IL-10 Regulates Liver Pathology in Acute Murine *Schistosomiasis mansoni* But Is Not Required for Immune Down-Modulation of Chronic Disease

Thomas A. Wynn, Allen W. Cheever, Megan E. Williams, Sara Hieny, Pat Caspar, Ralf Kühn, Werner Müller and Alan Sher

*J Immunol* 1998; 160:4473-4480; [http://www.jimmunol.org/content/160/9/4473](http://www.jimmunol.org/content/160/9/4473)
IL-10 Regulates Liver Pathology in Acute Murine Schistosomiasis mansoni But Is Not Required for Immune Down-Modulation of Chronic Disease

Thomas A. Wynn,* Allen W. Cheever,† Megan E. Williams,* Sara Hieny,* Pat Caspar,* Ralf Kühn,‡ Werner Müller,‡ and Alan Sher*

We have used IL-10 gene knockout mice (IL-10T) to examine the role of endogenous IL-10 in the down-modulation of hepatic granuloma formation and lymphocyte responses that occurs in chronic infection with the helminth parasite Schistosoma mansoni. Although IL-10-deficient animals showed 20 to 30% mortality between 8 and 14 wk postinfection, they displayed no alterations in their susceptibility to infection and produced similar numbers of eggs as their wild-type littermates. The IL-10T mice displayed a significant increase in hepatic granuloma size at the acute stage of infection, which was associated with increased IFN-γ, IL-2, IL-1β, and TNF-α mRNA expression in liver and elevated Th1-type cytokine production by lymphoid cells. Despite developing an enhanced Th1-type cytokine response, the IL-10T mice showed no consistent decrease in their Th2-type cytokine profile. Surprisingly, although granulomatous inflammation was enhanced at the acute stage of infection, the livers of IL-10T mice displayed no significant increase in fibrosis and underwent normal immune down-modulation at the chronic stage of infection. Moreover, the down-modulated state could be induced in IL-10T mice by sensitizing the animals to schistosome eggs before infection, further demonstrating that the major down-regulatory mechanism is not dependent upon IL-10. We conclude that while IL-10 plays an important role in controlling acute granulomatous inflammation, it plays no essential role in the process of immune down-modulation in chronic schistosome infection. The Journal of Immunology, 1998, 160: 4473–4480.

IL-10 is an important cytokine produced by a variety of cell types, including CD4+ and CD8+ T cells, monocytes/macrophages, mast cells, keratinocytes, eosinophils, and various tumor cells (1). It was originally characterized by its ability to down-regulate Th1 response development, but is now known to be a major immunoregulatory cytokine influencing Th cell development as well as the production of numerous proinflammatory cytokines. More recent work has begun to investigate the role of IL-10 in the regulation of immune responses induced by a variety of important human pathogens, and the results from these studies, primarily performed in murine models, have clearly identified IL-10 as an important regulatory cytokine in infectious disease. IL-10 was shown to play a critical host-protective role in both Toxoplasma gondii and Trypanosoma cruzi infection, since IL-10-deficient mice died more rapidly than their wild-type (wt)2 counterparts (2, 3). The increased mortality in both situations was attributed to the development of an uncontrolled and pathologic type 1 immune response in the IL-10-deficient animals, which was associated with the overproduction of IFN-γ, TNF-α, and IL-12. IL-10 is also important in suppressing inflammation in a murine model of allergic bronchopulmonary aspergillosis (4) and reduces lung injury and increases survival in Pseudomonas aeruginosa pneumonia (5). Recent studies have shown that the cytokine can suppress both Th1- and Th2-associated cytokine responses in the lungs of mice exposed to either schistosome eggs (6) or the opportunistic fungal pathogen Aspergillus fumigatus (4). Thus, IL-10 appears to play a critical role in regulating the host responses to a wide variety of different pathogens.

In Schistosomiasis mansoni, CD4+ lymphocytes are central in orchestrating the formation and growth of hepatic granulomas, and cytokines direct the inflammatory response around the schistosome eggs. Th2 cytokine expression becomes dominant shortly after egg laying begins, with IL-4, IL-5, IL-10, and IL-13 being the principal cytokines secreted by lymphoid cells after stimulation with schistosome egg Ags (7). The secretion of Th1 cytokines, IFN-γ and IL-2, is concurrently down-regulated at the time when Th2 responses are reaching their peak, and it has been postulated that the down-regulation of the Th1 response begins the transition into the chronic stage of infection, which is noted by reduced granuloma formation around newly deposited eggs and reduced cytokine expression by CD4+ T cells. This change in pathology mirrors a similar alteration in tissue responsiveness that occurs in infected patients. Numerous mechanisms have been postulated for this down-modulation, including a role for CD8+ T cells (8), anti-idiotypic Abs (9), B cells (10), and IL-10 (11). Interestingly, the down-regulated state can be reversed in mice by treatment with exogenous IL-2, IL-4, or TNF-α, suggesting that the down-modulatory mechanism is linked to the expression of these cytokines (12–14).

Given the known counter-regulatory role of IL-10 in Th1 response development, it was hypothesized that the cytokine probably plays a critical role in the down-modulatory process. Neutralization studies performed in vitro have shown that blocking
IL-10 can restore IFN-γ production in spleen cells from infected mice (15). IL-10 was demonstrated to act primarily on the APC population, since neutralization of IL-10 increased costimulatory molecule expression and restored the ability of granuloma-derived macrophages to activate egg-specific Th1 clones (16, 17). Moreover, exogenous IL-10 has been shown to significantly suppress pulmonary granuloma formation (18).

Because schistosomiasis causes significant morbidity and mortality, and severe disease is associated with defective regulation in the human population that it affects, a thorough understanding of the process of immune down-modulation might lead to more effective strategies for immunologic intervention of disease. Therefore, in the present study we have focused on the role of IL-10 in regulating hepatic granuloma formation and fibrosis induced by schistosome infection. In these studies, wild-type and IL-10-deficient (IL-10T) mice (19) were infected and compared for changes in infection intensity, egg production, granulomatous inflammation, hepatic fibrosis, and Th1/Th2 cytokine profiles both in vitro and in vivo. Moreover, acute and chronic infections were investigated to determine whether IL-10 plays a critical role in the process of immune down-modulation, as has been hypothesized.

Materials and Methods
Mice, parasites, and Ag preparations
The IL-10-deficient mice used in these studies were originally generated on the 129J background and were back-crossed on the C57BL/10 background (19). The colony was maintained by intercrossing the fifth generation male and female heterozygous offspring. The wt, heterozygous (HET), and IL-10T littersmates between 8 and 14 wk of age were used in all experiments. All mice were housed in a National Institutes of Health American Association for the Accreditation of Laboratory Animal Care-approved animal facility and were maintained on water containing antibiotics (Baetrim) (Roche Laboratory, Nutley, NJ) to reduce the occurrence of enterocolitis. Signs of enterocolitis and rectal prolapse were detected in approximately 20% of IL-10-deficient animals by 20 wk of age. We observed no increased or decreased frequency of enterocolitis as a result of *S. mansoni* infection. Nevertheless, it is remains possible that the enterocolitis in some mice could in part be contributing to the findings reported in infected IL-10T mice. All data, including measurements of fibrosis, infection intensity, tissue eosinophilia, and liver cytokine mRNA levels, were compared to those from uninfected wt and IL-10T age-matched controls. Cercariae of *S. mansoni* (NMRI strain) were obtained from infected *B. glabrata* snails (Biomedical Research Institute, Rockville, MD). Soluble egg (SEA) and soluble worm (SWAP) Ag preparations were derived from homogenized eggs and adult parasites as previously described (20).

Parasitology and histopathology
Mice were infected by percutaneous exposure of tail skin for 40 min in water containing between 20 and 25 cercariae. In some experiments, animals were sensitized to schistosome eggs before infection by injecting 5000 purified eggs i.p. on three occasions separated by 2 wk (20). No mortality was detected in either egg-sensitized wt or IL-10-deficient animals up to 8 wk postinfection. Mice were killed with i.p. pentobarbitol (18 mg; Sigma, St. Louis, MO) at the weeks indicated, spleens and mesenteric lymph nodes were pooled from four or five animals per group, while spleens were processed individually. Cells were plated in 24-well tissue culture plates at a final concentration of 3 × 10⁶ cells/ml in RPMI supplemented with 2 mM glutamine, 25 mM HEPES, 10% FCS, 50 μM 2-ME, penicillin, and streptomycin. Cultures were incubated at 37°C in an atmosphere of 5% CO₂. Cells were stimulated with SEA at 20 μg/ml, with SWAP at 50 μg/ml, or with Con A at 5 μg/ml. Supernatant fluids were harvested at 72 h and assayed for cytokine activity. IFN-γ, IL-5, and IL-10 were measured as described previously (20). IL-4 levels were determined by proliferation of CT.4S cells. Cytokine levels were calculated using standard curves constructed using recombinant murine cytokines.

RT-PCR detection of cytokine mRNAs
Two 25-μg portions of each liver were combined and homogenized in 1 ml of RNA STAT60 using a tissue Polytron (Omni International, Waterbury, CT), and total RNA was isolated as recommended by the manufacturer. The RNA was resuspended in diethylpyrocarbonate-treated water and quantitated spectrophotometrically. A RT-PCR procedure was performed as previously described (22) to determine the relative quantities of mRNA for IFN-γ, IL-2, IL-1β, IL-4, IL-5, IL-10, TNF-α, and hypoxanthine phosphoribosyl transferase. The primers and probes for all genes were previously described (22, 23). The PCR conditions and cycle number were strictly optimized for each cytokine primer pair, such that a linear relationship between input RNA and final PCR product was obtained. Positive and negative controls were included in each assay to confirm that only cDNA PCR products were detected and that none of the reagents was contaminated with cDNA or extraneous PCR products. The amplified DNA was analyzed by electrophoresis, Southern blotting, and hybridization with cytokine-specific probes. The chemiluminescent signals were quantitated using a 600 ZS scanner (Microtek International, Torrance, CA). The amount of PCR product was determined by comparison of signal density to that of standard curves generated from simultaneously amplified stepwise dilutions of cDNA obtained from samples with a high amount of specific cytokine mRNA. Fold increases for individual samples were calculated as the reciprocal of the equivalent dilution of control (uninfected mouse liver) cDNA. Amplification of hypoxanthine phosphoribosyl transferase served as an internal control for the amount of RNA and cDNA from each sample.

Statistics
Schistosome worm and egg numbers, changes in cytokine mRNA, and values for secreted cytokine proteins were compared using Student’s two-tailed *t* test. Hepatic fibrosis was compared by analysis of covariance, using the log of total liver eggs as the covariate and the log of hydroxyproline per gram liver. IL-4 levels were determined by proliferation of CT.4S cells. Cytokine mRNA levels were calculated using standard curves constructed using recombinant murine cytokines.

Results
IL-10-deficient mice display enhanced hepatic pathology during acute infection, but undergo normal immune down-modulation in chronic infection
To evaluate the regulatory role of IL-10 in *S. mansoni* egg-induced liver pathology, we infected wt, HET, and IL-10T littermates percutaneously with 25 *S. mansoni* cercariae. The animals were sacrificed 8 and 14 wk postinfection and examined for several parasitologic and immunologic parameters. As shown in Table I, all three groups of mice harbored similar worm numbers, and tissue eggs produced per worm pair did not vary among the groups at any time point examined. At 8 wk postinfection, the time of the peak tissue response, the IL-10T mice displayed between a 30 and 40% increase in the average volume of hepatic granulomas compared with their wt littermates (Fig. 1). Furthermore, the HET litter mates developed a number of granulomas intermediate between those of the wt and IL-10T mice, suggesting a possible gene dosage effect for IL-10 activity. As is characteristic for acute granulomas, the lesions in wt and HET mice contained large numbers of eosinophils (Table I). Nevertheless, the granulomas in IL-10T animals exhibited a significant reduction in their eosinophil composition. There was also an increase in central necrosis in granulomas.
surrounding the eggs in IL-10T vs wt mice. The degree of fibrosis (measured by hydroxyproline) was evaluated in the animals, and again, despite having a significantly enhanced inflammatory response, the levels of hydroxyproline were consistently, but not significantly, lower in IL-10T vs wt mice.

To determine whether IL-10 plays a role in the down-regulation of the inflammatory response that is seen as the infection becomes chronic, additional animals were sacrificed at 14 wk, and the size and cellular composition of the granulomas were compared with those observed at 8 wk. As expected, wt mice displayed a marked (30%) reduction in the size of the newly formed granulomas at wk 14 compared with those at 8 wk (Fig. 1). Although the granulomas were still somewhat larger in the IL-10T vs wt mice at 14 wk, the IL-10T mice also displayed a highly significant reduction in the size of their lesions (46% reduction from 8 wk). There were no obvious differences in the cellular composition of the granulomas in any of the groups at 14 wk postinfection, and despite seeing higher fibrosis in involuting granulomas, there were no significant differences detected between wt and IL-10T mice in this parameter (Table I).

### IL-10-deficient mice display an altered cytokine response during the acute, but not the chronic, stage of infection

We have shown previously that the development of pulmonary schistosome egg granulomas is highly dependent upon type 2 cytokine production (24). Nevertheless, the role of Th1 vs Th2-associated cytokines in hepatic lesion formation during infection remains highly controversial (11, 13, 20, 21, 25–27). To determine whether an altered cytokine response might be playing a role in the enhanced granulomatous response seen at 8 wk in IL-10T mice, we isolated the mesenteric lymph nodes and spleens from 8- and 14-wk-infected mice; cultured the isolated lymphocytes in the presence of SEA, SWAP, or mitogen (Con A); and then assayed the supernatants for the production of IFN-γ, IL-5, IL-4, and IL-10. As expected, wt lymphocytes obtained from lymph nodes (Fig. 2A) and spleens (not shown) exhibited a highly polarized type 2 cytokine pattern in response to SEA, SWAP, and mitogen at 8 wk, displaying high levels of IL-4, IL-5, and IL-10, but very low levels of IFN-γ. In contrast, the lymphocytes from IL-10T mice demonstrated a mixed Th1/Th2 profile of cytokine expression, since IFN-γ levels were increased in response to both Ags and mitogen, while levels of IL-4 and IL-5 were modestly, but variably, decreased compared those in their wt littermates. A similar mixed profile of cytokine expression was reported in the lymph nodes draining the lungs of IL-10T mice injected i.v. with schistosome eggs (6). The HET mice displayed a profile of cytokine expression that was either similar to that of their wt littermates or intermediate between those of wt and IL-10T mice.

At 14 wk, IFN-γ production was virtually undetectable in all three groups of mice, showing a dramatic reduction from the levels observed at 8 wk in the IL-10T mice (Fig. 2B). IL-10 production was similarly down-regulated in both wt and HET animals by 14 wk, and in the experiment shown was completely absent in the response to both Ag and mitogen restimulation. IL-4 and IL-5 production also tended to be lower at 14 vs 8 wk in wt and HET mice and was not significantly or consistently different in the IL-10T animals at this later time point. Thus, although the wt and IL-10T mice displayed dramatically altered cytokine profiles at the acute stage, as the infection proceeded into the chronic phase, these differences were diminished, and all wt, HET, and IL-10T mice developed reduced, but polarized, Th2-type cytokine responses.

#### Table I. Parasitological and histological measurements in infected IL-10-deficient mice

<table>
<thead>
<tr>
<th>Week postinfection</th>
<th>Group</th>
<th>Parasite Recovery</th>
<th>Granuloma Formation</th>
<th>Fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Worm pairs</td>
<td>Eggs/worm pair (×1000)</td>
<td>Eos (%)*</td>
</tr>
<tr>
<td>8</td>
<td>A wt (n = 7)</td>
<td>2.42 ± 0.34c</td>
<td>5.09 ± 0.47</td>
<td>52 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>B HET (n = 7)</td>
<td>3.57 ± 0.96</td>
<td>5.25 ± 1.00</td>
<td>60 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>C IL-10T (n = 6)</td>
<td>5.00 ± 0.63</td>
<td>4.78 ± 0.58</td>
<td>31 ± 7.6*</td>
</tr>
<tr>
<td>14</td>
<td>A wt (n = 8)</td>
<td>3.25 ± 0.70</td>
<td>16.90 ± 1.86</td>
<td>46 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>B HET (n = 8)</td>
<td>3.75 ± 0.67</td>
<td>12.40 ± 1.60</td>
<td>48 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>C IL-10T (n = 5)</td>
<td>3.80 ± 0.99</td>
<td>18.50 ± 3.99</td>
<td>40 ± 5.0</td>
</tr>
</tbody>
</table>

* Percentage eosinophils within granulomas.
* Central necrosis within granulomas was scored from absent (0) to most severe (4).
* SEM.
* Means are significantly different from those in group A and B (p < 0.05).

**FIGURE 1.** The reduction in granuloma formation seen in chronic schistosome infection occurs in the absence of IL-10. The wt, Het, and IL-10-deficient (IL-10T) mice were infected with 25 cercariae of *S. mansoni* and then sacrificed at 8 wk (filled bars) or 14 wk (open bars) postinfection to evaluate the sizes of the hepatic granulomas. The data shown are the mean ± SEM of five to eight animals per group at each time point, and the numbers shown above the 14 wk points indicate the average percent reduction in granuloma for that group vs that at 8 wk. The reduction in granuloma formation in IL-10T mice at 14 vs 8 wk was highly significant (p < 0.05) by Student’s t test.
Presensitization of mice with schistosome eggs prevents the exacerbation of the granulomatous response observed in acutely infected, IL-10T mice

Our previous studies have indicated that prior exposure to schistosome eggs can decrease acute stage granuloma formation and reduce fibrosis in animals subsequently infected with *S. mansoni* (20). Thus, egg sensitization may provide a means to more rapidly induce the down-modulated state typically observed at more chronic stages of infection with the parasite. To further determine whether immune down-modulation could be established in mice deficient in IL-10, we sensitized groups of wt and IL-10T mice to the parasite eggs three times before exposing them to a percutaneous infection. As shown in Figure 4, nonsensitized IL-10T mice again showed a markedly increased granulomatous response compared with that of their wt littermates at 8 wk postinfection. Egg sensitization had only a modest suppressive effect on the size of granulomas in the wt mice, while the IL-10T mice displayed a highly significant reduction in the size of their lesions as a result of i.p. egg sensitization, again further demonstrating that immune down-modulation can be induced in the absence of IL-10.

To determine whether the egg-induced decrease in hepatic granuloma formation in IL-10T mice was associated with specific alterations in the patterns of cytokine expression, mesenteric lymph node and spleen cells were analyzed for their Ag-induced responses. Again, nonsensitized wt mice displayed a polarized Th2-type response, while IL-10T mice exhibited a mixed Th1/Th2-type response, showing the most dramatic changes in the expression of IFN-γ (Fig. 5). Egg sensitization appeared to have little or no effect on Th2-associated cytokine expression in both wt and IL-10T mice, while in contrast, the production of IFN-γ in IL-10T mice was significantly decreased as a result of prior egg exposure. Similar changes in cytokine expression were observed in the spleen (data not shown).

To determine whether other Th1-associated cytokines were affected by prior egg sensitization, we again subjected liver mRNA to RT-PCR analysis and analyzed the changes in expression of several Th1- and Th2-associated cytokine mRNAs. The wt mice displayed the typical Th2-polarized pattern of cytokine expression, while IFN-γ, IL-2, IL-1β, and TNF-α were all increased in the IL-10T mice (Fig. 6). As seen in the spleens and mesenteric lymph nodes, egg sensitization had little or no effect on the pattern of cytokine expression in wt mice, while in IL-10-deficient mice, prior egg sensitization had a dramatic and highly significant suppressive effect on nearly every cytokine mRNA examined, except TNF-α, for which the decreases observed were not statistically significant.

### Discussion

Infection of mice with *S. mansoni* is associated with hepato-intestinal disease, which is characterized by the formation of granulomas around the parasite eggs that become lodged in host tissues. In humans, the chronic granulomatous response in the liver can ultimately lead to severe tissue scaring, fibrosis, and, in some individuals, portal hypertension, bleeding, and death. The murine model of schistosomiasis has been used extensively to study the mechanism of granuloma formation and its immunologic regulation. In mice, the egg-induced liver pathology evolves through an acute phase at approximately 8 wk postinfection, which is characterized by vigorous granulomatous inflammation and peak cytokine expression by CD4+ lymphocytes, followed by a chronic period characterized by extensive liver fibrosis, reduced egg-associated inflammation, and decreased cytokine production by CD4+ T cells (28). It has been hypothesized that defective or incomplete
immune down-modulation might explain the development of severe disease in some individuals infected with the parasite. Thus, an important focus of current research has been to elucidate the immunologic mechanisms underlying the down-modulation of pathology (11).

Early experiments analyzing the Th1/Th2 balance in schistosome infection revealed that Th2-type cytokines dominate the response at the acute (patent) stage of infection, while Th1-associated cytokines are expressed preferentially at earlier time points, before or in the first few days of egg laying (7, 29). The switch from Th1 to Th2 cytokine dominance coincides with patency and has been attributed to the development of an immune response to the eggs that are first produced by the adult female parasites between 4 and 5 wk postinfection. In vitro studies have suggested that IL-10 is in large part responsible for the suppression of IFN-γ expression in infected mice (15), a finding that has also been observed with PBMC obtained from infected humans (30). One

FIGURE 3. Changes in liver cytokine mRNA expression in acutely infected wt and IL-10T mice. The wt, HET, and IL-10-deficient mice were infected with 25 cercariae of S. mansoni and then sacrificed at 8 wk postinfection to evaluate the changes in expression of several lymphokines previously shown to be modulated during infection. mRNA levels were measured in the livers by RT-PCR and expressed relative to levels detected in uninfected controls (given an arbitrary value of 1). The fold changes in expression shown are the individual values for six to eight animals per group, and the bar indicates the average within each group. The asterisk indicates that the data are significantly different from those for the wt group as determined by Student’s t test (p < 0.05).

FIGURE 4. Granuloma formation is reduced in IL-10-deficient mice that have been presensitized with schistosome eggs before infection. Control PBS-treated (PBS) and i.p. egg-sensitized (Egg) wt (+/+ ) and IL-10T (−/−) mice were infected with 25 cercariae of S. mansoni and then sacrificed at 8 wk postinfection to evaluate the volume of hepatic granulomas. The data shown are the average granuloma volumes from 10 to 11 animals/group, and the bar indicates the average within each group. The reduction in granuloma formation seen in egg-sensitized vs nonsensitized IL-10T mice was significant (p < 0.05, by Student’s t test).

FIGURE 5. Schistosome egg-sensitized IL-10T mice after infection display reduced IFN-γ production and unaltered Th2-type cytokine expression. Control PBS-treated (PBS) and i.p. egg-sensitized (Egg) wt (+/+ ) and IL-10T (−/−) mice were infected with 25 cercariae of S. mansoni and then sacrificed at 8 wk postinfection to evaluate cytokine secretion in mesenteric lymph nodes. Pooled single cell suspensions from four or five animals per group were incubated in 24-well plates (3 × 10⁶/well) and stimulated with medium alone, with SEA at 20 μg/ml, or with SWAP at 50 μg/ml as indicated in the figure. IFN-γ, IL-5, IL-4, and IL-10 levels were measured 72 h later as described in Materials and Methods. An uninfected wt group was also included as a control for Ag-specific cytokine production. These experiments were repeated with similar results.
hypothesis is that the early Th1-type response is an essential component of the acute granulomatous response and that immunomodulation occurs as a result of the down-regulation of the Th1 response (11). IL-10, produced in large part by granuloma-derived macrophages, was therefore hypothesized to be the major factor leading to T cell anergy and reduced Th1 cytokine expression in chronic schistosomiasis infection. IL-10 was suggested to operate in this manner by down-regulating the expression of costimulatory molecules on APCs (16, 17).

In the current study, the role of IL-10 in murine schistosomiasis was directly investigated by analyzing the host response in mice genetically deficient in the cytokine (19). In our studies, we found that steady state levels of IL-10 mRNA were significantly increased in the livers of wt mice 8 wk postinfection and that both spleen and mesenteric lymph node cells secreted the cytokine in response to egg Ag restimulation in vitro. Interestingly, although IL-10-deficient animals displayed a markedly altered cytokine response and exhibited enhanced hepatic granuloma formation at 8 wk postinfection, the mice underwent normal immune down-modulation at the chronic stage. At 8 wk postinfection, IFN-γ, IL-2, TNF-α, and IL-1β mRNA expressions were significantly elevated in IL-10T vs wt mouse livers undergoing down-modulation at the chronic stage. At 8 wk postinfection, IFN-γ, IL-2, TNF-α, and IL-1β mRNA expressions were significantly elevated in IL-10T vs wt mouse livers undergoing granuloma formation, and expression of IFN-γ was similarly increased in the mesenteric nodes and spleens, indicating that an enhanced Th1-type immune response was induced in the cytokine-deficient mice. Despite having an increased Th1 response, the animals displayed only a partial reduction in the production of Th2 cytokines, which dominated the response in the wt animals during the acute stage. By 14 wk, the differences between the wt and IL-10T mice in terms of both cytokine production and granuloma size were much less obvious, and both groups displayed primarily a Th2-dominated response and reduced IFN-γ expression and granuloma formation compared to those at 8 wk (Fig. 2).

Although these data fail to demonstrate a necessary role for IL-10 in immune down-modulation, they clearly demonstrate that IL-10 can regulate early acute granuloma formation in schistosomiasis and suggest that in certain situations, maximal inflammation associates with a mixed Th2/Th1 response. Nevertheless, the particular requirement for IFN-γ in hepatic granulomatous inflammation remains unclear. A recent report examining schistosomiasis infection in IFN-γ-deficient mice (C57BL/6 background) found virtually unaltered granuloma formation in the livers at 8 wk postinfection, and the animals underwent normal immune down-modulation at the chronic stage (31), while pulmonary granuloma formation was enhanced in IFN-γ-depleted i.e. egg-challenged mice (24). Moreover, mice sensitized with eggs and IL-12 before infection exhibited much smaller granulomas while displaying a marked increase in IFN-γ production (20). Thus, it is currently unclear whether the enhanced inflammatory response in IL-10T mice is due to increased IFN-γ production or possibly to the altered expression of other factors involved in the granulomatous response. IL-1β and TNF-α are likely candidates given their enhanced expression in IL-10T mice (Fig. 3), and TNF-α in particular has already been demonstrated to play an important role in the granulomatous response (14, 27). Somewhat surprisingly, the production of several Th2-associated cytokines was only minimally down-regulated in the IL-10T mice (Figs. 2 and 3). Therefore, it is also possible that the reduced, but persistent, Th2 response in combination with an enhanced Th1 response contribute to the increased lesion formation observed during the acute stage.

Surprisingly, despite having a markedly enhanced inflammatory response at wk 8, there was no corresponding increase in tissue eosinophilia or fibrosis in the IL-10T vs wt mice at 8 wk. IFN-γ is known to suppress collagen synthesis by fibroblasts (32), and exogenous IFN-γ has been shown to suppress fibrosis in schistosome-infected mice (33). Therefore, although the absence of IL-10 enhances the inflammatory response, the corresponding increase in IFN-γ may, in turn, prevent exacerbated fibrosis and suppress the influx of eosinophils. In support of this conclusion, previous studies using IL-12/egg-sensitized mice have shown that altering the
Th cell response toward a Th1 response markedly decreased hepatic fibrosis (20). Interestingly, while IFN-γ was significantly increased in IL-10T mice, Th2-associated cytokines were only partially and variably decreased. In contrast to these observations, IL-12/egg-sensitized mice showed a sustained polarization in the Th1 direction and showed no significant increase in Th2-type cytokine expression even at late time points (20). Moreover, in addition to developing less liver fibrosis, the latter animals generated smaller egg-induced granulomas. Thus, reduced fibrosis appears to correlate more with the presence of IFN-γ, while egg-induced inflammation may be more dependent upon the production of Th2 cytokines. These findings add to the growing evidence arguing for a partial dissociation of granuloma size and hepatic fibrosis (34). IFN-γ depletion studies in the IL-10 knockout mice could be used to determine more directly whether the changes in granuloma formation and/or collagen synthesis are directly controlled by this important immunoregulatory cytokine.

In general, the observations regarding cytokine regulation in infected IL-10T mice presented here are similar yet at the same time distinct from observations recently described in a pulmonary granuloma model (6). In both cases, the mutant animals developed a mixed or unpolarized Th1/Th2-type response to schistosoma eggs. Surprisingly, however, the effects of IL-10 deficiency on the inflammatory response in both locations were strikingly different. In the lung, where the inflammatory response is driven primarily by Th2 cytokines (24), the increased IFN-γ response in IL-10T mice resulted in an average 30% reduction in the size of the egg-induced lesions (6). The resulting decrease in lesion size was attributed to an increased IL-12/IFN-γ response, since IL-12 neutralization restored granuloma formation to wt levels, while markedly decreasing IFN-γ production. By contrast, in schistosome-infected IL-10T mice, in which granuloma formation occurs in liver and intestines, the mixed Th1/Th2 response was associated with increased granuloma size during the acute stage of infection (Table I). These observations therefore may help explain some of the conflicting results that have been reported in studies examining granuloma formation in the lungs vs livers of egg-injected or infected mice, respectively (35). Despite these differences, in both cases IFN-γ was associated with reducing the major pathologic manifestation found at each site. In the lung, increased Th1 responses were associated with less inflammation (24), while in the liver, less fibrosis was observed (20). Together, these findings underscore the pitfalls in studying mechanisms of disease pathogenesis in schistosomiasis based solely on experiments involving the measurement of either pulmonary or hepatic granuloma formation.

The current report as well as the previous study examining granuloma formation in the lung both demonstrate that IL-10 production is critical for polarizing the egg-induced Th2 response (6). Although IL-10 was not required for the development of Th2 cytokine production, the continued expression of Th2-associated cytokines despite markedly increased IFN-γ production probably contributed to the survival of schistosome-infected IL-10T mice. This conclusion is based in part on the findings of a recent study examining schistosome infection in IL-4-deficient mice (C57BL/6 background) (36). These mice failed to develop a significant Th2 response and, as a result, lost weight and exhibited signs of severe acute cachexia linked to an increased Th1-type response. We also observed weight loss and mortality in infected IL-10T mice, 20 to 30% of which succumbed between wk 8 and 14 postinfection (data not shown). IFN-γ production was also augmented in the IL-10T mice, to levels even higher than those reported in IL-4-deficient animals (25, 26). Nevertheless, the degree of mortality did not reach the levels reported in the IL-4T mice, which all died between 9 and 11 wk postinfection (36). We hypothesize that these differences are due to the continued presence of a reduced, but significant, Th2 response in the IL-10T mice. Thus, although Th2 cytokine expression is potentially damaging in terms of triggering excessive fibrosis, a more modest response is necessary for granuloma formation and appears to play an essential host-protective role in schistosomiasis.

Together, these data demonstrate that while IL-10 is not essential for immune down-modulation in schistosomiasis, the cytokine has an important role in regulating early acute disease as well as the character of the developing immune response. Interestingly, not only did IL-10-deficient mice show normal immune down-modulation during chronic infection, but we were able to accelerate the down-modulated state by sensitizing these animals to the parasite eggs before infection (Fig. 5). The sensitized mice showed reduced cytokine mRNA expression in their livers as well as markedly reduced granuloma size at wk 8 compared with their unsensitized IL-10T littermates. The most striking finding in these experiments was the marked suppression of IL-2 mRNA expression, which was reduced to almost background levels in the egg-sensitized IL-10T animals. These findings are particularly important since previous studies have shown that the down-modulated state can be reversed in chronically infected mice by exogenous rIL-2 (12). Identifying the IL-10-independent mechanism for suppressing IL-2 production could lead to a more thorough understanding of the process of immune down-modulation. Numerous other mechanisms to explain immunomodulation have been described, including a potential role for CD8+ T cells and immunoregulatory Ids (8, 9, 37). B cell depletion studies have suggested a role for these cells as well in immunomodulation (10), and recent studies examining chronic schistosome infection in B cell-deficient mice (38) have identified a potentially critical role for the humoral response in immunomodulation. Thus, although these data demonstrate that IL-10 is an important immunoregulatory cytokine in acute schistosomiasis, other mechanisms clearly play a more critical role in the important process of immune down-modulation.

Acknowledgments

We thank Fred Lewis and Barbara Clark at Biomedical Research Institute for providing the S. mansoni eggs and cercariae, and Dragan Jankovic and Marika Kullberg for their helpful discussions and their critical review of the manuscript.

References


