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Review

Immunotherapeutic Approaches for Alzheimer's Disease

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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 (Aβ) and tau proteins. Oligomeric forms of Aβ 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 Aβ. However, there is very limited evidence in human studies of the 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 need to be balanced against risks of stimulating excessive autoimmune toxic inflammation. For greater efficacy the next generation of vaccines needs to focus more on concurrently targeting all the intermediate toxic conformers of oligomeric Aβ and tau species.

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. 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 (Aβ) plaques and tau protein aggregates in the form of intracellular neurofibrillary tangles (NFTs) (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 Aβ in plaques correlates poorly with the clinical status of patients (Nelson et al., 2012; Terry, 1996).

Soluble oligomeric forms of Aβ 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 Aβ 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 Aβ 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 also has revealed that Aβ- and tau-related pathology can, in certain scenarios, "seed" or transmit each other (Ashe and Aguzzi, 2013; Jucker and Walker, 2011). 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 Goñi, 2012; Wisniewski and Goñi, 2014). With a central role for Aβ in AD pathogenesis under the "amyloid cascade" model, several strategies directed toward the eradication of Aβ 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 Goñi, 2014). Though Aβ-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 arisen 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 autoimmune 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 Aβ- and tau-related pathology simultaneously? This article reviews current preclinical and clinical data for Aβ 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

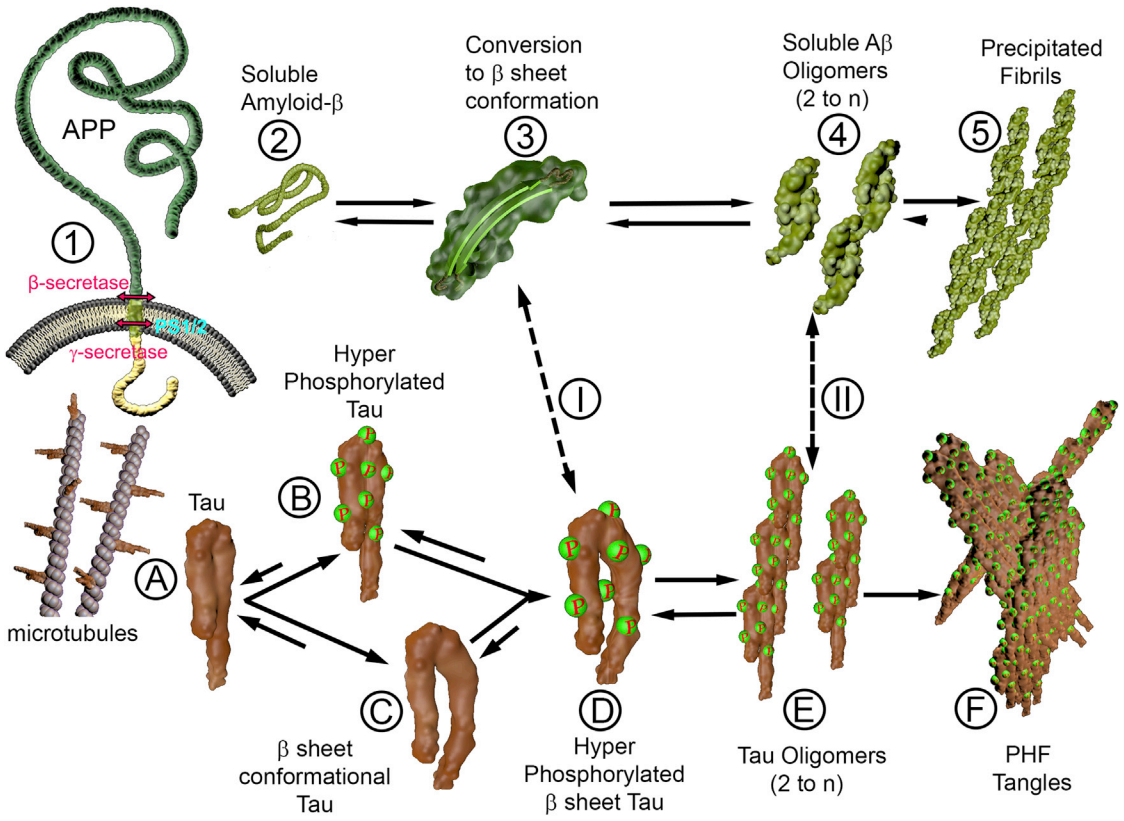

Figure 1. Aβ and Tau Conformational Changes in AD

(1–5) (1) APP undergoes normal cleavage by β and γ-secretase (PS is part of the γ-secretase complex) to produce the (2) normal sAβ. sAβ can undergo a conformational change to (3) a β sheet-rich conformer that further aggregates to form (4) soluble, toxic Aβ oligomers. These also may precipitate to form (5) relatively inert fibrils in amyloid plaques and congophilic amyloid angiopathy.

(A–F) (A) Tau is a microtubule-binding protein. Tau can undergo (B) hyperphosphorylation or (C) a conformational change to a β sheet conformer. These species can both further change to (D) hyperphosphorylated tau in a β sheet-rich form that is predisposed to further aggregation into (E) toxic, tau oligomers. These can precipitate to form (F) PHFs in the form of NFTs.

(I and II) 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, 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.

is defined by the deposition of extracellular Aβ as senile neuritic plaques and congophilic angiopathy (CAA), as well as intracellular hyperphosphorylated fibrillar tau accumulation in the form of 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 <65 years) and the much more common sporadic late-onset (LOAD) form (onset at >65 years). EOAD is related to mutations in presenilin 1 (PS1), presenilin 2 (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 suggest 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 Aβ (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 Khoury, 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 Aβ aggregation, especially in its toxic oligomeric form, is the principal insult that produces neuronal toxicity and triggers downstream signaling events that in turn lead to the 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 Aβ (sAβ). These include but are not limited to apoE, especially its E4 isoform; ∝1-antichymotrypsin (ACT); and C1q complement factor (Potter and Wisniewski, 2012). Histological and biochemical evidence suggests that "pathological chaperone" proteins co-localize with fibrillar Aβ 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 sAβ and/or chaperone proteins is achieved. A critical concentration of the precursor proteins would favor a conformational change, and drive the formation of toxic Aβ oligomers and subsequent activation of downstream signaling cascades. Mechanisms implicated for achieving this in sporadic AD could be a permutation of impaired clearance of Aβ from the brain as a consequence of aging and/or inflow of serum Aβ into the CNS (Holtzman et al., 2012).

Several studies in familial AD (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 toward overproduction of sAβ or the generation of specific species of sAβ (such as Aβ1–42) that are more prone to aggregation (Hardy, 2006). In addition, a rare APP mutation (first reported in an Icelandic population) that 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 Aβ 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 Aβ and tau are co-expressed reveal that Aβ deposition predates formation of tau aggregates, supporting the concept that NFT formation is downstream from Aβ aggregation (Götz et al., 2001; Oddo et al., 2003; Wisniewski and Sigurdsson, 2010). Lastly, enhancement of Aβ clearance in transgenic mouse models with overexpression 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 also has revealed that the inhibition of Aβ in animal models with overexpression of mutant APP and tau not only prevents the 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 Aβ is well established, definitive evidence regarding Aβ's central function in sporadic LOAD is more limited. A number of autopsy studies have indicated that medial temporal and subcortical tau pathology precedes Aβ pathology in the majority of patients (Jack 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 Aβ in LOAD, the levels of biochemically extracted Aβ peptides from the brains of people with sporadic AD correlate well with cognitive deficits (Näslund et al., 2000). Further, Aβ peptide dimer/oligomer extracts derived from sporadic AD brains have been shown to disrupt synaptic structure, function, and plasticity, which are critical cellular correlates of memory (Shankar et al., 2008). Interestingly, exogenous injections of Aβ 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 Aβ load relative to non-immunized controls. Regardless of these encouraging results, no improvements in long-term survival outcome, time to severe dementia, and cognitive function were seen among the immunized groups. Two recent, large phase III trials of passive immunization for AD also have 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 Goñi, 2014).

One also could 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 Aβ and tau pathways downstream of itself is also possible (Castellani et al., 2008; Small and Duff, 2008). We hypothesize that Aβ 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 suggest that, in many cases, a regionally limited tauopathy precedes Aβ pathology; however, for the tau pathology to evolve to AD, it requires the concomitant presence of Aβ pathology (Braak and Del Tredici, 2011; Crary et al., 2014; Duyckaerts, 2011; Jack et al., 2013). Hence tau and Aβ 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 Aβ- 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 have been used to target both Aβ 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 Aβ in Humans

Preliminary work that suggested a strong role for immunotherapy for AD revealed that anti-Aβ-targeting antibodies were capable of preventing Aβ 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 Aβ first as an active immunogen and then to assess if it could prevent pathology in mouse models of Aβ-related AD pathology. The first in vivo immunization trial, reported in a seminal paper by Schenk et al. (1999) demonstrated that full-length, aggregated Aβ1–42 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 Aβ1–42 or Aβ homologous peptides along with Freund's or alum adjuvants not only prevented Aβ plaque pathology, but also protected against the development of cognitive deficits (Asuni et al., 2006; Janus et al., 2000; Lemere, 2013; Morgan et al., 2000; Sigurdsson et al., 2001, 2004).

Biochemical assays identified the first 15 amino acids of the Aβ peptide as the site of the principal epitope. Further, immunohistochemical assays also revealed that antibodies generated in mice toward Aβ can label amyloid plaques on human AD brain sections, raising the possibility of such immune intervention in humans. Interestingly, peripheral injections of anti-Aβ monoclonal antibodies (mAbs) into the systemic circulation also could reduce Aβ 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 Goñi, 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 Aβ with a strong adjuvant was the appropriate immunogen, another view speculated that the use of non-fibrillogenic, nontoxic Aβ homologous peptides together with adjuvants that activate primarily the humoral, Th-2 response (rather than the Th-1 cell-mediated response) might be more effective and reduce potential toxicity (Lemere et al., 2001; Sigurdsson et al., 2001, 2002; Wisniewski and Goñi, 2014). The design for the Aβ homologous peptide immunogens, albeit with a few appropriate amino acid substitutions, is based on the fact that the major B cell epitopes that are within the first 15 amino acids of Aβ 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., 2001, 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 Aβ1–42 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 Aβ1–42 peptide with QS21. Multiple doses were tested and it was demonstrated that 53% of patients could mount an anti-Aβ 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 Aβ1–42. 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 IIa trial was conducted in October 2001 and involved 372 patients; 300 of 372 enrolled patients were part of the arm that received a higher formulation of QS21 (aggregated Aβ1–42 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 of 298 subjects with no placebo patients developing this complication), the trial was concluded early in January 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 (ARIAs). 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., 2003, 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 Aβ, 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., 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 double-edged sword, and raised the concern that the potential beneficial effects of the vaccine were being counterbalanced 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 Aβ 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, 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 Aβ 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 Aβ vaccination trials in either Phase I or II (http://www.clinicaltrials.gov) are currently ongoing (Table 1). 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 Aβ fragment (Aβ1–6), in this case along with an adjuvant carrier that is derived from multiple copies of the coat protein of bacteriophage Qβ. Mild to moderate probable AD subjects with Mini Mental State Examination (MMSE) scores ranging from 16 to 26 were enrolled in the trial. They were randomized to two cohorts, the first that received three injections of 50 μg CAD106 (24 test and 7 placebo subjects, cohort 1) or a second cohort that received 150 μg 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-Aβ IgM titers in cohorts 1 and 2, respectively, while 67% and 82% developed anti-Aβ 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 cerebrospinal fluid (CSF) biomarkers was noted in the CAD106 subjects. However, some differences were seen in cohort 2 subjects compared to controls for plasma Aβ1–40. 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 February 2013, are yet to be reported.

Another ongoing active Phase II immunization trial, being conducted by Janssen and Pfizer, is ACC-001, which uses the same Aβ(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 Aβ N terminus in their AD02 trials (Schneeberger et al., 2009). Affiris AG also has started another Phase I trial with the same technology to target a pyroglutamic-3-modified Aβ N terminus, a post-translational modified version of Aβ. This post-translational modification of Aβ that renders it more prone to aggregation is believed to happen after its deposition in plaques or vascular amyloid (Frost et al., 2013; Saido et al., 1995). Interestingly, pyroglutamic-3-modified Aβ, 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 Aβ 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 Aβ 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 ongoing active immunization trials have yet to be reported. These second-generation active immunization strategies were designed to more specifically target pathological conformers of Aβ that hopefully decrease chances of autoimmune toxicity. However, as the immunogens are still derived from the Aβ sequence, some element of cross-reactivity to normal Aβ peptides is to be expected, with the plausible risk of inflammatory toxicity. Moreover, none of these approaches directly addresses 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 host immune system with agents like pre-formed antigen, a process that is called as active immunization (Figure 2). One of the easiest ways to provide anti-Aβ antibodies without increasing the chances of uncontrolled Th-1-mediated antibody is passive transfer of exogenous monoclonal anti-Aβ antibodies. Importantly, studies have shown that AD Tg model mice treated by this method developed significantly reduced Aβ 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 (BBB) 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-Aβ antibodies can lead to direct Aβ disassembly by targeting Aβ deposits in the brain. Microglial activation by antibodies in the brain also can target plaques and clear them. In addition, blockage of Aβ toxicity or sequestration of Aβ monomers by antibodies prevents their aggregation in the CNS. The "peripheral sink effect" has been proposed as an

Table 1. Active and Passive Trials for Immunotherapeutical Approaches to Treat AD

Pharmaceutical Company	Trial	Stage	Status	
ACTIVE				
				Aβ Target
ELAN	AN1792	Phase II	(2000–2002) halted, no improvement, encephalitis 6%	Aggregated Aβ1–42, QS21, Polysorbate80
Novartis	CAD106	Phase I	Aβ titers, no change biomarkers	Aβ1–6/Bacteriophage Qβ
		Phase II	Not reported	
Janssen/Pfizer	ACC-001	Phase II	Not reported, finishing	Aβ1–6-QS21
Affinis AG/GSK	AFFITOME AD02	Phase II	Not reported	Mimotope of unmodified Aβ N terminus
Affinis AG	AFFITOME	Phase I	Ongoing	Mimotope of pyroglutamic-3 modified Aβ N terminus
AC Immune	ACI-24	Phase I/IIa	Not reported	β-sheet conformation of Aβ peptide
				Tau Target
Axon	AAD 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	No clinical improvement, ARIA, trend to efficacy	Humanized 3D6, anti-Aβ1–5, six infusions, different dosages
		Phase III	No improvement, halted	
Eli Lilly	Solaneuzumab	Phase III(2)	No improvement overall, secondary analysis slight improvement in early AD; ongoing extension in early AD	Humanized mAb266 Anti-Aβ16–24
Janssen/Pfizer	Bapineuzumab AAB-003	Phase I	Ongoing	Hu IgG4 3D6 to reduce ARIA
Hoffman-La Roche	Gantenerumab DIAN	Phase III	Ongoing, future: in autosomal dominant AD	Antibody to fibrillar form of Aβ. Positions 3–12; 18-27
Eli Lilly	Solaneuzumab A4	Phase III	Starting: asymptomatic AD with positive PET	Humanized mAb266. Anti-Aβ16–24
AC Immune/Genentech	Crenezumab	Phase II/III		Hu IgG4 anti-multiple epitopes Aβ1–40
	ABBY	Phase II	Failed to meet clinical co-primary endpoints	
	BLAZE	Phase II	No difference in biomarkers/ FDG-PET	
	API	Phase III	Ongoing in EOAD families with PS1 mut. E280A	
Octapharma	IVIG Octapharma	Phase II	Safe, no improvement	Three different doses of naturally occurring anti-Aβ
Baxter	IVIG Gammagrad	Phase III	No improvement, data not released	Naturally occurring anti-Aβ
Baxter	IVIG newGAM	Phase II	Ongoing	Naturally occurring anti-Aβ

Source: http://www.clinicaltrials.gov.

important mechanism via which anti-Aβ antibodies can block Aβ deposition, namely by binding sAβ circulating in the blood stream and reducing free sAβ levels, leading to sAβ being drawn out for the brain. Additional studies are needed in this area for understanding 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 mAb 3D6, which has an epitope of

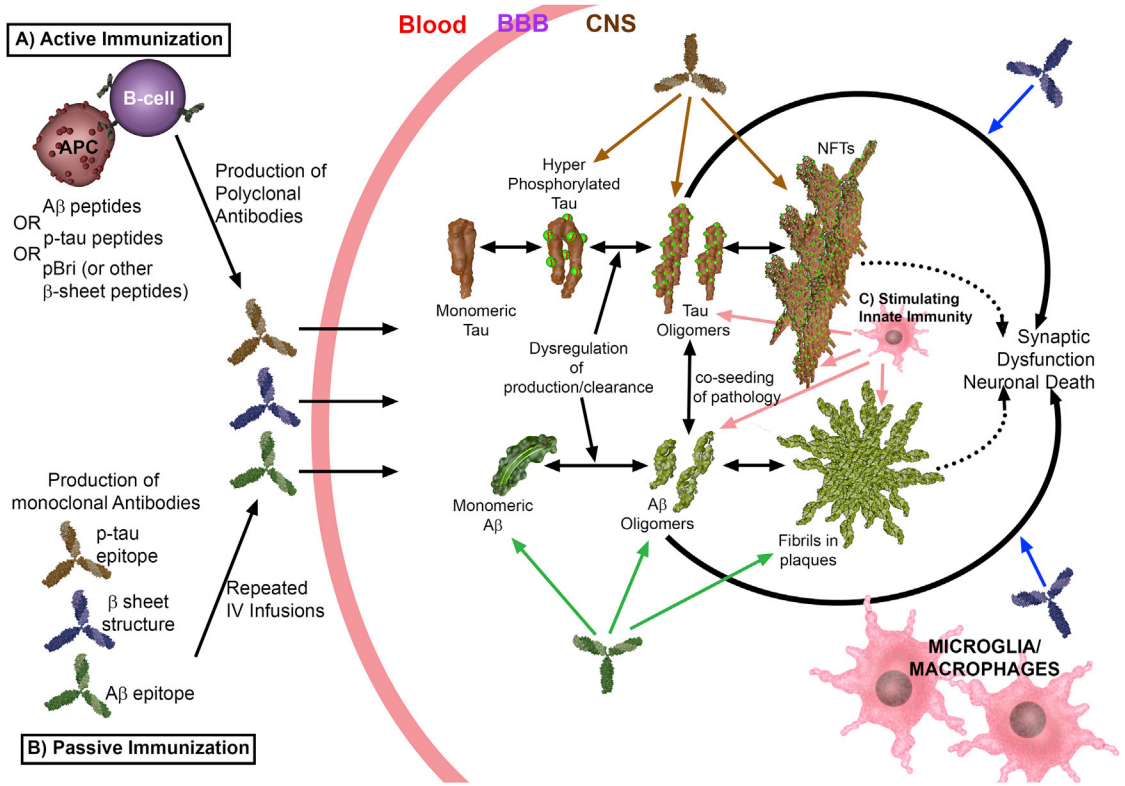

Figure 2. Different Immunotherapeutic Approaches to Ameliorate AD Pathology

(A) Active immunization can be performed using Aβ peptides, phosphorylated tau (ptau) peptides, or preparations such as pBri as an immunogen. These immunogens are presented to B cells by antigen-presenting cells (APC). Use of Aβ peptides or ptau peptides will give rise to the production by B cells of antibodies to Aβ 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 Aβ and tau pathological conformers (but not normal monomeric sAβ or tau proteins).
(B) Passive immunization can be performed by the production of mAbs that bind to Aβ, 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). Antibodies to Aβ will recognize normal sAβ, oligomeric Aβ, and/or deposited fibrillar Aβ (with varying preference depending on the type[s] of antibodies to Aβ). Similarly, antibodies to ptau will recognize monomeric ptau species, oligomeric tau, and/or NFTs, with varying preference depending on the specific anti-ptau antibody(ies). Antibodies to β sheet will simultaneously act to ameliorate both Aβ and tau pathologies by specifically binding pathological conformers, without binding to normal sAβ or tau.
(C) Stimulation of innate immunity also can be used to ameliorate AD pathology by enhancing microglia/macrophage function via TLRs or related pathways. Microglia/macrophages are stimulated similarly by the immune complexes produced using active or passive immunization approaches.

residues 1–5 of Aβ. 3D6 is known to cross the BBB, and, in Tg mouse studies, it was shown to bind plaques in the brain and elicit Fc-receptor-mediated, microglial phagocytosis of Aβ plaques (Bard et al., 2000, 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 four different doses (0.15, 0.5, 1, and 2 mg/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 were documented on the ADAS-cog, Neuropsychological Test Battery (NTB), MMSE, and Clinical Dementia Rating (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 positron emission tomography (PET) imaging in a subset of participants (Rinne et al., 2010).

Some of the noteworthy complications in these trials were related to ARIA (Sperling et al., 2012). The abnormalities included the fluid-attenuated inversion recovery (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 the presence of apoE4 allele. Of eight symptomatic patients, seven were apoE4 carriers and six 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 Aβ (Farlow and Brosch, 2013; Sperling et al., 2012). A higher CAA burden has been characterized previously 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 (0.5 mg/kg limit) to apoE4 carriers and to restrict the maximum dose in apoE4 non-carriers to 1.0 mg/kg. A total of 1,121 patients were involved in this phase III trial, and infusions were given every 13 weeks for a total of six infusions in 1.5 years. No clinical improvement was noted in either the apoE4 carrier or non-carrier groups. In 15% of the 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) that has mutations in the Fc domain to reduce effector function and reduce ARIA, is in two Phase I clinical trials (http://www.clinicaltrials.gov).

A humanized version of mAb 266, known as Solanezumab, which has an epitope at residues 16–24 of Aβ, is also under study. In Tg mouse models, 266 was shown to bind specifically with monomeric sAβ, thereby lowering amyloid pathology while increasing total sAβ levels in the plasma (Bard et al., 2000, 2003). A reduction of free circulating sAβ and Aβ 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 400 mg of Solanezumab (~5.7 mg/kg) every 4 weeks. Cognition was studied at 80 weeks and no differences were noted compared to controls. 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 Aβ 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 FAD, as well as utilize Gantenerumab, a mAb that selectively binds fibrillar Aβ and is in an ongoing 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 (Anti-Amyloid Treatment for Asymptomatic Alzheimer's Disease), which includes ~1,000 patients that lack symptoms of AD but are positive for amyloid on PET scan.

The Alzheimer's Prevention Initiative (API) is a 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 Aβ 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 Aβ (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-Aβ 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 eight 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 Aβ levels increased transiently, with CSF Aβ being decreased after 6 months. Moreover, the MMSE increased after 6 months by an average of 2.5, and returned to baseline level after washout. A total of 23 AD biomarkers were studied in these patients by collecting CSF from spinal taps before the initiation of therapy, 6 months afterward, and after 3-month washout. Of eight study subjects, significant improvement in biomarkers was seen in six subjects after 6 months of 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 6 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 had new asymptomatic microhemorrhages. Studies by Baxter Healthcare Corporation included an 18-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 that have been described above might lack an essential element that is the ability to specifically target Aβ oligomers, which are the most deleterious components of Aβ. The prior approaches target either both the normal and pathological conformers of Aβ or only the sAβ (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 sAβ can interfere with its crucial physiological functions like neuroprotection, modulation of long-term potentiation, and innate immunity. This also may increase the risk of autoimmune complications (Giuffrida et al., 2009; Puzzo et al., 2008; Soscia et al., 2010; Wisniewski and Goñi, 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 buildup for them to be therapeutically beneficial. Prior studies have

demonstrated that the appearance of the earliest clinical signs of AD corresponds to peak Aβ 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 to have a significant effect, amyloid-directed therapy targeting sAβ alone, or both the sAβ and deposited Aβ, 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

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 has been considerable interest in targeting phosphorylated tau for immunomodulation in AD (Boutajangout and Wisniewski, 2014; Kayed and Jackson, 2009; Noble et al., 2009; Sigurdsson, 2008; Yoshiyama et al., 2013). Some work has shown that tau pathology precedes formation of amyloid plaques, appearing first in the locus coeruleus and then spreading to other brain stem nuclei and the entorhinal cortex (Braak and Del Tredici, 2011; Elobeid et al., 2012; Jack 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, making tau 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 and 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 PHF formation (Eidenmüller et al., 2001; Fath et al., 2002). This model develops NFTs 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 reduce tau-related pathology with active immunization (Asuni et al., 2007). These results were 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 be initially 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, and 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 also has 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 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 compared to controls (d'Abramo et al., 2013). In this report, the authors compared DA31 (a pan-tau antibody), PHF1 (detects pSer396/404), and MC1 (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 MC1 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 MC1 from 6 to 14 months of age versus control Tg mice (d'Abramo et al., 2013). Previously, it had been shown that PHF1 is able to decrease tau-related pathology when treatment is started prior to the onset of disease (Boutajangout et al., 2011). Together, these results suggest

that, although immunotherapy directed toward 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 Aβ-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 previously has been shown to be critical in modulating Aβ deposition. TLR4-deficient mice displayed increases of diffuse Aβ and fibrillar Aβ deposits compared with control mice (Tahara et al., 2006), suggesting that TLR4 signaling is involved in Aβ clearance (Jin et al., 2008). Microglia deficient in TLR2, TLR4, or the co-receptor CD14 are not activated by Aβ 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 Aβ 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 Aβ levels, without apparent toxicity, and improved cognitive function, with a recent study in 3xTg mice showing the same approach also can reduce tau-related pathology in association with cognitive benefits (Scholtzova et al., 2009, 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 Aβ and tau pathologies.

Targeting Abnormal Protein Conformation Rather Than Aβ- or Tau-Related Pathology Individually

The most pathological conformers of Aβ 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 Aβ amyloid pathology, therapeutic interventions that impede Aβ 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 Aβ 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 Aβ oligomers reflect the potentially powerful role of this approach and warrant further attention (Lambert et al., 2007, 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 Aβ or tau is that the normal physiological function of these proteins remains intact. A recently proposed approach used conformationally specific antibodies or active immunization that aimed 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 Aβ- and tau-related pathologies.

Our group has been actively engaged in this approach for the last several years (Wisniewski and Goñi, 2014). To accomplish this goal, we developed a therapeutic immunomodulation, specific for pathological β sheet conformation, shared by Aβ 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, which results in a stabilized, predominately β sheet oligomeric form that does not form fibrils and that we term pBri (Goñi et al., 2010, 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 Aβ, 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 Aβ. An immunomodulatory approach of such design will have a decreased risk of causing autoimmune 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 Aβ is highly effective in reducing amyloid plaques and produces cognitive rescue (Goñi 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 Aβ- and tau-related pathologies. It decreases the disease pathology of both deposited conformers, but most importantly the soluble oligomeric levels of Aβ and tau, leading to improvement in cognitive deficits (Goñi et al., 2013).

With this approach, the reductions of deposited Aβ and tau are most likely related to interfering with the intermediate oligomeric forms of Aβ and tau before they fibrillize, rather than directly acting on the plaques and NFTs.

Conclusions

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 Aβ 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 toward 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 pre-existing pathology. Many studies have shown that, even at the mild cognitive impairment stage of AD, extensive amyloid and tau pathologies are already present (Nelson et al., 2012). Also, it has been proposed that, in sporadic LOAD, tau pathology is not simply downstream of Aβ-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 Aβ and tau toxic oligomers concurrently might be most efficacious in symptomatic AD. This might be possible by seeking similarities in the tau and Aβ toxic conformers to actively induce a humoral immune response via conformational mimicry. An active immunization approach of the same type also can be used for the development of mAbs, 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, offering potential promise for multiple conformational neurodegenerative diseases.

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