ANTIPRION IMMUNOTHERAPY: TO SUPPRESS OR TO STIMULATE?

Adriano Aguzzi and Christina J. Sigurdson

Abstract | Although human prion diseases are rare, they are invariably fatal, and treatments remain elusive. Hundreds of iatrogenic prion transmissions have occurred in the past two decades, and the bovine spongiform encephalopathy epidemic has raised concerns about prion transmission from cattle to humans. Research into therapeutics for prion disease is being pursued in several centres and prominently includes immunological strategies. Currently, the options that are being explored aim either to mobilize the innate and adaptive immune systems towards prion destruction or to suppress or dedifferentiate the lymphoreticular compartments that replicate prions. This article reviews the pathophysiology of prion diseases in mouse models and discusses their relevance to immunotherapeutic and immunoprophylactic antiprion strategies.

ENCEPHALOPATHIES
A general term for diseases that affect the brain, including metabolic, toxic, traumatic, infectious and neoplastic disorders. Although it is used to describe the lesions that are caused by prion diseases, transmissible spongiform encephalopathies also affect the spinal cord.

Institute of Neuropathology, University Hospital Zürich, Schmelzbergstrasse 12, CH8091 Zürich, Switzerland. Correspondence to A.A. or C.J.S.
e-mails: adriano@pathol.unizh.ch; christina.sigurdson@usz.ch
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Prion diseases are fatal neurodegenerative conditions that affect humans and a wide variety of animals (TABLE 1). Although prion diseases have some morphological and pathophysiological similarities to other progressive ENCEPHALOPATHIES, such as Alzheimer's and Parkinson's diseases[1], they are unique in that they are transmissible. Typically, intracerebral inoculation of healthy individuals with affected brain tissue reproduces the disease. This important fact was recognized more than half a century ago for scrapie[2], a prototypical prion disease that affects sheep and goats. Therefore, prion diseases are also known as transmissible spongiform encephalopathies (TSEs), a term that emphasizes their infectious character.

Less than 1% of all reported cases of Creutzfeldt–Jakob disease (CJD) in humans can be traced to a defined infectious source. However, the identification of bovine spongiform encephalopathy (BSE)[3] and its subsequent epizootic spread has highlighted prion-contaminated meat-and-bone meal as an extremely efficient vector for bovine prion diseases[4] (BOX 1), which does not completely lose its infectious potential even after extensive autoclaving[5].

Human prion diseases typically have a long latency period (the time between infection and the manifestation of clinical symptoms), from 2 years to more than 30 years for some cases of kuru. From the viewpoint of interventional treatment approaches, there is a potential window of intervention after infection has occurred but before brain damage has been initiated. For much of this latency time, prions are executing neuroinvasion, which is the process of reaching the nervous system after entering the body from peripheral sites[6,7]. During this process, little or no damage occurs to the brain, and as we discuss, it is hoped that interruption at this stage can prevent neurodegeneration. Here, we focus on mouse models of TSEs, which have proven eminently useful for understanding prion pathogenesis and might help in the testing of prophylactic and therapeutic strategies. The extent to which this information can be extrapolated to natural human infections needs further investigation.

Prion nomenclature

Newcomers often complain that prion nomenclature and prion-related concepts are complex. However, many insiders also find the nomenclature to be inconsistent, and confusion has increased owing to the many alternative nomenclatures that have been proposed over time. We adhere to the following convention: the term prion denotes the transmissible principle that causes TSEs, without assigning any specific physical

Table 1 | **Prion diseases of humans, animals and animal models**

Disease	Natural host species	Transmission route or disease-induction mechanism	Other susceptible species	References
Creutzfeldt–Jakob disease	Humans	Sporadic, familial (*PRNP* germline mutation) or iatrogenic	Primates, hamsters, human *PRNP*-transgenic mice and wild-type mice	127–131
Variant Creutzfeldt–Jakob disease	Humans	Ingestion of BSE-contaminated food	Human *PRNP*-transgenic mice and wild-type mice	130,132,133
Kuru	Humans	Ingestion or ritualistic cannibalism	Primates	127,134
Fatal familial insomnia	Humans	Familial (*PRNP* germline mutation)	Wild-type mice	135
Gerstmann–Sträussler–Scheinker syndrome	Humans	Familial (*PRNP* germline mutation)	Primates and mutated *Prnp*- transgenic mice	136,137
Scrapie	Sheep and goats	Horizontal and possibly vertical	Primates, hamsters, ovine *Prnp*-transgenic mice and wild-type mice	127,138–141
Chronic wasting disease	Mule deer, white-tailed deer and Rocky Mountain elk	Horizontal and possibly vertical	Ferrets	141
Bovine spongiform encephalopathy	Cattle	Ingestion of BSE-contaminated MBM	Primates, bovine *Prnp*-transgenic mice, human *PRNP*-transgenic mice and wild-type mice	132,138,142–144
Spongiform encephalopathy of zoo animals	Zoological bovids and primates	Ingestion of BSE-contaminated MBM	Wild-type mice	145
Feline spongiform encephalopathy	Zoological and domestic felids	Ingestion of BSE-contaminated MBM	Wild-type mice	146
Transmissible mink encephalopathy	Mink	Ingestion; origin of epidemics unclear	Hamsters	147

BSE, bovine spongiform encephalopathy; MBM, meat-and-bone meal; *PRNP*, gene that encodes the cellular prion protein, PrP^{C}.

characteristics to it. Instead, we refer to PrP^{Sc} as the prion protein that is associated with disease — a modified form of the cellular prion protein (PrP^{C}) that accumulates in TSE-affected individuals[8].

PrP^{C} is anchored in the plasma membrane by a glycosylphosphatidylinositol anchor[9] and is expressed by a wide variety of cells[10,11]. Despite the availability of PrP^{C}-deficient mice for the past 12 years[12], the normal function of PrP^{C} is still unclear. Several subtle abnormalities have been described for PrP^{C}-deficient mice[13,14], but the molecular basis of these is undefined. PrP^{C} has similarities to membrane-anchored signal peptidases[15], but this observation has not been substantiated by functional data. The speculation that PrP^{C} might be a SUPEROXIDE DISMUTASE[16,17] is particularly attractive because of its multiple copper-binding sites, and it was recently suggested that amino-proximally truncated PrP^{C} might reduce endogenous dismutase activity[18]. However, PrP^{C} does not make any measurable contribution to dismutase activity *in vivo*[19,20].

PrP^{Sc} was originally defined as protease-resistant, aggregated PrP, and considerable evidence indicates that PrP^{Sc} is the prion[21]. However, protease resistance is merely a surrogate marker for the structural characteristics of PrP^{Sc}, and PrP^{C} can undergo modifications that are distinct from protease resistance[22,23]. Therefore, PrP^{Sc} might be best defined on the basis of generic disease-associated structural modifications rather than protease resistance. Furthermore, PrP^{Sc} might not be identical to the prion[24]. Instead, the infectious principle might consist of one of the following: a subspecies of protease-resistant PrP^{Sc} molecules; an unstable intermediate, provisionally known as PrP* (REF. 25); a complex of PrP^{Sc} with another protein(s), which might be host-derived[26]; or a complex of PrP^{Sc} with non-protein compounds[27], which might include non-coding nucleic acids[28].

Pathogenesis of prion disease

Despite two decades of intensive research, we still do not know whether the PRION HYPOTHESIS is correct[23]. If the prion consists entirely of PrP^{Sc}, it might propagate by TEMPLATE-DIRECTED REFOLDING or by SEEDING (also known as nucleation)[29] (FIG. 1). Does PrP^{Sc} catalyse conversion of PrP^{C} to more PrP^{Sc} by direct action, or does it take advantage of as-yet-unknown accessory molecules? The second possibility is plausible, because numerous attempts to 'grow' prion infectivity in chemically defined cell-free systems have failed. Genetic evidence indicates that at least one as-yet-undefined locus distinct from *PRNP* (the gene that encodes PrP^{C}) controls prion replication[26], but no physical evidence of a protein encoded by another locus has been found. In the future, advances in mass spectrometry, together with PrP^{Sc} baits that are composed of easily identifiable molecular tags linked to PrP^{Sc} (REF. 30), might facilitate the resolution of this question.

Regardless of the mechanism, the accumulation of PrP^{Sc} is fundamental to prion diseases[31]. The abnormally folded protein forms insoluble aggregates that are partially resistant to proteolytic digestion[32]. It is thought that such aggregates, or more probably some type of metastable conversion intermediate[25] or oligomeric structure[33], wreak havoc on the central nervous system (CNS), causing neurodegeneration by

SUPEROXIDE DISMUTASE
An antioxidant enzyme that contains zinc, manganese or copper, and protects cells from damage by superoxide radicals.

PRION HYPOTHESIS
Maintains that transmissible spongiform encephalopathies are infectious diseases caused by the conversion of a host-encoded, protease-sensitive protein — known as the prion protein (PrP) — to an abnormal, protease-resistant isoform. The hypothesis also states that the infectious agent is completely devoid of nucleic acids. The abnormal or disease-associated PrP (known as PrP^{Sc}) self-aggregates and forms amyloid fibrils. PrP^{Sc} has been identified as the main component of the infectivity.

TEMPLATE-DIRECTED REFOLDING
A model for prion conversion in which a single molecule of exogenously introduced abnormal (disease-associated) prion protein (PrP^{Sc}) interacts with and converts endogenous cellular prion protein (PrP^{C}) to a PrP^{Sc} conformation. Spontaneous conversion is potentially prevented by a high-energy barrier, the crossing of which is required for conversion to amyloidogenic PrP^{Sc}.

SEEDING
A prion-conversion model (also known as nucleation), in which cellular prion protein (PrP^{C}) and abnormal (disease-associated) prion protein (PrP^{Sc}) are in a thermodynamic equilibrium that favours the PrP^{C} conformation. A highly ordered 'seed' of PrP^{Sc} might form, by which monomeric PrP^{Sc} can be recruited and stabilized, eventually forming an amyloid fibril. Fragmentation of the PrP^{Sc} aggregates increases the number of seeds and the potential for recruiting additional monomeric PrP^{Sc} molecules.

unknown mechanisms and, ultimately, death. Transgenic mice in which PrP^{C} is solely expressed by astrocytes[34] are readily susceptible to prion disease. In addition, the depletion of PrP^{C} only in neurons leads to a reversal of spongiform changes in prion-inoculated mice[35]. Consequently, ablation of the *Prnp* gene[36] abolishes prion replication[37] and pathogenesis[38], and mice with half of the normal level of PrP^{C} expression have a marked delay in disease development[39,40].

Involvement of the immune system. In many prion diseases — including variant CJD (vCJD), scrapie in sheep and goats, and chronic wasting disease (CWD) in deer — PrP^{Sc} accumulates in the lymphoid tissues before CNS involvement[41–44]. Indeed, some lymphoreticular involvement seems to occur in most TSEs that have been studied, although the degree of involvement is highly variable. For example, when cattle are experimentally inoculated with BSE, the ileal Peyer's patches accumulate low levels of PrP^{Sc} and have low levels of infectivity[45,46]. Also, splenic PrP^{Sc} is detectable in at least one-third of patients with sporadic CJD[47], albeit in small amounts. Even scrapie is heterogeneous in this respect, because sheep with certain genotypes accumulate much less lymphoid PrP^{Sc} than others[48]. However, the mouse model of scrapie has been used to study prion-disease pathogenesis for decades, and it shows a marked early phase of prion replication in lymphoid tissues[49]. Therefore, the immunotherapeutic strategies that have been developed using the mouse model of scrapie might be of greatest relevance for treating TSEs that have an extensive lymphoid-amplification phase, and it is unknown whether some natural human infections have this property.

Box 1 | **From BSE to vCJD and beyond**

The bovine spongiform encephalopathy (BSE) crisis was recognized in 1986 as a limited epidemic occurring in cattle of the United Kingdom, and it was soon defined as a new form of transmissible spongiform encephalopathy[3]. Since then, BSE has affected cattle worldwide, with the first case of BSE in the United States being reported in December 2003.

Most disturbingly, since 1996, a variant form of Creutzfeldt–Jakob disease (vCJD) has been reported in humans[113–115]. Extensive epidemiological, biochemical and pathogenetic evidence indicates that vCJD is caused by the ingestion of BSE-contaminated food. vCJD mainly affects young patients, has a distinctive histopathological profile of brain lesions and displays a biochemical 'signature' that is similar to BSE in cattle. In addition, prions are more prone to accumulate in the lymphoid tissues of patients with vCJD[116] than in those with sporadic CJD[47].

Although millions of people have undoubtedly been exposed to BSE, less than 150 individuals have developed vCJD so far. It is probable that an unknown number of additional individuals have been infected with BSE prions but have not yet developed overt disease or have developed a permanent subclinical carrier state without the symptoms of spongiform encephalopathy. Such permanent carrier states have been described in experimentally infected animals[117]. These infected individuals might, however, transmit infection to others: for example, by blood transfusion[110,111]. Importantly, sheep infected with BSE can efficiently transmit the agent to other sheep by blood transfusion[118,119]. Therefore, it is imperative to assess the prevalence of subclinical infection, and anonymous prevalence studies to this effect are underway in the United Kingdom[41] and in Switzerland[120].

In a discussion of the immunological aspects of prion diseases, it is important to distinguish between an early extraneural phase and a late CNS phase of pathogenesis[6]. There is no doubt that components of the immune system participate in pathogenesis in both compartments in mice, but their respective functional implications are strikingly different. In the CNS, prion pathology involves large-scale activation and proliferation of microglial cells[50] — the specific macrophages present in the brain. In addition, it has been reported that T cells also infiltrate the CNS to some extent[51], which leads to the hypothesis of atypical CNS inflammation, because the expression of both pro-inflammatory and effector cytokines are upregulated in prion infection[52]. However, microglial activation is a characteristic of almost all CNS pathologies, and its importance should not be overinterpreted. More importantly, intracerebral administration of prions has been shown to induce TSE in mice that have various immune deficiencies, including lack of B cells, T cells, interferon (IFN) receptors, tumour-necrosis factor (TNF), lymphotoxins (LTs) or their receptors[53], receptors for IgG (FcγRs), complement factors or their receptors[54], TOLL-LIKE RECEPTORS (TLRs)[55] or chemokine receptors[56]. In all of these models, pathogenesis after intracerebral prion delivery proceeded with the same kinetics as in wild-type immunocompetent mice, and in terminally sick mice, intracerebral prion concentrations were unchanged[57]. This evidence indicates that, after prions are present in the CNS, the disease course cannot be modified by manipulating immunological parameters.

In the extraneural immune system, however, the situation is different. More than thirty years ago, when the term prion had not been coined and the protein-only hypothesis had only recently been proposed[58], it was discovered using bioassays that lymphoreticular organs contain prion infectivity[49,59]. For scrapie in sheep and goats, infectivity was assessed in a broad range of tissues using experimentally inoculated goats as donors and uninfected goats as recipients[60], and both spleen and lymph nodes were shown to transmit scrapie (FIG. 2).

In the decades following these discoveries, our understanding of the extraneural immune phase advanced considerably, and an impressive collection of data has accrued that provides clues about peripheral prion pathogenesis and 'neuroinvasion' — the process by which prions gain entry to the nervous system. However, many important aspects of the pathogenesis are still unclear. For example, even after prions have entered the nervous system, it is unknown how prions travel along neuronal processes and 'jump' from one process to another.

The lymphoid system in prion disease

In several TSEs, such as BSE, vCJD and kuru, prions are transmitted by ingestion (TABLE 1). An *in vitro* study has shown that microfold cells (M cells) — which are specialized intestinal epithelial cells that transfer antigens, including pathogens, through the intestinal epithelium — can transport infectious prions from the

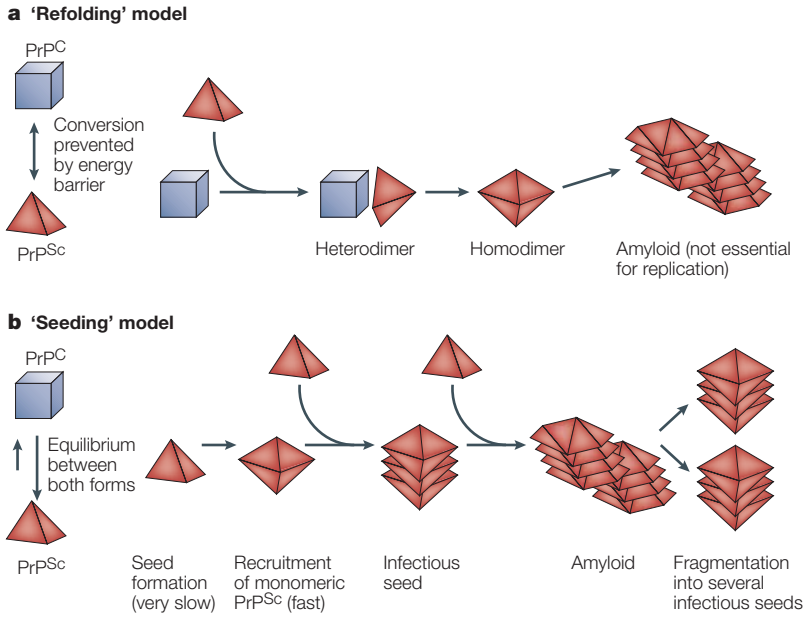

Figure 1 | **The prion hypothesis. a** | The 'refolding' or template-directed assistance model postulates an interaction between exogenously introduced disease-associated prion protein (PrP^{Sc}) and endogenous cellular prion protein (PrP^{C}), which is induced to transform itself into more PrP^{Sc}. A high-energy barrier might prevent the spontaneous conversion of PrP^{C} to PrP^{Sc}. **b** | The 'seeding' or nucleation–polymerization model proposes that PrP^{C} and PrP^{Sc} are in a reversible thermodynamic equilibrium. So, only if several monomeric PrP^{Sc} molecules are mounted in a highly ordered seed can more monomeric PrP^{Sc} be recruited and eventually aggregate to form amyloid. In such a crystal-like seed, PrP^{Sc} becomes stabilized. Fragmentation of PrP^{Sc} aggregates increases the number of nuclei, which can recruit more PrP^{Sc}, and so seems to result in replication of the agent. In sporadic prion disease, fluctuations in the local PrP^{C} concentration might (exceptionally rarely) trigger spontaneous seeding and self-propagating prion replication. This figure is modified with permission from *Nature Reviews Molecular Cell Biology* REF. 121 © (2001) Macmillan Magazines Ltd.

apical to the basolateral surface of the intestinal epithelium[61]. It is possible that additional cells, including epithelial cells, dendritic cells (DCs) and lymphocytes, are involved in prion trafficking past the epithelial barrier. Subsequently, prions can be amplified in the lymphoid tissue: Peyer's patches seem to be required for prion-disease development after oral exposure of mice[62], and they also accumulate PrP^{Sc} early after the infection of deer or sheep that are orally exposed to CWD[44] or scrapie[63], respectively. Therefore, lymphoid accumulation might be a general mechanism in prion pathogenesis. However, the identity of the cells that are involved in early transport from the intestinal mucosa to the Peyer's patches or draining lymph nodes remains unclear. DCs are obvious candidates, and there is evidence that indicates they might be involved[64,65]. However, even if they do have a role, DCs might not account for all of the transport of prions, and other cells, including tingible-body macrophages (phagocytic cells in lymphoid germinal centres)[66], might be involved in the trafficking of prions to draining lymphoid tissues and/or directly to nerve endings. Many studies using mouse models of scrapie have shown a delay or prevention of disease after interference with lymphoid prion accumulation[67,68].

Some of the lymphoid participants that are crucial to prion accumulation have been revealed. Functional FOLLICULAR DENDRITIC CELLS (FDCs) are important[67,69], but it is not clear whether they are the only site of lymphoreticular prion replication or accumulation[70,71]. B cells are also required[53] because they provide maturation signals for FDCs (FIG. 3). There is also an ill-characterized bone-marrow-derived cell population that supports prion replication[70,72], although this might not apply to all scrapie-inducing prion strains[73].

The microarchitecture of lymphoid tissue crucially controls the efficacy of prion neuroinvasion: for example, manipulation of the distance between FDCs and major splenic nerves affects the velocity of neuroinvasion[56]. CXC-chemokine receptor 5 (CXCR5) is usually required for the homing of lymphocytes to specific microcompartments of lymphoid organs[74], so in contrast to wild-type mice — in which FDCs reside antipodal to follicular vessels[56] — mature FDCs of CXCR5-deficient mice are associated with germinal centres and are thereby immediately adjacent to central arterioles. Although the SYMPATHETIC NERVE processes in the spleens of CXCR5-deficient mice are normal, the ablation of CXCR5 juxtaposes FDCs with major splenic nerves[56]. Intraperitoneal prion delivery then results in an accelerated incubation period in CXCR5-deficient mice, and transmission and/or neuroinvasion is therefore more efficient than in wild-type mice. Because prion replication is unaltered in the lymphoreticular and CNS compartments, the data indicate that shortening the distance between FDCs and nerves selectively increases the velocity of neuroinvasion. The efficiency of prion transfer between the immune and nervous systems seems to be one of the main determinants of the efficiency and velocity of neuroinvasion. But does this increased velocity result from the passive diffusion of prions from FDCs to nerves, or are mobile cells involved? This question has yet to be answered.

Morphological evidence from time-course studies indicates involvement of the vagal nerve and the sympathetic nervous system as routes of peripheral prion transport to the CNS[75,76]. Glatzel *et al.*[77] have shown that the sympathetic nervous system is crucially involved in neuroinvasion: mice with sympathectomy show a considerably prolonged incubation period. Moreover, transgenic mice that overexpress a nerve growth factor transgene and thereby have sympathetic hyperinnervation of lymphoid organs show an accelerated incubation period after peripheral prion exposure, indicating the importance of these peripheral nerves in prion pathogenesis[77].

Therapeutic prospects

Is it possible to exploit the roles of the lymphoid system and immune cells in prion pathogenesis for therapeutic or prophylactic purposes? At least four alternatives can be envisaged on the basis of the information that we have discussed: removal of functional FDCs and therefore ablation of lymphoid prion-replication sites; stimulation of the innate immune system; enhancement of elimination of PrP^{Sc} using PrP-specific antibodies; or binding of available PrP^{C} or PrP^{Sc} so that they are unavailable for

TOLL-LIKE RECEPTORS (TLRs). A rapidly growing family of receptors that recognize pathogen-associated molecular patterns, which are conserved molecular patterns that are common to large groups of microorganisms and/or viruses. For example, TLR4 recognizes bacterial lipopolysaccharide, and TLR5 recognizes bacterial flagellin. Activation signals from TLRs are relayed through cytoplasmic adaptor proteins, leading to the transcription of genes that encode cytokines.

FOLLICULAR DENDRITIC CELLS (FDCs). Cells with a dendritic morphology that are present in the lymphoid germinal centres, where they present intact antigens (which are either held in immune complexes or associated with complement receptors) to B cells. FDCs are of non-haematopoietic origin and are not related to dendritic cells.

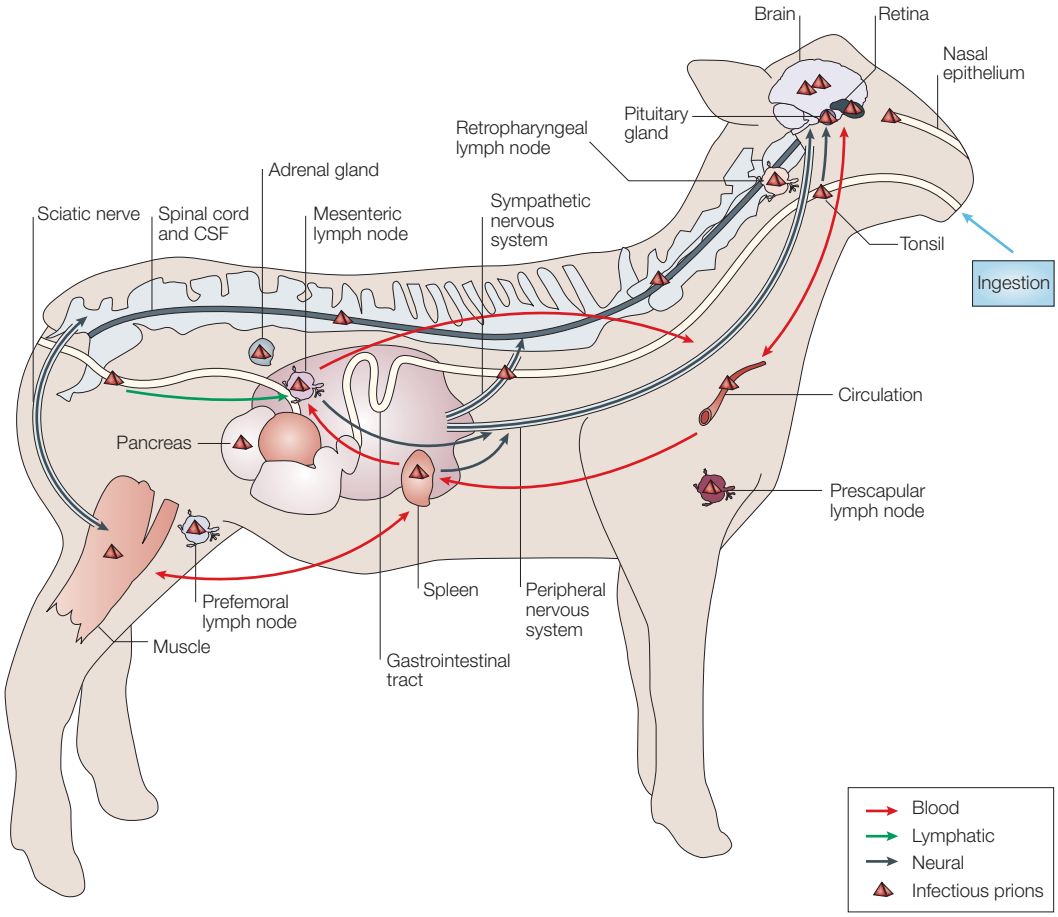

Figure 2 | **Dissemination of prions in scrapie-infected sheep or goats.** Tissues that accumulate infectious prions include the central and peripheral nervous systems, the spleen, the lymph nodes[122] and some non-lymphoid organs. In various animal models, these non-lymphoid organs include the pituitary gland[60], adrenal gland[60], pancreas[59], nasal mucosa[123], intestine[123], muscle[60] and eye (retina)[124]. How do prions reach these distant sites? Although nerves[60], blood[118] and lymph might contain infectivity, the precise carrier mechanisms are unclear. Routes of prion shedding and transmission in sheep and goats are also still unclear: ingestion of scrapie-infected placenta can transmit scrapie[125], but it is not the sole route of horizontal prion spread. Potential routes are indicated by arrows. CSF, cerebrospinal fluid.

conversion (FIG. 4). All of these approaches, which include both suppression and stimulation of the immune system, are now being tested in suitable *in vivo* systems. These immunomodulatory approaches to prion-disease prevention and therapy have mostly been studied in mice that are experimentally infected with mouse-adapted scrapie. However, because involvement of the lymphoid system has been found for almost all forms of TSE, we think that it is reasonable to presume that mouse-adapted scrapie provides a realistic generic model for TSE therapy.

Although this review focuses on immunological antiprion strategies that target the early window of prion lymphoid amplification, it should be noted that many other strategies are being tested for their potential to disrupt prion replication. Many substances seem to be capable of influencing the maintenance of a prion-infected state by cultured cells: these include Congo red[78], amphotericin B[79], anthracycline derivatives[80], sulphated polyanions[81], pentosan polysulphate[82], soluble LT-β receptors (LT-βRs)[67], porphyrins[83], branched polyamines[84] and β-sheet-breaker peptides[85].

Targeting FDCs. In the mouse model of scrapie, some forms of immunodeficiency impair prion replication and delay disease development, showing the important contribution of the early lymphoid phase. For example, mice that lack B cells and mature FDCs — such as severe combined immunodeficient (SCID) mice, recombination-activating gene 1 (RAG1)-deficient mice or μMT mice (which have a targeted disruption of the immunoglobulin μ-chain gene) — resist intraperitoneal prion infection[53], except after exposure to extremely high doses[86]. Replacement of B-cell populations, regardless of their expression of PrP^{C}, restores prion infectibility, possibly because of the key role of mature B cells in the induction of FDC maturation by providing TNF and LTs (FIG. 3). Typically, PrP^{Sc} 'decorates' FDC membranes in secondary follicles of the spleen[87], lymph nodes, tonsils and Peyer's patches (FIG. 5). This phenomenon seems to occur to a variable extent in several prion diseases, including vCJD[41], scrapie[87] and CWD[88], as well as in mouse models of scrapie and CJD[87,89].

SYMPATHETIC NERVE
A nerve that is part of the functional division of the autonomic nervous system that innervates the heart, lungs, gastrointestinal tract and sweat glands.

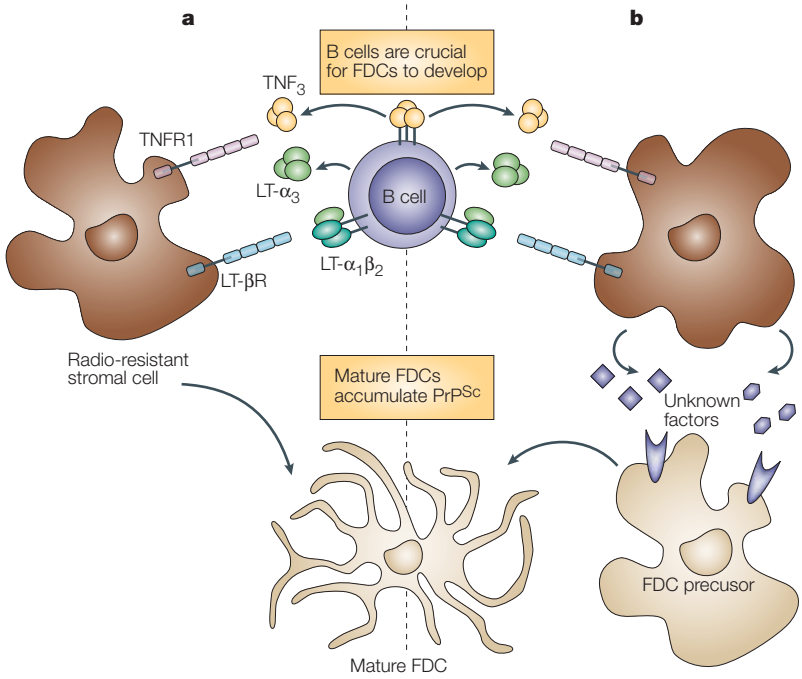

Figure 3 | **Models of the signalling pathways required for the establishment and maintenance of functional follicular dendritic cells.** For the formation of follicular dendritic cell (FDC) networks in the follicles of secondary lymphoid organs, both the tumour-necrosis factor (TNF)- and the lymphotoxin-β (LT-β)-signalling pathways are required. However, maintenance of mature FDCs seems to depend solely on continuous activation of the LT-β pathway. Soluble TNF_3 homotrimers, and membrane-bound TNF_3 homotrimers that are tethered to B cells, signal through the TNF receptor (TNFR1; also known as TNFRp55), whereas the LT-$\alpha_1\beta_2$ heterotrimer signals through the LT-β receptor (LT-βR). These ligands are provided by follicular B cells, and radio-resistant stromal cells need to express the cognate receptors (both TNFR1 and the LT-βR). Two different FDC-maturation models are conceivable: FDC-precursor cells are radio-resistant stromal cells that differentiate into mature FDCs by activation of both the TNFR1- and LT-βR-signalling pathways (**a**); or radio-resistant stromal cells that differ from FDC precursors are activated by TNFR1- and LT-βR-signalling pathways and produce molecules that stimulate the maturation of FDC precursors (**b**). PrP^{Sc}, disease-associated prion protein. This figure is modified with permission from *Nature Reviews Molecular Cell Biology* REF. 121 © (2001) Macmillan Magazines Ltd and REF. 126 © (1999) The Rockefeller University Press.

So, would ablation of functional FDCs impair the formation of these PrP^{Sc} depots? To address this question, the LT-signalling pathways (mediated by both LT-α_3- and LT-$\alpha_1\beta_2$) were inhibited using a soluble LT-βR–immunoglobulin (Ig) fusion protein — known as the LT-βR–Ig immunoadhesin — which effectively disbands mature FDC networks within 1 day through dedifferentiation and loss of the FDC markers FDC-M1, FDC-M2 or complement receptor 1, and later disrupts B-cell follicles, modifies splenic marginal-zone macrophages[90] and reduces DC numbers[91]. Administration of soluble LT-βR–Ig before inoculation with prions abolished splenic prion replication and delayed neuroinvasion[67,69]. Moreover, after prion inoculation, treatment with soluble LT-βR–Ig also led to a modest delay in disease development, although splenic prion infectivity was detectable at 8 weeks after inoculation[67]. Recent data from our laboratory are encouraging and are in agreement with Mabbott *et al.*[68]: treatment of mice with LT-βR–Ig for 1 week before a low-dose intraperitoneal prion inoculation and then each week for only 2 weeks after inoculation has so far completely protected mice from disease, with no wild-type mice developing scrapie at more than 500 days after inoculation (M. Heikenwaelder and A.A., unpublished observations).

After oral exposure to prions, however, it might be more difficult to prevent neuroinvasion: the administration of LT-βR–Ig, even as early as 14 days after exposure, failed to modify prion-disease susceptibility or survival time[68]. This might indicate that — at least for the oral-infection model — neural entry occurs early after prion ingestion. Nevertheless, Peyer's patches still seem to be crucial, because mice that lack them are resistant to orally administered prions[62].

In summary, the data from studies using LT-βR–Ig indicate that post-exposure treatment of humans with LT-βR–Ig could only be considered as a possible prophylaxis at well-defined early time points in cases of known prion exposure. These cases might include recipients of blood transfusions from patients with CJD, and researchers, pathologists, neurologists, neurosurgeons and technicians after accidental CJD injection. In these situations, we think that a case could be made for experimental use of post-exposure prophylaxis with LT-βR–Ig, although it is not known how soon after exposure LT-βR–Ig would need to be administered. Therefore, prophylaxis would need to be provided as soon as possible. It should be considered that LT-βR–Ig will not offer any relief to patients with overt CJD, for whom neural entry has already taken place.

Stimulating the innate immune system. For a long time, we wondered whether members of the TLR family might be involved in the innate recognition of prions. This idea was enticing because, theoretically, the ordered aggregate state of PrP^{Sc} might render it recognizable as a PATHOGEN-ASSOCIATED MOLECULAR PATTERN. However, the kinetics of prion pathogenesis in MyD88 (myeloid differentiation primary-response protein 88)-deficient mice that are inoculated intraperitoneally with prions are identical to those of wild-type mice[55], indicating that TLR1, -2, -6 and -9, which signal through the adaptor protein MyD88 (REF. 92), are probably not involved in prion recognition and signalling.

However, targeting TLR9 might have other beneficial effects. TLR9 recognizes DNA sequences that are over-represented in bacterial DNA. For example, UNMETHYLATED CpG MOTIFS, which are commonly found in bacteria, can stimulate mouse and human lymphocytes through TLR9 (REF. 93). Repetitive CpG motifs that are present in synthetic oligodeoxynucleotides (CpG ODNs) mimic unmethylated nucleic-acid sequences in bacteria, thereby stimulating the innate immune system through TLR9, which is expressed by various immune cells — such as monocytes, macrophages and DCs. Treatment with CpG ODNs has been discussed as a therapy to delay prion disease on the basis of promising results in mouse models of scrapie[94]. In these studies, mice were treated with a multi-dose regimen — receiving CpG ODNs or a control each day for 20 days — with the result that prion-disease development was delayed for more than 149 days in the group treated with CpG ODNs.

PATHOGEN-ASSOCIATED MOLECULAR PATTERN
A molecular pattern that is found in pathogens but not mammalian cells. Examples include terminally mannosylated and polymannosylated compounds, which bind the mannose receptor, and various microbial products, such as bacterial lipopolysaccharides, hypomethylated DNA, flagellin and double-stranded RNA, which bind Toll-like receptors.

So, what mechanism underlies the observed antiprion effect of treatment with CpG ODNs? It has been proposed by Sethi *et al.*[94] that prion-specific antibodies are induced after exposure to prions and administration of CpG ODNs, which are a highly effective adjuvant. However, we have not found evidence of PrP-specific immunoreactivity after repetitive CpG ODN treatment (REF. 95 and M. Polymenidou and A.A., unpublished observations). Instead, the delay in development of prion disease might be due to destruction of lymphoid follicles, which are the main site of peripheral prion amplification (FIG. 6). Alternatively, it cannot be discounted that the massive expansion of the macrophage and DC compartments that is evident after repetitive CpG ODN treatment might lead to enhanced PrP^{Sc} degradation or prion sequestration. It is possible that macrophages could function as prion carriers when they are overloaded, but they are also known to destroy infectivity when prion concentrations are manageable[96].

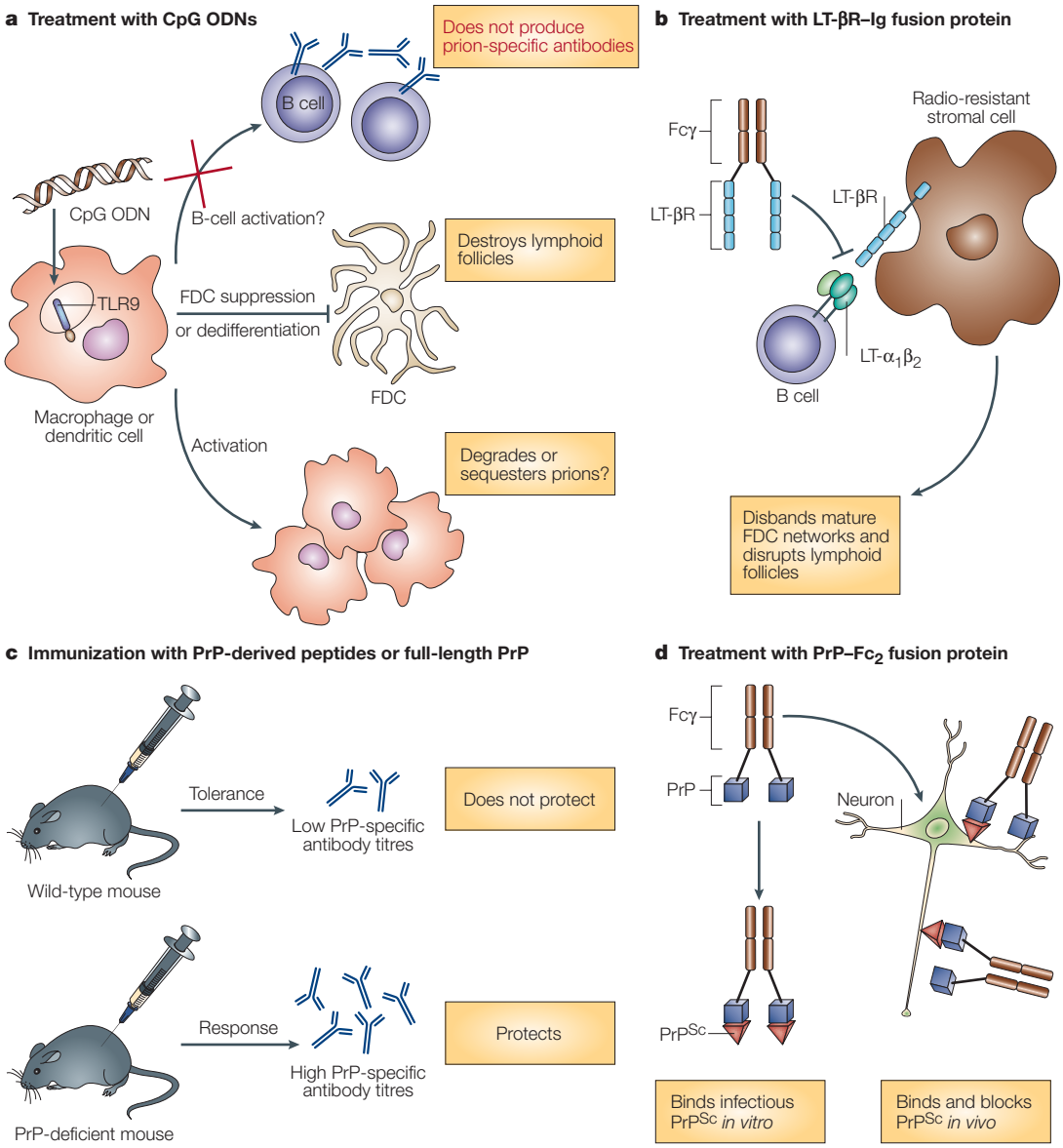

Figure 4 | **Immunotherapeutic strategies for prion disease. a** | Ablation of mature follicular dendritic cells (FDCs) delays the development of prion disease in mice. However, treatment with multiple doses of CpG-containing oligodeoxynucleotides (CpG ODNs) produces severe unwanted side-effects, including immunosuppression, liver necrosis and thrombocytopaenia. **b** | Treatment with the lymphotoxin-β receptor (LT-βR)–Ig fusion protein seems to be better tolerated, but the best protection is achieved when the fusion protein is administered immediately after exposure to prions. **c** | Vaccination against a self-protein is difficult because of immune tolerance, and it has the potential to induce autoimmune disease. Mice devoid of the prion protein (PrP) develop high PrP-specific antibody titres after immunization with PrP-derived peptides or full-length PrP; however, tolerance in wild-type mice allows the induction of only low titres of PrP-specific antibodies. **d** | Treatment with dimeric full-length PrP fused to the Fc portion of human IgG1 ($PrP–Fc_2$) delays the development of prion disease in transgenic mice, most probably owing to its interaction with the disease-associated PrP (PrP^{Sc}). LT-$\alpha_1\beta_2$, LT heterotrimer; TLR, Toll-like receptor.

UNMETHYLATED CpG MOTIFS
DNA containing an unmethylated cytosine–guanosine sequence. Such sequences are prevalent in bacterial DNA but rare in mammalian DNA. Unmethylated CpG is endocytosed by cells of the innate immune system and interacts with Toll-like receptor 9, activating a signalling cascade that results in the production of pro-inflammatory cytokines.

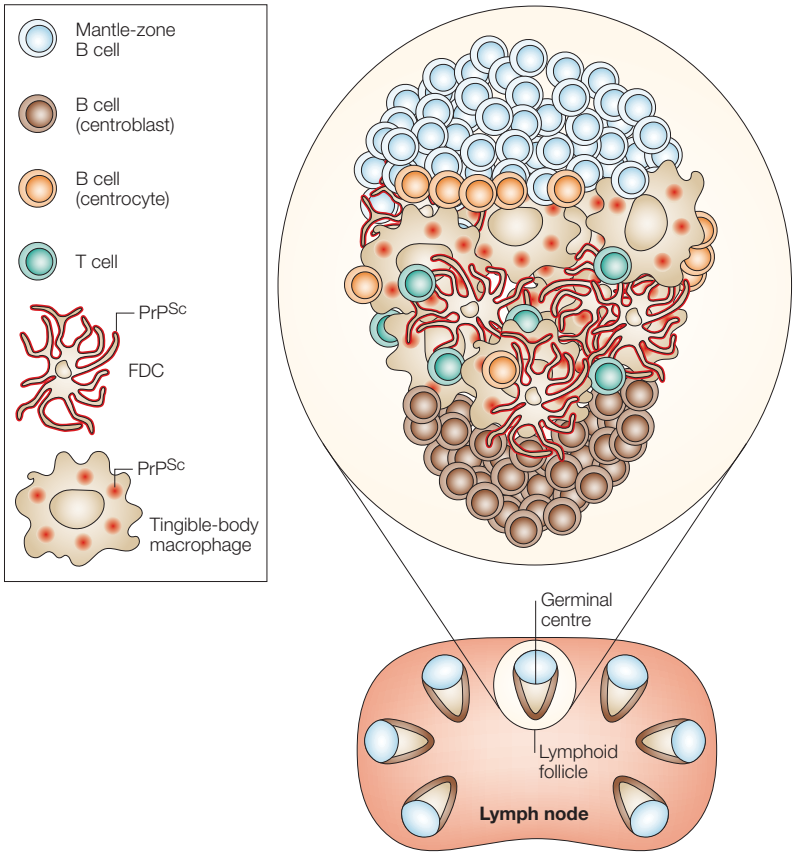

Figure 5 | **PrP^{Sc} accumulates in the germinal centres of lymphoid follicles in prion-infected lymph nodes.** As shown schematically, disease-associated prion protein (PrP^{Sc}) aggregates (red) are detectable at the plasma membrane of follicular dendritic cells (FDCs) and in the cytoplasm of tingible-body macrophages, using immunohistochemistry and electron microscopy[42,43,48]. Other lymphoid cells might also contain PrP^{Sc}, but the nature of these cells is still being debated.

CpG ODNs, however, are unlikely to be a viable antiprion therapy because of the severe toxic side-effects that are associated with their repeated administration. Although one or two doses of CpG ODNs are beneficially immunostimulatory, the frequent administration of CpG ODNs has marked side-effects, which range from lymphoid follicular destruction and impaired antibody class switching, to ascites, hepatotoxicity and thrombocytopaenia[95]. All of these effects are strictly TLR9 dependent, because TLR9-deficient mice are unaffected by the administration of CpG ODNs. Lymphoid follicular destruction and hepatotoxicity are, at least in part, consequences of the CYTOKINE STORM that is unleashed by excessive TLR signalling, because mice that are deficient in the IFN-α receptor, the IFN-γ receptor or the TNF receptor suffer from less lymphoid and hepatic destruction than wild-type mice.

Immunizing against prion disease. Antibodies specific for PrP^{Sc} are not generated during the course of prion infections, but artificial induction of humoral immune responses to PrP^{C} and/or PrP^{Sc} might be protective. However, vaccination against the prion protein has proven extremely challenging. First, wild-type mice are highly tolerant to PrP as an immunogen. This is not surprising because PrP^{C} is expressed almost ubiquitously and is found at the surface of both B and T cells, where it has even been claimed to participate in the T-cell-receptor signalling complex by interacting with ZAP70 (ζ-chain-associated protein kinase of 70 kDa)[97]. Second, it was generally thought (with good reason) that antibodies specific for PrP^{C}, if they could be elicited at all, might lead to severe systemic immune-mediated diseases, because PrP is expressed by many cell types[98,99]. Third, PrP-specific antibodies would be unlikely to cross the BLOOD–BRAIN BARRIER in therapeutic concentrations.

In 2001, it was reported that mice transgenic for the μ-chain of a PrP-specific antibody were protected against prion disease after exposure by a peripheral route (that is, intraperitoneal inoculation)[100]. In parallel, many other publications addressed prion immunoprophylaxis, both *in vitro* and *in vivo*[101,102]. White *et al.*[103] showed that passive immunization with PrP-specific antibodies after prion exposure delayed disease and markedly increased the incubation period compared with non-immunized mice. However, so far, active-immunization efforts have not led to high concentrations of PrP-specific antibodies due to immune tolerance to self-antigens. Moreover, intracranial delivery of PrP^{C}-specific antibodies has recently been shown to result in neuronal apoptosis in the cerebellum and hippocampus — most probably through clustering of PrP^{C}, which is thought to trigger an abnormal signalling pathway[104]. These results are alarming and certainly reinforce the concept that adequate *in vivo* safety studies must be carried out before prion immunoprophylaxis trials take place in humans. However, it should be noted that neuronal loss occurred only when using antibodies specific for a subset of PrP epitopes (those present in amino acids 95–105 of the PrP sequence) and only at extremely high concentrations of these antibodies. So, we do not consider that these data rule out the possibility of antiprion immunization, but further study is required.

PrP–Fc$_2$: a sink for pathogenic PrP^{Sc}. To study PrP^{C}–PrP^{Sc} complexes and 'fish' for potential PrP^{C} ligands, a transgenic mouse was developed that expresses soluble full-length mouse PrP rendered dimeric by fusion with the Fc portion of human IgG1 (known as PrP–Fc$_2$). It was thought that this model would provide a stable and soluble, tagged form of PrP^{C} for tracking potential interaction partners for PrP^{C} and PrP^{Sc} *in vivo*. However, after prion inoculation, these mice were surprisingly resistant to prion disease[30]. The PrP–Fc$_2$ was not converted to a prion-disease-causing isoform. Moreover, when the transgenic mice expressing PrP–Fc$_2$ were back-crossed with wild-type mice and then inoculated with prions, they showed marked retardation in the development of prion disease, which was equivalent to a 10^5-fold reduction in titre of the prion-infected inoculum. This antiprion effect

CYTOKINE STORM
A sudden surge in the circulating levels of pro-inflammatory cytokines, such as interleukin-1, interleukin-6, tumour-necrosis factor and interferon-γ.

BLOOD–BRAIN BARRIER
A barrier that is formed by tight junctions between endothelial cells and markedly limits the entry to the central nervous system of leukocytes and all large molecules, including (to some extent) immunoglobulins, cytokines and complement proteins.

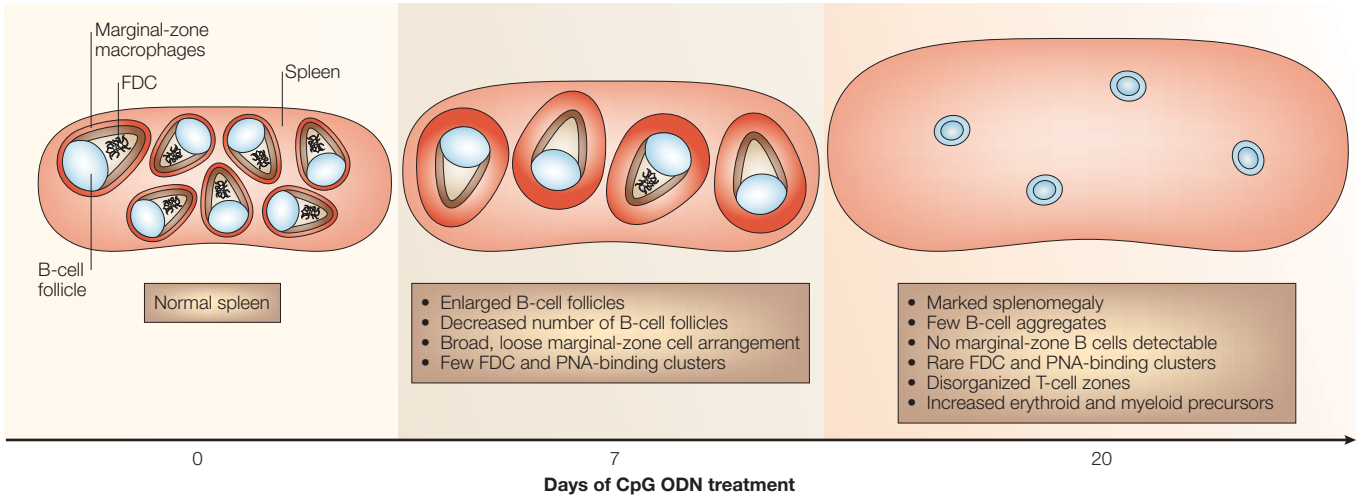

Figure 6 | **Treatment with CpG ODNs leads to lymphoid germinal-centre destruction.** Treatment of mice with a multi-dose regimen of immunostimulatory CpG-containing oligodeoxynucleotides (CpG ODNs) (20 doses of 60 μg each day) prolongs the onset of prion disease but at a high cost[95]. As the daily injections continue, the severity of follicular destruction in the spleen increases, until day 20 of treatment when the spleen is markedly enlarged but has few follicles and only rare follicular dendritic cell (FDC)-M1-positive cells. PNA, peanut agglutinin.

occurred in two different lines of PrP–Fc_2-expressing transgenic mice after either intracerebral or intraperitoneal infection with scrapie. Therefore, it seems that PrP–Fc_2 effectively antagonizes prion accumulation in the spleen and brain.

In this study, the PrP–Fc_2 was bound to PrP^{Sc} in complexes with properties similar to LIPID RAFTS. Therefore, it seems that PrP–Fc_2 competes directly with PrP^{C} for binding to PrP^{Sc}. Because PrP–Fc_2 cannot be converted to the protease-resistant, disease-causing isoform, it might be effectively functioning as a 'sink' for PrP^{Sc}, by binding PrP^{Sc} and preventing the binding and conversion of PrP^{C}.

Although the precise mechanisms that occur at the molecular level remain a matter of speculation, all biochemical evidence indicates that PrP–Fc_2 might bind PrP^{Sc} and prevent conversion. Further studies using this innovative therapy could reveal its potential to treat prion disease.

Which therapy? It is important to note that none of the immunotherapies being developed is close to being used for the treatment of humans or other animals. But, which of these therapies has the most potential? In our opinion, therapy using LT-βR–Ig is the only candidate that could be considered at present, and in humans, it would need to be used under the 'compassionate/experimental' label. Because LT-βR–Ig might prevent prion disease only if it is administered early in infection, its potential usefulness is restricted to incidents in which the time of prion exposure is well defined, such as needle-stick accidents that involve cerebrospinal fluid from patients with CJD. In our experience, such incidents occur more frequently than might be expected, and our current inability to offer a prophylactic option to the victims is frustrating.

Theoretically, germinal-centre disruption, owing to the breakdown of FDC networks that is caused by LT-βR–Ig, might have unwanted effects on immune function. However, manipulation of the LT pathway has been carried out in macaques without apparent side-effects: primary antibody responses to keyhole limpet haemocyanin were found to be unaltered during a 20-day period[105]. Moreover, LT-fusion proteins are already entering clinical trials as a treatment for rheumatoid arthritis. Hopefully, the results of such studies will become available in the foreseeable future.

PrP–Fc_2 is also a promising compound that might be developed as a therapy. However, further research is needed to establish whether it is effective not only as a transgene but also as a biopharmaceutical. In addition, it remains to be established whether the current form of PrP–Fc_2 has the strongest antiprion properties: the introduction of dominant-negative mutations analogous to those described for *Prnp*[106] might considerably augment its efficacy.

Our prognosis for the administration of CpG ODNs is less favourable. At least in the mouse model, the toxic effects of frequent and repeated administration of CpG ODNs seem to be prohibitive. One might argue that severe side-effects could be tolerable in the case of a disease that is invariably fatal. However, if the antiprion action of CpG ODNs is mediated by indiscriminate immune suppression, then there are alternative ways to achieve this goal that would at least not lead to hepatotoxicity.

At present, it is unclear whether vaccination approaches will be translated to clinical practice. PrP^{C}-specific antibodies are clearly effective at eliminating prions from cultured cells[101,102], but the predictive value of such *in vitro* tests is limited. In transgenic mice that express PrP^{C}-specific antibodies, peripheral (but not central) prion pathogenesis is impaired. But, at present,

LIPID RAFTS
Specialized membrane domains that are enriched in cholesterol, glycosphingolipids and proteins that function in signal transduction. Rafts are often equated with 'detergent-resistant membranes', which can be isolated by density-gradient centrifugation as a function of their high buoyancy.

the main unresolved problem is that tolerance to PrP^C is high. Although PrP-deficient mice generate high concentrations of prion-specific antibodies[107,108], it is extremely difficult to elicit a strong prion-specific antibody response in mice that express PrP^C (REF. 109). Although it is not impossible to immunize wild-type mice so that they generate antibodies able to recognize bacterially expressed PrP, we have reason to suspect that considerable protection can only be provided by antibodies that react with natural PrP^C displayed on eukaryotic-cell membranes in the context of its molecular partners[148]. Finally, the interface between lymphoreticular organs and nerves has just begun to be explored with respect to prion pathogenesis[56]. Although it is clear that prions exploit peripheral nerves to accomplish neuroinvasion[70,77], it is currently unclear whether this property can be targeted to develop therapeutic or prophylactic approaches.

The future of antiprion therapeutics

The extent of exposure of the European population to BSE is unknown. It could be that millions of people have been exposed to the causative agent of BSE, considering that the prevalence of subclinical BSE in slaughtered cattle might have peaked at ~20% in the United Kingdom and was also high in other European countries. Fortunately, so far, only 147 cases of vCJD have been recorded worldwide (until July 2004) (see National CJD Surveillance Unit in online links box).

Since the recent BSE crisis, BSE has become a rare disease among cattle, and several redundant safety measures have been instituted throughout the human food chain; therefore, at present, the danger of contracting a prion infection from cattle is, arguably, minimal. However, it must be assumed that an unknown number of humans are currently subclinically infected with BSE prions. Although some of these individuals might progress to overt disease (that is, vCJD or other as-yet-unrecognized phenotypic manifestations of BSE infection), an unknown proportion of infected individuals might establish a permanent subclinical carrier state. It is a high public-health priority to determine both whether these individuals could inadvertently transmit the agent to others and the ways in which this might occur (BOX 1).

For example, the possibility of prion transmission by receiving a blood transfusion from individuals with preclinical vCJD has prompted radical measures to prevent this scenario. Consequently, the United Kingdom and many other countries have introduced universal leukodepletion of blood units. However, it is unknown whether this costly measure is adequate or necessary, because the way in which prion infectivity partitions in blood is unclear. In addition, the United Kingdom is sourcing its entire supply of plasma and plasma-derived products from abroad. Most recently, recipients of blood transfusions have been banned from making further donations. By contrast, labile blood products, such as thrombocytes and erythrocytes, are too scarce to be outsourced. Nonetheless, a vCJD case has recently emerged in the United Kingdom that might have resulted from transfusion of a blood unit from a donor with preclinical vCJD[110]. Unfortunately, records show that 48 individuals in the United Kingdom have received blood transfusions from donors who later developed vCJD[111].

Given these unknowns, it is essential that we continue to develop therapies for prion diseases. Until approximately 40 years ago, it was thought that prion diseases were completely independent of the immune system. The lack of overt inflammation in spongiform lesions provided support for this view. However, it is now obvious that there is a close and multifaceted relationship between the infectious agent and the various components of the immune system[57].

Hopefully, this accrued knowledge will eventually be put into useful practice. So, what prevention strategy could be used for individuals who are known to have been exposed to prions? And what treatment strategy could be attempted to curb prion diseases during the clinical phase? In this regard, it is encouraging to note that many of the steps in prion transport that have been discussed here seem to be rate limiting in experimental scrapie models. Consequently, these steps could be targets for interventions, which might be either therapeutic or prophylactic[112]. The tremendous interest in this field has attracted researchers from various neighbouring disciplines, including immunology, genetics and pharmacology, and therefore it is to be hoped that rational and efficient methods for managing prion infections are developed in the future.

1. Aguzzi, A. & Haass, C. Games played by rogue proteins in prion disorders and Alzheimer's disease. *Science* **302**, 814–818 (2003).
2. Cuille, J. & Chelle, P. L. Experimental transmission of trembling to the goat. *C. R. Hebd. Seances Acad. Sci. D, Sci. Nat.* **208**, 1058–1160 (1939).
3. Wells, G. A. *et al*. A novel progressive spongiform encephalopathy in cattle. *Vet. Rec.* **121**, 419–420 (1987).
4. Weissmann, C. & Aguzzi, A. Bovine spongiform encephalopathy and early onset variant Creutzfeldt–Jakob disease. *Curr. Opin. Neurobiol.* **7**, 695–700 (1997).
5. Taylor, D. M. Inactivation of transmissible degenerative encephalopathy agents: a review. *Vet. J.* **159**, 10–17 (2000).
6. Aguzzi, A. Neuro-immune connection in spread of prions in the body? *Lancet* **349**, 742–743 (1997).
7. Nicotera, P. A route for prion neuroinvasion. *Neuron* **31**, 345–348 (2001).
8. Bolton, D. C., McKinley, M. P. & Prusiner, S. B. Identification of a protein that purifies with the scrapie prion. *Science* **218**, 1309–1311 (1982).
Discovery of the proteinase-K-resistant prion, PrP^{Sc}, in a hamster infected with scrapie.
9. Stahl, N., Borchelt, D. R., Hsiao, K. & Prusiner, S. B. Scrapie prion protein contains a phosphatidylinositol glycolipid. *Cell* **51**, 229–240 (1987).
10. Moudjou, M., Frobert, Y., Grassi, J. & La Bonnardiere, C. Cellular prion protein status in sheep: tissue-specific biochemical signatures. *J. Gen. Virol.* **82**, 2017–2024 (2001).
11. Miele, G. *et al*. Embryonic activation and developmental expression of the murine prion protein gene. *Gene Expr.* **11**, 1–12 (2003).
12. Büeler, H. R. *et al*. Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. *Nature* **356**, 577–582 (1992).
13. Collinge, J. *et al*. Prion protein is necessary for normal synaptic function. *Nature* **370**, 295–297 (1994).
14. Tobler, I. *et al*. Altered circadian activity rhythms and sleep in mice devoid of prion protein. *Nature* **380**, 639–642 (1996).
15. Glockshuber, R. *et al*. Prion protein structural features indicate possible relations to signal peptidases. *FEBS Lett.* **426**, 291–296 (1998).
16. Brown, D. R., Schulz-Schaeffer, W. J., Schmidt, B. & Kretzschmar, H. A. Prion protein-deficient cells show altered response to oxidative stress due to decreased SOD-1 activity. *Exp. Neurol.* **146**, 104–112 (1997).
17. Brown, D. R. *et al*. Normal prion protein has an activity like that of superoxide dismutase. *Biochem. J.* **344**, 1–5 (1999).
18. Sakudo, A. *et al*. Impairment of superoxide dismutase activation by N-terminally truncated prion protein (PrP) in PrP-deficient neuronal cell line. *Biochem. Biophys. Res. Commun.* **308**, 660–667 (2003).
19. Waggoner, D. J. *et al*. Brain copper content and cuproenzyme activity do not vary with prion protein expression level. *J. Biol. Chem.* **275**, 7455–7458 (2000).

20. Hutter, G., Heppner, F. L. & Aguzzi, A. No superoxide dismutase activity of cellular prion protein *in vivo*. *Biol. Chem.* **384**, 1279–1285 (2003).
21. Prusiner, S. B. Novel proteinaceous infectious particles cause scrapie. *Science* **216**, 136–144 (1982).
Classic enunciation of the protein-only hypothesis of prion disease by Stanley Prusiner.
22. Safar, J. *et al*. Eight prion strains have PrP^{Sc} molecules with different conformations *Nature Med*. **4**, 1157–1165 (1998).
23. Aguzzi, A. & Heikenwalder, M. Prion diseases: cannibals and garbage piles. *Nature* **423**, 127–129 (2003).
24. Chesebro, B. *et al*. Identification of scrapie prion protein-specific mRNA in scrapie-infected and uninfected brain. *Nature* **315**, 331–333 (1985).
Describes the cloning of the gene that encodes PrP^{C}.
25. Weissmann, C. Spongiform encephalopathies. The prion's progress. *Nature* **349**, 569–571 (1991).
26. Telling, G. C. *et al*. Prion propagation in mice expressing human and chimeric *PrP* transgenes implicates the interaction of cellular PrP with another protein. *Cell* **83**, 79–90 (1995).
27. Weissmann, C. A 'unified theory' of prion propagation. *Nature* **352**, 679–683 (1991).
28. Deleault, N. R., Lucassen, R. W. & Supattapone, S. RNA molecules stimulate prion protein conversion. *Nature* **425**, 717–720 (2003).
29. Aguzzi, A. & Weissmann, C. Prion research: the next frontiers. *Nature* **389**, 795–798 (1997).
30. Meier, P. *et al*. Soluble dimeric prion protein binds PrP^{Sc} *in vivo* and antagonizes prion disease. *Cell* **113**, 49–60 (2003).
31. Aguzzi, A. & Polymenidou, M. Mammalian prion biology. One century of evolving concepts. *Cell* **116**, 313–327 (2004).
32. McKinley, M. P., Bolton, D. C. & Prusiner, S. B. A protease-resistant protein is a structural component of the scrapie prion. *Cell* **35**, 57–62 (1983).
33. Kayed, R. *et al*. Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science* **300**, 486–489 (2003).
34. Raeber, A. J. *et al*. Astrocyte-specific expression of hamster prion protein (PrP) renders *PrP* knockout mice susceptible to hamster scrapie. *EMBO J*. **16**, 6057–6065 (1997).
35. Mallucci, G. *et al*. Depleting neuronal PrP in prion infection prevents disease and reverses spongiosis. *Science* **302**, 871–874 (2003).
Shows that depletion of PrP from the neurons of transgenic mice prevents the development of prion disease in infected mice.
36. Basler, K. *et al*. Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene. *Cell* **46**, 417–428 (1986).
37. Büeler, H. R. *et al*. Mice devoid of PrP are resistant to scrapie. *Cell* **73**, 1339–1347 (1993).
Shows that PrP is required for susceptibility to prion infections. An important milestone in prion research.
38. Brandner, S. *et al*. Normal host prion protein necessary for scrapie-induced neurotoxicity. *Nature* **379**, 339–343 (1996).
39. Büeler, H. *et al*. High prion and PrP^{Sc} levels but delayed onset of disease in scrapie-inoculated mice heterozygous for a disrupted *PrP* gene. *Mol. Med.* **1**, 19–30 (1994).
40. Manson, J. C., Clarke, A. R., McBride, P. A., McConnell, I. & Hope, J. *PrP* gene dosage determines the timing but not the final intensity or distribution of lesions in scrapie pathology. *Neurodegeneration* **3**, 331–340 (1994).
41. Hilton, D. A. *et al*. Specificity of lymphoreticular accumulation of prion protein for variant Creutzfeldt–Jakob disease. *J. Clin. Pathol.* **57**, 300–302 (2004).
42. Heggebo, R., Press, C. M., Gunnes, G., Gonzalez, L. & Jeffrey, M. Distribution and accumulation of PrP in gut-associated and peripheral lymphoid tissue of scrapie-affected Suffolk sheep. *J. Gen. Virol.* **83**, 479–489 (2002).
43. Jeffrey, M., McGovern, G., Martin, S., Goodsir, C. M. & Brown, K. L. Cellular and sub-cellular localisation of PrP in the lymphoreticular system of mice and sheep. *Arch. Virol. Suppl.* **16**, 23–38 (2000).
44. Sigurdson, C. J. *et al*. Oral transmission and early lymphoid tropism of chronic wasting disease PrP^{res} in mule deer fawns (*Odocoileus hemionus*). *J. Gen. Virol.* **80**, 2757–2764 (1999).
45. Terry, L. A. *et al*. Detection of disease-specific PrP in the distal ileum of cattle exposed orally to the agent of bovine spongiform encephalopathy. *Vet. Rec.* **152**, 387–392 (2003).
46. Wells, G. A. *et al*. Preliminary observations on the pathogenesis of experimental bovine spongiform encephalopathy (BSE): an update. *Vet. Rec.* **142**, 103–106 (1998).
47. Glatzel, M., Abela, E., Maissen, M. & Aguzzi, A. Extraneural pathologic prion protein in sporadic Creutzfeldt–Jakob disease. *N. Engl. J. Med.* **349**, 1812–1820 (2003).
48. Houston, E. F., Halliday, S. I., Jeffrey, M., Goldmann, W. & Hunter, N. New Zealand sheep with scrapie-susceptible *PrP* genotypes succumb to experimental challenge with a sheep-passaged scrapie isolate (SSBP/1). *J. Gen. Virol.* **83**, 1247–1250 (2002).
49. Fraser, H. & Dickinson, A. G. Pathogenesis of scrapie in the mouse: the role of the spleen. *Nature* **226**, 462–463 (1970).
Shows that PrP accumulates in lymphoid organs in the mouse model of scrapie.
50. Heppner, F. L., Prinz, M. & Aguzzi, A. Pathogenesis of prion diseases: possible implications of microglial cells. *Prog. Brain Res.* **132**, 737–750 (2001).
51. Betmouni, S., Perry, V. H. & Gordon, J. L. Evidence for an early inflammatory response in the central nervous system of mice with scrapie. *Neuroscience* **74**, 1–5 (1996).
52. Perry, V. H., Cunningham, C. & Boche, D. Atypical inflammation in the central nervous system in prion disease. *Curr. Opin. Neurol.* **15**, 349–354 (2002).
53. Klein, M. A. *et al*. A crucial role for B cells in neuroinvasive scrapie. *Nature* **390**, 687–690 (1997).
54. Klein, M. A. *et al*. Complement facilitates early prion pathogenesis. *Nature Med.* **7**, 488–492. (2001).
55. Prinz, M. *et al*. Prion pathogenesis in the absence of Toll-like receptor signalling. *EMBO Rep.* **4**, 195–199 (2003).
56. Prinz, M. *et al*. Positioning of follicular dendritic cells within the spleen controls prion neuroinvasion. *Nature* **425**, 957–962 (2003).
57. Aguzzi, A. Prions and the immune system: a journey through gut, spleen, and nerves. *Adv. Immunol.* **81**, 123–171 (2003).
58. Griffith, J. S. Self-replication and scrapie. *Nature* **215**, 1043–1044 (1967).
A mathematician proposes that the scrapie agent might not be a traditional infectious agent but, instead, an infectious protein.
59. Pattison, I. H. & Millson, G. C. Further observations on the experimental production of scrapie in goats and sheep. *J. Comp. Pathol.* **70**, 182–193 (1960).
60. Pattison, I. H. & Millson, G. C. Distribution of the scrapie agent in the tissues of experimentally inoculated goats. *J. Comp. Pathol*. **72**, 233–244 (1962).
61. Heppner, F. L. *et al*. Transepithelial prion transport by M cells. *Nature Med.* **7**, 976–977 (2001).
62. Prinz, M. *et al*. Oral prion infection requires normal numbers of Peyer's patches but not of enteric lymphocytes. *Am. J. Pathol.* **162**, 1103–1111 (2003).
63. Heggebo, R. *et al*. Detection of PrP^{Sc} in lymphoid tissues of lambs experimentally exposed to the scrapie agent. *J. Comp. Pathol.* **128**, 172–181 (2003).
64. Huang, F. P., Farquhar, C. F., Mabbott, N. A., Bruce, M. E. & MacPherson, G. G. Migrating intestinal dendritic cells transport PrP^{Sc} from the gut. *J. Gen. Virol.* **83**, 267–271 (2002).
65. Aucouturier, P. *et al*. Infected splenic dendritic cells are sufficient for prion transmission to the CNS in mouse scrapie. *J. Clin. Invest.* **108**, 703–708 (2001).
66. Prinz, M. *et al*. Lymph nodal prion replication and neuroinvasion in mice devoid of follicular dendritic cells. *Proc. Natl Acad. Sci. USA* **99**, 919–924 (2002).
67. Montrasio, F. *et al*. Impaired prion replication in spleens of mice lacking functional follicular dendritic cells. *Science* **288**, 1257–1259 (2000).
68. Mabbott, N. A., Young, J., McConnell, I. & Bruce, M. E. Follicular dendritic cell dedifferentiation by treatment with an inhibitor of the lymphotoxin pathway dramatically reduces scrapie susceptibility. *J. Virol.* **77**, 6845–6854 (2003).
69. Mabbott, N. A., Mackay, F., Minns, F. & Bruce, M. E. Temporary inactivation of follicular dendritic cells delays neuroinvasion of scrapie. *Nature Med.* **6**, 719–720 (2000).
70. Blättler, T. *et al*. PrP-expressing tissue required for transfer of scrapie infectivity from spleen to brain. *Nature* **389**, 69–73 (1997).
Finds that PrP^{C} is required for prions to reach the CNS after a peripheral exposure (either intraperitoneal or intravenous).
71. Kaeser, P. S., Klein, M. A., Schwarz, P. & Aguzzi, A. Efficient lymphoreticular prion propagation requires PrP^{C} in stromal and hematopoietic cells. *J. Virol.* **75**, 7097–7106 (2001).
72. Klein, M. A. *et al*. PrP expression in B lymphocytes is not required for prion neuroinvasion. *Nature Med.* **4**, 1429–1433 (1998).
73. Brown, K. L. *et al*. Scrapie replication in lymphoid tissues depends on prion protein-expressing follicular dendritic cells. *Nature Med.* **5**, 1308–1312 (1999).
74. Forster, R. *et al*. A putative chemokine receptor, BLR1, directs B cell migration to defined lymphoid organs and specific anatomic compartments of the spleen. *Cell* **87**, 1037–1047 (1996).
75. Beekes, M., Baldauf, E. & Diringer, H. Sequential appearance and accumulation of pathognomonic markers in the central nervous system of hamsters orally infected with scrapie. *J. Gen. Virol.* **77**, 1925–1934 (1996).
76. Beekes, M., McBride, P. A. & Baldauf, E. Cerebral targeting indicates vagal spread of infection in hamsters fed with scrapie. *J. Gen. Virol.* **79**, 601–607 (1998).
77. Glatzel, M., Heppner, F. L., Albers, K. M. & Aguzzi, A. Sympathetic innervation of lymphoreticular organs is rate limiting for prion neuroinvasion. *Neuron* **31**, 25–34 (2001).
78. Caughey, B. & Race, R. E. Potent inhibition of scrapie-associated PrP accumulation by Congo red. *J. Neurochem.* **59**, 768–771 (1992).
79. Pocchiari, M., Schmittinger, S. & Masullo, C. Amphotericin B delays the incubation period of scrapie in intracerebrally inoculated hamsters. *J. Gen. Virol.* **68**, 219–223 (1987).
80. Tagliavini, F. *et al*. Effectiveness of anthracycline against experimental prion disease in Syrian hamsters. *Science* **276**, 1119–1122 (1997).
81. Caughey, B. & Raymond, G. J. Sulfated polyanion inhibition of scrapie-associated PrP accumulation in cultured cells. *J. Virol.* **67**, 643–650 (1993).
82. Farquhar, C., Dickinson, A. & Bruce, M. Prophylactic potential of pentosan polysulphate in transmissible spongiform encephalopathies. *Lancet* **353**, 117 (1999).
83. Priola, S. A., Raines, A. & Caughey, W. S. Porphyrin and phthalocyanine antiscrapie compounds. *Science* **287**, 1503–1506 (2000).
84. Supattapone, S. *et al*. Branched polyamines cure prion-infected neuroblastoma cells. *J. Virol.* **75**, 3453–3461 (2001).
85. Soto, C. *et al*. Reversion of prion protein conformational changes by synthetic β-sheet breaker peptides. *Lancet* **355**, 192–197 (2000).
86. Fraser, H. *et al*. Replication of scrapie in spleens of SCID mice follows reconstitution with wild-type mouse bone marrow. *J. Gen. Virol.* **77**, 1935–1940 (1996).
87. Jeffrey, M., McGovern, G., Goodsir, C. M., Brown, K. L. & Bruce, M. E. Sites of prion protein accumulation in scrapie-infected mouse spleen revealed by immuno-electron microscopy. *J. Pathol.* **191**, 323–332 (2000).
88. Sigurdson, C. J. *et al*. PrP^{CWD} lymphoid cell targets in early and advanced chronic wasting disease of mule deer. *J. Gen. Virol.* **83**, 2617–2628 (2002).
89. Kitamoto, T., Muramoto, T., Mohri, S., Doh-ura, K. & Tateishi, J. Abnormal isoform of prion protein accumulates in follicular dendritic cells in mice with Creutzfeldt–Jakob disease. *J. Virol.* **65**, 6292–6295 (1991).
90. Mackay, F. & Browning, J. L. Turning off follicular dendritic cells. *Nature* **395**, 26–27 (1998).
91. Wu, Q. *et al*. The requirement of membrane lymphotoxin for the presence of dendritic cells in lymphoid tissues. *J. Exp. Med.* **190**, 629–638 (1999).
92. Adachi, O. *et al*. Targeted disruption of the *MyD88* gene results in loss of IL-1- and IL-18-mediated function. *Immunity* **9**, 143–150 (1998).
93. Hemmi, H. *et al*. A Toll-like receptor recognizes bacterial DNA. *Nature* **408**, 740–745 (2000).
94. Sethi, S., Lipford, G., Wagner, H. & Kretzschmar, H. Postexposure prophylaxis against prion disease with a stimulator of innate immunity. *Lancet* **360**, 229–230 (2002).
95. Heikenwalder, M. *et al*. Lymphoid follicle destruction and immunosuppression after repeated CpG oligodeoxynucleotide administration. *Nature Med.* **10**, 187–192 (2004).
96. Beringue, V. *et al*. Role of spleen macrophages in the clearance of scrapie agent early in pathogenesis. *J. Pathol.* **190**, 495–502 (2000).
97. Mattei, V. *et al*. Prion protein is a component of the multimolecular signaling complex involved in T cell activation. *FEBS Lett.* **560**, 14–18 (2004).
98. Manson, J. *et al*. The prion protein gene: a role in mouse embryogenesis? *Development* **115**, 117–122 (1992).
99. Cashman, N. R. *et al*. Cellular isoform of the scrapie agent protein participates in lymphocyte activation. *Cell* **61**, 185–192 (1990).
100. Heppner, F. L. *et al*. Prevention of scrapie pathogenesis by transgenic expression of anti-prion protein antibodies. *Science* **294**, 178–182 (2001).

101. Enari, M., Flechsig, E. & Weissmann, C. Scrapie prion protein accumulation by scrapie-infected neuroblastoma cells abrogated by exposure to a prion protein antibody. *Proc. Natl Acad. Sci. USA* **98**, 9295–9299 (2001).
102. Peretz, D. *et al*. Antibodies inhibit prion propagation and clear cell cultures of prion infectivity. *Nature* **412**, 739–743 (2001).
103. White, A. R. *et al*. Monoclonal antibodies inhibit prion replication and delay the development of prion disease. *Nature* **422**, 80–83 (2003).
104. Solforosi, L. *et al*. Cross-linking cellular prion protein triggers neuronal apoptosis *in vivo*. *Science* **303**, 514–516 (2004).
105. Gommerman, J. L. *et al*. Manipulation of lymphoid microenvironments in nonhuman primates by an inhibitor of the lymphotoxin pathway. *J. Clin. Invest.* **110**, 1359–1369 (2002).
106. Perrier, V. *et al*. Dominant-negative inhibition of prion replication in transgenic mice. *Proc. Natl Acad. Sci. USA* **99**, 13079–13084 (2002).
107. Brandner, S. *et al*. Normal host prion protein (PrP^C) is required for scrapie spread within the central nervous system. *Proc. Natl Acad. Sci. USA* **93**, 13148–13151 (1996).
108. Prusiner, S. B. *et al*. Ablation of the prion protein (*PrP*) gene in mice prevents scrapie and facilitates production of anti-PrP antibodies. *Proc. Natl Acad. Sci. USA* **90**, 10608–10612 (1993).
109. Koller, M. F., Grau, T. & Christen, P. Induction of antibodies against murine full-length prion protein in wild-type mice. *J. Neuroimmunol.* **132**, 113–116 (2002).
110. Aguzzi, A. & Glatzel, M. vCJD tissue distribution and transmission by transfusion — a worst-case scenario coming true? *Lancet* **363**, 411–412 (2004).
111. Llewelyn, C. A. *et al*. Possible transmission of variant Creutzfeldt–Jakob disease by blood transfusion. *Lancet* **363**, 417–421 (2004).
112. Aguzzi, A., Glatzel, M., Montrasio, F., Prinz, M. & Heppner, F. L. Interventional strategies against prion diseases. *Nature Rev. Neurosci.* **2**, 745–749 (2001).
113. Will, R. G. *et al*. A new variant of Creutzfeldt–Jakob disease in the UK. *Lancet* **347**, 921–925 (1996).
114. Chazot, G. *et al*. New variant of Creutzfeldt–Jakob disease in a 26-year-old French man. *Lancet* **347**, 1181 (1996).
115. Aguzzi, A. & Weissmann, C. Spongiform encephalopathies: a suspicious signature. *Nature* **383**, 666–667 (1996).
116. Hill, A. F., Zeidler, M., Ironside, J. & Collinge, J. Diagnosis of new variant Creutzfeldt–Jakob disease by tonsil biopsy. *Lancet* **349**, 99–100 (1997).
117. Frigg, R., Klein, M. A., Hegyi, I., Zinkernagel, R. M. & Aguzzi, A. Scrapie pathogenesis in subclinically infected B-cell-deficient mice. *J. Virol.* **73**, 9584–9588 (1999).
118. Houston, F., Foster, J. D., Chong, A., Hunter, N. & Bostock, C. J. Transmission of BSE by blood transfusion in sheep. *Lancet* **356**, 999–1000 (2000).
Shows that BSE prion infectivity circulates in the blood and is transmissible by transfusion. It was subsequently reported (reference 111) that a probable case of vCJD was contracted through blood transfusion.
119. Hunter, N. *et al*. Transmission of prion diseases by blood transfusion. *J. Gen. Virol.* **83**, 2897–2905 (2002).
120. Glatzel, M. *et al*. Human prion diseases: epidemiology and integrated risk assessment. *Lancet Neurol.* **2**, 757–763 (2003).
121. Aguzzi, A., Montrasio, F. & Kaeser, P. S. Prions: health scare and biological challenge. *Nature Rev. Mol. Cell Biol.* **2**, 118–126 (2001).
122. Hadlow, W. J., Kennedy, R. C., Race, R. E. & Eklund, C. M. Virologic and neurohistologic findings in dairy goats affected with natural scrapie. *Vet. Pathol.* **17**, 187–199 (1980).
123. Hadlow, W. J. *et al*. Course of experimental scrapie virus infection in the goat. *J. Infect. Dis.* **129**, 559–567 (1974).
124. Buyukmihci, N., Rorvik, M. & Marsh, R. F. Replication of the scrapie agent in ocular neural tissues. *Proc. Natl Acad. Sci. USA* **77**, 1169–1171 (1980).
125. Pattison, I. H., Hoare, M. N., Jebbett, J. N. & Watson, W. A. Spread of scrapie to sheep and goats by oral dosing with foetal membranes from scrapie-affected sheep. *Vet. Rec.* **90**, 465–468 (1972).
126. Endres, R. *et al*. Mature follicular dendritic cell networks depend on expression of lymphotoxin-β receptor by radioresistant stromal cells and of lymphotoxin-β and tumor necrosis factor by B cells. *J. Exp. Med.* **189**, 159–168 (1999).
127. Gibbs, C. J. Jr, Amyx, H. L., Bacote, A., Masters, C. L. & Gajdusek, D. C. Oral transmission of kuru, Creutzfeldt–Jakob disease, and scrapie to nonhuman primates. *J. Infect. Dis.* **142**, 205–208 (1980).
128. Gibbs, C. J. Jr & Gajdusek, D. C. Infection as the etiology of spongiform encephalopathy (Creutzfeldt–Jakob disease). *Science* **165**, 1023–1025 (1969).
129. Liberski, P. P. & Mori, S. The Echigo-1: a panencephalopathic strain of Creutzfeldt–Jakob disease: a passage to hamsters and ultrastructural studies. *Folia Neuropathol.* **35**, 250–254 (1997).
130. Taguchi, Y., Mohri, S., Ironside, J. W., Muramoto, T. & Kitamoto, T. Humanized knock-in mice expressing chimeric prion protein showed varied susceptibility to different human prions. *Am. J. Pathol.* **163**, 2585–2593 (2003).
131. Tateishi, J. Transmission of human prion diseases to rodents. *Semin. Virol.* **7**, 175–180 (1996).
132. Asante, E. A. *et al*. BSE prions propagate as either variant CJD-like or sporadic CJD-like prion strains in transgenic mice expressing human prion protein. *EMBO J.* **21**, 6358–6366 (2002).
133. Brown, D. A., Bruce, M. E. & Fraser, J. R. Comparison of the neuropathological characteristics of bovine spongiform encephalopathy (BSE) and variant Creutzfeldt–Jakob disease (vCJD) in mice. *Neuropathol. Appl. Neurobiol.* **29**, 262–272 (2003).
134. Gajdusek, D. C., Gibbs, C. J. Jr & Alpers, M. Transmission and passage of experimenal 'kuru' to chimpanzees. *Science* **155**, 212–214 (1967).
Shows that a human TSE, kuru, is transmissible to chimpanzees. Kuru was endemic in the Fore people of Papua New Guinea and transmitted between individuals through ritualistic cannibalism of the dead.
135. Tateishi, J. *et al*. First experimental transmission of fatal familial insomnia. *Nature* **376**, 434–435 (1995).
136. Tateishi, J., Sato, Y., Nagara, H. & Boellaard, J. W. Experimental transmission of human subacute spongiform encephalopathy to small rodents. IV. Positive transmission from a typical case of Gerstmann–Straussler–Scheinker's disease. *Acta Neuropathol.* **64**, 85–88 (1984).
137. Baker, H. F., Duchen, L. W., Jacobs, J. M. & Ridley, R. M. Spongiform encephalopathy transmitted experimentally from Creutzfeldt–Jakob and familial Gerstmann–Straussler–Scheinker diseases. *Brain* **113**, 1891–1909 (1990).
138. Bruce, M. E. *et al*. Strain characterization of natural sheep scrapie and comparison with BSE. *J. Gen. Virol.* **83**, 695–704 (2002).
139. Prusiner, S. B., Cochran, S. P. & Alpers, M. P. Transmission of scrapie in hamsters. *J. Infect. Dis.* **152**, 971–978 (1985).
140. Vilotte, J. L. *et al*. Markedly increased susceptibility to natural sheep scrapie of transgenic mice expressing ovine PrP. *J. Virol.* **75**, 5977–5984 (2001).
141. Bartz, J. C., Marsh, R. F., McKenzie, D. I. & Aiken, J. M. The host range of chronic wasting disease is altered on passage in ferrets. *Virology* **251**, 297–301 (1998).
142. Castilla, J. *et al*. Early detection of PrP^{res} in BSE-infected bovine *PrP* transgenic mice. *Arch. Virol.* **148**, 677–691 (2003).
143. Bons, N. *et al*. Natural and experimental oral infection of nonhuman primates by bovine spongiform encephalopathy agents. *Proc. Natl Acad. Sci. USA* **96**, 4046–4051 (1999).
144. Lasmezas, C. I. *et al*. BSE transmission to macaques. *Nature* **381**, 743–744 (1996).
145. Bruce, M. *et al*. Transmission of bovine spongiform encephalopathy and scrapie to mice: strain variation and the species barrier. *Philos. Trans. R. Soc. Lond. B* **343**, 405–411 (1994).
146. Fraser, H. *et al*. Transmission of feline spongiform encephalopathy to mice. *Vet. Rec.* **134**, 449 (1994).
147. Bessen, R. A. & Marsh, R. F. Identification of two biologically distinct strains of transmissible mink encephalopathy in hamsters. *J. Gen. Virol.* **73**, 329–334 (1992).
148. Polymenidou, M. *et al*. Humoral immune response to native eukaryotic prion protein correlates with anti-prion protection. *Proc. Natl Acad. Sci. USA* 3 August 2004 (doi:10.1073/pnas.0404772101).

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