10 Prions

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Transmissible spongiform encephalopathies (TSEs), or prion diseases, are neurodegenerative diseases characterized by spongiform changes, neuronal death, astrocytosis, and accumulation of the pathologic protein PrP^{Sc} in the brain and to a lesser extent in other organs; they 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). From an epidemiologic viewpoint, CJD can be classified as sporadic (sCJD), familial (fCJD), iatrogenic (iCJD), and variant (vCJD). Even the most frequent form, sCJD, is very rare and appears to be evenly distributed worldwide: Those countries that carry out surveillance report, quite uniformly, an incidence of approximately 0.6 to 1.2×10^{-6} per year.[1] The etiology of sCJD is unknown: No exogenous or endogenous causes 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 the past decades. Most of these have been attributed to transplantation of tissues or administration of pituitary 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 by the replacement of natural by recombinant peptide hormones in the mid-1980s, and yet individual patients are developing the disease even now—owing to the long incubation times involved.

Most recently a patient developed vCJD after having received a blood transfusion derived from another vCJD patient.[8] Although it cannot be formally excluded that both patients developed prion disease independently, it is very likely that this case represents the first identified instance of blood-borne CJD transmission.[9]

Biochemical and histopathologic 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, evoking fears of a large upcoming epidemic. Since then, however, the incidence of vCJD in the United Kingdom appears to be stabilizing and may actually be even falling. Hence, there is substantial hope that the total number of vCJD victims will be relatively small.[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 fluctuated between 1.3×10^{-6} and 1.4×10^{-6} per year. However, in 2001 and 2002 the incidence was 2.6×10^{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: In addition to 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 Creutzfeldt-Jakob Disease

Clinically, 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 sCJD, however, must usually await the analysis of central nervous tissue, at biopsy or postmortem. "Probable CJD" cases are diagnosed mainly on the basis of clinical symptoms, when no histopathologic or biochemical confirmation is available. Such "probable CJD" cases may contaminate mortality statistics in countries that register CJD cases on the basis of surrogate markers, including elevation of protein 14-3-3 in the cerebrospinal fluid (CSF).[16,17]

In the case of vCJD disease, firm diagnosis can often be obtained by the biopsy of tonsils, which have been shown to harbor significant amounts of PrP^{Sc} in germinal centers.[18] Highly sensitive methods have revealed that at least one third of patients with sCJD deposit PrP^{Sc} in skeletal muscle and/or in spleen.[19] Although the sensitivity of 30% is insufficient for routine diagnostics, these data open the possibility of minimally invasive diagnostics for sCJD, perhaps in combination with more sensitive methods in the future.

Magnetic resonance imaging (MRI) 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 type of neuroradiologic changes have been described.[21,22]

The onset of CJD is often heralded by mental changes.[23–25] Dementia is the most common finding early in the course of disease and is universal by the late stages of illness. Other presentations include personality changes with unusual or abnormal behaviors, disordered sleep, and distorted vision. Motor symptoms commonly emerge during the course of illness and may consist of any combination of cerebellar, pyramidal, and extrapyramidal abnormalities. Myoclonus, especially a heightened startle response to unexpected sound or light, is a characteristic feature of the mid to late stages of CJD. In most instances, CJD is rapidly fatal, with the mean duration of illness less than 1 year. Three laboratory tests can often add confidence to the clinical diagnosis: electroencephalogram (EEG), MRI, and CSF examination. The EEG is almost always abnormal because of diffuse slow wave activity; periodic triphasic complexes are especially characteristic in CJD. MRI brain scans reveal increased signal on fluid-attenuated inversion recovery (FLAIR) and diffusion-weighted imaging (DWI) sequences in basal ganglia, thalamus, and/or cerebral cortex that correlate with the clinical symptoms and that presumably reflect the underlying spongiform degeneration of gray matter.[26,27] There are no inflammatory cells in the CSF, but measures of neuronal degeneration are markedly elevated. These include the 14-3-3 protein, although whether this measure is diagnostic for CJD remains debated. The most distinctive finding, however, is a greatly elevated level of tau, the microtubule-associated protein.[28]

Genetics and the Incidence of Creutzfeldt-Jakob Disease

Although 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[29] and are exemplified by the "octapeptide repeat expansion" mutants of both mouse[30] and man.[31,32]

In addition to disease-causing mutations, polymorphisms in *PRNP* can have a profound effect on susceptibility to prion disease. Thus, all cases of vCJD have the met/met, rather than the val/val or met/val configuration at position 129.[33,34] Moreover, humans heterozygous at this site are largely protected from CJD: This effect is so important that it may have exercised selective evolutionary pressure.[35] A lys, rather than a glu residue at position 219, is thought to be protective against sCJD.[36]

However, it is becoming increasingly apparent that genetic susceptibility markers and modifiers are not limited to polymorphisms in the PrP-encoding reading

frame, as revealed by the identification of several quantitative trait loci affecting incubation time in the mouse.[4–7] It is presently not clear what these modifiers might be. The possible protective effect against vCJD of a certain MHC class-II constellation[37] has been disputed.[38] Nonetheless, based on all that is known about the critical role of the immune system in peripheral prion infection,[39] immunity-controlling genes are likely to be represented among endogenous modifiers.

Given that a large proportion of the British population may have been exposed to BSE infectivity, that animal experiments indicate that the infectious dose (ID_{50}) for oral cross-species transmission of BSE is relatively low (500 mg of brain tissue sufficed to cause disease in sheep[40]), and that only approximately 150 humans have contracted vCJD, it is likely that vCJD susceptibility is controlled by endogenous and/or exogenous factors other than the amount of infectious agent ingested.[41]

The Timeline of Transmissible Spongiform Encephalopathy Research

In one or the other form, prions have captured a sizable mind share for almost 2 centuries (Table 10-1). Scrapie—the prototypic prion disease affecting sheep and goats—had been a concern since the 19th century. This is understandable given the importance of the wool textile business in the industrial revolution. However, the crucial breakthrough was already achieved in the 1930s by the experimental transmission of scrapie to goats.[42] Little happened in the 2 following decades, until Carleton Gajdusek showed that Kuru, which was decimating the aborigines of Papua New Guinea,[43] was a TSE. Interestingly, the first attempts at transmitting Kuru to primates failed for the same reason as experimental transmission of scrapie among sheep had failed for decades: The incubation time of the disease was longer than the patience of the investigators.[44] Following a concise suggestion by William Hadlow that Kuru resembled scrapie, and hence might exhibit a very long incubation time,[45] Gajdusek achieved transmission of Kuru to chimps[46,47] and, shortly thereafter, transmission of CJD.[48]

It is remarkable and somewhat sobering to note that some of the questions that had already been formulated in the 19th century are still open. For example, is sheep scrapie a predominantly genetic or infectious disease? If the latter is true, how does it spread among flocks? The wildfire-like epizootic of chronic wasting disease in North American cervids,[49] as well as the "scrapie eradication plan" of the European Union (which aims at selective breeding of purportedly scrapie-resistant sheep genotypes), bears the most recent witness to the general importance of these issues.

The Nature of the Prion

It is widely accepted that the TSE agent, or prion, is not a typical microorganism consisting of agent-specific nucleic acid encoding one or more agent-specific proteins, such as a bacterium or virus, although some dissent on even this point continues. Notably, no immune responses are elicited on infection.

TABLE 10–1 Essential Chronology of Prion Research

Mid-18th century	Earliest description of scrapie recorded
1898	Neuronal vacuolation discovered in brains of scrapie-sick sheep
1918	Contagious spread of scrapie in natural conditions suspected
1920	First cases of CJD described[165,166]
1937	Scrapie epidemic in Scotland following administration of formalin-treated louping ill vaccine prepared from sheep brain
1939	Experimental transmission of scrapie reported[42]
1955–1957	Kuru discovered among Fore people of Papua New Guinea[43]
1959	Similarities between Kuru and scrapie noted[45]
1961	Multiple strains of scrapie agent described[167]
1961	Scrapie transmitted to mice[168]
1963	Transmission of Kuru to chimpanzees reported[46]
1966	Scrapie agent found to be highly resistant to ionizing radiation and ultraviolet light[169,170]
1967	First enunciation of the protein-only hypothesis[171]
1968	CJD transmitted to chimpanzees[48]
	Description of *Sinc* gene affecting scrapie incubation period in mice[172]
1974	First documented iatrogenic prion transmission (corneal graft)[173]
1980	Protease resistant, highly hydrophobic protein discovered in hamster brain fractions highly enriched for scrapie infectivity[174]
1982	Prion concept enunciated[175]
1985	Gene encoding PrP^{C} cloned[176,177]
1986	PrP^{C} and PrP^{Sc} isoforms shown to be encoded by same host gene[178]
1987	Linkage between *Prnp* and scrapie incubation period in mice[179]
	First report of BSE in cattle[180]
1989	Mutation in PrP linked to Gerstmann-Sträussler syndrome[3]
	Importance of isologous PrP^{C}/PrP^{Sc} interactions established[181]
1992	Ablation of *Prnp* by gene targeting in mice[62]
1993	$Prnp^{o/o}$ mice are resistant to scrapie inoculation[61,67]
	Structural differences between PrP^{C} and PrP^{Sc} isoforms noted[182]
1994	Cell-free conversion of PrP^{C} to protease-resistant PrP[52]
1996	New variant of CJD identified[183]
	BSE prion strain carries a distinct glycotype signature[184]
	First NMR structure of core murine PrP^{C} solved[185]
1997	Evidence that nvCJD is caused by the BSE agent[10,11]
	B-lymphocytes necessary for peripheral prion pathogenesis[96]
1998	Genes controlling incubation period are congruent with *Prnp*.[186]
1999	Discovery of the PrP^{C} homologue[187]
2000	Temporary depletion of lymphoid FDCs impairs prion replication[71]
	Experimental transmission of BSE in sheep by blood transfusion[188]
2001	Complement involved in prion pathogenesis[98,101]
2003	Transgenic expression of soluble PrP inhibits prion replication[156]

BSE, Bovine spongiform encephalopathy; CJD, Creutzfeldt-Jakob disease; FDC, follicular dendritic cell; NMR, nuclear magnetic resonance; nvCJD, new variant Creutzfeldt-Jakob disease.

One of the most striking and characteristic features of the disease is the deposition, mainly in the brain but to a lesser degree also in other tissues, of a partially protease-resistant protein, designated PrP^{Sc} or PrP-res, a beta-sheet-rich conformational isomer of the protease-sensitive, alpha-helix-rich ubiquitous host protein PrP^{C}. Biochemical and genetic evidence link PrP and its gene to the disease. PrP^{Sc} copurifies with infectivity and vice versa, familial forms of CJD are invariably linked to mutations in the PrP gene.

By and large, the available data and the failure to identify a disease-specific nucleic acid support the "protein-only" hypothesis. As enunciated by S. Prusiner, this hypothesis proposes that the infectious agent consists of PrP^{Sc}, that it is devoid of nucleic acid, and that its "replication" comes about by PrP^{Sc}-mediated, autocatalytic conversion of PrP^{C} to PrP^{Sc}.[50] However, it is not clear that the infectious entity is PrP^{Sc}, operationally defined as a protease-resistant, aggregated form of PrP, rather than some other conformer, generically designated as PrP*,[51] nor has the requirement for other components been excluded. The critical experiment of converting purified PrP^{C}, be it recombinant or from a natural source, into an infectious form has not been reported so far, although conversion of PrP^{C} into PrP^{Sc} has been achieved.[52–55] 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.[56–59]

In 1983, Stanley Prusiner and coworkers described a crucial property of the prion: its remarkable resilience against proteolytic degradation.[60] Digestion with 50 µg/mL of proteinase K (PK) at 37°C for 2 hours would not degrade the carboxy proximal domain of PrP^{Sc} nor decrease the infectious titer of the prion preparation. However, PrP^{Sc} is not "unbreakable" and can eventually be digested by more vigorous enzymatic treatment—in which case prion infectivity titers will also subside. This remarkable discovery identified PrP^{Sc} as the first reliable surrogate marker of prion infection. The impact of this technology was phenomenal: even now—20 years after its original description—the detection of PK-resistant prion protein (termed $PrP^{27\text{-}30}$ because of its molecular weight after hydrolysis of its PK-sensitive amino terminal domain) remains the gold standard for biochemical diagnosis of prion diseases and forms the basis for all of the currently marketed BSE tests.

Charles Weissmann then verified a crucial prediction of Prusiner's protein-only hypothesis. If PrP^{Sc} multiplies by imparting its conformation onto host-borne PrP^{C}, organisms devoid of PrP^{C} should be resistant to prion infection.[61] *Prnp*$^{o/o}$ mice are alive and well,[62] notwithstanding some minor abnormalities[63–65]—some of which may not even be causally related to the prion gene.[66] The excitement was considerable as it became gradually clear that inoculation of *Prnp*$^{o/o}$ mice with brain homogenate from scrapie-sick mice failed to induce disease of any kind[61] or elicit any subclinical replication of the agent.[67]

The study of Büeler and colleagues[61] has sometimes been invoked as the "final proof" of the protein-only hypothesis. That is certainly not the case: The knockout experiment was designed to *disprove* Prusiner's hypothesis—and it would have certainly done so if *Prnp*$^{o/o}$ mice had developed disease. As always with negative results, alternative interpretations can be offered.[68] Those skeptical of the prion hypothesis were quick in pointing out that PrP^{C} may be a receptor for a hitherto unidentified virus, whose ablation would confer antiviral resistance. Yet it is fair to say that the resistance to scrapie of *Prnp* knockout mice constitutes one of the

most stringent challenges to the protein-only hypothesis. Hence its failure is very significant.

The availability of *Prnp*$^{o/o}$ mice has triggered a cascade of technologic and conceptual advances. For example, it emerged that PrP^{C}, in addition to controlling prion replication, is necessary for neuronal damage: *Prnp*$^{o/o}$ neurons adjacent to infected *Prnp*$^{+/+}$ brain grafts do not incur damage.[69] PrP^{C} is also involved in the transport of the infectious agent from peripheral sites to the central nervous system (CNS): Its expression appears to be needed in a sessile compartment,[70] which is likely to be congruent with stromal components of the lymphoreticular tissue[71] and of the peripheral nervous system.[72] The microenvironment of lymphoid organs appears to control the velocity of neuroinvasion.[73]

PrP^{C} is not only produced by neurons: its expression is, in fact, quite ubiquitous, notably including lymphocytes[74] and stromal cells of lymphoid organs.[75] As a result, wild-type mice enjoy an extremely tight immunologic tolerance against PrP^{C}, which had rendered the production of high-affinity immunoreagents very difficult. Instead, the immunization of *Prnp*$^{o/o}$ mice yielded large numbers of very-high affinity antibodies, some of which form the basis for the current crop of BSE tests.

Still, it proved difficult to generate conformational antibodies discriminating between PrP^{C} and PrP^{Sc}. This is surprising in view of the dramatic structural differences between these two isoforms and their differential binding to serum proteins.[76] Does the failure of the immune system to generate antibodies specific for PrP^{Sc} indicate that all relevant neoepitopes of PrP^{Sc} that are newly exposed by the conversion of the protein to its disease-associated state are inaccessible? Early claims of discriminatory antibodies, such as Prionics' 15B3 clone,[77] have not lived up to the expectations. A recently developed antibody against a characteristic tripeptide (YYR) exposed in PrP^{Sc}, but not in PrP^{C}, may be more promising.[78] However, the YYR motif is certainly not specific to PrP^{Sc}, and the usefulness of this antibody awaits independent confirmation.

Extraneural PrP^{Sc}

Refinements in the technologies for detection of PrP^{Sc} have prompted a renaissance of studies of the distribution of the disease-associated prion protein in extracerebral organs of patients. These studies revealed that extraneural PrP^{Sc} is more widespread than previously thought. Zanusso and colleagues found that PrP^{Sc} is readily detectable in the olfactory mucosa of sCJD victims.[79] Glatzel and colleagues[19] have found that approximately one third of the Swiss sCJD patients display PrP^{Sc} in their skeletal muscle and another third (partially overlapping) had PrP^{Sc} in lymphoid organs. Further investigations are under way to determine whether these findings are universally valid for CJD patients or represent a specific characteristic of the Swiss CJD collective. If the latter were true, one might speculate that the abnormal peripheral pathogenesis of CJD in Swiss patients points to a specific etiology.

The UK vCJD cases are likely to be primary transmissions from cattle BSE. However, experimental transmission studies show that TSE strain characteristics can change on serial passages after the original primary transmission.[80] Therefore, horizontal vCJD transmission among humans could result in a different pheno-

type than vCJD. This scenario calls for innovative studies aimed at developing and validating classical and emerging, up-to-date prion strain typing tools.

Pathogenic Mechanisms in Prion Diseases

The damage wrought by prions is mainly evident in the CNS, although pathologic changes in the spleen of nonhuman primates have also been noted (C. Lasmezas, personal communication). Because PrP^{Sc} accumulates in the CNS and in some instances is deposited as an amyloid, it is has been indicted as the toxic entity causing neuronal apoptosis and eliciting disease. The finding that peptides derived from the PrP region 106-126 form aggregates and are toxic to cultured neuronal cells[81,82] has been adduced in support of this contention, although there has been some dispute as to the reproducibility of the phenomenon.[83] It is, however, not evident that the pathogenicity of the oligomerized peptides on cultured cells mimic the properties of PrP^{Sc} accumulating in the CNS.

PrP^{Sc} produced by a prion-infected, PrP-expressing neuronal graft in the brain of PrP knockout mouse did not cause disease nor did it result in damage to neighboring neuronal tissue devoid of PrP.[69] 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 days after intracerebral (i.c.) inoculation, in contrast to 150 days in wild-type mice, although they accumulate levels of PrP^{Sc} similar to those of wild-type animals by 150 days after infection.[84] Therefore, PrP^{Sc} is likely to be responsible for CNS pathology only in neurons that express PrP^{C}.

Gain of toxic function by a PrP moiety that is different from PrP^{Sc} is a distinct possibility. Over several years, a lively debate has unfolded on the role of abnormal PrP^{C} topologies. Targeting of PrP to the cytosol was reported to result in rapidly lethal neurodegeneration (albeit without accumulation of PrP^{Sc}), and proteasome inhibition induces a slightly protease-resistant, cytoplasmic PrP species in cultured cells.[85,86] Therefore, prion toxicity was proposed to start with retrotranslocation of PrP^{C} 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.[87] Moreover, the toxicity of cytosolic PrP has been contested.[88,89] Lingappa found that PrP^{C} assumes a transmembrane topology (^{Ctm}PrP), whose concentration correlates with neurotoxicity.[90,91] These data have been taken to suggest that ^{Ctm}PrP represents a major toxic moiety.

From the previous discussion, it becomes apparent that further work is needed to understand the role of alternative PrP topologies in prion neurotoxicity. Moreover, the biochemical pathways leading to pathogenicity, triggered be it by PrP^{Sc}, cytoplasmic PrP, or ^{Ctm}PrP, are still obscure.

SPREAD OF PRIONS

Prion pathogenesis can be broken down into spatially and temporally distinct phases: (1) infection and peripheral replication, (2) migration from the periphery to the CNS (neuroinvasion), and (3) neurodegeneration. The resistance to prions

of mice that lack PrP^C expression is amply documented.[61,69,92,93] PrP^C expression is required for transporting the infectious agent from the peripheral sites to the CNS (as monitored by PrP^C-expressing neurografts)[70] and within the CNS.[94] However, reconstitution of *Prnp*$^{o/o}$ mice with wild-type (wt) bone marrow is insufficient to restore neuroinvasion in engrafted *Prnp*$^{o/o}$ mice,[70] although the capacity of the spleen to accumulate prions of the RML strain is reconstituted.[70,95] This suggests that hematopoietic cells transport prions from the entry site to the lymphoreticular system (LRS), which accumulates and replicates prions, but that PrP^C expression in an additional compartment, presumably the peripheral nervous system, is required. B lymphocytes (not necessarily expressing PrP^C) are crucial for peripheral prion spread and neuroinvasion.[96,97]

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^{Sc} following scrapie infection,[75] and maturation of FDCs requires signaling by B cells expressing LTα/LTβ trimers on their surface. Indeed, blockade of LT-β signaling via administration of soluble LTβR-Ig ablates mature FDCs and significantly impairs neuroinvasion and accumulation of peripheral PrP^{Sc} and infectivity.[71,98] FDCs are crucial to disease progression after oral scrapie challenge but only within a short time window.[99,100]

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[101] or mice depleted of the C3 complement component[98] 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.

However, just which cell types are involved in neuroinvasion? The innervation pattern of lymphoid organs is primarily sympathetic.[102] 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.[72] Although there is no physical contact between FDCs and sympathetic nerve endings,[103] the distance between FDCs and splenic nerves affects the velocity of neuroinvasion.[104] 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

On oral challenge, an early rise in prion infectivity is observed in the distal ileum of infected organisms. This applies to several species but was most extensively investigated in sheep. Western blot analysis has shown that Peyer's patches (PPs) accumulate PrP^{Sc}. This is true also in the mouse model of scrapie, in which administration of mouse-adapted scrapie prions (RML strain) induces a surge in intestinal prion infectivity as early as a few days after inoculation.[100,105,106] Indeed, immune cells are crucially involved in the process of neuroinvasion after oral application: Mature FDCs, located in PPs, may be critical for the transmission of scrapie from the gastrointestinal tract.[105]

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[107] and in hematogenous spread[108] 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 some hundred fold.[109] Anti-PrP antibodies were found to inhibit formation of protease-resistant PrP in a cell-free system.[110] Also, antibodies[111,112] and F(ab) fragments directed against PrP[113] 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 proved exceedingly difficult to elicit humoral immune responses, because the mammalian immune system is largely tolerant to PrP of the same species. Mice devoid of PrP[62] show no tolerance and are highly susceptible to immunization with recombinant PrP[93] or PrP^C-expressing cells.[94]

Tolerance is typically brought about by activation-induced cell death (AICD), 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. They expressed an immunoglobulin/B-cell receptor μ chain containing the epitope-interacting region of 6H4, a high-affinity anti-PrP monoclonal antibody.[77] The transgenic μ chain associated with endogenous κ and λ chains; some pairings led to reactive moieties and, consequently, to anti-PrP^C titers in $Prnp^{o/o}$ and $Prnp^{+/+}$ mice. The buildup of anti-PrP^C titers, however, was more sluggish in the presence of endogenous PrP^C, suggesting that clonal deletion was actually occurring. B cell clones with the highest affinity to PrP^C are probably eliminated by tolerance, whereas clones with medium affinity are retained. The latter sufficed to block prion pathogenesis on intraperitoneal (i.p.) prion inoculation.[114] Hence, B cells are not intrinsically tolerant to PrP^C and can—in principle—mount a protective humoral response against prions.

The challenges to practical antiprion immunization, however, are enormous. Although providing an encouraging proof of principle, transgenic immunization cannot easily be reduced to practice. Passive immunization failed to confer protection if treatment was started after the onset of clinical symptoms, suggesting that it might be a better candidate for prophylaxis rather than for therapy of TSEs. Active immunization, like in most antiviral vaccines, may be more effective but is rendered exceedingly difficult by the stringent tolerance to PrP^C.[115,116]

A recent report has outlined 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.[117] Because monovalent Fab fragments did not elicit these responses, it is likely that crosslinking of PrP^C by bivalent IgG antibodies is neurotoxic in vivo—maybe by eliciting some deleterious signaling event. Although these results put a cautionary note on the prospect of using antibodies against clinically overt prion diseases, it

is possible that anti-PrP Fab fragments are capable of reducing infectious titers[113] without exerting a toxic effect.[117] 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 on chronic administration to scrapie-infected mice.[118] 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.[96,97,105,119] In addition, MyD88$^{-/-}$ mice undergo normal prion pathogenesis despite abrogation of TLR9 signaling.[120] Hence, more detailed studies will be needed to understand the basis of the antiprion effect of CpG-ODN. The realization that repeated administration of CpG oligodeoxynucleotides 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.[121]

Search for Therapeutic Agents

Devising approaches to the therapy of TSEs, or prion diseases, is beset by many difficulties. For one, the nature of the infectious agent, the prion, is only understood 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 CSF or brain tissue. Finally, because the disease is mostly only recognized 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.

Screening for putative therapeutic agents has been conducted at various experimental levels. Based on the assumption that PrP^{Sc} is either the infectious agent or at least the pathogenic entity, compounds have been sought that in a *cell-free system* would stabilize PrP^{C}, destabilize PrP^{Sc}, or prevent conversion and thereby decrease the level of PrP^{Sc}. Bis-ANS (4,4′-dianilino-1,1′-binaphthyl-5,5′-sulfonate) was described as potently inhibiting PrP aggregation,[122] whereas so-called β-sheet breaker peptides[123] and branched polyamines[124] partially disassembled PrP^{Sc} to a protease-sensitive form. However, compounds identified by this type of screen, although potentially of interest, still face high hurdles to qualify as drug candidates: They must be able to reach the appropriate cellular compartment; provide an acceptable therapeutic index (i.e., ratio of toxic to therapeutic dose); exhibit pharmacokinetics that allow the build-up of a sufficiently high concentration in the biophase, which implies the capacity of the compound to cross the BBB effectively; and be accessible in sufficient quantity by chemical synthesis or from biologic sources.

A *yeast-based screen* has been reported in which the capacity of compounds to diminish the propagation of "yeast prions" is assessed.[125] Because the yeast proteins involved have a sequence 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.[126] *Scrapie-infected cells,* in particular the murine neuroblastoma derived N2a line, have been used as targets for prospective drugs, assessing the decrease of PrP^{Sc} levels as measure for therapeutic activity. The steady state level of PrP^{Sc} is determined by the rate of formation relative to that of degradation. Although originally thought to be very stable, PrP^{Sc} in murine neuroblastoma cells has a half-life in the order of a day or so, and inhibition of its formation leads to its elimination within a few days. This is the case after inhibition of PrP^{C} synthesis, for example, by siRNA,[127] as well as sequestration or depletion of PrP^{C} from the cell surface by binding of anti-PrP antibodies,[111,112] Fab fragments,[113] aptamers,[128] or compounds such as biquinoline[129] or suramine.[130] Interference with the conversion reaction has been attributed to the binding of compounds such as heparan mimetics,[131,132] Congo red,[133] or phthalocyanine tetrasulfonate[134] to PrP^{Sc} and/or PrP^{C}. Accelerated degradation of PrP^{Sc} is attributed to the consequence of its interaction with branched polyamines.[124,135] Polyene antibiotics such as amphotericin B are believed to interact with detergent-resistant microdomains or rafts[136] and inhibit generation of PrP^{Sc} of at least some prion strains by interfering with the trafficking of PrP^{C}.[137] Recently, a screen of 2000 compounds using scrapie-infected N2a cells yielded 17 candidates that were inhibitory at 10 mM or less. Interestingly, only polyphenols were inhibitory in the cell-free conversion system.[138]

A more stringent screen, mostly applied to compounds 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 so-called 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.[139] Thus, $Prnp^{o/o}$ mice transgenic for bovine or human PrP genes become susceptible to BSE and CJD prions, respectively.[140] Interestingly, however, some strains of wild-type mice are far more susceptible to human vCJD prions than mice transgenic for the human PrP gene.[80]

Drug candidates have been administrated before, concomitantly, early, or late after inoculation with prions, mostly, for convenience, i.p., but occasionally i.c. to overcome the BBB. A critical variable is also the site of prion inoculation, which is usually i.p. or i.c. More rarely peroral i.p. inoculation requires prion doses with orders of magnitude higher than i.c. inoculation, and incubation times are typically twice as long. The important consideration here is that i.p. or peroral 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 10-2, prolong the incubation time in animal models when administered before or early after infection. Among these are sulfated polyanions,[141–145] Congo red D,[146] polyene antibiotics,[145,147–149] tetracyclic compounds,[150] and tetrapyrroles.[134,151,152] Copper added to the drinking water of scrapie-infected hamsters has been reported to delay clinical disease of scrapie-infected hamsters,[153] but a similar effect was reported

TABLE 10–2 Representative Compounds Used in Attempts at Therapy of Prion Disease

Compound	Cell-Free	Cell Culture	Animal	Proposed Mechanism	References
Acridines					
Quinacrine	Binds weakly to helix3 of PrP^{C} (Km ca. 1mM) No effect on PrP^{Sc}	Reduces PrP^{Sc} levels in ScN2a cells (IC_{50} 300 nM), less so in ScGT cells	No effect on CJD/mouse model. Permeate BBB	Accumulation in lysosomes, binding to PrP^{Sc}?	189, 190
Bis-acridine derivatives		Reduce PrP^{Sc} levels in ScN2a cells (IC_{50} 25–40 nM)		Binding to unknown receptor?	191
Anthracyclines					
4′-iodo-4′-deoxy-doxorubicin			Inoculum and drug coinjected prolonged survival of scrapie-infected Syrian hamster	Binding to PrP^{Sc} ?	150

Table continued

TABLE 10–2 Representative Compounds Used in Attempts at Therapy of Prion Disease—cont'd

Compound	Cell-Free	Cell Culture	Animal	Proposed Mechanism	References
Anti-PrP antibodies					
Fab D18	Binds PrP^{C} 132-156	Reduce PrP^{Sc} levels in ScN2a cells (IC_{50} 9 nM)		Impedes PrP^{C}-PrP^{Sc} interaction	113
6H4	Binds PrP^{C} 144-152	Reduce PrP^{Sc} levels in ScN2a cells (IC_{50} ca. 10 nM)	Protects mice against i.p. infection when light chain expressed from transgene	Impedes PrP^{C}-PrP^{Sc} interaction	111, 114
ICSM18	Binds PrP^{C} 146-156	Reduce PrP^{Sc} levels in ScN2a cells	i.p. administration protects mice against i.p. prion inoculation	Impedes PrP^{C}-PrP^{Sc} interaction	192
Aptamers					
DP7	Binds PrP^{C}	Reduce PrP^{Sc} levels in ScN2a cells		Impede PrP^{C}-PrP^{Sc} interaction	128
SAF93	Binds PrP^{Sc}, prevents conversion			Impede PrP^{C}-PrP^{Sc} interaction	193, 194
Cyclic tetrapyrrols					
PcTS, TMPP-Fe^{3+}	Prevent conversion		Increased survival time in mouse injected i.p.	Unknown	151

TABLE 10–2 Representative Compounds Used in Attempts at Therapy of Prion Disease—cont'd

Compound	Cell-Free	Cell Culture	Animal	Proposed Mechanism	References
Peptides					
PrP106-128, PrP113-141	Inhibits conversion	Reduces PrP^{Sc} levels in ScN2a cells		Impedes conversion by binding to PrP^{C} or PrP^{Sc}	195
β-sheet breaker iPrP13	Partial disassembly of PrP^{Sc}		Pretreatment of inoculum decreases infectivity 1–1.5 logs in mice	Disassembly of PrP^{Sc}	123
Polyamines					
Polypropyleneimine (PPI)	Renders PrPSc of some prion strains susceptible to PK digestion	Reduces PrP^{Sc} levels and infectivity in ScN2a cells		Destabilizes PrP^{Sc}	124, 125
Polyanions (heparan mimetics)					
Pentosan polysulfate (PPS)	Stimulates in vitro conversion!	Reduces PrP^{Sc} levels in ScN2a cells	Increases survival time of hamsters and mice infected i.p. when given hours after infection; when administered intraventricularly also late after infection. Treatment of human vCJD under way	Interference with PrP–glucosaminoglycans interaction; stimulation of PrP^{C} endocytosis	143, 144, 155, 196–198

Table continued

TABLE 10–2 Representative Compounds Used in Attempts at Therapy of Prion Disease—cont'd

Compound	Cell-Free	Cell Culture	Animal	Proposed Mechanism	References
Dextran sulfate, HM2602		Reduces PrP^{Sc} levels in ScN2a cells	Increases survival time of hamsters and mice infected i.p. when given hours after infection	Interference with PrP–glucosaminoglycans interaction	132, 199, 200
Polyene antibiotics					
Amphotericin B	Modifies rafts	Reduces PrP^{Sc} levels in ScN2a and GT1 cells	Prolongs survival in 263 k-infected hamsters and mice also after late administration. Ineffective in human CJD		147, 149, 163, 201
MS 8209				Prevents uptake of prions in periphery?	202
Filipin	Disrupts lipid rafts	Reduces PrP^{Sc} levels in ScN2a cells		Disrupts lipid rafts, reduces endocytosis, causes release of PrP^{C} from cell surface	203, 204

TABLE 10–2 Representative Compounds Used in Attempts at Therapy of Prion Disease—cont'd

Compound	Cell-Free	Cell Culture	Animal	Proposed Mechanism	References
Polysulfonated small-molecular-weight compounds					
Suramin			Modest increase in survival time of i.p. inoculated hamsters	Misfolds PrPC at plasma membrane, endocytosis, intracellular retention and degradation	130, 143, 205
Congo red	Inhibits conversion, stabilizes PrP^{Sc}	Reduces PrP^{Sc} levels in ScN2a cells	Modest increase in survival time of i.p. inoculated hamsters	Inhibits conversion, stabilizes PrP^{Sc}	133, 200, 206, 207
Quinolines					
2,2′-biquinoline	Binds to $rPrP^{C}$	Reduces PrP^{Sc} levels in ScN2a cells (IC_{50} 1–100 nM)	Some extension of survival in i.c. inoculated mice after intraventricular infusion		129
Recombinant proteins					
PrP-Fc(2)	Binds to $rPrP^{Sc}$		Disease retarded in mice expressing the transgene		156

BBB, Blood-brain barrier; CJD, Creutzfeldt-Jakob disease; i.c., intracerebral; IC_{50},half maximal inhibitory concentration; i.p., intraperitoneal; vCJD, variant Creutzfeldt-Jakob disease.

for the copper chelator D-(-)-penicillamine in scrapie-infected mice[154]; 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^{Sc} deposition even when administered late after infection, whereas antimalarial drugs such as quinacrine showed no significant effect. At excessive doses, adverse effects such as hematoma formation were observed.[155] Intraventricular infusion of biquinoline derivatives also resulted in moderate extension of the survival period.[129]

A PrP-Fc_2 fusion protein that was found to compete with PrP^{C} for PrP^{Sc} had a protective effect against i.p. scrapie infection of mice when expressed from a transgene.[156] It will be interesting to determine whether PrP-Fc_2 is also active when delivered as a drug. If that proves true, soluble prion protein mutants may represent useful prionostatic compounds.

Attempts at Human Therapy

The earliest therapeutic attempts in 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.[157]

QUINACRINE

Quinacrine, chlorpromazine, and some tricyclic derivatives with an aliphatic side chain were described as efficient inhibitors of PrP^{Sc} formation in murine neuroblastoma cells chronically infected with the Chandler scrapie isolate.[158,159] Because quinacrine and chlorpromazine have been used in human medicine as antimalarial and antipsychotic drugs, respectively, and because they cross the BBB, they have been proposed as therapeutic agents for CJD patients.[159] No therapeutic effect was seen following quinacrine treatment of 20 patients[160] (A. Alperovich, quoted in reference 161), although some transient improvement was occasionally seen.[162] Subsequent animal experiments failed to demonstrate efficacy in the treatment of TSEs,[161] even after intraventricular infusion.[155]

AMPHOTERICIN B

Amphotericin B and some of its analogues were shown to delay the appearance of spongiosis, astrogliosis, and PrP^{Sc} accumulation in the brain of scrapie-infected hamsters.[145] However, an attempt to treat a CJD patient with amphotericin B remained unsuccessful.[163] In view of its high systemic toxicity, these results dampen any hopes that amphotericin B will prove useful in prion disease therapy.

PENTOSAN POLYPHOSPHATE

In late 2002, data were presented at two prion meetings and published recently,[155] suggesting that intraventricular administration of PPS to intracerebrally infected mice was effective at prolonging incubation time. PPS is marketed in certain

countries as a treatment for interstitial cystitis and as an anticoagulant, although its side effects include hemorrhage and hypersensitivity reactions.

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, Dame Butler-Schloss, heard the evidence of Doh-Ura, the Japanese researcher who had performed the animal studies; that of a neurosurgeon willing to administer the novel treatment; and the opinion of a number of respected neurologists who expressed reservations regarding this experimental treatment. Dame Butler-Schloss 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 those lacking capacity for personal decisions).[164] Treatment has been initiated and the patient experienced prolonged survival, albeit in a severely disabled state. A further patient treated with an almost 10-fold higher dose did not experience any improvement and died after 16 months of disease, which is not significantly different from the 14-month median survival of all vCJD patients.[208]

Physicians can thus come under pressure from the courts to allow new treatments to be used without having been tested in clinical trials, although from the ruling described previously, 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.

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