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Is vaccination against transmissible spongiform encephalopathy feasible?

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Summary

Prion diseases are a unique category of illness, affecting both animals and humans, where the underlying pathogenesis is related to a conformation change of the cellular form of a normal, self-protein called a prion protein (PrP^{C} [C for cellular]) to a pathological and infectious conformation known as scrapie form (PrP^{Sc} [Sc for scrapie]). Currently, all prion diseases are without effective treatment and are universally fatal. The emergence of bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease has highlighted the need to develop possible therapies. In Alzheimer's disease (AD), which has similarities to prion diseases, both passive and active immunisation have been shown to be highly effective at preventing disease and cognitive deficits in model animals. In a human trial of active vaccination in AD, despite indications of cognitive benefits in patients with an adequate humoral response, 6% of patients developed significant complications related to excessive cell-mediated immunity. This experience highlights that immunotherapies designed to be directed against a self-antigen have to finely balance an effective humoral immune response with potential autoimmune toxicity. Many prion diseases have the gut as a portal of infectious agent entry. This makes mucosal immunisation a potentially very attractive method to partially or completely prevent prion entry across the gut barrier and to also produce a modulated immune response that is unlikely to be associated with any toxicity. The authors' recent results using an attenuated *Salmonella* vaccine strain expressing the prion protein show that mucosal vaccination can partially protect against prion infection from a peripheral source, suggesting the feasibility of this approach.

Keywords

Bovine spongiform encephalopathy – Chronic wasting disease – Conformational disorder – Mucosal vaccine – Prion – Salmonella – Transmissible spongiform encephalopathy – Variant Creutzfeldt-Jakob disease.

Introduction

Prion disease occurs both in humans and in various animals such as cows, sheep, goats, mink, deer and elk. These diseases are also known as transmissible spongiform encephalopathies or prionoses. They are a unique category of illness in that they can be infectious or transmitted genetically and are sporadic in occurrence. Abundant evidence has made it clear that these slow infections are neither caused by a virus nor any nucleic acid containing particle. A comprehensive body of evidence has presented compelling data that the transmissible pathogen for these diseases is a proteinaceous infectious particle (hence the term 'prion') (37, 38). All prion diseases result from a conformational alteration of the same host-derived prion protein (PrP^{C} [C for cellular]) to a disease-associated conformer called PrP^{Sc} (Sc for scrapie). This conversion can be precipitated by an exogenous, infectious source of PrP^{Sc}, a mutation in the prion protein that predisposes to such a conformational change, or a spontaneous conformational change, as occurs in sporadic prion disease.

The human forms are kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease (GSS) and fatal familial insomnia. In animals these diseases include bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep and goats, chronic wasting disease (CWD) in deer and elk and transmissible mink encephalopathy (42). Neuropathologically, these different forms of the disease are all characterised by spongiform change, neuronal loss and astrocytosis; in addition amyloid deposition may occur. However, the regional pattern of brain lesions and the extent of prion amyloid deposition vary within and between species. Within species, these differences depend on the strain of prion causing the infection. A barrier exists limiting transmission of prions across species, but once this barrier is overcome a new, stable and distinct pattern of infection can develop in the new host species.

Bovine spongiform encephalopathy, variant Creutzfeldt-Jakob disease and chronic wasting disease

Interest in prion disease has greatly increased since the emergence of BSE in the United Kingdom (UK) and the resulting appearance of variant CJD (vCJD) in human populations. Bovine spongiform encephalopathy arose from the feeding of cattle with prion-contaminated meat and bone meal products, while vCJD developed following entry of BSE into the human food chain (8). Since the original report in 1996 (60) a total of 182 confirmed cases of vCJD have been diagnosed, 156 in the UK, 17 in France, 3 cases in Ireland and one each in Italy, Canada, Japan, the Netherlands, Saudi Arabia and the United States of America (USA). The patients from these countries resided in the UK during a key exposure period of the population to the BSE agent. It has been difficult to predict the expected future numbers of vCJD. Mathematical analysis has predicted that between 1,000 and 136,000 individuals will eventually develop the disease. This broad range reflects a lack of knowledge regarding the time of incubation and the number of patients who could be infected from a given dosage of BSE agent. Because the vCJD agent is present at high levels in the lymphatic tissue, screening for PrP^{Sc} was performed on sections from lymph nodes, tonsils, and appendices taken from archives in the UK. Three out of 12,674 randomly selected samples showed evidence of subclinical infection, leading to a prediction that about 4,000 further cases of vCJD may occur in the UK. However, there is much uncertainty about such a predication, as it is not known if all subclinical infections will progress or whether such screening of lymphoid tissue would capture all subclinical cases. The initially predicted epidemic of vCJD does not seem to be materializing, as the number of cases in the UK has declined from a peak of 28 in 2000 to 17 in 2002, with only 5 cases in 2005 (8). A complicating factor for estimating future numbers of vCJD is the occurrence of several transfusion-associated cases. These occurred after incubation periods of 6 to 8 years. One of these disease-associated donations was made more than 3 years before the donor became symptomatic, suggesting that vCJD can be transmitted from silently infected individuals (11). The estimated risk for new cases of vCJD in other European countries is much lower. In the UK, 200,000 cases of BSE were reported (it is estimated that four times this number entered the food chain), compared to a combined total of approximately 500 BSE cases in other European countries. This suggests a significantly lower exposure of these populations to BSE prions. A few cases of BSE have also been reported in other parts of the world, such as Japan, the USA and Canada.

Of greater concern in North America is CWD. This disease is now endemic in Colorado, Wyoming and Nebraska and continues to spread to other parts of the USA. Cases have been reported in the Midwest and it has now been detected as far east as New York State (61). Most vulnerable to CWD infection are white-tailed deer, and the disease is now found in areas with large populations of these animals, which indicates that its prevalence can be expected to increase substantially in the future. Occurrence of CJD among three young deer hunters raised speculation that CWD could be transmitted to humans (7), but autopsy of these three subjects did not show the extensive amyloidosis characteristic of vCJD and CWD (25). However, like BSE, CWD is transmissible to non-human

primates and transgenic mice expressing human PrP^C (41, 54, 58). Therefore, the possibility of such transmission needs to be closely monitored. Chronic wasting disease is similar to BSE in that the peripheral titres of the prion agent are high. PrP^{Sc} has been detected in both the muscle and saliva of CWD-infected deer (1, 30).

Biology of the prion protein

PrP^C is expressed in many types of cells; however, the highest level of expression is found in central nervous system (CNS) neurons (21, 24). A knowledge of the molecular anatomy of PrP^C is crucial for understanding its malfunction in prion diseases. The whole protein is located on the outer surface of the cell anchored to the cell membrane by phosphotidylinositol glycolipid (GPI) attached to its C-terminus. The central portion of the peptide contains one short α-helical segment (α-helix A) flanked by two short β-strands. The N-terminus is unstructured and extends into the intracellular space. The N-terminus harbours five octapeptide repeats. Histidines located within the octapeptides bind copper ions (9). It has been postulated recently that the possible function of PrP^C is to capture, store, and present copper to the neuron (9, 39, 40). The copper binding state of PrP^C influences its conformation and copper chelation has been shown to inhibit PrP^{Sc} infection (48). The exact function of PrP^C remains to be elucidated. The protein is not essential since Prnp knock-out mice (12) did not show a significant disease phenotype. Minor abnormalities in synaptic physiology (14) and in circadian rhythm (55) have been described in these knock-out mice.

Prion diseases and other conformational disorders

The prion diseases belong to a broader category of conformational diseases (43). The etiology of each of the conformational diseases is related to a specific protein that can exist in at least two distinct forms associated with either health or disease. The most common conformational disorder is Alzheimer's disease (AD), in which the disease state is associated with the accumulation of an endogenously expressed peptide, the amyloid-β peptide, in a β-sheet structure within neuritic plaques. Other conformational disorders include Parkinson's and Huntington's diseases. The pathological conformer of PrP^C is PrP^{Sc}, which due to its increased β-sheet content demonstrates increased resistance to proteolysis and the ability to aggregate and polymerize. Although the insolubility of PrP^{Sc} has prevented crystallographic conformational studies, less exact structural methods such as circular dichroism and Fourier transform infrared spectroscopy indicate a β-sheet content as high as 45% (compared with 3% in PrP^C) and a α-helix content of 30% (40% in PrP^C) (3).

Understanding the mechanism that converts PrP^C into PrP^{Sc} is another intriguing aspect of prion diseases. One of the most crucial features of PrP^{Sc} is its ability to bind to PrP^C: this initiates a self-perpetuating vicious cycle and enables prion diseases to be transmitted (38). It has been demonstrated in cellular models that the PrP is transported to the membrane in the PrP^C form and that the conversion of PrP^C to PrP^{Sc} occurs at the cell surface. Neurons produce native PrP^C (24) and transport it to the cellular surface where it can encounter PrP^{Sc}, leading to its conformational change into a high β-sheet content state. During progression of the disease, the amount of PrP^C produced remains stable, whereas the amount of PrP^{Sc} increases. The homozygosity of PrP^C facilitates prion replication. This has been observed in humans with respect to the codon 129 polymorphism, as well as in sheep with respect to the VRQ/VRQ polymorphisms. Evidence from transgenic animals expressing various segments of PrP^C indicates that residues 90-150 are required for the interaction with PrP^{Sc} leading to conversion of PrP^C into PrP^{Sc}. The spontaneous conversion of PrP^C into PrP^{Sc} has been demonstrated in sheep and probably is the major cause of scrapie and sporadic CJD.

The immune system and prion infection

The prion protein is a self-antigen; hence, prion infection is not known to elicit a classical immune response. In fact, the immune system is involved in the peripheral replication of the prion agent and its ultimate access to the CNS (4, 50). Paradoxically, immune suppression with, for example, splenectomy or immunosuppressive drugs, increases the incubation period. This incubation period, during which time the prion agent replicates peripherally without producing any symptoms, is 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 (15). Lymphatic organs such as the spleen, tonsil, lymph nodes or gut-associated lymphoid tissue (GALT) contain high concentrations of PrP^{Sc} long before PrP^{Sc} replication starts in the brain (10, 26). Cells found to be particularly important for peripheral PrP^{Sc} replication are the follicular dendritic cells and the migratory bone-marrow derived dendritic cells (5, 26). Dendritic cells from infected animals are capable of spreading the disease (5). An emerging therapeutic approach for prion infection is immunomodulation (44, 50).

Vaccination for prion infection

Currently there is no treatment that would arrest and/or reverse progression of prion disease in non-experimental settings, although many approaches have been tried (56). In AD model mice it has been definitively shown that immunotherapy can prevent the onset of cognitive deficits and the development of amyloid lesions (31, 63). Significantly, this method of treatment is associated with consistent cognitive benefits in the mice (2, 20, 32, 49). An antibody-mediated response is probably critical for a therapeutic response, since similar results have been obtained with passive immunisation (6). Active immunisation for AD has recently been tried in humans by Elan Pharmaceuticals, with significant toxicity resulting from the vaccine (18, 62, 63). In the human phase 2A clinical trial of the vaccine (called AN-1792) 18 out of 372 patients worldwide developed symptoms of meningitis or meningoencephalitis, with symptoms apparently responding to immunosuppression in most patients (12 patients out of the 18 responded fully) (18). Recent evidence suggests that patients who developed anti-Aβ titres benefited cognitively from vaccination, including patients among the 12 that initially had complications (18, 19) and that vaccination resulted in amyloid clearance as judged by three autopsies performed in vaccinated patients (two autopsies from patients with encephalitis and one without complications) (17, 28, 33). Hence, it appears that if safety issues can be addressed, a vaccine approach will prove to have important therapeutic value in patients (58, 63) and it is the subject of new ongoing trials.

In part because of this success in AD models, similar experiments with anti-PrP antibodies were initiated in prion infectivity culture models and active and passive immunisation studies were carried out in rodent models. Earlier *in vivo* studies had shown that infection with a slow strain of PrP^{Sc} blocked expression of a more virulent fast strain of PrP, mimicking vaccination with a live attenuated organism (27). In tissue culture studies anti-PrP antibodies and antigen binding fragments directed against PrP have been shown to inhibit prion replication (16, 34, 35). One study demonstrated that active immunisation with recombinant PrP delayed the onset of prion disease in mice, but the therapeutic effect was relatively modest and eventually all the mice succumbed to the disease (46). This limited therapeutic effect may be explained by the observation that antibodies generated against prokaryotic PrP often do not have a high affinity towards PrP^{C} (36), although in studies carried out by the authors the increase in the incubation period correlated well with the antibody titres against PrP^{C}. The follow-up passive anti-PrP immunisation study confirmed the importance of the humoral response, showing that anti-PrP antibodies are able to prolong the incubation period (47). Subsequently, other investigators, using a much higher antibody dosage, were able to completely prevent disease onset in mice exposed to PrP^{Sc}, provided passive immunisation was initiated within a month of exposure (59). This type of approach could be used immediately following accidental exposure in humans to prevent future infection. However, passive immunisation has not been found to be effective closer to the clinically symptomatic stages of prion infection. Moreover, passive immunisation would be too costly an approach for animal prion diseases.

In the development of immunotherapeutic approaches targeting a self-antigen, designing a vaccine avoiding autoimmune related toxicity is a major concern. The emerging data from AD-targeting immunisation is that toxicity is due to excessive cell-mediated immunity within the CNS, while the therapeutic response is linked to humoral immunity. In addition, toxicity could be partially related to the immunogen and/or to the adjuvant used; in the human AD vaccination trial fibrillar Aβ1-42 was used as an immunogen. This peptide is well known to be toxic. Hence, the authors have been promoting the use of nonamyloidogenic derivatives as immunogens for protein conformational disorders, including AD and prion diseases (45, 49, 63) and interestingly a recent study indicated that α-helical PrP elicited an antibody response whereas an amyloidgenic β-sheet form of PrP favored a cytotoxic T-cell response (51). How significant an issue direct toxicity of the immunogen may be for prion vaccination remains unclear. Unlike the amyloid β peptide used for vaccination in AD models, direct application of recombinant PrP has not been shown to be toxic. However, this issue has not been investigated as thoroughly as in the Alzheimer's field. One study has shown that cytosolic accumulation of PrP was toxic (52), whereas other investigators observed that PrP was neuroprotective in another cell culture model (22).

A potential ideal means of using immunomodulation to prevent prion infection is mucosal immunisation. One important reason for this is that the gut is the major route of entry for many prion diseases such as CWD, BSE and vCJD. Furthermore, mucosal immunisation can be designed to induce primarily a humoral immune response, avoiding the cell-mediated toxicity that was seen in the human AD vaccine trail. Recently, the authors have been developing prion vaccines that target gut-associated tissue, the main site of entry of the prion agent. One of their approaches is to express PrP in attenuated *Salmonella* strains as a live vector for oral vaccination. Live attenuated strains of *Salmonella enterica* have been used for many years as vaccines against salmonellosis and as a delivery system for the construction of multivalent vaccines, with broad applications in human and veterinary medicine (29). One of the main advantages of this system is that the safety of administering live attenuated *Salmonella* has been extensively confirmed in humans and animals (23, 53).

Ruminants and other veterinary species can be effectively immunised by the oral route using live *Salmonella*, to induce humoral mucosal responses (13, 57). The authors are currently exploring ways to increase the efficacy even further. In these studies, the mucosal IgA anti-PrP titre correlates well with the delay or prevention of prion infection, further supporting the importance of the humoral response for the therapeutic effect. *Salmonella* target M-cells, antigen sampling cells in the intestines, which may also be important for uptake of PrP^{Sc} (26, 50). Hence, this approach is more targeted than prior vaccination studies, which probably explains the improved efficacy. By exploring other strains of attenuated *Salmonella*, using different bacteria or oral adjuvants, and/or by altering the expression levels or sequence of the PrP antigen, it is likely that the percentage of uninfected animals can be improved. The authors' recent work utilising this approach indicates that complete protection to clinical prion infection via an oral route is possible. Overall, this approach holds great promise as an inexpensive prophylactic immunotherapy to prevent the spread of prion disease, particularly in animals at risk and perhaps eventually in certain high-risk human populations.

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La vaccination contre l'encéphalopathie spongiforme transmissible est-elle une option réaliste ?

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Résumé

Les maladies à prion constituent une catégorie unique de pathologies affectant aussi bien les animaux que l'homme et dont la pathogénèse est associée à une conversion de la protéine de l'hôte, appelée protéine prion, de sa forme cellulaire normale PrP^{C} (C pour cellulaire) en une conformation pathogène et infectieuse appelée PrP^{Sc} (Sc pour *scrapie*, tremblante en anglais). À l'heure actuelle, il n'existe aucun traitement efficace contre les maladies à prion, dont l'issue est toujours fatale. L'émergence de l'encéphalopathie spongiforme bovine et de la variante de la maladie de Creutzfeldt-Jakob exige la mise au point de nouveaux traitements. Dans des expérimentations portant sur la maladie d'Alzheimer (qui présente des similitudes avec les maladies à prion), l'immunisation passive et active s'est révélée efficace pour prévenir la maladie chez les animaux de laboratoire et pour limiter les troubles cognitifs qui en résultent. Lors d'une série d'essais de vaccination active contre la maladie d'Alzheimer chez l'homme, une amélioration des fonctions cognitives a été obtenue chez des patients présentant une bonne réponse humorale, mais 6 % des patients ont souffert de complications graves, liées à une réponse à médiation cellulaire trop importante. Cette expérience met en exergue la nécessité, dans le domaine des immunothérapies dirigées contre un antigène autologue, de parvenir à un difficile équilibre entre la recherche d'une immunité humorale et le souci d'éviter toute toxicité auto-immune. Pour de nombreuses maladies à prion, l'intestin est l'organe par où l'agent pathogène pénètre dans l'organisme. De ce fait, l'immunisation muqueuse est une méthode particulièrement prometteuse qui vise à empêcher totalement ou partiellement le prion de franchir la paroi intestinale tout en produisant une réponse immunitaire ciblée et exempte de toxicité. Les résultats obtenus par les auteurs

en utilisant une souche vaccinale atténuée de *Salmonella* exprimant la protéine prion montrent que la vaccination muqueuse confère une protection partielle contre l'infection à prion à partir d'une source périphérique, ce qui paraît confirmer la faisabilité de cette démarche.

Mots-clés
Cachexie chronique – Encéphalopathie spongiforme bovine – Encéphalopathie spongiforme transmissible – Immunisation mucosale – Prion – Salmonella – Trouble de la conformation – Variante de la maladie de Creutzfeldt-Jakob.

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¿Es factible la vacunación contra la encefalopatía espongiforme transmisible?

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Resumen
Las enfermedades priónicas constituyen una singular categoría de dolencias que afectan tanto a los animales como al hombre y cuya patogénesis guarda relación con el cambio de conformación de una proteína del propio organismo, que pasa de la llamada forma celular (PrP^{C} [proteína priónica celular]) a una conformación patológica e infecciosa denominada forma priónica (PrP^{Sc} [en inglés, "*scrapie form*"]). En la actualidad no hay tratamiento eficaz para ninguna de esas enfermedades, que resultan invariablemente fatales. La aparición de la encefalopatía espongiforme bovina y de la variante de la enfermedad de Creutzfeldt-Jakob ha hecho más necesario que nunca encontrar posibles terapias. En el caso de la enfermedad de Alzheimer, que presenta similitudes con las afecciones priónicas, se ha demostrado que en modelos animales la inmunización tanto pasiva como activa resulta muy eficaz para prevenir la enfermedad y las consecuentes deficiencias cognitivas. En el curso de un ensayo de vacunación activa contra la enfermedad realizado en seres humanos, y pese a ciertos signos que indicaban beneficios cognitivos en pacientes con una buena respuesta humoral, se observaron importantes complicaciones ligadas a una respuesta excesiva de inmunidad celular en un 6% de los pacientes. Esa experiencia pone de manifiesto que las terapias inmunológicas dirigidas contra un autoantígeno deben hallar un delicado equilibrio entre la búsqueda de eficacia de la respuesta inmunitaria humoral y el riesgo de toxicidad autoinmune. En muchas enfermedades priónicas el intestino es la vía de entrada del agente infeccioso, lo que hace de la inmunización de las mucosas un método en potencia muy atractivo para prevenir, parcial o totalmente, la penetración de un prión a través de la barrera intestinal y también para inducir una respuesta inmunitaria modulada poco susceptible de generar toxicidad. Los resultados obtenidos recientemente por los autores (con una cepa vacunal de salmonelas atenuadas que expresan la proteína priónica) demuestran que la inmunización de las mucosas puede conferir protección parcial contra las infecciones priónicas procedentes de una fuente periférica, lo que lleva a suponer que se trata de un método viable.

Palabras clave
Anomalía de conformación – Caquexia crónica – Encefalopatía espongiforme bovina – Encefalopatía espongiforme transmisible – Inmunización de mucosas – Prión – Salmonella – Variante de la enfermedad de Creutzfeldt-Jakob.

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