Neuron, Vol. 24, 503–506, November, 1999, Copyright ©1999 by Cell Press

Prion Protein and the Transmissible Spongiform Encephalopathy Diseases

Minireview

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Transmissible spongiform encephalopathy (TSE) diseases or prion diseases are rare fatal neurodegenerative diseases of humans and other animals. In the past decade, a high awareness of TSE diseases has developed due to the appearance of bovine spongiform encephalopathy (BSE) or "mad cow disease" in the United Kingdom. Due to the potential for human infection, in Europe BSE has influenced medical, agricultural, economic, and political issues, perhaps even to a greater extent than has AIDS.

Primary symptoms of TSE diseases in humans are dementia and ataxia. These diseases are usually characterized by spongiform degeneration of the brain accompanied by appearance of activated astrocytes and accumulation of abnormal aggregates consisting of protease-resistant prion protein (PrP), which sometimes forms amyloid-like plaques. TSE diseases are transmissible by inoculation or ingestion of infected tissues. Incubation periods prior to clinical symptoms range from months to years, and in the case of some kuru patients may have been as long as 40 years.

TSE diseases in humans can be divided into three groups: sporadic, familial, and iatrogenic (or infectious) (Brown et al., 1994). Sporadic Creutzfeldt-Jakob disease (CJD) is not associated with any known mutations and occurs world-wide at an incidence of around 1 per 2 million. The source of this disease is completely unknown. Familial TSE diseases are all associated with different mutations in the PrP gene, and include familial CJD, Gerstmann-Sträussler-Scheinker syndrome (GSS), and fatal familial insomnia (FFI). Infectious or iatrogenic TSE diseases include kuru, which was spread by ritual cannibalism in New Guinea tribesmen, CJD, which is spread by transplantation or inoculation with brain tissues or extracts from unsuspected CJD patients, and variant CJD (vCJD), which is apparently due to infection of humans with the agent of BSE.

Prion Protein (PrP)

Besides transmissibility, the other hallmark of TSE diseases is the presence of abnormal protease-resistant PrP (PrP-res, also known as PrP^{Sc}), detectable by immunoblot or immunohistochemistry in the brains of afflicted individuals. PrP-res is believed to be responsible for the pathogenic effects in the TSE diseases, either by direct toxicity or indirect effects due to interactions with the normal PrP expressed on many brain cells. PrP-res is generated posttranslationally by an unknown mechanism from normal protease-sensitive PrP (PrP-sen, also known as PrP^{C}), which is expressed as a GPI-linked cell surface protein in a variety of tissues and cell types both within and outside the nervous system (Caughey and Chesebro, 1997). The main difference between PrP-res and PrP-sen appears to be in their folded structures. The structure for PrP-sen has recently been solved by NMR and has an N-terminal flexible domain followed by a C-terminal globular domain with three α helices (Riek et al., 1996). In contrast, the precise structure of PrP-res is not known, but biophysical measurements indicate a high amount of β sheet structure. Furthermore, PrP-res often forms fibrils that show birefringence after binding of Congo red. This property is a well-known characteristic of β sheet–rich non-PrP amyloid proteins found in amyloidosis associated with other diseases, such as type II diabetes, Alzheimer's disease, tuberculosis, rheumatoid arthritis, and various cancers.

The generation of PrP-res from PrP-sen has been studied in scrapie-infected mouse cells as well as under cell-free conditions in test tubes (Caughey and Chesebro, 1997). The PrP-res generated in these cell-free laboratory studies mimics closely the material produced in brains of scrapie-infected animals and humans. However, because these cell-free experiments contain large amounts of infectivity associated with the PrP-res used to initiate the conversion process, it has not yet been possible to prove that the abnormal PrP formed in vitro is actually infectious.

The normal function of PrP-sen is not known. PrP is not essential for viability, as some lines of PrP knockout mice are fertile and neurologically normal, although some sleep abnormalities have been observed. Other lines of PrP knockout mice have shown cerebellar degeneration, but this difference may be due to disruption of adjacent genes. Interestingly, expression of PrP-sen is required for susceptibility to TSE diseases, and propagation of infectivity is eliminated or drastically reduced in the absence of the PrP gene (Weissmann, 1999). This has been interpreted to imply that PrP is either a receptor for the infectious agent or a component of the agent.

Natural and Experimental Scrapie in Sheep

Scrapie has been recognized as a disease in sheep for over 2 centuries, and was the first TSE disease to be shown as experimentally transmissible. Thus, sheep scrapie provides an unusual opportunity to compare natural and experimental TSE disease processes. Although in animals there are no known genetic cases of TSE disease comparable to those seen in humans, allelic variations in the sheep PrP sequence do occur, and variation at several residues in the PrP sequence influences susceptibility to both natural and experimental scrapie infection. The mechanism of these effects is not known, but these data show clearly that PrP is an important susceptibility factor for TSE diseases. A similar effect may also occur in humans, where variation at PrP codon 129 does not induce a familial TSE disease but instead appears to influence susceptibility to sporadic CJD.

Although the infectious nature of sheep scrapie has been long recognized, the mode of transmission within a flock is not clear. Direct physical contact between

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individuals is not required, and there are numerous reports of transmission by pasturing sheep on ground previously occupied by a flock with a high scrapie incidence. Such pastures appear to be contaminated by scrapie infectivity perhaps derived from placental tissue, fetal membranes, or decomposing carcasses.

Bovine Spongiform Encephalopathy (BSE)

In the past decade, the BSE epidemic in the UK has brought international attention to the TSE family of diseases (reviewed by Collee and Bradley, 1997). BSE was spread by feeding of protein supplements contaminated with the rendered tissues of BSE-positive cattle. Several laboratory tests, including PrP-res banding patterns in protein gels and comparative titration in various mouse strains, identify similarities in BSE from all sources tested, as compared to most commonly known isolates of sheep scrapie. However, it remains unclear whether BSE originated by adaptation from an unusual strain of sheep scrapie or from an unrecognized bovine TSE case.

BSE has also been transmitted to other species by feeding of contaminated meat and bone meal to ungulates and large felines in zoos and probably also to domestic cats. Transmission to humans has also been suggested by the appearance of vCJD in a small group of younger humans, primarily in the UK. The similarity in pathology and in laboratory tests lends support to this interpretation. At present, one cannot be certain whether the vCJD cases represent the beginning of a much larger epidemic in humans or whether they are unusual cases of transmission across a rather resistant species barrier. In contrast to BSE, there is no evidence of spread of sheep scrapie to humans. Therefore, there may be important fundamental differences between scrapie and BSE in their interactions with different hosts.

Chronic wasting disease (CWD) in deer and Rocky Mountain elk in Colorado and Wyoming and transmissible mink encephalopathy (TME) on mink ranches are two other examples of TSE diseases of unknown etiology. CWD is now also recognized to be a serious problem in many "game farms" in other regions of the USA. Its spread appears to be enhanced by the abnormal population densities found in such facilities.

Experimental Models

TSE diseases have been studied experimentally in several laboratory species, including mice, rats, hamsters, and nonhuman primates. In general, TSE diseases show a preference for transmission to the species of origin or a closely related species. Most noteworthy was the original demonstration of the transmissibility of CJD and kuru from humans to chimpanzees (Gajdusek and Gibbs, 1971). Transmission to a less closely related species is also possible and appears to involve a progressive adaptation during serial passage in the new host.

Early experiments identified the *Sinc* gene as important in host susceptibility to scrapie (Dickinson et al., 1968), and subsequently *Sinc* was found to be the gene encoding PrP. Experiments with PrP transgenic mice where expression of hamster PrP was found to render transgenic mice susceptible to hamster-specific scrapie strains also identified PrP as a susceptibility factor for cross-species transmission (Prusiner, 1998). In molecular studies, the use of chimeric PrP molecules in transgenic mice has identified the importance of amino acid residues in the central portion of PrP in species-specific interactions between the inoculated TSE agent and the host animal. Similar studies extended these results using scrapie-infected mouse neuroblastoma cells, where differences at PrP amino acid residue 138 were identified as critical for cross-species resistance between mice and hamsters as measured by PrP-res formation. Interestingly, this residue is homologous to a polymorphic residue at position 142 in goat PrP, which was previously found to influence resistance to BSE and certain sheep scrapie strains in vivo.

Similar studies have shown that transgenic mice expressing human PrP have increased susceptibility to many human TSE disease isolates. This has broadened the possibilities for studying human isolates in less expensive and more rapid rodent models suitable for screening of possible therapeutic drugs. However, in spite of extensive knowledge of PrP sequences from a variety of species, the extent of species-specific resistance to TSE diseases remains impossible to predict solely by analysis of PrP sequences. This is of critical importance in the matter of human susceptibility to BSE.

Transgenic mice expressing PrP using tissue-specific promoters have been studied to analyze the roles of different PrP-expressing cells on susceptibility to TSE disease. PrP expression in either neurons or astrocytes is sufficient for induction of clinical disease and typical scrapie neuropathology. This would appear to imply that generation of a toxic PrP-res or peptide moiety in the brain may be independent of the brain cell type producing the normal PrP. Testing of mice expressing PrP in microglia or oligodendroglia will be required to examine this matter more thoroughly. In contrast to the above results, PrP expression in liver, T lymphocytes, and B lymphocytes did not induce scrapie susceptibility, suggesting perhaps that the toxic PrP product must be produced in the brain to have a pathogenic effect.

Routes of Neuroinvasion

In mouse scrapie, functional B lymphocytes are necessary for neuroinvasion after infection at peripheral sites, but PrP expression on B lymphocytes is not required. In recent experiments, PrP-negative B lymphocytes may have influenced neuroinvasion indirectly by allowing the development of mature spleen follicular dendritic cells (FDCs) as important sites of agent replication and/or PrP-res accumulation (Klein et al., 1998). Although in mice replication of scrapie in spleen may be required to amplify titers after peripheral scrapie infection, splenic infection was by itself unable to induce transport of infectivity from spleen to brain. The importance of PrP-positive peripheral nerves in neuroinvasion was recently demonstrated when transgenic mice expressing PrP only in neurons were shown to develop scrapie after oral or i.p. infection with high doses of agent (Race et al., 2000). Blood-borne transport of infectivity to the brain remains difficult to exclude completely; however, in most model systems infectivity levels in blood are extremely low, and transmission by blood transfusion in animals has only rarely been effective. Nevertheless, when considering the issue of blood safety for human transfusion, it is important to remember that different animal species and TSE strains might differ in infectivity levels of blood.

Transgenic Mouse Models of Familial Human TSE

None of the animal TSE diseases mentioned above serve as models for the familial forms of human TSE diseases, which are strongly associated with different PrP mutations. However, PrP with the mutation from proline to

leucine at position 102, found in human GSS patients, has been expressed in transgenic mice. More recently, human PrP with extra amino acid octarepeat regions, also associated with familial TSE disease, has been generated in transgenic mice. For both the Leu-102 PrP mutant and the extra amino acid octarepeat PrP mutant, mice expressing high amounts of the mutant PrP develop a fatal neurological disease with neuropathology similar but not identical to TSE disease. However, in neither model is there generation of abundant PrP-res with the high degree of protease resistance found in the human counterparts of these models. Furthermore, the transmissibility of the diseases produced in these mouse models remains questionable. For the octarepeat mutant, there is no transmission data yet available (Chiesa et al., 1998). For the Leu-102 mutant, the disease cannot be transmitted to normal PrP (Pro-102) mice but can be transmitted to transgenic mice expressing Leu-102 PrP at levels too low to induce disease spontaneously (Hsiao et al., 1994). This result has been interpreted by the authors as evidence for transmission, but it clearly does not mimic the transmission of known TSE diseases including the human familial Leu-102 PrP disease, which is in fact transmissible to monkeys and mice expressing only Pro-102 PrP. Thus, in this mouse model the brain disease induced lacks the two critical hallmarks of TSE disease, PrP-res and transmissibility, and may in fact be a disease due to overexpression of a mutant protein rather than a true TSE disease.

To avoid artifacts due to abnormal transgene copy number and abnormal integration sites, PrP with the Pro-102-Leu mutation has been recently substituted for the normal mouse PrP gene by homologous recombination (Manson et al., 1999). In contrast to the previous transgenic mice, such recombinant mice fail to develop spontaneous CNS disease. However, they do have an increased susceptibility to infection by a human GSS isolate. These results suggest that mutant Pro-102-Leu PrP may be an important susceptibility factor rather than a direct cause of GSS.

TSE Strains

The existence of biologically different scrapie strains in inbred animals with a single type of PrP gene remains an interesting enigma. Disease induced by scrapie strains can differ in the clinical symptoms produced, the regions of brain affected, and the incubation period prior to clinical onset. These differences might be explained by mutations in a nucleic acid genome if the infectious agent is a virus, but no genomes have yet been identified. In contrast, the existence of strains is difficult to explain if the infectious agent is a protein. However, structural variations in PrP-res might "encode" strain-specific properties, and recent data suggest that PrP-res structures might be capable of conferring such properties on newly formed PrP-res in a template-like fashion.

Biophysical Inactivation and the Viral Hypothesis

In the past decade, there has been a massive increase in knowledge concerning many aspects of the TSE diseases largely due to the discovery of PrP. Nevertheless, there continues to be a paucity of information concerning the structure and composition of the infectious agent. Early ultrafiltration studies suggested that the infectious particle was small and might be a virus. However, the infectivity showed a strong resistance to sterilization by heat and chemicals, which led Griffith in 1967 to propose that the agent might be a self-replicating protein (Griffith, 1967). Following the discovery of scrapie-associated fibrils (Merz et al., 1981) and the identification of PrP as a major component of infectious fractions, the "protein-only" hypothesis was refined into the "prion" hypothesis (Prusiner, 1982). So far, neither the viral nor the protein-only model has been proved or disproved, and the nature of the infectious agent remains a central mystery in the TSE field (Chesebro, 1998).

Inactivation studies by irradiation, heat, and chemicals have led to conflicting conclusions regarding the uniqueness of TSE infectivity. Using heat or hypochlorite, the majority of infectivity actually shows a kinetics of inactivation and a small resistant fraction (0.1%–0.01%) that is similar to known viral examples, such as bacteriophage fd (Rohwer, 1991). This minor resistant fraction should not be used to infer unique properties in the majority of the infectivity.

The spectrum for inactivation of scrapie infectivity by UV irradiation suggested that the critical target was neither protein nor nucleic acid, but instead appeared to be lipidic in nature (Alper et al., 1978). However, in past virological experiments using nonpenetrating radiation such as UV, shielding of the critical target molecule of the infectious agent by other molecules in the mixture or attached to the agent has been known to influence the results. In fact, the unusual inactivation spectrum for scrapie was similar to intact tobacco mosaic virus, a well-characterized RNA virus, whereas the isolated RNA from this virus had peak inactivation at a wavelength predicted for a typical nucleic acid. Thus, in view of these issues and the difficulty in purification of the scrapie agent, UV studies may not provide definitive information as to the nature of the scrapie infectivity.

Scrapie infectivity has also been studied using more penetrating radiation like X rays, where shielding or blocking of the radiation is not an issue. Many experiments have resulted in similar inactivation rate constants; however, different groups have varied markedly in their interpretation of these results (Rohwer, 1991). Using target theory calculations, some workers have concluded that the maximum genome size would be very small. In contrast, others making empirical comparisons to viruses with known genomes have arrived at a genome size consistent with a small (2–4 kb) virus. However, both of these interpretations might underestimate the genome size if the TSE agent had a means of repairing damaged nucleic acid during replication. Such a situation occurs with retroviruses, like HIV, in which the two RNA genomes in each particle can be partially damaged and then repaired during reverse transcription, thus giving a higher resistance to x-irradiation than that predicted by genome size alone.

These data are only indirect evidence that may be consistent with the viral hypothesis. In spite of many efforts, there are still no data supporting any candidate viruses. Furthermore, although small nucleic acid molecules have been found in purified infectious scrapie samples, efforts to identify an intact nucleic acid molecule of potential genome size have met with failure. Therefore, if such a genome exists, it would have to be capable of regeneration from small fragments by a copy choice mechanism during transcription as described

above. In addition, the role of mutant and nonmutant PrP in the case of a viral etiology remains hypothetical. To explain the very high correlation of TSE disease in persons with certain PrP mutations, one would have to speculate that the mutant PrP might serve as an efficient susceptibility factor or receptor for a viral agent that might be relatively common in the population. In contrast, the normal nonmutant PrP would have to be much less efficient than mutant PrP in this role in order to account for the extremely low incidence of sporadic CJD. The recent experiments showing an increased sensitivity to human GSS in mice expressing the Pro-102-Leu mutation might be an example of this situation (Manson et al., 1999). Similar effects probably occur for many viral diseases in humans where there is a high incidence of infection compared to the incidence of clinical disease. Examples include HTLV I and B19 parvovirus. However, at present this possibility remains speculative as applied to TSE diseases in the absence of additional supportive data using actual candidate viruses.

The Protein-Only Hypothesis

Although the discovery of PrP has led to a vast increase in knowledge concerning the role of PrP in susceptibility and pathogenesis of TSE diseases, the question of whether PrP-res is an integral component of the infectious agent remains unresolved. The most important evidence supporting this concept is the finding that PrP is the predominant macromolecule found in fractions of purified infectious agent. However, two caveats persist regarding this matter. First, because of the presence of aggregated PrP, the agent is difficult to purify, and the best fractions still contain detectable nucleic acid molecules. These fractions might also conceivably contain other components relevant to infectivity. Second, in purified fractions the ratio of PrP molecules to infectious units is extremely high ($\sim$100,000), and this fact has led to speculation that only a subfraction of the PrP-res, referred to as PrP*, is the infectious form (Weissmann, 1999). However, it remains unclear how to identify or distinguish biochemically the proposed infectious and noninfectious forms of the protease-resistant PrP.

Among the most puzzling aspects of the protein-only hypothesis is the comparison of TSE diseases to other amyloid diseases. In all these diseases, protein misfolding is a prominent part of the pathogenesis, and in many amyloid diseases interactions between the normal and abnormal proteins can lead to formation of additional abnormal protein. This is similar to the situation with TSE diseases. However, only the TSE diseases appear to be easily transmissible experimentally. This suggests that the protein interactions common to all amyloid diseases probably do not explain the unique transmissibility of TSE diseases (Chesebro, 1998). The existence of this dilemma does not imply that the protein-only or viral hypotheses are incorrect, but rather that we are lacking some crucial information to explain the differences between TSE diseases and the nontransmissible amyloid diseases.

Lastly, there is still only limited information regarding the origin of sporadic CJD in humans. The various animal models appear to be similar to the human diseases in the accidental or iatrogenic infection group; however, animal TSE diseases so far have not adequately modeled sporadic human TSE disease. For sporadic TSE disease, the protein-only hypothesis has proposed rare spontaneous misfolding as a possible primary event, but presently there is no evidence to support this proposal. Misfolded PrP molecules formed by denaturation and renaturation in vitro have not generated any infectivity de novo. Furthermore, in Australia and New Zealand, where scrapie has been eradicated, there is no evidence of spontaneous recurrence of sheep scrapie. In addition, in humans the peak age incidence of sporadic CJD is 55–60 years, and if spontaneous misfolding were the primary event, one might expect a continuously increasing incidence with age, since more time might allow more opportunity for rare misfolding events.

Conclusions

Research in the past 15 years has provided a wealth of new information on the TSE diseases. Particularly noteworthy has been the identification of PrP as a factor in disease susceptibility, the species barrier, and many aspects of disease pathogenesis. Further understanding of the structure of PrP-res and of amyloids in similar diseases, such as Alzheimer's disease, may provide clues as to the differences and similarities among these protein folding diseases. The problem of the nature of the TSE agents remains an enigma. Proof of the protein-only hypothesis may require generation of biologically active transmissible agent in a cell-free environment where a virus cannot replicate. Conversely, proof of a viral etiology will require identification and isolation of a candidate virus. Future efforts should not neglect this fascinating and important area.

Selected Reading

Alper, T., Haig, D.A., and Clarke, M.C. (1978). J. Gen. Virol. *41*, 503–516.

Brown, P., Gibbs, C.J., Jr., Rodgers-Johnson, P., Asher, D.M., Sulima, M.P., Bacote, A., Goldfarb, L.G., and Gajdusek, D.C. (1994). Ann. Neurol. *35*, 513–529.

Caughey, B., and Chesebro, B. (1997). Trends Cell Biol. *7*, 56–62.

Chesebro, B. (1998). Science *279*, 42–43.

Chiesa, R., Piccardo, P., Ghetti, B., and Harris, D.A. (1998). Neuron *21*, 1339–1351.

Collee, J.G., and Bradley, R. (1997). Lancet *349*, 636–641.

Dickinson, A.G., Meikle, V.M., and Fraser, H. (1968). J. Comp. Pathol. *78*, 293–299.

Gajdusek, D.C., and Gibbs, C.J., Jr. (1971). Nature *230*, 588–591.

Griffith, J.S. (1967). Nature *215*, 1043–1044.

Hsiao, K.K., Groth, D., Scott, M., Yang, S.L., Serban, H., Rapp, D., Foster, D., Torchia, M., DeArmond, S.J., and Prusiner, S.B. (1994). Proc. Natl. Acad. Sci. USA *91*, 9126–9130.

Klein, M.A., Frigg, R., Raeber, A.J., Flechsig, E., Hegyi, I., Zinkernagel, R.M., Weissmann, C., and Aguzzi, A. (1998). Nat. Med. *4*, 1429–1433.

Manson, J. (1999). EMBO J., in press.

Merz, P.A., Somerville, R.A., Wisniewski, H.M., and Iqbal, K. (1981). Acta Neuropathol. (Berl.) *54*, 63–74.

Prusiner, S.B. (1982). Science *216*, 136–144.

Prusiner, S.B. (1998). Proc. Natl. Acad. Sci. USA *95*, 13363–13383.

Race, R., Oldstone, M., and Chesebro, B. (2000). J. Virol., in press.

Riek, R., Hornemann, S., Wider, G., Billeter, M., Glockshuber, R., and Wuthrich, K. (1996). Nature *382*, 180–182.

Rohwer, R.G. (1991). In Current Topics in Microbiology and Immunology. Volume 172. (Berlin: Springer-Verlag), pp. 195–232.

Weissmann, C. (1999). J. Biol. Chem. *274*, 3–6.