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Exposure time determines the protective effect of *Trichinella spiralis* on experimental colitis

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Abstract

Several studies demonstrate the protective effect of *Trichinella spiralis* (*T. spiralis*) on autoimmune diseases, however the optimal exposure time remains unexplored. This study aimed to determine whether pre-exposure of mice to *T. spiralis* conferred greater protection than introduction of the parasite in the acute phase of experimental colitis. We compared the effect of *T. spiralis* on dextran sodium sulfate (DSS)-induced colitis using two exposure paradigms: introduction three weeks prior to, or immediately after the induction period. Inflammation scores, morphological changes and cytokine profiles in serum and colonic tissue were assessed. At a parasite dose of 300 cysts, post exposure had a more pronounced effect on cytokine profiles, improved gross appearance of colon tissue, and reduced inflammatory symptoms. In addition, we demonstrate that regardless of cyst number, pre-exposure to *T. spiralis* did not confer protective benefits when compared to parasite introduction in the acute phase of DSS-induced colitis. Moreover, our data indicates that the underlying mechanisms of action involve an IL-17/TNF-alpha synergistic reaction, suppression

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Author contributions statement

Conceptualization, X.W., X.X. and X.Y.; methodology, W.Z., M.Z.; software, W.Z.; validation, M.Z.; formal analysis, X.S.; investigation, Y.H.; resources, B.L.; data curation, Y.W.; writing—original draft preparation, W.Z. and M.Z.; writing—review and editing, X.X.; visualization, Q.L.; supervision, X.W.; funding acquisition, X.Y. and X.W.

Declaration of competing interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.micpath.2020.104263>.

of Th1 and Th2 responses, and an upregulation of the regulatory cytokines IL-10 and TGF-beta 1. Our results demonstrate that moderate exposure to *T. spiralis* in the acute phase of DSS-induced colitis improves disease associated inflammation and tissue disruption.

1. Introduction

Ulcerative colitis (UC) is a long-term inflammatory ulcerative condition of the colon and rectum characterized by abdominal pain and diarrhea mixed with blood [41]. Long-standing UC can lead to a series of complications such as megacolon, inflammation of the eye, joints, or colon cancer [40]. As one of the most severe complications contributing substantially to the morbidity and mortality [3]. Another meta-analysis which concentrated on patients in Europe and North America revealed that 25 years of chronic UC increased colorectal cancer risk by as much as 34% [15].

Standard treatment for UC depends on the extent of involvement and disease severity. It can be treated with various medications such as 5-aminosalicylic acid, corticosteroids, immunomodulators and biologics. For patients with symptoms such as exsanguinating hemorrhage, intestinal perforation or strongly suspected carcinoma, surgery is the most common treatment option. However, both chronic medication and multi-step surgeries can cause side effects. Since UC usually begins in young adulthood and lasts throughout life [12], patients with UC have a significantly impacted quality of life and it is especially difficult for younger patients to accept long-term medication.

The idea that exposure to certain infections may decrease the risk of developing an allergy is not new, however it wasn't until the hygiene hypothesis was formally proposed by Strachan that researchers and especially immunologists began to explore the possible protective effect of parasites on autoimmune disease [36]. Joel V. Weinstock and David E. Elliott state that raising children in extremely hygienic environments negatively affects immune development, predisposing them to immune diseases such as Crohn's disease and possibly UC later in life [37]. Moreover, they conclude that early exposure to helminths promotes Th2-type immune responses that may prevent the development of excessive Th1-type inflammatory reactions that cause autoimmune disease in genetically predisposed people [37].

Early exposure to parasites is crucial to decrease the risk of atopy and allergy-related diseases, an effect substantiated by several longitudinal placebo-controlled studies. A multitude of studies focused on a treatment known as deworming in an effort to confirm whether early-life exposure to parasites affects pathophysiological and immunological processes and whether in-utero or early childhood infection is more important with regard to the development of allergy-related diseases later in life. Data from the literature suggests that the timing of host exposure to parasites is critical. However, most of the pre-clinical studies investigating the relationship between helminths and colitis were conducted using two different exposure paradigms; 1) pre-infecting animals with parasites such as *Taenia crassiceps* [28] and *Trichuris muris* [39] to determine if chemically-induced colitis could be inhibited, or 2) chemical induction of colitis and subsequent treatment with parasites or parasite-derived products [43]. To investigate the time-dependent differences of these

exposure paradigms on colitis development and severity, we performed head-to-head comparisons of pre-colitis parasite exposure vs. exposure to parasites in the acute phase of colitis development.

We chose *Trichinella spiralis* (*T. spiralis*) for our study as it was the first identified parasite belonging to the *trichinella* genus and is the most well-characterized member. In its life cycle of infective muscle larvae, it occupies the host's muscle cell with minor harm due to mixed Th2/Treg regulate immune response [9], and in contrast to other intestinal nematodes, this parasite is able to successfully persist in rodents for their entire life span or in humans for up to several months or years [24]. The severity of symptoms of human trichinellosis varies according to the number of larvae ingested. Ingestion of a low number of larvae can be asymptomatic while a few hundred larvae can result in obvious gastrointestinal symptoms [19].

In this study, we aimed to verify whether pre-existing parasitic infection or parasitic intervention in the acute phase of DSS-induced colitis impact prognosis. This data is valuable in determining the optimal time frame to introduce parasites or parasite-derived products. In addition, by investigating the cytokine profiles in both serum and colonic tissue, a clearer picture can be formed regarding the mechanisms underlying the therapeutic benefits of intestinal nematodes on colitis.

2. Methods and materials

2.1. Experimental model and subject details

Experiment design is shown in Fig. 1. All experimental procedures were performed in accordance with, and with the approval of, the local ethics committee for the use of animals at Central South University (Changsha, China). *T. spiralis* (ISS 533) was originally isolated from swine and then maintained in female Kunming mice. Female C57BL/6 mice aged 47–51 days were provided by the Experimental Animal Center of Central South University, free of specific pathogens, housed in ventilated cages and were fed a normal chow diet. They had free access to food and water throughout the experiment, with the exception of a 24 h-fasting period prior to *T. spiralis* exposure.

2.2. Experiment design

2.2.1. Colitis model—Dextran sodium sulfate (DSS, 36, 000 to 50, 000 Da, MP Biomedicals) were dissolved into double distilled water (DDW) to make a solution with a concentration of 2.5% and presented to mice as drinking water for 7 days continuously to induce colitis. Fresh solutions were made, and turbidity was checked every day. Body weight for each animal was recorded daily and occult blood tests were conducted daily during the 7-day DSS-exposure period and once a week for 21 days after the exposure period.

2.2.2. Introduction of *T. spiralis* into mice—Kunming mice were infected with *T. spiralis*. The C57BL/6 mice were divided into five groups: two pre-DSS exposure groups with 30 or 300 *T. spiralis* larvae, DSS only group, two post-DSS exposure groups with 30 or 300 *T. spiralis* larvae. For the two pre-DSS exposure groups, the freshly harvested

diaphragm containing infectious encysted larvae of *T. spiralis* were made flatten and counted and cut into pieces with 30 or 300 *Trichinella* larvae under the microscope. Then, the pieces of infected diaphragm were embedded into food sandwich and we watched single housed experimental C57BL/6 mouse eat the food sandwich to ensure that the right number of larvae was introduced. The infected diaphragm will be digested by the gastric juice into single larva, which then enters the intestine through the digestive tract. This is the traditional way of *Trichinella* infection. These two pre-DSS exposure groups of mice were allowed to recover until day 21 post-infection. Then all mice received 2.5% DSS for 7 days to induce colitis. For the two post-DSS exposure groups, mice consumed 30 or 300 *T. spiralis* larvae on day 28 similar to the two pre-DSS exposure groups. All mice were euthanized at day 49 from the first time we introduced the infectious larvae of *T. spiralis*.

2.3. Sample collecting

Mice were anesthetized with 10% chloral hydrate, via intraperitoneal injection (0.06 mL/10 g body.

weight) 6–8 min following unconsciousness and serum and colon tissue were collected and

2.4. treated as follows

2.4.1. Serum—0.8–1 mL peripheral blood was collected per animal through orbital vein puncture with glass capillaries. The blood was placed at 37 °C for 1 h followed by centrifugation at 1200 rpm for 15 min. Serum was then collected and stored at –20 °C until further use.

2.4.2. Colon tissue—Whole colon tissue (0.5 cm away from the anus to the distal end of the appendix) was collected. 1 mL syringes without needles were used to gently wash out the intestinal contents. Filter paper was used to absorb the extra water. 1 cm colon tissue (2.5–3.5 cm away from the distal end of appendix) was cut and fixed in 4% formalin, embedded with paraffin and sliced at 5 µm for H&E staining.

2.4.3. Colon homogenate—All colon tissue (except for the 1 cm colon prepared for histopathological observation) were homogenized with a glass homogenizer. During the process of homogenization, a total amount of 5 mL phosphate buffer solution (PBS) was added, and homogenization time was limited to 5 min.

2.5. Stool condition scoring and histological scoring

2.5.1. Stool score—Stool condition was evaluated by the stool character and fecal occult blood test results, which is a modified version based on the work of Cooper et al. [10] (according to Supplemental Table 1), partial score of these two aspects were added to get the final score.

2.5.2. Microscopic score and macroscopic score—After the mice killed, macroscopic score was based on colon length, hyperemia, wall thickening, ulceration, inflammation extension and damage these parameters as Supplemental Table 3 described. Slices for each mouse were stained with hematoxylin and eosin (H&E) and assessed for

general extent of inflammation and crypt damages, using a method from L. A. Dieleman (Dieleman LA. et al., 1998). 2 features were quantified by the percentage involved. Briefly, each section was graded for each feature separately by multiplying the score for that feature and the percentage involvement (in a range from 0 to 12 for extent of inflammation and 0 to 16 for crypt damages) (Supplemental Table 2). For example, around 40% transmural damage and 60% damage restricted within mucosa will make “extent” score $2*3 + 3*1 = 9$; and if 40% crypts are basal 2/3 damaged and 60% crypts are intact, then crypt damage score is $2*2 + 3*0 = 4.2$ experienced examiners performed the scoring blind according to the grading scale shown as Supplemental Table 2.

2.6. Cytokine quantification

ELISA assay for IL-1 β (Cat#: E-EL-M0037c), IL-4 (Cat#: E-EL-M0043c), IL-10 (Cat#: E-EL-M0046c), IL-17 (Cat#: E-EL-M0047c), TGF- β 1 (Cat#: E-EL-M0051c), TNF- α (Cat#: E-EL-M0049c) from Elabscience[®] were used for quantification of cytokine levels. When running ELISA assay for homogenate supernatant, the total protein concentration was determined using BCA kit (Cat#: CW0014S, CWBIO, China). Assays were performed following protocols supplied by vendors.

2.7. Statistical analysis

GraphPad Prism 7.04 was used for data analysis and representation. Two-way ANOVA and Tukey’s multiple comparisons test were applied and multiplicity adjusted P values for each comparison were annotated. Three cohorts were conducted. Data is shown as mean $\pm$ SD and a P value of $p < 0.05$ was considered significant.

3. Results

3.1. Body weight and stool score

During the 7 days of DSS administration, mice developed significant and typical manifestations of acute colitis, such as weight loss, messy fur, poor mental state due to severe diarrhea, and bloody stools. Weight loss was more obvious in the last 3 days of the colitis induction time. Groups without pre-infection of *T. spiralis* appeared to have a greater reduction, although not statistically significant, in body weight compared to animals exposed to the parasite prior to DSS treatment (Fig. 2A). We used a scoring scale of stool condition based on the scoring scale of Disease Activity Index (DAI) introduced by Harry. S. Cooper [9,10]. The total score of the stool character and the positive degree in occult blood test is evaluated blind (scale shown as Supplemental Table 1 in the methods section). Although stool status in all groups deteriorated gradually, there appeared to be no differences between the pre-infected group and those not infected in the first 7-day period. However, after 7 days of induction, acute colitis was established and the stool status of both pre-infected and post-infected groups tended to alleviate more rapidly than mice with only DSS treated control group (Fig. 2B). Acute colitis will clear up on its own if the DSS-DDW alternating regime is not repeated to induce chronic colitis, and while stool status of mice with DSS only appeared to improve after 21 days, it’s not as striking compared to groups which have received their dosage of *T. spiralis*, especially the post-infection groups (Fig. 2B).

3.2. Gross appearance and weight of colon

We evaluated the gross appearance of the colon 21 days after acute colitis induction. The colon length of DSS only mice was significantly shorter than untreated controls (Fig. 3A). Moreover, severe intestinal/peritoneal adhesions were observed along with thickened intestinal walls, intense edema, and fragility. Colon length was improved in all *T. spiralis* treated groups compared to DSS only group with the exception of the group pre-infected with only 30 cysts. The colons of animals from this group remained shortened with edema and thickened intestinal walls. The percentage of colon/body weight of post-300 group was significant higher than both post-30 and pre-300 groups (Fig. 3B).

3.3. Histopathological analysis of colons

In an untreated control colon, intestinal glands with abundant goblet cells can be seen (Fig. 4A). In contrast, abnormal crypt structure and disrupted mucous membranes were observed in the colons of DSS only mice (Fig. 4B). In addition, loss of epithelial cells and infiltration of inflammatory cells including neutrophils and mononuclear cells were apparent in several areas in DSS only mice, however when mice were treated with *T. spiralis*, infiltration was observed only in focal lesion areas (Fig. 4B&CDEF). Disruption of crypts and multifocal cellular infiltration was still apparent in all groups at 21 days after the induction of colitis, whether animals were exposed to *T. spiralis* or not, however the re-epithelialization process as well as the regeneration of crypt structure was more apparent in groups post-infected with *T. spiralis* (Fig 4EF). In addition, *T. spiralis* pre-treated groups exhibited more mucous membrane lesions and cellular infiltration as well as extensive edema deep into the submucosa and partially into the muscularis externa (Fig 4CD).

Microscopically we evaluated the crypt damage and the extent of inflammation with a modified histological score system based on previous studies [18]. We discarded the grading of inflammation and regeneration properties as the evaluation levels for these two is less informative compared to the extent of inflammation and crypt damage. Crypt damage and the extent of inflammation were translated to percentage involvement (Fig. 5), according to the scale shown in Supplemental Table 2.

3.4. Cytokine analysis

Analysis of cytokine levels using an ELISA assay revealed a significant decrease in serum levels of the Th1 pro-inflammatory cytokine IL-1 β , when *T. spiralis* was introduced in the acute phase of colitis immediately following the 7-day period of DSS-induction compared to the DSS only group without parasite treatment (Fig. 6A). In contrast, pre-exposing mice to a low dose of larvae significantly increased serum IL-1 β (Fig. 6A). Circulating IL-10, a representative Th2 cytokine, was significantly higher in animals post-infected with 300 cysts compared to those infected with only 30 cysts, as was the case for serum TGF- β 1 (Fig. 6B). Interestingly, serum proinflammatory cytokine IL-17 was decreased in comparison to the DSS only group when mice were exposed to parasites during the acute phase of colitis at a dose of 300 cysts. Serum IL-17 was unaltered in the other 3 parasite-exposed groups compared to DSS only group (Fig. 6C). No significant differences between pre and post infection were observed in serum TGF- β 1 following infection with 30 cysts, however

introducing a higher number of larvae in the acute phase upregulated serum TGF- β 1 level (Fig. 6D).

A similar trend to serum IL-1 β was observed in colonic tissue homogenate levels of proinflammatory cytokine TNF- α (Fig. 6F). Interestingly, while infection with 300 cysts had no effect on IL-4, a representative Th2 cytokine in local colon lesions, the other three *T. spiralis* infected groups exhibited a significant increase in IL-4 compared to DSS only mice (Fig. 6E). In colon tissues, a significant upregulation of IL-17 was observed in both groups pre-exposed to *T. spiralis* regardless of cyst load and at the low parasite dose when exposed during the acute phase (Fig. 6G). Similar to serum TGF- β 1, no significant differences between pre and post infection were observed in tissue TGF- β 1 following infection with 30 cysts, however introducing a higher number of larvae in the acute phase upregulated colon tissue TGF- β 1 level (Fig. 6H).

4. Discussion

The hygiene hypothesis postulates an inverse correlation between the incidence of enteric infections including parasitic infections and chronic inflammatory bowel diseases (IBD). However, the direct use of helminths as a supplementary treatment for colitis is still controversial due to inconsistent results observed in animal models of colitis and human studies. In animals, while *Hymenolepis diminuta* treatment was detrimental in an animal model of oxazolone-induced colitis [22], benefits of using *Hymenolepis diminuta* treatment have been observed in both 2,4,6-trinitrobenzene sulfonic acid (TNBS) and DSS models of colitis. In a population-based cohort study conducted in Denmark, early enterobiasis did not protect children from chronic inflammatory diseases including IBD [23]. However, clinical studies with the porcine whipworm *Trichuris suis* or human hookworm *Necator americanus* showed efficacy in amelioration of disease in IBD patients. Thus, further confirmation of the safety and therapeutic potentials of different helminth species is needed.

Here, we explored several parameters of disease progression in a mouse model of DSS-induced colitis using two parasite exposure paradigms: pre-exposure with *T. spiralis* (to test hygiene hypothesis) or exposure in acute phase of the disease (to test therapeutic potentials). Improvements in inflammatory outcomes and colon appearance were observed in groups infected with *T. spiralis* in the acute phase of experimental colitis compared to animals exposed before disease onset. These improvements included alleviated symptoms (weight loss, diarrhea, bloody stool, etc.) during DSS progression, as well as improvements in the gross and microscopic appearance of colon tissue 21 days after colitis was established. Moreover, the cytokine profiles of animals post-infected with *T. spiralis* at a dose of approximately 300 cysts were consistent with reports from previous studies including a significant decrease in the Th1 pro-inflammatory cytokine IL-1 β in the serum and a downregulation in TNF- α in colon homogenate. In addition, while we observed increases in the regulatory cytokine TGF- β , IL-17- another proinflammatory cytokine important in many autoimmune diseases - was significantly decreased in mice post-infected with 300 cysts.

In contrast to previous studies using *T. spiralis*-derived products [8,14,35], we used complete parasites in our intervention paradigm to exclude the possibility of missing effective

component(s) that could be crucial for immunomodulation of inflammation, as different *T. spiralis* proteins have different biological and immunological effects on immune system modulation [25,32].

Recently, two tumor necrosis factor (TNF) antagonists, adalimumab and golimumab, received market authorization. Both antagonists block TNF- α from binding to its receptor, thereby inhibiting the NF- κ B and mitogen-activated protein (MAP) kinase downstream signaling cascade thus promoting mucosal healing. Mice post-infected with *T. spiralis* at a dose of 30 cysts exhibited a more significant decrease in colon lesion TNF- α than mice pre-infected with the same dose, however when compared to the colitis only control group, only an infection dose of 300 cysts was high enough to significantly reduce TNF- α . However, an increase in the occurrence of opportunistic infections has been reported in human studies using TNF antagonists for treating colitis. Here, mesalamine, azathioprine, and corticosteroids were often prescribed. Therefore, cumulative toxicity should also be considered when evaluating parasitic intervention as a treatment for colitis.

Although the loci identified might only partly contribute to IBD heritability [Uhlir HH. et al., 2014], differences in the genetic characteristics of Crohn's disease (CD) and UC have been reported. Notably, 30 of the 163 susceptibility loci for IBD are unique to CD while 23 of the 163 susceptibility loci are unique to UC [30]. Moreover, the mucosal immunity of UC and CD differs in that UC involves both the Th2 immune response and, to a lesser extent, Th1 and Th17 responses [4,20]. Another interesting finding of this study is that while hosts are typically protected from parasites or parasite-derived products by inducing a strong Th2 response [25], *T. spiralis* suppresses the Th2 response as noted by significantly lower IL-4 levels in colon tissue of mice post infected with 300 cysts compared to mice pre-infected with the same amount. However, IL-4 levels of both groups were still higher than the DSS-control group. Further research is needed to understand the complicated interplay of IL-4 and the Th2 response in *T. spiralis* treatment of colitis. Goblet cell hyperplasia is believed to be an important factor in the ability of hosts to expel *T. spiralis* [7,44], and IL-4 is a key mediator of this process [17,27]. However studies also report that Th2 cytokines such as IL-5 stimulate cytotoxicity of eosinophils, in vitro [29], which also contributes to worm expulsion [38] or NBL death [26] during infection. Interestingly, IL-10 is also produced by activated eosinophils, and inhibits the activation of macrophages and neutrophils that release nitrogen oxide [21], which is necessary for *T. spiralis* survival. Further researches are needed to interpret the complex interplay between the effect of *T. spiralis* and DSS, compared to the lower dose of parasites, a higher parasitic dose of 300 cysts may act in an immunomodulatory manner to abrogate DSS-induced Th2 response. Although minor, modulation of the Th2 immune response even to a small extent appears to sufficiently offset the detrimental effects of DSS. However, previous studies didn't observe a significant effect on IL-4 in the 2,4,6-trinitrobenzenesulfonic acid (TNBS) induced colitis model post-infected with parasites and in DSS-induced colitis model post-infected with hookworm [5,34].

DSS-induction of colitis is a well-established model with several symptoms resembling human IBD, particularly UC. However chronic UC results in prominent lymphoid aggregates, fissuring ulceration, and focal inflammation which more closely resembles CD [1,16]. In a study published this year using a rat model, Catana and colleagues concluded

that DSS-induction of colitis was a more appropriate model for the study of human UC than TNBS induction [6]. However, the two induction methods do have some similarities. Another possible reason for the discrepancies between our findings and previous reports could be the variability in the species of mice used in these experiments. Along this line, a recent study using BALB/c mice revealed that Th2/Th17/Treg polarized immunity might protect against DSS-induced colitis [42].

The pathological process of human trichinellosis involves two main phases; an intestinal phase and a muscle phase [33]. When raw or undercooked meat is infected and eaten, cystic muscle larvae are released from the nursing muscle cells. In the acidic environment of the stomach, they migrate and burrow into the intestinal mucosa, settling into epithelial cells to mature and reproduce. One study in BALB/c mice shows that procreation of newborn larvae (NBL) happens 3–7 days post infection (dpi) and that adult *T. spiralis* are expelled from the intestine of mice within 10–17 dpi [13]. The intestinal phase is the crucial phase that determines both the process and the consequences of trichinellosis [24]. Experimental colitis can be alleviated by either crude muscle larvae or by the main component of excretory/secretory (ES) products of muscle larvae [14,31]. Additionally, ES antigen of the adult worm could also be an effective treatment [Yang X. 2014]. Previous studies have shown that the ES antigen induces IL-10 and TGF- β secretion from macrophages in vitro [2,25]. It's possible that the immunomodulatory effect of *T. spiralis* may have been initiated in the intestinal stage as the regulatory cytokines IL-10 and TGF- β were higher when parasites were introduced after the induction of colitis. This effect was more pronounced when the number of cysts increased, suggesting that a certain threshold parasite dose is necessary to balance the immune response of the host while maintaining viability during an upregulated Th2 response.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Experimental design. Kunming mice were infected with *T. spiralis*. The C57BL/6 mice were divided into five groups: 30-pre: the mice infected with 30 *T. spiralis* 21 days previously then induced with DSS 7 days; 300-pre: the mice infected with 300 *T. spiralis* 21 days previously then induced with DSS 7 days; 30-post: the mice induced with DSS 7 days then introduced 30 *T. spiralis* 21 days; 300-post: the mice induced with DSS 7 days then introduced 300 *T. spiralis* 21 days; only UC: the mice induced with DSS 7 days then free drinking.

Fig. 2.

Body weight and stool score during DSS-induction of UC. Weight loss observed in the last 3 days in UC induction time, groups without pre-infection of *T. spiralis* decreased more steeply than groups with *T. spiralis* (A); stool status of both pre-infected and post-infected groups tended to alleviate more rapidly than mice with only colitis after 7-day period (B). scoring scale of stool condition based on Cooper et al., n = 5 in each group. Data is represented as mean $\pm$ SD.

Fig. 3.

Gross appearance and weight of colon 21 days after establishment of DSS-induced UC. Shortened and fragile colons with thickened edematous intestinal walls were observed in UC-control mice (A). Pre-exposure appeared to abrogate colon shortening and edema and restore colon weight (B). Data is represented as mean $\pm$ SD, n = 5 per group. Significant differences are indicated by asterisks; p < 0.01 **, ns denotes no significance.

Fig. 4.

H&E staining of colon cross-sections 21 days after UC induction. Cross-section of colon shown in circles ($\times 40$ magnification), representative damage of crypts or epithelium shown as squares ($\times 400$ magnification). Normal crypt structure and intact mucous membrane, intestinal glands with abundant goblet cells clearly observed in normal (wildtype) colon (A), Abnormal crypt structure and disrupted mucous membrane observed in UC-only mice (B). Loss of epithelial cells and infiltration of inflammatory cells observed in focal lesion areas at varying degrees of severity in mice treated with *T. spiralis* (B–F). More lesions in the mucous membrane, along with cellular infiltration and extensive edema was observed in the pre-treated groups (C&D), while re-epithelialization and regeneration of crypt structure was more apparent in groups post-infected with *T. spiralis* (E&F), Three different cross-sections of colon per mouse were stained and assessed; $n = 5$ per group.

Fig. 5.

Crypt damage score and extent of inflammation score of colons. The extent of inflammation and crypt damage were quantified to percentage involved based on previous studies of Dieleman et al. [11]. Timing of intervention seemed to be more important than the number of cysts. Data is represented as mean $\pm$ SD. Significant differences are indicated by asterisks, $p < 0.05$, $p < 0.01$, were annotated in the figure as *, **, separately, ns denotes no significance ($n = 5$ in each group).

Fig. 6.

Serum and colon tissue homogenate cytokine quantification. (A–D) Serum levels of IL-1 β , IL-10, IL-17 and TGF- β 1, and (E–H) colon tissue levels of IL-4, TNF- α , IL-17 and TGF- β 1 were quantified using an ELISA assay. Circle pattern means inoculation with 30 *Trichinella spiralis*, square means inoculation with 300 *Trichinella spiralis*. Data is represented as mean $\pm$ SD. Significant differences are indicated by asterisks, $p < 0.05$, $p < 0.01$, $p < 0.001$, $p < 0.0001$ were annotated in the figure as *, **, ***, **** separately, ns denotes no significance ($n = 5$ in each group).