A prion primer

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Abstract

BY BIOLOGICAL AND MEDICAL CRITERIA, prions are infectious agents; however, many of their properties differ profoundly from those of conventional microbes. Prions are "encoded" by alterations in protein conformation rather than in nucleic acid or amino acid sequence. New epidemic prion diseases (bovine spongiform encephalopathy and new variant Creutzfeldt–Jakob disease) have recently emerged under the active surveillance of the modern world. The risk of contracting prion disease from blood products or other biologicals is now a focus of worldwide concern. Much has been discovered about prions and prion diseases, but much remains to be done.

Résumé

LA BIOLOGIE ET LA MÉDECINE considèrent les prions comme des agents infectieux, mais beaucoup de leurs caractéristiques diffèrent profondément de celles des microbes classiques. Les prions sont «codés» par des altérations de la conformation des protéines plutôt que dans la séquence des acides nucléiques ou des acides aminés. De nouvelles maladies épidémiques à prions (encéphalopathie spongiforme bovine et nouvelle variation de la maladie de Creutzfeldt–Jakob) ont fait leur apparition récemment sous la surveillance active du monde moderne. Le risque d'être atteint d'une maladie à prions transmise par des produits sanguins ou d'autres tissus biologiques soulève maintenant des préoccupations mondiales. On a découvert de nombreuses caractéristiques des prions et des maladies à prions, mais il reste encore beaucoup à faire.

Prions and prion diseases are like nothing else we have encountered in biology or medicine. The causative agents of the transmissible spongiform encephalopathies (TSEs) are clearly infectious by the standards of disease transmission and particle replication in susceptible hosts. However, many properties of these agents differ profoundly from those of conventional microbes. Dr. Stanley Prusiner, who was awarded the 1997 Nobel Prize in Medicine and Physiology, developed the prion hypothesis, which asserts that infectivity resides in a protein — a *pro*teinaceous *in*fectious particle (the middle letters were transposed for euphony).[1,2] Consistent with this notion, the infectious agent is highly resistant to treatments that damage or destroy nucleic acids, such as ultraviolet radiation and nucleases, but can be inactivated by exposure to treatments that destroy or denature proteins, such as chaotropic ions or denaturing detergents.

Pursuing the prion hypothesis, Prusiner and colleagues purified and partially sequenced a protease-resistant protein fragment (PrP 27-30) from scrapie-infected hamster brain that co-purified with scrapie infectivity, allowing for the molecular cloning and sequencing of its cognate cDNA. Surprisingly, this infectious protein was found to be encoded by an ancient, highly conserved host gene.[3] The data suggested that the same nucleic acid and amino acid sequence could give rise to 2 proteins with exceedingly different properties: a normal cellular isoform (PrP^{C}) — expressed in normal brain, readily soluble and protease sensitive — and an abnormal, scrapie-associated isoform (PrP^{Sc}) — accumulating in TSE brain, poorly soluble, partially protease resistant and associated with infectious activity. Subsequent work has demonstrated that these 2 isoforms cannot be explained by differential mRNA splicing or covalent post-translational modifications. More recent work has suggested that major conformational differences ex-

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BSE	bovine spongiform encephalopathy
CJD	Creutzfeldt–Jakob disease
FFI	fatal familial insomnia
GSSD	Gerstmann–Sträussler–Scheinker disease
TSE	transmissible spongiform encephalopathy
vCJD	variant CJD

ist between the isoforms; PrP^C possesses a high content of α helices and a small region of antiparallel β-sheet structure, whereas PrP^{Sc} displays a high β-sheet content and few or no α helices.[2,4] The 3-dimensional structure of either isoform has not been completely solved.

The prion hypothesis thus asserts that infectivity resides in a PrP^C-to-PrP^{Sc} "recruitment reaction" that occurs on a post-translational level, inherent in changes in protein conformation (Fig. 1). The absolute dependence of PrP^{Sc} generation on PrP^C has been demonstrated in vivo in PrP knockout mice, which are entirely resistant to experimental scrapie,[5] and has now been shown in a cell-free system by Caughey and colleagues.[6] "Strains" of the prion agent, with distinct pathology and incubation period in experimental animals, have been attributed to multiple forms of abnormal PrP folding.[7] Does PrP^{Sc} acquire a conformase activity, like a chaperone, to mold PrP^C into its structural likeness,[2] or do PrP^{Sc} aggregates behave like crystals, recruiting monomers from the PrP^C-saturated local environment at the cell surface?[6] These and other hypotheses of prion infectivity are now being actively explored.

Human transmissible spongiform encephalopathies

The history of human prion diseases begins with Dr. D. Carleton Gajdusek's pivotal descriptions of the stone-age Fore people in the highlands of Papua New Guinea (dramatically popularized in a recent book by Richard Rhodes[8]). The Fore people in the 1950s were remarkable to the outside world for 2 characteristics: their practice of ritual cannibalism (a sign of respect for deceased loved ones) and their susceptibility to kuru, which means "shaking with fear" in the Fore tongue. Kuru is a progressive ataxic syndrome with late dementia and other neurological impairments, and is neuropathologically characterized by noninflammatory neurodegeneration (loss of neurons), gliosis (activation and proliferation of astrocytes and microglial cells), spongiform change (microscopic vacuoles in the brain parenchyma) and accumulation of an abnormal protease-resistant protein in brain plaques (subsequently found to be PrP^{Sc}). The veterinary pathologist Hadlow recognized that kuru may be a human form of scrapie, and his letters prompted experimental transmission of kuru to subhuman primates by Gajdusek and Gibbs. Continued transmission studies at the US National Institutes of Health revealed that kuru was the prototype for a group of diseases occurring in sporadic, familial and iatrogenic forms throughout the world. Creutzfeldt–Jakob disease (CJD) and Gerstmann–Sträussler–Scheinker disease (GSSD) were first transmitted because of clear neuropathological resemblance to kuru; other prion disorders have been recognized more recently on the basis of more subtle pathological features or by mutations in the prion protein gene. The rubric of prion diseases now includes such syndromes as fatal familial insomnia (FFI) and new variant CJD (vCJD). It seems safe to predict that other prion-related diseases will be discovered. Moreover, we should not be surprised if prion gene polymorphisms, somatic mutations or conformational abnormalities are linked to developmental, inflammatory and neoplastic diseases, or other disorders.

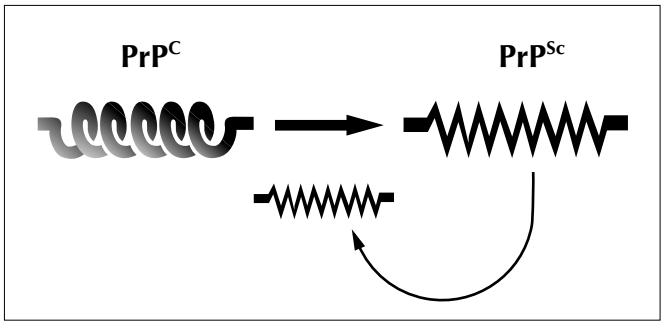

Fig. 1: Prion "recruitment reaction": PrP^C (left) is converted into PrP^{Sc} (right), which can act as a catalyst for further conversion. PrP^C is a normal, protease-sensitive, highly soluble cell-surface protein that is predominantly α-helical; its conformational isoform PrP^{Sc} has a high β-sheet content and is abnormal, protease resistant, poorly soluble and disease specific.

Prion diseases: clinical features and diagnosis

The clinical phenotypes of the human prion diseases are appalling. The usual presentation of CJD — the most common human TSE — is a rapidly progressive dementing syndrome in the sixth to eighth decade of life, at some phase accompanied by myoclonus. Involvement of the pyramidal and extrapyramidal motor systems generally produces spasticity, rigidity and tremor. Occasional severe involvement of the occipital lobes may produce cortical blindness, rare in other dementing diseases. Motor neuron involvement may result in weakness, fasciculations and amyotrophy. In about one-third of the cases of sporadic CJD, initial involvement of the cerebellum produces ataxia, dysarthria and dysmetria; cerebellar symptoms occur usually late in two-thirds of CJD cases with dementing onset. Death in sporadic CJD is mercifully rapid, usually occurring within 3 to 12 months. Rarely, a person with CJD may live 10 years or more after symptom onset. GSSD usually presents as a cerebellar syndrome at first, with subsequent involvement of other regions of the nervous system, terminating in death after a protracted course. Early thalamic degeneration in FFI and related disorders produces the distressing symptom complex of progressive insomnia with late dementia.

Definitive diagnosis of human TSEs is achieved through neuropathological examination of the brain at autopsy. Paraclinical testing useful in the diagnosis includes electroencephalography, which may reveal periodic triphasic complexes at 1 per second, classic of the disease. MRI is able to reveal changes in the density of deep grey nuclei in only a minority of people with CJD.[9] Recently the advent of genetic testing has provided diagnostic insight into CJD and other TSEs: to date, all confirmed cases of autosomal dominant familial prion diseases can be linked to mutations in the protein coding sequence of the prion protein gene.[10] Genetic testing in patients with "sporadic" CJD has also resulted in surprises: between 10% and 25% of tested individuals possess mutations in the prion gene. In addition, a polymorphism of methionine or valine at codon 129 of the human prion protein may contribute to CJD susceptibility; sporadic CJD is usually homozygous at this site. Recently, a test of cerebrospinal fluid has been advanced to assist CJD diagnosis, based on the appearance of neuronal cell signaling protein 14-3-3.[11] Protein 14-3-3 is detectable in disorders with a rapid rate of neuronal loss (e.g., CJD, viral encephalitis, cerebral infarct and even some cases of Alzheimer's disease) and can presumably produce negative test results in slowly progressive prion disease (e.g., GSSD).

The world is waiting for a sensitive and specific diagnostic test of prion disease. Unlike microbial particles, the prion agent does not contain specific nucleic acid sequences: the genetic information that can be so readily detected and amplified in conventional agents is simply not part of the TSE infectious process. Moreover, the prion agent, being a modified host protein, does not prompt an immune response (e.g., serum antibodies) that could be used in diagnostic testing. With prion disease, these conventional tools of the microbiologist and the epidemiologist are rendered useless, making it difficult to detect infection, define a presymptomatic phase of the illness, track transmission and type strains. In the future, diagnostic tests will probably emerge from sensitive detection of PrP^{Sc}, which may be identical with the prion agent itself, or a reliable surrogate of infection.

Epidemic prion disease in the UK

TSEs are not new in the annals of veterinary practice; scrapie of sheep and goats has been recognized for centuries. Some of the long-standing mystery involved in the transmission of scrapie by flocks in the field has been solved by the recent identification of PrP^{Sc} in sheep mites.[12]

The animal prion disease of most recent renown is bovine spongiform encephalopathy (BSE) — popularly known as "mad cow disease" — which has affected to date over 150 000 cattle in the UK and continental Europe.[13] This major epidemic probably originated in the supplementation of cattle feed by offal (including high-infectivity tissues such as brain and spleen) from sheep and cattle. It has been theorized that changes in practices of rendering sheep carcasses allowed the sheep scrapie agent to infect British cattle. However, the spontaneous crossing of species barriers for sheep scrapie is probably quite rare, despite the existence of many strains of sheep agent adapted for laboratory rodents. The near-simultaneous emergence of BSE across the UK suggests that the epidemic may have originated from supplements derived from one or a few contaminated cattle, for which there is no species barrier. Canada has recently joined the US in the ban of ruminant-to-ruminant feeding in an effort to avoid BSE introduction into North America.

Through the regulation of cattle feed (e.g., the prohibition of ruminant supplement to ruminants) and selective culling of affected herds, the number of new cases of BSE has declined progressively since 1993. The BSE epidemic will likely be extinguished early in the next millennium.[14] However, BSE may only be the beginning of a series of prion epidemics in other species. Unlike sheep scrapie, the BSE prion agent seems particularly suited for interspecies transmission. Consumption of contaminated cattle material has been linked to prion disease in zoo cats, exotic ungulates, domestic cats, swine and even an ostrich (the first reported avian spongiform encephalopathy, still to be confirmed). Disconcertingly, BSE can also be transmitted to sheep.

Unfortunately, humans appear to be one of the species susceptible to interspecies transfer of the BSE agent. In April 1996, Will and colleagues[15] reported 10 cases of a new strain of human CJD in the UK, differentiated clinically from conventional CJD by young age at onset (some of the patients were teenagers), psychiatric and ataxic presentation, and a marginally slower rate of progression. Periodic complexes on electroencephalograms were not observed in the 10 cases. This vCJD is a distinct neuropathological entity, characterized by markedly increased brain accumulation of PrP^{Sc}, some of which is accumulated in discrete plaques surrounded by prominent spongiosis ("daisy plaques" [see page 1385]). Spongiform change elsewhere in the brain is minimal compared with that in conventional CJD. Indeed, the histological appearance of vCJD is so distinct from that of conventional CJD that no case can be deemed confirmed without the pathognomonic neuropathology. Since the initial report, 14 additional cases have been identified from the UK and continental Europe. The timing and location of the vCJD epidemic, the transmission of BSE to marmosets with near-identical neuropathology to human vCJD[16] and the conservation of protease-resistant molecular species between human and BSE PrP^{Sc17} support the hypothesis that

vCJD represents a new human strain of prion disease contracted from BSE-infected material.

How many people are likely to contract vCJD? The calculations are affected by many factors impossible to clarify at present. Interspecies transmission of prion disease is less efficient than intraspecies transmission, and recruitment of human PrP^{C} by bovine PrP^{Sc} is very low in transgenic mice and in vitro. The oral route of transmission for prion disease is less efficient than direct brain inoculation in all tested species. The incubation period of the illness is unknown; if kuru can provide any guidance, periods of 10 to 15 years are typical, and incubation up to 30 years has been recorded. An incubation period of 10 to 15 years is typical in peripheral inoculation of contaminated cadaveric human pituitary hormones. It is impossible to predict with confidence the final extent of the vCJD epidemic, although most analyses would suggest that the number of affected individuals will range from the dozens to hundreds, rather than thousands to millions. Canada is participating in an international surveillance program for vCJD.

I must emphasize that vCJD is a new entity. It is possible that all of our carefully garnered assumptions regarding the transmissibility of conventional CJD — such as the apparent low risk from blood and blood products — are not applicable to vCJD. Clearly, a great deal of work must be performed to define the medical, social and economic implications of this new human prion disease.

Transmission of prion disease by blood and blood products

It is well established that CJD can be transmitted iatrogenically:[18] by contaminated and inadequately sterilized neurosurgical instruments, by transplantation of dura mater onto the surface of the brain, by peripheral injections of growth hormone and gonadotropin prepared from cadaveric pituitaries and by corneal transplantation.

Can CJD be transmitted by blood and blood products? In an accompanying editorial in this issue (see page 1367) Dr. Maura Ricketts reviews the epidemiological evidence for blood-borne transmission of CJD; I will focus on the biological background of this question. Bioassay measurement of titres in tissues of experimentally infected animals reveals that the scrapie agent replicates first in the spleen and other lymphoid tissues, but eventually reaches highest titres in the brain before death.[19] Infectivity detected in the spleen by bioassay depends on the expression of PrP^{C} by spleen cells and does not accumulate by nonspecific "carry-over" from the original inoculum.[20] Within the cellular elements of blood, prion infectivity has been found predominantly or exclusively in mononuclear cells (lymphocytes and monocytes in distinction to granulocytes or erythrocytes[21]). Consistent with the absolute requirement for PrP^{C} in the generation of PrP^{Sc},[5,6] monocytes and lymphocytes express PrP^{C} as a surface membrane molecule, but mature erythrocytes and granulocytes do not.[22] Human CD34+ bone marrow multipotential stem cells — the progenitor cells for erythrocytes, granulocytes and mononuclear cells — also express cell surface PrP^{C}.[23] In addition, recent data have suggested that experimentally inoculated scrapie agent can be co-purified with some plasma proteins.[24]

In Canada, the Laboratory Centre for Disease Control (LCDC) has initiated a 3-year study of CJD and other TSEs to determine the risk of iatrogenic transmission of human prion diseases through the transfusion of blood or blood products. The study will include field interviews, hospital record extraction, human prion gene sequencing for familial disease and a neuropathology reference laboratory. All clinicians (neurologists, psychiatrists, geriatricians and neuropathologists) who encounter cases in which the differential diagnosis includes CJD are asked to contact the LCDC to report their cases (CJD study, tel 800 489-2999).

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Medical images

Focusing on new variant CJD

These images represent the pathological changes seen in the cerebral cortex of a person who died of new variant Creutzfeldt–Jakob disease. In the top image, hematoxylin–eosin staining reveals distinct "florid plaques" (arrows), characterized by a central eosinophilic core with a feathery peripheral ring surrounded by a halo of spongiform change. Spongiform change is also apparent in the surrounding neuropil (arrowheads). In the image below, immunoperoxidase staining reveals extensive deposition of protease-resistant prion protein, both in the florid plaques (arrows) and as small punctate deposits (arrowheads). (Original magnification x600, increased by 148%).

Histological material kindly provided by Dr. Catherine Bergeron, University of Toronto, from Dr. James Ironside, CJD Surveillance Unit, Edinburgh.