Acta Neuropathol (1989) 79:129–136

Acta Neuropathologica

Regular papers

Ferritin immunohistochemistry as a marker for microglia*

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Summary. An immunohistochemical analysis of formalin-fixed, paraffin-embedded brain sections was performed with antisera against holoferritin and the light(L)-subunit of ferritin. Sections immunostained using anti-glial fibrillary acidic protein (GFAP), *Ricinus communis* agglutinin-1 (RCA-1) stain for microglia and iron stain (Berlin blue stain) were compared. The L-subunit of ferritin was purified from normal human spleen according to the modified scrapie-associated fibrils purification, and the antiserum was raised in a rabbit. Both ferritin antisera positively stained resting and, more markedly, reactive microglia, both of which were also stained with RCA-1 but not with GFAP. Ferritin-positive resting microglia were seen more abundantly in cerebral and cerebellar cortices than in white matter. The advantages of ferritin antisera over RCA-1 are as follows. (1) RCA-1 heavily stains blood vessels, while anti-ferritin does not, hence the microglial cells are more readily visualized with ferritin immunohistochemistry. (2) Reactive microglia and macrophages are more strongly stained with anti-ferritin. (3) The staining intensity of ferritin is independent of the length of tissue fixation in formalin. However, anti-ferritin is inferior to RCA-1 in staining resting microglia with a scanty cytoplasm, especially in the white matter, probably because the former recognizes cytoplasmic components, while the latter recognizes cell membrane. Iron stain only gave a reaction to microglial cells in brains with neurosyphilis and to hemosiderin-laden macrophages. Thus, in addition to RCA-1, ferritin antisera are useful as a microglia marker in formalin-fixed, paraffin-embedded sections.

* Supported in part by Dr. A. Kondo, Department of Neuropathology, Neurological Institute, Kyushu University

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Key words: Ferritin – Microglia – Immunohistochemistry – Scrapie-associated fibrils

The origin and function of microglia have been debated since their first identification by del Rio-Hortega using silver carbonate staining [34]. Many authors supported the idea, based on a variety of techniques [16, 23, 33], of a mesodermal derivation of microglia. In contrast there is the view that microglia derive from neuroectodermal cells [20]. The relationship between resting microglia, reactive microglia, brain macrophages and blood monocyte is not clearly understood.

Although microglia and macrophages do not always share antigenic and enzymatic properties, their functions are similar. Microglia have phagocytic activity, in vivo and in vitro [35, 43]. Microglia have been stained immunologically [8, 11, 14, 28], but most reacted only on frozen sections and paraffin sections were of little use. Among the various immunohistochemical and cytochemical markers for microglia, *Ricinus communis* agglutinin-1 (RCA-1) has been used most often for routine paraffin sections [21, 26, 29]. We have applied anti-ferritin to paraffin sections of normal and diseased brains and compared the findings of ferritin immunohistochemistry with immunostain with anti-glial fibrillary acidic protein (GFAP), RCA-1 stain for microglia and iron stain of serial sections.

Materials and methods

Isolation and purification of the light(L)-subunit of ferritin

Ferritin has been purified by various methods [2, 5, 9]. In this study, a partial purification of ferritin was performed using the modified scrapie-associated fibrils (SAF) purification of a

normal human spleen, removed at autopsy. Spleen tissue (wet tissue 1 g) was homogenized in 4 ml of 40 mM Tris-HCl pH 7.9/10 mM NaCl/6 mM MgCl and DNase I (BDH Chemicals, Poole, England) was added to a final concentration of 500 μg. The mixture was incubated at 37° C for 60 min. After adding 5 ml of 10% solution of sarkosyl (Tokyo Kasei Kogyo, Tokyo, Japan) adjusted to pH 7.4 with NaH_2PO_4, the preparation was then centrifuged at 22000 *g* for 30 min. The supernatant was then centrifuged at 215000 *g* for 120 min and the pellet was resuspended in 1 ml of 1% sarkosyl/10% NaCl/50 mM Tris-HCl pH 7.6 and sonicated. The suspended pellet was centrifuged at 215000 *g* for 60 min, resuspended and sonicated in 1 ml of 1% sarkosyl/10% NaCl/50 mM Tris-HCl pH 7.6 and 1 μg of proteinase K (E. Merck, Darmstadt, FRG) was added. The mixture was incubated at 37° C for 120 min and then centrifuged at 215000 *g* for 60 min. DNase I was added to the tissue homogenate because the large amount of DNA in the homogenate prevented pellet formation when sarkosyl was used as the detergent. The final pellet was solubilized with 2% sodium dodecyl sulfate (SDS)/50 mM Tris-HCl pH 7.0 and 5% β-mercaptoethanol (β-ME) and applied to a CL-Sepharose 4B (Pharmacia Fine Chemicals, Sweden) column (1.5 × 95 cm) previously equilibrated with 50 mM Tris-HCl pH 7.0/0.2% SDS. The major protein peak was collected, dialyzed against distilled water, and lyophilized. The lyophilized protein was solubilized with 2% SDS/50 mM Tris-HCl and 5% β-ME and applied to high performance liquid chromatography (HPLC, TSK 3000 SW, 0.75 × 60 cm) previously equilibrated with 50 mM Tris-HCl pH 7.0/0.1% SDS. The major protein peak was collected, dialyzed and lyophilized. The molecular weight of this protein was determined by SDS polyacrylamide gel electrophoresis (PAGE). Electrophoresis was performed in 15% polyacrylamide gel, according to Laemmli [22]. For amino acid sequence analysis, this protein was digested with lysyl-endopeptidase and fragmented peptides were isolated with reverse-phase HPLC.

Generation and characterization of antisera

Antiserum against ferritin was raised in a rabbit. Anti-ferritin antibody was affinity purified as follows. The purified L subunit of ferritin was used for the subsequent conjugation to formyl-cellulofine (Chisso Chemicals, Tokyo, Japan). The antiserum (2 ml) was diluted with 50 mM Tris-HCl pH 7.6/0.5 M NaCl (28 ml) and was circulated overnight on an affinity column. The column was extensively washed with 50 mM Tris-HCl pH 7.6/0.5 M NaCl. The antibody was then eluted with 0.2 M glycine-HCl pH 2.5 and neutralized with 0.2 M Tris-HCl pH 8.6. After affinity purification, the specificity of the antisera was checked by Western blotting. Samples taken from frozen, postmortem, normal human spleen, liver and heart were dissolved in 1% SDS, 50 mM Tris-HCl pH 7.6 at a concentration of 50 mg wet tissue/ml. Proteins from these samples were separated by SDS-PAGE, 500 μg wet tissue/lane of the gel, and transferred to a Durapore membrane GVHT (Millipore Corporation, Bedford, Mass) according to Towbin et al. [38]. The Durapore strips were incubated overnight at 37° C with diluted antibodies to holoferritin from liver (Dako, 1:2000) and affinity-purified L-ferritin antibody (0.1 μg/ml). After washing, the strips were incubated at 37° C for 30 min in alkaline phosphatase-conjugated anti-rabbit IgG (Promega Biotec, 1:7500) and color was developed with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (ProtBrot, Promega Biotec).

Immunohistochemistry, lectin histochemistry and iron stain

Formaldehyde-fixed, paraffin-embedded tissues were serially sectioned at an thickness of 6 μm. We examined specimens from eight normal human brains, five patients treated for neurosyphilis, ten with Creutzfeldt-Jakob disease (CJD), three with Alzheimer disease (AD), two with senile dementia of Alzheimer type (SDAT) and one each with cerebral infarction, intracerebral hemorrhage and viral encephalitis. The age distribution of patients with normal brains was from 5 to 61 years. Tissue from brains of patients with CJD, AD or SDAT had been fixed for periods from 2 weeks to 19 years. Normal human blood smears, spleen, liver and tonsils were also examined.

Deparaffinized sections were incubated in methanol containing 0.3% H_2O_2 for 30 min to block endogenous peroxidase. After washing, sections were incubated overnight at 4° C with diluted anti-human holoferritin (1:2000), affinity-purified L-ferritin antibody (0.3 μg/ml) and anti-GFAP ([25], 1:1000). They were then exposed to a biotinylated anti-rabbit IgG antiserum, streptavidin-horseradish peroxidase complex (Stravigen, Biogenix Laboratories, Dublin, Calif) and the colored reaction product was developed with 0.01% 3.3′-diamino-benzidine tetrahydrochloride. Lectin histochemistry was performed according to Mannoji et al. [26]. After deparaffinization and blocking of the endogenous peroxidase activity, sections were incubated with RCA-1 (Vector Laboratories, 25 μg/ml) for 45 min at room temperature. The streptavidin-peroxidase complex and chromogenic substrates were used as described above. Sections were counterstained with hematoxylin. Methanol-fixed blood smears were also stained with anti-L-ferritin, using the avidin-biotin-peroxidase complex method.

Iron stain was performed using the Berlin blue method [6] on tissue from the brains of three patients with CJD, five with neurosyphilis and one with viral encephalitis.

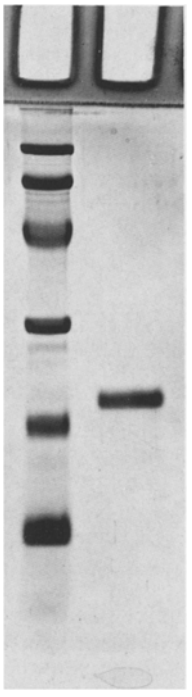

Fig. 1. Polyacrylamide gel electrophoresis profile of protein purified from the spleen. A single band of 22 kDa is apparent. *Left lane* shows the standard proteins, the molecular mass of which were 94, 67, 43, 30, 20 and 14 kDa

Results

Characterization of anti-L-ferritin

During application of the modified SAF purification, sarkosyl-soluble and proteinase K-digested proteins

SSQIRQNYSTDVEAAVNYLVNLYLYASYTYLSLGFYFDNYDVALEGVSHFFRELAEEKREGYERL

LKMQNQRGGRALFQDIKKPAEDEWGKTPDAMKAAMALEKKLNQALLDLHALGSARTDPHLCDFLE

THFLDEEVKLIKKMGDHLTNLRKLGGPEAGEYLFERLTLKHD

Amino acid code Gly:G, Ala:A, Val:V, Leu:L, Ile:I, Ser:S, Thy:T, Asp:D, Asn:N, Glu:E, Gln:Q, Lys:K, Met:M, His:H, Arg:R, Phe:F, Tyr:Y, Trp:W, Cys:C, Pro:P

Fig. 2. Amino acid sequence of the light (L) subunit of the human spleen. *Underline* shows three peptides fractionated by reverse-phase HPLC after lysyl-endopeptidase digestion of a protein of 22 kDa

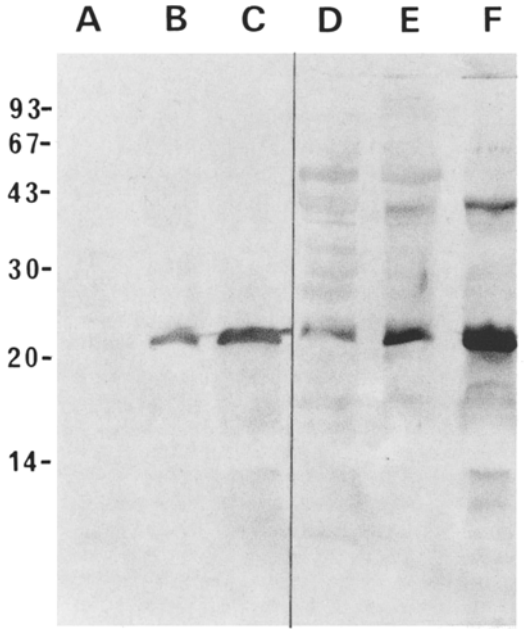

Fig. 3. Immunoblots of normal human homogenates (lanes A and D; heart: lanes B and E; liver: lanes C and F; spleen) probed with anti-ferritin antisera (lanes A, B and C; affinity-purified L-ferritin antibody 0.1 μg/ml: lanes C, D and E; anti-holoferritin antibody; Dako, 1:4000). 500 μg wet tissue was electrophoresed for each sample. Affinity-purified L-ferritin antibody reacted with a single band of 22 kDa on lanes B and C but no band on lane A. Anti-holoferritin reacted with several bands, including a slightly larger band of 22 kDa, probably the heavy subunit

were removed from spleen tissue. SDS-soluble proteins from the final pellet were applied to a CL-Sephalose 4B column and subsequently HPLC. The molecular mass to the main peak was estimated to be about 21 to 22 kDa (Fig. 1). This fraction was used for immunization, affinity purification of antibody and amino acid sequence analyses. Because the N terminus of the purified 22-kDa protein was blocked, this protein was digested with lysyl-endopeptidase and the three peptide peaks were fractionated by reverse-phase HPLC. These three peptides corresponded to the amino acid sequences of the L subunit of human spleen apoferritin (Fig. 2) [42].

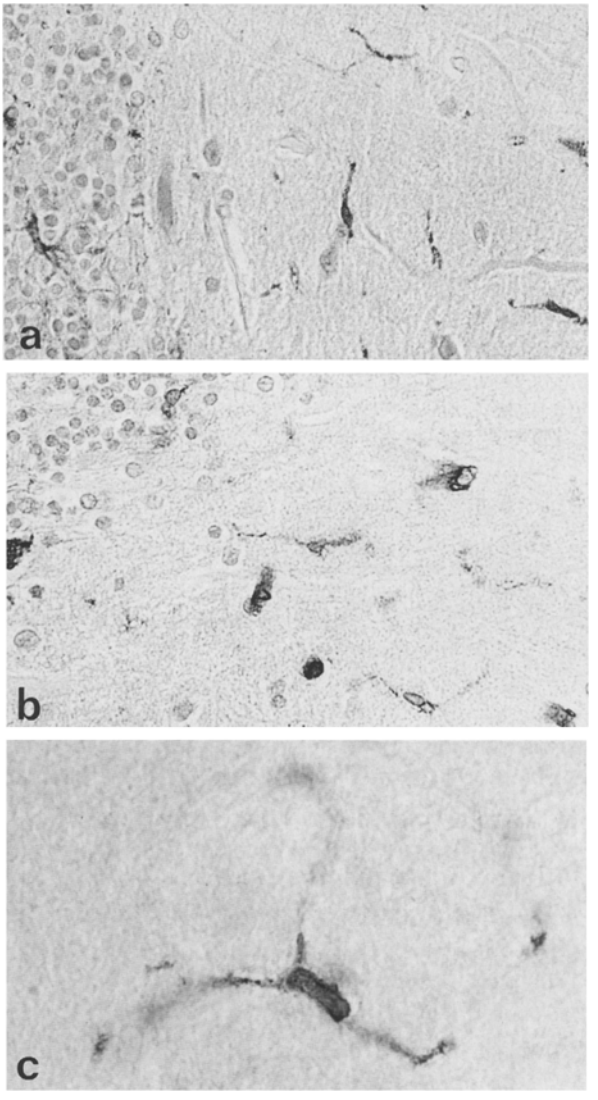

Fig. 4a–c. Visualization of resting microglia with anti-ferritin (**a** and **c**) and RCA-1 (**b**) in normal human CNS. **a** Ferritin-positive resting microglia in the cerebellar molecular layer. Small cells with a scanty cytoplasm and delicate, branching processes are scattered. **b** RCA-1-positive microglia. Note dense staining of endothelial and blood cells. **c** High magnification of a microglial cell, note the rod-shaped nucleus and long, slender processes. **a, b** ×400, **c** ×1000

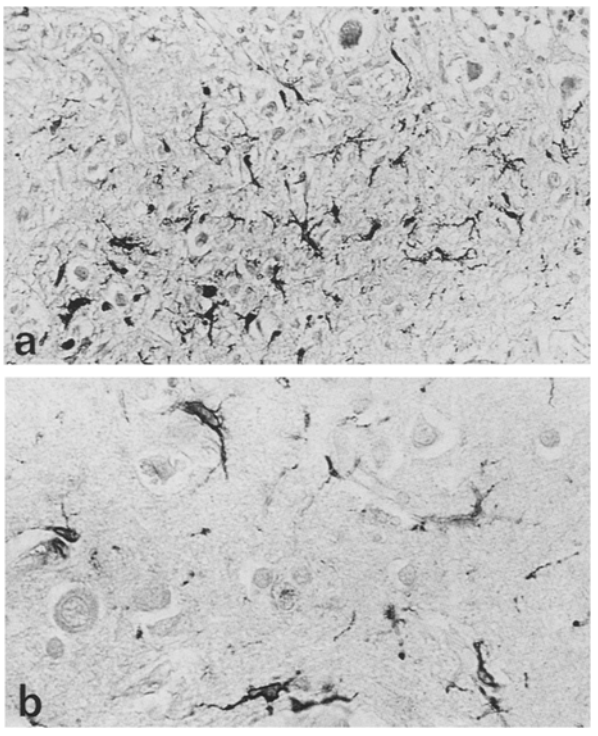

Fig. 5a, b. Reactive microglia stained with affinity-purified L-ferritin. **a** Markedly increased number of microglial cells in the cerebellar molecular layer of the Creutzfeldt-Jacob disease (CJD)-infected brain. **b** Cerebral cortex of the brain with neurosyphilis. These dendritic cells become larger and more intensely stained with anti-ferritin as compared to findings in the normal brain. **a** ×200, **b** ×400

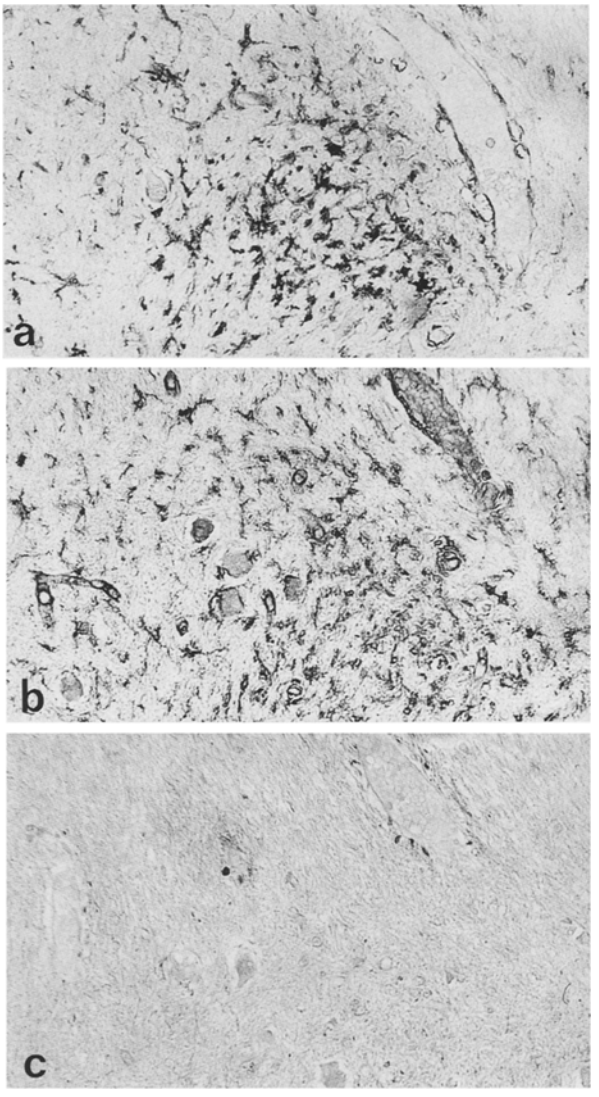

Fig. 6a–c. Glial nodule in the brain of a patient with viral encephalitis, examined using (**a**) affinity-purified L-ferritin antibody, (**b**) RCA-1 and (**c**) anti-GFAP in serial sections. Anti-ferritin and RCA-1 but not anti-GFAP reacted to clustered microglial cells and macrophages. **a–c** ×200

The specificity of the affinity-purified antibody against the L subunit of spleen ferritin was confirmed with Western blotting of tissues from the human heart, liver and spleen. This antiserum gave a single 22-kDa band in liver and spleen but none in the heart tissue. The anti-holoferritin from the liver yielded several bands in the liver, spleen and heart tissue, presumably the L and H subunit and other proteins (Fig. 3).

Immunohistochemistry and lectin histochemistry

Holoferritin and L-ferritin antisera were applied to paraffin sections of normal and pathological brains. In all the tissues from the normal brains, small, bipolar or multipolar cells with a scanty cytoplasm, presumably resting microglia were stained. In the serial sections, these cells were also stained with RCA-1 but were not positive for GFAP (Fig. 4) or with Berlin blue. Ferritin-positive microglia were more prominent in the cerebral and cerebellar cortices, basal ganglia, and thalamus than in the white matter. Some of these cells lay close to capillaries and others, in the gray matter, close to neuron bodies. Anti-ferritin demonstrated strongly stained, and an increased number of, microglia in pathological brain tissues (Fig. 5). Most had a plump body with elongated processes and were considered to be reactive microglia. In brain tissue from a patient with a viral encephalitis, microglial cells increased diffusely and focally throughout the CNS. In the serial sections, glial nodules and other microglial cells were strongly stained with anti-ferritin and less strongly with RCA-1 but were negative for GFAP (Fig. 6) and with Berlin blue. Reactive microglia were also seen around a considerable number of senile plaques in the SDAT brain and kuru plaques in the CJD-infected brains. Surrounding

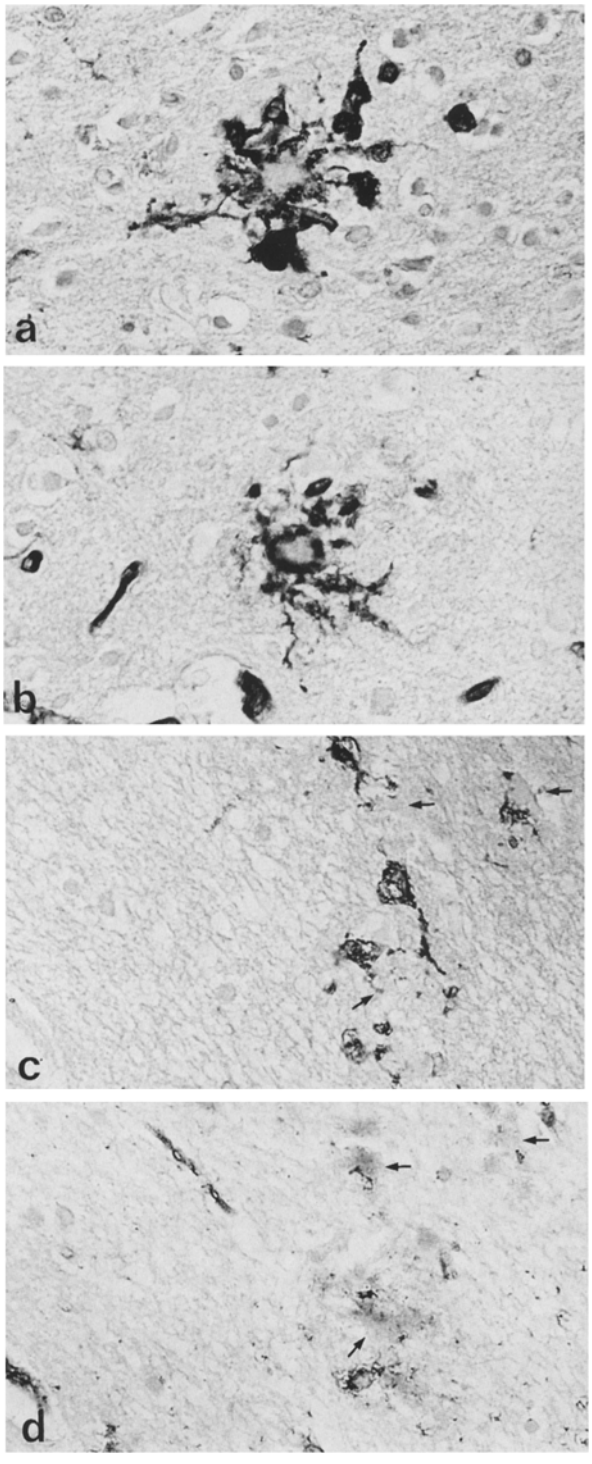

Fig. 7a–d. Microglia around the senile plaque in the cerebral cortex from a senile dementia of Alzheimer type brain (**a** and **b**) and the kuru plaque in the cerebral cortex from a CJD brain (**c** and **d**). **a**, **c** Affinity-purified L-ferritin antibody stained microglia, but not endothelial cells and blood cells. **b, d** RCA-1 reacted not only to microglia but also to endothelial cells, blood cells and kuru plaque (**d**). **c, d** Serial sections and *arrow* shows kuru plaque. **a–d** ×400

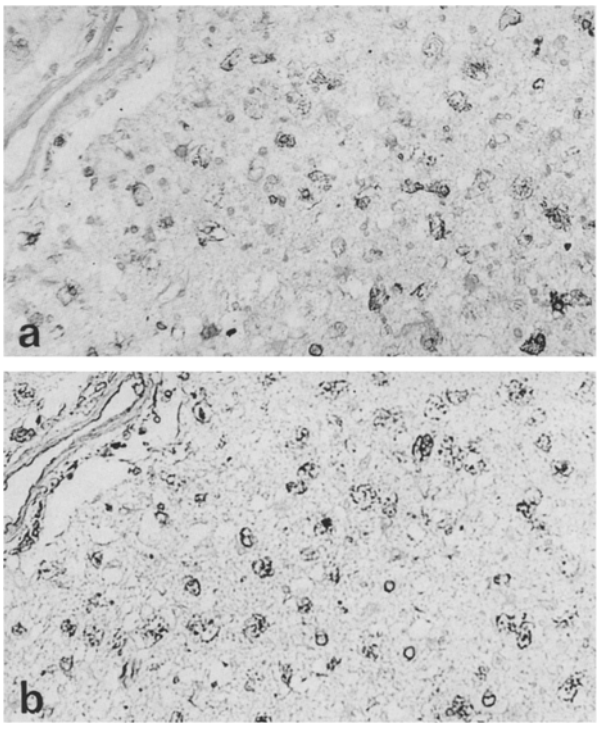

Fig. 8a, b. Lipid-laden macrophages infiltrating the cerebral white matter in a CJD-infected brain. **a** Affinity-purified L-ferritin antibody. **b** RCA-1. **a, b** ×200

microglia were stained to much the same extent with anti-L-ferritin and RCA-1, and the latter also stained the cores of kuru plaques, at a weak to moderate intensity (Fig. 7). There was an accumulation of ferritin-positive microglia at the periphery of the infarction.

In addition to the microglial cells, ferritin antisera reacted with monocytes on blood smears, macrophages (including lipid-laden and hemosiderin-laden ones) in both the lymphoid organs and CNS tissues (Fig. 8), Kupffer cells and a fair number of hepatocytes. Ferritin antisera reacted with a cytoplasmic component of positively stained cells. Although Purkinje cells, some oligodendrocytes and myelinated fibers in the white matter reacted weakly with anti-ferritin, the endothelial cells were not stained. There was no significant difference in the staining intensity of microglial cells by either ferritin antisera, but the background staining was remarkably decreased with the affinity-purified L-ferritin antibody. Compared with RCA-1, which reacted strongly to endothelial cells, the microglia were more readily visible with ferritin immunohistochemistry. Cell bodies of reactive microglia and macrophages were more intensely visualized with ferritin antisera than with RCA-1. On the other hand, resting microglia were more visible

with RCA-1 than with ferritin antisera, particularly in the white matter, partly because the latter reacted weakly with myelinated fibers. Ferritin antisera reacted with the microglia regardless of the length of tissue fixation. RCA-1 did not react with tissues which had been in long-term fixation (more than several years).

Berlin blue stain showed iron pigments in hemosiderin-laden macrophages and microglial cells in two of five cases of neurosyphilis. Most other microglia which stained with anti-ferritin were negative for Berlin blue stain.

Discussion

To purify ferritin, we used a modification of the SAF method for isolation and purification of SAF or "prion" protein from scrapie- or CJD-infected brains [7, 18]. Previously, we obtained several antisera against SAF fractions purified from CJD brains [19]. One of these antisera immunolabeled microglia and macrophages, but not kuru plaques. This antiserum reacted with the 22-kDa protein from cerebral and splenic tissues, but not with SAF or prion protein, in Western blotting. The purification protocol was re-examined and modified to isolate and identify this 22-kDa protein from the spleen. The final step of the SAF purification was altered from centrifugation at 22000 *g* to 215000 *g* because ferritin was less likely than amyloid to precipitate under high ionic strength solution (data not shown). The amino acid sequence analyses of this 21–22-kDa protein was performed and the L-ferritin fragment was identified.

Ferritin is an intracellular iron-storage protein found in most but not all eukaryotic cells. It has a molecular mass of 450 kDa [13] and consists of 24 subunits of two types with molecular mass of about 19 kDa (L) and 21 kDa [heavy (H)] [1]. These two subunits share extensive sequence homology, but are derived from different genes, the H subunit mainly from chromosome 11, and the L subunit mainly from chromosome 19 [4, 15, 41]. The proportion of these two subunits varies with the tissue [2, 40]. In the liver and spleen the principle component is the L subunit, while in heart tissue it is the H subunit. Thus, the L subunit was purified from spleen by a modified SAF purification. Immunoreactive products were not detected with anti-L-ferritin in Western blotting of heart tissue. Since ferritin is known to be resistant to protease [24, 31], we could isolate ferritin by the modified SAF purification, presumably because of this protease resistance and sarkosyl insolubility.

Ferritin plays a central role in recycling iron for synthesis of heme and other proteins. Hepatocytes, macrophages and red cells of embryos are the cells specialized for iron storage. About 90% of the iron is converted to ferritin by macrophages, then the iron is slowly released to apotransferrin [37]. The mean ferritin concentration in monocytes is 7.5 times the concentration in other leukocytes [36]. In addition, ferritin in cells of the monocytes-macrophage lineage increases during maturation in vitro, and alveolar macrophages have the highest ferritin content [1]. In the CNS, brain iron exists in forms of ferritin, transferrin as iron binding protein [3] and others. About one third of brain iron is ferritin [12] and ferritin concentrations in normal human and rat brains were determined to be 38.1 and 49.1 μg/g (wet tissue), respectively, both of which were less than one third of the ferritin concentrations of normal rat liver [10]. There are data that transferrin is located specifically in oligodendrocytes [3]. Reports on the distribution of ferritin in the CNS are few. Ferritin was evidenced in the neuronal cytoplasm of Purkinje cells of the rat [27]. Koeppen and Dentinger described ferritin-positive microglia in five cases of superficial siderosis, the pathogenesis of which was thought to be the ability of the brain to biosynthesize ferritin in response to prolonged contact with hemoglobin iron [21]. We present evidence in this paper that ferritin antisera reacts with microglia from both normal brains and those with a pathology. While the function of ferritin in microglia is unknown, it can be inferred from functional homology between macrophages and microglia. Except for iron storage for self-utilization, there are two possible functions: one is iron storage for other cells (recycling iron), and the other is sequestration of excess iron [37]. In addition, microglia might also be related to hemosiderin formation, because hemosiderin is thought to be the oxidative product of ferritin [32]. Iron storage in microglia and macrophages was noted with Berlin blue stain in some tissues from patients with cerebral hemorrhage or neurosyphilis. This stain is, however, much less sensitive than the anti-ferritin immunostain.

RCA-1 and LN-1 are reagents capable of revealing microglia in paraffin sections [26, 30]. LN-1 is a B cell-specific monoclonal antibody which stains microglia but not macrophages. However, the suitability of LN-1 as a specific marker of microglia is questionable, since this antibody did not react to microglia in 5 of 27 tissues from both normal and diseased brains [30]. RCA-1 binds specifically to β-D-galactosyl residues on the cell membrane and hence blood cells, endothelial cells and even resting microglia with a scanty cytoplasm, as well as reactive microglia, can be visualized with RCA-1. However, the staining intensity with RCA-1 is greatly influenced by the length of tissue fixation. It has been reported that scrapie amyloid plaques can be labeled with lectins [39]. In our study,

RCA-1 reacted with a considerable number of kuru plaques in CJD brains which were not stained with anti-ferritin. On the other hand, since ferritin is present in cell sap and lysosomes of the cytoplasm [17], reactive microglia and macrophages may be stained with ferritin antisera more clearly than resting microglia.

We obtained evidence that the microglia contained larger amounts of ferritin than other brain cells, thus the use of ferritin antisera as a microglia marker in formalin-fixed, paraffin-embedded thin sections, in addition to RCA-1, will be of benefit.

Acknowledgements. We thank the kindness of Miss K. Hatanaka in assisting our laboratory works and M. Ohara for the comments on the manuscript.

References

1. Andreesen R, Osterholz J, Bodemann H, Bross KJ, Costabel U, Lohr GW (1984) Expression of transferrin receptors and intracellular ferritin during terminal differentiation of human monocytes. Blut 49:195–202
2. Arosio P, Adelman TG, Drysdale JW (1978) On ferritin heterogeneity. J Biol Chem 253:4451–4458
3. Bertrand B, Popovici T, Levin MJ, Tuil D, Kahn A (1985) Transferrin gene expression visualized in oligodendrocytes of the rat brain by using in situ hybridization and immunohistochemistry. Proc Natl Acad Sci USA 82:6706–6710
4. Caskey JH, Jones C, Miller YE, Seligman PA (1983) Human ferritin gene is assigned to chromosome 19. Proc Natl Acad Sci USA 80:482–486
5. Cham BE, Roeser HP, Nikles A, Ridgway K (1985) A procedure for the purification of ferritin from human liver by heating a methanol-treated homogenate. Anal Biochem 151:561–565
6. Dacie JV, Doniach I (1947) Basophilic property of iron-containing granules in siderocytes. J Pathol Bacteriol 59: 684–686
7. Diringer H, Hilmert H, Simon D, Werner E, Ehlers B (1983) Towards purification of the scrapie agent. Eur J Biochem 134:555–560
8. Esiri MM, Booss J (1984) Comparison of methods to identify microglial cells and macrophages in the human central nervous system. J Clin Pathol 37:150–156
9. Fielding J, Bothwell TH, Cook JD, Crichton RR, Crosby WH, Dallmann PR, Drysdale JW, Hallberg L, Halliday J, Harrison PM, Hershko C, Layrisse M, Ramsay WNM, Worwood M (1984) Preparation, characterization and storage of human ferritin for use as a standard for the assay of serum ferritin. Clin Lab Haematol 6:177–191
10. Fleming J, Joshi JG (1987) Isolation of aluminum-ferritin complex from brain. Proc Natl Acad Sci USA 84:7866–7870
11. Franklin WA, Mason DY, Pulford K, Falini B, Bliss E, Gatter KC, Stein H, Clarke LC, McGee JO'D (1986) Immunohistological analysis of human mononuclear phagocytes and dendritic cells by using monoclonal antibodies. Lab Invest 54:322–335
12. Hallgren B, Sourander P (1958) The effect of age on the nonhaem iron in the human brain. J Neurochem 3:41–51
13. Harrison PM, Fischbach FA, Hoy TG (1967) Ferric oxyhydroxide core of ferritin. Nature 216:1188–1190
14. Hayes GM, Woodroofe MN, Cuzner ML (1987) Microglia are the major cell type expressing MHC class II in human white matter. J Neurol Sci 80:25–37
15. Hentze MW, Keim S, Papadopoulos P, O'Brien S, Modi W, Drysdale J, Leonard WJ, Harford JB, Klausner RD (1986) Cloning, characterization, and chromosomal localization of a human ferritin heavy-chain gene. Proc Natl Acad Sci USA 83:7226–7230
16. Hickey WF, Kimura H (1988) Perivascular microglial cells of the CNS are bone marrow-derived and present antigen in vivo. Science 239:290–292
17. Iancu TC, Neustein HB (1977) Ferritin in human liver cells of homozygous beta-thalassaemia: ultrastructural observations. Br J Haematol 37:527–535
18. Kitamoto T, Hikita K, Tashima T, Tateishi J, Sato Y (1986) Scrapie-associated fibrils (SAF) purification method yields amyloid proteins from systemic and cerebral amyloidosis. Biosci Rep 6:459–465
19. Kitamoto T, Tateishi J (1988) Immunohistochemical confirmation of Creutzfeldt-Jakob disease with a long clinical course with amyloid plaque core antibodies. Am J Pathol 131:435–443
20. Kitamura T, Miyake T, Fujita S (1984) Genesis of resting microglia in the gray matter of mouse hippocampus. J Comp Neurol 226:421–433
21. Koeppen AH, Dentinger MP (1988) Brain hemosiderin and superficial siderosis of the central nervous system. J Neuropathol Exp Neurol 47:249–270
22. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685
23. Ling EA, Kaur C, Wong WC (1982) Light and electron microscopic demonstration of non-specific esterase in amoeboid microglial cells in the corpus callosum in postnatal rats: a cytochemical link to monocytes. J Anat 135:385–394
24. Listowsky I, Blauer G, Englard S, Betheil JJ (1972) Denaturation of horse spleen ferritin in aqueous guanidinium. Biochemistry 11:2176–2181
25. Mannoji H, Takeshita I, Fukui M, Kitamura K (1981) Glial fibrillary acidic protein in medulloblastoma. Acta Neuropathol (Berl) 55:63–69
26. Mannoji H, Yeger H, Becker LE (1986) A specific histochemical marker (lectin *Ricinus communis* agglutinin-1) for normal human microglia, and application to routine histopathology. Acta Neuropathol (Berl) 71:341–343
27. Martin TL, Switzer RC, Joshi J, Zimmerman A (1985) Ferritin localization in rat brain by immunocytochemistry. 15th Annual Meeting, Society for Neuroscience. Dallas, TX, October 20–25. 11:502 [abstr]
28. Mathew RC, Gupta SK, Katayama I, Curtis J, Turk JL (1983) Macrophage-specific antigen is expressed by resting microglia in the CNS but not by Langerhans cells in the skin. J Pathol 141:435–440
29. Michaels J, Price RW, Rosenblum MK (1988) Microglia in the giant cell encephalitis of acquired immune deficiency syndrome: proliferation, infection and fusion. Acta Neuropathol (Berl) 76:373–379
30. Miles JM, Chou SM (1988) A new immunoperoxidase marker for microglia in paraffin section. J Neuropathol Exp Neurol 47:579–587
31. Niitsu Y, Ishitani K, Listowsky I (1973) Subunit heterogeneity in ferritin. Biochem Biophys Res Commun 55:1134–1140
32. O'Connell MJ, Baum H, Peters TJ (1986) Haemosiderin-like properties of free-radical-modified ferritin. Biochem J 240:297–300

33. Oehmichen M, Wietholter H, Gencic M (1980) Cytochemical markers for mononuclear phagocytes as demonstrated in reactive microglia and globoid cells. Acta Histochem 66:243–252
34. Rio-Hortega P del (1919) El tercer elemonto de los centros nerviosos. I. La Microglia en estado normal. II. Intervencion de la microglia en los procesos patologicos. III. Naturaleza probable de la microglia. Bol Soc Esp Biol 9:69–120
35. Streit WJ, Kreutzberg GW (1988) The response of endogenous glial cells to motor neuron degeneration induced by toxic ricin. J Comp Neurol 268:248–263
36. Summers M, Worwood M, Jakobs A (1974) Ferritin in normal erythrocytes, lymphocytes, polymorphs, and monocytes. Br J Haematol 28:19–26
37. Theil EC (1987) Ferritin: structure, gene regulation, and cellular function in animals, plants, and microorganisms. Annu Rev Biochem 56:289–315
38. Towbin H, Staehelin T, Gordon J (1978) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheet: procedure and some applications. Proc Natl Sci USA 76:4350–4354
39. Vorbrodt AW, Dobrogowska DH, Kim YS, Lossinsky AS, Wisniewski HM (1988) Ultrastructural studies of glycoconjugates in brain micro-vessels and amyloid plaques of scrapie-infected mice. Acta Neuropathol (Berl) 75:277–287
40. Watanabe N, Drysdale J (1983) Studies on heterogeneity in ferritin subunits. Biochem Biophys Acta 743:98–105
41. Worwood M, Brook JD, Cragg SJ, Hellkuhl B, Jones BM, Perera P, Roberts SH, Shaw DJ (1985) Assignment of human ferritin genes to chromosomes 11 and 19q13.3–19qter. Hum Genet 69:371–374
42. Wustefeld C, Crichton RR (1982) The amino acid sequence of human spleen apoferritin. FEBS Lett 150:43–48
43. Zucker-Franklin D, Warfel A, Grusky G, Frangione B, Teitel D (1987) Novel monocyte-like properties of microglial/astroglial cells. Lab Invest 57:176–185

Received March 19, 1989/Revised, accepted May 19, 1989