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Difference between the tau protein of Alzheimer paired helical filament core and normal tau revealed by epitope analysis of monoclonal antibodies 423 and 7.51

(neurofibrillary tangles/neurodegeneration)

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ABSTRACT **The microtubule-associated protein tau that is incorporated into paired helical filaments (PHFs) undergoes some form of aberrant posttranslational processing in Alzheimer disease. Difficulties in deciding which changes are critical for PHF formation stem in part from the lack of immunochemical markers specific for PHF tau. The only monoclonal antibody (mAb) that is known to react with PHF tau but not with the predominant normal adult tau species is mAb 423. Another mAb (7.51, described in this paper) recognizes a segment of tau that is included in the minimal recognition unit required by mAb 423. Unlike 423, which is PHF tau-specific, mAb 7.51 recognizes all PHF core-derived tau as well as native soluble tau and recombinant tau expressed in bacteria and so serves as a generic tau marker. Both epitopes are in the 12-kDa fragment released from the Pronase-resistant core of the PHF (which encompasses the tandem repeat region). The mAb 7.51 epitope requires segments located in the last two repeats, which are common to all tau isoforms. The mAb 423 epitope requires sequences located near both the N and the C terminus of the 12-kDa fragment common to three- and four-repeat tau isoforms. Fragments denatured by concentrated formic acid and SDS regain 423 reactivity when denaturing agents are removed. Since the primary amino acid sequences of PHF tau and normal tau are identical in the repeat region, we conclude that 423 reactivity also requires a modification(s) occurring within an ≈90-residue segment that are not present in tau proteins so far described in the human brain.**

The microtubule-associated protein tau is a constituent of the paired helical filaments (PHFs) (1, 2) that are characteristic of the neurofibrillary pathology of Alzheimer disease (3). This protein is incorporated into the PHF in such a way that the N-terminal domain of some 200 amino acids projects into a Pronase-sensitive fuzzy coat, whereas the repeat region of the molecule is embedded within the protease-resistant core of the filament (1, 4, 5). Thus the same region of the molecule that functions as the microtubule-binding domain (6–8) is also integral to the PHF core. It has been suggested that the modification of tau responsible for PHF formation is abnormal phosphorylation (9–12, 16), which may be associated with the appearance in soluble brain extracts of a tau species of slightly lower than normal gel mobility that is recognized by monoclonal antibody (mAb) Alz50 (13–21). The only available markers of abnormal phosphorylation of tau in Alzheimer disease, including Alz50 (16), map to epitopes located outside the repeat region of tau (11, 19, 21). There is, however, no evidence that these parts of the tau molecule are important for PHF assembly.

Anti-PHF markers generally available are not actually specific for PHF tau (18–22). This makes it impossible to distinguish, for example, between normal soluble tau, soluble tau processed abnormally in the somatodendritic compartment after PHF formation, and tau species modified in a way that is unique to the PHF. In earlier studies (1, 4, 23) we reported a mAb, 423, that recognizes the PHF core and PHF core-derived tau but not normal mammalian or human tau. The mAb 423 epitope is therefore specifically associated with PHF tau. In this study we report the mapping of the mAb 423 epitope within the 12-kDa fragment that represents the PHF core-binding region of tau and describe another mAb, 7.51, whose epitope maps in the same region of both normal and PHF-associated tau. The epitope analysis indicates that PHF tau contains posttranslational modifications within a 90-residue segment that are not present in tau proteins so far described in the human brain.

MATERIALS AND METHODS

Enrichment of PHFs and Isolation of tau Protein Fragments from the Pronase-Resistant Core of the PHF. The tangle-enriched, Pronase-digested PHF fraction termed ifII was prepared as described (1). The F5.5 fraction, in which PHF core-derived tau fragments are substantially enriched, was also prepared as described (1). In this protocol, contaminants soluble at pH 5.5 are first extracted by sonication. The PHF core-derived tau fragments then become soluble at pH 5.5 only after a further formic acid sonication step. The pellet left after this extraction can be brought into solution at pH 11.0 and is termed N11. In an alternative protocol, the pellet left after the pH 5.5 sonication is brought into suspension by sonication in 100 mM NH_4HCO_3 at pH 8.5. The supernatant produced by centrifugation at 12,000 × *g* for 10 min is termed ABCsup and contains a suspension of short morphologically recognizable PHF fragments (24, 25).

Protein Sequence Analysis. Sequence analyses of the 12-kDa band or its fragments were performed by transferring bands resolved by SDS/PAGE to poly(vinylidene difluoride) (PVDF) membrane (Immobilon; Millipore) (26). Blotted bands were excised and sequenced using an Applied Biosystems gas-phase sequencer with on-line HPLC detection of phenylthiohydantoin derivatives of amino acids.

mAbs. The hybridoma NOAL 6/66.423.2, which produces mAb 423, has been described (1, 23). The hybridoma NOAL 7.51 (mAb 7.51) was derived by using the spleen of a BALB/c mouse hyperimmunized with the N11 subfraction as a source of cells for fusion with the NSO myeloma cell line, as described (27). NOAL 7.51 was selected on the basis of

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Abbreviations: PHF, paired helical filament; mAb, monoclonal antibody; PVDF, poly(vinylidene difluoride).

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strong staining of normal human tau preparations and PHF core-derived tau fragments by immunodot assay.

Proteolytic Digestion of PHFs and tau Fractions. F5.5 and recombinant tau variants (28) were proteolyzed with endoproteinase Asp-N (sequencing grade; Boehringer Mannheim) for 10 min at room temperature (substrate/protease ratio, 200:1, wt/wt) or with *Staphylococcus aureus* V8 protease (ICN), also for 10 min at room temperature (substrate/protease ratio, 100:1, wt/wt). The digestion was terminated by boiling in SDS sample buffer, and the resulting fragments were separated by SDS/PAGE in a 10–20% gradient minigel and either were immunoblotted against mAb 7.51 or mAb 423 (hybridoma supernatant) or were blotted to PVDF membrane for N-terminal amino acid sequencing. Additionally, a performic acid-treated PHF preparation (ABCsup) was adjusted to pH 2.0 with 10% (vol/vol) formic acid and digested overnight at room temperature with pepsin (Sigma) (substrate/protease ratio, 100:1, wt/wt) then treated as above.

Gel Electrophoresis and Immunoblots. SDS/PAGE (29) was carried out using a 10–20% gradient minigel (30). Two-dimensional separation involved isoelectric focusing (pH 3.0–10) followed by SDS/PAGE in an 18% gel. Transfer of bands for immunoblotting was carried out in 10 mM Caps buffer (pH 12.0) for 1 hr at 150 mA. Following transfer, or after spotting of antigen for immunodot assay on PVDF membrane incubated in methanol and then phosphate-buffered saline (125 mM sodium chloride/25 mM phosphate, pH 7.4), the PVDF membrane was treated with 1% (wt/vol) gelatin in phosphate-buffered saline (pH 7.4), incubated for 4 hr at room temperature with hybridoma supernatant, and washed extensively with phosphate-buffered saline. Bound antibody was detected by the peroxidase technique using 4-chloro-1-naphthol (Sigma) as the chromogen. Immunodot assays were developed similarly.

Immunoaffinity Purification. mAb 423, purified to homogeneity by protein A column (Pharmacia) chromatography of ascites grown in (C3H × BALB/c) F_1 mice, was coupled to a CNBr-activated Sepharose 4B column (Pharmacia). After the F5.5 fraction was loaded, material bound to mAb 423 was eluted by salt gradient (successively 1 M, 2 M, 3 M, and 4 M sodium thiocyanate). The flow-through and salt-eluted fractions, monitored by immunodot assay, were also examined by immunoelectron microscopy using mAbs 423 and 7.51.

Immunoelectron Microscopy. Immunoelectron microscopy was carried out (4) using 423 and 7.51 hybridoma supernatants or Alz50 kindly supplied by P. Davies (Albert Einstein College of Medicine, Bronx, NY).

Preparation of Normal Human tau. Normal human tau protein was isolated from human brain by three cycles of temperature-dependent assembly–disassembly of microtubule proteins (31), followed by heating to 100°C for 5 min in 50 mM Tris·HCl, pH 7.5/200 mM NaCl. Isolation and purification of recombinant-expressed human tau isoforms has been described (28).

RESULTS

NOAL 6/66.423.2 (mAb 423) was raised against a highly enriched Pronase-treated PHF preparation called ifII (1, 23). mAb 423 decorates isolated Pronase-treated PHFs (Fig. 1*A*), and recognizes PHF core-derived tau extracts in immunoblots after denaturation by boiling in SDS/2-mercaptoethanol and electrotransfer (Fig. 2). However, mAb 423 does not recognize tau isolated from adult bovine (1) or human (Fig. 2, lane 8, and refs. 23–25) sources, nor does it recognize any of the six known isoforms of human tau that have been expressed in *Escherichia coli* (Fig. 2). Furthermore, no digestion product of native or expressed tau has been produced that acquires 423 reactivity.

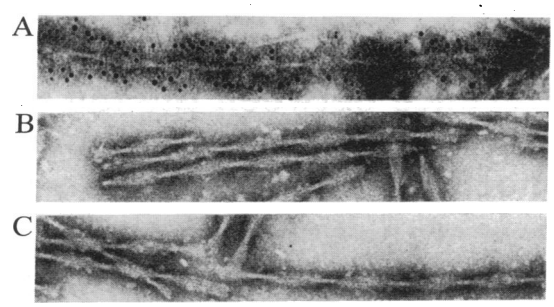

FIG. 1. Immunoelectron microscopy of Pronase-treated PHFs with mAbs 423 (*A*), 7.51 (*B*), and Alz50 (*C*). Only mAb 423 labels the Pronase-resistant core of the PHF. mAb 7.51 recognizes PHF-core tau only after release (see Figs. 2 and 3). In each case, PHFs were prepared by the ifII protocol (1). (×110,000.)

NOAL 7.51 (mAb 7.51), raised against a Pronase-treated PHF core subfraction (N11, see *Materials and Methods*), recognizes the same PHF core-derived tau extracts in immunoblots as mAb 423 (Fig. 2). However, 7.51 does not decorate Pronase-treated PHFs (Fig. 1*B*) or untreated PHFs (data not shown). Unlike 423, mAb 7.51 does recognize all tau proteins isolated from mammalian sources (data not shown), as well as native and expressed human tau proteins (Fig. 2). mAb 7.51 therefore behaves as a generic tau marker.

mAb 423 and 7.51 Immunoreactivity in PHF Core-Derived tau. The F5.5 fraction is an enriched preparation of tau fragments released from the Pronase-resistant core of the PHF that encompass only the tandem repeat region (1). It is composed of a series of bands originally estimated at 9.5, 12, 24, and 28 kDa (1). In view of revised molecular size markers (32, 33), we now refer to the 9.5- and 12-kDa fragments as 12 and 14 kDa, respectively. Immunoblots of F5.5 with mAbs 423 and 7.51 reveal an almost identical pattern (Figs. 2 and 3), the only detectable difference being the occasional presence of minor components running ahead of the prominent 12-kDa band. When present, these minor components are recognized by mAb 7.51 but not mAb 423 (Fig. 3 *A* and *D*).

Immunoaffinity purification of F5.5 material with mAb 423 showed that essentially all of the mAb 7.51-reactive material was retained on, and could be eluted from, a 423-Sepharose 4B column, as detectable either by immunodot assay (data not shown) or by immunoelectron microscopy (Fig. 3 *B*, *C*, *E*, and *F*).

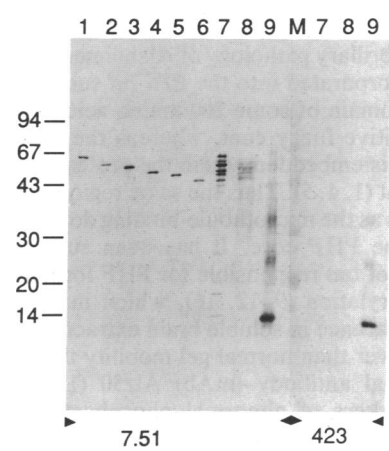

FIG. 2. Immunoblots of bacterially expressed (lanes 1–7), native (lane 8), and PHF core-derived (lane 9) human tau probed with mAbs 7.51 and 423. mAb 7.51 recognizes expressed, native, and PHF core-derived preparations of human tau protein. By contrast, mAb 423 recognizes only PHF core-derived tau protein. Molecular size markers are shown in lane M (molecular masses in kilodaltons are at left).

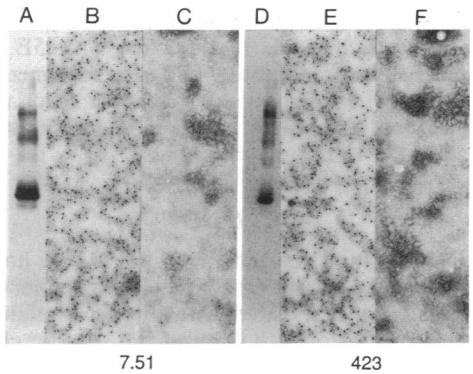

FIG. 3. (*A* and *D*) Immunoblots of PHF core-derived tau fractions showing that all the major species in F5.5 are recognized by mAbs 7.51 (*A*) and 423 (*D*). (*B*, *C*, *E*, and *F*) Immunoelectron microscopy of mAb 423 immunoaffinity-purified PHF-core tau extracts (column loaded with F5.5) shows that the retentate is reactive with mAbs 7.51 (*B*) and 423 (*E*), whereas the column flow-through is essentially unreactive with either 7.51 (*C*) or 423 (*F*). F5.5-derived tau material appears structurally amorphous on electron microscopy. (×40,000.)

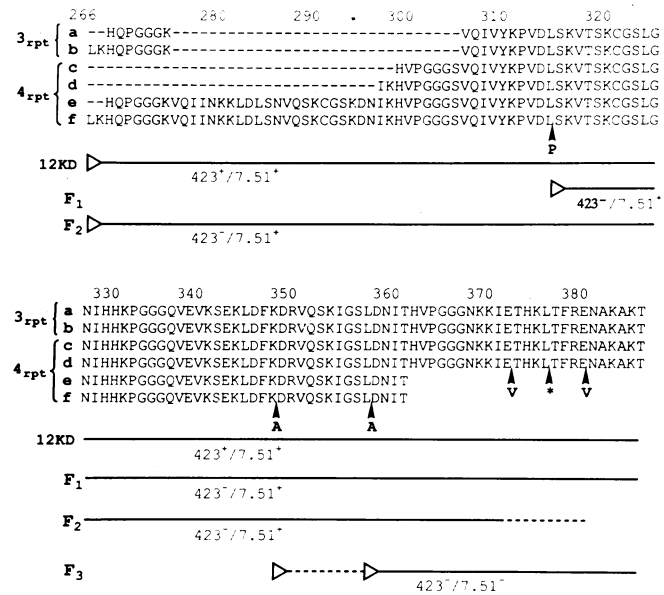

FIG. 4. N-terminal sequence analysis of the 12-kDa (12KD) band has shown the presence of multiple peptides derived from the repeat region of three- and four-repeat tau isoforms (34). These peptides encompass repeats 1, 2, and 3 of three-repeat (3rpt species a and b) and four-repeat (species e and f) tau isoforms and repeats 2, 3, and 4 (species c and d) of four-repeat tau. These peptides are aligned according to the known cDNA sequences of human tau (34, 35, 37). The starting 12-kDa species is reactive with mAbs 423 and 7.51 (see Fig. 5, lanes 5, 8, and 12). F_1 denotes the 8-kDa fragment released after oxidized PHF core fragments are further digested with pepsin (Fig. 5, lane 4). The N-terminus of this fragment is at arrowhead **P**, and this material is recognized by mAb 7.51 (Fig. 5, lane 7) but only very weakly by mAb 423 (Fig. 5, lane 6). F_2 denotes an 8-kDa fragment produced by digesting F5.5 with V8 protease (Fig. 5, lane 9). In this case, the major N-terminal species remain intact, but material is lost from the C terminus, probably at either of the positions denoted **V**. F_2 is recognized by mAb 7.51 (Fig. 5, lane 9), but not by mAb 423 (Fig. 5, lane 11). The C terminus at Leu-376 of a recombinant tau fragment (residues 1–376) that retains mAb 7.51 reactivity is shown by an asterisk. F_3 denotes mAb 7.51-negative fragments of 2–4 kDa (Fig. 5, lane 14) with N termini at Asp-348 and Asp-358 produced by endoproteinase Asp-N digestion of an mAb 7.51-positive recombinant tau fragment (residues 250–441; Fig. 5, lane 13). The entire mAb 7.51 epitope is contained between Ser-315 and Leu-376. Residues within the 32-amino acid stretch between Ser-315 and Lys-348 are necessary for reactivity but may not be sufficient. mAb 423 recognizes a configuration of the repeat region that requires the integrity of segments located near both ends of the 12-kDa fragment common to three- and four-repeat isoforms.

These results do not support the proposal that there are two immunochemically distinct forms of tau in PHFs (22). On the contrary, our results show that virtually all tau species present in F5.5, as defined by the generic marker mAb 7.51, are also recognized by the PHF-specific mAb 423. Thus the major tau species present in F5.5 are reactive either with both antibodies or with neither.

Further epitope mapping was restricted to the predominant 12-kDa band. N-terminal sequence analysis of this band (R.J., M.N., P.E., and C.M.W., unpublished work) has shown the presence of multiple tau peptides that are derived from at least two distinct tau isoforms (34–37). Fig. 4 shows their sequences. The C terminus of the fragments has not been defined unequivocally; however, the presence of a chymotrypic fragment (FRENAKAKT) defines the minimum length as 90 residues (1). We have attempted to resolve the fragments present in the 12-kDa band by two-dimensional gel electrophoresis. Both antibodies recognize at least four spots (data not shown), although their identity with respect to the sequences shown in Fig. 4 remains to be established. That mAb 423 recognizes multiple 12-kDa peptides makes it unlikely that the epitope is restricted to a particular three- or four-repeat tau isoform or to a specific N-terminal cleavage site.

mAb 7.51 Epitope. Smaller fragments produced by enzymatic digestion of PHF core preparations have been used for mapping studies. A fragment with a gel mobility of 8 kDa and a minor species of lower gel mobility (higher molecular mass) can be produced by pepsin digestion (Fig. 5, lane 4). The 8-kDa fragment starts at Ser-315, shown by arrowhead **P** in Fig. 4 (F_1), and presumably excludes fragments derived from species e and f in Fig. 4, which are shorter at the C terminus. It is not known whether these species are included in the minor components. F_1 is recognized by mAb 7.51 (Fig. 5, lane 7).

We have produced a different fragment by V8 protease digestion of F5.5 that also has a gel mobility of 8 kDa. The N-terminal sequence of this band is identical to the N-terminal sequence of the parent 12-kDa fragment. Therefore, the molecular mass loss must have resulted from C-terminal cleavage. In view of the known specificity of V8 protease (38), the fragment should extend to Glu-372 or possibly to Glu-380, as shown by arrowheads **V** in Fig. 4 (F_2). This fragment also retains mAb 7.51 reactivity (Fig. 5, lane 9).

A recombinant tau fragment produced in bacteria (36) terminates at Leu-376, shown by an arrowhead with an asterisk in Fig. 4. This is also recognized by mAb 7.51 (data not shown). Another recombinant tau fragment (residues 250–441; ref. 36) was, not surprisingly, mAb 7.51-positive (Fig. 5, lane 13). When this fragment was digested with endoproteinase Asp-N the immunoreactivity was lost (Fig. 5, lane 14), even though the preparation was shown to contain fragments between 2 and 4 kDa starting at Asp-348 and -358, shown by arrowheads **A** in Fig. 4 (F_3).

We conclude that the entire mAb 7.51 epitope is contained between Ser-315 and Leu-376. Residues within the 32-amino acid stretch between Ser-315 and Lys-348 are necessary for 7.51 reactivity but may or may not be sufficient.

mAb 423 Epitope. The F_1 pepsin fragment, strongly recognized by mAb 7.51 (Fig. 5, lane 7), has only residual reactivity with mAb 423 (lane 6), suggesting that residues located between the N terminus of the 12-kDa fragment and Leu-316 are necessary but not sufficient to retain full mAb 423 reactivity. Immunoreactivity also requires a segment near the C terminus of the fragment, missing from F_2 in Fig. 4, since the 8-kDa fragment produced by V8 protease diges-

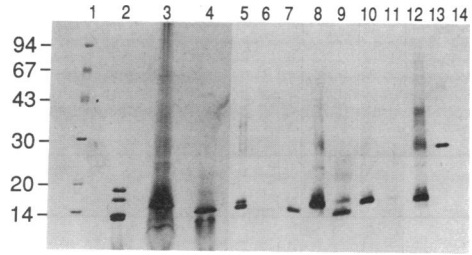

FIG. 5. Immunoblotting of peptide fragments used to map the mAb 423 and 7.51 epitopes in F5.5 Coomassie-stained F5.5 12-kDa and 14-kDa species are shown before (lane 3) and after (lane 4) pepsin digestion. The 8-kDa pepsin fragment, denoted F_1 in Fig. 4, was produced by digesting Pronase-treated PHF fragments in the ABC-sup fraction after performic acid oxidation. Whereas the intact F5.5 doublet is recognized by mAbs 423 (lane 5) and 7.51 (lanes 8 and 12), the 8-kDa fragment is recognized strongly by mAb 7.51 (lane 7) but only very weakly by mAb 423 (lane 6). V8 digestion of F5.5 produced another 8-kDa species, F_2 in Fig. 4, which is recognized by mAb 7.51 (lane 9) but not at all by mAb 423 (lane 11). The recombinant tau fragment (residues 250–441) is recognized by mAb 7.51 (lane 13), but after endoproteinase Asp-N digestion, which gives rise to the fragments denoted F_3 in Fig. 4, mAb 7.51 immunoreactivity is lost (lane 14). Molecular size markers are shown in lanes 1 and 2.

tion (Fig. 5, lanes 9 and 11), in which the N terminus is known to be intact from direct sequence analysis, is not recognized by mAb 423.

These results demonstrate that full 423 reactivity requires the integrity of segments located near both the N and the C end of the 12-kDa fragment. Fragments with various N-terminal sequences (Fig. 4) are recognized by mAb 423, making it most unlikely that the N termini generated by Pronase digestion are part of the epitope.

mAb 423 Immunoreactivity and Potential Posttranslational Modifications. The configuration recognized by mAb 423 is capable of regeneration after denaturation in concentrated formic acid, lyophilization, and further denaturation in boiling SDS solution. Since the primary amino acid sequence of fragments from PHF tau and normal tau are identical, mAb 423 reactivity also requires a modification(s) not present in normal tau, occurring within the minimal recognition unit. However, mAb 423 immunoreactivity is unchanged by treatment of F5.5 with alkaline phosphatase (Fig. 6), which has been shown to modulate Tau-1 immunoreactivity in tangles (9). Likewise, mAb 423 immunoreactivity is unaffected by treatment with broad spectrum glycohydrolases (data not shown).

Alz50 immunoreactivity, thought to be associated with abnormal phosphorylation of tau (16), was not detected in

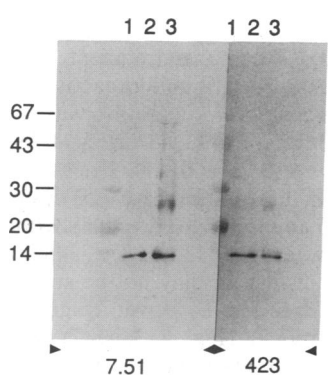

FIG. 6. Immunoblots of F5.5 before (lanes 2) and after (lanes 3) alkaline phosphatase treatment, probed with mAbs 7.51 and 423. There is no change in immunoreactivity with either mAb 7.51 or 423 after alkaline phosphatase treatment. Molecular mass markers are shown in lane 1.

F5.5 (data not shown). Another potential tau modification is ubiquitination, which would be detected by 5.25E, a mAb raised against PHF preparations that also recognizes ubiquitin peptides (39). This antibody does not react with the 12-kDa band (data not shown), and no ubiquitin sequence has been found in the 12-kDa band (1).

DISCUSSION

tau protein that is incorporated into the PHF core undergoes a complex form of aberrant processing in Alzheimer disease. This change is not associated with the production of any abnormal tau mRNA or with any isoform-specific change in message levels (34, 35, 37), although increased overall levels of tau message have been reported in hippocampus (40). Likewise no differences in primary amino acid sequence have been found in tau peptides derived from PHF preparations (1, 2, 41). Therefore, any abnormal processing of tau that is associated which PHF assembly must occur posttranslationally.

Several studies have claimed that tau protein is antigenically modified in Alzheimer disease (10–19, 21). However none of the anti-PHF antibodies generally available discriminate between the predominant soluble and PHF-associated tau species (11, 18, 19). Furthermore, after PHF assembly, portions of the tau molecule that are located outside the repeat region may be modified in ways that are characteristic of the somatodendritic compartment but irrelevant as regards PHF assembly. For example, the N-terminal 200-amino acid domain of tau and the C-terminal domain beyond the repeat region are accessible to proteolytic enzymes both *in vivo* (42) and *in vitro* (1, 4, 43), despite incorporation into the PHF. Likewise, fuzzy-coat tau domains can be substrates for alkaline phosphatase (9), and presumably also for kinases, in the assembled PHF. This leaves open the possibility that abnormal phosphorylation of PHF tau domains outside the repeat region and other soluble tau proteins caught in the somatodendritic compartment could be the consequence rather than the cause of neurofibrillary degeneration in Alzheimer disease.

In this report we show that the repeat region of tau, which is tightly bound within the core structure of the PHF and is exposed after Pronase digestion (1, 4), is a better candidate for detecting a tau modification that is specifically associated with the PHF. A major 12-kDa species, released from the Pronase-resistant PHF core by formic acid treatment, consists of a mixture of fragments derived from three- and four-repeat isoforms (1). All of these encompass only three repeats (R.J., M.N., P.E., and C.M.W., unpublished work). We now show that multiple PHF-core tau species are recognized by two antibodies with contrasting immunochemical profiles, mAbs 423 and 7.51. Whereas PHF core-derived tau species react with both mAbs 423 and 7.51, preparations of normal adult tau proteins described so far are recognized only by mAb 7.51. Thus mAb 423 detects a form of tau that is specifically associated with the Alzheimer PHF core, whereas 7.51 recognizes an epitope common to all known tau isoforms.

mAb 7.51 reacts with all known isoforms of bacterially expressed tau. It is therefore most unlikely to depend on posttranslational modifications. We have localized the 7.51 epitope to a short segment contained in the last two repeats that is common to all tau isoforms. This epitope is hidden when tau is bound to the core of the PHF, and it is exposed only after release (see also ref. 25). The penultimate repeat is known to be critical for microtubule binding (7, 8). The same repeat, part of which is necessary for 7.51 reactivity, may also be important in PHF core formation.

We have shown that segments located near both ends of an ≈90-amino acid fragment of tau are needed for full mAb 423

reactivity. We do not know whether these segments contribute directly or indirectly to the conformation of the epitope. Since multiple distinct peptides in the 12-kDa band are 423-positive, it is unlikely that mAb 423 reactivity depends on any specific N- or C-terminal cleavage site created by Pronase digestion. This is also supported by labeling of intact rat fetal tau by mAb 423 (43), immunohistochemical staining of intracellular tangles by mAb 423 in sections not treated with proteases (42), and immunogold labeling of fuzzy PHFs not treated with proteases (4, 42, 44). However, the epitope is more accessible following Pronase digestion of PHFs (4, 42). The three-dimensional structure of PHF tau detected by 423 is capable of regeneration after concentrated formic acid and boiling SDS denaturation (1) and persists in solution after release (24, 25). We have been unable to find any condition of the predominant species of human native, or partially Pronase-digested (23), adult tau that acquires 423 reactivity either before or after denaturation. In spite of extensive sequence analysis (1, 2), no difference in primary sequence between mAb 423-reactive and -unreactive tau has been detected. We conclude that the most likely explanation is that PHF tau contain a modification(s) not present in the isoforms of normal human tau described so far. These modifications either force an alternative tertiary structure, or provide antigenic determinants absent from normal tau, or both. Neither phosphorylation that is sensitive to alkaline phosphatase nor ubiquitination appears to be required for 423 reactivity.

A tau protein(s), A68, isolated from some [but not all (18)] Alzheimer brains has been described to be associated with neurofibrillary pathology (18–20) and very recently with A68-derived PHFs (21). However, A68 is recognized by mAb 7.51 but not by 423 (C. Caputo, V. Lee, C.M.W., and M.N., unpublished work). It will now be important to determine the relationship between A68 and the 12-kDa tau fragment from the PHF core. It will also be important to determine whether phosphorylated residues unavailable to alkaline phosphatase are present in the core fragment. In any event, 423 remains the only described mAb that is specifically and consistently associated with PHF tau and does not crossreact with the predominant tau proteins normally found in the adult brain. On the other hand, the immunoreactivity detected by mAb 7.51 in brain homogenates is found only in normal tau proteins, since the epitope remains hidden when tau is bound to the PHF. These reagents therefore provide excellent immunochemical discrimination between normal and PHF-associated tau protein. In the following paper (25), ELISAs that have been developed on the basis of the combined use of mAbs 423 and 7.51 (24) are shown to discriminate unequivocally between the predominant normal soluble tau proteins and PHF tau in the same brain tissue extracts.

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