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Rationale for Peptide and DNA Based Epitope Vaccines for Alzheimer's Disease Immunotherapy

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Abstract

Amyloid-beta (A β) immunotherapy has received considerable attention as a promising approach for reducing the level of A β in the CNS of Alzheimer's disease patients. However, the first Phase II clinical trial, for the immune therapy AN1792, was halted when a subset of those immunized with A β ₄₂ developed adverse events in the central nervous system. In addition, data from the trial indicated that there was a low percentage of responders and generally low to moderate titers in the patients that received the vaccine. Generated antibodies reduced β -amyloid deposits in the parenchyma of patients' brains, but no reduction in soluble A β or significant improvements in cognitive function of patients were observed. These data and data from pre-clinical studies suggest that reduction in the most toxic oligomeric forms of A β is important for prevention or slowing down of the progression of cognitive decline, and that vaccination should be started prior to irreversible accumulation of the oligomeric A β , at the early stages of AD. Protective immunotherapy requires a development of safe and effective strategy for A β immunotherapy. In this review, the rationale for developing epitope vaccines for the treatment of AD will be discussed. We believe that an epitope vaccine will induce an adequate anti-A β antibody response in the absence of potentially adverse self T cell-mediated events, making it possible to start immunization at the early stages of AD.

Keywords

Alzheimer's Disease; peptide vaccine; DNA vaccine; epitope vaccine; immunotherapy

OVERVIEW OF ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is one of the most devastating diseases of the century, and the risk of this disease increases drastically along with advancing age. AD is characterized clinically by an insidious onset and progressive cognitive decline that impacts memory, language, judgment and orientation in time and space. According to a study led by researchers at the Johns Hopkins Bloomberg School of Public Health, more than 26 million people worldwide were estimated to be living with Alzheimer's disease in 2006 [1]. The researchers concluded that the global prevalence of Alzheimer's disease will grow to more than 106 million by 2050. By that time, 43 percent of those with Alzheimer's disease will need high-level care, equivalent to that of a nursing home. Even modest improvement in prevention rates or in a delay of the onset and progression of the disease will have enormous economical impact on public health care system.

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The neuropathological features of the disease include neurofibrillary tangles (NFT), deposition of A β in senile plaques, and neuronal loss in affected brain regions [2]. These pathological changes result in a profound loss of synapses over the course of the disease, thereby contributing to a progressive reduction in the functional capacity of the patient. The origins of these neuropathological changes are largely unknown and likely heterogeneous. It is known that approximately 1% of the AD cases are associated with mutations in amyloid- β precursor protein (APP), presenilin-1 and presenilin-2[3]. However, 99% of AD cases are not associated with known mutations and the nature of the etiological agent(s) is not yet understood [4]. Nevertheless, independent of these causative factors, the consequences are very common and lead to the same neuropathological changes.

A critical aim in developing therapeutic interventions for AD is the identification of suitable targets. According to the Amyloid Cascade Hypothesis adopted by the majority of AD researchers, an over-production of A β is the primary pathological event, which precedes tau accumulation and leads to neurofibrillary degeneration and dementia [5,6]. The A β peptide is cleaved from the integral membrane amyloid precursor protein (APP) by β -secretase to yield a secreted fragment of APP β and a C-terminal fragment (CTF) of APP (CTF β). CTF β is then cleaved by γ -secretase within the membrane to yield a smaller C-terminal fragment (CTF γ) and A β fragments of various lengths [7–9]. Over-production of these peptides, as well as the failure of their degradation by enzymes such as neprilysin and insulin degrading enzyme, lead to their oligomerization and aggregation over time to produce senile plaques. Therefore, therapeutic interventions have been aimed at preventing A β over-production or accelerating its degradation. Scientists have long considered A β peptides to be byproducts of APP cleavage and investigated mostly the toxic features of these peptides. Recent studies clearly demonstrated that this proteolytic pathway is a physiological process and A β becomes toxic when its levels are excessive. It has been shown that neuronal activity increases the formation of A β and that increased A β leads to depression of excitatory synaptic transmission [10]. These two findings have led to a hypothesis that A β may normally serve as a negative feedback signal that maintains neuronal activity within a normal dynamic range: too much neuronal activity leads to formation of more A β , which depresses excitatory synapses and reduces neuronal activity.

Currently it is known that genetically-derived familial AD is a consequence of over-production of A β . In contrast, sporadic AD may be a result of age-related impairment of A β elimination from the brain. Several pathways for elimination of A β have been identified: microglia-mediated clearance [11,12], degradation by neprilysin and insulin degrading enzyme [13,14], efflux from the brain by active transport across the blood brain barrier (BBB) as a complex with low density lipoprotein receptor-related protein 1 (LRP)[15], or drainage with interstitial fluid along capillary and arterial blood vessel walls [16,17]. When age-related accumulation and aggregation of A β occur due to failure of these pathways, the immune system recognizes these aggregated proteins as “foreign” and attempts to remove them. Studies have demonstrated the presence of anti-A β antibodies in the blood and CSF of healthy humans and AD patients. However the titers of these antibodies are lower in AD patients compared with healthy individuals, suggesting that the immune system may be capable of protecting against AD [18–20].

In summary, when developing therapeutic drugs aimed to decrease A β production, one should have in mind that A β has a role to play in the normal function of the nervous system. The presence of anti-A β antibodies in healthy, elderly people supports the concept that immunotherapy may be a way to decrease the level of overproduced A β peptide and prevent its oligomerization and aggregation without complete inhibition of its constitutive production and physiological function.

A β IMMUNOTHERAPY

Pre-Clinical Studies

In 1999, Schenk and colleagues reported that a vaccine that contained fibrillar A β_{42} when injected into APP-transgenic mice prevented the deposition of A β , as well as the development of dystrophic neurites and astrogliosis in their brains. Other researchers have reproduced and extended the original findings to include studies showing that active A β immunization with different peptide immunogens and routes of administration can generate anti-A β antibodies capable of lowering the cerebral A β burden and blocking the loss of cognitive abilities in APP/Tg mice as they accumulate A β with aging [21,22]. Studies of peripheral administration of anti-A β antibodies showed the presence of these antibodies in the brain [22,23] and the reduction of cerebral A β load if given before robust plaque deposition. Passive immunization improves behavior in APP/Tg mice, even in aged animals having cerebral A β loads unchanged *via* immunization [24–27]. These results clearly demonstrated that anti-A β -specific that anti-A β -specific antibodies are sufficient for clearing amyloid deposits from the brains of APP/Tg mice. Importantly, the reductions in neuropathology were associated with an improvement of behavioral functions in the immunized animals. At the time when Elan and Wyeth, encouraged by these data, started clinical trials for AN-1792, the only adverse responses observed in mouse AD models following A β immunotherapy were the three to four-fold increase of cerebral amyloid angiopathy (CAA) severity and the occurrence of microhemorrhages within the brain vasculature of very aged APP/Tg mice injected weekly with high doses of anti-A β monoclonal antibodies [28]. The sites of microhemorrhage have been co-localized with cerebral vascular A β deposits. Similar results were obtained in different strains of APP Tg mice [27,29,30]. Only later was it reported that active immunizations of APP/Tg mice with fibrillar A β_{42} formulated in a strong conventional adjuvant (CFA/IFA) could also increase microhemorrhages in the brains of vaccinated animals [31].

Outcomes from the AN-1792 Clinical Trials

In the Phase I clinical trial initiated by Elan Pharmaceuticals in 2000, patients with mild and moderate AD were injected with fibrillar A β_{42} formulated in strong Th1-type adjuvant QS21. 53% of patients generated positive antibody response without any obvious adverse events. A Phase II trial started in 2001, which was unexpectedly terminated before the subjects received all of the planned vaccinations when 6% of the subjects developed meningoencephalitis.

Positive outcome—The most important positive outcome of this trial is the evidence that anti-A β antibodies are capable of changing AD pathology. Post-mortem analysis of the brains of eight immunized patients has shown lower A β load compared with unimmunized controls. There was no evidence of a relationship between the degree of A β plaque removal and AN-1792 dose, whereas the mean antibody response during the treatment period was inversely correlated with post-mortem A β load [32]. In the subset of antibody responders, CSF tau was reduced compared with baseline level and the change was greater than in the placebo group. There was an interesting observation that in regions where plaques have been removed, tau-containing plaque-associated dystrophic neuritis was absent. Nevertheless, no effect was seen on CSF levels of A β_{42} . Importantly, a correlational trend in regression of the neuropsychological test battery (NTB) composite Z-scores and the geometric mean antibody titers was observed. Although this correlation was not significant, the direction of the trend favored higher IgG antibody titers [33]. Follow-up studies of a Zurich cohort of 30 patients with mild and moderate AD showed significantly slower rates of decline of cognitive functions in responders compared to patients without anti-A β plaque recognizing antibodies. Importantly, there was a dose-response relationship between the increase in serum antibodies against A β plaques and the clinical outcome [34]. Overall, even low/moderate titers of anti-A β antibodies generated in a small subset of immunized patients (19.7%) were capable of reducing parenchymal amyloid

pathology [35–41] and slowing the progressive cognitive decline associated with the disease [33,34].

Negative outcome—Although the results from the Phase I trial showed good tolerability, in the phase IIa portion of the AN-1792 immunotherapy a subset of individuals developed adverse events in the central nervous system [34–38,41–43]. Importantly, only vaccinated participants (n=18) developed meningoencephalitis, whereas none of the control patients (n=72) injected with placebo developed adverse events [42]. Postmortem examination of the brains from two vaccinated AD patients with neuroinflammation [35,36] showed an infiltration of T cells in the leptomeninges, densest in the areas with amyloid angiopathy. There was also sparse T cell involvement in the cerebral cortex, perivascular spaces and within the parenchyma. In the first case report, infiltrated T cells (CD3⁺, CD45RO⁺) were primarily CD4⁺, although a few CD8⁺ cells were also detected. B cells (CD79a⁺ and CD20⁺) were not present in the brain. In addition, diffuse abnormalities in the cerebral white matter were observed, with a marked reduction in the density of myelinated fibers and extensive macrophage infiltration [35]. The second case report generally agreed with the first one, although there were some distinct differences, such as the presence of mostly CD8⁺ instead of CD4⁺ mature T cells, multi-nucleated giant cells filled with dense deposits of A β , and multiple cortical hemorrhages [36]. Another case report on an AD patient without clinical presentation of encephalitis also demonstrated some lymphocytic infiltration in the leptomeninges [37]. These results indicate that anti-A β -specific T cells may induce significant side effects in AD patients vaccinated with full-length A β ₄₂. The immunized cases had severe CAA compared with unimmunized cases. Although the pathophysiology of these side effects is not completely clear, the main feature was T cell infiltration associated with the meninges being affected by CAA.

Importantly, the same case reports indicated that anti-A β antibodies were not responsible for the observed adverse effects after active vaccination, and that the AN1792 vaccine was beneficial for AD patients because it significantly reduced amyloid β -plaque load. However a recent assessment of the relationship between A β ₄₂ immune response, degree of plaque removal and long-term clinical outcomes demonstrated that clearance of amyloid plaques in the brains of AD patients is not accompanied by the prevention of progressive neurodegeneration [32]. These data suggest that plaque removal is not enough to stop the progression of neurodegeneration and to improve cognitive function. It is possible that amyloid plaques may represent a defense mechanism that removes potentially neurotoxic soluble/oligomeric forms of A β peptide. If this is true, the disruption of amyloid plaques without the efficient removal of soluble A β , as was observed in the AN1792 trial [32,38] could increase adverse events such as CAA and microhemorrhages without improvement in cognitive functions.

Conclusions from the AN-1792 clinical trials—The results of the first vaccination of elderly AD patients with the AN-1792 vaccine confirmed that anti-A β antibodies are beneficial for AD patients and may at least slow the progression of disease. However this trial raised concerns about the safety and the efficacy of the active immunization strategy with A β ₄₂ self-peptide. The low number of vaccine responders, the relatively low antibody titers generated even after multiple immunizations suggest that alternative immunotherapeutic strategies should be pursued. New generation vaccines should be able to induce high titers of anti-A β antibodies without the generation of autoreactive T cell activation. Th2-type rather than Th1-type adjuvant should be used to avoid inflammation in the brain. In addition, lessons from the AN-1792 trial showed that even anti-A β antibodies may reduce the amyloid plaques in the brain of vaccinated subjects, the increase of soluble A β in result of the solubilization of these plaques may have more toxic effect on neurons [38]. Therefore AD immunotherapy may be most effective if started before the accumulation of toxic forms of A β peptide in people with

early pre-clinical stages of AD or even in healthy people with family history or genetic susceptibility to AD. Hopefully, the diagnosis of AD at very early stages will be possible in the nearest future by measuring biomarkers such as the Tau/A β and pTau/A β ratios in CSF [44–46] and/or detecting accumulation of A β in the brains using PIB-PET scans [47]. The availability of a safe and effective vaccine at that time would allow the prevention or at least a delay in the onset and progression of AD.

NEW GENERATION AD VACCINES

As discussed above, one challenge associated with the clinical use of the self A β_{42} peptide as a vaccine is the potential for the development of unwanted anti-A β /APP Th1 immune responses [35,36,42]. The molecular basis for this reactivity is the uptake of fibrillar A β_{42} (fA β_{42}) by antigen-presenting cells (APC) and presentation of self-epitopes to T cells through MHC class molecules. If these epitopes are recognized by specific Th1 lymphocytes, the T cells will become activated and produce proinflammatory cytokines. In addition, activated microglia that are a major source of inflammatory factors and are associated with A β plaques in AD brain samples, can mediate the permeability of the BBB and recruit immune cells from the periphery. Subsequently, activated microglia expressing MHC class II molecules may process and present A β peptide to A β -specific Th1 cells that have crossed the BBB, and induce additional inflammation. Although the activation of microglia as a response to signals from injured neurons is a protective process [48], chronic inflammation may be harmful and lead to neurodegeneration [48]. Neurons have only a limited capacity for repair and cannot tolerate long-term inflammation, so inflammatory processes must be tightly regulated [49].

Different types of T cells may have different effects on microglial cell activation and amyloid clearance. Th1 cells can promote microglia activation and inflammation through secretion of IFN γ and up-regulation of MHC class II molecules by microglial cells. The anti-inflammatory cytokines produced by Th2 cells have been shown to exhibit neuroprotective properties [49] and appear to support eventual microglia apoptosis [50] following an inflammatory stimulus, and have been demonstrated to act as an important feedback mechanism to turn off an inflammatory response *in vivo* [51]. Glial cells, in turn, can facilitate apoptosis of infiltrating T cells in order to reduce brain inflammation. Interestingly, one report demonstrated that Th1, but not Th2 cells underwent apoptosis after such activation [52]. Recently, interesting studies have been performed to demonstrate the effect of T cells and related cytokines on A β degradation in human primary cultures of macrophages and microglia. These studies demonstrated that proinflammatory cytokines inhibited, whereas anti-inflammatory and regulatory cytokines (IL-4, IL-10 and TGF β) enhanced A β degradation in macrophages. IFN γ and TNF α directly suppressed the expression of A β degradation enzymes in monocyte-derived macrophages [53]. These data also support the suggestion that generation of Th2 response in AD patients is more beneficial and less harmful than Th1 response.

Studies show that cognitively normal elderly and AD patients have an increase in auto-reactive T cells specific for the A β peptide [52]. It is possible that AD patients developing meningoencephalitis in the AN-1792 clinical trial had stronger autoimmune T cell responses and inflammation in the brain that was elicited by immunization with fA β_{42} formulated in the strong Th1-type adjuvant, QS21. Thus, it may be important to induce anti-inflammatory Th2 cell responses instead of triggering additional activation of Th1 cells that induce an increased inflammatory environment in the brain. Another way to avoid additional activation of autoreactive T cells is the replacement of self A β Th cell epitope with a foreign Th cell epitope while keeping the self-B cell epitope intact. Fortunately, studies mapping B and T cells epitopes of A β_{42} demonstrated that these two epitopes are distinct (see next section).

B cell epitope/s of A β ₄₂

Several groups have endeavored to map murine A β B cell epitopes using small peptides derived from A β ₄₂ and antisera from mice immunized with fibrillar A β ₄₂ [54–58]. For example, Lemere *et al.*, [56,57] described intranasal vaccination of PDAPP mice and determined that among six overlapping peptides, only A β _{1–15} abolished immunoreactivity of experimental sera with AD plaques in tissue sections. Using four peptides spanning aa 1–16, 10–20, 20–29, and 29–40 of A β ₄₀, another group [55] demonstrated that antisera from PS1/Tg mice recognize A β _{1–16} along with A β ₄₀ and A β ₄₂ peptides. We also demonstrated that A β _{1–15} was the major epitope recognized by antibodies raised in wild type and APP/Tg mice immunized with fibrillar A β ₄₂ formulated in different adjuvants [58]. These results concur with other reports, and support that the A β _{1–15} peptide represents the major B cell epitope of A β ₄₂ in mice of different haplotypes and in APP/Tg 2576 animals. In addition, these data have been confirmed using proteolytic cleavage of intact, immobilized immune complexes between A β ₄₂ and polyclonal antibodies from APP/Tg mice immunized with protofibrillar aggregates of A β ₄₂ followed by high-resolution Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) [59]. This analysis revealed AP4–10 as the smallest region of the A β peptide that was recognized with high affinity by these antibodies. Part of this antigenic epitope (3EFRH₆) was found in amyloid plaques *in situ* and antibodies to this epitope had therapeutic effects *in vitro* [60–63]. It has also been demonstrated that antisera against aa 1–11, 1–7, and 1–5 of A β ₄₂ are more potent in binding to β -amyloid plaques and triggering *ex vivo* phagocytosis than antibodies to aa 3–7, 5–11, and 11–26 [64]. In addition, these experiments were confirmed by using polyclonal rabbit antibodies to the fibrillar A β ₄₂ [65]. Such antibodies bound to the linear A β _{1–8} sequence with relatively high affinity. Importantly, epitope mapping of antibodies generated after AN-1792 vaccination are also primarily N-terminal specific [66].

T cell epitopes of A β ₄₂—Two human immunodominant cytotoxic and T helper epitopes predicted by computer models are located at the central and C-terminal regions, respectively [67–69]. Monson *et al.* mapped the major T cell epitope in the A β _{15–42} peptide, which is segregated from the dominant B cell epitopes identified in A β _{1–15} is [70]. Fine mapping using five overlapped peptides within A β _{15–42} peptide showed that T cell epitopes were most frequently localized in the A β _{16–30} region.

We mapped T helper responses to A β ₄₂ in immune BALB/c mice and demonstrated *in vitro* T cell activation after re-stimulation with peptides A β _{6–20}, A β _{1–28}, or A β _{1–40}, but not with A β _{1–15} [58]. Although these data with wild type animals of H2^d haplotype indicate that smaller overlapping peptides might be used to map the T cell epitope of A β ₄₂ more precisely, it is clear that the A β _{1–15} peptide does not possess the T cell epitope of A β ₄₂ in these mice. However, Das *et al.* demonstrated that the major T cell epitope in APP/Tg mice, as well as HLA-DR3 and HLA-DQ8 Tg mice, is A β _{1–16}. T cells from HLA-DR4 mice responded to both A β _{1–16} and A β _{11–28} whereas HLA-DQ6 Tg mice failed to mount significant B and T cell responses to A β _{1–42} [71]. These data indicated that T cell epitopes recognized by different HLA haplotypes may be localized in different regions of the A β peptide. Therefore, it is reasonable to minimize the size of the B cell epitope to avoid the activation of autoreactive T cells in humans bearing specific MHC class II haplotypes. One way of achieving this is to design a vaccine that includes a self A β B cell epitope plus a foreign MHC class II T cell epitope that would provide T help for the generation of an effective anti-A β antibody response. This approach might avoid the generation of T cell immunity against A β and preclude the concerns raised in the AN-1792 clinical trial.

Epitope Vaccines

To estimate the impact of antibodies against different parts of the A β peptide in reduction of plaque burden and neuronal protection, Bard *et al.* immunized PDAPP mice with different N-

terminal fragments ($A\beta_{1-5}$, $A\beta_{3-9}$, $A\beta_{5-11}$) or a fragment derived from an internal region of the peptide ($A\beta_{15-24}$) conjugated with a T cell epitope derived from ovalbumin. This study demonstrated that antibodies specific to the N-terminal region of $A\beta$ are important for plaque clearance and neuronal protection *via* an Fc-mediated mechanism [64]. Based on these data, different groups started to work on the design and further refinement of epitope vaccines composed of various N-terminal fragments of $A\beta$ and non-self T cell epitopes.

First generation epitope vaccines tested in mice were based on the $A\beta_{1-15}$ peptide [81]. We decided to incorporate a foreign Th cell epitope into the $A\beta_{1-15}$ vaccine to activate non-self antigen-specific T lymphocytes which would initiate and direct the formation of anti- $A\beta$ antibodies. Incorporation of a promiscuous foreign T cell epitope into the $A\beta$ -immunogen provides a number of significant advantages to $A\beta$ -immunotherapy design. We selected PADRE (aK-Cha-VAAWTLKAAa, where “a” is D alanine, “Cha” is L-cyclohexylalanine), a synthetic, non-natural Pan HLA DR-binding Epitope [72,73], as our candidate foreign T cell epitope. The potency of this molecule to generate Th cells that induce strong antibody responses against many different antigens is well established [74–78]. When combined with disease-specific antigens, PADRE induces important co-stimulatory signals that amplify the antigen-specific immune response, which generates high titer and high affinity IgG antibodies [72, 73,75]. PADRE tested against human T cells in a proliferation assay was found to be 100-fold more potent on a molar basis than a tetanus-derived universal epitope [79]. In addition, PADRE binds with high affinity to 15 of 16 of the most common HLA-DR types tested to date and with high/intermediate affinity to mouse I-A^{b/d} and I-E^{b/d} MHC haplotypes [72,73,75]. Because of its binding promiscuity, PADRE is likely to overcome the problems posed by the extreme polymorphism of HLA-DR molecules in the human population. Several other advantages of PADRE made it preferable as a T cell epitope over other carrier-proteins (OVA, KHL, Tetanus Toxoid-derived universal epitope, or MTB) traditionally used to make vaccines: (i) PADRE can be easily synthesized; (ii) the antibody responses are generated not to PADRE, but to the B cell epitope only; (iii) it has been used in human clinical studies and has been shown to be safe and well tolerated [78]. Thus, we coupled the foreign T cell epitope PADRE with an $A\beta$ B cell epitope and predicted that the vaccine would: (1) activate non-self PADRE-specific Th lymphocytes; (2) induce therapeutic anti- $A\beta$ antibody formation; and (3) help to generate a more potent antibody production than with the self-T cell epitope of $A\beta$.

PADRE- $A\beta_{1-15}$ was synthesized on a 4-branched multiple antigenic peptide (MAP) backbone to generate PADRE- $A\beta_{1-15}$ -MAP. MAPs are capable of inducing high titer antibody responses specific to many different antigens because multiple copies of immunogen attached to the core matrix are significantly more potent [80]. BALB/c mice, known as moderate responders to the PADRE T cell epitope [77], generated robust anti- $A\beta_{42}$ humoral immune responses after immunization with the PADRE- $A\beta_{1-15}$ -MAP epitope vaccine formulated in the Th2- type adjuvant, alum. The antibody titers were equivalent to titers in mice immunized with $A\beta_{1-33}$, which contains both the B and T cell epitopes of $A\beta_{42}$. Thus, even though BALB/c mice are low responders to PADRE, they still generated a strong anti- $A\beta_{42}$ response to the prototype epitope AD vaccine. The antibodies induced by PADRE- $A\beta_{1-15}$ -MAP were specific to $A\beta$, because they did not bind to the MAP backbone or PADRE in ELISA assays [81]. As we expected, PADRE- $A\beta_{1-15}$ -MAP formulated in the Th2 adjuvant, alum, induced anti- $A\beta$ antibodies of mainly the IgG1 isotype in H-2^d mice. Finally, anti- $A\beta$ antibodies generated after immunization of mice with PADRE- $A\beta_{1-15}$ -MAP were potentially therapeutic because they bound to amyloid plaques in brain tissue from an AD case [81]. Analysis of T cell proliferation in splenocytes from animals immunized with our epitope vaccine demonstrated that mice generated robust T cell responses only to the PADRE but not to the $A\beta$ peptide or MAP backbone. The predominant cytokine produced by splenocytes from immunized mice was IL-4. These results were not surprising, since antigen was formulated in alum, a Th2-type adjuvant, and we immunized BALB/c mice that are normally Th2-dominant [75,82]. Thus, this $A\beta$

epitope vaccine was capable of activating PADRE-specific T cells that stimulated B cells to produce anti-A β ₁₋₁₅ antibodies.

Intranasal immunization of mice with other epitope vaccines based on A β ₁₋₁₅ generated high concentrations of therapeutically potent anti-A β antibodies of IgG1 and IgG2b isotypes without the activation of A β -specific T cells [83]. Two principally different vaccines have been designed: a tandem repeat of A β ₁₋₁₅ peptide linked by two lysines (2 $\times$ A β ₁₋₁₅) or A β ₁₋₁₅ peptide conjugated with a T helper cell epitope from the HIV IIIB gp120 glycoprotein (T1-A β ₁₋₁₅). Surprisingly, 2 $\times$ A β ₁₋₁₅ is formulated in mutant *Escherichia coli* heat-labile enterotoxin LT adjuvant was more potent at generating anti-A β antibodies than T1-A β ₁₋₁₅+LT. Both vaccines generated robust T cell responses specific to the individual vaccines and no responses were generated against full length A β ₄₂ or A β ₁₋₁₅. The authors speculated that the absence of T cell responses against A β ₁₋₁₅ after immunization with the 2 $\times$ A β ₁₋₁₅ immunogen may be attributable to the formation of a new, unidentified T cell epitope that is not present in a single sequence of A β ₁₋₁₅. Such new epitopes that work very well in mice would not necessarily work in humans, therefore epitope vaccines should be carefully designed in order to avoid unanticipated responses. Four copies of the A β ₁₋₁₅ epitope (4 $\times$ A β ₁₋₁₅) were used for generating an AD vaccine based on an adenoviral vector [84]. Immunization of 12 month old Tg 2576 mice induced high titers of anti-A β antibodies, reduced cerebral insoluble A β levels and behavioral impairment in old Tg 2576 mice, however did not change the level of soluble A β in the brain. Like the epitope vaccine 2 $\times$ A β ₁₋₁₅ described by Maier *et al.*, splenocytes from immunized mice were activated *in vitro* by 4 $\times$ A β ₁₋₁₅, but not with A β ₄₀. Although the authors did not use A β ₁₋₁₅ for re-stimulation of splenocytes, it is likely that again in this case, a new T cell epitope was generated due to the use of multiple copies of the A β peptide. DNA prime and adenovirus boost regimens have been used to induce responses against the small epitope A β ₁₋₆. Even the presence of 11 copies of this epitope induced only very low titers of anti-A β antibodies, probably due to the absence of a potent T cell epitope [85]. Smaller parts of the N-terminal region of the A β peptide were used in the design of vaccines based on viral-like particles (VLP). Incorporation of the A β B cell epitope into a viral capsid protein allows the expression of this epitope on the surface of VLP in a repetitive and ordered array. Such organization of the epitope may induce T cell-independent B cell activation and production of high titers of anti-A β antibodies. On the other hand, T cell epitopes from the viral proteins may help B cells to induce T cell-dependent humoral response. Vaccination of mice and rabbits with papillomavirus VLP that displayed A β ₁₋₉ on the viral capsid surface induced measurable titers of anti-A β antibodies in rabbits and moderate titers in APP/PS1 Tg mice [86]. Generated antibodies were therapeutically potent and recognized amyloid plaques in brain tissue sections from an AD case. Even the moderate titers of anti-N-terminal antibodies reduced the A β load in the cortex and hippocampus of immunized mice, yet the total A β in brain homogenates were largely unchanged, although a trend towards lower levels was observed. Another papillomavirus VLP containing A β ₁₋₉ was generated through the streptavidin bridge technique [87]. Immunization of C57BL/6 mice with VLPs formulated in IFA induced high titers of anti-A β antibodies of predominantly IgG1 isotype without the generation of A β -specific T cell responses. The same antigen incorporated into the bacteriophage Qp induced even higher antibody response [87]. Another Qp-based vaccine comprising the A β ₁₋₆ epitope (CAD106) covalently linked to VLPs was tested in various species [88,89]. CAD106 injected without any adjuvant induced high titers of antibodies in mice, rabbits and monkeys. The therapeutic potency of generated antibodies was shown *in vitro* and *in vivo*. Antibodies bound to amyloid deposits in human and APP Tg mouse brain sections, and protected neurons from oligomeric and fibrillar toxicity *in vitro*. Immunization of APP23 and APP24 Tg mice with CAD106 prevented cortical and vascular plaque deposition. Immunization of aged mice with advanced amyloid pathology reduced the amyloid deposition without inducing microhemorrhages. Currently, Novartis is investigating the safety, tolerability and A β -specific antibody responses in a group of patients with mild to moderate

AD following three subcutaneous injections of CAD106[89]. Although the report from this trial is not available yet, a VLP-based vaccine is already approved for human papillomavirus, if the safety of QP-VLP based vaccines is established, this platform may serve as an effective advanced delivery system for A β epitopes.

All of the epitope based vaccines described above, with the exception of our first generation epitope vaccine based on the A β _{1–15} B cell epitope and synthetic, non-natural epitope PADRE, either do not contain any well characterized T cell epitope or are based on multiple T cell epitopes from viral proteins in VLP. Although we did not observe anti-A β _{1–15} specific T cells in our experiments with the APP/Tg mice, in our second generation epitope vaccine we decided to shorten the B cell epitope to aa 1–11, based on the report that APP/Tg and HLA class II Tg mice immunized with fibrillar A β ₄₂ recognized the A β _{1–16} peptide [71]. In addition, our new generation epitope vaccines contain 2 or 3 copies of the A β B cell epitope, significantly increasing the antibody response and affinity of antibody-antigen binding [63,99,100 and unpublished data].

DNA and peptide epitope vaccines were designed based on A β _{1–11} and PADRE epitopes. The peptide epitope vaccine was synthesized on a 4-branched MAP backbone to generate 2A β _{1–11} PADRE-MAP immunogen and was formulated in Quil A adjuvant. MAPs provide multiple copies of immunogen attached to the core matrix and significantly enhance antibody responses [80,90]. 2A β _{1–11}-PADRE-MAP formulated in QuilA induced robust anti-A β antibody responses in wild-type and two different APP Tg mice: 3 $\times$ Tg-AD and Tg 2576, without activation of autoreactive T cells. However, some problems associated with the development of safe and effective epitope vaccines still exist. For example, a peptide epitope vaccine is immunogenic when it is synthesized on a MAP backbone but cannot be easily scaled up for clinical studies. Another issue associated with a peptide epitope vaccine strategy is the requirement for a potent conventional adjuvant. The only adjuvant currently approved for use in humans is alum, which may not be capable of inducing strong anti-A β immunity in elderly patients as was previously shown in mice [91].

An alternative to the peptide vaccination approach is DNA immunization. DNA-based vaccination provides a unique method of vaccination [92], exhibiting properties that may be advantageous for the development of vaccines against a variety of pathogens as well as for human diseases including cancer, autoimmune disorders, and Alzheimer's disease. A unique property of DNA-based vaccination is the ability to induce prolonged, endogenous antigen synthesis and processing within the subject's own cells. DNA immunization has been shown to generate protective humoral and cellular immune responses against multiple viral, bacterial and tumor antigens [93–98]. This approach also permits rational inactivation or removal of sequences encoding potentially toxic or oncogenic protein domains as well as the inclusion of molecular adjuvants, such as cytokines, which can direct T helper cell responses toward the desired pathway.

Thus, we designed and constructed a new generation DNA epitope vaccine composed of macrophage derived chemokine (MDC) as a molecular adjuvant, 3 copies of the A β _{1–11} B cell epitope, and the promiscuous T cell epitope, PADRE. Both wildtype and 3 $\times$ Tg-AD mice immunized with the DNA epitope vaccine induced robust anti-A β humoral and Th2-polarized immune responses. Importantly, the DNA epitope vaccine, like the peptide epitope vaccine, did not generate autoimmune cellular responses specific to A β [63,99,100]. MDC molecular adjuvant incorporated into the vaccine design not only facilitates the internalization of antigen into endo-lysosomal compartments of target CCR4⁺ APC, and increases the efficiency of processing and presentation of antigens, but also plays a critical role in the antigen-induced recruitment of Th2 cells *via* chemotaxis and activation of CCR4-expressing Th2-type CD4⁺ T cells [101–103], followed by B-cell activation [104].

In vitro characterization of therapeutic features of anti-A β_{1-11} antibodies generated after immunization of 3xTg-AD mice with the DNA epitope vaccine demonstrated that the antibodies bound to different forms of A β_{42} , although the affinity of binding to oligomers is higher than to fibrils. The antibodies bound to amyloid plaques in the brain sections of an AD case, reduced amyloid plaque burden after intracranial injection of APP Tg mice, and inhibited the fibril-mediated cytotoxicity of A β_{42} . Substoichiometric concentrations of purified anti-A β_{1-11} antibody prevented aggregation of A β_{42} , and induced disaggregation of preformed A β_{42} fibrils down to a non-filamentous and nontoxic species. Although reduced oligomer-mediated cytotoxicity was observed upon preincubation of A β oligomers with the anti-A β_{1-11} antibody, antibodies could not disaggregate oligomers though they did delay A β_{42} oligomer formation. These *in vitro* observations suggest that therapeutic vaccination cannot disrupt *in vivo* toxic oligomers and may only minimally inhibit preexisting AD pathology and support the hypothesis that immunotherapy should be started before A β accumulation in the vasculature and parenchyma of brains induces an unalterable process. In other words, if AD pathology is associated with accumulation of oligomeric forms of A β_{42} in the brain, the therapeutic vaccination strategy will likely not be effective. To verify this concept, we compared data generated after immunization of the APP Tg mice with or without pre-existing AD-like pathology at the start of immunization [99,100]. In both cases, vaccination significantly decreased the A β depositions in the brains of mice. A potential problem of immunotherapy could be the fact that a reduction of insoluble A β may lead to increased levels of soluble forms of this peptide [38], primarily oligomers, the most toxic form for neurons, and impair cognitive function [105]. In these studies we demonstrated that early vaccination prevented the accumulation of both insoluble and soluble/oligomeric A β and prevented cognitive dysfunction in immunized animals, whereas vaccination started in mice with pre-existing AD-like pathology reduced insoluble but not soluble/oligomeric A β . In both cases vaccination with the epitope vaccine stimulated less activation of microglia compared with non-immunized controls and did not induce microhemorrhages. These data and the fact that the AN-1792 vaccine was not clinically effective in AD patients, suggest that AD immunotherapy may be most effective not as a therapeutic measure but as a prophylactic measure for the treatment of people with early pre-clinical stages of AD.

Currently Elan Corporation is conducting a Phase 2 clinical trial (ACC-001) using as an immunogen the A β_{1-7} epitope conjugated with the carrier protein CRM197 and formulated in QS21 adjuvant. The ACC-001 trial was temporarily suspended due to vasculitis (inflammation of the blood vessels) detected in one patient. It is not yet clear if the skin lesions were related to the antigen, the alternative adjuvant QS-21, or to something else entirely. The results of this trial will provide important perspective regarding the potential efficacy of an epitope vaccine approach for AD. However, some safety concerns may still remain due to the use of the strong inflammatory QS21 adjuvant.

FUTURE DIRECTIONS

One important obstacle for active vaccination of AD patients is hypo-responsiveness to vaccines in the elderly. It is known that immunosenescence in the elderly contributes to the poor outcome of vaccination and increased susceptibility of the elderly to infectious disease. The T cell population shifts to a lower ratio of naïve to memory cells, and fewer naïve T cells are produced by the thymus with age [106,107]. The involution of the thymus is almost complete at the age of 60 years and new T cells can no longer be generated. In addition, the *in vivo* production of proinflammatory cytokines is upregulated in the elderly [107,108]. This problem could potentially be overcome by regular vaccination of people with very early-stage AD, which can be diagnosed by measuring tau/A β and ptau/A β ratio in the CSF and/or by screening for accumulation of A β in the brains using PIB-PET scan [44–47], and subsequently providing yearly booster vaccinations. Another possible strategy to counteract

immunosenescence is to recruit previously generated memory T cells produced during childhood vaccination or during prior exposure to human pathogens. The majority of elderly people already possess a broad panel of specific memory T cells. Vaccination of these people with a recombinant vaccine composed of a B cell epitope of the desired antigen (A β peptide in the case of AD vaccination) and Th epitopes from conventional vaccines or common pathogens, may boost the pre-existing memory T cells to expand rapidly and differentiate into effector T cells, which would lead to a faster and stronger antibody response specific to the fusion protein antigen. Tetanus toxin (TT), diphtheria toxin (DT), influenza virus, HBV, measles are examples of such conventional vaccines/pathogens. As a result of mandatory vaccination against TT, DT, measles, yearly vaccination and/or infection with influenza, and vaccination against HBV, almost everyone should have memory CD4⁺ T cells specific to epitopes in these vaccines. Thus, a booster injection with an epitope vaccine containing these conventional Th epitopes along with a strong Th2 molecular adjuvant should be enough for the rapid activation of pre-existing anti-TT, anti-DT, anti-HBV and anti-flu memory Th cells, resulting in the induction of strong and potent anti-A β antibody production in the elderly.

Nevertheless, even if effective strategies are developed to increase antibody responses in elderly people, analysis of pre-clinical and clinical data accumulated over the past decade suggest that vaccination will be effective if started before the accumulation of toxic forms of β -amyloid or at least at the early stages of AD. Antibodies may prevent or delay the oligomerization of A β ₄₂, but it is very difficult to disrupt preformed oligomers [63]. Of course, a prophylactic vaccine must be very safe. Incorporation of universal foreign T cell epitopes, as well as a molecular adjuvant that could be much safer than currently available conventional adjuvants, into a DNA epitope vaccine provides such a possibility. Another effective way to develop safe AD vaccines is with an epitope vaccine composed of a B cell epitope mimicking the structure of toxic forms of A β ₄₂ and a foreign T cell epitope. Such a vaccine has the potential to be safe even for vaccination of young, healthy individuals and could prevent the accumulation of toxic β -amyloid peptides. Thus, the generation of peptides mimicking toxic forms of A β peptide (mimotopes) is one the most promising strategies for the development of a safe and effective AD vaccine.

CONCLUSIONS

In summary, we believe that AD progression could be safely prevented or delayed if (i) the vaccine induces high titers of antibodies specific to toxic forms of A β without activation of harmful autoreactive T cells and induction of inflammation; (ii) vaccination starts before or at least at the early stages of the accumulation of toxic forms of β -amyloid peptide. Present-day achievements in development of diagnostic and prognostic methodologies make this vaccination scheme possible.

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ABBREVIATIONS

A β	Amyloid-beta
AD	Alzheimer's disease
APC	Antigen-presenting cells

APP	Amyloid precursor protein
BBB	Blood brain barrier
CAA	Cerebral amyloid angiopathy
CSF	Cerebral spinal fluid
CTF	C-terminal fragment
LRP	Lipoprotein receptor-related protein 1
MAP	Multiple antigenic peptide
MDC	Macrophage derived chemokine
NFT	Neurofibrillary tangles
PADRE	Pan DR epitope
TT	Tetanus toxin
VLP	Viral like particles

References

1. Ziegler-Graham K, Brookmeyer R, Johnson E, Arrighi HM. Worldwide variation in the doubling time of Alzheimer's disease incidence rates. *Alzheimers Dement* 2008;4:316–23. [PubMed: 18790458]
2. Price DL, Sisodia SS. Cellular and molecular biology of Alzheimer's disease and animal models. *Annu Rev Med* 1994;45:435–46. [PubMed: 8198393]
3. Campion D, Dumanchin C, Hannequin D, Dubois B, Belliard S, Puel M, Thomas-Anterion C, Michon A, Martin C, Charbonnier F, Raux G, Camuzat A, Penet C, Mesnage V, Martinez M, Clerget-Darpoux F, Brice A, Frebourg T. Early-onset autosomal dominant Alzheimer disease: prevalence, genetic heterogeneity, and mutation spectrum. *Am J Hum Genet* 1999;65:664–70. [PubMed: 10441572]
4. Iqbal K, Grundke-Iqbal I. Alzheimer neurofibrillary degeneration: significance, etiopathogenesis, therapeutics and prevention. *J Cell Mol Med* 2008;12:38–55. [PubMed: 18194444]
5. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 2002;297:353–6. [PubMed: 12130773]
6. Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. *Science* 1992;256:184–5. [PubMed: 1566067]
7. Selkoe DJ. The molecular pathology of Alzheimer's disease. *Neuron* 1991;6:487–98. [PubMed: 1673054]
8. Selkoe DJ. Alzheimer's disease: a central role for amyloid. *J Neuropathol Exp Neurol* 1994;53:438–47. [PubMed: 8083687]
9. Esler WP, Wolfe MS. A portrait of Alzheimer se-cretases-new features and familiar faces. *Science* 2001;293:1449–54. [PubMed: 11520976]
10. Kamenetz F, Tomita T, Hsieh H, Seabrook G, Borchelt D, Iwatsubo T, Sisodia S, Malinow R. APP processing and synaptic function. *Neuron* 2003;37:925–37. [PubMed: 12670422]
11. Paresce DM, Chung H, Maxfield FR. Slow degradation of aggregates of the Alzheimers disease amyloid beta-protein by microglial cells. *J Biol Chem* 1997;272:29390–7. [PubMed: 9361021]
12. Qiu WQ, Ye Z, Kholodenko D, Seubert P, Selkoe DJ. Degradation of amyloid beta-protein by a metalloprotease secreted by microglia and other neural and non-neural cells. *J Biol Chem* 1997;272:6641–6. [PubMed: 9045694]
13. Iwata N, Tsubuki S, Takaki Y, Watanabe K, Sekiguchi M, Hosoki E, Kawashima-Morishima M, Lee HJ, Hama E, Sekine-Aizawa Y, Saido TC. Identification of the major Abeta1-42-degrading catabolic pathway in brain parenchyma: suppression leads to biochemical and pathological deposition. *Nat Med* 2000;6:143–50. [PubMed: 10655101]

14. Qiu WQ, Walsh DM, Ye Z, Vekrellis K, Zhang J, Podlisny MB, Rosner MR, Safavi A, Hersh LB, Selkoe DJ. Insulin-degrading enzyme regulates extracellular levels of amyloid beta-protein by degradation. *J Biol Chem* 1998;273:32730–8. [PubMed: 9830016]
15. Shibata M, Yamada S, Kumar SR, Calero M, Bading J, Frangione B, Holtzman DM, Miller CA, Strickland DK, Ghiso J, Zlokovic BV. Clearance of Alzheimer's amyloid-ss(1–40) peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier. *J Clin Invest* 2000;106:1489–99. [PubMed: 11120756]
16. Schley D, Carare-Nnadi R, Please CP, Perry VH, Weller RO. Mechanisms to explain the reverse perivascular transport of solutes out of the brain. *J Theor Biol* 2006;238:962–74. [PubMed: 16112683]
17. Weller RO, Massey A, Newman TA, Hutchings M, Kuo YM, Roher AE. Cerebral amyloid angiopathy: amyloid beta accumulates in putative interstitial fluid drainage pathways in Alzheimer's disease. *Am J Pathol* 1998;153:725–33. [PubMed: 9736023]
18. Du Y, Dodel R, Hampel H, Buerger K, Lin S, Eastwood B, Bales K, Gao F, Moeller HJ, Oertel W, Farlow M, Paul S. Reduced levels of amyloid beta-peptide antibody in Alzheimer disease. *Neurology* 2001;57:801–5. [PubMed: 11552007]
19. Moir RD, Tseitin KA, Soscia S, Hyman BT, Tri-zarry MC, Tanzi RE. Autoantibodies to redox-modified oligomeric Abeta are attenuated in the plasma of Alzheimer's disease patients. *J Biol Chem* 2005;280:17458–63. [PubMed: 15728175]
20. Weksler ME, Relkin N, Turkenich R, LaRusse S, Zhou L, Szabo P. Patients with Alzheimer disease have lower levels of serum anti-amyloid peptide antibodies than healthy elderly individuals. *Exp Gerontol* 2002;37:943–8. [PubMed: 12086704]
21. Morgan D, Diamond DM, Gottschall PE, Ugen KE, Dickey C, Hardy J, Duff K, Jantzen P, Di-Carlo G, Wilcock D, Connor K, Hatcher J, Hope C, Gordon M, Arendash GW. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* 2000;408:982–5. [PubMed: 11140686]
22. Janus C, Pearson J, McLaurin J, Mathews PM, Jiang Y, Schmidt SD, Chishti MA, Home P, Heslin D, French J, Mount HT, Nixon RA, Mercken M, Bergeron C, Fraser PE, St George-Hyslop P, Westaway D. A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature* 2000;408:979–82. [PubMed: 11140685]
23. Bard F, Cannon C, Barbour R, Burke RL, Games D, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Lieberburg I, Motter R, Nguyen M, Soriano F, Vasquez N, Weiss K, Welch B, Seubert P, Schenk D, Yednock T. Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nat Med* 2000;6:916–9. [PubMed: 10932230]
24. Dodart JC, Bales KR, Gannon KS, Greene SJ, DeMattos RB, Mathis C, DeLong CA, Wu S, Wu X, Holtzman DM, Paul SM. Immunization reverses memory deficits without reducing brain Abeta burden in Alzheimer's disease model. *Nat Neurosci* 2002;5:452–7. [PubMed: 11941374]
25. Kotilinek LA, Bacskai B, Westerman M, Kawarabayashi T, Younkin L, Hyman BT, Younkin S, Ashe KH. Reversible memory loss in a mouse transgenic model of Alzheimer's disease. *J Neurosci* 2002;22:6331–5. [PubMed: 12151510]
26. Wilcock DM, Rojiani A, Rosenthal A, Levkowitz G, Subbarao S, Alamed J, Wilson D, Wilson N, Freeman MJ, Gordon MN, Morgan D. Passive amyloid immunotherapy clears amyloid and transiently activates microglia in a transgenic mouse model of amyloid deposition. *J Neurosci* 2004;24:6144–51. [PubMed: 15240806]
27. Wilcock DM, Rojiani A, Rosenthal A, Subbarao S, Freeman MJ, Gordon MN, Morgan D. Passive immunotherapy against Abeta in aged APP-transgenic mice reverses cognitive deficits and depletes parenchymal amyloid deposits in spite of increased vascular amyloid and microhemorrhage. *J Neuroinflammation* 2004;1:24. [PubMed: 15588287]
28. Pfeifer M, Boncristiano S, Bondolfi L, Stalder A, Deller T, Staufenbiel M, Mathews PM, Jucker M. Cerebral hemorrhage after passive anti-Abeta immunotherapy. *Science* 2002;298:1379. [PubMed: 12434053]
29. DeMattos RB, Boone LI, Hepburn DL, Parsadanian M, Bryan MT, Ness DK, Pirooz KS, Holtzman DM, Bales KR, Gitter BD, Paul SM, Racke M. *In vitro* and *in vivo* characterization of beta-amyloid

- antibodies binding to cerebral amyloid angiopathy (CAA) and the selective exacerbation of CAA-associated microhemorrhage. *Neurobiol Aging* 2004;25:577.
30. Wilcock DM, Alamed J, Gottschall PE, Grimm J, Rosenthal A, Pons J, Ronan V, Symmonds K, Gordon MN, Morgan D. Deglycosylated anti-amyloid-beta antibodies eliminate cognitive deficits and reduce parenchymal amyloid with minimal vascular consequences in aged amyloid precursor protein transgenic mice. *J Neurosci* 2006;26:5340–6. [PubMed: 16707786]
  31. Wilcock DM, Jantzen PT, Li Q, Morgan D, Gordon MN. Amyloid- β vaccination, but not nitro-NSATD treatment, increases vascular amyloid and microhemorrhage while both reduce parenchymal amyloid. *Neuroscience* 2007;144:950–60. [PubMed: 17137722]
  32. Holmes C, Boche D, Wilkinson D, Yadegarfar G, Hopkins V, Bayer A, Jones RW, Bullock R, Love S, Neal JW, Zotova E, Nicoll JA. Long-term effects of Abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. *Lancet* 2008;372:216–23. [PubMed: 18640458]
  33. Gilman S, Koller M, Black RS, Jenkins L, Griffith SG, Fox NC, Eisner L, Kirby L, Rovira MB, Forette F, Orgogozo JM. Clinical effects of Abeta immunization (AN 1792) in patients with AD in an interrupted trial. *Neurology* 2005;64:1553–62. [PubMed: 15883316]
  34. Hock C, Konietzko U, Streffer JR, Tracy J, Signorell A, Muller-Tillmanns B, Lemke U, Henke K, Moritz E, Garcia E, Wollmer MA, Umbricht D, de Quervain DJ, Hofmann M, Maddalena A, Papassotiropoulos A, Nitsch RM. Antibodies against beta-Amyloid slow cognitive decline in Alzheimer's disease. *Neuron* 2003;38:547–54. [PubMed: 12765607]
  35. Nicoll JA, Wilkinson D, Holmes C, Steart P, Markham H, Weller RO. Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. *Nat Med* 2003;9:448–52. [PubMed: 12640446]
  36. Ferrer I, Rovira MB, Guerra MLS, Rey MJ, Costa-Jussa F. Neuropathology and pathogenesis of encephalitis following amyloid-beta immunization in Alzheimer's disease. *Brain Pathol* 2004;14:11–20. [PubMed: 14997933]
  37. Masliah E, Hansen L, Adame A, Crews L, Bard F, Lee G, Seubert P, Games D, Kirby L, Schenk D. Abeta vaccination effects on plaque pathology in the absence of encephalitis in Alzheimer disease. *Neurology* 2005;64:129–31. [PubMed: 15642916]
  38. Patton RL, Kalback WM, Esh CL, Kokjohn TA, Van Vickie GD, Luehrs DC, Kuo YM, Lopez J, Brune D, Ferrer I, Masliah E, Newel AJ, Beach TG, Castano EM, Roher AE. Amyloid-beta peptide remnants in AN-1792-immunized Alzheimer's disease patients: a biochemical analysis. *Am J Pathol* 2006;169:1048–63. [PubMed: 16936277]
  39. Nitsch, RM.; Hock, C. Immunotherapy against beta-amyloid in Alzheimer's disease. 8th International Conference on Alzheimer's and Parkinson's disease: progress and new perspectives; Salzburg: Austria. 2007.
  40. Boche, D.; DeBeer, S.; Cox, A.; Wilkinson, D.; Holmes, C.; Neal, J.; Love, S.; Esiri, M.; Bridges, L.; Weller, R.; Nicoll, JA. Evidence of a transient increase in cerebral amyloid angiopathy after Abeta42 immunization in human Alzheimer's disease. 8th International Conference on Alzheimer's and Parkinson's disease: progress and new perspectives; Salzburg: Austria. 2007.
  41. Nicoll JA, Barton E, Boche D, Neal JW, Ferrer I, Thompson P, Vlachouli C, Wilkinson D, Bayer A, Games D, Seubert P, Schenk D, Holmes C. Abeta species removal after abeta42 immunization. *J Neuropathol Exp Neurol* 2006;65:1040–8. [PubMed: 17086100]
  42. Orgogozo JM, Gilman S, Dartigues JM, Laurent B, Puel M, Kirby LC, Jouanny P, Dubois B, Eisner L, Flitman S, Michel BF, Boada M, Frank A, Hock C. Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization. *Neurology* 2003;61(1):46–54. [PubMed: 12847155]
  43. Bayer AJ, Bullock R, Jones RW, Wilkinson D, Paterson KR, Jenkins L, Millais SB, Donoghue S. Evaluation of the safety and immunogenicity of synthetic Abeta42 (AN 1792) in patients with AD. *Neurology* 2005;64:94–101. [PubMed: 15642910]
  44. Fagan AM, Roe CM, Xiong C, Mintun MA, Morris JC, Holtzman DM. Cerebrospinal fluid tau/beta-amyloid(42) ratio as a prediction of cognitive decline in nondemented older adults. *Arch Neurol* 2007;64:343–9. [PubMed: 17210801]

45. Fagan, AM.; Roe, CM.; Xiong, C.; Mintun, MA.; Morris, JC.; Holtzman, DM. Cerebrospinal fluid bio-markers of early stage Alzheimer disease. 8th International Conference on Alzheimer's and Parkinson's disease: progress and new perspectives; Salzburg: Austria. 2007.
46. de Jong D, Jansen RW, Kremer BP, Verbeek MM. Cerebrospinal fluid amyloid beta42/ phosphorylated tau ratio discriminates between Alzheimer's disease and vascular dementia. *J Gerontol A Biol Sci Med Sci* 2006;61:755–8. [PubMed: 16870640]
47. Klunk WE, Engler H, Nordberg A, Wang Y, Blomqvist G, Holt DP, Bergstrom M, Savitcheva I, Huang GF, Estrada S, Ausen B, Debnath ML, Barletta J, Price JC, Sandell J, Lopresti BJ, Wall A, Koivisto P, Antoni G, Mathis CA, Langstrom B. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Ann Neurol* 2004;55:306–19. [PubMed: 14991808]
48. Neumann H. Control of glial immune function by neurons. *Glia* 2001;36:191–9. [PubMed: 11596127]
49. Hanisch UK. Microglia as a source and target of cytokines. *Glia* 2002;40:140–55. [PubMed: 12379902]
50. Yang MS, Park EJ, Sohn S, Kwon HJ, Shin WH, Pyo HK, Jin B, Choi KS, Jou I, Joe EH. Interleukin-13 and -4 induce death of activated microglia. *Glia* 2002;38:273–80. [PubMed: 12007140]
51. Shin WH, Lee DY, Park KW, Kim SU, Yang MS, Joe EH, Jin BK. Microglia expressing interleukin-13 undergo cell death and contribute to neuronal survival *in vivo*. *Glia* 2004;46:142–52. [PubMed: 15042582]
52. Monsonego A, Imitola J, Zota V, Oida T, Weiner HL. Microglia-mediated nitric oxide cytotoxicity of T cells following amyloid beta-peptide presentation to Th1 cells. *J Immunol* 2003;171(5):2216–24. [PubMed: 12928365]
53. Yamamoto M, Kiyota T, Walsh SM, Liu J, Kipnis J, Dcezu T. Cytokine-mediated inhibition of fibrillar amyloid-beta peptide degradation by human mononuclear phagocytes. *J Immunol* 2008;181:3877–86. [PubMed: 18768842]
54. Town T, Tan J, Sansone N, Obregon D, Klein T, Mullan M. Characterization of murine immunoglobulin G antibodies against human amyloid- β 1–42. *Neurosci Lett* 2001;307:101–4. [PubMed: 11427310]
55. Dickey CA, Morgan DG, Kudchodkar S, Weiner DB, Bai Y, Cao C, Gordon MN, Ugen KE. Duration and specificity of humoral immune responses in mice vaccinated with the Alzheimer's disease-associated beta-amyloid 1–42 peptide. *DNA Cell Biol* 2001;20:723–9. [PubMed: 11788050]
56. Lemere CA, Maron R, Selkoe DJ, Weiner HL. Nasal vaccination with beta-amyloid peptide for the treatment of Alzheimer's disease. *DNA Cell Biol* 2001;20:705–11. [PubMed: 11788048]
57. Lemere CA, Maron R, Spooner ET, Grenfell TJ, Mori C, Desai R, Hancock WW, Weiner HL, Selkoe DJ. Nasal A β treatment induces anti-A β antibody production and decreases cerebral amyloid burden in PD-APP Mice. *Ann NY Acad Sci* 2000;920:328–31. [PubMed: 11193172]
58. Cribbs DH, Ghochikyan A, Tran M, Vasilevko V, Petrushina I, Sadzikava N, Kesslak P, Kieber-Emmons T, Cotman CW, Agadjanyan MG. Adjuvant-dependent modulation of Th1 and Th2 responses to immunization with beta-amyloid. *Int Immunol* 2003;15:505–14. [PubMed: 12663680]
59. McLaurin J, Cecal R, Kierstead ME, Tian X, Phinney AL, Manea M, French JE, Lambermon MH, Darabie AA, Brown ME, Janus C, Chishti MA, Home P, Westaway D, Fraser PE, Mount HT, Przybylski M, St George-Hyslop P. Therapeutically effective antibodies against amyloid-beta peptide target amyloid-beta residues 4–10 and inhibit cytotoxicity and fibrillogenesis. *Nat Med* 2002;8:1263–9. [PubMed: 12379850]
60. Solomon B, Koppel R, Hanan E, Katzav T. Monoclonal antibodies inhibit *in vitro* fibrillar aggregation of the Alzheimer beta-amyloid peptide. *Proc Natl Acad Sci USA* 1996;93:452–5. [PubMed: 8552659]
61. Solomon B, Koppel R, Frankel D, Hanan-Aharon E. Disaggregation of Alzheimer beta-amyloid by site-directed mAb. *Proc Natl Acad Sci USA* 1997;94:4109–12. [PubMed: 9108113]
62. Frenkel D, Balass M, Katchalski-Katzir E, Solomon B. High affinity binding of monoclonal antibodies to the sequential epitope EFRH of beta-amyloid peptide is essential for modulation of fibrillar aggregation. *J Neuroimmunol* 1999;95:136–42. [PubMed: 10229123]
63. Mamikonyan G, Necula M, Mkrtichyan M, Ghochikyan A, Petrushina I, Movsesyan N, Mina E, Kiyatkin A, Glabe C, Cribbs DH, Agadjanyan MG. Anti-A β 1–11 antibody binds to different beta-amyloid species, inhibits fibril formation, and disaggregates preformed fibrils, but not the most toxic oligomers. *J Biol Chem* 2007;282:22376–86. [PubMed: 17545160]

64. Bard F, Barbour R, Cannon C, Carretto R, Fox M, Games D, Guido T, Hoenow K, Hu K, Johnson-Wood K, Khan K, Kholodenko D, Lee C, Lee M, Motter R, Nguyen M, Reed A, Schenk D, Tang P, Vasquez N, Seubert P, Yednock T. Epitope and isotype specificities of antibodies to beta-amyloid peptide for protection against Alzheimer's disease-like neuropathology. *Proc Natl Acad Sci USA* 2003;100:2023–8. [PubMed: 12566568]
65. Miller DL, Currie JR, Mehta PD, Potemski A, Hwang YW, Wegiel J. Humoral immune response to fibrillar beta-amyloid peptide. *Biochemistry* 2003;42(40):11682–92. [PubMed: 14529278]
66. Lee M, Bard F, Johnson-Wood K, Lee C, Hu K, Griffith SG, Black RS, Schenk D, Seubert P. Abeta42 immunization in Alzheimer's disease generates Abeta N-terminal antibodies. *Ann Neurol* 2005;58:430–5. [PubMed: 16130106]
67. Sigurdsson EM, Knudsen E, Asuni A, Fitzner-Attas C, Sage D, Quartermain D, Goni F, Frangione B, Wisniewski T. An attenuated immune response is sufficient to enhance cognition in an Alzheimer's disease mouse model immunized with amyloid-beta derivatives. *J Neurosci* 2004;24:6277–82. [PubMed: 15254082]
68. Singh H, Raghava GP. ProPred: prediction of HLA-DR binding sites. *Bioinformatics* 2001;17:1236–7. [PubMed: 11751237]
69. Singh H, Raghava GP. ProPred1: prediction of promiscuous MHC Class-I binding sites. *Bioinformatics* 2003;19:1009–14. [PubMed: 12761064]
70. Monsonego A, Zota V, Kami A, Krieger JI, Bar-Or A, Bitan G, Budson AE, Sperling R, Selkoe D, Weiner HL. Increased T cell reactivity to amyloid beta protein in older humans and patients with Alzheimer disease. *J Clin Invest* 2003;112(3):415–22. [PubMed: 12897209]
71. Das P, Chapoval S, Howard V, David CS, Golde TE. Immune responses against Abeta1–42 in HLA class II transgenic mice: implications for Abeta1–42 immune-mediated therapies. *Neurobiol Aging* 2003;24:969–76. [PubMed: 12928057]
72. Alexander J, Sidney J, Southwood S, Ruppert J, Oseroff C, Maewal A, Snoke K, Serra HM, Kubo RT, Sette A. Development of high potency universal DR-restricted helper epitopes by modification of high affinity DR-blocking peptides. *Immunity* 1994;1:751–61. [PubMed: 7895164]
73. Alexander J, Fikes J, Hoffman S, Franke E, Sacchi J, Appella E, Chisari FV, Guidotti LG, Chesnut RW, Livingston B, Sette A. The optimization of helper T lymphocyte (HTL) function in vaccine development. *Immunol Res* 1998;18:79–92. [PubMed: 9844827]
74. del Guercio MF, Alexander J, Kubo RT, Arrhenius T, Maewal A, Appella E, Hoffman SL, Jones T, Valmori D, Sakaguchi K, Grey HM, Sette A. Potent immunogenic short linear peptide constructs composed of B cell epitopes and Pan DR T helper epitopes (PADRE) for antibody responses in vivo. *Vaccine* 1997;15:441–8. [PubMed: 9141216]
75. Alexander J, del Guercio MF, Maewal A, Qiao L, Fikes J, Chesnut RW, Paulson J, Bundle DR, DeFrees S, Sette A. Linear PADRE T helper epitope and carbohydrate B cell epitope conjugates induce specific high titer IgG antibody responses. *J Immunol* 2000;164:1625–33. [PubMed: 10640784]
76. La Rosa C, Wang Z, Brewer JC, Lacey SF, Villacres MC, Sharan R, Krishnan R, Crooks M, Markel S, Maas R, Diamond DJ. Preclinical development of an adjuvant-free peptide vaccine with activity against CMV pp65 in HLA transgenic mice. *Blood* 2002;100:3681–9. [PubMed: 12393676]
77. Wei WZ, Ratner S, Shibuya T, Yoo G, Jani A. Foreign antigenic peptides delivered to the tumor as targets of cytotoxic T cells. *J Immunol Methods* 2001;258:141–50. [PubMed: 11684131]
78. Weber JS, Hua FL, Spears L, Marty V, Kuniyoshi C, Celis E. A phase I trial of an HLA-A1 restricted MAGE-3 epitope peptide with incomplete Freund's adjuvant in patients with resected high-risk melanoma. *J Immunother* 1999;22:431–40. [PubMed: 10546159]
79. Panina-Bordignon P, Tan A, Termijtelen A, Demotz S, Corradin G, Lanzavecchia A. Universally immunogenic T cell epitopes: promiscuous binding to human MHC class II and promiscuous recognition by T cells. *Eur J Immunol* 1989;19:2237–42. [PubMed: 2481588]
80. Tam JP. Synthetic peptide vaccine design: synthesis and properties of a high-density multiple antigenic peptide system. *Proc Natl Acad Sci USA* 1988;85:5409–13. [PubMed: 3399498]
81. Agadjanyan MG, Ghochikyan A, Petrushina I, Vasilevko V, Movsesyan N, Mkrtichyan M, Saing T, Cribbs DH. Prototype Alzheimer's disease vaccine using the immunodominant B cell epitope from

- beta-amyloid and promiscuous T cell epitope pan HLA DR-binding peptide. *J Immunol* 2005;174:1580–6. [PubMed: 15661919]
82. Moore A, McCarthy L, Mills KH. The adjuvant combination monophosphoryl lipid A and QS21 switches T cell responses induced with a soluble recombinant HIV protein from Th2 to Th1. *Vaccine* 1999;17:2517–27. [PubMed: 10418898]
  83. Maier M, Seabrook TJ, Lazo ND, Jiang L, Das P, Janus C, Lemere CA. Short amyloid-beta (Abeta) immunogens reduce cerebral Abeta load and learning deficits in an Alzheimer's disease mouse model in the absence of an Abeta-specific cellular immune response. *J Neurosci* 2006;26:4717–28. [PubMed: 16672644]
  84. Zou J, Yao Z, Zhang G, Wang H, Xu J, Yew DT, Forster EL. Vaccination of Alzheimer's model mice with adenovirus vector containing quadrivalent foldable Abeta(1–15) reduces Abeta burden and behavioral impairment without Abeta-specific T cell response. *J Neurol Sci* 2008;272:87–98. [PubMed: 18571202]
  85. Kim HD, Jin JJ, Maxwell JA, Fukuchi K. Enhancing Th2 immune responses against amyloid protein by a DNA prime-adenovirus boost regimen for Alzheimer's disease. *Immunol Lett* 2007;112:30–8. [PubMed: 17686533]
  86. Zamora E, Handisurya A, Shafti-Keramat S, Borchelt D, Rudow G, Conant K, Cox C, Troncoso JC, Kirnbauer R. Papillomavirus-like particles are an effective platform for amyloid-beta immunization in rabbits and transgenic mice. *J Immunol* 2006;177:2662–70. [PubMed: 16888028]
  87. Chackerian B, Rangel M, Hunter Z, Peabody DS. Virus and virus-like particle-based immunogens for Alzheimer's disease induce antibody responses against amyloid-beta without concomitant T cell responses. *Vaccine* 2006;24:6321–31. [PubMed: 16806604]
  88. Staufenbiel, M.; Wiederhold, K-H.; Tissot, AC.; Frey, P.; Fulurija, A.; Hiestand, P.; Jennings, GT.; Luond, R.; Mayer, P.; Ortmann, R. Immunization with Abeta1–6 coupled to the viral-like particle Qbeta (CAD 106) efficiently removes beta-amyloid without inducing Abeta-reactive T cells. *Proceedings of 10th ICAD Alzheimer's and Dementia*; 2006. p. S20
  89. Jennings GT, Bachmann MF. The coming of age of virus-like particle vaccines. *Biol Chem* 2008;389:521–36. [PubMed: 18953718]
  90. Nardin EH, Calvo-Calle JM, Oliveira GA, Clavijo P, Nussenzweig R, Simon R, Zeng W, Rose K. Plasmodium falciparum polyoximes: highly immunogenic synthetic vaccines constructed by chemoselective ligation of repeat B-cell epitopes and a universal T-cell epitope of CS protein. *Vaccine* 1998;16:590–600. [PubMed: 9569470]
  91. Ghochikyan A, Mkrtychyan M, Petrushina I, Movsesyan N, Karapetyan A, Cribbs DH, Agadjanyan MG. Prototype Alzheimer's disease epitope vaccine induced strong Th2-type anti-Abeta antibody response with Alum to Quil A adjuvant switch. *Vaccine* 2006;24:2275–82. [PubMed: 16368167]
  92. Donnelly JJ, Liu MA, Ulmer JB. Antigen presentation and DNA vaccines. *Am J Respir Crit Care Med* 2000;162:S190–3. [PubMed: 11029393]
  93. Tang DC, Devit M, Johnston SA. Genetic immunization is a simple method for eliciting an immune response. *Nature* 1992;356:152–4. [PubMed: 1545867]
  94. Agadjanyan, MG.; Ugen, K.; Wang, B.; Villafana, T.; Merva, M.; Petrushina, I.; Williams, WW.; Weiner, DB. DNA inoculation with an HTLV-I envelope DNA construct elicits immune responses in rabbits. In: Chanock, RM.; Ginsberg, HS.; Brown, F.; Lerner, RA., editors. *Vaccines '94: Modern Approaches to New Vaccines Including Prevention of AIDS*. Cold Spring Harbor Laboratory Press; Cold Spring Harbor, NY: 1994. p. 47–53.
  95. Pardoll DM, Beckerleg AM. Exposing the immunology of naked DNA vaccines. *Immunity* 1995;3:165–9. [PubMed: 7648389]
  96. Wang B, Ugen KE, Srikantan V, Agadjanyan MG, Dang K, Refaeli Y, Sato AI, Boyer J, Williams WV, Weiner DB. Gene inoculation generates immune responses against human immunodeficiency virus type 1. *Proc Natl Acad Sci USA* 1993;90:4156–60. [PubMed: 8483929]
  97. Lu S, Arthos J, Montefiori DC, Yasutomi Y, Manson K, Mustafa F, Johnson E, Santoro JC, Wissink J, Mullins JJ, Haynes JR, Letvin NL, Wyand M, Robinson HL. Simian immunodeficiency virus DNA vaccine trial in macaques. *J Virol* 1996;70:3978–91. [PubMed: 8648735]
  98. Boyer JD, Ugen KE, Wang B, Agadjanyan M, Gilbert L, Bagarazzi ML, Chattergoon M, Frost P, Javadian A, Williams WV, Refaeli Y, Ciccarelli RB, McCallus D, Coney L, Weiner DB. Protection

- of chimpanzees from high-dose heterologous HIV-1 challenge by DNA vaccination [see comments]. *Nat Med* 1997;3:526–32. [PubMed: 9142121]
99. Petrushina I, Ghochikyan A, Mkrtichyan M, Mamikonyan G, Movsesyan N, Davtyan H, Patel A, Head E, Cribbs DH, Agadjanyan MG. Alzheimer's disease peptide epitope vaccine reduces insoluble but not soluble/oligomeric A β species in amyloid precursor protein transgenic mice. *J Neurosci* 2007;27:12721–31. [PubMed: 18003852]
  100. Movsesyan N, Ghochikyan A, Mkrtichyan M, Petrushina I, Davtyan H, Olkhanud PB, Head E, Biragyn A, Cribbs DH, Agadjanyan MG. Reducing AD-like pathology in 3xTg-AD mouse model by DNA epitope vaccine—a novel immunotherapeutic strategy. *PLoS ONE* 2008;3:e21–4.
  101. Bonecchi R, Bianchi G, Bordignon PP, D'Ambrosio D, Lang R, Borsatti A, Sozzani S, Allavena P, Gray PA, Mantovani A, Sinigaglia F. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. *J Exp Med* 1998;187:129–34. [PubMed: 9419219]
  102. Sallusto F, Lenig D, Mackay CR, Lanzavecchia A. Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. *J Exp Med* 1998;187:875–83. [PubMed: 9500790]
  103. Imai T, Nagira M, Takagi S, Kakizaki M, Nishimura M, Wang J, Gray PW, Matsushima K, Yoshie O. Selective recruitment of CCR4-bearing Th2 cells toward antigen-presenting cells by the CC chemokines thymus and activation-regulated chemokine and macrophage-derived chemokine. *Int Immunol* 1999;11:81–8. [PubMed: 10050676]
  104. Biragyn A, Belyakov IM, Chow YH, Dimitrov DS, Berzofsky JA, Kwak LW. DNA vaccines encoding human immunodeficiency virus-1 glycoprotein 120 fusions with proinflammatory chemoattractants induce systemic and mucosal immune responses. *Blood* 2002;100:1153–9. [PubMed: 12149191]
  105. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid β -peptide. *Nat Rev Mol Cell Biol* 2007;8:101–12. [PubMed: 17245412]
  106. Candore G, Balistreri CR, Colonna-Romano G, Grimaldi MP, Lio D, Listi F, Scola L, Vasto S, Caruso C. Immunosenescence and anti-immunosenescence therapies: the case of probiotics. *Rejuvenation Res* 2008;11:425–32. [PubMed: 18442326]
  107. Weinberger B, Hernler-Brandstetter D, Schwanninger A, Weiskopf D, Grubeck-Loebenstein B. Biology of immune responses to vaccines in elderly persons. *Clin Infect Dis* 2008;46:1078–84. [PubMed: 18444828]
  108. Franceschi C, Olivieri F, Marchegiani F, Cardelli M, Cavallone L, Capri M, Salvioli S, Valensin S, De Benedictis G, Di Iorio A, Caruso C, Paolisso G, Monti D. Genes involved in immune response/inflammation, IGF1/insulin pathway and response to oxidative stress play a major role in the genetics of human longevity: the lesson of centenarians. *Mech Ageing Dev* 2005;126:351–61. [PubMed: 15621218]