

RESEARCH

Open Access



Tau passive immunization blocks seeding and spread of Alzheimer hyperphosphorylated Tau-induced pathology in 3 × Tg-AD mice

Chun-ling Dai[†], Wen Hu[†], Yunn Chyn Tung, Fei Liu, Cheng-Xin Gong and Khalid Iqbal*

Abstract

Background: Accumulating evidence indicates that Tau pathology can spread from neuron to neuron by intake and coaggregation of the hyperphosphorylated Tau (p-Tau) seeds with the host neuron protein. Thus, clearance of Tau seeds by immunization with Tau antibodies could provide a potential therapeutic opportunity to block the spread of the pathology in Alzheimer's disease (AD) and other tauopathies. We report prevention of the seeding and spread of tau pathology with mouse monoclonal antibody 43D against the N-terminal projection domain of Tau (Tau 6–18) in triple-transgenic AD (3 × Tg-AD) mice.

Methods: Female 11- to 12-month-old 3 × Tg-AD mice were intravenously immunized weekly for 6 weeks with 15 µg/ injection of mouse monoclonal antibody 43D or with mouse immunoglobulin G as a control. AD p-Tau isolated from a frozen autopsied AD brain was unilaterally injected into the right hippocampus on the day of the second dose of immunization. Tau pathology and its effect on Aβ pathology were assessed by immunohistochemical staining.

Results: We found that the injection of AD p-Tau into the hippocampus of 11- to 12-month-old 3 × Tg-AD mice time-dependently induced Tau aggregation in the hippocampus and promoted the spread of Tau pathology to the contralateral hippocampus. Tau pathology was observed as early as 6 weeks after AD p-Tau injection. Tau pathology templated by AD p-Tau was thioflavin-S-positive and was about two-fold greater than that seen in naive 18-month-old 3 × Tg-AD mice; Tau pathology in the latter was thioflavin-S-negative. Immunization with Tau antibody 43D dramatically blocked AD p-Tau seeding in the ipsilateral hippocampus and inhibited its propagation to the contralateral side in 3 × Tg-AD mice. Furthermore, AD p-Tau injection enhanced the amyloid plaque load in the ipsilateral side, and immunization with 43D showed a tendency to attenuate it.

Conclusions: These findings indicate that AD p-Tau-injected 3 × Tg-AD mice represent a practical model to study the seeding and spread of Tau pathology, their effect on Aβ pathology, and the effect of Tau immunotherapy on both Tau and Aβ pathologies. Immunization with Tau antibody 43D to Tau 6–18 can prevent the seeding and spread of Tau pathology, making it a potential therapeutic treatment for AD and related tauopathies.

Keywords: Tauopathy, Alzheimer's disease, Immunotherapy, Tau transmission, Amyloid plaques

* Correspondence: khalid.iqbal.ibr@gmail.com

[†]Equal contributors

Department of Neurochemistry, Inge Grundke-Iqbal Research Floor, New York State Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY 10314, USA



© The Author(s). 2018 **Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

Background

Alzheimer's disease (AD), characterized by progressive loss of memory and other cognitive functions, is the most common cause of dementia. The two major histopathological hallmarks in the AD brain are extracellular senile plaques, consisting of amyloid- β (A β) peptides [1], and intracellular neurofibrillary tangles (NFTs), composed of abnormally hyperphosphorylated Tau (AD p-Tau) protein [2]. Tau, a microtubule-associated protein, normally stabilizes neuronal microtubules and promotes their assembly. However, in certain pathological conditions, hyperphosphorylated Tau causes the formation of insoluble aggregates that are toxic to neurons [3–6]. The Tau pathology made up of the p-Tau is also a hallmark of several neurodegenerative disorders known as *tauopathies*, which include frontotemporal dementia, corticobasal degeneration, progressive supranuclear palsy, Pick disease, Guam Parkinsonism-dementia complex, and chronic traumatic encephalopathy. Currently, there are no effective treatments available for AD and related tauopathies.

Studies using autopsied brains indicate that neurofibrillary pathology in the brains of patients with AD starts in the entorhinal/perirhinal cortex and spreads anatomically in a defined pattern to the limbic system and eventually the isocortex [7, 8]. Only recently, the stereotypical pattern of Tau pathology progression similar to that in AD was shown experimentally in mice. Injection of brain extract from P301S mutated Tau-expressing mice harboring NFTs or brain homogenates from human tauopathies into the brain of transgenic wild-type human Tau (h-Tau)-expressing mice induced the assembly of wild-type h-Tau into filaments and the spread of pathology from the site of injection to neighboring brain regions [9, 10]. Intracerebral inoculation of young PS19 mice overexpressing P301S mutated h-Tau with synthetic preformed aggregates assembled from recombinant full-length Tau or truncated Tau containing four microtubule-binding repeats resulted in rapid induction of NFT-like inclusions that propagated from injected sites to connected brain regions in a time-dependent manner [11]. The spread of Tau pathology in brains was further confirmed in the mouse models expressing P301L mutated h-Tau restricted to the entorhinal cortex (EC), in which Tau pathology progressed from EC transgene-expressing neurons to neurons without detectable transgene expression, first to EC neighboring cells, followed by propagation to neurons downstream in the synaptic circuit, such as the dentate gyrus, cornu ammonis (CA) fields of the hippocampus, and cingulate cortex [12, 13]. Taken together, these data suggest that a prion-like spread mechanism may drive Tau pathology, leading to synaptic and cognitive deficits in human AD and related tauopathies.

The abnormally hyperphosphorylated/oligomeric Tau released into the extracellular space from the affected neurons is suspected to serve as the seed for the spread of Tau pathology by the ingesting cells [9, 14]. Therefore, immunotherapy against Tau may clear extracellular Tau that is involved in the spread of the pathology and rescue memory deficits. Treatment with antibodies that block the seeding activity in vitro and anti-Tau pS396 and pT231 were reported to inhibit the spread of Tau pathology in Tau-transgenic mice [15, 16].

In a recent study, we found that intrahippocampal injection with AD p-Tau isolated from a frozen autopsied AD brain produced numerous p-Tau tangles and neuropil threads locally and in neocortex lateral to injection and upstream of the hippocampus in h-Tau-transgenic mice [17]. We discovered that immunization with monoclonal antibody 43D against Tau 6–18 reduced both Tau and A β pathologies, as well as rescued cognitive impairment in moderate to severe stages of Tau pathology in triple-transgenic AD (3 \times Tg-AD) mice [18, 19].

In this article, we report that, in addition to seeding and spread of Tau pathology, AD p-Tau also enhances A β plaque load and that immunization with antibody 43D prevents the seeding and propagation of Tau pathology and the promotion of A β pathology in 3 \times Tg-AD mice. These findings suggest that immunization with 43D can efficiently block both Tau and A β pathologies promoted by extracellular Tau seeds. Thus, immunization targeting proximal N-terminal Tau domain Tau 6–18 offers a potential therapeutic opportunity for AD and related tauopathies.

Methods

Transgenic mice

The homozygous 3 \times Tg-AD mice harboring human APP_{SWE} and Tau_{P301L} transgenes with knock-in PS1_{M146V} under the control of the mouse Thy1.2 promoter, created in the laboratory of Dr. Frank LaFerla [20], were obtained from The Jackson Laboratory (Bar Harbor, ME, USA; <https://www.jax.org/strain/004807>). Both homozygous male and female 3 \times Tg-AD mice with the mixed C7BL/6;129X1/Sv;129S1/Sv genetic background were bred in the animal colony of the New York State Institute for Basic Research in Developmental Disabilities (Staten Island, NY, USA). Mice had access to food and water ad libitum and were housed (four or five animals per cage) in a pathogen-free facility with 12-h/12-h day/night cycles. The female 3 \times Tg-AD mice were reported to develop amyloid plaques starting at \sim 9 months of age and NFTs starting at \sim 12 months of age, respectively, and the pathologies are predominantly restricted to the hippocampus, amygdala, and cerebral cortex [20–22]. Unlike females, male 3 \times Tg-AD mice show inconsistent and weak pathology [23–26], and

hence only female animals were used in the present study.

To minimize the variation, mice used in this study were first grouped by body weight and age, and then the mice from each litter were evenly assigned to different study groups. The grouped mice were randomized into three study groups: (1) AD p-Tau intracerebral injection plus six weekly doses of 43D (AD p-Tau/43D; $n = 6$), (2) AD p-Tau intracerebral injection plus six weekly doses of mouse immunoglobulin G (IgG) (AD p-Tau/IgG; $n = 7$), and (3) saline (vehicle) intracerebral injection plus six weekly doses of 43D (saline/43D; $n = 6$) (Fig. 1a). A separate group of 11- to 12-month-old female 3 × Tg-AD mice that received AD p-Tau injections as above were killed with a 3-week interval to determine the time-dependent AD p-Tau seeding at the injection site ($n = 2$

at each time point). To compare Tau pathology produced by templation with AD p-Tau with that produced by spontaneous development, we employed 18-month-old naive female 3 × Tg-AD mice ($n = 3$).

AD brain samples

Frozen brain tissue samples from autopsied and histopathologically confirmed AD cases were obtained from the Harvard Brain Tissue Resource Center, McLean Hospital (Belmont, MA, USA).

Preparation of Tau seeds

Oligomeric, AD p-Tau was isolated from the cerebral cortex of a frozen autopsied AD brain as described by us previously [6, 17, 27]. Briefly, 10% brain homogenate prepared in the buffer (20 mM Tris-HCl, pH 8.0, 0.32 M

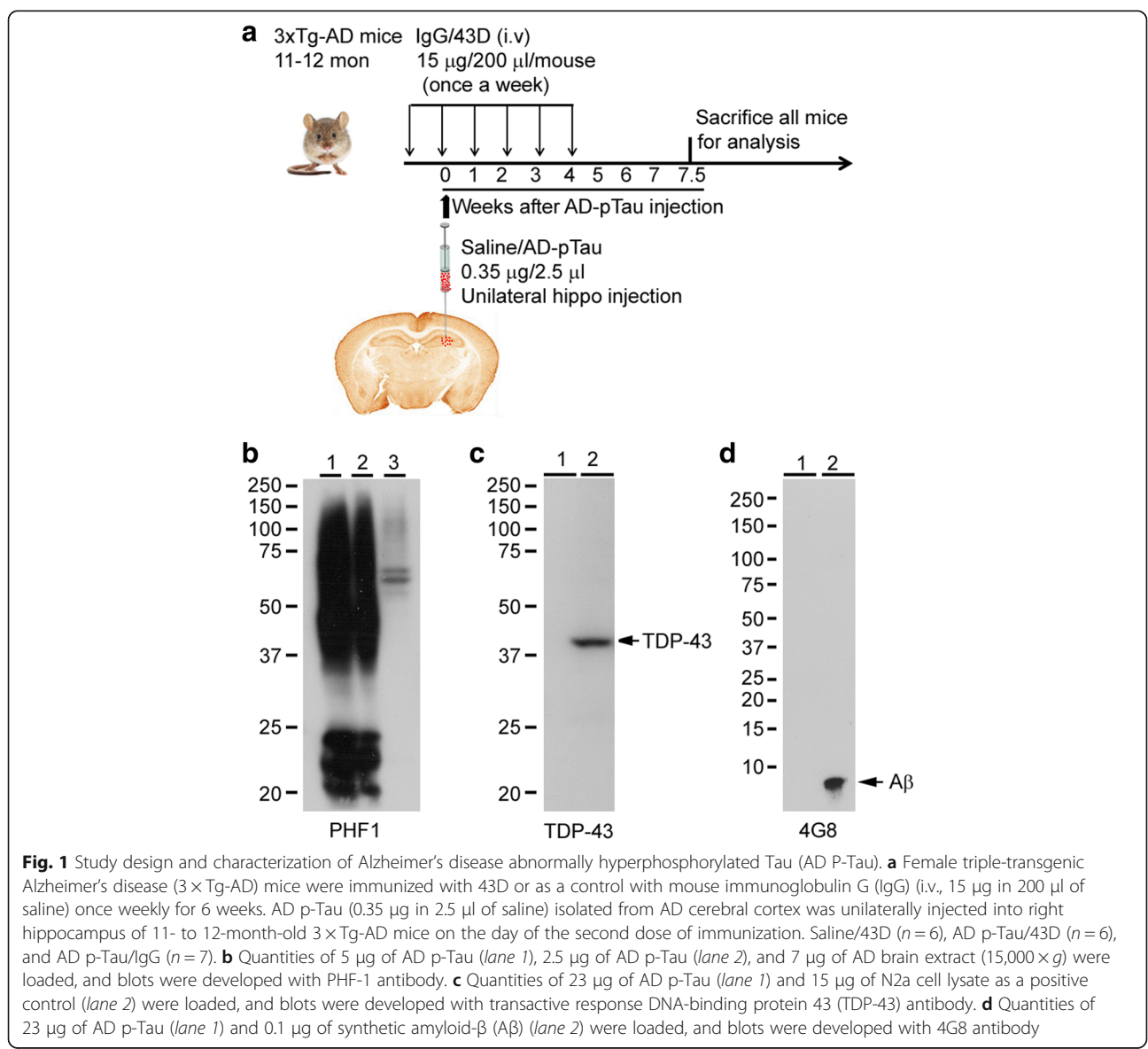


Fig. 1 Study design and characterization of Alzheimer's disease abnormally hyperphosphorylated Tau (AD P-Tau). **a** Female triple-transgenic Alzheimer's disease (3 × Tg-AD) mice were immunized with 43D or as a control with mouse immunoglobulin G (IgG) (i.v., 15 µg in 200 µl of saline) once weekly for 6 weeks. AD p-Tau (0.35 µg in 2.5 µl of saline) isolated from AD cerebral cortex was unilaterally injected into right hippocampus of 11- to 12-month-old 3 × Tg-AD mice on the day of the second dose of immunization. Saline/43D ($n = 6$), AD p-Tau/43D ($n = 6$), and AD p-Tau/IgG ($n = 7$). **b** Quantities of 5 µg of AD p-Tau (lane 1), 2.5 µg of AD p-Tau (lane 2), and 7 µg of AD brain extract (15,000 × g) were loaded, and blots were developed with PHF-1 antibody. **c** Quantities of 23 µg of AD p-Tau (lane 1) and 15 µg of N2a cell lysate as a positive control (lane 2) were loaded, and blots were developed with transactive response DNA-binding protein 43 (TDP-43) antibody. **d** Quantities of 23 µg of AD p-Tau (lane 1) and 0.1 µg of synthetic amyloid-β (Aβ) (lane 2) were loaded, and blots were developed with 4G8 antibody

sucrose, 10 mM β -mercaptoethanol, 5 mM MgSO_4 , 1 mM ethylenediaminetetraacetic acid [EDTA], 10 mM glycerophosphate, 1 mM Na_3VO_4 , 50 mM NaF with phosphatase and protease inhibitors was centrifuged at $27,000 \times g$ for 30 minutes. The supernatant was further centrifuged at $235,000 \times g$ for 45 minutes, and the resulting pellet (AD p-Tau) was collected and washed three times and then resuspended in saline. The AD p-Tau was bath-sonicated using three bursts of 10 seconds each.

Intracerebral injections of AD p-Tau

As previously reported by us [17], mice were deeply anesthetized with 1.25% tribromoethanol (Avertin; Sigma-Aldrich, St. Louis, MO, USA) and placed in a stereotactic frame. After a craniotomy 1 mm in diameter was made with a motorized minidrill, the Tau seeds were injected using a 10- μl Hamilton syringe custom-made with a 30-gauge/0.5-inch cemented needle (Hamilton Syringe Co., Reno, NV, USA). AD p-Tau was unilaterally injected into the right hippocampus (0.35 μg of Tau in 2.5 μl of saline) of 11- to 12-month-old female $3 \times \text{Tg-AD}$ mice. The coordinates were as follows: -2.5 mm anterior/posterior, $+2.0$ mm medial/lateral to bregma, and -1.8 mm dorsal/ventral to the dura surface. Tau seeds were injected at a rate of 1.25 $\mu\text{l}/\text{minute}$, and the needle was kept in position for 3 minutes before slow withdrawal to prevent leakage of the liquid infused. An identical volume of saline was also injected into the hippocampus of $3 \times \text{Tg-AD}$ mice as vehicle controls. The skin was sutured after injection, and the mice were allowed to completely recover on a soft heating pad before they were returned to their home cages.

Immunizations with Tau antibodies

Female $3 \times \text{Tg-AD}$ mice (six to seven mice/group) aged 11 to 12 months old were immunized intravenously through the tail vein with 15 μg of 43D or as a control with mouse IgG in 200 μl of saline once weekly for 6 weeks. One week after the first dose of immunization, mice received an intracerebral injection of AD p-Tau or saline as a vehicle control (Fig. 1a).

Tissue process

Mice were anesthetized and transcardially perfused with 30 ml of PBS, followed by 20 ml of 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were removed and postfixed in 4% paraformaldehyde in 0.1 M phosphate buffer for 48 h and then processed through 30% sucrose in 0.1 M phosphate buffer until the brain tissue sank to the bottom of the tube. Serial 40- μm coronal brain sections were collected and used in the present study.

Immunofluorescence and thioflavin-S staining

Free-floating coronal sections were washed in 10 mM PBS (three times, 15 minutes each) and then incubated in 0.3% Triton X-100 for 30 minutes. The sections were again washed in 10 mM PBS (three times, 15 minutes each) and blocked by incubation in blocking solution (5% normal goat serum, 0.1% Triton X-100, and 0.05% Tween 20 in PBS) for 60 minutes. Sections were then incubated with AT8 (phospho-tau at $\text{Ser}^{202}/\text{Thr}^{205}$, 0.2 $\mu\text{g}/\text{ml}$; Thermo Fisher Scientific, Rockford, IL, USA) or PHF-1 (phospho-tau at $\text{Ser}^{396}/\text{Thr}^{404}$, 1:1000, a gift from Dr. Peter Davies) or both AT8 and rabbit monoclonal antibody against $\text{A}\beta$ (D54D2, 1:300; Cell Signaling Technology, Danvers, MA, USA) at 4°C overnight. On the second day, sections were washed in 10 mM PBS (three times, 15 minutes each), followed by incubation with Alexa Fluor 555-conjugated goat anti-mouse IgG (1:500; Thermo Fisher Scientific), or in case of double immunostaining, in combination with Oregon Green 488-conjugated goat anti-rabbit IgG (1:500; Thermo Fisher Scientific), in 10 mM PBS with 0.05% Tween 20 for 2 h at room temperature. Sections were subsequently washed, mounted onto Superfrost Plus slides (Fisher Scientific, Pittsburgh, PA, USA) and allowed to air-dry for 3 h at room temperature. Sections on the slide were stained with 0.05% thioflavin-S in PBS in the dark for 8 minutes and then washed in 50% ethanol twice for 1 minute each and in water for 3 minutes. Finally, the sections were coverslipped using ProLong Gold Antifade Mounting Medium (Thermo Fisher Scientific). In the study to investigate the time-dependent AD p-Tau seeding in $3 \times \text{Tg-AD}$ mice, AT8 antibody and Alexa Fluor 488-conjugated goat antimouse antibodies (1:500; Thermo Fisher Scientific) were used. Images were acquired using a Nikon Eclipse 90i microscope equipped with an EZ C1 laser-scanning confocal microscopy system (Nikon Instruments, Melville, NY, USA). No primary antibody staining was used as a control for all immunohistochemical studies.

Quantitation of tangles with AT8 and PHF-1 high-intensity staining

The templated Tau pathology by AD p-Tau exhibited a bright immunofluorescence signal as compared with markedly dimmer phospho-Tau staining of nontemplated Tau pathology in the hippocampus. We set high-intensity staining with the gain at 6.40B for $\times 10$ and 6.10B for $\times 20$ objectives in the confocal system, a setting that filtered out the phospho-Tau staining of nontemplated Tau pathology in the hippocampus. In this way, we were able to easily count the number of templated tangles in the mouse brain sections.

Serial whole-brain coronal sections (40 μm) were collected from bregma $+0.4$ mm to -3.4 mm. The number

of tangles in from 22–25 serial coronal sections per mouse (every fourth section of the brain) that stained positive with both thioflavin-S and intensely with AT8 or PHF-1 were counted at $\times 10$ magnification by an experimenter who was blind to this study.

Immunohistochemical staining

To study the morphology of templated NFT-like pathology, we performed immunohistochemical staining of mouse brain sections with antibodies against different phospho-tau epitopes (i.e., pSer²⁰²/pThr²⁰⁵ [AT8] and pSer^{396/404} [PHF-1]), and we visualized the immunostaining with 3,3'-diaminobenzidine (DAB). We used brain sections of naive 3 \times Tg-AD mice at 18 months of age as a control to examine spontaneously developed Tau pathology in this mouse model.

Free-floating brain sections were washed with PBS and permeabilized with 0.3% Triton X-100 in PBS. Endogenous peroxidase activity was abolished by treatment with 0.3% H₂O₂ in PBS. The sections were then blocked with normal goat serum, followed by incubation either with AT8 or with antibody PHF-1 overnight at 4 °C, followed by washing in PBS and incubation with horseradish peroxidase-conjugated goat antimouse IgG (1:2000; Jackson ImmunoResearch, West Grove, PA, USA) for 2 h at room temperature. After washing with PBS, sections were developed with 0.05% DAB plus 0.015% H₂O₂ in PBS. Sections were then mounted on pre-cleaned, positively charged microscopic slides; counterstained with Mayer's hematoxylin; dehydrated; cleared in Histo-Clear (National Diagnostics, Atlanta, GA, USA); and coverslipped. Stained sections were visualized, and photomicrographs were taken using a Nikon 90i research microscope. Staining was color-thresholded using ImageJ for Windows software (<https://imagej.nih.gov/ij/>), and the percentage of area occupied by AT8 or PHF-1 staining in the somatodendritic compartment of individual CA1 neurons with Tau inclusions was quantified at $\times 60$ magnification.

Western blot analysis

For Western blot analysis, AD brain homogenate (10%) prepared in 20 mM Tris-HCl, pH 8.0, 0.32 M sucrose, 10 mM β -mercaptoethanol, 5 mM MgSO₄, 1 mM EDTA, 10 mM glycerophosphate, 1 mM Na₃VO₄, and 50 mM NaF with phosphatase and protease inhibitors was centrifuged at 15,000 $\times g$ for 30 minutes [18]. The extract and the AD p-Tau samples were boiled in 2 \times Laemmli buffer for 5 minutes.

N2a cells (the mouse neuroblastoma cell line) were harvested in the exponential growth phase and lysed in Laemmli SDS sample buffer with Roche cOmplete Mini Protease Inhibitor mixture (Roche Diagnostics, Indianapolis, IN, USA) and directly boiled for 5 minutes.

A β _{1–42} used for Western blotting was synthesized commercially (a gift from Dr. David Miller).

Protein concentration was measured by using the PierceTM 660 nm protein assay (Thermo Fisher Scientific, Rockford, IL, USA). The samples were resolved in 10% SDS-PAGE and electrotransferred onto Immobilon-P membrane (MilliporeSigma, Burlington, MA, USA). The blots were then probed with primary antibodies PHF-1 and transactive response DNA-binding protein 43 (TDP-43) (Cell Signaling Technology) and developed with the corresponding horseradish peroxidase-conjugated secondary antibody and enhanced chemiluminescence (ECL) kit (Thermo Fisher Scientific). For A β , the samples were resolved in 5–20% gradient SDS-PAGE and electrotransferred onto nitrocellulose membrane. The blots were then probed with A β antibody 4G8 (BioLegend, San Diego, CA, USA) and developed with the corresponding horseradish peroxidase-conjugated secondary antibody and ECL kit (Thermo Fisher Scientific).

Statistical analysis

Data were analyzed using Prism version 5.0 software (GraphPad Software Inc., La Jolla, CA, USA) and one-way or two-way analysis of variance (as appropriate) followed by a Bonferroni post hoc test. Inter-group comparisons were performed using paired or unpaired two-tailed *t* tests. All data are presented as mean \pm SEM, and *P* < 0.05 was considered a statistically significant difference.

Results

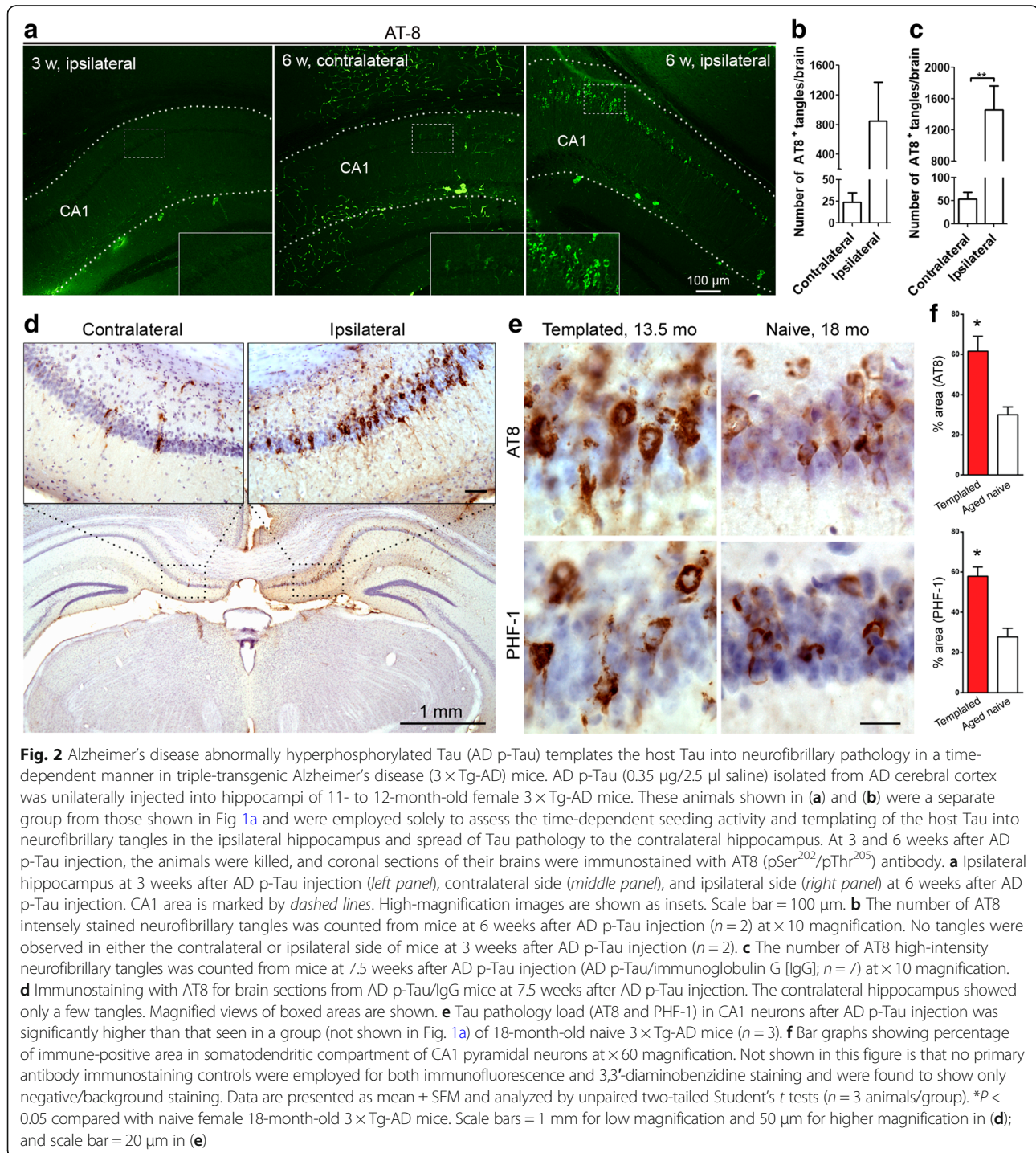
AD p-Tau seeds Tau pathology in a time-dependent manner in 3 \times Tg-AD mice

In a recent study, we showed that intrahippocampal injection of oligomeric AD p-Tau into the hippocampus of 3-month-old h-Tau-transgenic mice produced spread of Tau pathology in the brain at 11 months after injection [17]. To develop a practical mouse model in which Tau spread can be studied in a relatively short period, we employed 11- to 12-month-old 3 \times Tg-AD mice. Unlike the original report of Oddo et al. [20], the 3 \times Tg-AD mice bred in our colony develop amyloid plaques at around 13 months of age and Tau pathology even later. To determine the potency of the AD p-Tau to template the host Tau into NFTs in 3 \times Tg-AD mice, we unilaterally injected 0.35 μ g of the oligomeric Tau protein into the right hippocampus of 11- to 12-month-old female 3 \times Tg-AD mice and evaluated Tau pathology at 3 and 6 weeks after the injection (Fig. 1a). Compared with AD brain extract, the AD p-Tau was highly enriched in hyperphosphorylated Tau as seen on Western blots developed with phospho-Tau antibody PHF-1 (Fig. 1b).

Furthermore, the AD p-Tau preparation did not have detectable levels of either A β or TDP-43 (Fig. 1c, d).

At 3 weeks after AD p-Tau injection, we found low-intensity AT8 staining, and at 6 weeks we found high-intensity AT8 staining, in the ipsilateral hippocampus, but only low-intensity AT8 staining at these two time points in the contralateral hippocampus (Fig. 2a). The number of the intensely stained neurons was several-

fold higher in the ipsilateral than in the contralateral hippocampus (Fig. 2b). These results indicate that AD p-Tau has seeding activity and can template host Tau into NFTs as early as 6 weeks after AD p-Tau injection in 11- to 12-month-old 3 \times Tg-AD mice. This process is faster than what we previously found in h-Tau mice, in which Tau seeded and NFTs were observed 9 months after injections [17].



AD p-Tau successfully seeds and templates host Tau into neurofibrillary tangles in 3 × Tg-AD mice

Female 3 × Tg-AD mice develop amyloid plaques starting at ~9–13 months and NFTs starting ~12–15 months of age, and the pathologies are restricted predominantly to the hippocampus, amygdala, and cerebral cortex [20–22]. Thioflavin-S staining that recognizes β -pleated sheets is commonly used to label amyloid plaques and NFTs [28, 29]. On the basis of our findings described above that showed overt Tau pathology 6 weeks after injection of AD p-Tau, we perfused the remaining study mice, which by then were 7.5 weeks post-AD p-Tau injection, to evaluate the seeding and spread of Tau pathology.

Immunohistochemical staining showed that AD p-Tau seeded robust NFT-like pathology in the ipsilateral hippocampus of 11- to 12-month-old 3 × Tg-AD mice at 7.5 weeks after unilateral AD p-Tau injection (Fig. 2c–f). The number of AT8 high-intensity positive tangles in the AD p-Tau-injected hippocampus were 0, 800, and 1500 at 3 (AD p-Tau/saline; $n = 2$), 6 (AD p-Tau/saline; $n = 2$), and 7.5 (AD p-Tau/IgG; $n = 7$) weeks after AD p-Tau injection, which clearly demonstrated that AD p-Tau time-dependently templated host Tau into tangles in 3 × Tg-AD mice (Fig. 2b, c). AT8 immunostained neurons were located mainly in CA1 and subiculum subfields of the hippocampus, whereas CA3 and dentate gyrus were spared from Tau pathology (Fig. 2d). In the contralateral hippocampus, however, fewer neurons were positive for AT8 or PHF-1 (Fig. 2d). Intriguingly, neurons with templated Tau pathology in 13.5-month-old 3 × Tg-AD mice showed even much higher, approximately twofold, load of tau inclusions compared with the spontaneously developed Tau pathology in 18-month-old naive female 3 × Tg-AD mice (Fig. 2e and f).

We employed double labeling with immunofluorescence and thioflavin-S staining to further characterize the templating of Tau pathology by AD p-Tau in 3 × Tg-AD mice. In the ipsilateral hippocampus of saline-injected mice, we found amyloid plaques with thioflavin-S-positive staining, but very few neurons showed high-intensity AT8 staining (Fig. 3a). Additionally, the few neurons that showed high-intensity AT8 staining were found negative with thioflavin-S staining (Fig. 3a). Importantly, the mice injected with AD p-Tau showed an increased density of neurons with high-intensity AT8 staining, and these neurons were also thioflavin-S-positive in the CA1 and subiculum areas (Fig. 3b). In contrast, the naive 18-month-old 3 × Tg-AD mice showed intense AT8 staining similar to that of the AD p-Tau-injected 13.5-month-old mice, but they were thioflavin-S-negative (Fig. 3c). To further characterize the identity of the filamentous intraneuronal inclusions templated by AD p-Tau preparation, we coimmunostained brain sections with AT8 and rabbit monoclonal

antibody against A β , which recognizes both A β_{40} and A β_{42} . We found that AT8-positive tangles were negative for A β (Fig. 3d). In addition, tangle-bearing neurons showed no increase in A β immunoreactivity in the somatodendritic compartment, as compared with tangle-free neurons (Fig. 3d). These results indicate that AD p-Tau can rapidly seed and template host tau into NFTs in the 11- to 12-month-old 3 × Tg-AD mice.

Immunization with 43D blocks AD p-Tau seeding and Tau pathology propagation in 3 × Tg-AD mice

Our previous studies showed that immunization with mouse monoclonal antibody 43D targeting the N-terminal projection domain of Tau decreased Tau pathology and improved cognitive deficits in moderate to severe stages of Tau pathology in 3 × Tg-AD mice [18, 19]. To investigate whether 43D monoclonal antibody can block AD p-Tau seeding and inhibit the spread of templated host Tau inclusions in 3 × Tg-AD mice, we immunized mice with six weekly intravenous doses of 43D or as a control with mouse IgG. On the day of the second dose of immunization, AD p-Tau (0.35 μ g/2.5 μ l) was unilaterally injected into the right hippocampus (Fig. 1a). The mice were killed at 7.5 weeks after AD p-Tau injection, and serial coronal sections were collected. No high-intensity AT8 staining was observed in either the ipsilateral or contralateral hippocampus of the mice with saline injection into the hippocampus and treated with 43D (Fig. 4a). However, many neurons in the ipsilateral hippocampus showed prominent Tau inclusions that were labeled with high-intensity AT8 staining in the mice with AD p-Tau injection and treated with mouse IgG (Fig. 4a), which indicates that injected AD p-Tau acts as Tau seeds and induces the host-overexpressed Tau to form NFTs in seeded neurons. Importantly, immunization with 43D dramatically decreased Tau pathology in the ipsilateral hippocampus of the mice injected with AD p-Tau compared with mouse IgG-treated controls. We counted the double AT8 and thioflavin-S-positive neurons from serial 22–25 coronal sections per mouse, which were every fourth section of the brain. The quantification data showed that immunization with 43D significantly decreased Tau pathology in the ipsilateral hippocampus (Fig. 4b).

To determine whether pathological Tau induced by AD p-Tau can spread to the different brain regions distant from the injection sites, we analyzed and compared Tau pathology between the ipsilateral and contralateral hippocampus. We found only a few AT8 intensely stained neurons in the mice injected with saline and treated with 43D, and there was no difference between ipsilateral and contralateral hippocampi (Fig. 4a and b). However, many AT8 intensely positive

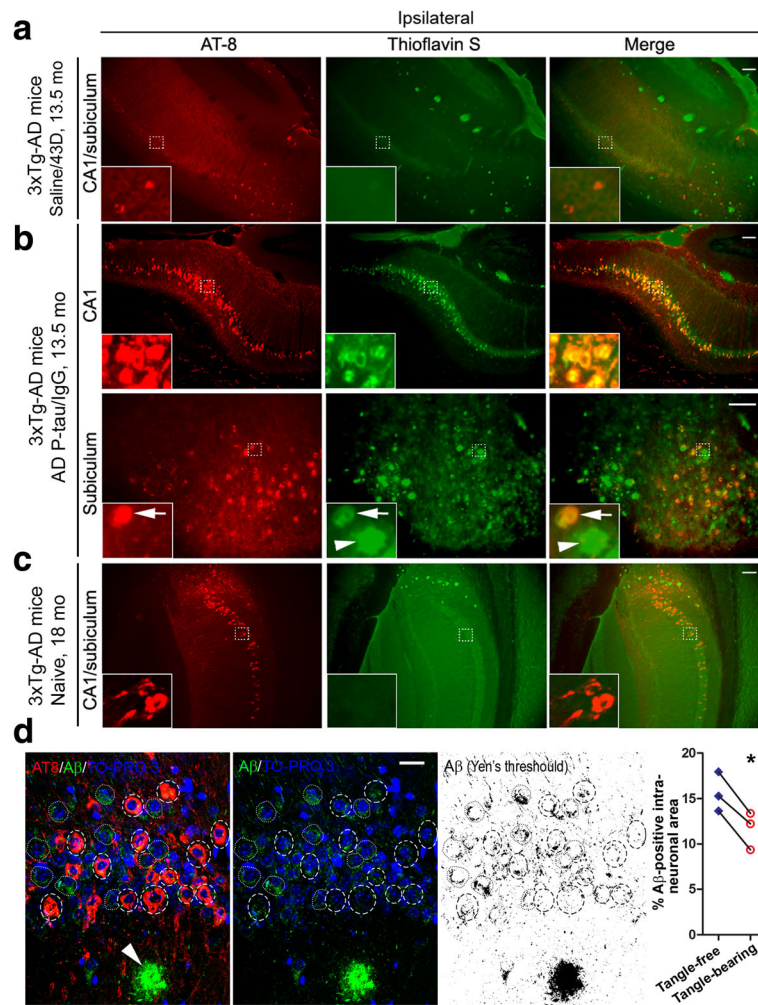
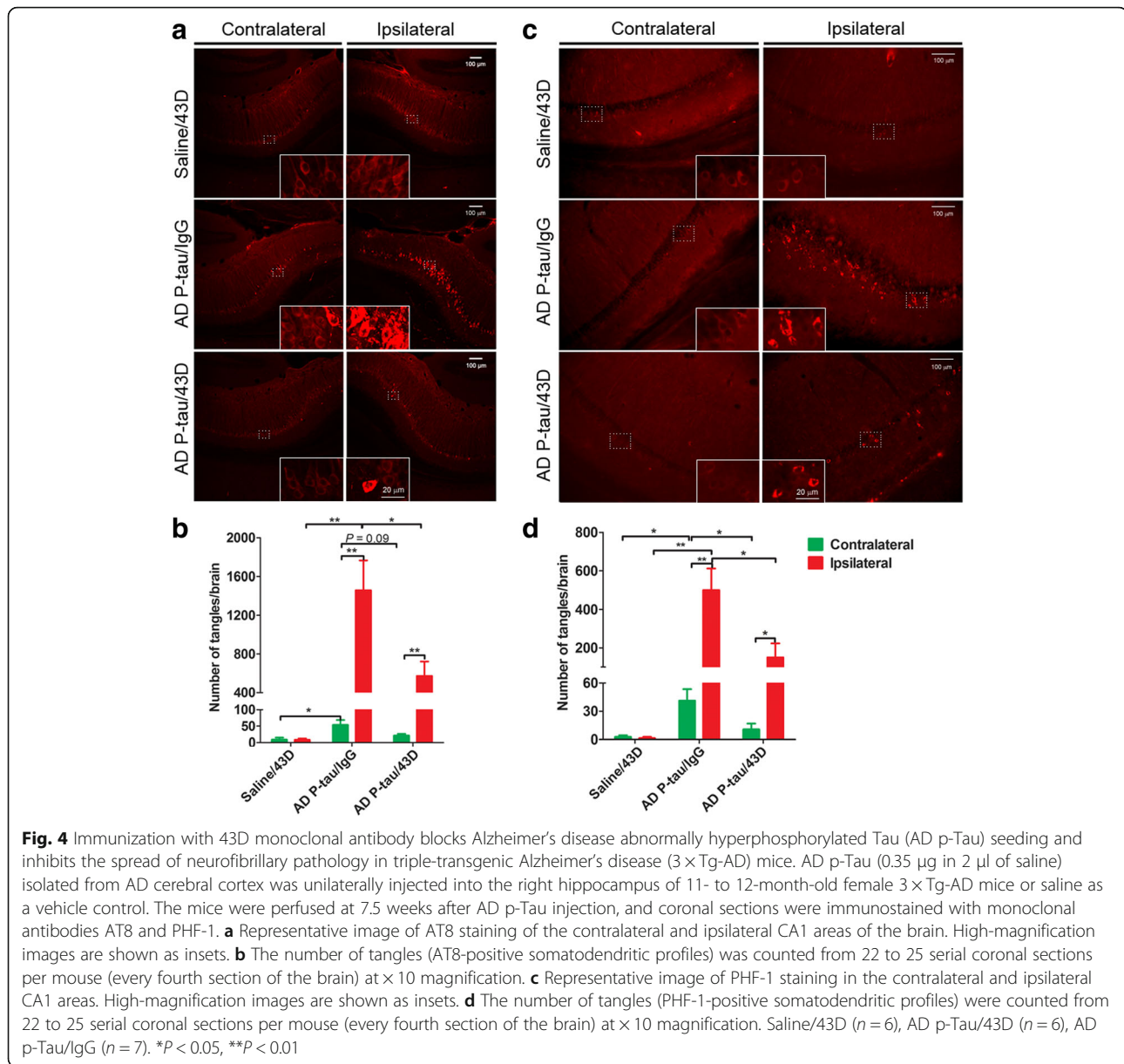


Fig. 3 Alzheimer's disease abnormally hyperphosphorylated Tau (AD p-Tau) seeds and templates the host Tau into neurofibrillary pathology at 7.5 weeks after AD p-Tau injection in triple-transgenic Alzheimer's disease (3 × Tg-AD) mice. AD p-Tau (0.35 μg/2.5 μl saline), or saline (2.5 μl) as vehicle control, was unilaterally injected into the right hippocampus of 11- to 12-month-old female 3 × Tg-AD mice, and the mice were perfused at 7.5 weeks after injection. **a** AT8 and thioflavin-S staining in the 3 × Tg-AD mice injected with saline and treated with 43D. **b** AT8 and thioflavin-S staining in CA1 and subiculum in the 3 × Tg-AD mice injected with AD p-Tau and treated with mouse immunoglobulin G (IgG). **c** AT8 and thioflavin-S staining in naive 18-month-old female 3 × Tg-AD mice. **d** Double-immunofluorescence staining with AT8 and anti-amyloid-β (anti-Aβ) (D54D2) in brain sections of mice injected with AD p-Tau and treated with control mouse IgG. Tangle-bearing neurons (*dashed circles*) showed neither Aβ-positive filamentous inclusions nor increased Aβ somatodendritic immunoreactivity as compared with tangle-free neurons (*dotted circles*). Aβ staining was thresholded using Yen's arithmetic and quantified using ImageJ software, and results were expressed as average percentage area occupied by Aβ staining in the somatodendritic compartment of tangle-bearing neurons compared with adjacent tangle-free neurons at × 60 magnification (n = 3 mice). High-magnification views of the boxed regions are shown as insets. *Arrows* = tangles; *arrowheads* = Aβ plaques. *P < 0.05, paired t test. Scale bars = 100 μm (**a-c**) and 20 μm (**d**)

NFTs were found in the contralateral hippocampus of the mice injected with AD p-Tau and treated with mouse IgG. The number of AT8 intensely stained neurons in the mice injected with AD p-Tau was several-fold higher than that in the mice injected with saline in both the ipsilateral and contralateral hippocampus, suggesting that AD p-Tau was taken up by neurons at the injection site and that the Tau pathology from the seeded neurons of the ipsilateral hippocampus spread to the contralateral hippocampus

(Fig. 4a and b). Compared with mouse IgG treatment, immunization with monoclonal antibody 43D showed a clear trend (P = 0.09) to decrease the number of AT8 intensely stained neurons in the contralateral hippocampus, though it did not reach statistical significance (Fig. 4a and b). Importantly, similar results were also found with PHF-1 staining (Fig. 4c and d). These findings indicate that immunization with 43D blocks the AD p-Tau induced seeding and inhibits the spread of Tau.



AD p-Tau accelerates amyloid plaque pathology

Previously, we discovered that immunization with Tau antibody 43D can inhibit not only Tau but also Aβ pathology in 3 × Tg-AD mice [18, 19]. To investigate whether AD p-Tau injection can promote the formation of amyloid plaques, we compared the amyloid plaque load with thioflavin-S staining between the ipsilateral and contralateral brain in AD p-Tau/IgG (n = 7) and AD p-Tau/43D (n = 6) mice. We found a higher amyloid plaque burden in the AD p-Tau-injected side of the brain than in the contralateral brain, but we found a similar level of amyloid plaque load between the saline-injected side and the contralateral side (Fig. 5a, b, e). Importantly, the AD p-Tau-injected side had more amyloid plaque load than

the saline-injected side (Fig. 5e). These data indicate that AD p-Tau can accelerate the development of Aβ pathology. Consistently, the amyloid plaque load was larger in the AD p-Tau-injected animals than in saline/43D and AD p-Tau/43D mice; 43D treatment showed a trend to decrease the plaque load in the AD p-Tau/43D mice compared with AD p-Tau/IgG mice (Fig. 5c and d).

Discussion

We recently discovered that immunotherapy targeting the N-terminal projection domain of Tau with mouse monoclonal antibody 43D (Tau 6–18) decreases both Tau and Aβ pathologies and rescues memory impairment in 3 × Tg-AD mice [18, 19]. However, whether

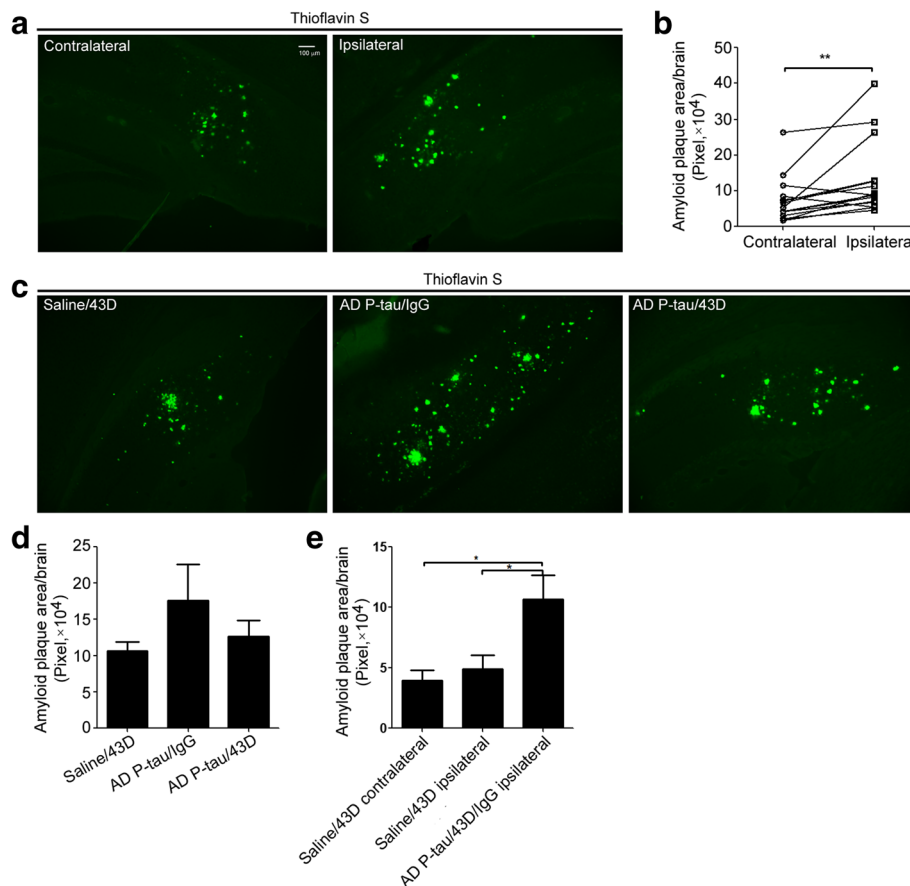


Fig 5 Alzheimer's disease abnormally hyperphosphorylated Tau (AD p-Tau) injection accelerates the development of amyloid- β (A β) plaques in triple-transgenic Alzheimer's disease (3 \times Tg-AD) mice. AD p-Tau (0.35 μ g in 2 μ l of saline) isolated from autopsied AD cerebral cortex was unilaterally injected into the right hippocampus of 11- to 12-month-old female 3 \times Tg-AD mice or saline as a vehicle control. The mice were perfused at 7.5 weeks after AD p-Tau injection, and the coronal sections were stained with thioflavin-S. **a** Representative images of thioflavin-S staining of plaques in the contralateral and ipsilateral subiculum of mice in the AD p-Tau/immunoglobulin G (IgG) group. **b** Plaque areas in the contralateral and ipsilateral sides of the brain were calculated at $\times 10$ magnification from 22 to 25 serial coronal sections per mouse in AD p-Tau/43D ($n = 6$) and AD p-Tau/IgG ($n = 7$) groups. ****** $P < 0.01$, paired t test. **c** Representative images of thioflavin-S staining of plaques in the ipsilateral subiculum of the mice in saline/43D, AD p-Tau/IgG, and AD p-Tau/43D groups. **d** Plaque areas were calculated at $\times 10$ magnification from 22 to 25 serial coronal sections per mouse (every fourth section of the brain) in mice treated with saline/43D ($n = 6$), AD p-Tau/IgG ($n = 7$), and AD p-Tau/43D ($n = 6$). **e** Plaque areas in the contralateral and ipsilateral hippocampus in saline/43D mice ($n = 6$) and in the ipsilateral hippocampus in AD p-Tau/43D or IgG ($n = 13$) were measured at $\times 10$ magnification from 22 to 25 serial coronal sections per mouse (every fourth section of the brain). A two-tailed t test was used for ipsilateral side between saline/43D and AD p-Tau/43D and AD p-Tau/IgG mice. A paired t test was used between contralateral and ipsilateral hippocampus of saline/43D mice. ***P < 0.05**

immunization with 43D can block AD p-Tau seeding and prevent the spread of Tau pathology *in vivo* was not known. The present study shows, for the first time to our knowledge, that immunization with 43D antibody can block AD p-Tau seeding and the consequent spread of neurofibrillary pathology in 3 \times Tg-AD mice.

It has been well established that 0.1–1% of circulating antibodies can enter the brain [30, 31]. Mouse IgG has a half-life of about 1 week in plasma [32, 33]. Researchers in several studies have reported that passive immunization with high doses of antibodies (15–30 mg/kg, *i.p.*) decreased Tau pathology and rescued memory dysfunction [15, 34, 35]; however, Lee et al. [36] did not find a dose-

dependent decrease of Tau pathology in Tau P301L mice treated once weekly with intraperitoneal injections of anti-pS409-Tau antibody at 3, 10, or 30 mg/kg for 3 months. In our studies, a dose of 43D antibody (100 μ g/mouse \sim 3.5 mg/kg, *i.p.*, once weekly for 4 weeks, or 15 μ g/mouse \sim 0.5 mg/kg, *i.v.*, once weekly for 6 weeks) decreased Tau pathology and rescued memory deficits [18, 19]. In the present study, we found that Tau antibody 43D at an *i.v.* dose of \sim 0.5 mg/kg blocks AD p-Tau-induced seeding and spread of Tau pathology in 3 \times Tg-AD mice. Consistent with our studies, passive immunization targeting Tau with a dose similar to the one we used was shown to improve memory in AD mouse models [37, 38]. A single

intravenous injection of 30 μ g of oligomeric Tau antibody was reported to reverse both locomotor and memory deficits in a mouse model of tauopathy [39].

In our previous work, we found that AD p-Tau templates host Tau into neurofibrillary pathology, which shows as both high-intensity AT8- and thioflavin-S-positive staining in h-Tau mice [17]. Therefore, in the present study, we employed positive high-intensity AT8 staining to determine the seeding and spread of Tau pathology in 3 \times Tg-AD mice. We found only weak AT8 staining in both the contralateral and ipsilateral hippocampus in saline-injected 13.5-month-old 3 \times Tg-AD mice. However, high-intensity AT8 staining that colocalized with thioflavin-S staining was apparent at 6–7.5 weeks after AD p-Tau injection in 3 \times Tg-AD mice. Interestingly, although we observed similar high-intensity AT8 staining in naive 18-month-old female 3 \times Tg-AD mice, these AT8-positive neurons were mostly thioflavin-S-negative. One possible explanation is that high-intensity AT8 staining, even in 18-month-old 3 \times Tg-AD mice, represents a nonfibrillized hyperphosphorylated Tau (i.e., a less advanced stage than NFTs). Thus, it appears that Tau pathology induced by AD p-Tau in 3 \times Tg-AD mice is similar to that seen in AD. The prion-like seeding and propagation of Tau pathology in the present study is consistent with previous studies in which investigators employed intracerebral injection of brain homogenates from Tau-transgenic mice [9, 40, 41] and humans with pathologically confirmed AD or related tauopathies [10, 42], or synthetic tau fibrils formed *in vitro* [11, 43], into h-Tau-transgenic mice or wild-type mice [10, 44] to show the conversion of the host Tau into insoluble NFT-like aggregates and their spread in recipient mice. The spread of Tau pathology in a progressive manner to the hippocampus was also shown in human P301L Tau-transgenic mice that express the transgene apparently specifically in layer II neurons of the EC [12, 13].

Previous studies showed that seeded Tau pathology can spread to anatomically connected areas via synaptic connection [12, 13, 40, 41, 43]. In a recent study, we found seeded somatodendritic Tau pathology in axonally connected upstream cortex areas, suggesting that hyperphosphorylated Tau seeds isolated from AD brain might have templated or been transported retrogradely to the somatodendritic compartment [17]. Because the left and right hippocampi innervate each other, we postulate that axonal connection could be the main approach through which Tau is spread to the contralateral side. However, the possibility of Tau to spread to the contralateral hippocampus through interstitial/ventricular fluid cannot be ruled out.

We previously found the spread of Tau pathology 11 months after AD p-Tau injection in h-Tau mice [17].

In the present study, we found the seeding and spread of Tau pathology induced by AD p-Tau as early as 6 weeks after AD p-Tau injection in a time-dependent manner similar to that observed in human tauopathies and its inhibition with Tau antibody 43D in 3 \times Tg-AD mice. These features make the AD p-Tau-injected 3 \times Tg-AD mice a practical *in vivo* model to screen Tau-based treatment approaches.

Recent studies have shown that Tau can be released from intact neurons into the extracellular space as a physiological process that is independent from neuronal death [45–47], which indicates that constitutive Tau release into extracellular space could play an important role in the spread of Tau pathology in AD and related tauopathies. Importantly, mostly N-terminal, but not C-terminal, fragments are found in extracellular Tau in AD cerebrospinal fluid (CSF) [48, 49]. Extracellular Tau seeds can enter neurons and further induce intracellular Tau accumulation and subsequent spreading of Tau pathology. Therefore, extracellular Tau plays a pivotal role in the pathogenesis of AD and related tauopathies and provides a potential target for the treatment of AD and related tauopathies. In the present study, we did not collect and analyze CSF or interstitial levels of Tau and thus do not know whether the inhibition of Tau pathology observed by immunization with 43D was due to clearance of only extracellular or both intracellular and extracellular Tau; only a fraction of the antibody is known to be taken up by neurons [50, 51]. Tau antibodies can enter neurons through clathrin-mediated endocytosis following binding to low-affinity Fc γ II or Fc γ III receptors on neurons [50, 51] or through other mechanisms [34, 52], and they interact with intracellular Tau that inhibits its aggregation and prevents its release into the extracellular space and subsequent spread in the brain [53, 54]. Thus, intracellular interaction between 43D antibody and Tau may also contribute to the inhibition of the seeding and spread of Tau pathology.

According to the A β cascade hypothesis, Tau pathology is believed to be downstream of A β pathology [55]. However, studies of human AD and control brains of various ages revealed that Tau pathology can also precede A β pathology in aged and AD brains [56, 57]. In the present study, we found that AD p-Tau injection enhanced the A β plaque load in the ipsilateral side compared with the contralateral side of the brain, and immunization with 43D shows a trend to reduce A β pathology. Previously, we found that immunization with 43D can decrease A β pathology in mice [18, 19], which was confirmed recently by Sigurdsson's group, who showed that active immunization with a Tau peptide reduces both Tau and A β pathologies in 3 \times Tg-AD mice [58]. These findings indicate that Tau pathology may affect A β pathology. The possible mechanism by which Tau pathology affects A β pathology is currently not understood.

Although we did not detect any A β in the AD p-Tau preparation, the presence of undetectable amounts of A β oligomers sufficient to induce A β pathology cannot be ruled out. Alternatively, Tau pathology could possibly enhance A β pathology by promoting neurodegeneration-associated amyloidogenic processing of APP and/or by inhibiting the degradation of A β . Similarly, inhibition of Tau pathology by immunization with Tau antibody 43D could have increased neuronal plasticity and consequently reduced A β pathology by reducing the amyloidogenic processing of APP. In our previous study, we found that immunization with 43D increased the levels of C1q, the first protein in the complement cascade, and C9, the late-stage activation marker, and promoted microglial activation and aggregation around A β plaques [18]. The complement system plays primary role in innate and adaptive immune response, and several studies indicate that complement may also play an important role in the pathogenesis and progression of AD [59]. C1q can protect cultured primary neurons against A β -induced neurotoxicity [60]. Knockout of C3 or overexpression of complement receptor-related protein γ (Crry), an inhibitor of complement C3 convertase, increases the amyloid load but reduces phagocytic microglia [61, 62]. Microglia are the phagocytes of the brain and express complement receptor CR3. C3 promotes plaque clearance via triggering of A β phagocytosis by microglia [63, 64]. Therefore, the activation of the complement system by passive immunization with monoclonal antibody 43D might be involved in eliminating aggregated and toxic proteins.

Conclusions

The present study shows that the 3 \times Tg-AD mouse intracerebrally injected with AD p-Tau provides a practical model of seeding and spread of tau pathology and acceleration of A β pathology. Tau pathology can promote A β pathology. Immunization with 43D blocks the AD p-Tau-induced seeding and the spread of Tau pathology and acceleration of A β pathology. These studies indicate that immunization with 43D against N-terminal projection domain of Tau (Tau 6–18) represents a potential treatment opportunity for AD and related tauopathies.

Abbreviations

A β : Amyloid- β ; AD: Alzheimer's disease; AD p-Tau: AD abnormally hyperphosphorylated Tau; CA: Cornu amonis; CSF: Cerebrospinal fluid; DAB: 3,3'-Diaminobenzidine; EC: Entorhinal cortex; ECL: Enhanced chemiluminescence; EDTA: Ethylenediaminetetraacetic acid; h-Tau: Human Tau; IgG: Immunoglobulin G; NFT: Neurofibrillary tangle; TDP-43: Transactive response DNA-binding protein 43; 3 \times Tg-AD: Triple-transgenic Alzheimer's disease mouse model

Acknowledgements

Tau antibodies 43D and 77E9 employed for this study were generated and characterized under the supervision of Dr. Inge Grundke-Iqbal, who died on September 22, 2012.

Funding

This work was supported by Alzheimer's Association grants 2015-NIRG-339945 and 2016-NIRG-397030 and by the New York State Office of People with Developmental Disabilities (OPWDD).

Availability of data and materials

The datasets generated during and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

CLD performed the main studies, analyzed the data, and wrote the manuscript. WH and YCT carried out the study. FL and CXG contributed to manuscript revision. KI designed the study, analyzed the data, and helped to revise the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All procedures involving mice were reviewed and approved by the Institute for Basic Research in Developmental Disabilities Animal Care and Use Committee (protocol number 198) in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals (revised February 15, 2015).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 17 August 2017 Accepted: 12 January 2018

Published online: 31 January 2018

References

- Glenner GG, Wong CW. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem Biophys Res Commun*. 1984;120:885–90.
- Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI. Abnormal phosphorylation of the microtubule-associated protein tau (τ) in Alzheimer cytoskeletal pathology. *Proc Natl Acad Sci U S A*. 1986;83:4913–7.
- Avila J, Lucas JJ, Perez M, Hernandez F. Role of tau protein in both physiological and pathological conditions. *Physiol Rev*. 2004;84:361–84.
- Alonso AC, Zaidi T, Grundke-Iqbal I, Iqbal K. Role of abnormally phosphorylated tau in the breakdown of microtubules in Alzheimer disease. *Proc Natl Acad Sci U S A*. 1994;91:5562–6.
- Alonso A, Zaidi T, Novak M, Grundke-Iqbal I, Iqbal K. Hyperphosphorylation induces self-assembly of tau into tangles of paired helical filaments/straight filaments. *Proc Natl Acad Sci U S A*. 2001;98:6923–8.
- Alonso AC, Grundke-Iqbal I, Iqbal K. Alzheimer's disease hyperphosphorylated tau sequesters normal tau into tangles of filaments and disassembles microtubules. *Nat Med*. 1996;2:783–7.
- Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol*. 1991;82:239–59.
- Braak H, Del Tredici K. The pathological process underlying Alzheimer's disease in individuals under thirty. *Acta Neuropathol*. 2011;121:171–81.
- Clavaguera F, Bolmont T, Crowther RA, Abramowski D, Frank S, Probst A, et al. Transmission and spreading of tauopathy in transgenic mouse brain. *Nat Cell Biol*. 2009;11:909–13.
- Clavaguera F, Akatsu H, Fraser G, Crowther RA, Frank S, Hench J, et al. Brain homogenates from human tauopathies induce tau inclusions in mouse brain. *Proc Natl Acad Sci U S A*. 2013;110:9535–40.
- Iba M, Guo JL, McBride JD, Zhang B, Trojanowski JQ, Lee VM. Synthetic tau fibrils mediate transmission of neurofibrillary tangles in a transgenic mouse model of Alzheimer's-like tauopathy. *J Neurosci*. 2013;33:1024–37.
- Liu L, Drouot V, Wu JW, Witter MP, Small SA, Clelland C, et al. Trans-synaptic spread of tau pathology in vivo. *PLoS One*. 2012;7:e31302.
- de Calignon A, Polydoro M, Suarez-Calvet M, Williams C, Adamowicz DH, Kopeikina KJ, et al. Propagation of tau pathology in a model of early Alzheimer's disease. *Neuron*. 2012;73:685–97.

14. Frost B, Jacks RL, Diamond MI. Propagation of tau misfolding from the outside to the inside of a cell. *J Biol Chem*. 2009;284:12845–52.
15. Sankaranarayanan S, Barten DM, Vana L, Devidze N, Yang L, Cadelina G, et al. Passive immunization with phospho-tau antibodies reduces tau pathology and functional deficits in two distinct mouse tauopathy models. *PLoS One*. 2015;10:e0125614.
16. Yanamandra K, Kfoury N, Jiang H, Mahan TE, Ma S, Maloney SE, et al. Anti-tau antibodies that block tau aggregate seeding in vitro markedly decrease pathology and improve cognition in vivo. *Neuron*. 2013;80:402–14.
17. Hu W, Zhang X, Tung YC, Xie S, Liu F, Iqbal K. Hyperphosphorylation determines both the spread and the morphology of tau pathology. *Alzheimers Dement*. 2016;12:1066–77.
18. Dai CL, Tung YC, Liu F, Gong CX, Iqbal K. Tau passive immunization inhibits not only tau but also A β pathology. *Alzheimers Res Ther*. 2017;9:1.
19. Dai CL, Chen X, Liu F, Gong CX, Grundke-Iqbal I, Iqbal K, et al. Passive immunization targeting the N-terminal projection domain of tau decreases tau pathology and improves cognition in a transgenic mouse model of Alzheimer disease and tauopathies. *J Neural Transm (Vienna)*. 2015;122:607–17.
20. Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kaye R, et al. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular A β and synaptic dysfunction. *Neuron*. 2003;39:409–21.
21. Blanchard J, Wanka L, Tung YC, del Carmen C-AM, LaFerla FM, Iqbal K, et al. Pharmacologic reversal of neurogenic and neuroplastic abnormalities and cognitive impairments without affecting A β and tau pathologies in 3xTg-AD mice. *Acta Neuropathol*. 2010;120:605–21.
22. Kazim SF, Blanchard J, Dai CL, Tung YC, LaFerla FM, Iqbal K, et al. Disease modifying effect of chronic oral treatment with a neurotrophic peptidergic compound in a triple transgenic mouse model of Alzheimer's disease. *Neurobiol Dis*. 2014;71:110–30.
23. Carroll JC, Rosario ER, Kreimer S, Villamagna A, Gentsch E, Stanczyk FZ, et al. Sex differences in β -amyloid accumulation in 3xTg-AD mice: role of neonatal sex steroid hormone exposure. *Brain Res*. 2010;1366:233–45.
24. Hirata-Fukae C, Li HF, Hoe HS, Gray AJ, Minami SS, Hamada K, et al. Females exhibit more extensive amyloid, but not tau, pathology in an Alzheimer transgenic model. *Brain Res*. 2008;1216:92–103.
25. Clinton LK, Billings LM, Green KN, Caccamo A, Ngo J, Oddo S, et al. Age-dependent sexual dimorphism in cognition and stress response in the 3xTg-AD mice. *Neurobiol Dis*. 2007;28:76–82.
26. Rodriguez JJ, Jones VC, Tabuchi M, Allan SM, Knight EM, LaFerla FM, et al. Impaired adult neurogenesis in the dentate gyrus of a triple transgenic mouse model of Alzheimer's disease. *PLoS One*. 2008;3:e2935.
27. Kopke E, Tung YC, Shaikh S, Alonso AC, Iqbal K, Grundke-Iqbal I. Microtubule-associated protein tau: abnormal phosphorylation of a non-paired helical filament pool in Alzheimer disease. *J Biol Chem*. 1993;268:24374–84.
28. Urbanc B, Cruz L, Le R, Sanders J, Ashe KH, Duff K, et al. Neurotoxic effects of thioflavin S-positive amyloid deposits in transgenic mice and Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2002;99:13990–5.
29. Sun A, Nguyen XV, Bing G. Comparative analysis of an improved thioflavin-S stain, Gallyas silver stain, and immunohistochemistry for neurofibrillary tangle demonstration on the same sections. *J Histochem Cytochem*. 2002; 50:463–72.
30. Shah DK, Betts AM. Antibody biodistribution coefficients: inferring tissue concentrations of monoclonal antibodies based on the plasma concentrations in several preclinical species and human. *MAbs*. 2013;5:297–305.
31. Wang W, Wang EQ, Balthasar JP. Monoclonal antibody pharmacokinetics and pharmacodynamics. *Clin Pharmacol Ther*. 2008;84:548–58.
32. Vieira P, Rajewsky K. The bulk of endogenously produced IgG2a is eliminated from the serum of adult C57BL/6 mice with a half-life of 6–8 days. *Eur J Immunol*. 1986;16:871–4.
33. Agadjanyan MG, Zagorski K, Petrushina I, Davtyan H, Kazarian K, Antonenko M, et al. Humanized monoclonal antibody armanezumab specific to N-terminus of pathological tau: characterization and therapeutic potency. *Mol Neurodegener*. 2017;12:33.
34. Collin L, Bohrmann B, Gopfert U, Oroszlan-Szovik K, Ozmen L, Gruninger F. Neuronal uptake of tau/pS422 antibody and reduced progression of tau pathology in a mouse model of Alzheimer's disease. *Brain*. 2014;137:2834–46.
35. Chai X, Wu S, Murray TK, Kinley R, Cella CV, Sims H, et al. Passive immunization with anti-Tau antibodies in two transgenic models: reduction of Tau pathology and delay of disease progression. *J Biol Chem*. 2011;286:34457–67.
36. Lee SH, Le Pichon CE, Adolfsen O, Gafner V, Pihlgren M, Lin H, et al. Antibody-mediated targeting of tau in vivo does not require effector function and microglial engagement. *Cell Rep*. 2016;16:1690–700.
37. Umeda T, Eguchi H, Kunori Y, Matsumoto Y, Taniguchi T, Mori H, et al. Passive immunotherapy of tauopathy targeting pSer413-tau: a pilot study in mice. *Ann Clin Transl Neurol*. 2015;2:241–55.
38. Boutajangout A, Ingadottir J, Davies P, Sigurdsson EM. Passive immunization targeting pathological phospho-tau protein in a mouse model reduces functional decline and clears tau aggregates from the brain. *J Neurochem*. 2011;118:658–67.
39. Castillo-Carranza DL, Sengupta U, Guerrero-Muñoz MJ, Lasagna-Reeves CA, Gerson JE, Singh G, et al. Passive immunization with Tau oligomer monoclonal antibody reverses tauopathy phenotypes without affecting hyperphosphorylated neurofibrillary tangles. *J Neurosci*. 2014;34:4260–72.
40. Ahmed Z, Cooper J, Murray TK, Garn K, McNaughton E, Clarke H, et al. A novel in vivo model of tau propagation with rapid and progressive neurofibrillary tangle pathology: the pattern of spread is determined by connectivity, not proximity. *Acta Neuropathol*. 2014;127:667–83.
41. Takeda S, Wegmann S, Cho H, DeVos SL, Commins C, Roe AD, et al. Neuronal uptake and propagation of a rare phosphorylated high-molecular-weight tau derived from Alzheimer's disease brain. *Nat Commun*. 2015;6:8490.
42. Boluda S, Iba M, Zhang B, Raible KM, Lee VM, Trojanowski JQ. Differential induction and spread of tau pathology in young PS19 tau transgenic mice following intracerebral injections of pathological tau from Alzheimer's disease or corticobasal degeneration brains. *Acta Neuropathol*. 2015;129:221–37.
43. Peeraer E, Bottelbergs A, Van Kolen K, Stancu IC, Vasconcelos B, Mahieu M, et al. Intracerebral injection of preformed synthetic tau fibrils initiates widespread tauopathy and neuronal loss in the brains of tau transgenic mice. *Neurobiol Dis*. 2015;73:83–95.
44. Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, Guerrero-Munoz MJ, Kiritoshi T, Neugebauer V, et al. Alzheimer brain-derived tau oligomers propagate pathology from endogenous tau. *Sci Rep*. 2012;2:700.
45. Chai X, Dage JL, Citron M. Constitutive secretion of tau protein by an unconventional mechanism. *Neurobiol Dis*. 2012;48:356–66.
46. Pooler AM, Phillips EC, Lau DH, Noble W, Hanger DP. Physiological release of endogenous tau is stimulated by neuronal activity. *EMBO Rep*. 2013;14:389–94.
47. Yamada K, Holth JK, Liao F, Stewart FR, Mahan TE, Jiang H, et al. Neuronal activity regulates extracellular tau in vivo. *J Exp Med*. 2014;211:387–93.
48. Bright J, Hussain S, Dang V, Wright S, Cooper B, Byun T, et al. Human secreted tau increases amyloid- β production. *Neurobiol Aging*. 2015;36:693–709.
49. Meredith Jr JE, Sankaranarayanan S, Guss V, Lanzetti AJ, Berisha F, Neely RJ, et al. Characterization of novel CSF Tau and ptau biomarkers for Alzheimer's disease. *PLoS One*. 2013;8:e76523.
50. Congdon EE, Gu J, Sait HB, Sigurdsson EM. Antibody uptake into neurons occurs primarily via clathrin-dependent Fc γ receptor endocytosis and is a prerequisite for acute tau protein clearance. *J Biol Chem*. 2013;288:35452–65.
51. Gu J, Congdon EE, Sigurdsson EM. Two novel Tau antibodies targeting the 396/404 region are primarily taken up by neurons and reduce Tau protein pathology. *J Biol Chem*. 2013;288:33081–95.
52. McEwan WA, Falcon B, Vaysburd M, Clift D, Oblak AL, Ghetti B, et al. Cytosolic Fc receptor TRIM21 inhibits seeded tau aggregation. *Proc Natl Acad Sci U S A*. 2017;114:574–9.
53. Wang Y, Mandelkow E. Tau in physiology and pathology. *Nat Rev Neurosci*. 2016;17:5–21.
54. Pedersen JT, Sigurdsson EM. Tau immunotherapy for Alzheimer's disease. *Trends Mol Med*. 2015;21:394–402.
55. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science*. 2002;297:353–6.
56. Braak H, Braak E. Frequency of stages of Alzheimer-related lesions in different age categories. *Neurobiol Aging*. 1997;18:351–7.
57. Braak H, Zetterberg H, Del Tredici K, Blennow K. Intraneuronal tau aggregation precedes diffuse plaque deposition, but amyloid- β changes occur before increases of tau in cerebrospinal fluid. *Acta Neuropathol*. 2013;126:631–41.
58. Rajamohamedsait H, Rasool S, Rajamohamedsait W, Lin Y, Sigurdsson EM. Prophylactic active tau immunization leads to sustained reduction in both tau and amyloid- β pathologies in 3xTg mice. *Sci Rep*. 2017;7:17034.
59. Bonifati DM, Kishore U. Role of complement in neurodegeneration and neuroinflammation. *Mol Immunol*. 2007;44:999–1010.

60. Pisalyaput K, Tenner AJ. Complement component C1q inhibits β -amyloid- and serum amyloid P-induced neurotoxicity via caspase- and calpain-independent mechanisms. *J Neurochem*. 2008;104:696–707.
61. Wyss-Coray T, Yan F, Lin AH, Lambris JD, Alexander JJ, Quigg RJ, et al. Prominent neurodegeneration and increased plaque formation in complement-inhibited Alzheimer's mice. *Proc Natl Acad Sci U S A*. 2002;99:10837–42.
62. Maier M, Peng Y, Jiang L, Seabrook TJ, Carroll MC, Lemere CA. Complement C3 deficiency leads to accelerated amyloid β plaque deposition and neurodegeneration and modulation of the microglia/macrophage phenotype in amyloid precursor protein transgenic mice. *J Neurosci*. 2008;28:6333–41.
63. Fu H, Liu B, Frost JL, Hong S, Jin M, Ostaszewski B, et al. Complement component C3 and complement receptor type 3 contribute to the phagocytosis and clearance of fibrillar A β by microglia. *Glia*. 2012;60:993–1003.
64. Choucair-Jaafar N, Laporte V, Levy R, Poindron P, Lombard Y, Gies JP. Complement receptor 3 (CD11b/CD18) is implicated in the elimination of β -amyloid peptides. *Fundam Clin Pharmacol*. 2011;25:115–22.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at
www.biomedcentral.com/submit

