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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-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 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. The Journal of Immunology, 2004, 172: 5616–5621.

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18-gauge, ball-tipped feeding tube and treated with an antihelmintic drug (pyrantel pamoate tartrate) 3 wk postinoculation. These mice were then reinoculated 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 (Beltsville strain; specimens on file at the U.S. National Helminthological Collection, U.S. Department of Agriculture, Beltsville, 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 muscle by peptic-HCl (1% each) digestion of eviscerated, 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). Feaces 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, 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 at 1 V (DVC 1000 voltage clamp; World Precision Instruments, Sarasota, FL), and the short-circuit current (Isc) was monitored continuously. In addition, 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 Isc were measured after the cumulative addition of acetylcholine, 5-hydroxytryptamine (serotonin (5-HT)), PGE2, histamine, and 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 Isc 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, 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 Isc 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 Isc 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 Isc 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...
on the epithelial cell. In uninfected WT mice, TTX reduced responses to 5-HT ($34.9 \pm 8.6$ vs $13.3 \pm 4.4 \mu A/cm^2$; $p < 0.05$) 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 ($94.7 \pm 8.2$ vs $82.4 \pm 15.4 \mu A/cm^2$) or PGE$_2$ (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 PGE$_2$ in nematode infection, indicating that this prosecretory effect of PGE$_2$ is dependent on nerves (Fig. 5B). A comparison of responses to PGE$_2$ (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 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 hoxose transport in the small intestine in Hp (19) and in hoxose or manitol 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). 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

**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>
</thead>
<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$^a$</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 μM; histamine (HIST), 1 μM; $n = 6$ in each group.

$^b$ $p < 0.05$ 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 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 5-HT (22). These reductions persisted after treatment with 5-HT 4 receptors on intrinsic primary afferent neurons in 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 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](http://www.jimmunol.org/)

**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, 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,
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 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. pylori, the rapid development and accumulation of infective larvae of T. sipho in the striated muscle, and the high fecundity of N. brachyale 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