Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 61–65, January 1990
Medical Sciences

Interleukin 5 is required for the blood and tissue eosinophilia but not granuloma formation induced by infection with *Schistosoma mansoni*

(cytokines/TH2 lymphocytes/granulomas/γ interferon/helminth infection)

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Communicated by Richard M. Krause, October 5, 1989

ABSTRACT **Eosinophils are thought to play a major role in the immunobiology of schistosomiasis. To investigate the immunologic basis of the eosinophil response and directly assess the function of eosinophils in egg-induced pathology, mice infected with *Schistosoma mansoni* were injected with a monoclonal antibody produced against interleukin 5 (IL-5), a cytokine previously shown to stimulate eosinophil differentiation *in vitro*. This treatment suppressed the generation of eosinophil myelocyte precursors in the bone marrow and reduced to background levels the numbers of mature eosinophils in the marrow, in circulation, and within acute schistosome egg granulomas. Nevertheless, granulomas in the anti-IL-5-treated/eosinophil-depleted mice at 8 weeks of infection were only marginally smaller than those in animals injected with control monoclonal antibody, and hepatic fibrosis was comparable in the two groups. Additional parameters such as worm burden, egg output, and serum IgE levels were unaltered by the anti-IL-5 treatment. In contrast, infected animals injected with monoclonal antibody against γ interferon (IFN-γ) displayed circulating eosinophil levels that were elevated with respect to control mice, possibly because of an enhanced release of mature eosinophils from the marrow, and developed egg granulomas that were indistinguishable in size and cellular composition from those in control animals. Immunologic assays revealed that lymphocytes from acutely infected mice produce large quantities of IL-5 but minimal IFN-γ when stimulated with either egg antigen or mitogen. Taken together, these results indicate that neither IL-5 nor eosinophils are essential for egg-induced pathology but suggest that lymphocytes that belong to the IL-5-producing TH2 subset predominate during acute infection and may induce granuloma formation by the production of other cytokines.**

Eosinophilia is a prominent feature of the cellular response to schistosomes. Infection with these trematode worms causes dramatic increases in levels of eosinophils in peripheral blood as well as in the granulomas induced in host tissues by the eggs of the parasite (1, 2). In addition, eosinophils are a major component in the tissue response against challenge infection larvae (schistosomula) in immune animals (3, 4).

Because of their prominence in immunopathological lesions, it has been hypothesized that eosinophils play a role both in the pathogenesis of egg granulomas as well as in protective immunity itself. This concept is supported by an extensive series of *in vitro* observations demonstrating selective killing of schistosomula by eosinophils in the presence of antibodies (5) as well as direct destruction of ova by the cells (6). Nevertheless, it has been more difficult to obtain convincing *in vivo* data supporting an effector function for eosinophils in the immune response to schistosomes. Thus, while infected mice treated with polyvalent antisera prepared against purified eosinophil preparations show decreased granuloma size (7), increased numbers of hepatic eggs (7), and defective resistance against challenge infection (8), the possibility that these effects are due to noneosinophil related activities in the antibody preparations has not been rigorously ruled out.

An alternative approach to evaluating the immunobiologic function of eosinophils in schistosomiasis would be to specifically block the production of the cells in the bone marrow induced by the infection. Schistosome-induced eosinophilia is known to be a T cell-dependent immune process based on its absence in athymic hosts (9). Moreover, antigen-stimulated T cells from infected mice and humans produce an activity (eosinophil stimulation promoter; ESP) that both stimulates the migration of and attracts eosinophils *in vitro* (9). Nevertheless, the molecule(s) in this supernatant material responsible for its biologic activity have not been formally identified.

The recent identification and molecular cloning of a cytokine, interleukin 5 (IL-5), that stimulates eosinophilopoiesis in bone marrow cultures, has suggested a more defined mechanism for the control of schistosome-induced eosinophilia. The IL-5 molecule, originally characterized as a T cell-replacing factor for certain B-cell responses (10), was later shown to be chemically identical to a T cell-produced factor (eosinophil differentiation factor; EDF) (11) that preferentially stimulates the differentiation of murine eosinophils in bone marrow cultures. Interestingly, among cloned $CD4^+$ T-cell lines, IL-5 is produced uniquely by clones of the TH2 subset, the same subset responsible for the synthesis of IL-4, which stimulates IgE responses (12). The formation of schistosome egg granulomas is also known to depend on $CD4^+$ T cells (13), thereby raising the question of a possible role for IL-5 in the pathogenesis of these eosinophil-enriched lesions.

Neutralizing monoclonal antibodies recently have been produced against the murine IL-5 molecule (14) and have been shown to block eosinophilia *in vivo* (15). As described in this report, treatment of schistosome-infected mice with these antibodies completely and selectively blocks both the circulating and tissue eosinophil response to the parasite without appreciably reducing egg-induced pathology. Thus, our findings establish a fundamental role for IL-5 in schistosome-induced eosinophilia, yet argue against a requirement for eosinophils in the induction of schistosomal disease.

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Abbreviations: IL-5, interleukin 5; IFN-γ, γ interferon; SEA, schistosome soluble egg antigen; ESP, eosinophil-stimulation promoter.

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MATERIALS AND METHODS

Parasite and Laboratory Host. Cercariae of *Schistosoma mansoni* (NMRI strain) were obtained from the Biomedical Research Institute (Rockville, MD). Six-week-old female C3H/HEN mice were obtained from the Division of Cancer Treatment, National Cancer Institute (National Institutes of Health). The animals were infected by percutaneous exposure of the tail to 25–40 cercariae per mouse. At least 80% of the infected mice in each experimental group remained alive at the termination of each experiment. Noninfected mice of the same age and sex were maintained as controls.

Anti-cytokine Monoclonal Antibodies and *in Vivo* Treatment. The following rat IgG_1 monoclonal antibodies were used: anti-mouse IL-5 (TRFK-5) (14); anti-mouse γ-interferon (IFN-γ) (XMG 1.2) (16), and anti-*Escherichia coli* β-galactosidase (GL113), used as an isotype control (gift of Dr. J. Abrams, DNAX). Antibodies were purified from tissue culture supernatants, and all preparations were 95–98% rat IgG_1. Infected mice, in groups of six or seven, were each injected intraperitoneally with 1 mg of antibody in 0.5 ml of saline at 5, 6, and 7 weeks after the initial cercarial exposure. This dose was selected based on previous depletion experiments with the two monoclonal antibodies (15, 17).

Hematologic and Parasitologic Assays. Mice were bled at weeks 7 and 8 postinfection by incision of the tail vein, and the blood was diluted in Turk's solution for total leukocyte counts and Discombe's solution (18) for eosinophil counts. In addition, smears were prepared and stained with Diff-Quick (American Scientific Products, McGaw Park, IL) for differential counts. Bone marrow precursors were enumerated by examination of cells flushed from the femurs of mice during autopsy, cytospin-smeared, and stained with fast green/neutral red. Total serum IgE was measured by ELISA as described (19).

Adult worms were enumerated after hepatic perfusion of 8-week infected mice immediately after sacrifice (20). Fecal egg numbers (21) were evaluated in stools collected during a 24-hr period immediately prior to termination of the experiment, and tissue egg counts were performed on digested liver and intestines as described (21).

Pathology. Half of the liver and small portions of the spleen and small intestine were fixed in Bouin–Hollande solution and processed routinely. The size of hepatic granulomas was determined in histological sections stained with hematoxylin/eosin. The diameters of 30 granulomas containing single eggs with a mature miracidium were measured in each liver by using an ocular micrometer. Granuloma volume was calculated assuming a spherical shape (21).

The percent of eosinophils and other cell types in hepatic and intestinal granulomas and inflammatory infiltrates was estimated in sections stained with Giemsa. Occasional sections were also stained with Litt's modification of the Dominici stain (22). The proportion of eggs containing immature, mature, or dead embryos (with or without infiltration of host cells) was estimated by examining 100 eggs in liver sections stained with hematoxylin/eosin or Giemsa.

Hepatic collagen in a 200-mg portion of the left lobe of the liver was measured as hydroxyproline by using technique B of Bergman and Loxley (23).

Measurement of T-Cell Cytokine Responses. Pooled splenocytes from three or four animals per group were cultured at 5×10^6 cells per ml in Dulbecco's minimal essential medium containing 4.5 g of glucose (Advanced Biotechnology, Silver Spring, MD) per ml, 10% (vol/vol) fetal calf serum, 2 mM glutamine, 100 units of penicillin per ml, 100 μg of streptomycin per ml, 25 mM Hepes, and 50 μM 2-mercaptoethanol at 37°C in a 5% CO_2/95% air Con A in the presence of either concanavalin A (Con A) (5 μg/ml) or soluble egg antigen (SEA). The SEA, prepared from schistosome eggs (kindly provided by Fred Lewis, Biomedical Research Institute, Rockville, MD) by an established procedure (24), was used at a concentration (10 μg/ml) previously shown to give optimal proliferative responses in splenocytes from 8-week infected mice. After 24 hr, culture supernatants were harvested and assayed for their concentration of cytokines by two-site ELISA using either a polyclonal antibody plus a monoclonal antibody (IFN-γ assay) (25) or two monoclonal antibodies recognizing distinct epitopes (IL-5 assay) (15, 25). Cytokine production was calculated by reference to

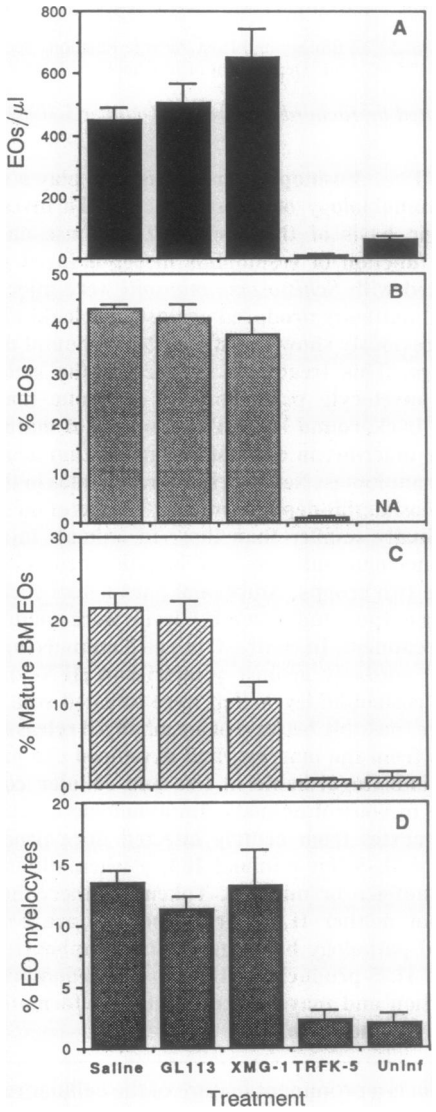

FIG. 1. Effect of *in vivo* treatment with anti-cytokine antibodies on *S. mansoni*-induced tissue and peripheral blood eosinophilia; data are means ± SEM. Infected mice were injected with saline, control monoclonal antibody (GL-113), anti-IFN-γ (XMG-1), or anti-IL-5 (TRFK-5) as described. At 8 weeks after the initial infection, the animals were bled and sacrificed 24 hr later. Peripheral blood eosinophil (EO) counts (*A*) were determined after direct staining in Discombe's fluid. The percentage of eosinophils in egg granulomas is shown in *B*. In addition, mature eosinophils (*C*) and eosinophil (EO) myelocytes (*D*) were enumerated in stained bone marrow smears. The results shown are pooled data from two separate experiments, each consisting of six to eight animals per group. In each panel the cell counts in the TRFK-5-treated infected mice are significantly different from the counts in the control (GL113-treated) group at $P < 0.001$ or less. In addition in *C*, the bone marrow (BM) eosinophil (Eos) counts in the XMG-1-treated group are significantly different from those of the GL113-treated group at $P < 0.01$. Control cell counts were performed on uninfected (Uninf) mice as indicated.

Table 1. Total and differential leukocyte counts in 8-week-infected mice treated with anti-cytokine antibodies

Animal group	Leukocytes, total × 10^{-6}	Lymphocytes		Neutrophils		Monocytes		Eosinophils	
		%	Total × 10^{-6}	%	Total × 10^{-6}	%	Total × 10^{-6}	%	Total × 10^{-6}
Infected									
+ saline	9.3 ± 1.1	62.7 ± 2.0	5.83	24.1 ± 2.0	2.24	2.4 ± 0.3	0.22	11.3 ± 0.8	1.05
+ cont mAb	11.8 ± 0.7	63.9 ± 1.0	7.54	22.7 ± 1.4	2.67	3.4 ± 0.5	0.40	10.1 ± 0.9	1.19
+ anti-IFN-γ	11.4 ± 0.6	56.9 ± 4.2	6.52	22.3 ± 1.8	2.55	3.1 ± 0.4	0.35	13.2 ± 1.1*	1.50
+ anti-IL-5	8.4 ± 0.4*	65.4 ± 3.2	5.51	31.3 ± 3.4*	2.63	2.1 ± 0.4	0.18	1.3 ± 0.2	0.11
Uninfected	9.8 ± 0.4	74.9 ± 2.5	7.32	19.9 ± 1.5	1.95	2.5 ± 0.4	0.24	2.4 ± 0.3	0.24

Data presented are pooled means and SEMs from two separate cytokine-depletion experiments, each consisting of five to seven animals per group.
*Values are significantly different ($P < 0.05$ or less) from the counts observed in control mice injected with control monoclonal antibody.

a standard curve constructed by assaying recombinant murine IFN-γ or IL-5.

RESULTS

Schistosome-infected mice treated with anti-IL-5 monoclonal antibody 5, 6, and 7 weeks after cercarial exposure displayed peripheral blood eosinophil levels measured at week 8 (or at week 7; data not shown) that were dramatically reduced with respect to either untreated mice or mice treated with control monoclonal antibody (GL113) of the same isotype (Fig. 1*A*). Indeed, the number of eosinophils in anti-IL-5-treated mice was significantly lower ($P < 0.05$) than that observed in uninfected (untreated) animals. In contrast, the percentage of neutrophils in the anti-IL-5 treated mice was significantly higher than that recorded in the control (GL113-treated) animals (Table 1). Nevertheless, because of a decreased total white cell count in the anti-IL-5 group, there was no change in the absolute numbers of neutrophils present.

Anti-IL-5 treatment had a similar effect on bone marrow precursors of eosinophils. Mature eosinophil levels in the bone marrow of infected mice were reduced to below the level recorded in uninfected mice as a result of anti-IL-5 administration (Fig. 1*C*). A comparable reduction was observed in the level of eosinophil myelocytes (Fig. 1*D*), indicating an effect of anti-IL-5 treatment on the generation of this precursor cell.

Because IFN-γ is a major product of cell-mediated responses, the effects of *in vivo* administration of a neutralizing monoclonal antibody (XMG1.2) against this cytokine were also evaluated. Direct (Fig. 1*A*) as well as differential (Table 1) eosinophil counts were found to be elevated in infected XMG1.2-treated animals and, in the case of the latter assay, were significantly different from the levels recorded in the mice injected with control monoclonal antibody (GL113). In contrast, the level of mature eosinophils in the bone marrow of XMG1.2-treated animals was significantly reduced with respect to that observed in the GL113-treated mice, while myelocyte precursor numbers remained unchanged (Fig. 1 *C* and *D*).

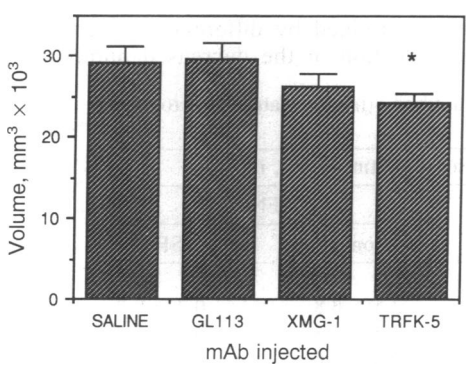

FIG. 2. Influence of anti-cytokine antibody treatment on acute granuloma formation. The data (means ± SEM) shown are pooled from two separate experiments consisting of six to eight animals per group. The asterisk indicates that the mean granuloma sizes of the TRFK-5- and GL-113-treated mouse groups were significantly different at the $P < 0.05$ level as determined by Student's *t* test. mAb, monoclonal antibody.

A major effect of anti-IL-5 treatment was observed on tissue eosinophil levels in egg granulomas in livers of mice infected for 8 weeks (Fig. 1*B*). The percentage of these cells dropped from >40% in the granulomas of control animals to <1% in the anti-IL-5 group. No other pronounced alterations were seen in the cellular composition of the lesions. Comparable effects of anti-IL-5 treatment were observed on intestinal granulomas, although moderate (but clearly reduced) numbers of eosinophils remained in the spleens of the IL-5-depleted mice. Liver and intestinal granuloma eosinophil levels were unaffected by treatment of the animals with anti-IFN-γ antibodies (Fig. 1*B*).

While causing a pronounced reduction in granuloma eosinophil levels, anti-IL-5 treatment had only a minor effect on the total size of the lesions (Fig. 2), reducing their mean volume by only 18% ($P < 0.05$) with respect to that measured for granulomas in the GL113-treated control animals. Similarly, the injection of anti-IFN-γ antibodies failed to ablate granuloma formation (Fig. 2) when using a dosage and regimen from a parallel experiment (not shown) in which healing of *Leishmania major* infection by resistant mice was completely blocked. Moreover, in both the anti-IL-5- and anti-IFN-γ-treated groups, significant effects on adult worm burden, tissue and fecal egg counts, egg viability, and fibrosis (as measured by tissue hydroxyproline levels) were absent (Table 2). Similarly, neither antibody treatment significantly altered the elevated total IgE levels observed in the infected mice (Table 2).

To evaluate the possible effect of antibody administration in T-lymphocyte responsiveness, spleen cells from mice in the different groups were stimulated with either SEA or Con A, and supernatants were harvested from the cultures 24 hr later. Both IFN-γ and IL-5 levels were then measured by ELISA (Table 3). While cells from uninfected mice produced marginal or undetectable levels of IL-5 after exposure to Con A or SEA, splenocytes from 8-wk-infected animals responded strongly to both stimuli, releasing >20-fold the level of cytokine synthesized by the control cells. In contrast, the spleen cells from the infected mice released only low levels of IFN-γ when stimulated with SEA (Table 3). Although the IFN-γ responses of these cells to Con A were somewhat higher than those observed with SEA, they did not differ significantly from the Con A responses obtained with splenocytes from uninfected mice. While pretreatment of infected animals with monoclonal anti-IL-5 or IFN-γ antibodies appeared to result in variations in cytokine responses (Table 3), these effects were not consistent in the different experiments performed.

Table 2. Parasitologic and pathologic parameters in 8-week-infected mice treated with anti-cytokine monoclonal antibodies (mAb)

Animal group	Worm pairs	Tissue eggs per pair $\times 10^{-3}$	Fecal eggs per pair per day	Hepatic fibrosis, μmol of hydroxyproline per liver	Serum IgE levels, μg/ml
Infected					
+ saline	9.3 ± 0.7	6.3 ± 0.3	84 ± 9	24.4 ± 1.4	233 ± 37
+ cont mAb	9.0 ± 1.1	5.4 ± 0.3	105 ± 15	23.4 ± 2.1	247 ± 46
+ anti-IFN-γ	9.3 ± 0.8	5.7 ± 0.5	98 ± 11	28.1 ± 2.9	348 ± 68
+ anti-IL-5	9.5 ± 0.9	5.5 ± 0.4	108 ± 16	21.9 ± 2.7	254 ± 57
Uninfected	0	0	0	2.2 ± 0.3	0.2 ± 0.06

Values shown are pooled means and SEMs from two experiments, each consisting of six to eight animals each. None of the differences are significant at $P = 0.05$, nor were differences noted in the percent of eggs in the liver (67%–68% in all groups), in liver weight (12% of body weight in all groups) or spleen weight (2.0–2.5% of body weight). cont, Control.

DISCUSSION

A major issue in the pathology of helminth-induced disease concerns the immunologic basis of the blood and tissue eosinophilia which accompanies infection with these parasites. In addition to IL-5, a number of mediators have been identified that either stimulate eosinophil differentiation in bone marrow cultures or promote migration of the cells *in vitro*. These agents include interleukin 3, granulocyte–macrophage colony-stimulating factor, eosinophil chemotactic factor of anaphylaxis (ECF-A), ESP, and the complement activation products C3a and C5a (9, 26). Nevertheless, it is only very recently that direct *in vivo* evidence has been obtained for a role of one of the above substances in helminth-induced eosinophilia. In that investigation (15), treatment with anti-IL-5 blocked the peripheral blood and pulmonary eosinophilia induced by the rodent nematode *Nippostrongylus brasilensis*. In the present study, we have confirmed and extended the evidence for the IL-5 dependency of helminth-induced eosinophilia to a medically important parasite, *Schistosoma mansoni*, and to a major pathologic lesion associated with schistosome infection, the egg granuloma.

As reported above, injection with anti-IL-5 monoclonal antibody completely abrogated both peripheral blood and tissue (granulomatous) eosinophilia and markedly reduced the formation of both mature eosinophils and myelocyte precursors in the bone marrow of 8-week-infected mice (Fig. 1). These effects were observed in the apparent absence of altered IgE levels (which could influence eosinophilia by triggering release of eosinophil chemotactic factor of anaphylaxis by mast cells) or significant changes in the levels of other leukocytes (Table 1). Indeed, the altered ratios of other cells (e.g., neutrophils and lymphocytes) observed in the peripheral blood (Table 1) and granulomas (not shown) can readily be explained by the depletion of the eosinophils, as reflected in lower total leukocyte counts (Table 1) or granuloma volume (Fig. 2), and a consequent increase in the percentage of noneosinophils present. Thus, the most simple interpretation of the data is that observed cellular effects result from a highly specific blockade of eosinophilopoiesis at the premyelocyte stage in the bone marrow with no compensatory increase in other leukocytes. Nevertheless, the results do not rule out the possibility that IL-5 may also play a role in the release from the bone marrow, recruitment into the tissues, and/or activation (27) of eosinophils in *S. mansoni*-infected mice.

Early studies (9) established the dependence of eosinophilia in murine schistosomiasis on T lymphocytes and described a supernatant activity, ESP, derived by stimulating T cells from infected mice with mitogen or antigen, which stimulates the migration of eosinophils *in vitro*. Recent experiments (29) now indicate that IL-5 is an important component of the biological activity of ESP. These observations, together with the results presented here, argue that IL-5 is a major lymphokine affecting eosinophil responses in mice and that at least one step in parasite-induced eosinophilia requires IL-5. Indeed, as shown in Table 3, IL-5 is produced in large quantities by stimulated T cells from mice with acute infections in comparison to cells from uninfected animals and is readily detected in supernatants obtained by culturing isolated egg granulomas (J. M. Grzych and A.S., unpublished data). It is noteworthy, that T cells from anti-IL-5-treated mice also produce high levels of IL-5 (Table 3), which confirms that the monoclonal antibody does not exert its effect on eosinophilia through the deletion of IL-5-secreting (i.e., TH2) lymphocytes.

As expected, treatment with anti-IFN-γ failed to reduce the levels of circulating and tissue eosinophils and, in the case of the peripheral blood counts, actually increased them (Fig. 1*A*, Table 1). This elevation, which was statistically significant when determined by differential leukocyte counting, may be a reflection of the decreased numbers of mature

Table 3. Cytokine responses of spleen cells from monoclonal antibody (mAb)-treated mice to mitogen (Con A) or SEA

Spleen cell donors	Cytokine concentration 24 hr after stimulation, ng/ml			
	IL-5		IFN-γ	
	Con A	SEA	Con A	SEA
Infected				
+ saline	18.5 ± 1.6	3.0 ± 0.2	2.5 ± 0.8	0.2 ± 0.2
+ cont mAb	26.8 ± 6.4	3.8 ± 0.3	3.5 ± 1.3	0.3 ± 0.3
+ anti-IFN-γ	19.3 ± 4.0	3.2 ± 0.3	3.6 ± 1.0	0.7 ± 0.4
+ anti-IL-5	33.3 ± 17.3	5.0 ± 1.8	2.7 ± 0.4	0.3 ± 0.3
Uninfected	0.7 ± 0.9	<0.1	3.4 ± 1.6	<0.1

Pooled splenocytes (5.0×10^6 per well) were stimulated with either Con A (10 μg/ml) or SEA (20 μg/ml), and the supernatants were collected 24 hr later. The concentration of IL-5 or IFN-γ were then measured by ELISA. The results shown are the means and SDs of two separate cytokine depletion experiments, each involving duplicate ELISA measurements. Background release levels have been subtracted from each point.

eosinophils but unaltered levels of myelocyte precursors observed in bone marrow smears from anti-IFN-γ-treated mice (Fig. 1 *C* and *D*). Thus, one explanation for the observed results is that IFN-γ plays a role in regulating the release of the mature cells from the marrow reservoir and that depletion of the cytokine results both in uncontrolled release and in a consequent elevation in circulating eosinophils. Alternatively, the observed elevations may reflect the previously documented influence of IFN-γ on IL-5-producing TH2 lymphocytes (28).

The major goal of the present study was to evaluate the role of IL-5 in the pathogenesis of the schistosome egg granuloma. We were surprised to find that while totally depleting eosinophils from these lesions, anti-IL-5 treatment caused only a marginal reduction in granuloma size and no alteration in hepatic fibrosis or numbers of eggs in the tissues or feces (Figs. 1*B* and 2; Table 3). In addition, microscopic examination (data not shown) revealed no apparent changes in granuloma cell composition other than the absence of eosinophils. The possibility that eosinophil depletion affects egg pathology and fibrosis in later (chronic) stages of infection was not examined in the present study.

The results presented here are in contrast to those obtained after treatment of infected mice with anti-eosinophil serum (AES), which led to a marked increase in mortality, an increase in the number of eggs in the liver, and a marked decrease in granuloma size (7). An important difference between the present and previous study is that anti-IL-5 treatment clearly affects eosinophilopoiesis in the marrow (Fig. 1), while AES appears to be directed primarily against the mature cells. Although unlikely based on the above arguments, it is possible that other cells compensate for eosinophils in granuloma formation in the anti-IL-5-treated but not in the AES-treated mice. Alternatively, the polyvalent AES reagent could be exerting its influence on egg pathology by a cross-reacting or contaminating reaction with other (noneosinophil) inflammatory cells or their precursors. It should also be noted that in our experiments, the absence of eosinophils in anti-IL-5-treated mice had no effect on the viability of embryos within the eggs contained in granulomas as determined by microscopic examination (data not shown) although the *in vitro* toxicity of eosinophils for eggs is well documented (6, 9). This discrepancy in the *in vivo* and *in vitro* findings may reflect the capacity of other (noneosinophil) cells to limit egg viability within granulomas.

Although anti-IL-5-mediated depletion of eosinophils failed to significantly alter egg pathology, it is clear both from the dramatic ablation of granuloma eosinophils induced by monoclonal antibody treatment (Fig. 1*C*) and the dominant IL-5 responses observed in SEA- or mitogen-stimulated lymphocytes from acutely infected mice (Table 3), that the IL-5-producing TH2 subset of $CD4^+$ cells plays a major role in the response to schistosome eggs. In this regard, it is important to note that treatment with anti-IFN-γ under a regimen that blocked healing of *L. major* infection had no effect on acute granuloma formation (Fig. 2) and that minimal (TH1 type) IFN-γ responses were observed upon SEA stimulation of 8-week-infected spleen cells (Table 3). Taken together, these results imply that the acute T-cell response to schistosome eggs occurs primarily in the TH2 subset and that granuloma formation is therefore likely to depend on cytokines, other than IL-5, produced by TH2 cells. Thus, the granulomatous response, traditionally thought to be a manifestation of classical delayed-type hypersensitivity, may instead be the result of an allergic, TH2 response to egg antigens.

We thank Drs. Stephanie James, Ed Pearce, Daniel Colley, and Franz von Lichtenberg for their helpful discussions and gratefully acknowledge the expert technical assistance of Jane Macedonia and Pat Natovitz, Brian Seymour, and Patricia Johnson. Dr. John Abrams kindly provided the GL113 monoclonal antibody.

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