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Squirrel monkeys infected with BSE develop Tau pathology

Pedro Piccardo^{1,2}, Juraj Cervenak¹, Oksana Yakovleva³, Luisa Gregori¹, Kitty Pomeroy¹, Anthony Cook⁴, F Salih Muhammad⁴, Torsten Seuberlich⁵, Larisa Cervenakova³, and David M Asher¹

¹Laboratory of Bacterial and TSE Agents, Center for Biologics Evaluation and Research, United States Food and Drug Administration (US FDA), Rockville, Maryland, USA ²Neuropathogenesis Division and The Roslin Institute R(D)SVS, University of Edinburgh, Easter Bush, Midlothian, UK ³Transmissible Diseases Department, Jerome H. Holland Laboratory for the Biomedical Sciences, American Red Cross, Rockville, Maryland, USA ⁴BIOQUAL, Inc., Rockville, Maryland, USA ⁵NeuroCentre, National and OIE Reference Laboratories for BSE and Scrapie, Vetsuisse Faculty, University of Bern, Bern, Switzerland

Abstract

Squirrel monkeys were experimentally infected with the classical bovine spongiform encephalopathy (BSE) agent. Two to four years later, six of the monkeys developed alterations in interactive behavior, cognition and other neurological signs typical of transmissible spongiform encephalopathy (TSE). At necropsy, all brains showed pathological changes similar to those described in variant Creutzfeldt-Jakob disease (vCJD) of humans, except that the squirrel monkey brains contained no PrP amyloid plaques typical of that disease. Constant neuropathological features included spongiform degeneration, gliosis, deposition of abnormal prion protein (PrP^{TSE}) and many deposits of abnormally phosphorylated tau protein (p-Tau) in several areas of the cerebrum and cerebellum. Western blots showed large amounts of proteinase-K-resistant prion protein in the central nervous system. The striking absence of PrP plaques (prominent in brains of cynomolgus macaques with both experimental BSE and vCJD and in patients with vCJD) reinforces the conclusion that the host plays a major role in determining the neuropathology of TSEs. Results of this study suggest that p-Tau, found in the brains of all BSE-infected monkeys, might play a role in the pathogenesis of TSEs. Whether p-Tau contributes to development of disease or appears as a secondary change late in the course of illness remains to be determined.

Keywords

bovine spongiform encephalopathy; prion disease; prion protein; tau protein

Introduction

Transmissible spongiform encephalopathies (TSEs) or prion diseases are neurodegenerative protein-misfolding disorders that affect humans and animals. TSEs are usually characterized by spongiform degeneration of the brain with neuronal loss, reactive gliosis and deposition

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Conflict of Interest

All authors declare no conflict of interest.

of abnormal disease-associated prion protein. The abnormal forms of PrP are generally called PrP^{Sc} (“scrapie-type” a PrP), PrP^{Res} (protease-resistant PrP) or PrP^d (comprising several different membrane-associated, aggregation states of PrP, and both protease-resistant and protease-sensitive forms). Here we use the general term PrP^{TSE} as proposed by the World Health Organization (WHO) to designate all forms of abnormal PrP, a terminology generally used by the United States Food and Drug Administration (FDA) (World Health Organization, 2006). PrP^{TSE} results from the post-translational misfolding of a normal host-encoded “cellular” isoform of the normal prion protein (PrP^C). TSE agents occur as distinct strains with different biological properties, transmitting infection relatively efficiently between members of the same species (Prusiner, 1982; Asher et al., 1993; Brown et al., 1994; Aguzzi et al., 2004). Although interspecies transmissions are usually less efficient, they also occur, as shown most dramatically by the development of variant Creutzfeldt-Jakob disease (vCJD) in humans infected by exposure to beef products from cattle with bovine spongiform encephalopathy (BSE) (Will et al., 1996). Recent reports show convincingly that vCJD has been transmitted from human to human by transfusions of red blood cells and by injections of a human plasma-derived product (Turner, et al., 2009).

BSE reached epidemic proportions in the UK in 1992 followed by a progressive decline attributed to implementation of safety measures to prevent the recycling of the infectious agent in contaminated animal feed. However, the geographic distribution of BSE has expanded, and the disease has been reported in indigenous cattle in 24 other countries of Europe, North America, the Middle East and Asia (OIE - World Organisation for Animal Health, 2011). BSE was thought to be caused by a single strain of agent (classical BSE). However, active surveillance led to the recognition of atypical forms of BSE (Biacabe et al., 2004; Casalone et al., 2004; Seuberlich et al., 2010). BSE infections have been transmitted either experimentally or naturally to various animal species including rodents, felines, bovine and non-bovine ruminants, pigs and non-human primates (Fraser et al., 1992; Pearson et al., 1992; Foster et al., 1993; Lasmezas et al., 1996; Ryder et al., 2000; Lasmezas et al., 2001; Bons et al., 2002; Lasmezas et al., 2005; Comoy et al., 2008; Plinston et al., 2011) as well as to humans. Monkeys have a special relevance as TSE models for risk assessment, experimental studies of pathogenesis, evaluation of diagnostic methods and trials of potential therapies (Brown et al., 1994; Lasmezas et al., 1996; Lasmezas et al., 2001; Bons, et al., 2002; Lasmezas et al., 2005; Williams et al., 2007; Comoy et al., 2008; Race et al., 2009). Although low transmission rates and long incubation periods are often observed when TSEs are transmitted to a new species, in what is commonly known as a “species barrier,” experience with squirrel monkeys (sensitive to almost all TSE agents affecting humans and to several animal TSEs as well) indicates that they have been an especially useful model species (Asher et al., 1993; Brown et al., 1994; Race et al. 2009). We report the transmission of BSE to six squirrel monkeys as a model with a consistent clinical and pathologic illness that may be useful to study TSE pathogenesis. The work presented here was carried out using brain suspension from a cow with the classical form of BSE, first described in 1987 (Wells et al., 1987), affecting the vast majority of all cattle with the disease reported to date, because experts from the European Centre for Disease Prevention and Control recently concluded that at present, the only TSE agent demonstrated to be zoonotic is the classical BSE agent (European Food Safety Authority (EFSA) Panel on Biological Hazards (BIOHAZ), 2011).

Materials and Methods

Animal model

Squirrel monkeys (*Saimiri sciureus*) were purchased from Osage Research Primates (Kaiser, MO, USA) and housed in a biosafety-level-3 (BSL-3) animal facility approved by the United States Department of Agriculture (USDA). Animals were screened for the absence of

common simian pathogens before initiation of studies. All experiments were reviewed and approved by the Institutional Animal Care and Use Committee of the Center for Biologics Evaluation and Research (CBER), FDA. Tissues of four other squirrel monkeys of roughly the same age that had died of pneumonia or sepsis but without TSE, confirmed by neuropathology, immunohistochemistry (IHC) and Western blotting, (WB), were used as controls. All monkeys were young females, but exact ages are unknown. Monkeys inoculated with the same sample were housed three to a cage in a BSL-3 room with a 12h light-dark cycle. Food and water were provided *ad libitum*.

Source of BSE agent and bioassay

The work presented here was carried out using brain from a cow with the classical form of BSE (Wells et al., 1987). A 10% v/w suspension of BSE-infected bovine brain in 250-mM sucrose was provided by the NeuroCentre, National and OIE Reference Laboratories for BSE and Scrapie, Bern, Switzerland. This material was derived from a clinically diseased Swiss cow with the classical type of BSE confirmed by WB and IHC in 2003. The infectivity titer of the BSE brain suspension in transgenic mice expressing bovine PrP (TgBo) mice was $5.0 \log_{10} \text{LD}_{50}/30\mu\text{l}$ of inoculum (Cervenakova unpublished). In May 2006, serial 10-fold dilutions of the same BSE inoculum previously titrated in TgBo mice were prepared (10^{-1} through 10^{-9} relative to original brain) and 0.15 ml aliquots injected intracerebrally (IC); 0.15 ml of the same inoculum was also injected intraperitoneally (IP). Three animals per group were inoculated with the BSE inoculum diluted 10^{-1} – 10^{-6} ; two animals per group were inoculated with the same material diluted 10^{-7} – 10^{-9} . Following inoculations, animals were carefully observed at least once daily. Observers assessed the monkeys' social behavior (interactions with cage-mates and caretakers), cognitive faculties (time spent exploring the cage, seeking food and handling toys) and neurological signs (coordination, motor impairment, tremors). Following onset of neurological signs, monkeys were separated from cage mates to protect them from aggression and housed in cages in the same room, in sight of the other monkeys. Squirrel monkeys showing overt neurological signs were maintained until they met previously determined humane criteria for euthanasia. No animal was allowed to become severely ill. When unable to feed, groom or ambulate easily, monkeys were anesthetized deeply and exsanguinated. Blood was collected and components stored at -80°C for future analysis. Tissues obtained at necropsy were used to detect PrP^{TSE} by WB using published protocols (Cervenakova et al., 2011); nitrocellulose membranes were probed with monoclonal antibody 6D11 directed against PrP amino acids 93–109 (Santa Cruz, Biotechnology, Santa Cruz, CA, USA). Histopathological and IHC studies employed formalin-fixed paraffin-embedded 5- μm tissue sections using published protocols (Piccardo et al., 2007). Sections for PrP IHC were probed with monoclonal antibodies 3F4 (1:3500) (Covance, Princeton, NJ, USA) directed against a conserved region (amino acids 109–112) of human PrP (Kascasak, 2010) and 6H4 monoclonal antibody epitope mapped to human PrP amino acids 144–152 (1:3500) (Prionics, Zurich, Switzerland). Astrogliosis was detected using a polyclonal antibody to bovine glial fibrillary acidic protein (GFAP) (1:400) (Dako North America, Carpinteria, CA, USA). Brain sections were also probed with monoclonal antibody 4G8 directed to amino acids 17–24 of β -amyloid (1:300) (Covance, Princeton, NJ, USA), anti-PHF-tau antibody AT8 directed to tau protein doubly phosphorylated at Ser202 and Thr205 (1:450) (Thermo Fisher Scientific, Rockford, IL, USA), and OC anti-fibrillar oligomer polyclonal antibody recognizing generic epitopes common to many amyloid fibrils and fibrillar oligomers, but not prefibrillar oligomers or natively folded proteins (1:1000) (Millipore, Billerica, MA, USA), anti-tau RD4 antibody recognizing 4-repeat tau isoform (1:1000) (Millipore), and an anti Apo-E antibody directed to recombinant human ApoE (1:100) (Chemicon, Temecula, CA, USA). Amounts of spongiform degeneration and PrP^{TSE} accumulation were scored semi-quantitatively by a commonly-used scale modified from Fraser and Dickinson (0: none; 1:

few/occasional/ mild; 2: moderate; 3: severe) in the cerebral cortex, striatum, thalamus, hypothalamus, hippocampus, cerebellum and brain stem of all animals (Fraser and Dickinson, 1967).

Results

Transmission of BSE to squirrel monkeys

Animals inoculated with 10^{-1} and 10^{-2} dilutions of brain suspension developed disease between 29 months and 46 months after inoculation. Disease evolved over periods of 2 months (monkeys 735, 738), 3 months (monkey 722), 4 months (monkey 721), and 5 months (monkeys 737, 739). Other squirrel monkeys inoculated with BSE brain extracts diluted a thousand-fold or more have remained asymptomatic for more than five years.

In four monkeys the disease began with ataxia. One animal (monkey 722) first developed mild weakness of the lower extremities, while another (monkey 721) showed repeated myoclonic jerks as the initial sign of disease. Monkeys then developed resting and intention tremors, marked loss of balance, jerky uncoordinated movements, bradykinesia, myoclonic jerking and generalized weakness. Animals had difficulty holding objects and transferring food from hand to mouth. Monkeys 737 and 738 became increasingly reluctant to move about the cage and came to spend most of the time sitting watchfully at the bottom of the cage. No animal appeared irritable, became aggressive or lost weight. Information on the onset and progression of neurological disease of all six animals is summarized in Table 1. Tables 2 and 3 compare our results with those of other studies comparing clinical and histopathologic findings in humans with vCJD and animals with natural or experimental vCJD and BSE (Supplemental 1, 2) (Lasmezas et al., 1996; Will et al., 1996; Wilesmith et al., 1988; Wilesmith et al., 1991; Lasmezas et al., 2001).

Histopathology, immunohistochemistry and immunoblot analysis of BSE in squirrel monkeys

Tissues of four control monkeys showed no signs of TSE by histopathological, IHC and WB methods. Monkeys infected with BSE had severe spongiform degeneration in several regions of the cerebral cortex, particularly in the frontal and occipital cortices seen in hematoxylin-and-eosin stained sections (Figures 1, 2). In these regions, confluent vacuoles sometimes resulted in cyst-like spaces in the neuropil. Spongiform degeneration was also observed in the caudate nucleus, thalamus, hypothalamus, cerebellum and the brain stem. Spongiform degeneration in the hippocampus was mostly limited to a discrete region of the CA1 area; no spongiform degeneration was observed in the subiculum or entorhinal cortex. Spongiform degeneration in the temporal cortex was variable, ranging from severe to minimal or absent. We identified no florid plaques in brains of squirrel monkeys with BSE a typical feature, although not specific, seen in brains of both patients with vCJD and cynomolgus macaques (*Macaca fascicularis*) infected with BSE and vCJD agents. However, plaques have not been detected in brains of cows or felines with BSE (Wells et al., 1987; Will et al., 1996; Lasmezas et al., 1996; Lasmezas et al., 2001; Lasmezas et al., 2005; Williams et al., 2007). The cerebellum showed spongiform degeneration in the molecular and granule cell layers with variable loss of Purkinje and granule cells. Gliosis, best demonstrated by immunostaining for GFAP, was seen in areas of the cerebral cortex, basal ganglia, cerebellum and in the white matter of all animals. (Intense perivascular GFAP staining in cortical areas may reflect the astrocytic foot processes adjacent to blood vessels).

Similarly to findings in vCJD in humans (Ironside et al., 2008), IHC of all six animals analyzed showed widespread accumulation of PrP^{TSE} in the gray matter with occasional PrP^{TSE} staining in the white matter. The distribution and patterns of PrP^{TSE} accumulation

were similar in sections probed with antibodies 3F4 and 6H4. The most intense PrP^{TSE} immunostaining was seen in the frontal and occipital cortices and the thalamus; the geniculate nucleus was also severely affected in five animals (Figure 2). Other brain areas also involved were the striatum, hypothalamus, cerebellum and brain stem. PrP^{TSE} deposits were seen in the CA1 area of the hippocampus. In the cerebellum, PrP^{TSE} deposits were observed in granule and Purkinje cell layers and in the molecular layer (Figure 4b). IHC and Congo red staining showed no PrP-amyloid plaques. Histopathological and IHC findings are displayed in Figure 1 and summarized in Table 1.

WB analyses of brain homogenates from all six squirrel monkeys with TSE showed the same PrP^{TSE} profile: high-molecular-mass glycoform predominating and a non-glycosylated protease-resistant fragment of approximately 20–21 kDa. Brain tissue extracts from both cattle with classical BSE and a patient with vCJD (WHO reference material 98–145) digested with proteinase K (PK) have yielded non-glycosylated protease-resistant fragments of approximately 18–19 kDa suggesting that PrP^{TSE} in the bovine brain inoculum and the brains of BSE infected squirrel monkeys had different proteolytic sites (Figure 3).

Sections probed with monoclonal antibody 4G8 directed to amino acid residues 17–24 of β -amyloid revealed a few positive plaques in the frontal and temporal cortices of a control squirrel monkey (659); examination of adjacent sections showed that isolated plaques were also detected by a conformation-dependent antibody (OC antibody) detecting generic epitopes common to many amyloid fibrils and fibrillar oligomers. No Apo-E was detected in the brain of that monkey and there was no Congo red birefringency, suggesting that the plaques are probably composed of pre-amyloid A β . 4G8-immunopositive A β plaques were not observed in any other squirrel monkey (721, 722, 735, 737, 738, 739, or in the remaining animals used as controls), with or without TSE disease. Brains of several monkeys (none of which had A β plaques), contained isolated intracellular accumulations of granular 4G8-immunopositive material, seen in areas of the gray matter of the cerebrum, in the cerebellum (limited to the Purkinje cell layer) and in brain stem. A similar pattern of intracellular staining, revealed by IHC using antibody 4G8, has been previously observed in brains of otherwise normal humans, including children as young as two years old with no neurological disease. Those observations suggest that IHC detected an A β -precursor protein rather than mature A β peptide itself (Aho et al., 2010). Selected brain sections from several other squirrel monkeys, both with BSE and controls, also showed no deposits of ApoE and no Congo red birefringent structures (data not shown).

IHC with anti-tau antibody AT8, used to detect hyperphosphorylated-tau protein (p-Tau), revealed abundant round and rod-shaped deposits of p-Tau in the cerebrum and cerebellum. We observed no neurofibrillary tangle-like deposits, neuropil threads or diffuse intracytoplasmic tau staining — forms that have comprised most tau deposits described in various human dementing diseases (Kovacs et al., 2011). In all BSE-infected primates described here, the cerebrum showed the largest amounts of p-Tau, usually present in the same cortical areas with the most abundant spongiform degeneration and PrP (Figure 2a, b and Figure 4a). p-Tau was also seen in the striatum, thalamus and hippocampus. In the hippocampus, most p-Tau was seen in the CA1 area, the area most consistently affected by spongiform degeneration and PrP deposition. The cerebellum contained rod-shaped deposits of p-Tau in the granule and molecular layers (Figure 4c). We found, in agreement with other reports (Reiniger et al., 2011), a correlation between degree of spongiform degeneration, PrP accumulation and p-Tau lesions. Sections stained with Congo red showed no birefringent deposits, suggesting that AT8 antibody detected diffuse pre-tangles, a lesion that precedes the formation of neurofibrillary tangles in patients with Alzheimer's disease (Baner et al. 1989). Similar p-Tau deposits were seen in brain sections of monkey #739 (with widespread spongiform degeneration and a massive accumulation of PrP) probed with RD4 antibody

directed to a four-repeat tau isoform. IHC revealed no p-Tau in the brains of any uninfected monkey (data not shown), in spite of an exhaustive search of many control sections.

Discussion

Studies of experimental BSE and vCJD infections in rodents have provided insight into pathogenesis, distribution of infectivity in tissues and elements of host resistance (Fraser et al., 1992). However, models in animals more closely related to humans are also informative. Although squirrel monkeys and some other non-human primates are highly susceptible to infection with several human TSE agents, BSE has not previously been characterized in squirrel monkeys. Here we report that inoculation of BSE agent into squirrel monkeys transmitted a characteristic clinico-pathologic illness with progressive behavioral, cognitive, motor and cerebellar signs. The brains of all affected monkeys showed severe spongiform encephalopathy with multiple types of prominent PrP deposits but no amyloid plaques, a typical feature, although not specific, seen in brains of both patients with vCJD and cynomolgus macaques (*Macaca fascicularis*) infected with BSE and vCJD agents. In addition, brains of all six monkeys with BSE showed consistent and abundant p-Tau deposits in several areas of the cerebrum and cerebellum.

We observed a difference between electrophoretic mobility of the non-glycosylated form of proteolytically-cleaved PrP^{TSE} extracted from brains of all squirrel monkeys with BSE (approximately 21 kDa) and non-glycosylated PrP^{TSE} from cattle with BSE and humans with vCJD (approximately 19 kDa). The original recognition of vCJD and its association with BSE in cattle was based, among other similarities, on what has been called “molecular strain typing.” Such analysis identified a purportedly unique glycoform of PrP^{TSE}, the non-glycosylated form of which had a molecular mass of approximately 19 kDa by WB in extracts from brains of both BSE-infected cattle and patients with vCJD. Those findings were interpreted as suggesting that vCJD patients and cows had been infected by the same stable and well-defined pathogen (Collinge et al., 1996; Parchi et al., 1997) identifiable by the single PrP^{TSE} isoform that it elicited. The finding by Lasmezas and colleagues of a similar 19-kDa-PrP^{TSE} isoform in extracts of brains from macaques with experimental BSE (Lasmezas et al., 2005) supported that conclusion. However, later studies detected non-glycosylated PrP^{TSE} fragments with a molecular mass of 21 kDa as well as the smaller form in extracts from brains of vCJD patients (Yull et al., 2006), and both forms have also been observed in other forms of CJD (Puoti et al., 1999; Yull et al., 2006; Uro-Coste et al., 2008; Jansen et al., 2010). Taken together, our results and those of others suggest that molecular strain typing has limitations and justify caution before inferring the origin of a TSE from the biochemical characteristics of PrP^{TSE}. In particular, it seems premature to rely on glycotyping alone to conclude that a TSE did or did not result from a BSE infection. In addition, our results reinforce the concept that the host plays an important role in the histopathologic presentation of TSEs elicited by the same infectious agent.

We observed extensive deposits of p-Tau in the cerebrum and cerebellum of all six squirrel monkeys with BSE but not in brains of four uninfected control monkeys. No clear differences in intensity or distribution of p-Tau deposits were observed in animals with incubation periods of BSE ranging from 29–46 months or in those with durations of clinical disease ranging from 2 to 5 months. However, most p-Tau occurred in areas with large amounts of PrP. Taken together, these findings suggest a correlation between accumulations of p-Tau and PrP^{TSE} independent of either incubation time or duration of clinical disease. Whether or not accumulation of p-Tau contributes to early disease or appears only late in BSE of monkeys remains to be determined. Asuni et al., using the ME7 strain of mouse-adapted scrapie agent, concluded that p-Tau appeared only in late-stage scrapie, suggesting that tau itself does not cause illness and is unlikely to serve as a useful early marker of

disease (Asuni et al., 2010). Further studies with squirrel monkeys during the incubation period of experimental BSE might allow a more direct investigation of the possible role of tau protein in the pathogenesis of TSEs.

p-Tau has been reported in brains of patients with Gerstmann-Sträussler-Scheinker (GSS) disease associated with one of a number of mutations in the *PRNP* gene: P105L, G131N, S132I, H187R, F198S, D202N, Q217R, Y218N and Q227X (Nochlin et al., 1989; Kitamoto et al., 1993; Tagliavini et al., 1993; Ghetti et al., 1996; Piccardo et al., 1998; Malucci et al., 1999; Panegyres et al., 2001; Ishizawa et al., 2002; Colucci et al., 2006; Alzualde et al., 2010; Jansen et al. 2010; Ghetti et al., 2011; Reiniger et al., 2011). p-Tau has also been described in brains of patients with Prion Protein Cerebral Amyloid Angiopathy associated with *PRNP* point mutations Y145X, Y160X and Y163X (Ghetti et al., 1996b; Holton et al., 2010; Jayadev et al., 2011). Both diseases are characterized by the presence of PrP amyloid; some patients with GSS disease show co-deposition of PrP with A β -amyloid (Ghetti et al., 1996a). Such findings suggest that tau pathology is more likely to be a secondary “downstream” pathogenic process resulting from the primary illness rather than causing extracellular amyloid to accumulate (Ghetti et al., 1996; Ishizawa et al., 2002). Tau pathology has also been described in brains of vCJD patients, experimental vCJD in mice and experimental BSE in transgenic mice expressing bovine PrP (Bautista et al., 2006; Giaccone et al., 2008; Sikorska et al., 2009; Reiniger et al., 2011). These observations suggest correlations between both distribution in brain tissue and time of appearance of PrP^{TSE} and p-Tau in both natural and experimental TSEs. The data gathered so far prompted the hypothesis that neuritic dystrophy (involving the formation of p-Tau) might represent a neuronal reaction to amyloid deposition and that the generation of neurotoxic amyloid forms of PrP changes fluidity of brain cell membranes, alters signal transduction pathways and leads to abnormal phosphorylation of tau (Giaccone et al., 2008). Our results differ somewhat from early reports of p-Tau in TSEs of transgenic and wild-type mice (Bautista et al., 2006; Giaccone et al., 2008). Bautista et al., using PrP — bovinized transgenic mice, observed particularly widespread accumulations of p-Tau in the hippocampus with smaller amounts in the cerebellum (Bautista et al., 2006). Giaccone et al., studying vCJD in wild-type mice, observed the largest amounts of p-Tau in the diencephalon (Giaccone et al., 2008). Interestingly, we observed the largest deposits of p-Tau deposition in the cerebral cortex and in some areas of the cerebellum, a distribution similar to that in patients with vCJD (Giaccone et al., 2008). This similarity is not surprising, considering that non-human primates are a species more closely related to humans than are rodents.

Tau pathology has been seen in the cerebellum (an area of the brain once thought to be refractory to tau pathology) of humans and animals with several forms of TSE (Giaccone et al., 2008; Sikorska et al., 2009; Furuoka et al. 2010; Reiniger et al., 2011) and of some patients with Alzheimer’s disease and other dementias (Kovacs et al., 2011; Sepulveda-Falla et al., 2011). Increased levels of p-Tau were reported by Goodall et al. (Goodall et al., 2006) in the CSF of several patients with vCJD and in one patient with sCJD; investigations are under way to determine whether detection of p-Tau in CSF might serve as a useful marker, in addition to 14-3-3 and other molecules in the CSF originating in the brain, to facilitate the antemortem diagnosis of vCJD (Croes et al., 2002; Green et al., 2010).

In conclusion, we transmitted BSE to six squirrel monkeys and compared the clinical and pathological findings. We established that squirrel monkeys are susceptible to experimental BSE infection, developing a TSE resembling, in several respects, vCJD in humans. Other squirrel monkeys inoculated with BSE brain extracts diluted a thousand-fold or more have remained asymptomatic for more than five years. We intend to maintain all surviving animals for at least five additional years and to study asymptomatic primates in search of early markers of disease. Given the nature of other TSEs (incubation periods occasionally

lasting up to 39 years in iatrogenic CJD (Croes et al., 2002) and over 50 years in kuru (Collinge et al., 2006), squirrel monkeys infected with the BSE might provide a model to help assess elements of risk and to understand the pathogenesis of TSEs, including the possible role of tau protein in cerebral degeneration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Lesion profile patterns of spongiform degeneration (a), PrP deposition (b), and gliosis (c) observed in brains of six squirrel monkeys inoculated with BSE agent (red). Data obtained with sections from the brain of an uninfected squirrel monkey used as control are in black. The profiles were produced from mean scores (with standard deviations) for nine brain areas: **F** frontal cortex, **T** temporal cortex, **O** occipital cortex, **GN** geniculate nucleus, **HI** hippocampus, **Crb** cerebellum, **BS** brain stem, **Wcx** white matter cerebral cortex, **Wcrb** white matter cerebellum.

Figure 2. Severe spongiform degeneration and PrP deposition in the frontal cortex of a squirrel monkey inoculated with the BSE agent. Fine punctate, perivacuolar, pericellular and linear PrP deposits (arrows) are shown in panel **b**. Panel **a** hematoxylin-eosin, panel **b** immunostained with anti-PrP antibody 6H4.

Figure 3.

Comparative Western blot (WB) analysis of prion protein in cBSE inoculum; human vCJD (World Health Organization Candidate Biological Reference CJD, material prepared and distributed by J. Cooper, K. Sadhani and P. Minor, UK National Institute for Biological Standards and Control (NIBSC); and brain extracts from squirrel monkeys with TSE disease confirmed neuropathologically (721, 722, 735, 737, 738 and 739). Brain from squirrel monkey 659 with no neurological illness or spongiform encephalopathy served as normal control. Non-PK-treated (–) and PK-treated samples (+). Molecular mass is indicated on the left of the panel. Filters were probed with anti-prion protein antibody 6D11.

Figure 4.

Abundant p-Tau-immunoreactive deposits in the cerebral cortex of a squirrel monkey 735 inoculated with BSE agent (**a**). PrP accumulation (**b**) and p-Tau profiles (**c**) in the cerebellum of squirrel monkey 739 inoculated with the BSE agent. p-Tau immunopositivity is seen in the molecular and granule cell layers of the cerebellum (arrows). p-Tau immunostaining using anti-phosphorylated tau protein AT8 shown in panels **a** and **c**, PrP immunostaining using anti-prion protein antibody 6H4 shown in panel **b**.

Table 1

Clinical and pathological findings in six squirrel monkeys inoculated with cBSE

<i>Monkey number</i>						
	739	738	737	735	722	721
Inoculum dilution	10 ⁻¹	10 ⁻¹	10 ⁻¹	10 ⁻²	10 ⁻²	10 ⁻²
Clinical sign at onset months after inoculation)	Ataxia 29	Ataxia 38	Ataxia 38	Ataxia 38	Weakness 46	Myoclonus 42
Clinical signs during course of illness (duration in months)	Tremor Weakness Dysdiadokinesia Bradykinesia 5	Tremor Bradykinesia Myoclonus Behavioral Dysmetria Withdrawal 2	Tremor Bradykinesia Behavioral Myoclonus Withdrawal 5	Tremor Myoclonus Behavioral Weakness 2	Tremor Ataxia 3	Bradykinesia Ataxia Weakness Seizure 4
SD PrP	+++ +++	++/+++ ++	++/+++ +/+++	++ ++	++/+++ +++	++ +++

cBSE: Classical form of BSE.

SD: Spongiform degeneration and PrP (detected by IHC with antibody 6H4) assessed by semiquantitative three-point score in areas of the cerebrum, cerebellum and brain stem. Results summarize overall intensity of lesion profiles (see text) from the cerebral cortex (area most consistently affected in all animals). Lesion profiles are displayed in Figure 1.