

# APPROACHES TO THERAPY OF PRION DISEASES

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**Key Words** transmissible spongiform encephalopathies, Creutzfeldt-Jakob disease, bovine spongiform encephalopathy, proteinopathy, neuroblastoma cells

■ **Abstract** Devising approaches to the therapy of transmissible spongiform encephalopathies, or prion diseases, is beset by many difficulties. For one, the nature of the infectious agent, the prion, is understood only in outline, and its composition, structure, and mode of replication are still shrouded in mystery. In addition, the mechanism of pathogenesis is not well understood. Because clinical disease affects mainly the brain parenchyme, therapeutic agents must be able to traverse the brain-blood barrier (BBB) or have to be introduced directly into the cerebrospinal fluid or brain tissue. And finally, because the disease is usually recognized only after onset of severe clinical symptoms, the question arises as to whether the neurodegenerative processes can be reversed to any extent after a successful eradication of the agent.

## THE DISEASE

Transmissible spongiform encephalopathies (TSEs) are neurodegenerative diseases characterized by spongiform changes, neuronal death, astrocytosis, and accumulation of the pathological protein PrP<sup>Sc</sup> (the “scrapie form” of PrP) in the brain and to a lesser extent in other organs. TSEs are by definition transmissible, although this criterion may be difficult to establish in some cases.

The most common human TSE, or prion disease, is Creutzfeldt-Jakob disease (CJD). Epidemiologically, CJD is classified as sporadic (sCJD), familial (fCJD), iatrogenic (iCJD), and variant (vCJD). Even the most frequent form, sCJD, is very rare. It appears to be evenly distributed worldwide; countries that carry out surveillance report, quite uniformly, an incidence of  $\sim 0.6\text{--}1.2 \times 10^{-6}$  per year (1). No exogenous or endogenous causes of sCJD have been identified yet. An endemic form of CJD, designated Kuru, occurred among the Aborigines in Papua New Guinea throughout the 1950s and 1960s. Kuru was horizontally transmitted by cannibalistic rituals and has not been observed in individuals born after cannibalism was abandoned (2).

Familial forms of CJD are transmitted as autosomal dominant traits and invariably cosegregate with mutations in *PRNP*, the gene that encodes the prion protein (3). Although experimental evidence from the mouse implies a role of additional factors (4–7), no genetic loci other than *PRNP* have been implicated in the pathogenesis of human prion diseases.

Several hundred cases of iCJD have been reported in recent decades. These have been attributed mainly to transplantation of tissues or administration of hormones derived from deceased individuals suffering from unrecognized TSEs, and, to a lesser extent, to the use of contaminated instruments in neurosurgical interventions. Infection by contaminated hormones was effectively eliminated when recombinant-peptide hormones replaced natural hormones in the mid-1980s, but individual patients are developing the disease even now—owing to the long incubation times involved.

Recently a patient developed vCJD after receiving a blood transfusion derived from another vCJD patient (8). Another transfusion recipient died of unrelated causes but was found at autopsy to harbor PrP<sup>Sc</sup> in his lymphoid system (8a). Although it cannot be formally excluded that both patients developed prion disease independently, it is very likely that these cases represent the first identified instances of blood-borne CJD transmission (9).

Biochemical and histopathological evidence suggests that vCJD represents transmission of bovine spongiform encephalopathy (BSE) prions to humans (10–12). The incidence of vCJD in the United Kingdom rose each year from 1996 to 2001 ([http://www.doh.gov.uk/cjd/cjd\\_stat.htm](http://www.doh.gov.uk/cjd/cjd_stat.htm)), evoking fears of a large upcoming epidemic. Since then, however, the incidence of vCJD in the United Kingdom appears to be stabilizing and may even be falling. Hence there is hope that the total number of vCJD victims will be smaller than originally feared (13).

In Switzerland, CJD has been a statutory notifiable disease since December 1987. A National Reference Center for Prion Diseases was established in 1995. Between 1996 and 2000, the incidence of CJD oscillated between  $1.3 \times 10^{-6}$  and  $1.4 \times 10^{-6}$  per year. However, in 2001 and 2002 the incidence was  $2.6 \times 10^{-6}$  per year (14), and this level appears to have been maintained through 2003 (15). The cause of this apparent surge in incidence is unknown; beside statistical fluctuations, TSEs of iatrogenic or zoonotic origin have been discussed. It is also plausible that an “awareness bias” may be contributing, at least in part, to the increased CJD reporting.

## Diagnosis of CJD

Patients suffering from CJD typically present with rapidly progressive cognitive decline, which may be fulminant and progress to akinetic mutism within weeks. Cerebellar signs are also very frequent and electroencephalographic recordings often visualize periodic sharp wave complexes. The definitive diagnosis of CJD, however, must usually await the analysis of central nervous tissue, bioptically or post mortem. “Probable CJD” cases are diagnosed mainly on the basis of clinical symptoms, when no histopathological or biochemical confirmation is available.

Such cases may contaminate mortality statistics in countries that register CJD cases based on surrogate markers, including elevation of protein 14.3.3 in the cerebrospinal fluid (16, 17). Highly sensitive methods have revealed that at least one third of patients with sCJD deposit PrP<sup>Sc</sup> in skeletal muscle and/or in spleen (19). The sensitivity of 30% is insufficient for routine diagnostics, but these data open the possibility of minimally invasive diagnostics for sCJD, perhaps in combination with future more sensitive methods.

A firm diagnosis of vCJD can often be obtained by biopsy of the tonsils, which have been shown to harbor significant amounts of PrP<sup>Sc</sup> in germinal centers (18). Magnetic resonance imaging has evidenced the frequent presence of hyperintensity in the posterior thalamus of vCJD patients (20). This “pulvinar sign” was originally thought to discriminate reliably between sCJD and vCJD, but cases of sCJD with the same neuroradiological changes have been described (21, 22).

## Genetics and the Incidence of CJD

While all fCJD cases cosegregate with *PRNP* mutations, it is possible that some *PRNP* mutations cause neurodegenerative disease that is not transmissible and therefore represents a proteinopathy rather than a prion disease. Many such instances have been described in the mouse (23) and are exemplified by the “octapeptide repeat expansion” mutants of both mouse (24) and man (25, 26).

Beside disease-causing mutations, *PRNP* may also comprise polymorphisms that have a profound effect on susceptibility to prion disease. Thus, all clinical cases of vCJD have the met/met rather than the val/val or met/val configuration at position 129 (27, 28). However, recently subclinical vCJD was diagnosticized post mortem in a patient with met/val heterozygosity who died of other causes, showing that infection is certainly possible in this genotype but that progression to clinical disease may be very much slower than in met/met homozygotes (8a). Humans heterozygous at position 129 are largely protected from CJD; this effect is so important that it may have exercised selective evolutionary pressure (29). A lys rather than a glu residue at position 219 is thought to be protective against sCJD (30).

However, it is becoming increasingly apparent that genetic susceptibility markers and modifiers are not limited to the known polymorphisms in the PrP-encoding reading frame, as revealed by the identification of several quantitative trait loci that affect incubation time in the mouse (4–7). It is not clear what these modifiers might be. The possible protective effect against vCJD of a certain MHC class II constellation (31) has been disputed (32). Nonetheless, based on all that is known about the critical role of the immune system in peripheral prion infection (33), immunity-controlling genes are likely to feature among endogenous modifiers.

A large proportion of the British population may have been exposed to BSE infection. Animal experiments indicate that the infectious dose (ID<sub>50</sub>) for oral cross-species transmission of BSE is relatively low [500 mg of brain tissue sufficed to cause disease in sheep (34)], yet only ~150 humans have contracted vCJD. Thus, it is likely that vCJD susceptibility is controlled by endogenous and/or exogenous factors other than the amount of infectious agent ingested (35).

## Extraneural PrP<sup>Sc</sup>

Refinements in the technologies for detection of PrP<sup>Sc</sup> have prompted a renaissance of studies of the distribution of the disease-associated prion protein in extracerebral organs. These studies in sCJD patients revealed that extraneural PrP<sup>Sc</sup> is more widespread than previously thought. Zanusso and colleagues found that PrP<sup>Sc</sup> is readily detectable in the olfactory mucosa of sCJD patients (36). Glatzel et al. found that approximately one third of the Swiss sCJD patients display PrP<sup>Sc</sup> in their skeletal muscle and another third (partially overlapping) have PrP<sup>Sc</sup> in lymphoid organs (19). Further investigations are under way to determine whether these findings are universally valid for CJD patients or whether they are specific to the Swiss CJD cohort. If the latter were true, one might speculate that the peripheral presence of CJD in Swiss patients points to a specific etiology.

In vCJD, it has been appreciated for several years that substantial amounts of PrP<sup>Sc</sup> are detectable in lymphoid organs, and tonsil biopsies often suffice to firmly establish the diagnosis (18). It remains to be established whether PrP<sup>Sc</sup> is present in skeletal muscle and other extraneural tissues of vCJD patients. The UK vCJD cases are likely to be primary transmissions from cattle with BSE. However, experimental transmission studies show that TSE strain characteristics can change upon serial passages after the original primary transmission (37). Therefore, horizontal vCJD transmission among humans could result in a different phenotype than zoonotic vCJD. This scenario calls for innovative studies to develop and validate classical and emerging tools for up-to-date prion strain typing.

## THE NATURE OF THE INFECTIOUS AGENT

It is widely (though not universally) accepted that the TSE agent, or prion, is not a typical microorganism like a bacterium or virus, consisting of agent-specific nucleic acid that encodes one or more agent-specific proteins; rather, it may consist of a misfolded host protein, perhaps associated with other components. Notably, prion infection elicits no immune response.

One of the most striking and characteristic features of TSE is the deposition, mainly in the brain but also in other tissues, of a partially protease-resistant protein designated PrP<sup>Sc</sup> or PrP-res, which is a beta-sheet-rich conformational isomer of the protease-sensitive, alpha-helix-rich ubiquitous host protein PrP<sup>C</sup>. Biochemical and genetic evidence link PrP and its gene to the disease. PrP<sup>Sc</sup> copurifies with infectivity and vice versa. Familial forms of CJD are invariably linked to mutations in the PrP gene, and mice with disabled PrP genes are resistant to prion disease and fail to propagate the agent (for a review see Reference 23). By and large, the available data and the failure to identify a disease-specific nucleic acid support the “protein-only” hypothesis. As enunciated by Prusiner, this hypothesis proposes that the infectious agent consists of PrP<sup>Sc</sup>, that it is devoid of nucleic acid, and that its “replication” comes about by PrP<sup>Sc</sup>-mediated, autocatalytic conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup> (38). However, it is not clear that the infectious entity is PrP<sup>Sc</sup>, operationally

defined as a protease-resistant, aggregated form of PrP, rather than some other conformer, generically designated as PrP\* (39)—nor has the requirement for other components been excluded. The critical experiment of converting purified PrP<sup>C</sup>, be it recombinant or from a natural source, into an infectious form has not been convincingly reported so far, although PrP<sup>C</sup> has been converted into PrP<sup>Sc</sup> in an in vitro system (40–43). The propagation of conformationally changed yeast proteins (so-called yeast prions) both in vitro and in vivo offers proof in principle of the “protein-only” hypothesis (44–47).

## PATHOGENIC MECHANISMS IN PRION DISEASES

The damage wrought by prions is mainly evident in the central nervous system (CNS), although pathological changes in the spleen of nonhuman primates have also been noted (C. Lasmezas, personal communication). Because PrP<sup>Sc</sup> accumulates in the CNS and in some instances is deposited as an amyloid, it has been indicted as the toxic entity that causes neuronal apoptosis and elicits disease. The finding that peptides derived from PrP region 106–126 form aggregates and are toxic to cultured neuronal cells (48, 49) has been adduced in support of this contention, although the reproducibility of the phenomenon has been disputed (50). It is, however, not evident that the pathogenicity of the oligomerized peptides on cultured cells mimics the properties of PrP<sup>Sc</sup> accumulating in the CNS.

PrP<sup>Sc</sup> produced by a prion-infected, PrP-expressing neuronal graft in the brain of PrP knockout mice did not cause disease, nor did it result in damage to neighboring neuronal tissue devoid of PrP (51). In addition, prion-infected mice carrying only a single PrP allele and producing half the wild-type level of PrP do not exhibit disease until about 450 d after intracerebral inoculation, in contrast to 150 d in wild-type mice, although they accumulate levels of PrP<sup>Sc</sup> similar to those of wild-type animals by 150 d after infection (52). Finally, depletion of PrP<sup>C</sup> in neurons of prion-infected mice by conditional knockout some weeks after prion inoculation prevents clinical disease despite massive accumulation of PrP<sup>Sc</sup> and infectivity in and around astrocytes (53). Therefore, PrP<sup>Sc</sup> is likely to be responsible for CNS pathology only in neurons that express PrP<sup>C</sup>.

Gain of toxic function by a PrP moiety other than PrP<sup>Sc</sup> is a distinct possibility. Over several years, a lively debate has unfolded on the role of abnormal PrP<sup>C</sup> topologies. Targeting of PrP to the cytosol was reported to result in rapidly lethal neurodegeneration (albeit without accumulation of PrP<sup>Sc</sup>), and proteasome inhibition induces a slightly protease-resistant, cytoplasmic PrP species in cultured cells (54, 55). Therefore, prion toxicity was proposed to start with retrotranslocation of PrP<sup>C</sup> from the endoplasmic reticulum to the cytosol, in conjunction with impaired proteasomal function. However, others have found that cytosolic PrP retains its secretory leader peptide and does not contain a glycosyl phosphatidyl inositol anchor, suggesting that it never enters the endoplasmic reticulum (56). Moreover, the toxicity of cytosolic PrP has been contested (57, 58). Lingappa and colleagues found that PrP<sup>C</sup> assumes a transmembrane topology (<sup>C<sub>tm</sub></sup>PrP) whose concentration

correlates with neurotoxicity (59, 60). These data have been taken to suggest that  $C^{tm}$ PrP represents a major toxic moiety.

Further work is needed to clarify the role of alternative PrP topologies in prion neurotoxicity. Moreover, the biochemical pathways that lead to pathogenicity, be it triggered by PrP<sup>Sc</sup>, cytoplasmic PrP, or  $C^{tm}$ PrP, are still obscure.

## SPREAD OF PRIONS

Prion pathogenesis can be broken down into spatially and temporally distinct phases: (a) infection and peripheral replication, (b) migration from the periphery to the CNS (neuroinvasion), and (c) neurodegeneration. The resistance to prions of mice that lack PrP<sup>C</sup> expression is amply documented (51, 61–63). PrP<sup>C</sup> expression is required for transporting the infectious agent from the peripheral sites to the CNS (as monitored by PrP<sup>C</sup>-expressing neurografts) (64) and within the CNS (65). However, reconstitution of *Prnp*<sup>0/0</sup> mice with wild-type bone marrow is insufficient to restore neuroinvasion in engrafted *Prnp*<sup>0/0</sup> mice (64), although the spleen's capacity to accumulate prions of the Rocky Mountain Laboratory strain is reconstituted (64, 66). This finding suggests that hematopoietic cells transport prions from the entry site to the lymphoreticular system, which accumulates and replicates prions, but that PrP<sup>C</sup> expression in an additional compartment, presumably the peripheral nervous system, is required. B lymphocytes (not necessarily expressing PrP<sup>C</sup>) are crucial for peripheral prion spread and neuroinvasion (67, 68).

The dependence on lymphotoxin (LT)-mediated signaling by B cells may explain—at least in part—the requirement for B cells in peripheral pathogenesis. Follicular dendritic cells (FDCs) accumulate PrP<sup>Sc</sup> following scrapie infection (69), and maturation of FDCs requires signaling by B cells that express  $LT\alpha/LT\beta$  trimers on their surface. Indeed, blockade of  $LT\beta$  signaling via administration of soluble  $LT\beta R$ -Ig ablates mature FDCs and significantly impairs neuroinvasion and accumulation of peripheral PrP<sup>Sc</sup> and infectivity (70, 71). FDCs are crucial to disease progression after oral scrapie challenge, but only within a short time window (72, 73).

FDCs play a role in antigen trapping and in binding opsonized antigens to the CD21/CD35 complement receptors. Two studies have demonstrated that the complement system is relevant to prion pathogenesis. Mice genetically engineered to lack complement factors (74) or mice depleted of the C3 complement component (71) exhibited enhanced resistance to peripheral prion inoculation. Because FDCs are most likely immobile cells, they are unlikely to be responsible for prion transport into the CNS.

But just which cell types are involved in neuroinvasion? The innervation pattern of lymphoid organs is primarily sympathetic (75). Sympathectomy delays the onset of scrapie, whereas sympathetic hyperinnervation enhances splenic prion replication and neuroinvasion, suggesting that innervation of secondary lymphoid organs is the rate-limiting step to neuroinvasion (76). Although there is no physical



contact between FDCs and sympathetic nerve endings (77), the distance between FDCs and splenic nerves affects the velocity of neuroinvasion (78). It remains to be determined whether this results from passive diffusion of prions or whether mobile cells (e.g., germinal center B cells) are involved in an active transport process.

## Oral Prion Uptake

Upon oral challenge, an early rise in prion infectivity occurs in the distal ileum of infected organisms. This has been observed in several species but most extensively investigated in sheep. Western blot analysis has shown that Peyer's patches accumulate PrP<sup>Sc</sup>. This is true also in the mouse model of scrapie, where administration of mouse-adapted scrapie prions (RML strain) induces a surge in intestinal prion infectivity as early as a few days after inoculation (73, 79, 80). Indeed, immune cells are crucial to the process of neuroinvasion after oral application. Mature FDCs, located in Peyer's patches, may be critical for the transmission of scrapie from the gastrointestinal tract (79).

Myeloid dendritic cells may be involved in the transport of infectious agent by this process, and in fact recent work has implicated dendritic cells as potential vectors of prions in oral (81) and hematogenous (82) spread of the agent. It is equally possible, however, that lymphatic colonization is followed by direct entry of prions into nerve terminals.

## ACTIVE AND PASSIVE VACCINATION

It was reported early on that anti-PrP antiserum reduces the titer of infectious hamster brain homogenates about a hundredfold (83). Anti-PrP antibodies were found to inhibit formation of protease-resistant PrP in a cell-free system (84). Also, antibodies (85, 86) and F(ab) fragments directed against PrP (87) can suppress prion replication in cultured cells.

These data suggest the feasibility of antiprion immunoprophylaxis, which could be implemented as passive immunization (transfer of antibodies) or active immunization (administration of antigens as vaccines). Active immunization is generally more effective, but it is exceedingly difficult to elicit humoral immune responses, because the mammalian immune system is largely tolerant of PrP of the same species. Mice devoid of PrP (88) show no tolerance and are highly susceptible to immunization with recombinant PrP (63) or PrP<sup>C</sup>-expressing cells (65).

Tolerance is typically brought about by activation-induced cell death, which is incurred by B or T lymphocytes undergoing very strong cross-linking of their antigen receptors. To determine whether the resilience of wild-type mice to antiprion immunization is attributable to the T- or B-cell compartment, transgenic mice were generated that expressed an immunoglobulin/B-cell receptor  $\mu$  chain containing the epitope-interacting region of 6H4, a high-affinity anti-PrP monoclonal antibody (89). The transgenic  $\mu$  chain associated with endogenous  $\kappa$  and  $\lambda$  chains; some pairings led to reactive moieties and, consequently, to anti-PrP<sup>C</sup>

titers in *Prnp*<sup>0/0</sup> and *Prnp*<sup>+/+</sup> mice. The buildup of anti-PrP<sup>C</sup> titers, however, was more sluggish in the presence of endogenous PrP<sup>C</sup>, which suggests that clonal deletion was actually occurring. B-cell clones with the highest affinity to PrP<sup>C</sup> are probably eliminated by tolerance, whereas clones with medium affinity are retained. The latter sufficed to block prion pathogenesis upon intraperitoneal prion inoculation (90). Hence, B cells are not intrinsically tolerant of PrP<sup>C</sup>, and can—in principle—mount a protective humoral response against prions. It was subsequently found that passive transfer of anti-PrP monoclonal antibodies (in admittedly heroic amounts) delays the onset of scrapie in mice infected with prions by intraperitoneal inoculation but is ineffective in mice infected by intracerebral inoculation (91), perhaps because insufficient levels of antibody are reached in the brain.

Although transgenic immunization provides an encouraging proof of principle, it cannot easily be reduced to practice. Passive immunization failed to confer protection if treatment was started after the onset of clinical symptoms, so it might be a better candidate for prophylaxis than for therapy of TSEs. Active immunization may be more effective, as in most antiviral vaccines, but it is rendered exceedingly difficult by the tolerance to PrP<sup>C</sup> (92, 93).

A recent report outlines a potentially serious obstacle to prion immunotherapy. Intracerebral injection of anti-PrP antibodies specific to certain epitopes at high concentrations provoked degeneration of hippocampal and cerebellar neurons (94). Because monovalent Fab fragments did not elicit these responses, it is likely that crosslinking of PrP<sup>C</sup> by bivalent IgG antibodies is neurotoxic *in vivo*; perhaps it elicits some deleterious signaling event. Although these results add a cautionary note to the prospect of using antibodies against clinically overt prion diseases, it is possible that anti-PrP Fab fragments are capable of reducing infectious titers (87) without exerting a toxic effect (94). Moreover, extraneural antibody administration may be useful for immunoprophylaxis of prion infections at early stages, before the agent reaches the brain.

## Immunostimulation and Antiprion Prophylaxis

Cytidyl-guanyl oligodeoxynucleotides (CpG-ODN), which bind Toll-like receptor 9 (TLR9) and stimulate innate immune responses, were reported to delay disease upon chronic administration to scrapie-infected mice (95). The contention that immune stimulation might protect against prions is difficult to reconcile with the observation that immune deficiencies of all kinds inhibit prion spread (67, 68, 74, 79, 96). Besides, *MyD88*<sup>-/-</sup> mice undergo normal prion pathogenesis despite abrogation of TLR9 signaling (97). Hence, more detailed studies will be needed to reveal the basis of the antiprion effect of CpG-ODN. The fact that repeated administration of CpG-ODN can derange the architecture of lymphoid germinal centers, which are sites of prion replication, suggests that the antiprion effect of these compounds may rely on their immunosuppressive rather than their immunostimulatory properties (98).



## SEARCH FOR THERAPEUTIC AGENTS

Screening for putative therapeutic agents has been conducted at various experimental levels. Based on the assumption that PrP<sup>Sc</sup> is the infectious agent, or at least the pathogenic entity, compounds have been sought that in a cell-free system would stabilize PrP<sup>C</sup>, destabilize PrP<sup>Sc</sup>, or prevent conversion and thereby decrease the level of PrP<sup>Sc</sup>. Bis-ANS (4,4'-dianilino-1,1'-binaphthyl-5,5'-sulfonate) was described as potentially inhibiting PrP aggregation (99), and so-called  $\beta$ -sheet breaker peptides (100) and branched polyamines (101) partially disassembled PrP<sup>Sc</sup> to a protease-sensitive form. However, compounds identified by this type of screen, though potentially of interest, still face high hurdles to qualify as drug candidates: They must reach the appropriate cellular compartment; provide an acceptable therapeutic index (that is, ratio of toxic to therapeutic dose); exhibit pharmacokinetics that allow the build-up of a sufficiently high concentration in the biophase, which implies a capacity to cross the BBB effectively; and, last but not least, be accessible in sufficient quantity by chemical synthesis or from biological sources. No compounds have taken these hurdles so far.

A yeast-based screen has been reported in which the capacity of compounds to diminish the propagation of "yeast prions" is assessed (102). Because the sequences of the yeast proteins involved are completely different from that of PrP, it is not clear how useful this screen will be to find compounds active on true prions.

A limited number of cell lines are susceptible to infection by prions (103). Scrapie-infected cells, particularly the murine neuroblastoma-derived N2a line, have been used as targets for prospective drugs; the decrease of PrP<sup>Sc</sup> levels serves as a measure for therapeutic activity. The steady-state level of PrP<sup>Sc</sup> is determined by the rate of formation relative to that of degradation. Although originally thought to be very stable, PrP<sup>Sc</sup> in murine neuroblastoma cells has a half-life on the order of a day or so, and inhibition of its formation leads to its elimination within a few days. This is also the case after inhibition of PrP<sup>C</sup> synthesis, for example by siRNA (104), as well as sequestration or depletion of PrP<sup>C</sup> from the cell surface by binding of anti-PrP antibodies (85, 86), Fab fragments (87), aptamers (105), or compounds such as biquinoline (106) or suramine (107). Interference with the conversion reaction has been attributed to the binding of compounds such as heparan mimetics (108, 109), Congo red (110), or phthalocyanine tetrasulfonate (111) to PrP<sup>Sc</sup> and/or PrP<sup>C</sup>. Accelerated degradation of PrP<sup>Sc</sup> is attributed to its interaction with branched polyamines (101, 112). Polyene antibiotics such as amphotericin B are believed to interact with detergent-resistant microdomains or rafts (113) and to inhibit generation of PrP<sup>Sc</sup> of at least some prion strains by interfering with the trafficking of PrP<sup>C</sup> (114). Recently, a screen of 2000 compounds using scrapie-infected N2a cells yielded 17 candidates that were inhibitory to PrP<sup>Sc</sup> accumulation at 10  $\mu$ M or less. Interestingly, only polyphenols were inhibitory in the cell-free conversion system (115). However, none of these were active in a prion-infected mouse model (115a).

Cell components other than PrP are believed to participate in or influence the conversion reaction, such as the laminin receptor precursor (LRP) (116). Indeed, siRNA against LRP mRNA inhibits PrP<sup>Sc</sup> accumulation in scrapie-infected N2a cells (117). A candidate for “protein X,” postulated to play a role in the conversion process on the basis of genetic evidence (118), has not been identified so far.

A more stringent screen, mostly applied to compounds that are active in the cell-based assay, is provided by animal models, usually mice or hamsters. Animals are usually, but not always, poorly susceptible to prions from heterologous species. However, repeated passaging may overcome this species barrier, yielding mouse- or hamster-adapted strains. Replacement of the endogenous PrP gene by the homologous gene of the prion donor may render mice susceptible to the foreign prions (119). Thus, *Prnp*<sup>0/0</sup> mice transgenic for bovine or human PrP genes become susceptible to BSE and CJD prions, respectively (120). Interestingly, however, some strains of wild-type mice are far more susceptible to human vCJD prions than are mice transgenic for the human PrP gene (37).

Drug candidates have been administered before, during, soon after, and long after inoculation with prions. For convenience they are usually given intraperitoneally (i.p.), but occasionally intracerebrally (i.c.) to overcome the BBB. A critical variable is the site of prion inoculation, which is usually i.p. or i.c., more rarely peroral. The i.p. route requires prion doses orders of magnitude higher than does i.c. inoculation, and incubation times are typically twice as long. The important consideration here is that i.p. or peroral prion inoculation provides a wide window of potential susceptibility to i.p. administration of drugs that are excluded from the CNS by the BBB. This window closes as neuroinvasion takes place.

Many compounds, representative examples of which are listed in Table 1, prolong the incubation time in animals when administered before or soon after infection. Among these are sulfated polyanions (121–125), Congo red D (126), polyene antibiotics (125, 127–129), tetracyclic compounds (130), and tetrapyrroles (111, 131, 132). Copper added to the drinking water of scrapie-infected hamsters has been reported to delay clinical disease (133), but a similar effect was reported for the copper chelator D(-)-penicillamine in scrapie-infected mice (134); such is life in the prion field. None of the compounds tested in animal models were effective when administered peripherally after onset of clinical symptoms. However, when infused intraventricularly, pentosan polysulfate (PPS) at high levels extended the survival of mice and decreased PrP<sup>Sc</sup> deposition even when administered late after infection, while antimalarial drugs such as quinacrine showed no significant effect. At excessive doses, adverse effects such as hematoma formation were observed (135). Intraventricular infusion of biquinoline derivatives also resulted in moderate extension of the survival period (106).

A PrP-Fc<sub>2</sub> fusion protein that was found to compete with PrP<sup>C</sup> for PrP<sup>Sc</sup> had a protective effect against i.p. scrapie infection of mice when expressed from a transgene (136). It will be interesting to determine whether PrP-Fc<sub>2</sub> is also active when delivered as a drug. If so, soluble prion protein mutants may represent useful prionostatic compounds.

TABLE 1 Representative compounds used in attempts at therapy of prion disease

Compound	Cell-free	Cell culture	Animal/Human	Proposed mechanism	References
Acridines					
Quinacrine	Binds weakly to helix 3 of PrP <sup>C</sup> (K <sub>m</sub> ca. 1 mM). No effect on PrP <sup>Sc</sup>	Reduces PrP <sup>Sc</sup> levels in ScN2a cells (IC <sub>50</sub> 300 nM), less so in ScGT cells	No effect on CJD/mouse model. Penetrates BBB	Accumulates in lysosomes, binds to PrP <sup>Sc</sup> ?	145, 146
Bis-acridine derivatives		Reduce PrP <sup>Sc</sup> levels in ScN2a cells (IC <sub>50</sub> 25–40 nM)		Binds to unknown receptor?	147
Anthracyclins					
4'-iodo-4'-deoxy-doxorubicin			Inoculum and drug coinjected prolonged survival of scrapie-infected Syrian hamster	Binds to PrP <sup>Sc</sup> ?	130
Anti-PrP antibodies					
Fab D18	Binds PrP <sup>C</sup> 132–156	Reduces PrP <sup>Sc</sup> levels in ScN2a cells (IC <sub>50</sub> 9 nM)		Impedes PrP <sup>C</sup> -PrP <sup>Sc</sup> interaction	87
6H4	Binds PrP <sup>C</sup> 144–152	Reduces PrP <sup>Sc</sup> levels in ScN2a cells (IC <sub>50</sub> ca. 10 nM)	Protects mice against i.p. infection when light chain is expressed from transgene	Impedes PrP <sup>C</sup> -PrP <sup>Sc</sup> interaction	85, 90

(Continued)

TABLE 1 (Continued)

Compound	Cell-free	Cell culture	Animal/Human	Proposed mechanism	References
ICSM18	Binds PrP <sup>C</sup> 146–156	Reduces PrP <sup>Sc</sup> levels in ScN2a cells	i.p. administration protects mice against i.p. prion inoculation	Impedes PrP <sup>C</sup> -PrP <sup>Sc</sup> interaction	91
Aptamers DP7	Binds PrP <sup>C</sup>	Reduces PrP <sup>Sc</sup> levels in ScN2a cells		Impedes PrP <sup>C</sup> -PrP <sup>Sc</sup> interaction	105
SAF93	Binds PrP <sup>Sc</sup> , prevents conversion			Impedes PrP <sup>C</sup> -PrP <sup>Sc</sup> interaction	148, 149
Cyclic tetrapyrrols PcTS, TMPP-Fe <sup>3+</sup>	Prevent conversion		Increase survival time in mouse injected i.p.	Unknown	131
Peptides PrP106-128, PrP113-141	Inhibit conversion	Reduce PrP <sup>Sc</sup> levels in ScN2a cells		Impede conversion by binding to PrP <sup>C</sup> or PrP <sup>Sc</sup>	150
$\beta$ -sheet breaker iPrP13	Partial disassembly of PrP <sup>Sc</sup>		Pretreatment of inoculum decreases infectivity 1–1.5 logs in mice	Disassembles PrP <sup>Sc</sup>	100
Polyamines polypropyleneimine (PPI)	Renders PrP <sup>Sc</sup> of some prion strains susceptible to proteinase K digestion	Reduces PrP <sup>Sc</sup> levels and infectivity in ScN2a cells		Destabilizes PrP <sup>Sc</sup>	101, 112

<p>Polyanions (heparan mimetics) Pentosan polysulfate (PPS)</p>	<p>Stimulates in vitro conversion!</p>	<p>Reduces PrP<sup>Sc</sup> levels in ScN2a cells</p>	<p>Increases survival time of hamsters and mice infected i.p. when given hours after infection or when administered intraventricularly also late after infection. Treatment of human vCJD under way</p>	<p>Interferes with PrP- glucosaminoglycans interaction; stimulates PrP<sup>C</sup> endocytosis</p>	<p>123, 124, 135, 139–142, 151–153</p>
<p>Dextran sulfate, HM2602</p>		<p>Reduces PrP<sup>Sc</sup> levels in ScN2a cells</p>	<p>Increases survival time of hamsters and mice infected i.p. when given hours after infection</p>	<p>Interferes with PrP- glucosaminoglycans interaction</p>	<p>109, 154, 155</p>
<p>Polyene antibiotics Amphotericin B</p>	<p>Modifies rafts</p>	<p>Reduces PrP<sup>Sc</sup> levels in ScN2a and GT1 cells</p>	<p>Prolongs survival in 263k-infected hamsters and mice after late administration. Ineffective in human CJD</p>		<p>127, 129, 143, 156</p>
<p>MS 8209</p>				<p>Prevents uptake of prions in periphery?</p>	<p>157</p>
<p>Filipin</p>	<p>Disrupts lipid rafts</p>	<p>Reduces PrP<sup>Sc</sup> levels in ScN2a cells</p>		<p>Disrupts lipid rafts, reduces endocytosis, causes release of PrP<sup>C</sup> from cell surface</p>	<p>158, 159</p>

(Continued)

TABLE 1 (Continued)

Compound	Cell-free	Cell culture	Animal/Human	Proposed mechanism	References
Polysulfonated small-molecular- weight compounds Suramin			Modest increase in survival time of i.p. inoculated hamsters	Misfolds PrP <sup>Sc</sup> at plasma membrane, endocytosis, intracellular retention and degradation	107, 123, 160
Congo red	Inhibits conversion, stabilizes PrP <sup>Sc</sup>	Reduces PrP <sup>Sc</sup> levels in ScN2a cells	Modest increase in survival time of i.p. inoculated hamsters	Inhibits conversion, stabilizes PrP <sup>Sc</sup>	110, 155, 161, 162
Quinolines 2,2'-biquinoline	Binds to rPrP <sup>C</sup>	Reduces PrP <sup>Sc</sup> levels in ScN2a cells (IC <sub>50</sub> 1– 100 nM)	Somewhat extends survival in i.c. inoculated mice after intraventricular infusion		106
Recombinant proteins PrP-Fc(2)	Binds to rPrP <sup>Sc</sup>		Retards disease in mice expressing the transgene		136



## ATTEMPTS AT HUMAN THERAPY

The earliest attempts to treat human prion disease, performed when the agent was generally assumed to be a virus, were carried out with antiviral drugs, such as amantadine, and were unsuccessful (137).

### Quinacrine

Quinacrine, chlorpromazine, and some tricyclic derivatives with an aliphatic side chain were described as efficient inhibitors of PrP<sup>Sc</sup> formation in murine neuroblastoma cells chronically infected with the Chandler scrapie isolate (138, 139). Because quinacrine and chlorpromazine have been used in human medicine as antimalarial and antipsychotic drugs, respectively, and because they cross the BBB, they were proposed as therapeutic agents for CJD patients (139). No therapeutic effect was seen following quinacrine treatment of 20 patients (140) (A. Alperovich, quoted in Reference 141), although some transient improvement occasionally occurred (142). Subsequent animal experiments failed to demonstrate efficacy in the treatment of TSEs (141), even after intraventricular infusion (135).

### Amphotericin B

Amphotericin B and some of its analogues delayed the appearance of spongiosis, astrogliosis, and PrP<sup>Sc</sup> accumulation in the brain of scrapie-infected hamsters (125). However, an attempt to treat a CJD patient with amphotericin B was unsuccessful (143). In view of its high systemic toxicity, these results dampen any hopes that amphotericin B will prove useful in prion disease therapy.

### Pentosan Polysulfate

Data presented at two prion meetings in 2002, and published recently (135), suggest that intraventricular administration of PPS to intracerebrally prion-infected mice prolonged incubation time. PPS is marketed in some countries as a treatment for interstitial cystitis and as an anticoagulant, although its side effects include hemorrhage and hypersensitivity reactions.

## ETHICAL CONSIDERATIONS

Recently a legal case was brought by two families whose children JS and PA, aged 18 and 16 respectively, suffered from vCJD [DS v JS and an NHS Trust and The Secretary of State for Health, intervenor; PA v JA and an NHS Trust and The Secretary of State for Health (2002) EWHC 2734 (Fam)]. They applied to the court to permit intraventricular administration of PPS, a treatment previously given only to rodents and dogs. The judge heard testimony from Doh-Ura, the Japanese researcher who had performed the animal studies; from a neurosurgeon

willing to administer the novel treatment; and from several respected neurologists who expressed reservations about it. The judge found that both young patients had “some enjoyment from life which is worth preserving” and that the treatment, as it was supported by medical opinion, would be in their “best interest” (the legal criterion for doctors to treat patients who lack capacity for personal decisions) (144). Treatment has been initiated, but no reports on the fate of the patients have been issued.

Physicians can thus come under pressure from the courts to allow new treatments to be used without having been tested in clinical trials, although the ruling described above implies that such decisions would have to withstand the “Bolam” test of being acceptable to a reasonable body of medical opinion. The ruling also upheld the application of the Human Rights Act in this area, citing Articles 2 and 8, the rights to life and to respect for family life. It is not inconceivable that such analysis could allow patients to circumvent clinical trials by asserting their rights to receive innovative therapy, and this development is of concern, particularly in the clinical field of human prion diseases.

We may at some stage be confronted with a therapy that can eradicate prion infection without reversing the neural damage, which in extreme cases could condemn patients to years or decades of severe disability and dementia. This would lead to an ethical dilemma as to whether treatment should be withheld if the disease has progressed to a severe stage. Such situations could be prevented if a diagnostic test could detect prion disease in its preclinical stage. Whether such a test, if it ever became available, would be applied to detect a disease with an incidence of 1 in a million per year is a matter of debate; clearly it would be practicable in the case of familial prion diseases.

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