

Introduction to the transmissible spongiform encephalopathies or prion diseases

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Sheep scrapie has been known for at least 200 years and was described as a transmissible disease over 100 years ago. Since then, three groups of transmissible spongiform encephalopathies or TSE diseases have been identified in humans including familial, infectious and sporadic types. The discovery of the prion protein (PrP) in the 1980s greatly accelerated knowledge of the biology and pathogenesis of TSE diseases as this protein was found to play a critical role in disease susceptibility and the TSE species-barrier and may also be a component of the infectious agent itself. Nevertheless, the nature of the TSE agents remains an enigma. Proof of the protein-only hypothesis may require generation of biologically active transmissible agent in a cell-free environment where a virus cannot replicate. Conversely, proof of a viral aetiology will require identification and isolation of a candidate virus. Further understanding of the structure of the disease-associated protease-resistant PrP should help elucidate the mechanism of PrP conversion from the normal to the abnormal form. Such information should open up new approaches to both diagnosis and therapy.

Transmissible spongiform encephalopathy (TSE) diseases or prion diseases are rare fatal neurodegenerative diseases of humans and other animals. In the past decade, TSE diseases have achieved enhanced visibility in the media due to the appearance of bovine spongiform encephalopathy (BSE) or 'mad cow disease' in the UK. Due to the potential for human infection, BSE has strongly influenced medical, agricultural, economic and political issues in Europe. North America has been spared the ravages of the BSE epidemic; however, there is growing concern over the high incidence of the cervid TSE, chronic wasting disease (CWD), in wild and captive populations of deer and elk in the Rocky Mountain and Mid-western areas of the US and Canada¹.

TSE diseases are transmissible by inoculation or ingestion of material contaminated by infected tissues. Incubation periods prior to clinical symptoms range from months to years and, in the case of some kuru patients, may have been as long as 40 years. Primary symptoms of TSE diseases in humans are dementia and ataxia. These diseases are usually

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characterised by spongiform degeneration of the brain accompanied by the appearance of activated astrocytes. Most distinctive, however, is the accumulation in the central nervous system of scrapie-associated fibrils² or prion rods³ consisting of abnormal protease-resistant forms of host-derived prion protein (PrP). Normally, PrP is a protease-sensitive sialoglycoprotein which is usually anchored to membranes via glycosylphosphatidylinositol (GPI). In scrapie, PrP^{res} is formed from the normal PrP by an apparent change in conformation and aggregation state. The biochemistry and structural aspects of PrP conversion from the normal to the abnormal forms have been previously reviewed⁴ and will also be covered in subsequent chapters in this volume. Much evidence suggests that these abnormal forms of PrP may be critical in the transmission and pathogenesis of TSE diseases (reviewed elsewhere⁵⁻⁷). Indeed, it has been proposed, but not yet proven, that an abnormal PrP, is the infectious TSE agent or prion⁸. TSE-associated forms of PrP have been termed PrP^{Sc}, PrP^{BSE}, PrP^{CJD}, *etc.*, according to the particular TSE involved or, more operationally, PrP^{res}, for protease-resistant PrP. The terms PrP^C or PrP^{sen} refer to normal protease-sensitive PrP.

History of transmission

Scrapie has been recognised as a disease in sheep in Europe for over 200 years, and sheep breeders were aware that scrapie-free flocks developed disease after introduction of new stock from infected flocks, which suggested that the disease might be transmissible. Experimental transmission was reported as early as 1899⁹; however, the unusually short 6-month incubation period observed suggested that these sheep might have been naturally infected prior to inoculation. Transmission was subsequently confirmed by Cuille and Chelle¹⁰, who later demonstrated the filterable nature of the agent. The mechanism of the natural transmission of scrapie remains uncertain. Placenta and other tissues can contaminate pastures at the time of birth¹¹, and uninfected flocks have developed the disease when maintained on such pastures without any direct contact with infected sheep. This would appear to explain the findings in Iceland that scrapie-free sheep became infected when introduced 3 years after eradication of infected flocks¹².

When human TSE diseases, such as Creutzfeldt-Jakob disease (CJD) and Gerstmann-Sträussler-Scheinker syndrome (GSS) were first described^{13,14} (see Richardson & Masters¹⁵ for a review), these diseases were not thought to be transmissible. The description of kuru in certain New Guinea tribesmen in 1957¹⁶ and the observation by Hadlow of the similarities between the pathology of kuru in humans and scrapie in sheep¹⁷ led to the successful transmission of kuru and later CJD from humans to chimpanzees

and other primates by Gajdusek and co-workers^{18,19}. From this point, the TSE diseases of animals and humans were recognised to belong to the same group and the causative agents, though uncharacterised, were shown to have similar unusual properties of resistance to inactivation.

Human TSE diseases

In humans, TSE diseases can be subdivided into three groups – infectious, sporadic and familial (Table 1). Diseases of all three groups can usually be transmitted to primates by ingestion or inoculation of brain tissue²⁰, thus fulfilling one of the main characteristics of TSE diseases.

Transmitted/iatrogenic TSE

Kuru and iatrogenic CJD

The transmitted/iatrogenic group consists of kuru, iatrogenic Creutzfeldt-Jakob disease (CJD) and variant CJD (vCJD). For kuru and iatrogenic CJD, it is clear that the patients are exposed to TSE agent by contact with brain tissues or extracts contaminated by TSE agent. In kuru, this occurs during the handling of brain tissue of relatives who died of kuru²¹. Iatrogenic CJD has been induced by transplantation of corneal or dural tissue from patients with TSE, or by neurosurgery using instruments incompletely sterilised following use on TSE patients^{22–25}. Iatrogenic CJD has also been detected after inoculation of growth hormone extracted from pituitary glands pooled from large groups of individuals²⁶. In this situation, the extracts were apparently contaminated with brain tissue from an undiagnosed CJD patient.

Table 1 Human TSE diseases

Sporadic	No clear exposure to infectious TSE agent Creutzfeldt-Jakob disease (CJD), sporadic fatal familial insomnia (FFI) No PrP mutation 1:2,000,000 incidence world-wide
Familial/genetic	No clear exposure to infectious TSE agent Familial CJD (dementia), GSS (ataxia), FFI (sleep abnormalities) Associated with PrP mutations
Infectious/iatrogenic	Kuru, variant CJD Neurosurgery, corneal transplant, growth hormone therapy

Clinical findings vary in the different forms of iatrogenic CJD. In kuru and in disease caused by inoculation of contaminated growth hormone extracts, cerebellar ataxia is the primary sign. Dementia is less prominent and usually occurs late in the disease course. The incubation period or latent period (time from exposure to the agent until clinical onset) is long, ranging from 2 years to greater than 10 years. Interestingly, in disease following corneal or dural transplant or use of contaminated neurosurgical instruments^{23,27} dementia is more prominent and the latency is shorter (1–2 years). It is likely that direct introduction of agent into the brain in the latter instances might account for the clinical differences observed. Mutation of the PrP gene is not usually found in this group of patients; however, susceptibility may be influenced by variant PrP codon 129 genotypes, Val/Met, Met/Met, and Val/Val, which segregate in the normal population^{24,28}.

Variant CJD (vCJD)

In 1996, authorities in the UK described vCJD, a new CJD which is now believed to be the human form of bovine spongiform encephalopathy (BSE)²⁹. This disease is not a familial disease associated with mutations in the PrP gene as described below, although most cases have the Met/Met genotype at PrP codon 129 which is a common genotype in the Caucasian population. vCJD can be distinguished from sporadic CJD, which is the usual form of human TSE disease recognised world-wide for many decades, by the early age of onset, absence of EEG changes typically found in CJD, and distinct neuropathological features³⁰. Predominant clinical presentation involves psychiatric symptoms including behavioural changes, anxiety, depression and withdrawal. This is followed in weeks to months by a cerebellar syndrome with ataxia, and subsequently myoclonus. Later in the course, there are memory disturbances which progress to severe cognitive impairment and finally akinetic mutism. Neuropathology shows spongiform change, neuronal loss and astrogliosis most prominently in the basal ganglia and thalamus. In addition, there are striking amyloid plaques containing protease-resistant PrP throughout the cerebrum and cerebellum, and these plaques are often surrounded by vacuoles giving rise to the characteristic unusual 'florid plaque' morphology.

The unusually young age range of these patients and their distinctive pathology suggested that they represented a new clinical TSE disease, and the initial occurrence of these patients in the UK suggested an association with BSE in cattle. Subsequent laboratory experiments indicated a strong similarity between BSE and vCJD based on patterns of infectable mouse strains, lesion distribution in mouse brain, PrP^{res} gel banding patterns and

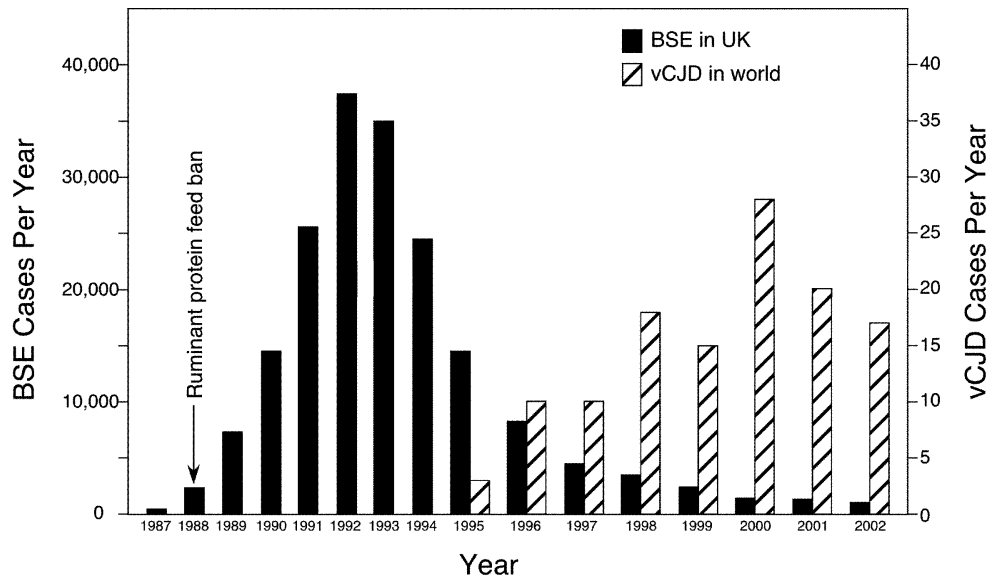


Fig. 1 Comparison of the yearly incidence of BSE in cattle in the UK and vCJD in humans world-wide.

neuropathology after transmission to cynomolgous macaques^{31–34}. Based on these data, most observers agree that vCJD represents spread of BSE from cattle to humans. Since there is no association of occupational exposure of vCJD patients to cattle on farms or in abattoirs, it is likely that spread may have occurred through consumption of BSE-contaminated meat products. As of 2003, there are over 130 cases of vCJD reported, mostly from the UK. At present it is not possible to predict accurately the expected number of cases in the future. This is partly due to the fact that neither the incubation period for transmission between cattle and humans nor the dose received by infected humans is known. However, the peak of the BSE epidemic in cattle occurred in the years 1992–1993, and the incidence has declined dramatically since that time due to regulations preventing feeding of ruminant meat and bone meal back to ruminants. In contrast, the incidence of vCJD in humans is low and it is unclear whether a peak incidence has occurred (Fig. 1). As of 2003, it has been 11 years since the BSE peak in the UK. This long time period without a clear peak in human yearly incidence suggests that humans might be partially resistant to BSE-induced disease and/or that the dose of BSE infectivity to which people have been exposed is quite low. In either case, it seems likely that many more humans have been exposed to infectious BSE than have developed the actual clinical disease.

There is now concern that some individuals exposed to BSE might be asymptomatic carriers of the infection^{35–37}, and these people might, in turn,

pose a risk of further transmission of the infection to others. Because of this potential problem, there has been an increased awareness of the need to use adequate sterilisation procedures for surgical instruments. The recommended use of high-temperature autoclaving plus sodium hydroxide³⁸ is difficult to achieve for some types of instruments. There has also been widely publicised concern that the blood supply might be contaminated with the vCJD agent. This possibility is supported by evidence that BSE in sheep can be transmitted by blood transfusion³⁹. Because of these findings, many countries have passed rules to diminish the use of blood from donors who might have been exposed to BSE/vCJD in the UK during the peak of the BSE epidemic.

Sporadic TSE

Sporadic CJD accounts for the majority of TSE disease cases in humans at present. This disease occurs at an incidence of 1 in 2×10^6 people world-wide. There is no association with a mutant PrP allele, nor is there any epidemiological evidence for exposure to TSE agent through contact with people or animals with TSE diseases^{24,40}. However, heterozygosity (Met/Val) at PrP codon 129 appears to be associated with a lower risk of sporadic CJD⁴¹. Lack of any routine laboratory test for preclinical diagnosis makes the search for agent sources and other risk factors extremely difficult. At present, the means of acquisition of TSE agent in these patients remains a mystery. Current hypotheses include exposure to an as yet unidentified virus, spontaneous generation of a non-viral agent through somatic cell mutation of PrP in each diseased individual, and stochastic initiation of spontaneous PrP^{res} formation without PrP mutation. So far, there is no evidence for spontaneous PrP^{res} formation in any animal or human TSE disease. Moreover, in New Zealand and Australia where scrapie has been eradicated, there is no evidence of spontaneous occurrence of sheep scrapie. In addition, in humans the peak age incidence of sporadic CJD is 55–60 years, and if spontaneous misfolding were the primary event, one might expect a continuously increasing incidence with age, since more time might allow more opportunity for rare misfolding events.

Clinical and pathological features of sporadic CJD are somewhat less variable than in infectious and familial TSE diseases (for reviews see Brown *et al.*²⁰ and Masters *et al.*⁴²). Males and females are affected at equal frequencies. The age of onset is usually between 50–70 years, but can range as low as 16 years and as high as 80 years. The primary clinical symptom is usually dementia which often presents with cognitive disturbances such as confusion, memory loss and bizarre behaviour. This usually progresses to a severe dementia which can also be associated with myoclonus, cerebellar symptoms such as ataxia, visual signs, seizures, and pyramidal and extrapyramidal signs. The EEG often shows a disease-specific pattern of periodic synchronous discharge (PSD) consisting of periodic triphasic

waves at 1–2 cycles/s, which is not usually seen in the familial or infectious TSE groups. Clinical duration is short, and terminates with death usually within 1–12 months.

Pathological findings consist primarily of astrocytosis and spongiform changes associated with loss of neurons, mostly in the grey matter. The abnormal protease-resistant form of PrP is detectable by Western blot analysis of brain homogenate in most cases, and can frequently also be detected directly by immunohistochemistry in properly treated brain sections. In 5% of cases, amyloid plaques containing PrP are also present²⁰.

Familial TSEs

Familial TSEs are associated with the presence of an autosomal dominant genetic alteration of the PrP gene^{43,44}. These diseases include familial CJD, Gerstmann-Sträussler-Scheinker syndrome (GSS) and fatal familial insomnia (FFI). There is variability in the clinical and pathological findings, the age of onset, and the duration depending on the particular PrP mutation involved. The primary clinical finding can be ataxia, dementia or sleep abnormality. Clinical variability occurs even within individual patients of the same family, suggesting that genes other than PrP or non-genetic factors also influence these diseases^{45–47}. For most PrP mutations, all positive individuals eventually develop the disease; however, for Lys 200, this is not the case, suggesting that other factors might be required to induce disease in these individuals⁴⁸. Some possibilities include infection by an exogenous viral agent, presence of additional genes, age of onset, and dietary or environmental factors.

In addition to point mutations in the PrP gene, insertions in the octapeptide repeat coding region of PrP have been associated with degenerative brain disease. Addition of 2, 5, 6, 7, 8, and 9 extra octapeptide repeats have been detected in various families. Clinical disease usually begins at an early age and is of long duration. Findings are variable and include dementia, ataxia and other features typical of TSE. Pathology is also variable and consists of PrP plaques, astrocytosis and spongiosis.

Animal TSE diseases

Natural and experimental scrapie in sheep

Scrapie has been recognised as a disease in sheep for over two centuries, and was the first TSE disease to be shown as experimentally transmissible. Thus sheep scrapie provides an unusual opportunity to compare natural and

experimental TSE disease processes. Although in animals there are no known genetic cases of TSE disease comparable to those seen in humans, allelic variations in the sheep PrP sequence do occur, and variation at several residues in the PrP sequence influences susceptibility to both natural and experimental scrapie infection⁴⁹. Although the mechanism of these effects is not certain, the susceptibility of sheep with these allelic variants to scrapie correlates with the relative efficiencies with which the respective PrP^C molecules convert to PrP^{res} in *in vitro* systems⁵⁰.

Bovine spongiform encephalopathy (BSE)

In the past decade, the BSE epidemic in the UK has brought international attention to the TSE family of diseases. BSE was spread by feeding of protein supplements contaminated with the rendered tissues of BSE-positive cattle. Several laboratory tests including PrP^{res} banding patterns in protein gels and comparative titration in various mouse strains, identify similarities in BSE from all sources tested, as compared to most commonly known isolates of sheep scrapie. However, it remains unclear whether BSE originated by adaptation from an unusual strain of sheep scrapie or from an unrecognised bovine TSE case.

BSE has also been transmitted to other species by feeding of contaminated meat and bone meal to ungulates and large felines in zoos and probably also to domestic cats. Transmission to humans has also been suggested by the appearance of vCJD in over 130 humans primarily in the UK. The similarity in pathology and in laboratory tests lends support to this interpretation^{31,32}. At the molecular level, the interactions between BSE (bovine) PrP^{res} and human PrP^C are rather inefficient⁵¹. However, this apparent 'molecular species barrier' is clearly only one of multiple factors that are likely to influence the extent of BSE transmissions to humans.

Chronic wasting disease (CWD)

CWD in deer and Rocky Mountain elk in the US and Canada is another example of a TSE disease of unknown origin. CWD is now recognised to be a serious problem in game farms and certain wild deer and elk populations in North America. Its spread appears to be enhanced by the abnormal population densities found in domestic facilities, although the actual mechanism of transmission is unknown^{1,52}. First recognised in captive animals, CWD has now also been detected at > 10% incidence in certain wild deer populations in south-eastern Wyoming and northern Colorado and at a much lower incidence in wild elk. In both species, the incidence of CWD in contaminated game farms can be much higher than in wild populations, and the commerce in live farmed deer and elk

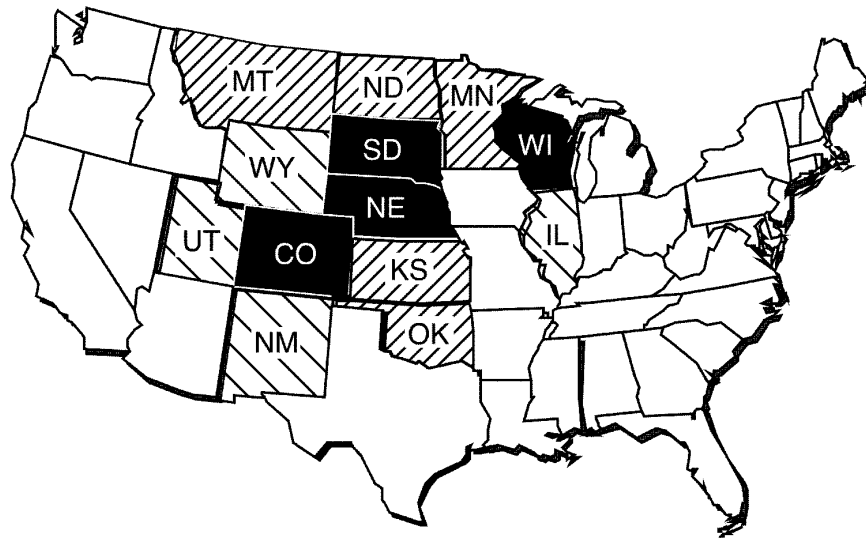


Fig. 2 Map of CWD incidence in the US. CWD in farmed deer and elk only (MT, ND, MN, KS, OK). CWD in wild deer and elk only (WY, UT, IL, NM). CWD in both wild and farmed deer and elk (CO, SD, NE, WI).

appears to account for the rapid spread to new sites in the US and Canada which are not always geographically contiguous with previous areas of infection (Fig. 2). The fact that CWD is found in wild ruminants on the same range as cattle and sheep raises concern over the possibility that CWD could be transmitted to domestic animals and possibly might also pose a risk for human infection similar to BSE.

Transmissible mink encephalopathy (TME)

TME is a TSE disease believed to be acquired by feeding animals tissues from scrapie-infected sheep or TSE-infected cattle. TME has been described in several mink ranches in the US⁵³. Although thought by many to be a mink-adapted form of sheep scrapie, there is anecdotal evidence to suggest that it might have arisen from a cattle TSE disease. However, TME has been readily transmitted to hamsters⁵⁴, whereas this has not been observed with BSE from Europe. Therefore, TME and BSE appear to be distinct TSE agents.

Species barrier and experimental animal models

TSE diseases have been studied experimentally in several laboratory species including mice, rats, hamsters and non-human primates. In general, TSE diseases show a preference for transmission to the species of origin or a closely related species. Most noteworthy was the original demonstration of the

transmissibility of CJD and kuru from humans to chimpanzees^{18,19}. Transmission to a less closely related species is also possible and appears to involve a progressive adaptation during serial passage in the new host. For example, scrapie from sheep or goats and BSE from cattle have produced typical TSE disease in mice, and mouse- and hamster-adapted agents from these and other sources have been used extensively for pathogenesis studies and characterisation of the agents.

Early experiments identified the *Sinc* gene as important in host susceptibility to scrapie⁵⁵, and subsequently *Sinc* was found to be the gene encoding PrP^{Sc}. More recent studies showed that expression of PrP^{Sc} is required for susceptibility to TSE diseases, and propagation of infectivity is eliminated in the absence of the PrP gene⁵⁷. This has been interpreted to imply that PrP is either a receptor for the infectious agent or an integral component of the agent.

PrP was identified as a susceptibility factor for cross-species transmission experiments with PrP transgenic mice where expression of hamster PrP was found to render transgenic mice susceptible to hamster-specific scrapie strains⁵⁸. The use of chimeric PrP molecules illustrated the importance of amino acid residues in the central portion of PrP in species-specific interactions between the inoculated TSE agent and the host animal⁵⁹. In particular, mouse PrP residues 138 and 154 were found to be most important in the species barrier between mice and hamsters^{60,61}. The changes between mouse and hamster PrP at residues 138 and 154 do not appear to alter the folded structure of PrP^{Sc} significantly^{62–64}, and thus it is unclear how these residues influence the species barrier. Knowledge of the molecular structure of PrP^{Sc} should give better insight into the mechanism of influence of these residues on cross-species PrP conversion. Interestingly, residue 138 is homologous to a polymorphic residue at position 142 in goat PrP which was previously found to influence resistance to BSE and certain sheep scrapie strains *in vivo*⁴⁹.

In similar studies, transgenic mice expressing human PrP have been shown to have increased susceptibility to human TSE disease isolates^{65–68}. These results have broadened the possibilities for studying human isolates in less expensive and more rapid rodent models suitable for screening of possible therapeutic drugs. However, in spite of knowledge of PrP^{Sc} sequences and structures from a variety of species, the extent of species-specific resistance to TSE diseases remains impossible to predict solely by analysis of PrP sequences and structure^{69,70}. This is of critical importance in the matter of human susceptibility to BSE.

Transgenic mice models of familial human TSE disease

Familial forms of human TSE diseases are strongly associated with different PrP mutations. Many of these disease-associated PrP mutations

have been expressed in tissue culture cell lines, and although the mutant PrP often shows abnormal properties, so far no transmissible infectivity has been generated *in vitro* in such systems. Interestingly, transgenic mice overexpressing either the Leu 102 PrP GSS mutant⁷¹ or the extra amino acid octarepeat PrP mutant⁷² develop a fatal neurological disease with neuropathology similar to TSE disease. However, in neither model is there generation of PrP^{res} with the high degree of protease-resistance found in the human counterparts of these models. Furthermore, the transmissibility of the diseases produced in these transgenic mouse models remains questionable. For the octarepeat mutant, transmission has so far not been successful⁷³. For the Leu 102 mutant, the disease could not be transmitted to normal Pro 102 PrP mice, but could accelerate disease onset in transgenic mice expressing low levels of Leu102 PrP transgene⁷⁴. This result was interpreted by the authors as evidence for transmission, but it clearly does not mimic the transmission of known TSE diseases including the human familial GSS disease associated with Leu102 PrP, which is in fact transmissible to monkeys and mice expressing only Pro102 PrP^{20,75}. Thus, the questionable transmissibility and the lack of PrP^{res} suggests that this transgenic model may in fact be a disease due to overexpression of a mutant protein rather than a true TSE disease. To avoid artefacts due to abnormal transgene copy number and abnormal integration sites, PrP with the Leu102 mutation has been substituted for the normal mouse PrP gene by homologous recombination⁷⁶. In contrast to the above transgenic mice, such recombinant mice fail to develop spontaneous CNS disease, and they also do not generate any infectious transmissible agent. This might be due to the fact that they are expressing only normal levels of the mutant PrP. However, they do have an altered susceptibility to infection by various TSE agents from different species⁷⁷. These results suggest that mutant Leu102 PrP may alter susceptibility for TSE diseases rather than acting as a direct cause of GSS.

Nature of the infectious agent

Viral hypothesis

In the past decade, there has been a massive increase in knowledge concerning many aspects of the TSE diseases largely due to the discovery of PrP. Nevertheless, there continues to be a paucity of information concerning the structure and composition of the infectious agent. Early ultrafiltration studies suggested that the infectious particle was small and might be a virus, and the viral hypothesis remains to this day an alternative which has been difficult to prove or disprove. Inactivation studies by irradiation, heat and chemicals have led to conflicting

Table 2 Caveats concerning TSE infectivity

Viral hypothesis	
	No virus or viral genome
	Unclear role of virus in genetic TSE diseases
	Resistance to chemical and physical inactivation
	Viruses survive in geothermal waters
Protein-only hypothesis	
	Nucleic acids in purified PrP ^{res}
	No clear mechanism for TSE strains
	Similar protein conversions in non-transmissible amyloid diseases
	Transgenic and knock-in mouse models of human GSS
	No PrP ^{res}
	No transmission to wild-type mice
	No spontaneous disease in knock-in mice

conclusions regarding the uniqueness of TSE infectivity⁷⁸. Using heat or hypochlorite, the majority of infectivity actually shows kinetics of inactivation and a small resistant fraction (0.1%) that is similar to known viral examples, such as bacteriophage fd^{79,80}. This minor resistant fraction should not be used to infer unique properties in the majority of the infectivity. Furthermore, the discovery of viruses in bacteria of geothermal acidic hot springs capable of living at temperatures as high as 93°C and pH as low as 1.0 has altered thinking about the ability of viruses and virus-replicating machinery to survive extreme physical conditions^{81,82}. Therefore, it might be difficult to rule out the presence of a virus in TSE infectivity based on resistance to inactivation by heat and acid (Table 2).

The spectrum for inactivation of scrapie infectivity by UV irradiation suggested that the critical target was neither protein nor nucleic acid, but instead appeared to be lipid in nature⁸³. However, in past virological experiments using non-penetrating radiation such as UV, shielding of the critical target molecule of the infectious agent by other molecules in the mixture or attached to the agent has been known to influence the results. In fact, the unusual inactivation spectrum for scrapie was similar to intact tobacco mosaic virus, a well-characterised RNA virus, whereas the isolated RNA from this virus had peak inactivation at a wavelength predicted for a typical nucleic acid⁸⁴. In view of these issues and the difficulty in purification of the scrapie agent, UV studies may not provide definitive information as to the nature of the scrapie infectivity.

Scrapie infectivity has also been studied using X-rays, where shielding or blocking of the radiation is not an issue. Many experiments have resulted in similar inactivation rate constants; however, different groups have varied markedly in their interpretation of these results⁷⁸. Using target theory calculations, some workers have concluded that the maximum genome size would be very small^{85,86}. In contrast, others making empirical comparisons

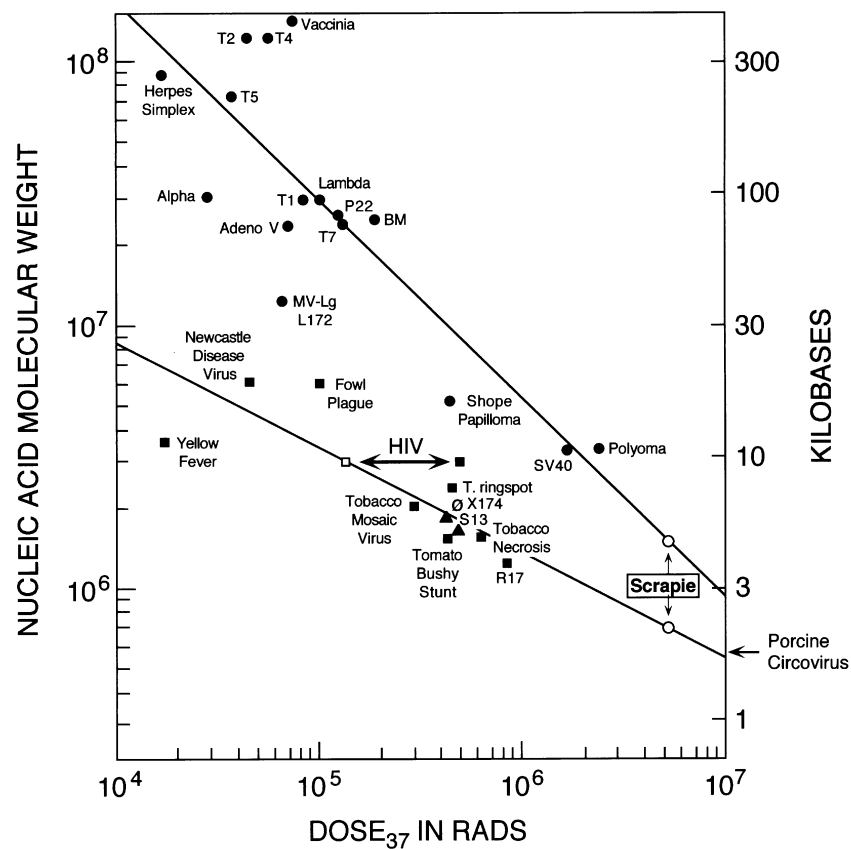


Fig. 3 Comparison of viral nucleic acid molecular weight to dose of X-ray irradiation required for 37% inactivation (D_{37}) for a variety of viruses and for the scrapie agent. The figure was adapted from Rohwer *et al*⁷⁸. The solid circles are double-stranded viruses, and the solid squares are single-stranded viruses. The best-fit lines for each of these groups are shown. The open square shows the D_{37} value predicted for HIV based on its genome size, and the adjacent solid square shows the actual value observed experimentally. Based on radiation inactivation experiments, the scrapie agent might be predicted to have a genome size of 2–4 kb which is larger than the genome size for one of the smallest known viruses, porcine circovirus (1759 nucleotides), shown for comparison.

to viruses with known genomes have arrived at a genome size consistent with a small (2–4 kb) virus (Fig. 3)^{78,79}. However, both these interpretations might underestimate the genome size if the TSE agent had a means of repairing damaged nucleic acid during replication. Such a situation occurs with retroviruses in which the two RNA genomes in each particle can be partially damaged and then repaired during reverse transcription, thus giving a higher resistance to X-ray irradiation than predicted by genome size alone (Fig. 3).

These data are only indirect evidence that may be consistent with the viral hypothesis. In spite of many efforts there are still no data supporting any candidate viruses (Table 2). Furthermore, although small nucleic acid molecules have been found in purified infectious scrapie samples, efforts to identify an intact nucleic acid molecule of potential genome size have met with failure⁸⁷. Therefore, if such a genome exists, it would have to be capable of regeneration from small fragments by a copy-choice mechanism during transcription as described above. In addition, the role of mutant and non-mutant PrP in the case of a viral aetiology remains hypothetical. To explain the very high correlation of TSE disease in persons with certain PrP mutations, one would have to speculate that the mutant PrP might serve as an efficient susceptibility factor or receptor for a viral agent. Such a putative virus would have to be relatively common in the population to account for the nearly 100% disease incidence in patients with certain PrP mutations. However, in order to account for the extremely low incidence of sporadic CJD in people lacking PrP mutations, the normal non-mutant PrP would have to be much less efficient than mutant PrP in its interactions with such a virus. Similar effects probably occur for many viral diseases in humans where there is a high incidence of infection compared to the incidence of clinical disease. Examples include HTLV I retrovirus and B19 parvovirus. However, at present, this possibility remains speculative as applied to TSE diseases in the absence of additional supportive data using actual candidate viruses.

Protein-only hypothesis

Because TSE infectivity shows a strong resistance to sterilisation by heat and chemicals, Griffith proposed in 1967 that the agent might be a self-replicating protein⁸⁸. Following the discovery of scrapie-associated fibrils² and prion rods³ and the identification of PrP as a major component of infectious fractions^{89,90}, the protein-only hypothesis was refined into the prion hypothesis⁸. Although the discovery of PrP has led to a vast increase in knowledge concerning the role of PrP in susceptibility and pathogenesis of TSE diseases, the question of whether PrP^{res} is an integral or sole component of the infectious agent remains unresolved. The most important evidence supporting this concept is the finding that PrP^{res} is the predominant macromolecule found in fractions of purified infectious agent. However, several caveats persist regarding this matter (Table 2). First, because of the presence of aggregated PrP, the agent is difficult to purify, and the purest fractions still contain detectable nucleic acid molecules. These fractions might also conceivably contain other components relevant to infectivity. Second, in

purified fractions the ratio of PrP molecules to infectious units is extremely high (approximately 100,000), and this fact led to the speculation that only a subfraction of the PrP^{res} (*i.e.* PrP*) is the infectious form⁹¹. However, it remains unclear how to identify or distinguish biochemically the proposed infectious and non-infectious forms of the protease-resistant PrP. Third, although circumstantial evidence suggests that an abnormal form of PrP alone may be infectious, so far cell-free *in vitro* conversion of PrP to a protease-resistant form has not generated *de novo* infectivity⁹². Fourth, the aforementioned lack of generation of infectivity in recombinant knock-in Leu102 PrP mice⁷⁶ raises the possibility that mutant PrP alone cannot generate a transmissible agent. Fifth, there is the puzzling comparison of TSE diseases to the classical amyloid diseases. In both disease groups, protein misfolding is a prominent feature of the pathogenesis and, in many amyloid diseases, interactions between the normal and abnormal proteins can lead to formation of additional abnormal protein. This is similar to the situation with TSE diseases. However, only the TSE diseases appear to be easily transmissible experimentally. This suggests that the protein interactions common to all amyloid diseases probably do not explain the unique transmissibility of TSE diseases⁹³. The existence of this dilemma does not imply that the protein-only or viral hypotheses are incorrect, but rather that we are lacking in some crucial information to explain the differences between TSE diseases and the non-transmissible amyloid diseases.

TSE strains

The existence of biologically different scrapie strains in inbred animals with a single type of PrP gene remains an interesting enigma⁹⁴. Disease induced by scrapie strains can differ in the clinical symptoms produced, the regions of brain affected and the incubation period prior to clinical onset. These differences might be explained by mutations in a nucleic acid genome according to the viral hypothesis, but no genomes have yet been identified. In contrast, the existence of strains may be difficult to explain by the protein-only hypothesis. However, structural variations in PrP^{res} might encode strain-specific properties, and recent data suggest that PrP^{res} structures might be capable of conferring such properties on newly formed PrP^{res} in a template-like fashion^{95,96}. This possibility is further supported by studies that have indicated that different strain-associated forms of PrP of the same amino acid sequence can differ in susceptibility to PK, secondary structure and other conformationally sensitive parameters^{32,97-99}. Nonetheless, it is unclear how strain specific properties or conformations might be preserved during passage between

species where numerous PrP amino acid differences exist¹⁰⁰ unless distinct PrP^{res} conformations can exist which propagate themselves independent of certain changes in primary sequence.

Unsolved issues

Despite the progress that has been made in describing the TSE diseases, fundamental uncertainties remain. First and foremost is the problem of the precise nature of the infectious agent and whether it is composed solely of PrP^{res}. A definitive answer to this question will likely require the *de novo* generation of TSE infectivity from PrP^{sen} derived from an uninfected source under cell-free conditions that do not allow the replication of any viruses. If this were to be demonstrated, it would distinguish the TSE diseases from other amyloidoses which have not proven to be transmissible by amyloid protein or fibrils themselves. A related issue is how multiple strains of TSE agents are defined at the molecular level and propagated in the host. Another issue most salient to human and animal health is the one of how and when various TSE agents cross species barriers. The appearance of vCJD in Europe has raised concerns about the safety of food products derived from BSE-positive animals with subclinical infections which are very difficult to detect. In addition, there is great concern that humans exposed to BSE might carry infectivity in blood or other tissues, and thus pose a risk to others by blood transfusion or contamination of surgical instruments.

The likelihood of extensive human exposure to BSE has made the development of effective TSE treatments even more urgent. Recent discoveries that several different types of molecules can inhibit PrP^{res} formation raise optimism that treatment of TSE diseases may be possible in the future. It is conceivable that treatment aimed at this apparently pathogenic product may be effective even in the absence of a precise understanding of the nature of the transmissible agent.

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