INFECTION AND IMMUNITY, May 1978, p. 393-397
0019-9567/78/0020-0393$02.00/0

Vol. 20, No. 2
Printed in U.S.A.

Suppression of Cellular Responses in Mice During *Trypanosoma cruzi* Infections

EDWIN C. ROWLAND AND RAYMOND E. KUHN*
Department of Biology, Wake Forest University, Winston-Salem, North Carolina 27109

Received for publication 6 December 1977

Immunosuppression has been reported to occur in several protozoan parasitic infections. The significance of this suppression on host resistance or on parasite avoidance of immune destruction has not, however, been determined. In the present study two strains of mice that differ with respect to resistance to *Trypanosoma cruzi* were examined during the course of infection for differences in expression of suppression of blastogenic responses to phytohemagglutinin and an antigen preparation of these parasites. It was found that in vitro blastogenic responses were suppressed in both strains of mice: on day 12 for C57BL/6 mice (resistant strain) and on day 17 for C3H(He) mice (susceptible strain). Neither C3H(He) nor C57BL/6 lymph node cells (LNC) responded to a crude sonically treated antigen of these parasites, although C57BL/6 LNC were inhibited by this antigen later in infection. There was no abrogation of suppression of LNC responses late in infection, when decreases in spleen weight or total spleen cell numbers occurred, in the resistant C57BL/6 mice. LNC from normal uninfected mice were found to be completely suppressed in responsiveness to phytohemagglutinin when cultured at a 2:1 ratio with LNC from C57BL/6 mice with 18-day infections. Attempts to characterize the cell type responsible for this suppression showed it to be a non-thy 1.2-bearing, nylon wool-adherent cell.

Suppression of cell-mediated responses has been observed in tumor systems and systemic infections (4). Recently, suppression of immune responses has also been shown to develop during the course of several protozoan and metazoan parasitic infections. Of the protozoans, *Trypanosoma congolense* (8), *Trypanosoma brucei* (1), *Trypanosoma musculi* (2), *Trypanosoma cruzi* (11), and *Toxoplasma gondii* (12) have been shown to nonspecifically suppress cellular immune responses in their mammalian hosts. The significance, if any, of this suppression phenomenon on the development of protective immunity to these parasites, however, has not been determined.

As part of continuing studies on the immunobiology of the host-parasite relationship in experimental Chagas' disease, we present here results of a study of the relationship of nonspecific immunosuppression to resistance to *T. cruzi* in two strains of mice (C3H and C57BL/6) that differ in their resistance to these parasites (9). Delayed hypersensitivity responses to an antigenic extract of the parasite and in vitro blastogenic responses to this antigen and phytohemagglutinin (PHA), as manifestations of cell-mediated immunity, were assayed during the course of infection. The cellular basis of the observed suppression was examined, and the relationship of resistance to the parasite and the suppressed state are discussed.

MATERIALS AND METHODS

Animals. C3H(He) Dub females were purchased from Flow Research Laboratories, Inc. (Dublin, Va.). C57BL/6J female mice were obtained from Jackson Laboratories (Bar Harbor, Me.). Animals were housed six to a cage and given Purina Laboratory Chow and water ad libitum. C57BL mice have been shown to be significantly more resistant to *T. cruzi* than are C3H mice (9; R. E. Kuhn, E. C. Rowland, and D. S. Cunningham, submitted for publication).

Antigen preparation. Culture forms of *T. cruzi* were grown in LIT medium (7), harvested, and washed three times in cold phosphate-buffered saline, and the final concentration was adjusted to 5×10^8 trypanosomes per ml and subjected to sonic treatment for 3 min at 20% intensity with a ⅜-inch (ca. 1.0 cm) probe (Biosonic, Bronwill Scientific Inc., Rochester, N.Y.). The result was a crude sonically treated antigen (CSA), quantified as 5×10^8 trypanosome equivalents per ml.

Delayed hypersensitivity assay. Dermal reactivity of mice during *T. cruzi* infection was examined by measuring the induration of the ear after injection of CSA. Preliminary experiments had shown that maximum responsiveness could be elicited with a challenge of 10^6 trypanosome equivalents; therefore, the left ear of a mouse was injected intradermally (27-gauge needle) with 10^6 trypanosome equivalents in phosphate-buffered saline in a total volume of 0.02 ml, and

the right ear received 0.02 ml of phosphate-buffered saline as a control. Ear thickness was measured before injection and 5, 24, and 48 h postinjection, using an engineer's micrometer.

Blastogenic assay. Lymph node cells (LNC) were obtained from both uninfected and infected C3H(He) and C57BL/6 mice from the axillary, brachial, inguinal, and mesenteric lymph nodes, which were teased apart in cold RPMI 1640 (Grand Island Biological Co., Grand Island, N.Y.) containing fetal bovine serum (10%). The resultant suspension was passed through a small-mesh (20-gauge) stainless steel screen. Culturing procedures followed the method of Colley (3). LNC (4×10^6) in 2 ml of RPMI 1640 fortified with fetal bovine serum (10%) plus penicillin G (100 μg/ml) and streptomycin sulfate (50 μg/ml) were cultured in plastic test tubes (Falcon 2003). Appropriate cultures received either 2 μl of PHA P (Difco Laboratories, Detroit, Mich.) or 5×10^6 trypanosome equivalents of CSA per tube. Cultures were incubated at 37°C in a 5% CO_2-95% air atmosphere for 3 days. Eight hours before harvesting the cultures, 2 μCi of [^{3}H]thymidine (specific activity, 2 Ci/nmol; New England Nuclear Corp., Boston, Mass.) was added to each culture. At the end of the labeling period the cultures were cooled to 4°C and washed twice with cold phosphate-buffered saline. Bovine serum albumin (0.5 mg per culture) was added as a carrier, and precipitation was effected by addition of 2 ml of cold 10% trichloroacetic acid. Two hours later the cultures were centrifuged, the resulting pellet was dissolved in 0.5 ml of 0.2 N NaOH, and 2 ml of cold 10% trichloroacetic acid was added for a second precipitation. After centrifugation the pellets were hydrolyzed with 0.5 ml of 0.5 N NaOH, neutralized with 4 drops of 5% acetic acid, and placed in scintillation vials containing toluene, phosphors, and 10% Biosolve BBS-3 (Beckman Instruments, Fullerton, Calif.). The samples were counted in a Beckman scintillation counter, and a stimulation index (SI) for the antigen or mitogen added to the experimental cultures was calculated as follows: SI = mean counts per minute of experimental cultures/mean counts per minute of unstimulated control cultures.

Lymphoid separation on nylon wool columns. Separation of LNC was done by the method of Gravely and Kreier (5). Briefly, 0.6 g of nylon wool (E. I. duPont de Nemours & Co., Wilmington, Del.) was packed into a 10-cm^3 plastic syringe and sterilized. The column was washed with 20 ml of RPMI 1640–fetal bovine serum (10%) and incubated at 37°C for 1 h. After rinsing with 5 ml of medium, 2 ml of the cell suspension was gently layered onto the column and overlaid with 1 ml of medium. The column was sealed and incubated for 45 min at 37°C. Twenty-five milliliters of medium was then passed through the column, and the nonadherent cells were collected. The nylon wool was removed from the syringe and placed in a petri dish with cold medium. Adherent cells were collected by gentle squeezing of the nylon wool with forceps. After harvesting, adherent and nonadherent cells were washed once by centrifugation, resuspended in fresh medium, and adjusted to the desired concentration.

Anti-thy 1.2 treatment. Anti-thy 1.2 antiserum produced in AKR mice against C58 thymocytes was kindly provided by Max Proffitt, Cleveland Clinic Foundation (Cleveland, Ohio), and selective T-cell depletion of LNC was done as described by Proffitt et al. (10). LNC from 24-day infected C57BL/6 mice were concentrated to 2×10^7 cells per ml of RPMI 1640. Five milliliters of this suspension was added to 5 ml of a 1:256 dilution of anti-thy 1.2 antiserum in RPMI 1640 and mixed continuously for 15 min at room temperature. The tube was then filled with cold RPMI 1640 and centrifuged, and the supernatant was removed. The pellet was suspended in Rabbit Low-Tox complement (Cedarline Laboratories, Hicksville, N.Y.) at a final dilution of 1:15 in RPMI 1640 and incubated at 37°C for 30 min. The tube was again filled with cold medium and centrifuged, and the supernatant was removed. The cells were treated for a second time with the anti-thy 1.2-plus-complement procedure, after which they were resuspended in RPMI 1640. (The final cell suspension was found by trypan blue dye exclusion to contain 73% viable cells.)

Statistics. Differences in in vitro responsiveness of LNC from normal or infected mice to PHA or CSA were determined using Student's t test and were considered significant when $P < 0.05$. Significant differences in dermal hypersensitivity to CSA in infected mice were determined with the Student-Newman-Keuls multiple comparison test. A single-factor analysis of variance was used to determine significant differences in the experiments using cell mixing or anti-thy 1.2 treatment of LNC (13).

RESULTS

Changes in spleen and lymph node weights (axillary and brachial pairs weighed together) in C3H(He) and C57BL/6 mice during *T. cruzi* infection are shown in Fig. 1. The spleens and lymph nodes from infected C3H(He) mice increased rapidly in weight until death. Infected C57BL/6 mice had undergone a fivefold increase in lymphoid organ weight by day 22 of infection, but the weight decreased thereafter.

Delayed hypersensitivity responses to antigen challenge in 14-day-infected C3H(He) mice and 14-, 21-, and 28-day-infected C57BL/6 mice were not significantly different from control responses to phosphate-buffered saline (Table 1). However, the group of C57BL/6 mice infected for 79 days exhibited a significant ($P < 0.01$) response within 24 h of the CSA challenge. Therefore, no in vivo dermal response to *T. cruzi* antigen challenge was observed in acutely infected mice.

The responses of LNC from C3H(He) and C57BL/6 mice to CSA and PHA are depicted in Fig. 2. LNC from normal C3H(He) mice displayed no response to CSA but responded significantly to PHA. Likewise, lymphocytes from infected C3H(He) mice did not display any proliferative response to CSA, whereas PHA induced significant responses in cultures from animals infected for 7 and 12 days. However, exposure of lymphocytes from mice with 17-day

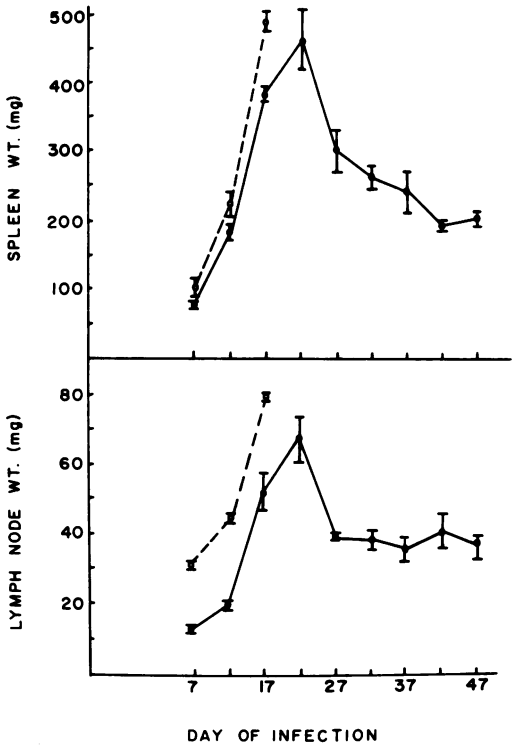

FIG. 1. *Spleen and lymph node weights of C3H(He) (○) and C57BL/6 (●) mice during infections with T. cruzi. Data presented are means (± standard error).*

infections to PHA resulted in blastogenic activity that was suppressed significantly below controls (SI = 0.37).

Uninfected C57BL/6 mice also showed no response to CSA but responded significantly to PHA (Fig. 2). Furthermore, the addition of CSA to cultures of infected cells resulted in a significant reduction of blastogenic activity below that in unstimulated control cultures. This suppression had developed as early as day 17 of infection and continued up to the time of death of C57BL/6 mice. The LNC from 7-day-infected mice proliferated in response to PHA, but from day 12 through the remainder of the infection the response was significantly lower than that of unstimulated controls (SI < 1.0).

The possibility that the reduction of the proliferation resulting from PHA stimulation was a dose-related phenomenon was examined by culturing C57BL/6 lymphocytes from day-22 infected animals with increasing concentrations of PHA. The results (Table 2) show that blastogenic activity of infected LNC was less than controls, regardless of the dose of PHA used.

To determine whether or not the suppressive activity of infected LNC (ILNC) stimulated by

TABLE 1. *Delayed hypersensitivity to a CSA in C3H(He) and C57BL/6 mice infected with T. cruzi*

Day of infection	Mouse strain	Treatment	Induration, mm (± standard error)		
			5 h	24 h	48 h
14	C3H(He)	PBS[a]	0.023 (0.003)	0.018 (0.003)	0.013 (0.008)
		CSA	0.025 (0.007)	0.015 (0.005)	0.010 (0.006)
14	C57BL/6	PBS	0.023 (0.005)	0.018 (0.009)	0.001 (0.011)
		CSA	0.048 (0.011)	0.025 (0.005)	0.015 (0.007)
21	C57BL/6	PBS	0.048 (0.003)	0.018 (0.008)	0.010 (0.018)
		CSA	0.048 (0.003)	0.028 (0.013)	0.025 (0.003)
28	C57BL/6	PBS	0.015 (0.015)	0.038 (0.013)	0.040 (0.010)
		CSA	0.023 (0.008)	0.050 (0.007)	0.027 (0.008)
79	C57BL/6	PBS	ND[b]	0.020 (0.011),	ND
		CSA	ND	0.090[c] (0.011)	ND

[a] PBS, Phosphate-buffered saline.
[b] ND, Not done.
[c] Significantly different from control ($P < 0.01$).

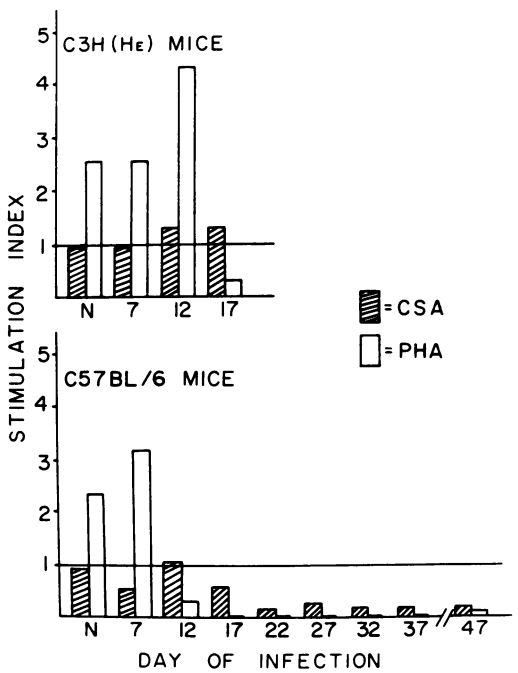

FIG. 2. *Blastogenic responses of C3H(He) and C57BL/6 mice to a CSA of T. cruzi culture forms and to PHA during the course of infection with T. cruzi.*

TABLE 2. *Blastogenic response of LNC from C57BL/6 mice to varying doses of PHA when tested on day 24 of infection with T. cruzi*

PHA (μl)	cpm (± standard error)	SI[a]
0	5,113 (460.5)	
0.5	656 (200.0)	0.13
2	232 (15.7)	0.05
10	197 (15.0)	0.04
50	138 (10.8)	0.03

[a] In each case, $P < 0.001$.

PHA could reduce the response of normal LNC (NLNC) to PHA, increasing numbers of LNC from C57BL/6 mice infected for 18 to 25 days with *T. cruzi* were added to cultures of NLNC. The results of two of these experiments are shown in Fig. 3. In experiment 1 (Fig. 3) the response of NLNC to PHA was not affected by the presence of either 1.25×10^5 ILNC per culture (a ratio of 32 NLNC:1 ILNC) or 5×10^5 ILNC (8 NLNC:1 ILNC). However, the addition of 2×10^6 ILNC (2 NLNC:1 ILNC) significantly reduced the PHA response of NLNC. In a repeat of this experiment (Fig. 3, experiment 2) similar results were obtained, with normal cells being suppressed when cultured at a ratio of 2 NLNC:1 ILNC.

To determine the response characteristics of populations of infected lymphocytes, LNC from C57BL/6 mice infected for 21 days were separated on a nylon wool column and assayed for their in vitro response to PHA. (Of the cells separated on the column, 60 to 70% were adherent and 30 to 40% were nonadherent.) The results indicate that adherent and nonadherent cells, when cultured together in the proper proportions (in this case, 1.2×10^6 adherent plus 0.8×10^6 nonadherent cells per ml), exhibited suppression in responsiveness to PHA (Fig. 4). When the adherent population of lymphocytes was cultured alone, PHA stimulation resulted in a significant reduction of proliferation, compared with controls (SI = 0.003). Alternatively, the nonadherent cells from infected mice responded significantly to PHA (SI = 6.94 [Fig. 4]).

In an effort to characterize the suppressor cells further, ILNC from C57BL/6 mice infected 24 days were treated with anti-thy 1.2 antiserum plus complement and assayed for their blastogenic response to PHA. The proliferative responses to PHA of both untreated and anti-thy 1.2-treated ILNC were suppressed (Fig. 5). It appears, therefore, that suppressor LNC were not sensitive to the anti-thy 1.2 treatment.

DISCUSSION

The observation that C3H(He) and C57BL/6 mice are both suppressed during the course of *T. cruzi* infection and that the more resistant C57BL/6 mouse is suppressed earlier and to a greater degree than the more susceptible

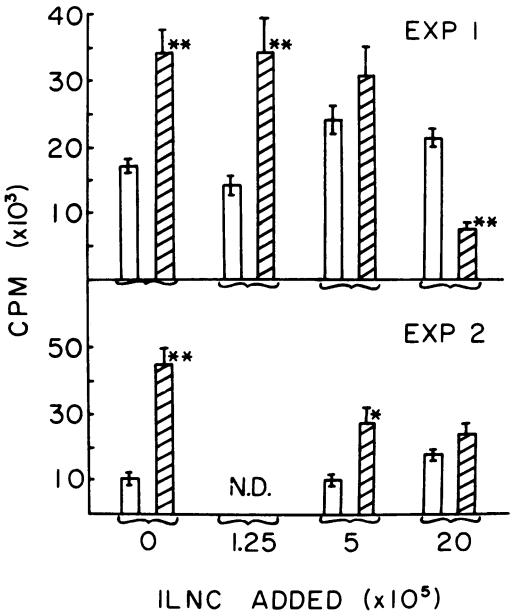

FIG. 3. *Effect of LNC from T. cruzi-infected mice on the in vitro blastogenic response of NLNC to PHA. ILNC were added to cultures of 4×10^6 NLNC. Data presented are mean counts per minute ± standard error for unstimulated control (open bars) and PHA-stimulated (striped bars) cultures. Significant differences from control cultures are denoted by * ($P < 0.05$) and ** ($P < 0.01$).*

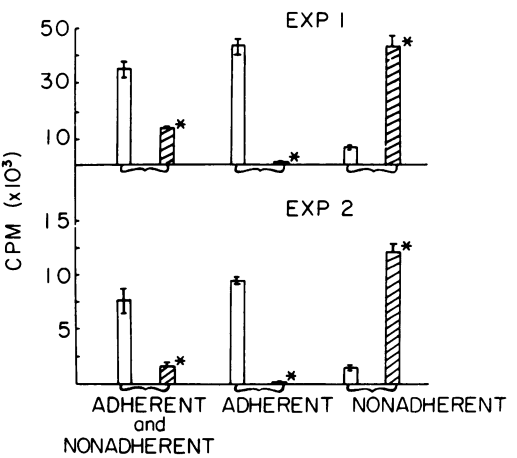

FIG. 4. *Blastogenic response to PHA of adherent and nonadherent LNC from T. cruzi-infected mice. Data reported are means (± standard error) for unstimulated control (open bars) and PHA-stimulated (striped bars) cultures. Significant difference from control cultures is denoted by * ($P < 0.002$).*

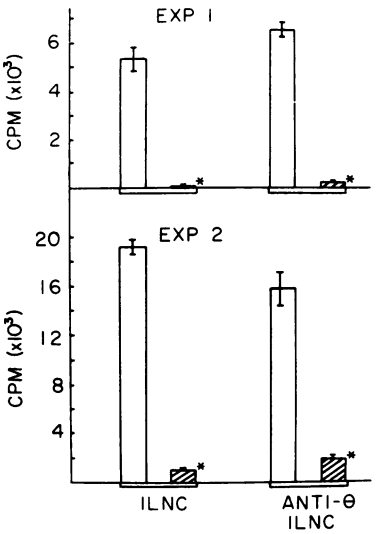

FIG. 5. *Effect of anti-thy 1.2 treatment on the blastogenic response of LNC from T. cruzi-infected mice to PHA. Data reported are means (± standard error) for unstimulated control (open bars) and PHA-stimulated (striped bars) cultures. Significant difference from control cultures is denoted by * ($P < 0.001$).*

C3H(He) mouse is enigmatic. Should the expression of immunosuppression of cellular responses during the course of protozoan parasitic infections be a means of parasite avoidance of immune destruction, it is not reflected in resistance or susceptibility to *T. cruzi* under the conditions used in these experiments.

The significance, if any, of immunosuppression during experimental Chagas' disease was not determined in this study. It was shown, however, that a suppressor cell was induced during the course of infection and was capable of suppressing NLNC responses to PHA. This suppressor cell was shown not to be a typical T-cell in that it was adherent to nylon wool and not sensitive to anti-thy 1.2 antiserum and complement. Jayawardina and Waksman (6) have found a similar situation in mice with *T. brucei* infections, as have J. F. Albright, J. W. Albright, and D. G. Dusanic (personal communication), studying *T. musculi* infections.

It was of interest that C57BL/6 mice showed no abrogation of immunosuppression well past the typical acute phase, when spleen weight and total spleen cell numbers decreased to normal values. This is in contrast to the report by Albright et al. (2), which showed that immune responsiveness of *T. musculi*-infected mice was reestablished as the spleen decreased in weight after the acute infection.

This observed difference in the *T. musculi* versus *T. cruzi* systems mentioned above points out that, although immunosuppression seems to be a phenomenon common to many host-parasite systems, considerable caution must be exercised in attempting to generalize the significance of this phenomenon, or mechanisms that regulate it, in inherently different host-parasite systems.

ACKNOWLEDGMENTS

This research was supported by Public Health Service grant AI 13128 from the National Institute of Allergy and Infectious Diseases.

We thank Malcolm Powell for technical assistance, Debbie Vogler for the preparation of figures, and Lila Spencer for preparation of the manuscript.

LITERATURE CITED

1. **Ackerman, S. B., and J. R. Seed.** 1976. Immunodepression during *Trypanosoma brucei gambiense* infections in the field vole, *Microtus montanus*. Clin. Exp. Immunol. **25**:152-158.
2. **Albright, J. F., J. W. Albright, and D. G. Dusanic.** 1977. Trypanosome-induced splenomegaly and suppression of mouse spleen cell responses to antigens. RES J. Reticuloendothel. Soc. **21**:21-31.
3. **Colley, D. G.** 1971. Schistosomal egg antigen-induced lymphocyte blastogenesis in experimental murine *Schistosoma mansoni* infection. J. Immunol. **107**:1477-1480.
4. **Fudenberg, H. H., D. P. Stites, J. L. Caldwell, and J. V. Wells.** 1976. Basic and clinical immunology. Lange Medical Publications, Los Altos, Calif.
5. **Gravely, S. M., and J. P. Kreier.** 1976. Adoptive transfer of immunity to *Plasmodium berghei* with immune T and B lymphocytes. Infect. Immun. **14**:184-190.
6. **Jayawardena, A. N., and B. H. Waksman.** 1977. Suppressor cells in experimental trypanosomiasis. Nature (London) **265**:539-541.
7. **Kuhn, R. E., R. T. Vaughn, and N. P. Iannuzzi.** 1974. The *in vivo* distribution of ^{51}Cr-labeled *Trypanosoma cruzi* in mice. Int. J. Parasitol. **4**:585-588.
8. **Manfield, J. M., and J. H. Wallace.** 1974. Suppression of cell mediated immunity in experimental African trypanosomiasis. Infect. Immun. **10**:335-339.
9. **Marr, J. S., and E. H. Pike.** 1967. The protection of mice by "Corpus Christi" strain of *Trypanosoma cruzi* when challenged with "Brazil" strain. J. Parasitol. **53**:657-659.
10. **Proffitt, M. R., M. S. Hirsch, I. F. C. McKenzie, B. Gheridian, and P. H. Black.** 1975. Immunological mechanisms in the pathogenesis of virus-induced murine leukemia. II. Characterization of autoreactive thymocytes. Int. J. Cancer **15**:230-240.
11. **Reed, S. G., C. L. Larson, and C. A. Speer.** 1977. Suppression of cell-mediated immunity in experimental Chagas' disease. Z. Parasitenkd. **52**:11-17.
12. **Strickland, G. T., A. Ahmend, and K. W. Sell.** 1975. Blastogenic response of toxoplasma-infected mouse spleen cells to T- and B-cell mitogens. Clin. Exp. Immunol. **22**:167-176.
13. **Zar, J. H.** 1974. Biostatistical analysis. Prentice-Hall, Inc., Englewood Cliffs, N.J.