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Could immunomodulation be used to prevent prion diseases?

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Abstract

All prion diseases are currently without effective treatment and are universally fatal. The underlying pathogenesis of prion diseases (prionoses) is related to an autocatalytic conformational conversion of PrP^{C} (C for cellular) to a pathological and infectious conformer known as PrP^{Sc} (Sc for scrapie) or PrP^{Res} (Res for proteinase K resistant). The past experience with variant Creutzfeldt–Jakob disease, which originated from bovine spongiform encephalopathy, as well as the ongoing epidemic of chronic wasting disease has highlighted the necessity for effective prophylactic and/or therapeutic approaches. Human prionoses are most commonly sporadic, and hence therapy is primarily directed to stop progression; however, in animals the majority of prionoses are infectious and, as a result, the emphasis is on prevention of transmission. These infectious prionoses are most commonly acquired via the alimentary tract as a major portal of infectious agent entry, making mucosal immunization a potentially attractive method to produce a local immune response that can partially or completely prevent prion entry across the gut barrier, while at the same time producing a modulated systemic immunity that is unlikely to be associated with toxicity. A critical factor in any immunomodulatory methodology that targets a self-antigen is the need to delicately balance an effective humoral immune response with potential autoimmune inflammatory toxicity. The ongoing epidemic of chronic wasting disease affecting the USA and Korea, with the potential to spread to human populations, highlights the need for such immunomodulatory approaches.

Keywords

amyloid β; chronic wasting disease; conformational disorders; Creutzfeld Jakob disease; prion; vaccine

Bovine spongiform encephalopathy, variant Creutzfeldt–Jakob disease & their similarities to chronic wasting disease

Interest in developing potential therapeutics for prionoses has greatly increased since the emergence of bovine spongiform encephalopathy (BSE) and the resulting appearance of variant Creutzfeldt–Jakob disease (vCJD) in human populations, as well as the more recent

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epidemic of chronic wasting disease (CWD). BSE was first identified among cattle in the UK in 1985, with its emergence being related to the practice of feeding meat-and-bone meal from animal carcasses to cattle [1,2]. The original source of the BSE is unknown, but is presumed to be either a sporadic BSE case or a more infectious strain of scrapie. The rendering of BSE contaminated bovine carcasses into meat-and-bone meal amplified transmission which peaked in 1992. During that time, more than 3000 clinical cases per month were being recorded [2]. This led to the emergence of vCJD with the first case being recognized in 1996 [3]. Since the original report in 1995, a total of 225 probable or confirmed cases of vCJD have been diagnosed, 176 in Great Britain, 25 in France, five cases in Spain, four cases in Ireland, three in the USA and few cases elsewhere [201]. It has been difficult to predict the expected future numbers of vCJD. The most recent modeling suggests approximately 390 further cases between 2010 and 2179 [3,4]. Since the vCJD agent is present at high levels in lymphatic tissue, screening for PrP^{Sc} was performed in 2004 on sections from lymph nodes, tonsils and appendices archived in the UK. Three out of 12,674 randomly selected cases (237 per million) showed evidence of subclinical infection, leading to a prediction that approximately 4000 further cases of vCJD may occur in the UK [5,6]. A second study of anonymous tonsil biopsies from the 1961–1985 birth cohort, performed in 2008, found one positive follicle out of 9672 samples using immunohistochemical methods [7], while interim data from an ongoing study using archived appendix tissue revealed four positive cases from 13,878 patients, giving a prevalence result similar to the 2004 study (288 per million; see [202]) [8]. There is much uncertainty over future predictions of vCJD prevalence, since there is a lack of knowledge regarding the time of incubation, the number of patients who could be infected from a given dosage of BSE agent and other factors which can govern an individual's susceptibility to clinical infection. In addition, it is not known if the reported screening of lymphoid tissue has captured all subclinical cases and whether all subclinical infections will progress to disease. A further complicating factor for estimating future numbers of vCJD are several transfusion-associated cases, which occurred after incubation periods of 6–8 years. One of these disease transmitting donations was made more than 3 years prior to the donor becoming symptomatic, indicating that vCJD is transmissible from asymptomatic infected individuals [9]. Furthermore, using data collected from the UK National Creutzfeldt–Jakob disease (CJD) Research and Surveillance Unit, it is possible to conclude that each of the four positive appendix cases, in the ongoing survey of appendix tissue, obtained their infection from currently unidentified vCJD subjects. An additional factor to consider is the influence of the methionine/valine (M/V) polymorphism at codon 129 of the *PRNP* gene. Homozygocity at this codon is associated with greater susceptibility to infection. So far all the clinically symptomatic cases of vCJD had the methionine/methionine (M/M) codon 129 genotype. However, two clinically nonsymptomatic patients with the M/V genotype were found to be infected (one from a blood transfusion and the other was found to be positive from a random appendix and tonsil specimen survey of the population) [10,11]. The finding of such carriers raises the possibility of a secondary spread of infection via the transfusion of blood/plasma products, surgical procedures or tissue transplants from individuals with the codon 129 M/V genotype, who likely have a much longer (or possibly life-long) asymptomatic infection [12,13]. At present, no effective method exists for screening blood for vCJD contamination [14,15], although such assays are in development [16,17]. Therefore, the risk of further cases of vCJD occurring due to blood transfusion remains a possibility. In the USA, cases of vCJD acquired elsewhere have been documented; hence, there is the possibility that asymptomatic carriers are donating to the US blood supply, particularly as surveillance methods are limited [18,19]. Furthermore, several atypical strains of scrapie (atypical/Nor98 scrapie) and BSE (higher and lower [BSE-H and BSE-L, respectively]) have been documented, which may be more easily transmissible to humans [20,21]. Similarities in the western blot pattern of atypical BSE PrPRes to a less common type of sporadic CJD (type MV2) have led to the suggestion that this form of CJD may have an infectious origin [20,22]. Another study noted the presence of approximately

14 and 7-kDa fragments of PrPRes in BSE-H, which are similar to those found in some CJD cases [23]. Hence it is possible that a percentage of CJD cases thought to be sporadic are in fact of infectious origin. Consistent with this hypothesis, it has been shown that BSE-L is transmissible to nonhuman primates (cynomolgus macaque monkeys) [24] and to transgenic mice expressing either normal or elevated levels of human M129 PrP with a higher transmission rate than that observed with classical BSE [21,25], suggesting that there is no significant species barrier between BSE-L in cattle and humans.

CWD appears to be the most infectious prionoses to date, affecting free-ranging and farmed ungulates (white-tailed deer, mule deer, elk and moose) [26–29]. CWD was first described in 1967 and was recognized to be a prion disease in 1978 on the basis of brain histopathology [26,30,31]. CWD has been detected in 19 states of the USA, three Canadian provinces and in South Korea [29]. Up to 90% of captive cervids have been reported to be prion positive, while in the wild the prion-infection prevalence has been reported to be as high as 50%. Transmission of CWD is mainly horizontal via the mucosal/oral route [29,32,33]. A report of sporadic CJD among three deer hunters from the same region, who were younger than typical sporadic CJD patients, raised the speculation of transmission of CWD to humans [34]. However, autopsy of these three subjects did not show the characteristic extensive PrP^{Res} amyloidosis as seen in vCJD and CWD [35]. On the other hand, CWD has been shown to be transmissible to nonhuman primates (squirrel monkeys) by two groups using intracerebral inoculation [36,37]. Significantly, the more recent study also showed CWD to be orally transmissible to squirrel monkeys [37]. Interestingly the clinical symptoms of CWD infection in CWD-infected monkeys resembled a wasting syndrome rather than more typical symptoms such as ataxia, this leads to the speculation that if CWD is transmitted to humans and presented in a similar manner, the disease may not be identified as a prion disease without neuropathological evaluation (something which is done on a very small proportion of patient deaths) [37]. CWD has also been shown to be transmissible to sheep, cattle, fallow deer and several North American rodents (prairie voles, mice and ferrets) that can scavenge on CWD carcasses [38–42]. These animals can enter the human food chain directly or indirectly by accidental inclusion in grain and forage. Large predators of cervids in the wild are not surprisingly preferentially killing incapacitated CWD-infected animals, raising the possibility of further cross-species spread [43]. Thus far, studies using transgenic mice expressing human PrP^{C} have failed to show transmission of CWD, indicating that this presents a more significant species barrier compared with BSE to human transmission [44–46]. On the other hand, two different strains of CWD have recently been identified with the likelihood that there are several more [47]. The possibility that these other strains of CWD have greater potential for human spread remains to be explored. To assess this risk, transmission studies using nonhuman primates and human PrP expressing Tg mice will need to be repeated with all known CWD strains. These future studies will also need to take into account the multiple PrP polymorphisms, which influence transmission rates, in the CWD inoculum and the host human PrP [48,49]. PrP^{CWD} has been found not only in the CNS of infected deer, but also in virtually all biological material including: blood, muscle, feces, fat, urine, antler velvet and saliva [33,50–56]. Therefore the possibility of transmission to humans needs to be closely monitored. A recent study using *in vitro* protein misfolding cyclic amplification showed that CWD PrP^{Res} can convert human PrP^{C} after the CWD prion strain was stabilized by passage with cervid PrP^{C}; this highlights the potential of CWD prions to infect humans [57]. The risk of transmission to humans of CWD is difficult to gauge [44]. The prevalence of CWD in free-range deer varies from up to 50% in some endemic areas to <1% in states in which CWD has only recently been discovered, such as West Virginia and New York (USA) [26,28,29]. It is estimated that over 6.6 million deer and 6.9 million cervid species are killed annually in the USA; hence, it is certain that human exposure has occurred and continues to occur, by direct contact with hunters and game processors, through the consumption of venison, and by contact with products from

cervids. An additional factor to consider is that the preclinical period of human prion infection via an oral route can be very long; in the case of kuru (a human prionoses related to endocannabolism) an incubation period of 56 years was documented [58]. In contrast to the distribution of BSE-infected beef, which would typically be greatly diluted in the food processing chain, it is more frequent that only a few family members and friends consume venison from a CWD-infected animal, thus producing exposure to a proportionally greater inoculum of PrP^{Res}. This increased risk of exposure is specific to hunters/consumers of deer, rather than the general population. CWD exposure to both humans and farm animals may also occur from contaminated environmental sources; however, there are no data available to estimate the extent of such exposure. The CWD agent is extremely stable in the environment, where it readily binds to soil [59–61] and has even been detected in the water of CWD-endemic areas [62]. Binding to certain types of soil has been shown to dramatically enhance CWD transmission [59,63]. The likely exposure of humans to CWD-infected tissue is difficult to estimate. In a 2006–2007 population survey conducted by FoodNet in the USA, it was found that of 17,373 survey responders 18.5% had hunted deer or elk, and 1.2% had hunted deer or elk in CWD-endemic areas [64]. Of the 11,635 responders who had consumed venison, 88.6% had obtained it from the wild [64]. Hence it is likely a large population is being exposed to CWD-infected food. A significant findings is that CWD can transmit nasally, with high efficacy, as an aerosol among cervid PrP transgenic mice [65]. This represents the first documentation of prion spread via this respiratory route; although, a subsequent study has shown that other prionoses may also have some limited ability to spread by aerosol [66]. Hence, if CWD were to cross the species barrier to humans, it would pose a major threat, likely far greater than vCJD, highlighting the need to develop better vaccination/immunomodulation approaches to prevent CWD transmission and uncontrolled spread. The development and testing of such potential approaches are discussed below.

The immune system & prion infection

PrP^{C} is expressed in T and B lymphocytes, NK cells, platelets, monocytes, dendritic cells (DCs) and follicular DCs (FDCs) [67]. This expression pattern and the lack of an immune response to a self-antigen, has resulted in the immune system being an active player very much involved in the peripheral and asymptomatic replication of the prion agent, and its eventual access to the CNS, which is associated with the clinical manifestations of prion infection [68,69]. Numerous studies have shown that immune suppression with, for example, splenectomy or immunosuppressive drugs, increases the incubation period [68], while nonspecific immunostimulation has the opposite effect [70]. This asymptomatic incubation period, during which time the prion agent replicates peripherally, can be quite long, lasting many months in experimental animals and up to 56 years in documented human cases associated with cannibalistic exposure to the prion agent [58]. In humans the duration of peripheral prion replication is not known in the absence of significant neuroinvasion. This critical period would be the time when various antiprion therapeutic agents, which do not cross the blood–brain barrier, would have a chance of success. Lymphatic organs including the spleen, tonsil, lymph nodes and gut-associated lymphoid tissue contain high concentrations of PrP^{Sc} long before PrP^{Sc} replication starts in the brain [32,71,72]. Immune cells found to be particularly important for peripheral PrP^{Sc} replication include the FDCs and the migratory bone-marrow derived DCs [32,72–75]. DCs from infected animals have been demonstrated to be capable of spreading the disease when passively transferred [74,75]. It is hoped that immunotherapeutic approaches that overcome the self tolerance of these immune cells will inhibit prion replication in the lymphorecticular system and ultimately prevent or delay significant neuroinvasion. However, a delicate balance has to be achieved in order to produce a qualitative immune response, while avoiding potential autoimmune inflammatory toxicity [76–78]. A further complication is that while in the majority of prion disease, infection and replication in the lymphorecticular system shortens

the incubation time and facilitates CNS invasion, this does not appear to be the case in most BSE cases, in sCJD and in some types of scrapie such as the drowsy form of hamster scrapie [79–81]. The potential beneficial effect of altering the immune response to PrP would therefore have to be tailored to each particular PrP^{Res} strain and may require an immune response within the CNS as well as peripherally in some cases. That such a goal is achievable is suggested by data from studies in Alzheimer's disease (AD) model animals and AD patients [82–87]. AD is another conformational neurodegenerative disorder where the pathogenesis is related to the accumulation of self proteins in an abnormal conformation within the CNS; in the case of AD these proteins are amyloid β (Aβ) and tau, which accumulate as toxic oligomers, as well as amyloid plaques and neurofibrillary tangles, respectively [88]. A number of different immune-modulating approaches with effects within the CNS have been shown to ameliorate this pathology [82–85,89–91].

In vitro studies

A precise understanding of the molecular mechanisms and pathways involved in the PrP^{C} to PrP^{Sc} conversion remains to be elucidated; however, there is abundant evidence of the primal importance of 'seeding' by aggregated PrP^{Sc} molecules acting as a template for PrP^{C} binding and the subsequent conversion to more PrP^{Sc} [92,93]. This interaction is critically dependent on the correct stereochemistry as supported by the existence of a species barrier for prion infection, related to minor differences in the primary sequence of PrP^{C} in different species. For example, it has recently shown that amino acid polymorphisms at positions 170 and 174, in the β2–α2 loop, are critical for transmission within and between species [94–96]. It is not surprising that antibodies that may alter or mask the critical epitopes on PrP^{C} and/or PrP^{Sc}, involved during the mutual conformational complementarity, required in prion propagation, will be inhibitory for prion replication. This was initially demonstrated in 1988 when an anti-PrP^{C} polyclonal antibody was used *ex vivo* on a prion preparation prior to inoculation; a 2-log reduction in infectivity was noted [97]. Using scrapie-infected cells it was later shown that an anti-PrP mAb, 6H4 directed to residues 144–152, was able to clear infection *in vitro* [98]. In the same year, Peretz *et al.* used a number of different PrP specific Fab fragments for scrapie clearance in chronically infected N2a cells [99]. They found D13 (directed to residues 95–103) and D18 (directed to residues 132–156) to be the most effective at scrapie clearance [99]. Kim *et al.* generated a large panel of antibodies raised to either recombinant mouse PrP or purified mouse PrP^{Sc} in PrP knockout mice and tested them therapeutically in a N2a scrapie-infected cell line [100,101]. They found that all anti-PrP antibodies that were able to bind to PrP^{C} on the cell surface, as judged by flow cytometry, were able to inhibit prion infection. We also tested a panel of anti-PrP mAbs to different epitopes of PrP in scrapie infected N2a cells and found the most effective to be 6D11, which is directed to residues 95–105 (hence similar to D13); however, antibodies directed to residues 130–140 and 143–155 were also quite effective [102]. These various studies suggest that therapeutic antibodies need to have high affinities of binding to PrP^{C} and/or PrP^{Sc}, as well as targeting specific critical PrP domains. We have also shown that 6D11 is able to inhibit prion infection *in vivo*, with a very significant prolongation of the scrapie incubation period and a reduction of the severity of symptomatic disease; however, all treated animals eventually succumbed to infection [102]. Interestingly, it has been reported that deleting residues 32–134, in the PrP molecule, but not residues 23–134, produces symptoms of neuropathology; suggesting that residues 23–31 (KKRPKPGGW), a positively charged segment, are required for a toxic phenotype [103]. This segment is involved in binding glycosaminoglycans that may inhibit toxicity most likely by interfering with a membrane-associated target site, consistent with the hypothesis that chaperones or facilitator molecules that interact with different parts of the PrP molecule are involved in its conformational transitions. Given the fact that so many parts of the PrP molecule are involved in different stages of the PrP^{C} to PrP^{Sc} conformational change, it is likely that in

the more complicated *in vivo* situation, an optimal strategy will be the concurrent use of two or more anti-PrP mAbs (or derivatives thereof) which target a number of the critical regions for the PrP^{C} to PrP^{Sc} conversion. This greater difficulty of obtaining *in vivo* therapeutic effects with passive immunization is highlighted by some subsequent studies where anti-PrP antibodies which were able to clear PrP^{Sc} infection in tissue culture, only produced a minimal (but statistically significant) delay in the incubation period when used *in vivo* [104].

Passive immunization for prion infection

In an initial passive anti-PrP immunization study using wild-type CD1 mice we showed using mAbs 8B4 (to mouse PrP residues 34–52) and 8H4 (to mouse PrP residues 175–185) given immediately after challenge with 139A scrapie by intraperitoneal injection (50 μg/week), that this resulted in a significant prolongation of the incubation period with 10% of the 8B4 treated animals remaining disease free in the group challenged with a lower dose of PrP^{Sc} [105]. In another study using higher doses (4000 μg/week intraperitoneally) of either ICSM 18 (to mouse PrP residues 146–158) or ICSM 35 (to mouse residues 95 to 105), it was shown that prion infection from a peripheral source could be completely prevented if treatment was continued for either 7 or 30 days immediately following PrP^{Sc} challenge [106]. This approach could be used immediately following accidental exposure in humans to prevent future infection. Unfortunately, passive immunization has not been found to be effective closer to the clinically symptomatic stages of prion infection. Furthermore, passive immunization would be too costly for animal prion diseases. As discussed above, passive immunization has also been shown to be effective at inhibiting prion infection when initiated immediately or within 30 days after peripheral prion infection [105–107]. Most likely, the lack of greater therapeutic efficacy of these passive immunization approaches is linked to the poor blood–brain barrier permeability of anti-PrP antibodies. Importantly, Song *et al.* demonstrated therapeutic efficacy with anti-PrP antibodies up to 120 days post inoculation, using direct intraventricular infusion [108]. However, a caveat with such an approach is that neuronal apoptosis has been reported to occur in response to some anti-PrP antibodies applied directly to the CNS [109,110], indicating that the specific characteristics of of binding, epitope) will be the antibodies to be used (e.g., K_D important factors determining the probability of toxic side effects. Therapeutically effective anti-PrP antibodies which are specific for PrP^{Res} would have less potential for autoimmune toxicity versus anti-PrP antibodies that target both PrP^{C} and PrP^{Res}. However, PrP^{C} remains a therapeutically viable therapeutic target as demonstrated by studies which have ablated expression of PrP^{C} in the presence of early spongiform pathology, resulting in reversal of this pathology without toxicity [111,112].

Active vaccination for prion infection

Using AD-model mice it has been definitively demonstrated in numerous studies that active immunization can prevent the onset of cognitive deficits and the development of amyloid lesions [84]. Importantly, this method of treatment is associated with consistent cognitive benefits in the mice [83,113–117]. A humoral immune response is critical for a therapeutic response, since similar results have been obtained with passive immunization in animal models [118]. Active immunization was initially tried in humans for AD by Elan, with significant toxicity resulting from the vaccine [119–121]. In the Phase IIa clinical trial of this AD active vaccine (called AN-1792), 18 out of 372 patients worldwide developed symptoms of meningitis or meningoencephalitis, with symptoms apparently responding to immunosuppression in the majority of patients (12 patients out of the 18 responded fully) [82,119]. Patients who developed anti-Aβ titers benefited cognitively from vaccination, including patients among the 12 that initially had complications, although these benefits were relatively modest [82,119,122]. The absence of more dramatic clinical benefits in these patients may be related to vaccination being initiated too late in the progression of the

disease process [82,123]. The limited autopsy data documented that vaccination resulted in both amyloid clearance and a reduction in tau-related pathology [82,124,125]. Therefore, if future protocols can resolve these important safety issues and, more effectively, time the point at which treatment should begin, such a vaccine approach will more likely to have a significant cognitive beneficial effect in patients [120,126]. A number of AD vaccination clinical trials are currently ongoing [82,127,128].

Partly due to this success in AD models, analogous experiments with anti-PrP antibodies were initiated in prion infectivity culture models, as well as active and passive immunization studies in animal prion models. The first *in vivo* studies of an active immunization-like approach had shown that challenge with a slow strain of PrP^{Sc} blocked the latter expression of a more virulent fast strain of PrP, mimicking vaccination with a live attenuated organism [129]. We first demonstrated that active immunization with recombinant PrP delayed the onset of prion disease in wild-type mice; however, the therapeutic effect was very modest and eventually all the mice succumbed to the disease [130]. The limited therapeutic effect could be explained by the observation that the antibodies generated against prokaryotic PrP often do not have a high affinity towards the critical portions of PrP^{C} that are involved in binding and replication and that the anti-PrP titers generated were low [131]. However, the anti-PrP^{C} titers correlated well with the prolongation of the incubation period. Other groups attempted to use active immunization in wild-type animals to prolong the incubation period, with some failing to break immunological tolerance and produce a therapeutic response [131], while in other studies active immunization was verified to have a partially therapeutic effect [132–135]. These conflicting results reflect methodological differences in terms of the immunogens, immunization methods, adjuvants, animal models and PrP^{Sc} strains used for challenge, as well as highlighting the difficulty in breaking tolerance to a self protein. The likelihood that immunological approaches, which reduce levels of PrP^{C} or limit its availability for conversion to PrP^{Sc}, may be more effective closer to symptomatic disease is consistent with another study. Here it was shown that the cognitive deficits, impaired neurophysiological function and characteristic early hippocampal spongiform pathology of prion infection could be completely reversed by genetically knocking out the expression of endogenous neuronal PrP^{C} [111].

A synthesis of the above data indicates that a departure from a classical vaccination approach and the use of some specific properties of antibodies would make it possible to interfere with one or more stages of the initiation or progression of prion disease. A paramount issue is that the self nature of PrP has to be accounted for. Specifically, immunomodulation for prion disease has to overcome tolerance to the PrP^{C} in order to raise antibodies that will interfere with PrP critical binding sites that are involved with one or more of the following functions: facilitation of invasion, the PrP^{C} to PrP^{Sc} interaction or interactions with host factors. Furthermore, any humoral or cellular autoimmune inflammatory effect has to be avoided or minimized. A fine balance between quality and quantity of therapeutically active immunoglobulin molecules has to be accomplished without knowing *a priori* what would be the ideal nature of the required immunogen and what may be the best route to present it to the immune system.

A potentially ideal means of using immunomodulation to prevent prion infection is by utilizing mucosal immunization. The most obvious reason for using this approach is that the gut is the major route of entry for many prion diseases such as CWD, BSE, transmissible mink encephalopathy and the initial cases of vCJD (future cases of vCJD are more likely to be related to other routes such as blood transfusion). In addition, mucosal immunization can be designed to primarily induce a humoral immune response with a secretory IgA response in the gut, avoiding the cell-mediated inflammatory response that was the major cause of toxicity in the initial human AD active vaccine trial. This type of secretory IgA response can

prevent entry of the prion agent into the body and has a greater potential to be highly effective, despite the relatively low anti-PrP antibody titers which can be generated. We have developed prion vaccines that target gut-associated tissue, the main site of entry of the prion agent. One approach has been to express PrP in attenuated *Salmonella* strains, where one or more genes responsible for virulence have been deleted, as a live vector for oral vaccination. Live attenuated strains of *Salmonella enterica* are very well characterized and have been used for many years as vaccines against salmonellosis in humans, as well as serving as a delivery system for the construction of multivalent vaccines with broad application in both human and veterinary medicine [136,137]. A significant advantage for this system is that the safety of human administration of live attenuated *Salmonella* has been extensively confirmed in humans and animals, in whom it has been shown to be able to penetrate the gut mucosa and specifically deliver protein products to immune presenting cells in lymphoid follicles [137–139]. A variety of animals have been effectively immunized by an oral route using live *Salmonella*, to induce humoral mucosal responses [140,141]. *Salmonella* targets M-cells, antigen sampling cells in the intestines, which importantly may also be critical for PrP^{Sc} uptake [72,76,142]. Therefore, this approach is more targeted than prior vaccination studies, providing a possible explanation for the improved efficacy [77,143]. The *Salmonella* vector can also express one or several repeating copies of PrP, producing and delivering a protein product that might simulate the three-dimensional sites critical for the PrP^{C} to PrP^{Sc} interaction [77]. This approach takes into account that if tolerance is broken, the majority of the B-cell response will be devoted to producing dimeric secretory IgA in the mucosa, with a more limited (in comparison to a conventional vaccination methodology) systemic IgG level, which will help to maintain an optimal level of anti-PrP systemic antibodies with a low risk of autoimmune pathology. With a mucosal immunization methodology the V regions selected for recombination, within the mesenteric lymphoid tissue, to produce neutralizing IgA are likely very distinct from a humoral response obtained systemically. Our past data, using 139A scrapie prions in wild-type CD-1 mice indicate that in animals that have a significant anti-PrP mucosal IgA response and a systemic anti-PrP IgG response, full protection against oral challenge with the PrP^{Sc} is possible [77,143]. Further refinement of mucosal immunization, aiming for greater specificity to critical epitopes rather than high anti-PrP levels, is likely to lead to an effective means of preventing prion disease in animal and human populations at risk for prion exposure. Because a fraction of the antibodies raised in a mucosal immunization will be effective at neutralizing PrP^{Sc} invasion, binding, conversion or progression; successfully vaccinated animals may be used to produce monoclonal antibodies with therapeutic potential for passive immunization. Such therapeutic monoclonal antibodies can be humanized and used to prevent infection following accidental exposure or progression of ongoing disease. Current evidence indicates that more than one monoclonal antibody would be needed to obtain full protection. Such an approach is being pursued in our laboratory, as well as by other groups.

Mucosal immunization to prevent CWD in white-tailed deer is ongoing. Similar to our experiments in mouse models of scrapie infection, we have used attenuated *Salmonella* strains; however, in this case they express deer PrP. The animals have been orally inoculated numerous times, including tonsil and rectal inoculations with the vaccination supplemented with polymerized recombinant deer PrP, which was produced by cross-linking PrP as described for the ABri peptide [117]. Control animals were given an attenuated *Salmonella* not carrying any foreign protein [144]. As expected, both groups of animals produced high levels of IgA anti-*Salmonella* in their plasma, saliva and feces. Significantly, the vaccinated group had a low titer of anti-PrP IgG and IgA in the plasma, as well as anti-PrP IgA in the saliva. The deer immunoglobulins were precipitated from plasma, saliva and feces and semi-purified. This purified IgG and IgA from deer vaccinated with *Salmonella*-expressing deer PrP reacted on western blots strongly against polymerized deer PrP, as well as *Salmonella*

antigens and to a lesser extent to monomers and dimers of mouse and sheep recombinant PrP. Control deer showed a reaction only against the *Salmonella* antigens in matching blots. Both deer groups were challenged with homogenized brains of terminal CWD deer orally via food bait. These preliminary results indicate for the first time that specific antibody responses against the self-antigen PrP can be produced in the biological fluids (gut and plasma) of large cervid mammals [144]. Quantitative results of infection progression will be available over the next year.

An alternative active immunization approach, which also has encouraging preliminary results, is to induce active immunization which specifically targets PrP^{Sc} or the shared β-sheet rich conformation of the pathological conformers/oligomers found in prionoses and other neurodegenerative diseases. One such approach is based on the hypothesis that in PrP^{Sc} certain epitopes are exposed, normally buried in PrP^{C}. Prior studies have shown that the conversion of recombinant PrP to a more PrP^{Sc}-like state is associated with increased exposure of tyrosine side chains [145,146]. Use of a peptide based on these exposed epitopes was tested in sheep and it gave rise to a PrP^{Sc} selective IgG immune response [147]. Another possible immunomodulatory method specifically targets pathological β-sheet rich conformations (of PrP^{Res} or other toxic species such as Aβ), by immunizing with a polymerized British amyloidosis related peptide which has no sequence homology to PrP, amyloid β or other human proteins. We demonstrated that the pABri peptide via a conformational mimicry induces a humoral immune response that recognizes both Aβ oligomers and neurofibrillary tangles – lesions that characterize AD [117]. This immunogen was able to cognitively rescue AD-model mice [117] and in human PrP-expressing transgenic mice, was able to induce an immune response to PrP [Wisniewski T, Goni F, Unpublished Data]. This type of immunomodulation has the potential to induce a humoral immune response to pathological conformers without the risk of an autoimmune response using a nonself immunogen, without any risk of autoimmune toxicity.

Expert commentary

Currently none of the conformational neurodegenerative disorders has a highly effective therapy. Numerous studies using AD models have shown that immunotherapeutic approaches can greatly reduce amyloid and tau-related pathology, resulting in a significant cognitive rescue. Recent autopsy and imaging data from human trials is also suggestive that this approach can ameliorate both AD plaque and tau pathology. Prion diseases are much less common than AD; however, the past outbreak of vCJD, originating from BSE, and the ongoing CWD epidemic, with its potential for human transmission, highlights the importance of developing therapies for this group of disorders. The specific self-replicating ability of the pathological PrP^{Sc} to convert physiological PrP^{C} depends on features present in different parts of the protein. Extensive *in vitro* and *in vivo* data using prion infection models have shown that immunomodulation is effective at preventing infection. Because many prion disease have the mucosa of the alimentary tract as a point of entry, mucosal immunization is particularly suitable for these forms of prion infection, with recent studies suggesting that prion infection via an oral route can be prevented by appropriate mucosal vaccination. This approach may be particularly suitable to stem the current epidemic of CWD with its specter of potential spread to large human populations. In the future, immunomodulation could also be the basis of delaying the onset, or preventing the progression, of known familial prionoses and the treatment of sporadic CJD (if methods for presymptomatic diagnosis are developed), provided that an immune response could be stimulated in the CNS targeting PrP^{Res} without inducing toxicity. A promising immunomodulatory therapeutic approach is the specific targeting of the PrP^{Sc} conformation or the shared β-sheet-rich pathological conformation that is found in toxic oligomers that are central to the pathogenesis of many neurodegenerative conditions.

Five-year view

It is possible that in the next 5 years initial cases of a novel human prion disease related to CWD will occur. Despite the considerable species barrier between cervids and humans, the large numbers of affected animals and the likely substantial ongoing human exposure, with relatively little monitoring, makes such an event possible in the near term future. It is unclear if such a new disease were to emerge, whether initial cases will be recognized as a prion disease given the atypical wasting syndrome symptoms seen in squirrel monkeys infected with CWD [37]. Such a zoonosis may emerge either directly from cervids or via an intermediate host such as cattle or sheep. This would be consistent with data showing that CWD can be passaged to nonhuman primates, as well as, cattle and the ability of PrP^{CWD} to convert human PrP to PrP^{Res} *in vitro* using protein misfolding cyclic amplification. What the properties of such a new human prion disease will have is difficult to predict. It would be hoped that such a human prionosis would not be as infectious as CWD is among cervids. Such an event would highlight the need for effective therapies for prion diseases, such as immunomodulation.

Initial trials of mucosal immunization to prevent prion disease in sheep and deer have been recently initiated. Within the next 5 years, data will be available to determine if such approaches are effective. It is also likely that anti-PrP antibodies and/or anti-pathological conformation targeting antibodies, which are effective at preventing or delaying prion disease in tissue culture and in animal models, will be humanized and engineered for nontoxicity to be tested in passive immunization trials for humans with CJD and familial prion disease.

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Key issues

- There is currently no effective therapy for any prion disease.
- Emerging data using tissue culture and animal models of prion infection have shown that some anti-PrP antibodies given passively, or produced by active immunization, are able to prevent or prolong the incubation period of prion infection.
- Currently there is an epidemic of a relatively newly recognized animal prion disease, chronic wasting disease, in the USA that has the potential to spread to human populations.
- Typically, most animal prion disease, such as chronic wasting diseases, spread via an oral route.
- Recent studies have shown that mucosal immunization can prevent prion infection via an oral route in a significant proportion of challenged animals.