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一般的名称	人全血液	研究報告の公表状況	Castilla J, Brun A, Diaz-San Segundo F, Salguero FJ, Gutierrez-Adan A, Pintado B, Ramirez MA, Del Riego L, Torres JM. J Virol. 2005 Jul;79(13):8665-8.	公表国
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研究報告の概要

○遺伝子導入マウスモデルにおいて評価したBSEプリオンの垂直感染

本稿では、ウシPrP(boTg)を発現させたトランスジェニックマウスにBSEプリオンを脳内投与した時の母子感染の証拠を示す。脳内PrP^{res}沈着がウエスタンブロット解析により検出できる発症少し前の時期に交配させた場合にのみ、感染した母マウスから生まれた新生児マウスの脳にPrP^{res}が検出された。boTgマウスの脳内接種後に乳汁中に感染性は検出できず、その他の組織がプリオン伝播キャリアとして関与していることが示唆された。本稿に示す結果から、マウスモデルにおいてBSEプリオンの感染が遠心性に中枢神経系から末梢組織に、また子マウスに広がることが証明される。また、これらの結果によりウシにおけるBSEの垂直感染の発生を支持する過去の疫学データを補足できるかも知れない。

使用上の注意記載状況・その他参考事項等

人全血液CPD「日赤」
照射人全血液CPD「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
遺伝子導入マウスモデルにおいてBSEプリオンの感染が遠心性に中枢神経系から末梢組織に、また子マウスに広がることが証明されたとの報告である。	今後も引き続き、プリオン病に関する新たな知見及び情報の収集に努める。

(N)

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Vertical Transmission of Bovine Spongiform Encephalopathy Prions Evaluated in a Transgenic Mouse Model

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In this work we show evidence of mother-to-offspring transmission in a transgenic mouse line expressing bovine PrP (boTg) experimentally infected by intracerebral administration of bovine spongiform encephalopathy (BSE) prions. PrP^{res} was detected in brains of newborns from infected mothers only when mating was allowed near to the clinical stage of disease, when brain PrP^{res} deposition could be detected by Western blot analysis. Attempts to detect infectivity in milk after intracerebral inoculation in boTg mice were unsuccessful, suggesting the involvement of other tissues as carriers of prion dissemination. The results shown here prove the ability of BSE prions to spread centrifugally from the central nervous system to peripheral tissues and to offspring in a mouse model. Also, these results may complement previous epidemiological data supporting the occurrence of vertical BSE transmission in cattle.

Prion diseases or transmissible spongiform encephalopathies (TSEs) belong to a class of infectious diseases characterized by the presence of an abnormally folded protein (PrP^{Sc}) that accumulates in the brains of affected individuals (24). TSEs may be of spontaneous, familial, or infectious origin. While spontaneous and familial etiologies have been described for the disease in humans (22, 23), infectious TSEs have been clustered mainly in domestic animals, from which sheep scrapie was the prototype of disease (17). The epidemic dimension of bovine spongiform encephalopathy (BSE) in the mid-1980s contributed to the spread of the disease to humans in the form of variant Creutzfeldt-Jakob disease (vCJD) (7, 8). It is now generally accepted that the consumption of contaminated meat and/or meat-derived products has been the most probable route of transmission of BSE prions to humans. Natural routes of transmission have been described for scrapie prions (16, 19, 20), although scant information is available regarding BSE natural routes of infection. The ability of scrapie prions to accumulate in placental tissues from genetically susceptible ewes (1, 25, 27) might be a contributing factor in scrapie epidemiology (16). However, this picture still remains diffuse for BSE. No PrP^{Sc} accumulation is detected in placentas from BSE-infected cattle (31), and neither blood nor milk from BSE-infected animals have yet been shown to be infectious, consistent with the apparent absence of the prion agent in peripheral tissues (3). Experiments to test maternal transmission in cattle showed that approximately 10% of calves born to cows with confirmed BSE developed disease (2). This transmission rate, however, was obtained in a scenario of disease prevalence, since some of the calves were born after the feed ban was fully effective.

The ability of prions to move from the central nervous system (CNS) through afferent nerve fibers has been described for several TSEs, including genetic and sporadic human prion diseases (14, 15)and scrapie (28), and was suggested for chronic wasting disease (CWD) (26). Recently, it has been shown how vCJD and Gerstmann-Sträussler-Scheinker syndrome (strain Fukuoka-1) prions retaining full infectivity can be detected in the blood of mice after intracerebral inoculation (6). To test the ability of BSE prions to spread from CNS to peripheral tissues, we studied the efficiency of BSE transmission from intracerebrally BSE-inoculated mothers to their offspring in a transgenic mouse line (boTg110) expressing bovine PrP (4). boTg110 mice express boPrP controlled by the mouse PrP promoter at a level eight times that of the level of bovine PrP in cattle brain as previously described (4). Groups of boTg110 females were intracerebrally infected with a BSE inoculum named BSE_1 consisting of a pool from 49 BSE-infected cattle brains (TSE/08/59) supplied by the Veterinary Laboratories Agency (New Haw, Addlestone, Surrey, United Kingdom). The titer of this inoculum was $\sim 10^8$ 50% infective dose units per gram of bovine brainstem when measured in the boTg110 mouse line (data not shown). At different times postinoculation, infected female mice were mated with healthy homologous males (Table 1). Group I female mice (mated at 195 and 223 days postinoculation [d.p.i.]) showed a strong PrP^{res} signal as judged by Western blot analysis of brain extracts (data not shown). In contrast, only mouse 09 from group II (mated at 160 d.p.i.) showed detectable brain PrP^{res} accumulation, in good agreement with the kinetics of PrP^{res} deposition in this mouse model (4).

PrP^{res} was clearly detected by Western blotting in 2 out of 10 mice born from group I females (mated at 195 and 223 d.p.i.) but in only 1 out of 40 in group II (mated at 160 d.p.i.). The PrP^{res} banding pattern observed for group I positive brains was similar to that for brains from Tg110 mice intracerebrally challenged with the BSE_1 inoculum, and no differences could be observed in their relative molecular weight mobilities (Fig. 1A)

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TABLE 1. Vertical transmission of BSE in bo-PrP-Tg110 mice after intracerebral inoculation

Group	Inoculum	Mouse	d.p.i. to:				Clinical signs	PrP^{res} in mothers[a]	No. of offspring with PrP^{res}/total no. of offspring (d.p.i.)
			Mating	Offspring	Milking	Culling			
I	BSE_1	01	195	246	256	274	Yes	−++	1/5 (622)
I	BSE_1	02	223	250	258	274	Yes	−++	1/5 (613)
II	BSE_1	09	160	182	190	237	Yes	+	1/13 (536)
II	BSE_1	12	160	182	190	210	No	−	0/14
II	BSE_1	14	160	182	190	210	No	−	0/13
III	None (control)	03	220	246	256	276	No	−	0/12
III	None (control)	05	220	246	256	276	No	−	0/10

[a] +++, strong PrP^{res} signal; ++, PrP^{res} accumulation detectable in brain; −, no detectable PrP^{res} in brain.

Deglycosylation experiments with *N*-glycosidase F (PNGase F) confirmed this observation (Fig. 1B). However, differences in the amounts of immunoreactive PrP^{res} were found between group I and II: PrP^{res} levels in mouse 09/02 from group II were found to be clearly lower than those in mice from group I. This fact might be explained by the shorter survival time of this mouse (time to death, 536 d.p.i.) relative to those of mice from group I, which died at 622 and 613 days postinfection. Differences in the percentages of PrP^{res}-positive offspring among groups I and II (20% versus 2.5%; $P_{r\ test} = 0.098$) might be related to the time after intracerebral BSE prion inoculation after which mating was allowed. Thus, higher transmission rates, defined by the presence of detectable PrP^{res}, are obtained if the accumulation of pathogenic PrP in brain is allowed to reach certain nonpathological levels without disturbing the reproductive competence of female mice. The high percentage of PrP^{res}-negative littermates could be attributed to the limited sensitivity of the Western blot technique (5). In addition, exploring the presence of PrP^{res} depositions by immunohistochemistry in brains from mice negative for PrP^{res} by Western blotting was consistently unsuccessful (data not shown). The lack of PrP^{res} detection, however, cannot exclude completely the existence of subclinical infections in the PrP^{res}-negative offspring. This assumption can be supported by the statistically significant differences ($P = 0.020$) observed in the survival times between offspring from infected (585 ± 60, 589 ± 71, 583 ± 36, 566 ± 63 and 608 ± 20 d.p.i.) and control (637 ± 57 d.p.i.) mothers (Fig. 2). Moreover, there was no difference between the survival times of PrP^{res}-positive and PrP^{res}-negative offspring mice. To confirm the fact of subclinical infection, works on second-passage experiments are in progress.

The fact that BSE prions delivered into mice brains can be transmitted to a next generation is indicative of their intrinsic ability to centrifugally spread from the CNS to other peripheral tissues. In fact, the ability of prions to move from CNS through afferent nerve fibers has been also described for other TSEs, including genetic and sporadic human prion diseases (14, 15) and scrapie (28), and was suggested for chronic wast-

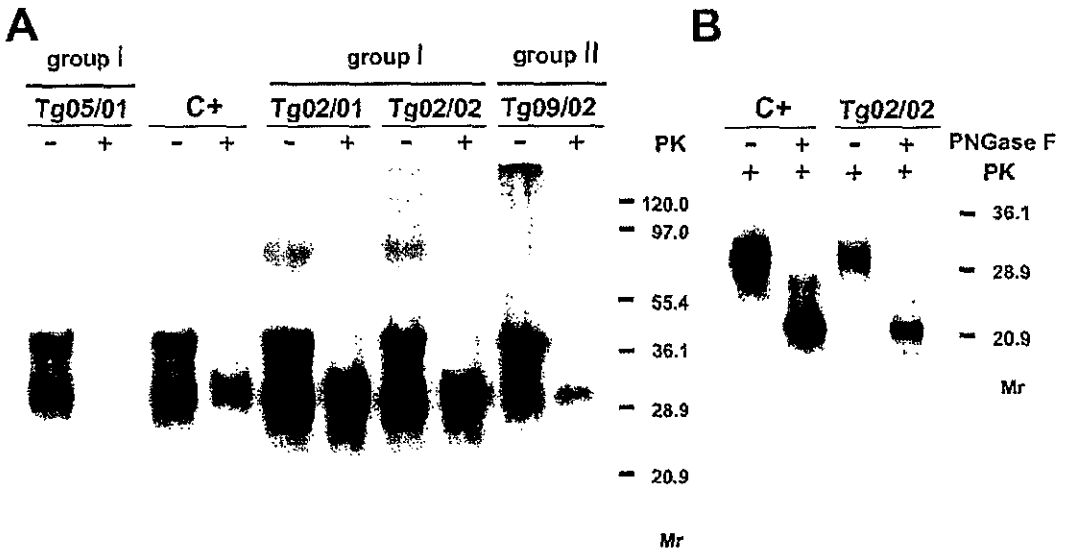

FIG. 1. (A) Comparison of Western blot profiles in brain detergent-insoluble fractions from PrP^{res}-positive offspring. Tg05/01, mouse born from the Tg05-uninfected female; Tg01/02 and Tg02/02, mice born from the Tg01 and Tg02 BSE_1 inoculum-infected females, respectively; Tg09/02, mouse born from the Tg09-infected female; C+, brain extract from a Tg110 mouse intracerebrally inoculated with BSE_1 inoculum; Mr, Relative molecular mass expressed in kilodaltons; PK, proteinase K treatment. Protein loads per lane are equivalent in progeny mice. In the Tg05/01 mouse the PK − lane shows soluble brain fraction. (B) Deglycosylation studies of PrP^{res} from control (C+) and progeny Tg02/02 brain extracts.

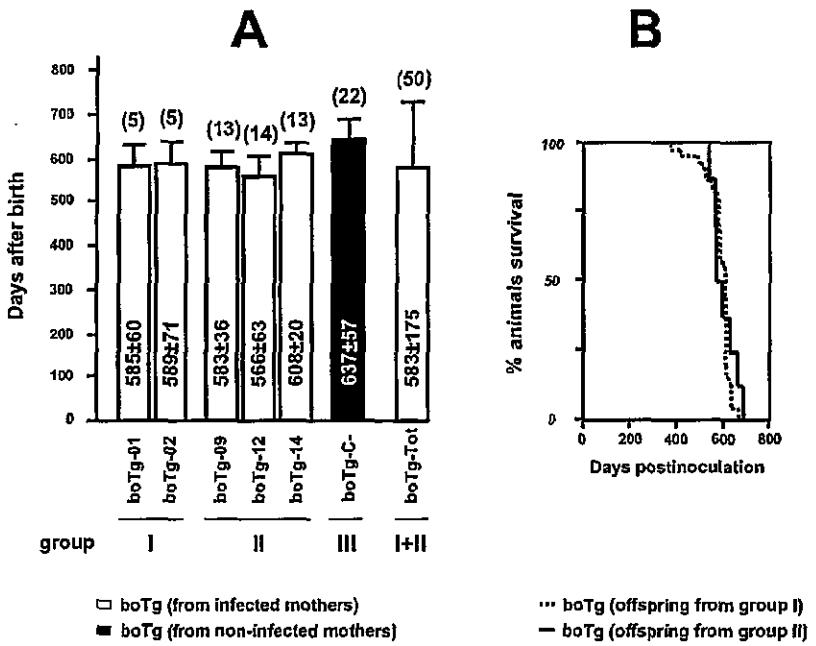

FIG. 2. Mean survival times of mice born from infected mothers. (A) Histograms showing survival times of the offspring of each female and of all offspring (boTg-Tot). BoTg-C-, offspring from uninoculated group III mothers. The values within the bars represent the days after inoculation ± standard deviations. The numbers of mice of each type inoculated are in parentheses. (B) Kaplan-Meier curves correspond to the overall groupings of the offspring (groups I and II)..

ing disease (CWD) (26). Recently it has been shown how vCJD and Gerstmann-Sträussler-Scheinker syndrome (strain Fukuoka-1) prions retaining full infectivity can be detected in the blood of mice after intracerebral inoculation (6). The role of blood in BSE prion dissemination became more evident after the demonstration of BSE transmission to sheep via blood transfusion even during the preclinical phase of an experimental oral BSE inoculation in sheep (18). Our results indicated that BSE prions could be transmitted to the offspring after intracerebral inoculation in a process that seems to be more efficient when detectable amounts of PrP^{res} are present in the brain. The way by which prion infectivity is transmitted through a next generation could be then, based on previous work, be identified as blood dissemination. Other investigated tissues (placenta, lymphoid tissues, and gastrointestinal tract) were negative for PrP^{res} either by Western blotting or by analysis with immunohistochemistry (data not shown). However, these negative results do not allow one to conclude that there is a lack of infectivity in these tissues. In our experimental model, other fluids cannot be disregarded as vehicles for prion spread. To asses whether the route of infection through milk feeding was involved, we carried out experimental inoculations of milk extracted from mothers. For this purpose, 0.5 ml of pooled milk extracted from both infected and uninfected mothers was delipidated and intracerebrally injected into boTg110 mice after a concentration step (centrifugation at 25,000 × *g* for 30 min). We estimate that the amount of milk used for the inoculations represents 25% of the milk intake during lactancy. Analysis of the survival times of mice inoculated or mock inoculated did not show any significant difference (Fig. 3). Brains from these mice were then analyzed with both histopathology and immunohistochemistry for the presence of PrP^{res}. Similarly, no PrP^{res} was detected (data not shown). This negative result does not exclude the potential of milk to transmit prions but suggests that the relevance of this fluid in infectivity might be very low if it exists at all. Thus, the centrifugal dispersion of prions together with the ability of blood to retain prion infectivity might account for the transmission of BSE prions to the offspring without excluding other possible ways.

With regard to BSE in cattle, previous fieldwork studies

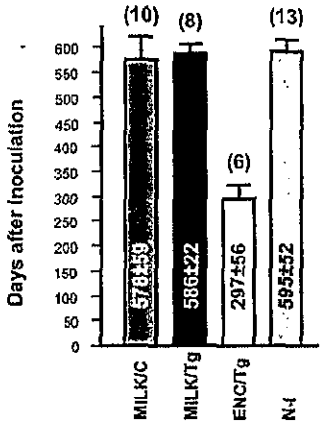

FIG. 3. Survival times of boTg110 mice inoculated with donor milk samples. Survival times for mice inoculated with milk from healthy female boTg110 mice (MILK/C) or milk (MILK/Tg) and brain (ENC/Tg) pools from BSE_I-infected female boTg110 mice are shown. The values within the bars indicate the days postinoculation ± standard deviations. The numbers of mice inoculated with each type of sample are indicated in parentheses.

suggested that the disease may be passed from cow to calf (29, 30). However, there has been controversy and uncertainties regarding whether or not maternal transmission has implications in the prevalence of this disease similar to those that it has for sheep scrapie (9, 10). Our results reveal an enhanced risk of disease in mice born from BSE-infected mothers at the end stage of the incubation time. The same type of risk may apply to the offspring from BSE-infected cattle, as has been suggested from the epidemiological data (9). However, it is necessary to point out here some differences between our transgenic mouse model and bovine species. Firstly, boTg110 mice express boPrP at a level eight times that of bovine PrP in cattle brain; therefore, there is more PrP^{C} substrate available for conversion to PrP^{Sc}. Secondly, there are some evident differences with respect to the architectural anatomies of mouse and cattle placentations. In cattle, the placenta is bridged to the uterus by a cotyledonary form of attachment, and the structure is of the syndesmochorial type, in which the embryo trophoblastic layer and the maternal uterine epithelium are not fused. In contrast, mouse embryonic and uterine epithelia are completely fused (hemochorial). This type of structure allows blood from the uterine endothelium to be in close contact with the fetal placenta, therefore facilitating the chances for prion dissemination and embryonic contamination.

The BSE agent can propagate efficiently in sheep (11), and the possibility of sheep flocks becoming infected with BSE was raised (21). However, in contrast to findings for sheep scrapie, no evidence of PrP^{Sc} has been found in the reproductive tissues of sheep infected with BSE (13), nor has BSE been reported in the offspring of experimentally infected ewes (12). Since transmission of BSE prions to the offspring occurs in the mouse model, it is reasonable to assume that host-specific restrictions may compromise the ability of BSE prions to be vertically transmitted.

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販売名（企業名）	ハプトグロビン注－ヨシトミ(ベネシス)					

研究報告の概要

慢性消耗病(CWD)はシカ及びヘラジカの新興のプリオン病である。シカ及びヘラジカの CWD の地域分布は広がっており、ヒトとの接触頻度の増加により伝播が起こる可能性がある。これまで CWD と関連付けられたヒトプリオン病の発生は無かったが、ヒトに対する CWD の異種間伝播のリスクは明確にされていない。ヒトへの CWD 伝播リスクを調べるためには、霊長類での感染実験が有用である。CWD のミュールジカの 20％（W/V）脳ホモジネート 200μl を 2 匹の成熟した雌のリスザルに大脳内へ接種した。接種されたリスザルは 2 匹とも進行性神経系疾患を発症し、疾患末期の 31 ヶ月及び 34 ヶ月目に安楽死させた。ウエスタンブロットによる分析の結果、脳における異常プリオン蛋白（PrP-res）量は、34 ヶ月目に安楽死させたリスザルの方が 31 ヶ月目に安楽死させたより多かった。31 ヶ月目に安楽死させたリスザルの脳、脳幹、脊髄の組織学的検査によって、CWD 感染による神経変性に一致する広範囲の海綿状変化が明らかになった。リスザルのプリオン病感染物質を脳内接種後の疾患末期までの期間は、ミンクの伝染性脳症（9～12 ヶ月）、スクレイピー（16 ヶ月）の病原体を接種されたものより長く、散発的 CJD(11～37 ヶ月)およびクール病(10～48 ヶ月)を接種された範囲内であった。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(1)略
1)略
2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

報告企業の意見	今後の対応
リスザルを用いたCWDの感染実験により、霊長類にCWDが感染する可能性を示唆した初めての報告である。これまで血漿分画製剤によってCWDを含むプリオン病が伝播したとの報告はない。しかしながら、万一CWD感染者の血液が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

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NOTES

Interspecies Transmission of Chronic Wasting Disease Prions to Squirrel Monkeys (*Saimiri sciureus*)

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Chronic wasting disease (CWD) is an emerging prion disease of deer and elk. The risk of CWD transmission to humans following exposure to CWD-infected tissues is unknown. To assess the susceptibility of nonhuman primates to CWD, two squirrel monkeys were inoculated with brain tissue from a CWD-infected mule deer. The CWD-inoculated squirrel monkeys developed a progressive neurodegenerative disease and were euthanized at 31 and 34 months postinfection. Brain tissue from the CWD-infected squirrel monkeys contained the abnormal isoform of the prion protein, PrP-res, and displayed spongiform degeneration. This is the first reported transmission of CWD to primates.

Chronic wasting disease (CWD) is a prion disease of elk and deer in North America that was first identified at cervid research facilities in Colorado and Wyoming in the late 1960s (17, 18). CWD has been identified on cervid game farms from Montana to New York and has been diagnosed in wild deer and elk in Colorado, Wyoming, Nebraska, South Dakota, Wisconsin, New Mexico, Illinois, and Utah and in Saskatchewan, Canada (1, 14, 15). The geographic distribution of CWD in deer and elk has been expanding and will likely result in an increase in human exposure to the CWD agent. Although there have been no cases of human prion disease linked to CWD infection, the risk of interspecies transmission of CWD to humans following consumption of CWD-infected tissues is uncertain (5, 13).

One approach to assess the susceptibility of humans to animal prion diseases is by experimental transmission to nonhuman primates (9–11). To investigate the susceptibility of nonhuman primates to CWD, two adult female squirrel monkeys (*Saimiri sciureus*) were intracerebrally (i.c.) inoculated with 200 μl of a 20% (wt/vol) brain homogenate from a female mule deer in the clinical phase of CWD (inoculum was a gift from Elizabeth Williams, Department of Veterinary Sciences, University of Wyoming, Laramie, WY). Both CWD-inoculated squirrel monkeys developed a progressive neurological disease and were euthanized at the terminal stages of disease at 31 and 34 months postinfection, respectively (data on clinical symptoms and the time to onset of disease were not available).

To determine whether the abnormal form of the prion protein, PrP-res, was present in the CWD-infected squirrel monkeys, brain homogenates were analyzed by Western blotting as previously described using the anti-PrP monoclonal antibody 6H4 (Prionics AG, Switzerland) (2). For this analysis, a 5% (wt/vol) brain homogenate in Dulbecco's phosphate-buffered saline (Mediatech, Inc.) from CWD-infected squirrel monkeys, a CWD-infected elk, or an uninfected mouse was either digested with proteinase K (PK) (4 U/ml; United States Biochemical) for 1 h at 37°C with agitation or was not digested with PK. In the samples that were not digested with PK, PrP migrated between 21 and 35 kDa in the CWD-infected squirrel monkeys (Fig. 1, lanes 1 and 2) and between 30 and 35 kDa in the CWD-infected elk (Fig. 1, lane 3) and in the uninfected mouse sample (Fig. 1, lane 4). In the samples that were digested with PK, PrP-res were detected in the two CWD-infected squirrel monkeys (Fig. 1, lanes 5 and 6) and in the CWD-infected elk sample (Fig. 1, lane 7). In the PK-digested uninfected mouse brain, PrP was not detected (Fig. 1, lane 8), indicating that PK digestion completely removed the PK-sensitive isoform of PrP. In both CWD-infected squirrel monkeys, the migration of the three PrP-res polypeptides on sodium dodecyl sulfate-polyacrylamide gels was similar. The diglycosylated PrP-res polypeptide migrated at 30 kDa similar to what has been reported for squirrel monkeys infected with sporadic Creutzfeldt-Jakob disease (CJD), kuru, and scrapie (4). The relative abundance of PrP-res in the brain from the squirrel monkey that was sacrificed at 34 months postinfection (Fig. 1, lane 5) was greater than that in the squirrel monkey sacrificed at 31 months postinfection (Fig. 1, lane 6) and may represent differences in the state of disease progression when the animals were sacrificed.

Histological examination of the brain, brain stem, and spinal cord from the squirrel monkey that was euthanized at 31 months postinfection revealed widespread spongiform changes that are consistent with CWD-induced neurodegeneration.

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