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Review

Scrapie pathogenesis in brain and retina: Effects of prion protein expression in neurons and astrocytes

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Brain damage in the transmissible spongiform encephalopathies or prion diseases is associated with the conversion of normal host prion protein to an abnormal protease-resistant isoform, and expression of prion protein is required for susceptibility to these diseases. This article reviews the data on studies using transgenic mice expressing prion protein in specific individual cell types to study the roles of these cell types in prion disease pathogenesis. Surprisingly damage to neurons in brain and retina appeared to require different prion protein–expressing cells, suggesting that different pathogenic mechanisms operate in these two neuronal tissues. *Journal of NeuroVirology* (2005) **11,** 476–480.

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Introduction

Transmissible spongiform encephalopathies (TSEs) or prion diseases are a family of naturally occurring transmissible neurodegenerative diseases, including scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, and chronic wasting disease (CWD) in North American deer and elk. The true origins of the transmissible infectivity in these diseases are not known, but current evidence suggests that the diseases are not spontaneous in origin (Chesebro, 2004). In humans three classes of prion diseases have been distinguished: sporadic Creutzfeldt-Jakob disease (sCJD) of unknown origin, familial CJD associated with inherited mutations in the prion protein gene, and acquired CJD, where the transmissible infection appears to have originated from an external source of sCJD, such as kuru in New Guinea, contaminated pituitary growth hormone preparations, or contaminated surgical instruments. The most recent example of acquired prion disease is variant CJD (vCJD) in a small number of humans probably infected by consumption of meat from BSE-infected cattle (see Chesebro, 2003; Aguzzi and Polymenidou, 2004; Weissmann, 1999; Prusiner, 1998 for reviews).

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Prion protein and TSE infectivity

All TSE diseases are characterized by the accumulation of abnormally folded protease-resistant prion protein (PrPres or PrP^{Sc}) derived from the protease-sensitive host-encoded prion protein (PrPsen or PrP^{C}) (for review see Caughey and Lansbury, 2003). Because partially purified PrPres is enriched for the transmissible infectious agent of these diseases, it is possible that PrPres itself can transmit the disease. This interpretation, known as the "protein-only" hypothesis (Griffith, 1967; Prusiner, 1982), is supported by *in vitro* experiments where PrPres can induce misfolding of PrPsen in a cell-free system by a seeded polymerization mechanism (Kocisko *et al*, 1994; Caughey, 2003). However, *de novo* generation of TSE infectivity, or prions, *in vitro* has been difficult to prove (Legname *et al*, 2004). An additional concern regarding the protein-only hypothesis is the fact that peptides or proteins involved in many nontransmissible amyloid diseases, such as Alzheimer's disease, can also undergo cell-free conversion to the amyloid state. Therefore, although this mechanism may be important in spread of the protein misfolding process within and between organs of one individual, it may not be relevant to the transmission of prion disease to new individuals. The alternative or "viral" hypothesis is currently less favored in the prion disease field due to lack of convincing data regarding any candidate viruses (Chesebro, 2003). However, viruses are difficult to exclude, and a viral genome would be an

attractive explanation for the occurrence and propagation of biologically disparate TSE strains. In addition, the continued finding in certain models of high infectivity titers with minimal PrPres requires explanation and may provide new insights into the nature of TSE infectivity.

Role of specific cell types in scrapie brain pathogenesis

In all TSE diseases the neurodegeneration is characterized by gliosis and vacuolation of the neuropil eventually leading to neuronal death. This pathology usually colocalizes with the presence of PrPres. Therefore, PrPres or its by-products are considered to be likely mediators of the pathogenesis. Expression of PrPsen, the precursor of PrPres, is required for *in vivo* replication of TSE infectivity and development of disease. However, PrP is normally expressed on a wide variety of cell types *in vivo*, and it is important to determine which of these cell types are required for scrapie replication and disease. Previous experiments have expressed PrPsen in transgenic mice using promoters with activity restricted to certain cell types. In these experiments PrP expression in neurons alone or in astrocytes alone resulted in susceptibility to scrapie infection and disease (Race *et al*, 1995; Raeber *et al*, 1997), whereas PrP expression in hepatocytes or T lymphocytes did not (Raeber *et al*, 1999) (Table 1).

After intracerebral scrapie inoculation of mice expressing PrP only in neurons (NSE Tg), disease was rapid (55 days) and appeared similar to disease in wild-type animals. However, disease was considerably slower (230–240 days) in mice where PrP was expressed only in astrocytes (GFAP Tg). This might be due either to the overall lower PrP level found in GFAP Tg mice or to the inefficiency of astrocytic PrP in inducing disease. Although the level of PrP expression undoubtedly plays a role, it seems likely that PrPres generated by astrocytes might be less efficient at inducing neuronal damage compared to direct neuronal PrPres. In support of this latter interpretation, unusually high PrPres levels were produced in brain prior to clinical disease in GFAP Tg mice (Kercher *et al*, 2004), suggesting that this PrPres might be less neurotoxic than PrPres produced directly in neurons. In ultrastructural studies of these mice, PrPres was found to colocalize with astrocytic plasma membranes and also was found in extracellular spaces of neurites found in close proximity to astrocytes (Jeffrey *et al*, 2004). Indeed the ultrastructural features of damage to these neurites, including loss of organelles and loss of integrity of the outer membranes of these neurites was also colocalized to areas around astrocytic processes. By ultrastructural analysis, vacuoles observed in the neuropil had typical features of TSE with detached and broken inner membranes. These features indicated that astrocytic PrPres was able to indirectly induce neuronal damage characteristic of prion diseases even when neurons expressed no detectable PrP. In contrast, astrocytes appeared to be activated, but there was no apparent morphological damage to astrocytes producing PrPres. Possibly the main neurotoxic factors generated by these astrocytes are PrPres-related peptides, aggregates, and fibrils (Caughey and Lansbury, 2003). However, it is still unclear whether these astrocytes might release other non-PrP neurotoxic factors and/or whether they might be incapable of carrying out their required activities such as maintaining normal glutamate homeostasis.

Table 1 Scrapie in transgenic mice expressing PrP in specific cell types

Cell types expressing PrP	*Promoter*	*Scrapie replication*	*Scrapie brain disease*
Astrocyte[a]	GFAP[e]	+	Slow (235d)
Neuron[b]	NSE[f]	+	Fast (55d)
Multiple types[c]	PrP	+	Fast (55d)
Hepatocyte[d]	Albumin	−	—
T lymphocyte[d]	Lck	−	—

[a]GFAP Tg mice (Raeber *et al*, 1997); [b]NSE Tg mice (Race *et al*, 1995); [c]Tg7 mice (Race *et al*, 2000); [d](Raeber *et al*, 1999); [e]GRAP, glialfrillary acid protein; [f]NSE, neuron-specific enalase.

Possible mechanisms of pathogenesis

Mechanisms of scrapie brain pathogenesis in GFAP Tg mice were also studied in a tissue culture system (Brown, 1999). In these experiments a PrP peptide p106–126, known to induce apoptosis in neurons expressing PrP, but not in PrP-null neurons, was used to stimulate a coculture of astrocytes from GFAP Tg mice plus neurons from PrP-null mice. Damage to PrP-negative neurons was induced indirectly by p106–126 only when PrP-expressing astrocytes from GFAP Tg mice were present in the cultures. One mechanism suggested by these data was inhibition of the ability of astrocytes to scavenge glutamate resulting in glutamate-mediated neuronal damage. However, p106–126 may not accurately mimic scrapie pathogenesis associated with PrPres formation. Thus, further experiments will be required to prove which mechanisms are responsible for the *in vivo* damage seen.

Interestingly, a different result was observed in experiments using Tg mice where neuronal PrP expression was spontaneously eliminated by Cre-Lox recombination at around 8 to 12 weeks of age (Mallucci *et al*, 2003). In such mice, after scrapie infection, PrPres was generated by astrocytes and perhaps also other non-neuronal cells, but no clinical disease was observed over a time period exceeding 600 days. The authors concluded that indirect damage to PrP-negative neurons by PrPres from astrocytes did not

occur at a level sufficient to cause disease. This interpretation disagreed with the conclusion suggested by *in vivo* experiments with GFAP Tg mice as discussed above. One possible explanation of these differences is that GFAP Tg mice might express levels of neuronal PrP that are undetectable, but nevertheless sufficient for transducing a neurotoxic signal from astrocytic PrPres. However, this seems an unlikely explanation because in cell culture experiments using PrP peptide p106–126, neurotoxic signals were in fact transmitted from PrP expressing astrocytes to neurons from PrP-null mice. Furthermore, high PrP expression levels are required for scrapie susceptibility *in vivo*, and PrP (+/−) heterozygous mice have a marked prolongation of the scrapie incubation period (Fischer *et al*, 1996). In addition, in GFAP Tg mice the path of scrapie neuroinvasion from the eye (see below) does not follow the usual optic tract neurons, indicating that neurons in these mice lack sufficient PrP expression to transport scrapie infectivity. Thus, although one cannot directly exclude low PrP expression on the neurons of GFAP Tg mice, this would not appear to be a likely explanation for the neuronal degeneration and disease observed.

Furthermore, these two scrapie systems differ in important aspects which might lead to opposing conclusions. In particular, the mouse RML/Chandler scrapie strain was used in the Cre-Lox experiment, whereas the hamster 263K strain was used in the GFAP Tg mice. These two scrapie strains differ markedly in incubation period in mice and hamsters respectively (RML/Chandler, 140 to 160 days [Eklund *et al*, 1967]; 263K, 75 days [Kimberlin and Walker, 1986]). The rapid disease induction by 263K suggests a more virulent phenotype and possibly different pathogenic mechanisms. In other experiments, the 263K strain was more directly neuroinvasive after intraperitoneal (IP) inoculation compared to the RML/Chandler strain, which required amplification in spleen prior to neuroinvasion (Race *et al*, 2000; Kimberlin and Walker, 1989). These differences between the two strains might contribute to a disparity in the potential pathogenic mechanisms, which could explain the conflicting aspects of these experiments.

Scrapie retinal pathogenesis

Retina has often been used as an easily accessible central nervous system tissue to study various aspects of disease pathogenesis. In the past retina has been infected by scrapie either using direct intraocular inoculation (Foster *et al*, 1986) or by retrograde infection via the optic nerve following intracerebral inoculation (Hogan *et al*, 1981). After intraocular inoculation progression of the infection and the pathology temporally followed the optic tract to the visual areas of the brain, indicating that optic nerves were the probable means of progression to the brain (Scott and Fraser, 1989). In these experiments retinal degeneration was observed with some, but not all scrapie strains (Foster *et al*, 1986).

Because the 263K hamster scrapie strain was known to induce severe retinal degeneration in infected hamsters, to study cell type effects on scrapie pathogenesis in the eye, we elected to use the 263K scrapie strain in the three lines of transgenic mice expressing hamster PrP which were described above. Following intraocular scrapie inoculation, PrPres deposition in retina was seen in all three transgenic mouse lines (Kercher *et al*, 2004). Subsequently the scrapie infection appeared to proceed to the brain, and mice from all three lines died after exhibiting typical evidence of scrapie brain disease, including weight loss, kyphosis, ataxia, and exaggerated high stepping gait. NSE Tg and Tg7 mice both died around 90 to 100 days, and the infection appeared to follow PrP-expressing neurons of the optic tract as reported previously in another model. In contrast, GFAP Tg mice died around 335 days. Although the infection apparently progressed up the optic nerve to the brain, after entering the brainstem, the infection did not remain strictly in the optic tracts, but instead spread within many nearby regions, not following any identifiable neuroanatomical pattern. The infection was not following the path of optic tract neurons, as was seen in mice whose neurons express PrP, but rather appeared to spread from cell to cell where it was localized adjacent to GFAP-positive astrocytes. Thus PrP expression only on astrocytes dramatically altered the pattern of neuroinvasion in GFAP Tg mice.

By histopathological analysis of retinas, gliosis and PrPres were extensive in Tg7 and NSE Tg mice, but were minimal in GFAP Tg mice, possible due to their low retinal PrPsen expression (Kercher *et al*, 2004). In spite of the similarities in retinal gliosis and PrPres generation in Tg7 and NSE Tg mice, Tg7 and NSE Tg retinas differed markedly in neurodegeneration and in evidence for apoptosis. Retinas of Tg7 mice had widespread apoptosis and were almost completely destroyed by the time of onset of clinical brain disease, whereas NSE Tg retinas showed minimal retinal apoptosis and no apparent retinal degeneration up to the time of death (Table 2). This surprising

Table 2 Comparison of 263K scrapie pathogenesis in brain and retina in transgenic mice expressing PrP in specific cell types

	Brain		*Retina*		
Prp expression	*Disease*	*PrPres*	*Degeneration*	*PrPres*	*Gliosis*
Astrocytes (GFAP Tg)	Slow	++++	No	+	No
Neurons (NSE Tg)	Fast	++	No	+++	Yes
Multiple cells (Tg7)	Fast	++	Yes	+++	Yes

From Kercher *et al*, 2004.

difference indicated that PrP expression and PrPres generation in retinal neurons was not alone sufficient to cause scrapie-induced retinal degeneration. This was in marked contrast to the situation in brain where neuronal degeneration was very prominent in both NSE Tg and Tg7 mice.

Comparison of scrapie pathogenesis in retina and brain

The explanation for the differences between retina and brain damage in these Tg mouse lines remains unknown. We speculate that the entire process of degeneration may be different in neurons of brain and retina. For example, in retina, apoptosis appears to be the primary mechanism of degeneration, whereas in brain apoptosis temporally follows vacuole formation and may be a later or secondary aspect of neurodegeneration. For retinal degeneration to occur, PrP expression in multiples cell types may be required, as expression in neurons alone was not sufficient. In contrast, in brain, PrP expression in either astrocytes or neurons was sufficient for degeneration. The PrP-expressing cell types likely required for retinal damage may include neurons plus astrocytes and/or microglia. Because PrPres is not localized near endothelial cells or oligodendroglia in this model, it would seem less likely that these cells play a major role in the disease process. Microglia have been identified as possible important contributors to scrapie retinal damage by recent experiments showing that PrP peptides or extracts of scrapie-infected cells could mediate local retinal damage in nontransgenic mice within a few days of intraocular inoculation (Marella and Chabry, 2004). Therefore, future experiments will have to consider neurons, astrocytes, microglia, and their products in trying to elucidate the complex reactions leading to scrapie-induced pathogenesis in both retina and brain.

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