Induction of CD8\(^+\) regulatory T cells in the intestine by Heligmosomoides polygyrus infection

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Metwali, Ahmed, Tommy Setiawan, Arthur M. Blum, Joseph Urban, David E. Elliott, Long Hang, and Joel V. Weinstock. Induction of CD8\(^+\) regulatory T cells in the intestine by Heligmosomoides polygyrus infection. Am J Physiol Gastrointest Liver Physiol 291: G253–G259, 2006; doi:10.1152/ajpgi.00409.2005.—This study determined whether Heligmosomoides polygyrus induces intestinal regulatory T cells. Splenic T cells proliferate strongly when cultured with anti-CD3 and antigen-presenting cells (APC). Lamina propria T cells from mice with H. polygyrus infection, lamina propria T cells from mice without H. polygyrus only modestly affected T cell proliferation. The worm-induced regulatory T cell was CD8\(^+\) and required splenic T cell contact to inhibit proliferation. The regulation also was IL-10 independent, but TAP-dependent, suggesting that it requires major histocompatibility complex (MHC) class I interaction. Additional studies employed mice with transgenic T cells that did not express functional TGF-\(\beta\) receptors. The lamina propria T regulator inhibited proliferation of these transgenic T cells nearly 100%, suggesting that TGF-\(\beta\) signaling via the T cell was not required. CD8\(^+\) T cells were needed for worms to reverse piroxicam-induced colitis in Rag mice (T and B cell deficient) reconstituted with IL-10-/- T cells. Thus H. polygyrus induces a regulatory CD8\(^+\) lamina propria T cell that inhibits T cell proliferation and that appears to have a role in control of colitis. 

inflammatory bowel disease; helminths; T cells; lamina propria 

HELMINTH INFECTION COULD BE a protective factor against development of inflammatory bowel disease (IBD). The prevalence of IBD is highest in industrialized nations where helminthic colonization is common (32). There also have been favorable clinical trials using helminths to treat human IBD (5, 28, 29). Animal models of IBD also suggest that helminths prevent the disease. Rodents treated with nonviable schistosome ova (6) and intestinal worms like Trichiura muris (8), Trichinella spiralis (14), Heligmosomoides polygyrus, or Hymenolepis diminuta (25) are protected from trinitrobenzene sulfonic acid (TNBS)-induced colitis. Mice deficient in IL-10 production develop a chronic Th1-type colitis that is prevented or reversed by colonization with T. muris or H. polygyrus (7, 8) or by exposure to nonviable schistosome ova.

Helminths exert a strong influence on the host immune system. Studies in humans suggest that exposure to various helminths results in decreased T cell signal transduction, lower expression of CD28 with increased expression of CTLA4, reduced proliferation to recall antigens/mitogens, decreased DTH responses (4), and raised IL-10 levels (2). In mice, the Th2 response to helminths can deviate Th1 antigenic immunity toward Th2 (15, 24, 27). People carrying helminths also can show immune bias away from the Th1 response normally elicited with tetanus vaccination (26) or in vitro mitogen stimulation (2). However, there can be concomitant dampening of Th2-type reactivity (1, 17, 31) and reduced lymphocyte responsiveness to various antigens and mitogens, suggesting that the effects of helminths on the immune system are not simply a change in the balance between Th1 and Th2.

Helminths may induce production of regulatory T cells in their host. T cells from the mesenteric lymph node (MLN) of H. polygyrus-colonized IL-10-deficient mice, unlike MLN cells from their worm-free IL-10 littermates, abrogate established colitis when transferred into IL-10-deficient recipients (7). Also, after H. polygyrus infection, lamina propria (LP) T cells from healthy wild-type (WT) mice make large amounts of regulatory cytokines like IL-10 and TGF-\(\beta\).

To extend these observations, this study determined whether H. polygyrus induces a functional regulatory T cell in the intestinal mucosa of mice that was likely to influence susceptibility to colitis. It was found that H. polygyrus enhanced expression of a CD8\(^+\) regulatory T cell in the intestine that blocks splenic T cell proliferation in a major histocompatibility complex (MHC) class I dependent fashion. The function of this regulatory cell required cell contact but did not need IL-10 or TGF-\(\beta\) signaling through the T cell. In a Rag-transfer colitis model of IBD, H. polygyrus required CD8\(^+\) T cells in vivo to reverse the disease process.

MATERIALS AND METHODS

Mice and H. polygyrus or Trichuris muris infection. This study used C57BL/6 WT (Jackson Laboratory, Bar Harbor, ME), IL-10-/-, TAP-/-, or Rag (T and B cell deficient) mice. Also used were transgenic C57BL/6 mouse expressing T cell-specific, dominant-negative TGF-\(\beta\) receptor type II (TGF-\(\beta\)-RII), rendering the cells unresponsive to TGF-\(\beta\) (provided by R. A. Flavell, Yale University). Breeding colonies for the mutant animals were maintained in specific pathogen-free facilities at the University of Iowa or at Tufts New England Medical Center.

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For most experiments, mice about 5 wk of age were colonized with 200 H. polygyrus third-stage larvae or with 150 T. muris ova by oral gavage. Infective, ensheathed H. polygyrus L3 (U.S. National Helminthological Collection, no. 81,930) were obtained (from fecal cultures of eggs) by the modified Baermann method and stored at 4°C. WT mice were studied after 2 wk of infection. Animals were housed and handled following national guidelines and as approved by our Animal Review Committee.

**Rag-transfer IBD model of IBD.** Each Rag mice (~5-wk-old) received either 4 x 10^6 unfractionated splenic T cells (Thy1.2+), 3.5 x 10^6 CD4+ T cells, or 3.5 x 10^6 CD4+ and 2 x 10^6 CD8+ T cells by intraperitoneal injection. One week later, animals received piroxicam (Sigma, St. Louis, MO) mixed in their food (NIH-31M) for 2 wk. They received 40 mg/250 g body wt of food during week 1 and 60 mg/250 g body wt food during week 2. Mice, subsequently, were placed on the normal rodent chow without piroxicam and, in some cases, colonized with H. polygyrus as described above. The colitis was evaluated 14 days after stopping the piroxicam.

**Histological analysis of colitis.** Colon sections (from the ileocecal valve to the mid descending colon) were obtained longitudinally and rolled up onto a glass rod. The tissue was fixed in 4% neutral-buffered formalin, removed from the glass rods without unrolling the tissue, and processed for sectioning. Tissue was sliced to obtain longitudinal sections of colon that were 6-μm-thick and then stained with hematoxylin and eosin for light microscopic examination. The inflammation was scored from 0–4 using the following criteria: grade 0, No change from normal tissue; grade 1, patchy mononuclear cell infiltrates in the LP; grade 2, more uniform mononuclear cell infiltration involving both the epithelium and LP; this was accompanied by minimal epithelial hyperplasia and slight-to-no depletion of mucus from goblet cells; grade 3, some epithelial and muscle hypertrophy with patchy lymphocytic infiltrates extending into the muscle layers; there was mucus depletion and occasional crypt abscesses and epithelial erosions; grade 4, lesions involved most of the intestinal section; the inflammation, which was composed mostly of lymphocytes and some neutrophils, was transmural and severe, and there was prominent thickening of both the epithelial and muscle layers, and there was mucus depletion and more frequent crypt abscesses. Ulcerations were frequent.

**Lamina propria mononuclear cell isolation.** Gut lamina propria mononuclear cells (LPMC) were isolated as described (7). Cell viability was 90% as determined by eosin Y exclusion.

**LP T cell enrichment.** LP T cells (Thy1.2+) were isolated by positive selection using antibody-coated, paramagnetic beads as described by the manufacturer (Dynal, New Hyde Park, NY). Flow cytometry was used after each separation to assure appropriate recovery and purity (>98%) of the Thy1.2+ T cells. The Thy1.2+ cells contained all the other expected leukocyte subsets but were thoroughly depleted of T cells (<1%).

To enrich for LP CD4+ or CD8+ T cells, CD4+ or CD8+ T cell subsets were deleted from dispersed LPMC with appropriate lytic MAb and complement (C) before paramagnetic bead isolation of the Thy1.2+ T cells. To lyse CD4+ or CD8+ T cells, LPMC (2 x 10^6 cells/ml) were incubated for 1 h at 4°C in 0.5 ml of Roswell Park Memorial Institute (RPMI) containing anti-CD4 (purified from GK-1.5 hybridoma, from ATCC) or anti-CD8 MAb (purified from TIB-211 hybridoma, from ATCC) at appropriate concentration. After incubation, the cells were washed by centrifugation at 4°C, suspended in an equal volume of a 1:10 dilution of Low-Tox-M rabbit C (Cedarlane Laboratories, Hornby, ON, Canada), and incubated again for 1 h at 37°C. Next, the cells were again treated with antibody and C as described above. After washing in RPMI, viability was determined with Trypan blue exclusion dye. Anti-CD4 and anti-CD8 MAb successfully depleted 99% of the appropriated T cell population as determined by cytometric flow analysis.

**Spleen cell enrichment.** Single cell suspensions of splenocytes were prepared by gentle teasing in RPMI 1640 medium (GIBCO, Grand Island, NY). The splenocytes were washed three times in RPMI. Splenic T cells were isolated by negative selection using the SpinSep enrichment procedure employing antibody-coated, dense particles as described by the manufacturer (no. 17031 and no. 17032; Stem Cell Technologies, Vancouver, BC, Canada). Non-T cells were irradiated for 3.4 min (3.5 RAD) in a gamma irradiator and used as antigen-presenting cells (APC). Flow cytometric analysis after each separation assured appropriate recovery and purity (>98%) of the Thy1.2+ T cells.

**Cell culture and cell proliferation assay.** For cell proliferation experiments, splenic cells (3 x 10^6 cells/well) and an equal number of irradiated splenic APC (3 x 10^6 cells/well) were cultured at 37°C for 48 h in 96-well microtiter plates (Corning, Cambridge, MA) in 200 μl of complete medium. The medium was RPMI containing 10% FCS, 25 mM HEPES buffer, 2 mM l-glutamine, 5 x 10^-5 M β-mercaptoethanol, 1 mM sodium pyruvate, 100 U/ml penicillin, 5 mg/ml gentamycin, and 100 mg/ml streptomycin (all GIBCO).

Some wells also received LP T cells (1.5-12 x 10^4 cells/well). The cells were cultured alone or with anti-CD3 MAb (2C11, ATCC) (1 μg/ml). All wells received 1 μCi of [3H]Tdr (PerkinElmer, Wellesley, MA) 16 h before harvesting. Cells were collected using a cell harvester, and incorporated [3H]Tdr was counted in a Tri-Carb β-scintillation counter (Packard, Palo Alto, CA).

**Transwell experiments.** Transwell experiments were performed in 96-well Transwell plates (0.4-μm-thick membrane; Nalg Nunc International, Rochester, NY). Splenetic T cells (3 x 10^6 cells/well) were mixed with equal numbers of irradiated APC and placed in the outer chamber. LP T cells (12 x 10^6 cells/well) were either added directly to the outer chamber or placed along with APC (3 x 10^6 cells/well) in the inner chamber of the Transwell system. All variables were cultured in quadruplicate. The cells were then stimulated with anti-CD3 MAb (1 μg/ml) for 48 h at 37°C. Proliferation was measured after a 16-h pulse with [3H]Tdr. The cells in the outer chamber were harvested as above, and incorporated [3H]Tdr was measured using the liquid scintillation counter.

**CFSE labeling of responder T cells.** Purified splenic T cells were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE) (Molecular Probes, Eugene, OR). Cells (2 x 10^7 cells/ml) were incubated with 10 μM CFSE in PBS at 37°C for 15 min. The cells then were washed twice with an equal volume of PBS and twice with culture medium.

**Flow cytometry analysis.** LPMC were washed twice and adjusted to 10^7 cells/ml in fluorescence-activated cell sorting (FACS) buffer (HBSS containing 1% FCS, 20 mM HEPES, and 0.02% sodium azide). The cell suspensions then were dispensed into microcentrifuge tubes, each containing 10^6 cells in 100 μL FACS buffer and stained with saturating amounts of conjugated antibodies for 30 min at 4°C. Following staining, cells were washed twice. Stained cells were analyzed on a FACS 440 flow cytometer (Becton-Dickinson, Mountain View, CA).

Before adding labeled MAb, each tube received 1 μg 2.4G2 antibody (anti-FcγR; ATCC) to block nonspecific binding of conjugated antibodies to Fc receptors. The other reagents used for staining were anti-CD4-Cy5 (RM2511; CalTag, Burlingame, CA), anti-Thy1.2-PE, and CD4-Cy5. Some wells also received LP T cells (1.5-12 x 10^4 cells/well). The cells were cultured alone or with anti-CD3 MAb (2C11, ATCC) (1 μg/ml). All wells received 1 μCi of [3H]Tdr (PerkinElmer, Wellesley, MA) 16 h before harvesting. Cells were collected using a cell harvester, and incorporated [3H]Tdr was counted in a Tri-Carb β-scintillation counter (Packard, Palo Alto, CA).

**Statistical analysis.** Data are means ± SE of multiple determinations. Difference between two groups was compared using the Student’s t-test. P values <0.05 were considered significant.
RESULTS

Splenic T cells cocultured with LP T cells proliferate weakly following polyclonal stimulation. WT splenic T cells mixed with irradiated splenic APC proliferated strongly when stimulated with soluble anti-CD3 MAb [3 × 10^4 cells/well; 94,376 ± 3,329 counts/min (cpm); n = 4]. LP T cells from the terminal ileum (TI) of healthy WT mice cocultured with APC and anti-CD3 MAb proliferated minimally (up to 12 × 10^4 cells/well; <1,500 cpm; n = 4). Subsequent experiments mixed splenic T cells with APC and various numbers of LP T cells during anti-CD3 stimulation. This resulted in a somewhat decreased rate of proliferation when splenic T cells and LP T cells were mixed at ratios of 1:2 or 1:4 (Fig. 1). WT splenic T cells also were mixed with LP T cells from the TI of mice colonized for 2 wk with *H. polygyrus*; they again were cocultured with irradiated APC and stimulated with soluble anti-CD3 MAb. This resulted in a much stronger inhibition of splenic responder T cell proliferation at cell mix ratios of 1:1 and higher (Fig. 1). LPMC depleted of T cells mixed with the splenic responder T cells at ratios up to 4:1 did not affect cellular proliferation (data not shown).

To determine the effect of LP T cells on splenic CD4 and CD8 T cell proliferation, experiments were performed using CFSE-labeled splenic T cells. Purified splenic T cells were labeled with CFSE and mixed with APC and LP T cells from mice colonized with *H. polygyrus*. Cells were stimulated to proliferate with soluble anti-CD3 MAb as before. Figure 2 shows that both the CD4 and CD8 T cell subsets proliferated poorly in the presence of LP T cells.

Additional experiments determined the requirement of APC in the regulatory process by using adherent anti-CD3 MAb in place of APC to stimulate T cell proliferation.
Mixing LP T cells from the TI of *H. polygyrus*-colonized mice with WT splenic T cells at ratios of up to 4:1 did not inhibit splenic responder T cell proliferation under these conditions (splenic T cells, 84,446 ± 943 cpm vs. LP T cells + splenic T cells at a 1:4 ratio, 90,333 ± 3,599 cpm; n = 3).

Inhibition of splenic T cell proliferation requires cell contact. A Transwell culture system was used to determine whether inhibition of proliferation required cell contact. As expected, LP T cells from *H. polygyrus*-infected mice strongly inhibited splenic T cells proliferation when cells were mixed together in the outer chamber of a Transwell culture plate (Fig. 3). However, LP T cells did not inhibit the proliferation of the splenic responder T cells when a semipermeable membrane separated the cell subsets.

Regulation of proliferation is not depended on IL-10 or T cell TGF-β signaling. Experiments were performed using T cells and APC from IL-10−/− mice. Like cells from WT controls, responder splenic T cells from IL-10−/− mice mixed with IL-10−/− APC proliferated strongly upon anti-CD3 stimulation (78,991 ± 3,106 cpm; 8 determinations from 2 experiments). IL-10−/− LP T cells from *H. polygyrus*-infected animals mixed with IL-10−/− APC proliferated poorly in response to anti-CD3. As seen in Fig. 4, mixing IL-10−/− splenic T cells and IL-10−/− APC from uninfected mice with IL-10−/− LP T cells from *H. polygyrus*-infected animals resulted in a nearly 85% decrease in anti-CD3-induced, responder splenic T cell proliferation.

Also used were splenic T cells from transgenic C57BL/6 mice expressing a T cell-specific, dominant-negative TGF-βRII, rendering the cells unresponsive to TGF-β. WT LP T cells were mixed with WT splenic APC and TGF-βRII transgenic responder splenic T cells followed by anti-CD3 stimulation. The WT LP T cells from *H. polygyrus*-colonized mice inhibited TGF-βRII transgenic responder T cell proliferation by more than 97% (Fig. 4). The responder splenic T cells proliferated well when mixed only with WT APC and anti-CD3 (90,717 ± 1,545 cpm; 8 determinations from 2 experiments).

The regulatory T cells are CD8+ T cells. To characterize the LP T cells from *H. polygyrus*-infected mice that limited splenic T cell proliferation, LP T cells (Thy1.2+CD8+) were fractionated into their CD4+ and CD8+ subsets. WT splenic T cells mixed with irradiated splenic APC and LP CD8+ T cells proliferated weakly with soluble anti-CD3 MAb stimulation. However, CD4+ LP T cells did not inhibit the proliferation of the splenic responder T cells (Fig. 5). Similar experiments also used isolated LP CD8+ T cells from the TI of healthy WT mice without *H. polygyrus* infection. These cells only minimally decreased the rate of splenic T cell proliferation. This was only evident when splenic T cells and LP T cells were mixed at ratios of 1:2 or higher.

Flow cytometry analysis of the isolated LPMC from the TI of *H. polygyrus*-infected mice showed that the unfractonated LPMC were 8.7 ± 1.1% Thy1.2+ CD8+ T cells. Less than 3% of these CD8+ T cells expressed CD25, and less than 1% expressed the natural killer cell marker DX5. In contrast, LPMC from healthy uninfected control animals were 13 ± 0.3% Thy1.2+ CD8+ T cells.

Regulation is TAP-dependent. Transporter associated with antigen processing (TAP)-deficient mice have difficulty forming class I peptide complexes. Experiments have used TAP-deficient mice to determine the potential importance of class I peptide complexes for the function of worm-induced, LP regulatory T cells. Splenic T cells from TAP−/− mice mixed with TAP−/−
Trichuris muris also induces intestinal regulatory T cells. As outlined previously, H. polygyrus induced expression of a CD8+ T cell in the LP that could inhibit the proliferation of splenic T cells in vitro. Previous experiments showed that T. muris, which is another intestinal helminth, also protects mice from colitis (8). It was determined whether this helminth induced similar CD8+ T cells in the intestinal mucosa. WT mice were given T. muris ova via gastric gavage. Two weeks later, WT splenic T cells were mixed with CD8+ LP T cells, cocultured with irradiated APC, and stimulated with soluble anti-CD3 MAb. This resulted in a strong inhibition of splenic responder T cell proliferation (87.2 ± 3.6% inhibition of proliferation; 3 independent experiments; cell mix, 4:1).

CD8+ T cells are required for worm-induced protection in the Rag IL-10−/− T cell transfer model of colitis. It was shown previously that Rag mice (T and B cell deficient) reconstituted with either unfractionated IL-10−/− splenic T cells or CD4+ IL-10−/− T cells develop a severe and persistent Th1-type colitis after NSAID (piroxicam) treatment (3). The Rag mouse protocol involved transferring IL-10−/− T cells (unfractionated splenic T cells or T cell subsets) by intraperitoneal injection into 5-wk-old Rag mice. The mice were given piroxicam orally for 2 wk starting 1 wk after cell transfer. Colitis was assessed 2 wk after stopping the piroxicam (total of 5 wk). In some instances, animals were colonized with H. polygyrus just after piroxicam administration. Figure 7 shows that worm colonization can reverse piroxicam-induced colitis when mice are reconstituted with unfractionated splenic IL-10−/− T cells or with purified CD4+ and CD8+ IL-10−/− T cells. However, there was little protection when mice were reconstituted with just CD4+ IL-10−/− T cells. Analysis of isolated LP T cells from worm-protected animals revealed the presence of CD8+ T cells that could inhibit splenic T cell proliferation in vitro.
**DISCUSSION**

It was determined whether worm colonization induces mucosal T cells with regulatory cell-like properties to further define potential mechanisms through which helminths limit mucosal immune responses. Here we show that LP T cells from *H. polygyrus*-infected mice, in contrast to LP T cells from uninfected controls, strongly suppress the proliferation of splenic T cells from uninfected animals. Experiments using CFSE-labeled indicator splenic T cells showed that both CD4<sup>+</sup> and CD8<sup>+</sup> T cells were subject to regulation by the LP T cell. Regulatory T cells can induce peripheral tolerance and constrain mucosal reactivity (13, 16, 30). Such regulatory cells can control self-reactive T cells and are functionally important in limiting inflammation in various animal models of IBD (20). There are several regulatory T cell phenotypes. For instance, some express CD4, whereas others express CD8 (10). There are naturally occurring CD4<sup>+</sup> CD25<sup>+</sup> regulatory T cells that develop in the thymus. Also described are CD4<sup>+</sup> regulatory T cells that produce high levels of IL-10 and/or TGF-β (Tr1 and Th3) (18). These are secondary suppressor cells that develop from CD4<sup>+</sup> CD25<sup>+</sup> T cells in the periphery.

LP T cells were fractionated into their CD4 and CD8 subsets to delineate the phenotype of the worm-induced regulatory cell. The ability to inhibit proliferation resided exclusively with the CD8<sup>+</sup> T cell subset. Flow analysis showed that enhanced CD8 regulatory activity following worm colonization was not simply secondary to recruitment of relatively more Thy<sup>+</sup> CD8<sup>+</sup> cells into the mucosa. Instead, the LP of *H. polygyrus*-colonized mice contained fewer CD8<sup>+</sup> T cells, but with augmented regulatory capacity.

In our studies, it is possible that the CD8<sup>+</sup> regulatory T cells controlled the activated splenic responder T cells through one or more mechanisms like secretion of regulatory lymphokines, recognition of transiently expressed nonpolymorphic membrane molecules unrelated to TCR or antigen, or detection of peptide MHC class I complexes on activated T cells.

Transwell experiments showed that the regulation required direct cognate interactions between the CD8 regulatory T cell and the activated responder T cells. This is akin to the natural regulatory T cells that mediate much of their suppressor activity through direct cell-to-cell contact (9).

Natural regulatory cells either secrete TGF-β or express on their surface latency-associated peptide, which is the amino-terminal domain of the TGF-β precursor peptide (23). Expression of TGF-β is an important mechanism through which these cells exercise suppression both in vitro and in vivo (21). It also may have an important role in the function of acquired T regulatory cells (e.g., Th3). Our experiments using T cells from TGF-βRII, dominant-negative transgenic mice suggested that TGF-β signaling in the responder splenic T cells was not essential for the CD8 regulatory T cell activity.

We also examined the importance of IL-10 for CD8 regulatory T cell action. IL-10 is a strong modulator of immune functions (19). IL-10 inhibits production of various important pro-inflammatory cytokines like TNF-α and IL-12 and can block dendritic and macrophage cell function. Transgenic mice lacking IL-10 develop a severe Th1-type colitis, illustrating the importance of IL-10 for mucosal protection. In the total absence of IL-10, *H. polygyrus* induced expression of CD8<sup>+</sup> regulatory T cells that blocked responder T cell proliferation similar to cells from WT controls. This suggests that IL-10 is not essential for either their regulatory activity or development.

T cell suppression mediated by CD8<sup>+</sup> T cells has been characterized in several animal models of inflammation. They participate in resistance to the autoimmune disease of experimental allergic encephalomyelitis (12). Also, CD8<sup>+</sup> T cells are involved in the downregulation of the CD4<sup>+</sup> T cell response to superantigen staphylococcal enterotoxin B (11). CD8 T cells can kill activated T cells (11) and B cells (22) through recognition of specific peptides associated with the nonclassical MHC class I molecule Qa-1 on these cells. They require priming by activated CD4<sup>+</sup> T cells during the primary immune response to regulate the secondary immune response. This is in contrast to the CD4<sup>+</sup> CD25<sup>+</sup> regulatory T cells that occur naturally and function during the primary phases of an immune reaction.

TAP is important for transporting peptides into the MHC class I complex. The CD8<sup>+</sup> LP regulatory T cells described here required TAP expression in the responder cells, suggesting that their function is dependent on MHC class I interactions. TAPI expression is required both in the responder T cell and the APC for full expression of CD8 ± LP regulatory T cell function, perhaps indicating that a cognate interaction is necessary for this activity, a notion consistent with the requirement for TAP.

In our experiments, it remains unsettled whether the LP CD8<sup>+</sup> regulatory T cell kills the responder T cell or functions through some other mechanism. The CFSE experiments suggest that killing of responder cells is not the predominant mechanism of action.

*Rag* mice reconstituted with unfraccionated IL-10<sup>−/−</sup> T cells, CD4<sup>+</sup> and CD8<sup>+</sup> T cells, or just with CD4<sup>+</sup> T cells are highly susceptible to piroxicam-induced colitis (3). We showed here that *H. polygyrus* infection reverses the inflammation, but only if mice receive CD8<sup>+</sup> T cells along with CD4. This suggests that protection from colitis is dependent on CD8<sup>+</sup> T cells. The appearance of CD8<sup>+</sup> regulatory T cells in the intestinal mucosa of these protected mice supports the notion that these regulatory T cells are important for this protective process.

There has been a dramatic increase in the prevalence of immune-mediated diseases in industrialized countries (1, 2) where previously common exposure to helminths is now rare (10). This suggests that helminth exposure may bias the immune response and afford protection from these diseases. One such possible biasing mechanism is induction of immune cells with regulatory function. Here we show that colonization with an intestinal helminth induces a novel mucosal CD8<sup>+</sup> T cell that inhibits proliferation of CD4<sup>+</sup> and CD8<sup>+</sup> T cells through a contact and TAP-dependent mechanism. Also, in an IBD model of intestinal inflammation, helminth suppression of colitis requires CD8<sup>+</sup> T cells, suggesting that such CD8<sup>+</sup> regulatory T cells may be important for this protection.

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