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Colonization with *Heligmosomoides polygyrus* Suppresses Mucosal IL-17 Production

David E. Elliott,†‡ Ahmed Metwali,†‡ John Leung,†‡ Tommy Setiawan,‡ Arthur M. Blum,‡ M. Nedim Ince,†‡ Lindsey E. Bazzone,§ Miguel J. Stadecker,§ Joseph F. Urban, Jr.,† and Joel V. Weinstock*  

Helminth exposure appears to protect hosts from inappropriate inflammatory responses, such as those causing inflammatory bowel disease. A recently identified, strongly proinflammatory limb of the immune response is characterized by T cell IL-17 production. Many autoimmune type inflammatory diseases are associated with IL-17 release. Because helminths protect from these diseases, we examined IL-17 production in helminth-colonized mice. We colonized mice with *Heligmosomoides polygyrus*, an intestinal helminth, and analyzed IL-17 production by lamina propria mononuclear cells (LPMC) and mesenteric lymph node (MLN) cells. Colonization with *H. polygyrus* reduces IL-17A mRNA by MLN cells and inhibits IL-17 production by cultured LPMC and MLN cells. Helminth exposure augments IL-4 and IL-10 production. Blocking both IL-4 and IL-10 alone, restores IL-17 production in vitro. Colonization of colitic IL-10-deficient mice with *H. polygyrus* suppresses LPMC IL-17 production and improves colitis. Ab-mediated blockade of IL-17 improves colitis in IL-10-deficient mice. Thus, helminth-associated inhibition of IL-17 production is most likely an important mechanism mediating protection from inappropriate intestinal inflammation. *The Journal of Immunology*, 2008, 181: 2414–2419.

Inflammatory bowel disease (IBD) is prevalent in highly developed industrialized countries. IBD is rare in less developed nations, but emerges as nations develop socioeconomically (1). This pattern of prevalence is opposite to that of parasitic worm exposure (2). Transmission of parasitic worms (helminths) is prevented by modern hygienic practices, so colonization is rare in developed countries. Helminths alter their host’s immune responses (3), which may limit development of pathologic-type inflammations like IBD (2, 4).

Helminth exposure can prevent or reverse colitis in animal models of IBD. Mice and rats develop colitis when rectally challenged with trinitrobenzenesulfonic (TNBS) acid in 50% ethanol (5). These animals are protected from TNBS-type colitis when exposed to *Schistosoma mansoni* (6, 7), *Trichuris muris* (2), *Trichinella spiralis* (8), *Hymenolepis diminuta* (9), or *Heligmosomoides polygyrus* (10). IL-10−/− mice develop severe chronic Th1-driven colitis in response to normal gut flora (11). Colonization with *T. muris* or *H. polygyrus* inhibits development of spontaneous colitis in IL-10−/− mice (2). Furthermore, colitis improves in IL-10−/− mice given *H. polygyrus* after inflammation is previously established (12). Exposure to helminths also protects mice from other immune-mediated inflammations such as experimental autoimmune encephalitis (EAE) (13, 14), reactive airway disease (15, 16), and autoimmune diabetes (17).

Recently identified is a novel, strongly proinflammatory limb of the immune response characterized by IL-17 production by Th17 cells (18). Inflammatory conditions once attributed to aberrant Th1 or Th2 responses may result from Th17 activity. IL-17 is important in colitis. IL-17 mRNA and protein expression is augmented in inflamed mucosa from patients with ulcerative colitis or Crohn’s disease, but not in disease controls (19, 20). Mice with TNBS colitis have increased mucosal IL-17 production (21). IL-17R-deficient mice are resistant to TNBS colitis (21). Colonic IL-17 mRNA expression is increased in immune-deficient (RAG−/−) mice that develop colitis after reconstitution with CD4 T cells from IL-10−/− mice and treatment with rIL-23 (22). Development of this colitis is inhibited with anti-IL-17 and anti-IL-6 Ab blockade (22).

IL-17 expression is increased in other immune-mediated diseases such as multiple sclerosis (MS) (23, 24) and asthma (25, 26). IL-17 expression is up-regulated in murine EAE (27), a model of MS. IL-17−/− mice are resistant to EAE (27). In mice, IL-17 expression is increased in reactive airway disease, and IL-17R-deficient mice are resistant to this model of asthma (28).

Murine colitis, EAE, and reactive airway disease all result from excessive IL-17 production. Because helminths protect mice from these inflammations, we investigated whether colonization with the intestinal nematode *H. polygyrus* would affect expression of IL-17. We find that intestinal lamina propria mononuclear cell (LPMC) and mesenteric lymph node (MLN) cell IL-17 production is strongly inhibited in *H. polygyrus*-colonized mice. Colonization results in augmented IL-4 and IL-10 production (10). We show that these cytokines, especially IL-4, regulate IL-17 release. This is...
the first report that helminths inhibit proinflammatory IL-17 production, which may explain why populations with high prevalence of helminth infections have low prevalence of autoimmune disease.

Materials and Methods

Mice and H. polygyrus infection

This study used C57BL/6 wild-type (The Jackson Laboratory) and C57BL/6 IL-10−/− mice. IL-10−/− mice were bred on site in specific pathogen-free animal facilities. At 6 wk of age, mice were colonized with 150 H. polygyrus third stage larvae (L3) by oral gavage. Infective, ensheathed H. polygyrus L3 (U.S. National Helminthological Collection no. 81930) were obtained (from fecal cultures of eggs) by the modified Baer/Phosphoribosyltransferase amplified using 5'-CACGTCAC-3' and 5'-GGCCAAGGACTTCCTCCAGA-3' primers and 5'-FAM-CAGACTCTCAGA3'-GCCGTTCGACGTCAC-3' as probe. The results were normalized to hypoxanthine phosphoribosyltransferase amplified using 5'-TGAAGAGCTACTGTTAGTACCTGACAAC-3' and 5'-GCAAAGCTGACGTTCAACATTAC-3' as primers and 5'-TET-TGGTCTCTCGTGAAGCTGACGTCACGCCC-3' as probe. Amplification was performed using Taqman universal master mix (Applied Biosystems) and analyzed with a ABI PRISM 7700 Sequence Detection System.

LPMC isolation

Terminal ileal intestinal tissue was washed extensively with RPMI 1640, and all visible Peyer’s patches were removed with a scissors. The intestine was opened longitudinally, cut into 5-mm pieces, and then incubated in 0.5 mM EDTA in calcium- and magnesium-free HBSS for 20 min at 37°C with shaking to release intraepithelial lymphocytes and epithelial cells. This was repeated after thorough washing. Tissue then was incubated for 20 min at 37°C in 20 ml of RPMI 1640 containing 1% FCS, 25 mM HEPES buffer, 2 mM l-glutamine, 5 × 10−5 M 2-ME, 1 mM sodium pyruvate, 100 U/ml penicillin, 5 mg/ml gentamicin, and 100 mg/ml streptomycin (all Life Technologies), and 1 mg/ml collagenase (Sigma-Aldrich no. 1130). At the end of the incubation, the tissue was subjected to further mechanical disruption using a 1-ml syringe. To remove debris, the LPMC preparations were washed through a prewet gauze layered in a funnel with RPMI 1640. Then, the LPMC were washed once and sieved through a prewet 2-cm nylon wool column gently packed into a 10-ml syringe. After washing, cells (up to 2 × 10^7) were layered onto a column of Percoll with a 30:70% gradient. Cells were spun at 2200 g at room temperature for 20 min. The LPMC collected from the 30:70 interface were washed and maintained on ice until used. Cell viability was 90% as determined by eosin Y exclusion.

Cell culture

For cytokine analysis, cells were cultured for 48 h in 96-well microtiter plates (Corning Glass) with 200 µl of medium (5 × 10^6 cells/well) at 37°C. The culture medium was RPMI 1640 containing 10% FCS, 25 mM HEPES buffer, 2 mM l-glutamine, 5 × 10−5 M 2-ME, 1 mM sodium pyruvate, 100 U/ml penicillin, 5 mg/ml gentamicin, and 100 mg/ml streptomycin (all Life Technologies). For most experiments, the cells were cultured alone or with anti-CD3 (2C11; 1 µg/ml; American Type Culture Collection) and anti-CD28 (PV1; 1 µg/ml; American Type Culture Collection). Cytokine-blocking experiments used anti-IL-12 mAb at 2.5 µg/ml (1B1;3; BD Pharmingen) and anti-IL-4 mAb at 2.5 µg/ml (11B11; American Type Culture Collection). Cytokine addition experiments used murine rIL-4 and/or rIL-10 each at 50 ng/ml (PeproTech).

ELISA and intracellular flow cytometry

Commercial ELISA was used to measure the concentration of IL-17A in culture supernatants. The capture Ab was MAB721 (R&D Systems), and detection Ab was biotinylated BAF421 (R&D Systems). Color development used streptavidin-HRP (Zymed Laboratories) and tetramethylbenzidine substrate (Pierce), and plates were read at 490 nm. Sensitivities of the ELISAs were 30 pg/ml.

For intracellular flow cytometric staining, MLN cells were cultured and stimulated overnight with PMA and ionomycin, as previously described (12). For the last 4 h of culture, brefeldin A (Golgi Plug; BD Pharmingen) was added to the cells. The cells were then washed twice and adjusted to 10^6 cells/ml in FACS buffer (HBSS containing 1% FCS and 0.02% sodium azide). The cell suspensions were then dispensed into microcentrifuge tubes, each containing 10^6 cells in 100 µl of FACS buffer and 1 µg of 2.4G2 Ab (anti-FcγR; American Type Culture Collection) to block nonspecific binding of Abs to FcRs. The cells were then fixed and stained with saturating amounts of anti-CD4-Cy5 (RM2511; Caltag Laboratories)- and anti-Thy-1.2-FITC (TS; Sigma-Aldrich)-conjugated Abs for 30 min at 4°C. To identify cytokine-secreting cells, cells were costained with anti-IL-17 PE (BD Pharmingen) using Cytofix/Cytoperm kit (BD Pharmingen), according to manufacturer’s instructions. Following staining, cells were washed three times resuspended for analysis on a BD Biosciences FACS 440 flow cytometer.

Induction and evaluation of colitis

To induce colitis, 5- to 6-wk-old IL-10−/− mice were given piroxicam (Sigma-Aldrich) mixed into their feed (NIH-31M) for 2 wk. They received 60 mg of piroxicam/250 g food during week 1 and 80 mg of piroxicam/250 g food during week 2. Mice were then placed on normal rodent chow without piroxicam. The colitis was evaluated 16 days after stopping piroxicam. For some experiments, piroxicam-treated IL-10−/− mice were colonized with H. polygyrus between 24 and 48 h after stopping the piroxicam. To grade intestinal inflammation, colons were removed at the indicated time point, rolled, fixed, and embedded in paraffin. The inflammation was scored from 0 to 4 using the following criteria: grade 0, no change from normal tissue; grade 1, patchy mononuclear cell infiltrates in the lamina propria; grade 2, more uniform mononuclear cell inflammation involving both the epithelium and lamina propria, and accompanied by epithelial hyperplasia and slight depletion of mucus from goblet cells; grade 3, some epithelial and muscle hypertrophy with patchy lymphocytic infiltrates extending into the muscle layers, mucus depletion, occasional crypt abscesses, and epithelial erosions; and grade 4, severe, transmural inflammation with prominent thickening of both the epithelial and muscle layers, mucus depletion, frequent crypt abscesses, and ulcerations (12). In some experiments, IL-10−/− colitic mice were treated with two i.p. injections of blocking anti-IL-17 mAb (BD TC11 18H10) given 1 wk apart at 0.5 mg/mouse or with an isotype control Ab.

Statistical analysis

Data are means ± SE of multiple determinations. Difference between two groups was compared using Student’s t test. Values of p < 0.05 were considered significant.

Results

Colonization with H. polygyrus reduces IL-17A mRNA and protein expression by MLN cells

Colonization with H. polygyrus protects mice from TNBS colitis (10). IL-17 expression increases in TNBS colitis, and inhibition of IL-17 signaling reduces colonic inflammation in that model (21). Therefore, we examined the effect of colonization with H. polygyrus on IL-17 production. Control mice were treated similarly, but received no H. polygyrus larvae. RNA extracted from freshly isolated MLN cells contains transcripts for IL-17A. Expression of IL-17 mRNA is significantly reduced in MLN cells isolated from mice colonized with H. polygyrus as measured by real-time PCR (Fig. 1A).

We also examined MLN cell expression of IL-17 after TCR stimulation with anti-CD3 mAb. MLN cells were isolated from helminth naive (control) or colonized mice and cultured for 48 h in the absence or presence of anti-CD3 mAb. Culture supernatants were tested for IL-17 content by ELISA. MLN cells from H. polygyrus-colonized mice made significantly less IL-17 as compared with naive mice (Fig. 1B).

Colonization with H. polygyrus reduces the frequency of IL-17-expressing MLN T cells

IL-17 is produced by a distinct lineage of CD4+ T cells (Th17). Inhibition of IL-17 production could result from a decrease in the
number of IL-17-expressing T cells in the MLN population. MLN cells were isolated from helminth naive and colonized mice, and then stimulated overnight with PMA and ionomycin to maximally activate T cells. Secretion of IL-17 was blocked by addition of brefeldin A for the last 4 hours of stimulation. IL-17 expression was evaluated by intracytoplasmic flow cytometry. The frequency of CD4+IL-17+ T cells was reduced by 50% in _H. polygyrus_-colonized as compared with naive mice (Fig. 1C).

Colonization with _H. polygyrus_ reduces IL-17 expression by intestinal LPMC

_H. polygyrus_ resides in the duodenum and proximal jejunum of mice. Colonization produces no histological change in the more distal ileum, but does alter IFN-γ, IL-4, IL-13, and IL-10 production by lymphocytes isolated from this site (10). We examined whether helminth exposure altered IL-17 production by ileal LPMC. LPMC were isolated for naive or helminth-colonized mice and cultured for 48 h in the absence or presence of anti-CD3 and anti-CD28 mAb. Culture supernatants were tested for IL-17 content by ELISA. LPMC from _H. polygyrus_-colonized mice made significantly less IL-17 as compared with naive mice (Fig. 1D).

IL-10 blockade does not release IL-17 production by MLN from helminth-colonized mice

Like IL-17, MLN and LPMC IFN-γ production is strongly inhibited in mice colonized with _H. polygyrus_. Colonization dramatically increases IL-10 production by cultured lymphocytes stimulated with anti-CD3. Previously, we found that blocking in situ IL-10 signaling in vitro with anti-IL-10R mAb (1B1.3) releases inhibition of IFN-γ (10). Thus, helminth-induced IL-10 actively inhibits IFN-γ release. We tested whether similar blockade of IL-10R would augment IL-17 release. MLN cells were isolated as above from helminth-colonized mice and were stimulated in the presence of blocking anti-IL-10R Ab or isotype control (Fig. 2A and B). Unlike IFN-γ, IL-17 production was not affected by IL-10 blockade alone.

Blockade of IL-4 and IL-10 synergizes to restore IL-17 production by MLN cells from helminth-colonized mice

In addition to IL-10, helminth exposure increases lymphocyte IL-4 production (10). IL-4 can suppress Th17 activity (30). We examined whether the in situ produced IL-4, which is enhanced in colonized mice, suppresses IL-17. MLN cells were isolated as above from helminth-colonized mice and were stimulated in the presence of blocking anti-IL-4 Ab (11B11). Addition of anti-IL-4 partially restored IL-17 production (Fig. 2C). Including anti-IL-10R Ab with anti-IL-4 further enhanced IL-17 release. Anti-IL-4, anti-IL-10R, or a combination of anti-IL-4 and anti-IL-10R did not increase IL-17 production by MLN cells from helminth naive mice (data not shown).

Colonization with _H. polygyrus_ suppresses IL-17 production from IL-10−/− mice

Helmint-associated suppression of IL-17 is not released by blocking IL-10 in culture. However, blocking IL-10 enhances IL-17 production if IL-4 is also blocked. We examined whether colonization with _H. polygyrus_ would effectively suppress IL-17 production in IL-10−/− mice. MLN cells were isolated from naive or

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**FIGURE 1.** IL-17 mRNA expression, cytokine production, and Th17 frequency are reduced in _H. polygyrus_-colonized mice. A, Total RNA was isolated from freshly isolated unstimulated MLN cells of C57BL/6 wild-type naive (control) or 2-wk helminth-colonized mice. IL-17A mRNA content was determined by real-time PCR using hypoxanthine phosphoribosyltransferase mRNA as reference. Data are from three separate experiments. B, MLN cells were isolated from B6 wild-type mice colonized for 2 wk with _H. polygyrus_. Control MLN cells were from helminth naive B6 wild-type mice. MLN cells were cultured for 48 h at 5 × 10^5 cells/well with or without anti-CD3 stimulation. Culture supernatant IL-17A content was measured by ELISA. Data are means ± SE from three separate experiments. C, MLN cells from helminth naive and 2-wk-colonized B6 wild-type mice were isolated and cultured with PMA/ionomycin and then Golgi Plug. Then cells were fixed, labeled, and prepared for intracytoplasmic flow cytometry. Cells were stained for IL-17 and CD4 expression. Results presented are representative of three experiments. D, LPMC were isolated from B6 wild-type mice colonized for 2 wk with _H. polygyrus_ or helminth naive B6 wild-type mice and cultured for 48 h at 5 × 10^5 cells/well in the absence or presence of anti-CD3/anti-CD28 stimulation. Culture supernatant IL-17 content was measured by ELISA. Data are mean ± SE from two separate experiments.
helminth-colonized IL-10−/− mice and cultured as above. MLN cells from H. polygyrus-colonized IL-10−/− mice made significantly less (p < 0.01) IL-17 as compared with naive mice (Fig. 3A). Addition of neutralizing anti-IL-4 mAb to these cultures significantly increased in vitro IL-17 production. Addition of anti-IL-4 mAb to cell cultures obtained from helminth naive mice does not increase IL-17 production (data not shown).

The Ab blockade experiments suggest that in situ generated IL-4 can suppress IL-17 production. We tested this by adding murine rIL-10 and/or rIL-4 to anti-CD3-stimulated MLN cell cultures obtained from helminth naive IL-10−/− mice (Fig. 3B). Addition of rIL-10 partially (26 ± 8%), but significantly reduces IL-17 production in these cultures. Addition of rIL-4 reduces IL-17 production more completely (73 ± 9%). Addition of both IL-4 and IL-10 to MLN cell cultures obtained from helminth naive wild-type mice showed similar significant (p < 0.05) suppression of IL-17 (34 ± 8% and 72 ± 11%, respectively).

IL-10−/− mice spontaneously develop colitis that is associated with increased mucosal IL-17 production (22). Synchronous uniform colitis can be induced in IL-10−/− mice by treating them with low-dose piroxicam (31), a nonselective inhibitor of cyclooxygenase. Low-dose piroxicam does not cause colitis in wild-type mice, and the colitis induced in IL-10−/− mice persists after piroxicam is withdrawn. Like spontaneous colitis, piroxicam-induced colitis is associated with a dramatic increase in LPMC IL-17 production (Fig. 4A). Colitis was scored on a 0- to 4-point scale, as described in Materials and Methods. The colitis score was 3.5 ± 0.4 (SE) for the piroxicam-treated helminth naive (control) group. After mice were rendered colitic with piroxicam, we colonized some with H. polygyrus. Colonization with H. polygyrus reverses established colitis in IL-10−/− mice (12). After 2 wk of worm colonization, we isolated LPMC from helminth naive and H. polygyrus-exposed mice. The colitis score was 0.6 ± 0.3 (SE, p < 0.01) for the piroxicam-treated helminth naive (control) group. After mice were rendered colitic with piroxicam, we colonized some with H. polygyrus. Colonization with H. polygyrus reverses established colitis in IL-10−/− mice (12). After 2 wk of worm colonization, we isolated LPMC from helminth naive and H. polygyrus-exposed mice. The colitis score was 0.6 ± 0.3 (SE, p < 0.01) for the piroxicam-treated helminth naive (control) group. After mice were rendered colitic with piroxicam, we colonized some with H. polygyrus. Colonization with H. polygyrus reverses established colitis in IL-10−/− mice (12). After 2 wk of worm colonization, we isolated LPMC from helminth naive and H. polygyrus-exposed mice. The colitis score was 0.6 ± 0.3 (SE, p < 0.01) for the piroxicam-treated helminth naive (control) group.
Blockade of IL-17A reverses established colitis in IL-10−/− mice

Colonization with *H. polygyrus* reverses colitis and reduces LPMC IL-17 production in IL-10−/− mice. This suggests that helminth-associated inhibition of IL-17 production may permit resolution of the persistent inflammation. To test this hypothesis, we treated colitic IL-10−/− mice with blocking anti-IL-17A mAb (Fig. 4B). IL-17 blockade significantly reduced inflammation in IL-10−/− mice with established colitis.

Discussion

IBD and other pathologic inflammations such as MS and asthma are associated with elevated IL-17 expression (19, 20, 23–26). IL-17 expression is increased in TNBS colitis (21) and in RAG-deficient mice rendered colitic by reconstitution with IL-10−/− CD4+ T cells and exogenous IL-23 treatment (22). IL-10−/− mice develop colitis spontaneously (32), and an identical colitis can be initiated in these mice by brief treatment with piroxicam, with the colitis persisting after piroxicam is discontinued (31). We found that mucosal IL-17 production is dramatically increased (~10-fold) in colitic IL-10−/− mice as compared with age-matched, noncolitic mice. Lamina propria T cells are the source of this IL-17 (data not shown). Blockade of IL-17 with anti-IL-17 mAb improves established colitis in this model. This extends previous observations that abrogation of IL-17 prevents initial development of colitis in the TNBS (21) and RAG reconstitution (22) models, and shows that maintenance of colitis requires ongoing IL-17 signaling.

Dysregulated immune responses produce pathologic inflammation like that of IBD. Immune-mediated diseases such as IBD, MS, and asthma have become prevalent in highly developed industrialized countries, but remain rare in less developed nations (3). This suggests that an environmental factor absent in highly industrialized countries protects individuals in the population from developing immune-mediated disease. One such environmental factor may be the absence of exposure to parasitic worms (2). Helminth carriage was previously universal (33) and has become rare in industrialized countries due to sewage treatment, highly regulated food industries, cement side walks, and other modern hygienic innovations.

Previously, we showed that colonization of colitic IL-10−/− mice with *H. polygyrus* results in resolution of the intestinal inflammation (12). Exposure to helminths reduces inflammation in TNBS-type colitis (2, 6, 8, 9) and in murine models of MS (13, 14) and asthma (15, 16). These inflammations are associated with increased IL-17 production. Therefore, we investigated whether colonization with *H. polygyrus* inhibits mucosal IL-17 expression in wild-type and IL-10−/− mice.

We found that colonization with *H. polygyrus* resulted in a 16-fold decrease in MLN cell IL-17 mRNA expression as compared with that of helminth naive mice. This experiment evaluated transcripts from freshly isolated and otherwise unstimulated lymphocytes and reflects the baseline cytokine skewing of the colonized animal. Unstimulated MLN cells from either colonized or naive mice did not release IL-17 in culture. However, when stimulated with anti-CD3 mAb, MLN cells do release IL-17, and mice harboring *H. polygyrus* showed a 3-fold decrease in production of this cytokine as measured by ELISA. This suggested that IL-17-producing T cells remained present in the MLN population of helminth-colonized mice, and could be recruited to express IL-17, but not to the level shown by cells from naive animals. Flow cytometric analysis of PMA- and ionomycin-stimulated CD4+ T cells showed that the frequency of IL-17-expressing cells (Th17) was decreased by nearly 50% in the MLN population of *H. polygyrus*-colonized mice.

Intestinal LPMC produced more IL-17 than did similarly cultured MLN cells. Unlike MLN cells, LPMC from helminth naive wild-type mice made IL-17 without additional stimulation. This unstimulated production was abrogated in LPMC from helminth-colonized mice. Treatment with anti-CD3 and anti-CD28 mAb augmented LPMC IL-17 production. Like MLN cells, anti-CD3-stimulated LPMC from *H. polygyrus*-exposed mice produced about one-third the amount of IL-17 as that made by lymphocytes from helminth naive mice. This reduction in capacity to make IL-17 could make the intestinal mucosa more resistant to pathologic inflammation.

Helminths induce MLN cell and LPMC IL-10 production in their hosts, which could regulate IL-17 production. Previously, we found that blocking IL-10 reversed helminth-inhibited IL-12/23p40 and IFN-γ release (10). Therefore, we tested whether in situ IL-10 production mediated the suppression of IL-17 production in cultured MLN cells from helminth-colonized mice. Unlike IFN-γ, IL-17 production was not enhanced by blocking IL-10 signaling. This suggests that helminthic modulation of IL-17 production occurs through a mechanism distinct from that of IFN-γ in wild-type mice.

Helminths induce strong Th2 responses typified by IL-4 production. IL-4 can inhibit IL-17 production by memory T cells (30). Therefore, we tested whether in situ IL-4 production mediated the suppression of MLN cell IL-17 production after helminth exposure. IL-4 blockade partially restored IL-17 production by MLN cells from helminth-colonized mice. Thus, IL-17 suppression due to Th2 skewing may be a mechanism of helminth-mediated protection from IL-17-driven inflammation. Blockade of IL-4 did not completely restore IL-17 production. Addition of anti-IL-10R Ab synergized with anti-IL-4 Ab to permit enhanced IL-17 release. Thus, helminth-induced IL-4 and IL-10 function in concert to maximally inhibit IL-17.

Blockade of IL-10 alone did not permit IL-17 production by MLN cells from colonized wild-type mice. Thus, IL-10 is not necessary or sufficient in itself to regulate IL-17 secretion. Consistent
with this observation, helminth exposure suppressed IL-17 production by MLN and LPMC from noncolitic IL-10−/− mice. Helminth exposure reverses established IL-10−/− colitis (12). The resolution in colitis is associated with a nearly 10-fold decrease in LPMC IL-17 production. The reduction in IL-17 production was not due to the presence of fewer T cells in culture because LPMC were cultured at the same number of cells/well whether isolated from colitic or noncolitic mice. Improvement in inflammation could result in less IL-17 production. In the absence of colitis, helminth colonization suppresses lamina propria cell IL-17 production in wild-type and IL-10−/− mice. Furthermore, we and others (22) find that blockade of IL-17 with mAb suppresses colitis. This suggests that helminth-associated inhibition of mucosal IL-17 contributes to resolution of colitis.

This is the first report that colonization with an intestinal helminth results in inhibition of IL-17 production and a decrease in Th17 cells. We measured IL-17 production by LPMC and MLN cells from mice actively colonized with H. polygyrus. These studies do not address whether mucosal IL-17 production remains suppressed after colonization has resolved. These results suggest that suppression of T cell IL-17 release is one of the mechanisms that protect helminth-colonized mice from inappropriate inflammation and reverses ongoing intestinal inflammation (12). IL-4 in conjunction with IL-10 are possible important helminth-induced regulatory cytokines for IL-17.

Disclosures
The authors have no financial conflict of interest.

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