Increased PrP mRNA expression in lymphoid follicles of the ileal Peyer’s patch of sheep experimentally exposed to the scrapie agent

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To understand the functional role of cellular prion protein (PrPC) in the initiation and maintenance of prion disease within the host, it is important to obtain a more detailed understanding of PrPC transcription in tissues during the development of disease. Using an experimental model with oral infection, we examined the effect of scrapie and the accumulation of the scrapie related form of the prion protein (PrPSc) on the expression level of PrP mRNA in the ileal Peyer’s patch of sheep. In the early phase of infection, prior to PrPSc accumulation, no effect on the PrP expression was detected. However, it was found that lambs with PrP genotypes associated with high susceptibility for scrapie generally had higher PrP mRNA levels than lambs with less susceptible genotypes. Further, in highly susceptible VRQ/VRQ sheep at a stage of disease with high accumulation of PrPSc, real-time RT-PCR and microdissection were used to investigate levels of PrP mRNA in four different tissue compartments. An increased level of PrP mRNA was found in lymphoid follicles of infected sheep compared with controls, indicating upregulation of PrP expression in the follicles to compensate for the loss of PrPC converted to PrPSc, or that PrPSc accumulation directly or indirectly influences the PrP expression. Still, the PrP expression level in the follicles was low compared with the other compartments investigated, suggesting that although increased PrP expression could contribute to PrPSc accumulation, other factors are also important in the processes leading to accumulation of PrPSc in the follicles.

INTRODUCTION

Scrapie in sheep is the prototype of a group of fatal neuro-degenerative prion diseases called transmissible spongiform encephalopathies (TSEs). These diseases are characterized by the accumulation of an abnormal protease-resistant form of the cellular prion protein (PrPC) named PrPSc, which is closely associated with infectivity (Prusiner et al., 1998). This change in protein structure results in heavy accumulation of PrPSc in the central nervous system (CNS), particularly at the terminal stage of the disease (Caughey et al., 2001) and also in peripheral lymphoid tissues earlier in the course of the disease (van Keulen et al., 1999).

The importance of PrPC in prion diseases has been shown in studies using PrP-knockout mice. PrPC expression in neurons is required for the development of neuropathology in the CNS (Brandner et al., 1996), and expression of PrPC in peripheral tissues is important for efficient transmission of infectivity from the periphery to the CNS (Blattler et al., 1997). In sheep, polymorphisms in the PrP gene at codons 136, 154 and 171 are strongly correlated with susceptibility and disease development (Goldmann et al., 1990; O’Rourke et al., 1997). Valine (V) at codon 136 is associated with enhanced susceptibility, while a change from glutamine (Q) to arginine (R) at codon 171 is associated with increased resistance to classical scrapie. However, while PrPC expression has been shown to be crucial for the presence or absence of disease, an understanding of PrPC transcription during the development of prion diseases is limited (Chiesa & Harris, 2001). Indeed, a functional role for PrPC in the initiation and maintenance of disease within a host remains to be determined (Caughey & Baron, 2006).

A recent epidemiological investigation of natural TSE infection in sheep, cattle and humans correlated susceptibility to prion disease with the development of gut-associated lymphoid tissue (GALT) (St Rose et al., 2006). The association demonstrated between the development of GALT and the risk of TSE infection adds support to the asserted importance of the oral route of infection for the natural pathogenesis of scrapie (Hadlow et al., 1982). The lymphoid follicles of GALT have been shown to be the first site of accumulation of PrPSc in sheep and mice (Kimberlin
& Walker, 1989; Andreoletti et al., 2000; Heggebø et al., 2000). Studies using the isolated gut loop model in sheep have called into question the postulated route of transport of PrP across the intestinal mucosa. Recently, Jeffrey et al. (2006) showed a disparity between route of uptake of scrapie-infected material from the gut lumen and the site of de novo generated disease-associated PrP subsequent to scrapie agent replication. While de novo generated PrPSc was demonstrated in ileal Peyer’s patch (PP) follicles 4 weeks after experimental exposure, which is a similar time scale to other experimental infection models in sheep [5 weeks post-inoculation; (Heggebø et al. 2003)], the immunohistochemical evidence suggested transport of the scrapie agent across the absorptive epithelium of the exposed gut segment rather than the follicle-associated epithelium proposed by others (Mabbott & Bruce, 2002; Press et al., 2004). Among the issues raised by the gut loop study was the nature of events occurring in ileal PP follicles accompanying PrPSc accumulation. The conversion and/or accumulation of PrPSc continues in advancing disease, and lymphoid tissues represent a major reservoir of peripheral infectivity in many TSEs (Hadlow et al., 1982; van Keulen et al., 2000).

The disease associated form of PrP is generated from PrPC and it is widely assumed that high expression of PrPC occurs at the site for conversion of PrPC to PrPSc (McBride et al., 1992). It is important to note that the sites of PrP accumulation do not necessarily reflect the location where normal PrP is converted to PrPSc. Consistent with this notion, studies of tissue-specific expression of PrP in various species including sheep and cattle have shown high expression of PrP in peripheral lymphoid tissues, in addition to tissues of the CNS (Tichopad et al., 2003; Ning et al., 2005a; Han et al., 2006). In a recent study, we reported levels and distribution of PrP mRNA in tissue compartments of the ileal PP of scrapie-free lambs using laser capture microdissection and real-time RT-PCR (Austbø et al., 2006). The results were supported by in situ hybridization and immunohistochemistry, and showed large differences between PrP mRNA levels in the different compartments with the lowest expression levels in lymphoid follicles of the ileal PP. This is noteworthy given the above assumption that potential sites of conversion and accumulation of the protein should display high levels of PrPSc expression. However, only a few studies have addressed PrP expression in TSE-infected tissue prior to or after the accumulation of PrPSc. Studies of prion disease neurotoxicity in cell culture systems have indicated that the disease process alters PrP gene expression (Fioriti et al., 2005; Ning et al., 2005b). To our knowledge, similar studies addressing disease processes in peripheral lymphoid tissues have not been performed. Skretting et al. (2004) documented 25 differentially expressed cDNA fragments in the ileal PP of lambs 1 week after oral inoculation with the scrapie agent but did not identify PrP as differentially expressed.

The aim of the present work was to compare the expression of PrP mRNA in the ileal PP of sheep experimentally infected with scrapie at an early and a later stage of disease with that of non-infected sheep. Thus, we have investigated whether infection and accumulation of PrPSc are associated with altered PrP mRNA expression in lymphoid tissues.

**METHODS**

**Animals.** The sheep investigated were of the Norwegian white breed Ryga. The number of animals and genotypes can be found in Table 1. Apart from one natural case, scrapie lambs were infected orally, as described previously, at an age of 6–8 weeks with brain homogenate from sheep with scrapie (Heggebø et al., 2000). Age- and genotype-matched 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. At different times after exposure (see Table 1) inoculated lambs and matched controls were necropsied and tissue samples were snap frozen in 1,1,1-trifluoroethane/1,1,2 tetrafluoroethane/ pentafluoroethane (R404A; Ausimont) chilled in liquid nitrogen and stored at −80 °C until use.

The sheep were categorized into two groups; (i) early lymphoreticular phase, 1 and 5 weeks after infection where little or no accumulation of PrPSc is observed [the PrPSc accumulation has been characterized in all except four animals by Heggebø et al. (2003)] and (ii) late lymphoreticular phase, 3–14 months after infection where PrPSc is immunohistochemically detectable in most lymphatic tissues (Table 1).

**Ethical aspects and safety provisions.** The experimental inoculations with scrapie-infected material were conducted in the confined and controlled isolation facilities of the Norwegian School of Veterinary Science in Sandnes, Norway. Legal and ethical national requirements and code of practice were implemented in the animal experiments.

**Laser capture microdissection.** Sections of 14 μm thickness were cut using a cryostat (Leitz Cryostat 1720) and mounted on special membrane slides for laser microdissection (Molecular Machines & Industries). The sections were air-dried at room temperature. Laser capture microdissection of tissue sections were stained with RNase-free haematoxylin and dried at room temperature. Laser capture microdissection of tissue sections

<table>
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<tr>
<th>Genotype</th>
<th>Status (n)</th>
<th>Time after exposure</th>
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<tr>
<td>ARQ/ARR Scrapie (1)/Control (1)</td>
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<td>ARR/VRQ Scrapie (1)/Control (1)</td>
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<td>ARQ/VRQ Scrapie (2)/Control (2)</td>
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<td>ARQ/VRQ Scrapie (4)/Control (4)</td>
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<td>VRQ/VRQ Scrapie (4)/Control (4)</td>
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<td>ARQ/VRQ Scrapie (4)/Control (4)</td>
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*Indicates natural scrapie case.

Table 1. Status and PrP genotype of sheep used in this study

Highly susceptible genotypes are indicated in bold. n, No. of animals.
was performed using the SLµCut laser microdissection system (Molecular Machines & Industries). The SLµCut equipment is provided with an automated UV dissection system coupled to video imaging. Tissue samples were securely removed using an adhesive membrane, which protects the tissue on the slide against cross contamination. To ensure that the material was representative and sufficient, several separate pieces of each of the desired compartments were microdissected to obtain an area corresponding to 1 × 10^6 µm^2 or approximately 500–1000 cells depending on the cell density, i.e. at least 10 different follicles from each animal.

**RNA extraction.** RNA from laser-captured tissue was isolated using the Absolutely RNA Nanoprep kit (Stratagene). The kit allows rapid purification of high-quality total RNA from extremely small samples of cells (1–10^6 cells) harvested by laser capture microdissection with an expected yield of up to 100 ng. The manufacturer’s protocol was followed including the optional DNase step. RNA was eluted into 20 µl elution buffer and stored at −80 °C. Total RNA from frozen tissue was isolated using the RNeasy Midi kit (Qiagen).

**Quantitative real-time RT-PCR.** Quantitative real-time RT-PCR was performed using a one-step qPCR core kit (Eurogentec). Primers were designed to span across intron sections using PrimerExpress 1.5 (Applied Biosystems). The expression level was measured with relative quantification by using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the reference gene. Each quantification target was amplified in triplicate samples and a control lacking the template for each master mix was always included in the experiments.

Primers and hybridization probes used for the quantitative real-time RT-PCR were as follows: Ovis aries PrP, forward 5′-TCCCGAGA-GACACAGATCCACCT-3′; reverse 5′-GATCGAACATGCCATATG- GCCCT-3′; probe 5′-FAM-ACCATGATGACTTCTATCTGCTGTG-ATTACGGT-TAMRA-3′; Ovis aries GAPDH, forward 5′-GTAGTCC- ACCATGGAAGTA-3′; reverse 5′-CCACGTACTCAAGCACCAGCAT- 3′; probe 5′-FAM-TCCACGCCACATGCAAGGACAAGA-TAMRA-3′. Real-time RT-PCR was carried out in an ABI Prism 7700 (Applied Biosystems) using the following uniform temperature profile: 30 min at 48 °C (reverse transcription), then 10 min at 95 °C (denaturation), followed by 40 cycles of 30 s at 95 °C, 15 s at 56 °C and 60 s at 60 °C. The same cycling profile was used for all real-time RT-PCRs. The data were analysed using Sequence Detection system v1.9.1 (Applied Biosystems).

**Statistics.** For datasets that are sampled from a Gaussian distribution, statistical differences were evaluated using the paired or non-paired t-test. For the other samples, the non-parametric Mann-Whitney test was used. The distribution of datasets was tested using the Kolmogorov–Smirnov normality test. Differences in expression between compartments were considered to be significant with values of probability P < 0.05 (InStat GraphPad Software).

**Immunolabelling for PrPSc on tissue sections.** The presence of PrPSc was verified using paraffin wax sections of tissues from VRQ/VRQ sheep, while serial frozen sections were utilized to detect PrPSc in lymphoid follicles from ARQ/VRQ sheep prior to microdissection. Paraffin wax sections (4 µm) were placed on positively charged slides (Superfrost Plus; Menzel-Glaser) and dried for at least 12 h at 37 °C. After a standard dewaxing procedure, sections were autoclaved in citrate buffer (10 mM citric acid monohydrate, pH 6.0) at 121 °C for 30 min, transferred to PBS, pH 7.4, and treated with 3% H2O2 in methanol for 20 min to inhibit endogenous peroxidase. The frozen sections (10 µm) were also placed on positively charged slides (Superfrost Plus; Menzel-Glaser). They were allowed to dry for 1 h followed by fixation in 10% formal-calcium for 15 min. To enhance binding of primary antibodies to PrPSc, the sections were immersed in 98% formic acid for 10 s and rinsed thoroughly in distilled water and PBS. In the subsequent steps, a signal amplification was performed (Tyramide Signal Amplification-indirect kit; NEN Life Science Products) on both types of sections as described previously (Austbø et al., 2006). Subsequent to the blocking step, the sections were incubated overnight at 4 °C with primary monoclonal antibodies of IgG1 isotype to detect PrP: L42, P4 (both kindly provided by Dr Martin H. Groschup, Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Germany) and 6H4 (Prionics). L42 was used on both paraffin wax and frozen sections, whereas P4 and 6H4 were only applied to frozen sections. After the final amplification step, antigen-bound streptavidin-horseradish peroxidase was detected with diaminobenzidine (paraffin wax sections) or aminoethylcarbazole (frozen sections) as substrates. Paraffin sections from animals known to be positive or negative for PrPSc were also used to evaluate the specificity of immunolabelling. Microphotographs were captured with a Spot RT Slider digital camera (Diagnostic Instruments) mounted on a Leica DMRXA microscope (Leica Microsystems).

**RESULTS**

**PrP mRNA expression in the ileal PP of sheep 1 and 5 weeks after experimental exposure to scrapie**

PrP mRNA expression was investigated in whole tissue blocks from the ileal PP of lambs 1 and 5 weeks after oral exposure to scrapie. Eighteen lambs of five different PrP genotypes were compared with 18 controls of the same age and PrP genotype (Table 1). Of the 36 lambs examined, 28 have previously been characterized by immunohistochemistry (Heggebo et al., 2000, 2003) and showed increased PrP levels in follicles of the ileal PP in the susceptible animals as early as 1 week after challenge, while the presence of PrPSc was only detectable 5 weeks after challenge. By using the real-time RT-PCR procedure, no significant changes in PrP mRNA expression levels were observed between the scrapie lambs and the controls. Comparisons were also made by grouping animals in one group with PrP genotypes associated with reduced susceptibility to scrapie (ARQ/ARQ, ARQ/ARQ and VRQ/ARQ) and another group with genotypes associated with high susceptibility (VRQ/VRQ and VRQ/ARQ) (Fig. 1). No significant differences in PrP expression were found between the lambs exposed to scrapie and the control lambs in either the high- or the low-susceptibility groups (Fig. 1), implying that there is no change in PrP expression as a consequence of scrapie infection at this early phase of infection. Interestingly, the general expression level of PrP was higher in lambs with highly susceptible PrP genotypes compared with the group with low susceptibility. When the 18 control lambs with high and low susceptibility were compared, the difference was not significant (P = 0.08). A significant difference (P = 0.01) was only present when all 36 animals, i.e. both the scrapie-infected and control lambs, were included in the test. One should notice that when including the infected animals in the comparison, any disease related change in the expression of infected animals might contribute to the significant difference observed for all 36 animals.
PrP mRNA levels in the ileal PP of sheep 8 and 10 months after experimental exposure to scrapie

PrP mRNA levels were studied in ileal PP of lambs 8 and 10 months after challenge. For this purpose, highly susceptible PrPVRQ/VRQ sheep characterized by significant PrPSc accumulations in peripheral lymphoid tissues were used. Accumulation of PrPSc was verified by immunohistochemistry for all animals exposed to scrapie (Fig. 2). In the ileal PP, there was an intense granular accumulation of PrPSc in the lymphoid follicles. Additionally, PrPSc was present in the myenteric plexuses and to a lesser degree in the dome and the interfollicular areas.

Eight to ten months after oral exposure to the scrapie agent, PrP mRNA expression in whole tissue blocks from the ileal PP of lambs was not significantly different from tissue expression levels in age and PrP genotype matched control lambs (Fig. 3). Because of our previously identified variability in PrP expression levels in tissue compartments of the ileal PP of normal uninfected lambs (Austbø et al., 2006), PrP mRNA expression was also measured in different tissue compartments of the ileal PP of scrapie-infected and control lambs. As for the previous study, expression levels were investigated by laser-assisted microdissection and quantitative RT-PCR. PrP mRNA expression was detected in all the examined compartments of the ileal PP, both of sheep experimentally exposed to scrapie and of scrapie-free control animals (Fig. 4). Laser-dissected follicle, interfollicular area (i.e. the T-cell area and the area of the submucosa immediately adjacent to the inner muscular layer), muscular layer and mucosal villi (lamina propria and epithelium) were examined for all eight animals. The lowest PrP mRNA levels were detected in the follicles and the villi, while the highest levels were found in the interfollicular area and the muscular layer. The PrP mRNA levels were two- to fourfold higher in the interfollicular area and the muscular layer compared with the PrP mRNA levels in the follicles. By comparing scrapie-infected and control animals, it was found that the PrP mRNA level was significantly increased in the follicles of the scrapie-infected animals (P=0.025). Due to the small sample size, the distribution of the datasets could not be tested and therefore the non-parametric Mann–Whitney test was used. Significant changes were not detected in the other compartments (Fig. 4).

PrP mRNA expression in follicles with high- or low-PrPSc accumulation in ARQ/VRQ sheep experimentally exposed to scrapie

In the period from 3 to 14 months after exposure to scrapie, the ileal PP of some animals with genotype ARQ/VRQ simultaneously displayed follicles with a high accumulation of PrPSc and follicles with no detectable accumulation of PrPSc. Immunohistochemistry was performed on frozen sections from the ileal PP with three different antibodies against PrP and subsequently...
lymphoid follicles were divided in two groups based on the presence of detectable levels of PrPSc (Fig. 5). Follicles without labelling for PrPSc were allocated to one group and follicles with high presence of typical tingible-body macrophages labelled for PrPSc inside the follicle area were allocated to the other group. For quantification of PrP mRNA levels, follicles belonging to the two groups were dissected from frozen sections using laser-assisted microdissection. Immunohistochemically labelled serial sections were used for the identification and group allocation of follicles. Paired \( t \)-test calculations of the quantitative real-time RT-PCR analyses did not demonstrate a significant increase \((P=0.09)\) in PrP mRNA expression in the follicles with high accumulation of PrPSc compared to follicles without detectable accumulation of PrPSc (Fig. 6).

**DISCUSSION**

No change in PrP mRNA levels in whole tissue from the ileal PP at early (1 and 5 weeks) or later times (8 and 10 months) was detected after oral inoculation with the scrapie agent. An analysis of the infected group of lambs based on PrP genotype did not reveal significant changes in
PrP expression either in lambs with low susceptibility to scrapie or in lambs with high susceptibility. However, a correlation was found between the general PrP mRNA levels and PrP genotype, with the highest levels present in sheep that are highly susceptible to scrapie (PrP genotypes ARQ/ARR, ARQ/ARQ and ARR/VRQ) and lower levels in the less susceptible sheep (PrP genotypes ARQ/VRQ and VRQ/VRQ). These results are consistent with a previous finding that peripheral blood mononuclear cells from scrapie-susceptible sheep expressed higher levels of cell-surface PrPSc than less susceptible sheep (Halliday et al., 2005). PrP mRNA levels probably influence the level of PrPSc, which can be further misfolded into PrPSc. Consequently, differences in PrP mRNA expression between sheep of high and low susceptibility might be a factor that could contribute to differences in susceptibility or at least to incubation time together with other variables such as route of infection, dose, prion strain and other genetic factors.

We recently reported considerable variation in the level of PrP expression between tissue compartments in the ileal PP of normal uninfected lambs (Austbø et al., 2006). To pursue this finding in scrapie-infected sheep, a detailed investigation of PrP expression in peripheral lymphoid tissue compartments was conducted at an advanced stage of disease in sheep of the highly susceptible VRQ/VRQ PrP genotype. By the use of laser-assisted microdissection and quantitative RT-PCR, expression of PrP in well-defined compartments of the ileal PP was investigated. The study showed that the PrPSc-laden lymphoid follicles from the ileal PP had increased PrP mRNA levels compared with follicles from age- and genotype-matched control lambs.

The finding that the other ileal PP compartments investigated displayed no significant change despite overall higher levels of expression, could indicate an association between the increase in PrP expression and the processes taking place at this stage of infection in the follicles, i.e. the compartment with the earliest and most pronounced PrPSc accumulation during scrapie development (Andreoletti et al., 2000; Heggebø et al., 2000). The relatively small increase observed in PrP mRNA levels may represent a cell response to infection in order to sustain natural levels of PrPSc in a situation where PrPSc is converted to PrPSc during the prolonged incubation period of scrapie. Alternatively, PrPSc itself, directly or indirectly, could influence the PrP expression. Another possibility is that the increase in PrP mRNA expression could reflect a functional change within the follicles or a change in the cell content, suggesting that there is an increase in the cellular source of PrPSc.

In a previous study of normal lambs, PrP mRNA levels were found to be low in the lymphoid follicles and substantially higher in the other compartments (Austbø et al., 2006). Although the follicles were the only compartment found to exhibit an increased PrP mRNA level after infection in the present study, the level of PrP mRNA in follicles was still low compared with the interfollicular area and the muscular layer. The findings of the present study suggest that high levels of PrP mRNA expression in cells or in a tissue do not imply accumulation of PrPSc in that same tissue and it is likely that other factors are involved in the accumulation of PrPSc (Caughey & Baron, 2006). Various cell types have been shown to differ in their content of proteolytic enzymes and their ability to degrade PrPSc (Luhr et al., 2004). This may influence the ability of a cell to resist or avoid accumulation of PrPSc. Another possible explanation for the accumulation of PrPSc in a tissue with low level of PrP expression is that the accumulation is not exclusively due to conversion of PrPSc produced by follicular dendritic cells in lymphoid follicles but could be supplemented by PrPSc transported into the follicles and subsequently trapped on follicular dendritic cells via receptors such as the complement receptor (Mabbott et al., 2001). Fevrier et al. (2004) reported that PrP-expressing cells before and after infection with sheep prions release both PrPSc and PrPSc, respectively, into the extracellular environment. The released proteins are associated with exosomes, membraneous vesicles with a potential ability for intercellular membrane exchange. Exosomes bearing PrPSc were found to be infectious. Several types of cells including B- and T-lymphocytes, dendritic cells, mast cells, intestinal epithelial cells, neurons and others have been reported to secrete exosomes (Fevrier et al., 2005; Faure et al., 2006). This could represent a mechanism for the spread of disease-associated PrP between cells in the body that is independent of the intrinsic production of PrP in the various cell types.

![Fig. 6. Internal comparison of PrP mRNA expression in ileal PP follicles with different degree of PrPSc accumulation. The two bars represent pairwise comparison of PrPSc-negative follicles (grey bar) and PrPSc-positive follicles (black bar) within five ARQ/VRQ sheep. Values on the y-axis were normalized relative to PrP mRNA levels in PrPSc-negative follicles. The PrP mRNA expression levels were obtained by relative quantitative real-time RT-PCR analysis using GAPDH. Error bar represents standard deviation.](image-url)
To obtain more information on the association between the process of PrP\textsuperscript{Sc} accumulation in lymphoid follicles and regulation of PrP expression, we investigated PrP mRNA levels in follicles from five ARQ/VRQ animals (3–14 months of age). The ileal PP of the selected animals possessed follicles with a high accumulation of PrP\textsuperscript{Sc} and follicles with no detectable accumulation of PrP\textsuperscript{Sc}, as has been reported by others (Ersdal et al., 2005). At later terminal stages of disease, the lymphoid follicles of most ARQ/VRQ sheep show heavy accumulations of PrP\textsuperscript{Sc} (Ersdal et al., 2005). In the present study, we observed increased expression of PrP in follicles with detectable PrP\textsuperscript{Sc} compared with PrP\textsuperscript{Sc}-negative follicles. Although the difference was not significant (P=0.09), the observed tendency supports the finding in VRQ/VRQ sheep, indicating that accumulation of PrP\textsuperscript{Sc} in follicles might be associated with increased levels of PrP mRNA.

This work has addressed the association between PrP mRNA expression and accumulation of PrP\textsuperscript{Sc} in the ileal PP. Although it has previously been shown that PrP\textsuperscript{C} expression in peripheral tissues of mice was controlled primarily at the transcriptional level (Ford et al., 2002), it is important to note that additional post-transcriptional regulation might occur in sheep at the translational level as well as by degrading enzymes, and the stability of PrP will also influence the level of expressed protein. Despite possible post-transcriptional regulation, we observed that PrP\textsuperscript{Sc}-laden lymphoid follicles showed a significant increase in PrP mRNA expression. The follicular compartment continued, however, to display low levels of PrP mRNA compared with other compartments, similar to what was previously shown in uninfected animals (Austbø et al., 2006). The relatively low-PrP expression level together with the strong ability to accumulate PrP\textsuperscript{Sc} could suggest that factors supporting PrP\textsuperscript{Sc} production and accumulation are more prevalent in the follicles than in the other compartments, or that the follicular cells lack efficient mechanisms for degradation or export of the disease related PrP that are present in other compartments, or possibly that PrP\textsuperscript{Sc} accumulation is the result of transport of PrP\textsuperscript{Sc} into the follicles from high-PrP expression compartments.

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