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\(^\text{c}\)) co-purifies with infectivity in diseased tissues (Bolton et al., 1982). This modified protein (PrP\(^\text{Sc}\)) 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\(^\text{c}\) (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., 1997a). Sporadic CJD in humans can be transmitted autogenously 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\(^\text{Sc}\) rapidly accumulate in lymphoid tissues (Kimberlin & Walker, 1979; Farquhar et al., 1994; Brown et al., 1999b; Beekes & McBride, 2000; Heggebo et al., 2000; Mabbott et al., 2000b), long before either is detectable in the central nervous system (CNS). Likewise, PrP\(^\text{Sc}\) 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\(^\text{Sc}\) is first detected in Peyer’s patches and gut-associated lymphoid tissues (Andréoletti et al., 2000; Heggebo 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.

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., 1999b; Beekes & McBride, 2000; Jeffrey et al., 2000; Mabbott et al., 2000b), 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\(^\text{c}\) (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\(^{-/-}\), β2-µ\(^{-/-}\), TCR\(^{α-\delta}\) 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)
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 (Raebet al., 1999a). There is also no evidence that B lymphocytes themselves secrete neuroinvasive or neurodegenerative factors during disease (Frigget 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., 1999b; Frigget 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 (Trachuk et al., 1998), and mice deficient in either TNFα (Pasparakis et al., 1996) or TNF-R1 (Matsumoto et al., 1996b) 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 PrPSc in their spleens (Mabbott et al., 2000b). In spleens from scrapie-challenged TNFα−/− mice, the absence of detectable infectivity and PrPSc in the spleen and reduced disease susceptibility coincide with an absence of mature FDCs (Brown et al., 1999b; Mabbott et al., 2000b). Lymphocytes are unlikely to be directly involved in disease pathogenesis, as both T and B lymphocytes are present and functional in lymphoid tissues of TNFα−/− mice (Pasparakis et al., 1996). Taken together, these results suggest that the accumulation of infectivity and PrPSc 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., 1999b; Mabbott et al., 2000b), with similar studies in immunodeficient mice lacking mature FDCs [TNF-R1−/− (Matsumoto et al., 1996b) and lymphotoxin (LT) β−/− (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 LTβ−/− 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 PrPSc accumulations have been detected in direct association with FDCs in the lymphoid tissues of patients with vCJD (Hill et al., 1997b, 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 $\alpha_1/\alpha_2$, which signals exclusively through the lymphotoxin $\beta_2$ receptor, and soluble tumour necrosis factor-$\alpha$ (TNF-$\alpha$), 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).
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 (Klatt et al., 1997; Klein et al., 1998; Brown et al., 1999b). 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., 1999b; Table 1). This provides strong evidence that FDCs themselves produce PrP 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., 1999b; 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

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

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Donor cells</th>
<th>PrP expressed by</th>
<th>Scrapie titre*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prnp+/+</td>
<td>–</td>
<td>FDCs Yes, Lymphocytes Yes</td>
<td>6·1, 6·3, 5·7, 6·2†</td>
</tr>
<tr>
<td>Prnp−/−</td>
<td>–</td>
<td>No FDCs, No Lymphocytes</td>
<td>Undetectable†</td>
</tr>
<tr>
<td>SCID/Prnp+/+</td>
<td>Prnp+/+ bone marrow</td>
<td>FDCs Yes, Lymphocytes Yes</td>
<td>6·0, 5·7†</td>
</tr>
<tr>
<td>SCID/Prnp−/−</td>
<td>Prnp−/− bone marrow</td>
<td>FDCs Yes, Lymphocytes No</td>
<td>5·5, 5·9†</td>
</tr>
<tr>
<td>SCID/Prnp+/+</td>
<td>Prnp+/+ lymphocytes</td>
<td>FDCs Yes, Lymphocytes Yes</td>
<td>4·8, 4·4‡</td>
</tr>
<tr>
<td>SCID/Prnp−/−</td>
<td>Prnp−/− lymphocytes</td>
<td>FDCs Yes, Lymphocytes No</td>
<td>4·8, 4·7‡</td>
</tr>
<tr>
<td>SCID/Prnp+/+</td>
<td>–</td>
<td>No FDCs, No Lymphocytes</td>
<td>Undetectable†</td>
</tr>
<tr>
<td>SCID/Prnp−/−</td>
<td>Prnp+/+ bone marrow</td>
<td>No FDCs, Lymphocytes Yes</td>
<td>Undetectable$</td>
</tr>
<tr>
<td>SCID/Prnp−/−</td>
<td>Prnp−/− bone marrow</td>
<td>No FDCs, No Lymphocytes</td>
<td>Undetectable†</td>
</tr>
</tbody>
</table>

* Infectivity titres in log intracerebral 50% infectious dose (units/g).
† Brown et al. (1999b).
‡ 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., 1999).

### 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, 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 (Klatt et al., 1997; Klein et al., 1998; Brown et al., 1999b). 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., 1999b; Table 1). This provides strong evidence that FDCs themselves produce PrP 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., 1999b; 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.
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., 1999b), 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). Intrasynosomal 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 PrPSc 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 PrPc only on T lymphocytes (Raeb et al., 1999b) 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 PrPc by FDCs (McBride et al., 1992; Brown et al., 1999b) is unlikely to be the only important property of these cells for scrapie accumulation and disease transmission. FDCs characteristically trap and retain antigens...
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 microorganisms. 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-Bf/C2−/− mice; alternative and classical pathway deficiencies; Botto et al., 1998) show a severe defect in the accumulation of PrPSc in the spleen and significantly prolong the incubation period after peripheral injection with scrapie.
receptors (Fc-)
depletion of circulating antibodies, or of individual antibody
antibody in the retention of infectivity by FDCs was found, as
classical pathway is antibody-mediated. Likewise, no role for
infections, it is unlikely that complement activation via the
production of antibodies to PrP
interact with complement components is not known. As the
pathogenesis if B lymphocyte maturation was unaffected
whether the classical or alternative complement activation
pathways are involved in the localization of scrapie to FDCs
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 PrPSc might
interact with complement components is not known. As the
production of antibodies to PrPSc 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- 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 PrPSc 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
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
LTαβ (Matsumoto et al., 1996a; 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.
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 PrPSc (1B3-positive cells; Farquhar et al., 1989).
Original magnification, × 400.

Fig. 5. Blockade of the LTβR signalling pathway by treatment with LTβR-Ig
Consistent 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 PrPSc and
infectivity in the spleen and significantly impairs neuroinvasion
(Mabbott et al., 2000a; Montrasio et al., 2000). These effects
are most likely due to a loss of mature PrPSc-expressing FDCs,
although effects on other cell types in lymphoid tissues cannot
be excluded. Therefore, as predicted, strategies that tem-
porarily inactivate FDCs, such as blockade of the LTβR
signalling pathway, may present an opportunity for early
intervention in peripherally transmitted TSE diseases.

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 PrPSc
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 PrPc 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 PrPc expressed within lymphoid tissues?**

While there is a wealth of information implicating a central role for PrPSc in the development of TSE disease, the normal function of PrPc, the cellular form of the prion protein, is not clear. PrPc is a highly conserved glycoprotein which is attached to the outer leaflet of the cell membrane via a glycosylphosphatidylinositol anchor (Baldwin et al., 1992). PrPc 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 PrPc 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).

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**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, PrPSc 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 PrPc (McBride et al., 1992; Brown et al., 1999b; Mabbott et al., 2000a), 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 PrPc 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 PrPc on both of these cell types suggests it may share a common function. Accumulating evidence suggests PrPc binds copper (Brown et al., 1997; Stockel et al., 1998) and may act as an antioxidant with superoxide dismutase activity (Brown et al., 1999a). It is possible that PrPc 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 PrPc 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 PrPc 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 PrPc 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 PrPc may only play a subtle role in the development or function of the immune system, or that the effects of PrPc 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 PrPsc-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 PrPc 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 LTR pathway (Mackay & Browning, 1998), block scrapie replication in the spleen and significantly delay neuroinvasion (Mabbott et al., 2000a; 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 PrPsc 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 PrPsc 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 PrPsc 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


Published ahead of print (15 June 2001) in JGV Direct as DOI 10.1099/vir.0.17771-0.