Cellular Phenotypes in the Abomasal Mucosa and Abomasal Lymph Nodes of Goats Infected with *Haemonchus contortus*

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Summary

The distribution of T-cell subsets (CD2, CD4, CD8, and γδ) and B cells (IgM) was examined at 3, 6, 10 and 13 days post-infection (dpi) in the abomasal mucosa and abomasal lymph nodes of goats primarily infected with *Haemonchus contortus*. In the abomasal mucosa a mild (3 and 6 dpi) or marked (10 and 13 dpi) increase of T cells, particularly CD4+ and γδ+ lymphocytes, was observed, whereas the increase in CD8+ cells was less pronounced. B cells and IgG+ plasma cells also showed a marked increase in the abomasal mucosa at 10 and 13 dpi. The abomasal lymph nodes showed an increase in size, particularly at 10 and 13 dpi, and a decrease in the proportion of T cells, particularly CD8+ lymphocytes, due to the increased proportion of B cells. The proportion of CD4+ and γδ+ lymphocytes did not change significantly during the infection in the abomasal lymph nodes, but their absolute numbers were augmented as a result of the enlargement of the nodes. The results revealed a strong cellular and humoral immune response during the early post-infection stages. However, as indicated by the worm burdens, this rapid host response was unable to induce larval expulsion.

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Keywords: goats; *Haemonchus contortus*; immune response; lymphocyte subsets

Introduction

*Haemonchus contortus* is a gastrointestinal nematode of particular importance in relation to small ruminant production in warm climates. Current control measures rely mainly on chemotherapy, but the high prevalence of anthelmintic resistance in goats necessitates the development of immunological methods of control. The protective immune response of ruminants to gastrointestinal nematodes ranges from strong to weak (Gasbarre et al., 1993), and knowledge of the mechanisms on which it is based is essential for the development of effective methods of immunization (Klei, 1997; Meeusen and Piedrafita, 2003). Despite numerous studies, the mechanisms of immunity against *H. contortus* in sheep remain unclear.

Gamble and Zajac (1992) reported that the protective response in a resistant sheep breed was mediated by the proliferation of mucosal mast cells, globule leukocytes and eosinophils. A specific antibody response, represented mainly by IgG1 and IgA, was also reported in resistant sheep (Gill et al., 1993a; Schallig et al., 1995). Proliferation of CD4+ T lymphocytes appears necessary for the generation of mucosal mast cell hyperplasia, tissue eosinophilia, and specific antibody production (Gill et al., 1993b). In naive sheep, proliferation of CD4+, γδ+ lymphocytes and B cells was reported in the abomasal mucosa (Balic et al., 2002). Two effector mechanisms of resistance have been described, namely rapid rejection of incoming larvae and delayed expulsion of the parasites from their tissue niche (Balic et al., 2002); both mechanisms seem to be associated with Th2 responses (Meeusen et al., 2005; Lacroux et al., 2006).

Only a few studies have investigated the host response to gastrointestinal nematodes in goats
Immune Response of Goats to *H. contortus*

(Huntley et al., 1995; Patterson et al., 1996). Protection was induced in goats by immunization with cysteine protease-enriched protein fractions from *H. contortus* (Ruiz et al., 2004). The abomasal lymph node infiltrate in immunized kids was studied by Jasmer et al. (2003) and Perez et al. (2001, 2003) studied the local cellular infiltrates in abomasal mucosa and abomasal lymph nodes of goats infected with single or multiple doses of *H. contortus*. The aim of the present study was to analyze T lymphocyte subpopulations and B cells (IgM) in the abomasal mucosa and abomasal lymph nodes during early post-infection stages in goats primarily infected with *H. contortus*.

**Materials and Methods**

**Experimental Design**

Fifteen 5-month-old male Florida goats, recently weaned, were obtained from an *H. contortus*-free farm. They were free of gastrointestinal nematodes as judged by faecal egg counts, and were drenched with fendectomized (Panacur; Hoechst Roussel Vet, Barcelona, Spain). The animals were housed indoors under worm-free conditions and fed goat pellets and lucerne chaff. They were divided into five groups (Table 1), one of which consisted of uninfected controls. Goats of the remaining groups (1–4) were infected orally with *H. contortus* larvae (L3) of caprine origin, kindly provided by Professor Dr. J. Alunda, Veterinary Faculty of Madrid. Details of infection and times of euthanasia are given in Table 1. Goats were killed by intravenous injection of a mixture of embutramide and mebezonium iodide (T61; Hoechst Roussel Vet). All animals were subjected to necropsy, and worm counts were performed as previously described (Perez et al., 2003).

**Tissue Collection**

Samples from the abomasum (cardiac, fundic and pyloric areas) and abomasal lymph nodes were collected at necropsy. The longitudinal diameter of the abomasal lymph nodes was recorded, the results being expressed as mean ± SD. The samples were snap-frozen in OCT (Miles, Elkhart, IN, USA) by immersion in 2-methylbutane (Merk, Darmstadt, Germany) cooled in liquid nitrogen. Frozen samples were fixed for 10 min in acetone and stored at −80°C. Serial sections (7 μm) were then cut with a cryostat at −25°C and stored again at −80°C until used.

**Immunohistochemistry (IHC)**

The avidin–biotin–peroxidase (ABC) method (Perez et al., 2001) was used on snap-frozen tissue sections. Endogenous peroxidase activity was blocked by incubation with phenylhydrazine (Sigma, St Louis, MO, USA) 0.05% in Tris-buffered saline (TBS), pH 7.2. Tissue sections were incubated with 2% normal goat serum (Vector Laboratories, Burlingame, CA, USA) for 30 min at room temperature. The primary antibodies (Table 2) were then applied overnight at 4°C. A biotinylated rabbit anti-mouse IgM antibody (Dako, Glostrup, Denmark) diluted 1 in 50 was applied to sections treated with the CD4 mAb. A biotinylated goat anti-mouse immunoglobulin serum (Dako) diluted 1 in 50 was applied to sections treated with the remaining mAbs. An ABC complex (Vector) diluted 1 in 50 was used as the third reagent. Tissue sections were then incubated for 1 min with 3,3′-diaminobenzidine tetrahydrochloride (Sigma) 0.035% in TBS containing hydrogen peroxide 0.1%, rinsed in tap water, lightly counterstained with Mayer’s haematoxylin, and mounted with Immumont (Shandon, Pittsburgh, PA, USA). For negative control purposes, the primary antibodies were replaced by phosphate-buffered saline (PBS) or non-immune isotypic sera were used. Lymph nodes of the control animals were used as positive controls for all the primary antibodies.

**Cell Counting and Statistical Analysis**

Immunoreactive cells were counted in 30 fields of 0.06 mm², randomly selected, in photomicrographs of the mucosa of the abomasal fundus. In the

**Table 1**

Infection of goats with *H. contortus*: experimental design and abomasal worm burdens

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of goats</th>
<th>Interval (days) between infection and killing</th>
<th>Infective dose (L3)</th>
<th>Worm burdens (mean ± SD)</th>
<th>Establishment rate (%) *</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>3</td>
<td>10 000</td>
<td>1239 ± 168</td>
<td>12.4</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>6</td>
<td>10 000</td>
<td>956 ± 202</td>
<td>9.6</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>10</td>
<td>20 000</td>
<td>1495 ± 356.1</td>
<td>7.48</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>13</td>
<td>20 000</td>
<td>2778 ± 403.8</td>
<td>13.89</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>3, 6, 10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*No significant differences between infected groups.
†Divided into two doses of 10,000, separated by an interval of 3 days.
Results

Abomasal Mucosa

Results of the immunohistochemical study are summarized in Table 3. CD2+ T lymphocytes were located mainly in the lamina propria at the base of the abomasal glands (Fig. 1). They increased dramatically in groups 3 and 4, in which intraepithelial CD2+ T cells were seen in the glands, and numerous CD2+ T lymphocytes surrounded some larvae in the abomasal mucosa. CD4+ T lymphocytes were observed in the lamina propria, mainly at the base of the abomasal glands (Fig. 2), whereas they were absent or sparse in the neck and isthmus of the glands. Groups 3 and 4 showed a marked increase of this cell type as compared with groups 1 and 2 and control animals. CD4+ T cells were also observed in the inflammatory infiltrate that surrounded larvae within the abomasal mucosa in groups 3 and 4. These two groups showed an increase in CD8+ T lymphocytes; this increase was, however, less marked than that of CD4+ T lymphocytes. The ratio CD4/CD8 was 1.7 (control group), 1.6 (group 1), 1.8 (group 2), 2.6 (group 3) and 2.5 (group 4). Occasional γδ T lymphocytes occurred in the controls and groups 1 and 2; these cells were significantly increased in groups 3 and 4 (Fig. 3), in which they were located mainly in the body, neck and isthmus areas of the glands, both in the lamina propria and epithelium.

The anti-IgM mAb reacted with surface IgM in lymphoid cells mainly located in isolated lymphoid follicles within the abomasal mucosa. In groups 3 and 4 a few IgM+ B cells were also found in the diffuse infiltrate located in the lamina propria.

Abomasal Lymph Nodes

The longitudinal diameter of the abomasal lymph nodes was 6.9 ± 1.4 mm (control group), 9.0 ± 1.6 mm (group 1), 10.2 ± 1.9 mm (group 2), 12.1 ± 2 mm (group 3) and 14.9 ± 2.1 mm (group 4). Hyperplasia of the lymphoid follicles and medullary cords was mild (group 1) severe (group 2) or very severe (groups 3 and 4), whereas hyperplasia of paracortical areas was less evident. The proportion of the different cell subsets is summarized in Table 4. The proportion of CD2+ T lymphocytes decreased in infected goats, particularly in groups 3 and 4. However, due to the enlargement of the abomasal lymph nodes, the absolute number of T cells increased. The proportion of CD4+ T lymphocytes increased slightly in groups 1

Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>CD2</th>
<th>CD4</th>
<th>CD8</th>
<th>γδ</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.4 ± 2.1</td>
<td>2.6 ± 0.9</td>
<td>1.5 ± 0.7</td>
<td>0.3 ± 0.15</td>
<td>0.2 ± 0.16</td>
</tr>
<tr>
<td>1</td>
<td>5.2 ± 2.6</td>
<td>3.2 ± 2.3</td>
<td>2.0 ± 1.5</td>
<td>1.5 ± 0.7</td>
<td>1.2 ± 0.7</td>
</tr>
<tr>
<td>2</td>
<td>6.1 ± 3.1</td>
<td>4.2 ± 2.4</td>
<td>2.3 ± 1.3</td>
<td>1.6 ± 0.9</td>
<td>2.1 ± 0.9</td>
</tr>
<tr>
<td>3</td>
<td>25.8 ± 4.6*</td>
<td>18.3 ± 4.3*</td>
<td>6.9 ± 3.1*</td>
<td>4.5 ± 2.1*</td>
<td>19.5 ± 0.4*</td>
</tr>
<tr>
<td>4</td>
<td>31.5 ± 6.0*</td>
<td>21.7 ± 5.8*</td>
<td>8.6 ± 5.5*</td>
<td>6.2 ± 2.3*</td>
<td>20.4 ± 3.9*</td>
</tr>
</tbody>
</table>

*Significantly different (P < 0.05) from groups 1 and 2 and control group.
and 2 and decreased slightly in groups 3 and 4 as compared with the control group. However, in the light of the marked reduction in the proportion of T cells and the enlargement of the abomasal lymph nodes, the absolute number of CD4⁺ T lymphocytes showed a striking increase, particularly in groups 2, 3 and 4. In contrast, the proportion of CD8⁺ T lymphocytes was reduced in all infected groups, particularly in groups 3 and 4, as compared with the control group. As infection progressed, the CD4/CD8 ratio increased (1.4 [control group], 2.4 [group 1], 2.6 [group 2], 2.9 [group 3] and 3.0 [group 4]). The γδ⁺ T lymphocytes were sparse and their proportion did not change significantly during the infection. 

The proportion of B cells increased in the infected groups, particularly in groups 3 and 4. The anti-IgM mAb labelled mainly the lymphoid cells of the follicles and to a lesser extent those of the medullary cords, in which plasma cells were also IgM⁺.

**Discussion**

The present study revealed an infiltration of CD2⁺ T lymphocytes which was slightly greater than the infiltration of CD3⁺ T lymphocytes found in a previous study based on the same groups (Perez et al., 2003). This discrepancy may have been due to the loss of antigens resulting from formalin-fixation of tissues in the previous study. The present results showed a rapid recruitment of lymphocyte subpopulations in the abomasal mucosa and abomasal lymph nodes of goats primarily infected with *H. contortus*. In a previous histopathological study, mast cells and eosinophils also increased rapidly in the abomasal mucosa, but
globule leucocytes appeared only during the late post-infection stages (Perez et al., 2001, 2003). Sheep primarily infected with *H. contortus* also showed rapid changes in the lymphocyte populations of the abomasal mucosa and abomasal lymph nodes (Balic et al., 2000, 2002), but sheep protected by immunization against *H. contortus* did not show such changes (Gorrell et al., 1988; Balic et al., 2002), suggesting that the strong lymphocyte recruitment was induced by the presence of larvae within the mucosa.

The striking increase in CD2 T lymphocytes in the abomasal mucosa at 10 and 13 days post-infection (dpi) was due mainly to an increase in CD4 T cells. These results accord with previous histopathological studies in which the abomasal lymphocytic infiltrate increased significantly at 11 dpi in goats (Al-Zubaidy et al., 1987) and 5–12 dpi in sheep (Hunter and MacKenzie, 1982; Balic et al., 2000) infected with *H. contortus*. The marked increase in CD4+ T lymphocytes in the abomasal infiltrate at 10 and 13 dpi suggests that this cell subset plays an important role in the early host response against *H. contortus*, which accords with the results reported in sheep and goats immunized with *H. contortus* gut antigens (Karanu et al., 1997) and in sheep infected with *H. contortus* (Gill et al., 1993b; Pena et al., 2006).

CD8+ T lymphocytes did not increase in the abomasal mucosa at 3 and 6 dpi, an observation that accords with previous results in sheep (Balic et al., 2000, 2002). However, in the present study this lymphocyte subset increased significantly at 10 and 13 dpi, an observation not documented in sheep. The increase of γδ T lymphocytes in the abomasal mucosa at 10 and 13 dpi accords with the results obtained in immunized sheep infected with *H. contortus* (Balic et al., 2002) and with *Trichostrongylus colubriformis* (McClure et al., 1992). Similarly, the increase of IgM+ lymphocytes and plasma cells in the abomasal mucosa at 10 and 13 dpi accords with the reported increase of IgM+ plasma cells in the abomasal mucosa of sheep infected with *H. contortus* (Gill et al., 1992; Balic et al., 2002). In sheep, the presence of B cells in abomasal mucosa is related to the production of specific antibodies against *H. contortus*, some of which are protective (Rainbird et al., 1998; Amarante et al., 2005).

In the abomasal lymph nodes (ALNs) the increased ratio CD4/CD8 at 6 dpi, and particularly at 10 and 13 dpi, and the pronounced increase in IgM+ cells, coinciding with an abundant infiltrate of Cd79a+B cells and IgG+ plasma cells (Perez et al., 2003), suggests a strong humoral immune response, a finding also reported in *H. contortus*-infected sheep (Balic et al., 2000). Similarly, a significant increase in CD4 T lymphocytes was reported in ALNs of kids partly protected by immunization with GA1 antigen from *H. contortus* (Balic et al., 2000). In contrast, naive calves infected with *Ostertagia ostertagi* showed a marked depletion of CD2+ and CD4+ T cells in the ALNs, whereas this depletion was not observed in immunized calves (Gasharre, 1994).

In conclusion, a rapid recruitment of T cells, particularly CD4+ T lymphocytes, and B cells, occurred in the abomasal mucosa and ALNs of *H. contortus*-infected goats at 10 and 13 dpi. However, this strong local immune response failed to induce rapid expulsion of larvae. The main difference from immunized sheep was a lower CD4/CD8 ratio in the infiltrates of the abomasal mucosa and ALNs in the goats of the present study. Future studies in immunized goats should include an analysis of cytokine profiles.

**Acknowledgments**

This work was supported by CICYT (Project N. AGF96-1132) and Junta de Andalucía (AGR137).

**References**


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[Received, May 3rd, 2007]
[Accepted, November 27th, 2007]