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Exuberant cellular reaction of the optic nerves in experimental Creutzfeldt-Jakob disease

Paweł P. Liberski[1], Beata Sikorska[1], Jolanta Bratosiewicz-Wąsik[1,2], Anna Waliś[1], Paul Brown[3], and **David Brown[4]**

[1]Department of Molecular Pathology and Neuropathology, Chair of Oncology, Medical University of Łódź, Poland; [2]Department of Virological Diagnostics, Chair of Molecular Biology, Biochemistry and Biopharmacy, Medical University of Silesia in Katowice, Poland; [3]Laboratory of Central Nervous System Studies, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, USA; [4]Department of Biology and Biochemistry, University of Bath, Bath, UK

Abstract. We report here on the exuberant glial reaction in the optic nerves affected by prion diseases. Optic nerves from CJD- and GSS-, and scrapie-infected mice and hamsters showed severe pathology. These lesions were qualitatively indistinguishable from each other but were more intense in the Fujisaki model than in the hamsters inoculated with Echigo-1. Exuberant cellular reaction comprised of macrophages containing numerous mitochondria, abundant rough endoplasmic reticulum, and secondary lysosomes filled with digested myelin debris, electron-dense material and occasionally, entire myelin-bound vacuoles were readily observed in both models. Macrophages actively digesting myelin fragments and containing lyre-like bodies and paracrystalline inclusions were frequently noted. Some macrophages extended long filopodia to form labyrinth-like structures, and within a few macrophages, concentric arrays of cisterns and channels sequestrated part of the cytoplasm. An analogous network of narrow cisterns was seen to surround whole segments of the myelinated fibers.

The correspondence should be addressed to P.P. Liberski, Email: ppliber@csk.am.lodz.pl

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INTRODUCTION

The prion diseases are a group of neurodegenerative disorders which include Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease (GSS) and fatal familial insomnia in man; scrapie in sheep and goats, and bovine spongiform encephalopathy (or "mad cow disease") in animals. According to the widely, but not universally accepted "prion theory", these disorders are caused by group of agents referred collectively to as "prions" (Brown et al. 1991, Collinge 2001, Gajdusek 1977, Ironside et al. 2002, Liberski and Jaskólski 2002, Liberski et al. 2002a,b, Prusiner 1998). According to the "prion theory", a prion is composed "predominantly or entirely" of an abnormal conformer of prion protein (PrP) which is a pathological isoform of a normal cellular glycoprotein. An alternative nomenclature uses the terms "transmissible spongiform encephalopathy" (TSE) and "infectious amyloid" rather than "prion" (Gajdusek, 1996). In prion-affected brain, a disease specific form of PrP (PrP^{sc}, PrP^{res} or Prp^{d}; depending on the nomenclature) accumulates in the form of amyloid plaques, diffuse punctate deposits and several other forms (Budka et al. 1995, Gonzalez et al. 2002, Ironside 2000, Liberski and Jaskólski 2002).

The involvement of the visual system is well recognized in prion diseases. Firstly, scrapie replicates in the eye (Buyukmihci et al. 1977, 1980, 1985a,b, Hogan et al. 1981, 1986) and PrP is expressed in the retina (Christi et al. 1997, Head et al. 2003). In variant CJD, PrP^{Sc} is expressed in the retina and the proximal part of the optic nerve at levels 2.5% and 25% of the brain concentration, respectively (Wadsworth et al. 2001). Secondly, if scrapie is inoculated intraocularly, infection travels along the optic nerves to target the superior colliculi and lateral geniculate bodies (Fraser 1982, Fraser and Dickinson 1985, Foster et al. 1990, Jeffrey et al. 1991, Kimberlin and Walker 1986, Liberski et al. 1990b, 2002, Scott and Fraser 1989a,b, Scott et al. 1991, 1992). Thirdly, the retina degenerates in several models of scrapie and CJD in rodents as a result of apoptosis of ganglion cells (Buyukmihci et al. 1977, 1983, 1985a,b, Foster et al. 1986a,b, Hogan et al. 1981, 1983) and cases of CJD and GSS with retinal or geniculate body degeneration or optic nerve atrophy have already been reported (Kitagawa et al. 1983, Lesser et al. 1979, Sato et al. 1992, Sugai et al. 2000). Fourthly, iatrogenic CJD cases following transplantation of cornea have been described (Duffy et al. 1974). However, in none of these studies was the pathology of the optic nerve studied in detail (Buyukmihci et al. 1982).

The classical and ultrastructural neuropathology of prion diseases has been well described (Jeffrey et al. 1995, Liberski and Jeffrey 2000, Liberski et al. 2002). It comprises spongiform change, astrocytic gliosis and neuronal loss brought about by the apoptotic process. The majority of cases of Creutzfeldt-Jakob disease (CJD) are classified as polioencephalopathic – i.e., affecting gray matter, but some two dozen cases have met the criteria of a panencephalopathic type where the white matter is predominantly affected. These white matter changes do not entirely result from Wallerian degeneration but retrograde changes will occur if ganglion cell bodies are lost (Fraser et al. 1997) because of the absence of a correlation with corresponding gray matter damage (Liberski et al. 1989b). The same phenomenon of primary degeneration of white matter in excess of neuronal drop-out has been described in experimental scrapie (Fraser 1979). The white matter changes are also relatively well understood at the molecular level (Kordek et al. 1996, Liberski et al. 1993).

We report here the ultrastructural pathology of the cellular reaction in the optic nerves in two models of human TSE in rodents. Preliminary observations on the Fujisaki strain of GSS have been already published (Waliś et al. 1997).

MATERIAL AND METHODS

Animals and strains

The Fujisaki strain of GSS, originally isolated from the brain tissues of a 56-year old man with progressive dementia (Tateishi et al. 1978), was passaged three times (at 1:10 dilution) in mice in this laboratory (Kingsbury et al. 1982, Liberski et al. 1989b).

Five weanling 4- to 6-week old NIH Swiss mice (Animal Production Area, Frederick Cancer Research Facility, Frederick, MD) were each inoculated intracerebrally with 0.03 ml of a 10% (w/v) clarified brain suspension of the Fujisaki strain of GSS (titer, 3.1×10^4 LD_{50} per 0.03 ml, by intracerebral route). The incubation period ranged from 16 to 18 weeks (Liberski et al. 1989b). Control animals were sham-inoculated with saline.

In addition, 5 hamsters were inoculated with the Echigo-1 strain of CJD (Liberski et al. 1999). This strain was isolated by Mori and colleagues (1989) from a 33 years-old female patient with a panencephalopathic

type of sporadic CJD. The inoculum prepared from her brain was repeatedly passaged in guinea pigs, and then re-isolated in hamsters. The incubation period following intracerebral inoculation of hamsters with 10 % (w/v) cleared suspension of the Echigo-1-affected brain was approximately six months. Appropriate control animals were sham-inoculated with saline. For each experiment, five terminally ill TSE-infected animals and three control animals were used. Additionaly, one brain from each experiment was immunolabeled with 6H8 anti-PrP antibodies to confirm the diagnosis (Kovacs et al. 2002).

Electron Microscopy

Following light anesthesia with ether, five CJD- and two sham-inoculated mice were killed by intracardiac perfusion using 180 ml of 1.5% glutaraldehyde and 1% paraformaldehyde prepared in phosphate buffer (pH 7.4). Five Echigo-1-infected and two sham-inoculated hamsters were each perfused with 100 ml of 1.25% glutaraldehyde and 1% paraformaldehyde prepared in cacodylate buffer (pH 7.4) followed by 50 ml of 5% glutaraldehyde and 4% paraformaldehyde. Both methods produced identical results with regard to tissue preservation (Liberski, unpublished data). From perfused animals, optic nerves were dissected and divided into three or four samples which were postfixed in 1% osmium tetroxide for 1 to 2 hours, dehydrated through a series of graded ethanol and propylene oxide, and embedded in Embed 812 (Electron Microscopy Sciences, Ft. Washington, PA). Semithin sections were stained with toluidine blue, and ultrathin sections were stained with lead citrate and uranyl acetate, coded prior to examination and photography with Zeiss EM 109 or JEM 100C transmission electron microscopes.

RESULTS

The optic nerves of control animals inoculated intracerebrally with homogenates of optic nerves or with saline appeared normal albeit some myelin sheaths exhibited subtle separation of lamellae. Axons contained moderate numbers of microtubules and neurofilaments, and astrocytic septa between clusters of fibers were readily visible.

In contrast, optic nerves from CJD- and GSS-, and scrapie-infected rodents showed severe pathology (Fig. 1). These lesions were qualitatively indistinguishable from each other but were more intense in the Fujisaki model than in the hamsters inoculated with Echigo-1.

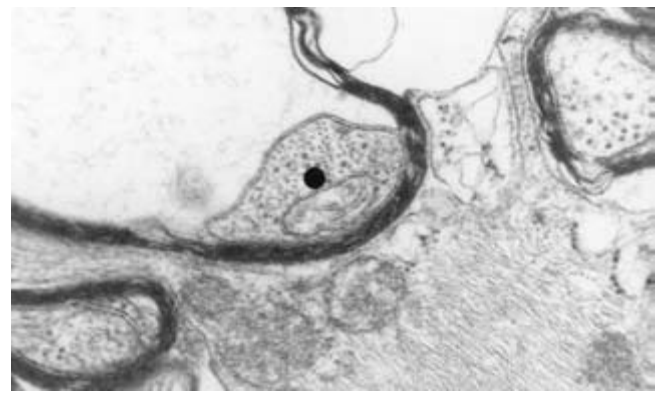

Fig. 1. A typical vacuole in a myelinated fibre in the optic nerve of a mouse inoculated with the Fujisaki strain of GSS. Note the shrunken axon attached to the innermost layer of the myelin (circle) and astrocytic process in close contact with the vacuole. Original magnification, x 30,000.

Exuberant cellular reaction comprised of macrophages containing numerous mitochondria, abundant rough endoplasmic reticulum, and secondary lysosomes filled with digested myelin debris, electron-dense material and occasionally, entire myelin-bound vacuoles were readily observed in both models (Fig. 2). Macrophages actively digesting myelin fragments and containing lyre-like bodies and paracrystalline inclusions were frequently noted (Fig. 3). On closer inspection, some macrophages showed small vacuoles the size of mitochondria that contained whorled myelin fragments (Fig. 3b). Still other macrophages extended long filopodia to form labyrinth-like structures (Fig. 4), and within a few macrophages, concentric arrays of cisterns and channels sequestrated part of the cytoplasm (Fig. 5). An analogous network of narrow cisterns was seen to surround whole segments of the myelinated fibers. Hypetrophic astrocytes were numerous and were often seen in close contacts with macrophages.

DISCUSSION

In a series of previous studies we evaluated the only existing model of panencephalopathic type of CJD, the Fujisaki strain of GSS passaged in mice (Liberski et al. 1989b, Tateishi et al. 1978); the present study describes a second panencephalopathic model of CJD available in small laboratory rodents. It is potentially important for use in comparative studies of different strains of agent in the same host (hamster). Thus far only mouse and ham-

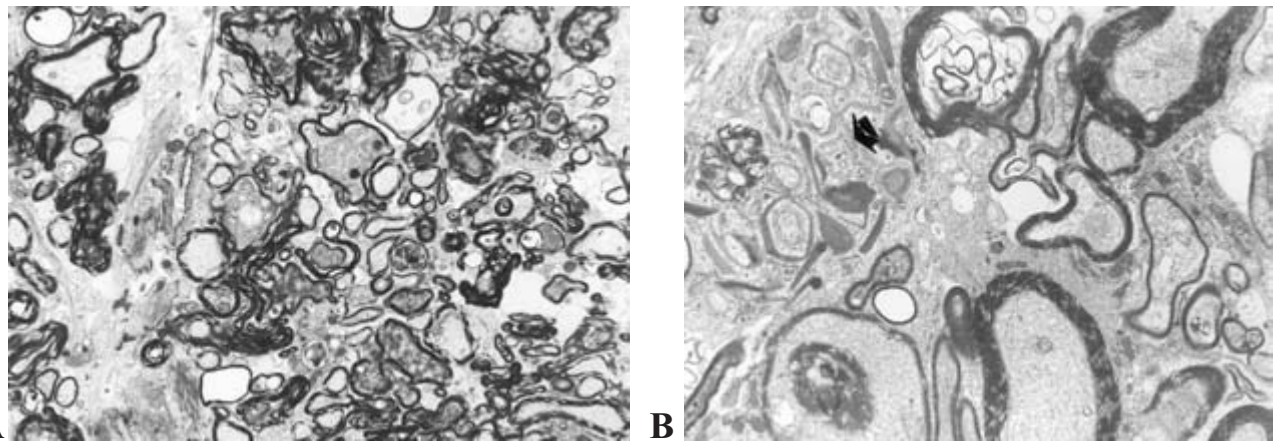

Fig. 2. Pathological changes in the optic nerves from hamster brain (A) infected with the Echigo-1 strain of CJD, magnification, x 4,400 and mouse brain (B) infected with the Fujisaki strain of CJD, magnification, x 12,000. Note widespread degeneration of the myelinated fibres and fragment of the cytoplasm of the macrophage (arrow) containing paracrystalloid inclusions in (B).

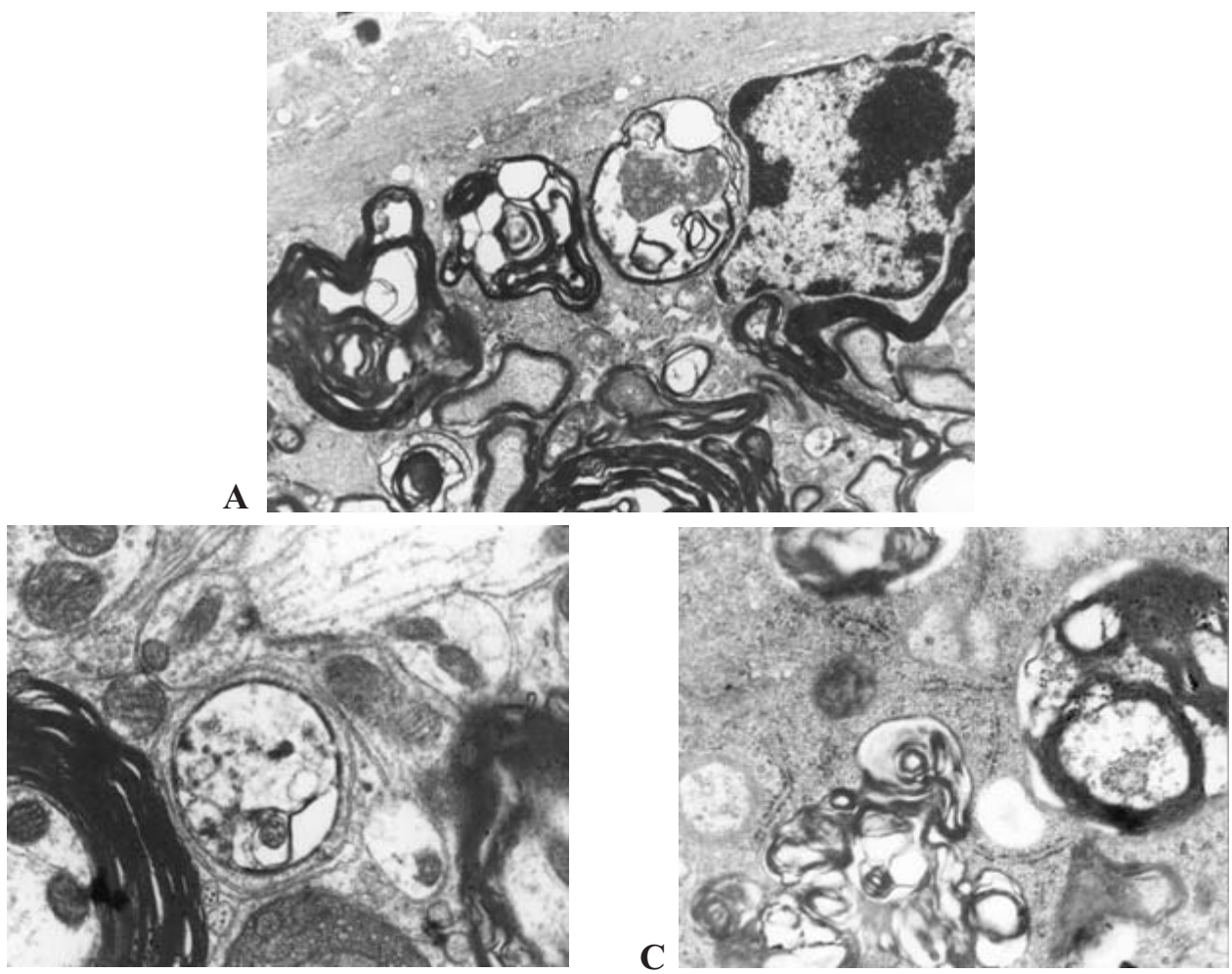

Fig. 3. (A) Myelined fragments within a macrophage in the optic nerve from a hamster inoculated with the Echigo-1 strain of CJD, magnification, x 7,000; (B) the cytoplasm of a macrophage containing several small vacuoles with whorled myelin fragments, magnification, x 12,000; (C) higher magnification of the cytoplasm of the macrophage showing abundant myelin segments undergoing digestion, magnification, x 30,000.

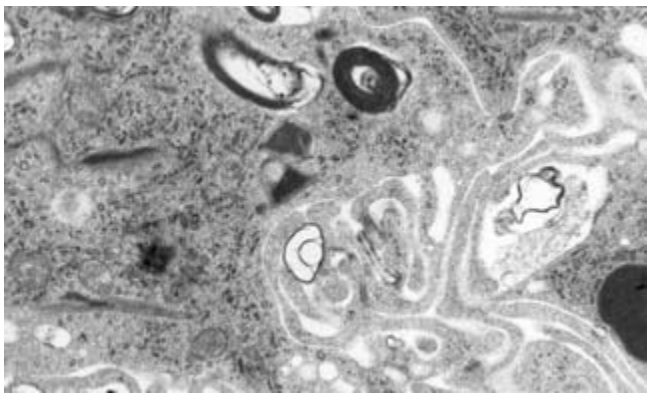

Fig. 4. A macrophage from a mouse infected with the Fujisaki strain of GSS containing digestive chambers with whorled myelin or whole segments of myelinated fibers. Note also elaborate network of filopedia. Magnification, x 12,000.

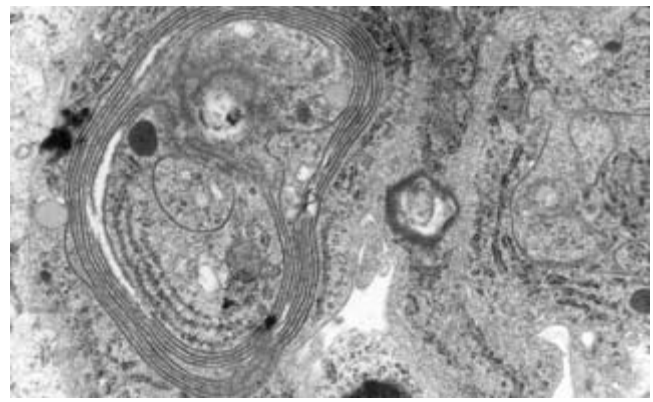

Fig. 5. An elaborated concentric array of narrow cisterns surrounding a part of the cytoplasm of a macrophage. Magnification, x 7,000.

ster models have been available for comparative studies (Liberski and Budka 1999, Liberski et al. 1989a, 1992, 1995).

Degeneration of the optic nerve seems to parallel that of retinal degeneration in several rodent models of prion diseases (Buyukmihci et al. 1982, Hogan et al. 1981, 1983). Degeneration of the optic nerve in mice infected with the Fujisaki strain of GSS (Hogan et al. 1983) exceeded that of hamsters infected with scrapie (Hogan et al. 1981) and our study confirmed these observations. In both models, however, degeneration of the outer retinal layer, with thinning of outer and inner photoreceptor elements and almost total loss of the outer plexiform layer was seen. Moreover, numerous macrophages were observed in the subretinal space and some pyknotic (apoptotic) cells were also detected (Hogan et al. 1981). The optic nerve is only briefly described in the study of Hogan et al. (1983); the changes described consist of vacuolar degeneration and astrocytosis. It is uncertain whether optic nerve degeneration follows retinal degeneration in prion diseases or *vice versa* (die-back phenomenon). Because both Wallerian degeneration and primary myelinated fiber degeneration were detected in the optic nerve described here, it is plausible that both processes take place concurrently. In BSE and in ME7 and 87V murine scrapie some white matter vacuolation occurs but neuronal loss is not a prominent feature in these models (Jeffrey et al. 1994). In the Fujisaki panencephalopathic strain of GSS, severe neuronal loss is generally not observed and the majority of neuronal cell bodies studied ultrastructurally looked entirely normal (Liberski et al. 1990a). However, parvalbumin-immunopositive neurons are reduced by 50% in the terminal stage if the disease (Guentchev et al. 1998) and that neuronal population may contribute to the die-back process. Thus, we speculate that Wallerian degeneration, and that retinal degeneration while vacuolation of the myelin sheath is the primary event, as in the white matter of panencephalopathic strains (Liberski et al. 1989b).

Discussion of the putative role of activated microglia/ /macrophages in prion diseases requires a few words about nomenclature. Microglia were reported in 1919 by Rio del Hortega on the basis of silver impregnation studies (Dickson and Lee 1997). However, as originally used by Rio del Hortega, the term covered not only microglia as currently understood but also oligodendrocytes and small astrocytes. The term has since evolved to cover "intrinsic cells of the CNS with a mesodermal origin and a characteristic morphology and antigenic phenotype consistent with a special type of mononuclear phagocyte" (Dickson and Lee 1997). There is another type of cell, the "brain macrophage" (foamy macrophage or "gitter" cells) which is derived from blood-circulating monocytes and which share with microglia several ultrastructural and antigenic similarities (i.e., expression of CD68 antigen). To confuse the problem even further, some macrophages are derived from activated microglia, and a distinct class of "perivascular microglia/macrophages" seems to be different from resting microglia. Indeed, perivascular microglia/ /macrophages rest outside the blood-brain barrier between the outer basement membrane and the glia limitans and they are derived from monocytes (Dickson and Lee 1997). Taken together, the relationship be-

tween all classes of cells mentioned above is still far from clear though all macrophages and microglia are of mesenchymal origin

In the present study, we employed stringent ultrastructural criteria to classify a cell as an activated microglia: the presence of dark cytoplasm and digestive vacuoles; the absence of intermediate filaments (which distinguishes them from astrocytes); and the absence or paucity of microtubules (which distinguishes them from oligodendrocytes) (Liberski et al. 2002).

We observed a robust microglia/macrophage activity in areas of degeneration of the myelin fibers in the optic nerves of both models of panencephalopathic CJD. This activation is not qualitatively different from that in the white matter earlier described in these models (Liberski et al. 1989) and is not surprising given the fact that optic nerves are extensions of the central white matter.

Panencephalopathic CJD is not a classic demyelinating condition because there is neither an initial immunologically-mediated phase nor an infiltration of activated lymphocytes into the brain parenchyma. However, the end-stage of disease characterized by myelin internalization and breakdown is virtually identical to the end-stage of multiple sclerosis (MS) or experimental allergic encephalomyelitis (EAE). According to the "graded response hypothesis" (Dickson and Lee 1997) when tissue damage is limited, only ramified microglia and perivascular microglia are activated, but when the damage is more widespread (like in MS), robust activation of resting microglia is assisted by the influx of blood-born macrophages. This is likely to be the case in panencephalopathic strains of CJD where myelin digestion is only too evident at the ultrastructural level. Parenthetically, at this stage monocyte- and microglia--originated macrophages cannot be discriminated solely on the basis of morphological ground.

The role of microglia/macrophages in prion disease is multifaceted (for review, see Rezaie and Lantos 2001). The most straightforward evidence would seem to be the presence of microglia in the amyloid plaques which we (Barcikowska et al. 1993, Guiroy et al. 1994) and others (Miyazono et al. 1991) have clearly demonstrated almost a decade ago. Whether microglia are merely scavenger cells or actively amyloid-processing cells has been a subject of fierce debate (Liberski and Jeffery 2000). There is no robust plaque formation in either CJD model reported here and, thus, the role of microglia in them must be different.

There is a second, well-documented role of microglia cells which mediate tissue destruction in prion disease. First, microglia are extensively activated in both CJD- and scrapie-affected brains (Baker et al. 1999, Muhleisen et al. 1995, Van Everbroeck et al. 2002, Williams et al. 1994, 1995) but the extent of this activation seems to differ between models (Baker et al. 1999). Collectively, microglia activation dominates in those areas which also exhibit a high level of spongiform change and apoptotic neurons as detected by the TUNEL method (Jesionek-Kupnicka et al. 2001). It is highly plausible that microglia contribute to tissue destruction by secretion of biologically active compounds like pro-inflammatory cytokines (Campbell et al. 1994, Kordek et al. 1996, Liberski et al. 1993) or chemokines (Baker et al. 1999) not unlike those forming "a cytokine arm" of HIV-1-infected brain encephalitis (Budka et al. 1991).

Microglia have been suggested to have a causative role in the neurodegeneration seen in prion disease. Evidence from studies on scrapie infected mice suggest that microglia increase in number and become activated in regions containing PrP^{Sc} (Giese et al. 1998). This activation precedes the majority of apoptotic events occurring in the neurons of these regions. However, modeling of microglial involvement has been dependent on *in vitro* studies of the toxicity of a fragment of the prion protein termed PrP106-126 (Forloni et al. 1993). This fragment has been shown to be neurotoxic to retinal neurons following injection in the eye of rats (Ettaiche et al. 2000). In neuronal culture the mechanism of toxicity of PrP106-126 requires two events. The first is a direct interaction with the target cell, the neuron. Soluble forms of the peptide bind to PrP^{c} on the cell surface inactivating the protein and inducing a profound reduction in cellular resistance to oxidative stress (Brown 2000, Brown et al. 1996). The peptide also has secondary effects, inhibiting astrocytic clearance of glutamate (Brown and Mohn 1999) and activating microglia (Brown et al. 1996). Microglia activated by PrP106-126 or PrP^{Sc} release superoxide and pro-inflammatory cytokines (Hafiz and Brown 2000). The release of cytokines in the presence of oxidative stress induces increased astroglial proliferation (Hafiz and Brown 2000). The combination of decreased astrocytic protection of neurons and the presence of toxic factors from microglia then trigger apoptosis in nearby neurons (Brown 1999, Brown et al. 1996). The combination of various glial disturbances resulting from interaction with PrP106-126 or PrP^{Sc} creates micro-environment oxidative stress that appears to be required for the initiation of apoptosis in this model

of apoptosis in prion disease. Thus, prion induced microglial activation in combination with compromised stress defenses in neurons are a probable cause of neuronal death.

CONCLUSIONS

The robust macrophagic reaction is a common finding in optic nerves affected by prion diseases but the intensity of it is dependent on the model used.

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