Diffuse Lewy body disease:
light and electron microscopic immunocytochemistry of senile plaques

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Summary. The nature of senile plaques (SP) in 27 cases of diffuse Lewy body disease (LBD) was investigated using immunocytochemistry and antibodies to beta amyloid protein synthetic peptides (BetaSP), ubiquitin (UBQ), paired helical filaments (PHF; Ab39) and a 68-kDa protein in Alzheimer brains (Alz50). Lewy bodies were present in widespread areas of the neocortex of all cases and were more easily detected with ubiquitin immunocytochemistry than with conventional stains. All cases had neocortical SP, but only six cases had neocortical neurofibrillary tangles (NFT). SP were very numerous in most cases and were usually "pale", "diffuse" or "very primitive" plaques with thioflavin S fluorescent microscopy. SP in diffuse LBD were immunostained with BetaSP. Several cases had extensive amyloid angiopathy that was also immunoreactive with BetaSP. SP in diffuse LBD were characterized by amyloid deposits with few or no neuritic elements that could be detected with thioflavin S, Bielschowsky's stain or double staining with BetaSP and Bodian's silver stain. They differed from plaques in Alzheimer's disease by lack of PHF-type neurites that could be stained with Ab39. In diffuse LBD, SP contained PHF-type neurites only in areas coexistent with NFT. Some SP had round, granular neurites that were immunoreactive with UBQ, but weakly argyrophilic with Bodian's stain and nonfluorescent with thioflavin S. Diffuse LBD lacked significant neuritic change in the neuropil that could be detected with UBQ, Ab39 and Alz50. The latter finding is a characteristic feature that distinguishes Alzheimer's disease from diffuse LBD.

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coexistent Alzheimer changes [32]; others have described demented individuals with diffuse LBD and minimal Alzheimer changes [23, 46, 56]. In most studies of diffuse LBD, including the cases previously reported by us, Alzheimer changes were assessed with conventional histochemical techniques. Using these methods, most cases had at least a few senile plaques (SP), but no neurofibrillary tangles (NFT) in the neocortex. Cases with both SP and neocortical NFT were the minority, possibly representing combined AD and diffuse LBD.

The purpose of this study was to determine the frequency of cortical LB in cases with brain stem LB and to characterize SP in diffuse LBD. Twenty-seven cases of diffuse LBD were studied with immunocytochemical methods and a battery of antibodies that react with paired helical filaments (PHF; Ab39) amyloid, a 68-kDa protein from Alzheimer brains or ubiquitin. The results are compared with normal elderly subjects and AD [8, 12]. Our findings suggest that SP in diffuse LBD are similar to SP in nondemented elderly individuals, but different from those in AD [12, 13]. Furthermore, diffuse LBD differs from AD by lack of PHF-type neuritic change in the gray matter.

Materials and methods

Study material included brains that were referred to our institution for evaluation of dementia and brains obtained from autopsies of prospectively evaluated individuals. Detailed longitudinal clinical data were available on 12 patients. Nine of the subjects were enrolled in the Teaching Nursing Home study, one subject was a member of the Bronx Aging Study, and one patient was a private patient of one of the authors (HAC).

In conjunction with diagnostic evaluation, sections from primary and association cortices (from two to six sections per case) and from the hippocampus were processed for paraffin embedding. Sections from the neocortex and hippocampus were stained with hematoxylin and eosin, thioflavin S, and a modified Bielschowsky stain. Under fluorescent illumination, thioflavin S-positive SP were counted in at least three contiguous fields at a ×10 magnification from the lateral sides of perpendicularly sectioned gyri of each section of cortex. The same procedure was used to count NFT, except at ×40. The average number of lesions was recorded for the available neocortical association areas of that case. Corresponding field sizes were used to count SP and NFT in the CA1 region of the hippocampus, and the average lesion count was recorded (see Table 1).

Seven-micrometer-thick hippocampal sections were cut and mounted on gelatin-coated glass slides for the 44 cases with brain stem LB. After deparaffinization, the sections were immunostained with either peroxidase-antiperoxidase (PAP) or avidin-biotin peroxidase (ABC) methods as previously reported [10]. Immunostaining with the antisera raised to a beta amyloid synthetic peptide (BetaSP) was performed on sections from all cases after pretreatment of sections with 90% formic acid, which has been shown to enhance detection of amyloid in tissue sections [30]. Some of the sections that had been previously immunostained with BetaSP were counterstained with Bodian’s silver method.

At least one section from each case, including the hippocampus and parahippocampal gyrus, was used in double-labeling immunocytochemistry. The deparaffinized sections were incubated for 20 min at room temperature with 5% nonfat milk containing 1% normal goat serum to block nonspecific antibody binding, followed by a cocktail composed of Ab39 (1:5) and UBQ (1:250) diluted in 5% nonfat milk. The sections were incubated for 16 h at 4°C. After extensive washing of the slides in phosphate-buffered saline (PBS), a cocktail containing biotinylated horse anti-mouse (1:200) and swine anti-rabbit (1:20) was applied for 30 min. The sections were then processed in sequential detection steps, using the ABC method and 3,3’-diaminobenzidine followed by rabbit PAP and 4-chloronaphthol.

Postmortem brain tissue that had been fixed in 10% phosphate-buffered formaldehyde from 12 cases of diffuse LBD was transferred to 30% sucrose before sectioning with the Vibratome. Forty-micrometer-thick Vibratome sections were cut from the cingulate gyrus at the level of the genu of the corpus callosum and from hippocampus at the level of the lateral geniculate nucleus. The sections were incubated in 5% nonfat milk in PBS before immunostaining to block nonspecific antibody binding. Sections incubated in the absence of the antibody or in the presence of irrelevant monoclonal antibodies were run as controls. Sections from normal brains without lesions and Alzheimer brains were used as negative and positive tissue controls, respectively. Free floating sections were stained using either PAP for rabbit antibodies or ABC for mouse monoclonal antibodies. The chromogen was 3,3’-diaminobenzidine.

In one case short postmortem interval autopsy tissue was available from an individual who was a participant in a prospective study of aging and dementia [7, 18] (case 1). In this case, in addition to frozen sections and formaldehyde-fixed tissue, tissue was also fixed in periodate-lysine-paraformaldehyde (PLP), 4% paraformaldehyde and Bouin’s fixative and sectioned with the Vibratome. Floating sections were processed for immunostaining by previously published methods [49–51].

Antibodies

Ab39. Ab39 is a mouse monoclonal IgG1 antibody that was raised to formalin-fixed Alzheimer NFT [54]. It recognizes PHF in AD [9], filaments in Pick bodies [9, 55], and straight filaments found in NFT of progressive supranuclear palsy [9], but does not recognize LB [9]. On Western blots of normal brain, it does not show significant binding to any normal protein, but it binds to proteins excluded from the stacking gels in SDS-polyacrylamide gel electrophoresis of Alzheimer brain homogenates [54].

Alz50. Alz50 is a mouse monoclonal IgM antibody that was raised to basal forebrain homogenates of AD. It was selected from hybridomas showing preferential immunoreactivity to Alzheimer brain compared to normals on enzyme-linked immunosorbant assay (ELISA) [50]. It immunostains a series of proteins in the 68-kDa range [49], and it also shows cross-reactivity with tau protein from human and animal brains [34]. It immunolabels NFT and neurons that may be vulnerable to NFT formation [26]. It also reacts with neurons in fetal Down’s syndrome brain [51]. Preliminary studies indicated that it reacts with SP in AD, but not in PD [49].

UBQ. UBQ is an affinity-purified rabbit antiserum raised to Keyhole limpet hemocyanin (KLH)-conjugated ubiquitin. It reacts with ubiquitin on Western blots and ELISA and shows no binding to denatured tau or neurofilament proteins on Western blots. Its characterization has been previously reported [36].