Role of Host Granulomatous Response in Murine Schistosomiasis Mansoni

EOSINOPHIL-MEDIATED DESTRUCTION OF EGGS

G. RICHARD OLDS and ADEL A. F. MAHMOUD, *Division of Geographic Medicine, Department of Medicine, Case Western Reserve University and University Hospitals, Cleveland, Ohio 44106*

ABSTRACT Eosinophils form 50% of cells in the host granulomatous response to *Schistosoma mansoni* eggs, but their functional role in these granulomas and their relation to egg destruction is unknown. We have studied the course of *S. mansoni* infection in mice treated with normal rabbit serum (NRS) or depleted of their eosinophils by monospecific anti-eosinophil serum (AES). At 6-wk of infection (after 2 wk of egg deposition) the AES-treated animals were similar to NRS-treated controls with the exception that hepatic granulomas in the AES-treated animals were 50% smaller and devoid of eosinophils. At 8 wk of infection, AES-treated mice had significantly higher mortality, spleen weight, portal pressure, and 80% more eggs retained in their livers. These data suggest that eosinophil depletion delayed egg destruction. We subsequently studied destruction of eggs injected into the pulmonary microvasculature of sensitized mice. 2,000 *S. mansoni* eggs were intravenously injected into the tail veins of mice treated with NRS, anti-neutrophil serum, AES or ATG (anti-thymocyte globulin); at time intervals the remaining eggs were recovered from the lungs by tissue digestion. Egg recovery from NRS- or anti-neutrophil serum-treated mice began to decrease by day 16 and the percent recovery of eggs at day 24 was 55 and 52%, respectively. In contrast, animals treated with AES had smaller lung granulomas that were devoid of eosinophils and a marked delay of egg destruction was seen. It took until day 44 for 50% of the eggs to be destroyed. In ATG-treated animals smaller granulomas were seen that had diminished lymphocytes and also 75% less eosinophils. ATG treatment apparently slowed egg destruction but was not statistically significant. Our data define the role of the eosinophils in destruction of schistosome eggs in vivo and delineates the protective function of these cells within the host granulomatous response.

INTRODUCTION

Eosinophilia is the hallmark of helminth infections, particularly those with a tissue migratory phase (1, 2). In murine schistosomiasis mansoni, the onset of peripheral blood and bone marrow eosinophilia coincides with egg deposition in the host tissue (6 wk after infection) (1). Recently, we have initiated a series of studies on the role of eosinophils in schistosomiasis and other helminth infections (1, 3, 4) after the development of a monospecific anti-eosinophil serum (AES)[1] (5). This investigative tool has enabled us to delineate in vivo the role of the eosinophil in protecting mice against the larval stage of *Schistosoma mansoni* (3) and *Trichinella spiralis* (4). Furthermore, the antiserum has been used to deplete the eosinophils from leukocyte suspensions and to study in vitro the consequences of their absence on destruction of the multicellular larvae of helminth parasites (6–8).

In the present investigation we turn our attention to the relationship between the eosinophil and the schistosome eggs retained in the host tissue. *S. mansoni* worms reach maturity 4–6 wk after infection and produce on the average 300 eggs/d per worm (9). Almost half of these eggs are carried to the outside with the

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[1] *Abbreviations used in this paper*: AES, anti-eosinophil serum; ANS, anti-neutrophil serum; ATG, anti-thymocyte globulin; NRS, normal rabbit serum.

fecal excreta; the remainder are retained in the host tissues (10). These trapped eggs elicit a delayed hypersensitivity granulomatous response that is several times its size (11). Investigations in this laboratory and others have elucidated the cell-mediated nature of the granulomatous response and its role in the pathogenesis of disease in schistosomiasis (11–14). Furthermore, it has been suggested that the host granulomatous response leads to egg destruction (15). These granulomas consist of several cell types, mainly eosinophils, macrophages, and lymphocytes (16). Their functional role and the contribution of their cellular components to the ultimate elimination of the eggs from the host tissues is unclear. Recently James and Colley (17–20) reported that co-culturing schistosome eggs and eosinophil-rich peritoneal exudates in vitro resulted in preferential adherence and invasion of their shells by the eosinophils. Their studies suggest, therefore, that eosinophils play a defined role in egg destruction in vitro.

The current investigations were designed to use AES to examine the effects of eosinophil depletion on the course of *S. mansoni* infection in the mouse. In the AES treated mice, increased morbidity and mortality was observed, as well as significant retention of schistosome eggs in their tissues. Furthermore, a marked delay in egg destruction in the lungs was observed in the eosinophil-depleted mice, but not in mice treated with anti-neutrophil serum (ANS), anti-thymocyte globulin (ATG) or normal rabbit serum. Our studies show that the presence of the eosinophils within the granulomatous response around *S. mansoni* ova serves a vital role related to destruction of the parasite eggs.

METHODS

Animals. Young adult female mice of CF-1 strain (22–26 g body wt) were obtained from Carworth Farms, Inc., New City, New York. Inbred female mice of the C57BL/6J strain (18–22 g) were purchased from Jackson Laboratories, Bar Harbor, Maine.

Antisera. Monospecific AES was raised in rabbits injected subcutaneously with purified suspensions of mouse eosinophils in complete Freund's adjuvant as described (5). Neutrophil-rich peritoneal exudates were stimulated by injecting normal CF-1 mice intraperitoneally with 3 ml of 3% proteose peptone in 0.85% NaCl; 15 h thereafter the animals were similarly injected and the peritoneal exudate cells harvested at 3 h. The cells were purified on a Hypaque density gradient, mixed with complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.) and injected into rabbits (21). The specificity of these antisera was evaluated in vitro and in vivo as described (21). Rabbit ATG was purchased from Microbiological Associates (Bethesda, Md.); the lyophilized material was reconstituted with 20 ml of 0.85% NaCl and aliquoted before freezing. All rabbit sera were heat-inactivated at 56°C for 30 min and were administered intraperitoneally in doses of 0.25 ml according to the schedules described below. Control mice were given similar injections of heat-inactivated normal rabbit serum (NRS).

Effect of eosinophil depletion on the course of S. mansoni infection. Mice were infected by the subcutaneous injection of 25 cercariae of a Puerto Rican strain of *S. mansoni* (22). At 4 wk after infection (i.e., before the onset of oviposition), half of the animals received three weekly injections of AES and the other half received NRS. The effects of antisera injections were also evaluated in groups of normal mice. During the subsequent 4–6 wk, all mice were observed for changes in body weight and mortality. Samples of peripheral blood were obtained weekly from six randomly selected mice, from each group, for determination of the total eosinophil and leukocyte counts.

At 8 wk of infection, randomly selected groups of AES- and NRS-treated animals were weighed and anesthetized with an intraperitoneal injection of sodium pentobarbital (Diamond Laboratories, Inc., Des Moines, Iowa). A laparotomy was performed and a needle connected to a pressure transducer was inserted into the portal vein and the blood pressure was recorded (23). When the needle was removed blood was collected for hematocrit determination. The chest was then opened, and the gastroesophageal junction was examined for the presence of varices. Adult schistosome worms were recovered from the portal circulation by perfusion with saline through the inferior vena cava just above the hepatic vein; the worms were collected in conical flasks and counted (24). The liver and spleen were removed and weighed. A small portion of the liver was preserved in 10% buffered formalin for histopathologic examination, and the remainder of the organ was reweighed and frozen for subsequent counting of eggs after tissue digestion in potassium hydroxide (25). Hematoxylin- and eosin-stained sections of the liver were used to determine the size of the granulomatous response around eggs deposited in that organ. Measurements of two perpendicular diameters of the inflammatory response around single schistosome eggs freshly deposited in the livers (as judged by the maturity and eosinophilic stain of the miracidium) were made by a Vickers image shearing measuring device, (Vickers Instruments, Inc., Woburn, Mass.). At least 20 granulomas were measured per liver, the mean diameter of the granulomas in each animal was computed, and the mean for each experimental group was used for statistical analysis using the Student's *t* test.

Effect of antisera on egg destruction in the lung. To study the effect of depletion of various leukocytes on the rate of egg destruction, we used the isolated lung granuloma model in which eggs are injected into the tail vein of mice and are trapped in the pulmonary microvasculature (26). At time intervals thereafter, eggs can be recovered by removing the lungs and digesting the tissues by KOH. If the animals were sensitized by pre-exposure to the eggs subcutaneously the number of eggs recovered decreased significantly (27). We, therefore, used egg sensitized animals to construct ova destruction curves in control and leukocyte-depleted mice. *S. mansoni* eggs were obtained from the livers of 8-wk infected mice and suspended in 0.85% NaCl at a concentration of 4,000 eggs/ml. C57BL/6J mice were sensitized by the injection of 2,000 eggs subcutaneously and 2 wk later a similar number of eggs was injected into the tail vein. AES, ANS, ATG, and NRS injections were started on the day before the intravenous injection of the eggs and were continued three times weekly for the duration of the experiment. At various time intervals after the intravenous egg injections, groups of treated and control mice were randomly selected and killed by overdose of sodium pentobarbital. The chest cavity was opened, the lungs were removed, and the organs from each mouse were placed in a Waring blendor (Waring Products Div., Dynamics Corp. of America, Hartford, Conn.) and ground at medium speed for 2 min with 25 ml of 0.85% NaCl. Lung fragments were transferred to a flask containing 50 ml of 80 g/liter

KOH and an additional 25 ml of NaCl was used for washing the blendor to bring the total volume to 100 ml. The flasks were then incubated at 37°C for 3 h with intermittent agitation; quadruplicate 1 ml aliquots were removed and the number of eggs counted on a Sedgwick-Rafter chamber (Curtin-Matheson Scientific, Houston, Tex.). The mean recovery for each group of mice was calculated and used for statistical comparison with other groups using the Students *t* test.

Size and cellular composition of pulmonary egg granulomas. 8 d after intravenous injection of eggs, groups of antisera-treated and control animals were anesthetized with intraperitoneal sodium pentobarbital. In some animals the lungs were inflated with buffered 10% formalin and processed for measuring granuloma size by πMc particle measurement computer (Millipore Corp., Bedford, Mass.) (28). In other experiments the lungs were inflated with 1 ml 0.85% NaCl, removed and homogenized in a Waring blendor at low speed for 30 s to separate the intact granulomas. The contents of the blendor were transferred to conical flasks, allowed to settle for a few minutes, and then washed twice with 200 ml of saline. Aliquots of the intact granulomas settling to the bottom of the conical flasks were aspirated and transferred to petri dishes. Granulomas were removed with a Pasteur pipette and counted; 50 intact granulomas were placed in a 12 × 25 mm plastic tube (No. 2054, Falcon Labware, Div., Becton Dickinson & Co., Oxnard, Calif.) to which 0.5 ml of 10 mg/ml collagenase, (Worthington Biochemical Corp., Freehold, N. J.), 0.05 ml of a 10 mg/ml pronase solution (Cal-Behring Corp., American Hoechst Corp., San Diego, Calif.), and 0.01 ml of heat-inactivated fetal calf serum (Flow Laboratories, Inc., Rockville, Md.) was added (16). After 60 min of intermittent agitation at 37°C, the granulomas were dispersed into their individual cellular components. The cell suspension was washed twice in phosphate-buffered saline supplemented with 10% fetal calf serum. Total cell counts were performed in a Coulter counter (Coulter Electronics Inc., Hialeah, Fla.). The counts obtained were divided by 50 to compute total cell numbers per granuloma. Differential counts were made on cytocentrifuge preparations (Shandon elliott cytospin, Shandon Southern Instruments Inc., Scientific, Sewickley, Pa.), fixed in methanol for 10 min, and were stained with tetrachrome stain.

RESULTS

Eosinophil depletion in mice infected with S. mansoni. The mean eosinophil counts in the peripheral blood of normal CF-1 female mice was 31±6/mm³. It increased in 7 wk *S. mansoni*-infected animals to 79±6/mm³ ($P > 0.01$) and by 9 wk it was significantly elevated to 235±63 eosinophil counts/mm³ ($P < 0.02$) (Fig. 1). In contrast, no eosinophils were detected in the peripheral blood of AES-treated animals through the 8 wk of infection, and at 9 wk the mean count was 31±24/mm³, which is only 13% of that detected in NRS-treated controls ($P < 0.02$). Mortality in AES-treated infected mice was first observed at week 7, when 20% of the animals were dead compared to none in the infected controls. The cumulative percent mortality in this group was 46 and 88 at 8 and 9 wk, respectively. Whereas none of the infected control mice died at 8 wk, only 5% mortality was observed 9 wk after infection. Serum treatment in uninfected mice for 4 wk (AES or NRS) was not associated with decreased survival.

For parasitologic and pathologic evaluation, four groups of animals were studied at 6 and 8 wk after cercarial exposure. At 6 wk of infection, in the *S.*

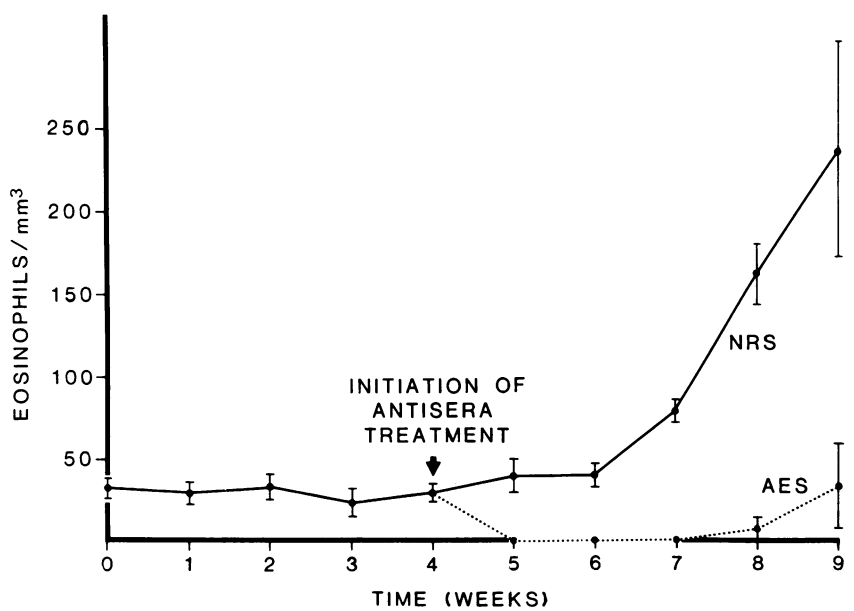

FIGURE 1 The effect of NRS or AES treatment on the total peripheral blood eosinophil counts of animals infected with 25 *S. mansoni* cercariae. Antisera treatment was started at week 4 and continued through week 9. Each point represents the mean counts of six randomly selected mice±SEM.

TABLE I

Effect of AES and NRS Treatment on Uninfected Mice and Animals Infected for 6 wk with 25 S. mansoni Cercariae

	n	Body weight	Liver weight	Spleen weight	Parameter: Portal pressure	Parameter: Hemato-crit	Eggs/liver	Diameter of granulomas
		g	mg	mg	cm H_2O	%		μm
Uninfected mice								
NRS-treated*	6	28±1.0‡	1,322±321	90±15	5.1±0.3	45±0.7	—	—
AES-treated	6	28±1.0	1,401±216	98±10	5.3±0.6	44±0.9	—	—
Schisto-infected mice								
NRS-treated	7	26±0.6	2,289±221	354±43	9.3±1.1	41±1.0	3,110±1,250	380±20
AES-treated	6	27±1.0	2,397±162	442±38	8.8±1.0	38±1.1	3,412±810	190±20§

* Antisera treatment was started at week 4 after infection and continued three times a week through week 6. All animals were then killed at week 6 and various pathological and parasitological parameters of disease were quantitated.

‡ Mean±SE.

§ Significantly different from NRS-treated controls, $P < 0.001$.

mansoni-infected animals treated with NRS, liver weight increased by 40% ($P < 0.05$) and spleen weight by 293% ($P < 0.005$) (Table I). The mean portal pressure of uninfected mice was 5.1±0.3 cm H_2O and increased by 82% to 9.3±1.1 cm H_2O in 6 wk infected animals ($P < 0.01$). The mean egg counts recovered from the livers of infected NRS-treated mice was 3,110 ±1,250 eggs/liver. The mean granuloma diameter around freshly deposited eggs was 380±20 μm. Examination of stained liver sections showed that ~50% of the cells constituting the egg granulomas were eosinophils. The major disease manifestations due to schistosomiasis mansoni in the eosinophil depleted mice at 6 wk were similar to those treated with NRS (Table I). The degree of hepatic and splenic enlargement (71 and 351%) and portal hypertension (66%) was not significantly different in the two infected serum-treated groups. In contrast, the eosinophil-depleted mice developed a mean granuloma diameter of 190±20 μm, which was 50% less than the NRS-treated controls ($P < 0.005$). Histopathological examination of the smaller granulomas in the AES-treated mice showed no detectable eosinophils. The intensity of infection was similar in the two groups as there was no difference in the mean number of worms recovered (Table I).

At 8 wk of infection NRS-treated mice manifested the advanced stage of hepatosplenomegaly due to schistosomiasis mansoni (Table II). The mean liver and spleen weights increased to 136 and 340% of

TABLE II

Effect of AES and NRS Treatment on Uninfected Mice and Animals Infected for 8 wk with 25 S. mansoni Cercariae

	n	Body weight	Liver weight	Spleen weight	Parameter: Portal pressure	Parameter: Hemato-crit	Eggs/liver	Diameter of granulomas	Adult worms/mouse
		g	mg	mg	cm H_2O	%		μm	
Uninfected mice									
NRS-treated*	6	29±1‡	1,352±192	102±10	5.5±0.8	46±0.9	—	—	
AES-treated	6	29±1	1,395±212	127±15	5.2±0.9	45±1.1	—	—	
Schisto-infected mice									
NRS-treated	9	28±1	3,189±181	449±37	11.5±0.6	31±2.0	7,476±809	425±30	15±1
AES-treated	12	29±1	3,344±234	632±30§	15.2±0.8§	28±1.5	12,429±2,617‖	260±15¶	15±2

* Antisera treatment was started at week 4 after infection and continued three times a week through week 8. All animals were then killed at week 8 and various pathological and parasitological parameters of disease were quantitated.

‡ Mean±SEM.

§ Significantly different from NRS-treated controls, $P < 0.01$.

‖ Significantly different from NRS-treated controls, $P < 0.025$.

¶ Significantly different from NRS-treated controls, $P < 0.001$.

control values in normal uninfected NRS-treated mice, respectively. Both values are significant at the 1% level. The mean portal pressure was 11.5±0.6 cm H_2O and the hematocrit dropped to 31±2%. An average of 7,476±809 eggs per liver was recovered from these animals and the mean granuloma diameter was 425 ±30 μm. Mice treated with AES and examined at 8 wk of infection showed no significant differences in the numbers of adult worms recovered from their portal circulation when compared to NRS-treated controls (Table II). The clinical disease in the eosinophil-depleted mice, however, was significantly different from NRS-treated controls. The mean spleen weight was 40% higher and the mean portal pressure was 32% higher ($P < 0.025$ and $P < 0.05$, respectively). Furthermore, the mean granuloma diameter in the livers of AES-treated mice was 39% smaller (260±15 vs. 425±30 μm, $P < 0.001$). The most striking difference between these two groups was in the number of eggs recovered from their livers. Whereas a mean of 7,476±809 eggs/liver was recovered from the NRS-treated mice, the mean value for the eosinophil-depleted animals was 80% higher (12,429±2,617 eggs/liver, $P < 0.025$).

Effect of leukocyte depletion on the rate of egg destruction in the lungs. A decay curve was constructed for the rate of disappearance of a known number of *S. mansoni* eggs injected into the pulmonary vasculature of previously sensitized mice (by a subcutaneous injection of 2,000 eggs). In control NRS-treated mice, of a mean of 2,000 eggs injected intravenously, the recovery of eggs 1 d later averaged 1,950±104 (Fig. 2). At day 8, a mean of 1,905±120 eggs was obtained which was not significantly different from day 1. By day 16, the first significant decrease in egg counts was detected; the percent recovery was 73 ($P < 0.025$) and it decreased to 43 on day 32 ($P < 0.001$). Lungs removed from animals at 44 d contained a mean of 18% of the eggs recovered on day 1 ($P < 0.001$). Regression analysis of the rate of disappearance of the schistosome eggs showed that previously sensitized mice destroy half of the eggs injected into their lungs in 27 d. On the other hand, mice treated with AES showed a significant delay in the rate of egg destruction (Fig. 2). During the first 24 d after egg injection, the mean numbers recovered from the eosinophil depleted animals at three time intervals (8, 16, and 24 d) were not significantly different from the number of eggs recovered on day 1. The average egg recovery at day 8 was 1,880±98, at day 16 it was 1,840±102, and at day 24 the mean was 1,610±185 or 83% of the day 1 value (1,900±100). A significant decrease of egg counts obtained from AES-treated mice was first observed on day 32 (percent recovery was 73, $P < 0.05$). Regression analysis of the rate of egg recovery in AES-treated mice showed that half the eggs were destroyed by day 44. This is 17 d longer than the half-life time of eggs in control mice ($P < 0.01$).

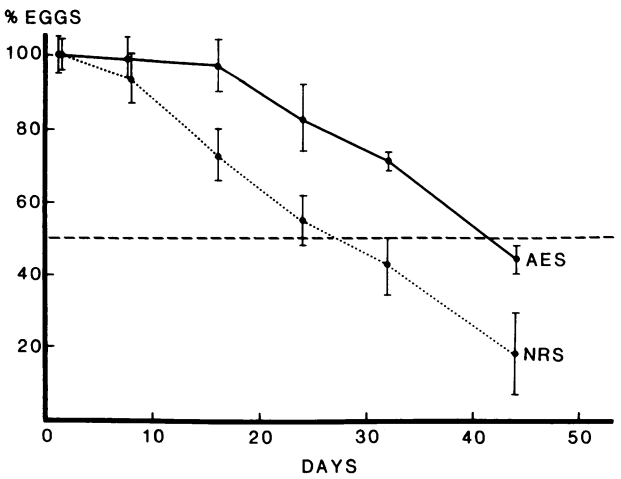

FIGURE 2 The effect of NRS or AES treatment on the rate of destruction of eggs injected intravenously into the lungs of mice previously sensitized with 2,000 *S. mansoni* eggs subcutaneously. Antisera treatment was started the day before the intravenous injection of 2,000 eggs and continued three times a week. At intervals, groups of mice (eight each) were killed, their lungs were removed, digested, and the number of recovered eggs was determined. Data represent mean±SEM for each experiment.

The effect of timing of eosinophil depletion on the rate of egg destruction was subsequently studied. Administration of AES during the first 2 wk decreased egg recovery at day 32 from 35±12% in control animals to 51±15% ($P < 0.05$). Similarly, if AES administration was initiated on day 16 and continued for 2 wk the recovery of eggs at day 32 was 59±10% compared to 35±12% in NRS-treated animals ($P < 0.01$). In mice receiving AES treatment for the full 32 d the recovery was 72±6%.

Granuloma formation and its cellular components were correlated with egg recovery in groups of antisera treated mice (Table III). In three separate experiments, the mean diameters of 8-d granulomas in NRS-, ANS-, ATG-, and AES-treated mice were 252, 257, 178, and 160 μm, respectively. Both ATG and AES treatment significantly reduced granuloma size ($P < 0.01$ and $P < 0.001$, respectively). The reduction in granuloma size was reflected in the total number of cells and the percentage of each cell recovered from the lesions (Table III). In ATG-treated mice, there was significant decrease in the total number of lymphocytes and eosinophils, but the latter constituted a higher percentage of cells (66%) in the granulomas. In contrast, the reduction in granuloma size observed in AES-treated mice was associated with a marked reduction of

TABLE III

*Effect of Antileukocyte Treatment on Granuloma Size and Cellular Composition in the Lungs of Mice**

Treatment	Granuloma diameter	Cell counts per granuloma × 10^3				
		Total	Eosinophils	Lymphocytes	Macrophages	Neutrophils
	μm					
NRS	252±26	12.1±1.9	7.1±0.3 (58)‡	2.9±0.2 (24)	1.5±0.2 (13)	0.6±0.2 (5)
ANS	257±14	12.7±1.5	6.7±0.4 (53)	3.7±0.7 (29)	2.3±0.4 (18)	0±0 (0)
ATG	178±34§	4.9±0.6§	3.3±0.5§ (66)	0.4±0.05‖ (8)	1.3±0.3 (25)	0.1±0.1 (2)
AES	160±22§	3.8±0.6§	0.04±0.01‖ (1)	2.4±0.4 (63)	1.3±0.3 (34)	0.1±0.1 (2)

* Groups of mice (eight each) were sensitized with 2,000 *S. mansoni* eggs subcutaneously 2 wk before intravenous injection with 2,000 eggs. Mice were killed at day 8 for granuloma measurement and enzymatic digestion to determine cell composition. All values are mean±SEM.

‡ Percentage of total cell count in parentheses.

§ Significantly different from NRS-treated controls, $P < 0.01$.

‖ Significantly different from NRS-treated controls, $P < 0.001$.

eosinophils (1%) in comparison to 58% in NRS-treated controls. Furthermore, the percentages of macrophages and lymphocytes were significantly higher in the granulomas recovered from AES-treated mice. Egg recovery was compared in the four experimental groups at 1, 16, 24, and 32 d (Table IV). Animals treated with either NRS or ANS showed a similar decrease in the rate of egg recovery. The mean number of eggs obtained by digestion of the lungs at day 24 was 1,066±138 and 990±151, respectively ($P < 0.05$). In contrast, the mean number of eggs recovered from AES-treated mice at day 24 was 1,610±185, which was not significantly different from the mean at day 1 (1,900±100). Egg recovery in ATG-treated animals at day 24 was intermediate (mean 1,268±148) between NRS- and AES-treated mice, but not significantly different from the values obtained from either group.

DISCUSSION

Since the description of the eosinophil polymorphonuclear leukocyte by Paul Ehrlich in 1879, an enormous literature has accumulated on the cell morphology, kinetics and production (29) but little has been learned of its function (30). The diversity of clinical and experimental conditions associated with eosinophilia (30) defied the development of a unifying concept for the functional role of these cells. Helminth infections represent unique models of infectious agents characteristically associated with tissue and peripheral blood eosinophilia (1, 2). In murine schistosomiasis mansoni the kinetics and time-course of eosinophilia has been well characterized (1). Furthermore, a protective role for the cells against the invading stage of *S. mansoni* has been suggested in studies using a monospecific antieosinophil serum (3). Mice depleted of their eosinophils lost their acquired immunity against subsequent challenge with *S. mansoni*. These in vivo observations on the protective role of the eosinophil were supported by data obtained from in vitro models using murine (8), rat (31), and human (6) cells.

This investigation concerns the role of eosinophils in the host granulomatous response around schistosome eggs retained in its tissues. The eosinophils increase in the peripheral blood of *S. mansoni*-infected animals along with the onset of oviposition (1) and they constitute ~50% of the cells forming the host granulomatous response (16). Data obtained from the kinetics of egg production by the worms (300/worm per day) and egg recovery from the tissues of infected animals showed that eggs accumulate until a steady state is reached by 12 wk of infection (23). After this phase, the rate of egg deposition almost equals or lags behind the rate of egg destruction (32). Although it has been suggested that the host destroys the retained eggs through the formation of granulomatous lesions (15), the role of the individual cell types within these granulomas is unknown. In vitro studies by James and Colley (17–20) suggested that the eosinophils, but not other cells, adhere to and invade the schistosome egg shells (17). They showed that egg destruction involves a specific anti-egg antibody found in immune mouse serum (18) and nonspecific activation of eosino-

TABLE IV

*Effect of Antileukocyte Treatment on Recovery of S. mansoni Eggs from the Lungs of Mice**

	Total egg counts/mouse lung			
	Day 1	Day 16	Day 24	Day 32
NRS	1,930±103	1,410±147 (73)‡	1,066±138 (55)	850±156 (44)
ANS	1,900±121	1,220±185 (64)	990±151 (52)	790±180 (42)
ATG	1,920±108	1,710±78 (89)	1,268±148 (66)	1,080±91 (56)
AES	1,900±100	1,840±102§ (97)	1,610±185§ (83)	1,420±55‖ (74)

* Groups of egg sensitized mice (eight each) were killed at various times after intravenous injection of 2,000 *S. mansoni* eggs.

‡ Percent reduction of egg counts as compared to day 1 recovery in parentheses.

§ Significantly different from NRS controls, $P < 0.05$.

‖ Significantly different from NRS controls, $P < 0.01$.

phils by lymphokines (19). Furthermore, antimycin A, an inhibitor of aerobic respiration, depletion of Ca^{++} ions and cytochalasin B inhibited egg destruction by eosinophils (20). These observations led to the present investigation where the role of the eosinophil within the host granulomatous response was explored by depletion studies. AES-treated mice succumbed sooner to a worm burden that does not usually lead to marked host mortality (23). These animals also showed marked pathophysiological changes such as the degree of splenomegaly and portal hypertension although their granulomas were smaller in size. This unexpected result may be due to the higher egg counts in their livers and the associated increase in the extent of tissue damage. The mortality and extent of pathology in these animals closely resemble what would be predicted if they were infected with approximately double the worm load (23). As the AES-treated mice had a worm load equal to the controls, the increased egg counts in the former group could be related to the absence of their eosinophils. The antiserum-induced eosinopenia may lead to an increased rate of egg production by the adult worms or a decreased rate of destruction by the host granulomas and consequently accumulation of eggs.

The next series of experiments were designed to explore specifically the role of the eosinophil in destruction of schistosome eggs in vivo. The lung granuloma model was used (26); sensitized mice were injected with a known number of eggs that lodge into their pulmonary microvasculature and could be recovered at time intervals thereafter (32). Half of the injected ova in NRS-treated mice were destroyed in 27 d. Similarly, in animals depleted of their neutrophils, 52% of the eggs were recovered at 24 d. In contrast, animals depleted of eosinophils had a marked impairment in the rate of egg destruction. The first significant decrease in egg recovery was noticed at day 32 and half the eggs were destroyed in 44 d. Eosinophil depletion affected the rate of egg destruction whenever it was induced, either during the first 2 wk after egg injection or during the subsequent 2 wk. The rate of egg destruction in animals treated with ATG appeared slower than the control animals although not significantly different. ATG treatment was associated with decreased eosinophil and lymphocyte content of the pulmonary granulomas. Eosinophilia in schistosomiasis and other helminth infections has been shown to be T lymphocyte-dependent (11); nude athymic mice form smaller granulomas that are deficient in eosinophils (14, 33). Lymphocyte depletion in ATG-treated mice resulted in reduction of the total numbers of eosinophils in the granulomas, which may be due to the role of lymphocytes in cell production, mobilization, or recruitment to sites of egg deposition (2, 29, 34).

Analysis of egg destruction curves show that in the AES-treated mice, after an initial delay period (16–24 d), the slope was parallel to that noted in control animals. This observation may be due to the involvement of multiple factors that bring about the final destruction of the eggs, though the eosinophils seem to be the more important contributing cell. Alternatively, the method we used to deplete the eosinophils was not absolute. The repeated injections of rabbit serum leads to the development of anti-rabbit anti-

bodies and the subsequent enhanced elimination of AES (35). Some eosinophils may, therefore, escape the effect of the antiserum and provide an explanation for the gradual but delayed destruction of the eggs in the AES-treated mice.

How the murine eosinophils that measure ~10 μm in diameter destroy the multicellular schistosome eggs with its nonliving shell (140 × 65 μm) is unknown. Although there are major differences between the different stages of schistosome worms inside the host, current investigations suggest that the eosinophils may be uniquely adapted to destruction of multicellular nonphagocytosable parasites. The eosinophils adhere firmly to complement or antibody opsonized schistosomula of *S. mansoni* (36) and discharge the contents of their granules at the cell-parasite interface. Release of some contents of the eosinophil granules such as major basic protein (37) or the generation of hydrogen peroxide (38) and other products of the peroxidative pathway has been demonstrated when the cells make contact with the parasites. Furthermore, purified major basic protein and eosinophil peroxidase have been shown to kill the schistosomula of *S. mansoni* in vitro (37, 39) (similar studies on mediators of egg destruction by human and murine eosinophils are now in progress). Our observations provide evidence for yet another unique and protective function of the eosinophils. The long-known association of eosinophilia and helminth infection has served as models for the description of the cell function, as well as the biochemical basis for host protective mechanisms against multicellular organisms.

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