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Tissue inhibitor of metalloproteinase-1 and interleukin-10 in serum from naïve and scrapie infected sheep

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ABSTRACT

Tissue inhibitor of metalloproteinase-1 (TIMP-1) and interleukin-10 (IL-10) were identified as potential biomarkers for ovine scrapie in a mouse model. The development of novel diagnostic methods to identify preclinical scrapie-infected animals is needed. In this study, ELISA was used to assess TIMP-1 and IL-10 levels in 158 serum samples from naïve and preclinical scrapie-infected sheep. Young (≤ 18 months) naïve sheep had significantly lower TIMP-1 levels compared with old (≥ 20 months) naïve and old infected sheep ($P < 0.04$). Young naïve sheep had lower IL-10 than old naïve sheep ($P < 0.001$). Both cytokines tended to have lower levels in young naïve sheep compared to infected sheep but this did not reach significance. A larger sample size will be helpful in determining the potential of these cytokines as a diagnostic tool.

1. Introduction

Transmissible spongiform encephalopathies (TSEs), or prion diseases, are fatal, neurodegenerative diseases where host-encoded normal cellular prion protein is misfolded into an alternative conformational isoform (PrP^{Sc}) which is considered to be a principle component of the infectious agent (Prusiner, 1998). Naturally occurring TSEs include scrapie in sheep and goats and is often acquired through oral exposure to the infectious agent (Andreoletti et al., 2002).

The current gold standard for antemortem diagnosis of scrapie in sheep is detection of PrP^{Sc} accumulations in lymphoid tissue of the rectoanal mucosa-associated lymphoid tissues in sheep at 14 months of age or older (Gonzalez et al., 2008). The availability of a quick, non-invasive method for screening large numbers of samples would result in substantial animal health and economic benefits. While measurable systemic immune response to scrapie infection was not detected, observations in transgenic mice overexpressing the valine allele of ovine *PRNP* (Tg338) suggest increased cytokine expression by central nervous system cells may serve as markers for an inflammatory response (Newsom et al., 2011). Specifically, tissue inhibitor of metalloproteinase-1 (TIMP-1) and interleukin-10 (IL-10) increased by two- to eight-fold in the brain, spleen, mesenteric lymph nodes and serum in preclinical and clinical scrapie-infected mice versus scrapie-naïve mice suggesting that these cytokines may be suitable biomarkers for scrapie infection. These observations led us to assess whether IL-10 and TIMP-1 cytokines could be used as serum biomarkers for scrapie in sheep, the natural host.

2. Materials and methods

TIMP-1 and IL-10 were analyzed in duplicate in 158 sheep serum samples from naïve and preclinical scrapie-infected sheep (Table 1 and Suppl. Figs. 1 and 2) using enzyme-linked immunosorbent assays (ELISA) following the manufacturer's protocol (TSZ Scientific LLC). Preclinical scrapie was confirmed by immunohistochemical examination of lymphoid tissues, notably the retropharyngeal lymph node or palatine tonsil, and rectal biopsies collected from animals with no neurologic signs at the time of cull. Experimental protocols were approved by the Washington State University Institutional Animal Care and Use Committee. Serum was obtained from blood by centrifugation for 20 min at 420 × *g* at 4°C. Genomic DNA was extracted from blood collected into EDTA anticoagulant using iPrep™ PureLink® gDNA Blood Kit (Invitrogen) following the manufacturer's instructions and haplotypes were determined by sequencing of the open reading frame of the

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Table 1
Distribution of the number of sheep samples analyzed by infection status, gender, and age in months (mo).

	Naïve sheep			Infected sheep		
	Pregnant	Non-pregnant	Male	Pregnant	Non-pregnant	Male
≤ 18 mo	14	16	17	6	5	4
≥ 20 mo	30	31	17	3	11	4
Total (n)	44	47	34	9	16	8

prion protein gene, *PRNP* (Alverson, O'Rourke, & Baszler, 2006). An analysis of variance was conducted using a mixed model with SAS 9.4 (SAS Inst. Inc., Cary, NC) for both cytokines. The model was reduced to only factors that were significant or were a main effect. Specifically, we included the fixed effects of age, gender, infection status, pregnancy status within gender, and prion haplotype and a random effect of assay replicate. The final model contained only the main effects as well as age by gender and age by infection status interactions. To adjust for unbalanced data, the Tukey-Kramer procedure was used to identify pairwise significant differences among least square means. The equation used is presented below:

$$Y_{ijklmn} = \mu + A_i + G_j + AG_{ij} + P(G)_k + D_l + AD_{il} + H_m + R_n + e_{ijklmnn},$$

in which Y_{ijklmn} is the observation of the *i*th age (young ≤ 18 months and old ≥ 20 months) of sheep, gender (*j*th), pregnancy status within gender (*k*th), infection status (*l*th), prion haplotype (*m*th), and random replicate (nth); μ is an intercept term common to all observations and e_{ijklmn} is the residual error.

3. Results and discussion

Serum TIMP-1 was found to be significantly lower in young naïve sheep compared with old naïve and old infected sheep ($P<0.04$; Fig. 1 and Suppl. Fig. 1). A similar trend was observed in young naïve sheep versus young infected sheep but was not statistically significant ($P>0.16$). This observation is consistent with lower TIMP-1 in naïve versus scrapie-infected Tg338 mice regardless of age tested (Newsom et al., 2011). TIMP-1 has been shown to be downregulated by estradiol in pregnant sheep and its absence may be associated with increased uterine protease activity during estrus (Nothnick, 2000). Whereas, in the current study, pregnancy was associated with lower TIMP-1 ($P<0.022$), the interaction between pregnancy and infection status was not significant ($P>0.8$) and removed from the model

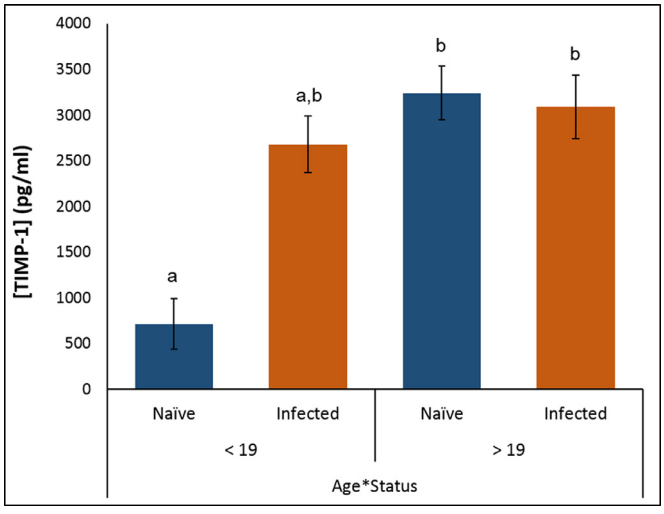

Fig. 1. TIMP-1 levels (pg/ml) determined within age vs infection status in sheep serum samples. The bars are the least squares means with the standard errors. Different letters were $P < 0.05$.

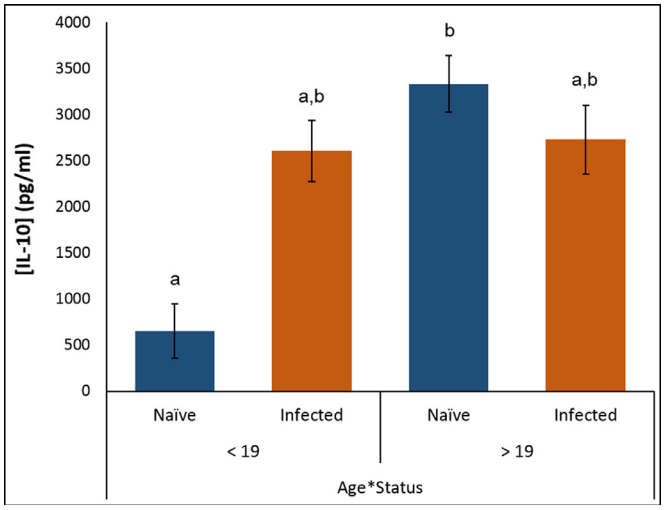

Fig. 2. IL-10 levels (pg/ml) determined within age vs infection status in sheep serum samples. The bars are the least squares means with the standard errors. Different letters were $P < 0.05$.

without further analysis. Similar to observations of TIMP-1, IL-10 was significantly lower in young naïve sheep when compared to old naïve sheep ($P<0.0001$; Fig. 2 and Suppl. Fig. 2). While IL-10 tended to be lower in young naïve sheep relative to young and old scrapie infected sheep, this observation was not significant ($P<0.19$). Studies have shown that IL-10, an inhibitor of T-lymphocyte helper 1 cytokines (de Silva, Begg, & Whittington, 2011), plays a role in regulation of TSEs as was demonstrated by rapid development of prion disease in IL-10 deficient mice (Thackray, McKenzie, Klein, Lauder, & Bujdoso, 2004). The lack of statistical concordance between this study and previous studies showing increased IL-10 in scrapie-infected mice relative to naïve mice (Newsom et al., 2011; Thackray et al., 2004) may be due to the sampling of an outbred population and may reach significance upon testing more animals. Previous studies indicate that polymorphisms at codons 136, 154, and 171 of the open reading frame of sheep *PRNP* influence resistance (ARR/ARR) or susceptibility (VRQ/VRQ, ARQ/ARQ) to scrapie (Baylis & Goldmann, 2004). In the current study, prion haplotype was not associated with serum levels of TIMP-1 ($P>0.07$) or IL-10 ($P>0.08$) (data not shown).

The development of non-invasive screening methods to identify preclinical scrapie-infected animals is highly desirable. This study demonstrates that the trend of the increase of TIMP-1 and IL-10 levels in scrapie-infected animals is similar to observations in infected-mice but lacked significance potentially due to sampling in the natural host (which is an outbred population) and/or the sample size tested. While studies of scrapie infected-mice are helpful in demonstrating infectivity and strain variation, studies of potential ovine biomarkers should be confirmed or performed directly in the natural hosts, since mice may not be universally reliable as a model for comparative studies of inflammatory diseases (Seok et al., 2013). Alternatively, the data suggests that the cytokines tested hereby might not be ideal biomarkers to be used as predictive diagnostic tools, especially for the preclinical stage, suggesting that additional candidates should be identified.

4. Conclusion

Currently, diagnosis of TSE is solely based on the use of techniques aimed at identifying the PrP^{Sc} protein, and it precludes the use of biomarkers, since so far none was identified for indicating preclinical infections. This study found that TIMP-1 and IL-10 levels tended to have lower levels in young naïve sheep compared to infected sheep but these differences did not reach statistical significance. Although the data gathered so far is insufficient to propose the use of biomarkers in the

diagnosis of scrapie, it supports the possible future development of such biomarker probes. Future studies using a larger sampling cohort will be needed to determine the potential value of these cytokines as a screening tool for scrapie-infected sheep.

Conflict of interest

The authors declare no conflict of interest.

Ethical statement

Experimental protocols were approved by the Washington State University Institutional Animal Care and Use Committee

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.vas.2019.100056.

References

Alverson, J., O'Rourke, K. I., & Baszler, T. V. (2006). PrPSc accumulation in fetal cotyledons of scrapie-resistant lambs is influenced by fetus location in the uterus. *The Journal of General Virology, 87*, 1035–1041. https://doi.org/10.1099/vir.0.81418-0.

Andreoletti, O., Lacroux, C., Chabert, A., Monnereau, L., Tabouret, G., Lantier, F., et al. (2002). PrP(Sc) accumulation in placentas of ewes exposed to natural scrapie: Influence of foetal PrP genotype and effect on ewe-to-lamb transmission. *The Journal of General Virology, 83*, 2607–2616. https://doi.org/10.1099/0022-1317-83-10-2607.

Baylis, M., & Goldmann, W. (2004). The genetics of scrapie in sheep and goats. *Current Molecular Medicine, 4*, 385–396. https://doi.org/10.2174/1566524043360672.

de Silva, K., Begg, D., & Whittington, R. (2011). The interleukin 10 response in ovine Johne's disease. *Veterinary Immunology and Immunopathology, 139*, 10–16. https://doi.org/10.1016/j.vetimm.2010.07.022.

Gonzalez, L., Dagleish, M. P., Martin, S., Dexter, G., Steele, P., Finlayson, J., et al. (2008). Diagnosis of preclinical scrapie in live sheep by the immunohistochemical examination of rectal biopsies. *The Veterinary Record, 162*, 397–403. https://doi.org/10.1136/vr.162.13.397.

Newsom, D. M., Liggitt, H. D., O'Rourke, K., Zhuang, D., Schneider, D. A., & Harrington, R. D. (2011). Cytokine antibody array analysis in brain and periphery of scrapie-infected Tg338 mice. *Comparative Immunology, Microbiology and Infectious Diseases, 34*, 387–397. https://doi.org/10.1016/j.cimid.2011.06.001.

Nothnick, W. B. (2000). Disruption of the tissue inhibitor of metalloproteinase-1 gene results in altered reproductive cyclicity and uterine morphology in reproductive-age female mice. *Biology of Reproduction, 63*, 905–912. https://doi.org/10.1095/biolreprod63.3.905.

Prusiner, S. B. (1998). Prions. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 13363–13383. https://doi.org/10.1073/pnas.95.23.13363.

Seok, J., Warren, H. S., Cuenca, A. G., Mindrinos, M. N., Baker, H. V., & Xu, W. Inflammation, Host Response to Injury, L.S.C.R.P.. (2013). Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proceedings of the National Academy of Sciences of the United States of America, 110*, 3507–3512. https://doi.org/10.1073/pnas.1222878110.

Thackray, A. M., McKenzie, A. N., Klein, M. A., Lauder, A., & Bujdoso, R. (2004). Accelerated prion disease in the absence of interleukin-10. *Journal of Virology, 78*, 13697–13707. https://doi.org/10.1128/JVI.78.24.13697-13707.2004.