Recent developments in the pathogenesis, diagnosis, and therapy of prion diseases

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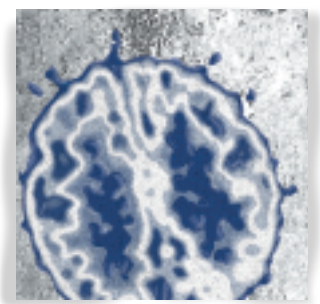

Prions continue to pose a formidable challenge to life sciences. While human prion diseases are still rare, the incidence of a new variant of Creutzfeldt-Jakob disease in the United Kingdom is increasing exponentially—raising fears that it might develop into a major epidemic. This disease is likely to represent the result of human infection with bovine prions. Therefore, understanding how prions replicate and damage the brain, and how their action may be possibly counteracted, has become a major public health issue. Here I examine some current hypotheses concerning the links between bovine and human prion diseases, and the mechanisms by which prions reach and damage the central nervous system after having entered the body at extracerebral sites.

Twenty years after the inferred beginning of the bovine spongiform encephalopathy (BSE) epidemic,[1,2] in-depth understanding of the impact of this zoonosis on human health has become more important than ever. As far as the actual status of BSE today goes, the news is mixed but not uniformly bad: the incidence of this disease within the British "national herd" (the total cattle population in the United Kingdom) reached a peak in 1992, and has declined ever since.[2,3] The first histopathological confirmation of BSE was reported in November 1986 for a case that had occurred in April 1985. Contaminated meat-and-bone meal (which had been used as a protein supplement in ruminant feed) was soon recognized as the predominant mode of transmission of the disease; this feeding practice was banned in 1988.

Given the average incubation time of the disease—which runs to several years—one could argue that the measures introduced were highly effective. However, there is much less reason to rejoice when one considers that several mathematical models proposed in the past few years predicted that the prevalence of the disease would level off to zero around the turn of the century—a prediction that has unfortunately proved untrue *(Figure 1)*.[4]

Switzerland, the country in which the authors of this article work, has the dubious privilege of being the nation with the largest number of reported BSE cases after the United Kingdom, Portugal, and Ireland.[5] Although the peak of the epidemic hit Switzerland some 3 years after it hit the United Kingdom, it has leveled off to relatively low but stable levels in the last 24 months. Of even more concern is the fact that in at least one country, ie, Portugal (as well as—at least formally—Ireland and France), the number of BSE cases is actually increasing.[6,7]

BSE and human prion diseases

While all of the above may be predominantly of concern to veterinary medicine, a peculiar new variant of Creutzfeldt-Jakob disease (CJD, the pendant to BSE in humans) was first described in 1996[8] and has, thus far, taken a toll of 83 lives *(Table I)*.[9] As detailed below, there is good reason to suspect that new-variant Creutzfeldt-

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Selected abbreviations and acronyms

BSE	*bovine spongiform encephalopathy*
CJD	*Creutzfeldt-Jakob disease*
ER	*endoplasmic reticulum*
FDC	*follicular dendritic cell*
LTβ	*lymphotoxin β*
nvCJD	*new-variant Creutzfeldt-Jakob disease*
PrP	*prion protein*
PrP^{C}	*normal prion protein*
PrP^{Sc}	*disease-associated prion protein*
sCJD	*sporadically occurring Creutzfeldt-Jakob disease*
TNF	*tumor necrosis factor*

Jakob disease (nvCJD) represents the result of infection of humans with the BSE agent.

Several striking characteristics of nvCJD set it aside from the "classical" sporadically occurring Creutzfeldt-Jakob disease (sCJD) that was described eight decades ago *(Table II)*.[10,11] For one thing, sCJD typically affects elderly persons, whereas nvCJD has so far predominantly hit very young people, with an age range spanning between 12 and 52 years. The reason for this age distribution remains unclear.[12] Also, the clinical course of the two diseases is radically different: sCJD is typically a rapidly progressing illness leading to severe dementia and ultimately death within months, and sometimes even weeks. On the other hand, nvCJD tends to develop over a much more protracted period. Also, the predominant initial symptoms in nvCJD are personality changes and psychosis, rather than dementia.[13]

Even under the microscope, the two diseases are very different from each other. sCJD is typically characterized by

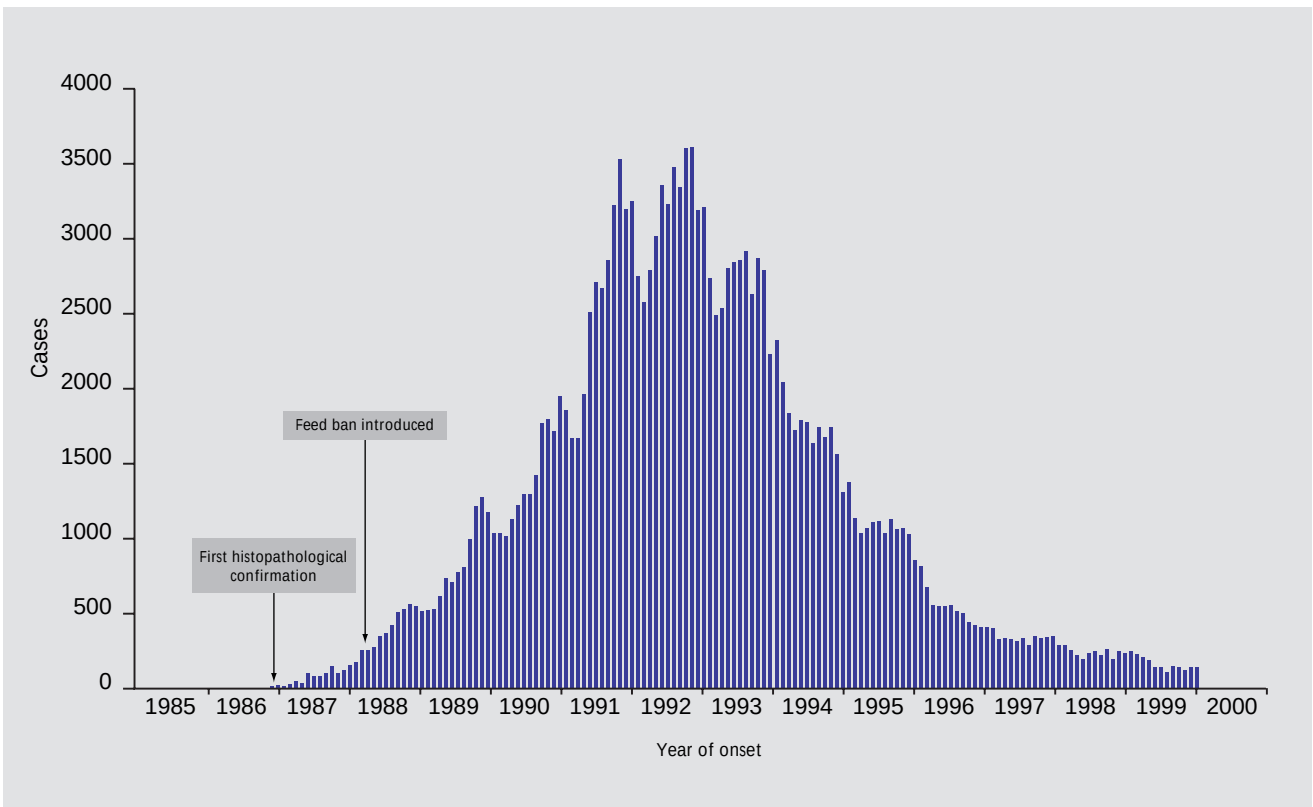

Figure 1. Confirmed cases of bovine spongiform encephalopathy (BSE) plotted by month and year of onset. Data valid to end of January 2000 (produced in June 2000). The good news is that the epizootic has been receding ever since 1992. The not-so-good news is that, despite several predictions, the incidence has not reached zero, and appears to be leveling off asymptotically at a low, but measurable, height. Reproduced from reference 3: http://www.maff.gov.uk/animalh/bse/bse-statistics/graph/epidem.pdf. Copyright © 2000, Ministry of Agriculture, Fisheries and Food, UK.

Year	sCJD	nvCJD*	Total**
1985	26		28
1986	26		26
1987	23		24
1988	22		24
1989	28		32
1990	28		33
1991	32		36
1992	43		51
1993	38		46
1994	51		59
1995	35	3	47
1996	40	10	60
1997	59	10	80
1998	63	18	88
1999	61	14	83
2000	25	27	52

Table I. Incidence of new-variant Creutzfeldt-Jakob disease (nvCJD) in the United Kingdom since 1985. Although data for 2000 were incomplete at the time of writing, the incidence of nvCJD appears to have surpassed that of sporadically occurring Creutzfeldt-Jakob disease (sCJD) for the first time.[9]
*Including probable deaths awaiting postmortem results, and living sufferers of probable nvCJD.
**Including hereditary and iatrogenic cases (not shown here).
Reproduced from reference 9:
http://www.doh.gov.uk/cjd/stats/sept00.htm.

I	• Progressive neuropsychiatric disorder
	• Duration of illness >6 months
	• Routine investigations do not suggest an alternative diagnosis
	• No history of potential iatrogenic exposure
II	• Early psychiatric symptoms
	• Persistent painful sensory symptoms
	• Ataxia
	• Myoclonus or chorea or dystonia
	• Dementia
III	• Electroencephalogram does not show the typical appearance of sCJD (or no electroencephalogram performed)
	• Bilateral pulvinar high signal on MRI scan
IV	• Positive tonsil biopsy

Table II. Diagnostic criteria for new-variant Creutzfeldt-Jakob disease (nvCJD).[9]
MRI, magnetic resonance imaging; sCJD, sporadically occurring Creutzfeldt-Jakob disease.
Reproduced from reference 9:
http://www.doh.gov.uk/cjd/stats/sept00.htm.

widespread vacuolation of the cortical neuropil, which, in its most extreme manifestation, makes the brain resemble a sponge (when observed under low-magnification microscopy), hence the designation "spongiform encephalopathy." Instead, the hallmark of nvCJD is the extremely prominent accumulation of small spherical protein deposits, termed plaques, in the brain of the affected individual. While some plaques can be seen in a minority of patients affected with sCJD, the plaques of nvCJD have a specific morphology that includes a characteristic rim of microvacuolated tissue. Further peculiarities of nvCJD include severe destruction of neurons in the thalamus, which is recognizable by noninvasive neuroimaging methods (the so-called pulvinar sign),[14] as well as generalized colonization of the lymphoid organs by the infectious agent and deposition of the disease-associated prion protein (PrP) known as PrP^{Sc} (see below) in the germinal centers of the lymph nodes, tonsils, and spleen.[15-17]

Finally, the new disease has so far exclusively struck patients whose prion gene homozygously encodes methionine at position 129. This group represents only one third of the population[18] and approximately two thirds of sCJD patients in the authors' experience.

For the sake of completeness, it has to be said that CJD of very young people is not a totally new phenomenon. In the last 20 years, development of the disease has been recorded in almost 100 children and teenagers. However, in the overwhelming majority of cases, this was the result of documented iatrogenic exposure to the agent, typically brought about by administration of growth hormone or pituitary gonadotrophins of cadaveric origin, which had been (sometimes liberally) administered to treat pituitary dwarfism and other conditions in the era preceding recombinant DNA technology.

What are the lines of evidence that the agent causing nvCJD is identical to that of BSE when transmitted to humans? None of the arguments that have surfaced to date are completely conclusive—yet each of them is certainly tantalizing, particularly when all are considered together. For one thing, much effort has been invested in attempts to characterize the "strain properties" of the agent affecting cows and humans. Because the molecular substrate underlying the nature of prion strains (which are inheritable phenotypic traits that can be reproduced upon serial passage through experimental animals) is not known, strain typing of prion has to rely on surrogate markers.

Two such markers have proved particularly useful. One is the distribution of vacuoles in the brain of affected animals: some strains will mainly target, for example, the cortical cerebral ribbon, while others will predominantly

affect the midbrain.[19] The BSE prion strain was shown to virulently and consistently attack the dorsal medulla and the superior colliculus (part of the optical pathway).[20] A careful study of these parameters yielded the disquieting result that BSE prions extracted from the brains of affected cows and nvCJD prions derived from the brains of British patients do indeed produce the same lesional patterns when transmitted to panels of susceptible mice.[20-22]

The second marker for strain typing of prions comes from the analysis of the biochemical properties of the disease-associated prion protein recovered from the brain of cattle and humans. These studies take advantage of the fact that different steric conformations (which, according to the most popular current hypothesis, account for the phenotypic strain properties) will expose different sites of the protein to the action of proteolytic enzymes, which, in turn, can be identified by the different molecular weights of the resulting fragments. When used in conjunction with the ratio of diglycosylated to monoglycosylated prion protein—another parameter that appears to correlate with strain properties—these traits were again indistinguishable between BSE and human nvCJD prions.[23,24]

A third line of argument relating BSE and human nvCJD concerns the epidemiology of the disease. To date, the total number of definite and probable cases of nvCJD amounts to 82 in the United Kingdom, 1 in Ireland, and 2 in France.[25] Assuming that the quality of the epidemiological surveillance is similar in these countries and in the rest of Europe, which has not reported cases of nvCJD, the conclusion that its incidence correlates with the prevalence of BSE is unavoidable.

One of the most powerful arguments for the pathogenesis comes from primate studies. In a classic experiment, the French group of Lasmézas and colleagues inoculated brain extracts from BSE-affected cows into cynomolgus macaques. After approximately 3 years, all inoculated primates (2 adults and 1 infant) developed spongiform encephalopathy. The histopathological appearance of the disease was identical to that of nvCJD and included the characteristic "florid plaques," which are never present in sCJD, but have been recognized in every single case of nvCJD.[26]

As impressive as the above list of arguments may seem, there is no denying that each is phenomenological rather than causal. Distribution of histopathological lesions, as well as morphology of plaque deposits, is certainly way downstream of the molecular events responsible for prion strain specificity. Measurements of the "glycotype ratio" may be more directly related to the essence of strains, but there is still no way to tell whether they may represent mere surrogate markers.

It is desirable to measure the conformation of the disease-associated prion protein in a more direct way. Some inroads have been made toward that goal with a method that exploits the relative affinities of antibodies against the normal mammalian prion protein (anti-PrP^C),[27,28] but to our knowledge this possibility is, so far, restricted to the differentiation of mouse and hamster prion proteins, and has not yet been applied to investigating BSE and nvCJD.

The question on everybody's mind, however, is how the numbers of nvCJD victims will change in the future. As terrible as the disease has been for the patients affected thus far, we have not yet seen a large-scale epidemic. Although many mathematical models have been generated,[29,30] the number of cases that have been identified so far is still too small to gain any certainty about future developments. That the number of cases diagnosed in the last 12 months amounts to 36 (up from 14 in the 12 months before that) is certainly cause for concern, if not for alarm.[31]

Another extremely important question relates to the possible existence of chronic subclinical disease, and the possibly of a permanent carrier status—in cows as well as in humans. Evidence that such a carrier status may be produced by the passage of the agent across species was first reported by Race and Chesebro,[32,33] and has recently been confirmed—at least for the passage between hamsters and mice.[34] Immune deficiency can also lead to a similar situation in which prions replicate silently in the body, even when there is no species barrier.[35] These hints may imply that the problem of animal transmissible spongiform encephalopathies (TSEs) could be more widespread than generally assumed, and may call for drastic measures in the realm of farming. It is not impossible that humans carrying the agent may transmit it horizontally.[36] The risks associated with the latter possibility can be met competently only if knowledge is accrued about the mode of transmission of the agent and the mechanism by which prions reach the brain upon peripheral inoculation into extracerebral sites. The rest of this review article is devoted to analyzing the progress that has been made in these fields.

The making of prions

A noncommittal, operational definition[37] says that the prion is the infectious agent that causes scrapie, BSE,

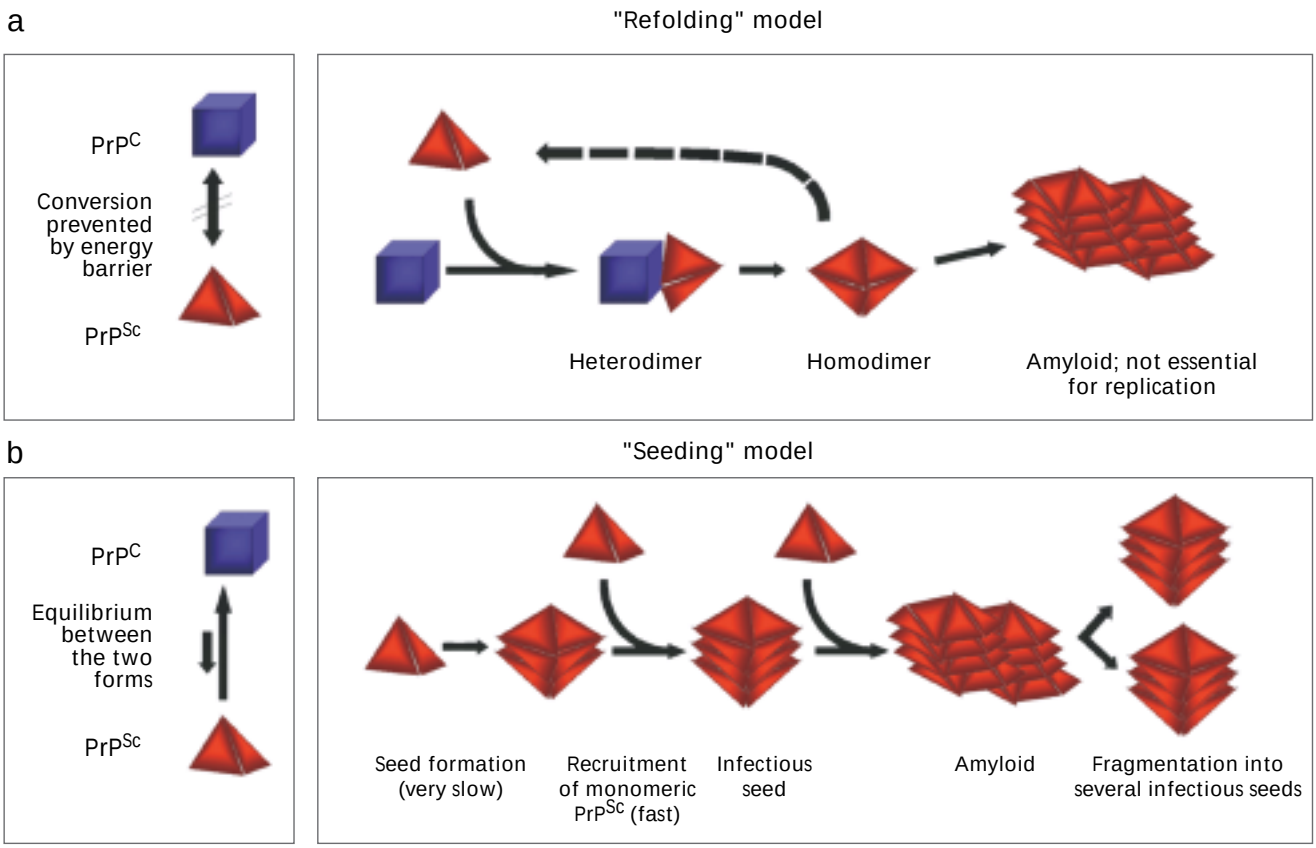

Figure 2. The "protein only" hypothesis and two popular models for the conformational conversion of normal prion protein PrP^{c} into disease-associated prion protein PrP^{Sc}. (a) The "refolding" or template assistance model postulates an interaction between exogenously introduced PrP^{Sc} and endogenous PrP^{c}, which is induced to transform itself into further PrP^{Sc}. A high energy barrier may prevent spontaneous conversion of PrP^{c} into PrP^{Sc}. (b) The "seeding" or nucleation-polymerization model proposes that PrP^{c} and PrP^{Sc} are in a reversible thermodynamic equilibrium. Only if several monomeric PrPSc molecules are mounted into a highly ordered seed, can further monomeric PrP^{Sc} be recruited and eventually aggregate to amyloid. Within such a crystal-like seed, PrP^{Sc} becomes stabilized. Fragmentation of PrP^{Sc} aggregates increases the number of nuclei, which can recruit further PrP^{Sc} and thus results in apparent replication of the agent.

CJD, other TSEs, such as chronic wasting disease of mule deer and elk, and other less common diseases that affect, for example, exotic ungulates and captive felids. Obviously, although this definition is useful in that it facilitates understanding, it says nothing about the true physical nature of the agent. A very different definition that has become rather popular among yeast geneticists centers around the structural biology of prions. According to this second definition, prions are proteins that can exist in at least two different conformations, one of which is capable of inducing the conversion of further individual prion molecules from one conformation into the other. Therefore, prion proteins can serve as true genetic elements even if they do not contain informational nucleic acids, in that they are self-perpetuating and heritable.[38] Nineteen years after the original formulation of the prion hypothesis by Stanley Prusiner *(Figure 2)*, and 4 years after he was awarded the Nobel Prize in 1997, there continues to be uncertainty about the question of whether these two definitions coincide in the case of mammalian prions. One further problem is that all amyloids and their precursors would fit the second definition, yet amyloid proteins themselves do not appear to be transmissible or infectious in vivo or in cell cultures. In the last few months, we have witnessed breathtaking advances in the understanding of prion phenomena in yeast, and there is no doubt that at least two yeast proteins exist that fulfill the above definition. It is generally

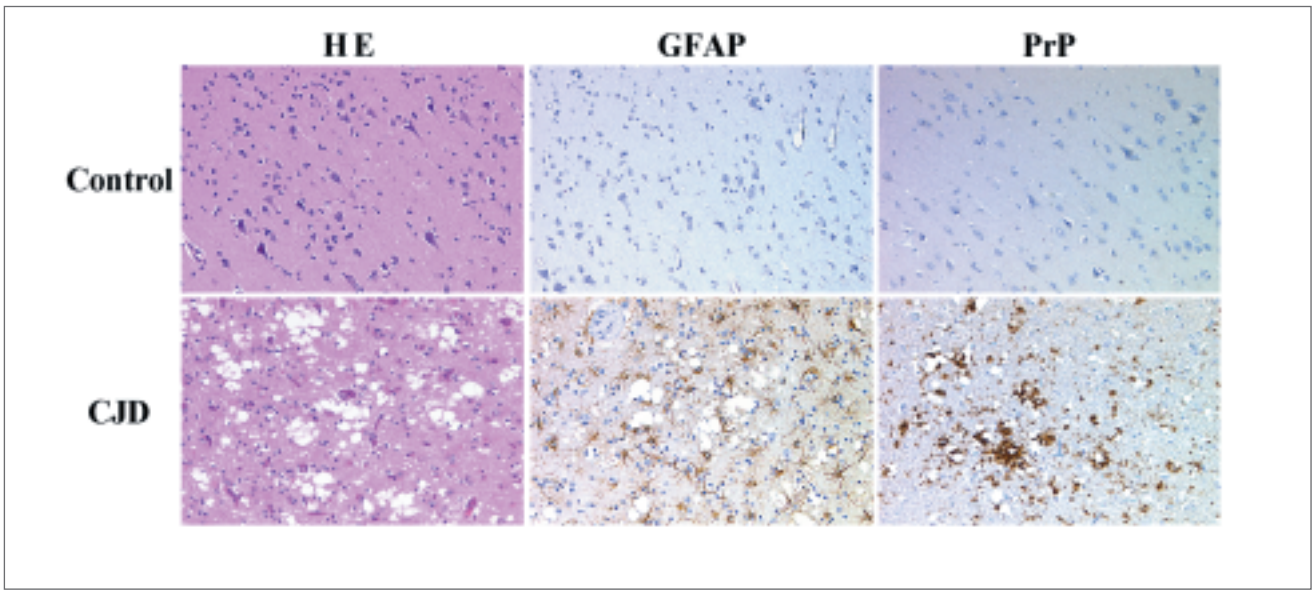

Figure 3. Neuropathological features of transmissible spongiform encephalopathies. Histology and immunohistochemical analysis of frontal cortex samples of the brain of a patient who died of noncerebral causes (upper row) and a patient suffering from Creutzfeldt-Jakob disease (CJD) (lower row). Brain sections were stained with hematoxylin-eosin (HE, left panels), with antibodies against glial fibrillary acidic protein (GFAP, middle panels), and with antibodies against the prion protein (PrP, right panels). Neuronal loss and prominent spongiosis are visible in the HE stain. Strong proliferation of reactive astrocytes (gliosis) and perivacuolar PrP deposits are detectable in the GFAP and PrP immunostains of the CJD brain samples.

believed that the ultimate experiment proving that a given protein is a prion is "in vitro conversion": this term defines a cell-free manipulation by which the noncontagious conformation is transformed into a transmissible agent. Ideally, this manipulation should occur without participation of the pathological, transmissible prion, in order to formally exclude the possibility of cross-contamination. Two recent papers have shown that these conditions can be met in the case of the yeast prions identified so far, Sup35[39,40] and Ure2p.[41,42]

Disappointingly, no such successes have ever been reported in the case of mammalian prions, despite the most intensive of efforts. In vitro conversion of the normal mammalian prion protein, PrP^{C} (C for cellular), can yield a moiety that displays many of the physical and chemical properties characteristic of the disease-associated prion protein, PrP^{Sc} (Sc for scrapie), such as aggregation into higher-order quasi-crystalline complexes that are birefringent when observed under polarized light (especially upon staining with amyloid dyes, such as Congo red), formation of fibrils that are identifiable by electron microscopy, and partial resistance to proteolytic enzymes as identified by digestion with proteinase K.[43] Intriguingly, in vitro conversion is subject to a distinct species barrier, just like "true" spongiform encephalopathies.[44,45]

However, the crucial element that is common to the two definitions mentioned above, and that is absolutely required for the classification of a protein as a prion, is transmissibility. None of the experimental procedures that have been reported thus far have unambiguously accomplished the transformation of the cellular prion protein PrP^{C} into a transmissible agent. There is no dearth of speculations about why this has not been possible: the requirement for additional cellular factors distinct from PrP^{C}, for example, has been invoked on the basis of genetic evidence,[46] but has never been proven. Universal consensus about the nature of the agent will predictably only be reached once synthetic reconstitution from noninfectious material will have been achieved.

How prions damage their hosts

Notwithstanding all the unresolved problems, a number of important properties of the infectious agent can be studied, even in the absence of ultimate certainty about its true physical nature. Perhaps the most obvious ques-

tion regards what accounts for the exquisite propensity of prions to damage the central nervous system (CNS), the only part of the body undergoing histopathologically and clinically detectable degeneration *(Figure 3)*.
Cellular models of prion disease may prove very useful for addressing this question. However, prions replicate inefficiently in most established cell lines. A large number of studies have been performed with a synthetic peptide obtained from the central region of the PrP^{C} molecule, which has been shown to spontaneously assemble into amyloid-like structures. Interestingly, this peptide can elicit in vitro many reactions of brain cells that resemble those seen in vivo during the late stages of prion disease: activation of microglia cells, stimulation of intermediate filament production by astrocytes, and even death of neurons, which appears to depend on the presence of the normal prion protein in target cells.[47,48]
Despite the amount of information that has been accrued, all of these studies suffer from the fundamental problem that it is not clear whether the phenomenon observed in conjunction with exposure of cells to this small amyloidogenic peptide bear much relevance to what is happening in vivo during the course of prion replication—a process that may arguably be very different. Moreover, some of the published data have recently been challenged.[49]
In order to ask the simple question of whether cerebral accumulation of PrP^{Sc} in the extracellular space suffices to damage nerve cells, we have undertaken fetal neuroectodermal transplantation experiments.[50] Histological analysis of PrP-deficient mice that had been grafted with brain cells derived from transgenic mice overexpressing PrP^{C} and subsequently infected with prions indicated that pathology is confined to the regions of the brain that express PrP^{C}. In the surrounding PrP-deficient brain, no pathological changes could be detected even though substantial accumulations of pathological PrP^{Sc} occurred.[50] While the interpretation of this experiment is liable to certain caveats (most notably the possibility that a threshold concentration of PrP^{Sc} is needed for induction of neurodegeneration and is not attained outside the grafted tissue), it is difficult to avoid the conclusion that the neuronal cytotoxicity of PrP^{Sc} is dependent on the expression of cellular PrP^{C} by target cells. Why should that be? Perhaps PrP^{C} acts as a receptor for PrP^{Sc}. However, it has never been possible to demonstrate an affinity between these two moieties. Alternatively, the conversion process of PrP^{C} into PrP^{Sc} itself, rather than exposure to the disease-associated prion protein, may constitute the primary deleterious event.
The latter possibility has been thoroughly investigated in a series of elegant papers by Lingappa and coworkers. These authors have identified an atypical form of PrP^{C} that undergoes a peculiar biogenesis *(Figure 4)*.[51-54] Most cellular PrP^{C} is secreted into the lumen of the endoplasmic reticulum (ER) by virtue of its secretory signal peptide, where it is routed to the cell surface as a glycophosphoinositol-linked membrane-associated protein. However, a small proportion of PrP^{C} remains stuck in the ER membrane as a transmembrane protein. Depending on their orientation, Lingappa and coworkers have termed these proteins ^{Ctm}PrP and ^{Ntm}PrP (for carboxy- and amino-terminal transmembrane, respectively).[55,56] By quantifying the production of ^{Ctm}PrP in pathological conditions, they found that it correlates very well with the neurodegenerative changes—in fact much better than the accumulation of PrP^{Sc} itself.[51,57] These observations formed the basis for the hypotheses that (i) ^{Ctm}PrP is a marker of prion-induced neurodegeneration; and (ii) the conversion process of PrP^{C} into PrP^{Sc} triggers the formation of ^{Ctm}PrP, which, in turn, is an effector of neurotoxicity. Strong circumstantial evidence favors the second hypothesis, although the putative signaling pathways involved are still completely obscure.

The neuroinvasion of prions

In most cases of prion infection of humans and animals, the port of entry is extraneural. In the case of BSE (and possibly of nvCJD), exposure is probably oral, while most iatrogenic cases of CJD have occurred by parenteral administration (for example, intramuscular injection). The mechanism by which prions administered to the periphery of the body reach the CNS are therefore of great interest. By analogy with neurotropic viruses, there may be two main pathways of neuroinvasion. Many viruses, for example, those causing rabies and herpes, exploit the anatomical connections provided by peripheral nerves, and reach the CNS via axonal transport. Human immunodeficiency virus (HIV), however, utilizes a totally different mechanism: it reaches cerebral microglial cells using a "Trojan horse" mechanism that involves infection of macrophages. The latter cells are in equilibrium with perivascular microglia and are the prime target of HIV infection in the CNS.
What about prions? The available evidence suggests that

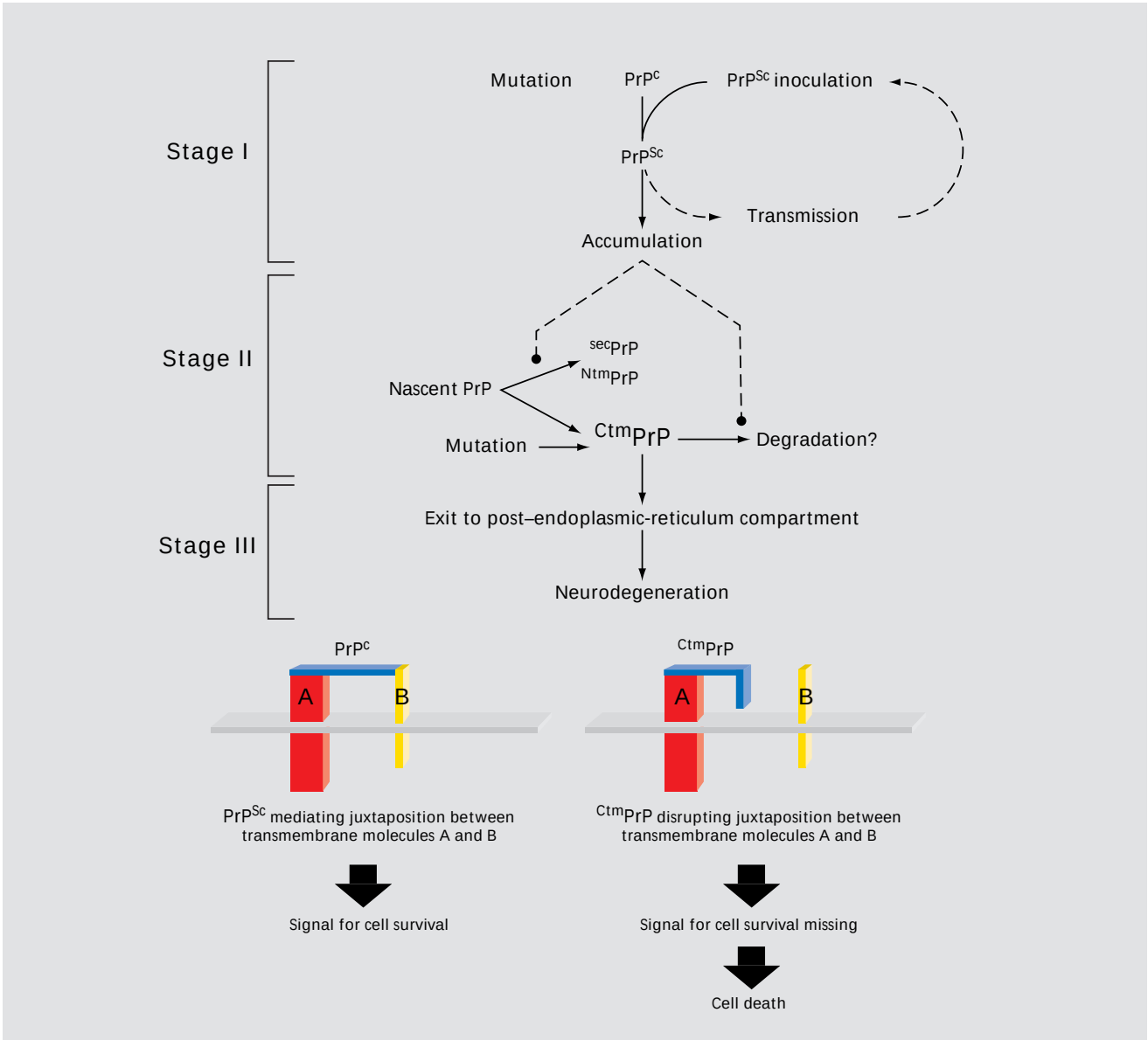

Figure 4. *Upper panel:* Three-stage model of prion pathogenesis (adapted from reference 51). Stage I represents the formation and accumulation of disease-associated prion protein PrP^{Sc}, initiated by either inoculation or spontaneous conversion of a mutated normal prion protein PrP^{C} into PrP^{Sc}. Stage II symbolizes the events involved in generating C-transmembrane prion protein ^{Ctm}PrP (see below), either by an unknown process involving PrP^{Sc} (characterized by dashed lines) or by certain mutations within PrP. Two distinct forms of PrP can be made at the endoplasmic reticulum (ER): one that is fully translocated (^{sec}PrP) and one that is transmembrane. Digestion with proteases of the latter results in two fragments: one is carboxy-terminal derived and glycosylated, the other is amino-terminal–derived and unglycosylated; thus, the first is termed C-transmembrane PrP (^{Ctm}PrP) with the carboxy-terminus in the ER lumen and the amino-terminus accessible to proteases in the cytosol, whereas the second is termed N-transmembrane PrP (^{Ntm}PrP) with an opposite conformation. Stage III depicts the hypothetical events involved in ^{Ctm}PrP-mediated neurodegeneration, possibly involving the exit of ^{Ctm}PrP to a post-ER compartment.
Lower panel: Possible role of ^{Ctm}PrP in cell death (adapted from reference 52): full-length PrP may act as a coreceptor on the cell surface, mediating the juxtaposition of two cell-surface transmembrane molecules A and B. This generates a signal for cell survival in the cytosol. Failure of ^{Ctm}PrP to bind B could induce cell death by not facilitating the association of A to B. This mechanism also could explain effects of expression of an amino-terminally truncated PrP^{53} as well as the Doppel gene product.[54]

both of these pathways may play a role. A wealth of evidence gathered in the last two decades indicates that prions are capable of colonizing the immune system; lymphocytes[58] and follicular dendritic cells (FDCs)[59] (which are located in the germinal centers of lymphoid organs) express sizable amounts of PrP^C. Blättler and colleagues have shown that extracerebral prion protein is required for neuroinvasion: *Prn-p* knockout mice harboring a PrP^C-expressing graft in their brain[50] consistently develop spongiform encephalopathy restricted to the neuroectodermal graft upon intracerebral inoculation,[60] but not upon intraocular, intraperitoneal, or even intravenous administration of the infectious agent.[61] At least in the case of intraocular inoculation, impairment of neuroinvasion is effected even when a specific transgenic manipulation prevents all antibodies against PrP^C from being generated.[62] Therefore, the absence of PrP^C, rather than an immune response against prions, prevents spread of the infectious agent within the body of a PrP^C-deficient mouse.[63]

From spleen to brain

The next obvious question relates to the identity of the cellular compartment that necessitates expression of PrP^C in order to support neuroinvasion. Reconstitution of the hematopoietic and lymphopoietic system with stem cells derived from wild-type or transgenic mice overexpressing PrP^C does not suffice to restore neuroinvasion,[64] implying that the crucial compartment that needs to express PrP^C is sessile and cannot be transferred by adoptive bone marrow reconstitution.[61] While the latter, negative results do not allow for unequivocal identification of the responsible compartment (for which there is a choice of at least two likely candidates, ie, peripheral nerves[65] and FDCs), titration experiments indicate that adoptive bone marrow transfer robustly reconstitutes the capability of the spleen to accumulate (and perhaps replicate) prions of the Rocky Mountain Laboratory (RML) strain after intraperitoneal inoculation.[61] This latter result was unexpected, and may suggest that hematopoietic cells (perhaps lymphocytes) may replicate prions, or may be otherwise involved in the transport of the agent from the site of inoculation to the spleen. Brown and colleagues have recently reported that, using a different prion strain called ME7, no accumulation of prions was detected in spleens of 13 PrP^C knockout mice reconstituted with PrP^C-positive hematopoietic cells and killed at unspecified "intervals through the incubation period."[66] Our laboratory has therefore repeated the experiments published previously and confirmed their unambiguous reproducibility in a large-scale study involving assessment of prion titers and PrP^{Sc} accumulation at 30, 60, 90, 120, and 270 days after inoculation in mice (P. Käser et al, unpublished results). Assuming that the experimental design of the Zurich and the Edinburgh studies is indeed comparable, the discrepancy between the Blättler results and those reported by Brown point to the possibility that different prion strains exhibit different tropisms for specific components of the immune system. There may be precedents for this: BSE prions are hardly detectable in lymphoid organs (with the possible exception of gut-associated lymphoid tissue for a transient period of time), while nvCJD prions extensively colonize human lymphoid organs. The identification of the molecular determinants of such differences in organ tropism may shed light on a basic mechanism of prion pathogenesis, and is also of prime public health interest for the reasons detailed above.

Anatomy of prion neuroinvasion

What are the cellular requirements for the lymphoinvasion of prions? This question was addressed by screening mouse strains with spontaneous and engineered deficiencies in various compartments of the immune system. From these studies, one clear-cut result emerged: any genetic defect that impairs the terminal differentiation of B lymphocytes completely blocks the colonization of lymphoid organs by prions, as well as the development of disease in the CNS upon peripheral inoculation.[67] This phenomenon is obviously due to a block of neuroinvasion, since B-cell–deficient mice display the same susceptibility to disease as wild-type mice when inoculated intracerebrally.[67] The dependence of neuroinvasion on B lymphocytes is absolute: we are not aware of any experimental design, mouse strain, or prion strain in which the spread of prions from the periphery to the CNS is not impaired by the ablation of B lymphocytes.
The necessity for the presence of B cells does not imply that they are sufficient for neuroinvasion. However, all attempts to identify additional necessary compartments have yielded less unambiguous results. A further candidate that is most likely required for neuroinvasion is certainly the FDC. FDCs have long been identified as the main site of accumulation of PrP^{Sc} in lymphoid organs.[59] However, experiments aimed at exploring the role of FDCs in peripheral prion pathogenesis have been less conclusive. So far, all the published material unanimously

indicates that accumulation of prions of intraperitoneally (IP) inoculated mice can only occur in spleens that have properly formed germinal centers and immunohistochemically identifiable FDCs: it has proven impossible to recover prions from spleens of IP inoculated mice deficient in tumor necrosis factor (TNF) receptor-1 (TNFR1)[67] (M. A. Klein et al, unpublished data) or TNF-α,[66] none of which contain identifiable FDCs in their spleens. In the case of the FDC-deficient lymphotoxin β (LTβ) knockout mice,[68] splenic infectivity was unfortunately not determined. Moreover, administration of soluble lymphotoxin β receptor (LTβR) very efficiently prevents the buildup of a splenic prion burden in wild-type mice,[69] a fact that was later confirmed to also be valid for the ME7 prion strain for scrapie,[70] despite its many alleged differences from the RML strain.

On the other hand, neuroinvasion—the development of brain disease after peripheral challenge—was completely unaffected in TNFR1[67] and LTβ[68] knockout mice, and could not even be fully repressed by the LTβR-Fc treatment.[69,70] Therefore, while the lack of LTβ signaling to FDCs is likely to account for some of the protection from peripheral prion inoculation observed in B-cell–deficient mice, all of the latter results point to an additional role of B cells in prion neuroinvasion, which is clearly independent of PrP expression[71] and must be distinct from LTβ/TNF signaling to FDCs. Because sympathetic nerve fibers do not appear to penetrate the germinal centers of lymphoid organs (M. Glatzel and A. Aguzzi, unpublished observation), lymphocytes may conceivably play a role in the migration of prions from FDCs to peripheral nerves.

Prions and blood

Because prions can be detected in lymphoreticular tissues of nvCJD patients, is there a risk of iatrogenic transmission via exposure to blood or tissues derived from preclinical nvCJD cases, and possibly from contaminated surgical instruments? Very thorough epidemiological surveys over two decades have not implicated blood transfusions or administration of blood products as risk factors for prion diseases. A small increase in relative risk for sCJD is associated with a history of surgery of all kinds,[72] and may indeed be indicative of unrecognized iatrogenic transmission.

However, the situation may not be as simple for nvCJD. For one thing, we do not know nearly as much about the epidemiology and iatrogenic transmissibility of this new disease as we do about sCJD. What is most unsettling is that the distribution of preclinical disease in the United Kingdom and other countries is totally obscure. The only available information is a retrospective immunohistochemical analysis of British appendices and tonsils[73]—a well-meant study, but of limited sensitivity.

Moreover, nvCJD appears to be much more "lymphoinvasive" than sCJD. In particular, nvCJD prions can be easily detected in lymphatic organs such as tonsils and appendix,[15,16,74] a fact that was previously demonstrated to be true for scrapie,[75-77] but not for sCJD prions. While all the available evidence points to FDCs as the prion reservoir in lymphatic organs, splenic lymphocytes of experimentally inoculated mice can be infected with prions.[78] Although prion infectivity of circulating lymphocytes appear to be at least two logs lower than that detected in splenic lymphocytes,[78] the possibility that circulating lymphocytes may be in equilibrium with their splenic counterparts calls for cautionary measures. The nature of the latter is matter of controversy: leukodepletion has been advocated, but there is still no certainty about its efficacy. In addition, even if blood prion infectivity were to be originally contained in lymphocytes in vivo, lysis of cells may lead to contamination with infectious "microparticles,"[78] which may be difficult to remove by any method, short of ultracentrifugation. On a more positive note, however, many of the virus removal steps involved in the manufacturing of stable blood products have some positive effects on prion removal. Therefore, the latter possibility can be regarded as a worst-case scenario.

A last consideration applies to secondary prophylaxis. Given the very large amount of infectious BSE material that has entered the human food chain, it is possible that many individuals harbor preclinical nvCJD. It is imperative and urgent to develop strategies that will help control spread of the agent and that will hopefully prevent the clinical outbreak of symptoms in these persons, and some promising approaches have been identified.[80,81] Possible targets for the interference with neuroinvasion are rate-limiting processes that control prion replication within the infected individual. In light of the knowledge discussed above, treatments that target the neuroimmune interface of prion replication and neuroinvasion[82] continue to represent a promising area for research aimed at postexposure prophylaxis. ❑

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Desarrollos recientes en la patogénesis, el diagnóstico y el tratamiento de las enfermedades causadas por priones.

Los priones continúan presentando un gran desafío para las ciencias biológicas. Aunque las enfermedades humanas por priones son todavía poco frecuentes, la incidencia de una nueva variante de la Enfermedad de Creutzfeldt - Jakob en el Reino Unido está aumentando exponencialmente, lo que hace temer que se pueda transformar en una gran epidemia. Es probable que esta enfermedad represente el resultado de una infección humana por priones bovinos. Por lo tanto, la comprensión del modo cómo los priones se replican y dañan el cerebro, y cómo su acción pudiera ser posiblemente contrarrestada, ha llegado a constituir un tema importante de salud pública. Aquí yo reviso algunas hipótesis actuales que se vinculan con las conexiones entre las enfermedades por priones de humanos y bovinos, y los mecanismos a través de los cuales los priones alcanzan y dañan el sistema nervioso central después de haber ingresado al cuerpo humano por sitios extracerebrales.

Développements récents dans la pathogenèse, le diagnostic et le traitement des maladies provoquées par les prions

Les prions continuent de représenter un défi redoutable pour les sciences de la vie. Alors que les maladies provoquées par les prions restent rares, l'incidence d'une nouvelle variante de la maladie de Creutzfeldt-Jakob au Royaume-Uni est en train d'augmenter de façon exponentielle, suscitant la crainte qu'elle pourrait évoluer vers une épidémie majeure. Cette maladie est probablement le résultat de l'infection humaine par les prions bovins. La compréhension du mécanisme par lequel le prion se réplique et lèse le cerveau, ainsi que des moyens possibles pour contrecarrer son action est de ce fait devenu un problème majeur de Santé publique. Cet article examine quelques-unes des hypothèses actuelles concernant les liens entre les maladies transmises par les prions chez l'homme et les bovidés, et les mécanismes par lesquels les prions atteignent le système nerveux central pour y provoquer des lésions après avoir pénétré dans le corps par des foyers extra-cérébraux.

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