Alzheimer's Neurofibrillary Tangles Contain Unique Epitopes and Epitopes in Common With the Heat-Stable Microtubule Associated Proteins Tau and MAP_2

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Ten monoclonal antibodies raised against Alzheimer's neurofibrillary tangles (ANTs) were characterized for reactivity with heat-stable microtubule fractions from bovine and human brain. Five of the antibodies showed very little reaction, but the other five reacted strongly with heat-stable microtubule associated proteins (MAPs). The proteins recognized by these antibodies have estimated molecular weights similar to those of known heat-stable MAPs, tau (52–68 kd) and MAP_2 (200–250 kd). That the proteins are indeed tau and MAP_2 is demonstrated by reaction of electroblotted proteins with antibodies raised in mouse and guinea pig against bovine brain tau and MAP_2. One anti-ANT antibody reacts only with tau, two bind strongly to tau and weakly to MAP_2, one recognizes both tau and MAP_2 equally well, and one primarily stains MAP_2. Extraction of ANT with 2% SDS does not remove tau or MAP_2 epitopes from ANT, indicating that epitopes shared with heat-stable MAPs are integral components of ANT. The existence of tau epitopes in ANT is also demonstrated by immunoblotting of ANT-enriched fractions with anti-tau antibodies. Most of the material recognized by anti-tau antibodies in ANT-enriched fractions is present in large molecules excluded by 3% polyacrylamide gel upon electrophoresis.

Anti-tau antibodies immunostain ANT in immunofluorescence and immunoperoxidase studies. The immunostaining can be blocked by absorption of anti-tau antibodies with purified tau proteins from bovine brain. Not all ANTs in any given tissue section or isolated Alzheimer perikarial preparations, however, are stained by anti-tau antibodies. These results are consistent with previous studies that have demonstrated heterogeneity of ANTs. Whether this heterogeneity is due to biochemical modification of MAPs or absence of MAPs in some ANTs is unknown. The significance of what appear to be shared epitopes recognized by monoclonal antibodies in tau and MAP_2, and the implications this may have on the pathogenesis of ANT formation, requires further investigation. (Am J Pathol 1987, 126:81–91)

ALZHEIMER'S DISEASE is characterized histopathologically by the presence of neurofibrillary tangles (ANTs) and neuritic plaques.[1] The insolubility of ANTs[2] has hampered the characterization of their constituent proteins. Information concerning the composition of ANTs has thus, of necessity, largely been obtained from immunochemical studies.[3-17]

It has been demonstrated that ANTs contain antigenic determinants in common with normal cytoskeletal proteins,[3-5,7-10,12-14,17-22] as well as unique antigenic determinants that are not present in normal neural and nonneural tissues.[6,11,15-16] The presence of

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neurofilament antigenic determinants in ANTs has been demonstrated by several groups using both polyclonal antisera and monoclonal antibodies.[5,8,10,12,14,17] The neurofilament epitopes in most cases appear to be phosphorylated.[14,17]

Early studies using antisera to twice-cycled microtubules suggested that ANTs might contain tubulin.[3] However, ample evidence now exists that it is not tubulin, but rather a minor component of the microtubule preparation, that shares antigenic properties with ANTs.[4] Microtubule preparations prepared by various methods have been shown to contain many proteins beside tubulin.[23,24] Some of these proteins enhance microtubule assembly *in vitro* and purify in stochiometric amounts with tubulin.[25] These proteins, known as "microtubule associated proteins" (MAPs),[25] may be classified on the basis of their molecular weight and stability to heat treatment. Of the two groups of high-molecular-weight MAPs, only those around 200 – 250 kd are heat-stable,[26] and they are called MAP_2.[26,27] A series of proteins in the mid-molecular weight range (52 – 68 kd) are also heat-stable, and they are referred to as tau proteins.[28]

We recently reported a series of 10 monoclonal antibodies raised against homogenates from Alzheimer brain referred to as anti-ANT antibodies.[15] Of this group of 10 antibodies, two recognized three or more proteins in the 50 – 70 kd range in supernatants from Tris buffer extracts of both normal and Alzheimer brains, and two reacted with proteins of similar molecular weight in normal brain. These antibodies also reacted with proteins of similar molecular weight in a microtubule fraction. Although incubation with a microtubule-enriched fraction carried out at that time did not significantly absorb the antibodies, as judged by immunofluorescence staining, the question of whether these mid-molecular weight proteins that cross-react with ANT are minor constituents of microtubule fractions was not resolved.

In this report we present evidence that the anti-ANT antibodies recognize tau proteins isolated from bovine brain. In addition, we demonstrate that some of our anti-ANT monoclonal antibodies react with MAP_2 and tau, whereas others show no reactivity to either of these proteins. The presence of tau epitopes in ANT is also demonstrated by reaction of ANT with antibodies raised against bovine brain tau.

Materials and Methods

Brain Tissue

Alzheimer's brains and normal brains were obtained at the time of autopsy and sections embedded in OTC compound for frozen sections. Other fresh and frozen brain tissue from Alzheimer's patients was obtained from the brain bank of Albert Einstein College of Medicine. Paraffin-embedded and formalin-fixed material was from tissue stored in the Rose F. Kennedy Center for Research in Mental Retardation and Human Development. All cases of Alzheimer's disease met diagnostic criteria set by an NIH consensus committee[30] and were studied with thioflavine-S fluorescent microscopy and Bodian stain.

Antibodies

The generation and characterization of anti-ANT monoclonal antibodies have been previously described.[15] They are twice-cloned mouse monoclonal antibodies raised against a crude Alzheimer's brain homogenate, half of which was fixed in formaldehyde before immunization. The monoclonal antibodies reactive against ANT were screened for immunofluorescence staining of isolated Alzheimer neuronal perikarya smeared on glass slides. Specificity of binding to ANT was assured by double labeling with thioflavine-S, a reagent that binds to tangles and emits green fluorescence under ultraviolet illumination.

A guinea pig polyclonal and a mouse monoclonal antibodies raised against tau proteins from bovine brain (see below) and a mouse monoclonal antibody to MAP_2 used in this study have been characterized in a previous study.[30] An additional mouse monoclonal antibody to tau proteins was generously provided by Dr. Lester Binder.[31]

Immunohistochemistry

The unlabeled antibody peroxidase anti-peroxidase (mouse monoclonal PAP, 1 : 100, Sternberger-Meyer, Jarrettsville, MD) or avidin – biotin – peroxidase (Vector Laboratories, Burlingame, Calif) method were applied to either formalin-fixed paraffin sections or frozen sections. Monoclonal supernatants were used straight, while other dilutions were 1 : 100 to 1 : 250 for guinea pig anti-tau, 1 : 100 for mouse monoclonal anti-tau, and 1 : 100 for mouse monoclonal anti-MAP_2. In most cases, diaminobenzidine was used as chromogen. To be certain that the structures stained by anti-tau antibodies were indeed tangles, some sections were stained with thioflavine-S after immunostaining. In these double-labeling experiments 4-chloro-naphthol was used as chromogen instead of diaminobenzidine, because the reaction products could be solubilized with xylene. After photography, the same slide was examined with thioflavine-S fluorescent microscopy.

Immunofluorescence

In addition to tissue sections, neuronal perikarya isolated from Alzheimer's brain and smeared on glass slides[4] were also used for testing the reactivity of anti-ANT and anti-tau antibodies. The slides were fixed with 100% methanol for 5 minutes, incubated with antibodies (1–2 hours, room temperature), and washed with PBS. The antigen–antibody binding was detected by rhodamine-labeled goat anti-mouse immunoglobulin (1 : 300) or by fluoroscein-labeled rabbit anti-guinea pig immunoglobulin (1 : 100) with the use of a Zeiss fluorescence microscope.

Absorption Studies

To determine whether the immunostaining of ANT by the anti-ANT antibodies, as shown in a previous report,[15] was due to anti-tau activity, each of the 10 monoclonal anti-ANT antibodies was incubated with purified tau proteins. The antibodies were used at 1 : 10 dilution. A total of 0.8, 4.5, or 8 μg of tau proteins (determined by the method of Lowry[33]) was added to 25 μl of diluted antibodies and incubated for 1 hour at room temperature. After centrifugation to remove immunoprecipitates, the absorbed and control unabsorbed anti-ANT antibodies were used in immunofluorescent studies of isolated Alzheimer neuronal perikarya as described above, and the intensity of the immunofluorescence was compared.

Heat-Stable Microtubule Associated Proteins

Microtubule fraction was prepared from bovine brains by two cycles of assembly and disassembly according to the method of Shelanski et al.[34] The sample was boiled for 5 minutes in the presence of 0.4 M NaC1 and 25 mM β-mercaptoethanol and centrifuged at 15,000 g for 30 minutes for removal of the precipitated heat-sensitive proteins.

A portion of the heat-stable sample was used for further purification of tau proteins with the use of the perchloric acid precipitation method.[35] The supernatant, which contains the tau proteins, and a sample containing heat-stable MAPs was analyzed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis by the system of Laemmli et al.[36] In all cases 10% polyacrylamide gel was used.

ANT-Enriched Preparation

A modified method of Iqbal et al[37] was used to purify a fraction that was 75–80% composed of thioflavine-S positive neuritic processes and ANT. The modification utilizes a simplified sucrose gradient in which the sample in 1.0 M sucrose is layered upon 1.2 M sucrose and spun at 800 g for 1 hour. The resulting pellet is resuspended and passed through a glass bead column.[38] The effluent is treated with 1% SDS for 10 minutes and centrifuged at 10,000 g. The pellet is resuspended in 0.1% SDS and layered over 1.0 M sucrose in an Eppendorf tube. This step is repeated three times. The major contaminant was lipofuscin. Amyloid cores were not present to any appreciable degree.

Dot Blots

One-half microliter of the ANT-enriched sample, containing about 0.5 μg of protein, was applied to nitrocellulose paper with the BioRad Dot-Blot apparatus. The blots were air-dried, then incubated with antibodies described above. Five percent nonfat dried milk in PBS was used to block nonspecific binding of antibodies to nitrocellulose paper. The antigen–antibody binding was detected by the avidin–biotin–peroxidase technique. Details of the procedure have been reported elsewhere.[15]

Western Blots

The ANT-enriched preparation, heat-stable MAPs, purified tau proteins from bovine brains, and molecular weight standards (Sigma) that had been denatured in SDS and electrophoretically separated on polyacrylamide gels were electroblotted on to nitrocellulose paper with the BioRad Transblot apparatus according to the method of Towbin et al.[38] The strip containing molecular weight standard was stained with 1% amido black. The remaining nitrocellulose strips were incubated with antibodies. The antigen-antibody binding was detected by the avidin–biotin–peroxidase technique.

To determine whether the epitopes in tau proteins, high-molecular-weight heat-stable MAPs, or ANT-enriched fractions contained phosphorylated epitopes, we treated some nitrocellulose strips with *Escherichia coli* alkaline phosphatase (2 U/ml for 2 hours at 37 C) prior to incubation with antibodies. This protocol has been shown to be effective in removing phosphate groups from neurofilament proteins blotted on nitrocellulose paper.[17]

Dephosphorylation was also carried out on samples that had not been exposed to SDS. In the ANT sample, this was done by incubation of about 15 μg of protein with alkaline phosphatase (5 U/ml for 16 hours at 37 C). The pellet, obtained after centrifugation at 12,000 g for 5 minutes, was solubilized in 2% SDS and then analyzed by gel electrophoresis and

Western blots. Heat-stable microtubule associated proteins were dephosphorylated in 0.05 M PIPES, 0.05 M Tris, 2 mM $MgCl_2$, 1 mM PMSF, pH 8.0, containing 10 U/ml of *E coli* alkaline phosphatase for 16 hours at 37 C.[30] The sample containing ANT, in contrast to those containing MAPs, could not be dissolved by the detergent and was loaded on the gel as a suspension.

Results

Immunoblotting of Anti-ANT Against Heat-Stable Microtubule Proteins

Tubulin, the major protein in the microtubule fraction, was removed by heat treatment, whereas MAP_2 and tau proteins remained soluble (Figure 1). The results of immunoblotting studies are shown in Figure 2. Five of 10 anti-ANT monoclonal antibodies recognized antigens present in the heat-stable microtubule fraction prepared from bovine brain. The staining pattern for each of these antibodies was not identical. One antibody (Ab175) stained five bands with molecular weights similar to tau proteins. These bands were recognized by polyclonal and monoclonal antibodies raised against tau. Two antibodies (Ab 705, 635) showed strong reactivity against tau, but also reacted weakly with several bands of molecular weight much higher than tau. These bands are almost certainly MAP_2 and its degradation products, because they are also stained by a monoclonal antibody raised against MAP_2. One antibody (Ab636) appeared to recognize tau and MAP_2 equally well, whereas another antibody (Ab322) stained mainly MAP_2. Tau was also stained by Ab322, but the reaction was very weak.

The staining pattern with anti-tau (Figure 3) or anti-ANT antibodies was not altered by pretreatment of electroblotted proteins with alkaline phosphatase. Dephosphorylation of the heat-stable microtubule fraction before gel electrophoresis did not affect the binding of anti-ANT antibodies to tau or MAP_2, although the largest tau protein was no longer visible (not shown). This band was missing in the Coomassie blue-stained gel as well. The immunoblotting pattern of dephosphorylated microtubule fractions with the anti-ANT antibody, Ab175, was comparable to that by anti-tau antibodies.

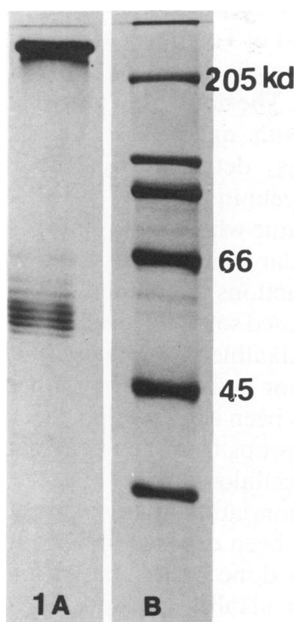

Figure 1—Gel electrophoretic profile of heat-stable microtubule fraction prepared from bovine brain **(A)** and molecular weight standard **(B)**. The gels (10% acrylamide) were stained with Coomassie blue.

Immunoabsorption of Anti-ANT Antibodies With Tau Proteins

ANTs present in the neuronal perikarya isolated from Alzheimer's brain, as we reported before,[15] were positive with all anti-ANT antibodies (1 : 10 diluted). Among the 10 antibodies tested, only the reactivity of one antibody (Ab175) could be removed by absorption with 0.8 μg of pure tau proteins per 25 μl of diluted antibody (Figure 4). Increasing the amount of tau to 8 μg removed the activity of Ab 705 but did not reduce the intensity of immunofluoresence of other anti-ANT antibodies to an appreciable degree.

Immunofluorescent and Immunoperoxidase Labeling of ANT by Anti-Tau Antibodies

Immunofluorescent studies of neuronal perikarya isolated from Alzheimer's brain showed that all three anti-tau antibodies bind to structures resembling ANTs. The immunolabeled elements were positive with thioflavine-S, indicating that they were indeed ANTs. Comparison of the number of ANTs stained by thioflavine-S with the number immunostained by antibodies revealed that anti-tau antibodies labeled only 70–85% of ANTs.

Immunohistochemistry of paraffin-embedded tissue from Alzheimer's brain showed staining of ANT in the hippocampus in all cases by the polyclonal antibody (Figure 5). Beside ANTs, neuritic processes of neuritic plaques and fine processes in the gray matter were also positive with the anti-tau antibody. Amyloid cores were not stained, and very weak staining was detected in axons in the white matter. Extraneuronal staining was not apparent. Double-labeling

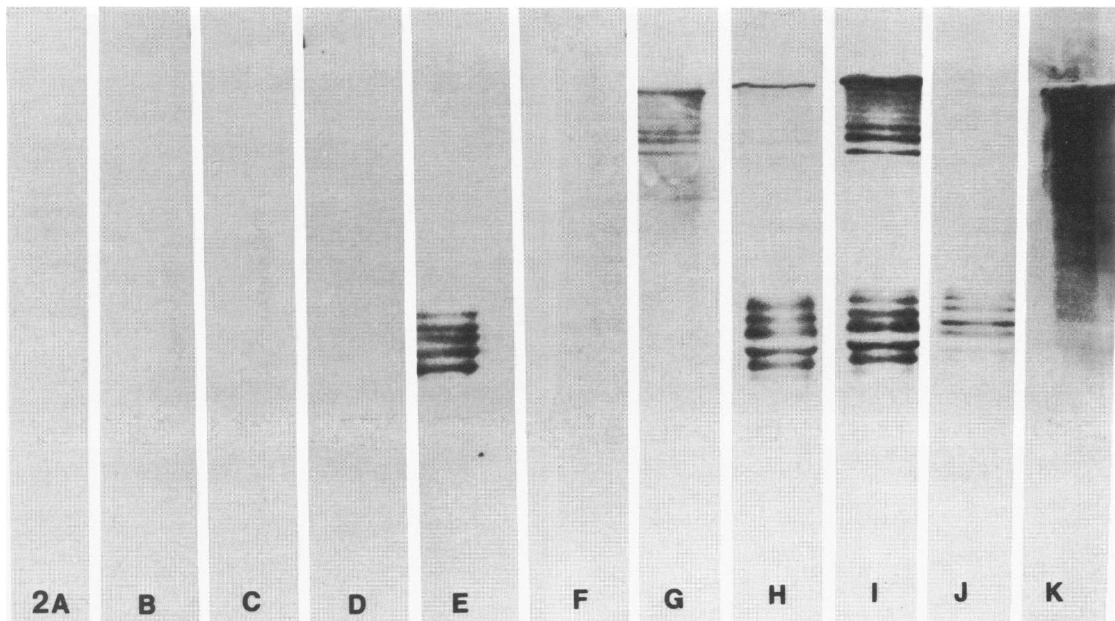

Figure 2—Immunoblots of a heat-stable microtubule fraction with anti-ANT antibodies. **A**—Ab39. **B**—Ab 64. **C**—Ab69. **D**—Ab117. **E**—Ab175. **F**—Ab 215. **G**—Ab322. **H**—Ab 635. **I**—Ab 636. **J**—Ab 705. **K**—Anti-MAP_2.

studies with 4-chloro-naphthol showed that not all of the ANTs were stained by the antibody. In one area of a section, most of the ANTs identified by thioflavine-S were immunostained. However, in a different area of the same section, only a small fraction of the ANTs were immunostained (Figure 6). Sections of brain from subjects who died of nonneurologic disease showed very little staining. The monoclonal antibodies did not react well with paraffin sections.

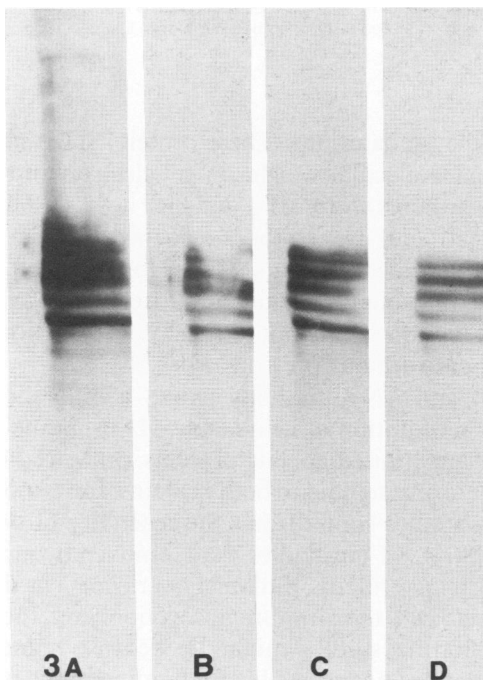

Figure 3—Immunoblots of a hear-stable microtubule fraction with guinea pig anti-tau antiserum (**A** and **B**) and a monoclonal anti-tau antibody (**C** and **D**). Lanes **B** and **D** were treated with *E coli* alkaline phosphatase after electrotransfer. Samples loaded on **A** and **C** were untreated.

Both polyclonal and monoclonal anti-tau antibodies bound to ANTs in frozen sections of Alzheimer brain. As we had observed in the paraffin sections, numerous fine processes in gray matter and the periphery of neuritic plaques reacted with the antibody. Glial cell processes, smooth muscle cells in the walls of blood vessels, and some seemingly normal neuronal perikarya were weakly stained by monoclonal antibodies. The intensity of staining with the polyclonal antibody, in general, was less than that with the monoclonal antibodies.

Immunoblotting of ANT-Enriched Preparation With Anti-Tau Antibodies

These studies were intended to test whether the binding of anti-tau antibodies to ANTs seen in immunocytochemical or immunofluorescence studies was due to epitopes integral to the ANTs or peripherally associated with them.

Dot blot analysis demonstrated that extraction of ANT sample with SDS did not remove the anti-tau reactive epitopes (Figure 7). In Western blots, all anti-tau antibodies bound to insoluble, high-molecular-weight substances excluded by 3% polyacrylamide gel (Figure 8). A band at the 68-kd region was very weakly stained by monoclonal antibodies and more intensely

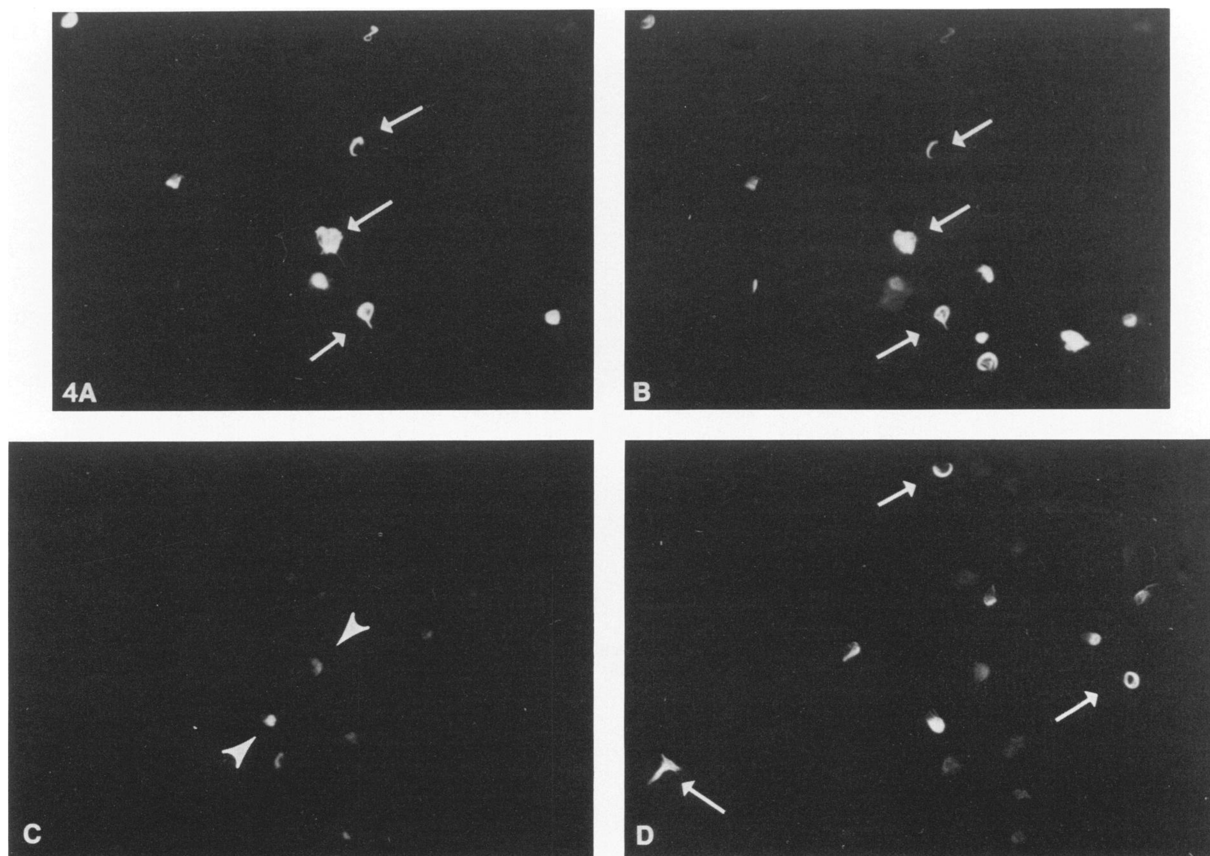

Figure 4—**A**—Immunofluorescent staining of ANTs in isolated perikarya with anti-ANT antibody, Ab 175. **B**—Staining of ANTs in **A** with thioflavine-S. Most of tha ANTs (indicated by *arrows*) are recognized by the antibody. (×160) **C**—Absorption of Ab 175 with pure tau proteins removed the anti-ANT activity. **D**—Staining of section in **C** with thioflavine-S. (×160) The fluorescent positive elements seen in the absorbed sample are lipofuscin (indicated by *arrowheads*).

stained by the polyclonal antibody. Two or three additional bands were detected by the polyclonal antibody.

Binding Between Anti-Bovine-Tau Antibodies and Heat-Stable Proteins of Human Origin

Supernatants from normal brain homogenates contained antigens that reacted with anti-tau antibodies (Figure 9). The epitopes were present in three to four bands migrating in the region similar to the molecular weight of bovine tau proteins. Boiling of the supernatant did not alter the immunoblot pattern, indicating that the proteins that contained the epitopes, similar to tau, were heat-stable.

Discussion

In this report we have demonstrated that 5 of 10 monoclonal antibodies raised against ANTs have reactivity against proteins present in bovine brain microtubule preparations. These proteins have similar apparent molecular weight on gel electrophoresis to the tau proteins and MAP_2, and they are also stable to heat treatment. The epitopes recognized by the anti-ANT antibodies are not sensitive to treatment with alkaline phosphatase, which indicates that phosphorylated epitopes are not critical for detection, in agreement with our previous work.[15]

Each anti-ANT antibody shows a different pattern of recognition of heat-stable MAPs. Some antibodies are limited to tau proteins or MAP_2 only; others are able to bind to both proteins. Immunofluorescent studies showed that the reactivity of two of these anti-ANT antibodies were removed by incubation with tau proteins, but others were not. The difference between the immunoabsorption and the immunoblotting studies might be because some tau epitopes were not accessible for binding in the native state but were exposed upon denaturation. However, incubation of antibodies with tau proteins that have been boiled in SDS with β-mercaptoethanpol and

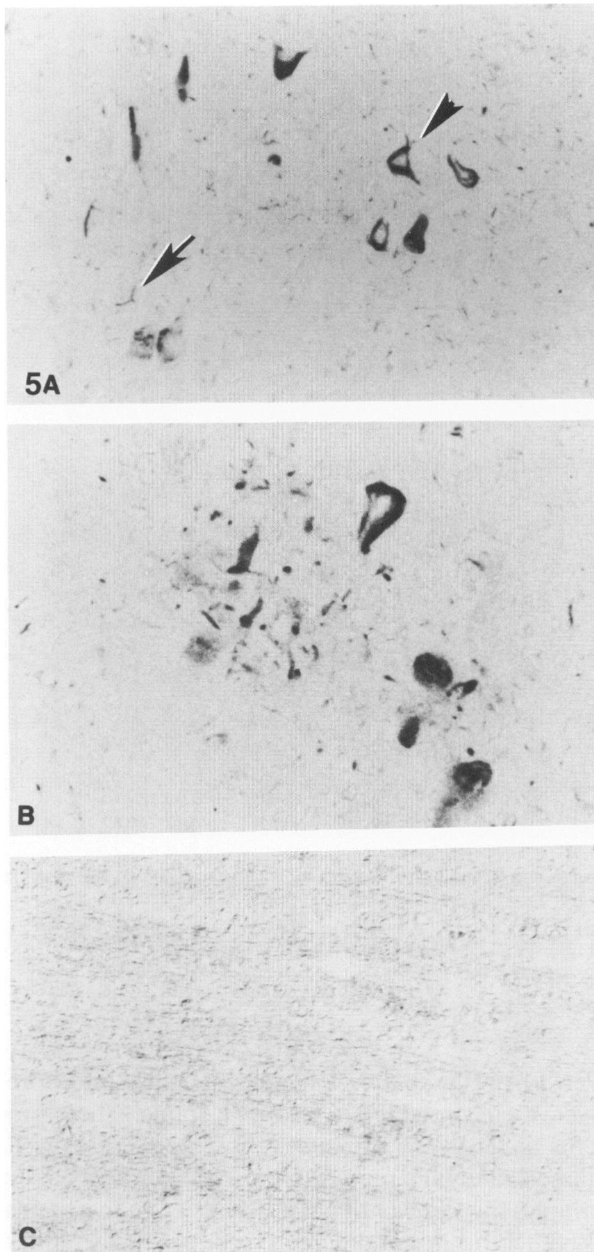

Figure 5—Immunoperoxidase staining of a paraffin section from Alzheimer's brain with guinea pig anti-tau antiserum. **A**—ANTs (indicated by *arrow heads*) and fine processes in gray matter (indicated by *arrows*). (×186) **B**—Neurites in neuritic plaque are stained. (×370) **C**—Axons in white matter fiber tracts are not well stained. (×370)

dialyzed against buffer did not absorb the antibodies. Further studies are necessary to give a definitive answer. The cross-reactivity between tau proteins and ANT is demonstrated further by immunocytochemical staining of isolated ANT or Alzheimer's brain sections with anti-tau antibodies and the blockade of this staining by purified tau proteins. Similar findings have been reported recently.[18-22]

Several issues, however, need to be addressed. First, based on double-labeling studies of ANT in tissue sections or isolated preparation, it appears that tau epitopes can be abundant in some ANT and undetectable in others. One may speculate that such heterogeneity is due to biochemical modifications of heat-stable MAPs due to disease processes and that only those that have not been modified are detected by anti-tau antibodies. Alternatively, the data may suggest that the incorporation of normal proteins (epitopes) into ANT or association of normal proteins with ANT is not essential for the ANT formation. The lack of binding of five of our anti-ANT antibodies to normal brain proteins could support this view.

Second, unlike observations in other species by Binder et al,[31] we did not find appreciable staining of normal human brain sections by anti-tau antibodies. In Alzheimer's brain sections anti-tau antibodies bind to ANTs, neurites in neuritic plaques, and fine processes in gray matter in areas with many ANTs. Most axons in human white matter are not stained well by anti-tau antibodies. This raises the possibility that epitopes to anti-tau antibodies in the nonpathologic state may be sensitive to autolysis during the extended postmortem interval and/or tissue processing involved in obtaining human autopsy material. Consequently, only epitopes associated with or incorporated into stable structures such as ANTs could be detected immunocytochemically. In this context, it is interesting to find that in ANT-enriched preparations most of the anti-tau reactive substance is excluded by gel electrophoresis. Other workers have interpreted the trace amounts of proteins in the 50–70-kd molecular weight range entering the gels from paired helical filament (PHF) preparation as "PHF proteins." It is still unclear whether they represent a limited solubility of the PHFs themselves or the release of absorbed cytoskeletal proteins. It should also be noted that anti-ANT antibodies do not stain brain sections from normal brains that have been fixed in formalin nor rat brain sections fixed briefly with absolute methanol,[15] but they stain rat tissue fixed by perfusion with paraforaldehyde and picric acid (A. Migheli, unpublished observations). The pattern of the later staining is similar but not identical to that reported by others.[30]

Information concerning the biochemical properties of tau has been derived primarily from studies of tau from nonhuman tissues or cells. A positive reaction between anti-bovine tau antibodies and ANT, therefore, is suggestive, but not conclusive, that the common epitopes detected in human material are in tau proteins. Using homogenates prepared from a normal human brain, we demonstrated that anti-tau reactive epitopes are found in proteins with molecular

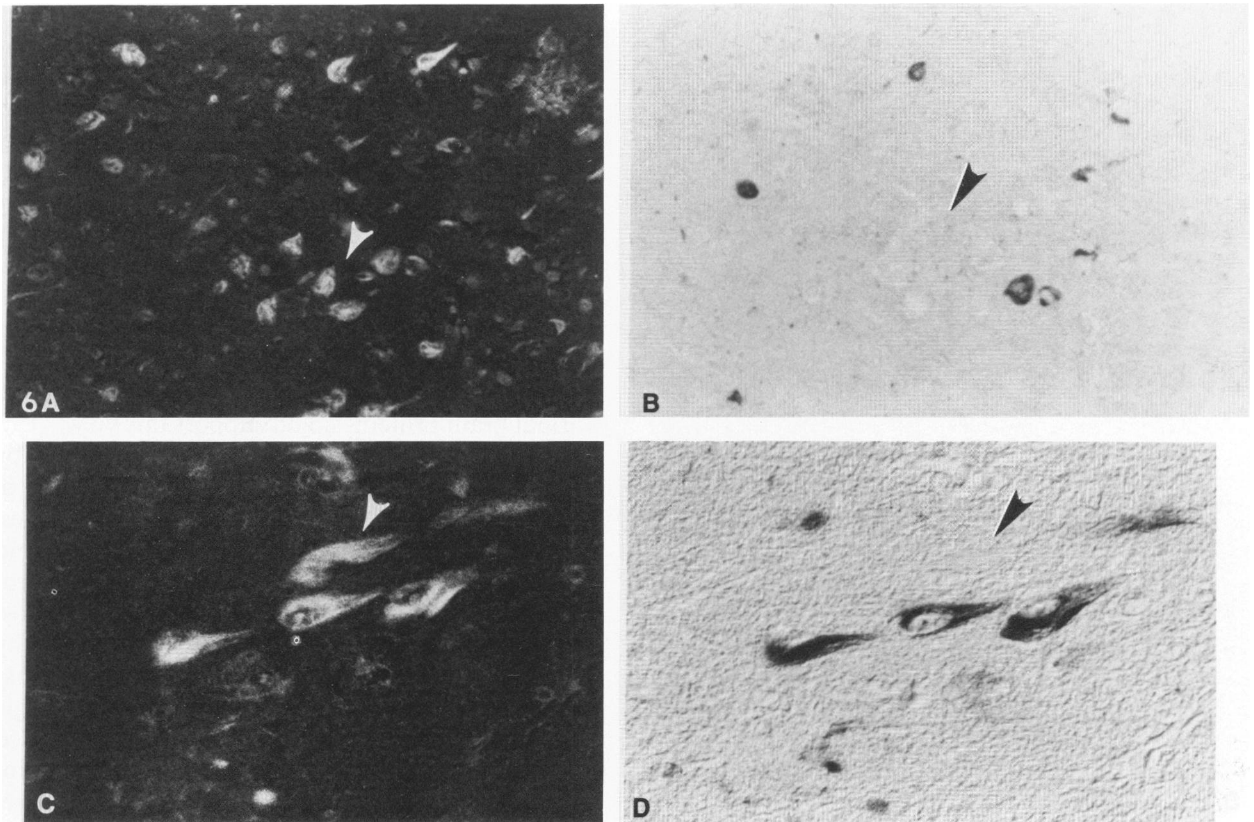

Figure 6—Double-labeling of Alzheimer's brain section (paraffin) with thioflavine-S (**A** and **C**) and guinea pig anti-tau antiserum (**B** and **D**). Not all ANTs (indicated by *arrowheads*) react with anti-tau antiserum. (**A** and **B**, ×187; **C** and **D**, ×370)

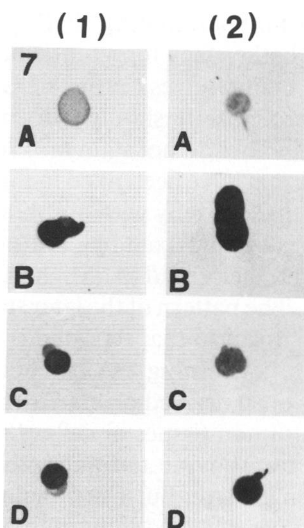

Figure 7—Dot blots of ANT-enriched fraction with guinea pig anti-tau antiserum, **(A)** monoclonal anti-tau antibody (Binder) **(B)**, monoclonal anti-tau antibody (Shelanski) **(C)**, and anti-ANT Ab 39 **(D)**. Samples in **1** are in Tris buffer, pH 7.6, and in **2** are in 2% SDS.

weights similar to those of bovine tau proteins. Moreover, these proteins are also heat-stable. These proteins are identified on immunoblots as three well-stained bands and one weakly stained band. In bovine samples the same antibodies usually stain five bands. Whether this minor difference is due to species difference or to postmortem effects on the human sample is not known.

Immunostaining of MAP_2 and its degradation products in the Western blots by some anti-ANT antibodies is interesting, because anti-MAP_2 monoclonal antibodies have been shown to stain ANT in frozen sections, but not paraffin sections, of Alzheimer's brain.[13] The epitopes recognized by our anti-ANT antibodies, unlike those shown by anti-MAP_2 antibodies, are integral components of ANT, because extraction with SDS did not remove the epitopes. In supernatant obtained from normal human brain homogenates we did not detect significant binding of anti-ANT antibodies nor, for that matter anti-MAP_2 antibody, to high-molecular-weight proteins. The

negative results could be due to the high susceptability of MAP_2 to proteolysis.[27]

Cross-reactivity between tau and MAP_2 demonstrated by several of our anti-ANT antibodies was unexpected, because tau and MAP_2 have different subcellular localizations.[30,38-41] Sharing of epitopes between tau and MAP_2 has not been reported in studies of nonhuman material with the use of anti-MAP_2 or anti-tau antibodies, and this has been put forward as an argument for tau proteins not merely being a degradation product of MAP_2.[27,40] The possibility that the cross-reactivity observed in our studies is due to the presence of two different antibodies is slim, because the monoclonal antibodies were produced from hybridomas that had been recloned at least two times. The sharing of epitopes between neurofilament proteins and MAP_2 or MAP_1 has been noticed in recent studies,[17,43] but the significance of these observations is not clear. Preliminary observations in our laboratory show immunoblots of certain anti-neurofilament antibodies against tau proteins, raising the possibility that at least some of the staining of ANT by anti-neurofilament antibodies could be related to anti-tau stains of ANT. MAP_2 and high-molecular-weight neurofilament proteins may function in bridging microtubule to neurofilament or other cytoplasmic organelles.[27,44,45] Little is known about the function of tau proteins.

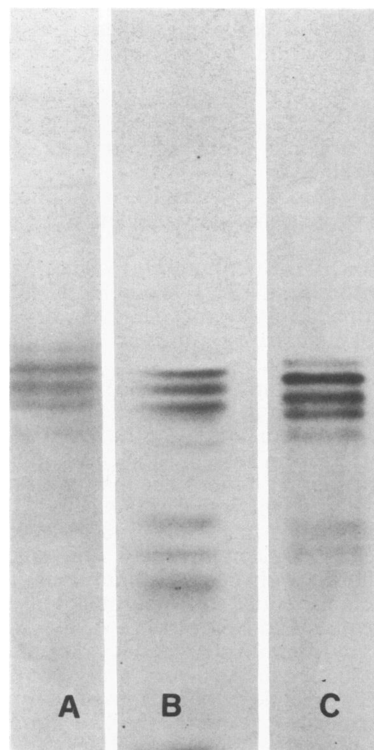

Figure 9—Immunoblots of supernatant from human brain homogenate (12,000g) with guinea pig anti-tau antibody (A) and monoclonal anti-tau antibody (B and C). The sample loaded on C has been heat-treated.

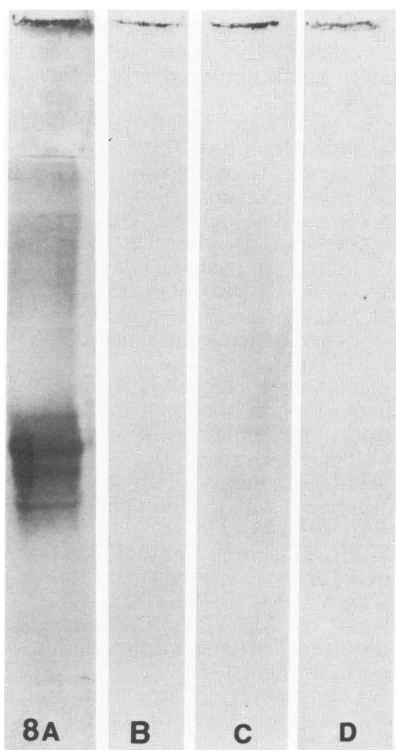

Figure 8—Immunoblots of an ANT-enriched fraction with guinea pig anti-tau antiserum (A), monoclonal anti-tau antibody (Shelanski) (B), monoclonal anti-tau antibody (Binder) (C), and anti-ANT 175 (D). All antibodies react with material excluded by stacking gel. Guinea pig antiserum, in addition, binds to bands migrating at the 52–68 kd region.

Our knowledge of the molecular nature of ANT has increased considerably in recent years by immunocytochemical and immunologic studies. The results of our studies emphasize further a close relationship between ANTs and MAPs. Interpretation of immunocytochemical and immunochemical data, however, demands caution. The question of whether or not these proteins or fragments of them are major components of ANTs will require dissolution of ANTs and the comparison of their amino acid sequences with normal cytoskeletal proteins.

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