Reduction of Soluble Aβ and Tau, but Not Soluble Aβ Alone, Ameliorates Cognitive Decline in Transgenic Mice with Plaques and Tangles*

Salvatore Oddo, Vitaly Vasilevko, Antonella Caccamo, Masashi Kitazawa, David H. Cribbs, and Frank M. LaFerla

From the Departments of Neurobiology and Behavior and Neurology, and Institute for Brain Aging and Dementia, University of California, Irvine, California 92697

Increasing evidence points to soluble assemblies of aggregating proteins as a major mediator of neuronal and synaptic dysfunction. In Alzheimer disease (AD), soluble amyloid-β (Aβ) appears to be a key factor in inducing synaptic and cognitive abnormalities. Here we report the novel finding that soluble tau also plays a role in the cognitive decline in the presence of concomitant Aβ pathology. We describe improved cognitive function following a reduction in both soluble Aβ and tau levels after active or passive immunization in advanced aged 3xTg-AD mice that contain both amyloid plaques and neurofibrillary tangles (NFTs). Notably, reducing soluble Aβ alone did not improve the cognitive phenotype in mice with plaques and NFTs. Our results show that Aβ immunotherapy reduces soluble tau and ameliorates behavioral deficit in old transgenic mice.

Alzheimer disease (AD) is clinically marked by a progressive deterioration of memory and other cognitive functions. Two obligate neuropathological lesions occur in the AD brain: amyloid plaques, mainly formed by a small peptide called amyloid-β (Aβ), and neurofibrillary tangles (NFTs) formed by the hyperphosphorylated microtubule-binding protein tau (1). The accumulation of Aβ plays a central role in the progression of the disease, and over the past several years, there has been a growing appreciation for the pathogenic effects of soluble assemblies of Aβ, which may be the predominant toxic species for neurons (2–4). The causes underlying AD-related memory loss and other cognitive changes are likely to be multifactorial, and although evidence suggests that soluble Aβ is an excellent candidate to be the initial trigger (4), other elements of AD neuropathology almost certainly contribute to the progressive deterioration in the cognitive faculties. In this regard, it is likely that the manifestation of tau pathology plays a pivotal role that further exacerbates the cognitive decline in the presence of Aβ and other AD-related alterations. Pathological assemblies of tau can induce neurodegeneration and dementia in the absence of Aβ, as occurs in disorders such as frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP17) (5). Elucidating the relationship between these two proteins (Aβ and tau) and their synergistic effects on cognition is facilitated by the utilization of a transgenic model that develops both Aβ and tau pathology.

The 3xTg-AD mice develop an age-dependent decline in the cognitive phenotype in both spatial and contextual learning and memory paradigms. The occurrence of intraneuronal Aβ appears to be a trigger for the onset of deficits in water maze spatial memory, an effect that is fully reversible by Aβ immunotherapy (6). However, as the mice age and extracellular Aβ and neurofibrillary pathology manifest, the mice show further cognitive decline, although the role that plaques and tangles play on the deterioration of the cognitive phenotype is still unresolved. Postmortem evaluation of AD brains has provided some correlational evidence linking these structures to the cognitive decline, with the general finding that tangles are a better correlate than plaques (7, 8). However, these types of studies have two inherent limitations. 1) They ignore the dynamic nature of clearance mechanisms; thus, it is possible that the guilty culprit (e.g. plaque or NFT) that induces cognitive decline is no longer present in the brain at the time of autopsy. 2) It is now recognized that soluble Aβ assemblies may directly cause cognitive dysfunction. Moreover, it remains to be established if and how soluble tau accumulation participates in the cognitive decline in the presence of Aβ pathology, which represents the focus of this study. Although increasing evidence suggests that soluble Aβ may be a key factor in inducing cognitive decline, to our knowledge, no firm experimental evidence has been presented to show a comparable role for soluble tau in an experimental mammalian system, particularly in the presence of Aβ pathology. A prior study involving regulatable tau transgenic mice found that NFTs are not sufficient to induce memory loss and neuronal loss, but no mechanism was defined or proposed (9).

Aβ immunotherapy, both active and passive, has been shown to be a valuable tool to decrease Aβ pathology and rescue cog-
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Introduction—While the human clinical trial involving an active immunization approach was suspended because 6% of the patients developed meningoencephalitis (15) this strategy still holds great potential because it is a disease-modifying intervention, in contrast with currently available treatments (16, 17). A critical issue that remains to be resolved is whether therapeutic interventions aimed at decreasing Aβ will suffice to improve cognition in the presence of established plaques and NFTs. Here we actively or passively immunized aged 3xTg-AD mice to determine whether it was possible to ameliorate their cognitive impairments in the presence of established plaques and NFTs. We report that reduction of both soluble Aβ and tau levels were required to rescue the cognitive impairments. Notably, decreasing soluble Aβ without affecting soluble tau levels did not improve cognition. Therefore, we conclude that Aβ immunotherapy represents an effective strategy for ameliorating cognitive decline even in aged brains marked by resistant amyloid plaques and NFTs. Moreover, these data suggest that soluble tau plays an important role in the cognitive impairments in aged 3xTg-AD mice and by extrapolation independent therapies aimed at further reducing its levels or restoring tau function may lead to even greater cognitive improvements in human patients.

Materials and Methods

Immunization—For active immunization, Aβ_{42} peptide was synthesized at the University of California Core Facility, and fibrillar Aβ_{42} (fAβ_{42}) was prepared as previously described (18) and delivered subcutaneously (s.c.). Blood was collected before the first immunization and 10 days after each boost from the retro-orbital sinus into EDTA-coated tubes. Tubes were centrifuged for 10 min at 4 °C, and the sera were collected as a supernatant and stored at −80 °C. For passive immunization, the mouse anti-Aβ monoclonal 20.1 antibody was prepared in low endotoxin format from a hybridoma kindly provided by Dr. William Van Nostrand (Stony Brook University, Stony Brook, NY). The 20.1 antibody is an IgG2b isotype and recognizes the N-terminal region of Aβ spanning amino acids 1–8. Mice were given weekly intraperitoneal injections of 300 μg of 20.1 antibody diluted in 300 μl of phosphate-buffered saline. Sera from the mice were collected before and at 24 h and 7 days after administration of the antibody, as well as at the end of experiment. The anti-Aβ antibody concentration as well as Aβ_{40} and Aβ_{42} peptide concentrations were measured.

Behavior—The passive inhibitory avoidance was conducted as previously described (6). The T-maze consisted of a central main arm with two side arms positioned perpendicular to the main arm. The central arm was 65-cm long, and the two side arms were 30-cm each. The maze width was 13.5 cm. The walls of the maze were made of transparent acrylic and were 20-cm tall. At the beginning of each test, the mice were placed in the main stem while one side arm was blocked by a barrier so that the mice were forced to make a choice. Once the mice entered the side arm, the entrance was blocked, thus retaining them in the side arm. Mice were left to explore that arm for 120 s at the end of which they were placed back in the main arm of the maze with both side arms open. Mice were free to choose the arm that they already explored or the new arm. Each animal was tested daily for 7 days and on each day we alternatively blocked one side arm. The numbers of alternations and the latency to make a choice during the free trial were recorded.

Immunological and Histological Staining—After completion of the behavioral tasks, the mice were trans-cardially perfused with ice-cold PBS. Following perfusion, each brain was sagittally and one-half of the brain was frozen in dry ice whereas the other half was fixed in ice-cold paraformaldehyde for 48 h. After fixation, brains were cut (50-μm thick) using a slicing vibratome (Pelco, Redding, CA), and sections were stored in 0.02% sodium azide in PBS. Immunohistochemical analysis was conducted as previously described (19). For thioflavine staining, sections were incubated in a 0.5% solution of ThioflavineS (Sigma Aldrich) in 50% ethanol for 10 min. Sections were then washed twice for 3 min each in 50% ethanol and twice for 3 min each in water. Quantification of the stained sections was done as previously described (20).

Western Blot and ELISA—Brains were homogenized in Tissue Protein Extraction reagent (Pierce) supplemented with a complete mini protease inhibitor tablet (Roche Applied Science) and phosphatase inhibitors (Calbiochem, San Diego, CA). The homogenized mixes were briefly sonicated to shear the DNA and centrifuged at 4 °C for 1 h at 100,000 × g. The supernatant was stored as the soluble fraction. The pellet was re-homogenized in 70% formic acid and centrifuged at 4 °C for 1 h at 100,000 × g. The supernatant was stored as the insoluble fraction. The extraction procedure was confirmed by Western blot, using APP as a marker of soluble proteins and flotillin as a marker of insoluble proteins (data not shown). Protein concentration was determined using the Bio-Rad protein assay, and samples were adjusted with T-PER to the same concentration. Western blot and Aβ ELISA experiments were done as previously described (19, 21). ELISA measurements of tau were conducted using a total tau ELISA kit from BIOSOURCE (Camarillo, CA) in accordance with the manufacturer’s instructions. The titers of anti-Aβ antibodies were measured as previously described (18).

Quantitative Cytokine Assay—Levels of different cytokines were quantitatively measured by Bio-Plex 200 (Bio-Rad) system using the manufacturer’s instructions.

Antibodies—The following antibodies were used in this study: anti-Aβ 6E10 (Signet Laboratories, Dedham, MA), anti-Aβ 1560 (Chemicon, Temecula, CA), anti-Aβ40 and anti-Aβ42 (BIOSOURCE, Camarillo, CA), anti-Aβ 35–40 (MM32-13.1.1, for Aβ_{40}) or anti-Aβ 35–42 (MM40-21.3.4, for Aβ_{42}), anti-β-actin (Sigma), anti-tau HT7, (Innogenetics, Belgium), AT8 and AT100 (Pierce), 8C11 and 16B5 were a generous gift from Dr. Peter Seubert. HT7, 8C11, and 16B5 recognize tau independent of its phosphorylation state.

Statistical Analysis—Data were analyzed using one-way analysis of variance (ANOVA) with Bonferroni post-test using GraphPad Prism version 3.00 for Windows, GraphPad Software, San Diego, CA.

Results

We first sought to determine whether aged mice harboring an extensive plaque and tangle burden would show improved...
learning and memory following active or passive Aβ immunization. For the active immunization study, thirty 18-month-old 3xTg-AD mice were randomly assigned to one of three groups: 1) untreated, 2) adjuvant only, and 3) fibrillar Aβ42. For the passive immunization, ten 20-month-old 3xTg-AD mice received 300 μg per injection of the anti-Aβ antibody 20.1, which is raised against the first 8 amino acids of the Aβ sequence. The active immunization trial was conducted over a period of 5 months, whereas the passive immunization trial was conducted over a period of 3 months (Fig. 1A). Consequently, all the mice were 23 months old at the conclusion of the experiment. Mice, actively immunized with fAβ42 had significant amounts of circulating anti-Aβ antibody (82.95 ± 37.09 μg/ml) after four injections and maintained uniform elevated levels of anti-Aβ antibodies. The antibody concentration 10 days after the last injection was 101.28 ± 63.61 μg/ml. In contrast, adjuvant-treated or unimmunized mice did not have detectable levels of anti-Aβ antibody during the experiment (data not shown). Average serum titers from the passively immunized mice were 1:53,000 and 1:5,400 at 24-h and 7-days postinjection, respectively.

Aβ Vaccination Ameliorates the Behavioral Phenotype despite Persistent Plaques and NFTs—At the end of the treatment period, we evaluated the behavioral phenotype of the 3xTg-AD mice using two different behavioral paradigms, the T-maze and the passive inhibitory avoidance. The former relies on the tendency of mice to alternate free choices in a T-maze during successive trials, and it is mainly based on working memory and is dependent on several brain regions including the basal forebrain, hippocampus, and prefrontal cortex (22). The passive inhibitory avoidance is a contextual learning and memory task, which is mainly dependent on the amygdala (23).

Aged 3xTg-AD mice that were either untreated or treated with adjuvant only performed at chance levels on the T-maze, i.e. they failed to alternate between the two arms of the maze on successive trials (47.6 ± 2.4% and 46.4 ± 5.2%, respectively, Fig. 1B). In contrast, we found that the percentage of alternations was significantly increased in both actively and passively immunized 3xTg-AD mice (63.5 ± 6.8% and 64.3 ± 2.7%, respectively, Fig. 1B) and was similar to that of age-matched non transgenic (NonTg) mice (71.43 ± 3.37% Fig. 1B). As the ability to successfully alternate with each successive trial is considered to reflect intact working memory (22), these results show that working memory can be restored in aged transgenic mice following active or passive immunization.

We next evaluated the mice on a contextual learning and memory task using passive inhibitory avoidance. During the initial training and the subsequent 3-h probe trial, all the groups...
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FIGURE 2. Reduction of soluble Aβ levels by active and passive immunization. A–F, photomicrographs of hippocampal sections from the brains of adjuvant-treated mice and actively or passively immunized mice stained for Aβ by 6E10 and thioflavine S. A and B, soluble and insoluble plaques are evident in the hippocampus of adjuvant-treated mice. C and D, plaque load was not significantly changed in the brains of actively immunized mice. E and F, in contrast, the number of plaques was reduced in the brain of passively immunized mice; nevertheless, numerous 6E10- and thioflavine S-positive structures are still apparent. G, imaging analysis of the thioflavine-stained sections revealed that the number of plaques was reduced only in the brains of passively immunized mice. H, consistent with the Aβ staining, detergent-insoluble Aβ40 and Aβ42 levels were unaltered in the brains of actively immunized mice compared with untreated- and adjuvant-treated mice. Chronic passive immunization, however, caused a significant decrease in Aβ40 and Aβ42 levels (p < 0.05). Soluble Aβ40 levels were significantly reduced only in the brains of passively immunized mice (p < 0.01), and no statistically significant differences were detected among the other groups. In contrast, soluble Aβ42 levels were significantly reduced following both active and passive immunization (p < 0.05 and p < 0.01, respectively). Soluble Aβ42 levels were also lower in the brains of passively versus actively immunized mice (p < 0.05). Abbreviations: Unt, untreated; Adj, adjuvant; Act, active; PasC, chronic passive. * indicates a significant decrease from the untreated, adjuvant, and actively immunized groups. # indicates a significant decrease from the untreated and adjuvant groups. Scale bar: 125 μm for A, C, and E; 85 μm for B, D, and F.

performed similarly (p < 0.05, Fig. 1C), indicating that the task was learned and retained during the first 3 h. For the 24-h probe, both control groups showed a reduced latency to cross over to the dark compartment, although the results did not achieve statistical significance compared with immunized mice (Fig. 1C). In contrast, analysis at the 36-h probe trial indicated that both the active and passive immunized groups performed similarly to NonTg mice (162.9 ± 17.1, 150.2 ± 19.8, and 173.9 ± 2.8 s), and significantly better than the unimmunized control groups (79.28 ± 17.5 and 93.1 ± 18 s, for untreated and adjuvant treated mice, respectively; Fig. 1C).

These results clearly indicate that 23-month-old 3xTg-AD mice can learn and briefly retain this information for the short term, but cannot form long term memories (i.e. 36 h), whereas immunized mice are able to retain the information throughout the duration of the experiment.

Reduction of Soluble Aβ Levels by Active and Passive Immunization—We next determined the effect of active and passive immunization on plaque burden in the aged mice. The number of thioflavine-positive plaques did not appear to be statistically reduced in aged 3xTg-AD mice immunized with fAβ42, as the overall burden was similar to the control groups (Fig. 2, A–D). In contrast, the number of thioflavine-positive plaques was significantly reduced in aged 3xTg-AD mice passively immunized (Fig. 2, E and F). The number of plaques per microscopic field was 29 ± 2.8 S.E., 29 ± 2.2 S.E., and 15 ± 2.1 S.E., for the adjuvant, active, and passive immunized mice, respectively (Fig. 2G).

Although the number of thioflavine-positive plaques was not significantly reduced in actively immunized mice, we still found that these mice performed significantly better than control mice in both the T-maze and the passive inhibitory avoidance tasks. Consequently, we analyzed brain extracts from these mice by sandwich ELISA to determine if total Aβ levels were reduced. Among all the groups analyzed, insoluble Aβ40 and Aβ42 were only significantly reduced in passively immunized mice (Fig. 2H), consistent with the immunohistochemical results. Notably, soluble Aβ42 levels were significantly lower in both the actively and passively immunized mice (Fig. 2H), whereas soluble Aβ40 levels were significantly decreased following passive immunization but not with active immunization (Fig. 2H). As the reduction of soluble Aβ42 levels was the only
species of Aβ consistently changed among all the immunized mice, it indicates that lowering this Aβ species even in mice with extensive plaque and tangle neuropathology can lead to improvements in cognition. These results are consistent with recent data showing that soluble Aβ better correlates with memory impairments in AD patients and transgenic mice (6, 24–29).

Reduction of Soluble but Not Insoluble Tau by Active and Passive Immunization—Because we immunized aged mice with very extensive plaque and tangle pathology, we next determined whether active or passive immunization had any beneficial downstream effects on the tau pathology. Although our previous studies showed that a single intrahippocampal injection of Aβ antibodies was able to reduce early, but not late hyperphosphorylated/aggregated tau in 1-year-old mice (19, 30), it remains to be established whether active or chronic passive immunization would be efficacious in aged mice with very advanced neuropathology. To address this issue, we immunostained sections with antibodies that recognize specific phospho-tau epitopes (AT100, AT8, PHF-1) or histologically evaluated sections following staining with Gallyas silver impregnation.

As expected, neither active nor passive immunization had any discernable effect on the number of Gallyas-positive neurons or on the number of PHF-1- and AT8-positive neurons (Fig. 3, A–L). In contrast, we found that the number of AT100-positive neurons was reduced following immunization compared with the control groups (Fig. 3, M–P). This finding was notable as AT100 is an early marker of tau pathology in the 3xTg-AD mice, whereas AT8- and PHF-1-positive neurons represent mid- and late-stage markers of tau pathology, respectively. M–P, active and passive immunization lead to a significant reduction ($p < 0.001$) of the number of AT100-positive neurons, which represents a marker of early stage tau pathology.

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FIGURE 3. Soluble tau levels were reduced by both active and passive immunization. A–C, histological visualization of NFTs in the hippocampus of immunized and control 3xTg-AD mice. D, quantification analysis shows that the number of Gallyas-positive neurons was unchanged following active or passive immunization compared with adjuvant-treated or untreated groups. E–K, serial sections to those presented in A–C, show that the number of PHF-1- and AT8-positive neurons was similar between immunized and control groups. Quantification analysis for PHF-1 and AT8 is presented in H and L, respectively. In the 3xTg-AD mice AT8- and PHF-1-positive neurons represent mid- and late-stage markers of tau pathology, respectively. M–P, active and passive immunization lead to a significant reduction ($p < 0.001$) of the number of AT100-positive neurons, which represents a marker of early stage tau pathology.
found that insoluble tau levels were unaltered among all the groups (Fig. 4E). Notably, soluble tau levels were significantly reduced in the actively and passively immunized mice compared with untreated or adjuvant-treated control mice (Fig. 4E). These results are consistent with our previous results showing that a single injection of an anti-\(A\beta\)/H9252 antibody is sufficient to clear not only \(A\beta\) but also early tau pathology (19, 30). The mechanism underlying the \(A\beta\) antibody-mediated reduction in tau pathology appears to be dependent on the proteasome, as inhibiting its activity prevented the \(A\beta\)-mediated clearance of tau (19).

Reducing Soluble \(A\beta\) without Reducing Soluble Tau Does Not Improve Cognition—Taken together, the data presented here demonstrate that it is possible to ameliorate the cognitive impairments in the 3xTg-AD mice by reducing soluble \(A\beta\)42 and tau levels, even in the presence of thioflavine-positive plaques and Gallyas-positive NFTs. It remains to be established, however, if the reduction of both soluble \(A\beta\)42 and tau levels is necessary to rescue the cognitive impairments or if the reduction of only soluble \(A\beta\)42 will suffice. We previously showed that the clearance of \(A\beta\) and tau pathology is hierarchical, with \(A\beta\) pathology cleared first and tau pathology reduced following the clearance of \(A\beta\) pathology (19). Moreover, recent data indicate that a single intraperitoneal injection of an anti-\(A\beta\) antibody was sufficient to rescue the behavioral deficit in a 4-day-long task. We found that acutely immunized mice failed to alternate in the T-maze and performed similarly to unimmunized age-matched 3xTg-AD mice (47.61 \(\pm\) 2.382\% and 51.40 \(\pm\) 3.733, respectively; Fig. 5B). Likewise, testing this group of mice on the passive inhibitory avoidance revealed that the latency to cross into the dark compartment was not statistically different compared with control mice for all three probe trials (Fig. 5C). Although we cannot exclude the possibility that a different acute immunization paradigm (e.g., allowing more time between treatment and testing) could ameliorate the cognitive problems, our data indicate that under the condition used, acute immunization with the \(A\beta\) antibody 20.1 is not sufficient to rescue the behavioral deficits in aged-3xTg-AD mice, harboring both thioflavine-positive plaques and Gallyas-positive NFTs.

To determine the consequences of the acute passive immunization on AD neuropathology, we stained sections from the brains of these mice for \(A\beta\) and tau. We found that the number of thioflavine-positive plaques was similar between the immunized and age-matched control mice (Fig. 6A). Consistent with these findings, insoluble \(A\beta\) levels, as measured by sandwich...
ELISA, were unchanged in the acutely immunized mice compared with control mice (Fig. 6B), whereas the levels of Aβ40 and Aβ42 were significantly lower in the immunized mice compared with age-matched control mice (Fig. 6B). Acute passive immunization had no effect on NFTs (Fig. 6C). Moreover, ELISA measurements and Western blot analysis showed that acute immunization with Aβ/H9252 antibody 20.1 had no effect on soluble and insoluble total tau levels (Fig. 6D); the phosphorylation profile of tau also appeared unaffected as determined with antibodies AT100, AT8, and PFH-1, which recognize early, mid-, and late-stage tau pathology in the 3xTg-AD mice, respectively (Fig. 6E). Notably, the acute Aβ immunization in these aged mice led to a 40% reduction in soluble Aβ42, which is comparable to the results obtained after chronic active immunization, which showed that soluble Aβ42 levels were reduced in the immunized mice 45 and 47% compared with the untreated and adjuvant group, respectively (Fig. 2H).

To determine whether altered levels of inflammatory cytokines underlie the behavioral changes following active and passive immunization, we quantitatively measured AD-related cytokine levels in the brain using Bio-Plex. We found that IL-1α, IL-1β, and MCP-1 levels, which have been shown to be significantly increased in AD brains as well as brains from animal models of AD, were not altered by immunization (Fig. 7, A, B, and D). Interestingly, passive immunization resulted in a reduction of IL-6 and TNFα (Fig. 7, C and E). Other cytokines (IL-4, IL-10, and IFNα) were undetectable in the Bio-Plex (data not shown). These results show that selective changes in cytokine levels likely do not account for the differential effect of acute and chronic immunization in cognition on the 3xTg-AD mice.

We next determined if Aβ oligomers were differentially affected by these three immunization paradigms. We found that a 56-kDa band, immunopositive with antibodies 6E10, 4G8, and 20.1, was selectively decreased in the immunized mice versus the unimmunized and the adjuvant-treated mice (Fig. 8, A and B). This band, which corresponds to the Aβ56 recently described by Ashe et al. (3), was not apparent when the samples were pretreated with 10% HFIP (Fig. 8A), consistent with the disruption of Aβ oligomers. Notably, this 56-kDa band was decreased equivalently in all the immunized mice, including the mice that were acutely passively immunized (Fig. 8, A and B); however cognitive impairments were ameliorated only when we observed a concomitant reduction in soluble tau levels.

**DISCUSSION**

Mounting evidence suggests that non-fibrillar assemblies of Aβ play a critical role in the pathogenesis of AD. Studies from transgenic AD models, organotypic, and cell culture models have provided convincing support that soluble Aβ can have detrimental consequences for many key neuronal activities. For example, using whole cell patch-clamp recordings, Walsh et al.
(36) showed that non-fibrillar Aβ assemblies, but not monomeric Aβ, block hippocampal LTP. More recently, we have shown that the onset of cognitive deficits in the 3xTg-AD mice is caused by the buildup of non-fibrillar intraneuronal Aβ, and its clearance via immunotherapy leads to cognitive improvement (6). Other groups have also shown that cognitive deficits in different transgenic models manifest before any overt plaque pathology (3, 37). Toward this end, it is important to note that even though Aβ seems to be the initial trigger of cognitive impairments and may facilitate the development of the tau pathology (at least in transgenic mice), once the tau pathology becomes established, it may further exacerbate the cognitive decline. The sum of these types of studies indicates that non-fibrillar forms of Aβ may be potent mediators of neurotoxicity in the aged brain. An unresolved question, however, relates to whether the same principle is applicable to tau, the other hallmark neuropathological protein of AD. In other words, does soluble tau play a role in the cognitive decline in AD, particularly in the presence of concomitant Aβ pathology? If so, reducing its levels within the brain should produce beneficial effects.

To address this question, we evaluated the cognitive phenotype following Aβ immunotherapy in advanced age mice. To our knowledge, this is the first report to investigate the effect of passive and active immunization on cognition in transgenic mice harboring both plaques and NFTs. Because of their advanced age, active immunization did not lead to a significant reduction in the plaque burden, consistent with previous reports (38) and likewise had no effect on the NFT load. In contrast, passive immunization significantly reduced the plaque burden but had no effect on NFTs, consistent with our previous study (19). Although neither immunization strategy reduced the NFT load, both approaches reduced soluble Aβ and tau levels, despite their advanced age, and this led to improved cognitive performance. Notably, we found a greater decrease in the steady-state levels of Aβ42 (as detected by ELISA) in the passively immunized mice compared with actively immunized mice, despite equivalent behavioral performance. These data indicate that total Aβ42 levels (monomeric and oligomeric) do not correlate with behavioral performance, which is consistent with previous results indicating that oligomeric Aβ correlates better with cognitive decline (3). Toward this end, we found that a 56-kDa band, immunopositive with antibodies 6E10, 4G8, and 20.1, was equally decreased in all immunized groups and correlates with the amelioration in cognition observed following active and passive immunization.

The data presented here strongly suggest that soluble tau plays an important role in cognition during advanced stages of the disease. Notably, reducing soluble Aβ only without reducing soluble tau did not improve cognitive performance. Although we cannot exclude that a selective decrease in soluble Aβ in a specific subregion of the brain may be necessary to rescue the behavioral deficit in the task used in this study, it is tempting to speculate that as certain soluble Aβ species appear to be more toxic than Aβ plaques (4), certain soluble

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**FIGURE 6.** Acute passive immunization lowers soluble Aβ but not soluble tau levels. **A,** representative histochemical staining showing that the plaque burden was unchanged between immunized and unimmunized mice. **B,** sandwich ELISA measurements, however, revealed that only soluble Aβ40 and Aβ42 levels were significantly reduced following acute passive immunization (p < 0.05). **C,** immunostaining with PHF-1 revealed that the number of NFTs were unchanged following acute passive immunization. **D,** similarly, ELISA measurements revealed that total tau levels in the soluble and insoluble fraction were unaltered between immunized and unimmunized mice. **E,** quantitative analysis of a series of Western blots indicated that the levels of phosphorylated tau (as measured by antibodies AT100, AT8, and PHF-1) were similar between immunized and control 3xTg-AD mice.
pathological forms of tau may be more detrimental than NFTs. However, the use of other interventions directly aimed at decreasing the NFT burden will be needed to determine their contribution, if any, to the cognitive decline during the late stages of AD. In this regard, the data presented here are not intended to exclude the possibility that further...
cognitive improvements will arise following the clearance or reduction of NFTs.

Remarkably, the reduction of soluble Aβ in APP transgenic mice was sufficient to rescue cognitive impairments suggesting that the accumulation of this Aβ species is responsible for the cognitive impairments in these mice (e.g. Refs. 25 and 35). These data are consistent with our previous results showing the Aβ accumulation triggers the learning and memory deficits in the 3xTg-AD mice (6). However, tau does not accumulate in APP transgenic mice, and our previous experiments were done at an age where in the 3xTg-AD mice there is no evident accumulation of tau pathology. Thus the results showing that reduction of soluble Aβ is sufficient to rescue cognitive decline in the absence of tau accumulation is consistent with the results presented here. Toward this end, our previous results (6) and those presented here indicate that although Aβ is the initial trigger of the cognitive decline in the 3xTg-AD mice, once soluble tau accumulates it further exacerbates the cognitive decline. At this stage, a reduction of both soluble Aβ and tau seems necessary to ameliorate memory impairments. The involvement of tau in microtubule stabilization and axonal transport is well established (39, 40). Hyperphosphorylated tau is known to detach from microtubules, thereby reducing their stability and eventually lead to impairments in axonal transport. Recent evidence suggests a new model for tau-induced neurodegeneration, whereby an excess tau clogs axonal transport by interfering with motor proteins, prior to NFTs formation (41). This view is supported by evidence from flies that overexpress tau and show progressive neurodegeneration and early death in the absence of NFTs (42) and by a recent report by Ashe and co-workers (9) suggesting that memory deficits and neuronal loss are to be independent of NFTs. The data presented here provide strong experimental evidence in a mammalian system to show that soluble tau levels play a major role during the cognitive decline observed in the 3xTg-AD mice.

Although the human clinical trial was suspended because of adverse side effects developed by 6% of the patients, some patients have since come to autopsy and reports describing their pathological load have been published. Aβ-immunized patients showed a slowing of cognitive decline compared with patients receiving the placebo (43, 44). At the neuropathological level, the three case reports so far published indicate that Aβ immunotherapy had some beneficial effect on clearing Aβ plaques but not in clearing NFTs (32–34). Notably, it has been shown that there is a decrease in soluble tau levels in the CSF of immunized patients (43). These human data are consistent with the results presented here and with the data that we have previously published (19).

In light of the results presented here and based on recent data suggesting that soluble tau levels in the CSF strongly correlate with the cognitive impairment in AD patients (45), soluble tau may be a valid therapeutic target for compounds aimed at decreasing the cognitive impairments in tauopathies including AD. The data reported in this article were obtained from a transgenic model and, as such, will need to be confirmed in humans.

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