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Efferent intestinal lymph protein responses in nematode-resistant, -resilient and -susceptible lambs under challenge with *Trichostrongylus colubriformis*

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ABSTRACT

The mechanisms underlying resistance to challenge by gastrointestinal nematode parasites in sheep are complex. Using DIGE, we profiled ovine lymph proteins in lambs with host resistance (R), resilience (Ri) or susceptibility (S) to a daily trickle challenge with the nematode *Trichostrongylus colubriformis*. Efferent intestinal lymph was collected prior to infection (day 1) and on days 5 and 10 post-infection. Eight proteins identified by LC-MS/MS, showed differences relating to host genotype. Of these, Serpin A3-3 and Serpin A3-7 have not been reported previously in the lymph proteome. Three acute phase proteins showed significant differences relating to interactions between breeding line and parasite challenge, including complement C3β, C3α and haptoglobin (Hp) β. In the R lambs C3α was significantly up regulated ($P < 0.05$) on day 10, while in the Ri lambs Hp β was significantly down regulated ($P < 0.05$). In the S lambs, levels of C3β were up regulated and levels of Hp β down regulated (both $P < 0.05$) on day 10. Hence we demonstrate that acute phase inflammation proteins contribute to differences in the innate immune response of sheep to challenge by *T. colubriformis*. The findings may lead to the development of new approaches to combat nematode infestations in sheep production systems.

Biological Significance
Breeding lines of sheep with resistance (R), resilience (Ri) or susceptibility (S) to nematode infections provide an experimental model to examine the biological mechanisms

Abbreviations: FEC, fecal egg counts; BV, breeding value; R, nematode resistant sheep breeding line; Ri, nematode resilient sheep breeding line; S, nematode susceptible sheep breeding line; DS, dag score; IFNγ, interferon gamma; IL, interleukin; TNF, tumor necrosis factor; DC, dendritic cells; APCs, antigen presenting cells; LWG, liveweight gain; BVA, biological variance analysis; REML, restricted maximum likelihood estimate; FA, formic acid; TCEP, tris(2-carboxyethyl)phosphine.

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underlying the ability of some sheep to expel worms and remain healthy without the use of an anthelmintic. Using proteomics we identified differences in the expression of acute phase lymph proteins in the R, Ri and S lambs. The results will assist the development of alternative control strategies to manage nematode infections in livestock.

1. Introduction

Infections of livestock with gastro-intestinal nematode parasites are commonly controlled by the use of anthelminthic drugs but their continuous application has resulted in the emergence of drug resistance in all major sheep producing countries. Drug resistance now presents a growing economic and welfare threat for the pastoral industry worldwide. Breeding sheep that are less reliant on anthelmintics to maintain health and productivity in the face of nematode challenge potentially offers an alternative control strategy to assist in overcoming this problem. Such breeding programs would be substantially enhanced if the biological basis of the traits involved in host resistance and/or resilience were better understood and easy-to-use marker based selection tools identified.

In New Zealand well characterized research flocks of sheep selectively bred for increased resistance (R), resilience (Ri) or susceptibility (S) to nematode infections were established some years ago [1–3]. Animals in the R flock were selected as lambs for low fecal nematode egg count (FEC) following a standardised exposure to natural nematode challenge on the assumption that this would indicate an ability to limit the establishment and/or subsequent development of an adult nematode burden [1]. Subsequent research confirmed this and also showed that this ability was generally associated with higher concentrations of tissue resident globule leukocytes, mast cells and eosinophils as well as elevated levels of *Trichostrongylus colubriformis*-specific antibodies, IgG_1, and IgM [4]. Moreover, the R genotype lambs were found to exhibit increased levels of both intestinal inflammation and interferon gamma (IFNγ) [5], as well as elevated IgE-levels [6,7]. In the R lambs increased resistance to infection is characterized by a predominant T helper-2 (Th-2) type cytokine response in cells that migrate in lymph from the intestinal tract to the lymph node draining the site of nematode infection [8,9], while in their S counterparts, the Th2 type response is much less pronounced [10]. In contrast to the R and S lambs, lambs in the Ri line were selected solely for their ability to withstand natural nematode challenge while grazing and so maintain acceptable growth rates with minimal anthelmintic intervention [11]. Genetic analyses showed that selection for this trait had led to higher growth rates and fewer dags under challenge, but had had no significant impact on resistance to infection as indicated by their FECs [3]. To date little research has been undertaken to elucidate the immunological or physiological basis of this trait.

The biological mechanisms that underlie resistance, resilience and susceptibility to nematode infections are clearly complex and probably involve both innate and acquired immunity. The host's antibody-mediated immune response to *T. colubriformis* infection demonstrates the involvement of the adaptive immune system reducing establishment of parasites [12]. In addition, cytokines associated with a protective Th-2 type response such as interleukin (IL)-5 and IL-13 are involved in the induction of allergic-type responses causing IgE secretion, which is typically found in sheep serum following parasite infection [6]. Eosinophils are associated with inflammation and expulsion of parasites and secrete chemotactic agents that attract various leukocyte subsets [13]. Th2-type cytokines are also involved in recruiting cell subsets such as mast cells or macrophages during parasitic infections. These cells then respond by secreting cytokines such as IL-1, IL-6 and IL-8, and TNF-α, which stimulate the liver and local tissue to produce acute-phase proteins.

The ability to limit nematode infection also involves the innate immune response, although the mode of action is not entirely understood. Several antigens displayed by *T. colubriformis* have been characterized but their direct involvement in acquired immunity or resistance to parasite infection has only been demonstrated for the carbohydrate larval allergen CarLA [14,15]. Nonetheless, ovine afferent lymph contains a diverse repertoire of antigen presenting cells (APCs) including dendritic cells (DC), macrophages and monocytes amongst other leukocytes [16,17]. DC residing in the upper small intestine [18,19], the site that harbors several economically important nematode species, traffic in afferent lymph upon stimulation with antigen. This induces adaptive immune responses [20].

While lymph fluid is closely related to blood serum and plasma, its composition may be altered by the metabolic activity of cells making up the tissue network in which it forms, as well as constituent cells in lymph nodes and the leukocytes which migrate in lymph. Thus, the proteome of mesenteric lymph of rodents and humans was found to differ significantly from blood plasma [21–23].

In the study reported here we investigated efferent intestinal lymph of sheep on the assumption that proteins associated with limiting nematode infections would be better represented here than in either blood or saliva. Our goal was thus to identify any systemic changes in secreted proteins that represent immune and inflammatory responses of sheep to nematode challenge in order to gain a better understanding of the biological mechanisms underlying the ability of sheep to limit the deleterious effects of gastrointestinal nematodes on their health and productivity. It was anticipated that this might ultimately lead to the possibility of manipulating the immune system to achieve improved nematode control and the identification of biomarkers to aid in the selection of genetically resistant and/or resilient animals.

2. Material and methods

The work flow showing the steps taken in the experiment and collection of data is summarized in Fig. 1.