

Leukotrienes Play a Role in the Control of Parasite Burden in Murine Strongyloidiasis¹

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It is clear that leukotrienes mediate inflammatory response; new aspects of leukotriene function have recently been described. In this study, we demonstrate that leukotrienes are key chemical mediators in the control of parasite burdens in mice infected with *Strongyloides venezuelensis*. High leukotriene levels were detected in the lungs and small intestines of Swiss mice. In infected Swiss mice treated with MK886, a leukotriene synthesis inhibitor, numbers of adult worms, and eggs/g/feces were greater than in infected-only animals. The MK886 treatment inhibited leukotriene B₄ production in the lungs and small intestines, albeit on different postinfection days. Similarly, parasite burdens and eggs/g/feces were greater in 5-lipoxygenase^{-/-} mice than in wild-type animals. These observations were confirmed by histopathological study of the duodena. We subsequently observed significant lower numbers of eosinophils and mononuclear cells in the blood, peritoneal cavity fluid, and bronchoalveolar lavage fluid of Swiss mice treated with MK886. In the lung parenchyma of infected animals, MK886 significantly inhibited synthesis of IL-5 at the beginning of infection, whereas levels of IL-12 increased progressively throughout the postinfection period. However, levels of leukotriene C₄, PGE₂, TNF- α , IL-3, IL-4, IFN- γ , and IL-10 were comparable between the treated and untreated groups. Nevertheless, IgE and IgG1 (but not IgG2a) synthesis was also significantly inhibited by MK886 administration. Therefore, in *S. venezuelensis*-infected mice, adult worm and egg burdens are leukotriene dependent. These findings indicate potential immunostimulatory strategies involving leukotriene administration, and may serve as an alert to physicians treating *Strongyloides stercoralis*-infected patients presenting asthma-like symptoms because use of 5-lipoxygenase inhibitors may worsen the infection. *The Journal of Immunology*, 2005, 175: 3892–3899.

The incidence of strongyloidiasis has increased dramatically, mainly in patients presenting altered immune status accompanied by malnutrition, having undergone organ or bone marrow transplantation, with acquired immunodeficiency syndrome, or receiving cancer chemotherapy (1–5). Many patients chronically infected with *Strongyloides stercoralis* are asymptomatic, whereas others present a variety of symptoms, most related to the skin or gastrointestinal system, and many *S. stercoralis* infections evolve to a cure (6). When the host-parasite balance is disturbed, as occurs in immunocompromised patients, intestinal worms may reproduce excessively and invade organs outside their normal migratory pathways. Such dissemination, if untreated, of-

ten results in the death of the host (6, 7). To date, the cause of this intense proliferation is unknown.

In human hosts and in murine models, the immune response to *Strongyloides sp* is characterized by intraepithelial and tissue increase of eosinophils (8, 9), as well as by intestinal mastocytosis (10, 11) and production of Th2-type cytokines such as IL-3, IL-4, and IL-5 (11–13). Production of IgA, IgE, IgG, and IgM Abs has also been demonstrated (14, 15). However, far less attention has been given to the role of leukotrienes in host defense during strongyloidiasis infection, in which they could act as mediators of antiparasitic activity. Leukotrienes are generated via the 5-lipoxygenase (5-LO)³ pathway in the arachidonic acid metabolism and have been implicated in many inflammatory and allergic diseases (16–19). Various authors have described new leukotriene immune response functions (20–23) and antimicrobial activity (24, 25). More recently, we demonstrated that leukotrienes are essential to efficient pulmonary antifungal host defense because they modulate NO and cytokine production during infection (26). The importance of leukotrienes in controlling helminth parasite burdens is still unknown, and very little information is available on the subject. It has been shown that levels of leukotriene B₄ (LTB₄) and leukotriene C₄ (LTC₄) are elevated in the intestinal mucosa of lambs infected with *Trichostrongylus colubriformis* (27–29). The authors suggested that these lipid mediators are associated with larval rejection and exclusion.

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³ Abbreviations used in this paper: 5-LO, 5-lipoxygenase; BALF, bronchoalveolar lavage fluid; LTB₄, leukotriene B₄; LTC₄, leukotriene C₄; PCF, peritoneal cavity fluid; WT, wild type.

In the present study, we investigated the effects of inhibition or absence of leukotrienes during *Strongyloides venezuelensis* infection in mice. Our results reveal for the first time that leukotrienes play an essential role in controlling parasite burdens, as well as in altering the parasite reproductive cycle and eliminating the parasites themselves.

Materials and Methods

Animals

Male Swiss mice weighing 16–25 g and male Wistar rats weighing 120–180 g were obtained from the animal facilities of the Central de Bioterismo da Universidade Estadual de Campinas and Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo. Male mice lacking the 5-LO enzyme gene (5-LO^{-/-}) and weighing 18–25 g were obtained from The Jackson Laboratory, and age-matched male wild-type (WT) mice (background, strain 129) were used as controls. All experiments were approved by and conducted in accordance with guidelines established by the Animal Care Committee of the Universidade Estadual de Campinas (Protocol 19-2). All infected and control animal strains were maintained under standard laboratory conditions.

Parasites

The *S. venezuelensis* strain was isolated from the wild rodent *Bolomys lasiurus* in April 1986 (30). The strain was maintained in *Rattus norvegicus* Wistar, routinely infected in the Parasitology Laboratory of the Instituto de Biologia da Universidade Estadual de Campinas.

Infection of mice with *S. venezuelensis* and treatment with MK886

S. venezuelensis third-stage infective larvae (L3) were obtained from charcoal cultures of infected rat feces. The cultures were stored at 28°C for 72 h, and the infective larvae were collected and concentrated by using a Baermann apparatus. The recovered larvae were then washed several times in PBS and counted. The number was subsequently adjusted to 10,000 and 15,000 L3 per ml of PBS for infection. Swiss mice were individually inoculated via s.c. abdominal injection with 100 μ l of PBS containing 1500 *S. venezuelensis* L3. Selected 5-LO^{-/-} and WT mice were similarly infected with 1000 L3. The Swiss mice were divided into two groups. Animals in the first group were treated orally by gavage with 0.5 ml of MK886 (2 mg kg⁻¹) at 1 h before infection and then daily until day 14 (final evaluation day). In the second group, the animals were treated orally by gavage with 0.5 ml of water, as described above. The last treatment was given 1 h before sacrifice.

Collection of blood, serum, bronchoalveolar lavage fluid (BALF), and peritoneal fluid

On postinfection days 1, 3, 5, 7, and 14, mice were anesthetized with 30 mg/kg s.c. tribromoethanol (Acros Organics), and blood samples were collected by cardiac puncture. Subsequently, the animals were sacrificed with an overdose of tribromoethanol. The chest cavity of each animal was carefully opened, and the trachea was exposed and catheterized. The catheter was tied in place, and sterile PBS/sodium citrate (0.5%) was infused in three 1-ml aliquots. The BALF was collected and placed on ice. The peritoneal cavity fluid (PCF) was obtained by injecting 3 ml of PBS in the peritoneal cavity. Total cell counts in blood, BALF, and PCF were immediately performed in a Neubauer chamber. Differential counts were obtained using Rosenfeld staining (31). Blood was then centrifuged, and the serum was stored at -70°C.

Alkaline parasite extracts

Alkaline extracts were prepared, as previously described (32). In brief, 1 ml of 0.15 M NaOH was added to ~170,000 *S. venezuelensis* larvae, which were maintained under gentle agitation for 6 h at 4°C. Subsequently, 0.3 M HCl was added until a pH of 7.0 was reached. This preparation was then centrifuged at 12,400 $\times$ g for 30 min at 4°C. Protein determination of the supernatant was 1.89 mg/ml, as detected by the Lowry method (33). The antigenic extract was used for ELISA.

Measurement of leukotrienes, PG, and cytokines

For determination of cytokine, LTB₄, LTC₄, and PGE₂ levels, lungs and duodena (10-cm sections) were removed on postinfection days 1, 3, 5, 7, and 14. Tissue samples were homogenized (Ultra-Turrax T8; IKA-Werke) in 1.5 ml (for lungs) or 2 ml (for duodena) of medium, centrifuged at

1500 $\times$ g, filtered, and stored at -70°C until assay. Eicosanoids were quantified using commercial ELISA kits obtained from Amersham Biosciences. Commercially available ELISA Abs were used to measure TNF- α , IL-3, IL-4, IL-5, IL-10, IL-12, and IFN- γ , according to manufacturer instructions (BD Pharmingen). Sensitivities were >10 pg/ml.

Measurement of Abs in sera

Specific IgE, IgG1, and IgG2a were determined in mice sera by ELISA, according to manufacturer instructions (Technical Data Sheet; BD Pharmingen). The plates were coated with *S. venezuelensis* alkaline extract at a concentration of 20 μ g/ml in carbonate buffer at pH 9.6 for 18 h at 4°C. After three washes in PBS containing 0.05% Tween 20 (pH 7.4), nonspecific binding sites were blocked with a buffer composed of the same PBS-Tween 20 solution plus 1% BSA for 1 h at 37°C. Serum samples (50 μ l each) were added to wells at a dilution of 1/20 (for measurement of IgG1 and IgG2a) or 1/5 (for measurement of IgE), both in blocking buffer. To measure IgG1 and IgG2a, plates were then incubated at room temperature for 1 h. For IgE measurement, however, the plate was incubated at 4°C for 24 h and washed five times. Subsequently, 50 μ l of biotin-labeled goat anti-mouse IgE, IgG1, and IgG2a (BD Pharmingen) was added to each well. The plates were incubated for 1 h at room temperature before being washed five times. NeutrAvidin-peroxidase conjugate (Pierce) was then added to each well. The samples were incubated for 1 h at room temperature and then washed eight times. The reactions were developed with SuperSignal Chemiluminescent, according to the manufacturer instructions (SuperSignal ELISA Pico Chemiluminescent; Pierce) at a dilution of 1/20. The reactions were measured on a chemiluminescent reader (FLX 800; Bio-Tek Instruments). The values of total IgE, IgG1, and IgG2a are expressed as relative light units.

Histology

Duodena (10-cm sections) were removed on postinfection days 5, 7, and 14. The tissue samples were then fixed in 10% Formalin and embedded in paraffin blocks. To count inflammatory cells and determine worm burdens, 5- μ m sections were stained with H&E and analyzed in a blinded fashion.

Filariform larvae, eggs, and adult worm counts

On postinfection days 1, 3, and 5, groups of mice were sacrificed and the lungs were harvested. The lungs were then tweezed so that migrating larvae could be collected and counted (34). On postinfection days 5, 7, 9, 11, and 14, the animals were placed individually on clean, moist absorbent paper and allowed to defecate. Eggs/g/feces were counted using the Cornell-McMaster quantitative method (35). The parasitological exam was performed twice, and the average of the two results was recorded. The animals were then sacrificed with tribromoethanol. To count the adult parasites, 10-cm duodenal sections and 11-cm jejunal sections were subsequently removed, placed on petri dishes containing saline, longitudinally sectioned, and incubated for 2 h at 37°C. The adult worms from the intestines and filariform larvae from lungs were counted under light microscopy at a magnification of $\times$ 100.

Statistical analysis

Each experiment was performed twice. The results of the experiments are expressed as mean $\pm$ SEM. Statistical variations were analyzed using ANOVA, followed by the Bonferroni test. Student's *t* test was used only in the analysis of parasite and egg numbers (Fig. 3 and Table I). The level of statistical significance was set at *p* < 0.05.

Results

Lung leukotrienes and PG production are increased in *S. venezuelensis*-infected mice, but only LTB₄ is inhibited with MK886

To confirm leukotriene release during *S. venezuelensis* infection, the lungs of infected Swiss mice, treated or not treated with MK886, were harvested daily from postinfection day 1 to postinfection day 14. Our results demonstrate that, on postinfection days 1 and 3, respectively, LTB₄ cell production in the lungs of *S. venezuelensis*-infected animals was 166 and 200% higher than that seen in uninfected controls. Treatment of infected animals with MK886 inhibited LTB₄ synthesis by 16% when compared with control animals and by 69% when compared with infected, untreated mice on postinfection day 1. However, on postinfection day

Table I. *Leukotriene depletion increases in numbers of adult worms in duodena and infective larvae recovered from lungs during S. venezuelensis infection*

Postinfection Day	Swiss Sv + H ₂ O		Swiss Sv + MK886		WT + Sv		5-LO ^{-/-} + Sv	
	Lungs ^a	Duodena ^b	Lungs ^a	Duodena ^b	Lungs ^c	Duodena ^d	Lungs ^c	Duodena ^d
1	260 ± 42		340 ± 76		51 ± 1		103 ± 1	
3	59 ± 6		67 ± 17		103 ± 1		105 ± 2	
5	0	4 ± 0.5	0	6 ± 1	2 ± 1	158 ± 14	2 ± 10	125 ± 10
7		3 ± 1		16 ± 3*		63 ± 13		117 ± 19*
9		3 ± 1		16 ± 3*		78 ± 1		168 ± 2*
11		3 ± 1		16 ± 3*		68 ± 1		178 ± 2*
14		0		0		70 ± 10		225 ± 38

^{a,c} Lungs were obtained on postinfection days 1, 3, and 5. To evaluate the number of infective larvae, the lungs were tweezed in saline and maintained at 27°C for 2 h, followed by centrifugation, and the pellet was examined under light microscopy at ×50 magnification for detection of parasites.

^{b,d} Duodenal sections were obtained on postinfection days 5, 7, 9, 11, and 14. To evaluate the number of adult worms in the duodena, 10 fields of each 5-μm section were counted under light microscopy at ×100 magnification. Data are expressed as mean ± SEM of one representative experiment (n = 5). *, p < 0.05; #, swiss Sv + H₂O vs swiss Sv + MK886 or WT + Sv vs 5-LO^{-/-} + Sv.

3, the percentage of inhibition when compared with infected animals was 49% (Fig. 1A). No significant differences were observed between postinfection days 5 and 14.

Levels of LTC₄ were also measured in the lungs of infected mice, treated or not treated with MK886, and in those of unin-

fected control mice. As demonstrated in Fig. 1B, there was no significant difference between infected animals, treated or not treated with MK886, and controls.

On postinfection days 1 and 3, levels of PGE₂ in the lungs of Swiss mice were comparable between those infected (treated or not treated with MK886) and those in the control group. Nevertheless, after postinfection day 5, there was a significant increase in PGE₂ production in infected mice (treated or not treated with MK886) when compared with the control animals. However, no significant difference was observed between infected animals treated with MK886 and infected, untreated animals (Fig. 1C).

Duodena leukotriene production is increased in S. venezuelensis-infected mice and is inhibited with MK886

During its cycle life in mice, this parasite lives in the duodena between postinfection days 5 and 14. In view of this fact, we measured LTB₄ in the duodena during this period. As shown in Fig. 2, *S. venezuelensis* infection induced a significant increase in LTB₄ synthesis in the small intestine when compared with uninfected mice. On postinfection days 5, 7, and 14, MK886 treatment inhibited LTB₄ synthesis by 31, 70, and 41%, respectively. However, this difference was significant only on postinfection day 7 (Fig. 2).

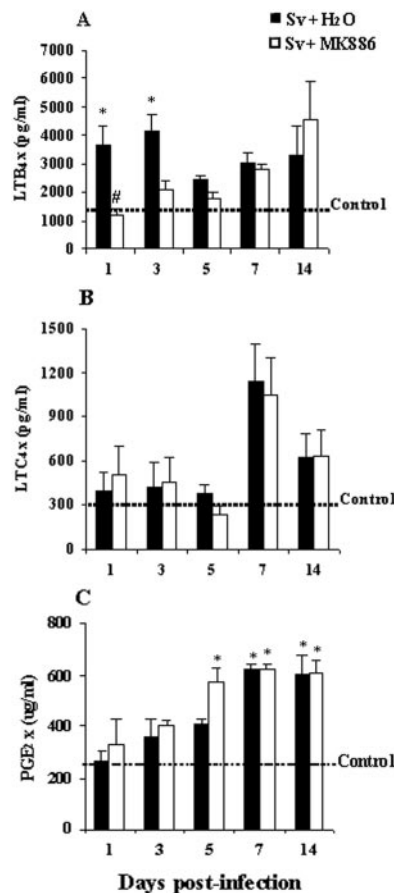


FIGURE 1. Effect of MK886 on LTB₄ (A), LTC₄ (B), and PGE₂ (C) synthesis in lung tissue. Leukotriene levels were determined by ELISA. Swiss mice were s.c. infected with *S. venezuelensis* (Sv) larvae and received daily treatment with water or MK886. Lungs were removed on postinfection days 1, 3, 5, 7, and 14. A group of uninfected animals was used as a control (dotted line). Data are expressed as the mean ± SEM of two independent experiments (n = 8–11 for LTB₄; n = 5–6 for LTC₄ and PGE₂). * or #, p < 0.05. *, Uninfected control mice vs either Sv + H₂O or Sv + MK886. #, Sv + H₂O vs Sv + MK886.

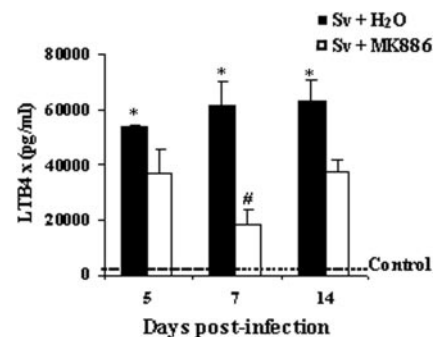


FIGURE 2. Effect of MK886 on LTB₄ synthesis in the duodenum. LTB₄ levels were measured by ELISA. Swiss mice were s.c. infected with *S. venezuelensis* (Sv) larvae, and treated daily with water or MK886. Duodena were removed on postinfection days 5, 7, and 14. A group of uninfected animals was used as a control (dotted line). Data are expressed as mean ± SEM (n = 5–8). * or #, p < 0.05. *, Uninfected control mice vs either Sv + H₂O or Sv + MK886. #, Sv + H₂O vs Sv + MK886.

Inhibition or absence of endogenous leukotrienes is associated with parasite burdens and female worm fertility

We recovered greater numbers of *S. venezuelensis* filariform larvae from the lungs of Swiss mice treated with MK886 than from those of infected-only mice. In addition, lungs from 5-LO^{-/-} mice presented more larvae than did those from WT mice (Table I). The inhibition of leukotriene synthesis also resulted in increased intestinal adult worm burdens on postinfection days 5, 7, 9, and 11, as well as increased numbers of eggs/g/feces on postinfection days 7, 9, and 11 (Fig. 3). On postinfection days 5, 7, 9, and 11, respectively, intestinal worm burdens in MK886-treated animals were 101, 58, 24, and 33% higher than those seen in infected-only mice (Fig. 3A). Significant increases in eggs/g/feces were observed on postinfection days 7 (92%), 9 (75%), and 11 (533%) (Fig. 3C). On postinfection day 14, no adult worms or eggs were recovered from any Swiss mice in either group. In 5-LO^{-/-} mice, the number of recovered adult worms presented a significant increase on postinfection days 9 (105%), 11 (162%), and 14 (200%) (Fig. 3B), and numbers of eggs/g/feces increased significantly on postinfection days 7 (196%), 9 (117%), 11 (77%), and 14 (11,700%) (Fig. 3D). In one experiment, parasite worms were also recovered and counted in the jejunum and ilea of infected Swiss mice (treated or not treated with MK886), 5-LO^{-/-} mice, and WT mice. In the ilea, worm counts were very low and were equal among the four groups of animals. Worm numbers in the jejunum were similar to those found in the duodena (data not shown).

Histopathological findings confirm quantitative results

Histopathological analysis of the duodena of infected Swiss mice treated with MK886 demonstrated numerous worms, located mainly beneath the epithelial layer, which were greater in number than in the infected-only mice (Fig. 4D). On postinfection days 7 and 14, infected 5-LO^{-/-} mice also presented greater number of worms in that same location than did infected WT mice (Fig. 4, F and H). In infected-only Swiss mice and WT mice (Fig. 4, C, E, and G), adult worms were accompanied by intense cellular infiltration into the lamina propria of the villi, and eosinophils were detected. The intense cellular infiltration and eosinophils

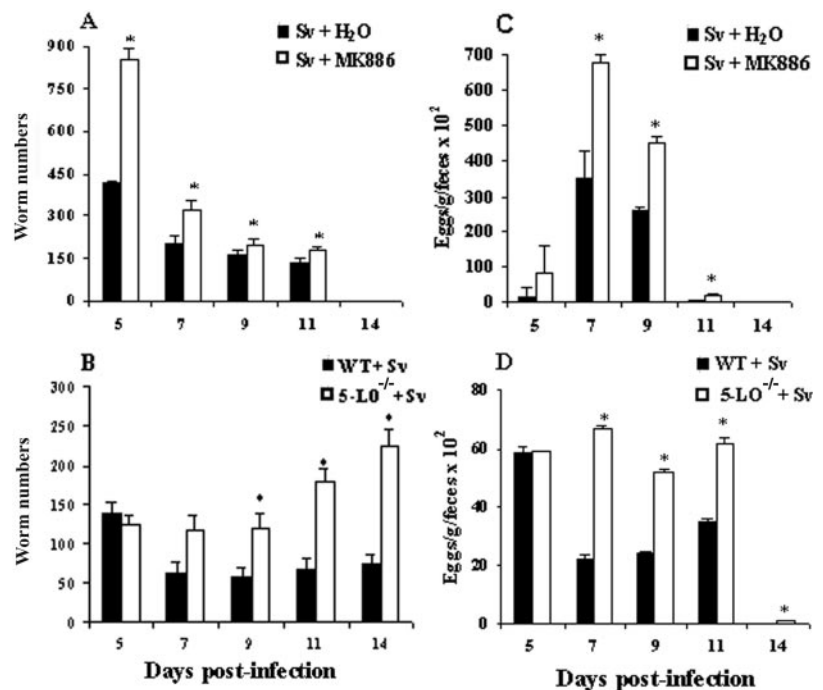
were not evident in the small intestines of 5-LO^{-/-} mice or of mice treated with MK886 (Fig. 4, D, F, and H). On postinfection day 14, no difference was seen in the numbers of inflammatory cells between infected Swiss mice (treated or not treated with MK886) and infected WT mice. In Swiss mice, adult worms were completely expelled by this stage of the infection (Fig. 3A and Table I). Nevertheless, on postinfection day 14, adult worms were observed in higher quantities in 5-LO^{-/-} mice than in WT mice (Fig. 4H).

MK886 inhibits leukocyte counts in blood, PCF, and BALF

As shown in Fig. 5, *S. venezuelensis*-infected mice developed systemic, peritoneal, and pulmonary eosinophilia (Fig. 5, A–C) and presented higher mononuclear cell counts (Fig. 5, D–F) throughout the period of infection. Between postinfection days 3 and 14, recruitment of eosinophils and mononuclear cells into the PCF and BALF was greater in infected mice than in uninfected control mice. In all compartments, cell numbers were highest on postinfection day 14 (the last observation day), with the exception of mononuclear cells in BALF, which peaked on postinfection day 5. No significant increase in neutrophil numbers was observed in any compartment studied.

Comparing *S. venezuelensis*-infected, MK886-treated animals with infected-only animals, eosinophil counts in the blood, PCF, and BALF (Fig. 5, A–C) were lower in the MK886-treated animals. The most significant reduction in eosinophil numbers (79% in blood, 67% in PCF, and 86% in BALF) was seen on postinfection day 14 (Fig. 5, A–C). However, on the same day, no alteration in mononuclear cell numbers was observed in the PCF or BALF from these mice. In BALF, MK886 treatment significantly inhibited mononuclear cells only on postinfection day 5 (Fig. 5F). In blood, MK886 treatment significantly inhibited mononuclear cells between postinfection days 3 and 14 (Fig. 5D). However, in general, MK886 treatment decreased cell numbers to levels equal to or lower than those seen in control mice, albeit at different stages of the infection.

FIGURE 3. Number of adult worms (A and B) and eggs/g/feces (C and D) recovered from Swiss, WT, and 5-LO^{-/-} mice after s.c. infection with *S. venezuelensis* (Sv) larvae. Only Swiss mice were treated (with water or MK886). Data are expressed as mean ± SEM (n = 5). *, p < 0.05. *, Sv + H₂O vs Sv + MK886 or WT vs 5-LO^{-/-}.



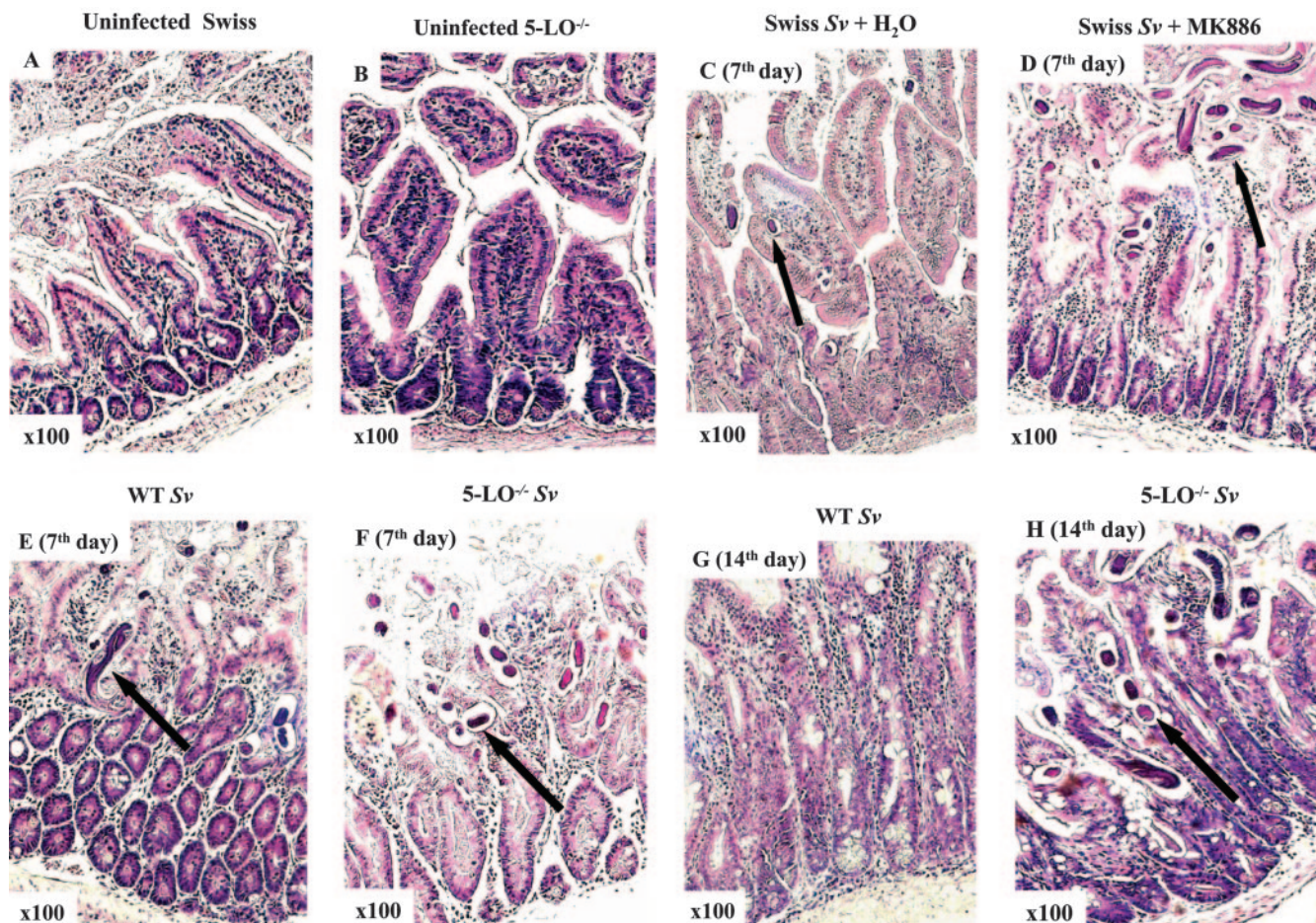


FIGURE 4. Histopathology of duodena from uninfected mice and from *S. venezuelensis* (Sv)-infected Swiss, 5-LO^{-/-}, and WT mice. Only infected Swiss mice were treated, either with MK886 (Sv + MK886) or water (Sv + H₂O). Representative H&E-stained duodenal sections from uninfected Swiss (A) and 5-LO^{-/-} (B) mice, from infected Swiss mice treated with water (C) or with MK886 (D), and from infected WT (E and G) and 5-LO^{-/-} (F and H) mice. The arrows indicate sections with adult worms. Magnification of panels, $\times 100$.

MK886 reduces IL-5, but increases IL-12 levels in the lung of S. venezuelensis-infected mice

We determined that MK886 treatment altered cytokines produced in the lung during *S. venezuelensis* infection. In *S. venezuelensis*-infected Swiss mice, IL-4, IL-5, IL-10, IFN- γ , and IL-12 levels in the lung tissue increased during infection, but with different time courses (Fig. 6). Levels of IL-4 and IL-5 in the lungs reached their peaks between postinfection days 1 and 3. Levels of TNF- α and IL-3 increased during the infection, although the difference was less than significant (Fig. 6A). In infected animals treated with MK886, IL-5 was inhibited only on postinfection days 1 and 3, and IL-4 was not altered at any point (Fig. 6, B and C).

Levels of IL-12 in the lungs of *S. venezuelensis*-infected animals were higher than in those of control mice only on postinfection day 14. Except on postinfection day 5, levels of IL-12 were higher in infected animals treated with MK886 than in infected-only animals (Fig. 6E). In all infected animals, levels of IFN- γ were higher than those seen in the control groups only between postinfection days 1 and 3. In both groups of infected mice, subsequent IFN- γ levels were similar to those seen in the controls (Fig. 6F). Levels of IL-10 in the lung tissue of *S. venezuelensis*-infected animals were higher than in the control mice on postinfection days 1 and 3, but decreased to levels comparable to those of the controls after postinfection day 5 (Fig. 6G). Treatment with MK886 did not alter IL-10 levels at any point.

MK886 reduces Ab production in serum of S. venezuelensis-infected mice

Parasite-specific IgG1, IgG2a, and IgE Abs were measured in serum from infected Swiss mice (treated or not treated with MK886) on postinfection days 1, 3, 5, 7, and 14, and these values were then compared with those found for uninfected controls (Fig. 7). The concentration of parasite-specific IgG1 in serum from *S. venezuelensis*-infected animals was higher than that seen in the control animals only on postinfection day 14. Nevertheless, treatment of infected animals with MK886 inhibited the synthesis of the IgG1 on all postinfection days (Fig. 7A). Levels of IgG2a were similar among these three groups of animals (Fig. 7B). As expected, concentrations of parasite-specific IgE in the serum of infected Swiss mice increased significantly only between postinfection days 7 and 14, peaking on day 14. Treatment with MK886 reduced IgE concentrations by 65 and 45%, respectively, on these 2 days.

Discussion

In the present study, we demonstrated that leukotrienes play an important role during *S. venezuelensis* infection. Our results show for the first time that *S. venezuelensis* infection induces greater LTB₄ release in the lungs and duodena than that seen in the tissue of uninfected animals (control group). The early augmentation in LTB₄ release in the lungs coincides with the L3 migration from the blood to the lungs, where L3 molt to fourth-stage larvae (L4) (6,

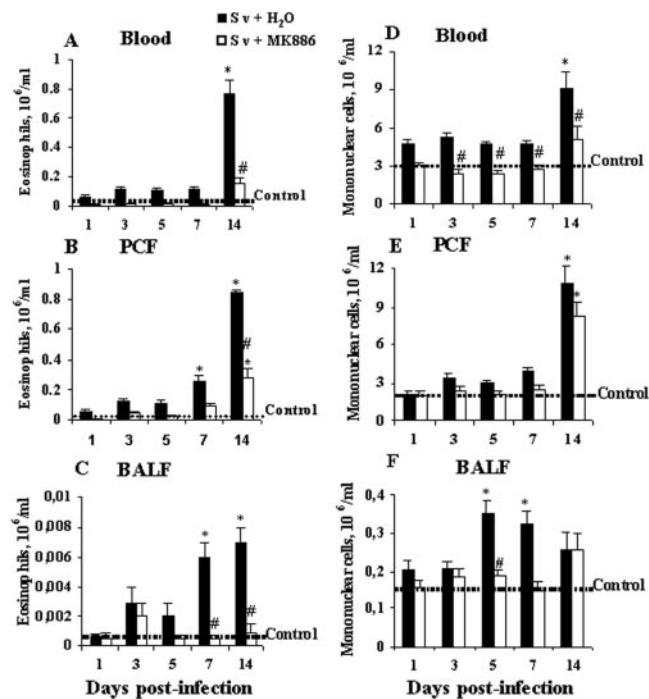


FIGURE 5. Effect of MK886 on inhibition of eosinophils and mononuclear cells in blood, PCF, and BALF. Cells were obtained from Swiss mice after infection with *S. venezuelensis* (Sv) larvae and daily per os administration of water or MK886. A group of uninfected animals was used as a control. Numbers of eosinophils (A–C) and mononuclear cells (D–F) were enumerated and identified through Rosenfeld staining. Data are expressed as mean $\pm$ SEM from two independent experiments ($n = 11$ –23). The dotted line represents the mean $\pm$ SEM of the cell numbers from uninfected animals (controls). * or #, $p < 0.05$. *, Controls vs either Sv + H₂O or Sv + MK886; #, Sv + H₂O vs Sv + MK886.

7). In addition, we detected an increase in LTB₄ levels in duodena of infected-only mice, at the same time that worms are detected in this tissue. Increase in leukotrienes in intestine has been demonstrated in other nematode-infected animals (27–29). Impairment of leukotriene synthesis, by either pharmacologic or genetic means, clearly resulted in increased numbers of worms and eggs recovered from infected animals, reflecting increased larvae survival during their passage through the lung and duodena. This may be attributable to altered resistance to parasite in the absence of LTB₄, as demonstrated in this study. *S. venezuelensis* infection also induced significant PGE₂ increase in lung from postinfection day 5. Moreover, at postinfection day 7, we observed discrete increase on LTC₄ level in the lungs of infected mice. Despite the fact that MK886 has been shown to inhibit LTC₄ and PGE₂ production (36–38), this drug had no noticeable effect on PGE₂ and LTC₄ production in this model of parasite infection. The relevance of this finding merits further investigation.

In mice infected with *S. venezuelensis*, MK886 treatment induced a significant decrease in eosinophil and mononuclear cell numbers in the BALF, PCF, and blood. It has been well established that leukotrienes are potent inflammatory mediators involved in eosinophil and mononuclear cell accumulation at the site of inflammation (39–43). Therefore, the inhibition of eosinophils that we observed in the blood, PCF, and BALF of *S. venezuelensis*-infected, MK886-treated mice was expected and strongly suggests that leukotrienes are released in serum of infected animals.

We also examined the effect of MK886 treatment on lung production of TNF- α , IL-3, IL-4, IL-5, IL-10, IFN- γ , and IL-12 during infection. The results demonstrated that MK886 treatment was

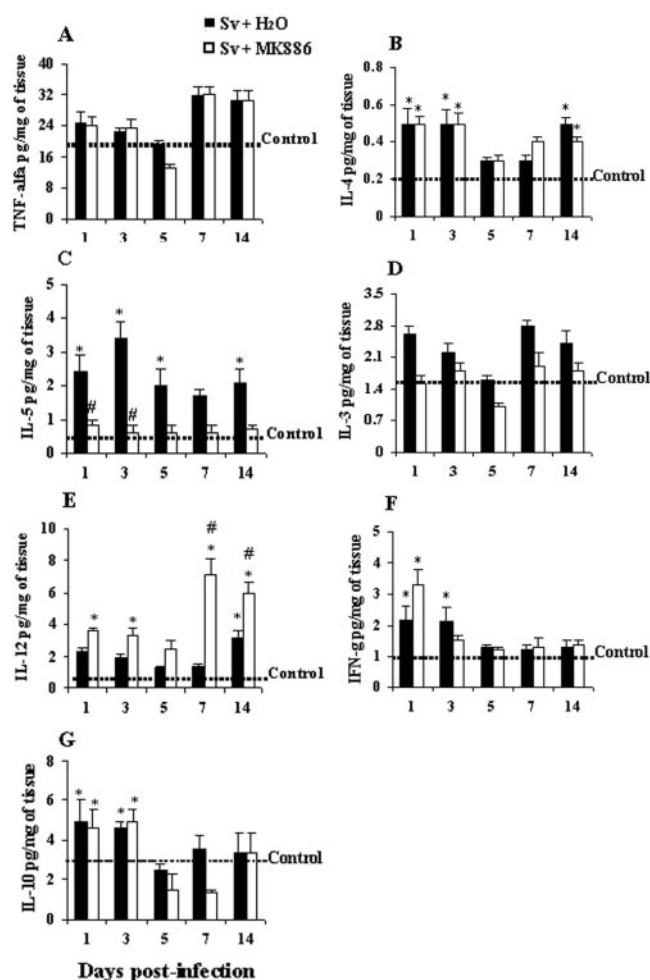


FIGURE 6. Cytokine levels in lung tissue. Mice were subjected to infection with *S. venezuelensis* (Sv) and treated daily per os with water or MK886. The dotted line represents a group of uninfected, untreated animals used as a control. Cytokine levels were determined by ELISA. The data are expressed as mean $\pm$ SEM of two independent experiments ($n = 6$ –11). * or #, $p < 0.05$. *, Controls vs either Sv + H₂O or Sv + MK886; #, Sv + H₂O vs Sv + MK886.

correlated with impairment of IL-5 production in lung tissue. In fact, lung levels of IL-5 were markedly lower in infected, MK886-treated mice than in infected, untreated animals. The results of previous studies show that IL-5 is essential to eosinophil differentiation and survival (44, 45), as well as to driving eosinophils from bone marrow to blood and tissue (31). Therefore, data in the literature have demonstrated that eosinophils contribute to the elimination of *S. venezuelensis* (9) and *S. stercoralis* in mice (46). Moreover, Korenaga et al. (8) demonstrated that host-protective immunity against tissue-migrating larvae was IL-5 dependent. Other authors have also demonstrated that LTB₄ regulates IL-5 production by human T lymphocytes (47). In this article, we verified the relevance of LTB₄ to IL-5 induction and demonstrated that this cytokine is crucial to the development of eosinophilia and to the elimination of *S. venezuelensis*. Therefore, maintenance of leukotriene levels during infection appears to be essential to regulating IL-5 release and the consequent eosinophil recruitment, as well as to evoking an appropriate effector mechanism (such as production of IgG1 and IgE Abs and IL-5) and inducing eosinophilia.

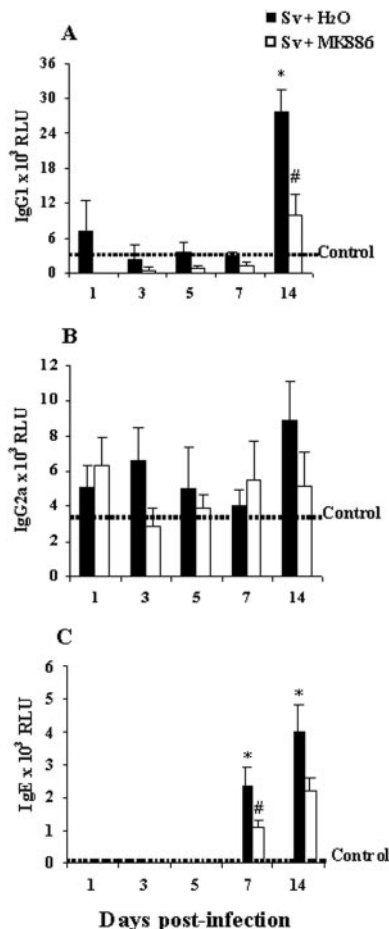


FIGURE 7. Specific Abs IgG1 (A), IgG2a (B), and IgE (C) in the serum of *S. venezuelensis* (Sv)-infected mice, treated or not treated with MK886. Abs were detected by ELISA and developed by chemiluminescence. The data are expressed as mean $\pm$ SEM of relative light units (RLU) of two independent experiments ($n = 6$ – 10 for IgG1 and IgG2a; $n = 5$ – 9 for IgE). The dotted line represents the values for serum from uninfected, untreated animals that were used as controls ($n = 6$ – 11). * or #, $p < 0.05$. *, Controls vs either Sv + H₂O or Sv + MK886; #, Sv + H₂O vs Sv + MK886.

Our data also indicate that IL-5 is involved in the production of protective IgG1 and IgE (although not IgG2a) Abs during infection. However, these data are not in total agreement with the findings of Herbert et al. (46), who showed that IL-5 is required for IgM production and not for IgG1. This discrepancy could be explained by the differences in the models used or by the higher IL-12 levels in *S. venezuelensis*-infected, MK886-treated mice. In another study, it was demonstrated that IL-12 administered to *S. stercoralis*-infected mice decreases eosinophil numbers and parasite-specific IgG1 levels, consequently nullifying the host Th2-dependent protective immune response (48). Also, IL-13 is an important growth factor for IgE-producing B lymphocytes (49). Although we do not measure IL-13 in our model, we may suggest that the level of this cytokine is also decreased in MK886-treated mice, because this drug decreases eosinophil numbers, a relevant source of IL-13 in Th2 immune response (50, 51). Thus, in our model, besides the fact that MK886 did not change IL-4 production, the decrease of IgE levels could be related with inhibition of IL-5 and IL-13 levels. Moreover, as has previously been demonstrated in studies of infection with other gastrointestinal nematodes, levels of IL-10 (52) and IL-4 (53) were higher in lungs of *S. venezuelensis*-infected mice than in uninfected controls.

In summary, we demonstrate for the first time that *S. venezuelensis* infection induces production of leukotrienes, which are essential for invoking a protective expulsion of parasites. We also observed that treatment of *S. venezuelensis*-infected mice with MK886 induces down-regulation of IL-5 and up-regulation of IL-12. This cytokine imbalance causes decreased IgG1 and IgE levels, as well as inhibiting production of eosinophils and mononuclear cells, thereby suppressing the appropriate effector mechanisms, amplifying parasite fecundity, and allowing greater larvae and worm survival. These findings indicate that leukotriene production is necessary for efficient effector mechanisms in this parasite infection. Moreover, these findings have immediate clinical importance because patients with strongyloidiasis may present airway hyperresponsiveness, as previously demonstrated in animal models (54). In *S. stercoralis*-infected patients presenting asthma-like symptoms, treatment of airway bronchoconstriction using 5-LO inhibitors may worsen the infection and promote hyperinfection.

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Disclosures

The authors have no financial conflict of interest.

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