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ORIGINAL ARTICLE

Schistosoma mansoni proteins attenuate gastrointestinal motility disturbances during experimental colitis in mice

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Abstract

AIM: To investigate the therapeutic effect of *Schistosoma mansoni* (*S. mansoni*) soluble worm proteins on gastrointestinal motility disturbances during experimental colitis in mice.

METHODS: Colitis was induced by intrarectal injection of trinitrobenzene sulphate (TNBS) and 6 h later, mice were treated ip with *S. mansoni* proteins. Experiments were performed 5 d after TNBS injection. Inflammation was quantified using validated inflammation parameters. Gastric emptying and geometric center were measured to assess *in vivo* gastrointestinal motility. Peristaltic activity of distal colonic segments was studied *in vitro* using a modified Trendelenburg set-up. Cytokine profiles of T-lymphocytes isolated from the colon were determined by real time reverse transcriptase-polymerase chain reaction.

RESULTS: Intracolonic injection of TNBS caused severe colitis. Treatment with *S. mansoni* proteins significantly ameliorated colonic inflammation after 5 d. TNBS did not affect gastric emptying but significantly decreased the geometric center and impaired colonic peristaltic activity 5 d after the induction of colitis. Treatment with *S. mansoni* proteins ameliorated these *in vivo* and *in vitro* motility disturbances. In addition, TNBS injection caused a downregulation of effector T cell cytokines after 5 d, whereas a *S. mansoni* protein effect was no longer observed at this time point.

CONCLUSION: Treatment with *S. mansoni* proteins attenuated intestinal inflammation and ameliorated motility disturbances during murine experimental colitis.

Key words: *Schistosoma mansoni*; Helminth proteins; Colitis; Peristalsis; Crohn's disease; Gastrointestinal motility; Trinitrobenzene sulphate

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INTRODUCTION

Crohn's disease and ulcerative colitis, the two most common forms of inflammatory bowel diseases (IBD), are idiopathic inflammatory disorders of the intestine. The current hypothesis states that IBD results from an uncontrolled immune response against intraluminal bacterial antigens in genetically predisposed individuals[1,2]. Genetic factors as well as environmental factors contribute to the development of the inappropriate immune response[3].

The incidence of Crohn's disease is highest in well-developed countries[4]. According to the hygiene hypothesis, this is directly related to the higher hygienic standards in these countries[5]. It is suggested that the lack of exposure to intestinal parasites (e.g. helminths) contributes to the susceptibility to Crohn's disease[6-8]. Several experimental and clinical studies showed the beneficial effect of helminth infections in IBD[9-12]. Current research is focusing on identifying helminth molecules with immunomodulatory function that exert this protective effect[13].

Patients with Crohn's disease often suffer from disturbed gastrointestinal motility leading to symptoms such as abdominal pain, cramps, diarrhea, weight loss, rectal bleeding and malnutrition[14,15]. It is well established that inflammation of the gut results in functional and structural changes of the enteric nervous system[16,17] and changes in smooth muscle contractility[18,19]. For instance, patients with ulcerative colitis have increased propagating contractions with lower peak amplitudes coupled with variable transit[20], whereas delayed gastric emptying and prolongation of orocecal transit time have been reported in patients with Crohn's disease[21-23]. Dysmotility can also occur in non-inflamed sites of the gastrointestinal tract. Gastroparesis often occurs in patients with inflammation restricted to the small or large intestine[14,24]. Motility disturbances may also persist in the period following an episode of gastrointestinal inflammation, resulting in the development of irritable bowel syndrome or functional dyspepsia[14,25].

The aim of this study was to investigate the therapeutic potential of *Schistosoma mansoni* (*S. mansoni*) soluble worm proteins (SmSWP) on gastrointestinal motility disturbances 5 d after induction of experimental colitis in mice both *in vivo* and *in vitro*. In addition, the inflammatory reaction was quantified and the balance between different T cell subsets was investigated based on their cytokine profile to elucidate the underlying immunological pathways.

MATERIALS AND METHODS

TNBS-induced colitis

Colitis was induced by intraluminal injection of 2,4,6-trinitrobenzene sulfonic acid (TNBS) as previously described[26]. Briefly, male Swiss mice (weight 26-28 g, Charles River, France) were fasted for 24 h and subsequently anesthetized by ketamine (90 mg/kg, ip) and xylazine (10 mg/kg, ip). Next, 100 μL of a 10 mg TNBS in 30% ethanol solution was injected intrarectally. Ethanol is required to break the intestinal epithelial barrier, whereas TNBS is a haptenating agent that immunogenizes autologous proteins. Control animals received an intrarectal injection of 100 μL saline. Afterwards, mice were held upside-down for 1 min to prevent leakage of TNBS solution. The Medical Ethical Committee on animal experimentation of the University of Antwerp, Belgium, approved all experiments.

Preparation of antigen mixtures

SmSWP were prepared as described previously[27]. Briefly, *S. mansoni* adult worms were recovered from mice (housed at the Queensland Institute of Medical Research, Brisbane, Australia), washed and homogenized in PBS and soluble proteins were extracted by centrifugation. Mice were treated with 25 μg SmSWP ip. Proteins were diluted to a final volume of 100 μL in PBS. Control animals were injected ip with 100 μL PBS.

Experimental protocol

In a previous study we investigated the time course of inflammation during TNBS colitis and found that inflammation peaked at day 3 and that colitis was self-limiting with near complete remission after 1 wk. In the present study, we wanted to evaluate the effect of helminth protein treatment after the peak of inflammation but when overt signs of colitis were still present.

In a first set of experiments, we scored the therapeutic effect of SmSWP on colonic inflammation 5 d after the induction of colitis. Six hours after TNBS injection, mice were treated once ip with 25 μg *S. mansoni* proteins or phosphate-buffered saline (PBS). Five days later, mice were sacrificed and inflammation was scored based on 5 parameters: clinical disease activity, macroscopic and microscopic inflammation score, extent of colonic inflammation and myeloperoxidase activity. Two different groups were studied: TNBS mice treated with PBS (TNBS-PBS) after 5 d and TNBS mice treated with 25 μg SmSWP (TNBS-SmSWP) after 5 d (n = 8-10 in each group).

In a second set of experiments, we investigated the effect of SmSWP treatment on *in vivo* gastrointestinal motility and *in vitro* colonic peristalsis 5 d after induction of colitis. Four different groups were studied: control-PBS mice, control mice treated with 25 μg SmSWP (control-SmSWP), TNBS-PBS mice and TNBS-SmSWP mice (n = 7-10 in each group). In preliminary experiments we also investigated the effect of colitis on gastrointestinal motility disturbances 3 d after the induction of colitis, and the effect of SmSWP treatment as these experiments were not performed previously.

In a third set of experiments, we investigated the

cytokine profile of colonic T cells 5 d after induction of colitis. Cytokine profiles were studied in 4 different groups: control-PBS, control-SmSWP, TNBS-PBS, TNBS-SmSWP (n = 5-8 in each group, for each n, colonic tissue of 3 mice was pooled).

Inflammatory scores

Briefly, the clinical disease score (0-8) was based on the following characteristic parameters (0-2 score each): weight loss, piloerection, immobility and blepharitis[27].

After sacrifice, the colon was removed and opened to score colonic damage macroscopically. Four parameters were taken into account: presence of adhesions, degree of colonic ulcerations, wall thickness, and degree of mucosal edema. The total score ranged from 0 to 12[28]. The extent of inflammation in the colon was also measured and expressed in cm. Tissue samples were harvested for histological assessment of the inflammatory infiltrate and for myeloperoxidase (MPO) assay. Colonic segments were fixed in 4% formaldehyde and embedded in paraffin for hematoxylin-eosin staining. Microscopic inflammation score ranged from 0 to 10 based on the following parameters: inflammatory infiltrate, number of gut wall layers infiltrated, loss of mucosal architecture, and edema[27]. MPO activity was measured to monitor the degree of myeloid cell infiltration in the colon. Colonic MPO activity was assayed according to published methods[29] and expressed as units MPO per gram tissue.

In vivo measurement of gastrointestinal motility: Evans blue technique

Mice were fasted for 18 h and *in vivo* semi-liquid meal motility was assessed according to published methods[30]. Briefly, mice received an intragastric injection of 0.1 mL Evans blue (50 mg/mL + 0.5% methylcellulose) *via* an orogastric cannula. Fifteen minutes later, mice were anesthetized and a laparotomy was performed. The stomach and small intestine were resected and the small intestine was divided into 5 segments of equal length. The amount of Evans blue in the segments was measured spectrophotometrically to assess gastric emptying (GE) and geometric center (GC): %GE = [ΣA_{565} (intestine 1-5)/ΣA_{565} (stomach + intestine 1-5)] × 100; GC = Σ (A_{565} of Evans blue per segment × segment number)/total A_{565}.

In vivo measurement of gastrointestinal motility: Solid beads technique

Mice were fasted for 18 h and *in vivo* solid meal motility was assessed as previously described[31]. Mice received an intragastric gavage of 25 green glass beads (0.4-0.5 mm in diameter) together with 0.5 mL H_2O solution *via* an orogastric cannula and were transferred to a wired bottom cage to prevent coprophagy[32]. Subsequently, 30, 120 and 360 min after gavage, mice were anesthetized, the gastrointestinal tract was resected and divided into different segments: stomach, 5 small intestinal segments, cecum, 2 colonic segments and feces. The number of beads in each segment was counted under a stereomicroscope and GE and GC were calculated by the following equations: %GE = [number of beads (small intestine 1-5 + cecum + colon 1-2 + feces)/total number of beads] × 100; GC = Σ (beads per segment × segment number)/total number of beads.

In vitro evaluation of colonic peristaltic activity

Assessment of colonic peristalsis was performed as previously described[31]. Briefly, mice were anesthetized, the colon was removed, flushed and put in cold aerated Krebs-ringer solution. The distal colon segment (3 cm in length) was mounted horizontally in an organ bath. For each segment, the oral end was connected to a perfusion pump for intraluminal infusion of Krebs solution and the other end was attached to a pressure transducer and a vertical tube of which the outlet could be raised in height. After 30 min of equilibration, the outlet was increased from 0 to 7.5 cm. Under these circumstances, spontaneous peristaltic contractions occurred. This activity was associated with regular pressure increases which were recorded by the pressure transducer and analyzed by a data-acquisition system (CED 1401, Cambridge Electronic Design, Cambridge, UK). After an equilibration period of 20 min, the mean amplitude (cmH_2O) of 3 consecutive peristaltic contractions as well as the mean time interval(s) between 4 subsequent peristaltic contractions were calculated and compared.

Investigation of T cell cytokine profiles

Colonic lamina propria mononuclear cells were isolated based on a 30%:70% gradient Percoll column as previously described[27,33]. Colonic lamina propria T cells were subsequently isolated by positive selection using the EasySep enrichment procedure employing antibody-coated, magnetic particles as described by the manufacturer (Stem Cell Technologies, Vancouver, Canada). Total RNA was extracted from isolated colonic T cells by using the Absolutely RNA microprep kit as described by the manufacturer (Stratagene, La Jolla, CA, USA).

Using real time reverse transcriptase-polymerase chain reaction (RT-PCR), we performed a quantitative analysis of the mRNA expression of different cytokines to determine the balance between T helper (Th) 1, Th17, Th2 and Treg (regulatory T) cells in colonic tissue. TaqMan Gene Expression assays (Applied Biosystems, Lennik, Belgium) specific for IFNγ produced by Th1 cells, IL17 produced by Th17 cells, IL-5 produced by Th2 cells and IL-10 produced by Treg cells were performed on a ABI Prism 7300 sequence detector system (Applied Biosystems, Lennik, Belgium) in 25 μL reaction volumes containing One step Universal PCR master mix (Applied Biosystems, Lennik, Belgium) as previously described[27].

Drugs

NaCl 0.9% (Plurule®, Baxter, Lessines, Belgium); 2,4,6 trinitrobenzene sulfonic acid solution (Fluka, Neu-Ulm, Germany); PBS (GIBCO BRL, Merelbeke, Belgium); diethyl ether, ethanol absolute, 30% hydrogen peroxide, methanol absolute, potassium dihydrogen phosphate, dipotassium hydrogen phosphate trihydrate (Merck, Darmstadt, Germany); xylazine (Rompun®, Bayer, Brussels, Belgium); ketamine (Ketalar®, Pfizer, Brussels, Belgium); hexadecyltrimethylammonium bromide, *o*-dianisidine dihydrochloride, FCS, collagenase, Percoll, Evans blue (Sigma Chemical, St. Louis, Missouri, USA); RPMI 1640, EDTA, HBSS, HEPES, L-glutamine, β-mercaptoethanol, sodium pyruvate, penicillin, streptomycin (Invitrogen, Merelbeke, Belgium), glass beads (0.4-0.5 mm in diameter) (VWR international, Leuven, Belgium) were purchased from the respective companies mentioned in parentheses. Helminth protein preparation was described earlier.

Presentation of results and statistical analysis

Data are presented as mean ± SE. Statistical analysis was performed in SPSS 16.0 for Windows. Analyses of the non-parametric data (clinical disease score, macroscopic and microscopic inflammation score) were performed by Mann-Whitney *U* tests. Parametric data (extent of inflammation, MPO, GE, GC, amplitude, interval and RT-PCR results) were analyzed by Student's *t*-tests or by two-way ANOVA (with TNBS colitis as factor 1 and protein treatment as factor 2). When the interaction was significant one-way ANOVA and Student-Newman-Keuls post hoc analysis was performed. P values ≤ 0.05 were considered to be significant.

RESULTS

Effect of SmSWP on TNBS-induced colitis after 5 d

The injection of TNBS caused an increase in all inflammatory parameters (Figure 1A-E) as compared to control mice that did not show any signs of inflammation (data not shown).

Treatment of TNBS-injected mice with SmSWP caused a significant decrease in clinical disease score (Figure 1A), macroscopic inflammation score (Figure 1B), extent of colonic inflammation (Figure 1C), microscopic inflammation score (Figure 1D) and a tendency to decrease the MPO activity (Figure 1E) as compared to TNBS-PBS mice.

Effect of TNBS-induced colitis per se on gastrointestinal motility

In preliminary experiments, 3 d after the induction of colitis, GE and GC of a semi-liquid Evans blue solution were not significantly different between control and TNBS mice: GE was 43% ± 9% in controls and 48% ± 10% in TNBS colitis mice and GC was 2.1 ± 0.3 in controls and 2.2 ± 0.3 in TNBS colitis mice (n = 7-9). We also evaluated the effect of colitis on gastrointestinal motility after intragastric gavage of 25 glass beads 3 d after the injection of TNBS. Experiments were performed 30 min, 120 min and 360 min after intragastric gavage of the marker: GE progressed over time in control mice (from 32% ± 12% to 61% ± 14% and 100% ± 0%, respectively) and in TNBS mice (from 42% ± 13% to 81% ± 9% and 97% ± 2%, respectively) but no significant differences between the control and TNBS groups were observed. The GC also increased over time from 1.5 ± 0.2 (30 min) to 3.2 ± 0.7 (120 min) and to 7.3 ± 0.2 (360 min) in control mice. This time-dependent increase in GC was also observed in mice with colitis (from 1.7 ± 0.3 to 2.9 ± 0.5 and 5.5 ± 0.6). When measured 360 min after gavage of the beads, GC in mice with colitis (5.5 ± 0.6) was significantly lower as compared to control mice (7.33 ± 0.2). Based on these preliminary results, further measurements studying the effect of worm protein treatment on GC were performed 360 min after intragastric gavage of 25 glass beads.

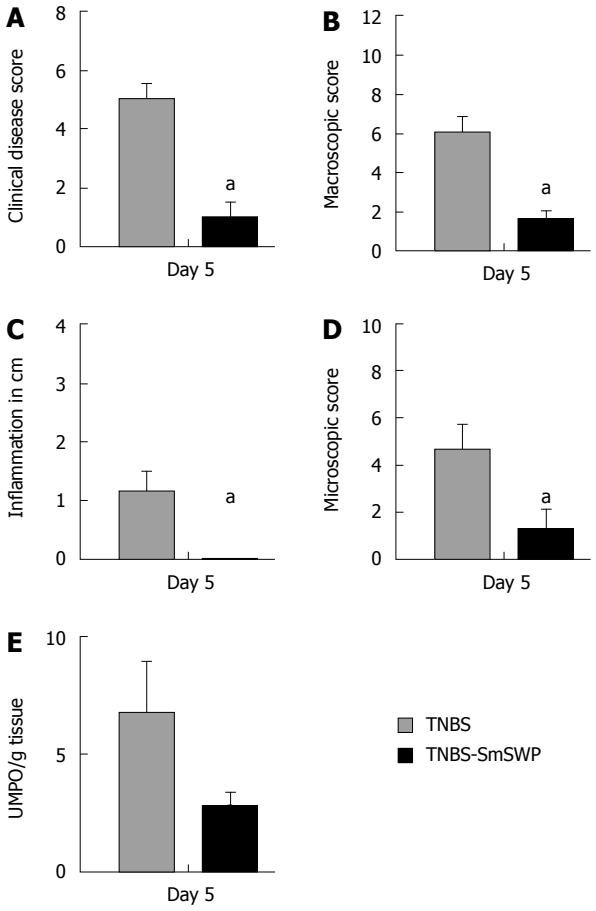

Figure 1 Effect of 25 μg Schistosoma mansoni soluble worm (SmSWP) proteins on clinical disease score (A), macroscopic score (B), extent of inflammation (C), microscopic score (D) and myeloperoxidase (MPO) activity (E) 5 d after trinitrobenzene sulphate (TNBS)-induced colitis. Grey bars represent phosphate-buffered saline (PBS)-treated TNBS mice; black bars represent SmSWP-treated TNBS mice. Data are presented as mean ± SE. Non-parametric data (A, B, D) were analyzed by the Mann-Whitney *U* test, parametric data (C, E) were analyzed by the Student's *t*-test; n = 8-10; $^{a}P \leq 0.05$, significant effect of SmSWP treatment.

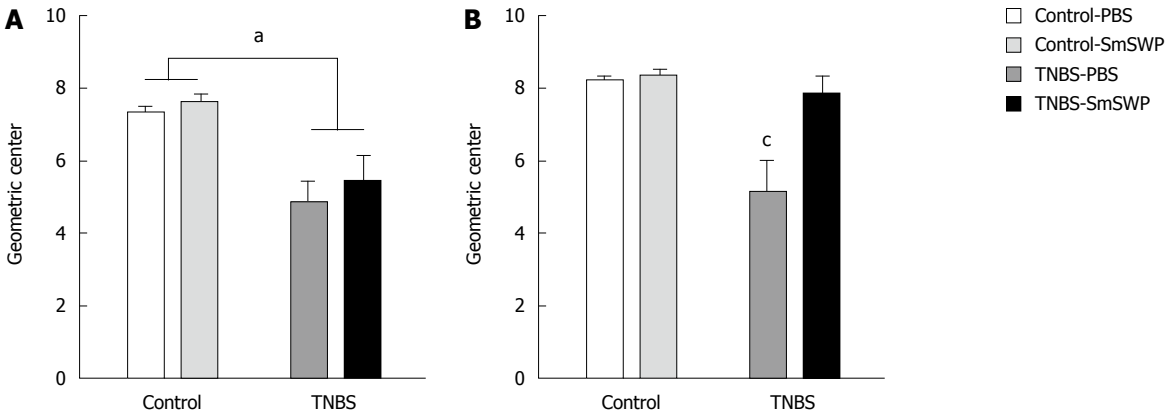

Figure 2 Effect of 25 μg *S. mansoni* proteins on geometric center 3 d (A) and 5 d (B) after the induction of colitis. Data were analyzed by two-way ANOVA with the Student-Newman-Keuls (SNK) post hoc test; n = 7-10; $^{a}P \leqslant 0.05$, significant colitis effect; $^{c}P \leqslant 0.05$, post hoc analysis showed a statistically significant difference from the other 3 groups.

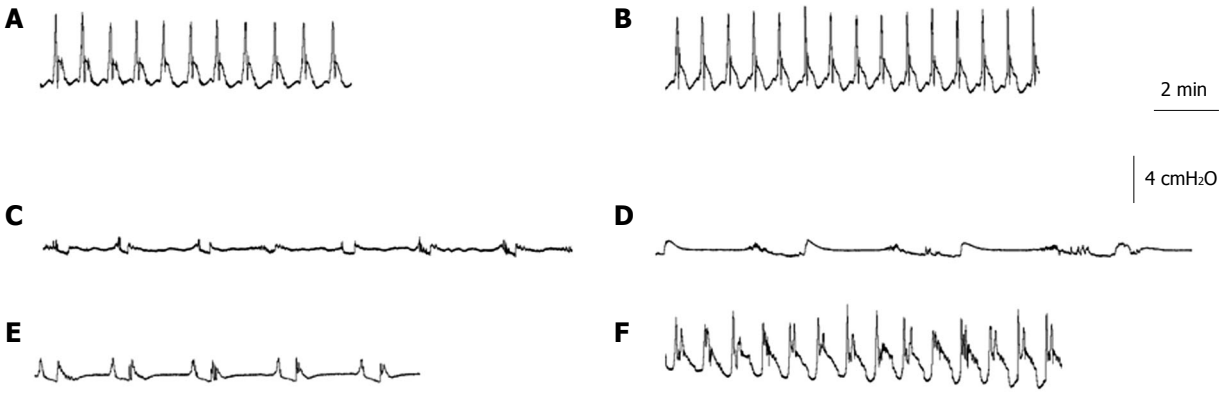

Figure 3 Peristaltic tracings as recorded in the control-PBS group (A), the control-25 μg SmSWP group (B), the TNBS-PBS group on day 3 (C), the TNBS-PBS group on day 5 (D), the TNBS-25 μg SmSWP group on day 3 (E) and the TNBS-25 μg SmSWP group on day 5 (F).

Effect of SmSWP treatment on delayed gastrointestinal transit during TNBS colitis

Experiments were performed 3 d (Figure 2A) and 5 d (Figure 2B) after TNBS injection. Treatment of control mice with SmSWP had no effect *per se* on GC at both time points (Figure 2A and B). TNBS-colitis significantly reduced GC 360 min after intragastric gavage of the beads both at day 3 and at day 5 (Figure 2A and B). Treatment of colitis mice with SmSWP had no effect on GC 3 d after the injection of TNBS (Figure 2A). However, treatment of colitis mice with SmSWP reversed the TNBS-induced decrease in GC at day 5 (Figure 2B).

Effect of SmSWP treatment on colonic peristalsis

Distention-induced peristaltic contractions were recorded (Figure 3) and, subsequently, the amplitude and the interval between the peristaltic waves were measured.

Peristaltic activity of distal colonic segments was measured 3 d (Figure 4A and B) and 5 d (Figure 4C and D) after TNBS enema. Treatment of control mice with SmSWP had no significant effect on colonic peristaltic activity at the two different time points (Figure 3B and Figure 4A-D). The induction of colitis caused significant impairment of peristaltic activity as shown by a significant decrease in amplitude and an increase in interval between the waves. These TNBS-induced disturbances in peristalsis were significant both on day 3 and on day 5 (Figure 3C and D, Figure 4A-D). Furthermore, it is important to note that in 4 of 8 TNBS-PBS mice on day 3 we were not able to measure any peristaltic activity whereas this was only the case in 1 of 8 TNBS-SmSWP mice.

Treatment with SmSWP did not ameliorate the disturbed peristaltic activity caused by intestinal inflammation after 3 d (Figure 3E, Figure 4A and B). However, 5 d after the induction of colitis the amplitude of the distension-induced peristaltic contractions was significantly increased to normal control values when mice were treated with SmSWP (Figure 3F and Figure 4C). At this time point, the mean interval between the waves remained increased after treatment with SmSWP as compared to control animals (Figure 4D).

Measurement of cytokine profiles in colonic T cells

We recently showed the importance of the differential roles of Th1, Th17, Th2 and Treg cells in colonic tissue 3 d after the induction of TNBS colitis and the effect of SmSWP treatment on these T cell subsets[27]. In this study we investigated the cytokine profiles of

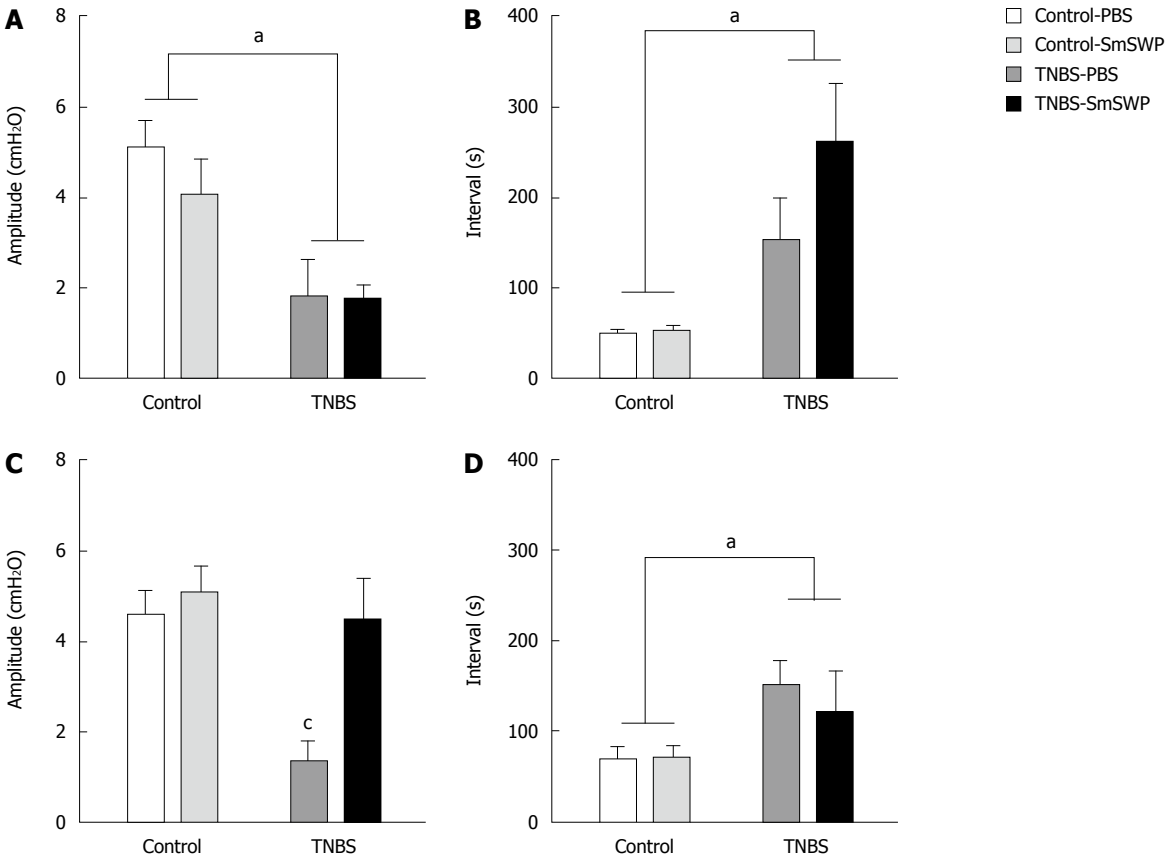

Figure 4 Effect of 25 μg *S. mansoni* proteins on the amplitude and interval of peristaltic waves 3 d (A, B) and 5 d (C, D) after the induction of colitis. Data are presented as mean ± SE. Data were analyzed by two-way ANOVA with SNK post hoc test; n = 7-9 (except for the TNBS-PBS group on day 3 n = 4) ; $^{a}P \leqslant 0.05$, significant colitis effect; $^{c}P \leqslant 0.05$, post hoc analysis showed a significant difference from the other 3 groups.

T cells isolated from colonic tissue on day 5 after the induction of colitis. As shown in Figure 5A, interferon (IFN)-γ mRNA expression was not significantly altered in colonic T cells 5 d after the induction of colitis. On the other hand, we found a significant downregulation of interleukin (IL)-17 and IL-5 expression 5 d after the induction of colitis in both PBS- and SmSWP-treated mice (Figure 5B and D). Investigating the Treg response, we found that injection of TNBS and treatment with SmSWP had no significant effect on IL-10 mRNA expression 5 d after TNBS injection (Figure 5C).

DISCUSSION

In this study we showed that treatment with SmSWP ameliorated *in vivo* and *in vitro* motility disturbances in a murine model of TNBS-induced colitis after 5 d.

Experimental and clinical data support the idea that helminths provide protection against IBD[34]. To avoid the possible disadvantages of a therapy with living parasites, current research is now focusing on the identification and characterization of helminth-derived immunosuppressive molecules that contribute to the protective effect[13]. Furthermore, it is well established that gut inflammation leads to disturbed gastrointestinal motility[14]. The model of TNBS-induced colitis is widely used to investigate motility disturbances occurring in the inflamed colon. We previously showed that contractility of colonic longitudinal smooth muscle strips was time-dependently decreased during TNBS colitis in rats and concurrent infection with *S. mansoni* abrogated these TNBS-induced contractility disturbances[10].

In a previous study we showed that TNBS colitis caused clear signs of inflammation 3 d after the induction of colitis and that our model of TNBS colitis was self-limiting with near complete remission after 1 wk[27]. In this study we focused on a later phase during murine TNBS-induced colitis. We showed that 5 d after TNBS injection, treatment with SmSWP also caused a significant decrease in clinical disease score, macroscopic inflammation score, extent of colonic inflammation and microscopic inflammation score at this later time point along the course of colitis. There was however no significant difference in MPO activity between TNBS-PBS and TNBS-SmSWP mice at this time point although a clear tendency of inhibition was observed. Taken together, these results indicate that treatment with helminth proteins ameliorated colonic inflammation leading to accelerated healing of colitis. A similar beneficial effect has been described previously by our group: rats with TNBS colitis showed spontaneous and complete healing of inflammation 4 wk after the induction of colitis and this was reduced to 2 wk

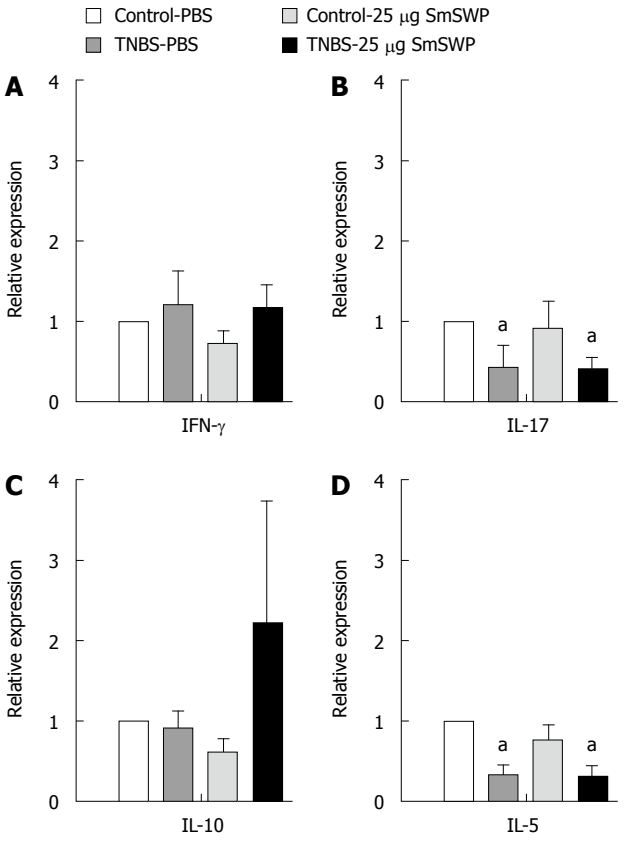

Figure 5 Interferon (IFN) and interleukin (IL) mRNA expression of T helper (Th) 1 (A), Th17 (B), regulatory T (Treg) (C) and Th2 (D) cells isolated from colonic tissue at day 5. Data are expressed as relative expression and the control-PBS group was chosen as calibrator. Data are presented as mean ± SE. Data were analyzed by two-way ANOVA with SNK post hoc test when appropriate; n = 5-8 (except for IL-17 n = 2-5); $^{a}P \leqslant 0.05$, significant colitis effect, no significant effect of worm protein treatment was shown at day 5.

in rats infected with *S. mansoni*[10].

Investigation of the effect of TNBS colitis on gastrointestinal motility failed to show an effect on gastrointestinal transit of a semi-liquid meal. In other words, colitis did not affect gastric emptying in our murine model. Nevertheless, delayed gastric emptying has been described in a clinical setting[21,23] as well as in the rat TNBS model[35]. Literature on TNBS-induced motility disturbances in mice is scarce and gastric emptying disturbances have not been reported so far. In addition to species differences, this lack of effect of colitis on gastric emptying in mice might be linked to the type and severity of inflammation induced and to the time point chosen to perform motility experiments.

On the other hand, assessment of gastrointestinal transit of a solid meal showed that the geometric distribution of solid beads in the gastrointestinal tract was significantly decreased 3 d after the induction of colitis and this decrease was still evident after helminth protein treatment by day 3. Five days after the induction of colitis, the geometric distribution was still significantly altered in mice with colitis but treatment with worm proteins significantly reversed transit to normal values at this time point. These results indicate that although the healing process of intestinal inflammation is ongoing in untreated TNBS mice by day 5, gastrointestinal motility of the gastrointestinal tract remains disturbed. Only when inflammatory signs are almost completely absent, as in the SmSWP-treated TNBS mice on day 5, was *in vivo* gastrointestinal motility of the distal gastrointestinal tract restored.

Comparable results were found on *in vitro* colonic peristalsis. The amplitude of distension-induced pressure waves as well as the interval between the waves were significantly altered in the colon of mice with TNBS-induced colitis, both at day 3 and day 5. Treatment with SmSWP did not have any ameliorating effect after 3 d whereas the amplitude was significantly increased to normal control values after 5 d. The interval between the waves was nevertheless still significantly augmented as compared to controls at day 5. This suggests that some signs of disturbed peristalsis persisted although inflammation is resolving and that the disturbed interval of *in vitro* peristaltic waves 5 d after colitis and worm treatment did not have any repercussion on *in vivo* colonic motility which, at that time point, was normalized in these mice.

With regard to the clinical setting, treatment of IBD patients with *Trichuris suis* ova caused clinical amelioration of both Crohn's disease and ulcerative colitis as shown by a decrease in Crohn's disease activity index and ulcerative colitis disease activity index[11,12]. This decrease in clinical disease scores might indicate that symptoms such as diarrhea and abdominal pain are less frequent after treatment with helminths. It is well known that infection with intestinal helminths can alter gastrointestinal motility thus contributing to worm expulsion[36]. The role of T cells in those circumstances was previously investigated, leading to the understanding that infection-induced intestinal muscle hypercontractility is CD4+ T cell-dependent[37].

Cytokines produced by mucosal leucocytes can also mediate neurogastrointestinal function. We previously showed that the pro-inflammatory cytokine IL-1β modulates gastrointestinal neuromuscular function[38]. In addition, Th2 cytokines IL-4 and IL-13 contribute to intestinal muscle hypercontractility[39]. Treatment with exogenous IL-10 has been shown to abrogate the delayed gastrointestinal transit during postoperative ileus[40]. Gastrointestinal inflammation during Crohn's disease is mediated *via* Th1 lymphocytes as well as through the recently described Th17 cells[41]. On the other hand, it is well established that helminths have the potential to evoke strong regulatory T cell responses with immunosuppressive properties[42]. In this way, we might hypothesize that infection with helminths induce Th2 and Treg immune responses that contribute to the amelioration of motility disturbances during colitis.

As such, we measured the cytokine profile of colonic T cells. We previously showed that a Th1 response (upregulation of IFN-γ) in the colon was evident 3 d after induction of TNBS colitis. This Th1 response

was significantly suppressed after administration of *S. mansoni* proteins. Treatment with SmSWP also caused an upregulation of regulatory T cell cytokines in the colon after 3 d[27]. In this study we identified the balance between the different T cell subsets in the colon at a later time point along the course of colitis, 5 d after the injection of TNBS. Our results showed there was no longer a significant effect on IFN-γ mRNA expression after the induction of colitis, indicating that the Th1 response seen on day 3 in the colon of TNBS-PBS mice had subsided by day 5. Furthermore, injection of helminth proteins decreased the expression of IL-17 after 3 d, both in control mice and in TNBS mice[27]. After 5 d we found decreased IL-17 mRNA expression due to a colitis effect instead of a protein effect. These differential results on IL-17 mRNA expression at both time points are interesting: at day 3 the effect on IL-17 expression was related to helminth protein, whereas it was colitis mediated at day 5. Although we did not detect a significant effect of colitis or worm protein treatment on IL-5 expression after 3 d, a significant downregulation of IL-5 expression on day 5 was apparent both in the PBS treated group and in the helminth protein treated group. One might hypothesize that the naturally occurring healing response leads to the production of regulatory cytokines which are able to suppress cytokines produced by T effector cells including IL-17 and IL-5. This coincides with the attenuation of inflammatory parameters at this time point as described above.

Experiments performed 3 d after the induction of colitis showed a significant upregulation of the mRNA expression of regulatory cytokines IL-10 and transforming growth factor-β after treatment with SmSWP that had subsided by day 5. Our results showed that the immunological effect of helminth protein treatment on Th1 and Treg cells, which is present after 3 d as shown previously[27], has diminished after 5 d. This might be explained by the fact that proteins were only injected once 6 h after TNBS or PBS injection and not repeatedly until day 5. Nevertheless, this single injection with helminth proteins evoked a protective effect that was almost immediate, leading us to assume that these proteins might also have an effect on innate immunity. It was previously reported that infection with *S. mansoni* prevented experimental colitis in mice by a mechanism dependent on macrophages[43]. Furthermore, dendritic cells are key regulators in the immune defence of the gut and are also influenced by helminth infections[44,45]. Investigation on how helminth proteins affect cells of the innate immune system might contribute to a better understanding of the immunological pathways by which helminth proteins suppress ongoing colonic inflammation.

In this study, we provide evidence that treatment with helminth proteins contributes to amelioration of gastrointestinal motility disturbances. Inhibition of inflammation and amelioration of motility disturbances after treatment with helminth proteins both appear at the same time. However, whether the beneficial effect of helminth protein treatment on gastrointestinal motility is directly or indirectly related to amelioration of inflammation needs to be further established. If helminth proteins provoke a reaction that not only leads to a reduction in inflammation but also influences the enteric nervous system and/or smooth muscle cells directly, these proteins might be useful in the treatment of gastrointestinal motility disturbances.

Taken together, we showed that treatment with *S. mansoni* proteins significantly attenuated the course of TNBS-induced colitis leading to reversal of *in vivo* gastrointestinal motility disturbances and amelioration of *in vitro* colonic peristalsis 5 d after induction. We conclude that SmSWP have therapeutic potential in gut inflammation leading to a marked reduction in inflammation and in gastrointestinal motility disturbances, accelerating the natural course of remission.

COMMENTS

Background

Gastrointestinal inflammation during inflammatory bowel diseases (IBD) results from an uncontrolled immune response against intraluminal antigens in genetically predisposed persons and might lead to motility disturbances with related symptoms. The lack of exposure to helminth infections, as a result of improved living standards and medical conditions, has contributed to the increased incidence of IBD in the developed world.

Research frontiers

Epidemiological, experimental and clinical data support the idea that helminths provide protection against IBD. However, treatment with living helminths may have serious drawbacks such as infection and/or invasion of the parasite to other tissues in the human host where they might cause pathology. Therefore, in this study the authors evaluated the therapeutic potential of helminth-derived proteins on inflammation and associated motility disturbances.

Innovations and breakthroughs

This study investigates the effect of TNBS colitis and exposure *to Schistosoma mansoni* proteins on murine gastrointestinal motility. This is a novel pursuit. The effects of inflammation and therapeutic interventions on gastrointestinal motility are largely ignored but critically important. The authors showed that treatment of experimental colitis with helminth proteins restored gastrointestinal motility.

Applications

Treatment with helminth soluble proteins attenuates inflammation and ameliorates motility disturbances during experimental colitis. These results suggest that helminth soluble proteins represent an attractive therapeutic option in the management of IBD.

Peer review

This is an interesting study dealing with the effect of *Schistosoma mansoni* proteins on inflammatory and motility response in a rat model of inflammatory bowel disease. The experimental methods are described comprehensively and the interpretations and conclusions justified by the results.

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