Review

Variant Creutzfeldt-Jakob disease

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It is clear that the prion strain causing bovine spongiform encephalopathy (BSE) in cattle has infected human beings, manifesting itself as a novel human prion disease, variant Creutzfeldt-Jakob disease (CjD). Studies of the incubation periods seen in previous epidemics of human prion disease and of the effect of transmission barriers limiting spread of these diseases between species, suggest that the early variant CJD cases may have been exposed during the preclinical phase of the BSE epidemic. It must therefore be considered that many cases may follow from later exposure in an epidemic that would be expected to evolve over decades. Since the number of people currently incubating this disease is unknown, there are concerns that prions might be transmitted iatrogenically via blood transfusion, tissue donation, and, since prions resist routine sterilisation, contamination of surgical instruments. Such risks remain unquantified. Although variant CJD can be diagnosed during life by tonsil biopsy, a prion-specific blood test is needed to assess and manage this potential threat to public health. The theoretical possibility that BSE prions might have transferred to other species and continue to present a risk to human health cannot be excluded at present.

The transmissible spongiform encephalopathies, or prion diseases, a group of neurodegenerative diseases that affect human beings and animals, have attracted much public and media attention. The unique biology of these previously obscure brain diseases have been the subject of long-standing and intense research and controversy. However, the appearance and rapid evolution to epidemic of the novel animal prion disease bovine spongiform encephalopathy (BSE), with the legitimate concerns of human transmission and a potentially severe threat to public health, have placed these diseases, and the people who have managed and studied them, under an unprecedented political and inquisitorial spotlight.

The human prion diseases have been traditionally classified into Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease, and kuru, and can be divided into three aetiological categories: sporadic, acquired, and inherited. The acquired prion diseases include iatrogenic CJD and kuru, and arise from accidental exposure to human prions through medical or surgical procedures or participation in cannibalism. Epidemiological studies show no association between sheep scrapie and the occurrence of CJD in human beings.[1] Sporadic CJD occurs in all countries, with a random case distribution and an annual incidence of one per million. Around 15% of human prion disease is inherited; all cases to date have been associated with coding mutations in the prion protein gene (*PRNP*), of which more than 20 distinct types are recognised.[2] The inherited prion diseases can be diagnosed by *PRNP* analysis, and the use of these definitive genetic diagnostic markers has enabled recognition of a wider phenotypic spectrum of human prion disease, which includes a range of atypical dementias and fatal familial insomnia.[3–5] No such pathogenic *PRNP* mutations are present in sporadic and acquired prion disease. However, a common prion protein (PrP) polymorphism at residue 129 (where methionine or valine can be encoded) is a key determinant of genetic susceptibility to acquired and sporadic prion diseases, which occur mostly in homozygous individuals.[6–8] This protective effect of *PRNP* codon 129 heterozygosity is seen also in some of the inherited prion diseases.[9,10]

Prion diseases of human beings and animals are associated with the accumulation in the brain of an abnormal partially protease-resistant isoform of a host-encoded glycoprotein known as prion protein. The disease-related isoform, PrP^{Sc}, is derived from its normal cellular precursor, PrP^{C}, by a post-translational process that involves a conformational change. PrP^{C} is rich in α-helical structure, whereas PrP^{Sc} seems to be composed mainly of a β-sheet structure. According to the "protein-only" hypothesis,[11] an abnormal PrP isoform[12] is the principal, and possibly the sole, constituent of the transmissible agent or prion. PrP^{Sc} is postulated to act as a conformational template that promotes the conversion of PrP^{C} to further PrP^{Sc}. PrP^{C} seems to be poised between two radically different folding states, and α and β forms of PrP can be interconverted in suitable conditions.[13] Soluble β-PrP aggregates in physiological salt concentrations to form fibrils with morphological and biochemical characteristics similar to PrP^{Sc}. A molecular mechanism for prion propagation can be proposed.[13] Prion replication, with recruitment of PrP^{C} into the aggregated PrP^{Sc} isoform, may be initiated by a pathogenic mutation (resulting in a PrP^{C} predisposed to form β-PrP) in inherited prion diseases, by exposure to a "seed" of PrP^{Sc} in acquired disease, or as a result of the spontaneous conversion of PrP^{C} to β-PrP (and subsequent formation of aggregated material) as a rare stochastic event in sporadic prion disease.

Although the existence of multiple strains or isolates of prions with distinct biological properties has provided a challenge to such a protein-only model of prion replication, prion strains can be clearly distinguished by differences in the biochemical properties of PrP^{Sc}.[11–15] Prion-strain diversity seems to be encoded by differences in PrP conformation and pattern of glycosylation.[16] A molecular approach to strain typing based on these characteristics has enabled the identification of four main types of CJD—sporadic and iatrogenic CJD are of PrP^{Sc}

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types 1–3, and all variant CJD cases are associated with a distinctive type 4 PrP^{Sc}.[16,19]

This novel biology of prion propagation explains the difficulty in studying and developing early diagnostic markers for these diseases. The high sensitivity and specificity of nucleic-acid-based diagnostic techniques are ineffective in the detection of an agent that seems to be devoid of an important nucleic-acid component. The lack of a humoral immune response, presumably because of immune tolerance to what are host proteins widely expressed in the immune system, has precluded a serological assay. Detection and classification of prion diseases had relied on descriptive pathological criteria and bioassay in laboratory animals, which relied on reaching the end stage of diseases with long incubation periods. Striking progress has, however, been made in the understanding of the molecular biology of these diseases and their pathogenic mechanisms. Such advances, seemingly slow under the scrutiny they have received, need to be considered in the context of the difficulties of research on the central nervous system and of the experimental time scale imposed by the slow pathogenesis of these diseases. Prion diseases are probably the best understood of the degenerative brain diseases and provide a paradigm and lead for research in the more common disorders such as Alzheimer's disease.

With respect to an epidemic of prion disease related to BSE in human beings, many conflicting opinions have been expressed and much uncertainty remains. However, key questions have been answered, and the next set of questions and the methods to address them have been clearly defined. The first key question is can and have BSE prions infected human beings? If the answer to this question is yes, two vital subsequent questions must be answered to inform public-health planning. First, how many human cases will occur, and over what time, as a result of historical dietary, occupational, and iatrogenic BSE exposure? Second, are there any remaining sources of exposure to fatal single or cumulative doses of the BSE agent from cattle or other animal species, including human beings?

Has the BSE agent infected human beings?

The transmissibility of the prototypic prion disease, scrapie, between sheep (and goats) was shown in 1936 by experimental inoculation,[20] and the neuropathological similarities between scrapie and the human disease kuru led to the suggestion that kuru may also be transmissible.[21] Kuru and then CJD were transmitted to primates in the 1960s.[22,23] Transmissible mink encephalopathy and chronic wasting disease of mule deer and elk were desribed from the 1940s onwards. The appearance of BSE in UK cattle from 1986 was widely attributed to transmission of sheep scrapie, endemic in the UK and many other countries, to cattle via contaminated feed prepared from rendered carcasses. It was argued that the multifocal onset of the BSE epidemic suggested exposure to a widely distributed agent, for which the scrapie agent was the only plausible candidate.[24] Subsequent characterisation of the prion strain that causes BSE, however, showed it to be distinct from that identified in sheep scrapie.[25] Further classic and molecular strain-typing studies[26] have not identified a BSE-like strain in sheep, which suggests that such a strain, if it originated at all in sheep, must be rare. Experimental transmission of sheep scrapie to cattle by the intracerebral route with US scrapie produced a disease type different from BSE.[27] Given the similarity in most other respects in these diseases across different mammals, it seems implausible that sporadic forms of prion disease will be unique to human beings. It could be argued, therefore, that unfortunate recycling of rare sporadic BSE cases led to the epidemic, since cattle carcasses were also rendered to produce cattle feed.

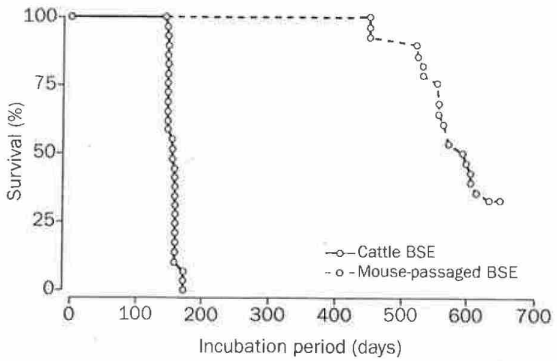
The assumption that BSE arose from sheep scrapie led to the further assumption that BSE would, like scrapie, pose little, if any, threat to human health, although the possibility that scrapie may develop new characteristics after passage in cattle was recognised. Irrespective of whether BSE originated from sheep scrapie, it was clear from 1990 onwards, with the occurrence of novel spongiform encephalopathies among domestic and captive wild cats, that its host range was different from scrapie. The suspicion that feline spongiform encephalopathy was related to BSE was confirmed by strain-typing studies.[16,25] Whether or not human beings would be encompassed by the novel host range of BSE could not be predicted or directly addressed experimentally. Many new animal species have developed spongiform encephalopathies coincident with or after the arrival of BSE, including greater kudu, nyala, Arabian oryx, Scimitar horned oryx, eland, gemsbok, bison, ankole, tiger, cheetah, ocelot, puma, and domestic cats (R Bradley, personal communication). Several of these have been confirmed to be caused by a BSE-like prion strain,[16,25] and most or all of these strains are likely to be related to the BSE prion.

A novel form of human prion disease, variant CJD, was recognised in the UK in 1996[28] and implied the arrival of a new risk factor for CJD.[29] These epidemiological studies suggested a link with BSE, which was strongly supported by molecular strain-typing studies[16] and subsequently by experimental transmission studies into transgenic and conventional mice,[30,31] which confirmed that variant CJD and cattle BSE are caused by the same prion strain. That variant CJD is a human manifestation of BSE is, therefore, supported by compelling experimental data.

Scale of the epidemic

Factors that are important in determining the probability of BSE transmission to an individual include dose, route of exposure, genetic susceptibility, and the height of the species barrier between cattle and human beings. The latter is expected to be different for individuals with different *PRNP* genotypes. In addition, the species barrier varies with prion-strain type, although BSE is thought to be caused by a single strain. To date, however, only nine cattle brains, from more than 170 000 collected, have been strain typed,[25,31] and the existence of other less common BSE strains is possible.

Attempts by statistical modelling to predict the eventual scale of any variant CJD epidemic have served only to emphasise the uncertainties.[32] Subsequent predictions, formed from a larger range of possible incubation periods of variant CJD in human beings, show even greater uncertainty of epidemic sizes, ranging from less than 100 to several million cases.[33] Despite the wide confidence limits on these epidemiological estimates, some workers have taken reassurance from the small number of additional variant CJD cases that have been identified since March, 1996. Although major uncertainties clearly exist, an assessment of previous epidemics of human prion disease



Typical effect of a species barrier: transmission of cattle BSE and mouse-passaged BSE to inbred FVB mice

Primary passage associated with long mean incubation period, wider range of incubation periods, and <100% animals developing clinical disease. Subsequent passages in mice associated with consistent short incubation and all animals succumb to disease.

and an appreciation of the biological effects of species barriers, does, in my opinion, challenge such optimism.

Epidemics of human prion disease

The kuru epidemic in the eastern highlands of Papua New Guinea provided the largest experience of acquired human prion disease.[34] Kuru was transmitted during cannibalistic feasts when deceased relatives were eaten by their close family and others in the immediate community. The epidemic is thought to have originated when a person who had developed sporadic CJD, which is known to occur at random in all populations, died and was eaten. The recycling of prions within this isolated population led to a substantial epidemic that became the major cause of death among children and adult women. Before the end of cannibalism in the late 1950s, such feasts were common and the multiple exposures that individual kuru patients may have had complicated precise estimates of incubation periods. However, studies of later cases with well-defined exposures provided more precise estimates.[35] Rare cases of kuru were recorded in children as young as 4·5 years, which suggests incubation periods of this length or shorter. Although dietary exposure to kuru was assumed to be the main route of transmission, inoculation with brain or other tissue, either via cuts or sores or to the conjunctiva (through eye rubbing) was also likely. Since such routes of transmission in laboratory animals result in shorter mean incubation periods than oral exposure, these cases of kuru with very short incubation periods may not represent oral transmission. At the other extreme, occasional cases of kuru are still occurring in the Fore region in patients exposed during some of the last cannibalistic feasts held in their villages, and are consistent with incubation of longer than 40 years (unpublished data). Mean incubation periods have been estimated to be around 12 years (M Alpers, personal communication).

More than 100 cases of acquired CJD have occurred as a result of intramuscular injection with human cadaveric pituitary-derived growth hormone, inadvertently contaminated with CJD prions. Multiple exposures, commonly over several years, complicate accurate estimation of incubation periods. Mean incubation periods are, however, estimated to be about 12 years.[36]

Together, these data suggest that incubation periods of human prions in human beings (in the absence of a species barrier) after peripheral inoculation or oral exposure, range from at least 4 years to 40 years, with a mean of about 10–15 years. Cases resulting from oral exposure may have longer mean incubation. Most of the earliest cases of CJD related to iatrogenic growth hormone in the UK were homozygous for valine at polymorphic *PRNP* codon 129, a genotype seen in about 11% of normal white people.[37] This effect of codon 129 genotype may affect the mean incubation period as well as overall susceptibility. Heterozygotes would be expected to have the longest mean incubation periods.

The species barrier

Transmission of prion diseases between different mammalian species is limited by a so-called species barrier.[38] On primary passage of prions from species A to species B, typically not all inoculated animals of species B would succumb; those that did would do so with longer and more variable incubation periods than with transmission of prions within the same species, on which, typically, all inoculated animals would succumb with a short and remarkably consistent incubation period. On second passage of infectivity to further animals of species B, transmission parameters resemble within-species transmissions, with most, if not all, animals developing the disease with short and consistent incubation periods. Species barriers can therefore be quantified by measurement of the fall in mean incubation period on primary and second passage in the new host species or, perhaps more rigorously, by comparative titration study. The latter involves inoculation of serial dilutions of an inoculum in the donor and the new host species, and comparison of the dilution required to kill 50% of animals (LD_{50}). The effect of a substantial species barrier (for instance that between hamsters and mice) is that few, if any, animals succumb to disease on primary passage and at incubation periods approaching the natural lifespan of the species concerned.

As an example, consider the species barrier that limits transmission of BSE from cattle to conventional mice. This barrier has been extensively studied experimentally because of the use of mice to assay BSE infectivity. BSE can be readily transmitted to mice, with most, if not all, inoculated animals succumbing to disease on primary passage (also known as a high attack rate). This relatively moderate species barrier has been formally measured by comparative titration studies of the same BSE isolate by intracerebral inoculation into cattle and mice, which have shown a barrier of about 1000-fold (ie, it takes 1000 times more BSE inoculum to kill a mouse than a cow).[39] The effect of this barrier on incubation periods is to increase mean incubation periods by around three-fold and to substantially increase the range of incubation periods (figure).

Such experiments are generally done by the most efficient route of transmission (intracerebral). A formal titration of BSE-infected material in mice to determine an oral LD_{50} has not been reported. However, oral challenge with about 10 g of BSE-affected cow brain killed most exposed mice.[40] If the bovine-to-human species barrier were similar to that to mice, that would suggest an oral LD_{50} in human beings of a similar magnitude to that for mice. Clearly, the hope is that the species barrier limiting transmission of BSE to human beings will be far higher. If we assume, however, that the barrier is similar (it remains possible that it could be lower), extrapolation would

suggest mean incubation periods of BSE in human beings of perhaps 30 years, with a range of 10 years to longer than a normal human lifespan. Such estimations, based on extensive transmission studies across species from many research groups over several decades, suggest the need for caution about optimistic assessments of the likely size of a human epidemic only 3 years after the recognition of variant CJD.

Early studies of the molecular basis of the species barrier suggested that it mainly resided in differences in PrP primary structure between the species from which the inoculum was derived and the inoculated host. Transgenic mice expressing hamster PrP were, unlike wild-type mice, highly susceptible to infection with hamster prions.[41] That most sporadic and acquired CJD occurred in individuals homozygous at *PRNP* polymorphic codon 129 supported the view that prion propagation proceeded most efficiently when the interacting PrP^{Sc} and PrP^{C} were of identical primary structure.[6,7] However, it has been long recognised that the type of prion strain affects ease of transmission to another species. With BSE prions, the strain component of the barrier seems to predominate, with BSE not only transmitting efficiently to a range of species, but maintaining its transmission characteristics even when passaged through an intermediate species with a distinct PrP gene.[25] For example, transmission of CJD prions to conventional mice is difficult, with few, if any, inoculated mice succumbing after long incubation periods, which is consistent with a substantial species barrier.[30,42] In sharp contrast, transgenic mice expressing only human PrP are highly susceptible to CJD prions, with 100% attack rate and consistent short incubation periods that are unaltered by second passage, consistent with a complete lack of species barrier.[42] However, variant CJD prions (comprising human PrP of identical primary structure) transmit much more readily to wild-type mice than do classic CJD prions, whereas transmission to transgenic mice is less efficient than with classic CJD.[30] The term species barrier does not seem appropriate to describe such effects, and species-strain barrier or transmission barrier may be preferable. PrP aminoacid sequence and strain type affect the three-dimensional structure of glycosylated PrP that will presumably, in turn, affect the efficiency of the protein-protein interactions thought to determine prion propagation. Contribution of other components to the species barrier are possible and may involve interaction of cofactors that mediate the efficiency of prion propagation, although no such factors have yet been identified.

Mammalian PrP genes are highly conserved. Presumably only a restricted number of different PrP^{Sc} conformations (that are stable and can therefore be serially propagated) are permissible thermodynamically and constitute the range of prion strains seen. PrP glycosylation may be important in stabilisation of particular PrP^{Sc} conformations. Although a substantial number of different such PrP^{Sc} conformations may be possible among the range of mammalian PrPs, only a subset would be allowable for a given single mammalian PrP. Substantial overlap between the favoured conformations for PrP^{Sc} derived from species A and species B might therefore lead to easy transmission of prion diseases between these two species, whereas two species with no preferred PrP^{Sc} conformations in common would have a large barrier to transmission (transmission would necessitate a change of strain type). According to such a model of a prion transmission barrier, BSE may represent a thermodynamically favoured PrP^{Sc} conformation that is permissive for PrP expressed in a wide range of species, which would account for the remarkable promiscuity of this strain in mammals.

The species barrier between cattle BSE and human beings cannot be directly measured but can be modelled in transgenic mice expressing human PrP^{C}, which produce human PrP^{Sc} when challenged with human prions.[42] When such mice, expressing human PrP valine 129 (at high concentrations) and mouse PrP, are challenged with BSE prions, three possibilities may occur: the mice could produce human prions, murine prions, or both. In fact, only mouse-prion replication could be detected. Although there are caveats for this model, especially that propagation of human prions in mouse cells may be less efficient than that of mouse prions, this result would be consistent with the bovine-human barrier being higher than the bovine-mouse barrier for this *PRNP* genotype. In the second phase of these experiments, mice expressing only human PrP were challenged with BSE. Although CJD isolates transmit efficiently to such mice at around 200 days, only infrequent transmissions at more than 500 days were seen with BSE, consistent with a substantial species barrier for this human *PRNP* genotype. The *PRNP* valine 129 genotype was studied initially in attempts to produce an animal model of human prion disease, since this genotype was over-represented among early cases of iatrogenic CJD,[6] which suggests increased susceptibility or shorter incubation periods in this genotype. These studies should, however, be repeated in mice expressing only human PrP methionine 129 and in heterozygotes. So far, BSE seems to have transmitted only to human beings who have the *PRNP* codon 129 methionine homozygous genotype.

Epidemiology of variant CJD

The time and source of exposure to BSE of the current cases of variant CJD are not known. Nor do we know whether infection was acquired from a single high-dose exposure or as a result of accumulation of dose, risk, or both after low-level BSE exposure, perhaps over many years. Only limited experimental data are available on cumulative dose in prion diseases.[43] One patient with confirmed variant CJD was, however, a strict vegetarian from 1985 onwards (unpublished). Although it is possible that there was occult exposure to BSE prions via prepared or processed foods, pharmaceuticals, or cosmetics, for example, a more plausible explanation is that exposure predated the overt BSE epidemic. Epidemiological modelling studies support such a conclusion.[33] About 50 000 BSE-infected cattle are estimated to have entered the human food chain before recognition of the first clinical BSE case in 1986, which was before the 1989 ban on inclusion of specified bovine offals in the human diet.[44] This finding suggests an incubation period of longer than 11 years, which is in the region of the shortest incubation periods suggested by the estimates cited in this paper. If we were to conclude that these early cases of variant CJD resulted from exposure to preclinical BSE before onset of the overt BSE epidemic, it would be logical to conclude that many more cases must result from the much larger dietary exposure that followed.

The highest exposure period of the UK population to BSE was possibly around 1989–90, when the specified

bovine offals ban was being introduced and the incidence of BSE in cattle was still rising rapidly. The exposure of the population to BSE depended, however, not only on the BSE epidemic curve itself and the timing of the specified bovine offals and other statutory bans designed to keep exposure to BSE to a minimum, but also on the extent to which these bans were effective. The effect of such bans is dependent on the extent to which high-titre tissues, especially brain, were actually used for human food products before the ban; much of this material may have been rendered for animal feed. Although it could be argued that the current cases of variant CJD were exposed to BSE near the peak period of exposure, perhaps around 1990, incubation periods of the earliest variant CJD cases would have to be 5 years or less, which corresponds to the shortest reported incubation periods of kuru, for which transmission did not involve a species barrier. This possibility seems implausible and contradictory, since cases arising from earlier exposure to BSE would then be expected to have occurred. Shorter incubation periods imply a correspondingly smaller species barrier, which in turn implies a larger epidemic size for a given population exposure. Arguments for longer incubation periods in the current variant CJD patients, with apparently reassuring implications for the species barrier and attack rate, have to place infection at a very early stage of the BSE epidemic when exposure was low compared with subsequent exposure.

Another important consideration is that the affected individuals to date have had no history of an unusual dietary or occupational exposure to BSE.[28] Transmission of prion diseases is highly dose dependent, and inoculation is a more efficient route of transmission than is the oral route in laboratory animals. Therefore, if BSE had low pathogenicity for human beings and only a small epidemic occurred, the few individuals who contracted variant CJD might be expected to have had inoculation injuries with BSE-infected material (eg, abattoir workers) or an unusual diet with known exposure to brain or other high-titre tissues. Instead, the lack of such history is suggestive of one or more key environmental cofactors or something unusual about these individuals that gives them a high innate sensitivity to BSE. Such susceptibility could be an increased sensitivity to infection with the agent, a shorter incubation period after infection, or both. The unremarkable history of exposure to BSE among patients with variant CJD to date suggests that these susceptibility factors are more important than the degree of exposure. Susceptibility could be genetic or related to one or more cofactors. All patients with variant CJD analysed to date have been *PRNP* codon 129 methionine homozygotes (refs 45, 46, and unpublished data). All cattle studied are homozygous for methionine at the corresponding bovine codon.[47] About 38% of the normal white population are, however, of this *PRNP* genotype.

Studies of inbred lines of mice, encoding the same *Prnp* allele, but with various incubation periods to the same prion strain, suggest that other genetic loci affect the incubation period. The human homologues of these unidentified disease-modifier loci are likely to be relevant to BSE incubation periods in human beings. Possible cofactors that might facilitate infection by the oral route include buccal lesions and tonsil and gastrointestinal infection. Coexistent gut infection with nematodes may predispose to scrapie infection of sheep.[48] It has been long established in natural sheep scrapie and experimental rodent scrapie that early prion replication occurs in the lymphoreticular system, with detectable neuroinvasion much later in the incubation period.[49] In cattle, BSE infectivity is first detectable in the distal ileum around 6 months after exposure, consistent with infection in Peyer's patches.[50] PrP^{Sc} is detectable in lymphoreticular tissues from all patients studied to date with variant CJD, in sharp contrast to other forms of CJD, which suggests a prominent lymphoreticular phase in human infection with BSE prions.[46,51,52] Such a cofactor could be relevant to the unexplained age distribution of variant CJD, since children would be expected to have more frequent infections activating such lymphoreticular tissues that may facilitate access, replication, or both of BSE prions. By contrast, immunosuppression may be protective against prion disease.[53,54]

To date, epidemiological studies have not identified any environmental risk factors for variant CJD other than that of UK residence. Identification of particular foodstuffs that might have contained high concentrations of the BSE prion and have caused variant CJD is severely hampered by lack of knowledge about the distribution of BSE-infected tissues in food products and the extreme difficulty of obtaining accurate and detailed dietary histories from years previously in anyone, let alone those with severe cognitive impairment. However, some hypothesis-driven questions could be pursued, for example, about histories of specific infectious diseases (that result in activation of the lymphoreticular system of the tonsils, gut, or both—or severe leucocytosis) and gastrointestinal disorders (that might affect gut permeability to prions) during the peak exposure of the population to BSE. These questions will also be challenging to an epidemiological approach, and experimental studies to confirm such cofactors in laboratory animals may be the only realistic way to address such hypotheses.

Finally, transmission studies in transgenic mice expressing human PrP valine 129 have suggested that transmission of BSE to human beings with the *PRNP* codon 129 valine homozygous genotype (and possibly heterozygotes) might lead to a clinical syndrome distinct from variant CJD, since a different PrP^{Sc} type (designated type 5) was produced on transmission of variant CJD to such animals.[30] It is unknown whether type 5 human prions would produce another new disease, such as variant CJD, which was quite distinct and easy to differentiate from classic CJD on clinical and pathological criteria, or produce a phenotype indistinguishable from classic forms of CJD. In the latter situation, such cases could still be distinguished by molecular-strain typing. No such cases have yet been reported.

Remaining routes of transmission of BSE and variant CJD

The substantial extension of measures to limit dietary exposure to BSE prions in March, 1996, especially the 30-month rule (whereby only animals younger than this age can be used for human foodstuffs), allied with the continued decline in the UK BSE epidemic, should have ensured that any cattle BSE entering the human diet is kept to a minimum, if significant at all, compared with earlier exposure. It is still theoretically possible, however, that BSE could have been transmitted to other

agricultural species via contaminated feed. The possibility that BSE may have been transmitted to, and has (like scrapie) become endemic in, sheep has caused concern. BSE that has been transmitted to sheep is clinically indistinguishable from scrapie and can be detected only by strain typing. Molecular strain typing for sheep, which seems feasible in preliminary studies[26] and which, unlike classic strain typing, could be applied rapidly to thousands of cases, has not yet been assessed in large-scale studies. Other theoretical concerns are that BSE infectivity could be present in cattle in a subclinical form and that subclinical transmission of BSE to other species might have occurred.[55,56] These possibilities could be investigated by screening brain tissue for PrP^{Sc} from relevant animals slaughtered for human consumption and from older animals in systematic studies.

Concern has arisen that blood and blood products from donors incubating variant CJD may pose a risk for iatrogenic transmission of the disease. Reports of infectivity of blood from patients with classic CJD are infrequent and have been questioned.[57] Infectivity of blood from patients in the clinical phase of variant CJD is unknown. PrP^{Sc} is, however, consistently found in the lymphoreticular system of patients who have variant CJD,[46] and lymphocytes express significant concentrations of PrP^{C};[58] in mice, B lymphocytes (although not necessarily expressing PrP^{C}) are required for prion neuroinvasion after peripheral inoculation.[59–61] UK policy is to move to routine leucodepletion of all whole blood, a practice already in use (for other health reasons) in some countries, and to source plasma for plasma products from outside the UK. The experimental basis for the latter action is less clear.

A further possible route of transmission of variant CJD is via contaminated surgical instruments. Iatrogenic transmission of classic CJD via neurosurgical instruments has been reported and normal hospital sterilisation procedures are not likely to completely inactivate prions. There is evidence that classic CJD may also be transmitted by other surgical procedures.[63] Although all surgical instruments used on patients with suspected CJD are quarantined and not reused unless a non-prion diagnosis is unequivocally confirmed, the extensive lymphoreticular involvement in variant CJD, which is probably present from an early preclinical stage, raises the possibility that instruments could be contaminated, especially during procedures that involve contact with lymphoreticular tissues. Common such procedures are tonsillectomy, appendicectomy, and lymph-node and gastrointestinal biopsy.

At present, the risks from blood and blood products and from contaminated surgical instruments cannot be quantified. The decision is whether to act now (and to what extent) to lessen any risk of iatrogenic prion transmission, how to balance such action against the substantial financial costs that may be involved, and the introduction of new risks from the alternative measures, or, at the other extreme to do nothing until sufficient data are available to guide a formal risk; benefit analysis has to be a political decision. Variant CJD can clearly be diagnosed in the appropriate clinical context by tonsil biopsy,[46] and PrP^{Sc} is probably present in tonsil and other lymphoreticular tissues from an early preclinical stage. Calculation of prevalence estimates of preclinical variant CJD may therefore be possible by anonymous analysis of routine tonsillectomy or appendicectomy samples, and several such studies are in progress. Clearly, a reliable, sensitive, blood-based diagnostic test to detect preclinical variant CJD would be invaluable. Current methods are not yet sufficiently sensitive to detect PrP^{Sc} in blood. The antibodies to PrP used do not distinguish between PrP^{C} and PrP^{Sc} and, therefore, a protease pretreatment step is needed. In addition, protease resistance is an uncertain marker of infectivity, and some prions may be less protease resistant than others. Some prion diseases have little or no detectable PrP^{Sc}.[5,64,65] Specific antibodies to PrP^{Sc} may offer routes to more sensitive detection methods. The only antibody reported to date to have such specificity, an IgM antibody designated 15B3,[66] has not yet been shown to offer any diagnostic advantages over existing methods. The development of high-affinity conformation-specific IgG antibodies remains an important goal. Another possible approach is in-vitro amplification of PrP^{Sc}, in which, ideally, PrP could, under suitable conditions, be seeded with tissue or tissue extracts from patients. Current in-vitro models of prion propagation are, however, of uncertain validity[67] and are highly inefficient (requiring a large molar excess of PrP^{Sc} to convert PrP^{C}).[68] The availability of highly purified, recombinant-derived, soluble β-PrP may offer new avenues for production of conformation-specific antibodies and in-vitro amplification systems for prion detection.[13] Sensitive diagnostic methods will be required not only for large-scale prevalence studies of preclinical variant CJD and to exclude blood and other tissue and organ donations from preclinical cases, but also to be able to reassure the "worried well". The earliest symptoms of variant CJD are ill-defined peripheral sensory disturbances, depression, and anxiety, and clear evidence of an emerging epidemic of variant CJD might lead many to seek medical advice and reassurance.

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