

Prion diseases and the gastrointestinal tract

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The gastrointestinal (GI) tract plays a central role in the pathogenesis of transmissible spongiform encephalopathies. These are human and animal diseases that include bovine spongiform encephalopathy, scrapie and Creutzfeldt-Jakob disease. They are uniformly fatal neurological diseases, which are characterized by ataxia and vacuolation in the central nervous system. Although they are known to be caused by the conversion of normal cellular prion protein to its infectious conformational isoform (PrP^{Sc}) the process by which this isoform is propagated and transported to the brain remains poorly understood. M cells, dendritic cells and possibly enteroendocrine cells are important in the movement of infectious prions across the GI epithelium. From there, PrP^{Sc} propagation requires B lymphocytes, dendritic cells and follicular dendritic cells of Peyer's patches. The early accumulation of the disease-causing agent in the plexuses of the enteric nervous system supports the contention that the autonomic nervous system is important in disease transmission. This is further supported by the presence of PrP^{Sc} in the ganglia of the parasympathetic and sympathetic nerves that innervate the GI tract. Additionally, the lymphoreticular system has been implicated as the route of transmission from the gut to the brain. Although normal cellular prion protein is found in the enteric nervous system, its role has not been characterized. Further research is required to understand how the cellular components of the gut wall interact to propagate and transmit infectious prions to develop potential therapies that may prevent the progression of transmissible spongiform encephalopathies.

Key Words: *Autonomic nervous system; Dendritic cells; Enteric nervous system; Lymphoreticular system; Prion*

Transmissible spongiform encephalopathies (TSEs) are a family of neurodegenerative diseases in which proteinaceous infectious agents, termed prions, accumulate in the central nervous system (CNS). TSEs have been documented in a variety of species, including Creutzfeldt-Jakob disease (CJD), variant CJD, kuru, Gerstmann-Sträussler-Scheinker syndrome and fatal familial insomnia in humans; chronic wasting disease in elk and mule deer; scrapie in sheep; and bovine spongiform encephalopathy (BSE) in cows. The discovery of several cases of BSE in cattle in Alberta (1) and chronic wasting disease in Saskatchewan and Alberta (2) has helped these diseases gain prominence in Canada in recent years, fuelling an increased

Les maladies à prions et le tube digestif

Le tube digestif joue un rôle important dans la pathogenèse des encéphalopathies spongiformes transmissibles. Il s'agit de maladies humaines ou animales, qui englobent notamment l'encéphalopathie spongiforme bovine et la maladie de Creutzfeldt-Jakob. Ce sont toutes des maladies neurologiques mortelles, qui se caractérisent par l'ataxie et la vacuolisation du système nerveux central. Même si l'on sait que la cause réside dans la transformation de la protéine normale du prion cellulaire en son isoforme conformationnelle infectieuse (PrP^{Sc}), on en connaît bien peu sur le processus de propagation et de transport de la protéine vers le cerveau. Les cellules M, les cellules dendritiques et peut-être les cellules entéro-endocriniennes sont des facteurs importants dans le passage des prions infectieux à travers l'épithélium gastro-intestinal. À partir de là, la propagation de la PrP^{Sc} nécessite l'intervention des lymphocytes B, des cellules dendritiques et des cellules dendritiques folliculaires des plaques de Peyer. L'accumulation précoce de l'agent pathogène dans les plexus du système nerveux entérique étaye l'assertion selon laquelle le système nerveux autonome joue un rôle important dans la transmission de la maladie. D'ailleurs, la présence de PrP^{Sc} dans les ganglions des nerfs sympathiques et parasympathiques du tube digestif ne fait qu'ajouter du poids à l'assertion. De plus, le système lymphoréticulaire a été mis en cause comme voie de transmission de la protéine depuis l'intestin jusqu'au cerveau. Même si la présence de la protéine normale du prion cellulaire a été observée dans le système nerveux entérique, son rôle n'a pas été caractérisé. Il faudrait donc faire plus de recherche pour comprendre comment les constituants cellulaires de la paroi intestinale participent au processus de propagation et de transmission des prions infectieux et pour élaborer des traitements susceptibles de prévenir l'évolution des encéphalopathies spongiformes transmissibles.

interest in both the etiology and pathogenesis of TSEs. TSEs are acquired through ingestion or inoculation of infectious prion protein, which upon accumulation in the CNS causes ataxia, neuropathological lesions with spongiform vacuolation, neuronal cell loss and, invariably, death (3).

TSEs can arise from the prion gene as a result of germ-line mutations (eg, hereditary CJD and fatal familial insomnia) or possibly somatic mutation (eg, sporadic cases of CJD) and most commonly due to the ingestion of materials that are contaminated with infective prions (4). The essential pathophysiological event, in all forms of orally acquired TSEs, occurs when the host's encoded prion protein (PrP^C) adopts the conformation of

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its infectious misfolded counterpart, PrP^{Sc} (5,6). In all cases of TSE, the misfolded prion protein is eventually transmitted to the CNS, where it leads to neuronal cell death and the formation of protease-resistant aggregates (5). Why other somatic tissues fail to undergo degeneration in TSEs remains unclear, given that PrP^C, the 'template' from which PrP^{Sc} is formed, is so widely expressed. Prion protein is found in a variety of tissues, although its highest expression is in the CNS. Outside of the brain and spinal cord, the gastrointestinal (GI) tract, autonomic and sensory nerves, spleen, thymus, heart and testes also express high levels of PrP^C (7). The role of PrP^C is not known, although it has been suggested (8-10) that it regulates synaptic function in neurons, possibly by regulating copper metabolism. There is also some evidence (11,12) suggesting that the prion protein may have a neuroprotective role in response to stimuli, such as oxidative stress.

Development of a murine model lacking the PrP^C-encoding gene was crucial for studying the normal physiological role of prion protein, as well as for demonstrating the necessity of endogenous PrP^C in prion propagation and disease induction. In all cases, following either oral ingestion or inoculation with infectious prions, transgenic mouse models lacking PrP^C were found to neither accumulate nor circulate PrP^{Sc} except in singular, extremely rare cases, during which prion aggregates still developed, albeit at much lower levels than in wild-type scrapie-infected mice (5,13-15). Thus, mice lacking the endogenous prion gene were found to be totally resistant to prion disease induction, regardless of the route of administration of the infective material. This finding represented a milestone in prion research and supported the infectious prion hypothesis of TSE pathogenesis.

Although TSEs manifest themselves primarily in the CNS of affected animals, the pathophysiology of TSEs does not exclusively involve components of the nervous system. It has been well documented (3,16,17) that cells and organs of the immune system, while themselves apparently remaining largely unaffected by accumulation of PrP^{Sc}, are key players in many of the pathophysiological events from the point of prion exposure to the accumulation of PrP^{Sc} in the CNS (18).

Early studies (16) linking the lymphoreticular system (LRS) to TSE pathogenesis involved splenectomy, thymectomy or genetic asplenia, or athymia in mice, and suggested that the spleen, not the thymus, was of crucial importance to scrapie pathogenesis in mice. Later, experiments (19) using severe combined immunodeficient mice further supported a role for lymphoid organs, because immunodeficiency significantly prolonged the disease's incubation time, and reconstitution with normal splenocytes was able to restore disease susceptibility (20). In studying human or animal TSEs, PrP^{Sc} accumulation has been repeatedly demonstrated in cells and organs of the LRS, often before accumulation in the CNS (21-24). It has also been shown that PrP^C expression by both stromal and hematopoietic components is important for optimal prion propagation and development of CNS disease (25). Acknowledging the importance of the LRS in TSE pathogenesis, recent research has focused considerable effort on characterizing the potential contributions of immune components in mediating infection, propagation and progression of TSEs.

While the role of the lymphoid system in TSE pathogenesis is unquestioned, components of the nervous system are also key players in disease progression. Recent research (26,27) has documented prion entry into the CNS via the enteric nervous

system (ENS), and in the autonomic and sensory innervation of the GI tract. Because PrP^C expression is necessary to maintain prion disease, the high PrP^C concentration found in enteric neurons and glial cells (7,28) raises the possibility of the ENS playing a vital role in prion disease, by providing an initial site for infectivity and prion generation, and by aiding the retrograde transport of the communicable agent to the brain along the efferent innervation supplied to the ENS.

ABSORPTION OF PRIONS FROM THE GI TRACT

Most TSEs are acquired orally, wherein prion protein is ingested and subsequently absorbed (29,30). However, despite its acknowledged importance, many aspects of the oral pathway of TSE transmission remain poorly understood (31).

The initial step, when prion protein transmigrates from the gut and into the lymphoid system, has been proposed to involve epithelial M cells, because these represent key sites of enteric antigen sampling by facilitating the entry and delivery of enteric pathogens into underlying lymphoid tissues via the process of transepithelial transport (32). An experiment in which artificial M cells, derived from carefully differentiated intestinal-like cells, were required for transepithelial prion transport *in vitro* indicated a role for M cells in prion absorption (33). However, there has been little additional research to further support this hypothesis, and the requirement for M cells in prion transport has yet to be documented using *in vivo* models.

Alternatively, prion absorption through the gut wall may involve enteroendocrine cells, which are scattered throughout the mucosa of the GI tract from the stomach to the rectum, and are responsible for endocrine and paracrine signalling in the GI tract (34). Due to their high basal concentration of secretory vesicles and their intimate relationship with enteric and extrinsic nerves located beneath the epithelium, these cells are more than adequately equipped to use transcytosis to convey prions across the epithelial barrier to nervous tissues. Enteroendocrine cells also express both PrP^C messenger RNA and protein on a level comparable with that of enteric neurons (7), suggesting an additional role for these cells in prion amplification. However, the mechanisms by which these cells mediate transepithelial transport or prion amplification have yet to be elucidated, due in part to the focus on the role of M cells, lymphocytes and phagocytes in prion infection.

Peyer's patches (PPs), follicle clusters located along the mammalian intestine, are closely associated with M cells and have been implicated in early stages of prion pathogenesis following oral challenge. Researchers (24,35-39) have repeatedly observed early rises in PrP^{Sc} titres in PPs from a variety of species including mice, sheep, hamsters, deer, cattle and primates. In mouse models, reduced PP numbers have been linked to strong resistance to orally acquired prion infection (40). The evident contribution of PPs in prion pathogenesis has reinforced other research citing involvement of PP-localized cells in TSE, and has also turned attention to a more specific characterization of the contribution of PPs and PP cells in TSE progression.

AMPLIFICATION AND REPLICATION OF PRIONS

Between prion absorption and TSE manifestation in the CNS, there is an incubation period in which the ingested prion is replicated and amplified within the host. The replication

phase can vary in length and nature depending on species, genotype, environment and host-specific factors (41).

Although some specific aspects of the pathophysiological step remain poorly characterized, there is general consensus that follicular dendritic cells (FDCs), located in germinal centres of mammalian PPs, play a key role at this point of disease progression. Researchers have characterized FDCs primarily as a source of prion replication, because prion proliferative centres have repeatedly been documented as postmitotic, long-lived, low-density cells of stromal origin (42,43). Further support for FDC-mediated prion replication comes from studies that have shown that PrP^c, whose expression is a requirement for prion replication (6), is highly expressed by FDCs (44), and its pathogenic counterpart, PrP^{Sc}, can be isolated from FDCs at early stages following peripheral infection (44-46). Additionally, in mouse models, lack of functional FDCs has been linked to resistance to neuroinvasion following peripheral prion challenge (47-49). Combined, this evidence strongly suggests that FDCs are key sites of prion replication and accumulation during TSE pathogenesis. While it is possible that other types of replicative centres exist, FDCs remain the most widely accepted sites of prion replication.

FROM THE GUT TO REPLICATIVE CENTRES

FDCs are unquestionably prominent players in the lymphoid component of TSE pathogenesis, but recent research has suggested that additional lymphoid components, namely B cells and dendritic cells (DCs), may also bear some importance. While FDCs are known to be sites of PrP^{Sc} retention and replication, the mechanism by which PrP^{Sc} reaches FDCs after crossing the intestinal barrier remains unclear (50).

B cells

A role for B cells, not T cells, in TSE pathogenesis was first suggested in early studies involving splenectomy, where it was shown that the spleen is of crucial importance to scrapie pathogenesis in mice (16). A recent important experiment by Klein et al (51) complemented these findings by showing that B cell deficiencies in mice conferred resistance to scrapie after intraperitoneal injection.

However, in a follow-up experiment (52) by the same group, it was shown that PrP^c expression on B cells was not required for prion neuroinvasion, which, coupled with evidence that prion replication requires PrP^c expression (6), suggested that B cells may play an indirect role in prion propagation. In light of these findings, it has been suggested that B cells may transport PrP^{Sc} to nerve endings in a PrP^c-independent manner; immunoglobulin-PrP complexes may facilitate the conversion of PrP^c to PrP^{Sc}; or that B cell-secreted cytokines and immunoglobulins may somehow facilitate prion access to the nervous system (51,52). To date, there has been little evidence to support such propositions. The most convincing and best supported evidence (31) has suggested that B cells are important mostly in FDC maintenance.

B cells have well-documented roles in both the maturation and maintenance of FDCs within germinal centres, and do so mainly through secretion of tumour necrosis factor-alpha and lymphotoxin-alpha/beta (LT α / β) (53,54). Knockout mice devoid of LT- α , LT- β or LT- β receptor genes have shown reduced efficiency of prion propagation after peripheral exposure (47), and immunoglobulin-mediated pharmacological blocking of LT- β receptor has been shown to impair TSE

pathogenesis (48,49), supporting assertions that B cell contributions may be limited to their roles in FDC maintenance. Further, in studying scrapie infection in mouse PPs, it was shown that reduced numbers of enteric lymphocytes, including the abundant B cells within PPs, did not reduce prion pathogenesis following oral infection (40). These observations, combined with evidence that prion replication occurs in mitotically inactive stromal cells (42,43), suggest that B cells act primarily in support of the main lymphoid prion replicative centre, the FDC.

Because many aspects of prion transmission remain poorly characterized, it is too early to suggest that B cells contribute solely to FDC maturation during prion transmission. M cells, which were previously mentioned as candidates for absorption of prions across the gut wall, are dependent on intraepithelial B cells for maturation (31), potentially giving B cells an indirect role in enteric prion uptake. Additionally, because mechanisms for transmission of PrP^{Sc} from the gut to the sessile FDC, and for the subsequent transmission of PrP^{Sc} to the nervous system remain poorly understood (31,50), it is not possible to conclusively rule out the mobile B cell as a candidate carrier in each of these processes. Little experimental evidence has supported such a role for B cells in prion pathogenesis, but the observation by Andreoletti et al (55) that PrP^{Sc} is detectable in sheep B follicles of PPs before appearing in FDCs provides some indication that B cells may have a variety of roles in PrP^{Sc} transport.

DCs

Recent searches for a cellular bridge for prion transmission between the gut and FDCs have directed attention to DCs, professional antigen sampling cells located immediately below the follicle-associated epithelium within PPs. DCs are found in both the lamina propria and in PPs, and are PrP^c-positive hematopoietic system-derived cells that migrate through tissues and have a surveillance role. DCs are ideal candidates for the process in that they are abundant in follicular domes, where M cell-mediated enteric antigen sampling occurs (56). They are mobile and continuously migrate from the intestinal wall to mesenteric lymph nodes (57), and are capable of transporting antigens absorbed from the intestinal lumen (58). Experiments (59) investigating DCs in TSE transmission have shown that DCs are likely capable of transporting PrP^{Sc} from the gut to lymphoid prion replicative centres, though it has not been shown that DCs can transfer infectivity. It has also been shown that DCs residing in mesenteric lymph nodes can transport immunocomplexes from the subcapsular sinus to FDCs (60), and it has been suggested that DCs could transport PrP^{Sc} between lymphoid organs (61).

Mechanistically, DCs are known to transmit pathogenic molecules to FDCs through exosomal transfer, which would be a plausible mechanism for the transfer of the glycosylphosphatidylinositol-anchored prion protein from cell to cell because glycosylphosphatidylinositol-anchored proteins are preferentially transferred exosomally in lipid rafts (50,62). A recent experiment by Defaweux et al (50) in mice using a double immunofluorescence staining showed the existence of cell to cell contacts between DCs and gut epithelium and between DCs and FDCs, providing further support for a role for DCs as a bridge between prion absorption and replication within the lymphoid system.

However, recent research (63) using CD40 ligand-deficient mice, where DC migration is inhibited, observed unimpaired prion invasion, indicating that prion transport is not necessarily contingent on the migratory capacity of DCs. In light of these results, it was suggested that FDCs may directly acquire gut-absorbed prions from the surrounding environment, independent of DC-mediated prion transfer.

Macrophages

Studies of lymphoid TSE pathogenesis have also directed their attention toward macrophages and, unlike B cells, FDCs and DCs, it appears as though macrophages play a role in the clearance of prions from the host than in the propagation of infectivity. Macrophages were initially implicated in TSE pathogenesis when it was shown that prion replicative centres, like macrophages, express PrP^c and are long-lived and radioreistant (64). Macrophages are additionally present in PPs, which have been previously mentioned as key sites for the uptake of orally administered prions in many species. A role for macrophages in prion clearance has been suggested by studies where it was shown that they carry PrP^{Sc} intralysosomally (65), that extended *in vitro* culture with macrophages that show they can reduce scrapie infectivity (66) and that *in vivo* macrophage depletion in mice quickens the rate of scrapie progression and increases accumulation of infectivity and PrP^{Sc} in the spleen (67). A recent experiment by Maignien et al (68) further confirmed a role for macrophages in TSE resistance by using the macrophage-suicide technique to correlate the degree of BSE and scrapie resistance to amounts of PrP^{Sc} isolated from macrophages. Thus, there is reasonable evidence to suggest that macrophages are implicated in TSE pathogenesis and have primarily preventative roles, likely through the elimination of infective prion particles.

THE ENS IN PRION DISEASES

Vitally important for coordinating normal gut function, the ENS comprises a vast network of enteric neurons and nerve processes that are sustained by an even greater number of enteric glia, located in two major plexuses in the gut wall (69,70). The two ganglionated plexuses are the submucosal plexus, located between the mucosa and circular muscle layer within the submucosa, and the myenteric plexus, found between the circular and longitudinal muscular layers of the wall of the alimentary canal. The ENS also receives both sympathetic and parasympathetic innervation and it has a rich primary afferent innervation from vagal and spinal afferents (70). Under hierarchical control of the CNS, the ENS receives input from autonomic nerves, which act as a potential conduit for transmission from the gut to the brain by axonal transport.

Enteric neurons have been found to express high levels of PrP^c messenger RNA and protein in histologically normal specimens (7), implying a role for the ENS in prion diseases, because the conformational change of PrP^c to PrP^{Sc} is essential to prion disease (5).

Although germinal centres of lymphoid follicles are acknowledged as being poorly innervated, ultrastructural studies have found a network of nerve fibres within PP nodules (71,72), placing FDCs in close contact with nerve endings. It was previously postulated that the zone of lymphoid follicles innervated by terminal unmyelinated nerve fibres represented the entry into the autonomic nervous system (73), and recent

evidence reinforcing the importance of FDCs supports this method of neuroinvasion.

DCs have also been implicated in neuroinvasion. Prion neuroinvasion is commonly accepted to proceed through nerves innervating PPs and draining lymph node of PPs and the spleen (74,50). Defaweux et al (50) observed cell to cell contacts between DCs and enteric nerve endings in PPs. Nerve fibres, like FDCs, are sessile, so in the absence of direct FDC-neuronal cell to cell connections, which were not observed, mobile DCs are an attractive candidate for bridging lymphoid prion accumulation and neuroinvasion.

There is already some evidence (75-78) that transmission of prions to the ENS occurs after oral challenge, as pathogenic PrP labelling is consistently found within the myenteric and submucosal plexuses and gut-associated lymphoid tissue, which includes PPs, at the earliest time of sacrifice after infection. Outbred Syrian hamsters orally infected with scrapie were sacrificed at various times between 69 days and 132 days postinfection; from the earliest time onwards, immunolabelling revealed PrP^{Sc} in the ENS taken from the duodenal and ileal muscularis and submucosa (76), indicating the ENS as a potential site of replication and invasion.

It is also possible that PrP^{Sc} residing in intestinal PPs could pass into mesenteric lymph nodes and proceed to the brain via migrating cells of the LRS, thus completely bypassing the ENS (26). However, the early accumulation of the abnormal prion protein in the ENS argues against such a proposition.

AUTONOMIC INNERVATION OF THE GI TRACT WITH RELATION TO PRIONS

Although extensive enteric reflex arcs exist, the CNS retains autonomic control of the gut. Input regarding the luminal environment is carried to the CNS by extrinsic primary afferent (sensory) neurons, whose cell bodies are located in the nodose and dorsal root ganglia (70). Parasympathetic efferent signals moving away from the CNS are carried from the dorsal motor nucleus of the vagus and sacral parasympathetic nuclei in the spinal cord toward enteric neurons in the gut (70). Sympathetic efferent signals arise from neurons in the spinal cord, which synapse in the abdominal prevertebral ganglia (eg, celiac ganglion), from where the gut innervation is derived.

The role of the parasympathetic and sympathetic nerve trunks in PrP^{Sc} transmission have been increasingly regarded as a route for infective prions to enter the CNS (79), because PrP^{Sc} accumulation has been detected in the dorsal motor nucleus of the vagus nerve and the nodose, dorsal root and celiac ganglia (75,80). To date, however, no one has performed vagotomy and assessed the extent of prion accumulation in the CNS following this lesion. It was previously unclear if other autonomic nerves found within the wall of the gut are capable of transmitting to the spinal cord, however, recent studies indicate a much wider range of PrP^{Sc} accumulation than that mediated solely via the vagus nerve. Thus, McBride and Beekes (75) found heavy labelling in the celiac-mesenteric ganglion complex and scant labelling in the vagus nerve, suggesting mainly sympathetic transport of PrP^{Sc}. In contrast, Sigurdson et al (80) found heavy labelling in the vagus nerve and no apparent labelling in the celiac ganglion, suggesting parasympathetic transport. However, in both studies, the authors agreed that both the sympathetic and parasympathetic divisions of the autonomic nervous system likely play a role in transmission to the CNS.

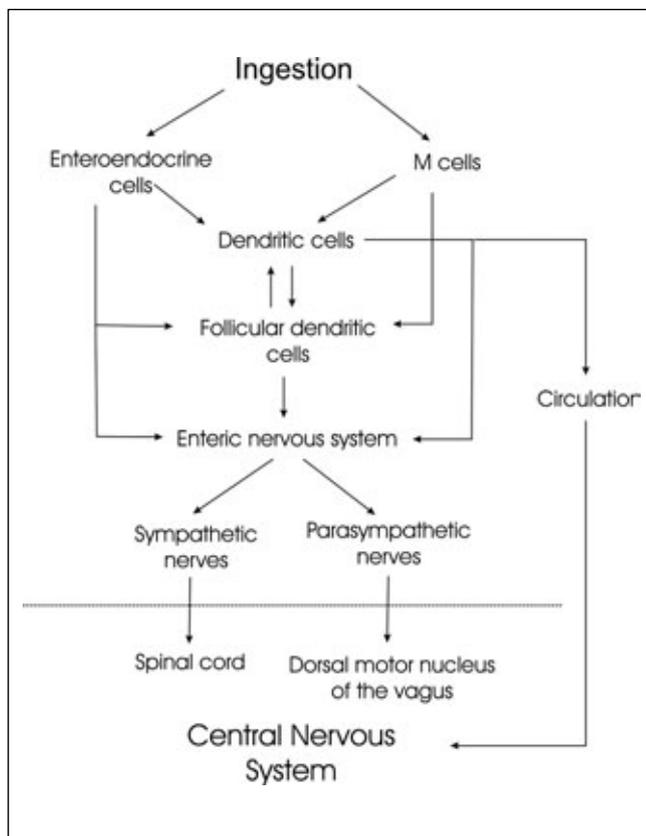


Figure 1) Possible routes of entry, propagation and neuroinvasion for cellular prion protein after introduction to the luminal environment of the gastrointestinal tract. M cells, dendritic cells and follicular dendritic cells are found in Peyer's patches. The dashed line indicates the central nervous system

CONCLUSIONS

The involvement of the immune and nervous system in the propagation and transmission of prions is summarized in Figure 1. While it is unquestionable that the lymphoid system is of crucial importance in TSE pathogenesis, many aspects regarding the nature of its contribution remain unclear.

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However, there is reasonable consensus that FDCs act as primary prion replicative centres, but the details of the lymphoid system's roles in prion absorption, transmission to replicative centres, and in potentially mediating neuroinvasion remain uncertain.

Further research into the precise sequence of events involved in neuroinvasion from the ENS to the brain is required before definitive statements about the pathways of in vivo transmission can be made. The relative roles of enteric glia and enteric neurons as reservoirs of PrP^c remains an area for investigation, as well, the effect of removing extrinsic neural pathways in the susceptibility to prion disease needs to be examined. These experiments will help to define the involvement of the autonomic nervous system in disease transmission.

There have been no reports of a spontaneous GI phenotype in mice lacking the prion gene; however, it is unlikely that intestinal structure and physiology of the PrP knockout has been subjected to close scrutiny. Examination of brain and behavioural activity in mice lacking PrP^c has helped shed light on the role of PrP^c in the CNS (81-83). By analogy, it is possible that study of the ENS in PrP^c knockout mice will elucidate a role for this protein in gut function. Hopefully, with further research into this area, a clearer idea of the role of PrP^c within the gut will be revealed, which in turn may lead to a better understanding of the mechanism of prion propagation, and the necessity of PrP^c to normal physiology.

The fatal nature of this disease, combined with the economic and political ramifications of TSEs in livestock, make the dissection of the mechanisms underlying infection and pathogenesis vitally important. A better understanding of the TSEs, and specifically, how prions infect organisms and how they spread is essential to the development of potential preventive measures and new treatments for this devastating disease.

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