Prion pathogenesis and secondary lymphoid organs (SLO): Tracking the SLO spread of prions to the brain

Citation for published version:
Mabbott, NA 2012, 'Prion pathogenesis and secondary lymphoid organs (SLO): Tracking the SLO spread of prions to the brain', Prion, vol. 6, no. 4, pp. 322-333.

Published In:
Prion

Publisher Rights Statement:
2012 Landes Bioscience

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
Prion pathogenesis and secondary lymphoid organs (SLO)

Tracking the SLO spread of prions to the brain

Neil A. Mabbott
The Roslin Institute and Royal (Dick) School of Veterinary Studies; University of Edinburgh; Midlothian, UK

Prion diseases are subacute neurodegenerative diseases that affect humans and a range of domestic and free-ranging animal species. These diseases are characterized by the accumulation of PrPSc, an abnormally folded isoform of the cellular prion protein (PrPC), in affected tissues. The pathology during prion disease appears to occur almost exclusively within the central nervous system. The extensive neurodegeneration which occurs ultimately leads to the death of the host. An intriguing feature of the prion diseases, when compared with other protein-misfolding diseases, is their transmissibility. Following peripheral exposure, some prion diseases accumulate to high levels within lymphoid tissues. The replication of prions within lymphoid tissue has been shown to be important for the efficient spread of disease to the brain. This article describes recent progress in our understanding of the cellular mechanisms that influence the propagation of prions from peripheral sites of exposure (such as the lumen of the intestine) to the brain. A thorough understanding of these events will lead to the identification of important targets for therapeutic intervention, or alternatively, reveal additional processes that influence disease susceptibility to peripherally-acquired prion diseases.

Prion diseases (transmissible spongiform encephalopathies) are sub-acute neurodegenerative diseases that affect both humans and animals. During prion disease aggregations of PrPSc, an abnormally folded isoform of the cellular prion protein (PrPC), accumulate in affected tissues. Prion infectivity co-purifies with PrPSc and is considered by some to constitute the major component of the infectious agent.1,2 Many prion diseases, including natural sheep scrapie, bovine spongiform encephalopathy (BSE), chronic wasting disease (CWD) in cervids and variant Creutzfeldt-Jakob disease (vCJD) in humans, are acquired peripherally such as by oral exposure. The transmissible nature of many prion diseases is an intriguing feature, when compared with other protein-misfolding diseases. However, recent data have started to challenge the opinion that this is restricted to prion diseases. Peripherally applied β-amyloid has been reported to induce cerebral β-amyloidosis in recipient APP23 transgenic mice.3 Similarly, pathological α-synuclein aggregates from the brain of a patient with Lewy body disease when injected into A53T α-synuclein transgenic mice induced the aggregation of endogenous α-synuclein in their enteric nervous systems.4

After exposure, many prion diseases first replicate within the secondary lymphoid organs (SLO) as they make their journey from the site of infection to the central nervous system (CNS) (a process termed “neuroinvasion”).5-9 Prion replication within SLO is critical for efficient neuroinvasion.5-7,10 From lymphoid tissues, prions appear to subsequently invade the CNS by spreading throughout the peripheral nervous system,11 although hematogenous spread cannot be entirely excluded.

Due to the availability of an ever increasing battery of immunodeficient and transgenic mouse, much attention in
recent years has focused on how prions are initially transported from the site of infection to the SLO, and onwards into the CNS. This article therefore focuses on the pathogenesis of the acquired prion diseases, and describes recent progress in our understanding of the cellular mechanisms that influence prion neuroinvasion from peripheral sites of exposure.

**Identification of the Cellular Sites of Prion Replication in SLO**

The stromal cells within SLO are a heterogeneous group of non-migratory cells with diverse functions including the organization and compartmentalization of SLO, the formation of the extracellular matrix, the guidance and survival of immune cells, and the formation of conduits through which small lymph-borne antigens may be delivered to the lymph node parenchyma. Follicular dendritic cells (FDC) are an important subset of these stromal cells and are situated within the primary B-cell follicles and germinal centers of SLO. FDC should not be confused with the bone marrow-derived classical dendritic cells (DC), as they are an entirely distinct, non-phagocytic and non-migratory stromal cell lineage. In the SLO of some sheep with natural scrapie, cervids with CWD and patients with vCJD, prions accumulate first upon FDC as they make their journey from the site of infection to the CNS (a process termed neuroinvasion). Many studies in mice have shown that prion accumulation upon FDC is important for efficient neuroinvasion after peripheral exposure. Furthermore, prion accumulation in the spleen and subsequent neuroinvasion are both impeded in immunodeficient mice that specifically lack FDC, or following their temporary de-differentiation. Although prion neuroinvasion from peripheral sites of exposure is dependent upon the presence of FDC in lymphoid tissues, it was uncertain whether these cells actually express PrPC and replicate prions. Expression cellular of PrPC is mandatory to sustain prion infection, and FDC appear to express high levels on their cell surfaces. Some studies have exploited the mesenchymal and non-hematopoietic-origin of FDC in order to attempt to address their role in prion pathogenesis. In these studies, mismatches were created in Prnp expression (which encodes PrP) between the FDC-containing stromal and lymphocyte- and leukocyte-containing hematopoietic compartments by grafting bone marrow cells from Prnp-deficient (Prnp–/–) mice into Prnp-expressing wild-type mice and vice versa. Using this approach FDC and all other stromal cells were derived from the recipient, whereas lymphocytes and other hematopoietic lineages were derived from the donor cells. Following peripheral exposure prion accumulation upon FDC was only detected in the spleens of mice with a Prnp-expressing stromal compartment.

While these studies clearly show that the presence of FDC is important for prion accumulation in the spleen, it was not possible to dissociate the Prnp expression status of FDC from that of the nervous system and all the other stromal and non-hematopoietic host-cell populations. The precise identification of the cell populations that facilitate prion replication in SLO is important for a number of reasons. Prion infection can occur within an inflammatory PrPC-expressing stromal cell population at sites of chronic inflammation that is phenotypically distinct from FDC. Most evidence indicates that FDC do not derive from hematopoietic precursors. However, the detection of donor hematopoietic cell-derived antigens on the surface of FDC in recipient mice was originally considered evidence of an FDC precursor cell population within the bone marrow. With hindsight these observations were most likely due to the FDC’s efficient ability to acquire exosome-associated antigens from neighboring cells. These microvesicles enable FDC to passively acquire and display proteins on their surfaces that they do not express at the mRNA level. Both PrPC and PrP are released from cells in association with exosomes. This suggests that FDC may likewise passively acquire PrPC and prions after their release in exosomes from other infected cells. The efficient ability of FDC to acquire exosome-associated proteins on their surfaces was recently highlighted in a detailed ultrastructural study of mouse Peyer’s patches. The intestinal epithelium-specific gene Gpa33 is expressed highly by enterocytes throughout the intestine and not by Peyer’s patch FDC. However, IHC analysis of Peyer’s patches shows FDC accumulate high levels of enterocyte-derived Gpa33 protein on their surfaces after being shed from the intestinal epithelium in association with exosomes.

To determine whether FDC also passively acquire PrPC embryonic day 15 spleen tissue from PrP-deficient (Prnp–/–) mice or wild-type (WT) controls was transplanted under the kidney capsule of WT recipient mice. Within the grafted spleens, the stromal FDC are of donor Prnp-genotype, whereas the majority of the hematopoietically-derived cells within the graft (lymphocytes, leukocytes, etc.) and all cell lineages within the rest of the mouse are of host Prnp-genotype. If FDC do acquire PrPC from other cell lineages one would expect to detect PrPC upon the surfaces of the FDC within the Prnp-deficient spleens grafted into WT mice (Prnp–/–→WT). Grafted spleen tissues within the recipient kidneys were analyzed by IHC 6 weeks after transplant and PrPC-expressing FDC were detected, as anticipated in the B cell follicles of the WT donor spleens grafted into WT recipients (WT→WT). However, the FDC within the Prnp–/– spleens grafted into WT mice (Prnp–/–→WT) lacked PrPC expression indicating that FDC do not simply acquire PrPC from other cell populations (Fig. 1).

FDC characteristically trap and retain native antigen on their surfaces for long periods in the form of immune complexes, consisting of antigen-antibody and/or complement components. FDC themselves express negligible levels of complement component C4 at the mRNA level, but the detection of large quantities of activated C4 protein on their surfaces by IHC illustrates their capacity to capture and retention of immune complexes. Prions also appear to be acquired by FDC as complement-opsonized complexes. Thus FDC may simply act as concentrating depots for prion-containing, complement-opsonized complexes. FDC and mature B cells express high levels of C42 which encodes the
complement receptors CR2/CR1 (CD21/35). Victoratos and colleagues illustrated how CD21-cre mice could be used to study FDC-specific gene function. Using this approach we recently created unique compound transgenic mice in which PrP<sup>C</sup> expression could be specifically "switched on" or "switched off" only on FDC. These mice where then used to establish whether FDC amplify prions, or simply acquire them from other infected host cells. In the first mouse line, PrP<sup>C</sup>-expression was switched on only on FDC. In the SLO of these mice only the FDC had the potential to replicate prions since they were the only cells in the body which expressed PrP<sup>C</sup>. Our data showed that the expression of PrP<sup>C</sup> only on FDC was sufficient to sustain high levels of prion replication upon FDC after peripheral exposure. These data confirm that FDC are the critical sites of prion replication in SLO.

We also created a second compound transgenic mouse model in which PrP<sup>C</sup> expression was specifically "switched off" only on FDC. If FDC are the critical sites of prion replication in the spleen, then one would also expect this to be blocked when PrP<sup>C</sup> expression was ablated only on FDC. Our data confirmed this to be the case (Fig. 2). As PrP<sup>C</sup> expression in all other host cells (e.g., nerves) in these mice was unaffected, these data clearly show that FDC do not simply acquire prions following release from other infected host cells, even in mice displaying clinical signs of prion disease in the brain.

Ultrastructural analysis of the cellular compartments within which PrP<sup>Sc</sup> localizes upon/within FDC has failed to detect any intracellular accumulation. Instead the PrP<sup>Sc</sup> appears to accumulate along the surfaces of their dendrites, increasing in density as the infection proceeds. This suggests that de novo PrP<sup>Sc</sup> conversion occurs upon the FDC surface.

The accumulation of high levels of PrP<sup>Sc</sup> within the brain ultimately leads to the development of neuropathology. Antigens trapped on the surface of FDC are considered to promote the development of high affinity antibody responses and to maintain immunological memory. Despite the detection of high levels PrP<sup>Sc</sup> upon the surfaces of FDC within SLO throughout almost the entire duration of the disease, no gross immunological defect has been reported. However, ultrastructural analysis of prion-affected SLO has revealed evidence of disease-associated morphological changes to FDC. These include adversely affected maturation cycles, abnormal dendritic folding and exacerbated accumulation of immune complexes between the FDC and isolated lymphoid follicles (ILF). In combination with the mesenteric lymph nodes, these tissues help protect the host from gastro-intestinal infections. However, within days of oral prion exposure early replication occurs upon FDC in Peyer's patches, and is obligatory for efficient neuroinvasion.

Further studies are necessary to determine whether these defects lead to subtle effects on FDC-dependent immune function such as impairments to antibody affinity maturation.

**Prions Spread from SLO to the Brain via the Peripheral Nervous System**

The SLO are highly innervated with sympathetic neurons, and following replication upon FDC prions subsequently infect the peripheral nervous system. The depletion of sympathetic nerves impedes prion neuroinvasion, indicating that following replication upon FDC, translocation to the CNS occurs via the peripheral nervous system. While the relative positioning of FDC to peripheral nerves has been shown to influence the rate of neuroinvasion, little is known of how the neuroinvasion actually occurs. Studies have also suggested that mono-nuclear phagocytes such as classical DC may transfer prions directly to the nervous system. However, whether these cells do play an important role in the transfer of prions from the immune to the peripheral nervous system in vivo is uncertain since subsequent data suggested this was unlikely.

**M Cells: Portals for Prions Across the Intestinal Epithelium**

The gut-associated lymphoid tissues (GALT) include the appendix, tonsils, Peyer's patches, colonic and cecal patches and isolated lymphoid follicles (ILF). In combination with the mesenteric lymph nodes, these tissues help protect the host from gastro-intestinal infections. However, within days of oral prion exposure early replication occurs upon FDC in Peyer's patches, and is obligatory for efficient neuroinvasion.
differentiation of M cells in the gut epithelium and maintains them in their differentiated state. However, the ability of M cells to mediate the transepithelial transfer of particulate antigens has been exploited by a range of pathogens, including bacteria and viruses, to enable them to gain entry into mucosal tissues.

Data from studies in which prions were immunohistologically traced after oral exposure, or using an in vitro system with “M cell-like” transcytotic properties, suggest that M cells are the initial sites of prion transcytosis across the intestinal epithelium. However, other studies using similar approaches suggested that this translocation occurred instead via enterocytes, independently of M cells. A further study showed that while some uptake of PrPSc was detectable within M cells, the majority appeared to localize to, and be transcytosed by, FAE-associated enterocytes. The significance of these observations for prion neuroinvasion from the intestine was uncertain.

The tumor necrosis factor (TNF) superfamily member receptor activator of NFκB ligand (RANKL) has been shown to be a critical factor that induces the differentiation of M cells in the gut epithelium and maintains them in their differentiated state. Within Peyer’s patches RANKL is expressed by subepithelial stromal cells beneath the FAE and signals via its receptor RANK (receptor activator of NFκB) which is expressed by epithelial cells throughout the intestine. M cells can be specifically depleted in vivo by RANKL neutralization, and are absent in RANKL-deficient mice. Data from recent experiments utilizing RANKL neutralization to specifically deplete M cells show that both the early prion accumulation upon FDC in Peyer’s patches and neuroinvasion were blocked in their absence at the time of oral exposure (Fig. 3). These data suggest that M cells are the important sites of prion uptake from the gut lumen into Peyer’s patches.

Whether prions are specifically acquired by M cells is uncertain. Their transcytosis could occur non-specifically via the bulk uptake of particulate antigen. However, molecules such as Gp2 which are highly and specifically expressed by M cells can be utilized by certain types of bacteria to aid their uptake. M cells also express high level of cellular PrPC, which is implicated in prion uptake from the gut lumen. Further studies are necessary to determine whether prions bind to the apical surface of M cells specifically or whether other molecules on the apical surface could act as a transcytotic receptor for other pathogenic microorganisms as suggested for the uptake of Brucella by macrophages.

Figure 2. The specific ablation of PrPC expression only on FDC blocks prion replication in the spleen. CD21-cre mice can be used to study FDC-specific gene function. (A and B) Using this approach we recently created a compound transgenic mouse in which PrPC expression could be specifically “switched off” only on FDC. (C and E) Following peripheral prion exposure high levels of PrPSc accumulate upon the surfaces of FDC in the spleens of control mice. (D and F) However, prion replication is blocked in the spleens of mice in which PrPC expression was specifically “switched off” only on FDC. Arrow heads in (D) show scavenged PrP within TBM. Arrows in (E) show PrPSc-positive FDC in the spleens of control mice. (A and B) scale bar = 100 μm; (C and D) scale bar = 20 μm; (E and F) scale bar = 500 μm. Adapted from McCulloch et al.

CD11c+ Cells Aid the Delivery of Prions to FDC in the GALT

Once antigens have been transcytosed by M cells they exit into the intraepithelial pocket beneath the basolateral membrane where they are processed by the lymphocytes and mononuclear phagocytes (MNPs, a heterogeneous population of macrophages and classical dendritic cells) within it or immediately below in the sub-epithelial dome. Migratory bone marrow-derived classical DC are centrally involved in the transport of antigens both within Peyer’s patches and into the mesenteric lymph nodes. Classical DC characteristically internalize antigens and process them into short peptides which they present to naïve T cells. Unlike macrophages, classical DC appear to be equipped with both degradative and nondegradative antigen uptake pathways to facilitate antigen presentation to both T and B cells. The ability of classical DC to capture and retain unprocessed
antigen, and migrate into B-cell follicles suggested classical DC are plausible candidates for the propagation of prions to and within SLO. Expression of the integrin α X (Itgax, CD11c) is commonly used to discriminate classical DC from macrophages. The use of transgenic mice in which CD11c+ cells can be specifically depleted has significantly extended our in vivo understanding of the immunobiology of classical DC. The depletion of CD11c+ cells prior to prion exposure impedes replication upon FDC in the draining SLO and delays neuroinvasion from the intestine, peritoneal cavity and skin. These data imply that following their transcytosis across the FAE by M cells, prions are subsequently propagated toward the FDC-containing B-cell follicles by classical DC. However, further studies are necessary to test this hypothesis. Our recent data illustrate how there is a distinct lack of absolutely unique cellular markers in the MNP system, and CD11c-expression alone does not specifically identify classical DC. Thus, whether the CD11c+ cells that influence prion pathogenesis are classical DC, or another MNP population within the Peyer’s patches, likewise remains to be determined.

Tunnelling nanotubes (TNT) have been proposed as a novel intercellular conduit through which prions and other pathogens such as HIV, may disseminate intracellularly between cells. Data also suggest that HIV may further utilize TNT to evade virus-specific antibody responses by shuttling virus-derived immunosuppressive factors from infected MNP to B cells. The protein M-Sec (encoded by Tnfaip2) is expressed at high levels by M cells and MNP and functions as a key regulator of TNT formation. Most M cells within the FAE of Peyer’s patches appear to have a one-to-one association with MNP which extend their dendritic processes into the basolateral pockets of M cells. This tight association raises the suggestion that prions may also be transferred intracellularly between M cells and MNP via TNT. It is also plausible that prions subsequently disseminate onwards from infected MNP to other cells via TNT.

**Mononuclear Phagocytes: A Double-edged Sword during Prion Pathogenesis**

While the nature of the CD11c+ cells implicated in prion propagation remains to be determined, it is clear from current data that depending on the context, MNP may act as a double-edged sword during prion pathogenesis. As described above, some MNP, perhaps those typical of classical DC, might behave as “Trojan horses” and provide a safe haven in which prions may be propagated to and within SLO. In contrast, highly phagocytic macrophages may facilitate their destruction.

Tingible body macrophages (TBM) are a subset of large MNP specific to the germinal centers of SLO. TBM characteristically contain many remnants of phagocytised apoptotic lymphocytes in various states of degradation (termed “tingible bodies”). Increased numbers of disease-specific PrP-containing TBM are found within the B-cell follicles of prion-affected animals. The detection of PrPSc only within the endosomal compartments of TBM, suggests that these cells scavenge and degrade prions from infected FDC. High levels of prions rapidly accumulate within the SLO within weeks of peripheral exposure. Following infection of the CNS the accumulation/replication of prions appears to occur exponentially until the death of the animal. However, the magnitude of the prion accumulation within the spleen rapidly reaches a plateau level which is maintained for the duration of the disease. How this plateau is maintained is uncertain. This may be due to the establishment of a competitive state whereby FDC act to amplify prions above the threshold required to achieve neuroinvasion, whereas macrophages act to destroy them. The ability of MNP to provide a protective response against prions is further illustrated when signaling pathways which stimulate pro-inflammatory responses by these cells are blocked. Prion accumulation in the spleen is enhanced in mice deficient in interferon regulatory factor 3 (IRF3), a key transcription factor of the MyD88-independent type I interferon production pathway. Similarly, tumor necrosis factor receptor 1-deficiency facilitates replication of RML scrapie prions within macrophages within SLO

Recent data challenge the opinion that M cells are major site of antigen transcytosis in the FAE. Ultrastructural analysis shows FAE enterocytes may also exocytose the intravacuolar contents of their late endosomes into the extracellular...
space of the SED.53 This potentially re-

presents a novel, previously unrecognized

important component of the normal

machinery for antigen presentation in the

GALT. These data also raise the ques-

tion of whether the route through which

antigens are transcytosed across the FAE

fundamentally influences the nature of

the immune response induced to those

antigens? Do antigens which are sampled

via M cells induce protective immune

responses, whereas a more tolerogenic

response is induced after transfer via FAE

enterocytes? After being exocytosed by

FAE enterocytes PrP80 appeared to be sub-

sequently detected within the endosomal

compartments of more macrophage-like

MNP cells rather than classical DC.33

In the absence of M cells after RANKL-

neutralization, prion neuroinvasion from

the GALT is blocked.66 Although possible

effects of RANKL-neutralization on FAE

enterocytes cannot be entirely excluded

in the above study,66 it is tempting to

speculate that following their transcytosis

by M cells or FAE enterocytes the fate of

the prions also differs: transfer via FAE

enterocytes, leading to phagocytosis and

destruction by MNP in the sub-epithe-

lial dome; transfer via M cells, facilitat-

ing uptake and transport by classical DC

toward FDC within the B-cell follicles.

Aging Dramatically Influences

Prion Pathogenesis within SLO

The ingestion of BSE contaminated meat

products is most likely the original source

of vCJD in humans.88 During the UK BSE

epidemic almost 500,000 infected cattle

were likely to have been slaughtered for

human consumption.89 Despite the proba-

ble widespread exposure of the UK human

population to the BSE agent, most clinical

cases of vCJD have occurred almost exclu-

sively in young adults (median age at onset

disease = 26 y; median age at death = 28 y). These data starkly contrast those for

sporadic CJD which have predominantly

occurred in the elderly (median age at onset of disease = 67 y).90 The suggestion

that this age-related incidence of vCJD

was simply due to exposure to greater

levels of BSE through dietary preference

has not been substantiated.91 This indi-

cated that other age-related factors were

also influencing the susceptibility to some

acquired prion diseases.

Host age significantly impairs immune

function. This raised the suggestion that

the effects of aging on the host’s immune

system may also influence the pathogen-

esis of many acquired prion diseases such

as natural sheep scrapie, CWD and vCJD

which replicate in SLO prior to neuroin-

vasion. This hypothesis is consistent with

data from a comparative study of Peyer’s

patches from sheep, cattle and humans

that suggested an age-related association

between Peyer’s patch development and

susceptibility to natural prion disease.32 In

aged (600-d-old) mice FDC are impaired, and

in contrast to those in young adult

spleens, appear atrophic and have lim-

ited capacity to trap and retain immune

complexes. As a consequence, aged mice

elicited poor antibody responses.93-96 Studies

using aged mice that show that coincident

with the effects of host age on FDC sta-
tus, the early replication of ME7 scrapie

prions upon FDC in the spleen was sig-
nificantly impaired.97 Furthermore, fol-

lowing peripheral exposure (via oral and

intraperitoneal routes), none of the aged

mice developed clinical prion disease dur-
ing their lifespans, although some dis-

played histopathological signs of disease

in their brains. A similar effect of host age on

susceptibility to peripheral infection with

RML scrapie prions has also been reported

in reference 98. Data also show that the

underdeveloped or reduced functional

status of FDC in neonatal mice likewise

coincides with impaired prion neuroinva-

sion following peripheral exposure.99,100

Whether a similar correlation is observed

in natural prion infections remains to be
determined.

Four cases of vCJD have also been

reported in recipients of blood or blood

products derived from vCJD-infected

donors.101-104 Interestingly, three of these

cases were reported in elderly patients: two

cases preclinical,102,104 one case clinical.101

This raised the possibility that intravenous

(i.v.) exposure may be more efficient in the

elderly when compared with other periph-

eral routes of exposure. Using an aged

mouse model, data show that, as in young

adult mice, the i.v. route is more efficient

than other peripheral routes of prion expo-
sure.105 However, disease pathogenesis in

i.v. exposed aged mice was significantly

impaired when compared with young mice

with most failing to develop clinical dis-

case during their lifespans.

The effects of host age on the ability of

FDC trap and retain immune com-

plexes are not simply due to the reduced

expression of complement receptors, or

reduced levels of opsonising complement

components in the serum, since these were

unaffected in aged mice.34 In the spleen

the marginal zone (MZ) forms a bar-

rier around the lymphocyte-containing

white pulp and plays an important role

in the capture of blood-borne antigens

and immune complexes, and facilitates

their efficient transport to FDC.12,106 The

B cells within the MZ continuously shuttle

between the MZ and B-cell follicles in the

underlying white pulp and play an impor-
tant role in the capture and transport of

blood-borne, complement-bound antigens

to FDC.106 Aging dramatically disturbs

the microarchitecture of the MZ (Fig. 4),

which impedes the delivery of immune

complexes to FDC.34 Since prions are like-

wise considered to be acquired by FDC as

complement-bound complexes,36-39 these

aging-related disturbances to the MZ

appear to impede the shuttling of prion-

containing complement-bound complexes

to FDC. As a consequence, prion replica-

cation on FDC is reduced and neuroinva-

sion impeded.34,97

The expression of PrP80 by FDC in the

spleens of aged mice is also dramatically

reduced.97 The mechanism responsible for

the downregulation of PrP80 expression

on aged FDC is uncertain. The expres-

sion of PrP80 by FDC in the spleen can be

stimulated by immune complex-trapping,

and is blocked in the absence of comple-

ment.107 Whether the reduced expression

of PrP80 by aged FDC is a consequence of

their reduced ability to trap complement-

opsonised immune complexes remains to

be determined.

The Presence of Prions

in SLO and Brain

Does Not Always Correlate

No prion-specific preclinical diagnostics

are currently available compounding the

problems for the assessment of disease-

incidence, treatment and eradication.
The detection of prions in blood,\textsuperscript{108} or lymphoid tissue biopsy specimens such as tonsils, appendix,\textsuperscript{19,109} and rectal-associated lymphoid follicles,\textsuperscript{110-113} have each been considered as useful preclinical diagnostics. Using transgenic mice expressing the ovine or human form of PrP\textsuperscript{C}, a recent study compared the ability of brain and SLO to replicate foreign, inefficiently transmitted prions. This might occur following the transmission of a prion disease between different species (termed “the species barrier”).\textsuperscript{114} The SLO in these transgenic mice were found to be consistently and markedly more permissive than the brain to cross-species infection with prions including CWD or BSE. These data illustrate how continued surveillance of SLO will be important to provide a realistic measure of the incidence of preclinical prion disease or “silent carriers,” within populations.

Analysis of the spleens and brains of prion-exposed aged mice likewise revealed that there was considerable variation in the detection of prions in the spleens of the clinically-negative aged survivors.\textsuperscript{34,97} While host age may represent an important barrier to the efficient transmission of peripherally acquired prion disease, data from these studies also suggest that there may be significant levels of subclinical prion disease in the elderly population.\textsuperscript{34,97} Although many prion-exposed aged mice displayed histopathological signs of prion disease in their brains, prions were absent in the spleens of some mice. Thus, while the analysis of SLO will aid our understanding of the prevalence prion diseases,\textsuperscript{114} such tests may be much less sensitive when used on elderly patients and livestock.

**Conclusions**

Recent advances in the study of the spread of prions to the brain suggest that after oral exposure neuroinvasion occurs via an elegant cellular relay (Fig. 5). After ingestion of a contaminated meal, prions appear to be actively transcytosed across the FAE from the gut lumen by M cells and FAE enterocytes.\textsuperscript{33} However, in the absence of M cells neuroinvasion is blocked.\textsuperscript{66} Once the prions have crossed the epithelium they are subsequently acquired by MNP (macrophages and classical DC). Current hypotheses suggest classical DC, in contrast to macrophages, act as Trojan horses and carry the prions onwards to the FDC in the B-cell follicles.\textsuperscript{77-79} The prions then infect and replicate upon FDC which are considered to amplify the prions above the threshold level required for neuroinvasion.\textsuperscript{10} Following their expansion upon FDC, prions subsequently infect neighboring nerve fibers of the enteric nervous system.\textsuperscript{33} Prions are mainly considered to spread to the CNS via the peripheral nervous system,\textsuperscript{11,49} (sympathetic and parasympathetic) although it is plausible that under some circumstances the hematogenous route may provide a parallel pathway of neuroinvasion.

Multiple factors other than the prion protein genotype of the host have been shown to significantly influence prion disease susceptibility, such as the effects of aging on the microarchitecture of the SLO.\textsuperscript{34,97} Studies show chronic inflammation may enhance prion uptake or expand their tissue distribution.\textsuperscript{7,28,115} Bacterial colitis also influences oral prion susceptibility.\textsuperscript{116} Infection with certain pathogenic bacteria has been shown to affect the density of M cells within the gut epithelium.\textsuperscript{117,118} Therefore, it is plausible that an expansion of M cells induced by concurrent infection with an intestinal pathogen
Figure 5. A potential cellular relay that mediates prion neuroinvasion after oral exposure. After ingestion of a contaminated meal, prions appear to be actively transcytosed into Peyer’s patches by M cells and enterocytes in the follicle-associated epithelium. In the absence of M cells neuroinvasion is blocked suggesting that M cells are the important sites of prion uptake from the gut lumen. The prions are subsequently acquired by mononuclear phagocytes (macrophages and classical DC) in the sub-epithelial dome of the Peyer’s patches. Current hypotheses suggest classical DC, in contrast to macrophages, act as Trojan horses and carry the prions to the FDC in the B cell follicles. The prions then infect and replicate upon FDC. Following their expansion upon FDC, prions subsequently infect enteric nerves. The prions then spread to the CNS via the peripheral nervous system (both sympathetic and parasympathetic).
or inflammatory stimuli may also influence oral prion susceptibility.

Recently, exposure via the nasal cavity has been proposed as another potentially important route of prion transmission. Furthermore, high levels of prions have been reported in the olfactory sensory epithelium. Furthermore, prions appear to be shed from the olfactory mucosa of affected animals. Interestingly, despite the presence of FDC within the nasal-associated lymphoid tissues, prion neuroinvasion appears to occur independently of SLO after exposure via the tongue or nasal cavity, implying direct infection of the nervous system. Further studies are clearly necessary to determine whether inhalation represents a significant natural route of prion transmission in domestic and free-ranging ruminants.

Finally, although significant progress has been made in our understanding of how some prion diseases establish infection within the SLO, little is known of how they subsequently infect the peripheral nervous system. A thorough understanding of this key event in neuroinvasion may identify important targets for therapeutic intervention, or alternatively, reveal additional processes that influence disease susceptibility to peripherally-acquired prion diseases.

Acknowledgments

The author is supported by project and Institute Strategic Programme Grant funding from the Biotechnology and Biological Sciences Research Council, Medical Research Council, European Commission (FP7) and University of Edinburgh Development Fund.

References


