TRICHINELLA SPIRALIS: CHANGES IN LEUCOCYTES DURING INFECTION

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Rats infected with Trichinella spiralis were examined during the course of infection for various changes in the leucocytic population. In each experiment rats were divided into three groups: Group A, inoculated with Escherichia coli B-5-lipopolysaccharide (LPS) administered four days before each experiment; Group B, infected with Trichinella spiralis; and Group C, untreated controls.

An extensive leucocytosis was observed in the peripheral blood and peritoneal cavity of infected rats. Regardless of the site (peripheral blood, bone marrow, peritoneal cavity), the most obvious change was an increase in eosinophils. Differential counts of peritoneal exudate cells also revealed a significant population of macrophages. Acid phosphatase activity, macrophage phagocytosis of polystyrene latex particles, and macrophage spreading revealed that peritoneal exudate cells from T spiralis-infected rats are activated from 6 to 48 days postinfection.

This paper serves to reinforce existing information on the changes and state of the various leucocytic populations during infection with T spiralis and aids in assessing the activity change of macrophages.

For many years, an association has been noted between helminth infection and increased levels of leucocytes. This relationship is particularly marked in patients with schistosomiasis, filariasis, ascariasis, trichinosis, and visceral larva migrans.[1-4] The present study was designed to observe and correlate the changes in the number and state of the various leucocytic populations in rats infected with Trichinella spiralis. The following parameters were examined: (1) leucocytic populations in peripheral blood, peritoneal cavity, and bone marrow during the course of infection with T spiralis, and (2) macrophage activation by means of analysis of acid phosphatase activity, measurement of peritoneal macrophage spreading, and determination of peritoneal macrophage phagocytic activity.

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MATERIALS AND METHODS

Experimental Hosts

Eighty-one female Sprague Dawley rats weighing 190 ± 10 g were used as experimental hosts. The rats were obtained from the animal breeding laboratory at the National Institutes of Health, Bethesda, Md, and housed in groups of three in steel-mesh-bottomed cages. A commercially prepared pellet diet was obtained from Wayne Laboratory Food, Chicago, Ill, and the rats were fed and watered ad libitum. The water bottles were cleaned daily and the cages and food cups steamed frequently to minimize algal growth and bacterial infection.

Preparation of Inocula

T spiralis larvae were obtained from 200 ± 7 g Sprague Dawley rats infected six weeks earlier with 2,000 larvae according to the modified methods of Gursch[5] and Bachman.[6]

The Parasite

T spiralis obtained from the Animal Parasitology Laboratory, Beltville, Md, was used as the experimental pathogen. This organism has been maintained by oral passage into rats for several years.[5]

In order to quantitate the parasites, 0.1 mL of the larval suspension was placed on a 75 × 25 mm microscope slide and covered with a 22 × 50 mm cover slip. The total number of larvae was counted under low power (100×) magnification. At least four counts were taken and the average number of larvae per 0.1 mL was then determined. Finally, a standardized inoculum was achieved by adjusting the volume with tap water to give the desired dosage for infection.

On day one of each experiment, rats were fed by esophageal cannulation (15 larvae per gram of body weight suspended in 1 to 1.5 mL of tap water using a blunted 18-gauge, 2-in needle attached to a 1-mL syringe). Prior to filling each syringe, the larvae suspension was gently agitated to ensure homogeneity of the mixture.

LPS Activation of Peritoneal Cells

Escherichia coli B-5 lipopolysaccharide (LPS) (Difco Laboratories, Detroit, Mich) was used for activation of peritoneal mononuclear cells and therefore served as the positive control.[7] One hundred mg of LPS were dissolved in 230 mL of sterile phosphate buffered saline (PBS) to a final concentration of 435 μg/mL and stored in aliquots of 10 mL at −10° C.

Four days prior to each experiment a group of rats were inoculated intraperitoneally with 0.2 mL of LPS (87 μg).

Peripheral Blood Counts

White blood cells were counted by using white blood pipettes and hemacytometers. Routinely, every six days (starting with day one of the experiment and continuing until day 48) blood was drawn from the rat's tail and diluted 1:20 with 0.1 *M* acetic acid.

Thin blood films for differential counts of leucocytes were made on standard (75 × 25 mm) glass slides using blood from the tail of the rats. The blood films were allowed to dry, then stained with Difco Quik Dip, a hematological stain that gives results similar to a Wright-Giemsa stain (Harleco, Gibbstown, NJ). One hundred leucocytes were counted under oil immersion at 970× magnification.

Peritoneal Leucocytes

The peritoneal leucocytes were harvested according to the method of Cohn and Benson.[8] Total leucocyte counts were assessed with a hemacytometer and white blood cell pipette (diluting fluid was 0.1 *M* acetic acid and the diluting factor was 1:20).

After harvesting and counting, the peritoneal leucocytes were centrifuged at 1,000 × g for 10 minutes and suspended in three parts fetal calf serum to one part Hank's Balanced Salt Solution. Finally, differential cell counts were obtained on Difco Quik Dip stained smears prepared on standard (75 × 25 mm) glass slides using oil immersion at 970× magnification.

Differential Counts of Bone Marrow Leucocytes

Femurs from freshly killed rats were employed for bone marrow leucocyte differential counts. The distal and proximal ends of the femurs were wiped with 70 percent ethanol and then all muscle tissue was clipped off with a pair of scissors. Using a 25-gauge needle attached to a 1-mL syringe, the femurs were flushed with Hank's Balanced Salt Solution into prechilled test tubes (0° C).

After harvesting, the bone marrow leucocytes were centrifuged at 1,000 × g for 10 minutes, the supernatant fluid was decanted, and the cell pellet was resuspended in a mixture of three parts fetal calf serum to one part deionized water. Differential counts were obtained on Difco Quik Dip stained smears as described previously.

Biochemical Assay for Acid Phosphatase Activity

An enzymatic spectrophotometric method was used for acid phosphatase determination. The acid phosphatase test kit (Sigma Chemical Company, St. Louis, Mo) was employed according to the adaptation of Andersch and Szczypinski.[9]

Phagocytosis by Peritoneal Leucocytes

Phagocytosis of polystyrene latex particles,

TABLE 1. ACID PHOSPHATASE ACTIVITY IN PERITONEAL LEUCOCYTES OF LPS-INOCULATED RATS: ACTIVATION OF CELLS AT VARIOUS TIME INTERVALS

Time (h)	Mean Absorbance (±SE)
0	0.22 ± 0.02
24	0.24 ± 0.05
48	0.29 ± 0.01
72	0.46 ± 0.06
96	0.59 ± 0.06
120	0.52 ± 0.04
144	0.43 ± 0.03

TABLE 2. TOTAL WHITE BLOOD CELL COUNTS IN PERIPHERAL BLOOD OF TRICHINELLA SPIRALIS-INFECTED, LPS-INOCULATED, AND NORMAL RATS (CELLS × $10^3/\mu L$ ± SE)

	Rat Treatment		
Days	T spiralis	LPS	Normal
6	12.3 ± 2	14.5 ± 1	8.9 ± 0.4
12	18.1 ± 1	16.2 ± 3	9.7 ± 0.9
18	29.4 ± 0.9	21.8 ± 0.4	11.3 ± 2
24	25.2 ± 3	19.4 ± 2	13.5 ± 0.4
30	21.1 ± 4	18.2 ± 1	10.7 ± 0.7
36	19.5 ± 1	23.2 ± 0.8	14.1 ± 0.8
42	20.2 ± 1	17.5 ± 2	12.4 ± 1
48	17.1 ± 0.7	20.2 ± 1	13.8 ± 1

0.822 μ in diameter (Dow Chemical Company, Indianapolis, Ind), was performed after a modified method of Wehinger and Hofacker.[10] Peritoneal cells were harvested and collected as described previously. Cell suspension in the amount of 0.02 mL was pipetted into Lab-Tek slide chambers (Naperville, Ill) and 0.1 mL of 1 percent suspension of latex particle in Hank's Balanced Salt Solution was added. The Lab-Tek slide chambers were then incubated at 37° C for 30 minutes. After incubation, the mixture was decanted from the slide's chamber and the slides were flushed carefully with saline and allowed to air dry. The Lab-Tek chambers were then removed and the slides stained with Difco Quik Dip stain and air dried. Cells were examined under oil immersion at 970× magnification; the percentage of macrophages with latex particle and the number of particles phagocytosed per cell were determined for every 100 cells.

Spreading of Peritoneal Macrophages

Peritoneal leucocytes were harvested and collected from each rat as described previously. Lab-Tek slides were prepared as for latex particle phagocytosis assay and incubated at 37° C for 30 minutes. The slides were allowed to air dry, fixed and stained as before.

The maximal length of 100 cells was measured with an ocular micrometer. This dimension was taken as a measure of the macrophage spreading. Cell measurements were performed under oil immersion of 970× magnification.

Statistical Interpretation

The data are presented as averages calculated from all experiments. Statistical treatment of the data was limited to expression of standard errors and application of Student's *t* test for significance of differences of means having a probability level of 5 percent or less.[11]

RESULTS

Preliminary Acid Phosphatase Determination

Various samples of LPS-activated peritoneal leucocytes were tested for acid phosphatase activity at daily intervals. Absorbance gradually increased from 24 to 72 hours, reaching a peak at 96 hours (Table 1). As a result, animals destined for experimentation were inoculated intraperitoneally with 0.2 mL of LPS four days before initiating these investigations. Untreated control rats, not shown, produced peritoneal cells with much lower phosphatase activity.

Peripheral Leucocytic Population

Table 2 shows the total white blood cell counts in the peripheral blood of T spiralis-infected, LPS-inoculated, and normal rats. With the exception of day 6, the infected rats had statistically higher white blood cell counts than noninfected control rats. The overall differences in percentage

TABLE 3. DIFFERENTIAL COUNTS OF PERIPHERAL LEUCOCYTES (±SE) IN TRICHINELLA SPIRALIS-INFECTED, LPS-INOCULATED, AND NORMAL RATS

Rat Treatment/ Cell Type	Days 6	12	18	24	30	36	42	48
T spiralis								
Lymphocyte %	55 ± 2	54 ± 2	56 ± 3	60 ± 2	57 ± 1	52 ± 4	65 ± 5	58 ± 2
Neutrophil %	29 ± 1	27 ± 1	21 ± 2	21 ± 1	28 ± 2	32 ± 3	26 ± 2	30 ± 1
Eosinophil %	12 ± 2	16 ± 2	19 ± 1	16 ± 1	12 ± 0	13 ± 1	8 ± 1	9 ± 1
Monocyte %	4 ± 1	3 ± 0	4 ± 2	2 ± 1	2 ± 1	3 ± 1	4 ± 0	2 ± 1
Basophil %	0	0	0	1 ± 0	1 ± 0	0	0	1 ± 0
LPS								
Lymphocyte %	64 ± 1	66 ± 1	69 ± 2	66 ± 3	66 ± 4	64 ± 0.9	54 ± 2	62 ± 4
Neutrophil %	27 ± 3	26 ± 2	20 ± 1	25 ± 3	23 ± 4	28 ± 3	38 ± 5	31 ± 3
Eosinophil %	6 ± 1	6 ± 1	7 ± 0	6 ± 0	8 ± 1	5 ± 1	6 ± 1	5 ± 1
Monocyte %	3 ± 1	2 ± 1	3 ± 2	3 ± 1	2 ± 1	2 ± 0	2 ± 1	2 ± 0
Basophil %	0	0	1 ± 1	0	1 ± 0	1 ± 0	0	0
Normal								
Lymphocyte %	63 ± 4	68 ± 5	66 ± 3	68 ± 2	65 ± 3	63 ± 1	62 ± 2	66 ± 4
Neutrophil %	33 ± 4	29 ± 3	28 ± 4	25 ± 3	27 ± 1	31 ± 4	30 ± 3	28 ± 1
Eosinophil %	1 ± 1	1 ± 1	1 ± 0	1 ± 0	2 ± 1	1 ± 0	2 ± 1	1 ± 1
Monocyte %	3 ± 1	2 ± 1	5 ± 2	6 ± 1	5 ± 1	4 ± 1	5 ± 1	4 ± 1
Basophil %	0	0	0	0	1 ± 0	1 ± 1	1 ± 1	1 ± 0

increases ranged from approximately 24 to 160 percent. When compared with normal animals, LPS-inoculated animals also showed significant increases in total white blood cell counts for all the days of observation. The maximum percentage recorded was 93 percent on day 18. Variegated results were obtained when LPS-inoculated rats were compared with infected animals on days 6 and 48; however, they were not significant. Significant differences between LPS-inoculated and infected animals were evident on days 18 and 36. On these days the percentage increase or decrease over the LPS samples represented a total white blood cell population of 7,600 and 3,700 cells/μL, respectively.

Table 3 presents the differential count of peripheral blood leucocytes.

Lymphocytes

A significant decrease in the relative levels of lymphocytes was observed when T spiralis rats were compared with either LPS-inoculated or normal rats. However, on day 42, T spiralis-infected rats had a higher lymphocytic count (65 percent) in contrast to LPS-inoculated rats (54 percent). No essential differences in the levels of lymphocytes were seen between LPS-inoculated and normal control rats.

Neutrophils

Except for days 18 and 42, LPS-inoculated, T spiralis-infected, and normal animals had similar levels of neutrophil.

Eosinophils

Differential counts revealed a marked eosinophilia in T spiralis-infected and LPS-inoculated rats compared with normal rats. In LPS-inoculated rats, the eosinophilic level ranged between 5 and 8 percent. In T spiralis-infected rats, the eosinophilic cell increase was progressive, ranging from 8 to 19 percent. The peak response was observed on day 18 of infection, after which a steady decrease was seen from day 24 to 48.

TABLE 4. TOTAL PERITONEAL LEUCOCYTE COUNTS IN TRICHINELLA SPIRALIS-INFECTED, LPS-INOCULATED, AND NORMAL RATS (CELLS × $10^3/\mu L \pm SE$)

Days	Rat Treatment		
	T spiralis	LPS	Normal
6	8.8 ± 0.34	14.2 ± 1.17	8.1 ± 0.29
12	9.2 ± 0.61	17.8 ± 5.33	7.0 ± 0.76
18	13.0 ± 0.87	16.3 ± 3.01	8.8 ± 0.72
24	11.0 ± 1.02	19.3 ± 1.67	8.3 ± 1.22
30	14.6 ± 0.58	18.8 ± 1.17	8.0 ± 1.26
36	12.8 ± 1.71	19.5 ± 1.9	7.8 ± 0.92
42	17.3 ± 1.42	19.3 ± 0.44	8.3 ± 2.89
48	12.3 ± 1.31	17.2 ± 2.13	10.2 ± 0.44

Monocytes

No significant change in the relative monocytic levels in LPS-inoculated and T spiralis-infected rats was observed when compared with control animals. In all groups, the levels of monocyte represented 2 to 6 percent of the leucocytic population.

Basophils

In all groups, basophils were too rare for statistical analysis.

Peritoneal Leucocytic Population

The total peritoneal leucocytic counts of T spiralis-infected, LPS-inoculated, and normal rats are shown in Table 4. Between days 6 and 48, rats inoculated with LPS revealed a statistically higher level in the total number of peritoneal leucocytes. The difference in the leucocytic population between these two groups ranged from 2,000 to 8,670 cells per microliter. Infected rats showed from 830 to 9,000 more cells per microliter than the uninfected controls. Advantages over the control varied from 10 to 108 percent. In comparison with controls, the total peritoneal leucocytic counts of LPS-inoculated animals were statistically different from day 6. The differences varied from 69 to 154 percent over the normal rats.

Differential counts of peritoneal leucocytes in LPS-inoculated, T spiralis-infected, and normal rats are displayed in Table 5.

Lymphocytes

Except for day 6, there were no significant changes in the peritoneal lymphocytic levels between the LPS-inoculated and T spiralis-infected rats. The percentage of lymphocytes in these two groups generally remained stable at a range of 0 to 5 percent throughout the study. In contrast, the lymphocytic levels of normal rats ranged from 11 to 16 percent.

Neutrophils

Similarly, there were no essential differences in the levels of neutrophils between the LPS-inoculated and normal animals. The average neutrophilic level of LPS-inoculated rats was 7.5 ± 3, whereas the level for the normal animals was 7.0 ± 2. In contrast to LPS-inoculated and normal rats, T spiralis-infected rats exhibited a biphasal neutrophilic population. A gradual decrease in the differential count occurred after day 6, reaching the lowest level on day 30. The differential count then increased on day 36, reaching a second peak on day 48.

Eosinophils

In comparison with LPS-inoculated and normal rats, leucocytic counts showed an increase in eosinophilic cells in T spiralis-infected animals. Peritoneal eosinophilic levels of 8 to 22 percent were observed in T spiralis-infected rats from

TABLE 5. DIFFERENTIAL COUNTS OF PERIPHERAL LEUCOCYTES (±SE) IN TRICHINELLA SPIRALIS-INFECTED, LPS-INOCULATED, AND NORMAL RATS

Rat Treatment/ Cell Type	Days							
	6	12	18	24	30	36	42	48
T spiralis								
Lymphocyte %	12 ± 1	1 ± 0	4 ± 1	1 ± 1	3 ± 2	3 ± 1	2 ± 0	2 ± 1
Neutrophil %	12 ± 1	7 ± 1	6 ± 1	7 ± 1	6 ± 1	10 ± 1	9 ± 1	13 ± 0
Eosinophil %	2 ± 1	8 ± 1	15 ± 1	22 ± 1	11 ± 1	12 ± 2	12 ± 3	16 ± 4
Macrophage %	74 ± 5	84 ± 2	75 ± 1	70 ± 3	80 ± 2	75 ± 2	77 ± 3	69 ± 5
LPS								
Lymphocyte %	2 ± 1	1 ± 0	5 ± 2	1 ± 1	0	0	3 ± 2	0
Neutrophil %	13 ± 8	6 ± 2	4 ± 2	8 ± 2	5 ± 2	6 ± 2	7 ± 1	11 ± 1
Eosinophil %	5 ± 2	3 ± 2	4 ± 3	9 ± 1	5 ± 1	3 ± 1	3 ± 0	4 ± 2
Macrophage %	80 ± 9	90 ± 4	87 ± 2	82 ± 3	90 ± 4	91 ± 2	87 ± 3	85 ± 2
Normal								
Lymphocyte %	16 ± 3	11 ± 1	14 ± 3	12 ± 0	12 ± 3	11 ± 2	11 ± 3	11 ± 1
Neutrophil %	7 ± 2	8 ± 2	8 ± 1	6 ± 1	9 ± 1	5 ± 2	5 ± 2	8 ± 0
Eosinophil %	2 ± 2	4 ± 1	2 ± 1	4 ± 1	2 ± 1	3 ± 1	2 ± 1	4 ± 2
Macrophage %	75 ± 1	77 ± 1	76 ± 3	78 ± 2	77 ± 3	81 ± 2	82 ± 2	77 ± 1

day 12 to day 48. Peritoneal eosinophils in normal and LPS-inoculated rats averaged 3 and 4 percent, respectively.

Macrophages

With the exception of days 12 and 48, macrophages in the peritoneal cavity of T spiralis-infected rats showed differential counts similar to that of normal rats. For most periods of observation, the relative level of peritoneal macrophages in LPS-inoculated rats was much higher than in the other two groups.

Differential Count of Bone Marrow Leucocytes

The percentages of bone marrow leucocytes in LPS-inoculated, T spiralis-infected, and normal rats are shown in Table 6.

Lymphocytes

LPS-inoculated and normal rats showed no significant differences in the level of bone marrow lymphocytes on six of the eight days of the collection period. The average level of lymphocytes for the LPS-inoculated rats was 21.5 ± 3 as opposed to 28.3 ± 2 for normal rats. For most periods of the study, the relative levels of bone marrow lymphocytes in LPS-inoculated and normal rats were much higher than in T spiralis-infected rats (mean value, 17.6 ± 2).

Neutrophils

Even though LPS-inoculated rats showed higher neutrophilic levels on most days of observation, there were no significant differences in the levels of neutrophils among the three groups. Bone marrow neutrophils in normal, LPS-inoculated, and T spiralis-infected rats averaged 62.6, 66.7, and 62.8 percent, respectively.

Eosinophils

Bone marrow eosinophils collected from LPS-inoculated rats showed no significant differences when compared with those of normal animals. The average level of eosinophils for LPS-inoculated and normal rats were 5.3 ± 2 and 5.8 ± 1, respec-

TABLE 6. DIFFERENTIAL COUNTS OF BONE MARROW LEUCOCYTES (± SE) IN TRICHINELLA SPIRALIS-INFFECTED, LPS-INOCULATED, AND NORMAL RATS

Rat Treatment/ Cell Type	Days 6	12	18	24	30	36	42	48
T spiralis								
Lymphocyte %	24 ± 4	18 ± 3	24 ± 4	11 ± 1	6 ± 2	19 ± 4	11 ± 0	28 ± 2
Neutrophil %	68 ± 6	58 ± 0	46 ± 2	65 ± 2	66 ± 1	62 ± 2	74 ± 4	64 ± 4
Eosinophil %	6 ± 2	17 ± 1	25 ± 3	20 ± 1	19 ± 3	16 ± 1	10 ± 3	5 ± 0
Monocyte %	2 ± 0	7 ± 2	5 ± 2	4 ± 1	9 ± 2	3 ± 2	5 ± 0	3 ± 2
LPS								
Lymphocyte %	23 ± 6	29 ± 1	15 ± 1	18 ± 2	25 ± 6	19 ± 6	23 ± 1	20 ± 1
Neutrophil %	65 ± 11	57 ± 3	66 ± 3	66 ± 1	70 ± 3	73 ± 2	72 ± 2	66 ± 1
Eosinophil %	5 ± 2	7 ± 2	10 ± 2	8 ± 3	3 ± 2	3 ± 3	0	6 ± 1
Monocyte %	7 ± 2	7 ± 2	9 ± 1	8 ± 2	2 ± 0	5 ± 1	5 ± 0	8 ± 2
Normal								
Lymphocyte %	31 ± 2	30 ± 2	37 ± 1	24 ± 1	28 ± 6	24 ± 1	26 ± 4	26 ± 3
Neutrophil %	63 ± 0	64 ± 2	52 ± 0	62 ± 1	62 ± 3	65 ± 2	69 ± 5	64 ± 1
Eosinophil %	2 ± 2	3 ± 1	4 ± 2	5 ± 1	3 ± 2	5 ± 1	0	5 ± 1
Monocyte %	4 ± 0	3 ± 2	7 ± 3	9 ± 2	7 ± 2	6 ± 0	5 ± 2	5 ± 0

tively. The highest percentage of eosinophils was observed in T spiralis-infected rats. At 18 days postinfection, the differential counts for T spiralis-infected rats were six times higher than those of normal rats and 2.4 times higher than those of LPS-inoculated rats.

Monocytes

There were no significant changes in the relative level of bone marrow monocytes in the LPS-inoculated and T spiralis-infected rats when compared with the noninoculated controls (2 to 9 percent of the leucocytic population).

Acid Phosphatase Activity in Peritoneal Leucocytes

Table 7 represents the acid phosphatase activity in peritoneal leucocytes of T spiralis-infected, LPS-inoculated, and normal rats.

In LPS-inoculated animals, acid phosphatase activity of peritoneal leucocytes was three to four times that of cells from normal rats throughout the periods of observation. The average level of activity of acid phosphatase in the LPS-inoculated rats was 237 percent higher than in normal rats. Compared with T spiralis-infected rats, peritoneal leucocytes from LPS-inoculated rats showed higher acid phosphatase activity from day 6 to day 48. At one week postinfection, the LPS-inoculated rats showed acid phosphatase activity that was 4.6 times that of T spiralis-infected rats. At later periods of observation, the average activity of LPS-inoculated rats varied from 1.2 to 1.7 times those of peritoneal cells from T spiralis-infected rats.

Peritoneal Macrophage Spreading

Table 8 depicts the spreading activity of macrophages harvested from LPS-inoculated, T spiralis-infected, and normal rats. Spreading activities of peritoneal macrophages obtained from T spiralis-infected rats, compared with those of normal rats, were 5 percent higher at day 6 postinfection. By day 24 postinfection, the spreading activity of these cells peaked to 212 percent. From day 30 to day 48, the spreading activity was lower and ranged from 72 to 197 percent. Macrophages from LPS-inoculated rats had an average spreading length that varied from two to three times higher than macrophages from normal rats. When com-

TABLE 7. ACID PHOSPHATASE ACTIVITY IN PERITONEAL LEUCOCYTES OF TRICHINELLA SPIRALIS-INFECTED, LPS-INOCULATED, AND NORMAL RATS (MEAN ABSORBANCE ± SE)

	Rat Treatment		
Days	T spiralis	LPS	Normal
6	0.13 ± 0.04	0.60 ± 0.02	0.19 ± 0.03
12	0.39 ± 0.02	0.62 ± 0.03	0.20 ± 0.02
18	0.44 ± 0.02	0.66 ± 0.03	0.22 ± 0.03
24	0.38 ± 0.05	0.65 ± 0.03	0.18 ± 0.04
30	0.41 ± 0.06	0.63 ± 0.03	0.20 ± 0.04
36	0.38 ± 0.04	0.65 ± 0.04	0.17 ± 0.03
42	0.53 ± 0.02	0.70 ± 0.03	0.17 ± 0.01
48	0.47 ± 0.03	0.64 ± 0.04	0.20 ± 0.01

TABLE 8. PERITONEAL MACROPHAGE SPREADING OF TRICHINELLA SPIRALIS-INFECTED, LPS-INOCULATED, AND NORMAL RATS

	Macrophage Length (μ ± SE)		
Days	T spiralis	LPS	Normal
6	10.3 ± 0.1	21.8 ± 0.2	9.8 ± 0.4
12	14.7 ± 0.3	20.5 ± 0.4	9.3 ± 0.2
18	20.9 ± 0.3	23.1 ± 0.2	8.9 ± 0.3
24	29.3 ± 0.2	25.4 ± 0.5	9.4 ± 0.1
30	31.2 ± 0.5	21.3 ± 0.2	10.5 ± 0.3
36	19.3 ± 0.3	25.1 ± 0.2	9.6 ± 0.2
42	17.5 ± 0.1	23.6 ± 0.5	10.2 ± 0.3
48	15.3 ± 0.2	25.0 ± 0.3	8.7 ± 0.1

pared with T spiralis-infected rats, spreading activities of macrophages from LPS-inoculated animals ranged from 20.5 to 23.1 during the first three observational periods. On days 24 and 30, there were significant increases in the spreading activity of macrophages from the T spiralis group compared with those of LPS-inoculated animals. However, from day 36 to 48 the activity of T spiralis-infected rats decreased steadily to values below those of LPS-inoculated rats.

Phagocytosis by Peritoneal Macrophages

Table 9 represents the degree of phagocytosis of polystyrene latex particles by peritoneal macrophages harvested from LPS-inoculated, 24-day T spiralis-infected, and normal rats. The 24-day T spiralis-infected rats had 22.3 percent more macrophages with latex particles compared with normal animals. LPS-inoculated rats had 36.9 percent more macrophages with latex particles compared with noninfected controls. In comparison with T spiralis-infected animals, LPS-inoculated rats had 14.6 percent more macrophages with latex particles.

A comparison of macrophages from 24-day T spiralis-infected and normal rats showed that the former had 4.6 more latex particles per macrophage. Macrophages from LPS-inoculated rats had 9.3 more particles per macrophage than normal rats. Compared with T spiralis-infected ani-

TABLE 9. PHAGOCYTOSIS OF POLYSTYRENE LATEX PARTICLES BY PERITONEAL MACROPHAGES OF TRICHINELLA SPIRALIS-INFECTED, LPS-INOCULATED, AND NORMAL RATS*

	Rat Treatment		
	T spiralis	LPS	Normal
Percent macrophage with polystyrene latex particle ± SE	60.6 ± 7	75.2 ± 4	38.3 ± 5
Mean of polystyrene particle phagocytized per macrophage ± SE	7.1 ± 0.3	11.8 ± 0.2	2.5 ± 0.2

*24 days postinfection

mals, LPS-inoculated rats had 4.7 more latex particles per macrophage.

DISCUSSION

The extensive leucocytosis observed in this study was consistent with previously published works on rats infected with T spiralis[12,13] and mimicked pathological changes observed in various other hosts including humans,[14,15] dogs,[16] and pigs.[17]

Similarly, among the leucocytes, eosinophils were obviously an essential component of the immune mechanism against T spiralis. The presence of increased eosinophilia less than one week postinfection paralleled the observations of Zaiman et al,[13] and the marked increase in circulating eosinophils throughout the duration of these investigations was comparable to the primary wave of circulating eosinophils reported by Despommier et al.[18]

This study did not include investigation of the mechanism of eosinophilia or the role of these activated macrophages in T spiralis infection. Consequently, it is impossible to suggest that the maturing nematode initiated the eosinophilic response[13,18,19] or that the juvenile forms serve as the primary stimulus[20] or that both forms stimulate eosinophilia.[18]

In the same regard, it is equally impossible to assess the action of the host's bone marrow[21] or other immunological reactions that cause eosinophil accumulation in vivo.[22-26] Eosinophils were the prominent cell type in the early stages of trichinosis and there was a close association between eosinophils and macrophages. However, the authors can only speculate that this association might have been mediated by antigen-antibody complexes on the macrophage surface.[3] Butterworth[4] recognized the magnitude of the problem when he reported that "in spite of the consistent nature of the relationship between eosinophils and helminths, it has proved difficult to attribute a functional role to the eosinophils in such infection."

The presence of mainly macrophages in the peritoneal fluid resembled the earlier results of Wing et al,[27] who reported that the macrophage increase coincided with the elimination of the adult worms from the gut. Earlier, Larsh and Race[28] found that macrophages and lymphocytes infiltrate the intestinal wall at the time of immune rejection of adult worms from the gut.

Further analysis of peritoneal macrophage activity revealed elevated levels of acid phosphatase in peritoneal macrophage of T spiralis-infected rats. This finding approximated results obtained in earlier studies using specific macrophage activators.[29,30]

Macrophages from rats infected with T spiralis also showed an increase in phagocytic activity, suggesting that activated macrophages or products of activated lymphocytes might increase particle phagocytosis.[31-33]

When a second function of activated macrophage was studied from rats infected with T spiralis, increased spreading activity was noted. This increased spreading activity parallels previous

studies with many antigenic substances that increased adherence and spreading of macrophage.[34-36] The results observed in this study on macrophage activity were in agreement with those of previous research on macrophage activity changes with extraneous agents both in vivo and in vitro.[27,37,38]

More questions have been posed than answered. Nonetheless, it is hoped that these queries may lead to more investigations on trichinosis.

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