Intracellular Neurofibrillary Tangle-like Aggregations

A Constantly Present Amyloid Alteration in the Aging Choroid Plexus

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Intracellular neurofibrillary tangles is one of the most characteristic findings in Alzheimer's disease and senile dementia of Alzheimer type. In the present paper the authors show that intracellular accumulation of paired helical filaments is also a constant finding in the epithelial cells of the choroid plexus of aging persons. Like the neurofibrillary tangles, the fibrils of the choroid plexus show staining properties typical of amyloid. The nature of the fibrils could not be clarified by electron microscopy or by immunohistochemistry with the use of antisera to γ-trace or to amyloid fibril proteins of AA and prealbumin type. Amyloid protein AP, found in all amyloid substances except for neurofibrillary tangles and amyloid of senile plaques in the brain, was not demonstrated in the inclusions of the choroid plexus. (Am J Pathol 1986, 125:124–129)

COLLECTION of bundles of fibrils intracellularly in cerebral neurons, light-microscopically known as neurofibrillary tangles, is one of the most characteristic structural changes in Alzheimer's disease and in senile dementia of Alzheimer type.[1-3] These fibrils are arranged more or less in parallel, and each fibril consists of two filaments twisted around one another.[4-11] A typical property of the neurofibrillary tangles is their affinity for Congo red with green birefringence in polarized light after such staining.[12] Neurofibrillary tangles also possess cross-β pleated sheet conformation, and they can therefore be regarded as a type of intracellular amyloid.[13]

Threadlike structures, demonstrable by silver impregnation, have long been known to occur in the epithelial cells of the choroid plexus.[14] These inclusions, which increase in number with age,[15] have been shown to bind to Congo red[16] and to have a fibrillar ultrastructure.[15,17]

In the present study, we show that choroid plexus inclusions with amyloid properties are invariably present after the age of about 45 years. Furthermore, we demonstrate a close morphologic resemblance of the inclusions to the neurofibrillary tangles seen in dementia of Alzheimer type.

Materials and Methods

Light Microscopy

The choroid plexus from 102 randomly chosen patients was saved at autopsy and fixed in 4% formalde-

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hyde solution. Pieces of tissue from the cerebrum, heart, lungs, kidneys, and adrenal glands were also collected. The tissues were embedded in paraffin, sectioned, stained with alkaline Congo red, and studied in polarized light.

Electron Microscopy

For electron-microscopic studies, small pieces of choroid plexus were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, containing 0.1 M sucrose and postfixed in 1% osmium tetroxide in the cacodylate buffer for 90 minutes. The tissue pieces were then treated with 1% uranyl acetate in 50% ethanol overnight and embedded in Agar 100 (Agar Aids, Stansted, Essex, England). Ultrathin sections were contrasted with lead citrate and studied in a JEOL 100 C electron microscope at 60 kv.

Immunohistochemical Methods

Immunohistochemical studies were performed on the choroid plexus of 6 patients. Antibodies to human prealbumin and to retinol binding protein were from DAKO (Copenhagen, Denmark). Rabbit anti-amyloid protein AA antiserum was obtained by bimonthly subcutaneous injections of purified human protein AA mixed with Freund's incomplete adjuvant after an initial immunization of protein AA and Freund's complete adjuvant. After absorption with purified protein AP (10 mg/ml), this antiserum showed immunohistochemical reaction only with amyloid of AA-type. Antiserum to the prealbumin-related amyloid protein ASc_1 was characterized previously.[18] Rabbit anti-γ-trace antiserum was a gift from Dr. A. Grubb, Malmö. Antiprotein AP (amyloid P-component) antiserum was obtained by bimonthly immunization of a rabbit with protein AP purified from amyloid saline washes as described.[19] These antibodies were further purified by affinity chromatography on an AP-Sepharose 4B column, obtained by coupling of protein AP to CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden). Coupling and elution followed the manufacturer's instruction.

Deparaffinized tissue sections were incubated with the primary antisera for 3 hours at room temperature and subjected to the peroxidase–antiperoxidase method.[20] For visualization of the reaction, 3-amino-9-ethylcarbazole (Sigma, St. Louis, Mo) was used. In one experiment, sections were treated with anti-prealbumin and anti-ASc_1 antibodies, respectively, and the reaction was

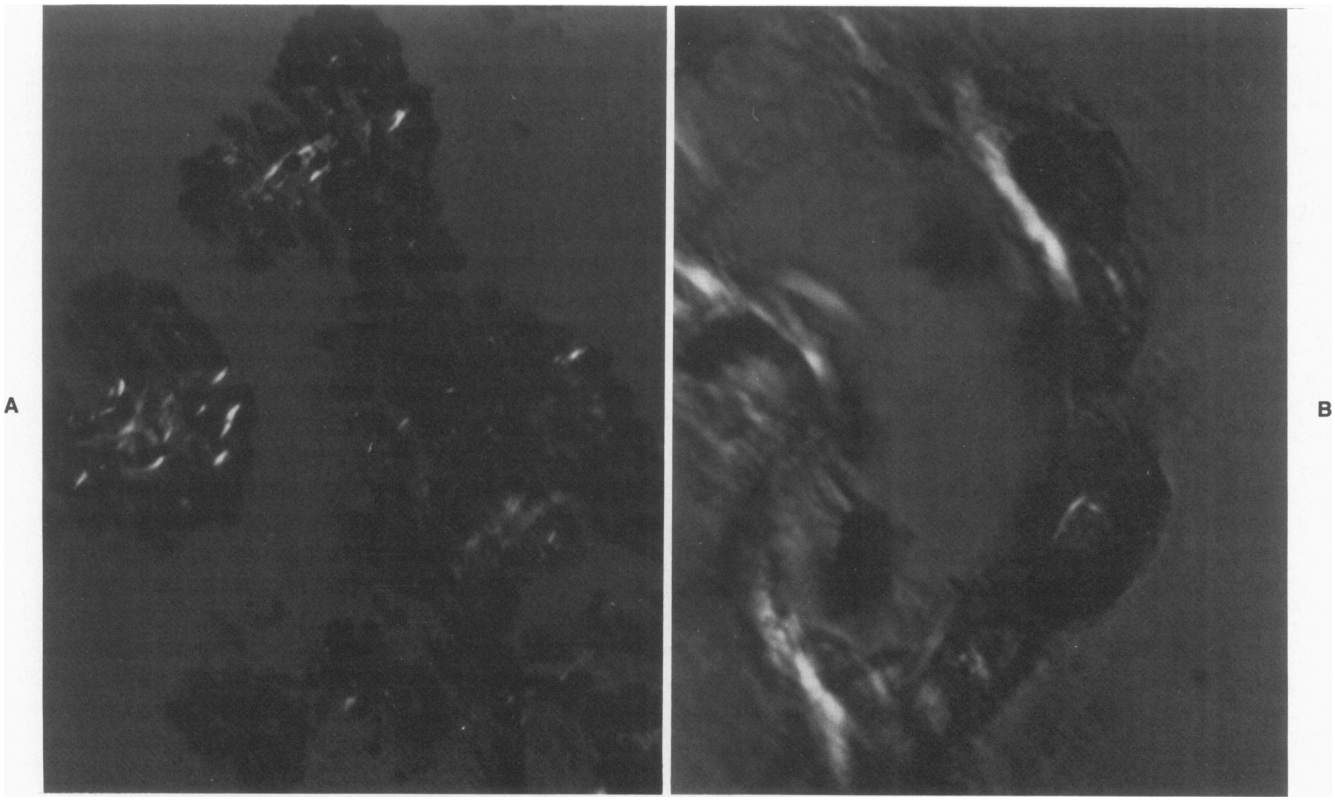

Figure 1—Small, needle-shaped amyloid fibril bundles exhibiting typical green birefringence in the epithelial cells of the choroid plexus. (Congo red, between crossed polars, **A**, ×500; **B**, ×1300)

Table 1—Frequency and Semiquantitative Estimation of Intracellular Amyloid in the Choroid Plexus

Age of patients (years)	Grade 0	Grade 1	Grade 2	Grade 3
<60 (n = 35)	26	6	3	0
60–79 (n = 37)	0	6	25	6
80– (n = 30)	0	2	22	6

* In Grade 1, only scattered cells with amyloid were found. In Grade 3, massive depositions were seen in over 50% of the epithelial cells.

visualized with 3′,3′-diaminobenzidine (Sigma). After that, the sections were stained briefly with Congo red in 50% ethanol. This allowed identification of the small amyloid deposits against the dark background (see below). All antisera were used in dilution 1:200 to 1:800. The purified anti-AP antibodies were used in concentrations of 4 and 8 μg/ml.

Results

Light-Microscopic Findings

Intracellular inclusions with affinity for Congo red and a bright green birefringence after such staining occurred in epithelial cells of the choroid plexus. The amyloid inclusions often were needle-shaped or crystallike (Figure 1). They often occurred solitarily, but sometimes cells filled with amyloid were seen. Deposits did not seem to occur outside the epithelial cells, although the existence of extracellular amyloid could not be ruled out. In one patient with systemic amyloidosis, deposits were seen in vessel walls. There was no correlation between the occurrence of intracellular amyloid deposits in the choroid plexus and amyloid in other organs, including other parts of the brain.

Frequency Study

The results of the frequency study are shown in Table 1. Intracellular choroid plexus amyloid was found in all patients over 52 years of age and was absent in only 26 patients, 45 years old or younger. There was a tendency of more pronounced amyloid deposits with increasing age.

Electron-Microscopic Findings

Choroid plexus from 4 patients with Grade 3 deposition (see Table 1) of amyloid was studied electron microscopically. In all cases, intracellular bundles of

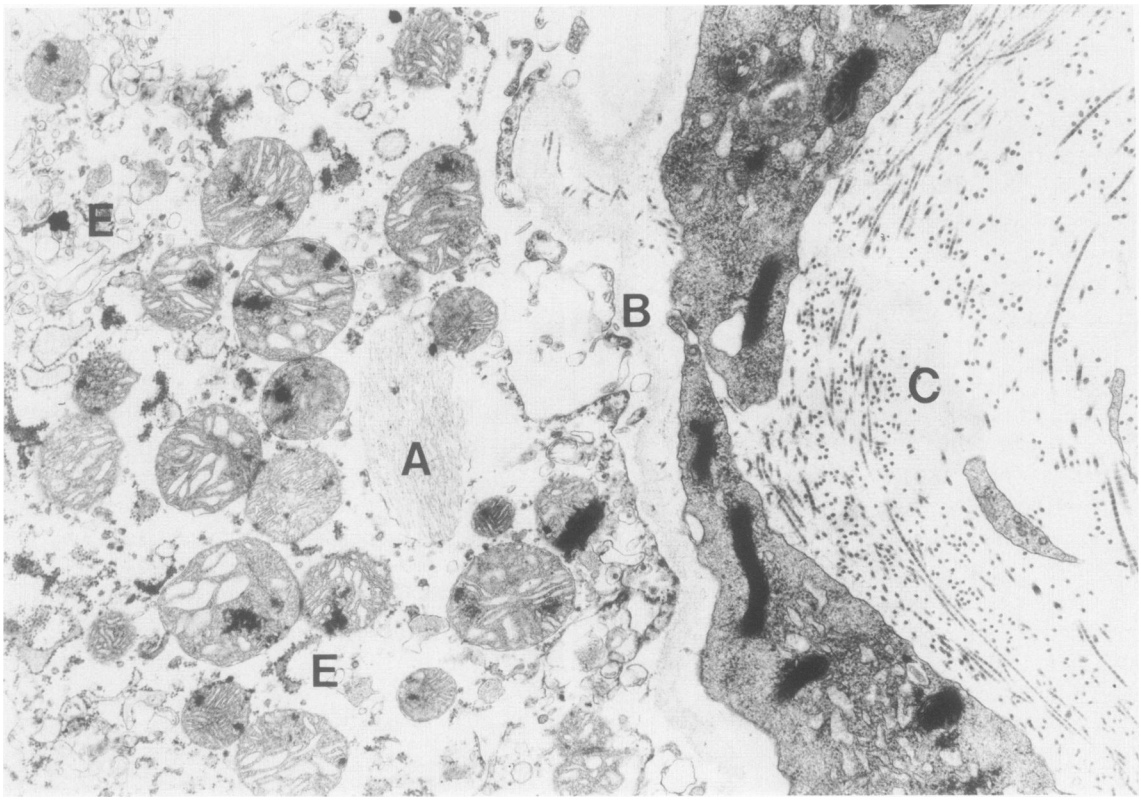

Figure 2—Choroid plexus epithelial cell (*E*) with a small amyloid inclusion (*A*). The epithelial cell is separated from the connective tissue (*C*) by a basement membrane (*B*). (×6500)

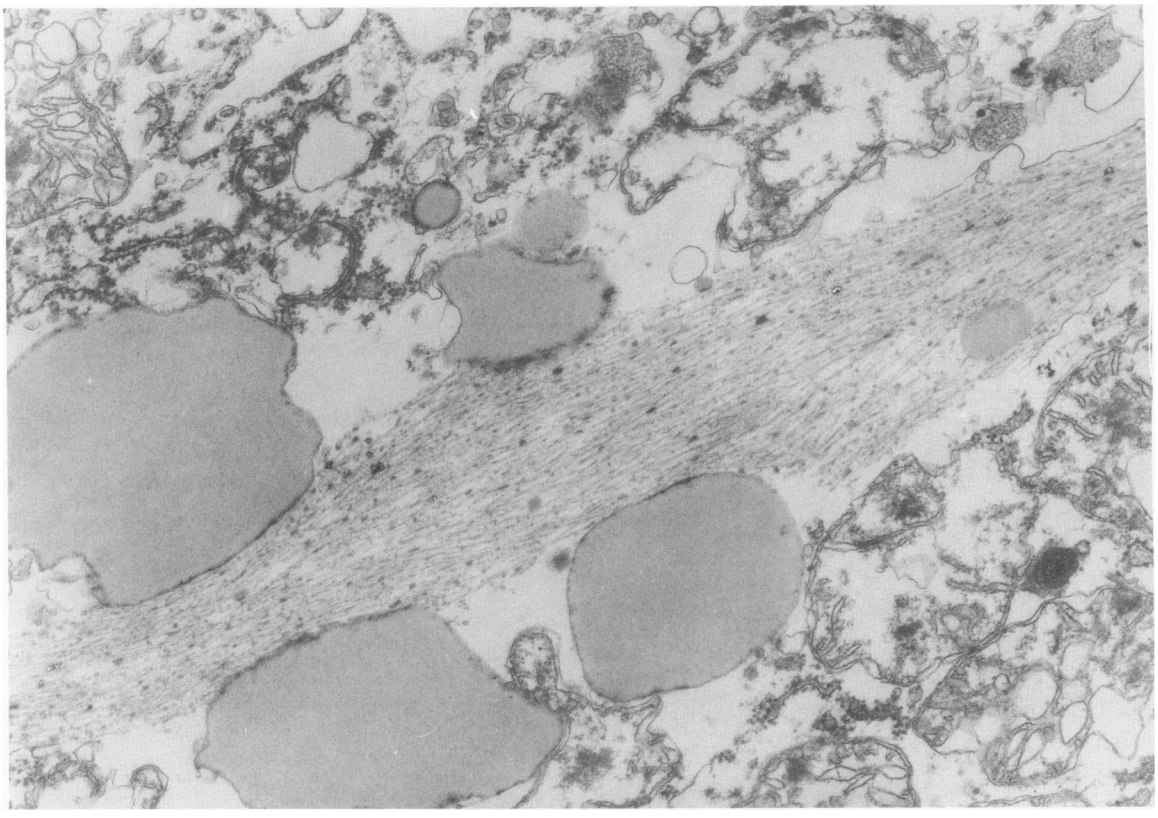

Figure 3—Obviously intracellular bundle of almost parallel amyloid fibrils. The bundle, which is partially limited by a membrane, is intermingled with small droplet-shaped structures and closely attached to residual bodies. (×15,000)

parallel fibrils were seen (Figure 2). The bundles were usually partially limited by a membrane and were intermingled with dropletlike structures of varying size (Figure 3). The fibrils, which were about 20 nm thick, seemed to consist of two filaments twisted around one another (Figure 4). The appearance resembled the neurofibrillary tangles seen in Alzheimer-like dementia, although no regular periodicity was seen with certainty.

Immunohistochemical Findings

A strong reaction was obtained within all epithelial cells of the choroid plexus when anti-prealbumin and anti-ASc_1 antibodies were used with the peroxidase–antiperoxidase method. This reaction was seen in all cases, irrespective of age or the occurrence of amyloid. The reaction product was granular and often seemed to be unevenly distributed in the cells. The amyloid was impossible to identify in sections developed with 3-amino-9-ethylcarbazole, but Congo red staining after development with 3′,3′-diaminobenzidine clearly showed that the amyloid did not bind anti-prealbumin or anti-ASc_1 antibodies.

Anti-retinol binding protein antibodies only gave a weak reaction in some cells of the choroid plexus. No positive reaction in plexus cells or in the amyloid was obtained when anti-protein AP antibodies. antiprotein-AA or anti-γ-trace antisera were used.

Discussion

Microdepositions of amyloid is very common in a variety of tissues in old age.[21] These amyloid deposits seem to vary highly in composition, but their exact chemical nature is for the most part, unknown. The deposits are usually supposed to be of no clinical importance, although they probably reflect a pathologic event.[22] It is possible, however, that the amyloid deposits may act as a diffusion barrier. Intracellular amyloid might also affect cellular function. In the present study, though, we were unable to show any correlation between amyloid deposits in the choroid plexus and other alterations. Patients with senile dementia of Alzheimer type did not differ from other patients with respect to choroid plexus amyloid.

Strictly intracellular depositions with amyloid properties are rare and are most commonly reported as neurofibrillary tangles in intracerebral neurons in dementia of Alzheimer type. Morphologically, the amyloid inclusions of the choroid plexus resembled the neuro-

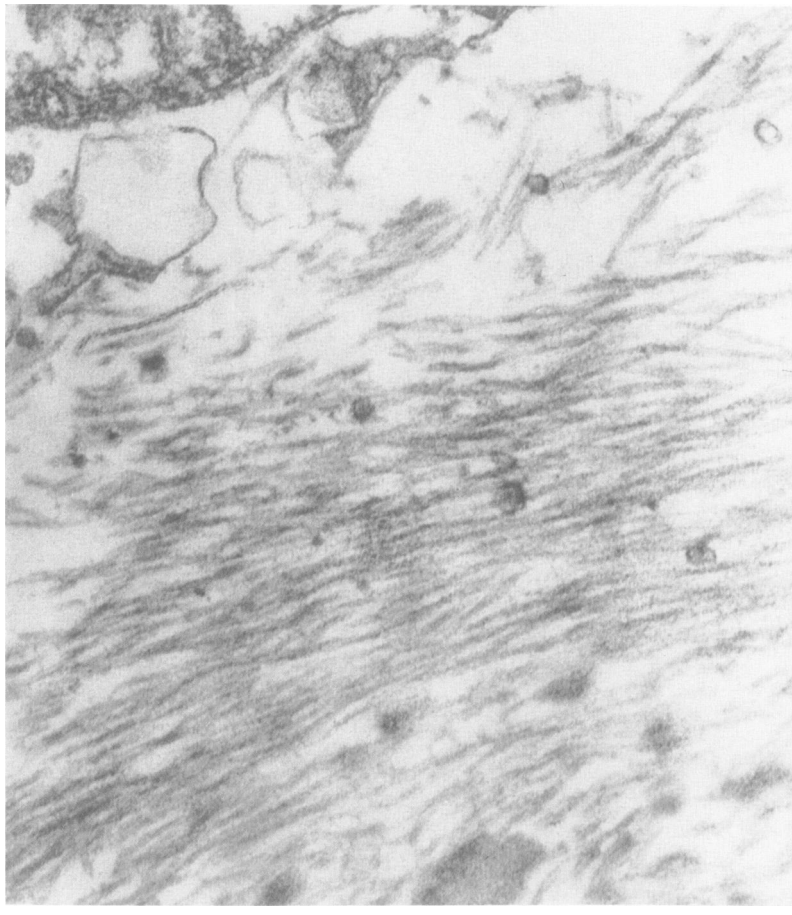

Figure 4—The parallel amyloid fibrils of the choroid plexus consist of two filaments twisted around one another, giving an appearance closely resembling the paired helical filaments of the neurofibrillary tangles. (×60,000)

fibrillary tangles. Thus, both consist of parallel bundles of fibrils containing two filaments twisted around one another. Although the amyloid deposits in the neurons and in the epithelial cells of the choroid plexus were morphologically similar, their occurrence in so different cells seems to make identical nature unprobable. However, the plaque core amyloid and the amyloid in Congophilic angiopathy have been demonstrated to share a fibril protein.[23-25] The same protein has been reported to be a major constitutent also of neurofibrillary tangles,[26] but contradictory results have been obtained by others,[25] and immunocytochemical studies indicate that the neurofibrillary tangles are derived from neurofilaments[6,8,11] or neurotubules.[5,7] The possibility of an identical nature of the amyloid of the choroid plexus and any of these amyloids is therefore under investigation in our laboratory.

The nature of the fibrils in the choroid plexus could not be clarified with the electron-microscopic findings or with the immunohistochemical study. No reaction was obtained with the antisera to the different amyloid fibril proteins. Of the known amyloid fibril proteins, prealbumin appeared as the potentially most probable subunit in the choroid plexus amyloid, because it has been shown that the plexus epithelial cells contain prealbumin.[27,28] It has also recently been shown that this protein is not simply transported transepithelially, but is actually synthesized in the choroid plexus.[29] However, both anti-prealbumin antibodies and the anti-protein ASc_1 antiserum failed to react with the choroid plexus deposits. This makes a prealbumin nature of the deposits highly unlikely, because all prealbumin-derived amyloids that we have studied have shown a reaction with at least one of these antisera (unpublished observations).

Amyloid P-component (protein AP) is a constituent of almost all human and animal amyloid.[19] Its significance in amyloid has been questioned, and at present most studies indicate that protein AP is passively absorbed to the amyloid fibrils. The amyloid of neurofibrillary tangles and of senile plaques in senile dementia of Alzheimer type has, however, been shown to lack protein AP.[30] To these we can now add the intracellular amyloid of the choroid plexus. It is thus clear that protein AP is not a necessary constituent in the formation of fibrils with amyloid properties.

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