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Research paper

Immune development and performance characteristics of Romney sheep selected for either resistance or resilience to gastrointestinal nematodes

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ABSTRACT

Immunological and performance characteristics were explored in Romney sheep from lines selected for either resistance or resilience to parasite infection. At a mean 78 days-of-age, twin lambs from a line selected for resistance (RT) and lambs from a line selected for resilience (RL) were infected with the intestinal nematode *Trichostrongylus colubriformis* for 100 days (I) while their twin remained as an uninfected control (C). Compared with RL, RT animals had lower levels of circulating $CD4^+$ T-cells (P = 0.003) but a greater proportion of these were activated ($CD4^+CD25^+$) in response to infection (P = 0.007). Differences between the lines in humoral immune responses to nematode infection varied with higher levels of *T. colubriformis* specific immunoglobulin (Ig) E in RT-I than RL-I (P = 0.002) but similar levels of both IgG (P = 0.926) and IgA (P = 0.321) responses. Temporal differences in the immune response also existed between the lines with RT-I animals displaying an earlier peak and more rapid reduction in FEC and an earlier peak in *T. colubriformis* specific IgA. In addition, compared with their RT-C and RL-C counterparts, infection caused a 22% reduction in feed intake from day 56 (P = 0.001) with total feed intake reduced by 15% and 9% for RT-I and RL-I, respectively. Cumulative liveweight gain was greatest for RL animals (P = 0.026) and relative to RT-C and RL-C was reduced by 5.8 kg and 4.9 kg for RT-I and RL-I, respectively. Overall, the selection lines appear to have differences in immunological characteristics that are both dependent on, and independent of parasite infection. Further, the difference in growth in the uninfected animals coupled with the similar cost of infection suggests the lower liveweight gain of RT-I compared with RL-I may be due to inherent differences between the lines in their growth potential, rather than a greater cost of infection in animals selected for resistance.

1. Introduction

Exploiting genetic divergence in the immunological responsiveness to gastrointestinal nematode parasites is an established tool to control what remains a significant threat to the health and welfare of grazing livestock. Typically, for sheep in temperate environments this is achieved through the two different approaches, being either the identification of animals that are resistant or that are resilient to gastrointestinal nematodes. Selection of animals for greater resistance is associated with an enhanced immunological capacity and both lower nematode burdens and concentrations of nematode eggs in the faeces while resilient animals have the ability to perform well in the face of larval parasite challenge despite at times harbouring a greater nematode burden and greater FEC (Morris et al., 1997). However, while the epidemiological advantages of selection for resistance are clear, the benefits of selection for reistance on animal performance have not always been apparent, leading to debate as to the wisdom of either approach (Greer, 2008; Williams 2011).

The acquisition of immunity to nematode parasites incurs a substantial nutritional cost to the host. The immune response *per se* has been calculated to be responsible for between 39% and 75% of the overall cost of parasite infection in grazing lambs subjected to predominantly *Trichostrongylus vitrinis* or *T. colubriformis* infections, respectively, (Blackburn et al., 2015; Dever et al., 2016) and the entire cost in indoor studies with lambs infected with either *T. colubriformis* or *Teladorsagia circumcincta* (Greer et al., 2005, 2008). This presumably reflects the recruitment of a number of immune effector cells and physiological changes in the gut which can be expected to increase nutrient demand following activation of the immune response to parasite antigens (Kimambo et al., 1988; MacRae, 1993; Coop and Holmes, 1996; Coop and Kyriazakis, 1999). The mass of protein associated with the immune response in peri-parturient sheep was

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estimated to increase metabolisable protein (MP) requirement by 5% (Houdijk et al., 2001) while respetive increases of 4% and 5% for metabolisable energy (ME) and MP requirements have been estimated in lines of Merino sheep seleted for resistance to *Haemonchus contortus* infection (Liu et al., 2005). In part, it may be expected greater resource allocation to the immune response may help explain the production advantage that is commonly observed in resilient compared with resistant selection lines in a temperate environment (Morris et al., 2000, 2005). However, what is less clear is how much of the difference in animal performance between these lines of animals selected for resistance or resilience can be attributed to greater diversion of nutrients for immune function in animals predisposed to mount a strong immune response or whether selection has resulted lines that exhibit differences in growth potential that are independent of parasite challenge. Given the importance of the acquisition phase of immunity on the impact on animal performance, of particular interest is whether these lines differ in their adaptive immune capacity and the strength and timing of response at the cellular and humoral levels to parasite infection which may contribute to a greater overall cost of immunity and subsequent diversion of nutrients away from growth.

2. Materials and methods

2.1. Animals and feeding

All proceedures were carried out with approval from, and in accordance with the Lincoln University Animal Ethics Committee, approval number LUAEC#274. Twenty twin mixed-sex Romney lambs representing all of the same-sex twins born in one year from lines that had undergone selection for either resistance (n = 10) or resilience (n = 10) to gastrointestinal nematodes were born and reared indoors to exclude exposure to gastrointestinal nematode infection. These lines were established by AgResearch in 1979 (Bisset et al., 1996; Morris et al., 2000) and were actively selected within line for divergance in faecal egg count (FEC) until transferred to Lincoln University in 2008 from which point the lines were maintained with replacement ewe and ram lambs randomly selected within each line each year. Lambs were offered a creep feed from four weeks-of-age and were weaned and penned individually from eight weeks-of-age from which time they were offered lucerne pellets *ad libitum* supplying 10.1 megajoules (MJ) of metabolisable energy (ME) per kg dry matter (DM) and containing 20% crude protein with access to fresh water and a low-copper mineral salt lick at all times.

2.2. Experimental design and treatments

At a mean age of 78 days (day 0) and mean live weight (LW) ± sem 15.7 ± 0.72 kg and 14.6 ± 0.38 kg for resistant and resilient lambs, respectively, one lamb from each twin pairing was trickle infected with the equivalent of 80 infective L3 *T. colubriformis* larvae per kg live weight per day for 100 days. Larvae were pippetted onto filter paper which was then rolled and adminstered using a balling gun in a three-times-weekly dosing regime. The remaining lamb from each twin pairing remained as an uninfected control in a 2 × 2 factorial design (n = 5) with selection line, *viz*, resistant (RT) or resilient (RL) and infection, *viz*, infected (-I) or not infected (-C) as factors.

2.3. Measurements and sampling

The amount of feed offered was recorded daily with individual feed refusals collected and weighed weekly. Sub-samples of feed offered and refused were taken for determination of DM after drying for 72 h at 90 °C. LW was recorded at weekly intervals as were faecal samples taken directly from the rectum for the determination of nematode faecal egg count (FEC; eggs per gram (epg)) (M.A.F.F, 1979) with a sensitivity of 100 epg. Blood samples were taken at days −1 and 6 relative to infection and fortnightly thereafter using jugular venipuncture into each of two 10 ml vacutubes (Becton Dickinson, VACUTAINER Systems, Rutherford, New Jersey, U.S.A). For serum collection plain vacutubes were used and stored at 4 °C for 24 h before centrifugation at 1250 × *g* for 10 min, with the serum separated and stored at −20 °C until analysis (described below). For the collection of peripheral blood mononuclear cells (PBMC) tubes containing lithuim heparin were used. Upon collection, tubes were placed on ice and immediately tranferred to the laboratory for the purification of WBC and subsequent labelling for surface markers (described below). Slaughter of infected animals on day 105 of infection, worm recovery from the first 5 m of small intestine and counting were as described by Donaldson et al. (2001).

2.4. Surface labelling of PBMC

The activation status of helper ($CD4^+$) T-cells within PBMC populations was assessed using the activation marker CD25. Five ml blood from lithium heparinised vacutainers was mixed with 5 ml PBS which was then layered gently to 3 ml Ficoll-Paque™ PLUS (GE Healthcare, Little Chalfont, UK) contained in a 15 ml centrifuge tube so as not to mix the samples. Following centrifugation at 900 × *g* at 20 °C for 30 min with no brake the band of PBMCs was removed and placed into a separate tube and washed twice with 5–10 ml cold FACS buffer (PBS with 10% heat inactivated FCS and 0.1% Na azide). For each wash the cell pellet was re-suspended and then centrifuged at 400 × *g* for 7 min at 4 °C. After the second wash the cells were re-suspended in 1 ml cold FACS buffer. Two aliquots of approximately 10^6 PBMC from each animal were collected, one incubated for 30 min with 1 µg of both anti-sheep CD4 (AbD Serotec MCA2213, clone 44.38 isotype mouse IgG2a) and anti-CD25 (interleukin (IL)-2 receptor alpha (IL-2R-α), AbD Serotec MCA 2430; clone ILA-111 isotype mouse IgG1) surface markers with the other aliquot incubated with 1 µg of the respective isotype controls; IgG1 negative control (AbD Serotec MCA928) and IgG2a negative control (AbD Serotec MCA929). After incubation the samples were washed twice with FACS buffer, each time being centrifuged at 400 x *g* for 1 min and the supernatant discarded. Following washing, 1 µg of the secondary antibodies goat anti mouse IgG1alexa flour 488 (Invitrogen A21121) and goat anti mouse IgG2a PE-Cy5.5 (Invitrogen M32218) was added in FACS buffer and incubated in the dark at 4 °C for 30 min. Cells were then washed twice with FACS buffer and once with PBS and maintained in the dark until analysis by flow cytometry (Beckman Coulter FC500 MPL). Data was analysed using CXP software version 2.1.

2.5. Serum analysis

Antibodies (Immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin E (IgE)) specific to *T. colubriformis* L3 s in serum were measured using an ELISA as described by Xie et al. (2004). Briefly, serum was diluted to 1:100 for IgG and 1:10 for both IgA, and IgE and then incubated in duplicate in 96 well plates that had been coated with 0.1 µg *T. colubriformis* L3 antigen per well. Following washing with PBS (pH 7.2 + 0.05% Tween 20), each well was incubated with 100 µl of the following conjugated antibodies: for L3 IgG horseradish peroxide (HRP) conjugated polyclonal rabbit anti-sheep immunoglobulins (Pierce Immunopure Antibodies, cat #31480, lot #GI959969) at a dilution of 1:4000, for L3 IgA HRP conjugated rabbit anti-sheep IgA (1.0 mg ml^{-1}, Bethyl Laboratories Inc, cat #A130-108P, lot #A130-108P-6) at a dilution of 1:2000 and for IgE 100 µl of mouse anti-sheep IgE monoclonal antibody (clone 2F1 Moredun Research Institute, Scotland) at a dilution of 1:200 followed by the addition of HRP conjugated goat anti-mouse IgG (STAR117P, AbD Serotec Ltd, lot #240107). Colour was developed using 100 µl of 0.05 M phosphate-citrate buffer adjusted to pH 5 with 0.02% of 30% H_2O_2 added and containing 100 µg of Tetramethylbenzidine dihydrochloride (TMB;