Modulation of inflammatory bowel disease in a mouse model following infection with *Trichinella spiralis*

Y. Zhao\(^a,1\), M.Y. Liu\(^a,\ast\), X.L. Wang\(^a,1\), X.L. Liu\(^a\), Y. Yang\(^a\), H.B. Zou\(^a\), S.M. Sun\(^a\), L. Yu\(^a\), B. Rosenthal\(^b\), H.N. Shi\(^c\), P. Boireau\(^d\), X.P. Wu\(^a\)

\(^a\) Key Lab for Zoonosis Research, Ministry of Education, First Hospital, Jilin University, Changchun 130062, PR China
\(^b\) Animal Parasitic Disease Laboratory, USDA, Building 1180, Beltsville, MD 20705, USA
\(^c\) Mucosal Immunology Laboratory, Pediatric Gastroenterology Unit, Massachusetts General Hospital East, Building 114, 18th Street, Charlestown, MA 02129, USA
\(^d\) ANSES, Laboratory for Animal Health, Maisons-Alfort, France

**Keywords:**
- *Trichinella spiralis*
- Inflammatory bowel disease
- Colonic immunity
- Immunomodulator

**Abstract**

Infection of mice with *Trichinella spiralis* redirects the mucosal immune system from a Th1 to a protective Th2 response with a reduction in the severity of trinitrobenzene sulfonic acid-induced colonic damage. *T. spiralis* infection induced IL-10 production in a dose-dependent manner in oxazolone (OXZ)-induced colitis. This phenomenon may be responsible for the lack of efficacy of *T. spiralis* in the treatment of OXZ-induced colitis. These results indicate that if the source of increased IL-10 production is identified and addressed, *T. spiralis* may alter the Th2 response.

**1. Introduction**

Inflammatory bowel disease (IBD) is a chronic, relapsing, inflammatory disorder of the gastrointestinal (GI) tract that includes both Crohn’s disease (CD) and ulcerative colitis (UC). Treatment of the disease includes conservative measures as well as surgical approaches in those who are non-responders to medical treatment (Triantafillidis et al., 2011). *Trichinella spiralis*, an intracellular nematode parasite, has been studied for its potential to modulate inflammation such as in the reduction of airway allergic inflammation in mice (Park et al., 2011). To test and explore immunoregulation of *T. spiralis* in IBD model mice, we investigated immune responses of the IBD model mice induced by trinitrobenzene sulfonic acid (TNBS) and oxazolone (OXZ).

**2. Materials and methods**

**2.1. Mice, parasitic infection, and induction of colitis**

Female BALB/c mice (6–8 weeks old) and *T. spiralis* were obtained from the Key Laboratory of Zoonoses, Ministry of Education, Institute of Zoonoses, Jilin University. The muscle larvae (ML) of *T. spiralis* were obtained from infected rodents 35 days post infection (p.i.). The protocol was approved by the Ethical Committee of Jilin University, China (Ref number 20090114). Five groups of mice (6 mice/group) were studied. Control mice were treated with 50% ethanol and all parasite-exposed groups were infected with 400 *T. spiralis* ML. The colitis groups were first treated with TNBS or OXZ, than infected with *T. spiralis* (TNBS; *T. spiralis* + OXZ) 21 days after chemical induction. Animals were sacrificed on day 3 or 7 after infection. To grade intestinal inflammation, colons were removed at the indicated time points and the damage...
was assessed macroscopically, histologically and biochemically for myeloperoxidase (MPO) activity according to published criteria (Boirivant et al., 1998; Fabia et al., 1993).

2.2. PCR and ELISA analyses of IFN-γ, IL-12, IL-4 and IL-10 mRNA and protein expression

Total RNA was extracted from colons and spleens using Trizol reagent (Invitrogen). RNA was quantified spectrophotometrically. RT-PCR was carried out in a total volume of 25 μl. The thermal cycling parameters and primers for β-actin (house-keeping gene), IFN-γ, IL-12, IL-4 and IL-10 have been previously described (Murray et al., 1990; Ulett et al., 2000; Cenac et al., 2005).

2.3. Cell culture

Lamina propria mononuclear cells (LPMCs) were isolated from freshly obtained colonic specimens using a modification of the technique described by Boirivant et al. (2005). After the LPMCs and spleen cells were incubated for 48 h, culture supernatants were harvested to measured concentrations of IFN-γ, IL-4, IL-10, and IL-12 with ELISA kits (U-CyTech) according to the manufacturer’s instructions.

2.4. Statistical analysis

All experiments were repeated three times. Data were analyzed using a one-way ANOVA followed by the Student–Newman–Keuls test and the chi-square test. A p value of <0.05 was considered as significant.

3. Results

3.1. Survival rates, colonic damage, and inflammation in T. spiralis-infected, colitis-induced mice

Compared to the TNBS group, mice subsequently infected with T. spiralis exhibited less mucosal damage, less thickening of the colonic wall, and less granulocyte infiltrate (Fig. 1a–d). Furthermore, T. spiralis-infected IBD mice survived at a significantly greater rate on day 3 and experienced significant reductions in the macroscopic damage score, histological score, and MPO activity on days 3 and 7 (Fig. 1e–h). Trichinella spiralis + OXZ mice exhibited no significant differences in any observed physiological parameters at any time points (Fig. 2).

3.2. Cytokines in colonic mucosa and spleens of T. spiralis-infected, colitis-induced mice

An increase of the IFN-γ and IL-12 levels was observed in TNBS mouse colons on both days 3 and 7. T. spiralis + TNBS mice exhibited delayed increases in IFN-γ and IL-12 (Fig. 3a and c). In spleens of T. spiralis + TNBS mice, the mRNA and protein levels of IL-12 on day 3 and IFN-γ on day 7 were significantly lower than in TNBS mice (Fig. 3b and d); however, the mRNA and protein levels of IFN-γ in T. spiralis + TNBS mice on day 3 were not significantly different from the TNBS mice.

As shown in Fig. 4a and d, the mRNA and protein levels of IL-4 and IL-10 in colons of T. spiralis + OXZ mice on days 3 and 7 were significantly higher than in uninfected OXZ mice (Fig. 4a and c). In the spleens of T. spiralis + OXZ mice, the mRNA and protein levels of IL-10 were significantly higher than in OXZ mice on day 3 (Fig. 4b and d). No differences were observed in the mRNA or protein levels of IL-4 or IL-10 in the spleens of mice on day 3. In contrast, the mRNA and protein levels of IL-4 and IL-10 in the spleens of T. spiralis + OXZ mice were significantly higher than in the OXZ group on day 7.

4. Discussion

Cytokines play key roles in IBD, helping polarize T cell differentiation to either Th1 or Th2. Cytokine levels influence the likelihood, severity, and timing of inflammation in IBD (Sanchez-Munoz et al., 2008). Concurrent infection with T. spiralis prevented this increase. In the spleen, worms decreased IFN-γ mRNA expression mainly on day 7 post-infection. Colons removed from mice after 50% ethanol treatment expressed IFN-γ, and this was significantly different compared to T. spiralis exposure alone. T. spiralis infection reduced the severity of TNBS-induced colitis based on the survival rates, macroscopic damage scores, histological scores, and MPO activities on days 3 and 7 post-infection. In the present study, mice infected with T. spiralis redirected the mucosal immune system response from a Th1 response toward a protective Th2 response, with an attendant reduction in the severity of TNBS-induced colonic damage and a balancing of the Th1/Th2 responses.

In spleens of OXZ-treated mice that were subsequently euthanized 7 days later, the levels of both IL-4 mRNA and protein were significantly higher than in those that did not receive parasite. The Th2 immune response is characterized by cells that make IL-4, IL-5, and IL-13. Interleukin-4 is a stimulatory molecule for B and T cells, and has known immunosuppressive effects in the intestine (Kugathasan et al., 2007). This may be responsible for the lack of efficacy of T. spiralis in the treatment of OXZ-induced colitis.

As in the murine model of IBD, IL-10 has suppressive anti-inflammatory activity in humans (Bouguen et al., 2011). Higher doses of IL-10 do not reduce inflammation and have been associated with systemic side effects, such as fever, headache, and malaise (Tilg et al., 2002). Thus, the immunoregulatory roles of IL-10 appear to be complex and dose-dependent. Proper doses of IL-10 can ameliorate inflammation, but high doses of IL-10 not only decrease the immune system’s ability to regulate excessive dysregulated Th2 responses, but also induce many proinflammatory cytokines. These results indicate that if the source and mechanism for elevated IL-10 levels in IBD can be identified, adult and/or newborn larval antigens of T. spiralis may be found that can control the levels of IL-10 and thereby alter the Th2 responses. Therefore, T. spiralis and its associated antigens may have bidirectional immunomodulatory effects, which deserve further exploration.
Fig. 1. Survival rates, colonic damage, and MPO activities in TNBS-induced mouse colitis (a–d): HE-stained colonic section of TNBS mice on 3 (a) and 7 (c) days (100×) and of *T. spiralis* + TNBS mice on 3 (b) (100×) and 7 (d) days (200×); (e) Survival rate in TNBS mice and *T. spiralis* + TNBS mice on 3 (b) and 7 (d) days; (f) MPO activity; (g) macroscopic damage assessment; (h) histological assessment.
Fig. 2. Survival rates, colonic damage, and MPO activities in OXZ-induced mouse colitis (a–d): HE-stained colonic section of OXZ mice on 3 (a) (40×) and 7 (c) (100×) and of T. spiralis + OXZ mice on 3 (b) (100×) and 7 (d) days (200×); (e) Survival rate in OXZ mice and T. spiralis + OXZ mice on 3 (b) and 7 (d) days; (f) MPO activity; (g) macroscopic damage assessment; (h) histological assessment.
Fig. 3. Expressed cytokines in colonic LPMC and splenic lymph cells in TNBS-induced mouse colitis IFN-γ (a) and IL-12 (c) production of colonic LPMC in TNBS mice treated or untreated with *T. spiralis* on days 3 and 7. IFN-γ (b) and IL-12 (d) production of splenic lymph cells in TNBS mice treated or untreated with *T. spiralis* on days 3 and 7.

Fig. 4. Expressed cytokines in colonic LPMC and splenic lymph cells in OXZ colitis model mice IL-4 (a) and IL-10 (c) production of colonic LPMC in TNBS mice treated or untreated with *T. spiralis* on days 3 and 7; IL-4 (b), and IL-10 (d) production of splenic lymph cells in TNBS mice treated or untreated with *T. spiralis* on days 3 and 7.
Conflict of interest statement

The authors declare that they have no competing interests.

Acknowledgements

This study was supported by MOST 2011AA10A215; NSFC 30825033, 31030064, 30972177, 30950110328, 81070311, 31072124; and NIH/NIDDK R01 DK082427.

References


