Immune Responses of CBA/J Mice to Graded Infections with *Toxocara canis*

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The immunological responsiveness of CBA/J mice infected with various numbers of the canine ascarid, *Toxocara canis*, was characterized after a single infection to ascertain the smallest infection capable of perturbing the immune system of the host. Mice receiving the lowest inoculations (5 eggs per mouse or 0.25 larvae per g of body weight) had detectable alterations in the number of circulating peripheral blood eosinophils and spleen weight-to-body weight ratios. Mice infected with 25 eggs each (1.25 larvae per g of body weight) showed augmented concanavalin A-elicited splenic lymphocyte transformation and a positive lymphocyte transformation in response to a toxocaral antigen preparation in addition to even higher eosinophil counts and heavier spleens. Spleen cells from mice receiving the two largest inocula (125 eggs and 250 eggs per mouse or 6.25 and 12.5 larvae per g of body weight, respectively) had in addition to the above responses a sixfold increase in spontaneous DNA synthesis. An enzyme-linked immunosorbent assay for mouse antibody responses to *T. canis* indicated that the time of onset as well as the magnitude of the antitoxocaral humoral response is directly proportional to the size of the inoculation used to initiate the infection. Finally, we showed that allowing the infection to become protracted results in some responses increasing somewhat in magnitude, but regardless of length of infection, the magnitude of any of the responses examined is proportional to the size of the infection. The results indicate that different host responses have different thresholds of sensitization and suggest that larvacidal reactions which require intricate interactions among several components of the immune system may not occur in very small infections.

Visceral larva migrans (VLM) is defined as the prolonged migration of nematode larvae through the soft tissues of the body exclusive of the skin (3, 4, 6). A frequent causative agent of VLM is the second stage larva of *Toxocara canis*, the ubiquitous roundworm of dogs (3-6, 14). The infection begins with the ingestion of fully embryonated ova in soil contaminated by the feces of infected canines. In dogs, the egg hatches in the stomach or small intestine or both and liberates a single larva measuring 20 by 400 μ m. The worm penetrates the gut wall and enters the lymphatic system or the hepatoportal circulation. From the liver the worm then passes to the heart and lungs where it breaks out of a bronchiole, ascends the respiratory tree, and is swallowed. After a series of molts, the larvae develop into mature, sexually dimorphic adults in the small intestine. Eggs will appear in the feces within 4 to 5 weeks of ingestion of the infected egg and will themselves be infective after a period of about 1 month after reaching the soil. Not all larvae that are ingested however, follow this pathway. Upon reaching the lungs, some larvae will enter the systemic circulation and distribute themselves throughout the viscera of the canine host (14).

T. canis eggs can also hatch in a large number of noncanid species, including human beings (4), and the larva then distribute themselves throughout the viscera (in a host-specific manner). Most often, these larvae become encapsulated in a granulomatous response (3-6, 8, 17, 20) or accumulate in the brain, where they elicit little or no histological reaction (4, 12). Interestingly, *T. canis* undergoes no development or maturation in a noncanid host, however, ingestion of an infected noncanid host by a canine will result in the estab-

lishment of a patent infection in the definitive canine host (31). The nonessential host, in which no development occurs but which acts to sustain the parasite through space and time until the definitive host ingests it, is said to be a paratenic host (3, 4) and offers the parasite a significant advantage in the competition to complete its life cycle.

One of the more severe sequela of a *T. canis* infection results from the invasion of the eye by a migrating larva, a condition referred to as ocular larva migrans (OLM). The ensuing inflammation may be so copious as to effect detachment and necrosis of the retina, leading to permanent blindness (14, 35). This lesion may present in such a manner that the unwary physician may mistake the lesion for a retinoblastoma and needlessly enucleate the eye (7, 27, 33). The typical patient with OLM rarely has systemic involvement, tends to be older (average age >7 years), has no history of pica, and does not have eosinophilia (13, 35).

Recently, a third form of the infection has been described in which there is a positive by enzyme-linked immunosorbent assay (ELISA) with or without eosinophilia. No other findings were noted and this form of toxocariasis was designated "clinically inapparent infection with or without eosinophilia" (2).

One hypothesis to explain the occurrence of these different forms of the disease holds that a large number of larvae may result in the marked eosinophilia and antibody levels usually associated with VLM. In contrast, infection with only a few larvae elicits little or no immune response and as a consequence, larvae may enter the systemic circulation and migrate throughout the host. Ocular infection would occur only as a random event and would be detected because of the acute perception by the patient of a visual deficit (14). The experiments herein were undertaken to

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evaluate several assays of the murine host cellular and humoral responses to a graded infection with *T. canis*. The *T. canis*-infected mouse model has been shown to be a good model of human VLM because infected mice have eosinophilia, pulmonary infiltrates, hyperglobulinemia, and eosinophil-rich granulomata like those seen in biopsy (4, 8, 20, 21). The findings suggest that the in vivo assays, namely eosinophil counts and spleen weight index, are sensitive enough to detect the smallest infection. This would seem to indicate that as few as five larvae are capable of priming the murine immune system.

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MATERIALS AND METHODS

Mice. Female CBA/J mice were obtained from Jackson Laboratories, Bar Harbor, Maine, and maintained on Purina Rodent Chow and water ad libitum in the Animal Health and Resources facilities of the College of Medicine. These facilities meet the National Institutes of Health guidelines established in the *Guide for the Care and Use of Laboratory Animals*. All mice were obtained as weanlings (12 to 15 g) and were allowed to adapt to the vivarium facilities for at least 7 days before being included in any experiment.

***T. canis*.** Procedures for culturing and inoculating this agent into mice have been previously described (18). Briefly, the fertilized eggs were cultured in 0.1 N H₂SO₄ until the infective second stage larvae developed. Mice were infected with graded doses of embryonated ova by oral intubation. The infective inocula were prepared by determining the number of ova in 25- μ l samples of a stock solution of ova with the aid of the light microscope. Smaller inocula were prepared by making appropriate dilutions of the largest size inoculum used.

Eosinophil counts. Freely flowing tail vein blood collected on paraffin film was diluted with Discombe's fluid (11) and counted on a hemacytometer. Blood was always collected between 0900 and 1100 h to minimize fluctuations due to diurnal cycles known to occur in circulating eosinophil levels. Eosinophil counts of 400 or less were considered to be normal.

Spleen weight indices. The procedure for determining the spleen weight index has been described elsewhere (18, 28). Briefly, the weight of the spleen of an infected mouse expressed as a ratio to the body weight of that mouse is divided by the same ratio for an uninfected, age-matched control animal. This value is taken to represent a measure of splenomegaly occurring in the infected animal. Thus, a spleen index of 2.0 would indicate a spleen that is twice as heavy as a control spleen.

Lymphocyte transformation assays. These procedures have been described previously (18). Briefly, single spleen cell suspensions were prepared aseptically in iced RPMI 1640 medium (Flow Laboratories, Rockville, Md.). Cells were cultured in 96-well, flat-bottomed microtiter plates (Flow) in 0.2 ml of RPMI 1640 medium containing 100 μ g of Gentamycin sulfate per ml, 5% (vol/vol) normal heat-inactivated human serum, and 2 μ g of concanavalin A (ConA) (Miles-Yeda, Ltd., Rehovot, Israel) per ml, 50 μ g of *Salmonella typhimurium* O901 endotoxin (lipopolysaccharide) (Difco Laboratories, Detroit, Mich.) per ml, or 1 μ g of *Toxocara* egg extract (TEE) antigen per ml prepared as described by Cypess et al. (10) and Glickman et al. (15). The culture plates were incubated under loose-fitting covers in a saturated

atmosphere of 5% CO₂ in air for 3 days (mitogens) or 5 days (antigen). The cultures were pulse-labeled during the last 8 h of incubation with [³H]thymidine ([³H]TdR) having a specific activity of 2.0 Ci/mmol (ICN, Cleveland, Ohio) and harvested onto glass fiber filter strips with a Brandel M24V Special automated cell harvester (Brandel Corporation, Gaithersburg, Md.). The amount of incorporated isotope was determined by immersing the filter strips in a preblended cocktail (3a70; RPI, Elk Grove Village, Ill.) and counting in a Beckman LS 6800 liquid spectrophotometer. All readings were corrected for quench and expressed as disintegrations per minute.

Determination of anti-toxocaral antibody by ELISA. *T. canis* exoantigen was obtained from larvae maintained in vitro as previously described (13). Antigen was diluted to a concentration of 20 μ g/ml with 0.1 M carbonate buffer, pH 9.6. A 50- μ l amount was added to each well of a 96-well polystyrene plate (Flow) and air-dried at 37°C overnight. After the plate was washed twice with a solution of 0.05% Tween 20 in phosphate-buffered saline, pH 7.4 (PBS-T20), 2% bovine serum albumin in PBS was added to the plate (100 μ l per well). After 10 min, the PBS–2% bovine serum albumin was removed, and each mouse serum was serially diluted in the plate 12 times in PBS-T20 with a 50- μ l diluter and incubated 1 h at 25°C. The plate was then washed three times with PBS-T20, and 50 μ l of 1:1,000 horseradish peroxidase-conjugated goat anti-mouse immunoglobulin G (IgG) and IgM (combined) (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Md.) diluted in PBS was added to each well. After 1 h at 25°C, the plate was washed three times with PBS-T20. Substrate solution (100 μ l) consisting of 0.4 mM 2,2'-azino-di(3-ethylbenzthiazoline sulfonic acid) (Sigma Chemical Co., St. Louis, Mo.) and 2 mM H₂O₂ diluted to final molarity in 0.05 M citrate buffer (pH 4.0) was added to each well. When the substrate conversion produced optimum color development, the reaction was stopped by adding 50 μ l of 1.0 N sodium fluoride to each well. Spectrophotometric absorbance was determined in individual wells with an MR580 Microelisa Auto Reader (Dynatech Laboratories, Inc., Alexandria, Va.) at 405 nm with a 490-nm reference. The results from groups of mice with equal infections at each time period were averaged, and dilutions were plotted against absorbance readings. This revealed that prozone reactions occurred frequently. Since no evidence of a prozone reaction was seen at dilutions greater than 1:64 all results were expressed from data pooled at dilutions of 1:128.

Statistical analysis. Eosinophil counts and splenic indices were determined to be nonparametric and were analyzed by Kruskal-Wallis single-factor analysis of variance by ranks with multiple paired comparisons. The remaining assays were parametric and were analyzed by single-factor analysis of variance and a Student-Newman-Keuls multiple range test. In all cases, a confidence level of $P \leq 0.05$ was considered significant (34).

RESULTS

Eosinophil counts. Groups of 22 mice were infected with 5, 25, 125, or 250 embryonated *T. canis* ova. Uninfected, age-matched mice served as controls. After 2 weeks, all mice were bled from the lateral tail vein, and the number of eosinophils per cubic millimeter of whole blood was determined. The results are depicted in Fig. 1, and it can be seen that there is an almost linear relationship between the size of infection and the magnitude of the peripheral eosinophil

FIG. 1. Peripheral blood eosinophil counts of uninfected CBA/J mice and mice infected with 5, 25, 125, or 250 embryonated *T. canis* ova on day 14 of infection. The data are expressed as mean $\pm$ the standard error. The asterisks indicate statistical significance with respect to uninfected controls.

response. Even the smallest infection (five eggs) resulted in a statistically significant rise in the number of eosinophils.

Spleen weight indices. Groups of 22 mice were infected with 5, 25, 125, or 250 embryonated *Toxocara* ova, and one group was left uninfected and served as a control. After 2 weeks of infection, the mice were weighed to the nearest 0.01 g and exsanguinated under ether vapor, and the spleens removed, trimmed free of extracapsular fat, and weighed to the nearest 0.1 mg. The mean spleen weight to body weight ratio for each group was determined and then divided by the ratio from the uninfected control group to determine the spleen weight index for that group. The results, seen in Fig. 2, indicate that mice receiving five eggs or more have splenomegaly. An inoculation size of 25 eggs results in a doubling of the weight of the spleen. As with the eosinophil counts, there is almost a linear relationship between the size of infection and the amount of splenomegaly occurring in mice infected with *T. canis*; even mice receiving the lowest inoculation dose showed a significant increase in spleen size.

FIG. 2. Spleen weight indices of uninfected CBA/J mice and mice infected with 5, 25, 125, or 250 embryonated *T. canis* ova on day 14 of infection. The data are expressed as mean $\pm$ standard error. Single asterisks implies statistical difference from controls, and double asterisks imply statistical differences from asterisks as well.

FIG. 3. Spontaneous DNA synthesis as measured by incorporation of [3 H]TdR thymidine by spleen cells from uninfected CBA/J mice and mice infected with 5, 25, 125, or 250 embryonated *T. canis* ova on day 14 of infection. The data are expressed as mean disintegrations per minute $\pm$ standard error. The numbers at the base of each bar indicate the number of animals in each group, and the asterisks imply statistical significance with respect to uninfected controls. DPM, Disintegrations per minute.

Mitogen-elicited lymphocyte transformation. Spleen cells from uninfected mice and mice infected with 5, 25, 125, or 250 eggs of *T. canis* were cultured in the presence or absence of the T-lymphocyte mitogen, ConA, or the B-cell mitogen lipopolysaccharide and allowed to incorporate [3 H]TdR for the final 8 h of culture. In the absence of exogenously added mitogen, spleen cells from uninfected controls and mice infected with 5 or 25 eggs incorporated approximately the same amount of isotope (Fig. 3). However, spleen cells derived from mice infected with the two largest inocula spontaneously incorporated over six times as much isotope as uninfected controls.

In mice infected with 25 eggs or more, the ConA response was over four times that of spleen cells taken from uninfected mice (Table 1). Lipopolysaccharide responses were not significantly elevated (data not shown).

Antigen-elicited lymphocyte transformation. Spleen cells from uninfected mice and mice infected with 5, 25, 125, or 250 eggs of *T. canis* were cultured in the presence or absence of TEE. The results of this experiment are depicted in Fig. 4. According to Oppenheim and Schecter (25) any response to an antigen that is two or more times the value of the unstimulated culture is considered positive; by that criterion, mice infected with 25 or more eggs for 14 days

TABLE 1. Augmented effect of *T. canis* infections of various sizes on ConA-elicited splenic lymphocyte transformation at day 14 postinoculation

Expt	Culture condition	[3 H]TdR incorporation (dpm) with the following infection size (eggs per mouse)				
		Uninfected	5	25	125	250
1 ^a	ConA (-)	678	1,043	2,934	6,852	7,152
	ConA (+)	74,375	90,346	260,824	390,272	227,332
2	ConA (-)	690	602	836	4,708	4,903
	ConA (+)	113,919	154,280	479,339	377,739	329,158
3	ConA (-)	215	833	1,358	3,109	7,088
	ConA (+)	121,845	141,338	536,333	473,878	552,833

^a Results of three of six experiments. Responses are derived from a single animal per infection size.

FIG. 4. [^3H]TdR incorporation by spleen cells from uninfected CBA/J mice and mice infected for 14 days with 5, 25, 125, or 250 embryonated *T. canis* ova in the presence or absence of TEE. Data are expressed as mean disintegrations per minute $\pm$ standard error. Numbers at the base of each bar indicate the number of animals in the group, and asterisks denote statistical significance of TEE-stimulated cultures from infected mice versus uninfected mice. DPM, Disintegrations per minute.

recognized TEE antigen. Uninfected mice and mice harboring five worms do not respond to this antigen. When the more stringent criterion of statistical analysis was applied to the data; however, only the responses of mice infected with 125 or 250 eggs were significantly different from uninfected control mice.

Antitoxocara antibody responses. Groups of three uninfected mice or mice infected with 5, 25, 125, or 250 *Toxocara* eggs were exsanguinated on days 14, 21, or 28 postinoculation. The blood was allowed to clot, and the sera was collected and frozen at -70°C until tested. The absorbance values from each group of three mice were pooled and are shown in Table 2. From the data in Table 2, two generalizations can be made. On each of the times examined, there appears to be proportionately more anti-*Toxocara* antibody as the size of the inoculation increases. For the three smaller sizes of inocula, the amount of antibody produced continued to rise from weeks 2 to 4, and the quantity of antibody elicited by the largest infection seemed to have peaked at 14 days and remained more or less constant.

Influence of longer infection times on graded infection-elicited responses. To answer the question whether the magnitude of the various immune responses would be similar if the experiment had been carried out beyond 14 days, groups of mice were infected with 5, 25, 125, or 250 *T. canis* eggs and

TABLE 2. Effect of increasing infection size on *T. canis* specific antibody (IgG and IgM) determined by ELISA at various times postinoculation

No. of eggs	Mean $\pm$ SE of A_{405}/A_{490} ($\times 10^{-3}$ U) ^a on day:		
	14	21	28
5	6 $\pm$ 4	6 $\pm$ 11	26 $\pm$ 6
25	28 $\pm$ 7	56 $\pm$ 8	136 $\pm$ 19
125	142 $\pm$ 24	111 $\pm$ 12	187 $\pm$ 4
250	161 $\pm$ 35	164 $\pm$ 8	161 $\pm$ 19
None	6 $\pm$ 5	3 $\pm$ 2	3 $\pm$ 1

^a All sera diluted 1:128. A_{405}/A_{490} , Ratio of absorbance at 405 nm/ absorbance at 490 nm. Mean $\pm$ standard error for three mice.

TABLE 3. Effect of graded *T. canis* infection on peripheral blood eosinophil counts and spleen indices at week 2 to 4 after inoculation

No. of eggs	Eosinophil counts ^a on day:			Spleen indices on day:		
	14	21	28	14	21	28
0	150 $\pm$ 76	83 $\pm$ 44	183 $\pm$ 44	1.0	1.0	1.0
5	783 $\pm$ 188	283 $\pm$ 67	350 $\pm$ 132	1.1	1.2	1.2
25	1,217 $\pm$ 257	450 $\pm$ 126	483 $\pm$ 164	1.2	1.2	1.2
125	3,267 $\pm$ 933	950 $\pm$ 250	700 $\pm$ 226	2.6	1.8	1.6
250	4,450 $\pm$ 1,450	1,316 $\pm$ 109	667 $\pm$ 73	3.6	2.3	1.9

^a Mean eosinophils per mm^3 of whole blood $\pm$ standard error of results for three mice.

evaluated on days 14, 21, and 28 postinoculation. These are the same mice that were used for the antibody determinations reported above. The value of the eosinophil counts and spleen weight indices are shown in Table 3. As regards the eosinophil counts, it can be seen that the trend of eosinophilia being proportional to the infection size persisted but at lower levels than those seen at 14 days postinoculation. The splenic indices suggest exactly the same thing. The functional lymphocyte studies are summarized in Table 4, 5, and 6. The spontaneous DNA synthesis assay suggests that with the smallest infective inoculum the amount of spontaneous synthesis increases from 14 days to 21 days postinoculation. With 25-egg infections the amount of synthesis held very constant, whereas the two largest infections peaked at 14 days and then declined dramatically over the ensuing 2 weeks. The ability of a *Toxocara* infection to elicit augmentation of ConA lymphocyte transformation also mirrored the spontaneous DNA synthesis in that the two largest infective inocula declined dramatically during week 3 and 4 of infection, whereas the smallest infection did not peak until week 3. The 25-egg infection held constant for 2 weeks and then declined to normal levels. As regards temporal lymphocyte transformation responses to TEE, the pattern of increasing responsiveness in 5-egg infections, sequentially similar responsiveness in 25-egg infections, and a sharp decline from 2-week peak responsiveness in 125- and 250-egg infections was observed again.

DISCUSSION

The results of this study clearly indicate that the number of eggs used to initiate a *Toxocara* infection in mice is an important criterion in determining the quality and quantity of the immune response this paratenic host mounts against the parasite. Mice infected with 125 or 250 ova (6 and 12 eggs per g of body weight, respectively) had substantial reactions in all of the assays examined. In contrast, only peripheral

TABLE 4. Effect of graded *T. canis* infection on spontaneous DNA synthesis^a week 2 to 4 postinoculation

No. of eggs	[^3H]TdR incorporation (dpm $\pm$ SE) ^b		
	14	21	28
0	1,299 $\pm$ 221	1,236 $\pm$ 27	2,018 $\pm$ 584
5	2,121 $\pm$ 55	3,604 $\pm$ 767	3,670 $\pm$ 1,437
25	3,236 $\pm$ 1,274	3,322 $\pm$ 502	2,969 $\pm$ 619
125	8,507 $\pm$ 1,220	2,315 $\pm$ 404	2,475 $\pm$ 306
250	16,012 $\pm$ 2,526	5,678 $\pm$ 2,173	2,927 $\pm$ 48

^a Splenic lymphocytes cultured in absence of exogenous stimulation for 72 h.

^b Results are mean $\pm$ standard error of triplicate cultures for three mice.

blood eosinophilia and a modest splenomegaly were observed in mice infected with five ova (0.25 eggs per g of body weight). Because *T. canis* does not replicate in mice like a bacterium or virus and because it can persist for long periods of time (4, 20), this model system is superbly suited for immunological studies of a chronic disease state. By choosing the number of eggs used to initiate infection it is possible to determine which host reactivities will be elicited. Even though *T. canis* does not replicate in mice, it does produce excretory or secretory antigenic materials or both which over time continue to stimulate the host. Thus, although *T. canis* can survive indefinitely in mice, a previous study (9) showed that when mice were infected with 200, 600, or 1,000 ova and the larval worm burdens were determined 7, 14, 28, and 56 days later, the larval recoveries tended toward the same low number. This study suggests that larger infections tend to elicit a more effective larvacidal reaction and perhaps accounts for the finding that with time most of the larvae accumulate in the brain where there appears to be little immunoreactivity (12).

These studies and those of others (1, 18, 24, 29, 30) suggest that most of the quantifiable antitoxocaral responses peak around day 14 of infection. It should be recalled however, that all of these studies employed infections that were equal to or much larger than those used in the present investigation. Previously, it was shown that mice infected with 250 *T. canis* ova have elevated levels of eosinophils, splenomegaly, increased spontaneous DNA synthesis, augmented T-cell mitogenic responses, and positive TEE lymphocyte transformation reactions as early as 6 days postinfection (18). All of these responses reached their maxima at or during week 2 of infection. The present studies utilized infection sizes smaller than any previous studies that we are aware of and our smallest infection size of five ova per mouse was chosen to overcome the technical difficulties of attempting an infection with a single ovum. The collective results suggest the response to a 5-ova infection is not nearly as rapid or substantial as is the response to infections 5 or 25 times as large (25 and 125 ova, respectively). In addition, the results suggest that infections of 125 ova or larger in CBA/J mice may elicit very active regulatory mechanisms that are manifested around week 2 of infection, and this may be the reason that most immune responses to larger infections appear to peak around week 2 of infection.

Aljeboori and Ivey (1) infected baboons with 1, 5, 10, or 20 *T. canis* ova per g of body weight and observed that the larger the worm burden, the higher the leukocyte count, the greater the percentage of eosinophils, and the higher the titer of antitoxocaral antibody. Similar results in which larger infections elicited larger responses were observed in a variety of macaque monkeys by Tomimura et al. (20). Olson and Schultz (24) initiated graded infections in guinea pigs

TABLE 6. Effect of graded *T. canis* infection on TEE lymphocyte transformation activity week 2 to 4 postinoculation

No. of eggs ^a	[³ H]TdR incorporation (dpm ± SE) on day ^b :		
	14	21	28
0 -	1,493 ± 287	2,169 ± 287	2,243 ± 935
+	2,735 ± 690	2,161 ± 74	3,230 ± 1,435
5 -	2,809 ± 109	5,880 ± 1,493	4,136 ± 1,739
+	5,558 ± 517	9,841 ± 1,373	7,088 ± 2,930
25 -	3,672 ± 1,163	5,274 ± 231	4,293 ± 1,169
+	8,198 ± 3,140	10,177 ± 527	6,799 ± 1,478
125 -	8,423 ± 1,980	3,629 ± 589	3,105 ± 425
+	15,677 ± 2,428	8,353 ± 1,443	6,412 ± 1,384
250 -	11,445 ± 673	7,047 ± 2,570	3,107 ± 358
+	39,416 ± 15,735	19,829 ± 5,224	8,228 ± 947

^a Splenic lymphocytes cultured in absence (-) or presence (+) of 1 µg of TEE per ml.

^b Results are mean ± standard error of triplicate cultures for three mice.

and observed a dose-dependent relationship between the number of eggs given and the magnitude of eosinophilia. These investigators also observed that smaller infections took a longer time to develop a given response and to reach their peak response. The results of the studies reported herein appear consistent with all of these studies when the different dosages and species of paratenic hosts are taken into account.

T. canis has been shown previously to elicit T-lymphocyte-mediated peripheral blood eosinophilia in the murine host (18, 29). Administration of antilymphocyte serum suppressed the peripheral blood eosinophilia and also prevented the formation of eosinophil-rich granulomata around the larvae. The finding in this study that mice infected with only five ova developed eosinophilia suggests that there was sufficient antigen to prime T lymphocytes. In agreement with others (4, 18, 24), we observed that a dose-dependent relationship exists between the number of eggs given per animal and the magnitude of peripheral eosinophilia.

Although the exact role of eosinophils in parasitic infections remains to be established, there is a large body of evidence to suggest that eosinophils may be helminthotoxic effector cells, especially as regards larval worms (26, 32). A common feature in many models of eosinophil-mediated larvacidal activity is the requirement for the presence of immune serum or antibody in the system (9, 16, 22, 23). In a review of 27 cases of human VLM, Beaver (4) observed a direct relationship in most of the cases between the degree of hypereosinophilia and hyperglobulinemia. Similar results were reported by Aljeboori and Ivey (1) and Tomimura et al. (30). On the basis of the present results it is clear that the number of worms is important in determining both the number of eosinophils in the circulation and the titer of antitoxocaral antibody, suggesting that the regulation of these two responses are intricately related.

Larva migrans due to infection with *Toxocara canis* can involve the eye only (OLM), the viscera only (VLM) or both (14). Based on considerations of patient histories, clinical findings, including the results of antigen-specific serological (ELISA) tests, and in some cases positive biopsies, Glickman and Schantz (14) proposed that OLM results from an infection with so few larvae that the patient fails to mount an immune response against the migrating larvae. As a result, these larvae continuously migrate unimpeded and are not detected unless they accidentally invade the eye and the resulting hemorrhage or acute inflammatory response or both disturbs the vision of the patient. Since the eye is part

TABLE 5. Effect of graded *T. canis* infection on augmentation of ConA responsiveness^a week 2 to 4 postinoculation

No. of eggs	[³ H]TdR incorporation (dpm ± SE) on day ^b :		
	14	21	28
0	111,491 ± 27,247	119,119 ± 14,779	80,692 ± 7,748
5	122,960 ± 16,181	217,009 ± 48,673	70,605 ± 24,030
25	312,437 ± 12,581	284,490 ± 14,594	127,128 ± 48,780
125	380,666 ± 31,112	74,068 ± 20,515	51,552 ± 11,463
250	519,388 ± 15,830	118,478 ± 46,282	49,994 ± 13,896

^a Splenic lymphocytes cultured with 2.5 µl of ConA per ml for 72 h.

^b Results are mean ± standard error of triplicate cultures for three mice.

of the central nervous system and therefore enjoys the protection of a blood-brain barrier, it is not known at the present time if inflammatory reactions to *T. canis* within the confines of the eye are more or less vigorous than they would be somewhere else in body given the same degree of immunological reactivity. Larger numbers of worms, however, do evoke immunological reactions, and most if not all worms are either destroyed or are encapsulated in fibrotic granulomata. This would be considered classical VLM. If the worm burden is exceptionally large, the combined form of the disease would be seen, with ocular involvement occurring as a random consequence of the large number of larvae systemically migrating throughout the body. The crux of this hypothesis seems to be whether there is a "critical antigenic mass" below which there is little or no immunological reaction to *T. canis*.

In attempting to relate the results of the current study to this hypothesis (14), the following points stand out. Based on the studies herein, very small infections (approaching single-worm infections) appear able to prime T lymphocytes for eosinophilia and cause mild splenomegaly. However, not all assays were positive at the same time, and presumably this reflects the failure of this size of infection to prime those aspects of the immune system for which these assays are thought to correlate. On the basis of our observation of a direct relationship of the size of the worm burden to both antibody response and eosinophilia and the similar observation by Beaver (4) of a relationship between eosinophilia and hyperglobulinemia, the mice in the present study receiving the smallest infections were producing little or no antibody by week 2 of infection. Thus, in the absence of any substantial humoral response, the eosinophils seen in mice harboring five-egg infections may have been rendered ineffective in trapping or killing these few larvae. It is interesting to consider that although the organisms themselves do not multiply in the paratenic host, they do continue to metabolize and therefore produce increasing amounts of antigenic material. Since this is apparently the case, the question then arises of how much antigen is necessary to elicit an effective immunity? Another question that becomes of interest is whether the host-parasite interaction would have been elaborated differently had immune serum of sufficient titer been available at the outset. Whether antibody is the critical missing link in determining an immunological milieu favoring OLM or VLM, is not known. In light of the fact that some but not all host responses are initiated in infections as small as five eggs, the data in the present studies are consistent with the aforementioned hypothesis in as much as any size *T. canis* infection is immunogenic. The size of infection apparently determines how many and at what time different immune mechanisms will be activated against the parasites, and it is this criterion that ultimately will determine which form of the disease will occur. However, it is also consistent with the possibility that all of the same mechanisms are elicited, but the sequence of activation is different. What the critical response to the migrating worms is, remains to be determined. At present, either antibody-mediated eosinophilic killing or granulomatous encapsulation seems most likely.

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