

## Research Article

# Research and Development of Vaccines for Alzheimer's Disease

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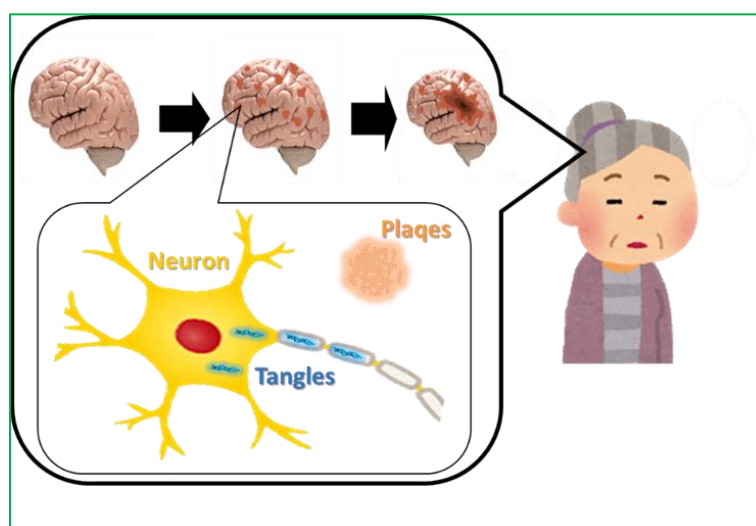
**Abstract:** Alzheimer's disease (AD), the most common cause of dementia, is a degenerative neurological disease including memory loss, language problems, and unpredictable behavior. AD is a growing concern as it appears almost exclusively in older adults. The number of cases will also increase as the number of older people rises. AD is a progressive condition, that is, the symptoms develop gradually over many years and eventually become more severe. It has not been made into a medicine to prevent or cure the disease although many studies have been conducted to overcome AD. The causes of AD are not fully understood and research continues to examine factors involved in the development of Alzheimer's disease. Pathological hallmarks of AD are plaques composed of accumulated amyloid  $\beta$  peptide ( $A\beta$ ) and neurofibrillary tangles due to the accumulation of hyperphosphorylated tau protein. In this context,  $A\beta$  and tau are considered as causes of AD, and studies on vaccines targeting them have been carried out. This review introduces studies on research and development of vaccines targeting  $A\beta$  and tau.

**Keywords:** Alzheimer's disease, Vaccine,  $A\beta$ , Tau.

## Introduction

Alzheimer's disease (AD) is one of dementia, beginning at the age of 40 to 50 years, and one in two people is said to develop this disease at the age of 80 and over. One of the major histopathological characteristics of AD is the presence of senile plaques, composed mainly of amyloid  $\beta$  peptide ( $A\beta$ ) aggregates (Figure 1) (Benilova *et al.*, 2012; Jongbloed *et al.*, 2015; Jeong, 2017; Cline *et al.*, 2018; Lane *et al.*, 2018).  $A\beta$  consists of 40 to 42 amino acids.  $A\beta$  is cut out from amyloid precursor protein (APP) by  $\beta$ -secretase and two enzymes called  $\gamma$ -secretase.  $A\beta$  forms soluble oligomers and the toxicity of oligomers to nerve cells is quite strong among them, so the  $A\beta$  oligomer has been considered to be a major cause of AD. Therefore, inhibition or elimination of formation of this soluble  $A\beta$  oligomer has been thought to lead to the prevention and treatment of AD. The possibility of an AD vaccine was first demonstrated by immunization experiments using  $A\beta$  and model mouse of AD (Schenk *et al.*, 1999). The model mouse is designed to overexpress human  $A\beta$  by recombinant DNA technology (Transgenic mouse) and senile plaques is observed in the mouse brain. The model mouse immunized with  $A\beta$  and a strong adjuvant induced antibodies against  $A\beta$  and reduced senile plaque formation in the transgenic mouse. In addition, learning behavior of mice was improved. In response to the result, clinical trials were conducted. A subsequent clinical trial, in which early AD patients received  $A\beta$  injections in combination with an adjuvant to improve the immune response, was suspended because the trial participants receiving the active vaccine developed encephalitis (Senior, 2002). Since then, the development of  $A\beta$  vaccines avoiding such side effects have been continued.

AD is thought to be caused by not only senile plaques composed of accumulated A $\beta$ , but also neurofibrillary tangles due to the accumulation of hyperphosphorylated tau protein. In AD, APP is cleaved to form A $\beta$ . Once formed, A $\beta$  stimulates refolding of more A $\beta$  peptides, which then assemble into amyloid fibers that form plaques. In patients with AD, A $\beta$  prions appear to begin multiplying deep in the temporal lobes of the brain and then spread to other regions. A $\beta$  also stimulates the misfolding of another protein called tau. Misfolded tau assembles into fibers that condense inside neurons to form tangles. Plaques and tangles are the pathological hallmarks of AD. Vaccines targeting A $\beta$  have been energetically carried out, but none of them has yet to come out of development. From such a situation, tau is considered as a potent target of AD vaccines and tau targeting vaccines have been promoted (Pedersen and Sigurdsson, 2015). In this review article, various studies concerning AD vaccines are introduced.



**Figure 1. Progression of AD**

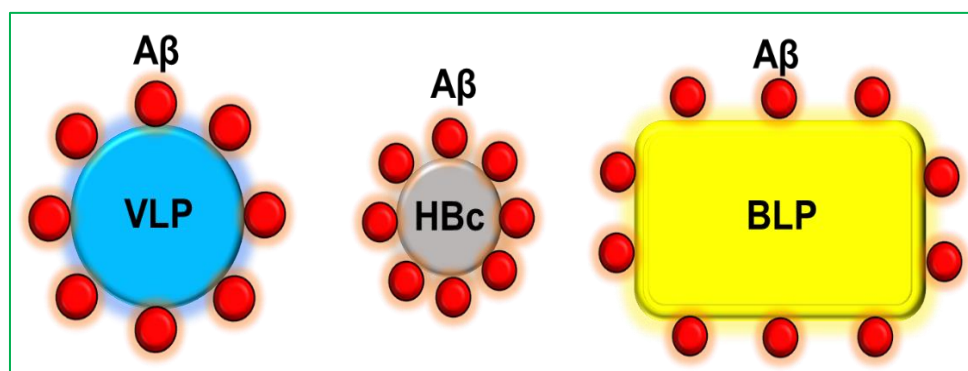
### Research and Development of A $\beta$ Vaccines

Schenk *et al.*, (1999) indicated the possibility of AD vaccine using A $\beta$ . This is the first report concerning AD vaccine. They showed that A $\beta$  (1-42 aa) and a strong adjuvant QS21 were inoculated to transgenic mice PDAPP in which brain senile plaques are formed by high human APP expression. As a result, it was found that amyloid plaque formation and memory behavior disorder were prevented at the early stage of administration (6 weeks of age), and reduction of senile plaque and amelioration of memory behavior disorder were observed at the later time of administration (11 weeks of age). This study indicated that immunization with A $\beta$  showed the possibility of being effective for prevention and treatment of AD. Based on these results, clinical trials were conducted, and there was no safety problem with phase I. However, the clinical study was discontinued due to the occurrence of meningoencephalitis in 6% of patients at the stage of phase II (Senior, 2002). The side effect is considered to be due to autoimmunity by T cells. Though the first clinical trial was discontinued due to meningoencephalitis, antibodies against A $\beta$  was observed in patients receiving A $\beta$  with adjuvant and senile plaque progression was also suppressed. It seemed likely that the A $\beta$  vaccine might have worked prophylactically and therapeutically in patients with AD (Schenk, 2012). In response to this result, researches on A $\beta$  vaccines that suppress cellular immunity have been processed so far. One approach is to delete T cell epitopes from A $\beta$  and to enhance immunogenicity of the shorten peptides. Petrushina *et al.*, (2007) designed the vaccine (PADRE-A $\beta$ ) consist of B cell epitope (A $\beta$  1-15 aa) in tandem and the universal T cell HLA DR epitope (PADRE). Immunization of BALB/c mice with the PADRE-A $\beta$  produced high

titers of anti-A $\beta$  antibodies. Splenocytes from immunized mice showed robust T cell stimulation in response to peptides containing PADRE. On the other hand, splenocytes from immunized mice were not reactivated by the A $\beta$  peptide. Their data suggest that PADRE-A $\beta$  could bias the immune response toward a Th2 phenotype and/or replace the A $\beta$  T cell epitope with a foreign T cell epitope and might prevent the adverse events that occurred during the first clinical trial.

Ghochikyan *et al.*, (2006) compared the induction of humoral immune responses with Quil A (Th1-type adjuvant) and aluminium salt (Th2-type adjuvant) adjuvants singly and in combination, using PADRE-A $\beta$ . Their data indicated that the combined use of both Th2-type adjuvant and Th1-type adjuvant enhanced the therapeutically relevant anti-A $\beta$  antibody production without inducing the potentially harmful Th1 immune response. Though alum is a Th2-type adjuvant, it is much less potent than the majority of Th1-type adjuvants including saponin (QS21). Therefore, they suggested that the combined use of both Th1- and Th2-type adjuvants could be more effective and safe vaccines for AD.

Wiessner *et al.*, (2011) designed CAD106 which is the virus-like particle (VLP) conjugated with A $\beta$  (Figure 2). In order to avoid activating A $\beta$ -specific T-cells, they chose the A $\beta$ 1–6 peptide (DAEFRH) as antigen, which is shorter than typical T-cell epitopes and resides outside the region of A $\beta$  reacting with T-cells. It was extended by a spacer (GGC) and covalently conjugated to the VLP derived from Escherichia coli RNA phage Q $\beta$ . Each VLP contains 350–550 A $\beta$  peptides. Immunization with CAD106 did not activate A $\beta$ -specific T-cells. In AD transgenic mice, CAD106 induced efficacious A $\beta$  antibody titers of different IgG subclasses mainly recognizing the A $\beta$ 3–6 epitope. CAD106 reduced brain amyloid accumulation in two AD transgenic mouse lines. In rhesus monkeys, CAD106 induced a similar antibody response as in mice. The antibodies stained amyloid deposits on tissue sections of mouse and human brain but did not label cellular structures containing APP. The antibodies reacted with A $\beta$  monomers and oligomers and blocked A $\beta$  toxicity in cell culture. CAD106 first-in-human study demonstrated a favorable safety profile and promising antibody response (Winblad *et al.*, 2012; Farlow *et al.*, 2015).



**Figure 2. Various forms of AD vaccine**

Hepatitis B virus core (HBc) particle was used for carrier protein of A $\beta$  (Jin *et al.*, 2014). They constructed HBc-A $\beta$  fusion protein (Figure 2). The fusion protein could form VLP displaying A $\beta$ 1–15 which is the B cell epitope of A $\beta$ . The A $\beta$ -HBc VLP was shown to elicit a strong immunogenicity in AD transgenic mice and the behavioral changes of mice were tested by Morris Water Maze. The escape latency of immunized mice was shorter than control. Immunohistochemistry results showed that A $\beta$ -HBc VLPs immunized mice had less amyloid deposit with less microglia in cortex and hippocampus.

Fu *et al.*, (2018) reported bacterium-like particle (BLP) carrying A $\beta$ 1-6 for AD vaccine. Different copy numbers of the A $\beta$ 1-6 peptide were specifically loaded on the surface of BLPs via fusion with a peptidoglycan anchoring domain (Figure 2). These four BLP-based A $\beta$  vaccines successfully induced high levels of A $\beta$ 42-specific antibodies in mice. However, none of the vaccines induced a T-cell-mediated immune response. Among the four vaccines, 6 copy-A $\beta$ 1-6-PA-BLP was the most effective in inducing A $\beta$ -specific antibodies, indicating that a suitable epitope copy number is critical for high immunogenicity of the BLP-based vaccine. Furthermore, high levels of serum A $\beta$ -specific antibodies could still be detected 3 months after the final administration of 6 copy-A $\beta$ 1-6 -PA-BLP. A simple technique of raising the immunogenicity of A $\beta$  has been reported (Matsuda *et al.*, 2009). They conducted the study on A $\beta$  vaccine without adjuvant. According to their report, cysteine-binding A $\beta$  could drastically induce antibody against A $\beta$  without using adjuvants. Only binding cysteine to C-terminal of A $\beta$  increased the immunogenicity. This cysteine binding effect was also observed with shorten A $\beta$  peptides. The cysteine-binding A $\beta$  could reduce accumulation of A $\beta$  by administration to AD transgenic mice. It is noteworthy that accumulation of soluble A $\beta$ , which is considered to be the cause of AD, could also be prevented. This simple technique would provide Inexpensive and safe AD vaccine.

### Research and Development of Tau Vaccines

Another target of AD vaccine is the tau protein. AD could be caused by not only A $\beta$ , but also hyperphosphorylated tau protein. A $\beta$  stimulates the misfolding of tau. Misfolded tau assembles into fibers that condense inside neurons to form tangles. It has reported that there is correlation between the generation of these neurofibrillary tangles and the progression of dementia. Therefore, tau protein has become an important focus of research into treatments for AD. Novak *et al.*, (2018) constructed AADvac-1 which consists of a synthetic peptide derived from amino acids 294 to 305 (tau 294–305) of the tau sequence coupled to keyhole limpet hemocyanin (KLH). Tau294–305 is a structural determinant of the truncated tau protein for the pathological tau-tau interaction. Tau294–305 is a short peptide with low immunogenicity. To develop a vaccine with tau 294–305 as an immunogen to induce high antibody titers, tau 294–305 should be combined with a carrier such as KLH. The Aavac-1 was shown to reduce tau pathology and improved sensorimotor function in transgenic animal. The Phase I study in humans has confirmed that AADvac1 is safe and well tolerated (Novak *et al.*, 2018). It induced a robust immune response and the cognition of the patients remained on average stable for the whole duration of the Phase I study. A similar approach has been conducted by Ji *et al.*, (2018). Tau 294–305 is a short peptide with low immunogenicity. In order for tau 294–305 to induce high antibody titers, they used HBc particle for a carrier. They genetically fused tau 294–305 to HBc (T294-HBc) forming VLP (Figure 3). Transgenic mouse P301S widely used tauopathy model was subcutaneously immunized with T294-HBc. T294-HBc elicited strong immune response and alleviated cognitive deficits and neuropathology progression in P301S mice.

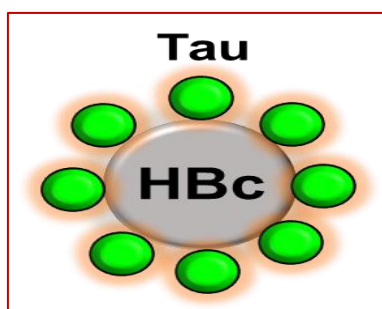


Figure 3. HBc particle expressing tau

Both of A $\beta$  and tau antigens could be required for AD vaccines. Matsumoto and Kohyama (2017) reported DNA vaccine targets both tau and A $\beta$ . Their DNA vaccine has four tandem repeats of A $\beta$ 1-42 and tau 379-408 that are connected to both ends of the Fc portion of immunoglobulin (Figure 4). The A $\beta$ -tau DNA vaccine was administrated to transgenic mice harboring plaques and tangles. They showed induction of a significant increase in antibodies against A $\beta$  and tau. The amount of A $\beta$ , total tau and phosphorylated tau was lower than untreated transgenic mice. Notably, neurotoxic phosphorylated tau was no longer detected after administration of the DNA vaccine.

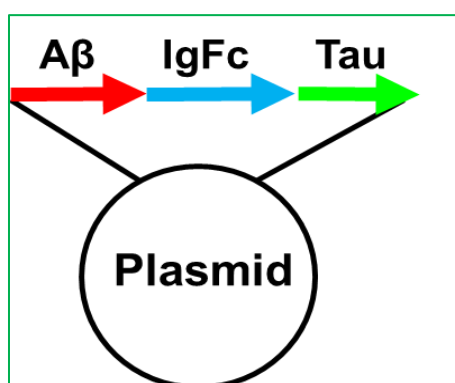


Figure 4. DNA vaccine expressing A $\beta$  and tau

### Conclusion

With aging, AD has been increasing. Overcoming this illness is very important for us to have a comfortable old age. Although many studies have been conducted to overcome AD, it has not been made into a medicine to prevent or cure the disease yet. A $\beta$  and tau are considered as causes of AD, and studies on vaccines targeting them have been carried out.

This review introduced studies on AD vaccines. Vaccines targeting A $\beta$  have been energetically carried out, but none of them has yet to come out of development. From such a situation, tau vaccine has been studied as a next candidate. It is not known at this time whether the vaccine against either A $\beta$  or tau is the final candidate. Vaccines against both antigens could be needed. In the situation where AD has been increasing, the vaccine development is a pressing issue.

**Conflicts of interest:** The authors declare no conflicts of interest.

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