Published in final edited form as:

Biochem Pharmacol. 2014 April 15; 88(4): 499–507. doi:10.1016/j.bcp.2013.12.020.

Immunotherapy for Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is the most common cause of dementia worldwide. In AD the normal soluble amyloid β (sA β) peptide is converted into oligomeric/fibrillar A β . The oligomeric forms of A β are thought to be the most toxic, while fibrillar A β becomes deposited as amyloid plaques and congophilic angiopathy, which serve as neuropathological markers of the disease. In addition the accumulation of abnormally phosphorylated tau as soluble toxic oligomers and as neurofibrillary tangles is a critical part of the pathology. Numerous therapeutic interventions are under investigation to prevent and treat AD. Among the more exciting and advanced of these approaches is vaccination. Active and passive Immunotherapy targeting only A β has been successful in many AD model animal trials; however, the more limited human data has shown much less benefit so far, with encephalitis occurring in a minority of patients treated with active immunization and vasogenic edema or amyloid-related imaging abnormalities (ARIA) being a complication in some passive immunization trials. Therapeutic intervention targeting only tau has been tested only in mouse models; and no approaches targeting both pathologies concurrently has been attempted, until very recently. The immune approaches tried so far were targeting a self-protein, albeit in an abnormal conformation; however, effective enhanced clearance of the disease associated conformer has to be balanced with the potential risk of stimulating excessive toxic inflammation. The design of future more effective immunomodulatory approaches will need to target all aspects of AD pathology, as well as specifically targeting pathological oligomeric conformers, without the use of any self-antigen.

Keywords

amyloid β ; tau; vaccination; immunomodulation; Alzheimer's disease; transgenic mice

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Introduction

Alzheimer's disease (AD) is the most common cause of dementia globally, affecting approximately 36 million people currently and ~115 million by 2050 [1]. The associated costs are enormous, being estimated in the USA alone to be ~\$200 billion in 2013. Presently available treatments have minimal, or no, effect on the course of disease. The neuropathology of AD consists of the accumulation of amyloid β (A β) as amyloid plaques and congophilic amyloid angiopathy (CAA), as well as the accumulation of aggregated, phosphorylated tau as neurofibrillary tangles [2]. The most toxic species of A β and aggregated tau are thought to be oligomeric, with both of these pathologies spreading via extracellular soluble oligomers, which under some conditions have been shown to use a "prion-like" mechanism [3–5]. A β and tau oligomers, as well as amyloid plaques and NFTs share many structural and biophysical properties, such as a high β -sheet content, resistance to proteolytic degradation and neuronal toxicity. It has also been shown that A β and tau related pathology can, under some conditions, "seed" or propagate each other [5].

At least in mouse models, immunotherapy has shown great effectiveness in preventing the development of both AD and prion diseases [6,7]. Numerous novel therapeutic strategies are being developed to potentially treat AD, with active and passive immunization being among the most advanced approaches [8–12]. Certainly in AD Tg mouse models A β directed immunization has been spectacularly successful using a wide variety of methods. However, translating these results effectively and without toxicity in humans has been challenging. Significant unanswered questions remain for the current and future human trials. What is the best design of a vaccine? What is the best target? How can auto-immune toxicity be avoided? When should therapy start? Also a key issue which needs to be addressed is the simultaneous targeting of both amyloid β (A β) and tau related pathology, as well as targeting the most toxic oligomeric forms of aggregated A β and tau.

Pathogenesis of Alzheimer's disease

The pathological hallmarks of AD are the accumulation of extracellular A β as neuritic plaques and congophilic angiopathy, as well as the intracellular accumulation of abnormally phosphorylated tau in the form of neurofibrillary tangles (NFTs). Missense mutations in APP or in the presenilin genes PRES 1 and 2 cause early onset, familial forms of AD (FAD) affecting <1% of AD patients [13,14]. The most common form of AD is sporadic and late-onset. The dominant theory for the causation of AD has been the amyloid cascade hypothesis [15–17]. This updated theory currently suggests that accumulation of A β peptides particularly in a highly toxic oligomeric form is the primary pathogenic driver, that downstream leads to tau hyperphosphorylation, NFT formation and ultimately to synaptic and neuronal loss. A number of proteins may actively promote the conformational transformation of soluble A β and stabilize pathological oligomeric conformers. Examples of such proteins in AD include apolipoprotein E (apo E), especially its E4 isoform [18], α 1-antichymotrypsin (ACT) [19] or C1q complement factor [20,21]. In their presence, the formation of A β fibrils in a solution of water-soluble A β is much more efficient [18,19]. These "pathological chaperone" proteins have been found histologically and biochemically in association with fibrillar A β deposits but not in preamyloid aggregates which are not associated with neuronal loss [22]. An important event in the pathomechanism of AD is thought to be reaching a critical concentration of water-soluble A β and/or chaperone proteins in the brain, at which point the conformational change occurs. This in turn leads to formation of A β aggregates, initiating a neurodegenerative cascade. In sporadic AD this may be related to any combination of an age-associated impaired clearance from the brain, and/or influx into the CNS of A β circulating in the serum [15]. Extensive evidence supports the amyloid cascade hypothesis in FAD patients and in models of FAD: 1) Inherited forms of

AD linked with mutations in the APP gene or in the *PRES1* or 2 genes are associated with changes in APP processing that favor over production of sA β or production of more aggregation prone forms of sA β such as A β 1–42 [23]. 2) Down's syndrome, where there is an extra copy of the APP gene due to trisomy 21, is associated with AD related pathology at a very early age [24,25]. 3) In transgenic and other models of co-expressed amyloid β and tau, amyloid β oligomer formation precedes and accentuates tau related pathology, consistent with the hypothesis that NFT formation is downstream from A β aggregation [26–29]. 4) In transgenic mouse models of mutant APP over-expression (where there is no tau pathology) therapeutic prevention and/or removal of A β is associated with cognitive benefits in experimental mice [26,30,31]. Importantly, in transgenic mouse models of both mutant APP and tau over-expression (with both amyloid and tau related pathology) prevention of A β pathology leads to both amelioration of cognitive deficits and tau related pathology [32–34]. However, evidence proving that A β is central in the common late-onset sporadic form of AD is more limited: 1) A correlation has been shown between biochemically extracted A β peptides species from sporadic AD brains with cognitive decline [35]. 2) Isolated A β peptide dimers/oligomers from sporadic AD brains have been documented to impair synaptic structure and function [36]. 3) A β extracted from sporadic AD patients has been shown to induce amyloid deposits when injected into transgenic mice [5,37]. Potential conflicting evidence to the amyloid cascade hypothesis comes from the autopsy data from the initial human active vaccination trial, which is further discussed below. Post-mortem analysis was available from nine subjects in the active immunization arm [38]. All these individuals showed a considerable degree of plaque removal and reduced A β load compared to comparable non-immunized controls. Despite this, there were no differences between placebo and active immunization groups in terms of longterm survival outcome, time to severe dementia and in outcome measures such as ADAS-Cog, MMSE or DAD. This might have been related to the immunization having begun too late in the disease process [9,15]; alternatively, one can use this data to suggest that the amyloid cascade hypothesis is an oversimplification. A number of investigators have suggested alternative theories, whereby accumulation of aggregated, toxic forms of A β and tau are dual pathways both downstream from a common upstream pathogenic deficit (which remains to be identified) [39–41]. In either of these scenarios it is essential for immunotherapy to address both of these pathologies to be highly effective in clinically symptomatic AD. In this review we will summarize the preclinical and clinical data for both A β and phosphorylated tau reduction immunotherapeutic approaches.

Active Immune Therapy Targeting A β in Humans

Initial studies supporting immunotherapy for AD showed that anti-A β antibodies could inhibit A β peptide fibrillization, disaggregate pre-formed fibrils and prevent cell culture based neurotoxicity [42,43]. This lead to Schenk et al. to use full length, aggregated A β 1–42 for active immunization with Freund's adjuvant to demonstrate that this could reduce plaque burden in an AD Tg model, without obvious toxicity [44]. Subsequent trials of active vaccination of AD Tg mice with A β 1–42 or A β homologous peptides co-injected with Freund's or alum adjuvants also demonstrated striking reduction in A β deposition, as well as prevention of cognitive impairments [26,30,45–49] (see Table 1). The predominant epitope from these active immunizations was mainly located in the first 15 amino acids of the A β peptide, with studies demonstrating that the generated anti-A β antibodies were able to label amyloid plaques on human AD brain sections. Similar effects on A β load and behavior were demonstrated in AD Tg mice by peripheral injections of anti-A β monoclonal antibodies indicating that the therapeutic effect of the vaccine is based primarily on eliciting a humoral response [26,30,50,51]. In these initial preclinical studies no toxicity was evident in the treated mice; however, some investigators suggested that use of non-fibrillogenic, non-toxic A β homologous peptides along with approaches that stimulate primarily humoral, Th-2

immunity, in contrast to a primary Th-1 cell mediated response might reduce potential toxicity [52–54]. These A β homologous peptide immunogens, with a few appropriate amino acid substitutions, were designed with the knowledge that the major B cell epitopes were within the first 15 amino acids of A β , while the T cell epitopes are in the mid and carboxyl terminus [48,49,53].

The dramatic biological effect of the original Schenk et al. vaccination in preclinical testing encouraged Elan/Wyeth in April 2000 to launch a randomized, multiple-dose, dose-escalation, double-blind Phase I clinical trial with a vaccine designated as AN1792, which contained pre-aggregated A β 1–42 and QS21 as an adjuvant. This type of vaccine design was aimed to induce a strong cell mediated immune response, since QS21 is known to be a strong inducer of Th-1 lymphocytes, which would produce an effect similar to that obtained in mice with the use of Freund's adjuvant (which is not approved for use in humans) [55]. The initial trial was conducted in the UK and involved 80 patients with mild to moderate AD [56]. This trial was designed to assess the antigenicity and the toxicity of multiple dose immunization with the full length A β 1–42 peptide with the QS21. 53% of patients developed an anti-A β humoral response. During the later stages of the phase I trial, the emulsifier polysorbate 80 was added to increase the solubility of the A β 1–42 immunogen, causing a greater shift from a Th2 biased response to a proinflammatory Th1 response [57]. In the subsequent phase IIa trial, begun in October 2001, 372 patients were enrolled with 300 receiving the aggregated A β 1–42 (AN1792) with QS21 in the polysorbate 80 formulation (AN1792 to placebo ratio of 4:1). This trial was prematurely terminated in January 11, 2002 when 6% of vaccinated patients manifested symptoms of aseptic meningoencephalitis (18 out of 298 subjects with no placebo patients developing this complication) [55,58,59]. The onset of this complication varied from 5 to 168 days after the last immunization. Most of these patients presented with increased confusion, lethargy and/or headache; however, some patients were asymptomatic. MRI findings showed white matter lesions with or without evidence of brain edema, termed amyloid-related imaging abnormalities (ARIA). Autopsies performed on a limited number of trial patients suggested that striking A β clearance of parenchymal plaques had occurred, similar to what had been reported in the animal studies, confirming the validity of this approach for amyloid clearance in humans [59–64]. In these cases extensive areas of cerebral cortex were devoid of plaques, with residual plaques having a "moth-eaten" appearance or persisting as "naked" dense cores. This amyloid clearance in most cases was in association with microglia that showed A β immunoreactivity, suggesting phagocytosis. Additional striking features were the persistence of amyloid in cerebral vessels, as well as unaltered tau immunoreactive NFTs and neuropil threads in regions of cerebral cortex where plaque clearing had apparently occurred, compared to regions without clearing [62–64]. Hence, this initial vaccination approach did not address vascular amyloid or NFT related pathology. Some cases also showed a deleterious T-cell reaction surrounding some cerebral vessels, suggestive of an excessive Th-1 immune response. It appeared that the immune reaction triggered by AN1792 was a double-edge sword, where the benefits of a humoral response against A β were overshadowed in some individuals by a detrimental T cell mediated inflammatory response [59,65]. The likely involvement of an excess cell mediated response in mediating toxicity was supported by analysis of peripheral blood mononuclear cells from trial patients, which were stimulated in vitro with the A β peptide, followed by quantification of cytokine secretion by enzyme-linked immunosorbent spot assay [57]. The cells of most responder trial patients mounted IL-2 and IFN- γ positive responses indicative of a Class II (CD4+) Th-1 type response [57]. The follow-up data from the Zurich cohort, who are a subset of the Elan/Wyeth trial [66,67], indicated that the vaccination approach may have some benefits for human AD patients. In agreement with the findings in the Zurich cohort, immune responders with high antibody titers in the multi-center cohort scored significantly better in composite scores of memory functions as compared to low- and non-responders or

to the placebo group of patients [57]. However, despite the apparent success in amyloid clearance indicated by the autopsy data, the clinical cognitive benefits were very modest when the active vaccination group was compared to the placebo group [68]. No difference between the antibody responders and the placebo group was found on multiple neuropsychological rating scales. This data may suggest that vaccination in this cohort was started too late; hence, tau related pathology was unaffected by vaccination and thus the cognitive benefits were small. Alternatively it may suggest that the amyloid cascade hypothesis must be an oversimplification of the pathogenesis of sporadic AD.

There are a number of next generation active A β vaccination trials in either Phase I or II (www.clinicaltrials.gov) (see Table 2). In 2012 Novartis Pharmaceuticals reported Phase I results for their active vaccine called CAD106 [9,69]. In this trial a small A β fragment (A β 1–6) was used which is a B-cell epitope, coupled to an adjuvant carrier formed by multiple copies of the coat protein of bacteriophage Q β . This Phase I trial enrolled mild to moderate probable AD subjects (MMSE 16–26). Patients were randomized to 3 injections of either 50 μ g CAD106 (24 subjects, cohort 1) or 150 μ g CAD106 (22 patients, cohort 2). Cohorts 1 and 2 had 7 and 5 placebo subjects, respectively. The study lasted 1 year, with a 2 year follow up period. In cohorts 1 and 2, 75% and 100% patients respectively, developed anti-A β IgM titers, while 67% and 82%, respectively developed anti-A β IgG titers. Although, nine patients reported serious adverse reactions, none were considered related to the immunogen. Significantly no cases of meningitis, meningoencephalitis or vasogenic edema occurred clinically or by MRI during the trial period or in the 2 year follow up. CAD106 subjects did not show significant differences in CSF biomarkers but differences were seen in cohort 2 in treated subjects compared to controls for free plasma A β 1–40. The study was not powered to show any clinical differences between treated and control subjects. Phase II CAD106 trials were completed in Feb 2013; the results have not been reported.

Other on-going active immunization trials include ACC-001 by Janssen and Pfizer. This Phase II trial uses an A β (1–6) fragment attached to a carrier protein, using the surface-active saponin adjuvant QS-21 [70]. Affiris AG in conjunction with GlaxoSmithKline (GSK) is using AFFITOME(R) technology, generating mimotopes (synthetic peptides that mimic specifically antigen epitopes) targeting the unmodified A β N-terminus in its AD02 trials [71]. Affiris AG is also in Phase I trials using this same technology to target a pyroglutamic-3 modified A β N-terminus. The latter represents a post-translational modification of A β which is thought to occur once it is deposited in plaques or vascular amyloid; this modification renders A β more aggregation prone [72,73]. Pyroglutamic-3 modified A β is found only in plaques and vascular amyloid deposits and not normally in plasma or CSF, except in therapeutic situations where deposited A β is being mobilized [74]. AC Immune is conducting Phase I/IIa trials to test ACI-24, which is designed to induce a humoral immune response to A β in a predominately β -sheet conformation. This is similar to the approach this group reported in an AD Tg model, that used a tetra-palmitoylated amyloid 1–15 peptide in a predominately β -sheet conformation as an immunogen [75]. The initial results of these on-going active immunization trials have not yet been reported. A potential advantage of these second generation active immunization approaches is that some of them more specifically target pathological conformers of A β ; this reduces the chances of autoimmune toxicity. However, the immunogens are still based on the A β sequence, so there will likely be some degree of cross reactivity to normal A β peptides, with the continued risk of inflammatory toxicity. Furthermore none of these active immunization trials directly address tau related pathology.

The Past Passive Immunization Experience for AD

Passive immunization consists of an injection of pre-prepared antibodies to patients, as opposed to active immunization where the immune system is stimulated to produce its own antibodies. Passive transfer of exogenous monoclonal anti-A β antibodies appears to be the easiest way to fulfill the goal of providing anti-A β antibodies without risk of uncontrolled Th-1 mediated autoimmunity. AD Tg model mice treated this way had a significantly reduced A β level and demonstrated cognitive benefit [50,51]. Potential problems with passive immunization include the need for repeated injections in a chronic disease, high cost, proper selection of antigen targets, blood-brain barrier penetration, the risk of hemorrhages and the development of an immune response to the injected antibodies.

There are a number of possible mechanisms of action by which antibodies can benefit AD pathology which are not mutually exclusive [30,76–78]. Anti-A β antibodies may target A β deposits in the brain leading to their direct disassembly. Antibodies within the brain may also activate microglia cells to clear plaques. This could be via eliciting Fc mediated phagocytosis or Fc-independent mechanism. Antibodies may also block A β toxicity or sequester A β monomers within the CNS preventing them from aggregating. An additional mechanism by which anti-A β antibodies can prevent A β deposition is the creation of a “peripheral sink” effect, where the removal of excess sA β circulating in the blood stream, leads to sA β being drawn out from the brain. Which of these mechanisms is most critical in the pre-clinical studies using transgenic AD model has not been fully elucidated, nor is this at all clear in the more limited human trials. Several passive immunization trials are underway; however, the two most advanced Phase III trials of both Bapineuzumab and Solanezumab were recently reported and both failed to show overall clinical improvement or disease modifying outcomes [78]. Bapineuzumab is a humanized version of the mouse monoclonal antibody 3D6, which is specific to residues 1–5 of A β . 3D6 was shown to cross the BBB, bind to plaques in the brain and induce Fc-mediated microglial phagocytosis of A β plaques in Tg mouse studies [50,79]. The Phase II trials of Bapineuzumab (study 201 with 234 patients and study 202 with 28 patients) involved six infusions every 13 weeks over 8 months at 4 different doses (0.15mg/kg, 0.5mg/kg, 1mg/kg and 2mg/kg) [78,80]. The trials showed no statistically significant outcomes but there were some trends toward efficacy on the Alzheimer’s Disease Assessment Scale-Cognitive subscale (ADAS-Cog) and the Disability Assessment for Dementia (DAD). Significant complications in these trials were related to amyloid-related imaging abnormalities (ARIA) [81]. These included FLAIR MRI signal abnormalities representing parenchymal vasogenic edema and sulcal effusions (ARIA-E) and MRI abnormalities seen on T2* weighted gradient echo sequences, that are thought to represent microhemorrhages and hemosiderosis (ARIA-H). 36 patients (17% of total patients) developed ARIA-E during treatment; however, this was symptomatic in 8 of these 36 patients (22% of patients with ARIA-E). Adverse events included headache, confusion, neuropsychiatric and gastrointestinal symptoms. ARIA-H occurred in 17 patients with ARIA-E and in 7 of 177 patients without ARIA-E [81]. There was an association of these adverse effects with both the presence of the apoE4 allele and increased dose. 7 out of the 8 symptomatic patients were apoE4 carriers and 6 out of the 8 were treated with the two highest doses of Bapineuzumab. A likely mechanism of these adverse events is related to removal of cerebral vessel A β associated with increased BBB permeability and microhemorrhages [78,81]. It is well characterized that apoE4 carriers have a higher CAA burden [22]. Based on these Phase II results, Phase III trials were designed to give the apoE4 carriers a lower dose (limited to 0.5mg/kg) and to limit the maximum dose in apoE4 non-carriers to 1.0mg/kg. This Phase III trial involved 1121 patients who were given infusions every 13 weeks for 6 infusions over 1 and half years. There was no clinical improvement in either the apoE4 carrier or non-carrier groups. ARIA occurred in 15% of the apoE4 group and in 9% and 4% of the 1.0mg/kg and 0.5mg/kg apoE4 non-carrier groups, respectively.

Based on these results clinical development of Bapineuzumab has been halted. However, AAB-003 is a humanized version of 3D6 (i.e. Bapineuzumab) with mutations in the Fc domain to reduce its effector function and possibly reduce ARIAs. AAB-003 is currently in two Phase I trials (www.clinicaltrials.gov).

Solanezumab is a humanized version of mAb 266 which has an epitope at residues 16–24 of A β . 266 was shown to bind to monomeric, soluble A β , lowering amyloid pathology in Tg mouse models in association with markedly increased A β levels in the plasma [50,79]. Its major mechanism of action is thought to be via peripheral A β sequestration and a peripheral sink. Two Phase III trials have been conducted with a combined number of ~800 mild to moderate AD patients in both control and treated groups. Treated patients were given 400mg of Solanezumab (~5.7mg/kg) every 4 weeks. Cognition was assessed at 80 weeks; there was no difference compared with controls overall. However, when mild AD patients were analyzed separately there was a statistically significant benefit in cognitive scores; albeit the effect was small [78]. Despite the higher dosage used of Solanezumab, compared to Bapineuzumab, ARIAs were not seen as a complication and an increase in plasma A β was documented [82]. As a result of these findings Solanezumab will be tested in two prevention or very early treatment trials. The Dominantly Inherited Alzheimer Network (DIAN) trial will be conducted in adult children of a parent with familial AD with a known mutation. In this trial Gantenerumab will also be tested. This mAb preferentially binds to fibrillar A β and is already in a Phase III prevention trial involving 770 patients who have a significant amyloid burden by PET scans but are without AD symptoms [31,83,84]. Solanezumab will also be tested in a prevention trial called A4 or the Anti-Amyloid Treatment for Asymptomatic Alzheimer's disease, among ~1000 patients 70 yr and older who are positive for amyloid by PET but who do not have symptoms of AD.

An additional prevention trial is the Alzheimer's Prevention Initiative (API) to be conducted in 300 individuals with a PS1 mutation (E280A) from a large Colombian kindred. This mutation is associated with an aggressive AD phenotype where robust A β deposition starts at ~25yrs. Patients 30 yr and older will be tested with Genentech's Crenezumab mAb which binds to soluble, oligomeric and fibrillar A β deposits [85]. Crenezumab has an IgG4 backbone which reduces effector function.

Similar in approach to the above passive immunizations trials, intravenous immunoglobulin (IVIG) are currently in several clinical trials for AD, with the rationale being that IVIG, produced from a large pool of donors, would contain a small fraction of naturally occurring anti-A β antibodies. IVIG is widely used as an immunosuppressant in a variety of autoimmune mediated neurological disorders, often with regular repeating doses and no significant side effects. It has been reported that patients who receive regular IVIG infusions have a reduced risk of subsequently developing AD [86]. In a pilot, open label study in 8 mild AD patients IVIg was infused over 6 months, discontinued and resumed for another 9 months [87]. Following each infusion the plasma A β levels increased transiently with CSF A β being decreased after 6 months. The MMSE increased an average of 2.5 after 6 months and returned to baseline after washout and remained stable with the subsequent IVIg infusions. The CSF of these patients was collected before therapy, after 6 months of therapy and after the 3 month wash-out. A panel of 23 putative diagnostic CSF AD biomarkers was analyzed in these patients. Six of the 8 subjects showing improvements in these markers after 6 months of therapy, which reverted to baseline after IVIG wash-out [88]. However, in two more recent trials no significant slowing of AD progression was demonstrated [30,89]. In the Octapharma IVIG trial 58 mild to moderate AD patients were infused at 3 different doses over 6 months. No significant improvement was noted on MRI volume measurements, FDG-PET or any cognitive measures. Six out of the 43 IVIG treated patients had new incident microhemorrhages, which were asymptomatic. Baxter Healthcare Corporation

conducted an 18 month Phase III trial of Gammagrad 10% IVIG in 390 mild to moderate AD subjects. The results of this trial have not been fully reported but no significant improvement in cognitive measures was noted [30].

All the above described passive immunization approaches have the short coming that they do not specifically target A β oligomers, which are known to be the most toxic species of A β . Either both the normal conformer and the pathological conformer of A β are targeted or in the case of Solanezumab only soluble A β is targeted [90]. This can be problematic as targeting normal sA β may inhibit its known physiological functions such as neuroprotection, modulation of long term potentiation and innate immunity, as well as having the risk of inducing autoimmune complications [91–93]. An additional issue is that such approaches would need to be started very early in the AD process to be clinically highly effective. Extensive neuropathological data has established that by the time the earliest clinical signs of AD emerge, A β deposition may be close to reaching its peak and that NFT formation and neuronal loss are substantial but have not yet reached peak levels [2,94]. This would suggest that amyloid directed therapy targeting soluble A β alone, or both soluble and deposited A β , would need to begin very early, even before the mild cognitive impairment stage, in order to have a major effect. Hence such approaches are not likely to be very useful for symptomatic AD.

Tau Related Pathology as an Immune Target

Neurofibrillary tangles (NFTs) are a major pathologic hallmark of AD. NFTs are intraneuronal inclusion bodies that consist of an accumulation of paired helical filaments (PHFs), which biochemically are mainly composed of abnormally phosphorylated tau. Recently there is increasing focus on phosphorylated tau as an immunotherapeutic target [94–98]. Although most thinking in the field suggests that tau pathology is downstream from A β deposition [2], some studies have indicated that tau pathology begins to deposit prior to amyloid plaques in the locus ceruleus and from there spreads to other brainstem nuclei and the entorhinal cortex [99,100]. Whether this early tau pathology is integral to AD related pathology development or part of the “normal” aging process is unclear [94]. In any case, numerous studies have shown that the degree of tau related pathology correlates better with the degree of dementia compared to amyloid plaque burden, making tau an attractive target in symptomatic AD patients [101–103]. The human amyloid directed vaccination experience summarized above, showing that amyloid burden reduction does not translate to cognitive improvements in symptomatic AD subjects, strengthens this point.

It has been shown that active immunization of Tg mice P301L with a phospho-tau peptide (containing the phosphorylated PHF-1 epitopes Ser 396, Ser 404) for two to five months could prevent tau related pathology when started prior to the onset of pathology [104]. These particular phosphorylation epitopes were chosen since these sites have been shown to increase the fibrillogenic nature of tau and contribute into paired helical filaments formation [105,106]. Histological and biochemical analyses showed a reduction of aggregated tau in the brain and improve performance on motor tasks [104]. This study clearly documented that it is possible to reduce tau related pathology with active immunization. Similar findings were reported in a htau/PS1 tau pathology model [107]. A caveat is that these studies use mice with tau pathology resulting in locomotor deficits, so cognition could not be assessed as a therapeutic endpoint.

At first examination it is difficult to understand how an antibody response to a protein which is accumulating intra-cellularly can have beneficial effects. However, such an outcome is supported by a study of immunization in a Parkinson's disease transgenic mouse model with α -synuclein showing a reduction of intracellular α -synuclein aggregates [108]. Recent

studies have indicated anti-tau antibodies can cross the BBB, can be taken up by neurons via low-affinity Fc receptors and bind to pathological tau within the endosomal/lysosomal system [109]. Furthermore evidence has shown that injection of fibrillar tau brain extract into the brains of transgenic wild-type expressing mice can induce the formation of human tau into filaments, as well as the spread of pathology from the site of injection into neighboring brain regions [110]. This type of "infectivity" of abnormal protein conformation from outside the cell has also been demonstrated for polyglutamine aggregates [111] and is well characterized in prion disease [5]. Hence if the spread of certain pathological forms of tau, that lead to PHF pathology in AD, occurs via such a prion like mechanism, anti-phosphorylated tau antibodies would not necessarily need to enter cells in order to be effective.

Active immunization using tau epitopes carries the risk of inducing encephalitis or neuronal apoptosis. In an early study it was shown that immunization of female C57BL/6 mice with full length recombinant tau produced neurological deficits, NFT-like changes, gliosis and an inflammatory infiltrate [112]. This risk also exists if the epitope is phosphorylated tau. A recent study showed that in E257T/P301S-tau Tg mice and in wild type mice repeatedly immunized with a mixture of three phospho-tau peptides resulted in neuroinflammation associated with neurological disability in the tau Tg mice [113]. This would suggest that a passive immunization approach with anti-phospho-tau directed mAbs might be a safer approach. Two passive immunization trials showed tau related pathology and motor deficits could be reduced when the antibody is administered at early time points prior to the onset of tau pathology [114,115]. Another study, using anti-tau antibodies which block tau aggregation, showed that intracerebroventricular administration of these antibodies could reduce pathology and reduce contextual fear conditioning deficits in P301S tau Tg mice when administered starting at 6 months of age over a 3 month period [116]. Although this study showed pathology could be ameliorated by administration of anti-tau antibodies at a point when pathology is already present, the intraventricular route is a major disadvantage. To date the only study which has administered anti-phosphorylated tau antibodies in mice at a point when pathology is already established failed to show any benefits on animal survival versus controls [117]. This study compared DA31 (a pan-tau antibody), PHF1 (detects pSer396/404) and MC1 (detects a pathological tau conformation in P301L Tg tau mice, which develop pathology from about 3 months of age. MC1 injected mice from 7 to 10 months showed a reduction of tau related pathology immunohistochemically and biochemically. However in mice injected with either PHF1 or MC1 from 6 to 14 months of age, there was no change in survival versus control Tg mice [117]. In a prior study, it was shown PHF1 is able to reduce tau related pathology when treatment is started prior to the onset of disease [114]. Hence although tau pathology directed immunotherapy holds some promise, there appears to be some risk of toxicity and it remains to be clearly shown that it could be effective at time points when significant tau pathology is already present. This will be critical for this type of therapeutic approach to be efficacious at symptomatic stages of AD, when substantial tau related pathology is already evident.

Targeting Abnormal Protein Conformation Instead of Aiming at A β or Tau Related Pathologies Individually

The most toxic species of A β and aggregated tau are thought to be oligomeric. Both of these pathological conformers have been shown to spread via extracellular soluble oligomers in a prion-like mechanism [3–5]. Interestingly it has been shown that therapeutic strategies that block A β oligomer toxicity can be effective at reversing cognitive deficits following a relatively short treatment schedule and in the presence of extensive preexisting amyloid A β pathology [118,119]. Hence, such therapeutic strategies might have the greatest chance of being effective even when significant pathology is already present. A β and tau oligomers,

share many structural and biophysical properties, such as a high β -sheet content, resistance to proteolytic degradation and neuronal toxicity. Active vaccination or use of monoclonal antibodies that specifically target A β oligomers, tau oligomers or preferably both would be an ideal way to block AD related toxicity. A small number of pre-clinical studies targeting A β oligomers suggest that this methodology is potentially powerful and in the need of further development [120–124]. Targeting only the oligomeric forms of A β or tau would also avoid the potential of interfering with the normal physiological functions of these proteins. A novel immunotherapeutic approach is to target the shared abnormal β -sheet conformation of amyloid proteins using conformationally specific antibodies or active immunization that favors such an anti-conformational response [120,121,125]. Such an approach has the advantage that both A β and tau related pathologies would be addressed concurrently. To achieve this aim we developed a therapeutic immunomodulation that specifically targets the pathological β -sheet conformation that is shared by A β and tau disease associated species. We used a polymerized British amyloidosis (pBri) related peptide in a predominantly β -sheet, oligomeric form, prepared by the use of glutaraldehyde as a crosslinker in a controlled manner. British amyloidosis (ABri) is a rare form of familial human amyloidosis associated with a missense mutation in a stop codon resulting in the transcription of an intronic sequence, leading to production of a highly amyloidogenic protein with a carboxyl terminus that has no sequence homology to any other native human protein, including A β and tau [126–128]. We hypothesized that through conformational mimicry the pBri peptide, which corresponds to the polymerized oligomeric 13 amino acids of the carboxyl terminus of ABri, would induce a conformation selective immune response that will recognize oligomeric A β , as well as aggregated, phosphorylated tau. Such an immunostimulatory approach would have a reduced risk of inducing auto-immune complications as it is specific to pathological conformers and the immunogen has no sequence homology to any known mammalian protein/peptide. We showed in an earlier study that this immunomodulation targeting a pathological conformation of A β was highly effective at reducing amyloid plaques, correlating with behavioral rescue [128]. The polyclonal antibody response obtained in the vaccinated mice specifically recognized plaques and dystrophic neurites in human brain tissue [128]. More recently we have shown that this approach directed to abnormal protein conformation is effective in both TgSwDI mice with abundant vascular pathology and in 3xTg mice with both A β and tau related pathology, reducing pathology, oligomer levels and producing cognitive rescue [129].

Conclusions and Future Directions

Numerous different active and passive immunization therapeutic approaches are under development. Approaches targeting A β peptides may have efficacy when begun very early in disease onset prior to any clinical symptoms. Such prevention clinical trials are underway. Tau pathology directed immunotherapy also has promise; however, toxicity is a potential issue and it remains unclear if this approach can be effectively used in the presence of extensive preexisting disease. Most patients even at the mild cognitive impairment stage of AD already have extensive pathology [2]. In addition, some investigators have suggested that in sporadic late-onset AD tau related pathology is not downstream from A β related pathology and that both of these pathologies are dual independent pathways [39,94]. In such a scenario it would be critically important for a therapeutic intervention to be highly efficacious, that both pathologies would be addressed concurrently.

Given the current situation, we hypothesize that in the future directing the immune system to clear the highly toxic abnormal, oligomeric conformers of both A β and tau concurrently may have the greatest potential to be efficacious in symptomatic AD. Such an approach may be achieved by determining the commonality in the tau and A β toxic conformers and using this information to actively modulate the humoral immune response through conformational

mimicry. This active immunization approach can also be used for the development of monoclonal antibodies or related immunotherapeutics, to be used alone or in combination with other agents, to treat the different stages of AD. Such a “ β -sheet buster” approach, directed to pathological protein conformation, has the potential to be efficacious in multiple conformational neurodegenerative diseases.

Acknowledgments

This manuscript was supported by NIH grants AG20245, AG08051 and NS73502, as well as Alzheimer's Disease Association grant IIRG-13-283707

It is also supported by the Seix Dow Foundation.

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Table 1

Active and Passive Immunotherapy in AD Mouse Models

Targeting A β pathology with cognitive benefit and/or amyloid related pathology			
Year	Mouse model	Target/action	References
1999	PDAPP	Aggregated A β 1–42-Freund's	[44]
2000-present	Multiple amyloid Tg Models	A β N-terminus, middle, or C-terminus Freund's or Alum; mAbs anti-A β all regions	[45–55] [76–80; 128]

Targeting Tau related pathology			
Year	Mouse model	Target/action	references
2007–2013	tauP301L, htau/PS1, tauE257T/P301S	Phosphorylated tau; active or passive Freund's or Alum	[95–99; 105, 107] [108;114–116]

Targeting both tau and A β pathology concurrently			
Year	Mouse model	Target/action	references
2010–3	APP/PS1, 3xTg, TgSwDI	Oligomeric forms of β -sheet peptides/proteins Non-self conformational mimicry; Alum	[128,129]

Table 2

Active and Passive Human Immunotherapy Trials for AD target Amyloid Plaques

ACTIVE				
Trial	Stage	Status	Aβ target	references
AN1792	Phase II	Halted, No improvement	Aggregated A β 1–42- QS 21	[56,57;66–68]
CAD106	Phase I/II	I no results II not reported	A β 1–6- bacteriophage QB	[9, 69]
ACC-001	Phase II	finishing	A β 1–6- QS 21	[70]
AFFITOME AD02	Phase II	Not reported	Mimotope of A β N-terminus	[71]
AFFITOME	Phase I	ongoing	Mimotope of PGA A β N-Terminus	[72,73]
ACI-24	Phase I/IIa	Not reported	A β with β -sheet conformation	[75]

PASSIVE				
Trial	Stage	Status	mAb/target	references
Bapineuzumab AAB-001	Phase III	No improvement	Humanized 3D6 anti A β 1–5	[78–81]
Solanezumab	Phase III	No improvement	Hum mAb266 anti-A β 16–24	[78;82–83]
Bapineuzumab AAB-003	Phase I	Ongoing	Humanized 3D6/IgG4	[81]
Gantenerumab DIAN	Phase II/III	Ongoing	Fibrillar A β positions 3–12; 18–27	[84–85]
Crenezumab API	Phase III/II	Ongoing	PS1 mutation E280A	[86]
IVIG Octapharma	Phase II	No improvement	Naturally occurring anti-A β	[87–90]
IVIG Gammagrad	Phase III	No improvement	Naturally occurring anti-A β	[87–90]
IVIG new Gam	Phase II	Ongoing	Naturally occurring anti-A β	[87–90]