Plasma protein loss in lambs during a mixed infection of *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* – a consequence of the immune response?

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ABSTRACT

The effect of corticosteroid-induced immuno-suppression on the plasma protein loss of lambs infected with gastrointestinal nematode parasites was investigated. Eight parasite naïve lambs received either a mixed infection of *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* (group IF) or received the same infection while concurrently immuno-suppressed with the corticosteroid methylprednisolone acetate (group IS). Concentrations of nematode eggs in the faeces of lambs increased to 1,500 epg in IF and 4,000 epg in IS by day 42, indicating successful immuno-suppression. Infection reduced plasma albumin concentration compared with pre-infection levels by 19% in IF animals ($p<0.05$), but only 7% (ns) in IS animals. Plasma loss (ml day^{-1}) into the gastrointestinal tract was estimated during two seven day periods through the appearance of intravenously infused ^{51}Cr in the faeces. Protein loss into the gastrointestinal tract (g d^{-1}) was calculated by multiplying the plasma loss (ml d^{-1}) by the total protein concentration in the plasma (g l^{-1}). Plasma protein losses at day 35 were increased by 310% in IF and by 258% in IS relative to pre-infection levels, and were not affected by immuno-suppression ($p>0.05$). These results suggest the leakage of plasma proteins into the alimentary tract are a consequence of intestinal pathology caused by the parasite, rather than detrimental components of the immune reaction. It is hypothesised the ability of IS animals to maintain plasma albumin is the result of a greater ability to replace leaked proteins as a consequence of the net catabolic actions of corticosteroids.

Keywords: Nematoda; immuno-suppression; plasma protein; sheep.

INTRODUCTION

Infections with gastrointestinal nematode parasites in young sheep are frequently observed to reduce the nutrient economy of the host (Sykes and Greer, 2003). In particular, both abomasal and small intestine parasites have consistently been observed to cause a reduction in the apparent nitrogen (N) digestibility that has resulted in the daily N balance of infected sheep being 3-5g less than their uninfected controls (Poppi *et al*., 1986). Increased endogenous N losses are partly due to leakage of plasma proteins into the lumen of the alimentary tract as a consequence of damage caused to both the abomasum and small intestine during infection, which if not replaced through commensurate synthesis, is reflected in a depression of serum albumin concentration (Steel *et al*., 1980). Recent reviews have suggested that many of the pathological changes in the gastrointestinal tract may be undesirable consequences of the immune response, rather than physical damage caused by the parasite *per se* (Hein *et al*., 2001; Colditz, 2002). This is supported by the findings of Lawrence *et al*. (2001), in which mast cell deficient mice had longer intestinal villi than their immunologically competent counterparts after 13 days of infection with *T. spiralis*, despite harbouring a greater worm burden. Furthermore, Greer *et al*. (2005) found corticosteroid-induced immuno-suppression prevented a reduction in serum albumin that was observed in immunologically normal animals during infection with the intestinal parasite *Trichostrongylus colubriformis*. The current experiment investigated the effect of corticosteroid induced immuno-suppression on plasma protein leakage during gastrointestinal nematode parasitism in order to examine the possibility that plasma N leakage may be a consequence of immuno-pathology rather than the mechanical damage caused by the parasite *per se*.

MATERIALS AND METHODS

Animals and treatments

Eight 6-month-old parasite naïve Coopworth ewe lambs were housed in metabolism crates at day -20. Animals were allowed to adjust to their surroundings before the measurement of plasma protein loss (described below) commencing on day -13. On day 0, lambs were allocated to one of two treatment groups that were balanced for pre-treatment losses of protein into the gastrointestinal tract. One group (group IF; n=4) received a challenge dose of 10,000 *Trichostrongylus*

colubriformis and 20,000 *Teladorsagia circumcincta* L3 infective larvae on day 0 followed by a trickle infection in a three-times weekly dosing regime on Monday, Wednesday and Friday of each week with the equivalent of 2000 L3 *T. colubriformis* larvae d^{-1} and 4000 *T. circumcincta* L3 larvae d^{-1} until day 42.

The remaining group (group IS; n=4) received the same infection while concurrently treated with a weekly intramuscular injection of 1ml 30 $kgLW^{-1}$ of the corticosteroid Depredone (Jurox Pty. Ltd., Rutherford, NSW, Australia, containing 40mg methylprednisolone acetate ml^{-1}) in order to suppress immune function.

Measurement of plasma loss

Plasma loss (ml day^{-1}) into the gastrointestinal tract was determined during two seven day periods commencing on day -13 and 35 of infection through the appearance of intravenously infused ^{51}Cr in the faeces, as described by Bown *et al*. (1991). Briefly, approximately 800 µCi of ^{51}Cr (Perkin Elmer Inc, Boston, USA) contained in 10ml physiological saline was infused into the jugular vein on day -13 over a period of 1-2 min to allow the distribution of the isotope throughout the plasma pool. After allowing 24 h for the anticipated excretion of ^{51}Cr that was not bound to plasma protein, blood samples were collected every 24 h using jugular venipuncture into lithium heparin vacutubes (Becton Dickinson, VACUTAINER Systems, Rutherford, New Jersey, USA) and were immediately centrifuged at 3000rpm for 10 minutes before the separation of plasma. Faecal output was measured every 24 h for seven days, with sub-samples taken and dried at 90ºC for 72 h to allow the calculation of total daily faecal dry matter (DM) production. Due to possible contamination of collected faeces with urinary ^{51}Cr, additional faecal samples were collected directly from the rectum twice daily for measurement of radioactivity per g DM. The amount of radioactivity in plasma and faecal samples was measured using a scintillation detector (LKB Wallac 1282 Compugamma, Universal gamma counter) and, after correction for background radiation, expressed as counts per second per ml of plasma and counts per second per g faeces DM, the latter of which was multiplied by the daily faecal DM production to give total daily faecal radioactivity output. The same procedure was repeated for the second measurement period commencing at day 35, with the exception that 400µ Ci $^{51}CrCl_3$ was used.

Enteric plasma volume and N loss into the gastrointestinal tract was calculated according to Herd (1971). The amount of daily total radioactivity (counts per second per g DM) excreted in the faeces was divided by plasma radioactivity (counts per second per ml) to give the total amount of plasma lost into the faeces for each 24 h period (ml d^{-1}). Protein loss into the gastrointestinal tract (g d^{-1}) was calculated by multiplying the plasma loss (ml d^{-1}) by the total protein concentration in the plasma (g l^{-1}). Plasma protein leakage for each individual was calculated daily and averaged for the seven days following the injection of ^{51}Cr and divided by 6.25 to give plasma N leakage.

Feeding and sampling

All animals had *ad libitum* access to fresh water and a 50:50 w/w mix of lucerne chaff and oat chaff, with the exception of the period of plasma protein loss measurements, during which time animals were offered solely lucerne chaff in an attempt to ensure adequate faecal $^{51}CrCl_3$ concentrations for reliable radioactivity detection. Individual feed refusals were collected and weighed daily during plasma protein loss measurement periods and weekly from day 0. Sub-samples of feed offered and refused were taken for determination of DM. Faecal samples were taken weekly from the rectum of each sheep from day 0 for the determination of faecal nematode egg concentration (FEC) using a modification of the McMaster method (Ministry of Agriculture, Fisheries and Food, 1979) and expressed as eggs per gram of fresh faeces (epg). Live weight was measured weekly. Weekly blood samples were collected from day -7 to 35, and the plasma separated and stored at -20º C until analysis for albumin and total protein concentrations with a Cobas Mira Plus Auto-analyser (Roche Diagnostics GmbH, Mannheim, Germany) using kits #1970569 and #1040901, respectively, with plasma globulins calculated by difference.

Statistical analysis

Data were analysed using GENSTAT statistical package (version 4.2, Lawes Agricultural Trust, Rothamstead Experimental Station, VSN International Ltd , 2001). Faecal egg counts were log10 (count + 1) transformed before analysis with back transformed means presented. Data for live weight, food intake, FEC, plasma albumin, globulin and total protein concentrations underwent sequential comparison of ante-dependence structures for repeated measures before being analysed by REML. Differences between plasma protein loss for periods beginning at day -15 and day 35 were analysed using a *t*-test.

This trial was undertaken within a designated radioactive laboratory with approval

from, and in accordance with the Lincoln University Animal Ethics Committee No. 68.

RESULTS

Faecal egg counts

Overall, there was a treatment x time interaction ($p<0.001$) that was reflected in an increase in the FEC of all individuals from day 20 that was greater in IS animals, reaching peaks of 1520 epg and 4069 epg on day 41 for IF and IS animals, respectively.

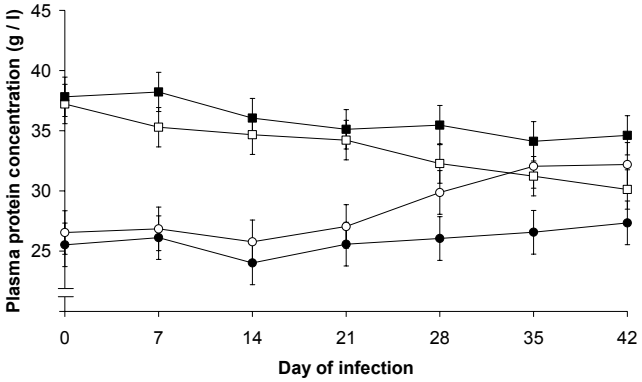
Food intake and live weight

There was a treatment x time interaction ($P<0.05$) in voluntary food intake that was reflected in IS animals maintaining intake while IF animals displayed a transient decrease relative to IS of 18-24% of dry matter intake from day 21 to day 34 of infection ($P<0.05$). Live weight was not significantly influenced by treatment, although IF lambs displayed a steady decrease in weight of 116g/d from day 13 of infection that was not observed in IS animals.

Plasma proteins

Total plasma protein concentration was not influenced by either treatment ($P=0.860$) or time ($P=0.195$). Mean plasma albumin and globulin concentrations are shown in Figure 1. For albumin there was a treatment x time interaction ($P=0.017$) that was reflected in a greater rate of decrease in IF than in IS animals, resulting in a 13% lower plasma albumin concentration in IF relative to IS at day 42 ($p<0.05$). Globulin concentration tended to increase with time ($p=0.054$) and was not influenced by immune suppression ($p=0.121$), nor was there a suppression x time interaction ($p=0.777$).

FIGURE 1: Mean plasma albumin (squares) and globulin (circles) concentrations (g l^{-1}) of **lambs** either infected with Teladorsagia circumcincta and Trichostrongylus colubriformis (IF; □, ○) or similarly infected and concurrently immuno-suppressed (IS; ■, ●).



Plasma N loss

Mean individual plasma N losses into the faeces are shown in Table 1. Infection significantly increased mean plasma N loss observed on day 35 compared with pre-infection levels on day -13 by 310% in IF and by 258% in IS groups ($p<0.05$).

TABLE 1: Individual and mean plasma N loss (g d^{-1}) on days -13 and 35 of lambs that were either infected with *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* (IF) or similarly infected and concurrently immuno-suppressed (IS)

		Start day for plasma loss	
Treatment	Sheep ID	-13	35
IF	1	0.70	2.56
	2	0.48	1.61
	3	0.63	1.72
	4	0.61	1.63
	Mean	**0.61±0.05**	**1.83±0.23**
IS	5	0.50	2.17
	6	0.59	1.39
	7	0.72	1.70
	8	0.59	0.94
	Mean	**0.60±0.04**	**1.55±0.26**

DISCUSSION

These results suggest plasma protein loss as a consequence of infection with *T. colubriformis* and *T. circumcincta* is not alleviated by corticosteroid induced immuno suppression.

The 800µCi of $^{51}CrCl_3$ administered to animals during the pre-infection plasma N loss measurement period was considerably larger than the 300µCi used by Bown *et al*. (1991). The larger dose of radioactive material in the current study was initially considered necessary to ensure adequate counts in faecal material, given the anticipated counting efficiency from previous experience (*unpublished*). However, it was apparent that much lower amounts of $^{51}CrCl_3$ were needed, consequently a smaller dose of 400 µCi $^{51}CrCl_3$ was chosen for the second plasma N loss measurement period. This was confirmed by maintenance of faecal counts during the second measurement period in excess of 10 x background radiation.

The success of this trial was dependant on the ability to establish a pathogenic infection and for the corticosteroid treatment to provide immuno-suppression. In the case of the latter, the much greater FEC of 4,000 epg in IS compared to the 1,500 epg observed in IF indicates that immuno-suppression was achieved in IS animals. Unfortunately, due to the radioactive nature of gut contents at the conclusion of the trial, confirmation of this through comparative worm burdens was not

undertaken. Despite no significant reduction in live weight, the 24% reduction in feed intake and 22% reduction in plasma albumin experienced by IF animals indicates a pathogenic infection was established. Further confirmation of this comes from the increase in plasma N loss from 0.61gN d^{-1} pre-infection to 1.83gN d^{-1} at week six in IF animals, a threefold increase comparable in magnitude to that observed after seven weeks of infection with 3,000 *T. colubriformis* and 3,000 *T. circumcincta* larvae d^{-1} by Bown *et al*. (1991).

Reductions in serum albumin are frequently observed during infection with both *T. colubriformis* and *T. circumcincta* (Poppi *et al*., 1986), and are believed to reflect increased plasma N leakage into the alimentary tract as a consequence of intestinal pathology that is not replaced by commensurate synthesis or globulin production (Steel *et al*., 1980). Corticosteroid-induced immuno-suppression in the current study prevented a 20% reduction in plasma albumin concentration that was apparent in IF animals. These findings are in agreement with those of Greer *et al*. (2005) who observed that a similar immuno-suppression regime prevented a 22% reduction in serum albumin in young lambs infected with *T. colubriformis*, despite much larger comparative worm burdens in immuno-suppressed animals compared with their immunologically normal counterparts.

The lack of a reduction in serum or plasma albumin in immuno-suppressed animals may indicate pathological changes in the gastrointestinal tract that are undesirable consequences of the immune response, as suggested by Hein *et al*. (2001). Indeed, Lawrence *et al*. (2001) reported mast cell deficient mice had longer intestinal villi than their immunologically competent counterparts after 13 days of infection with *T. spiralis*, despite harbouring a greater worm burden. It was hypothesised by Lawrence *et al*. (2001) that one of the roles of the immune response was to act directly on the gastrointestinal tissue, making an unfavourable environment for the parasite and, as a consequence, becoming the major cause of intestinal pathology. However, the comparable increase in plasma N appearance in the faeces of IS to that of IF animals indicates that immuno-suppression did not reduce the leakage of plasma proteins into the alimentary tract. Furthermore, these results suggest that similar levels of disruption to intestinal integrity were caused as a consequence of infection in IS compared to IF animals.

The similar levels of plasma N appearance were evident despite FEC in excess of 4,000 epg in IS animals that were considerably greater than the 1,500 epg observed in IF animals, possibly indicating a larger parasite burden in immuno-suppressed animals. Moreover, increases in the sequestration of leucine in the gut of 24% have been reported during *T. colubriformis* by Yu *et al*. (2000) that presumably reflect an increased amino acid demand for the repair of gastrointestinal tissue (Colditz, 2002). Such demand for amino acids for repair of the damage to the alimentary tract would not be expected in IS animals due to the net catabolic actions of the corticosteroid compounds. Turini *et al*. (2003) observed reduced fractional synthesis rates of 19% in the gut mucosa of rats that were treated with the corticosteroid dexamethasone. However fractional synthesis rates in the liver of these rats were increased by 61%, resulting in a 17% increase in liver weight. Reduced muscle tissue deposition has also been observed in sheep that received the same corticosteroid regime to that used in the current study and which was accompanied by larger livers and an increase in serum urea concentration, all of which are consistent with an increased supply of amino acids for deamination as a consequence of the catabolic effects of corticosteroids (Greer *et al*., 2005).

It seems probable that the lack of a reduction of plasma albumin in IS animals observed here and by Greer *et al*. (2005) is a consequence of an enhanced availability of amino acids which enabled the replacement of lost plasma proteins, rather than as a consequence of reduced intestinal pathology.

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