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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)

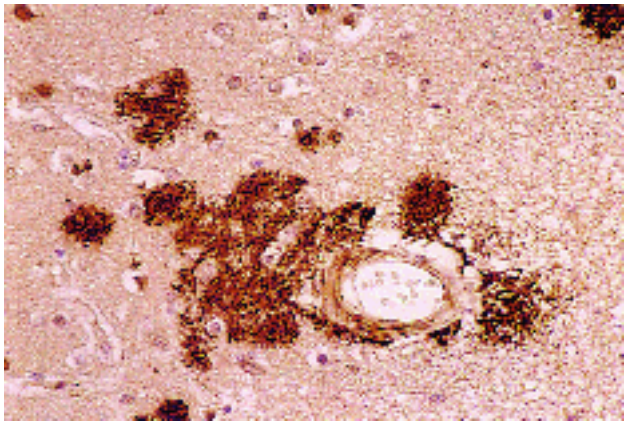
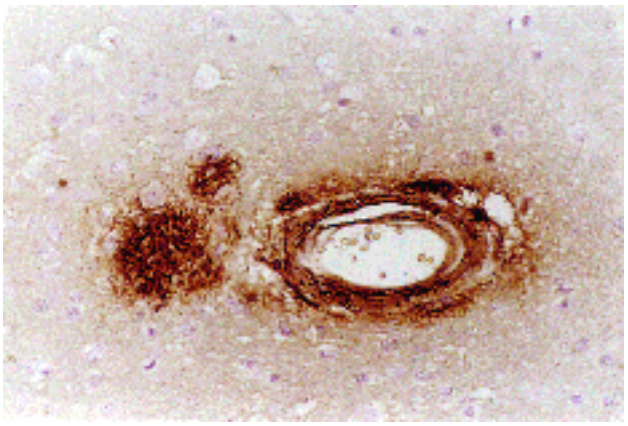
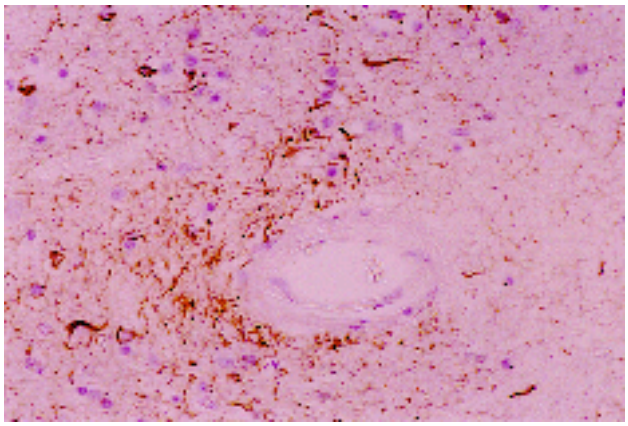
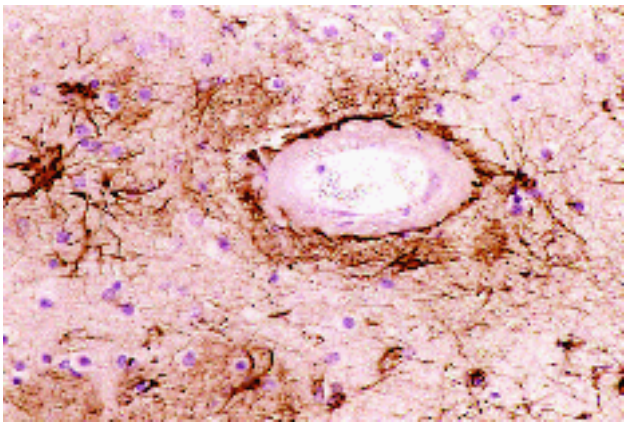
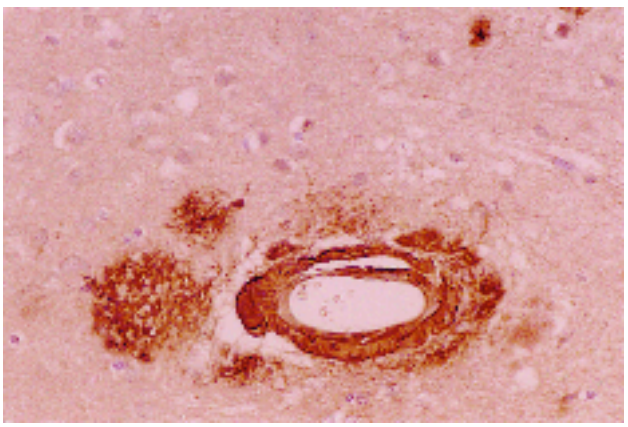
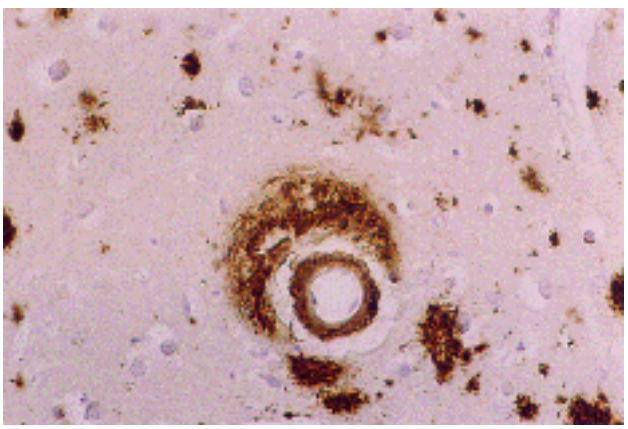
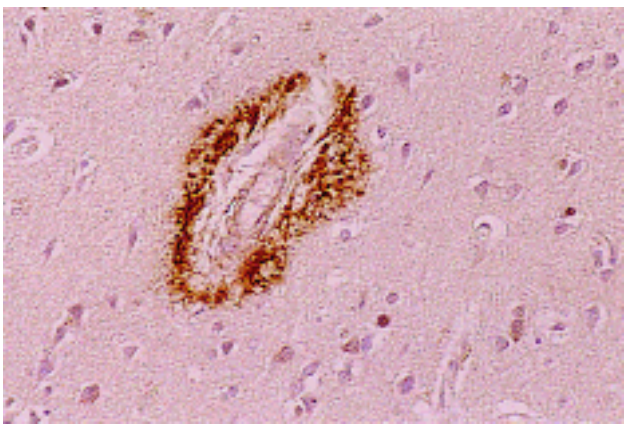
		Rating*								
		SP								
		Mature plaque		Diffuse plaque						
		Aβ42+		Aβ42+		CAA		PP		
Patient		Aβ40+	AT8	Aβ40–	AT8	Aβ42	Aβ40	Aβ42	Aβ40	AT8
Alzheimer's disease	1	+++	+	++	–	+	+	+	+	+
	2	+++	+	+++	–	++	++	++	+	+
	3	+++	+	+++	–	++	++	++	+	+
	4	++	+	+++	–	+	+	+	+	+
	5	++	+	+++	–	+	+	+	+	+
	6	+	+	+++	–	+	+	+	+	+
	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

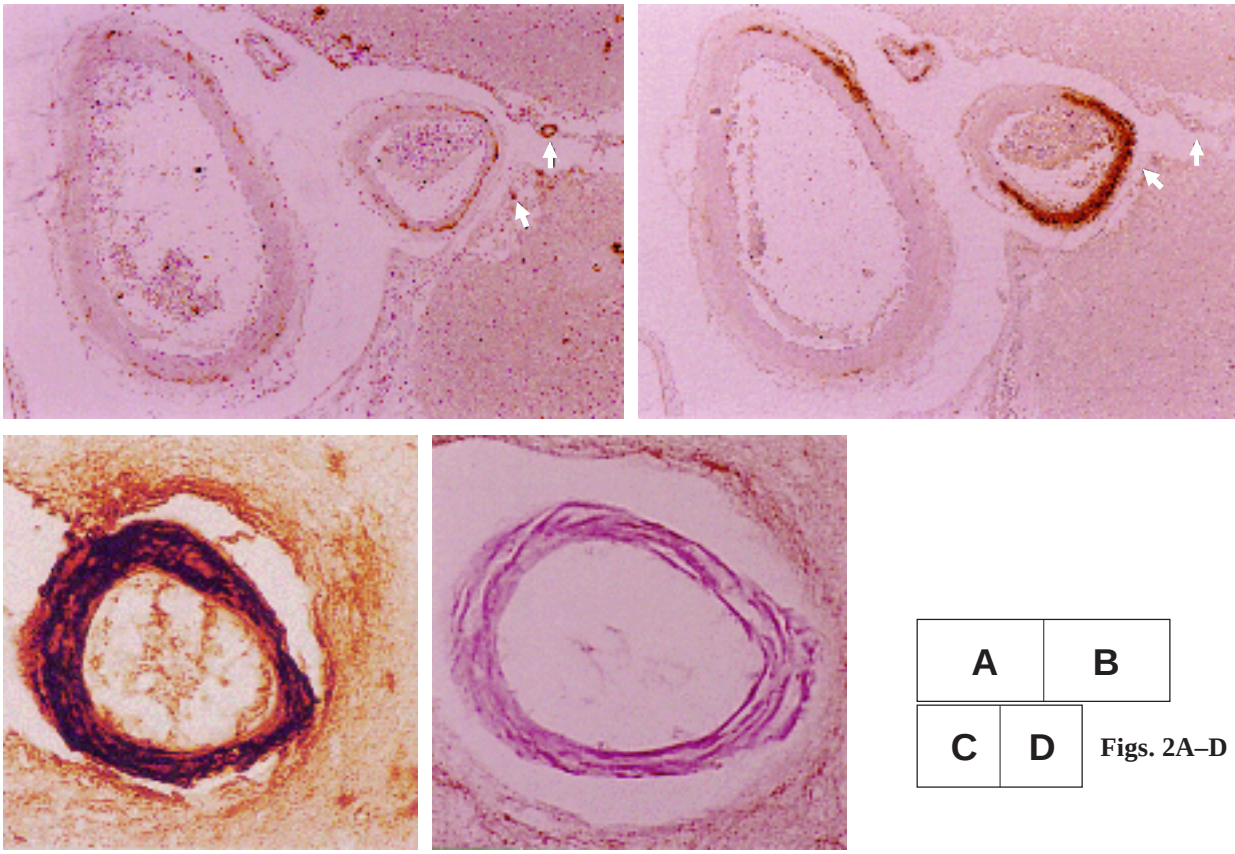


Fig. 2A–D. Immunohistochemistry of cerebral amyloid angiopathies (CAAs).
A: Immunostaining for amyloid β42 (Aβ42). In larger meningeal arteries, small positive areas are limited to the media adjacent to the adventitia but smaller cortical arteries are intensely stained in their whole walls (arrows). 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 abscence 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 angiopathy 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 Aβ deposits like SPs and CAAs in AD and CAA patients. They were always immunostained with Aβ42 and Aβ40, but their positive-staining areas were always larger in Aβ42 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 PPs may play an important role in the pathogenesis of dementia, especially in CAA-D, and that there is a close relationship between the formation of PPs and the development of CAAs.

Neither the exact origin of Aβ in PPs and CAAs nor the mechanism by which it is deposited has as yet been resolved. Mandypur (1975) described that amyloid appeared in the neuropil along arterioles or in the Virchow-Robin spaces in the cerebral cortex, but the vessel wall itself was not necessarily involved. Some recent studies raised the hypothesis that Aβ was deposited initially in periarterial interstitial fluid drainage pathways of the cerebral cortex and contributed to CAAs in AD (Weller et al., 1998, 2000; Yow et al., 2002). Our results support this hypothesis from 2 aspects. First, PPs were also found around non-CAA arteries in AD and CAA patients, although they were frequently seen around varying degrees of CAAs. This finding suggests that the formation of PPs precedes the development of CAAs. Second, the main component of Aβ in PPs was Aβ42, and the early Aβ deposits in the vascular wall was also Aβ42, suggesting that Aβ in the vascular wall came from PPs, which was subsequently followed by Aβ40. 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 Aβ deposit pathway described by Weller et al. (1998, 2000).

In summary, the present study demonstrated some immunohistochemical characteristics of PPs: they are immunopositive for Aβ42 and Aβ40, predominant for Aβ42, 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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References

1 Alonzo NC, Hyman BT, Rebeck GW, Greenberge SM. Progression of cerebral amyloid angiopathy: accumulation of amyloid β 40 in affected vessels. J Neuropathol Exp Neurol 1998;57:353–359.

2 Cohen DL, Hedera P, Premkumar DRD, Friedland RP, Kalaria RN. Amyloid-β protein angiopathies masquerading as Alzheimer's disease? Ann NY Acad sci 1997;826:390–395.

3 Frautschy SA, Cole GM, Baird A. Phagocytosis and deposition of vascular β-amyloid in rat brains injected with Alzheimer β-amyloid. Am J Pathol 1992; 140:1389–1399.

4 Griffiths RA, Martimer TF, Oppenheimer DR, Spalding JMK. Congophilic angiopathy of the brain: a clinical and pathological report on two siblings. J Neurol Neurosurg Psychiatry 1982;45:396–408.

5 Iwatsubo T, Odaka A, Suzuki N, Mizusawa H, Nukina N, Ihara Y. Visulization of Aβ42 (43) and Aβ40 in senile plaques with end-specific Aβ monoclonals: evidence that an initially deposited species is Aβ42 (43). Neuron 1994;13:45–53.

6 Iwatsubo T, Mann DMA, Odaka A, MSc, Suzuki N, Ihara Y. Amyloid β protein (Aβ) deposition: Aβ42 (43) precedes Aβ40 in Down syndrome. Ann Neurol 1995;37:294–299.

7 Jellinger KA. Alzheimer disease and cerebrovascular pathology: an update. J Neural Transm 2002; 109:813–836.

8 Kalaria RN. Small vessel disease and Alzheimere's dementia: pathological considerations. Cerebrovasc Dis 2002;13 Suppl 2:48–52.

9 Kumar-Singh S, Cras P, Wang R, Kros JM, Swieten JV, Lubke U, et al. Dense-core senile plaques in the Flemish Variant of Alzheimer's disease are vasocentric. Am J Pathol 2002;161:507–520.

10 Mandybur TI. The incidence of cerebral amyloid angiopathy in Alzheimer's disease. Neurology 1975;25:120–126.

11 Mandybur TI. Cerebral amyloid angiopathy: the vascular pathology and complications. J Neuropathol Exp Neurol 1986;45:79–90.

12 Miyakawa T, Shimoji A, Kuramoto R, Higuchi Y. The relationship between senile plaques and cerebral blood vessels in Alzheimer's disease and senile dementia. Virchows Arch (Cell Pathol) 1982;40:121–129.

13 Morel F, Wildi E. General and cellular pathochemistry of senile and presenile alterations of the brain. In: Proceedings of the First International Congress of Neuropathology, vol 2. Torino: Rosenberg & Sellier; 1952. p. 347–374.

14 Neumann MA. Combined amyloid vascular changes and argyrophilic plaques in the central Nervous system. J Neuropathol Exp Neurol 1960;19:370–382.

15 Plant GT, Révész T, Barnard RO, Harding AE, Gautier-Smith PC. Familial cerebral amyloid angiopathy with nonneuritic amyloid plaque formation. Brain 1990;113:721–747.

16 Scholz W. Studien zur Pathologie der Hirngefässe. II. Die drusige Entartung der Hirnarterien und Kapillaren. Zeitschrift für die Gesamte Nuerologie und Psychiatrie (Berlin) 1938;162:694–715.

17 Uyematsu S. On the pathology of senile psychosis. The differential diagnostic significance of Redlich-Fisher's miliary plaques. J Nerv Ment Dis 1923;57: 1–25, 131–156, 237–260.

18 Vidal R, Calero M, Piccardo P, Farlow MR, Unverzagt FW, Méndez E, et al. Senile dementia associated with amyloid β protein angiopathy and tau perivascular pathology but not neuritic plaques in patients homozygous for the APOE-ε4 allele. Acta Neuropathol 2000;100:1–12.

19 Vinters HV, Wang ZZ, Secor DL. Brain parenchymal and microvascular amyloid in Alzheimer's disease. Brain Pathol 1996;6:179–195.

20 Weller RO, Massey A, Newman TA, Hutchings M, Kuo YM, Roher AE. Cerebral amyloid angiopathy amyloid β accumulates in putative interstitial fluid drainage pathways in Alzheimer's disease. Am J Pathol 1998;153:725–733.

21 Weller RO, Massey A, Kuo YM, Roher AE. cerebral amyloid angiopathy: accumalation of Aβ in interstitial fluid drainage pathways in Alzheimer's disease. Ann NY Acad Sci 2000;903:110–117.

22 Yamada M, Itoh Y, Suematsu N, Otomo E, Matsushita M. Vascular variant of Alzheimer's disease characterized by severe plaque-like β protein angiopathy. Dement Geriatr Cogn Disord 1997;8: 163–168.

23 Yamaguchi H, Yamazaki T, Lemere CA, Frosch MP, Selkoe DJ. β amyloid is focally deposited within the outer basement membrane in the amyloid angiopathy of Alzheimer's disease: an immunoelectron microscopic study. Am J Pathol 1992;141:249–259.

24 Yow HY, Weller RO. A role for cerebrovascular disease in determining the pattern of β-amyloid deposition in Alzheimer's disease. Neuropathol Appl Neurobiol 2002;28:149.

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