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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,*‡ Viktoriya 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). Additionally, IL-25 is proposed to act in the initiation and amplification of allergic airway pathology that has striking similarities to nematode infection (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 (STAT6/2), IL-4 (IL-4/2), and IL-13 (IL-13/2) 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 Finkelman). Mice deficient in IL-25 (IL-25/2) 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. *Heligmosomoides polygyrus* and *Trichuris muris* infections were described previously (13, 15). Appropriate age-matched controls were performed for each infection. Adult worms were detected quantitatively by scanning the intestinal surface with a dissecting scope. In general, WT mice completely expel worms by day 9 in BALB/c or day 10 in C57BL/6 after inoculation, and therefore, the presence 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 an individual animal were averaged. Changes in short-circuit current were determined in response to the cumulative addition of acetylcholine to the serosal side.

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

The modified microsnap well system is a miniaturized version of the standard Ussing chamber that has been engineered to measure the transepithelial 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 microsnap well system. Two hundred fifty milliliters 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, Kalamazoo, 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 CapsSure 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 30 s, 30 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 1) 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 sections 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 Ab (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, Burlingame, CA) and digitally photographed using an Olympus microscope and Fluov View 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 treated 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 NaH2PO4, 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.

**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...
IMMUNE-REGULATED IL-25 EXPRESSION IN THE SMALL INTESTINE

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 ± 0.1 in vehicle versus 8.0 ± 2.0 in 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).

*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.

### 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, CAGCAAAGAGCAAGAAC</td>
</tr>
<tr>
<td></td>
<td>Reverse, CACAATTCCTGCGCCCT</td>
</tr>
<tr>
<td>IL-17RA</td>
<td>Forward, ACTGTGGAGCTTCGAG</td>
</tr>
<tr>
<td></td>
<td>Reverse, CCGCTTGCAGGAGAGAG</td>
</tr>
<tr>
<td>IL-17RB</td>
<td>Forward, AGCCATGCTGGCAATC</td>
</tr>
<tr>
<td></td>
<td>Reverse, CCGCTTGCAGGAGAGAG</td>
</tr>
<tr>
<td>IL-26</td>
<td>Forward, AGATGAAGGAGACAGAG</td>
</tr>
<tr>
<td></td>
<td>Reverse, GCAGAGAAGTGGTAAAG</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Forward, TGGCTGTTCCTGCTTAC</td>
</tr>
<tr>
<td></td>
<td>Reverse, AGGTTGGTATCATAGAG</td>
</tr>
<tr>
<td>GATA3</td>
<td>Forward, CTGAGACGCACCAACC</td>
</tr>
<tr>
<td></td>
<td>Reverse, GTGAGAGTGGAGCTGAGT</td>
</tr>
<tr>
<td>T-bet</td>
<td>Forward, GTATCTGGTTCCAGCCG</td>
</tr>
<tr>
<td></td>
<td>Reverse, ACTGTGGTCGAGAGTCC</td>
</tr>
</tbody>
</table>

(1) 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). 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 ± 0.1 in vehicle versus 8.0 ± 2.0 in N. brasiliensis infected mice).
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).

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). These changes in gut function were similar to those reported previously in response to nematode infection or exogenous administration of IL-4/IL-13 (13, 17, 21, 24, 25).

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
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. BALB/c mice were injected i.v. with 0.7 μg IL-25 daily and studied at day 7 postinjection. A, C, and D, qPCR was performed to measure the mRNA expression of major cytokines of (A) Th2, (C) Th1, and (D) IL-25 and IL-17RB. B, ELISA was performed to validate in situ IL-13 production in the small intestine. The fold increases for mRNA expression 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.
Infection, as comparable increases were observed in *N. brasiliensis* and *H. polygyrus* infection of the small intestine and in *T. muris* infection of the colon. IL-25 is proposed to act as both an initiating and amplification factor for the Th2 immune response in allergic airway inflammation (10). During the course of *N. brasiliensis* infection, IL-25 mRNA expression peaks at day 9 postinfection, which follows the upregulation of IL-13 and is coincident with the maximal changes in intestinal function (23). These data indicate that IL-25 may act more as an amplification factor than an initiator of the Th2 response during nematode infection. The mechanisms underlying the regulation of IL-25 remain relatively unexplored.

In the current study, exogenous IL-25 increased IL-4/IL-13 expression, yet nematode infection or exogenous IL-13 also upregulated IL-25 expression by a STAT6-dependent mechanism. This reciprocal control between IL-25 and IL-13 constitutes a double positive feedback mechanism that acts to amplify the Th2 immune response in vivo and may play an important role in developing and maintaining the host defense against nematode infection. This is a novel role for IL-13, as IL-25 is thought to be important primarily for the initiation of the Th2 response, an effect confirmed in the current study by the increased generation of IL-13 in response to exogenous IL-25. The biological activity of IL-13 is mediated by IL-13Rα1, which forms the type 2 IL-4R complex with IL-4Rα, linked to STAT6 (28). Both IL-4Rα and IL-13Rα1 are expressed constitutively on epithelial cells (29). Taken together, these data indicate that epithelial-derived IL-25 binds to local immune cells leading to the release of IL-13, which binds to the type 2 IL-4R to activate STAT6, resulting in an increased epithelial cell production of IL-25.

Nematode infection is associated with STAT6-dependent changes in both epithelial and smooth muscle function that facilitate worm expulsion (13, 17, 24, 25). IL-13 is the dominant effector molecule that coordinates the host immune response to *N. brasiliensis* infection, and mice deficient in IL-13 exhibit impaired worm clearance (30). Indeed, many of the infection-induced changes in gut function are largely dependent on IL-13 (13). In the current study, however, the infection-induced changes in smooth muscle function were dependent entirely on IL-25. Although smooth muscle contains receptors for IL-25, IL-17RA, and IL-17RB, the fact that exogenous IL-25 could not induce worm clearance or hypercontractility in IL-13−/− mice indicated that these effects were mediated by the downstream upregulation of IL-13. This is consistent with data showing that IL-13 was required for IL-25-mediated protection against experimental autoimmune encephalomyelitis and Th17 suppression (3). Because the primary source of IL-25 appears to be epithelial cells, a direct effect of IL-25 on smooth muscle in vivo is unlikely. These data emphasize the importance of IL-25 upregulation of IL-13, which then induces a hypercontractility of smooth muscle directly via STAT6-dependent gene expression, as well as indirectly through alternatively activated macrophages (23). The ability of IL-25 to downregulate expression of proinflammatory Th1 and Th17 cytokines, which are associated with a hypercontractility of smooth muscle, may also contribute to the indirect effects of IL-25 on smooth muscle. The function of IL-17RB on smooth muscle and its interaction with IL-17RA is unclear, as the downstream signaling of these receptors has not been fully elucidated but may function to influence the activity of other cytokines that bind these subunits on smooth muscle.

IL-13 is not expressed by structural cells; therefore, the effects of IL-25 on IL-13 can be attributed to IL-17RA/IL-17RB on immune cells. Identification of the cell types that respond to IL-25 and generate Th2 cytokines, especially in the gut, is an area of active research. Early studies indicated these cells were perhaps a novel population of IL-4/IL-5/IL-13–producing non-B and non-T cells (1). Four more recent papers showed that several previously unidentified cell populations may also respond to IL-25, including “multipotent progenitor cell population” (31), “natural helper cells” (32), “nuocytes” (33), and “Th2 cells” (34). It remains to be determined whether any of these cell types contribute to IL-25–induced production of Th2 cytokines that impact intestinal function.

The current study showed that exogenous IL-25 mimicked the effects of nematode infection or exogenous IL-4 and IL-13 on epithelial cell permeability and secretion (17, 24, 25). IL-25 also plays a role in nematode infection–induced alterations in epithelial function, as the infection-induced epithelial hyposecre- tion in response to acetycholine was aborted in deficient mice, and the increased permeability was modestly attenuated. Previous studies demonstrated that these two nematode-induced effects were STAT6-dependent; therefore, the inability of IL-25 deficiency to completely prevent the infection-induced changes in permeability was surprising. This result may be attributed to a direct effect of worms on intestinal permeability that is independent of IL-25–mediated upregulation of Th2 cytokines. Previous studies showed that mice deficient in IL-25 were unable to expel worms, whereas administration of IL-25 restored the host immunity against *T. muris* in genetically susceptible mice or induced rapid worm expulsion in *N. brasiliensis*-infected WT mice (2, 11). The presence of worms in the intestines of deficient mice supports the hypothesis of a direct effect.

In conclusion, to our knowledge this is the first study to show the importance of IL-25 in nematode infection–induced changes in intestinal function that contribute to worm expulsion. Although IL-25 is implicated in the host immune response to nematode infection through regulation of Th2 cytokines (2, 11), the expression of IL-25 and the receptors are also regulated by Th2 cytokine activation of STAT6. This reciprocal regulation plays an important role in initiation and maintenance of the Th2 protective immunity during nematode infection. It is likely that IL-25 produced by epithelial cells acts on receptors, primarily IL-17RB, located on immune cells to amplify the local expression of IL-13. Indeed, IL-13 plays a critical role in the biological activities of IL-25 in host immunity against enteric nematode infection. The ability of IL-25 to downregulate the expression of proinflammatory cytokines supports a key role for this epithelial-derived cytokine in mucosal homeostasis and may provide a novel therapeutic target for the treatment of gut pathologies, such as inflammatory bowel disease.

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