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Schistosoma japonicum-Infected Mice Show Reduced Hepatic Fibrosis and Eosinophilia and Selective Inhibition of Interleukin-5 Secretion by CD4$^+$ Cells after Treatment with Anti-Interleukin-2 Antibodies

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***Schistosoma japonicum*-infected mice were injected with antibodies to interleukin-2 (IL-2) and/or IL-2 receptor to clarify the role of IL-2 on the granulomatous reaction around schistosome eggs in the liver. Granulomas were of normal or slightly increased size in animals subjected to IL-2 blockade, but hepatic fibrosis was markedly decreased in treated animals 10 weeks after infection. Anti-IL-2 treatment significantly decreased the in vitro secretion of IL-5 by antigen-stimulated spleen cells, and peripheral eosinophilia and tissue eosinophilia were diminished. Secretion of IL-2, IL-4, and gamma interferon was unaffected. Our results indicate that IL-2 is not an essential determinant of granuloma size in *S. japonicum*-infected mice but that, as in *Schistosoma mansoni* infection, the development of hepatic fibrosis is critically dependent on IL-2 levels and granuloma size and hepatic fibrosis are differentially regulated.**

Most hepatic pathology in murine schistosome infections is caused by the granulomas, and subsequent fibrosis, around eggs laid by the parasites. *Schistosoma japonicum* worms live in the portal venous system and begin to lay eggs 4 weeks after infection. Both the size of the granulomas formed and the degree of fibrosis in schistosome-infected mice are mediated by T cells, at least partly through a complex interaction of cytokines (6, 9, 22). We have speculated that the *S. japonicum* granuloma is mediated predominantly by T-helper 2 (Th2) lymphocytes because we found interleukin-4 (IL-4) and IL-5 to be the predominant cytokines secreted by spleen cells of *S. japonicum*-infected mice, while the Th1-related cytokines, IL-2 and gamma interferon (IFN-γ), were present in only small amounts (21). IL-2 is a pleiotropic cytokine which affects growth of both Th1 and Th2 lymphocyte subsets (15) and the expression of message for IL-4, IL-5, IL-6, IL-9, and granulocyte-macrophage colony-stimulating factor in human peripheral T cells (10). We recently reported markedly decreased hepatic fibrosis in *Schistosoma mansoni*-infected mice treated with antibodies to IL-2 and IL-2 receptor (IL-2R), and Perrin and Phillips (17) and Mathew et al. (14) have reported that treatment of *S. mansoni*-infected mice with recombinant IL-2 restored the size of immunologically modulated granulomas. In the present study, we examined the effects of monoclonal antibodies (MAbs) against IL-2 and IL-2R on hepatic pathology in *S. japonicum*-infected mice and on the secretion of cytokines by splenic cells from these mice. Granuloma size was slightly increased by anti-IL-2 treatment, and by 10 weeks hepatic fibrosis was significantly less in treated than in untreated mice. In vivo anti-IL-2 treatment significantly inhibited IL-5 secretion by spleen cells. We speculate that the diminished fibrosis may be related to down-regulation of Th2 responses by anti-IL-2 treatment.

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MATERIALS AND METHODS

Parasites. Snails infected with a Philippine (Lowell) strain of *S. japonicum* were received from Yung-san Liang, Lowell Tropical Medicine Institute, Lowell, Mass. Cercariae from snails crushed and placed in dechlorinated water were counted under a dissecting microscope and injected subcutaneously through a 22-gauge needle in a volume of 0.1 to 0.3 ml to infect mice (21).

Mice. C3H/HeN female mice were from the Division of Cancer Treatment, National Cancer Institute, Frederick, Md. Mice, 4 to 6 weeks of age, were exposed to 10 to 15 *S. japonicum* cercariae and killed 7 or 10 weeks later. Granulomas around *S. japonicum* eggs are of maximum size at 7 weeks and are substantially down-regulated at 10 weeks.

MAbs. MAbs S4B6 (anti-IL-2, of immunoglobulin G2a [IgG2a] isotype) and PC61 (anti-IL-2R, IgG1) were prepared from ascites obtained from nude mice injected with the respective cell lines. After ammonium sulfate precipitation, the MAbs were further purified on DEAE-cellulose columns (DE-52; Whatman Biosystems Ltd., Maidstone, Kent, England). Control MAbs (CMAbs) J1.2 (IgG2b [no control IgG2a being available]) or GL113 (IgG1) were similarly prepared, except that GL113 was not column purified. Two milligrams of MAb was injected intraperitoneally weekly beginning 4 weeks after infection, just as egg-laying began, and continued until 1 week before the animals were killed.

Antigens and antibodies. Soluble egg antigen (SEA) was prepared from purified eggs by the technique of Boros and Warren (2). SEA was as mitogenic as concanavalin A (ConA) in blastogenesis assays (data not shown), but it did not stimulate cytokine secretion by spleen cells from uninfected mice. Soluble adult worm antigen (SWAP) was prepared as previously described (21). Serum IgE levels were measured by enzyme-linked immunosorbent assay (8).

Parasitology and pathology. Mice were killed by the intraperitoneal injection of 10 mg of pentobarbital containing 50

TABLE 1. Parasitologic findings 7 and 10 weeks after infection in mice treated with anti-IL-2 or anti-IL-2R[a]

Treatment	No. of mice	No. of WPs[b]	No. of eggs/WP in the tissues (10^3)	No. of eggs/WP in the feces[c]	IgE (μg/ml)[c,d]
At 7 weeks					
CMAb or saline	41	4.1 ± 0.4	45 ± 2	759 ± 127	63 ± 7
Anti-IL-2R	11	5.0 ± 1.3	46 ± 4	647 ± 113	ND[e]
Anti-IL-2	21	3.7 ± 0.5	45 ± 2	444 ± 144	55 ± 9
Anti-IL-2 + anti-IL-2R	21	4.2 ± 0.5	45 ± 3	537 ± 138	64 ± 10
At 10 weeks					
CMAb or saline	25	4.4 ± 0.5	84 ± 3	2,302 ± 1,022	123 ± 13
Anti-IL-2R	5	2.8 ± 0.4	68 ± 8	885 ± 234	ND
Anti-IL-2	13	3.8 ± 0.5	80 ± 4	1,484 ± 426	147 ± 8
Anti-IL-2 + anti-IL-2R	13	3.4 ± 0.5	82 ± 3	1,504 ± 158	232 ± 44

[a] Combined results from four experiments are shown.
[b] WP, worm pair. All of the mice in each group were examined.
[c] About half of the mice in each group were examined.
[d] The only statistically significant differences in the data are those for IgE at 10 weeks after infection.
[e] ND, not done.

U of heparin or by inhalation of methoxyflurane after injection of heparin only. Bone marrow was examined as previously described (18). Mice were then perfused to recover adult schistosomes (7). If mice were killed without pentobarbital, 2 mg of pentobarbital per ml was then added to the perfusion fluid to facilitate recovery of the worms. Approximately half the liver was used for the counting of schistosome eggs after digestion in 4% KOH for 18 h (3). About 200 mg of liver was used for the estimation of collagen as hydroxyproline by method B of Bergman and Loxley (1), and the remainder was fixed in Bouin-Hollande solution and used to prepare histological sections which were stained with Litt's modification of the Dominici stain (13). A 1-cm portion of small intestine was examined histologically, and the remainder, together with the colon, was digested for the counting of eggs. Feces collected over a 24-h period were fixed in 10% formalin, and eggs were counted as previously described (6).

The diameters of granulomas containing a single egg with a mature miracidium were measured in histologic sections with an ocular micrometer. The diameters of granulomas containing three to five eggs, at least one of which contained a mature embryo, were measured separately, and their size is a convenient, if arbitrary, measure of granulomas around multiple eggs.

Cytokine responses of treated mice. Spleens from three to five mice were removed aseptically, and cell suspensions were prepared to determine responses to *S. japonicum* SEA, SWAP, or ConA. Cells from individual mice were incubated at a concentration of 7.5×10^6 cells per ml in a 2-ml volume in 5-ml polystyrene tubes and stimulated with 5 μg of ConA per ml, 20 μg of SEA per ml, or 40 μg of SWAP per ml in RPMI culture medium containing 10% fetal bovine serum, 2 mM glutamine, 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), 50 μM 2-mercaptoethanol, penicillin, and streptomycin. Cells were incubated at 37°C in an atmosphere of 5% CO_2–95% air. Supernatants were harvested at 24 h for determination of IL-2 and IL-4 and at 72 h for determination of IL-5 and IFN-γ. IL-2 levels were determined by proliferation of the CTLL-2 line in the presence of MAb to IL-4 (11B11), and IL-4 levels were determined by the proliferation of CT4S cells (12) in the presence of MAb to IL-2 (S4B6). Neither cell line proliferated significantly when both 11B11 and S4B6 MAbs were added to the medium. Cytokine levels were calculated by using standard curves constructed with recombinant murine cytokines (21).

Statistics. Granuloma size and hepatic fibrosis (per egg or per worm pair) decreased with increasing intensity of infection (worm pairs) in these and previous (6) experiments. These variables were therefore compared by analysis of covariance by using the total number of liver eggs as the covariate and logarithms of the variables and of the covariate. Results within treatment groups did not differ significantly in the four experiments done, and the results have been pooled for most analyses (see Table 1). Mice treated with control MAbs did not differ significantly from those given saline, and the results from these two groups have also been pooled. Variables which did not change with infection intensity were compared by one-way analysis of variance or by Student's *t* test, results being considered significant for a P of ≤ 0.05. The Mann-Whitney U test was used to compare the levels of cytokines secreted by cells, and Fisher's combination (omnibus) test was used when testing the significance of results pooled from more than one experiment.

RESULTS

Parasitologic findings. Anti-IL-2 treatment did not affect the number of worms recovered or the number of parasite eggs in the tissues or feces (Table 1).

Pathologic findings. Hepatic granulomas were generally larger in mice treated with anti-IL-2 and/or anti-IL-2R and the differences were significant for granulomas around single eggs 10 weeks after infection and for granulomas around multiple eggs at 7 weeks ($P < 0.05$ and $P < 0.01$, respectively, by analysis of covariance); however, granuloma size in the various treatment groups did not differ in a consistent pattern (Fig. 1). Granuloma size decreased comparably between weeks 7 and 10 in mice given CMAb or anti-IL-2 treatment.

The hepatic fibrosis per egg was not significantly decreased by anti-IL-2 treatment 7 weeks after infection ($P = 0.06$) but was markedly decreased in treated mice at 10 weeks (Fig. 2; $P < 0.0001$, by analysis of covariance). The percentage of eosinophils in the granulomas of mice receiving anti-IL-2 plus anti-IL-2R was less than half that of infected control mice ($P < 0.0001$ by ANOVA), and eosinophils were less affected in animals receiving anti-IL-2 or

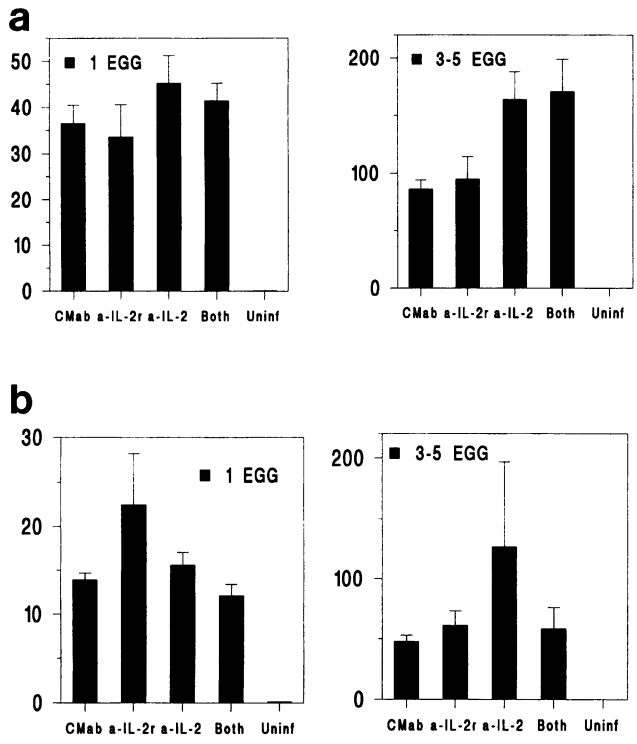

FIG. 1. Granuloma volume in various treatment groups. The volumes of granulomas around single eggs or clusters of three to five eggs (in cubic millimeters $\times 10^{-3}$) are shown 7 (a) and 10 (b) weeks after infection for the various treatment groups. Abbreviations: CMAb, CMAb GL113; a-IL-2r, anti-IL-2R; a-IL-2, anti-IL-2; both, anti-IL-2 plus anti-IL-2R; Uninf, uninfected.

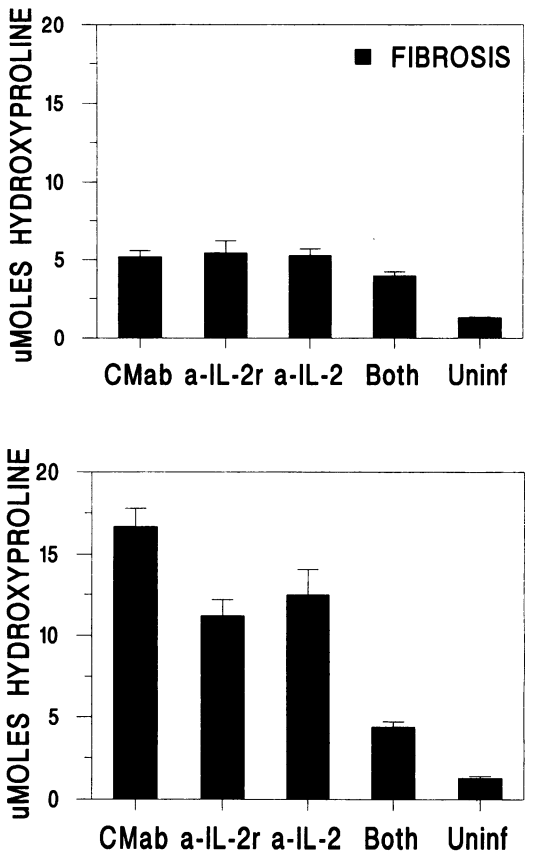

FIG. 2. Hepatic fibrosis in various treatment groups. Liver fibrosis is expressed as micromoles of hydroxyproline per liver. Abbreviations are as described in the legend to Fig. 1. Differences were analyzed by covariance by using micromoles of hydroxyproline per 10,000 eggs as the indicator of fibrosis and total liver eggs as the covariate. Results differ significantly at 10 weeks ($P < 0.0001$) but not at 7 weeks ($P < 0.06$).

anti-IL-2R separately (Fig. 3). Peripheral eosinophils, examined in two to three mice per group, averaged 253/mm^3 at 7 weeks after infection in animals treated with the CMAb, 140/mm^3 in anti-IL-2 treated mice, 60/mm^3 in mice given anti-IL-2 and anti-IL-2R, and 72/mm^3 in uninfected mice. Mature eosinophils in the bone marrow averaged 28% in mice treated with CMAb, 13% in anti-IL-2 antibody-treated mice, and 7% in mice given anti-IL-2 and anti-IL-2R ($P < 0.05$ by analysis of variance).

Cytokine secretion. IL-5 secretion from cultured spleen cells was significantly decreased by anti-IL-2 treatment, while IL-4 was variably and not significantly affected (Fig. 4). The secretion of Th1-related cytokines, IL-2 and IFN-γ, was slightly but not significantly increased by IL-2 blockade (Fig. 5). IL-2 secretion after SWAP, but not ConA or SEA, stimulation diminished significantly between weeks 7 and 10 of infection, i.e., temporal modulation of IL-2 secretion was independent of IL-2 blockade ($P < 0.01$ for mice treated with GL113 or anti-IL-2, by Fisher's omnibus test using results of the Mann-Whitney U test).

Serum IgE levels. Total serum IgE was comparably elevated in all infected animals 7 weeks after infection. By 10 weeks, animals treated with anti-IL-2 plus anti-IL-2R showed significantly higher levels of IgE (Table 1; $P < 0.02$ by Student's t test, allowing for two comparisons by using Bonferroni's correction).

DISCUSSION

Anti-IL-2 treatment of *S. japonicum*-infected mice led to a slight increase in the size of circumoval granulomas but a marked decrease in the subsequent hepatic fibrosis. The combined effect of anti-IL-2 plus anti-IL-2R was generally greater than the effect of either antibody alone. MAb PC61 blocks only the α-chain of the high-affinity IL-2R, and an intermediate-affinity IL-2R may still be functional. The binding of local IL-2 by anti-IL-2 MAb should thus reinforce the effect of anti-IL-2R.

Similar effects of anti-IL-2 treatment were seen in *S. mansoni*-infected mice in which there was a slight but significant decrease in granuloma size and a marked decrease in hepatic fibrosis 8 weeks after infection (5). A dissociation between granuloma size and hepatic fibrosis in *S. japonicum* infection has been noted previously among mouse strains (4), after treatment with anti-IL-5 or anti-IFN-γ antibodies (6) and with time during the course of infection (16).

The magnitude and pattern of IL-2 secretion by spleen cells in our experiment are similar to those reported by Stavitsky and Harold, who noted decreased IL-2 secretion by spleen or granuloma cells in chronically infected mice in parallel with decreased blastogenic responses to SEA in these same mice (19, 20). Our results, on the contrary, indicate that regulation of granuloma size is unlikely to be wholly attributed to the decreased availability of IL-2 since granulomas were slightly larger at both 7 and 10 weeks in our

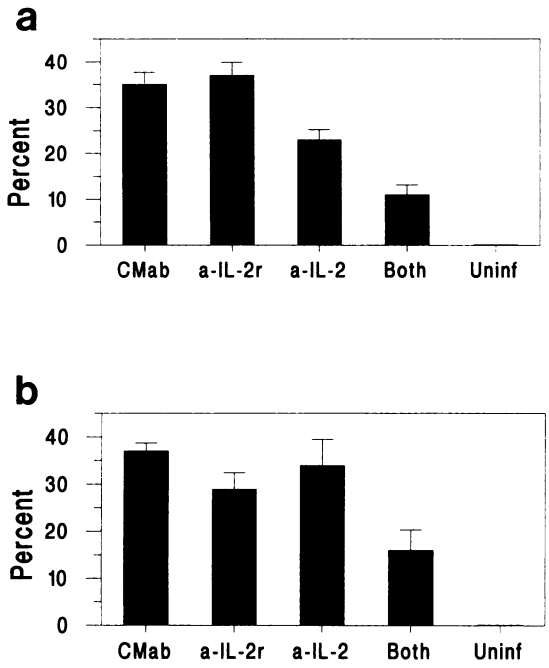

FIG. 3. Eosinophils present in granulomas. The percent eosinophils in the granulomas differed significantly among the groups at 7 (a) and 10 (b) weeks.

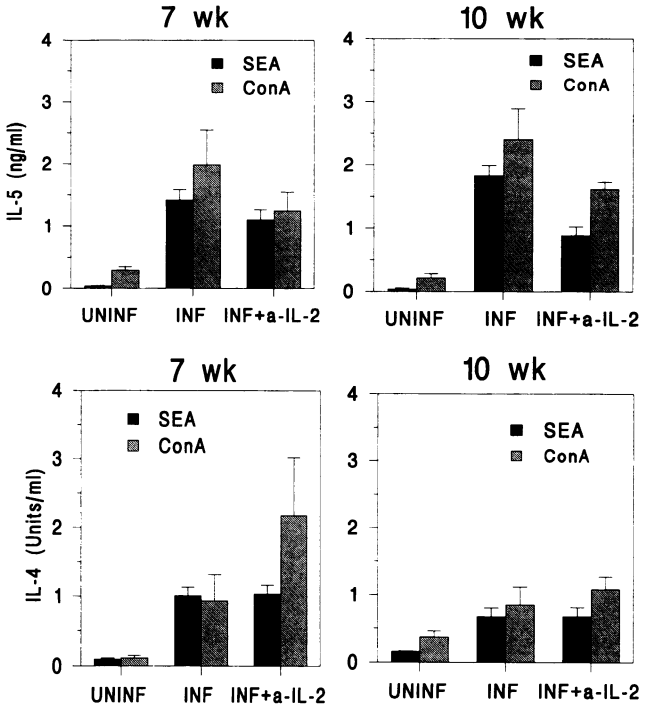

FIG. 4. Th2 cytokine secretion from spleen cells stimulated with SEA, SWAP, and ConA. Almost no IL-5 or IL-4 was secreted by normal spleen cells. IL-5 secretion in response to SEA and SWAP was significantly decreased in anti-IL-2 treated mice. The combined results of two experiments in which three mice from each group were examined at 7 and 10 weeks are shown. Significant and more marked diminution of IL-5 secretion was found in anti-IL-2-treated mice in a third experiment, and an even more marked effect was found in spleen cells from mice treated with both MAbs.

anti-IL-2-treated mice than in those treated with CMAb. The work of Olds et al. indicates that modulation of collagen synthesis has not yet begun in untreated mice 10 weeks after infection (16), a time at which we found a marked effect of anti-IL-2 treatment on hepatic fibrosis. Our results differ from those of Kresina (11), who noted a marked reduction in the size of *S. japonicum* egg granulomas in C57BL mice treated with contra-IL-2, an IL-2 antagonist. The difference might be related to his earlier examination of mice, 5 weeks after infection; he may have achieved a more marked suppression of IL-2 activity than we did; or contra-IL-2 may have effects other than those against IL-2.

Anti-IL-2 treatment, as in *S. mansoni*-infected mice (5), partially suppressed peripheral and tissue eosinophilia and significantly suppressed in vitro IL-5 production by spleen cells stimulated with *S. japonicum* SEA and SWAP. In vivo suppression is probably more marked than that measured in vitro since no anti-IL-2 was present during the 3-day incubation of spleen cells in vitro. In previous experiments, IL-5 production was found to be dependent on $CD4^+$ lymphocytes (21). Secretion of another Th2 cytokine, IL-4, was not significantly affected, and the unchanged to increased levels of serum IgE in anti-IL-2-treated mice suggest that IL-4 production was normal to increased in vivo. This may reflect a dissociation in the effects of anti-IL-2 treatment of secretion of these cytokines by Th2 cells or the production of IL-4 by other cell types. For example, most IL-4 produced in vitro by antigen-stimulated spleen cells from *S. mansoni*-infected mice is derived from non-B, non-T cells rather than $CD4^+$ cells (20a).

We speculate that the effect of anti-IL-2 treatment on

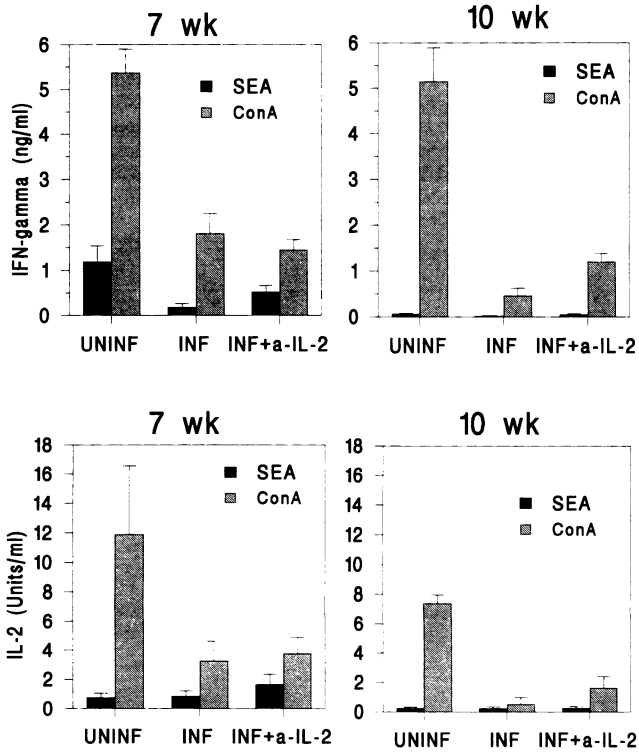

FIG. 5. Th1 cytokine secretion. IFN-γ and IL-2 responses to ConA were markedly decreased in both groups of infected mice. Anti-IL-2 treatment had no significant effect. IFN-γ levels averaged 1 to 2 ng/ml in supernatants of unstimulated cells at 7 weeks.

hepatic fibrosis may be caused by the down-regulation of Th2 cells. The effect is not directly related to depletion of IL-5 or of eosinophils since anti-IL-5 treatment of *S. japonicum*-infected mice, which completely depleted granuloma eosinophils, had no effect on hepatic fibrosis (6). Anti-IL-4 treatment of either *S. mansoni*- or *S. japonicum*-infected mice also results in decreased hepatic fibrosis (3a). This is consistent with a role for Th2 cells in fibrosis, but IL-4 secretion itself was unaffected in the present experiments.

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