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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,¶ Ilidy M. Katona,*† Joseph F. Urban, Jr.,¶ and Terez Shea-Donohue2††

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, and that these 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. The Journal of Immunology, 2004, 172: 5616–5621.

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-4Rα 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, ensheathed, 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
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, 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 5 ml of Krebs buffer. Agar-salt bridges and electrodes were used 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, or histamine to the serosal side. To determine whether nematode-induced alterations in Iₑ were due to a direct effect on the epithelial cell, an indirect effect mediated by nerves, or a combination of both mechanisms, responses to acetylcholine, 5-HT, PGE₂, and histamine were compared in the presence and the absence of tetrodotoxin (TTX; 2 μM), a potent neurotoxin that inhibits conduction by blocking the sodium channel in nerves. After the peak response to the final concentration of each secretagogue was recorded, the Krebs buffer on each side of the chamber was replaced, and the tissue was allowed to equilibrate for 30 min. Upon re-equilibration, concentration-dependent changes in Iₑ were measured in response to the cumulative addition of glucose to the mucosal side.

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; acetylcholine was dissolved in ultrapure water (1 μM) and frozen in water. 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. Hp² mice exhibited a significant decrease in resistance (Fig. 1), as reported previously (15), and resistance was reduced similarly in Tsp¹ and Nb¹ 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 in the presence and the absence of TTX, a potent neurotoxin. A significant change in response in the presence of TTX demonstrates a contribution of nerves, whereas no change in the response in the presence of TTX indicates a direct effect.
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.

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 PGE$_{2}$ are dependent on enteric nerves, whereas the antisecretory effects of acetylcholine, 5-HT, PGE$_{2}$, 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 hextose transport in the small intestine in Hp (19) and in hextose or mannitol transport in Tsp (20–22) and with the observed increase in luminal fluid in the jejuna of Nb rats on days 7 and 10 postinfection (23).

Table I. STAT6 dependence of nematode-induced alterations in epithelial cell secretion to PGE$_{2}$ and histamine

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Hp</th>
<th>Tsp</th>
<th>Nb</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>WT</td>
<td>STAT6</td>
<td>WT</td>
<td>STAT6</td>
</tr>
<tr>
<td>PGE$_{2}$</td>
<td>34 $±$ 5</td>
<td>42 $±$ 5</td>
<td>70 $±$ 14</td>
<td>59 $±$ 10</td>
</tr>
<tr>
<td>HIST</td>
<td>48 $±$ 9</td>
<td>50 $±$ 8</td>
<td>45 $±$ 1</td>
<td>58 $±$ 14</td>
</tr>
</tbody>
</table>

$^a$ Values are the mean $±$ SE. PGE$_{2}$, 10 $μ$M; histamine (HIST), 1 $μ$M; $n = 6$ in each group.

$^b$ $p < 0.05$ WT vehicle.
PGE2 and histamine by a mechanism that was dependent on both previously that IL-4, but not IL-13, increased responses to both regulation of intestinal epithelial cell secretion. We demonstrated fluid can be increased by up-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). Alternately, 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 development 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 PGE2 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 both PGE2 and histamine. These reductions persisted after infection and appeared to be part of the generic response to enteric nematode infection. The prosecretory effects of PGE2 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 PGE2 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-HT1 receptor in 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](http://www.jimmunol.org/)

FIGURE 3. Segments of muscle-free intestinal mucosa were mounted in Ussing chambers to measure concentration-dependent changes in Isc 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](http://www.jimmunol.org/)

FIGURE 4. Segments of muscle-free intestinal mucosa were mounted in Ussing chambers to measure concentration-dependent changes in Isc 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 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 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, they do prevent fluid loss through diarrhea (27, 28). In the present study diarrhea was not a consequence of infection with any of the three nematodes.

The potent effect of GI nematode infection on the secretory response to acetylcholine is consistent with the importance of this mediator not only in mammals, but also in nematodes. Evidence for the critical nature of acetylcholine is supported by the existence of nematode acetylcholinesterases first detected and subsequently quantified in the esophageal and excretory glands of Nb (29–31). Since that initial discovery, it has been shown that Nb, Hp, and Tsp all secrete distinct acetylcholinesterases (32–36) that hydrolyze the acetylcholine secreted by the host (37–39), thereby modulating epithelial cell function and neural regulatory feedback loops. In addition to acetylcholinesterase of nematode origin, host acetylcholinesterase expression is up-regulated at distinct sites on the basement membrane of intestinal epithelial cells in rats infected with Nb (40), a response that may serve to limit the hyperstimulation of the enteric nervous system by the presence of worms in the lumen. From an evolutionary standpoint, it is of interest that both hosts and parasites elaborate acetylcholinesterases, which may serve to antagonize neurally mediated prosecretory reflexes. Such mutual acetylcholinesterase activity in situ would be beneficial to both the parasite and the host by not only limiting the accumulation of fluid in the lumen that would ordinarily facilitate worm expulsion, but also dampening the potential debilitating effects of excess fluid loss on the host through diarrhea.

The biological significance of a stereotypic intestinal epithelial cell response should not be underestimated. In response to enteric nematode infection, up-regulation of immune mediators initiates a set of defined effects on mucosal physiology. In this manner the host is provided with a defense mechanism that may be linked to preprogrammed changes in intestinal function that ultimately contribute to the expulsion of the parasite. Although the three GI nematodes studied in this experiment (Hp, Tsp, and Nb) exhibit different life cycles, all share an enteral phase that evokes similar alterations in intestinal epithelial cell function that depend upon IL-4 and/or IL-13 signaling through the STAT6 pathway. Studies performed by Khan et al. (41) and Akiho et al. (42) have underscored the critical roles of IL-4, IL-13, and STAT6 in mediating the intestinal smooth muscle hypercontractility observed in Tsp-infected mice. In this regard recent work from our laboratory suggests that parasite-specific refinement of the generalized host response to GI nematode infection also involves stereotypic alterations in gut motility. In these studies the relative importance of IL-4 and IL-13 in the immune response to either Hp or Nb correlated with the effects of those cytokines on smooth muscle contractility (43).

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,

![Figure 5](image-url)  
**FIGURE 5.** Segments of muscle-free intestinal mucosa were mounted in Ussing chambers to measure concentration-dependent changes in I_{sc} in response to histamine (A) or PGE₂ (B) in uninfected or Hp-, Nb-, or Tsp-infected WT mice in the presence or the absence 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.

![Figure 6](image-url)  
**FIGURE 6.** Schematic diagram of reflex secretory pathway via submucosal plexus (adapted from Cooke (14)).
nematode-induced alterations in responses to PGE₂, 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 PGE₂ 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 antiserotonin 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 *Hp*, the rapid development and accumulation of infective larvae of *Tp* in the striated muscle, and the high fecundity of *Nh* 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