Curly Fiber and Tangle-like Inclusions in the Ependyma and Choroid Plexus—A Pathogenetic Relationship with the Cortical Alzheimer-type Changes?

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Abstract. The question of whether thread- and tangle-like inclusions of the choroid plexus (known as Biondi inclusions) are related to the cortical lesions in Alzheimer disease (AD) has been debated for almost a century, yet remains unanswered. Recently β-amyloid protein was biochemically isolated from the plexus, indicating a possible pathogenetic relationship between the degenerative changes of the cerebral cortex and those of the plexus. The goal of the present study was to analyze whether or not a significant correlation exists between the occurrence of the cortical AD-type changes and those in the ependyma and choroid plexus. In 292 consecutive autopsy cases several cortical areas, the ependyma, and the choroid plexus were analyzed to look for AD-type changes and Biondi inclusions using histochemical staining techniques and immunohistochemistry. A semiquantitative analysis of the density of cortical AD-type changes showed that of the 292 cases, 63 had severe cortical changes, 23 moderate changes, and 142 discrete changes. In 64 cases no plaques or neurofibrillary tangles were found. The number of cases with thread- and tangle-like elements in the plexus and ependyma was more than 90% in the 5 groups with cortical AD-type lesions, but low in the group without AD-type cortical changes (19%). The pathological argyrophilic filamentous structures accumulating in the ependymal layer and plexus had histochemical properties of amyloid and were immunoreactive with antibodies to β-component, ubiquitin, β-interactin, and tau protein. They did not react with antibodies to neurofilament proteins. Ultrastructurally, they consisted of densely packed straight and paired helical filaments and closely resembled neurofibrillary tangles and neuritic threads. The highly significant correlation (χ², p = 0.001; R = 0.85) between the occurrence of AD-type changes in the cortex and those in ependyma and plexus suggests a pathogenetic relationship.

Key Words: Alzheimer disease; Amyloid; Choroid plexus; Curly fibers; Ependyma; Neurofibrillary tangles.

INTRODUCTION

Recently, intracellular tangle-like inclusions were reported to occur in astrocytes in Alzheimer disease (AD), indicating that the formation of tangles is not unique to neurons (1). These intracellular tangle-like inclusions in glial cells were immunopositive for Tau and ubiquitin (2), and an electron microscopic study revealed the presence of paired helical filaments (PHF) and straight tubules, which were indistinguishable from those seen in neurons (1).

The accumulation of helically or ring-shaped, slightly undulating filaments, sometimes with curved end, in the ependymal layer of the cerebral ventricles was described at the beginning of this century (3). Similar pathological argyrophilic structures (called Biondi filaments, rings or bodies) were reported to occur in the epithelial lamina of the choroid plexus (4). Similar to neurofibrillary tangles, Biondi bodies have affinity for Thioflavin S, and when stained with Congo red show the typical green-yellow birefringence by polarizing microscopy (5, 6).

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Based on the morphological, histochemical, and ultrastructural similarities between neurofibrillary tangles and the argyrophilic thread and tangle-like structures of the plexus, in 1943 Zimman suggested a pathogenetic relation between the formation of Biondi filaments and the Alzheimer neurofibrillary degeneration (7). Furthermore, Schwartz proposed that “in some instances Biondi bodies are the earliest manifestations of cerebral amyloidosis” (5).

Recently, a positive reaction of Biondi filaments for β-amyloid protein has been reported (8) and β-amyloid protein was biochemically isolated from the choroid plexus (9). These observations further suggested the possibility that the same pathological basic process may be responsible for the degenerative changes in the cortex and in the choroid plexus. As this question is still open, the aim of this study was to analyze whether or not a significant correlation exists between the occurrence of the cortical AD-type lesions and the Biondi inclusions in the plexus. If a strong association exists, this would suggest a similar pathogenetic origin of the formation of these structures.

MATERIAL AND METHODS

Autopsy Cases Investigated

For this study 292 autopsied brains were studied. In all cases a semiquantitative analysis of senile plaques was performed on Thioflavin-S and on β-amyloid immunostained sections. Thioflavin S and Gallyas silver stained sections were used for neurofibrillary tangles and neuritic threads. The semiquantitative analysis of the ependymal and choroid plexus changes was made on Thioflavin-S-stained and on Jones’ silver-stained (10)
Fig. 1. Illustration of the morphological basis of the semiquantitative analysis used in the present study. A–D: β-amyloid immunostained sections showing senile plaques. E–H: Gallyas-stained sections showing neurofibrillary tangles. I–L: Jones' -stained sections with thread (arrowheads) and tangle-like (arrows) inclusions in the choroid plexus (I–K) and in the ependymal layer (arrows on L). Photomicrographs A, E, I and L show very high density (++++) of plaques (A), tangles (E) plexus (I) and ependymal (L) inclusions. B, F and J illustrate high density (+++); C, G and K moderate density (+) of plaques, tangles and Biondi inclusions, respectively. Sections with only a few plaques and tangles (D, H) correspond to low density (+). Sections without any cortical, plexus or ependymal changes were considered as "-" (not illustrated here). Several cortical areas (entorhinal cortex, hippocampus, frontal and parietal associative areas) were rated. This semiquantitative analysis was used also for the staging of the severity of the Alzheimer-type cortical changes following Braak (11). Bars: 100μm. Bar in A is the same for B–D and bar on E is the same for F–L.
Fig. 2. Photomicrographs illustrating neurofibrillary tangles and neuropil threads but also curly fibers and/or tangle-like filaments in the ependymal layer and epithelial cells of the choroid plexus in AD cases. They all exhibit strong yellow fluorescence when stained with Thioflavin-S for amyloid. A: Curly fibers in the neuropil of the temporal cortex of a familial AD case. Curly fibers (arrows) exhibit the same yellow fluorescence as neurofibrillary tangles and senile plaque. Paraffin section. B: Tangle and
sections. All sections were examined independently by 2 investigators. The results were compared, and in the few cases having a discrepancy in gradation, both investigators reviewed sections. The use of 2 staining techniques decreased errors due to staining artifacts. For the semiquantitative analysis, the densities of senile plaques, tangles, and neuropil threads (like those of the ependymal and plexus inclusions) were graded in the following way: absence of lesion = −; low = +; moderate = ++; high = +++; and very high = ++++ (Fig. 1). The semiquantitative analysis was made on 3 different brain areas, namely, the temporal cortex, which included the hippocampus and the entorhinal cortex, the frontal, and the parietal associative cortical areas. After rating these cortical areas, they were correlated and staged following Braak et al. 1993 (11). In addition, in all cases the histological criteria of the neuropathological diagnosis of AD as proposed by Khachaturian (12) were

curly fiber-like Biondi filaments in epithelial cells of the choroid plexus at the level of temporal horn of the lateral ventricle. Thioflavin-S stained frozen section from a familial AD case. C: Tangle-like Biondi filaments in the epithelial cells of the choroid plexus in a sporadic AD case, at high magnification. Paraffin section. D: Curly fibers, sometimes forming rings or loops accumulating in the ependyma of the temporal horn of the lateral ventricle, in a sporadic AD case. Bar is the same for A and D and represents 10 μm. 15 μm in B and 5 μm in C.

Fig. 3. Photomicrographs A–E illustrate curly fibers in the cortex but also similar argyrophilic filaments in the choroid plexus and in the ependyma in cases with AD. A–D are paraffin sections, E is a frozen section. The paraffin sections were stained with the Gallyas silver impregnation technique and the frozen sections with a modified Globus-Hortega stain similar to the Bielschowsky technique. A–D: Silver impregnated neuropil threads in the cerebral cortex (A, D) and Biondi filaments in the choroid plexus (B, C) in an AD case. In B and C curly fiber-like Biondi filament apparently seems to be partly intra- and partly extracellularly located. Note the similar morphology of the cortical curly fibers and of the Biondi filaments. The morphological similarity is even more pronounced if one compares the argyrophilic filaments in plexus epithelial cells in C with cortical curly fibers in D. E: Ependymal layer of the lateral ventricle showing neuropil thread-like filaments accumulated in the ependymal layer. For an optimal staining of the pathological filaments in the ependyma and plexus we would recommend to use the Jones' metenamine silver technique (10). Bar in A is the same as for B and corresponds to 20 μm. Bar in C is the same for D and E and represents 10 μm.
also tested. For this purpose paraffin sections stained with Thioflavin-S and immunostained for β-amyloid were used. All plaques visualized by these techniques were counted.

Of the 292 brains, 228 contained AD-type histological changes. In the remaining 64 cases the brains were without any senile plaques, neurofibrillary tangles, neurplil threads, or β-amyloid deposition (AD –). Based on the semi-quantitative analysis of the cortical AD-type changes and on the staging procedures, the 228 cases with AD-type cortical changes were divided into 3 groups. There were 63 cases with severe (AD ++, Braak stages V and VI), 23 cases with moderate (AD +++, Braak stages III and IV) and 142 cases with discrete AD-type changes (AD ++; Braak stages I and II). The AD +++ group included 4 familial AD (FAD) and 2 Down syndrome (DS) cases. In 5 cases we observed curly fibers in the cortex without plaques or tangles that were included in the AD + group. In all cases of the AD + group the histological criteria for the neuropathological diagnosis of AD proposed by Khachaturian were fulfilled. In 60 of these 63 cases the clinical record mentioned dementia. In the remaining AD + + cases neuropsychological examination was not performed.

**Histochemical and Immunohistochemical Techniques**

For the neuropathological analysis of the AD-type histological changes, blocks were taken from at least 3 different cortical areas (frontal, parietal, and temporal) of all the 292 formalin fixed brains. The blocks taken from the temporal lobe included the hippocampus, part of the entorhinal cortex, as well as the ependymal region and the choroid plexus of the temporal horn of the lateral ventricle. At autopsy, in 17 of the 292 cases, similar samples (frontal, parietal, and temporal), including the ependymal region and the choroid plexus, were taken before fixation of the brain in formalin. These samples were frozen in liquid N2 and stored at –80°C until processing. Paraaffin and frozen sections cut from all these blocks were stained with the Gallyas silver technique, Thioflavin S, Congo red, and were immunostained with monoclonal antibody to β-amyloid protein (DAKO, M 872, dil: 1:100). Paraaffin sections of 35 cases (25 with AD-type changes and 10 control cases) were also stained with the Jones’ (10) and the Galloc silver techniques and were immunostained using a monoclonal antibody directed against the 70 kD and 200 kD neurofilament proteins (Bio-Science, 01020, dil: 1:500) (13).

Unfixed frozen sections were also immunostained with polyclonal antibodies to P component (DAKO, A-302, dil: 1:500), fibronectin (DAKO, A 245, dil: 1:3000), ubiquitin (DAKO, Z 0458, dil: 1:500) and after 4% paraformaldehyde fixation with the same anti-neurofilament protein antibody (Bio-Science, 01020, dil: 1:30) as used for paraaffin sections.

Frozen sections taken from the temporal region of the brains of the 17 cases where frozen material was stored at –80°C (12 AD + + cases and 5 AD-cases) were fixed in 4% paraformaldehyde and were immunostained for Tau protein. These sections included the hippocampus, the entorhinal cortex, as well as the choroid plexus and the ependymal layer of the temporal horn of the lateral ventricle. In this way, we were able to compare the Tau-reactivity of the neurons with those of the epithelial cells of the choroid plexus. Two monoclonal antibodies (Sigma T-5530, Clone Tau-2, dil: 1:1000, Chemicon MAB375, Clone: Tau-2, dil: 1:500), and a polyclonal antibody (Sigma, T-6402, dil: 1:500) were used. The monoclonal anti-Tau antibody (Sigma T-5530) reacts with chemically heterogenous Tau (55kD-62kD) and binds Tau proteins in either their phosphorylated or nonphosphorylated forms. These antibodies do not show cross-reaction with other microtubule-associated proteins.

For Tau-immunostaining, as for the other antigens, the avidin-biotin-peroxidase technique was used, but here the DAB reaction was revealed in black by nickel-ammonium sulfate and the sections were not counterstained with hematoxylin. Control sections without primary antibody or with irrelevant mono- or polyclonal antibodies (i.e. anti-Cytomegalovirus monoclonal antibody, DAKO M757, dil: 1:100) were also used.

**Electron Microscopy**

For ultrastructural analysis, small samples taken from the temporal cortex, the choroid plexus, and the ependymal layer of the lateral ventricles. Samples were postfixed in a 2% aqueous solution of osmium tetoxide, dehydrated in a graded ethanol series, and embedded in epon. The ultrathin sections were contrasted with uranyl acetate and lead citrate and were examined using a Philips CM-10 electron microscope.

**Statistical Analysis**

Contingency tables (2 x 2; 2 x 4; 5 x 5) were computed using FREQ procedure from SAS Institute Inc. (SAS/STAT User’s Guide. 1990,Version 6). The Fischer’s test was used to test the significance of the association between the cortical AD-type changes and the presence of curly fiber and tangle-like inclusions in the ependymal layer and choroid plexus. Where possible the significance of the χ² was calculated together with the Pearson correlation coefficient.

**RESULTS**

**Histochemical and Immunohistochemical Investigation**

In 225 of the 228 cases with AD-type histological changes in the cerebral cortex (Fig. 2A), including the 4 FAD and 2 DS cases, we found accumulation of neuropil threads in the ependymal layer (Fig. 2D) and intracellular congophilic inclusions in the epithelial cells of the choroid plexus (Fig. 2B, C). In only 1 case, we observed them in the plexus but not in ependyma. We disregarded this exception for the statistical analysis; therefore it was possible to group together the ependymal changes and those of the plexus (EP&PL). We did not find alterations in the ependyma or the plexus in only 3 cases of the 228 cases with AD-type cortical changes. In 12 of the 64 control cases, without any cortical AD-type histological changes, similar amyloid inclusions were observed; however, there were none present in the choroid plexus or the ependymal layer in the other 52 control cases.

In the ependymal layer the morphology and the localization of the thread-like Thioflavin-S positive structures were similar to those of the curly fibers. Frequently, they formed small rings similar to those of the Bioni rings. These structures in regions of the ventricular surface,
CURLY FIBER AND TANGLE-LIKE INCLUSIONS IN THE PLEXUS

which were denuded from ependyma due to granular ependymitis, were absent.

The size and shape of the Biondi inclusions in the choroid plexus varied. Some resembled silver grains, curly fibers (Fig. 3A–D), or tangles (Fig. 2B, C), and frequently they had a ring-like or globular appearance. Occasionally the filamentous inclusions seemed partly extracellularly located (Fig. 3B, C).

These structures exhibited a strong yellow fluorescence when stained with Thioflavine S (Fig. 2B, C, D). They bound Congo red and then exhibited a bright green birefringence. These thread and tangle-like inclusions were easily and consistently visualized by the Jones' (Fig. 1L) and Grocott methenamine silver methods both on frozen and paraffin sections. They stained with the Gallyas silver technique (Fig. 3B, C), particularly on frozen sections. These curly fiber and ring-like inclusions were found to be immunoreactive with antibodies to P component, ubiquitin, fibronectin, but also with the mono and polyclonal anti-Tau antibodies (Fig. 4A, C). The granular staining of Biondi rings, filaments, and globular structures with anti-Tau antibodies was similar to that seen in tangle bearing neurons (Fig. 4D), but in contrast to neurons they lacked immunoreactivity to neurofilament proteins. They were frequently associated with small lipofuscin granules.

Ultrastructural Analysis

Electron microscopic analysis of the thread and tangle-like inclusions of the choroid plexus (Fig. 5) showed paired helical filaments of about 20–25 nm in diameter (Fig. 5B). Twisted and straight forms were both observed. They were distributed in the cytoplasm in small bundles of about 0.3–1-μm-wide of dense, broad group of oriented fibrils (Fig. 5A). The shape of these filament bundles was either thread-like or in the form of incomplete or complete rings (Fig. 5C). They were frequently in close relation with small lipid and lipofuscin granules. In the ring-like bodies, the lipid droplets were centrally located (Fig. 5C). Sometimes less closely packed, more

Fig. 4. Photomicrographs showing Tau immunoreactivity in epithelial cells of the choroid plexus in a case with sporadic AD. A: Frozen section immunostained with monoclonal antibody against Tau protein, showing dark granular staining of the epithelial cells of the choroid plexus. Several immunostained rings, thread and globular structures are visible. B: Biondi inclusions as visualized by the Jones' method in the choroid plexus of the same case. In A, arrow points to an immunoreactive thread-like inclusion, arrowheads and asterisks in A and B to ring-like and tangle-like inclusions, respectively. C: Arrows point to Biondi inclusions showing granular immunostaining. At this higher magnification the granular staining of the epithelial cells and their inclusions is well visible and is similar to those of the tangle bearing neurons (D). Photomicrograph D was taken from a frozen section of the temporal cortex of the same AD case. The same anti-Tau monoclonal antibody was used as for the plexus (see A and C). Bar in A corresponds to 20 μm and is the same for B. Bar in C represents 10 μm and is the same for D.
Fig. 5. A–C illustrates the ultrastructure of the paired helical filaments of the thread and ring-like Bioni inclusions lying in the epithelial cells of the choroid plexus in a sporadic AD case. A: Arrow points to a thread-like inclusion lying in the cytoplasm of a plexus epithelial cell. B: Illustrates that the inclusion in A, when seen with higher magnification is made up from paired helical filaments. C: Ultraphotomicrograph showing that the bundles of paired helical filaments sometimes form ring like structure, here curved around a large lipid droplet. The scale bar corresponds to 2μm in A, to 0.1μm in B, and to 1μm in C.

dispersed filaments were also seen. We did not find similar ultrastructural changes in the choroid plexus of 2 control cases.

Statistical Analysis

There were 228 cases with AD-type cortical changes (AD+, AD++ and AD+++ groups together) and 64 cases without cortical changes (AD−). When these 2 groups were checked for the presence (EP&PL+) or the absence (EP&PL−) of thread- and tangle-inclusions in ependyma and choroid plexus, the proportion of cases with thread- and tangle-like elements in ependyma and plexus was over 98% in the group with cortical AD-type lesions and lower than 19% in the AD− group. In contrast, the proportion of cases without thread-like structures in ependyma and plexus was very low (less than 2%) in the group with pathological lesions of AD-type but very high in the AD− group (more than 81%). A χ² test, using the 2 × 2 contingency table (64 controls vs 228 brains with AD-type changes) showed that the association between the Alzheimer-type histological changes in the cerebral cortex, the ependymal curly fibers and
Fig. 6. Comparison of the frequencies of cases with AD-type changes in groups with and without thread and tangle-like inclusions in the ependyma and choroid plexus. A: The percentage of cases with thread and tangle-like elements in the ependyma and plexus (EP&PL+) is very high (more than 96%) in the 3 subgroups with severe (AD++, 63 cases), moderate (AD+, 23 cases) or discrete (AD+) AD-type cortical changes, and low (18.7%) in the control group (AD−, 64 cases). The 100% corresponds to the total number of cases in each subgroup. On the contrary, the percentage of cases without thread-like structures in the ependyma and plexus (EP&PL−) is extremely low (less than 3.2%) in the subgroups with AD-type lesions, but very high in the AD− group (more than 81%). The graphics indicate that the presence of ependymal and plexus inclusions is associated with the cortical AD-type changes (Fisher test p < 0.0001). B: The association was also significant when the ependymal and plexus changes were divided into 5 subgroups according to the severity of these lesions (EP&PL+, ++, +++; ++++ and EP&PL−). The percentage of cases with cortical changes (AD+, AD+; AD+++) was very high in the subgroup with severe ependymal and plexus changes (EP&PL−++) and progressively diminished with the decreasing density of ependymal and plexus changes. In the EP&PL− group the occurrence of the cortical AD-type changes was very low.

the Biondi inclusions of the choroid plexus is highly significant ($\chi^2 = 208.9; p = 0.001$), with a high value for the Pearson correlation coefficient ($R = 0.85$).

The frequency of the AD-type changes in ependyma and plexus was higher than 96% in all of the 3 subgroups with severe, moderate, and discrete AD changes (Fig. 6A). The occurrence of AD-type changes in cortex and the presence of curly fiber or tangle-like inclusions in the plexus was significantly correlated ($R = 0.54, \chi^2 = 209; Fischer's p < 0.0001$). The correlation was also significant (Fischer's $p < 0.0001$) when the ependymal and plexus changes were subdivided into 5 groups according to the severity of the changes (Fig. 6B). In cases with severe ependymal and plexus changes the percentage of cases with cortical changes was very high and progressively decreased with the density of ependymal and plexus changes. In cases without EP&PL changes the occurrence of the cortical AD-type changes is very low. A similar correlation was found between cortical, ependymal, and plexus changes when the severity of senile plaques, neurofibrillary tangles, or neuropil threads were considered (Fig. 7 A–C). In cases without ependymal and plexus inclusions (EP&PL− group) the percentage of cases with senile plaques, neurofibrillary tangles, or neuropil threads was lower than 4%.

**DISCUSSION**

Whether or not the formation of the Biondi inclusions in the choroid plexus is related to the degenerative process responsible for the appearance of the Alzheimer-type lesions in the cerebral cortex has been debated for several decades. Recently, immunohistochemical and biochemical detection of β-amyloid protein in the amyloid inclusions of the choroid plexus (8, 9) indicates that the basic process responsible for the degenerative process in the cerebral cortex and choroid plexus may be similar.

A systematic study of the AD-type changes in the cerebral cortex and of the thread and tangle-like inclusions of ependyma, and choroid plexus of 292 consecutive autopsy cases was performed in order to analyze whether or not a significant correlation exists between the cortical, the ependymal, and the plexus changes. In almost all cases
with cortical AD-type changes, we found thread-like structures in the ependymal layer and Biondi filaments and rings in the plexus. Only 3 cases out of the 228 cases with cortical AD lesions did not show these thread and ring-shaped inclusions. In these 3 cases a severe granular ependymitis was observed with wide destruction of the ependymal layer.

There is some evidence in the literature for an occasional extrusion of Biondi bodies into the ventricle (14). The seemingly, partly extracellular location of some Biondi filaments we have observed in this study is surprising and difficult to explain. For conclusive evidence further studies (e.g., using confocal microscopy analysis) would be necessary.

The statistical analysis of the results indicates a highly significant correlation between the AD-type cortical lesions and the amyloid inclusions of ependyma and choroid plexus. It is interesting to notice the high frequency and the high density of ependymal and plexus changes not only in brains with severe or moderate, but also with discrete AD-type cortical changes. The percentage and the severity of the cortical changes are progressively decreasing with those of the ependymal and plexus changes. Finally, in cases without ependymal and plexus inclusions this percentage and density is very low. In 18.7% of the 64 cases without AD-type changes in the cortex, thread and tangle-like structures in the ependyma and plexus were already present. These findings suggest that the ependymal and plexus changes may be 1 of the earliest manifestations of the degenerative process. We have found that these "curly fibers" and "tangles" in the ependymal layer and in the extraneural choroid plexus were immunoreactive with antibodies to ubiquitin, P component, and fibronectin, thus exhibiting similar immunohistochemical features as neurofibrillary tangles (2, 15).

Highly phosphorylated microtubule-associated protein Tau is a major antigenic component of paired helical filaments of neurofibrillary tangles (16). Tau proteins accumulate also in a nonfilamentous and phosphorylated

![Graphs showing percentage of cases with different densities of senile plaques, tangles or neurit threads with respect to the densities of the ependymal and plexus changes. A, B and C. Percentage of cases with different densities of senile plaques (SP), tangles (NFT) and neurit threads (NT), like those of the ependymal and plexus inclusions were graded in the following way: absence of lesion = - ; low = + ; moderate = ++ ; high = +++ ; and very high = +++++. The total number of cases in each subgroup represents 100%. The severity of the EP&PL changes was clearly associated with the severity of the AD-type cortical changes (SP, NFT, NT). In the subgroup with severe plexus changes (EP&PL+++), the percentage of cases with very high density of SP, NFT or NT was very high and progressively diminished with decreasing densities of EP&PL changes. In cases without ependymal and plexus inclusions (EP&PL− group) this percentage was lower than 4%.](https://jnen.oxfordjournals.org/content/57/11/1210)

**Fig. 7.** Comparison of frequencies of cases with different densities of senile plaques, tangles or neurit threads with respect to the densities of the ependymal and plexus changes. A, B and C. Percentage of cases with different densities of senile plaques (SP), tangles (NFT) and neurit threads (NT), like those of the ependymal and plexus inclusions were graded in the following way: absence of lesion = - ; low = + ; moderate = ++ ; high = +++ ; and very high = +++++. The total number of cases in each subgroup represents 100%. The severity of the EP&PL changes was clearly associated with the severity of the AD-type cortical changes (SP, NFT, NT). In the subgroup with severe plexus changes (EP&PL+++), the percentage of cases with very high density of SP, NFT or NT was very high and progressively diminished with decreasing densities of EP&PL changes. In cases without ependymal and plexus inclusions (EP&PL− group) this percentage was lower than 4%.
form in perikarya and dendrites at early, "pre-tangle" stages in AD. Our results showing a positive immunoreaction of plexus epithelial cells with anti-Tau antibodies suggest that similar to neurofibrillary degeneration, the Tau protein could possibly participate in the formation of Biondi inclusions of the epithelial cells of the plexus. This would be in agreement with recent reports that non-neuronal cells may well show immunoreactivity to hyperphosphorylated Tau, namely, in inclusion body myositides vacuolated muscle fibers containing paired helical filaments were reported to be immunoreactive (17, 18).

Further analyses, including biochemical and molecular biological studies will be necessary for a more precise molecular characterization of the thread and tangle-like inclusions of plexus and ependyma.

Both paired helical and straight filaments are components of neurofibrillary tangles and neuritip threads in the AD brain (19) and were reported to be present also in astrocytes (1), in epithelial cells of the choroid plexus, and in the adrenal cortical cells (20). These observations are in agreement with our electron microscopic analysis showing that the curly fiber and tangle-like inclusions found in choroid plexus showed paired helical filaments, and both twisted and straight filaments. The fact that they express histochemical, immunohistochemical, and ultrastructural properties similar to neurofibrillary tangles and curly fibers in the AD brain, indicates that the formation of these structures is not unique to the central nervous system.

The monoclonal antibody against neurofilament proteins 70 kD and 200 kD used for the present study recognizes neurofibrillary tangles, but does not label tangle or curly fiber-like inclusions in the choroid plexus. This would be in harmony with the observations that phosphorylated neurofilament proteins are associated with neurofibrillary tangles but are not the building blocks of these structures. Indeed, monoclonal antibodies generated against isolated neurofibrillary tangles do not always react with neurofilament proteins (21). Their positivity in tangle bearing neurons is proposed to be secondary to the damage of the perikaryon by the degenerative process (22). The phosphorylation of neurofilament proteins in the perikaryon, which in normal condition is not phosphorylated, was reported in other pathologies as well, including those due to simple retrograde changes following axonal interruption (22, 23).

These findings together with the statistical analysis which shows a strong association between the frequency of the AD-type cortical lesions and Biondi inclusions suggest that the pathogenesis of the degenerative changes in the cortex, ependyma, and plexus may be similar.

In conclusion, in 228 cases with AD-type cortical changes (of 292 autopsy cases), pathological argyrophilic filaments with morphological and ultrastructural similarity to curly fibers and tangles and with histochemical properties of amyloid, occurred also in the epithelial cells of the choroid plexus. These extraneuronal curly fibers and tangles were immunoreactive with antibodies to ubiquitin, P component, fibronectin (2, 10), and Tau-protein, but negative for neurofilament proteins. The cortical AD-type histological changes are strongly correlated with the occurrence of thread-, tangle- and ring-like inclusions of the ependyma and choroid plexus. These results, together with the observations of other authors, are in favor of a pathogenetical relationship between the degenerative changes of cortex, ependyma, and plexus. They also suggest that the ependymal and plexus changes may well be 1 of the earliest manifestations of the degenerative process in the central nervous system.

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