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Intestinal infection with *Trichinella spiralis* induces distinct, regional immune responses

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ABSTRACT

The aim of this study was to evaluate differences between the small and large intestines (SI and LI) with regard to colonization and immunity during infection with *Trichinella spiralis*. In orally infected C57BL/6 mice, the gender ratios of worms differed among the SI, cecum, and LI. Mucosal mastocytosis developed in the SI but not in the LI, consistent with reduced IL-9 and IL-13 production by explants from the LI. Despite these differences, worms were cleared at the same rate from both sites. Furthermore, IL-10 production was reduced in the LI, yet it was instrumental in limiting local inflammation. Finally, passive immunization of rat pups with tyvelose-specific antibodies effectively cleared fist-stage larvae from all intestinal regions. We conclude that despite regional differences in immune responsiveness and colonization, immune mechanisms that clear *T. spiralis* operate effectively throughout the intestinal tract.

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1. Introduction

Regions of the vertebrate intestine differ with regard to size, physiology, and microbial flora. In addition, the small and large intestine (SI and LI) constitute distinct immune environments with differences in regulatory T cell phenotypes and the production of IL-10 and TGF-β (Autenrieth et al., 1997; Maynard et al., 2007). Endothelial cells displaying MAdCAM-1 recruit α4β7-expressing lymphocytes throughout the intestine, while CCR9-bearing lymphocytes are selectively recruited to the SI by locally produced CCL25/TECK (Gorfu et al., 2009). In this way, immune responses can be directed to the SI. Although many parasites are restricted in their distribution in the gastrointestinal tract, *Trichinella spiralis* colonizes both the small and large intestines (Tyzzer, 1916; Roth, 1938) providing an opportunity to investigate variation in regional immune responses and the impact this may have on infection.

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2. Materials and methods

2.1. Animals

C57BL/6^{NHsd} and BALB/c^{NHsd} were purchased (Harlan, USA) and IL-10 deficient (B6.129P2-IL10^{tm1Cgn}) mice and Albino Oxford (AO) rats were bred and maintained under specific pathogen-free conditions according to the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care. Experiments were performed with the approval of the Cornell University Institutional Animal Care and Use Committee.

2.2. Parasite and infections

Maintenance of *Trichinella spiralis* (pig strain), L_1 recovery, and intestinal worm burden estimation were carried out as described (Blum et al., 2009; Gagliardo et al., 2002). Adult mice (7–10 weeks old) or suckling rat pups (12–16 days old) received 400 or 200 L_1 by gavage, respectively. Crude L_1 parasite extract (cAg) was prepared as described (Appleton and Usack, 1993).

2.3. Tissue collection, preparation, and evaluation

Mice were euthanized with $C0_2$. The SI and LI were removed and cut longitudinally, prepared as swiss rolls (Moolenbeek and Ruitenberg, 1981), fixed in Carnoy's solution, and sectioned for staining with Alcian Blue (pH 0.4) and Nuclear Fast Red. Alternatively, tissues were fixed in formalin prior to sectioning and H & E staining. Mast cells in Alcian Blue-stained sections were estimated per crypt-villus unit (CVU) in a minimum of 50 CVU per section. Scoring of enteropathy in H & E-stained sections was as follows: epithelial hyperplasia (0–3), severity of inflammation (0–4). The sum of these two scores was multiplied by a value assigned to the distribution of inflammatory foci (0–3) for a total score ranging from 0 to 21. Severity of inflammation was defined as follows: no significant inflammation – 0; cellular infiltrate within the lamina propria, mild – 1, moderate – 2, severe and extending into the submucosa – 3; severe with crypt abscess, goblet cell depletion, and ulceration – 4. Neutrophil infiltration was given a score from 0 (no infiltration) to 3 (severe infiltration). Microscopy and image capture were performed with an Olympus BX51 microscope and DP25 camera, using Microsuite Basic Edition software.

2.4. Antibody treatment of rat pups

Monoclonal tyvelose-specific IgG1 (clone 9D4) and polyclonal IgG (nIgG) were prepared as described previously (Appleton and McGregor, 1987; Appleton et al., 1988). Rat pups were treated with 2.5 mg of antibody per 20 g of body weight by gavage, challenged 1 h later, and intestinal parasite burdens estimated after 24 and 48 h (Blum et al., 2009).

2.5. Cytokine measurement

Five-mm pieces of jejunum, ileum, or LI were weighed prior to processing for explant cultures, as described (Egan et al., 2011). Explants were cultured with 50 μg/mL of cAg for 16–18 h at 37 °C. Explant supernatants were centrifuged at 138 × *g* and assayed for IL-4, IL-5, and IL-10 by ELISA as described previously (Beiting et al., 2007). The same protocol was applied to measure IL-9 (BD Biosciences: 2.5 μg/mL capture clone D8402E8, 0.25 μg/mL detection antibody clone D9302C12), IL-13 (Ebioscience: 2 μg/mL capture clone ebio13A, 0.2 μg/mL detection antibody clone eBio1316H), IL-17A (BD Biosciences: 2 μg/mL capture clone TC11-18H10, 0.17 μg/mL detection antibody clone TC11-8H4.1), and IFN-γ (BD Biosciences: 1 μg/mL capture antibody clone AN-18; Ebioscience: 0.125 μg/mL detection antibody clone XMG1.2). Recombinant cytokine standards were purchased (Ebioscience).

2.6. Statistical analysis

Experiments were performed twice and data were evaluated using Student's *t* test or ANOVA with Tukey's post hoc test for multiple means. *P*-values less than 0.05 were considered to be statistically significant.

3. Results

3.1. Distribution of T. spiralis in the intestinal tract

Previous reports have documented the presence of *T. spiralis* in the large intestine; however, the location of the worms in the tissue has not been described. Ten days post-infection (dpi), adult worms occupied an epithelial habitat similar to that observed in the small intestine (Fig. 1A) (Wright, 1979). Peak worm burdens occurred prior to day 5 in the SI, on day 9 in the cecum, and on day 13 in the LI (Fig. 1B–D). Once established, worms were expelled at comparable rates from each site. Results obtained from C57BL/6 and BALB/c mice were indistinguishable.

The ratio of female to male *T. spiralis* colonizing the SI has been reported to be approximately 2:1, shifting to 1:1 as worms are expelled (Gursch, 1949). Fig. 1 (panels E, F, G) shows the expected transition in gender ratio in the SI of C57BL/6 mice, while equal numbers of male and female parasites were observed at all times in the cecum and LI. Similar results were obtained from BALB/c mice (not shown). These results suggest that females are cleared more rapidly from the SI and are less successful than males in colonizing the distal compartments.

3.2. Cytokine production and mast cell response

Assay of cytokines in explant cultures revealed no significant differences in IL-4, IL-5, or IFN-γ among the jejunum, ileum, and LI (Fig. 2A). Although colonization of the sites was not synchronous, the response kinetics were similar for these cytokines across the sites. In contrast, IL-9, IL-10, and IL-13 were significantly lower in LI explant cultures, and the modest output of these cytokines was delayed compared to the SI; IL-17 was reproducibly lower in the LI only at 15 dpi.

In the context of *T. spiralis* infection, intestinal mastocytosis in the SI of mice is driven by IL-9 and IL-10 (Faulkner et al., 1997; Helmby and Grencis, 2003), and mast cells are believed to be essential to the mechanism of worm expulsion (Ha et al., 1983; Knight et al., 2000). The absence of a significant increase in Alcian Blue positive cells in the LI during infection (Fig. 2B) correlated with reduced production of IL-9 and IL-10 in that compartment. Despite these deficiencies, worms were expelled at a rate similar to that observed in the SI. Thus, the immune response to *T. spiralis* in the LI is significantly different from that of the SI, and does not feature mastocytosis, yet expulsion occurs in a timely manner.

3.3. Enteropathy and the influence of IL-10

In C57BL/6 mice, inflammation in the SI increased dramatically during infection, while no reproducible inflammatory response was detected at any time in the LI (Fig. 3). In contrast, inflammation increased significantly on day 10 of infection in the LI of IL-$10^{-/-}$ mice, and this inflammation resolved by day 15. We concluded that IL-10 is a key regulator of inflammation in the LI during *T. spiralis*

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