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VACCINATION AS A THERAPEUTIC APPROACH FOR ALZHEIMER'S DISEASE

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Abstract

Alzheimer's disease (AD) is the most common cause of dementia worldwide. AD is a member of a broad range of neurodegenerative diseases characterized pathologically by the conformational change of a normal protein into a pathological conformer with a high β-sheet content that renders it neurotoxic. In the case of AD the normal soluble amyloid β (sAβ) peptide is converted into oligomeric/fibrillar Aβ. The oligomeric forms of Aβ have been hypothesized to be the most toxic, while fibrillar Aβ becomes deposited as amyloid plaques and congophilic angiopathy, which both serve as neuropathological markers of the disease. In addition the accumulation of abnormally phosphorylated tau as soluble toxic oligomers and as neurofibrillary tangles is a critical part of the pathology. Numerous therapeutic interventions are under investigation to prevent and treat AD. Among the most exciting and advanced of these approaches is vaccination. Immunomodulation is being tried for a range of neurodegenerative disorders with great success being reported in most model animal trials; however, the much more limited human data has shown a more modest clinical success so far, with encephalitis occurring in a minority of patients treated with active immunization. The immunomodulatory approaches for neurodegenerative diseases are targeting a self-protein, albeit in an abnormal conformation; hence, effective enhanced clearance of the disease associated conformer has to be balanced with the potential risk to stimulate excessive toxic inflammation within the CNS. The design of future immunomodulatory approaches that are more focused is dependent on addressing a number of questions such as: When is the best time to start immunization? What are the most appropriate targets for vaccination? Is amyloid central to the pathogenesis of AD or is it critical to target tau related pathology also? In this review we discuss the past experience of vaccination for AD and the development of possible future directions that target both amyloid β and tau related pathologies.

Keywords

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

Alzheimer's disease is the most common cause of dementia worldwide, affecting approximately 37 million people currently. In the USA, AD is the 6th leading cause of

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death, with an estimated 5.3 million Americans having AD. By 2050, according to some estimates, 1:85 persons worldwide will be affected by AD (1). Currently available treatments for AD provide largely symptomatic relief with only minor effects on the course of the disease. There is an urgent need for better therapeutic interventions. Besides immunomodulation, numerous other approaches are being studied, which include anti-Aβ aggregation agents, secretase inhibitors/modulators blocking Aβ production, tau aggregation blockers, agents targeting mitochondria, stem cell therapies and various neuroprotective strategies (2). Perhaps the greatest hope for an intervention that shall significantly impact disease progression in the near future comes from the vaccination approaches (3,4). Certainly in AD Tg mouse models Aβ directed immunization has been spectacularly successful using a wide variety of methods. However significant unanswered questions remain for the current and future human trials as to what is the best design of a vaccine, what is the best target and when should therapy start? A key issue which needs to be addressed is the targeting of both amyloid β (Aβ) and tau related pathology.

PATHOGENESIS OF FAMILIAL AND SPORADIC ALZHEIMER'S DISEASE

The pathological hallmarks of AD are the accumulation of Aβ as neuritic plaques and congophilic angiopathy, as well as accumulation of abnormally phosphorylated tau in the form of neurofibrillary tangles (NFTs). Missense mutations in APP or in the presenilin genes PRES 1 and 2 can cause early onset, familial forms of AD (FAD) affecting <4% of AD patients. The most common form of AD is sporadic and late-onset. The dominant theory for the causation of AD has been the amyloid cascade hypothesis (5,6). This theory currently suggests that accumulation of Aβ peptides particularly in a highly toxic oligomeric form is the primary pathogenic driver, that downstream leads to tau hyperphosphorylation, NFT formation and ultimately to synaptic and neuronal loss. Extensive evidence supports this hypothesis in FAD patients and in models of FAD: 1) Inherited forms of AD linked with mutations in the APP gene or in the PRES1 or 2 genes are associated with changes in APP processing that favor over production of sAβ or production of more aggregation prone forms of sAβ such as Aβ1-42 (7). 2) Down's syndrome, where there is an extra copy of the APP gene due to trisomy 21, is associated with AD related pathology at a very early age (8). 3) In transgenic and other models of co-expressed amyloid β and tau, amyloid β oligomer formation precedes and accentuates tau related pathology, consistent with the hypothesis that NFT formation is downstream from Aβ aggregation (9-11). 4) In transgenic mouse models of mutant APP over-expression (where there is no tau pathology) therapeutic prevention and/or removal of Aβ is associated with cognitive benefits in experimental mice (12-15). Importantly, in transgenic mouse models of both mutant APP and tau over-expression (with both amyloid and tau related pathology) prevention of Aβ pathology leads to both amelioration of cognitive deficits and tau related pathology (16-18). However, evidence proving that Aβ is central in the common late-onset sporadic form of AD is more limited: 1) A correlation has been shown between biochemically extracted Aβ peptides species from sporadic AD brains with cognitive decline (19). 2) Isolated Aβ peptide dimers/oligomers from sporadic AD brains have been documented to impair synaptic structure and function (20). 3) Aβ extracted from sporadic AD patients has been shown to induce amyloid deposits when injected into transgenic mice (21). A significant problem for the amyloid cascade hypothesis comes from the autopsy data from the initial human active vaccination trial, which is further discussed below. Post-mortem analysis was available from nine subjects in the active immunization arm (22). All these individuals showed some degree of plaque removal and reduced Aβ load compared to comparable non-immunized controls. Despite this, there were no differences between placebo and active immunization groups in terms of long-term survival outcome, time to severe dementia and in outcome measures such as ADAS-Cog, MMSE or DAD. This may be related to immunization having begun too late in the disease process; alternatively, one can use this data to suggest that the amyloid

cascade hypothesis is an oversimplification. A number of investigators have suggested alternative theories, whereby accumulation of Aβ and tau hyperphosphorylation are dual pathways both downstream from a common upstream pathogenic deficit (which remains to be identified) (23-25). In such a scenario it is essential for immunotherapy to address both of these pathologies to be highly effective.

Hence in this review we will summarize the preclinical and clinical data for both Aβ and phosphorylated tau reduction immunotherapeutic approaches.

PAST ACTIVE IMMUNIZATION HUMAN EXPERIENCE TARGETING AB

Initial data supporting immunotherapy for AD showed that anti-Aβ antibodies could inhibit Aβ peptide fibrillization/oligomerizaton and prevent cell culture based neurotoxicity (26,27). This lead to vaccination of AD Tg mice with Aβ1-42 or Aβ homologous peptides co-injected with Freund's adjuvant which demonstrated striking reductions in Aβ deposition and as a consequence elimination of behavioral impairments (12-15,28,29). Similar effects on Aβ load and behavior have been demonstrated in AD Tg mice by peripheral injections of anti-Aβ monoclonal antibodies indicating that the therapeutic effect of the vaccine is based primarily on eliciting a humoral response (30,31). In the initial preclinical studies no toxicity was evident in the treated mice; however, some investigators suggested that use of non-fibrillogenic, non-toxic Aβ homologous peptides along with approaches that stimulate primarily humoral, Th-2 immunity, in contrast to a primary Th-1 cell mediated response might reduce potential toxicity (32-34). The dramatic biological effect of vaccination in preclinical testing encouraged Elan/Wyeth in April 2000 to launch a randomized, multiple-dose, dose-escalation, double-blind Phase I clinical trial with a vaccine designated as AN1792, which contained pre-aggregated Aβ1-42 and QS21 as an adjuvant. This type of vaccine design was aimed to induce a strong cell mediated immune response, since QS21 is known to be a strong inducer of Th-1 lymphocytes (35). The initial trial was conducted in the UK and involved 80 patients with mild to moderate AD (36). This trial was designed to assess the antigenicity and the toxicity of multiple dose immunization with the full length Aβ1-42 peptide with the QS21. 53% of patients developed an anti-Aβ humoral response. During the later stages of the phase I trial, the emulsifier polysorbate 80 was added causing a greater shift from a Th2 biased response to a proinflammatory Th1 response (37). In the subsequent phase IIa trial, begun in October 2001, 372 patients were enrolled with 300 receiving the aggregated Aβ1-42 (AN1792) with QS21 in the polysorbate 80 formulation. This trial was prematurely terminated in January 2002 when 6% of vaccinated patients manifested symptoms of acute meningoencephalitis (18 out of 298 subjects) (35,38,39). Autopsies performed on a limited number of trial patients suggested that striking Aβ clearance of parenchymal plaques had occurred, similar to what had been reported in the animal studies, confirming the validity of this approach for amyloid clearance in humans(39-44). In these cases extensive areas of cerebral cortex were devoid of plaques, with residual plaques having a "moth-eaten" appearance or persisting as "naked" dense cores. This amyloid clearance in most cases was in association with microglia that showed Aβ immunoreactivity, suggesting phagocytosis. Additional striking features were the persistence of amyloid in cerebral vessels, as well as unaltered tau immunoreactive NFTs and neuropil threads in regions of cerebral cortex where plaque clearing had apparently occurred, compared to regions without clearing (42-44). Hence, this initial vaccination approach did not address vascular amyloid or NFT related pathology. Some cases also showed a deleterious T-cell reaction surrounding some cerebral vessels, suggestive of an excessive Th-1 immune response. It appeared that the immune reaction triggered by AN1792 was a double-edge sword, where the benefits of a humoral response against Aβ were overshadowed in some individuals by a detrimental T cell mediated inflammatory response (39,45). The likely involvement of an excess cell mediated response in mediating

toxicity was supported by analysis of peripheral blood mononuclear cells from trial patients, which were stimulated in vitro with the Aβ peptide, followed by quantification of cytokine secretion by enzyme-linked immunosorbent spot assay (37). The cells of most responder trial patients mounted IL-2 and IFN-γ positive responses indicative of a Class II (CD4+) Th-1 type response (37). Not all patients who received AN1792 responded with antibody production. The majority mounted a humoral response and showed a modest but statistically significant cognitive benefit demonstrated as an improvement on some cognitive testing scales comparing to baseline and a slowed rate of disease progression comparing to the patients who did not form antibodies (36,46). The follow-up data from the Zurich cohort, who are a subset of the Elan/Wyeth trial (46,47), indicated that the vaccination approach may be beneficial for human AD patients. In agreement with the findings in the Zurich cohort, immune responders with high antibody titers in the multi-center cohort scored significantly better in composite scores of memory functions as compared to low- and non-responders or to the placebo group of patients (37). However, it is striking that despite the apparent success in amyloid clearance indicated by the autopsy data, the clinical cognitive benefits were very modest when the active vaccination group was compared to the placebo group (48). No difference between the antibody responders and the placebo group was found on the ADAS-Cog, Disability Assessment for Dementia, Clinical Dementia Rating scale, MMSE or on the Clinical Global Impression of Change. It was only on a nine-item composite NTB that antibody responders had a slight benefit compared to the placebo group. This data can be used to suggest that vaccination in this cohort was started too late; hence, tau related pathology was unaffected by vaccination and thus the cognitive benefits were small. Alternatively it can be suggested that the amyloid cascade hypothesis must be an oversimplification of the pathogenesis of sporadic AD. The latter view is supported by the follow up study of the 80 patients in the initial phase I AN1782 trial, of whom 8 came to autopsy (22). This study showed that despite evidence of very significant amyloid plaque removal in 6 out of the 8 autopsy subjects, which correlated with the anti-Aβ titer, in the overall group there was no evidence of improved survival or an improvement in the time to severe dementia (22).

PAST PASSIVE IMMUNIZATION EXPERIENCE FOR AD

Passive immunization consists of an injection of pre-prepared antibodies to patients, as opposed to active immunization where the immune system is stimulated to produce its own antibodies. Passive transfer of exogenous monoclonal anti-Aβ antibodies appears to be the easiest way to fulfill the goal of providing anti-Aβ antibodies without risk of uncontrolled Th-1 mediated autoimmunity. AD Tg model mice treated this way had a significantly reduced Aβ level and demonstrated cognitive benefit (30,31). Potential problems with passive immunization include the need for repeated injections in a chronic disease, high cost, proper selection of antigen targets, blood-brain barrier penetration, the risk of hemorrhages and the development of an immune response to the injected antibodies. Several passive immunization trials are underway with the most advanced being the Phase III Bapineuzumab trial begun in Dec 2007 (4). The Phase II trial using this anti-Aβ monoclonal antibody was a randomized, double-blind, placebo controlled trial testing 3 doses in 240 participants. In each of the escalating doses of the antibody, approximately 32 subjects received active agent and 28 placebos. Although the study did not attain statistical significance on the primary efficacy endpoint in the whole study population, in the sub-group of non-apoE4 carriers clinically significant benefits were documented using a number of scales including the Mini Mental State Examination (MMSE) and the Alzheimer's Disease Assessment Scale Battery, over the 18 month trial period. In addition among non-apoE4 carriers, evaluation of the MRI results showed less loss of brain volume in treated versus control patients. However, it was reported that some patients in the treatment group

developed vasogenic edema, a significant adverse reaction. The Phase III trial is targeting to recruit 800 patients and run until December 2010.

Using a somewhat similar approach, IVIg is currently in clinical trial for AD, with the rationale being that IVIg contains some anti-Aβ antibodies. In a pilot, open label study in 8 mild AD patients IVIg was infused over 6 months, discontinued and resumed for another 9 months (49). Following each infusion the plasma Aβ levels increased transiently with CSF Aβ being decreased after 6 months. The MMSE increased an average of 2.5 after 6 months and returned to baseline after washout and remained stable with the subsequent IVIg infusions. These promising initial findings clearly need to be repeated in a larger cohort. The attraction of IVIg use is that there is extensive experience using IVIg safely for multiple neurological disorders; however, it is a very expensive treatment and the percentage of anti-Aβ antibodies in IVIg is extremely low so this is not likely to be neither very specific nor highly effective form of treatment.

A particular concern in association with passive immunization is cerebral microhemorrhage. The mechanism of this hemorrhage is thought to be related to Aβ deposition in the form of congophilic amyloid angiopathy (CAA) that causes degeneration of smooth muscle cells and weakening of the blood vessel wall. A number of reports have shown an increase in microhemorrhages in different AD mouse models following passive intra-peritoneal immunization with different monoclonal antibodies with high affinity for Aβ plaques and CAA (50-52). Microhemorrhages following active immunization in animal models have also been reported but only in two studies, hence this appears to be less of a problem with this approach (53,54). In transgenic mouse models, Aβ antibodies can both prevent the deposition of vascular amyloid, and remove it thus contribute to vascular repair. On the other hand, the autopsies from the AN1792 trial indicated no clearance of vascular amyloid and in one of these cases numerous cortical bleeds were found, which are typically rare in AD patients, (41). This is an important issue as CAA is present in virtually all AD cases, with approximately 20% of AD patients having “severe” CAA (55). Furthermore CAA is present in about 33% of cognitively normal elderly, control populations (56). The need for vascular repair and regeneration during Aβ immunotherapy represents another argument for early treatment as well as an argument favoring subtle clearance over a longer time period.

PHOSPHORYLATED TAU AS AN IMMUNE TARGET

Neurofibrillary tangles (NFTs) are a major pathologic hallmark of AD. NFTs are intraneuronal inclusion bodies that consist of an accumulation of paired helical filaments (PHFs), which biochemically are mainly composed of abnormally phosphorylated tau. Recently there is increasing focus on phosphorylated tau as an immunotherapeutic target (57-59). In the CNS, human tau is expressed in 6 isoforms arising from alternative mRNA splicing from a single gene on chromosome 17q21, containing 16 Exons (see Figure 1) (60,61). The size range of the six isoforms is between 352 and 441 amino acids, which differ by the absence or presence of 29 (Exon 2) or 58 (Exon 2 + Exon 3) amino acids inserts in the amino-terminal. The carboxy-terminal half of tau contain three or four semi-homologous repeat of 31 or 32 amino acids, encoded by Exon 10. The repeats (3R, 4R) correspond to the microtubule binding region of protein tau. (see Figure1). Stabilization of microtubules by tau is essential for the maintenance of neuronal cell morphology and transport of organelles. In addition, tau has other roles such as interactions with kinesin -1 and the complex dynactin/dynein (62,63). Tau plays also a crucial role in neuronal cell architecture by interacting with plasma membrane or cytoskeleton proteins such as actin, spectrin and neurofilament proteins. Several mutations have been detected in the tau gene in FTDP-17 and other tauopathies, however none have been linked to AD (64). Most of these mutations affect the binding of tau to microtubules or enhance the aggregation of tau into fibrils. Other

intronic mutations that affect the splicing of Exon 10 induce an increase of isoforms with 4 repeats. In AD, tau is hyperphosphorylated at all phosphorylated sites with 9 phosphates per molecule in comparison to normal brain tau that has 2 to 3 phosphorylated residues (65). Other studies suggested that changes in tau splice forms are related to neurodegeneration. In some animal models expressing mutated Tau there is an increase of 4R versus 3R tau (66). The functional significance of a shift in the 3Rtau/4Rtau ratio remains unclear, but four - repeat tau binds microtubules with a higher affinity than three- repeat tau (67).

Normal tau and PHF tau differ in molecular weight and banding pattern as seen in Figure 1. Normal tau has 6 bands between 45 and 68 kDa, while PHF-Tau has 4 bands between 60 and 74 kDa (see Figure 1) (68,69). The diversity of tau isoforms is related to various post-translational modifications such as phosphorylation, glycosylation, glycation, ubiquitination, nitration (70). The splicing regulation of the tau gene and the relative expression of isoforms is not significantly changed in sporadic AD (Figure 2) (71). Tau has multiple phosphorylation sites that were characterized using phospho-tau dependant antibodies (see Figure 3). 71 out of the 85 potential phosphorylated sites have been shown to be phosphorylated in physiological or pathological conditions (72,73). More than 20 protein kinases have been implicated in the phosphorylation of tau proteins, with glycogen synthase kinase-3β (GSK-3β) and cyclin-independent kinase (cdk5) thought to play the most important role in phosphorylation under pathological condition (72-75).

Several transgenic mice models that express human tau with FTDP-17 mutation have been produced (see Table 1). Some of these mice display neurofibrillary tangles, neuronal death and behavioral deficits (76-85) except a Tg mice model that expresses a mutated (N279K) tau that shows behavioral deficits without formation of NFTs or neuronal loss (86). In these models there is disruption of axon transport due to the tau expression that induces synaptic and neuronal loss. Another Tg tau mice model was developed expressing the mutated P301S tau which shows synaptic loss that precedes tangles formation (84). The distribution of neurofibrillary tangles in most of these tauopathies models are in contrast to Alzheimer's disease, since NFT is localized in different brain regions such as the brain stem, spinal cord or in fronto-temporal cortex instead of the entorhinal region, hippocampus and neocortex as observed in AD (87). In order to generate a more ideal model for AD, other researchers have used a single wild-type human tau to generate a transgenic model; however, most of these models did not develop NFT, with the except of two models: One expressing ON3R wild-type tau with few NFTs in aged animals (80) and another with abundant NFT that expresses all 6 human tau isoforms on a knockout background for murine tau (88,89). The absence of tangles in mice that expressed a single wild-type human tau is likely due to the endogenous tau inhibiting the formation of NFT-like pathology.

Recently, it has been shown that active immunization of Tg mice P301L with a phospho-tau peptide (containing the phosphorylated PHF-1 epitopes Ser 396, Ser 404) for 2 to 5 months could prevent tau related pathology (90,91).

These particular phosphorylation epitopes were chosen since these sites have been shown to increase the fibrillogenic nature of tau and contribute into paired helical filaments formation (92,93). Histological and biochemical analyses showed a reduction of aggregated tau in the brain and improve performance on motor tasks(90). This study clearly documented that it is possible to reduce tau related pathology with active immunization.

At first examination it is difficult to understand how an antibody response to a protein which is accumulating intra-cellularly can have beneficial effects. However, such an outcome is supported by a study of immunization in a Parkinson's disease transgenic mouse model with α-synuclein showing a reduction of intracellular α-synuclein aggregates (94). An additional

study has shown that antibodies against Aβ can be internalized in AD neuronal culture models of Aβ accumulation and clear intra-neuronal Aβ aggregates via the endosomal–lysosomal pathway (95). Furthermore recent evidence has shown that extracellular tau aggregates can be internalized and promote the fibrillization of intracellular full length tau in a tissue culture model (96) and that injection of fibrillar tau brain extract into the brains of transgenic wild-type expressing mice can induce the formation of human tau into filaments, as well as the spread of pathology from the site of injection into neighboring brain regions (97). This type of "infectivity" of abnormal protein conformation from outside the cell has also been demonstrated for polyglutamine aggregates (98) and is well characterized in prion disease (99,100). Hence if the spread of PHF pathology in AD occurs via a prion like mechanism, anti-phosphorylated tau antibodies would not need to enter cells in order to be effective.

QUESTIONS TO ADDRESS FOR A NEW GENERATION OF AD VACCINES

An initial question which needs to be addressed is when to begin a vaccination protocol. Extensive neuropathological data has established that by the time the earliest clinical signs of AD emerge, Aβ deposition may be close to reaching its peak and that NFT formation and neuronal loss are substantial but have not yet reached peak levels (101,102). This would suggest that amyloid directed therapy would need to begin very early, perhaps even before the mild cognitive impairment stage, in order to have a maximal effect.

This is consistent with the albeit limited autopsy data from the initial AN1792 study that showed that despite evidence of very significant amyloid plaque removal in 6 out of the 8 autopsy subjects, which correlated with the anti-Aβ titer, in the overall group there was no evidence of improved survival or an improvement in the time to severe dementia (22). Hence there is a need for identification of markers predicting the conversion from normal aging to very mild dementia/mild cognitive impairment. These include CSF biomarkers such as increased p-tau/Aβ42 ratios in cognitively normal individuals which enhance the probability of conversion to MCI (103). Early FDG-PET changes in hippocampal glucose metabolism can predict the conversion of normal cognition to pathologically verified AD (104). Studies in AD Tg models suggest that paramagnetic amyloid binding ligands utilizing magnetic resonance imaging have potential for early amyloid detection and following treatment effects (105,106). However currently, direct imaging of amyloid depositions with agents such as PIB, using PET imaging, is the most promising method for identification of early amyloid deposits and identifying patients who will likely convert to MCI from normal aging and MCI to early AD (107,108). An alternative approach is to immunize targeting both Aβ deposition and tau related pathology.

Such an approach has a higher probability of having a more clear effect on the clinical course, even if started when clinical symptoms are evident. Furthermore if, as discussed above, tau pathology is not downstream from amyloid deposit but represents a parallel pathology related to a common upstream cause, it will be essential to target tau related pathology regardless of how early vaccination treatment is initiated.

Another significant issue which needs to be addressed in future studies is the development of better models for pre-clinical testing of vaccination approaches. There are many shortcomings to current Tg models of AD pathology. These include the fact that Tg amyloid deposits typically lack the extensive post-translational modifications of AD amyloid and thus are much more soluble, presumably allowing them to be cleared more easily (109). The rodent immune system is quite different from the human immune system, leading to significant differences in the toxic responses to amyloid deposition (110). Most current tau Tg models reflect FTDP related pathology in contrast to AD tau pathology, as discussed

above (87). Relatively few of the vaccination approaches being developed have been tested in non-human primates or other non-transgenic models of AD which may provide more accurate models of the type of immune response that might be elicited in aged humans as well as better reflecting the combination of true human Aβ and tau related pathology(111-113). These models include the rhesus monkey, the vervet monkey, mouse lemurs and aged beagles (113-118). It is striking that in a recent 22 month active immunization trial in aged beagles, despite an ~80% reduction in cortical Aβ immunoreactivity, little cognitive improvement on multiple measures of learning and memory could be detected (119). However, improvements in some executive functions were found, mirroring the modest improvements seen only in the z-score of the Neurological Test Battery among patients in the AN1782 trial. These results reinforce the need to begin immunomodulation very early in the disease progression focusing on preventing Aβ deposition rather than clearance of preexisting lesions, as well as the likely need to target tau related pathology concurrently.

Active vaccination approaches under development are aiming to avoid the excessive Th1 stimulation that resulted in encephalitis in a proportion of the AN1792 patients. Concurrently the formulation of any active vaccine also has to overcome the problem of immunosenescence in the target patient population. One promising approach taken by several investigators is to alter the sequence of the Aβ peptide immunogen in order to remove or alter the major Th1 stimulator sites in the carboxyl terminus and the middle portion of Aβ, while focusing on the major Th2 stimulator site in the amino terminus (28,33,120-122). These Aβ homologous peptide immunogens can be combined with various co-stimulator epitopes. An example of this approach is a combination with a synthetic, non-natural Pan HLA DR-binding epitope PADRE (122) or linkage to viral-like particles (VLP) (123-125) to induce a primarily humoral immune response. These can be further combined with other immunostimulator carriers. For example the Aβ Th2 amino terminal epitope can be combined with PADRE and macrophage derived chemokine (MDC) in a DNA epitope vaccine to drive robust Th2 responses (126). The choice of adjuvant is also an important consideration. The use of polysorbate 80, a strong Th1 stimulate adjuvant, in the AN1792 trial is one likely contributing factor to the encephalitis in a minority of patients. Use of adjuvants such as alum which drive primarily a Th2 response is preferable (29,119). The route of immunization also plays an important role. Stimulating mucosal immunity by vaccinating nasally, via the gut or transcutaneously has been shown to drive strong Th2 responses (127-129). An alternative, non-mutually exclusive approach to enhance vaccine design is to stimulate innate immunity and enable microglia/macrophages to phagocytose amyloid deposits. Over 20 yrs ago, H.Wisniewski noted that while brain-resident macrophages were unable to phagocytose amyloid, brain-infilrating macrophages are plaque competent (130). A number of recent studies suggest that only a small percentage of plaques are associated with peripheral origin macrophages and that these are required for plaque clearance (131-133). Vaccination approaches based on this knowledge are now being developed. Stimulation of peripheral macrophages to enter the CNS and phagocytose amyloid has been achieved by stimulation of the Toll-like receptor 9 using CpG (134,135), via blockade of the CD40/CD40L interaction (136) and by blockade of TGFβ-Smad2/3 innate signaling pathway (137). These innate immunity stimulatory approaches can be used alone or in combination with adaptive immunity stimulation. Stimulating the innate immune system has the added potential advantage that it could be effective against both Aβ and tau related pathologies.

Another important issue for future vaccination approaches is what is the best target for either active or passive immunization? Abundant evidence both in vivo and in vitro suggests that the most toxic species of Aβ are oligomers or Aβ derived diffusible ligands (ADDLs) (138,139) with a similar line of evidence suggesting that tau oligomers are the most toxic

form of phosphorylated tau (59,84). Active vaccination or use of monoclonal antibodies that specifically target Aβ oligomers, tau oligomers or preferably both would be an ideal way to block AD related toxicity.

A small number of pre-clinical studies targeting Aβ oligomers suggest that this methodology is potentially powerful and in the need of further development (140-144). An additional important factor to consider is that emerging evidence suggests that monomeric Aβ peptides have normal physiological functions in the brain such as neuroprotection and modulating LTP (145,146), with phosphorylated tau also having a normal role (58). Targeting only oligomeric Aβ or tau would avoid the potential of interfering with these physiological functions. A novel immunotherapeutic approach is to target the shared abnormal β-sheet conformation of amyloid proteins using conformationally specific antibodies or active immunization that favors such a conformational response (140,141,147). Such an approach has the advantage that both Aβ and tau related pathologies would be addressed concurrently.

CONCLUSION

Numerous therapeutic approaches are under development for AD; however, harnessing the immune system to clear both Aβ and tau related pathology is perhaps the most promising and advanced approach. Abnormal protein conformation is thought to be not only the underlying pathogenesis of AD but also of a long list of neurodegenerative conditions, such as prion disease, Parkinson's disease and Huntington's chorea, with immunomodulation having the potential to be a disease altering therapeutic approach for all these disorders. For example it has been shown that prion directed mucosal vaccination can prevent infection from an exogenous source (148,149). Ultimately, directing the immune system to clear the highly toxic abnormal oligomeric conformers that characterize multiple neurodegenerative diseases has the potential to dramatically alter the course of a wide spectrum of age associated diseases.

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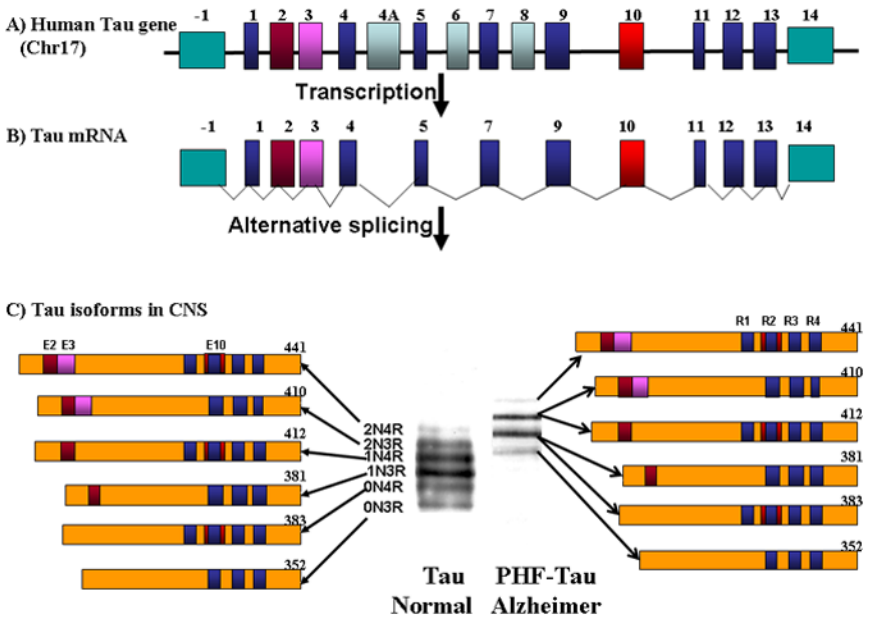

Figure 1.

Shows a schematic representation of the human tau gene which is located on chromosome 17q21, and spans more than 130 kb. This gene is composed of 16 Exons. (A). Exons 1 and 14 are transcribed but not translated (turquoise color). The Exons 4A, 6 and 8 are not transcribed in human (light blue/charcoal color) (B). In the human brain, 6 tau isoforms ranging from 352 to 441 amino acids are generated by alternative splicing of Exons 2, 3, and 10 (shown in brown/red, pink and red respectively) from a single gene. Exons 1, 4, 5, 7, 9, 11, 12 and 13 (blue color) are included in all isoforms. Exon 3 is always included with Exon 2. The microtubule binding domains are indicated by R1, R2, R3 and R4, which correspond respectively to Exon 9, 10, 11 and 12, respectively. (C). Extraction of tau proteins and PHF-Tau from normal and Alzheimer brain respectively, shows by immunoblotting six bands between 45-68 kDa which correspond to different tau isoform in normal brain, while in PHF-tau 4 bands are detected between 60-74 KDa which corresponds to the aggregation of 6 hyperphosphorylated tau isoforms in the AD brain.

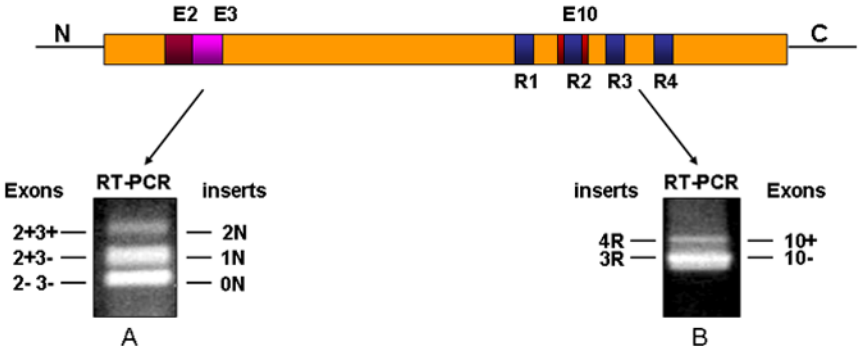

Figure 2.

Shows the electrophoresis of RT-PCR amplification products of the 5' domain of tau mRNAs (A), of the 3' domain of tau mRNAs (B). Extraction of RNA was performed in from the cerebellar cortex of a sporadic Alzheimer's disease patient. The expression of mRNA by RT-PCR shows different isoforms of human tau detected in the N-terminal (0N, 1N, 2N) and in the C-terminal (3R, 4R). Symbol (+) indicates with exon, while (-) indicates without exon.

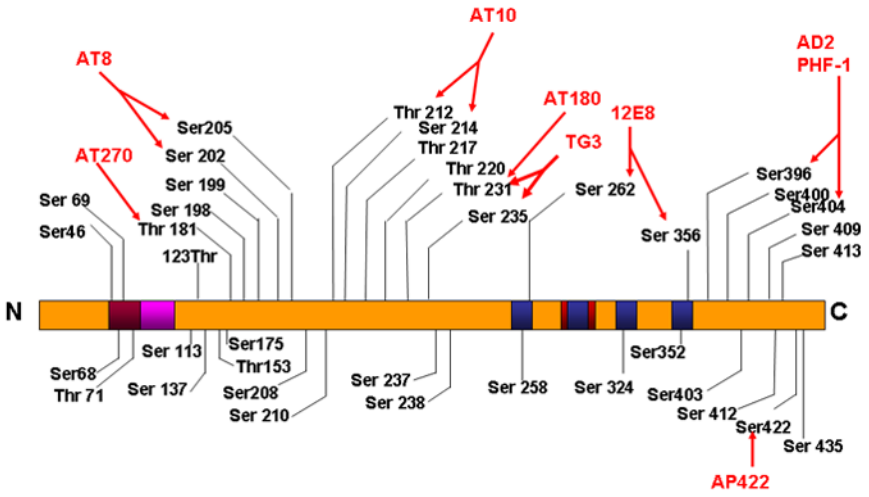

Figure 3.

In the human Alzheimer brain, more than 40 phosphorylation sites on tau have been identified and localized in the proline-rich domain and in the C-terminal region. Phosphorylated sites are identified with 8 phospho-tau specific antibodies as indicated in figure.1C, with a red color. It has been suggested that the phosphorylation at Ser 262/356 is responsible for the detachment of tau from microtubules.

Table1

Shows the different transgenic models used to study tau related pathology which express wild-type (WT) tau or mutated tau that has been identified in various FTDP-17 pedigrees.

Tau Isoform	Tau Mutation	Presenilin cDNA	APP cDNA	Promotor	NFT	Citation
2N4R	WT	—	—	Thy-1	NO	(150)
2N4R	WT	—	—	Thy-1.2	NO	(151)
2N4R	WT	—	—	Thy-1.2	NO	(152)
0N3R	WT	—	—	HMG-CoAR	NO	(153)
0N3R	WT	—	—	PrP	NO	(154)
3R	WT	—	—	Mouse tubulin Tα1	NO	(155)
0N3R	WT	PS1 M146L	—	HMG-CoAR	NO	(156)
0N3R	WT	PS1 M146L	APP 751(SL)	HMG-CoAR and Thy-1	NO	(157)
2N4R	WT/KoKI	—	—	Thy-1	No	(158)
6 isoforms human	WT	—	—		Yes	(88)
0N4R	P301L	—	—	PrP	Yes	(159)
0N4R	P301L	—	APPsw	PrP	Yes	(79)
0N4R	P301S	—		Thy1.2	Yes	(160)
0N4R	P301L	PS1 M146V	APPsw	Thy1.2	Yes	(10)
2N4R	P301L	—	—	Thy-1.2	Yes	(9)
2N4R	G272V	—	—	PrP	Yes	(161)

Tau Isoform	Tau Mutation	Presenilin cDNA	APP cDNA	Promotor	NFT	Citation
2N4R	M337V	—	—	PDGF	Yes	(81)
2N4R	R406W	—	—	CaMKII	Yes	(162)
2N4R	R406W P301L G272V	—	—	Thy-1	Yes	(163)
2N4R	P301L	—	—	Thy-1	Yes	(158)
1N2R	G272V P301S			Thy1.2	Yes	(164)