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Migrating intestinal dendritic cells transport PrP^{Sc} from the gut

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Bovine spongiform encephalopathy, variant Creutzfeldt–Jakob disease (vCJD) and possibly also sheep scrapie are orally acquired transmissible spongiform encephalopathies (TSEs). TSE agents usually replicate in lymphoid tissues before they spread into the central nervous system. In mouse TSE models PrP^{c}-expressing follicular dendritic cells (FDCs) resident in lymphoid germinal centres are essential for replication, and in their absence neuroinvasion is impaired. Disease-associated forms of PrP (PrP^{Sc}), a biochemical marker for TSE infection, also accumulate on FDCs in the lymphoid tissues of patients with vCJD and sheep with natural scrapie. TSE transport mechanisms between gut lumen and germinal centres are unknown. Migratory bone marrow-derived dendritic cells (DCs), entering the intestinal wall from blood, sample antigens from the gut lumen and carry them to mesenteric lymph nodes. Here we show that DCs acquire PrP^{Sc} *in vitro*, and transport intestinally administered PrP^{Sc} directly into lymphoid tissues *in vivo*. These studies suggest that DCs are a cellular bridge between the gut lumen and the lymphoid TSE replicative machinery.

The transmissible spongiform encephalopathies (TSEs), or 'prion' diseases, are neurodegenerative disorders which include Creutzfeldt–Jakob disease (CJD) and kuru in humans, bovine spongiform encephalopathy (BSE), transmissible mink encephalopathy, chronic wasting disease (CWD) in mule deer and elk, and scrapie in sheep and goats. Replication of the infectious TSE agent depends critically on the host prion protein (PrP^{c}), which accumulates as an abnormal, detergent-insoluble, relatively proteinase-resistant isoform, PrP^{Sc}, in diseased tissues (Bolton *et al.*, 1982; Bueler *et al.*, 1992). The precise nature of the infectious agent is uncertain, but PrP^{Sc} co-purifies with infectivity and is considered to be a major component (Farquhar *et al.*, 1998; Prusiner *et al.*, 1982).

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The consumption of BSE-contaminated meat products is the most likely cause of variant (v) CJD in humans (Bruce *et al.*, 1997; Hill *et al.*, 1997), and ingestion has been implicated in the transmission of other TSE diseases. The timing of events in TSE pathogenesis, as determined by tracking PrP^{Sc} accumulation, varies depending on agent strain, host genotype and the route of infection (Farquhar *et al.*, 1994, 1996). However, soon after experimental intragastric or oral exposure of rodents with scrapie, infectivity and PrP^{Sc} accumulate first in Peyer's patches, gut-associated lymphoid tissues and ganglia of the enteric nervous system (Beekes & McBride, 2000; Kimberlin & Walker, 1989), long before their detection in the central nervous system (CNS). Likewise, following experimental oral exposure of mule deer fawns with CWD, PrP^{Sc} is also detected first in lymphoid tissues draining the gastro-intestinal tract (Sigurdson *et al.*, 1999). How and when sheep become infected with natural scrapie is not known, but the detection of PrP^{Sc} in Peyer's patches and gut-associated lymphoid tissues (Andréoletti *et al.*, 2000; Heggebø *et al.*, 2000) prior to detection within the CNS (van Keulen *et al.*, 1999) suggests that this disease is also acquired orally.

Early PrP^{Sc} accumulation takes place on follicular dendritic cells (FDCs) within germinal centres in lymphoid tissues of patients with vCJD (Hill *et al.*, 1999), sheep with natural scrapie (van Keulen *et al.*, 1996) and rodents inoculated with scrapie by peripheral routes (Brown *et al.*, 1999; Mabbott *et al.*, 2000b; McBride *et al.*, 1992). In mouse scrapie models, mature FDCs are critical for scrapie replication and PrP^{Sc} accumulation in lymphoid tissues, and in their absence neuroinvasion following peripheral challenge is significantly impaired (Brown *et al.*, 1999; Mabbott *et al.*, 2000a, b; Montrasio *et al.*, 2000).

The transport mechanisms by which TSE agents reach the germinal centres from the gut lumen are not known. Migratory bone marrow-derived dendritic cells (DCs) are centrally involved in transport of proteins both within Peyer's patches and on into mesenteric lymph nodes (Banchereau *et al.*, 2000). These cells are a distinct lineage from FDCs, which are tissue-resident and are not considered to be of haemopoietic origin (Endres *et al.*, 1999; Kapasi *et al.*, 1993). DCs enter the intestinal

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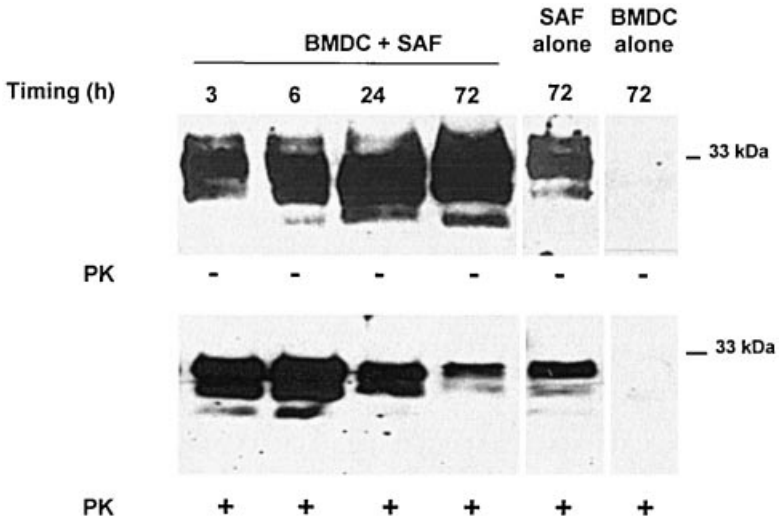

Fig. 1. BMDCs acquire PrP^{Sc} following *in vitro* culture with SAF. BMDCs (1×10^6 cells) were cultured in the absence (BMDC alone) or presence of SAF (equivalent to 10 mg infected brain tissue) for the times indicated. Immunoblots show the accumulation of detergent-insoluble, relatively proteinase K-resistant PrP^{Sc} within BMDC lysates. Treatment of lysates in the presence (+) or absence (−) of proteinase K (PK) is indicated. SAF (equivalent to 10 mg infected brain tissue) was incubated in medium alone as a control. Following PK treatment, a typical three-band pattern was observed between molecular mass values of 20 and 30 kDa, representing unglycosylated, monoglycosylated and diglycosylated isomers of PrP (in order of increasing molecular mass). SAF equivalent to 50 μg infected brain tissue and/or BMDCs equivalent to 10^4 cells were loaded per lane.

wall from the bloodstream, sample antigens from the gut lumen, and then migrate via lymph to mesenteric lymph nodes (Liu & MacPherson, 1993). These observations suggested to us that migrating DCs might provide a cellular bridge between the gut lumen and the secondary lymphoid tissues in which TSE agents replicate.

To test the hypothesis that DCs can acquire TSE agents, we first investigated the uptake of PrP^{Sc} by DCs *in vitro*. Rat bone marrow-derived DCs (BMDCs) were prepared as previously described (Huang *et al.*, 2000) and cultured at 1×10^6 cells/ml in RPMI 1640 medium supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 40 ng/ml murine GM-CSF and 1500 U/ml rat IL-4. Medium and cytokines were replaced every 72 h, and by day 10 to 12 of culture 90% of cells had characteristic DC morphology and expressed MHC class II, B7 and CD11c. Scrapie-associated fibrils (SAF), highly infective fibrillar aggregates of PrP^{Sc}, were prepared from the brains of mice terminally affected with the mouse-passaged ME7 strain as previously described (Hope *et al.*, 1986), sonicated in PBS, and a suspension equivalent to 10 mg infected brain tissue (wet weight) was added to each BMDC culture for the times indicated. Following incubation, culture medium was aspirated and cells lysed with 0·1% *N*-laurylsarcosine. Lysates were treated in the presence or absence of 50 μg proteinase K for 30 min at 37 °C, subjected to electrophoresis through 12% SDS–polyacrylamide gels (Bio-Rad) and proteins transferred to polyvinylidine difluoride membranes (Bio-Rad). PrP was detected with rabbit polyclonal antiserum 1B3 specific for PrP (Farquhar *et al.*, 1989) and bound antibody visualized by enhanced chemiluminescence (Amersham).

Detergent-insoluble, relatively proteinase K-resistant PrP^{Sc} accumulations were detected in BMDC lysates within 3 h of culture with SAF, peaking at around 6 h of culture (Fig. 1). No PrP^{Sc} accumulations were detected at any time in lysates from BMDCs treated with PBS (Fig. 1) or SAF equivalent preparation from normal uninfected brain (data not shown). Neither was uptake identified when B or T lymphocytes were incubated with SAF *in vitro* (data not shown). After antigen acquisition by DCs, a large proportion is degraded in endosomal/lysosomal compartments for presentation to T lymphocytes on MHC class II (Banchereau *et al.*, 2000). We have shown, however, that DCs, unlike macrophages, can retain some protein antigens in native, non-degraded form for at least 36 h (Wykes *et al.*, 1998). After 24 h of culture of BMDCs with SAF, the level of PrP^{Sc} detected had declined moderately (Fig. 1), implying that BMDCs acquire PrP^{Sc}, some of which is subsequently catabolized but a considerable proportion of which is retained intact. An increased proteinase K-sensitive PrP signal was also detected after 24 h incubation (Fig. 1), which may also represent the break-up of SAF aggregates within the DC and the revealing of more epitopes as the PrP is digested.

We next sought to demonstrate whether DCs can acquire and transport PrP^{Sc} *in vivo* to mesenteric lymph nodes after delivery of SAF by intra-intestinal injection. PVG ($RT1^c$) rats bred and maintained under specific-pathogen-free conditions were mesenteric lymphadenectomized as previously described (Liu *et al.*, 1998; Pugh *et al.*, 1983). Six weeks later, when the afferent lymphatics (lacteals) draining the intestine had joined the efferent mesenteric lymphatics, SAF (equivalent to 10 mg infected brain tissue per rat) or PBS (as a control) was injected into the jejunum. Cells that would normally have been trapped in the mesenteric lymph nodes in intact animals were then collected by thoracic duct cannulation over 8 to 16 h. Lymph DCs (> 90% pure) were isolated by a combined density centrifugation and magnetic antibody cell sorting protocol as previously described (Huang *et al.*, 2000), while T and B lymphocytes (> 99% pure) were isolated by magnetic antibody cell sorting alone.

Immunocytochemical analysis showed that after intra-intestinal SAF exposure, large amounts of PrP were present as

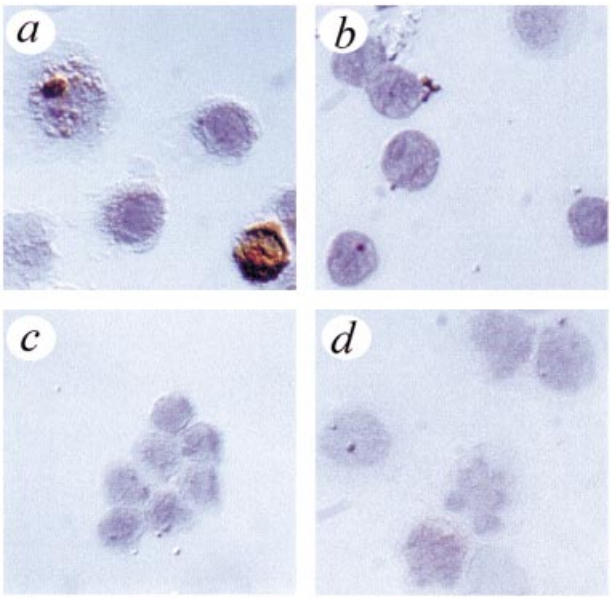

Fig. 2. DCs transport intestinally injected SAF to mesenteric nodes via lymph. Lymph was collected 8 to 16 h after intestinal injection of SAF and strong cytoplasmic inclusions of PrP were detected by immunocytochemistry in a small proportion of DCs (a) but not B (b) or T (c) lymphocytes in the thoracic duct pseudo-lymph of mesenteric lymphadenectomized rats. Only endogenous PrP was detected in DCs from PBS-injected control animals (d). Magnification $\times 1000$. In all panels, PrP was detected using the PrP-specific polyclonal antiserum 1B3 (Farquhar *et al.*, 1989).

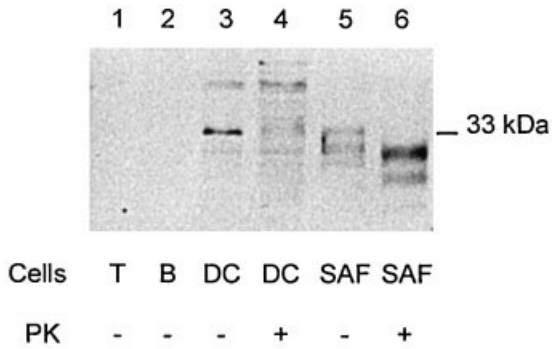

Fig. 3. Immunoblot analysis of pooled cell lysates (1×10^6 cells per lane) from SAF-treated rats confirmed the presence of PrP^{Sc} in lymph DCs (lane 4) but not in T or B lymphocytes. SAF equivalent to 2 or 4 μg of infected brain tissue was loaded in lanes 5 and 6, respectively. Treatment of samples in the presence (+) or absence (−) of proteinase K (PK) before electrophoresis is indicated. PrP dimers are seen at approximately 60 kDa in lanes 3 and 4.

conspicuous cytoplasmic inclusions in 4 to 5% of lymph DCs (Fig. 2a). No such deposits were identified within T or B lymphocyte populations (Fig. 2b, c, respectively). Much weaker PrP staining was seen around lymph DCs (Fig. 2d) and B lymphocytes (data not shown) from PBS-treated controls, indicative of membrane-associated endogenously expressed rat PrP^c. Immunoblot analysis confirmed the presence of detergent-insoluble, relatively proteinase K-resistant PrP^{Sc} in lymph DC lysates selected from SAF-injected rats (Fig. 3, lane 4). However, the characteristic molecular mass shift in the three-band PrP^{Sc} signature after proteinase K digestion was not seen (Fig. 3, lane 6; Hope *et al.*, 1986). The multiple bands detected may indicate a difference in PrP processing either in the intestine or within DCs. No PrP was detected in lysates of T or B lymphocytes selected from SAF-injected rats (Fig. 3, lanes 1, 2, respectively). Neither was PrP detected in lysates from DCs, T or B lymphocytes from mesenteric lymphadenectomized rats treated with PBS or an SAF equivalent preparation from normal uninfected brain as a control (data not shown).

We next attempted to estimate scrapie infectivity levels in cell populations by animal bioassays. Pooled cell lysates were prepared from DCs, T lymphocytes or B lymphocytes from SAF-treated rats and injected intracerebrally into groups of 12 assay mice (approximately $2{\cdot}5 \times 10^5$ cells per mouse). Despite the detection of PrP^{Sc} in lymph DCs by immunoblot (Fig. 3) and immunocytochemical (Fig. 2a) analysis, infectivity levels were below the level detectable by bioassay. This most probably reflects the sensitivity of the assay given that only a small number of cells were available for injection per assay mouse. Of those, only a small subset of the DCs had acquired PrP^{Sc} (approximately 1×10^4 cells per mouse). This small number of cells represents the maximum we could collect; each assay mouse (usually 12 per group) receiving DCs from two cannulated rats. As infectivity bioassays are more sensitive than PrP immunoblots, the failure to detect scrapie infectivity in DC lysates despite positive detection of PrP^{Sc} by immunoblot is most likely because greater numbers of cells were analysed in the immunoblot study (1×10^6 cells per lane). All other cell populations and concentrated cell-free lymph plasma ($\times 75$ using Microcon concentrators, Amicon) from SAF-injected rats were also negative.

In this study we show that DCs can acquire PrP^{Sc} *in vitro* and that a small sub-population of migrating DCs can take up and transport PrP^{Sc} from the gut lumen through the lymphatics to lymphoid tissue. We have also shown that the uptake of PrP^{Sc} from the gut lumen is restricted to DCs, as no PrP^{Sc} was detected in other lymph cells or cell-free lymph plasma. The small numbers of cells involved, perhaps in addition to partial intracellular degradative mechanisms, may explain the longer incubation periods, and reduced efficiency of infection, following oral exposure in comparison with other peripheral routes. Within lymphoid tissue FDCs play a critical role in the amplification of TSE infectivity outside the CNS (Brown *et al.*, 1999; Mabbott *et al.*, 2000a, b; Montrasio *et al.*, 2000). Our findings suggest that following infection via the gastrointestinal tract, DCs act as a cellular bridge between the gut lumen and the lymphoid TSE replicative machinery.

Within the intestine, DCs have been described in the lamina propria (Maric *et al.*, 1996), and in Peyer's patches where they form a dense layer of cells in the subepithelial dome, just beneath the follicle-associated epithelium and in close contact with M cells (Kelsall & Strober, 1996). Following oral challenge

of rodents with scrapie, heavy pathological PrP accumulations are detected within cells of the follicle-associated epithelium with morphology consistent with M cells (Beekes & McBride, 2000), which have the potential to transcytose infectivity *in vitro* (Heppner *et al.*, 2001). However, further studies are necessary to determine whether DCs acquire PrP^{Sc} after it has been internalized by M cells, or by direct uptake across the mucosal epithelium as recently shown for the transport of apoptotic intestinal epithelial cells (Huang *et al.*, 2000) or bacteria (Rescigno *et al.*, 2001). In addition, our studies do not exclude the possibility of direct uptake into PrP-expressing enteric nerves (Shmakov *et al.*, 2000).

The detection of infectivity within lymphoid tissues (Bruce *et al.*, 2001) and PrP accumulation upon FDCs of patients with vCJD (Hill *et al.*, 1999) suggest that this disease shares a similar pathogenesis to rodent TSE models. Immunomodulation alters susceptibility to TSEs in rodent models (Mabbott *et al.*, 1998) and gut inflammation markedly stimulates DC traffic from the intestine (MacPherson *et al.*, 1995). Our studies suggest that it will be important to investigate where TSEs are taken up in the human gastro-intestinal tract, and whether this can be exacerbated by inflammatory conditions that stimulate DC migration.

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