Distribution of prion protein in the ileal Peyer’s patch of scrapie-free lambs and lambs naturally and experimentally exposed to the scrapie agent

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A sensitive immunohistochemical procedure was used to investigate the presence of prion protein (PrP) in the ileal Peyer’s patch of PrP-genotyped lambs, including scrapie-free lambs and lambs naturally and experimentally exposed to the scrapie agent. The tyramide signal amplification system was used to enhance the sensitivity of conventional immunohistochemical procedures to show that PrP was widely distributed in the enteric nervous plexus supplying the gut wall. In scrapie-free lambs, PrP was also detected in scattered cells in the lamina propria and in the dome and interfollicular areas of the Peyer’s patch. In the follicles, staining for PrP was mainly confined to the capsule and cells associated with vascular structures in the light central zone. In lambs naturally exposed to the scrapie agent, staining was prominent in the dome and neck region of the follicles and was also found to be associated with the follicle-associated epithelium. Similar observations were made in lambs that had received a single oral dose of scrapie-infected brain material from sheep with a homologous and heterologous PrP genotype 1 and 5 weeks previously. These studies show that the ileal Peyer’s patch in young sheep may be an important site of uptake of the scrapie agent and that the biology of this major gut-associated lymphoid tissue may influence the susceptibility to oral infection in sheep. Furthermore, these studies suggest that homology or heterology between PrP genotypes or the presence of PrP genotypes seldom associated with disease does not impede uptake of PrP.

Introduction

In scrapie, a transmissible subacute spongiform encephalopathy of sheep and goats, the alimentary tract is considered to be a major route of entry of infection (Hadlow et al., 1982). However, the involvement of the gut-associated lymphoid tissues in the uptake of the scrapie agent and in the early pathogenesis of the infection has been addressed predominantly in rodents (Beekes & McBride, 2000). Experimental studies in rodent models have shown that while spread of the agent directly to peripheral nerves probably occurs (Fraser et al., 1996), replication and accumulation of the agent in peripheral lymphoid tissues appear to facilitate neuroinvasion (Lasmézas et al., 1996). Follicular dendritic cells have been implicated as the cells of the lymphoid system that sustain replication of the agent in transmissible spongiform encephalopathies (McBride et al., 1992; Kitamoto et al., 1991; Brown et al., 1999), although the presence of mature B-lymphocytes has been shown to influence the course of infection in mice (Klein et al., 1997, 1998). Studies in sheep have shown that the abnormal isoform of the prion protein PrP, termed PrP<sup>Sc</sup>, which is strongly associated with disease (Bolton et al., 1982), localizes to germinal centres in lymphoid tissues such as the spleen and lymph node (van Keulen et al., 1996), consistent with the distribution of follicular dendritic cells in sheep.

Another important experimental observation for the early pathogenesis of scrapie is the need for expression of the normal cellular prion protein, termed PrP<sup>C</sup>. Studies have shown that
transgenic mice not expressing PrP<sup>C</sup> are resistant to infection by the scrapie agent (Büeler et al., 1993) and that PrP<sup>C</sup>-expressing tissue is required for transmission of infectivity from the periphery to the central nervous system (Blättler et al., 1997). While biochemical and genetic investigations have mapped expression of PrP<sup>C</sup> at the tissue and organ level in sheep (Brown et al., 1990; Horiiuchi et al., 1995; Goldmann et al., 1999), few immunohistochemical studies of lymphoid tissues have addressed the distribution of PrP<sup>C</sup>. Most immunohistochemical protocols have been directed toward detecting the accumulation of PrP<sub>Sc</sub> that occurs during disease (van Keulen et al., 1996; Miller et al., 1993). Indeed, the diagnostic usefulness of these procedures is based on the inability of conventional immunohistochemical protocols to detect normal cellular PrP.

Gut-associated lymphoid tissue refers to the solitary and aggregated lymphatic follicles, subepithelial lymphocytes, plasma cells and macrophages and intraepithelial lymphocytes that are distributed along the whole length of the alimentary tract (Landsverk et al., 1991; Landsverk & Press, 1998). In sheep, during the first months of life, a major continuous aggregation of lymphatic follicles, termed the ileal Peyer’s patch, is present in the ileum and terminal jejunum but by 18 months of age this major lymphoid organ has undergone involution and is all but non-existent (Carlens, 1928; Reynolds & Morris, 1983). During its life, the ileal Peyer’s patch is responsible for generating the vast majority of B-lymphocytes in the circulation and peripheral lymphoid tissues (Reynolds & Morris, 1983; Gerber et al., 1986; Press et al., 1996) and for diversification of the pre-immune antibody repertoire (Reynaud et al., 1991; Lucier et al., 1998). Accordingly, the lymphatic follicles of the ileal Peyer’s patch consist predominantly of B-lymphocytes supported by an extensive network of mesenchymal stromal cells including follicular dendritic cells and reticular cells along with a population of tingible body macrophages (Nicander et al., 1991; Halleraker et al., 1990; Press et al., 1992). T-lymphocytes, macrophages and dendritic cells are a significant presence in the lymphoid tissues overlying and adjacent to the follicles, namely the dome and interfollicular areas (Halleraker et al., 1990; Press et al., 1992). The ileal Peyer’s patch also possesses another feature of possible relevance to the pathogenesis of scrapie, namely a follicle-associated epithelium that can take up a wide spectrum of macromolecules and particles (Landsverk, 1987, 1988). The uptake of pathological agents across this specialized epithelium has been implicated in a number of diseases including salmonellosis and paratuberculosis infection (Wolf & Bye, 1984; Momotani et al., 1988; Landsverk et al., 1990a).

Infection with the scrapie agent is considered to occur in young sheep, less than 9 months of age (Hourigan et al., 1979). The apparent susceptibility of lambs to infection is influenced by a number of factors including PrP genotype, strain of agent and environmental factors such as artificial or natural rearing (Hunter et al., 1996; Elsen et al., 1999). During this period of high susceptibility to infection, the ileal Peyer’s patch is the major gut-associated lymphoid tissue possessing an extensive bed of follicular dendritic cells and a specialized epithelium actively engaged in the uptake and transcytosis of macromolecules from the gut. However, studies of the distribution of PrP<sub>Sc</sub> in the lymphoid tissues of sheep with scrapie have mostly focused on the spleen and lymph nodes or investigated mucosa-associated lymphoid tissues at readily accessible sites such as the tonsils (Schreuder et al., 1998). The present study was undertaken to investigate the distribution of PrP in the ileal Peyer’s patch of sheep. The distribution of PrP in lambs that had been exposed to scrapie-infected material either naturally or experimentally was compared with the distribution of PrP<sup>C</sup> in scrapie-free lambs.

### Methods

**Sheep and tissue collection.** The sheep investigated were of the Norwegian white breeds (Steiger, Rygja and Dala); their PrP genotypes designated according to the major disease-linked polymorphisms at codons 136 (alanine/valine), 154 (arginine/histidine) and 171 (glutamine/arginine) are presented in Tables 1–3. The PrP genotypes of the confirmed natural cases of scrapie are presented in Table 2. Tissue samples were collected at an abattoir (Fellesslakteriet, Oslo) from 12 randomly selected healthy lambs, 5 months of age. About 70% of the sheep received at this abattoir originate from counties where scrapie has never been diagnosed (Hedmark, Oppland and Østfold), and 30% originate from a county where scrapie has been diagnosed once and in one sheep only (1998). Passive abattoir surveys in the region (obex sectioning on healthy sheep over 3.5 years of age) through the years 1997–1999 have also been negative on histopathology and immunohistochemistry (Bratberg et al., 2000). The four counties have very few sheep of the Rygja and Steiger breeds (between 0.3% and 5%) (Anon., 2000), which are the breeds that have been affected in more than 90% of the scrapie cases in Norway (Tranulis et al., 1999).

### Table 1. Status and PrP genotype of scrapie-free lambs collected from the abattoir

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PrP genotypes are designated according to the major disease-linked polymorphisms at codons 136 (alanine/valine), 154 (arginine/histidine) and 171 (glutamine/arginine).
Experimental oral exposure to brain material from natural cases of scrapie: AV<br>136RR154QQ171 (two cases) and VV136RR154QQ171 (one case)

PrP genotypes are designated as in Table 1.

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Tissue samples were collected from a further nine lambs, aged from 2–8 weeks, that had been born in a flock of sheep while being held in an isolation facility. This flock of sheep was maintained in the isolation facility while the diagnosis of scrapie was confirmed in three adult sheep with clinical symptoms. The nine lambs were born from clinically healthy and histologically normal mothers. The PrP genotypes of the lambs and the clinical cases from this flock are presented in Table 2.

An experimental oral infection of 11 lambs of known PrP genotype (Table 3) was performed. Each lamb was given a single dose of 15 ml of a 30% (w/v) homogenate of brain tissue (5 g brain tissue) by stomach tube. The homogenate for each PrP genotype contained frozen sheep brain tissue (−20°C; transverse section of cerebrum anterior to hippocampus) from clinical cases of scrapie with the appropriate PrP genotype. The frozen tissues were pooled and mixed with physiological saline in a stomacher. The brain tissue from clinical cases with the PrP genotypes VV136RR154QQ171 and AV136RR154QQ171 had shown extensive histopathological changes typical for scrapie. The brain tissue from the clinical case with the PrP genotype AA136RR154QQ171, which is a PrP genotype that is seldom associated with disease, had only minor histopathological changes, restricted to the cerebellum. In all clinical cases, the diagnosis of scrapie was confirmed using immunohistochemistry as described by van Keulen et al. (1995), with polyclonal antibodies kindly provided by L. J. M. van Keulen (DLO-Institute for Animal Science and Health, Lelystad, The Netherlands) and Western blotting (National Veterinary Institute, Oslo, Norway).

One week after oral exposure to the scrapie agent, four lambs aged 7–9 weeks were necropsied and tissue samples collected. Five lambs were necropsied and tissue samples collected 5 weeks after oral dosing at an age of 11–13 weeks. Two lambs were necropsied 11 months after oral dosing. Tissue samples were collected from control lambs that were the same age and had the same PrP genotype as the lambs orally exposed to the scrapie agent. The control lambs received an oral dose of physiological saline on the same day and in the same manner as the lambs exposed to the scrapie agent and were necropsied on the same day after oral dosing, namely 1 week (four lambs), 5 weeks (five lambs) and 11 months (one lamb) (Table 3).

<table>
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<td>1 week</td>
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<td>AA136RR154QQ171</td>
<td>Homology</td>
<td>11 months</td>
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<tr>
<td>AA136RR154QQ171</td>
<td>AA136RR154QQ171</td>
<td>Control</td>
<td>11 months</td>
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For the three groups of lambs, namely abattoir-collected control lambs, and naturally and experimentally exposed lambs, tissue samples were collected from the ileal Peyer’s patch near the insertion of the ileocaecal fold. The tissue samples were frozen in 1,1,1-trifluorotane/pentfluorotane (R404A, Ausimont Via S. Pietro, Bollate, Italy), chilled in liquid nitrogen and stored at −70 °C. To protect the mucosa during freezing and sectioning, the tissues were placed with the mucosa down onto pieces of liver. Sections 8 µm in thickness were cut on a cryostat and stored at −70 °C.

### PrP genotyping

PrP genotyping was performed on blood or frozen tissues as described previously (Tranulis et al., 1999). Briefly, tissue samples were thawed and gently detached from the supporting liver tissues and thoroughly washed in lysis buffer (10 mM Tris–HCl pH 8.0; 100 mM EDTA; 1%, w/v, SDS) by vortexing followed by centrifugation at 10000 g for 30 s and removal of the supernatant to allow addition of fresh lysis buffer. This procedure was repeated five times to ensure adequate removal of any contaminant DNA from the liver slices. PrP polymorphisms were detected by automated DNA sequencing, using dye-terminator cycle sequencing of a PCR-generated product covering codons 93 to 216 of the PrP open reading frame (GenBank accession no. M31313). Samples were analysed by capillary electrophoresis on an ABI Prism 310 Genetic Analyser (Perkin Elmer).

### Immunohistochemistry

An avidin–biotin–peroxidase immunohistochemical method ( Vectastain ABC Kit, Vector Laboratories) was used to detect PrP in frozen tissue sections. This protocol did not distinguish between PrP\(^+\) and PrP\(^-\). The protocol incorporated an avidin–biotin blocking step (Avidin/Biotin Blocking Kit, Vector Laboratories) and was combined with a tyramide signal amplification system (TSA-Indirect, NEN Life Science Products) to increase the sensitivity of the protocol over that of conventional immunohistochemical procedures.

The frozen sections were allowed to dry for at least 2 h and were fixed in 10% formal-calcium for 10 min. The sections were washed in Tris-buffered saline (TBS) pH 7.5 for 10 min and then incubated with a blocking reagent (TSA-Indirect) containing 17% avidin (Vector Laboratories) for 20 min to block endogenous biotin. The blocking reagent from the TSA kit was used as diluent in all further steps in the protocol. The blocking serum was tapped off the slides, and the primary antibody containing 17% biotin (Vector Laboratories) was applied directly to the sections. The mouse monoclonal anti-PrP antibodies used in the present study were P4 (Harmeyer et al., 1998), L42 (Hardt et al., 2000) and eH4 (Korth et al., 1997), and all had an IgG1 isotype. P4 and eH4 both recognize an epitope in the same domain of ruminant PrP while L42 recognizes a different epitope in the first a-helix of ruminant PrP. The sections were incubated overnight at 4 °C. After washing for 10 min in TBS, the sections were incubated with a biotinylated secondary antibody (Vectastain ABC Kit) for 30 min, washed and then incubated for 10 min with 1% H2O2 in methanol to inhibit endogenous peroxidase. Following washing, the sections were incubated for 30 min with peroxidase-conjugated biotin–avidin complex (Vectastain ABC Kit). After washing, the sections were incubated with biotinyl tyramide (TSA-Indirect) for 5 min, washed and then incubated with streptavidin–horseradish peroxidase for 30 min. Peroxidase activity was detected using 3-aminop-9-ethyl carbazole (Sigma) for 10 min. The reaction was stopped by washing the sections in distilled water. The sections were counterstained with haematoxylin, mounted in polyvinyl alcohol and cover-slipped before examination. As a control for the anti-PrP antibodies used, the primary antibody was replaced with the blocking reagent containing 17% biotin, or with an irrelevant antibody of the same isotype as the primary antibody. A mouse monoclonal IgG1 antibody against rainbow trout immunoglobulin (K. Falk, personal communication) was used. Furthermore, sections from a confirmed scrapie case in an adult sheep were included in each immunohistochemical run. As a control for the tissues, an IgG1 monoclonal antibody against a sheep T-lymphocyte marker (SBU-T1) (Beya et al., 1986) was used.

For sections that were not subjected to tyramide signal amplification, the initial 30 min incubation with the peroxidase-conjugated biotin–avidin–complex was followed by washing and incubation with the aminoethylcarbazole solution, as above.

### Results

Within the various groups of lambs, namely control scrapie-free lambs and lambs naturally and experimentally exposed to the scrapie agent, the three anti-PrP monoclonal antibodies showed similar patterns of immunolabelling in the ileal Peyer’s patch (Fig. 1). However, antibody eH4 tended to give some diffuse staining in the lamina propria and in the interfollicular areas of the Peyer’s patches. Examination of control sections showed that this reactivity was not related to endogenous biotin or endogenous peroxidase or nonspecific interactions of the antibody isotype.

### Control scrapie-free lambs

In sections not subjected to tyramide signal amplification, staining for PrP was only detected in the peripheral nervous tissue of the enteric plexus. This staining was predominantly confined to the myenteric plexus between the muscle layers of the gut wall (Fig. 2A). Staining was faint in some individuals.

With tyramide signal amplification, staining was more prominent in all individuals (Fig. 2B). There was stronger staining in the myenteric plexus and staining was also detected in the capsule of the Peyer’s patch follicles (Fig. 2D). In the lamina propria and interfollicular area, long, thin processes, possibly representing nerves or smooth muscle cells, showed staining for PrP (Fig. 2C). Some large, mononuclear cells in these areas also showed staining. In follicles, cells associated with blood vessels or stromal elements located in the light central zone showed some staining (Fig. 2D). Many follicles showed little or no staining.

### Lambs naturally exposed to scrapie

As with the control scrapie-free lambs, in sections not subjected to tyramide signal amplification, staining for PrP was detected only in the peripheral nervous tissue of the enteric plexus. With tyramide signal amplification, a prominent feature in lambs from the naturally exposed group was staining in the dome and the neck region of follicles (Fig. 3A). Small focal areas of staining were also observed to be associated with the luminal border of some epithelial cells in the follicle-associated epithelium and in some regions of the adjacent absorptive epithelium. A granular punctate pattern of staining was evident in the domes as was strong staining in the cytoplasm of large mononuclear cells. Strong staining was also present in large,
Fig. 1. Immunohistochemical detection of PrP in the ileal Peyer’s patch of a lamb with PrP genotype VV_{136}RR_{154}QQ_{171} following experimental oral exposure to the scrapie agent from a donor with PrP genotype VV_{136}RR_{154}QQ_{171} 1 week previously. Sections were stained with anti-PrP monoclonal antibodies L42 (A), P4 (B), 6H4 (C) and an irrelevant isotype control antibody (D). Note consistent granular staining in the light central zone of follicles a and c of panels (A)–(C) while there is little or no staining in follicle b. Frozen tissue. Bar, 88 µm.

Single mononuclear cells in the interfollicular areas (not shown). In some follicles, the granular pattern of staining extended into the follicle and staining was prominent throughout the light central zone of the follicle (Fig. 3B). The dark peripheral zone of the follicle, close to the capsule, tended to show little or no staining. The level of staining varied between follicles and between individuals.

Lambs experimentally exposed to scrapie

As with the other two groups of lambs, tyramide signal amplification was needed to detect PrP in areas other than the enteric nerve plexus of the intestinal wall. With signal amplification, the age- and genotype-matched control lambs showed patterns of staining that were similar to the patterns in the control scrapie-free lambs. One and five weeks after experimental exposure, the lambs showed patterns of staining that were similar to those of the naturally exposed lambs. However, in the experimentally exposed lambs, the levels of staining in the domes and neck region of the follicles and in the interfollicular areas and lamina propria were less prominent (Fig. 4A). More evident in the experimentally exposed lambs was the difference in levels of staining between follicles (Figs 1 and 4B). Most follicles showed no staining while some showed a weak granular pattern in the neck region that extended into the light central zone of the follicle. However, a small number of follicles showed very strong staining throughout the light central zone and extending almost to the capsule. This very strong staining in a small number of follicles was most prominent in the tissues collected from the lambs 1 week after oral exposure to the scrapie agent. These lambs also showed some staining associated with the brush border of cells in the absorptive epithelium.

In the ileal Peyer’s patch of the two lambs collected 11 months after oral dosing, strong staining was present only in the peripheral nervous tissue of the enteric plexus. Most follicles showed little or no staining, except for the capsule of the follicles.
Fig. 2. Immunohistochemical detection of PrP in the ileal Peyer's patch of scrapie-free lambs. (A) Lamb with PrP genotype AA136RR154RR171 shows weak staining for PrP (red) in the myenteric plexus (arrow) between the muscle layers of the gut wall following a conventional immunohistochemical procedure. Frozen tissue. Bar, 15 µm. (B) A section from the same tissue sample as panel (A) stained using tyramide signal amplification (TSA). Note the stronger presence of staining (arrow) for PrP in the myenteric plexus. Frozen tissue. Bar, 15 µm. (C) Lamb with PrP genotype AV136RR154QQ171 shows staining for PrP (red) in the lamina propria (arrow), a, absorptive epithelium; d, dome. Frozen tissue. Bar, 45 µm. (D) A section from the same tissue sample as panel (C) showing staining for PrP (red) in the capsule (thin arrows) of the lymphoid follicles (f) and associated with blood vessels (arrowheads) or stromal elements within the follicles. Some cells in the submucosal interfollicular zone (i) also show staining (curved arrow). m, muscularis mucosa. Frozen tissue. Bar, 45 µm.

Discussion

In this study, a sensitive immunohistochemical protocol was used to show that PrP has a wide distribution in the ileal Peyer's patch of lambs during the first 6 months of life and that the distribution is altered following exposure to the scrapie agent. To enhance the overall sensitivity of detection of PrP in the early pathogenesis of transmissible spongiform encephalopathies, an immunohistochemical protocol that did not distinguish between PrP$^C$ and the abnormal isoform of PrP
PrP in Peyer’s patch of lambs with scrapie

associated with disease in scrapie-infected animals, PrP\textsuperscript{Sc}, was used. The PrP epitopes recognized by antibodies that are currently available for use in immunohistochemistry are not specific for PrP\textsuperscript{C} or PrP\textsuperscript{Sc}. Traditionally, stringent immunohistochemical protocols are adopted that diminish PrP\textsuperscript{C} and enhance the immunodetection of PrP\textsuperscript{Sc} (van Keulen \textit{et al.}, 1996; Miller \textit{et al.}, 1993; Hardt \textit{et al.}, 2000). However, by using frozen tissues and permissive immunohistochemical techniques, PrP can be detected in tissues outside the central nervous system of scrapie-free animals (McBride \textit{et al.}, 1992). The increased sensitivity of the present protocol, which was achieved using the tyramide signal amplification system (Egan \textit{et al.}, 1996; Hunyady \textit{et al.}, 1996), enabled PrP to be detected in control scrapie-free lambs and in lambs only a short time after exposure to scrapie-infected material.

PrP was consistently detected within the enteric nervous system of the intestinal wall from the three groups of lambs examined in the present study. The enteric nervous system associated with the ileal Peyer’s patch includes a myenteric plexus between the muscle layers, a submucosal plexus between the lymphoid follicles and nerve fibres extending into interfollicular areas and domes of follicles and to the lamina propria (Krammer & Künnel, 1993). PrP\textsuperscript{Sc} has been detected in the peripheral nervous system of scrapie-diseased sheep (Groschup \textit{et al.}, 1999) and has recently been reported in the myenteric and submucosal plexus of sheep with natural scrapie (van Keulen \textit{et al.}, 1999). The wide distribution of PrP described in the present study supports the suggestion that the autonomic nervous system of the gut wall may be involved in the early pathogenesis of scrapie. However, the cells and nerve fibres of the enteric nervous system were not the only sites of PrP in control scrapie-free lambs. Mononuclear cells, presumably macrophages within the lamina propria and dome and interfollicular areas, also showed staining. Interestingly, there was little staining for PrP in the follicles of the ileal Peyer’s patch. The detection of PrP in the follicle capsule and in cells associated with vascular structures in the light central zone suggests an association in scrapie-free lambs with certain subpopulations of reticular fibroblasts rather than follicular dendritic cells (Nicander \textit{et al.}, 1991). A number of morphological and possibly functional differences have been described between follicular dendritic cell populations in the ileal Peyer’s patch and the populations in germinal centres of lymph nodes and spleen. Indeed, it is debatable whether the

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**Fig. 3.** Immunohistochemical detection of PrP in the ileal Peyer’s patch of a lamb with PrP genotype AA\textsubscript{151}HR\textsubscript{154}QQ\textsubscript{171} naturally exposed to the scrapie agent. (A) The dome (d) of a lymphoid follicle shows prominent staining (red) for PrP, particularly in the cytoplasm of large mononuclear cells (arrows) immediately beneath the follicle-associated epithelium. Note the presence of focal staining (arrowhead) on the luminal border of the follicle-associated epithelium. a, absorptive epithelium. Frozen tissue. Bar, 22 \( \mu \text{m} \). (B) A granular pattern of staining (arrows) is present in the neck region of a follicle (f). Staining (red) is also present in the dome region (d). e, follicle-associated epithelium. Frozen tissue. Bar, 22 \( \mu \text{m} \).
follicular dendritic cells of the ileal Peyer’s patch are ‘true’ follicular dendritic cells (Nicander et al., 1991).

An important observation in the present study was the prominence of staining in the dome and neck regions of follicles in lambs naturally exposed to the scrapie agent. The extent of the granular pattern of staining and the presence of staining in large mononuclear cells indicate that PrP was present in a diverse cell population, which could include nerves, smooth muscle cells, macrophages, dendritic cells and reticular fibroblasts. The presence of staining associated with the luminal border of cells in the follicle-associated epithelium suggests uptake of PrP from the gut lumen. Studies conducted predominantly in calves have shown that the follicle-associated epithelium of the ileal Peyer’s patch possesses a homogeneous population of specialized epithelial cells (Landsverk, 1981) that is capable of internalizing macromolecules and particles including pathological agents (Reynolds & Morris, 1983; Landsverk, 1987, 1988; Landsverk et al., 1990b). This specialized epithelium is also able to eliminate residual bodies by exocytosis, bringing transcytosed material into contact with the well-developed subepithelial rim of macrophages and other cell populations in the dome (Landsverk, 1981). The ileal Peyer’s patch is a large organ in young lambs, extending for up to 2.5 m and estimated to contain over 100,000 follicles (Reynolds & Morris, 1983). In these young animals, the continuous Peyer’s patch in the region of the ileum accounts for about 90% of the aggregated follicles in the whole alimentary tract. Thus, the demonstration in the present study that the follicle-associated epithelium appears to be involved in the uptake of PrP from the gut lumen identifies a significant site of entry for the scrapie agent. The involution of the ileal Peyer’s patch at puberty (about 12–18 months in sheep) (Reynolds & Morris, 1983) and the accompanying drastic reduction in the number of follicles (and follicle-associated epithelia) may contribute to the reduced susceptibility to infection by the scrapie agent observed in older animals (Hourrigan et al., 1979; Hunter et al., 1996; Elsen et al., 1999).

The extent of exposure of the naturally exposed group of lambs to the scrapie agent was unknown. The lambs had been in close contact with scrapie-infected sheep (Brotherson et al., 1968; Dickinson, 1974) but whether the variation in staining observed between lambs was the result of differences in duration and levels of exposure or the ages of the lambs or

Fig. 4. Immunohistochemical detection of PrP in the ileal Peyer’s patch of a lamb with PrP genotype AV136RR154QR171 following experimental oral exposure to the scrapie agent from a donor with PrP genotype VV136RR154QQ171, 1 week previously. (A) Staining (red) is present in the dome of a follicle (d). a, absorptive epithelium; e, follicle-associated epithelium. Frozen tissue. Bar, 45 μm. (B) Strong staining (red) in the light central zone of a follicle (f) appears to be associated with blood vessels (thin arrow) and stromal elements. Strong staining is also present in the cytoplasm of some large mononuclear cells, possibly representing tingible body macrophages (curved arrows). An adjacent follicle shows little or no staining. The follicle capsule is indicated by an arrowhead. Frozen tissue. Bar, 45 μm.
their PrP genotype is unknown. The three confirmed cases of scrapie in the affected flock that were held in isolation with the lambs had PrP genotypes that were typical for Norwegian cases of scrapie, namely AV136RR134QQ171 and VV136RR134QQ171 (Tranulis et al., 1999). However, none of the nine lambs from this flock carried either of these two PrP genotypes. Indeed, these lambs carried PrP genotypes not previously identified in scrapie cases in Norway (Tranulis et al., 1999). While the resistant PrP genotype of these lambs probably reduces the likelihood of the development of clinical disease (Hunter et al., 1996), the present results suggest that these animals have at least taken up the scrapie agent, as evidenced by changes in the distribution of PrP. Recent studies investigating the existence of carrier animals, that is, animals which have a latent infection and do not show signs of clinical scrapie during a natural lifespan but have the ability to pass on the infection to other sheep, have focused on sheep with resistant PrP genotypes (Clouscard et al., 1995; Bosser et al., 1996; O’Rourke et al., 1997). The present study provides information on the accumulation of PrP in the extraneurale tissues of naturally exposed sheep with QR171 and QQ171 genotypes (O’Rourke et al., 1997). However, the presence of a carrier state of infection would need to be confirmed by bioassay.

To address under more controlled conditions the uptake of PrP in sheep with different combinations of PrP genotypes in donor and recipient, groups of lambs with PrP genotypes strongly associated with disease or seldom associated with disease were exposed experimentally to brain material from clinical scrapie-cases (Table 3). One and five weeks after a single oral administration of scrapie-infected brain material, staining for PrP was prominent in the dome and neck regions of follicles, albeit at lower levels than observed in some naturally exposed lambs. Nevertheless, this pattern of staining was not detected in the age- and PrP genotype-matched control lambs and supports the observations in the naturally exposed lambs. Even so, it should be noted that from the present study it is not known whether the prominent staining for PrP in the naturally and experimentally exposed lambs was associated with infectivity for the scrapie agent that would eventually lead to disease. Eleven months after oral exposure to brain material from clinical cases with homologous PrP genotypes strongly associated with disease, there was little or no staining in ileal Peyer’s patch follicles. Sigurdson et al. (1999) used repeated oral exposure to a relatively large amount of inoculum over 5 days and were able to detect chronic wasting disease PrPres in the gut-associated lymphoid tissues of mule deer fawns 6 weeks after exposure. Whether the absence of follicular staining in ileal Peyer’s patch follicles 11 months after oral exposure to scrapie-infected brain material in the present study is the result of using a too small dose will need to be addressed in further experiments.

A feature that was more obvious in the experimentally exposed lambs than in the naturally exposed lambs was the variation in the staining for PrP between neighbouring follicles in the ileal Peyer’s patch. The reason for the variation in staining for PrP between follicles is not known, although these striking differences were not observed in the age- and PrP genotype-matched controls, suggesting an involvement of the scrapie agent. It is interesting to note that genetic analysis of immunoglobulin light-chain rearrangement in the ileal Peyer’s patch of sheep has shown that the follicles are colonized by only a limited number of B-lymphocyte clones having rearranged one light-chain allele (two to three per follicle) (Reynaud et al., 1991). Whether the immunoglobulin genotype of B-lymphocyte clones in Peyer’s patch follicles influences the distribution of PrP following exposure to the scrapie agent or whether other factors such as selective uptake (Landsverk, 1987, 1988; Wolf & Bye, 1984), disturbances in PrP metabolism (Jeffrey et al., 1998) or maturational status of mesenchymal stromal cells within the follicles (Nicander et al., 1991) are more relevant will need to be addressed in future studies.

The present study did not distinguish between the normal cellular form of PrP (PrPc) and the abnormal isoform associated with disease (PrPSc). Both forms exist within an infected animal and both forms would have been transferred in the infected brain material used for the experimental exposures. Distinguishing between these two forms represents a challenge for immunohistochemical investigations of the early pathogenesis of scrapie and antibodies suitable for immunohistochemistry that recognize epitopes exclusively on the abnormal isoform are needed (Korth et al., 1997). Nevertheless, the present study has shown that enhancing the sensitivity of conventional immunohistochemical approaches may prove to be a useful tool in the investigation of the early pathogenesis of scrapie and argues for the wider application of these approaches to methods such as histoblotting and PET-blotting (Schulz-Schaeffer et al., 2000). This study also shows that experimental infection of scrapie-free lambs of defined age and PrP genotype may yield further insights into the nature of the uptake and dissemination of PrP in scrapie-infected sheep.

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References


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