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IL-4Rα-responsive smooth muscle cells increase intestinal hypercontractility and contribute to resistance during acute Schistosomiasis

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[1]*International Centre for Genetic Engineering and Biotechnology and Division of Immunology, Institute of Infectious Disease and Molecular Medicine, Health Sciences Faculty;* [2]*Department of Clinical Laboratory Sciences Anatomical, Division of Pathology, Health Sciences Faculty; and* [3]*Department of Anatomy and Physiology, Health Sciences Faculty, University of Cape Town, Cape Town, South Africa*

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Marillier RG, Brombacher TM, Dewals B, Leeto M, Barkhuizen M, Govender D, Kellaway L, Horsnell WG, Brombacher F. IL-4Rα-responsive smooth muscle cells increase intestinal hypercontractility and contribute to resistance during acute Schistosomiasis. *Am J Physiol Gastrointest Liver Physiol* 298: G943–G951, 2010. First published April 1, 2010; doi:10.1152/ajpgi.00321.2009.—Interleukin-(IL)-4 and IL-13 signal through heterodimeric receptors containing a common IL-4 receptor-α (IL-4Rα) subunit, which is important for protection against helminth infections, including schistosomiasis. Previous studies demonstrated important roles for IL-4Rα-responsive hematopoietic cells, including T cells and macrophages in schistosomiasis. In this study, we examined the role of IL-4Rα responsiveness by nonhematopoietic smooth muscle cells during experimental acute murine schistosomiasis. Comparative *Schistosoma mansoni* infection studies with smooth muscle cell-specific IL-4Rα-deficient (SM-MHC^{cre}IL-4Rα$^{-/flox}$) mice, heterozygous control (IL-4Rα$^{-/flox}$) mice, and global IL-4Rα-deficient (IL-4Rα$^{-/-}$) mice were conducted. *S. mansoni*-infected SM-MHC^{cre}IL-4Rα$^{-/flox}$ mice showed increased weight loss and earlier mortalities compared with IL-4Rα$^{-/flox}$ mice, despite comparable T_H2/type 2 immune responses. In contrast to highly susceptible IL-4Rα-deficient mice, increased susceptibility in SM-MHC^{cre}IL-4Rα$^{-/flox}$ mice was not accompanied by intestinal tissue damage and subsequent sepsis. However, both susceptible mutant mouse strains failed to efficiently expel eggs, demonstrated by egg reduction in the feces compared with control mice. Reduced egg expulsion was accompanied by impaired IL-4/IL-13-mediated hypercontractile intestinal responses, which was present in the more resistant control mice. Together, we conclude that IL-4Rα responsiveness by smooth muscle cells and subsequent IL-4- and IL-13-mediated hypercontractility are required for host protection during acute schistosomiasis to efficiently expel *S. mansoni* eggs and to prevent premature mortality.

Schistosoma mansoni; helminth; intestine; murine; *Nippostrongylus brasiliensis*

SCHISTOSOMA MANSONI INFECTION is a major cause of morbidity and mortality in tropical countries (36). Following infection, adult *S. mansoni* worms reside in the blood vessels and fail to elicit a strong host immune response. However, egg production from mating pairs of adult worms, which starts approximately 5 wk postinfection, is highly immunogenic. Egg movement from the mesenteric system into the intestine and to the liver causes considerable tissue damage and drives a predominant antigen-specific T_H2 cytokine response characterized by increased host production of IL-4, IL-5, and IL-13 and a subsequent type 2 antibody response characterized by high levels of IgE and IgG1. Both IL-4 and IL-13 signal via receptors containing the IL-4 receptor α (IL-4Rα) subunit (13), inducing a STAT6-dependent gene transcription that polarizes host immunity to a T_H2 response (3, 15, 27, 28, 32, 34). Experimental *S. mansoni* infections using transgenic mice have demonstrated key roles for IL-4Rα responsiveness, which is crucial for host granuloma formation around the egg and host survival during *S. mansoni* infection (18, 21). Subsequent studies in mice with cell specific disruption of IL-4Rα expression have further dissected the role of IL-4 and IL-13 target cells for host protection during schistosomiasis. Here evidence was provided that IL-4/IL-13-activated alternative macrophages (aaMph) are crucial for the survival of acute schistosomiasis by controlling egg-induced intestinal pathology and downregulating type 1 responses. Mechanistically it has been shown that, in the absence of aaMph, mice (LysM^{cre}IL-4Rα$^{-/lox}$ strain) succumbed to acute schistosomiasis due to severe intestinal pathology, subsequent gut leakage, and bacterial infiltration, resulting in LPS-induced sepsis (14, 18, 19). More recent data demonstrated similar roles for aaMph in other immune responses (26, 33).

Moreover, infection studies in CD4$^+$ T cell-specific IL-4Rα-deficient mice [Lck^{cre}IL-4Rα$^{-/lox}$ strain (37)] showed evidence that IL-4-promoted T_H2 cells are not crucial for survival of acute schistosomiasis but are involved in liver granuloma formation (24). The increased susceptibility by pan-T cell-specific IL-4Rα-deficient mice (iLck^{cre}IL-4Rα$^{-/lox}$ mice) further suggests that IL-4Rα-responsive non-CD4$^+$ T cells do substantially contribute to survival during acute schistosomiasis (9). These and other studies clearly demonstrated the importance of IL-4Rα expression on hematopoietic cells in reducing morbidity and increasing survival from *S. mansoni*-infection (18, 19, 24, 35).

The possible role of IL-4Rα-responsive nonhematopoietic target cells during schistosomiasis is less well defined. Smooth muscle cells in particular have been implicated in a number of helminth associated diseases. In vivo and in vitro studies have shown that STAT-6-deficient smooth muscle cells from helminth-infected mice have reduced hypercontractile responses (1, 22). Activation of the transcription factor STAT6 is considered to be an important cellular response to IL-4Rα activa-

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tion, suggesting that IL-4/IL-13 responsiveness is required for increased contractility. *Nippostrongylus brasiliensis* infection studies in mice lacking the IL-4Rα on smooth muscle cells [C.Cg-Il4ra^{tm1Fbb}/Il4ra^{tm2Fbb}Tg(Myh11-cre)5013Gko (synonym used in this manuscript: SM-MHC^{cre}IL-4Rα$^{-/flox}$)] resulted in delayed worm expulsion. This was associated with reduced T_H2 cytokine responses, goblet cell hyperplasia, and reduced mRNA expression of muscarinic acetylcholine receptor (M3/mAchR), an important mediator of smooth muscle contractility (20). *S. mansoni* infection has also been associated with increased intestinal hypercontractility (6, 12, 30, 31), accompanied by increased proliferation of smooth muscle cells during the acute phase of infection (30), which may result in functional changes in the muscularis during chronic schistosomiasis. These and other studies (6, 17, 30, 31, 39) prompted us to determine whether IL-4/IL-13 responsiveness by smooth muscle cells has any impact in schistosomiasis. Our approach to address this question was based on comparative *S. mansoni* infection studies using SM-MHC^{cre}IL-4Rα$^{-/flox}$ mice, global IL-4Rα$^{-/-}$ mice, and heterozygous IL-4Rα$^{-/lox}$ controls. Infected SM-MHC^{cre}IL-4Rα$^{-/flox}$ mice showed increased susceptibility, which was accompanied with impaired hypercontractile responses and egg expulsion into the gut lumen.

MATERIALS AND METHODS

Mice. Mice were kept in individually ventilated cages under specific pathogen-free conditions within the biomedical animal facility of the Health Science Faculty, University of Cape Town (UCT). Mice were free of MHV, Reo3, Theiler, PVM, Sendai, MVM, MPV, Ektromelie, L CM, Adeno (Mad K87), Polyoma, Hanta, M CMV, Rota (EDIM), MNV, MiceThymic V., *Mycoplasma pulmonis*, and *Clostridium piliforme* tested by ELISA antigen screening (BioDoc, Hannover, Germany) and of *Citrobacter freundii*, *Bordetella bronchiseptica*, *Corynebacterium* sp., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptobacillus moniliformis*, *Salmonella* spp., and endo- and exoparasites screened in sentinels (Pathcare, Vetlab, Cape Town, South Africa). The sentinels were positive for *Helicobacter* sp., *Pasterella pneumotropica*, and MNV. All experiments were approved by the independent Animal Ethics Research Board at UCT, approval number 005/040 and 008/027. Mice were age (6–10 wk) and sex matched for each experiment. Il4ra^{tm1Fbb}/Il4ra^{tm1Fbb} (IL-4Rα$^{-/-}$) (29) and C.Cg-Il4ra^{tm1Fbb}/Il4ra^{tm2Fbb}Tg(Myh11-cre)5013Gko (SM-MHC^{cre}IL-4Rα$^{-/lox}$) mouse strains (20) were generated or backcrossed at least to F9 on BALB/c genetic background. Cre transgenic negative littermates (IL-4Rα$^{-/lox}$) were used as controls in all experiments.

Schistosoma mansoni infection. Mice were percutaneously infected with 70–100 live cercariae of a Puerto Rican strain of *S. mansoni* obtained from infected *Biomphalaria glabrata* snails (kindly provided by Dr. A. P. Mountford, University of York, York, UK) as previously described (18).

Nippostrongylus brasiliensis infection. Mice were subcutaneously infected with ~750 infective stage three larvae (a kind gift from Klaus Erb, Wurzburg, Germany) as previously described (5).

ELISA and serum analysis. Single-cell suspensions were prepared from spleens and mesenteric lymph nodes removed from infected and uninfected mice. We cultured 1×10^6 cells/ml in IMDM (GIBCO) medium supplemented with 10% fetal calf serum (GIBCO) at 37°C in 96-well plates precoated with either PBS or 20 mg/ml anti-CD3 (clone 145-2C11) or *S. mansoni* egg antigen (SEA; BioGlab). After 72 h, cells were centrifuged at 1,200 rpm for 5 min and the supernatants were collected. Cytokine concentration was determined by ELISA as described previously (29). Serum LPS were determined by using the QCL-1000 kit for endotoxin determination (Lonza) in combination with an endotoxin standard (Sigma).

Antibody analysis. Analysis of antigen-specific IgG1, IgG2a and b, and IgE and cytokine determination production were carried out by capture ELISA, as previously described (18). In brief, Nunc Maxisorp 96-well plates were coated with 10 μg/ml of *S. mansoni* SEA overnight at 4°C in borate buffer (50 mM), pH 9.6. Plates were washed and blocked with blocking buffer (10% milk powder) overnight. Samples were diluted in dilution buffer and added in serial dilutions, and the plates were incubated overnight at 4°C. Parasite-specific antibodies were detected by using alkaline phosphatase-conjugated goat anti-IgG1 (B020-NK20), goat anti-IgG2b (F659-UF89), and rat anti-IgE (23G3) (all from Southern Biotechnology Associates). Total IgE was determined with monoclonal antibodies 84.1C and alkaline phosphatase-conjugated rat anti-IgE (23G3) used for detection. The plates were subsequently washed and incubated with *p*-nitrophenyl phosphate (1 mg/ml) (Boehringer Mannheim), and the enzyme reaction was read at 405 nm via a Versamax microplate reader (Molecular Devices, Sunnyvale, CA).

Determination of S. mansoni egg expulsion from mouse fecal material. Analyses of fecal egg samples for the presence of eggs was performed using the modified Kato-katz technique as previously described (10, 11). Briefly: ten 40-mg fecal pellets were suspended in isotonic solution overnight. The pellets were disrupted by aspiration in a syringe without a needle and filtered through a 150-μm mesh sieve. Each sample was counted in triplicate using an inverted light microscope at ×100 magnification and values were averaged and normalized according to the resuspension volume and the weight of the feces collected.

Measurement of contraction in whole tissue. Whole tissue sections, 1 cm long, were dissected from the ileum (*S. mansoni*) or jejunum (*N. brasiliensis*) region of the small intestine and suspended in a four-chamber automatic organ bath system in oxygenated Krebs buffer at a resting tension of 0.5 g as previously described (38). Data acquisition and analysis was conducted by the ADInstruments Powerlab and the LabChart analysis software. In brief, all tissue was stimulated with 50 mM potassium chloride (KCl) prior to acetylcholine (−9 to −3 log M) stimulation, washed and equilibrated for 10 min between each dose, and at the end of the experiment tissue was air dried, weighed, and contractile force expressed in millinewtons per milligram of tissue.

Histology and histopathology. Tissue samples were fixed in a neutral buffered formalin solution. Following embedding in paraffin, samples were cut into 5- to 7-μm sections. Sections were stained with hematoxylin and eosin (H&E), periodic acid Schiff reagent, or chromotrope 2R and aniline blue solution and counterstained with Wegert's hematoxylin for collagen detection. Granulomas were measured by use of NIS elements Basic Research software, 4D experiment ability (IMP Scientific & Precision). The area (μm^2) of each granuloma containing a single egg was measured by subtracting the area of the egg from the area of the whole granuloma (μm^2) by using the software indicated above (8). An average of 25 granulomas per mouse were measured and included in the analysis. All histological examinations were scored by the same individual in a blind fashion to obtain consistency.

All the digital images were captured with a Nikon 5.0 Mega Pixels Color Digital Camera (Digital SIGHT DS-SMc).

Statistics. Data are presented as means ± SE, and the significant differences were determined by two-tailed Student's *t*-test and ANOVA (Prism software, http://www.prism-software.com). *P* values of less than 0.05 were considered significant.

RESULTS

SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice infected with S. mansoni show earlier mortality and weight loss. To determine whether IL-4Rα-responsive smooth muscle cells are required for host sur-

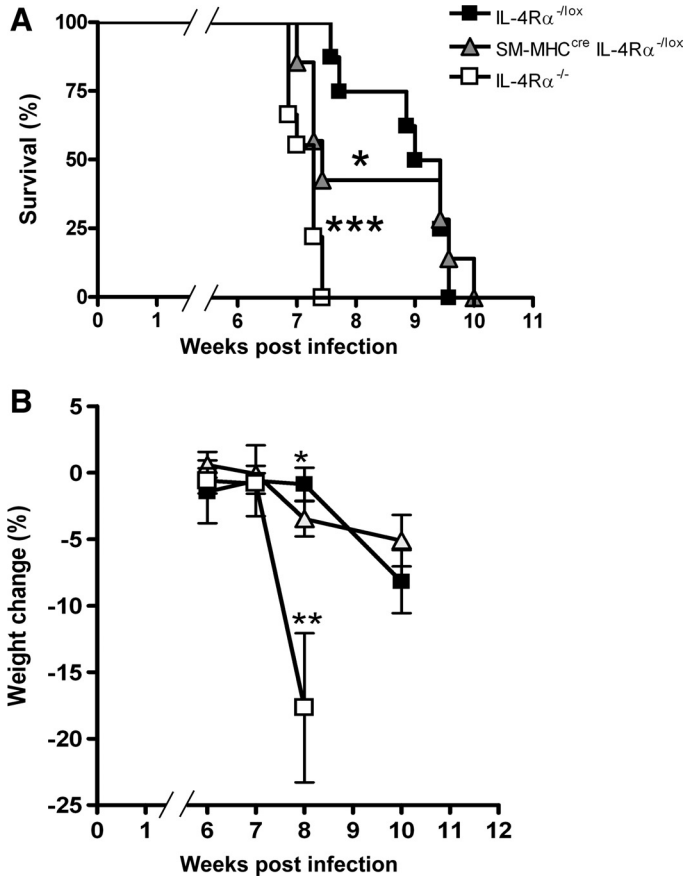


Fig. 1. Survival (*A*) and body weight loss (*B*) reduced in *Schistosoma mansoni*-infected SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice. *A*: IL-4Rα$^{-/lox}$ control mice (black) $n = 9$, SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice (gray) $n = 7$, and IL-4Rα$^{-/-}$ mice (white) $n = 8$ (data are pooled from 3 experiments). *B*: *S. mansoni* infected with 100 cercariae. Mice were weighed weekly. Data (means ± SE) are representative of 3 independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ (significantly different from IL-4Rα$^{-/lox}$ control mice).

vival, IL-4Rα$^{-/lox}$, SM-MHC^{cre}IL-4Rα$^{-/lox}$ and IL-4Rα$^{-/-}$ mice were infected with 100 *S. mansoni* cercariae and monitored for disease outcome. As previously described, global IL-4Rα$^{-/-}$ mice succumbed to infection rapidly from *week 7* postinfection (pi). SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice also showed significantly increased mortality than control IL-4Rα$^{-/lox}$ mice during the first 8 wk of infection (Fig. 1*A*). Earlier mortality at *week 8* correlated to significant weight loss in SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice compared with control mice (Fig. 1*B*).

The observed enhanced mortality of SM-MHC^{cre}IL-4Rα$^{-/flox}$ mice was also present at lower infectious doses with 10 and 37.5% of SM-MHC^{cre} IL-4Rα$^{-/lox}$ mice dying at *week 8*, compared with 0 and 12.5% mice in control IL-4Rα$^{-/lox}$ mice infected with 70 and 80 cercariae, respectively (Table 1).

Together, these data show increased susceptibility against acute schistosomiasis in the absence of the IL-4Rα on smooth muscle cells only.

Unaltered schistosoma-specific cytokine and antibody responses in infected SM-MHC^{cre} IL-4Rα$^{-/lox}$ mice. The shift from a weak T_H1 against the *S. mansoni* worm to a dominant T_H2 response against the eggs, peaking at *week 8* pi, is essential for host protection during acute schistosomiasis (36, 42, 43). To determine whether the *Schistosoma*-specific immune response was altered in SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice, we examined antigen-specific cytokine responses from mesenteric lymph node (MLN) cells and splenocytes at *week 8* pi. As expected, SEA-restimulated MLN cells (Fig. 2*A*) and splenocytes (Fig. 2*B*) from control IL-4Rα$^{-/lox}$ mice showed a predominant T_H2-type response with increased IL-4, IL-13, and IL-10, as well as reduced IFN-γ, whereas global IL-4Rα$^{-/-}$ mice showed a shift to T_H1-type cytokine response with increased IFN-γ but reduced IL-4, IL-13, and IL-10. SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice showed an equivalent T_H2-type response as observed in control IL-4Rα$^{-/lox}$ mice, although there was a trend toward lower IL-10 levels in the systemic response of SM-MHC^{cre}IL-4Rα$^{-/lox}$ splenocytes, these differences reached no statistical significance. As expected from the observed cytokine responses, control IL-4Rα$^{-/lox}$ mice and SM-MHC^{cre}IL-4Rα$^{-/flox}$ mice presented a predominantly type 2 antibody response with increased SEA-specific IgG1 and total IgE antibodies but reduced SEA-specific IgG2 antibody responses. In contrast, global IL-4Rα$^{-/-}$ mice showed a shift to a type 1 response with increased SEA-specific IgG2a and IgG2b responses but impaired IgG1 and IgE antibodies (Fig. 2*C*). Together, these results demonstrate that SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice reveal an unaltered type 2 immune response during *S. mansoni* infection.

Impaired egg expulsion in infected SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice. Schistosoma eggs get trapped in the liver and intestine, causing considerable pathology. Eggs traverse the intestine to reach the gut lumen for expulsion. This egg deposition induces granuloma formation by the host, leading to inflammation and fibrosis and the subsequent histopathology associated with morbidity during schistosomiasis (7, 18, 21). As expected at *week 8* pi, control IL-4Rα$^{-/lox}$ mice showed mature liver granuloma surrounding Schistosoma eggs, which was accompanied by massive collagen deposition (Fig. 3*A*). As previously shown, granuloma formation in global IL-4Rα$^{-/-}$ mice was also present but diminished (18, 25). Liver granulomas and collagen deposition in SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice were similar to those presented in control IL-4Rα$^{-/lox}$ mice.

Control IL-4Rα$^{-/lox}$ and SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice were characterized by prominent intestinal granulomatous formation, but little collagen deposition (data not shown) as well as diffuse inflammatory infiltrates in the intestine (Fig. 3*B*). The

Table 1. *Infection dose-related survival at 8 wk postinfection*

	70 Cercariae		80 Cercariae		100 Cercariae	
Mouse	mice died/total	%	mice died/total	%	mice died/total	%
IL-4Rα$^{-/lox}$	0/5	0	1/8	12.5	2/11	18.2
SM-MHCcre IL-4Rα$^{-/lox}$	1/10	10	3/8	37.5	4/11	36.4
IL-4Rα$^{-/-}$	5/7	71.4	2/3	66.7	9/12	75.0

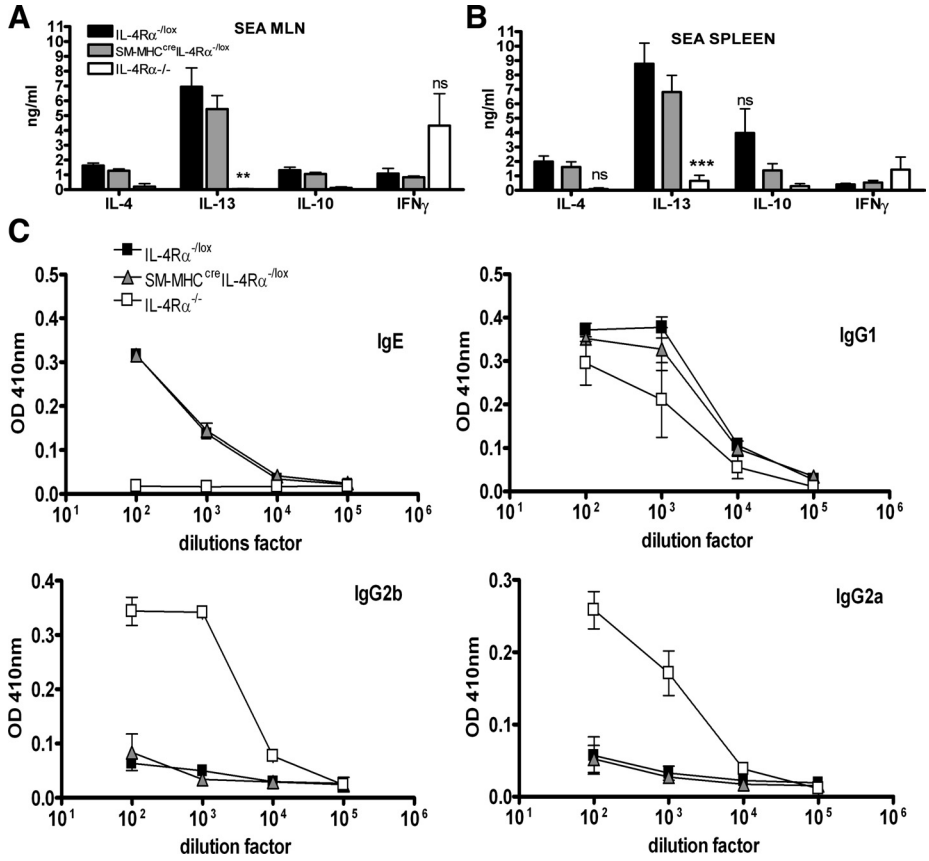


Fig. 2. Intact immune response in *S. mansoni*-infected SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice. *A* and *B*: cytokine responses: Draining mesenteric lymph node (MLN) cells or spleen cells of 8-wk-infected IL-4Rα$^{-/lox}$ (black), SM-MHC^{cre}IL-4Rα$^{-/lox}$ (gray) or IL-4Rα$^{-/-}$ (white) were re-stimulated with SEA and cytokine production into the supernatant was determined. *C*: antibody responses: SEA-specific antibody production (IgG1, IgG2a, IgG2b) and total IgE antibody production were determined from sera of 8-wk-infected IL-4Rα$^{-/lox}$ (black), SM-MHC^{cre}IL-4Rα$^{-/lox}$ (gray) and IL-4Rα$^{-/-}$ (white). $N = 4$ mice per group. Data (means ± SE) are representative of 3 independent experiments. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with IL-4Rα$^{-/lox}$ control mice.

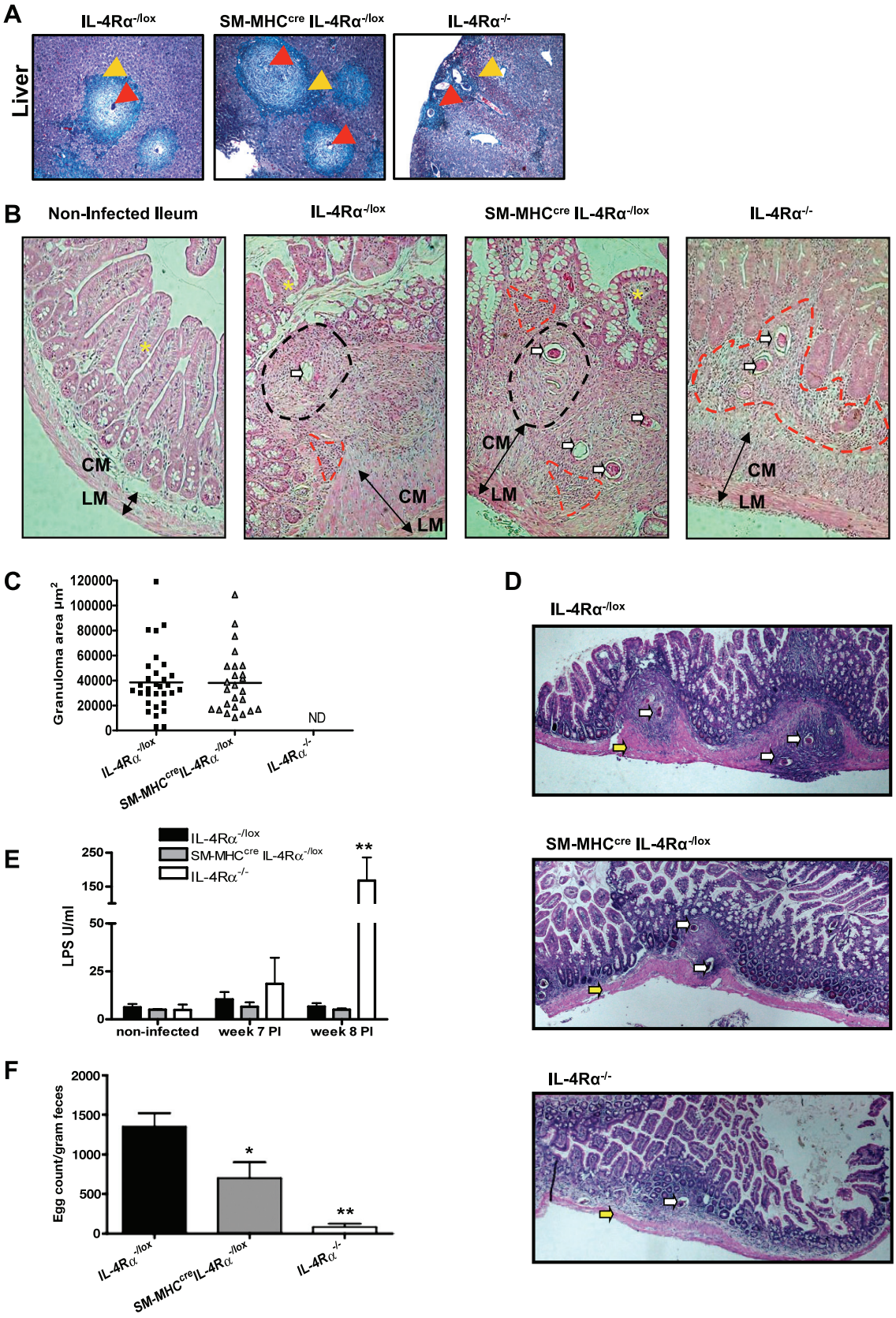
granuloma size was similar between the two strains but poorly developed in IL-4Rα$^{-/-}$ mice (Fig. 3, *C* and *D*). Global IL-4Rα$^{-/-}$ mice showed severe enteritis throughout the lamina propria (Fig. 3*B*), leading to tissue injury and bacterial infiltration, resulting in strikingly increased blood LPS concentrations (Fig. 3*E*) and resulting in septic shock at *week 7* (see Fig. 1), as previously shown (18). All infected mouse strains developed hyperplasia of muscularis propria, which was closely associated with egg deposition (Fig. 3, *B* and *D*). The length and area of the muscular layer on nonsequential serial sections from the small intestine were quantified in individual mice on cross sections to obtain the average thickness (NIS-elements software program from Nikon, Japan). Thickness varied between 60 and 120 μm without significant differences observed between 8-wk-infected SM-MHC^{cre}IL-4Rα$^{-/lox}$ and control mice (data not shown).

To determine whether egg retention in the tissue influenced expulsion, fecal egg numbers were quantified. This revealed significantly lower numbers of eggs in the feces of SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice and global IL-4Rα$^{-/-}$ mice compared with IL-4Rα$^{-/lox}$ mice (Fig. 3*F*). Together, these results show that SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice have an impaired egg expulsion, suggesting that IL-4Rα responsiveness by smooth muscle cells is important for optimal egg expulsion.

N. brasiliensis and S. mansoni infection induce hypercontractility impaired in infected SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice. The results obtained in this study demonstrated IL-4Rα signaling in smooth muscle cells to play a significant role in the excretion of eggs through host intestinal tissue. A potential host response that may facilitate this movement through the intestine are T_H2-associated intestinal hypercontractile responses (1). Such responses have been implicated in the expulsion of intestinal helminths such as *Trichuris muris*, *Heligmosomoides polygyrus*, and *N. brasiliensis* (16).

To investigate whether IL-4Rα responsiveness is important for cholinergic contraction, *N. brasiliensis*-infected SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice, global IL-4Rα$^{-/-}$, and control IL-4Rα$^{-/lox}$ were infected with ~750 L3 larvae and killed at *day 10* postin-

Fig. 3. Egg expulsion impaired in *S. mansoni*-infected SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice. *A*: liver granuloma. Representative chromotrope 2R and aniline blue solution (CAB)-stained liver tissue sections (×100 magnification) with collagen fibers in granuloma in blue; red arrows indicate egg, blue arrow indicate granuloma. *B*: intestine granulomatous formation. Muscularis propria from the small intestine with enteritis and hyperplasia [hematoxylin and eosin (H&E), original magnification ×100] indicated with black arrow; circular (CM) and longitudinal (LM) muscle layer; yellow asterisks indicate villi; white arrows indicate egg; lines demarcate examples of granuloma (black) or enteritis (red). *C*: liver granuloma size. Granuloma size was determined from H&E-stained ileum sections by using a computerized morphometric analysis program (NIS elements by NIKON) by measuring 25 granuloma/mouse. ND, not detectable. *D*: diffuse inflammatory granulomata. Small intestine sections (H&E, original magnification ×40) with thickening of the muscularis propria (yellow arrow) in the area closely surrounding by eggs (white arrow). *E*: sepsis. Lipopolysaccharide (LPS) units in sera from individual mice measured by QCL-1000 kit for endotoxin determination. *F*: fecal egg output. Eggs from fecal pellets from individual mice counted in data (means ± SE) are representative of 2 (*E* and *F*) and 3 (*A*—*D*) individual experiments from 8-wk-infected mice ($n = 4$ per mouse strain) with 100 cercariae. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with IL-4Rα$^{-/lox}$ control mice.



fection, and the jejunum was isolated. A longitudinal segment of the intestine tissue was suspended in an organ bath and the level of contraction in response to concentration-dependent cholinergic stimulation was measured. Infection with *N. brasiliensis* enhanced tension to acetylcholine significantly in control IL-4Rα$^{-/lox}$ mice compared with uninfected IL-4Rα$^{-/lox}$ mice (Fig. 4*A*). In contrast, jejunum isolated from *N. brasiliensis* infected IL-4Rα$^{-/-}$ mice (Fig. 4*B*) did not respond with increased tension to acetylcholine. Interestingly, jejunum from infected SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice was responsive to acetylcholine but contraction were attenuated and variable between individual mice and did not reach statistical significance compared with uninfected mice (Fig. 4*C*). These results confirm a direct role of IL-4Rα responsiveness for cholinergic-induced contraction and suggest that IL-4Rα responsiveness by smooth muscle cells is important to increase contraction.

Because contractile mechanisms may also facilitate *Schistosoma* egg traversal through the intestine to the gut lumen, we measured the ability of intestinal (ileum) contraction in *S. mansoni*-infected mice in response to a cholinergic stimulus at *week 8*, when worms showed efficient egg production. The ileum was used because this is the region most dominantly disrupted by infection and gastrointestinal motility has previously been shown to be affected by infection in this region (30, 40, 41). As observed with *N. brasiliensis*, acetylcholine stimulation following infection with *S. mansoni* caused significant concentration-dependent increase of tension in control IL-4Rα$^{-/lox}$ mice compared with uninfected control mice (Fig. 4*D*). This was abrogated in global IL-4Rα$^{-/-}$ mice (Fig. 4*E*) and strongly attenuated in the ileum from SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice (Fig. 4*F*). An independent cumulative experiment at *week 9* pi and noncumulative experiment at *week 12* pi showed similar results (data not shown). Together, these results demonstrate that IL-4Rα responsiveness on smooth muscle cells is important for efficient *N. brasiliensis*- and *S. mansoni*-induced cholinergic contraction, which is associated with optimal *Schistosoma* egg expulsion and efficient *Nippostrongylus* worm expulsion.

DISCUSSION

Morphological and physiological changes in the gastrointestinal system during helminth infections may be important contributors to host defense mechanisms. One important change is the intestinal hypercontractility that is driven by smooth muscle cells. In this study, we directly examined the role of IL-4- and IL-13-mediated hypercontractility during acute infection with *S. mansoni* in smooth muscle cell-specific IL-4Rα-deficient mice (SM-MHC^{cre}IL-4Rα$^{-/lox}$). We demonstrate that IL-4Rα responsiveness by smooth muscle cells is required for *S. mansoni*-induced intestinal hypercontractility, facilitates egg expulsion and prevents premature mortality during acute schistosomiasis.

Mechanical movement of the gut by hypercontractility has been suggested to aid in the expulsion of worms during gut-dwelling helminth infections (1). Previous studies have shown that the IL-4/IL-13/IL-4Rα/STAT6-mediated pathway is necessary for hypercontractility of smooth muscle cells (1, 2, 44). We recently demonstrated that IL-4Rα responsiveness on smooth muscle cells is required for optimal expulsion of the nematode *N. brasiliensis*. Delayed expulsion in infected SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice was associated with reduced host T_H2 immune responses, cholinergic receptor expression and goblet cell hyperplasia (20). In this study, we extend this observation by demonstrating that hypercontractility is mainly dependent on IL-4Rα responsiveness by smooth muscle cells, as intestinal tissue of infected SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice showed attenuated acetylcholine-mediated hypercontractile responses.

In contrast to the gut-dwelling *N. brasiliensis* and *Trichinella spiralis*, *S. mansoni* is a blood fluke, where female worms deposit their eggs intravascular within the mesenteric veins. Eggs that succeed in penetrating the walls of the mesenteric veins transit through the small and large intestinal tract, where they pierce the mucosal lining and eventually reach the gut lumen and are excreted with the feces. Only about half the eggs reach the lumen; the remainder are retained mainly within the gut wall and liver, leading to chronic inflammation with type 2 granulomatous formation, the cause for morbidity and mortality in mice and humans (40). Interestingly, liver histopathology by egg-induced granuloma formation and gut wall pathology by egg-induced diffuse inflammation and granulomatous formation were similarly presented in both infected SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice and control mice (see Fig. 3). Smooth muscle cell hyperplasia, presented by thickening of the muscularis propria, was variable in control mice (6, 30) and similarly variable in smooth muscle cell IL-4Rα-deficient mice (see Fig. 3*D*). Smooth muscle cell IL-4Rα-deficient mice also responded with a comparable type 2 immune response to the egg infiltration as observed in control mice. This was in striking contrast to *N. brasiliensis* infection, where smooth muscle cell-specific IL-4Rα-deficient mice showed reduced T_H2-type cytokine responses compared with infected control mice (20). Despite similar adaptive immune responses, smooth muscle cell-specific IL-4Rα-deficient mice started to die earlier than control mice, irrespective of the infectious dose applied. Early mortality was concomitant with reduced egg expulsion, also observed in highly susceptible global IL-4Rα-deficient mice (18) and in other mutant mouse strains, where the IL-4/IL-13 pathway is impaired (14). In human schistosomiasis, a large proportion of infected individuals suffer from motility-related gastrointestinal problems (23), including abdominal pain and diarrhea that can be life threatening in infants and immune-deficient individuals. These symptoms suggest that the intestinal physiological responses are also affected by infection and that changes induced by infection have relevance in host protection or susceptibility. In the mouse it has been found that ileal contractility, measured by neuromuscular function of longitudinal muscle strips, were increased during chronic schistosomiasis (30, 31). In this study, we showed in control mice that *S. mansoni* infection induced an increase in cholinergic gut motility during acute schistosomiasis (see Fig. 4*A*). Moreover, we showed that this hypercontractility was dependent on IL-4Rα responsiveness and that smooth muscle cells were the major cellular source, as in *N. brasiliensis*-induced hypercontractility. The observed lack of IL-4/IL-13-mediated gut hypercontractility and subsequent slower output of transferring eggs from the gut wall tissue to the lumen has likely caused or contributed to the earlier mortality observed in SM-MHC^{cre}IL-4Rα$^{-/lox}$ and global IL-4Rα-deficient mice. *S. mansoni* is also able to induce contractility of the portal vein (4). Because vein contractility is mediated by smooth muscle cells, it is possible that infected SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice may have experienced reduced vein contractility, particular within the intestine and portal

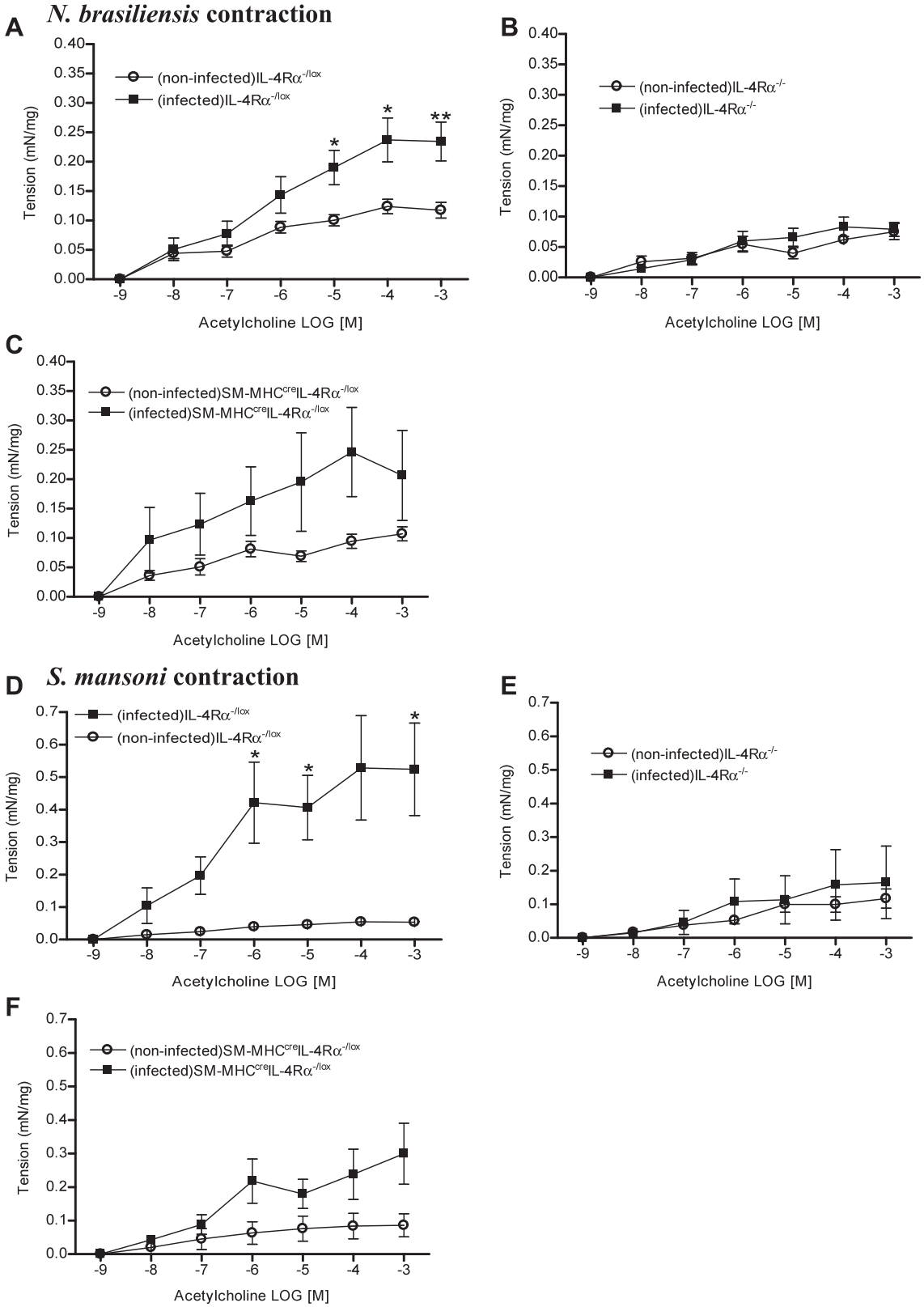


Fig. 4. *Nippostrongylus brasiliensis*- and *S. mansoni*-induced and IL-4Rα-mediated tissue hypercontractility. *A–C*: improved noncumulative dose-response curves of acetylcholine (−9 to −3 mM)-induced jejunum contraction from mice infected with *N. brasiliensis* (10 days postinfection) compared with noninfected mice. Data (means ± SE) from $n > 10$ mice per group. These are a representation of 4–5 independent experiments that have been pooled according to statistical allowance. *D–F*: noncumulative dose-response curve of acetylcholine-induced ileum contraction from mice infected with 80 *S. mansoni* cercariae compared with noninfected mice. Data (means ± SE) from $n = 4$ mice per group. Tissue contractile results are shown as means ± SE for individual dose points; $^{*}P < 0.05$; $^{**}P < 0.01$.

veins, which may have contributed to delayed egg expulsion, increased congestion of eggs in the blood stream and in the tissue, possible hypertension, and subsequent increased mortality. We and others have shown that early mortality of highly susceptible IL-4Rα-deficient mice is mainly caused by septic shock, apparent by the strikingly increased blood LPS titers in moribund mice. This is due to the severe destruction of the intestinal wall caused by the host-induced type 1 inflammation in response to the egg retention in the gut wall. Downregulation of this detrimental T_H1-mediated immunopathology is controlled by aaMph, as previously shown in highly susceptible macrophage/neutrophil-specific IL-4Rα-deficient mice (18). The less severe mortality observed in SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice compared with global or macrophage/neutrophil-specific IL-4Rα-deficient mice can be explained by the presence of the egg-induced host T_H2 response and IL-4/IL-13-activated alternative macrophages (20) in infected SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice. The reduced life span of infected SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice compared with control mice is likely caused by the observed reduced output of transferring eggs from the gut wall tissue to the lumen due to attenuated smooth muscle cell hypercontractility. This may have tipped the sensitive balance of a combination of present pathological features to a shorter life span in infected SM-MHC^{cre}IL-4Rα$^{-/lox}$ mice compared with the morbidity of infected control mice, which also died eventually.

In conclusion, these results suggest that IL-4/IL-13 responsive smooth muscle cells play a role in hypercontractility by improving parasite egg excretion thereby reducing intestinal driven morbidity, mortality egg migration, and excretion during the acute phase, thereby preventing early mortality. We provided further evidence that the host mediates egg expulsion and that both hematopoietic and nonhematopoietic cells are required for host protection and survival.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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