Cell-surface expression of PrP<sup>C</sup> and the presence of scrapie prions in the blood of goats

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Although host-encoded prion protein (PrP<sup>C</sup>) expression in ovine PBMCs and prion infectivity in scrapie-infected sheep blood have been demonstrated, such studies have not been reported in goats. Therefore, this study characterized cell-surface expression of PrP<sup>C</sup> on PBMC subsets derived from normal goats and sheep, by flow cytometry, and determined prion infectivity in blood from a scrapie-infected goat using a transfusion bioassay in goat kids. Cell-surface PrP<sup>C</sup> expression was detected on all subsets of goat PBMCs. The highest PrP<sup>C</sup> cell-surface expression was found in CD2<sup>+</sup> T lymphocytes in goats. Transmission of infection was detected in all three recipients who received whole blood from a goat with classical scrapie. It was concluded that caprine PBMCs express PrP<sup>C</sup> similarly to sheep but with relative differences among PBMCs subsets, and that blood-borne infectious prions can be detected in scrapie-infected goats. Thus, similar to sheep, goat blood may be a suitable diagnostic target for the detection of scrapie infection.

Prion diseases, or transmissible spongiform encephalopathies, are unique fatal neurodegenerative disorders that affect a number of different species including goats and sheep (scrapie), cattle (bovine spongiform encephalopathy, BSE), deer, elk and moose (chronic wasting disease), humans (Creutzfeldt–Jakob disease) and mink (transmissible mink encephalopathy). The infectious agent is largely proteinaceous and consists primarily of a conformational isoform (PrP<sup>Sc</sup>) of the host-encoded prion protein PrP<sup>C</sup> (Prusiner, 1982). A characteristic feature of transmissible spongiform encephalopathies is the accumulation of PrP<sup>Sc</sup> in the central nervous system (Bolton et al., 1982). In goats and sheep with classical scrapie disease, PrP<sup>Sc</sup> also accumulates in the lymphoreticular system (Valdez et al., 2003; van Keulen et al., 1996). Detection of PrP<sup>Sc</sup> in brain and lymphoid tissues by immunoassay has been a reliable diagnostic marker for prion disease.

Accumulation of PrP<sup>Sc</sup> in host tissues requires cellular expression of PrP<sup>C</sup> (Büeler et al., 1993). PrP<sup>C</sup> is a glycosylphosphatidylinositol-anchored protein attached predominantly on the extracellular surface of the plasma membrane (Stahl et al., 1987). In sheep, PrP<sup>C</sup> is widely expressed by many cell types including neurons and leukocytes (Gossner et al., 2009; Halliday et al., 2005; Herrmann et al., 2001). In goats, only expression of PrP<sup>C</sup> in brain tissue (Vorberg et al., 1999), cells in the mammary gland (Didier et al., 2008) and milk (Didier et al., 2008; Franscini et al., 2006) has been reported to date.

The early detection of PrP<sup>Sc</sup> in lymph nodes of goats and sheep with classical scrapie, humans with variant Creutzfeldt–Jakob disease, cervids with chronic wasting disease and in most rodent scrapie models suggests that infectious prions are disseminated in extraneural tissues via the circulatory and lymphatic systems. The presence of prions in the blood of sheep with classical scrapie or experimentally induced BSE has been confirmed in transfusion bioassays in which recipient lambs were inoculated with whole blood, purified leukocytes, plasma or platelets from infected donor sheep (Houston et al., 2000, 2008; Hunter et al., 2002; McCutcheon et al., 2011). It is noteworthy that, although BSE-infected sheep blood is infectious to sheep, BSE-infected cattle blood is not infectious to cattle. Using the same bioassay procedure, we recently determined in sheep with classical scrapie that relatively high levels of infectivity are associated with the CD72<sup>+</sup> pan-B lymphocytes and also with the CD21<sup>+</sup> subset (Dassanayake et al., 2011). However, prion infectivity could not be detected in serum or blood clots derived from goats with classical scrapie in an early study utilizing non-transgenic mice as bioassay recipients (Hadlow et al., 1980).
The present study was conducted to compare the cell-surface expression of PrP<sup>C</sup> on peripheral blood cell fractions of goats and sheep, and also to determine whether infectious prions are indeed present in the blood of goats with classical scrapie, as they are in sheep, by utilizing goat kids as bioassay recipients.

All experimental protocols used in this study were approved by the Institutional Animal Care and Use Committee (IACUC) at Washington State University (WA, USA) before the onset of the study. Six normal goats and six normal sheep were selected for cell-surface PrP<sup>C</sup> expression studies (see Supplementary Methods, available in JGV Online). PBMCs, polymorphonuclear leukocytes (PMNs), platelets and red blood cells (RBCs) were prepared and labelled with anti-PrP<sup>C</sup> mAb 5B2 (Li <i>et al.</i>, 2000) or 6H4 (Korth <i>et al.</i>, 1997) (2 µg ml<sup>–1</sup> final concentration) alone or with the anti-PrP<sup>C</sup> mAb and a cell subset-specific mAb (15 µg ml<sup>–1</sup> final concentration), followed by a fluorochrome-labelled secondary antibody for cell-surface PrP<sup>C</sup> expression analysis by flow cytometry, as described previously (Herrmann <i>et al.</i>, 2001). The PBMC subset-specific mAbs used in this study are listed in Table S1. As has been reported in sheep (Halliday <i>et al.</i>, 2005; Herrmann <i>et al.</i>, 2001), flow cytometry analysis revealed that cell-surface PrP<sup>C</sup> expression was clearly detectable in PBMCs from goats (Fig. 1). Previous studies carried out by our group (Herrmann <i>et al.</i>, 2001) and others (Halliday <i>et al.</i>, 2005) have demonstrated a lack of cell-surface PrP<sup>C</sup> expression on ovine PMNs, RBCs and platelets. In the blood of goats, cell-surface PrP<sup>C</sup> expression was similarly not detectable on PMNs and RBCs; however, a slight shift in the PrP<sup>C</sup> fluorescence histograms for platelets was detected (data not shown). This observation suggested that circulating platelets of goats express relatively lower levels of cell-surface PrP<sup>C</sup>.

As most cell-surface PrP<sup>C</sup> expression is limited to the PBMCs of goats and sheep, we performed a more detailed comparison of PrP<sup>C</sup> expression levels between PBMCs isolated from the blood of six goats and six sheep. Two goats were homozygous for the wild-type <i>PRNP</i> haplotype encoding proline at codon 240 (240PP), whilst four goats were heterozygous for 240P and the alternative wild-type allele encoding serine at codon 240 (240PS). Four sheep were homozygous for the <i>PRNP</i> haplotype encoding alanine at codon 136 and arginine at codons 154 and 171 (i.e. ARR/ARR), whilst two sheep were heterozygous for arginine and glutamine at codon 171 (i.e. ARQ/ARR). The mean fluorescence intensity (MFI) of cell-surface PrP<sup>C</sup> expression on PBMCs from goats with mAb 5B2 was 70 ± 6 and with mAb 6H4 was 170 ± 9. The MFI of cell-surface PrP<sup>C</sup> expression on PBMCs from sheep with mAb 5B2 was 49 ± 5 and with mAb 6H4 was 86 ± 9. We noted that the PrP<sup>C</sup> MFI of PBMCs was significantly higher when using mAb 6H4 than when using mAb 5B2 in both goats and sheep, even though mAb 5B2 has two potential binding sites at the N terminus (between residues 37 and 55) on PrP<sup>C</sup> (Li <i>et al.</i>, 2000). Although not explored here, this unexpected finding might be attributed to differences in the affinity and/or avidity of the mAbs for the respective epitopes. Human PrP<sup>C</sup> is known to undergo proteolytic processing between His-111 and Met-112 residues, resulting in the release of a soluble N-terminal fragment (N1) and retaining a membrane-bound C-terminal fragment (C1) (Chen <i>et al.</i>, 1995). This cleavage occurs constitutively in 10–50 % of PrP<sup>C</sup> molecules that are attached to the plasma membrane. The possibility of such cleavage in ovine PrP<sup>C</sup> has also been observed with transgenic ovinized mice brain tissues (Nicot & Baron, 2010). Therefore, the observed higher MFI values with mAb 6H4 in both goat and sheep PBMCs might also be attributable to such proteolytic cleavage of PrP<sup>C</sup>, resulting in losing 5B2 epitopes with soluble N1 fragments.

Quadrant analysis of the dot plots revealed that >90% of each major PBMC population expressed PrP<sup>C</sup> on their cell surface (data not shown). We further determined the relative levels of PrP<sup>C</sup> surface expression by PBMC subsets within goats and sheep. A generalized linear mixed model (GLIMMIX procedure, SAS version 9.2; SAS Institute) was used to analyse, within each species, the dependence of the mAb-specific MFI of PrP surface expression on PBMC subset (the fixed effect), conditioning the regression by including the random effects of blood donor and repeated sampling as well as model residuals. In agreement with previous findings in sheep (Halliday <i>et al.</i>, 2005; Herrmann <i>et al.</i>, 2001), cell-surface PrP<sup>C</sup> expression was observed in all major PBMC subsets of sheep and also in goats but with some significant expression differences between the two species (Table 1). Whether measured using mAb 5B2 or 6H4, the MFI of PrP<sup>C</sup> surface expression on caprine PBMCs was statistically significantly higher in CD2<sup>+</sup> T lymphocytes; however, statistical separation of other PBMC subset populations was dependent on the anti-PrP mAb used. The relatively low MFI of PrP<sup>C</sup> surface expression by other PBMC subsets were statistically similar as measured using mAb 5B2, but some statistical grouping was achieved using mAb 6H4 (Table 1). Perhaps of greatest comparative interest was the relatively low level of PrP<sup>C</sup>.

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![Fig. 1. Cell-surface PrP<sup>C</sup> expression patterns from normal goat and sheep PBMCs. PBMCs were labelled with isotype-matched control mAb (grey shaded histograms) or anti-prion mAb 5B2 (black-line histograms). The experiment was repeated three times with PBMCs from six goats and six sheep and the results of a representative experiment are shown. R-PE, R-phycoerythrin.](image-url)
Table 1. Comparison of cell-surface PrP^C expression on PBMCs subsets from goats and sheep

Up and down arrows indicate the direction of difference [non-overlapping 95 % confidence limits (CL)] for a given PBMC type within a column between goats and sheep.

<table>
<thead>
<tr>
<th>Species</th>
<th>PBMC type*</th>
<th>5B2 MFI (mean ± 95 % CL)†</th>
<th>P_adj group‡</th>
<th>6H4 MFI (mean ± 95 % CL)§</th>
<th>P_adj group§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat</td>
<td>CD2</td>
<td>↑ 79±7</td>
<td>A</td>
<td>↑ 192±17</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>GD</td>
<td>58±7</td>
<td>B</td>
<td>144±17</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>CD14</td>
<td>↑ 44±7</td>
<td>B</td>
<td>↑ 124±17</td>
<td>B C</td>
</tr>
<tr>
<td></td>
<td>B-B2</td>
<td>49±7</td>
<td>B</td>
<td>95±17</td>
<td>C D</td>
</tr>
<tr>
<td></td>
<td>CD21</td>
<td>↓ 44±7</td>
<td>B</td>
<td>↓ 84±17</td>
<td>D</td>
</tr>
<tr>
<td>Sheep</td>
<td>GD</td>
<td>67±6</td>
<td>a</td>
<td>126±11</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>CD21</td>
<td>71±6</td>
<td>a</td>
<td>125±11</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>CD2</td>
<td>60±6</td>
<td>a</td>
<td>92±11</td>
<td>b</td>
</tr>
<tr>
<td></td>
<td>BB2</td>
<td>42±6</td>
<td>b</td>
<td>75±11</td>
<td>b c</td>
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<tr>
<td></td>
<td>CD14</td>
<td>30±6</td>
<td>c</td>
<td>54±11</td>
<td>c</td>
</tr>
</tbody>
</table>

*PBMC subpopulation as determined by flow cytometry using PBMC type-specific mAbs targeting CD2, γδ (GD), CD14, B-B2 or CD21 antigens.
†MFI determined by flow cytometry using anti-PrP mAb 5B2, as determined by least-squares estimation and shown as the mean ± 95 % CL.
‡Statistical grouping (within column and species) as determined by pair-wise comparisons using a modified Bonferroni procedure to give adjusted P-values (P_adj). Shown within a column are the statistically different groupings for goats and for sheep, indicated by capital letters for goat groupings and small letters for sheep groupings.
§MFI determined by flow cytometry using anti-PrP mAb 6H4, as determined by least-squares estimation and shown as the mean ± 95 % CL.

Surface expression by CD21^+ B lymphocytes in the blood of goats. In sheep, the MFI of PrP^C surface expression was highest in CD21^+ B lymphocytes and γδ T lymphocytes (Table 1). Higher expression of PrP^C in the CD21^+ B-lymphocyte subset has been previously reported (Halliday et al., 2005). PrP^Sc has been detected from a subpopulation of B lymphocytes including CD21^+/− cells from scrapie-infected sheep but not from CD21^+ T lymphocytes or monocytes (Edwards et al., 2010). Furthermore, our recently concluded study demonstrated that both pan-B lymphocytes and the CD21^+ subset of B lymphocytes harbour prion infectivity (Dassanayake et al., 2011). However, prion infectivity in goat blood or PBMC subsets has not yet been studied. A previous study (Halliday et al., 2005) reported lower expression of PrP^C in sheep γδ T lymphocytes. However, higher PrP^C expression in γδ T lymphocytes from all six sheep (ARR/ARR and ARQ/ARR) and all six goats was observed in this study. The mAb 86D (Mackay et al., 1989) used by Halliday et al. (2005) recognizes the T19 molecule and the GB54 mAb used in this study recognizes the WC1 molecule on γδ T lymphocytes. Later studies have revealed that these two molecules are identical (Hanby-Flarida et al., 1996). Therefore, the basis for the apparent higher expression of PrP^C on both the sheep and goat γδ T lymphocyte subsets is not clear at this stage.

Given the relatively high levels of PrP^C surface expression on caprine PBMCs and the previous detection of prion infectivity in the blood of sheep (Houston et al., 2000, 2008; Hunter et al., 2002; McCutcheon et al., 2011), we attempted to detect prion infectivity in the blood of a goat with classical scrapie infection with a transfusion bioassay using goat kids. The age of the blood donor goat was 38 months old at the time of blood transfusion. The Nubian donor goat was PRNP 240PS and showed clinical signs of classical scrapie at the time that whole blood (~300 ml) was collected. The blood was immediately transfused (100 ml each) into three recipient Saanen goat kids that were homozygous wild type for the PRNP 240P allele. This genotype is not associated with polymorphisms that delay the incubation period (Goldmann et al., 2004; Vaccari et al., 2009). Recipient infection status was evaluated by serial biopsies of the rectoanal mucosa-associated lymphoid tissue (RAMALT), as described previously (Espenes et al., 2006; González et al., 2005). Transmission of infection was not detected at 12 months post-transfusion in any of the three recipients; however, transmission of infection was confirmed in two goats (nos 4189 and 4190) at 18 months post-transfusion by initial detection of PrP^Sc in the RAMALT (Fig. 2). The remaining goat (no. 4188) was again biopsied at 25 months post-transfusion and PrP^Sc was detected in the RAMALT (Fig. 2). As the last goat was not biopsied between 18 and 25 months post-transfusion, it was difficult to predict when the PrP^Sc accumulation began in lymphoid follicles. Konold et al. (2008) reported the early stage of lateral transmission of scrapie following the detection of PrP^Sc in control lambs after mixing with scrapie milk recipient lambs. Although it is more likely that this particular goat also got the scrapie infection through the blood transfusion, we cannot rule out possibility of lateral transmission of scrapie from the two scrapie-infected recipient goats due to the lack of negative-control animals along with blood transfusion recipients. However, this same-species transfusion bioassay allowed us to confirm the presence of infectious prions in the blood of the goat with classical scrapie disease.
The presence of PrPSc in the lymphoreticular system and detection of prion infectivity in blood suggest that peripheral blood might be a suitable target for pre-clinical diagnostic test development. Our future studies will be directed towards identification of caprine PBMC subsets and other blood components with detectable levels of PrPSc.

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References


Fig. 2. Immunolabelling of PrPSc in the RAMALT of whole-blood recipient goats. Serial rectal biopsies were taken from three recipient goats following blood transfusion and the tissues were fixed in formalin, embedded in paraffin and 3 μm sections were cut and immunolabelled with anti-prion mAb F99/97.6.1. (a–c) PrPSc immunolabelling (dark red) was visible in the RAMALT follicles of all three whole-blood recipient goats: goat 4188 (a), goat 4189 (b) and goat 4190 (c). (d) PrPSc immunolabelling was not observed in RAMALT follicles when using an isotype-matched control mAb [same tissue block as shown in (c)]. Bar, 200 μm.


