Visceral Hyperalgesia and Intestinal Dysmotility in a Mouse Model of Postinfective Gut Dysfunction

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Background & Aims: **We established the concept that transient enteric infection may lead to persistent gut dysfunction, evident in vitro, in nematode-infected mice. The present study determined whether gut dysfunction in this model involves motor and sensory changes reminiscent of changes found in patients with postinfective irritable bowel syndrome (PI-IBS) and investigated underlying mechanisms.** ***Methods:*** **Mice infected up to 70 days previously with *Trichinella spiralis* (*Tsp*) underwent videofluoroscopy with image analysis to assess upper gastrointestinal motility. Pseudoaffective responses to colorectal distention (CRD) were assessed using a barostat and validated by single fiber recordings from spinal nerves during CRD. Tissues were examined at different time points for histology, immunohistochemistry, and cytokine analysis. Some mice received dexamethasone intraperitoneally on days 23–25 PI or *Tsp* antigen orally on days 29, 43, and 57 PI.** ***Results:*** **From day 28 PI, no discernible inflammation was present in the gut. Frequency and propagation velocity of intestinal contractions decreased, and retroperistalsis increased at days 28 to 42 PI. CRD induced an allodynic and hyperalgesic response in PI mice, which was accompanied by increased single unit discharge. Gavage of *Tsp* antigen induced T-cell responses and sustained gut dysfunction for 70 days PI. Administration of dexamethasone postinfection normalized dysmotility and visceral hyperalgesia.** ***Conclusions:*** **Long-lasting gut dysmotility and hyperalgesia develop in mice after transient intestinal inflammation. These changes are maintained by luminal exposure to antigen and reversed by corticosteroid treatment. The findings prompt consideration of this as a model of PI-IBS.**

Our limited conceptualization of irritable bowel syndrome (IBS) contributes to the high socioeconomic impact of this disorder;[1] until recently, no animal models have been constructed to develop and test hypotheses regarding gut dysfunction and to develop new therapeutic strategies or diagnostic approaches.[2] An important contribution to our understanding of IBS is the recent recognition that acute gastroenteritis is the highest risk factor recognized to date for the development of IBS.[3] Prospective studies indicate that between 7% and 31% of patients with acute gastroenteritis subsequently develop symptoms of IBS.[4–8] A recent study indicates that the majority of patients with postinfective IBS (PI-IBS) are symptomatic for at least 6 years.[9] Although stress and behavioral factors are important in the expression of IBS, the long natural history suggests that environmental factors play a role in maintaining gut dysfunction.[4,9,10] This may arise from the lumen because patients with PI-IBS have increased intestinal permeability and evidence of immune activation.[11]

In a previous study, we have shown that in NIH Swiss mice infection with *Trichinella spiralis* results in altered muscle contractility that persists for up to 42 days postinfection (PI).[12] This occurs despite the fact that the mucosal compartment of the gut is restored to normal and that there is no overt inflammation. These findings provided proof of concept that transient infection leads to persistent gut dysfunction—a concept on which the pathogenesis of PI-IBS is based.[10]

To achieve greater face validity of the nematode-infected mouse as a model for PI-IBS, the present study examined whether sensory and motor changes seen in PI-IBS[5,13] occur in this model in vivo. The study also examined mechanisms whereby altered gut function induced by *T. spiralis* infection is sustained over time. Specifically, we examined whether intestinal exposure to crude *T. spiralis* antigen PI could prolong intestinal dysfunction and whether this was accompanied by evidence of immune activation, as occurs in patients with PI-IBS.[11] Finally, we determined whether changes in gut sensory and motor function could be reversed by corti-

Abbreviations used in this paper: CRD, colorectal distension; EMG, electromyographic; IBS, irritable bowel syndrome; ICC, interstitial cells of Cajal; IL, interleukin; MPO, myeloperoxidase; PI, postinfection; PI-IBS, postinfective irritable bowel syndrome; *Tsp*, *Trichinella spiralis*.

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costeroids given after recovery from infection, as predicted on the basis of our in vitro study.[14]

Materials and Methods

Animals

The experimental procedure was approved by the Animal Welfare Committee of McMaster University Medical Center. Specific pathogen-free female NIH Swiss mice, 6–8 weeks old, were purchased from National Cancer Institute (Frederick, MD). Groups of mice were assigned to motility, colorectal distention (CRD), and single unit discharge recording experiments. Both motility and CRD experiments were performed in additional mice after *T. spiralis* antigen challenge. Mice were killed at different time points during the protocols to obtain tissues for inflammation grading and cytokine and immunohistochemical analysis. Intestinal samples were taken from mid jejunum and colonic samples from the splenic flexure.

Trichinella spiralis

T. spiralis cultures originated in the Department of Zoology at the University of Toronto. The colony was maintained through alternating infection between Sprague-Dawley rats and CD1 mice. Larvae of *T. spiralis* were obtained from mice infected for more than 60 days.

Experimental Treatment

T. spiralis larvae (350–400 larvae per mouse) or placebo were administered intragastrically. Dexamethasone (0.5 mg/kg in 0.2 mL solution; ICN, Saint-Laurent, Quebec, Canada) or placebo (0.2 mL saline) was administered intraperitoneally on days 23 to 25 PI once daily. Additional groups of mice received either crude *T. spiralis* antigen (10 μg of protein in 0.2 mL solution) or placebo (0.2 mL saline) by intragastric gavage on days 29, 43, and 57 PI.

Inflammation

Acute inflammation was assessed by myeloperoxidase (MPO) activity in intestinal tissue. The assay was performed on frozen samples as described previously.[12] MPO activity is expressed in units per mg of tissue, where 1 unit of MPO is defined as a quantity of the enzyme able to convert 1 μmol of hydrogen peroxide to water in 1 minute at room temperature.

Mucosal damage and the presence of an acute and chronic inflammatory infiltrate were graded blindly by 2 investigators on a scale from 0 to 3 for each parameter in H&E-stained sections. Total scores were calculated by adding the 3 individual parameter scores (range, 0–9). A mean total score from the 2 investigators was obtained.

Gastrointestinal Motility

Mice were trained for 10 minutes per day in custom-built restrainers for a period of 2 weeks to minimize the impact of restraint stress. The experiments were performed before and on days 7, 21, 28, and 42 after *T. spiralis* infection (n = 25). In another part of the study designed to investigate the effect of specific antigen challenge, mice (n = 20) were studied before infection and on days 28 and 70 PI. Gastric and intestinal motility were assessed by using video image analysis as described previously.[15,16] Briefly, mice were gavaged intragastrically by 0.2 mL of iodine-based solution (Hypaque; Sanofi Winthrop, Markham, Ontario, Canada), placed in a Plexiglas restrainer, and continuously fluoroscoped for 3 minutes. Video images were then digitized and analyzed by using public-domain NIH Image 1.62 software (developed at the U. S. National Institutes of Health and available at: http://rsb.info.nih.gov/nih-image) with custom-made routines. Pixels from the longitudinal axis of the distal stomach and proximal small intestine were extracted from the single images and stacked to create spatiotemporal maps. These maps were used to assess the frequency and propagation velocity of contractions and the incidence of retroperistalsis.

Pseudoaffective Response to CRD

Electromyographic (EMG) electrodes were surgically implanted under sterile conditions in the anterior abdominal wall muscle of mice (n = 40) anesthetized with ketamine (Ketalean; Bimeda-MTC, Cambridge, ON, Canada; 90 mg/kg) and xylazine (Rompun; Bayer, Toronto, ON, Canada; 20 mg/kg) intraperitoneally, and a chronic fistula was exteriorized. Mice were then allowed to recover for a period of at least 7 days.

The response to CRD was assessed using a method described previously.[17] Mice were briefly anesthetized with enflurane (Enflurane USP; Abbott Laboratories, Saint-Laurent, Quebec, Canada), and a custom-made balloon catheter (20 × 10 mm) was gently inserted into the distal colon. A recording cable was connected to the chronic fistula, and mice were placed into Bollman restrainers. After connecting the catheters and cables to the barostat and EMG acquisition system, respectively, the mice were allowed a 5-minute rest. CRD was then performed in a stepwise fashion. Each 10-second distention was followed by a 5-minute resting period. Each level of distention (10, 30, 40, and 60 mm Hg) was repeated 3 times. EMG activity of the abdominal muscle was continuously recorded using customized software (Acquire 5.0, A. Bayatti). The area under the curve was calculated for 5 seconds before and after the beginning of each distention period by using customized software (GrafView 4.1, A. Bayatti). The median value for each distention level per mouse was then calculated.

Single Unit Discharge Recording

Mice (n = 8, 4 infected) were fasted overnight and anesthetized with ketamine/xylazine IP (as described above). A laminectomy was performed to expose the sixth left lumbar dorsal root ganglion and its root. The skin and connective tissue were used to create a receptacle, in which nerve tissue was immersed in mineral oil at 37°C to protect spinal cord and exposed sixth left lumbar dorsal root ganglion and its root. Bipolar tungsten electrodes were used to record single unit discharge and were connected to the AI-402 headstage (Axon

Instruments, Foster City, CA). The signal was amplified via Programmable Signal Conditioner (Cyber Amp 380, Axon Instruments) and fitted with bandwidth of 10–100 Hz. The amplified signal were acquired via an A/D and D/A converter at a sampling frequency of 1000 Hz and analyzed on-line by workbench software (Data Wave Technologies, Longmont, CO). The spike with a <5% variation in amplitude and shape was considered to represent a single unit discharge. A balloon catheter was inserted into the distal colon and distended with 0.2 mL of air and responses recorded.

Splenocytes Cultures and Cytokine Production

Cytokine production in splenocytes culture was assessed as described previously.[18] Briefly, mice were killed at day 70 PI and single-cell suspensions of spleen were prepared in RPMI containing 10% fetal calf serum, 5 mmol/L L-glutamine, 100 U of penicillin/mL, 100 μg of streptomycin/mL, 25 mmol/L HEPES, and 0.05 mmol/L 2-mercaptoethanol (all from Gibco-BRL, Burlington, Ontario, Canada). Cells were incubated in the presence of concanavalin A (5 μg/mL) or *Tsp* antigen (10 μg/mL). Culture supernatants were harvested after 24 hours and interleukin (IL)-4, interferon γ concentrations in the supernatants were measured by an enzyme immunoassay using commercially available kits purchased from R&D Systems (Minneapolis, MN).

Immunohistochemistry for CD3+ Cells

Samples of jejunum and colon were collected at day 70 PI, and immunostaining for CD3+ cells was performed on paraffin sections by using a modified method described previously.[19] We used rabbit anti-mouse CD3 (Dako A/S, Glostrup, Denmark; 1:300 dilution) as primary antibodies followed by biotinylated swine anti-rabbit (Dako A/S; 1:300 dilution) and streptavidin peroxidase conjugate (Dako A/S; 1:600 dilution). The antibodies were visualized by using 3-amino-9-ethylcarbazole, and the tissue was then counterstained with Mayer's hematoxylin. The number of CD3+ cells per visual field was counted (×250 magnification).

Statistics

Data are presented as means with 95% confidence intervals. Statistical testing was performed by using analysis of variance (ANOVA) with Dunnet post hoc test or *t* test for paired or unpaired data, as appropriate. *P* value lower than 0.05 was considered as statistically significant.

Results

Inflammation

MPO activity in the jejunum increased from 0.34 ± 0.3 in uninfected mice to 6.6 ± 3.5 units/g tissue on day 7 PI ($P < 0.01$). MPO values remained elevated on days 14 (1.3 ± 1.7 units/g) and 21 PI (1.6 ± 0.7 units/g, $P < 0.05$ vs. uninfected) but normalized by days 28 (0.71 ± 0.5 units/g) and 42 PI (0.68 ± 0.2 units/g). MPO activity in the colon increased from 0.29 ± 0.3 units/g in uninfected mice to 0.74 ± 0.5 units/g on day 14 PI ($P < 0.05$) and normalized thereafter (Figure 1).

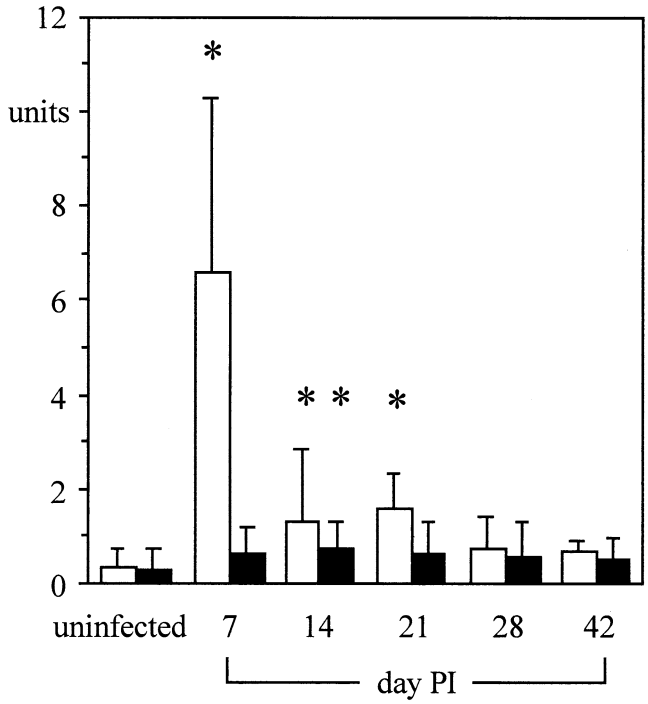

Figure 1. MPO activity in the jejunum and colon. MPO activity in the jejunum (*open bars*, n = 10 per group) increased significantly at day 7 PI and remained elevated until day 21 PI. MPO activity in the colon (*solid bars*, n = 10 per group) increased significantly at day 14 PI and normalized thereafter. Data are displayed as means and 95% confidence intervals. ANOVA test, $*P < 0.05$ vs. uninfected.

H&E staining of the jejunum showed a marked infiltration by neutrophils and eosinophils on day 7 PI. Acute inflammation gradually decreased from day 14 to 28 PI, when no overt inflammatory infiltrate was observed. A transient acute inflammatory infiltrate was also present in the colon on day 14 PI but disappeared by day 28 (Table 1). No discernible inflammation was present in the gut at day 70 PI after challenge with *T. spiralis* antigen.

Gastrointestinal Motility

The frequency of gastric contractions increased on day 7 PI and normalized on day 21 PI. However, the propagation velocity of gastric contractions was not affected by *T. spiralis* infection (Table 2). Dexamethasone treatment did not affect frequency or velocity of gastric contractions.

Regular and coordinated peristalsis was observed in the small intestine of uninfected mice, occasionally interrupted by short periods of retroperistalsis (Figure 2*A*). In infected mice, the occurrence of retrograde contrac-

Table 1. Inflammation Grading in H&E-Stained Sections

	Uninfected	Day 7	Day 14	Day 21	Day 28	Day 42
Jejunum	0.13 ± 0.2	4.8 ± 1.6[a]	1.6 ± 0.9[a]	1.1 ± 0.2[a]	0.5 ± 0.5	0.33 ± 0.3
Colon	0.10 ± 0.1	0.75 ± 0.6	1.0 ± 1.2[a]	0.56 ± 0.5	0.28 ± 0.3	0.11 ± 0.2

[a]ANOVA, $P < 0.05$ vs. uninfected.

tions increased, and ectopic dominant pacemakers appeared in the proximal small intestine (Figure 2*B*).

The frequency of contractions in the small intestine was 44.1 ± 1.3 cycles/minute in uninfected mice, and it decreased in infected mice at day 14 and 21 PI. In mice treated with placebo, the frequency of intestinal contractions at 28 and 42 days PI was lower (41.3 ± 2.1 and 41.6 ± 0.9 cycle/minute, respectively, both $P < 0.05$) when compared with uninfected mice. Dexamethasone administration normalized the frequency of intestinal contractions on day 42 (Figure 2*C*).

The aboral velocity of intestinal contractions increased from 10.9 ± 1.0 mm/s in uninfected mice to 15.7 ± 1.9 mm/s on day 7 PI ($P < 0.05$). In mice treated with placebo, the propagation velocity normalized on day 21 PI (9.9 ± 1.1 mm/s) but continued to decrease to 9.3 ± 0.8 mm/s on day 28 and to 8.8 ± 1.1 mm/s on day 42 PI (both $P < 0.05$ vs. uninfected). Intestinal contractions propagated orally at a velocity of 4.4 ± 1.1 mm/s in uninfected mice. On day 7 PI, the oral velocity increased to 6.9 ± 1.6 mm/s ($P < 0.05$), normalized to 5.0 ± 0.8 mm/s at day 21 PI, and remained stable thereafter. Dexamethasone treatment did not affect aboral or oral propagation velocity.

Retrograde propagation of contractions in the small intestine occurred 18.9% ± 7.6% of the time in uninfected animals. On day 7 PI, retroperistalsis was observed 16.1% ± 6.8% of the time, increasing on day 21 PI ($P < 0.05$ vs. uninfected). In placebo-treated mice, the occurrence of retroperistalsis was more frequent on days 28 and 42 PI (both $P < 0.01$ vs. uninfected). In dexamethasone-treated mice, the occurrence of retrograde contractions on day 28 PI was increased when compared with uninfected mice but normalized on day 42 PI (Figure 2*D*).

Pseudoaffective Response to CRD

CRD induced a volume-dependent response as measured by EMG of the abdominal muscle. In uninfected mice, the area under the curve was 0.31 ± 0.08, 0.39 ± 0.15, 1.05 ± 0.29, 1.38 ± 0.41, and 1.34 ± 0.38 mV/min for 0, 10, 20, 30, and 60 mm Hg of distention, respectively. No change was observed day 10 PI. However, on day 28 PI, the responses increased by 50%–60% compared with uninfected mice (Figure 3). As shown in Figure 3*B*, treatment with dexamethasone on days 23–25 PI normalized CRD responses by day 28 PI.

Single Unit Discharge

In control mice, resting single unit discharge was 4.74 ± 2.14 Hz and increased to 16.75 ± 1.01 Hz ($P < 0.05$) with 0.2 mL of colorectal distention. At day 28 PI, resting activity tended to be higher, and the response to colonic distention increased significantly to 39.97 ± 6.56 Hz ($P < 0.01$ vs. uninfected) (Figure 4).

Effect of Antigen Administration on Motility and Colorectal Sensitivity in the PI State

The frequency of intestinal contractions decreased from 47.8 ± 3.6 cycles/min in uninfected mice to 42.3 ± 4.3 cycles/min at day 28 PI ($P < 0.05$). At day 70 PI, the frequency of intestinal contractions normalized in placebo-treated mice. In *Tsp* antigen-treated mice, the frequency of intestinal contractions remained lower when compared with before *Tsp* infection (43.4 ± 3.1 cycles/min, $P < 0.05$ vs. before infection). Similarly, the ocurrence of retroperistalsis increased from 15.2% ± 7.3% in uninfected mice to 43% of the time at day 28 PI ($P < 0.05$). At day 70 PI, retroperistalsis normalized in mice treated with placebo but remained elevated in *Tsp* antigen-treated mice (40.2% ± 20.1%, $P < 0.05$ vs. before infection) (Figure 5).

At day 70 PI, the pseudoaffective response to CRD was significantly increased in mice treated with *Tsp* antigen compared with before infection ($P < 0.05$). However, no difference in CRD response was found in *T.*

Table 2. Gastric Motility

	Uninfected	Day 7	Day 21	Day 28	Day 42
Frequency (*cycle/min*)	4.7 ± 0.9	6.0 ± 0.6[a]	5.2 ± 0.8	4.8 ± 0.7	4.8 ± 0.9
Velocity (*mm/min*)	1.13 ± 0.14	0.99 ± 0.11	1.16 ± 0.14	1.13 ± 0.10	0.98 ± 0.11

[a]ANOVA, $P < 0.05$ vs. uninfected.

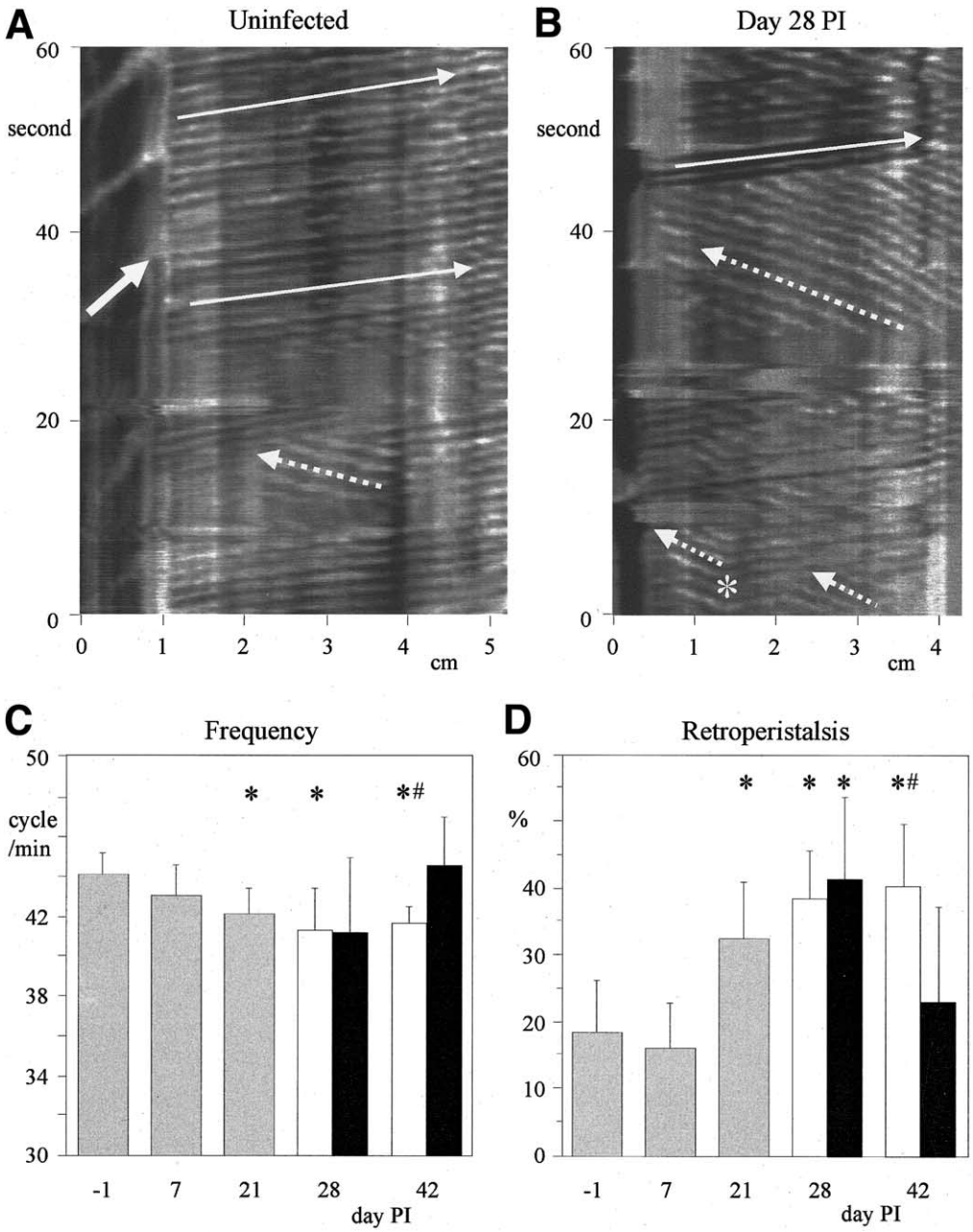

Figure 2. Effect of *T. spiralis* infection on motility patterns in vivo. (*A*) Example of spatiotemporal map from uninfected mouse showing regular contractions propagating aborally in the stomach (*thick arrow*) and small intestine (*thin arrow*). Occasionally, retrograde contractions appeared in the small intestine (*thick dashed arrow*). (X-axis = position along the intestine, y-axis = time; pylorus located at 1 cm.) (*B*) Example of spatiotemporal map from mouse at day 28 PI. Regular peristalsis in the small intestine (*thin arrow*) is replaced by orally propagating contractions (*thick dashed arrows*). Ectopic pacemakers appear in proximal small intestine (*). (*C*) Frequency of contraction in the small intestine decreased by day 21 PI and remained lower in mice treated with placebo (*open bars*). Frequency of contraction in dexamethasone-treated mice (*solid bars*) normalized by day 42 PI. (*D*) Retroperistalsis appeared 19% of the time in uninfected mice but increased 2-fold at day 21 PI. Retroperistalsis remained high in placebo-treated mice, and it normalized in dexamethasone-treated mice by day 42 PI. ANOVA, $*P < 0.05$ vs. uninfected; *t* test, $\#P < 0.05$ vs. dexamethasone treatment.

spiralis–infected mice treated with placebo compared with before infection (Figure 5).

Effect of Antigen Administration on Cytokine Production by Splenocytes

Tsp antigen-stimulated IL-4 production was 50.3 ± 46.8 pg/mL in uninfected mice and 147.8 ± 108.9 pg/mL in *Tsp* placebo-treated mice. In *Tsp* antigen-treated mice, IL-4 production increased to 216.2 ± 94.4 pg/mL ($P < 0.05$ vs. uninfected). Concanavalin A–stimulated IL-4 production was 660.9 ± 238.9 pg/mL in uninfected mice and 943.6 ± 703.9 pg/mL in *Tsp* placebo-treated mice. IL-4 production increased to 2124.5 ± 1218.2 pg/mL in *Tsp* antigen-treated mice ($P < 0.05$ vs. uninfected and $P = 0.07$ vs. placebo).

Tsp antigen-stimulated interferon γ production was undetectable in uninfected mice, 58.5 ± 71.2 pg/mL in *Tsp* placebo-treated mice, and 81.2 ± 85.7 pg/mL in *Tsp* antigen-treated mice (both $P < 0.05$ vs. uninfected). After nonspecific stimulation with concanavalin A, interferon γ production was 803.2 ± 357.0 pg/mL in uninfected mice, and 941.8 ± 734.9 pg/mL in *Tsp* placebo-treated mice and tended to increase to 1385 ± 354.2 pg/mL in *Tsp* antigen-treated mice ($P = 0.08$ vs. uninfected).

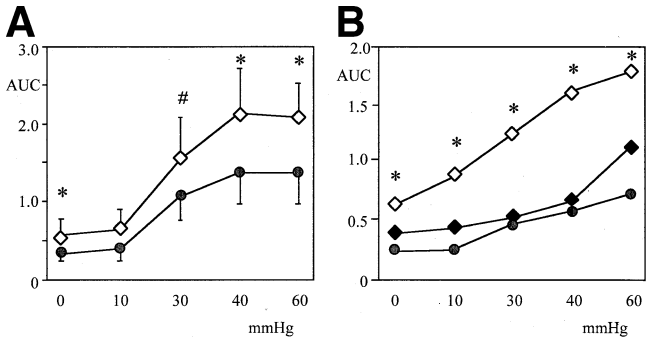

Figure 3. Pseudoaffective response to CRD. (*A*) There was a dose-dependent response to CRD in mice before infection (*shaded circles*), which increased by 50%–60% in mice at day 28 PI (*open diamonds*). *T* test for paired data, $*P < 0.05$ and $\#P = 0.08$ vs. before infection. (*B*) At 28 days PI, previously infected mice treated with placebo (*open diamonds*) had a higher pseudoaffective response than uninfected controls (*shaded circles*). Previously infected mice treated with dexamethasone normalized their response to CRD (*solid diamonds*). ANOVA, $*P < 0.05$ vs. uninfected controls.

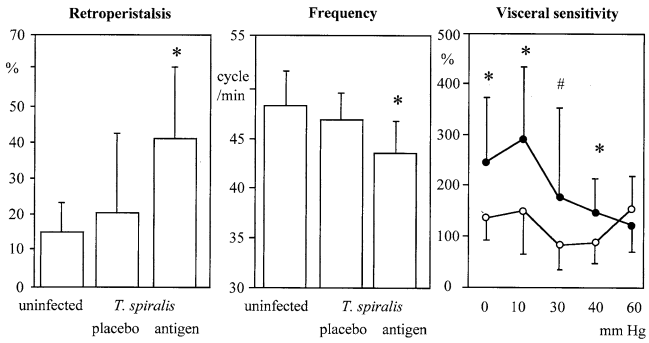

Figure 5. Effect of *T. spiralis* antigen administration on gut function at day 70 PI. (*A*) Retroperistalsis appeared 40% of the time in mice treated with *T. spiralis* antigen, whereas it normalized in mice receiving placebo. (*B*) Frequency of contractions was lower in mice treated with *T. spiralis* antigen, whereas it normalized in mice receiving placebo. (*C*) Pseudoaffective response to CRD was increased in mice treated with *T. spiralis* antigen (*solid circles*), whereas it was unchanged in placebo-treated mice (*open circles*). Data are expressed as percentage of the response in the same mice before *T. spiralis* infection. *T* test for paired data, $*P < 0.05$ vs. before infection, $\#P = 0.08$ vs. before infection.

Effect of Antigen Administration on Density of Intestinal CD3+ Cells

In the jejunum, the density of CD3+ cells/field was 73.3 ± 19.4 in uninfected mice and 84.7 ± 16.9 in *Tsp* placebo-treated mice. In *Tsp* antigen-treated mice, the density of CD3+ cells increased to 106.2 ± 17.0 cells/field ($P < 0.05$ vs. both uninfected and placebo). In the colon, the density of CD3+ cells/field was 35.0 ± 29.9 in uninfected mice and tended to increase to 64.8 ± 31.4 in *Tsp* placebo-treated mice and to 64.5 ± 42.4 cells/ field in *Tsp* antigen-treated mice (both $P = 0.10$ vs. uninfected).

Discussion

There is growing evidence that infection and/or inflammation in the gut play an important role in symptom generation in at least a subset of patients with IBS.[10,20] Understanding the mechanisms through which low-grade inflammation affects gut function is critical for designing future therapeutic targets. This study shows that altered intestinal motility and visceral hyperalgesia persist in mice that have recovered from a nematode parasite infection. The profile of altered gut physiology and the absence of overt inflammation in the colonic mucosa are reminiscent of PI-IBS.[5,13] The ability of antigen to prolong gut dysfunction provides a putative mechanism for the maintenance of this syndrome that can last more than 6 years PI in >50% of cases.[9] Of potential clinical interest is the finding that a window of therapeutic opportunity exists PI when anti-inflammatory therapy prevented the development of hyperalgesia and reversed most of the altered motility in this model.

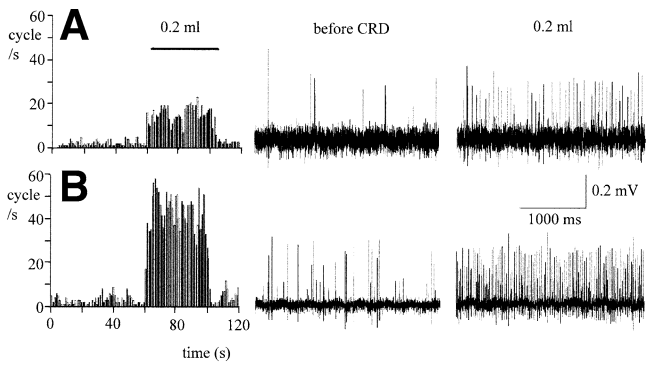

Figure 4. Single unit discharge in response to colorectal distention (CRD). (*A*) Single unit discharge before and during 0.2 mL distention in uninfected mouse (*left*). Detailed recordings of single fiber firing before (*middle*) and during CRD (*right*). (*B*) At day 28 PI, the single unit discharge increased significantly during 0.2 mL distention.

Gut motility was markedly altered during and after acute *T. spiralis* infection. The frequency of gastric contractions and propagation velocity of intestinal contractions increased during the acute phase and resulted in the passage of loose stools. This likely reflects the host's inflammatory response to infection, which we believe contributes to the eviction of the parasite from the gastrointestinal tract.[21] In contrast to gastric motility that normalized in the PI period, the frequency of intestinal contractions was decreased for up to 6 weeks post–*T. spiralis* infection. This was paralleled by a slower propagation of contractions and an increased occurrence of retroperistalsis, likely resulting in prolonged intestinal transit. In healthy mice, retrograde peristalsis appeared 10%–20% of the time and is probably involved in the

mixing of luminal contents. These contractions increased to 40% of the time in the PI period, suggesting the development of abnormal coordination between segments of intestine. The frequency of contractions in the gut is determined by slow waves generated by interstitial cells of Cajal (ICCs). A frequency gradient of slow waves exists along the gut, which results in propagation of contractions preferentially in the aboral direction.[22] We have shown previously that acute *T. spiralis* infection in C57Bl6 mice disrupts the network of ICCs, and ectopic dominant pacemakers with lower frequency appear distally.[16] This can result in oral propagation and decreased frequency of contractions. The propagation velocity of the intestinal contractions remained lower despite dexamethasone treatment, possibly because of irreversible functional or structural changes in a population of ICCs similar to those described in *Nippostrongylus brasiliensis* infection in the rat.[23] The function of the enteric nervous system has been shown to be altered during the acute infection with *T. spiralis* in several animal models,[24–26] and treatment with antinerve growth factor antibody prevented the development of intestinal dysmotility in rats.[27] Thus, part of the altered motor patterns observed in our study could be a consequence of long-term damage to ICCs and the enteric nervous system; steroid-sensitive component is likely caused by suppression of altered function. Interestingly, a recent study using high-resolution manometry found increased incidence of retrograde contractions in the duodenum during the postprandial period, both in diarrhea and constipation-predominant patients with IBS. Furthermore, the degree of intestinal retroperistalsis was related to the severity of their symptoms.[28]

Altered mechanosensitivity with lower discomfort and pain thresholds has been documented in patients with IBS.[29,30] We found that mice previously infected with *T. spiralis* had a hyperalgesic response to CRD, which persisted for at least 6 weeks PI. These mice also exhibited allodynia, in that they displayed an increased response to the presence of the catheter in the lumen, in the absence of any balloon distention.

We acknowledge that our assessment of visceral sensitivity in this model could be influenced directly by the parasite. During its life cycle, larvae encyst in skeletal muscle and produce a local inflammatory response that likely alters the excitability of the muscle. This in turn may influence EMG from skeletal muscle, which is a component of the visceromotor response. However, we do not believe that this is the basis for our interpretation that the increased EMG recorded from abdominal muscle reflects visceral hyperalgesia. The fact that single unit firing frequency in dorsal root ganglia during CRD increased 2.5-fold in mice during the PI period when compared with uninfected controls supports our interpretation of a hyperalgesic state.

In the small intestine, which is the primary habitat of *T. spiralis*, MPO activity peaks at day 10 PI.[21] During the eviction of the parasite from the gut, we observed a small and transient rise in colonic MPO activity on day 14 PI. By day 28 PI, when hyperalgesia appeared, there was no evidence of colitis as reflected by histology or MPO activity. We believe that the sensitization of sensory nerves, induced by the acute inflammatory and immune activation following infection, is maintained by local mediator production in the neuromuscular tissues[14] and is enhanced by further immune activation after exposure to luminal antigen. This scenario is consistent with the ability of corticosteroid to suppress hyperalgesia when administered shortly after recovery from the initial infection in this model. It is not known whether a similar window of therapeutic opportunity exists in humans. A recent study failed to show symptomatic improvement following a short course of prednisone in patients with PI-IBS.[31] However, the steroid was given at different intervals PI, and the window of opportunity may have been missed.

That immune activation plays a role in gut dysfunction in, at least a subset of patients with IBS, has recently been suggested by various clinical studies. IBS patients with previously diagnosed gastroenteritis have higher numbers of lymphocytes and mast cells and increased IL-1β messenger RNA expression in colonic biopsies.[11,32] It has also been suggested that IBS patients are genetically more susceptible to inflammatory stimuli than healthy controls.[33] Finally, recent studies in patients with an insidious onset of IBS symptoms have shown signs of mucosal immune activation with increased CD25+ and CD3+ cell counts in colon[34] and chronic low-grade inflammation in full-thickness jejunal biopsy specimens.[35] Although these findings suggest that ongoing immune activation occurs in IBS, they do not identify the stimulus. It is possible that exposure of gut tissues to luminal antigen, with epitopes similar to those of the initial infective agent, could maintain immune activation and sustain gut dysfunction in PI-IBS. The nature of the antigen could be dietary or derived from the intestinal microbiota; acute infection could lead to a breakdown of oral tolerance to these antigens. Identification of the precise antigen in the clinical setting would be difficult because of the numerous possible candidates. Therefore, as a proof of concept, we challenged mice with structurally related antigen at 2-week

intervals. Experiments were performed 1 week after the last challenge to avoid the immediate hypersensitivity reaction. At 70 days PI, gut function in placebo-treated mice had returned to normal. However, *T. spiralis* antigen-treated mice still displayed gut dysmotility and hyperalgesia. These functional abnormalities were accompanied by evidence of immune activation, as reflected by increases in CD3+ve cells in the gut. There is an interesting parallel with asthma; about which studies have identified a relationship between viral infections and the subsequent development of airway hyperreactivity.[36] In this model, a subset of chronically activated T-memory cells sensitized against allergenic, occupational, or viral antigens drive and maintain hyperreactivity in the bronchial tree.[37] We do not believe that antigens originating from encysted larvae affect the validity of this model. *Tsp* antigen is not detected in serum of *Tsp*-infected rats at any time point, independently of the infective dose, despite the presence of a robust antibody response.[38] Systemic exposure to the antigen is therefore minimal, if any, at the PI state.

In conclusion, our results show that transient nematode enteritis induces long-term intestinal dysmotility and colonic hypersensitivity in mice in vivo. These functional abnormalities are immune driven, and they are normalized by early and potent anti-inflammatory treatment. Furthermore, gut dysfunction can be sustained over time by administration of antigen related to the triggering infection. We suggest that exposure to bacterial or dietary antigens related to the triggering infection may maintain symptoms in patients with PI-IBS.

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