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MINIREVIEW

The spread of prions through the body in naturally acquired transmissible spongiform encephalopathies

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Transmissible spongiform encephalopathies are fatal neurodegenerative diseases that are caused by unconventional pathogens and affect the central nervous system of animals and humans. Several different forms of these diseases result from natural infection (i.e. exposure to transmissible spongiform encephalopathy agents or prions, present in the natural environment of the respective host). This holds true also for scrapie in sheep, bovine spongiform encephalopathy in cattle, chronic wasting disease in elk and deer, or variant Creutzfeldt–Jakob disease in humans, all of which are assumed to originate predominantly from peroral prion infection. This article intends to provide an overview of the current state of knowledge on the spread of scrapie, chronic wasting disease, bovine spongiform encephalopathy and variant Creutzfeldt–Jakob disease agents through the body in naturally affected hosts, and in model animals experimentally challenged via the alimentary tract. Special attention is given to the tissue components and spreading pathways involved in the key stages of prion routing through the body, such as intestinal uptake, neuroinvasion of nerves and the central nervous system, and centrifugal spread from the brain and spinal cord to peripheral sites (e.g. sensory ganglia or muscles). The elucidation of the pathways and mechanisms by which prions invade a host and spread through the organism can contribute to efficient infection control strategies and the improvement of transmissible spongiform encephalopathy diagnostics. It may also help to identify prophylactic or therapeutic approaches that would impede naturally acquired transmissible spongiform encephalopathy infections.

Prion diseases: transmissible spongiform encephalopathies of animals and humans

Scrapie in sheep and Creutzfeldt–Jakob disease (CJD) in humans were the first reported examples of an emerging family of transmissible, unconventional diseases that affect a range of animal species and humans. The group includes Gerstmann–Sträussler–Scheinker syndrome, kuru and variant CJD (vCJD) of humans, bovine spongiform encephalopathy (BSE) of cattle, chronic wasting disease (CWD) of captive or

Abbreviations
BSE, bovine spongiform encephalopathy; CJD, Creutzfeldt–Jakob disease; CNS, central nervous system; CWD, chronic wasting disease; DC, dendritic cell; ENS, enteric nervous system; FAE, follicle-associated epithelium; FDC, follicular dendritic cell; GALT, gut-associated lymphoid tissue; IHC, immunohistochemistry; LRS, lymphoreticular system; LTα/β, lymphotoxin α/β; LTβR-Ig, lymphotoxin β receptor-immunoglobulin fusion protein; M cell, microfold cell; PK, proteinase K; PNS, peripheral nervous system; PrP, prion protein; PrP^{C}, normal cellular isoform of PrP; PrP^{res}, protease-resistant form of PrP; PrP^{Sc}, disease-associated isoform of PrP, considered as a key component of infectious TSE agents according to the prion hypothesis; PrP^{sen}, protease-sensitive form of PrP; PrP^{TSE}, disease-associated prion protein from TSE-affected individuals; TME, transmissible mink encephalopathy; TNF-α, tumor necrosis factor-α; TSE, transmissible spongiform encephalopathy; vCJD, variant Creutzfeldt-Jakob disease.

free-ranging deer and transmissible mink encephalopathy (TME) of captively reared mink. All of these diseases, collectively called the transmissible spongiform encephalopathies (TSEs) or prion diseases, cause a progressive degeneration of the central nervous system (CNS) that is eventually fatal. Pathological features include often, but not invariably, gliosis, neuronal cell loss and spongiform change. However, the pathognomonic feature of all members of this group of diseases is the deposition in the CNS of an aberrant form of the prion protein (PrP) with a pathologically altered folding and/or aggregation structure. According to the prion hypothesis, the causative agents of prion diseases are proteinaceous infectious particles ('prions') which are composed essentially – if not entirely – of misfolded PrP, referred to as PrP^{Sc}. The 'protein-only model' of the prion hypothesis postulates that TSE agents replicate through a molecular mechanism in which abnormally folded PrP^{Sc} acts as a catalyst or template nucleus, which recruits cellular PrP and transforms it into its own 'infectious' spatial structure [1,2].

The normal cellular isoform of PrP, which is expressed in neurons, lymphoid cells and other tissues of mammals, has been designated as PrP^{C}, whereas for the disease-associated PrP from TSE-affected individuals the pragmatic term PrP^{TSE} was recently introduced [3] in order to avoid confusion resulting from increasingly complex PrP nomenclatures (e.g. PrP^{Sc}, PrP^{BSE}, PrP^{CJD}, PrP^{CWD}, PrP^{sen}, PrP^{res}, etc. [3]) and their mingling with etiological concepts such as the prion hypothesis. Accordingly, throughout this review the descriptive term PrP^{TSE} will be used to designate disease-associated PrP which can be detected in affected animals and humans by analytical methods such as western blotting [4,5], immunohistochemistry (IHC) [6,7] or paraffin-embedded tissue blotting [8]. Western- and paraffin-embedded tissue blotting detect partially proteinase K (PK)-resistant forms of PrP^{TSE}, and IHC visualizes aggregated deposits of this protein. PrP^{TSE} was established in many studies as a reliable biochemical marker for the transmissible causative agent of TSEs [4,9–14]. However, the gold standard for the direct demonstration of TSE infectivity has been bioassays in reporter animals.

Scrapie is the archetype of all TSEs [15], and its hosts (sheep and goats) are thought to acquire the disease naturally via horizontal transmission between animals and via vertical transmission from ewe to lamb. The emergence (in the 1980s) and transmission of a new animal TSE agent, distinct from the established scrapie agents in sheep and goats, led to an epidemic of BSE, or 'mad cow disease', in cattle [16]. Although the origin of the BSE agent remains unclear [17,18], the route of its propagation and dissemination is less elusive. It appears that BSE was transmitted within the bovine population by feeding cows and oxen a contaminated meat and bone meal protein supplement derived from BSE-infected cattle ([19,20]; reviewed in [16]). The BSE agent was also transmitted, again probably via the alimentary route, to domestic and large captive cats in which it caused feline spongiform encephalopathy [21], and to a variety of ungulates in zoos [22]. TSEs in animals also include TME [23], and CWD [24] of captive and free-ranging red deer [known in North America as wapiti or elk, for example Rocky mountain elk (*Cervus elaphus nelsoni*) and whitetail deer (*Oedocoilus virginianus*)]. The recent rapid spread of CWD through several states of the USA has caused increased attention and concern over contagion and/or the apparent ease of its transmissibility.

Human TSEs are differentiated into sporadic, hereditary and acquired forms [25]. Human CJD occurs with a worldwide relatively constant incidence of 1–1.5 cases per million inhabitants per year. The majority of cases (about 85–90%) arise spontaneously (i.e. without any recognizable external origin), mainly in patients over 50 years of age (sporadic CJD). However, in about 5–10% of patients, CJD is associated with an autosomal-dominant hereditary predisposition caused by various mutations in the PrP gene (familial CJD). A small number of classic CJD cases can be attributed to transmission as a result of medical intervention (iatrogenic CJD). The emergence of a new variant of Creutzfeldt–Jakob disease (termed vCJD) affecting mainly young individuals (average age 28 years, range 14–74 years) was reported from the UK in 1996 [25,26]. vCJD differs significantly from classic forms of CJD in its distinct etiology, pathophysiology and clinical manifestation and, as such, represents a new, independent entity within the family of TSEs. According to the current state of knowledge, the vast majority of vCJD cases diagnosed to date (in the UK, 161 as of July 2006 [27]) can almost certainly be attributed to transmission via BSE-contaminated foodstuffs. However, two reports published in 2004, and a further report communicated in 2006, raised the possibility that vCJD-infected human blood could also transmit the vCJD agent from human to human [28–30]. Other known TSEs in humans are the Gerstmann–Sträussler–Scheinker syndrome, fatal familial insomnia and kuru [25]). Like familial CJD, Gerstmann–Sträussler–Scheinker syndrome and fatal familial insomnia are associated with characteristic mutations in the PrP gene and subject to autosomal-dominant inheritance. In contrast, kuru (now obsolete) was limited to several

distinct areas of Papua-New Guinea where ritual cannibalism of the Fore tribe formerly disseminated the disease [31,32].

A characteristic feature of the pathogenesis of all TSEs, including sporadic and hereditary forms, is the consistent, reproducible and restricted replication of the TSE pathogen in specific tissue sites, but particularly within the brain. When transmitted to another individual, this pathogen can induce a TSE in the new host; hence the term 'infectious TSE agent'.

Routes of infection in naturally acquired prion diseases

Scrapie, CWD, BSE and vCJD represent the most relevant forms of naturally acquired prion diseases that are caused by exposure to TSE pathogens in the normal living environment of the respective host. Substantial evidence suggests that many, if not the majority, of cases of ovine scrapie [33–36], BSE [19,37] and purportedly TME [38–40] and CWD [41–43], are caused by ingestion of TSE agents and subsequent invasion of the organism via the alimentary tract. This also holds true for the two human TSEs (i.e. kuru and vCJD). Kuru was reportedly transmitted by ritualistic cannibalism [44,45], and the linkage of vCJD to BSE [46,47] is now generally acknowledged to be through consumption of BSE-contaminated foodstuffs.

In contrast to other TSEs, there is evidence that scrapie and CWD are not only transmissible but contagious. Peroral infection in horizontal or vertical scrapie transmissions is thought to occur via infected placenta or other carriers (e.g. abraded skin, flesh of dead animals) that may either be ingested or taint the ground long after the contaminated tissue has disintegrated [48,49]. In addition, mites [50], as well as fly larvae and pupae [51], have been suggested as living harbours of ingestible infectivity. Recently, prions were also detected in the saliva of CWD-infected cervids [52]. As well as ingestion, scarification of skin or gums has been shown to provide an efficient portal of entry for scrapie agent into the body [53,54], and transdermal (or conjunctival) invasion of infectious agent has been suggested as an alternative natural pathway for the transmission of kuru agent [31,55].

Exploration of the systemic spread of infection in naturally acquired TSEs

The BSE epidemic and subsequent emergence of vCJD effectively highlighted the risks of TSE agents to public health, and the identification of the oral route as a key pathway for the transmission of the agents causing naturally acquired TSEs emphasized the need for systematic studies on the pathogenesis of these diseases. In order to implement an efficient infection control, it is essential to identify – either directly by bioassay, or indirectly by detection of the agent's biochemical marker, PrP^{TSE} – the reservoirs of infectivity in the body at presymptomatic and clinical stages of incubation. Such information should also facilitate the development of improved TSE diagnostics that allow pathogen detection at an early stage without requiring CNS samples. Furthermore, an improved understanding of the pathways of spread and mechanisms of invasion used by prions may help to identify approaches for prophylactic or therapeutic intervention. Historically, the majority of studies addressing the spread of infection through the body in acquired TSEs used mouse and hamster models of experimental scrapie in conjunction with parenteral routes of infection, such as intraperitoneal (i.p.) or intravenous administration of agent. Although this shed light on many fundamental aspects of TSE pathogenesis [56–58], it was recognized that such approaches could not properly reflect the transmission of scrapie, BSE, CWD or vCJD as it would occur naturally in the affected hosts. Additionally, it became increasingly evident 'that the requirements for oral and i.p. pathogenesis differ profoundly' and 'that the pathophysiology of prion infection after oral uptake relies on mechanisms and cellular components significantly different from the established requirements for the intraperitoneal route...' [59]. The ultimate routing of infection in naturally acquired prion diseases, such as scrapie, CWD, BSE and vCJD, may depend on a variety of factors that include strain and dose of the agent, or species and PrP genotype of the host. Thus, the spread of agent through the body of vCJD-, BSE-, CWD- and scrapie-affected individuals must be investigated by complementary studies in experimentally challenged animals where such variables can be controlled, as well as in naturally infected hosts.

Prion routing following natural infection or experimental peroral challenge: involved tissue components and pathways of spread

From the data outlined above, it is clear that TSE routing depends on a number of variables. However, a wealth of findings has revealed that the routing of TSE agents through the body follows characteristic phases that may partly operate in parallel (Fig. 1), specifically (a) accumulation of infectious agent in lymphoid tissue, (b) spread to the peripheral nervous system (neuro-

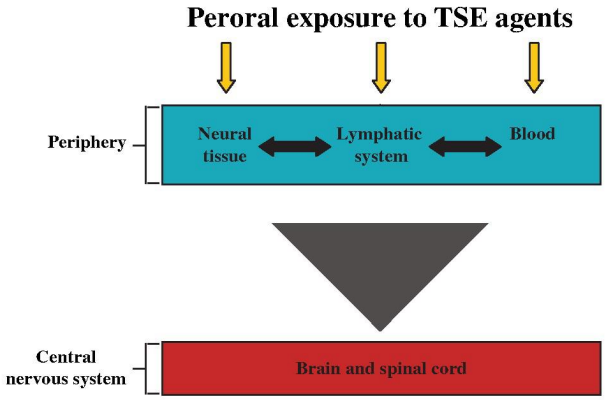

Fig. 1. Possible pathways of neuro- and central nervous system invasion following natural infection or experimental peroral challenge with prions.

invasion), (c) ascension to and dissemination within the brain and spinal cord, and (d) centrifugal spread from the CNS to further peripheral sites such as muscles. The involvement of a hematogenous phase is also conceivable in certain native and experimental hosts at both preclinical and clinical stages of incubation.

The purpose of this review was to overview current knowledge on the spread of scrapie, CWD, BSE and vCJD through the body in naturally affected hosts and in animals experimentally challenged via the alimentary tract. Major insights into the spread of infection through the body were obtained from experimental studies using laboratory rodents orally challenged with TSE agents. The findings from such studies were reviewed together with pathophysiological observations on the spread and targeting of TSE pathogens in native host species of scrapie, CWD, BSE and vCJD, both after natural and experimental peroral infection. The aim was to show how present pathophysiological concepts have emerged from progressing investigations and to highlight that which remains to be achieved in this complex area of research.

Lymphoid involvement in pathogenesis

Scrapie and BSE in laboratory rodents

Kimberlin & Walker [60] provided the first detailed analysis of the spread of scrapie to the CNS following uptake of infectivity via the alimentary tract. After an intragastric challenge of mice, an almost immediate uptake of agent and onset of replication in the intestine was observed that preceded replication in cervical lymph nodes and spleen [60]. Mice fed with scrapie or BSE agent showed initial PrP^{TSE} deposition in Peyer's patches and mesenteric lymph nodes prior to infection of other lymphoid tissues, including the spleen [61]. Splenectomy following intragastric infection of mice had no effect on the incubation period [60]. Thus, consistent with findings in hamsters perorally challenged with scrapie [4,14], there is substantial evidence that – other than for the intraperitoneal route – for this route of infection, the spleen plays little or no role in neuroinvasion. Rather, after alimentary uptake of infectivity, intestinal (and in small ruminants also oropharyngeal) components of the gut-associated lymphoid tissue (GALT) and GALT-draining lymph nodes appear to play a more significant role in the early stages of pathogenesis.

In hamsters fed with 263K scrapie, intestinal lymph nodes and Peyer's patches were identified simultaneously with enteric neurones as the first sites of PrP^{TSE} deposition [62]. Initial infection of the alimentary canal predominantly occurs at the level of the ileum and caudal jejunum (D. Krüger & M. Beekes, unpublished results). Mesenteric lymph nodes draining the jenunal and ileal lymphatic nodules and Peyer's patches were also found to contain PrP^{TSE} in early preclinical incubation. Within the lymphoid follicles, PrP^{TSE} accumulated on the processes of follicular dendritic cells (FDCs), in dome and tingible body macrophages (TBMs), in the follicle-associated epithelium (FAE), possibly associated with microfold cells (M cells), and in cells with dendritic cell (DC) morphology [6,62,63]. Owing to the shortness of the incubation period in this hamster model it was not possible to determine the relative temporal sequence of the appearance of pathological PrP in these GALT elements. Following infection of the GALT, scrapie agent was found at later stages of incubation, in lymphoreticular system (LRS) components such as the spleen [4,63] or submaxillary lymph nodes [63]. The data obtained from this hamster model suggested three options for the involvement of the GALT in neuroinvasion. GALT and/or other non-neuronal gut components are (a) obligatory key players, (b) optional mediators, or (c) bystanders of neuronal infection after oral uptake of infectivity.

The involvement of M cells, DCs, macrophages and FDCs has been investigated comprehensively in other morphological and functional rodent studies [64]. M cells were shown to have the potential to transcytose infectious TSE agent *in vivo* [65], and studies in rats revealed that migrating DCs can take up and transport PrP^{TSE} *in vivo* to mesenteric lymph nodes after the administration of scrapie-associated fibrils into the jejunum [66]. In Peyer's patches, DCs form a layer of cells in the subepithelial dome beneath the FAE and are in close contact with M cells [67].

Accordingly, DCs could potentially act as cellular bridges between the gut lumen and the lymphoid TSE replicative machinery.

Macrophages of the GALT may also have a role in the peripheral pathogenesis of scrapie, CWD, BSE and vCJD. It has been reported that peritoneal macrophages have the ability to reduce infectivity when isolated and co-incubated with TSE agent [68], and PrP^{TSE} has been demonstrated within the lysosomes of splenic TBMs in scrapie-infected mice [69]. Chemical depletion of gut-associated macrophages with particles containing clodronate led to an earlier appearance and to increased amounts of PrP^{TSE} in Peyer's patches after oral infection of mice with scrapie or BSE [70]. The authors of the latter study concluded that the macrophages had fulfilled a function of clearing a proportion of TSE agent that had crossed the gut barrier. As a result, these cells might influence the kinetics of infection by reducing the effective dose available in the germinal centres.

In order to maintain their differentiated state, germinal centre FDCs require lymphotoxin α/β ($LT\alpha/\beta$) signals from B lymphocytes (or T and natural killer cells) and tumor necrosis factor-α (TNF-α) [59,71]. Their role in neuroinvasion was investigated using lymphotoxin β receptor-immunoglobulin fusion protein ($LT\beta R$-Ig)-induced dedifferentiation. With this approach, Mabbott *et al.* [71] observed that mature FDCs appeared to be essential for the spread of infection from the gastrointestinal tract. Treatment of mice with $LT\beta R$-Ig before oral scrapie challenge blocked PrP^{TSE} accumulation in Peyer's patches and mesenteric lymph nodes and prevented neuroinvasion. However, treatment 14 days after oral challenge did not alter the susceptibility or survival time compared with non-$LT\beta R$-Ig treated control mice, suggesting that by this period of time, infectivity had already spread to the enteric nervous system. Prinz *et al.* [59] used a different panel of orally challenged knockout mice to address the involvement of mucosa-associated lymphoid tissue in intestinal neuroinvasion. In β_7 integrin-deficient ($\beta 7^{-/-}$) mice, which exhibit a marked reduction of B cells, T lymphocytes and FDCs in Peyer's patches, scrapie pathogenesis was unimpaired. The authors also found a residual population of FDCs in the Peyer's patches of scrapie-resistant μMT mice and of scrapie-resistant RAG-$1^{-/-}$ mice, which are deficient for B cells, or B and T lymphocytes, respectively. TNF-$\alpha^{-/-} \times LT\alpha^{-/-}$ mice lacking the two cytokines TNF-α and $LT\alpha$ also showed complete resistance to peroral scrapie infection, as did μMT and RAG-$1^{-/-}$ mice. Whereas Peyer's patches of $\beta 7^{-/-}$ mice are atrophic but normal in number, Peyer's patches were found only in reduced numbers in μMT and RAG-$1^{-/-}$ mice, and not at all in TNF-$\alpha^{-/-} \times LT\alpha^{-/-}$ mice. From these and other findings, Prinz *et al.* [59] concluded that FDCs are unlikely to be rate limiting for the gut-mediated uptake of orally ingested scrapie agent. Their results suggested that absolute resistance to oral scrapie infection could be achieved by the absence of Peyer's patches or B cells. The apparent discrepancy between the findings of Mabbott *et al.* [71] and Prinz *et al.* [59] remains to be resolved.

An intact GALT does not appear to be invariably necessary for peroral TSE infection. Neither FDCs nor $CD11c^{+}$ DCs were essential for neuroinvasion in mice perorally challenged with high doses of RML scrapie agent [72]. This observation is consistent with previous findings in rodent models of parenteral infection where reduced susceptibility of immunodeficient mice to TSE challenge was overcome by inoculation with high doses of infectivity [73–76]. Additionally, certain TSE agents, such as the hamster-adapted DY strain of TME, can induce a non-LRS-related neuroinvasion after inoculation into highly innervated peripheral tissues, such as the tongue [77]. Furthermore, transgenic mice, which expressed hamster PrP^{C} under the control of a promotor for neuron-specific enolase in peripheral nerves, but not in lymphoid cells, were susceptible to orally administered hamster scrapie [78]. Together with the conspicuous lack of LRS involvement in the pathogenesis of natural BSE (see below), these findings in murine models suggest that, after an oral challenge, progression of infection can occur in the absence of detectable lymphoid infection.

One way that this may be mediated is by uptake via nerve endings or enterocytes. PrP^{TSE}–protein complexes were found to be transcytosed in vesicular structures across an *in vitro* model of the human intestinal epithelial cell barrier [79]. However, enterocytes were not identified as a site of initial PrP^{TSE} accumulation in mice infected orally with a murine-adapted strain of BSE agent [80].

These collective observations in rodent models suggest that direct infection of the nervous system is possible after alimentary challenge: by high doses of agent, by inoculation into highly innervated tissue, or by exposure to highly neuroinvasive TSE strains. Alternatively, amplification of agent in the GALT and other LRS components might operate as a prerequisite or facilitating factor for neuroinvasion following oral uptake of lower doses of infectivity or of less neuroinvasive strains.

Scrapie and BSE in small ruminants

Alimentary tract involvement in TSE pathogenesis of ruminants has long been suspected. In fundamental

experiments using a bioassay to detect infectivity in the tissues of naturally infected sheep, Hadlow and colleagues showed the early appearance of scrapie agent in tonsil, retropharyngeal and mesenteric lymph nodes, and intestine [34]. Later, a series of comprehensive studies investigated the temporal-spatial appearance and final distribution of PrP^{TSE} deposition in lymphoid and neural tissues of naturally infected Texel sheep (homozygous for VRQ at PrP codons 136, 154 and 171) [36,81–83]. These revealed the palatine tonsil and Peyer's patches of the caudal jejunum and ileum, and the GALT-draining lymph nodes (such as the retropharyngeal, caudal jejunal and ileocaecal lymph nodes), as the first sites where PrP^{TSE} could be detected. GALT tissues of the oropharynx and the gut thus appeared as the sites of primary replication of the scrapie agent [83]. Similar findings have been reported [35] for natural scrapie in VRQ homozygous Romanov sheep. In van Keulen's studies [83], PrP^{TSE} was found initially in TBMs of lymphoid nodules and subsequently associated with FDCs and in unidentified cells of the dome beneath the FAE – possibly dendritic cells or dome macrophages (MΦ). In the GALT-draining lymph nodes, PrP^{TSE} was then found to be associated with free ranging cells in the cortical and paracortical sinuses. Following infection of the GALT and GALT-draining lymph nodes, PrP^{TSE} deposition started in a variety of non-GALT lymphatic tissues, including the spleen, before invasion of the enteric nervous system was observed. An essential role of FDCs and mononuclear cells (presumed to represent macrophages) of the dome in ovine scrapie was also supported by the findings of Heggebø *et al.* [84,85] in naturally infected ARQ homozygous Suffolk sheep. Van Keulen *et al.* [83] suggested that DCs or MΦs may be carrying the scrapie agent from M cells in the follicle-associated epithelium to the germinal centres of lymphoid follicles. After interaction with lymphocytes, they possibly undergo apoptosis and release their cargo, which may be phagocytosed by TBMs or might infect FDCs. Furthermore, DCs or MΦs could spread to cortical and paracortical sinuses in GALT-draining lymph nodes. When PrP^{TSE}-positive free-ranging cells in those sinuses gain access to the efferent lymph stream and subsequently to blood, this may eventually cause a blood borne dissemination of the scrapie agent to non-GALT-associated lymphoid tissues.

When isolated intestinal loops of sheep were experimentally inoculated with scrapie agent, the findings suggested that PrP^{TSE} can be transported across villous mucosa in sheep that have both scrapie-susceptible and scrapie-resistant PrP genotypes [86]. Jeffrey *et al.* [86] interpreted their findings as indicative of transport of inoculum by dendritic cells or macrophages and discussed that infectivity and PrP^{TSE} may be carried across the FAE – at least partially – via different routes.

Despite the consistency of the findings reported by van Keulen *et al.* [36,81–83] and Andréoletti *et al.* [35], it must be emphasized that in sheep scrapie the tropism and distribution of infectious agent and PrP^{TSE} may be influenced by the ovine PrP genotype. Different genotypes of sheep replicate infectivity less efficiently than others, both in terms of incubation period and distribution of infectivity and PrP^{TSE} in peripheral tissues. In sheep carrying the $PrP^{VRQ/ARR}$ genotype, CNS invasion was reported (although not shown) to occur without prior infection of the lymphoid tissue [83]. VRQ/ARR sheep replicate infectivity in the periphery, but less frequently than occurs in conventionally scrapie-susceptible genotypes [87,88]. Finally, Suffolk sheep with the $PrP^{ARR/ARQ}$ or $PrP^{ARR/ARR}$ genotype have been found in British flocks to be largely resistant to scrapie infection and to deposition of PrP^{TSE} in the LRS and CNS [85]. However, pathophysiological findings on the spread of prions through the body may depend not only on the strain of agent or the genotype of the host, but also on the infectious dose. This may hold true, particularly when determining the temporal-spatial course of agent replication and PrP^{TSE} deposition in sheep with highly scrapie-resistant PrP genotypes.

Sheep orally infected with BSE show widespread lymphoreticular deposition of PrP^{TSE} [89,90]. In a time-course study with $PrP^{ARQ/ARQ}$ homozygous Romney sheep that had been perorally inoculated with BSE agent, early lymphoid PrP^{TSE} deposition was detected in varying sites, including retropharyngeal lymph nodes, ileal Peyer's patches and spleen [91]. Within germinal centres, PrP was first found in the cytoplasm of TBMs and then associated with FDCs. Subsequently, infection appeared to spread rapidly throughout the LRS, eventually affecting a broad range of GALT and non-GALT lymphoreticular tissues. However, in some of those BSE-infected sheep, CNS invasion occurred in the absence of detectable PrP^{TSE} in the lymphoid system [91].

CWD in elk and deer

As observed for scrapie-infected sheep, in mule deer experimentally challenged with CWD agent via the oral route, PrP^{TSE} was first detected in alimentary tract-associated lymphoid tissues, such as tonsil, retropharyngeal lymph node, Peyer's patch and ileocecal lymph node [92]. In the tonsils and retropharyngeal

lymph nodes of those animals, PrP^{TSE} was found primarily within germinal centres [93]. Here, it accumulated on or outwith FDC membranes, in the cytoplasm of TBMs and possibly on B lymphocytes. The preclinical cellular distribution of PrP^{TSE} in the lymphoid system was similar to that found in advanced disease.

BSE in cattle

In BSE-affected cattle, the distribution of infectivity and PrP^{TSE} in the lymphoreticular system is relatively limited. To date, field cases of BSE have shown the presence of infectious agent nearly exclusively in CNS tissue and the retina [16]. Following experimental peroral challenge, infectivity was detected during preclinical and clinical phases of incubation in the distal ileum, an area rich in LRS tissue of Peyer's patches [37,94]. Within this tissue, PrP^{TSE} could be identified, mainly in macrophages, in a small proportion of the follicles of Peyer's patches [95]. However, such intestinal PrP^{TSE} immunostaining was not observed in naturally occurring clinical BSE [95]. Only recently have minute traces of infectivity been found, by intracerebral bioassay in cattle, in palatine tonsil tissue from a preclinically infected donor animal experimentally challenged via the oral route [96]. In the context of this atypical finding, the authors of the study emphasized that there is no evidence for widespread lymphatic or hematogenous spread of the BSE agent in affected cattle.

BSE in primates

An immunohistochemical examination of two clinically ill lemurs, from a French zoo, which had both been infected accidentally with BSE-contaminated feed, revealed conspicuous PrP staining (presumed by the authors to indicate infectious BSE agent) in tonsil, spleen and the gastrointestinal tract [97]. Furthermore, PrP^{TSE} was visualized after experimental oral challenge of macaques with BSE [98] in LRS tissues, such as tonsils and spleen, and in the entire gut from the duodenum to the rectum. Here, PrP^{TSE} was found in individual intestinal lymphoid follicles as well as in Peyer's patches, but was not reported to be present in enterocytes.

VCJD in humans

In vCJD patients, infectious agent has been detected by bioassays in tonsils and in the spleen [99]. Further studies [5,100] revealed PrP^{TSE} in tonsils as well as in other components of the lymphatic system (spleen, lymph nodes, and appendix-associated lymphoid tissue) colocalized with FDCs [101] and also macrophages [7]. Most interestingly, PrP^{TSE} was detected in preserved appendix samples removed from patients up to 2 years before the onset of vCJD symptoms and 4 years before death [102–104].

Figure 2 provides a schematic representation of tissue components and pathways found to be involved in

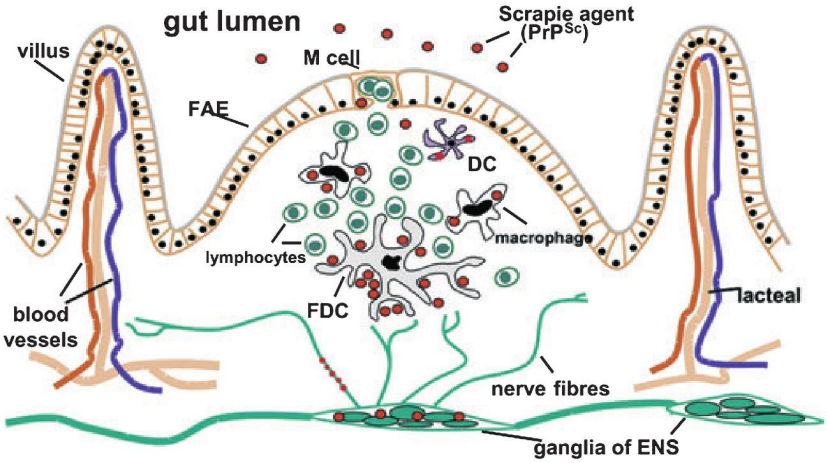

Fig. 2. Intestinal cell types and tissue components showing deposition of disease-associated prion protein from transmissible spongiform encephalopathy-affected individuals (PrP^{TSE}) after exposure of the alimentary tract to transmissible spongiform encephalopathy agents. Microfold cells (M cells) in the follicle-associated epithelium (FAE), dendritic cells (DCs), macrophages, and follicular dendritic cells (FDCs) of the gut-associated lymphoid tissue (GALT), as well as fibres and ganglia of the enteric nervous system (ENS), may be involved in the uptake, replication and spread of prions. Adapted from Mabbott & Bruce [162]. (Note, although not shown here, nerve fibres also extend to contact the lacteal epithelium and villous enterocytes [107]).

the crossing of the gut wall, GALT-related spread of infection and intestinal neuroinvasion following ingestion of TSE agents.

Neuroinvasion, sympathetic and parasympathetic spread to the CNS, and propagation from the brain and spinal cord to peripheral nervous system components

The expression of PrP^C is a prerequisite for cells to sustain TSE infection [105], and the spread of prions from a peripheral site of infection to the brain is dependent on PrP expression in a tissue compartment between the LRS and the CNS [106]. This tissue compartment turned out to be the peripheral nervous system. However, the mechanisms by which ingested TSE agents pass from the GALT or other sites of the alimentary tract to nerve tissue has yet to be elucidated. They may involve interaction or contact between immune cells and nerves [107–109]. Lymphoid organs are innervated largely by the sympathetic nervous system and, more specifically, by branches of the the splanchnic nerve [110,111]. Sensory fibres of the vagus nerve are widely distributed in the gastrointestinal tract and communicate chemically with activated DCs [112,113]. Furthermore, vagal efferents synapse in intrinsic ganglia of the enteric nervous system (ENS) which innervates numerous targets in the intestinal wall, including the mucosa and submucosa [114]. Thus, the alimentary tract provides a variety of candidate sites and pathways for neuroinvasion, including FDC–nerve contacts, anatomical connections between DCs and the peripheral nervous system (PNS), or transfer through exosomes [64].

Scrapie in laboratory rodents

Neural spread of infection from the gastrointestinal tract via the enteric and sympathetic nervous system to the spinal cord after alimentary infection was first suggested by Kimberlin & Walker [60], based on findings from infectivity studies in mice intragastrically challenged with scrapie. More detailed information on the spread of infection from the intestine to the CNS was obtained from chronological studies on the temporal-spatial pattern of PrP^{TSE} deposition in hamsters orally challenged with the 263K scrapie agent [4,6,14, 62,115,116]. In this animal model, initial neuronal deposition of PrP^{TSE} – and thus neuroinvasion – was observed in myenteric and submucosal ENS ganglia of the small intestine [6,62]. The stomach, small intestine and ascending colon are innervated, partly via ganglia of the ENS, by the parasympathetic vagus and sympathetic splanchnic nerves, which thereby constitute a 'CNS–Gut axis'. Whether infection of this neural axis depends on components of the LRS or other intermediate structures, or may occur by direct infection of nerves which abut onto villous epithelium [107], remains to be established. When the dynamics of PrP^{TSE} deposition in the ENS, splanchnic nerve circuitry (celiac and mesenteric ganglion complex–intermediolateral grey column–dorsal root ganglia) and vagus nerve circuitry (dorsal motor nucleus of the vagus nerve–commissural nucleus of the solitary tract–nodose ganglia), as well as the subsequent pattern of PrP^{TSE} deposition in the PNS and CNS, were established in greater detail, this shed further light on how scrapie agent spreads to the CNS [6,115,116]. The findings suggested that the infection ascended retrogradally via autonomic ganglia and efferent fibres of the vagus and splanchnic nerves innervating the gut, to the dorsal motor nucleus in the brain, and to the intermediolateral grey column in the thoracic spinal cord, respectively. From these sites of initial CNS invasion at the level of the thoracic spinal cord and the medulla oblongata, the infection propagated, apparently along defined neuroanatomical projections and in a specific sequence, within the spinal cord and brain in both ascending and descending directions. Centrifugal spread from the CNS appeared to be responsible for subsequent infection of sensory nodose or dorsal root ganglia of the vagus and splanchnic nerve circuitries, respectively (although direct routing from the viscera along sensory fibres to the nodose and dorsal root ganglia cannot be ruled out formally).

A detailed pictorial representation summarizing these observations on the involvement of the enteric nervous system and the splanchnic and vagus nerve circuitries in the routing of infection to the CNS, as well as to sensory nodose and dorsal root ganglia, is given in Fig. 3.

Scrapie and BSE in sheep

Comprehensive studies on the pathogenesis of ovine scrapie, which addressed the question of neuroinvasion and prion propagation to the CNS, were performed by van Keulen *et al.* [36,82,83] in naturally infected Texel sheep. Previously, infectivity had been detected in the peripheral nerves of scrapie-affected sheep [34,117]. Using PrP^{TSE} as a biochemical marker for infectivity, van Keulen *et al.* [36,83] identified the enteric nervous system, at the level of the duodenum and ileum, as the first neural tissue to be invaded by the scrapie agent. The authors discussed that the proximity of Peyer's patches and the submucosal and myenteric plexuses of

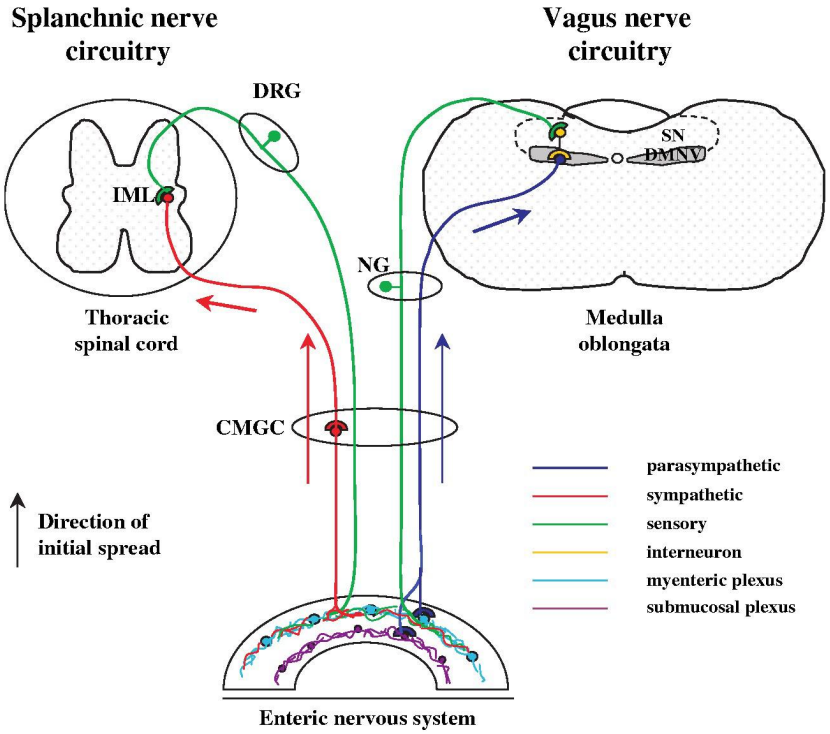

Fig. 3. Neuronal pathways involved in the centripetal spread of prions from the intestine to the brain and spinal cord after peroral infection. As established in great detail in hamsters orally challenged with 263K scrapie [4,6,14,62,115,116], and in sheep with natural scrapie [36,83], initial spread to the central nervous system occurs in a retrograde direction along parasympathetic and sympathetic fibres of the vagus and splanchnic nerves. Enteric and abdominal ganglia are involved early in pathogenesis. CMGC, celiac and mesenteric ganglion complex; DMNV, dorsal motor nucleus of the vagus nerve; DRG, dorsal root ganglion; IML, intermediolateral cell column; NG, nodose gangion; SN, solitary tract nucleus. Adapted from McBride *et al.* [6].

the ENS may facilitate intestinal neuroinvasion. From the ENS, further spread of infection occurred along parasympathetic and sympathetic efferent neuronal pathways of the vagus and splanchnic nerves to the brain and – via the celiac and mesenteric ganglion complex – to the spinal cord, respectively. Initial portals of CNS entry were the dorsal motor nucleus of the vagus nerve in the brain and the intermediolateral grey column in the spinal cord. Subsequently to the dorsal motor nucleus of the vagus nerve, cerebral PrP^{TSE} deposition occurred in the solitary tract nucleus and vestibular nuclei. From the early foci of infection in the CNS, the agent showed further spread in both ascending and descending directions. After PrP^{TSE} had accumulated in the CNS, deposition of the protein was detected in sensory nodosal ganglia and dorsal root ganglia of the vagus and splanchnic nerve circuitry, respectively.

Sheep experimentally infected with BSE agent also exhibited PrP^{TSE} in autonomic and other parts of the peripheral nervous system, such as celiac ganglia, vagus nerve (classified as preliminary positive) and dorsal root ganglia, as well as in the enteric nervous system [90,91].

CWD in elk and deer

The dorsal motor nucleus of the vagus nerve was similarly identified as the first site of PrP^{TSE} deposition in the brain in deer orally challenged with CWD [118]. For CWD, involvement of vagus and splanchnic nerve circuitries in the spread of the agent through the body was further corroborated by immunohistochemical detection of PrP^{TSE} in myenteric ENS ganglia, the cervical vagosympathetic trunk containing parasympathetic vagal nerve fibres, in nodose ganglia, the celiac ganglion and in the intermediolateral grey column of naturally infected deer with clinical disease [42].

BSE in cattle

In a naturally infected cow preclinically incubating BSE, comprehensive paraffin-embedded tissue blot analyses revealed the dorsal motor nucleus of the vagus nerve as the only brain region showing deposition of PrP^{TSE} [119]. This finding pointed to the vagus nerve as a route for initial brain invasion also in BSE after presumed exposure to infectious agent via the alimentary tract.

In contrast to scrapie or BSE in sheep, CWD in deer and vCJD in humans, for natural BSE in cattle the neuronal presence of infectious agent or PrP^{TSE} has been confirmed, until recently, only in CNS tissue and retina [16], and for the distal ileal myenteric plexus [95], respectively. However, a report published in 2006 on three cows that preclinically incubated BSE after natural infection [120] described the detection of

PrP^{TSE} additionally in satellite and ganglionic cells of dorsal root ganglia and in peripheral nerves. After an experimental peroral challenge of cattle with BSE agent, PrP^{TSE} was also detected in myenteric neurons [95] additionally to infectivity in dorsal root ganglia and the trigeminal ganglion [37].

BSE in primates

Clinically diseased nonhuman primates, orally challenged with BSE agent, showed PrP^{TSE} in the enteric nervous system, autonomic sympathetic fibres and peripheral locomotor nerves [98].

VCJD in humans

In vCJD patients, PrP^{TSE} was found in sympathetic celiac and superior mesenteric ganglia [121], and in dorsal root and trigeminal ganglia [101]. Gut ganglia and parasympathetic ganglia also showed positive imunohistochemical staining, but the author stressed that these findings need to be interpreted with caution [101].

Infection of muscles

The finding that in peroral or otherwise naturally acquired TSEs prions spread centripetally to, and centrifugally from, the brain and spinal cord through peripheral nerves, suggested that, following peroral infection, they may eventually also propagate via neural pathways to target tissues other than the lymphoreticular and nervous systems. Muscles from animals provide an important component of human food and have therefore been examined in several studies for the presence of TSE infectivity or PrP^{TSE}. Until recently, this did not reveal any evidence for significant amounts of TSE agents in this type of tissue [34,122], apart from a single report [123]. However, in 2002, a study by Bosque *et al.* [124] described the detection of substantial amounts of infectivity and PrP^{TSE} in hindlimb muscles from mice that had been intracerebrally infected with scrapie.

Following the report by Bosque *et al.* [124], a study using hamsters orally challenged with scrapie [125] detected substantial amounts of PrP^{TSE} in a variety of muscles, including tongue. This provided, for the first time, direct experimental evidence for the spread of infection to muscle tissue in a perorally acquired prion disease. Subsequently, PrP^{TSE} was also detected in the muscles of orally challenged hamsters already prior to the onset of clinical scrapie symptoms, and the presence of infectivity was confirmed in muscle tissue by titration in bioassays [126].

Because the hamster model of oral challenge had previously been shown to provide baseline information about the peripheral routing of infection in naturally occurring ruminant TSEs and other orally acquired prion diseases, the findings from these examinations highlighted the need to thoroughly investigate whether prions can be found in the muscles of animals entering the human food chain. The first results from such studies were reported in 2004 by Andréoletti and coworkers. These authors found PrP^{TSE} accumulation in muscle tissue of naturally infected and of perorally challenged sheep during both preclinical and clinical phases of incubation [127]. Subsequently, PrP^{TSE} was further detected in tongue specimens from preclinically and clinically affected sheep naturally infected with scrapie [128]. Furthermore, bioassays in transgenic reporter mice showed prion infectivity in skeletal muscles of CWD-infected deer [129], and – although at apparently only a very low level – in the musculus semitendinosus of a cow in the clinical stage of naturally acquired BSE [130].

Prion invasion of muscle tissue was also observed in TSEs with an origin other than peroral infection. Bartz *et al.* [131] found PrP^{TSE} in tongue tissue after intracerebral inoculation of hamsters with six different prion strains, whereas Thomzig *et al.* detected pathological PrP in the muscles of hamsters and mice with intracerebrally transmitted rodent-adapted BSE or vCJD [132]. Furthermore, PrP^{TSE} was detected in patients with sporadic CJD [133,134], including those affected by inclusion body myositis [135], and in patients with iatrogenic CJD [134].

Regarding the question of via which pathways prions invade muscle tissue, findings in the hamster model of peroral scrapie infection provided new conceptual pathophysiological insights: they suggested centrifugal spread of infection from spinal or cranial motor neurons via efferent projections to myofibres (Fig. 4) [126]. In skeletal muscle of scrapie infected sheep, however, Andréoletti *et al.* [127] observed PrP^{TSE} deposition in muscle spindles (i.e. in mechanoreceptors innervated by efferent and sensory nerve fibres). Upon intracerebral infection of hamsters with the hyper strain of TME agent, Mulcahy *et al.* [136] observed PrP^{TSE} deposition in the tongue at the neuromuscular junction, as well as associated with sensory nerve fibres in the lamina propria below the mucosal epithelium. This indicated invasion of the tongue via the motor innervation of lingual muscles and, additionally, spread of infection via sensory nerve fibres that project into the epithelial cell layers of the tongue. In more recent studies (Schulz-Schaeffer & Beekes, unpublished results), PrP^{TSE} was also detected in muscle spindles of

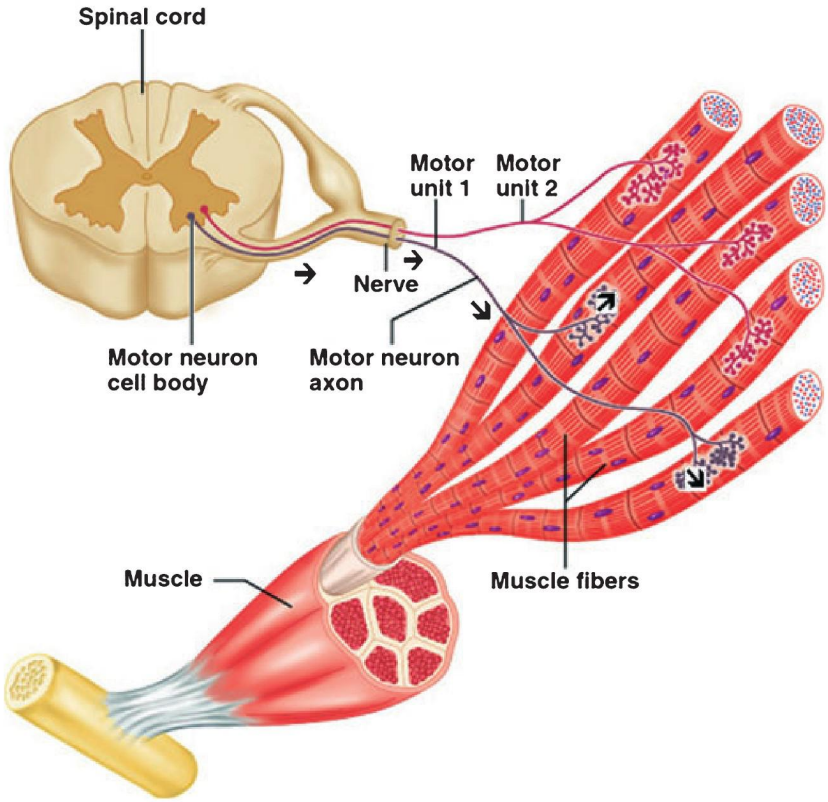

Fig. 4. Centrifugal spread of infection to muscles. The intramuscular location and distribution pattern of disease-associated prion protein (PrP^{TSE}), from transmissible spongiform encephalopathy-affected individuals together with the protein's late occurrence in muscles, suggest a centrifugal spread of infection, as indicated by arrows from spinal (or cranial) motor neurons via efferent projections of motor units to neuromuscular junctions and from there postsynaptically into muscle fibres [126]. As PrP^{TSE} was also found in muscle spindles which act as mechanoreceptors [127], sensory nerve fibres may provide an additional pathway for infection (not shown in this figure). Adapted from a figure by Pearsons Education, Inc. (http://www.people.virginia.edu/~dp5m/phys_304/pix.html).

perorally challenged scrapie hamsters, consistent with infection of muscles via afferent nerves additionally to efferent invasion. Muscle specimens from patients with vCJD were also found to be positive for PrP^{TSE}, but here deposition appeared to be confined to nerve fibres [134].

Blood borne dissemination of prions

When prions obtain access to the bloodstream – for example via infected free-ranging lymphoid cells that cross to the efferent lymph in the cortical and paracortical sinuses of GALT-draining lymph nodes – this may disseminate the infectious agent throughout the body after ingestion of TSE agents. However, several lines of evidence suggest that hematogenous spread does not contribute substantially to the infection of the brain in experimental goat scrapie [137,138], TME of mink [139], and in kuru [44,140] or vCJD patients [141]. However, TSE infectivity was detected at preclinical and clinical stages of incubation, in the blood of sheep naturally infected with scrapie or perorally challenged with BSE agent [142,143]. In addition, human blood donations from donors who preclinically incubated vCJD obviously also contained infectious agent [28–30]. Previously, a low-level 'viremia', lasting for at least 40 days after an intraperitoneal challenge with 263K scrapie, was observed in hamsters [144]. In the latter study, the animals showed infectivity in the brain only 5 days after intraperitoneal injection, and this cerebral infection persisted without replication of the agent during a 40-day observation period. It was therefore suggested by Kimberlin [56] that transport of agent via blood to the CNS may cause a limited infection of a subpopulation of non-neuronal brain cells (e.g. capillary endothelial cells), without replication in, or spread to, other cerebral cell types. Accordingly, hematogenous spread would not constitute a relevant pathway mediating neuroinvasion of infectivity in the brain and spinal cord. This is consistent with findings in hamsters orally challenged with scrapie, where a highly defined temporal-spatial targeting of infection in the CNS, and a consistent lack of early PrP^{TSE} labelling in brain sites with a compromised blood–brain barrier (such as the area postrema or choroid plexus), rather argued against a hematogenous infection of neuronal brain tissue [6]. Further evidence against blood borne infection of neuronal tissue in the CNS comes from studies showing that prion neuroinvasion is not compromised by a deficiency for T cells but depends on the sympathetic innervation of LRS organs in mice peripherally inoculated with prions via parenteral routes [64].

The influence of inflammation on the propagation of prion infection

A further aspect to be considered in the context of invasive prions and their tissue tropism has started to unfold only during the past few years: Jeffrey *et al.* [145] observed, in scrapie-infected sheep, PrP^{TSE} in foci of inflammation in the abomasum which were caused by alimentary parasites. This raised the possibility that the perforations and inflammatory foci induced by those parasites might provide portals of scrapie infection. The influence of inflammation as a factor that possibly enhances the uptake of ingested TSE agents in the alimentary tract [146], and may lead to a more widespread and pronounced accumulation of infectivity in the body, was confirmed in further studies. The latter effect was observed not only in parenterally infected mice [147] but also in naturally acquired sheep scrapie [148]. This is of particular concern because inflammatory processes may facilitate the shedding of TSE agents from infected hosts, for example via urine – as demonstrated in mice that were intraperitoneally infected with scrapie and suffering from a lymphocytic nephritis [149].

Mechanisms of prion propagation in the nervous system

Although comprehensive research efforts have achieved substantial progress in dissecting the spreading pathways of scrapie, CWD, BSE and vCJD infections through the body in naturally affected hosts and animals experimentally challenged via the oral route, the cellular and molecular components and mechanisms that mediate the propagation of prions along nerve fibres of the ENS, PNS and CNS remain elusive. With respect to the nervous system, in principle, several different modes of spread are conceivable, such as (a) axonal transport [150,151], (b) passive translocation in perineural lymphatics, (c) cell-free or cell-associated spread in neural interspaces, (d) sequential infection of Schwann cells [152], or (e) domino-like conversion of PrP^{C} into PrP^{TSE} along nerves [153]. In one report [6], evidence was described which was compatible with translocation of PrP^{TSE} by established axonal transport processes, and, in another study, PrP^{TSE} deposits were detected in nerve fibres of parenterally infected scrapie hamsters between myelin sheet and axon [154]. However, pharmacological segregation of axonal neurofilaments and microtubuli by β,β′-iminodiproprionitril did not influence the apparent rate of spread of infection along peripheral nerves in hamsters after administration of scrapie agent into the footpad [155]. Similarly, transgenic mice overexpressing four-repeat tau with a reported impairment of axonal transport showed incubation times comparable to those of control mice following internerval infection with scrapie [156]. Also, the incubation time after peripheral prion infection was not affected in mice heterozygous for a dynein mutation [157]. Thus, it remains to be established whether prions are transported within axons, or whether their neural spread is mediated by other mechanisms.

Concluding remarks and outlook

Although prion diseases are caused by transmissble agents that differ profoundly from all known conventional pathogens, such as bacteria, viruses, fungi or parasites, the pattern of their retrograde spread from the alimentary tract to the CNS along efferent fibres of autonomic nerves shows a striking similarity to that of pseudorabies virus [115] or to reovirus serotype 3 [62]. Like these viruses, prions spread along well-defined synaptically linked neuronal pathways. However, other than for pseudorabies virus and reovirus, the molecular basis of prion propagation along neuronal processes is still practically unknown. Thus, a better insight into the mechanisms of prion propagation in the nervous system is much needed. This might not only indicate new approaches for interfering with the spread of infection in the PNS, but also provide therapeutic avenues for inhibiting disease progression in the brain and spinal cord.

The prophylaxis or therapy of TSEs is a notoriously challenging task. Prophylactics against nonexperimental TSEs are not yet available, and it is not currently possible to treat these diseases in a causal manner [158,159]. However, during the past few years, vaccines have been under development which exhibited certain protective effects against intraperitoneally or perorally transmitted prion diseases under laboratory conditions [160,161]. In particular, mucosal vaccination with an attenuated *Salmonella* vaccine strain expressing mouse PrP has been found to cause significant delay or prevention of prion disease in mice later orally exposed to scrapie agent [161]. The authors discussed that the observed protective effect probably predominantly resulted from a reduction of the infective inoculum via immunoglobulin A neutralization in the gut. Although it is currently too early to speculate whether mucosal vaccination against peroral prion transmission provides an immunoprophylactic approach that may potentially contribute to the prevention, or even eradication, of naturally transmitted diseases such as scrapie, BSE and CWD, this possibility certainly merits further exploration.

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