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Toward Therapy of Human Prion Diseases

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Abstract

Three decades after the discovery of prions as the cause of Creutzfeldt-Jakob disease and other transmissible spongiform encephalopathies, we are still nowhere close to finding an effective therapy. Numerous pharmacological interventions have attempted to target various stages of disease progression, yet none has significantly ameliorated the course of disease. We still lack a mechanistic understanding of how the prions damage the brain, and this situation results in a dearth of validated pharmacological targets. In this review, we discuss the attempts to interfere with the replication of prions and to enhance their clearance. We also trace some of the possibilities to identify novel targets that may arise with increasing insights into prion biology.

PRIONS AND PRION DISEASES

Prion diseases, or transmissible spongiform encephalopathies (TSEs), are caused by the ordered aggregation of the proteinase K-resistant form of the prion protein, PrP^{Sc}, a misfolded version of the cellular prion protein PrP^{C}. Because similar mechanisms are operative in many other neurodegenerative and systemic diseases, the protein aggregates causing the latter were termed prionoids (1, 2). By our definition, prionoids operate similarly to prions at the molecular level but have not (yet) been shown to be transmissible from one individual to another. In the case of prion disease, the aggregation is self-sustaining and therefore transmissible between individuals—which renders TSEs infectious (3–6). These diseases comprise Creutzfeldt-Jakob disease (CJD), kuru, fatal familial insomnia, and genetic TSEs in humans (7); the latter are caused by mutations in the *PRNP* gene encoding PrP^{C}. Neuropathologically, nonspecific signs (astrogliosis, neuronal loss, and amyloid deposition) are accompanied by spongiform changes: intraneuronal and intraneuritic vacuoles occasionally containing degenerating organelles (8). The spongiform degeneration of neurons is highly specific to prion diseases and typically allows for a definitive diagnosis.

The presence of cellular prion protein is necessary not only for the de novo generation of prions but also for the host organism to experience prion-related neurotoxicity (9). Mice ablated for PrP^{C} do not acquire the disease after exposure to prions. The availability of PrP^{C} seems to be rate limiting, as prion-infected mice containing only a single allele of the *Prnp* gene encoding PrP^{C} develop the disease much later than wild-type mice.

Prion diseases are rare, with 1.5–2 reported cases per million people per year. Yet they are invariably fatal, and currently there is no effective treatment. Identification of any potential antiprion therapy could also pave the way for treatment of misfolding disorders induced by prionoids. Here we focus on the therapeutics that in our opinion may have the potential to succeed and on the challenges awaiting them.

TARGETING PRION CONVERSION

The cellular PrP^{C} is expressed on the plasma membrane, where it is sorted into detergent-resistant membrane domains (10). The presence of certain mutations may induce PrP^{C} to adopt, through poorly understood mechanisms, a pathological and ultimately infectious conformation leading to disease (11). Ordered PrP^{Sc} aggregates can seed the nucleation of further prions. PrP^{Sc} can assume a broad variety of compositions ranging from large, insoluble aggregates and plaques to small oligomers (6).

Polyanionic compounds and amyloidotropic dyes can abrogate the conversion of PrP^{C} to PrP^{Sc} in vitro (12, 13) but could not be translated into therapies owing to toxicity, poor pharmacokinetics, and low efficacy (14, 15). Treatment of prion-infected neuroblastoma cells with branched polyamines resulted in clearance of PrP^{Sc} (16). The compounds are protonated at acidic pH and may act on prion conversion in endosomes and lysosomes (16). However, none of these compounds had any beneficial effect in vivo.

Dendrimers are synthetic molecules comprised mainly of branched polyamines with modifiable end groups (17). Phosphorous dendrimers were effective antiprion agents in vitro and cleared PrP^{Sc} significantly, yet they were not developed further (18). Pentosan polysulfate prolonged the survival of prion-infected mice and was thought to interfere with the conversion of PrP^{C} to PrP^{Sc} but did not have any reproducible effect in prion-affected humans (19, 20). Amantadine, originally used prophylactically against influenza virus, was suggested to have ameliorated the clinical course of CJD in a variety of reports, with anecdotal survival times of up to several years after the first symptoms occurred (21). Other reports, however, failed to reproduce beneficial effects of amantadine in CJD patients (22, 23). Another antiviral drug, acyclovir, was ineffective

in two patients suffering from CJD (24, 25), as was interferon in a case series of two patients (26). Flupirtine, an aminopyridine commonly used as an analgesic, was used in a placebo-controlled, double-blind study that suggested amelioration of cognitive deficits in sporadic CJD (sCJD) and genetic CJD despite unchanged survival times (27).

Quinacrine is an antiprotozoal drug that was introduced in the 1930s as an antimalarial agent, and during the variant CJD (vCJD) epidemic at the turn of the new millennium, the lack of efficient antiprion compounds compelled researchers to recruit patients for clinical studies (28). An open compassionate-use trial of quinacrine in 30 sCJD and two vCJD patients did not significantly prolong their survival or ameliorate the functional impairments and did not show beneficial effects on brain pathology (29). PRION-1, a prospective, patient-preference trial of quinacrine, also failed to significantly prolong the survival or improve cognitive deficits in the 107 patients enrolled (30). The study conductors hypothesized that low levels of the drug in the cerebrospinal fluid might have been responsible for the failure to reach clinical endpoints, although application of the drug showed an overall acceptable safety profile (30). Because compassionate-use trials cannot detect small effects of a molecule owing to the lack of a placebo arm, a randomized, double-blind, placebo-controlled trial of quinacrine in sCJD patients was performed thereafter (31). Fifty-one patients were eventually included for functional and survival analyses. Although patients in the quinacrine arm performed slightly better in terms of functional scores during the early treatment course, no survival benefit was observed upon quinacrine administration, leading to its elimination as a prion disease therapeutic (31).

We have reported the generation of reactive oxygen species to be a downstream effector of prion-induced neurotoxicity, and administration of antioxidants such as acetylated hydroxytyrosol effectively extended the survival of prion-diseased mice. One case report suggested beneficial effects of neurological disease in a CJD patient who received a complex mixture of antioxidants, including vitamin E and alpha lipoic acid, although he succumbed to disease 22 months after onset of symptoms (32).

A pilot compassionate-use trial of doxycycline showed beneficial effects on patient survival independent of age, gender, and codon 129 polymorphism of the *PRNP* gene (33). In a first-of-its-kind, multicentric, prospective, placebo-controlled, randomized, Phase II study, doxycycline did not show superiority in the first interim analysis when compared to placebo, and the study was stopped (34). Although the latter study provided class 1 evidence that doxycycline does not extend survival in prion disease patients, a subsequently published case report suggested an unusually long survival time (>5 years) of a patient suffering from variably protease-sensitive prionopathy who was treated with doxycycline (35). These reports indicate the helplessness of clinicians who prescribe antiprion drugs despite their proven lack of efficiency. Compound B, IND24, and anle138b were among other compounds that showed no effect against human prions (34).

These disappointing results raise questions on the viability of strategies to identify prion therapeutics. In particular, it has become evident that cell culture models are poorly predictive of effectiveness in vivo. A plausible reason lies in the fact that it is generally difficult to maintain prion infectivity in immortalized, continuously growing cells. For such cultured cells to remain chronically infected with prions, replication would have to be at least as rapid as cell division: A negative differential would inevitably result in loss of infectivity over time. In addition, prion replication may impose a fitness cost on infected cells, resulting in noninfected cells (which may arise because of inhomogeneously infected cultures or acquired resistance) overgrowing the system (36). These characteristics lead us to predict that infected cell cultures are inherently unstable systems—a prediction verified experimentally by the observation that most chronically infected cell lines spontaneously gravitate toward lower infectivity titers over time (unpublished observations by members of the Aguzzi lab). One could therefore argue that the crucial issue with N2A

cells is not how to cure them but rather how to maintain them in an infected state. In this interpretative frame, it is not surprising that agents capable of curing cultured cells almost always prove entirely ineffective when tested in vivo, where brain-resident cells undergo much slower turnover rates and spontaneous resistance has hardly any chance to develop (**Table 1**).

Cerebellar organotypic cultured slices (COCS) seem to represent a more realistic system for testing antiprion compounds and are indeed garnering a much better record than N2A cells in predicting in vivo antiprion efficacy (37–42). The main disadvantage of COCS over N2A cells, however, is the laboriousness of their production, which precludes their feasibility for high-throughput screens. Instead, COCS are best suited as secondary screens for intermediate validation of compounds identified in vitro.

One key consideration in designing an effective therapeutic option is to consider the frangibility (i.e., the propensity to break) of the pathological protein aggregates. Prion aggregate frangibility is the most important parameter in determining prion infectivity; these predictions from first principles were largely confirmed in animal models (40). By using β-sheet-breaking compounds that convert large aggregates into many small oligomers, one might inadvertently create more propagons (43). It follows that maybe an effective therapy should aspire not to break down PrP^{Sc} aggregates but rather to hyperstabilize said aggregates (**Figure 1**). Luminescent conjugated polythiophenes (LCPs) appear to act as such prion hyperstabilizers. LCPs bind to cross-β spines in PrP^{Sc} (44, 45). LCPs can detect PrP^{Sc} aggregates with great sensitivity, and their emission spectra can differentiate between different amyloids (45) and prion strains (46).

LCPs reduce prion infectivity in samples containing prion aggregates from brains of infected mice (47). The binding of the LCPs to amyloid fibrils was resolved at the atomic level and was found to rely on cooperative electrostatic interactions. However, digestion with proteinase K, which is a proxy for fibril stability, was enhanced by LCPs, whereas infectivity of the same prion preparation was decreased dose dependently (47). These observations are consistent with the hypothesis that LCPs indeed decrease the infectivity of prions by hyperstabilizing PrP^{Sc} aggregates. The structural elucidation of the interaction between LCPs and amyloid allowed us to design new LCPs with stronger binding. These showed higher efficacy in prolonging the survival of prion-infected mice (48).

TARGETING CELLULAR PATHWAYS FOR PRION THERAPY

The ubiquitin-proteasome system (UPS) maintains quality control in cells by degrading misfolded or damaged proteins (49). Early studies revealed the presence of ubiquitin within protein aggregates (50, 51). An elevated level of ubiquitinated proteins in the brains of prion-infected mice is associated with a dysfunctional UPS (52), which may contribute to neurotoxicity. PrP^{Sc} can bind to the external leaflet of the 20S proteasomal subunit and may impair its function (53). Other studies have postulated that prion oligomers inhibit the catalytic B subunit or prevent substrate entry into the proteolytic core (54). These hypotheses may explain the failure of proteasomes in prion infections (52, 55), but it is difficult to conceive how PrP, which resides in the lumen of the endoplasmic reticulum (ER) and in the extracellular space, could be driven to encounter proteasomes in the cytosol. The idea that PrP undergoes conspicuous ER-associated protein degradation (ERAD) is plausible (56, 57) but has been challenged (45).

Inhibition of ubiquitin carboxy-terminal hydrolase 14 (USP14), a deubiquitinase attached to the 19S proteasome subunit, results in clearance of aggregation-prone proteins (58). A small molecule targeting USP14 accelerates the degradation of proteins associated with neurodegenerative diseases, such as TDP43, tau, and ataxin. An as-yet-unexplored strategy targeting misfolded proteins in neurodegenerative diseases is the generation of small-molecule compounds, which direct the endogenous E3 ubiquitin ligases to their substrates. PROTACs (proteolysis-targeting chimeric

Table 1 Chemical compounds targeting prion disease

Compound name or class	Disease model and inoculation route	Administration route	Author conclusion	Reference
6-OHDA	i.p. and i.c. inoculation of mice with RML4.1	i.p.	Significant prolongation of incubation times through sympathectomy	146
Amantadine	Human patients suffering from CJD (ns, $n = 4$)	Oral	EEG changes in 2/4 treated patients, transient amelioration of neurological symptoms, no differences in survival	21
Astemizole	RML- or 22L-infected PK1 cells, i.c. inoculation of mice with RML	Addition to cell culture medium, i.p.	Inhibition of prion replication, prolonged survival in mice	88
Congo red	i.p. and i.c. inoculation of hamsters with 263K and 139H (i.c. only)	i.p.	Delay of mean incubation period in scrapie-inoculated hamsters	14
	i.c. and i.p. inoculation of hamsters with 263K	Subcutaneous, i.c.	Prolonged survival time of scrapie-inoculated hamsters	15
Dendrimers	22L-infected N2A cells, i.p. inoculation of mice with C506M3	Addition to cell culture medium, i.p.	Clearance of PrP^{Sc} in scrapie-infected cells and reduction of infectivity in mice spleens	17
DMSO	Scrapie-infected N2A cells	Addition to cell culture medium	Interference with PrP^{Sc} formation	96
Doxycycline	Human patients diagnosed with probable CJD ($n = 21$)	Oral	Significantly prolonged survival of doxycycline-treated patients compared to untreated controls	33
	Human patients suffering from sCJD ($n = 114$), iCJD ($n = 2$), vCJD ($n = 1$), and gCJD ($n = 4$)	Oral	No significant differences in terms of survival times or neuropathological changes	34
Flupirtine	Human patients suffering from sCJD ($n = 12$) and gCJD ($n = 2$)	Oral	Significantly less deterioration in dementia tests, no differences in survival	27
Glycerol, TMAO	Scrapie-infected N2A cells	Addition to cell culture medium	Interference with PrP^{Sc} formation	96
GSK2606414	i.c. inoculation of mice with RML	Oral gavage	Abrogation of clinical prion disease in mice	73
Imatinib	Chronically 22L-infected N2A cells, i.p. inoculation of mice with RML5	Addition to cell culture medium, i.p.	Clearance of PrP^{Sc}, prolonged survival	92
LCPs	SCEPA, MPA, RML6-infected COCS	Addition to cell or slice culture medium	Reduction of PrP^{Sc} through hyperstabilization of aggregates	47
	i.c. inoculation of mice with RML6	Intraventricular	Prolonged incubation period through hyperstabilization of PrP^{Sc} aggregates	48
Lithium	Persistently RML-infected N2A cells	Addition to cell culture medium	Clearance of PrP^{Sc}	87

(*Continued*)

Table 1 (*Continued*)

Compound name or class	Disease model and inoculation route	Administration route	Author conclusion	Reference
Pentosan polysulfate	i.c. inoculation of mice with 263K, RML, and Fukuoka-1	Intraventricular	Prolongation of incubation time in scrapie-infected mice	19
	Neuropathological workup of brains from human patients suffering from iCJD ($n = 1$), sCJD ($n = 2$), and GSS ($n = 1$) from Reference 161	Intraventricular	Uncertain therapeutic effects of pentosan polysulfate infusions	20
Polyamines	Scrapie-infected N2A cells, incubation with brain homogenates from scrapie-infected mice	Addition to cell culture medium, co-incubation with brain homogenate	Clearance of PrP^{Sc}, probably due to interference with prion propagation in endosomes and lysosomes	16
Quinacrine	Human patients suffering from sCJD ($n = 30$) and vCJD ($n = 2$)	Oral	No differences in functional status, brain lesions, or survival	29
	Human patients suffering from iCJD ($n = 2$), sCJD ($n = 45$), vCJD ($n = 18$), and gPrD (ns, $n = 42$)	Oral	10% of patients taking quinacrine had transient amelioration of neurological symptoms, no differences in survival	30
	Human patients suffering from sCJD ($n = 51$)	Oral	Quinacrine-treated patients deteriorated less on 2/3 functional rating scales, no differences in survival	31
Rapamycin	Tg(PrP-A116V) mouse model of GSS	i.p.	Extended survival in rapamycin-treated mice	89
STI571	Scrapie-infected N2A cells	Addition to cell culture medium	Clearance of PrP^{Sc}	85
Tacrolimus	RML- or 22L-infected PK1 cells, i.c. inoculation of mice with RML	Addition to cell culture medium, i.p.	Inhibition of prion replication, no prolonged survival in mice	88
Trehalose	Chronically 22L-infected N2A cells	Addition to cell culture medium	Decrease of newly synthesized PrP^{Sc} particles	91

Abbreviations: 6-OHDA, 6-hydroxydopamine; CJD, Creutzfeldt-Jakob disease; COCS, cerebellar organotypic cultured slices; DMSO, dimethyl sulfoxide; EEG, electroencephalogram; gCJD, genetic CJD; gPrD, genetic prion disease; GSS, Gerstmann-Sträussler-Scheinker syndrome; i.c., intracerebral; iCJD, iatrogenic CJD; i.p., intraperitoneal; LCP, luminescent conjugated polythiophene; ns, not specified; MPA, misfolded protein assay; PrP^{Sc}, proteinase K-resistant form of the prion protein; RML*X*, *x*th passage of the Rocky Mountain Laboratory prion strain; SCEPA, scrapie cell endpoint assay; sCJD, sporadic CJD; TMAO, trimethylamine *N*-oxide; vCJD, variant CJD.

molecules) consist of a peptide that recognizes a specific ubiquitin ligase chemically linked to a small molecule that recognizes the target protein (59). Once bound to the target protein, it creates spatial proximity between the substrate and ubiquitin ligase, promoting polyubiquitination and enhanced degradation of the target substrate. Researchers carried out extensive studies to characterize PROTACs for cancer treatment (60) and determined they can perhaps be used to target misfolded prions. A new strategy involves a combination of chaperone proteins and small-molecule compounds. The small molecule acts as a guide to the substrate, whereas the chaperone engages with the misfolded proteins and renders them amenable to proteasomal degradation (61). Such a strategy was implemented in spinal-bulbar muscular atrophy and amyotrophic lateral sclerosis (62).

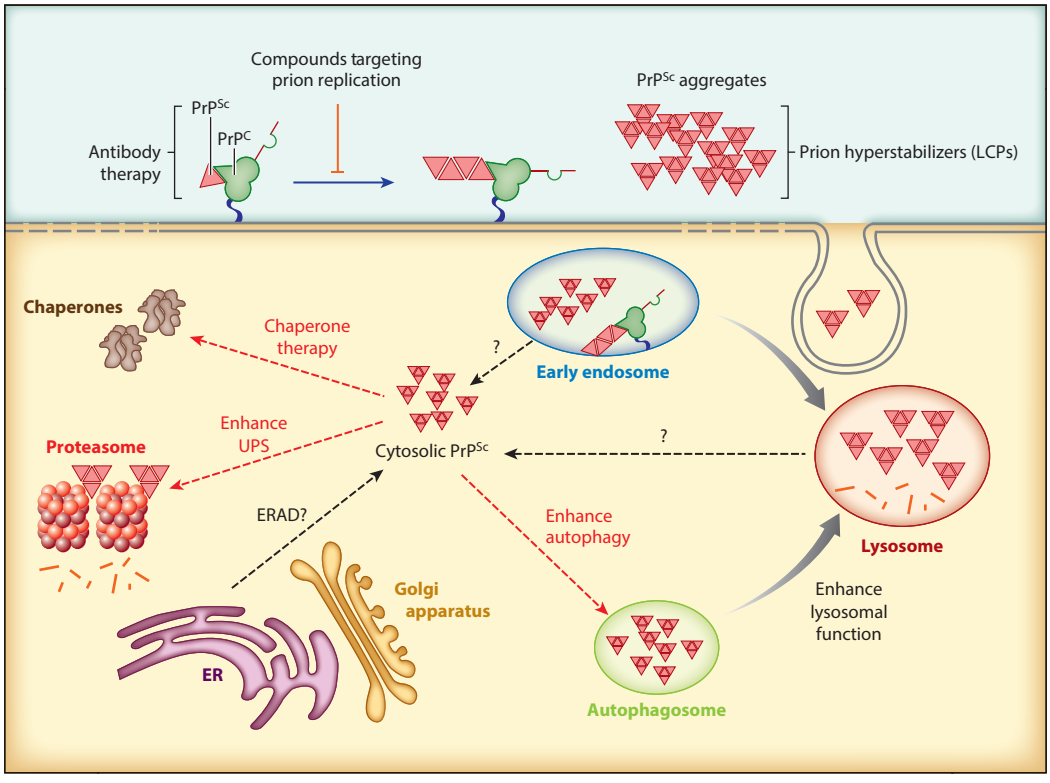

Figure 1

Upon encountering a prion propagon, the cellular PrP^{C} is converted and incorporated into PrP^{Sc}. The conversion of PrP^{C} to PrP^{Sc} probably begins on the plasma membrane and continues throughout the endocytic pathway. PrP aggregates have also been observed in the cytoplasm and may originate through ERAD, leakage from defective endosomes and lysosomes, or both. Potential therapeutic points of intervention include prion clearance (antibody therapy) and prion replication, including hyperstabilization of aggregates. Intracellular targets include enhancers of autophagy and of lysosomal function, as well as modulators of the UPS and chemical chaperones. Abbreviations: ERAD, endoplasmic reticulum–associated protein degradation; PrP^{C}, cellular prion protein; PrP^{Sc}, proteinase K-resistant form of the prion protein; UPS, ubiquitin-proteasome system.

TARGETING THE UNFOLDED PROTEIN RESPONSE

A common event in protein misfolding disorders is the upregulation of the unfolded protein response (UPR), also referred to as ER stress (63). Over 30% of all cellular proteins traverse the ER before being modified and disseminated to their final destinations. The ER controls a complex set of cellular processes by which proteins are synthesized, folded, and posttranslationally modified (64). Disturbances in the function of the ER may lead to the accumulation of misfolded proteins or alteration in calcium homeostasis, resulting in the induction of stress.

UPR can restore cellular proteostasis by shutting down global translation and thereby reduce the load of misfolded proteins in the ER (65). Also, UPR enhances the synthesis of chaperones and other proteins that assist in protein folding to repair the misfolded proteins in the ER (66). The misfolded ER proteins can be retrotranslocated to the cytosol, where they are degraded by the ERAD pathway (67). The major transducers of UPR are protein kinase RNA-like ER kinase (PERK), inositol-requiring enzyme-1 (IRE1), and activating transcription factor-6 (ATF6). PERK is a transmembrane protein essential for the attenuation of the translation by phosphorylation of

the eukaryotic translation initiation factor (eIF2α), whereas IRE1 and ATF6 are mainly involved in the synthesis of chaperones necessary for protein folding (68).

Elevated levels of the ER chaperones GRP94, GRP78, and GRP54 were observed in prion-infected humans and mice (69). Prion infection disrupts calcium homeostasis in the cell, affecting the ER; cells exposed to purified PrP^{Sc} displayed activation of UPR and calcium release from the ER, along with upregulation of chaperones identified in CJD patients (70). Furthermore, there exists a complex interplay between UPS and ER stress, and it is widely believed that inhibition of proteasomal function elicits UPR (71).

Prion-infected mice show sustained activation of PERK and phosphorylation of eIF2α, resulting in downregulation of global protein translation through eIF2α phosphorylation (72, 73), which leads to decreased synaptic proteins and neuronal death. Overexpression of the eIF2α-specific phosphatase GADD34 rescues synaptic defects and neuronal loss, at least for a while (73). Pharmacological inhibition of PERK restores translation and provides some neuroprotection (73). The integrated stress response inhibitor B, which targets the translational inhibition downstream of eIF2α, was also shown to ameliorate prion pathology (74).

In contrast to PERK inhibitors, guanabenz and its derivative sephin 1 prevented neurodegeneration in a mouse amyotrophic lateral sclerosis model by interfering with GADD34 and enhancing the phosphorylated status of eIF2α (75). This long-term translational arrest may prevent synthesis of new propagons and thereby provide neuroprotection. Guanabenz has previously been shown to enhance prion clearance (76), yet severe side effects have restricted its use so far. This problem illustrates the basic conundrum of UPR-based therapies: The process of interfering with a general control mechanism of translation is inevitably ridden with deleterious, unintended consequences (**Figure 1**).

TARGETING LYSOSOMAL DEGRADATION AND AUTOPHAGY

The conversion of PrP^{C} to PrP^{Sc} occurs at the plasma membrane (77) and in the endocytic pathway, including through the recycling of endosomes and multivesicular bodies (78, 79). The accumulation of misfolded prions in the endocytic compartments may alter the composition of the vesicular compartments and their functioning. Lysosomes are the major sites for degradation of cellular PrP^{C}, and PrP^{Sc} can accumulate in lysosomes (80). In cell cultures, PrP^{Sc} can be cleared by lysosomes; however, other defects arising in the endolysosomal machinery and PrP^{Sc} overload may ultimately render lysosomes nonfunctional. Indeed, prion infection results in reduced levels of membrane-bound rab7, affecting the maturation of lysosomes and their capacity to degrade proteins (81).

Another key delivery route of PrP^{Sc} to lysosomes for degradation is autophagy (82). In autophagy, the cytosolic constituents are engulfed by a double-membrane structure, the autophagosome, which fuses with lysosomes releasing their contents for degradation. Giant multivesicular bodies and autophagic vesicles (AVs) are observed in neurons of prion-infected mice, in prion-infected cell cultures, and in genetic prion models (83). Autophagy may play a protective role by scooping up aggregates and delivering them for degradation. Researchers originally believed that spongiform vacuoles observed in prion diseases are AVs, yet these vacuoles do not have the membrane characteristics of AVs, nor do they display any autophagy markers. Impairing autophagy pharmacologically or by siRNA inhibits the capacity of cells to degrade PrP^{Sc} (84). Hence, promoters of autophagy and lysosomal degradation could be therapeutic against prions (85, 86). Lithium has been shown to enhance the clearance of PrP^{Sc} in cultured cell lines by inducing autophagy (87) and reduces cellular PrP^{C} levels slightly. Rapamycin and tacrolimus, which also promote autophagy, showed similar results (88, 89).

Trehalose is an alpha-linked disaccharide synthesized by fungi and plants to protect them against environmental stress conditions by preventing protein denaturation. In cell culture, trehalose induces autophagy and may improve the clearance of misfolded proteins (90). PrP^{Sc} from prion-infected cell cultures was cleared rapidly by treatment with trehalose (91). Similarly, imatinib, another autophagy-promoting compound, abolished PrP^{Sc} levels in cell cultures (92).

CHAPERONE THERAPY

Molecular chaperones interact with other proteins and assist them in attaining a stable conformation. They represent an important quality control system that prevents misfolding and aggregation. In yeast, the heat shock protein 104 (Hsp104) disaggregase can solubilize cytosolic aggregates of Sup35, the yeast prion Ψ (93). Researchers identified a triad of Hsp110/70/40 as a mammalian minimal disaggregase (94). Upregulation of Hsp70 alone afforded neuroprotection in model systems (95). However, it is unclear whether this triad could be exploited therapeutically against prion diseases.

Chemical chaperones are small molecules that bind to proteins and restore their function by refolding them and letting them attain a stable structure. In spite of their nonspecific mode of action and low affinity, their ability to eliminate protein aggregates makes them attractive as therapeutics. Methylamines and glycerol have been effective in blocking the conversion of PrP^{C} to PrP^{Sc} in cell culture models (96). Anthracyclines, porphyrins, and diazo dyes were also effective in blocking prion replication in the in vitro assays (97), yet in vivo results were discouraging.

ACTIVE IMMUNOTHERAPY AGAINST PRION DISEASE

Immunization strategies have shown promise in various protein misfolding disorders (98). Active immunization against prions is hindered by the widespread expression of the cellular prion protein PrP^{C} in the body, leading to self-tolerance. Immunization with small prion fragments designed to fit into known grooves of major histocompatibility complex class II binding pockets elicited anti-PrP^{C} immunity, and antibodies derived thereof reduced proteinase K–resistant PrP^{Sc} levels in a prion-infected tumor transplant (99). Active immunization of mice with recombinant prion protein delayed prion disease when the immunogen was administered prophylactically and, to a lesser extent, when animals were already infected (100). Clinical disease induced through orally administered prions was attenuated after vaccination of mice (101, 102) and deer (103). However, another report failed to show differences in disease susceptibility through prophylactic prion vaccination in deer suffering from chronic wasting disease, a prion disease of deer and elk (104). A modest disease delay was achieved after immunization with recombinant prion protein fragments and intraperitoneal prion inoculation (105, 106). Attempts to break self-tolerance using a combined DNA and protein vaccination regime yielded mixed results (107, 108).

Because the immune system is tolerant to self-antigens, antibodies derived from immunizations often lack the affinity needed for effective therapy. Addressing the molecular whereabouts of PrP^{C} self-tolerance, one study found that even small amounts of extraneuronal PrP^{C} abolished an efficient immune response (109). A delay in disease onset was achieved by Freund's adjuvant, suggesting a benefit through an unspecific activation of the immune system (110). Another study suggested a strongly neuroprotective effect through post hoc immunostimulation against prions using repetitive administration of CpG oligodeoxynucleotides (CpG-ODNs) that are suggested to stimulate innate immunity (111). A chronic CpG-ODN treatment, however, was shown to induce profound immunosuppression with lymphoid follicle destruction, hepatotoxicity, and hemorrhagic ascites (112). Moreover, repetitive immunization of mice increases their susceptibility to

peripherally induced prion disease through reduced prion clearance, reduced size of follicular dendritic cell (FDC) networks, or both, suggesting that individual immune states (e.g., hyperactivated or depressed) may predispose mice to prion disease vulnerability (113).

PASSIVE IMMUNOTHERAPY AGAINST THE PRION PROTEIN

The first proof of concept for prion immunotherapy demonstrated a reduction in prion infectivity through exposure of cell-free, purified prions with PrP-specific antisera (114). Passive prion immunotherapy through diminishing PrP^{Sc} levels in vitro was exhibited when the monoclonal anti-PrP antibodies 6H4, SAF32, and SAF61 or Fab-fragments of the PrP-specific antibodies D13, D18, R1, and R2 were given to chronically prion-infected N2A neuroblastoma cells (115–118) (for a complete list of antibodies tested, see **Table 2**). When D13 was given as a bivalent antibody (D13-IgG), widespread neuronal apoptosis was observed, suggesting neuronal death occurred through cross-linking of PrP^{C}, a finding that was not seen with the holo-IgG molecule of D18 (119). When a single-chain fragment of D18 was engineered into the adeno-associated virus 9 vector and transduced into Rocky Mountain Laboratory–infected mice brains, prolonged survival of inoculated mice was observed (120). The toxic effects of D13 were reproduced in a second study (41).

Transgenic overexpression of an IgM^{a} μ chain of the anti-PrP antibody 6H4 reduced prion infectivity and levels of PrP^{Sc} in prion-infected mice (121), and peripheral injections with the monoclonal anti-PrP antibodies 8B4, 8H4, and 8F9 led to a decrease in clinical disease onset (122), as did injections with the PrP-α1 helix targeting antibody 31C6 (123). 31C6 was reported to be protective against prion disease when given as late as when clinical signs had already manifested, albeit through intraventricular application (124).

The safety profile of the anti-PrP antibodies ICSM18 and ICSM35 is highly controversial (125). One report found no drug-related toxicity of both compounds after stereotaxic injections of 2 μg of antibody in mice (126). However, in a dose-escalation study with ICSM18, the allegedly safe dosage of 2 μg of antibody showed drug-attributable neurotoxic effects, raising concerns about the suitability of ICSM18 in clinical trials (127). Of note, POM1, a monoclonal antibody directed against a similar epitope as ICSM18, shows severe neurotoxicity ex vivo and in vivo (39, 41, 128, 129).

Human autoantibodies recognizing the mutant prion fragment $PrP^{A117V}{}_{106–126}$ from commercially available, pooled immunoglobulins were proposed to be protective against $PrP^{A117V}{}_{106–126}$-induced neuronal death in vitro through microglial uptake of the mutated fragment (130, 131). However, $PrP^{A117V}{}_{106–126}$ does not exist in nature, and therefore such speculations are implausible.

Encouraging neuroprotection after prion challenge was obtained with monoclonal antibodies targeting the epitopes in octapeptide repeat domain (OR) of the prion protein. PrP^{Sc} formation was inhibited in N2A cells by two such anti-OR antibodies, mab110 and SAF34. More recently POM2, a high-affinity monoclonal antibody targeting the OR, conferred protection against prion infection in COCS (**Figure 2**) (41). Although prion titers were not affected, downstream cytotoxic events downstream of prion replication were selectively suppressed (39). Interestingly, however, anti-PrP^{C}-OR antibody 4H11 did not ameliorate the prion disease in mice, although it had been shown to clear PrP^{Sc} in cell culture experiments (132). Instead, 4H11-injected animals showed behavioral deficits and heightened neuronal loss and astrogliosis (132). It will be important to understand the differences between 4H11 and other anti-OR antibodies in order to understand why 4H11 antibody treatment failed to confer protection whereas intracerebral injections of the anti-OR antibody POM2 did not elicit toxicity (127).

4H11 was raised against an artificial murine PrP dimer that was hypothesized to represent a misfolded intermediate during the pathological conversion between PrP^{C} and PrP^{Sc} (132) while

Table 2 Therapeutic antibodies against PrP ex vivo and in vivo

Clone	Epitope region	Prion inoculation	Administration route	Assessment of toxicity and protection	Results	Reference(s)
6H4	α1	RML5, i.p.	Transgenic expression of μ chain	Mouse bioassay	No transgene-expressing animal succumbed to the disease	121
D13	CC2	NA	Stereotactic injection	Histology	Innocuous at 1 μg, toxic at 2 μg	119
			Stereotactic injection	Histology	Innocuous at up to 2 μg	126
			Stereotactic injection	Histology, MEMRI	Toxic at 6 μg	41
			Stereotactic injection	Histology, MEMRI	Innocuous at 2 μg, toxic at 6 μg; estimated upper limit of safe dose: 3.7–5.4 μg	127
D18	End of HC–α1	NA	Stereotactic injection	Histology	Innocuous at 2 μg	119
		NA	Stereotactic injection	Histology	Innocuous at up to 2 μg	126
		RML (ns), i.p.	AAV9	Survival	Disease delay in inoculated animals receiving the AAV9-mediated antibody	120
8B4	Between CC1 and OR	139A, i.p.	i.p.	Survival	Significant disease delay	122
8H4	α2					
8F9	α3 and beginning of GPI					
ICSM18	α1–3	RML (ns)	i.p.	Survival, PrP^{Sc} levels	Extended survival in i.p. but not i.c. inoculated mice treated with ICSM18, decreased levels of PrP^{Sc}	125
		NA	Stereotactic injection	Histology	Innocuous at up to 2 μg	126
		NA	Stereotactic injection	Histology, MEMRI	Innocuous at 2 μg, toxic at 6 μg; estimated upper limit of safe dose: 3.1 μg	127
ICSM35	End of OR–CC2	RML (ns)	i.p.	Survival, PrP^{Sc} levels	Extended survival in i.p. but not i.c. inoculated mice treated with ICSM18, decreased levels of PrP^{Sc}	125
		NA	Stereotactic injection	Histology	Innocuous at up to 2 μg	126

(*Continued*)

Table 2 (*Continued*)

Clone	Epitope region	Prion inoculation	Administration route	Assessment of toxicity and protection	Results	Reference(s)
31C6	α1	Obihiro, Chandler	Intraventricular	Survival, histology, PrP^{Sc} levels	Extended survival when given shortly after inoculation and when clinical symptoms were present	124
		Chandler	Intravenous		Extended survival of prion-infected, 31C6-treated mice	123
4H11	OR	6PB1	Intraventricular	Survival, histology, behavior	No prolonged survival of 4H11 in prion-infected mice, 4H11 induced behavioral deficits and neuronal loss	132
POM1	α1–3	NA	Stereotactic injection	Histology, MEMRI	Severe neurotoxicity	41
			COCS	Biochemistry, microarray	Induction of toxic pathways similar to bona fide prions	38, 39
			COCS, i.c.	Survival, histology, PrP^{Sc} levels	No generation of infectivity	129
POM2	OR	NA	Stereotactic injection	Histology, MEMRI	Innocuous at up to 2 μg of scFvPOM2 (molar equivalent to 12 μg holo-IgG)	41
		RML6	COCS	Histology, PrP^{Sc} levels	Neuroprotection by POM2 in prion-infected COCS	38, 39

Abbreviations: α1–3, alpha-helices 1–3 of PrP; AAV9, adeno-associated virus 9; CC1/2, charged cluster 1/2 of PrP; COCS, cerebellar organotypic cultured slices; GPI, glycosylphosphatidylinositol; HC, hydrophobic core of PrP; i.c., intracerebral; i.p., intraperitoneal; MEMRI, manganese-enhanced magnetic resonance imaging; NA, not applicable; ns, not specified; OR, octapeptide repeat of PrP; PrP, prion protein; PrP^{Sc}, proteinase K-resistant form of the prion protein; RML*X*, *x*th passage of the Rocky Mountain Laboratory prion strain.

POM2 was raised from immunization of PrP^{C} knock-out mice (133). One possible explanation for the discrepancy between the two OR-binding antibodies might be off-target effects of 4H11 due to immunization with a nonnatural protein.

TARGETING THE PERIPHERAL REPLICATION AND NEUROINVASION OF PRIONS

Genetic blockade of B cell maturation ablated the onset of prion disease after peripheral prion inoculation (134). In light of these findings, one might speculate that pharmacological ablation of B cells (e.g., through the anti-CD20 antibody rituximab) could afford postexposure prophylaxis. Initial prion accumulation occurs in secondary lymphoid organs prior to neuroinvasion (135), whereas other prion strains—so-called neurotropic prions—can primarily invade the central nervous system without the need for peripheral replication (136). Early studies have argued for the

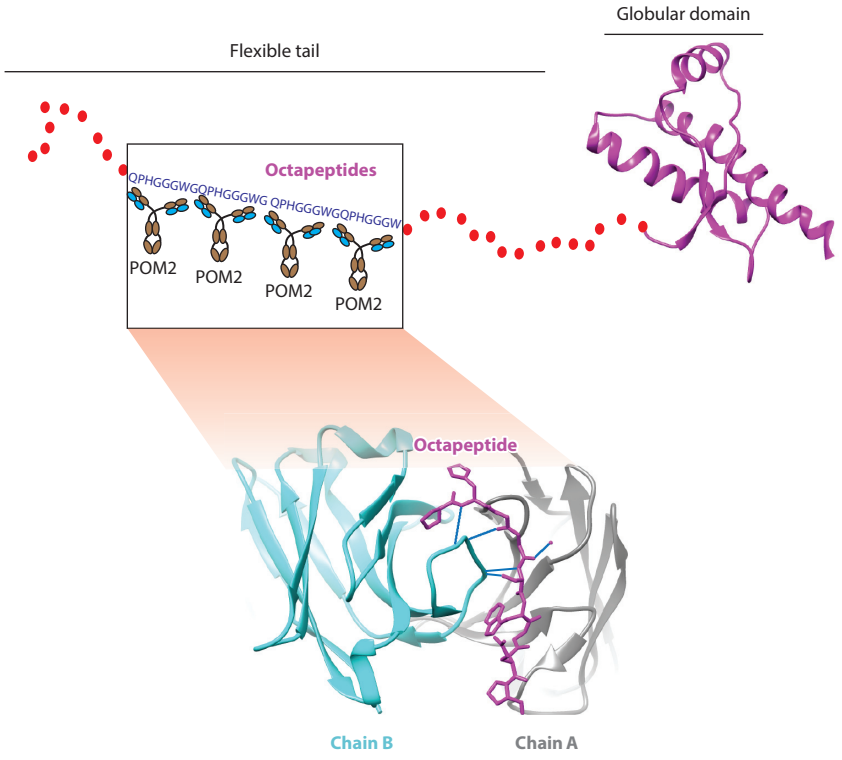

Figure 2

Immunotherapy is quickly evolving as an attractive therapeutic strategy against prion disease. The monoclonal antibody POM2 binds to a degenerate epitope in the octapeptide repeat region of PrP^C and protects against prion-induced neurodegeneration. The red dots indicate the N terminus of prion protein, also called the flexible tail, which is intrinsically disordered. The ordered globular domain of PrP^C is represented in magenta. The interaction between an $F(ab)_1$ fragment of the POM2 antibody (*cyan* and *gray*) and its cognate epitope on PrP^C (*purple*) is visualized in the inset. Blue lines indicate the interactions. Abbreviation: PrP^C, cellular prion protein.

requirement of mature PrP^C-expressing FDCs for prion neuroinvasion: Ablation of differentiated B cells prevented peripheral scrapie pathogenesis due to the lack of FDC maturation signals secreted by B cells (134), and mice lacking either expression of PrP^C on mature FDCs or mature FDCs did not succumb to peripherally initiated prion disease (137). As FDCs depend on lymphotoxins and tumor necrosis factor (TNF) from B cells for development and maintenance, they provide an opportunity to target prion replication (138). Administration of a hybrid protein consisting of lymphotoxin β receptor and human immunoglobulin (LTβR-Ig) dedifferentiated FDCs through inhibition of the lymphotoxin α/β pathway and led to a delay of prion disease upon peripheral inoculation (139), even when LTβR-Ig was given late during the disease course—but not upon intracerebral inoculation (140). Dedifferentiation of FDCs through a single injection of soluble human TNF receptor linked to the Fc portion of human immunoglobulin IgG1 also led to decreased disease susceptibility to peripherally administered prions (141).

FDCs trap immune complexes by binding to Fcγ receptors. They also bind opsonized antigens via the complement receptors CD21/CD35. Pharmacological and genetic ablation of the complement factor C3 or its receptor CD21/CD35 prolonged incubation times in peripherally

prion-inoculated mice (142, 143). Hence, complement activation through PrP^{Sc} may lead to more FDC-bound PrP^{Sc} and favor prion replication. Neither membrane-bound nor secreted immunoglobulins altered prion neuroinvasion (143). Circulating immune complexes bound to PrP^{Sc} may not play a role in prion pathology because deletion mutants of a variety of Fcγ receptors had no effect on prion incubation times (143).

The sympathetic nervous system (SNS) innervates secondary lymphoid organs, and experimental evidence has pointed to an involvement of the SNS in prion pathogenesis, as splanchnic nerves are an early replication site after peripheral prion inoculation (144) and prions accumulate in sympathetic and sensory ganglia as well (145). A transient pharmacological ablation of the SNS through injection of 6-hydroxydopamine or anti–nerve growth factor antibodies led to delayed scrapie onset after peripheral inoculation (146).

In a study addressing the cells responsible for conveying prions to the gut-associated lymphoid tissue after oral exposure, microfold cells (M cells) (147), specialized epithelial cells, were depleted through application of a monoclonal antibody against receptor activator of nuclear factor κB ligand (148). M cell depletion led to reduced prion uptake into FDCs without modifying FDC status and prevented disease onset after oral prion exposure (148).

THERAPIES AGAINST PRION DISEASE IN HUMANS

To date, no clinical trial against prion diseases has succeeded. The low prevalence of prion diseases inherently limits researchers' ability to conduct double-blind, randomized, placebo-controlled, multicenter trials on large patient groups. Rare diseases are less likely to be funded through industry, and indeed, a systematic review found only one out of seven trials in CJD had an industrial sponsor, in contrast to an overall average of three out of four industry-sponsored studies (149, 150).

Owing to the lack of a prion disease–specific disease rating scale, initial clinical studies were performed using cognitive test batteries not specifically designed to address prion disease phenotypes (27) or using survival as an outcome measure (151). Limited sample sizes and heterogeneous endpoints lead to therapeutic interventions being published as case reports. Yet case reports are intrinsically flawed by publication bias: An exceptional treatment success is more likely to be published than a treatment failure. Extension of the endpoint-based primary outcome (i.e., survival) in prion disease trials to neuropsychological, psychiatric, and other functional ranking systems may improve power calculations for future trials (152).

PALLIATIVE CARE IN PRIONOPATHIES

With no effective therapy currently available against prion diseases, all medical care is essentially supportive and palliative. Primarily, nursing efforts are intended to keep the patients safe (i.e., by providing walking assistance through walkers and wheelchairs and—during the terminal stage—a hospital bed with regular skin and mouth care and assistance with food intake) (153). Specifically, pyrexia (i.e., broad variations in body temperature), which was suggested to be a common symptom that, if left untreated, may lead to enhanced agitation, could be alleviated through the use of fans and tepid sponge baths (154). Further distressing symptoms that need to be addressed carefully are myoclonic jerking, heightened sensory sensitivity, shortness of breath, incontinence, and constipation (154).

Strict preventive measures in agriculture and in human medicine have reduced the incidence of vCJD to a near-complete disease extinction (155). Although the current World Health Organization *Tables on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies* (156)

do not formally deem blood or urine from non-vCJD diseased patients as infectious, some reports suggest detectable infectivity of urine from sCJD patients and transmissibility of blood from human genetic prion disease patients to primates (157, 158). Hence, preventive actions have to be followed by all personnel working with non-vCJD prionopathies as well, including but not limited to wearing appropriate protective gear and gaining knowledge about the relative infectivity of different human tissues (159). On another note, as CJD patients need both palliative and mental health care, the development of multidisciplinary guidelines can improve patient care through the development of sophisticated treatment schemes (160).

DISCLOSURE STATEMENT

Adriano Aguzzi is a founder and director of Mabylon Inc., a company devoted to the development of human antibodies to intractable diseases including prion diseases. The authors are not aware of any other affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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