Effect of *Schistosoma mansoni*-induced granulomatous inflammation on murine gastrointestinal motility

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Moreels, Tom G., Joris G. De Man, Johannes J. Bogers, Benedicte Y. De Winter, Gunther Vrolix, Arnold G. Herman, Eric A. Van Marc, and Paul A. Pelckmans. Effect of *Schistosoma mansoni*-induced granulomatous inflammation on murine gastrointestinal motility. Am J Physiol Gastrointest Liver Physiol 280: G1030–G1042, 2001.—In *Schistosoma mansoni*-infected mice, gastrointestinal transit was measured in vivo and the neuromuscular function of longitudinal muscle strips of inflamed ileum and noninflamed gastric fundus was assessed in vitro. Eight weeks after infection, the ileal wall was acutely inflamed, as shown by a mucosal inflammatory infiltrate, leading to an increase in mucosal thickness, in myeloperoxidase (MPO) activity, and in interleukin (IL)-1β production. At that time, both gastrointestinal transit and in vitro ileal contractility were normal. Twelve weeks after infection, chronic granulomatous inflammation led to proliferation of the muscle layer and to a further increase in MPO activity, whereas IL-1β production normalized. Gastrointestinal transit was decreased, whereas in vitro ileal contractility was increased irrespective of the contractile stimulus. In vitro incubation with IL-1β (10 ng/ml for 60 min) significantly increased ileal contractility only at 8 wk after infection. Indomethacin, tetrodotoxin, and atropine had no differential effect on ileal contractility in controls and infected mice. In vitro contractility of noninflamed gastric fundus was normal both 8 and 12 wk after infection. We conclude that intestinal schistosomiasis 8 wk after infection is associated only with structural changes of the ileum, whereas 12 wk after infection, both structural and functional changes are present. These changes are characterized by increased ileal wall thickness, decreased gastrointestinal transit, and increased smooth muscle contractility restricted to the inflamed gut segment.

**Schistosomiasis** is a chronic parasitic disease caused by a trematode blood fluke of the genus *Schistosoma*. It is the second most prevalent parasitic disease after malaria, affecting over 200 million people in 74 countries (44). Human schistosomiasis can lead to several chronic syndromes, among which is intestinal schistosomiasis, which is essentially caused by *Schistosoma mansoni* (for reviews see Refs. 15, 17, 43). Approximately 7 wk after the initial transcutaneous infection, egg production starts in the tributaries of the inferior mesenteric vein. Deposited intravascularly, the eggs penetrate the vessel wall to reach the terminal ileum and the colon, leading to acute inflammation. After penetrating the mucosa, the eggs are excreted along with the feces. However, ~50% of the eggs remain entrapped within the gut wall. Because of the release of antigenic substances, egg deposition leads to chronic granulomatous inflammation, resulting in intestinal lesions (43). The granulomatous response in the gut wall is characterized by a collagenous matrix with macrophages, eosinophilic granulocytes, and lymphocytes of both T and B lineages surrounding the eggs (25, 42, 43). The clinical manifestations of human intestinal schistosomiasis range from nausea and abdominal cramps to diarrhea and dysentery (15, 17, 22). Because there is little evidence of significant malabsorption in schistosomiasis, it is suggested that clinical manifestations may be caused by gastrointestinal neuromuscular dysfunction resulting from the inflammatory granulomatous infiltration of the gut wall (15).

The present study was undertaken to investigate the effect of *S. mansoni* infection on gastrointestinal transit and on the electrical and pharmacological contractile properties of longitudinal muscle strips of the gastrointestinal tract of the mouse. To investigate the hypothesis that motility disturbances not only occur in macroscopically inflamed regions but also in remote unaffected regions of the gastrointestinal tract, both inflamed (terminal ileum) and noninflamed (gastric fundus) tissues were studied. To investigate the time course of gastrointestinal neuromuscular dysfunction, the effect of *S. mansoni* infection was studied in the acute phase (8 wk after infection) and in the chronic phase (12 wk after infection) of inflammation.

**MATERIALS AND METHODS**

*S. mansoni* Infection

The local Ethics Committee of the University of Antwerp approved all experiments. The laboratory maintenance of...
S. mansoni and the transcutaneous infection of mice with S. mansoni were described previously (25, 27). In brief, 20 male Swiss mice at 7 wk of age were put in groups of 5 animals in a plastic tank filled with 1 cm of aquarium water and were allowed to adapt for 15 min. Then infectious cercariae of a Puerto Rican strain of S. mansoni were added in the tank in a ratio of 100 cercariae per mouse, and the mice were spontaneously infected via the transcutaneous route. The animals were kept in the tank for 60 min, after which they were transferred back to their cages. The effect of S. mansoni infection on the gastrointestinal motility was studied 8 wk (10 mice) and 12 wk (10 mice) after infection. Previous studies showed that on the basis of granuloma formation and fibrogenesis, one could differentiate between an acute phase of inflammation 8 wk after the primary infection and a chronic phase of inflammation 12 wk after infection (21, 24, 27, 41, 43). Eight weeks after the primary infection, adult worms start to produce eggs, of which a substantial portion remain entrapped within the host's tissues. This leads to an acute inflammatory reaction. Subsequently, antigenic substances of the entrapped eggs induce a chronic granulomatous inflammation. This chronic phase of inflammation is fully established 12 wk after the primary infection.

Measurement of Gastrointestinal Transit

To study gastrointestinal motility under fasting conditions and to exclude possible motility disturbances induced by (non)allergenic food antigens (18), mice were fasted with free access to drinking water 24 h before the day of the experiment. The measurement of gastrointestinal transit was modified from the method of De Winter et al. (13). In brief, mice received an intragastric injection of 0.1 ml of Evans blue (50 mg in 1 ml of 0.9% sodium chloride with 0.5% methylcellulose) via a specially designed orogastric cannula introduced into the stomach. Fifteen minutes later, the mice were killed by a carotidotomy under ether anesthesia and gastrointestinal transit was measured from the pylorus to the most distal point of migration of Evans blue and expressed in centimeters. It was previously shown that short-lasting ether anesthesia does not influence gastrointestinal transit (13).

Tissue Preparation

After the mice were killed, the stomach and an ~9-cm-long ileal segment located 5 cm proximal to the ileocolonic junction were rapidly removed and placed in ice-cold Krebs-Ringer solution (in mM: 118.3 NaCl, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 2.5 CaCl2, 25 NaCHO3, 0.026 CaEDTA, and 11.1 glucose). From the isolated stomach, the gastric fundus was cut in two halves. One half was used for histology; two longitudinal muscle strips from the other half were prepared for pharmacological contractility studies. The ileal segment was used in all experiments in the following way. The most distal ~3 cm was used for the myeloperoxidase (MPO) activity assay; from the middle ~3 cm the mucosal layer was removed to determine mucosal interleukin (IL)-1β levels, and from the remaining muscle layers two 1-cm-long longitudinal muscle strips were prepared for pharmacological experiments. One of the two muscle strips was always used as a control, receiving the vehicle of the drug that was administered to the second muscle strip. The most proximal ~3 cm was used for histological examination.

Histology

Immediately after the mice were killed, tissue samples from the liver were snap-frozen in liquid nitrogen and frozen sections were prepared to verify the state of infection. Tissue samples from the gastric fundus and the terminal ileum were fixed in 4% formaldehyde and embedded in paraffin. Transverse sections, 4 μm thick, were cut from the paraffin-embedded segments and stained with Masson's trichrome or hematoxylin-eosin. Morphometric analysis to assess the thickness of the different layers of the gut wall and to assess the cross-sectional area (CSA) of the longitudinal muscle layer was performed in a blinded fashion using an image analysis system (Fenestra, Kinetic Imaging, Liverpool, UK). To avoid investigator's bias, wall thickness and CSA were measured on granuloma-free sections.

MPO Activity Assay

Tissue MPO activity, which is directly related to the number and activity of myeloid cell infiltrates in the inflamed tissue, was assayed to monitor the degree of inflammation (5, 32). Full-thickness ileal segments were blotted dry, weighed, and placed in a potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide at 5 g of tissue per 100 ml of buffer. The samples were placed on ice, minced, and homogenized for 30 s (PRO 200, PRO Scientific, Monroe, CT). The homogenate was subjected to two sonication and freeze-thawing cycles. The suspension was centrifuged at 15,000 g for 15 min at 4°C. Aliquots (0.1 ml) of the supernatant were added to 2.9 ml of o-dianisidine solution (16.7 mg of o-dianisidine in 1 ml of methyl alcohol, 98 ml of 50 mM potassium phosphate buffer, pH 6.0, and 1 ml of 0.05% H2O2 solution as a substrate for the MPO enzyme). The change in absorbance was read at 460 nm over 60 s with a Spectronic Genesys 5 spectrophotometer (Milton Roy, Rochester, NY). One unit of MPO activity was defined as the quantity able to convert 1 μmol of H2O2 to H2O per minute at 25°C, and activity was expressed in units per gram of tissue. Because of the limited amount of tissue, MPO activity was not assayed in the gastric fundus.

IL-1β Protein Assay

IL-1β is considered a mediator in many inflammatory diseases and in the production of systemic acute phase responses (14). Granulomas caused by schistosomiasis have been shown to secrete IL-1β during the early phase of granuloma formation (12), and IL-1β has been shown to modulate gastrointestinal neuromuscular function (10). To investigate the role of IL-1β in gastrointestinal dysmotility during murine schistosomiasis, mucosal IL-1β levels were assayed by a commercially available four-member solid-phase sandwich ELISA kit (Cytoscreen mIL-1β, Biosource International, Camarillo, CA). According to the manufacturer's instructions, there is no cross-reactivity to other murine acute phase cytokines such as tumor necrosis factor-α and IL-6. Ileal segments were cut along the mesentery, and the mucosa was removed by sharp dissection under a stereomicroscope. The remaining underlying muscle layers were used for contractility studies. The dissected mucosa was snap-frozen in liquid nitrogen and stored at −70°C for later processing. To determine the level of IL-1β, the tissue samples were thawed, gently blotted dry, weighed, and placed in ice-cold Tris-EDTA (10 mM Tris-HCl and 1 mM EDTA, pH 7.4) buffer containing 0.05% sodium azide, 1% Tween 80, 2 mM phenylmethylsulfonyl fluoride, and 1 μg/ml of each of the protease inhibitors antipain, aprotinin, leupeptin, and pepstatin A at 100 mg of tissue per 1 ml of buffer (6). The mucosal samples were placed on ice, minced, homogenized for 20 s (PRO 200, PRO Scientific), and centrifuged at 11,000 g for 10 min at 4°C. The supernatants were collected and filtered (0.45-μm Acrodisc...
syringe filter, Pall, Ann Arbor, MI). Finally, IL-1β levels were assayed with the Cytoscreen mL-1β ELISA kit according to the manufacturer’s instructions. Mucosal IL-1β content was expressed in nanograms of protein per gram of mucosa.

Pharmacological Studies

Tissue preparation. After removal of the mucosa, two longitudinal muscle strips, exactly 1.0 cm in length, of both the gastric fundus and the terminal ileum were mounted in organ baths (5 ml) filled with Krebs-Ringer solution maintained at 37°C and aerated with a mixture of 5% CO₂ and 95% O₂. One of the two muscle strips was always used as a control, receiving the vehicle of the drug that was administered to the second muscle strip. At the end of the experiment, muscle strips were blotted and weighed.

Isometric tension recording. One end of the muscle strip was anchored to a glass rod and pulled through two platinum ring electrodes. The other end was connected to a strain gauge transducer (Statham UC2) for continuous recording of isometric tension. The muscle strips were brought to their optimal point of length-tension relationship using 0.1 μM carbachol and then allowed to equilibrate for at least 60 min before experimentation (31). During the equilibration period the muscle strips were washed every 15 min with fresh Krebs-Ringer solution.

Experimental protocols. In a first series of experiments, contractions of the muscle strips of both the gastric fundus and the ileum were induced in three different ways. First, non-receptor-mediated contractions were induced by 50 mM KCl in the presence of the neurotoxin tetrodotoxin (TTX, 1 μM). Secondly, receptor-mediated contractions were studied by means of different contractile receptor agonists. They were randomly induced by cumulative concentrations of ACh (0.1 nM–0.1 mM), serotonin (5-HT, 0.1 nM–1 μM), and the ileum were induced in three different ways. First, non-receptor-mediated contractions were induced by 50 mM KCl in the presence of the neurotoxin tetrodotoxin (TTX, 1 μM). Secondly, receptor-mediated contractions were studied by means of different contractile receptor agonists. They were randomly induced by cumulative concentrations of ACh (0.1 nM–0.1 mM), serotonin (5-HT, 0.1 nM–1 μM), PGF₂α (0.1 nM–1 μM), and substance P (SP, 0.1 nM–0.1 μM). Between the responses to the different contractile receptor agonists, tissues were washed four times with an interval of 15 min. The second muscle strip of each mouse was used as a time control, receiving the vehicle of the drug that was administered to the first muscle strip. Receptor-mediated contractions were expressed by negative logarithm of the molar concentration inducing 50% of the maximal contractile response (pD₂) and maximal contraction (E max) values. Finally, ideal contractility was studied by means of electrical field stimulation (EFS; 0.5–16 Hz, 0.3-ms pulse duration, 10-s pulse trains) of the myenteric neurons. In the gastric fundus, myenteric neurons were stimulated by EFS with the following parameters: 0.5–8 Hz, 10-ms pulse duration, and 10-s pulse trains. Preliminary experiments showed that these EFS parameters selectively depolarized enteric neurons, because the elicited contractions were completely blocked by pretreatment with 1 μM TTX. The responses to stimulation at higher frequencies were not completely blocked. EFS-induced contractions were expressed by their respective E F₅₀ (frequency inducing 50% of the contractile response at 8 Hz in the fundus and to 16 Hz in the ileum) and E max values at 8 and 16 Hz. Responses were always measured at the top of the contractile peak. The contractility of muscle strips of 8- and 12-wk infected mice was compared with the contractility of muscle strips from age-matched control mice.

In a second series of experiments the in vitro effect of different modulators of contractility was studied. In muscle strips of controls and 8-wk and 12-wk infected mice, the effect on pharmacological contractility of IL-1β (10 ng/ml, 1-h incubation time), the cyclooxygenase inhibitor indomethacin (2 μM), the neurotoxin TTX (1 μM), and the cholinergic muscarinic receptor antagonist atropine (1 μM) was investigated. All experiments were performed in parallel, muscle strips serving as time controls and receiving the vehicle of IL-1β, indomethacin, TTX, or atropine. Contractions of the time controls remained constant over the time course of the experiment. In the gastric fundus, contractions were expressed in grams of contraction per milligram of tissue weight. In the ileum, contractions were expressed in grams of contraction per square millimeter of CSA of the longitudinal muscle layer. The CSA was determined on histological sections of the ileal segment immediately proximal to the segment used for the contractility study.

Drugs Used

The following drugs were used: atropine sulfate, 30% hydrogen peroxide, indomethacin sodium trihydrate, and sodium azide (Merck, Darmstadt, Germany); ACh chloride, antipain, aprotinin, bovine serum albumin, carbachol, Evans blue, hexadecyltrimethylammonium bromide, leupeptin, o-dianisidine dihydrochloride, pepstatin, phenylmethylsulfonyl fluoride, and SP (Sigma Chemical, St. Louis, MO); serotonin creatinine sulfate monohydrate (Janssen Chimica, Geel, Belgium); TTX (Alomone Labs, Jerusalem, Israel); PGF₂α (Dinolytic; purchased from Upjohn, Puurs, Belgium as a sterile aqueous solution containing 5 mg/ml PGF₂α, and 9 mg/ml benzyl alcohol); and human recombinant IL-1β (R&D Systems, Abingdon, UK) dissolved in sterile phosphate-buffered saline (GIBCO BRL, Merelbeke, Belgium) with 0.1% bovine serum albumin, aliquoted, and frozen at −70°C.

Presentation of Results and Statistical Analysis

Morphometric analysis. The microscopic measurement of the thickness of the gut wall is expressed in micrometers. The CSA of the longitudinal muscle layer is expressed in square millimeters. Values are shown as means ± SE for the number of mice indicated. For graphical analysis, one-way analysis of variance followed by Dunnett’s test was used to compare the results from 8- and 12-wk infected mice with control mice. P values of <0.05 were considered significant. Because there was no statistically significant difference between the age-matched control group for the 8-wk infected mice and the age-matched control group for the 12-wk infected mice, the control groups were pooled into one group.

MPO activity assay. MPO activity is expressed in units per gram of tissue. Values are shown as means ± SE for the number of mice indicated. For statistical analysis, one-way analysis of variance followed by Dunnett’s test was used to compare the results from 8- and 12-wk infected mice with control mice. P values of <0.05 were considered significant. Because there was no statistically significant difference between the age-matched control group for the 8-wk infected mice and the age-matched control group for the 12-wk infected mice, the control groups were pooled into one group.

IL-1β protein assay. Mucosal IL-1β levels are expressed in nanograms of protein per gram of mucosa. Values are shown as means ± SE for the number of mice indicated. For statistical analysis, one-way analysis of variance followed by Dunnett’s test was used to compare the results from 8- and 12-wk infected mice with control mice. P values of <0.05 were considered significant. Because there was no statistically significant difference between the age-matched control group for the 8-wk infected mice and the age-matched control group for the 12-wk infected mice, the control groups were pooled into one group.
Gastrointestinal transit. Because the total length of the small intestine was not statistically different among the three groups, gastrointestinal transits are expressed in centimeters of migration of Evans blue, measured from the pylorus to the most distal point of migration. Values are shown as means \(\pm\) SE for the number of mice indicated. For statistical analysis, one-way analysis of variance followed by Dunnett’s test was used to compare the results from 8- and 12-wk infected mice with control mice. \(P\) values of \(<0.05\) were considered significant. Because there was no statistically significant difference between the age-matched control group for the 8-wk infected mice and the age-matched control group for the 12-wk infected mice, the control groups were pooled into one group.

Pharmacological studies. In the gastric fundus, contractions are expressed as grams of contraction per milligram of tissue weight. In the ileum, contractions are expressed as grams of contraction per square millimeter of CSA of the longitudinal muscle layer, which was determined on histological cross-sections of the ileal segment immediately proximal to the segment used in the contractility studies. The receptor-mediated contractions induced by the different agonists are characterized by the respective \(pD_2\) (in \(-\log M\)) and \(E_{\text{max}}\) values, calculated by Graphpad Prism software. Values are shown as means \(\pm\) SE for the number of mice indicated. Only the first muscle strip of the ileum and the gastric fundus of each mouse was used to determine the mean. The second muscle strip served as a time control. For statistical analysis, one-way analysis of variance followed by Dunnett’s test was used to compare the results from 8- and 12-wk infected mice with control mice. Student’s \(t\)-test for paired values was used when the effect of the modulators IL-1\(\beta\), indomethacin, TTX, and atropine was studied. \(P\) values of \(<0.05\) were considered significant. Because there was no statistically significant difference between the age-matched control group for the 8-wk infected mice and the age-matched control group for the 12-wk infected mice, the control groups were pooled into one group.

Fig. 1. Cross sections from the ileum of control mice (A) and mice after 8 (B) and 12 (C) wk of infection with *Schistosoma mansoni*. The images are printed at the same final magnification; calibration bar represents 100 \(\mu\)m. The tunica mucosa is thickened in 8- and 12-wk infected mice, whereas the tunica muscularis propria is only thickened in 12-wk infected mice. The villi in the infected specimens are broadened and blunted, and an increased cellular infiltrate can be seen. In the center of the granuloma, remnants of the egg shell (arrowheads) can be seen surrounded by inflammatory cells. Granuloma-free sections were used to measure wall thickness and cross-sectional area (CSA) of the longitudinal muscle layer (hematoxylin-eosin, \(\times\) 20).
RESULTS

Histology

Eight weeks after the initial infection with *S. mansoni*, there was an acute diffuse mucosal ileal inflammation (Fig. 1B) as well as an early granulomatous response surrounding entrapped eggs. The mucosal inflammatory infiltrate consisted mainly of eosinophilic and neutrophilic granulocytes and scarce lymphocytes. During this acute inflammatory phase, the scarce granulomas consisted of macrophages and eosinophilic granulocytes surrounded by lymphocytes. After 12-wk infection, the mucosa was characterized by blunted villi and increased crypt depth (Fig. 1C). Granulomas were very numerous, indicating that the inflammatory response was chronic. The total ileal wall thickness of infected mice was increased (Fig. 2). In 8-wk infected mice this was caused by a thickening of the mucosal layer, whereas in 12-wk infected mice the thickness of both the mucosal and the muscular layers was increased (Fig. 2). Similarly, the CSA of the longitudinal muscle layer was significantly increased in 12-wk infected mice (Fig. 2). This illustrates the morphological differences between acute and chronic phases of inflammation. The gastric fundus of infected mice did not show signs of inflammation.

MPO Activity Assay

As illustrated in Fig. 3, infection with *S. mansoni* caused a significant and time-dependent increase in MPO activity in the ileum. During the acute inflammatory phase, MPO activity was significantly increased compared with controls. During the chronic phase, when granulomas were numerous, MPO activity was even further increased, to 10 times the control value.

IL-1β Protein Assay

Fig. 4 shows that the ileal mucosa of control mice contains 2.02 ± 0.09 ng IL-1β/g mucosa (*n* = 10). This is comparable with IL-1β production in the rabbit colon (11). During the acute inflammatory phase, the mucosal IL-1β level was significantly increased to more than double the control value. However, during the chronic phase, the mucosal IL-1β level was nearly normalized.

Effect of *S. mansoni* Infection on Gastrointestinal Transit

The total length of the small intestine of control mice was 47.8 ± 0.9 cm (*n* = 10). This was not statistically different from the length of the small intestine in 8-wk infected mice (46.6 ± 1.0 cm; *n* = 10) and 12-wk infected mice (45.4 ± 0.8 cm; *n* = 10). The gastrointestinal transit of Evans blue in control mice was 25.2 ± 1.7 cm (*n* = 10). After 8-wk infection the transit was not significantly altered compared with controls (Fig. 5). However, after 12-wk infection, the transit was significantly decreased to 18.2 ± 1.8 cm (*n* = 7; Fig. 5). These results indicate that only chronic intestinal

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**Fig. 2.** Effect of *S. mansoni* on the thickness of ileal sections and on the CSA of the longitudinal muscle layer of 8 (8w)- and 12 (12w)-wk infected mice. Thickness is expressed in μm, and CSA is expressed in mm². All results are shown as means ± SE. *P < 0.05, significantly different from control (con) mice, 1-way analysis of variance followed by Dunnett’s test (*n* = 5–6).

**Fig. 3.** Effect of *S. mansoni* infection on ileal myeloperoxidase (MPO) activity 8 and 12 wk after infection. Results are expressed in units/g of tissue and shown as means ± SE. *P < 0.05, significantly different from the MPO activity in control mice, 1-way analysis of variance followed by Dunnett’s test (*n* = 7–10).
schistosomiasis was associated with an inhibition of the gastrointestinal transit.

Effect of S. mansoni Infection on Contractility of Ileum

Spontaneous basal activity of ileal muscle strips progressively increased in 8- and 12-wk infected mice compared with controls (Fig. 6). In ileal muscle strips of control mice, 50 mM KCl induced a contraction of $5.95 \pm 0.72$ g/mm$^2$ ($n = 10$). During the acute inflammatory phase, the contractile response was $6.74 \pm 1.01$ g/mm$^2$ ($P > 0.05; n = 10$). During the chronic inflammatory phase, the response to KCl was significantly increased to $9.34 \pm 0.96$ g/mm$^2$ ($P < 0.01; n = 7$).

Receptor-mediated contractions were studied with different contractile agonists. Under control conditions, all contractile agonists induced dose-dependent contractions and the rank order of the maximal response was ACh > PGF$_{2\alpha}$ > SP > 5-HT, the latter being a weak contractile agonist (Figs. 7 and 8). This rank order was maintained 8 and 12 wk after infection (Figs. 7 and 8). However, compared with controls, the $E_{\text{max}}$ values of each respective agonist significantly increased 12 wk after infection (Figs. 7 and 8). The rank order of the pD$_2$ values in muscle strips of control mice was SP > 5-HT > PGF$_{2\alpha}$ > ACh, which was maintained after 8 and 12 wk (Fig. 8). Compared with controls, there was no significant change in pD$_2$ values of each respective agonist after 8 and 12 wk (Fig. 8).

EFS (0.5–16 Hz, 0.3-ms pulse duration, 10-s pulse trains) of the enteric neurons induced frequency-dependent contractions in the ileum of control mice and 8-wk and 12-wk infected mice. These contractions were of cholinergic origin because they were completely blocked by the muscarinic receptor antagonist atropine (1 µM) or by the neurotoxin TTX (1 µM) (data not shown). The response to EFS in 8-wk infected mice did not significantly differ from the response in controls (Fig. 9). However, 12 wk after infection, the response to EFS was significantly increased compared with controls (Fig. 9). These results indicate that both receptor-mediated and non-receptor-mediated contractility of the ileal longitudinal muscle layer nonselectively increased during the chronic phase of intestinal schistosomiasis. In a series of additional experiments, the contractility of the ileum was also investigated after 40 wk of infection. Forty weeks after infection, the ileal contractions to EFS (0.5–8 Hz), ACh (0.1 nM–100 µM), and KCl (50 mM) were still significantly increased compared with controls and the responses were comparable to those obtained at 12 wk after infection (controls vs. 40-wk postinfection, EFS 2 Hz: from $1.14 \pm 0.26$ to $3.12 \pm 0.64$ g/mm$^2$; 1 µM ACh: from $3.36 \pm 0.39$ to $5.98 \pm 1.44$ g/mm$^2$; 50 mM KCl: $5.95 \pm 0.72$ to $8.50 \pm 1.98$ g/mm$^2$; $P < 0.05; n = 3$).

Effect of Modulators of Neuromuscular Function on S. mansoni-Induced Contractility Alterations in Ileum

Granulomas caused by schistosomiasis have been shown to secrete IL-1β during the early phase of granuloma formation (12), and IL-1β (10 ng/ml, 1-h incubation) has been shown to modulate gastrointestinal neuromuscular function (10). In control mice, IL-1β significantly inhibited the contractile response to ACh: $E_{\text{max}}$ values decreased from $3.89 \pm 0.29$ to $2.70 \pm 0.21$ g/mm$^2$ ($P < 0.01; n = 10$; Table 1). However, the contractile response to SP in control mice increased after incubation with IL-1β: $E_{\text{max}}$ values were significantly enhanced from $2.24 \pm 0.26$ to $3.13 \pm 0.59$ g/mm$^2$.
These results are in accordance with previously reported observations (2). The contractile response to PGF$_{2\alpha}$, 5-HT, and KCl in control mice was not significantly altered by IL-1$\beta$. In 8-wk infected mice, incubation of ileal muscle strips with IL-1$\beta$ increased receptor-mediated contractility, independent of the agonist under study (Table 1), whereas IL-1$\beta$ had no influence on the KCl-induced contraction. In 12-wk infected mice, both receptor-mediated and non-receptor-mediated contractions were increased compared with controls. However, incubation of ileal muscle strips of 12-wk infected mice with IL-1$\beta$ had no influence on the KCl-induced contraction and did not increase the receptor-mediated contractility to ACh, PGF$_{2\alpha}$, SP, or 5-HT.

Endogenous prostaglandins have been shown to be important mediators of both granuloma formation (35) and gastrointestinal motility in control conditions and during inflammation (3, 16). Therefore, the cyclooxygenase inhibitor indomethacin was used to study the role of prostaglandins in the S. mansoni-induced contractility alterations of the ileum. Indomethacin (2 $\mu$M) decreased the spontaneous activity of muscle strips of both control and infected mice, indicating that endogenous prostaglandins are necessary for the maintenance of the inherent tone of the muscle strips (Fig. 6). However, indomethacin was not able to reverse the increased contractility induced by the different receptor agonists or KCl in 8- and 12-wk infected mice (Fig. 6).

To study the role of enteric neurons, the muscle strips were incubated with the neurotoxin TTX (1 $\mu$M). TTX blocked the response to EFS completely in both control mice and infected mice. However, TTX did not influence the spontaneous activity of the muscle strips. TTX did not alter the receptor-mediated and non-receptor-mediated contractility of muscle strips of control mice and did not reverse the increased contractility caused by S. mansoni infection (data not shown). Also, incubation with the cholinergic muscarinic receptor antagonist atropine (1 $\mu$M) did not alter the spontane-
ous contractility of the tissue preparations (data not shown).

**Effect of S. mansoni Infection on Contractility of Gastric Fundus**

EFS (0.5–8 Hz, 1-ms pulse duration, 10-s pulse trains) of the enteric neurons only induced very weak contractions in control conditions. They were completely blocked by atropine (1 μM) or TTX (1 μM), and they were increased when nitric oxide synthase was blocked by Nω-nitro-L-arginine (100 μM; data not shown). However, exogenous ACh induced strong contractions of the gastric fundus. The rank order of the maximal contractile response to different receptor agonists was PGF$_{2\alpha}$ > ACh > SP > 5-HT, the latter being a very weak contractile agonist (Fig. 10). This indicates that the mouse gastric fundus contains very few cholinergic neurons, although functional cholinergic receptors are present on the smooth muscle cell membrane. Neither the receptor-mediated contractions nor the KCl-induced contractions of the gastric fundus were significantly altered by intestinal schistosomiasis of the ileum and the colon (Fig. 10).

IL-1β selectively increased the E$_{\text{max}}$ values of ACh in muscle strips of the gastric fundus of control mice (from 0.35 ± 0.03 to 0.42 ± 0.04 g/mg; $P < 0.01$; $n = 10$), and the same was found for the muscle strips of 8-wk (from 0.35 ± 0.03 to 0.43 ± 0.04 g/mg; $P < 0.01$; $n = 10$) and 12-wk (from 0.35 ± 0.06 to 0.44 ± 0.08 g/mg; $P < 0.01$; $n = 7$) infected mice. IL-1β had no effect on other receptor-mediated contractions or KCl-induced contractions (data not shown).
These results indicate that the contractility of the gastric fundus is not altered by intestinal schistosomiasis affecting more distal regions of the gut.

DISCUSSION

In the present study, the effect of intestinal *S. mansoni* infection of the mouse on in vivo gastrointestinal motility and on in vitro contractility of longitudinal muscle strips was examined. On the basis of studies of granuloma formation and fibrogenesis in the liver, an acute and a chronic phase of schistosomiasis-induced inflammation are distinguished: 8 wk after the initial infection, a diffuse inflammatory infiltrate is present and hepatic granuloma formation is maximal; 10–16 wk after the infection, hepatic granuloma size gradually decreases and collagen production progressively increases (21, 24, 27, 41). On the other hand, intestinal granulomas are not subject to such modulation (41). To study gastrointestinal motility during the acute and the chronic inflammatory phase, the present study was performed 8 and 12 wk after the initial infection.

The inflammatory reaction to *S. mansoni* infection was assessed by histology, by MPO activity assay, and by IL-1β protein assay. Eight weeks after infection, a significant increase in total ileal wall thickness was noted, which was caused by a diffuse inflammatory infiltration of the mucosa. No trophic changes of the muscle layers were found. At that time, both MPO activity and mucosal IL-1β level were significantly increased. These data indicate that 8 wk after infection the ileal mucosal layer is acutely inflamed. However, the structure of the muscle layers was preserved and neither the gastrointestinal transit nor the contractility of ileal and gastric longitudinal smooth muscle strips was altered. On the other hand, 12 wk after infection, the increase in mucosal thickness was less pronounced but the thickness of the smooth muscle layers was nearly doubled compared with controls, resulting in a significant increase in the CSA of the longitudinal muscle layer. Also, granulomas were present in all layers of the intestinal wall. They consisted of macrophages, eosinophilic granulocytes, and lymphocytes. MPO activity was further increased compared with the acute inflammatory phase. However, mucosal IL-1β levels were normalized. During the chronic inflammatory phase, the gastrointestinal tran-
Means described (39) and myenteric plexus destruction leads to logical alterations of the myenteric plexus have been -induced motor abnormalities, although morpho-
motility was not primarily involved in the tractility or the increased contractile response, indicat-
neurotoxin TTX did not influence the spontaneous con-
ations of the number or the sensitivity of the agonist of the smooth muscle cells increased without alter-
counted for on the basis of trophic changes alone. Our
muscle layer, the increased contractility cannot be ac-
out the infection. Because contractions were expressed
tractile receptor agonists. The pD 2 values of the con -
shown by the enhancement of Emax values of all con-
irrespective of the contractile stimulus used. This was
phase of inflammation, the contractility was increased
the enteric neurons and by KCl. During the chronic
contractions. Contractions were also induced by EFS of
selective increase of the ileal longitudinal muscle strip
sit was significantly decreased, with a concurrent non-
selective increase of the ileal longitudinal muscle strip contractility. The contractility of the gastric fundus remained normal throughout the course of the infect-
In the present study, different contractile receptor agonists induced dose-dependent contractions of ileal longitudinal muscle strips in control animals. ACh and PGF2α were shown to be the most effective contractile receptor agonists, whereas 5-HT induced only weak contractions. Contractions were also induced by EFS of the enteric neurons and by KCl. During the chronic phase of inflammation, the contractility was increased irrespective of the contractile stimulus used. This was shown by the enhancement of Emax values of all contractile receptor agonists. The pD2 values of the contractile receptor agonists remained unaltered throughout the infection. Because contractions were expressed in terms of force per CSA of the longitudinal smooth muscle layer, the increased contractility cannot be accounted for on the basis of trophic changes alone. Our results indicate that the intrinsic contractile capacity of the smooth muscle cells increased without alterations of the number or the sensitivity of the agonist receptors. Pretreatment of the muscle strips with the neurotoxin TTX did not influence the spontaneous contractility or the increased contractile response, indicating that the neuronal component of gastrointestinal motility was not primarily involved in the Schistosoma-induced motor abnormalities, although morphological alterations of the myenteric plexus have been described (39) and myenteric plexus destruction leads to an increase in intestinal muscle thickness (23). Indeed, morphometric analysis of the ileum of 12-wk infected mice showed a significant increase of the CSA of the longitudinal muscle layer. Therefore, the effect of S. mansoni infection on the smooth muscle function might be indirect through alterations of the myenteric plexus. The present study shows that both morphological and functional alterations of the longitudinal smooth muscle layer lead to gastrointestinal motor dysfunction in the infected host. The role of the myenteric plexus in schistosomiasis-induced alterations of gastrointestinal motility remains to be elucidated. Because the increased muscle contractility does not depend on the type of contractile stimulus, it is possible that this overall hypercontractility abolishes the integrated propulsive activity of the normal gut, leading to a dysfunction of peristalsis and decreased gastrointestinal transit. In addition, there is little evidence of significant malabsorption in schistosomiasis, indicating that the clinical intestinal manifestations are caused by a dysfunction of the gastrointestinal motor system (15).

Our results indicate that, during the acute inflammatory phase of intestinal schistosomiasis, gastrointestinal motor function is not substantially altered, even in the presence of mucosal inflammation. However, during the chronic phase of inflammation, when granulomas are present, the gastrointestinal transit is decreased, with a concurrent increase in ileal longitudinal smooth muscle contractility. This hypercontractility was also still evident after 40 wk of infection. The present study on intestinal schistosomiasis reveals marked differences compared with the vast amount of data available on gastrointestinal motor function in nematode-infected rodents. Nippostrongylus brasiliensis and Trichinella spiralis are nematodes that develop in the small intestine (7). As has been shown extensively, these intraluminal parasites induce acute and chronic inflammatory changes of the small intestine

Table 1. Effect of interleukin-1β on contractility of ileal longitudinal muscle strips of control mice and mice infected with Schistosoma mansoni

<table>
<thead>
<tr>
<th></th>
<th>IL-1β</th>
<th>Control</th>
<th>8 wk</th>
<th>12 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCl</td>
<td>5.95±0.72</td>
<td>6.74±0.11</td>
<td>9.34±0.96</td>
<td></td>
</tr>
<tr>
<td>ACh</td>
<td>3.89±0.29</td>
<td>5.11±0.53</td>
<td>8.37±0.58</td>
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</tr>
<tr>
<td>PGF2α</td>
<td>3.99±0.61</td>
<td>5.21±0.68*</td>
<td>5.64±0.22</td>
<td></td>
</tr>
<tr>
<td>SP</td>
<td>2.24±0.26</td>
<td>2.66±0.42</td>
<td>4.26±0.23</td>
<td></td>
</tr>
<tr>
<td>5-HT</td>
<td>3.13±0.59*</td>
<td>4.19±1.09*</td>
<td>4.16±0.44</td>
<td></td>
</tr>
<tr>
<td>5-HT</td>
<td>1.70±0.14</td>
<td>2.31±0.33</td>
<td>4.74±1.20</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7–10. Interleukin-1β (IL-1β, 10 ng/ml) was incubated over 60 min. SP substance P; 5-HT, serotonin. Contractions are expressed in g contraction/mm² cross-sectional area of the longitudinal muscle layer. The receptor-mediated contractions are characterized by their respective maximal contraction (Emax) values. P < 0.05 Emax significantly different from Emax without IL-1β, Student’s t-test for paired values.
leading to intestinal motor dysfunction (for reviews, see Refs. 7, 10, and 38). In the nematode-infected small intestine the transit is initially decreased, but it progressively increases as the course of the infection progresses (19, 34). The increased intestinal transit plays an active role in host defense by means of increased worm expulsion (for reviews, see Refs. 7, 10, and 38). The stimulation of peristalsis is reflected in the increased force generation by intestinal longitudinal smooth muscle (36), alterations in neuronal excitability (30), and alterations of neurotransmitter release (40), which are under direct control of substances released by T lymphocytes (10, 38), eosinophilic granulocytes (37), and mast cells (20, 29, 33). The nematode-infected rodents were shown to be worm free after 2- to 4-wk infection (36). Apparently, the hypothesis of motility-related host defense does not hold in intestinal schistosomiasis. Several possibilities can be put forward to explain the different inflammatory modulation of the intestinal motor function. *S. mansoni* is a blood fluke. The adult worms mate in the small vessels of the portal circulation of the liver. They then migrate by 10.220.33.3 on June 26, 2017 http://ajpgi.physiology.org/ Downloaded from

Fig. 10. Effect of *S. mansoni* infection on *E* max and pD2 values of different contractile agents inducing contractions of longitudinal muscle strips of the gastric fundus 8 and 12 wk after infection. *E* max values (left) are expressed in g contraction/mg tissue of the longitudinal muscle layer and shown as means ± SE. pD2 values (right) are expressed as the negative logarithm of the molar concentration and shown as means ± SE. No significant differences between infected mice and control mice were found using 1-way analysis of variance followed by Dunnett's test (n = 7–10). Because only 1 concentration of KCl was studied (50 mM), the pD2 value was not calculated.
phages (42), whereas other types of inflammatory cells play a pivotal role in the nematode-infected gut (38). Therefore, other cytokines may be involved, leading to a different inflammatory modulation of the motor function.

We addressed the role of IL-1β, which is an important granulomatous factor and which is secreted by macrophages during schistosomiasis (9, 12). IL-1β is important in the early recruitment stages of granuloma formation, and its secretion by macrophages is rapidly downregulated after granuloma elicitation (9). In the present study, we showed that mucosal IL-1β levels are indeed elevated during the acute inflammatory phase, whereas they are normalized during the chronic granulomatous phase of inflammation. The mucosal IL-1β content of the ileum of control mice was ~2 ng protein/g tissue, which is very similar to the IL-1β content of the rabbit colonic mucosa (11). IL-1β was also shown to be of major importance in the modulation of neuromuscular function during gastrointestinal inflammation (10). To study the effect of IL-1β on ileal neuromuscular function, we incubated ileal muscle strips with human recombinant IL-1β. This led to different results in controls and 8-wk infected mice. In muscle strips of control mice, IL-1β decreased the contractile response to ACh and increased the response to SP. These results are in accordance with previous reports (2). However, 8 wk after infection, incubation with IL-1β significantly increased the response to all contractile receptor agonists. Interestingly, the non-receptor-mediated contraction induced by KCl was not increased after IL-1β incubation. This suggests that after 8-wk infection gastrointestinal neuromuscular function is altered compared with that in control mice. After 12-wk infection, IL-1β did not affect the contractility of the ileum. The reason why exogenously administered IL-1β differently alters the contractility according to the time course of the infection remains speculative. However, we showed that endogenous IL-1β production is only increased during the early acute inflammatory phase, and it was previously shown that the endogenous IL-1β secretion by granuloma-associated macrophages is rapidly downregulated once the granuloma is formed during the acute inflammatory phase (9). In addition, the onset of the secretion of the IL-1 receptor antagonist in schistosomiasis-induced granulomas was shown to coincide with the decrease of IL-1β secretion during the chronic inflammatory phase (8). This may explain why exogenously administered IL-1β was maximally active during the acute inflammatory phase (8-wk infection), whereas during the chronic phase of inflammation (12-wk infection) the effect of exogenous IL-1β might be reduced because of the presence of the endogenous IL-1 receptor antagonist. Further studies are ongoing to elucidate the mechanism of action of IL-1β and the IL-1 receptor antagonist in intestinal schistosomiasis.

Other arachidonic acid metabolites, such as prostaglandins, have also been shown to be important granulomagenic factors during schistosomiasis (35). Their secretion by macrophages is constant throughout the infection. Indomethacin, a blocker of cyclooxygenase activity, inhibited the spontaneous contractility of the ileal muscle strips both in control mice and in infected mice, indicating that endogenous prostaglandins play a role in the maintenance of the inherent tone of the tissue preparation. However, the effect of intestinal schistosomiasis on the receptor-mediated and non-receptor-mediated contractions was not reversed by indomethacin, indicating that endogenous prostaglandins did not mediate the increased contractile response to receptor agonists and KCl in the ileum of S. mansoni-infected mice.

Previous studies in humans and in animal models showed that the neuromuscular function of remote noninflamed parts of the gastrointestinal tract is disturbed during inflammation (1, 26, 28). In the present study, intestinal schistosomiasis did not involve the gastric fundus histologically. Pharmacological contractility studies revealed that the neuromuscular function of the gastric fundus of infected mice did not differ from that of control mice. Also, the modulatory effect of IL-1β in the gastric fundus was similar in controls and in infected mice. Therefore, it is concluded that intestinal schistosomiasis thoroughly alters ileal contractility, but the motor abnormalities seem to remain confined only to the inflamed regions of the gastrointestinal tract.

In summary, we demonstrated the role of smooth muscle alterations in the pathophysiology of gastrointestinal motor abnormalities caused by S. mansoni infection. Our results indicate that the mouse model of intestinal schistosomiasis is an appropriate model to study the effect of granulomatous inflammation on gastrointestinal motility. Gastrointestinal transit is inhibited during chronic infection. This is associated with an increase in in vitro contractility of longitudinal muscle strips of the ileum caused by both trophic and functional changes at the smooth muscle cell level, whereas the contractility of the gastric fundus remains normal. Our results support the hypothesis that the clinical symptoms of intestinal schistosomiasis may be caused by a dysfunction of gastrointestinal motor function. We also demonstrated a modulatory role of IL-1β in this model, but this requires further study.

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