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CD25⁺CD4⁺ Cells Contribute to Th2 Polarization during Helminth Infection by Suppressing Th1 Response Development¹

Amy S. McKee² and Edward J. Pearce³

Mice infected with Schistosoma mansoni develop polarized Th2 responses in which Th1 responses are prevented by IL-10-mediated suppression of IL-12 production. We show that dendritic cells from infected mice are primed to make IL-12 in response to CD40 ligation, and that IL-10 acts by inhibiting this process. In infected mice, two subpopulations of CD4⁺ cells, separable by their expression of CD25, make IL-10. CD25⁺CD4⁺ cells expressed forkhead box P3, inhibited proliferation of CD4⁺ T cells, and made IL-10, but little IL-5. In contrast, CD25⁻CD4⁺ cells failed to express forkhead box P3 or to inhibit proliferation and accounted for all the IL-5, IL-6, and IL-13 produced by unseparated splenic populations. Thus, CD25⁺ and CD25⁻ subpopulations could be characterized as regulatory T cells (Treg cells) and Th2 cells, respectively. Consistent with their ability to make IL-10, both CD25⁺ and CD25⁻CD4⁺ T cells from infected mice were able, when stimulated with egg Ag, to suppress IL-12 production by CD40 agonist-stimulated dendritic cells. Additionally, in adoptive transfer experiments, both CD4⁺ subpopulations of cells were able to partially inhibit the development of Th1 responses in egg-immunized IL-10⁻/⁻ mice. The relationship of Treg cells in infected mice to natural Treg cells was strongly suggested by the ability of CD25⁺CD4⁺ cells from naive mice to inhibit Th1 response development when transferred into egg-immunized or infected IL-10⁻/⁻ mice. The data suggest that natural Treg cells and, to a lesser extent, Th2 cells play roles in suppressing Th1 responses and ensuring Th2 polarization during schistosomiasis. The Journal of Immunology, 2004, 173: 1224–1231.

Infections with schistosomes are chronic and are characterized immunologically by an early Th1 response that switches to a Th2-dominated response after the onset of parasite egg production (1). Primary Th2 responses fail to eliminate schistosomes from the host, but permit host survival during acute infection by preventing potentially lethal inflammatory pathology (2–4). How schistosome eggs induce Th2 responses is unclear, but appears to be linked to their inherent ability to avoid directly inducing dendritic cell (DC) maturation and IL-12 production (5). Nevertheless, during patent infection with schistosomes, DC undergo a form of maturation, mediated by CD40 ligation, that is crucial for Th2 response initiation (6, 7). The failure of this pathway, which in many other systems elicits IL-12 production (8–10), to precipitate Th1 responses was surprising and suggested the existence of an effective regulatory mechanism. Consistent with the hypothesis that signals for Th1 response development are suppressed after exposure to parasite eggs, mice lacking IL-10 develop schistosome egg Ag-specific Th1 responses during infection (11–14). This is directly the result of unregulated IL-12 production, since mice deficient for IL-12 and IL-10 fail to polarize in this fashion and develop excessively Th2-skewed responses (14). The lack of IL-10 leads to increased morbidity and mortality during the acute phase of infection (13) and can result in hepatic granulomatous pathology of increased severity in those animals that survive into the chronic disease phase (15).

Based on the findings outlined above, we initiated a search for an IL-10-based regulatory system that could suppress the ability of DC to make IL-12 in response to CD40 ligation. Although myeloid APCs can make IL-10 and may be important sources of this cytokine during infection (16), the results of our recent studies directed us toward an analysis of Ag receptor-expressing cells that are capable of responding to schistosome Ag by making IL-10 (17). In particular, we were interested in whether regulatory T (Treg) cells play any role during infection. There has been prominent discussion about the possible role of Treg cells in helminth infections and their involvement in the prevention of overt allergic or autoimmune pathologies in helminth-infected individuals (18, 19). Moreover, a recent report has implicated Treg cells specifically in the suppression of Th1 responses induced by mature DC (20). Although a cardinal feature of Treg cells is their ability to suppress the proliferation of conventional CD4⁺ cells (21), they are also noted for their ability to suppress Th1-mediated pathologies by a process that is dependent upon IL-10 (22, 23). Indeed, IL-10 appears to play a central role in preventing overtly pathological Th1 or Th2 responses in a variety of settings (24), and studies in mice and humans have shown decreased susceptibility to atopy and autoimmunity during schistosomiasis (25–28), which in some cases has been correlated with increased IL-10 production (26, 29). Thus, there appeared to be a strong case for a role for Treg cells during schistosome infection.
We report in this study evidence that infection leads to the development of a population of CD25^+ CD4^+ T cells that express the Treg cell transcription factor forkhead box P3 (Foxp3), inhibit T cell proliferation, make IL-10 but little of the signatures Th2 cytokines, and potently suppress IL-12 production by CD40 agonist-activated DC. The possibility that these cells arise from natural Treg cells is suggested by the finding that rigorously purified CD25^+ CD4^+ T cells from uninfected mice potently suppressed Th1 response development in infected IL-10^-/- mice. Treg cells from infected mice share some properties with Th2 cells, notably their ability to make IL-10. Our data suggest that this ability to make IL-10 allows both Treg and Th2 cells to suppress the development of Th1 responses to schistosoma egg Ag, and it seems likely that these cells cooperate with each other to enforce the Th2 polarization that characterizes the immune response to schistosome infected mice.

Materials and Methods

Animals and parasites

C57BL/6 (B6), B6 Thy1.1, B6 Thy1.2, B6 IL-10^-/-, BALB/c, and BALB/c IL-10^-/- mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and/or bred at University of Pennsylvania (Philadelphia, PA). IL-10-deficient and age-matched control mice were provided with antibiotic-containing water (Bactrim; HiTech Pharmaceutical, Amityville, NY). For infection, mice were each injected s.c. with ~70 Schistosoma mansoni cercariae (NMRI strain) and used 6–8 wk later. The majority of experiments were repeated with cells from B6 and BALB/c mice. However, for egg injection experiments, BALB/c WT CD25^- and CD25^+ cells were adoptively transferred into BALB/c IL-10^-/- mice; for adoptive transfer experiments using infected mice, B6 mice were used as donors and recipients.

Cell isolation and Ag preparation

Endotoxin-free schistosome eggs and schistosoma egg Ag (SEA) were prepared as described previously (30). Single-cell suspensions of splenic or lymph node (LN) cells were prepared by pushing lymphoid organs through sterile 70-μm pore size nylon mesh. DC were isolated, as previously described (17), by incubating splenocytes for 15 min with CD11c-specific microbeads (Miltenyi Biotec, Auburn, CA), followed by passage through a MACS magnet, or by using AutoMACS (Miltenyi Biotec). CD25^- and CD25^+ cells from the CD11c^+ fraction or from a negatively selected CD4^- fraction were MACS-sorted using a CD25^+ cell isolation kit (Miltenyi). In some experiments CD4^- or B cells were depleted using purified mAbs GK1.5 or RA3.3A1, respectively, followed by sheep antirat Ig Dynabeads (Dynal Biotech, Lake Success, NY) and magnetic sorting. Cell purity was always assessed by flow cytometry to ensure that targeted populations had been depleted or very highly enriched. For these experiments, cells were always resuspended to occupy a volume equivalent to that which they would have occupied before depletion or purification. For adoptive transfer experiments, CD25^- CD4^- and CD25^- CD4^- populations were FACs-sorted using a DiVa flow cytometer (BD Biosciences, Mountain View, CA); cells were routinely >99% pure for these experiments.

Isolated splenic DC (5 × 10^7/well of a 96-well plate) were cultured in RPMI 1640 containing 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine (all from Mediatech, Herndon, VA), 5 × 10^-5 M 2-ME (Sigma-Aldrich, St. Louis, MO), and 10% FCS (HyClone, Logan, UT). Agonistic anti-CD40 Ab (10 μg/ml; 3/23 LE; BD Pharmingen, San Diego, CA) was added to DC for 15 min at 4°C. CD1c^- cells (2 × 10^5) were then recombined with unwashed DC, and SEA (50 μg/ml) was added as required. Final culture volumes were 200 μl. In some experiments a neutralizing anti-IL-10 mAb (2A5) or normal rat IgG was added to cells at 50 μg/ml. Cells were cultured for 24 h at 37°C in 5% CO2. To measure egg-specific Th1 responses, mesenteric LN cells from infected mice, or peritoneal exudate cells (PEC) from egg-injected mice, were resuspended at 5 × 10^5/ml in CTM (DMEM (Mediatech) containing 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, 2 mM HEPES, 5 × 10^-5 M 2-ME, and 10% FCS) and were cultured for 21 h in the absence or the presence of SEA (50 μg/ml) or plate-bound anti-CD3 (0.5 μg/well). Cytokines in cell supernatants were measured by ELISA, as previously described (30).

Proliferation assay

Responder Thy1.2 splenocytes were labeled with CFSE (Molecular Probes, Eugene, OR) and mixed at a ratio of 2:1 with isolated CD25^- or CD25^- CD4^- cells from uninfected or infected B6 Thy1.1 mice in CTM in the presence or the absence of mAb anti-CD3 (0.5 μg/ml). Cells were cultured for 4 days at 37°C in 5% CO2, harvested, and stained with anti-Thy1.2 and anti-CD4 mAb. CFSE dilution was analyzed in cells staining positively for both these markers, using a FACSCalibur flow cytometer and FlowJo software (Tree Star, San Carlos, CA).

Flow cytometry

Cells were stained with Abs diluted in DPBS and 1% FCS for 30 min on ice, washed, and fixed in DPBS, 1% formaldehyde, and 0.05% sodium azide (Sigma-Aldrich). For detection of IL-12 after surface staining for CD11c and CD8α, DC were fixed in 3% paraformaldehyde for 30 min on ice and washed in saponin buffer (PBS and 0.075% saponin). Permeabilized cells were incubated in saponin buffer with 5% normal mouse serum for 15 min on ice. PE-labeled anti-IL-12p40 mAb or PE-IgG1 (isotype control), diluted in saponin buffer with 5% normal mouse serum, were added for 30 min. The cells were washed in saponin buffer, followed by DPBS. The expression of surface markers and of intracellular IL-12 was analyzed on a FACSCalibur flow cytometer using FlowJo software (Tree Star). All fluorochrome-labeled Abs were obtained from Pharmingen.

Adaptive transfer experiments

FACS-sorted populations (>99% pure) of mesenteric LN-derived CD25^- CD4^- or CD25^- CD4^- cells were washed and resuspended at 2.5 × 10^6/ml in HBSS (Mediatech). Cells were sorted from either uninfected mice or mice that had been infected for 7 wk. IL-10^-/- mice that had been infected for 6 wk were each injected i.p. with 250 μl of HBSS alone or containing 5 × 10^5 CD25^- CD4^- or CD25^- CD4^- cells from uninfected mice. Two weeks later, mesenteric LN from the recipients was harvested, and T cell responses were assessed as described above. For egg injection experiments, uninfected IL-10^-/- mice were each injected i.p. with 250 μl of HBSS alone or containing 5 × 10^5 CD25^- CD4^- or CD25^- CD4^- cells from uninfected or infected wild-type (WT) mice and 1 day later were each injected i.p. with 2.5 × 10^7 FT schistosome eggs. Unmanipulated WT and IL-10^-/- mice were also injected with eggs as controls. Ten days later, PEC were recovered from these mice by peritoneal lavage, as described previously (31), and egg Ag-specific T cell responses were assessed as described above.

RT-PCR

RNA was isolated from purified CD25^- CD4^- and CD25^- CD4^- cells using RNeasy (Qiagen, Valencia, CA), cDNA was reverse transcribed using Superscript II (Invitrogen, Carlsbad, CA), and different samples were equalized using RT-PCR for hypoxanthine phosphoribosyltransferase in the presence of a competitor. To amplify Foxp3 transcripts, we used previously specified primers (32) and 28 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s. PCR products were resolved in 1% agarose and visualized by ethidium bromide staining.

Statistical analysis

Student's t test was used to calculate the significance of differences between means.

Results

Infection-induced IL-10 regulates IL-12 production by DCs

The development of Th2 responses against schistosome Ag requires ligation of CD40 on DC (6, 7). To investigate the possibility that IL-12 production by CD40L-activated DC is regulated during schistosome infection, we purified cells that stained positively for both these markers, using a FACSCalibur flow cytometer and FlowJo software (Tree Star, San Carlos, CA), suggesting that they had been primed to make IL-12. However, when SEA was added to cells from infected mice, the percentages
of CD8α− and CD8α+ DC making IL-12 were significantly reduced (p < 0.01) to background levels (Fig. 1A, D). We examined the possibility that SEA, which can bind to DC-specific intercellular adhesion molecule-grabbing nonintegrin (33), is acting directly on DC to suppress IL-12 by measuring IL-12 produced by isolated DC cultured with CD40 agonist plus SEA in the absence of CD11c+ cells. Although SEA did cause a drop in the percentage of IL-12+ cells, in all cases the decrease was slight (not shown). Together, these data suggest that SEA-responsive CD11c+ cells within the spleens of infected mice inhibit the ability of DC to make IL-12 in response to CD40 ligation.

IL-10 is an important suppressor of IL-12 production by DC (34) and has been implicated in the control of Th1 responses during schistosomiasis (11–14). Consequently, we measured IL-10 in the supernatants of DC plus CD11c+ cells in the presence or the absence of SEA to ascertain whether there was a correlation between suppressed IL-12 production and the presence of IL-10. When SEA was added, IL-10 levels significantly increased in cultures of cells from infected, but not uninfected mice (p < 0.01; Fig. 1B, E). DC themselves produced little IL-10 in response to CD40 agonist (Fig. 1B and not shown). These data suggested that IL-10 from SEA-responsive CD11c+ cells could be the factor that suppresses the ability of DC to make IL-12 in response to CD40 ligation. To experimentally address this issue, we purified DC from the spleens of infected IL-10−/− mice and measured IL-12 produced in the absence or the presence of SEA-stimulated autologous CD11c+ cells. DC isolated from infected IL-10−/− mice and from infected WT mice made similar amounts of IL-12 in response to CD40 ligation (Fig. 1C, left panel, ■). Strikingly, however, whereas splenic CD11c+ cells from infected WT mice were able to respond to SEA and significantly (p < 0.05) inhibit IL-12 production by CD40 agonist-stimulated autologous DC, CD11c+ cells from infected IL-10−/− mice were unable to do so (Fig. 1C, right panel). Consistent with this finding, SEA-activated CD11c+ cells from infected WT mice were unable to limit CD40 agonist-induced production of IL-12 by autologous DC when the activity of IL-10 was inhibited with a neutralizing Ab (Fig. 1D). These data indicate that IL-10 is the factor produced by SEA-stimulated CD11c+ cells that prevents IL-12 production by CD40-agonist-stimulated DC in vitro.

To examine whether IL-10 is playing a significant role in inhibiting DC activation in vivo, we isolated DC from the spleens of infected IL-10−/− mice and compared their maturation status to that of DC from uninfected IL-10−/− animals and from infected and uninfected WT mice. As noted previously (17), schistosome infection in WT animals leads to a slight overall increase in the expression of MHC class II, CD80, and CD40 on DC (Fig. 2A), indicating that the cells have received a maturation signal; we know this to be CD40/CD40L dependent (17). In comparison, the increase in expression of each of these markers as a result of infection was consistently found to be greater in IL-10−/− mice (Fig. 2A). More detailed analysis of the major splenic DC subsets revealed, to a greater or lesser extent, increased expression of MHC class II, CD80, CD86, and CD40 on both CD8α+ and CD8α− DC as a result of S. mansoni infection in IL-10−/− vs WT mice (Fig. 2B). Taken together, the data shown in Figs. 1 and 2 indicate that during infection with S. mansoni, DC maturation and activation to make IL-12 are tightly regulated by IL-10.

FIGURE 1. The ability of DC from S. mansoni-infected mice to make IL-12 in response to CD40 ligation is suppressed by IL-10 from Ag-responsive CD11c+ cells. A, Isolated splenic DC and CD40 agonist (αCD40)-treated DC from uninfected (control) or infected (S.m.) mice were reunited with sorted CD11c+ cells from autologous spleens with (■) or without (□) SEA and stained for CD11c, CD8α, and intracellular IL-12 after 24 h of culture. The percentages of IL-12-positive CD8α+ and CD8α− DC were assessed by flow cytometry. B, Cells were cultured as described in A for 24 h, and secreted IL-10 in culture supernatants was measured. C, Left panel, Purified CD11c+ DC from uninfected (−) or infected (+) WT and IL-10−/− mice were incubated with medium (□) or CD40 agonist (■). Right panel, CD11c+ DC from uninfected (−) or infected (+) WT and IL-10−/− mice were reunited with CD11c+ cells from autologous mice with (■) or without (□) SEA for 24 h, after which IL-12 in culture supernatants was measured. D, CD11c+ cells from infected mice were cocultured with DC or CD40 agonist-stimulated DC (αCD40 DC) from autologous spleens in the presence of medium (■) or with SEA (□ and not detected (ND)), either with neutralizing anti-IL-10 Ab (αIL-10) or control rat IgG (Rat IgG). B–D, Cytokines in 24 h culture supernatants were measured by ELISA. Results are representative of three experiments. These experiments were performed more than three times each, with consistent results.

FIGURE 2. IL-10 mediates suppression of DC maturation during schistosomiasis. A, Splenocytes from infected (■) and uninfected (□) WT and IL-10−/− mice were stained for CD11c, MHC class II, CD80, CD86, and CD40, and the relative expression of each marker was analyzed on gated CD11c+ MHC class II+ cells by flow cytometry. Staining with isotype control Ab is indicated by the light gray line. Cells were pooled from three mice per experimental group. B, Fold increase in the expression of MHC II, CD80, CD86, and CD40 on DC from infected WT (□) or IL-10−/− (■) mice, compared with DC pooled from three uninfected genotype-matched controls, was determined by measuring the change in mean fluorescence intensity on gated CD8α+ and CD8α− DC. Bars indicate the mean fold change in mean fluorescence intensity for cells assessed from three individual mice per group, with SE bars. Data are from one experiment and are representative of two additional experiments.
Two subpopulations of CD4+ cells make IL-10 and suppress IL-12 production by CD40 agonist-stimulated DC

In addition to T cells, B cells have been reported to be capable of playing an important role in the regulation of immunopathology by producing IL-10 (35). To identify the Ag-responsive CD11c− cell type responsible for making IL-10 during schistosomiasis, we depleted CD4− cells or B220+ cells from infection-derived CD11c− cells and reunited the depleted populations with autologous CD40 agonist-treated DC plus SEA. In these experiments, depletion of CD4 cells caused a profound decrease in IL-10 levels, whereas B cell depletion had little effect (Fig. 3A). Consistent with the view that IL-10-producing CD4 cells are responsible for inhibiting the production of IL-12 by DC, CD11c− spleen cells that had been depleted of CD4 cells failed to inhibit the production of IL-12 by CD40 agonist-stimulated autologous DC, whereas B cell-depleted populations were effective (p < 0.01) suppressors (Fig. 3B).

At least two distinct populations of CD4 cells can produce IL-10: Th2 cells (36) and Treg cells (21). Although schistosomiasis is known to induce strong Th2 responses (5), little is known about Treg responses during this infection, and it is possible that either Th2 or Treg cells could play a role in the regulation of DC activation. CD25 expression is a robust marker of Treg cells (37), and analysis of the expression of this marker on mesenteric LN CD4+ revealed that infection leads to an expansion of the CD25+ CD4+ population (Fig. 4A); the majority of the CD25+ cells were additionally CD45RB low (not shown), which also is an indicator of Treg cells (38). We MACS-sorted CD25+ CD4+ and CD25− CD4+ cells (Fig. 4A), and used RT-PCR to examine the expression of Foxp3 in the two subsets; Foxp3 was recently shown to program the development and function of CD25+ CD4+ Treg cells (32, 39, 40) and to be expressed in Treg, but not Th2, cells (32). Sorted CD25+ CD4+ cells were Foxp3 positive, whereas CD25− CD4+ cells were either weakly positive (uninfected mice) or negative (Fig. 4B), suggesting that the CD25+ subpopulation did not contain any Treg cells, whereas the CD25− subpopulation did. We next cultured sorted CD25+ CD4+ and CD25− CD4+ cells from uninfected or infected mice with isolated, CD40 agonist-treated DC from autologous spleens in the presence or the absence of SEA and measured secreted IL-10. In addition, we measured the Th2 cytokines IL-5, IL-6, and IL-13. Cells from uninfected mice failed to make IL-10 in response to SEA (Fig. 5A), whereas both CD25+ and CD25− fractions from infected mice produced equivalent amounts of IL-10 when stimulated with SEA (Fig. 5A). In marked contrast, the major source of IL-5, IL-6, and IL-13 was the CD25+ population (Fig. 5B). Thus, egg Ag-responsive CD4+ cells could be functionally separated based on their expression of CD25.

To establish the relative contribution of CD25+ CD4+ cell-derived IL-10 to the SEA-induced suppression of IL-12 production by DC, we measured IL-12 in the supernatants of CD40 agonist-treated DC cultured in the presence of autologous MACS-sorted CD25+ CD4+ or CD25− CD4+ cells. SEA-responsive CD25+ CD4+ cells were capable of inhibiting IL-12 production in these assays, and their effect in this regard was as potent as that of unfractionated splenic populations (Fig. 6, C and A, respectively). Additionally, consistent with the finding that they, too, make IL-10, CD25− CD4+ cells were found to be able to respond to SEA by suppressing IL-12 production by DC (Fig. 6B). We noted that in the presence of SEA, CD25+ CD4+ cells were less effective (p < 0.05) than CD25− CD4+ cells at suppressing IL-12 production (compare Fig. 6, B and C), and that in the absence of egg Ag, IL-12 levels were higher in cultures that lacked CD25+ CD4+ cells (compare Fig. 6, B with A and C).

CD25+ CD4+ cells, but not CD25− CD4+ cells, suppress T cell proliferation

To further characterize the CD25+ and CD25− subsets of CD4+ cells, we assessed whether they were capable of limiting the proliferation of other T cells. We found that MACS-sorted CD25+ CD4+ cells from infected mice were more effective than CD25− CD4+ cells from uninfected mice at suppressing T cell proliferation (Fig. 7). In contrast, CD25− CD4+ cells from infected
or uninfected mice were completely incapable of this regulatory function (Fig. 7).

**CD25\^*CD4\^+ and CD25\^−CD4\^+ cells suppress the development of egg Ag-specific Th1 responses**

To examine whether Treg cells could regulate Th1 response development in vivo, we adoptively transferred FACS-sorted CD25\^*CD4\^+ cells and CD25\^−CD4\^+ cells from infected WT mice into IL-10\^−/− mice and 1 day later injected these mice i.p. with schistosome eggs; we have reported previously that this immunization pathway induces the development of an egg Ag-specific Th2 response within the peritoneal cavity (31). Ten days postimmunization we assessed Th1 and Th2 responsiveness using an established protocol (31) in which the ability of cells recovered from the site of Ag deposition to make IFN-\(\gamma\) and IL-5, as markers of Th1 and Th2 responses, respectively, in response to restimulation with SEA in vitro was measured. We found that cells from egg-injected IL-10\^−/− animals made IL-5 (Fig. 8A), but additionally made significantly more (\(p < 0.05\)) IFN-\(\gamma\) than did cells from egg-injected WT mice (Fig. 8A), reflecting the inability of IL-10\^−/− mice to polarize their egg-specific response in a Th2 direction. Consistent with a role for CD25\^*CD4\^+ cells in the prevention of Th1 response development, transfer of CD25\^*CD4\^+ cells from infected WT mice into IL-10\^−/− mice resulted in a \(\geq 50\%\) diminution (\(p < 0.05\)) in the egg-specific Th1 response, as measured by IFN-\(\gamma\) production (Fig. 8A), but had no discernible effect on the egg-specific Th2 response, as indicated by equivalent IL-5 levels (Fig. 8A). CD25\^−CD4\^+ cells from infected mice were also found to be effective at inhibiting Th1 response development in this immunization system (Fig. 8A), but, additionally, significantly (\(p < 0.05\)) enhanced Th2 responses (Fig. 8A), consistent with the fact that they were adding to the pool of Th2 cells in the recipient mice.

To examine whether the CD25\^*CD4\^+ cells in infected mice could be related to naturally arising Treg cells, we compared the ability of CD25\^*CD4\^+ cells sorted from naive uninfected WT animals or from infected WT animals to limit egg Ag-specific Th1 response development in egg-injected IL-10\^−/− mice and found that cells from uninfected and infected mice were equally capable in this regard (Fig. 8B). To extend these studies, we next examined the effect of adoptively transferred CD25\^*CD4\^+ and CD25\^−CD4\^+ cells from naive uninfected mice on Th1 and Th2 response development during infection in the absence of IL-10. For these experiments we used as recipients mice that had been infected for 6 wk and that would be expected to be at the very earliest stage of responding immunologically to egg Ag (1) and assessed egg Ag-specific responses 2 wk later. As reported previously (13), splenocytes from infected IL-10\^−/− mice respond to SEA by making more IFN-\(\gamma\) and more IL-5 than do cells from infected WT mice (Fig. 8C). Strikingly, CD25\^*CD4\^+ cells transferred from uninfected mice abrogated IFN-\(\gamma\) production almost completely in the infected IL-10\^−/− mice, reducing it to levels seen in infected WT mice (Fig. 8C). In this system, transferred CD25\^*CD4\^+ cells were also effective in limiting IFN-\(\gamma\) production, but to a lesser extent (\(p < 0.05\)) than CD4\^*CD25\^+ cells, whereas splenocytes from infected IL-10\^−/− recipients of CD25\^*CD4\^+ cells made 5 times as much IFN-\(\gamma\) in response to SEA as did splenocytes from infected IL-10\^−/− recipients of CD25\^−CD4\^+ cells (Fig. 8C). Interestingly, there was no measured effect of CD25\^*CD4\^+ or CD25\^−CD4\^+ cells on IL-5 production in the infected IL-10\^−/− recipients (Fig. 8C). Taken together, the data from the adoptive transfer experiments indicate that CD25\^*CD4\^+ cells can function in a highly regulatory capacity to prevent Th1 response development, while having little effect on Th2 responses. The ability to suppress Th1 responses is shared by Th2 cells.

**Discussion**

In this study we initiated a search specifically for cells that, by producing IL-10 to suppress the ability of DC to make IL-12 in response to CD40 ligation, might play a role in facilitating Th2 polarization in mice infected with schistosomes. We show that infection leads to the development of two subpopulations of CD4\^+ cells that are able to make IL-10 and inhibit CD40 agonist-initiated IL-12 production. One subpopulation is CD25\^+ and definable as Th2 on the basis of its ability to make Th2 cytokines in addition to IL-10. The other subpopulation is CD25\^+ and on the basis of several criteria, including surface phenotype, Foxp3 expression, and ability to suppress T cell proliferation, is definable as Treg.

Several types of Treg have been described, and the relationship between the adaptive Treg cells that emerge during infection and those that arise naturally in the thymus and are considered self-Ag responsive is not yet clear (41–43). However, our finding that CD25\^*CD4\^+ cells transferred from naive WT mice severely curtail the ability of infected recipient IL-10\^−/− mice to mount Th1 responses against egg Ag strongly supports a model in which natural Treg cells play a role in preventing the expression of Th1 responses during infection. This is consistent with the recent report...
that natural Treg cells are activated during infection with the protozoan parasite *Leishmania major*; in this model, Treg cells function analogously to those in schistosome-infected mice by producing IL-10 and limiting parasite-specific Th1 response development (44).

As Treg cells overexpress a subset of Th2 gene transcripts (45), it seems likely that Treg and Th2 cells will share certain regulatory abilities, and consistent with this hypothesis, we found both CD25+ CD4+ and CD25− CD4+ populations from infected mice to produce IL-10 and suppress IL-12 production in vitro. Although the relative contributions of Treg and Th2 cells to Th1 response suppression during *S. mansoni* infection in WT mice remain to be determined, it is clear from our data that both can suppress Th1 responses in vivo in IL-10−/− mice (Fig. 8). We noted that CD25+ CD4+ cells, but not CD25− CD4+ cells, from infected WT mice contribute to the expression of a stronger Th2 response in egg-injected, IL-10-deficient recipient animals, presumably because they are an additional source of Th2 cytokines and/or provide IL-4 to promote Th2 polarization of the ongoing recipient response. Taken together, the data indicate that CD25+ and CD25− CD4+ cells can regulate the development of egg Ag-specific Th1 response development. However, CD25+ CD4+ cells were found to be more capable than CD25− cells in this regard, especially when transferred into infected IL-10−/− animals. As CD25+ and CD25− cells make similar levels of IL-10, we hypothesize that characteristics in addition to the potential to produce this cytokine must be important for Th1 response suppression in vivo. Possibly the distinction between CD25+ and CD25− cells lies in the unique ability of the former to inhibit the proliferation of other T cells. These issues are the subjects of ongoing investigation.

Although CD25 expression is a good marker for Treg cells in naive mice, it might have been expected that it would additionally have identified activated conventional CD4+ cells in infected animals. Nevertheless, we found that lack of CD25 expression is an excellent indicator of Th2 cells in schistosome-infected animals. Additional support for this view has come from experiments using mice transgenic for a bicistronic IL-4–GFP reporter (46), where infection has been clearly seen to lead to the expansion of a population of CD25− CD4+ IL-4−GFP− cells (J. Taylor, M. Mohrs, and E. J. Pearce, unpublished observations). Thus, in schistosome-infected mice, CD25 is a robust marker for distinguishing between Th2 CD4+ cells and an additional subset of CD4+ cells that has the hallmarks of Treg cells.

Although Treg cells are implicated in the regulation of Th2-mediated pathologies in diseases such as asthma (47), and our data (Fig. 8) and those of others (14) indicate that Th2 as well as Th1 responses can be enhanced in the absence of IL-10, we noted in our adoptive transfer experiments that any effect of CD25+ CD4+ cells on Th2 response intensity was significantly less impressive than the observed effects on Th1 response development. Perhaps this reflects the relative sensitivity of Th1 and Th2 responses to suppression by IL-10, with the former sensitive to lower concentrations that could be provided by the small numbers of transferred cells. We are considering the possibility that the ability of Th2 cells to make IL-6, which is able to subvert Treg inhibitory activity (48), makes them more resistant than Th1 cells to Treg-mediated suppression.

A surprising finding is that CD25+ CD4+ cells from infected mice are responsive to SEA, as indicated by the ability of this Ag to induce IL-10 production when added to cocultures of these cells plus DC. As schistosomes are metazoan parasites, in which many genes share considerable homology to mammalian genes, there are possibly some peptide sequences in common between parasite and host that can be recognized by T cells. However, at this time it is not clear from our studies whether SEA-responsive CD25+ CD4+ cells are cross-reactive for self-Ag. Regardless, SEA-responsive CD25+ CD4+ cells are not detectable in uninfected mice, so we
can conclude that schistosome infection stimulates the accumulation, clonal expansion (49), or simply the functional maturation of CD25+CD4+ regulatory cells with TCR that recognize SEA. Presumably, part of this process involves acquisition of the ability to produce IL-10. The regulatory activity of natural Treg cells appears to stem from their expression of Foxp3, a transcription factor that acts as a master regulator of the Treg phenotype (39). Natural Treg cells and naive CD25+CD4+ cells, in which Foxp3 is expressed by viral transduction, fail to make IL-10 in response to TCR ligation in vitro (32, 40). Nevertheless, these cells are capable of ameliorating severe autoimmune pathologies through a mechanism that is dependent upon IL-10 (50, 51), suggesting that under appropriate in vivo circumstances, Treg cells undergo an additional activation/differentiation step that allows them to express the IL-10 gene, a conclusion supported by recent reports (52) and the data presented in this study.

Neither the kinetics of the development of the Treg-like activity nor the factors that promote this response during infection are clear from the studies reported in this paper. Recent observations have suggested that, as might be anticipated, APCs can play a central role in promoting the development of adaptive Treg cell responses (41, 53–55). Of particular importance in this regard is the report that schistosome egg-derived lyso-phosphatidylserine activates DC via TLR2 to invoke the development of IL-10-producing adaptive Treg cells (56). Perhaps a mechanism such as this explains the strong Treg response that develops during schistosomiasis.

The consequences of failing to control DC activation are apparent in schistosome-infected IL-10−/− mice, where splenic DC exhibit an exaggeratedly mature phenotype, Th1 responses develop, and disease severity is significantly increased. We believe that in addition to their ability to regulate T cell proliferation, a crucial function of Treg cells is to tightly control immune system intrinsic pathways of DC activation during chronic infection. In this way these cells contribute to the prevention of excessive proinflammatory Th1 responses and allow sustained Th2 response polarization, an outcome that is crucial for host survival during schistosomiasis.

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