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平成22年度　研究成果に関する刊行一覧表
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Accumulation of L-type Bovine Prions in Peripheral Nerve Tissues

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We recently reported the intraspecies transmission of L-type atypical bovine spongiform encephalopathy (BSE). To clarify the peripheral pathogenesis of L-type BSE, we studied prion distribution in nerve and lymphoid tissues obtained from experimentally challenged cattle. As with classical BSE prions, L-type BSE prions accumulated in central and peripheral nerve tissues.

Bovine spongiform encephalopathy (BSE) is a fatal neurodegenerative disorder of cattle characterized by accumulation of a protease-resistant form of a normal cellular prion protein (PrPres) in the central nervous system. The scientific literature in general has assumed that BSE in cattle is caused by a uniform strain (classical BSE). However, different neuropathologic and molecular phenotypes of BSE (atypical BSEs) have recently been reported from various countries (*1*). Recent data from Western blot analyses of field cases of atypical BSEs are characterized by a higher (H-type BSE) or lower (L-type BSE) molecular mass of the unglycosylated form of PrPres than is classical BSE (*2*). The origins of atypical BSEs remain obscure; unlike classical BSE, atypical BSE has been detected mainly in aged cattle and suggested as a possible sporadic form of BSE (*3*).

Several lines of evidence demonstrate that classical BSE and a variant form of Creutzfeldt-Jacob disease are most likely caused by the same agent (*4,5*). Transmission of classical BSE to humans has been proposed to result from ingestion of contaminated food. Whether atypical BSEs are transmissible to humans remains uncertain; however, human susceptibility to L-type BSEs is suggested by recent experimental transmission in primates (*6*) and mice transgenic for human prion protein (PrP) (*7*) by using the most effective route of intracerebral inoculations of prions. The L-type BSE prion is much more virulent in primates and in humanized mice than is the classical BSE prion, which suggests the possibility of zoonotic risk associated with the L-type BSE prion. These findings emphasize the critical importance of understanding tissue distribution of L-type BSE prions in cattle because, among the current administrative measures for BSE controls, the specified risk materials removal policy plays a crucial role in consumer protection.

In Japan, atypical BSE was detected in an aged Japanese Black cow (BSE/JP24) (*8*). We recently reported the successful transmission of BSE/JP24 prions to cattle and showed that the characteristics of these prions closely resemble those of L-type BSE prions found in Italy (*9*). In this study, we report the peripheral distribution of L-type BSE prions in experimentally challenged cattle.

The Study

The Animal Ethics Committee and Animal Care and Use Committee of the National Institute of Animal Health approved the study. Five Holstein calves 2–3 months of age were intracerebrally injected with 1 mL of 10% (w/v) brain homogenates prepared from the medulla oblongata of BSE/JP24. In our earlier report, experimentally challenged cattle appeared to display clinical signs indicative of BSE at 11 months postinoculation (mpi) (*9*). Animals were sequentially euthanized before and after the onset of clinical signs (cattle identification codes 8515 and 496 at 10 and 12 mpi, respectively) and at the terminal stage of the disease (cattle identification codes 528, 1061, and 5566 at 16 mpi). A wide range of tissues was sampled at subsequent necropsy. We provisionally categorized the adrenal gland as nerve tissue because of the presence of chromaffin cells in the medulla of the gland.

Western blot analysis for PrPres was performed on obex tissue samples as described previously by using anti-PrP monoclonal antibody T2 (*9*). PrPres was detectable in all obex samples obtained 10, 12, and 16 mpi, suggesting that transmission of L-type BSE prions to these animals was successful. Dilution of the protease-treated brain sample and analysis of Western blot results showed that the detection threshold for PrPres was 1.25 μg of brain tissue equivalent (data not shown).

A variety of nerve and lymphoid tissue samples were investigated for accumulation of PrPres by Western blot analysis by using phosphotungstic acid precipitation, as described previously (*10*); examples of cattle tissue samples obtained 10 and 16 months mpi (codes 8515 and

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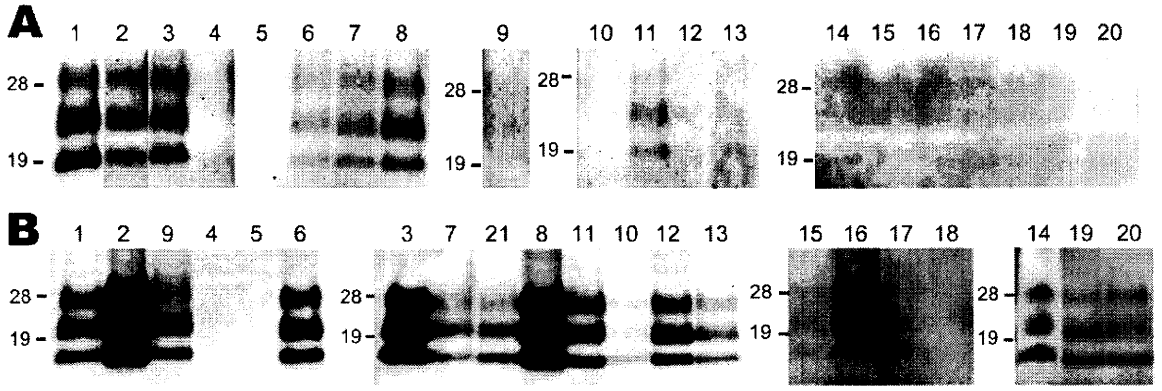

Figure 1. Western blot analysis of a protease-resistant form (PrPres) of a normal cellular prion protein in nerve tissue samples obtained from cattle 10 (A) and 16 (B) months postinoculation (cattle identification codes 8515 and 1061, respectively). The nerve tissues tested are shown above the lanes: 1, trigeminal ganglia; 2, pituitary gland; 3, anterior cervical ganglion; 4, facial nerve; 5, hypoglossal nerve; 6, cranial mesenteric ganglia; 7, vagus nerve (cervical part); 8, stellate ganglia; 9, adrenal gland; 10, phrenic nerve; 11, vagus nerve (pectoral part); 12, vagosympathic trunk (pectoral part); 13, vagosympathetic trunk (lumbar part); 14, accessory nerve; 15, suprascapular nerve; 16, brachial nerve plexus; 17, median nerve; 18, radial nerve; 19, sciatic nerve; 20, tibial nerve, 21, middle cervical ganglion. The equivalent of 100 mg of tissue was loaded. Western blots were probed with monoclonal antibody T2 to detect PrPres. Molecular mass standards (kDa) are indicated on the left of each panel.

1061, respectively) are shown in Figure 1. In cattle at the preclinical stage, PrPres was detectable in all tested ganglia and barely detectable in the vagus nerve and vagosympathic trunk. In cattle at the terminal stage, PrPres was barely detectable in the forelimb nerves (suprascapular nerve, brachial nerve plexus, median nerve, and radial nerve), whereas substantial amounts of PrPres were present in other nerve tissues except for facial and hypoglossal nerves (Table). A broader nerve tissue distribution of PrPres was observed in cattle at 16 mpi than at 10 and 12 mpi. Contrary to what we found in nerve tissues, we detected no PrPres from tests performed on lymphoid tissues obtained from any of the 5 cattle studied.

Infectivity of selected nerve tissues (including the obex, sciatic nerve, adrenal gland, brachial nerve plexus, and vagus nerve) obtained from cattle euthanized at 10, 12, and 16 mpi (codes 8515, 498, and 5566, respectively) was analyzed by intracerebral injection into mice transgenic for bovine prion protein, as described previously (*11*). As a negative control, mice were injected with cells from the brainstem of a normal cow. The presence of PrPres in the brains of all mice used in the experiment was determined by Western blot analysis. Infectivity was detected in all nerve tissues tested, regardless of the presence of detectable PrPres (Figure 2). Control mice showed no apparent abnormality >500 days postinoculation.

Conclusions

We report accumulation of L-type atypical BSE prions in peripheral nerve tissues sampled from intracerebrally challenged cattle. Our study demonstrated that almost all of the peripheral nerve tissues tested became PrPres positive in a time-dependent manner, whereas no PrPres was detectable in lymphoid tissues, even in cattle with fatal atypical BSE. Our results suggest the possibility that, like classical BSE prions, L-type BSE prions propagated in the central nervous system and were spread centrifugally by nerve pathways (*11,12*). In

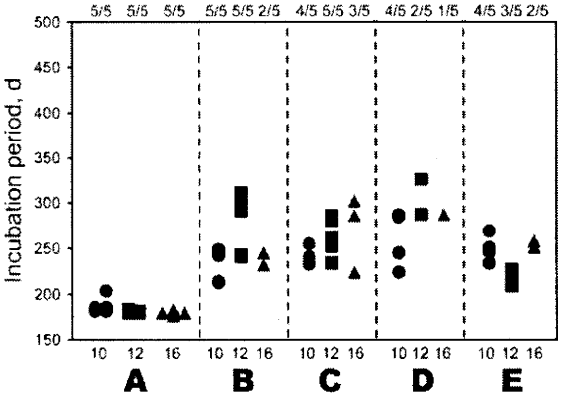

Figure 2. Bioassay using nerve tissues obtained from bovine spongiform encephalopathy JP24 prion-inoculated cattle. Inocula from selected tissues—obex (A), sciatic nerve (B), adrenal gland (C), branchial nerve plexus (D), and vagus nerve cervical part (E)—were prepared from cattle euthanized at 10 (code 8515, circle), 12 (code 498, square), and 16 (code 5566, triangle) months postinoculation were and inoculated intracerebrally into mice transgenic for bovine prion protein. Ratios above graph indicate number of prion-diseased mice/number of inoculated mice at 500 d postinoculation.

Italy, L-type BSE prions have been characterized in detail by using cattle challenged intracerebrally. However, PrPres was not detected in their peripheral tissues, including the peripheral nerves (*13*). The reason for the discrepancy in PrPres detection is unclear. In view of the similarities between the L-type and BSE/JP24 prion characteristics (*9*), this discrepancy may result from differences in the methods used for PrPres detection.

We detected infectivity in the nerve tissue samples (including samples from the obex, sciatic nerve, adrenal gland, brachial nerve plexus, and vagus nerve) obtained 10, 12, and 16 mpi. On the basis of the incubation time of 223 ± 25 (mean ± SD) days in mice injected with a 1,000-fold dilution of the obex homogenate, infectious titers in peripheral nerve tissues appeared to be 1,000 × lower than those estimated in the obex during endpoint titration of infectivity.

Our results demonstrate that L-type atypical BSE prions can be distributed in the peripheral nerve tissues of intracerebrally challenged cattle. These findings are useful for understanding L-type BSE pathogenesis and accurately assessing the risks associated with this disease. Investigations of prion distribution in cattle that have been orally challenged with L-type BSE prions are critical.

Table. Western blot detection of PrPres in tissue samples obtained from cattle intracerebrally challenged with BSE/JP24 prion*

Tissue samples	Cattle identification codes				
	8515 (10 mpi)	498 (12 mpi)	528 (16 mpi)	1061 (16 mpi)	5566 (16 mpi)
Nerve tissues					
Obex	+	+	+	+	+
Spinal cord	+	+	+	+	+
Cauda equina	+	+	+	+	+
Optic nerve	+	+	+	+	+
Pituitary gland	+	+	+	+	+
Trigeminal ganglia	+	+	+	+	+
Cranial cervical ganglia	+	+	+	+	+
Stellate ganglia	+	+	+	+	+
Vagosympathic trunk	+	+	+	+	+
Cranial mesenteric ganglia	+	+	+	+	+
Vagus nerve	+	+	+	+	+
Facial nerve	–	–	–	–	–
Hypoglossal nerve	–	–	–	–	–
Phrenic nerve	–	+	+	+	+
Accessory nerve	–	+	+	+	+
Suprascapular nerve	–	–	+	+	+
Brachial nerve plexus	–	–	+	+	+
Median nerve	–	–	+	+	–
Radial nerve	–	–	+	+	–
Sciatic nerve	–	+	+	+	+
Tibial nerve	–	+	+	+	+
Adrenal gland	–	+	+	+	+
Lymphoid tissues					
Spleen	–	–	–	–	–
Tonsil	–	–	–	–	–
Parotid lymph nodes	–	–	–	–	–
Lateral retropharyngeal lymph nodes	–	–	–	–	–
Mandibular lymph nodes	–	–	–	–	–
Brachiocephalic lymph node	–	–	–	–	–
Anterior cervical lymph node	–	–	–	–	–
Axillary lymph nodes	–	–	–	–	–
Superficial inguinal lymph nodes	–	–	–	–	–
Subiliac lymph nodes	–	–	–	–	–
Popliteal lymph nodes	–	–	–	–	–
Splenic lymph nodes	–	–	–	–	–
Hepatic lymph nodes	–	–	–	–	–
Internal iliac lymph nodes	–	–	–	–	–
External iliac lymph nodes	–	–	–	–	–
Mesenteric lymph nodes	–	–	–	–	–

*PrPres, prion protein resistant; BSE, bovine spongiform encephalopathy; mpi, months postinoculation; +, positive for PrPres; –, negative for PrPres.

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Dr Iwamaru is a veterinarian and senior researcher with the Prion Disease Research Center, National Institute of Animal Health. His research focuses on the molecular mechanisms underlying neurodegenaration in prion diseases.

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Predominant Involvement of the Cerebellum in Guinea Pigs Infected with Bovine Spongiform Encephalopathy (BSE)

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Summary

This study reports the experimental transmission of bovine spongiform encephalopathy (BSE) to guinea pigs and describes the cerebellar lesions in these animals. Guinea pigs were inoculated intracerebrally with 10% brain homogenates from BSE-affected cattle. These animals were designated as the first passage. Second and third passages were subsequently performed. All guinea pigs developed infection at each passage. The mean incubation period of the first passage was 370 days post-infection (dpi) and this decreased to 307 dpi and 309 dpi for the second and third passages, respectively. Mild to severe spongiform degeneration and gliosis were observed in the cerebral cortex, thalamus and brainstem. In addition, the affected animals had marked pathological changes in the cerebellum characterized by severe cortical atrophy associated with Bergmann radial gliosis of the molecular layer and reduction in the width of the granular cell layer. Immunohistochemically, intense PrP^{Sc} deposition and scattered plaque-like deposits were observed in the molecular and granular cell layers. Cerebellar lesions associated with severe atrophy of the cortex have not been reported in animal prion diseases, including in the experimental transmission of PrP^{Sc} to small rodents. These lesions were similar to the lesions of human kuru or the VV2 variant of sporadic Creutzfeldt–Jakob disease, although typical kuru plaques or florid plaques were not observed in the affected animals.

Keywords: BSE; cerebellum; experimental transmission; guinea pig

Introduction

Scrapie in sheep and goats, bovine spongiform encephalopathy (BSE), chronic wasting disease (CWD) in deer and Creutzfeldt–Jakob disease (CJD) (sporadic, familial and transmitted or acquired forms), Gerstmann–Sträussler–Sheinker disease or syndrome (GSS) and kuru in man are neurodegenerative disorders belonging to a group of prion diseases or transmissible spongiform encephalopathies (TSEs). They are characterized by the accumulation of abnormal protease-resistant prion protein (PrP^{Sc}), which is an isoform of the cellular protease-sensitive prion protein (PrP^{C}). These accumulations are the result of post-translational modifications resulting in an increase in the presence of the β-sheet conformation of the protein in the brain (Prusiner, 1998).

The most characteristic finding of TSEs in animals is widespread spongiform degeneration of the central nervous system (CNS) accompanied by reactive astrocytic gliosis and microglial activation. In human prion diseases, including kuru and the VV2 type of sporadic Creutzfeldt–Jakob disease (sCJD-VV2), severe pathological changes are observed in the cerebellum with the loss of granular and Purkinje cells, fusiform swelling of the proximal portion of Purkinje cell axons (torpedoes) and intense Bergmann radial gliosis (DeArmond and Prusiner, 1997; DeArmond *et al.*, 2004). These cerebellar lesions have not been reported in animal prion diseases, including in the

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experimental transmission of PrP^{Sc} to small rodents, although PrP^{Sc} immunolabelling of varying intensity or deposition patterns was reported in the cerebellar cortex in BSE (Wells *et al.*, 1991; Orge *et al.*, 2000; Debeer *et al.*, 2003), scrapie (Wood *et al.*, 1997; González *et al.*, 2002) and CWD (Spraker *et al.*, 2002; Williams, 2005).

In the study of the transmission of BSE to various species of small rodents for development of an animal model, guinea pigs showed high susceptibility for the BSE prion. In addition, the cerebellar lesions in infected guinea pigs resembled the lesions observed in kuru or sCJD-VV2, although there were no typical amyloid plaques or florid plaques. The aim of this study was to describe the neuropathology of the cerebellar lesions in guinea pigs inoculated intracerebrally with BSE.

Materials and Methods

BSE Sample, Animals and Experimental Design

BSE cattle (BSE/JP4) used in this study were slaughtered at an abattoir in Japan for meat consumption and diagnosed by means of a rapid enzyme-linked immunosorbent assay (ELISA) for BSE (Grassi *et al.*, 2001). A sample of brain from the pons to the medulla oblongata, including the obex, was sectioned sagittally along the midline. One half was sampled for the rapid ELISA and confirmatory western blotting, while the other half was fixed in 15% neutral buffered formalin. That sample was submitted to our laboratories and re-examined for confirmation by histological and immunohistochemical analyses and western blotting.

Guinea pigs (3–4-week-old female Hartley strain; SLC, Shizuoka, Japan) and mice (Jcl:ICR strain; Clea, Tokyo, Japan) were inoculated intracerebrally with 10% brain homogenates in phosphate-buffered saline (PBS) from BSE-affected cattle (BSE/JP4); these animals were designated as first passage. For the second passage, guinea pigs and mice were inoculated with 10% brain homogenate in PBS from the first passage animals that developed clinical signs. Guinea pigs and mice for the third passage were inoculated with 10% brain homogenate in PBS from the second passage animals that developed clinical signs. Five guinea pigs and five mice were used in each passage. Animals inoculated with 10% normal mouse or guinea pig brain homogenate in PBS were used as controls. When animals showed clinical signs of the terminal stage of the disease, they were sacrificed under anaesthesia and the brain was removed and used for histopathological, immunohistochemical and immunoblot examinations. The experiments were conducted in accordance with the guidelines of the University Animal Care and Use Committee.

Neuropathology

Brains were fixed in 15% neutral buffered formalin for 1 week and then immersed in 98% formic acid for 1 h to reduce the risk of prion infectivity. Samples were embedded in paraffin wax and sections (4 μm) were stained with haematoxylin and eosin (HE), luxol fast blue-HE (LFB-HE) or the Bielschowsky silver method.

Immunohistochemistry (IHC) was performed with anti-PrP monoclonal antibody (mAb) 110 (recognizing mouse prion protein residues 56–90; Furuoka *et al.*, 2007; diluted 1 in 500) and anti-glial fibrillary acidic protein (GFAP; DAKO, Carpinteria, California; diluted 1 in 1,000) as the primary antibodies, and a secondary antibody conjugated to horseradish peroxidase-labelled polymer (DAKO Envision Kit; DAKO). The pretreatment method to retrieve PrP^{Sc} immunoreactivity was the 135DWHA method as previously reported (Furuoka *et al.*, 2004, 2007). Endogenous peroxidase activity was blocked by incubation in H_2O_2 3% for 5 min at room temperature. The sections were exposed to each primary antibody for 1 h at room temperature and then incubated with the second antibody for 30 min at room temperature. The signals were detected using diaminobenzidine (Simple stain DAB; Nichirei, Japan) followed by counterstaining with Mayer's haematoxylin.

Immunoblotting

Frozen brain samples from cattle (BSE/JP4), Jcl:ICR mice and guinea pigs infected with BSE prions were stored at −80°C and investigated for the presence of PrP^{Sc} by immunoblotting. PrP-enriched fractions were prepared from affected brain tissues by sarcosyl extraction, differential centrifugation and proteinase K (PK) digestion as previously described (Horiuchi *et al.*, 2002). For western blotting, the purified PrP^{Sc} was separated by 12% sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes (Immobilon-P, Millipore, Massachusetts). Membranes were blocked with 5% non-fat dry milk in PBS for 60 min and incubated with primary antibody mAb110 (1 in 1,000) in PBS containing 0.1% Tween 20 for 60 min. After washing with PBS, the membranes were incubated with horseradish peroxidase-conjugated anti-mouse immunoglobulin (Ig) G antibody (Amersham Biosciences, Buckinghamshire, UK) for 60 min. They were washed again and developed with enhanced chemiluminescence (ECL) western blotting detection reagents (Amersham) and the bands were visualized by exposure to X-ray film (RX-U FUJI Medical, Japan).

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Results

BSE cattle (BSE/JP4) showed a mild form of spongiform change in the dorsal nucleus of the vagus (DNV), the nucleus of the solitary tract (NST) and the marginal zone of the reticular formation. Immunohistochemically, PrP^{Sc} was distributed in the vestibular nucleus, DNV, NST, spinal trigeminal nucleus, nucleus hypoglossal nerve, nucleus of facial nerve, reticular nucleus and nucleus olivaris, which showed fine granular, focal aggregation or dot-like deposits. Western blotting showed that the relative amounts of di-, mono- and non-glycosylated forms were approximately 70, 20 and 10% of the total amount of PK-resistant PrP^{Sc}, respectively (Fig. 1), and confirmed that BSE/JP4 was typical of BSE.

In this transmission study of guinea pigs inoculated intracerebrally with BSE prions, the mean incubation period of the first passage was 370 dpi. This decreased to 307 dpi and 309 dpi for the second and third passages, respectively, with a statistically significant difference between the incubation period of the first passage guinea pigs and that of the second and third passage guinea pigs ($P < 0.05$ in t test). The incubation period of primary transmission in the guinea pigs was much shorter than that in the Jcl:ICR mice (mean incubation period 456 dpi); however, the incubation period of the guinea pigs in the second or third passage was not as significantly shortened as that of Jcl:ICR mice in the second or third passage (mean incubation period of 171 or 174 dpi, respectively; Table 1). Fig. 2 shows the survival curve of those guinea pigs and mice.

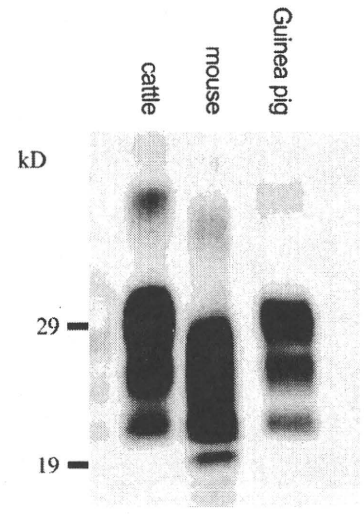

Fig. 1. Western blot analysis of the PrP^{Sc} types present in cattle, mice and guinea pigs infected with BSE. Molecular mass markers are indicated. Glycoform and molecular size of PrP^{Sc} detected in the guinea pig are similar to that of cattle.

Table 1
Incubation periods in guinea pigs and Jcl:ICR mice

Passage	*Mean incubation period (days)**	
	Guinea pigs	*Jcl:ICR mice*
First	370 ± 20.8 (5/5)†	456 ± 18 (5/5)
Second	307 ± 6.6 (5/5)	171 ± 5.6 (5/5)
Third	309 ± 5.1 (5/5)	174 ± 3.7 (5/5)

*Mean ± SD.
†Number of PrP^{Sc}-positive animals/number of animals inoculated.

In affected mice, neuropil vacuolation without amyloid plaques was associated with astrogliosis and microglial proliferation was observed throughout all areas of the brain. Although vacuolation was scattered throughout the molecular and granular layers and the cerebellar medulla, there was no astrogliosis of the molecular layer or loss of the granular layer in the cerebellar cortex. PrP^{Sc} deposits were diffusely distributed in the cortex, thalamus, hippocampus and medulla oblongata. Additionally, plaque-like deposits were observed in the cortex and thalamus. In the cerebellum, aggregate PrP^{Sc} deposition associated with gliosis was observed in the granular layer, but was not prominent in the molecular layer (Fig. 3a, b). Perineuronal or fine granular PrP^{Sc} deposition was present in the cerebellar nucleus and medulla. These lesions and patterns of PrP^{Sc} deposition are very similar to those previously described in BSE-infected C57BL mice (Fraser *et al.*, 1992; Green *et al.*, 2005).

The guinea pigs infected with BSE prions showed mild to severe spongiform degeneration characterized by large rounded vacuoles or small irregular shaped vacuoles and had astrogliosis in the cerebral cortex, thalamus and brainstem. In addition, as compared with control animals (Fig. 4a, c), the affected guinea pigs, especially those from the second or third passage

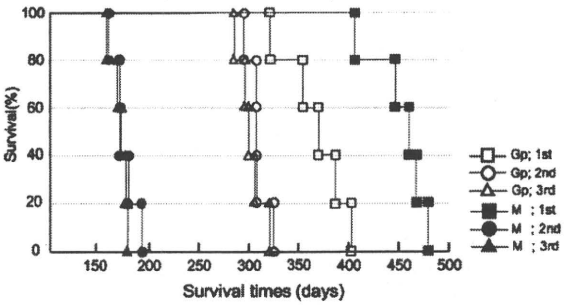

Fig. 2. The graph shows survival curves. Gp, guinea pig; M, mouse; 1st, animals inoculated intracerebrally with 10% brain homogenates in PBS from BSE-affected cattle; 2nd, animals inoculated with 10% brain homogenate in PBS from the first passage guinea pigs or mice; 3rd, animals inoculated with 10% brain homogenate in PBS from the second passage animals.

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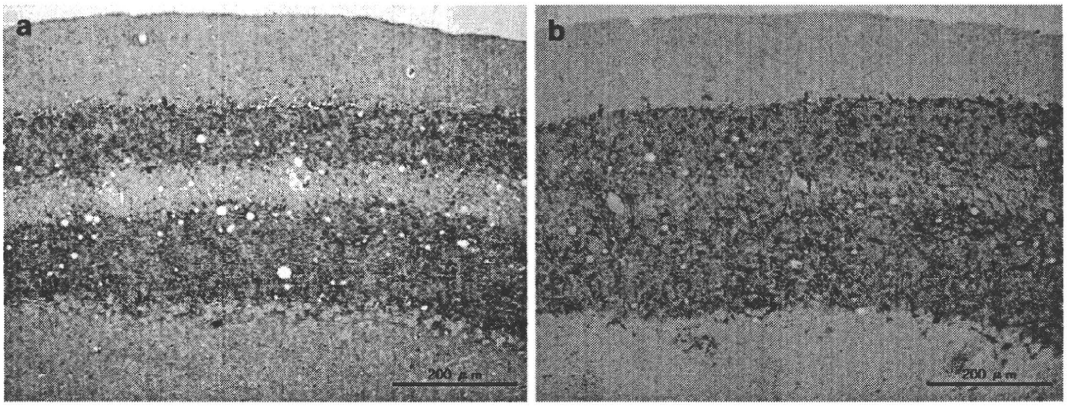

Fig. 3. Coronal sections of cerebellar cortex from third passage mice inoculated with BSE prion. (a) PrP^{Sc} deposition showing granular or focal aggregate patterns is observed in the granular layer. (b) This is associated with astrogliosis. IHC.

groups, had severe atrophy of the cerebellar cortex (Fig. 4b). The area of the molecular layer associated with the proliferation of hypertrophic astrocytes was reduced in width (Fig. 4d). A noticeable decrease in small granular cells resulted in a reduction of the granular layer. The Purkinje cells were relatively well preserved compared with the other two layers in the cerebellar cortex. The cerebellar white matter and cerebellar nuclei showed severe spongiform degeneration with hypertrophic astrocytosis. Dendritic

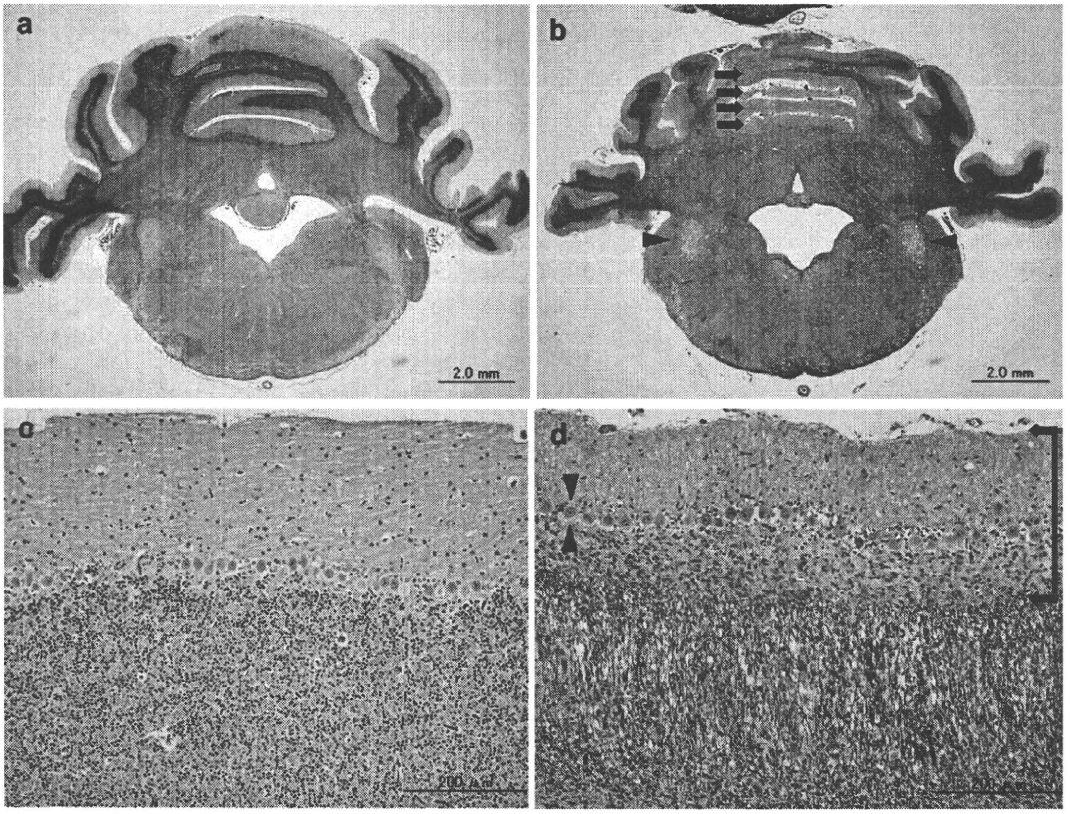

Fig. 4. Coronal sections at the level of the cerebellum and pons from (a) control and (b) affected guinea pigs. Cerebellar lobules of the affected animal show thinning and disappearance of the granular cell layer (arrows). Symmetrical areas of pallor characterized by moderate to severe spongiosis of the cerebellar medulla and peduncle are observed (arrowheads). Cerebellar cortex from (c) control and (d) affected guinea pigs. In the affected animal there is a reduction in width of the molecular and granular layers with severe gliosis (paired arrow), although the Purkinje cells are well preserved (paired arrowhead). LFB-HE.

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expansion in the molecular layer (Fig. 5a, b) and axonal torpedoes in the granular layer (Fig. 5c, d) were frequently observed with HE staining and the Bielschowsky silver staining method. No kuru plaques or florid plaques were observed in any area of the brain. PrP immunolabelling showed diffuse PrP^{Sc} deposition and plaque-like or glial-type PrP^{Sc} deposition associated with spongiform changes. Additionally, there was no different PrP^{Sc} immunoreactivity between the inoculation site and the opposite side. In the cerebellum, intense PrP^{Sc} deposition was observed in the molecular and granular layers (Fig. 6a) and diffuse PrP^{Sc} deposition with granular and perineuronal patterns was observed in the cerebellar nucleus and medulla. In mild lesions, PrP^{Sc} deposition showed diffuse synaptic-type immunoreactivity in the molecular layer and coarse granular labelling in the granular layer (Fig. 6b). In severe lesions characterized by the disappearance of the granular layer, intense accumulation of PrP^{Sc} was detected in the molecular layer (Fig. 6c). In the mild lesions showing no apparent cortical atrophy, scattered plaque-like deposits were found (Fig. 6d). GFAP immunohistochemistry demonstrated the proliferation of Bergmann glia and fibrous astrocytes in the molecular and granular layers, respectively (Fig. 6e, f).

In western blot analysis, the PrP^{Sc} bands in cattle infected with BSE prions revealed typical glycoform ratios characterized by overrepresentation of the high molecular mass glycoform. Although the apparent molecular sizes and glycoform ratios of PrP^{Sc} from mice were different from that of cattle, guinea pigs had a profile similar to that found in cattle (Fig. 1).

Discussion

The cerebellar cortex in guinea pigs infected intracerebrally with BSE prions showed severe atrophy characterized by marked depletion of granular cells and gliosis of the molecular layer associated with intense PrP^{Sc} deposition and plaque-like immunohistochemical deposits. The PrP^{Sc} that originated from the BSE prion was preferentially labelled in the granular layer with some extension into the molecular layer. This is supported by the fact that the granular layer showed more intense PrP^{Sc} immunoreactivity than the

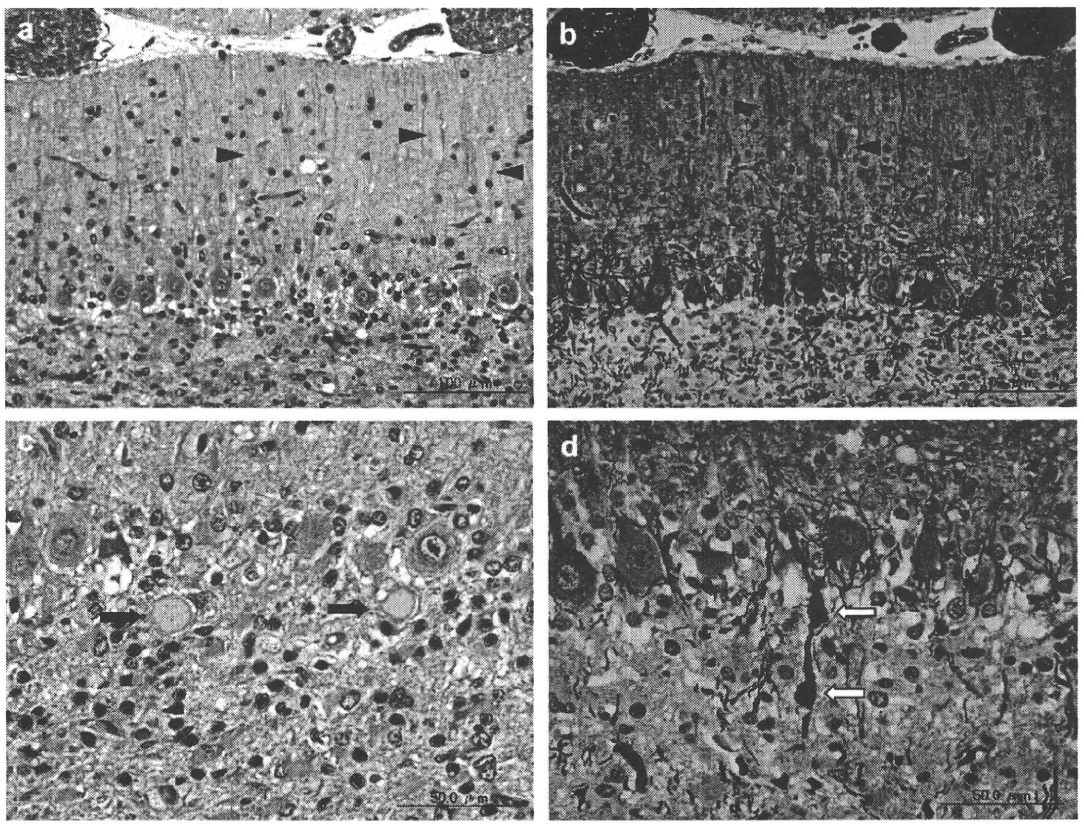

Fig. 5. (a) Numerous pale swollen dendrites (dendritic expansion) (arrowheads) in the molecular layer. (b) These are argyrophilic (arrowheads). (c) Proximal axonal swellings (arrows) and (d) torpedoes (arrows) of Purkinje cells are observed in the granular layer. (a) and (c) HE. (b) and (d) Bielschowsky silver staining method.

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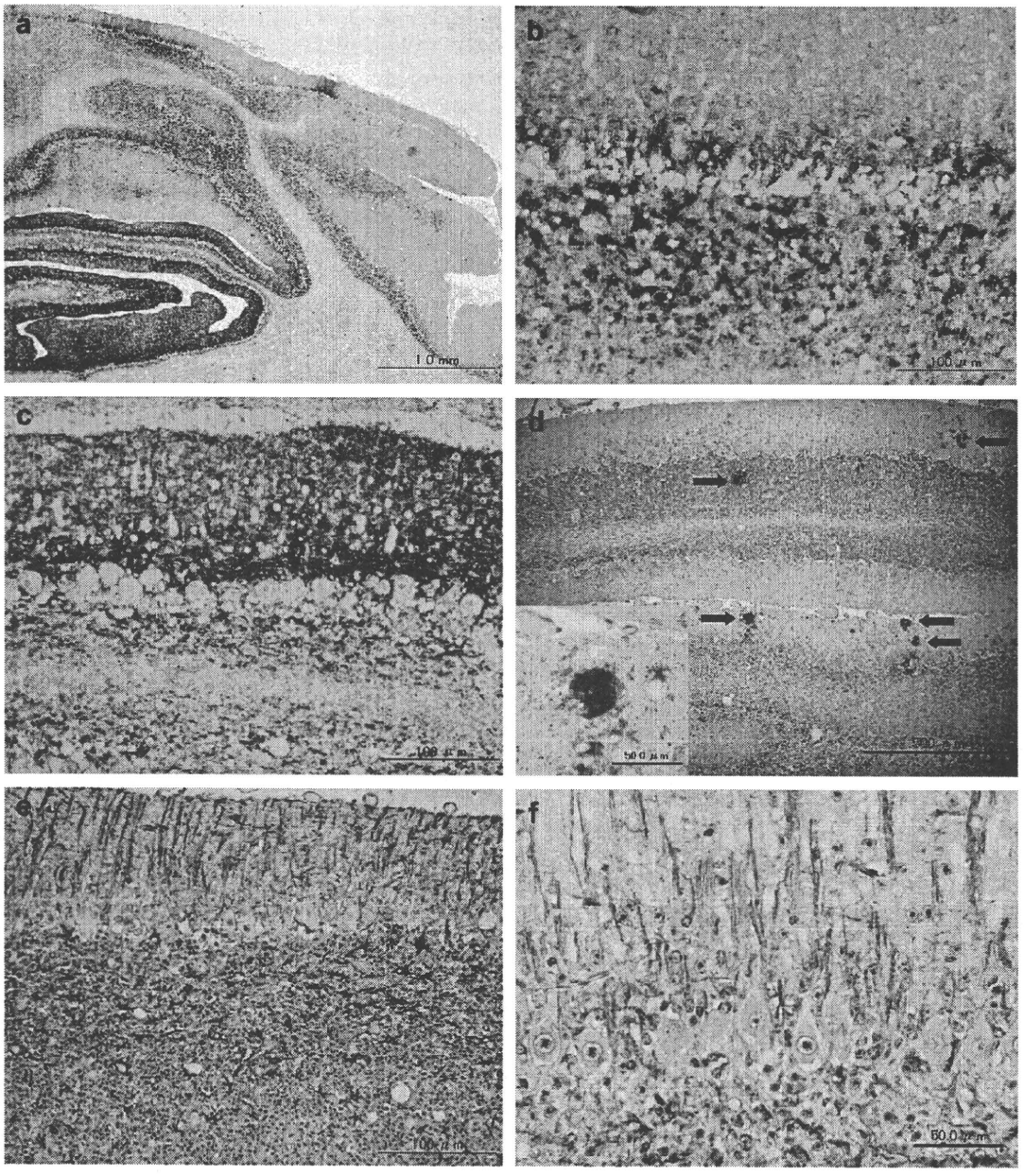

Fig. 6. (a) The cerebellum shows strong PrP^{Sc} deposition localized to the granular and molecular layers. (b) In mild lesions, synaptic deposition is observed in the granular layer with only mild deposition in molecular layer. (c) In severe lesions showing the disappearance of the granular layer, the molecular layer shows widespread PrP^{Sc} immunoreactivity. (d) Several plaque-like immunohistochemical deposits are observed in a relatively mild lesion in the cerebellar cortex (arrows). Inset. High magnification of plaque-like structure. (e, f) GFAP immunolabelling shows the striking proliferation of Bergmann glia and fibrous astroglia in the molecular and granular layers, respectively. IHC.

molecular layer in the lesions without severe cerebellar cortical atrophy. Although the Purkinje cells were well preserved, dendritic expansions and axonal torpedoes caused by severe PrP^{Sc} deposition were frequently observed. The 22L strain of scrapie inoculated into mice has been reported to produce severe vacuolation of the granular layer associated with a significant decrease in the Purkinje cells (Fraser, 1979; Kim *et al.*, 1987; Cunningham *et al.*, 2005). Similarly, a strain of CJD derived from serial passage in guinea pigs and hamsters is also known to form many vacuoles in the granular layer when inoculated into rats (Manuelidis *et al.*, 1997). Additionally, the deposition of PrP^{Sc} or vacuolar degeneration associated with

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