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# Widespread Cytoskeletal Pathology Characterizes Corticobasal Degeneration

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***Corticobasal degeneration (CBD) is a rare, progressive neurological disorder characterized by widespread neuronal and glial pathology. Using immunohistochemistry and laser confocal microscopy, we demonstrate that the nonamyloid cortical plaques of CBD are actually collections of abnormal tau in the distal processes of astrocytes. These glial cells express both vimentin and CD44, markers of astrocyte activation. Glial pathology also includes tau-positive cytoplasmic inclusions, here localized to Leu 7-expressing oligodendrocytes. In addition, a wide array of neuronal pathology is defined with tau-positive inclusions in multiple domains of a variety of cortical neurons. CBD thus exhibits widespread glial and neuronal cytoskeletal pathology, including a novel structure, the astrocytic plaque. CBD is a disease of generalized cytoskeletal disruption affecting several cell types and multiple domains of these cells. The further definition of CBD pathology refines the diagnosis and pathophysiological understanding of this unique disease and has important implications for other neurodegenerative diseases, like Alzheimer's disease, characterized by abnormal tau deposition. (Am J Pathol 1995, 146:1388–1396)***

Corticobasal degeneration (CBD) is a progressive degenerative disease affecting both cortical and subcortical systems. The original patients described by Rebeiz and colleagues[1,2] presented with asymmetric abnormalities of motor function including prominent involuntary movements. Mental function remained relatively intact until late in the course of the disease. Additional reports described similar patients who, in addition, demonstrated supranuclear gaze palsies and Parkinsonian features.[3–5] Although the combination of asymmetric motor disturbances with cortical sensory loss and apraxia without marked cognitive dysfunction has been felt to be relatively specific for CBD,[6] neuropathologically documented presentations as progressive supranuclear palsy (PSP) and Parkinson's disease make antemortem diagnosis difficult. In addition, cases presenting first with dementia have been reported.[7–9]

Given that the clinical findings often mimic better known disorders, the diagnosis of CBD is often made only at autopsy. Typical gross pathological findings include marked, asymmetric frontoparietal atrophy. In contrast to Pick's disease, temporal structures are generally spared. Prominent cell loss and marked gliosis involve white as well as gray matter. A characteristic, though nonspecific, feature of CBD pathology is the swollen achromatic neuron, or ballooned cell, present in affected cortical areas. These cells are enlarged, with eccentric nuclei and loss of Nissl substance. Like Pick cells, these neurons contain phosphorylated neurofilament epitopes[10] but have been reported not to stain with anti-tau antisera.[6,11] Ballooned neurons occur primarily in affected cortical areas, although small numbers may be seen in other regions. Subcortical nuclei are variably affected in CBD, with the most consistent site of pathology being the substantia nigra. Severe cell loss with pigmentary incontinence and marked gliosis are usual. Other areas, including the lateral thalamic nucleus, globus pallidus, claustrum, subthalamic nucleus, red nucleus, striatum, and midbrain tegmentum, are less commonly involved.

Immunohistochemical techniques have revealed additional pathology in CBD not suspected with routine histological methods. Neurofibrillary tangles and other tau-immunoreactive inclusions have been described in brainstem and cortical neurons.[3,7,9,12,13] Neurofibrillary tangles are abnormal filamentous structures composed, at the electron microscopic level, predominantly of paired helical filaments

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(PHFs). Argyrophilic PHF inclusions are found in a variety of degenerative diseases, most notably Alzheimer's disease (AD), and appear to be composed primarily of hyperphosphorylated tau, a microtubule-binding protein. Antibodies recognizing the abnormal PHF-tau have been extremely useful in characterizing cytoskeletal pathology in AD and other neurodegenerative disorders.

Glia in CBD demonstrate prominent cytoskeletal alterations. Argyrophilic, tau-immunoreactive inclusions have been observed in cells identified by morphological criteria as oligodendroglia.[9,13] Electron microscopic visualization of the tau-positive neurons and glia revealed inclusions composed of 15-nm-wide straight filaments similar to those found in PSP.[9] Analysis of purified filaments from CBD tissue demonstrated 13- to 26-nm twisted filaments.[13]

An intriguing pathological feature of CBD is the presence of amyloid-negative cortical plaques. Clusters of short, thread-like tau-immunoreactive structures surround an unstained central area, resembling an AD plaque; however, the cores do not contain amyloid.[14] We demonstrate here that these plaques are collections of abnormal tau in the distal processes of activated astrocytes. We further localize tau-positive structures to specific neuronal types and to domains within neurons. In addition, the oligodendroglial nature of glial cells containing inclusions is confirmed by double-label immunohistochemistry. The further definition of CBD pathology not only has important implications for understanding underlying pathophysiology but also helps to refine diagnostic criteria for this rare disorder.

## Materials and Methods

Brain tissue from 11 patients with CBD was examined. Cases included eight females and three males; patients ranged in age from 61 to 85 years old with a mean age of 75. Postmortem intervals were from 3.5 to 18 hours. Most patients presented with behavioral changes and cognitive impairment. Ideomotor apraxias and aphasias were also present in many patients, but in only one case was aphasia documented in the absence of significant intellectual impairment. In addition, two patients exhibited prominent Parkinsonian symptoms, and one presented with features of PSP. The diagnosis of CBD was made on the basis of pathological findings of neuronal loss in cortex and substantia nigra, ballooned neurons in cortex, basal ganglia, and brainstem, as well as astrocytic plaques and characteristic tau-positive neuronal and glial inclusions.[14,15] AD control tissue was from an 89-year-old female with a postmortem interval of 6 hours. The diagnosis of AD was based on the modified, age-adjusted quantitative criteria of Khachaturian.[16]

### Immunohistochemistry

Brain tissue obtained at the time of autopsy was immersion-fixed for 12 to 16 hours in 4% paraformaldehyde, stored in 30% sucrose, and subsequently sectioned with a Vibratome at a thickness of 40 μ. For immunostaining, sections were preincubated with 5% nonfat milk to block nonspecific antibody binding and then incubated with primary and secondary antibodies as described.[17] Primary antibodies were obtained from the following sources and used at the indicated concentrations: rabbit polyclonal antibodies to neurofilaments (1:200; Alex Chiu, Albert Einstein College of Medicine); monoclonal antibodies recognizing PHF-tau (Alz 50,[18,19] 1:5; PHF1,[20] 1:100; TG-3,[21] 1:5; Peter Davies, Albert Einstein College of Medicine; AT-8,[22] 1:1000; Innogenetics, Ghent, Belgium); rabbit polyclonal antibodies to amyloid-$\beta$[23] (1:250); rabbit polyclonal antibodies to amyloid P component (1:100; Dako Corp., Carpinteria, CA); monoclonal mouse immunoglobulin (Ig)G to apolipoprotein E (1:200; Biodesign, Kennebunk, ME); rabbit polyclonal antibodies to glial fibrillary acidic protein (GFAP) (1:400; BioGenex, San Ramon, CA); monoclonal mouse IgG to vimentin (3B4, 1:50; Boehringer-Mannheim, Indianapolis, IN); monoclonal mouse IgG to CD44 (BU52, 1:200; Binding Site, San Diego, CA); rabbit polyclonal antibodies to S-100 (1:400; Dako); monoclonal mouse IgM to LN-3 (1:10; ICN Biochemicals, Costa Mesa, CA); monoclonal mouse IgG to calbindin (CL-300, 1:20; Sigma Chemical Co., St. Louis, MO); rabbit polyclonal antibodies to calretinin (1:50; Chemicon, Temecula, CA); and monoclonal mouse IgM to Leu 7 (1:20, Becton Dickinson, San Jose, CA). Antibody binding was detected with peroxidase- or alkaline phosphatase-conjugated, isotype-specific secondary antibodies. For immunofluoresence, sections were processed as above with fluorescein isothiocyanate- or rhodamine-conjugated secondary antibodies. Sections used were from the frontal or parietal lobe in CBD and hippocampus in AD, except where otherwise noted.

## Results

Numerous ballooned neurons in affected cortical areas were present in all cases. As expected, neurofilament antibodies reacted strongly with the swollen chromatolytic cells (Figure 1, A and B). Many ballooned neurons also contained tau protein. Immunoreactivity ranged from diffuse, patchy cytoplasmic

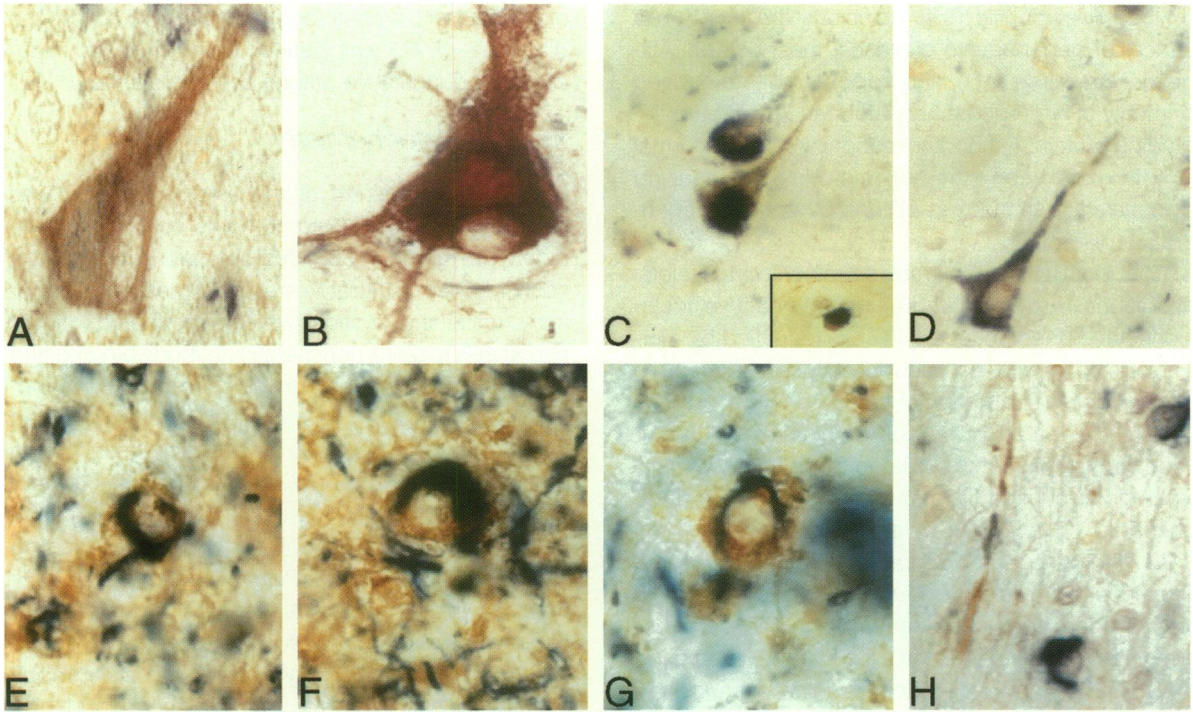


**Figure 1.** *Double-label immunostaining of neurons and oligodendroglia in CBD with anti-tau antiserum.* **A** *and* **B:** *Ballooned neurons in a section of cortex are intensely positive with antibodies directed to phosphorylated neurofilament (brown) and variably positive for tau (blue).* **C** *and* **D:** *Neurons in a cortical section stained with anti-neurofilament antibodies (brown) have a variety of dense and diffuse tau inclusions (blue). Inset in* (**C**) *shows a small nonpyramidal neuron with a dense, round tau-immunoreactive inclusion (blue).* **E** *to* **G:** *Oligodendroglia from white matter labeled with antibodies recognizing Leu 7 (brown) have crescent-shaped tau-positive inclusions (blue) extending into the proximal oligodendroglial cell process in* (**E**). **H:** *White matter immunolabeled for neurofilament (brown) and tau (blue) demonstrating focal tau immunoreactivity in an axon. Note also adjacent tau-positive oligodendroglial inclusion.*

staining (Figure 1A) to intense labeling in the cell body (Figure 1B). The cytoplasmic tau immunoreactivity was not as uniform as neurofilament staining and usually did not extend into swollen proximal processes of ballooned neurons.

CBD differed from AD by the presence of extensive white matter pathology. Figure 2A shows the gray-white junction from a patient with AD reacted with an antibody, PHF1, that recognizes abnormal tau protein. Similar results were obtained with other antibodies that recognize abnormal tau, including Alz 50, AT-8, and TG-3. These antibodies recognize epitopes along the length of the tau molecule.[18–22] Likewise, these antibodies all recognized the entire complement of abnormal tau-immunoreactive inclusions detailed below, demonstrating that the tau-immunoreactive structures contain tau protein. Extensive deposition of abnormal tau occurred in the cortical gray matter but spared the white matter. In contrast, a similar section from a patient with CBD demonstrates extensive tau-positive inclusions in both gray and white matter (Figure 2B). The tau-positive white matter structures represent both axonal threads and oligodendroglial inclusions (see below).

Figure 2, C and D, illustrates an additional feature of CBD pathology. Vibratome sections (40 μ) of CBD tissue stained for abnormal tau revealed the presence of numerous plaque-like structures consisting of an annulus of tau-positive structures surrounding a clear central core. Because these regions resembled Alzheimer's neuritic plaques, stains for amyloid were performed. A variety of methods, including thioflavin staining and amyloid-$\beta$ and amyloid P component immunohistochemistry, detected no amyloid in CBD plaques. Antibodies against apolipoprotein E, an additional component of AD plaques, also failed to react with CBD material. Control sections from AD brains contained abundant amyloid-$\beta$, amyloid P component, and apolipoprotein E immunoreactivity (data not shown).

Tau-positive inclusions also appeared in gray matter between CBD plaques; however, the morphology of plaque tau-positive structures differed from other neuropil tau-immunoreactive material. Tau-positive structures in the plaques were thicker and brush-like, in contrast to the more thread- and grain-like forms in the nonplaque neuropil (Figure 2D, inset). Similar neuropil threads were also characteristic of the gray

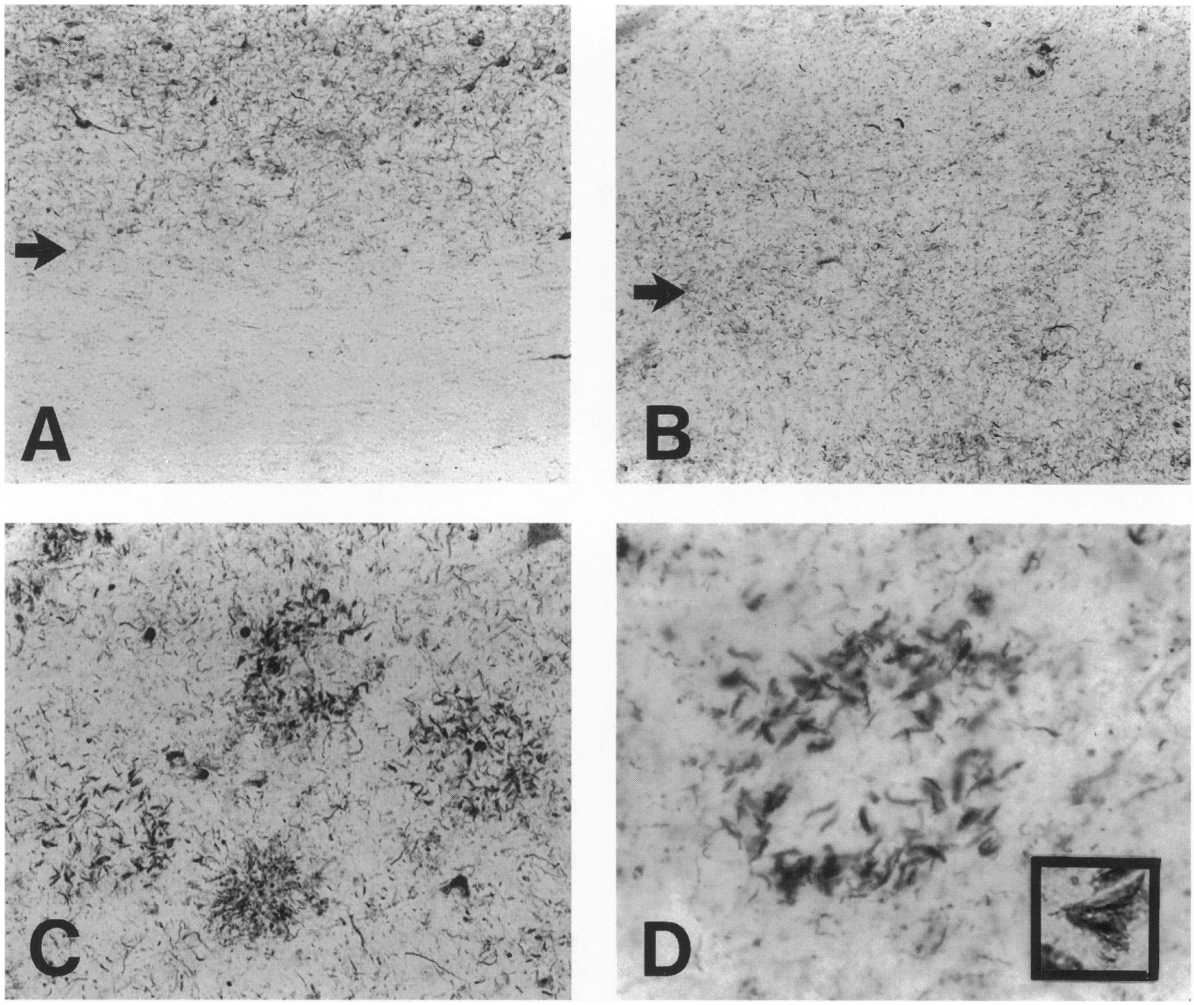


**Figure 2.** **A** *and* **B**: *The gray-white junction in CBD* (**A**) *and AD* (**B**) *immunolabeled with antibodies that recognize abnormal tau demonstrate extensive white matter pathology in CBD. The arrow indicates the transition between gray and white matter.* **C** *and* **D**: *Vibratome section of cortex from a case of CBD immunolabeled with antibodies recognizing abnormal tau shows several CBD plaques.* **D: inset** *High power view illustrating the brush-like morphology of the tau-immunoreactive structures.*

matter in AD; however, they tended to have a more clearly laminar (layers III and V) predominance in AD compared with CBD.

As CBD plaques did not surround an amyloid core, we investigated other potential organizing principles. Double immunostaining with anti-tau and anti-GFAP antibodies revealed that the tau-positive structures appeared at the distal tips of intensely GFAP-positive astrocytes (Figure 3A). Comparisons with sections double stained for tau and microglia with the specific marker LN-3[24] showed no apparent association (Figure 3B). Although astrocytes surrounded by tau-positive structures tended to react more strongly with the GFAP antiserum, not all GFAP-positive astrocytes were surrounded by abnormal tau. In contrast, a monoclonal antibody recognizing vimentin was highly specific for the astrocytes in the plaques (Figure 3C). Only those astrocytes reacting with the vimentin antibody were surrounded by abnormal tau. An additional astrocyte marker, CD44, was also used to stain astrocytes. Again, the anti-CD44 was highly specific for astrocytes in CBD plaques. Unlike vimentin, which stained predominantly astrocytes surrounded by numerous tau-positive structures, CD44 was also expressed by astrocytes associated with fewer tau-immunoreactive threads (Figure 3E). Cells with astrocytic morphologies expressing vimentin and CD44 also expressed GFAP as determined by double-label immunohistochemistry, confirming their identity as astrocytes. Both plaque and nonplaque astrocytes expressed S-100, and no appreciable enhancement of S-100 immunolabeling occurred in the plaque astrocytes.

With peroxidase and alkaline phosphatase detection, the tau-positive structures and the astrocyte processes appeared closely associated, and careful in-

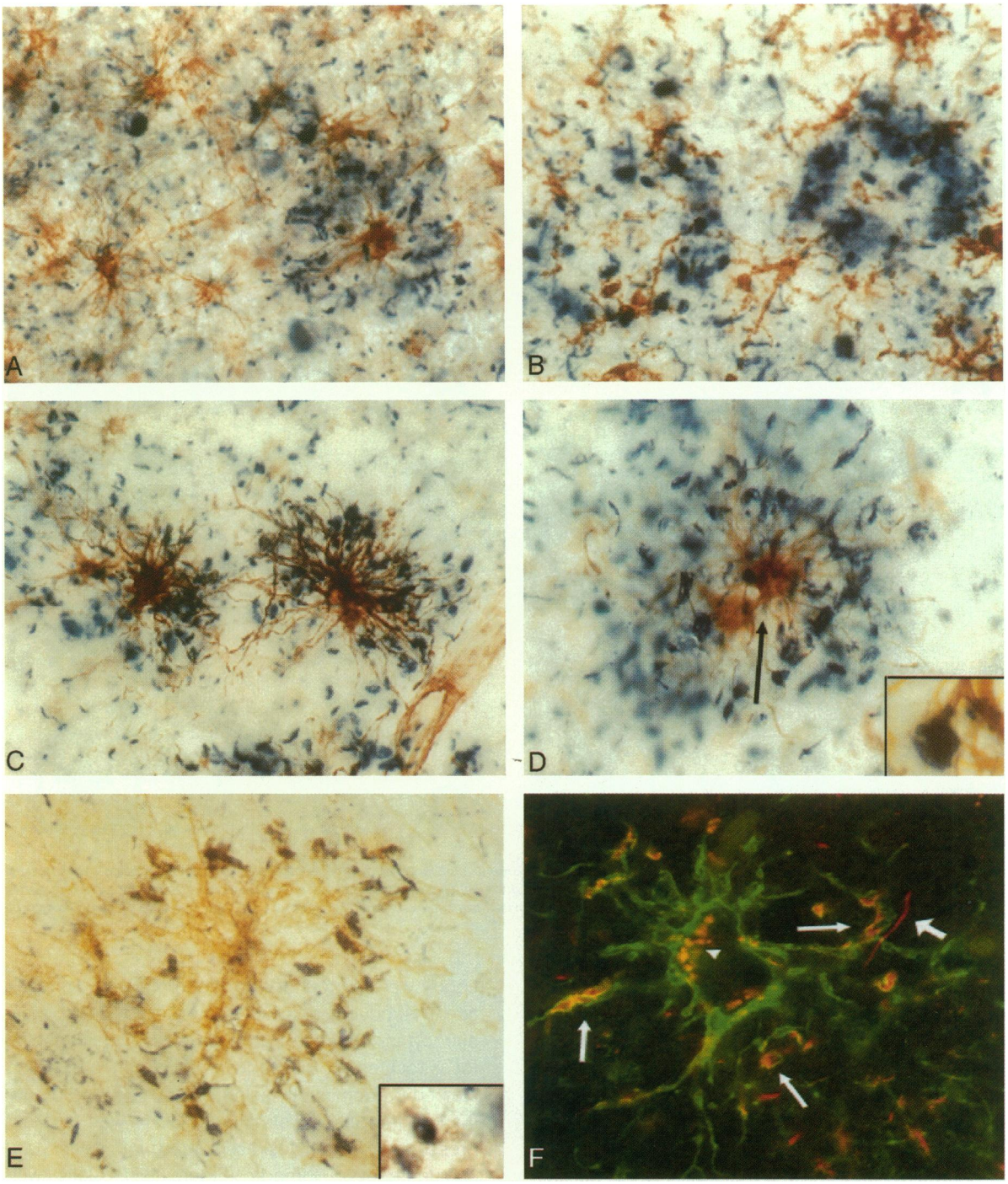


**Figure 3.** *Double-label immunostaining of glia and tau in the cortex of CBD.* **A:** *Subset of GFAP-positive astrocytes (brown) are surrounded by abnormal tau (blue).* **B:** *Microglia immunolabeled with LN-3 (brown) have no obvious association with the tau plaques (blue).* **C** *and* **D:** *Astrocytes stained with anti-vimentin antibodies (brown) are surrounded by tau inclusions (blue). Arrow in* (**D**)*points to focal tau immunoreactivity in astrocyte cell body.* (**D inset:** *Vimentin-positive astrocytic process (brown) apparently contiguous with a tau-positive inclusion (blue).* **E** *and* **F:** *Astrocytes double labeled with anti-CD44 to demonstrate the astrocyte plasma membranes and antibodies to abnormal tau.* **E:** *Astrocytes expressing CD44 (brown) are surrounded by tau-positive inclusions (blue).* **E inset:** *Tau-positive inclusion in a single dilatation of an astrocytic process.* **F:** *Optical sections through an astrocyte labeled with anti-CD44 (green) and abnormal tau (red) demonstrate overlap of the two markers (yellow) and tau-positive material within astrocytic processes (thin arrows). A tau-positive thread (red) not within the astrocyte is also evident (thick arrow). The arrowhead points to autofluorescent pigment in the astrocyte cell body.*

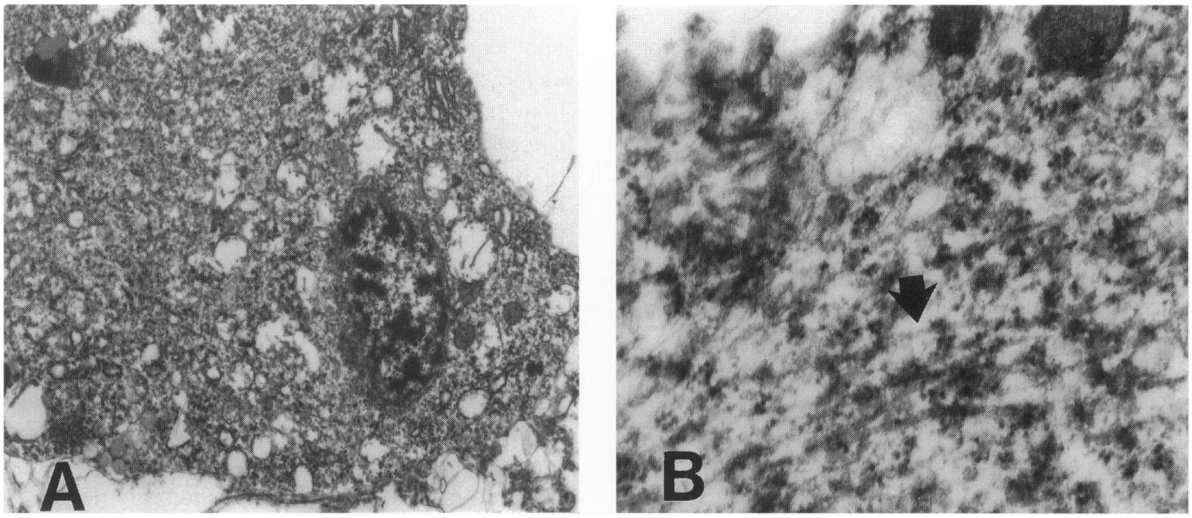


**Figure 4.** *Electron microscopic analysis of abnormal neurons from the cortex in CBD.* **A:** *A neuronal cell body contains an abnormal accumulation of filaments.* **B:** *A higher power view of the filaments (arrow). The filaments range from 20 to 25 nm in diameter.*

spection suggested that the tau-positive material was localized in distal astrocyte processes (Figure 3, D and E). Occasional focal deposits of tau were also observed in astrocyte cell bodies (Figure 3D, arrow). To confirm these observations, we analyzed serial optical sections through the astrocytes with confocal microscopy. Sections through tissue double labeled for tau and GFAP and for tau and vimentin revealed close association, but not overlap, of the abnormal tau proteins and the glial filaments. The abnormal tau protein and glial filaments either occupied spatially distinct domains of the same cell or were located in separate cells. To distinguish these possibilities, the astrocyte cell surface marker CD44 was used to define the limits of the glial processes. When the astrocyte plasma membrane was delimited by the anti-CD44 antibody, abnormal tau was revealed within focal dilatations of distal astrocytic processes (Figure 3F, thin arrows). Similar results were obtained in an analysis of multiple plaques. Occasional longer, thinner tau-positive structures clearly outside labeled astrocytes were also observed near plaques and in nonplaque neuropil (Figure 3F, thick arrow).

Although glial pathology was striking, widespread neuronal pathology also occurred. In addition to the ballooned neurons described above, numerous pyramidal and small nonpyramidal neurons throughout the affected cortical gray matter contained tau-positive neuronal inclusions (Figure 1, C and D). Certain classes of neurons may have been spared from abnormal tau deposition because double-label immunohistochemistry revealed no inclusions in small calbindin- or calretinin-expressing neurons (data not shown). The tau-immunoreactive neuronal material ranged in appearance from discrete globular or crescent-shaped forms to diffuse deposits extending into proximal processes. Abnormal tau also deposited in distal neuronal processes as shown by the presence of tau-positive threads in neurofilament reactive structures in the white matter (Figure 1H). Ultrastructurally, abnormal 20- to 25-nm-wide straight filaments were present in the cell bodies of cells also containing smaller filamentous structures consistent with neurofilaments (Figure 4, A and B).

Numerous glial cytoplasmic inclusions were also present in the white matter. These inclusions were present in oligodendrocytes as shown by the colocalization of the Leu 7 antigen to cells containing tau-positive inclusions (Figure 1, E-G). Leu 7 specifically labels oligodendroglia and choroid plexus epithelium.[25] These glial inclusions were typically crescent shaped and often extended into proximal oligodendroglial processes (Figure 1E).

## Discussion

We report the immunohistochemical findings of 11 patients with the rare disorder of CBD. Few of the present cases demonstrated clinical features generally considered typical of CBD. Although varied clinical presentations have been reported in the past,[7–9] this relatively large series suggests that the majority of patients meeting neuropathological criteria for CBD will not be diagnosed antemortem. The present results emphasize the need for additional clinicopathological studies and a greater recognition of pertinent pathological features that are characteristic of CBD. The further definition of CBD pathology discussed below should facilitate additional diagnoses.

CBD displays several unique pathological features that, in aggregate, make a differential diagnosis from similar neurodegenerative diseases possible.[15] Ballooned, or swollen achromatic, neurons are a hallmark of CBD. Although also found in Pick's disease, pellagra, and occasionally AD, they comprise an apparently invariant feature of CBD. We show here that, contrary to some previous reports,[6,11] ballooned neurons accumulate tau protein (Figure 1, A and B). Ballooned neurons in Pick's disease may also contain abnormal tau.[26]

Another distinctive pathological feature of CBD is prominent white matter involvement. Histological sections demonstrate myelin pallor with marked gliosis, and specific immunohistochemistry with antibodies to abnormal tau show that white matter in CBD contains numerous tau-positive threads and glial inclusions. Although occasional neurofibrillary tangles may be detected in superficial white matter, such extensive white matter pathology is not seen in AD. Occasional argyrophilic, tau-positive thread- and grain-like structures may occur in white matter connecting affected subcortical areas in multisystem atrophy[27,28] and PSP[29,30] but are typically sparser and more limited in distribution. In multisystem atrophy, glial inclusions are ubiquitin positive but inconsistently react with tau antibodies, and they are more common in subcortical rather than cortical white matter.[27,28,31–33]

The most striking feature of CBD pathology is the amyloid-negative cortical plaque. We demonstrate that these structures actually represent abnormal tau accumulations in distal processes of astrocytes that express GFAP, vimentin, and CD44. Using these three astrocyte markers and optical sectioning with confocal microscopy to localize antigen precisely in thin optical tissue sections, we determined that focal dilatations of distal astrocyte processes contain dense deposits of tau-immunoreactive material (Figure 3F). We suggest the term astrocytic plaques for these unique structures. Several lines of evidence suggest that tau-containing astrocytes differ from surrounding glia. First, the cells express abundant GFAP and are specifically stained with antibodies to vimentin and CD44, the astrocytic hyaluronic acid receptor (Figure 3, C-F).[34] Enhanced CD44 expression may be an early event in astrocytic plaque formation because astrocytes containing just a few processes with abnormal tau deposits react with the CD44 antibody. In contrast, only intensely tau-positive glia express high levels of vimentin. The hypertrophied appearance and antigenic properties of tau-positive astrocytes suggests reactive astrocytosis, which occurs in response to a wide variety of central nervous system insults.[35]

Astrocytic tau has been demonstrated in PSP[36–38] and occasionally in AD and Pick's disease.[39,40] The astrocytic pathology described here differs markedly from that reported previously. So-called tufted astrocytes in PSP contain tau-positive material localized primarily to the cell body and proximal processes. Focal punctate accumulations of tau in astrocyte cell bodies does occur occasionally in CBD (Figure 3D), but the majority of the material accumulates in distal processes.

Glial pathology in CBD is not limited to astrocytic tau deposition. Oligodendroglia also contain tau-positive inclusions. Previously, glial cells with the appearance of oligodendrocytes were shown to contain abnormal tau in CBD.[9,13] We confirm this localization using double-label immunohistochemistry (Figure 1, E-G). Oligodendroglia contain argyrophilic, variably tau-positive inclusions in a variety of neurodegenerative disorders including multisystem atrophy,[27,28,31–33] argyrophilic grain dementia,[41] AD, and Pick's disease.[39,40] The relationship between oligodendroglial inclusions in these neurodegenerative disorders awaits further definition.

Along with diverse glial pathology, abundant neuronal pathology also exists in CBD. Both pyramidal and small nonpyramidal neurons contain tau-positive inclusions (Figure 1, A-D and H). Abnormal tau is present in cell bodies, proximal dendrites, and axons. The absence of inclusions in calbindin- and calretinin-expressing neurons suggests some cellular specificity. Calbindin neurons have variably been described as spared in Parkinson's disease[42] and preferentially affected in AD.[43,44] Quantitative analysis of calbindin and calretinin expression in CBD would be required to confirm the exclusion of tau from neurons containing calcium-binding proteins.

Tau, a microtubule-binding protein, has generally been considered specific for neurons and deposition of abnormal tau in neurodegenerative diseases either a cause or consequence of neuronal dysfunction.[45] Our analysis of CBD invites a re-evaluation of these assumptions. We demonstrate tau-positive inclusions in both gray and white matter, neurons and glia, and in multiple cellular domains. We are confident that the tau-immunoreactive material we are detecting does represent tau protein because a variety of antibodies recognizing epitopes along the length of the tau molecule all recognize the various inclusions (see Materials and Methods and Results). Limited glial tau deposition has been described previously with the use of immunohistochemistry, but controversy regarding its site of synthesis has remained because *in situ* hybridization studies have localized tau mRNA to neurons,[46–48] although tau mRNA has been detected

in cultured astrocytes.[49] Our demonstration of extensive glial tau-immunoreactive inclusion formation suggests that glia can synthesize tau and make mechanisms such as phagocytosis appear unlikely. However, without RNA localization studies, we cannot definitively identify the cells synthesizing the tau protein forming abnormal inclusions in CBD.

Abnormal tau deposition appears to be a global phenomenon in CBD, and corresponding cellular dysfunction is suggested by extensive neuronal cell loss, white matter gliosis, and astrocyte abnormalities. Together with similar but more restricted findings in other studies, our results suggest that generalized cytoskeletal pathology is a common feature of many neurodegenerative diseases. Theories regarding the pathogenesis of such disorders must account for these widespread alterations. The present characterization both expands the scope of pathology expected in diverse neurodegenerative diseases and helps to define the diagnostic criteria necessary for their differentiation.

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