Brief Definitive Report

EOSINOPHILS AND RESISTANCE TO *TRICHINELLA SPIRALIS**

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Although eosinophilia has long been associated with helminth infections, the function of the eosinophil granulocyte has remained obscure. Depletion of eosinophils by a recently developed monospecific anti-eosinophil serum (AES) (1) has provided a means by which the role of these cells might be elucidated. Schistosomiasis has been the only worm infection so far studied by this method. By using AES to eliminate eosinophils from the cells added to an in vitro assay of antibody-dependent cellular cytotoxicity, it was found that the absence of these cells prevented radiochromium release from tagged schistosomula (2). Of particular significance was the demonstration in vivo that the acquired resistance of mice with chronic schistosomiasis mansoni to challenge infection was abrogated by administration of AES (3). To verify these important observations and to extend them to another helminth infection, the present studies of trichinosis were undertaken.

In trichinosis there are two phases in which eosinophils might affect the outcome of an infection. Firstly, after the ingestion of muscle larvae, adult worms develop in the intestines where they remain for approximately 2 wk and then are spontaneously eliminated. Secondly, first-stage larvae produced during this period penetrate the gut mucosa and pass via the bloodstream to the skeletal muscles where they mature into infective third-stage larvae over 3 wk. We have examined the effect of AES with its attendant marked depletion of circulating and tissue eosinophils on both the intestinal and muscle phases of a primary infection of mice with *Trichinella spiralis*.

Materials and Methods

Monospecific AES was prepared in rabbits immunized with eosinophils derived from mice infected with *Schistosoma mansoni* as previously described (1). 100 CF_1 Swiss albino mice (Carworth Farms, N. Y.), 20–22 g in weight, were each infected by oral intubation with 350 larvae of *T spiralis* (from a strain originally supplied by Dr. W. C. Campbell, Merck Institute for Medical Research, N. J.) obtained by acid-pepsin digestion of infected mice (4). Each mouse was given 0.25 ml of AES or normal rabbit serum (NRS) by intraperitoneal injection twice weekly beginning on the 3rd day after infection. Groups of mice were killed at weekly intervals. Adult worms were recovered from the small intestine which was removed intact, chopped into 2-cm pieces, and then digested with 0 04% NaOH (5). Muscle larvae were obtained by homogenization of the whole carcass in a Waring blendor followed by digestion in 1% acid-pepsin (4). Peripheral blood and bone marrow absolute eosinophil counts were performed (6). Biopsies of the hamstring muscles were stained with hematoxylin and eosin for measurement of muscle inflammation with a π MC particle measurement computer system (Millipore Corp., Bedford, Mass.) (7), while muscle and small intestinal biopsies were stained for tissue eosinophils using May-Grunwald stain (8).

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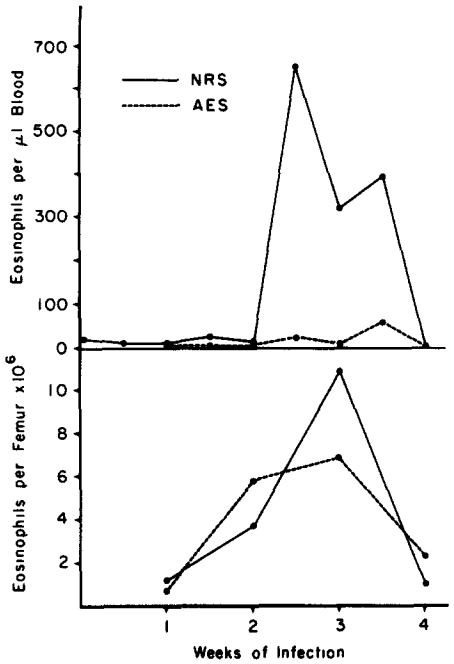

FIG 1. Geometric mean absolute peripheral blood eosinophil counts and bone marrow eosinophils per femur in mice treated with NRS (●---●) and AES (●—●). Peripheral blood eosinophilia was suppressed by treatment with AES Bone marrow eosinophilia occurred earlier in mice treated with AES.

Results

The peripheral eosinophilia which was observed between 17and 25 days after infection in mice treated with NRS was completely suppressed in animals treated with AES (Fig. 1). Mice treated with AES, which has no activity against immature eosinophils (6), showed significantly higher bone marrow eosinophil counts 2 wk after infection ($P < 0.001$, t test) than animals given NRS. 3 wk after infection, however, the bone marrow eosinophil counts were higher in mice given NRS ($P < 0.01$, t test).

The moderate infiltration of the small intestinal mucosa by eosinophils in infected mice treated with NRS was markedly suppressed in the animals treated with AES. While the inflammatory infiltrate around muscle larvae in mice treated with NRS consisted of 10–20% eosinophils, eosinophils were reduced approximately fivefold in the animals treated with AES. There was a commensurate reduction in the area of inflammation around muscle larvae; the geometric mean area of inflammation around cysts in animals given NRS was 3,450 μm^2 (range 480–24,600) compared with 3,040 μm^2 (450–20,500) in mice treated with AES, but this difference was not statistically significant.

Intestinal worm counts at 1, 2, and 3 wk after infection were similar in mice treated with AES or NRS (Table I). Muscle larvae at 3 and 4 wk after infection in mice treated with AES, however, were almost double the number found in animals treated with NRS ($P < 0.01$ and 0.001, respectively) (Table I).

TABLE I

Intestinal Adult Worm Counts and Muscle Larvae Counts in Mice at Varying Periods after a Primary Infection with T. spiralis

Duration	Treatment	Numbers of animals	Mean	SE	Probability*
Intestinal adult counts					
1 wk	AES	10	160	12	NS
	NRS	10	166	13	
2 wk	AES	10	4	3	NS
	NRS	10	19	12	
3 wk	AES	9	3	2	NS
	NRS	6	1	1	
Muscle larval counts					
3 wk	AES	6	21,400	3,400	<0 01
	NRS	9	11,000	770	
4 wk	AES	20	62,500	4,600	<0 001
	NRS	20	32,700	1,800	

Treatment with AES did not influence the expulsion of adult worms from the gut, but impaired resistance to the larval stages was evidenced by increased numbers of muscle larvae

* Student's *t* test

Discussion

As in other systemic helminth infections, eosinophilia is one of the hallmarks of trichinosis. The rise in peripheral eosinophils was shown by Basten and Beeson to be dependent upon an intact cellular immune system (9, 10). Furthermore, it has been demonstrated that lymphocytes obtained from animals with trichinosis when exposed to soluble *Trichinella* larval antigens secrete a lymphokine, eosinophil stimulation promoter (11). This substance may be one of the factors responsible for the migration of eosinophils into the major areas of inflammation observed in trichinosis: the intestinal mucosa where the adult worms reside between the columnar epithelial cells of the villi (12) and the muscles where the larvae undergo encystation. It has been suggested that the intestinal inflammation, of which eosinophils are a prominent component, is responsible for the expulsion of worms from the gut (13). The present study, however, indicates that eosinophils do not play a role in this response, as virtual elimination of these cells from the intestinal inflammatory infiltrate did not alter the rate of expulsion of the adult worms.

It appears, though, that eosinophils are an important factor in resistance to the systemic phase of this infection as mice treated with AES had double the number of muscle larvae found in animals given NRS. Since the length of residence of adult worms in the gut, and hence the time available for larval production was identical in the two groups, the numbers of muscle larvae should have been similar. Although it is possible that the fecundity of the female worms in the eosinophilopenic animals could have been increased, it seems more likely that the increased number of muscle larvae in the AES-treated mice was related to facilitation of either the passage of the first-stage larvae through the gut mucosa and the bloodstream or of their development into mature larvae in the skeletal muscles. The small reduction in the area of inflammation around the muscle larvae was consistent with markedly decreased numbers of eosino-

phils in the lesions. These observations parallel those found in schistosomiasis where depletion of eosinophils with AES resulted in a corresponding decrease in the area of inflammation around schistosome eggs (6).

Mahmoud et al. (3) showed that eosinophils are an essential factor in the resistance of mice with schistosomiasis mansoni to a challenge infection. The present experiments reveal that eosinophils play a role in resistance to the systemic phase of a primary *T. spiralis* infection in mice. Whether their effect is nonspecific or is related to the development of specific acquired immunity is as yet uncertain. Thus, the long-recognized association of eosinophils with helminth infections has been further defined. In a second helminthiasis, eosinophils have been shown to be an important factor in protection against infection.

Summary

Mice infected with *Trichinella spiralis* were depleted of eosinophils by repeated administration of rabbit anti-mouse eosinophil serum. There was no effect on the spontaneous expulsion of adult worms from the small intestines, but the numbers of larvae in the muscles were almost doubled. It is concluded that eosinophils contribute to resistance to the systemic phase of trichinosis.

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References

1. Mahmoud, A. A. F., K. S. Warren, and D L. Boros. 1973. Production of a rabbit antimouse eosinophil serum with no cross-reactivity to neutrophils. *J. Exp. Med.* 137:1526.
2. Butterworth, A. E., R. F. Sturrock, V. Houba, A. A. F. Mahmoud, A. Sher, and P. H. Rees. 1975. Eosinophils as mediators of antibody-dependent damage to schistosomula. *Nature (Lond.).* 256:727.
3. Mahmoud, A. A. F., K. S. Warren, and P. A. Peters. 1975. A role for the eosinophil in acquired resistance to *Schistosoma mansoni* infection as determined by antieosinophil serum. *J. Exp. Med.* 142:805.
4. Gould, S. E. 1970. Trichinosis in man and animals. S. E. Gould, editor. Charles C Thomas, Publisher. Springfield, Ill. 190.
5. Campbell, W. C. 1965. Immunizing effect of enteral and enteral-parenteral infections of *Trichinella spiralis* in mice. *J. Parasitol.* 51:185.
6. Mahmoud, A. A. F., K. S. Warren, and R. C. Graham, Jr. 1975. Antieosinophil serum and the kinetics of eosinophilia in schistosomiasis mansoni. *J. Exp. Med.* 142:560.
7. Grove, D. I., and K. S. Warren. 1976. Effects on murine trichinosis of niridazole, a suppressant of cellular but not humoral immunological responses. *Ann. Trop. Med. Parasitol.* 70:449.
8. Manual of histological and special staining technics of the armed forces institute of pathology, second edition. 1960. McGraw-Hill Book Company, New York 177.
9. Basten, A., M. H. Boyer, and P. B. Beeson. 1970. Mechanism of eosinophilia. I. Factors affecting the eosinophil response of rats to *Trichinella spiralis*. *J. Exp. Med.* 131:1271.
10. Basten, A., and P. B. Beeson. 1970. Mechanism of eosinophilia. II. Role of the lymphocyte. *J. Exp. Med.* 131:1288.
11. Warren, K. S., R. Karp, R. P. Pelley, and A. A. F. Mahmoud. 1976. The Eosinophil

Stimulation Promoter test in murine and human *Trichinella spiralis* infection. *J. Infect. Dis.* 134:277.

12. Race, G. J., J. E. Larsh, J. H. Martin, and N. F. Weatherly. 1974. Light and electron microscopy of the intestinal tissue of mice parasitized by *Trichinella spiralis*. *In* Trichinellosis: Proceedings of the Third International Conference on Trichinosis. C. W. Kim, editor. Intext Educational Publishers, New York. 75.
13. Larsh, J. E., and G. J. Race. 1975. Allergic inflammation as a hypothesis for the expulsion of worms from tissues: a review. *Exp. Parasitol.* 37:251.