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Ultrastructural studies of experimental scrapie and Creutzfeldt-Jakob disease in hamsters. II. Astrocytic and macrophage reaction towards axonal destruction

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Abstract. We report here the microglial (macrophage) and astrocytic reaction in several models of transmissible spongiform encephalopathies (TSEs) or prion diseases. With the low power electron microscopy it was readily apparent that myelinated vacuoles were surrounded by cells and their processes. The latter belonged either to hyperplastic reactive astrocytes or to macrophages. Typically, reactive astrocytes exhibited cytoplasm filled with innumerable glial filaments and, occasionally, other organelles (like cilia) and abundant tortuous intercellular junctions of adhesive plaque junction type. Desmosome-like junctions connecting astrocytic elements were also seen. As described earlier, astrocytic processes were occasionally interdigitated with oligodendroglial cells and their processes. Two types of macrophages were readily described. The majority of them exhibited electron-dense cytoplasm and numerous "empty" vacuoles (digestive chambers) containing cellular debris. Occasional vacuoles were surrounded by a thin collar reminiscent of "lyre-like inclusions" of the second type of macrophages. Several mylinated fibres were clearly engulfed by the cytoplasm of a macrophage containing unusual annulate lamellae.

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INTRODUCTION

The view of transmissible spongiform encephalopathies (TSEs) or prion diseases has changed. They were considered to be unorthodox diseases in which cellular reactions including the immune response are paradygmatically absent. Now, these diseases are considered to be consisting of atypical cellular reactions in which major roles are played by cells diversely involved in a smorgasbord of immunologic responses (Brown 1997, Klein et al. 2000, Collinge, Hawke 1998, Klein and Aguzzi 2000, Collinge 2001, Mabbott and Bruce 2001, Mabbott et al. 2002, Heppner et al. 2001a, Weissmann et al. 2001). To this end, we evaluated four models of TSEs in rodents - the panencephalopathic type of Creutzfeldt-Jakob disease (CJD) either in hamsters (the Echigo-1 strain) or in mice (the Fujisaki strain of CJD) and polioencephalopathic types of the 263K and 22C-H strains of scrapie passaged in hamsters. We selected those models for two reasons. First, because they cover the only two readily available and well characterized models of CJD in small laboratory rodents widely used in the past in many experiments (Mori et al. 1989, Liberski et al. 1998, Liberski et al. 1989; Liberski, Mori 1997) including those of blood infectivity. Secondly, because we search for cellular reactions towards the well defined phenomena of alterations of myelinated fibres irrespective whether occurring in polio- or in panencephalopathic models (Liberski et al. 1989a). We report here on the microglial (macrophage) and astrocytic reactions in those models of TSE. The accompanying paper describes the alterations to myelinated fibres.

METHODS

All strains of TSEs and animals used in this study along with all electron microscopic procedures were reported in detail in the first part of this paper. Briefly, Ten hamsters were inoculated with the 263 K or 22C-H strains of scrapie. These strains are widely used as experimental tools primarily because of relatively short incubation periods which, for mice, ranged from 16 to 18 weeks and for hamsters from 9 to 10 weeks for the 263K strain and 24-26 weeks for the 22C-H strain. In addition, hamsters were inoculated with the Echigo-1 strain of CJD (Liberski et al. 1997). The Echigo-1 strain of CJD was isolated by Mori and colleagues (1989) from a case of 33 years-old female with a panencephalopathic type of CJD. The inoculum prepared from a CJD patient's brain passaged to guinea pigs with incubation periods (IP) of 728 days at the primary and approximately 400 days at subsequent passages. A substrain with substantially reduced IP (254 days) was isolated from an animal which exibited active running and an excessive response to external stimuli . This strain was re-isolated in hamsters with IP of 141 days at the 3rd passage. An incubation period following intracerebral inoculation of hamsters with 10% cleared suspension of the Echigo-1--affected brain was approximately six months. Appropriate control animals were sham-inoculated with saline.

Electron Microscopy

Following intracardiac perfusion with 150 ml of 2.5% glutaraldehyde and 1% paraformaldehyde in cacodylate buffer (pH 7.2), the brains were removed and rinsed in cold fixatives overnight. Samples [1 mm^3] of the right parietal cortex and adjacent corpus callosum were dissected, rinsed in phosphate buffer, postfixed in 1% osmium tetroxide, dehydrated through a graded series of ethanols and propylene oxide and embedded in Embed (Electron Microscopy Sciences, Ft. Washington, PA). Ultrathin sections were stained with lead citrate and uranyl acetate, and specimens were examined using Zeiss 109 or JEM 100 C transmission electron microscopes.

RESULTS

Scrapie-infected hamster brains

In a companion paper we reported on alterations of the myelianted fibres in hamsters infected either with the 263K or 22C-H strains of scrapie or by Echigo-1 strain of CJD. Here we describe cellular reactions aimed at myelinated fibres undergoing either Wallerian degeneration or primary damage.

Even with low power electron microscopy it was readily apparent that myelinated vacuoles were surrounded by cells and their processes (Fig. 1). The latter belonged either to hyperplastic reactive astrocytes or to macrophages.

Astrocytic reaction

Robust astrocytic reaction in the forms of proliferation and hypetrophy is regarded as a hallmark of TSEs

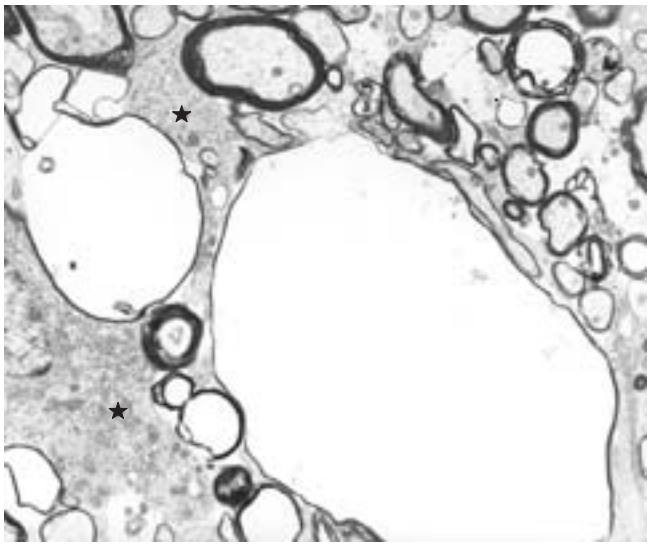

Fig. 1. Low power electron micrograph of two mylinated vacuoles from a hamster brain infected with the 263K strain of scrapie. Note that axons are completely missing from this plane of section. Between vacuoles, many cellular elements are visible (stars). Original magnification, x 4,400.

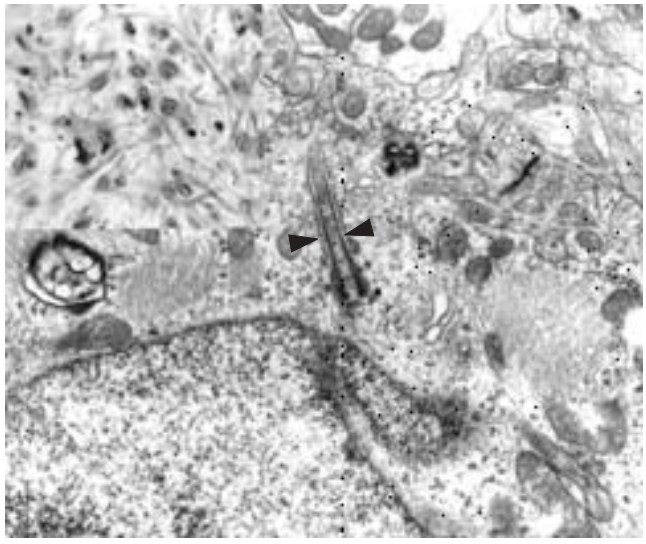

Fig. 2. A typically hypertrophic astrocyte from a hamster brain infected with the 63K strain of scrapie. Note an intracytoplasmic cilium (arrowheads). Original magnification, x 12,000. Inset, exuberant gliosis as visualized by light microscopy and H & E staining.

and it will be only briefly described here. Typically, reactive astrocytes exhibited cytoplasm filled with innumerable glial filaments and, occasionally, other organelle, like cilia (Fig. 2) and abundant tortuous intercellular junctions of adhesive plaque junction type (Fig. 3); desmosome-like junctions connecting astrocytic elements were also seen (Fig. 4). As described earlier (Liberski et al. 1997), astrocytic processes were occasionally interdigitated with oligodendroglial cells and their processes (Fig. 4).

Macrophages

Two types of macrophages were readily described. The majority of them exhibited electron-dense cytoplasm and numerous "empty" vacuoles (digestive chambers)

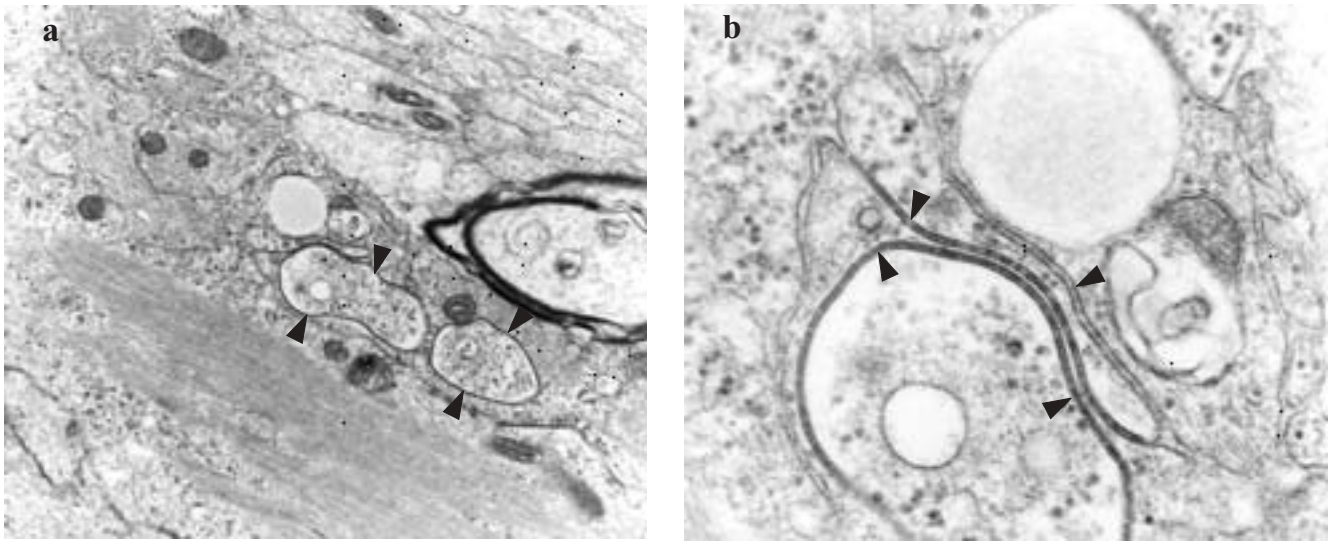

Fig. 3. Low (a) and high (b) power electron micrograph of adhesive plaque junctions (arrowheads) from a hamster brain infected with the 22C-H strain of scrapie. Note that the cytoplasm contains both glial filaments arranged in bundles and abundant glycogen granules. Original magnifications, (a), x 12,000; (b), x 50,000.

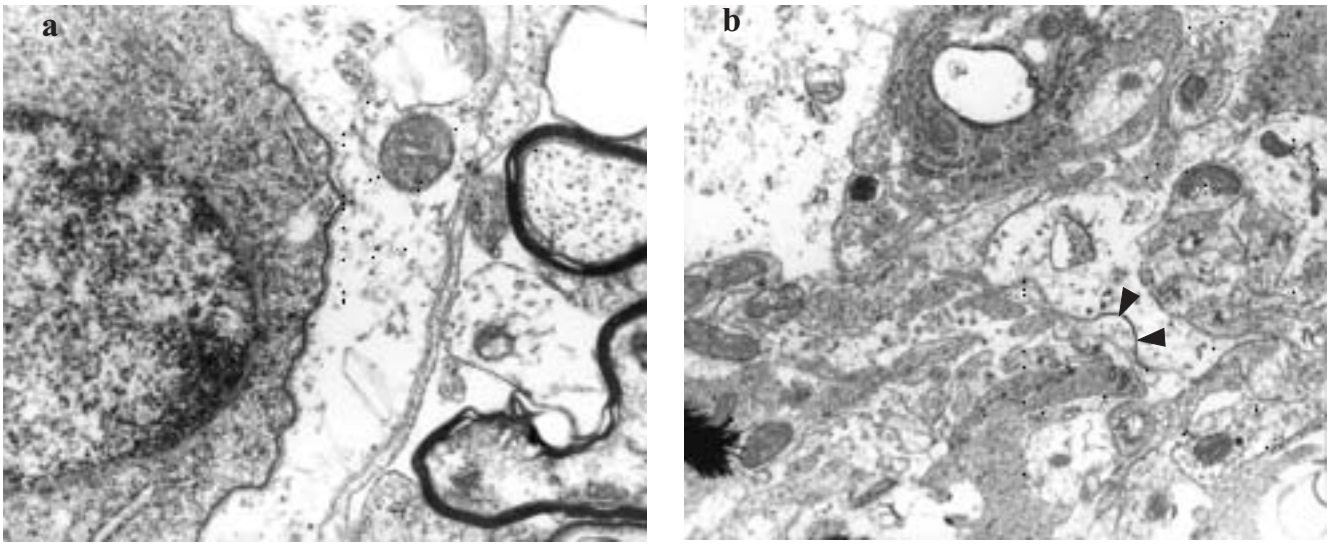

Fig. 4. Astrocytic processes characterized by cytoplasm of low electron density and the presence of adhesive plaque junctions meshed with oligodendroglial cells (a) and their processes (b). Note that the cytoplasm and the finger-like projections of another astrocyte are connected with the desmosome-like junction (arrowheads).

containing cellular debris (Fig. 5). Occasional vacuoles were surrounded by a thin collar reminiscent of "lyre-like inclusions" of the second type of macrophages (Liberski et al. 1989; Fig. 6). The latter were rare and characterized by numerous "lyre-like" inclusions (Fig. 6). Several mylinated fibres were clearly engulfed by the cytoplasm of a macrophage (Fig. 7). Of note, in the cytoplasm of a macrophage unusual annulate lamellae were found (Fig. 8).

DISCUSSION

The majority of TSEs are polioencephalopathies (diseases of the gray matter) and corresponding fine structural changes are relatively well known (Jeffrey et al. 1995b). However, the panencephalopathic type of CJD characterized by predominant involvement of the white matter has been also reported (Tateishi et al. 1978) and

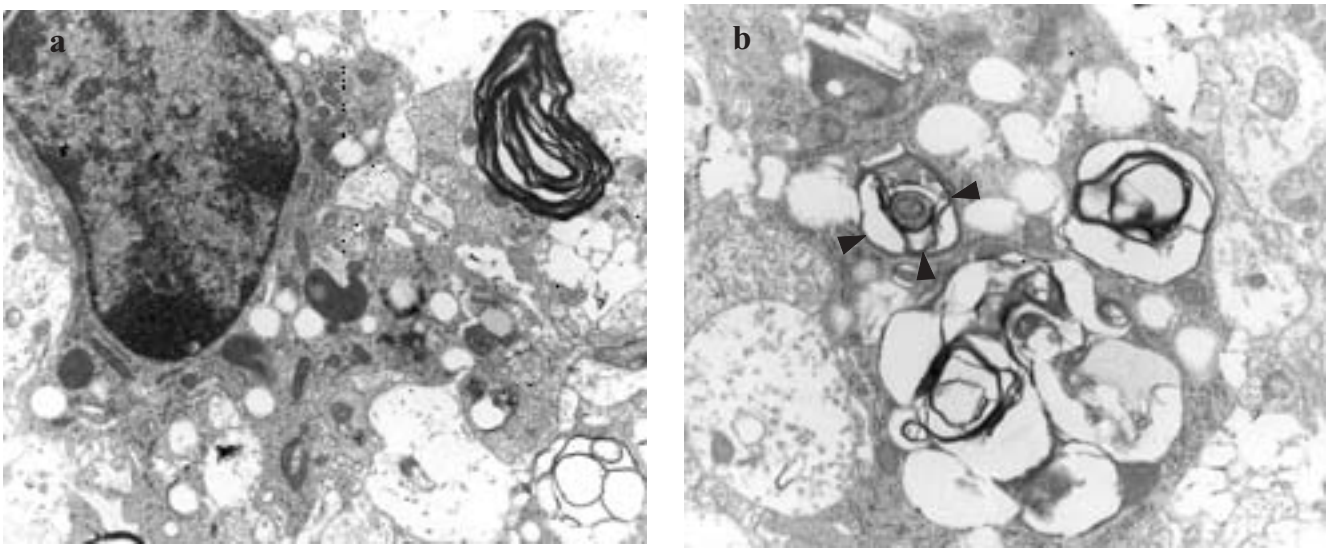

Fig. 5. Low (a) and high (b) power electron micrograph of macrophages from a hamster brain infected with the 22C-H strain of scrapie. Note abundant "empty" vacuoles containing cellular debris (better visible at "b"). Note that occasional vacuoles exhibit a "collar" reminiscent of "lyre-like inclusions" (arrowheads). Original magnifications, (a), x 7,000; (b), x 12,000.

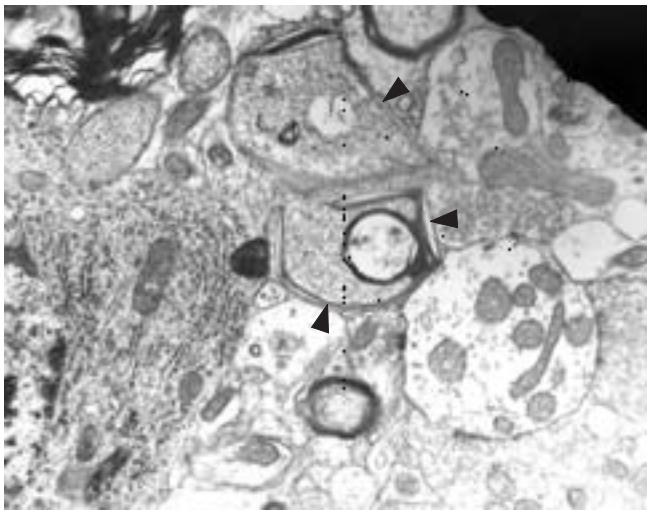

Fig. 6. A macrophage containing two "lyre-like" inclusions (arrowheads) from a hamster brain infected with the 263K strain of scrapie. Original magnification, x 12,000.

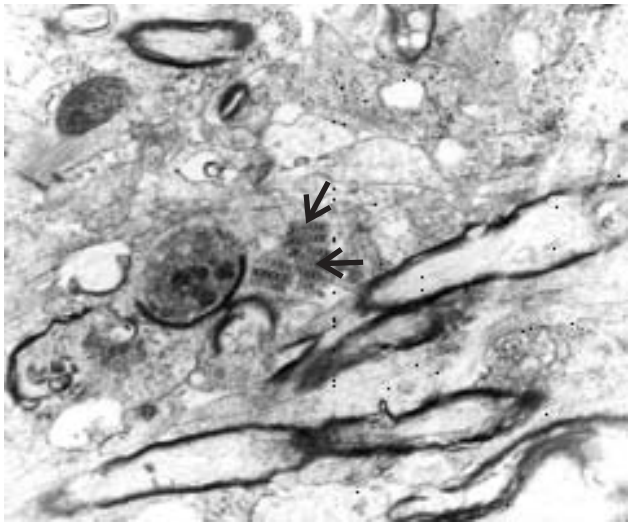

Fig. 8. The cytoplasm of a macrophage containing cellular debris and arrays of annulatae lamellae (arrows). Original magnification, x 12,000.

axonal and myelin pathology at the ultrastructural level have been described (Liberski et al. 1989).

The pattern of changes of myelinated fibres in the Echigo-1 strain of CJD and in two scrapie models, is similar to that of mouse brain infected with the Fujisaki strain of CJD albeit quantatively less robust. It consists of the presence of intramyelin vacuoles, numerous alterations of myelinated axons and an exuberant cellular reaction composed of astrocytes and macrophages. Of note, macrophages contain electron-lucent vacuoles

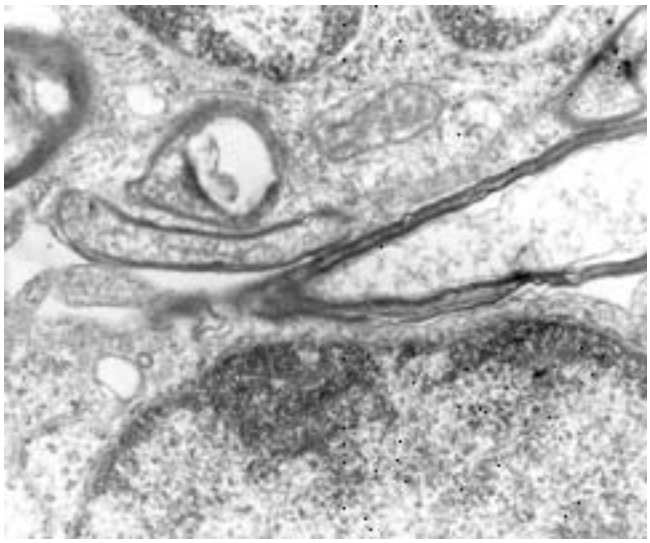

Fig. 7. Two mylinated fibres engulfed within the cytoplasm of a macrophage from a hamster brain infected with the 263K strain of scrapie. Original magnification, x 20,000.

with cellular debris while lyre-like bodies, so prominent in mouse brains infected with the Fujisaki strain of CJD (Fig. 9), were observed only occasionally. As it is reported in the companion paper, the true demyelination, was never seen in any model of hamster TSE.

The final pattern of damage to the myelinated fibres and severe cellular reactions toward there are partially reminiscent of those encountered in some demyelinating conditions including experimental allergic encephalo-myelitis (EAE) and multiple sclerosis (Raine 1985). In contrast to TSEs, the neuropathology of MS and EAE is characterized by focal perivascular lymphocytic infiltrates and macrophages associated with areas of demyelination and disappearance of oligodendroglial cells. The latter are perspicuously absent from the majority of TSEs but it has been reported under some circumstances (Museteanu and Diringer 1981). The significance of the latter finding, if any, is unknown.

In the demyelinating conditions, the terminal stage of destruction to myelinated fibres is non-specifically mediated by diverse cyto- and chemokines secreted from monocytic cells and macrophages; one better known player is a proinflammatory cytokine, tumor necrosis factor-α (TNF-α) (Locksley et al. 2000); another one is Il-1 (Lee et al. 1987, Lai and Baumann 1996).

In the brain, TNF-α is produced by both microglial cells and astrocytes (Robbins et al. 1987, Lieberman et al. 1987, Lau and Liu 2001). TNF-α damages oligodendroglial cells *in vitro* and it is a mitogen for

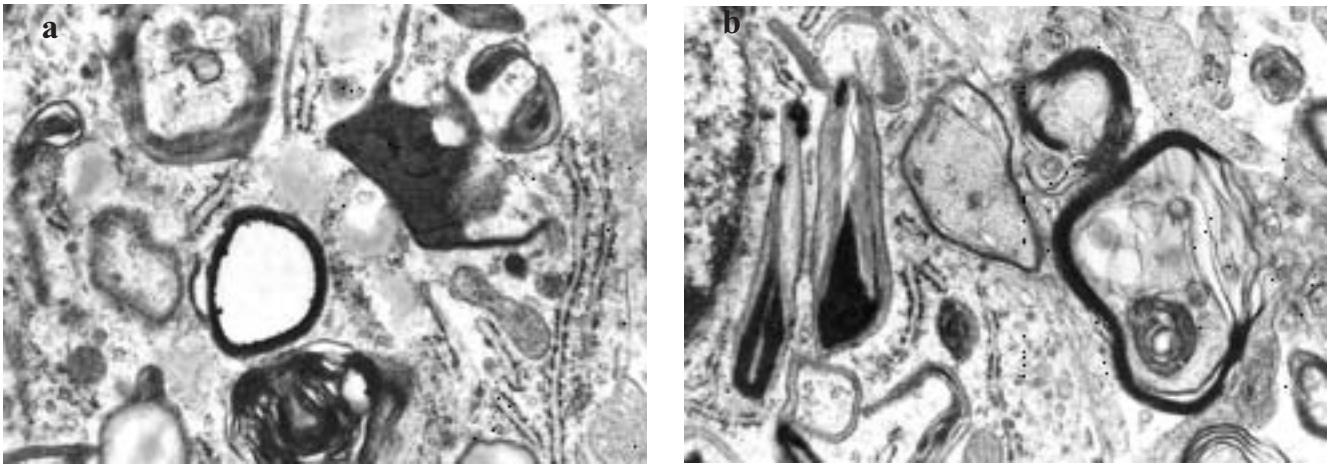

Fig. 9. (a, b) Typical lyre-like inclusions from mouse brain infected with the Fujisaki strain of CJD. Original magnification, x 20,000.

astrocytes but not for oligodendrocytes (Lachman et al. 1987). Reactive astrocytes also produce prostaglandins and IL-1 (Fontana et al. 1982). These cells may also express class II major histocompatibility complex (MHC) antigens following exposure to interferon-γ (IFN-γ) probably by increasing expression of a receptor for TNF-α (Fierz et al. 1985, Benveniste et al. 1989.

TNF-α is merely the "violin section" from a large "orchestra" of cytokine molecules, which may act synergically or in opposition to each other. TNF-α induces production of IL-1, IL-6, TGF-α and other cytokines, and may thus activate multiple cell types in the inflammatory reaction (Philip and Epstein 1986). In addition, IL-1 may induce production of TNF-α (Campbell et al. 1993) and further amplify the reaction. Transgenic mice with IL-6 over-expression show hippocampal lesions similar to those observed in scrapie (Gulian et al. 1988), although IL-6 was not shown to be over-expressed in this disease (Campbell et al. 1994, Walsh et al. 2001). Intracerebral inoculation with IL-1 α or IF-γ causes extensive astrocytosis (Gulian et al. 1988, Olsson 1995). IL-1α is mitogenic for astrocytes but is similiar to TNF-α in causing a decrease in the number of oligodendrocytes. Collectively, activated astrocytes and macropohages may release a smorgasbord of biologically active substances which further amplify this reaction.

TNF-α may also be responsible for some of the neuronal cell loss which occurs in CJD and in scrapie and BSE. TNF-α may play a role inducing apoptosis (Van Everbroeck et al. 2002, Verhuis et al. 2002). In experimental CJD and in scrapie and also in experimental BSE, neuronal autophagic vacuoles, similar to those observed in cells undergoing apoptosis, were observed (Liberski et al. 1992, Jeffrey et al.1992, 1995).

The potential role of TNF-α in development of neuropathological changes has also been studied in TSEs (Liberski et al. 1993, 1995, Kordek et al. 1996). Recombinant murine TNF-α injected into the vitreous of the mouse eye produced myelin ballooning in the optic nerve (Liberski et al. 1993). This myelin dilatation is ultrastructurally indistinguishable from that observed in the panencephalopathic type of CJD. Identical experiments with the same results were performed in hamsters; this means that TNF- α action on the optic nerve is not species-specific (Liberski, unpublished observations). Furthemore, TNF-α immunoreactivity in astrocytes in the brain tissues of scrapie- and CJD-infected mice has also been shown (Liberski et al. 1995, Kordek et al. 1996). Along the same lines, Campbell et al. (1993) in their sequential study of experimental scrapie demonstrated that the mRNA of TNF-α, IL-1α and IL-1β are over-expressed in scrapie-affected brains, the phenomena being brain-specific.

Similar results were obtained by us in another experimental TSE - the panencephalopathic type of CJD (Kordek et al. 1996). In that study, low intense bands for TNF-α increased significantly after 15 week PI, being unchanged in the brains of controls. Analogous results were obtained also for IL-1α. In contrast, bands for PrP and β-actin were similar in both infected and uninfected brain tissue and did not show any correlation with the disease progression. These resluts were consequently confirmed by Western and Northern blot analysis, and

by immunohistochemistry, confirming hypertrophic astrocytes as TNF-α -secreting cells. These data were further confirmed by finding not only upregulation of mRNA of IL-α, IL-β and TNF-α but also an increased activity of NF-κB (Kim et al. 1999, Walsh et al. 2001) which is the major transcriptional factor for proinflammatory cytokines, and by direct visualization of IL-β and TNF-α in the scrapie-infected mouse brains (Williams et al. 1997). However, mice devoid of TNF-α are susceptible for TSE infection albeit only following intracerebral inoculation (Mabbott et al. 2000) while those devoid of lymphotoxin alpha are also susceptible following an oral challenge (Oldstone et al. 2002).

Collectively, these studies demonstrated that TNF-α and IL-1 α are upregulated in scrapie- and CJD-affected brains. Thus, these cytokines may serve as molecular mediators of the white matter degeneration observed in experimental CJD. On the other hand, the over-expression of TNF- α in diseases as diverse as CJD, AIDS vacuolar myelopathy and MS may suggest that pro-inflammatory lymphokines may merely act as the final end-stage and totally non specific mediators of any axon and myelin damage, irrespective of its cause. Accumulation of PrP may initiate a cascade of events which induces release of TNF-α, IL-1 α and β and probably other cytokines, which produce secondary astrocytosis and microglial infiltration. Release of these cytokines in turn further amplifies cytokines resulting in the damage of white brain tissue.

The relation between gliosis, astrocytes, microglia and PrP appear more intricate and elaborate than suspected. Astrocyte changes are the hallmark of TSEs (Liberski 1987) because they not only became hypertrophic but also proliferate (Biernat et al. 1995) and PrP primes astrocytes to proliferate in response to biologically active substances, like IL1 and IL-6, secreted from microglia (Brown et al. 1998, Peyrin et al. 1999, Hafiz and Brown 2000). PrP^{Sc} and GFAP colocalized to astrocyte (Ye et al. 1998) but knock-out mice devoid of GFAP ($GFAP^{-/-}$) are susceptible to scrapie (Gomi et al. 1995, Tatzelt et al. 1996) which suggest that astrocytes are not directly involved in propagation of the infectivity. Furthermore, when PrP transgene was expressed in $PrP^{-/-}$ null mice exlusively in astrocytes using the GFAP promoter, these mice were also susceptible to scrapie (Raeber et al. 1997). Astrocytes can be neurotoxic and exert this role following exposure to neurotoxic peptide PrP106-126 *in vitro* by inhibiting glutamate clearance from the vicinity of synapses (Brown 1999). However, changes in astrocytes that would lead to such neurotoxic behaviour have been shown to be mediated by microglia (Hafiz and Brown 2000).

In summary, we demonstrated here, using thin-section electron microscopy, that astrocytic and macrophage reaction in hamster brains infected either with polioencephalopathic strains of scrapie or panenecephalopathic strains of CJD are qualitatively similar while quantitatively different. The vast amount of data on the involvement of these cells in pathogenesis of TSEs warrant their further studies, in particular, at the molecular level.

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