Yonago Acta medica 2003;46:9–16

An Immunohistochemical Study of Perivascular Plaque in Alzheimer's Disease and Cerebral Amyloid Angiopathy

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Immunohistochemical study of perivascular plaques (PPs) in 7 patients with Alzheimer's disease (AD) and 4 with cerebral amyloid angiopathy (CAA)(CAA with dementia in 3 patients and CAA with massive cerebral hemorrhage in 1 patient) demonstrated that PPs were also a common form of amyloid β (Aβ) deposits, like SPs and CAAs, in both AD and CAA, and that they had similar immunostaining characteristics to mature senile plaques (SPs), manifesting as always positive for both Aβ42 and Aβ40 but predominant for Aβ42. In addition, PPs were always associated with AT8-positive degenerated neurites and GFAP-positive astrocytes and fibers. These findings suggest that PPs as mature plaques contribute to the development of dementia, especially in CAA with dementia which lack AD pathology. Moreover, 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, suggesting that there was a close relationship of the formation of PPs and the development of CAAs. PPs were also found around non-CAA arteries, although they were frequent around varying degrees of CAAs, suggesting that the initial Aβ42 deposits in PPs contribute to the development of CAAs.

Key words: Alzheimer's disease; cerebral amyloid angiopathy; immunohistochemistry; perivascular plaque; senile plaque

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; Vidal et al., 2000; Kalaria, 2002). In the pathological investigations of AD and CAA, a great number of studies have focused on SPs and CAAs (Alonzo et al., 1998; Iwatsubo et al., 1994, 1995), and elucidated that Aβ42 deposition is predominant in SPs and Aβ40 in CAAs. However, another 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 vas-

Abbreviations: Aβ, amyloid β protein; ABC, avidin-biotin-peroxidase complex; AD, Alzheimer's disease; ApoE, apolipoprotein E; CAA, cerebral amyloid angiopathy; CAA-CH, CAA with massive cerebral hemorrhage; CAA-D, CAA with dementia; GFAP, glial fibrillary acidic protein; NFT, neurofibrillary tangle; PBS, phosphate-buffered saline; PP, perivascular plaque; SP, senile plaque

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, Bielshowsky, 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 serial sections were made. The sections were immunostained with the following primary antibodies: anti-pan Aβ (Aβ-1) (polyclonal, Wako, Osaka, Japan; dilution 1:1000); anti-Aβ42 (BC05) and -Aβ40 (BA27) (monoclonal, Wako; Aβ Immunohistostain Kit); anti-human apolipoprotein E4 (ApoE4) (5B5) (monoclonal, IBL, Fujioka, Japan; dilution 1:10); anti-human smooth muscle actin (1A4) (monoclonal, DAKO A/S, Copenhagen, Denmark; dilution 1:50); anti-glial fibrillary acidic protein (GFAP) (polyclonal, DAKO A/S; dilution 1:1000); and anti-human phosphorylated tau (AT8) (monoclonal, Innogenetics, NV, Ghent, Belgium; dilution 1:1000). Immunostainings were performed by the avidin-biotin-peroxidase complex (ABC) method. Sections mounted on poly-L-lysine-coated glass slides were deparaffinized, rehydrated in a graded ethanol series, washed in distilled water for 10 min, and treated with 3% hydrogen peroxide diluted in distilled water for 30 min to block endogenous peroxidase activity in the tissue. After washing in 0.01 M phosphate-buffered saline (PBS) pH 7.4 for 10 min, the sections were resubjected to blocking with 10% normal serum (Nichirei, Tokyo, Japan) for 30 min to avoid nonspecific binding of secondary antibodies. The sections were then incubated overnight with the primary antibodies in a moist chamber at 4˚C. After washing 3 times with PBS (5 min each) the sections were incubated with appropriate secondary antibodies for 60 min at room temperature, treated with the ABC reagent for 60 min, exposed to 0.5% 3,3'-diaminobenzidine-0.005% hydrogen peroxide. For enhancement of Aβ and ApoE4 immunostaining, deparaffinized tissue sections were pretreated with 96% fomic acid for 5 min. The sections were heated up to 110˚C for 10 min to improve ApoE4 immunostaining. Finally, sections were counterstained with hematoxylin.

Neurofibrillary tangles (NFTs) were counted in 5 nonselected × 100 fields in 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

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

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

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 $10 \times$ 40 fields in the occipital cortex.

with Aβ. The rating of PPs severity was determined as follows: +, 1–3; ++, 4–6 and +++, ≥ 7 in 10 non-selected $\times$ 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 Bielshowsky 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 leptomengeal and parenchymal blood vessels. CAA-

Table 2. Immunohistochemical features of senile plaque (SP), cerebral amyloid angiopathy (CAA) and perivascular plaque (PP)

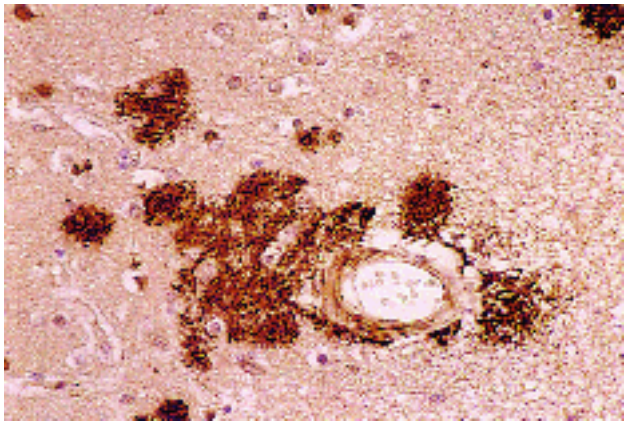
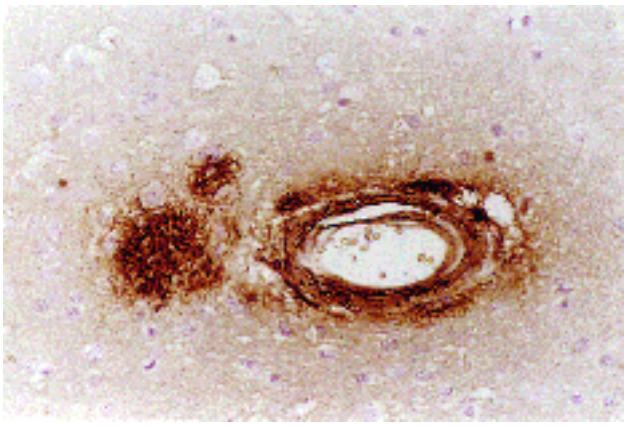
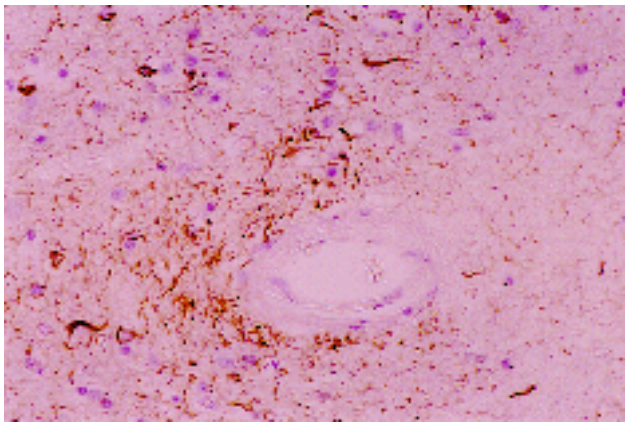
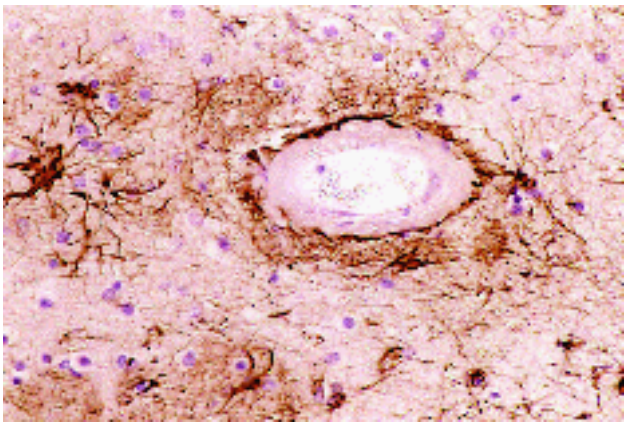
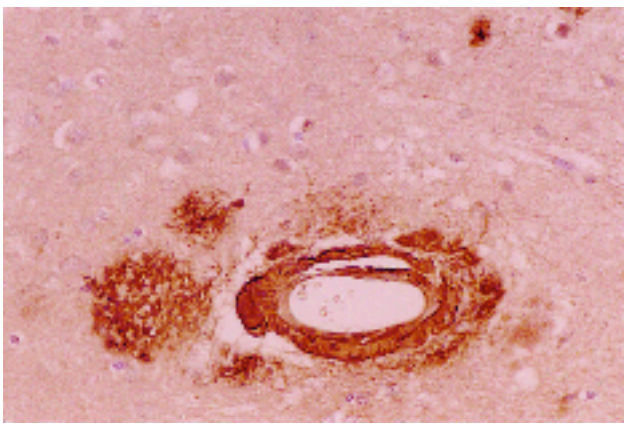
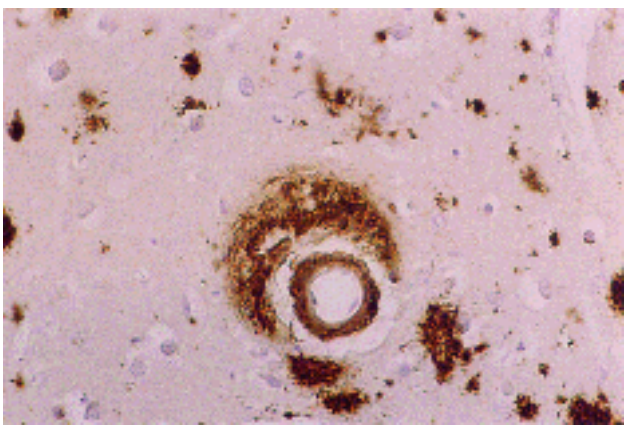
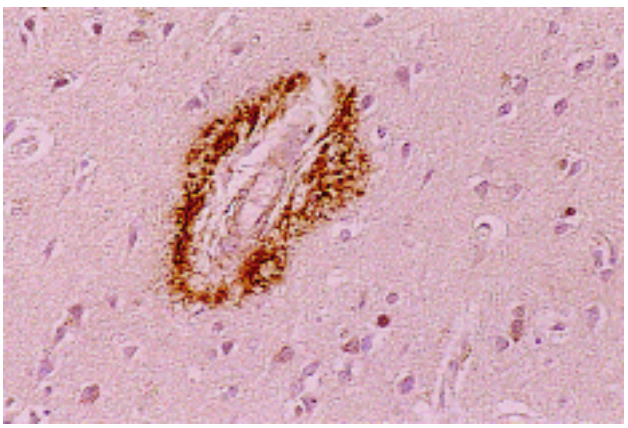
		Rating*								
		SP								
		Mature plaque		Diffuse plaque						
		Aβ42+		Aβ42+		CAA		PP		
Patient		Aβ40+	AT8	Aβ40–	AT8	Aβ42	Aβ40	Aβ42	Aβ40	AT8
Alzheimer's disease	1	+++	+	++	–	+	+	+	+	+
	2	+++	+	+++	–	++	++	++	+	+
	3	+++	+	+++	–	++	++	++	+	+
	4	++	+	+++	–	+	+	+	+	+
	5	++	+	+++	–	+	+	+	+	+
	6	+	+	+++	–	+	+	+	+	+
	7	++	+	+++	–	++	++	++	++	+
CAA with dementia (D)	1	–	–	++	–	+++	+++	+++	+++	+
	2	–	–	+	–	+++	+++	+++	+++	+
	3	–	–	+	–	+++	+++	+++	+++	+
CAA with massive cerebral hemorrhage (CH)										
	1	–	–	+	–	++	++	++	++	+

Aβ40, amyloid β40 protein; Aβ42, amyloid β42 protein; AT8, anti-human phosphorylate α tau.
* 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 cortices.
PP: +, 1– 3; ++, 4– 6; +++, ≥7 in 10 × 40 fields in the occipital cortices.
AT8: +, positive; –, negative.

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.

A	B
C	D
E	F
G	

Figs. 1A–G