INFECTION AND IMMUNITY, Oct. 1978, p. 155-160
0019-9567/78/0022-0155$02.00/0
Copyright © 1978 American Society for Microbiology
Vol. 22, No. 1
Printed in U.S.A.

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

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

Received for publication 4 August 1978

C57BL/6 mice exhibit low parasitemias and often survive *Trypanosoma cruzi* infections, whereas C3H(He) mice die during the acute phase with relatively high parasitemias. The present study showed that both strains of mice develop nonspecific immunosuppression to challenge with sheep erythrocytes during the course of infection. Several major differences in immunosuppression-related phenomena between the two strains of mice were determined, yet there is no apparent relationship between immunosuppression and resistance to *T. cruzi*. Both the number of direct plaque-forming cells and the titer of 2-mercaptoethanol-sensitive agglutinating antibody were significantly lower on day 11 for C57BL/6 mice and day 9 for C3H(He) mice. The number of indirect plaque-forming cells and the titer of mercaptoethanol-resistant agglutinating antibody were reduced by day 36 of infection in C57BL/6 mice and 13 days postinfection in C3H(He) mice. In both strains the degree of humoral response suppression of mice increased concomitant with the period of infection, but was not correlated with the changes in spleen cell numbers. Preliminary experiments designed to explore the mechanism underlying the induction and maintenance of immunosuppression in this host-parasite model disclosed the presence of suppressor substance in the serum of *T. cruzi*-infected mice. The passive transfer of serum from infected mice to syngeneic recipients elicited a state of immunosuppression to sheep erythrocytes, but did not diminish anti-erythrocyte activity in allogeneic recipients. The induction of immunosuppression in normal mice was further found to be dependent on the interval between serum transfer and challenge with antigen. No quantitative differences existed between the magnitude of suppressed humoral responses in mice infected for varying lengths of time and recipients of serum collected from similarly infected mice.

A number of animal parasites, both metazoan and protozoan, have been shown to induce cellular and/or humoral immunosuppression to heterologous antigens during the course of infection in mammals (2, 3, 6, 10, 19, 23). Of the protozoan parasites known to effect a state of nonspecific immunosuppression in hosts, the extracellular trypanosomes, especially the African trypanosomes, have received considerable attention (1, 5, 8, 12, 13, 18).

We report here results of experiments on the onset and development of immunosuppression of humoral responses during the course of experimental American trypanosomiasis, a disease caused by the obligate, intracellular protozoan *Trypanosoma cruzi*. These experiments were done in two strains of mice which differ in susceptibility to these hemoflagellates, in an attempt to determine any relationships between susceptibility to infection and onset and/or magnitude of immunosuppression. While no such correlation was evident, it was found that sera from mice infected with *T. cruzi* contain a host-specific suppressor substance which interferes with the induction of immune responsiveness to heterologous erythrocytes.

MATERIALS AND METHODS

Animals. Eight- to 10-week-old C57BL/6 (Jackson Laboratories, Bar Harbor, Me.) and C3H(He) (Flow Laboratories, Dublin, Va.) female mice were used in all experiments. They were housed in plastic cages in groups of six and maintained in a temperature-controlled animal room. Purina laboratory chow and water were supplied ad libitum.

Infections. Mice were infected by the intraperitoneal route with 10^3 blood-form trypomastigotes in 0.1 ml of 0.1 M phosphate-buffered saline (pH 7.0). At this infection inoculum, C57BL/6 mice usually live 45+ days and often survive, whereas C3H(He) mice die on about day 24. A Brasil strain of the parasite maintained in C3H(He) mice was used in all infections (16).

Immunizations. Mice were immunized intraperitoneally with 0.2 ml of a 10% sheep erythrocyte (SRBC) suspension (4×10^8 cells) in phosphate-buffered saline.

Serum and spleen cells. Animals were bled from the retro-orbital plexus and killed by cervical dislocation. Individual blood samples remained at room temperature for 1 h for clot formation and for 2 h at 4°C for clot retraction, and were centrifuged at $1,200 \times g$ for 15 min. The resultant sera were inactivated at 56°C for 30 min and stored at −80°C until use. After mice were killed, their spleens were removed, weighed, and gently teased apart with forceps in 2.5 ml of Eagle minimal essential medium (Gibco, Grand Island, N.Y.) fortified with heat-inactivated fetal calf serum (5%). The cells were deaggregated by drawing once through a 23-gauge needle into a plastic syringe and expelling through a 26-gauge needle. The cell suspension was centrifuged at $340 \times g$ for 5 min, then resuspended in 2.5 ml of Eagle minimal essential medium plus fetal calf serum. Nucleated spleen cells were counted on a hemacytometer, using Turk solution as a diluent. Spleen cells from different mice were not pooled.

Serum transfer studies. Sera collected throughout the course of *T. cruzi* infections in C3H(He) and C57BL/6 mice were inactivated at 56°C for 30 min and stored at −80°C until use. Transfer of serum to normal recipients was done via the retro-lateral tail vein in 0.2-ml aliquots. Unless designated otherwise, C57BL/6 mice were challenged with SRBC 2 days, and C3H(He) mice 4 days, after receiving serum from infected mice. Dilution of serum for passive transfer was done in phosphate-buffered saline.

Hemolytic plaque assay. Direct and indirect plaque-forming cells (DPFC and IPFC, respectively) were determined 100 and 202 h post-SRBC immunization, respectively. A slide modification of the Jerne plaque technique was employed (14) using goat anti-mouse immunoglobulin G heavy- and light-chain antiserum (Cappel Laboratories, Downington, Pa.) at a 1/800 dilution in phosphate-buffered saline for the expression of IPFC. Guinea pig serum (Gibco) at a 1:30 dilution in phosphate-buffered saline was used as a source of complement.

Hemagglutination. A microtiter hemagglutination assay was used for the determination of 2-mercaptoethanol (2-ME)-sensitive and 2-ME-resistant agglutinating antibody titers (22).

Adsorption of suppressor serum with SRBC. Sera from C57BL/6 mice which had been infected for 28 days were pooled, heat inactivated, and adsorbed two times at 0°C with 0.2 ml of thrice washed, packed SRBC per 1.0 ml of serum. Each adsorption was done for 15 min, and SRBC were separated from the serum by centrifugation at $750 \times g$ for 5 min.

Kinetics. DPFC and IPFC and 2-ME-sensitive and 2-ME-resistant hemagglutinin titers were determined at various times during the course of infection in C57BL/6 and C3H(He) mice. DPFC and 2-ME-sensitive hemagglutination measurements were made 3, 4, 5, and 6 days after SRBC immunization; IPFC and 2-ME-resistant hemagglutination measurements were made 7, 8, 9, and 10 days after SRBC immunization.

Statistics. All results were considered statistically significant at a $P < 0.05$ level. Because PFC data are non-normal, a $\log_2$ transformation was utilized to normalize the data (11). An analysis of variance and Dunnett's multiple comparison test were used for statistical evaluation in all experiments (24).

RESULTS

The effect of *T. cruzi* on the host response to SRBC. *T. cruzi* infections in both the susceptible C3H(He) and resistant C57BL/6 mice resulted in suppression of humoral responses to SRBC (Fig. 1 and 2). While the numbers of direct and indirect PFC and levels of 2-ME-resistant and 2-ME-sensitive antibody were depressed in both animals, it was found that IPFC and 2-ME-resistant antibody showed an unexplained (and reproducible) initial enhancement in C57BL/6 mice before these parameters of responsiveness became significantly reduced later in the infection. No such amplification was detected in infected C3H(He) mice. Also, neither the observed suppression in either strain of mouse nor the apparent amplified early responses in C57BL/6 mice can be attributed to

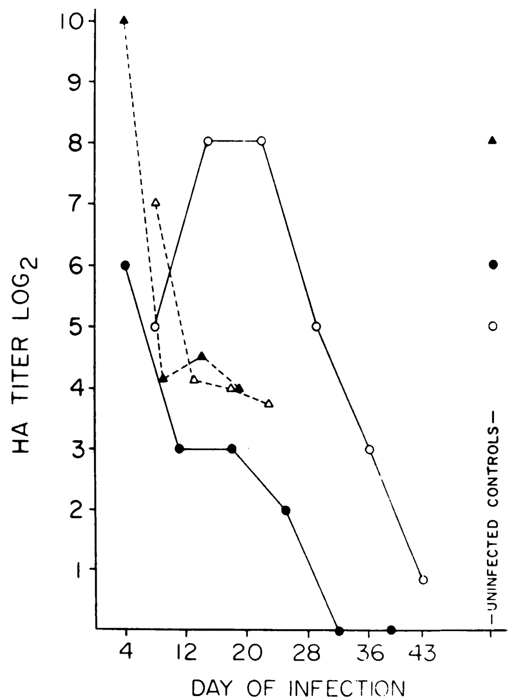

FIG. 1. *Anti-SRBC antibody titers in C3H(He) (▲, △) and C57BL/6 (●, ○) mice during the course of T. cruzi infection. Each point represents day of infection on which anti-SRBC antibody was measured. SRBC challenge was done 4 and 8 days prior to assay for 2-ME-sensitive (○, △) and 2-ME-resistant (●, ▲) antibody, respectively. Multiple comparison standard errors for 2-ME-sensitive and 2-ME-resistant antibody were 0.1096 and 0.2146, respectively.*

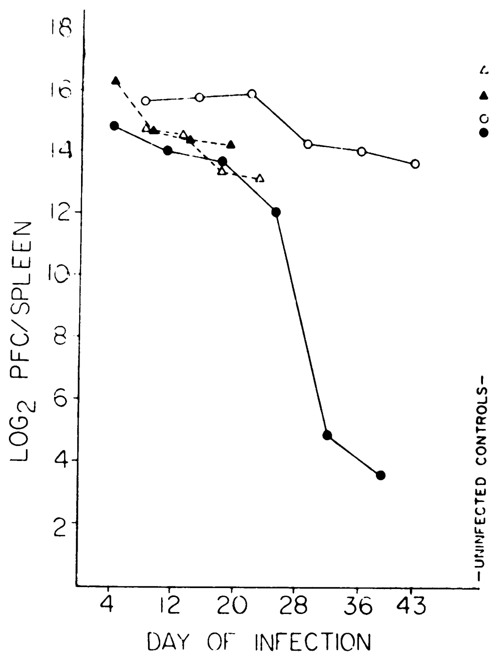

FIG. 2. *Anti-SRBC DPFC (closed symbols) and IPFC (open symbols) responses in C3H(He) (▲, △) and C57BL/6 (●, ○) mice during the course of T. cruzi infection. Each point represents day of infection on which PFC response was measured. SRBC challenge was done 4 and 8 days prior to assay for DPFC and IPFC, respecitvely. Multiple comparison standard errors for DPFC and IPFC were 0.1117 and 0.2583, respectively.*

changes in kinetics of the responses to SRBC. Regardless of stage of infection, magnitude of suppression, or strain of mouse, maximal anti-SRBC responses occurred on day 4 (100 h postchallenge) for immunoglobulin M and DPFC production and day 8 (202 h postchallenge) for 7S antibody and IPFC production (Fig. 3).

Relationship between immunosuppression and splenomegaly. Albright et al. (2) found that mice infected with *Trypanosoma musculi* exhibit suppressed humoral responses and that the suppression is progressively reduced as the animals self-cure. This abrogation of suppression was correlated with reduction in spleen size and spleen cell numbers. No such relationship was observed in the present investigation (data not shown) or in a previous study on suppression of cellular responses during the course of *T. cruzi* infection in mice (20).

Soluble suppressor substance in sera of *T. cruzi*-infected mice. Suppressor cells (8, 18) as well as circulating suppressor substances (17) are known to be mediators of various nonspecific immunosuppression phenomena. In investigating the possibility of soluble suppressor substances being generated during the course of *T. cruzi* infections, it was found in the present study that serum from infected mice could passively immunosuppress normal syngeneic recipients, rendering them incapable of normal anti-SRBC responses. Serum from C3H(He) and C57BL/6 mice infected with *T. cruzi* for at least

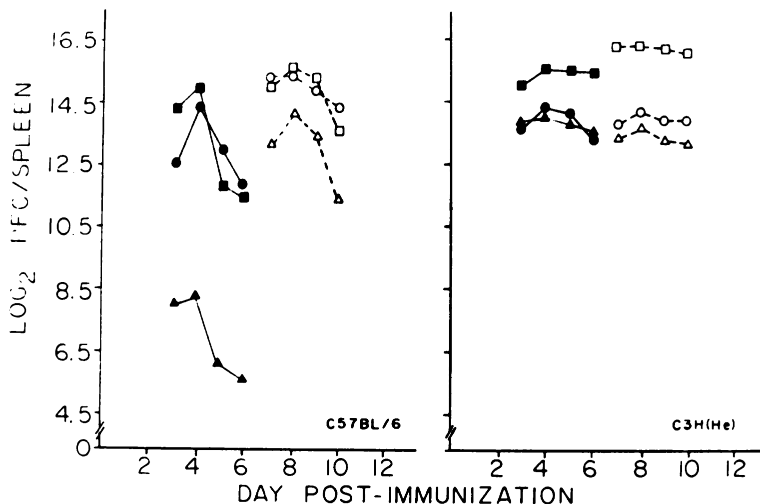

FIG. 3. *Kinetics of anti-SRBC DPFC (closed symbols) and IPFC (open symbols) responses in normal (■, □) and T. cruzi-infected mice. C57BL/6 mice were infected for 11 (●, ○) or 25 (▲, △) days and C3H(He) mice were infected for 6 (●, ○) or 12 (▲, △) days when challenged. Multiple comparison standard errors for C57BL/6 DPFC and IPFC and C3H(He) DPFC and IPFC were 0.3014, 0.1960, 0.2701, and 0.2146, respectively.*

9 days and 14 days, respectively, passively suppressed anti-SRBC DPFC in syngeneic recipients. The degree of suppression effected by the transferred sera correlated with the degree of suppression in the infected donor mice (see Fig. 1 and 2), suggesting an increase in the serum concentration of the suppressor substance with time of infection (Fig. 4). It was noted as well that C57BL/6 mice were more completely suppressed by transfer of dilutions of "suppressor serum," although the net reduction in numbers of responsive spleen cells in the two strains was similar (Fig. 5).

Of special interest was the observation that serum from infected mice, known to be suppressive in the syngeneic recipient, could not suppress humoral responses in the allogeneic recipient (Fig. 4).

The in vivo conditions optimal for the induction of immunosuppression were critically dependent on the temporal relationship between passive transfer of serum and challenge with SRBC. Passive immunosuppression was maximal in C56BL/6 mice when suppressor serum was administered 2 days prior to challenge with SRBC, whereas a lapse of 4 days was optimal

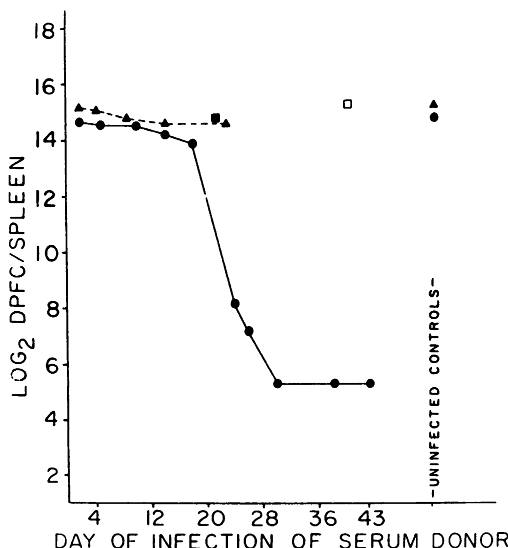

FIG. 4. *Effect of passive transfer of serum from T. cruzi-infected mice on the anti-SRBC DPFC response of normal recipients. Recipients received 0.2 ml of serum (intravenously) for syngeneic [C57BL/6 → C57BL/6, ●; C3H(He) → C3H(He), ▲] or allogeneic [day 22 C3H(He) serum → C57BL/6, ■; day 40 C57BL/6 serum → C3H(He), □] donors. Uninfected controls received normal, syngeneic serum. Multiple comparison standard errors for C57BL/6 and C3H(He) syngeneic transfers were 0.1814 and 0.2281, respectively.*

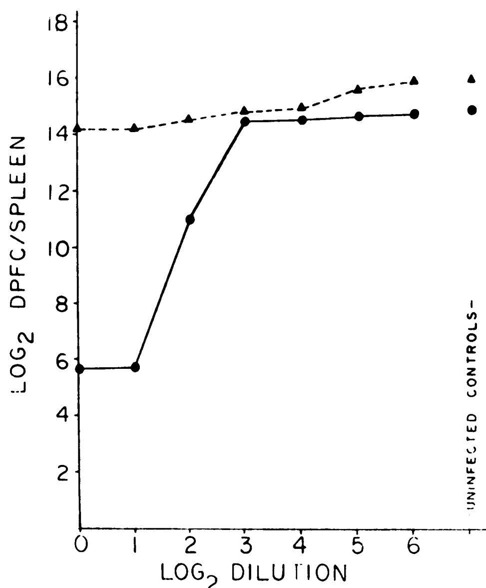

FIG. 5. *Effect of passive transfer of dilutions of serum from T. cruzi-infected mice [day 20 C3H(He); day 36 C57BL/6] on the anti-SRBC DPFC response of normal syngeneic recipients [C57BL/6, ●; C3H(He), ▲]. Multiple comparison standard errors for C57BL/6 and C3H(He) mice were 0.4132 and 0.2901, respectively.*

for suppression in C3H(He) mice (Fig. 6). Suppression of responsiveness did not occur when the suppressor serum was given on the same day as or after challenge with SRBC.

It was possible, especially with the C56BL/6 mice which develop high background anti-SRBC PFC during the course of infection, that the observed passive immunosuppression was due to cross-reactive anti-SRBC antibody in the suppressor serum. To examine this possibility, serum from *T. cruzi*-infected C56BL/6 mice (known to be suppressive) was adsorbed with SRBC prior to transfer into syngeneic recipients. The mice were then challenged with SRBC two days later, and their anti-SRBC responses were determined. It was found that there was no abrogation of suppressor activity in the suppressor serum as a result of adsorption with SRBC. Control mice had mean DPFC per spleen and hemagglutination titers of 31,250 and 1/64, respectively, whereas recipients of adsorbed or nonadsorbed suppressor serum had DPFC per spleen of 75.83 and 79.67, respectively, and undetectable hemagglutination titers.

DISCUSSION

Several studies have suggested that antibody plays a significant role in immunity to *T. cruzi*

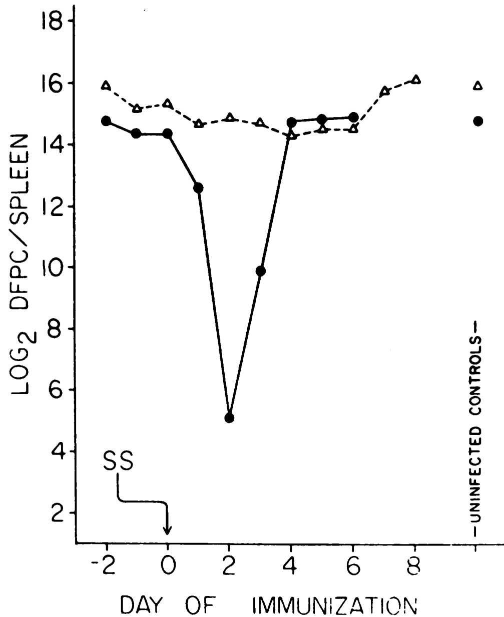

FIG. 6. *Effect of passive transfer of suppressor serum (SS) from T. cruzi-infected mice [day 22 C3H(He); day 36 C57BL/6] on the development of the anti-SRBC DPFC response in normal syngeneic recipients [C57BL/6, ●; C3H(He), △] administered SRBC before, after, or on the day of serum transfer. Multiple comparison standard errors for C57BL/6 and C3H(He) mice were 0.1487 and 0.1623, respectively.*

in mammals (7, 15, 21). However, it is apparent from the present study and an earlier report by Clinton et al. (4) that humoral responses are impaired in *T. cruzi*-infected mice. Suppressed responses to heterologous erythrocytes were observed in C3H(He) mice, which are highly susceptible to these parasites, and in the relatively resistant C57BL/6 mice. Indeed, infected C57BL/6 mice persist with levels of suppression exceeding those of C3H(He) mice, which die early in the acute phase of the disease. It does not appear, therefore, that immunosuppression of humoral responses, in and of itself, is responsible for the differences in susceptibility to *T. cruzi* in C3H(He) and C57BL/6 mice.

It may be determined in future studies that the unexplained initial amplification of numbers of IPFC and level of 7S antibody in infected C57BL/6 mice is important in resistance to this parasite. One might have anticipated similar amplification in DPFC numbers and 19S antibody responsiveness, although the necessity for this correlation is questionable (9). It was noted during the course of these experiments that *T. cruzi*-infected C57BL/6 mice, but not C3H(He), developed high levels of background anti-SRBC PFC (ca. 2^{12} DPFC; 2^{11} IPFC), suggesting that these more resistant animals may be sensitized to SRBC via recognition of cross-reacting antigens on the parasite and that the initial amplification of the IPFC is actually a secondary response. This, however, may be an unlikely possibility since, as shown in Fig. 2, normal C3H(He) mice were found to be more responsive to SRBC than were C57BL/6 mice.

An important observation in the present study was the determination that sera of infected mice contain a host-specific substance capable of mediating suppression of humoral responses when passively transferred to normal, syngeneic recipients. Although the role of this suppressor substance in immunity to the parasite was not determined, the fact that the inductive phase of antibody production is affected has profound implications on the development of resistance to *T. cruzi*. If, as with African trypanosomes, different clones or subpopulations of parasites arise during infections, or if antigenic modulation occurs in *T. cruzi*, then the host would have impaired responses to antigenic variants developing in the latter stages of infection and may explain, in part, the persistence of the parasite in the immune host.

Studies are in progress to define the nature of the suppressor substance, including its origin (host, parasite, or both), specificity with respect to host H-2 haplotypes, and physical characteristics. Preliminary results show it to have a molecular weight of about 200,000 on Sephadex G-200 gel filtration.

ACKNOWLEDGMENTS

We appreciate the help of D. Vogler and C. Smith in preparing the figures, and L. Spencer in preparing the manuscript.

This research was supported by Public Health Service grant no. AI-13128 from the National Institute of Allergy and Infectious Diseases.

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 antigen and mitogens. RES J. Reticuloendothel. Soc. **21**:21-31.
3. **Barriga, O.** 1975. Selective immunodepression in mice by *Trichinella spiralis* extracts and infections. Cell. Immunol. **17**:306-309.
4. **Clinton, B. A., L. Ortiz-Ortiz, W. Garcia, T. Martinez, and R. Capin.** 1975. *Trypanosoma cruzi*: early immune responses in infected mice. Exp. Parasitol. **37**:417-425.
5. **Corsini, A. C., C. Clayton, B. A. Askonas, and B. M. Ogilvie.** 1977. Suppressor cells and loss of B-cell potential in mice infected with *Trypanosoma brucei*. Clin. Exp. Immunol. **29**:122-131.

6. **Crandall, C. A., and R. B. Crandall.** 1976. *Ascaris suum*: immunosuppression in mice during acute infection. Exp. Parasitol. **40**:363–372.
7. **Culbertson, J. T., and M. H. Kolodny.** 1938. Acquired immunity in rats against *Trypanosoma cruzi*. J. Parasitol. **24**:83–90.
8. **Eardley, D. D., and A. N. Jayawardena.** 1977. Suppressor cells in mice infected with *Trypanosoma brucei*. J. Immunol. **119**:1029–1033.
9. **Gearhart, P. J.** 1977. Non-sequential expression of multiple immunoglobulin classes by isolated B-cell clones. Nature (London) **269**:812–813.
10. **Good, A. H., and K. L. Miller.** 1976. Depression of the immune response to sheep erythrocytes in mice infected with *Taenia crassiceps* larvae. Infect. Immun. **14**:449–456.
11. **Gottlieb, C. F.** 1974. Application of transformations to normalize the distribution of plaque-forming cells. J. Immunol. **113**:51–57.
12. **Hudson, K. M., C. Byner, J. Freeman, and R. J. Terry.** 1976. Immunodepression, high IgM levels and evasion of the immune response in murine trypanosomiasis. Nature (London) **264**:256–258.
13. **Jayawardena, A. N., and B. H. Waksman.** 1977. Suppressor cells in experimental trypanosomiasis. Nature (London) **265**:539–541.
14. **Jerne, N. K., C. Henry, A. A. Nordin, H. Fugi, A. M. C. Koros, and I. Lefkovits.** 1974. Plaque forming cells: methodology and theory. Transplant. Rev. **18**:130–191.
15. **Kierszenbaum, F., and J. G. Howard.** 1976. Mechanisms of resistance against experimental *Trypanosoma cruzi* infection: the importance of antibodies and antibody-forming capacity in the Biozzi high and low responder mice. J. Immunol. **116**:1208–1211.
16. **Kuhn, R. E., and J. E. Murnane.** 1977. *Trypanosoma cruzi*: immune destruction of parasitized mouse fibroblasts *in vitro*. Exp. Parasitol. **41**:66–73.
17. **Lee, S. T., and F. Paraskevas.** 1977. Soluble factors in mouse immune sera. III. Origin, nature, and target of small molecular weight suppressive factors isolated from serum during primary responses. Cell. Immunol. **32**:171–182.
18. **Mansfield, J. M., and O. Bagasra.** 1978. Lymphocyte function in experimental African trypanosomiasis. I. B cell responses to helper T cell-independent and-dependent antigens. J. Immunol. **120**:759–765.
19. **Poels, L. G., and C. C. van Niekerk.** 1977. *Plasmodium berghei*: immunosuppression and hyperimmunoglobulinemia. Exp. Parasitol. **42**:235–247.
20. **Rowland, E. C., and R. E. Kuhn.** 1978. Suppression of the cellular responses in mice during *Trypanosoma cruzi* infections. Infect. Immun. **20**:393–397.
21. **Rubio, M.** 1959. Natural and acquired immunity against *Trypanosoma cruzi* in the hamster (*Cricetus auratus*). Biologica **2718**:95–116.
22. **Scott, D. W., and R. K. Gershon.** 1970. Determination of total and mercaptoethanol-resistant antibody in the same serum sample. Clin. Exp. Immunol. **6**:313–316.
23. **Strickland, G. T., and P. C. Sayles.** 1977. Depressed antibody responses to a thymus-dependent antigen in toxoplasmosis. Infect. Immun. **15**:184–190.
24. **Zar, J. H.** 1974. Biostatistical analysis. Prentice-Hall, Inc., Englewood Cliffs, N.J.