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Transcriptional profiling of the ovine abomasal lymph node reveals a role for timing of the immune response in gastrointestinal nematode resistance

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ABSTRACT

Gastrointestinal nematodes are a serious cause of morbidity and mortality in grazing ruminants. The major ovine defence mechanism is acquired immunity, with protective immunity developing over time in response to infection. Nematode resistance varies both within and between breeds and is moderately heritable. A detailed understanding of the genes and mechanisms involved in protective immunity, and the factors that regulate this response, is required to aid both future breeding strategies and the development of effective and sustainable nematode control methods. The aim of this study was to compare the abomasal lymph node transcriptome of resistant and susceptible lambs in order to determine biological processes differentially expressed between resistant and susceptible individuals.

Scottish Blackface lambs, with divergent phenotypes for resistance, were challenged with 30,000 *Teladorsagia circumcincta* larvae (L3), and abomasal lymph nodes recovered at 7 and 14 days post-infection (dpi). High-throughput sequencing of cDNA from the abomasal lymph node was used to quantitatively sample the transcriptome with an average of 32 million reads per sample. A total of 194 and 144 genes were differentially expressed between resistant and susceptible lambs at 7 and 14 dpi respectively. Differentially expressed networks and biological processes were identified using Ingenuity Pathway Analysis. Genes involved in the inflammatory response, attraction of T lymphocytes and binding of leukocytes were more highly expressed in resistant animals at 7 dpi and in susceptible animals at 14 dpi indicating that resistant animals respond to infection earlier than susceptible animals. Twenty-four Single Nucleotide Polymorphisms (SNP) within 11 differentially expressed genes, were tested for association with gastrointestinal nematode resistance in the Scottish Blackface lambs. Four SNP, in 2 genes (*SLC30A2* and *ALB*), were suggestively associated with faecal egg count.

In conclusion, a large number of genes were differentially expressed in the abomasal lymph node of resistant and susceptible lambs responding to gastrointestinal nematode challenge. Resistant Scottish Blackface lambs appear to generate an earlier immune response to *T. circumcincta*. In susceptible lambs this response appears to be delayed. SNP in 2 differentially expressed genes were suggestively associated with faecal egg count indicating that differentially expressed genes may be considered candidate loci for mediating nematode resistance.

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1. Introduction

Gastrointestinal nematodes (GIN) are a serious cause of morbidity and mortality in grazing ruminants. Infected lambs have a reduced ability to absorb nutrients from the gastrointestinal tract, resulting in ill-thrift and, occasionally, death. Sub-clinical infection adds to the production losses in the form of reduced growth rate and light, under-finished carcasses. Anthelmintic drenching has been the method of choice for nematode control for the last 50 years; however, consumer concerns about food products from animals subjected to chemical treatment, combined with the inevitable evolution of anthelmintic resistant nematodes, means alternative, sustainable methods of parasitic nematode control are required.

Resistance to GIN is moderately heritable ($h^2 \sim 0.3$) (Bishop and Morris, 2007; Safari et al., 2005), therefore a sustainable method of nematode control is to select for genetically resistant individuals (Kemper et al., 2009). Selection using phenotypic traits, such as faecal egg count (FEC), requires prior exposure to GIN, whereas selection could be simplified through the identification of molecular markers. A detailed understanding of the genes and mechanisms involved in expressing a resistant phenotype and the factors that regulate this response would facilitate the identification of candidate markers.

Transcriptome analysis is a powerful method for the identification and quantification of genes expressed during a physiological perturbation. A number of previous studies have been undertaken to characterise the duodenal (Diez-Tascon et al., 2005; Keane et al., 2007; Keane et al., 2006), abomasal mucosal (Knight et al., 2011; Rowe et al., 2009) and lymph node and lymph fluid transcriptome (Andronicos et al., 2010; Gossner et al., 2013; Knight et al., 2010; MacKinnon et al., 2009) and have led to the identification of genes and biological processes associated with the host response to GIN. As a result of these studies, a number of pathways have been postulated to be involved in the development of a resistant phenotype; however, no clear consensus has emerged. In Perendale selection lines, susceptible lambs were found to have increased intestinal mucosal expression of genes involved in the stress response, while resistant animals had increased expression of Major Histocompatibility Complex (MHC) class II, free radical scavenging and fatty acid metabolism genes (Keane et al., 2007; Keane et al., 2006). Transcriptomic analysis of the abomasal lymph node of Texel (resistant) and Suffolk (susceptible) lambs suggested that a balanced T helper (Th) cell response was associated with resistance (Ahmed, 2013). A comparison of the abomasal lymph node transcriptome of resistant and susceptible Scottish Blackface lambs also identified Th cell differentiation and polarisation as important in the development of a resistant phenotype (Gossner et al., 2013). Differences between the studies may reflect biological or technical variation in the experimental design such as tissue sampled, lamb age, nematode exposure history, the magnitude and species of the nematode challenge, or the transcriptomic platform. Alternatively, the differences may reflect physiological differences between breeds and individuals in how they develop resistance.

Resistance to GIN may be manifested by controlling worm burden, worm fecundity or a combination of both (Stear et al., 1996b). The majority of previous studies concerning gene expression in resistant and susceptible animals have been based on a model where resistant and susceptible animals differ significantly in worm burden (Ahmed, 2013; Gossner et al., 2013; Keane et al., 2007; Keane et al., 2006; Pernthaner et al., 2005; Zaros et al., 2014). However, the genes and pathways involved in regulating worm fecundity may differ from those involved in controlling worm burden. We previously described a method to reliably identify repeatable within-breed variation in the ability of Scottish Blackface lambs to resist GIN infection (McRae et al., 2014). Resistant lambs were found to display lower FEC, lower worm fecundity and a higher level of anti-nematode IgA in both serum and mucosa. The physiological response to infection, as indicated by anti-nematode antibody levels, haematology and pepsinogen, was most pronounced at 7 and 14 days post-infection (dpi), although the phenotype (reduced FEC) was not yet evident at this time point.

The aim of the present study was to use high-throughput sequencing of cDNA to sample the transcriptome of the abomasal lymph node of Scottish Blackface lambs with divergent phenotypes for GIN resistance in order to identify genes and biological processes associated with the ability to express resistance. In this breed, repeatable differences among individuals in FEC, were positively associated with both increased worm burden and increased worm fecundity (McRae et al., 2014; Stear et al., 1995). Differentially expressed (DE) genes were considered candidate genes for mediating resistance and markers in these genes were tested for association with FEC in a larger Scottish Blackface cohort.

2. Materials and methods

2.1. Ethical approval

The animal procedures described in this study were conducted under experimental licence from the Irish Department of Health in accordance with the Cruelty to Animals Act 1876 and the European Communities (Amendments of the Cruelty to Animals Act 1976) Regulations, 1994.

2.2. Animals

Purebred male Scottish Blackface lambs (n = 92) were sourced from the flock at the Teagasc Hill Sheep Farm, Leenane, Co. Mayo in 2010. Lambs were managed from birth on improved lowland pasture where the major nematode species is *Teladorsagia circumcincta* (B. Good, unpublished data). All lambs received an oral benzimidazole anthelmintic treatment at 5 weeks of age to control *Nematodirus battus* infection.

Flock FEC (eggs per gram (epg)) was monitored weekly, from when lambs were approximately 8 weeks of age, using the FECPAK method (Fecpak). Eggs were distinguished as *Nematodirus* spp. (FEC_{NEM}) and 'other trichostrongyles' spp (FEC_{OT}). When FEC_{OT} reached approximately 600 epg the lambs were individually sampled twice (FEC1A and FEC1B), 1 week apart, and FEC was determined for each sample using the modified McMaster method (Anon, 1986). $FEC1A_{OT}$ and $FEC1B_{OT}$ were averaged to give $FEC1_{OT}$, the first phenotypic measurement of resistance. Following FEC1B the lambs were treated with a non-persistent macrocyclic lactone (ML, Oramec, Merial Animal Health Ltd.) in accordance with manufacturer's recommendations. Flock FEC was again monitored weekly until FEC_{OT} reached approximately 600 epg when 2 more FEC (1 week apart) per individual were completed (FEC2A and FEC2B) and the average computed to generate $FEC2_{OT}$, the second phenotypic measurement of resistance. This cohort of animals constituted grazing group 1. This process was replicated in 2011 with male (n = 76) and female (n = 90) lambs in grazing groups 2 and 3, resulting in 2 phenotypic FEC measurements from 258 animals which were used for genetic association studies.

2.3. Experimental infection

For the animals born in 2010 (n = 92), individual animal values for $\ln(FEC_{OT} + 25)$ were used to identify the most resistant (subsequently known as "LowFEC") and susceptible (subsequently known as "HighFEC") lambs, using mixed model procedures (SAS® v9.1). Data for each natural infection ($FEC1_{OT}$ and $FEC2_{OT}$) were analysed separately using a model that included rearing type (single or twin)