Virchows Arch (2002) 441:358–367
DOI 10.1007/s00428-002-0643-8

ORIGINAL ARTICLE

**Atsushi Sasaki · Mikio Shoji · Yasuo Harigaya
Takeshi Kawarabayashi · Masaki Ikeda
Makoto Naito · Etsuro Matsubara · Koji Abe
Yoichi Nakazato**

Amyloid cored plaques in Tg2576 transgenic mice are characterized by giant plaques, slightly activated microglia, and the lack of paired helical filament-typed, dystrophic neurites

Received: 19 December 2001 / Accepted: 21 February 2002 / Published online: 1 May 2002

Abstract We examined the brains of Tg2576 transgenic mice carrying human amyloid precursor protein with the Swedish mutation and Alzheimer's disease (AD) by means of immunohistochemistry and electron microscopy to clarify the characteristics of amyloid-associated pathology in the transgenic mice. In 12- to 29-month-old Tg2576 mice, congophilic cored plaques in the neocortex and hippocampus were labeled by all of the Aβ1-, Aβ40- and 42-specific antibodies, as seen in the classical plaques in AD. However, large-sized (>50 μm in core diameter) plaques were seen more frequently in the older mice (18–29 months) than in those with AD (approximately 20% vs 2% in total cored plaques), and Tg2576 mice contained giant plaques (>75 μm in core diameter), which were almost never seen in the brain of those with AD. Neither thread-like structures nor peripheral coronas were observed in the cored plaques of the transgenic mice in the silver impregnations. Immunohistochemically, plaque-accompanied microglia showed a slight enlargement of the cytoplasm with consistent labeling of Mac-1 and macrosialin (murine CD68), and with partial labeling of Ia antigen and macrophage-colony stimulating factor receptor. Ultrastructurally, the microglia surrounding the extracellular amyloid fibrils in the large, cored plaques showed some organella with phagocytic activity, such as secondary lysosomal, dense bodies, but intracellular amyloid fibrils were not evident. Dystrophic neurites in the plaques of the transgenic mice contained many dense multilaminar bodies, but no paired helical filaments. Our results suggest that giant cored plaques without coronas or paired helical filament-typed, dystrophic neurites are characteristic in Tg2576 mice, and that plaque-associated microglia in transgenic mice are activated to be in phagocytic function but not sufficient enough to digest extracellularly deposited amyloid fibrils.

Keywords Alzheimer's disease · Amyloid β protein · Microglia · Senile plaques · Transgenic models

A. Sasaki (✉) · Y. Nakazato
Department of Pathology, Gunma University School of Medicine,
3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan
e-mail: achie@med.gunma-u.ac.jp
Tel.: +81-27-2207972, Fax: +81-27-2207978

Y. Harigaya · T. Kawarabayashi · M. Ikeda
Department of Neurology, Gunma University School of Medicine,
Maebashi, Japan

M. Naito
Second Department of Pathology,
Niigata University School of Medicine, Niigata, Japan

M. Shoji · E. Matsubara · K. Abe
Department of Neurology,
Okayama University Graduate School of Medicine,
Okayama, Japan

Introduction

It is important to establish an animal model of Alzheimer's disease (AD) to study its pathogenesis and develop therapeutic drugs. Recent studies in transgenic mice have indicated that overproduction of mutant β-amyloid protein precursor (APP) causes increases in Aβ deposition forming senile plaques [2, 5, 9, 10, 14, 15, 17, 20, 27, 29]. Of these transgenic models, Tg2576 mice carrying human APP with the Swedish mutation are considered as a good model for AD, since the Tg2576 mice develop age-dependent behavioral deficits and elicit amyloid plaques with a dense core that are similar to the classic neuritic plaques seen in AD [6, 31]. Thus, a detailed pathological assessment of Tg2576 mice is necessary to evaluate how close the pathology associated with dense amyloid deposits in the mice fits that seen in AD.

Activated microglia were consistently observed in primitive and classic plaques in the brains of AD patients [16, 21, 25]. In vitro studies showed the production of cytokine and neurotoxic molecules by activated microglia [8, 11, 22, 23]. These data support an important role of microglia in the disease process of AD. In addition, a

Table 1 Antibodies used in the study. GFAP glial fibrillary acidic protein, *SR* scavenger receptor, *CSF-1R* colony stimulating factor-1 receptor, *M-CSFR* macrophage-colony-stimulating factor receptor

Antibody	Specificity	Type	Dilution	Source
Ab-9204	Amyloid beta 1–6 aa	Rabbit serum	×500	T. Saido
S40	Amyloid beta 36–40 aa	Rabbit serum	×500	Y. Harigaya
S42	Amyloid beta 38–42 aa	Rabbit serum	×500	Y. Harigaya
Anti-GFAP	GFAP (40–55 kD)	Rabbit serum	×1000	Y. Nakazato
Mac-1	CD11b/CD18, CR3	Rat monoclonal antibody	×20	Behringer-Mannheim
F4/80	Pan-macrophages	Rat monoclonal antibody	×1	S. Gordon
M5/114	Mouse Ia antigen	Rat monoclonal antibody	×20	Behringer-Mannheim
C-20	C-fms/CSF-1 R (M-CSFR)	Rabbit serum	×1000	Santa-Cruz
2F8	SR class AI/II	Rat monoclonal antibody	×250	S. Gordon
FA-11	SR class D (macrosialin/CD68)	Rat monoclonal antibody	×1000	M. Naito
ER-MP20	Macrophage precursor antigen	Rat monoclonal antibody	×100	M. Naito
ER-MP58	Macrophage precursor antigen	Rat monoclonal antibody	×100	M. Naito

recent study has shown that passively administered antibodies against Aβ peptide reduced the extent of plaque deposition in a transgenic mouse model of AD and suggested that microglia are responsible for clearing plaques through Fc receptor-mediated phagocytosis and/or astroglia-derived, transforming growth factor β1 (TGFβ1) production [3, 26, 32].

In the present study, to clarify the similarities and differences between AD and Tg2576 plaques, and to characterize microglial activation in Tg2576 at light microscopic and ultrastructural levels, we studied the brains of Tg2576 mice using immunohistochemistry with various microglia/macrophage markers, immunoelectron microscopy, and transmission electron microscopy.

Materials and methods

Animals

Tg2576 mice expressing $\beta APP695_{K595N/M596L}$ used in this study have been described previously [14, 29]. We used a total of ten Tg2576 mice (five male and five female) between 12 months and 29 months of age. Non-transgenic littermates served as controls. The animals were sacrificed by ether, and the organs were immediately extracted and processed according to the following procedure.

AD brains

We examined the brains of 11 clinically and histopathologically confirmed AD cases (age 66–84 years), which were obtained at autopsy.

Light microscopy and immunohistochemistry

For light microscopy and immunohistochemistry, the brains from Tg 2576 mice were fixed in 4% paraformaldehyde (PFA) with 0.1 M phosphate-buffered saline (PBS, pH 7.6) and embedded in paraffin, and the autopsy brains of the AD cases were fixed in buffered formalin and embedded in paraffin. The paraffin-embedded sections were cut 5 μm thick and stained using hematoxylin and eosin (HE), Congo red, Bodian, methenamine-silver (M-S), Gallyas, and modified Bielshowsky methods. Some blocks of the mice brains were fixed with a periodate–lysine–PFA (PLP) fixative at 4°C for 4–6 h, washed with PBS containing 10, 15, and 20% sucrose, and embedded in OCT compound (Miles, Elkhart, USA). The specimens were frozen in liquid nitrogen and cut into 6- 8-μm-thick sections using a cryostat. The primary antibodies used in immunohistochemistry are summarized in Table 1. Aβ immunohistochemistry was performed on the paraffin sections using a Vectastain Elite ABC kit (Vector), while immunohistochemical analyses for the other primary antibodies were performed on cryostat-cut tissue sections. Some of the frozen sections were fixed in 4% PFA for 30 min at 4°C. After incubation with the anti-mouse monoclonal antibodies described in Table 1, biotinylated goat-anti-rat Ig (1:50 diluted, Tago, Inc., Camerillo, Calif.) was used as a secondary antibody, followed by the addition of streptavidine/biotin-peroxidase complex (1:1, Nichirei Corp, Tokyo). After visualization with 3, 3′-diaminobenzidine, the sections were briefly stained with hematoxylin for nuclear staining. For semiquantitative analysis, the size of the amyloid core was measured using a ×10 eye piece micrometer and a ×40 objective lens (0–250 μm in diameter) in the cerebral sections stained with Aβ40- or Aβ42-specific antibodies in the brains of Tg2576 mice and AD cases.

Immunoelectron microscopy

The brain tissue samples from 20-month-old Tg2576 mice were fixed in PLP for 6 h at 4°C. After washing in PBS containing a graded series of sucrose, the tissues were embedded in OCT compound and rapidly frozen in liquid nitrogen. Frozen sections (6–8 μm) of the cerebral cortices were incubated with Mac-1 antibody followed by the addition of horseradish peroxidase-conjugated goat anti-rat IgG (H+L) (Tago). After immunostaining, the sections were embedded in Quetol 812 (Nisshin EM), and ultrathin sections were cut. They were contrasted with uranyl acetate and examined using electron microscopy.

Transmission electron microscopy

Cerebral cortical tissues of 20-month-old Tg2576 mice were cut into small pieces, immersed in fixative (2.5% glutaraldehyde, 0.1 M phosphate buffer, pH 7.4) for 4 h and washed several times in 0.1 M phosphate buffer containing 7% sucrose. They were post-fixed in 2% osmium tetroxide, dehydrated in a graded series of ethanol and propylene oxide, and embedded in Quetol 812. Ultrathin sections were cut, then stained with uranyl acetate and lead acetate prior to electron microscopy.

Results

Dense-cored plaques in Tg2576 mice were labeled with Congo red and Aβ immunostaining, and associated with astrogliosis, as in classic plaques of AD

Dense-cored plaques labeled with either Aβ40 or Aβ42 were present in the Tg2576 brain at 12 months. Only a few β-amyloid deposits were observed in 12-month-old

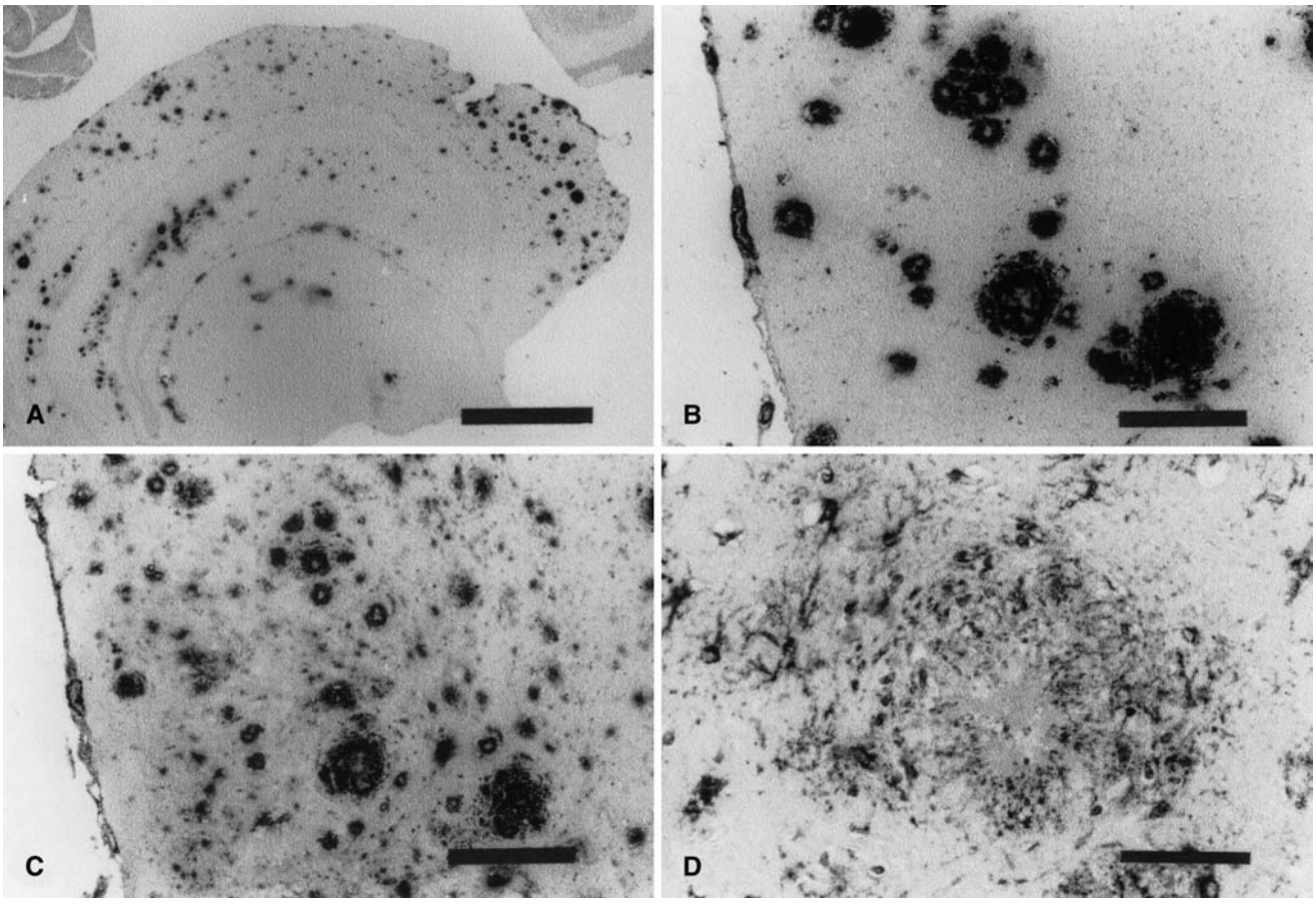


Fig. 1 Amyloid deposits in the Tg2576 mouse brain. Senile plaques with large dense cores and amyloid angiopathy in 23-month-old transgenic mice were visualized by means of Aβ immunohistochemistry using Aβ1 (**a**), Aβ40 (**b**), and Aβ42 (**c**) antibodies. Note astrogliosis in close proximity to the dense-cored plaques in 20-month-old Tg2576 mice visualized using double staining of Congo red and glial fibrillary acidic protein immunohistochemistry (**d**). *Bars* 1 mm (**a**), 200 μm (**b**, **c**), 50 μm (**d**)

Table 2 Quantification of Aβ40-positive, cored plaques in Tg2576 mice. The cerebral hemisphere was stained with anti-Aβ40 antiserum, and the immunopositive plaques were counted per section. Numbers in parentheses show the percentage of total cored plaques in the section

Mouse number	Age (months)	Total plaques	Plaques in different core size		
			<50 μm	50–75 μm	>75 μm
1	12	17	14 (82.35)	1 (5.88)	2 (11.76)
2	14	23	21 (91.30)	1(4.34)	1 (4.34)
3	15	14	13 (92.85)	0 (0)	1 (7.14)
4	15	38	33 (86.84)	3 (7.89)	2 (5.26)
5	18	56	41 (73.21)	11 (19.64)	4 (7.14)
6	18	72	58 (80.55)	7 (9.72)	7 (9.72)
7	23	111	73 (65.76)	30 (27.02)	8 (7.20)
8	29	96	69 (71.87)	21 (21.87)	6 (6.25)

Tg2576 mice, while the density of β-amyloid deposits considerably increased at 18 months (Table 2). In 20-, 23-, and 29-month-old Tg2576 mice, large numbers of dense-cored plaques which were labeled with Aβ1, Aβ40 or Aβ42 were observed in the neocortex and hippocampus (Fig. 1a–c). Congophilic, β-amyloid deposits were observed not only in the amyloid plaques, but also in the vascular wall of the meninx and cerebral parenchyma. Increased astrocytic processes of astrogliosis around the amyloid core, which is a common finding of classic plaques in AD, were detectable in the cored plaques in Tg2576 mice by glial fibrillary acidic protein immunohistochemistry (Fig. 1d). Diffuse plaques with Aβ40-negative and Aβ42-positive immunohistochemistry were observed in Tg2576 (Fig. 1b, c), although diffuse plaques were not a predominant form of senile plaques in Tg2576 in contrast to AD. Neither Aβ immunostaining nor positivity for Congo red staining could be detected in the brains of the controls.

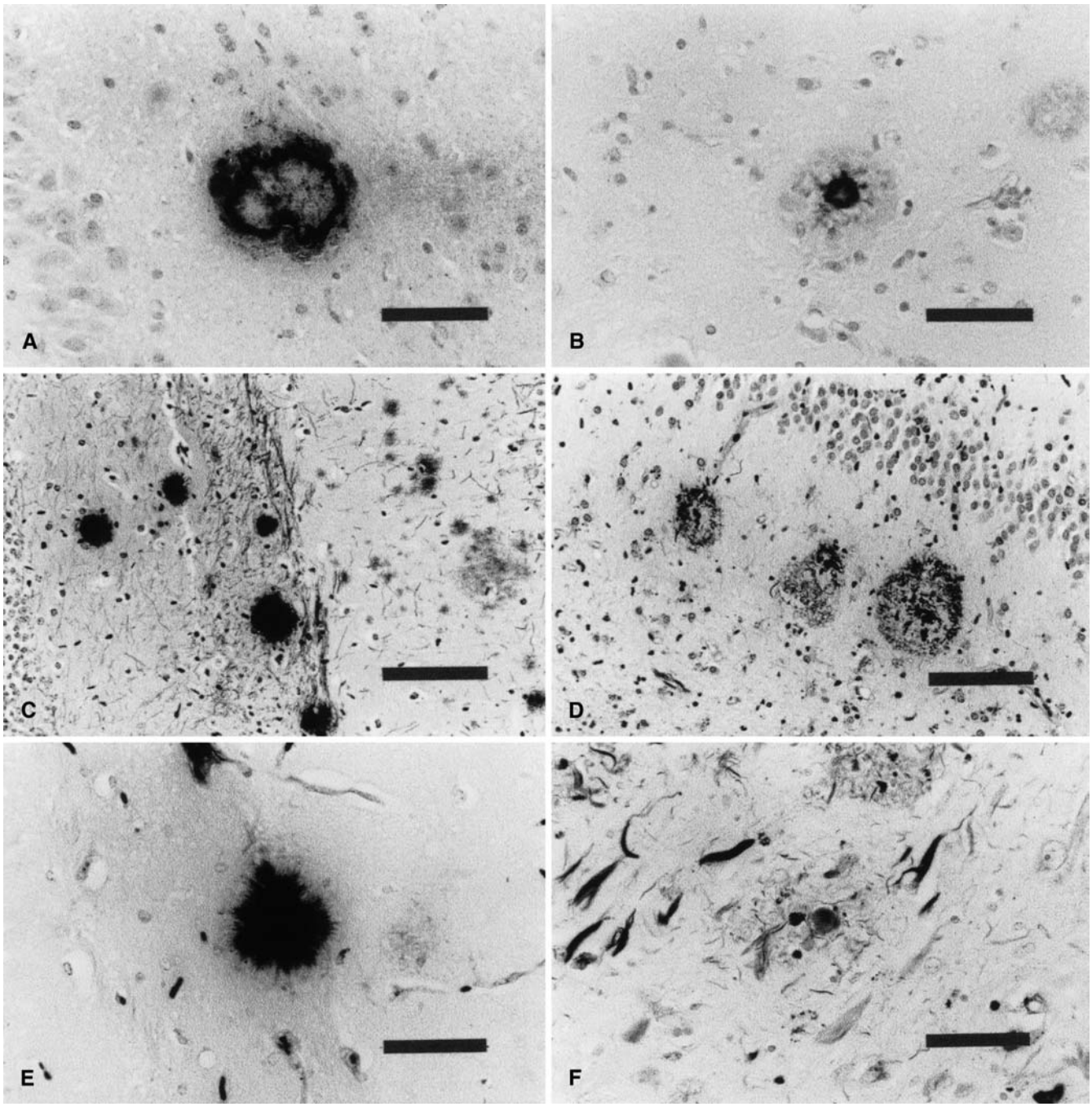


Fig. 2 Comparison of dense-cored plaques in the hippocampal region between the Tg2576 mice (**a**, **c**, **e**) and those with Alzheimer's disease (AD; **b**, **d**, **f**). Twenty-month-old Tg2576 mice showed amyloid plaques with large dense cores lacking corona and Gallyas-positive neurites, while those with AD contained smaller classic plaques and compact plaques with corona and tuft-shaped neurites. Neurofibrillary tangles were also observed by Gallyas impregnation in those with AD. Aβ40 immunohistochemistry (**a**, **b**), methenamine-silver (**c**, **d**) and Gallyas (**e**, **f**) stainings. *Bars* 50 μm (**a**, **b**, **e**, **f**), 100 μm (**c**, **d**)

Compared with AD, Tg2576 mice showed larger plaques, including giant plaques, and neither thread-like structures nor peripheral coronas

Quantitative data of cored plaques in different ages of Tg2576 mice are summarized in Table 2. Giant plaques (>75 μm in the core diameter) were present at 12 months, and increased between 18 months and 23 months, comprising approximately 5–10% of the total cored plaques (Fig. 1b–d, Fig. 2a). Plaques larger than 50 μm in diameter of the core represented approximately 20% (19.3±3.4%, mean±SEM; n=8) of all dense-core plaques in Tg2576 mice, while those plaques were ap-

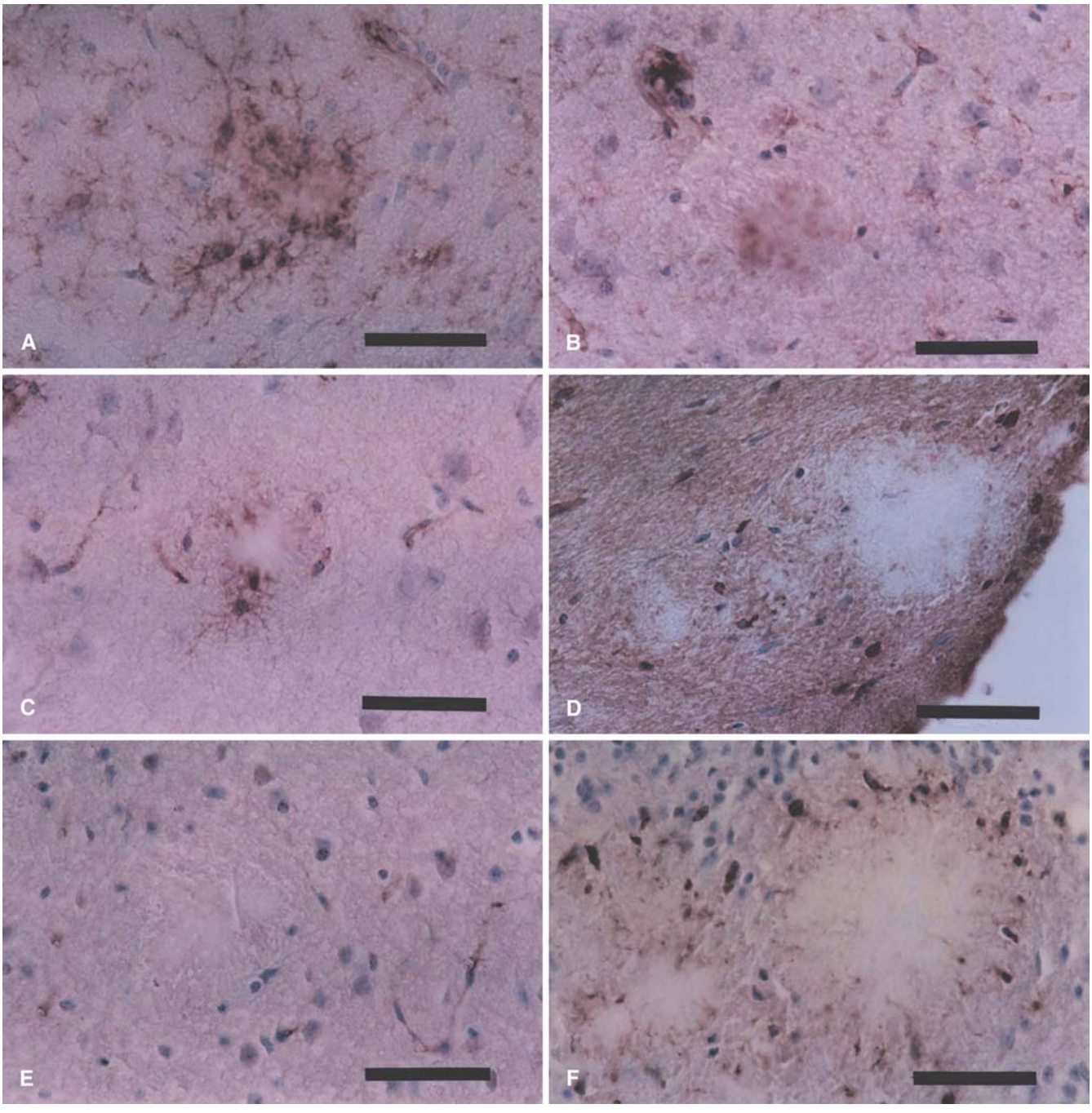


Fig. 3 Cored plaque-related activation of the microglia in transgenic mice. Immunocytochemical analysis of 20-month-old Tg2576 brain showed that the microglia surrounding dense amyloid core had consistent labeling of Mac-1 (**a**) and macrosialin (**f**), and with partial labeling of Ia antigen (**c**) and macrophage-colony stimulating factor receptor (**d**). Note a weak staining of F4/80 (**b**) and the absence of scavenger receptor A (**e**). *Bars* 50 μm (**a**–**f**)

proximately 2% (1.7±0.8%, mean±SEM; n=11) in those with AD. Giant plaques were almost never seen in the brains of those with AD (Fig. 2b). In the dense-cored plaques of the Tg2576 mice, amyloid cores were positively stained with Bodian, M-S (Fig. 2c), and Gallyas (Fig. 2e) stainings, as in classic plaques in AD. In contrast to AD, however, neither peripheral coronas nor thread-like structures were observed in the cored plaques of the mice in M-S and Gallyas preparations, respectively (Fig. 2c–f).

Cored plaque-associated microglia showed immunophenotypical activation

Clusters of Mac-1-stained microglia were found around the cores of amyloid plaques in all the regions containing compact amyloid plaques throughout the neocortex and hippocampus (Fig. 3a). The F4/80 antibody showed less

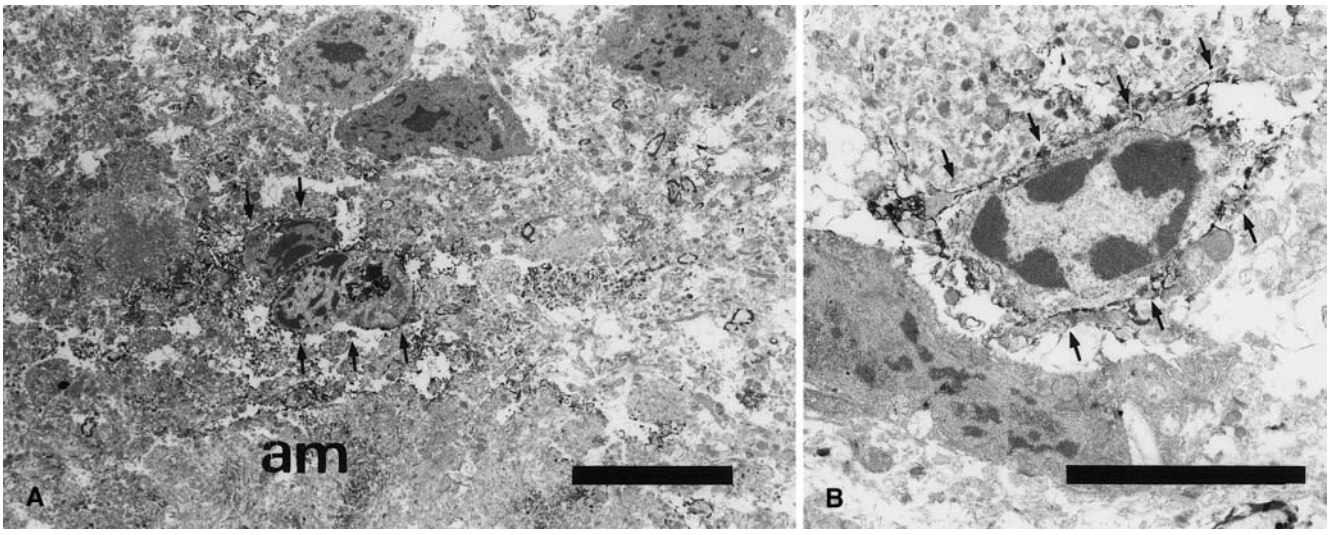


Fig. 4 Immunoelectron micrograph using Mac-1 antibody in Tg2576 transgenic mice. Mac-1 immunostaining revealed labeling of the microglial cell surface (*arrows*) at the periphery of the amyloid plaque with bundles of amyloid fibrils (*am*). *Bars* 10 μm (**a**), 5 μm (**b**)

intensity of microglial staining than Mac-1 (Fig. 3b). A subpopulation of microglia surrounding the compact plaques was positive for Ia antigen (Fig. 3c). Only a small portion of plaque-associated microglia showed a positive reactivity for the macrophage-colony-stimulating factor (M-CSF) receptor (Fig. 3d). With regard to the expression of the scavenger receptor (SR), SR class A reactivity was not found in the plaque-associated microglia, while SR class D – macrosialin (murine CD68) – was found in those cells (Fig. 3e, f). The plaque-associated microglia stained with each of the above markers showed a mild increase in the cytoplasm, but no ameboid shape, at the light microscopic level. No reactivity of monocyte/macrophage-precursor cell markers, ER-MP20 and ER-MP58, was found within the activated microglia (data not shown).

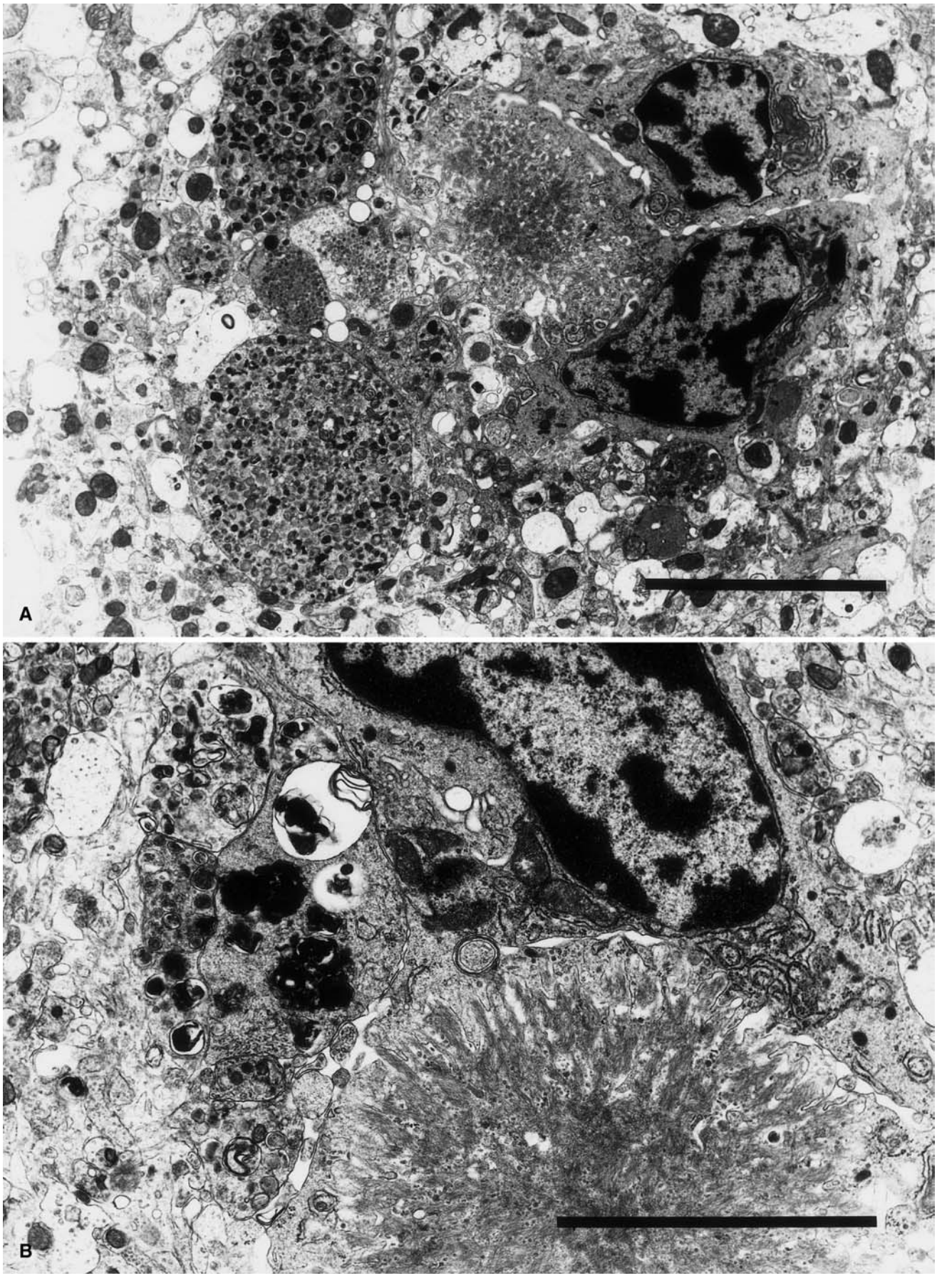
Microglia at the interface with amyloid fibrils showed characteristics of phagocytosis but no intracellular amyloid fibrils

Immunoelectron micrographs of Mac-1-immunostained sections confirmed Mac-1 specificity to the surface of microglial cell bodies and processes at the periphery of amyloid plaques (Fig. 4a, b). The nucleus had a block of heterochromatin under the nuclear membrane, and the cytoplasm was narrow with few developed cell organelles.

Ultrastructure of small amyloid plaques revealed mild to moderate amyloid deposits in the center, and the presence of dystrophic neurites and increased microglia in the periphery (Fig. 5a). The amyloid–microglia interface was partly ruffled, and the microglial cell membrane formed a close association with the radially oriented, amyloid fibrils (Fig. 5b). In the large plaques, an amyloid core of very dense extracellular amyloid fibrils, numerous dystrophic neurites, neuronal cell bodies, astrocytic processes, and microglial cells was embedded in the plaque. The amyloid fibrils consisted of filaments of approximately 10 nm in diameter. The microglia in contact with the dense amyloid fibrils had typical characteristics of phagocytosis of lipofuscin-like, lysosomal inclusion bodies in the cytoplasm (Fig. 6). In both the small and large cored plaques, amyloid fibrils were absent within the microglial cytoplasm. The dystrophic neurites contained numerous structures such as dense bodies, lamellar structures, mitochondria and Hirano body-like changes. No paired helical filaments (PHFs) were observed.

Discussion

This study demonstrated age-related β-amyloid deposition as cerebral amyloid angiopathy and senile plaques in the neocortex and hippocampus, and that cored plaques, not diffuse plaques, appear early and are the predominant form of plaques. These findings are almost compatible with the observations previously described in APP transgenic mice [17, 27]. The cellular components of the cored plaques in Tg2576 mice were very similar to those of classic plaques in AD with regard to a core of dense amyloid fibrils, dystrophic neurites, activated microglia, and astrocytic processes. However, this study showed that the cored plaques of Tg2576 lacked peripheral amyloid coronas (the crown) of the plaque, which are stained by Aβ immunohistochemistry and silver impregnation methods in classic plaques of the cerebrum in AD [33, 34]. Moreover, in contrast to AD, the formation of large plaques, including giant plaques more than 75 μm in the core diameter, was characteristic in the cored plaques of Tg2576 mice. As a comparative study with regard to the size of the cored plaques in the hippocampus between Tg2576 mice and AD brains, this study indicated that


A
B

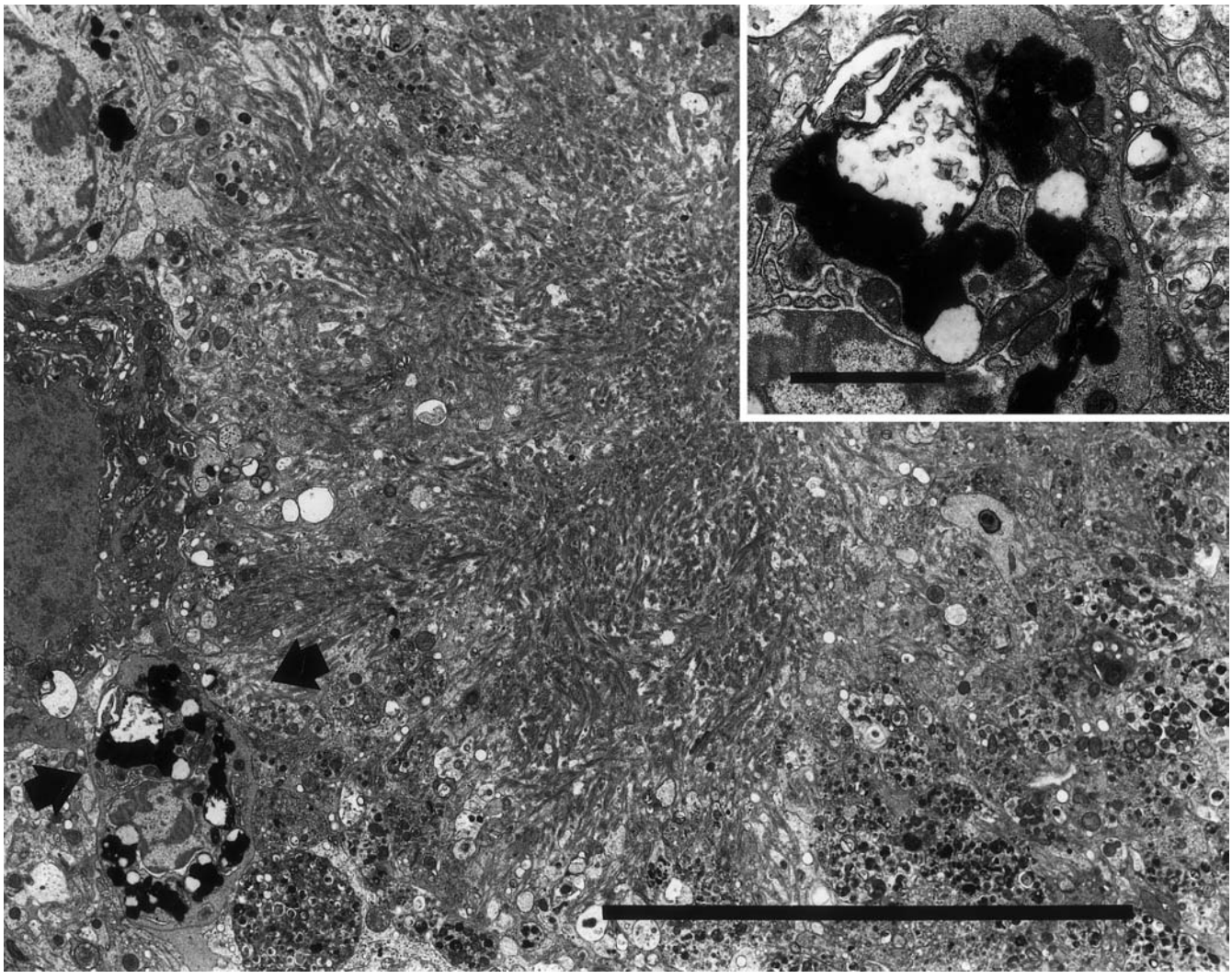


Fig. 6 Ultrastructure of large amyloid plaques in Tg2576 mice. *Inset* Higher magnification of the area indicated by *arrows*. The microglia surrounding abundant very dense amyloid cores shows typical characteristics of phagocytosis such as vacuoles and inclusion bodies. *Bars* 20 μm (*a*), 2 μm (*inset*)

Tg2576 mice contained large plaques of more than 50 μm, approximately tenfold those of AD, and that the giant plaques are a distinct feature in Tg2576 mice. Our quantitative data showed that giant plaques were present in 12-month-old mice, and increased in aged mice, from 18 months of age, correlating with markedly increased numbers of cored plaques. Plaque size in APP transgenic mice has not been investigated in detail in previous studies, but previous studies using different APP transgenic mice (Tg2576, APP23, PDAPP) have demonstrated similar histological findings of dense-cored plaques [2, 5, 9, 20, 27]. Thus, these characteristics appear common to each of the APP transgenic mice.

◀ **Fig. 5** Ultrastructure of small amyloid plaques in Tg2576 mice. **a** An amyloid core was surrounded by two microglial cells and dystrophic neurites containing abundant electrondense multilaminar bodies. **b** The microglia made close contact with radially oriented, amyloid fibrils but contained no amyloid fibrils in the cytoplasm. *Bars* 6 μm (**a**), 5 μm (**b**)

To better understand the microglial reaction, we evaluated its activation state in the brains of Tg2576 mice using various types of microglia/macrophage markers, light microscopy and electron microscopy. Using Mac-1 antibody, an increased cell number and size of microglial cells around cored amyloid plaques was observed in this study. These observations are similar to findings in the brains of APP transgenic mice and AD patients [5, 9, 16, 21, 25, 27]. From the Mac-1 staining, most of the plaque-associated microglia were considered to be activated slightly or moderately at light microscopic levels. The mechanism for microglial proliferation and activation in APP transgenic mice remains unclear. This study first demonstrated the expression of the M-CSF receptor on plaque-associated microglia in APP transgenic mice. However, the receptor does not seem to be a strong candidate as an important mediator of microglial activation at plaques in the brains of Tg2576 mice, since only a subpopulation of microglia showed upregulation of the M-CSF receptor in this study.

Regarding the microglia surrounding the amyloid cores, Ia antigen (MHC class-II protein) immunohistochemistry showed less reactivity than Mac-1 staining, as described in a previous study of APP23 transgenic mice [5]. In plaque-associated, activated microglia, the MHC class-II antigen expression of the transgenic mice seemed less marked than that of human AD. It is important to note that MHC class II in mice is much less easily induced on microglia than that in humans [1, 18], and that phagocytic microglia in non-human gray matter are generally not MHC class-II positive, if phagocytosis is not associated with a strong, immune-response-mediated activation [4, 28]. This possibility is supported by the observation that we did not obtain any evidence of lymphocytic infiltration in the gray matter of Tg2576.

The macrophage scavenger receptor (MSR) family has recently been extended and is now divided into seven types, including classes A and D [13, 24]. The broad ligand specificity of the receptor suggests multifunctional roles in various physiological and pathological conditions, such as athelosclerosis and AD. In this study, we examined the expression of two types of MSR – class A type I/II and class D (CD68/macrosialin) – on plaque-associated microglia. The MSR class-A expression on plaque-associated microglia seemed to be absent or very low in Tg2576 mice. In contrast to class A type I/II, plaque-associated microglia accumulated macrosialin in nearly all the compact amyloid plaques in this study. Increased levels of CD68 indicate that the cells are phagocytically active and digest internalized material because they accumulate lysosomal vacuoles [30]. Resting microglia show a low macrosialin expression, as described here and in a previous study [5]. Taken together, an increase in macrosialin indicates evidence of a phagocytic capability of the microglia in mice. Consistent with these results, we demonstrated microglia with typical phagocytic morphology at the ultrastructural level. Our immunohistochemical and ultrastructural studies indicated that plaque-associated microglia have the function of phagocytic removal using a lysosomal system, but no evidence of digesting amyloid fibrils.

Our electron microscopic study consistently demonstrated dystrophic neurites with large numbers of dense laminar bodies around the small and large cored plaques. These dystrophic neurites correspond to spherical (type I) or dystrophic-type neurites. Another type of neurites, fusiform (type II) neurites or PHF-type neurites, were absent in this study and in PDAPP transgenic mice [20]. Dystrophic-type neurites are found in senile plaques in AD and in transgenic mice, whereas PHF-type neurites are specific to humans and highly characteristic of AD [7, 19]. Tau phosphorylation had been reported in the plaques with giant cores in our previous study using Tg2576 mice [29]. The conversion of tau into PHF might need longer periods.

The earliest amyloid deposits in humans are so-called diffuse plaques, while amyloid appears in the transgenic mice to form as an initial dense-cored plaques. In most AD patients, moreover, very little Aβ40 is deposited in the brain [12]. It is not clear why diffuse amyloid deposits are not a prominent feature of the transgenic model and why large amounts of Aβ40 are deposited and giant plaques are formed in the Tg2576 model. It may be that more than one factor or some combination of factors, such as species, strain, promotor, expression level, or mutated transgene, might cause these discrepancies. Our study suggests that the lack of phagocytizing amyloid fibrils in the microglia may contribute to further enlargement of the dense-cored plaques leading to the formation of giant plaques.

In summary, the present results demonstrate that Tg2576 mice share many histological and ultrastructural alterations of dense-cored plaques with classic plaques of AD that make them a valuable model in which to study the mechanisms of local tissue pathology associated with dense amyloid deposits in AD. Furthermore, some morphological characteristics of plaques containing dense amyloid core in the transgenic animal model were presented as follows: (1) the plaques containing amyloid cores and dystrophic neurites often form giant plaques and lack the corona of the plaque, (2) plaque-associated microglia are activated but not sufficiently enough to digest extracellular amyloid fibrils, and (3) dystrophic neurites of plaques are lacking in PHF-type neurites. These results could be helpful in the assessment for monitoring the effects of local tissue pathology when therapeutic drugs (e.g., anti-amyloid or anti-inflammatory drug treatment) are administered in experimental model mice.

Acknowledgements We thank Prof. Haruyasu Yamaguchi (Gunma University School of Health Sciences, Maebashi, Japan) for his helpful comments on this manuscript. The expert technical assistance of Machiko Yokota and Kohji Isoda is gratefully acknowledged. Supported by Grants-in Aid for the Primary Amyloidosis Research Committee (S. Ikeda and T. Ishihara), surveys and research on special disease from Ministry of Health, Labor and Welfare of Japan, and by Grants-in Aid for Scientific Research (C) (12670592, 12670593) and Scientific research on Priority Areas (C) – Advanced Brain Science Project – from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

1. Andersson PB, Perry VH, Gordon S (1991) The kinetics and morphological characteristics of the macrophage-microglial response to kainic acid-induced neuronal degeneration. Neuroscience 42:201–214
2. Apelt J, Schliebs R (2001) β-amyloid-induced glial expression of both pro- and anti-inflammatory cytokines in cerebral cortex of aged transgenic Tg2576 mice with Alzheimer plaque pathology. Brain Res 894:21–30
3. Bard F, Cannon C, Barbour R, Burke RL, Games D, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Lieberburg I, Motter R, Nguyen M, Soriano F, Vasquez N, Weiss K, Welch B, Seubert P, Schenk D, Yednock T (2000) Peripherally administered antibodies against amyloid β-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. Nat Med 6:916–919
4. Bohatschek M, Jones LL, Kreutzberg GW, Raivich G (1998) Expression of immunoregulatory molecules MHC1, MHC2 and B7-2 in the axotomized mouse facial motor nucleuss. Clin Neuropathol 17:286

5. Bornemann KD, Wiederhold KH, Pauli C, Ermini F, Stalder M, Schnell L, Sommer B, Jucker M, Staufenbiel M (2001) Aβ-induced inflammatory processes in microglia cells of APP23 transgenic mice. Am J Pathol 158:63–73
6. Chapman PF, White GL, Jones MW, Cooper-Blacketer D, Marshall VJ, Irizarry M, Younkin L, Good MA, Bliss TVP, Hyman BT, Younkin SG, Hsiao KK (1999) Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. Nat Neurosci 2:271–276
7. Dickson DW, Farlo J, Davies P, Crystal H, Schenk D, Games D (1988) A double-labeling immunohistochemical study of senile plaques. Am J Pathol 132:86–101
8. EI Khoury J, Hickman SE, Thomas CA, Cao L, Silverstein SC, Loike JD (1996) Scavenger receptor-mediated adhesion of microglia to β-amyloid fibrils. Nature 382:716–719
9. Frautschy SA, Yang F, Irrizarry M, Hyman B, Saido TC, Hsiao K, Cole GM (1998) Microglial response to amyloid plaques in APPsw transgenic mice. Am J Pathol 152:307–317
10. Games D, Adams D, Alessandrini R, Barbour R, Berthelette P, Blackwell C, Carr T, Clemens J, Donaldson T, Gillespie F, Guido T, Hagopian S, Johnson-Wood K, Khan K, Lee M, Leibowitz P, Lieberburg I, Little S, Masliah E, McConlogue L, Montoya-Zavala M, Mucke L, Paganini L, Penniman E, Power M, Schenk D, Seubert P, Snyder B, Soriano F, Tan H, Vitale J, Wadsworth S, Wolozin B, Zhao J (1995) Alzheimer-type neuropathology in transgenic mice overexpresing V717F β-amyloid precursor protein. Nature 373:523–527
11. Giulian D, Haverkamp LJ, Yu JH, Karshin W, Tom D, Li J, Kirkpatrick J, Kuo LM, Roher AE (1996) Specific domains of β-amyloid from Alzheimer plaque elicit neuron killing in human microglia. J Neurosci 16:6021–6037
12. Gravina SA, Ho L, Eckman CB, Long KE, Otvos Jr L, Younkin LH, Suzuki Y, Younkin SG (1995) Amyloid β protein (Aβ) in Alzheimer's disease brain. Biochemical and immunocytochemical analysis with antibodies specific for at Aβ40 or Aβ42(43). J Biol Chem 270:7013–7016
13. Holness CL, da Silva RP, Fawcett J, Gordon S, Simmons DL (1993) Macrosialin, a mouse macrophage-restricted glycoprotein, is a member of the lamp/lgp family. J Biol Chem 268:9661–9666
14. Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G (1996) Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. Science 274:99–102
15. Irizarry MC, McNamara M, Fedorchak K, Hsiao K, Hyman BT (1997) APPsw transgenic mice develop age-related Aβ deposits and neuropil abnormalities, but no neuronal loss in CA1. J Neuropathol Exp Neurol 56:965–973
16. Itagaki S, McGeer PL, Akiyama H, Zhu S, Selkoe D (1989) Relationship of microglia and astrocytes to amyloid deposits of Alzheimer disease. J Neuroimmunol 24:173–182
17. Kawarabayashi T, Younkin LH, Saido TC, Shoji M, Hsiao K, Younkin SG (2001) Age-dependent changes in brain, CSF, and plasma amyloid β protein in the Tg2576 transgenic mouse model of Alzheimer's disease. J Neurosci 21:372–381
18. Lawson LJ, Frost L, Risbridger J, Fearn S, Perry VH (1994) Quantification of the mononuclear phagocyte response to Wallerian degeneration of the optic nerve. J Neurocytol 23:729–744
19. Masliah E, Mallory M, Deerinck T, deTeresa R, Lamont S, Miller A, Terry RD, Carragher B, Ellisman M (1993) Re-evaluation of the structural organization of neuritic plaques in Alzheimer's disease. J Neuropathol Exp Neurol 52:619–632
20. Masliah E, Sisk A, Mallory M, Mucke L, Schenk D, Games D (1996) Comparison of neurodegenerative pathology in transgenic mice overexpressing V717F β-amyloid precursor protein and Alzheimer's disease. J Neurosci 16:5795–5811
21. Mackenzie IR, Hao C, Munoz DG (1995) Role of microglia in senile plaque formation. Neurobiol Aging 16:797–804
22. McDonald DR, Brunden KR, Landreth GE (1997) Amyloid fibrils activate tyrosine kinase-dependent signaling and superoxide production in microglia. J Neurosci 17:2284–2294
23. Meda L, Cassatella MA, Szendrei GI, Otvos Jr L, Baron P, Villalba M, Ferrari D, Rossi F (1995) Activation of microglial cells by β-amyloid protein and interferon-r. Nature 374:647–650
24. Naito M, Kodama T, Matsumoto A, Doi T, Tkahashi K (1991) Tissue distribution, intracellular localization, and in vitro expression of bovine scavenger receptor. Am J Pathol 139:1411–1423
25. Sasaki A, Yamaguchi H, Ogawa A, Sugihara S, Nakazato Y (1997) Microglial activation in early stages of amyloid β protein deposition. Acta Neuropathol 94:316–322
26. Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Liao Z, Lieberbung I, Motter R, Mutter L, Soriano F, Shopp G, Vasquez N, Vandevert C, Walker S, Wogulis M, Yednock T, Games D, Seubert P (1999) Immunization with amyloid-β attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature 400:173–177
27. Stalder M, Phinney A, probst A, Sommer B, Staufenbiel M, Jucker M (1999) Association of microglia with amyloid plaques in brains of APP23 transgenic mice. Am J Pathol 154:1673–1684
28. Streit WJ, Graeber MB, Kreutzberg GW (1989) Expression of Ia antigen on perivascular and microglial cells after sublethal and lethal motor neuron injury. Exp Neurol 105:115–116
29. Tomidokoro Y, Harigaya Y, Matsubara E, Ikeda M, Kawarabayashi T, Okamoto K, Shoji M (2001) Aβ amyloidosis induces the initial stage of tau accumulation in APPsw mice. Neurosci Lett 299:169–172
30. Walker DG (1998) Inflammatory markers in chronic neurodegenerative disorders with emphasis on Alzheimer's disease. In: PL Wood (ed) Neuroinflammation, mechanisms and management. Humana Press, Inc. Totowa, pp 61–90
31. Westerman MA, Cooper-Blacketer D, Gomez-Isla T, Mariash A, Ashe KH (2000) Age-dependent behavioral deficit in a transgenic mouse model of Alzheimer's disease. Soc Neurosci Abstr 26:1318
32. Wyss-Coray T, Lin C, Yan F, Yu G-Q, Rohde M, MaConlogue L, Masliah E, Mucke L (2001) TGF-β1 promotes microglial amyloid-β clearance and reduces plaque burden in transgenic mice. Nat Med 7:612–618
33. Yamaguchi H, Hirai S, Morimatsu M, Shoji M, Ihara Y (1988) A variety of cerebral amyloid deposits in the brains of the Alzheimer-type dementia demonstrated by β protein immunostaining. Acta Neuropathol 76:541–549
34. Yamaguchi H, Nakazato Y, Shoji M, Takatama M, Hirai S (1991) Ultrastructure of diffuse plaques in senile dementia of the Alzheimer type: comparison with primitive plaques. Acta Neuropathol 82:13–20