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Mary P. Lambert
Northwestern University, Evanston, USA

Shasta Sabo
Northwestern University, Evanston, USA

Chi Zhang
Northwestern University, Evanston, USA

S. Ather Enam
Aga Khan University, ather.enam@aku.edu

William L. Klein
Northwestern University, Evanston, USA

Pergamon

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Constitutive Alzheimer's-Type Tau Epitopes in a Neuritogenic Rat CNS Cell Line

MARY P. LAMBERT, SHASTA SABO, CHI ZHANG, S. ATHER ENAM AND WILLIAM L. KLEIN[1]

Department of Neurobiology and Physiology, 2153 N. Campus Drive, Northwestern University, Evanston, IL 60208

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LAMBERT, M. P., S. SABO, C. ZHANG, S. A. ENAM AND W. L. KLEIN. *Constitutive Alzheimer's-type tau epitopes in a neuritogenic rat CNS cell line.* NEUROBIOL AGING **16**(4) 583-589, 1995. – Paired helical filaments (PHFs) of Alzheimer's disease (AD) largely comprise hyperphosphorylated forms of the cytoskeletal protein tau. AD-type tau phosphoepitopes, detected by various monoclonal antibodies, are absent from normal adult neurons, but recent studies have shown that their expression may contribute to neuritogenesis and axon differentiation in the developing nervous system. Therefore, we have examined a brain nerve cell line that is spontaneously neuritogenic for possible expression of AD-type tau epitopes. The neuritogenic rat brain cell line B103 was found to constitutively produce two AD-related epitopes of tau, detected by cellular immunofluorescence studies with the PHF-1 and Alz-50 monoclonal antibodies. Biochemical studies showed that the antibodies bound to proteins within the molecular weight range expected for phosphorylated tau isoforms. Further verification was established by use of tau antisense oligomers, which eliminated cellular immunofluorescence due to the AD-related monoclonals and polyclonal anti-tau but did not eliminate fluorescence due to anti-tubulin. Cells treated with tau antisense were not neurite-free. Neurites that remained, however, were abnormal, generally short and wavy in appearance. Cellular distribution of the tau epitopes was found to be particularly interesting. Alz-50 recognized only cytoplasmic tau whereas PHF-1 recognized nuclear tau as well as cytoplasmic. Thus, the two epitopes are morphologically segregated within the cell. Because subcellular segregation of tau is compromised in Alzheimer's disease, mechanisms that segregate AD-type phosphotau epitopes in B103 cells may have relevance to this neurodegenerative disorder.

Alzheimer's disease PHF-1 Alz-50 Antisense Tau protein Nuclear tau Neuroblastoma Cell line

PAIRED helical filaments (PHFs) are known to be the principal structures of neurofibrillary tangles, a neuronal abnormality characteristic of Alzheimer's disease (AD) (46). PHFs comprise aberrantly phosphorylated forms of the cytoskeletal protein tau (14,35,28). Tau normally can exist in various states of phosphorylation, but the level of phosphorylation is considerably elevated in Alzheimer's-afflicted brain (23,26,17). Various phosphoepitopes found in Alzheimer's-type tau can be discerned by monoclonal antibodies, a number of which have been raised against paired helical filaments obtained from Alzheimer's brain tissue. Two of these antibodies, Alz-50 (51) and PHF-1 (20,21,22), have been found to be highly specific for degenerating neurons in the adult human brain (19,27,13). The Alz-50 epitope is at the amino terminal of tau. It comprises amino acids 2–10 of tau (numbering based on the longest form of adult human tau), a sequence that appears to be conformationally sensitive to the hyperphosphorylation associated with AD tau (32,16). The PHF-1 epitope is carboxy to the microtubule binding domains and depends on phosphorylation of ser396 in AD tau (4). Alz-50 and PHF-1 epitopes also are found transiently in the developing brain where their expression has been attributed to the massive nerve cell death that occurs during embryogenesis and brain development (reviewed in 5). These epitopes also can be induced in mature neurons that are degenerating due to experimental manipulations (41,8).

The AD-type tau epitopes, however, are not always associated with cell death. Recent studies of the PHF-1 phosphotau epitope in developing chick brain neurons, for example, have shown it is expressed transiently by viable cells undergoing neuritogenesis and axon differentiation. The PHF-1 Alzheimer's-type posttranslational modification of tau thus is highly conserved in evolution (43), complementing the highly conserved nature of tau itself (34), and is linked to neuritogenesis in immature neurons.

Because AD-type tau phosphoepitopes can occur in viable immature neurons, they also are likely to occur in cloned nerve cell lines, which typically express multiple features of immature neurons. Such cell lines provide homogeneous experimental systems, and they would be of use for investigations of the regulatory cell biology of Alzheimer's type tau phosphorylation. The current work has used the PHF-1 and Alz-50 antibodies to investigate

[1]To whom correspondence should be addressed.

tau epitopes expressed by the B103 rat nerve cell line. This line is of particular interest because it is spontaneously neuritogenic and is derived from the central rather than the peripheral nervous system (47). Results here show that the B103 rat cells constitutively produce both the Alz-50 and PHF-1 tau epitopes. The Alz-50 epitope is exclusively extranuclear, but the PHF-1 epitope is abundant in the nuclear as well as extranuclear compartments.

METHOD

Cell Culture

The B103 rat CNS cell line was a generous gift of D. Schubert of the Salk Institute, CA. Cultures are maintained in Dulbecco's Modified Eagle medium, antibiotics (streptomycin, penicillin, and fungizone, Gibco/BRL), and 10% fetal calf serum (Gibco/BRL). For experiments, cells are plated on poly L-lysine-coated glass coverslips or Permanox LabTek plates (8 wells/plate, Nunc) at a density of 9×10^5 cells/cm^2 in a total volume of 0.4 ml Cells are allowed to grow for 24 h before fixing.

Immunocytochemistry

Cells are fixed with 3.7% formaldehyde for 15 min at 37°C and permeabilized with 0.1% Triton X-100 for 5 min at room temperature. After washing with PBS containing 1% bovine serum albumin and 1% DMSO (PBS+), the cells are blocked with 10% normal goat serum in PBS+ for 15 min. After another wash with PBS+, primary antibodies (PHF-1, Alz-50, and others) are added and allowed to react for 1.5 h at 37°C. After washing for 3 × 5 min with PBS+, FITC-conjugated anti-mouse IgG is added and allowed to react for 1.5 h in the dark. A final 3 × 5 min wash with PBS+ and then one with PBS are done before mounting. Images are taken using a Hamamatsu 10-bit digital CCD camera with the MetaMorph imaging system (Universal Imaging, Westchester, PA) on a Nikon Diaphot microscope equipped with epifluorescence capability. The MetaMorph system is also used to analyze the images for fluorescence intensity. PHF-1 (2.7 mg/ml) and Alz-50 (300 μg/ml) monoclonal antibodies were generous gifts of Jonathan Chong of Abbott Laboratories. They were used at 1:200 and 1:100 dilution, respectively, for immunocytochemistry and 1:4000 for immunoblots. Polyclonal anti-tau (against embryonic chick brain) was obtained from Sigma Chemical and used at 1:200 for immunocytochemistry and 1:4000 for immunoblots. This antibody does not cross-react with MAP1, MAP2, and tubulin, according to the company literature, and shows wide cross-reactivity amongst mammalian species (43,44). Polyclonal anti-tubulin (against embryonic chick brain) was obtained from ICN Biomedical, and used at 1:200. Tau-1 antibody was obtained from Boehringer Mannheim and used at 1:1000.

Radioimmunoprecipitation Assay

Cultures are grown as described for 24 h in a 60 mm dish. After rinsing with Hanks basic salt solution (37C, Gibco/BRL) and met/cys free DMEM (ICN, supplemented with 0.584 mg/ml glutamine) at 37°C, cells are incubated in the met/cys free DMEM for 20 min. 150 mCi of 35S-methionine/cysteine (NEN, Express) is added and the culture is grown overnight at 37C. After removal of the medium and two washes with chilled PBS, cells are harvested in 10 mM Tris-HCl, pH 7.4 containing 0.8 M NaCl, 3 mM EGTA, and 1 mM PMSF (fresh). After brief homogenization, the cell lysate is centrifuged at 30,000 × g for 20 min at 4°C. The supernatant cell lysate can be frozen at this point. 100 μl of lysate is used for each precipitation. 5 μl of PHF-1 antibody (4.6 mg/ml) is added to the lystate and incubated for 1.5 h on ice. Meanwhile, a solution of protein A Sepharose is prepared in PBS containing 0.1% SDS, 1% Triton X-100, fresh deoxycholate, PMSF and aprotinin (PBS-TDS). 200 μl of protein A Sepharose is added to each sample and the sample is shaken at 1600 rpm for 1.5 h at 4°C. The Sepharose is then washed 3 × with PBS-TDS. 25 μl of Laemmli buffer (30) is added to each sample and the sample is boiled for 5 min. Samples are then centrifuged and run on 8% SDS-PAGE gels (Novex). The gel is fixed in 10% acetic acid and 25% isopropanol for 30 min and soaked in Amplify (Amersham) for 30 min before being dried and exposed to X-ray film.

Alz-50 Immunoprecipitation

Cells are grown in poly L-lysine-coated 60 mm dishes for 24 h and harvested with cold PBS and 0.02% EDTA. Then cells are briefly centrifuged and lysed with lysis buffer (150 mM NaCl; 50 mM Tris-HCl, pH 7.5; 1% Triton × 100; 0.1% SDS; 2 mM EDTA; added cocktail of protease inhibitors before use: 2 mM PMSF, 0.2 TIU/ml aprotinin, 1 mg/ml leupeptin and 50 mM NaF, 1% deoxycholate acid, 30 mM NaH_2PO_4). After centrifugation at 14,000 × g for 10 min, the supernatant is assayed for protein content, and then stored in 100 μg aliquots. For immunoprecipitation, 2 μl of Alz-50 antibody is added and allowed to incubate for 2 h on ice. Then, 200 μl of protein A Sepharose prewashed with lysis buffer is added to each tube and the tube is shaken in the cold for 1.5 h. Unbound proteins are removed by 4 washes with lysis buffer. Bound proteins are solubilized in 20 μl Laemmli buffer (30), boiled for 5 min, separated by electrophoresis on a 14% SDS-PAGE gel (Novex), and then transferred to nitrocellulose using a Hoeffer transfer apparatus. The nitrocellulose is then blocked for 2 h in PBS+ 0.1% Tween 20 and 3% nonfat milk incubated with Alz-50 antibody (1:100 in PBS+ 0.1% Tween 20) for 1 h, washed 3 × 15 min with PBS+ 0.1% Tween 20, and incubated with mouse IgG secondary antibody (Vector Laboratories, Inc) for 30 min. After washing, bound proteins are treated with ABC reagent for 30 min and visualized with DAB.

ECL immunoblot and immunoprecipitation. Cells were grown in 60 mm dishes in DMEM, 10% FCS, for 24 h, and harvested in 0.01 M Tris, pH 7.4, supplemented with 1 mM EDTA, 1 mM EGTA, 0.15 M NaCl, 1% Triton X-100, and a cocktail of protease inhibitors made up fresh (2 mM PMSF, 0.2 TIU/ml aprotinin, 1 mg/ml leupeptin, 50 mM NaF, and 1 mg/ml pepstatin A). Cells were lysed by homogenization and the protein content was determined. For an immunoblot, 20 μg of protein was added to an equal volume of Laemmli buffer and boiled for 5 min. This sample was then loaded on an 8% polyacrylamide gel (Novex) and electrophoresed under denaturing conditions. The separated proteins were then transferred to a nitrocellulose membrane in the cold using a Hoeffer transfer apparatus. The membrane was then washed, blocked with 10% nonfat dry milk and probed with primary antibody for 1.5 h. After extensive washing, the antibody was conjugated to HRP-labeled secondary for 1 h and visualized with ECL reagents. For an immunoprecipitation, 250 μg of protein was first precleared with Protein A Sepharose which has been washed with PBS containing 1% Triton-X-100, 0.1% SDS, 1% BSA, and 5 mg/ml fresh deoxycholate for 30 min. Primary antibody (13 μg) was then added and the solution allowed to sit on ice for 1 h with occasional shaking. Protein A Sepharose was then added and the solution was shaken at 1600 rpm overnight at 4°C. The Sepharose was precipitated by centrifugation and then washed three times with the above collection buffer to remove unbound proteins. Bound proteins were then solubilized in 25 of Laemmli buffer by boil-

ing for 5 min. The solution was briefly centrifuged and the entire supernate was loaded on an 8% Novex gel and electrophoresed as described above. Visualization of the separated, transferred proteins was also as described above, using the ECL protocol.

Antisense Addition

Cultures are plated at 2×10^5 cells/ml in a total volume of 0.2 ml/well in DMEM supplemented with the N2 mixture of Bottenstein and Sato (2). A 25 base deoxyoligonucleotide corresponding to the inverse complement of the sequence from −14 to +11 of rat tau (generously provided by Abbott Laboratories) is added to the medium at 50 μM concentration 10 min after plating and at 20-h intervals thereafter. At 60 h, cultures are fixed and permeabilized for 5 min with 0.2% Triton X-100. They are double-labeled for PHF-1 and tubulin or PHF-1 and tau as described above. The secondary antibodies used are FITC-conjugated anti-rabbit IgG and Texas Red-conjugated anti-mouse IgG.

RESULTS

The B103 rat neuroblastoma cell line, derived from a rat CNS tumor, has various enzymatic characteristics of neuronal cells and spontaneously produces neurites in culture (47). Because axon formation and differentiation correlate with PHF-1 tau expression in the developing CNS, we asked if the B103 cell line might also express AD-related tau epitopes. B103 cells were grown for 24 h, fixed, and probed for these epitopes with PHF-1 or Alz-50 monoclonal antibodies. Visualization was with FITC-conjugated anti-mouse IgG. As shown in Fig. 1, both PHF-1 and Alz-50 antigens were expressed strongly in every cell observed, both in the cell bodies and neuritic processes. Neuritic expression of PHF-1 tau was uniform out to the growth cone area, although as cell polarity developed, PHF-1 tau appeared more abundant in the thin, axon-like neurite (Fig. 1B, arrow). Alz-50 staining was stronger in proximal areas of the neurite, in many instances tapering off near the growth cone, with no evidence of polarity.

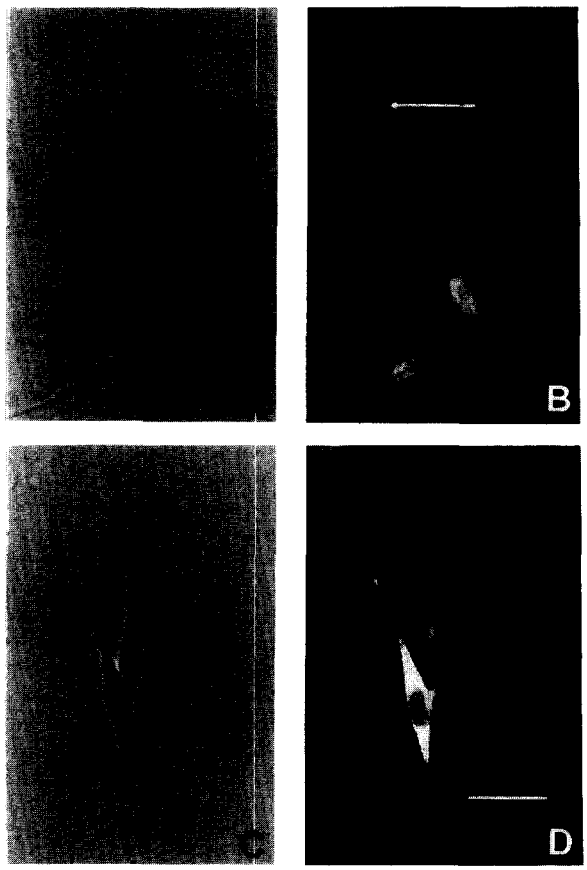

FIG. 1. PHF-1 and Alz-50 immunoreactivity are present in newly plated B103 cells. (A,B) Phase and fluorescent micrographs, respectively, of PHF-1 immunoreactivity in B103 cells; (C,D) Phase and fluorescent micrographs, respectively, of Alz-50 immunoreactivity in similar cells. Arrow indicates long process with more intense PHF-1 immunoreactivity. Bar in (D) equals 25 microns. B103 cells were cultured and immunostained for PHF-1 and Alz-50 tau as described in the Method section.

Of particular interest, the location of these tau antigens differed within the soma. The PHF-1 epitope was found in both the nucleus and cytoplasm, while the Alz-50 epitope was excluded from the nucleus. Antigens to polyclonal tau antibody were found in all cellular compartments (11, see also the tau sense control in Fig. 4F). Nuclear PHF-1 immunoreactivity was relatively more abundant than cytoplasmic , although the difference in intensity may be due partially to the relative thickness of the nucleus. In addition, several days after plating, two populations of cells could be observed, those which had both nuclear and cytoplasmic PHF-1 labeling and those which had only cytoplasmic labeling (data not shown). The decrease in nuclear PHF-1 immunoreactivity did not correlate with any apparent morphological property. Alz-50 immunofluorescence did not show any changes with respect to location or intensity during this time.

The Alz-50 antibody recognizes an epitope that depends on the 2–10 amino acid residues of tau (16). To verify that Alz-50 immunostaining found in B103 cells is also due to tau, proteins from B103 cell lysate were immunoprecipitated with Alz-50 antibody. This immunoprecipitate was then solubilized, separated by electrophoresis and reprobed with Alz-50 antibody. The result is shown in Fig. 2. Three proteins in cell lysate reacted with the Alz-50 antibody. Their apparent molecular weights are approximately 51, 53, and 60 kDa. The two lower bands are in the range of fetal tau (29,15); the upper band is that of the lowest molecular weight form of PHF-tau (28,17,35).

Because the calcium ionophore A23187 has been shown to affect the expression of Alz-50 in hippocampal cells (41), the effect of this agent on B103 cells was also assessed. A concentration-dependent morphological effect was seen after 90 min of incubation, with 5 μM ionophore causing a retraction of neurites while 0.5 μM had no effect (11, data not shown). Cell bodies changed from a rounded shape to one more triangular or fusiform and the neurites assumed a dystrophic or wavy appearance. In the presence of 50 μM ionophore, most of the cells detached from the substratum. The remaining attached cells had almost completely retracted neurites. Despite significant morphological changes, Alz-50 immunofluorescence was unchanged.

PHF-tau proteins, including those recognized by the PHF-1 antibody, have molecular weights in the 60-68 kDa range (28, 17,35). The PHF-1 antibody also recognizes an epitope in fetal tau, the smallest form of tau with a molecular weight of 55–58 kDa (4,8) and epitopes in cultured peripheral neuroblastoma cells from 50–58 kDa (44,36,40,11). Attempts to analyze the Mr of the PHF-1 antigen in B103 cell lysate by western blot showed no specific bands. Therefore, a more sensitive radioimmunoprecipitation analysis was performed. B103 cells were grown overnight in cys/met free medium with 35S-methionine. Protein was precipitated from cell lysate using the PHF-1 anti-

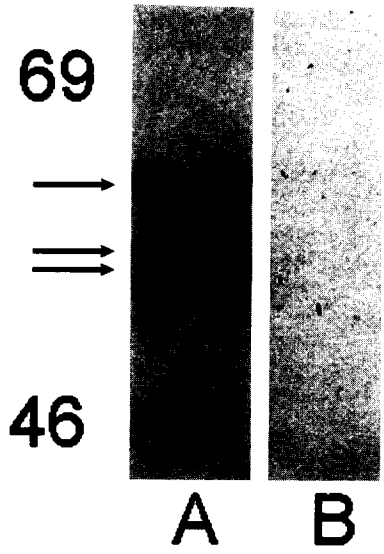

FIG. 2. Alz-50 immunoblot of B103 cell lysate shows three bands in the molecular weight range of 50 to 60 kDa. Left: molecular weight standards. Lane A: Alz-50 antibody, Lane B: No primary. Arrows indicate the bands precipitated by the Alz-50 antibody at approximately 51, 53, and 60 kDa, respectively.

body and adsorption to Protein A Sepharose. The major specific protein which was immunoprecipitated by the PHF-1 antibody had a molecular weight of 60 kDa and was not present when the primary antibody was omitted from the procedure (Fig. 3). Although multiple forms of the tau protein were not observed, the molecular weight was in the range for adult phosphorylated

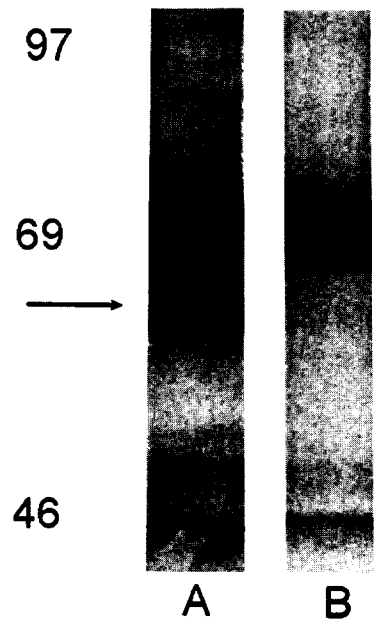

FIG. 3. RIPA analysis shows a 60 kDa band with the PHF-1 antibody in B103 cells grown for 24 h. Left: molecular weight standards. Lane A: PHF-1 antibody, Lane B: buffer alone. Arrow indicates the major 60 kDa band precipitated with PHF-1 antibody.

tau. Because the band found with radioimmunoprecipitation was faint and immunoblots were unsuccessful, additional studies were done to verify the nature of PHF-1 immunoreactivity by eliminating all tau proteins from the cells by transiently introducing antisense tau DNA. Previous work showed that tau antisense deoxyoligonucleotides administered from the time of plating eliminated tau expression in cultured cerebellar macroneurons and caused them to maintain an array of minor neurites without forming morphologically distinct axons and dendrites (7,6). In these same cells, exposure to tau antisense after establishment of an axon-like neurite caused the retraction of this neurite. To determine if tau antisense deoxyoligonucleotides caused any changes in B103 cultures, cells were given a 25 base deoxyoligonucleotide corresponding to the inverse complement of the sequence from -14 to +11 of rat tau. After 60 h, cultures were fixed and processed for immunofluorescence using various antibodies. Cells administered tau antisense deoxyoligonucleotides had only minor neurites (Fig. 4A), most of which appeared wavy or abnormally shaped, whereas cells administered tau sense deoxyoligonucleotides had essentially normal neurites (Fig. 4 B,D,F,H) compared to no addition controls.

Compared to cells given tau sense deoxyoligonucleotides, the cells given tau antisense had dramatically decreased PHF-1 and polyclonal tau staining (Fig. 4 C,D,E,F). Nuclear as well as cytoplasmic staining was eliminated. In contrast, tau antisense had no effect on immunolabeling for the cytoskeletal protein tubulin, illustrating selectivity of the response (Fig. 4 G,H). Note that the apparent nuclear staining for tubulin in occasional cells was due to label associated with a spindle apparatus; although the B103 cells spontaneously make neurites, they are not postmitotic. Absence of PHF-1 and tau immunofluorescence but retention of tubulin staining strongly suggest that the immunofluorescence seen with the PHF-1 antibody is due to tau protein. This conclusion is verifed by the nuclear immunoprecipitation experiment presented next.

The nuclear concentration of PHF-1 tau immunostaining and its lack of reaction in western blots led us to the speculation that PHF-1 antibody was reacting with nuclear tau. Accordingly, a nuclear fraction prepared by the procedure of Loomis et al. (38), was probed with antibody against polyclonal tau; additionally, PHF-1-reactive protein was immunoprecipitated from the nuclear fraction and probed with polyclonal tau antibody. The polyclonal tau antibody used for these experiments previously has been characterized by Pope et al. (44) and shown to specifically recognize tau isoforms cross-reactive with PHF-1 and Tau-1. As shown in Fig. 5, the polyclonal tau antibody recognized tau in the B103 nuclear extract (Lane 2), with the highest of three molecular weight forms present in greatest amount. Most important, the proteins that were immunoprecipitated from the nucleus by the PHF-1 tau antibody also were recognized by the polyclonal tau antibody (Lane 3). Again, the most prominent form was the highest molecular weight band at approximately 60 kDa. The total number of forms recognized cannot yet be determined because the regularity of the bands indicate some degradation may have taken place. As expected, proteins from the whole cell lysate (Lane 1) also were recognized by polyclonal tau antibody. In a separate gel, both the nuclear extract and the total lysate also gave a positive signal with Tau-1 antibody (data not shown). Results were similar to polyclonal anti-tau in the nuclear extract, with the higher of two molecular weight forms more prominent. The immunoprecipitation and immunoblot data thus verify that B103 cells express in their nucleus a PHF-1-reactive form of tau protein.

Several other cell lines also were examined for PHF1-tau immunofluorescence (11, data not shown). The F3 variant of PC12

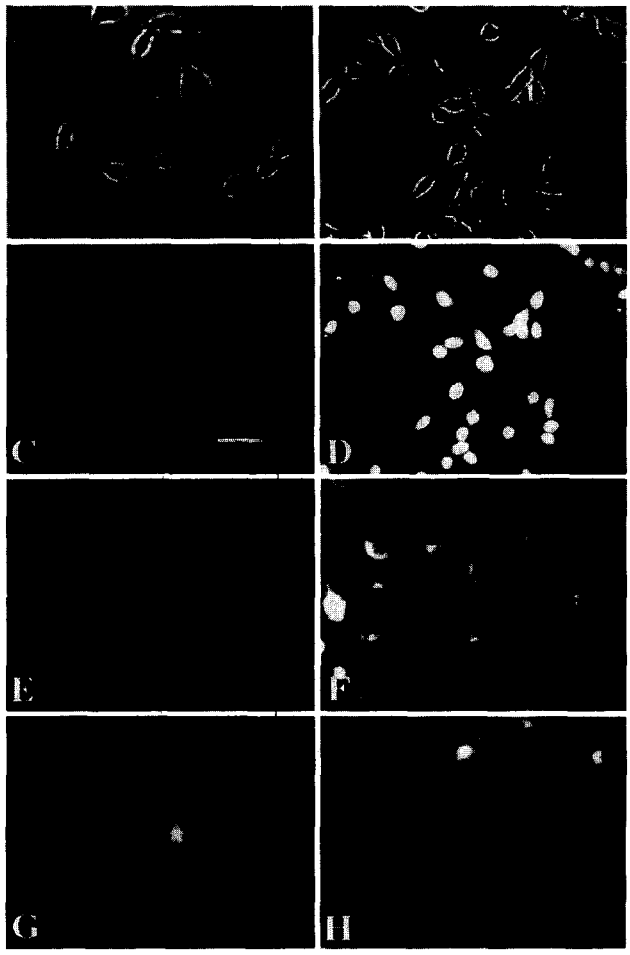

FIG. 4. Exposure to antisense tau in the medium causes loss of neurites and loss of immunolabeling after 60 h. B103 cells were plated on poly L-lysine and exposed to sense or antisense tau DNA (50 μM) for 60 h before fixing and observation with the Diaphot. (A,B) Phase micrographs: antisense and sense tau, respectively; (C,D) PHF-1 tau immunofluorescence: antisense and sense tau, respectively. (E,F) Polyclonal tau immunofluorescence: antisense and sense tau, respectively. (G,H) Tubulin immunofluorescence: antisense and sense tau, respectively. Note the absence of PHF-1 tau and polyclonal tau immunoreactivity with antisense tau DNA (C,E). Bar in (C) equals 50 microns.

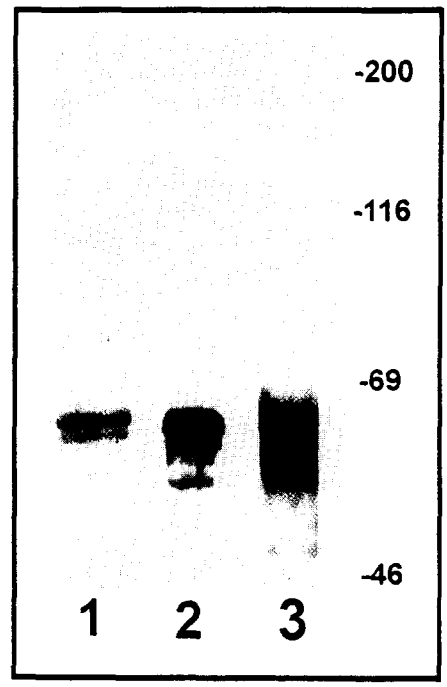

FIG. 5. Nuclear proteins immunoprecipitated with PHF-1 are recognized by polyclonal tau antibody. Lane 1: B103 whole cell lysate was immunoblotted with polyclonal anti-tau. Lane 2: Proteins from a nuclear fraction of B103 cells prepared according to Loomis et al. (38) were immunoblotted with polyclonal anti-tau. Lane 3: Another aliquot of this fraction was immunoprecipitated with PHF-1 antibody and then reprobed with polyclonal anti-tau. Molecular weight markers are shown on the right. The results indicate cross reactivity is seen between nuclear tau and PHF-1 antibody.

cells had very weak Alz-50 and PHF-1 labeling. The mouse neuroblastoma line N1E-115 had a heterogenous expression of Alz-50 tau, with some cells having high levels of antigen and others having relatively lower levels. The human neuroblastoma HCN-1A cells, differentiated or un-differentiated, did not express Alz-50 or PHF-1 tau protein. The SH-SY5Y human neuroblastoma cell line spontaneously expressed the PHF-1 epitope (40), while NTera 2 cells expressed PHF-1 tau only after differentiation with retinoic acid (45). These findings demonstrate that expression of the PHF-1 and Alz-50 epitopes varies widely in different cell lines of neural origin.

DISCUSSION

Data presented here have shown that the PHF-1 and Alz-50 epitopes of tau are present constitutively in B103 rat neuroblastoma cells. The B103 cell line is of particular interest because of its CNS origin and its ability to spontaneously produce neurites. Several other lines that were examined also expressed the PHF-1 epitope. Only B103 cells, however, showed robust levels of Alz-50 immunoreactivity. The Alz-50 epitope was exclusively cytoplasmic; the PHF-1 immunoreactivity occurred throughout the cytoplasm but also was found in the nucleus, where it showed cross-reactivity with nuclear tau. The data confirm that these Alzheimer's-type tau epitopes are not simply the products of dying cells. Of particular interest, their subcellular distribution patterns indicate differences in trafficking and, presumably, function.

AD-type tau epitopes have been seen previously in cell culture systems. Alz-50 immunoreactivity has been reported in association with various neurodegeneration paradigms (41). Recently, in rat hippocampal cells, Alz-50 immunoreactivity was found to increase during the degenerative cascade evoked by β/A4 peptides (49). A similar association with B/A4-evoked degeneration has been seen in postmitotic SH-SY5Y human neuroblastoma cells (31). Prior to degeneration, these various cell culture systems expressed the Alz-50 epitope at low levels in contrast to B103 cells. The robust expression of Alz-50 tau in the B103 line shows that it is not exclusively the product of dying cells. Similar to the association of the Alz-50 epitope with viable B103 cells, the expression of the PHF-1 epitope also is not dependent on neurodegeneration. In the developing CNS, PHF-1 tau localizes to immature axons of viable neurons developing in vivo and in culture (43). A recent report also has shown that the PHF-1 epitope is spontaneously present in the LAN human neuroblastoma cell line, where it is downregulated by heat shock (8).

Phosphorylation, including the sort seen in Alzheimer's-afflicted neurons (42,39), decreases the ability of tau to stabilize microtubules, thereby increasing cytoskeletal plasticity. This phenomenon applies to the PHF-1 epitope (4) as well as other sites of tau phosphorylation (1,18,27). Excessive destabilization may give rise to the dystrophic neurites found in AD brain tissue. Physiologically, phosphorylation-evoked cytoskeletal plasticity is associated with mitosis (48) as well as neuritogenesis. Available data suggest the kinase cascades of either mitosis or neuritogenesis could lead to elevated levels of tau phosphorylation. In proliferating SH-SY5Y human neuroblastoma cells, PHF-1 immunoreactivity varies as a function of the cell cycle, with upregulation observed at mitosis (44). In differentiated SH-SY5Y cells, stimulation of integrins, a known neuritogenic receptor, causes a marked increase in PHF-1 immunofluorescence (40). In vitro experiments have shown that kinases coupled to the cell cycle will phosphorylate tau, with cdc2 kinase and mitogen activated protein (MAP) kinase producing AD-type epitopes, including the PHF-1 epitope (33,10,9). Interestingly, cdc2 kinase, MAP kinase, and PHF-1 immunoreactivity all downregulate in vivo following neuronal differentiation (3,43, 24). Similar decreases in cdc2 kinase and PHF-1 immunoreactivities have been observed in association with retinoic acid-induced differentiation in neuroblastoma cell lines (12,40). In AD-brain, upregulation of cdc2 kinase (33) is consistent with the idea that postmitotic brain cells in AD may be responding aberrantly to a mitogenic signal. Ectopic mitogenic signals have been hypothesized to evoke apoptosis in postmitotic cells, such as neurons (25), and apoptosis has been linked to Alzheimer's pathogenesis in cell culture studies (37)

It is not understood why tau is in the nucleus, nor why it contains the PHF-1 but not the Alz-50 epitope. Loss of immunofluorescence by antisense tau oligonucleotides eliminates the possibility that the immunoreactivity is an artifact. Nuclear tau previously has been reported to occur in proliferating primary cell cultures and in two cell lines (38,50). This tau was correlated with expression of a 2 kDa mRNA, the untranslated region of which was hypothesized to target the protein to regions and functions not associated with microtubules. The current results extend these previous observations by showing that nuclear tau is present in other human neural cell lines and is phosphorylated to generate an AD-type epitope.

Nuclear tau and its phosphorylation may have a bearing on tau anomalies seen in Alzheimer's disease. Normally in mature neurons, tau is an axonal protein, but in AD, hyperphosphorylated tau accumulates ectopically in the somatodendritic compartment. Hypothetically, ectopic somatic tau in AD could derive from disruptions in pathways that transport tau to the nucleus and cause its nuclear phosphorylation. The nature of such disruptions is unknown, but recent studies of B/A4-evoked neurodegeneration have shown that neuronal apoptosis, which leads to breakdown of nuclear structure, could be involved (37). The B103 rat brain cell line should prove to be a useful model for future studies of the regulatory cell biology of tau transport and phosphorylation and their relationship to mechanisms of Alzheimer's pathogenesis.

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