Research Article

Th17: A New Participant in Gut Dysfunction in Mice Infected with *Trichinella spiralis*

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*Trichinella spiralis* infection in rodents is a well-known model of intestinal inflammation associated with hypermotility. Our aim was to elucidate if Th17 cells were involved in the development of gastrointestinal hypermotility in this experimental model. Intestinal inflammation was observed by hematoxylin-eosin (HE) staining. Jejunal smooth muscle contractility was investigated in response to acetylcholine (Ach). The effects of IL-17 on jejunal smooth muscle contractility were explored. Flow cytometry was used to analyze the proportion of Th17 cells in jejunum. The levels of IL-17, IL-23, and TGF-β1 in jejunum were measured by Western blot. Our results showed that the inflammation in jejunum was severe at 2 weeks postinfection (PI), which was not discernible at 8 weeks PI. Jejunal smooth muscle contractility was increased at 2 weeks PI and kept higher at 12 weeks PI. The proportion of Th17 cells and the expression of IL-17 were upregulated in jejunum at 2 weeks PI and normalized at 8 weeks PI. When jejunal smooth muscle strips were cultured with IL-17, contractions elicited by Ach were enhanced in a concentration-dependent manner. Our data suggest that Th17 cells are increased during acute infection with *Trichinella spiralis* and IL-17 may contribute to jejunal muscle contractility in mice.

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1. Introduction

Intestinal motor alterations are associated with clinical symptoms such as diarrhea, constipation, and abdominal pain. Studies demonstrated that visceral hypersensitivity and persistently altered intestinal muscle dysfunction existed in mice infected with *Trichinella spiralis* [1, 2]. In fact, the adaptive response to intestinal parasites has been suggested as a paradigmatic defense response of the intestine against external pathogens. For these reasons, experimental parasite infection has been commonly used as model to understand pathogenesis of intestinal dysfunction [3–5].

The immune changes in *Trichinella spiralis* infection appear to be T helper (Th) cell dependent, as have been shown to be prevented by cyclosporine [6]. According to their capability of producing cytokines, Th cells were classified as three distinct subsets, Th1, Th2, and Th17. Characteristically, Th1-type cells produce interleukin (IL)-12 and IFN-γ; Th2-type cells synthesize IL-4, IL-5, IL-6, IL-9, and IL-10. Th17-type cells, newly discovered subset of CD4+ effort T cells, produce IL-17 distinctively.

Although studies have been performed on the roles of Th2 cells and related cytokines in intestinal dysfunction during infection of *Trichinella spiralis* [7, 8], it is unknown about the functionality of Th17 in this process. Previously, TGF-β1 has been commonly considered as an anti-inflammatory cytokine but now found to be critical in the differentiation of Th17 cells [9]. Recent work by D. Yen showed that IL-23 stimulated Th17 to generate the proinflammatory mediator IL-17, which is important to maintain the chronic intestinal inflammation [10]. The aim of this study is to analyze the roles of Th17 cells and related cytokines in gut dysfunction in mice infected with *Trichinella spiralis*.

2. Materials and Methods

2.1. Mice. Studies were performed on male inbred National Institute of Health (NIH) mice obtained from the Institution of Biological Product (Wuhan, China) and used between 6 and 8 weeks of age. Mice were bred in an accredited facility at the Institute for Animal Health maintained at 23–24°C in
were incubated at 37°C and L-glutamine (200 μM, Invitrogen). The culture dishes contained penicillin (100 units/mL)/streptomycin (100 μg/mL, Gibco) and were incubated at 37°C for 5 hours. The isolated serosal surface was washed several times with 0.85% NaCl and suspended in 2% of Agar (Sigma-Aldrich). Mice were infected by the oral administration of 300 larvae in 0.2 mL of 0.85% NaCl. Jejunum was thoroughly washed with PBS and then cut into 0.5 cm pieces. The epithelium was removed by incubation with 1 mM DTT (Sigma-Aldrich) and 1 mM EDTA (Sigma-Aldrich) for 30 min with gently shaking. After repeating this step twice, the tissue was cut into smaller pieces and then digested with 1 mg/mL collagenase D (Roch) for 37°C for 90 min. Lamina propria cells were harvested by discontinuous 40/70 percoll gradient (Amersham Biosciences).

2.4. Histological Study. Samples of jejunum were obtained, fixed for 48 hours in 10% neutral buffered formalin, embedded in paraffin, cut into 5 μm sections, and stained with hematoxylin-eosin (H&E) according to standard procedures.

2.5. Tissue Preparation and Organ Culture Procedure. The method used was described in detail by Ohama et al. [12]. Briefly, mice were executed by cervical vertebra disjointing. A 10 cm segment of the jejunum was detached from mesenterium and placed in sterile Hanks’ balanced salt solution. Strips were teased along the natural line of cleavage from the longitudinal smooth muscle and then transferred to culture dishes with Medium 199 (Invitrogen) supplemented with penicillin (100 units/mL)/streptomycin (100 μg/mL, Gibco) and L-glutamine (200 μM, Invitrogen). The culture dishes were incubated at 37°C in an atmosphere of 95% air and 5% CO₂. The incubation medium was replaced every day. Jejunum longitudinal muscle was preincubated with or without IL-17 (0.1-10 ng/mL, Peprotech) for 2 days. Freshly isolated smooth muscle strips were prepared as described above but not under sterile conditions.

2.6. Measurement of Muscle Contractility. Longitudinal muscle strips were cut by 3 mm × 10 mm and then placed in 10 mL organ bath containing warm (37°C) oxygenated (95% O₂, 5% CO₂) Krebs solution. The upper end of each strip was attached to an isometric force transducer (Forte-10, WPI, USA), which was connected to an amplifier. The digitized data were collected by a computer equipped with Acknowledge 3.7.1 software (BIOPAC system, USA). After an equilibration period of 60 minutes with flushing in every 15 minutes at a load of 0.25 g, the length–tension relationship was recorded. Muscle strips were stretched by load increments of 0.25 g exposed to 10⁻⁶ M Ach. The degree of applied tension producing the maximum response to Ach was identified as optional tension. The area under curve (AUC: g·s) was measured in time intervals of 5 min after Ach addition. The response in different groups was quantified by calculating the AUC when muscles were stretched by application of optional tension.

2.7. Isolation of Intestinal Lamina Propria Mononuclear Cells. Jejunum was thoroughly washed with PBS and then cut into 0.5 cm pieces. The epithelium was removed by incubation with 1 mM DTT (Sigma-Aldrich) and 1 mM EDTA (Sigma-Aldrich) in RPMI 1640 medium supplemented with 5% FCS at 37°C for 30 min with gently shaking. After repeating this step twice, the tissue was cut into smaller pieces and then digested with 1 mg/mL collagenase D (Roch) at 37°C for 90 min. Lamina propria cells were harvested by discontinuous 40/70 percoll gradient (Amersham Biosciences).

2.8. Surface and Intracellular Cytokine Staining. Cells obtained from dissection of lamina propria were incubated for 4 h with 50 ng/mL PMA (Alexis), 750 ng/mL Ionomycin (Sigma-Aldrich), and 10 mg/mL Brefeldin A (Biolegend) in a tissue culture incubator at 37°C. After surfaces staining with the phycoerythrin-conjugated antismouse CD4 antibody (Biolegend), the cells were fixed and permeabilized with Fixation/Permeabilization solution (Biolegend). Then the cells were stained intracellularly with allogum cynalin-conjugated antimouse IL-17 antibody (Biolegend). Cytokine staining was performed as the manufacturer’s protocol. Samples were acquired on a LSR II (BD Biosciences) and data were analyzed by FACSDiVa software (BD Biosciences).

2.9. Western Blot Analysis. A total of 80 μg of protein lysates derived from jejunal tissue samples were loaded on 15% SDS-PAGE gels. Membranes were probed overnight at 4°C with antibodies against IL-17A (R&D System), TGF-β1 (Biovision), IL-23p19 (Santa Cruz), or β-Actin (Pierce) antibodies, followed by the appropriate species-specific horseradish peroxidase conjugate (Pierce) and developing in the SuperSignal West Pico Substrate (Pierce). Band intensities were quantitated by the Quantity One 4.6.2 software (BioRad).

2.10. Statistical Analysis. Data are expressed as means ± SD. Statistical significance was calculated with the Kruskal-Wallis or Mann-Whitney test as appropriate using SPSS 11.0 software. The correlation between gut contraction and expression of IL-17 was analyzed using Spearman’s rho-test. All P value of .05 or less was considered significant.

3. Results

3.1. Morphology. At 2 weeks PI, H&E staining of the jejunum showed hyperemia, swelling, and decrease in villus height.
An intense inflammatory response with mixed infiltration of neutrophil cells, eosinophil cells, and lymphocytes affecting the mucosal and submucosal layers was induced by T. spiralis infection. There was no discernible inflammation presented in the gut at 8 and 12 weeks PI (Figure 1).

### 3.2. Jejunal Smooth Muscle Contraction Response to Acetylcholine

Increased contractile responses to Ach were noted in longitudinal muscle strips from 2 weeks to 12 weeks PI in the T. spiralis infected mice (Figure 2). At 2 weeks after infection, the AUC in the infected mice was significantly increased over control when maximum response was generated by longitudinal muscle response to ACh (1.63 ± 0.19 g·s versus 1.34 ± 0.18 g·s, \(P = .026\)). Longitudinal muscle contraction response kept higher at 8 weeks PI (1.60 ± 0.17 g·s versus control, \(P = .041\)) and 12 weeks PI (1.74 ± 0.10 g·s versus control, \(P = .026\)).

### 3.3. The Proportion of Th17 Cells in Mucosal Lamina Propria

In jejunum, a significant proportional increase was observed for IL-17 producing cells in the infected mice at 2 weeks PI compared with controls (9.13 ± 2.73 versus 3.78 ± 1.97, \(P < .01\)) and normalized at 8 weeks (5.38 ± 1.37) and 12 weeks (4.68 ± 1.48) (Figure 3).

### 3.4. Cytokine Expression

IL-17 is the key cytokine secreted by Th17 cells characteristically [13]. To explore whether Th17 cells take effect during infection, we analyzed the content of IL-17 in jejunum at various time points. The results showed that the expression of IL-17 was significantly elevated (\(P < .01\)) at 2 weeks PI and turned to normal thereafter (Figure 4).

Though studies have showed that TGF-\(\beta_1\) and IL-23 take part in the differentiation and expansion of Th17 cells [9, 10], but it is not clear whether TGF-\(\beta_1\) and IL-23 are involved in the inflammation in mice infected with Trichinella spiralis, so the levels of these two cytokines in the gut were studied at each time point. In jejunum, a higher level of TGF-\(\beta_1\) in infected mice was noted at 2 weeks PI compared with uninfected mice (0.31 ± 0.03 versus 0.17 ± 0.05, \(P < .01\)), and normalized at 8 and 12 weeks PI (0.26 ± 0.07 and 0.24 ± 0.07, resp.) (Figure 4). The expressions of IL-23 in the jejunum at 2, 8, and 12 weeks PI were 0.16 ± 0.02, 0.14 ± 0.04, and 0.11 ± 0.03, respectively, and no significant changes were found compared with control during infection (Figure 4).

### 3.5. Association between Gut Contraction and Expression of IL-17

To explore whether the jejunum hypercontractility in the infected mice was affected by the expression of IL-17, the relationship of jejunal muscle contraction and expression of IL-17 was analyzed. We found that the expression of IL-17 in jejunum was correlated significantly with jejunal longitudinal muscle contraction (\(r = 0.773, P = .039\)) at 2 weeks PI (Figure 5), while the correlations could not be observed at 8 or 12 weeks PI.

### 3.6. IL-17-Induced Muscle Hypercontractility

To determine the effect of IL-17 on muscle contractility, jejunal longitudinal muscle isolated from normal mice was preincubated
long time after intestinal inflammation recovered [1, 2].

observed at the early stage of infection and last for a

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After the larvae transferred to skeletal muscle, no discernible

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skeletal muscle to encyst at about 1 month PI, which is

known as muscle stage. In our study, histology investigation

4. Discussion

A number of parasite infections in rodents cause intestinal

flammation, such as *Trichinella spiralis*. During a relatively

brief intestine stage for 1-2 weeks, adult female worms

release newborn larvae that rapidly enter mesenteric venule

[14], disseminate throughout the host, and eventually enter

skeletal muscle to encyst at about 1 month PI, which is

known as muscle stage. In our study, histology investigation

showed that the presence of adult worms and larvae in

the jejunal mucosa cause a severe inflammatory response

at 2 weeks PI, which persists until eviction of the parasite.

After the larvae transferred to skeletal muscle, no discernible

inflammation was presented at 8 and 12 weeks PI.

Parasites have provided excellent models for studying

the intestine dysfunction during and after infection of pathogens. Intestinal muscle hypercontractility can be

observed at the early stage of infection and last for a

long time after intestinal inflammation recovered [1, 2].

Previous studies performed on NIH Swiss mice showed that

contractile response of jejunum longitudinal muscle strips

was remarkably increased during acute *Trichinella spiralis*

infection [1]. By 21 days postinfection, the adult worms

leave the host and the acute inflammation normalizes, but

functional alterations of the small intestine persist for at

least a further 21 days [15]. In our study, the NIH mice

were used, which are genetically closely related to but not

identical to the NIH Swiss mice and behave immunologically

very similarly to the NIH Swiss mice [16]. It should be

recognized that we not only have proven that NIH mice

infected with *Trichinella spiralis* showed the similar motility

abnormalities which others have demonstrated using this

model in NIH Swiss mice [1] but also have proven that the

hypercontractility lasted for 12 weeks PI.

Recent studies demonstrated that altered intestinal motility

in infected mice was associated with the increased T

cell in gut, which can be reversed by a corticosteroid

treatment [15]. T cells also mediate the hypercontractile

state of muscle during *Trichinella spiralis* infection [17, 18].

Furthermore, reports show that Th2 cytokines can induce

muscle hypercontractility by a direct action on smooth

muscle cell [8], while data about Th17 cells are not available.

We analyzed the proportion of Th17 cells isolated from

mucosal lamina propria and the expression of IL-17 in

jejenum in mice infected with *Trichinella spiralis*. The results

showed that the proportion of Th17 cells and the level of IL-

17 were increased at 2 weeks PI compared with the controls

and recovered at 8 weeks PI. Meanwhile, we found that

the expression of IL-17 correlated with the jejunal smooth

muscle contraction at 2 weeks PI, which implied that the

hypercontractility of intestine smooth muscle was affected

by the content of IL-17 in intestine. To confirm the effect

of IL-17 on muscle contraction, we investigated the jejunum

longitudinal smooth muscle contractility preincubated with

or without IL-17. We found that IL-17induced increase

of contractile forces in a concentration-dependent manner.

Similar finding was observed in another study that IL-17

also has a role in airway hypersensitivity responses, such

as asthma and chronic obstructive pulmonary disease [19,

20], which are associated with the increased number of

neutrophils and linked to IL-17. But how IL-17 alerts smooth

muscle contraction, by regulating excitation-contraction

coupling, by inducing other cytokines, or by other means?
The mechanism underlying the influence of IL-17 on the

contractility of intestine smooth muscle in mice during

infection needs to be further investigated. It should be noted

that the jejunal hyper contractility lasted for 12 week PI when

IL-17 had normalized, which suggested that other cytokines

or cells might work in this period of time.

Weinstock JV reported that helminth infection down-

regulates IL-17 production by lamina propria mononuclear

cells (LPMCs) and mesenteric lymph node cells at 2 weeks PI

[21]. In our study, increased number of Th17 and content

of IL-17 in jejunum were observed at 2 weeks PI and

normalized thereafter. It has been shown that the time course

during infection varies in different pathogen strains of mice

and using different doses of larvae [22, 23]. This study used

C57BL/6 mice infected with 150 H. polygyrus [21], while our

study used NIH mice infected with 300 T. spiralis. Therefore

the results may be different.

Figure 2: Responses of the jejunal longitudinal muscle to 10−6

M Ach-induced contraction. Responses were obtained from tissues

stretched when optional tention was applied. The upper and lower

whiskers indicate the maximum and minimum values, respectively.

Box lines represent the 25th (bottom), 50th (middle), and 75th

(top) percentile value; ΔP < .05 indicate differences between control

and *Trichinella spiralis*-infected mice.
Polarization of T cells subset can be influenced by several factors, including the cytokine microenvironment, differential antigen processing, and antigen characteristics. TGF-β1 is a pleiotropic cytokine made by multiple cell types [24], which has been in the spotlight because of its emerging roles in the differentiation of Th17 cells from naive T cells [9]. TGF-β1 not only has a critical function as an antagonist of Th1 development affecting IFN-γ as well as T-bet [25], but also interferes with Th2 differentiation [26], thus allowing the diversion to IL-17 T cell differentiation. In our study, the level of TGF-β1 in jejunum was increased at 2 weeks PI, in accompany with the upregulating of IL-17 and Th17 cells and then recovered at 8 weeks PI. The results showed that Th17 cells might be induced by TGF-β1 but not sustained during *Trichinella spiralis* infection.

IL-23 is expressed in the intestine in various models of intestinal inflammation [27, 28], which could act by reinforcing the Th17 response to form IL-23-Th17 axis in colitis, although it is not required during the differentiation of Th17 [29]. We also observed the change of IL-23 expression in the jejunum of the *Trichinella spiralis* infected mice at different time points. It is interesting that there was no significant change in the level of IL-23 in the jejunum of infected mice, which indicated that the function of IL-23 in nematode infection is not as important as it does in intestine...
inflammation induced by IL-10 knockout [10] or pathogenic CD4 T-cell transfer [30].

In summary, the present study demonstrated that Th17 cells influenced the intestine smooth muscle contractility during intestinal infection with *Trichinella spiralis*. TGF-β1 might induce differentiating of Th17 cells during infection, while IL-23 was not involved in this process. These results not only have implications for host defense against nematodes but also may have broader implications for clinical gastroenterology.

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