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Critical Role of IL-25 in Nematode Infection-Induced Alterations in Intestinal Function

Aiping Zhao,*† Joseph F. Urban, Jr.,‡ Rex Sun,* Jennifer Stiltz,*† Motoko Morimoto,§ Luigi Notari,*† Kathleen B. Madden,¶ Zhonghan Yang,*† Viktoria Grinchuk,*† Thirumalai R. Ramalingam,‖ Thomas A. Wynn,‖ and Terez Shea-Donohue*†

IL-25 (IL-17E) is a member of the IL-17 cytokine family. IL-25-deficient mice exhibit impaired Th2 immunity against nematode infection, implicating IL-25 as a key component in mucosal immunity. The sources of IL-25 and mechanisms responsible for the induction of Th2 immunity by IL-25 in the gastrointestinal tract remain poorly understood. There is also little information on the regulation of IL-25 during inflammation or its role in gut function. In the current study, we investigated the regulation of IL-25 during Nippostrongylus brasiliensis infection and the contribution of IL-25 to the infection-induced alterations in intestinal function. We found that epithelial cells, but not immune cells, are the major source of IL-25 in the small intestine. N. brasiliensis infection-induced upregulation of IL-25 depends upon IL-13 activation of STAT6. IL-25−/− mice had diminished intestinal smooth muscle and epithelial responses to N. brasiliensis infection that were associated with an impaired Th2 protective immunity. Exogenous IL-25 induced characteristic changes similar to those after nematode infection but was unable to restore the impaired host immunity against N. brasiliensis infection in IL-13−/− mice. These data show that IL-25 plays a critical role in nematode infection-induced alterations in intestinal function that are important for host protective immunity, and IL-13 is the major downstream Th2 cytokine responsible for the IL-25 effects. The Journal of Immunology, 2010, 185: 000–000.

Interleukin-25, also called IL-17E, is a cytokine member of the IL-17 family, which includes IL-17A through F. Whereas other members of the IL-17 family have biological activities similar to Th1 inflammatory cytokines, IL-25 appears to be involved primarily in the promotion of Th2 immunity, including allergy, asthma, and enteric nematode infection (1). Of equal importance is that IL-25 also inhibits proinflammatory Th1 and Th17 cytokine responses (2). IL-25−/− animals develop severe intestinal inflammation during nematode infection or are highly susceptible to experimental autoimmune encephalomyelitis (3, 4). In addition, IL-25 is downregulated in the colon in patients with inflammatory bowel disease, and exogenous IL-25 ameliorates experimental colitis in mice (5). Studies in isolated cell populations showed that IL-25 is produced by a variety of immune cells, such as activated Th2 cells (1), mast cells (6), macrophages (7), and CD4+ and CD8+ T cells (2). More recently, it was demonstrated that peripheral human eosinophils and basophils secrete IL-25 (8). There is a low constitutive expression of IL-25 in several tissues, with the highest expression in gastrointestinal tract, lung, and testis (1). IL-25 mRNA is upregulated in the gut after Nippostrongylus brasiliensis infection or in the lung in response to Aspergillus fumigatus infection (9), but the principal source of IL-25 in infection remains to be identified. In addition, the mechanisms underlying the regulation of IL-25 expression during nematode infection are relatively unexplored.

Enteric nematode infection induces a polarized Th2 immune response, including elevated production of IL-4, IL-5, and IL-13, which contributes to worm expulsion. There is strong evidence supporting a role for IL-25 in the Th2-mediated protective immunity, as mice with IL-25 deficiency cannot expel worms efficiently and develop a Th1- and Th17-related chronic inflammation (2, 10, 11). Expulsion of enteric nematodes is associated with stereotypic changes in intestinal function including smooth muscle hypercontractility, epithelial cell hyposecretion, and increased mucosal permeability. These changes are dependent primarily on IL-4/IL-13 and receptor-mediated activation of STAT6 signaling pathways (12, 13). The effects of IL-25 on gut function are unknown.

Given the potential anti-inflammatory effects of IL-25 and its importance in the development of Th2 immunity, the current study was designed to 1) investigate the role of IL-4/IL-13 and STAT6 signaling in N. brasiliensis infection-induced upregulation of IL-25; 2) identify the specific cell population that produces/expresses IL-25 in the small intestine; 3) determine the contribution of IL-25 in the N. brasiliensis infection-induced alterations in intestinal smooth muscle and mucosal function; and 4) elucidate the downstream molecules that mediate the IL-25 effect on intestinal smooth muscle function. This study is the first to show,
to our knowledge, that epithelial cells are the major sources of IL-25 in the small intestine during infection, that nematode-induced upregulation of IL-25 depends upon IL-13 activation of STAT6, and that IL-25 has effects on gut function that are mediated primarily by IL-13.

Materials and Methods

**Mice**

BALB/c, C57BL/6, and SCID mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice deficient in STAT6 (STAT6-/-), IL-4 (IL-4/-), and IL-13 (IL-13/-/-) on either BALB/c or C57BL/6 background were obtained from the breeding colonies at the University of Cincinnati (a generous gift from Dr. Fred Finkelmann). Mice deficient in IL-25 (IL-25/-/-) were generated by Regeneron Pharmaceuticals (Tarrytown, NY) and were backcrossed to the C57BL/6 background for 10 generations. These studies were conducted in accordance with principles set forth in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, revised 1996) and by the Beltsville Animal Care and Use Committee.

**Administration of IL-25 or IL-13**

Mice (n = 5/group) were injected i.v. with 0.7 μg mouse recombinant IL-25 or 10 μg IL-13 (R&D Systems, Minneapolis, MN) in 100 μl saline daily for 7 d or as otherwise indicated. Control mice were given an injection of saline (for IL-13 treatment) or 35 μg BSA (for IL-25 treatment). The amount of cytokine administered was based on the observation that daily injection of immune-competent BALB/c mice with this dose of IL-13 enhances worm expulsion (14) or on the minimum dose of IL-25 that induced a prominent Th2 immune response from a previous study (1).

**Enteric nematode infection and worm expulsion**

Infective, third-stage larvae of *N. brasiliensis* (specimens on file at the U.S. National Parasite Collection, U.S. National Helminthological Collection, Collection 81930, Beltsville, MD) were propagated and stored at room temperature in fecal/charcoal/peat moss culture plates until used (13). Groups of mice were inoculated s.c. with 500 third-stage larvae. Except for the time-course experiment, mice were euthanized and studied at day 9 (BALB/c) or 10 (C57BL/6) postinfection. The timing of the studies after infection with *N. brasiliensis* correlated with the time of the maximal effects on gut function and coincided with worm expulsion, based on the results from our previously (13) or currently performed time-course experiment on wild-type (WT) mice. *H. polygyrus* and *T. muris* infections were described previously (13, 15). Appropriate sections of adult worms in the intestine indicates delayed expulsion.

**In vitro smooth muscle contractility in organ baths**

In vitro smooth muscle contractility was measured as described previously (13). Smooth muscle responses to electric field stimulation (EFS), 5-hydroxytryptamine (5HT), or acetylcholine and the amplitude of spontaneous contractions were determined. Tension was expressed as force per cross-sectional area (16).

**In vitro epithelial cell ion transport in Ussing chambers**

Muscle-free segments of small intestine were mounted in Ussing chambers as described previously (17). After a 15-min period, concentration-dependent changes in short-circuit current were determined in response to the cumulative addition of acetylcholine to the serosal side. Responses from all tissue segments exposed to acetylcholine from a previous study (1).

**Microscope well assay for mucosal transepithelial electrical resistance**

The modified microscope well system is a miniaturized version of the standard Ussing chamber that has been engineered to measure the trans-epithelial electrical resistance (TEER) of intestinal fragments exposed to various stimuli (18). A decrease in TEER reflects increased tissue permeability. Briefly, segments of mouse intestine stripped of both muscle and serosal layers were placed in the microscope well system. Two hundred fifty microliters of DMEM containing 4.5 g/l glucose, 4 mM l-glutamine, 50 U/ml penicillin, 50μg/ml streptomycin, and MEM with 1 mM nonessential amino acids was added to the mucosal side. Three milliliters of the same medium was added to the serosal side. The system was incubated at 37°C with 5% CO₂ for 30 min to stabilize the pH, and the baseline TEER was measured.

**Preparation of frozen tissue blocks and sectioning for laser-capture microdissection**

Frozen tissue blocks were prepared as described (19). Four-micrometer tissue sections used for laser-capture microdissection (LCM) were obtained from frozen blocks using plain uncoated slides and an HM505E cryostat (Richard-Allan Scientific, Kalamaazoo, MI). Sections of frozen tissue were stained with H&E to assess changes in tissue morphology. Cryosectioned tissue was stained with H&E and dehydrated. LCM was performed with a PicCell II (Arcturus Engineering, Mountain View, CA). Cells were captured from the region of the epithelium, lamina propria, and smooth muscle in the small intestine and transferred to CapSure LCM Caps (Arcturus Engineering).

**RNA extraction, cDNA synthesis, and real-time quantitative PCR**

Total RNA was extracted from intestine whole tissue or from LCM samples as described previously (19). RNA samples (2 μg) were reverse-transcribed to cDNA using the First Strand cDNA Synthase Kit (MBI Fermentas, Hanover, MD) with random hexamer primer. Real-time quantitative PCR (qPCR) was performed on an iCycler detection system (Bio-Rad, Hercules, CA). PCR was performed in a 25-μl volume using SYBR Green Supermix (Bio-Rad). Amplification conditions were: 95°C for 3 min, 50 cycles of 95°C for 15 s, 60°C for 15 s, and 72°C for 20 s. The fold-changes in mRNA expressions for targeted genes were relative to the respective vehicle groups of mice after normalization to 18S rRNA. Primer sequences (Table I) were designed by using Beacon Designer 7.0 (Premier Biosoft International, Palo Alto, CA), and synthesized by the Biopolymer Laboratory of the University of Maryland (Baltimore, MD).

**Immunofluorescence staining**

Frozen blocks of midjejunum were prepared using the Swiss-roll technique and stored at −80°C. Tissue sections (4 μm) were cut from frozen blocks using an HM505E cryostat (Richard-Allan Scientific). Tissue slides were fixed in cold acetone for 30 min and blocked with 10% normal rat serum in PBS for 1 h at room temperature, incubated with anti–IL-25 Abs (1:50; R&D Systems) overnight at 4°C, and then incubated with Alexa 488 rabbit anti-goat IgG. The slides were coverslipped with Vectorshield (Vector Laboratories, Burlingham, CA) and digitally photographed using an Olympus microscope and Fluoview confocal software (Olympus America Inc., Central Valley, PA). The images were taken by establishing settings for the samples from the individual vehicle groups and using the same conditions to evaluate the samples from the infected or treated groups. Comparisons were made only among slides prepared on the same day.

**Solutions and drugs**

Krebs buffer contained (in mM) 4.74 KCl, 2.54 CaCl₂, 118.5 NaCl, 1.19 NaH₄PO₄, 1.19 MgSO₄, 25.0 NaHCO₃, and 11.0 glucose. All drugs were obtained from Sigma (St. Louis, MO) unless otherwise indicated. On the day of the experiment, 5HT was dissolved in water, and appropriate dilutions were made. All experiments were performed in the absence of drugs.

**Data analysis**

Agonist responses were fitted to sigmoid curves (GraphPad, San Diego, CA). Statistical analysis was performed using one-way ANOVA followed by Newman-Keuls test to compare the responses and gene expression among the different treatment groups.

**Results**

**Nematode-induced upregulation of IL-25 is IL-13- and STAT6-dependent**

Previous studies showed that *N. brasiliensis* infection increased mRNA expression of IL-25 in the small intestine (9). To determine the temporal changes in IL-25 expression during nematode infection, we infected WT BALB/c mice with *N. brasiliensis* and collected tissue at days 5, 7, 9, and 14 postinfection. Real-time PCR analysis
Notably, the infection-induced upregulation of IL-25 in mice was not specific to *N. brasiliensis*, nor was it restricted to the small intestine, as similar results were observed in *H. polygyrus*-infected small intestine (fold change, 1.0 ± 0.2 in vehicle versus 3.1 ± 1.0 in *N. brasiliensis*, *n* = 5, *p* < 0.05), peaked at day 9 postinfection (Fig. 1A), and returned to control levels by day 14 postinfection (data not shown).

There was a similar mRNA expression of IL-25 in all strains of uninfected mice after *N. brasiliensis* infection. As the time course experiment on WT BALB/c mice showed that IL-25 mRNA expression peaked at day 9 postinfection, this time point was therefore selected for the studies to investigate the immune regulation of IL-25 expression. There was a similar mRNA expression of IL-25 in all strains of uninfected mice (data not shown). The infection-induced upregulation of IL-25 in WT mice was present in IL-4−/− mice but absent in STAT6−/− and IL-13−/− mice, indicating a dependence on IL-13 activation of STAT6 (Fig. 1A). The importance of IL-13 is supported further by the finding that exogenous administration of IL-13 upregulated IL-25 expression (Fig. 1B).

*Epithelial cells, but not immune cells, are the major cellular source of IL-25 in the small intestine*

To determine the source of IL-25 in the small intestine, we used qPCR in epithelial, smooth muscle, and lamina propria captured by LCM. As described previously, mRNA expression of the specific molecular markers for epithelial (villin), smooth muscle (α smooth muscle actin), and immune cells (leukocyte common Ag) was analyzed first on the respective LCM to ensure the purity of the samples (19). Intestinal epithelial cells from control mice expressed detectable levels of IL-25 mRNA that were further upregulated by *N. brasiliensis* infection (Fig. 2A). Of interest is that expression of IL-25 was undetectable in either lamina propria or smooth muscle of small intestine from control or nematode-infected mice, suggesting that immune or smooth muscle cells do not express measurable levels of IL-25 mRNA (Fig. 2A). As a positive control for RNA recovery, IL-13 was detectable in LCM-captured lamina propria from both control and infected mice. There was also no IL-25 mRNA expression in mesenteric lymph nodes in either control or *N. brasiliensis*-infected mice. Consistent with a non-T/non-B cell source of IL-25, we found that exogenous administration of IL-13 upregulated IL-25 expression in SCID mice (Fig. 1B).

To confirm that IL-25 was expressed selectively on intestinal epithelial cells, we used immunofluorescent staining of frozen sections of small intestine with anti–IL-25 mAb. Similar to mRNA data from LCM samples, IL-25 staining was evident only in epithelial cells, but not in the lamina propria or smooth muscle. Staining of surface cells was significantly increased in intensity in *N. brasiliensis*-infected mice (Fig. 2B). The specificity of the Ab to IL-25 was validated by a preliminary experiment showing no staining on tissue slide when the Ab was omitted. Moreover, no staining was revealed when the same Ab was used for the intestinal tissue slides from either IL-25 KO control or infected mice.

**Immune-regulated IL-25R expression**

The activity of IL-25 requires the receptor subunit IL-17RB, which binds IL-25, as well as IL-17RA. Interestingly, however, IL-17RA does not appear to interact directly with IL-25 but does bind other members of the IL-17 family (20). To determine the potential IL-25-responsive cells in the small intestine, we measured IL-25R mRNA expression at baseline and after nematode infection. In whole tissue, only IL-17RB was upregulated significantly in response to infection by a mechanism that was STAT6-dependent (Fig. 3A). In addition, the elevated expression was observed

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### Table I. Primer sequences for real-time qPCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequences (5′ to 3′)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-25</td>
<td>Forward, CAGCAAAGAGCAGAAAGCCTC CGAGGCAGAAGCAG</td>
</tr>
<tr>
<td>IL-17RA</td>
<td>Reverse, ACGCGGGACACCGCCGACCAGAAGCAGGAG</td>
</tr>
<tr>
<td>IL-17RB</td>
<td>Forward, CAGCGGGACACCGCCGACCAGAAGCAGGAG</td>
</tr>
<tr>
<td>IL-23a</td>
<td>Forward, CAGCGGGACACCGCCGACCAGAAGCAGGAG</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Forward, CAGCGGGACACCGCCGACCAGAAGCAGGAG</td>
</tr>
<tr>
<td>GATA3</td>
<td>Forward, CAGCGGGACACCGCCGACCAGAAGCAGGAG</td>
</tr>
<tr>
<td>T-bet</td>
<td>Forward, CAGCGGGACACCGCCGACCAGAAGCAGGAG</td>
</tr>
</tbody>
</table>

*Table I* showed that IL-25 mRNA expression in the small intestine began to rise at day 7 (fold change, 1.0 ± 0.2 in vehicle versus 3.1 ± 1.0 in *N. brasiliensis*, *n* = 5, *p* < 0.05), peaked at day 9 (Fig. 1A), and returned to control levels by day 14 postinfection (data not shown).

![FIGURE 1](http://www.jimmunol.org/) Nematode-induced IL-25 is IL-13−/− and STAT6-dependent. Mice were infected with *N. brasiliensis* and studied at day 9 postinfection (A) or injected i.v. with 10 µg mouse recombinant IL-13 daily and studied at day 7 postinfection (B). qPCR was performed to measure mRNA expression in the small intestine. The fold changes were relative to the individual vehicle groups after normalization to 18S rRNA. *n* ≥ 5 for each group. *p* < 0.05 versus the respective vehicle group.

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**H. polygyrus, n = 5, *p* < 0.05** or *T. muris*-infected colon (fold change, 1.0 ± 0.2 in vehicle versus 2.8 ± 0.6 in *T. muris*, *n* = 5, *p* < 0.05). In addition, the expression of IL-25 was independent of the genetic background of the mouse, as similar effects were seen in both BALB/c and C57BL/6 mice (data not shown).

We next determined the role of IL-4/IL-13 activation of STAT6 on the upregulation of IL-25 using IL-4−/−, IL-13−/−, or STAT6−/− mice after *N. brasiliensis* infection. As the time course experiment on WT BALB/c mice showed that IL-25 mRNA expression peaked at day 9 postinfection, this time point was therefore selected for the studies to investigate the immune regulation of IL-25 expression. There was a similar mRNA expression of IL-25 in all strains of uninfected mice (data not shown). The infection-induced upregulation of IL-25 in WT mice was present in IL-4−/− mice but absent in STAT6−/− and IL-13−/− mice, indicating a dependence on IL-13 activation of STAT6 (Fig. 1A). The importance of IL-13 is supported further by the finding that exogenous administration of IL-13 upregulated IL-25 expression (Fig. 1B).

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in infected IL-4−/− or IL-13−/− mice, indicating that either Th2 cytokine alone can elevate the expression (Fig. 3A). LCM revealed constitutive expression of both IL-17RA and IL-17RB in epithelial and smooth muscle cells, with the highest expression of IL-17RA in epithelial cells and the highest expression of IL-17RB in smooth muscle cells (Fig. 3B). Unlike IL-25, both IL-17RA and IL-17RB were expressed constitutively on immune cells in the lamina propria. Nematode infection increased expression of IL-17RB in epithelial cells and in the lamina propria but did not further increase expression in smooth muscle (Fig. 3B). In contrast, expression of IL-17RA was reduced in the entire cross section of the small intestine, including cells from lamina propria, epithelium, and smooth muscle (Fig. 3B).

**Contribution of IL-25 to nematode infection-induced intestinal smooth muscle and mucosal function**

In animals with nematode infection, a measurement of successful Th2 protective immunity is the effective clearance of adult worms, which is completed by day 9 in BALB/c and day 10 in C56BL/6 WT mice after inoculation. Expulsion was impaired in IL-25−/− mice on the C56BL/6 background, as significant numbers of worms remained in the small intestine at day 10 (Fig. 4A). Changes in gut function facilitate worm clearance; therefore, we compared smooth muscle and epithelial cell function in WT and IL-25−/− mice. *N. brasiliensis* infection induced a characteristic intestinal smooth muscle hypercontractility in WT mice as shown previously (13, 21–23). The enhanced smooth muscle responses to acetylcholine, electric field stimulation, and 5HT were absent completely in infected IL-25−/− mice (Fig. 4B–D). In addition, the increased amplitude of spontaneous contractions in infected WT mice (4247 ± 1275 in vehicle versus 8517 ± 1851 in infected, n ≥ 5, p < 0.05) was abrogated as well in IL-25−/− mice (4476 ± 1378 in vehicle versus 4037 ± 424 in infected, n ≥ 3).

IL-25 also plays a conspicuous role in infection-induced changes in epithelial cell function. Although the increase in mucosal permeability (decrease in TEER) was attenuated modestly in IL-25−/− mice (Fig. 4E), the stereotypic reduction in epithelial secretion in response to acetylcholine was not observed in IL-25−/− mice (Fig. 4F).

**Exogenous IL-25 induced similar changes in intestinal epithelial and smooth muscle function to nematode infection**

To investigate further the effects of IL-25 on gut function, we administered recombinant IL-25 to mice daily for 7 d. Exogenous IL-25 induced a smooth muscle hypercontractility in response to
acetylcholine (Fig. 5A), electrical field stimulation (Fig. 5B), and 5HT (Fig. 5C) and increased the amplitude of spontaneous contractions (Fig. 5D). Epithelial responses were also altered by IL-25, including increased mucosal permeability (Fig. 6A) and hyposecretion in response to acetylcholine (Fig. 6B). For epithelial functional studies, muscle-free mucosa was mounted in (E) microsnap well for the measurement of TEER or in Ussing chambers for epithelial cell response to (F) acetylcholine (1 mM), n ≥ 3 for each group. *p < 0.05 versus (A) WT: N. brasiliensis or (B–F) the respective vehicle; †p < 0.05 versus the respective WT.

IL-25–induced changes in gut function were associated with a significant upregulation of the Th2 cytokines, IL-4 and IL-13, and master Th2 transcription factor, GATA3 (Fig. 7A). The ability of IL-25 to increase IL-13 mRNA was validated further by ELISA, which showed an elevation in in situ IL-13 production in the intestine of mice treated with IL-25 (Fig. 7B). The major Th1 cytokines, IL-12p40 and IL-23a, were significantly downregulated in IL-25–treated mice, while IFN-γ and the Th1 master transcription factor, T-bet, remained unchanged (Fig. 7C). Notably, IL-25 administration upregulated the expression of IL-25 itself and its receptor, IL-17RB, suggesting a positive feedback mechanism that could significantly amplify the effect of IL-25 during infection (Fig. 7D).

**Exogenous IL-25 was unable to restore the impaired host immunity against N. brasiliensis infection in IL-13−/− mice**

Previous studies proposed that the biological activities of IL-25 are mediated by the downstream effects on Th2 cytokines IL-4, IL-5, IL-9, and IL-13 (11), but whether this involves a specific cytokine or a combination of cytokines is unknown. To address this question, we administered IL-25 daily to N. brasiliensis–infected IL-13−/− mice, using the same dose that evoked a significant increase in smooth muscle contractility in WT mice (Fig. 5). The infection–induced intestinal smooth muscle hypercontractility to acetylcholine and the increased amplitude of spontaneous contractions, observed in WT mice (13, 21, 23), was absent in infected IL-13−/− mice and was not restored by IL-25 (Fig. 8). Finally, exogenous IL-25 did not reinstate the host defense against...
**FIGURE 5.** Exogenous IL-25 induced intestinal smooth muscle hypercontractility in mice. BALB/c mice were injected i.v. with 0.7 μg IL-25 daily and studied at day 7 postinjection. Intestinal strips were suspended longitudinally in organ baths for in vitro contractility studies in response to (A) acetylcholine (10 nM to 0.1 mM), (B) EFS (1–20 Hz, 80 V), (C) 5HT (100 μM), or (D) for spontaneous contraction. n = 5 for each group. *p < 0.05 versus the respective vehicle.

**FIGURE 6.** Exogenous IL-25 increased mucosal permeability and induced stereotypic changes in mucosal epithelial function in mice. BALB/c mice were injected i.v. with 0.7μg IL-25 daily and studied at day 7 postinjection. Muscle-free mucosa was mounted in (A) microsnap well for the measurement of TEER or in (B) Ussing chambers for epithelial secretion in response to acetylcholine (10 nM to 1 mM). n = 5 for each group. *p < 0.05; **p < 0.01 versus the respective vehicle.

*N. brasiliensis* infection in IL-13−/− mice, as both vehicle-treated and IL-25–treated IL-13−/− mice harbored comparable numbers of adult worms in the small intestine, whereas C57BL/6 WT mice expelled worms completely at day 10 postinfection (data not shown).

**Discussion**

IL-25 is emerging as a key regulator of inflammation in the gut mucosa because of its ability to promote Th2, while suppressing Th1 and Th17, cytokine responses. In the current study, we demonstrated that there is a constitutive expression of IL-25 in intestinal epithelial cells, but not in immune cells, and which is upregulated significantly during nematode infection. IL-17RB, the receptor that binds IL-25, is located on both structural and immune cells and is also upregulated during infection. Infection-induced increases in both IL-25 and IL-17RB expression involved activation of STAT6. Furthermore, IL-13 is the major downstream Th2 cytokine responsible for the IL-25–mediated changes in gut function that are important for protective immunity against nematode infection.

Low levels of IL-25 mRNA are found in many tissues with the highest expression in gastrointestinal tract (1). There are, however, controversial reports regarding the cell types that produce IL-25. For example, Fort et al. (1) showed that IL-25 was expressed exclusively by Th2 cells, whereas Wang et al. (8) demonstrated that eosinophils and basophils, but not other immune cells (T cells, B cells, monocytes, dendritic cells, etc.), secreted IL-25. In response to *N. brasiliensis* infection, various immune cells including macrophages, dendritic cells, eosinophils, and Th2 cells are activated and recruited to the intestinal lamina propria. Previous results showed that certain isolated immune cells, including highly polarized Th2 cells, could be induced to express IL-25 (1, 2, 6, 7). Notably, we observed that cells from intestinal lamina propria and mesenteric lymph nodes had undetectable levels of IL-25 mRNA constitutively and during nematode infection, suggesting that immune cells in the gut are not the major source of IL-25 in vivo. The finding that epithelial cells produce IL-25 is consistent with previous reports in both the colon (4) and lung (8). Of interest is the ability of nematode infection or exogenous IL-13 to upregulate epithelial cell expression of IL-25, which was coincident with...
a reduction in proinflammatory cytokines IL-23 and IL-12p40. Others showed a downregulation of IL-25 in colonic epithelial cells in the absence of commensal bacteria that was associated with an overexpression of IL-23 and IL-12p35 (4). These data support an important role for epithelial-derived IL-25 in immune homeostasis in both the small intestine and colon, acting in particular to control the development of Th17-driven pathologies. To our knowledge, this is the first report showing that epithelial cells are the major source of IL-25 in the small intestine during infection; however, we cannot rule out the possibility that immune cells from other portions of the gut, or under other conditions in the small intestine, can express IL-25.

The receptor for IL-25 consists of IL-17RB, which binds IL-25 and is a 56-kDa single-transmembrane protein expressed abundantly in kidney, intestine, and other peripheral organs (26), and IL-17RA, which appears to be necessary for the biological activity of IL-25 in the lung (20). Whereas IL-25 mRNA was found only in epithelial cells, both structural and immune cells in the small intestine express IL-17RA and IL-17RB. Infection upregulated IL-17RB expression by a mechanism involving IL-4/IL-13 and STAT6, an effect similar to that observed for IL-25. The observation that exogenous IL-25 also upregulated its own expression, as well as IL-17RB, suggests a novel positive feedback mechanism in which epithelial-derived IL-25 can maintain its own expression. In the lung, part of the IL-25 effect was attributed to upregulation of IL-17A, which binds to IL-17RA (20). The role of IL-17RA in IL-25–mediated effects in the small intestine is less evident, as neither nematode infection nor exogenous IL-25 altered the already constitutively low expression of IL-17A (Z. Yang, V. Grinchuk, J. Stiltz, R. Sun, L. Notari, J. Urban, T. Shea-Donohue, and A. Zhao, unpublished data). Indeed, IL-25 downregulated IL-17RA, suggesting that the major effects of IL-25 in the intestine appear to be mediated through IL-25 binding to IL-17RB.

In the current study, we extend previous findings and show that the upregulation of IL-25 is perhaps a common feature in nematode infection.
Interleukin 25 regulates type 2 cytokine-dependent immunity and limits chronic inflammatory bowel disease.

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Disclosures
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References


