Modulation of Particle Uptake in Trichinella spiralis-Infected Mice

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Mice were infected with 170 Trichinella spiralis larvae, and their blood clearance of carbon particles was studied 4, 7, 13, 32, and 135 days later and compared with that of noninfected control mice. Clearance in mice with a 4-day-old infection was comparable to that in the controls; clearances in mice with 7- and 13-day-old infections were increasingly accelerated and significantly different from that in the controls; clearances in mice with 32- and 135-day-old infections were proportionally inhibited. Trichinellosis in mice does not modulate phagocytosis during the intestinal stage, but causes enhancement during the migratory period and inhibition during the muscle phase.

Recent papers have demonstrated that Trichinella spiralis infections or extracts interfere with immune responses to unrelated antigens in mice (reviewed in reference 1). Other reports have suggested that the nematode infection enhances phagocytic activity (2, 9). Lubiniecki et al. (6) found that the distribution of phagocytized sheep erythrocytes changed in T. spiralis-infected mice, and Wing et al. (12) reported that macrophages were more abundant in the peritoneal cavity of similarly infected mice. My observations (J. Parasitol., in press) indicate that, simultaneously with a depression of the T cell-dependent responses, inoculation of T. spiralis extracts in mice enhances the processing of unrelated antigens, presumably by activation of phagocytic cells.

To continue these investigations, I decided to study the phagocytic activity in T. spiralis-infected mice by measuring the rate of clearance of particles from the peripheral blood at different times of infection. To avoid interference due to cross-reactivity of the particles with immune reactions to antigens of the nematode, I selected a nonantigenic material, carbon.

MATERIALS AND METHODS

Female Swiss white mice, about 4 months of age and 30 g of weight, were used. The parasites were obtained by HCl-pepsin digestion of carcasses of similar animals, infected 30 to 40 days previously. Infection was done by intragastric administration of 170 larvae. Carbon clearance was studied in control mice after a mock infection with 0.15 M NaCl (saline) and in infected mice at 4 (group 14), 7 (17), 13 (113), 32 (132), or 135 (1135) days after the actual infection. The carbon suspension was injected intracardially (0.1 ml/10 g of mouse) in animals previously anesthetized with 0.6 mg of sodium pentobarbital per 10 g of body weight by intraperitoneal injection. Ten successive 20-μl samples of blood were obtained from the sectioned tip of the tail with a micropipette wetted in heparin (200 IU/ml) at 1 and then every 2 min, starting at the end of the injection. Each sample was hemolyzed in 2.0 ml of 0.1% NaCO3 and read in a spectrophotometer at 675 nm. Only the samples of mice that did not show effusion of the carbon suspension into the thoracic cavity after recovering from the anesthesia were considered adequate.

The carbon suspension was prepared by dialyzing black India ink (Pelikan, Hannover) in 100 volumes of 25% methanol for 12 h twice and 100 volumes of saline for 12 h twice, centrifuging at 500 x g for 10 min, and recovering the supernatant fluid. This fluid was diluted in 0.5% gelatin in saline until the optical density at a further dilution at 1:1,000 in distilled water read 0.55 at 675 nm; the suspension in gelatin was then used for injection into the mice.

The mean and standard error of the optical densities of the samples at each time were obtained for each group, and the statistical significance was studied by analysis of variance. The difference among groups was assessed by the Duncan multiple range test. Only P < 0.05 was considered a significant difference.

RESULTS

The time course of the carbon clearance in noninfected control mice and in infected mice is shown in Fig. 1. The results in the control group and group 14 were statistically similar at all times. Clearances in group 17 and 113, on the contrary, were always significantly faster than in the control group; group 113 showed the fastest clearance of all groups up to 11 min but became comparable to the clearance of group 17 thereafter. Group 132 exhibited a significantly reduced clearance as compared with the control group, beginning at 7 min, and group 1135 presented the same phenomenon after 1 min. Groups 1135 and 132 yielded comparable results.
at 3, 7, and 9 min, but the former group had a slower clearance at all other times.

**DISCUSSION**

Since carbon particles have not been demonstrated to be antigenic or to activate the complement system, my results probably indicate alteration of the inherent activity of phagocytic cells rather than enhanced uptake mediated by cross-reactive antibodies or complement factors bound to the particles. Any nonspecific adsorption of these macromolecules onto the carbon particles should have been comparable in control and infected mice.

Harley and Gallicchio (4) reported that larvae of *Trichinella* are found first in the circulation of rats on day 4 of infection, peak on days 9 to 10, and are absent after day 14. A similar timing must occur in mice since Podhajecky (10) found the first larvae in the blood on day 6, and I have observed them on day 5 (Am. J. Vet. Res., in press). By day 32 of infection, the larvae are fully grown, infective, and encapsulated in skeletal muscles (3, 11). Accordingly, my observations on day 4 of infection indicate the influence of developing and adult intestinal parasites on phagocytic activity; those on days 7 and 13 show the influence of the migratory larvae and those on days 32 and 135 detect the influence of muscle parasites.

Observation of the clearance curves demonstrates that intestinal *T. spiralis* do not modify the particle uptake but that migratory larvae cause an increasing enhancement of the ability of the host to remove nonantigenic particles from its blood stream. The muscle stage of the parasite, on the contrary, produces an inhibition of the particle uptake that becomes more pronounced with time. That encapsulated parasites still release biologically active substances (which might affect phagocytic function) is demonstrated by the persistence of manifestations of specific immunity in the host for years after the infection (5).

Modulation of the phagocytic activity has been reported in other parasitic infections, notably malaria (7) and toxoplasmosis (8). The mechanisms or the biological significance of this phenomenon in trichinellosis are not known yet, but there is evidence that *T. spiralis* infections or extracts enhance the disposal of *Listeria monocytogenes* (3) and *Trypanosoma* spp. (9) and the production of IgM antibodies to nonrelated antigens (Barriga, J. Parasitol., in press); both events are likely to be mediated by phagocytic hyperactivity. On the other hand, most deaths in human trichinellosis occur after week 4 of infection and are frequently associated with bronchopneumonia; this may be explained by the failure of the macrophages to eliminate contaminants from the air passages during the chronic stage of trichinellosis.
At any rate, the biphasic modulation of the uptake of particles in trichinellosis is bound to have important repercussions in the ability of the host to produce defensive responses against the homologous and intercurrent infections.

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LITERATURE CITED