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Review article

New vaccine therapy for Alzheimer's disease.

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Abstract

One of the main hallmarks of Alzheimer's disease is the deposition of neuritic plaques in the brain. Preventive measures for Alzheimer's disease have been explored; the first-line therapy would be to prevent the accumulation of amyloid β protein (Aβ), which is the main component of senile plaques. This is because all three of the causative genes of early onset Alzheimer's disease are involved in the pathway of Aβ formation. Accumulation occurs long before nerve cell death, as is known. Therefore, vaccine therapy is expected to be the ideal measure to prevent and treat Alzheimer's disease. This review precisely reports, explaining differences in adverse effects between oral and subcutaneous administration, that there is a new edible vaccine, which employed plant-expressed amyloid β as an antigen and successfully achieved a reduction of senile plaques.

KEY WORDS: Alzheimer's Disease, oral vaccine therapy, amyloid β protein, gene expression in plant

Introduction

There are currently no complete therapeutics for Alzheimer's disease (AD), which is characterized by neuronal cell death caused by the accumulation of amyloid β protein (Aβ). Among well-known AD risks, major factors are life-style diseases such as diabetes mellitus and hypertension. Therapeutic agents for these diseases have been reported to have a large number of findings for the prevention of AD. Diverse symptomatic therapies for AD are recognized. However, dead brain cells are rarely regenerated, so symptoms cannot be improved as long as neurogenesis does not occur. Even an agent that is the most effective at delaying the aggravation of symptoms is not a causal treatment to eliminate the root of the disease [1].

A new vision for AD currently places emphasis on prevention rather than treatment. Vaccine therapy is gaining significant attention to prevent the accumulation of Aβ. Aducanumab, which is an antibody drug, is being developed and is now in clinical testing. The interim report of the clinical trial announced that Aβ deposition was reversibly inhibited [2]. We have explored active immunotherapy to prevent Aβ deposits within the brain, employing crop-expressed amyloid β and examining the effects of oral vaccines. We have succeeded in the reduction of Aβ in the brain through oral immunization in Tg2576 mouse model bearing familial AD, where Capsicum annuum var. angulosum [3, 4] and rice [5-7] expressing Aβ were orally administered to mice. We report these trials as well as outcomes of an experiment in medicinal herb expressing Aβ.

Alzheimer's disease and vaccine therapy

Alzheimer's disease (AD), which is characterized by decreased cognitive functions, is a neurodegenerative disease that is caused by the deposit of senile plaques and the gradual loss of nerve cells. Neurogenesis is infrequent in humans once nerve cells are dead, so the irreversible symptoms severely and drastically accumulate. Consequently, it is difficult to preserve human dignity. The increase in the proportion of elderly persons aged 65 or older is expected to

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exceed 40% of the population in Japan by 2050. Therefore, the aging population will inevitably raise the costs of health maintenance and nursing care. Under these circumstances, citizens are encouraged to extend healthy life expectancy through the improvement of life style such as eating a healthy diet and regular exercise [1].

Regarding the attempt to develop agents inhibiting Aβ deposition, there have not yet been any reports of agents with an outstanding effect or a complete response. As a new approach, vaccine therapy against AD is regarded as an ideal preventive measure, which is efficient and cost-effective. The problem is that costs tend to be very high for antibody drugs. To increase antibody titers safely and at a low cost, an oral vaccine is considered to be the best choice [8]. However, it is challenging to increase the quantity of antibody titers for oral vaccines, and it is necessary to utilize an oral adjuvant as a simultaneous administration for the increase of antibody titers in animal testing. Here, in this review, we report the current state of edible vaccines on which we are conducting an investigation, as well as a newly developed method to increase antibody titers without the use of oral adjuvant.

Molecular mechanisms underlying Alzheimer's disease onset

The difference between Alzheimer's disease (AD) and dementia is shown in ***Fig. 1***. AD is a neurodegenerative disease, which accounts for a major part of dementia, and is defined as the presence of cerebral atrophy, neuritic plaques (main component: Aβ) and neurofibrillary tangles (main component: phosphorylated tau protein) in the brain. Early onset AD has genetic mutations in the pathway of the formation of Aβ, which is the main component of neuritic plaques. Mutations exist in genes of either presenilin 1, presenilin 2 or amyloid precursor protein. Presenilin 1 and presenilin 2 are subunits of enzyme γ secretase producing Aβ, and amyloid precursor is its substrate. Contrarily, mutations in the pathway of tau phosphorylation for neurofibrillary tangles also lead to cognitive impairment.

As for a gene responsible for late-onset Alzheimer disease in older people around the age of 65, apolipoprotein E (ApoE) was identified in the early 1980s. ApoE exists as three isoforms. Among them, the E4 version of the ApoE gene increases a risk for developing late-onset Alzheimer disease. Even having one E4 gene could lead to dementia. It was found that ApoE4 homozygote is 11.6 times as likely to develop AD in comparison with E3 homozygote. However, this does not mean that E4 always results in the onset of AD; E4 is a factor of being at risk to develop AD. ***Figure 2*** shows the genetic risk of AD, including genes which are unlikely to result in AD.

History of Alzheimer's vaccine

There are two vaccine types of immunization: active and passive immunity. Active immunotherapy provides antigen and passive immunotherapy provides antibody in itself. Passive immunity has already been performed for cancer treatments as a humanized antibody or humanized monoclonal antibody with side-effect management. Passive immunity is short-lived, in general, requiring monthly administration in many cases. In addition, passive immunity has more side effects to the body, such as nausea, thrombus, haemorrhagia and damage to organs. Additionally, their high cost is a problem. Aside from this, rather than an antibody, transplantation of antigen-specific T cells could be a countermeasure, but this cannot be applied as a preventive measure for people who do not have a disease.

From this point of view, the active immunity vaccine therapy is necessary for treatment of chronic diseases; this therapy is financially advantageous. A large number of people can receive benefits due to its safety and affordability. In addition, this could contribute to the prevention of diseases.

Among a large number of human clinical trials for AD that have been performed so far, almost all trials were prematurely terminated. Although several trials are now being conducted, they have not achieved sufficient results (some are now in phase 3 clinical testing). For this reason, several problems are indicated below.

The first problem is adjuvant. The first trial, a clinical trial of AN1792 regarding Aβ42 oligomer as an antigen, employed purified saponin QS21 as an adjuvant. Saponin QS21 triggered severe inflammatory reactions, which resulted in meningoencephalitis of 6% in 300 subjects. In addition, surface active agent sorbic acid, which was used for the elevation of adjuvant effects, was also a problem, as was pointed out.

Secondly, the antigen was a problem. Almost all clinical trials chose an Aβ N-terminal as epitope; B cell epitope for Aβ oligomer exists in Aβ N-terminal 1-14. However, this resulted in the expression of a T helper-dominated inflammatory response or the presence of microbleeds. Consequently, the trials were prematurely terminated or suspended.

The third problem was animal models. Almost all animal experiments used transgenic mice which showed a remarkable accumulation of Aβ. Many researchers have the opinion that animal models which have a gradual accumulation of Aβ along with longevity must be observed before moving to human clinical trials with confirmation of its effectiveness.

Other than these factors, the fact that older persons are unlikely to produce antibodies against the antigen is one of the reasons that trial success cannot be achieved. Generally, simultaneous performance of direct immunization and DNA vaccine is favorable, but this type of clinical trial has never been conducted. Furthermore, the concomitant use of Aβ and tau vaccine is not being conducted at present.

Outcomes of injection immunity and antibody administration therapies indicated a direct proportion between antibody titer and therapeutic effect. However, elevation of antibody titer inescapably requires an increase of T cell response, which is like a double-edged sword. Re-examining the administration methods is necessary to solve this problem.

Primary reason for failures and improvement plan for Alzheimer' vaccine

It has been said that the first Alzheimer' vaccine trial, AN1792, was a failure due to the adverse effect of

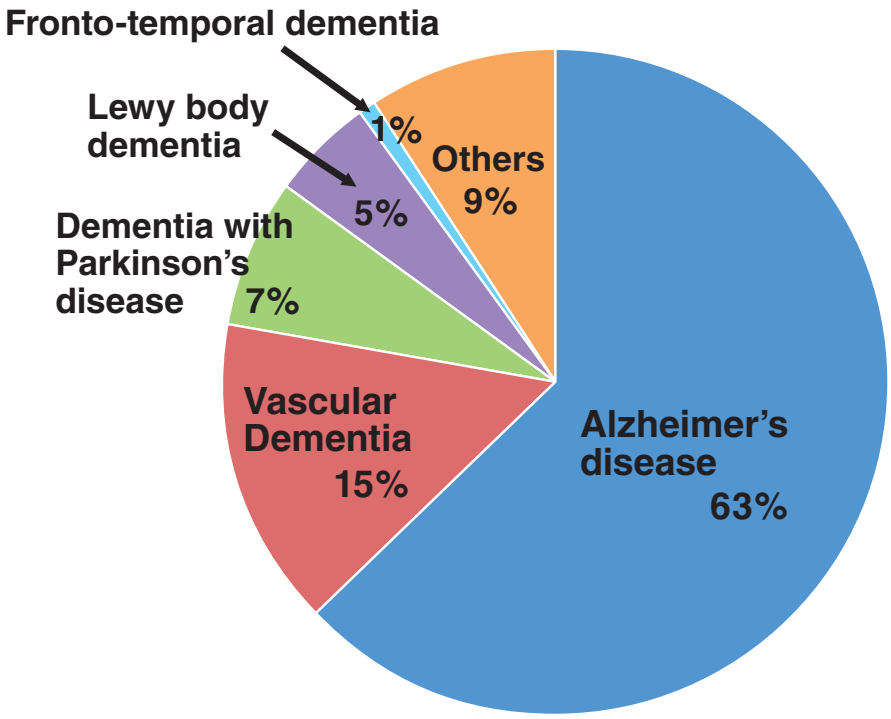


Fig. 1. **Alzheimer's disease and dementia.**

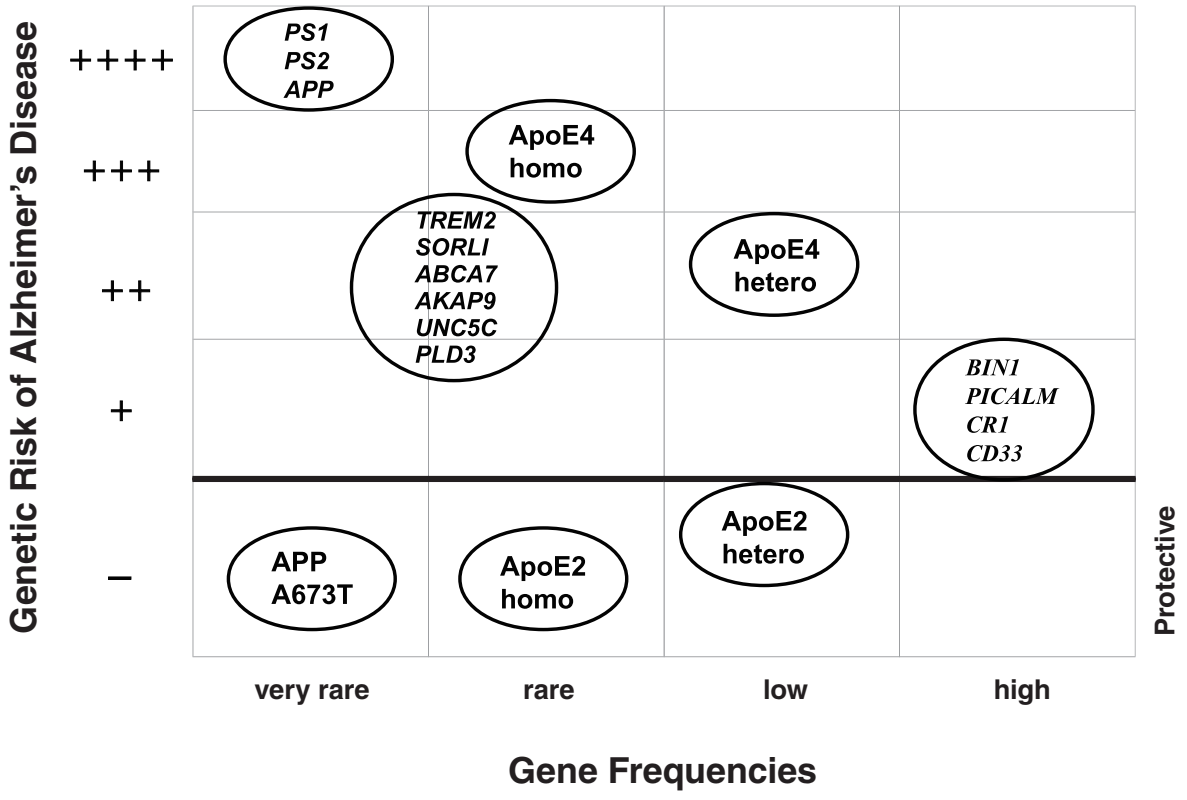


Fig. 2. **Genetic mutation of Alzheimer's disease.**

meningoencephalitis. However, the findings indicated that the decrease in cognitive function was reduced in subjects who had increased antibody titers. Therefore, this vaccine has a potential to be an effective treatment, if its adverse effects are mitigated [9]. Furthermore, there was a fundamental problem there. The ability to identify who would develop dementia in humans is unreliable. It is clear that vaccine administration must start in the stage before the loss of neurons occurs. Using conventional methods, focusing on people who have ApoE4 homozygous genes is a way to help the greatest number of people, as they have greater risk for the onset of dementia. However, this approach is not convincing; due to individual differences, reliable examinations cannot be performed on vaccine efficacy. Therefore, a new proposal is considered. Measuring Aβ accumulation in the brain plays the role of a biomarker, using amyloid positron emission tomography (PET). Amyloid PET here is a neuroimaging tool which measures the quantity of the Aβ accumulation in the brain using positron emission tomography. The inspection method is performed as follows; radiolabeling PIB, which is a chemical compound that binds to senile plaques, administering an intravenous injection and then taking tomographic images using PET. This inspection enables researchers to quantify the Aβ accumulation in the brain.

To conduct a clinical trial, it is necessary to examine both the quantity of Aβ in the brain and cognitive function. Further investigation must pursue the development of an antigen that is efficient for production of antibodies, to identify a condition where soluble Aβ oligomer, which is harmful, must not become solubilized from senile plaques, and to induce anti-inflammatory Th2 response instead of inflammatory Th1 response (the adjuvants must be thoroughly examined). The key to success is to create a preventive vaccine therapy to inhibit Aβ accumulation prior to deposition, not targeting insoluble Aβ, which have been already deposited. It is reported that a candidate antigen would be a fusion protein of Aβ and an antigen of influenza or others. The reason is that antibody could be easily produced against pieces of Aβ, which have B cell epitope, when fusion proteins are produced; surface antigen of hepatitis B, tetanus toxin and diphtheria toxin, which have an experience of immunity, have T cell epitope.

Approach of edible oral vaccine

We started vaccine design and development with a novel and promising therapeutic approach around the year 2000, when human clinical trials started to be performed for an AD vaccine. Our new therapy was an oral vaccine. Accordingly, it was suggested that edible vaccines, unlike injection, controlled inflammatory Th 1 response almost completely [4, 7].

We employed two types of rice-based vaccine: one was rice expressing GFP-Aβ42 (in this case, commercially available cholera toxin subunit B was used as an adjuvant) and the other was an adjuvant-free rice with tandem connection between cholera toxin B sub-unit and Aβ42, which was produced by Dr. Fumio Takaiwa from the National Institute of Agrobiological Sciences [10, 11]. In this review, we primarily describe the former.

Aβ42 was sufficiently expressed in rice (8 μg per grain). Tg2576 transgenic mice were administered four times with 10 μg every other week. Three weeks after the final administration, 0.5 μg of Aβ42 with incomplete Freund's adjuvant was administered by booster injection. We performed two types of administration for comparative study: one was oral administration (in this case, cholera toxin subunit B was used as an oral adjuvant) and the other was subcutaneous administration (in this case, incomplete Freund's adjuvant was used.)

Furthermore, to induce immune tolerance against rice protein, a pregnant mouse was fed rice and an immune tolerance was induced via breast-feeding in infant mice. Later on, the progeny of the lactating mother mice were administered with Aβ42 in an experiment. It would be far from acceptable if humans with an antibody against rice protein should develop food allergy. The results of the investigation indicated that mice which were not induced to immune tolerance showed an increase in the antibody titer against protein with molecular weight of 15-19kDa (unidentified but glutenin basic subunit or prolamine), while the prepared mice produced no antibody against the protein. It was concluded that antibody against protein was not produced and immune tolerance was induced in the prepared mice (***Fig. 3***) [6]. However, behavioral testing did not show significant effects in a Y-maze test, while differences were shown in spontaneous motor activity. In Tg2576 mice that had Aβ42 accumulation in their brain, spontaneous motor activity increased, which was in direct proportion to the quantity of Aβ42. The present experiment of oral administration indicated a decrease in quantity of Aβ42 and a decrease in spontaneous motor activity. No difference was detected in the arm with hypodermic injection.

The investigation showed another important finding; in comparison between oral and subcutaneous administration, there were distinct differences in T cell response. ***Figure 4*** showed outcomes of the *Capsicum annuum* var. angulosum vaccine, which was performed prior to the rice vaccine (Aβ42-GFP was expressed in a leaf of *Capsicum annuum* var. angulosum and the vaccine was administered both orally and subcutaneously). The effectiveness of oral administration was clearly presented. Oral administration inhibited Th1 response for the most part and Th2 response was predominant, while subcutaneous administration showed strong Th1 response [4]. Thinking logically, it would be an annoyance if an antibody against food is produced causing an inflammatory reaction. It was concluded that an antibody was gradually produced via oral administration, which led to successful outcomes.

In the construct that was connected in tandem between cholera toxin B subunit and Aβ42, which was provided by Dr. Fumio Takaiwa, Aβ protein was expressed in protein body. By using this rice, Aβ was not degraded by pepsin in the gagster and reached intestinum; an antibody was produced by gut immunity. This rice also showed, as well as in the experiment of Senescence-Accelerated Mouse-Prone 8 (SAMP8), that effectiveness of Aβ42 reduction was confirmed. SAMP8 were administered with this vaccine every other week for ten months, starting at three months of age, which confirmed the increase in Aβ42 antibody titer and the decrease in the quantity of soluble Aβ42 in the brain [11].

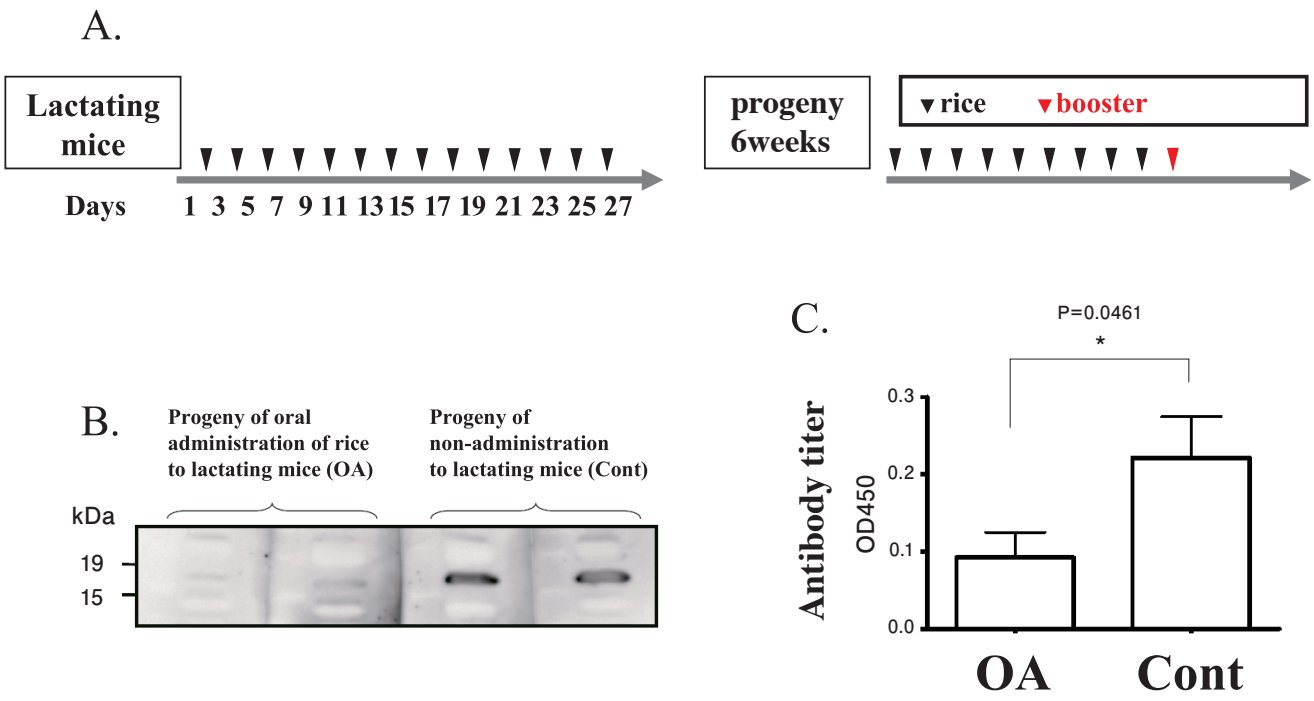
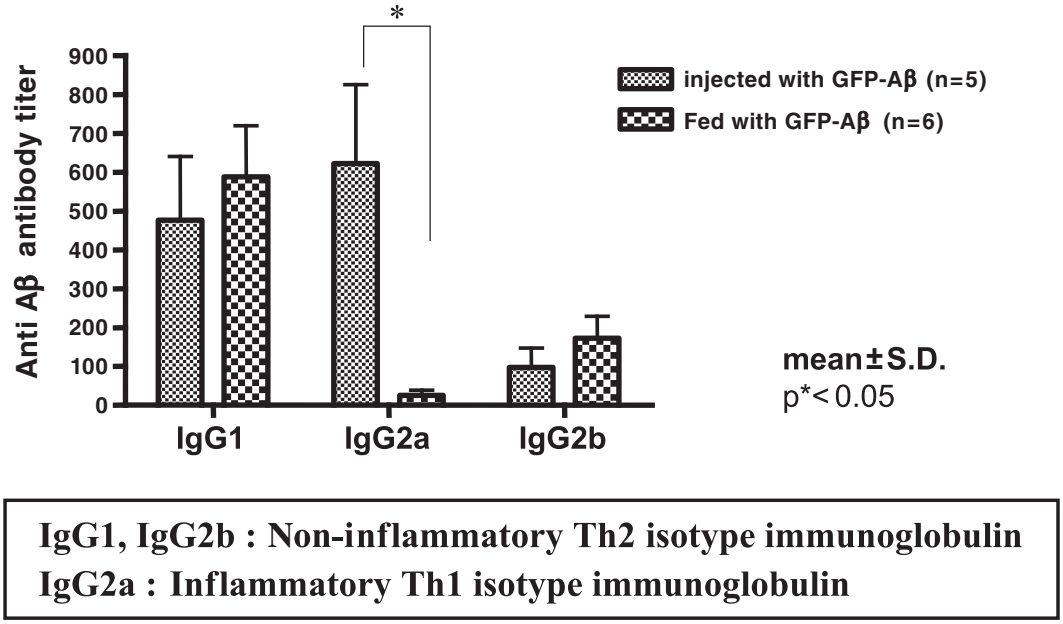


Fig. 3. **Production of anti-rice storage protein antibodies in mice.**

A. Experimental protocol: lactating mothers were orally administered wild rice (0.2 g/day) from delivery until weaning.

B.Westernblot of rice storage protein. **C.** Amount of anti-bodyagainst rice whole protein. OA, oral administration of rice; Cont, control.



IgG1, IgG2b : Non-inflammatory Th2 isotype immunoglobulin
IgG2a : Inflammatory Th1 isotype immunoglobulin

Fig. 4. **IgG isotyping of anti-Aβ antibodies in wild-type immunized mice.**

Aβ, β-amyloid; SD, standard deviation.

Herb expressing Aβ and future vaccine for Alzheimer's disease

Ruta chalepensis L. is a medicinal herb which grows naturally in Okinawa prefecture. This is commonly called kohenruda or *Ishanakashi-gusa*, which means "an herb which makes a doctor cry or keeps the doctor away." The place of origin is the Mediterranean Region [12]. It is known that this herb has beneficial effects such as antiphlogistic, sedation and detoxification. It is said that this herb is effective for nerve pain and rheumatism [13, 14].We performed Aβ42 expression in *Ruta chalepensis* L., which has anti-aging effects and drug efficacy. When *Ruta chalepensis* L. expressing Aβ42 was administered to mice, elevation of anti-antibody titer against Aβ42 was confirmed in the half of mice without the use of an adjuvant. This suggested that *Ruta chalepensis* L. contains a certain chemical compound that had the effect of an adjuvant. This is a new hope for development in the edible vaccine. Our Aβ42 expression in *Ruta chalepensis* L. is the first time it has been achieved in the world. An edible herb expressing the Aβ vaccine is anticipated to be developed in the near future. (A paper by Yoshida, Watanabe and Ishiura is in press in Proc. Japan Acad.)

Conclusion

Considering the increasing number of patients with Alzheimer's disease and the need of prolonged nursery care [15], there is not much desire for expensive medical therapy in present society, where prevention is better than a cure. It is common knowledge that pharmaceutical pricing of health care spending oppresses the administration of the Ministry of Health, Labour and Welfare. Further, it is also a well-known fact that safe and affordable methods for prevention are sought. It would be a worthwhile idea to re-examine oral vaccines as a promising and safe measure for prevention therapy, although the effects of an oral vaccine do not appear immediately [8].

There is one point that we felt was unusual in the oral administration experiments during these several years. That is, antibody titer and the removal effect of senile plaques do not increase proportionally, at least in mouse models. There were a large number of cases indicating that even a small rise of antibody titer induced removal effects. Originally, in oral administration, the extent in antibody titer rise is small. However, it would be important to take advantage of this.

Acknowledgments

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Conflict of interest

The authors claim no conflict of interest in this study.

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