Prions: Quisling Proteins

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In the midst of the current BSE scare veterinarians are being called on for their opinion. In order to give it, members of the profession must first understand the etiology of BSE and the possibility of its transmission to other species.

What are transmissible spongiform encephalopathies?

The transmissible spongiform encephalopathies (TSE) are a group of neurodegenerative diseases that constitute a subset of what were called slow diseases. TSE comprise a complex group of human and animal syndromes (Tables 1 and 2) that includes genetic, infectious and sporadic forms. The common characteristics are spongiform (vacuolar) degeneration of neurons, reactive glial changes, variable amyloid plaque formation in the C.N.S., transmissibility to experimental animals and very prolonged incubation periods (months, years), whether the diseases are acquired naturally or experimentally. **In recent years it has emerged that another reason for classifying them together is because the aetiology and the pathogenic mechanisms that lead to clinical signs in the various diseases are related to modifications of a single cellular protein called the prion protein (PrP).**

Table 1. Transmissible spongiform encephalopathies in humans

Disease	Aetiology	Source
Kuru	Infection	Ritual cannibalism
Creutzfeldt-Jakob disease (CJD)		
Iatrogenic	Infection	Injection of pituitary extracts Contaminated neurosurgical instruments Corneal transplants
Sporadic	Unknown	
Familial	Mutation of prion gene	
Gerstmann-Sträussler-Scheinker Syndrome (GSS)	Mutation of prion gene	
Fatal familial insomnia	Mutation of prion gene	

What is a slow disease?

The concept of slow diseases was introduced in 1954 by Sigurdsson, an Icelandic veterinarian, who observed that diseases that run a protracted course can be divided into two broad groups: chronic diseases and slow diseases. The course of a chronic disease tends to be irregular and is unpredictable. In contrast, a slow disease has a long latent period that may last several months to several years but, once the clinical signs appear, the disease progresses inexorably, albeit slowly, to its predictable termination in death. For instance, scrapie in sheep is the prototypic slow disease that manifests as a TSE,: it has a latent (incubation) period of from several months to two years and a clinical course of from two to 12 months. Although the time scales are somewhat different, this pattern is retained when the scrapie agent is transmitted experimentally across the species barrier into susceptible laboratory animals.

Table 2. Transmissible spongiform encephalopathies of animals

Disease	Natural Host
Scrapie	Sheep, goats
Bovine spongiform encephalopathy (BSE)	Cattle
Feline spongiform encephalopathy (FSE)	Cats, puma, cheetah
Transmissible mink encephalopathy (TME)	Mink
Chronic wasting disease	Mule deer, elk
Exotic ungulate encephalopathy	Eland, oryx, nyala, kudu, gemsbuk.

What is the species barrier?

Scientists who tried to transmit slow diseases to new host species found that it was not always possible, that the range of susceptible species was very small, and that

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the initial trans-species passage required a very extended incubation period which was reduced significantly on further passage between members of the new host species. These experiences fostered the idea that there is a species barrier to the spread of slow diseases.

So, the concept of a species barrier is of theoretical interest rather than of practical importance?

On the contrary, it is a major safeguard against a cataclysmic upsurge in the prevalence of human TSE. In the past, it has protected the consumers of mutton from the scrapie agent which we know has been active in sheep for more than two centuries, at least. More recently, it appears to have protected the pig from the infective agent acquired by cattle from contaminated meat and bone meal, a product that was fed to both species and which, in fact, was consumed in proportionately greater quantities by pigs. Experimentally, it has been possible to breach the barrier by injecting massive quantities of infectivity directly into the pig's brain but not by administering the same dose by the oral route; in other words, the barrier was almost absolute and it was breached only in quite extraordinary circumstances that would never occur in nature.

What is the transmissible agent in scrapie?

The precise nature of the agent has not been elucidated. There is good evidence for the existence of different "strains" that exhibit different incubation periods and produce different histopathological patterns when transmitted to susceptible laboratory animals. Historically, three likely candidates have been proposed: virus, virino, prion.

The transmissible agent can pass through a filter with pores small enough to retain all but **viruses**. However, it is not inactivated by procedures that normally destroy viruses (boiling, ionising or ultraviolet radiation, etc) and efforts to find a virus have not been successful.

A **virino** is a composite structure that contains a host protein and a host-independent molecule (possibly a small nucleic acid). In this model the nucleic acid determines the strain of the agent while the host protein (probably prion protein) provides a coat that protects the nucleic acid from the immune system of the host and from various degradative processes. However, a nucleic acid has not been found in infective material; moreover, infectivity is not reduced by nucleases or by ionising radiation.

Prion (pronounced "preeon" by Prusiner, who baptized it) is an acronym for a "*pro*teinaceous *in*fectious particle which resists inactivation by procedures which modify nucleic acids". The infectious particle (designated scrapie prion: PrP^{Sc}) is a modified form of a normal cell-surface protein (designated cellular prion: PrP^{c}) that is attached to the outer surface of neurons, glial cells and, to a lesser extent, lymphocytes. Apparently, the helical backbone of the cellular prion switches to beta-strands in PrP^{Sc}. The β-strands tend to aggregate into protease-resistant sheets that constitute the scrapie-associated fibrils (SAF) seen when amyloid plaques develop in the brain of affected animals. According to the prion model, the infective agent propagates itself by contacting normal PrP^{c} molecules on which it "imposes" its own conformation (Figure 1). This change initiates an exponential cascade of conversion to the infective form that will not stop until the host cell dies. Nowadays, there is an acceptance that prion protein is associated with the events that lead to frank TSE but opinion is divided as to whether or not the prion proteins is **the** infectious agent. Those who have doubts point out that the prion model does not explain why different strains of the scrapie agent are associated with different incubation periods and different pathological patterns in the same inbred strain of mouse (that encodes a single amino acid sequence for its PrP^{c}). Nevertheless, it is evident that the disease cannot progress without the involvement of the host PrP^{c}. Recently, this has been confirmed by experiments in mice in which the gene for the prion protein had been inactivated; they were resistant to mouse-adapted scrapie.

Does the prion model take account of the species barrier?

As mentioned, the nub of the prion hypothesis is the ability of the infective prion (PrP^{Sc}) to induce a cascade of conversion of newly-synthesized host cellular prion protein (PrP^{c}) to the PrP^{Sc} conformation. Cross-species changes in confirmation are difficult to achieve. It is known that the conversion is most efficient when the amino acid sequences of the PrP^{c} and PrP^{Sc} are identical. Transmission across the species barrier demands interaction between two prion proteins with non-identical amino acid sequences. Heterogeneity between PrP^{c} and PrP^{Sc} at particular locations in their amino acid sequences prevents the conformational change and endows the host with an absolute species barrier to that particular strain. It is only when the infective prion is able to impose its will on the host cellular prion that the species barrier is crossed and if that is to happen it usually requires a prolonged incubation period to induce the necessary conformational change. It has been suggested that the shorter incubation time on further passage within the new host species is facilitated by convergence of the amino acid sequences of PrP^{c} and PrP^{Sc}.

Is the prion model compatible with the familial and sporadic forms of TSE?

Yes. The hypothesis is that in the sporadic forms of the diseases (e.g., CJD, GSS, natural scrapie) the self-replicating conversion of cellular prion protein is triggered by a rare chance that produces the first PrP^{Sc} in a host that has a genetic susceptibility to prion dis

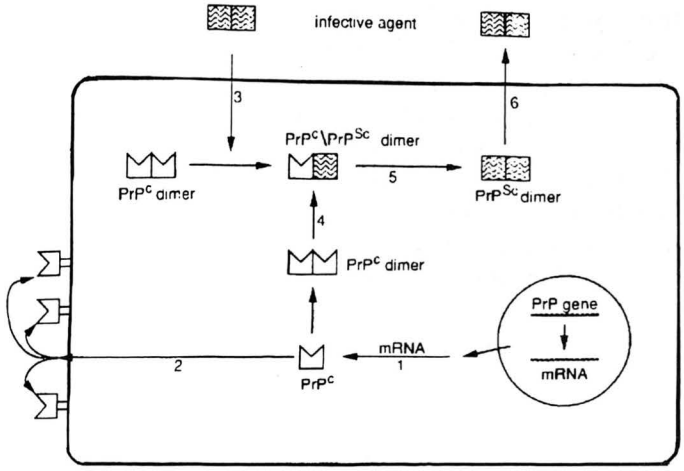

Figure 1. Depiction of the Prion Model - The PrP gene is responsible for the production of a normal cellular prion (PrP^c) which becomes anchored to the outside of the plasma membrane of a neuron or a glial cell (1,2). Its function is not known (proponents of the virus and virino models suggest that it may act as a receptor for the infective agent: virus or virino, respectively). When an infective prion enters a cell (3) it contacts a PrP^c dimer in the cell interior, forms an PrP^c/PrP^{Sc} heterodimer, and then induces a conformational change in the PrP^c component (4) to yield a PrP^{Sc} homodimer (the infective agent: 5,6). The conformational change is always a post-translational event (i.e., it always occurs after the synthesis of PrP^c). A rare chance conformational change in PrP^c yields the first PrP^{Sc} that initiates the cascade (4,5,6) that leads to the sporadic form of TSE, while a point mutation or deletion in the PrP^c gene is responsible for a similar chain of events in familial TSE.

ease. There are several familiar syndromes in humans (variants of CJD, GSS) that have been attributed to specific mutations in the prion protein gene (Table 1). It is believed that the mutations cause the diseases rather than being responsible for susceptibility to an exogenous transmissible agent.

How are TSE transmitted in nature?

The recognized modes of transmission are:

(a) genetic - in an autosomal dominant pattern in human families that carry certain mutations in the prion gene: CJD, GSS

(b) from infected mother to offspring: scrapie

(c) by cannibalism: kuru

(d) by contaminated feed: TME, BSE, FSE, TSE of exotic ungulates etc.

In recent times scepticism has been expressed about the perceived wisdom that scrapie is maintained in the sheep population largely by maternal transmission from infected dam to her lambs and, via infected placental tissues, to other members of the flock. There has been no unequivocal evidence of maternal transmission in any of the other TSE. The possibility of iatrogenic transmission should not be overlooked. There have been human cases of CJD that have been traced to the use of material from cadavers (e.g., growth hormone from the pituitary gland; dura mater used for surgical repair) or the failure of routine sterilization procedures to decontaminate instruments used in neurosurgery.

What decontamination procedures are effective against the transmissible agent?

The agent is resistant to boiling, ultraviolet light, ionising radiation, fixation in formalin, formaldehyde or glutaraldehyde, domestic detergents, iodophores, hydrogen peroxide or routine autoclaving at 121°C for 15 minutes. The recommended cycle for surgical equipment is 134°C at 30lbs psi for 18 minutes (or six cycles of three minutes each).

The disinfectant of choice is sodium hypochlorite containing 20,000 ppm available chlorine for at least one hour, with the proviso that on open surfaces repeated wetting is necessary over the treatment period.

Which tissues from clinical cases are infective for other animals?

Until very recently one could feel confident in citing literature that stated that milk, meat, blood, faeces and urine from clinical cases were either not infected at all or only to a level that posed no risk of transmission of the disease. On the other hand, there was ample evidence to incriminate nervous and lymphatic tissues. This was particularly evident in kuru, which was acquired from those tissues by women and children while the adult males, who feasted on muscle, escaped the disease. The recent report of a cluster of CJD cases in Britain with a putative link to BSE may (or may not) raise doubts about the validity of the perceived wisdom. In general, it is believed that the following organs are likely to have the highest level of infectivity: brain, spinal cord, spleen, thymus, gut-associated lymphoid tissue (Peyers patches), peripheral lymph nodes, pituitary gland, dura mater, eye. **Tissues regarded as unlikely to carry infectivity are: muscle, kidney, skin, milk, semen, faeces, urine and saliva.**

The Importance of a Species Barrier

The species barrier concept is of practical importance in assessing the risk for humans from consumption of animal products that might contain the scrapie agent.

It is instructive to consider the background to two major "outbreaks" of TSE within the past 40 years: kuru in the stone-age Fore tribe in Papua-New Guinea and BSE in cattle in Britain. Kuru was restricted to the Fore

people, amongst whom it was transmitted orally (and, perhaps, transcutaneously) during ritual cannibalism of their dead. It is suspected that the infective agent was bequeathed to the tribe by a victim of sporadic CJD: thus, the agent was not restrained by a species barrier and it caused a major problem amongst the tribe. There is strong anecdotal evidence that sporadic cases of what later became known as BSE had occurred in Britain for several years before the disease was identified as a specific clinical entity. Since meat and bone meal was derived from both cattle and sheep carcasses, it is possible that it contained a cattle-adapted agent whose propensity to cause spongiform encephalopathy was not restrained by the species barrier. Thus, in both diseases the victims did not have protection provided by a species barrier and the exposure to the infective agent was facilitated by cannibalism-ritualistic amongst the Fore people, enforced in British cattle - which, in effect, serially passaged the agent within the same species.

A Comparison of Methods of Assessing Copper and Selenium Status in Cattle in the Field

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Abstract

Clinical copper deficiency in cattle is common throughout the United Kingdom where there are significant intakes of molybdenum and sulphur and insufficient copper. Several methods for the assessment of copper status have been advocated over the years. These have included the measurement of plasma, serum or liver copper concentrations or the assessment of a number of copper containing enzymes. Selenium deficiency is also a common problem, but unlike copper, there are no dietary interactions that affect the assessment of selenium status in cattle. In an extensive study from September 1994 through to September 1995 a total of 1571 blood samples were obtained from cows that had possible trace element deficiencies. These animals comprised dairy cows at various stages of lactation and suckler cows. In this study the copper parameters measured were plasma copper (PI-Cu) and TCA-soluble plasma copper (TCA-Cu) concentrations, erythrocyte superoxide dismutase (SOD) and serum caeruloplasmin (CP) activities. Selenium status was measured by the activity of the erythrocyte selenoenzyme glutathione peroxidase (GSHPx). The results are expressed as the distribution of the population according to whether the copper status could be below 8 μmol/l, marginal was 8-12, normal was 12-23 and toxic was anything over 23 μmol/l. Based on this distribution of PI-Cu concentrations, 3.2% of cattle were classified as deficient, 20.7% marginal, 74.7% normal and 1.4% as toxic. The TCA-Cu concentrations showed a similar pattern with 3.5% deficient, 22.7% marginal, 72.5% normal and 1.3% toxic. The plasma copper enzyme, caeruloplasmin indicated that 11.0% of cattle were deficient whilst 89.0% were normal. The erythrocyte copper enzyme, SOD, showed a similar distribution with 2.3% of cattle being classified as deficient and the rest being in the normal range. However, when the copper status was expressed as the ration CP/PI-Cu as described by Telfer *et al.* (1996), only 28.6% of cattle were regarded as being adequate and not requiring supplemental copper. Selenium status as measured by GSHPx activity showed that 28.3% of cattle were deficient in selenium whilst the rest were all in the normal category. These results show that copper status as measured by the current conventional methods indicates an adequate status in 75% of the cows. However, when status is based on the CP/PI-Cu ratio this diagnosis is completely different with just 25% of the cows being adequate. Feedback from veterinary surgeons is indicating that copper supplementation of those cows diagnosed as inadequate by the CP/PI-Cu ration have shown clinical responses to copper supplementation.

KEYWORDS: Copper, selenium, diagnosis, caeruloplasmin, superoxide dismutase, glutathione peroxidase

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