Immunohistochemical Localization of Amyloid Beta-protein Deposits in Extracerebral Tissues of Patients with Alzheimer’s Disease*

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ABSTRACT

An autopsy study was conducted on patients with Alzheimer’s disease in order to explore the possibility of amyloid beta-protein deposits in tissues other than the brain. Immunoperoxidase staining techniques were employed using an amyloid beta-protein antiserum as primary antibody; paraffin sections of kidney, liver, heart, lung, spleen, bone marrow, colon, stomach, adrenal, thyroid, and brain were examined. Our autopsy cases were divided into two groups. The first group comprised 10 patients with Alzheimer’s disease. The second group consisted of eight control cases, of age-matched individuals, that died of unrelated causes and showed no clinicopathologic evidence of Alzheimer’s disease. Identification was made of strong, although focal, positive staining in two kidneys and one lung of three different patients with Alzheimer’s disease. All other tissues, including the control cases, showed negative staining.

Introduction

Amyloid beta-protein (ABP) is a principal component of senile plaques and cerebral congophilic angiopathy which, along with neurofibrillary tangles, constitute the pathologic hallmark of Alzheimer’s disease (AD). It is now widely accepted that ABP derived from beta-Amyloid Precursor Protein (B-APP) through enzymatic cleavage. The anatomic location and the cell types involved in such interactions are still unknown.16

Whether deposition of ABP occurs exclusively in the brain or also occurs in other organs of patients with AD is a subject of controversy. Joachim and coworkers8 first reported the detection of deposits of ABP in tissues outside the brain including skin, subcutaneous tissues, and intestine, and suggested that AD is a systemic disorder. A second, related study, by Arai and coworkers,1 investigated the
distribution of B-APP in neural and non-neural tissues. By immunohistochemistry and Western Blotting, limited amounts of B-APP were identified in non-neural tissues such as myocardium, adrenal gland, bone marrow, and megakaryocytes.

To investigate the possibility of the deposition of ABP in tissues other than the brain of victims of AD, an autopsy study was performed by examining tissue sections from most organ systems.

Materials and Methods

Two groups of autopsy cases were selected for this study. The first group consisted of 10 cases of AD. The 10 patients with AD, with an age range from 67 to 91 years, had a documented clinical history of dementia and a clinical diagnosis of AD which was confirmed by neuropathologic examination of the brain. A diagnosis of AD was reached by adhering to the “minimum microscopic criteria” previously proposed by a panel of experts.10 These criteria require the identification of 10 to 15 or more senile plaques, for patients older than 65, in any one sq mm field (microscopic magnification of ×200 field). The modified Bielchowski silver stain was employed for identifying and counting the senile plaques. The second group consisted of eight control autopsy cases of individuals that died of unrelated causes such as car accidents, myocardial infarcts, and carcinomas. Their age ranged from 65 to 84. None of these cases had a clinical history of dementia and no changes of AD were seen on examination of the brain.

Our primary antibody (1:2000 dilution) was an amyloid beta-protein polyclonal antiserum.* This rabbit antiserum was raised against a synthetic ABP peptide comprising residues 1 to 40 (aspartic acid through valine). The production and characterization of this antiserum is similar to that described for antiserum Y by Joachim et al.8 Formalin-fixed, paraffin-embedded sections from the kidney, heart, lung, spleen, liver, bone marrow, colon, stomach, adrenal, thyroid, brain frontal and parietal cortex, and hippocampus were immunohistochemically stained in all 18 cases. A standard ABC immunoperoxidase method was employed for staining. The procedure included deparaffinization, quenching of endogenous peroxidase with 0.5 percent hydrogen peroxide in methanol, followed by a blocking step with normal goat serum (1:5 dilution in 0.05M PBS, pH 7.4). Tissue sections were then sequentially incubated, in room temperature, with primary antiserum (1:2000 dilution) for one hour, biotinylated secondary antibody (1:200 dilution) for 30 minutes, and ABC reagent† for 30 minutes. Between each step of incubation the slides were thoroughly rinsed with 0.05M PBS containing two percent normal sheep serum, pH 7.4. As a final step, tissue sections were rinsed with 0.05M phosphate buffer, pH 7.4, color developed with diaminobenzidine, and counterstained with Harris hematoxylin. Appropriate positive and negative controls were used in all of our procedures. Hippocampus brain sections from patients known to have AD were used as positive tissue controls. Substituting of primary antibody with normal rabbit serum was used for negative controls. To probe the specificity of the positive immunoreaction, preabsorbed primary antibody was applied to all slides that demonstrated positive immunostaining. Absorption of the primary antibody was carried out by

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† Vector Laboratories, Burlingame, CA.
incubating (room temperature for two hours) the primary antibody was lyophilized Alzheimer's brain cortex known to contain numerous amyloid plaques. The mixture was centrifuged at 12,000 rpm for 20 minutes and the supernatant was used for immunostaining. In order to enhance the sensitivity of our staining procedure, all examined tissues were pretreated with formic acid, according to previously published guidelines. A second set of sections from all tissues was predigested with the proteolytic enzyme pronase by incubating the slides with 0.1 percent pronase† in 0.5M Tris buffer, pH 7.5 for 10 minutes in room temperature. As an additional means of detecting tissue amyloid deposits, the alkaline congo red method was applied to another set of all tissue sections. Kidney and heart sections from a systemic amyloidosis case were used as positive congo red staining controls.

Results

All 10 cases of AD showed large numbers (>15 per 1 sq mm) of brain neocortex and hippocampus immunoreactive plaques. Strong positive immunostaining was also observed in several meningeal and superficial brain cortex blood vessel walls of all patients with AD. Pretreatment with formic acid resulted in significant improvement in the intensity of brain plaque immunostaining (figure 1), when compared to pronase pretreated cases. The control group (eight non-AD cases) generally showed few brain immunoreactive plaques or complete absence of immunostaining. All examined extracerebral tissues of the control group, showed negative immunostaining.

Tissue from three of 10 patients with AD demonstrated focal, although clear-cut, positive immune reactions in organs outside the neuroaxis. More specifically, kidney sections from two different patients with AD revealed similar positive immunostaining in the interstitium of the kidney medulla (figures 2 and 3). Both of these sections were predigested with pronase. Parallel/deeper sections of the same two, positive when predigested with pronase, kidney tissues, stained negative when pretreated with formic acid. The third positive case was a lung section that showed weak perivascular positive staining but focally strong peribronchiolar positive immunoreaction (figure 4). This case was pretreated with formic acid. Deeper sections of the same lung tissue, positive when pretreated with formic acid, stained negative when predigested with pronase. The con-

† Sigma, St. Louis, MO.
spicuous peribronchiolar distribution of the positive staining raised questions about possible cross-reaction with some antigenic determinant of the basement membrane.

No other basement membrane, besides the one shown in figure 4, displayed similar positive staining. Treatment with preabsorbed primary antibody of parallel/deeper sections of all immunopositive cases greatly diminished or abolished the immune reactions in the brain as well as in the three positive extracerebral sites. Examination under a polarizing microscope of the congo red stained tissues, revealed the characteristic positive apple-green color in the walls of several cerebral and meningeal vessels as well as in a variable number of neuritic plaques of all 10 patients with AD. The number of congo red positive plaques was clearly less than the positive immunoreactive plaques. It has been shown, however, that congo red stains positively only the neuritic or presumably more mature plaques. The less mature amyloid deposits or diffuse plaques are undetectable with congo red. Congo red was also positive in few neuritic plaques of some control, non-AD cases. All extraneural tissues of both the group with AD and the control group yielded negative results with congo red.

**Discussion**

The majority of the various types of amyloidoses are systemic disorders. Forms of localized amyloidoses include the senile cardiac amyloidosis and the amyloid deposits in medullary carcinoma of the thyroid. Alzheimer's disease is recognized as a distinct type of amyloidosis owing to excessive deposition of a unique form of amyloid, presumably restricted to the brain. This, along with...
the primarily neurological clinical manifestations, suggests that AD may be a localized form of amyloidosis.4,6 This view was initially challenged by several studies12,14,20 which reported significant biochemical cellular alterations in non-neural cells, including erythrocytes, platelets and skin fibroblasts of patients with AD. Several other investigators studied the distribution of B-APP in non-neural tissues. Immunoreactivity of B-APP was demonstrated in extracts of various cell type cultures,9 human muscle,3,19 salivary gland,3 and adenohypophysis.3 Joachim and colleagues8 demonstrated extraneural ABP deposition in the large intestine, skin and subcutaneous tissues of patients with AD, but did not mention whether they examined any additional tissues.

Our study did not include skin and subcutaneous tissue examination. Unlike Joachim and colleagues, positive staining in the large intestine was not observed by us. Positive ABP immunohistochemical reaction, however, was identified by us in other extraneural tissues, in three of 10 patients with AD. Although focal and sparse, it is our opinion that our findings are of potential importance. A likely cause for the sparsity of positive immunostaining as well as for the negative large intestine staining, can be attributed to the nature of our primary antiserum. It has been reported before8 that antisera raised against the synthetic ABP peptide (similar to the antibody used in this study), detected non-neural amyloid deposits much less sensitively than antisera raised against the native ABP peptide. It is therefore suggested that the use of antisera raised against the native ABP peptide, may be more efficient in detecting extracerebral ABP deposits.

Overfixation of autopsy samples in formalin is not an uncommon event and may result in antigenic crosslinking and masking of immunoreactive epitopes.2,5 The use of proteases has been shown13,17 to offer significant immunostaining improvement by unmasking "buried" epitopes of at least some antigens. It is worth noting that both of our positive kidney sections were predigested with pronase. The produced positive immune reaction was sharp, with no background staining and no evidence of tissue necrosis that could have resulted in a false positive reaction.

The occurrence of massive deposits of ABP in extracerebral tissues of patients with AD is unlikely. The subclinical deposition of small amounts of ABP remains, however, a distinct possibility. The findings of our study as well as those of Joachim and coworkers8 support this contention. Larger studies, employing methodologies refined to detect trace amounts of deposits of ABP, may be rewarding. Such efforts may provide additional support for the hypothesis that AD is a systemic disorder, and improve
our understanding of the pathogenesis of this disease. Additionally, the ability to detect ABP in extraneural sites may provide the means for tissue diagnosis of AD during life.

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References


