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Heligmosomoides polygyrus Infection Can Inhibit Colitis through Direct Interaction with Innate Immunity

Long Hang,* Tommy Setiawan,* Arthur M. Blum,* Joseph Urban,† Korynn Stoyanoff,* Seiji Arihiro,‡ Hans-Christian Reinecker,‡ and Joel V. Weinstock*

Less developed countries have a low incidence of immunological diseases like inflammatory bowel disease (IBD), perhaps prevented by the high prevalence of helminth infections in their populations. In the Rag IL-10−/− T cell transfer model of colitis, Heligmosomoides polygyrus, an intestinal helminth, prevents and reverses intestinal inflammation. This model of colitis was used to explore the importance of innate immunity in H. polygyrus protection from IBD. Rag mice briefly exposed to H. polygyrus before reconstitution with IL-10−/− colitogenic T cells are protected from colitis. Exposure to H. polygyrus before introduction of IL-10−/− and OT2 T cells reduced the capacity of the intestinal mucosa to make IFN-γ and IL-17 after either anti-CD3 mAb or OVA stimulation. This depressed cytokine response was evident even in the absence of colitis, suggesting that the downmodulation in proinflammatory cytokine secretion was not just secondary to improvement in intestinal inflammation. Following H. polygyrus infection, dendritic cells (DCs) from the lamina propria of Rag mice displayed decreased expression of CD80 and CD86, and heightened expression of plasmacytoid dendritic cell Ag-1 and CD40. They were also less responsive to lamina propria, producing less IL-12p40 and IL-10. Also diminished was their capacity to present OVA to OT2 T cells. These experiments infer that H. polygyrus does not require direct interactions with T or B cells to render animals resistant to colitis. DCs have an important role in driving both murine and human IBD. Data suggest that phenotypic alternations in mucosal DC function are part of the regulatory process.

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Abbreviations used in this paper: DC, dendritic cell; IBD, inflammatory bowel disease; KO, knockout; LP, lamina propria; LPMC, LP mononuclear cell; PDCA, plasmacytoid dendritic cell; Ag, T cell, terminal ileum.

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Inflammatory bowel disease (IBD) and other immune-mediated illnesses are rare in tropical, less-developed countries. Helminth infections common in such countries may prevent IBD (1). Various helminth species used in several animal models of intestinal inflammation can avert or limit disease activity. For instance, rodents receiving nonviable schistosome OVA (2) or intestinal helminths like Trichuris muris (3), Trichinella spiralis (4), Heligmosomoides polygyrus, or Hymenolepis diminuta (5) are protected from trinitrobenzene sulfonic acid-induced colitis. T. muris or H. polygyrus infection, or schistosome OVA exposure prevents or reverses the chronic Th1-type colitis of IL-10−/− (IL-10−/−) mice (3, 6).

At least part of the protective process involves induction of regulatory T cells and cytokines in the host. H. polygyrus is a murine intestinal helminth. IL-10−/− mice develop colitis spontaneously. T cells from the mesenteric lymph nodes of H. polygyrus-infected IL-10−/− mice abrogate established colitis when transferred into IL-10−/− recipients (6). Helminth colonization induces FoxP3 expression in mesenteric lymph nodes and lamina propria (LP) T cells. In a Rag-transfer colitis model of IBD, H. polygyrus required CD8+ T cells in vivo to reverse the disease process (7). H. polygyrus infection also elicits a regulatory T cell population able to down-regulate allergic-induced lung pathology (8). Also, after H. polygyrus infection, LP T cells from healthy wild-type mice make large amounts of regulatory cytokines like IL-10 and TGF-β (9).

Interactions with cells of the innate immune system could be part of the protective process. For instance, schistosomes protect BALB/c mice from dextran sulfate sodium enteritis via a macrophage-dependent mechanism not requiring regulatory T cells (10). Protection in animal models of asthma may involve alternatively activated macrophages (11).

We used a Rag IL-10−/− T cell transfer murine model of IBD to further explore the importance of innate immunity in H. polygyrus protection from IBD. This investigation showed that direct interaction alone with the innate immune system is sufficient to allow H. polygyrus to impede intestinal Ag-specific responses and to protect mice from colitis. Data suggest that changes in dendritic cell (DC) function contribute to this regulatory process.

Materials and Methods

Mice

This study used C57BL/6 Rag2, OT2, and IL-10−/− mice (The Jackson Laboratory, Bar Harbor, ME). Breeding colonies were maintained in specific pathogen-free facilities at Tufts University. Animals were housed and handled following national guidelines and as approved by our Animal Review Committee.

Colitis model

Rag mice of similar age were reconstituted with 106 IL-10−/− splenic T cells and 3 × 107 OT2 splenic T cells given i.p. One week later, the animals were administered piroxicam (Sigma-Aldrich, St. Louis, MO) mixed into their feed for 2 wk (piroxicam at 40 mg/250 g chow, week 1; 60 mg/250 g chow, week 2) to induce colitis. The piroxicam was then stopped. Two weeks later, the mice were sacrificed; their colons were examined microscopically for colitis; and LP mononuclear cells (LPMCs) were isolated for culture (Fig. 1).
H. polygyrus infection

Infective, ensheathed H. polygyrus L3 (U.S. National Helminthological Collection 81930) were obtained from fecal cultures of eggs by the modified Baermann method and stored at 4°C. Mice were colonized with 125 H. polygyrus third-stage larvae by oral gavage.

For some experiments, animals were infected for 2 wk with H. polygyrus after induction of colitis (end of piroxicam treatment) and then sacrificed to assess colitis severity (Fig. 1). In other experiments, Rag mice first were infected with H. polygyrus or just received a sham infection for 2 wk, and then both the infected and control groups received a single dose of pyrantel pamoate (0.5 mg/mouse; Sigma-Aldrich) via oral gavage to eliminate H. polygyrus. One week after deworming, the mice were reconstituted with T cells, and some were treated with piroxicam, as described above, to induce colitis, whereas others were observed without piroxicam treatment. In separate control experiments, deworming was confirmed by documenting the absence of adult H. polygyrus in the small bowel 1 wk after receiving the drug.

Dispersion of splenocytes, and splenic T cell enrichment

Single-cell suspensions of splenocytes were prepared by gentle teasing in RPMI 1640 medium (Life Technologies, Grand Island, NY). The cells were washed three times in RPMI 1640. Splenic T cells or CD4+ T cells were isolated by negative selection using the EasySep mouse T cell enrichment kit, as outlined by the manufacturer (19751; StemCell Technologies, Vancouver, Canada). Viability was determined using exclusion of trypan blue dye.

LPMC isolation and LP cell fractionation

Gut LPMCs were isolated from the terminal ileum (TI), as described (6). Cell viability was 90%, as determined by trypan blue exclusion. LP DCs (CD11c+), macrophages (F4/80+), and NK cells (DX5+) were isolated from dispersed LPMCs using appropriate mAbs and FACs. Cells were surface stained with fluorochrome-labeled anti-CD11c, anti-F4/80, and/or anti-DX5 mAb before sorting (see below).

Cell culture

LPMCs from T cell-reconstituted Rag mice were cultured (2 × 10⁵ cells/well) for 48 h in 96-well round-bottomed plates. Cells were cultured along with OVA (50 μg/ml; Sigma-Aldrich) or anti-CD3 mAb (2C11; American Type Culture Collection, Manassas, VA) and anti-CD28 mAb (BD Pharmingen, San Diego, CA), each at 0.5 μg/ml. 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, Gaithersburg, MD). After culture, the supernatants were assayed for IFN-γ and IL-17A using ELISA (described below).

In the Rag LPMC/OT2 T cell mix experiments, OT2 Thy1.2 splenic T cells were mixed with LPMCs from Rag mice at the ratio of 1:3. Cells (2 × 10⁵) were cultured in RPMI 1640 complete medium for 48 h. Some cultures contained OVA at up to 1000 μg/ml to stimulate cytokine release. Supernatants were assayed for IFN-γ and IL-17 after the incubation using ELISA.

In some experiments, OT2 Thy1.2 T cells (1.5 × 10⁵) were mixed with isolated CD11c+ F4/80+, and/or DX5+ LPMCs (~4 × 10³ of each) from Rag mice cultured 48 h, as above, with up to 50 μg OVA before the supernatants were assayed for IFN-γ and IL-17.

In other experiments, isolated CD11c+ LP DCs were cultured for 48 h alone with or without 100 ng/ml highly purified LPS derived from Escherichia coli 026:B6 (Sigma-Aldrich), or mixed with OT2 T cells and stimulated with OVA (50 μg/ml). Supernatants were assayed for IL-12p40, IL-10, and TGF-β using ELISA. Cells were again reconstituted in RPMI 1640 complete medium except for TGF-β determinations in which the cultures contained 1%, rather than 10%, FCS with 1 mg/ml albumin (AMRESCO, Solon, OH).

Sandwich ELISAs

ELISAs were performed using paired Abs (R&D Systems, Minneapolis, MN), according to manufacturer’s instructions. IL-17 ELISA was done using primary capture Ab from (R&D Systems) and biotinylated anti-IL-17A Ab (R&D Systems). IL-12 (p40) ELISA was performed using primary capture Ab (BD Pharmingen, Franklin Lakes, NJ) and biotinylated secondary Ab (BD Pharmingen). IL-10 was captured with anti-IL-10 mAb (R&D Systems) and detected with biotinylated mAb (R&D Systems). To measure IFN-γ, plates were coated with a mAb to IFN-γ (HB170; American Type Culture Collection) and incubated with supernatant. IFN-γ was detected with polyclonal rabbit anti–IFN-γ (gift from Dr. M. Wilson, University of Iowa, Iowa City, IA), followed by biotinylated goat anti-rabbit IgG (Axell, Westbury, NY). Total TGF-β was measured using acid-treated supernatant and mAb240 for capture and biotinylated chicken IgY BAF240 for detection (both R&D Systems).

Flow cytometry analysis

LPMCs were washed twice, adjusted to 10⁶ cells/ml in FACS buffer (lymphocyte growth medium), and stained with saturating amounts of conjugated mAbs for 30 min at 4°C. Following staining, cells were washed twice and resuspended in lymphocyte growth medium for analysis on a FACScanCalibur using CellQuest software (BD Biosciences, Mountain View, CA). Before adding labeled mAb, each tube received 1 μg anti-Flc mAb (eBioscience, San Diego, CA) to block nonspecific binding of conjugated Abs to FeRs. The mAbs used for staining or cell sorting were anti-Thy-1.2-FITC, or -PECy5, or -allophycocyanin; anti-CD11c-PE-Cy7; anti-F4/80-PE-Cy5; anti-CD4-PE or –PE-Cy5; anti–DX5-PE (all from eBioscience).

Statistical analysis

Data are means ± SE of multiple determinations. Difference between two groups was compared using Student t test. Multiple group comparisons used analysis of variation and Dunnent r test. The p values <0.05 were considered significant.

Results

Development of an IBD model in which gut LPMCs respond to OVA Ag

Rag mice reconstituted with IL-10−/− T cells develop severe colitis after piroxicam exposure (12). Intestinal luminal Ags that drive LP T cells are poorly defined. To permit the study of Ag-specific responses in the intestine LP, we reconstitute Rag mice with IL-10−/− T cells mixed with OT2 T cells bearing MHC class II-dependent TCR that recognize OVA. These mice were highly susceptible to colitis upon piroxicam administration. LPMCs isolated from such mice cultured with OVA in vitro released large amounts of IFN-γ and IL-17, which were secreted minimally in the absence of OVA (Fig. 2). LPMCs from mice reconstituted only with IL-10−/− T cells did not respond to OVA, suggesting that the cytokine response was OT2 T cell dependent.

H. polygyrus infection stops colitis and reduces the LPMC cytokine response to OVA

H. polygyrus can reverse colitis in Rag mice reconstituted with IL-10−/− T cells (6, 13). Using the Rag IL-10 knockout (KO)/OT2 T cell reconstitution model of IBD, we determined whether infection with H. polygyrus after induction of colitis could suppress intestinal inflammation and decrease cytokine release from LPMCs isolated from these mice. Fig. 3 shows that H. polygyrus markedly suppressed the intestinal inflammation and that isolate LPMCs from H. polygyrus-infected animals produced much less IFN-γ and IL-17 after OVA stimulation compared with the sham-infected controls. No IL-4 was detected.

Additional experiments reconstituted Rag mice with IL-10 KO and OT2 T cells, as above, but 1 wk later, the animals were infected with H. polygyrus and never given piroxicam to induce colitis. Two weeks postinfection, LPMCs isolated from the TI and cultured as in Fig. 3 also made much less IFN-γ and IL-17 after OVA stimulation compared with the sham-infected controls. No IL-4 was detected.

Rag mice exposed to H. polygyrus only before T cell reconstitution are protected from colitis

Immune effector and regulatory pathways of both innate and adaptive immunity help drive colitis. Experiments explored whether H. polygyrus interacting exclusively with cells of the innate immune system would suffice to control disease. Rag mice devoid of func-
H. polygyrus exposure before introduction of T cells was sufficient to block inflammation and to suppress both anti-CD3/CD28 mAb- and OVA-induced cytokine responses from the LPMCs. Once more, there was no detectable IL-4.

Additional experiments determined whether the reduced LPMC cytokine response to OVA was only secondary to improvement in inflammation. Following a brief infection with H. polygyrus and reconstitution of adaptive immunity, as described above, mice were maintained for 3 wk, but they were not given piroxicam to induce colitis. LPMCs isolated from these animals made substantially less IFN-γ and IL-17 and no IL-4 after either OVA or anti-CD3/CD28 mAb stimulation in vitro compared with the uninfected controls (Fig. 5).

In Rag mice without T cell reconstitution, H. polygyrus exposure alters intestinal DC function, cell surface protein expression, and cytokine secretion

In Rag mice without T cell reconstitution, H. polygyrus exposure alters intestinal DC function, cell surface protein expression, and cytokine secretion. It was assumed that H. polygyrus interactions with cellular components of innate immunity caused the above described protection from colitis and alterations in cytokine production, to test this hypothesis, Rag mice were infected with H. polygyrus for 2 wk. After 2 wk of infection, LPMCs isolated from Rag mice were mixed with splenic OT2 T cells and then cultured in vitro with or without various concentrations of OVA to stimulate cytokine release. After incubation for 48 h, the supernatants were assayed for IFN-γ and IL-17 content. LPMCs isolated from age-matched Rag mice that never had H. polygyrus infection served as controls. Fig. 6 shows that LPMCs from Rag mice with previous H. polygyrus infection supported OVA-induced IFN-γ production poorly. IL-17 secretion was minimally affected. LPMCs cultured without OT2 T cells and OT2 T cells cultured without LPMCs released no detectable IFN-γ or IL-17 upon OVA or LPS stimulation. IL-4 was not detected in any cultures.

The alteration noted in the supportive role of isolated LPMCs in the OT2 T cell IFN-γ response could have reflected H. polygyrus-induced changes in the cellular composition of the isolated, dispersed LPMCs. Flow analysis of the isolated LPMCs from H. polygyrus-infected and control mice revealed no significant alterations in the composition of DCs (CD11c<sup>-</sup>), macrophages (F4/80<sup>+</sup>), or NK cells (DX5<sup>+</sup>) (Fig. 7).

Additional experiments explored the potential role of these three cell types in modulating the OT2 IFN-γ response. Using FACS, CD11c<sup>+</sup> DCs were isolated from the dispersed LPMCs from Rag mice previously exposed to H. polygyrus or from mice that never received this infection. CD11c<sup>+</sup> DCs were mixed with OT2 T cells and then cultured with OVA for 48 h. CD11c<sup>+</sup> intestinal DCs from mice previously infected with H. polygyrus were less proficient at supporting the IFN-γ response compared with DCs from the controls, whereas there was no change in IL-17 secretion (Fig. 8). F4/80<sup>+</sup> and DX5<sup>+</sup> LP cells from either H. polygyrus-infected or control Rag mice mixed with OT2 T cells individually or in combination did not support OVA-induced IFN-γ or IL-17 secretion. Adding F4/80<sup>+</sup> macrophages or DX5<sup>+</sup> NK cells to the CD11c<sup>+</sup> LP DC/OT2 T cell mix did not affect OVA-induced IFN-γ or IL-17 secretion either.

DCs support or modulate T cell responses by cell-to-cell contact and through secretion of cytokines. Flow cytometric analysis was used to study cell surface proteins associated with DC function. We examined CD11b, MHCII, CD8a, CD103, CD80, CD86, plasmacytoid dendritic cell Ag (PDCA)-1, and CD40. DCs from the TI of Rag mice exposed to H. polygyrus, compared with DCs from control Rag animals, displayed a relative decrease in surface expression of CD80 and CD86 with enhanced expression of PDCA-1 and CD40 (Fig. 9). We also examined CD11c<sup>+</sup> DCs in LPMCs isolated from the colon. These cells showed a similar pattern of changes in cell surface protein expression. DCs can produce various cytokines, such as IL-12, IL-10, and TGF-β, that can stimulate, modulate, and/or inhibit T cell development and secretion. To see whether H. polygyrus exposure altered the capacity of intestinal CD11c<sup>+</sup> DCs to produce cytokines, DCs from Rag mice previously infected with H. polygyrus or DCs from control animals that never experienced this infection were cultured alone or with LPS for 48 h to stimulate cytokine release. DCs isolated

![FIGURE 2](http://www.jimmunol.org/) Rag mice were reconstituted with IL-10<sup>−/−</sup> T cells (10<sup>7</sup>) or IL-10<sup>−/−</sup> T cells (10<sup>5</sup>) and OT2 T cells (3 × 10<sup>5</sup>) given i.p. One week later, piroxicam was mixed in their chow and administered for 2 wk to induce colitis. Two weeks after stopping the piroxicam, LPMCs were isolated for culture. Cultures contained 2 × 10<sup>5</sup> LPMCs/well maintained for 6 h in vitro with or without OVA (50 μg/ml). ELISAs measured IL-17 and IFN-γ. Data are mean ± SE from three independent experiments. OT2 + OVA versus all other comparisons, p < 0.01.

![FIGURE 3](http://www.jimmunol.org/) H. polygyrus infection (125 larvae) reversed colitis, and LPMCs isolated from the TI of H. polygyrus-infected mice released less IFN-γ and IL-17 upon OVA stimulation (50 μg/ml). Rag mice were reconstituted with 10<sup>6</sup> IL-10<sup>−/−</sup> KO T cells and 3 × 10<sup>5</sup> OT2 T cells given i.p. One week later, piroxicam was mixed in their chow and administered for 2 wk to induce colitis. After stopping the piroxicam, the treatment group was infected with H. polygyrus. The control group received no infection (Fig. 1). Two weeks later, the colons were examined microscopically for colitis, and LPMCs were isolated for culture. Cultures contained 2 × 10<sup>5</sup> LPMCs/well maintained for 48 h in vitro with or without OVA (50 μg/ml). ELISAs measured IL-17 and IFN-γ. LPMCs made little or no IFN-γ or IL-17 without OVA stimulation (<50 pg). Histology was scored blinded on a 4-point scale. Original magnification ×40. All data are mean ± SE from three independent experiments. Control versus H. polygyrus, p < 0.01.
from mice previously exposed to *H. polygyrus* released less IL-12p40 and IL-10 in response to LPS than did comparable DCs isolated from the control animals (Table I). In other cultures, the DCs from either *H. polygyrus*-exposed or unexposed Rag mice were mixed with OT2 cells and then cultured with OVA (100 μg/ml). After OVA stimulation, supernatants from cultures containing *H. polygyrus*-exposed DCs also contained less IL-10 and IL-12p40. In all experiments, cellular supernatants did not contain measurable amounts of TGF-β.

**Discussion**

The most important observation of this study is that Rag mice briefly exposed to *H. polygyrus* before reconstitution with IL-10−/− colitogenic T cells are protected from colitis. Moreover, the protection is as profound as that seen in Rag mice exposed to *H. polygyrus* after T cell reconstitution. T cells drive the pathology in this animal model of IBD (12). The data presented in this study suggest that *H. polygyrus* regulates the pathogenic T cell response that causes IBD via direct interaction with cellular components of the innate immune system requiring no direct interface with T cells.

The intestinal mucosal immune system responds to many molecules within the gut. It is difficult to study the control of T cell responses to intraluminal Ags in the LP because a multitude of poorly defined Ags shapes mucosal immunity. To overcome this limitation, we injected OT2 transgenic T cells, which recognize OVA in a class II-dependent fashion, into the IL-10−/− murine model of IBD. When introduced at the time of IL-10−/− T cell transfer, OT2 T cells appear in the LP, and isolated LPMCs cultured in vitro with OVA produce large amounts of IFN-γ and IL-17. IFN-γ and IL-17 are proinflammatory cytokines incriminated in driving colitis in both human and many murine models of IBD (13).

Exposure to *H. polygyrus* before introduction of IL-10−/− and OT2 T cells reduced the capacity of the intestinal mucosa to make IFN-γ and IL-17 after either anti-CD3 mAb or OVA stimulation. This depressed cytokine response was evident even in the absence of colitis, suggesting that downmodulation in proinflammatory cytokine secretion was not just secondary to improvement in intestinal inflammation.

It was assuming that innate immunity, influenced by *H. polygyrus* exposure, rendered mice resistant to colitis and the LP less prone to secrete colitogenic cytokines like IFN-γ and IL-17. The dispersed LPMCs from these mice were comprised mostly of DCs, macrophages, and NK cells, which were the focus of this study. *H. polygyrus* infection did not alter the relative proportion of these three cell subsets in the dispersed LPMC preparations. Our in vitro studies using various combinations of these three cell subsets revealed that DCs poorly supported OVA-specific IFN-γ production after *H. polygyrus* exposure. Neither the macrophages nor the NK cells had a substantial effect on this Ag-specific response.

DCs within the intestines function to limit local immune responses to the mostly harmless luminal Ags (14). Disruption of DC function may be one of the factors promoting IBD. The aim of this study was not to categorize all the various DC subsets expressed in the gut after *H. polygyrus* infection, but to determine whether *H. polygyrus* exposure substantially affected the phenotypic and functional expression of intestinal DCs in Rag mice expressing no functional T or B cells. We examined the state of DC activation after 2 wk of infection because the Rag mice were dewormed at that time point.
*H. polygyrus* infection had a prominent effect on the capacity of DCs to display various surface proteins and to secrete cytokines. There was decreased expression of the costimulatory molecules CD80 and CD86 associated with a decrease in IL-12p40 secretion. These alterations in DC function could explain why the intestinal DCs isolated from mice with *H. polygyrus* infection were less efficient at presenting OVA to OT2 T cells, stimulating IFN-γ production. IL-10 suppresses IL-12 secretion, depresses Th1 cell function, and has other immunoregulatory properties important for maintaining mucosal immune homeostasis (15). *H. polygyrus* exposure actually decreased, rather than increased, the capacity of the DC population to produce IL-10, suggesting that IL-10 did not participate in the downregulation of DC CD80/86 expression, IL-12 secretion, or OT2 stimulation. Soluble TGF-β, another important mucosal immunoregulatory cytokine (15), and IL-4 were not detected within culture supernatants, suggesting that these cytokines were not critical for this regulation. Our study did not rule out the possible participation of cell surface-bound TGF-β in the regulatory process.

PDCA-1 is a marker of plasmacytoid DCs and was more widely expressed on the intestinal DCs after *H. polygyrus* infection. Activation of T cells by plasmacytoid versus conventional DCs appears to favor generation of regulatory-type T cells (16). Thus, the appearance of more intestinal DCs expressing PDCA-1 after *H. polygyrus* colonization could be of regulatory significance.

It is notable that *H. polygyrus* exposure did not affect the overall level of MHC class II expression, which is centrally important for Ag-induced OT2 cell activation. Also notable was the failure of *H. polygyrus* infection to alter the number of DCs displaying CD8, CD11b, and CD103, which are markers of intestinal DC subsets reported to influence Th and regulatory T polarization (14).

Expression of CD40 on DCs increased after *H. polygyrus* colonization. DCs express CD40 upon activation following interactions with some pathogens and microbial products (17). Some data suggest that the expression of CD40 on DC subsets is more dependent on the nature of the stimulus rather than on the phenotype of the DCs. Ligation of CD40 can result in enhanced expression of costimulatory molecules and proinflammatory molecules like IL-12 and has been implicated in DC-mediated induction of effector T cell responses (18). The physiological significance of *H. polygyrus* induction of CD40 on the intestinal DCs remains obscure.

It is assumed, although not yet proven, that changes in DC function lead to the protection. It still remains possible, although less likely, that the intestinal DCs assimilated *H. polygyrus* Ags after the infection and that this retained Ag drove the protection.

*H. polygyrus* mostly inhabits the proximal small intestine in our Rag mice. We evaluated the function of DCs from the distal TI. It remains unknown how *H. polygyrus* communicated with the DCs in distal regions of the intestine. DCs can extend dendrites across

**Table I.** LP DCs from *H. polygyrus*-infected mice make less IL-12p40 and IL-10

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<tr>
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<th>IL-12 p40</th>
<th>IL-10</th>
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<td></td>
<td>No <em>Hp</em></td>
<td><em>Hp</em></td>
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<tr>
<td>CD11c</td>
<td>27 ± 0.3</td>
<td>16 ± 0.7</td>
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<tr>
<td>CD11c + LPS</td>
<td>259 ± 21</td>
<td>147 ± 3.3</td>
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Rag mice were colonized with *H. polygyrus* for 2 wk, whereas control mice received only a sham infection. Isolated DCs (4 × 10⁵) were cultured in vitro for 2 d with or without LPS (100 ng/ml) to stimulate IL-12p40 and IL-10 production. Data are mean ± SE, n = 3.
References


Disclosures

The authors have no financial conflicts of interest.

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