

Soluble amyloid β -protein dimers isolated from Alzheimer cortex directly induce Tau hyperphosphorylation and neuritic degeneration

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Edited* by Gregory A. Petsko, Brandeis University, Waltham, MA, and approved February 25, 2011 (received for review November 13, 2010)

Alzheimer disease is a major cause of cognitive failure, and a pathogenically related but more subtle process accounts for many cases of mild memory symptoms in older humans. Insoluble fibrillar plaques of amyloid β -proteins ($A\beta$) and neurofibrillary deposits of hyperphosphorylated tau proteins are the diagnostic lesions of AD, but their temporal mechanistic relationship has long been debated. The recent recognition that small, diffusible oligomers may be the principal bioactive form of $A\beta$ raises the key question of whether these are sufficient to initiate cytoskeletal change and neurite degeneration. A few studies have examined the effects of oligomers of synthetic $A\beta$ peptides of one defined length at supra-physiological concentrations, but the existence of such assemblies in the AD brain is not established. Here, we isolated $A\beta$ dimers, the most abundant form of soluble oligomer detectable in the human brain, from the cortices of typical AD subjects and found that at subnanomolar concentrations, they first induced hyperphosphorylation of tau at AD-relevant epitopes in hippocampal neurons and then disrupted the microtubule cytoskeleton and caused neuritic degeneration, all in the absence of amyloid fibrils. Application of pure, synthetic dimers confirmed the effects of the natural AD dimers, although the former were far less potent. Knocking down endogenous tau fully prevented the neuritic changes, whereas overexpressing human tau accelerated them. Coadministering $A\beta$ N-terminal antibodies neutralized the cytoskeletal disruption. We conclude that natural dimers isolated from the AD brain are sufficient to potently induce AD-type tau phosphorylation and then neuritic dystrophy, but passive immunotherapy mitigates this.

Alzheimer disease (AD) and its harbinger, mild cognitive impairment—amnesic type, comprise the most prevalent late-life cognitive disorder in humans. The aging of the population in developed nations has led to predictions that the prevalence of Alzheimer-type dementia will rise substantially during the next few decades. Intensive research over almost 30 y has led to the hypothesis that progressive cerebral accumulation of the 42-residue amyloid β -protein ($A\beta$) may precipitate the synaptic dysfunction and cytoskeletal changes that underlie the symptoms of AD (1). Although insoluble amyloid plaques are one of the two neuropathological hallmarks of AD, recent studies suggest that these are in equilibrium with small, diffusible oligomers of $A\beta$ that may serve as the principal synaptotoxic form of the protein (2).

A major unresolved question about AD pathogenesis is the relationship of $A\beta$ deposits to the other cardinal lesion of the disease, the neurofibrillary tangle. These two lesions occur together in virtually all cases of AD, but whether $A\beta$ build-up is directly responsible for the neurofibrillary degeneration of AD is the subject of debate. Specifically, the growing experimental evidence that key features of the AD phenotype, such as dendritic spine loss, altered hippocampal synaptic plasticity, and impaired memory can be triggered by $A\beta$ oligomers (3–9)—including those isolated directly from patients' brains (10)—raises the question of whether soluble $A\beta$ oligomers are responsible by themselves for inducing altered tau phosphorylation, cytoskeletal change, and degeneration of neurites. Here, we address this central issue by isolating $A\beta$ dimers, the major form of soluble oligomer that can

be detected and isolated from human brain (10), from the cerebral cortex of typical AD cases and showing that they first induce tau phosphorylation at specific epitopes characteristic of AD in primary hippocampal neurons, and then produce cytoskeletal collapse and neuritic degeneration, but knock-down of endogenous tau fully prevents this phenotype. Two key advantages of our approach are: (i) it examines natural oligomers of the heterogeneous $A\beta$ peptides that exist at low nanomolar concentrations in AD patients, and (ii) it uses a cell-culture system to apply in a systematic fashion biochemically fractionated and well-defined $A\beta$ species, something not possible in *in vivo* mouse models, where a complex array of $A\beta$ assembly forms coexist.

Results

Soluble $A\beta$ Oligomers Isolated from the AD Cortex Induce Marked Cytoskeletal Abnormalities at Subnanomolar Concentrations in Primary Hippocampal Neurons. To determine whether soluble $A\beta$ oligomers are sufficient to trigger neurofibrillary degeneration, even in the absence of amyloid plaques, we isolated $A\beta$ dimers, the most abundant detectable form of soluble oligomer, from the AD cerebral cortex as recently described (10). These dimers are principally composed of $A\beta_{42}$ (10). Soluble (Tris-buffered saline, TBS) extracts from cortical homogenates of humans diagnosed with clinically and neuropathologically typical AD (AD-TBS extracts) or age-matched non-AD subjects (Cont-TBS extracts) were immunoprecipitated with 3D6, a monoclonal antibody specific for the free N terminus (Asp-1) of human $A\beta$, and the immunoprecipitate was eluted with lithium dodecyl sulfate (LDS) and subjected to size exclusion chromatography (a method we refer to as IP-SEC). $A\beta$ monomers and LDS-stable dimers could be separated and detected in the SEC fractions of the AD-TBS extracts but not in the corresponding fractions of Cont-TBS, as expected (Fig. 1A). Importantly, SDS-stable higher oligomers, including dodecamers, were not detectable by IP-SEC in AD-TBS extracts (Fig. 1A); accordingly, we focused on the dimers, cortical levels, of which were recently shown by others to correlate strongly with several features of the AD phenotype (Mini-Mental State Examination score; Blessed cognitive score; Braak score; synaptic protein levels) (11). We prepared primary cultures of hippocampal neurons from E18 rat embryos. After 18 d in culture, mature hippocampal neurons were exposed to either the monomer-rich or the dimer-rich SEC fractions of the AD-TBS

Author contributions: M.J. and D.J.S. designed research; M.J., N.S., T.Y. and G.C. performed research; D.W. contributed new reagents/analytic tools; M.J. analyzed data; and M.J. and D.J.S. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

Freely available online through the PNAS open access option.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1017033108/-DCSupplemental.

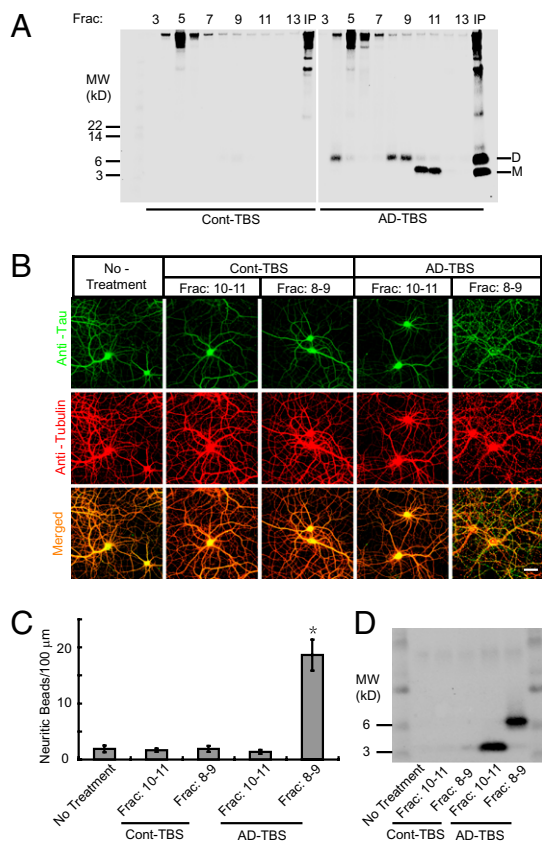


Fig. 1. Cytoskeletal abnormalities induced in primary hippocampal neurons by soluble A β oligomers isolated by SEC from the AD cerebral cortex. (A) AD-TBS (Right) or Cont-TBS (Left) was immunoprecipitated with 3D6 (3 μ g/mL), eluted with sample buffer containing 4% LDS, and subjected to SEC. SEC of the immunoprecipitation of AD-TBS resolves A β dimers (fractions 8–9) from monomers (fractions 10–11), as detected by Western blot with 6E10 + 2G3 + 21F12. IP, 3D6 immunoprecipitates of the starting Cont-TBS or AD-TBS extracts used for this SEC. (B) Confocal images showing the tau (green) and Tubulin (red) immunoreactivities of the cytoskeleton of hippocampal neurons [days in vitro (DIV) 21] after 3-d treatment with A β monomers (SEC fractions 10–11) or A β dimers (SEC fractions 8–9) isolated from AD-TBS or the corresponding fractions from Cont-TBS. (Scale bar, 50 μ m.) (C) Histograms represent the average number of tau-positive beads along 100- μ m lengths of Tubulin-positive neurites under different conditions. Asterisk indicates data significantly different from those of neurons without treatment ($P < 0.01$ by Student t test). Error bars, SEM. (D) After a 3-d incubation on primary neurons, the conditioned media were immunoprecipitated with A β antiserum AW7. A β monomers and dimers were precipitated from medium that contained the reconstituted IP-SEC fractions of AD-TBS but not from that with Cont-TBS.

extracts [or the corresponding fractions of Cont-TBS, which share other cortical proteins with the AD-TBS SEC fractions (Fig. 1A) but contain no A β] for 3 d, at which time the cultures were fixed for immunocytochemistry. Application of IP-SEC fractions 8–9 of the AD-TBS extract, which contain soluble, SDS-stable A β dimers but few or no monomers (Fig. 1A), induced a marked degeneration of neurites after 3 d of exposure, as indicated by extensively beaded, dystrophic neurites and an abnormal microtubule cytoskeleton revealed by Tubulin and tau immunocytochemistry (Fig. 1B and C, and Fig. S1). In contrast, applications of the A β monomer-rich fractions (fractions 10–11) of AD-TBS from the same SEC run or else either of the corresponding SEC fractions (fractions 8–9, 10–11) of Cont-TBS induced no significant neuritic alteration (Fig. 1B and C). The application of the A β dimers to hippocampal neurons for 2 d did not induce significant neuritic degeneration, but at higher magnification we observed some

slightly beaded neurites (Fig. S1), suggesting the initiation of a progressive neurite degeneration. Following the 3-d treatment, we were able to recover residual A β monomers or dimers by immunoprecipitating the conditioned media (Fig. 1D), thereby verifying the continuous exposure of the neurons to these soluble A β species. The concentration of human A β dimers in the final culture medium was around 0.5 nM, as determined by ELISA or quantitative Western blot, values similar to those observed for A β in the AD cortex (10). Although exposure to the A β dimers invariably disrupted the neuritic cytoskeleton, neuronal cell bodies generally survived the 3-d treatment, and there was no significant increase in the very low baseline levels of apoptotic cells in the cultures, as revealed by TUNEL staining (Fig. S2). The collapse of the cytoskeleton was A β -dependent, because prior immunodepletion of the dimer-rich SEC fractions by the A β antiserum AW7 fully prevented the effects of the dimers, whereas its preimmune serum was without effect (Fig. S3).

Importantly, when hippocampal neurons were cultured for 7 d, application of the soluble A β dimers did not induce a collapse of the cytoskeleton or other changes (Fig. S4A), in accord with evidence that the neurotoxicity of synthetic A β (at much higher concentrations) requires the maturation of neurons in vitro (12, 13). To explore the basis for this intriguing selectivity, we performed quantitative Western blotting of lysates from hippocampal neurons cultured for 7 or 18 d and found that in the 18-d neurons, the expression of mature tau (~55 kDa) was significantly increased, but the phosphorylation of the tau kinase GSK3 β at serine 9, which is a negative regulator of GSK3 β activity (14), was decreased (Fig. S4B). These results suggest that the time-dependent maturation of tau and of GSK3 β activity (the latter creates the AT8 phosphoepitope on tau; see below) is associated with the ability of A β dimers to induce neurite degeneration. However, more work is needed to identify additional changes underlying the selective vulnerability of older cultured neurons to A β oligomers.

Pure Synthetic A β Dimers Induce Cytoskeletal Disruption Similar to That Caused by Natural Dimers Isolated from the AD Brain. In light of several studies describing the neurotoxic effects of synthetic A β aggregates or cell-secreted A β oligomers (3, 5–8, 15–17), we assessed the effects of such preparations on the neuritic cytoskeleton in an attempt to confirm the changes described above with natural dimers isolated from the AD cortex. We examined a synthetic human A β 40 peptide in which serine 26 is mutated to cysteine (A β 40 S26C), enabling the formation of stable, disulfide-bonded dimers under oxidizing conditions (10, 18). This pure dimer was sufficient to induce an increasing alteration of the microtubule cytoskeleton and neuritic architecture in a dose-dependent manner, with a strong effect similar to that of the AD-TBS extracts observed at 500-nM concentration (Fig. S5A and B). A minor degree of neuritic beading could be observed at A β 40 S26C dimer concentrations as low as 100 nM (Fig. S5B). Importantly, the synthetic dimers always required much higher concentrations (>100-fold) to induce cytoskeletal effects comparable to those of the natural dimers isolated from AD cortex.

Disruption of the Neuritic Cytoskeleton Induced by Soluble A β Oligomers Is Dependent on Tau Expression. Neurofibrillary tangles and dystrophic neurites, the key cytopathological lesions of neurons in AD, contain abnormal filaments of hyperphosphorylated tau proteins (19–21). Numerous studies suggest that the tau protein is a mediator of the neuronal degeneration induced by supraphysiological concentrations of synthetic A β fibrils in vitro (8, 16, 22) and of the memory deficits of transgenic mice expressing mutant human amyloid precursor protein (APP) (23). Accordingly, we asked whether the neuritic cytoskeletal disruption caused by natural A β oligomers in the absence of amyloid fibrils was dependent on tau expression. Two weeks after neurons were transduced with

lentivirus encoding siRNA against tau, we detected a highly significant decrease (>85%) of tau expression compared with control siRNA or no transduction (Fig. 2*A*). When SEC fractions containing soluble A β dimers from AD-TBS or else pure synthetic A β 40 S26C dimers (500 nM) were applied, these each induced neuronal cytoskeletal disruption in control siRNA-treated neurons, but had no significant effect on the tau knock-down neurons (Fig. 2*B*).

Acceleration of the Neuritotoxic Effect of A β Oligomers by Expressing Human Tau. A recent analysis of transgenic mice expressing human tau revealed deficits in neural plasticity and memory, suggesting that the presence of human tau is sufficient to impair certain synaptic and cognitive functions (24). Our results above show that natural A β dimer-induced neuritic disruption is dependent on endogenous rodent tau. We asked whether expression of human

tau in rat neurons enhances the neurotoxic effect of A β dimers. We transduced hippocampal neurons with lentivirus encoding the longest splice form of human tau fused to EYFP (hTau-EYFP), or just EGFP as a control. One week after transduction, we documented the expression of human tau by both blotting (Fig. 3*A, Right*) and immunostaining (Fig. 3*A, Left*). After treatment with soluble dimers isolated by SEC from AD-TBS, the neurites of neurons (> DIV 18) expressing human tau were substantially disrupted after only 2 d of exposure, at which time point the neurites of treated neurons expressing EGFP or not transduced were still morphologically intact (Fig. 3*B* and Fig. S1), as in all our previous experiments. A β monomers from the same SEC run had no discernable effect at 2 or 3 d of exposure (Fig. 3*B*).

Soluble A β Oligomers Alter the Phosphorylation of Tau at AD-Relevant Epitopes. During AD pathogenesis, tau undergoes abnormal hyperphosphorylation that contributes to neurodegeneration (1, 8, 25, 26). The phosphorylation states of several epitopes within tau are increased in transgenic mice coexpressing mutant human APP, presenilin, and tau (27). Because we found above that tau plays a permissive role in the cytoskeletal alteration induced by soluble A β oligomers, it became important to elucidate which phosphoepitopes on tau may be altered by the natural oligomers. We transduced rat hippocampal neurons with EGFP or hTau-EYFP. After being cultured for 18 to 19 d, neurons were exposed to SEC-isolated human A β dimers for just 1 d, and the phosphorylation state of both the exogenous human and endogenous rat tau proteins was assayed by quantitative Western blotting with epitope-specific antibodies (the phosphorylated bands were normalized to the respective total tau signal in the

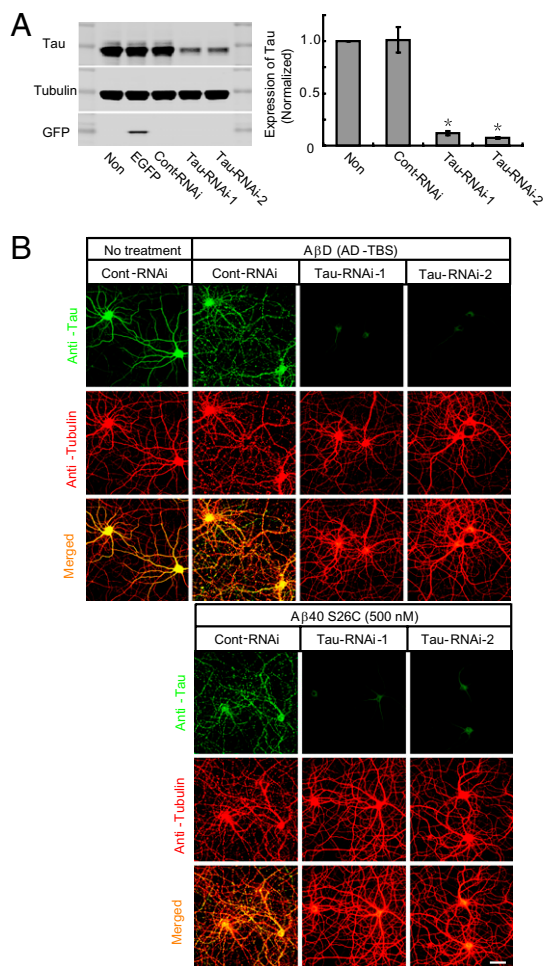


Fig. 2. Disruption of the neuritic cytoskeleton by soluble A β oligomers is dependent on tau expression. (*A*) Representative Western blots showing the expression of endogenous tau in primary hippocampal neurons (DIV18) transduced with lentivirus encoding EGFP or scrambled RNAi (Cont-RNAi) or RNAi against rat tau (Tau-RNAi-1, Tau-RNAi-2). Western blotting of Tubulin or GFP served as a control. Histograms represent the average expression level of tau, normalized to values in parallel cultures without lentiviral transduction. Asterisks indicate data significantly different from those of neurons without transduction ($P < 0.01$ by paired Student t test). Data are from five independent experiments; error bars, SEM. (*B*) Confocal images showing the tau (green) and microtubule (red) cytoskeleton of primary hippocampal neurons (DIV21) transduced with lentivirus encoding Cont-RNAi or Tau-RNAi-1 or Tau-RNAi-2 after 3-d treatment with A β D from AD-TBS or pure A β 40 S26C (500 nM). (Scale bar, 50 μ m.)

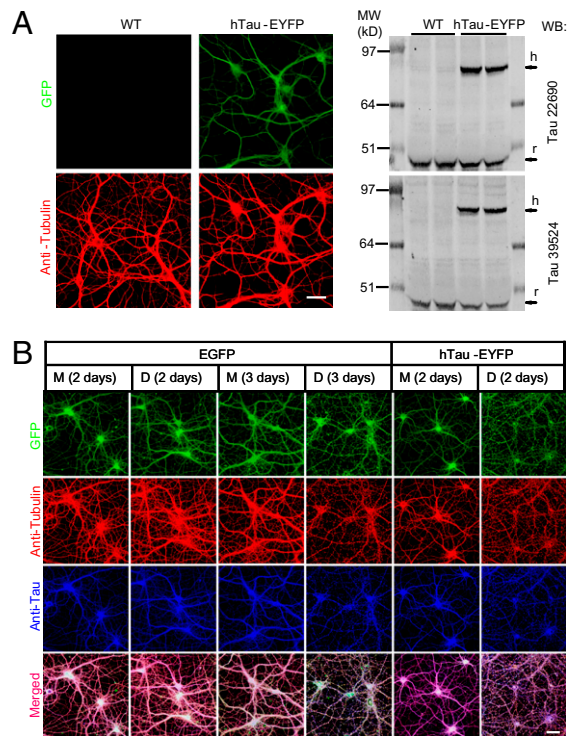


Fig. 3. The neuritotoxic effect of soluble A β oligomers is accelerated by expressing human tau. (*A*) Confocal images (*Left*) and Western blotting (*Right*) show the expression of hTau-EYFP in primary hippocampal neurons (DIV7) after transduction with lentivirus encoding hTau-EYFP. (*B*) Confocal images show the GFP fluorescence (green), microtubule (red) and total tau (blue) cytoskeleton of hippocampal neurons (DIV20 or DIV21) transduced with lentivirus encoding EGFP or hTau-EYFP after 2 or 3 d treatment with A β M or A β D from AD-TBS. (Scale bars, 50 μ m.)

same neurons) (Fig. 4 and Fig. S6). The cultures were also examined by immunocytochemistry (Fig. S7). Soluble A β dimers isolated from AD-TBS and pure A β 40 S26C dimers each induced substantial increases in tau phosphorylation at Ser202/Ser205 (AT8 epitope) and at Ser262 (12E8 epitope), a moderate increase at Thr181 (AT270 epitope), and no significant changes at Ser231/Thr235 (AT180 epitope) or Ser396 (PHF-1 epitope). Identical application of the A β dimers to hippocampal neurons cultured for just 8 d did not induce any increase in tau phosphorylation at the AT8 or 12E8 epitopes (Fig. S6), in accord with the lack of neuritic effects at this age (see above). As an important control, A β monomers from the same SEC run of the same AD-TBS extracts did not significantly alter tau phosphorylation. As a positive control for the phosphoepitope quantification, treatment of the neuronal cultures with the phosphatase inhibitor, okadaic acid (200 nM, 2 h), consistently increased phosphorylation levels (Fig. S6). Immunodepleting A β from the dimer-rich SEC fractions before their application prevented the hyperphosphorylation of tau at the AT8 and 12E8 epitopes, indicating that A β dimers were necessary for the effect (Fig. S8). It is of interest that the oligomer-induced increases in phosphorylation showed some difference between human and rat tau; in the same neurons, human tau phosphorylation was more sensitive to the application of A β oligomers, so that the degree of increase at the AT8, 12E8, and AT270 epitopes of human tau (all known to be hyperphosphorylated in AD neurons) was greater than those of rat tau (Fig. 4).

Specific Immunological Neutralization of the Cytoskeletal Alterations Induced by Human A β Dimers. Immunotherapy against A β by both active (vaccination) and passive (antibody infusion) approaches has reached advanced clinical testing in AD patients. In light of all of the results above, we asked whether monoclonal antibodies directed at specific A β epitopes could modify the cytoskeletal al-

teration and neuritic degeneration caused by soluble dimers from the human (AD) brain. We tested three monoclonal A β antibodies: 3D6 to the free Asp-1 N terminus (a humanized version of which is in Phase 3 human trials); 82E1, another Asp-1 specific N-terminal antibody; and 21F12 to the free Ile-42 C terminus of A β 42. Each antibody was coadministered (final concentration, 3 μ g/mL) with the dimer-rich SEC fraction to mature (≥ 18 DIV) primary hippocampal cultures. As before, monomer-rich SEC fractions from the same chromatography served as a negative control. We found that when either 3D6 or 82E1 was present with the soluble dimers, no significant alteration of neuritic architecture and tau and Tubulin immunostaining were observed (Fig. 5 A and B). In contrast, 21F12 had no significant neutralizing effect (Fig. 5 A and B). This relative efficiency of neutralization was further confirmed by pull-down of A β dimers by protein G agarose (PGA) beads from the conditioned media of the antibody-treated neurons. Substantially larger amounts of A β dimers were pulled down by 3D6 and 82E1 than by 21F12, whereas PGA beads applied in the absence of antibodies pulled down no dimers (Fig. 5C).

Discussion

Understanding the relationship of the two pathognomonic changes of AD, amyloid accumulation and neurofibrillary degeneration, represents an ongoing goal of research on the disease. Here, we show that natural oligomers, principally dimers, isolated directly from the cortex of typical, late-onset AD patients are sufficient to induce tau hyperphosphorylation at AD-relevant epitopes and then disrupt the microtubule cytoskeleton and cause neuritic dystrophy. Soluble dimers from the human brain appear to have a conformation that is highly potent in inducing these neuronal

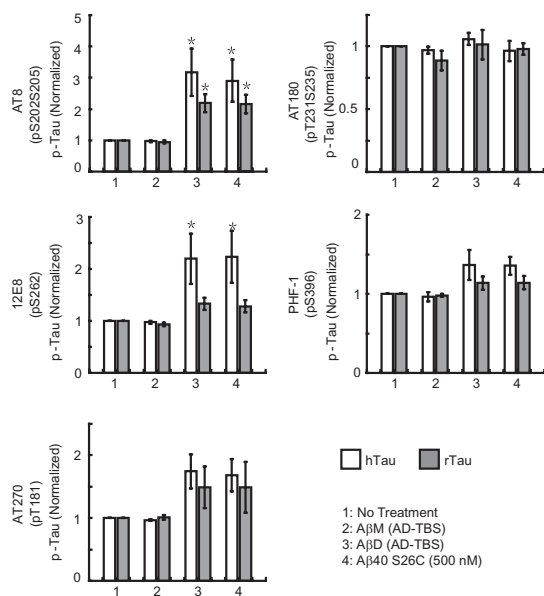


Fig. 4. Alteration of the phosphorylation state of tau at AD-relevant epitopes by soluble A β oligomers. Primary hippocampal neurons (DIV19) transduced with lentivirus encoding hTau-EYFP were treated under different conditions, as indicated in the key. Histograms represent the average levels of phosphorylation of human (h) or rat (r) tau at specific epitopes (AT8: Ser202/Ser205; 12E8: Ser262; AT270: Thr181; AT180: Ser231/Thr235; PHF-1: Ser396), normalized to the values in parallel cultures without treatment. Data are means of three independent experiments. Asterisks indicate data significantly different from those of neurons without treatment ($P < 0.05$ by paired Student t test). Error bars, SEM.

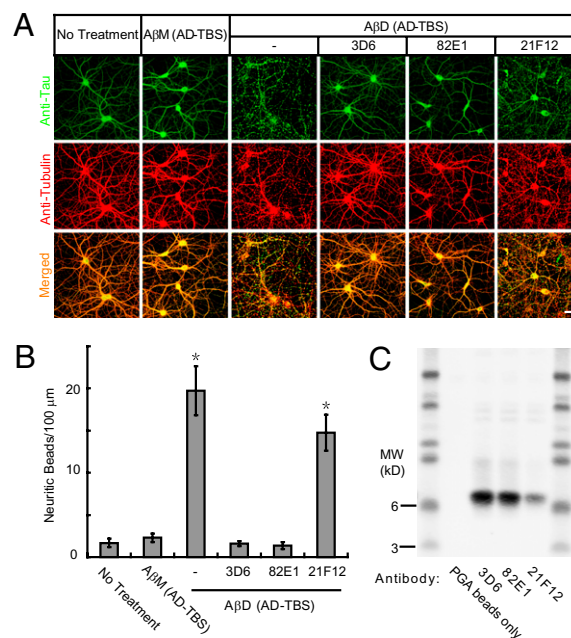


Fig. 5. Immunological neutralization of the cytoskeletal alterations induced by human A β dimers. (A) Confocal images showing the tau (green) and microtubule (red) cytoskeleton of primary hippocampal neurons (DIV21) after 3 d treatment with A β M or A β D from AD-TBS with or without monoclonal antibodies (3D6, 82E1, or 21F12) against human A β (each at 3 μ g/mL). (Scale bar, 50 μ m.) (B) Histograms represent the average number of tau-positive beads along 100- μ m lengths of Tubulin-positive neurites under different conditions. Asterisks indicate data significantly different from those of neurons without treatment ($P < 0.01$ by Student t test). Error bars, SEM. (C) Pull-down of the A β dimers by PGA beads added to the conditioned media of the neurons after the 3-d treatment, as detected by Western blot with 6E10 + 2G3 + 21F12.

changes, as concentrations in the subnanomolar range caused cytoskeletal collapse, whereas levels of pure synthetic dimers at least two orders of magnitude higher were needed to produce otherwise indistinguishable effects in the same experiments.

Numerous controls confirmed that the cytoskeletal changes we report are attributable specifically to human A β dimers [the most abundant form of soluble oligomers recoverable from AD cortex (10, 11, 28)]: (i) monomers from the same size-exclusion chromatography at equal or higher amounts had no effect; (ii) corresponding SEC fractions from age-matched control brains lacking A β were negative; (iii) the SDS-stable dimers were recoverable from the media at the end of the treatment, proving they were present throughout the exposures that resulted in alteration of the tau cytoskeleton; (iv) pure, synthetic A β dimers produced closely similar effects to AD-brain derived dimers (albeit at much higher concentrations); (v) immunodepletion of natural A β dimers from the SEC fractions precluded any subsequent neuritic injury; and (vi) coadministering highly specific monoclonal antibodies to the N terminus but not the C terminus of A β prevented the effects.

Intracerebral injection of synthetic A β fibrils into mice transgenic for mutant human tau can induce tau hyperphosphorylation and local neurofibrillary changes (29). Moreover, the coexpression of mutant human APP and mutant human tau leads to enhanced neuronal tau accumulation and dystrophic neurites in double-transgenic mice (30). Another mouse line transgenic for both mutant human APP and tau undergoes tau alterations that can be temporarily reversed by microinjecting anti-A β antibodies (31). Conversely, the crossing of tau knock-out mice to APP transgenic mice ameliorates the memory deficits that occur in the latter (23, 32), demonstrating the importance of endogenous tau in mediating adverse responses to A β . These and other mouse studies support a pathogenic relationship between A β accumulation and tau-mediated neuronal alteration, but in such *in vivo* models, one is unable to specify which assembly form of A β is responsible for any neuronal changes, as abundant monomers, dimers, higher oligomers, and amyloid fibrils exist in complex mixtures simultaneously. To specify which forms of A β can induce tau alteration and cytoskeletal degeneration, one must use a cell-culture system, which allows the controlled application of biochemically defined A β species that can be recovered and confirmed immediately after the exposure. To our knowledge, the present study is unique in providing evidence that natural A β oligomers, and specifically dimers isolated from the AD brain, are sufficient to induce tau hyperphosphorylation at AD-relevant epitopes, microtubule disruption, and neuritic degeneration, thus directly linking the accumulation of soluble oligomers *per se* to neurofibrillary degeneration.

The neuronal effects we describe clearly depend on the expression of tau: they were prevented by knocking down endogenous tau and were accelerated by expressing wild-type human tau. Our combined use of mature (≥ 18 DIV) cultures, biochemically isolated and defined human A β species, and an array of epitope-specific tau antibodies strongly supports the concept that soluble A β oligomers can induce AD-type cytoskeletal impairment in the absence of amyloid plaques. This result does not mean that plaques play no role in the fibrillary degeneration of neurons and their processes, as there is clear evidence that peri-plaque A β assemblies (type unspecified) are associated with local dendritic spine loss (33) and neuritic dystrophy in AD brains (34). Indeed, the presence of bioactive dimers within amyloid plaque cores (10) suggests that plaques serve as local reservoirs of small oligomers that can diffuse away from them and cause surrounding neuritic/synaptic injury.

Our observation on the potential of different monoclonal antibodies to neutralize oligomer effects on the tau cytoskeleton has relevance in light of current advanced trials of passive immunotherapy in AD. We find that two distinct antibodies to the free Asp-1 of A β , one of which (in humanized form) is in Phase 3

trials (35), are more potent in preventing the effects of soluble dimers from the AD cortex on the tau cytoskeleton than is a C-terminal specific antibody. This result suggests that endogenous dimers and other oligomers with an exposed N terminus (Asp-1) have a conformation that is particularly able to induce neurofibrillary degeneration (this article) and synapse loss (10). Active immunization with synthetic A β induces principally an N-terminal region antibody response in humans (36), and Phase 2 trials of a vaccine comprising an N-terminal A β fragment are underway. An earlier vaccine trial using full-length A β was halted prematurely because of occurrence of a self-limited meningoencephalitis in 6% of the 300 recipients (36). Nonetheless, those recipients having A β antibody responses showed less subsequent decline on some tests of verbal memory and an apparent decrease in CSF phospho-tau levels (36). A postmortem follow-up of a small subset of recipients from the Phase 1 trial of this full-length A β peptide vaccine, which suggested that some subjects could undergo marked clearance of A β plaques but still die with advanced dementia (37), is inconclusive, as it documented only two such subjects from a trial that originally included 80, and residual levels of A β oligomers in the brains were not assessed.

Tau has been found to be phosphorylated at over 30 serine/threonine residues in the human brain (38, 39), and approximately half of these are canonical sites for proline-directed protein kinases, including certain members of the MAP kinase, cyclin-dependent kinase and glycogen synthase kinase 3 (GSK3) families (26, 40). On cultured hippocampal neurons, synthetic A β oligomers (sometimes called ADDLs) or AD brain extracts have been shown to induce tau phosphorylation at several epitopes (41). A recent study showed that synthetic ADDLs can induce missorting of tau into dendrites, tau phosphorylation, and disruption of microtubules (8), but this work used 5- μ M concentrations of the synthetic ADDLs compared with the subnanomolar levels of natural human brain dimers used here. The mechanism by which diffusible extracellular oligomers of A β bind to neurons and lead to increased activity of select kinases that phosphorylate tau at some but not other epitopes remains unclear. Ittner et al. reported that tau protein, in addition to its principally axonal locus, is sorted in small amounts to dendrites and that this helps mediate the postsynaptic targeting of the src kinase Fyn, substrates of which are certain NMDA receptors (32). The authors postulated that A β -mediated enhanced targeting of tau to dendrites could alter this normal function. However, there are numerous different ways in which an interaction between A β oligomers and tau could occur in AD brains (42), and clarifying precisely how this occurs is the next major step for the approach we report here. In this regard, we consistently observed that hippocampal neurons cultured for ≤ 7 d were resistant to the cytoskeletal injury induced by the A β dimers, suggesting that signaling programs that develop in more mature neurons are required for expression of this phenotype. In their analyses of APP-only transgenic mice, Roberson et al. (23) saw no change in phosphoepitopes of endogenous murine tau at age 4 to 6 mo but did see phosphotau-positive punctae in periplaque dystrophic neurites at >20 mo. Here, we observed altered phosphorylation of endogenous rat tau (albeit less robustly than transfected human tau). Numerous APP transgenic mouse lines that do not also express human tau show neuritic dystrophy but no AD-type neurofibrillary tangles, suggesting that human tau may be necessary for full-blown tangle formation *per se*.

Our findings with natural dimers isolated directly from AD patients, coupled with the wide availability of postmortem brain tissue from AD and non-AD subjects, recommends the use of endogenous oligomers isolated from the human cortex (10, 43), as the most biologically relevant approach to learn how A β oligomers alter tau phosphorylation and cytoskeletal function. Various aggregated forms of synthetic A β designated ADDLs (3) or protofibrils (44, 45) and generated from high concentrations

of a single, defined A β peptide, have not been proven to occur as such in the human brain, whereas heterogeneous dimers, trimers (10, 11, 28), slightly larger low-n oligomers (11), and dodecamers (28) have. Deciphering the mechanisms of these natural oligomers will require purifying them to homogeneity from the AD cortex, labeling them, and exposing primary neurons or brain slices to them to identify in unbiased fashion their molecular targets. We hypothesize that these targets are likely to be plasma membrane lipids (which would be expected to avidly bind the highly hydrophobic oligomers) rather than the hydrophilic ectodomains of protein receptors. Oligomer binding could perturb the fine structure of the lipid bilayer, and this could lead to secondary biophysical effects on the structure and function of various transmembrane receptors (e.g., the NMDA, AMPA, insulin, and α 7-nicotinic receptors implicated to date) that may then contribute to the profound changes in the tau cytoskeleton documented here.

Materials and Methods

See *SI Materials and Methods* for detailed descriptions.

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