PRIONS: HEALTH SCARE AND BIOLOGICAL CHALLENGE

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Although human prion diseases are rare, the incidence of 'new variant' Creutzfeldt–Jakob disease in the United Kingdom is increasing exponentially. Given that this disease is probably the result of infection with bovine prions, understanding how prions replicate — and how to counteract their action — has become a central issue for public health. What are the links between the bovine and human prion diseases, and how do prions reach and damage the central nervous system?

EPIZOOTIC
Refers to a disease that is temporally prevalent and widespread in a population of animals.

PRION-ONLY HYPOTHESIS
States that the prion is devoid of informational nucleic acid and consists of protein (or glycoprotein) as its essential pathogenic component.

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Twenty years after the inferred beginning[1,2] of the bovine spongiform encephalopathy (BSE) epidemic, in-depth understanding of the effects of this EPIZOOTIC on human health has become more important than ever. The incidence of BSE in the British 'national herd' (the total cattle population in the United Kingdom) reached a peak in 1992 and has declined 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 food) was soon recognized as the main mode of transmission of the disease, and this feeding practice was banned in 1989.

Given the average incubation time of the disease — a matter of years — one could argue that the measures introduced were highly effective. However, several mathematical models proposed over the past 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. Projections were highest if the agent was assumed to transmit horizontally, and — counterintuitively — lowest if maternal transmission was assumed (FIG. 1).

Switzerland has the dubious privilege of being the nation with the largest number of reported BSE cases after the United Kingdom (and, recently, Portugal and Ireland)[4]. Although the peak of the epidemic hit Switzerland some three years after it hit the United Kingdom, it has leveled off to relatively low but stable levels over the past 24 months. Of even more concern is the fact that in Portugal, Ireland, France and, most recently, Germany, the number of BSE cases is actually increasing[5] (and see link to the World Organization for Animal Health), and that BSE is now being seen in BARB ('born after the real ban') cows that were born after 1996 — when a very rigid 'zero tolerance' meat-and-bone meal ban became effective.

The molecular basis of prion diseases

To understand why this is happening, much research is going into the molecular events that underlie replication of the infectious agent — the prion. According to the terminology adopted here, the term 'prion' does not have structural implications, other than that a protein is an essential component. This can be defined[6] as the agent that causes BSE, scrapie (a prion disease in sheep), Creutzfeldt–Jakob disease (CJD) in humans, and other transmissible spongiform encephalopathies, such as chronic wasting diseases of mule deer and elk, and other less common diseases that affect exotic ungulates such as kudu and nyalas, and captive large cats. This definition has been useful to foster understanding, but it says nothing about the true physical nature of the agent. A different definition that has become popular among yeast geneticists centres on the structural biology of prions. According to this second definition, prions are proteins that can exist in at least two conformations, one of which can induce the conversion of further prion molecules from one conformation into the other. So prion proteins can act as true genetic elements — even though they do not con-

tain informational nucleic acids — in that they are self-perpetuating and heritable[7].

Eighteen years after Stanley Prusiner formulated his PRION-ONLY HYPOTHESIS (FIG. 2) — for which he was awarded the Nobel Prize in 1997 — there is still uncertainty as to whether these two definitions coincide in the case of mammalian prions, as it has not yet been unequivocally established that the disease-associated protein isolated by Prusiner and termed PRP^{Sc} represents the infectious agent. Another problem is that, although all AMYLOID PROTEINS and their precursors would fit the second definition, these proteins do not seem to be transmissible or infectious *in vivo* or in cell culture. Although several yeast proteins have been shown to fulfil both of these criteria (BOX 1), no such successes have been reported for mammalian prions.

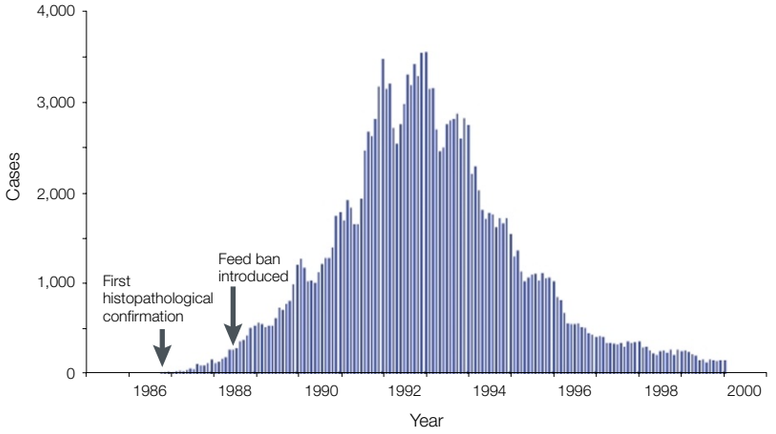

Figure 1 | **Confirmed cases of BSE plotted by month and year of onset.** Data valid to end of January 2000 (produced in June 2000). The good news is that the epizootic has been receding since 1992. The not-so-good news is that, despite several predictions, the incidence has not reached zero, and seems to be levelling off asymptotically at a low, but measurable, height. (Source: British Ministry of Agriculture, Fisheries and Food.)

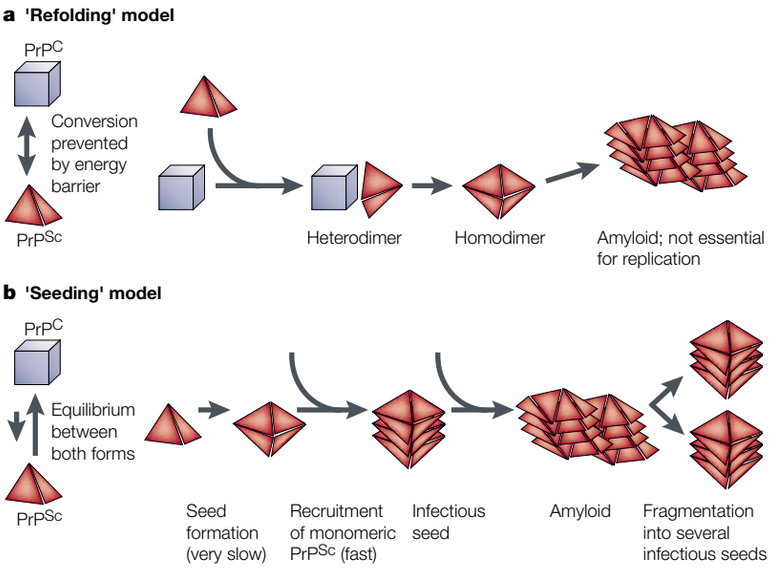

Figure 2 | **The 'protein-only' hypothesis and two popular models for the conformational conversion of PrP^{C} into PrP^{Sc}. a** | The 'refolding' or template-assistance model postulates an interaction between exogenously introduced PrP^{Sc} and endogenous PrP^{C}, which is induced to transform itself into further PrP^{Sc}. A high-energy barrier might 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 PrP^{Sc} 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. In sporadic prion disease, fluctuations in local PrP^{C} concentration might — exceedingly rarely — trigger spontaneous seeding and self-propagating prion replication.

What is a prion?

The normal mammalian prion protein is known as PRP^{C}. *In vitro* conversion of this protein can yield a moiety that has many of the physico-chemical properties that are characteristic of PrP^{Sc}, the disease-associated prion protein. These include aggregation into higher-order quasi-crystalline complexes that are birefringent when observed under polarized light (especially when stained 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[8–10].

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 reported so far has unambiguously accomplished transformation of the cellular prion protein PrP^{C} into a transmissible agent. Speculations abound as to 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[11], but has never been proved. Universal consensus about the nature of the agent will predictably be reached only once a synthetic reconstitution has been done from non-infectious material.

How the prion damages its host

Notwithstanding all the unresolved problems, certain properties of the infectious agent can be studied. Perhaps the most obvious question is this: how do prions damage the central nervous system, which is the only compartment of the body that undergoes histopathologically (FIG. 3) and clinically detectable degeneration in prion diseases?

Cellular models of prion disease might be useful to address this question, although prions replicate inefficiently in most established cell lines. Many studies have been done using a synthetic peptide from the central region of the PrP^{C} molecule, which spontaneously assembles into amyloid-like structures. *In vitro*, this peptide can elicit reactions that resemble those seen in brain cells during the late stages of prion disease. These include activation of microglial cells, stimulation of the production of intermediate filaments by astrocytes, and even the death of neurons, all of which seem to depend on the presence of the normal prion protein in target cells[12,13]. But all of these studies suffer from a fundamental problem. 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 prion

PRP^{SC} OR PRP-RES
An 'abnormal' form of the mature *Prnp* gene product found in tissue of transmissable spongiform encephalopathy sufferers, operationally defined as being partly resistant to proteinase K digestion under defined reaction conditions. It is believed to differ from PrP^{C} only (or mainly) conformationally, and is rich in β-sheet structure. Within the framework of the protein-only hypothesis it is often considered to be the transmissible agent or prion.

AMYLOID PROTEINS
A term introduced by Rudolf Virchow a century ago to designate proteins that show birefringence under polarized light when stained with Congo red. A more modern concept defines amyloids as proteins that attain their energy minimum in a highly ordered, aggregated state.

PRP^{C} OR PRP-SEN
The naturally occurring form of the mature *Prnp* gene product.

Box 1 | **Yeast prion studies**

Over the past several months, there have been breathtaking advances in the understanding of prion phenomena in yeast, and there is no doubt that at least two yeast proteins fulfil the molecular definition of a protein that can propagate its conformational state to its siblings in an 'infectious' manner. The ultimate experiment to show that a given protein is a prion is '*in vitro* conversion': this term defines a cell-free manipulation by which the non-contagious conformation is transformed into a transmissible agent. Ideally, this manipulation should occur without participation of the pathological, transmissible prion, to exclude the possibility of cross-contamination. These conditions can be met in the case of the yeast prions identified so far, Sup35 (REFS 83,84) **and** Ure2p (REFS 85,86)**.**

The prion state (denominated ψ^{+}) of the Sup35 protein brings the cell into a state of 'translational infidelity' that allows ribosomal read-through across stop codons. In the long run this cannot be a good thing for the cell, but a tantalizing conceptual development has just been proposed by Susan Lindquist[87] — ψ+ might provide a crucial evolutionary buffer that allows cells to explore, for a limited period of time, a large variety of combinatorial mutational hits in the hope of finding traits that provide selective advantage in a fluctuating environment.

replication — a process that may be very different. Moreover, some of the published data have been challenged recently[14].

To ask whether cerebral accumulation of PrP^{Sc} is enough to damage nerve cells, PrP-deficient mice[15] (which are resistant to scrapie[16] and do not replicate prions[17]) have been grafted with brain cells derived from transgenic mice that overexpress PrP^{C}. These mice were subsequently infected with prions[18], and the pathology was confined to the regions of the brain that expressed PrP^{C}. In the surrounding, PrP-deficient brain, no pathological changes were detected — even if there was substantial accumulation of pathological PrP^{Sc} (REF. 18). Although interpretation of this experiment requires certain caveats (notably, the possibility that a threshold concentration of PrP^{Sc} is needed for neurodegeneration, and that this level is not reached outside the grafted tissue), it is difficult to avoid the conclusion that the neuronal cytotoxicity of PrP^{Sc} depends on the expression of cellular PrP^{C} by target cells. Why should that be? Perhaps PrP^{C} acts as a receptor for PrP^{Sc}. There is some evidence that this might be the case[19]. Alternatively, the process of converting PrP^{C} into PrP^{Sc} — rather than exposure to the disease-associated prion protein — might be the main deleterious event.

This second possibility has been investigated in a series of elegant papers by Lingappa and co-workers. These authors have identified an atypical form of PrP^{C} that undergoes a peculiar biogenesis. Most cellular PrP^{C} is translocated into the lumen of the endoplasmic reticulum (ER) by virtue of its secretory signal peptide. The PrP^{C} is then routed to the cell surface as a glycophosphatidylinositol-linked membrane-associated protein. But a small proportion of PrP^{C} is made as a transmembrane form that later leaves the ER. Lingappa and co-workers have named these forms of PrP^{C} according to their orientation[20,21] — ^{Ctm}PrP and ^{Ntm}PrP (carboxy- and amino-terminal transmembrane prion protein, respectively, FIG. 4). Lingappa and colleagues found that levels of ^{Ctm}PrP correlate well with the neurodegenerative changes in pathological conditions — in fact, the correlation is much better than that with the accumulation of PrP^{Sc} itself[22,23]. These observations formed the basis for two hypotheses: that ^{Ctm}PrP might be a marker of prion-induced neurodegeneration; or that the conversion of PrP^{C} into PrP^{Sc} might trigger the formation of ^{Ctm}PrP, which might, in turn, be an effector of neurotoxicity. Strong circumstantial evidence from transgenic mice and from patients with the hereditary prion disease, Gerstmann–Sträussler–Scheinker syndrome, favours the second hypothesis, although there is no information about putative signalling pathways that might be involved.

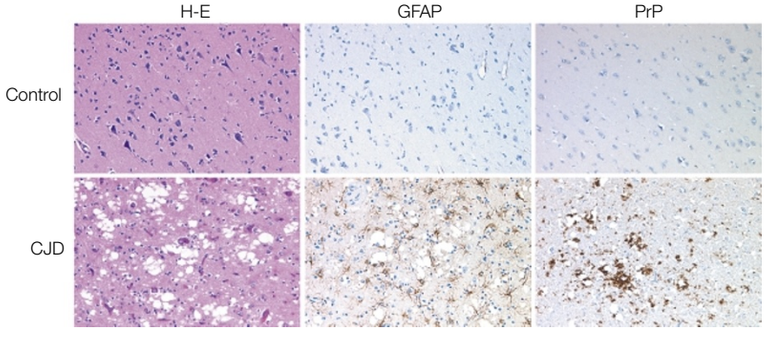

Figure 3 | **Neuropathological features of transmissible spongiform encephalopathies.** Histological and immunohistochemical analysis of frontal cortex samples from the brain of a patient who died of non-cerebral causes (upper row) and of a patient suffering from CJD (lower row). Brain sections were stained with hematoxylin-eosin (H-E, 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 H-E stain. Strong proliferation of reactive astrocytes (gliosis) and perivacuolar prion protein deposits are detectable in the GFAP and PrP immunostains of the CJD brain samples.

The march of prions ...

In most cases of prion infection in both humans and animals, the point of entry is outside the nervous system. In the case of BSE (and also, possibly, of new variant (nv) CJD, which develops owing to infection of humans with the BSE agent), exposure might be oral. By contrast, most cases of CJD brought about by medical procedures have occurred by parenteral administration (for example, intramuscular injection) of prion-contaminated growth hormone and gonadotropins of human cadaveric origin — in the age preceding recombinant DNA technology. But how do prions that are administered to the periphery of the body reach the central nervous system (CNS)? By analogy with viruses that affect the nervous system, there might be two main pathways of neural invasion. Many viruses — for example those that cause rabies and herpes — exploit the anatomical connections provided by peripheral nerves, and reach the CNS

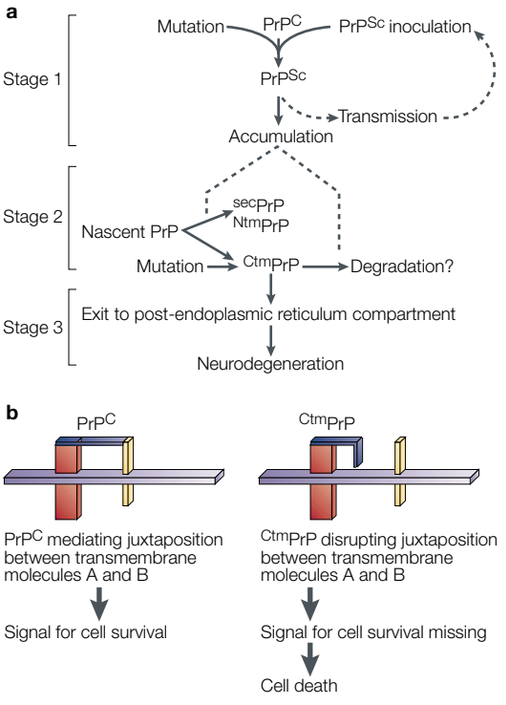

Figure 4 | **Three-stage model of prion pathogenesis, and possible role of ^{Ctm}PrP in cell death. a** | Stage 1 represents the formation and accumulation of PrP^{Sc}, initiated by either inoculation or spontaneous conversion of a mutated PrP^{C} to PrP^{Sc}. Stage 2 symbolizes the events involved in generating ^{Ctm}PrP, either by an unknown process that involves 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 a transmembrane form. Digestion with proteases of the transmembrane form results in two fragments. One is derived from the carboxyl terminus and is glycosylated (designated C-transmembrane PrP; ^{Ctm}PrP). Its carboxyl terminus is in the ER lumen and the amino terminus is accessible to proteases in the cytosol. The other fragment is derived from the amino terminus and is unglycosylated (termed N-transmembrane PrP; ^{Ntm}PrP), and has the opposite conformation. Stage 3 depicts the hypothetical events involved in ^{Ctm}PrP-mediated neurodegeneration, possibly involving the exit of ^{Ctm}PrP to a post-ER compartment (adapted from REF. 29). **b** | Possible role of ^{Ctm}PrP in cell death. Full-length PrP might act as a co-receptor 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[89], as well as the Doppel gene product[90] (Figure adapted from REF. 88).

through axonal transport. Human immunodeficiency virus (HIV), however, uses a different mechanism: it reaches cerebral microglial cells using a 'Trojan horse' mechanism that involves infection of macrophages.

What about prions? Available evidence indicates that both pathways are involved: prions can colonize the immune system as well as lymphocytes[24] and follicular dendritic cells[25] (which are located in the GERMINAL CENTRES and express considerable amounts of PrP^{C}) (FIG. 5). Blättler and colleagues have shown[18] that extracerebral prion protein is required for neural invasion: prion knockout (*Prnp*) mice that harbour a PrP^{C}-expressing graft in their brains consistently develop spongiform encephalopathy (restricted to the graft) upon intracerebral inoculation with the infectious agent[26]. However, they do not develop such encephalopathies upon intra-ocular, intraperitoneal or even intravenous administration of the agent[27]. In the case of intra-ocular inoculation, neural invasion is impaired even when a specific transgenic manipulation prevents all antibodies against PrP^{C} from being generated[28]. In other words, it is the absence of PrP^{C} — rather than an immune response against prions — that prevents the spread of the infectious agent within the body of a PrP^{C}-deficient mouse[29].

... from spleen to brain

In which cellular compartment must PrP^{C} be expressed to support neural invasion? Reconstitution of the haematopoietic and lymphopoietic system with stem cells derived from wild-type or transgenic mice that overexpress PrP^{C} is not enough to restore neural invasion[30]. These results imply that the crucial compartment is sessile, and that it cannot be transferred by adoptive bone-marrow reconstitution[27]. There are at least two likely candidates for this compartment — the peripheral nerves[31] and the follicular dendritic cells. However, titration experiments indicate that adoptive bone-marrow transfer reconstitutes the ability of the spleen to accumulate (and, perhaps, to replicate) prions of the Rocky mountain laboratory (RML) STRAIN after intraperitoneal inoculation[27]. This unexpected result might indicate that haematopoietic cells — perhaps lymphocytes — can replicate prions, or that they transport the infectious agent from the site of inoculation to the spleen.

Our laboratory has repeated these experiments and confirmed their unambiguous reproducibility (P.S.K., Michael A. Klein, Petra Schwarz and A.A., unpublished observations). But Brown and colleagues reported[32] that, using a different prion strain called ME7, they could not detect any accumulation of prions in the spleens of PrP^{C}-knockout mice that had been reconstituted with PrP^{C}-positive haematopoietic cells and killed at unspecified intervals through the incubation period. Assuming that the experimental design of the Blättler study using RML prions and the Brown experiments using ME7 is indeed comparable, the discrepancies between the outcomes might point to the possibility that different prion strains show a varying propensity to colonize specific components of the immune system. There might be precedents for this: BSE prions are hardly detectable in lymphoid organs of cows (with the possible exception of gut-associated lymphoid tissue over a transient period of time), whereas nvCJD prions extensively colonize human lymphoid organs. By identifying the molecular determinants of such differences in organ tropism,

GERMINAL CENTRES
Specialized areas of lymphoid organs that are important for affinity maturation of B cells.

PRION STRAINS
Prion strains have different phenotypes — for example, incubation times, distribution of lesions in the brain, relative abundance of mono-, di- and unglycosylated PrP^{Sc}, and electrophoretic mobility of the protease-resistant part of PrP^{Sc}. But they can all be propagated in the same inbred mouse strain, indicating that, within the framework of the protein-only hypothesis, the same polypeptide chain can mediate different strain phenotypes.

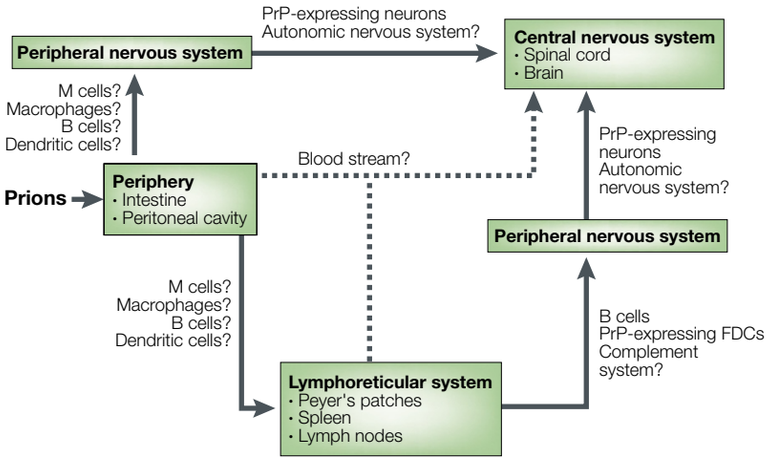

Figure 5 | **Model of prion neuroinvasion in mice.** After peripheral inoculation, prions colonize the lymphoreticular system. Possible elements that might contribute to the transport into the lymphoreticular system are M cells, macrophages, B cells and dendritic cells. Required elements of secondary lymphoid organs for prion replication are functional follicular dendritic cells (FDCs) and B cells. In the progress of the disease, prions find access to the central nervous system, probably through elements of the autonomic nervous system. Alternatively, direct invasion of the central nervous system by nerves might occur after peripheral infection. Central nervous system infection through the blood cannot be excluded, but is, in our opinion, unlikely.

we might learn more about the basic mechanism of prion pathogenesis.

Anatomy of prion neuroinvasion

What are the cellular requirements for invasion of prions into lymphoid tissues? This question has been addressed by screening mouse strains with spontaneous and engineered deficiencies in various compartments of the immune system, and one clear-cut result has emerged: any genetic defect that impairs the terminal differentiation of B-cell precursors into antibody-secreting cells completely blocks the colonization of lymphoid organs by prions. Such defects also block the development of disease in the CNS upon peripheral inoculation[33]. This phenomenon is obviously due to a block of neuroinvasion, because B-cell-deficient mice show the same susceptibility to disease as wild-type mice when inoculated intracerebrally[33]. This dependence of neuroinvasion on B cells 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 ablation of B cells.

The necessity for B cells does not imply that they are sufficient for neuroinvasion. However, attempts to identify other necessary compartments have yielded ambiguous results. One candidate is the follicular dendritic cell (FDC), which is the main site for accumulation of PrP^{Sc} in lymphoid organs[25] and functions as an antibody-dependent antigen trap. But again, experimental evidence has not been completely conclusive. All published data indicate that, in intraperitoneally inoculated mice, prions can accumulate only in spleens with properly formed germinal centres and immunohistochemically identifiable FDCs. Nobody has recovered prions from the spleens of intraperitoneally inoculated mice deficient in tumour necrosis factor receptor 1 (TNFR1) (REF. 33), TNF-α[32], or lymphotoxin-β[34], none of which contain identifiable FDCs in their spleens. Moreover, administration of soluble lymphotoxin-β receptor efficiently prevents the build-up of a splenic prion burden in wild-type mice[35]. This result is also valid for the ME7 prion strain[36], despite its many alleged differences from the RML strain.

The molecular mechanisms by which FDCs capture prions are, of course, of immediate interest. Capture of conventional antigens by FDCs occurs through Fcγ receptors and complement receptors: there is reason to believe that the same systems are operational in prion capture when limiting amounts of infectivity are introduced[37], but further molecules are likely to be involved in this process.

On the other hand, neuroinvasion — the development of brain disease after peripheral challenge — is completely unaffected in TNFR1 (REF. 33) and lymphotoxin-β[34] knockout mice, and cannot even be repressed fully by treatment with an antibody against the lym-

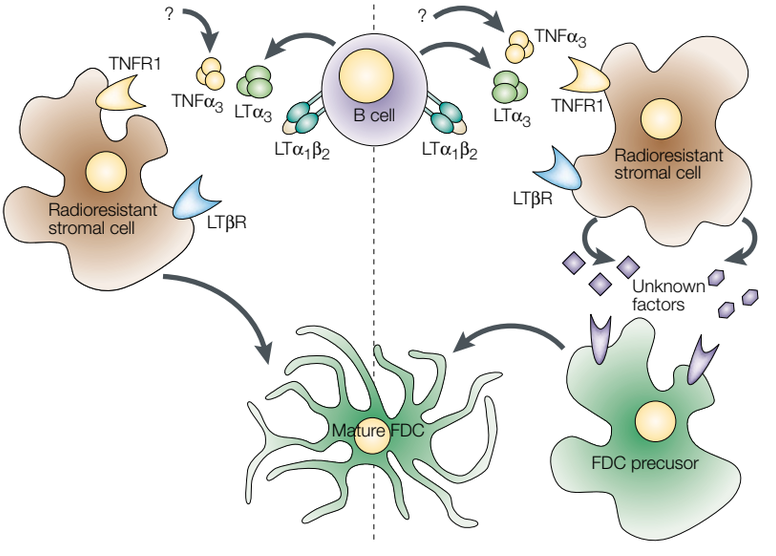

Figure 6 | **Models of the signalling pathways required for the establishment and maintenance of functional follicular dendritic cells.** For the formation of follicular dendritic cell (FDC) networks in the follicles of secondary lymphoid organs, both the tumour necrosis factor (TNF) and the lymphotoxin (LT)-β signalling pathways are necessary. However, maintenance of mature FDCs seems to depend solely on the continuous activation of the lymphotoxin-β pathway. Soluble and membrane-bound forms of $TNF\alpha_3$ homotrimers and $LT\alpha_3$ homotrimers, tethered to B cells, signal through the TNFR1, whereas the $LT\alpha_1\beta_2$ heterotrimer signals through the LTβR. Follicular B cells provide the ligands, whereas expression of both TNFR1 (TNFRp55) and LTβR is required on radioresistant stromal cells. Two alternative FDC-maturation models are conceivable: FDC precursor cells are stromal radioresistant cells that differentiate into mature FDCs by activation of both TNFR1 and LTβR signal pathways; or radioresistant stromal cells, which differ from FDC precursors, are activated by TNFR1 and LTβR signalling pathways and produce molecules that stimulate the maturation of FDC precursors. (Figure adapted from REF. 91.)

Table 1 | **Incidence of nvCJD since 1985***

Year	Sporadic CJD	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	89
1999	61	15	84
2000	38	25¶	72

* In the United Kingdom. ‡ Including probable nvCJD cases awaiting autopsy results, or that are still alive. ‖ Including hereditary and iatrogenic cases.
¶ Counted as of 3 January 2001.

photoxin-β receptor[35,36]. Therefore, although the lack of signalling from lymphotoxin-β to FDCs probably accounts for some of the protection from peripheral prion inoculation that is observed in B-cell-deficient mice, B cells probably have an additional role in prion neuroinvasion. This function is clearly independent of PrP expression[38], and it must also be distinct from lymphotoxin-β/TNF signalling to FDCs (FIG. 6).

The arm of neuroinvasion that takes prions from germinal centres to the central nervous system has been less intensely studied. Peripheral nerves, probably belonging to the autonomic nervous system, are likely to be important[39–41], and lymphocytes might be involved in the migration of prions from FDCs to peripheral nerves.

From bench to clinic

How have all of these molecular studies helped us to understand transmissible spongiform encephalopathies? For example, as prions can be detected in lymphoreticular tissues (such as spleen, lymph nodes, tonsils and appendix) of patients with nvCJD, is there a risk of IATROGENIC transmission through exposure to blood or tissues from people with preclinical nvCJD, or from exposure to contaminated surgical instruments? Epidemiological surveys over the past two decades have not implicated blood transfusions or administration of blood products as risk factors for prion diseases. However, a small increase in relative risk for the 'classical', sporadically occurring disease (sCJD) is associated with surgery of all kinds[42], and it might indeed indicate unrecognized iatrogenic transmission.

In the case of nvCJD the situation might not be as simple — for one thing, we do not know as much about the epidemiology and iatrogenic transmissibility of this new disease as we do about sCJD. New variant CJD was first described in 1996 (REF. 43), and has claimed almost 100 lives in the United Kingdom and in France so far[44] (TABLE 1). Several striking characteristics of nvCJD set it apart from sCJD, which was described 80 years ago[45,46] (TABLE 2). For one thing, sCJD typically affects elderly people, whereas nvCJD has mainly hit very young people, with a range of between 12 and 52 years of age. The reason for this age distribution remains unclear[47]. Also, the clinical courses of the two diseases are different. Whereas sCJD is typically a rapidly progressing illness, leading to severe dementia and then death within months or even weeks, nvCJD tends to develop over a more protracted period. Also, the initial symptoms of nvCJD are usually personality changes and psychosis, rather than dementia[48].

Even under the microscope, the two diseases are very different. One feature is common to sCJD and nvCJD: widespread vacuolation of the cortical neuropil (the meshwork of axons and dendrites) which, in its most extreme manifestation, makes the brain resemble a sponge under low-magnification microscopy — hence the designation 'spongiform encephalopathy'. Another hallmark of nvCJD is the prominent accumulation of small spherical protein deposits termed plaques in the brains of affected people. Although some plaques can be seen in a minority of patients with sCJD, the plaques of nvCJD have a specific morphology that includes a characteristic rim of microvacuolated tissue[49]. Further peculiarities of nvCJD include severe destruction of neurons in the THALAMUS . The latter can be recognized by non-invasive neural imaging methods, such as the so-called pulvinar sign[50] (a hyperintense area in the posterior thalamus that can be visualized by magnetic resonance imaging). In nvCJD there is generalized colonization of lymphoid organs by the infectious agent, and immunochemical methods (typically western blots) have shown deposition of disease-associated PrP^{Sc} in germinal centres of lymph nodes, tonsils and spleen[51–53].

The new disease has so far exclusively struck patients whose prion gene is homozygous for methionine at position 129. In our experience, this group represents only one-third of the population[54] and around two-thirds of patients with sCJD. For completeness, we

Table 2 | **Diagnostic criteria for nvCJD**

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 sporadic CJD (or no electroencephalogram done) • Bilateral pulvinar high signal on MRI scan
IV	• Positive tonsil biopsy

IATROGENIC
Caused by medical treatment.

THALAMUS
A conglomerate of neuronal groups in the diencephalon.

CORTICAL CEREBRAL RIBBON
The grey matter located underneath the surface of the brain.

FLORID PLAQUES
Deposits of prion protein surrounded by a rim of vacuolated brain tissue.

should also mention that CJD is not a totally new phenomenon in very young people[55]. Over the past 20 years, CJD has been recorded in almost 100 children and teenagers. But in most of these cases, this was a result of documented iatrogenic exposure to the infectious agent. Typically, it came from growth hormone or pituitary gonadotropins from corpses, which were given to children to treat pituitary dwarfism and other conditions in the era before recombinant DNA technology.

BSE: a human prion disease?

What is the evidence that the agent causing nvCJD might be identical with that of BSE when transmitted to humans? So far, none of the arguments is conclusive, but each — and particularly when they are all considered together — is tantalizing.

Much effort has gone into characterizing the 'strain properties' of the agent that affects cows and humans. Because the molecular substrate that underlies the nature of prion strains (which are heritable phenotypic traits that can be reproduced upon serial passage through experimental animals) is not known, strain typing of prions has to rely on surrogate markers.

Two such markers have been particularly useful. One is the distribution of neuronal vacuoles in the brains of affected animals: for example, whereas some strains target the CORTICAL CEREBRAL RIBBON, others mainly affect the midbrain[56]. The BSE prion strain attacks the dorsal medulla and the superior colliculus (a part of the optical pathway) virulently and consistently[57]. Worryingly, BSE prions (extracted from the brains of affected cows) and nvCJD prions derived from the brains of British patients produce the same lesional patterns when transmitted to panels of susceptible mice[49,57,58].

The second marker for strain typing of prions comes from analysing the biochemical properties of disease-associated PrP recovered from the brains of cattle and humans. Different steric conformations of PrP (which, according to the most popular hypothesis, account for the phenotypic strain properties) expose different sites to the action of proteolytic enzymes. These sites, 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 PrP (the glycotype ratio) — another parameter that seems to correlate with strain properties — these traits were again found to be indistinguishable between BSE and human nvCJD prions[59,60].

A third line of argument that relates BSE and human nvCJD concerns epidemiology of the disease. So far, the total number of definite or probable cases of nvCJD is 82 in the United Kingdom, one in Ireland and two in France[61]. 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 unavoidable conclusion is that the incidence of nvCJD correlates with the prevalence of BSE.

One of the most powerful arguments is the study of pathogenesis in primates. In a classical experiment, Lasmézas and colleagues[62] inoculated brain extracts from BSE-affected cows into cynomolgus macaques. After about three years, all inoculated primates (two adults and one infant) developed spongiform encephalopathy. The histopathological appearance of the disease was identical to that of nvCJD and included characteristic FLORID PLAQUES, which have been recognized in every case of nvCJD. Characteristically, nvCJD plaques are surrounded by a rim of microvacuolated brain tissue — a feature that they share with the deposits in scrapie. But this feature is not seen in classical CJD, nor in any other human spongiform encephalopathy.

However, impressive as all of these arguments might seem, each is phenomenological rather than causal. Distribution of histopathological lesions, as well as morphology of plaque deposits, is downstream of the molecular events that are responsible for prion strain specificity. Measurements of the 'glycotype ratio' might be more directly related to the essence of strains, but there is still no way to tell whether they might simply be surrogate markers. It would be desirable to measure the conformation of disease-associated PrP more directly. Some inroads have been made with a method that exploits the relative affinities of antibodies against PrP^C (REFS 63,64), but, to our knowledge, this possibility is restricted to differentiation of mouse and hamster PrPs, and has not yet been applied to investigating BSE and nvCJD.

So how many nvCJD victims will there be in the future? Terrible as the disease has been for patients, we have not yet seen a large-scale epidemic. And, although many mathematical models have been generated[65,66], the number of cases is still too small to predict future developments with any certainty. The number of cases diagnosed in the 12 months that preceded the writing of this article has risen to 36 (from 14 in the 12 months before) — certainly a cause for concern, if not for alarm.

Another question relates to the possibility of chronic subclinical disease or a permanent 'carrier' status in cows as well as in humans. Evidence that such a carrier status might be produced by the passage of the infectious agent across species was first reported by Race and Chesebro[67,68], and has recently been confirmed[69] — at least for the passage between hamsters and mice. Immune deficiency can also lead to a similar situation in which prions replicate silently in the body, even when there is no species barrier[70]. So the problem of animal transmissible spongiform encephalopathies could be more widespread than is assumed, and might call for drastic measures in farming. Moreover, people carrying the infectious agent might transmit it horizontally[71], and the risks associated with this possibility can be met only if we know more about how the agent is transmitted and how prions reach the brain from peripheral sites.

Preventive measures

What can be done in the meantime to prevent the spread of the disease? As discussed above, nvCJD seems to be much more 'lymphoinvasive' than sCJD. In particular, nvCJD prions can be detected easily in lymphatic organs such as tonsils and the appendix[51,52,72]; this is also the case for scrapie[73–75] but not for sCJD prions.

Although prion infectivity of circulating lympho-

cytes seems to be at least two orders of magnitude lower than that detected in splenic lymphocytes[76], the possibility that circulating lymphocytes might be in equilibrium with their splenic siblings call for cautionary measures when dealing with blood products. But what should these be? Leukodepletion — a filtering process that aims to reduce the number of leukocytes in transfused blood units — has been advocated, but there is still no certainty about its efficacy. In addition, even if blood prion infectivity is initially contained in lymphocytes *in vivo*, lysis of cells might lead to contamination of blood units with infectious 'microparticles'[77], which might be difficult to remove by any method (short of ultracentrifugation). However, many of the virus-removal steps involved in the manufacture of stable blood products have some positive effects on prion removal, so the possibility of such contamination can be regarded as a worst-case scenario.

A final consideration applies to secondary prophylaxis. Given the large amount of infectious BSE material that has entered the human food chain, it is possible that many people harbour preclinical nvCJD. Unfortunately, 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[78] — a well-meant study, but of limited sensitivity. Most available assays for PrP^{Sc} are breathtakingly insensitive, although the identification of proteins that selectively bind PrP^{Sc} (but not PrP^{C}) might herald some developments in this field[79]. Once subclinical carriers are identified, it will be imperative to develop strategies that will help to control spread of the agent and prevent the outbreak of symptoms in these people. Indeed, some promising approaches have been identified[80,81]. Possible targets for interfering with neural invasion are rate-limiting processes that control prion replication in the infected person. In light of the knowledge discussed above, treatments that target the neuroimmune interface of prion replication and neural invasion[82] continue to be a promising area for research.

Links

DATABASE LINKS Creutzfeldt-Jakob disease | PRP^{C} | Prp | Gerstmann-Sträussler-Scheinker syndrome | HIV | TNFR1 | TNF-α | lymphotoxin-β | lymphotoxin-β receptor | Sup35 | Ure2p

FURTHER INFORMATION World Organization for Animal Health | The Official Mad Cow Disease Home Page | Department of Health CJD page

ENCYCLOPEDIA OF LIFE SCIENCES Amyloidosis

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