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Immunotherapeutic Approaches for Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is the most prevalent form of dementia worldwide and is an emerging global epidemic. It is characterized by an imbalance between production and clearance of amyloid β (A β) and tau proteins. Oligomeric forms of A β and tau are believed to be the most toxic. Dramatic results from AD animal models showed great promise for active and passive immune therapies targeting A β . However, there is very limited evidence in human studies of clinical benefits from these approaches. Immunotherapies targeting only tau pathology have had some success but are limited so far to mouse models. The majority of current methods is based on immunological targeting of a self-protein; hence, benefits needed to be balanced against risks of stimulating excessive auto-immune toxic inflammation. For greater efficacy the next generation of vaccines will need to focus more on concurrently targeting all the intermediate toxic conformers of oligomeric A β and tau species.

Keywords

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

Introduction

Alzheimer's disease [AD] affects more than 20 million people worldwide currently, with about 135 million people expected to develop it by 2050. The staggering numbers affected by this global health epidemic translates into significant direct and indirect health care expenses, with direct costs for the USA alone estimated to be about \$214 billion in 2014.

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Historically, AD has been characterized as a neurodegenerative disease chiefly defined by its pathological signature including β amyloid deposits in the form of extracellular amyloid β (A β) plaques and tau protein aggregates in the form of intracellular neurofibrillary tangles (NFT) (Nelson et al., 2012). A central mechanism underlying the formation of both amyloid plaques and NFTs in AD is pathogenic cerebral protein aggregation. Though both amyloid plaques and aggregated tau have an essential role in AD pathology and are part of the neuropathological definition of the disease, numerous studies suggest that in these precipitated forms they are relatively biologically inert. Hence, the accumulation of aggregated A β in plaques correlates poorly with the clinical status of patients (Nelson et al., 2012; Terry, 1996). Soluble oligomeric forms of A β and tau, which may replicate via a “prion-like” mechanism, are thought to be the chief mediators of cytotoxicity in AD (Ashe and Aguzzi, 2013). These oligomeric species of A β initially accumulate intraneuronally, eventually leading to cell death and the extracellular deposition of amyloid plaques (D’Andrea et al., 2001; Gouras et al., 2000). Both A β and tau oligomers have similar but not identical structural and biophysical properties including a high β -sheet content, some resistance to proteolytic degradation and neuronal toxicity. Recent work has also revealed that A β and tau related pathology can, in certain scenarios, “seed” or transmit each other (Ashe and Aguzzi, 2013; Jucker and Walker, 2011). Current existing therapies have either no or minimal disease modifying benefit. Hence, a number of novel therapeutic strategies are currently under investigation, many of which involve modulating the immune system.

Preclinical studies in transgenic mouse models have shown great efficacy of immunotherapy in the prevention of both AD and prion diseases (Wisniewski and Goni, 2012; Wisniewski and Goni, 2014). With a central role for A β in AD pathogenesis under the “amyloid cascade” model, several strategies directed towards eradication of A β and downstream targets via small molecules or immunotherapies have been and are being explored (Huang and Mucke, 2012; Morgan, 2011; Ozudogru and Lippa, 2012; Wisniewski and Goni, 2014). Though A β directed immunization via multiple approaches has shown promising results in AD Tg mouse models, the translation to safe and efficacious therapy for humans still remains a challenge. Insights from these studies have raised further issues that need to be addressed in current and future studies. Questions that have come up from previous work which are critical for the development of successful immunotherapy include identification of the ideal target and the timing of therapy. What is the best design for a vaccine that is both specific and safe? How could we avoid auto-immune toxicity? Would the ideal approach be active or passive immunization? Is stimulation of the innate immune system a viable option? Can a single vaccine be designed to target both amyloid β (A β) and tau related pathology simultaneously? This article reviews current preclinical and clinical data for A β and phosphorylated tau reduction immunotherapy and discusses how this information may lead to the next generation of more effective vaccines.

Pathogenesis of Alzheimer’s disease

AD is a complex neurodegenerative disease characterized clinically by a progressive deterioration of memory. Pathologically it is defined by deposition of extracellular A β as senile neuritic plaques and congophilic angiopathy (CAA), as well as, intracellular hyperphosphorylated fibrillar tau accumulation in the form of neurofibrillary tangles (NFTs)

(Figure 1). Genetic studies have shown that AD is a heterogeneous disorder that includes the early-onset (EOAD) form (<5% of all AD patients, with onset at <65yrs) and the much more common sporadic late-onset form (LOAD, with onset >65yrs). EOAD is related to mutations in presenilin 1, presenilin 2 (PS1 and PS2) or the amyloid precursor protein (APP), when associated with autosomal dominant inheritance (Bertram and Tanzi, 2012; Guerreiro and Hardy, 2014; Karch et al., 2014). Epidemiological data suggests that apparent autosomal dominant transmission is found in only ~10% of all EOAD cases (<1% of all AD cases), leaving the genetic association of the majority of EOAD unexplained (Guerreiro and Hardy, 2014; Wingo et al., 2012). LOAD afflicts >95% of patients with AD and is related to both genetic and environmental factors (Bertram and Tanzi, 2012; Guerreiro and Hardy, 2014; Karch et al., 2014; Kim et al., 2014). Some of the known environmental risk factors for LOAD include level of physical activity, educational status, diabetes mellitus, hypertension and head injury (Beydoun et al., 2014; Di Marco et al., 2014). The strongest identified genetic risk factor for LOAD is the inheritance of the apolipoprotein (apo) E4 allele (Kanekiyo et al., 2014; Potter and Wisniewski, 2012). The role of apoE in AD is complex but includes isotype specific effects on the aggregation and clearance of brain A β (Kanekiyo et al., 2014). Recently, rare variants of another gene that encodes the triggering receptor expressed on myeloid cells 2 (TREM2; located on 6p21.1) have been reported as a significant risk factor for LOAD, with an odds ratio similar to apoE4 (Boutajangout and Wisniewski, 2013; Hickman and El, 2014). Several hypotheses have been postulated for the development of plaques and tangles, which lead to synaptic and neuronal loss and subsequent decline of memory and cognition in AD. The most favored theory currently in the field that has served as the platform for many therapeutic strategies is the amyloid cascade hypothesis (Hardy and Selkoe, 2002; Holtzman et al., 2012). The central idea proposed in the hypothesis is that A β aggregation, especially in its toxic oligomeric form, is the principal insult which produces neuronal toxicity and triggers downstream signaling events that in turn lead to hyperphosphorylation of tau and development of NFTs (Figure 1). A multitude of “chaperone” proteins have been described that stabilize pathological oligomers and mediate a conformational change of soluble A β . These include but are not limited to apolipoprotein E (apo E), especially its E4 isoform, α 1-antichymotrypsin (ACT) or C1q complement factor (Potter and Wisniewski, 2012). Histological and biochemical evidence suggests that “pathological chaperone” proteins co-localize with fibrillar A β deposits but not with preamyloid aggregates that are not linked to neuronal loss. From a biochemical perspective, a seminal event in the development of pathologic aggregates is the point at which a critical concentration of soluble A β and/or chaperone proteins is achieved. A critical concentration of the precursor proteins would favor a conformational change, drive formation of toxic A β oligomers and subsequent activation of downstream signaling cascades. Mechanisms implicated for achieving this in sporadic AD could be a permutation of impaired clearance of A β from the brain as a consequence of aging and/or inflow of serum A β into the CNS (Holtzman et al., 2012). Several studies in familial Alzheimer’s disease (FAD) patients and in models of FAD have provided evidence in favor of the amyloid cascade hypothesis. The first line of evidence comes from functional analyses of APP gene or in the PS1 or 2 genes that are associated with inherited forms of AD. Mutations in these genes show concomitant changes in APP processing biased towards over production of soluble A β (sA β) or generation of specific species of sA β such as A β 1-42 that are more

prone to aggregation (Hardy, 2006). In addition, a rare APP mutation first reported in an Icelandic population which protects against AD is supportive of the amyloid cascade (Jonsson et al., 2012). This mutation (A673T) acts by reducing amyloidogenic processing of APP and also mildly decreasing A β peptide aggregation (Maloney et al., 2014). The next line of evidence stems from the association of Down's syndrome with AD related pathology at a very young age. Here, an extra copy of the APP gene secondary to trisomy 21 provides excellent *in vivo* gain-of-function evidence supporting the amyloid hypothesis (Hartley et al., 2014). Further, animal models where amyloid β and tau are co-expressed reveal that A β deposition predates formation of tau aggregates, supporting the concept that NFT formation is downstream from A β aggregation (Gotz et al., 2001; Oddo et al., 2003; Wisniewski and Sigurdsson, 2010). Lastly, enhancement of A β clearance in transgenic mouse models with over-expression of mutant APP, but with no tau pathology, has been shown to improve cognitive function in mice (Lemere, 2013; Wisniewski and Sigurdsson, 2010). Subsequent work has also revealed that inhibition of A β in animal models with overexpression of mutant APP and tau not only prevents development of tau related aggregates but also improves cognitive deficits (Blurton-Jones and LaFerla, 2006; McKee et al., 2008; Oddo et al., 2006). In contrast to the genetic forms of AD where the role of A β is well established, definitive evidence regarding A β 's central function in late-onset sporadic AD is more limited. A number of autopsy studies have indicated that medial temporal and subcortical tau pathology precedes A β pathology in the majority of patients (Jack, Jr. et al., 2013). Genome-wide association studies (GWAS) in LOAD have implicated a number of different genes involved in innate immunity, cholesterol metabolism and endocytosis, suggesting greater etiological heterogeneity (Karch et al., 2014). Supporting a role of A β in LOAD the levels of biochemically extracted A β peptides from brains of people with sporadic AD, correlate well with cognitive deficits (Naslund et al., 2000). Further, A β peptide dimer/oligomer extracts derived from sporadic AD brains have been shown to disrupt synaptic structure, function and plasticity that are critical cellular correlates of memory (Shankar et al., 2008). Interestingly, exogenous injections of A β extracts from sporadic AD patients can induce amyloid aggregates in transgenic mice (Ashe and Aguzzi, 2013; Meyer-Luehmann et al., 2006).

One of the significant concerns with the amyloid cascade hypothesis comes from the post mortem analyses from the active vaccination trials in humans. (Holmes et al., 2008). Individuals from the active immunization or the 'test' arm revealed a significant decrease in plaque burden and strikingly reduced A β load relative to non-immunized controls. Regardless of these encouraging results, no improvement in long-term survival outcome, time to severe dementia and cognitive function was seen among the immunized groups. Two recent, large phase III trials of passive immunization for AD have also ended with no evidence of clinical benefit, although post-hoc analysis suggested a positive trend in a subpopulation of early AD patients in the Solanezumab trial (Doody et al., 2014; Salloway et al., 2014). One plausible explanation here is that immunization was conducted in the late stage of the disease process, possibly out of the window to translate into a meaningful clinical benefit (Holtzman et al., 2012; Wisniewski and Goni, 2014). One could also theorize that the amyloid hypothesis represents only part of the complete story. The existence of a currently unknown upstream factor(s) or insult that triggers both the A β and tau pathways

downstream of itself is also possible (Castellani et al., 2008; Small and Duff, 2008). We hypothesize that A β and tau abnormal conformers interact; however, the importance of individual abnormal conformers to this interaction and their influence on pathology might differ from patient to patient (Figure 1). Recent data suggests that in many cases a regionally limited tauopathy precedes A β pathology; however, for the tau pathology to evolve to AD it requires the concomitant presence of A β pathology (Braak and Del, 2011; Crary et al., 2014; Duyckaerts, 2011; Jack, Jr. et al., 2013). Hence tau and A β may be independent processes that show pathological synergy in the evolution of AD. Regardless of the heterogeneous pathogenesis of LOAD, immunotherapy remains an attractive and potentially effective strategy, if it is targeted to the common pathways of both A β and tau related pathology in early stages, as well as, in clinically symptomatic AD. Here, we will review both active and passive immunotherapeutic approaches along with preclinical and clinical data that has been used to target both A β and phosphorylated tau. From past and current experiences we will try to foresee possible pathways to effectively treat AD and related neurodegenerative disorders.

Active Immune Therapy Targeting A β in Humans

Preliminary work that suggested a strong role for immunotherapy for AD revealed that anti-A β targeting antibodies were capable of preventing A β peptide fibrillization, disrupting preformed fibrils and thus, thwarting fibril dependent neurotoxicity in *in vitro* cell culture based assays (Solomon et al., 1997). This initial work motivated further *in vivo* studies to test the role of A β first as an active immunogen and then to assess if it could prevent pathology in mouse models of A β related AD pathology. The first *in vivo* immunization trial, reported in a seminal paper by Schenk et al. demonstrated that full length, aggregated A β 1-42 in conjunction with Freund's adjuvant could reduce plaque load *in vivo*, which at the time was hypothesized to be the main culprit in AD pathology (Schenk et al., 1999). No obvious toxicity was reported in this trial. Results along the same line were confirmed and extended in later studies, where active immunization with A β 1-42 or A β homologous peptides along with Freund's or alum adjuvants not only prevented A β plaque pathology, but also protected against development of cognitive deficits (Asuni et al., 2006; Janus et al., 2000; Lemere, 2013; Morgan et al., 2000; Sigurdsson et al., 2001a; Sigurdsson et al., 2004). Biochemical assays identified the first 15 amino acids of the A β peptide as the site of the principal epitope. Further, immunohistochemical assays also revealed that antibodies generated in mice towards A β can label amyloid plaques on human AD brain sections, raising the possibility of such immune intervention in humans. Interestingly, peripheral injections of anti-A β monoclonal antibodies into the systemic circulation could also reduce A β plaque burden and behavior, suggesting that the therapeutic effect of the vaccine was likely mediated by generating a humoral response (Bard et al., 2000; DeMattos et al., 2001; Lemere, 2013; Wisniewski and Goni, 2014). These pilot preclinical trials revealed no evidence of toxicity in the immunized mice. However, there is some debate about the type of immune response involved in mediating the beneficial effects by these peptides. Besides the classical view at the time, that fibrillar A β with a strong adjuvant was the appropriate immunogen, another view speculated that the use of non-fibrillogenic, non-toxic A β homologous peptides together with adjuvants that activate primarily the humoral, Th-2

response rather than the Th-1 cell mediated response primarily might be more effective and reduce potential toxicity (Lemere et al., 2001; Sigurdsson et al., 2001b; Sigurdsson et al., 2002; Wisniewski and Goni, 2014). The design for the A β homologous peptide immunogens, albeit, with a few appropriate amino acid substitutions, is based on the fact that the major B cell epitopes which are within the first 15 amino acids of A β form the principal B cell epitopes, while the mid and carboxyl terminus are the chief site for the T cell epitopes (Asuni et al., 2006; Sigurdsson et al., 2001b; Sigurdsson et al., 2004).

The striking results from these preclinical studies served as the launching pad for Elan/Wyeth's group to launch a randomized, multiple-dose, dose-escalation, double-blind Phase I clinical trial. This trial, started in April 2000 used the AN1792 vaccine, which was comprised of pre-aggregated A β 1-42 and QS21 as an adjuvant. The vaccine was designed to generate a strong cell mediated immune response. A strong inducer of Th-1 lymphocytes, QS21 produces an effect similar to that obtained in mice with the use of Freund's adjuvant (which is not approved for use in humans) (Wisniewski and Frangione, 2005). The pilot study performed in the UK involved 80 patients with mild to moderate AD (Bayer et al., 2005). The primary aim here was to determine the efficacy and safety of full length A β 1-42 peptide with QS21. Multiple doses were tested and it was demonstrated that 53% of patients could mount an anti-A β humoral response. In the later segment of the phase I trial, polysorbate 80, which acts as an emulsifier, was added to increase the solubility of A β 1-42. The increased emulsifier concentration caused a greater shift from a Th2 humoral response to a proinflammatory Th1 response (Pride et al., 2008). A follow up phase II trial was conducted in October 2001 that involved 372 patients. 300 out of 372 enrolled patients were part of the arm that received a higher formulation of QS21 (aggregated A β 1-42 with QS21 in the polysorbate 80 formulation (AN1792 to placebo ratio of 4:1). As 6% of immunized patients developed symptoms of aseptic meningoencephalitis (18 out of 298 subjects with no placebo patients developing this complication), the trial was concluded early in January 11, 2002 (Boche and Nicoll, 2008; Wisniewski, 2005; Wisniewski and Frangione, 2005). The spectrum of onset of symptoms, which included confusion, lethargy and headache ranged from 5 to 168 days after the last immunization the patient had received. Neuroimaging revealed white matter lesions with or without evidence of brain edema, termed amyloid-related imaging abnormalities (ARIA). Consistent with data from animal models, post-mortem analyses in a sub-group of trial patients revealed apparent dramatic clearance of plaques in the brain parenchyma, thus validating the efficacy of this approach for precipitated amyloid fibril clearance in humans (Boche and Nicoll, 2008; Bombois et al., 2007; Ferrer et al., 2004; Masliah et al., 2005a; Nicoll et al., 2005; Nicoll et al., 2006). Histopathology revealed that there were broad stretches of cerebral cortex devoid of plaques, interspersed with areas that had residual plaques. These persistent plaques had a "moth-eaten" appearance or seemed to have a "naked" dense core. Additionally, these plaques were seen along with microglia, that were immunoreactive for A β , suggesting that amyloid clearance here was in association with phagocytosis. Other notable features included the presence of unchanged tau reactive NFTs and the persistence of amyloid in cerebral vessels, as well as neuropil threads in apparent regions of plaque clearing, suggesting that this preliminary approach had not targeted vascular amyloid or tau related pathology (Masliah et al., 2005a; Nicoll et al., 2005; Nicoll et al., 2006). In some plaques, a T-cell reaction

surrounding some cerebral vessels was observed, reminiscent of an overstimulated deleterious Th-1 immune response. These features suggested that the immune response seemed to be working as a two-edged sword and raised the concern that the potential beneficial effects of the vaccine were being counter balanced by a deleterious auto-toxic T-cell response (Boche and Nicoll, 2008; Sadowski and Wisniewski, 2007). Further support in favor of a toxic response came from in vitro studies, where peripheral blood mononuclear cells from patients in the trial were analyzed after stimulation with the A β peptide. Quantification of cytokines by enzyme-linked immunosorbent spot assays revealed that cells of responder patients could generate IL-2 and IFN- γ positive responses suggestive of a Class II (CD4+) Th-1 type response (Pride et al., 2008). Also, follow-up results from the Zurich cohort, a subgroup of the Elan/Wyeth trial (Hock et al., 2002; Hock et al., 2003), indicated that immunization as a strategy might be beneficial for some AD patients. In accordance with the data from the Zurich cohort, the results from a multi-center cohort demonstrated that people with high antibody titers or immune responders performed better in outcome measures that scored memory functions relative to low- and non-responders or to the placebo group of patients (Pride et al., 2008). Though the pathology results with dramatic clearance of plaque burden have been striking, the benefits observed clinically in cognitive function have been very minimal (Gilman et al., 2005). No change in function was noted between the antibody responders and the placebo group when assessed via multiple neuropsychological rating scales. One possibility is that the timing of intervention was incorrect. Thus, there were no effects on NFT pathology with subsequent mild benefits in cognitive deficits. Furthermore, reducing the plaque burden may be inconsequential in the presence of existing widespread neuronal death and dysfunction that is related more directly to toxic oligomeric species of both A β and tau. Another explanation is that this approach does not target the complete pathology of AD and other factors driving cognitive dysfunction are not addressed. Thus, the amyloid hypothesis might be an oversimplification of the pathogenesis of sporadic AD.

A number of next generation A β vaccination trials in either Phase I or II (www.clinicaltrials.gov) are currently ongoing. Novartis Pharmaceuticals launched a Phase I trial with an active vaccine called CAD106 (Winblad et al., 2012; Wisniewski, 2012). The vaccine CAD106 is designed to target only a B cell epitope, the small amino-terminal A β fragment (A β 1-6) in this case along with an adjuvant carrier that is derived from multiple copies of the coat protein of bacteriophage Q β . Mild to moderate probable AD subjects with MMSE scores ranging from 16–26 were enrolled in the trial. They were randomized to two cohorts, the first which received 3 injections of either 50 μ g CAD106 (24 test and 7 placebo subjects, cohort 1) or a second cohort which received 150 μ g CAD106 (22 test and 5 placebo subjects, cohort 2). With a treatment time span of 1 year and a 2 year follow up period, the study revealed that 75% and 100% patients developed anti-A β IgM titers in cohorts 1 and 2 respectively, while 67% and 82% developed anti-A β IgG titers respectively in cohorts 1 and 2. Nine patients reported serious adverse reactions, but none were thought to be secondary to the immunogen. In support of this idea, no cases of meningitis, meningoencephalitis or vasogenic edema were identified clinically or by imaging during the initial trial or 2 year follow up period. No significant change in CSF biomarkers was noted in the CAD106 subjects. However, some differences were seen in cohort 2 treated subjects compared to

controls for plasma A β 1-40. A limitation of this trial was that it was not sufficiently powered to demonstrate a significant clinical difference between the treatment and control arms. The results from the Phase II CAD106 trial, which was completed in Feb 2013, are yet to be reported.

Another on-going active Phase II immunization trial being conducted by Janssen and Pfizer is ACC-001, that uses the same A β (1-6) fragment coupled to a carrier protein, and the surface-active saponin adjuvant QS-21 (Ryan and Grundman, 2009). Further, Affiris AG together with GlaxoSmithKline (GSK) has utilized AFFITOME(R) technology to generate synthetic antigenic peptides called mimotopes to target the unmodified A β N-terminus in their AD02 trials (Schneeberger et al., 2009). Affiris AG has also started another Phase I trial with the same technology to target a pyroglutamic-3 modified A β N-terminus, a post-translational modified version of A β . This post-translational modification of A β that renders it more prone to aggregation is believed to happen after its deposition in plaques or vascular amyloid (Frost et al., 2013; Saïdo et al., 1995). Interestingly, pyroglutamic-3 modified A β , which is normally present in plaques and vascular amyloid deposits but is not detectable in the CSF or plasma, is only found in these biological fluids during therapeutic interventions where deposited A β has been mobilized (DeMattos et al., 2012). AC Immune has initiated Phase I/IIa trials with their product, ACI-24, which works by generating a humoral immune response to A β in a primarily β -sheet conformation. The design is based on previous work by this group in an AD Tg model, where a tetra-palmitoylated amyloid 1–15 peptide that exists chiefly in a β -sheet conformation was used as an immunogen (Hickman et al., 2011; Muhs et al., 2007). The preliminary results from these on-going active immunization trials have yet to be reported. These second generation active immunization strategies were designed to more specifically target pathological conformers of A β that hopefully decreased chances of auto-immune toxicity. However, as the immunogens are still derived from the A β sequence, some element of cross reactivity to normal A β peptides is to be expected, with the plausible risk of inflammatory toxicity. Moreover, none of these approaches directly address tau related pathology.

The Past Passive Immunization Experience for AD

The process of injecting pre-made antibodies to provide host immunity is known as passive immunization. This is in contrast to the process of stimulating the immune system of host by agents like pre-formed antigen, a process that is called as active immunization (Figure 2). One of the easiest ways to provide anti- A β antibodies without increasing chances of uncontrolled Th-1 mediated antibody is passive transfer of exogenous monoclonal anti- A β antibodies. Importantly, studies have shown that AD Tg model mice treated by this method, developed significantly reduced A β level and showed cognitive benefit (Bard et al., 2000; DeMattos et al., 2001). Passive immunization has been associated with problems like: difficulty in selection of appropriate antigen targets, expensive costs, need for repeated injections in chronic diseases, blood –brain barrier penetration, hemorrhagic risk and the triggering of immune response to the antibodies that are injected.

Interestingly, studies have shown a number of possible congruent mechanisms of action, which can benefit AD pathology (Farlow and Brosch, 2013; Lemere, 2013; Moreth et al.,

2013). Anti- A β antibodies, can lead to direct A β disassembly by targeting A β deposits in the brain. Microglial activation, by antibodies in the brain can also target plaques and clear them. In addition, blockage of A β toxicity or sequestration of A β monomers by antibodies prevents their aggregation in the CNS. The “peripheral sink effect” has been proposed as an important mechanism via which anti-A β antibodies can block A β deposition, namely by binding sA β circulating in the blood stream and reducing free sA β levels, leading to sA β being drawn out for the brain. Additional studies are needed in this area for understanding, as to which of these mechanisms is (are) the most important in the transgenic AD mouse model, or in the more limited human trials. At present, several passive immunization trials are under study. Recently reported trials of both Bapineuzumab and Solanezumab are the two most advanced phase III trials in this field, unfortunately both failed to show overall clinical improvement or any clear disease modifying results (Doody et al., 2014; Salloway et al., 2014). Bapineuzumab is a humanized version of the mouse monoclonal antibody 3D6, which has an epitope of residues 1–5 of A β . 3D6 is known to cross the BBB, and in Tg mouse studies, it was shown to bind plaques in the brain and elicits Fc receptor mediated, microglial phagocytosis of A β plaques (Bard et al., 2000; Bard et al., 2003). The Phase II trials of Bapineuzumab (study 201 with 234 patients and study 202 with 28 patients) involved six infusions that were done every 13 weeks at 4 different doses (0.15mg/kg, 0.5mg/kg, 1mg/kg and 2mg/kg) (Farlow and Brosch, 2013; Salloway et al., 2009). Even though these trials have not showed statistically significant results overall, a *post-hoc* analysis restricted to subjects who received all infusions showed significant improvement in the pooled treated group compared to controls on the Disability Assessment of Dementia (DAD) and the Alzheimer’s Disease Assessment Scale- Cognitive subscale (ADAS-Cog) (Salloway et al., 2009). In addition, among non-apoE4 carrier patients significant (but small) benefits was documented on the ADAS-cog, NTB, MMSE and CDR scales. The Bapineuzumab treated subjects showed a reduction in cortical fibrillar amyloid deposits compared to both baseline measures and controls over the 78 weeks of the trial as evaluated by 11C-PiB PET imaging in a subset of participants (Rinne et al., 2010). Some of the noteworthy complications in these trials were related to amyloid related imaging abnormalities (ARIA) (Sperling et al., 2012). The abnormalities include the FLAIR MRI signal abnormalities due to parenchymal vasogenic edema and sulcal effusions (ARIA-E) and MRI abnormalities due to microhemorrhages and hemosiderosis as seen on the T2* weighted gradient echo (ARIA-H). Though 36 patients (17% of total patients) developed ARIA-E during treatment, it was symptomatic only 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 (Sperling et al., 2012). An association of these side effects was seen with both the increased dose and presence of apoE4 allele. Out of 8 symptomatic patients 7 were apoE4 carriers and 6 were treated with the two highest doses of Bapineuzumab. A possible mechanism for these adverse events is increase in BBB permeability and microhemorrhages, due to removal of cerebral vessel A β (Farlow and Brosch, 2013; Sperling et al., 2012). A higher CAA burden has previously been characterized in apoE4 carriers (Potter and Wisniewski, 2012). On the basis of these Phase II results, the Phase III trials were created, with the aim of giving a lower dose (0.5mg/kg limit) to apoE4 carriers and to restrict the maximum dose in apoE4 non-carriers to 1.0

mg/kg. A total of 1121 patients were involved in this phase III trial, and infusions were given every 13 weeks for total of 6 infusions in one and a half years. No clinical improvement was noted in either the apoE4 carrier or non-carrier groups. In 15% of apoE4 group ARIA occurred, while in apoE4 non-carrier groups, occurrences were 9% and 4%, of the 1.0 mg/kg and 0.5 mg/kg categories, respectively. In light of these observations, the clinical development of Bapineuzumab has been halted. However, AAB-003 a humanized version of Bapineuzumab (3D6), which has mutations in the Fc domain to reduce effector function and reduce ARIA is in two Phase I clinical trials (www.clinicaltrials.gov).

A humanized version of mAb 266, known as Solanezumab, which has an epitope at residues 16–24 of A β is also under study. In Tg mouse models, 266 was shown to bind specifically with monomeric soluble A β , thereby lowering amyloid pathology, while increasing total sA β levels in the plasma (Bard et al., 2000; Bard et al., 2003). A reduction of free circulating sA β and A β sequestration in the CNS are proposed as the major mechanisms of action. A total of roughly 800 AD patients with mild to moderate disease, in both control and treated groups have been followed in two Phase III trials. The treatment group was given 400mg of Solanezumab (~5.7 mg/kg) every 4 weeks. Cognition was studied at 80 weeks and no differences were noted compared to controls. Of note when patients with mild AD were studied separately, a small but statistically significant benefit was noted in the cognitive scores (Doody et al., 2014; Farlow and Brosch, 2013). Importantly, even though a high dose of Solanezumab was used (compared to Bapineuzumab), ARIAs were not found as a complication and an increase in plasma A β was found (Farlow et al., 2012). Inspired by these results, Solanezumab will be used in two preventive or very early treatment trials. The Dominantly Inherited Alzheimer Network (DIAN) trial will target adult children in families with known mutations and a diagnosis of familial AD; as well as utilizing Gantenerumab, a mAb that selectively binds fibrillar A β and is in an on-going Phase III prevention trial, involving ~770 patients that lack clinical AD symptoms but on PET scan have appreciable amyloid disease (Bohrmann et al., 2012). Another significant prevention trial, aiming to test Solanezumab is the A4 or the Anti- Amyloid Treatment for asymptomatic Alzheimer's disease, which includes ~ 1000 patients that lack symptoms of AD but are positive for amyloid on PET scan.

The Alzheimer's Prevention Initiative (API) is a further prevention trial, to be performed in ~ 300 people of a Colombian kindred with PS1 mutation (E280A). A very severe AD phenotype is seen in this mutation, characterized by A β deposition from ~ 25 years. The study aims to test patients 30 years and older, using Genetech's Crenezumab mAb. This antibody interacts with multiple species of A β (Adolfsson et al., 2012). The effector function of Crenezumab is reduced by using a IgG4 backbone.

Another avenue of active research in passive immunization trials is the role of intravenous immunoglobulin (IVIG) in AD. The basis for its use is that IVIG obtained from a large cohort of donors, contains a small, but significant amount of naturally acting anti-A β antibodies. In a number of autoimmune neurological disorders, IVIG is used as an immunosuppressant and even with multiple successive doses, has no major side effects. Remarkably, a decreased risk of developing dementia is seen in patients who receive regular IVIG infusions (Fillit et al., 2009). In a phase I, open label study in 8 mild AD patients IVIG

was infused over 6 months, followed by an interruption and then resumed for another 9 months (Relkin et al., 2009). Following each infusion the plasma A β levels increased transiently, with CSF A β being decreased after 6 months. Moreover the MMSE increased after 6 months by an average of 2.5, returned to baseline level after washout. A total of 23 AD biomarkers were studied in these patients by collecting CSF from spinal taps before initiation of therapy, 6 months afterward and after 3 month wash-out. Out of eight study subjects, significant improvement in biomarkers was seen in six subjects after six month therapy, which gradually returned to baseline levels after IVIg washout (Shayan et al., 2012). Nonetheless, in two recent trials, no significant slowing of AD progression could be documented (Dodel et al., 2013; Lemere, 2013). In the Octapharma, IVIG trial with ~60 mild to moderate AD patients, infusions were done over six months at three different doses. Study methodologies such as MRI volume measurement, FDG-PET or cognitive measures did not show any significant improvement. Of the 43 IVIG treated patients, six patients had new, asymptomatic, microhemorrhages. Studies by Baxter Healthcare Corporation included an eighteen month phase III trial of Gammagrad 10% IVIG in ~ 400 AD patients with mild to moderate disease. The results from this trial have not been fully released but so far no significant improvement in cognitive measures has been detected (Lemere, 2013).

The passive immunization approaches which have been described above might lack an essential element that is the ability to specifically target A β oligomers, which are the most deleterious components of A β . The prior approaches target either both the normal and pathological conformers of A β or only the soluble A β (i.e. Solanezumab) (Panza et al., 2012). The lack of specificity to toxic forms is a major setback in these therapeutic approaches as targeting normal sA β , can interfere with its crucial physiological functions like neuroprotection, modulation of long term potentiation and innate immunity. This may also increase the risk of autoimmune complications (Giuffrida et al., 2009; Puzzo et al., 2008; Soscia et al., 2010; Wisniewski and Goni, 2014). The apparent transient effects of these therapies also implies the need for a large number of administrations with a tremendous burden on health care systems and the increased risk of developing an immune response to the infused immunoglobulins. Another crucial aspect is that these therapies may have to be started very early in AD pathology build up, for them to be therapeutically beneficial. Prior studies have demonstrated that the appearance of the earliest clinical signs of AD corresponds to peak A β deposition, along with substantial NFT formation and neuronal loss, which have still not yet reached peak levels (Nelson et al., 2012; Yoshiyama et al., 2013). It is postulated that in order to have a significant effect, amyloid directed therapy targeting soluble A β alone, or both the soluble A β and deposited A β , should be started early, preferably even before cognitive impairment starts. It can be safely said that as of now, these therapeutic approaches have limited utility in symptomatic AD.

Tau Related Pathology As An Immune Target

Neurofibrillary tangles (NFTs), a pathognomonic feature of AD, are intracellular inclusion bodies that consist of deposits of paired helical filaments (PHFs), which are primarily composed of hyperphosphorylated tau. Recently, there is considerable interest on targeting phosphorylated tau for immunomodulation in AD (Boutajangout and Wisniewski, 2014; Kaye and Jackson, 2009; Noble et al., 2009; Sigurdsson, 2008; Yoshiyama et al., 2013).

Some recent work has shown that tau pathology precedes formation of amyloid plaques, appearing first in the locus coeruleus and then spreading to other brainstem nuclei and the entorhinal cortex (Braak and Del, 2011; Elobeid et al., 2012; Jack, Jr. et al., 2013). In addition, work by multiple groups has shown that the degree of tau related pathology is better correlated with the degree of dementia when compared to the amyloid plaque burden; hence, making tau as a desirable target in symptomatic AD patients (Arriagada et al., 1992; Bancher et al., 1993; Terry, 1996). Further support for this idea is provided by the results from the human immunization trials (as reviewed above), where the reduction in amyloid plaque load, did not produce cognitive benefits in symptomatic AD subjects.

In animal models, treatment with a phospho-tau peptide (containing the phosphorylated PHF-1 epitopes Ser 396, Ser 404) given prior to the onset of pathology was able to prevent development of tau aggregates in the Tg P301L mouse tau model (Asuni et al., 2007). Phosphorylation at these specific epitopes has been shown to increase the fibrillogenic character of tau and enhances paired helical filaments formation (Eidenmuller et al., 2001; Fath et al., 2002). This model develops neurofibrillary tangles in several brain regions and the spinal cord. Two groups were immunized from 2 to 5 months and from 2 to 8 months. Immunohistochemical analysis using PHF-1 and MC1 antibodies showed a significant reduction in tau related pathology, compared to controls. In addition, an amelioration in the vaccinated groups was seen on a number of sensorimotor tasks. Antibodies generated by this vaccination were found to cross the BBB, bind to phosphorylated tau and reduce pathology without significant adverse effect; thus, providing strong support in favor of the idea that it is possible to also reduce tau related pathology with active immunization (Asuni et al., 2007). These results were also confirmed in a similar study done in an htau/PS1 tau pathology model (Boutajangout et al., 2010). As the transgenic mice used in these studies had severe locomotor deficits, a major limitation of this work was that cognition could not be assessed as a therapeutic endpoint.

How an antibody response to a protein that has intracellular inclusions could have beneficial effects can initially be difficult to understand. However, support for this idea is lent from immunization studies done in a transgenic mouse model of Parkinson's disease where a reduction in intracellular α -synuclein aggregates was demonstrated (Masliah et al., 2005b). Studies done recently by multiple groups have suggested that anti-tau antibodies can cross the BBB, are translocated inside neurons via low-affinity Fc receptors where they can bind to pathological tau within the endosomal/lysosomal system (Congdon et al., 2013). Additionally, injection of fibrillar tau brain extract into the brains of transgenic wild-type tau expressing mice can push the induction of tau into filaments, along with the spread of pathology from the injection site into adjacent brain regions (Clavaguera et al., 2009). Such an "infectivity" of abnormal protein conformation from outside the cell has also been established for polyglutamine aggregates (Ren et al., 2009) and is well described in prion disease (Colby and Prusiner, 2011). Hence, one can reason that if certain pathological forms of tau can spread and lead to PHF pathology in AD via a "prion like" replicative mechanism, anti-phosphorylated tau antibodies might not necessarily need to enter cells in order to be effective.

With active immunization using tau epitopes, there exists a risk of inducing encephalitis or neuronal apoptosis. This line of thought is backed by an early study, where immunization of female C57BL/6 mice with full length recombinant tau produced neurological deficits, NFT-like changes, gliosis and an inflammatory infiltrate (Rosenmann et al., 2006). Even with phosphorylated tau as an epitope, the possibility of deleterious effects still persists. This risk is evident in a study where E257T/P301S-tau Tg mice and wild type mice were repetitively immunized with a mixture of three phospho-tau peptides producing neuroinflammation, in conjunction with significant neurological disability in the tau Tg mice (Rozenstein-Tsalkovich et al., 2013). Hence, one might speculate that a passive immunization approach with anti-phospho-tau directed mAbs might be safer. Two trials have been conducted where passive immunization was chosen as the targeting strategy, and revealed that tau related pathology and motor deficits were reduced if the timing of the antibody administration was prior to the onset of tau pathology (Boutajangout et al., 2011; Chai et al., 2011). Another study, with serial intracerebroventricular administration of anti-tau antibodies (starting at 6 months of age over a 3 month period), demonstrated a decrease in pathology and contextual fear conditioning deficits in P301S tau Tg mice (Yanamandra et al., 2013). Even though this study demonstrated that administration of anti-tau antibodies at a point when pathology is already present could improve behavior, the intraventricular route used in this case is a major disadvantage. Further, the only study to date to show improvement in pathology after its onset has been unable to show any benefits on animal survival as compared to controls (d'Abramo et al., 2013). In this report, the authors compared DA31 (a pan-tau antibody), PHF1 (detects pSer396/404) and MCI (detects a pathological tau conformation) in P301L Tg tau mice, (which have an onset of pathology at about 3 months of age). Mice injected with MCI revealed a reduction of tau related pathology immunohistochemically and biochemically from 7 to 10 months. However, there was no change in survival between mice injected with either PHF1 or MCI from 6 to 14 months of age versus control Tg mice (d'Abramo et al., 2013). Previously, it has been shown that PHF1 is able to decrease in tau related pathology when treatment is started prior to the onset of disease (Boutajangout et al., 2011). Together, these results suggest that though immunotherapy directed towards tau holds promise, there is some risk of toxicity. For best results, more work needs to be done to clearly define the tau form to be used and the optimal timing of the directed immunotherapy.

Innate Immunity Stimulation as a Means to Ameliorate AD Pathology

Studies conducted over 20 years ago had suggested the potential critical role of microglia for both the formation and clearance of amyloid lesions in AD (Frackowiak et al., 1992; Wisniewski et al., 1992; Wisniewski and Wegiel, 1994). The importance of inflammatory pathways affecting the function of microglia for the pathogenesis of AD is highlighted by the results of GWAS, where many of the implicated genes have a major role in immunological processes, as well as the recent linkage to AD of a rare variant of TREM2, a gene that regulates phagocytosis and the activation state of microglia/macrophages (Boutajangout and Wisniewski, 2013; Karch et al., 2014). Microglia play a critical role in the innate immune system of the CNS and one of the most potent ways to stimulate this system is via the Toll-like receptors (TLRs). Neuroinflammation can contribute to cognitive impairment and play a significant role in AD progression (Lampron et al., 2013; Lee et al.,

2013); however, it is increasingly recognized that tightly regulated stimulation of innate immunity processes and specific microglia activation can be neuroprotective depending on the stimulus and the environment (Schwartz et al., 2013). Microglia lose their A β clearing capabilities as AD progresses (Fiala et al., 2005; Lai and McLaurin, 2012; Majumdar et al., 2007). Senescence of microglia function has been suggested to play a fundamental role in both AD and other neurodegenerative diseases (Streit and Xue, 2014). Modulation of innate immunity via TLR2, 4 and 9 signaling pathways has previously been shown to be critical in modulating A β deposition. TLR4 deficient mice displayed increases of diffuse A β and fibrillar A β deposits compared with control mice (Tahara et al., 2006), suggesting that TLR4 signaling is involved in A β clearance (Jin et al., 2008). Microglia deficient in TLR2, TLR4, or the co-receptor CD14 are not activated by A β and do not show a phagocytic response (Reed-Geaghan et al., 2009). Furthermore, stimulation of microglial cells with TLR2-, TLR4-, or TLR9- specific agonists accelerates A β clearance both in vitro and in vivo (Michaud et al., 2013). It has been shown that the administration of the TLR9 agonist CpG oligonucleotides (ODN) containing unmethylated CpG sequences to AD model Tg2576 mice induced a reduction of cortical and vascular A β levels without apparent toxicity and improve cognitive function, with a recent study in 3xTg mice showing the same approach can also reduce tau related pathology in associate with cognitive benefits (Scholtzova et al., 2009; Scholtzova et al., 2014). Various CpG DNA drugs that are TLR9 agonists have been shown to be safe for both humans and rodents (Vollmer and Krieg, 2009). Hence, these preclinical studies indicate that modification of microglial function in neurodegeneration is a viable therapeutic target to ameliorate both A β and tau pathologies.

Targeting Abnormal Protein Conformation Rather Than A β or Tau Related Pathologies Individually

The most pathological conformers of A β and aggregated tau have been proposed to be oligomeric. Both of these entities have been demonstrated to be soluble, very mobile forms that spread extracellularly using prion like replicative mechanisms. Notably, recent studies have shown that in the presence of A β amyloid pathology, therapeutic interventions that impede A β oligomer toxicity can reverse cognitive deficits within a remarkably short treatment duration (Barry et al., 2011; Chung et al., 2010). It can be safely concluded that these molecular targets have great potential even when significant pathology is present. A number of structural and biophysical properties are shared between A β and tau oligomers, like a high β -sheet content, neuronal toxicity and imperviousness to proteolytic degradation. A limited number of studies using antibodies that specifically target A β oligomers reflect the potentially powerful role of this approach and warrant further attention (Lambert et al., 2007; Lambert et al., 2009; Lee et al., 2006; Mamikonyan et al., 2007; Moretto et al., 2007; Rasool et al., 2013). Another benefit of targeting only the oligomeric form of A β or tau is that the normal physiological function of these proteins remains intact. A more recent proposed approach uses conformationally specific antibodies or active immunization which aims to target the shared abnormal β -sheet confirmation of amyloid proteins (Lee et al., 2006; Moretto et al., 2007; Wisniewski et al., 2009). This approach has the benefit of simultaneously targeting both the A β and tau related pathologies. Our group is actively engaged in this approach for the last several years (Wisniewski and Goni, 2014). In order to

accomplish this goal, we developed a therapeutic immunomodulation, specific for pathological β sheet conformation, shared by A β and tau disease associated species. In our studies, we employed a polymerized peptide derived from the carboxyl terminus of the British amyloidosis (ABri) peptide prepared by the use of glutaraldehyde as a cross linker, that results in a stabilized, predominately β sheet oligomeric form that does not form fibrils, which we term pBri (Goni et al., 2010; Goni et al., 2013). One of the rare forms of familial human amyloidosis is ABri, which is associated with a missense mutation in a stop codon that leads to transcription of an intronic sequence, thereby causing production of a highly amyloidogenic protein, with a carboxy terminus that lacks sequence homology to A β , tau or any other native human proteins (Rostagno et al., 2005; Vidal et al., 1999). We proposed that via conformational mimicry, the pBri peptide, in its stabilized oligomeric form, can initiate a conformation selective immune response, which is specific to pathological aggregated/oligomeric conformers of phosphorylated tau and A β . An immunomodulatory approach of such design will have a decreased risk of causing auto-immune complications, as it is specific to pathological conformers and the immunogen does not have sequence homology to any mammalian peptide. Our past studies have shown that this immunomodulation targeting, of pathological conformation of A β is highly effective in reducing amyloid plaques and produces cognitive rescue (Goni et al., 2010). Our recent studies have demonstrated that our approach of targeting abnormal protein confirmation is effective in both the TgSwDI mice, which have a high burden of vascular pathology, and in 3xTg mice which have both A β and tau related pathology, decreasing the disease pathology of both deposited conformers, but most importantly the soluble oligomeric levels of A β and tau, leading to improvement in cognitive deficits (Goni et al., 2013). With this approach the reductions of deposited A β and tau are most likely related to interfering with the intermediate oligomeric forms of A β and tau before they fibrillize, rather than directly acting on the plaques and NFTs.

Summary

It is an exciting time, with many different active and passive immunization therapeutic approaches currently either under development or in trials. In addition pre-clinical studies are exploring innate immunity stimulation. Strategies that target A β peptides could be effective if used very early in disease onset before the development of any clinical dysfunction and are currently in ongoing prevention trials. Though immunotherapy targeted towards tau pathology has shown some promise, it bears the risk of toxicity. With the current knowledge, it remains undefined if it can be used effectively in symptomatic AD where there is preexisting pathology. Many studies have shown that even at the mild cognitive impairment stage of AD, extensive amyloid and tau pathology is already present (Nelson et al., 2012). In addition, it has been proposed that in sporadic late-onset AD, tau pathology is not simply downstream of A β related pathology but that these pathologies could be generated by dual pathways that can interact synergistically (Small and Duff, 2008; Yoshiyama et al., 2013). If this holds true, it would be essential to devise an approach that could simultaneously and effectively target both pathologies.

We postulate that such a strategy that can harness the immune system to clear both A β and tau toxic oligomers concurrently might be most efficacious in symptomatic AD. This might

be possible by seeking similarities in the tau and A β toxic conformers to actively induce a humoral immune response via conformational mimicry. An active immunization approach of the same type can also be used for the development of monoclonal antibodies, to be used alone or as a panel, possibly with other agents in different stages of AD. This “ β -sheet buster” approach represents a unifying therapeutic methodology, where pathological protein conformation is being targeted. This offers potential promise for multiple conformational neurodegenerative diseases.

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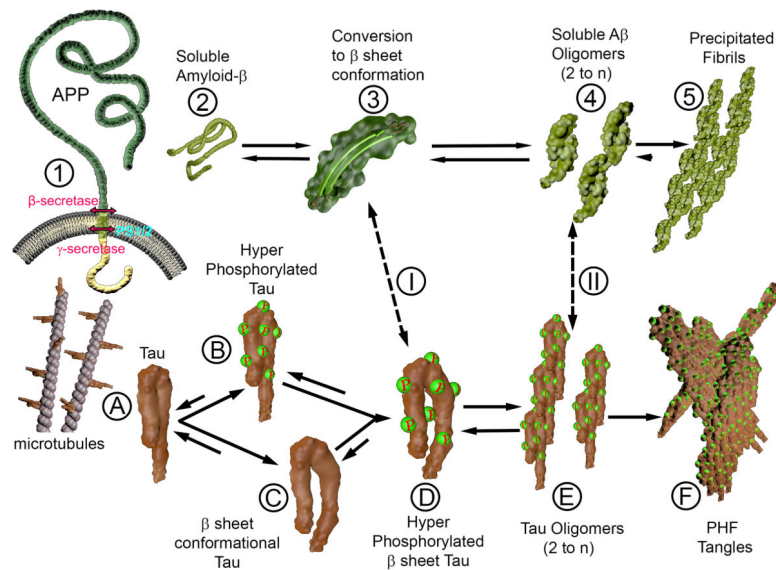


Figure 1. Aβ and tau Conformational Changes in Alzheimer's disease

1) Amyloid precursor protein (APP) undergoes normal cleavage by β and γ-secretase (presenilin [PS] is part of the γ-secretase complex) to produce the normal soluble Aβ (2). Soluble Aβ can undergo a conformational change to a β-sheet rich conformer (3) that further aggregates to form soluble, toxic Aβ oligomers. These may also precipitate to form relatively inert fibrils in amyloid plaques and congophilic amyloid angiopathy.

A) Tau is a microtubule binding protein. Tau can undergo hyperphosphorylation (B) or a conformational change to a β-sheet conformer. These species can both further change to hyperphosphorylated tau in a β-sheet rich form (D) that is predisposed to further aggregation into toxic, tau oligomers. These can precipitate to form paired helical filaments (PHF) in the form (F) of neurofibrillary tangles.

The Aβ β-sheet conformers and Aβ oligomers may cross seed, under some circumstances, with intermediate tau species in a β-sheet conformation and with tau oligomers (I and II), to synergistically exacerbate AD pathology.

The most effective immunotherapeutic approaches for AD will need to be able to concurrently reduce levels of the toxic Aβ and tau oligomeric species.

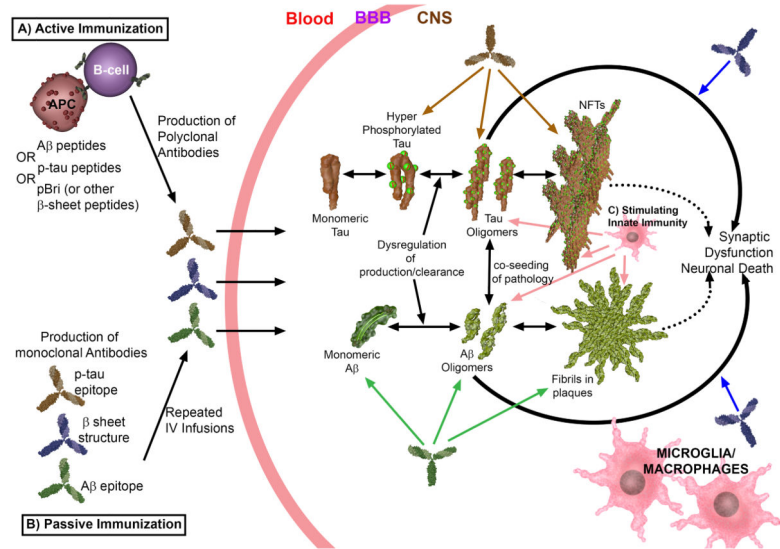


Figure 2. Different Immunotherapeutic Approaches to Ameliorate AD Pathology

A) Active immunization can be performed using A β peptides, phosphorylated tau (ptau) peptides or preparations such as polymerized Bri peptide (pBri) as an immunogen. These immunogens are presented to B-cells by antigen presenting cells (APC). Use of A β peptides or ptau peptides will give rise to the production by B-cells of antibodies to A β or ptau epitopes, respectively. Use of pBri (or equivalent preparations of an immunogen that is a non-self peptide, in a stabilized, oligomeric β -sheet conformation) will lead to the production of antibodies that recognize both A β and tau pathological conformers (but not normal monomeric sA β or tau proteins).

B) Passive immunization can be performed by the production of monoclonal antibodies (mAb) that bind to A β , ptau or β -sheet pathological conformations. These antibodies need to be infused systemically in concentrations sufficient for adequate BBB penetration (typically only ~0.1% of a systemically injected mAb will cross the BBB).

Once antibodies cross the BBB (using either active or passive immunization) they will act to enhance the clearance and degradation of their targets. Additional or alternative mechanisms may include disaggregation or neutralization of their target (i.e. blocking of toxicity). Abs to A β will recognize normal sA β , oligomeric A β and/or deposited fibrillar A β (with varying preference depending on the type(s) of Abs to A β). Similarly, Abs to ptau will recognize monomeric ptau species, oligomeric tau and/or neurofibrillary tangles, with varying preference depending on the specific anti-ptau Ab(s). Abs to β -sheet will simultaneously act to ameliorate both A β and tau pathology by specifically binding pathological conformers; without binding to normal sA β or tau.

C) Stimulation of innate immunity can also be used to ameliorate AD pathology by enhancing microglia/macrophage function via Toll-like receptors (TLRs) or related pathways. Microglia/macrophages are stimulated similarly by the immune complexes produced using active or passive immunization approaches.

Table 1

Active and passive trials for immunotherapeutic approaches to treat AD (www.clinicaltrials.gov)

ACTIVE			
Pharmaceutical Company	Trial	Stage	Status
			Aβ Target
ELAN	AN1792	Phase II	(2000–2002) halted, no improvement, encephalitis 6%
Novartis	CAD106	Phase I Phase II	A β titers, no change biomarkers Not reported
Janssen/Pfizer	ACC-001	Phase II	Not reported, finishing
Affinis AG/GSK	AFFITOME AD02	Phase II	Not reported
Affinis AG	AFFITOME	Phase I	Ongoing
AC Immune	ACI-24	Phase I/IIa	Not reported
			Tau Target
Axon	AAAD vac1	Phase I	Ongoing continuation, tolerable, safe, antibody titers
			Synthetic mutant tau peptide coupled to KLH-ALUM
PASSIVE			
Monoclonal antibody/target			
Janssen/Pfizer	Bapineuzumab AAB-001	Phase II Phase III	No clinical improvement, ARIA, trend to efficacy No improvement, halted
Eli Lilly	Solaneuzumab	Phase III(2)	No improvement overall, secondary analysis slight improvement in early AD; ongoing extension in early AD
Janssen/Pfizer	Bapineuzumab AAB-003	Phase I	Ongoing
Hoffman-La Roche	Gantenerumab DIAN	Phase III	Ongoing, future: in autosomal dominant AD
Eli Lilly	Solaneuzumab A4	Phase III	Starting: asymptomatic AD with positive PET
AC Immune/Genentech	Crenezumab ABBY BLAZE API	Phase II/III Phase II Phase II Phase III	Failed to meet clinical co-primary endpoints No difference in biomarkers/FDG-PET Ongoing in EOAD families with PSI mut. E280A
Octapharma	IVIG Octapharma	Phase II	Safe, no improvement
Baxter	IVIG Gammagrad	Phase III	No improvement, data not released
Baxter	IVIG newGAM	Phase II	Ongoing
			Three different doses of naturally occurring anti-A β
			Naturally occurring anti-A β
			Naturally occurring anti-A β