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Genetic profile of adaptive immune traits and relationships with parasite resistance and productivity in Scottish Blackface sheep

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ABSTRACT

Gastrointestinal (**GI**) parasites cause significant production losses in grazing ruminants which can be mitigated by breeding animals resistant to disease. Lymphocyte cytokine production and parasite-specific Immunoglobulin A (**IgA**) are adaptive immune traits associated with immunity to GI parasites. To explore the utility of these traits for selective breeding purposes, this study estimated the genetic parameters of the immune traits in sheep and assessed their relationship with disease and productivity traits. Whole blood stimulation assays were performed on 1 040 Scottish Blackface lambs at two months of age in 2016–2017. Blood was stimulated with either pokeweed mitogen (**PWM**), a non-specific activator of lymphocytes, and *Teladorsagia circumcincta* (**T-ci**) larval antigen to activate parasite-specific T lymphocytes. The type of adaptive immune response was determined by quantifying production of cytokines interferon-gamma (**IFN-γ**), interleukin (**IL**)-4, and IL-10, which relate to T-helper type (**Th**) 1, Th2 and regulatory T cell responses, respectively. Serum T-ci specific IgA was also quantified. Heritabilities were estimated for each immune trait by univariate analyses. Genetic and phenotypic correlations were estimated between different immune traits, and between immune traits vs. disease and productivity traits that were recorded at three months of age. Disease phenotypes were expressed as faecal egg counts (**FEC**) of nematode parasites (Strongyles and Nematodirus), faecal oocyst counts (**FOC**) of coccidian parasites, and faecal soiling score; production was measured as lamb live weight. Significant genetic variation was observed in all immune response traits. Heritabilities of cytokine production varied from low (0.14 ± 0.06) to very high (0.77 ± 0.09) and were always significantly greater than zero ($P < 0.05$). IgA heritability was found to be moderate (0.41 ± 0.09). Negative associations previously identified between IFN-γ production and FOC, and IL-4 production and strongyle FEC, were not evident in this study, potentially due to the time-lag between immune and parasitology measures. Instead, a positive genetic correlation was found between FOC and PWM-induced IFN-γ production, while a negative genetic correlation was found between FOC and T-ci induced IL-10. Live weight was negatively genetically correlated with IFN-γ responses. Overall, IFN-γ and IL-4 responses were positively correlated, providing little evidence of cross-regulation of Th1 and Th2 immunity within individual sheep. Furthermore, T-ci specific IgA was highly positively correlated with PWM-induced IL-10, indicating a possible role for this cytokine in IgA production. Our results suggest that while genetic selection for adaptive immune response traits is possible and may be beneficial for parasite control, selection of high IFN-γ responsiveness may negatively affect productivity.

Implications

Recently, adaptive immune traits based on cytokine production from stimulated blood have been associated with resistance to strongyle and coccidian parasites in sheep. This study estimated genetic parameters of these traits alongside parasite-specific Immunoglobulin A, and their association with parasitology and production parameters. All immune traits exhibited low–high heritabilities. However, expected associations between immune and parasitology traits were not observed, potentially due to the timing of immune and parasitology measures. Furthermore, interferon-γ production was negatively associated with live weight. This study has identified immune traits which may be useful for selective breeding; however, such selection should consider both parasite resistance and productivity.

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Introduction

Gastrointestinal (**GI**) infections with coccidian (protozoan) and nematode parasites pose a serious constraint on sheep production. Ranging from subclinical weight loss to lethal pathologies, GI infections result in sub-optimal performance which has been estimated to cost for the British farming industry around £84 million, making it the most costly disease in ruminant farming (Nieuwhof and Bishop, 2005; Charlier et al., 2014). Infections with these parasites are widespread in grazing sheep, and co-infection with coccidian and nematode parasites is common (Burgess et al., 2012). While both types of parasite infect the GI tract, there are considerable differences in their interaction with the host, with single cell coccidian parasites infecting and replicating in epithelial cells and larger multicellular nematode parasites residing within the GI lumen or closely associated with the GI mucosa (Chartier and Paraud, 2012; McRae et al., 2015).

The success or failure of immune response of the host to infection depends on a range of different factors, such as pathogen burden and the scale of the immune response itself, with the latter being regulated by the activity of T-helper cells (London et al., 1998). These cells are recognised as key coordinators of the adaptive immune response which recognises and specifically responds to pathogens (Eagar and Miller, 2019). Two of the most important subsets of T-helper cells are T-helper type (**Th**) 1 and Th2, from which naïve T cells differentiate following antigen presentation. Different functions are associated with Th1 and Th2: while the former are primarily involved in inflammatory responses and controlling intracellular pathogens, the latter are mainly involved in inducing humoral responses, typically to extracellular pathogens (Mosmann et al., 1986). Among the cytokines involved in Th1 immune responses are interferon-gamma (**IFN-γ**), interleukin (**IL**)-2, and Tumour Necrosis Factor-alpha, whereas Th2 immunity is associated with production of IL-4, IL-5, IL-9 and IL-13 (Zhu and Paul, 2010). In the context of ovine GI parasite infections, coccidian parasites are thought to be controlled by Th1 immune responses (Engwerda et al., 2014), whereas Th2 cells play a key role in controlling parasitic nematode infections (McNeilly and Nisbet, 2014).

Of critical importance to the adaptive immune response is another subset of T cells, referred to as regulatory T cells (**Treg**). Treg are responsible for regulating the immune responses by preventing or inhibiting immune responses, partially through production of inhibitory cytokines such as IL-10 and Transforming Growth Factor-beta. Treg play a key role in preventing over-activation of the immune response and subsequent immunopathology (Thornton, 2010), but may also be actively induced by certain parasitic ovine nematodes as part of their immune evasion strategy (Grainger et al., 2010).

These three Th subtypes (Th1, Th2 and Treg) are characterised by the secretion of key prototypic cytokines following T cell activation. One of the main cytokines produced by Th1 cells is IFN-γ, which is recognised as a key limiting factor in coccidian infections (Ovington et al., 1995; Ozmen et al., 2012). A direct role for this cytokine in coccidian immunity is through classical activation of macrophages which clear the parasite within the intestinal mucosa via phagocytosis (Taubert et al., 2009). This cytokine also promotes Th1 differentiation and is an inhibitor of Th2 cell proliferation (O'Shea et al., 2019).

A key cytokine involved in Th2 immune responses against nematodes is IL-4 (McNeilly and Nisbet, 2014). This cytokine is produced by Th2 polarised cells and is involved in promoting antibody responses and B cell class switching to immunoglobulin (**Ig**) E. It also serves as an inhibitor of Th1 immunity and classical macrophage activation while promoting the Th2 responses through a positive feedback loop (O'Shea et al., 2019).

Initially believed to be produced by Th2 cells (Fiorentino et al., 1989), IL-10 has been shown to have an immunomodulatory role, controlling and mediating inflammatory responses during infections by a wide range of pathogens including protozoa and nematodes (Ng et al., 2013) but also key in controlling autoimmune diseases and allergy (Hawrylowicz, 2005). This cytokine exerts an antagonistic effect on Th1 and Th2, affecting both the innate and adaptive responses (Haritova and Stanilova, 2012). IL-10 is able to actively supress IFN-γ induced macrophage activity against both intracellular and extracellular pathogens allowing their survival (Gazzinelli et al., 1992). In addition to immune-regulatory functions, IL-10 is also involved in promotion of plasma cell differentiation and antibody production (Maseda et al., 2012). Parasite-specific antibodies, in particular IgA, are also key effectors of the immune response against gastrointestinal (**GI**) parasites (de la Chevrotière et al., 2012). IgA is the most abundant antibody isotype at mucosal surfaces and nematode-specific IgA is known to be linked to resistance to nematodes in sheep, being associated with reduced worm size and fecundity in natural infections (Stear et al., 1995).

Recently, we have shown that independent of age, sex, and each other, production of IL-4 from T cell mitogen-stimulated whole blood negatively predicted Gl nematode faecal egg count (**FEC**) in a wild population of Soay sheep, while production of IFN-γ negatively predicted coccidian faecal oocyst count (**FOC**) (Corripio-Miyar et al., 2022). Additionally, in our previous study in Scottish Blackface sheep, we found significant positive genetic correlations between FEC and FOC (Pacheco et al., 2021), suggesting that Th1 and Th2 responses may also be positively correlated at the genetic level. Together, these data suggest that Th1 and Th2 immune traits derived from circulating lymphocytes may be useful selection markers for co-selection of resistance to both coccidian and nematode parasites. As cellular immune traits can be obtained from routine blood samples and have been shown to be repeatable in other species (Denholm et al., 2017), these may be more useful selection markers than faecal parasitology measures, which are variable over time and can be difficult to record at scale. However, while parasite-specific IgA is known to be moderately heritable in sheep, the genetics underlying variation in different types of Th immune responses and how these relate to productivity and disease is currently unknown.

The aims of the present study were to (i) evaluate T cell cytokine production from whole blood (as a measure of Th polarisation) and nematode parasite-specific IgA levels in lambs, (ii) examine the host genetic background for these traits and (iii) assess the relationship of immune traits with animal disease and production traits. In this regard, in an extension to preliminary results presented in abstract form (Pacheco et al., 2019), we determined the immune status of more than 1 000 pedigree sheep, estimated the amount of genetic variance between animals and the trait heritability, and derived genetic and phenotypic correlations with parasitic infection phenotypes and live weight of animals.

Material and methods

Animals and traits

Blood samples for cellular and serum antibody analyses were collected from a total of 1 040 Scottish Blackface lambs at two approximately months of age, on average, and born in 2016 and 2017, belonging the SRUC experimental hill farm flock, Midlothian, Scotland. Faecal samples were collected from the same individuals one month later for welfare reasons, as collection of both blood and faecal samples at the same time was considered to result in

unacceptable harm in lambs of this age. Animals were managed in typical hill farm conditions throughout the year and continually exposed to natural GI infections. The flock has been continually monitored since 1990 for aspects of performance and health from which several genetic studies have been published (e.g. Conington et al., 2001; Lambe et al., 2014). The flock was split into distinct selection lines: 'Selection', 'Control' and 'Industry' lines. The Selection and Control lines were selected based on the selection index described by Conington et al. (2001) as high- or average-performing, respectively. The Industry line was selected based on visual appearance, disregarding performance data. The population includes a subgroup of lambs that were born from a selection of ewes from across the three previously mentioned genetic lines that were mated to bought-in rams, linking the flock with the Scottish Blackface industry breed improvement programme.

At the first sampling time-point (2-mo of age), whole blood stimulation assays were used to characterise the adaptive immune response traits of this flock in response to Pokeweed mitogen (**PWM**) and the common GI nematode *Teladorsagia circumcincta* (**T-ci**) by measuring release of the cytokines IFN-γ, IL-4, and IL-10, which relate to Th1, Th2 and Treg, respectively. PWM is a mitogenic lectin which stimulates B and T lymphocytes irrespective of antigenic specificity (Janossy and Greaves, 1971), while *T. circumcincta* somatic antigen from fourth stage larvae (**Tci-L4**) was used to activate parasite-specific lymphocytes. Levels of *T. circumcincta*-specific IgA in serum were also quantified by ELISA. Additionally, at the second sampling time-point (3-mo of age), individual animal data were collected on faecal counts of Strongyle ($\mathbf{FEC_S}$) and Nematodirus ($\mathbf{FEC_N}$) eggs and Coccidia oocysts (FOC), along with records on faecal soiling (dagginess (**DAG)** score) and live weight (**LWT**) as described in (Pacheco et al., 2021). Designations for the different cytokines and respective stimulants are shown in Table 1.

Whole blood stimulation assays

Blood was collected aseptically into serum and lithium heparin vacutainers (Becton Dickinson, Oxford, UK) by jugular venepuncture. Whole blood stimulation assays were carried out by mixing 100 µl of whole blood with 100 µl of complete medium [RPMI-1640 (Gibco, ThermoFisher Scientific, UK) supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin and 50 µM 2-mercaptoethanol (all from Sigma-Aldrich, UK)] containing 10 µg/ml final concentration of PWM, 5 µg/ml T-ci-L4 or phosphate buffered saline (**PBS**) as control to account for any non-specific cytokine secretion. Samples were plated in triplicate in tissue culture grade round bottom 96-well plates (Corning Costar, Sigma-Aldrich, UK). The plates were then incubated at 37 °C with 5% of CO_2 in air for 48 h. After the incubation period, plates were spun at 1500 rpm for 5 min and supernatants stored at −20 °C for cytokine analysis.

Cytokine ELISA

Capture ELISAs were performed to examine the secretion of IFN-γ, IL-4 and IL-10, following stimulation with PWM or T-ci-L4. All incubations were carried out at room temperature unless stated otherwise. IL-4 and IFN-γ were quantified using commercial ELISA kits according to the manufacturer's instructions (MABTECH AB, Augustendalsvägen, Sweden). Mouse monoclonal anti-bovine IL-10 capture and detection antibodies (clones CC318 and CC320b, respectively, BioRad, UK) and standard curves produced using supernatants from COS-7 cells transfected with bovine IL-10 (Kwong et al., 2002) were used to quantify IL-10 secretion. Washing steps for all ELISAs were performed six times with 350 µl washing buffer (PBS + 0.05% Tween20) using a Thermo Scientific Wellwash™ Versa (ThermoFisher Scientific). High-binding capacity ELISA plates (Immunolon™ 2HB 96-well microtiter plates, ThermoFisher Scientific) were incubated with coating antibodies overnight at 4 °C. Plates were then washed and blocked for 1 h with PBS containing 0.05% Tween 20 (Sigma-Aldrich, UK) and 0.1% BSA Bovine Serum Albumin (BSA, Sigma-Aldrich, UK) for IL-4, IFN-γ or PBS containing 3% of BSA for IL-10. Following a further washing step, 50 µl of supernatants or standards were added in duplicate for 1 h. Subsequently, plates were washed and detection antibodies added for 1 h. This was followed by washing and addition of Streptavidin-HRP (Dako, Agilent, Santa Clara, US) for 45 min. After the final washing step, SureBlue TMB substrate (Insight Biotechnology, London, UK) was added and the reaction was stopped by the addition of TMB stop solution (Insight Biotechnology, London, UK). Absorbance values were read at O.D. 450 nm. Standard curves were included in all plates and were constructed using seven serial dilutions of recombinant cytokines ranging from 400 to 6.25 pg/ml for IFN- γ (MABTECH AB); 2 000 to 62.5 pg/ml for IL-4 (MABTECH AB) and 13.2 to 0.206 BU/ml for IL-10 (Kwong et al., 2002).

Table 1
Cytokine and stimulant combination designations for sheep whole blood stimulation assays.

Stimulant	Cytokine	Designation
Pokeweed mitogen (PWM)	IFN-γ	$\text{IFN-}\gamma_{PWM}$
	IL-4	IL-4_{PWM}
	IL-10	IL-10_{PWM}
T. circumcincta L4 antigen (T-ci-L4)	IFN-γ	$\text{IFN-}\gamma_{T\text{-}ci}$
	IL-4	$\text{IL-4}_{T\text{-}ci}$
	IL-10	$\text{IL-10}_{T\text{-}ci}$

Abbreviations: IFN-γ = interferon-gamma; IL = interleukin.

Ovine T. circumcincta-specific immunoglobulin A ELISA

Indirect ELISAs were carried out to detect antigen-specific T-ci IgA in serum samples. Briefly, high-binding capacity ELISA plates (Immunolon™ 2HB 96-well microtiter plates, ThermoFisher Scientific) were incubated with 5 µg/ml of parasite antigen (*T. circumcincta* L3 somatic antigen in 0.5 M bicarbonate buffer, pH 9.6) at 4 °C overnight. Washing steps were carried out as detailed for cytokine ELISAs and incubations carried out at 37 °C unless stated differently. Following overnight incubation, plates were washed and blocked with 200 µl of blocking buffer (PBS + 3% fish gelatin, Sigma-Aldrich, UK) for 1 h. Following a further washing step, sera samples diluted 1:4 in dilution buffer (PBS + 0.5% Tween80 + 0.5 M NaCl) were added in duplicate and incubated for 1 h. Each plate also included a positive control serum sample. Following washing, 100 µl of 1:15 000 polyclonal rabbit anti-ovine IgA conjugated to horse radish peroxidase (AHP949P, BioRad, UK) was added to all wells and incubated for 1 h. After a final wash, 100 µl of TMB substrate (TMB substrate kit, ThermoFisher Scientific) was added and reaction stopped after 5 min by the addition of TMB stop solution provided within the TMB substrate kit. Absorbance values were read at OD 450 nm. All values were then normalised using the positive control.

Data analysis

Preliminary statistical analyses were carried out to determine significant fixed effects affecting the immunological traits of study. A stepwise backward elimination approach was followed, leaving only significant fixed effects in the model. Fixed effects with *P*-values <0.05 were included in the final model for each trait. Significant fixed effects for each immunological trait are summarised in Table 2, with estimates and SE for significant fixed effects shown in

Table 2
Details of significant fixed effects and *P*-values for sheep immunological traits.

Fixed effect	IFN-γ_{PWM}	IL-4_{PWM}	IL-10_{PWM}	IFN-$\gamma_{T\text{-}ci}$	IL-$4_{T\text{-}ci}$	IL-$10_{T\text{-}ci}$	IgA
Year	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	n.s.
Sex	n.s.	<0.001	<0.001	n.s.	n.s.	n.s.	n.s.
Birth-rearing rank	n.s.	<0.001	n.s.	0.047	n.s.	n.s.	n.s.
Grazing location	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.007
Lamb age at recording	<0.005	n.s.	<0.001	0.033	n.s.	n.a.	<0.001
Dam age	n.s.	n.s.	n.s.	n.s.	n.s.	0.004	0.042
Year/grazing location interaction	<0.001	n.s.	<0.001	0.003	n.s.	n.s.	0.002

Abbreviations: IFN-γ_{PWM}, IL-4_{PWM}, IL-10_{PWM} = production of interferon-gamma (IFN-γ), interleukin (IL)-4 and IL-10, respectively, following stimulation of whole blood with pokeweed mitogen; IFN-$\gamma_{T\text{-}ci}$, IL-$4_{T\text{-}ci}$, IL-$10_{T\text{-}ci}$ = production of IFN-γ, IL-4 and IL-10, respectively, following stimulation of whole blood with *T. circumcincta* L4 antigen; IgA = serum *T. circumcincta* L3 – specific immunoglobulin A; n.s. = non-significant.

Supplementary Table S1. Subsequently, the following mixed model was used to derive (co)variance components and genetic parameters:

$$y = X\beta + Za + e,$$

where

- y is the trait record of each animal (IFN-γ, IL-4, IL-10 and IgA)
- β is the vector of significant fixed effects
- a is the vector of additive genetic effects, including the animal pedigree
- e is the vector of random residual effects
- X and Z are the design matrices linking records to fixed and random effects, respectively.

The pedigree in this population included 6 458 animals in total and all of these individuals were included in the respective relationship matrix. The categorical fixed effects used in this study included sex of the animal, grazing location birth-rearing rank (single or twins), year of birth, genetic line, and age of dam at parturition. The number of animals per class of categorical fixed effect is shown in Table 3. Age of lambs at the time of sampling was fitted as a covariate. When appropriate, significant interactions were also fitted. Immunological data were log-transformed (Log + 1) prior to all analyses in order to ensure normality of distribution.

Univariate analyses were first performed for each immunological trait separately in order to derive estimates of genetic variance. Trait heritability estimates were then derived as the proportion of phenotypic variance explained by genetic variation between individuals (random effect in model above). Subsequently, bivariate analyses were conducted in order to estimate the genetic and phenotypic correlations among immune traits, and between immune traits and disease (FEC_S, FEC_N, FOC, DAG) and production (LWT) traits (Pacheco et al., 2021). All analyses above were performed using ASReml v3.0 (Gilmour et al., 2009) software.

Results

Heritability estimates of cytokine expression from univariate analyses (Table 4) varied considerably between cytokine types and the stimulation assay. Cytokine expression varied considerably between cytokines analysed and stimulation protocol used, ranging from 0.14 ± 0.06 and 0.77 ± 0.10, corresponding to IL-4_{PWM} and IL-$4_{T\text{-}ci}$, respectively. All estimates were significantly greater than zero ($P < 0.05$). Average heritability estimates from bivariate analyses are also presented in Table 4 and were shown to be broadly similar (ranging from 0.14 ± 0.06 and 0.77 ± 0.10, corresponding to IL-4_{PWM} and IL-$4_{T\text{-}ci}$, respectively). Additional details on bivariate estimates of heritability are provided in Supplementary Tables S2–S4.

Table 3
Number of individual sheep per categorical fixed effect.

Fixed effect	Level	Number of individuals
Year of birth	2016	578
	2017	562
Sex	Male	539
	Female	501
Genetic line	Selection	320
	Control	309
	Industry	313
	L	96
Age of dam (years)	2	274
	3	309
	4	286
	5	169
	n.a.	2
Birth-Rearing Rank	11 – single F/reared as single	439
	21 – single M/reared as single	73
	22 – single M/ reared as twin	506
	29 – single M/artificially reared	2
	31 – twin born/reared as single	6
	32 – twin born/reared as twin	13
	n.a.	1
Grazing location	111 – Castlelaw Heft hill	133
	112 – Castlelaw Howgate hill	117
	113 – Castlelaw Front hill	66
	122 – Castlelaw Front field	41
	123 – Castlelaw Dipper field	119
	124 – Castlelaw First paddock	66
	127 – Castlelaw West-park	209
	601 – Castlelaw Hill 4	212
	n.a.	17

Abbreviations: n.a. = data not available; L = subgroup of lambs born from a selection of ewes mated to bought-in rams linking the flock with the Scottish Blackface industry breed improvement programme (https://signetdata.com/technical/genetic-notes/hill-sheep-breeding-index/); M = male; F = female.

Table 4
Immune trait heritability estimates (h^2) and their respective SE (in brackets) for Scottish Blackface lambs.

Traits	[1]Univariate h^2	[2]Avg. Bivariate h^2
IFN-γ_{PWM}	0.33 ± 0.10	0.31 ± 0.10
IL-4_{PWM}	0.77 ± 0.09	0.71 ± 0.10
IL-10_{PWM}	0.16 ± 0.07	0.26 ± 0.09
IFN-$\gamma_{T\text{-}ci}$	0.27 ± 0.08	0.23 ± 0.07
IL-$4_{T\text{-}ci}$	0.14 ± 0.06	0.11 ± 0.06
IL-$10_{T\text{-}ci}$	0.22 ± 0.08	0.25 ± 0.09
IgA	0.41 ± 0.09	0.34 ± 0.10

Abbreviations: IFN-γ_{PWM}, IL-4_{PWM}, IL-10_{PWM} = production of interferon-gamma (IFN-γ), Interleukin (IL)-4 and IL-10, respectively, following stimulation of whole blood with pokeweed mitogen; IFN-$\gamma_{T\text{-}ci}$, IL-$4_{T\text{-}ci}$, IL-$10_{T\text{-}ci}$ = production of IFN-γ, IL-4 and IL-10, respectively, following stimulation of whole blood with *T. circumcincta* L4 antigen; IgA = serum *T. circumcincta* L3 – specific immunoglobulin A.
[1] Univariate h^2 = heritability estimates ± SE from univariate analyses.
[2] Avg. Bivariate h^2 = average heritability estimates ± SE from bivariate analyses.

Table 5 summarises estimates of genetic correlations ($\mathbf{r_G}$) between traits. We found a strong and positive r_G between FOC and IFN-γ_{PWM} (0.67 ± 0.30), and a stronger, but in this case, negative r_G between FOC and IL-$10_{T\text{-}ci}$. Live weight (LWT) had an overall negative r_G with IFN-γ, with significant negative genetic correlations between LWT and both IFN-γ_{PWM} and IFN-$\gamma_{T\text{-}ci}$. Overall, Th1 and Th2 were positively genetically correlated (0.57 ± 0.15 between IFN-γ_{PWM} and IL-4_{PWM}; 0.74 ± 0.21 between IFN-$\gamma_{T\text{-}ci}$ and IL-$4_{T\text{-}ci}$; and 0.50 ± 0.15 between IFN-$\gamma_{T\text{-}ci}$ and IL-4_{PWM}). We also found some evidence of genetic correlations between Th2 and regulatory immune responses (0.53 ± 0.23 between IL-10_{PWM} and IL-$4_{T\text{-}ci}$). Finally, IgA was moderately correlated with IL-4_{PWM} (0.32 ± 0.17) and strongly correlated with IL-10_{PWM} (0.85 ± 0.17).

In the case of phenotypic correlations (Table 6), we found no significant correlations between immune and disease traits, but there was evidence of antagonism between IFN-γ_{PWM} and LWT (−0.09 ± 0.04), and a positive association between IL-10_{PWM} and LWT (0.10 ± 0.04), although these correlations were weak. There was also evidence that Th1 and Th2 responses were positively correlated at the phenotypic level. IgA was found to be positively correlated with all cytokines released following polyclonal T cell activation with PWM at the phenotypic level.

Discussion

Our results show that there is significant heritable genetic variability in all immunological traits investigated in this study, suggesting that individual animals vary in their genetic capacity to mount adaptive immune responses under similar conditions of natural parasite infection. Heritability estimates for *T. circumcincta*-specific IgA were similar to those reported previously (Stear et al., 1995; Fairlie-Clarke et al., 2019), whereas significant heritability estimates reported for the cellular (cytokine) traits have not been previously reported in sheep. Contrary to our expectations that Th1- and Th2-immunity would negatively regulate each other, we found that Th-1- and Th-2-associated cytokine measures were favourably correlated. We found no evidence of any association between any of the immune measurements and nematode FEC at either the genetic or phenotypic levels but did see some significant associations between IFN-γ and IL-10 release and FOC at the genetic level (correlations of 0.67 ± 0.30 between FOC vs. IFN-γ_{PWM} and −0.84 ± 0.31 between FOC and IL-$10_{T\text{-}ci}$). Importantly, significant antagonistic genetic correlations were found between IFN-γ production and LWG, suggesting that selection for higher IFN-γ production, for example to increase resistance to intracellular pathogens, would come with productivity costs.

While there has been limited information on the genetic control of cytokine production in sheep, a number of similar studies have been performed in humans which have reported varying levels of genetic control dependant on the specific cytokine and the type of immune response measurement. For example, studies in humans have shown that serum levels of Th-associated cytokines can be moderately heritable, with heritability estimates of 0.49, 0.22 and 0.46 for IL-4, IL-10 and IFN-γ, respectively (Brodin et al., 2015). In other studies in humans, heritability estimates of IFN-γ release from stimulated blood leukocytes range from 0.0 to >0.9, depending on the bacterial, fungal or other immune stimulus used (Li et al., 2016). Interestingly, in this latter study, heritability of IFN-γ release from T cell mitogen-stimulated whole blood, analogous to the PWM assay employed in this study, was estimated to be ∼0.47, which is similar to our estimate of 0.33 ± 0.10 for IFN-γ_{PWM} in the present study.

In ruminant livestock, genetic studies of cellular traits have generally focused on the total numbers of different blood leukocyte populations, or the proportions of these different cell types within the total leukocyte population. These studies have identified moderate heritability estimates for T cell subsets in cattle of 0.46 and 0.41 for % CD4+ and CD8+ T cells, (Denholm et al., 2017) and between 0.22 and 0.5 for total blood lymphocytes (Leach et al., 2013; Chinchilla-Vargas et al., 2020). While such studies point to significant genetic control of T cell numbers, they do not consider functional diversity within the T cell populations. A more functional approach to cellular immune trait analysis in cattle has focused on measuring immune responses following immunisation with antigens known to induce either Th1 or Th2 polarised responses (Thompson-Crispi et al., 2012). Genetic analysis of these responses indicated that both Th-1 and Th-2 responses were moderately heritable, ranging from 0.16 to 0.38. Taken together, studies in humans and cattle indicate a key role for host genetics in controlling adaptive immune responses, which is also supported by the moderate to high heritability estimates for Th-associated cytokine release reported in our study.

In the present study, blood leukocytes were stimulated with either a polyclonal T cell mitogen (PWM) or a nematode parasite antigen (Tci-L4), to phenotype total and parasite-specific circulating T cell populations, respectively. Our results identified a significant but weak phenotypic correlation between the two stimuli within each cytokine and no genetic correlation, giving confidence that polyclonal and antigen-specific cytokine release assays were evaluating distinct T cell phenotypes and were under different genetic control. This may be due to differences in the mechanism of cellular activation between the two stimuli, with PWM

Table 5
Genetic correlation estimates between immunological traits, disease traits (FEC_S, FEC_N, FOC and DAG) and production traits (LWT) and the respective SE (in brackets) in Scottish Blackface lambs.

	IFN-γ_{PWM}	IL-4_{PWM}	IL-10_{PWM}	IFN-$\gamma_{T\text{-}ci}$	IL-$4_{T\text{-}ci}$	IL-$10_{T\text{-}ci}$	IgA
FEC_S	−0.20 (0.34)	−0.18 (0.24)	0.01 (0.33)	−0.27 (0.33)	0.01 (0.40)	−0.16 (0.34)	−0.17 (0.32)
FEC_N	−0.16 (0.43)	0.17 (0.33)	−0.16 (0.44)	0.02 (0.40)	0.01 (0.51)	0.20 (0.42)	0.29 (0.40)
FOC	0.67 (0.30)*	−0.17 (0.27)	0.51 (0.41)	−0.28 (0.35)	−0.09 (0.48)	−0.84 (0.31)*	0.59 (0.39)
DAG	0.10 (0.38)	0.39 (0.24)	−0.43 (0.34)	0.34 (0.33)	0.31 (0.46)	−0.03 (0.40)	0.27 (0.31)
LWT	−0.54 (0.18)*	−0.11 (0.18)	0.03 (0.26)	−0.51 (0.20)*	−0.26 (0.32)	0.02 (0.27)	−0.07 (0.25)
IFN-γ_{PWM}	–	–	–	–	–	–	–
IL-4_{PWM}	0.57 (0.15)*	–	–	–	–	–	–
IL-10_{PWM}	0.36 (0.28)	0.23 (0.29)	–	–	–	–	–
IFN-$\gamma_{T\text{-}ci}$	0.19 (0.25)	0.50 (0.15)*	−0.22 (0.24)	–	–	–	–
IL-$4_{T\text{-}ci}$	−0.06 (0.33)	0.41 (0.26)	−0.53 (0.23)*	0.74 (0.21)*	–	–	–
IL-$10_{T\text{-}ci}$	0.00 (0.27)	0.03 (0.20)	−0.37 (0.26)	0.25 (0.25)	0.01 (0.35)	–	–
IgA	0.39 (0.23)	0.32 (0.17)*	0.85 (0.17)*	−0.06 (0.25)	−0.15 (0.32)	0.43 (0.23)	–

Abbreviations: FEC_S = strongyle faecal egg count; FEC_N = Nematodiris faecal egg count; FOC = faecal coccidian oocyst count; DAG = faecal soiling score; LWT = Live weight; IFN-γ_{PWM}, IL-4_{PWM}, IL-10_{PWM} = production of interferon-gamma (IFN-γ), Interleukin (IL)-4 and IL-10, respectively, following stimulation of whole blood with pokeweed mitogen; IFN-$\gamma_{T\text{-}ci}$, IL-$4_{T\text{-}ci}$, IL-$10_{T\text{-}ci}$ = production of IFN-γ, IL-4 and IL-10, respectively, following stimulation of whole blood with *T. circumcincta* L4 antigen; IgA = serum *T. circumcincta* L3 specific immunoglobulin A.

* Estimates statistically different from zero ($P < 0.05$).

Table 6
Phenotypic correlation estimates between immunological traits, disease traits (FEC_S, FEC_N, FOC and DAG) and production traits (LWT) and the respective SE (in brackets) in Scottish Blackface lambs.

	IFN-γ_{PWM}	IL-4_{PWM}	IL-10_{PWM}	IFN-$\gamma_{T\text{-}ci}$	IL-$4_{T\text{-}ci}$	IL-$10_{T\text{-}ci}$	IgA
FEC_S	−0.04 (0.04)	−0.04 (0.04)	−0.03 (0.04)	−0.05 (0.03)	−0.04 (0.03)	−0.03 (0.04)	0.00 (0.04)
FEC_N	0.04 (0.04)	−0.06 (0.04)	−0.05 (0.03)	−0.06 (0.03)	−0.04 (0.03)	−0.03 (0.03)	−0.03 (0.04)
FOC	−0.01 (0.04)	−0.05 (0.04)	0.04 (0.04)	0.03 (0.04)	−0.02 (0.03)	−0.01 (0.04)	0.03 (0.04)
DAG	0.02 (0.04)	0.06 (0.04)	0.01 (0.03)	0.04 (0.03)	0.03 (0.03)	−0.02 (0.03)	0.05 (0.04)
LWT	−0.09 (0.04)*	0.07 (0.04)	0.10 (0.04)*	0.01 (0.04)	−0.02 (0.36)	0.05 (0.04)	−0.04 (0.04)
IFN-γ_{PWM}	–	–	–	–	–	–	–
IL-4_{PWM}	0.32 (0.04)*	–	–	–	–	–	–
IL-10_{PWM}	−0.03 (0.04)	0.13 (0.04)*	–	–	–	–	–
IFN-$\gamma_{T\text{-}ci}$	0.24 (0.04)*	0.31 (0.04)*	−0.03 (0.04)	–	–	–	–
IL-$4_{T\text{-}ci}$	0.08 (0.04)*	0.18 (0.04)*	−0.04 (0.04)	0.34 (0.03)*	–	–	–
IL-$10_{T\text{-}ci}$	−0.01 (0.04)	0.06 (0.04)	0.18 (0.04)*	0.24 (0.03)*	0.06 (0.03)	–	–
IgA	0.08 (0.04)*	0.14 (0.04)*	0.11 (0.04)*	0.02 (0.04)	0.07 (0.04)	0.07 (0.04)	–

Abbreviations: FEC_S = strongyle faecal egg count; FEC_N = Nematodiris faecal egg count; FOC = faecal coccidian oocyst count; DAG = faecal soiling score; LWT = Live weight; IFN-γ_{PWM}, IL-4_{PWM}, IL-10_{PWM} = production of interferon-gamma (IFN-γ), Interleukin (IL)-4 and IL-10, respectively, following stimulation of whole blood with pokeweed mitogen; IFN-$\gamma_{T\text{-}ci}$, IL-$4_{T\text{-}ci}$, IL-$10_{T\text{-}ci}$ = production of IFN-γ, IL-4 and IL-10, respectively, following stimulation of whole blood with *T. circumcincta* L4 antigen; IgA = serum *T. circumcincta* L3 specific immunoglobulin A.
* Estimates statistically different from zero ($P < 0.05$).

activation a result of direct binding to T cell surface molecules (Yokoyama et al., 1977), whereas antigen-specific activation requiring presentation of processed antigen via major histocompatibility complex molecules (Mallone and Nepom, 2004).

Contrary to our expectations that Th1 and Th2 immune responses would negatively regulate each other (Cox, 2001), we found significant positive correlations between Th1 and Th2 cytokine measures (IFN-γ and L-4, respectively) at both the phenotypic and genetic levels. Correlations were stronger at the genetic level where moderate to strong correlations were seen (r_G = 0.74 between IFN-γ_{TCI} and IL-4_{TCI}), indicating Th1 and Th2 traits were partially under the same genetic control. Antagonism between Th1- and Th2-immunity is well established in laboratory immunology (Kaiko et al., 2008); however, we have seen similar strong positive associations between Th1 and Th2 cytokine responses in the St. Kilda Soay sheep population using the same whole blood stimulation assays (Corripio-Miyar et al., 2022), and work in wild rodent populations has found synergistic rather than antagonistic associations between Th1 and Th2 phenotypes (Arriero et al., 2017; Young et al., 2020). One explanation for this observation is that the immune response is highly compartmentalised, meaning that while local antagonism between Th1- and Th2-immunity within specific anatomical locations may exist, animals may be able to mount different types of Th response at different sites of infection (Kelly et al., 1991). Furthermore, challenge with a variety of intra- and extracellular pathogens, and the need for plasticity in the immune response could lead to genetic selection of individuals better able to mount effective immune responses to different types of parasites.

We found a moderate negative r_G between IL-10_{PWM} and IL-$4_{T\text{-}ci}$ (−0.53 ± 0.23), which might be indicative of the regulatory function of IL-10. Evidence has shown that nematodes are able to initiate the expansion of immune-regulatory cells that act on supressing both Th1 and Th2 immune responses in order to promote their survival in the host (Turner et al., 2008, McNeilly et al., 2013). We also found a strong positive genetic correlation between IL-10_{PWM} and *T. circumcincta*-specific IgA (0.85 ± 0.17), which is consistent with data from humans and mice showing that IL-10 can promote IgA class switching and production (Cerutti, 2008).

In the present study, we found a significant negative correlation between IL-10_{TCI} and FOC at the genetic level, and while this was partly unexpected as IL-10 has been shown to interfere with immunity to coccidian parasites due to its immuno-regulatory effects (Ozmen et al., 2012), studies in mice and chickens have also shown a positive association between resistance to coccidian parasites and IL-10 production (Wakelin et al., 1993; Boulton et al., 2018). Contrary to our previous study (Corripio-Miyar et al., 2022) in which IFN-γ and IL-4 were negatively correlated with FOC and FEC_S, respectively, this study found no significant negative correlations between these cytokine measures and parasite egg/oocyst counts. Indeed, IFN-γ_{PWM} was significantly positively correlated with FOC at the genetic level (0.67 ± 0.30). However, in the previous study, parasitology measures were recorded at the same time as the cytokine measures whereas in this study, parasitology measures were recorded one month after cytokine analysis. Furthermore, in our previous study, immune phenotyping of lambs occurred at around 4 months of age, whereas in this study, lambs were around 2 months old, an age at which immunity to GIN, and in particular nematode parasites, is not fully developed (McRae et al., 2015; Greer and Hamie, 2016). It is also known that the immune response to GI parasites following initial exposure in lambs is highly dynamic and involves a complex interplay between different types of Th response which varies over time, meaning that associations between Th responses and parasite burden may only be apparent at specific time-points post-infection (Hassan et al., 2011; Liu et al., 2022). Thus, the lack of and/or unexpected associations between our Th1 and Th2 cytokine measurements with the parasitology data may be due to either immune phenotyping lambs at an age before anti-parasite immunity has fully developed, or due to the time-lag between the immune and parasitology measures. This would also explain the lack of association between FEC_S and IgA in this study: while IgA is a trait which has been consistently shown to be negatively associated with FEC_S, sufficient parasite exposure is required prior to the IgA measurement for this association to be detectable (Shaw et al., 2012).

Given the significant genetic variance estimated in the present study and our previous observations of Th1 and Th2 traits being associated with protection against nematode and coccidian parasites, respectively (Corripio-Miyar et al., 2022), immunological traits can be included in genetic selection programmes aiming to enhance the animals' inherent resistance to parasites. In this regard, consideration should be taken of how these traits correlate with productivity traits (de la Chevrotière et al., 2012). Here, we found that IFN-γ production, both to PWM and Tci-L4, at two months of age is adversely associated with LWT at three months of age. Thus, selecting for increased IFN-γ may compromise weight and LWT, potentially through increased Th1 mediated immunopathology (Venturina et al., 2013). This is consistent with a recent meta-analysis of heritable traits associated with GI parasite resistance in sheep which concluded that adaptive immune

traits are generally negatively correlated with performance traits (Hayward, 2022). Use of selection index methodology would be advisable here to effectively combine and improve two genetically antagonistic traits. Additionally, there is some risk that selection for Th2 immune responses may affect LWG as there is a positive r_G between Th1 (IFN-γ) and Th2 (IL-4) type immunity, indicating some level of shared genetic control is shared between IL-4 and IFN-γ traits.

One limitation of this study is the relatively small sample size, which is likely to have impacted on the precision of the genetic correlations. Indeed, many of the identified correlations that looked substantial were not significant. This partly reflects the complexity of the immune trait measurements, which require fresh whole blood and considerable laboratory input. However, the sample size of this study (1 040 lambs) is similar or greater than similar studies in humans involving restimulation of blood leukocytes such as Brodin et al. (2015) and Li et al. (2016) which analysed a total of 210 and 700 individuals, respectively. Nevertheless, it would be important to validate this study on a larger number of individuals before these immune traits were considered for selective breeding purposes.

In conclusion, our results show that there is substantial genetic variability among individual lambs with regard to all immunological traits, although it is not clear if selecting for these traits is favourable regarding live weight. Our results shed light on the complex mechanism of the adaptive immune response in growing lambs. Firstly, we found evidence that both Th1 and Th2 immune responses are partially under the same genetic control, demonstrating the lack of a clear Th1/Th2 dichotomy. Furthermore, consistent with other studies in non-laboratory settings, there was no marked biased polarisation towards a specific immune response. Additionally, there is evidence to suggest that Th1 immune responses at 2 months of age could be impacting the capacity for the animal to gain weight, translating in animals with lower weights at 3 months. Our results form the basis of future studies that continue to build upon the groundwork laid here, including exploring the timing of adaptive immune and parasitology trait measurements, and their association with parasite resistance and productivity. This is particularly important as this study involved a relatively small sample size, which may have influenced the precision of our heritability and correlation estimates. Importantly, these results do not support genetic selection on single immune traits, which may result in unexpected negative consequences such as reduced LWT. Instead, immune traits should be considered as part of a comprehensive selection index including other animal traits of interest.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2023.101061.

Ethics approval

All experiments were approved by the SRUC Animal Experiments Committee and were performed according to Home Office Guidelines under Project Licence numbers 60/4358 and P90111799.

Data and model availability statement

The genetic model used for the analyses was not deposited in an official repository. Data on animal performance, pedigree and health traits are maintained in a secure SQL database at SRUC and are available upon request from the corresponding author.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence-assisted technologies in the writing process.

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Declarations of interest

None.

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