Enteric Nematodes Induce Stereotypic STAT6-Dependent Alterations in Intestinal Epithelial Cell Function

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Enteric Nematodes Induce Stereotypic STAT6-Dependent Alterations in Intestinal Epithelial Cell Function

Kathleen B. Madden,* Karla Au Yeung,§ Aiping Zhao,†¶ William C. Gause,‡ Fred D. Finkelman,* Ildy M. Katona,*† Joseph F. Urban, Jr.,¶ and Terez Shea-Donohue*†¶

Infection with gastrointestinal nematodes exerts profound effects on both the immune and physiological responses of the host. We showed previously that the Th2 cytokines, IL-4 and IL-13, induce STAT6-dependent changes in intestinal epithelial cell permeability, absorption, and secretion that are similar to those observed in a secondary infection with Heligmosomoides polygyrus. In the current study we investigated whether nematode-induced effects on epithelial cell function were 1) generic, 2) dependent upon STAT6, and 3) attributable to direct effects on the epithelial cells themselves or mediated by effects on enteric nerves. Our results demonstrate that infection of BALB/c mice with three different gastrointestinal nematodes (H. polygyrus, Nippostrongylus brasiliensis, and Trichinella spiralis) alters intestinal epithelial cell function by decreasing resistance, glucose absorption, and secretory responses to 5-hydroxytryptamine and acetylcholine, two critical mediators in the submucosal reflex pathway. These modified responses are dependent on STAT6 and are the result of both direct effects and indirect effects mediated through enteric nerves.


Gastrointestinal (GI) nematode infection induces a type 2 immune response (1) that is distinguished by up-regulation of the Th2 cytokines IL-4, IL-5, IL-9, and IL-13, which results in elevated serum IgE levels, eosinophilia, and increased numbers of mucosal mast cells (MMC) (2–4). Among the Th2 cytokines, IL-4 and IL-13 exhibit functional overlap in many of their biological activities, which can be attributed to their binding to the type 2 IL-4R, comprising the IL-4Rα and IL-13Rα chains (5, 6). When IL-4 binds to either its primary receptor (type 1 IL-4R, which comprises the IL-4Rα chain and the common cytokine receptor γ-chain) or the type 2 receptor that it shares with IL-13, Janus kinase-dependent tyrosine phosphorylation of the IL-4R α-chain and STAT6 is initiated (7–9).

The intestinal epithelium serves as a physical barrier between the external environment (the lumen) and the internal milieu, and transports nutrients, ions, and fluid. To maintain physiological homeostasis, the amount of fluid in the lumen is regulated by the mucosa (by modifying nutrient and ion absorption and ion secretion), which is modulated by the enteric nervous system. The characteristic intestinal response to nematode infection is termed “weep and sweep” and describes the increased amount of fluid in the lumen and the hypercontractility of smooth muscle that promote worm expulsion. MMC release a number of soluble mediators, such as PGE2 and histamine (10), which increase epithelial cell secretion (11–13). Epithelial secretion is also enhanced by enteric nerves though a reflex pathway that involves serotonin (5-hydroxytryptamine (5-HT)) stimulation of sensory afferent pathways that are linked to cholinergic efferent pathways (14).

Changes in intestinal fluid homeostasis observed in mice infected with intestinal nematode parasites facilitate worm expulsion. We demonstrated previously that a secondary infection of BALB/c mice with the GI nematode Heligmosomoides polygyrus (Hp), which evokes a strong Th2 cytokine response in the affected host (2), increased intraluminal fluid (15). It is unclear, however, whether these effects are unique to this parasite or are a general feature of enteric infection. The aims of the current study were to investigate 1) whether enteric nematodes induce stereotypic alterations in intestinal epithelial cell function, and 2) the role of STAT6 and enteric nerves in nematode-induced changes in function.

Materials and Methods

Animals

Male and female 8- to 12-wk-old BALB/c mice were purchased from The Jackson Laboratory (Bar Harbor, ME). STAT6-deficient (STAT6−/−) mice on a BALB/c background were bred at Uniformed Services University of the Health Sciences (Bethesda, MD) and were age- and sex-matched with controls in all experiments.

These studies were conducted in accordance with the principles set forth in the Guide for Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council, revised 1996.

Parasites

Infected, encephalitic, third-stage larvae (L3) of Hp (specimens on file at the U.S. National Helminthological Collection, U.S. Department of Agriculture, Beltsville, MD) were propagated and maintained described previously (16) and were stored at 4°C until use. For a secondary infection (Hp 2°), STAT6−/− or BALB/c mice were inoculated orally with 200 L3 via an ingestion route.
18-gauge, ball-tipped feeding tube and treated with an antihelmintic drug (pyrantel pamoate tartrate) 3 wk postinoculation. These mice were then reinjected orally with 200 Hp L3 (d0 Hp 2°) 12–20 days after drug treatment and were studied 12 days after the second inoculation. A secondary Hp infection was used in the present study because the primary infection is persistent, unlike Nb and Trichinella spiralis (Tsp), and only a memory response to a challenge infection is sufficient to induce expulsion like that observed in Nb and Tsp.

Tsp (Beltville strain; specimens on file at the U.S. National Helminthological Collection, U.S. Department of Agriculture, Beltville, MD) were propagated and maintained as described previously (17). Briefly, Tsp were passed serially in BALB/c or Swiss-Webster mice, and first-stage larvae (L1) were recovered from infected mouse by peptic-HCl (1% each) digestion of evacuated, ground mouse carcasses. After washing with several changes of H2O, BALB/c or STAT6−/− mice were inoculated orally with 50 L1 Tsp (Tsp 1°) suspended in 0.2% Bacto Agar (Difco, Detroit, MI) via an 18-gauge, ball-tipped feeding tube and were studied 12 days after infection.

Nippostrongylus brasiliensis (Nb) L3 were propagated and maintained as described previously (18). Feeces collected daily from Nb-infected mice from days 6 through 8 and incubated afterward as a slurry for 8 days served as a stock source of L3; BALB/c or STAT6−/− mice were inoculated s.c. with 500 L3 Nb (Nb 1°) via a 23-gauge needle and were studied 9 days after infection.

**Ussing chambers**

To optimize our analyses of intestinal epithelial cell function, assay time points were selected on the basis of data collected by us and others regarding the times of peak worm expulsion and concomitant Th2-dependent effector mechanisms (maximal levels of serum IgE and peripheral blood eosinophilia, neutrophilia, and mucosal mastocytosis) in normal, STAT6-sufficient mice (reviewed in Ref. 2). Four 1-cm segments of mucosa were stripped of muscle and mounted in Ussing chambers that exposed 0.126 cm² of tissue to 5 ml of Krebs buffer. Agar-salt bridges and electrodes were used to measure the potential difference. Every 50 s the tissues were short-circuited to measure the potential difference. Every 50 s the clamp voltage was adjusted to 1 V for 10 s to allow calculation of tissue resistance using Ohm’s law.

After the 15-min equilibrium period, tissue resistance, a measure of tissue permeability, was determined. After a second 15-min period, concentration-dependent changes in Iₑ were measured after the cumulative addition of acetylcholine, 5-hydroxytryptamine (serotonin (5-HT)), PGE₂, histamine, glucose, and TTX were made using distilled water.

**Solutions and drugs**

Krebs buffer contained 4.74 mM KCl, 2.54 mM CaCl₂, 118.5 mM NaCl, 1.19 mM NaH₂PO₄, 1.19 mM MgSO₄, and 25.0 mM NaHCO₃ on each side. The tissues were allowed to equilibrate for 15 min in Krebs buffer containing 12 mM glucose on the serosal side and 10 mM mannitol on the mucosal side. All drugs were obtained from Sigma-Aldrich (St. Louis, MO) unless stated otherwise. Stock solutions were prepared as follows. TTX was dissolved in citrate buffer to a stock solution of 1 mM, and acetylcholine was dissolved in ultrapure water (1 µM) and frozen. On the day of the experiment, 5-HT, PGE₂, and histamine were dissolved in water, and appropriate dilutions of acetylcholine, 5-HT, PGE₂, histamine, glucose, and TTX were made using distilled water.

**Data analysis**

For each mouse, responses for each secretagogue were performed in a single tissue; therefore, the n for each group reflects the number of mice. Responses from all tissue segments exposed to glucose from an individual mouse were averaged to yield a mean response per animal. Similarly, resistances were calculated for all tissue segments from each mouse and were averaged to yield one mean per animal. Statistical analysis was performed using one-way ANOVA to compare resistance and maximal Iₑ responses among groups. Cumulative dose responses were compared using multiple ANOVA with post-hoc analysis for multiple comparisons. A value of p < 0.05 was considered significant.

**Results**

**Effects of enteric nematode infection on epithelial cell resistance and sodium-linked glucose absorption in wild-type (WT) and STAT6−/− mice**

The effects of gastrointestinal nematode infection on epithelial cell resistance were evaluated in STAT6−/− and BALB/c (WT) mice (n = 8–10). Resistance, a measure of tissue permeability, was similar in untreated WT and STAT6−/− mice. Hp 2° mice exhibited a significant decrease in resistance (Fig. 1), as reported previously (15), and resistance was reduced similarly in Tsp 1° and Nb 1° infections. This decrease was not observed in STAT6−/− mice. To assess the effects of GI nematode infection on substrate-linked sodium absorption, glucose was added to the mucosal (luminal) side of the tissue. Infection with any one of the three nematodes (n = 5–8) significantly decreased Iₑ responses to glucose in WT, but not STAT6−/−, mice (Fig. 2). The STAT6 dependence of nematode-induced reduction in resistance and glucose absorption indicates that these effects are immune-mediated.

**STAT6 dependence of effects of enteric nematode infections on epithelial cell secretion**

Responses to all secretagogues (n ≥ 5) were similar in uninfected WT and STAT6−/− mice (Table I), indicating a lack of constitutive regulation by STAT6. Enteric infection induced a stereotypic decrease in the Iₑ responses to both 5-HT (Fig. 3) and acetylcholine (Fig. 4). In contrast, nematode infection of WT mice uniformly induced a STAT6-dependent elevation in the maximal responses to PGE₂ compared with uninfected controls (Table I). Peak responses to histamine were unchanged by any nematode infection (Table I).

**Contribution of enteric nerves to the nematode-induced changes in intestinal secretion**

We compared responses to these secretagogues in uninfected and infected WT and STAT6−/− mice (Table I), indicating a lack of constitutive regulation by STAT6. Enteric infection induced a stereotypic decrease in the Iₑ responses to both 5-HT (Fig. 3) and acetylcholine (Fig. 4). In contrast, nematode infection of WT mice uniformly induced a STAT6-dependent elevation in the maximal responses to PGE₂ compared with uninfected controls (Table I). Peak responses to histamine were unchanged by any nematode infection (Table I).

![FIGURE 1](http://www.jimmunol.org/) Segments of muscle-free intestinal mucosa were mounted in Ussing chambers to measure changes in tissue resistance (an index of epithelial permeability) in WT or STAT6−/− uninfected control or Hp-, Nb-, or Tsp-infected mice. Values are the mean ± SE (n ≥ 8–10 in each group). *, p < 0.05; **, p < 0.01 vs WT control.
FIGURE 2. Segments of muscle-free intestinal mucosa were mounted in Ussing chambers to measure concentration-dependent changes in $I_{sc}$ in response to glucose in uninfected or Hp-, Nb-, or Tsp-infected WT mice (A) or in uninfected or Hp-, Nb-, or Tsp-infected STAT6-/- mice (B). Values are the mean ± SE ($n = 5–8$ in each group). ***, $p < 0.01$ vs WT control.

on the epithelial cell. In uninfected WT mice, TTX reduced responses to 5-HT (34.9 ± 8.6 vs 13.3 ± 4.4 μA/cm²; $p < 0.05$) and histamine (Fig. 5A) significantly, but not completely, indicating a direct effect of 5-HT and histamine on epithelial cells as well as a significant contribution of enteric nerves. In contrast, there was no difference between responses to acetylcholine (94.7 ± 8.2 vs 82.4 ± 15.4 μA/cm²) or PGE₂ (Fig. 5B) in the presence or the absence of TTX, indicating that both secretagogues work primarily by a direct effect on the epithelial cell. The partial dependence of 5-HT, but not acetylcholine, on enteric nerves is consistent with the model of the secretory reflex pathway diagrammed in Fig. 6.

The dramatic nematode-induced inhibition of responses to 5-HT and acetylcholine was not altered further by TTX in the few tissues in each infection that exhibited any measurable response (data not shown), indicating that the inhibition of secretion observed in Figs. 3 and 4 is due to a direct effect of nematodes on the epithelial cell. In contrast, TTX abolished the increased secretion to PGE₂ in nematode infection, indicating that this prosecretory effect of PGE₂ is dependent on nerves (Fig. 5B). A comparison of responses to PGE₂ (Fig. 5B) in the presence of TTX, however, shows a significant decrease in all three nematode infections consistent with a direct antisecretory effect that is normally masked by enteric nerves. A similar direct antisecretory effect was observed for histamine in Hp- and Tsp-infected mice (Fig. 5A).

Discussion

Our results demonstrate that infection of BALB/c mice with any of three GI nematodes, Hp, Tsp, or Nb, evokes uniform changes in epithelial cell function, characterized by decreased intestinal epithelial cell resistance and Na⁺-linked glucose absorption. These combined effects make the major contribution to the increased intraluminal fluid characteristic of enteric infection. Nematodes, however, also have dichotomous effects on intestinal secretion. The prosecretory effects of PGE₂ are dependent on enteric nerves, whereas the antisecretory effects of acetylcholine, 5-HT, PGE₂, and histamine are due to direct effects on epithelial cells. The changes observed in epithelial cell function were uniformly dependent on the STAT6 signaling pathway. IL-4/IL-13 expression is up-regulated during the Th2-type response to infection with GI nematodes, and these two cytokines are believed to signal through STAT6 (2–4, 7–9). These data demonstrate that enteric infection elicits a IL-4/IL-13R-mediated activation of STAT6 signaling and induction of stereotypic epithelial cell responses that facilitate worm expulsion.

Although the increase in luminal fluid that is a feature of nematode infection has been assumed to derive, at least in part, from enhanced secretion, excess fluid can arise from increased secretion and/or decreased absorption. The present study demonstrates that nematode infection consistently decreased sodium-linked absorption of glucose. This occurs despite differences in the impact of the infection on intestinal morphology; however, the inoculation doses were chosen to induce a vigorous host response that leads to parasite expulsion without overt clinical expression of disease. These data agree with work of others reporting alterations in hexose transport in the small intestine in Hp (19) and in hexose or mannitol transport in Tsp (20–22) and with the observed increase in luminal fluid in the jejunum of Nb rats on days 7 and 10 postinfection (23). We reported previously that treatment of BALB/c mice with either IL-4 or IL-13 induced a significant reduction in glucose absorption (24). In the current study we show that the nematode-induced inhibition of glucose absorption is also dependent on IL-4- and IL-13-mediated activation of STAT6 signaling pathways. This Th2- and STAT6-dependent effect on intestinal function can be linked to worm expulsion. We reported earlier that a primary infection with Hp, which does not clear spontaneously, also inhibited glucose absorption, but the effect was not nearly as dramatic as that observed during a secondary Hp infection (15), which induces rapid production of IL-4 and increased levels of IL-13 (25). Of interest is that administration of IL-4C over 7 days to primary Hp-infected mice induced worm expulsion (26) and was associated

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° Values are the mean ± SE. PGE₂, 10 μM; histamine (HIST), 1 μM; $n = 6$ in each group.

° $p < 0.05$ WT vehicle.
with further reduction in glucose absorption (15), consistent with a role for this IL-4/IL-13-mediated effect on intestinal function in worm clearance.

The nematode-induced inhibition of glucose absorption observed in our study was also associated with decreased tissue resistance, resulting in enhanced intestinal permeability. In this regard, others showed recently that increased intestinal epithelial cell permeability, measured by the transit of [14C]mannitol, is evident in both primary and secondary *Tsp* infections (22). In these studies increased permeability was linked to parasite expulsion and required mouse mast cell protease-1 (22). *Tsp*-infected mice displayed not only a disruption of the normal jejunal expression of an important tight junction protein, occludin-1, but also quantitatively less of this protein, suggesting that it may serve as a substrate for the enzymatic activity of mouse mast cell protease-1 (22). Alternatively, increased mucosal permeability may allow protective proteins from lymph and serum to gain access to the gut lumen, where they might be able to damage the parasite. Increased permeability might also facilitate the absorption of worm Ags, which could promote the developmental of a protective immune response. The present study indicates that increased permeability is also a stereotypic feature of nematode infection.

In addition to changes in permeability and absorptive capacity of the intestinal epithelium, luminal fluid can be increased by up-regulation of intestinal epithelial cell secretion. We demonstrated previously that IL-4, but not IL-13, increased responses to both PGE_2_ and histamine by a mechanism that was dependent on both STAT6 and MMC (24). In the present study all three nematode infections induced a STAT6-dependent increase in secretion in response to PGE_2_, but had no effect on responses to another mast cell mediator, histamine. The lack of an effect on histamine was observed previously in *Hp* infection (15) and appears to be part of the generic response to enteric nematode infection. The prosecretory effects of PGE_2_ were dependent enteric nerves as well, because they were not observed in the presence of TTX. In addition, in the absence of nerves, an antisecretory effect of histamine was revealed in *Hp* and *Tsp*-infected mice. Mast cell and nerve interactions are well documented and are considered to be enhanced by the mastocytosis induced by nematode infection (12). It is of interest, then, that in the absence of nerves, nematode infection induced inhibition of responses to both PGE_2_ and histamine. These data suggest that these prosecretory effects can be attributed to the amplification of neuroimmune interactions and that they normally mask the direct antisecretory effects of nematodes on epithelial cell responses to these mast cell mediators.

The neural regulation of epithelial cell secretion involves activation of a reflex pathway that includes actions of 5-HT and acetylcholine in pathways within the submucosal plexus of the enteric nervous system (Fig. 6). The reflex is initiated by release of 5-HT from enterochromaffin cells in response to a variety of stimuli, including mucosal stroking. The released 5-HT binds to 5-HT_1A/5-HT_4 receptors on intrinsic primary afferent neurons in the submucosal plexus, subsequently releasing sensory neuropeptides such as substance P, which, in turn, activate secretomotor neurons within the submucosal plexus (14). These secretomotor neurons release transmitters, including acetylcholine, which bind to their respective to postsynaptic receptors on intestinal epithelial cells, resulting in chloride secretion (14). One would expect, therefore, that the presence of worms in the lumen would elicit an increase in reflex-mediated secretion. Surprisingly, we observed a uniform decrease in the secretion in response to both 5-HT and acetylcholine that was dependent on STAT6. These reductions persisted after

**FIGURE 3.** Segments of muscle-free intestinal mucosa were mounted in Ussing chambers to measure concentration-dependent changes in $I_{sc}$ in response to 5-HT in uninfected or *Hp*, *Nb*, or *Tsp*-infected WT mice (A) or in uninfected or *Hp*, *Nb*, or *Tsp*-infected STAT6$^{+/−}$ mice (B). Values are the mean ± SE ($n$ ≥ 5 in each group). ***, $p$ < 0.01 vs WT control.

**FIGURE 4.** Segments of muscle-free intestinal mucosa were mounted in Ussing chambers to measure concentration-dependent changes in $I_{sc}$ in response to acetylcholine in uninfected or *Hp*, *Nb*, or *Tsp*-infected WT mice (A) or in uninfected or *Hp*, *Nb*, or *Tsp*-infected STAT6$^{+/−}$ mice (B). Values are the mean ± SE ($n$ ≥ 5 in each group). ***, $p$ < 0.01 vs WT control.
chemical blockade of enteric nerves by TTX, suggesting that these effects are the result of a direct effect on the epithelial cell. We showed previously that treatment with exogenous IL-4 or IL-13 induced a STAT6-dependent reduction in the intestinal epithelial cell secretory response to 5-HT, but not to acetylcholine (24). In fact, nematode-induced inhibition of responses to acetylcholine was one of the few effects of enteric infection that was not mimicked by exogenous IL-4 or IL-13. The reduced acetylcholine responses, however, were STAT6 dependent, indicating that this inhibitory response requires activation of both IL-4 and IL-13 signaling pathways and suggesting that the effect is downstream of STAT6. Although negative effects of worm infection on neurally mediated epithelial secretion do not promote rapid worm clearance, the presence of TTX. Values are the mean ± SE (n ≥ 5 in each group). *p < 0.05 vs respective control in the absence of TTX; **p < 0.05 vs uninfected WT control in the presence of TTX.

In summary, we have shown that infection of BALB/c mice with any of three different GI nematodes has dramatic effects on intestinal epithelial cell function, eliciting a stereotypic host response that is driven through the STAT6 signaling pathway shared by IL-4 and IL-13. These alterations include decreased resistance (a measure of tissue permeability) and decreased absorption of glucose. These effects make the major contribution to the increased intraluminal fluid characteristic of enteric infection. In addition,
nematode-induced alterations in responses to PGE$_2$, reflect a significant prosecretory effect that is mediated by enteric nerves. In the absence of nerves, however, nematodes have direct inhibitory effect on the epithelial cell response to both PGE$_2$ and histamine, showing the contribution of nerves to the host response. Of interest was the overall STAT6-dependent inhibition of secretion in response to 5-HT and acetylcholine. These antisecretory effects benefit the host by limiting the accumulation of fluid in the lumen that could lead to diarrhea. Parasite biology has apparently adapted in different ways to the stereotypic response of the host intestine, including the chronic primary infection established by $H_p$, the rapid development and accumulation of infective larvae of $T_s$ in the striated muscle, and the high fecundity of $N_b$ during its brief residency in the gut. Our results reflect a dynamic equilibrium between effects detrimental to the worm vs effects detrimental to the host, with the ultimate goal of parasite expulsion with minimal disruption to the physiological homeostasis of the GI tract.

Acknowledgments

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References