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Identification of Phosphorylation Sites in PHF-TAU from Patients with Guam Amyotrophic Lateral Sclerosis/Parkinsonism-dementia Complex

MADHUMALTI MAWAL-DEWAN, PHD, M. LUISE SCHMIDT, PHD, BRIAN BALIN, PHD, DANIEL P. PERL, MD, VIRGINIA M.-Y. LEE, PHD, AND JOHN Q. TROJANOWSKI, MD, PHD

Abstract. Guam Amyotrophic Lateral Sclerosis/Parkinsonism-Dementia Complex (Guam ALS/PDC) is a progressive neurodegenerative disorder characterized by abundant neurofibrillary tangles (NFTs) composed of aggregated paired helical filaments (PHFs). These abnormal filaments resemble the PHFs in neurofibrillary lesions of classic Alzheimer's disease (AD), and recent studies demonstrated that tau in Guam ALS/PDC is aberrantly phosphorylated and biochemically similar to the abnormal tau proteins (PHFtau) in classic AD. However, unlike PHFtau in AD, there is little information on the specific sites of phosphorylation in PHFtau from Guam ALS/PDC. Thus, to address this important issue, we examined tangle-rich Guam ALS/PDC and AD brains by Western blot, immunoelectron microscopy and immunohistochemistry using 13 antibodies to defined phosphate-dependent or -independent epitopes distributed throughout AD PHFtau. These studies identified 7 previously unknown sites of phosphorylation in PHFtau from Guam ALS/PDC (i.e. Thr181, Thr231, Ser262, Ser396, Ser404, Ser422, and the site defined by monoclonal antibody AT10), all of which also are found in AD PHFtau. Indeed, the Western blot, light and immunoelectron microscopic data suggest that NFTs, PHFs and PHFtau in Guam ALS/PDC are very similar to their counterparts in classic AD. Thus, insights into mechanisms leading to the accumulation of neurofibrillary lesions in Guam ALS/PDC may advance understanding of the pathogenesis and biological consequences of these lesions in classic AD.

Key Words: Alzheimer's disease; Guam ALS/PDC; Neurofibrillary tangles; Tau.

INTRODUCTION

Lytico-bodig is a neurodegenerative condition endemic to native Chamorros of Guam which takes the form of a motor neuron disease (lytico) similar to amyotrophic lateral sclerosis (ALS) and a form of parkinsonism accompanied by severe dementia (bodig) (1–3). Accordingly, this spectrum of neurodegenerative disease is often referred to as Guam ALS/parkinsonism-dementia complex (Guam ALS/PDC). While individual patients show clinical features similar to ALS, Parkinson's disease (PD) and Alzheimer's disease (AD), the most characteristic lesions of Guam ALS/PDC are widespread neurofibrillary tangles (NFTs) and related neurofibrillary lesions (1–14). The tangles in Guam ALS/PDC are particularly abundant in the entorhinal cortex and hippocampal formation, and the accumulation of NFTs is accompanied by a profound loss of neurons in the same areas. However, in contrast to classical AD, the brains of patients with Guam ALS/PDC contain few or no senile plaques (1–3, 5, 6, 9, 10, 13), and lesions (e.g. Lewy bodies) found in other neurodegenerative diseases also are not seen in this disorder (1–14).

By immunohistochemical and ultrastructural criteria, the NFTs of Guam ALS/PDC are indistinguishable from those found in classic AD (1–4, 7–9, 11–16). For example, the NFTs in Guam ALS/PDC contain straight filaments as well as paired helical filaments (PHFs), and they react with antibodies to tau proteins and ubiquitin just like the straight filaments and PHFs in AD NFTs. However, the NFTs in the brains of Guam ALS/PDC patients are more widespread and they occur in locations (e.g. spinal cord) where NFTs are rare or not observed at all in AD or other neurological diseases (10, 11).

Although β-amyloid (Aβ) immunoreactive NFTs and some Aβ-positive diffuse and neuritic plaques have been identified by sensitive immunohistochemical methods in Guam ALS/PDC brains (5, 6, 10, 13), the invariable abundance of NFTs and the relative paucity of Aβ deposits, Lewy bodies and other neurodegenerative inclusions in these brains strongly suggest that NFTs are directly involved in the dysfunction and degeneration of neurons in this unusual disorder. Since neuropathological and immunochemical studies have demonstrated that the NFTs in Guam ALS/PDC contain PHFs that are immunologically similar to the PHFs and abnormal tau proteins (PHFtau) in the NFTs of classic AD (1–4, 7–9), investigation of the biochemistry and pathogenesis of NFTs and the PHFtau proteins that form these tangles in the brains of Chamorros with Guam ALS/PDC provides a unique opportunity to elucidate the biological significance of these intraneuronal inclusions. Indeed, Buée-Scherrer et al (4) recently demonstrated that PHFtau in

From the Department of Pathology and Laboratory Medicine, Division of Anatomic Pathology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 (MMD, MLS, VM-Y, JQT); the Departments of Neurobiology and Pathology, Medical College of Pennsylvania and Hahnemann University School of Medicine, Broad and Vine St., New College Building, Rm. 5412, Philadelphia, PA 19102 (BB); and the Departments of Pathology and Psychiatry, Mt. Sinai School of Medicine, New York, NY 10029 (DPP).

Correspondence to: John Q. Trojanowski, MD, PhD, Department of Pathology and Laboratory Medicine, Division of Anatomic Pathology, HUP, Maloney Bldg., Room A009, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-4283.

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Guam ALS/PDC is aberrantly phosphorylated and exhibits biochemical as well as immunological properties similar to PHFtau in AD. However, it is not yet clear if PHFtau in Guam ALS/PDC and AD are phosphorylated at similar amino acid residues because, unlike AD PHFtau, there is little information on the specific sites of phosphorylation in PHFtau from Guam ALS/PDC. Accordingly, the present studies were undertaken to identify sites of phosphorylation in PHFtau in Guam ALS/PDC, and to compare these phosphorylation sites with those in PHFtau from classic AD patients using immunological methods and 13 antibodies that recognize defined phosphate-independent or -dependent epitopes extending from amino to the carboxy terminal domains of PHFtau in AD. Here, we identify 7 previously unknown sites of phosphorylation in PHFtau from Guam ALS/PDC (i.e. Thr181, Thr231, Ser262, Ser396, Ser404, Ser422 and the site defined by monoclonal antibody AT10). Further, using this panel of antibodies, including a new antibody (pS422) that recognizes phosphorylated Ser422 in AD PHFtau, we show by Western blots as well as by light and immunoelectron microscopy that NFTs, PHFs and PHFtau in Guam ALS/PDC are very similar to their counterparts in classic AD.

MATERIALS AND METHODS

Isolation of Tau and PHFtau from Brain Tissue

Autopsy-derived normal human fetal and adult brain tau was extracted from previously characterized individuals free of neurological disease as described (17–20). PHFtau was purified from postmortem neocortex of well-characterized classic AD patients and from previously studied Guam ALS/PDC patients (4, 7, 8) as reported earlier (17–20).

Immunoblot Analysis

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) and Western blot analysis of tau proteins were performed according to methods described in previously published reports using 10% SDS-PAGE (17–20). The epitope-specific anti-tau/PHFtau polyclonal and monoclonal antibodies (MAbs) used in this study have been extensively characterized in a series of immunohistochemical and immunochemical studies of tau and/or PHFtau isolated from normal adult, fetal and AD postmortem brains as well as from biopsy-derived normal fragments of human cortex in addition to tau peptides and wild type and mutant recombinant tau proteins (17–33). These antibodies included three MAbs (Alz50, T14, T46) and one polyclonal antibody (133) that are specific for phosphorylation-independent epitopes (17–20, 26, 32) as well as six MAbs (PHF1, AT8, AT10, AT270, AT180, 12E8) and two polyclonal antibodies (T3P, pS422) that are specific for phosphorylation-dependent epitopes (17–20, 23–25, 27–32) in tau and/or PHFtau as summarized in the schematic in Figure 1. Further, we also used the MAb T1 that recognizes amino acid residues 189–207 when all the serines within this domain are nonphosphorylated (17–21, 32). Aside from AT8 and T1 (4), none of the other antibodies to tau and/or AD PHFtau that we used in this study have even been exploited before to characterize the phosphorylation sites in PHFtau from Guam ALS/PDC. Bound antibody was detected using enhanced chemiluminescence (Dupont NEN) according to the instructions of the vendor. The protein concentrations in the samples were monitored using bicinchoninic acid as a dye reagent with bovine serum albumin as standard (17, 18).

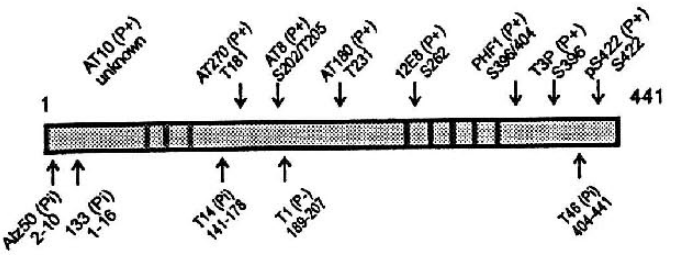

Fig. 1. This schematic illustration of the 441-amino-acid-long tau isoform (not drawn to scale) shows the location of the epitopes recognized by the anti-tau antibodies used here. The code names of the antibodies are identified in bold characters, and the numbers below the name of the antibody indicate the amino acid sequence within which the epitope resides that is recognized by the antibody. As in the text, the numbering system used here is for the largest human brain tau isoform (41). The relative locations of the amino terminal inserts and the carboxyl-terminal microtubule (MT) binding repeats are shown. The MT binding repeats are continuous, and the space between each one here is merely used to facilitate the visualization of each repeat. Antibodies T14, T46 Alz50 and 133 are phosphorylation independent (Pi), while 12E8, AT8, PHF1, pS422, AT270, AT10, T3P and AT180 recognize their epitopes in a phosphorylated state (P+) and T1 recognizes its epitope when it is not phosphorylated (P-). The epitope(s) recognized by AT10 (also designated as AT100 in some reports; 20) is unknown, and its placement in the figure is arbitrary. See text for information on the specificities of these antibodies.

Immunoelectron Microscopy of Isolated PHFs from Guam ALS/PDC Brains

Immunolabeling of enriched fractions of PHFs from Guam ALS/PDC brains was accomplished using previously described methods (19, 34). Briefly, following biochemical isolation of dispersed PHFs (19, 34), the samples were adsorbed onto carbon-coated grids and blocked for 10 minutes (min) with 0.1% cold water fish gelatin (Sigma) in Tris buffered saline (TBS) to eliminate nonspecific background labeling. The grids were incubated with several different mouse MAbs and a rabbit polyclonal antiserum at dilutions that ranged from 1:500 to 1:1000 for 30 min, rinsed for 30 min in TBS containing 0.1% cold water fish gelatin and blocked a second time for 15 min in TBS containing 0.1% cold water fish gelatin. Following the second blocking step, the grids were incubated with secondary antibodies diluted 1:5 to 1:10 in TBS containing 0.1% cold water fish gelatin for 30 min. The secondary antibodies were goat antisera to rabbit or mouse IgG that were conjugated to 5 or 10 nm gold particles (Amersham). Following the final incubation, the grids were rinsed in TBS and negatively stained in 0.5 to 1% aqueous uranyl acetate. Controls for each immunolabeling experiment included: (a), incubation of the grids without a primary antibody and (b), substitution of MAbs and the antiserum with spent medium from a nonsecreting mouse myeloma cell line (SP2).

Tissue Collection, Diagnostic Assessment and Immunohistochemistry

The brain samples used here were obtained and characterized as described in several previous publications (4, 7, 8, 17–20, 24, 26, 30, 35–38). Brain tissues derived from 7 Guamanian Chamorro patients were available for study, and the clinical histories for these cases were obtained from the medical records of the Marianas Health Study and the Guam Memorial Hospital. Of these cases, 5 showed predominantly parkinsonian signs with accompanying dementia, and clinically all of these patients were thought to represent typical examples of Guam PDC. An additional case showed progressive weakness and limb atrophy consistent with a diagnosis of Guam ALS. Finally, the brain of a 101-year-old female Guam native who remained free of evidence of neurologic dysfunction during life also was available for study here. Brain samples from well-characterized AD patients (17–20, 24, 26, 30, 35–38) also were available for the studies described here.

At autopsy, the brains of the Guam subjects were fixed in 10% buffered formalin, and referred to the Neuropathology Division of the Mount Sinai Medical Center for neuropathologic characterization and diagnosis. For 3 of the Guam PDC cases and the single asymptomatic subject, portions of the hippocampus and temporal cortex, frozen at autopsy at −70° C (at postmortem intervals of 3 to 5 hours), were available for biochemical analysis. Data from these 4 cases have been reported previously (4, 7), and all of the brains from the Guam PDC patients showed the typical features of this condition, i.e. widespread accumulations of NFTs with accompanying loss of neurons and gliosis including severe involvement of the substantia nigra. In one of these specimens, there was diffuse senile plaque formation in the neocortex, but neocortical NFTs predominated in layers II/III, rather than in layer V as in classic AD (7, 8). The remainder of the Guam PDC cases were virtually free of amyloid plaque accumulation. The single Guam case with progressive weakness showed motor neuron degeneration with lateral column demyelination accompanied by widespread hippocampal and neocortical NFTs consistent with Guam ALS. Finally, the asymptomatic normal control case (i.e. the 101-year-old female Guam native) showed rare NFTs, consistent with normal age-related changes. The acquisition and processing of AD case material from patients followed at the University of Pennsylvania as well as brain samples from normal fetal and adult controls have been described previously (17–20, 24, 26, 30, 35–38).

Fresh brain tissues were frozen and stored at −70° C until they were used for biochemical analysis, while all of the fixed brain samples were embedded in paraffin, and 6-μm-thick sections were cut for immunohistochemical analysis. Immunostaining was carried out with and without microwave pretreatment using the peroxidase anti-peroxidase (PAP) procedure for MAbs, and the avidin-biotin detection method (Vector kit, Burlingame, CA) for the rabbit antiserum according to previously published procedures (17–20, 32, 35–40).

The studies conducted here were performed in accordance with National Institutes of Health guidelines for the study of human subjects and the studies were approved by the University of Pennsylvania Medical Center.

RESULTS

Western Blot Comparison of Tau in Guam ALS/PDC and AD PHFtau

To determine whether or not tau in Guam ALS/PDC brains is phosphorylated at sites previously identified in AD PHFtau, we performed comparative Western blot studies of tau from the brains of Guam ALS/PDC cases, normal fetal and adult subjects, and classic AD patients using the panel of antibodies summarized above and in Figure 1. Representative data from these studies are shown in Figure 2. For example, Figure 2A documents the immunodetection of normal fetal and adult tau, AD PHFtau and PHFtau from Guam ALS/PDC using the phosphate-independent MAbs T14 and T46. Although tau from the Guam ALS/PDC brains migrated much more slowly than autopsy-derived normal fetal and adult brain tau, the triplet of PHFtau proteins from the Guam ALS/PDC brains comigrated almost exactly with PHFtau from AD brains (compare lanes τ_1 to τ_3 with lanes Aτ and PHFτ in Fig. 2A). Like AD PHFtau, PHFtau from Guam ALS/PDC was immunoreactive with MAb 12E8, which is specific for an epitope containing Ser262 (the numbering system used here and below corresponds to the longest adult human brain tau protein; 41) when it is phosphorylated (Fig. 2B), but PHFtau from Guam ALS/PDC was not labeled by MAb T1 (Fig. 2C), which recognizes tau when the Ser or Thr residues within the amino acid sequence 189–207 are not phosphorylated. Additionally, the Guam ALS/PDC-derived PHFtau proteins were labeled by several other antibodies that recognize AD PHFtau including AT8 (which binds to PHFtau phosphorylated at Ser202 and Thr205; Fig 2D), PHF1 (which recognizes PHFtau phosphorylated at Ser396/404; Fig 2E), pS422 (which recognizes PHFtau phosphorylated at Ser422; Fig 2F), AT270 (which recognizes PHFtau phosphorylated at Thr181; Fig 2G), AT180 (which recognizes PHFtau phosphorylated at Thr231; Fig 2H), AT10 (which recognizes an undefined epitope in PHFtau that is not present in normal adult or fetal brain tau; Fig. 2I), and T3P (which has a specificity similar to PHF1; data not shown). Thus, in addition to the Ser202/Thr205 site demonstrated earlier by Buée-Scherrer et al (4), we have identified 7 previously unknown sites of phosphorylation (i.e. Thr181, Thr231, Ser262, Ser396, Ser404, Ser422 and the site defined by monoclonal antibody AT10) in PHFtau from Guam ALS/PDC. Taken together, these findings suggest that PHFtau proteins in the Guam ALS/PDC brains are phosphorylated at the same sites as AD PHFtau.

Immunoelectron Microscopy of PHFs Isolated from Guam ALS/PDC Brains

To determine if PHFs in the Guam ALS/PDC brains are immunologically similar to PHFs in classic AD, we

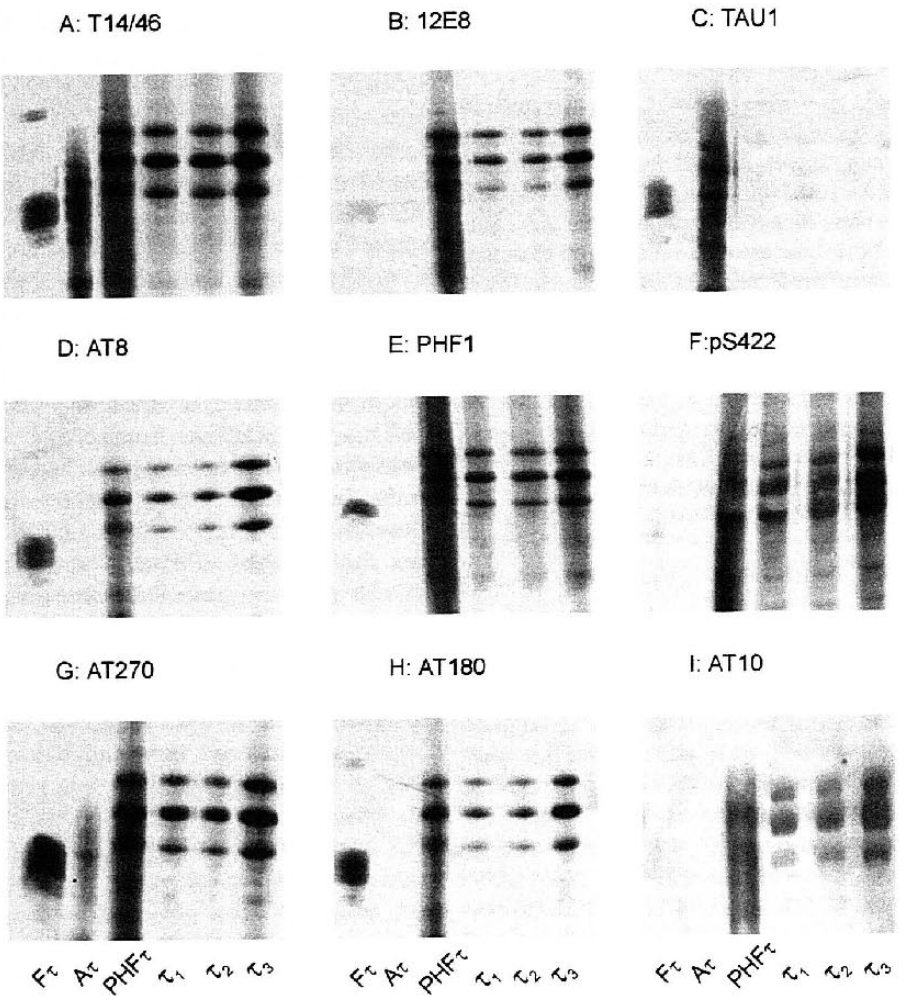

Fig. 2. The representative Western blots in panels A-I illustrate the sites and state of phosphorylation of tau isolated from Guam ALS/PDC brains (in the lanes labeled τ_1 to τ_3) for comparison with normal human fetal tau (in the lanes labeled Fτ), normal human adult tau (in the lanes labeled Aτ) and AD PHFtau (in the lanes labeled PHFτ) from postmortem brains. Samples of fetal tau, adult human tau, PHFtau and tau from Guam ALS/PDC brain were loaded onto SDS-polyacrylamide gels for immunoblot analysis as described in the methods section here. The antibodies used for the detection of these tau proteins are shown above each of the immunoblot panels (A-I). To obtain comparable levels of T14/46 tau or PHFtau immunoreactivity in the different tau preparations shown in each of the lanes, we performed preliminary studies of different protein loads per lane and then loaded 1.5 μg of the fetal tau (Fτ), 5 μg of the adult tau (Aτ), 2 μg of the PHFtau (PHFτ), and 10 μg of the Guam ALS/PDC tau (τ_1 to τ_3) preparations in the corresponding lanes of panel A. Although the same amount of PHFtau was loaded for all of the antibodies, twice the amount of fetal tau, adult tau and Guam ALS/PDC tau was loaded in the lanes shown in the subsequent panels for antibodies 12E8, AT8, PHF1, pS422, AT270, AT10 and AT180.

probed enriched samples of PHFs isolated from the Guam ALS/PDC brains with several anti-PHFtau antibodies (i.e. AT8, PHF1, 12E8, pS422), and the immunolabeled PHFs were examined by electron microscopy (see Fig. 3). These studies demonstrated variable labeling of the PHFs isolated from the Guam ALS/PDC brains with these anti-PHFtau antibodies. For example, the PHFs stained by AT8, 12E8 and PHF1 showed strong but discontinuous or periodic decoration of these filaments, while pS422 did not decorate these PHFs as extensively as the other antibodies. However, an antiserum to an epitope in the microtubule binding domain of tau (i.e. antibody 135) did not label any of these filaments (data not shown). Since these immunoelectron microscopic data are consistent with the Western blot findings, we infer that the PHFs from Guam ALS/PDC brains are similar to the PHFs that aggregate into NFTs and other neurofibrillary lesions in classic AD.

Immunohistochemical Comparison of NFTs in Guam ALS/PDC and AD Brains

The Western blot results were confirmed and extended by immunohistochemical analysis of the NFTs in the brains of patients with Guam ALS/PDC. For example, Figure 4 shows representative data produced with several

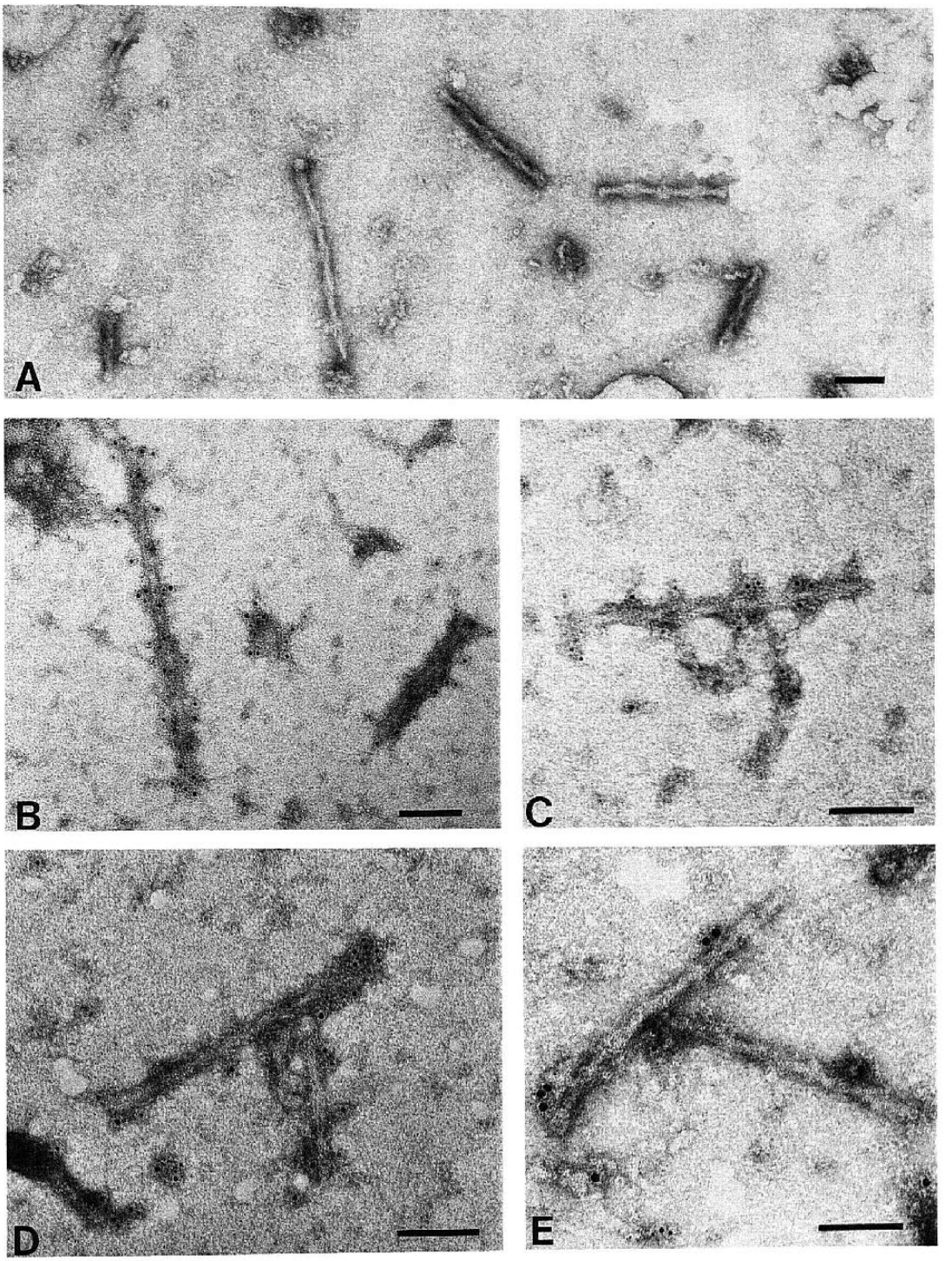

Fig. 3. Panels A–E show representative electron micrographs of PHFs isolated from tangle-rich cortical regions of Guam ALS/PDC brains. The PHFs in panel A were not subjected to immunoelectron microscopy but were stained with 1% aqueous uranyl acetate as described in the methods section to reveal PHFs that have the same morphology and dimensions as the PHFs typically observed in AD. Panels B–E show similar PHFs that were probed with AT8 (B), PHF1 (C), 12E8 (D), and pS422 (E) followed by staining with 1% aqueous uranyl acetate as described in the methods section. These immunoelectron microscopy studies demonstrate immunoreactive phosphate-dependent PHFtau epitopes for each of these antibodies that are distributed along the length of the PHFs from the Guam ALS/PDC brains. The labeling pattern varies from periodic (AT8, PHF1) to random (12E8, pS422) and the labeling intensity is variable. The bar = 100 nm in each panel.

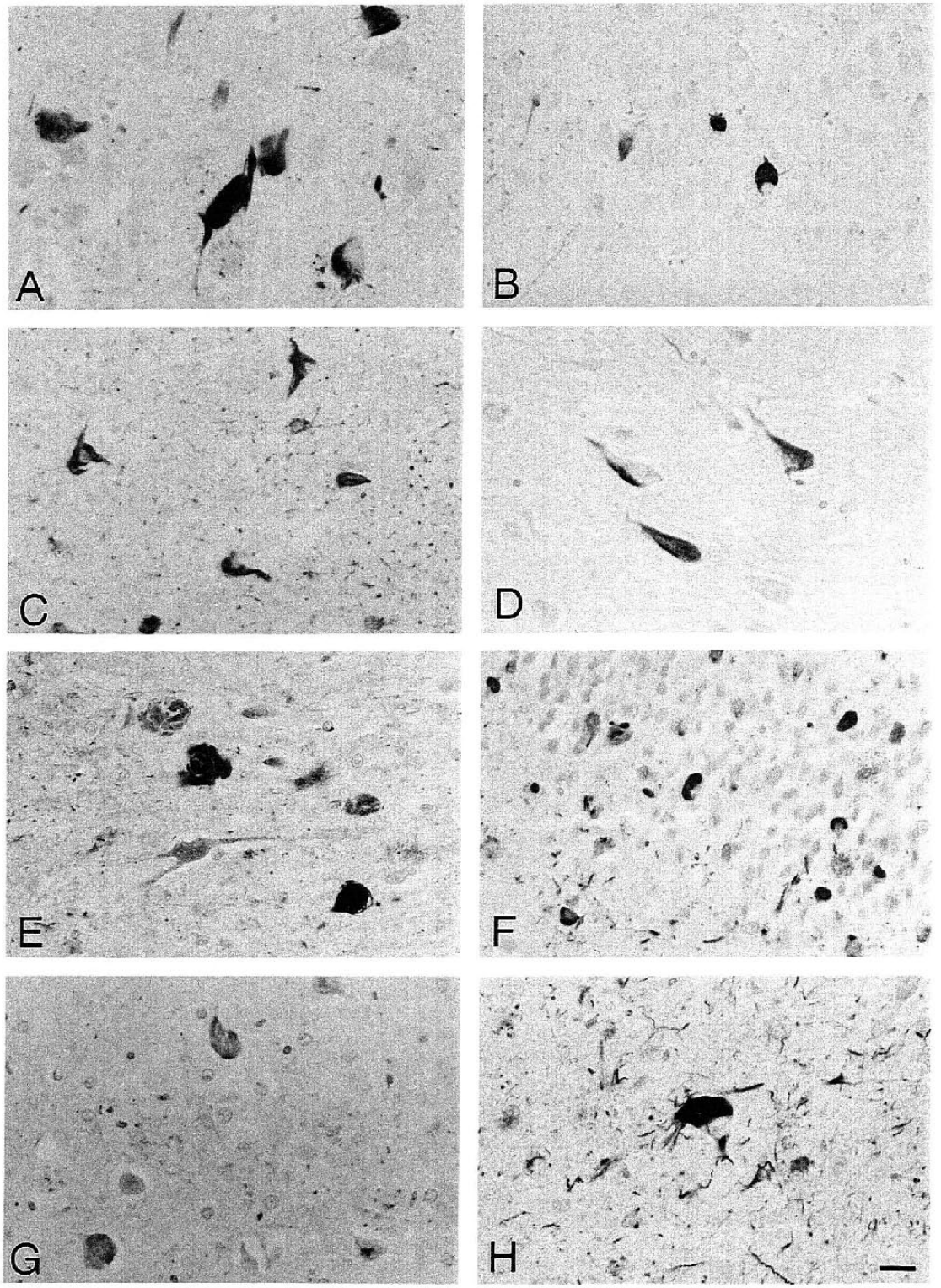

Fig. 4. The panels shown here illustrate representative findings from the immunohistochemical studies of the Guam ALS/PDC cases using antibodies that are known to recognize defined epitopes in tau or AD PHFtau. Panels A–H show paraffin sections through the hippocampus of Guam ALS/PDC brains stained with the following antibodies: 133 (A), ALZ50 (B), AT8 (C), 12E8 (D), T3P (E), PHF1 (F), T46 (G), and pS422 (H). The photomicrographs illustrate immunoreactive NFTs in Ammon's horn (A-E, G, H) and the dentate gyrus (F). Neuropil threads also are labeled and these neurofibrillary lesions are most prominent in panels A, C, E, F and G. The sections were lightly counterstained with hematoxylin, and all of the panels are at the same magnification (bar in H = 10 μm).

of the antibodies (e.g. 12E8, PHF1, AT8, and T3P) illustrated in the Western blots shown in Figure 2. Notably, all of these antibodies stained NFTs and a variable number of neuropil threads in the Guam ALS/PDC brains similar to the results obtained with these antibodies in previous studies of classical AD brains (17–20, 24, 30). Although some of the antibodies (e.g. T14, AT270, AT180) used in these immunohistochemical studies did not label NFTs and neuropil threads in brain tissues fixed with 10% NBF as well as in the ethanol-fixed samples even after microwave treatment (42), all of the anti-tau antibodies that stained neurofibrillary lesions in the 10%-NBF-fixed AD brains also labeled numerous tangles and abnormal neurites in all of the 10%-NBF-fixed brain samples from the Guam ALS/PDC patients (see Fig. 4A–H). In contrast, only rare NFTs were labeled by these antibodies in immunohistochemical studies of sections from the normal 101-year-old Guam native, and this was consistent with diagnostic workup of this case using conventional neuropathological methods including silver stains for the detection of plaques and tangles (data not shown).

In addition to intraneuronal and extracellular NFTs, reactive astrocytes also were labeled by several of these antibodies (e.g. T3P, PHF1 and AT8) in the Guam ALS/PDC brains, and these tau-positive astrocytes were seen throughout the gray and white matter (data not shown). While it was not often clear if small tau-positive profiles in cells in the gray matter were neurons or astrocytes, the abundance and morphology of these cells in white matter and severely gliotic regions enabled recognition of these cells as astrocytes. Although PHFtau-positive glial tangles may be abundant in the brains of patients with neurodegenerative diseases (15, 43), the cytoplasmic PHFtau immunoreactivity in the astrocytes of the Guam ALS/PDC brains was not fibrillar, and additional studies are needed to characterize the ultrastructure of these PHFtau-positive profiles. Finally, while Aβ-positive SPs were infrequent in the Guam ALS/PDC brains, polyclonal antibodies specific for Aβ (e.g. 2332) did stain a variable number of SPs and diffuse plaques as well as extracellular ("ghost") tangles in these cases (data not shown) as reported earlier (13).

DISCUSSION

Since Guam ALS/PDC is characterized by a clinical phenotype similar to ALS, PD and AD as well as by abundant AD-like neurofibrillary pathology (1–3), this disorder provides a unique opportunity to determine the role that tau-rich NFTs and dystrophic neurites play in the dysfunction and degeneration of neurons in inherited and sporadic forms of classic AD. Although the recent study by Buée-Scherrer et al (4) demonstrated that PHFtau in Guam ALS/PDC is aberrantly phosphorylated and exhibits biochemical as well as immunological properties similar to PHFtau in AD, it was not clear if PHFtau in Guam ALS/PDC and AD is phosphorylated at the same or different sites. Thus, it was important to assess the extent to which the abnormal phosphorylation of tau in this disorder recapitulates the properties of PHFtau in classic AD. For this reason, we extended earlier studies of the properties of PHFtau in Guam ALS/PDC by identifying 7 previously unknown sites of phosphorylation in PHFtau from Guam ALS/PDC (i.e. Thr181, Thr231, Ser262, Ser396, Ser404, Ser422 and the site defined by the monoclonal antibody AT10). Notably, all of these sites also are phosphorylated in AD PHFtau. Accordingly, based on the present series of immunological studies, we conclude that the abnormal tau proteins in the NFTs and related neurofibrillary lesions of Guam ALS/PDC are very similar if not identical to PHFtau in classic AD. These conclusions are supported by the concordance of our Western blot studies of tau proteins isolated from Guam ALS/PDC brains, the immunohistochemical analysis of Guam ALS/PDC neurofibrillary pathology, and the immunoelectron microscopic characterization of PHFs purified from the Guam ALS/PDC brains. Indeed, using a large and extensively characterized panel of antibodies to defined phosphate-dependent or -independent epitopes that span nearly the entire length of AD PHFtau (11 of which have never been used before to characterize PHFtau from Guam ALS/PDC), we could not detect any substantive biochemical or immunochemical differences between AD PHFtau and the abnormal tau proteins in Guam ALS/PDC brains.

The recognition that AD is a heterogeneous group of dementing disorders (15, 16) with common clinical (i.e. progressive memory loss) and pathological features (i.e. neuron loss, amyloid plaques, neurofibrillary lesions) may make it increasingly valuable to investigate disorders like Guam ALS/PDC in order to elucidate the biological significance of specific AD lesions. For example, the apolipoprotein E (APOE) ε4 allele is a risk factor for sporadic and familial AD (FAD), but FAD is linked to at least 3 other genes on chromosomes 1, 14 and 21, and other FAD genes are likely to exist (15, 16, 44–46). Additionally, the co-occurrence of AD in patients with antecedent PD has prompted speculations that genes involved in the pathogenesis of PD may play a role in the pathogenesis of some forms of AD (47, 48). Accordingly, heterogeneity in the AD genotype and phenotype may complicate efforts to elucidate the role of individual hallmark AD lesions.

Despite persistent uncertainties about the precise role specific AD lesions play in the onset and progression of this disorder, our study complements the findings from previous investigations of Guam ALS/PDC (1–13) by providing additional support for the notion that the accumulation of PHFtau in neurofibrillary lesions may underlie the dysfunction and degeneration of neurons in AD. Specifically, the abundance of NFTs and the paucity

of amyloid plaques as well as the absence of Lewy bodies (LB) or other lesions in Guam ALS/PDC brains strongly suggest that NFTs are directly involved in the dysfunction and degeneration of neurons in Guam ALS/PDC regardless of the clinical phenotype of the disease. Further, the present study confirms and significantly extends reports demonstrating that the NFTs in Guam ALS/PDC contain PHFs, and that these PHFs and their subunit proteins are structurally and immunologically similar if not identical to the PHFs and PHFtau proteins seen in the NFTs of classical AD patients (1, 4, 7, 8). Finally, taken together with recent immunohistochemical studies of Guam ALS/PDC (13), our data suggest that the generation of AD-like PHFtau and the formation of NFTs occurs independently of the deposition of Aβ.

Since AD-like neurofibrillary lesions and neuron loss are the dominant if not sole lesions in the brains of patients with Guam ALS/PDC, it is highly likely that NFTs and related neurofibrillary pathology play a central role in the pathogenesis of this disorder. Thus, we conclude that efforts to gain insights into the mechanisms leading to the generation of AD-like PHFtau in Guam ALS/PDC may clarify the role that PHFtau and neurofibrillary lesions play in the dysfunction and massive loss of neurons in classic AD.

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