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Can Alzheimer disease be prevented by amyloid- β immunotherapy?

Cynthia A. Lemere and Eliezer Masliah

Center for Neurologic Diseases, Department of Neurology, Brigham and Women's Hospital, New Research Building 636F, 77 Avenue Louis Pasteur, Boston, MA 02115, USA

Department of Neurosciences, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0624, USA

Abstract

Alzheimer disease (AD) is the most common form of dementia. The amyloid- β (A β) peptide has become a major therapeutic target in AD on the basis of pathological, biochemical and genetic evidence that supports a role for this molecule in the disease process. Active and passive A β immunotherapies have been shown to lower cerebral A β levels and improve cognition in animal models of AD. In humans, dosing in the phase II clinical trial of the AN1792 A β vaccine was stopped when ~6% of the immunized patients developed meningoencephalitis. However, some plaque clearance and modest clinical improvements were observed in patients following immunization. As a result of this study, at least seven passive A β immunotherapies are now in clinical trials in patients with mild to moderate AD. Several second-generation active A β vaccines are also in early clinical trials. On the basis of preclinical studies and the limited data from clinical trials, A β immunotherapy might be most effective in preventing or slowing the progression of AD when patients are immunized before or in the very earliest stages of disease onset. Biomarkers for AD and imaging technology have improved greatly over the past 10 years and, in the future, might be used to identify presymptomatic, at-risk individuals who might benefit from A β immunization.

Introduction

Alzheimer disease (AD) is the leading cause of dementia, affecting more than 26 million people worldwide.¹ Clinically, the disease is characterized by progressive memory loss and a decline in cognitive abilities. Several symptomatic treatments are in use for AD; however, no disease-modifying therapies are currently available. The two major pathological hallmarks of AD are extra cellular amyloid plaques, which are formed mainly from the amyloid- β (A β) peptide, and intracellular neurofibrillary tangles (NFTs), which contain hyperphosphorylated tau. Other pathological changes in the brain include gliosis, inflammation, neuritic dystrophy, neuron loss, and changes in neurotransmitter levels.^{2,3} In AD, the development of pathology in the brain is thought to precede cognitive symptoms and, hence, diagnosis of the disease, by many years.

The A β peptide, which comprises 40–42 amino acids, is generated following proteolytic cleavage of the amyloid precursor protein (APP).⁴ Several findings suggest that A β ,

particularly the 42 amino acid form ($A\beta_{1-42}$), is a major factor in the pathogenesis of AD. Mutations in *APP* and in the genes that encode presenilins 1 and 2, (proteins involved in cleavage of APP) are associated with AD in a small number of families. Furthermore, $A\beta$ is deposited in plaques and blood vessels in the brain early in the disease process. Finally, $A\beta$ oligomers and fibrillar aggregates are toxic to neurons.⁵⁻⁷

The ‘amyloid cascade hypothesis’ (Box 1) emphasizes a central role for $A\beta$ in the pathogenesis of AD. Thus, $A\beta$ has become a major therapeutic target, with various anti- $A\beta$ strategies being pursued. These strategies include lowering the production of the peptide by inhibiting the enzymes responsible for $A\beta$ generation, preventing the formation of $A\beta$ aggregates, and increasing the rate of $A\beta$ clearance from the brain. $A\beta$ immunotherapy uses anti- $A\beta$ antibodies, generated following vaccination or introduced passively, to increase the rate of clearance and prevent aggregation of this peptide (Figure 1).

Over the past 10 years, $A\beta$ immunotherapy has emerged from preclinical studies in transgenic mouse models of AD to enter clinical trials in humans. Presently, at least 13 different $A\beta$ immunotherapies are in clinical trials worldwide.⁸ Adverse events, including meningoencephalitis and vasogenic edema, have been noted in some of these clinical trials. Nevertheless, studies of active and passive $A\beta$ immunotherapies are continuing to move forward, with an estimated total enrollment of >9,000 patients. On the basis of results from preclinical and clinical studies, we believe that $A\beta$ immunotherapy has strong potential for preventing AD if patients are immunized before disease onset or in the earliest stages of the disorder. In this Review, we will provide an overview of the preclinical studies in animal models that supported a role for $A\beta$ immunotherapy in the prevention of AD pathogenesis and cognitive deficits. We also will summarize the details of the AN1792 vaccine trial and deliver an update on the active and passive $A\beta$ immunotherapies currently in clinical trials. Lastly, we will outline what we perceive to be important considerations in the development of $A\beta$ immunotherapy for preventing AD.

Preclinical evidence

In the mid-1990s, Solomon and colleagues demonstrated that anti- $A\beta$ monoclonal antibodies dissolved $A\beta$ aggregates and prevented $A\beta$ monomers from aggregating *in vitro*.^{9,10} Subsequently, anti- $A\beta$ antibody-based therapies for AD have emerged.

Active immunization

In 1999, Schenk *et al.* reported the first evidence that active $A\beta$ immunotherapy could reduce $A\beta$ pathology *in vivo*. The researchers vaccinated PDAPP mice—transgenic animals that exhibit amyloid plaque pathology—with pre-aggregated, synthetic $A\beta_{1-42}$. The anti- $A\beta$ antibodies generated following immunization both prevented plaque deposition and led to a reduction of the number of preestablished plaques in the brains of these animals.¹¹ In addition, Vaccination attenuated gliosis and neuritic dystrophy. Numerous studies have confirmed and extended these findings in several transgenic AD mouse models, using various immunogens, adjuvants and routes of administration. In such models, active $A\beta$ immunization initiated before or at the onset of AD-like pathogenesis lowered brain $A\beta$ levels effectively¹²⁻¹⁶ and attenuated behavioral deficits.^{17,18} These findings demonstrated the potential of active $A\beta$ immunization for preventing cerebral $A\beta$ accumulation and cognitive impairment.

The effects of active $A\beta$ immunotherapy on transgenic animals exhibiting well-established AD-like pathology have been variable. In plaque-bearing PDAPP mice (aged 11–15 or 18 months), vaccination with $A\beta_{1-42}$ prevented the formation of new plaques and, presumably, led to a reduction in established plaques.¹¹ In Tg2576 mice (a plaque-bearing transgenic mouse model of AD), however, although $A\beta_{1-42}$ vaccination in young, pre-plaque animals prevented

plaque deposition, the same vaccine was much less effective in clearing pre-established plaques in aged mice.¹⁴ In 18 month-old 3xTg-AD mice—transgenic animals that exhibit both amyloid plaques and NFTs— active vaccination with fibrillar A β _{1–42} did not reduce plaque number, or the levels of insoluble A β or insoluble tau. Immunization did, however, lead to an improvement in behavioral performance and a reduction in soluble A β levels in these animals, suggesting that soluble A β species (for example, oligomers) might be directly linked to the behavioral impairment observed in the 3xTg-AD model.¹⁹ By contrast, in aged beagles that exhibited diffuse plaques and cognitive deficits, A β vaccination led to a reduction in plaque burden but did not alter cerebral A β oligomer levels or improve cognition.²⁰

In general, the anti-A β antibodies described in the studies above recognized various B cell epitopes within the first 15 amino acids of A β ,^{12,21–25} whereas the major T cell epitopes were mapped mainly to the middle region and carboxyl terminus of the peptide.^{24,26} An A β -specific T cell epitope has, however, been reported within the first 16 amino acids of the peptide in Tg2576 mice on a C57BL/6 background and in some human leukocyte antigen transgenic mice.²⁷

Key points

- Preclinical studies support the idea that Alzheimer disease (AD) can be prevented by amyloid- β (A β) immunotherapies
- Adverse events, but some efficacy, were observed in clinical trials of the AN1792 A β vaccine and the passive A β immunotherapy bapineuzumab
- At least 13 A β immunotherapies are in clinical trials in patients with mild to moderate AD
- Second-generation A β vaccines that avoid the adverse events observed with AN1792 and improve antibody generation might improve A β immunotherapy efficacy
- A β immunization might be considerably more efficacious in AD if administered before A β aggregation, thereby protecting the brain from downstream neurodegenerative effects

Box 1

The amyloid cascade hypothesis

The ‘amyloid cascade’ hypothesis places the formation of early, toxic amyloid- β (A β) oligomers and the accumulation of A β aggregates at the center of Alzheimer disease pathogenesis. This hypothesis states that over time, an imbalance in A β production and/or clearance leads to gradual accumulation and aggregation of the peptide in the brain, initiating a neurodegenerative cascade that involves amyloid deposition, inflammation, oxidative stress, and neuronal injury and loss.^{3,102,103} Supporting this hypothesis, *in vitro* and *in vivo* studies in animal models have shown that oligomeric and fibrillar forms of A β cause long-term potentiation impairment^{104,105} and synaptic dysfunction,^{106–108} and accelerate the formation of neurofibrillary tangles that eventually cause synaptic failure and neuronal death.¹⁰⁹

Passive immunization

In young PDAPP mice, passive immunization with an anti-A β monoclonal antibody (m266), which recognized an epitope in the middle region of the peptide (A β 13–28), prevented plaque

deposition, and led to a reduction in the levels of soluble and insoluble A β , as well as an increase in the levels of A β –anti-A β antibody complexes in the blood. These findings supported a role for passive A β immunotherapy in the prevention of AD.²⁸ In older plaque-bearing PDAPP mice, only passive immunization with monoclonal antibodies that recognized the amino-terminal epitopes of A β (that is, not the mid-region or carboxy-terminal epitopes) led to a reduction in pre-established plaques.²⁹ Generally, studies in aged transgenic mice have reported that passive A β immunotherapy, when initiated after the onset of pathology, has lowered the plaque burden and cerebral A β 1–42 levels,³⁰ reduced neuritic dystrophy,²⁹ and reversed behavioral deficits (in some studies behavioral improvements occurred in the absence of changes in brain A β levels).^{31,32} Systemic injections of some anti-A β antibodies, however, while lowering A β -plaque burden, led to an increase in the occurrence of microhemorrhages in areas of cerebral amyloid angiopathy (CAA).^{33,34} In one study, passive immunization with anti-A β monoclonal antibodies directed against the carboxyl terminus of the peptide (A β 28–40) led to a reduction in plaque burden and an improvement in behavioral deficits, despite an increase in microhemorrhage.³⁵ Direct intra hippocampal injection of anti-A β monoclonal antibodies lowered the plaque burden and reduced the rate of early aggregation of phosphorylated tau in 12 month-old 3xTg-AD mice.³⁶ This treatment did not, however, have any effect on more advanced tau pathology, such as NFTs, in older 3xTg-AD mice,³⁶ suggesting that early lowering of A β might have downstream effects on tau pathology.

Passive A β immunization has been shown to have beneficial effects on synaptic plasticity and neuronal function. Intracerebroventricular infusion of anti-A β antibodies protected against synaptic loss and gliosis in Tg2576 transgenic mice.^{37,38} Furthermore, monoclonal anti-A β antibodies against the amino terminus and the middle region of A β prevented synaptic plasticity disruption induced by intracerebroventricular infusion of naturally occurring, cell-derived A β oligomers,³⁹ or of A β dimers from human AD cerebrospinal fluid (CSF).⁴⁰ An anti-A β monoclonal antibody (3D6) that recognizes the free amino terminus was shown to protect dendritic spines and improve structural plasticity in the brains of PDAPP mice.⁴¹

Taken together, preclinical studies of A β immunotherapy suggest that the overall efficacy of this treatment approach might be higher when immunization occurs before the aggregation of A β and tau, and before amyloid accumulates in cerebral blood vessels. Several of the first-generation A β immunotherapies that have been tested in animal models have already moved into the later phases of clinical trials. Furthermore, numerous second-generation treatments are under investigation in animal models and early clinical trials (Box 2). The mechanisms of A β clearance following immunization might vary depending on the stage of disease (Box 3).

Clinical evidence

The AN1792 trials

Design—In the late 1990s, elan and Wyeth developed an A β vaccine for use in humans. This vaccine, AN1792, consisted of a synthetic form of the A β 1–42 peptide and the surface-active saponin adjuvant QS-21. To date, the data from the two AN1792 trials are the only results of major active A β immunization clinical trials that have been made widely available. The first AN1792 trial, initiated in December 1999, was a phase I safety study conducted in 80 patients with mild to moderate AD. These individuals were randomly assigned to one of four treatment groups: AN1792, AN1792 without QS-21, QS-21 only, or placebo. Over the subsequent 6 month period, patients in each group received four intramuscular vaccinations. This phase I trial did not report any notable adverse events.⁴²

Following the results of the phase I study, a phase IIa 15 month trial was initiated in October 2001 to evaluate the efficacy of AN1792 plus QS-21 in AD. A total of 372 patients with mild to moderate AD were enrolled in this double-blind, placebo-controlled, multicenter study. Of

the 300 patients who received AN1792 (plus QS-21), 223 completed the study. In the other arm of the trial, 53 of the 72 patients who received placebo completed the study. This trial was stopped in January 2002 after ~6% of patients developed aseptic meningoencephalitis and leukoencephalopathy.^{43,44} The trial design was subsequently revised to determine the safety and tolerability of AN1792 at 9 months after the last dose of vaccine. The results of the AN1792 trials are summarized below.

Immune response—QS-21 strongly induces T-helper (T_H) 1 lymphocytes. Thus, the AN1792 vaccine was designed to induce a strong cell-mediated immune response, which is needed in the elderly to generate a robust antibody response.⁴⁵ In the phase I trial, however, overall serum anti-A β antibody titers were low, reaching above 1:1,000 in only ~23% of the patients.⁴² During the later stages of the phase I trial, the addition of polysorbate 80 to enhance solubility of the peptide increased the number of antibody responders to almost 60% and modified the immune response from a predominantly T_H2 response to a proinflammatory T_H1 response.⁴⁶

In the phase IIa study, a slight improvement in the antibody titers following AN1792 immunization was obtained, with ~20% of the patients showing serum antibody titers above 1:2,200 after between one and three vaccinations. In these patients, immunization resulted in generation of anti-A β antibodies targeting the amino terminus of A β .⁴⁷ This trial, as stated earlier, was discontinued after 18 of the 300 patients developed meningoencephalitis following between one and three immunizations. This finding led to the conclusion that AN1792 promoted the production of A β -reactive autoimmune T cells, which was probably related to activation of T_H1 lymphocytes. The T_H1 response might have been related to the adjuvant (QS-21) or to the presence of T cell and B cell recognition epitopes in the A β peptide. The B cell epitope (within the first 11–15 amino acids of A β) is considered to be important for generation of anti-A β antibodies, whereas the most common T cell epitope (within amino acids 15–42 of A β)⁴⁸ has been proposed to have initiated the T responses that triggered the meningoencephalitis in some AN1792-vaccinated patients.

Box 2

Second-generation amyloid- β immunotherapies

Efforts are underway to develop novel active and passive amyloid- β (A β) immunotherapies that overcome the safety concerns associated with the AN1792 vaccine and exhibit an increase in efficacy. Most of the treatments described below are currently in preclinical stages of investigation, although some have reached human clinical trials.

An ideal A β vaccine would stimulate a T-helper (T_H) 2 immune response (rather than a T_H1 -mediated cellular immune response) to generate robust anti-A β antibody production that prevents or slows cognitive decline. Examples of second-generation active A β vaccines include: nontoxic, soluble A β derivative immunogens;¹⁵ phage display of A β_{3-6} ;¹¹⁰ short amino-terminal A β fragments that target the B cell epitope while avoiding T cell activation;^{16,111–113} herpes simplex virus amplicons coding for A β ;¹¹⁴ the T_H2 -biased amino-terminal UBITH® A β vaccine (United Biomedical, Hauppauge, NY, USA);¹¹⁵ nonviral DNA A β vaccines;¹¹⁶ DNA vaccines encoding A β amino-terminal fragments, a promiscuous T cell epitope and molecular adjuvants;^{117,118} and A β ‘retroparticles’ (A β_{1-15} displayed on retrovirus-like particles fused to the transmembrane domain of platelet-derived growth factor).¹¹⁹ In addition to the different types of vaccines, various adjuvants and routes of vaccine delivery (such as oral, intranasal and transcutaneous delivery) are under investigation to improve the safety, efficacy and ease of use of these immunotherapies.

Passive A β immunotherapies are also evolving to include not only site-directed humanized monoclonal antibodies (directed to the amino terminus, carboxyl terminus or middle region

of A β), but also conformation-specific antibodies. The latter group includes antibodies that recognize toxic, soluble A β oligomers¹²⁰ and A β -derived diffusible ligands.¹²¹ This group also comprises short-chain variable fragment anti-A β antibodies,^{122–124} anti-A β intrabodies, which target intraneuronal A β ,¹²⁵ and antibodies directed against toxic, modified amino-terminal species of A β .¹²⁶

Box 3

Mechanisms of amyloid- β clearance and immunotherapy efficacy

Several mechanisms could explain how antibodies directed at amyloid- β (A β) promote the clearance of this peptide *in vivo*. When anti-A β antibodies form complexes with A β peptides, the Fc portion of the antibodies might bind the Fc receptor on microglia, inducing phagocytosis of these complexes.³⁰ This mechanism would require that anti-A β antibodies cross the blood–brain barrier (BBB) and bind A β within the CNS. Some evidence has been reported to support this mechanism; however, two studies have demonstrated that Fc-mediated phagocytosis is not required for immunotherapy-induced A β clearance.^{127,128} In an alternative mechanism, anti-A β antibodies in the blood might cause a shift in the concentration gradient of A β across the BBB, thus resulting in an increase in A β efflux from the brain to the periphery.^{28,129} A β antibodies might bind and remove small A β aggregates, thereby neutralizing the effects of toxic A β species on synapses.³⁹ One study suggests that certain antibodies (for example, a midregion A β antibody, m266) sequester monomeric A β inside the brain, facilitating clearance of the peptide and preventing build up of toxic, aggregated forms of A β .¹³⁰

These proposed mechanisms of A β clearance by immunotherapy are not mutually exclusive and might be disease stage-dependent. For example, a preventive vaccine administered before cerebral amyloid accumulation might not require that the antibodies cross the BBB to enhance A β clearance and maintain A β in its monomeric state. By contrast, a therapeutic vaccine (delivered once plaque deposition is well underway) would probably benefit from the transport of A β antibodies into the CNS to induce A β phagocytosis and neutralize local A β toxicity. Circulating anti-A β antibodies in the periphery might pull A β from brain to blood for clearance, thereby preventing further deposition.

Cognitive, MRI and biochemical outcomes—The phase I study was designed to test the safety of the AN1792 vaccine; however, during the trial, immunized patients were noted to display a tendency towards slower cognitive decline than controls.⁴² Thus, in the phase trial, the primary end points included safety, tolerability, and pilot efficacy measures—multiple cognitive measures, volumetric MRI, and CSF levels of A β _{1–42} and phosphorylated tau (phospho-tau). In a single-center analysis of a subgroup of 30 patients involved in the phase IIa trial, improvements were reported in some measures of cognitive performance in six patients with high antibody titers.⁴⁹ Nevertheless, in the phase IIa trial as a whole, no significant effects on cognitive performance—as measured by several neuropsychological tests—were reported in the antibodyresponder group.⁴⁴ A few of the tests related to memory (for example, the z-score across the neuro psychological test battery) showed that the antibody responders exhibited a slower rate of memory deterioration than did the placebo-treated patients ($P = 0.02$). A follow-up study in a subset of patients 4.6 years after AN1792 dosing was halted demonstrated that most of the antibody responders still had detectable anti-A β titers (17 of 19 individuals tested). Moreover, these individuals had a markedly reduced rate of functional decline on the Disability Assessment for Dementia Scale and the Dependence Scale compared with placebo-treated patients.⁵⁰

Volumetric MRI studies revealed that antibody responders had increased brain volume loss compared with controls, and that a dissociation existed between brain volume loss and cognitive function in these individuals.⁵¹ One possible explanation for this dissociation could be that the removal of amyloid by immunotherapy might have occurred late in the progression of the disease at a point when the neurodegenerative pathology and atrophy in the hippocampus was very advanced. Alternatively, the MRI results might have indicated that amyloid removal was accompanied by dynamic changes in fluids, which led to an extensive reduction in hippocampal volume. The dissociation between brain volume loss and cognitive performance might also reflect some negative effects of the vaccination on fiber or white matter volume. Of note, although the hippocampal volume was small in the ANe observed difference in p-tau levels in this subset of patients might not be representative of the entire study. CSF A β ₁₋₄₂ levels in the immunized patient subset were not markedly altered from baseline by AN1792. This lack of difference might be explained by inter-patient variability and the small number of individuals assessed. In conclusion, although the AN1792 trial was halted, this study provided the first indication that A β immunization might affect the pathology of AD, as was predicted by studies in animal models.

Pathological outcomes—Experimental studies in APP transgenic mice showed clear evidence that A β immunotherapy led to a reduction in A β pathology; thus, most case reports of AN1792-vaccinated patients have focused on investigating the effects of the treatment on plaques. In the consideration of these reports, and in the future assessment of A β immunotherapy-treated patients, other aspects of AD neuro pathology, as well as indicators of neuro degeneration, should be considered. AD is a complex neuro degenerative disorder that specifically damages limbic structures, the association neocortical pathways⁵²⁻⁵⁴ and the cholinergic system.^{55,56} Amyloid plaques² and NFTs⁵⁷ are key neuropathological diagnostic features of AD; however, the neuro degenerative process in AD might be initiated by damage to the synaptic terminals.^{58,59} Indeed, early synaptic pathology has been postulated to lead to axonal abnormalities,⁶⁰ dendritic spine⁶¹ and dendrite atrophy⁶² and, eventually, neuronal loss.⁵⁸

Of the 80 patients enrolled in the phase I trial, 42 died before or during follow-up. eight of these AN1792vaccinated individuals were analyzed neuro pathologically.⁶³ This study showed that the A β load in the AN1792 group (2.1% area of neuropil covered by amyloid) was consistently reduced relative to the age-matched, unvaccinated AD group (5.1%). Furthermore, the extent of plaque removal was significantly associated with the mean anti-A β antibody titer for up to 84 weeks after the first dose of vaccine (Kruskal–Wallis test, $P = 0.02$). Despite plaque removal, the eight vaccinated individuals exhibited severe dementia at the time of death. A number of factors might account for this discrepancy between amyloid load and extent of dementia. Most of these patients only received one or two vaccinations and did not complete the trial. Moreover, the removal of pre-existing amyloid deposits might not be sufficient to ameliorate established memory deficits. In addition, by the time the amyloid was removed, the ongoing neurodegenerative pathological processes might have been at an advanced stage and, hence, irreversible.

Neuropathological studies were initially conducted on the brains from 3 of the 18 individuals who developed meningoencephalitis during the AN1792 trial. These studies reported the presence of an unusual form of meningoencephalitis and leukoencephalopathy, with numerous T cells and macrophages infiltrating the white matter and perivascular spaces in these brains.^{64,65} Amyloid plaques were sparse or absent throughout areas of the neocortex in these patients (suggesting a favorable clearance of A β), while other hallmarks of an AD brain, including CAA and NFTs, were identified in the CNS. Similarly, in a single case report of a patient with dementia with Lewy bodies, who exhibited globally stable functional and cognitive features,

A β immunization resulted in a marked clearance of amyloid deposits, with tau and synuclein pathology remaining unchanged.⁶⁶

A neuropathological case study was performed on the brain of a 71 year-old patient with AD who was immunized with AN1792 but did not develop encephalitis (Figure 2).⁶⁷ No amyloid plaques were noted in the frontal cortex in this case, although abundant A β -immunoreactive macrophages were observed in this region. The presence of NFTs and CAA was an indication that the pathology was ongoing. In this patient, the white matter seemed normal, and minimal lymphocytic infiltration of the leptomeninges was observed. Consistent with the findings from this case, a subsequent study of three immunized patients revealed extensive clearance of amyloid and tau-containing neurites. However, other features of tau pathology (NFTs and neuropil threads) as well as CAA were still present.⁶⁵ In fact, a previous study had showed that although parenchymal amyloid was focally disaggregated, vascular deposits were relatively preserved or even increased in two patients with AD approximately 1 year after immunization with AN1792.⁶⁸ Immunoassays revealed that the total soluble A β levels in the gray and white matter were sharply increased in these vaccinated patients compared with unimmunized patients with AD and healthy controls.⁶⁸ In another study, brains were examined from nine AD patients 4 months to 5 years after AN1792 vaccination. The brains from these individuals had markedly increased cerebrovascular A β _{1–42} and A β _{1–40} deposition compared with brains from unimmunized patients with AD.⁶⁹ Remarkably, a complete absence of both plaques and CAA was noted in the brains of two patients who died 4 and 5 years after the first immunization,⁶⁹ raising the possibility that, given time, A β is eventually cleared from the cerebral vasculature.

Current trials

Passive immunization—At least seven passive AD immunotherapies are in clinical trials in patients with mild to moderate AD (Table 1).⁸ In 2008, elan and Wyeth reported that a phase IIa trial of bapineuzimab (AAB-001)—a humanized monoclonal antibody that recognizes the amino terminus of A β —in patients with mild to moderate AD did not meet the study's end points for cognitive efficacy, although a trend for cognitive stabilization was observed.⁷⁰ Post hoc analysis, however, demonstrated significant cognitive benefits from this treatment in multiple tests for patients who did not carry the apolipoprotein E (*APOE*) ϵ 4 allele—a major genetic risk factor for AD. By contrast, only a trend towards benefit was observed in *APOE* ϵ 4 carriers, possibly because of accelerated pathogenesis in these individuals. The outcomes from the post hoc analysis are encouraging; however, these results should be interpreted with caution, as the use of multiple comparisons in post hoc analyses is controversial.

Ongoing clinical trials that include the separation of *APOE* ϵ 4 carriers into different treatment groups, as well as additional cognitive measures, should help to resolve whether bapineuzimab demonstrates efficacy in AD. Several large phase III bapineuzumab trials are currently underway. In five of these studies, more than 4,000 patients have been stratified according to *APOE* ϵ 4 status to see whether this genetic difference affects the efficacy of the drug. In these trials, carriers of *APOE* ϵ 4 are only receiving the lowest dose (0.5 mg/kg) of the treatment as a result of incidences of transient vasogenic edema observed in the phase II study, particularly in this subgroup. An extension study involving 1,350 patients with AD who participated in early bapineuzumab trials is also ongoing. Participants in this study will receive bapineuzumab by intravenous injection for 2.5 years. In April 2009, elan and Wyeth dropped the highest of the three bapineuzumab doses (2 mg/kg) in *APOE* ϵ 4 non-carriers because of the risk of vasogenic edema; these patients are now being dosed with 1 mg/kg.⁷¹ In addition, subcutaneous injection of bapineuzumab is being investigated in 120 patients with AD in a phase II clinical trial.

In 2008, eli Lilly reported that a 12 week phase II trial of solanezumab (LY2062430)—a humanized monoclonal antibody that recognizes the middle region of A β and binds soluble

forms of the peptide—successfully met the safety and tolerability end points. In this study, solanezumab was administered to 52 patients with mild to moderate AD in doses of up to 400 mg per week. The Alzheimer Disease Assessment Scale-Cognition scores and CSF tau levels were unchanged in these patients.^{72,73} Interestingly, amino-terminal truncated A β species (including pyroglutamate-modified A β) were detected in patients' blood following, but not before, passive immunization, suggesting a sequestration of A β from the brain to the periphery.⁷⁴ Two large phase III studies of solanezumab in a total of 2,000 patients with mild to moderate AD are underway.

Another strategy for passive immunization is the injection of intravenous immunoglobulin (IvIg), a pooled mixture of natural human immunoglobulins that include A β antibodies (those recognizing A β oligomers and fibrils, among others).⁷⁵ IvIg antibodies have been shown to interfere with the oligomerization and fibrillization of A β ,^{76,77} protect neurons against A β -mediated toxicity,⁷⁶ and promote A β clearance from the brain.⁷⁸ In a pilot study, IvIg treatment led to a reduction in total A β levels in CSF, an increase in serum A β levels, and stabilization of cognition in five patients with AD.⁷⁹ In one 18 month study in eight patients with mild AD, IvIg therapy was administered for 6 months, stopped for 3 months, and then resumed for 9 months. IvIg-treated patients showed signs of cognitive improvement after 6 months. However, cognitive function declined to baseline during the washout period, and then stayed at baseline during the subsequent 9 months.⁸⁰ A β levels in CSF were reduced only during the periods of IvIg infusion. In addition, anti-A β antibodies were detected in the CSF of the patients following IvIg treatment, indicating that IvIg antibodies might cross the blood–brain barrier and lower A β levels in the brain. Baxter Healthcare and the Alzheimer's Disease Consortium Study have initiated a phase III IvIg study. One other IvIg study, sponsored by Octapharma, is in progress. In addition, several phase I trials of other passive A β immunotherapies are underway (Table 1).

Active immunization—Following halting of the dosing in the phase II AN1792 trial for safety reasons, the second-generation A β vaccines currently in clinical trials have been designed to avoid stimulating adverse immune responses (Table 2).⁸ elan and Wyeth's A β vaccine ACC-001, an A β aminoterminal immunoconjugate, was shown to be safe in a phase I study and is currently in phase II clinical trials in ~360 patients with mild to moderate AD. This phase II trial was put on hold briefly because of a skin lesion in one patient; however, none of the adverse effects observed in the AN1792 trial have been observed in this new study.⁸¹

Novartis also has an A β vaccine (CAD-106) in clinical trials. CAD-106 comprises multiple copies of A β _{1–6} coupled to Qb virus-like particles. A first-in-man trial of CAD-106 was conducted in Sweden in 58 patients with mild to moderate AD. Individuals in this trial received three 50 μ g or 150 μ g doses of the vaccine.⁸² Low antibody titers were observed with the 50 μ g dose of CAD-106; however, at the 150 μ g dose, antibody titers were twofold higher. No differences were observed in the exploratory outcome measures—clinical assessment, A β CSF levels or whole brain volume by MRI—between CAD-106-treated and placebo-treated patients. A phase II trial of this vaccine is underway in ~84 patients with mild AD.

Affiris AG have reported that in separate phase I clinical trials, each conducted in 24 patients with mild to moderate AD, two peptide vaccines mimicking parts of the aminoterminal of the A β sequence, AD01 and AD02, were found to be safe after four single-dose vaccinations were given 4 weeks apart.⁸³ Long-term tolerability phase Ib studies are underway for these vaccines. Merck and United Biochemical both also have active A β vaccines in phase I trials (Table 2).

Improving amyloid- β immunotherapy

Predicting Alzheimer disease

A β immunotherapy has a high potential for lowering cerebral A β levels and protecting cognition; however, as indicated in animal models, this approach to therapy seems to be more effective when administered early in the disease course. Thus, one of the biggest hurdles to preventing AD by immunization is our ability to identify people at risk of developing the disease beyond those individuals bearing genetic mutations in *APP* or the presenilin genes. Currently, a definitive diagnosis of AD is made at autopsy, on the basis of neuropathological presence of amyloid plaques and NFTs—lesions that accumulate over a number of years before neuronal loss and clinical dementia. Progress in biomarkers and imaging technology, combined with more-sophisticated neuro psychological testing has increased the likelihood that cohorts of healthy, middle-aged and elderly individuals at risk of developing AD can be identified. The presence of an *APOE* $\epsilon 4$ allele increases the risk of AD but does not determine exactly when, or indeed if, an individual will develop the disease. Fagan and colleagues have shown that in individuals with very mild or mild AD (Clinical Dementia Ratings 0.5 and 1, respectively), CSF levels of A β_{1-42} are reduced compared with healthy aged-matched controls, while levels of CSF tau and p-tau₁₈₁ are both increased.⁸⁴ A similar profile of CSF changes was reported in patients with mild cognitive impairment (MCI) who later converted to AD.⁸⁵ In another study, low CSF A β_{1-42} levels were associated with a reduction in whole-brain volume, as measured by structural MRI. This finding suggested that as A β aggregates and forms cerebral plaques, brain volume undergoes atrophy.⁷³ In this study, increases in the levels of CSF tau and p-tau₁₈₁ occurred after CSF A β levels decreased. Moreover, these increases in tau levels were more strongly associated with neuronal damage and cognitive decline than were CSF A β levels. One study has reported that 20–40% of elderly individuals without dementia had at least some, if not complete, neuropathological findings of AD at autopsy. Moreover, this study revealed that the presence of AD lesions was associated with cognitive dysfunction in specific tests of episodic memory, semantic knowledge, visual spatial ability, and/or executive function.⁸⁷

Brain imaging, when combined with the afore mentioned biomarkers, greatly increases our ability to predict who is at risk of developing AD several years before disease onset. Brain volume measurements by structural MRI can discriminate between MCI and AD and predict conversion of MCI to AD with high accuracy.⁸⁸ ventricular volume, as measured by MRI, was shown to predict MCI in healthy elderly individuals followed up for 15 years.⁸⁹ Another advance in AD brain imaging is PeT with ¹¹C-labeled Pittsburgh compound B (PIB), which labels amyloid in the brains of living individuals.⁹⁰ In AD, PIB binding has been associated with brain atrophy,⁹¹ a reduction in CSF A β_{1-42} levels,⁸⁴ and a decrease in glucose metabolism.⁹² Somewhat surprisingly, high PIB binding has been reported in healthy elderly individuals, suggesting that cerebral amyloid deposition precedes cognitive impairment in AD.^{93–96} Thus, in the future, a combination of imaging (for example, MRI and/or PIB-PeT) and biomarkers (such as low CSF A β_{1-42} levels and high CSF tau and phospho-tau₁₈₁ levels) might be used to identify and define groups of individuals for AD prevention trials.

Immunotherapy considerations

A β immunization looks like a promising approach for combating AD, yet a number of issues pertaining to the treatments themselves need to be considered for this strategy to succeed, including the type of immunotherapy (Box 4). Indeed, long-term prevention might be more feasible with active than with passive immunization, especially when one considers vaccinating very large populations over long periods of time. Strategies targeting specific A β conformations that are known to be toxic to neurons might benefit from antibodies directed against the amino terminus (immunodominant epitope of A β), given the poor accessibility of

other portions of A β in aggregates.²⁵ Whether dissolving existing plaques will result in the release of soluble toxic A β species (for example, oligomers) and, if so, for how long, remains unclear. Immunization before plaque deposition might avoid this problem. As a result of A β immunotherapy-induced microhemorrhage in transgenic mouse models of AD with CAA and vasogenic edema in humans (especially in *APOE* $\epsilon 4$ carriers), immunization might need to be administered before the build up of amyloid in blood vessel walls to avoid these adverse effects. Intraneuronal A β might act as a nidus for A β deposition (intra neuronally and extracellularly) in the soma and along processes and terminals of affected neurons. Thus, limiting intra neuronal A β formation at an early stage of AD development might be protective.^{97,98} An immunological approach for the reduction of A β formation by targeting the β -secretase cleavage site of APP has been proposed.⁹⁹

Box 4

Active versus passive amyloid- β immunization

Both active and passive amyloid- β (A β) immunotherapies have their advantages and disadvantages. Active A β immunotherapy has the potential to be more cost-effective and long-lasting, with fewer visits to the doctor, than passive immunization, which requires monthly infusions of costly humanized monoclonal antibodies. Active therapies might have additional benefits. One study has reported that A β vaccination in nonhuman primates led to an increase in the production of crossreactive, potentially protective A β autoantibodies.¹³¹ Such antibodies are typically lower in patients with AD than in age-matched healthy individuals;¹³² therefore, boosting the levels of these proteins might be beneficial.

Vaccination usually involves delivery of a strong adjuvant to boost antibody production. Such adjuvants could potentially induce an undesirable immune response, especially in elderly individuals in whom proinflammatory cytokines are already above normal levels.¹⁰⁰ In these cases, a passive A β immunotherapy might be beneficial. One way to circumvent this problem for A β vaccines could be to use anti-inflammatory T helper 2-biased adjuvants, which might help avoid unwanted adverse effects while still increasing antibody production. Another problem for active therapies is that if an adverse event does occur, the immune response to the active vaccine can be difficult to stop quickly. Passive A β immunotherapies, on the other hand, can be stopped at any time. Another advantage to the passive therapeutic approach is the use of antibodies that are specific for particular A β conformations or species thought to be most toxic, thereby avoiding removal of all A β from the brain. One disadvantage of the passive approach is the potential for patients to eventually develop neutralizing antibodies against the passive therapy.

The ability of the human body to generate a strong humoral response or fight infection declines with aging, a condition known as immunosenescence.^{100,101} In addition, the immune system might participate in AD pathogenesis. Chronic exposure of humans and transgenic mice to A β aggregates seems to lead to cellular and humoral hyporesponsiveness to the peptide, which could, in turn, contribute to the disease process.²⁶ Thus, a preventive AD vaccine started before the build up of aggregated A β and/or immunosenescence (for example, at 50–60 years of age) might lead to a more robust and long-lasting humoral immune response and, hence, high levels of production of A β -lowering antibodies. A prevention trial, however, could take many years and incur high costs. Identifying individuals at risk of developing AD might improve the feasibility of such trials.

Many healthy middle-aged individuals with a family history of AD might be willing to participate in a clinical AD prevention trial; however, before any such trial could take place, safety and efficacy of the immunotherapy in patients with AD will probably be required. Unfortunately, little evidence exists that A β immunotherapy can reverse cognitive impairment

in patients with mild to moderate AD. Indeed, stopping the progression of neuro degeneration and cognitive decline once it is well underway might be difficult, even in the context of plaque clearing,⁶³ suggesting again that early immunization might improve the efficacy of A β immunotherapy. Ongoing clinical trials should provide some further clarification about whether A β immunotherapy is effective in patients who already have AD. These trials, if negative, will still not determine whether AD can be prevented if A β is lowered presymptomatically, before the onset of substantial neurodegeneration.

Conclusions

A β immunotherapy continues to show promise as a strategy for preventing AD or treating the disease in its early stages. Preclinical studies in animal models have shown clear evidence that A β immunization can prevent cerebral amyloid pathology, synaptic degeneration and cognitive deficits. Early human clinical trials of the AN1792 A β vaccine were associated with adverse effects but also showed some signs of efficacy. Indeed, on the basis of these studies, at least 13 A β immunotherapies are currently in human clinical trials, mostly in patients with mild to moderate AD. If successful, these trials could lead to the first evidence of a disease-modifying treatment for AD. Moreover, such an outcome would indicate successful translation of preclinical studies in animal models to clinical efficacy in humans.

A β immunotherapy might, in the future, be an effective treatment for preventing AD. Imaging and bio markers have improved dramatically over the past 10 years, increasing the probability of identifying at-risk individuals before clinical symptom onset and allowing the A β immunotherapy response to be monitored. If given early, before AD pathogenesis is well underway, A β immunization might be able to prevent aggregation of neurotoxic forms of A β , thereby preventing downstream effects, such as synaptic dysfunction, neuronal damage and cognitive impairment.

Review criteria

Articles for review were identified from PubMed and Google searches using the following terms: “A β immunotherapy”, “immunotherapy and Alzheimer’s disease”, “Alzheimer’s vaccine”, “A β vaccine”, “A β immunization”, “AN1792”, “CAD106”, “bapineuzumab”, “solaneuzumab”, “Alzheimer’s disease clinical trials”, “affitope”, and “Alzheimer’s disease treatments”. Only articles published in English and from 1995 were retrieved and considered for review. Information regarding A β immunotherapy clinical trials was found on clinicaltrials.gov and company websites. Abstracts and reports from meetings were used to find the most recent results of clinical trials.

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Figure 1.

Active and passive immunization approaches. **a** | Vaccination (active immunization) activates the body's immune system to produce antigen-specific antibodies. In AD, full-length Aβ or a fragment of Aβ conjugated to a foreign T cell epitope carrier protein can be used as an antigen, which is delivered into the body alongside an immune system booster (adjuvant). The humoral immune response is generated when APCs, which take up and process the antigen, present T cell epitopes to naive T_H lymphocytes, activating the latter (first signal). Binding of co-stimulatory molecules on the surfaces of APCs and T cells provides a secondary signal that enhances T cell activation. Meanwhile, the soluble antigen binds to B cell receptors, via the B cell epitope, and this antigen is presented to activated T cells to help the B cell make antibodies against the antigen. Activated T cells also produce cellular immune responses. A T_H1 cellular immune response leads to the release of pro-inflammatory cytokines, whereas a T_H2 response causes release of anti-inflammatory cytokines. **b** | Passive immunization bypasses the need for the body to mount an immune response to produce antigen-specific antibodies. In both active and passive Aβ immunization, anti-Aβ antibodies bind Aβ, targeting the peptide for clearance. Abbreviations: Aβ, amyloid-β; APC, antigen presenting cell; T_H , T helper.

Figure 2.

Neuropathological findings in an AN1792-immunized patient with Alzheimer disease. A 71 year-old male patient with a 10 year history of dementia was administered three doses of AN1792. He died a year later due to failure to thrive, but did not develop meningoencephalitis.

a | Gross morphology of the brain showed preservation of the limbic structures and cortical ribbon. **b** | In the frontal cortex, although amyloid- β immunoreactive macrophages were abundant, no amyloid plaques were detected. **c** | Cortical vessels exhibited persistent amyloidosis despite removal of the surrounding amyloid plaques. **d** | CD4-positive T cells (indicated by brown staining) were frequently located around blood vessels. **e** | In areas where amyloid was removed, the neuritic network (labeled with an anti-neurofilament antibody) appeared preserved.

Table 1

Ongoing clinical trials of passive A β immunotherapies

Therapy	Sponsors	Antibody	Phase and number of trials	Estimated patient enrollment	treatment duration	Primary outcome measures	Estimated completion date of final trial
Bapineuzumab (AAB-001)*	Elan; Wyeth; JANSSEN Alzheimer Immunotherapy	Anti-A β amino terminal MAb	III; one trial	1,350	2.5 years	Safety and efficacy [‡]	July 2012
Bapineuzumab (AAB-001)*	Elan; Wyeth; JANSSEN Alzheimer Immunotherapy	Anti-A β amino terminal MAb	III; five trials	4,650 [§]	18 months	Cognition and global function	April 2011
Solanezumab (LY2062430)	Eli Lilly	Anti-A β mid-region MAb	III; two trials	2,000	19 months	Cognition and global function	July 2012
Gammagard™ IVIg (10%) [¶]	Baxter Healthcare; Alzheimer's Disease Consortium Study	Pooled human antibodies	III; one trial	360	18 months	Cognition and global function	July 2011
Bapineuzumab (AAB-001) [¶]	Wyeth	Anti-A β mid-region MAb	II; one trial	120	6 months	Treatment-related adverse effects	March 2010
PF-04360365	Pfizer	Anti-A β MAb	II; two trials	211	12–18 months	Safety, tolerability and pharmacokinetics	November 2011
R1450	Hoffman-LaRoche	Fully human MAb	II; one trial	60	3–12 months	Safety and tolerability	May 2009
IVIg (10%)	Octapharma	Pooled human antibodies	II; one trial	56	6 months	Changes in plasma A β levels	September 2009
GSK933766A	GlaxoSmithKline	Anti-A β antibody	I; one trial	122	12 months	Safety, tolerability and treatment-related adverse effects	August 2010

* Delivered by intravenous injection.

[‡] Long-term extension of earlier AAB-001 phase II trial.[§] Participants stratified according to apolipoprotein E genotype.[¶] Gammagard produced by Baxter Healthcare, Deerfield, IL, USA.^{¶¶} Delivered by subcutaneous injection. Abbreviations: A β , amyloid- β ; IVIg, intravenous immunoglobulin; MAb, monoclonal antibody.

Table 2

Ongoing clinical trials of active amyloid- β immunotherapies

Therapy	Sponsors	Vaccine	Phase and number of trials	Estimated patient enrollment	treatment duration	Primary outcome measures	Estimated completion date of final trial
ACC-001	Elan; Wyeth	A β amino-terminal conjugate	II; three trials	360	24 months	Safety, tolerability and treatment-related adverse effects	May 2012
CAD-105	Novartis	A β ₁₋₅ coupled to Qb virus-like particles	II; three trials	84	12–24 months	Safety and tolerability	June 2011
Affitope AD01; Affitope AD02	Affiris AG; GlaxoSmithKline	A β amino-terminal mimotope $\pm$ adjuvant	Ib; two trials	48	12 months	Safety and tolerability*	December 2009
V950	Merck	A β amino-terminal peptides conjugated to ISCO-MATRIX [‡]	I; one trial	124	48 months	Safety and tolerability	April 2014
UB311	United Biochemical	A β ₁₋₁₄ using UBITH [§]	I; one trial	18	7 months	Safety and tolerability	December 2010

* Long-term extension of previous phase I trial.

[‡] ISCO-MATRIX[®] produced by CSL Behring, King of Prussia, PA, USA.

[§] UBITH[®] produced by United Biomedical, Hauppauge, NY, USA. Abbreviation: A β , amyloid- β .