Abstract  To evaluate glial lesions in cases of dementia with Lewy bodies (DLB), we studied the brains of four patients with DLB. Astrocytic star-like inclusions, which resembled tufted astrocytic fibrillary tangles in shape, were found in the cortex of two of these cases. In addition, coiled bodies were found in the white matter of the cerebrum in two cases. The astrocytic star-like inclusions were immunohistochemically negative for tau protein, ubiquitin and \( \alpha \)-synuclein. The coiled bodies were immunohistochemically negative for tau protein but immunopositive for ubiquitin and \( \alpha \)-synuclein. These results suggest that in DLB a primary degenerative process takes place in both glial cells and neurons.

Key words  Dementia with Lewy bodies · Astrocytic star-like inclusions · Coiled bodies · Tau · \( \alpha \)-Synuclein

Introduction

Dementia with Lewy bodies (DLB) has been recognized as a clinical entity of primary degenerative dementia [14]. DLB is pathologically characterized by the emergence of Lewy bodies (LBs) in the neurites of cortical, subcortical, and brain stem structures. In DLB, ubiquitin-positive neurites in the CA2–3 sector of the hippocampus, and microvacuolation (spongy state) in the parahippocampus have been reported as specific pathological findings. However, studies on glial lesions in DLB have been limited [1] and the occurrence of astrocytic star-like inclusions (ASIs) in DLB has not been reported so far.

To our knowledge, this is the first report of tau-immunonegative ASIs being detected in the cerebral cortices of two patients with DLB. These ASIs resembled tufted astrocytic fibrillary tangles (AFTs) in shape. However, they differed from tufted AFTs in that they were immunonegative for tau protein. In this report, we examined glial lesions, including ASIs, in four patients with DLB.

Materials and methods

We analyzed the postmortem brains, obtained 2–8 h after death, of four patients with DLB. All of the cases had been pathologically diagnosed as sporadic DLB, based on the pathological criteria for that disease [14]. The characteristics of the cases are listed in Table 1. The clinical characteristics and histopathological findings of our cases have been described previously [10, 11, 12], including a preliminary report of one case [8].

Histology

The brains were formalin-fixed, and 6-\( \mu \)m-thick paraffin-embedded sections were prepared from a number of different brain regions. Bodian’s stain, Bielschowsky’s stain, methenamine silver stain and, after pretreatment with 0.3% KMnO\(_4\), Gallyas-Braak’s (GB) stain were employed in addition to routine histochemical stains.

Immunohistochemical assays

Some of the sections were subjected to immunohistochemical examinations. The following primary antibodies and dilutions were used: mouse monoclonal antibody against phosphorylated tau (AT-8; Innogenetics; 1:500), mouse monoclonal antibody against tau, phosphorylation independent (T46; Zymed; 1:200), rabbit polyclonal antibody against \( \alpha \)-synuclein (Chemicon; 1:1,000), rabbit polyclonal antibody against ubiquitin (Chemicon; 1:200), and rabbit polyclonal antibody against glial fibrillary acidic protein (GFAP; DAKO; 1:200).

After incubation with one or other of the primary antibodies, sections were treated with biotinylated secondary antibodies. Sections were subsequently treated with an avidin-biotin complex (ABC; Vector Laboratories, diluted 1:100) and then visualized with 0.01% diaminobenzidine tetrahydrochloride (DAB).
### Results

Distributions of LBs and Alzheimer-type pathology

In all four cases, LBs were present in cortical, subcortical, and brain stem structures. Numerous LBs were observed in neurons, especially in the insula, cingulate gyri and temporal lobes of the cerebrum. The neurons with LBs were small and found mainly in the deep layers of the cortices. In some cases, many LBs were found in the frontal lobes and parietal lobes, but very few were identified in the occipital lobes.

In cases 1 and 2, very few neurofibrillary tangles (NFTs) were found in the transentorhinal cortex, and no senile plaques were observed. Therefore, cases 1 and 2 were classified as pure diffuse Lewy body disease (DLBD) [9]. On the other hand, in the neocortices of cases 3 and 4, the burden of senile plaques was similar to that observed in an age-matched Alzheimer’s disease (AD) patient. In case 3, NFTs were confined to the temporal lobe, and in case 4 the burden of neocortical NFTs was comparable to that usually observed in AD. Therefore, cases 3 and 4 comprise the common form of DLBD [9]. The characteristics of the cases are listed in Table 2.

### Astrocytic star-like inclusions

In case 1, which lacked Alzheimer-type pathology (Alz-P), and in case 3, in which Alz-P was observed, glial star-like inclusions (GSIs) were recognized in the cerebral cortices in sections stained with the GB stain (Fig. 1a, 1c).

The GSIs were composed of thin, argyrophilic radiating fibrils (Fig. 1a), and had large and lightly stained nuclei in the central area. Paired nuclei were also often observed (Fig. 1c). The nuclei were accompanied by little cytoplasm, and it is likely, therefore, that they were glial, and not neuronal in nature.

Double labelling using the anti-GFAP antibody and the GB-hematoxylin and eosin (H & E) method revealed that the central nuclei were astrocytic (Fig. 1b). Therefore, the GSIs observed were considered to be ASIs. GB-stained sections showed that these ASIs resembled tufted AFTs in shape.

GP-staining revealed ASIs very clearly, although they were also partially observed following methenamine silver staining. They were not detected following Bodian or Bielschowsky silver staining. The ASIs were immunonegative for the anti-ubiquitin, anti-α-synuclein, and anti-tau antibodies (including AT-8, tau46).

The distribution of ASIs in the cortex was most dense in the temporal lobe, including the entorhinal and transentorhinal cortices. The amygdaloid complex, insula, cingulate gyri and fronto-orbital cortices were also sites of predilection for the formation of ASIs. However, very few ASIs were found in the hippocampal formation, and none were found in the basal ganglia, thalamus and brain stem. Neocortical involvement was limited to the temporal and frontal cortices.

ASIs were distributed broadly in the cortical layers and were more abundant in the deep layers than in the superficial layers, but they were seldom found in the white matter.

### Coiled bodies

Silver staining by the GB method revealed circular or arc-shaped argyrophilic inclusions (coiled bodies; CBs) around the small nuclei in cells of the midbrain in all cases, but the densities of CBs were quite different in each case. In case 1, which lacked Alz-P, and case 4, which exhibited Alz-P, GB staining also revealed the presence of CBs in the white matter of the temporal lobe (Fig. 1d, 1e).

The nuclei that were surrounded by CBs were small and there was little or no cytoplasm (Fig. 1d, 1e). Double labelling using the anti-GFAP antibody and GB-H & E method failed to show any association of CBs with GFAP-positive astrocytes (Fig. 1d).

CBs in the midbrain of all four cases and the temporal lobe of case 1 were immunonegative for tau protein, but

<table>
<thead>
<tr>
<th>Case</th>
<th>BW (g)</th>
<th>Pathological diagnosis</th>
<th>Alz-P</th>
<th>ASIs</th>
<th>CBs in midbrain</th>
<th>CBs in temporal lobe</th>
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<td>+</td>
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<td>–</td>
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**Table 1** Clinical characteristics (SDAT senile dementia of Alzheimer type)

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<th>Case</th>
<th>Sex</th>
<th>Age at death (years)</th>
<th>Age at onset (years)</th>
<th>Duration (years)</th>
<th>Symptom at onset</th>
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<td>63</td>
<td>5</td>
<td>Forgetfulness</td>
<td>Presenile dementia</td>
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<tr>
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<td>67</td>
<td>2</td>
<td>Forgetfulness</td>
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<tr>
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<td>55</td>
<td>6</td>
<td>Forgetfulness</td>
<td>Alzheimer’s disease</td>
</tr>
</tbody>
</table>

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**Table 2** Pathological characteristics (BW brain weight, Alz-P Alzheimer-type pathology, ASIs astrocytic star-like inclusions, CBs coiled bodies, DLBD diffuse Lewy body disease)