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Alimentary prion infections

Touchdown in the intestine

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Abbreviations: PrP^{c}, cellular prion protein; PrP^{Sc}, scrapie prion protein; TSE, transmissible spongiform encephalopathy; LRP/LR, non-integrin laminin receptor; M-cells, microfold cells; FDCs, follicular dendritic cells; CJD, Creutzfeldt-Jakob disease; BSE, Bovine Spongiform Encephalopathy; CWD, Chronic Wasting disease; ENS, enteric nervous system; CNS, central nervous system; Aβ, amyloid-β

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Neurodegenerative diseases are caused by proteinaceous aggregates, usually consisting of misfolded proteins which are often typified by a high proportion of β-sheets that accumulate in the central nervous system. These diseases, including Morbus Alzheimer, Parkinson disease and Transmissible Spongiform Encephalopathies (TSEs)—also termed prion disorders—afflict a substantial proportion of the human population and, as such, the etiology and pathogenesis of these diseases has been the focus of mounting research. Although many of these diseases arise from genetic mutations or are sporadic in nature, the possible horizontal transmissibility of neurodegenerative diseases poses a great threat to population health. In this article we discuss recent studies that suggest that the "non-transmissible" status bestowed upon Alzheimer and Parkinson diseases may need to be revised as these diseases have been successfully induced through tissue transplants. Furthermore, we highlight the importance of investigating the "natural" mechanism of prion transmission including peroral and perenteral transmission, proposed routes of gastrointestinal uptake and neuroinvasion of ingested infectious prion proteins. We examine the multitude of factors which may influence oral transmissibility and discuss the zoonotic threats that Chronic Wasting disease (CWD), Bovine Spongiform Encephalopathy (BSE) and Scrapie may pose resulting in vCJD or related disorders. In addition, we suggest that the 37 kDa/67 kDa laminin receptor on the cell surface of enterocytes, a major cell population in the intestine, may play an important role in the intestinal pathophysiology of alimentary prion infections.

Many different mechanisms exist which underlie the etiology of the numerous neurodegenerative diseases affecting the human population. Amongst the most prominent are Morbus Alzheimer, prion disorders, Parkinson disease, Chorea Huntington, frontotemporal dementia and amylotrophic lateral sclerosis. The molecular mechanisms underlying these diseases vary; however, all neurodegenerative diseases share a common feature: they are caused by protein aggregation. The only neurodegenerative diseases proven to be transmissible are prion disorders. In contrast to frontotemporal dementia, recent evidence suggests that Alzheimer and Parkinson diseases may also be transmissible. Pre-symptomatic Alzheimer disease (APP23) mice exhibited an increase in the Alzheimer phenotype when brain homogenate of autopsied human Alzheimer disease patients and older, amyloid beta-(Aβ-) laden APP23 mice was injected into their hippocampi.[1] These findings suggest that the Aβ-abundant brain homogenate of Alzheimer disease patients may possess the ability to induce or supplement the overproduction of Aβ, possibly leading to the onset of Alzheimer disease.

The pathological feature associated with Parkinson disease is the formation of Lewy bodies in cell bodies and neuronal processes in the brain.[2] The main component of these protein aggregates is α-synuclein (reviewed in ref. 2). Autopsies of Parkinson disease patients revealed that Lewy bodies had formed on healthy

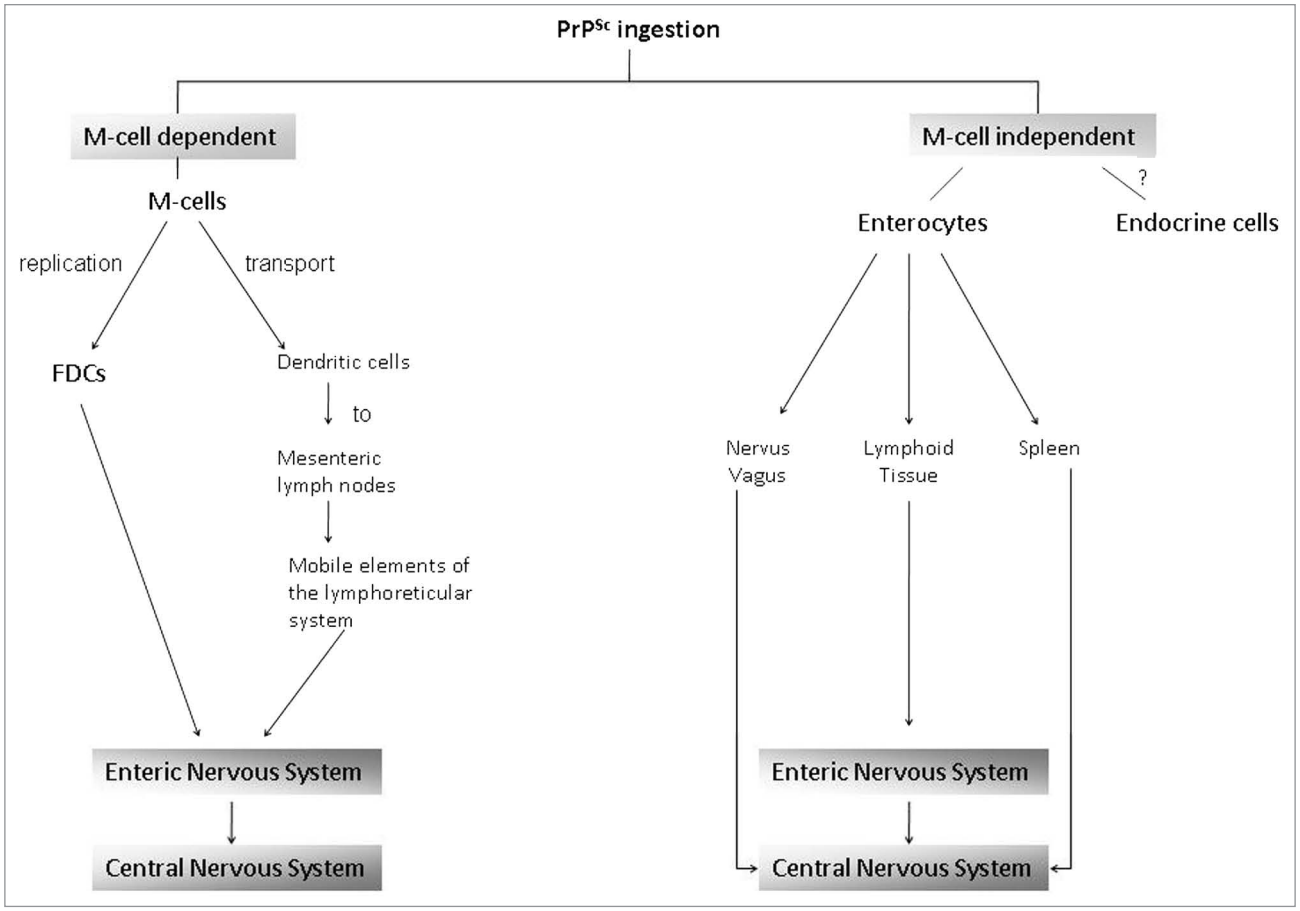

Figure 1. Proposed routes of gastrointestinal entry of ingested infectious prions (PrP^{Sc}) as well as possible pathways of amplification and transport to the central nervous system.

embryonic neurons that had been grafted onto the brain tissue of the patients several years before (prior to said examination).[3-5] It may thus be proposed that α-synuclein transmission is possible from diseased to healthy neurons, suggesting that Parkinson disease may be transmissible from a Parkinson disease patient to a healthy individual. These findings imply that Alzheimer and Parkinson diseases may be transmissible through tissue transplants and the use of contaminated surgical tools.[6]

Prion disorders, also termed Transmissible Spongiform Encephalopathies (TSEs), are fatal neurodegenerative diseases that affect the central nervous system (CNS) of multiple animal species. In lieu of the social, economic and political ramifications of such infections, as well as the possible intra- and interspecies transmissibility of such disorders, various routes of experimental transmission have been investigated including intracerebral, intraperitoneal, intraventricular, intraocular, intraspinal and subcutaneous injections (reviewed in ref. 7–9). However, such routes of transmission are not representative of the "natural" mechanism as the majority of prion disorders are contracted through ingestion of infectious prion (PrP^{Sc}) containing material. Thus, the peroral and perenteral prion transmission is of greatest consequence with respect to TSE disease establishment. Moreover, the presence of PrP^{Sc} in the buccal cavity of scrapie-infected sheep[10] (reviewed in ref. 11) and the possible horizontal transfer as a result hereof, as may be similarly proposed for animals suffering from other TSEs, may further contribute to the oral transmissibility of TSEs.

A number of model systems have been employed to study TSE transmissibility. Owing to ethical constraints, TSE transmissibility to humans via the oral route may not be directly investigated and as a result hereof, alternative model systems are needed. These may include the use of transgenic mice, cell lines which are permissive to infection[12] and experimental animals such as sheep, calves, goats, minks, ferrets and non-human primates (reviewed in ref. 9).

Intestinal entry of PrP^{Sc} has been proposed to occur via two pathways, the membranous (M) cell-dependent and M cell-independent pathways (**Fig. 1**).[13,14] The former involves endocytic M (microfold)-cells, which cover the intestinal lymphoid follicles (Peyer's patches)[14] and may take up prions and thereby facilitate the translocation of these proteins across the intestinal epithelium into the lymphoid tissues (reviewed in ref. 9) as has been demonstrated in a cellular model.[13] Following such uptake by the M cells, the prions may subsequently pass to the dendritic cells and follicular dendritic cells (FDCs) (**Fig. 1**), which allow for prion transport to the mesenteric lymph nodes and replication, respectively.[15] The prion

proteins may subsequently gain access to the enteric nervous system (ENS) and ultimately the central nervous system (CNS).[15]

However, prion intestinal translocation has been observed in the absence of M cells and has been demonstrated to be as a result of the action of polar, 37 kDa/67 kDa LRP/LR (non-integrin laminin receptor; reviewed in ref. 16–18) expressing enterocytes. Enterocytes are the major cell population of the intestinal epithelium and due to their ability to endocytose pathogens, nutrients and macromolecules,[19] it has been proposed that these cells may represent a major entry site for alimentary prions (**Fig. 1**).

Since enterocyte prion uptake has been demonstrated to be dependent on the presence of LRP/LR on the apical brush border of the cells,[14,20] the interaction between varying prion protein strains and the receptor[21-23] may be employed as a model system to study possible oral transmissibility of prion disorders across species as well as the intestinal pathophysiology of alimentary prion infections.[24] Moreover, the blockage of such interactions through the use of anti-LRP/LR specific antibodies has been reported to reduce PrP^{Sc} endocytosis[19] and thus these antibodies may serve as potential therapeutics to prevent infectious prion internalization and thereby prevent prion infections. It must be emphasized that the adhesion of prion proteins to cells is not solely dependent on the LRP/LR-PrP^{Sc} interactions;[24] however, this interaction is of importance with regards to internalization and subsequent pathogenesis.

We applied the aforementioned cell model to study the possible oral transmission of PrP^{BSE}, PrP^{CWD} and ovine PrP^{Sc} to cervids, cattle, swine and humans.[24] The direct transmission of the aforementioned animal prion disorders to humans as a result of dietary exposure and the possible establishment of zoonotic diseases is of great public concern. It must however be emphasized that the study investigated the co-localization of LRP/LR and various prion strains and not the actual internalization process.

PrP^{BSE} was shown to co-localize with LRP/LR on human enterocytes[24], thereby suggesting that PrP^{BSE} is transmissible to humans via the oral route which is widely accepted as the manner by which variant CJD originated. This suspicion was previously investigated using a macaque model, which was successfully perorally infected by BSE-contaminated material and subsequently lead to the development of a prion disorder that resembles vCJD.[25] These results, due to the evolutionary relatedness between macaques and humans, allowed researchers to confirm the oral transmissibility of PrP^{BSE} to humans. PrP^{BSE} may also potentially lead to prion disorder establishment in swine,[24] livestock of great economic and social importance.

The prion disorder affecting elk, mule deer and white-tailed deer is termed CWD. Cases of the disease are most prevalent in the US but are also evident in Canada and South Korea.[26,27] As the infectious prion isoform is reported to be present in the blood[28] and skeletal muscle,[29] hunting, consumption of wild venison and contact with other animal products derived from CWD-infected elk and deer may thereby pose a public health risk. Our studies demonstrate that PrP^{CWD} co-localizes with LRP/LR on human enterocytes[24] thereby suggesting a possible oral transmissibilty of this TSE to humans. This is, however, inconsistent with results obtained during intra-cerebral inoculation of the brains and spinal cords of transgenic mice overexpressing the human cellular prion protein (PrP^{c}),[26,27] which is essential for TSE disease establishment and progression. Further, discrepancies have also been reported with respect to non-human primates, as squirrel monkeys have been successfully intracerebrally inoculated with mule-deer prion homogenates,[30] while cynolmolgus macaques were resistant to infection.[31] CWD has been transmitted to ferrets, minks and goats[32] and as these animals may serve as domestic animals or livestock, secondary transmission from such animals to humans, through direct contact or ingestion of infected material, may be an additional risk factor that merits further scientific investigation.

Ovine PrP^{Sc} co-localization with LRP/LR on human and bovine enterocytes may be indicative of the infectious agents' ability to effect cross-species infections. The oral transmissibility of Scrapie has been confirmed in hamsters fed with sheep-scrapie-infected material.[33]

The discrepancies with regards to the transmissibility of certain infectious prion proteins when assessed by different model systems may be due to the experimental transmission route employed. Oral exposure often results in significantly prolonged incubation times when compared to intracerebral inoculation techniques and thus failure of transgenic mice and normal experimental animals to develop disease phenotypes after being fed TSE-contaminated material may not necessarily indicate that the infection process failed.[14] Apart from the route of infection, numerous other factors may influence transmission between species, including dose, PrP polymorphisms and genetic factors, the prion strain employed as well as the efficacy of prion transport to the CNS.[34] The degree of homology between the PrP^{c} protein in the animals serving as the infectious prion source and recipient has also been described as a feature limiting cross-species transmission.[34] The negative results, as referred to above, obtained upon prion-protein inoculation of animal models may have resulted due to the slow rate at which the infectious prion induces conformational conversion of the endogenous PrP^{c} in the animal cells and this in turn results in low levels of infectious prion replication and symptom development.[27]

Furthermore, even in the event that certain prion disorders are not directly transmissible to humans, most are transmissible to at least a single species of domestic animal or livestock. The infectious agents properties may be altered in the secondary host such that it becomes transmissible to humans (reviewed in ref. 35). Thus, interspecies transmission between animals may indirectly influence human health.

It is noteworthy to add that although the oral route of PrP^{Sc} transmission may result in prolonged incubation times, it may broaden the range of susceptible hosts. A common constituent of food is ferritin, a protein that is resistant to digestive enzyme hydrolysis and, due to its homology across species, it may serve as co-transporter of PrP^{Sc} and facilitate enterocyte internalization of the infectious prion.[36] It may thus be proposed

that prion internalization may occur via a ferritin-PrP^{Sc} complex even in the absence of co-localization between the infectious agent and LRP/LR such that many more cross-species infections (provided that the other infection factors are favorable) may be probable.[37] In addition, digestive enzymes in the gastrointestinal tract facilitate PrP^{Sc} binding to the intestinal epithelium and subsequent intestinal uptake[36] and thus depending on the individuals' digestive processes, the susceptibility to infection and the rate of disease development may vary accordingly. As a result hereof, though laboratory experiments in cell-culture and animal models may render a particular prion disorder non-infectious to humans, this may not be true for all individuals.

In lieu of the above statements, with particular reference to inconsistencies in reported results and the multiple factors influencing oral transmissibility of TSEs, further transmission studies are required to evaluate the zoonotic threat which CWD, BSE and Scrapie may pose through ingestion.

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