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Effect of *Trichinella spiralis* intervention on TNBS-induced experimental colitis in mice

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ABSTRACT

Inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease (CD), are chronic autoimmune diseases with a high recurrence rate. Epidemiological data have shown that the incidence of IBD increases annually because of improved sanitary conditions and reduced parasitic infection rates. In this experiment, experimental colitis was induced in mice by administering 2,4,6-trinitrobenzene sulfonic acid (TNBS) 28 days after they were infected with *Trichinella spiralis* to confirm that *T. spiralis* infection could alleviate the severity of TNBS-induced colitis.

Thirty-six male BALB/c mice aged 6–8 weeks were randomly divided into four groups: control group (with 50% ethanol, Control), *T. spiralis*-infected group (TS-Control), TNBS-induced colitis model group (Colitis), and *T. spiralis*-pre-infected and TNBS-induced colitis group (TS-Colitis). The mice were sacrificed 3, 7, and 14 days after the model was established. Changes in various colitis indicators to investigate the effect of *T. spiralis* infection on TNBS-induced murine CD model.

Results showed that the weight, DAI score, and macroscopic and microscopic colon damage in the TS-Colitis significantly decreased compared with those in the Colitis. ELISA revealed that the IFN-γ expression decreased and the IL-4 expression increased in the TS-Colitis compared with those in the Colitis. Western Blotting results revealed that the NF-κB expression increased in the Colitis and higher than those in the TS-Colitis. And Flow cytometry results revealed that the percentage of CD4 + CD25 + Foxp3 + Treg cells significantly increased in the TS-Colitis.

T. spiralis-infected mice induced Th2 immune responses and balanced Th1 immune responses stimulated by TNBS to ameliorate intestinal inflammation.

1. Introduction

Inflammatory bowel disease (IBD) is a chronic and recurrent intestinal autoimmune disease whose main clinical manifestations include emaciation, abdominal pain, diarrhea, and bloody stools (Strober et al., 2007; Cho, 2008). According to different pathological changes, IBD is divided into ulcerative colitis (UC) and Crohn's disease (CD) (Blumberg and Strober, 2001; Braegger and Macdonald, 1994).

The pathogenesis of IBD remains unclear, although current studies have proposed that this disorder is caused by genetic, environmental, immune, and other factors. Epidemiological data have shown a clear genetic predisposition in patients with IBD (Kyle, 1992). "Hygiene hypothesis" can explain the effect of environmental factors. With rapid developments in the society, medical standards and living conditions of humans have been improved. As a result, the degree of exposure of the human body to microorganisms and their products has reduced, and the number of infections has decreased remarkably. This phenomenon leads to inadequate stimulation of the immune system, especially in children, thereby compromising the efficiency of the immune system and resulting in an abnormal immune system and disordered inflammatory-related cytokines; thus, the incidence of IBD has increased (Halme et al., 2006).

Immunological evidence has demonstrated that the pathogenesis of IBD may be related to the imbalance between Th1 and Th2 cells in the intestinal mucus (Rocken et al., 1996; Adorini and Sinigaglia, 1997; Markus, 1995). These cells regulate the proliferation and differentiation of each other by secreting cytokines and maintaining their balance. Colitis caused by TNBS is similar to CD in terms of pathogenesis,

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pathological features, and clinical manifestations. This condition elicits a Th1-type immune response, and Th1-type cells express a large number of cytokines, such as IL-12 and IFN-γ (Markus, 1995; Strober, 2002).

Trichinella spiralis balances its relationship with its host's immune system as it parasitizes its host to escape host immunity without seriously damaging its host (Else, 2005). Therefore, *T. spiralis* infection is mainly characterized by Th1 and Th2 immune responses, that is, early infection, or intestinal stage, mainly induces a Th1 immune response, whereas late infection, or muscular stage, triggers a Th2 immune response (Mosmann, 1991). Th2 cells produce IL-4 that can promote the self-secretion of Th2 cells and inhibit the proliferation of Th1 cells. Therefore, the effect of *T. spiralis* infection on the immune system can help prevent and treat autoimmune diseases, including IBD. To understand the mechanism of "worm treatment," we measured the expression levels of Th1, Th2 cytokines and nuclear transcription factor NF-κB and the percentage of CD4 + CD25 + Foxp3 + Treg cells in this experiment. We found that the interaction between cytokines affected the development of IBD and provided a basis for the continuous improvement of treatments.

2. Materials and methods

2.1. Animals

Male BALB/c mice (specific pathogen free, SPF) aged 6–8 weeks (Animal Center of Harbin Medical University) were allowed to acclimatize for 4 days before the experiment and given free access to food and water under standard conditions. All of the procedures were in strict accordance with the Chinese National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.2. Experimental grouping

The experimental mice were randomly divided into four groups, with more than nine mice in each group: control group (50% ethanol, Control), *T. spiralis*-infected group (TS-Control), TNBS-induced colitis model group (Colitis), and *T. spiralis*-pre-infected TNBS-induced colitis group (TS-Colitis). At the beginning of the experiment, each mouse in the TS-Control and TS-Colitis was orally infected with 400 individual *T. spiralis* muscle larvae. After 28 days of infection, the Control and TS-Control were administered with 50% ethanol, and the Colitis and TS-Colitis were intrarectally injected with TNBS solution. The mice were sacrificed under euthanasia on days 3, 7, and 14. The weight of the mice in the four groups was determined daily.

2.3. Collection and oral administration of T. spiralis larvae in mouse muscles

T. spiralis (ISS3) was obtained from the Department of Parasitology, Northeast Agricultural University, and its host is a Heilongjiang Xunke pig. After the mice were sacrificed, their muscle tissues were harvested and bones were removed. The muscles were treated with artificial digestion solution (1% pepsin and 1% concentrated hydrochloric acid) for 4 h at 37 °C. Afterward, the mixture was filtered using an 80-mesh filter and rinsed thrice. The larvae were subsequently harvested in accordance with the modified Bellman's method. Their vitality was observed, and their number was counted under a microscope. The mice in the TS-Control and in the TS-Colitis were orally infected with 400 individual larvae.

2.4. TNBS-induced colitis

Stallmach et al. (2004) established the TNBS-induced colitis model approach. The mice in each group were anesthetized by intraperitoneally injecting with 3% sodium pentobarbital 24 h after fasting. A 1.0 mm thin catheter was inserted into the colon. The mice in the Colitis and TS-Colitis were rapidly injected with 150 μl of 2 mg of TNBS (Sigma, USA) in 50% ethanol solution (5% [m/v] TNBS dissolved in 50% ethanol). The mice in the Control and TS-Control were injected with the same amount of 50% ethanol solution. After injection, their tails were extracted for 3–4 min to prevent the release of liquid. When the anesthesia wore off, the mice were given free access to food and water. The colitis mouse model elicited a Th1-type immune response, which was similar to that in a human CD model. Inflammation was determined based on belowed parameters: clinical disease activity, macroscopic and microscopic inflammation score in the colon tissue. The grades were conveyed by three investigators blinded for the treatment of the mice.

2.5. T. spiralis infection

After the mice were sacrificed, their diaphragms were examined under a microscope, and our results indicated that *T. spiralis* successfully infected the mice. The success rates of infection and parasitic density were calculated.

2.6. Disease activity index evaluation

The mice were observed daily in terms of the changes in their mental status, activity, hair gloss, appetite, and defecation (presence of blood, stool pattern, defecation frequency). Each group of mice was given a disease activity index (DAI) score according to international standards: DAI = (weight loss score + stool score + blood stool score)/3. To detect fecal occult blood, we followed Benzidine method.

2.7. Macroscopic and microscopic assessment of colon injury

After the mice were sacrificed by cervical dislocation, the abdominal wall was opened and the intestine was exposed. The entire segment of the colon from the rectum to the cecocolic junction was removed, opened and rinsed thoroughly with normal saline. The isolated colon was examined for the macroscopic damage. Scores were assessed by using the following damage scoring system (Morris and Beck, 1989): 0: no damage; 1: localized hyperaemia without ulcers; 2: linear ulcers with no significant inflammation; 3: linear ulcer with inflammation at one site; 4: ulcer and inflammation at two or more places; 5: two or more major sites of inflammation and ulceration or one major site of inflammation and ulceration extending more than 1 cm along the colon. Then, colon specimens were fixed in 10% paraformaldehyde for hours and made into paraffin sections. Hematoxylin and eosin (HE) staining was performed. The pathological sections of the colon were observed under optical microscope. For histological damage, using the criteria of Wallace and Keenan (1990): 0: intact tissue construction with no apparent damage; 1: damage limited to surface epithelium; 2: localized ulcer confined to mucosa; 3: focal, transmural inflammation and ulceration; 4: extensive transmural ulceration and inflammation adjacent to normal mucosa; 5: extensive transmural ulceration and inflammation involving entire section. All datas are from three separate experiments and the scores assigned by three observers without knowing the state of the mice.

2.8. ELISA was used to detect the expression of IL-4 and IFN-γ in colon tissues

Colon tissues were cut and weighed. The lysate was added and homogenized with a glass homogenizer. After completion of lysis, 10,000–14,000 g was centrifuged for 3–5 min to obtain the supernatant, following the ELISA kit manual for cytokine detection (Bioss). The amount of cytokines were expressed per mg of total protein. All datas are from three separate experiments.

2.9. Western blotting of NF-κB p65 in colon tissues

Fifty micrograms of colon homogenate proteins were boiled with 5 × SDS-PAGE Sample Loading Buffer and separated by 12% polyacrylamide gel electrophoresis. Proteins were blotted onto a nitrocellulose filter (NC) membrane. The membrane was placed into blocking buffer (5% non-fat milk) for 2 h at room temperature. And the membrane was incubated with the Anti-NF-κB and Anti-β-Actin (1:5000 diluted in blocking buffer, Bioss) at 4 °C overnight. After being washed using PBST, the membrane was incubated with a peroxidase conjugated secondary antibody, which was diluted in 5% non-fat milk on a shaker for 1 h at room temperature. Being washed, the membrane was exposed using exposure equipment after dropping ultra-sensitive ECL chemiluminescence reagent (Alphabiotech). The bands were quantified by densitometry.

2.10. Flow cytometry was used to detect the expression of CD4 + CD25 + Foxp3 + treg in spleen and MLN

Spleen and Mesenteric lymph node (MLN) lymphocyte single cell suspension was prepared and dispensed into 1×10^6 cells in each tube. The cells were resuspended in PBS and incubated for 30 min in the dark with FITC-Rat-anti-mouse CD4 and APC-Rat-anti-mouse CD25 (SUNGENE BIOTECH). Fixation/permeabilization solution (Invitrogen, USA) was added to the resuspended cells to incubate for 30–60 min, and solution was washed again. After resuspension of cells, PE-Rat-anti-mouse Foxp3 (SUNGENE BIOTECH) was added, and the solution was incubated for 20 min, then washed and resuspended again for Flow cytometry testing. All datas are from three separate experiments.

2.11. Statistical analysis

All results were expressed as the mean ± standard error. Data were evaluated using one-way ANOVA analysis and SPSS 13.0 software. $P < 0.05$ was considered as statistically significant. All statistical analysis were performed using GraphPad Prism software.

3. Results

3.1. Survival rate

The survival rate of mice in the Colitis was only 66.67%, which was significantly lower than the survival rate of the TS-Colitis (86.67%; $P < 0.05$). Moreover, the survival rate of mice in the Control and TS-Control were 100%.

3.2. Weight change

The day of TNBS model established was experimental day 0. The body weight of mice in each group was recorded daily from the beginning until the end of experiment. The results were showed in Fig. 1. Due to pre-infection with *T. spiralis*, the initial weight of mice in the TS-Control was significantly lower than the other groups but was gradually increasing. The weight of mice in the Colitis was significantly lower that of the Control. The weight of mice in the Colitis continued to decrease. On day 7, the weight of the mice decreased to 2.77% and gradually increased. On day 13, the weight of the mice increased by 2.09%. The weight of the mice in the TS-Colitis decreased relatively slowly compared with that in the Colitis. On day 7, the weight reduced from 2.22% to the lowest value. Afterward, the weight gradually increased until it reached 3.13% on day 13.

3.3. DAI score

Twenty-four hours after modeling, mice in the Colitis manifested lethargy, sluggishness, appetite loss, rough hair, diarrhea, bloody stools

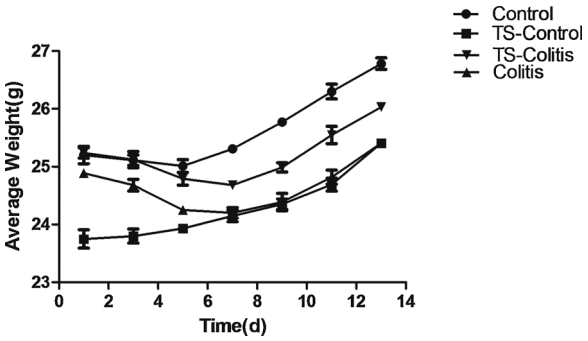

Fig. 1. Changes in weight of the mice in each group. The weight of the mice in the three other groups except TS-Control decreased until day 3. Afterward, they slowly regained weight. Data are shown as mean ± SD of 3 mice per group.

or fecal occult blood (+ ~ + +), and weight loss. These symptoms were most evident 3 days after modeling. All the symptoms in mice of the TS-Colitis were lower than those in the Colitis, and significantly different on day 1 and 3 ($P < 0.05$). The conditions of the surviving mice in both groups started to improve gradually on day 4, and the mice were relieved on day 7. In contrast to the mice in the Colitis, the mice in the TS-Colitis were still recovering. The DAI scores of the three groups at different time points were shown in Fig. 2.

3.4. Colon pathological changes

3.4.1. Macroscopic assessment of colon injury

On day 3 after modeling, macroscopic damage of colon showed that the intestinal wall was thickened, the intestine was congestive and narrow and formed a wide range of ulcerations, and in severe cases, the organ was transmural and could adhere to the surrounding tissues. The mucosa and the submucosal hyperemia and edema was segmental and could affect the whole colon. The damage score was 4.33 ± 0.34. Seven days after modeling, the colon injury of mice in the Colitis was alleviated; the ulcer area was reduced, and submucosal congestion and edema were also lessened; the stenosis of the intestine cavity rarely occurred, and the injury score was 2.44 ± 0.20. On day 14

Disease Activity Index Score Parameters

Weight loss (%)	Stool	Bloody stool	index
0-1 %	normal	none	0
1-5 %	soft and shaped	between	1
5-10 %	loose	slight	2
10-15 %	between	between	3
>15 %	diarrhea	gross bleeding	4

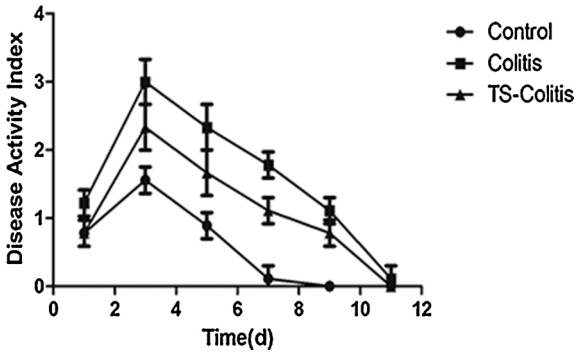

Fig. 2. DAI assessment of the TS-Colitis was lower than that of the TS-Control. Data are shown as mean ± SD of 3 mice per group.

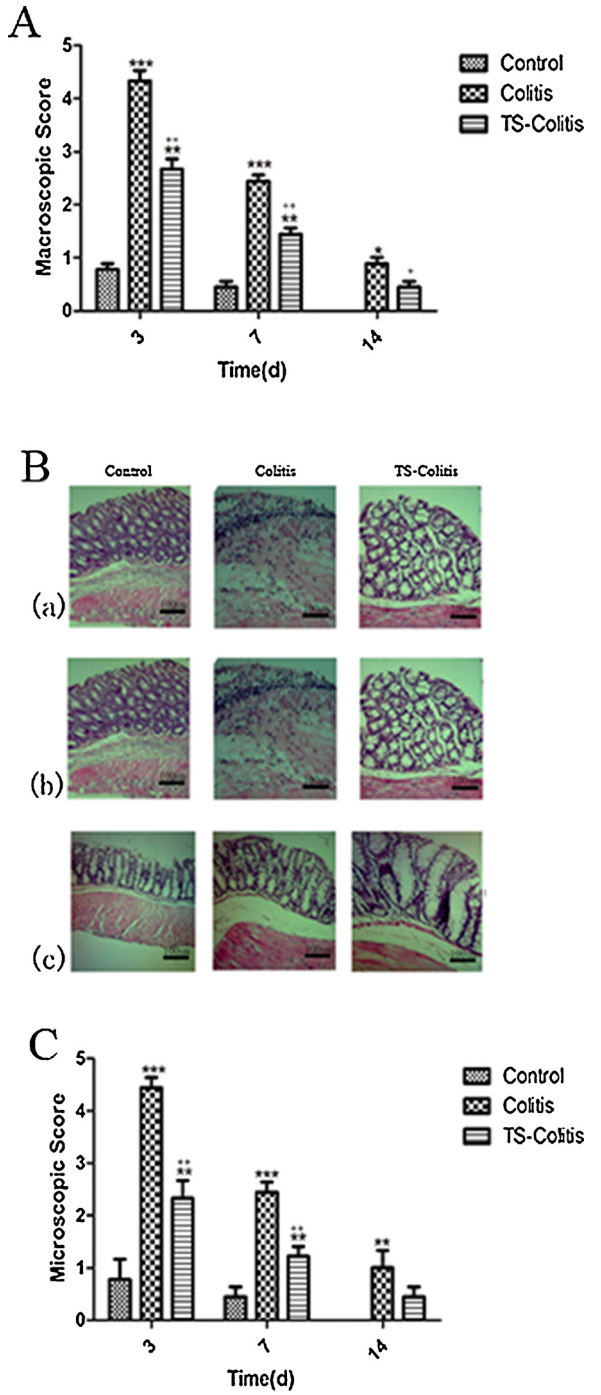

Fig. 3. Macroscopic scores (A) and microscopic scores (C) of the colons of the TS-Colitis were lower than those of the Colitis. Data are shown as mean ± SD of 3 mice per group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus Control of the same day, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.0001$ versus TS-Control of the same day and $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ versus TS-Colitis of the same day. Light micrograph of HE-stained colonic section of the Control, the Colitis, and the TS-Colitis 3 (a), 7 (b), and 14 days (c) after administration of TNBS. Scale bar represents 100 μm (B).

(0.89 ± 0.19), inflammatory symptoms basically disappeared. The mice pre-infected with *T. spiralis* had a significant improvement ($p < 0.01$) in terms of macroscopic injury compared with the Colitis on days 3 (2.67 ± 0.34) and 7 (1.44 ± 0.20). The colon was detected with slight congestion and edema and was diagnosed to have ulcer in the epithelium, or close to normal. In the Control, slight inflammatory changes were observed in the colon 3 days after modeling (0.78 ± 0.19), and normal conditions were restored on day 7 (0.44 ± 0.20) (Fig. 3(A)).

3.4.2. Microscopic assessment of colon injury

On day 3 after modeling, severe inflammatory reaction symptoms could be observed: intestinal epithelial cells were necrotic and shedding, intestinal villi were shorten and fused, and crypt was extended. Meanwhile, a large number of lymphocytes, neutrophils, and eosinophils were infiltrated into the mucosa and submucosa. The mucosal glands were not aligned or damaged. The score of injury was 4.44 ± 0.20, which was significantly different from the score of the Control (0.78 ± 0.39; $p < 0.001$). In the TS-Colitis, the lesion was confined to the mucosal layer, the infiltration of inflammatory cells was weakened, and the edema was mild. The score of injury was 2.33 ± 0.34, which was significantly reduced compared with the Colitis ($P < 0.01$). On day 7 after modeling (1.22 ± 0.19), the symptom of inflammatory reaction was relieved ($P < 0.01$). On day 14 (0.44 ± 0.20), the manifested symptoms basically returned to normal ($P > 0.05$) (Fig. 3(C)).

3.5. Production of IFN-γ and IL-4 in the colon

ELISA revealed that IFN-γ production in the colon tissues of the TS-Colitis was lower than that of the Colitis 3 days after modeling ($P < 0.01$). This parameter was significantly lower in the TS-Colitis than in the Colitis on days 7 ($P < 0.01$) and not significantly lower on days 14 ($P > 0.05$; Fig. 4(A)). At the same time, compared with the Colitis, the expression of IL-4 in the colon tissues of the mice in the TS-Colitis significantly increased on days 3 ($P > 0.05$) and 7 ($P < 0.05$) and remained high until day 14 ($P < 0.05$) (Fig. 4(B)). The IL-4 expression in the colon of mice that were only infected with *T. spiralis* was

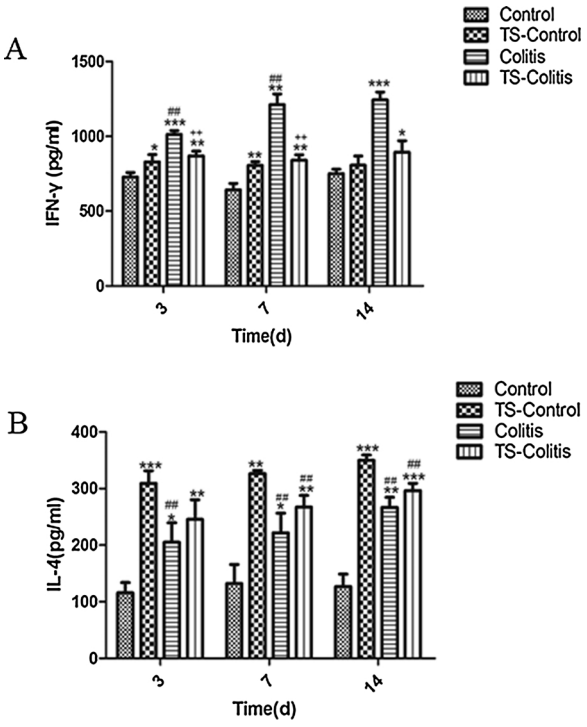

Fig. 4. IFN-γ (A) and IL-4 (B) concentration in the colon homogenate supernatants was tested by using ELISA. IFN-γ concentration increased in the Colitis and decreased in the TS-Colitis. IL-4 level decreased in the Colitis and increased in the TS-Colitis. Data are shown as mean ± SD of 3 mice per group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus Control of the same day, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.0001$ versus TS-Control of the same day and $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ versus TS-Colitis of the same day.

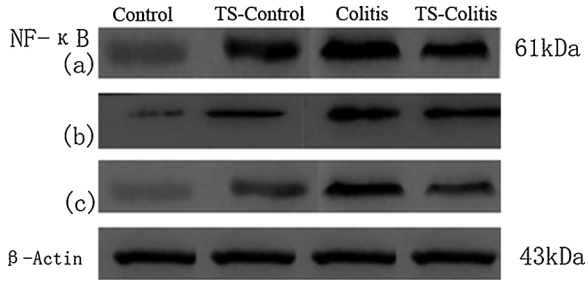

Fig. 5. The expression of NF-κB in colon tissues of four groups on 3 (a), 7 (b), and 14 days (c).

high, whereas the IFN-γ expression in the TNBS-induced colitis model was higher than that in the other groups.

3.6. Nuclear level of NF-κB p65 protein in colonic samples

Western blotting resules revealed that the NF-κB expression in the colon tissues of the TS-Colitis was significantly higher than that of the Control ($P < 0.01$) and lower than that of the Colitis 3 days after modeling ($P < 0.05$). This parameter was significantly lower in the TS-Colitis than in the Colitis on days 7 and 14 ($P < 0.05$) (Fig. 5).

3.7. Changes of t lymphocytes and CD4 + CD25 + Foxp3 + Treg cells in spleen and MLN

The mice were sacrificed 3, 7, and 14 days after TNBS modeling. The spleens and MLNs of each group were removed, and the expression of T lymphocytes and CD4 + CD25 + Foxp3 + Treg cells was detected by flow cytometry (Fig. 6). The number of T lymphocytes in spleens was significantly different between the Colitis and the TS-Colitis on the 3rd and 7th day ($P < 0.05$). On day 3, the percentage of CD4 + CD25 + Foxp3 + Treg cells in T cells in the spleens of the TS-Colitis was increased compared with the Colitis ($P < 0.05$). Moreover, the percentage of CD4 + CD25 + Foxp3 + Treg cells still increased on days 7, but no significant differences were observed ($P > 0.05$). And on days 14, the TS-Colitis compared with the Colitis ($P < 0.05$) was also increased. The percentage of CD4 + CD25 + Foxp3 + Treg cells throughout the experiment increased of the Colitis and the TS-Colitis ($P < 0.01$).

The trend of the change of T lymphocytes and Treg cells in MLN is similar to that of spleen. The number of T lymphocytes in MLN of the Colitis was significantly increased on 3rd and 7th day ($P < 0.05$) and significantly higher than that of the TS-Colitis ($P < 0.01$). And the number of CD4 + CD25 + Foxp3 + Treg cells of the TS-Colitis was significantly increased on 3rd, 7th ($P < 0.01$) and 14th ($P < 0.05$) than the Colitis (Fig. 7).

4. Discussion

Infection from worms can elicit varying degrees of intervention effects on IBD model (Wang et al., 2008). Elliott et al. (2000) first proposed that *Schistosoma mansoni* infection can relieve the mouse colitis model. Reardon et al. (2001) demonstrated that infection with *Taenia solium* can improve DSS-induced intestinal inflammation. Khan et al. (2002) confirmed that *T. spiralis* infection can relieve the severity of DNBS-induced colitis. Elliott et al. (2003) reported that *Schistosoma* eggs can be used to treat TNBS-induced colitis. Although the effects of *T. spiralis* on a TNBS-induced colitis model have been investigated, previous research mainly focused on different expression levels of Th1 and Th2 cytokines. Therefore, the present experiment was based not only on Th1 and Th2 immune responses but also on changes in the

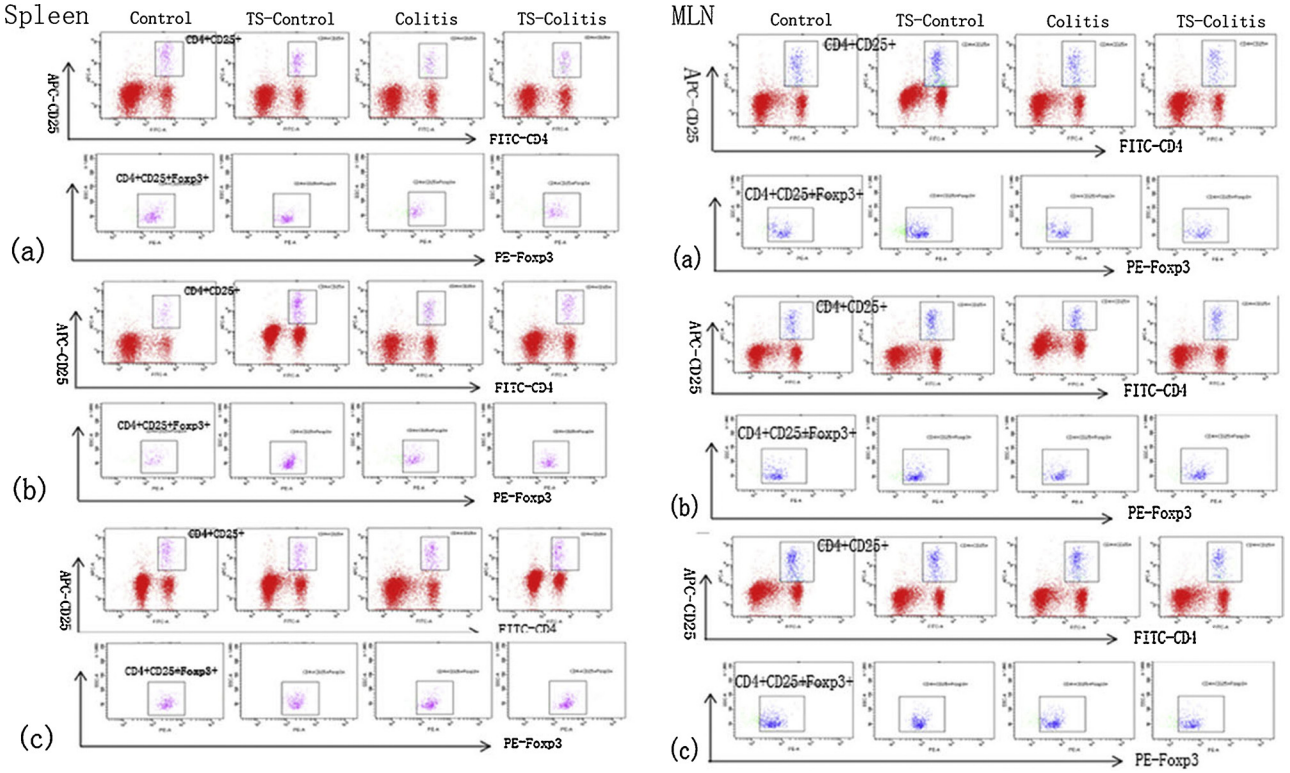

Fig. 6. Demonstration of the gating strategy for the flow cytometric analysis of mouse CD4 + CD25 + Foxp3 + Treg from spleens and MLNs 3 (a), 7 (b), and 14 days (c) after administration of TNBS. In this experiment a single cell suspension was prepared from the spleen and MLN of each group of mouse and stained with CD4 (FITC), CD25 (APC) and Foxp3 (PE) based on surface and intracellular staining protocols, respectively. Data were collected with FACSDiva flow cytometer and analyzed. Lymphocytes are identified by their scatter properties (FSC-A x SSC-A plot). Treg subpopulations were characterized by CD4 and CD25 surface expression, intracellular Foxp3 staining.

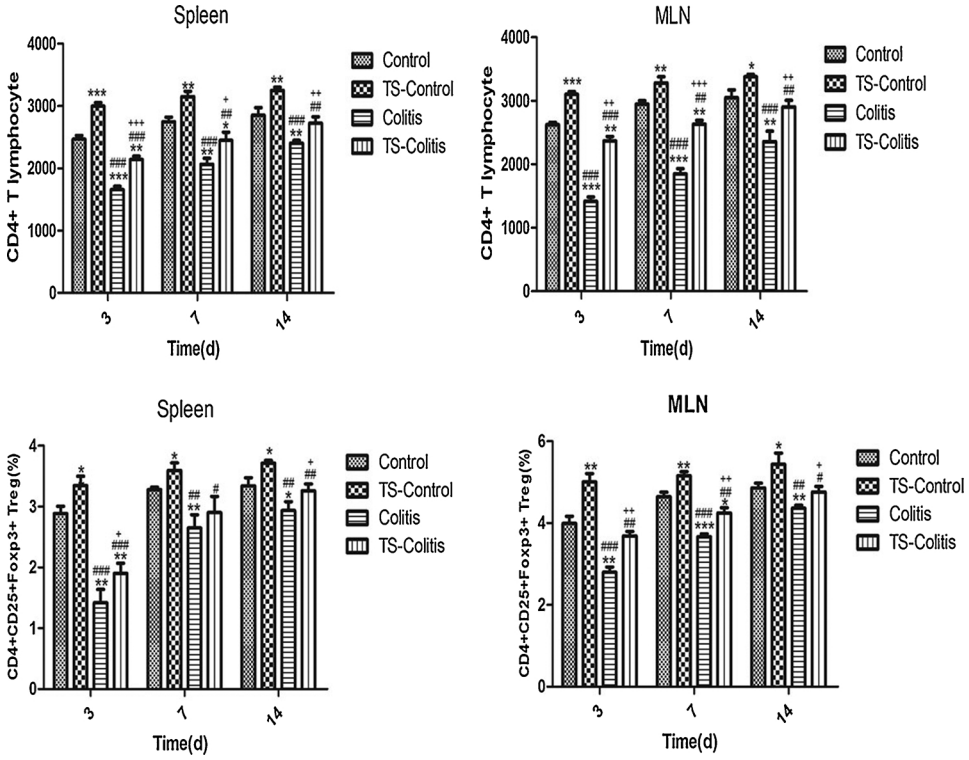

Fig. 7. The number of T lymphocytes in spleens and MLNs of four groups on 3, 7, and 14 days (A). And analysis of CD4 + CD25 + Foxp3 + Treg cells in spleens and MLNs of each group (B). The number of CD4 + CD25 + Foxp3 + Treg cells in the TS-Colitis increased compared with that of the Colitis. Data are shown as mean ± SD of 3 mice per group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus Control of the same day, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.0001$ versus TS-Control of the same day and $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ versus TS-Colitis of the same day.

number of Treg cells. This work aimed to investigate the role of Treg cells during the immune regulation of *T. spiralis* infection in TNBS-induced colitis.

The TS-Colitis showed an increased survival rate, reduced extent of intestinal damage, decreased DAI score, and significantly reduced intestinal inflammatory symptoms compared with those of the TS-Control. Therefore, infection with *T. spiralis* could relieve TNBS-induced colitis, and this finding was consistent with that observed in other helminth infections.

The TNBS-induced CD model stimulated the onset of other local intestinal mucosal immune disorders. Mononuclear cells in the mesentery lamina propria express increased IFN-γ levels, accompanied by systemic immune dysfunction, increased expression of the anti-inflammatory cytokine IL-4, decreased expression of the pro-inflammatory cytokine IFN-γ, and promoted development of diseases (Akazawa, 2002; Indaram, 2002). The mechanism of worm infection in treating CD is complicated and generally considered to balance Th1- and Th2-type immune responses. Th1 cells mainly secrete IL-2, IFN-γ, IFN-α, TNF-β, and other cytokines, whereas Th2 cells mainly release IL-4, IL-5, IL-6, and IL-10. In this experiment, IFN-γ and IL-4 were selected as a representative of Th1- and Th2-type cytokines.

ELISA demonstrated that infection with *T. spiralis* reduced the expression of IFN-γ and increased the expression of IL-4 in the mice of the Colitis, indicating that this infection caused the CD mucosal immune response to shift from Th1 to Th2. These responses reduced the intensity of the Th1-type immune response and thus relieved intestinal inflammation. These outcomes were consistent with the expected experimental results. From another perspective, CD also stimulated the shifting of the Th2-type immune response induced by *T. spiralis* larval infection to a Th1-type immune response, reduced the intensity of Th2-type immune response, and possibly alleviated the pathological changes attributed to parasitic infection in the body.

NF-κB is closely related to IBD. Previous experiment found that TNF-α and activated NF-κB were up-regulated and IκB was down-regulated in mouse colitis model. Another experiment showed that the NF-kB expression in active IBD patient's mononuclear cells in intestinal lamina propria was increased, above all suggested that the activation of NF-κB played an important role in intestinal mucosal immune response disorder of IBD, and the activated NF-κB promoted the expression of multiple proinflammatory cytokines and increased the recruitment of inflammatory cells, played an important role in the cell signal transduction of intestinal inflammation (Karin et al., 2004; Tak and Firestein, 2001). Western blotting results in this experiment showed that the expression of NF-κB in the colon tissue of the Colitis group was significantly higher than the Control group, and lower than the TS-Colitis group, these results suggested that overexpression of NF-κB aggravated the symptoms of intestinal inflammation, and pre-infection of Trichinella spiralis can reduce the activation of NF-κB and the secretion of proinflammatory cytokines, thus relieved intestinal inflammation, provided a strategy for the treatment of IBD.

The occurrence and development of IBD are also associated with a large number of intestinal mucosal immune regulatory cells, and the role of Treg cells in IBD has been extensively investigated. Treg cells are T cell subsets that can perform immunomodulatory functions, inhibit the activation and proliferation of other immune cells, maintain the body's immune balance, and prevent the development of autoimmune diseases (Maggi et al., 2012; Josefowicz and Lu, 2012). Foxp3 is a surface-specific marker of CD4 + CD25 + Treg cells produced in the thymus and peripheral blood. Huan et al. (2005) verified that Foxp3 is an important transcriptional regulator of CD4 + CD25 + Treg cell development and immune suppressive function and implicated in promoting the expression, maturation, and function of CD4 + CD25 + Treg cells (Kamikozuru et al., 2009), but CD4 + CD25 + Treg cells that express Foxp3 elicit immunomodulatory effects (Fonteont and Rudensky, 2005; Hori and Sakaguchi, 2004). During parasitic infection, host immunity is suppressed in its developmental stage to establish a parasitic life, resulting in an increased number of Treg cells in hosts. Beiting et al. (2007) detected an increased number of CD4 + CD25 + Foxp3 + Treg cells in the muscle of *T. spiralis*-infected mice, demonstrating that Treg cells participated in the survival of *T. spiralis* larvae in host skeletal muscle.

The results showed that the number of T lymphocytes and CD4 + CD25 + Foxp3 + Treg cells in spleen of the mice of the Colitis were significantly lower than that of the Control. Simultaneously, the

number of T lymphocytes and CD4 + CD25 + Foxp3 + Treg cells of the TS-Colitis were higher than that of the Colitis on the 3rd, 7th and 14th day. During the recovery period, T lymphocytes and CD4 + CD25 + Foxp3 + Treg cells all increased significantly. Due to the similar trend of T lymphocyte number and CD4 + CD25 + Foxp3 + Treg cell number, it is not certain whether the number of T lymphocyte changed leaded to the change of CD4 + CD25 + Foxp3 + Treg cells number. Therefore, the effect of T.spiralis intervention on IBD acted on T lymphocytes or Treg cells directly need to be further explored. At the same time, the overall trend of T lymphocyte number and CD4 + CD25 + Foxp3 + Treg cell number in MLN was similar to that of spleen.

Although Th1, Th2, and Treg cells interact with one another through the cytokine network, different transcriptional pathways involved in this process should be further investigated. Although parasitic infection could effectively alleviate the severity of autoimmune diseases, such as IBD, oral administration of eggs and acceptance of the idea that a worm is living inside your body could be difficult for patients. Therefore, we should develop a new research strategy that aims to use parasitic products as a substitute for parasitic infections in the treatment of IBD.

5. Conclusions

In summary, the infection of *T. spiralis* larvae caused a Th2-type immune response in the human body, resulting in an increase in IL-4 expression and a decrease in IFN-γ expression in colon tissues. And the expression of NF-κB was decreased. The number of T lymphocytes and CD4 + CD25 + Foxp3 + Treg cells also increased. Therefore, pre-infection with *T. spiralis* could relieve the TNBS-induced CD model of Th1-type intestinal inflammation.

Conflict of interest

The authors declare no conflict of interests.

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References

Adorini, L., Sinigaglia, F., 1997. Pathogenesis and immunotherapy of autoimmune diseases. IHlnlUlloI Today 18, 209–211.
Akazawa, A., 2002. Increased expression of tumor necrosis factor-alpha messenger RNA in the imestinal mucosa of inflammatory bowel disease, particularly inpatients with disease in the active phase. J. Oastroenterol. 37, 345.
Beiting, D.P., Gagliardo, L.F., Hesse, M., Bliss, S.K., Meskill, D., Appleton, J.A., 2007. Coordinated control of immunity to muscle stage Trichinella spiralis by IL-10, regulatory T Cells, and TGF-β. J. Immunol. 178 (2), 1039–1047.
Blumberg, R.S., Strober, W., 2001. Prospects for research in inflammatory bowel disease. JAMA 285 (5), 643–647.
Braegger, C.P., Macdonald, T.T., 1994. Immune mechanisms in chronic inflammatory bowel disease. Ann. Allergy 72 (2), 135–141.
Cho, J.H., 2008. The genetics and immunopathogenesis of inflammatory bowel disease. J. Nat. Rev. Immunol. 8, 458–466.
Elliott, D.E., Urban, J.F.J.R., Argo, C.K., Weinstock, J.V., 2000. Does the failure to acquire helminthic parasites predispose to Crohn's disease? FASEB J. 14 (12), 1848–1855.
Elliott, D.E., Li, J., Blum, A., Chowdhury, S.K., Omsted, P., Deng, Y., Collins, S.M., 2003. Exposure to Schistosome eggs protects mice from TNBS-induced colitis J. Am. J. Physiol. 284 (3), G385–G391.
Else, K.J., 2005. Have gastrointestinal nematode outwitted the immune system. J. Parasite Immunol. 27 (10–11), 407–415.
Fonteont, J.D., Rudensky, A.Y., 2005. A well adapted regulatory contrivance: regulatory T cell development and the forkhead family transcription factor Foxp3. J. Nat. Immunol. 6 (4), 331–337.
Halme, L., Paavola-Sakki, P., Turunen, U., Lappalainen, M., Farkkila, M., Kontula, K., 2006. Family and twin studies in infiammatory bowel disease. J. World J. Gastroenterol. 12, 3668–3672.
Hori, S., Sakaguchi, S., 2004. Foxp3: a critical regulator of the development and function of regulatory T cells. J. Microbes Infect. 6 (8), 745–751.
Huan, J., Culbertson, N., Spencer, L., Bartholomew, R., Burrows, G.G., Chou, Y.K., Bourdette, D., Ziegler, S.F., Offner, H., Vandenbark, A.A., 2005. Decreased Foxp3 levelsin multiple sclerosis patients. J. Neurosci. Res. 81, 45–52.
Indaram, A.V., 2002. Mucosal cytokine production in radiation-induced moiditis compared with inflammatory bowel disease. J. AMJ Gastroenterol. 95, 1221.
Josefowicz, S.Z., Lu, L.F., 2012. Regulatory T cells: mechanisms of differentiation and function. Annu. Rev. Immunol. 30, 531–564.
Kamikozuru, K., Fukunaga, K., Hirota, S., Hida, N., Ohda, Y., Yoshida, K., Yokoyama, Y., Tozawa, K., Kawa, K., Iimuro, M., Nagase, K., Saniabadi, A.R., Nakamura, S., Miwa, H., Matsumoto, T., 2009. The expression profile of functional regulatory T cells, CD4 + CD25high + / forkhead box protein P3 +, in patients with ulcerative colitis during active and quiescent disease. J. Clin. Exp. Immunol. 156 (2), 320–327.
Karin, M., Yamamoto, Y., Liotta, F., Wang, Q.M., 2004. The IKK NF-kappa B system: a treasure trove for drug development. Nat. Rev. Drug Discov. 3, 17–26.
Khan, W.I., Blennerhasset, P.A., Varghese, A.K., Chowdhury, S.K., Omsted, P., Deng, Y., Collins, S.M., 2002. Intestinal nematode infection ameliorates experimental colitis in mice. J. Infect. Immun. 70 (11), 5931–5937.
Kyle, 1992. Crohn's disease in the northeastern and northern Isles of Scotland: an epidemiological review. Gastroenterology 103 (2), 392–399.
Maggi, E., Cosmi, L., Liotta, F., Romagnani, P., Romagnani, S., Annunziato, F., 2012. Thymic regulatory T cells. Autoimmun. Rev. 4, 579–586.
Markus, E.N., 1995. Antibodies to interleukin 12 abrogate established experimental colitis in mice. J. Exp Med. 182 (9), 1281–1290.
Morris, G.P., Beck, P.L., 1989. Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterology 96, 795–803.
Mosmann, T.R., 1991. Cytokine secretion patterns and cross-regulation of T cell subsets. J. Immunol. Res. 10 (3–4), 183–188.
Reardon, C., Sanchez, A., Hogaboam, C.M., McKay, D.M., 2001. Tapeworm infection reduces epithelial ion transport abnormalities in murine dextran sulfate sodium-induced colitis. J. Infect. Immun. 69 (7), 4417–4423.
Rocken, M., Racke, M., Shevach, E.M., 1996. IL-4-induced immune deviation as antigen-specific therapy for infiammatory autoimmune disease. Immun. D Today 17, 225–231.
Stallmach, A., Marth, T., Weiss, B., 2004. An interleukin 12 p40-Ig G2b fusion protein abrogates T cell mediated inflammation: anti-inflammatory activity in Crohn's disease and experimental colitis in vivo. Gut 53 (3), 339–345.
Strober, W., Fuss, I., Mannon, P., 2007. The fundamental basis of inflammatory bowel disease. J. Clin. Invest. 117, 514–521.
Strober, W., 2002. The immunology of mucosal models of inflammation. J. Annu. Rev. Immunol. 20, 495–549.
Tak, P.P., Firestein, G.S., 2001. NF-kappaB: a key role in inflammatory diseases. Clin. Invest. 7, 7–11.
Wallace, J.L., Keenan, C.M., 1990. An orally active inhibitor of leukotriene synthesis accelerates healing in a rat model of colitis. J. Am. J. Physiol. 258, 527–534.
Wang, L.J., Cao, Y., Shi, H.N., 2008. Helminth infections and intestinal inflammation. J. World J. Gastroenterol. 14 (33), 5125–5132.