine and its production. (A) To make a secreted form of Aβ, a signal sequence of APP is ligated to Aβ1-43 or 1-21 cDNA. (B) AAV vaccine carrying Aβ cDNA was produced in HEK293 cells transfected with 3 plasmids shown in the figure.

the age of 56 weeks all mice were anesthetized with Nembutal, and their brains were fixed in 4% paraformaldehyde with 0.1 M phosphate buffer, pH 7.6 for immunohistochemical analysis.

Oral vaccination with AAV/Aβ43 or AAV/Aβ21 resulted in marked reduction of Aβ deposition in all treated groups compared to the control examined at age 56 weeks (Fig. 2). Quantitative image analyses in three different regions of the brain showed a significant decrease of Aβ burden in all vaccinated mice compared to control mice (Fig. 3).

HE staining of the brain sections of the treated mice showed no lymphocytic infiltration in either leptomeninges or cerebral white matter, and there was no hemorrhagic lesion in the brain. Immunohistochemical studies did not reveal any cellular infiltration positive for CD3, CD4, CD86, CD19, and CD11b in brain sections. Iba-1$^+$ activated microglia were more numerous in vaccinated mice, and some microglia cells containing phagocytosed Aβ were observed. In contrast, GFAP$^+$ cells were less frequent in vaccinated mice.

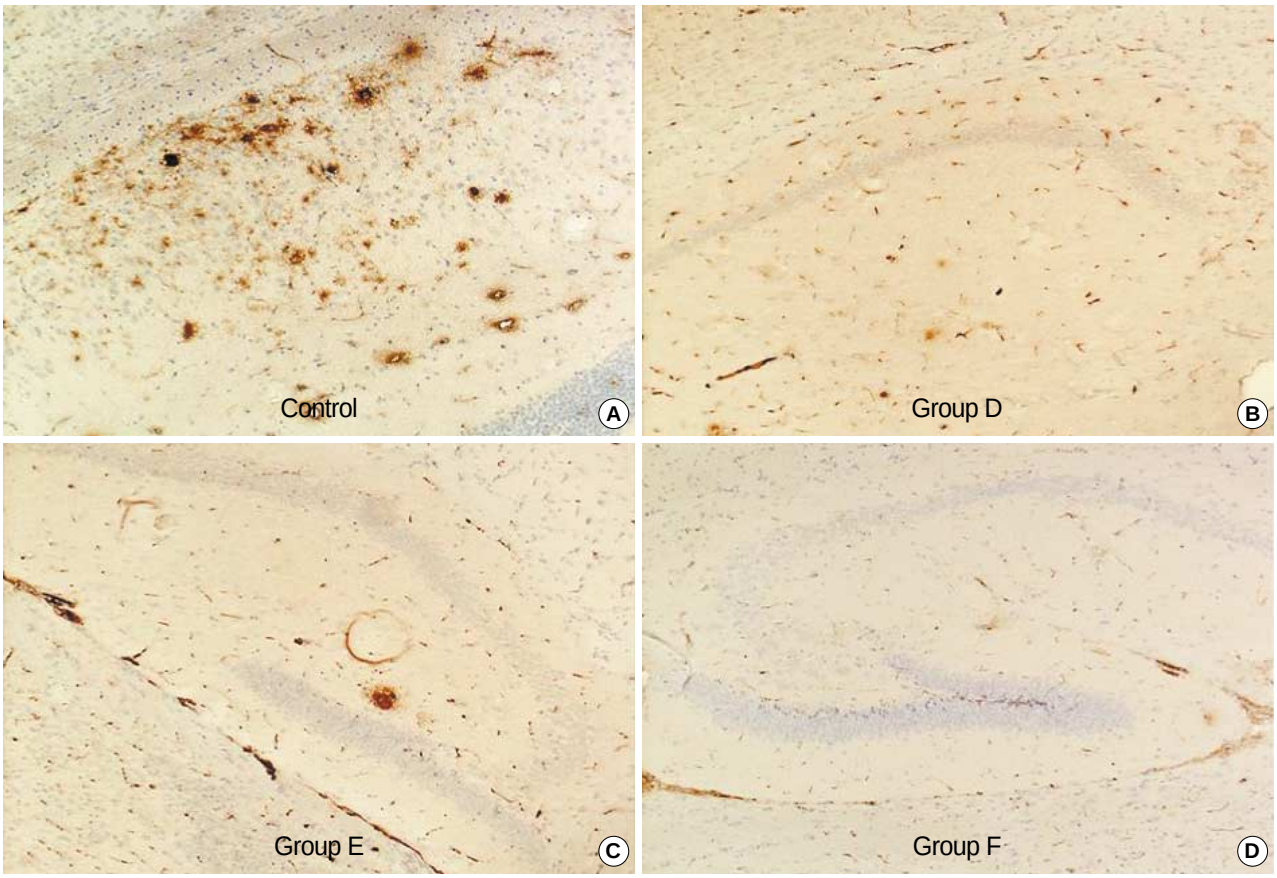

Fig. 2. Aβ burden in the brain of control and vaccinated mice. Aβ burden was significantly reduced in mice received AAV/Aβ vaccine at 15 weeks (B), 30 weeks (C) and 45 weeks (D) old than control (A). A disrupted plaque showing a group of activated microglia positive for Aβ staining is seen in C.

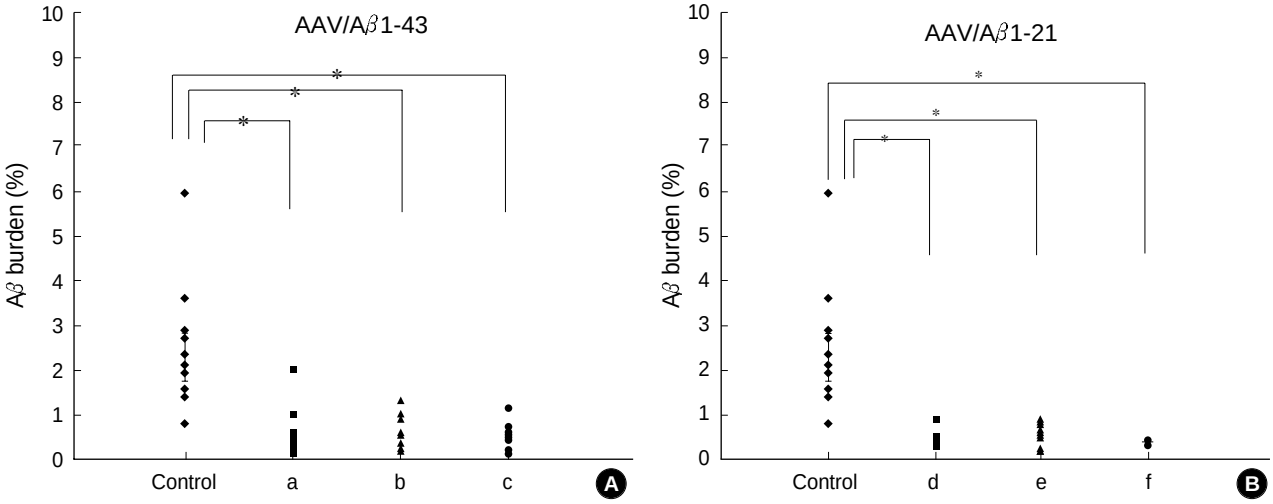

Fig. 3. Quantitative analysis of Aβ burden in the brain of control and vaccinated mice. Quantitative analyses show significant reduction of Aβ burden in vaccinated mice with both Aβ1-43 (A) and Aβ1-21 vaccine (B). Reduction of Aβ burden in mice treated with AAV/Aβ vaccine. Vaccine was given at 15 weeks (a, d), 30 weeks (b, e) and 45 weeks (c, f) of age and Aβ burden was examined at 56 weeks of age.

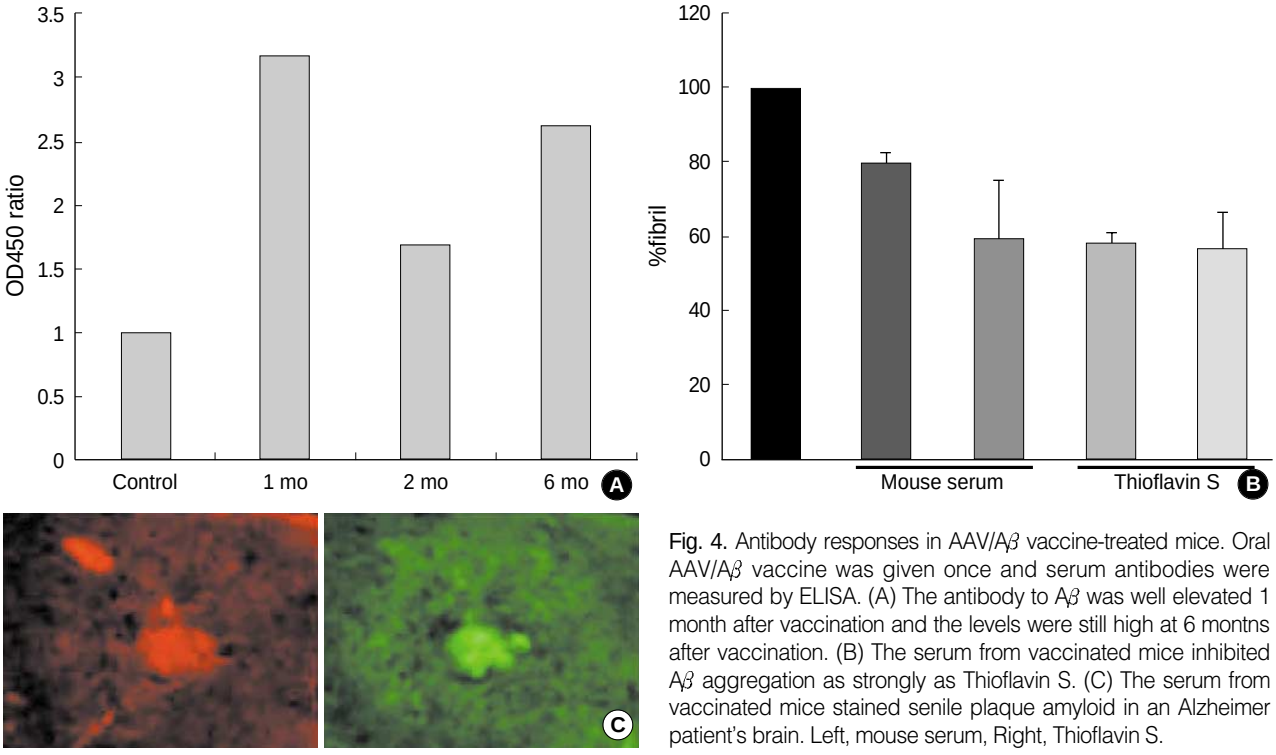

Fig. 4. Antibody responses in AAV/Aβ vaccine-treated mice. Oral AAV/Aβ vaccine was given once and serum antibodies were measured by ELISA. (A) The antibody to Aβ was well elevated 1 month after vaccination and the levels were still high at 6 montns after vaccination. (B) The serum from vaccinated mice inhibited Aβ aggregation as strongly as Thioflavin S. (C) The serum from vaccinated mice stained senile plaque amyloid in an Alzheimer patient's brain. Left, mouse serum, Right, Thioflavin S.

Immune responses to Aβ

In the treated Tg2576 mice, IgG antibodies were detected in the serum at 4 weeks, and kept elevated for more than 6 months (Fig. 4A). The antibody isotypes were mainly IgG1 and to a lesser amount IgG2b, but IgG2a was not detected and IgA was low. The immune sera from vaccinated mice stained the amyloid plaques in the brain (Fig. 4C). The proliferative response of spleen T cells against Aβ peptide was not detected in the vaccinated mice as well as in control mice.

Aβ vaccine-mediated meningoencephalitis

The exact mechanism of meningoencephalitis in patients who received AN-1792 vaccine is not known yet. The autopsied brain showed cellular infiltrates composed manly of

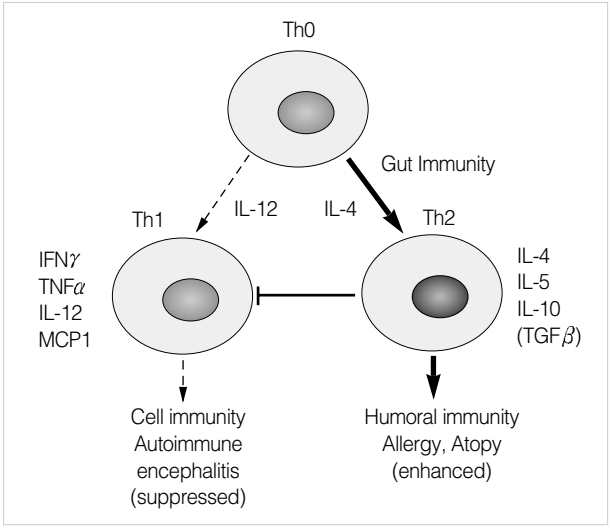

Fig. 5. Gut immune system. The gut immune system is strongly shifted to Th2, which helps humoral immune response and suppresses Th1 response.

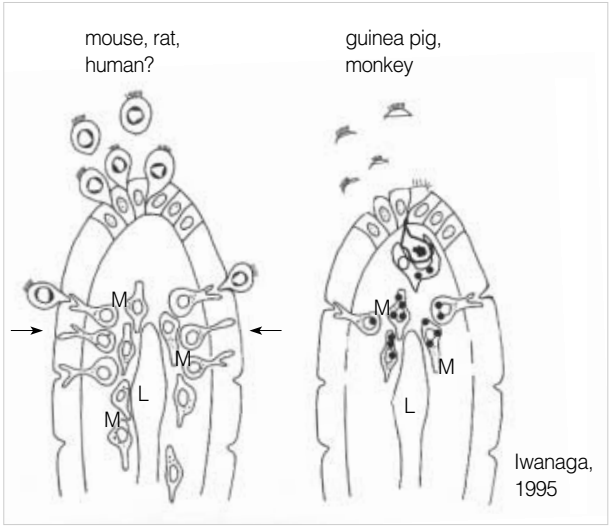

Fig. 6. Renewal of gut epithelium. Gut epithelial cells are renewed in a few days by exfoliations in mice, rats and humans, while they are engulfed by macrophages in guinea pigs and monkeys. Illustration was made from Iwanaga, 1995.

$CD4^+$ T cells and $CD8^+$ T cells. The MRI findings were similar to post-vaccinal encephalomyelitis, although gray matter lesions were more pronounced in AN-1792-related meningoencephalitis. It has been reported that there exist Aβ-reactive T cells in the human peripheral blood, and the frequencies are higher in the elderly[14]. Thus, it is highly probable that the AN-1792-related meningoencephalitis is autoimmune encephalitis, probably due to Th1 immune responses to Aβ. If this is the case, it may be possible to induce similar conditions experimentally in animals. Although there is a report showing experimental meningoencephalitis in B6 mice immunized with Aβ[15], we and others could not confirm their observation. However, Monsonego *et al*. could induce encephalitis in APP mice crossed with interferon-γ transgenic mice which have augmented Th1 immune responses[16]. Thus, it is reasonable to think that the meningoencephalitis is mediated by autoimmune Th1 T cells reactive to Aβ.

Gut immune system and advantage of AAV/Aβ vaccine

It is well known that the gut immune system is strongly shifted to Th2 (Fig. 5). There are two types of T helper cells, Th1 and Th2. Th1 T cells mainly help cellular immune responses and suppress Th2 cells. It is known that effector T cells for autoimmune encephalomyelitis are Th1 type. On the other hand, Th2 T cells mainly help humoral immune responses and suppress Th1 cells. Thus, the use of the gut immune system has a big advantage to induce autibodies and suppress adverse T cell immune responses. Since anti-Aβ antibodies were continuously elevated for more than six months in mice which received our oral Aβ vaccine, it might be sufficient for patients to take the oral vaccine once or twice a year. In addition, AAV was detected only in the gut without spreading over in other organs including germ cells. Adeno-associated viral DNA normally does not integrate into the cellular genome, instead it remains in the episomal region. Moreover, since the turnover of epithelial cells of the GI tract is relatively quick, the recombinant AAV is eliminated along with the course of renewal of the epithelial cells, suggesting a lower risk in case of an unexpected event. It is interesting to know that the majority of epithelial cells of the murine and probably human gut is exfoliated into the gut lumen, while those of the guinea pig and monkey gut are engulfed by macrophages in the lamina propria[17] (Fig. 6). Thus, majority of Aβ cDNA in the epithelial cells seems to be deleted along with exfoliation of gut epithelial cells in humans.

The mechanism of Aβ vaccine

The mechanism by which Aβ vaccine clears β amyloid from the brain tissue is still unknown. There are several hypotheses. First, Fc receptor-mediated uptake of Aβ-antibody complexes by activated microglia[4]. Second, antibody-mediated disaggregation of amyloid fibrils[18]. Several reports

indicated therapeutically active antibodies mainly recognize the residue 4-10 of Aβ peptide and these antibodies inhibit Aβ fibrillogenesis and cytotoxicity[19-21]. Third, DeMattos *et al*.[22] hypothesized that injected antibodies sequestrate Aβ from the peripheral blood and eventually pull Aβ out of the brain. In our vaccinated mice, some activated microglia contained Aβ, and sera from vaccinated mice showed inhibition of Aβ aggregation (Fig. 4B). Thus, all three mechanisms seem to be likely in our vaccine.

In conclusion, this oral Aβ vaccine seems to be safe and beneficial for AD. Currently we are testing this vaccine in monkeys.

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