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Neurofibrillary Tangles in Progressive Supranuclear Palsy Contain the Same Tau Epitopes Identified in Alzheimer's Disease PHFtau

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Abstract. Neurofibrillary tangle (NFT)–rich brain samples from patients with progressive supranuclear palsy (PSP) or Alzheimer's disease (AD) were probed with a large panel of anti-tau antibodies to compare the species of tau present in PSP and AD NFTs by immunohistochemistry and Western blot methods. These antibodies have been shown to recognize phosphate-independent or -dependent epitopes that extend from the amino to the carboxy terminal domains of normal brain tau and the abnormal tau in the paired helical filaments (PHFs) of AD NFTs (PHFtau). The immunohistochemical studies showed that all of the tau epitopes detected in brainstem PSP NFTs also were found in hippocampal AD NFTs and vice versa. While Western blots demonstrated 2 PHFtau-like immunobands in PSP brainstem, a triplet of PHFtau proteins were seen in the AD and PSP hippocampus.

Despite differences in the distribution, ultrastructure and immunoblot profile of NFTs in PSP and AD, the same constellation of tau epitopes is present in the abnormal tau proteins in PSP and AD NFTs. Thus, the generation of abnormal tau proteins in PSP (PSPtau) and AD (PHFtau) may have similar adverse biological consequences in both diseases.

Key words: Alzheimer's disease; Neurofibrillary lesions; Progressive supranuclear palsy; tau.

INTRODUCTION

Neurofibrillary tangles (NFTs) are intraneuronal inclusions that are hallmark lesions of the Alzheimer's disease (AD) brain (1). However, abundant NFTs also are found in the brains of patients with progressive supranuclear palsy (PSP), and similar tangles are the major or sole brain abnormalities in a number of other well-characterized neurodegenerative diseases (1). AD-NFTs are composed of paired helical filaments (PHFs), although straight filaments also may be seen in these lesions, and PSP-NFTs are composed predominantly of straight filaments or a mixture of straight filaments and PHF-like structures (2–7). In spite of their different morphologies, both AD-NFTs and PSP-NFTs are made up of hyperphosphorylated tau proteins (8–15). However, NFTs in PSP and AD exhibit some immunological and biochemical differences (10, 13). For example, in Western blot analyses performed with anti-tau antibodies, the abnormal tau in PSP-NFTs (PSPtau) has been demonstrated to contain two tau immunobands, while the tau in PHFs (PHFtau) in AD-NFTs is composed primarily of 3 immunobands (11, 12). Thus, it is not yet clear whether or not PSPtau contains fragments of tau or full-length tau proteins, and it is not known if some or all of the tau epitopes found in AD PHFtau also are found in PSPtau. To address this question, antibodies to phosphate-dependent and -independent epitopes spanning the entire tau molecule were employed here in immunohistochemical and Western blot studies of well-characterized brain samples from patients with AD or PSP. These studies showed that all of the anti-tau antibodies that detected NFTs in the AD brain also detected NFTs in the PSP brain, including brainstem. Further, although brainstem PSPtau was characterized by 2 immunobands, the PSP and AD hippocampus showed the same 3 immunobands that typify PHFtau. These data prompt us to speculate that the generation of abnormal tau proteins in PSP (PSPtau) and AD (PHFtau) involves similar mechanisms and has similar adverse biological consequences.

MATERIALS AND METHODS

Patients

Brain tissue samples from 7 patients with classical PSP were used in this study. The diagnosis of PSP was based on the clinical presentation and by the presence of numerous NFTs in the basal ganglia and brainstem (13) in accordance with diagnostic criteria for PSP proposed by the National Institute of Neurological Disorders and Stroke (16). As controls, the brains of four age-matched AD patients and one patient without evidence of a neurological disease were used. The diagnosis of AD was based on established consensus criteria (17). The diagnosis, age, sex, and postmortem interval of each patient are listed in Table 1.

Tissue Collection and Immunohistochemistry

Postmortem brain tissue samples (trimmed in the fresh state to a thickness of 3 to 5 mm) were obtained from many brain regions including the hippocampus, pons, and medulla oblongata, and they were fixed overnight by immersion in either

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TABLE 1
Patients Examined in This Study

Patient	Histology	Blot	Age	Sex	PMI
Progressive supranuclear palsy					
Case 1	Yes	Yes	77	F	9
Case 2	Yes	No	69	M	2.5
Case 3	Yes	Yes*	65	M	8
Case 4	Yes	No	67	F	3
Case 5	Yes	No	78	M	5
Case 6	Yes	No	82	F	10
Case 7	Yes	Yes	48	M	12
Alzheimer's disease					
Case 8	Yes	No	76	M	7.5
Case 9	Yes	No	63	F	4
Case 10	Yes	No	76	F	11
Case 11	No	Yes	90	F	15.5
Case 12	No	Yes	89	F	10
Normal control					
Case 13	No	Yes	49	M	5

Abbreviations: F = female; M = male; PMI = postmortem interval; * = both the brainstem and the hippocampal complex of this patient were subjected to immunoblot analysis.

TABLE 2
Antibodies Used in This Study

Antibody name	Epitope	Dilution	References
Monoclonal anti-tau antibodies			
12E8	ser262 (P+)	0.25 μg/ml	(34)
AT8	ser202/thr205 (P+)	1:500	(22, 25, 32, 34)
AT10	unknown	1:6,500	(31, 32)
AT180	thr231 (P+)	1;500	(24, 31)
AT270	thr181 (P+)	1:500	(24, 31)
Alz50	aa2-10 (Pi)	1:10	(20, 27, 36)
PHF1	ser396/404 (P+)	1:250	(28, 30, 31, 33)
T14	aa141-178 (Pi)	1:1,000	(20, 21, 29, 35)
T46	aa404-441 (Pi)	1:8,000	(20, 21, 29, 35)
Polyclonal anti-tau antibodies			
133	aa1-16 (Pi)	1:500	(23, 26)
189	aa76-87 (Pi)	1:1,000	(20, 26)
304	aa45-73 (Pi)	1:500	(20, 26)
T3P	ser396 (P+)	1:200	(20, 30, 31)
Monoclonal anti-ubiquitin antibody			
1510	ubiquitin	1:20,000	(37)

Abbreviations: aa = amino acids; P+ = phosphorylation dependent site; Pi = phosphorylation independent site; ser = serine; thr = threonine. One or more key references describing the characterization and specificity of the antibodies in this Table are listed in the far right column.

Bouin's solution or 70% ethanol containing 150 mM sodium chloride as described (8, 13, 18). Subsequently, the tissues were embedded in paraffin, and 6-μm-thick sections were cut and affixed to glass slides. The diagnostic evaluation of the tissue samples was performed as described earlier (13, 18) except that a modification of the Thioflavin S method was used which includes incubations in potassium permanganate and oxalic acid to quench lipofuscin autofluorescence (19). Immunostaining was carried out using the peroxidase antiperoxidase or avidin-biotin complex procedure with diaminobenzidine as chromogen according to recently described modifications of earlier procedures (18). The anti-tau antibodies used here have been characterized in detail using postmortem AD and normal adult and fetal brain samples as well as biopsy-derived normal brain samples (20–36). The names of these antibodies as well as their specificities and several key publications describing them are summarized in Table 2. In addition, a previously characterized anti-ubiquitin antibody (37) was also used here (Table 2).

Tau Isolation and Immunoblot Analysis

Autopsy-derived normal human tau was extracted as described (8, 20). Partially purified AD PHFtau from postmortem neocortical samples from 2 AD patients as well as brainstem samples from 3 PSP patients and hippocampus from 1 PSP patient were prepared exactly as reported previously (20, 21). Both the AD and PSP samples were examined in parallel using the same Western blot methods. Briefly, nitrocellulose replicas of gels containing the electrophoresed tau samples were prepared using 10% SDS-PAGE gels, and the replicas were then probed with a cocktail of two different phosphate independent anti-tau antibodies (i.e. T14 and T46). Similar amounts of each sample (10 to 20 μg) from the PSP and AD brains were loaded in each lane of the gels. The bound antibodies were detected with the peroxidase antiperoxidase method to visualize the protein bands recognized by these antibodies (20, 21, 31, 38). Recombinant human tau was a gift from Dr M. Goedert, and it was used as an additional control sample in the Western blots as described (20).

RESULTS

Immunohistochemistry

The panel of monoclonal (MAB) and polyclonal antibodies to defined epitopes spanning the amino to the carboxy terminal regions in tau (Table 2) was used to probe tangle-rich brainstem sections from seven PSP patients. Six of these anti-tau antibodies recognize phosphate independent tau epitopes and six other antibodies recognize epitopes that harbor phosphorylated serine or threonine residues scattered throughout the middle region of the tau molecule. One additional antibody, AT10, recognizes an undefined phosphate-dependent epitope that is present in AD PHFtau, but not in normal fetal or adult (both biopsy- and autopsy-derived) tau proteins (31). All of these anti-tau antibodies, including AT10, immunostained PSP-NFTs throughout the brainstem and medulla oblongata of all of the PSP patients studied here as summarized in Table 3. In addition, these antibodies also stained PSP tangles in glial cells (so-called glial tangles) and the numerous neuropil threads found in association with PSP NFTs (Fig. 1). In control experiments, the same panel of antibodies was applied to hippocampal sections from

TABLE 3
Summary of Immunohistochemical Results

Case	Region	133	ALZ50	304	189	T14	AT270	AT8	12E8	AT180	T3P	PHF1	T46	AT10
PSP														
1	Medulla	+	+	+	+	+	+	+	+	+	+	+	+	+
2	Pons	+	+	+	+	−	+	+	+	+	+	+	+	+
2	Medulla	+	+	+	+	+	+	+	+	+	+	+	+	+
3	Pons	+	+	+	+	+	+	+	+	+	+	+	+	+
3	Medulla	+	+	+	+	+	+	+	+	+	+	+	+	+
4	Pons	+	+	+	+	+	+	+	+	+	+	+	+	+
4	Medulla	+	+	+	+	+/−	+	+	+	+	+	+	+	+
5	Pons	+	+	+	+	+	+	+	+	+	+	+	+	+
5	Medulla	+	+	+	+	+	+	+	+	+	+	+	+	+
6	Pons	+	+	+	+	−	+	+	+	+	+	+	+	+
6	Medulla	+	+	+	+	+	+	+	+	+	+	+	+	+
7	Pons	+	+	+	+	+	+	+	+	+	+	+	+	+
7	Medulla	+	−	+	+	+	+	+	+	+	+	+	+	+
AD														
8	HIPP	NA	NA	+	NA	NA	+	+	NA	NA	NA	NA	NA	NA
9	HIPP	+	+	+	+	−	+	+	NA	+	+	+	+	+
10	HIPP	+	+	+	+	+	+	+	+	+	+	+	+	+

Abbreviations and symbols: AD = Alzheimer's disease; HIPP = Hippocampus; NA = not available; PSP = progressive supranuclear palsy; + = anti-tau positive lesions present; − = anti-tau positive lesions not present; +/− = anti-tau positive lesions very weakly immunostained.

three AD patients, and all of these antibodies recognized AD NFTs, dystrophic plaque neurites and neuropil threads (data not shown).

To identify samples of PSP telencephalon that might be suitable for Western blot analysis, sections containing the hippocampus and entorhinal cortex from each of the seven PSP patients were probed with MAB PHF1. These studies revealed that PSP cases 3 to 6 had large numbers of NFTs in both their hippocampi and entorhinal cortices. Notably, extracellular neurofibrillary tangles (E-NFTs) were found in all of these entorhinal cortices and also in some hippocampi of these 4 PSP patients. In contrast to AD brains where E-NFTs are always present, E-NFTs are not seen in the brainstem (i.e. pons and medulla oblongata) of PSP patients. Further, MAB 1510, which recognizes free or conjugated ubiquitin, immunodecorated a portion of the NFTs in the AD and PSP samples, and the tangles in both AD and PSP were Thioflavin S positive. Although we have noted differences in the Thioflavin S staining of PSP NFTs versus AD NFTs (13), similar differences were not seen with the modified Thioflavin S staining protocol used here which includes a step to quench lipofuscin autofluorescence (19).

Immunoblots

Nitrocellulose replicas of tau isolated from the brainstem of 3 PSP patients (cases 1, 3, and 7 in Table 1) as well as AD PHFtau, adult tau, and recombinant tau were probed with the anti-tau MABs AT10, AT8 (data not shown), and T14/T46. These blots showed a decrease in electrophoretic mobility of both the PSPtau and the AD PHFtau as compared to normal adult tau or recombinant tau indicating that both PSPtau and AD PHFtau were hyperphosphorylated. In addition, tau from the brainstem of PSP patients showed 2 tau bands with an apparent molecular weight (Mr) of 64 and 69 kD, while PHFtau from AD patients showed 3 immunobands with an Mr of 60, 64, and 69 kD (Fig. 2A). Tau also was isolated from the hippocampus and parahippocampal cortex of one PSP patient (case number 3), and analysis of this sample by Western blot using MABs T14/T46 revealed 3 immunoreactive bands that were indistinguishable from PHFtau in the AD brain (Fig. 2B).

DISCUSSION

The present study significantly extends previous investigations of the composition of tau proteins in the NFTs of PSP patients (9–15) by probing these lesions with a large panel of anti-tau antibodies to defined phosphate-dependent and -independent epitopes that span the entire length of tau proteins (20–36). Significantly, these studies clearly demonstrate that the NFTs in the brains of PSP and AD patients display the exact same constellation of tau epitopes by immunohistochemistry despite differences in the immunoblot profile of PSP brainstem versus AD hippocampus. Indeed, the antibodies that stained PSP NFTs also include the AT10 MAB, which recognizes a unique epitope that is present in PHFtau from the AD brain, but not in any fetal or adult forms of normal brain tau (31). Although our panel of anti-tau antibodies did not detect any differences in the composition of NFTs

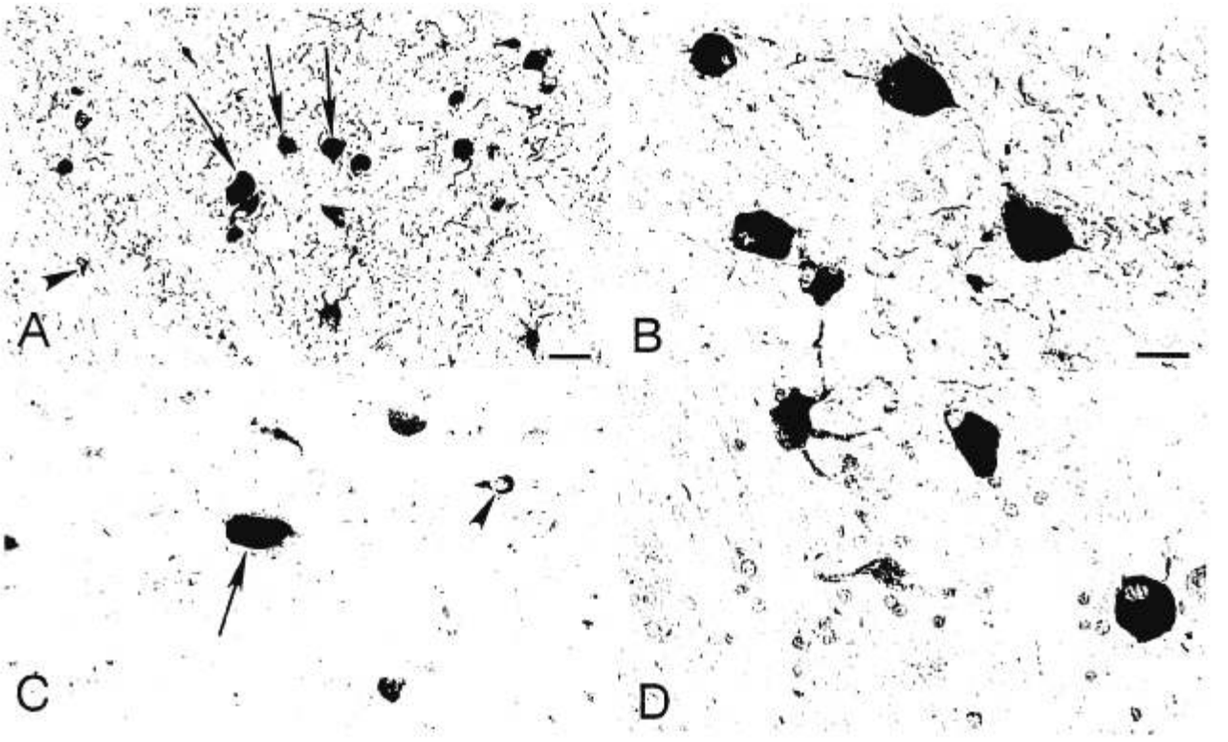

Fig. 1. Immunohistochemistry of PSP lesions. A. Pons of a PSP patient immunostained with MAB PHF1. Many large intraneuronal NFTs (arrows) and numerous neuropil threads are stained with PHF1, while PHF1 positive glial tangles (the arrowhead identifies one of several of these tangles in this field) are much smaller than intraneuronal NFTs. Scale bar = 20 μm. B. A different area of the same section as in panel A at higher magnification illustrates five intraneuronal NFTs and numerous neuropil threads. Scale bar = 20 μm. C. Medulla of a PSP patient immunostained with MAB 12E8. An intraneuronal NFT (arrow) and a glial tangle (arrowhead) are illustrated. Magnification is the same as in panel B. D. Pons of a PSP patient immunostained with MAB AT10 showing three intraneuronal NFTs and a diffusely stained neuron (center) without a fibrillary structure. Magnification is the same as in panel B.

from the brains of patients with PSP and AD, immunohistochemical and ultrastructural differences have been noted between the NFTs that accumulate in the brains of patients with these two distinct neurodegenerative disorders (2–7, 9, 10, 13, 39, 40). For example, AD NFTs display epitopes found in neurofilament proteins while similar epitopes are not present in PSP NFTs (13). It is unlikely, however, that the incorporation of fragments of neurofilament proteins into AD NFTs is responsible for the ultrastructural differences between the PHFs in AD NFTs and the straight filaments in PSP NFTs, since only a fraction of AD NFTs contain neurofilament protein epitopes (41). Thus, other modifications of tau could account for these ultrastructural differences as well as for the differences in the electrophoretic profiles of PSPtau in the brainstem of PSP cases and PHFtau isolated from AD brains documented here and in other studies (11, 12). For example, there are conflicting reports on whether or not PSP NFTs are ubiquitinated (42, 43), and the extent to which tau is ubiquitinated in PSP could modify the biochemical and structural properties of tau in the NFTs of PSP cases compared with NFTs in the AD brain. While the studies described here do not exclude this possibility, we show here that the anti-ubiquitin MAB 1510 immunostains at least a fraction of brainstem PSP NFTs in agreement with a previous report (44).

Although immunoblots of tau extracted from the brainstem of PSP patients demonstrated 2 immunobands with an Mr of 64 and 69 kD, neocortical extracts of tangle-rich AD brain contained 3 PHFtau bands with an Mr of 60, 64, and 69 kD. However, our studies of hippocampus and parahippocampal cortex from one PSP patient (case 3, Table 1) suggest that the abnormal tau proteins in the telencephalic NFTs of the PSP brain may be more similar to AD PHFtau than to the abnormal tau proteins recovered from the brainstem of PSP patients. Specifically, the immunoblots of PSP hippocampus and parahippocmpal cortex showed 3 PHFtau bands that were indistinguishable from PHFtau obtained from tangle-rich AD brains. Indeed, the PSP hippocampus contained E-NFTs, which are not seen in the PSP pons or medulla oblongata, but do occur in the AD hippocampus. Finally, although this observation is in agreement with a previous report (11), abnormally phosphorylated tau that migrates as 2 bands

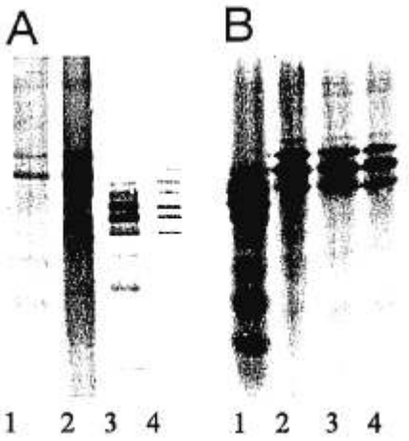

Fig. 2. Immunoblots of tau from PSP patients and controls. Tau was isolated, electrophoresed, transferred to a nitrocellulose membrane, and immunostained with MABs T14/T46 as described in the text. A. Lane 1: PSPtau from the brainstem; lane 2: AD PHFtau from hippocampus; lane 3: normal adult postmortem brain tau; lane 4: recombinant tau. B. Lane 1: normal adult postmortem brain tau; lane 2: AD PHFtau; lane 3: tau profile from the hippocampus and parahippocampal gyrus from a PSP patient; lane 4: tau profile from hippocampus and amygdala from the same PSP patient as in lane 3 (see Table 1).

with an Mr of 64 and 69 kD also has been detected in neocortical areas of PSP brains (11).

However, the biochemical basis for the presence of two abnormal tau bands in the PSP brainstem and three PHFtau bands in the PSP hippocampus as well as in the AD brain may be difficult to elucidate since all of the PHFtau epitopes detected in AD-NFTs also were present in PSP-NFTs. Further, since the hippocampal NFTs in elderly PSP patients probably represent a mixed population of PSP-induced tangles and age-related tangles, the biochemical profile of abnormal tau proteins in Western blots of PSP hippocampus may vary with the age of the patient and with the relative abundance of NFTs due to PSP. In contrast, similar problems do not appear to encumber analysis of the pons in PSP (45). Whether or not these differences in the electrophoretic profile of PSPtau from brainstem and PHFtau from the AD brain reflect differences in the expression and metabolism of normal tau proteins in these different brain regions or important pathological processes remains to be determined.

In summary, our data demonstrate that brainstem NFTs from PSP cases and telencephalic NFTs from AD patients share all of the same tau epitopes detected by the library of anti-tau antibodies used here. Thus, the abnormal tau proteins found in brainstem PSP tangles span the entire tau molecule, and they also are phosphorylated at all of the same serine and threonine residues as PHFtau in AD NFTs. These similarities are in contrast to some of the well-documented differences between the NFTs in PSP and AD. Thus, it will be important to determine if the NFTs in PSP and AD arise from similar or different mechanisms and if they have similar or different effects on the function and survival of affected neurons in these two neurodegenerative conditions.

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