

## THE ROLE OF CYTOKINES IN THE PATHOGENESIS OF HEPATIC GRANULOMATOUS DISEASE IN *SCHISTOSOMA MANSONI* INFECTED MICE

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*Cytokines are important in the cell-mediated response to Schistosoma mansoni eggs. We have found that Th2 cytokine responses (e.g. IL-4 and IL-5) are augmented after egg laying begins while Th1 responses (IL-2 and IFN- $\gamma$ ) are down regulated in S. mansoni infected mice. Treatment of mice with anti-IL-5 monoclonal antibodies (Mab) suppressed the eosinophil response almost completely but did not affect granuloma size and slightly increased hepatic fibrosis. Anti-IL-4 treatment abolished IgE responses in infected mice and decreased hepatic fibrosis slightly. Anti-IFN- $\gamma$  treatment had no effect on hepatic pathology. Anti-IL-2 treatment decreased granuloma size significantly and decreased hepatic fibrosis markedly. Anti-IL-2 treatment dramatically decreased IL-5 secretion by splenic cells in vitro and decreased peripheral blood and tissue eosinophilia. In contrast IL-4 secretion was unaffected and serum IgE was normal or increased. IL-2 and IFN- $\gamma$  secretion by splenic cells of treated mice were slightly but not significantly increased suggesting that anti-IL-2 treatment is affecting Th2 rather than Th1 responses.*

Key words: *Schistosoma mansoni* – cytokines – pathogenesis – IL-2 – IL-4 – IL-5 – IFN- $\gamma$

Granulomas inflammation around *Schistosoma mansoni* eggs is mediated by CD4<sup>+</sup> lymphocytes (Mathew et al., 1990) and the size of newly formed granulomas is immunologically down-regulated by CD8<sup>+</sup> lymphocytes in chronically infected mice (Chensue et al., 1981; Perrin & Phillips, 1988). Much of the interaction of lymphocytes with other cells is mediated through cytokines. It is therefore of interest to examine the effects of cytokines, and of anti-cytokine antibodies on the circumoval granulomas in schistosome infected animals.

Murine CD4<sup>+</sup> lymphocytes have recently been divided into Th1 and Th2 (Mosmann & Coffman, 1989) subclasses based on their patterns of cytokine secretion. Th1 cells secrete, among other cytokines, IFN- $\gamma$  and IL-2 while Th2 cytokines include IL-4 and IL-5 which are responsible, respectively, for switching B cells to IgE production and the stimulation of eosinophil production by the bone marrow, among other functions. Because of the prominence of IgE and eosinophils in schistosome infected animals we were particularly interested in the possible role of Th2 cells in the formation of schistosomal granulomas.

Examination of the pattern of cytokine

secretion by splenic lymphocytes during the course of *S. mansoni* and *S. japonicum* infection showed an abrupt increase in IL-4 and IL-5 production and a down-regulation of IL-2 and IFN- $\gamma$  at the inception of egg deposition in the liver (Grzych et al., 1991; Xu et al., 1991). The down-regulation of Th1 cytokines is at least partly mediated by IL-10 (Sher et al., 1991) which is produced by many cell types including Th2 lymphocytes.

We report here the effects of treatment of *S. mansoni* infected mice with monoclonal antibodies (mAb) against IL-5 and IFN- $\gamma$  and preliminary results of treatment with anti-IL-4, anti-IL-2 and anti-IL-2 receptor (IL-2R) antibodies.

### METHODS

*Parasites and mice* – Cercariae of a Puerto Rican (NMRI) strain of *S. mansoni* were obtained from the Biomedical Research Institute, Rockville, MD. Weanling female mice were from the Division of Cancer Treatment, NCI, Frederick, MD and exposed to 40 to 50 *S. mansoni* cercariae percutaneously.

*Antigens and antibodies* – Soluble egg antigen (SEA) and soluble adult worm antigen

were prepared as described by Grzych et al. (1991). mAb against IFN- $\gamma$  (XMG), IL-5 (TRFK-5), IL-2 (S4B5), IL-2R (PC61) and a control mAb (GL113) were prepared from ascites produced in nude mice injected intraperitoneally (i.p.) with the respective cell lines. Antibodies were precipitated with ammonium sulfate and in some instances further purified on DE52 columns. Anti-IL-4 (11B11) was precipitated from culture supernates using ammonium sulfate. Mice examined at 8 weeks were injected i.p. with mAb once a week, beginning 3 to 4 weeks after infection and continuing to the 7th week, in the following quantities: anti-IFN- $\gamma$  and anti-IL-4, 1mg; anti-IL-2 and anti-IL-2R, 2mg and anti-IL-4 10mg in the first injection followed by 5mg weekly.

*Cytokine responses of treated mice* – Spleen cells from each of 3-5 mice were incubated in 1ml volumes at a concentration of  $7.5 \times 10^6$  cells per ml and supernatant fluids collected after 24 hours for determination of IL-2 and IL-4, which were assayed by proliferation of CTLL-2 and CT4S cells, respectively, in the presence of neutralizing antibody to IL-4 and IL-2 respectively. IL-5 and IFN- $\gamma$  were determined by 2 site ELISA assays (Grzych et al., 1991). Standard curves were constructed using murine recombinant cytokines.

*Parasitology and pathology* – Mice were killed by i.p. injection of 10 mg pentobarbital containing 50 units of heparin. Adult schistosomes were recovered by perfusion (Duvall & DeWitt, 1967) and eggs in the liver and gut

counted after digestion of the tissues in KOH (Cheever, 1970). Portions of the liver were also used for determination of collagen as hydroxyproline (Bergman & Loxley, 1963) or fixed for histologic examination. The diameters of granulomas containing a single egg with a mature miracidium were measured with an ocular micrometer in slides stained with Litt's modification of the Dominici stain (Litt, 1963).

*Statistics* – Most results were compared by one way analysis of variance or t test. Granuloma volume and hepatic fibrosis per egg decreased with increasing intensity of infection. These variables were therefor evaluated by analysis of covariance using total liver eggs as the covariate.

## RESULTS

Worm numbers and worm fecundity were not altered by any of the anti-cytokine regimens. The results for short-term and long-term *S. mansoni* infections are shown in Table for anti-IL-5 and anti-IFN- $\gamma$ .

Blood eosinophils in anti-IL-5 treated mice were below the levels seen in uninfected mice (data not shown) and virtually no eosinophils were present in circumoval granulomas in short-term infections (Fig. 1) or in more prolonged infections (Fig. 2). Granuloma volume was not affected by anti-IL-5 or anti-IFN- $\gamma$  treatment but hepatic fibrosis was slightly increased (Figs 1 and 3), significantly so for anti-IL-5 treated mice killed 17 and 19 weeks

TABLE

Parasitologic data from mice treated with anti-IL-5, anti-IFN- $\gamma$  or control mAb

| Mouse strain, treatment | Weeks           | Worm pairs $\pm$ SE | Eggs per worm pair in the tissues (1000s) | Number of mice |
|-------------------------|-----------------|---------------------|---|----------------|
| C3H Control Mab         | 8               | 3.63 $\pm$ 0.62     | 5.84 $\pm$ 0.56                           | 11             |
| C3H a-IL-5              | 8               | 3.81 $\pm$ 0.64     | 6.66 $\pm$ 1.05                           | 11             |
| C3H Control mAb         | 17 <sup>a</sup> | 2.25 $\pm$ 0.29     | 18.7 $\pm$ 2.1                            | 12             |
| C3H a-IL-5              | 17              | 2.57 $\pm$ 0.34     | 16.2 $\pm$ 3.1                            | 7              |
| C3H a-IFN- $\gamma$     | 17              | 2.25 $\pm$ 0.23     | 15.2 $\pm$ 3.4                            | 8              |
| CBA Control Mab         | 19              | 1.42 $\pm$ 0.13     | 34.6 $\pm$ 3.3                            | 14             |
| CBA a-IL-5              | 19              | 1.41 $\pm$ 0.21     | 30.8 $\pm$ 3.5                            | 9              |
| CBA a-IFN- $\gamma$     | 19              | 1.66 $\pm$ 0.21     | 35.2 $\pm$ 3.4                            | 10             |

<sup>a</sup>: recovery of adult worms in this experiment was erratic.

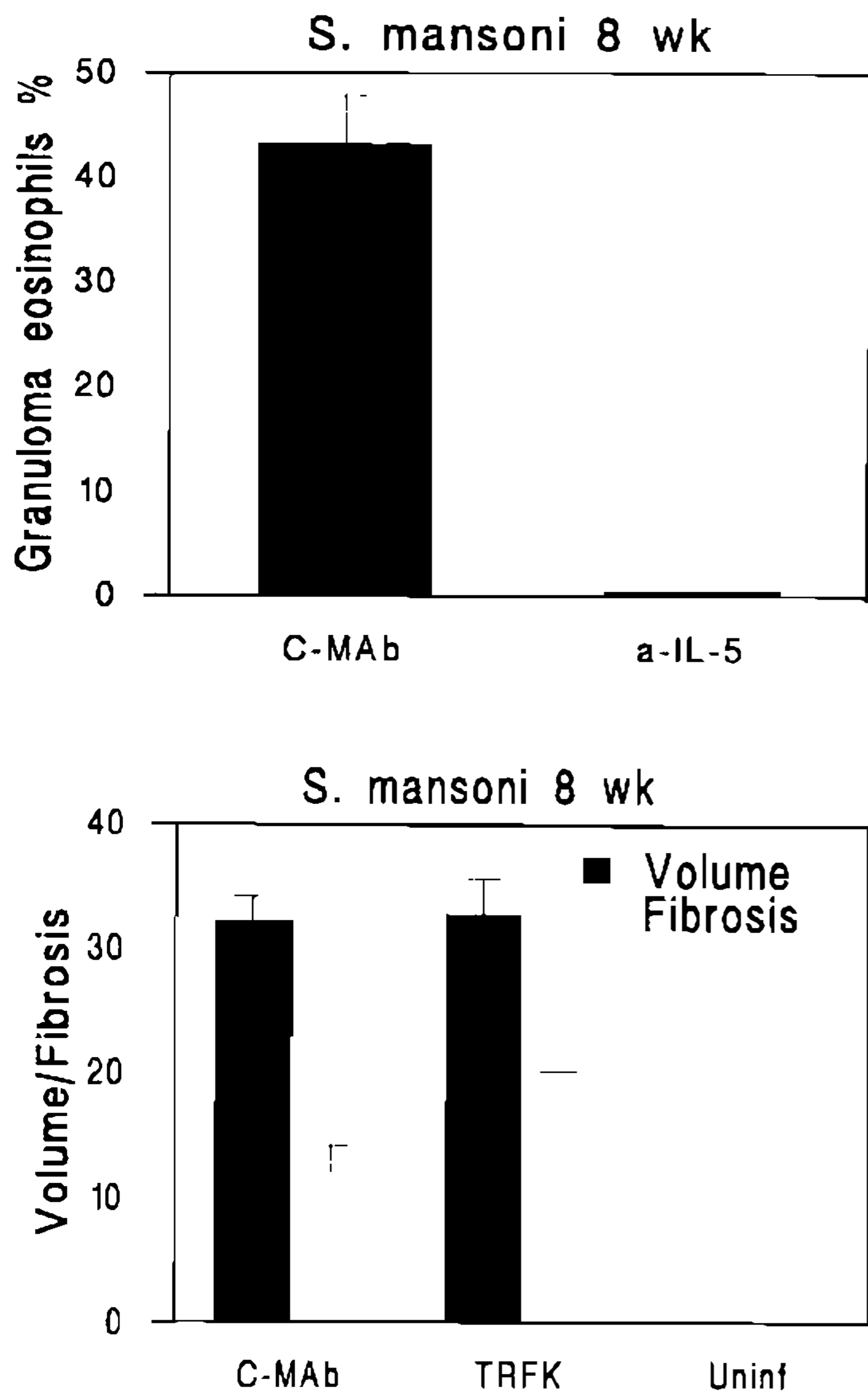


Fig. 1: granuloma eosinophils, volume and fibrosis in mice treated with anti-IL-5. The results are pooled from two previously unpublished experiments using 5-6 mice per experiment. Top panel - Granuloma eosinophils are dramatically reduced in mice infected with *Schistosoma mansoni* for 8 weeks and treated with anti-IL-5. Bottom panel - Granuloma volume ( $\text{mm}^3 \times 10^{-3}$ ) and hepatic fibrosis ( $\mu\text{moles per liver}$ ) are unaffected by anti-IL-5 (TRFK) treatment.

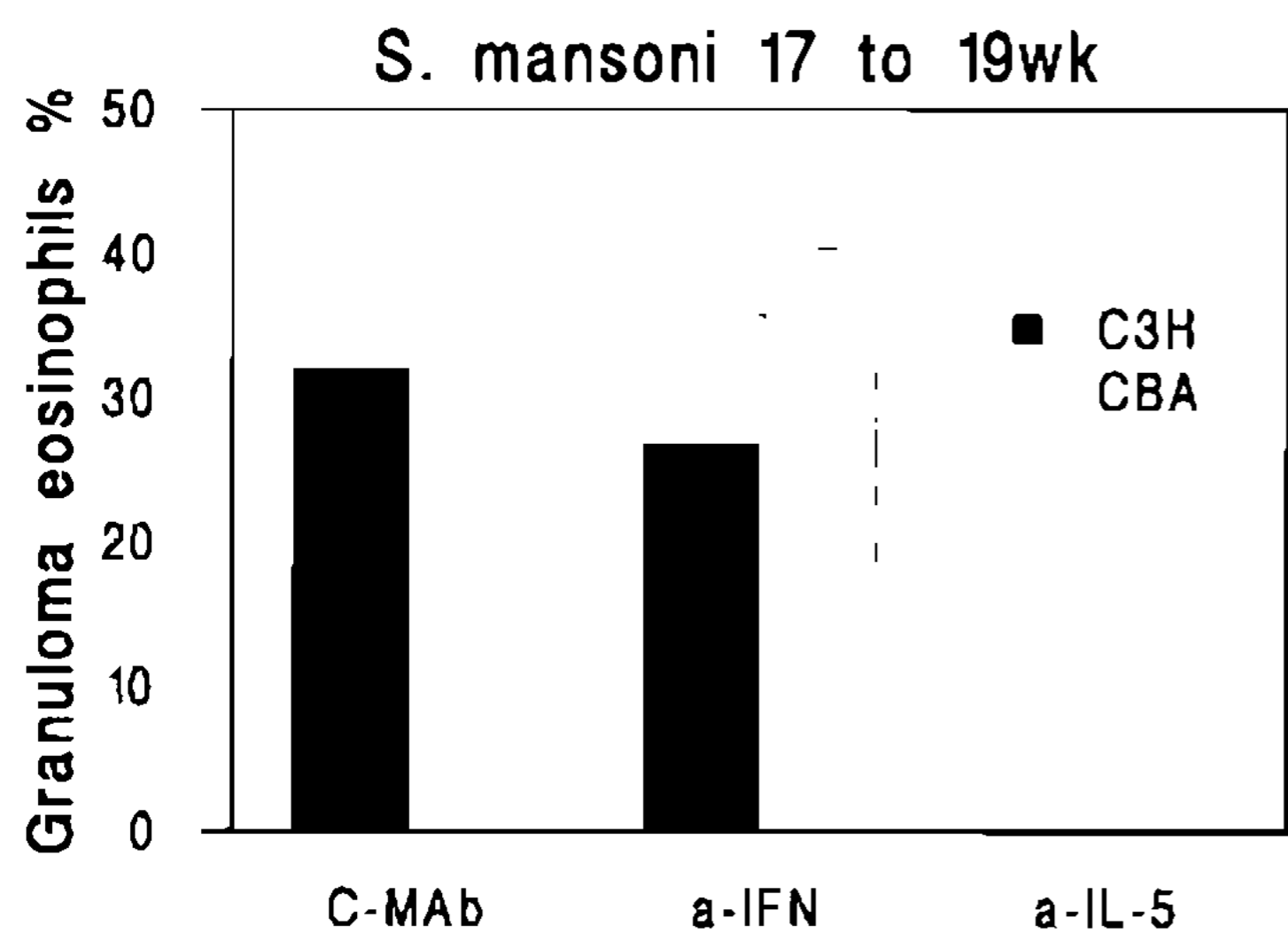


Fig. 2: granuloma eosinophils in chronically infected mice treated with anti-IL-5, anti-IFN- $\gamma$  or a control mAb. Granuloma eosinophils are unaffected by treatment with anti-IFN- $\gamma$  but are virtually absent after anti-IL-5 treatment in mice infected with *Schistosoma mansoni* for 17 or 19 weeks. mAb were injected for 6 weeks ending 1 week before mice were killed.

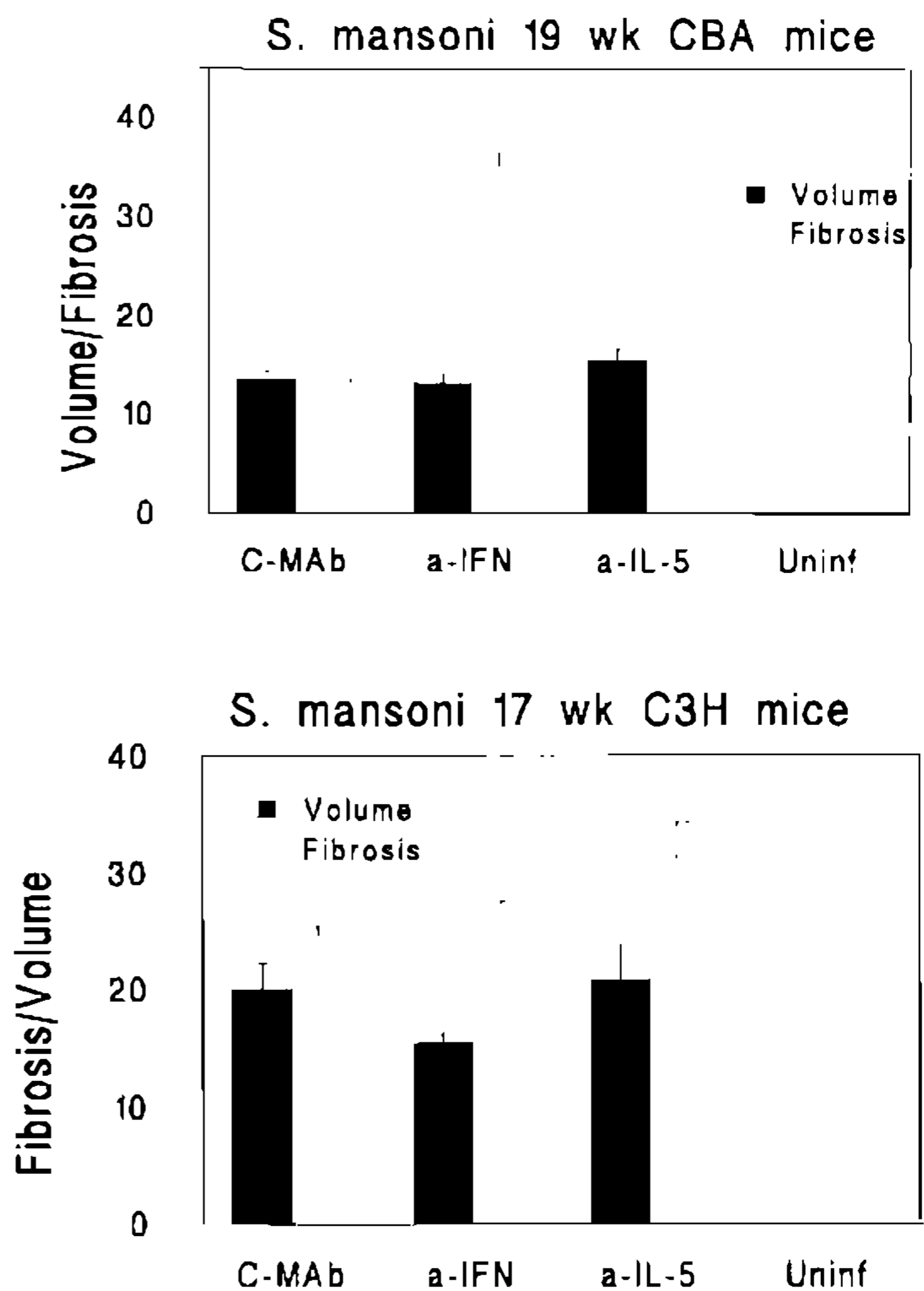


Fig. 3: granuloma volume and fibrosis in mice chronically infected with *Schistosoma mansoni* and treated with anti-IL-5 or anti-IFN- $\gamma$  mAb. Granuloma volume ( $\text{mm}^3 \times 10^{-3}$ ) was unaffected by anti-IFN- $\gamma$  or anti-IL-5 treatment in chronically infected mice. Hepatic fibrosis (plotted in  $\mu\text{moles per liver}$  but analyzed after correction for infection intensity) was significantly different among the groups of C3H mice (lower panel) but not among CBA mice (analysis of covariance using log total liver eggs as the covariate against log fibrosis per egg). Allowing for two comparisons, the effect of anti-IL-5 on fibrosis was significant in direct comparison with mice treated with the control mAb ( $p < 0.05$ ) while that of anti-IFN- $\gamma$  was not.

after infection (treated with mAb from 11-16 weeks and 12-18 weeks respectively). Combined treatment with anti-IL-4 and anti-IL-5 did not affect granuloma volume or fibrosis in mice killed 8 weeks after infection (data not shown). The secretion of cytokines by cultured spleen cells was unaffected following in vivo treatment with anti-IL-5 or anti-IFN- $\gamma$ .

Treatment with anti-IL-4 did not affect granuloma volume and produced a modest 20% decrease in hepatic fibrosis in each of 2 experiments (data not shown). As noted above, no effect of anti-IL-4 was noted when anti-IL-4 and anti-IL-5 treatments were combined in one of these experiments.

The treatment showing the most marked effect on hepatic fibrosis, and the only treatment significantly affecting granuloma volume, was anti-IL-2 with or without anti-IL-2R. Granuloma volumes were decreased by about 25% and hepatic fibrosis by more than 50% (data not shown). Anti-IL-2 treatment also reduced peripheral eosinophils by more than 50% and reduced the percent of eosinophils in granulomas significantly. Spleen cells from anti-IL-2 treated mice stimulated *in vitro* with SEA or Con A produced minimal IL-5 compared to mice treated with control mAb but produced normal levels of IL-4. Serum IgE was normal to increased in anti-IL-2 treated mice. The secretion of the Th1-related cytokines, IL-2 and IFN- $\gamma$ , was not affected.

#### DISCUSSION

Although the development of granulomas is temporally associated with vigorous Th2 responses and suppressed Th1 responses we were unable to markedly affect schistosome granulomas with mAb against IL-4 or IL-5. It is surprising that the exclusion of eosinophils from the granulomas in short term infections or their removal in chronic infections had no apparent effect other than a slight increase in hepatic fibrosis. In our previously reported results with anti-IL-5 treatment a minimal but significant decrease in granuloma volume was noted with no effect on hepatic fibrosis (Sher et al., 1990).

Anti-IL-5 treatment was equally effective in preventing blood and tissue eosinophilia in short-term and chronic infections in our experiments. This contrasts with the *in vitro* results of el-Cheikh et al. (1991) in which the development of eosinophilopoiesis in cells from chronically infected mice was dependent on a cytokine other than IL-5. It is possible that the adherent cells shown to produce this factor require IL-5 *in vivo* for their development or simply that the peripheral eosinophils are insufficient in number or state of maturation to respond to the factor.

The effects of IL-2 on granuloma formation and regulation are complex (Perrin & Phillips, 1989; Mathew et al., 1990) and the information on IL-2 secretion during the course of infection is contradictory (Mathew et al., 1990; Grzych et al., 1991; Henderson et al., 1991). Our present results suggest that IL-2 effects on the granulomas are mediated by Th2 cells as anti-IL-2 affects IL-5 production but

not the production of the Th1 cytokines measured by us.

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