PRION DISEASES

BSE and Prions: Uncertainties About the Agent

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Humans and domestic animals are tragically susceptible to a family of rare, fatal brain diseases called transmissible spongiform encephalopathies (TSEs) or prion diseases. A prominent example is the recent epidemic of bovine spongiform encephalopathy (BSE) in the United Kingdom. In March 1996, a new variant of a human TSE, Creutzfeldt-Jakob Disease (nvCJD), was reported in the United Kingdom in a small group of people, all of whom were much younger than most individuals with CJD (*1*). The unusual nature of these outbreaks prompted ready speculation that the causative agent for BSE was transmitted from cattle to humans, triggering the nvCJD cases. Although no definitive answer to this question has yet been obtained, several recent papers shed new light on this issue.

Brain damage in prion diseases is thought to occur when abnormal prion protein (PrP) molecules gain access to the brain and cause normal PrPs to take on the abnormal, disease-causing form. Raymond *et al.* (*2*) mimicked this process in a test tube and, in their cell-free biochemical system, analyzed interactions between normal and abnormal PrP molecules obtained from various species. Positive interactions between prions from different species, leading to formation of additional abnormal protein, usually predict the susceptibility of the different species to cross-species transmission of the prion diseases. Using abnormal, protease-resistant PrP from cattle with BSE, these workers found positive interactions with normal PrP of cattle, sheep, and mice (all of which are susceptible to experimental BSE), and negative interactions with hamster (which is resistant to BSE). Normal human PrP gave a weak, but detectable, positive interaction, a result that suggests that the biochemical potential for BSE transmission to humans exists, but that humans may be more resistant than other known susceptible species.

Two other groups have focused their efforts on nvCJD to test its possible relation to BSE. Bruce *et al.* (*3*) found that when brain tissue from three of the individuals with nvCJD and from BSE-infected cattle was injected into mice, all exhibited the same patterns of lesions in their brain. Furthermore, when brain tissue from eight of the BSE cases and three nvCJD cases (*3*, *4*) was injected into several different mouse strains, all required similar incubation periods before the disease was evident. In a separate communication, Hill *et al.* observed that the pattern of PrP bands detected by protein immunoblot analysis in mice inoculated with six nvCJD brain extracts was similar to that observed in BSE-inoculated mice (*5*). These similarities suggest that a similar strain of transmissible agent causes both BSE and nvCJD, but does not provide any indication of how BSE might have been transmitted from cattle to humans.

Prior to the above results, epidemiology had provided the strongest evidence that nvCJD is a form of a human prion disease related to BSE. Although the BSE epidemic itself is well past its peak, it is still uncertain whether the very low number of nvCJD cases detected since the initial report in 1996 reflect the gradual onset of a much larger incidence to be expected in the future. In this regard, data by Cousens *et al.* recently reveal an increased incidence of CJD in the United Kingdom in certain groups of farm workers exposed to cattle (*6*). However, these patients did not exhibit the type of plaques typical of the pathology of nvCJD, and the pattern of PrP bands detected was also not the same as that of nvCJD. Thus, this increase in CJD incidence is likely due to factors other than BSE, a conclusion supported by the finding of a similar increase in CJD in dairy farmers in Italy where BSE has not been found.

Although most interesting, these recent findings fall short of proof and illustrate the extreme difficulty of establishing the susceptibility of humans to the BSE agent. The dilemma is accentuated by the fact that the causative agents of all the TSEs remain an enigma. Although the notion that "protein only" can account for the infectious agent has received considerable publicity as a result of the Nobel prize award to S. Prusiner for the discovery of prions, the fact remains that there are no definitive data on the nature of prions. Prions continue to be vaguely defined, and for the most part this term is used as an operational term for the transmissible agent, but without structural implications (*7*). There are arguments both for and against the hypothesis that abnormal PrP itself is the transmissible agent (see the table below), but on either side of this controversy no argument is as yet completely convincing (*8*).

The central unresolved issue is the difference between TSEs and nontransmissible amyloid diseases such as Alzheimer's disease, type II diabetes, and amyloidoses associated with various cancers and inflammatory diseases (see table on next page). In both groups of diseases, a normal or mutant precursor protein or peptide is incorporated into an aggregated, noncovalently linked polymeric fibril-like structure (amyloid), often with a high level of β-sheet folding. This ordered aggregate can induce further polymerization of additional precursor protein, thus providing a possible basis for propagation or transmission. Furthermore, in the case of PrP, striking species and TSE strain specificity operates in this induced polymerization process, which might explain the species barrier effects and agent strains in vivo (*2*, *8*, *9*). However, none of the non-PrP amyloid dis-

IS ABNORMAL PRION PROTEIN THE TRANSMISSIBLE AGENT?

	For	Against
Amyloid diseases	•Amyloid fibrils can induce polymerization of precursors	•Amyloid diseases not transmitted by amyloid fibrils
Cell-free conversion	•PrP^{res} induces conversion of PrP^{sen}	•Do not know if infectivity is generated
Scrapie strains	•PrP^{res} structural variations	•Too many strains (20) in mice
PrP null mice	•No disease or agent replication	•PrP important as agent cofactor or receptor; involved in pathogenesis
Genetics	•TSE (both genetic and infectious) •PrP mutant causes disease?	•Retroviruses (both genetic and infectious) •PrP mutant = susceptibility gene?
Transgenic mice	•Overexpression of mutant PrP (Leu^{102}) causes brain disease	•No PrP^{res} by protein immunoblot •Transmission? •No disease if no overexpression
Irradiation	•Target size too small	•Target size = small virus
Composition	•Infectious samples have PrP^{res} •May be too little DNA for a virus •No virus found so far	•> 10^5 PrP^{res} molecules per infectious unit •Infectious samples have nucleic acids •New viruses can be difficult to find

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COMPARISON OF TRANSMISSIBILITY OF AMYLOID DISEASES

Disease	Amyloid protein	Transmissible by amyloid	Transmissible agent
Alzheimer's disease	β A4	No	None
Multiple myeloma	Ig L chain	No	None
Diabetes (type 2)	Amylin (IAPP)	No	None
Familial amyloidotic polyneuropathy	Transthyretin (TTR)	No	None
Chronic dialysis	β_2 Microglobulin	No	None
Thyroid carcinoma	Calcitonin	No	None
Secondary amyloidosis	SAA and SAP	No	*M. tuberculosis*, *M. leprae*, malaria
TSE	PrP^{res}	?	?

eases has been found to be transmissible by injection or ingestion of the polymers themselves in spite of extensive attempts [see the table above and (*10*)]. Thus, there is no precedent for the transmission of TSE by the abnormal polymeric PrP. Either we are missing some vital and unique factor that distinguishes the polymers of TSE from those of other amyloid diseases, or the agent of transmission may not be the abnormal polymers themselves.

The alternative is that a virus is the transmissible agent. It is certainly conceivable that a virus might induce polymerization of a normal or mutant cell protein, as has been shown for B19 parvovirus and sickle cell hemoglobin (*8*, *11*). Although no direct evidence for a TSE virus has been obtained, searching for unknown viruses is not straightforward. Even with the powerful methods of molecular biology, without a specific probe or unique sequence, new viruses can be extremely difficult to detect. However, this should not deter future search efforts as new viruses (Borna and hepatitis), continue to be discovered.

Support for the protein-only hypothesis also comes from the apparent spontaneous generation of a neurodegenerative disease in transgenic mice that overexpress a mutant form of PrP associated with familial TSE disease in humans (*12*, *13*). These results are inconclusive for several reasons. First, these mice differ from all known TSE models in that no abnormal protease-resistant PrP is detectable in diseased brain tissues by protein immunoblot. Second, brain disease is produced only when the mutant transgene is overexpressed, but not when it is present as a single-copy gene in the normal PrP locus. Third, transmission of this disease occurs only in transgenic mice expressing low levels of the same mutant PrP transgene, and not in normal nontransgenic mice. Thus, "transmission" could be due to transfer of cytokines or other molecules from diseased brain, which up-regulate mutant PrP transgene expression in the recipient mice. Nor have the transmitted materials been shown to have the typical properties of TSE agents, such as unusual resistance to inactivation by heat and harsh chemicals. To explain the lack of transmission to normal mice, it has been proposed that the PrP mutation (Leu^{102}) in the transgene causes a barrier to transmission to normal (Pro^{102}) mice. Although theoretically possible, it is unlikely because effective transmission can occur from humans with Leu^{102} PrP humans to both monkeys and mice with Pro^{102} (*14*, *15*).

Hopefully, open-minded future research will resolve the mysteries regarding the nature of the TSE agent. Additional information on this subject must be obtained to better facilitate development of drugs. Evidence suggests that treatment with drugs that interact with the abnormal PrP can prolong survival in experimental mouse or hamster scrapie, a TSE disease originally derived from sheep. Other approaches—gene therapy with TSE-resistant PrP alleles or elimination of the PrP gene itself—are also possible, particularly for domestic animals. Clearly, we are in the very early stages of exploration of this subject. It would be tragic if the recent Nobel Prize award were to lead to complacency regarding the obstacles still remaining. It is not mere detail, but rather the central core of the problem, that remains to be solved.

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