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Differential expression of intercellular adhesion molecule-1 (ICAM-1) in the Aβ-containing lesions in brains of patients with dementia of the Alzheimer type

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Abstract Inflammatory processes have been implicated in the formation of senile plaques in the cerebral cortex of patients with dementia of the Alzheimer type (DAT), since several inflammation-induced proteins are present within these plaques. The relation between inflammatory components and other amyloid β protein (Aβ)-containing lesions of the DAT brain [cerebrovascular amyloidosis (CA) and cerebellar senile plaques] is unclear. We studied the distribution of the inflammation-inducible protein intercellular adhesion molecule-1 (ICAM-1) in CA and in senile plaques of the cerebellum, using an immunohistochemical approach. We observed striking differences in ICAM-1 reactivity between the different types of Aβ-containing lesions. ICAM-1 was only expressed in classic senile plaques in the granular and Purkinje cell layer of the cerebellum, and not in diffuse senile plaques of the molecular layer. Also, ICAM-1 was not associated with CA; only when the vascular amyloid extended into the neuropil (dyshoric angiopathy) was perivascular ICAM-1 reactivity observed. This is in contrast to the putative primary involvement of inflammation in the formation of cerebrocortical classic and diffuse senile plaques. Our findings indicate that ICAM-1 expression, which may be an indicator of an inflammatory reaction, is induced in the neuropil depending on the specific site of Aβ production.

Key words Alzheimer's disease · Cerebellum · Intercellular adhesion molecule-1 · Cerebrovascular · Inflammation

Introduction

The amyloid β protein (Aβ) is the major component of senile plaques and cerebrovascular amyloidosis (CA) [12], two of the histopathological hallmarks of dementia of the Alzheimer type (DAT) [22]. Proteolytic processing of the membrane-spanning amyloid precursor protein (APP) may result either in the formation of soluble APP or in the production of the 4-kDa protein Aβ [15, 21, 42, 46]. Aβ is produced in a soluble form, but can also aggregate to form fibrils. Both forms are present in DAT brains.

The majority of extracellular Aβ found in CA is aggregated into Congo red-positive fibrils, although some non-fibrillar Aβ has been demonstrated intracellularly in smooth muscle cells [10]. In contrast, most of the Aβ formed in the cerebral cortex is deposited as diffuse senile plaques in a non-fibrillar form, although this view has been recently debated [6]. The diffuse senile plaque is supposed to mature into the classic senile plaque, consisting of a core of fibrillar Aβ and a corona of dystrophic neurites [40]. Senile plaques are also formed in the cerebellum. In the molecular layer they are exclusively of the diffuse type, but in the Purkinje cell and granular layer classic senile plaques have been identified [19, 24, 53, 55]. Beside differences in the aggregation state of Aβ, the exact length of Aβ accumulating in CA or senile plaques has been described to vary between these lesions [26, 28, 30, 32].

Regarding these differences, it is not clear if the pathogenesis of Aβ formation in CA and in the several types of senile plaques is identical. The presence or absence of inflammatory proteins may help to explain the differences observed in physical and chemical composition of CA and senile plaques. The importance of inflammatory processes in DAT pathogenesis is illustrated by the presence of several inflammation-induced proteins in cerebrocortical senile plaques, such as α_1-antichymotrypsin (α_1-ACT) [1, 36, 41], C-reactive protein (CRP) [16], complement factors [9] and intercellular adhesion molecule-1 (ICAM-1) [47], as well as by the intimate association of clusters of activated microglial cells with classic senile plaques [7, 34, 45, 48]. Inflammation might play an important role in the putative maturation of diffuse senile plaques into classic senile plaques, during which transformation of non-fibrillar Aβ into fibrils occurs. α_1-ACT has indeed been

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demonstrated to accelerate the process of Aβ fibril formation in vitro [23]. Moreover, a growing amount of clinical data is available indicating that therapy with anti-inflammatory drugs may be beneficial for DAT patients [4, 31].

Most of the immunohistochemical studies on the distribution of inflammation-associated proteins in DAT brains have focused on the cerebral cortex. It is unclear to what extent inflammatory processes are linked to other types of Aβ deposition. Therefore, we investigated the association of the inflammation-inducible protein ICAM-1 with Aβ deposition in the cerebellum of patients with DAT and CA using an immunohistochemical analysis of DAT and control brain tissues.

Materials and methods

Tissue samples

Brain tissue from patients with clinically diagnosed and neuropathologically confirmed DAT and from normal controls were obtained from autopsy material. A definite diagnosis of DAT was based on a combination of neuropathological [27] and clinical criteria. Tissue samples from frontal, parietal and temporal cortex were obtained from 9 control patients [6 female and 3 male; age 73.7 ± 14.1 years; post-mortem delay (PMD) 3.1 ± 1.2 h] and 32 DAT patients (20 female and 12 male; age 77.5 ± 11.3 years; PMD 2.3 ± 1.6 h). Samples from cerebellum was obtained from 8 control patients (5 female and 3 male; age 74.5 ± 15.0 years; PMD 2.9 ± 0.5 h) and 16 DAT patients (11 female and 5 male; 75.4 ± 11.7 years; PMD 3.3 ± 0.9 h). Tissue samples were frozen in liquid nitrogen immediately after removal.

Antibodies

mAb CL203 (anti-ICAM-1 domain 4) was a kind gift of Dr. S. Ferrone (Valhalla, N.Y.); mAb CA7 (anti-ICAM-1 domain 5) was a kind gift of Dr. R. Rothlein (Boehringer Ingelheim, Ridgefield, Colo.); mAb 6C6 (anti-Aβ) was a kind gift of Dr. D.L. Schenk (Athena Neurosciences, San Francisco, Calif.). The mAbs PN-E4 (anti-ICAM-1 domain 1), PN-E8 (anti-ICAM-1 domain 1) and PN-E12 (anti-ICAM-1 domain 1 or 2) have been described before [47].

Immunohistochemical staining

Cryosections (4 μm) were fixed in acetone for 5 min and subsequently in acetone containing 0.15% H_2O_2 for another 5 min to block endogenous peroxidase activity. Sections were incubated overnight at 4°C with primary antibodies and successively with the ABC detection kit (Vector, Burlingame, Calif.) according to the manufacturer's description. Each incubation was followed by washing with phosphate-buffered saline (PBS). Diaminobenzidine (DAB) was used as chromogen and the sections were counterstained with hematoxylin. Staining with the five different anti-ICAM-1 antibodies was similar, with variations only in the intensity of staining. Congo red staining was performed by successive dehydration of acetone-fixed tissue sections (4 μm) with increasing concentrations of ethanol. After a 20-min preincubation period in a solution of 3% NaCl in 80% ethanol, sections were incubated for 20 min in the same solution containing 0.5% Congo red. Finally, the sections were dehydrated in ethanol and xylol.

Results

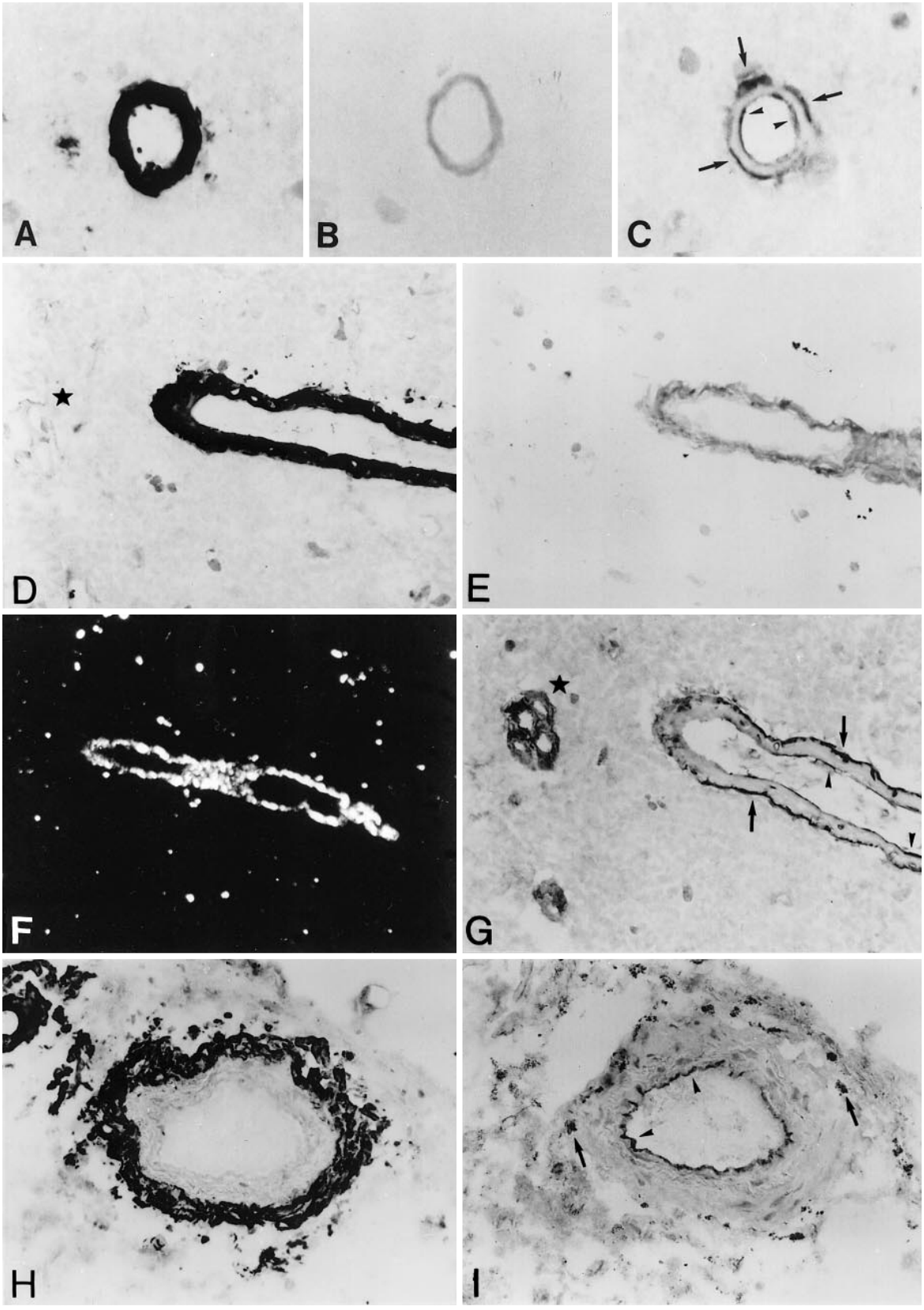
Cerebrovascular amyloidosis

CA was detected in capillaries and small to medium-sized arterioles in tissue sections of cerebral cortex in 19 out of 32 DAT patients (59%) by staining with the anti-Aβ mAb 6C6 (Fig. 1A, D). Serial section analysis revealed that many of the Aβ-positive vessels were also identified by Congo red staining (Fig. 1B, E), with concomitant bright birefringence under polarized light (Fig. 1F), although, in comparison with Aβ immunostaining, a smaller part of the vessel wall was stained for Congo red. ICAM-1, however, was only expressed by endothelial cells and by cells in a perivascular position, and was not associated with Aβ deposition in the vessel wall (Fig. 1C, G). There seemed to be an increased endothelial and perivascular ICAM-1 staining in Aβ-containing vessels compared to the staining observed in unaffected vessels in DAT (Fig. 1G) or control brain tissue (not shown). CA was also demonstrated by 6C6 staining in leptomeningeal arteries and arterioles of 9 out of 16 DAT cases (56%) (Fig. 1H), where the deposition of Aβ was observed in the tunica media. As in cortical vessels, these deposits were not labeled by any of the five anti-ICAM-1 mAbs. ICAM-1 staining was restricted to endothelial cells and some perivascular granular staining was also observed, probably reflecting the presence of macrophages (Fig. 1I). CA was neither observed in the cerebral cortex nor in the leptomeninges of any of the control patients.

In the cerebellum, CA was found in the leptomeningeal vessels of 8 out of 16 DAT patients with 6C6 staining (50%, Fig. 2A). Aβ-positive cortical vessels were demonstrated in 6 of these cases (38%). Similarly, as described above for cerebrocortical blood vessels, the majority of the cerebellar Aβ-positive vessels were identified with Congo red staining (Fig. 2B), but ICAM-1 staining was never observed in association with the Aβ deposits (Fig. 2C). In general, the pattern of ICAM-1 expression on endothelial cells and perivascular cells was similar to the patterns observed in unaffected vessels of DAT or control cerebellum. In the cerebellum of one control patient, CA was demonstrated in a few leptomeningeal vessels (not shown).

Dyshoric angiopathy

Dyshoric angiopathy (DA), or fine radiating deposits of amyloid extending into the neighboring parenchyma, was demonstrated only around parenchymal vessels in cerebrocortical sections of DAT patients (12 out of 32 cases, 38%) (Fig. 3A) and was not observed in the cerebellum of any of these patients. In control patients, DA was neither observed in the cerebellar nor in the cerebral cortex. In contrast to the absence of ICAM-1 reactivity in the Aβ-containing vessel walls (see Fig. 1C, G), perivascular tissue affected by DA was immunopositive for ICAM-1 (Fig. 3B).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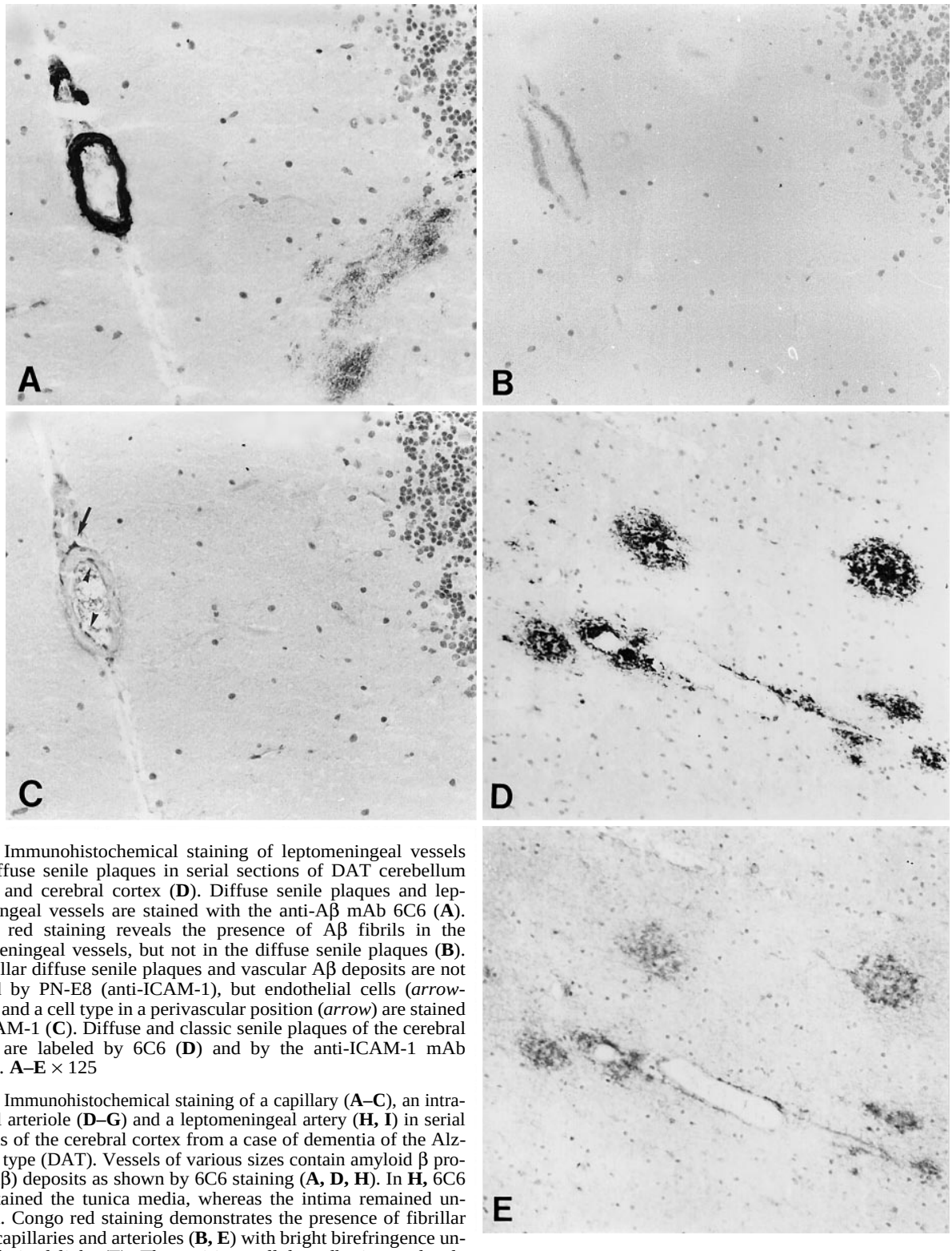

Fig. 2 Immunohistochemical staining of leptomeningeal vessels and diffuse senile plaques in serial sections of DAT cerebellum (**A–C**) and cerebral cortex (**D**). Diffuse senile plaques and leptomeningeal vessels are stained with the anti-Aβ mAb 6C6 (**A**). Congo red staining reveals the presence of Aβ fibrils in the leptomeningeal vessels, but not in the diffuse senile plaques (**B**). Cerebellar diffuse senile plaques and vascular Aβ deposits are not labeled by PN-E8 (anti-ICAM-1), but endothelial cells (*arrowheads*) and a cell type in a perivascular position (*arrow*) are stained for ICAM-1 (**C**). Diffuse and classic senile plaques of the cerebral cortex are labeled by 6C6 (**D**) and by the anti-ICAM-1 mAb CL203. **A–E** × 125

◀ **Fig. 1** Immunohistochemical staining of a capillary (**A–C**), an intracortical arteriole (**D–G**) and a leptomeningeal artery (**H, I**) in serial sections of the cerebral cortex from a case of dementia of the Alzheimer type (DAT). Vessels of various sizes contain amyloid β protein (Aβ) deposits as shown by 6C6 staining (**A, D, H**). In **H,** 6C6 only stained the tunica media, whereas the intima remained unstained. Congo red staining demonstrates the presence of fibrillar Aβ in capillaries and arterioles (**B, E**) with bright birefringence under polarized light (**F**). The anti-intercellular adhesion molecule (ICAM)-1 mAb PN-E8 stained endothelial cells (*arrowheads*, **C, G, I**) as well as cells in a perivascular position (*arrows*) (**C, G**). Unidentified perivascular granular ICAM-1 reactivity was observed in **I** (*arrows*). A group of small vessels, unaffected by Aβ deposition (*asterisk*, **D**), is also stained for ICAM-1 (*asterisk*, **G**). Note the absence of ICAM-1 reactivity in the Aβ deposits. **A–C,** × 430; **D, E, G,** × 240; **F**, × 120; **H, I,** × 170

Cerebellar senile plaques

Senile plaques were demonstrated by 6C6 staining in the molecular layer of the cerebellum in 10 out of 16 DAT patients (63%) (Fig. 2A). All these plaques were of the diffuse type, as demonstrated by the complete absence of

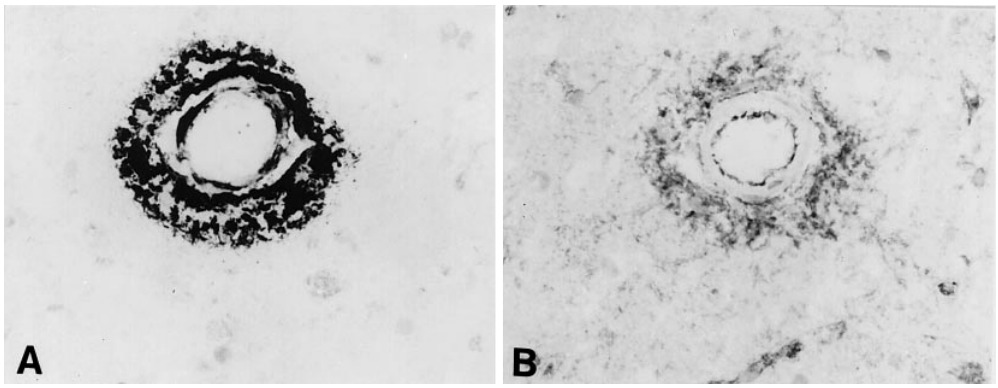

Fig. 3 Immunohistochemical staining of dyshoric angiopathy in serial sections of DAT cerebral cortex with the anti-Aβ mAb 6C6 (**A**) and the anti-ICAM-1 mAb PN-E8 (**B**). **A, B** × 250

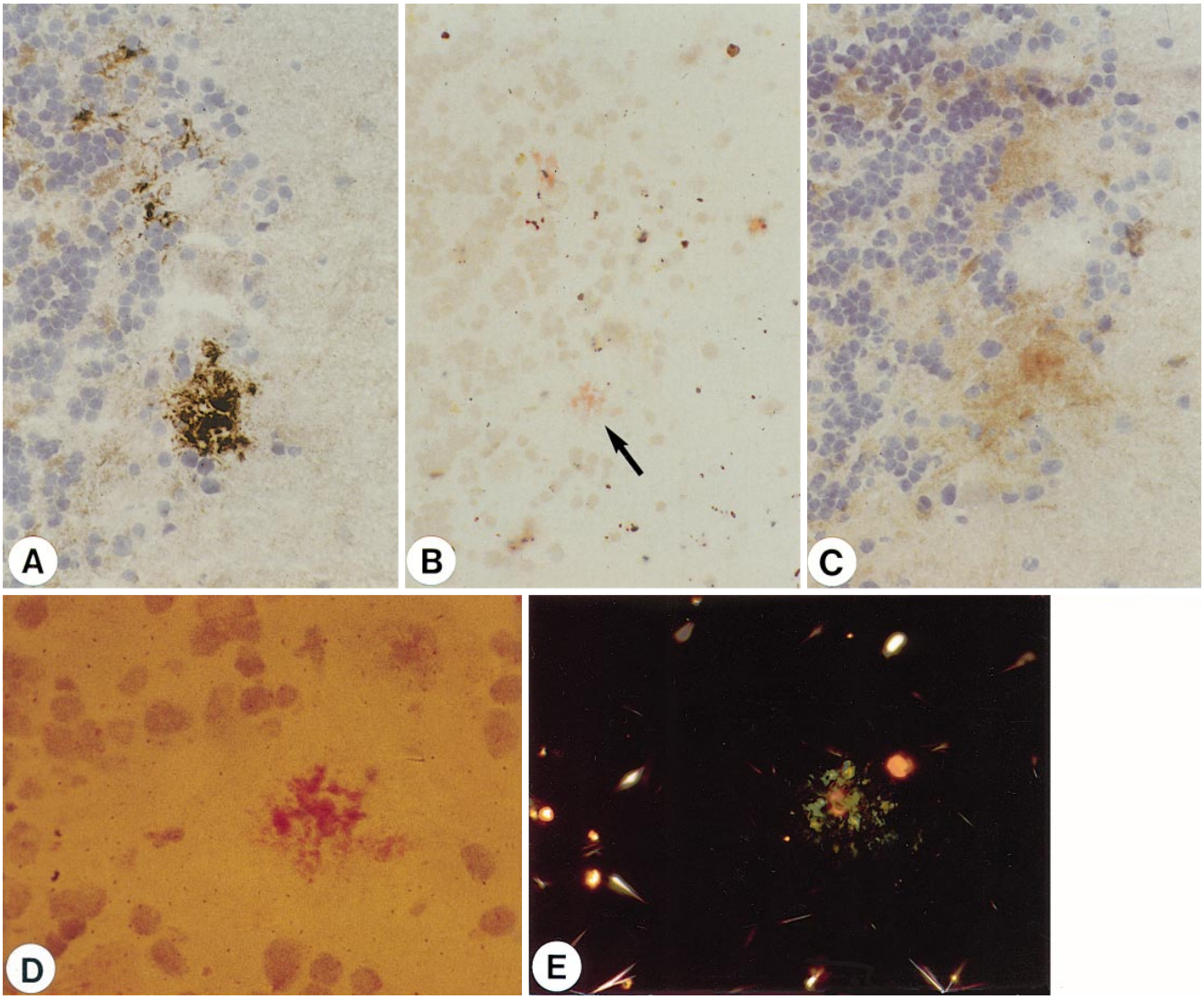

Fig. 4 Immunohistochemical staining of classic senile plaques in serial sections of DAT cerebellum with the anti-Aβ mAb 6C6 (**A**), Congo red (**B, D, E**) and the anti-ICAM-1 mAb CL203 (**C**). A high-power magnification (**D**) of the classic senile plaque, indicated by the *arrow* in **B**, is shown under polarized light in **E**. **A–C,** × 250; **D, E** × 850

Congo red staining in serial sections. Blood vessels affected by CA in the same sections, however, were strongly stained by Congo red (Fig. 2B). The diffuse senile plaques in the cerebellum were not stained for ICAM-1 (Fig. 2C). A small number of classic senile plaques was detected by 6C6 and Congo red staining of serial sections in the granular and Purkinje cell layers of the cerebellum in

only 3 DAT patients (19%) (Fig. 4A, B, D, E). These cerebellar classic senile plaques were diffusely stained for ICAM-1 (Fig. 4C). ICAM-1 staining was found in areas extending beyond the Aβ deposits. In cerebellar tissue sections from two control patients a few diffuse senile plaques were demonstrated, but classic senile plaques and senile plaque-associated ICAM-1 were completely absent from these sections. As a positive control for our ICAM-1 staining procedures, numerous senile plaques of both diffuse and classic type from the cerebral cortex of DAT patients were positively stained for ICAM-1 (Fig. 2D, E).

Discussion

Our findings demonstrate striking differences between the pathological lesions in the cortex and cerebellum in DAT brains. Consistent with other reports [19, 24, 53], we observed that, unlike in the cerebral cortex, classic senile plaques with a central amyloid core are completely absent from the cerebellar molecular layer. Diffuse senile plaques in this cerebellar layer were devoid of ICAM-1 reactivity, whereas ICAM-1 could be identified in the classic senile plaques in the Purkinje cell and granular layer of the cerebellum. As described previously [47], ICAM-1 reactivity in these classic senile plaques extended beyond the site of Aβ deposition, indicating that ICAM-1 is expressed in tissue areas larger than would be anticipated on the basis of Aβ staining. The distribution of ICAM-1 in the DAT cerebellum is in contrast to the situation in the cerebral cortex, where both types of senile plaques are invariably accompanied by ICAM-1 reactivity [47].

Similar to α_1-ACT [36], ICAM-1 was absent from cerebrovascular Aβ deposition in both the cerebral and the cerebellar cortex. Although a study reported the presence of inflammatory compounds in CA [41], we conclude that the formation of CA, in contrast to senile plaque formation in the cerebral cortex, is not associated with the production of an inflammation-inducible protein like ICAM-1. Many of the Aβ-positive cerebral and cerebellar vessels contained fibrillar material, indicating that Aβ fibrillization in the cerebrovasculature may occur in the absence of inflammatory proteins. In vitro studies have suggested that Aβ fibril formation may be accelerated by α_1-ACT [23], but this process seems irrelevant to CA formation. It may be possible that specific characteristics of vascular cells or components of the vascular basement membrane, especially HSPG [43] or apolipoprotein E [38, 51, 54] influence the formation of Aβ fibrils. In addition, the Aβ produced in the cerebrovasculature may differ in length from Aβ produced in the neuropil [26, 30, 32], possibly leading to a preferential production of the more amyloidogenic Aβ1–42 [18], but several conflicting reports have been published concerning this subject [17, 26, 28, 30, 32]. Strikingly, if the amyloid deposition extended beyond the vessel wall into the nearby neuropil (DA or *drusige Entartung*), we observed ICAM-1 immunoreactivity, similar to that described for α_1-ACT [36], indicating that interaction between Aβ and neuroglial tissue is essential for its induction.

The cellular source of the Aβ-associated ICAM-1 is yet uncertain. A number of cells may express ICAM-1, either constitutively or after proper stimulation. In the brain vasculature, endothelial cells and pericytes can express ICAM-1 [8, 33, 47, 49], and in the brain parenchyma, astrocytes and neural cells can do so [2, 3, 47]. ICAM-1 expression can be induced by a variety of inflammatory mediators, such as lipopolysaccharide, interferon-γ, tumor necrosis factor-α and interleukin-1 (IL-1) [8, 49, 50, 52]. Microglial cells are capable of IL-1 production [11, 39], and it has been demonstrated that in DAT brains these cells show enhanced IL-1 immunoreactivity [5, 7, 13, 14]. Astrocytes are the most likely source of ICAM-1 and α_1-ACT in DAT brains [2, 29], and it is possible that astrocytic ICAM-1 and α_1-ACT production is induced by IL-1 secreted by microglial cells [5]. Apparently, vascular cells, such as pericytes and endothelial cells, are not stimulated to produce ICAM-1 to the same extent as astrocytes in the neuropil.

Complement factors and α_1-ACT have been demonstrated in the diffuse senile plaques of hereditary cerebral hemorrhage with amyloidosis – Dutch type [37], but reports about their expression in either cerebral or cerebellar diffuse senile plaques of DAT brains are still contradictory [20, 35, 41]. Similar to ICAM-1, the proteoglycan HSPG is found in all types of senile plaques in the cerebral cortex, but not in diffuse senile plaques of the cerebellum [44]. Our results indicate that in the cerebellum ICAM-1 production may play a prominent role at the level of classic senile plaque formation, whereas it probably only marginally contributes to the formation of diffuse senile plaques in this brain region. It has recently been speculated [5] that microglial cells of the cerebellum, in contrast to their counterparts in the cerebral cortex, are relatively defective in producing the proper stimulating factors for astrocytic production of α_1-ACT which may also relate to ICAM-1 production. Interactions between microglial cells and astrocytes may be essential for the induction of inflammatory reactions in senile plaques. These interactions may not occur in the molecular layer of the cerebellum, in contrast to the situation in the cerebral cortex. Accordingly, neural and glial cells remain morphologically unchanged in diffuse senile plaques in the cerebellum [19, 24, 25, 53]. In the Purkinje cell and granular layer of the cerebellum, however, microglial cells and astrocytes may interact more closely, resulting in the induction of ICAM-1.

We conclude that ICAM-1 production is not uniformly involved in the process of Aβ formation, the involvement being basically dependent on the site of Aβ production in the DAT brain and the possibility of local interactions between microglial cells and astrocytes. It can be argued that production of inflammatory compounds like α_1-ACT and ICAM-1 occurs as a reaction to Aβ production in the brain, but it is important to note that in that case these reactions only take place in specific regions of the brain that do not include the cerebellar molecular layer and the brain

vasculature. Furthermore, it seems unlikely that production of inflammatory compounds occurs as a reaction to Aβ fibril formation, since inflammation-induced proteins are abundantly present in diffuse senile plaques in the cerebral cortex that are devoid of fibrils. Conversely, our findings are compatible with the hypothesis that inflammatory reactions may contribute to the process of Aβ fibrillization, although this remains to be established in the case of ICAM-1 production. However, other factors may also contribute to the formation of fibrillar Aβ, such as HSPG and apolipoprotein E, and this may be of particular importance for the pathogenesis of CA.

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References

1. Abraham CR, Selkoe DJ, Potter H (1988) Immunochemical identification of the serine protease inhibitor alpha-1-antichymotrypsin in the brain amyloid deposits of Alzheimer's disease. Cell 52: 487–501
2. Akiyama H, Kawamata T, Yamada T, Tooyama I, Ishii T, McGeer PL (1993) Expression of intercellular adhesion molecule (ICAM)-1 by a subset of astrocytes in Alzheimer disease and some other degenerative neurological disorders. Acta Neuropathol 85: 628–634
3. Birdsall HH, Lane C, Ramser MN, Anderson DC (1992) Induction of VCAM-1 and ICAM-1 on human neural cells and mechanisms of mononuclear leukocyte adherence. J Immunol 148: 2717–2723
4. Breitner JCS, Gau BA, Welsh KA, Plassman BL, McDonald WM, Helms MJ, Anthony JC (1994) Inverse association of anti-inflammatory treatments and Alzheimer's disease: initial results of a co-twin control study. Neurology 44: 227–232
5. Das S, Potter H (1995) Expression of the Alzheimer amyloid-promoting factor antichymotrypsin is induced in human astrocytes by IL-1. Neuron 14: 447–456
6. Davies CA, Mann DMA (1993) Is the "preamyloid" of diffuse plaques in Alzheimer's disease really nonfibrillar? Am J Pathol 143: 1594–1605
7. Dickson DW, Lee SC, Mattiace LA, Yen SC, Brosnan C (1993) Microglia and cytokines in neurological disease, with special reference to AIDS and Alzheimer's disease. Glia 7: 75–83
8. Dustin ML, Rothlein R, Bhan AK, Dinarello CA, Springer TA (1986) Induction by IL-1 and interferon: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). J Immunol 137: 245–254
9. Eikelenboom P, Stam FC (1982) Immunoglobulins and complement factors in senile plaques. An immunohistochemical study. Acta Neuropathol (Berl) 57: 239–242
10. Frackowiak J, Zoltowska A, Wisniewski HM (1994) Non-fibrillar β-amyloid protein is associated with smooth muscle cells of vessel walls in Alzheimer disease. J Neuropathol Exp Neurol 53: 637–645
11. Giulian D, Baker TJ, Shih LN, Lachman LB (1986) Interleukin 1 of the central nervous system is produced by ameboid microglia. J Exp Med 164: 594–604
12. Glenner GG, Wong CW (1984) Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun 120: 885–890
13. Griffin WST, Stanley LC, Ling C, White L, MacLeod V, Perror LJ, White III CL, Araoz C (1989) Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. Proc Natl Acad Sci USA 86: 7611–7615
14. Griffin WST, Sheng JG, Roberts GW, Mrak RE (1995) Interleukin-1 expression in different plaque types in Alzheimer's disease: significance in plaque evolution. J Neuropathol Exp Neurol 54: 276–281
15. Haass C, Selkoe DJ (1993) Cellular processing of β-amyloid precursor protein and the genesis of amyloid β-peptide. Cell 75: 1039–1042
16. Iwamoto N, Nishiyama E, Ohwada J, Arai H (1994) Demonstration of CRP immunoreactivity in brains of Alzheimer's disease: immunohistochemical study using formic acid pretreatment of tissue sections. Neurosci Lett 177: 23–26
17. Iwatsubo T, Odaka A, Suzuki N, Mizusawa H, Nukina N, Ihara Y (1994) Visualization of Aβ42(43) and Aβ40 in senile plaques with end-specific monoclonals: evidence that an initially deposited species is Aβ42(43). Neuron 13: 45–53
18. Jarrett JT, Berger EP, Lansbury PT (1993) The carboxy terminus of the β amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease. Biochemistry 32: 4693–4697
19. Joachim CL, Morris JH, Selkoe DJ (1989) Diffuse senile plaques occur commonly in the cerebellum in Alzheimer's disease. Am J Pathol 135: 309–319
20. Kalaria RN, Perry G (1993) Amyloid P component and other acute-phase proteins associated with cerebellar Aβ-deposits in Alzheimer's disease. Brain Res 631: 151–155
21. Kang J, Lemaire H, Unterbeck A, Salbaum JM, Masters CL, Grzeschik K, Multhaup G, Beyreuther K, Müller-Hill B (1987) The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature 325: 733–736
22. Khachaturian ZS (1985) Diagnosis of Alzheimer's disease. Arch Neurol 42: 1097–1104
23. Ma J, Yee A, Brewer BJ, Das S, Potter H (1994) Amyloid associated proteins α1-antichymotrypsin and apolipoprotein E promote assembly of Alzheimer β-protein into filaments. Nature 372: 92–94
24. Mann DM, Jones D, Prinja D, Purkiss MS (1990) The prevalence of amyloid (A4) protein deposits within the cerebral and cerebellar cortex in Down's syndrome and Alzheimer's disease. Acta Neuropathol 80: 318–327
25. Mattiace LA, Davies P, Yen S-H, Dickson DW (1990) Microglia in cerebellar plaques in Alzheimer's disease. Acta Neuropathol 80: 493–498
26. Miller DL, Papayannopoulos IA, Styles J, Bobin SA, Lin YY, Biemann K, Iqbal K (1993) Peptide composition of the cerebrovascular and senile plaque core amyloid deposits of Alzheimer's disease. Arch Biochem Biophys 301: 41–52
27. Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS, Hughes JP, Belle G van, Berg L (1991) The consortium to establish a registry for Alzheimer's Disease (CERAD). II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 41: 479–486
28. Murphy GMJ, Forno LS, Higgins L, Scardina JM, Eng FL, Cordell B (1994) Development of a monoclonal antibody specific for the COOH-terminal of β-amyloid 1-42 and its immunohistochemical reactivity in Alzheimer's disease and related disorders. Am J Pathol 144: 1082–1088
29. Pasternack JM, Abraham CR, Van Dyke BJ, Potter H, Younkin SG (1989) Astrocytes in Alzheimer's disease gray matter express α1-antichymotrypsin mRNA. Am J Pathol 135: 827–834

30. Prelli F, Castaño E, Glenner GG, Frangione B (1988) Differences between vascular and plaque core amyloid in Alzheimer's disease. J Neurochem 51: 648–651
31. Rogers J, Kirby LC, Hempelman SR, Berry DL, McGeer PL, Kaszniak AW, Zalinski J, Cofield M, Mansukhani L, Wilson P, Kogan F (1993) Clinical trial of indomethacin in Alzheimer's disease. Neurology 43: 1609–1611
32. Roher AE, Lowenson JD, Clarke S, Woods AS, Cotter RJ, Gowing E, Ball MJ (1993) β-Amyloid-(1-42) is a major component of cerebrovascular amyloid deposits: implications for the pathology of Alzheimer disease. Proc Natl Acad Sci USA 90: 10836–10840
33. Rothlein R, Dustin ML, Marlin SD, Springer TA (1986) A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1. J Immunol 137: 1270–1274
34. Rozemuller JM, Eikelenboom P, Stam FC, Beyreuther K, Masters CL (1989) A4 protein in Alzheimer's disease: primary and secondary cellular events in extracellular amyloid deposition. J Neuropathol Exp Neurol 48: 674–691
35. Rozemuller JM, Stam FC, Eikelenboom P (1990) Acute phase proteins are present in amorphous plaques in the cerebral but not in the cerebellar cortex of patients with Alzheimer's disease. Neurosci Lett 119: 75–78
36. Rozemuller JM, Abbink JJ, Kamp AM, Stam FC, Hack CE, Eikelenboom P (1991) Distribution pattern and functional state of α1-antichymotrypsin in plaques and vascular amyloid in Alzheimer's disease. Acta Neuropathol 82: 200–207
37. Rozemuller JM, Bots GTAM, Roos RAC, Eikelenboom P (1992) Acute phase proteins but not activated microglial cells are present in parenchymal β/A4 deposits in the brains of patients with hereditary cerebral hemorrhage with amyloidosis-Dutch type. Neurosci Lett 140: 137–140
38. Sanan DA, Weisgraber KH, Russell SJ, Mahley RW, Huang D, Saunders A, Schemel D, Wisniewski T, Frangione B, Roses AD, Strittmatter WJ (1994) Apolipoprotein E associates with β amyloid peptide of Alzheimer's disease to form novel monofibrils. J Clin Invest 94: 860–869
39. Sébire G, Emilie D, Wallon C, Héry C, Devergne O, Delfraissy J, Galanaud P, Tardieu M (1993) In vitro production of IL-6, IL-1β and tumor necrosis factor-α by human embryonic microglial and neural cells. J Immunol 150: 1517–1523
40. Selkoe DJ (1991) The molecular pathology of Alzheimer's disease. Neuron 6: 487–498
41. Shoji M, Hirai S, Yamaguchi H, Harigaya Y, Ishiguro K, Matsubara E (1991) Alpha 1-antichymotrypsin is present in diffuse senile plaques. Am J Pathol 138: 247–257
42. Shoji M, Golde TE, Ghiso J, Cheung TT, Estus S, Shaffer LM, Cai X-D, McKay DM, Tintner R, Frangione B, Younkin SG (1992) Production of the Alzheimer amyloid β protein by normal proteolytic processing. Science 258: 126–129
43. Snow AD, Sekiguchi R, Nochlin D, Fraser P, Kimata K, Mizutani A, Arai M, Schreier WA, Morgan DG (1994) An important role of heparan sulfate proteoglycan (Perlecan) in a model system for the deposition and persistence of fibrillar A beta-amyloid in rat brain. Neuron 12: 219–234
44. Snow AD, Sekiguchi RT, Nochlin D, Kalaria RN, Kimata K (1994) Heparan sulfate proteoglycan in diffuse plaques of hippocampus but not of cerebellum in Alzheimer's disease brain. Am J Pathol 144: 337–347
45. Tooyama I, Kimura H, Akiyama H, McGeer PL (1990) Reactive microglia express class I and class II major histocompatibility complex antigens in Alzheimer's disease. Brain Res 523: 273–280
46. Van Nostrand WE, Wagner SL, Suzuki M, Choi BH, Farrow JS, Geddes JW, Cotman CW, Cunningham DD (1989) Protease nexin-II, a potent anti-chymotrypsin, shows identity to amyloid β-protein precursor. Nature 341: 546–549
47. Verbeek MM, Otte-Höller I, Westphal JR, Wesseling P, Ruiter DJ, Waal RMW de (1994) Accumulation of intercellular adhesion molecule-1 in senile plaques in brain tissue of patients with Alzheimer's disease. Am J Pathol 144: 104–116
48. Verbeek MM, Otte-Höller I, Wesseling P, Van Nostrand WE, Sorg C, Ruiter DJ, Waal RMW de (1995) A lysosomal marker for activated microglial cells involved in Alzheimer classic senile plaques. Acta Neuropathol 90: 493–503
49. Verbeek MM, Westphal JR, Ruiter DJ, Waal RMW de (1995) T lymphocyte adhesion to human brain pericytes is mediated via VLA-4/VCAM-1 interactions. J Immunol 154: 5876–5884
50. Westphal JR, Willems HW, Schalkwijk CJM, Ruiter DJ, Waal RMW de (1993) A new 180-kDa dermal endothelial cell activation antigen: in vitro and in situ characteristics. J Invest Dermatol 100: 27–34
51. Wisniewski T, Castaño EM, Golabek A, Vogel T, Frangione B (1994) Acceleration of Alzheimer's fibril formation by apolipoprotein E in vitro. Am J Pathol 145: 1030–1035
52. Wong D, Dorovini-Zis K (1992) Upregulation of intercellular adhesion molecule-1 (ICAM-1) expression in primary cultures of human brain microvessel endothelial cells by cytokines and lipopolysaccharide. J Neuroimmunol 39: 11–22
53. Yamaguchi H, Hirai S, Morimatsu M, Shoji M, Nakazato Y (1989) Diffuse type of senile plaques in the cerebellum of Alzheimer-type dementia demonstrated by beta protein immunostain. Acta Neuropathol 77: 314–319
54. Yamaguchi H, Ishiguro K, Sugihara S, Nakazato Y, Kawarabayashi T, Sun X, Hirai S (1994) Presence of apolipoprotein E on extracellular neurofibrillary tangles and on meningeal blood vessels precedes the Alzheimer β-amyloid deposition. Acta Neuropathol 88: 413–419
55. Yamazaki T, Yamaguchi H, Nakazato Y, Ishiguro K, Kawarabayashi T, Hirai S (1992) Ultrastructural characterization of cerebellar diffuse plaques in Alzheimer's disease. J Neuropathol Exp Neurol 51: 281–286