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REVIEW ARTICLE

The immunobiology of TSE diseases

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Introduction

The transmissible spongiform encephalopathies (TSEs) are a group of fatal neurodegenerative diseases which include Creutzfeldt–Jakob disease (CJD) and kuru in humans, bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease (CWD) in mule deer and elk, and scrapie in sheep and goats. Although the precise nature of the infectious TSE agent is uncertain, an abnormal, detergent-insoluble, relatively proteinase-resistant isoform of a host cellular prion protein (PrP^c) co-purifies with infectivity in diseased tissues (Bolton *et al.*, 1982). This modified protein (PrP^{Sc}) is considered to be a major component of the infectious agent (Prusiner *et al.*, 1982). To maintain TSE infection, host cells must express the cellular isomer of the prion protein, as mice deficient in PrP^c (*Prnp*$^{-/-}$ mice) do not develop disease (Bueler *et al.*, 1992; Manson *et al.*, 1994).

Natural TSE infections are most often acquired by peripheral exposure. For example, the consumption of contaminated feed was most likely involved in the spread of BSE amongst cattle (Wilesmith *et al.*, 1991). Furthermore, consumption of BSE-infected meat is thought to be responsible for the emergence of variant (v) CJD in humans (Bruce *et al.*, 1997; Hill *et al.*, 1997*a*). Sporadic CJD in humans can be transmitted iatrogenically through transplantation of CJD-contaminated tissues or pituitary-derived hormones, but so far there is no indication that vCJD has been transmitted in this manner. Most of our understanding of the pathogenesis of TSEs has come from the study of experimental sheep or rodent scrapie models. Following experimental peripheral infection with scrapie, infectivity and PrP^{Sc} rapidly accumulate in lymphoid tissues (Kimberlin & Walker, 1979; Farquhar *et al.*, 1994; Brown *et al.*, 1999*b*; Beekes & McBride, 2000; Heggebø *et al.*, 2000; Mabbott *et al.*, 2000*b*), long before either is detectable in the central nervous system (CNS). Likewise, PrP^{Sc} is first detected in lymphoid tissues draining the gastrointestinal tract following experimental oral inoculation of mule deer fawns (*Odocoileus hemionus*) with CWD (Sigurdson *et al.*, 1999). Although the infection route of natural sheep scrapie is not known, PrP^{Sc} is first detected in Peyer's patches and gut-associated lymphoid tissues (Andréoletti *et al.*, 2000; Heggebø *et al.*, 2000) prior to detection within other lymphoid tissues and the CNS (van Keulen *et al.*, 1999), implying that this disease is also acquired orally.

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Lymphoid tissues play an important role in transmission in some TSE models, as genetic asplenia or splenectomy of mice, shortly before or after a peripheral scrapie challenge, significantly extends the incubation period (Fraser & Dickinson, 1978). The involvement of lymphoid tissues in TSE pathogenesis may be TSE strain-dependent, as BSE in cattle (Somerville *et al.*, 1997) and sporadic CJD in humans (Hill *et al.*, 1999) appear to be confined to nervous tissues. However, in patients with vCJD (Hilton *et al.*, 1998; Hill *et al.*, 1999; Bruce *et al.*, 2001), most sheep with natural scrapie (van Keulen *et al.*, 1996) or rodents experimentally infected with scrapie (McBride *et al.*, 1992; Brown *et al.*, 1999*b*; Beekes & McBride, 2000; Jeffrey *et al.*, 2000; Mabbott *et al.*, 2000*b*), infectivity accumulates in lymphoid tissues and abnormal forms of PrP are readily detected on follicular dendritic cells (FDCs) and tingible body macrophages within germinal centres (GCs). For many years FDCs have been considered likely targets for TSE replication in lymphoid tissues as they appear to express high levels of PrP even in uninfected mice (McBride *et al.*, 1992). But as FDCs are intimately associated with lymphocytes, which also express PrP^c (Cashman *et al.*, 1990; Mabbott *et al.*, 1997), further research was necessary to differentiate the roles of FDCs, macrophages and lymphocytes in scrapie pathogenesis.

The role of T lymphocytes in TSE pathogenesis

Evidence that T lymphocytes were not involved in scrapie pathogenesis came first from studies showing that thymectomy had no effect on the incubation period of the disease following peripheral infection (McFarlin *et al.*, 1971; Fraser & Dickinson, 1978). Since then, additional studies with transgenic and immunodeficient mice have shown that deficiencies in the T lymphocyte compartment alone ($CD4^{-/-}$, $CD8^{-/-}$, $\beta2\text{-}\mu^{-/-}$, $TCR\alpha^{-/-}$ or *Perforin*$^{-/-}$ mice) have no effect on disease susceptibility or the accumulation of infectivity in the spleen (Klein *et al.*, 1997, 1998).

The role of B lymphocytes in TSE pathogenesis

The accumulation of scrapie infectivity in the spleen and subsequent neuroinvasion are significantly impaired in mice deficient in B lymphocytes alone (μMT mice; Klein *et al.*, 1997)

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or jointly deficient in both mature B and T lymphocytes [severe combined immunodeficient (SCID) mice, *Rag*-1$^{-/-}$, *Rag*-2$^{-/-}$ and *Agr*$^{-/-}$ mice; Fraser *et al*., 1996; Klein *et al*., 1997]. There could be several explanations for this. B lymphocytes might physically deliver TSE infectivity to the CNS, but as no infectivity was detected on circulating blood lymphocytes in a model where high levels are readily detected on splenic lymphocytes this is unlikely (Raeber *et al*., 1999*a*). There is also no evidence that B lymphocytes themselves secrete neuroinvasive or neurodegenerative factors during disease (Frigg *et al*., 1999), and mice deficient in B lymphocytes are as susceptible as immunocompetent mice when injected with scrapie directly into the brain (Fraser *et al*., 1996; Taylor *et al*., 1996; Klein *et al*., 1997; Brown *et al*., 1999*b*; Frigg *et al*., 1999). However, B lymphocytes provide important signals for the maturation and maintenance of other cell types in GCs (Kosco-Vilbois *et al*., 1997; Chaplin & Fu, 1998). As a consequence, mice deficient in mature B lymphocytes (μMT, SCID, *Rag*-1$^{-/-}$ or *Rag*-2$^{-/-}$ mice) are indirectly deficient in FDCs, which require stimulation from B lymphocytes to maintain their differentiated state. Sub-lethal whole body γ-irradiation, which eliminates actively dividing B lymphocytes, T lymphocytes and monocytes, has no effect on scrapie pathogenesis when administered before or after peripheral scrapie challenge (Fraser & Farquhar, 1987). Therefore, as FDCs are radioresistant and mitotically inactive, B lymphocytes could conceivably contribute to scrapie pathogenesis indirectly, through their effects on FDC maturation. Of course, the γ-irradiation study (and other studies discussed above) does not exclude a direct contribution from terminally differentiated B lymphocytes, or other cells such as tingible body macrophages, which contain heavy accumulations of pathological PrP in spleens of infected mice (Jeffrey *et al*., 2000).

Role of FDCs in TSE pathogenesis

Studies using immunodeficient mice

Some of the signals between lymphocytes and FDCs are mediated via cytokines, which can play an important role in the organization of GCs (Kosco-Vilbois *et al*., 1997; Chaplin & Fu, 1998). For example, tumour necrosis factor-α (TNFα) secretion by lymphocytes is important for maintaining FDC networks (Fig. 1). Signalling is mediated through the TNF-receptor 1 (TNF-R1) expressed on the FDC and/or its precursor (Tkachuk *et al*., 1998), and mice deficient in either TNFα (Pasparakis *et al*., 1996) or TNF-R1 (Matsumoto *et al*., 1996*b*) lack mature FDCs. Despite the absence of GC structure in these immunodeficient mice, B lymphocytes are still able to respond to antigen stimulation and antibody class-switching can still occur. Interleukin-6 (IL-6) secretion by FDCs is also important for maintaining GC reactions, as in its absence, FDCs are able to mature but GC development is diminished (Kopf *et al*., 1998). Deficiencies in these and other cytokine signalling pathways have been used to distinguish the separate roles of the FDCs and lymphocytes in scrapie pathogenesis. Consistent with the γ-irradiation studies described earlier (Fraser & Dickinson, 1978), deficiencies in GCs alone do not affect scrapie pathogenesis. Peripherally challenged IL-6-deficient mice develop disease at the same time as wild-type mice, and accumulate high levels of scrapie infectivity and PrP^{Sc} in their spleens (Mabbott *et al*., 2000*b*). In spleens from scrapie-challenged TNF$\alpha^{-/-}$ mice, the absence of detectable infectivity and PrP^{Sc} in the spleen and reduced disease susceptibility coincide with an absence of mature FDCs (Brown *et al*., 1999*b*; Mabbott *et al*., 2000*b*). Lymphocytes are unlikely to be directly involved in disease pathogenesis, as both T and B lymphocytes are present and functional in lymphoid tissues of TNF$\alpha^{-/-}$ mice (Pasparakis *et al*., 1996). Taken together, these results suggest that the accumulation of infectivity and PrP^{Sc} in the spleen and subsequent neuroinvasion are dependent upon mature FDCs.

There are many laboratory mouse-passaged TSE strains that have distinct incubation periods and neuropathological characteristics. In our own laboratory we use the ME7 scrapie strain derived from natural sheep scrapie. Comparisons of our results described above (Brown *et al*., 1999*b*; Mabbott *et al*., 2000*b*), with similar studies in immunodeficient mice lacking mature FDCs [TNF-R1$^{-/-}$ (Matsumoto *et al*., 1996*b*) and lymphotoxin (LT) $\beta^{-/-}$ (Koni *et al*., 1997) mice] suggest some TSE strains may target different cell populations in peripheral tissues. For example, experiments in Professor Adriano Aguzzi's laboratory (Zürich, Switzerland) using the Rocky Mountain Laboratory (RML) scrapie strain (derived from experimental goat scrapie) show that TNF-R1$^{-/-}$ mice were as susceptible as immunocompetent mice to peripheral challenge (Klein *et al*., 1997). The high dose of scrapie used in this study may have bypassed the need for replication in lymphoid tissues, as has been previously shown following peripheral challenge of SCID mice with ME7 scrapie (Fraser *et al*., 1996). However, it is also possible that in the absence of mature FDCs in TNF-R1$^{-/-}$ mice, neuroinvasion of RML scrapie occurs via an FDC-independent pathway, which is not a consequence of the higher dosage of infection. Differences in the pathogenesis of mouse models of CJD have also been implied. SCID mice are refractory to infection with the mouse-passaged CJD strain Fukuoka-1, consistent with a role for FDCs (Kitamoto *et al*., 1991). In contrast, using LT$\beta^{-/-}$ mice, little evidence for FDC involvement by the mouse-passaged CJD strain Fukuoka-2 has been reported even though accumulations of pathological PrP are detected on FDCs in spleens of infected immunocompetent controls (Manuelidis *et al*., 2000). Current evidence suggests a similar variation in natural TSE diseases. Infectivity and heavy PrP^{Sc} accumulations have been detected in direct association with FDCs in the lymphoid tissues of patients with vCJD (Hill *et al*., 1997*b*, 1999; Hilton *et al*., 1998; Bruce *et al*., 2001), but not of patients with sporadic or even iatrogenic CJD, where infection is introduced via the periphery (Hill *et al*., 1999). However, to allow accurate interpretation of the cellular requirements of different TSE strains, the experimental con-

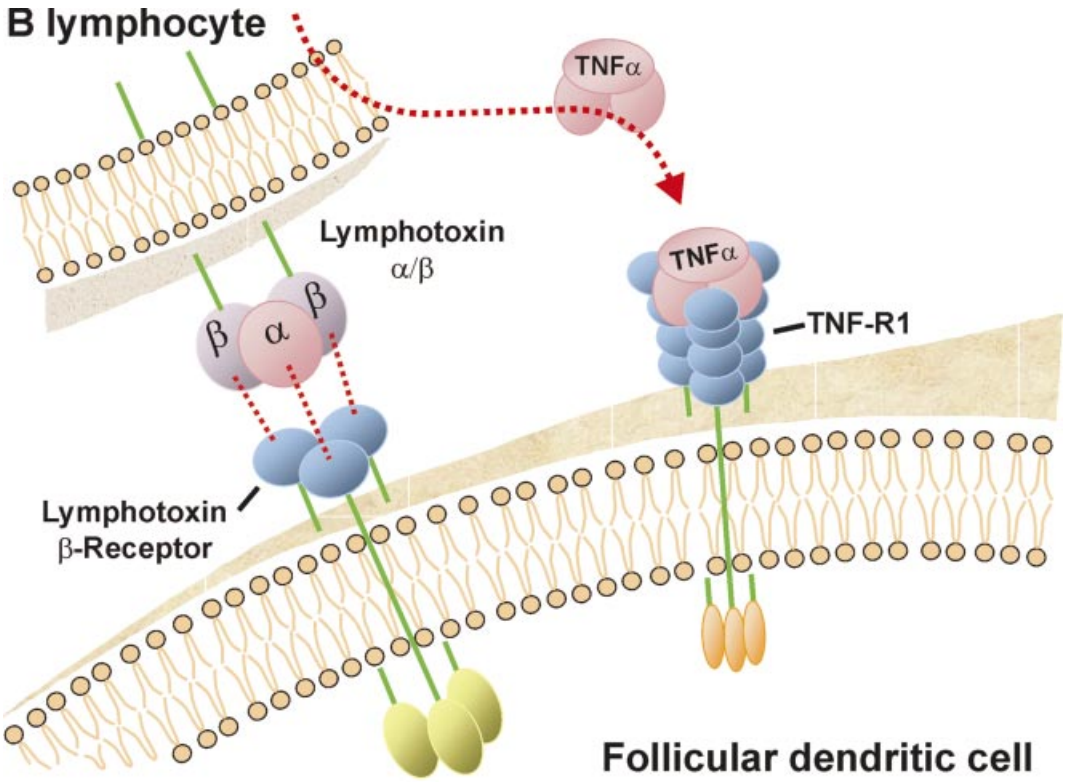

Fig. 1. B lymphocyte-derived cytokines are critical for maintaining the maturation of FDCs. These include membrane lymphotoxin α_1/β_2, which signals exclusively through the lymphotoxin β receptor, and soluble tumour necrosis factor-α (TNFα), which signals through the TNF-receptor 1 (TNF-R1). Both receptors are expressed on the FDC and/or its precursor cell. In the absence of either of the above cytokines or their corresponding receptors, FDCs do not differentiate.

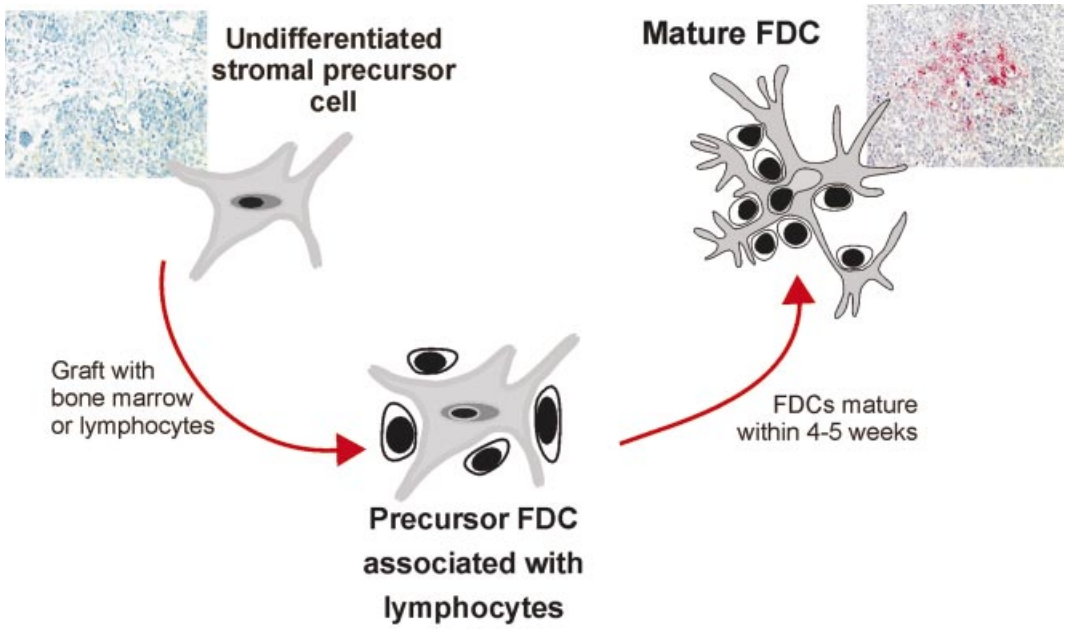

Fig. 2. Induction of FDC maturation in the spleens of SCID mice. Due to an absence of mature lymphocytes, the lymphoid tissues of SCID mice are indirectly deficient in mature FDCs (inset top left). Unlike lymphocytes, FDCs are not considered to be haemopoietic in origin. Current theories suggest FDCs derive from undifferentiated stromal precursor cells within lymphoid tissues. Within 4–5 weeks of grafting SCID mice with lymphocytes or bone marrow (as a source of lymphocytes), the lymphocytes of donor origin induce the differentiation of stromal precursor cells to form mature FDC networks (inset top right). In both inset panels, spleen sections are stained with the FDC-specific antiserum FDC-M1 (red; Kosco *et al.*, 1992).

Table 1. Following infection with the ME7 scrapie strain, high levels of infectivity accumulate in the spleen only in the presence of mature PrP^c-expressing FDCs

Where indicated recipient mice were grafted with bone marrow or splenic lymphocytes and challenged 28 days later with the ME7 scrapie strain (20 µl of 1% ME7 scrapie brain homogenate). Spleens were removed 70 days after scrapie inoculation, and infectivity titre was assessed by intracerebral inoculation of homogenates into groups of 12 assay mice.

Recipient	Donor cells	PrP^c expressed by: FDCs	PrP^c expressed by: Lymphocytes	Scrapie titre*
Prnp$^{+/+}$	–	Yes	Yes	6·1, 6·3, 5·7, 6·2†
Prnp$^{-/-}$	–	No	No	Undetectable†
SCID/*Prnp*$^{+/+}$	–	No FDCs	No lymphocytes	Undetectable†
SCID/*Prnp*$^{+/+}$	*Prnp*$^{+/+}$ bone marrow	Yes	Yes	6·0, 5·7†
SCID/*Prnp*$^{+/+}$	*Prnp*$^{-/-}$ bone marrow	Yes	No	5·5, 5·9†
SCID/*Prnp*$^{+/+}$	*Prnp*$^{+/+}$ lymphocytes	Yes	Yes	4·8, 4·4‡
SCID/*Prnp*$^{+/+}$	*Prnp*$^{-/-}$ lymphocytes	Yes	No	4·8, 4·7‡
SCID/*Prnp*$^{-/-}$	–	No FDCs	No lymphocytes	Undetectable†
SCID/*Prnp*$^{-/-}$	*Prnp*$^{+/+}$ bone marrow	No	Yes	Undetectable†§
SCID/*Prnp*$^{-/-}$	*Prnp*$^{-/-}$ bone marrow	No	No	Undetectable†

* Infectivity titres in log intracerebral 50% infectious dose (units/g).
† Brown *et al.* (1999*b*).
‡ N. A. Mabbott (unpublished data).
§ In contrast to mice challenged with the ME7 scrapie strain, following injection with RML scrapie, high levels of scrapie infectivity are detected in the spleen in the absence of PrP expression by FDCs so long as PrP is expressed by lymphocytes or other bone marrow-derived cells (Blättler *et al.*, 1997).

ditions including the dose of inoculum, route of injection and mouse strains used must be standardized.

Studies using chimeric mice with a mismatch in PrP status between FDCs and other cells of the immune system

An alternative approach used in the study of the pathogenesis of both ME7 and RML scrapie strains has been to use mice in which PrP^c, the host prion protein, is expressed on FDCs but not lymphocytes and vice versa. The ontogeny of FDCs is still uncertain (Kapasi *et al.*, 1998; Tkachuk *et al.*, 1998; Endres *et al.*, 1999), but in adult mice, they are not considered to be haemopoietic in origin but to derive from stromal precursor cells in the spleen and lymph nodes. FDC maturation in mice deficient in or depleted of lymphocytes can be induced by grafting with lymphocytes or haemopoietic cells (bone marrow or foetal liver cells) as a source of lymphocytes (Fig. 2; Kapasi *et al.*, 1993). As a consequence, the lymphocytes of graft origin induce the maturation of FDC precursor cells of recipient origin. By grafting bone marrow from PrP-deficient (*Prnp*$^{-/-}$) mice into PrP-expressing (*Prnp*$^{+/+}$) mice, and vice versa, we and others have created chimeric mice with a mismatch in PrP expression between FDCs and surrounding lymphocytes (Blättler *et al.*, 1997; Klein *et al.*, 1998; Brown *et al.*, 1999*b*). Using these models, immunolabelling for PrP on FDCs was only seen when the recipient expressed a functional PrP gene, and was independent of the PrP status of the lymphocytes (Brown *et al.*, 1999*b*; Table 1). This provides strong evidence that FDCs themselves produce PrP^c rather than acquiring it from other PrP-expressing cells. Following peripheral challenge of these mice with the ME7 scrapie strain, high levels of infectivity accumulate in the spleen only in the presence of PrP-expressing FDCs (Brown *et al.*, 1999*b*; Table 1). This accumulation is independent of PrP expression by the donor bone marrow or lymphocytes, and provides further evidence that FDCs are critical for the pathogenesis of the ME7 scrapie strain.

Consistent with the theory that some TSE strains may target different cells in the periphery, similar studies using the RML scrapie strain suggested the pathogenesis of this strain might be different from that of the ME7 strain. Following

infection with the RML scrapie strain, high levels of infectivity also accumulate in spleens of mice with PrP-expressing FDCs in the absence of PrP expression by lymphocytes (Klein *et al.*, 1998). However, in contrast to ME7 scrapie, high levels of RML scrapie also accumulated in the spleen in the absence of PrP expression by FDCs so long as PrP was expressed by lymphocytes or other haemopoietically derived cells (Blättler *et al.*, 1997). Although the origin of FDCs is unresolved, it is possible that under certain circumstances FDC precursors may be present within bone marrow or foetal liver cells (Kapasi *et al.*, 1998). Therefore, as foetal liver cells were mainly used as a source of haemopoietic cells in the RML scrapie study (Blättler *et al.*, 1997), and bone marrow cells in the ME7 scrapie strain study (Brown *et al.*, 1999*b*), technical differences could explain the observed discrepancies between these studies. However, as high levels of RML scrapie infectivity accumulate in the spleen following reconstitution of PrP-deficient mice with PrP-expressing foetal liver or bone marrow cells (Blättler *et al.*, 1997), this raises the possibility that the RML scrapie strain, unlike ME7, may target both PrP-expressing FDCs and lymphocytes (Table 1).

The role of macrophages in TSE pathogenesis

Within GCs, tingible body macrophages scavenge apoptotic B lymphocytes, endocytose FDC iccosomal antigen (immune complex-coated bodies) and are considered to regulate the GC reaction (Smith *et al.*, 1991, 1998). Intralysosomal PrP accumulations have been found within tingible body macrophages in spleens of scrapie-infected mice (Jeffrey *et al.*, 2000). Depletion of macrophages before or shortly after a peripheral scrapie infection increases the accumulation of infectivity and PrP^{Sc} in the spleen and shortens the incubation period (Beringue *et al.*, 2000). Likewise, scrapie infectivity decreases following extended *in vitro* culture with macrophages (Carp & Callahan, 1982). Taken together, these data suggest macrophages may sequester scrapie infectivity and impair early scrapie agent replication. Therefore, in the spleen a dynamic competitive state may be operating between destruction of infectivity by macrophages and accumulation by FDCs. The molecular mechanisms contributing to infectivity clearance by macrophages are not known.

Molecular interactions between TSEs and FDCs

Studies using transgenic mice which express high levels of PrP^{c} only on T lymphocytes (Raeber *et al.*, 1999*b*) or B lymphocytes (Montrasio *et al.*, 2001) indicate that other cellular characteristics are required to maintain TSE disease as these mice cannot replicate scrapie in lymphoid tissues. Therefore, the expression of high levels of PrP^{c} by FDCs (McBride *et al.*, 1992; Brown *et al.*, 1999*b*) is unlikely to be the only important property of these cells for scrapie accumulation and disease transmission. FDCs characteristically trap and retain antigens

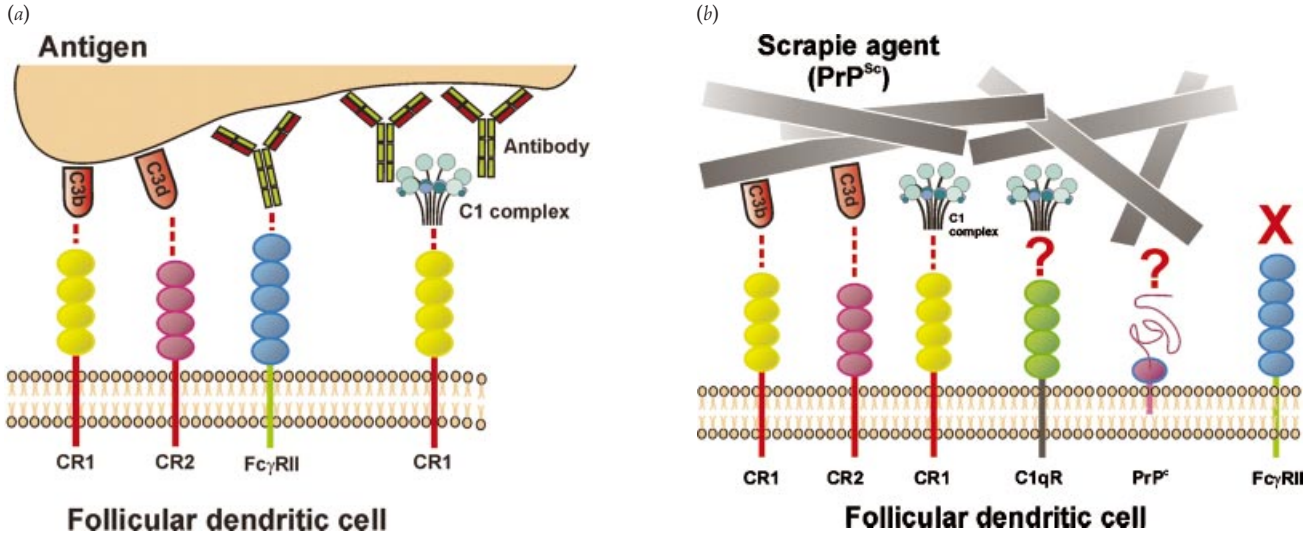

Fig. 3. (*a*) Molecular mechanisms of immune complex trapping by mouse FDCs. Soon after a micro-organism or antigen enters the body it is bound by antibody and/or complement components, in particular C3 fragments (C3b or C3d) or C1q. On the surface of the FDC, complement receptors CR1 and CR2 are important for trapping immune complexes via activated C3 fragments. CR1 may also bind directly to C1q. Following immunization, the binding of antibody molecules to Fc-γ receptor II (FcγRII) may also trap immune complexes. Reviewed in van den Berg *et al.* (1995). (*b*) Potential molecular interactions between scrapie and FDCs. Recent evidence suggests complement and complement receptors play an important role in the localization and retention of scrapie infectivity to FDCs following peripheral challenge (Klein *et al.*, 2001; Mabbott *et al.*, 2001). FDCs may retain the PrP^{Sc}-associated scrapie agent indirectly through interactions between C3 and CR1 or CR2, C1q and CR1 or perhaps between C1q and the specific C1q receptor (C1qR). Neither antibody nor antibody receptors (Fc-γ receptors) are likely to be involved. These studies do not rule out possible interactions between PrP^{Sc} and PrP^{c} on the surface of the FDC.

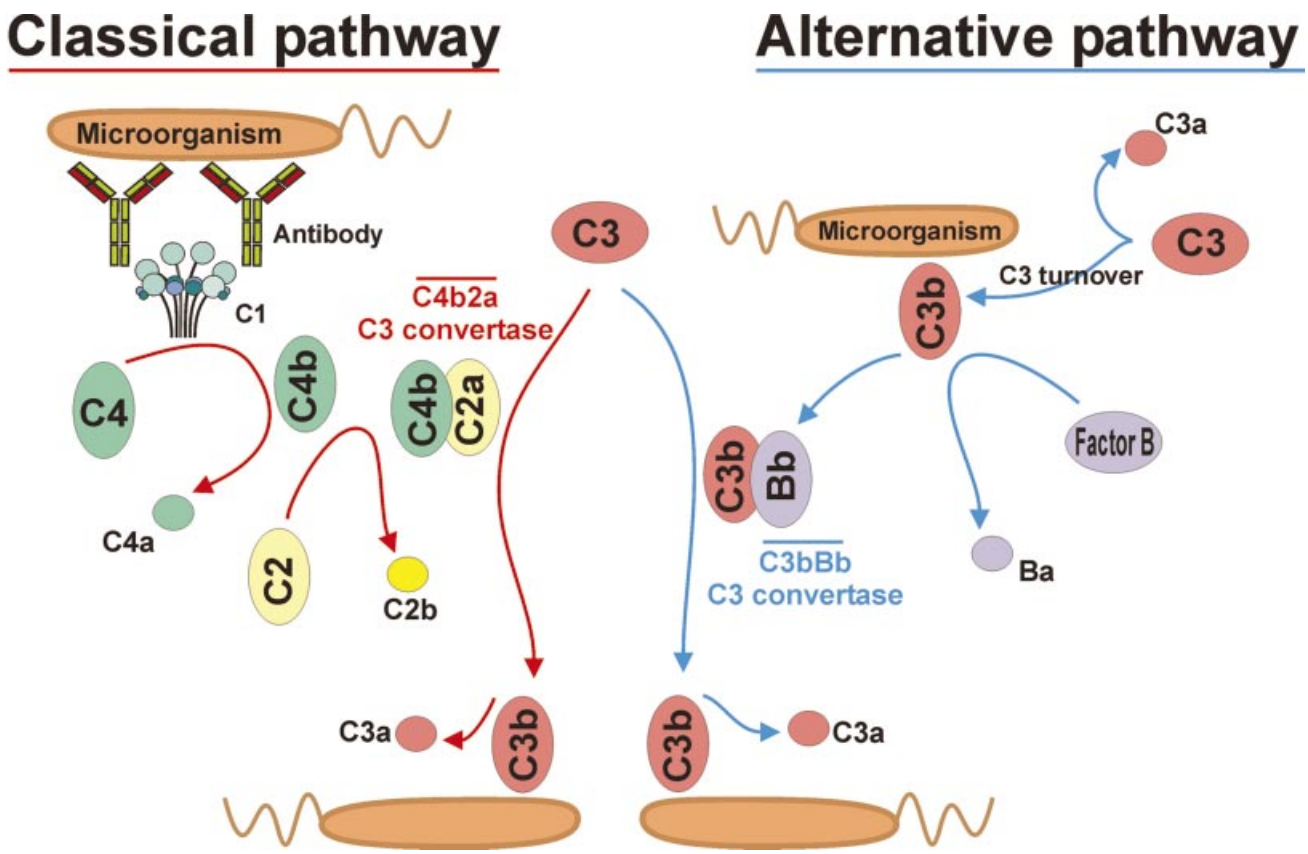

Fig. 4. Brief overview of the activation of C3 via the classical and alternative complement activation pathways. The classical activation pathway (red arrows) is activated by the binding of the C1 complex to either antibody-bound antigen or, in the absence of antibody, by a variety of polymeric antigens. Activated C1 then cleaves C4, releasing C4b and the anaphylatoxin C4a. Most of the C4b is hydrolysed, but some is deposited on the activating surface (e.g. immune complex or micro-organism). C2 is then cleaved, releasing the smaller C2b fragment, whereas the C2a fragment remains bound to C4b and forms the classical pathway C3 convertase ($\overline{C4bC2a}$). The alternative activation pathway (blue arrows) is antibody-independent and is based on the continuous turnover of C3 in tissue fluids, to produce a molecule structurally and functionally similar to C3b, which binds factor B, releasing the smaller fragment Ba. Activating surfaces (e.g. the surfaces of micro-organisms) stabilize C3b and facilitate its binding to factor B to form the alternative activation pathway C3 convertase ($\overline{C3bBb}$). Each C3 convertase cleaves more C3 and results in the binding of many C3b molecules to the same surface. Reviewed in Morley & Walport (2000).

on their surfaces in the form of immune complexes composed of antigen, antibodies and/or the third component of complement (C3). Complement activation and binding to cellular complement receptors are essential for localizing antigens on FDCs (Pepys, 1976; van den Berg *et al.*, 1995; Nielsen *et al.*, 2000), where they are retained for long time periods for recognition by B lymphocytes (Fig. 3*a*). Recent evidence suggests complement also plays an important role in the localization and retention of scrapie to FDCs during the first few days after infection (Klein *et al.*, 2001; Mabbott *et al.*, 2001; Fig. 3*b*). In these studies, both genetic deficiency in C3 (Klein *et al.*, 2001) or transient C3 depletion (Mabbott *et al.*, 2001) impair the accumulation of PrP^{Sc} in the spleen and significantly prolong the incubation period after peripheral injection with scrapie.

Distinct pathways are involved in C3 activation (reviewed in Morley & Walport, 2000; Fig. 4). The classical pathway is initiated primarily by the interaction of C1q with antibody bound to antigen, or in an antibody-independent manner by polymers or cell surface components of pathogenic micro-organisms. Following the initial activation of C1q, subsequent interactions with C4 and C2 are critical for amplification and progression of the classical pathway. In contrast, the alternative pathway is antibody-independent, and is initiated by the direct covalent binding of C3 to a diverse range of polymeric substances including zymosan, bacterial lipopolysaccharide and teichoic acid. Here, factor B and factor D are critical for the progression of the alternative pathway. Deficiencies in any of the above complement proteins lead to a complete deficiency in the relevant activation pathway, and severely impair antigen localization to FDCs.

Mice deficient in C1q ($C1qa^{-/-}$ mice; classical pathway deficiency; Botto *et al.*, 1998), and double knockouts of factor B and C2 ($H2\text{-}Bf/C2^{-/-}$ mice; alternative and classical pathway

deficiency; Taylor *et al.*, 1998) have been used to determine whether the classical or alternative complement activation pathways are involved in the localization of scrapie to FDCs after peripheral infection. These experiments demonstrated that the classical complement activation pathway was most likely to be involved in C3 activation during scrapie infection, as the incubation period was markedly prolonged in both *H2-Bf/C2*$^{-/-}$ and *C1qa*$^{-/-}$ mice (Klein *et al.*, 2001; Mabbott *et al.*, 2001). These studies also suggested C1q itself may interact directly with the CR1 complement receptor (Tas *et al.*, 1999) or the specific C1q receptor (Norsworthy *et al.*, 1999), as the effect of C1q deficiency on scrapie pathogenesis was greater than deficiencies in other down-stream complement components (C2 and C3). The mechanism through which PrP^{Sc} might interact with complement components is not known. As the production of antibodies to PrP^{Sc} has not been detected in TSE infections, it is unlikely that complement activation via the classical pathway is antibody-mediated. Likewise, no role for antibody in the retention of infectivity by FDCs was found, as depletion of circulating antibodies, or of individual antibody receptors (Fc-γ receptors) had no effect on the scrapie pathogenesis if B lymphocyte maturation was unaffected (Klein *et al.*, 2001).

The evidence to date would suggest that FDCs are ideal sites for scrapie replication in lymphoid tissues because they are long-lived cells that express high levels of PrP^{c} and are specialized to trap and retain unprocessed antigens (Fig. 3). Conventional viruses including human immunodeficiency virus type 1 (Racz & Tenner-Racz, 1995), porcine circovirus (Rosell *et al.*, 2000) and bovine viral diarrhoea virus (Collins *et al.*, 1999) have also been detected in association with FDCs, suggesting TSEs may not be the only infectious agents to exploit the unique characteristics of these cells.

Are FDCs potential targets for therapeutic intervention in TSE disease?

Once a TSE infection has spread to the CNS, the neurodegeneration it causes is most likely irreversible. But treatments that interfere with the early stages of infection in peripheral tissues can significantly impair or prevent the spread of disease to the CNS and decrease scrapie susceptibility (Farquhar & Dickinson, 1986; Farquhar *et al.*, 1999). The identification of FDCs as critical cells in the peripheral pathogenesis of TSE diseases suggests these cells could be potential targets for therapy during the interval between exposure to infection and neuroinvasion.

Another cytokine produced by B lymphocytes which is critical for the development of mature FDCs is membrane $LT\alpha_1\beta_2$ (Matsumoto *et al.*, 1996*a*; Koni *et al.*, 1997; Chaplin & Fu, 1998; Fig. 1). Signals are mediated through the LTβ receptor (LTβR; Endres *et al.*, 1999) expressed on the FDC or its precursor (Fig. 1). Specific neutralization of the LTβR signalling pathway through treatment with a fusion protein consisting of LTβR and human immunoglobulin (LTβR-Ig; Force *et al.*, 1995) leads to the temporary disappearance of mature FDCs within 72 h for approximately 28 days (Mackay & Browning, 1998; Fig. 5). Experiments have shown that a single treatment with LTβR-Ig before or shortly after peripheral scrapie challenge blocks the accumulation of PrP^{Sc} and infectivity in the spleen and significantly impairs neuroinvasion (Mabbott *et al.*, 2000*a*; Montrasio *et al.*, 2000). These effects are most likely due to a loss of mature PrP^{c}-expressing FDCs, although effects on other cell types in lymphoid tissues cannot be excluded. Therefore, as predicted, strategies that temporarily inactivate FDCs, such as blockade of the LTβR signalling pathway, may present an opportunity for early intervention in peripherally transmitted TSE diseases.

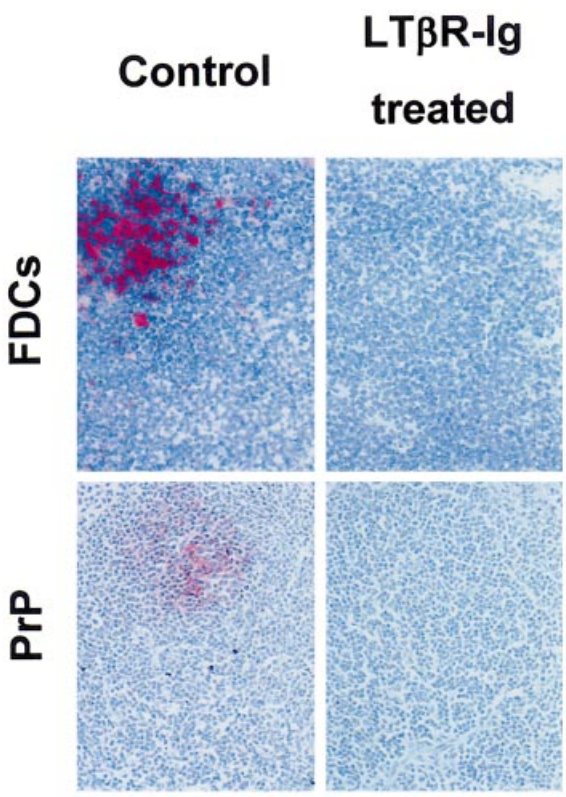

Fig. 5. Blockade of the LTβR signalling pathway by treatment with LTβR-Ig temporarily de-differentiates FDCs within 72 h for approximately 28 days. Mice were treated with a single dose of LTβR-Ig (100 μg) or human immunoglobulin (100 μg) as a control. Spleens were obtained 72 h after treatment and sections stained for FDCs (FDC-M2-positive cells; Kosco-Vilbois *et al.*, 1997) and PrP (1B3-positive cells; Farquhar *et al.*, 1989). Original magnification, ×400.

Recently, experiments in mice suggest that vaccination against the amyloid-β peptide may prevent and possibly be used to treat Alzheimer's disease (Janus *et al.*, 2000; Morgan *et al.*, 2000). This raises speculation that antibodies against PrP^{Sc} may be effective in treating TSE diseases (Thompson, 2001). However, since FDCs are most likely to acquire TSE infectivity in the same way they trap and retain immune complexes (Klein *et al.*, 2001; Mabbott *et al.*, 2001; Fig. 3), such an approach

might lead to the exacerbation of some TSE diseases, by enhancing the localization of infectivity on FDCs.

How do TSEs reach the CNS from the lymphoid tissues?

Although very low levels of infectivity have been detected in the blood-stream in some TSE models (reviewed in Brown, 1996), there is no evidence of haematogenous spread of infection from peripheral tissues to the CNS. Neuroinvasion is crucially dependent on PrP expression in a non-haemopoietic tissue compartment between the lymphoid tissues and CNS, such as peripheral nerves (Blättler *et al.*, 1997; Glatzel & Aguzzi, 2000; Race *et al.*, 2000), which express PrP^c in humans and animals (Heggebø *et al.*, 2000; Shmakov *et al.*, 2000). Following intragastric or oral challenge of rodents with scrapie, the infectious agent first accumulates in Peyer's patches, gut-associated lymphoid tissues and ganglia of the enteric nervous system (Kimberlin & Walker, 1989; Beekes & McBride, 2000). Within the Peyer's patch, abnormal PrP is readily detectable in FDCs, macrophages and cells of the follicle-associated epithelium with morphology consistent with that of M cells (Beekes & McBride, 2000; Fig. 6). Subsequently, infection most likely spreads to the CNS via the enteric nervous system, or splanchnic or vagus nerves (Kimberlin & Walker, 1989; Beekes *et al.*, 1996, 1998; Baldauf *et al.*, 1997; McBride & Beekes, 1999; Beekes & McBride, 2000). However, since the GCs within lymphoid tissues are poorly innervated it is not known how infectivity reaches peripheral nerve endings from FDCs.

Why is PrP^c expressed within lymphoid tissues?

While there is a wealth of information implicating a central role for PrP^{Sc} in the development of TSE disease, the normal function of PrP^c, the cellular form of the prion protein, is not clear. PrP^c is a highly conserved glycoprotein which is attached to the outer leaflet of the cell membrane via a glycophosphatidylinositol anchor (Baldwin *et al.*, 1992). PrP^c mRNA and protein are expressed at high levels in neurones of the adult CNS (Kretzschmar *et al.*, 1986) and peripheral nervous system (Shmakov *et al.*, 2000). Within the CNS, studies from PrP-deficient mice suggest PrP^c may play an important role in neurotransmission (Manson *et al.*, 1995; Collinge *et al.*, 1996), and circadian activity rhythms and sleep patterns (Tobler *et al.*, 1996).

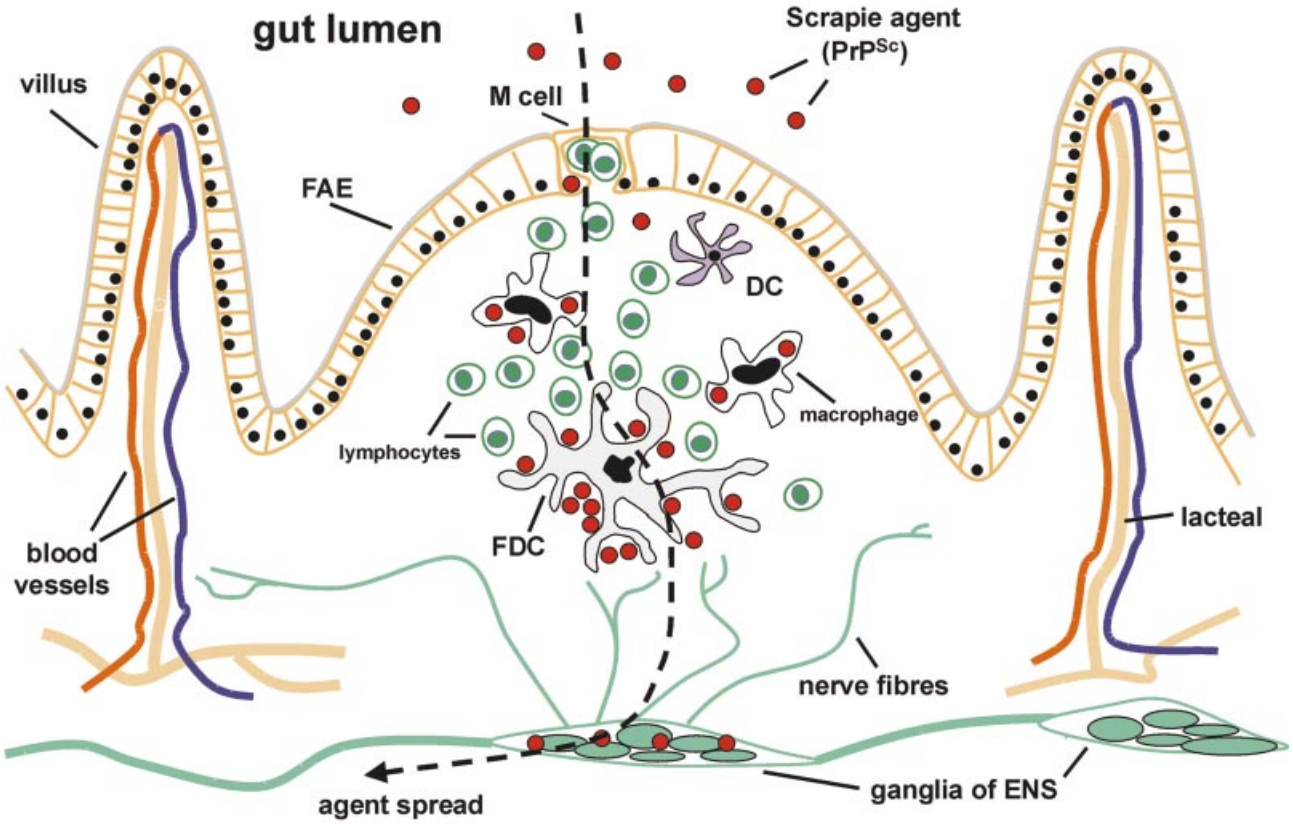

Fig. 6. Possible spread of scrapie infectivity from the gut lumen to the nervous system following oral infection (route indicated by dotted line). Soon after ingestion, PrP^{Sc} is detected readily within Peyer's patches upon FDCs, within macrophages, within cells with morphology consistent with that of M cells and within ganglia of the enteric nervous system (ENS). These observations suggest that following uptake of scrapie infectivity from the gut lumen infectivity accumulates upon FDCs in Peyer's patches and subsequently spreads via the ENS to the CNS. DC, Dendritic cell; FAE, follicle-associated epithelium.

In the lymphoid tissues, FDCs express high levels of PrP^c (McBride *et al.*, 1992; Brown *et al.*, 1999*b*; Mabbott *et al.*, 2000*a*), whereas expression on lymphocytes is low or undetectable (Cashman *et al.*, 1990; Mabbott *et al.*, 1997; Kubosaki *et al.*, 2001; Liu *et al.*, 2001). The function of PrP^c in the normal cellular physiology of lymphoid tissues is also unknown. Both neurones and FDCs are long-lived, mitotically inactive cells and the expression of high levels of PrP^c on both of these cell types suggests it may share a common function. Accumulating evidence suggests PrP^c binds copper (Brown *et al.*, 1997; Stockel *et al.*, 1998) and may act as an antioxidant with superoxide dismutase activity (Brown *et al.*, 1999*a*). It is possible that PrP^c may play a role in the long-term survival of FDCs within lymphoid tissues through protection from oxidative stress. Another study suggests that cell surface expression of PrP^c by FDCs could play a role in the non-specific binding of immune complexes by these cells (Jeffrey *et al.*, 2000). Ultrastructural analysis of spleens from scrapie-infected mice shows abnormal PrP accumulation occurs upon the highly convoluted FDC processes in regions associated with immune complex trapping. Furthermore, the hyperplastic appearance of the FDCs and complexity of their dendritic processes would suggest these cells are highly stimulated in TSE-infected lymphoid tissues (Jeffrey *et al.*, 2000).

Likewise, little is known about the role PrP^c plays in lymphocyte function (Mabbott *et al.*, 1997; Liu *et al.*, 2001), although studies have shown expression is regulated during lymphocyte development in the bone marrow and thymus (Kubosaki *et al.*, 2001; Liu *et al.*, 2001), and following mitogen activation (Cashman *et al.*, 1990; Mabbott *et al.*, 1997). Whatever the function of PrP^c in the host immune system is, the lymphoid tissues of PrP-deficient mice appear to develop normally (Bueler *et al.*, 1992; Manson *et al.*, 1994). Therefore, it is possible that PrP^c may only play a subtle role in the development or function of the immune system, or that the effects of PrP^c are compensated by another gene in PrP-deficient mice.

Conclusions

Collectively the evidence suggests that FDCs are critically required for the pathogenesis of some TSE diseases, as in their absence the accumulation of infectivity in lymphoid tissues and subsequent neuroinvasion following peripheral challenge is significantly impaired. Antigens are trapped and retained on the surface of FDCs through interactions between complement and cellular complement receptors, and recent experiments show these molecules also play an important role in the localization of the PrP^{Sc}-associated scrapie agent to lymphoid follicles (Klein *et al.*, 2001; Mabbott *et al.*, 2001). Therefore, it is likely that FDCs are ideal sites for scrapie replication in lymphoid tissues because they are long-lived cells that express high levels of PrP^c that are specialized to trap and retain unprocessed antigens. FDCs could be potential targets for therapeutic intervention, at least in some natural TSE diseases. Indeed, strategies which down-regulate FDC maturation, such as specific inhibition of the LTβR pathway (Mackay & Browning, 1998), block scrapie replication in the spleen and significantly delay neuroinvasion (Mabbott *et al.*, 2000*a*; Montrasio *et al.*, 2000). Likewise, temporary depletion of complement C3 also delays neuroinvasion (Mabbott *et al.*, 2001). However, some studies suggest that neuroinvasion of TSEs may occur through FDC-independent pathways, particularly after high dose challenge. Natural TSE diseases may also display a similar variation as vCJD is the only human TSE disease in which infectivity and PrP^{Sc} are detectable in tissues outside the CNS (Hilton *et al.*, 1998; Hill *et al.*, 1999; Bruce *et al.*, 2001). Within the lymphoid tissues of vCJD patients, the accumulation of PrP^{Sc} has been demonstrated in association with FDCs (Hill *et al.*, 1999), arguing for a key role for these cells in the pathogenesis of vCJD. Since its first description in 1996 (Will *et al.*, 1996), there have been 100 cases of vCJD in the UK (UK CJD Surveillance Unit, 2001). One particular concern is the recent experimental demonstration of BSE transmission between sheep via blood transfusion (Houston *et al.*, 2000), illustrating a potential risk of iatrogenic spread of vCJD in humans by blood transfusion or treatment with blood products. Therefore, the development of a reliable diagnostic test and retrospective studies of PrP^{Sc} accumulation in human lymphoid tissues (Ironside *et al.*, 2000) may help to provide an estimate of the potential magnitude of the vCJD epidemic and determine the risk of iatrogenic spread.

References

Andréoletti, O., Berthon, P., Marc, D., Sarradin, P., Grosclaude, J., van Keulen, L., Schelcher, F., Elsen, J.-M. & Lantier, F. (2000). Early accumulation of PrP^{Sc} in gut-associated lymphoid and nervous tissues of susceptible sheep from a Romanov flock with natural scrapie. *Journal of General Virology* **81**, 3115–3126.

Baldauf, E., Beekes, M. & Diringer, H. (1997). Evidence for an alternative direct route of access for the scrapie agent to the brain bypassing the spinal cord. *Journal of General Virology* **78**, 1187–1197.

Baldwin, M. A., Stahl, N., Hecker, R., Pan, K.-M., Burlingame, A. L. & Prusiner, S. B. (1992). Glycosylinositol phospholipid anchors of prion proteins. In *Prion Diseases of Humans and Animals*, pp. 380–397. Edited by S. B. Prusiner, J. Collinge, J. Powell & B. Anderton. London: Ellis Horwood.

Beekes, M. & McBride, P. A. (2000). Early accumulation of pathological PrP in the enteric nervous system and gut-associated lymphoid tissue of hamsters orally infected with scrapie. *Neuroscience Letters* **278**, 181–184.

Beekes, M., Baldauf, E. & Diringer, H. (1996). Sequential appearance and accumulation of pathognomonic markers in the central nervous system of hamsters orally infected with scrapie. *Journal of General Virology* **77**, 1925–1934.

Beekes, M., McBride, P. A. & Baldauf, E. (1998). Cerebral targeting indicates vagal spread of infection in hamsters fed with scrapie. *Journal of General Virology* **79**, 601–607.

Beringue, V., Demoy, M., Lasmezas, C. I., Gouritin, B., Weingarten, C., Deslys, J.-P., Adreux, J.-P., Couvreur, P. & Dormont, D. (2000). Role

of spleen macrophages in the clearance of scrapie agent early in pathogenesis. *Journal of Pathology* **190**, 495–502.

Blättler, T., Brandner, S., Raeber, A. J., Klein, M. A., Voigtländer, T., Weissmann, C. & Aguzzi, A. (1997). PrP-expressing tissue required for transfer of scrapie infectivity from spleen to brain. *Nature* **389**, 69–73.

Bolton, D. C., McKinley, M. P. & Prusiner, S. B. (1982). Identification of a protein that purifies with the scrapie prion. *Science* **218**, 1309.

Botto, M., Dell'Agnolla, C., Bygrave, A. E., Thompson, E. M., Cook, H. T., Petry, F., Loos, M., Pandolfi, P. P. & Walport, M. J. (1998). Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. *Nature Genetics* **19**, 56–59.

Brown, P. (1996). The risk of blood-borne Creutzfeldt–Jakob disease. In *Transmissible Subacute Spongiform Encephalopathies: Prion Diseases*, pp. 447–450. Edited by L. Court & B. Dodet. Paris: Elsevier.

Brown, D. R., Qin, K. F., Herms, J. W., Madlung, A., Manson, J., Strome, R., Fraser, P. E., Kruck, T., von Bohlen, A., Schulz-Schaeffer, W., Giese, A., Westaway, D. & Kretzschmar, H. (1997). The cellular prion protein binds copper in vivo. *Nature* **390**, 684–687.

Brown, D. R., Wong, B. S., Hafiz, F., Clive, C., Haswell, S. J. & Jones, I. M. (1999*a*). Normal prion protein has an activity like that of superoxide dismutase. *Biochemical Journal* **344**, 1–5.

Brown, K. L., Stewart, K., Ritchie, D., Mabbott, N. A., Williams, A., Fraser, H., Morrison, W. I. & Bruce, M. E. (1999*b*). Scrapie replication in lymphoid tissues depends on PrP-expressing follicular dendritic cells. *Nature Medicine* **5**, 1308–1312.

Bruce, M. E., Will, R. G., Ironside, J. W., McConnell, I., Drummond, D., Suttie, A., McCardle, L., Chree, A., Hope, J., Birkett, C., Cousens, S., Fraser, H. & Bostock, C. J. (1997). Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. *Nature* **389**, 498–501.

Bruce, M. E., McConnell, I., Will, R. G. & Ironside, J. W. (2001). Detection of variant Creutzfeldt–Jakob disease (vCJD) infectivity in extraneural tissues. *Lancet* **358**, 208–209.

Bueler, H., Fischer, M., Lang, Y., Bluethmann, H., Lipp, H.-P., DeArmond, S. J., Prusiner, S. B., Aguet, M. & Weissmann, C. (1992). Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. *Nature* **356**, 577–582.

Carp, R. I. & Callahan, S. M. (1982). Effect of mouse peritoneal macrophages on scrapie infectivity during extended in vitro incubation. *Intervirology* **17**, 201–207.

Cashman, N. R., Loertscher, R., Nalbantoglu, J., Shaw, I., Kascsak, R. J., Bolton, D. C. & Bendheim, P. E. (1990). Cellular isoform of the scrapie agent protein participates in lymphocyte activation. *Cell* **61**, 185–192.

Chaplin, D. D. & Fu, Y.-X. (1998). Cytokine regulation of secondary lymphoid organ development. *Current Opinion in Immunology* **10**, 289–297.

Collinge, S. B., Collinge, J. & Jefferys, J. G. R. (1996). Hippocampal slices from prion protein null mice: disrupted Ca^{2+}-activated K^+ currents. *Neuroscience Letters* **209**, 49–52.

Collins, M. E., Desport, M. & Brownlie, J. (1999). Bovine viral diarrhea virus quasispecies during persistent infection. *Virology* **259**, 85–98.

Endres, R., Alimzhanov, M. B., Plitz, T., Futterer, A., Kosco-Vilbois, M. H., Nedospasov, S. A., Rajewsky, K. & Pfeffer, K. (1999). Mature follicular dendritic cell networks depend on expression of lymphotoxin β receptor by radioresistant stromal cells and of lymphotoxin β and tumour necrosis factor by B cells. *Journal of Experimental Medicine* **189**, 159–168.

Farquhar, C. F. & Dickinson, A. G. (1986). Prolongation of scrapie incubation period by an injection of dextran sulphate 500 within the month before or after infection. *Journal of General Virology* **67**, 463–473.

Farquhar, C. F., Somerville, R. A. & Ritchie, L. A. (1989). Post-mortem immunodiagnosis of scrapie and bovine spongiform encephalopathy. *Journal of Virological Methods* **24**, 215–222.

Farquhar, C. F., Dornan, J., Somerville, R. A., Tunstall, A. M. & Hope, J. (1994). Effect of *Sinc* genotype, agent isolate and route of infection on the accumulation of protease-resistant PrP in non-central nervous system tissues during the development of murine scrapie. *Journal of General Virology* **75**, 495–504.

Farquhar, C., Dickinson, A. & Bruce, M. (1999). Prophylactic potential of pentosan polysulphate in transmissible spongiform encephalopathies. *Lancet* **353**, 117.

Force, W. R., Walter, B. N., Hession, C., Tizard, R., Kozak, C. A., Browning, J. L. & Ware, C. F. (1995). Mouse lymphotoxin-beta receptor. *Journal of Immunology* **155**, 5280–5288.

Fraser, H. & Dickinson, A. G. (1978). Studies on the lymphoreticular system in the pathogenesis of scrapie: the role of spleen and thymus. *Journal of Comparative Pathology* **88**, 563–573.

Fraser, H. & Farquhar, C. F. (1987). Ionising radiation has no influence on scrapie incubation period in mice. *Veterinary Microbiology* **13**, 211–223.

Fraser, H., Brown, K. L., Stewart, K., McConnell, I., McBride, P. & Williams, A. (1996). Replication of scrapie in spleens of SCID mice follows reconstitution with wild-type mouse bone marrow. *Journal of General Virology* **77**, 1935–1940.

Frigg, R., Klein, M. A., Hegyi, I., Zinkernagel, R. M. & Aguzzi, A. (1999). Scrapie pathogenesis in subclinically infected B-cell-deficient mice. *Journal of Virology* **73**, 9584–9588.

Glatzel, M. & Aguzzi, A. (2000). PrP^C expression in the peripheral nervous system is a determinant of prion neuroinvasion. *Journal of General Virology* **81**, 2813–2821.

Heggebø, R., Press, C. M., Gunnes, G., Lie, K. I., Tranulis, M. A., Ulvund, M., Groschup, M. H. & Landsverk, T. (2000). Distribution of prion protein in the ileal Peyer's patch of scrapie-free lambs and lambs naturally and experimentally exposed to the scrapie agent. *Journal of General Virology* **81**, 2327–2337.

Hill, A. F., Desbruslais, M., Joiner, S., Sidle, K. C. L., Gowland, I. & Collinge, J. (1997*a*). The same prion strain causes vCJD and BSE. *Nature* **389**, 448–450.

Hill, A. F., Zeidler, M., Ironside, J. & Collinge, J. (1997*b*). Diagnosis of new variant Creutzfeldt–Jakob disease by tonsil biopsy. *Lancet* **349**, 99–100.

Hill, A. F., Butterworth, R. J., Joiner, S., Jackson, G., Rossor, M. N., Thomas, D. J., Frosh, A., Tolley, N., Bell, J. E., Spencer, M., King, A., Al-Sarraj, S., Ironside, J. W., Lantos, P. L. & Collinge, J. (1999). Investigation of variant Creutzfeldt–Jakob disease and other prion diseases with tonsil biopsy samples. *Lancet* **353**, 183–189.

Hilton, D., Fathers, E., Edwards, P., Ironside, J. & Zajicek, J. (1998). Prion immunoreactivity in appendix before clinical onset of variant Creutzfeldt–Jakob disease. *Lancet* **352**, 703–704.

Houston, F., Foster, J. D., Chong, A., Hunter, N. & Bostock, C. J. (2000). Transmission of BSE by blood transfusion in sheep. *Lancet* **356**, 999.

Ironside, J. W., Hilton, D. A., Ghani, A., Johnston, N. J., Conyers, L., McCardle, L. M. & Best, D. (2000). Retrospective study of prion protein accumulation in tonsil and appendix tissues. *Lancet* **355**, 1693–1694.

Janus, C., Pearson, J., McLaurin, J., Mathews, P. M., Jiang, Y., Schmidt, S. D., Chishti, M. A., Horne, P., Heslin, D., French, J., Mount, H. T. J., Nixon, R. A., Mercken, M., Bergeron, C., Fraser, P. E., St George-Hyslop, P. & Westaway, D. (2000). Aβ peptide immunization reduces

behavioural impairment and plaques in a model of Alzheimer's disease. *Nature* **408**, 979–982.

Jeffrey, M., McGovern, G., Goodsir, C. M., Brown, K. L. & Bruce, M. E. (2000). Sites of prion protein accumulation in scrapie-infected mouse spleen revealed by immuno-electron microscopy. *Journal of Pathology* **190**, 323–332.

Kapasi, Z. F., Burton, G. F., Schultz, L. D., Tew, J. G. & Szakal, A. K. (1993). Induction of functional follicular dendritic cell development in severe combined immunodeficiency mice. *Journal of Immunology* **150**, 2648–2658.

Kapasi, Z. F., Qin, D., Kerr, W. G., Kosco-Vilbois, M. H., Schultz, L. D., Tew, J. G. & Szakal, A. K. (1998). Follicular dendritic cell (FDC) precursors in primary lymphoid tissues. *Journal of Immunology* **160**, 1078–1084.

Kimberlin, R. H. & Walker, C. A. (1979). Pathogenesis of mouse scrapie: dynamics of agent replication in spleen, spinal cord and brain after infection by different routes. *Journal of Comparative Pathology* **89**, 551–562.

Kimberlin, R. H. & Walker, C. A. (1989). Pathogenesis of scrapie in mice after intragastric infection. *Virus Research* **12**, 213–220.

Kitamoto, T., Muramoto, T., Mohri, S., Doh-Ura, K. & Tateishi, J. (1991). Abnormal isoform of prion protein accumulates in follicular dendritic cells in mice with Creutzfeldt–Jakob disease. *Journal of Virology* **65**, 6292–6295.

Klein, M. A., Frigg, R., Flechsig, E., Raeber, A. J., Kalinke, U., Bluethmann, H., Bootz, F., Suter, M., Zinkernagel, R. M. & Aguzzi, A. (1997). A crucial role for B cells in neuroinvasive scrapie. *Nature* **390**, 687–691.

Klein, M. A., Frigg, R., Raeber, A. J., Flechsig, E., Hegyi, I., Zinkernagel, R. M., Weissmann, C. & Aguzzi, A. (1998). PrP expression in B lymphocytes is not required for prion neuroinvasion. *Nature Medicine* **4**, 1429–1433.

Klein, M. A., Kaeser, P. S., Schwarz, P., Weyd, H., Xenarios, I., Zinkernagel, R. M., Carroll, M. C., Verbeek, J. S., Botto, M., Walport, M. J., Molina, H., Kalinke, U., Acha-Orbea, H. & Aguzzi, A. (2001). Complement facilitates early prion pathogenesis. *Nature Medicine* **7**, 488–492.

Koni, P. A., Sacca, R., Lawton, P., Browning, J. L., Ruddle, N. H. & Flavell, R. A. (1997). Distinct roles in lymphoid organogenesis for lymphotoxins α and β revealed in lymphotoxin β-deficient mice. *Immunity* **6**, 491–500.

Kopf, M., Herren, S., Wiles, M. V., Pepys, M. B. & Kosco-Vilbois, M. H. (1998). Interleukin 6 influences germinal center development and antibody production via a contribution of C3 complement component. *Journal of Experimental Medicine* **188**, 1895–1906.

Kosco, M. H., Pflugfelder, E. & Gray, D. (1992). Follicular dendritic cell-dependent adhesion and proliferation of B cells *in vitro*. *Journal of Immunology* **148**, 2331–2339.

Kosco-Vilbois, M. H., Zentgraf, H., Gerdes, J. & Bonnefoy, J.-Y. (1997). To 'B' or not to 'B' a germinal center? *Immunology Today* **18**, 225–230.

Kretzschmar, H. A., Prusiner, S. B., Stowring, L. E. & De Armond, S. J. (1986). Scrapie prion proteins are synthesized in neurons. *American Journal of Pathology* **122**, 1–5.

Kubosaki, A., Yusa, S., Nasu, Y., Nishimura, T., Nakamura, Y., Saeki, K., Matsumoto, Y., Itohara, S. & Onodera, T. (2001). Distribution of cellular isoform of prion protein in T lymphocytes and bone marrow, analyzed by wild-type and prion protein gene-deficient mice. *Biochemical and Biophysical Research Communications* **282**, 103–107.

Liu, T., Li, R., Wong, B., Liu, D., Pan, T., Petersen, R., Gambetti, P. & Sy, M.-S. (2001). Normal cellular prion protein is preferentially expressed on subpopulations of murine hemopoietic cells. *Journal of Immunology* **166**, 3733–3742.

Mabbott, N. A., Brown, K. L., Manson, J. & Bruce, M. E. (1997). T lymphocyte activation and the cellular form of the prion protein. *Immunology* **92**, 161–165.

Mabbott, N. A., Mackay, F., Minns, F. & Bruce, M. E. (2000*a*). Temporary inactivation of follicular dendritic cells delays neuroinvasion of scrapie. *Nature Medicine* **6**, 719–720.

Mabbott, N. A., Williams, A., Farquhar, C. F., Pasparakis, M., Kollias, G. & Bruce, M. E. (2000*b*). Tumor necrosis factor-alpha-deficient, but not interleukin-6-deficient, mice resist peripheral infection with scrapie. *Journal of Virology* **74**, 3338–3344.

Mabbott, N. A., Bruce, M. E., Botto, M., Walport, M. J. & Pepys, M. B. (2001). Temporary depletion of complement component C3 or genetic deficiency of C1q significantly delays onset of scrapie. *Nature Medicine* **7**, 485–487.

McBride, P. A. & Beekes, M. (1999). Pathological PrP is abundant in sympathetic and sensory ganglia of hamsters fed with scrapie. *Neuroscience Letters* **265**, 135–138.

McBride, P., Eikelenboom, P., Kraal, G., Fraser, H. & Bruce, M. E. (1992). PrP protein is associated with follicular dendritic cells of spleens and lymph nodes in uninfected and scrapie-infected mice. *Journal of Pathology* **168**, 413–418.

McFarlin, D. E., Raff, M. C., Simpson, E. & Nehlsen, S. H. (1971). Scrapie in immunologically deficient mice. *Nature* **233**, 336.

Mackay, F. & Browning, J. L. (1998). Turning off follicular dendritic cells. *Nature* **395**, 26–27.

Manson, J. C., Clarke, A. R., Hooper, M. L., Aitchison, L., McConnell, I. & Hope, J. (1994). 129/Ola mice carrying a null mutation in PrP that abolishes mRNA production are developmentally normal. *Molecular Neurobiology* **8**, 121–127.

Manson, J. C., Hope, J., Clarke, A. R., Johnston, A., Black, C. & MacLeod, N. (1995). PrP gene dosage and long term potentiation. *Neurodegeneration* **4**, 113–115.

Manuelidis, L., Zaitsev, I., Koni, P., Lu, Z. Y., Flavell, R. A. & Fritch, W. (2000). Follicular dendritic cells and dissemination of Creutzfeldt–Jakob disease. *Journal of Virology* **74**, 8614–8622.

Matsumoto, M., Lo, S. F., Carruthers, C. J. L., Min, J., Mariathasan, S., Huang, G., Plas, D. R., Martin, S. M., Geha, R. S., Nahm, M. H. & Chaplin, D. D. (1996*a*). Affinity maturation without germinal centres in lymphotoxin-α-deficient mice. *Nature* **382**, 462–466.

Matsumoto, M., Mariathasan, S. V., Nahm, M. H., Baranyay, F., Peschon, J. J. & Chaplin, D. D. (1996*b*). Role of lymphotoxin and the type 1 TNF receptor in the formation of germinal centers. *Science* **271**, 1289–1291.

Montrasio, F., Frigg, R., Glatzel, M., Klein, M. A., Mackay, F., Aguzzi, A. & Weissmann, C. (2000). Impaired prion replication in spleens of mice lacking functional follicular dendritic cells. *Science* **288**, 1257–1259.

Montrasio, F., Cozzio, A., Flechsig, E., Rossi, D., Klein, M. A., Rulicke, T., Raeber, A. J., Vosshenrich, C. A. J., Proft, J., Aguzzi, A. & Weissmann, C. (2001). B-lymphocyte-restricted expression of the prion protein does not enable prion replication in PrP knockout mice. *Proceedings of the National Academy of Sciences, USA* **98**, 4034–4037.

Morgan, D., Diamond, D. M., Gottschall, P. E., Ugen, K. E., Dickey, C., Hardy, J., Duff, K., Jantzen, P., Dicarlo, G., Wilcock, D., Connor, K., Hatcher, J., Hope, C., Gordon, M. & Arendash, G. W. (2000). Aβ peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* **408**, 982–985.

Morley, B. J. & Walport, M. J. (2000). The complement system. In *The Complement Facts Book*, pp. 7–22. Edited by B. J. Morley & M. J. Walport. London: Academic Press.

Nielsen, C. H., Fischer, E. M. & Leslie, R. G. Q. (2000). The role of complement in the acquired immune response. *Immunology* **100**, 4–12.

Norsworthy, P. J., Taylor, P. R., Walport, M. J. & Botto, M. (1999). Cloning of the mouse homolog of the 126 kDa human C1q/MBL/SP-A receptor, C1qR(p). *Mammalian Genome* **10**, 789–793.

Pasparakis, M., Alexopoulo, L., Episkopou, V. & Kollias, G. (1996). Immune and inflammatory responses in TNFα-deficient mice: a critical requirement for TNFα in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centres, and in the maturation of the humoral immune response. *Journal of Experimental Medicine* **184**, 1397–1411.

Pepys, M. B. (1976). Role of complement in the induction of immunological responses. *Transplant Reviews* **32**, 93–120.

Prusiner, S. B., Bolton, D. C., Groth, D. F., Bowman, K. A., Cochran, S. P. & McKinley, M. P. (1982). Further purification and characterisation of scrapie prions. *Biochemistry* **21**, 6942–6950.

Race, R., Oldstone, M. & Chesebro, B. (2000). Entry versus blockade of brain infection following oral or intraperitoneal scrapie administration: role of prion protein expression in peripheral nerves and spleen. *Journal of Virology* **74**, 828–833.

Racz, P. & Tenner-Racz, K. (1995). Germinal centre tropism of HIV-1 and other retroviruses. In *Follicular Dendritic Cells in Normal and Pathological Conditions*, pp. 159–181. Edited by E. Heinen. Bethesda, MD: R. G. Landes.

Raeber, A. J., Klein, M. A., Frigg, R., Flechsig, E., Aguzzi, A. & Weissmann, C. (1999*a*). PrP-dependent association of prions with splenic but not circulating lymphocytes of scrapie-infected mice. *EMBO Journal* **18**, 2702–2706.

Raeber, A. J., Sailer, A., Hegyi, I., Klein, M. A., Rulicke, T., Fischer, M., Brandner, S., Aguzzi, A. & Weissmann, C. (1999*b*). Ectopic expression of prion protein (PrP) in T lymphocytes or hepatocytes of PrP knockout mice is insufficient to sustain prion replication. *Proceedings of the National Academy of Sciences, USA* **96**, 3987–3992.

Rosell, C., Segales, J., Ramos Vara, J. A., Folch, J. M., Rodriguez Arrioja, G. M., Duran, C. O., Balash, M., Plana Duran, J. & Domingo, M. (2000). Identification of porcine circovirus in tissues of pigs with porcine dermatitis and nephropathy syndrome. *Veterinary Record* **146**, 40–43.

Shmakov, A. N., McLennan, N. F., McBride, P., Farquhar, C. F., Bode, J., Rennison, K. A. & Ghosh, S. (2000). Cellular prion protein is expressed in the human enteric nervous system. *Nature Medicine* **6**, 840–841.

Sigurdson, C. J., Williams, E. S., Miller, M. W., Spraker, T. R., O'Rourke, K. I. & Hoover, E. A. (1999). Oral transmission and early lymphoid tropism of chronic wasting disease PrP^{res} in mule deer fawns (*Odocoileus hemionus*). *Journal of General Virology* **80**, 2757–2764.

Smith, J. P., Lister, A. M., Tew, J. G. & Szakal, A. K. (1991). Kinetics of the tingible body macrophage response in mouse germinal center development and its depression of age. *Anatomical Record* **229**, 511–520.

Smith, J. P., Burton, G. F., Tew, J. G. & Szakal, A. K. (1998). Tingible body macrophages in regulation of germinal center reactions. *Developmental Immunology*, 285–294.

Somerville, R. A., Birkett, C. R., Farquhar, C. F., Hunter, N., Goldmann, W., Dornan, J., Grover, D., Hennion, R. M., Percy, C., Foster, J. & Jeffrey, M. (1997). Immunodetection of PrP^{Sc} in spleens of some scrapie-infected sheep but not BSE-infected cows. *Journal of General Virology* **78**, 2389–2396.

Stockel, J., Safar, J., Wallace, A. C., Cohen, F. E. & Prusiner, S. B. (1998). Prion protein selectively binds copper(II) ions. *Biochemistry* **37**, 7185–7193.

Tas, S. W., Klickstein, L. B., Barbashov, S. F. & Nicholson-Weller, A. (1999). C1q and C4b bind simultaneously to CR1 and additively support erythrocyte adhesion. *Journal of Immunology* **163**, 5056–5063.

Taylor, D. M., McConnell, I. & Fraser, H. (1996). Scrapie infection can be established readily through skin scarification in immunocompetent but not immunodeficient mice. *Journal of General Virology* **77**, 1595–1599.

Taylor, P. R., Nash, J. T., Theodoridis, E., Bygrave, A. E., Walport, M. J. & Botto, M. (1998). A targeted disruption of the murine complement factor B gene resulting in loss of expression of three genes in close proximity, factor B, C2, and D17H6S45. *Journal of Biological Chemistry* **273**, 1699–1704.

Thompson, C. (2001). In search of a cure for CJD. *Nature* **409**, 660–661.

Tkachuk, M., Bolliger, S., Ryffel, B., Pluschke, G., Banks, T. A., Herren, S., Gisler, R. H. & Kosco-Vilbois, M. H. (1998). Crucial role of tumour necrosis factor receptor 1 expression on nonhematopoietic cells for B cell localization within the splenic white pulp. *Journal of Experimental Medicine* **187**, 469–477.

Tobler, I., Gaus, S. E., Deboer, T., Achermann, P., Fischer, M., Rulicke, T., Moser, M., Oesch, B., McBride, P. A. & Manson, J. C. (1996). Altered circadian activity rhythms and sleep in mice devoid of prion protein. *Nature* **380**, 639–642.

UK CJD Surveillance Unit (2001). CJD statistics. http://www.cjd.ed.ac.uk/figures.htm.

van den Berg, T. K., Yoshida, K. & Dukstra, C. D. (1995). Mechanisms of immune complex trapping by follicular dendritic cells. *Current Topics in Microbiology and Immunology* **201**, 49–63.

van Keulen, L. J. M., Schreuder, B. E. C., Meloen, R. H., Mooij-Harkes, G., Vromans, M. E. W. & Langeveld, J. P. M. (1996). Immunohistological detection of prion protein in lymphoid tissues of sheep with natural scrapie. *Journal of Clinical Microbiology* **34**, 1228–1231.

van Keulen, L. J. M., Schreuder, B. E. G., Vromans, M. E. W., Langeveld, J. P. M. & Smits, M. A. (1999). Scrapie-associated prion protein in the gastro-intestinal tract of sheep with scrapie. *Journal of Comparative Pathology* **121**, 55–63.

Wilesmith, J. W., Ryan, J. B. & Atkinson, M. J. (1991). Bovine spongiform encephalopathy: epidemiological studies of the origin. *Veterinary Record* **218**, 199–203.

Will, R. G., Ironside, J. W., Zeidler, M., Cousens, S. N., Estibeiro, K., Alperovitch, A., Poser, S., Pocchiari, M. & Hofman, A. (1996). A new variant of Creutzfeldt–Jacob disease in the UK. *Lancet* **347**, 921–925.

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