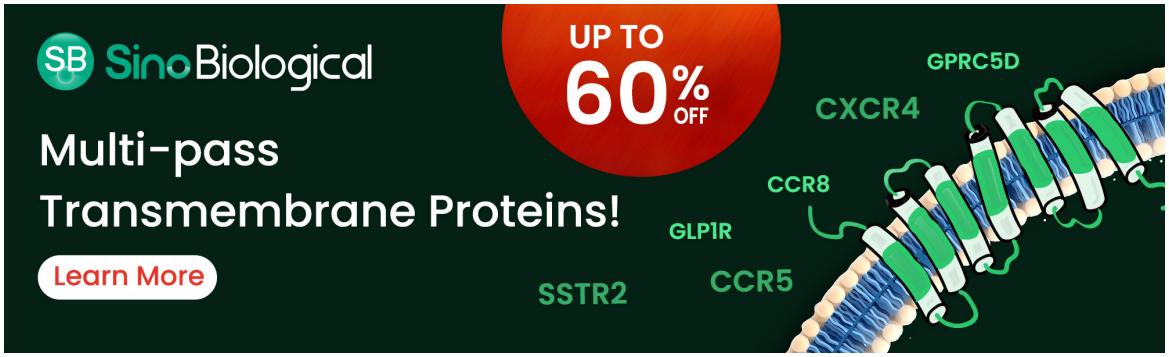

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The Pathogenesis of Schistosomiasis Is Controlled by Cooperating IL-10-Producing Innate Effector and Regulatory T Cells

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IL-10 reduces immunopathology in many persistent infections, yet the contribution of IL-10 from distinct cellular sources remains poorly defined. We generated IL-10/recombination-activating gene (RAG)2-deficient mice and dissected the role of T cell- and non-T cell-derived IL-10 in schistosomiasis by performing adoptive transfers. In this study, we show that IL-10 is generated by both the innate and adaptive immune response following infection, with both sources regulating the development of type-2 immunity, immune-mediated pathology, and survival of the infected host. Importantly, most of the $CD4^+$ T cell-produced IL-10 was confined to a subset of T cells expressing CD25. These cells were isolated from egg-induced granulomas and exhibited potent suppressive activity in vitro. Nevertheless, when naive, naturally occurring $CD4^+CD25^+$ cells were depleted in adoptive transfers, recipient IL-10/RAG2-deficient animals were more susceptible than RAG2-deficient mice, confirming an additional host-protective role for non-T cell-derived IL-10. Thus, innate effectors and regulatory T cells producing IL-10 cooperate to reduce morbidity and prolong survival in schistosomiasis. ***The Journal of Immunology*, 2004, 172: 3157–3166.**

Pathogen-triggered immune responses that are not regulated appropriately can cause significant damage to host tissues when they persist. Such is the case with schistosomiasis, a major infectious disease of the tropics, in which chronic egg-induced inflammation in the liver can lead to fibrosis, portal hypertension, bleeding, and eventual death. Schistosomiasis is caused by egg-laying adult parasites that reside in the mesenteric veins of their vertebrate hosts (1). In the case of *Schistosoma mansoni*, mature female worms produce hundreds of eggs a day, of which many exit the body through the gut. However, a substantial number are trapped in host tissues such as the liver and intestine. These trapped eggs induce a local inflammatory response and, as shown in the mouse model of the disease, Th2-associated cytokines play a prominent role (2–5). Indeed, when the egg-induced Th2 response is deviated to a more dominant Th1 response, parasite and egg burdens are unchanged, but granulomatous inflammation and fibrosis decrease substantially (6, 7).

In schistosome-infected mice and humans, IL-10 is elevated following infection, and its production has been linked with disease progression (8–11). In mice, IL-10 profoundly influences the polarization of the egg-induced Th2 response. In contrast to Th2-polarized immunocompetent wild-type (WT)[5] mice, infected IL-10-deficient animals generate a mixed Th1- and Th2-type immune response, which is sustained through chronic infection (12–14). As a consequence of the mixed response, the IL-10 mutant mice display severe liver damage and increased morbidity and mortality during the acute phase of the immune response (13). Moreover, in recent studies conducted with IL-$4^{-/-}$IL-$10^{-/-}$ and IL-$12^{-/-}$IL-$10^{-/-}$ double-knockout (KO) mice, we showed that IL-10 also protects schistosome-infected mice from the tissue-damaging and highly lethal effects of polarized immune responses (12, 13). The results from these studies and others suggest that IL-10 exhibits many important functional activities in vivo that go beyond its original description as a suppressive cytokine for Th1 responses (15).

IL-10 controls the growth and differentiation of a wide variety of immune cells and regulates immune responses by several distinct mechanisms (15). It suppresses the accessory cell activity of both macrophages and dendritic cells (DCs) by inhibiting production of cytokines like IL-12, IL-18, and TNF. It down-regulates MHC class II, CD40, and expression of several costimulatory molecules such as CD80 and CD86 (16–18). IL-10 also antagonizes the classical activation of macrophages (19) and helps drive an IL-4/IL-13-induced alternative state of activation where arginase-1, rather than NO synthase-2 expression, dominates (20, 21). Finally, IL-10 can affect T cell effector function by inhibiting T cell cytokine production and proliferation (22–24).

Although originally described as a product of $CD4^+$ Th2 cells (25), it is now known that a variety of cell types produce and

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[5] Abbreviations used in this paper: WT, wild type; KO, knockout; DC, dendritic cell; BMDC, bone marrow-derived DC; RAG, recombination-activating gene; SEA, soluble egg Ag; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HPRT, hypoxanthine-guanine phosphoribosyltransferase; Tr1, T regulatory 1.

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respond to IL-10. There is accumulating evidence that macrophage/dendritic- rather than T cell-derived IL-10 may be of particular importance in immunity to certain pathogens (26, 27). However, recent studies also suggest that specific populations of regulatory $CD4^+$ T cells with high production of IL-10 may be involved (28–30). These regulatory T cells can be either naturally occurring $CD4^+CD25^+$ T cells or $CD4^+$ T cells induced during the course of an immune response, with distinct subsets of DCs modulating their development (31). Several studies have shown their role in the control of organ-specific autoimmunity (32). Nevertheless, their involvement in the progression of schistosomiasis and other infectious diseases remains mostly unexplored (33). Thus, the relative importance of innate effector cell-derived IL-10 vs IL-10 produced by cells of the adaptive immune response is unclear and remains the subject of significant debate.

To examine the function of T cell- vs non-T cell-derived IL-10 in the pathogenesis of schistosomiasis, we developed an adoptive transfer model that allowed us to control the source of IL-10 in vivo. By adoptively transferring WT or IL-10-deficient $CD4^+$ T cells to recombination-activating gene $(RAG)^{-/-}$ or $RAG^{-/-}IL\text{-}10^{-/-}$ recipients, we restricted IL-10 expression to the T cell or non-T cell compartment (see Table II). Strikingly, both populations exhibited regulatory activity following infection with *S. mansoni* and were critical for host survival beyond the acute stage of infection. When T cells from granulomatous livers were isolated and stimulated with egg Ag (soluble egg Ag (SEA)), both $CD4^+CD25^-$ and $CD4^+CD25^+$ T cells produced IL-10, although the $CD4^+CD25^+$ population produced 3-fold higher amounts than the $CD4^+CD25^-$ T cells. Only granulomatous $CD4^+CD25^+$ T cells controlled the proliferation of naive T cells in vitro. Furthermore, when naturally occurring naive $CD4^+CD25^+$ T cells were depleted in adoptive transfers, recipient $IL\text{-}10^{-/-}RAG2^{-/-}$ mice were more susceptible to disease than $IL\text{-}10^{-/-}RAG^{-/-}$ mice that had been reconstituted with total $CD4^+$ T cells, demonstrating a specific host-protective effect of naturally occurring regulatory T cells. We also found significant differences in survival between recipient $RAG^{-/-}$ and $IL\text{-}10^{-/-}RAG^{-/-}$ mice, revealing a substantial host-protective role for non-T cell-derived IL-10 in schistosomiasis.

Materials and Methods

Mice, parasites, and Ag preparations

Female 42-day-old C57BL/6Ai, C57BL/10SgSnAi, C57BL/10SgSnAi-[KO]IL-10, C57BL/10SgSnAi-[KO]RAG2 were obtained from Taconic Farms (Germantown, NY). C57BL/10SgSnAi-[KO]IL-10 and C57BL/10SgSnAi-[KO]RAG2 were crossed for the generation of IL-10/RAG2 double-deficient mice and housed in a barrier facility (Taconic Farms). After delivery to the National Institutes of Health, all mice were housed under specific pathogen-free conditions in a National Institutes of Health American Association for the Accreditation of Laboratory Animal Care-approved animal facility. Cercariae of a Puerto Rican (Naval Medical Research Institute) strain of *S. mansoni* (Biomedical Research Institute, Rockville MD) were obtained from infected *Biomphalaria glabrata* snails. *S. mansoni* eggs were extracted from the livers of infected mice and enriched for mature eggs. SEA was obtained from homogenized *S. mansoni* eggs as described (6).

Immunizations, infections, and cell transfers

For percutaneous infections, groups of 10 mice were exposed by tail for 40 min in water containing 25–30 cercariae. In designated experiments, mice were sacrificed 8–9 wk after infection. In survival studies, infected mice were monitored for up to 100 days after infection, and all mice were bled from the tail vein on wk 8 after infection to measure the concentration of liver transaminases in their serum. Pulmonary granulomas were induced as described elsewhere (34)

For cell transfers, freshly prepared single-cell suspensions from spleens of naive, female C57BL/10SgSnAi and C57BL/10SgSnAi[KO]IL-10 mice were incubated with CD4-specific, magnetic microbeads (Miltenyi Biotec, Auburn, CA). $CD4^+$ cells were positively selected on magnetic separation$^+$ columns according to the manufacturer's protocol (Miltenyi Biotec). The purity of $CD4^+$ cells was >95% in all experiments. Between 1.0 and 1.5×10^6 $CD4^+$ cells in 0.5 ml of PBS were injected i.p. into the designated recipient mice. In infection experiments, the cells were transferred during the third week after infection, and in pulmonary granuloma studies, the cells were injected i.p. simultaneously with eggs during the primary egg inoculation. For depletion of $CD4^+CD25^+$ T cells, splenocytes from naive C57BL/10 mice were negative selected for $CD4^+$ T cells, using a magnetic bead mixture (anti-CD8, I-A^b, and NK) on the AutoMACS magnetic separation system (Miltenyi Biotec). The isolated $CD4^+$ T cells were incubated with anti-CD25-PE Ab (BD PharMingen, San Diego, CA), then with anti-PE magnetic beads, followed by a sensitive depletion protocol on the AutoMACS (Miltenyi Biotec). The purity of the $CD4^+$ T cell population was generally >98%, and the CD25 depletion was complete. The sorted cells were transferred at the time of infection with 10^6 cells per animal.

Histopathology, fibrosis measurement, and liver transaminases

Approximately half of the liver was fixed in Bouin-Hollande solution. Histologic sections were processed and stained with Giemsa (Histo-Path of America, Clinton, MD). The diameter and cell composition of granulomas (30 per mouse) surrounding single eggs were measured using an ocular micrometer, and the volume of each granuloma was calculated assuming a spherical shape. An experienced pathologist estimated the percentage of eosinophils. Only granulomas around mature, viable eggs were measured in the liver. The collagen content of the liver samples, determined as hydroxyproline, was analyzed as described previously (35).

The liver transaminases glutamic oxalacetic transaminase/aspartate aminotransferase (AST) and glutamic pyruvic transaminase/alanine aminotransferase (ALT) were measured in individual serum samples, taken from the tail vein of infected mice 8 wk after infection. Transaminase levels were measured by a modified protocol from a commercially available kit (Sigma-Aldrich, St. Louis, MO) as described elsewhere (36).

Gene expression analysis by real-time PCR

Tissue samples from the livers of infected mice or the lungs of egg-challenged mice were stored in Stat-60 at −80°C. RNA was extracted from homogenized tissue by the Stat-60 method (Tel-Test, Friendswood, TX) and further purified using the RNeasy mini-kit from Qiagen (Valencia, CA). Individual sample RNA (1 μg) was reverse-transcribed using Superscript II (Invitrogen, Carlsbad, CA) and a mixture of oligo(dT) and random primers. Real-time PCR was performed on an ABI Prism 7900 sequence detection system (Applied Biosystems, Foster City, CA). Primers (Table I) were designed using PrimerExpress software (Applied Biosystems). Relative quantities of PCR product was determined using SYBR Green PCR Master Mix (Applied Biosystems) and by the comparative threshold cycle method as described by Applied Biosystems for the ABI Prism 7700/7900

Table I. *Primer list*

Gene	Sense	Antisense
IL-4[a]	ACG AGG TCA CAG GAG AAG GGA	AGC CCT ACA GAC GAG CTC ACT C
IL-5[a]	TGA CAA GCA ATG AGA CGA TGA GG	ACC CCC ACG GAC AGT TTG ATT C
IL-10[b]	ATA ACT GCA CCC ACT TCC CAG TC	CCC AAG TAA CCC TTA AAG TCC TGC
IL-13[a]	GGC AGC ATG GTA TGG AGT GTG	TGG GTC CTG TAG ATG GCA TTG
HPRT[a]	GCC CTT GAC TAT AAT GAG TAC TTC AGG	TTC AAC TTG CGC TCA TCT TAG G

[a] Generated from mouse genomic databases by PrimerExpress (Applied Biosystems).
[b] From Ref. 27.

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sequence detection systems. Thereby, each sample was normalized to hypoxanthine-guanine phosphoribosyltransferase (HPRT) and expressed as a fold increase or decrease vs uninfected controls.

Cell isolation, flow cytometry, and cell sorting

Granuloma cells were isolated from liver tissue of infected C57BL/6Ai mice as described (14). Briefly, livers were perfused with sterile PBS to remove the blood, and then tissue was passed through a sterile stainless sieve and washed twice with cold PBS. The pellet of granulomas was resuspended in RPMI 1640 containing 1 mg/ml collagenase D (Roche, Basel, Switzerland) and 4 U/ml DNase I (Sigma-Aldrich) and digested for 20–30 min at 37°C. The digested material was passed through a cell strainer (100 μm) and washed twice with RPMI 1640. Erythrocytes were lysed with ACK buffer (BioWhittaker, Walkersville, MD), and cells were washed two times with RPMI 1640. Spleens and mesenteric lymph nodes from the same mice were passed through cell strainers (100 μm), washed twice with RPMI 1640, and treated with ACK buffer (BioWhittaker).

Cell sorting was performed on a FACSVantage (BD Biosciences, Mountain View, CA). Viable isolated granuloma cells were stored overnight at 4°C in sterile PBS and stained the next morning with anti-CD4-CyChrome (clone H129.19) or anti-CD4-TriColor (Caltag Laboratories, Burlingame, CA) and anti-CD25-PE (clone PC61) Abs (BD PharMingen). Sorting gates were set on $CD4^{bright}CD25^{dull}$ and $CD4^{bright}CD25^{bright}$ cells. The purity of isolated cell fractions was >98%.

Cytokine detection

Bone marrow-derived DCs (BMDCs) were prepared from IL-$10^{-/-}$ mice as described (37). Cells were recovered after 6–7 days. $CD4^+CD25^+$ and $CD4^+CD25^-$ cells were isolated and sorted from liver granulomas of chronically infected animals (at least 12 wk postinfection). A total of 1×10^5 sorted granuloma cells was incubated with 3.3×10^4 BMDCs in the presence of 20 μg/ml SEA in 96-well U-bottom microtiter plates in triplicate. After 3 days, the supernatants were removed, stored at −20°C, and later analyzed for cytokines.

Cytokines were analyzed using a mouse cytokine multiplex assay from Linco Research (St. Charles, MO). The assay was performed according to the manufacturer's protocol. Briefly, 25 μl of cell supernatant was incubated with Ab-coated bead mix for 16 h at 4°C. The beads were washed twice and incubated with secondary biotinylated Ab mix for 1 h at room temperature. Streptavidin-PE was added and incubated for additional 30 min at room temperature. The beads were washed three times and then analyzed on a Luminex 100 platform (Luminex, Austin TX). Cytokine concentrations were calculated from standard curves.

In vitro suppression assay

Proliferation assays were performed by culturing freshly isolated naive $CD4^+$ T cells (5×10^4) in U-bottom microtiter plates (0.2 ml) with irradiated, T cell-depleted spleen cells as APCs ($1–2 \times 10^5$), and titrated numbers of freshly isolated and FACS-sorted $CD4^+CD25^+$ or $CD4^+CD25^-$ T cells for 72 h at 37°C in complete medium. Cultures were polyclonally stimulated by soluble anti-CD3 and pulsed with [^{3}H]TdR for the last 12 h. For cytokine analysis, supernatants were taken at 72 h, and the production of IL-10 was measured as described above, using the Luminex technology.

$CD4^+$ responder T cells were prepared from spleens or lymph nodes of adult C57BL/6 mice. Single-cell suspensions were prepared as described above, and responder T cells were purified either by negative (depletion of $B220^+$, CD4, and I-A^b-positive cells) or positive selection (using CD4 magnetic beads) on the AutoMACS magnetic separation system (Miltenyi Biotec).

Statistical analysis

Hepatic fibrosis per egg decreases with increasing intensity of infection (total number of hepatic eggs) in the infection experiments (38). Therefore, these variables were compared by analysis of covariance, using the log of total liver eggs as the covariate and the log of hydroxyproline per egg. Survival of infected mice was analyzed and compared by log rank test using GraphPad Prism, version 4.0 (GraphPad, San Diego, CA). Change in body weight was analyzed using a two-way ANOVA with Bonferroni posttest (GraphPad Prism). All other variables were compared by Student's *t* test with Welch's correction (GraphPad Prism). Results were considered significant for $p < 0.05$.

Results

T cells and non-T cells produce IL-10 independently

By genetically crossing $RAG^{-/-}$ and IL-$10^{-/-}$ mice, we developed an adoptive transfer model to control the source of IL-10 during infection with *S. mansoni* (Table II). Mice from all eight experimental groups were sacrificed on wk 8 postinfection, the peak of the acute egg-induced inflammatory response in the liver. Total RNA was isolated from individual liver samples and analyzed by real-time PCR to quantify IL-10 mRNA levels within the granulomatous tissues. A marked induction of IL-10 mRNA was observed in $RAG^{-/-}$ mice, confirming the expression of IL-10 by a non-T cell source following infection (Fig. 1). IL-10 mRNA expression was also at comparable levels with the infected immunocompetent WT controls despite the absence of T cells in the $RAG^{-/-}$ mice. As expected, no IL-10 mRNA was detected in the infected IL-$10^{-/-}RAG^{-/-}$ group that had not received T cells. However, when infected IL-$10^{-/-}RAG^{-/-}$ mice were reconstituted with naive WT $CD4^+$ T cells, IL-10 mRNA was induced, albeit to a lesser extent than in all other IL-10-competent groups. These findings indicate that IL-10-producing T cells can develop in the absence of an innate source of IL-10 and migrate effectively to the granulomatous tissues. The results also confirm that IL-10 can be induced independently in both T cell and non-T cell populations.

T cells and non-T cells producing IL-10 both contribute to the development of polarized Th2 cytokine responses in vivo

Expression of mRNA for the Th2-associated cytokines IL-4, IL-5, and IL-13, was also assessed in the granulomatous livers. Because T cell-deficient mice fail to develop significant granulomas (39), not surprisingly, expression of all three cytokines was nearly undetectable in the control $RAG^{-/-}$ (−) and IL-$10^{-/-}RAG^{-/-}$ (−) groups not receiving T cells, thus confirming $CD4^+$ Th2 cells as the major source of these mediators (Fig. 2, *A–C*). However, following reconstitution with WT $CD4^+$ T cells, both groups expressed mRNA for IL-4, IL-5, and IL-13, although the pattern in the $RAG^{-/-}$ mice more closely mirrored the response in WT animals. Indeed, although IL-4 and IL-13 mRNA increased in the IL-$10^{-/-}RAG^{-/-}$ mice after reconstitution, the levels were significantly lower than in the WT $CD4^+$ reconstituted $RAG^{-/-}$ group. These data suggest that non-T cell-derived IL-10 contributes to the development of the Th2 response. Nevertheless, the results from these studies also indicate an important, albeit lesser role, for T cell-derived IL-10. In nearly every case, when IL-10KO $CD4^+$ T cells were used instead of WT $CD4^+$ cells, the mice

Table II. *Adoptive transfer model to control the cellular source of IL-10*

Recipient Mice[a]	Transferred[b] $CD4^+$ Cells	CD4 T Cell Response	Cellular Source of IL-10	
			T cells	Non-T cells
WT		+	+	+
IL-10 KO		+	−	−
RAG KO		−	−	+
RAG KO	WT	+	+	+
RAG KO	IL-10 KO	+	−	+
IL-10/RAG KO		−	−	−
IL-10/RAG KO	WT	+	+	−
IL-10/RAG KO	IL-10 KO	+	−	−

[a] Groups of 10 control or recipient mice (all C57BL/10) were infected percutaneously with 30 cerceriae.

[b] Naïve $CD4^+$ cells were isolated from spleens of WT or IL-$10^{-/-}$ donor mice (all C57BL/10) by a magnetic bead assay, and between 1.0 and 1.5×10^6 cells were injected i.p. into recipient animals during the third week after infection.

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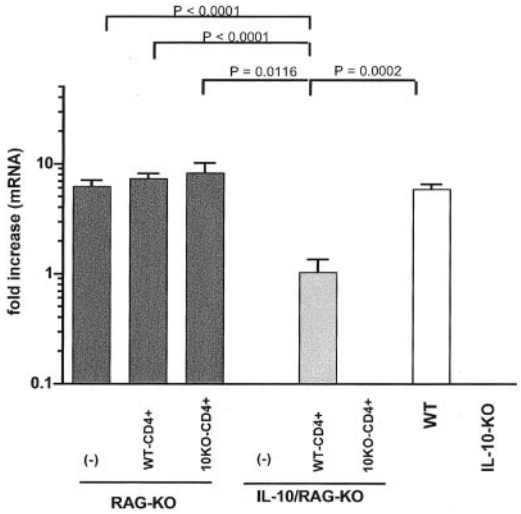

FIGURE 1. T cells and non-T cells express IL-10 mRNA independently. Groups of 10 mice were exposed to 30 cercariae, and $1.0–1.5 \times 10^6$ naive $CD4^+$ T cells were transferred to recipient mice 3 wk postinfection. Liver samples were taken from individual mice at the time of sacrifice (wk 8). Total RNA was isolated from randomly chosen samples of two independent experiments and analyzed individually by real-time PCR for relative quantities of IL-10 mRNA. Results were normalized to expression of HPRT and are expressed as fold increase over average IL-10 mRNA expression in naive WT mice ($n = 4$) on a $\log_{10}$ scale. Results are shown as group mean ± SEM (n = 7–10/group). Significance was determined by Student's t test ($p < 0.05$).

displayed reduced Th2 cytokine mRNA expression, and interestingly, it did not matter whether the recipient animals were RAG- or IL-10/RAG-deficient. This was particularly evident for IL-5 and IL-13, although similar findings were seen with IL-4 in the IL-$10^{-/-}$RAG$^{-/-}$ animals. Together, these data strongly indicate that both T cell- and non-T cell-derived IL-10 contribute to the development of polarized Th2 responses in vivo.

However, there were some differences among the Th2 cytokines. In particular, expression of IL-5 mRNA appeared to be controlled to a greater extent by T cell-derived IL-10. This was corroborated by the tissue eosinophil response, where reduced numbers of eosinophils were found in the groups receiving IL-$10^{-/-}$ $CD4^+$ T cells compared with mice receiving WT $CD4^+$ T cells. In contrast, no significant difference in either IL-5 (Fig. 2*C*) or tissue eosinophils (*F*) was noted when the responses in corresponding RAG$^{-/-}$ and IL-$10^{-/-}$RAG$^{-/-}$ mice were compared, regardless of whether WT vs IL-$10^{-/-}$ $CD4^+$ T cells were transferred. Surprisingly, however, despite the marked decrease in several Th2 cytokines (Fig. 2, *A–C*) and the obvious change in the cellular makeup of the lesions (*F*), granuloma volumes did not differ appreciably between the $CD4^+$-reconstituted RAG$^{-/-}$ and IL-$10^{-/-}$RAG$^{-/-}$ mice (*D*). Indeed, although granuloma development was strictly dependent on the presence of $CD4^+$ T cells (39), the IL-10-producing ability of the transferred cells appeared relatively unimportant (Fig. 2*D*). Similar conclusions were generated when liver hydroxyproline levels (a measure of tissue fibrosis) were compared (Fig. 2*E*). Here again, the differences between T cell- and non-T cell-derived IL-10 appeared minor, although a consistent increase in fibrosis was observed in the groups receiving IL-$10^{-/-}$ $CD4^+$ T cells, mirroring the pattern observed between WT and IL-$10^{-/-}$ controls. As reported recently in a related paper (40), decreased expression of the IL-13 decoy receptor likely explains the increase in IL-13-dependent fibrosis in these mice. Real-time PCR results from the adoptive transfer study showed reduced IL-13Rα2 levels in the groups receiving IL-$10^{-/-}$ $CD4^+$ T cells, further supporting this conclusion (data not shown).

T cells are major IL-10 producers controlling egg-induced morbidity and mortality

Serum concentrations of liver transaminases were measured as a highly sensitive indicator of liver damage. In this model, liver toxicity results from both the direct actions of secreted egg Ags and from the indirect effects of the overexuberant and sustained local immune response (41). Consistent with previous findings (13), IL-$10^{-/-}$ mice developed significant increases in both ALT and AST activity relative to WT mice (Fig. 3, *A* and *B*), which was attributable to their elevated Th1-associated cytokine response (13). In contrast, only marginal increases in ALT/AST levels were observed in infected RAG$^{-/-}$ and IL-$10^{-/-}$RAG$^{-/-}$ controls compared with naive controls, confirming the critical role of $CD4^+$ T cells in the development of egg-mediated pathology and liver damage (39). Consistent with the more modest responses reported in WT mice vs IL-$10^{-/-}$ controls, mice receiving WT $CD4^+$ cells consistently displayed smaller increases in serum ALT/AST than did animals receiving IL-$10^{-/-}$ $CD4^+$ T cells. The findings were similar in RAG- and IL-10/RAG-deficient mice, suggesting a more critical role for IL-10-producing T cells in the hepatoprotective response.

In addition to measuring transaminase levels, we examined the survival of T cell-reconstituted animals in three separate experiments. The pooled data from all experiments showed that most infected C57BL/10 WT mice survived well through chronic infection (70%), whereas IL-$10^{-/-}$ mice displayed 80% mortality by wk 10 (Fig. 3*C*). The infected RAG$^{-/-}$ and IL-$10^{-/-}$RAG$^{-/-}$ mice that had not received $CD4^+$ T cells also displayed a high degree of susceptibility with nearly 100% mortality in both groups by wk 14, although the median survival time of the RAG$^{-/-}$ mice (67 days) was slightly but consistently longer than the IL-$10^{-/-}$ RAG$^{-/-}$ controls (64 days) (Fig. 3*C*). When RAG$^{-/-}$ (median survival, 58 days) (Fig. 3*D*) and IL-$10^{-/-}$RAG$^{-/-}$ (57 days) (*E*) mice were reconstituted with IL-$10^{-/-}$ $CD4^+$ T cells, the mortality rates were virtually indistinguishable and reduced relative to the IL-$10^{-/-}$ controls (63 days) (*C*). In marked contrast, when adoptive transfers were performed with WT $CD4^+$ T cells, a significant number of mice survived through 100 days of infection, and it did not matter whether the recipient mice were RAG- (Fig. 3*D*) or IL-10/RAG-deficient (*E*). When the survival of T cell-recipient mice was compared, we found significant differences between WT $CD4^+$ and IL-$10^{-/-}$ $CD4^+$ T cell transfers for each strain ($p = 0.0001$ for RAG$^{-/-}$; $p = 0.011$ for IL-$10^{-/-}$RAG$^{-/-}$). Furthermore, the difference in survival between the IL-$10^{-/-}$ $CD4^+$ T cell-recipient RAG-deficient mice and WT $CD4^+$ T cell-recipient IL-10/RAG$^{-/-}$ mice was also highly significant ($p = 0.0059$). Thus, the presence of an IL-10-competent T cell is essential for host survival in acute schistosomiasis. Nevertheless, although not reaching significance in every study, we also noted greater median survival times in RAG$^{-/-}$ vs IL-$10^{-/-}$RAG$^{-/-}$ mice receiving WT $CD4^+$ T cells (74 vs 64 days). Together, these data and the results in Fig. 3*C* indicate an additional but less pronounced contribution by the non-T cell population as well.

It is important to point out that the survival of all adoptively transferred animals was never expected to fully duplicate the pattern observed with WT mice, because B cells, Abs, and other non-T cell related factors are known to contribute to host survival as the infection becomes chronic (42). Therefore, it is not surprising that infected WT controls survive much longer even though

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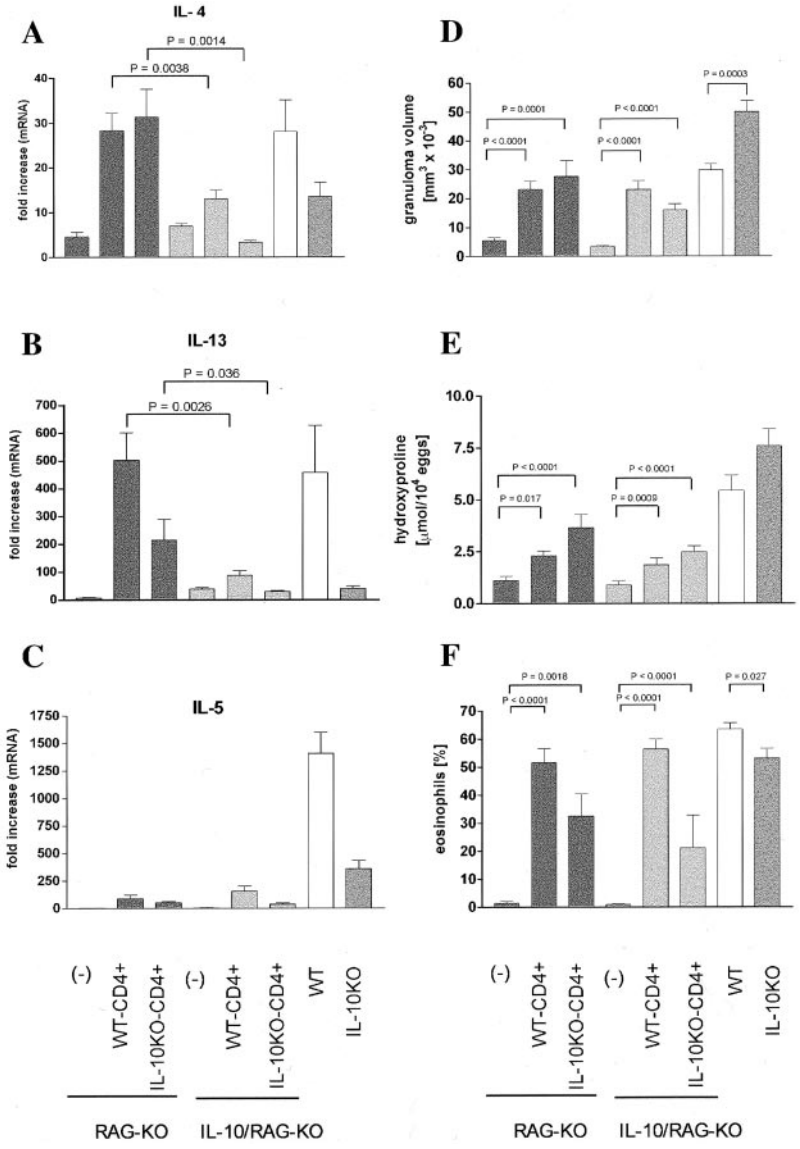

FIGURE 2. Even though the Th2 response is controlled by both T cell- and non-T cell-derived IL-10, liver pathology is influenced more by the T cell component. Groups of 10 mice were infected with 30 cercariae, and 1.0–1.5 $\times$ 10^6 naive $CD4^+$ T cells were transferred as in Fig. 1. IL-4 (*A*), IL-13 (*B*), and IL-5 (*C*) mRNA levels were determined by real-time PCR using total liver RNA from the same samples used in Fig 1 (n = 7–10/group). Results were normalized to individual HPRT expression and are expressed as fold increase over expression in naive control mice (n = 4 for each strain). Results shown are group mean $\pm$ SEM. Liver tissues of individual mice were also analyzed for granuloma volumes (*D*), liver fibrosis (*E*), and tissue eosinophilia (*F*). Fibrosis was quantified by analysis of hydroxyproline content and expressed as micromoles of hydroxyproline per 10,000 eggs. Results are shown as group mean $\pm$ SEM. For liver fibrosis measurements, significance was determined by analysis of covariance ($p < 0.05$). All other data were analyzed by Student's t test ($p < 0.05$). Similar pathology data were obtained in a second experiment.

they show similar or higher transaminase levels at wk 8 postinfection compared with the more susceptible $CD4^+$ T cell recipient mice or T and B cell-deficient control animals.

A subset of $CD4^+$ T cells is the main producer of T cell-derived IL-10 and exhibit regulatory activity

Given the relative importance of T cell-derived IL-10, we examined whether IL-10 was produced equally by all granuloma-associated $CD4^+$ T cells. There is increasing evidence that not all T cells have the same ability to produce IL-10. In particular, subpopulations of $CD4^+$ T cells that exhibit regulatory activity and produce IL-10 have been described recently (43), including naturally occurring regulatory T cells that constitutively express CD25, the IL-2Rα-chain. In the absence of more specific markers, we isolated inflammatory cells from the livers of chronically infected WT mice (>12 wk infected), sorted for $CD4^+CD25^-$ and $CD4^+CD25^+$ cells, and analyzed their cytokine expression profile. Both fractions of $CD4^+$ cells were cultured with freshly generated BMDCs from IL-10-deficient donors in the presence of SEA and then assayed for cytokine production by multiplex bead ELISA. The results show that each population produced similar amounts of all Th2-associated cytokines except IL-10 (Fig. 4*A*). Little production of Th1-associated cytokines was observed. Strikingly, the $CD4^+CD25^+$ T cell population produced on average three times more IL-10 than $CD4^+CD25^-$ T cells. Thus, although both populations were skewed toward a Th2-dominant response, IL-10 was unique in that the $CD25^+$ T cell population produced it at exceedingly high levels.

A hallmark of regulatory T cells is their ability to control T cell expansion (44, 45). To determine whether $CD25^+$ T cells from schistosome-infected mice exhibit regulatory activity, $CD4^+CD25^+$ and $CD4^+CD25^-$ cells were purified from the livers of chronically infected mice and cultured in vitro with naive T cells in increasing numbers. For assessment of proliferation, the naive T cells were stimulated with soluble anti-CD3. Proliferation rates were determined after 72-h incubation (Fig. 4*B*). Importantly, $CD4^+CD25^-$ T cells displayed no inhibitory activity, even when large numbers of cells were included. In marked contrast, purified $CD4^+CD25^+$ T cells inhibited the proliferation of naive T cells in a dose-dependent manner (Fig. 4*B*). Moreover, when anti-IL-10R Ab was included in the assay, T cell proliferation was partially restored in the $CD4^+CD25^+$ group (Fig. 4*C*). In contrast, the Ab had no significant effect when $CD4^+CD25^-$ T cells were used.

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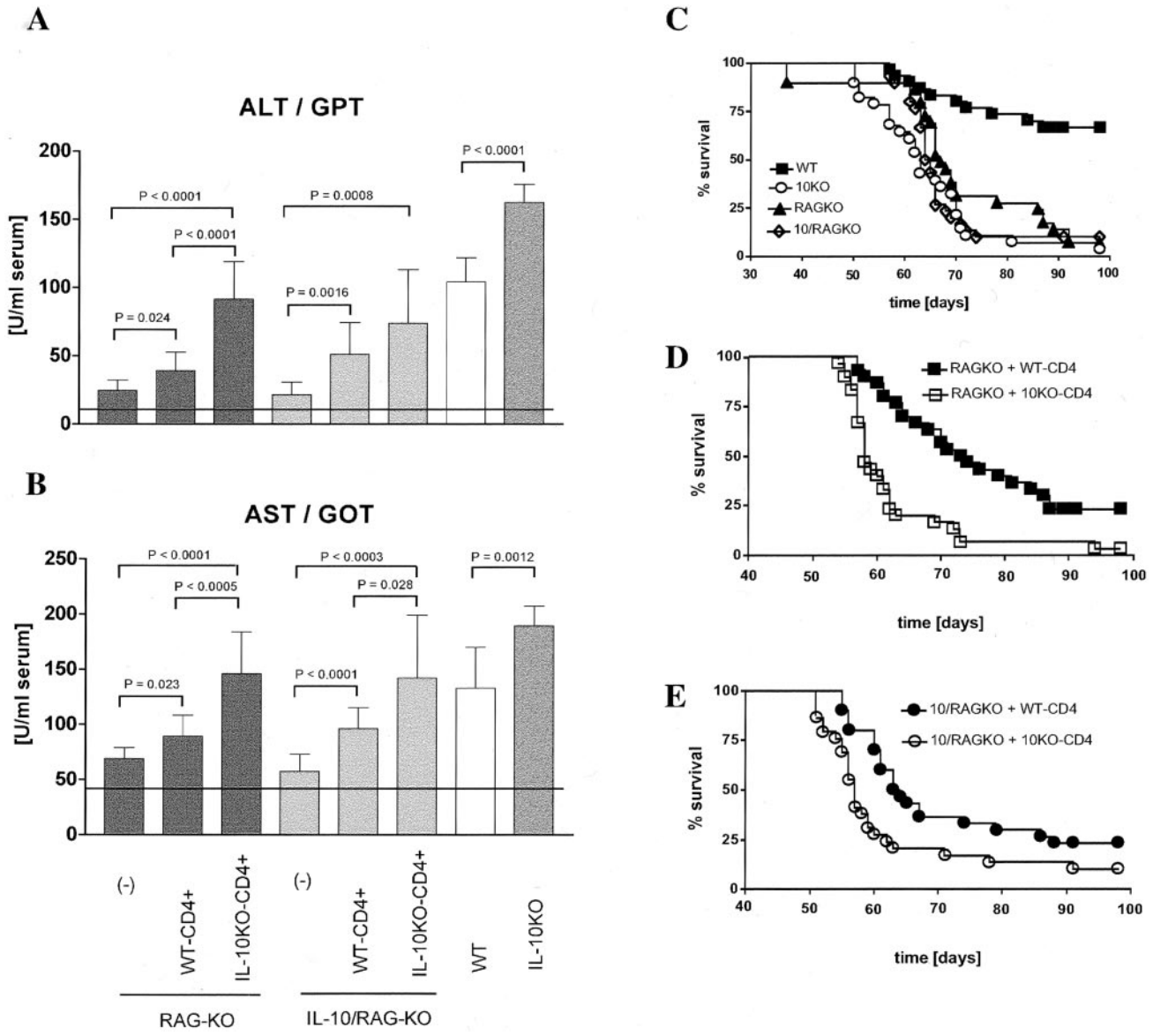

FIGURE 3. Egg-induced hepatotoxicity and survival of infected mice is controlled primarily by T cell-derived IL-10. Groups of 10 mice were infected with 30 cercariae, and 1.0–1.5 $\times$ 10^6 naive $CD4^+$ T cells were transferred as in Fig. 1. Liver ALT/AST levels were determined in individual serum samples (*A* and *B*) prepared 8 wk postinfection. The data are expressed as group mean $\pm$ SEM (n = 7–10). The line denotes the mean transaminase levels in all naive controls (n = 4/strain). Significance was determined by Student's t test ($p < 0.05$). The results are representative of two experiments. Survival of infected mice in all eight experimental groups was monitored in three independent experiments for up to 98 days. *C—E*, Shown are the pooled data for each group from the three experiments (n = 30/group): survival of WT, IL-$10^{-/-}$, $RAG^{-/-}$, and IL-$10^{-/-}RAG^{-/-}$ mice (*C*); survival of $RAG^{-/-}$ mice receiving IL-$10^{-/-}$ $CD4^+$ or WT $CD4^+$ T cells (*D*); and survival of IL-$10^{-/-}RAG^{-/-}$ mice receiving IL-$10^{-/-}$ $CD4^+$ or WT $CD4^+$ T cells (*E*). Survival data were analyzed for significant differences by GraphPad Prism 4.0 software, using a log rank test to compare survival curves ($p < 0.05$).

IL-10-producing innate effectors and $CD4^+CD25^+$ T cells cooperate to reduce liver damage and prolong survival during acute S. mansoni *infection*

CD25 is not a perfect marker of naturally occurring regulatory T cells, because CD25 can also be expressed by both activated T cells and induced regulatory T cells. Therefore, it is not an exclusive phenotypic marker for naturally occurring regulatory T cells. In an attempt to specifically determine the contribution of naturally occurring $CD4^+CD25^+$ regulatory T cells to protection from lethal egg-induced pathology, adoptive transfer experiments were performed with naive WT $CD4^+$ T cells depleted of $CD25^+$ cells. In addition, to establish whether non-T cell-derived IL-10 was required for the development of the protective T cell response, $RAG^{-/-}$ and $RAG^{-/-}$IL-$10^{-/-}$ mice were both used as recipient hosts. WT mice were included as controls, and as expected, they appeared normal, displayed no signs of cachexia, gained weight throughout the infection (not shown), and showed only a modest increase in serum transaminase levels (Fig. 5*D*), and most survived up to day 100 postinfection (*A*). Generally, lymphopenic mice reconstituted with CD25-depleted $CD4^+$ T cells spontaneously develop autoimmunity; however, B6/B10 genetic backgrounds show a greater resistance to autoimmunity, with only a minor form of intestinal inflammation/wasting disease appearing in animals receiving $CD4^+CD25^-$ T cells (46). To control for the relative contribution of autoimmune disease induced in our system, uninfected $RAG^{-/-}$ and IL-$10^{-/-}RAG^{-/-}$ mice were injected with 1 $\times$ 10^6

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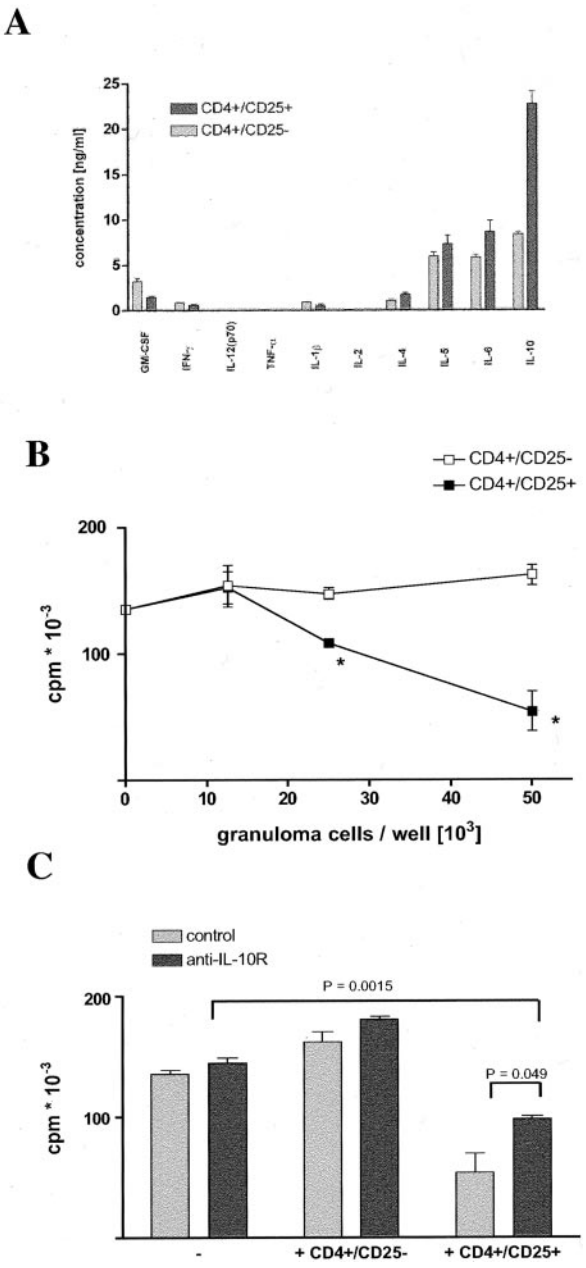

FIGURE 4. In hepatic granulomas, $CD4^+CD25^+$ T cells are the main producers of T cell-derived IL-10 and control the proliferation of naive T cells in part by IL-10. *A*, For cytokine analysis, hepatic granuloma cells were isolated from 10 chronically infected C57BL/6 mice (>12 wk postinfection). Cells were stained for CD4 and CD25 and then sorted for $CD4^+CD25^-$ and $CD4^+CD25^+$ expression. Purity of the cell fractions was >98%. Sorted cells (1×10^5) were cocultured with BMDCs (3.3×10^4) from IL-10 KO mice in the presence of 20 μg of soluble egg extract (SEA) for 72 h. Supernatants were analyzed for cytokines by a mouse cytokine multiplex bead assay. Results shown are means of triplicate samples and were confirmed in a second experiment. *B*, For proliferation assays, single-cell suspensions from liver granulomas of 10 chronically infected C57BL/6 mice were sorted for $CD4^+CD25^-$ and $CD4^+CD25^+$ cells. Increasing numbers of sorted cells were added to naive $CD4^+$ T cells (5×10^4) and irradiated spleen cells (2×10^5). The naive T cells were stimulated with soluble anti-CD3 Ab. After 72 h, T cell proliferation was determined by incorporation of radioactive [^{3}H]thymidine for an additional 12-h incubation. Data are displayed as mean counts per minute of triplicates. *C*, In designated wells, 25×10^3 sorted granuloma cells were plated with naive T cells as above, and IL-10 binding was blocked with a neutralizing IL-10R Ab or isotype control. Cell proliferation was determined and is denoted as mean counts per minute from triplicate wells. Significance was determined by Student's *t* test ($p < 0.05$). The results are representative of two experiments.

$CD25^+$-depleted $CD4^+$ cells and monitored for signs of wasting disease. These animals showed no signs of cachexia and no mortality up to 100 days after cell transfer (data not shown), confirming that host morbidity and mortality were attributed specifically to schistosome egg-induced liver damage and not resulting from an autoimmune response. Also consistent with earlier experiments, the infected $RAG^{-/-}$ and IL-$10^{-/-}RAG^{-/-}$ controls that had not received $CD4^+$ T cells both displayed low serum AST responses on wk 8, although there was a slight yet significant increase in the IL-$10^{-/-}RAG^{-/-}$ infected animals (Fig. 5*D*). Nevertheless, as the infections progressed, the mean survival time of the $RAG^{-/-}$ controls was significantly greater than that of the IL-$10^{-/-}RAG^{-/-}$ mice (almost 2 wk longer), confirming a role for non-T cell-derived IL-10 in host survival (Fig. 5*A*). Although infected $RAG^{-/-}$ mice receiving $CD25^+$-depleted $CD4^+$ T cells displayed significant increases in serum AST (Fig. 5*D*) and more weight loss (*E*) than the $RAG^{-/-}$ mice receiving nondepleted cells, the survival of both groups was similar (*B*). However, the results in the IL-$10^{-/-}RAG^{-/-}$ mice were different and much more striking. Consistent with infected $RAG^{-/-}$ mice, serum AST responses were significantly greater in the IL-$10^{-/-}RAG^{-/-}$ mice receiving CD25-depleted cells (Fig. 5*D*). They also displayed a more precipitous drop in weight between wk 7 and 8 postinfection than did IL-$10^{-/-}RAG^{-/-}$ mice given nondepleted T cells (Fig. 5*F*). However, in contrast to infected $RAG^{-/-}$ mice in which both populations of $CD4^+$ T cells extended the mean survival time, administering CD25-depleted $CD4^+$ T cells to the IL-$10^{-/-}RAG^{-/-}$ mice completely failed to provide protection, with 100% mortality observed by day 60 postinfection (Fig. 5*C*). In contrast, when whole $CD4^+$ T cells were transferred to the infected IL-$10^{-/-}RAG^{-/-}$ mice, survival was lengthened when compared with the CD25-depleted group ($p = 0.002$) (Fig. 5*C*), but never to the extent observed in $RAG^{-/-}$ mice ($p = 0.042$) (*B*).

Discussion

Triggering the immune system for defense against pathogenic organisms can pose a major dilemma for the host. The immune response must be potent enough to stop the invader but also specific and fine-tuned so that unwanted side effects, such as immune-mediated pathology, are avoided or minimized. In situations where the invading pathogen is not removed easily, as with many persistent parasitic infections, development of tissue pathology may be unavoidable and a necessary compromise to establish a successful and chronic host-parasite relationship. The mammalian immune system has a variety of mechanisms that appear to be designed to curtail innate and adaptive immune responses, so that an effective equilibrium is generated between the required host defense and the development of disease. Prominent among these mechanisms appears to be the requirement for the immunosuppressive cytokine IL-10.

The requirement for IL-10 is critical in several important human diseases including schistosomiasis, wherein marked increases in host morbidity and mortality are observed when IL-10 levels are low or absent (47–52). In murine schistosomiasis mansoni, IL-10 reduces hepatocyte damage induced by the parasite's eggs and is essential for maintaining a nonlethal chronic infection (13). IL-10 is produced by a variety of cells following infection, including activated T cells and macrophages, yet the specific contribution of IL-10 from these distinct cellular sources was previously unexplored. The generation of mice deficient in both IL-10 and RAG2 provided an ideal model system to address this issue, because their immune deficiencies could be manipulated selectively by adoptively transferring purified populations of WT, IL-10-deficient, or CD25-depleted $CD4^+$ T cells.

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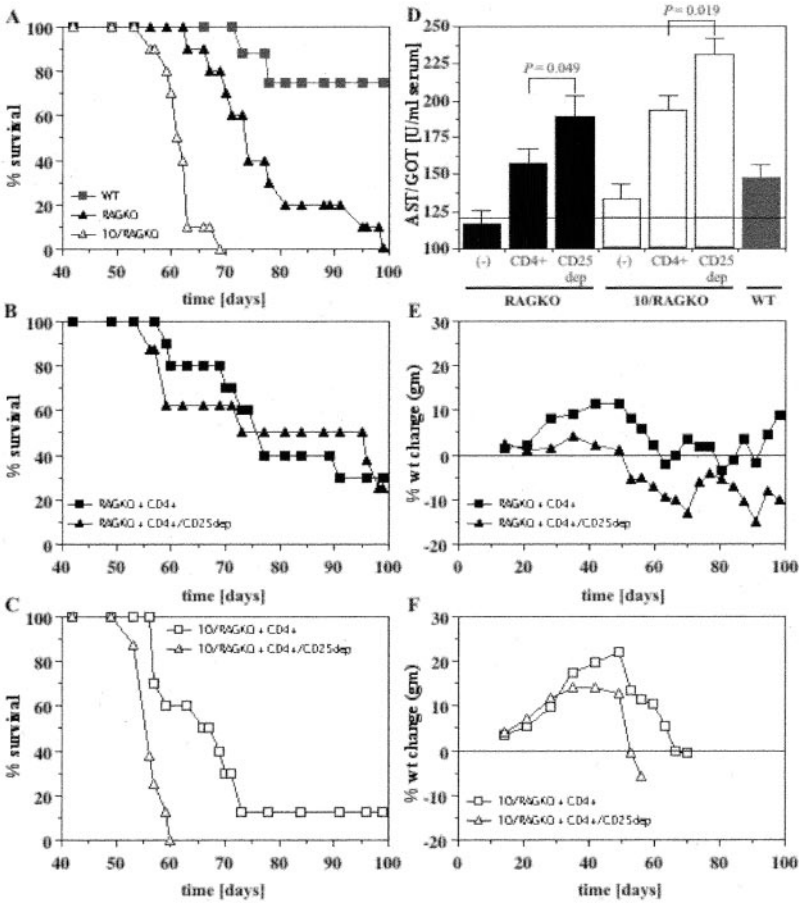

FIGURE 5. $CD4^+CD25^+$ T cells are required to protect RAG- and IL-10/RAG-deficient mice from the lethal effects of schistosome infection. A total of 1.0×10^6 naive $CD4^+$ T cells ($CD25^+$) or 1.0×10^6 naive $CD4^+$ T cells depleted of CD25-expressing cells ($CD25^-$) were transferred to recipient mice at the time of infection. All mice were exposed to ~30 *S. mansoni* cercariae. The survival of all infected mice was monitored over a period of 14 wk. *A*, Survival of WT ($n = 8$), $RAG^{-/-}$ ($n = 10$), and IL-10/$RAG^{-/-}$ control mice ($n = 10$). *B*, Survival of $RAG^{-/-}$ mice receiving whole $CD4^+$ ($n = 10$) or CD25-depleted $CD4^+$ T cells (CD25dep) ($n = 8$). *C*, Survival of IL-$10^{-/-}RAG^{-/-}$ mice receiving whole $CD4^+$ ($n = 10$) or CD25-depleted $CD4^+$ T cells ($n = 8$). Survival data were analyzed using GraphPad Prism 4.0 software, calculating survival fractions by the Kaplan-Meier method and using a log rank test to compare different curves ($p < 0.05$). *D*, Serum AST levels were determined in all mice on wk 8, and the values shown are averages ± SEM, with relevant *p* values shown. *E* and *F*, The weight of individual RAGKO (*E*) and IL-10/RAGKO (*F*) mice administered whole $CD4^+$ or CD25-depleted $CD4^+$ T cells was monitored at least twice weekly following infection, and the values shown represent the average change in weight as a percentage vs wk 1 postinfection.

Strikingly, our data from $RAG^{-/-}$ and IL-$10^{-/-}RAG^{-/-}$ control mice confirmed that much of the early acute egg-induced pathology is attributable to the $CD4^+$ T cell-mediated inflammatory response (39). Our initial adoptive transfer studies using IL-10-competent and IL-10-deficient $CD4^+$ T cells demonstrated that IL-10 is expressed by both $CD4^+$ T cell and non-T cell populations following infection (Fig. 1). Moreover, the ability to express IL-10 by each population appeared to be independent. Indeed, a significant IL-10 response was detected in the tissues of $RAG^{-/-}$ mice even without $CD4^+$ T cell reconstitution. Similarly, IL-$10^{-/-}$ $RAG^{-/-}$ mice that received only WT $CD4^+$ T cells also developed IL-10 responses, albeit at lower levels than the other groups. This latter finding was particularly important, because it illustrated two novel points: 1) that IL-10-producing $CD4^+$ T cells develop in the absence of any other source of IL-10, and 2) that IL-10-producing T cells are efficiently recruited to inflammatory sites, because little T cell-derived IL-10 was detected in peripheral lymphoid organs (data not shown), yet significant levels were found within the granulomatous tissues (Fig. 1).

One of the many essential functions of IL-10 during an immune response is to regulate the development of the $CD4^+$ T cell response. Several cytokine blocking and gene knockout studies showed that IL-10 is in large part responsible for the establishment of the polarized Th2 response that characterizes helminth infections (12, 13, 53–55). The Th2-associated cytokines IL-4, IL-5, and IL-13 all play important roles in the pathogenesis of schistosomiasis, with IL-4 and IL-13 directing granuloma formation (56), IL-13 as the primary stimulus for tissue fibrosis (57, 58), and IL-5 necessary for the prominent tissue eosinophil response (59, 60). Although many in vitro studies examining the regulation of Th1/Th2 responses by IL-10 suggested $CD4^+$ T cells might serve as a key autocrine source of IL-10, our adoptive transfer studies showed that non-T cell-derived IL-10 also has an immunoregulatory role. Although it is difficult to fully duplicate WT responses in T cell-reconstituted RAG2-deficient hosts, our $RAG^{-/-}$ mice developed Th2 responses that were comparable with those of WT controls when given WT $CD4^+$ T cells (Fig. 2). In contrast, when IL-10KO $CD4^+$ T cells were used in the transfer, a significant reduction in the most abundant Th2 cytokine, IL-13, was observed in $RAG^{-/-}$ recipients. Nevertheless, this was not accompanied by any consistent change in the levels of IL-4 or IL-5 mRNA, indicating that $CD4^+$ T cell-derived IL-10 might play a lesser role in Th2 polarization than previously hypothesized. Strikingly, the most dramatic reduction in the Th2 response was observed in the IL-$10^{-/-}RAG^{-/-}$ mice administered IL-10-deficient T cells. Although WT $CD4^+$ T cells partially restored IL-4, IL-5, and IL-13 production in the IL-$10^{-/-}RAG^{-/-}$ mice, when $RAG^{-/-}$ mice were used in the adoptive transfer, the Th2 response was much more robust. These findings demonstrate that, although both sources of IL-10 participate in Th2 response development, the non-T cell-derived component appears to have more impact.

Surprisingly, when host morbidity and mortality were considered, the IL-10-producing $CD4^+$ T cell population was more critical for host survival. When granuloma size, fibrosis, tissue eosinophilia, and serum transaminase levels (a measure of morbidity) were compared between the control (−), WT $CD4^+$, or IL-$10^{-/-}$ $CD4^+$ T cell groups, respectively, no significant differences were seen between the two recipient strains. However, when WT $CD4^+$ and IL-$10^{-/-}$ $CD4^+$ T cell responses were compared within each strain, marked differences were observed, most notably in survival, tissue eosinophil responses, and serum AST/ALT levels. Transferring IL-10-deficient T cells to the $RAG^{-/-}$ and IL-$10^{-/-}RAG^{-/-}$

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animals reduced the number of granuloma-associated eosinophils (Fig. 2*F*), increased serum transaminase levels (Fig. 3, *A* and *B*), and accelerated their time to death (Fig. 3, *D* and *E*). In fact, the mice receiving IL-10-deficient cells succumbed to the infection at a rate closely matching the mortality of the IL-10KO controls (Fig. 3*C*). The combined increase in pathology and liver damage likely explains the decreased survival time of IL-10$^{-/-}$ T cell recipient mice compared with WT T cell recipients. Finally, although there was evidence for a transient protective effect of non-T cell-derived IL-10 (Fig. 3*C*), statistical comparisons made between the RAG$^{-/-}$ and IL-10$^{-/-}$RAG$^{-/-}$ mice receiving either WT CD4$^+$ or IL-10$^{-/-}$ CD4$^+$ T cells (*D* and *E*) indicated no significant contribution beyond the acute stage of infection. Why T cell-derived IL-10 plays a more important role in this model remains unclear, although improved, prolonged, or more targeted delivery of IL-10 by regulatory T cells vs non-T cells at the site of granuloma formation might play a role.

Flow-cytometric sorting of granuloma cells revealed that a distinct subset of infiltrating T cells were responsible for a large fraction of the T cell-derived IL-10 (Fig. 4*A*). Interestingly, these cells stained brightly for CD4 and CD25, and functional assays revealed that they inhibited the proliferation of naive T cells in a dose-dependent manner. Furthermore, the suppressive activity was restricted to the CD25$^+$-expressing CD4$^+$ population (Fig. 4*B*) and partially inhibited by anti-IL-10R Abs, confirming a role for IL-10 (*C*). However, it is important to point out that CD25 is expressed on activated effector cells, naturally occurring regulatory T cells, and on some T regulatory 1 (Tr1) cell populations. By sorting for CD4$^+$CD25$^+$ cells in the absence of other characteristic markers of regulatory T cells, the Th2 cytokines we detected in our regulatory cell population could be derived from either one or a mix of these T cells.

We were able to directly demonstrate that naturally occurring CD4$^+$CD25$^+$ T cells play an important role in the regulation of host protection in this model by transferring naive CD25-depleted or whole CD4$^+$ T cells to RAG-deficient mice. Strikingly, the results from these studies showed that regulatory CD4$^+$CD25$^+$ T cells markedly protected the liver from egg-induced damage. Regardless of the mouse strain used, serum AST/ALT levels increased significantly when CD25-depleted CD4$^+$ T cells were transferred (Fig. 5*D*). The CD25-depleted CD4$^+$ T cell-recipient RAG- (Fig. 5*E*) and IL-10/RAG-deficient (*F*) hosts also lost more weight throughout the infection. However, this contrasted with the IL-10$^{-/-}$RAG$^{-/-}$ mice, where the pre-existing population of naive CD25$^+$ T cells significantly prolonged host survival during the acute stage of infection (Fig. 5*C*). One striking observation was that the endogenous population of regulatory T cells appeared to play no role in host survival when transferred to the IL-10-competent RAG$^{-/-}$ mice (Fig. 5*B*). These data together with the results in Fig. 3, *C—E*, strongly indicate that protective immunoregulation in schistosomiasis results from a coordinated cooperation between IL-10-producing innate effector and regulatory T cells. As it has recently been shown that a unique subset of IL-10-producing DCs can induce Tr1 cells and Ag-specific unresponsiveness (31), one important role for IL-10-producing innate effector cells in this model may be to generate Tr1 cells specific for parasite Ags.

In summary, the IL-10$^{-/-}$RAG$^{-/-}$ mice generated for these studies allowed us to examine the relative importance of T cell- vs non-T cell-derived IL-10 in the pathogenesis of schistosomiasis. Unexpectedly, although IL-10-producing cells associated with both the innate and adaptive immune response regulated Th2 response development and egg-induced pathology following infection, the generation of an IL-10-producing regulatory T cell population was particularly important for host survival. This regulatory T cell population was, in part, derived from the naturally occurring CD4$^+$CD25$^+$ population, but also very likely contained Tr1 (or Th2 cells) cells induced by IL-10-producing DCs (31) and possibly other IL-10-producing innate effector cells. To our knowledge, this is the first study to demonstrate an important functional role for regulatory T cells during helminth infection. Thus, therapies or vaccines that preferentially trigger the development of IL-10-producing T cells may prove highly effective for a variety of diseases in which overexuberant and/or sustained immune responses contribute to host morbidity and mortality.

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References

1. Ross, A. G., P. B. Bartley, A. C. Sleigh, G. R. Olds, Y. Li, G. M. Williams, and D. P. McManus. 2002. Schistosomiasis. *N. Engl. J. Med. 346:1212.*
2. Grzych, J. M., E. Pearce, A. Cheever, Z. A. Caulada, P. Caspar, S. Heiny, F. Lewis, and A. Sher. 1991. Egg deposition is the major stimulus for the production of Th2 cytokines in murine schistosomiasis mansoni. *J. Immunol. 146:1322.*
3. Pearce, E. J., P. Caspar, J. M. Grzych, F. A. Lewis, and A. Sher. 1991. Downregulation of Th1 cytokine production accompanies induction of Th2 responses by a parasitic helminth, *Schistosoma mansoni*. *J. Exp. Med. 173:159.*
4. Wynn, T. A., I. Eltoum, A. W. Cheever, F. A. Lewis, W. C. Gause, and A. Sher. 1993. Analysis of cytokine mRNA expression during primary granuloma formation induced by eggs of *Schistosoma mansoni*. *J. Immunol. 151:1430.*
5. Kaplan, M. H., J. R. Whitfield, D. L. Boros, and M. J. Grusby. 1998. Th2 cells are required for the *Schistosoma mansoni* egg-induced granulomatous response. *J. Immunol. 160:1850.*
6. Wynn, T., A. Cheever, D. Jankovic, R. Poindexter, P. Caspar, F. Lewis, and A. Sher. 1995. An IL-12-based vaccination method for preventing fibrosis induced by schistosome infection. *Nature 376:594.*
7. Hoffmann, K. F., P. Caspar, A. W. Cheever, and T. A. Wynn. 1998. IFN-γ, IL-12, and TNF-α are required to maintain reduced liver pathology in mice vaccinated with *Schistosoma mansoni* eggs and IL-12. *J. Immunol. 161:4201.*
8. Bosshardt, S. C., G. L. Freeman, Jr., W. E. Secor, and D. G. Colley. 1997. IL-10 deficit correlates with chronic, hypersplenomegaly syndrome in male CBA/J mice infected with *Schistosoma mansoni*. *Parasite Immunol. 19:347.*
9. Wynn, T. A., A. W. Cheever, M. E. Williams, S. Hieny, P. Caspar, R. Kuhn, W. Muller, and A. Sher. 1998. IL-10 regulates liver pathology in acute murine schistosomiasis mansoni but is not required for immune down-modulation of chronic disease. *J. Immunol. 160:4473.*
10. Montenegro, S. M., P. Miranda, S. Mahanty, F. G. Abath, K. M. Teixeira, E. M. Coutinho, J. Brinkman, I. Goncalves, L. A. Domingues, A. L. Domingues, et al. 1999. Cytokine production in acute versus chronic human schistosomiasis mansoni: the cross-regulatory role of interferon-γ and interleukin-10 in the responses of peripheral blood mononuclear cells and splenocytes to parasite antigens. *J. Infect. Dis. 179:1502.*
11. van den Biggelaar, A. H., R. van Ree, L. C. Rodrigues, B. Lell, A. M. Deelder, P. G. Kremsner, and M. Yazdanbakhsh. 2000. Decreased atopy in children infected with *Schistosoma haematobium*: a role for parasite-induced interleukin-10. *Lancet 356:1723.*
12. Wynn, T., R. Morawetz, T. Scharton-Kersten, S. Hieny, H. C. Morse III, R. Kuhn, W. Muller, A. Cheever, and A. Sher. 1997. Analysis of granuloma formation in double cytokine-deficient mice reveals a central role for IL-10 in polarizing both T helper cell 1- and T helper cell 2-type cytokine responses in vivo. *J. Immunol. 159:5014.*
13. Hoffmann, K. F., A. W. Cheever, and T. A. Wynn. 2000. IL-10 and the dangers of immune polarization: excessive type 1 and type 2 cytokine responses induce distinct forms of lethal immunopathology in murine schistosomiasis. *J. Immunol. 164:6406.*
14. Hesse, M., M. Modolell, A. C. La Flamme, M. Schito, J. M. Fuentes, A. W. Cheever, E. J. Pearce, and T. A. Wynn. 2001. Differential regulation of nitric oxide synthase-2 and arginase-1 by type 1/type 2 cytokines in vivo: granulomatous pathology is shaped by the pattern of L-arginine metabolism. *J. Immunol. 167:6533.*
15. Moore, K. W., R. de Waal Malefyt, R. L. Coffman, and A. O'Garra. 2001. Interleukin-10 and the interleukin-10 receptor. *Annu. Rev. Immunol. 19:683.*
16. Fiorentino, D. F., A. Zlotnik, T. R. Mosmann, M. Howard, and A. O'Garra. 1991. IL-10 inhibits cytokine production by activated macrophages. *J. Immunol. 147: 3815.*

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17. de Waal Malefyt, R., J. Haanen, H. Spits, M. G. Roncarolo, A. te Velde, C. Figdor, K. Johnson, R. Kastelein, H. Yssel, and J. E. de Vries. 1991. Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. *J. Exp. Med. 174:915*.
18. Ding, L., P. S. Linsley, L. Y. Huang, R. N. Germain, and E. M. Shevach. 1993. IL-10 inhibits macrophage costimulatory activity by selectively inhibiting the up-regulation of B7 expression. *J. Immunol. 151:1224*.
19. Bogdan, C., Y. Vodovotz, and C. Nathan. 1991. Macrophage deactivation by interleukin 10. *J. Exp. Med. 174:1549*.
20. Modolell, M., I. Corraliza, F. Link, G. Soler, and K. Eichmann. 1995. Reciprocal regulation of the nitric oxide synthase/arginase balance in mouse bone marrow-derived macrophages by TH1 and TH2 cytokines. *Eur. J. Immunol. 25:1101*.
21. Munder, M., K. Eichmann, and M. Modolell. 1998. Alternative metabolic states in murine macrophages reflected by the nitric oxide synthase/arginase balance: competitive regulation by $CD4^+$ T cells correlates with Th1/Th2 phenotype. *J. Immunol. 160:5347*.
22. de Waal Malefyt, R., H. Yssel, and J. E. de Vries. 1993. Direct effects of IL-10 on subsets of human $CD4^+$ T cell clones and resting T cells: specific inhibition of IL-2 production and proliferation. *J. Immunol. 150:4754*.
23. Schandene, L., C. Alonso-Vega, F. Willems, C. Gerard, A. Delvaux, T. Velu, R. Devos, M. de Boer, and M. Goldman. 1994. B7/CD28-dependent IL-5 production by human resting T cells is inhibited by IL-10. *J. Immunol. 152:4368*.
24. Taga, K., H. Mostowski, and G. Tosato. 1993. Human interleukin-10 can directly inhibit T-cell growth. *Blood 81:2964*.
25. Fiorentino, D. F., M. W. Bond, and T. R. Mosmann. 1989. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J. Exp. Med. 170:2081*.
26. Kane, M. M., and D. M. Mosser. 2001. The role of IL-10 in promoting disease progression in leishmaniasis. *J. Immunol. 166:1141*.
27. Lang, R., R. L. Rutschman, D. R. Greaves, and P. J. Murray. 2002. Autocrine deactivation of macrophages in transgenic mice constitutively overexpressing IL-10 under control of the human CD68 promoter. *J. Immunol. 168:3402*.
28. Belkaid, Y., C. A. Piccirillo, S. Mendez, E. M. Shevach, and D. L. Sacks. 2002. $CD4^+CD25^+$ regulatory T cells control *Leishmania major* persistence and immunity. *Nature 420:502*.
29. Annacker, O., R. Pimenta-Araujo, O. Burlen-Defranoux, T. C. Barbosa, A. Cumano, and A. Bandeira. 2001. $CD25^+CD4^+$ T cells regulate the expansion of peripheral CD4 T cells through the production of IL-10. *J. Immunol. 166:3008*.
30. Kullberg, M. C., D. Jankovic, P. L. Gorelick, P. Caspar, J. J. Letterio, A. W. Cheever, and A. Sher. 2002. Bacteria-triggered $CD4^+$ T regulatory cells suppress *Helicobacter hepaticus*-induced colitis. *J. Exp. Med. 196:505*.
31. Wakkach, A., N. Fournier, V. Brun, J. P. Breittmayer, F. Cottrez, and H. Groux. 2003. Characterization of dendritic cells that induce tolerance and T regulatory 1 cell differentiation in vivo. *Immunity 18:605*.
32. Shevach, E. M., R. S. McHugh, A. M. Thornton, C. Piccirillo, K. Natarajan, and D. H. Margulies. 2001. Control of autoimmunity by regulatory T cells. *Adv. Exp. Med. Biol. 490:21*.
33. Pearce, E. J., and A. S. MacDonald. 2002. The immunobiology of schistosomiasis. *Nat. Rev. Immunol. 2:499*.
34. Hesse, M., A. W. Cheever, D. Jankovic, and T. A. Wynn. 2000. NOS-2 mediates the protective anti-inflammatory and antifibrotic effects of the Th1-inducing adjuvant, IL-12, in a Th2 model of granulomatous disease. *Am. J. Pathol. 157:945*.
35. Cheever, A. W., M. E. Williams, T. A. Wynn, F. D. Finkelman, R. A. Seder, T. M. Cox, S. Hieny, P. Caspar, and A. Sher. 1994. Anti-IL-4 treatment of *Schistosoma mansoni*-infected mice inhibits development of T cells and non-B, non-T cells expressing Th2 cytokines while decreasing egg-induced hepatic fibrosis. *J. Immunol. 153:753*.
36. Marshall, A. J., and E. Y. Denkers. 1998. *Toxoplasma gondii* triggers granulocyte-dependent cytokine-mediated lethal shock in D-galactosamine-sensitized mice. *Infect. Immun. 66:1325*.
37. Lutz, M. B., N. Kukutsch, A. L. Ogilvie, S. Rossner, F. Koch, N. Romani, and G. Schuler. 1999. An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. *J. Immunol. Methods 223:77*.
38. Cheever, A. W. 1986. The intensity of experimental schistosome infections modulates hepatic pathology. *Am. J. Trop. Med. Hyg. 35:124*.
39. Mathew, R. C., and D. L. Boros. 1986. Anti-L3T4 antibody treatment suppresses hepatic granuloma formation and abrogates antigen-induced interleukin-2 production in *Schistosoma mansoni* infection. *Infect. Immun. 54:820*.
40. Chiaramonte, M. G., M. Mentink-Kane, B. A. Jacobson, A. W. Cheever, M. J. Whitters, M. E. Goad, A. Wong, M. Collins, D. D. Donaldson, M. J. Grusby, and T. A. Wynn. 2003. Regulation and function of the interleukin 13 receptor $\alpha 2$ during a T helper cell type 2-dominant immune response. *J. Exp. Med. 197:687*.
41. Hoffmann, K. F., T. A. Wynn, and D. W. Dunne. 2002. Cytokine-mediated host responses during schistosome infections; walking the fine line between immunological control and immunopathology. *Adv. Parasitol. 52:265*.
42. Jankovic, D., A. Cheever, M. Kullberg, T. Wynn, G. Yap, P. Caspar, F. Lewis, R. Clynes, J. Ravetch, and A. Sher. 1998. $CD4^+$ T cell-mediated granulomatous pathology in schistosomiasis is downregulated by a B cell-dependent mechanism requiring Fc receptor signaling. *J. Exp. Med. 187:619*.
43. Shevach, E. M. 2001. Certified professionals: $CD4^+CD25^+$ suppressor T cells. *J. Exp. Med. 193:F41*.
44. Thornton, A. M., and E. M. Shevach. 1998. $CD4^+CD25^+$ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. *J. Exp. Med. 188:287*.
45. Piccirillo, C. A., and E. M. Shevach. 2001. Cutting edge: control of $CD8^+$ T cell activation by $CD4^+CD25^+$ immunoregulatory cells. *J. Immunol. 167:1137*.
46. Sakaguchi, S., N. Sakaguchi, M. Asano, M. Itoh, and M. Toda. 1995. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol. 155:1151*.
47. Gazzinelli, R. T., M. Wysocka, S. Hieny, T. Scharton-Kersten, A. Cheever, R. Kuhn, W. Muller, G. Trinchieri, and A. Sher. 1996. In the absence of endogenous IL-10, mice acutely infected with *Toxoplasma gondii* succumb to a lethal immune response dependent on $CD4^+$ T cells and accompanied by overproduction of IL-12, IFN-γ and TNF-α. *J. Immunol. 157:798*.
48. Hunter, C. A., L. A. Ellis-Neyes, T. Slifer, S. Kanaly, G. Grunig, M. Fort, D. Rennick, and F. G. Araujo. 1997. IL-10 is required to prevent immune hyperactivity during infection with *Trypanosoma cruzi*. *J. Immunol. 158:3311*.
49. Li, C., I. Corraliza, and J. Langhorne. 1999. A defect in interleukin-10 leads to enhanced malarial disease in *Plasmodium chabaudi chabaudi* infection in mice. *Infect. Immun. 67:4435*.
50. Falcao, P. L., L. C. Malaquias, O. A. Martins-Filho, A. M. Silveira, V. M. Passos, A. Prata, G. Gazzinelli, R. L. Coffman, and R. Correa-Oliveira. 1998. Human schistosomiasis mansoni: IL-10 modulates the in vitro granuloma formation. *Parasite Immunol. 20:447*.
51. King, C. L., A. Medhat, I. Malhotra, M. Nafeh, A. Helmy, J. Khaudary, S. Ibrahim, M. El-Sherbiny, S. Zaky, R. J. Stupi, et al. 1996. Cytokine control of parasite-specific anergy in human urinary schistosomiasis: IL-10 modulates lymphocyte reactivity. *J. Immunol. 156:4715*.
52. Schopf, L. R., K. F. Hoffmann, A. W. Cheever, J. F. Urban, Jr., and T. A. Wynn. 2002. IL-10 is critical for host resistance and survival during gastrointestinal helminth infection. *J. Immunol. 168:2383*.
53. Sher, A., D. Fiorentino, P. Caspar, E. Pearce, and T. Mosmann. 1991. Production of IL-10 by $CD4^+$ T lymphocytes correlates with down-regulation of Th1 cytokine synthesis in helminth infection. *J. Immunol. 147:2713*.
54. Flores Villanueva, P. O., S. M. Chikunguwo, T. S. Harris, and M. J. Stadecker. 1993. Role of IL-10 on antigen-presenting cell function for schistosomal egg-specific monoclonal T helper cell responses in vitro and in vivo. *J. Immunol. 151:3192*.
55. Boros, D. L., and J. R. Whitfield. 1998. Endogenous IL-10 regulates IFN-γ and IL-5 cytokine production and the granulomatous response in *Schistosomiasis mansoni*-infected mice. *Immunology 94:481*.
56. Chiaramonte, M. G., L. R. Schopf, T. Y. Neben, A. W. Cheever, D. D. Donaldson, and T. A. Wynn. 1999. IL-13 is a key regulatory cytokine for Th2 cell-mediated pulmonary granuloma formation and IgE responses induced by *Schistosoma mansoni* eggs. *J. Immunol. 162:920*.
57. Chiaramonte, M. G., D. D. Donaldson, A. W. Cheever, and T. A. Wynn. 1999. An IL-13 inhibitor blocks the development of hepatic fibrosis during a T-helper type 2-dominated inflammatory response. *J. Clin. Invest. 104:777*.
58. Fallon, P. G., E. J. Richardson, G. J. McKenzie, and A. N. McKenzie. 2000. Schistosome infection of transgenic mice defines distinct and contrasting pathogenic roles for IL-4 and IL-13: IL-13 is a profibrotic agent. *J. Immunol. 164:2585*.
59. Sher, A., R. L. Coffman, S. Hieny, and A. W. Cheever. 1990. Ablation of eosinophil and IgE responses with anti-IL-5 or anti-IL-4 antibodies fails to affect immunity against *Schistosoma mansoni* in the mouse. *J. Immunol. 145:3911*.
60. Brunet, L. R., E. A. Sabin, A. W. Cheever, M. A. Kopf, and E. J. Pearce. 1999. Interleukin 5 (IL-5) is not required for expression of a Th2 response or host resistance mechanisms during murine schistosomiasis mansoni but does play a role in development of IL-4-producing non-T, non-B cells. *Infect. Immun. 67:3014*.

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