Transmissible spongiform encephalopathies: transmission, mechanism of disease, and persistence

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Prion protein is central to the control of development of all transmissible spongiform encephalopathies. Controversy exists as to whether the protein itself is responsible for disease manifestation, in one of perhaps several isoforms, or whether an additional informational molecule must be involved in conjunction with the protein. Recent studies have been trying to resolve these issues.

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Abbreviations

BSE	bovine spongiform encephalopathy
CSF	cerebrospinal fluid
CJD	Creutzfeldt–Jacob disease
CNS	central nervous system
LRS	lymphoreticular system
PrP	prion protein
PrP^{c}	protease-sensitive PrP
PrP^{Sc}	protease-resistant PrP
TSE	transmissible spongiform encephalopathy
vCJD	'new variant' CJD

Introduction

The group of diseases known collectively as transmissible spongiform encephalopathies (TSEs) includes, in animals, natural scrapie of sheep and goats, bovine spongiform encephalopathy (BSE), feline spongiform encephalopathy, chronic wasting disease of elk and mule deer, and transmissible mink encephalopathy (TME). In humans, TSEs include kuru, Gerstmann–Staussler–Scheinker syndrome (GSS), fatal familial insomnia (FFI) and Creutzfeldt–Jacob disease (CJD), of which there are sporadic, iatrogenic and new variant forms. The prion protein (PrP) is central to the control of development of all TSEs. All TSEs are associated with a conformationally altered form of the normal protease-sensitive host protein, PrP^{C}. In diseased individuals the altered PrP^{C} becomes protease resistant (known as PrP^{Sc}) and as such is a TSE-specific marker. PrP^{C} exists in many variant forms in different species and within the same species. Transgenic mouse studies have shown that polymorphic and mutated forms of the PrP gene and protein apparently control both host range of TSEs and incubation period of experimental disease, and mice that lack a functioning PrP gene are completely resistant to scrapie [1].

The causal agent of TSEs remains the subject of much controversy. Two contrasting theories have been formulated to try to explain how these diseases arise. The inability to recover any infection-specific nucleic acid from TSE preparations and the extreme resistance of TSEs to treatments that usually destroy nucleic acids led to the postulation of the prion hypothesis. This hypothesis maintains that TSEs are 'protein only' in origin with PrP^{Sc} as the causal agent, which acts as a seed in the continuous conversion of healthy PrP^{C} into the disease-associated PrP^{Sc} and thus apparently replicating infectivity. One of the major drawbacks for this idea is the need to explain how a protein molecule can specify the biological properties of different TSE strains; however, strain characteristics may be 'encoded' by the conformation and/or the level of glycosylation of PrP. The other major hypothesis centres on a more conventional virus-like agent, called a virino, with its own replicating informational molecular structure allowing it to develop different strains of disease and occasionally to change characteristics by mutating. The virino would be closely associated with PrP^{Sc}, which would protect it from a range of nucleic acid deactivating procedures. Neither of these hypotheses, however, has been entirely proved or refuted.

The purpose of this review is to deal briefly with some of the more recent findings surrounding the transmission of TSEs and to assess some of the mechanisms that are involved with their pathogenesis. We will also relate some of the issues surrounding the controversy of the nature of the TSE agent.

Transmission

Bovine spongiform encephalopathy

One of the best known of the TSE diseases is natural scrapie of sheep, which has been present in the UK for 250 years. It now seems most probable that this disease has led to the outbreak of BSE as a result of changes to the manufacturing processes of animal feed, whereby ruminant remains were rendered into feed [2]. Attempts in the US to mimic a BSE-like condition in cattle by inoculating them with brain homogenate from cattle which had been experimentally infected with natural scrapie from American sheep have proved difficult to interpret [3]. It is possible, however, that the scrapie which gave rise to BSE in the UK may have had quite different transmission properties to the US scrapie source used in these experiments. The BSE epidemic is undoubtedly on the wane, although whether it will ever be completely eradicated from the UK national herd depends on its natural means of disease propagation, such as maternally [4] or genetically [5] linked risk factors,

playing a significant role in maintaining a reservoir of infection.

The cost of BSE to the agricultural sector of the UK economy is widely recognised. A much greater cost is becoming clear, with BSE apparently having spread to the human population as a result of their dietary exposure.

Creutzfeldt–Jacob disease

'New variant' CJD (vCJD) has been recognised since March 1996 by its distinctive pathological features in the brains of affected people [6]. The most convincing evidence that vCJD is caused by the same agent that caused BSE in cattle came from transmission studies in which brain recovered from vCJD victims was inoculated into inbred laboratory mouse strains [7••]. This reproduced the pattern of disease characteristics in the mice that was previously thought to be unique to that produced by BSE in mice. Classic, or sporadic, CJD transmits to mice in a completely different manner. Biochemical analysis of PrP^{Sc} protein glycosylation patterns apparently confirmed the similarity between BSE and vCJD [8], and *in vitro* studies showed that human PrP^{C} (protease-sensitive) could be converted into the protease-resistant isoform by adding PrP^{Sc} from scrapie sheep and BSE cattle, albeit at a much lower frequency than homologous systems [9•].

As in mice, sheep and goats, the human PrP gene seems to control TSE susceptibility. Humans have a common polymorphism at codon 129 of their PrP gene, which encodes either valine (V) or methionine (M). So far only individuals with homozygous MM genotypes, have featured as vCJD cases [10]. Sporadic CJD occurs most often in individuals with either homozygous MM or VV genotypes, with MV appearing to give some protection from the disease. It may follow that if, in the future, vCJD develops in people with either VV or MV genotypes, the incubation of disease could be much longer than in individuals carrying MM and, indeed, may have different clinical symptoms. The iatrogenic spread of sporadic CJD is well documented among recipients of human growth hormone therapy some years ago [11]. Other routes of transmission have been emerging for some time, with recent cases occurring after cadaveric dura mater grafting [12] and corneal transplantation [13]. Many of these infected individuals are homozygous at codon 129 (either MM or VV) of the PrP gene.

As with all TSEs early diagnosis is particularly important. Recognition of the disease isoform PrP^{Sc} by Western blotting and immunohistochemistry on tissue of a tonsil biopsy from a vCJD patient has proved possible [14] and is a relatively noninvasive technique compared to brain biopsy, which can produce spurious results [15]. PrP^{Sc} has not been found so far in sporadic CJD tonsil biopsies and so immunohistochemistry on archived human tissue is now being used to confirm indeterminate diagnosis [16].

The prospect of therapeutic intervention in human TSE is still speculative even though considerable evidence is accumulating that chemicals such as Amphoteracin-B in hamsters and selected polyanions in mice can significantly prolong the onset of clinical experimental scrapie [17]. The chemical therapy was administered to the rodents around the time of experimental challenge and this may be crucial [17]. In humans the timing of infection is often unknown and once symptoms appear it is not yet possible to reverse them.

Natural scrapie

A recurrent area of debate in TSE research is whether natural scrapie in sheep is an infectious condition governed by alleles of the host PrP gene, or whether it is purely a genetic disease. Investigations of the ovine PrP gene have identified sheep with highly susceptible genotypes, closely linked with the development of natural scrapie: $VV_{136}RR_{154}QQ_{171}$ and $VA_{136}RR_{154}QQ_{171}$ in Cheviot sheep, and $AA_{136}RR_{154}QQ_{171}$ in Suffolk sheep. (The single letter amino acid designation is as follows: A is alanine, Q is glutamine, R is arginine and V is valine; the subscript refers to codon number.) Sheep of these genotypes exist in the scrapie-free countries of Australia and New Zealand, and can also be reared in the UK well past the normal age of death from scrapie (2–3 years) [18•]. The implication is that natural scrapie is not a genetic disease but requires some form of infectious agent in addition to the appropriate PrP genotype in order to develop. The incidence of natural scrapie in the UK is largely unknown. In addition to the genotypes mentioned above, a recent study has shown sheep of the PrP genotype $VA_{136}RR_{154}QR_{171}$ can sometimes develop disease [19] but it is not known if apparently healthy and resistant animals can act as subclinical reservoirs or carriers of infection.

If attempts to eradicate scrapie are to be successful some sort of 'early warning' system will have to be found so that sheep incubating the disease can be targeted. To achieve this at least two elements are required: firstly, knowledge of the PrP genotype, and secondly, a means to identify the disease before the appearance of clinical signs. The spleen, as an integral part of the lymphoreticular system, is also known to harbour PrP^{Sc} in selected experimental sheep scrapie models [20]. Tissues more amenable to biopsy are also being investigated; for example, in sheep incubating scrapie, PrP^{Sc} was detected by immunohistochemistry in biopsied palatine tonsil well before clinical signs appeared [21].

The availability of an antibody specific for the scrapie isoform of the PrP (see below) will aid diagnosis of TSE [22••]. Further work with cattle with BSE [23] and with a CJD patient [24] has linked the cerebrospinal fluid (CSF) protein 14-3-3 with disease, although similar studies have not yet been extended to sheep with scrapie. Another CSF protein, S-100, is also known to exhibit significantly raised levels in CJD patients [25].

Mechanism of disease

PrP^C and PrP^{Sc}

The involvement of PrP^{Sc} in TSE pathogenesis has been known for a number of years. The function of the normal protein PrP^C is not understood, although some studies in mice suggest roles in neuronal electrophysiology and the regulation of sleep patterns [26]. A good deal of work on the genesis of PrP^{Sc} from PrP^C has been undertaken, not only in tissue culture and cell-free systems (reviewed in [27••]) but also *in situ* using murine brain slices [28], in order to try to understand the mechanisms involved in the conversion and perhaps, in future, to interfere with the process therapeutically.

The tertiary structure of PrP is thought to vary between the normal form (PrP^C) and the scrapie isoform (PrP^{Sc}), with the former comprising mostly an α-helix state and the latter predominantly β sheets [29]. Nuclear magnetic resonance (NMR) spectroscopy of the PrP has refined the perception of its molecular architecture [30•]. The PrP molecule has two glycosylation sites, which can produce different banding patterns on polyacrylamide electrophoresis gels. The ratios of the intensity of these bands, or glycoforms, can apparently be specific to, and therefore perhaps diagnostic of, different strains of TSE, such as BSE [31]. Other studies using various sources of scrapie and BSE-derived material, however, have given a much more complex picture and do not support these conclusions [32•]. The potential of the glycosylation sites in the specification of TSE strain characteristics is also being tested in transgenic mice [33].

The essence of the prion hypothesis is that PrP^C is changed into PrP^{Sc} with no nucleic acid-like molecular intervention, and that the particular structure of PrP^{Sc} can result in different patterns of disease. This particular structure would be maintained by the PrP^{Sc} acting as a template in the conversion of more PrP^C. Transgenic mice with PrP genes containing patched-together mouse and hamster PrP gene sections (chimeric transgenes) have shown that there are distinct, species-specific sites along the PrP^C protein molecule, which are important in allowing hamster scrapie to infect mice. It is at these species-specific sites that invading PrP^{Sc} could interact with PrP^C in the host animal and this could help to explain the species barrier to infection [34].

Strains of TSEs

Detailed studies at the Neuropathogenesis Unit, Edinburgh over many years have provided evidence of discrete strains of TSE [35]. The properties of these strains, which include length of incubation period of disease and patterns of brain degeneration, are highly reproducible and depend on the *Sinc* (scrapie incubation period) genotype, also known as *Prni*, of the mouse strain used for transmission. It is now known that the *Sinc*/*Prni* gene and the *Prnp* gene, which denotes the gene producing PrP protein in mice, are one and the same [36•]. PrP therefore controls the incubation period of the disease as well as the species barrier. The existence of strains is not disputed by the prion hypothesis, which suggests strains are generated through different tertiary structures of the PrP^{Sc} protein [37] and variations in its glycosylation. The prion hypothesis concedes, however, that the PrP protein by itself may not be sufficient to explain strain diversity and has implicated protein X, probably a chaperone molecule, as the means by which disease characteristics are controlled [38•]. On the other hand, the virino hypothesis suggests the conventional involvement of a nucleic acid and is viewed as a plausible explanation for the identification of discrete strains of scrapie isolated in laboratory rodents [39,40]. Unfortunately for the virino supporters no trace of a candidate scrapie-specific nucleic acid or equivalent molecule has ever been discovered. By the same token, infectivity has never been generated *de novo* from PrP^C. It is interesting that there are precedents of proteins apparently transmitting heritable characteristics in yeast [41].

Spread of disease

The experimental infection of laboratory rodents with scrapie is normally achieved by intracerebral inoculation, except in PrP-null mice, which lack a functioning PrP gene and in which no infection is generated [42]. Even when tissue from PrP-expressing ($PrP^{+/+}$) mice is neurografted into the brains of PrP-null mice and the grafts infected with scrapie, histological scrapie changes only occur in the graft tissue and do not penetrate surrounding PrP-null tissue [43]. This demonstrates that PrP-expressing cells must be present for infection to be initiated. The $PrP^{+/+}$ grafts in PrP-null mice do not become infected even after the animals have been reconstituted with PrP-expressing lymphohaemopoetic stem cells [44]. An intermediate tissue or cell type which is unaffected by reconstitution is therefore implicated in the transport or processing of infectivity between the lymphoreticular system (LRS) and central nervous system (CNS). Studies in severely combined immunodeficient (SCID) mice suggest that follicular dendritic cells may be involved [45], although other results would seem to indicate that it is differentiated B cells that are of major importance in the neuroinvasive process following peripheral challenge [46••]. These conflicting results may be explained by the different scrapie strains used by each research group, targeting different cells in the LRS in a similar way to those targeted in the CNS. The additional important question of the possibility of transmission of TSE infection in blood remains unanswered [47].

However infection reaches the CNS, the mechanism for neuroinvasion of infectivity is becoming clearer following experimental studies in hamsters, which show that after intraperitoneal inoculation infectivity enters the spinal cord at specific thoracic vertebrae [48•]. An alternative route bypassing the spinal cord and entering the brain at

the medulla oblongata by way of the dorsal vagus nerve is also implicated in the spread of infection [48•].

With regard to the naturally occurring TSEs, the oral ingestion of infected material is considered one of the most probable routes of transmission. This long-held contention has been supported recently by the positive transmission from experimental oral challenge of scrapie in sheep [49], and also with BSE in cattle [2]. In the United States, a scrapie-like disease of unknown aetiology has emerged in free-ranging deer and elk; this may also be an example of naturally occurring oral transmission [50].

Persistence of infection

Following the ban on feeding meat and bone meal to ruminants and following the selected cull of cattle over thirty months of age, any reservoir of BSE infection in the UK cattle population must be declining rapidly [51]. We must also recognise the possibility, however, that BSE may be persisting as a very low incidence endemic disease of cattle and that it may also have entered the UK national sheep flock. It is impossible as yet to make definitive statements about either of these suppositions, nor for that matter to affirm that BSE could persist as a covert infection never to manifest as a clinical condition in the lifetime of the animal. There is evidence of such a carrier state in ruminants; however, the definitive experiments are only now being carried out.

Nothing is known about the possibility of infective material being excreted in waste from animals harbouring BSE. Evidence from experimental and epidemiological studies of sheep scrapie are not supportive of this as a means of transmission, although placentae recovered from parturient sheep infected with scrapie can transmit disease to other sheep by the oral route. BSE appears to be limited to the CNS in naturally infected cattle, unlike sheep scrapie, which has been found in many tissues [21]. BSE infectivity can, however, be isolated from spleens of sheep experimentally infected with BSE [52], suggesting that BSE in sheep may have a similar pathogenesis to scrapie in sheep.

Conclusions

The appearance of BSE in cattle and more importantly its apparent zoonosis as vCJD in humans has shown dramatically how important the group of TSEs has become. Strenuous efforts are being made to establish diagnostic tests so that the disease can be identified during the preclinical stages. These are concentrating on the identification of the disease-associated form of the PrP protein, PrP^{Sc}. The use of genetic markers, which are particularly relevant to natural scrapie in sheep and are linked to disease susceptibility/resistance, is already helping to lower the incidence of disease by enabling us to select resistant animals. This may not, however, eradicate subclinical infection.

In humans the progression of disease as vCJD is still uncertain. Measures have been taken to minimise as far as possible the likelihood of BSE-infected material entering the food chain. The great unknown is the number of people who could already be incubating the disease before their clinical presentation and whether these individuals could contribute to the epidemic through, for example, blood transfusions. In the meantime current research is indicating some possible areas of therapy, such as PrP gene ablation and chemical disaggregation of PrP^{Sc}. The uncertainty lies in extrapolation from experimental animal models of the disease to the human situation.

Finally, the debate over whether prion diseases have an aetiology entirely related to the PrP or whether replicating informational molecules are also involved may remain unresolved until, for example, a transmissible disease can be created *in vitro* by manipulation of the normal form of the PrP or the additional molecular component can be isolated.

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