

A ROLE FOR THE EOSINOPHIL IN ACQUIRED RESISTANCE TO *SCHISTOSOMA MANSONI* INFECTION AS DETERMINED BY ANTIEOSINOPHIL SERUM*

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Resistance to reinfection with *Schistosoma mansoni* has been demonstrated in several animal species including mice, rats, and rhesus monkeys (1-3). In the mouse, partial immunity develops 10-12 wk after primary infection which appears to be directed against the immature schistosomes (schistosomula) during the first few days of their development in the host. Using an assay system in which schistosomula are counted on their journey through the lungs, Sher et al. demonstrated that passive transfer of serum from partially immune donors to normal recipients conveyed significant resistance to reinfection (4). The mechanism of the serum-mediated response in vivo, and the possible role of effector cells remain unknown.

In vitro systems for assaying immunologic activity against schistosomula have demonstrated in the sera of infected humans (5), rhesus monkeys (6), rats (7), or guinea pigs (8) antibodies which are either directly lethal to schistosomula or require neutrophils. In addition, Butterworth et al. (9) have described killing of radiolabeled schistosomula on incubation with de complemented serum from schistosome-infected patients and normal peripheral leukocytes from uninfected individuals. Preliminary observations have suggested that maximum cytotoxicity was associated with eosinophil-rich polymorphonuclear leukocytes (9). This cytotoxicity was subsequently inhibited by the selective destruction of eosinophils with a monospecific rabbit antihuman eosinophil serum (10) supplied by our laboratories (11).

The above findings stimulated the present series of experiments in which the role of effector cells in acquired immunity to schistosomiasis in mice was studied in vivo by the use of monospecific antileukocyte sera. Immune animals lost their resistance to challenge infection when eosinophils—but not neutrophils—lymphocytes, or monocytes were depleted. Furthermore, the passive transfer of resistance by immune sera was blocked when mice were treated with antieosinophil serum. These results suggest that an antibody-dependent eosinophil-mediated mechanism is responsible for acquired resistance to schistosomiasis in living animals.

Materials and Methods

Animal Infection. Young adult female mice (18-22 g in weight) of the outbred CF-1 line were obtained from Carworth Div., Becton, Dickinson & Co., New York. For induction of acquired resistance, groups of mice were injected subcutaneously with 10 cercariae of *S. mansoni*

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obtained from a pool of *Australorbis glabratus* snails infected with a Puerto Rican strain of the parasite (12). Exposure to greater numbers of cercariae leads to high mortality from 10 wk of infection on. Experiments on acquired resistance were done 16 and 32 wk after the animals were infected. At the time of the challenge infection 90% of the 16-wk infected and 66% of the 32-wk infected animals had primary bisexual infections as determined by finding eggs in their livers. Those animals without eggs in the livers were discarded from the experiment.

Antisera. Monospecific antieosinophil serum (AES)¹ was prepared in rabbits injected subcutaneously with purified suspensions of mouse eosinophils in complete Freund's adjuvant as described by Mahmoud et al. (13). Rabbit antimouse neutrophil serum (ANS) was prepared by a modification of the method described by Simpson and Ross (14). Neutrophil-rich peritoneal exudates were stimulated by injecting normal CF-1 mice intraperitoneally with 3 ml of 3% proteose peptone; 15 h thereafter the animals were similarly injected and the peritoneal exudate cells were harvested 3 hr later. The cells were purified on a Hypaque density gradient (1 ml Hypaque and 2.1 ml distilled water), mixed with complete Freund's adjuvant, and injected into rabbits according to the protocol described for the AES (13). No cross-reactivity was detected between AES and ANS in vitro or in vivo (unpublished observations).

Antimacrophage serum (AMS) was raised in rabbits against mouse peritoneal macrophages as described by Boros and Warren (15). The antiserum used in these experiments was extensively absorbed with lymphocytes. The antilymphocyte serum (ALS) used was raised against mouse thymocytes in goats and was kindly provided by Dr. Mark A. Mandel of Case Western Reserve University School of Medicine, Cleveland, Ohio (16).

All antisera were administered intraperitoneally in doses of 0.25 ml starting 1 day before the challenge infection and continuing every other day for five doses. Differential leukocyte counts on peripheral blood samples were performed at frequent intervals to monitor the effects of the antisera.

Assay of Challenge Infection. Before examination of the resistance of infected immune mice to challenge infection, the percentage recovery of schistosomula in groups of five normal CF-1 mice was determined daily from day 4 to day 8; recovery was highest at day 6.

Challenge infection was done by exposing individual mice to 500 *S. mansoni* cercariae percutaneously as described previously (17). Recovery of the schistosomula from the lungs was performed as described by Sher et al. (2) and counts were performed in Sedgwick-Rafter chambers (Curtin-Matheson Scientific, Cleveland, Ohio) at a magnification of 100.

Groups of five immune mice were used to study the effect of AES on the recovery of schistosomula 4, 6, and 8 days after the challenge infection. Normal rabbit serum (NRS) was administered to control groups. Recovery of schistosomula at day 6 was then used in comparing the effects of AES to ALS, AMS, and ANS in groups of five mice.

The effect of AES administration was also assayed by adult worm recovery. 32 wk after a primary exposure to 10 *S. mansoni* cercariae, groups of six mice were challenged with 100 cercariae percutaneously as described above. Control mice received only primary or secondary exposures. The challenged animals were treated with NRS or AES 1 day before the challenge exposure and every other day thereafter for five doses. 6 wk later, adult worms were recovered by perfusion of the portal circulation (17) and the livers were crushed between glass plates and examined for residual worms and eggs. Large numbers of eggs revealed a primary bisexual infection.

Passive Transfer of Immune Sera. Mice exposed to 10 cercariae of *S. mansoni* were bled from the retro-orbital plexus 16 wk after infection. Blood collected from a group of 20 infected animals was pooled, allowed to clot at room temperature for 15 min and at 4°C for an additional hour. The tubes were then centrifuged and the serum collected and stored at -20°C until used. 10 uninfected, nonimmune mice were bled in a similar manner to provide control serum. Four groups of six to nine animals treated with NRS or AES received 0.5 ml of immune or nonimmune serum intravenously via a tail vein 1 day before challenge infection.

Results

Recovery of Schistosomula from the Lungs of Normal Mice and Effect of

¹ Abbreviations used in this paper: AES, antieosinophil; ALS, antilymphocyte serum; AMS, antimacrophage serum; ANS, antineutrophil serum; NRS, normal rabbit serum.

TABLE I
Recovery of Schistosomula from the Lungs of Normal Control Mice and those Treated with AES after a Percutaneous Exposure to 500 S. mansoni Cercariae

| Days after challenge infection | No. of schistosomula recovered | |
|--------------------------------|--------------------------------|-------------|
| | Normal controls | AES treated |
| | <i>mean ± SE</i> | |
| 4 | 73 ± 6.1 | 61 ± 5.0 |
| 5 | 93 ± 4.0 | — |
| 6 | 108 ± 6.4 | 98 ± 6.4 |
| 7 | 66 ± 3.9 | — |
| 8 | 41 ± 6.8 | 38 ± 4.5 |

AES. The number of schistosomula recovered from the lungs of normal mice 4–8 days after a percutaneous exposure to 500 cercariae is shown in Table I. The recovery increased from 15% at day 4 to 22% at day 6, and then declined to 8% at day 8. The table also illustrates the effect of AES injections on the recovery of schistosomula from the lungs of normal mice. No significant difference was demonstrated at any point on the curve, and the pattern of recovery was similar to control animals.

Recovery of Schistosomula from the Lungs of Immune Mice and Effect of Antisera. After challenge exposure to 500 *S. mansoni* cercariae the recovery of schistosomula from the lungs of mice with bisexual infections of 16 wk duration was 40% less than previously uninfected control mice at 4 days (41 ± 2.4 vs. 73 ± 6.1 schistosomula) and 38% less at 6 days (66 ± 3.9 vs. 108 ± 6.4 schistosomula). Treatment of the 16-wk infected animals with AES abrogated their immunity (Fig. 1): the counts of schistosomula at 4, 6, and 8 days rose to or above the levels seen in the previously uninfected animals (Table I) and in each case were significantly higher than those obtained from immune animals ($P < 0.001$). AES treatment did not appear to alter the rate of migration of the schistosomula, as the curves were similar to those seen in the untreated and NRS-treated animals in which a decline in recovery of the organisms was seen at 8 days. The effect of AES was also tested in a group of mice with bisexual infections of 32 wk duration. In the NRS-treated animals the counts of schistosomula on day 6 averaged 69 ± 10.0 ; the corresponding figure in the AES-treated mice was 115 ± 8.0 ($P < 0.01$).

The effect of AES was then compared to that of antisera against other white cell types. As shown in Table II, AES, ANS, and ALS specifically reduced the numbers of the corresponding cells in the peripheral blood. AMS virtually eliminated peripheral monocytes, but also induced a moderate degree of lymphopenia (Table II). Animals with bisexual *S. mansoni* infections of 16 wk duration which were treated with NRS showed 40% fewer schistosomula 6 days after challenge than previously uninfected mice. Recoveries of schistosomula from previously infected animals treated with ALS, AMS, or ANS were similar to those in the NRS-treated mice (Fig. 2). AES treatment, however, reversed the resistance of the previously infected mice: the mean recovery at 6 days rose

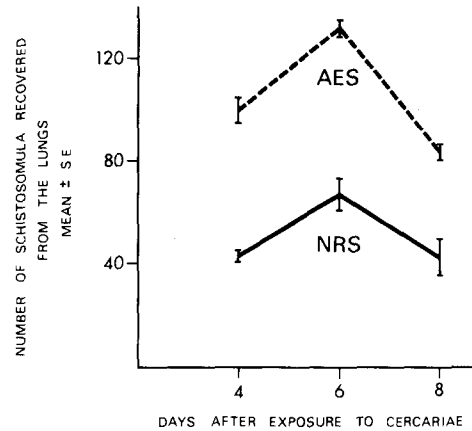


FIG. 1. Recovery of schistosomes from the lungs of partially immune mice treated with NRS or AES 4, 6, and 8 days after percutaneous exposure to 500 *S. mansoni* cercariae.

TABLE II
Differential Leukocyte Counts after Percutaneous Infection with 500 Cercariae of S. mansoni of Control Uninfected Mice and Previously Infected Mice Treated with NRS, ALS, AMS, ANS, and AES on days -1, 1, 3, 5, and 7.

| Experimental group | Days after cercarial challenge | Differential leukocyte count | | | |
|----------------------|--------------------------------|------------------------------|-------------|-------------|-----------|
| | | Lymphocytes | Neutrophils | Eosinophils | Monocytes |
| | | <i>mean % ± SE</i> | | | |
| Uninfected-untreated | 3 | 64 ± 1.3 | 29 ± 1.1 | 1 ± 0.6 | 6 ± 0.5 |
| | 6 | 57 ± 1.4 | 34 ± 1.4 | 3 ± 0.8 | 6 ± 1.2 |
| Infected-NRS | 3 | 57 ± 3.1 | 31 ± 1.1 | 15 ± 2.4 | 0.4 ± 0.1 |
| | 6 | 50 ± 1.7 | 36 ± 1.9 | 12 ± 1.0 | 2 ± 0.6 |
| Infected-ALS | 3 | 15 ± 1.5 | 62 ± 3.2 | 18 ± 2.9 | 5 ± 1.1 |
| | 6 | 19 ± 3.1 | 58 ± 2.1 | 15 ± 2.3 | 8 ± 1.4 |
| Infected-AMS | 3 | 36 ± 1.3 | 50 ± 2.1 | 13 ± 2.8 | 0.4 ± 0.1 |
| | 6 | 30 ± 2.7 | 56 ± 1.3 | 14 ± 2.0 | 0.7 ± 0.1 |
| Infected-ANS | 3 | 72 ± 4.0 | 10 ± 1.2 | 15 ± 4.2 | 3 ± 1.1 |
| | 6 | 69 ± 1.8 | 12 ± 0.7 | 12 ± 2.9 | 7 ± 2.9 |
| Infected-AES | 3 | 55 ± 2.0 | 40 ± 3.1 | 1.0 ± 0.3 | 4 ± 0.7 |
| | 6 | 57 ± 1.4 | 37 ± 1.4 | 0.5 ± 0.1 | 6 ± 1.2 |

above that seen in the previously uninfected mice and was significantly higher than that of the immune controls ($P < 0.001$).

Recovery of Schistosomula from the Lungs of Mice Rendered Immune by the Passive Transfer of Serum and Effect of AES. Sera collected from donor mice with bisexual *S. mansoni* infections of 16 wk duration transferred partial resistance to recipient mice. At 6 days the mean recovery of schistosomula after challenge infection with 500 cercariae of mice treated with serum from unin-

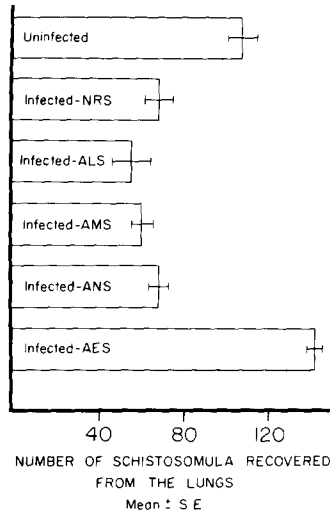


FIG. 2. Recovery of schistomula 6 days after percutaneous exposure to 500 cercariae of *S. mansoni* of control, uninfected mice, and partially immune previously infected mice treated with NRS, ALS, AMS, ANS, and AES.

ected mice plus NRS or AES was 90 ± 6.9 and 96 ± 6.4 , respectively. Mice injected with serum from the infected mice and treated with NRS showed significant immunity with a mean recovery of schistomula of 56 ± 4.5 ($P < 0.01$). AES treatment nullified the partial resistance to reinfection conferred by the transfer of immune serum as evidenced by a mean recovery of schistomula of 86 ± 5.7 ($P < 0.01$).

Recovery of Adult Worms from the Portal System of Immune Mice and Effect of AES. The worm burden of control mice which had received only the primary exposure to 10 cercariae 38 wk previously was 4 ± 0.6 . The mice challenged with 100 cercariae at 32 wk and treated with NRS had a worm burden at 38 wk of 16 ± 4.2 , which was a mean increment of 12 schistosomes. In contrast, similarly challenged mice treated with AES had a worm burden 6 wk later of 36 ± 7.7 , which was a mean increment of 32 schistosomes. The difference between the NRS- and AES-treated mice was highly significant statistically ($p < 0.001$). Previously uninfected control animals exposed to 100 cercariae showed a mean of 39 ± 9.2 schistosomes 6 wk later.

Discussion

Eosinophilia has been associated with allergic reactions, helminthic infections, and neoplasms for almost a century. Nevertheless, as Zucker-Franklin commented in a recent review, "Whether these cells fulfill a detrimental or beneficial function is still open to question," (18). In the past it was suggested that eosinophils break down histamine released in the tissues (19), and that they may be involved in the induction of antibody formation (20); these two theories have not been substantiated. With respect to allergic reactions, an eosinophil chemotactic factor of anaphylaxis has been described; this material has recently been found to be released by some neoplasms (21, 22). Eosinophils have also been

shown to be attracted to antigen-antibody complexes (23) and to phagocytize and destroy these potentially harmful entities (24). Recently the eosinophilia induced by helminths has been related to cell-mediated mechanisms (25, 26). Thus, while there are many potentially harmful or helpful immunological reactions with which eosinophils have been associated, none of them has been connected directly either to disease-related hypersensitivity reactions or to resistance to reinfection.

One of the major tools by which the different types of white cells have been associated with immunity or immunopathology has been the development of highly specific antisera against these cells. Thus the availability of ALS has provided much information about the role of lymphocytes, and even more specific antisera directed against B or T lymphocytes have added further to our knowledge (27). ANS has elucidated the role of the neutrophil in immune complex reactions (28). When a monospecific rabbit antimouse eosinophil serum (AES) was produced in our laboratories (13) it was hoped that it might provide information on the roles of the eosinophil. This antiserum agglutinated and was cytotoxic only to eosinophils *in vitro* and its activity could be absorbed out only with eosinophils, even the closely related polymorphonuclear neutrophil having no effect. It was also demonstrated in mice with schistosomiasis and recently in mice with trichinosis (unpublished data) that a single intraperitoneal injection of AES would cause a virtual disappearance of peripheral eosinophils for almost 5 days. Subsequent investigations have been performed on the effect of AES on the eosinophilic response to schistosome eggs, the major parasitic factor responsible for the development of schistosomal disease (29). Repeated doses of AES aborted the peripheral eosinophilic response and destroyed the mature eosinophils in the bone marrow, but induced the proliferation of eosinophil precursors. Most important, the granulomatous tissue lesions around the eggs contained no eosinophils and were consequently considerably reduced in size. This may have an effect on the rate of egg destruction, since eosinophils have been suggested as the major phagocytic cells involved in this process (30). However, the delayed hypersensitivity-type granulomas (31) in schistosomiasis are made up largely of macrophage and eosinophil effector cells, and removal of either of these cells (15, 29) diminishes the size of the lesion and, thus, the amount of tissue injury.

In the present investigation, we have turned our attention to immunity in schistosomiasis. Partial resistance to reinfection has been demonstrated in several species of rodents and primates (1). Only recently has this resistance been transferred *in vivo* and then with serum (4) and not with cells. *In vitro* systems involving the cultivation of immature schistosomes (schistosomula) have demonstrated a direct lethal antibody in antisera from immune animals (6). Another system has been described in rats, however, which involves antiserum, complement, and neutrophils (3). Using radiolabeled schistosomula and human antiserum and cells, an antibody-dependent cell-mediated killing system has been reported by Butterworth *et al.* (9). In subsequent studies using our rabbit antihuman eosinophil and neutrophil sera (32) Butterworth has gained further evidence that the eosinophil is the effector cell in his elegant *in vitro* system (11). Moreover, indirect evidence that eosinophils may function in immunity *in vivo* has recently appeared: a rhesus monkey repeatedly immunized by

X-irradiated cercariae on challenge with normal cercariae showed destruction of schistosomula associated with large numbers of eosinophils in the skin (33).

In our system, partially immune mice, as determined by a marked reduction in the numbers of schistosomula and adult worms, were exposed to AES. This treatment completely nullified the immunity as demonstrated by restoration to the numbers seen in control nonimmune mice of the schistosomula passing through the lungs at three different time periods and of the adult worms in the portal venous system. The total lack of effect of ANS, AMS, and ALS indicated that the eosinophil was the only significant effector cell in this *in vivo* system. Furthermore, the ineffectiveness of ALS indicated that a specific cell-mediated immunological reaction was not involved in the resistance to reinfection evinced by these animals. This was confirmed when antiserum from 16-wk infected mice passively transferred resistance to reinfection to uninfected mice; this immunity was again nullified by AES.

Thus AES appears to have defined a helpful role for the eosinophil. For the first time it has been shown definitively that the eosinophil is an essential factor in immunity *in vivo*, in this case, resistance to reinfection by the major helminth parasite of man.

Summary

Partial immunity to schistosomiasis *mansoni* has been demonstrated in mice and has recently been transferred passively with serum, but not with cells. *In vitro* studies using human and rodent materials have demonstrated antibody-dependent cell-mediated damage to immature schistosomes (schistosomula); the cell involved in some of these *in vitro* systems appears to be the neutrophil and in others the eosinophil is suspected. In the present study the effect of antileukocyte sera on partial immunity to schistosomiasis was tested *in vivo* using quantitative assay systems for schistosomula in the lungs and adult worms in the portal venous system. Mice infected with 10 cercariae of *Schistosoma mansoni* 16 and 32 wk before challenge with 500 cercariae showed reductions in the recovery of schistosomula at 4 and 6 days of approximately 40%; adult worm recovery was reduced by 60%. Treatment with antilymphocyte, antimacrophage, or antineutrophil serum had no effect on the numbers of schistosomula recovered from the lungs of immune animals, but in the mice treated with antieosinophil serum the numbers of schistosomula and adult worms recovered increased to the levels seen in normal nonimmune animals. Furthermore, sera collected from the partially immune mice and passively transferred to uninfected mice conferred a marked resistance to infection as measured by recovery of schistosomula; this was also abrogated by treatment with antieosinophil serum. These studies suggest that antibody-dependent cell-mediated immunity to schistosomiasis occurs *in vivo*, and also establishes a role for the eosinophil in immune systems.

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