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Microglia in Diffuse Plaques in Hereditary Cerebral Hemorrhage with Amyloidosis (Dutch). An Immunohistochemical Study

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Abstract. In hereditary cerebral hemorrhage with amyloidosis (Dutch) (HCHWA-D) β/A4 amyloid deposition is found in meningocortical blood vessels and in diffuse plaques in the cerebral cortex. Diffuse plaques putatively represent early stages in the formation of senile plaques. Microglia are intimately associated with congophilic plaques in Alzheimer's disease (AD), but microglial involvement in diffuse plaque formation is controversial. Therefore, we studied the relationship between microglia and diffuse plaques in the cerebral cortex of four patients with HCHWA-D using a panel of macrophage/microglia markers (mAbs LCA, LeuM5, LeuM3, LN3, KP1, OKIa, CLB54, Mac1, Ki-M6, AMC30 and the lectin RCA-1). Eight AD patients, one demented Down's syndrome (DS) patient and four non-demented controls were included for comparison. In controls and HCHWA-D patients ramified or "resting" microglia formed a reticular array in cortical gray and subcortical white matter. Microglial cells in or near HCHWA-D diffuse plaques retained their normal regular spacing and ramified morphology. In AD/DS gray matter more microglial cells were stained than in controls and HCHWA-D patients. Intensely immunoreactive microglia with enlarged cell bodies and short, thick processes clustered in congophilic plaques. In contrast to the resting microglia, these "activated microglia" strongly expressed class II major histocompatibility complex antigen, HLA-DR, and were AMC30-immunoreactive. These findings support the view that microglia play a role in the formation of congophilic plaques but do not initiate diffuse plaque formation. Another finding in this study is the presence of strong monocyte/macrophage marker immunoreactivity in the wall of cortical congophilic blood vessels in HCHWA-D.

Key Words: Amyloid; Cerebral amyloid angiopathy; Diffuse plaques; Hereditary cerebral hemorrhage with amyloidosis (Dutch); Immunohistochemistry; Microglia.

INTRODUCTION

Hereditary cerebral hemorrhage with amyloidosis (Dutch) (HCHWA-D) is an autosomal dominant cerebral β/A4 amyloid disease, characterized by lobar hemorrhages between the ages of 40 and 60 and dementia (1). It is caused by a G to C point mutation in the amyloid precursor protein (APP) gene on the long arm of chromosome 21 (2, 3). The pathological hallmark is amyloid angiopathy of meningocortical blood vessels (4). In addition diffuse plaques, which are commonly believed to represent early stages in plaque formation (5–8), are found in the cerebral cortex. Neurofibrillary pathology is absent and congophilic plaques are rare (9, 10) in contrast to Alzheimer's disease (AD), another cerebral β/A4 amyloid disease.

Congophilic plaques in AD are intimately associated with "activated" microglial cells (5, 11–18). Microglial cells in the normal central nervous system have a small polymorphic nucleus, scanty cytoplasm, and delicate ramifying processes (19), hence their name "ramified" microglia. In support of the hypothesis that they are derived from monocytes (20), microglia express monocyte/macrophage antigens (16, 21–23). Microglial cells may show morphological and immunological evidence of activation: the cell body enlarges, the processes become shorter and thicker, and the expression of monocyte/macrophage antigens, in particular of the class II major histocompatibility antigen HLA-DR, is induced or increases (5, 11, 13–18, 22, 23). As opposed to these "activated" microglia, ramified microglia are often denoted as "resting." However, if HLA-DR represents a true marker for microglial activation, the morphology of the microglial cell may not reflect its functional state (23, 24), since ramified or resting microglia can also express HLA-DR.

In contrast to the well-established association between activated microglia and congophilic plaques in AD, the association between microglia and diffuse plaques is debated. The light microscopical observations vary from the total absence of microglia (5, 16, 25, 26) to the presence of ramified, and in several studies HLA-DR-positive, microglia in part of (15, 27) or in virtually all (12, 28, 29) cerebral and cerebellar diffuse plaques. Microglia were observed ultrastructurally in diffuse plaques by Wisniewski et al (30). The function of microglia in plaque formation is unknown. It is suggested that they are engaged in the secretion (31–34) or phagocytosis (15) of amyloid fibrils in congophilic plaques or, on the other hand, that they play a primary role in plaque formation, initiating the formation of diffuse plaques (28, 30).

The present immunohistochemical study of four patients with HCHWA-D, eight patients with AD, one demented patient with Down's syndrome (DS) and four

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TABLE 1
Patient Data

Patient no.	Sex/Age (yr)	Diagnosis	Brain weight (g)	Post-mortem delay (h)	Paraffin (P) Cryostat (C)
1	f/47	HCHWA-D	1,250	<24	P
2	m/40	HCHWA-D	1,500	<24	P C
3	m/51	HCHWA-D	1,400	13	C
4	m/52	HCHWA-D	1,150	8	P C
5	m/48	AD	1,435	5.5	C
6	m/55	AD	1,020	<3	P
7	m/83	AD	1,180	2.5	P
8	f/84	AD	1,000	4.5	C
9	f/84	AD	760	<4	P C
10	f/83	AD	1,025	1.5	P C
11	m/71	AD	1,145	3	C
12	m/65	AD	1,360	<3	C
13	m/57	DS	900	<24	P
14	f/51	control	1,190	3	C
15	m/81	control	1,310	<24	P
16	m/36	control	1,500	11.5	P C
17	m/65	control	1,310	5	C

non-demented controls was performed to assess with a large panel of monocyte/macrophage markers the involvement of microglia in diffuse plaques in HCHWA-D, compared with congophilic senile plaques in AD.

MATERIAL AND METHDODS

Brain tissue was obtained at autopsy from four patients with HCHWA-D, eight patients with AD, one aged patient with DS and four non-demented controls without cerebral disorders (Table 1). Pieces of tissue were taken from several cortical regions and fixed in 10% buffered formalin (duration of fixation: 24 hours, 1 month, or unknown) for paraffin sections or frozen in liquid nitrogen for cryostat sectioning. Routine histological staining (hematoxylin and eosin, luxol fast blue, alkaline Congo red, methenamine silver [MS] [35], modified Bielschowsky and Holmes, Palmgren and Bodian stains) was performed on 10 μm thick sections from formalin-fixed, paraffin-embedded tissue.

Immunohistochemistry

The primary antibodies used in this study are listed in Tables 2–4 together with their specificity, sources, selected references (36–48) and the immunohistochemical technique which was used. For immunohistochemical staining on formalin-fixed tissue 4–6 μm thick paraffin sections were mounted on poly-L-lysine or chromalum gelatin glass slides, dehydrated in ethanol and preincubated in 0.3% H_2O_2 to block endogenous peroxidase. Immunohistochemical staining for β/A4 protein on paraffin sections was enhanced by treatment with 85% formic acid for 15 minutes (min). Immunohistochemical staining for the markers LN3 and KP1 on paraffin sections was enhanced by trypsinization (0.5% trypsin in 0.5% $CaCl_2$). For immunohistochemical staining on frozen tissue, 6 μm thick cryostat sections were mounted on poly-L-lysine or chromalum gelatin glass slides, air-dried and fixed in acetone for 10 min before use. The specificity of each antibody against macrophages was evaluated in lymphoid tissue (spleen, tonsil). The same technique as used for each antibody on lymphoid tissue was applied on brain tissue. All antibodies were appropriately diluted in phosphate-buffered saline (PBS), pH 7.4, containing 1% bovine serum albumin. Secondary antisera and reagents were tested for crossreactivity and nonspecific staining. Using the avidin-biotin-peroxidase complex (ABC) technique (Vector Labs., Burlingame, CA), deparaffinized rehydrated sections or acetone-fixed cryostat sections were preincubated with normal rabbit serum followed by overnight incubation at 4°C with the primary antibodies. After washing in PBS, sections were incubated with biotinylated rabbit anti-mouse immunoglobulin for 30 min and ABC for 60 min. The biotinylated lectin RCA-I was incubated only with ABC. In the peroxidase-anti-peroxidase (PAP) technique, deparaffinized and rehydrated sections or acetone-fixed cryostat sections were preincubated with normal swine serum and incubated overnight at 4°C with the primary rabbit antiserum. After washing in PBS, sections were incubated with swine anti-rabbit immunoglobulins (DAKO, Glostrup, Denmark) for 30 min. In the third step, sections were incubated with rabbit PAP complex for 30 min. Peroxidase activity was visualized using 3,3'-diaminobenzidine (DAB) (5 mg DAB in 10 ml PBS, pH 7.4, containing 0.02% H_2O_2) for 3–5 min. A cobaltchloride-enhanced DAB solution was used occasionally. Some sections, if not pretreated with formic acid, were counterstained with Congo red. After Congo red staining, sections were dehydrated and mounted. Double staining was performed on cryostat sections incubating simultaneously with rabbit polyclonal β/A4 antibody and monoclonal antibody (mAb) EBM11, followed in the second step by an incubation with alkaline phosphatase-labeled goat anti-rabbit (Tago) and biotinylated horse anti-mouse (Vector) immunoglobulins. In the third step, sections were incubated with peroxidase-labeled ABC. Peroxidase activity was visualized with the DAB method. Alkaline phosphatase was revealed using naphthol AS-MX phosphate as substrate and Fast Blue BB as coupling agent. Negative controls included staining with deletion of one or more steps or staining with nonspecific antibodies of the same immunoglobulin class. Sections of HCHWA-D and AD brains with macrophage-containing remnants of infarcts or hemorrhages served as positive controls.

RESULTS

Staining for Plaques, Neurofibrillary Tangles, Neuropil Threads, and Cerebral Amyloid Angiopathy

Non-demented Controls: Occasional diffuse plaques were observed in the two oldest control patients. Neurofibrillary degeneration or cerebral amyloid angiopathy (CAA) were not present.

HCHWA-D: Diffuse plaques (up to 25/mm²) were found in the cerebral cortex of all patients with HCHWA-D. These plaques were β/A4-immunoreactive, Congo red-negative and they showed silver staining with the MS (Fig. 1) and modified Bielschowsky stains, while silver staining according to Holmes, Palmgren and Bodian and MigT4 immunostaining did not reveal degenerating neu-

TABLE 2
Specificity of Macrophage/Microglia Markers and Selected References

Antibody (antigen)	CD number	Predominant specificity	References
LCA[a]	CD 45	leukocyte lineage	Warnke et al (36)
LeuM5[b]	CD 11c	iC3b receptor(?)	Schwarting et al (37)
LeuM3[b]	CD 14	monocytes, macrophages, dendritic cells	Dimitriu-Bona et al (38)
LN3[c]	HLA-DR	B cells, activated T cells (Ig2a)	Marder et al (39)
KP1[a]	CD 68	monocytes, macrophages	Pulford et al (40)
EBM11[d]	CD 68	mononuclear phagocytic lineage	Bliss et al (41)
OKIa[e]	—	HLA-DR (class II MHC)	Reinherz et al (42)
CLB54[f]	CD 18	β chain of LFA_1, LeuM5 and Mac1	Miedema et al (43)
Mac1[g]	CD 11b	iC3b receptor α chain	Todd et al (44)
Ki-M6[h]	CD68	monocytes, macrophages	Parwaresch et al (45)
RCA-1[i]	—	microglia	Mannoji et al (46)
AMC30[j]	—	"reactive microglia," macrophages	Cras et al (47)

[a] Dakopatts, Denmark.
[b] B&D, Becton and Dickinson, Inc.
[c] Clonab, Biotest, Germany.
[d] Gift, K Lennert, Klinikum der Christian-Albrechts-Universität, Kiel.
[e] Ortho-Mune.
[f] Gift, Central Lab. Netherlands Red Cross Transfusion Service, Amsterdam.
[g] Coulter Immunology.
[h] Gift, L Poulter, Royal Free Hospital, London.
[i] Vector.
[j] Gift, NV Innogenetics sa, Belgium.

rites. Neuropil threads (NT) or neurofibrillary tangles (NFT) were not observed. All patients showed prominent leptomeningeal and cortical CAA. Amyloid deposition, in the form of radially arranged "crystalloid substance" (50), generally affected the media and adventitia of cortical blood vessels. However, dyshoric angiopathy, that is vascular amyloid invading the parenchyma (51), was not observed.

AD/DS: Classical and primitive plaques were encountered in all patients in addition to diffuse plaques. Dystrophic neurites, NT and NFT were revealed by the appropriate silver stains and MigT4 immunostaining. Six patients showed a slight degree of meningocortical congophilic angiopathy of Pantelakis (52); two patients showed dyshoric angiopathy (51).

TABLE 3
Technique

Antibody (antigen)	Raised in	Dilution		Technique
		Crysotat	Paraffin	
LCA	mouse	1:100		ABC
LeuM5	mouse	1:25		ABC
LeuM3	mouse	1:50		ABC
LN3	mouse	1:50	1:25/10	ABC
KP1	mouse (IgM)	1:150	1:50	ABC
EMB11	mouse	1:50		ABC
OKIa	mouse	1:200		ABC
CLB54	mouse	1:1,500		ABC
Mac1	mouse	1:100		ABC
Ki-M6	mouse	1:2,000		ABC
AMC30	mouse		1:20,000	ABC
RCA-1 (lectin)	—		1:250/500	ABC

Macrophage/Microglia Markers

The results are summarized in Table 5.

Non-demented Controls: Cortical gray matter. Evenly dispersed cells with the morphology of ramified microglia were observed, showing moderate staining with mAbs LCA, LeuM5, LN3, KP1, and EBM11 (Fig. 2A). Monoclonal antibodies LeuM3, OKIa, CLB54, Mac1, and Ki-M6 yielded no staining at all. Monoclonal antibody KP1 stained more microglial cells in cryostat than in paraffin sections.

Subcortical white matter. Monoclonal antibodies LCA, LeuM5, LN3, KP1, EBM11, OKIa, and Mac1 stained regularly spaced cells with the morphology of ramified microglia. The cell density was higher and the staining was stronger and more distinct than in the cortical gray matter. Monoclonal antibodies LeuM3 and Ki-M6 yielded weak staining, CLB54 none.

Blood vessels. Single, elongated and flat cells, immunostained with mAbs LCA, LeuM5, LeuM3, LN3, KP1, EBM11, OKIa, and Ki-M6, were regularly observed extending along the wall of cortical blood vessels.

HCHWA-D: Cortical gray matter. The results were virtually identical to those in the non-demented controls (Fig. 2B), except for weaker staining with mAb LN3 and weak staining with mAb Ki-M6. Staining with the lectin RCA-1 confirmed the presence of a reticulum of cells with the morphology of ramified microglia (Fig. 3A).

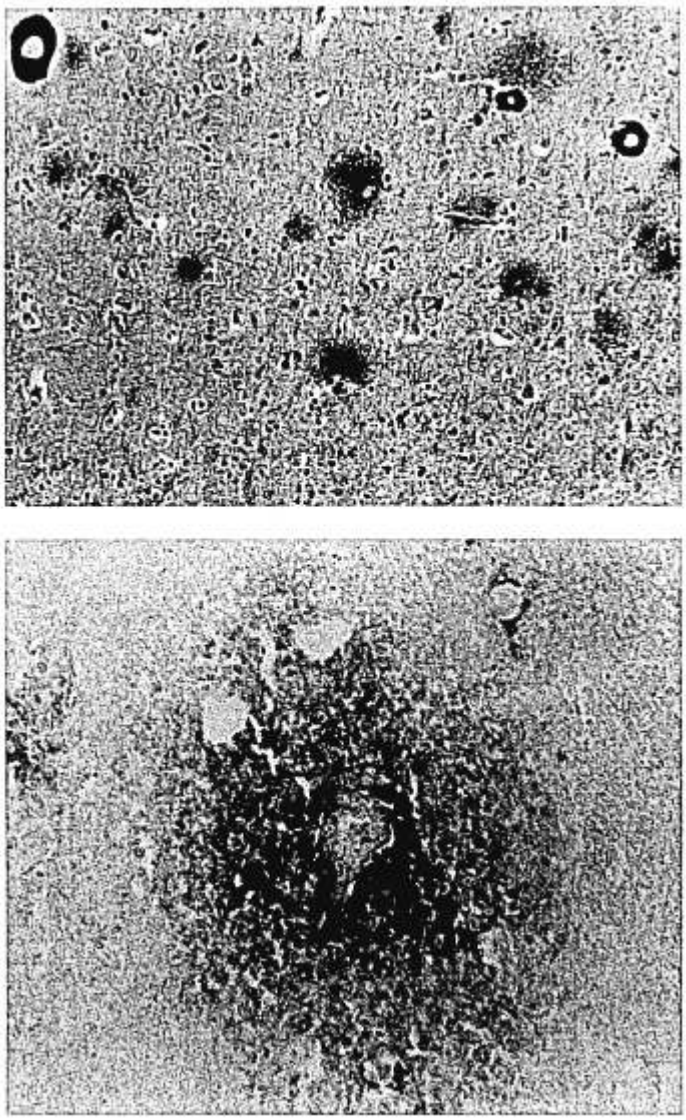

Fig. 1. Paraffin section of the cerebral cortex of a 52-year-old HCHWA-D patient. MS staining shows angiopathy and diffuse plaques, ×50.

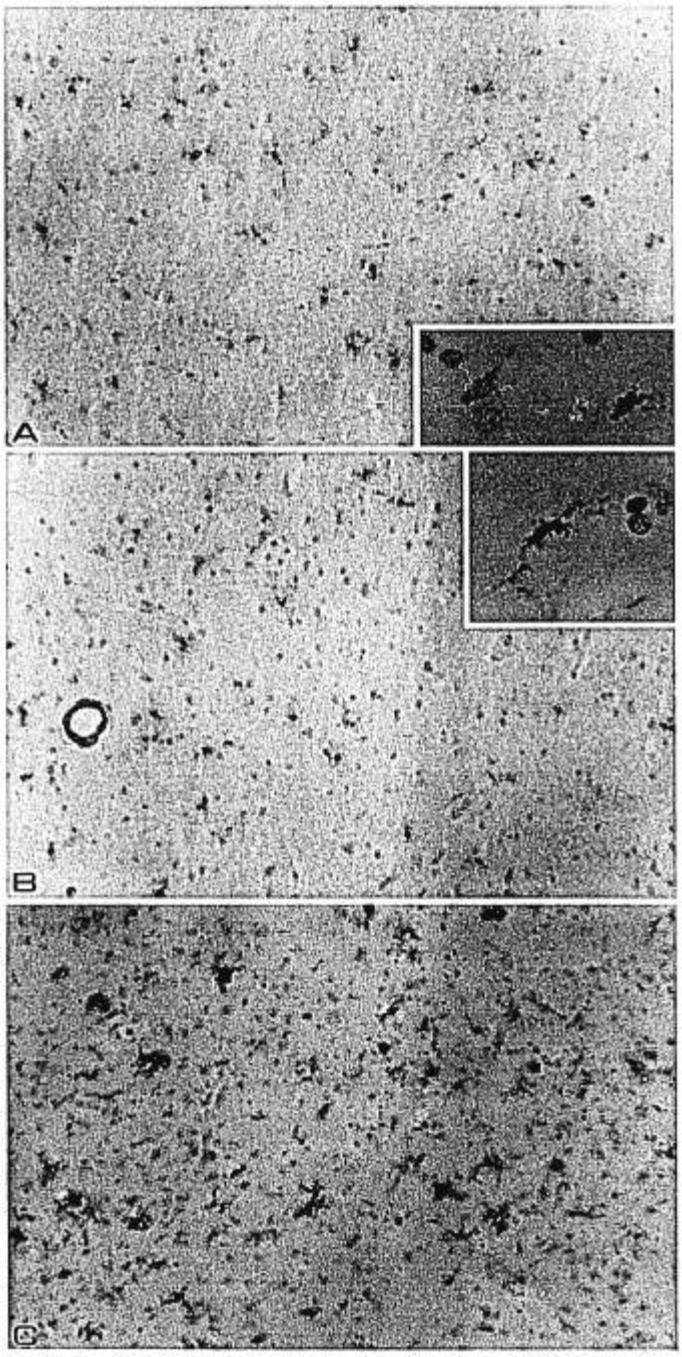

Fig. 2. A. Cortical gray matter of a 36-year-old non-demented control patient, KP1 immunostaining. Cryostat section demonstrating a reticulum of regularly spaced and moderately staining microglial cells, ×50. The inset shows their ramified morphology in a paraffin section, ×200. B. Cortical gray matter of a 40-year-old HCHWA-D patient, KP1 immunostaining. Cryostat section, counterstained with Congo red, showing regularly spaced microglial cells and a congophilic blood vessel, ×50. The picture is comparable to 2A. The inset shows the ramified microglial morphology in a paraffin section, ×200. C. Cortical gray matter of a 48-year-old AD patient. Cryostat section, stained with mAb LeuM5, demonstrating numerous microglial cells, showing strong immunostaining and local clustering, ×50.

Fig. 3. Same patient as in Figure 1. Cryostat section, double-stained with anti-β/A4 antibodies and mAb EBM11. Occasional EBM11-positive microglial cells are present near a β/A4-immunoreactive diffuse plaque, ×200.

Clustering of immunoreactive or RCA-1-positive cells or processes was not observed. Likewise, the double labeling experiments with anti-β/A4 antibodies and mAb EBM11 showed that microglial cells and/or their processes did not cluster in the β/A4-immunoreactive plaques. Only single EBM11-immunoreactive cells with the morphology of ramified microglia and EBM11-immunoreactive processes were sometimes found in the vicinity or within the confines of the β/A4 immunoreactive plaques (Fig. 4). These cells retained their regular spacing throughout the cortical gray matter.

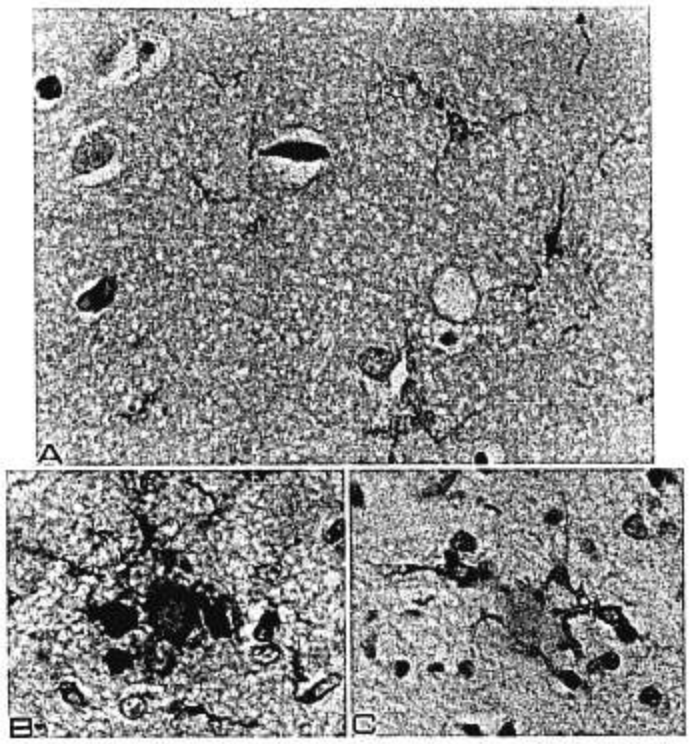

Fig. 4. A. Same patient as in Figure 2B. Paraffin section, stained with RCA-1, showing evenly dispersed ramified microglia. Blood vessels are also stained. B. Cortical gray matter of an 83-year-old AD patient. Paraffin section, stained with RCA-1, Congo red counterstain. Intensely staining, swollen microglia cluster around the congophilic core of a senile plaque, ×200. C. Cortical gray matter of a 57-year-old DS patient. Paraffin section, stained with mAb AMC30, Congo red counterstain. Several microglial cells are clustered in a congophilic senile plaque, ×200.

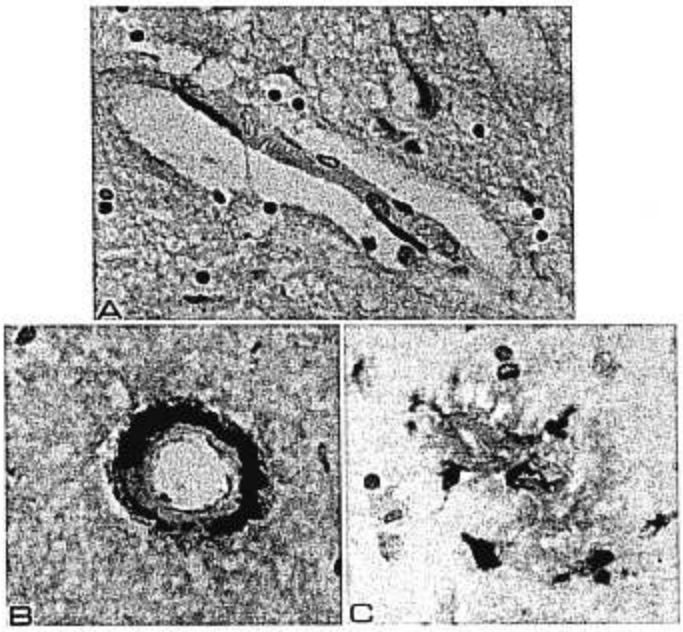

Fig. 5. A. Cerebral cortex of an 84-year-old AD patient. Paraffin section, stained with mAb KP1, showing flat, elongated cells extending along a blood vessel wall, ×200. B. Same patient as in Figure 3. Cryostat section, stained with mAb OKIa, Congo red counterstain. The congophilic wall of a cortical blood vessel shows strong immunoreactivity within its periphery, ×200. C. Cerebral cortex of a 65-year-old AD patient. 24 hour formalin-fixed paraffin section, stained with mAb KP1, using cobaltchloride enhancement, Congo red counterstain. Microglial cells cluster around dyshoric blood vessels, ×200.

Subcortical white matter. The same results were obtained as in the non-demented controls, except for mAbs LeuM3 and Ki-M6 which yielded stronger staining and mAb CLB54 yielding weak though inconsistent staining. The lectin RCA-1 revealed a network of cells with the morphology of ramified microglia.

Blood vessels and CAA. Similar flat cells as described in the non-demented controls were found along the walls of non-congophilic and congophilic blood vessels. Strong, circular or semicircular immunostaining, in particular with mAbs LeuM5, LN3, OKIa and Mac1 (Fig. 5B), was observed in the peripheral part of the congophilic wall of a number of cortical blood vessels. Microglia in the cortical parenchyma surrounding congophilic blood vessels did not show obvious clustering. They retained their ramified morphology.

Macrophages. Prominent staining with mAbs LCA, LeuM5, LeuM3, LN3, KP1, EBM11, OKIa, CLB54, Mac1, and Ki-M6 and the lectin RCA-1 was observed in rounded macrophages without processes, present in remnants of infarcts and hemorrhages. Similar cells were found in the perivascular space of cortical arterioles in the vicinity of such lesions.

AD/DS: Cortical gray matter. Clusters of microglial cells around congophilic amyloid deposits were visualized with all markers (Fig. 3B, C), except with LeuM3 and Mac1. In addition, all markers except LeuM3, Mac1 and CLB54 stained a large number of microglia with an irregular distribution throughout the gray matter (Fig. 2C). The cells often showed morphological evidence of activation, their body being swollen and their processes short and thick. The staining intensity varied among cells but was generally strong, especially in the microglia gathered around the amyloid deposits. Like in HCHWA-D, microglia did not cluster in diffuse plaques. Immunostaining with mAb LN3 was stronger in cryostat sections than in 24 hour formalin-fixed paraffin sections, though the number of stained cells seemed roughly equal. In 1 month formalin-fixed paraffin sections, mAb LN3 only stained microglia gathered around congophilic amyloid deposits and occasional dispersed cells. Monoclonal antibody KP1 stained more microglial cells in cryostat than in paraffin sections.

Subcortical white matter. The results were comparable to those in the non-demented controls, except for weak

TABLE 4
Other Antibodies

Antibody (against)	Raised in	Specificity	Dilution	Technique	Reference
β/A4 protein	rabbit	antiserum to synthetic peptide residue 1–28	1:500	PAP	Masters et al (48)
MigT4[a]	mouse	tangles, dystrophic neurites	1:50	ABC	Gheuens et al (49)

[a] NV Innogenetics sa, Belgium.

staining observed with mAbs CLB54 and Mac1 and stronger staining with mAb Ki-M6. Occasional cells were labeled by mAb AMC30.

Macrophages. Rounded macrophages were encountered in a few small infarcts in paraffin sections stained with mAbs LN3, KP1 and AMC30. They showed strong immunoreactivity with these mAbs.

Blood vessels and CAA. Like in the non-demented controls and the patients with HCHWA-D, immunostained, flat, elongated cells were present along cortical blood vessels (Fig. 5A). Immunostaining of the peripheral part of congophilic blood vessel walls was not observed. Swollen microglial cells clustered around blood vessels displaying dyshoric angiopathy (Fig. 5C).

DISCUSSION

This study demonstrates 1) the absence of clustering and activation of microglial cells in diffuse plaques in HCHWA-D in contrast to clustering and activation of microglial cells in congophilic plaques in AD/DS; 2) monocyte/macrophage marker immunoreactivity, including HLA-DR expression, of elongated, flat cells along the wall of cortical blood vessels in controls and all patients; and 3) strong immunostaining with monocyte/macrophage markers of the peripheral part of cortical congophilic blood vessel walls in HCHWA-D.

TABLE 5
Staining of Microglia with Macrophage/Microglia Markers

	HCHWA-D		AD/DS			Controls		
	GM	WM	CCP	GM	WM	GM	WM	mph
Cryostat								
LCA	+	+	+	+	+	+	+	+
LeuM5	+	+	+	+	+	+	+	+
LeuM3	−	+	−	−	±	−	±	+
LN3	±	+	+	+	+	+	+	+
KP1	+	+	+	+	+	+	+	+
EBM11	+	+	+	+	+	+	+	+
OKIa	−	+	+	+	+	−	+	+
CLB54	−	±	+	−	±	−	−	+
Mac1	−	+	−	−	±	−	+	+
Ki-M6	±	+	±	±	+	−	±	+
Paraffin								
RCA-1	+	+	+	+	+	np	np	+
AMC30	np	np	+	+	−	−	−	+
LN3	np	np	+	+	+	np	np	+
KP1	+	+	+	+	+	+	+	+

HCHWA-D: hereditary cerebral hemorrhage with amyloidosis (Dutch); AD: Alzheimer's disease; DS: Down's syndrome; mph: macrophages; GM: gray matter; WM: white matter; CCP: microglial clusters around congophilic plaques; + = positive staining; − = no staining; ± = weak staining; np = not performed.

Whereas diffuse plaques were abundantly present in the cerebral cortex of the HCHWA-D patients, microglial cells did not cluster in the plaques nor did they show evidence of activation in the sense of changes in their morphology and/or enhanced expression of monocyte/macrophage markers, including HLA-DR, in comparison to the non-demented controls. These findings confirm our previous observations in HCHWA-D with a limited number of macrophage/microglia markers (53). We now extend these findings by using double labeling with anti-β/A4 antibodies and mAb EBM11, which demonstrated the occasional presence of single immunoreactive microglial cells or their processes within or near diffuse plaques in HCHWA-D cortex. These cells retained their ramified morphology and regular spacing as part of a reticular array of ramified microglia in cortical gray and subcortical white matter similar to that observed in the controls of the present study.

The finding of a reticulum of ramified microglia in our control patients with monocyte/macrophage markers (mAbs LCA, LeuM5, KP1, and EBM11) is in agreement with previous studies of control brain (16, 21–23). Anti-HLA-DR-immunoreactive microglia are sparse in normal cortical gray matter (15, 18, 23) in contrast to the presumed constitutive HLA-DR expression by subcortical white matter microglia (23). Accordingly, subcortical white matter microglia in all our controls and patients strongly expressed HLA-DR in cryostat sections stained with mAbs OKIa and LN3. Cortical gray matter microglia of the HCHWA-D and control patients were negative with mAb OKIa and they showed only weak to moderate HLA-DR expression with mAb LN3. Clinical factors such as chronic debilitating diseases, which may apply to our control patient who died of carcinoma of the lung, presumably induce or enhance the expression of HLA-DR on gray matter microglia (23). Furthermore, the detection of HLA-DR as well as other monocyte/macro-

phage markers on microglia also depends on technical factors (23), which is illustrated in the present study by the differences in results obtained with mAb LN3 used on cryostat or paraffin sections. In this regard, Perlmutter et al (54) recently suggested that HLA-DR-expressing microglia may be a more common feature of human neocortical gray matter than has been previously described.

Microglia showing clear evidence of activation in view of their morphology and strong monocyte/macrophage marker, in particular anti-HLA-DR, immunoreactivity clustered around congophilic amyloid deposits in AD/DS. These cells also showed strong staining with mAb AMC30, a marker for "reactive" microglia (47), and with the lectin RCA-1. This confirms the observations of many other studies that activated microglia cluster around congophilic senile plaques (5, 11, 13–18, 22, 23).

From the foregoing observations it appears that microglia are present in plaques from an early stage onward. However, as microglial cells are parts of a uniformly spaced reticular array, their initial presence in diffuse plaques may well be coincidental. Clustering of microglia and evidence of their activation by changes in morphology and by clearly enhanced expression of macrophage/microglia markers do not appear until presumably later plaque stages. This implies that microglia do not play a primary role in plaque formation; that is, they do not initiate the formation of diffuse plaques. Rather, they play a secondary role, becoming activated at some point in the slow process of plaque formation.

The elongated cells, observed in this study along non-congophilic blood vessels of both controls and patients as well as along congophilic blood vessels, are most likely identical to the perivascular cells described in normal human brain by Graeber (55). These cells are located between the vascular and parenchymal basement membranes and constitutively express HLA-DR class II antigen (55). They are shown to be of bone marrow origin (56). The strong immunostaining with mAbs LeuM5, LN3, OKIa and Mac1 in the peripheral part of the congophilic wall of a number of cortical blood vessels in HCHWA-D may be related to these perivascular cells. This relationship may be a primary one, if, as proposed by Wisniewski et al (57), perivascular cells are the manufacturers of the amyloid fibrils of vascular amyloid. On the other hand, if, for instance, vascular amyloid fibrils are formed within the vascular basement membrane, outside of cells (58), the immunostaining may be a secondary phenomenon, possibly representing a reaction of perivascular cells to amyloid once it penetrates between the vascular and parenchymal basement membranes. In this way, the virtual absence of such staining in blood vessels showing congophilic angiopathy of Pantelakis, as observed in six of our AD/DS patients, may be explained by the less advanced degree of amyloid deposition in these as compared to HCHWA-D blood vessels. In the same way, the clustering of microglial cells around dyshoric blood vessels, as observed in two of our AD patients, may represent the microglial reaction to vascular amyloid finally invading the nervous parenchyma.

Taken together, the clustering of activated microglia around congophilic senile plaques in AD/DS and the absence of this phenomenon in diffuse plaques in HCHWA-D support the view that microglia play a role in the formation of congophilic plaques but do not initiate diffuse plaque formation. The strong macrophage/microglia marker immunoreactivity found in the wall of congophilic blood vessels in HCHWA-D is suggestive of a relationship between perivascular cells and vascular amyloid analogous to the one between microglia and the amyloid core of senile plaques.

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