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Passive immunization targeting pathological phospho-tau protein in a mouse model reduces functional decline and clears tau aggregates from the brain

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Abstract

Targeting hyperphosphorylated tau by immunotherapy is emerging as a promising approach to treat tauopathies such as Alzheimer's disease and frontotemporal dementia. We have previously reported that active tau immunization clears tau aggregates from the brain and attenuates or prevents functional impairments in two different tangle mouse models. Here, we assessed the efficacy of passive immunization with the PHF1 antibody, which targets a phospho-epitope within one of our active immunogens. Homozygous female tangle mice (JNPL3, 2–3 months) were injected intraperitoneally once per week with PHF1 or pooled mouse IgG (250 μg/ 125 μL; $n = 10$ per group) for a total of 13 injections. Their behavior was assessed at 5–6 months of age and brain tissue was subsequently harvested for analyses of treatment efficacy. The treated mice performed better than controls on the traverse beam task ($p < 0.03$), and had 58% less tau pathology in the dentate gyrus of the hippocampus ($p = 0.02$). As assessed by western blots, the antibody therapy reduced the levels of insoluble pathological tau by 14–27% (PHF1, $p < 0.05$; PHF1/total tau, $p < 0.0001$) and 34–45% (CP13 or CP13/total tau, $p < 0.05$). Levels of soluble tau and sarkosyl soluble tau were unchanged, compared with controls, as well as total tau levels in all the fractions. Plasma levels of PHF1 correlated inversely with tau pathology in the brainstem ($p < 0.01$), with a strong trend in the motor cortex ($p < 0.06$) as well as with insoluble total tau levels ($p < 0.02$), indicating that higher dose of antibodies may have a greater therapeutic effect. Significant correlation was also observed between performance on the traverse beam task and PHF1 immunoreactivity in the dentate gyrus ($p < 0.05$) as well as with insoluble PHF1/total tau ratio on western blots ($p < 0.04$). These results show that passive immunization with tau antibodies can decrease tau pathology and functional impairments in the JNPL3 model. Future studies will determine the feasibility of this approach with other monoclonals and in different tangle models in which thorough cognitive assessment can be performed.

Keywords

behavior; immunotherapy; mice; PHF1; tau; tangles

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Conflict of interest
Patent on tau immunotherapy pending.

An emerging therapy for Alzheimer's disease (AD) is immune modulation to clear amyloid-β (Aβ) (Schenk *et al.* 1999), which is likely to be antibody-mediated (Solomon *et al.* 1997; Bard *et al.* 2000; DeMattos *et al.* 2001; Sigurdsson *et al.* 2001, 2004; Bacskai *et al.* 2002; Das *et al.* 2003; Lemere *et al.* 2003), and improves cognition in animal models (Dodart *et al.* 1999; Janus *et al.* 2000; Morgan *et al.* 2000; Kotilinek *et al.* 2002). Unfortunately, the first clinical trial on this approach was halted because of encephalitis in 6% of patients (Schenk 2002), but it is currently being refined in animal models and in several new clinical studies. Some degree of cognitive stabilization was observed in the first trial (Hock *et al.* 2003; Gilman *et al.* 2005) and autopsies suggested removal of Aβ plaques (Nicoll *et al.* 2003, 2006; Ferrer *et al.* 2004; Masliah *et al.* 2005a). However, recent findings from this trial indicate that plaque clearance did not halt or slow the progression of dementia, emphasizing the need for alternative targets (Holmes *et al.* 2008).

Another important target for immunization in AD patients is pathological tau protein that is also the primary target in various tauopathies. Our published findings indicate that active immunization with an AD specific phosphorylated tau epitope, in JNPL3 P301L tangle model mice (Lewis *et al.* 2000), reduces brain levels of aggregated tau and slows progression of the tangle-related behavioral phenotype (Asuni *et al.* 2007). Clearance of extracellular tau/tangles may reduce associated damage and prevent the spread of tau pathology (Sigurdsson *et al.* 2002; Clavaguera *et al.* 2009; Frost *et al.* 2009; Sigurdsson 2009). Our findings (Asuni *et al.* 2007) and numerous reports of neuronal uptake of antibodies suggest that intracellular tau aggregates are also being cleared (Sigurdsson 2009).

Specifically, we have shown that these antibodies enter the brain and bind to pathological tau within neurons based on their colocalization with AD specific tau antibodies (Asuni *et al.* 2007). Furthermore, we have demonstrated that this approach reduces tau aggregates and prevents cognitive decline in three different tests in another tangle model (Boutajangout *et al.* 2010b). Others have reported that immunization with α-synuclein in transgenic mice clears these intraneuronal aggregates (Masliah *et al.* 2005b), and that Aβ antibodies are internalized in cultured neurons and clear intracellular Aβ aggregates (Tampellini *et al.* 2007). These studies support our findings and interpretations. Most recently, the promise of tau immunotherapy has been confirmed by others (Boimel *et al.* 2010). Although the active approach has certain advantages, it may have autoimmune side effects that can be avoided with passive immunization.

Here, we determined in the JNPL3 P301L mouse model, whether the repeated administration of a monoclonal tau antibody, PHF1, would have a therapeutic effect as assessed by functional, histological and biochemical measures. A part of this work was reported previously at the Alzheimer's Association International Conference on Alzheimer's Disease 2010 (Boutajangout *et al.* 2010a).

Materials and methods

Animals and antibody injections

Homozygous female JNPL3 mice (n = 10; obtained from Taconic, USA) were injected intraperitoneally (i.p.) with PHF1, a monoclonal tau antibody generously provided by Dr. Peter Davies, that recognizes neurofibrillary tangles and pre-tangles in Alzheimer's disease and various tangle mouse model, including the JNPL3 model (Lewis *et al.* 2000). It recognizes tau that is phosphorylated on serine amino acids 404 and 396 on C-terminus of tau (Greenberg *et al.* 1992). The antibody dose was 250 μg/125 μL, dissolved in phosphate buffered saline. Identical controls (n = 11) were injected i.p. with the same dose of mouse IgG in phosphate buffered saline (Equitech-Bio Inc., Kerrville, TX, USA). The mice received their first injection at 9–12 weeks of age and then at 7 day intervals for a total of 13

injections, followed by behavioral testing at 5–6 months and subsequent tissue analysis at 6–7 months. One control mouse died prior to behavioral analysis. All animals housed at NYU School of Medicine animal facilities are cared for by the Division of Laboratory Animal Resources veterinary staff in an AAALAC approved facilities. All the animal procedures were approved prior to experimentation by the IACUC committee of the university, and are in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals. These guidelines meet or exceed the issued ARRIVE guidelines.

Antibody levels in plasma

The mice were bled before the commencement of the study and periodically throughout the experiment. Plasma levels of PHF1 were detected by ELISA in which 0.5 μg of a peptide, Tau379–408[$PSer_{396, 404}$], that contains the phosphoepitope recognized by the antibody, was coated per well (Immulon 2HB; Thermo Electron Corp., Milford, MA, USA). The phospho-peptide was synthesized at the Keck Foundation at Yale University. For detection, goat antimouse IgG linked to a horseradish peroxidase (GE Healthcare, Little Chalfont, UK) was used at 1 : 3000 dilution. Tetramethyl benzidine (Pierce, Rockford, IL, USA) was the substrate.

Behavioral studies

At 5–6 months of age, animals went through several behavior tests 1 month prior to tissue harvesting to determine if therapy diminished age-related motor deficits that develop as tau pathology advances in the JNPL3 model. Sensorimotor tests that were performed were traverse beam, rotarod and locomotor activity.

Sensorimotor tests

The mice were adapted to the room with lights on for 15 min prior to testing.

Traverse beam—This task tests balance and general motor coordination and function integration. Mice were assessed by measuring their ability to traverse a graded narrow wooden beam to reach a shaded goal box. A bright light near the starting position on the beam guided the mice towards the goal box. The beam (1.1 cm wide and 50.8 cm long) was placed between two identical columns at 30 cm in height, and under the beam was a foam cushion to prevent injury. Mice were placed on the beam in perpendicular position for habituation, and were then monitored for 60 s. The number of foot slips each mouse had before falling or reaching the goal box were recorded for each of four successive trials and defined as errors. If the mouse fell of the beam before reaching the goal box, the animal was placed back in its position before falling.

Rotarod—Rotarod is used to measure forelimb and hind limb motor coordination and balance. This procedure was designed to assess motor behavior without a practice confound. Mice were first habituated in two trials to reach a baseline level of performance, and subsequently tested in three additional trials with 15 min between trials (Rotarod 7650 accelerating model; Ugo Basile, Biological Research Apparatus, Varese, Italy). Animals were placed on the rod (3.6 cm in diameter) with initial speed set at 1.5 rpm that was then raised every 30 s by 0.5 rpm. A soft foam cushion was placed under the rod to prevent injury from falling. The rod was cleaned with 30% ethanol after each session. To assess the performance, the speed of the rod was recorded when the mouse fell or inverted (by clinging) from the top of the rotating barrel.

Locomotor activity—Mouse activity was recorded over 15 min in a circular open field activity chamber (70 cm in diameter). Mice were first habituated in the chamber few at a

time for 15 min and then each mouse was tested for 15 min. After each session, the field was cleaned with 30% ethanol. A camera above the chamber automatically recorded horizontal movements in each dimension (i.e. *x, y* and two *z* planes) by measuring movement of the animal (white) relative to black background (San Diego Instruments, San Diego, CA, USA). Results are reported as distance traveled (cm), mean resting time and velocity (average and maximum) of the mouse.

Tissue processing and histology

Following behavioral testing, mice were deeply anesthetized with ketamine/xylazine (250 mg/50 mg per kg body weight, i.p.). The brain was subsequently removed without perfusion and processed as previously described (Sigurdsson *et al.* 1996). The left hemisphere was snap frozen and stored at −80°C until processed for western blots. Coronal brain sections (40 μm) of the right hemisphere were saved for histological staining with (i) mouse monoclonal tau antibody that stains pathological tau the [PHF1 (1 : 1000, recognizes phosphorylated serines 396 and 404 of the tau protein) (Otvos *et al.* 1994), generously provided by Peter Davies], (ii) rabbit polyclonal antibody against glial fibrillary acidic protein (GFAP 1 : 500; Dako, Carpinteria, CA, USA) in astrocytes, and (iii) tomatolectin (10 μg/mL; Vector Laboratories, Burlingame, CA, USA) to detect microglia. The sectioned series were placed in ethylene glycol cryoprotectant and stored at −20°C until used for immunohistochemistry. Staining was performed as previously described (Sigurdsson *et al.* 1996, 2001; Asuni *et al.* 2006), using mouse-on-mouse immunodetection kit (Vector) for the monoclonals, with every 10th section stained. To verify that reduced tau immunoreactivity in immunized mice was not caused by epitope masking by the injected PHF1 antibody, unmasking procedure was performed as described previously (Asuni *et al.* 2007).

Image analysis

Tau pathology in brain sections was quantified blindly with the Bioquant program (Bioquant Image Analysis Corp., Nashville, TN, USA) as described previously (Asuni *et al.* 2007). Every 10th section, randomly chosen, was stained with each antibody. For quantitative image analysis of immunohistochemistry, we initially selected the granular layer of the dentate gyrus, which consistently contained intraneuronal tau aggregates (pre-tangles and tangles). We analyzed the motor cortex and brainstem in these animals as well because tau pathology in those regions may relate to the motor abnormalities observed in this model. An individual blind to the experimental conditions of the study performed all procedures. For the PHF1 stained sections, we sampled every 10th section randomly from the mouse brain. In the dentate gyrus, the measurement was the percentage of area in the measurement field at 200× magnification occupied by reaction product with the tip of the dentate gyrus at the left edge of the field. In the motor cortex, the measurement was the percentage of neuronal staining in the field at 100× magnification with the thickest region of the cingulum positioned at the lower left edge of the field. In the brainstem, the measurement was the percentage of neuronal staining in the field at 100× magnification with the center of the top edge of the field positioned below the Aqueduct of Sylvius. Five sections were analyzed per animal for each brain regions.

Rating of microgliosis and astrogliosis—Semi-quantitative analysis was based on the severity of microgliosis throughout the brain (0, predominantly resting microglia; 1+, a few ramified and/or phagocytic microglia; 2+, moderate number of ramified/phagocytic microglia; and 3+, numerous ramified/phagocytic microglia). The rating of the GFAP sections was based on the complexity of astrocytic branching throughout the brain (1+, resting astrocytes, few processes; 2+, reactive astrocytes, moderate branching; and 3+, reactive astrocytes, extensive branching).

Western blots

Brains were weighed and homogenized (10% w/v) in Tris-buffered saline, pH 7.4 [TBS: 10 mM Tris/150 mM NaCl, containing protease inhibitors (one tablet of complete protease inhibitor cocktail (Roche Molecular Biochemicals, Indianapolis, IN, USA) per 50 mL of TBS, 1 mM NaF, 0.4 μM Na_3 VO_4 and 0.5 μM okadaic acid)]. The homogenate was centrifuged at 20 800 *g* for 5 min (4°C) and supernatants collected. For sarkosyl extraction, 11% sarkosyl solution was added to 300 μL supernatant for a final concentration of 1% and then incubated for 1 h at 37°C. Sarkosyl extracted supernatant and supernatant without sarkosyl were then centrifuged at 100 000 *g* for 1 h at 4°C in Beckman TL-100 ultracentrifuge, and the high-speed supernatants were collected and used for western blot analysis. Sarkosyl extraction results in dissociation of insoluble proteins including aggregated tau proteins. However, paired helical filaments, the end stage of tau pathology are not soluble in sarkosyl (Greenberg *et al.* 1990).

For the insoluble fraction, the pellet was re-suspended in the same volume of buffer without protease and phosphatase inhibitors, but that contained 1% (v/v) Triton X-100 and 0.25% (w/v) deoxycholate sodium. It was then ultracentrifuged at 50 000 *g* for 30 min to obtain a detergent extracted supernatant that was analyzed as an insoluble fraction (Boutajangout *et al.* 2002, 2004).

The supernatants from these three fractions were heated at 100°C for 5 min and the same amount of protein was electrophoresed on 12% (w/v) polyacrylamide gel. The blots were blocked in 5% nonfat milk with 0.1% Tween-20 in TBS and incubated with different antibodies overnight, and then washed and incubated at 21–23°C for 1 h with peroxidase-conjugated, anti-mouse or anti-rabbit IgG. Subsequently, the bound antibodies [monoclonal PHF1, monoclonal CP13 (P-Ser202) generously provided by Peter Davies (Albert Einstein College of Medicine, Bronx, NY) and polyclonal B19 (total tau raised against bovine tau) (Brion *et al.* 1991), generously provided by Jean-Pierre Brion (Free University of Brussels, Belgium)] were detected by enhanced chemiluminescence (ECL) (Pierce). Densitometric analysis of immunoblots was performed by the National Institutes of Health ImageJ program and the levels of pathological tau were normalized relative to total tau protein.

Data analysis

All data were analyzed with GraphPad Prism 4.3. Locomotor activity (distance traveled, maximum velocity, average speed and resting time), tau aggregates on western blots and immunoreactivity on brain sections within the dentate gyrus, motor cortex and brainstem were analyzed with unpaired *t*-test. Welch correction was used if the data failed a test of equal variance. When data failed two out of three normality tests (KS, D'Agostino & Pearson omnibus, and Shapiro–Wilk normality tests) non-parametric Mann–Whitney test was used. The tests were one-tailed as it was assumed based on our past findings that the immunotherapy would lead to clearance of tau pathology that would slow the progression of behavioral impairments. Total tau levels were not expected to change based on our previous studies and, therefore, were analyzed with two-tailed analysis. Two of the behavioral test, the traverse beam test and the rotarod test were analyzed by unpaired *t*-test or Mann–Whitney (trials combined) and two-way ANOVA (treatment *x* trials). Correlation between behavioral tests and tau pathology and between the plasma levels of PHF1 antibodies versus tau pathology or behavior was analyzed with Pearson *r* correlation or Spearman rank correlation when the data failed two out of three normality tests described above.

Results

To determine the feasibility of passive immunotherapy, homozygous P301L mice received 13 weekly i.p. injections with PHF1, a monoclonal tau antibody that recognizes neurofibrillary tangles and pre-tangles in various tangle mouse models, including the JNPL3 model employed in the present study, as well as in AD and other tauopathies (Lewis *et al.* 2000). PHF1 recognizes tau that is phosphorylated on serine amino acids 396 and 404 on the C-terminus of tau (Greenberg *et al.* 1992). It is, therefore, a monoclonal analog of the prototype of our active immunization approach (Asuni *et al.* 2007; Boutajangout *et al.* 2010b), Tau379–408[$PSer_{396, 404}$] that contains the PHF1 antibody epitope.

As assessed by western blots at the end of the study (Fig. 1a–d), the antibody therapy reduced the ratio of pathological to total tau by 27% (PHF1/B19; $p < 0.0001$) and 45% (CP13/B19; $p < 0.05$), but the levels of soluble tau and sarkosyl soluble tau were unchanged, compared with controls. Total tau levels did not differ significantly between the groups in any of the three fractions. In concord with the ratio analysis, levels of PHF1 tau only differed between the groups in the insoluble fraction (14% reduction, $p < 0.05$). Likewise, CP13 tau levels were decreased in the immunized mice in the insoluble fraction (34% reduction, $p < 0.05$). For comparison, representative samples from the control and immunized groups were run on the same gel, clearly showing PHF1-mediated clearance of the insoluble tau fraction (Fig. 1e)

Histological analysis indicated that the treated mice had 58% less PHF1 tau pathology in the dentate gyrus of the hippocampus ($p = 0.02$), and there was a strong trend for a decrease in PHF1 tau in the motor cortex (43% reduction, $p = 0.12$), but not in the brainstem that has more advanced tau pathology (Fig. 2a–m). These findings are in line with the western blots. Similar degree of micro and astrogliosis was observed in both groups (Fig. 3), which is analogous to our prior studies with active tau immunotherapy (Asuni *et al.* 2007; Boutajangout *et al.* 2010b).

Plasma levels of PHF1 were measured prior to the first injection (T0), 24 h after the 12th injection (T1), then 7 (T2) and 14 (T3) days after the 13th injection. As expected, time dependent decline in plasma PHF1 was observed, and the rate of decline seemed to fit with typical half-life of injected IgG (Fig. 4a). Interestingly, T1 plasma levels of PHF1 correlated inversely with tau pathology in the brainstem ($r = -0.72$; $p < 0.01$), with a strong trend for a similar pattern in the motor cortex ($r = -0.53$; $p < 0.06$), but not in the dentate gyrus (Fig. 4b–d), indicating that a higher dose of antibodies may have a greater therapeutic effect. In support of the histological analysis, insoluble total tau levels correlated inversely with T1 plasma levels ($r = -0.68$, $p < 0.02$, Fig. 4e). Significant correlation was also observed between performance on the traverse beam task and PHF1 immunoreactivity in the dentate gyrus ($r = 0.39$; $p < 0.05$) as well as insoluble PHF1/total tau ratio on western blots ($r = 0.40$; $p < 0.04$).

On the behavioral tasks, the treated mice performed better than controls on the traverse beam task ($p < 0.03$; Fig. 5a), but the groups did not differ on the rotarod or on the locomotor activity measures (Fig. 5b–f). As reflected by the behavioral analysis and lack of increased astrogliosis, there were no obvious detrimental effects of the antibody injections. One of the control mice died during the study for unknown reasons.

These results indicate that passive immunization with tau antibodies can decrease tau pathology and functional impairments in the JNPL3 P301L model. Future studies will determine the feasibility of this approach with other monoclonals and in different tangle models that more closely resembles AD.

Discussion

Our previous findings indicate that active immunization with a disease-related phosphorylated tau epitope, in two different tangle mouse models reduces brain levels of aggregated tau proteins and slows progression of the tauopathy-related behavioral phenotype (Asuni *et al.* 2007; Boutajangout *et al.* 2010b). Antibody-mediated clearance of extracellular tau aggregates/tangles may prevent spread of tau pathology (Sigurdsson *et al.* 2002; Clavaguera *et al.* 2009; Frost *et al.* 2009; Sigurdsson 2009), and reduce associated toxicity. Furthermore, numerous reports of neuronal uptake of antibodies suggest that intracellular tangles and pre-tangles may also be directly affected (Sigurdsson 2009). In support of that notion, we have previously shown that purified antibodies from high titer immunized mice enter the brain and bind to pathological tau based on their colocalization with tauopathy-specific tau antibodies (Asuni *et al.* 2007). Likewise, these antibodies generated in response to the vaccine stain both pathological tau in human AD brain and tangle Tg mouse brain (Asuni *et al.* 2007).

PHF1 is a monoclonal antibody that recognizes phosphoserine in positions 396 and 404 of the tau molecule (Greenberg *et al.* 1992), both of which are contained within our prototype phospho-tau immunogen, Tau379–408[PSer396, 404] (Asuni *et al.* 2007; Boutajangout *et al.* 2010b). In the present study, multiple injections of this antibody were associated with clearance of tau pathology as assessed by immunohistochemistry and western blots. Some functional improvements were observed as well. Analogous to our previous active tau immunization studies, tau pathology correlated well with antibody levels (Asuni *et al.* 2007) and behavioral outcome (Asuni *et al.* 2007; Boutajangout *et al.* 2010b), further supporting the validity of this approach.

When these findings are compared with our prior report using active immunization encompassing the same epitope in the same homozygous JNPL3 model (Asuni *et al.* 2007), the passive approach appears to be less efficacious. For example, PHF1 mediated clearance of tau pathology was more prominent in less affected regions such as the dentate gyrus compared with regions with more advanced tau pathology such as the brainstem. Likewise, soluble PHF1 tau levels were not altered with the passive immunotherapy, likely reflecting a more gradual clearance of tau aggregates. Western blots of individual brain regions might have clarified this further but at the same time complicated analysis of insoluble tau, which is limited in this model at the age analyzed. With regard to functional measures, therapeutic improvements were only observed in the traverse beam test, whereas with the active approach in our prior study in this model, benefits were also detected on the rotarod task. However, those previous improvements were less pronounced at 8 months compared with 5 months of age. Here, the mice were only tested at 5–6 months of age and appeared to have less functional impairments than the active immunization study group. Hence, it may be more difficult to detect therapeutic benefits when functional deficits are more modest in the control group.

One reason for less efficacy of PHF1 passive immunotherapy compared with our previous comparable active approach is likely the presumably greater fluctuation in antibody levels following weekly i.p. injections, compared with multiple active immunizations. Furthermore, the polyclonal response to the tau immunogen results in generation of antibodies that target a larger portion of the tau molecule than PHF1. These endogenous antibodies are of various classes, isotypes and affinities that change over time with repeated immunizations. All these differences may have added therapeutic benefits. Prior studies in the Aβ field have shown that efficacy of antibody-mediated clearance of Aβ can be isotype-dependent (Sigurdsson 2006). It is also conceivable that certain type of T-cell activation may have beneficial effects, analogous to when Aβ is being targeted by immunotherapy

(Fisher *et al.* 2010; Schwartz and Shechter 2010). However, a key safety feature of passive immunization is that it avoids a potentially detrimental T-cell response. Such adverse effects halted the first Aβ immunotherapy trial (Orgogozo *et al.* 2003), which makes the passive approach attractive for proof-of-concept clinical trials. For chronic therapy, the active approach would be more appropriate and it can be tailored to minimize autoimmune side effects.

There are two known pathways for clearance of tau within cells. The proteosome system handles soluble normal tau and misfolded soluble tau. Aggregates of misfolded tau are too large for proteosomal degradation and should be removed via the autophagy/lysosomal system. Macroautophagy has been shown to be likely involved in tau clearance (Berger *et al.* 2006; Hamano *et al.* 2008), and lysosomal processing influences tau aggregation and clearance in an inducible cellular model of tauopathy (Wang *et al.* 2009). Other studies have demonstrated that inhibition of lysosomes increase tau levels (Bendiske and Bahr 2003), lysosomal tau is detected in AD and control brains (Ikeda *et al.* 1998) and numerous autophagic and lysosomal vesicles are observed by electromicroscopy in the JNPL3 model (Lin *et al.* 2003), and in neuronal cultures with tau mutations (Lim *et al.* 2001). It is interesting to note as well that early pathological changes occur in the lysosomal system in AD (Nixon 2007), that may be associated with attempted clearance of tau and/or Aβ aggregates. Antibody-mediated disassembly of these aggregates should facilitate access and subsequent degradation by lysosomal enzymes, and thereby promote neuronal health. Importantly, antibodies have been visualized in lysosomes by immunoelectroscopy (Meeker *et al.* 1987). Our preliminary findings in a brain slice tangle model support this scenario. We have observed in this slice model with confocal imaging and biochemical measurements that anti-tau antibodies applied to the culture colocalize with tau aggregates and endosomal/lysosomal markers within neurons (Krishnamurthy *et al.* 2010).

No obvious toxic effects were observed following the multiple injections of the PHF1 antibody. The only mouse that died during the study belonged to the control group. Astrogliosis, which is a sensitive indicator of neurotoxicity, was comparable in the treated and control mice. The antibody-mediated clearance of the tau aggregates is likely to be gradual as reflected in similar degree of microgliosis in the immunized mice versus controls. These results mirror our previous active tau immunization studies (Asuni *et al.* 2007; Boutajangout *et al.* 2010b). Likewise, as in those prior studies, normal tau is not affected following PHF1 therapy. This is not particularly surprising as this pool of tau is soluble in the cytosol and should not be accessible to the antibodies based on the proposed mechanism of action. Furthermore, PHF1 and the polyclonal antibodies generated towards the disease-related phospho-tau epitope, should primarily target pathological tau protein.

In summary, these findings show for the first time that disease-specific tau antibodies can clear tau aggregates from the brain which reduces related functional impairments. Overall, passive or active tau immunotherapy is promising for various tauopathies and should be assessed in clinical trials in the near future.

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Abbreviations used

Aβ	amyloid-β

AD Alzheimer's disease

TBS Tris-buffered saline

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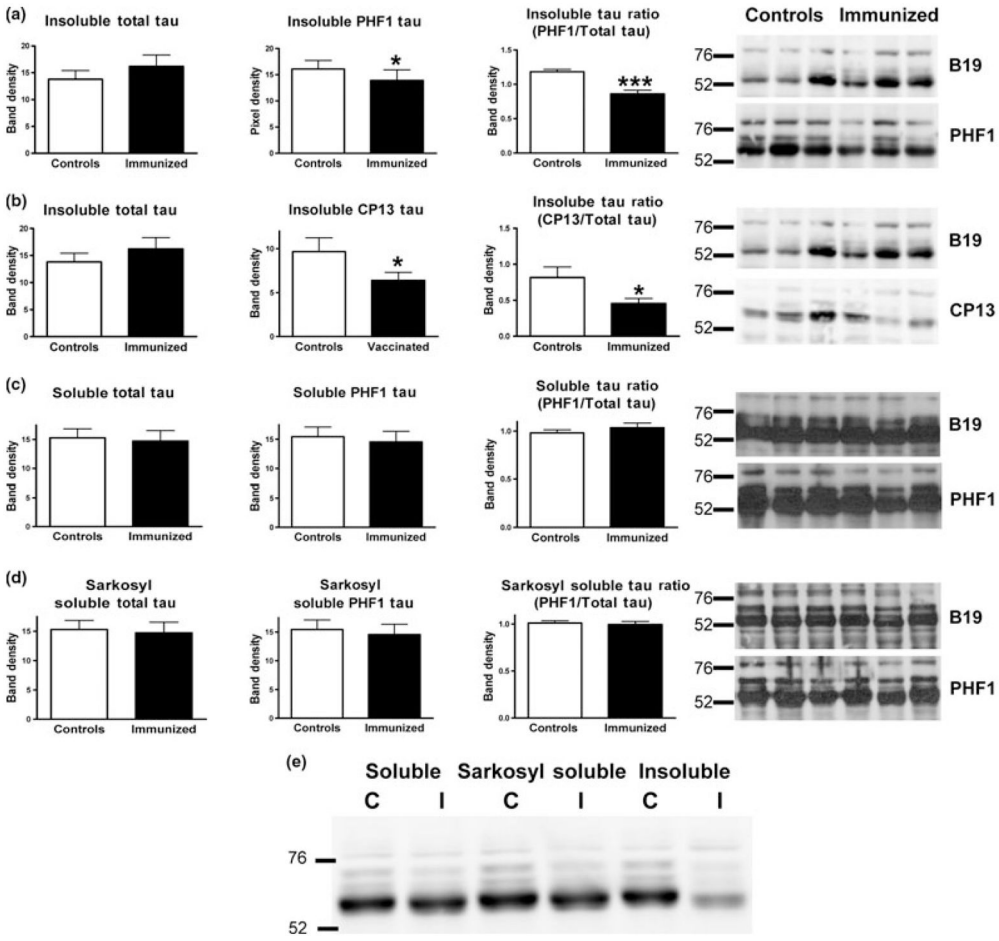

Fig. 1.

PHF1 immunotherapy reduced levels of insoluble tau in the brain. As assessed by western blots of the left hemisphere, the antibody therapy reduced insoluble pathological tau protein (a) PHF1/B19, 27% reduction, $p < 0.0001$; PHF1, 14% reduction, $p < 0.05$. (b) CP13/ B19, 45% reduction, $p < 0.05$; CP13, 34% reduction, $p < 0.05$, but levels of soluble (c) and sarkosyl soluble tau (d) were unchanged compared with controls. $n = 10$ per group. (e) Shows representative samples from the control and immunized groups run on the same gel, clearly showing PHF1-mediated clearance of the insoluble tau fraction. *$p < 0.05$; ***$p < 0.001$.

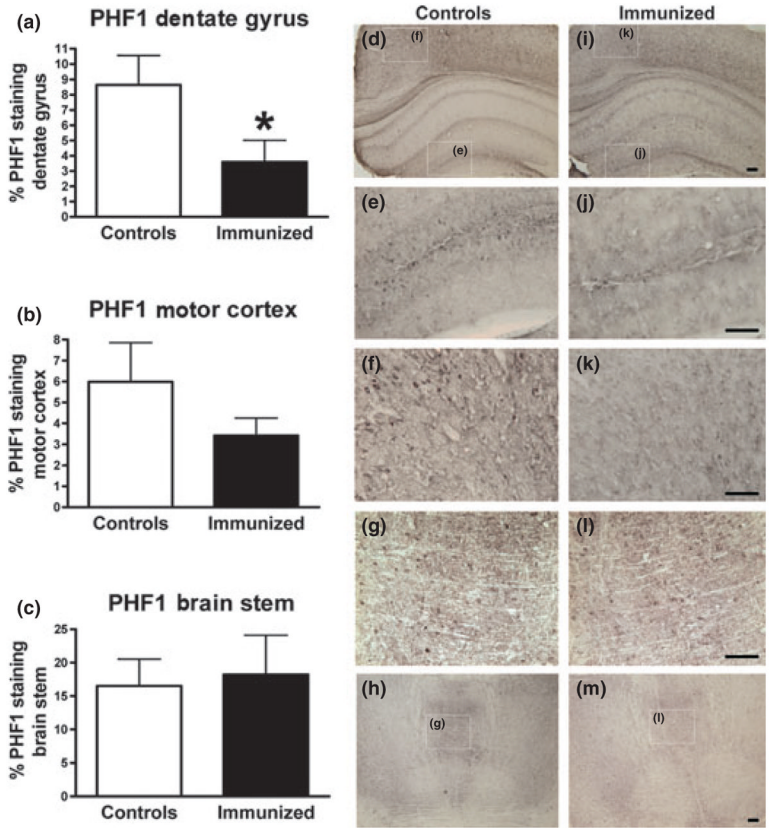

Fig. 2.

(a–c) PHF1 immunized P301L mice had less tau pathology in the dentate gyrus with a strong trend for decrease in the motor cortex, but not in the brainstem. Immunized animals had 58% and 43% less PHF1 stained tau pathology than controls in the dentate gyrus ($p = 0.02$) and motor cortex ($p < 0.12$), respectively. The degree of tau pathology was similar between the groups in the brainstem. (d–m) Representative images from a control (d–h) and an immunized mouse (i–m) show clearance of tau pathology with immunization. Higher magnifications of the boxed areas in d, h, i and m are shown in e, f, g, j, k and l, respectively. $n = 10$ per group and five sections were analyzed per animal. *$p < 0.05$. Scale = 100 μm.

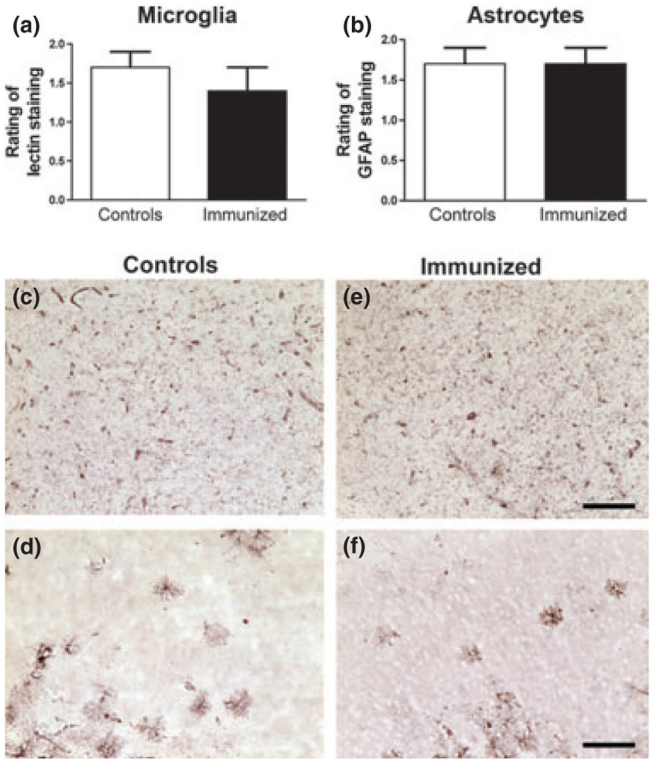

Fig. 3.

(a and b) Similar degree of micro and astrogliosis was observed in both groups. Representative cortical images for a control (c and d) and an immunized mouse (e and f) are shown. $n = 10$ per group, 15–20 sections were analyzed per animal (every 10th section of the brain). Scale = 100 μm.

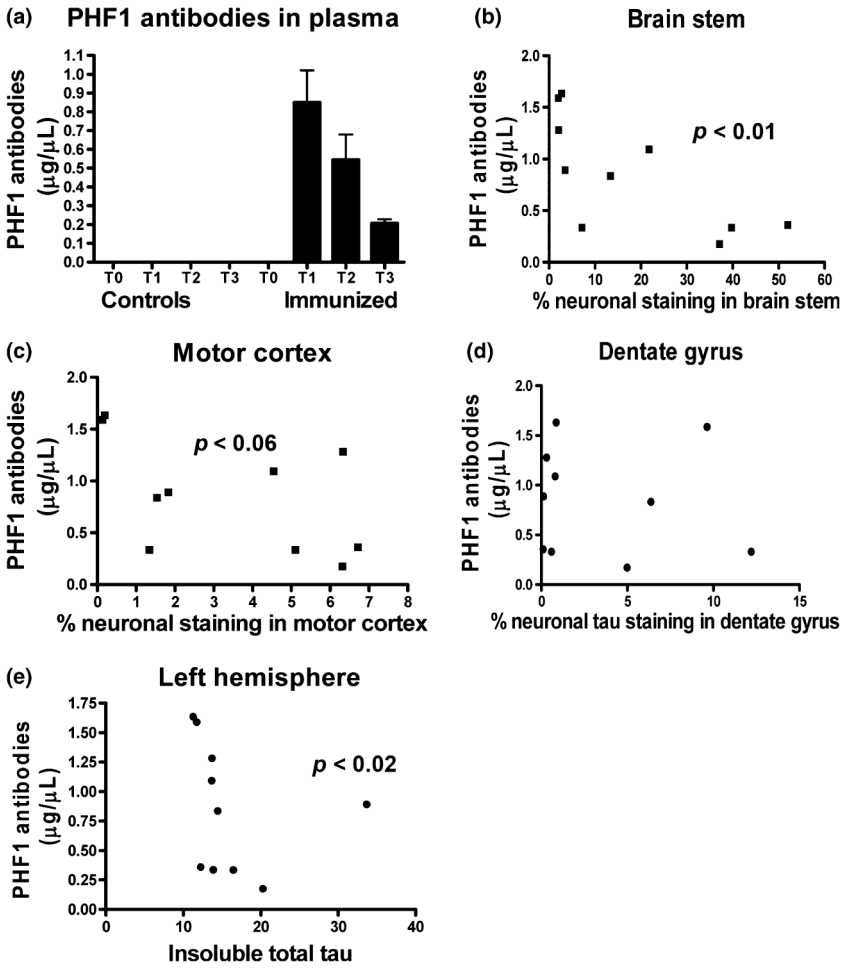

Fig. 4.

(a) PHF1 antibodies were cleared relatively quickly from plasma. No detectable antibodies were observed in controls, whereas the levels in immunized animals decreased over time. The rate of clearance appeared to be faster than for endogenous IgG (typical half-life of IgG is 21–28 days), as is often observed for therapeutic monoclonals (Keizer *et al.* 2010). Each bar represents the average values for the immunized mice + SEM. T0: prior to first immunization, T1: 24 h after the 12th injection, T2: 7 days after the 13th and last injection, T3: 14 days after last injection. The ELISA plates were coated with Tau379–408[P-$Ser_{396, 404}$]. $n = 10$ per group per each time point. (b–e) Plasma levels of PHF1 antibodies correlated inversely with tau pathology. Significant correlation was observed in the brainstem (b; $p < 0.01$), and a strong trend for correlation in the motor cortex (c; $p = 0.06$). A similar pattern, albeit with an outlier, was observed in the dentate gyrus (d). Similar pattern was detected when antibody levels were compared with levels of insoluble total tau on western blots (e; $p < 0.02$). $n = 10$ per group.

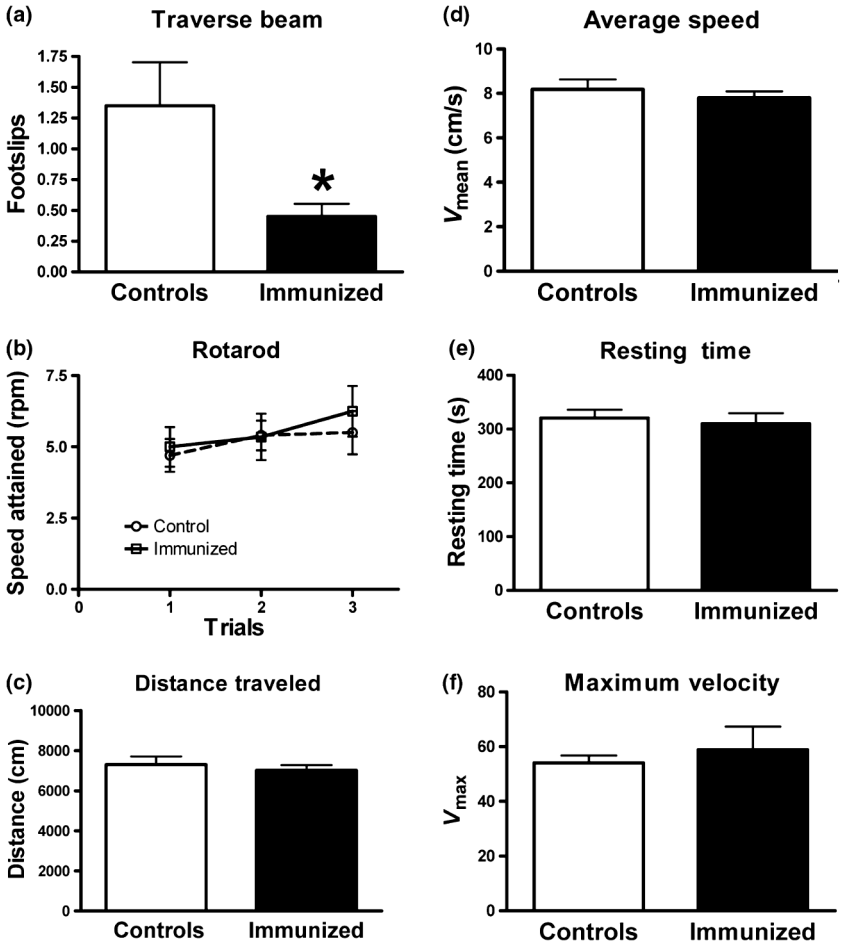

Fig. 5.

(a) PHF1 immunized P301L mice performed better on the traverse beam compared with controls. There was a significant difference between IgG injected controls and PHF1 immunized animals on the number of foot slips on the traverse beam with control animals having more foot slips when crossing the beam (trials combined, $p = 0.03$). (b–f) The groups did not differ significantly on the rotarod (b) or on various locomotor activity measures (c–f). $n = 10$ per group. *$p < 0.05$.