SCIENCE CHINA
Chemistry

• MINI REVIEWS •
• SPECIAL TOPIC • Chemistry from Chinese Female Chemists

March 2015 Vol.58 No.3: 383–389
doi: 10.1007/s11426-014-5310-9

New progress in active immunotherapy targeting to amyloid beta

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Received October 22, 2014; accepted November 24, 2014; published online January 16, 2015

Alzheimer's disease (AD) is one of the most common types of dementia whose hallmarks include neurofibrillary tangles and senile plaques. The latter are mainly composed of amyloid-β proteins (Aβ), and it's suggested that Aβ may be the causative factor in AD pathogenesis. Immunotherapy targeting Aβ for preventing aggregation of Aβ and mildly clearing amyloid plaques has been a hot topic since 1999. Although the first clinical trial of Aβ vaccine, AN-1792, failed in phase II, its results suggested some key points in the design of Aβ vaccines. Avoiding the possible toxic Aβ specific T cell response and inducing a T_h2 type cellular immune response may be beneficial for Aβ immunotherapy. Many associations and research groups are working on Aβ vaccine and some progress has been made in recent years. In this review, we have provided a detailed summary of past Aβ vaccines, which have been sorted by the immunogen, and we also discuss some recent progress and future perspectives.

Alzheimer's disease, amyloid beta, active immunotherapy, peptide vaccine, DNA vaccine

1 Introduction

It was reported that in 2010, there were about 36 million people worldwide who were affected by Alzheimer's disease (AD) [1], a kind of neurodegenerative disease. And it was estimated that by 2050, the number of patients will reach 115.4 million [1]. Many associations and groups have made great efforts to find a cure for AD, but there are still no effective disease-modifying therapies [2]. From previous research, we know that the hallmarks of AD include neurofibrillary tangles (NFTs) related with tau protein and senile plaques (SPs) which are mainly composed of β-amyloid (Aβ) proteins [3].

The relationship between tau and Aβ remains to be elucidated. Amyloid cascade hypothesis, one of the most widely accepted hypotheses, suggests that Aβ is the causative factor of AD and tau-related NFTs are downstream pathological features [4]. Considering the toxicity of many Aβ species, especially Aβ oligomers [5], many groups have focused on clearing Aβ species or preventing the aggregation of Aβ [6,7]. Among these potential therapies, immunotherapy stands out as an effective method, because several clinical trials based on active or passive immunotherapy have reached phase III or phase II [8]. Some clinical trials based on passive immunotherapies failed in recent years, and some newly developed antibodies are being tested [2,8]. In this review, we focus on active immunization.

In 1999, Elan Pharmaceuticals reported that after immunizing PDAPP mice with Aβ42, one of the most abundant Aβ species in the brain of AD patients, the amyloid burden in the brain was significantly reduced [9]. Based on this encouraging result, the first clinical trial of the anti-Aβ vaccine, known as AN-1792, was conducted. Unfortunately, during phase II trials, approximately 6% of patients treated with AN-1792 developed meningoencephalitis [10]. Considering the safety issue of the vaccine, the clinical trial was

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terminated.

Further studies on AN-1792 suggested that the reason of meningoencephalitis is hard to be illustrated clearly. However, it was suggested that Aβ specific T cells may play an important role in the adverse effect [10–12] and that antibodies targeting Aβ species may not cause meningoencephalitis because passive immunization of bapneuzumab did not induce the same adverse effect [13].

To simultaneously generate anti-Aβ antibodies and prevent the toxic Aβ specific T cell response, new vaccines for AD may need to use the B cell epitope of Aβ and exclude the epitope that induces the toxic Aβ specific T cell response [10,14]. According to previous research, the B cell epitope of Aβ42 is mainly in Aβ1–15, while the T cell epitope is mainly in Aβ16–42 [15–17].

Another factor that may affect the immune response is adjuvant. In the clinical trials of AN-1792, QS21, which may shift the cellular immunity towards T_h1 phenotype [16], was used as the adjuvant. T_h1 helper cells are known to elicit the production of the pro-inflammatory cytokine IFN-γ, which may aggravate inflammation. Although T_h1 cell response may increase the clearance of amyloid plaques by promoting the activation of microglial cells [18], designing a vaccine that generates T_h2 biased cellular response would be a more effective method for preventing adverse effect.

Currently, the research of second-generation Aβ vaccines is focusing on optimizing the structure of the vaccine and choosing the appropriate adjuvant.

2 Second-generation Aβ vaccine

There are many sound reviews on AD immunotherapy, in which different vaccines, based on the considerations mentioned above, have been summarized [2,8,19]. Here, we sort these vaccines into three categories: peptide/protein vaccines, DNA vaccines, and recombinant vaccines.

2.1 Peptide/protein vaccines

A typical second-generation Aβ vaccine developed by Cribbs and coworkers [20] consisted of the B cell epitope, Aβ1–15, and a non-self T cell epitope, pan HLA DR-binding epitope. Multiple peptide system was used to increase the size of the peptide, and Alum adjuvant, which was shown to be a T_h2 type adjuvant [16], was mixed with the peptide. Mice were immunized subcutaneously. The results suggested that PADRE-Aβ1–15-MAP was efficient in producing high titers of anti-Aβ antibodies without inducing anti-Aβ T cell response. Moreover, the Alum adjuvant shifted the cellular immunity towards a T_h2 phenotype.

Another strategy is conjugating part of Aβ sequence to a carrier protein, such as diphtheria toxoid (DT) [21], keyhole limpet hemocyanin (KLH) [22]. And the clinical trial named vanutide cridificar (ACC-001) conducted by Pfizer and Janssen took this strategy. In this trial, multiple copies of Aβ1–7 were conjugated to CRM197, a non-toxic mutant of DT, and combined with adjuvant QS21 [23]. Unfortunately, Pfizer announced that this clinical trial would discontinue in August, 2013 for unknown reasons (http://www.pfizer.com/sites/default/files/product-pipeline/pipeline_080913_0.pdf).

Other groups took similar approaches but changed the peptide sequence (different B and T cell epitope, or different carrier protein) and the adjuvant to achieve a stronger immune response while avoiding the adverse effects [2,8,19].

2.2 DNA vaccines

Unlike immunization with proteins/peptides, plasmid DNA encoding antigens are transfected into animal cells for DNA vaccination [24]. DNA vaccines have some advantages over traditional vaccines, as they are adjuvant free and are adjustable to either T_h1 or T_h2 phenotype, which make them suitable for Aβ immunotherapy.

Cribbs and coworkers [25] have done some work on DNA vaccines. They constructed a plasmid that expressed 3 copies of Aβ1–11 linked by the amino acids G and S, PADRE, and a chemokine (MDC/CCL2), which served as a molecular adjuvant. Transgenic mice were immunized in the abdominal skin using a gene gun. The result was similar to their previous work [16] where high titers of anti-Aβ antibodies were produced and a T_h2 type immune response was induced by the molecular adjuvant. Because transgenic mice model was used, they also observed a decrease in brain amyloid load and prevention of behavioral deficits.

Similar to the peptide vaccines, DNA vaccine development involved changing the sequence of the plasmid to change the sequence of the peptide expressed [2,8,19].

2.3 Recombinant vaccines

In this review, we regard recombinant vaccines as vaccines containing parts of viruses or bacteria in addition to the antigen peptides or DNAs. This type of vaccine has some common features with DNA vaccines, as they both use DNA to express the antigen, whereas the DNA vaccines require sophisticated technology, such as a gene gun to deliver the plasmid to the host cells.

Fukuchi and coworkers [26] constructed an adenovirus vector vaccine, which can induce an immune response through nasal inoculation. The adenovirus vector contained a cDNA expressing 11 copies of Aβ1–6 and PEDI (the receptor-binding domain of *Pseudomonas exotoxin* A). The PEDI domain was used to ensure the fused protein would be secreted, and thereby may induce a T_h2 type cellular response [27]. Their results suggested that nasal inoculation of the vaccine can efficiently induce a T_h2 type immune

response and reduce the amyloid burden in an AD mouse model. Their study also suggested a possible relationship between upregulation of IL-10 and T_h2 type cellular response.

Another study by Sigurdsson and coworkers [28] adopted viable Salmonella for the design of an Aβ vaccine. They transfected a plasmid encoding 4 copies of K6Aβ1–30 and TetC (a part of the tetanus toxin) into *Salmonella typhimurium*, which can express sufficient antigens for inducing an immune response in the body. Young Tg2576 mice were immunized with the Salmonella based vaccine by oral gavage. Because young mice were used for the study, the results suggested that prophylactic treatment by oral immunization of the vaccine may be efficient in preventing AD.

The clinical trial CAD106, which has reached phase II (NCT01097096), used virus like particle Qβ (bacteriophage) as a vector to carry many copies of Aβ1–6 [29]. According to the 2013 annual report of Novartis, further clinical investigation will begin in 4 years (http://www.novartis.com/downloads/investors/reports/novartis-annual-report-2013-en.pdf).

3 New progress in active immunotherapy

In recent years, many new strategies have emerged with respect to Aβ immunotherapy. Unlike traditional second-generation vaccines, these new approaches use either novel methods of vaccination or new antigenic peptides.

3.1 New methods of vaccination

Different inoculation methods may influence the cellular immune response, and it has been reported that transcutaneous immunization may induce a T_h2-biased immune response [30]. Nakagawa and coworkers [31] wanted to test transcutaneous immunization in an AD mouse model by using dissolving microneedle array as a new immunization method, which would cause less damage to the skin. Unfortunately, they did not observe significant improvement in behavioral experiments and the immune response was a T_h1 and T_h2 mixed type, although a significant decrease in amyloid plaques was observed. Therefore, other factors unrelated to the immunization method may influence the cellular immune response.

Another study by Wang and coworkers [32] demonstrated the efficiency of coimmunization with Aβ42 and a plasmid expressing Aβ42. They immunized APP_{695} mice intramuscularly with a mixture of Aβ42 and pVAX1-Aβ42 which could express Aβ42. Their study revealed that in addition to inducing high titers of anti-Aβ antibodies and reducing amyloid plaque formation, this strategy could induce high levels of regulatory T cells, which can inhibit Aβ-specific T cells. Thus, this method of immunization may provide a new way for suppressing inflammation in AD immunotherapy.

3.2 New antigenic peptides

Except for using short Aβ peptides as B cell epitopes, many groups are expanding the possible antigenic library of Aβ vaccination.

Although short Aβ peptides may not contain the T cell epitope and then prevent the toxic Aβ specific T cells response, they are still self-antigens which may be less effective than foreign peptides in inducing an immune response. In order to induce strong anti-Aβ immune response with a foreign peptide, mimotope can be used for Aβ immunotherapy. A mimotope is often a peptide which mimic the structure of the antigenic determinants of a specific antigen. This peptide can bind to the antibody targeting the antigen and may induce an immune response towards the antigen after immunization.

A group led by Martin *et al*. [33,34] reported that their SDPM1 peptide was efficient in working as a mimotope for Aβ. SDPM1 contains 20 amino acids and was used as an inhibitor for Aβ oligomerization in their previous work [35]. In 2010, they immunized APPswePSEN1 mice intraperitoneally with SDPM1 which was conjugated to streptavidin through its N-terminal biotin [33]. TiterMax Gold adjuvant was used in the first boost while IFA was used in subsequent boosts. The result indicated a T_h2 type cellular immune response, and high titers of anti-SDPM1 and anti-Aβ antibodies. In addition, there was significant reduction of soluble and insoluble Aβ besides and amyloid burden in the brain. However, differences in behavioral results between young and old mice indicated that immunization with this vaccine may only provide a preventative effect and not improve cognitive function in advanced patients. Therefore, in 2014, the components of the vaccine were changed [34]. SDPM1-4E was used as the antigen peptide and Alhydrogel (Alum adjuvant) was used as adjuvant. Results revealed that both young and old mice had significant improvements in cognitive function after immunization. Based on these results, SDPM1 may be effective in Aβ immunotherapy as it can induce a stronger immune response than Aβ peptides without involving toxic Aβ specific T cells.

The company AFFiRiS AG is currently developing this type of Aβ vaccine. The Affitope AD02 clinical trial is currently in phase II (NCT01117818). To our knowledge, the vaccine contains six amino acids that mimic the N-terminal of Aβ and Alum adjuvant [36].

Other possible antigens, such as Aβ oligomers, and N-terminal truncated/modified Aβ, are also being studied by some groups. Gevorkian and coworkers [37,38] reported the use of pyroglutamate-modified Aβ as a new antigen in Aβ

immunotherapy. Zvirbliene and coworkers [39] showed the size of Aβ oligomers may influence the immune response. Glabe and coworkers [40] used a 20 amino acids peptide as the mimetic of Aβ oligomers and coupled the peptide to colloidal gold particles.

Terry Jr. and coworkers [41] used another strategy involving RAGE/Aβ complex for Aβ immunotherapy. RAGE, receptor for advanced glycation endproducts, plays an important role in AD pathogenesis. RAGE can bind to Aβ and form high molecular weight complexes, thereby may increase the immunogenicity of the antigens. Mice immunized with the RAGE/Aβ complex orally were found to have higher titers of anti-Aβ and anti-RAGE antibodies than Aβ42 alone.

4 Summary and perspective

AD is a serious disease worldwide that shortens both patients' life expectancy and quality of life. Unfortunately, there are still no disease-modifying therapies, and even the high cost antibodies against Aβ have met problems in some clinical trials [8]. Developing vaccines against Aβ may be an inexpensive and effective method for treating AD. After failure of the clinical trial for AN-1792, researchers have tried to optimize the structure and components of the vaccine. Here, we have summarized the various Aβ vaccines developed in the past three years (Table 1).

After many years of research with Aβ active immunotherapy, it remains unknown what type of vaccine is effective in AD patients as there is still no successful clinical trial. However, these trials have provided insights on areas that require further research.

(i) Exploring new antigens, such as Aβ oligomers and truncated/modified Aβ species. Clearance of these Aβ species may be important for the prevention and treatment of AD.

(ii) Developing or choosing new adjuvant and new immunization methods for decreasing adverse effects and increasing the effectiveness of the vaccine (see Ref. [61]).

(iii) Many scientists recommend that early intervention may be important in preventing AD [2,8], which suggests

Table 1 Summary of Aβ vaccines in the past three years [2]

Authors (year)	Vaccine type	Immunogens	Adjuvant	Animal model	Principal results	Ref.
Wiley *et al.* (2012)	Peptide vaccine	aggregated Aβ42	monophos-phoryl lipid A	non-human primates	Preventive Aβ immunization may be safe and effective	[42]
Yao *et al.* (2012)	Peptide vaccine	Aβ15-GPGPG-Aβ1-15 vs 2Aβ1-15-GPGPG		C57BL/6 mice	Linker in middle site may be more effective	[43]
Fukuchi *et al.* (2012)	DNA prime-adenovirus boost	AdPEDI-$(Aβ1–6)_{11}$+Aβ1–8-KLH+ simvastatin		TgAPPswe/PS1dE9 mice	Simvastatin may reduce inflammation	[22]
Cao *et al.* (2012)	DNA vaccine	10Aβ3-10	IL-4 (molecular adjuvant)	BALB/c	T_h2-biased immune response	[44]
	DNA vaccine	10Aβ3-10	Melatonin	TgAPPswe/PSEN1dE9	Reduced Aβ deposits and cognitive impairment	[45]
	Adenovirus vector vaccine	10Aβ3-10	CpG motif (molecular adjuvant)	APPswe/PSEN1dE9 mice	Reduced Aβ deposits and cognitive impairment	[46]
Biragyn *et al.* (2012)	DNA vaccine	Aβ1–11 exposed on the surface of HBsAg particles		3xTgAD mice	Reduced Aβ plaques, ameliorated cognitive impairments, extended life-span	[47]
Glabe *et al.* (2012)	Peptide vaccine	Oligomer mimetic	IFA	Tg2576 mice	Reduces Aβ plaques, improved cognitive performance	[40]
Yao *et al.* (2012)	Peptide vaccine	4Aβ1-15	CFA/IFA	C57BL/6 mice	T_h2-biased immune response	[48]
Terry *et al.* (2012)	Peptide vaccine	RAGE/Aβ complex		APPSWE-PS1 mice	Higher titer than Aβ42, statistically significant improvement in cognition	[41]
Zvirbliene *et al.* (2013)	Peptide vaccine	Various Aβ1-42 oligomers	1st CFA, then PBS	BALB/c mice	1–2 nm Aβ1-42 oligomers induce highest titer and high specificity	[39]
Agadjanyan *et al.* (2013)	Peptide vaccine	3Aβ1-12 separated by P2 and P30	CFA/IFA, Quil-A, Alhydrogel. Tg2576 mice, Guinea pigs, Cynomolgus monkeys		Reduced Aβ plaques; preexisting memory T cells for tetanus toxoid strengthened immune response	[49]
Jin *et al.* (2013)	Peptide vaccine	2Aβ1-15 conjugated to HBc, formed VLPs		BALB/c mice	T_h2-biased immune response	[50]
Yao *et al.* (2013)	Peptide vaccine	4Aβ1-15	MF59 adjuvant	APP/PS1 mice	Reduces Aβ plaques, improved acquisition of memory	[51]
Cao *et al.* (2013)	DNA vaccine	10Aβ3-10	C3d-p28 molecular adjuvant	TgAPPswe/PSEN1dE9 mice	Reduced Aβ plaques, improved cognitive function	[52]

(*To be continued on the next page*)

(*Continued*)

Authors (year)	Vaccine type	Immunogens	Adjuvant	Animal model	Principal results	Ref.
Lemere *et al.* (2013)	Peptide vaccine	Aβ1-15 conjugated to DT	MAS-1 adjuvant	APPswe/PS1ΔE9 mice	Reduced Aβ plaques, improved cognitive function; antibodies for Aβ N terminus (not mid or C)	[21]
Kohyama *et al.* (2013)	DNA vaccine	IgLAβx4-huFc-huIL-4	B6C3-Tg 85Dbo/J mice; New Zealand white rabbits; Cynomolgus monkeys		Reduced Aβ plaques. Aβ1-42, AβpE3-42, Aβ oligomer, Aβ fibrils, significant reduction	[53]
Singer *et al.* (2013)	Peptide vaccine	3B epitopes: tau229–237[pT231/pS235], AβpE3–8, Aβ37/38–42/43 5 T epitopes, Alu-GelS adjuvant Three inbred wild-type mouse strains, P301S (Tau) and Tg2576 (Aβ) Immunized with mixture of different T-B two component vaccine				[54]
Nisizawa *et al.* (2013)	Peptide vaccine	B epitope: Aβ1-13 T epitope: Gag298-312 or DiTox382-401 Structure: RGD-T-KK-B		C57BL/6 and Balb/c mice	Induction of anti-Aβ antibodies without adjuvant	[55]
Sun *et al.* (2013)	DNA vaccine	6Aβ15 conjugated to PADRE or tox-in-derived carrier proteins		BALB/c and C57/BL6 mice PDAPPV717I	Reduced Aβ plaques, prevented cognitive dysfunction	[56]
Sun *et al.* (2014)	Peptide vaccine	6Aβ15 conjugated to PADRE or tox-in-derived carrier proteins. Adjuvant: Alhydrogel		BALB/c and C57/BL6 mice PDAPPV717I	Reduced Aβ plaques, prevented cognitive dysfunction. Antibodies highly bound to oligomers	[57]
Prisco *et al.* (2014)	Peptide vaccine	Aβ1-11 fusion on E2 (VLP)	Alhydrogel 2% or AddaVax	B6C3/F1 mice	T_h2-biased immune response	[58]
Martin *et al.* (2014)	Peptide vaccine	SDPM1-4E peptide	Alhydrogel	C57BL/6 mice APPswePSEN1dE9 mice	Anti-SDPM1 and anti-Aβ antibodies; reduced Aβ plaques, improves learning and memory	[34]
Wang *et al.* (2014)	Coimmunization with Aβ42 and plasmid expressing Aβ42			C57BL/6 mice APP695 mice	T_h1-suppressive response, induced high levels of iTreg; reduced plaque formation, improved behavior	[32]
Nakagawa *et al.* (2014)	Peptide vaccine	Aβ1–35-Cys, Aβ1–42	Cholera toxin	APPPS1 mice	Transcutaneous immunization using dissolving microneedle array failed to meet expectations	[31]
Agadjanyan *et al.* (2014)	Recombinant vaccine	Recombinant influenza virus, expressing Aβ1–10 (WSN-Aβ1–10)		C57Bl/6mice	Boosting of mice primed with WSN-WT with WSN-Aβ1–10 failed to enhance anti-Aβ antibody response	[59]
Jin *et al.* (2014)	Recombinant vaccine	Cholera toxin B subunit -Aβ42 fusion protein expressed in silkworm pupae		APPswe/PSEN1dE9 mice	Reduced Aβ plaques, prevented cognitive dysfunction	[60]

that prophylactic immunization in asymptomatic elderly individuals could be effective. Because in patients with mild-to-moderate AD, it may be too late to clear the amyloid burden after irreversible damage has been caused to neurons [8].

This work was supported by the National Basic Research Program of China (2013CB910700, 2012CB821600), the National Natural Science Foundation of China (21472109, 21102082) and the Research Project of Chinese Ministry of Education (113005A).

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