Local Immune Response of Canarian Majorera Goats Primary Infected With *Teladorsagia circumcincta*

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Abstract

Due to increased anthelmintic resistance, alternative methods to drugs are necessary to control gastrointestinal nematodes (GIN). Some of the most promising alternatives for their sustainability are based on the immune response of the host, such as the selection of genetically resistant breeds or the use of vaccines against these parasites. Given the limited information available on the immune response against GIN in goats, this study was carried out on the local immune response of goat kids of a Canary Islands indigenous breed (Majorera breed) experimentally infected with *Teladorsagia circumcincta*, one of the most pathogenic and prevalent GIN species.

For this purpose, the relationship between different parasitological and immunological parameters at local level (both related to the humoral and cellular immune responses) have been analyzed at early (1 week post-infection –wpi-) and late (8 wpi) stages of infection.

Primoinfection of goat kids with *T. circumcincta* L3 generates a complex immune response that could be defined as Th2 type, characterized by an increase of infiltration in abomasal tissues of several effector cells as well as a progressive presence of specific antibodies against parasitic antigens in the gastric mucus. These cellular responses could be evidenced from 1 wpi onward, showing an increase in the gastric mucosa of antigen presenting cells and various lymphocyte subsets. The complexity of the responses developed is evidenced by the statistically significant changes in numbers of all these subpopulations, as well as in the evolution of the relative cytokine gene expression.

From a functional point of view, negative associations were observed between the number of most of these cells (CD4, IgA, IgG, and CD45R cells) and parameters that could be related to the fecundity of worms, a phenomenon that was especially evident when the number of IgG or CD45R cells or the specific IgA levels of the mucus were compared with parasitological parameters such as the length of female worms or faecal egg counts at 8wpi.

Introduction

Gastrointestinal nematodes parasites (GIN) are responsible for important economic losses in livestock farming worldwide, mainly in extensive production systems. Among other effects, they compromise animal welfare and can reduce productivity in terms of milk production and growth rates, carcass quality and reproductive disorders [1]. Even in subclinical infections, the presence of these nematodes results in an inadequate use of feeding resources and contributes to the development of other infectious diseases. Among the various nematodes that affect the gastrointestinal tract of small ruminants, *Teladorsagia circumcincta* (*T. circumcincta*) outstands for its wide distribution and pathogenicity [2].

At present, control of these parasitic infections in ruminants relies heavily on treatments with anthelmintic drugs. However the emergence of parasite resistance to these products [3], as well as consumer concerns about the presence of chemical residues in meat and milk, have stimulated research towards new control strategies [4].

Through these alternatives, selection of resistant breeds [5], and improvement of the host immune system, by inducing protective responses [6], have been considered. Therefore, it would be of great interest to advance knowledge about the immunological mechanisms generated against ruminant GIN, information that would be very useful to optimize the development of vaccines against this group of parasites [7].

Though, there is a in increased interest in goat production as demonstrated by the continuous rise in this livestock population worldwide [8], most recent studies on the immune response to gastrointestinal nematodes have been conducted in cattle and sheep [9], and much less information is available for goats. In addition, information obtained in cattle or sheep can hardly be directly extrapolated to goats [10], due to GIN infections in goats are more severe than in other ruminants, there is a delay in the development of the immune response that makes it less effective [11, 12] and finally, different goat breeds have shown genetic variability in resistance to GIN infections [13, 14]. Therefore, it would be necessary to carry out specific studies to elucidate the immune response generated in goats against this group of nematode parasites.

Particular in goats, the GIN *T. circumcincta* constitutes one of the most relevant species due to: i) its high prevalence in many regions of the world [15, 16]; ii) its pathogenic potential also in subclinical infections [17]; iii) and its particular tendency to develop resistance to anthelmintics associated with different factors such as the off-label use of these drugs in goats [18].

Therefore, the aim of this study is contribute to the knowledge of the local immunological responses in primary infections with *T. circumcincta* in goats, as a preliminary step in the recognition of the mechanisms involved in resistance against this nematode species.

Materials And Methods

T. circumcincta strain used in this study was originally isolated from a sheep naturally infected with this nematode and, was kindly provided by Dr. Uriarte from the Centre for Agricultural Research and Technology of Aragón (CITA, Zaragoza, Spain).

Fifteen healthy goat kids (Majorera milk aptitude breed), three months old, were used in this study. These animals were reared under nematode-free conditions, until the beginning of experiment, when all goats were 6 months old. experiment started at 6 months of age. Afterward, all animals were randomly allocated in one of the following groups: group 1 (1 week infection) (n = 5), group 2 (8 weeks infection) (n = 5), group 3 (uninfected control, n = 5). Animals from groups 1 and 2 were orally inoculated with 8,000 *T. circumcincta* L3, and slaughtered at 1 (group 1) or 8 (group 2) weeks after infection (wpi) respectively. Group 3 animals were kept as uninfected controls and were also euthanized at 8 wpi.

In group 2 and 3, faecal samples were collected three times per week, from day 0 pi (post-infection) to the end of the study (8 wpi), to determine fecal egg counts.

At the end of the study, abomasa of all the animals were removed to perform parasitological (immature and adult worms counts), immunological (specific antibody levels in gastric mucus and antigenic recognition of proteins from *T. circumcincta* adult worms by immunoblot) as well as histological and immunohistochemical analysis. In addition, samples were also collected from gastric mucosa and abomasal lymph nodes to analyze the relative expression of cytokines in both tissues.

Parasitological Analysis

Fecal egg counts were determined by the modified McMaster technique [19] and expressed as number of eggs per gram of faeces (EPG). At the end of the experiment, the abomasa of all the animals were washed with distilled water, and 200 mL samples were collected and preserved with formalin to determine the number of immature or adult worms (males and females). Thirty adult female worms from each animal were measured with a calibrated ocular scale, and the number of intrauterine eggs per female was microscopically determined. Finally, immature worm burden was established by digestion of mucosal scrapings with pepsin-HCl at 37 °C. Digestion was stopped with formalin, aliquots of the digestion mix were examined to calculate the number of immature worms per gram of mucosa [20].

Analysis of Humoral Responses in Mucus

The antigen used to analyse the humoral responses was obtained from adult worms collected from the abomasum of monospecific infected donor animals. The worms were homogenized on ice with a homogenizer (Ultra-Turrax T8, IKA® WERKE) in a solution of PBS (0.01M) containing EDTA (1mM) and phenylmethylsulfonyl fluoride (PMSF) (10mM) (Sigma-Aldrich, USA). The homogenate was centrifuged at 5000 x *g* for 20 min at 4 °C and the supernatant, containing the soluble somatic antigen, was filtered and its protein concentration determined using a colorimetric method (Pierce BCA Protein Assay, ThermoFisher Scientific, USA).

Mucus samples used to determine the levels of specific local immunoglobulins, were obtained by superficial scraping of the abomasal mucosa and diluted in a buffer containing proteinase inhibitors (0.1M sodium phosphate, 0.05M sodium chloride, 3mM sodium acid, 1mM PMSF and 5mM EDTA; pH 7.1) at a rate of 2.5 mL buffer/gram of mucus. Samples were centrifuged at 18 000 x *g* for 30 minutes at 4 °C and supernatant used for the determination of specific antibody levels [21].

ELISA test

Optimal test conditions were established based on the results obtained from two pools of positive and negative mucus samples. The final concentrations of somatic antigen used for the determination of specific IgG or IgA levels in mucus was 3.0 µg/mL or 5.0 µg/mL respectively. Samples were diluted (1:100 -IgG- or 1:25 -IgA-) in PBS, and conjugate (anti-goat IgG-peroxidase, -Sigma-Aldrich Inc., USA- or anti-goat IgA-peroxidase, -Acris GmbH, Germany- was diluted in PBS and used at a 1:1000 or 1:5000 dilution respectively.

A citric acid-phosphate buffer containing 0.04 % (w/v) OPD and 0.1% (v/v) H₂O₂ was used as substrate. All samples were analyzed in duplicate, and optical densities (OD) were determined at a wavelength of 492 nm (Thermo Labsystems, Multiskan Ascent 354, USA) [21].

Immunoblot

Somatic antigens of *T. circumcincta* adult worm were fractionated by SDS-PAGE into a 12% (w/v) acrylamide gel under non-reducing conditions. The protein fractions were then electrotransferred onto a 0.22-μm-pore nitrocellulose filter membrane (Pure Nitrocellulose Blotting Membrane, Bio Trace™ NT, Life Sciences, USA), which was subsequently blocked with a 3% (w/v) solution of bovine serum albumin in PBS. After washing nitrocellulose strips with PBS-Tween 20 at 0.05% (w/v), they were subjected to an immuno-enzymatic reaction as previously described [22], in which gastric mucus samples were analyzed (diluted 1:1 in PBS). A 1/1000 dilution in PBS of the same conjugates used in ELISA test was employed. The immunorecognition was evidenced by incubation in a solution containing the chromogen 3-amino-9-ethylcarbazole (AEC Staining Kit, Sigma-Aldrich, USA), following the manufacturer's instructions.

Histology and Immunohistochemistry

Tissue samples from abomasal mucosa were cut (4 μm thick) and stained with Giemsa and hematoxylin-eosin stain in order to determine the number of eosinophils, globule leukocytes and mast cells. Cell counts were carried out at a 500x magnification in 40 randomly selected fields of 0.044 mm², at the upper and lower third of the mucosa. Eosinophils and globule leukocytes were counted in the hematoxylin-eosine stained sections and mast cells in the Giemsa sections. The results were expressed as number of cells/mm² [23].

For immunohistochemical analysis, sections (4 μm thick) from the abomasal mucosa were transferred to poly-L-lysine hydrobromide (Sigma-Aldrich Inc., USA) covered slides. Primary monoclonal antibodies against CD4, CD8, CD45R, γδ, MHCII and WC-1 lymphocytes were diluted at 1:15, 1:15, 1:5, 1:10, 1:20 and 1:5, respectively in 20 % v/v foetal bovine serum in PBS. Polyclonal anti-goat IgG (Vector Labs., USA) and anti-human IgA (Agilent Technologies, USA) sera were used at a dilution of 1:5000 and 1:500 respectively. All samples were incubated for 90 min at 25 °C. Positive reactions were demonstrated by incubation with biotinylated rabbit anti-mouse immunoglobulins (Agilent Technologies, USA) diluted 1:20 in RPMI medium (Sigma-Aldrich Inc., USA) and a solution of avidin-biotin peroxidase (ABC) complex at a 1:100 dilution in PBS. Finally, slides were reacted with 0.035% (w/v) 3–3'-diaminobenzidine tetrahydrochloride (Sigma-Aldrich) containing 0.01 % (v/v) hydrogen peroxide. Counterstaining was performed using Harris' hematoxylin stain, and immunoreactive cells were counted in 40 fields located in upper and lower third of the mucosa [21].

Determination of Relative Cytokines Gene Expression by Real Time PCR (RT-PCR)

Abomasal lymph node (approximately 0.5 g) and abomasal mucosa samples (0.5x1x1 cm) were preserved in Trizol (TRI-Reagent, Sigma-Aldrich, USA) at -80°C prior to RNA isolation. Total RNA extraction

was performed using a double phenolic extraction technique [24]. To eliminate possible contamination with genomic DNA, the samples were treated with DNase (RQ1 RNase-Free DNase Promega, USA). RNA samples were quantified using a spectrophotometer (NanoDrop-1000 Spectrophotometer, ThermoFisher Scientific, USA) at 260 nm, and their purity was estimated by determining the OD ratio 260/280 nm, while RNA integrity was determined by means of the RNA6000 Pico Kit (Agilent Technologies, USA).

cDNA synthesis was performed on a total volume of 20 µL, using 1 µg of total RNA and a reverse transcriptase kit following the manufacturer's instructions (iScript™ cDNA Synthesis Kit, BioRad Lab., USA). The resulting cDNA was diluted in 80 µl of DNA-free water (dilution 1:5) before amplification.

RT-PCR was performed using the GoTaq® qPCR Master Mix kit containing BRYT Green™ dye as fluorophore (Promega, USA). The reaction mixture was prepared according to the manufacturer's instructions, however, for most assays a higher concentration of magnesium chloride (MgCl₂) was used (Table 1). The amplification process was performed on an iCycler thermal cycler (BioRad, USA) fitted with a MyiQ™ Single Color Real-Time PCR Detection System. Process monitoring was carried out using the iQ5 Optical System Software Version 2.0 (BioRad, USA). Quantification was performed after 45 denaturation cycles at 94°C for 15 seconds, annealing at 61°C for 20 seconds and elongation at 72 °C for 15 seconds.

Table 1

Sequence of primers used in qPCR (Accession Number/Reference), size (in base pairs –bp-), melting temperatura (in °C –T_m-) of amplified products, and MgCl₂ concentrations used in each reaction.

	Primers 5' a 3'	Size (bp)	MgCl₂ (mM)	T_m (°C)	Accession Number/Ref.
B-act	CCAACCGTGAGAAGATGACCC CCCAGAGTCCATGACAATGCC	122	5	85	AF481159
IL-2	GTGAAGTCATTGCTGCTGGA TGTTCAAGTTTTTGCTTGGA	202	3	81	Craig et al., 2007
IL-4	GCTGGTCTGCTTACTGGTATG CGATGTGAGGATGTTTCAGC	100	5	80	FJ936316
IL-10	GTGGAGCAGGTGAAGAGAGTC TGGGTCGGATTTTCAGAGG	198	3	82	AF458378
INFγ	AGATAACCAGGTCATTCAAAGGAG GGCGACAGGTCATTCATCAC	180	3	82.5	U34232
IL-17	TGCTACTGCTTCTGAGTCTGGTGGC TGACCCTCACATGCTGTGGGAAGTT	111	0	83.5	Yan et al., 2011

The sequence of the primers used for the evaluated cytokines gene expression (IL-2, IL-4, IL-10, INF- γ and IL-17) and β -actine (housekeeping gene), the size and melting temperature (T_m) of the amplified products, as well as the concentration of $MgCl_2$ used in each reaction are detailed in Table 1. A relative quantification of gene expression was performed following the method $\Delta\Delta C_t$ [25]. The data were normalized using β -actine gene as housekeeping gene.

Statistical analysis

Data were analyzed statistically using IBM SPSS Statistics software for Windows, version 22.0 (IBM Corp. USA). The differences between the experimental groups in single day parameters were determined using the nonparametric Mann-Whitney U test. The Spearman correlation test was used to analyse the the association between different parameters assessed in the study. Probabilities of $p < 0.05$ were considered as significant.

Results

Parasitological Analysis

Infected animals slaughtered at 1 wpi (group 1) showed counts of $1,450 \pm 228.1$ larvae (mean $\pm$ SEM) in the gastric contents, with a mean length of 3.14 ± 0.22 mm. After digestion of the gastric mucosa, 77 ± 33.4 larvae/gram of mucosa were detected.

In group 2, slaughtered at 8 wpi, the mean value ($\pm$ SEM) of adult worms was $2,039 \pm 480.75$, of which 63% were female worms which showed a mean length ($\pm$ SEM) of $8.32 \text{ mm} \pm 0.63 \text{ mm}$, as well as a mean number of intrauterine eggs ($\pm$ SEM) of 9.03 ± 2.1 . Number of larvae in the mucosa was 6.6 ± 1.6 larvae/gram. Correlation analysis by Spearman's test (r) of all these data, showed a noticeable trend of negative association between the number of worms in the gastric content with their length ($r = -0.7$) and the number of intrauterine eggs ($r = -0.5$), although a positive association between length and number of intrauterine eggs showed a statistical significance ($r = 0.9$; $p < 0.05$).

The prepatent period of experimental infection in group 2 was 25 days post-infection (dpi). However, one of the animals showed fecal egg counts later on, in day 35 post-infection. The maximum mean value was reached at 47 dpi (620 epg) showing a mean FEC of $370 \text{ epg} \pm 80.5$, at the end of the experiment (Fig. 1).

Analysis of Humoral Responses in Mucus

Specific IgA anti-T. circumcinta

At a local level, the presence of specific IgA in the gastric mucus was much more evident in infected animals in which the worms reached the adult stage (group 2), when compared to group slaughtered at one wpi (group 1) and the uninfected control group (Fig. 2). Mucus samples from group 1 and 3, developed a similar and poor antigenic recognition against somatic proteins of adult worms by

immunoblot. In contrast, in animals slaughtered at 8 wpi (group 2), IgA anti-*T. circumcincta* developed a strong reaction against antigenic fractions with molecular weights of approximately 22, 39, 50, 84 and > 120 kDa (Fig. 3). In this group 2, the levels of specific antibodies of the IgA isotype present in the gastric mucus were negatively associated with the length of the female worms and the fecal counts of eggs at the end of the study ($r = -0.9$; $p < 0.01$).

Specific IgG anti-*T. circumcincta*

As described for IgA response to somatic antigens, the highest specific IgG levels were detected in samples obtained from group 2 (8 wpi), while animals from group 1 and uninfected controls showed a poor specific response (Fig. 2). These levels of mucus-specific IgGs in group 2 were negatively associated with worm length and fecal egg counts at slaughter ($r = -0.6$), but were highly variable within the group, so no statistical significance could be proven. Despite relatively high levels of specific IgG isotype recorded in group 2, recognition by immunoblot could only be clearly evidenced with protein fractions with a molecular weight of approximately 39 kDa.

Histological and Immunohistochemical Analysis

Infected groups 1 and 2 showed a mean number of eosinophils infiltrated in the gastric mucosa clearly higher than the uninfected control group, but those differences did not reach statistical significance. The counts of globule leukocytes and mast cells were also higher in the gastric mucosal from animals of the infected groups ($p = 0.06$) (Fig. 4). It was also remarkable the negative correlation observed between the number of globule leukocytes and the length of immature worms in the animals from group 1 (1 wpi) ($r = -0.894$; $p < 0.05$). However, no statistical association could be established between the number of eosinophils and mast cells and the parasitological parameters studied.

The immunohistochemical analysis showed that the monoclonal antibodies used displayed immunoreactivity with different cell subpopulations of the abomasal mucosa of goat kids. In both infected groups (groups 1 and 2), all the subpopulations analyzed were increased with respect to the control group, with statistical significance in the number of WC1, CD4⁺, MHCII⁺ and IgG⁺ cells in group 1, and $\gamma\delta$ ⁺, WC1⁺ and IgG⁺ cells in group 2 (Fig. 5).

The analysis of the correlations between these subpopulations and different parasitological parameters showed that there was a relation between the presence of high number of worms in the abomasums and the infiltration of various cellular subsets in the gastric mucosa. Thus, significant positive associations could be observed ($p < 0.05$) between the number of IgA⁺ cells and the number of immature worms at 1 wpi, as well as between CD4⁺, $\gamma\delta$ ⁺ and IgG⁺ cells and female worms at 8 wpi. In addition lymphocyte populations CD4⁺, $\gamma\delta$ ⁺, IgG⁺, IgA⁺ and CD45R⁺ were negatively associated with the length of the worms (both at 1 or 8 weeks p. i.) as well as the fecal egg counts (at 8 weeks p. i.), although these correlations only showed statistical significance in the group 2 when the relationship between FEC and IgG⁺ ($r = -0.9$; $p < 0.05$) or CD45R⁺ ($r = -0.9$; $p < 0.01$) lymphocytes were determined.

Relative Cytokines Gene Expression

All experimental groups showed detectable levels of gene transcription of IL-2, IL-4, IL-10, IL-17 and INF γ , in both lymph nodes and gastric mucosa, and although some trend in the relative gene expression between infected and control groups was observed, these differences did not reach statistical significance. In particular, it was remarkable the up-regulation of IL-4- and IL-17-gene transcription (4-fold and 6-fold respectively, in comparison with controls) which was detected in gastric lymph nodes one wpi (group 1), whereas this increased gene expression was not detected once the adult worms had developed (group 2). In this latter group, only a slight increase in INF- γ - gene transcription (2-fold in relation with control group) was detected in the gastric mucosa.

Discussion

Despite the homogeneity of the animals included in the different experimental groups in terms of age, sex, breed, origin or feeding, the different parasitological parameters analyzed in the present study showed a high variability, as it has been observed in other experimental infections with *T. circumcincta* in canarian goats [22, 26, 27]. Variability within a group was reflected by high SEM values in some of the parameters assessed, such as larvae and adult worm counts at the end of the experiment, indicating that immunoprotection against the parasite may differ among individuals, as it has been reported in some ovine breeds infected with *T. circumcincta* [28–30]. This finding has also been described in other goat breeds infected by different GIN species [31], which would indicate that important individual factors are involved on the natural defense mechanisms developed against GIN in goats.

Similarly, the prepatency period in our study was approximately 25 dpi, which seems to indicate a slight delay in larval development compared to sheep, which has also been observed in other trials in goats using other isolates of *T. circumcincta* [10], a phenomenon which could be linked to the use of sheep-adapted strains in both cases. The prepatent period reached 35 days in one of the experimentally infected animals, furthering the idea of the presence in this animal population of a different degree of natural resistance that could be manifested by a delay in the endogenous cycle of the parasite.

Among the mechanism associated with resistance to *T. circumcincta* infections, at least in sheep, is the reduction in FEC, mainly linked to decreased female fecundity rather than number of parasites [32]. In our study, the fecundity was assessed by the intrauterine egg counts of the female worms, which showed a logical negative correlation with the length of these worms, a feature found in other studies for this parasitic species [29], and the faecal egg counts observed at the end of the experiment. All of those parasitological parameters were negatively associated with the specific humoral immune response of the two analyzed isotypes (IgG and IgA), pointing to the possible role of the humoral response in the fecundity regulation of *T. circumcincta* in goats. Negative correlations reaching statistical significance when analyzing the association between specific IgA levels and length of females.

This predominant role of IgA in resistance to infection against *T. circumcincta* has been also observed in sheep infected with this parasite [33–36]. In sheep have also shown that some of those protective responses are associated with an increased antigenic recognition of parasite proteins by IgA immunoglobulins [37]. The prominent IgA level in the gastric mucus in the current trial could be related with the higher number of antigenic fractions recognized, in relation with IgG, shown by means immunoblot analyses. Accordingly, no association between IgG in mucus and parameters related to resistance to parasite development could be evidenced. The scarce local humoral response detected at the earliest stages of the infection, which does not seem to play a relevant protective role, supports the lack of previous sensitization of the animals.

The humoral response observed in the gastric mucus would match with a Th2 immune response, which is considered a common feature in GIN infections. In group 2, during the 8 weeks of the study, a development of this type of response characterised by an increase of antibodies of different isotypes (including IgA, IgG) at local level (as can be seen when comparing these parameters between infected animals and non-infected controls). This type of immune response is usually accompanied by increased mucosal eosinophil infiltration, as well as mast cell and globule leukocyte hyperplasia [38], also observed in this experimental group 2, where the increase in tissue eosinophil counts was close to statistical significance, although no relationship could be established of this tisular eosinophilia as a defensive mechanism against the parasite, in contrast to reports in *T. circumcincta* infected sheep [39].

The increase of effector cells, such as eosinophils and mast cells in the gastric mucosa, has been related as mechanisms of resistance against GIN [40]. Both cell populations increased in our study, especially at 8 weeks p.i. The increase in the number of these effector cells in the gastric mucosa one wpi was also observed and could be associated with innate defensive mechanisms [41]. Mast cells and eosinophils were also rapidly increased in the abomasal mucosa from goats primoinfected with *H. contortus* [42, 43]. However, these authors could not determined an increase in the number of globule leukocytes in the early stages as it was observed in our study. This increase shown an interesting negative association with the length of the immature worms, whose potential role as a natural resistance mechanism in this goat breed should be evaluated [44]. Eosinophil infiltration, although enhanced throughout the study -a phenomenon associated with a certain degree of resistance to *H. contortus* infection in some sheep breeds [23]-, could not be associated with any effect on the parasitological parameters investigated.

The present results also showed a rapid recruitment of lymphocyte subpopulations in the abomasal mucosa of goats primarily infected with *T. circumcincta*, similar to those found in goats infected with other gastric nematodes, such as *H. contortus* [45]. This increase, already observed in animals one week after experimental infection, was associated with the number of worms and with cell types involved in Th2 responses against GIN in sheep, such as antigen presenting cells (MCHII⁺) and CD4⁺ lymphocytes [46, 47]. This celular filtration, from a functional point of view, highlights a negative association between several populations such as CD4⁺, $\gamma\delta$ ⁺, IgA⁺, IgG⁺ and CD45R⁺, and fecundity reflected by a reduction in female worm length and FEC, as observed when the local humoral immune response was analyzed. The presence of a significant CD8⁺ lymphocyte infiltrate observed here was also previously detected in goats

and sheep infected with *H. contortus* from 7–10 days post-infection, but similar to what occurs in our study, no relationship could be established with the parasitological data [23, 45].

The infiltration of $\gamma\delta$ + lymphocytes and their WC1 + subpopulation in the gastric mucosa from the first week of infection was also evident here. These cells constitute an important percentage of lymphocytes in goats, especially in young animals [48], and are considered to have important defensive functions, playing a relevant role in the interrelation between innate and adaptive responses against bacteria, viruses, and protozoan parasites, but there is not much information available on its functionality against nematode parasites in ruminants [49]. As it has been observed in goats primarily infected by *H. contortus* [45], this cell population also displayed an increase in the gastric mucosa from the first wpi., showing a negative association with the length of immature worms (group 1) and female worms (group 2). This finding has also been observed in canary sheep resistant to *H. contortus*, a phenomenon associated, as in current trial, to the local humoral response developed by specific IgA antibodies [23] as it was previously discussed. However, this observation contrasts with results derived from sheep infected with *T. colubriformis*, where no significant defense mechanisms attributed to $\gamma\delta$ + cells could be identified [50], which could indicate differential biological functions of this cell type on the basis of parasite species, tisular location and/or host particularities.

Although cytokine expression in lymph nodes and gastric mucosa in infected and control animals was not conclusive, a rapid onset in IL-4 expression was observed -as previously described in sheep [51]-, a cytokine that could stimulate B-cell proliferation and infiltration of CD4, MCH-II and IgG cells in the gastric mucosa, as observed in our study. Regarding the observed increase in IL-17 expression, although it is a cytokine for which not much information is yet available on its role against ruminant GIN, it appears to have an important regulatory function in the defensive responses necessary to maintain immune tolerance [52].

When the experimental infection progressed, although a humoral response could be detected and some effector cell subpopulations were increased, the gene expression of mediators of the immune response was even less evident than during the first week of infection. Thus, only a slight increase in the relative expression of INF- γ in the gastric mucosa of group 2 compared to control group was observed. This finding, which has also been reported in the course of *H. contortus* or *O. ostertagi* infection in goats and calves, respectively, could be contradictory if we take into account the inhibitory effect of INF- γ on Th2 responses [53]. Such observation has been explained as the beginning of the shift towards an antagonistic Th1 response, which could occur in susceptible animals to GIN after a period of development of Th2 responses, as a result of a mechanism of evasion of the immune response [54–56], or even the expression of a mixed Th1/Th2 response that could be effective against GINs [57]

In conclusion, the primoinfection of goat kids with *T. circumcincta* L3 generates a complex immune response that could be defined as Th2 type, characterized by an increase of infiltration in abomasal tissues of several effector cells (eosinophils, mast cells and globule leukocytes) as well as a progressive presence of specific antibodies against parasitic antigens in the gastric mucus. These cellular responses

could be evidenced from 1 wpi onward, showing an increase in the gastric mucosa of antigen presenting cells (MCHII+) and various lymphocyte subsets (CD4, CD8, $\gamma\delta$, CD45R, IgA, and IgG). The complexity of the responses developed is evidenced by the statistically significant changes in numbers of all these subpopulations, as well as in the evolution of the relative cytokine gene expression observed in the abomasum and abomasal lymph nodes at 1 week and 8 weeks p.i.

From a functional point of view, negative associations were observed between the number of most of these cells (CD4, IgA, IgG, and CD45R cells) and parameters that could be related to the fecundity (such as the length of female worms or FEC at the end of the experiment), a phenomenon that was especially evident when the number of IgG or CD45R cells or the specific IgA levels of the mucus were compared with parasitological parameters such as the length of female worms or FEC at 8 wpi.

Although more studies (including a higher number of experimental animals and serial infections) would be necessary, all this information should be taken into account to evaluate alternative control strategies against *T. circumcincta* based on the host immune response of the host, such as selection programs for resistant goat breeds or vaccination protocols against this nematode species.

Declarations

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Authors' contributions

AR and JMM participated in the design of the study and JMM coordinated it. LO and JQ carried out the experimental infection and sampling. LO, JQ, AR, MMCF, OF and JMM carried out parasitological, humoral and cytokine gene expression assays. LO, JQ, MMCF, OF and FR with post-mortem procedures as well as the histological and immunohistochemical analysis. All authors participated in data analysis. LO, AR, MMCF, OF, FR and JMM drafted the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Experiments were approved by the Animal Welfare Ethics Committee of the Universidad de Las Palmas de Gran Canaria and from the local authorities, following the rules of the Spanish (RD 53/2013) and European (Directive 2019/63/EU) legislation.

Competing interests

The authors declare that they have no competing interests.

References

- [1] Taylor MA, Coop RL, Wall RL. Parasites of sheep and goats (Parasites of the digestive system). In: Taylor MA, Coop RL, Wall RL, editors. *Veterinary Parasitology*, 4th Edition. New Jersey, USA: John Wiley & Sons, Ltd; 2016. p. 436-474.
- [2] Stears MJ, Boag B, Cattadori I, Murphy L. Genetic variation in resistance to mixed, predominantly *Teladorsagia circumcincta* nematode infections of sheep: from heritabilities to gene identification. *Parasite Immunol.* 2009;31:274-82.
- [3] Papadopoulos E, Gallidis E, Ptochos S. Anthelmintic resistance in sheep in Europe: a selected review. *Vet. Parasitol.* 2012;189:85–8.
- [4] Vande Velde F, Charlier J, Claerebout E. Farmer behavior and gastrointestinal nematodes in ruminant livestock—Uptake of sustainable control approaches. *Frontiers in Veterinary Science* 2018;5:255.
- [5] González JF, Hernández A, Molina JM, Fernández A, Raadsma HW, Meeusen EN et al. Comparative experimental *Haemonchus contortus* infection of two sheep breeds native to the Canary Islands. *Vet. Parasitol.* 2008;153:374-8.
- [6] Stears MJ, Doligalska M, Donskow-Schemelter K. Alternatives to anthelmintics for the control of nematodes in livestock. *Parasitol.* 2007;134:139-51.
- [7] Miller JE, Horohov DW. Immunological aspects of nematode parasite control in sheep. *J. Anim. Sci.* 2006;84:124-32.
- [8] FAOSTAT. Food and Agricultural Organization of the United Nations (FAO) data set. 2020. Available at <http://www.fao.org>
- [9] Jackson F, Miller J. Alternative approaches to control—quo vadit? *Vet. Parasitol.* 2006;139:371–84.
- [10] Macalodowie C, Jackson F, Huntley J, McKellar A, Jackson E. A comparison of larval development and mucosal mast cell responses in worm-naïve goat yearlings, kids and lambs undergoing primary and secondary challenge with *Teladorsagia circumcincta*. *Vet. Parasitol.* 2003;114:1-13.

- [11] Hoste H, Torres-Acosta JF, Aguilar-Caballero AJ. Parasite interactions in goats: is immunoregulation involved in the control of gastrointestinal nematodes? *Parasite Immunol.* 2009;30:79–88.
- [12] Hoste H, Sotiraki S, Landau SY, Jackson F, Beveridge I. Goat-nematode interactions: think differently. *Trends Parasitol.* 2010;26:376–81.
- [13] Mandonnet N, Aumont G, Fleury J, Arquet R., Varo, H, Gruner L et al. Assessment of genetic variability of resistance to gastrointestinal nematode parasites in Creole goats in the humid tropics. *J. Animal Scien.* 2001;79:1706–12.
- [14] Onzima RB, Mukiibi R, Ampaire A, Benda KK, Kanis E. Between-breed variations in resistance/resilience to gastrointestinal nematodes among indigenous goat breeds in Uganda. *Trop. Anim. Health. Prod.* 2017; 49:1763–9.
- [15] Chartier C, Reche B. Gastrointestinal helminths and lungworms of French dairy goats: prevalence and geographical distribution in Poitou-Charentes. *Vet. Res. Commun.* 1992;16:327–55.
- [16] Molina JM, Gutiérrez AC, Rodríguez-Ponce E, Viera JA, Hernández S. Abomasal nematodes in goats from the subtropical island of Grand Canary (Spain). *Vet. Res.* 1997;28:259–70.
- [17] Zajac AM. Gastrointestinal nematodes of small ruminants: life cycle, anthelmintics, and diagnosis. *Vet. Clin. North. Am. Food. Anim. Pract.* 2006; 22:529–41.
- [18] Paraud C, Chartier C. Facing anthelmintic resistance in goats. In: Simões J, Gutiérrez C, editors. *Sustainable Goat Production in Adverse Environments: Volume I.* New York, USA: Springer International Publishing; 2017. p. 267-92.
- [19] Thienpont D, Rochette F, Van Parijs OFJ. Diagnosing helminthiasis by coprological examination. Beerse, Belgium: Janssen Research Foundation; 1979.
- [20] MAFF. *Manual of Veterinary Parasitological Laboratory Diagnostic Techniques.* 3rd ed. London, UK: Ministry of Agriculture, Fisheries and Food; 1989.
- [21] Molina JM, Hernández YI, Martín S, Ferrer O, Rodríguez F, Ruiz A. Immune response in goats vaccinated with thiol-binding proteins from *Haemonchus contortus*. *Parasite Immunol.* 2018;40:e12569.
- [22] Molina JM, Ruiz A, Gutiérrez AC, Rodríguez E, González J, Hernández S. Cross-reactive antigens of *Haemonchus contortus* adult worms in *Teladorsagia circumcincta* infected goats. *Vet. Res.* 1999;30:393-9.
- [23] González JF, Hernández A, Meeusen ENT, Rodríguez F, Molina JM, Jaber JR et al. Fecundity in adult *Haemonchus contortus* parasites is correlated with abomasal tissue eosinophils and $\gamma\delta$ -T cells in resistant Canarian Hair Breed sheep. *Vet. Parasitol.* 2011;78:286-92.

- [24] Sambrook J, Fritsch JF, Maniatis T. Molecular Cloning: A Laboratory Manual. New York, USA: Cold Spring Harbour Laboratory Press; 1989.
- [25] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 2001;25:402-8.
- [26] González JF, Molina JM, Rodríguez-Ponce E, Conde de Felipe MM, Ruiz A. Análisis de diversos parámetros hematológicos y parasitológicos en una infección experimental con *Teladorsagia circumcincta* en cabras de la agrupación caprina canaria. *Rev. Can. Cienc. Vet.* 2003;1:16-21.
- [27] Molina JM, Hernández Y, Ruiz A, González JF, Argüello A, Ferrer O et al. Preliminary study on the use of a *Teladorsagia circumcincta* bulk milk ELISA test in dairy goats under experimental conditions. *Vet. Parasitol.* 2009;166:228-34.
- [28] Stears MJ, Bairden K, Bishop SC, Duncan JL, Karimi SK, McKellar QA et al. Different patterns of faecal egg output following infection of Scottish Blackface lambs with *Ostertagia circumcincta*. *Vet. Parasitol.* 1995;59:29-38.
- [29] Beraldi D, Craig BH, Bishop SC, Hopkins J, Pemberton JM. Phenotypic analysis of host parasite interactions in lambs infected with *Teladorsagia circumcincta*. *Int. J. Parasitol.* 2008;38:1567-77.
- [30] Nisbet AJ, McNeilly TN, Wildblood LA, Morrison AA, Barteley DJ, Bartley Y et al. Successful immunization against a parasitic nematode by vaccination with recombinant proteins. *Vaccine* 2013;31:4017-23.
- [31] McBean D, Nath M, Kenyon F, Zile K, Bartley DJ, Jackson F. Faecal egg counts and immune markers in a line of Scottish Cashmere goats selected for resistance to gastrointestinal nematode parasite infection. *Vet. Parasitol.* 2016;229:1-8.
- [32] Stears MJ, Henderson, NG, Kerr A, McKellar QA, Mitchell S, Seeley C et al. Eosinophilia as a marker of resistance to *Teladorsagia circumcincta* in Scottish Blackface lambs. *Parasitology* 2002;124:553-60.
- [33] Strain SAJ, Bishop SC, Henderson NG, Kerr A, McKellar QA, Mitchell S et al. The genetic control of IgA activity against *Teladorsagia circumcincta* and its association with parasite resistance in naturally infected sheep. *Parasitology* 2002;124:545-52.
- [34] Halliday AM, Routledge CM, Smith SK, Matthews JB, Smith WD. Parasite loss and inhibited development of *Teladorsagia circumcincta* in relation to the kinetics of the local IgA response in sheep. *Parasite Immunol.* 2007;29:425-34.
- [35] Shaw RJ, Morris CA, Wheeler M, Tate M, Sutherland IA. Salivary IgA: a suitable measure of immunity to gastrointestinal nematodes in sheep. *Vet. Parasitol.* 2012;186:109-17.

- [36] Aboshady HM, Stear MJ, Johansson A, Jonas E, Bambou JC. Immunoglobulins as biomarkers for gastrointestinal nematodes resistance in small ruminants: A systematic review. *Nature.com/Scientific Report* 2020;10:7765.
- [37] Smith SK, Nisbet AJ, Meikle LI, Inglis NF, Sales J, Beynon RJ et al. Proteomic analysis of excretory/secretory products released by *Teladorsagia circumcincta* larvae early post-infection. *Parasite Immunol.* 2009;31:10-9.
- [38] Meeusen ENT, Balic A, Bowles V. Cells, cytokines and other molecules associated with rejection of gastrointestinal nematode parasites. *Vet. Immunol. Immunopathol.* 2005;108:121-5.
- [39] Henderson NG, Stear MJ. Eosinophil and IgA responses in sheep infected with *Teladorsagia circumcincta*. *Vet. Immunol. Immunopathol.* 2006;112:62-6.
- [40] Balic A, Bowles VM, Meeusen ENT. The immunobiology of gastrointestinal nematode infections in ruminants. *Adv. Parasitol.* 2000;45:181–241.
- [41] Hendawy SHM. Immunity to gastrointestinal nematodes in ruminants: effector cell mechanisms and cytokines. *J. Parasit. Dis.* 2018;42:471–82.
- [42] Pérez J, García PM, Hernández S, Martínez-Moreno A, de las Mulas JM, Cámara S. Pathological and immunohistochemical study of the abomasum and abomasal lymph nodes in goats experimentally infected with *Haemonchus contortus*. *Vet. Res.* 2001;32:463-73.
- [43] Pérez J, García PM, Hernández S, Mozos E, Cámara S, Martínez-Moreno A. Experimental haemonchosis in goats: effects of single and multiple infections in the host response. *Vet. Parasitol.* 2003;111:333-42.
- [44] Williams AR. Some observations on the role of bradykinin in immunity to *Teladorsagia circumcincta* in sheep. *J. Parasitol. Res.* 2012;2012:569287.
- [45] Pérez J, Zafra R, Buffoni L, Hernández S, Cámara S, Martínez-Moreno A. Cellular phenotypes in the abomasal mucosa and abomasal lymph nodes of goats infected with *Haemonchus contortus*. *J. Comp. Path.* 2008;138:102-7.
- [46] Gill, H. S., Watson, D. L. and Brandon, M. R. 1993. Monoclonal antibody to CD4+ T cells abrogates genetic resistance to *Haemonchus contortus* in sheep. *Immunology* 2001; 78:43-9.
- [47] Balic A, Bowles VM, Meeusen ENT. Mechanisms of immunity to *Haemonchus contortus* infection in sheep. *Parasite Immunol.* 2002;24:39-46.
- [48] Yirsaw A, Balwing CL. Goat $\gamma\delta$ T cells. *Dev. Comp. Immunol.* 2021;114:103809.

- [49] Guerra-Maupome M, Slate JR, McGill JL. Gamma-Delta T cell function in ruminants. *Vet. Clin. Food Anim.* 2019;35:453–69.
- [50] McClure SJ, Davey RJ, Lloyd JB, Emery DL. Depletion of IFN-gamma, CD8+ or Tcr gamma delta+ cells in vivo during primary infection with an enteric parasite (*Trichostrongylus colubriformis*) enhances protective immunity. *Immunol. Cell Biol.* 1995;73:552-5.
- [51] Craig NM, Miller HRP, Smith WD, Knight PA. Cytokine expression in naïve and previously infected lambs after challenge with *Teladorsagia circumcincta*. *Vet. Immunol. Immunopathol.* 2007;120:47-54.
- [52] McRae KM, Stear MJ, Good B, Keane OM. The host immune response to gastrointestinal nematode infection in sheep. *Parasite Immunol.* 2015;37:605–13.
- [53] Gill HS, Altmann K, Cross ML, Husband A. J. Induction of T helper 1- and T helper 2-type immune responses during *Haemonchus contortus* infection in sheep. *Immunology* 2000;99:458–63
- [54] Gasbarre LC, Leighton EA, Sonstegard T. Role of the bovine immune system and genome in resistance to gastrointestinal nematodes. *Vet. Parasitol.* 2001;98:51–64.
- [55] Muñoz-Guzmán MA, Cuéllar-Ordaz JA, Valdivia-Anda, AG, Buendía-Jiménez, J A, Alba-Hurtado F. Correlation of parasitological and immunological parameters in sheep with high and low resistance to haemonchosis. *Can. J. Anim. Sci.* 2006;86:363–71.
- [56] Nehra AK, Gowane GR, Kuriyal A, Chaurasiya A, Kumar R, Bhinsara DB et al. Immune response against subclinical haemonchosis in Himalayan hill goats. *Vet. Parasitol.* 2019;267:47-53.
- [57] Aboshady HM, Mandonnet N, Félicité Y, Hira J, Fourcot A, Barbier, C et al. Dynamic transcriptomic changes of goat abomasal mucosa in response to *Haemonchus contortus* infection. *Vet. Res.* 2020;51:44.

Figures

Figure 1

Eggs per gram of faeces (EPG) expressed as mean EPG $\pm$ SEM in goats orally infected with 8,000 *T. circumcincta* L3 (group 2).

Figure 2

Total IgG and IgA anti-T. circumcincta in mucus from groups 1, 2 (orally infected with 8,000 T. circumcincta L3 and slaughtered at 1 or 8 weeks p.i., respectively) and uninfected control group. Results are mean optical density (OD) at 492 nm ± SEM.

Figure 3

Immunorecognition of mucosal IgA of *T. circumcincta* adult worms somatic antigen in goats from group 2 (orally inoculated with 8,000 *T. circumcincta* L3) (P) and uninfected control group (C) at 8 weeks post-infection.

Figure 4

Levels of effector cells (eosinophils, mast cells and globule leucocyte counts) in the gastric mucosa in goats from groups 1, 2 (orally infected with 8,000 *T. circumcincta* L3 and slaughtered at 1 or 8 weeks p.i., respectively) and uninfected control group. Results are mean number cells/mm² ± SEM.

Figure 5

Levels of cellular subpopulations in the gastric mucosa in goats from groups 1, 2 (orally infected with 8,000 *T. circumcincta* L3 and slaughtered at 1 or 8 weeks p.i. respectively) and uninfected control group. Results are mean number cells/mm² $\pm$ SEM. *Significantly different from uninfected control group at $p < 0.05$.

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