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Harnessing the Immune System for Treatment and Detection of Tau Pathology

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Abstract

The tau protein is an attractive target for therapy and diagnosis. We started a tau immunotherapy program about 13 years ago and have since demonstrated that active and passive immunotherapies diminish tau pathology and improve function, including cognition, in different mouse models. These findings have been confirmed and extended by several groups. We routinely detect neuronal, and to a lesser extent microglial, antibody uptake correlating with tau pathology. Antibodies bind tau aggregates in the endosomal/lysosomal system, enhancing clearance presumably by promoting their disassembly. Extracellular clearance has recently been shown by others, using antibodies that apparently are not internalized. As most pathological tau is neuronal, intracellular targeting may be more efficacious. However, extracellular tau may be more accessible to antibodies, with tau-antibody complexes a target for microglial phagocytosis. The extent of involvement of each pathway may depend on numerous factors including antibody properties, degree of pathology, and experimental model. On the imaging front, multiple tau ligands derived from β -sheet dyes have been developed by several groups, some with promising results in clinical PET tests. Postmortem analysis should clarify their tau specificity, as in theory and based on histological staining, those are likely to have some affinity for various amyloids. We are developing antibody-derived tau probes that should be more specific, and have in mouse models shown in vivo detection and binding to pathological tau after peripheral injection. These are exciting times for research on tau therapies and diagnostic agents that hopefully can be applied to humans in the near future.

Keywords

Tau; Immunotherapy; Imaging; Antibodies

Tau as a Target for Immunotherapy

The earliest use of immunotherapy as potential treatment for Alzheimer's disease (AD) began with the successful attempt to clear amyloid- β (A β) plaques in mice [1]. Earlier in vitro work indicated that A β antibodies could prevent the formation of and promote

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disassembly of A β fibrils [2, 3]. Vaccination with A β peptides and derivatives resulted in reduced plaque burden and cognitive improvement in transgenic mice, suggesting that active immunization might be an effective option [1–8]. However, an early trial (AN-1792) using full-length A β 1-42 vaccine in humans was halted because of encephalitis in 6 percent of the participants receiving the treatment [9]. Subsequent analyses indicated that although the immunization reduced amyloid burden, clinical progression was not affected. This suggests that, in humans, reduction in plaque burden alone is insufficient for altering the course of the disease [10], at least when therapy is initiated after cognitive impairments are clearly evident. More recently, passive immunization strategies have been utilized in clinical trials with the most advanced studies being on Bapineuzumab and Solanezumab, monoclonal antibodies recognizing different sites on A β . Findings from Phase III trials have been disappointing with only modest efficacy. Some subgroups showed small but significant slowing of memory deterioration with Solanezumab. However, these did not translate into overall cognitive benefits [11].

Plaque clearance in the AN-1792 trial had limited effect on tau pathology, although it appeared to clear some plaque associated tau lesions [10, 12–17]. Limited autopsy data is available from the passive trials with one study reporting apparent alterations in A β composition but no change in plaque density or tau levels in three Bapineuzumab treated subjects, compared to controls [18]. More comprehensive data is available from the A β antibody trials on tau analyses in cerebrospinal fluid. In Phase II or Phase III Solanezumab trials, no change was observed in the levels of total tau or phospho-tau in treated subjects [19, 20]. In Phase II Bapineuzumab trial, significant decrease in phospho-tau was detected in treated individuals [21,22]. In the larger Phase III trial, reductions in phospho- and total tau have been reported both within the treatment group over time and compared to controls. These effects depend to some extent on apoE genotype [23]. Overall, these findings from the active and passive trials indicate that antibody-mediated clearing of A β does not have sufficient effect on tau pathology for clinical benefits. Several other active and passive A β targeting immunotherapies are in earlier stage clinical trials with the focus now shifting to prophylactic treatment in familial cases or earlier stage therapy in sporadic cases.

Because it appears that reducing A β pathology alone does not alter disease course, at least after cognitive deficits are clearly established, an alternative target for therapy is required. The other major hallmark of the disease, pathological tau protein is an attractive candidate for AD drug development and the lead target for other tauopathies. Our group has demonstrated the potential of active immunization with the Tau379-408[P-Ser396, 404] peptide in two different transgenic mouse models of disease, the JNPL3 and htau/PS1 lines [24, 25]. In both cases, aggregated tau burden was reduced and attenuation in the severity of behavioral and cognitive phenotypes was observed. The efficacy of active immunization against the P-Ser396, 404 epitope has been confirmed by other researchers as well [26, 27]. In addition, peptides comprising other pathological tau epitopes have been successfully utilized including P-Ser202/P-Thr205, P-Thr212/P-Ser214, P-Thr231, and P-Ser422 [28, 29], as well as P-Ser262 in our preliminary studies [30]. In all reports, levels of pathological tau were reduced.

Although there have been limited negative complications observed with active immunization strategies in animal models, the possibility of adverse reactions in human patients suggests a role for alternatives. Passive immunotherapy using antibodies which recognize both phospho and conformational epitopes have also been used successfully. As in active immunization protocols, targeting the P-Ser396, 404 epitope has been an effective strategy for reducing tau pathology [31–34]. Reduction of pathological tau and improvement in tangle-related behavioral deficits were achieved using PHF1 and novel tau antibodies raised against that region. Conformational antibody MC1 has also been shown to reduce pathological tau relative to control animals [33, 35]. Additionally, antibodies targeting total non-pathological tau have shown promising results in mouse models and in cell culture [36, 37].

Mechanism of action is another factor to consider in the development of therapeutic approaches. Tau lesions develop within neurons and thus may be more difficult to target directly, compared to A β plaques which are found extracellularly. In this context, it is interesting to note that multiple groups have shown that tau pathology is transmissible, suggesting that it could be targeted extracellularly. In transgenic animal models, tau pathology begins in specific areas before spreading to anatomically connected regions [38–40]. Spread of tau pathology can also be observed through the addition of exogenous tau injected into the brain, or to culture media. Abnormal tau is secreted and taken up by other cells via endocytosis, providing a template to recruit the native tau into aggregates [36, 41–48]. Thus, both intra and extracellular tau provide potential targets for antibodies as we have discussed previously [24, 49]. It may be possible to prevent spread through either intracellular clearance or by blocking uptake. Our results and those of others show brain entry of tau antibodies in tangle mice, their subsequent neuronal uptake into the endosomal/lysosomal system, where they colocalize with tau aggregates, and such uptake appears to be a prerequisite for acute tau clearance [24, 50–54]. Specifically, we have recently demonstrated that uptake is to a large extent receptor-mediated and occurs through low affinity Fc receptors, with a smaller fraction being taken up via bulk-mediated endocytosis. Long-term effects can also be envisioned with the antibodies leading to disassembly of tau fibrils which should facilitate access of lysosomal enzymes for their degradation. Support for clearance of intracellular aggregates can be seen in other model systems as well. Groups using immunotherapy focused on α -synuclein, amyloid- β , and superoxide dismutase have reported reduction in intracellular protein aggregates [55–58]. Extracellular tau may also be an important target when attempting to halt the spread of pathology. When added to culture media, pan-tau antibodies can prevent the propagation of tau pathology without the need to enter the cells [36]. Such tau antibodies are also able to target extracellular tau in vivo. In transgenic animals, infusion of pathological brain extract can induce aggregation, and addition of these antibodies in the extracellular space can prevent seeding of tau pathology [37]. Ideally, for the most efficacious approach assuming no safety concerns, both intra- and extracellular clearance would be incorporated into treatment strategies for antibody development going forward.

Potential difficulties in transitioning this promising research into clinical therapeutics arise from the nature of the tau protein and AD itself. We have seen that multiple epitopes, phospho, conformational, and total tau can be targeted successfully in animal models.

However, in human tauopathies the relative prevalence and types of epitopes are to some degree subject dependent and are likely to change over time, based on postmortem brain analyses. Thus, the efficacy of a given treatment may be dependent on the individual and the stage of the disease. In addition, whether the chosen epitope is disease specific is an important consideration, as targeting normal tau may prove detrimental. However, to determine the extent of this issue, more research on the fate of intracellular antibodies is needed. For example, if antibodies taken up by endocytosis remain in the endosomal/lysosomal compartment, it may not be harmful to utilize antibodies recognizing non-pathological tau. In fact, by broadening the range of tau targeted, clearance may be improved. If, on the other hand, antibodies are released into the cytosol then a pan-tau antibody may target non-pathological tau for degradation and possibly disrupt normal cell functions. Likewise, if the secreted non-pathological tau has a biological function, targeting disease epitopes would be preferred.

The choice of vaccination methodology also raises issues of efficacy and safety. In the case of active immunization, the choice of adjuvant and related danger of T-cell mediated reactions are factors to consider. In experiments with wild type animals, immunization with recombinant tau protein in complete Freund adjuvant supplemented with pertussis toxin produced tau pathology and neurological symptoms [59]. Similar results were recently found following immunization with phospho-tau peptides using these strong adjuvants. Animals developed anti-tau antibodies and in wild type animals this partially correlated with the degree of neuroinflammation [60]. Although such adjuvant combination would never be used in humans, these findings re-emphasize the view that active immunization against self-antigens should be limited to Th2-type adjuvants that primarily promote antibody response as opposed to Th1-type adjuvants promoting cell-mediated immunity [24, 61]. The effect of immunization on glial cells is also a potential source of concern. In our experiments with both active and passive tau immunization, we have not observed significant increases in microgliosis or astrocytosis [24, 25, 32], presumably because most of tau is being cleared intracellularly and over long treatment period. Overall, our data suggests a limited role for glia in antibody uptake and tau clearance. When examining antibody uptake, glia represent a minority of antibody containing cells and inhibition of phagocytosis does not reduce uptake [50, 52]. Other groups have observed a similar lack of effect on glial cells following immunization [27, 33]. However, Bi et al. [26] found a trend towards increased astrocytosis, and an increased number of microglia were observed by Biomet et al. (2010) [28]. Differences in observed glial effects may stem from different experimental and detection/analyses methods, all of which must be taken into consideration. Together, these findings and the results from the human trials of active A β immunization, highlight potential safety concerns. Because active vaccination has been successfully utilized with peptides representing multiple epitopes, it is tempting to suppose that multiple epitope vaccination targeting tau would prove even more effective. However, whether this would increase the possibility of adverse reaction in humans has not yet been determined. Adjuvant selection, minimization of T-cell response, and determination of ideal epitope size are still areas to explore.

Another possible route of development of therapeutics is the use of single chain variable fragments (scFv). Recent studies have utilized such fragments against A β in cell culture and

transgenic mouse models [62–65]. These fragments reduced amyloid aggregation and toxicity in cells, as well as amyloid deposits in some brain regions. Our group has developed numerous scFv derived from monoclonal tau antibodies, and their properties may provide some advantages over using intact whole antibodies. Their smaller size may promote greater brain penetrance and ease of entry into neurons, thus a better access to tau aggregates. However, the mechanism of cellular uptake is likely to differ between scFv and whole antibodies, and if Fc-mediated uptake is important for subsequent clearance, this could reduce scFv efficacy. In addition, extensive neuronal uptake of scFv may have adverse effects that have yet to be detected.

Imaging as a Diagnostic Tool to Track Tau Pathology

Because plaques and tangles may be present for years before the onset of symptoms, and the location of protein deposits can be used to identify disease stage, neuroimaging is an area which promises to aid in early detection of AD. Further, identifying the relative concentrations of A β plaques, neurofibrillary tangles and their precursors may provide clues as to which treatments may be most effective. As with AD immunotherapies, the development of imaging agents was first focused on A β . Various different chemical classes were evaluated including derivatives of Congo Red [66, 67], Thioflavin-T [68–70], stilbene [71–74], vinylbenzoxazole [75, 76] and several others. Of these, the best known and characterized is Pittsburgh compound B (PiB) which has been used in multiple studies on human subjects [68, 69, 77–79]. Recently, other compounds have been approved for use in humans, or are undergoing clinical trials. Of these, ¹⁸F-florbetapir (AMVViD) has been used in patients for PET imaging in cognitively impaired adults undergoing evaluation for AD and other causes of cognitive decline to estimate plaque density [71, 72, 80–83]. It is now approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Florbetaben (also known as BAY94-9172) is under review by the FDA and the EMA. In human studies, florbetaben was capable of binding to A β lesions and discriminating between patients with mild AD, frontotemporal lobar degeneration, and healthy controls [73, 84, 85]. Phase III trials are underway for ¹⁸F-NAV4694 a benzofuran derivative [86]. Vizamy, also known as flutemetamol, has recently been given FDA approval for analyzing A β plaque density in human patients [87] and is under review by the EMA. In addition to compounds meant for use in PET imaging, research has also been done on developing agents for near- infrared fluorescence imaging such as AO1987 [88]. However, due to the limited success of A β targeting clinical trials, expanding AD imaging agents to tau is an essential step. Tau imaging agents also allow the possibility of identifying different tauopathies in living patients, detecting the presence of these lesions before the onset of symptoms, and to follow the efficacy of tau-targeting therapies.

The nature of neurofibrillary tangles poses additional challenges to the development of contrast agents. Unlike plaques, all but very late stage tangles are found intracellularly. Thus, compounds must be capable of crossing not only the blood brain barrier but penetrating the cell membrane as well. Charge, molecular weight, and hydrophobicity are all factors to consider when developing tau binding ligands. Hydrophobic molecules less than 180 kDa are more likely to cross into the CNS when administered peripherally. Additionally, compounds must specifically bind tau lesions, a target rich in post-

translational modifications and containing multiple isoforms. This becomes more difficult when one considers that pathological protein aggregates in many diseases often share a very similar secondary structure, such as the cross β -sheet structures seen in amyloid diseases. Additionally, neurons contain large amounts of non-pathological tau that could also interfere with successful imaging [89, 90].

Several groups have identified potential candidates to serve as tau imaging agents. In a screen of over 2000 compounds, quinolone and benzimidazole derivatives were shown to selectively bind aggregated tau relative to A β . Using the quinolone scaffold, novel ligand THK523 showed good affinity to a number of binding sites on recombinant tau relative to amyloid fibrils. Human AD tissue analyzed through autoradiography and histofluorescence revealed that THK523 co-localizes with tau lesions but not with plaques. In tests using micro-PET analysis, ^{18}F -THK523 showed greater retention in the brains of tau transgenic mice compared to wild type littermates [92, 93]. In human subjects, THK523 showed binding in areas associated with disease such as the hippocampus, temporal and orbitofrontal lobes. However, there was concern over binding in white matter [94]. Furthermore, promising results were generated in vitro and initial mouse tests with compounds derived from rhodamine, thiohydantoin, and benzimidazole [95, 96]. T807 and T808 were found in a screen of over 900 compounds, and showed strong preference for tau lesions in human brains sections and the ability to cross the blood-brain barrier in mice [97, 98]. Recently, they have been utilized in small scale tests in human subjects. ^{18}F -T807 showed rapid entry into the brain, in both control and pathological groups. In control subjects, fast clearance from the white matter and cortex was observed, indicating that the compound does not bind normal tau. In contrast, ^{18}F -T807 accumulated in the brains of subjects with mild cognitive impairment and in AD patients [99]. ^{18}F -T808 has shown promise in human trials as well, with ligand binding correlating well with diagnosis and pathological findings at autopsy. However, there is concern about false labeling in the putamen, an area which showed high signal in PET scans but limited tau pathology postmortem [100]. Screening by others produced another class of ligands, phenyl/pyridinyl-butadienyl-benzothiazoles/benzothiazoliums (PBBs), which have been used to visualize tau inclusions in mice as well as in patients with AD and other tauopathies [101]. Sensitive detection of tau lesions was accomplished in vivo in transgenic mice by PET and two photon imaging. For human trials, normal elderly, AD patients, and one patient with corticobasal degeneration were given ^{11}C -PBB. Subjects were also given ^{11}C -PiB to gauge the selectivity of PBB. Results showed that PBB readily enters the brain, with low signal in control subjects. In the subject with CBD, PiB retention was similar to that seen in control subjects while PBB accumulation was seen in the neocortex and subcortical structures. In AD patients, PBB signal changed with disease stage and showed patterns distinct from those observed with PiB. Overall, these compounds show promise as diagnostic agents. Additionally, specific tau binding agents could be used to further explore the relationship between the development of tau pathology and disease progression as well as to monitor treatment efficacy.

All of the compounds described above are derivatives of β -sheet binding dyes and are therefore likely to have some affinity towards various pathological, and possibly normal, β -sheet containing proteins in human patients. This may not become an issue but it does raise some concerns regarding specificity and adverse reactions. In search for probes with greater

specificity, another option for consideration are scFv. Antibody fragments are being studied in cancer models for use as agents in PET and infrared imaging. Early in vitro studies show that scFv can be made to target tau oligomers and can be used to differentiate non-diseased and AD tissue samples [102]. Our group has generated high affinity scFv using combinatorial phage display. Administered peripherally they can cross the blood brain barrier and bind to intraneuronal tau aggregates in transgenic mice. Further, these scFv co-localize with markers of the endosome/autophagosome/lysosome pathway. When used for imaging by In Vivo Imaging System (IVIS), brain signal was significantly higher in tauopathy mice relative to wild type controls [103]. Using such scFv opens up the possibility of creating probes for particular tau species, and these ligands may then be tailored to certain disease stages. Such specificity may thereby provide a more accurate diagnosis that may help with selecting the most appropriate treatment strategy, including which epitopes to target.

Because tau pathology is a better correlate than A β for disease progression in AD, it is an attractive target for disease modifying therapy and to improve diagnostic accuracy of all tauopathies in conjunction with A β imaging ligands. Overall, such strategies focused on tau have made great strides in recent years and are very promising to combat and diagnose AD and other tauopathies.

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