Prions and the blood and immune systems

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Prion diseases take a number of forms in animals and humans. They are caused by conformational change in widely expressed prion protein leading to the formation of intracellular aggregates. Although the main focus of disease is the central nervous system, it is known that involvement of the immune system occurs in peripherally transmitted disease in particular. Animal experiments suggest that in some prion diseases follicular dendritic cells in the germinal centers are a major site of initial accumulation, and that abnormal prion protein and infectivity are detectable in peripheral lymphoid tissue from the earliest phase of disease. This raises the possibility that in a human peripherally transmitted prion disease like variant Creutzfeldt-Jakob disease, further transmission could occur through blood or tissue products or contamination of surgical instrumentation. Indeed two recent reports confirm that this disease has been transmitted by blood, raising significant public health concerns.

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Prion diseases have a long and interesting history. Scrapie, the prototypic prion disease was first described in sheep over 300 years ago and was recognized to be experimentally transmissible in 1936. This disorder is endemic in sheep and goat populations throughout most of the world but has never been shown to have been transmitted to humans. Chronic wasting disease (CWD) is endemic in elk and deer in Colorado, Wyoming and adjoining states in the USA and appears to be increasing in both incidence and geographic distribution. Transmission of these prion diseases occurs under natural circumstances though whether through direct contact or environmental contamination remains unclear. Both transmissible mink encephalopathy and bovine spongiform encephalopathy (BSE) are thought to be transmitted though the oral route. In particular the practice of using ruminant-derived meat and bone meal for animal feed is thought to have resulted in the epidemic of BSE in the UK since 1985[1] and the transmission of this agent to over 20 other species including domestic and exotic cats, and exotic ungulates such as gemsbok and nyala. Several forms of human prion disease have been described among which sporadic Creutzfeldt-Jakob disease (CJD)[2,3] and a variety of genetic prion diseases including familial CJD, Gerstmann-Sträussler-Scheinker (GSS) disease and fatal familial insomnia. Although sporadic CJD has an unknown etiology and an incidence of around 1/million population per annum throughout the world, there are examples of acquired prion disease in man. Kuru was described in the Fore people of Papua New Guinea in the late 1950s and is thought to have been transmitted orally through ritual cannibalistic funeral rites.[4] Many instances of iatrogenic CJD transmission have been recorded in which disease was acquired through use of prion contaminated neurosurgical instruments and stereotactic EEG electrodes, or transplantation of tissues (cornea, dura mater) or preparations (pituitary-derived growth hormone, follicular stimulating hormone) from sporadic CJD-affected cadavers.[5] Most recently, of course, variant CJD was first described in 1996 in the UK,[6] and represents transmission of BSE across the species barrier from cattle to humans.[7] Thus far 153 clinical cases have been described in the UK,[8] 9 in France, 2 in Ireland and one in each of Italy, Saudi Arabia, USA, Canada and Japan. Classically prion diseases cause significant damage to the central nervous system but there is little evidence of pathology elsewhere. However the existence of *natural* prion disease transmission raises the issues of the route(s) by which infection reaches the central nervous system and the distribution of infectivity in peripheral tissues. These factors have

important practical implications for our ability to diagnose and manage pre- and sub-clinical prion diseases and, from the public health perspective, inform risk assessments on likely transmission by blood or tissue transplantation or surgical instrumentation.

Molecular pathology of prion diseases

The development of prion disease is associated with a change in the conformation of a normal protein widely expressed in both animals and man called prion protein (PrP). The normal cellular form of PrP, PrP^{c}, is a 30-35 kDa glycoprotein with two N-glycosylation sites; it is anchored to the cell membrane through a glycosylphosphatidylinositol (GPI) anchor.[9,10] The molecule is expressed on the cell surface with a half-life of around 6 hours; it is internalized via clathrin-coated pits with some undergoing degradation and the rest being recycled to the cell surface.[11] The secondary structure of PrP^{c} consists of α helices amounting to about 40% of the molecule and a single β-pleated sheet[12,13] making up 3-5%. The function of PrP^{c} remains uncertain, but studies suggest it is a copper-binding protein[14] and interaction with the 37 kDa/67 kDa laminin receptor has been described.[15] PrP-deficient mice display some evidence of neurological disturbance[16] but are otherwise healthy.[17,18] Development of prion diseases is associated with changes in the secondary and tertiary conformations of the PrP molecule, namely an increase in the amount of β-pleated sheet to 40-50% of the molecule.[12,19] This changes both the physico-chemical and biological characteristics of PrP making it relatively resistant to proteinase digestion and leading to accumulation of PrP^{Sc} in affected tissues. The mechanism by which this conformational change occurs remains uncertain. The transmission of nucleic acids linked with this disorder has not been demonstrated.[20] The prion hypothesis[21] was first proposed by Prusiner in 1982 and suggests that the abnormally conformed PrP^{Sc} itself precipitates the conformational change in normal PrP^{c} through a process of homodimerization or of nuclear polymerization. This process can be reproduced to a certain extent in *in vitro* cell-free systems.[22,23] Indeed, Prusiner's group recently described data from experiments demonstrating that recombinant PrP, refolded into the disease-specific form *in vitro*, has the potential to transmit disease *in vivo*.[24]

The role of the immune system in the pathophysiology of disease

In experimental animal models the peripheral transmission of prions leads to the presence of PrP^{Sc} and infectivity in peripheral lymphoid tissue from a very early stage of disease.[25] In rodents inoculated peripherally with scrapie prions, splenectomy[26] and immunosuppression[27] delay neuroinvasion, whereas thymectomy[26,28] and irradiation[29] have no effect. Studies have shown that severe combined immunodeficient mice which lack B lymphocytes and T lymphocytes are resistant to peripheral prion infection,[30] but susceptibility can be restored following allogeneic bone marrow transplantation from a normal mouse.[31] Further studies using a variety of immunodeficient mice initially suggested a primary role for B lymphocytes in prion neuroinvasion as mice lacking them (μMT mice, RAG-$1^{-/-}$, RAG-$2^{-/-}$) were resistant to peripheral disease transmission, whereas mice lacking functional T lymphocytes alone (CD$4^{-/-}$, CD$8^{-/-}$, β2-$\mu^{-/-}$, *Perforin*$^{-/-}$, TCR$\alpha^{-/-}$) were not.[32] However, follicular dendritic cells (FDC) are dependent on the presence of B lymphocytes for essential maturation signals such as lymphotoxin. Therefore, mice that lack B lymphocytes or lymphotoxin also lack FDC. FDC can be temporarily depleted by blocking the lymphotoxin β receptor signaling pathway through treatment with LTβR-Ig.[33] This treatment depletes FDC and reduces susceptibility to peripherally inoculated prions.[34-37] While the effects of lymphotoxin blockade on prion pathogenesis argue strongly for a role of FDC in the initial accumulation of prions in lymphoid tissues, other potential effects of treatment should not be overlooked. Treatment with LTβR-Ig inhibits or prevents the development of experimental autoimmune encephalomyelitis by impairing T-lymphocyte responses and migration.[38] LIGHT is a transmembrane protein produced by activated T-lymphocytes that also binds to LTβR.[39] However, as prion pathogenesis is unaffected in T-lymphocyte-deficient mice, the effects of LTβR-Ig treatment are unlikely to be due to impaired LTβR- or LIGHT-mediated T lymphocyte responses or migration.[26,28,32,40] Signaling via LTβR has been shown to be important for the presence of migratory dendritic cells in the spleen.[41] Therefore, LTβR-Ig treatment might affect cell trafficking or prion transportation to lymphoid tissues. However, as treatment with LTβR-Ig up to 42 days after inoculation significantly extends survival time[34,37] the effects of treatment on pathogenesis are unlikely to be due to effects on cell trafficking as dendritic cells migrate to draining lymphoid tissues within the first few hours of antigen encounter.[42] Collectively these observations suggest it is highly unlikely that the major effects of LTβR-Ig-treatment on prion pathogenesis are independent of its effects on FDC maturation. FDC trap and retain antigens on their surfaces through interactions between complement components and cellular complement receptors. The expression of PrP^{c} by FDC,[40,43,44] and the ability of these cells to capture complement-bound complexes[45,46] appear critical for the accumulation of scrapie prions on the cells.

A prominent role for FDC in the initial replication of

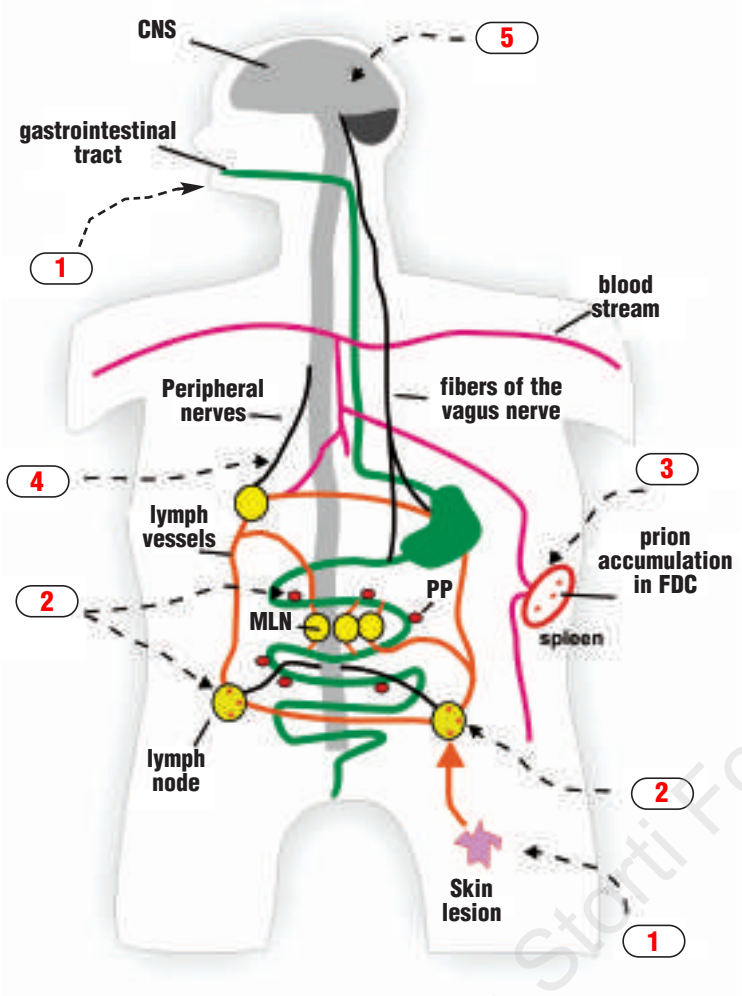

Figure 1. Route of prion neuroinvasion following peripheral exposure. 1. Natural prion diseases are often acquired by peripheral exposure such as orally or through skin lesions. 2. Following exposure, prions often first accumulate in follicular dendritic cells (FDC) in the germinal centres of local lymphoid tissues eg: draining lymph node following inoculation via the skin, or Peyer's patches (PP) and mesenteric lymph nodes (MLN) following oral exposure. How prions are initially transported to these tissues is not known. 3. Shortly afterwards prions are disseminated to most other lymphoid tissues including the spleen. This distribution most likely involves the blood and lymph. 4. After accumulating in FDC, prions infect sympathetic nerves and fibres of the vagus nerve, and spread along these to the central nervous system (CNS). 5. The accumulation of prions in the brain is accompanied by neurodegeneration and ultimately the death of the host.

prion diseases has been observed in both experimental and naturally infected hosts. In the secondary lymphoid tissues of mule deer fawns orally inoculated with CWD[47] and some sheep with natural scrapie,[48-50] PrP^{Sc} accumulation is seen predominantly on FDC in the secondary lymphoid tissue.[43,50,51] This finding is also seen in patients with variant CJD in whom PrP^{Sc} accumulation on FDC has been detected in the lymphoid tissue of most patients thus far examined.[52,53] In two cases, PrP^{Sc} was detected within appendix tissue samples from patients who underwent appendicectomy eight months and two years before the onset of clinical disease.[54,55] Thus both experimental and clinical data support the probability that infectivity is present in the peripheral lymphoid tissue of patients with variant CJD before the development of clinical neurological disease. A recent estimate of the likely prevalence of pre- or sub-clinical variant CJD prion infection in the UK based on a retrospective surveillance study of PrP^{Sc} accumulation in surgically removed tonsillectomy and appendicectomy samples suggests a frequency of around 3/12,674 in 10-30 year old individuals.[55] However, studies have also shown that PrP^{Sc} is not always detectable in the appendix from patients with variant CJD,[56,57] suggesting the prevalence of infection may be even higher than that suggested by the retrospective study. Many prion strains that differ in their species of origin, incubation period and neuropathological targeting have been identified by passage into experimental rodents. Increasing evidence suggests that some of these prion strains may also differ in their cellular tropism in lymphoid tissues. For example, whereas ME7 scrapie prions accumulate in lymphoid tissues only in the presence of PrP^{c}-expressing FDC,[43,44] RML scrapie prions appear to be able to target also PrP^{c}-expressing bone marrow-derived cells.[40,58,59] Indeed, macrophages can accumulate high levels of RML scrapie prions in the FDC-deficient lymph nodes of $TNFR1^{-/-}$ mice.[60] FDC may make only a small contribution towards the neuroinvasion of the Fukuoka-2 mouse-passaged CJD prion strain.[61,62]

Natural TSE diseases also appear to differ in their targeting of peripheral lymphoid tissues within the same host species. Although variant CJD accumulates in lymphoid tissues prior to neuroinvasion,[54,55] there is little evidence of this in patients with sporadic or iatrogenic

CJD.[53] However, by using a highly sensitive detection method PrP^{Sc} has been detected in spleen and muscle samples from some Swiss patients with sporadic CJD in the end stage of the disease.[63]

Transportation of prions to and from lymphoid tissues

How prions are initially transported from the site of exposure, such as from the intestinal lumen or the skin, to the germinal centers in which they replicate is not known. FDC could directly trap cell-free PrP^{Sc}, or other agent associated molecules, in a complement-bound complex,[45,46,64] but it is also possible that mobile cells transport the agent to lymphoid follicles. Several cells could potentially transport prions, including lymphocytes, macrophages and migratory bone marrow-derived dendritic cells (DC). The ability of macrophages to destroy infectivity and degrade PrP^{Sc} makes them an unlikely and inefficient transport candidate.[65,66] Furthermore, macrophages appear to impede the early accumulation of prions within lymphoid tissue. Lymphocytes are also unlikely candidates for the delivery of prions to and from germinal centers. Pathogenesis is unaffected in the absence of T lymphocytes, and prion infectivity was undetectable in association with circulating lymphocytes in a model in which high levels were detected in the spleen.[67]

Migratory bone marrow-derived DC are a distinct lineage from tissue-fixed, stromal-derived FDC.[68,69] These DC sample antigens in the periphery and deliver them to lymphoid tissues.[42] Unlike macrophages, DC can also retain some protein antigens in a native, non-degraded form.[70] These characteristics suggest migratory DC might also deliver prions to lymphoid tissues. Indeed, research has shown that the prion protein fragment PrP106-126 is a chemoattractant for monocyte-derived DC.[71] Others have demonstrated that bone marrow-derived DC can acquire PrP^{Sc} *in vitro*[72,73] and that a sub-population of migratory DC can transport intestinally injected PrP^{Sc} to mesenteric lymph nodes via the lymph.[72] Despite these observations direct evidence for the involvement of DC in the initial delivery of prions to lymphoid tissues is currently lacking.

Once prions have accumulated on FDC, infection spreads along peripheral nerves to the central nervous system.[74] Neuroinvasion along both sympathetic nerves and fibers of the vagus nerve has been implicated,[74-77] but how prions initially spread from FDC to nerves in lymphoid tissues is not known. This could involve transfer directly across an FDC-nerve synapse, but in spleens of wild-type mice contacts between FDC and sympathetic nerves are rare as these cells occupy separate anatomical sites: sympathetic nerves associate with splenic blood vessels, whereas FDC are located in the periphery of the white pulp within B-cell follicles. In an experimental system migratory bone marrow-derived DC have been proposed as a potential mechanism through which prions could be delivered to nerves within lymphoid tissues.[78] Intercellular transfer of prions within exosomes has also been proposed.[79]

Some chemokines provide signals which direct lymphocytes and FDC to their specific microenvironments in the spleen. In mice deficient in the chemokine receptor CXCR5 ($CXCR5^{-/-}$ mice), FDC are abnormally situated around the central arteriole in close association with splenic nerves. Neuroinvasion of scrapie prions from the spleens of $CXCR5^{-/-}$ mice is faster than that from the spleens of wild type-mice, suggesting that the distance between FDC and neurones influences the rate of prion transfer to the nervous system.[80] While it still remains to be determined how TSE agents are transferred from FDC to nerves in the spleen, these data suggest prion neuroinvasion might occur rapidly from sites where FDC are closely associated with nerve fibers.

Peripheral blood infectivity

Experimental animal models have provided important information on the presence and distribution of infectivity in the peripheral blood during the pre-clinical and clinical stages of prion disease. For example, data from mice infected with a human prion strain (mouse-adapted Fukuoka-1 strain of GSS disease) suggest that blood during the clinical phase of disease contains 100 infectious units (IU) of prion infectivity per mL in buffy coat, and approximately 10 IU per mL in plasma.[81-83] Much lower levels were detected in buffy coat during the pre-clinical phase, with infectivity undetectable in plasma. A similar level and distribution of infectivity had been shown in mice infected with variant CJD.[84] When individual blood components from prion-infected mice were fractionated, low levels of prion infectivity were detected in buffy coat, plasma, cryoprecipitate, and fraction I+II+III.[81] No infectivity was detected in association with fractions IV or V[81] or highly purified platelets.[85] Most attempts to demonstrate prion infectivity in blood from naturally infected sheep and goats with scrapie, mink with transmissible spongiform encephalopathy and cattle with BSE have failed.[86] The low titer of prion infectivity detected in blood, coupled with the low sensitivity of cross-species bioassays and the small volume of blood that can be injected into the brains of indicator animals may help to explain why these and other studies have inconsistently detected infectivity in blood. However, two studies show prion transmission between sheep through blood transfusion from donor sheep with natural scrapie or experimentally inoculated with BSE.[87,88] Whole blood or

buffy coat drawn during the pre-clinical and clinical phases of disease transmitted disease to at least 10% of the transfusion recipients.[87,88] These experiments provide important evidence that there are sufficient levels of prion infectivity present in the peripheral blood of some pre-clinical hosts to transmit disease to recipients by transfusion.

Blood from four patients with sporadic CJD and one with iatrogenic CJD was reported to have transmitted disease after intracerebral inoculation into experimental rodents but the validity of each transmission has been questioned.[89] There has also been no convincing evidence of transmission of sporadic CJD by blood or blood products.[90] Indeed, a large number of epidemiological case control, look-back and surveillance studies on sentinel populations such as hemophiliacs have failed to demonstrate any increased risk of sporadic CJD through blood transfusion or exposure to plasma products.[90-93] Of course, the possibility that rare transmissions of sporadic CJD may have occurred through blood or blood products cannot be entirely excluded. The lack of convincing evidence to suggest that sporadic CJD has been transmitted by blood transfusion and the failure to demonstrate PrP^{Sc} in lymphoid tissues during the preclinical stages of this disease suggests that the level of infectivity and PrP^{Sc} in lymphoreticular tissues is lower in sporadic compared to variant CJD.[53]

Mouse bioassays failed to detect infectivity in blood from two variant CJD patients.[94] Eighteen patients with variant CJD have previously been blood donors. Fifty recipients of blood components from these individuals have so far been identified of whom 17 are still alive. Two cases of probable transmission of variant CJD prions by blood transfusion have been described. In the first of these,[95] a blood donation was made in 1996 and the donor, who was well at the time, went on to develop clinical variant CJD, confirmed in 2001. Non-leukodepleted red cell concentrate from this donation was administered to a patient who developed variant CJD in 2003. A second case was recently described:[96] the donation was made in 1999 and the donor went on to develop variant CJD and died in 2001. A single unit of non-leukodepleted red cell concentrate derived from this donor was administered to a patient who died of unrelated causes in 2004. Post-mortem examination revealed evidence of abnormal PrP^{Sc} accumulation in the spleen and one of the cervical lymph nodes but not elsewhere. Western blot analysis confirmed that the mobility and glycoform ratio of the PrP^{Sc} detected was typical of that from variant CJD patients, and distinct from that of sporadic CJD. These cases demonstrate without much residual doubt that variant CJD prions are transmissible by blood transfusion from donors with pre- or sub-clinical infection. The second case is also interesting because whereas all patients with clinical variant CJD have thus far proved homozygous for methionine at codon 129 of the PRNP gene, this patient was heterozygous for methionine/valine at this locus.[96] However, as PrP^{Sc} was only detectable in the spleen and cervical lymph node of the recipient, this study also highlights the potential of retrospective analyses of tonsillar and appendicectomy tissues to underestimate the number of individuals currently incubating variant CJD.[56,57] To reduce the risk of potential transmission of variant CJD by blood transfusion the UK implemented universal leukodepletion in 1999. The rationale for this decision was based on observations that PrP^{Sc} could be detected in lymphoid tissues of variant CJD patients[52,53] implying that cells such as lymphocytes might potentially contaminate the blood-stream with prions. The effectiveness of leukodepletion in removing prion infectivity from blood has been tested using a human-sized unit of pooled blood from scrapie-infected hamsters. Although leukodepletion removed 42% of the total prion infectivity in blood, it was not itself sufficient to remove all blood-borne infectivity.[97] This is consistent with the detection of both cell-associated and soluble prion infectivity in peripheral blood.[81-83]

By way of a caveat, the conditions pertaining to these experiments may be quite different from those in humans with sub-clinical variant CJD. Both cases of transfusion-related variant CJD appear to have been transmitted by transfusion of non-leukodepleted red cells.[95,96] It is possible that even a low level of reduction in peripheral blood prion infectivity may suffice to reduce the number of people infected or prolong the incubation period in the recipients. Two companies are currently working on the development of prion reduction filters whch may be able to reduce prion infectivity by 3-4 log.

Concluding remarks

The presence of prion infectivity in the peripheral blood and immune systems of animals and humans with prion diseases raises public health concerns about the possible transmission of these diseases from animals to man, or between humans through blood or plasma products, tissue and organ transplantation or via contaminated surgical instruments. On the other hand it also raises the possibility of diagnostic or therapeutic intervention in infected individuals prior to the development of clinical disease. Countries in which these diseases are prevalent need to monitor these disorders and take necessary precautionary measures.

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