

Tau aggregates as immunotherapeutic targets

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1. ABSTRACT

Pathological aggregation of the microtubule-associated protein tau and accumulation of neurofibrillary tangles (NFT) and other inclusions containing hyperphosphorylated tau are defining histopathological features of Alzheimer disease (AD) and many other neurodegenerative diseases collectively known as tauopathies. The toxicity of tau aggregates has been demonstrated *in vitro* and *in vivo*; thus, their clearance by immunotherapy holds clinical promise. Published studies, which are limited in number, have exclusively focused on the clearance of hyperphosphorylated large tau aggregates, e.g., NFT. However, recent studies using human tissues and mouse models have questioned the toxicity and the presumed role of NFT in the progression of tauopathies and challenged the view of tangles as toxic species in the brain. Together, these novel studies have demonstrated that prefilamentous tau oligomers rather than NFT play a crucial role in these disorders. Here, we summarize recent advances in this new field, highlight the role of tau oligomers and their potential as a therapeutic target for the treatment of AD and other neurodegenerative tauopathies, and discuss the challenges that lie ahead.

2. INTRODUCTION

The aggregation and accumulation of the microtubule-associated protein tau in the human brain are a hallmark of tauopathies. These include Alzheimer disease (AD), Pick disease, progressive supranuclear palsy, corticobasal degeneration, and frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) (1, 2). With improved survival from chronic diseases and the aging of populations in developing and developed countries, dramatic increases in the incidences of tauopathies, especially AD, are predicted, with dire economic and social consequences (3). Hence, the development of therapies is an urgent research priority (4).

The neuropathological lesions in AD are deposits of amyloid- protein (A β) in plaques and the accumulation of hyperphosphorylated tau protein to form neurofibrillary tangles (NFT), neuropil threads, and neurite plaques (5-7). The amyloid hypothesis proposes that A β is the causative agent in AD and that tau pathology has a secondary role (8). Misfolding, aggregation, and deposition of A β give rise to the amyloid plaques that are present in AD patients, and in some degree in aging. However, the lack of a correlation

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between plaque accumulation and the clinical progression of AD diminishes the proposed pathological impact of plaque accumulation (9-11). On the other hand, the correlation of tau pathology with cognitive decline and neurodegeneration (5, 7, 12-14) and the discovery of mutations in the tau gene as a cause of disease directly implicate tau aggregation in the neurodegenerative process (15, 16). Interestingly, amyloid plaques are absent in individuals with FTL^{D17}, which suggests that tau aggregates rather than amyloid plaques play the key role in the progression of tauopathies (1, 2, 6, 17).

To date, there is no treatment to stop the progression of AD and the other tauopathies. Available drugs are effective only in alleviating symptoms (18). Immunotherapy for AD was initiated more than a decade ago with active immunization targeting A β (19). Despite the evidence supporting the role of tau in AD, therapeutic approaches primarily targeted A β . Although A β immunotherapy has shown beneficial effects in animal models (20-23), the first clinical trial was stopped because of side effects and disappointing results (24-28). Given the concerns regarding A β immunotherapy, therapeutic approaches have focused on reducing tau level and the formation of hyperphosphorylated insoluble tau aggregates in the brain (29, 30). They include interference with the splicing machinery to decrease the four-repeat tau isoforms, activation of proteolytic or proteasomal degradation pathways, prevention/reduction of tau hyperphosphorylation through the use of inhibitors of tau kinases, pharmacological stabilization of microtubule networks, and inhibition of tau aggregation through the use of small molecules (29).

An emerging and promising therapeutic approach, described by only a few published reports, to AD and other neurodegenerative diseases is immunomodulation to clear toxic aggregates of tau (31-34). Research in this area is expected to become intensive within the next few years. This review emphasizes the critical role of tau oligomers in the aggregation process.

3. TAU AGGREGATION

Tau protein is abundant in axonal projections of neurons at the central nervous system (35). It is encoded by the MAPT gene (microtubule-associated protein tau), which is located on chromosome 17q21 and contains 16 exons (16, 36, 37). Six tau isoforms have been identified in brain tissue, ranging from 352 to 441 amino acids. The isoforms result from alternative splicing of exons 2, 3, and 10 and can contain three or four binding domain repeats (3R and 4R). Under physiological conditions, tau protein is highly soluble and is normally phosphorylated. Tau isoforms have 30 phosphorylation sites (36) at multiple Ser and Thr residues (38, 39). In tauopathies such as AD, tau is hyperphosphorylated, which reduces its affinity for microtubules and promotes the formation of insoluble aggregates in cell bodies and dendrites (40-42).

The formation of tau aggregates, which occurs *in vitro* and *in vivo*, has been the focus of numerous studies;

however, the exact mechanism of tau aggregation remains unclear. Mechanistic studies *in vitro* of aggregation and filament formation by full-length tau protein have revealed striking similarities to A β aggregation. Tau aggregation after the addition of the tau monomer to the nucleus/template (template-assisted growth) (43, 44) leads to the formation of insoluble fibrillar structures termed “paired helical filaments” (PHF), which consist of two or more protofilaments wound around each other (45). Such structures are the main component of NFT isolated from the sarkosyl-insoluble fractions of AD brain homogenates (46, 47). Additionally, there is some evidence that nucleated oligomer conformation conversion leads to the formation of tau oligomers and other prefibrillar tau intermediates (48, 49). The results of this method, which does not involve the addition of tau monomeric species, support the assembly of all monomers into oligomers before the formation of fibrils, similar to the mechanisms proposed for sup35 prion and recently for A β (50, 51). Monomeric tau proteins associate with each other to form dimers/trimers, which leads to drastic changes in conformation and the generation of β -sheet-rich tau oligomers (49). These oligomers are highly toxic when applied extracellularly to cultured neuronal cells (49, 52, 53). Moreover, we recently demonstrated in an animal study that only tau oligomer prepared from full-length recombinant tau—not tau fibrils or tau monomer—leads to memory impairment, neurodegeneration, and synaptic and mitochondrial dysfunction *in vivo* (54).

4. TAU OLIGOMERS IN HUMAN BRAIN

The published literature indicates that NFT formation alone is insufficient for neurodegeneration and suggests that soluble tau aggregates are the most toxic and pathologically significant tau species (30, 52, 55-62). Stereological studies of human AD pathology have shown that neuronal loss in fact precedes NFT formation (14, 63-65). Furthermore, it has been estimated that hippocampal neurons containing NFT can survive for decades (61). However, tau oligomers have been biochemically characterized in human brain samples and a correlation between disease progression and the accumulation of granular tau oligomers in the brains of AD patients has been reported. Moreover, increased levels of tau oligomers are detected in the frontal cortex at very early stages of AD, before clinical manifestations of either AD or NFT are believed to develop (66, 67). In addition, tau-positive fine granules (TFGs) resembling tau oligomers were found in the cerebral white matter of postmortem tissue from individuals with the parkinsonism-dementia complex of Guam (PDC) tauopathy (68).

The pathological role of prefibrillar tau aggregates, e.g., tau oligomeric intermediates, in tauopathies is poorly understood, in part because of methodological challenges. Fortunately, two exciting, recent studies have reported the development of novel antibodies that enable the study of tau oligomers *in vivo* (69, 70). In the first report, Patterson *et al.* generated a monoclonal antibody that selectively recognizes tau dimers and higher-order oligomeric aggregates but that shows little

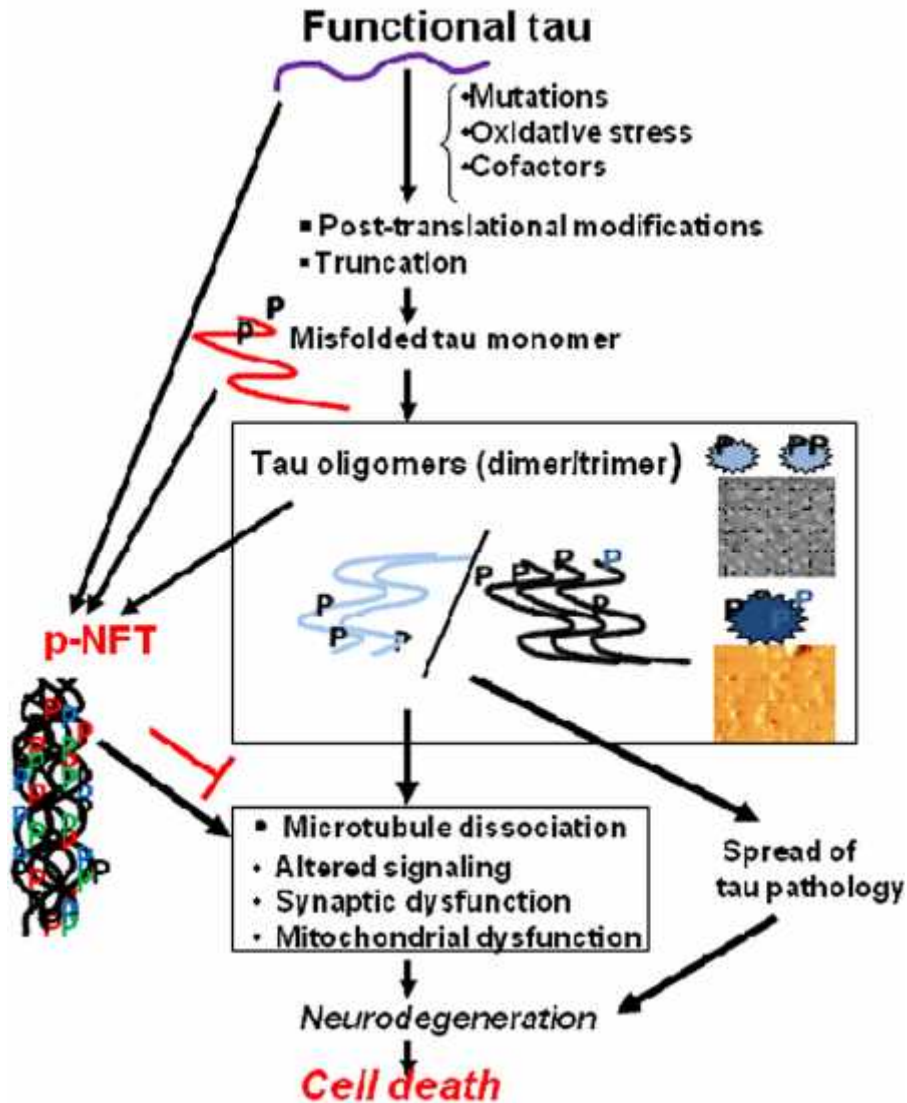


Figure 1. A schematic illustrating the central role of tau oligomers in neurodegeneration. Recently, studies from multiple laboratories have provided compelling evidence for the formation and pathogenic role of a tau species other than monomeric tau or hyperphosphorylated NFT (p-NFT). This tau intermediate aggregate (tau oligomers) is the toxic tau entity and the source of tau pathology and its propagation in neurodegenerative tauopathies; thus, it represents the ideal target for anti-tau immunotherapeutic approaches.

reactivity to tau filaments. Immunostaining of AD and control brain tissues with this antibody revealed that tau dimers/oligomers are markedly elevated in AD, appearing in early pathological inclusions such as neuropil threads and pre-tangle neurons as well as colocalizing with other early markers of tau pathogenesis (70). In the second report, we engineered a novel tau oligomer-specific antibody, T22, and used it to elucidate the temporal course and biochemical features of oligomers during NFT development in AD brain. We found that tau oligomers in human AD brain samples were fourfold higher than in controls. We also demonstrated that oligomeric tau conformers play a role in pre-tangle, neuritic plaques and neuropil threads in frontal cortex tissue from AD brains;

our analysis uncovered a consistent code that governs tau oligomerization with regard to degree of neuronal cytopathology. Moreover, we showed that the formation of tau oligomers is not limited to AD, since we found elevated levels of tau oligomers in progressive supranuclear palsy brain tissues compared with age-matched controls (69). Finally, in this study we detected areas exclusively containing tau oligomers, others mainly containing hyperphosphorylated NFT, and a third subset that seems to represent the transition of oligomers to NFT (69). It is intriguing to consider these data in light of novel studies suggesting that aggregated tau can “transmit” a misfolded state from one cell to another and indicating that tau oligomers could play a key role in this process (71-73). In

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AD, NFT appear first in the hippocampus, the basal nucleus of Meynert, and the brainstem (13, 74-76), and subsequently spread to other brain regions, including the neocortex, in a stereotypical pattern. But at the cellular level, it is unclear how tau pathology spreads, and further work will be necessary to define this process. Nevertheless, our results provide strong evidence that tau oligomers progress in an orderly sequence in AD brain (69).

This large body of evidence supports the notion that soluble tau oligomers—rather than hyperphosphorylated insoluble NFT—are the acutely toxic structures of tau (Figure 1).

5. TAU OLIGOMERS IN TAU MOUSE MODELS

Many tau rodent models have been developed, using different human tau isoforms; some of the models assemble key events in tau aggregation and neuronal loss (77-80). The first tau transgenic mouse model was obtained by expressing the long human tau isoform under the regulation of the hThy1 promoter. The mice showed early changes that were associated with the hyperphosphorylated state of tau in neural cell bodies, axons, and dendrites, but NFT were absent. This model showed pre-tangle changes similar to those that precede the full neurofibrillary pathology in AD (81). When mutations in the MAPT gene were discovered and linked with neurodegeneration—P301L, P301S, G272V, and R406W, among others—a new generation of transgenic mice was generated. The alterations seen varied from model to model, and the presence of memory deficits, slow axonal transport, hyperphosphorylated tau, PHF, NFT, and/or neuronal loss in the brain and spinal cord (58, 82-90).

Innovative work using these well-characterized mouse models suggests that tau oligomers play a key role in neurodegeneration and in eliciting behavioral impairments. The phenotypes are concurrent with the accumulation of soluble aggregated tau species and are dissociated from the accumulation of NFT (56, 58, 91). Tangles and neuronal cell death were reported in aged mice expressing nonmutant human tau in the absence of mouse tau (htau mice). In these mice, neurons containing phosphorylated tau displayed morphological alterations, such as vacuolization and neurodegeneration. However, not all senescent neurons contained NFT (92, 93). This suggests that tau toxicity is not necessarily related to NFT, at least in this mouse model. Moreover, Polydoro *et al.* demonstrated that 12-month-old htau mice (which showed moderate stages of tau pathology), but not 4-month-old htau mice (with early-stage tau pathology), developed learning and memory deficits resembling deficits in human AD. These results provide evidence that a process upstream of NFT formation may underlie the synaptic dysfunction and, perhaps, the cognitive decline in this mouse model (94). In a recently reported study, rTg4510 transgenic mice, a conditional model in which a tetracycline-responsive element occurs upstream of human mutant tau P301L, developed progressive NFT accumulation, neuronal loss, and behavioral impairments, including loss of memory.

When mutant tau expression was suppressed, the memory function was recovered but NFT continued to accumulate. This suggests that NFT are not the toxic species (58). Moreover, in this model region-specific dissociation of neuronal loss and neurofibrillary pathology was demonstrated by quantitative analysis (91). Another report showed that inhibition of tau hyperphosphorylation in JNPL3 transgenic mice, in which human mutant tau P301L is overexpressed, led to a delay in motor dysfunction and neuronal loss. Surprisingly, the formation of NFT was persistent, suggesting that toxic tau intermediates accumulate before the formation of NFT (95). In P301S transgenic mice, in which the human tau mutated gene is overexpressed, hippocampal synaptic losses were detected at 3 months of age. Before the formation of tangles, the mice showed microglial activation and synaptic dysfunction. Thus, microgliosis may be the earliest manifestation of neurodegenerative tauopathies (96). None of these reports provided direct evidence for the formation of tau oligomers and their role in the phenotypes.

Nevertheless, a few reports describe the detection and characterization of tau oligomers in mouse models of tauopathy. In rTg4510 mice, oligomers/multimers were detected and biochemically characterized. Western blots using mouse brain extracts, clearly showed the multimers at molecular weights of 140 and 170 kDa. The multimers were SDS resistant even after boiling. When the multimers were exposed to hydrofluoric acid, the 170-kDa multimer disappeared, but not the 140-kDa multimer (56). In another model, a bigenic mouse overexpressing full-length TTBK1 and the P301L tau mutant, there was enhanced tau phosphorylation in neuronal cell bodies of the cortex and the hippocampus. The mice displayed a loss of neurons and concurrent astrogliosis in the spinal cord. In an extract of proteins from the spinal cords of 5- to 6-month-old mice, sarkosyl-soluble oligomers with molecular weights of about 170 and 140 kDa were found (97). Collectively, the findings from all these studies suggest that tau oligomer accumulation, neuronal loss, and behavioral deficits precede the formation of NFT, and support the potential of these mouse models for evaluating therapeutic approaches that target tau oligomers.

6. TAU IMMUNOTHERAPY

Given the preeminence of the amyloid hypothesis (8) in the AD field, an extensive body of work has targeted various forms of A aggregates for drug development. Approaches have included reduction and alteration of the processing of the amyloid precursor protein, prevention of A misfolding and aggregation, minimization or elimination of A neurotoxicity, and acceleration of A clearance and degradation (98-103). Arguably, the most promising treatment approach to AD is the recently developed approach of anti-A immunotherapy, which is in clinical trials. The approach can be broadly classified as active or passive vaccination strategies to remove amyloid deposits (104). In active immunotherapy an immunogen is administered to stimulate an immune response, whereas in passive immunotherapy an antibody or antibodies are administered to provide short-term protection against infection or a clinical condition.

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Table 1. Active immunomodulation of tau pathology

Mouse model	Immunogen	Phospho-site recognized	Effects on tau pathology	Reference
C57BL/6	Recombinant human tau	Non	Induction of neurological deficits, NFT, axonal damage and inflammation	(108)
JNPL3	Tau379-408	Ser 396-404	Reduction of NFT and early aggregates recognized by PHF1 and MC1, respectively Mice performed better on locomotor task	(109)
tau [K257T/P301S]	Tau 195-213 Tau207-220 Tau224-238	P 202-205 P 212-214 P231	40% reduction of NFT in several brain areas, including cortex, hippocampus, brain stem. Increase of microglial burden.	(110)
htau/PS1	Tau379-408	Ser 396, 404	Reduction of NFT (57% less PHF1 immunoreactivity) Mice performed better on memory task.	(112)

Table 2. Passive immunomodulation of tau pathology

Mouse model	Antibodies	Phospho-site recognized	Effects on tau pathology	Reference
JNPL3	TOMA	Non	Reduction of Tau oligomers in brain and spinal cord. Mice performed better on Rotarod test	(116)
JNPL3	PHF1	Ser 396, 404	58% reduction of NFT in the dentate gyrus of the hippocampus. Mice performed better on traverse beam test.	(113)
JNPL3 and P301S	PHF1 MC1	Ser 396, 404 Non	Reduction of NFT in cortex/forebrain in P301L Reduction of neurospheroids in spinal cord of P301S	(115)

Results published to date from an ongoing randomized, placebo-controlled phase II clinical trial in patients with Alzheimer disease of the anti-A humanized monoclonal antibody bapineuzumab support the reduction of amyloid plaque load as detected by positron-emission tomography using carbon-11-labeled Pittsburgh compound B (11C-PiB), a marker of cortical fibrillar A load *in vivo* (28). There was a statistically significant reduction (modified intention-to-treat analysis) in the mean retention of 11C-PiB in the patients who intravenously given bapineuzumab compared with the placebo group. However, there was no significant difference between the groups in measures of cognition. Despite other evidence of amyloid plaque reduction (105), postmortem analyses of brains from patients who participated in earlier anti-A clinical trials failed to demonstrate changes in tau pathology, neuropil threads, synaptic dysfunction, or cerebral amyloid angiopathy (25, 106, 107). These postmortem results suggest that the clearance of tau pathology may be critical for memory improvement. In contrast to the extensive laboratory and animal investigations and the clinical testing of anti-A immunotherapy, immunotherapeutic approaches that target tau are still in very early stages. Next, we discuss the few published reports of anti-tau immunotherapy.

6. 1. Tau active vaccination

Immunomodulation to clear tau pathology is an exciting approach for the treatment of AD and other tauopathies. To date, there have been four published studies on anti-tau active immunotherapy (Table 1). In the first report, Rosenmann *et al.* immunized C57BL/6 mice with recombinant human tau protein. Unfortunately, these animals developed NFT-like structures, axonal damage, gliosis, mononuclear infiltrates, and motor phenotypes (108), suggesting that active immunotherapy may present some risks. However, recent data from active immunotherapy in tau transgenic mouse models using phosphorylated short tau fragments have demonstrated positive effects.

In 2007, Asuni *et al.* showed that using active immunization with phospho-tau epitopes clears tau aggregates in a mouse model of tauopathy. The investigators used the P301L mouse model (89), which overexpresses mutant tau and

develops pathology in motor cortex, brainstem, and spinal cord. In this study, mice were immunized for two to five months with a phospho-epitope containing the residues 379–408 of tau protein, while the controls received the adjuvant alone. The immunogen peptide includes the phosphorylation sites at Ser396 and Ser404, which are commonly associated with NFT. Locomotor activity tests showed improvement in treated mice at early stages of tau pathology (5 months of age) and late-stage pathology (8-month-old P301L mice). This study demonstrated that antibodies against this immunogen were able to cross the blood-brain barrier (BBB) and bind to phosphorylated tau (109).

Boimel *et al.* used a transgenic mouse model that they called the NFT-pathology model and the enhanced-NFT-pathology model, which overexpresses a double-mutated tau (K257T/P301S). This mouse model develops NFT at 6 months of age. Mice were immunized twice using peptides containing different phospho-epitopes, such as Tau195-213 (P-202/205), Tau207-220 (P-212/214), and Tau224-238 (P-231). This approach successfully reduced tau aggregates in multiple brain regions, including the cortex, hippocampus, and brain stem; they showed a 40% reduction in NFT load (110).

Subsequently, Boutajangout *et al.* showed that tau immunotherapy prevents cognitive decline in an AD mouse model (htau/PS1). They developed this mouse model by crossing htau mice (93) with a model carrying the human presenilin-1 (PS1) M146L mutation (111). The htau/PS1 mice, which show early onset of tau pathology, received multiple intraperitoneal injections starting at three and four months of age. The immunogen used in this active vaccination study was Tau379-408 (P-Ser 396,404). As result of the immunotherapy, the mice showed reduced levels of the tau aggregates reactive to PHF1 antibody compared to controls. When a learning and memory test was given, the htau/PS1 mice showed improvements after treatment (112).

6.2. Tau passive immunization

Two reports describing anti-tau passive immunotherapy were published in 2011 (Table 2). Boutajangout *et al.* used two- and three-month-old

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homozygous JNPL3 mice (89). Animals received 13 weekly intraperitoneal injections with PHF1 antibody. PHF1 is an antibody that recognizes the phospho-epitopes at Ser (396 and 404). This is the same phospho-epitope used in their previous work using active immunotherapy. They showed that treated mice had less PHF1 pathology in the hippocampus compared to a control group. In addition, immunized mice performed better in the traverse beam test (113). These results suggest that passive immunization with anti-tau antibody can decrease the pathological tau structures recognized by PHF1 antibody. It is also known that PHF1 antibody recognizes mainly extraneuronal NFT, which represent the late stage of tau pathology according to the well-established staging of NFT formation (114).

The second report on passive immunotherapy is the work of Chai *et al.* Here they used PHF1 and MC1 antibodies that recognize neurofibrillary inclusions. As mentioned above, PHF1 recognizes the phosphorylation at serine (396 and 404) and MC1 is a conformation-specific antibody that recognizes the amino acids 312–342 of tau protein. Both antibodies were used to treat two well-established tau transgenic models, the JNPL3 (89) and P301S (96) models. The 2-month-old JNPL3 mice were treated for four months with PHF1 and MC1 (15 mg/kg three times per week for two months, then 10 mg/kg twice per week). The 2-month-old P301S mice were treated for three months with twice-weekly doses (15 mg/kg) of both PHF1 and MC1. Using immunohistochemistry and ELISA they reported that there was a reduction of tangles in cortex/forebrain in the JNPL3 model, but with variable results in the P301S model. In the spinal cord of P301S there was a reduction of neurospheroids in the treated group compared to controls, which received an equal amount of a generic IgG. Moreover, the P301S-treated mice performed better on the rotarod locomotor test compared to controls (115).

Interestingly, the work done so far concerning immunotherapy against tau aggregates has had favorable results on the removal of NFT; unfortunately, the effects of these approaches on tau oligomers and other pre-filament tau species were not evaluated. Therefore, it is difficult at this stage to interpret the results and determine if these strategies are targeting the toxic tau species. One possible explanation is that the immunization eliminated both NFT and tau oligomers, so that the results are favorable.

Although active vaccination against tau has demonstrated its efficacy in animal models, it is always risky, especially when the target is an endogenous protein such as tau, so that autoimmunity remains the main concern. On the other hand, the advantage of passive immunization is that treatment can be discontinued at any time without secondary effects. Moreover, it has been estimated that the half-life of an IgG is short, whereas the effects of active immunotherapy are irreversible.

The development of tau oligomer-specific antibodies (69,70) is an exciting development, since these antibodies can sequester tau oligomers without affecting functional monomeric tau. This presents many exciting

opportunities to test anti-tau oligomer immunotherapeutic approach in the available animal models. Recently, we engineered an anti-tau oligomer-specific antibody (TOMA) that does not recognize monomeric functional tau or mature NFT and has a high affinity for tau oligomers. Passive immunization of the 8-month-JNPL3 mouse model with a single intracerebroventricular (ICV) injection of 1 µg per animal reversed the phenotypes in these animals. Biochemical and immunohistochemical analyses demonstrate a reduction of tau oligomers in the immunized animals compared to the controls, which received a non-specific IgG (116).

7. MECHANISMS FOR TAU CLEARANCE BY IMMUNOTHERAPY

To date, the exact mechanism of tau aggregates clearance by immunotherapy is not fully understood. Antibodies can enter the brain through a deficient BBB (117, 118). It is also well known that antibodies can be actively transported via adsorptive mediated transcytosis (119). Additionally, antibodies can get into the brain through antibody-secreting-cells that may secrete the antibodies locally (120). Also, the evidence suggests that antibodies can enter the central nervous system by means of endocytosis (121, 122). Once antibodies reach the brain, they can bind to extracellular aggregates and trigger microglia-related clearance. On the other hand, as disease progresses the NFT increase in number, filling the cell and eventually appear like an extracellular deposit termed “ghost tangles.” Tau is a cytoplasmic protein; however, tau protein is detected as well in cerebrospinal fluid (123). New data suggest that the N-terminal of tau is involved in tau secretion to the extracellular space and adjacent neurons *in situ*, at relatively low levels of overexpression (124). Recently, tau has been identified in brain interstitial fluid (ISF) of wild-type mice, suggesting that tau is released in the absence of neurodegeneration. Interestingly, ISF tau levels in the P301S transgenic mouse were fivefold higher than endogenous murine tau, but decrease with age, suggesting that extracellular monomeric tau is in equilibrium with extracellular or intracellular tau aggregates (125). Importantly, extracellular tau aggregates appear to be taken up into cells, induce intracellular tau misfolding, and thus cause the spread of tau pathology throughout the brain (71, 126). Thus, the clearance of tau aggregates can prevent the spread of tau pathology. It is possible that the clearance of extracellular aggregates (oligomers) by antibodies may facilitate further secretion and thereby indirectly clear intracellular tau aggregates.

Recently, a mechanistic study of antibody-mediated clearance of tau aggregates using an *ex vivo* brain slice model demonstrated that the anti-tau antibodies were internalized and the clearance of tau oligomers occurred via the endosomal/lysosomal pathway (127). Additional possible mechanisms for the clearance of tau aggregates may involve microglia-mediated clearance, autophagy-mediated clearance, and the peripheral sink mechanism; all these mechanisms have been implicated in the clearance of A deposits (104, 128, 129).

8. CONCLUSIONS

AD and related tauopathies are multifactorial diseases in which the brain degeneration and clinical manifestations arise from several different and perhaps related molecular events, in which tau plays a critical role. As such, it has been widely suggested that an efficient therapy would have to combine targeting the problem at different stages with distinct strategies. Therefore, studies designed to evaluate the benefits of immunotherapeutic approaches targeting tau aggregates are critical and should not be viewed as alternative approaches to A immunotherapy, but rather as complementary strategies, these strategies can be tested and developed in a relatively short period if we take advantage of the wealth of information generated by the A immunotherapy studies. So far, the results from tau immunotherapy in different animal models are encouraging. Nevertheless, many questions need to be answered before we move forward in the development of a safe vaccine or an effective passive immunization strategy. To date, the real concerns remain: Are we hitting the right target and removing the real toxic tau aggregates without affecting functional tau? What are the potential side effects? What are the mechanisms involved in the clearance of tau aggregates? Can the removal of tau aggregates reverse the disease manifestations and in the long term prevent the spread of tau pathology? Innovative work is needed to answer these critical questions and to advance immunotherapeutic applications targeting toxic tau aggregates.

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Abbreviations: AD: Alzheimer disease; NFT: neurofibrillary tangles; FTDP-17: frontotemporal dementia with parkinsonism linked to chromosome 17; A_β: amyloid-β; MAPT: microtubule-associated protein tau; 3R: three binding domain repeats; 4R: four binding domain repeats; PHF: paired helical filaments; TFGs: tau-positive fine granules; kDa: kilodaltons; ICV: intracerebroventricular injection; p-NFT: hyperphosphorylated NFT

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