It has been established that amyloid β (Aβ) deposits in the brain, as senile plaques (SPs) and cerebral amyloid angiopathies (CAAs), are histopathological hallmarks of Alzheimer’s disease (AD) (Mandybur, 1975; Griffiths et al., 1982; Miyakawa et al., 1982; Vinters et al., 1996; Jellinger, 2002), and that CAA alone may give rise to dementia in the elderly without AD pathology (Cohen et al., 1997; Yamada et al., 1997; Vinters et al., 1996; Jellinger, 2002), and that CAA alone may give rise to dementia in the elderly without AD pathology. However, other form of Aβ deposition, perivascular plaques (PPs) have not yet been studied thoroughly. PPs were first described by Uematsu (1923) as perivascular form of SPs and then by Scholz (1938) as *drusige Entartung* (in German). Morel and Wildi (1952) used the term dyshoric angiopathy which meant a breakdown of the blood-brain barrier. Previous researchers thought that amyloid in PPs extended from the vasi...
cular wall of CAAs to the surrounding parenchyma in the cerebral cortex (Neumann, 1960; Mandybur, 1986; Plant et al., 1990). Some recent studies have raised the hypothesis that Aβ is eliminated along perivascular interstitial fluid drainage pathways of the brain and progressively accumulates to form PPs, and further contributes to CAAs (Weller et al., 1998, 2000; Kalaria, 2002; Kumar-Singh, 2002; Yow and Weller, 2002). However, immunohistochemical studies focusing on PPs have not ever been reported. For the purpose of clarifying the neuropathological significance of PPs, we examined the brains of patients with AD and CAA by immunohistochemistry, and investigated the relationship of PPs to SPs and CAAs.

**Subjects and Methods**

Brain tissues were obtained from 7 patients with AD, 3 patients with CAA with dementia (CAA-D) and 1 patient with CAA with massive cerebral hemorrhage (CAA-CH). All patients were brought to autopsy and neuropathological examination in the Department of Neuropathology, Institute of Neurological Sciences, Tottori University Faculty of Medicine, Japan. All tissue specimens were fixed in 10% formalin for 2 weeks, embedded in paraffin and cut into 6-μm-thick sections. Routine neuropathological examinations were carried out with hematoxylin and eosin, Klüver-Barrera, Bielschowsky, Gallyas-Braak and Holzer stains. Summary of clinical and routine neuropathological features are shown in Table 1.

For immunohistochemistry in the present study, samples were selected from the occipital and temporal lobes including the Ammon’s horn and subiculum in Bielschowsky stain, and were rated as follows: +, 1–10; ++, 11–50 and ++++, ≥ 51. Quantitative analysis of SPs was performed based on the number of mature and diffuse plaques in the temporal cortices in Aβ42 and Aβ40 immunostainings in 10 nonselected × 100 fields. The abundance of SPs was rated as follows: +, 1–10; ++, 11–50 and ++++, ≥ 51. The rating of CAA severity was made based on the number of pan Aβ-positive vessels in the occipital cortices with 10 nonselected × 40 fields: +, 1–5 positive vessels; ++, 6–10 positive vessels and ++++, ≥ 11 positive vessels and at least 1 vessel showing complete replacement of the media.
with Aβ. The rating of PPs severity was determined as follows: +, 1–3; ++, 4–6 and +++; ≥7 in 10 non-selected ×40 fields in the occipital cortices.

Results

Immunohistochemistry of PPs

PPs were found in all 11 patients. In AD, they were small in number compared with SPs, but were numerous in CAA-D and CAA-CH in which mature plaques were absent and only a small number of diffuse plaques were found in 3 of the 4 patients (Table 2).

PPs were always immunostained for both Aβ42 and Aβ40, but their number and positive-staining areas were always greater in Aβ42 staining (Table 2, Figs. 1A and B), suggesting earlier deposition of Aβ42 than Aβ40 similar to mature plaques. All PPs were always associated with varying degrees of neuritic degeneration evidenced by AT8 immunostaining (Fig. 1C) as well as Gallyas-Braak and Bielskowsky stains and with GFAP-positive cells and fibers within or around them as well (Fig. 1D). These features are again similar to mature plaques. PPs were ApoE4-positive in AD patients 1 and 2 and CAA-D patient 1, and in these 3 patients SPs and CAAs were also positive for ApoE4 (Fig. 1E). Thus, PPs resembled mature plaques in that they were always positive for both Aβ42 and Aβ40, and that they were always associated with degenerated neurites and GFAP-positive cells and fibers. Their incidence was not proportional to that of mature and diffuse plaques but proportional to CAAs (Table 2). Although they were more frequent around varying degrees of CAAs (Figs. 1A, B and F), they were also found around non-CAA vessels (Fig. 1G), suggesting that PPs were formed earlier than CAAs.

Immunohistochemistry of CAAs

Different numbers of CAAs were found in all 7 AD patients: moderate in 3 and mild in 4 patients. They were marked in 3 CAA-D patients and moderate in 1 CAA-CH patient (Table 1). There was a good correlation in the severity of CAAs between the leptomingeal and parenchymal blood vessels. CAA-

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Table 1. Summary of clinical and pathological features of 11 cases examined

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at death (year)</th>
<th>Gender</th>
<th>Duration of illness (year)</th>
<th>Brain weight (g)</th>
<th>NFT</th>
<th>SP</th>
<th>CAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alzheimer’s disease</td>
<td>1</td>
<td>57</td>
<td>F</td>
<td>5</td>
<td>—</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>67</td>
<td>F</td>
<td>7</td>
<td>—</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>75</td>
<td>F</td>
<td>2</td>
<td>1180</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>56</td>
<td>F</td>
<td>4</td>
<td>—</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>89</td>
<td>F</td>
<td>6</td>
<td>1060</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>83</td>
<td>M</td>
<td>4</td>
<td>1400</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>79</td>
<td>F</td>
<td>8</td>
<td>860</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>CAA with dementia (D)</td>
<td>1</td>
<td>76</td>
<td>M</td>
<td>5</td>
<td>1205</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>2</td>
<td>68</td>
<td>M</td>
<td>16</td>
<td>1360</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>62</td>
<td>F</td>
<td>7</td>
<td>1360</td>
<td>+</td>
<td>+</td>
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<tr>
<td>CAA with massive cerebral hemorrhage (CH)</td>
<td>1</td>
<td>75</td>
<td>F</td>
<td>0.25</td>
<td>860</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

CAA, cerebral amyloid angiopathy; F, female; M, male; NFT, neurofibrillary tangle; SP, senile plaque; —, not weighed.

*NFT: +, 1–10; ++, 11–50; +++; ≥51 in 5 × 100 fields in the Ammon’s horn and subiculum.

SP: +, 1–10; ++, 11–50; +++; ≥51 in 10 × 100 fields in the temporal cortices.

CAA: +, 1–5; ++, 6–10; +++; ≥11 in 10 × 40 fields in the occipital cortex.
Table 2. Immunohistochemical features of senile plaque (SP), cerebral amyloid angiopathy (CAA) and perivascular plaque (PP)

<table>
<thead>
<tr>
<th>Patient</th>
<th>SP</th>
<th>Diffuse plaque</th>
<th>CAA</th>
<th>PP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mature plaque</td>
<td>Diffuse plaque</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aβ42+</td>
<td>Aβ40+</td>
<td>AT8</td>
<td>Aβ42+</td>
</tr>
<tr>
<td>Alzheimer’s disease 1</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>Alzheimer’s disease 2</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>Alzheimer’s disease 3</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>Alzheimer’s disease 4</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>Alzheimer’s disease 5</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>Alzheimer’s disease 6</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>Alzheimer’s disease 7</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>CAA with dementia (D) 1</td>
<td>–</td>
<td>–</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>CAA with dementia (D) 2</td>
<td>–</td>
<td>–</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>CAA with dementia (D) 3</td>
<td>–</td>
<td>–</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>CAA with massive cerebral hemorrhage (CH)</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

* SP: +, 1–10; ++, 11–50; +++ ≥51 in 10 × 100 fields in the temporal cortices.

CAAs were always labeled with both Aβ42 and Aβ40 (Table 2). Their staining intensity and positive-staining areas were, however, greater with Aβ40 in larger cortical and leptomeningeal arteries (Figs. 1A and B; Figs. 2A and B), but were greater with Aβ42 in smaller cortical arteries (Figs. 2A and B; arrows). In the same vessel wall, the 2 Aβ species were sometimes detected in different areas. In larger leptomeningeal arteries, early small deposits of Aβ42 were always observed at the media adjacent to the adventitia (Fig. 2A) or sometimes at the associated vasculopathies such as double barreling and clusters of multiple arteriolar lumina were seen in the 3 CAA-D patients.

CAAs were always labeled with both Aβ42 and Aβ40 (Table 2). Their staining intensity and positive-staining areas were, however, greater with Aβ40 in larger cortical and leptomeningeal arteries (Figs. 1A and B; Figs. 2A and B), but were greater with Aβ42 in smaller cortical arteries (Figs. 2A and B; arrows). In the same vessel wall, the 2 Aβ species were sometimes detected in different areas. In larger leptomeningeal arteries, early small deposits of Aβ42 were always observed at the media adjacent to the adventitia (Fig. 2A) or sometimes at the

Figs. 1A–G (p. 13). Immunohistochemistry of perivascular plaques (PPs).

A: Immunostaining for amyloid β42 (Aβ42), showing positive PPs. The occipital cortex in Alzheimer’s disease (AD) (Patient 2).

B: An adjacent section to A immunostained with Aβ40. Positive areas are smaller in the PPs but larger in the artery compared with A.

C: An adjacent section to A immunostained with AT8, showing degenerated neurites within or around the PPs.

D: GFAP-immunostaining of another serial section, showing numerous positive cells and fibers within and around the PPs.

E: A serial section of A immunostained with ApoE4. The staining pattern of the PPs is similar to B but the artery is intensely positive.

F: Immunostaining for Aβ42, showing positive PPs and artery. The occipital cortex of AD (Patient 7).

G: Immunostaining for pan Aβ (Aβ42 and Aβ40), showing PP around non-CAA artery.

A–G: original magnification, ×100. AT8, anti-human phosphorylated tau; ApoE4, apolipoprotein E4; CAA, cerebral amyloid angiopathy; GFAP, glial fibrillary acidic protein.
Immunohistochemistry of perivascular plaque

Figs. 1A–G
Fig. 2A–D. Immunohistochemistry of cerebral amyloid angiopathies (CAAs).

A: Immunostaining for amyloid β42 (Aβ42). In larger meningeal arteries, small positive areas are limited to the media adjacent to the adventitia but smaller cortical arteries are intensely stained in their whole walls (arrows). The occipital cortex in Alzheimer’s disease (AD) (Patient 2).

B: An adjacent section immunostained with Aβ40. Positive areas are larger than Aβ42 but smaller cortical arteries are negative (arrows). A, B: original magnification, × 40.

C: Immunostaining for Aβ40 of an artery in the occipital cortex, showing complete replacement of the wall by Aβ40. The occipital cortex from AD (Patient 3).

D: An adjacent section to C, showing complete absence of α-smooth muscle actin immunoreactivity in the media. C, D: original magnification, × 100.

adventitia as well. As the amount of amyloid deposition increased, the deposits extended more to part of the media and eventually to the whole vessel wall, which were clearly demonstrated as absence of smooth muscle actin immunoreactivity in the media (Figs. 2C and D). Early small amyloid deposits were never observed in the vicinity of the endothelium or the internal elastic lamina.

The relationship of PPs to SPs and CAAs

In AD patients, there were many mature plaques not only in the neuropil but also around blood vessels or adjacent to PPs, but diffuse plaques were not associated with blood vessels. There was no correlation between the number of PPs and that of SPs, but a good correlation was noted between the number of PPs and CAAs in AD and CAA patients (Table 2).
Discussion

Uematsu (1923) first described PPs as a perivascular form of SPs. Thereafter Scholz (1938) described PPs as *drusige Entartung* in German, SP-like angio-pathy in English. He reported the small argyrophilic material resembling SPs initially deposits at the outer part of the media of the cortical arteries, extends to the adventitia and then eventually to the perivascular brain tissues. After Scholz, there have been few studies focusing on PPs and the neuropathological significance of PPs has not yet been elucidated.

In this immunohistochemical study, we found PPs were also a common form of $\alpha\beta$ deposits like SPs and CAAs in AD and CAA patients. They were always immunostained with $\alpha\beta_{42}$ and $\alpha\beta_{40}$, but their positive-staining areas were always larger in $\alpha\beta_{42}$ staining. In addition, they were always associated with AT8-positive, degenerated neurites and also with GFAP-positive astrocytes and astrocytic fibers within and around them. All of these immunohistochemical features are the same as mature SPs, indicating that PPs are another form of mature plaque.

PPs were much more frequent in CAA, particularly in CAA-D in which CAAs were remarkably numerous but the number of both NFTs and SPs was within range of physiological aging. Furthermore, our semiquantitative analysis revealed no correlation between the number of PPs and that of SPs but a good correlation between the number of PPs and that of CAAs. These findings suggest that the formation of PPs precedes the development of CAAs. Second, the main component of $\alpha\beta$ in PPs was $\alpha\beta_{42}$, and the early $\alpha\beta$ deposits in the vascular wall was also $\alpha\beta_{42}$, suggesting that $\alpha\beta$ in the vascular wall came from PPs, which was subsequently followed by $\alpha\beta_{40}$. Yamaguchi et al. (1992) also reported the same results in an immunoelectron microscopic study. In addition, Frautschy et al. (1992) also supported the hypothesis from their observations in animal experiments where direct injection into rat brains of isolated amyloid plaque cores had migrated to vessel walls and ventricular linings, implying that the distribution of injected amyloid is not necessarily comparable to the initial site of its deposition. Our 2 findings, together with other previous reports, were consistent with the $\alpha\beta$ deposit pathway described by Weller et al. (1998, 2000).

In summary, the present study demonstrated some immunohistochemical characteristics of PPs: they are immunopositive for $\alpha\beta_{42}$ and $\alpha\beta_{40}$, predominant for $\alpha\beta_{42}$, and always associated with degenerated neurites and reactive astrocytosis. These findings suggest that PPs were another form of mature plaque and that they may contribute to the development of dementia, particularly in CAA-D, and to the formation of CAAs.

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