INFECTION AND IMMUNITY, Feb. 1981, p. 524-529
0019-9567/81/020524-06$02.00/0
Vol. 31, No. 2

Trypanosoma cruzi Infection in B-Cell-Deficient Rats

ANA-MARIA RODRIGUEZ,[1]* FERRUCIO SANTORO,[1]† DANIEL AFCHAIN,[1] HERVÉ BAZIN,[2,3] AND ANDRÉ CAPRON[1]

Centre d'Immunologie et de Biologie Parasitaire[1] and Service d'Immunologie Expérimentale,[2] Institut Pasteur, 59019 Lille Cédex, France, and the Experimental Immunology Unity, Faculty of Medicine of Louvain, Brussels 1200, Belgium[3]

The effect of neonatally initiated injections of anti-μ rabbit antiserum on immunity of rats against *Trypanosoma cruzi* infection was investigated in vivo. Anti-μ treatment resulted in a loss of immunoglobulin M (IgM) and IgG2a synthesis and, subsequently, of antibody production. These rats so treated were shown to be significantly more susceptible to the acute phase of the infection than the control rats treated with normal rabbit serum, as measured by increased parasitemia and mortality. These results indicate the essential role of antibodies, probably in association with complement or effector cells or both, in immunity to acute Chagas' disease.

Both humoral and cell-mediated immunity have been shown to occur during infection with *Trypanosoma cruzi*, the causative agent of Chagas' disease (22, 24). In fact, the passive transfer of specific anti-*T. cruzi* antibodies can protect mice against an acute infection (8, 11, 16). Moreover, several in vitro antibody-mediated cytotoxic mechanisms against culture and bloodstream forms of *T. cruzi* have been demonstrated (1, 14, 10). Evidence for the occurrence of cell-mediated immunity in Chagas' disease also has been provided in recent years. A variety of immunosuppressive treatments, such as X-irradiation (19), neonatal thymectomy (5, 23), and the use of athymic (nu/nu) mice (10), resulted in an exacerbation of experimental acute Chagas' infection. Furthermore, the passive transfer of spleen cells from *T. cruzi*-infected mice has effectively protected normal recipients against the infection (25). All of these findings strongly suggest the involvement of antibodies, probably in association with certain cellular populations, in the control of *T. cruzi* infection. However, no definitive conclusion on the role of humoral immune response in in vivo situations can be made at present.

The suppression of immunoglobulin synthesis by neonatally initiated injection of anti-μ antibodies in rats was reported recently (4). As in chickens and mice, this treatment was demonstrated to be effective on all the rat immunoglobulin classes.

By taking advantage of this model, we have studied comparatively the pattern of *T. cruzi* infection in normal and immunoglobulin-depleted rats to evaluate in vivo the role of antibodies in the control of the acute disease.

† Present address: Department of Pathology, New York University Medical Center, NY 10016.

MATERIALS AND METHODS

Anti-μ antiserum. The anti-μ antiserum was raised in rabbits by immunization with purified monoclonal rat IgM (IR 202 protein) emulsified in complete Freund adjuvant, as previously described (4). Myeloma proteins were purified from ascite fluid by gel filtration on AcA22 and AcA34 (LKB, Sweden), diethylaminoethyl chromatography, and zone electrophoresis in agarose gels (3). Appropriate absorption of the antiserum with germfree rat serum and the pseudoglobulin fraction of normal rat serum provided its monospecificity. Antiserum and control normal rabbit serum (NRS) were absorbed subsequently with liver and kidney cells and then shown not to bind to rat thymic cells by indirect immunofluorescence, using a fluorescein isothiocyanate-conjugated goat antiserum to rabbit IgG (Nordic Laboratories, Telburg, The Netherlands) as developing reagent (4). Finally, both the anti-μ antiserum and NRS were dialyzed against phosphate-buffered saline and sterilized by filtration with a 0.22-μm Millipore filter (Millipore Corp., Bedford, Mass.).

Rats and suppression protocol. Male and female axenic neonate rats born to completely inbred parental Fischer rats were obtained under sterile conditions and maintained in sterile, plastic isolators (La Calhene, Paris) during all the experiments. A total of 17 neonate rats divided into two groups was used in this work. Neonates of the first group (8 rats, 6 females and 2 males) were injected by the intraperitoneal route every other day, from day 0 to day 12, and three times a week, from day 12 to day 60 (anti-μ-treated rats). The dosage was 0.2 ml of anti-rat μ-chain for each injection during the first 12 days and 0.3 ml afterwards. A total of 26 injections was administered. Neonates of the second group (nine rats, five females and four males) were treated with NRS under the same exper-

imental conditions with the same protocol and dosage of injection as described above.

T. cruzi infection. The Tehuantepec strain of T. cruzi was maintained by serial passages of infected blood in male Fischer rats by the method previously described in mice (18). Bloodstream forms of trypomastigotes were obtained from the blood of injected Fischer rats after they were bled by cardiac puncture. The blood was mixed with heparin and diluted in Alsever solution to have 1×10^4 trypomastigotes per ml. Infection was performed by intraperitoneal injection of 1.0 ml of the dilution. All steps of rat infection were done under sterile conditions: the room was disinfected, the materials used for obtaining infective trypomastigotes were sterilized, and infection was done in a laminar flow hood. The rats were maintained in sterile, plastic isolators during all the experiments.

Injections of both anti-μ and NRS were continued in both groups for 2 weeks after infection. All of the rats were individually bled through the retro-orbital sinus every week to check only parasitemia and every 2 weeks to perform all of the other tests.

Parasitemia. To determine parasitemias, 5.0 μl of blood was taken from each infected rat with a heparinized pipette and placed on smears. The parasites were counted immediately by microscopic examination. Sixty fields were counted, and the results were converted and expressed in trypomastigotes per cubic millimeter by the method described by Pizzi and Prager (18) and Brenner (6).

Measurement of serum IgM and IgG2a. Serum IgM and IgG2a concentrations were determined by single radial immunodiffusion (4). IgM and IgG2a standards were LOU/C monoclonal purified proteins of the same subclass but different from those used for raising the antisera, which were obtained as previously described by Bazin et al. (2).

Passive hemagglutination. Formolized sheep erythrocytes, sensitized with a soluble antigen extract of T. cruzi epimastigotes, were used for the passive hemagglutination test, with glutaraldehyde as a coupling agent. Results were expressed as the reciprocal of highest dilution (titer) giving specific agglutination.

Complement studies. Levels of C4 and of components of the alternative complement pathway in rat serum were determined on hemolytic plates, as described by Lachmann and Hobart (13). These assays measure complement by a single radial diffusion technique in which the zone of hemolysis represents the endpoint. C4 levels were determined by allowing whole rat serum to diffuse into agarose, incorporating sensitized sheep erythrocytes and C4-deficient guinea pig serum (colony maintained at the Institut Pasteur) in complement fixation diluent (Oxoid Ltd., London, England).

Total alternative pathway activity was measured by allowing whole rat serum to diffuse into agarose, incorporating unsensitized guinea pig erythrocytes in a diluent containing 10 mM ethylene glycol-bis(β-aminoethyl ether)-N,N-tetraacetic acid and 7 mM magnesium. This diluent allows the agarose to activate the alternative pathway, with consequent bystander lysis of the guinea pig erythrocytes.

Statistical evaluation. Results were analyzed by Student's t test and the two-way analysis of variance.

RESULTS

Immunoglobulin levels. Litters of newborn axenic rats were injected by the intraperitoneal route with either sterile anti-μ or NRS by the schedule described above. The rats were bled at week 0 (1 day before infection) and every 2 weeks after infection with T. cruzi. Levels of IgM and IgG2a are shown in Table 1. Significant increases of these immunoglobulins were observed in NRS-treated rats during the course of the infection ($P < 0.001$). In anti-μ treated rats, no detectable levels of IgM and IgG2a could be observed, within the limits of our experimental conditions, either 1 day before infection or in weeks 2, 4, or 6 after T. cruzi infection.

Antibodies. Specific anti-T. cruzi antibodies were investigated by the passive hemagglutination test with T. cruzi-sensitized sheep erythrocytes in both the anti-μ- and NRS-treated rats described above. The rats were also classified according to sex (Fig. 1). Significant titers of anti-T. cruzi antibodies were observed 2 weeks after infection in NRS-treated male rats. At this same period, only one NRS-treated female rat showed a weak titer of anti-T. cruzi antibodies. However, 4 weeks after infection, all of the NRS-treated female rats were positive by the hemagglutination test. By contrast, in the anti-μ-

TABLE 1. *Comparison of IgM and IgG2a levels in NRS- and anti-μ-treated rats at different weeks after infection with T. Cruzi*

Animal treatment group	Immunoglobulin level (mg/ml) at following week after infection[a]:				P value[b]
	0	2	4	6	
IgM					
NRS	0.31 ± 0.25	2.37 ± 1.16	3.06 ± 1.14	4.22 ± 0.18	<0.001
anti-μ	0	0	0	0	
IgG2a					
NRS	0	0.44 ± 0.94	2.04 ± 0.92	3.98 ± 0.55	<0.001
anti-μ	0	0	0	0	

[a] Results are expressed as geometric means ± standard deviation.

[b] P value is between NRS- and anti-μ treated rats (student's t test after analysis of variance).

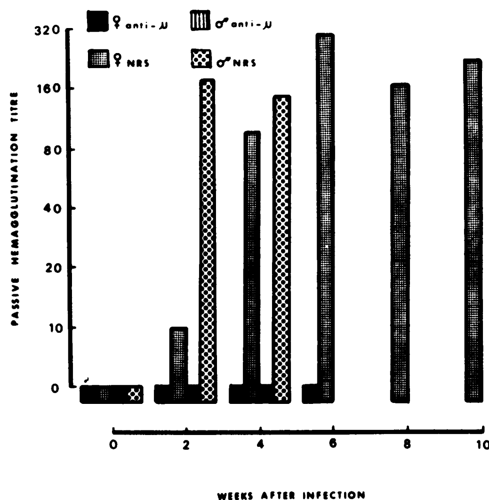

FIG. 1. *Comparison of anti-T. cruzi antibodies evaluated by passive hemagglutination in control (NRS)- and anti-μ-treated rats at different weeks after T. cruzi infection.*

treated rats (male and female), even with undiluted sera, the hemagglutination reaction was negative (Fig. 1).

Complement levels. Levels of C4 and of the whole alternative complement pathway were investigated by the hemolytic plate assay in all sera of anti-μ- and NRS-treated rats at weeks 0, 2, 4, and 6 after *T. cruzi* infection. Results are expressed as the percentage of C4 or alternative complement pathway as compared with the respective levels obtained with untreated Fischer normal rats (Fig. 2). Infection of NRS-treated rats was followed by a significant decrease of both the C4 and alternative complement pathway levels ($P < 0.001$). The same decrease in the alternative complement pathway level was also observed with anti-μ treated rats during the course of infection. By contrast, there was no change in C4 levels after *T. cruzi* infection in anti-μ-treated rats.

Parasitemia and mortality. The number of trypomastigotes per cubic millimeter of blood was evaluated by microscopic examination (Fig. 3). For parasitemia, the NRS- and anti-μ-treated rats also were separated by sex.

In the male NRS-treated rats, parasites were detected only 4 weeks after infection. In the male anti-μ-treated rats, parasitemia appeared earlier (3 weeks after infection) (Fig. 3). After this time, all of the NRS and anti-μ-treated male rats developed high parasitemias and died around week 5 of infection.

In the female NRS-treated rats, parasites were detected only 4 weeks after infection. These rats showed only a weak transient parasitemia, which

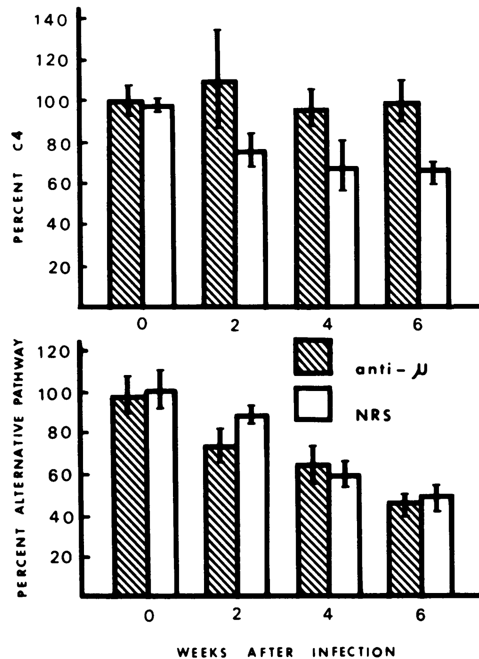

FIG. 2. *Levels of C4 and of the alternative complement pathway measured on hemolytic plates during infection with T. cruzi of control (NRS)- and anti-μ-treated rats.*

disappeared, in our experimental animals, at week 7 after infection. In this case, all the NRS-treated female rats survived, at least, until week 18 of infection, the end of these observations. Whereas in the female anti-μ-treated rats, the parasitemia appeared earlier (3 weeks after infection) than that noticed in NRS-treated rats (Fig. 3). The female anti-μ-treated rats developed high parasitemias and died at week 6 after infection. These rats so treated were shown to be significantly more susceptible to the acute phase of infection than were the control rats.

In the anti-μ-treated rats (females and males), parasitemia appeared earlier (3 weeks after infection) than that noticed in NRS-treated rats (male and female) (Fig. 3). Furthermore, all of the anti-μ treated rats developed high parasitemias and died at week 5 or 6 after infection. Also, in this case, female rats, which died around week 6, appeared more resistant than did anti-μ-treated male rats.

DISCUSSION

The present results demonstrated the necessity for B-cell effector mechanisms, probably involving antibodies, for resistance against acute Chagas' disease. In fact, the B-cell-deficient rats were hypersusceptible to *T. cruzi* infection in comparison with the natural susceptibility of the

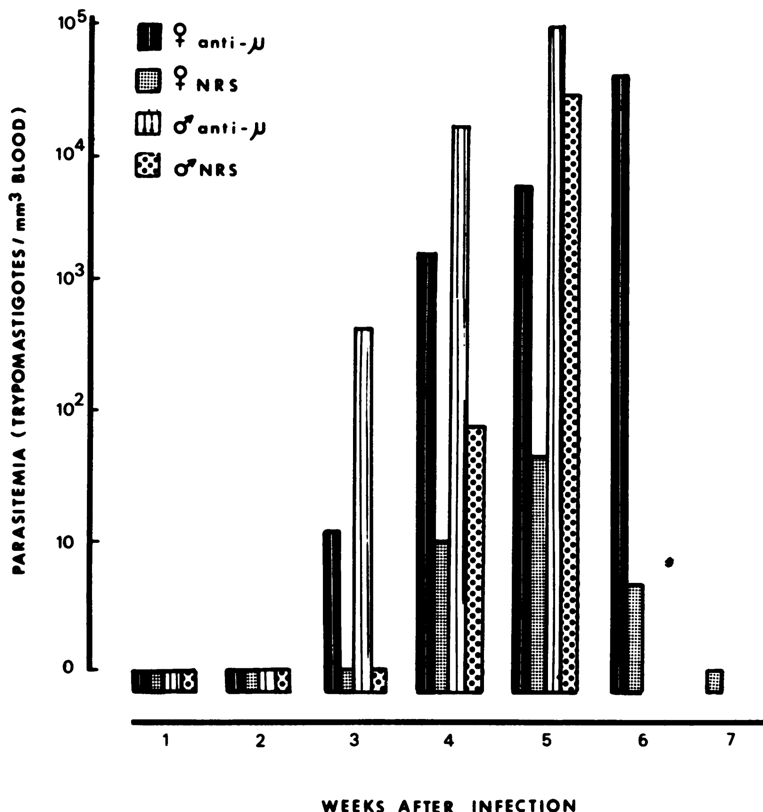

FIG. 3. *Geometric mean parasitemia in control (NRS)- and anti-μ-treated rats during the course of infection with 10^4 trypomastigotes of T. cruzi.*

control rats. Animals used in this study were maintained in an axenic status, and any other external influence, except the specific treatment, was excluded. The only difference between the two groups of animals used was the presence of anti-μ antibodies. Thus, the injection of anti-μ antibodies to deplete B-cells was an isolated action, and only B-cell-dependent mechanisms were abolished. If the suppression of immunoglobulin synthesis is now well documented, no effect has ever been observed on T-cell immunity in mice (15).

The efficiency of anti-μ treatment for immunoglobulin suppression in rats was excellent since, within the limits of the technique used, no detectable levels of IgM and IgG2a could be found (Table 1). This was in agreement with previously reported results (3, 4). In addition, failure to find any anti-*T. cruzi* hemagglutinating antibodies during the course of infection suggested strongly the lack of antibody-producing capacity in anti-μ-treated rats. Finally, the stability of C4 levels during the infection of B-cell-deficient rats in comparison with the decrease observed in control rats, allowed us to suggest that the classical complement pathway was activated by the antibodies, probably in a complexed form, appearing in *T. cruzi*-infected, NRS-treated rats.

The alternative complement pathway was consumed in both groups of rats infected with *T. cruzi* (Fig. 2). These results suggest that the alternative complement pathway is also activated in vivo during the course of acute Chagas' disease. Moreover, this activation does not require the presence of immunoglobulins, since after the infection of anti-μ-treated rats, a significant decrease of the alternative complement pathway was also observed. Thus, activation of complement during *T. cruzi* infection appears to occur by both pathways. Nevertheless, only activation of the classical complement pathway involves the presence of antibodies. All of these data should be related to the activation of both complement pathways in vitro by bloodstream forms of *T. cruzi* (21).

A large increase of IgM and IgG2a levels was found during infection of NRS-treated rats. These same increases were previously reported in mice infected with *T. cruzi* (7). These immu-

noglobulins were, moreover, associated with the appearance of anti-*T. cruzi* antibodies in the same rats (Fig. 1). When these animals were separated by sex, anti-*T. cruzi* antibodies were found, at high levels in male rats, before their detection in female rats. However, there was no significant difference in levels of IgM and IgG2a between male and female rats. As male rats appeared much more susceptible to infection than female rats (Fig. 3), one could suggest that these agglutinating antibodies which appear later in male rats (2 weeks after infection) are not involved directly in the control of the infection, even if we cannot be sure of the role of these antibodies in female rats. Other factors, probably also linked to the sex, appear to play an important role in the control of *T. cruzi* infection. In previous studies with mice, the influence of the sex in susceptibility to this disease also was reported (17).

The role of antibodies in the control of acute *T. cruzi* infection has been suggested by a number of workers in the last few years (11, 16, 20). In this study, B-cell-deficient rats presented a high susceptibility to this infection. In mice genetically selected for low antibody responsiveness, it was also shown that there is a particular susceptibility to acute Chagas' disease (9). The interpretation of these findings is that B lymphocytes, and especially their produced antibodies, are essential in the expression of immunity against infection with *T. cruzi*. If the involvement of antibodies in this immunity is likely, the mechanism(s) underlying the mode of anti-trypanosome action remains to be elucidated. Possibly, complement (12, 21) and effector cells (1, 14, 20) should collaborate with antibodies in killing *T. cruzi* in vivo.

ACKNOWLEDGMENTS

This investigation was supported by Centre National de la Recherche Scientifique (ERA 422) and Institut National de la Santé et de la Recherche Médicale (U 167). The skillful technical assistance of Marie-Ange Hospied, Marie-Christine Liebart, and Pierre Billaut is gratefully acknowledged. We also thank Marie-France Massard for typing this manuscript.

Ana-Maria Rodriguez, lecturer in Venezuela, is presently supported by a grant from the University of Los Andes (Merida).

LITERATURE CITED

1. **Abrahamsohn, I. A., and W. D. Dias da Silva.** 1977. Antibody-dependent cell-mediated cytotoxicity against *Trypanosoma cruzi*. Parasitology 75:317–323.
2. **Bazin, H. A., A. Beckers, and P. Querinjean.** 1974. Three classes and four subclasses of rat immunoglobulins: IgM, IgA, IgE and IgG1, IgG2a, IgG2b, IgG2c. Eur. J. Immunol. 4:44–48.
3. **Bazin, H., A. Capron, M. Capron, M. Joseph, J. P. Dessaint, and R. Pauwels.** 1980. Effect of neonatal injection of anti-μ antibodies on immunity to schistosomes (*S. mansoni*) in the rat. J. Immunol. 124:2373–2377.
4. **Bazin, H., B. Platteau, A. Beckers, and R. Pauwels.** 1978. Differential effect of neonatal injections of anti-μ or anti-ε antibodies on the synthesis of IgM, IgD, IgE, IgA, IgG1, IgG2a, IgG2b and IgG2c immunoglobulin classes. J. Immunol. 121:2083–2087.
5. **Behbehani, M. K.** 1971. *Trypanosoma (schizotrypanum) cruzi* infection in X-irradiated and thymectomized mice. Trans. R. Soc. Trop. Med. Hyg. 65:265.
6. **Brener, Z.** 1962. Observações sôbre a imunidade a superinfecções em camundongos experimentalmente inoculados com *Trypanosoma cruzi* e sumetidos a tratamento. Rev. Inst. Med. Trop. Sao Paulo 4:119–123.
7. **Capbern, A., P. Mattern, and R. Pautrizel.** 1974. Etude comparative du taux des protéines sériques au cours de trypanosomoses à *Trypanosoma gambiense* et à *Trypanosoma cruzi* chez la souris. Exp. Parasitol. 35:86–91.
8. **Kagan, I. G., and L. Norman.** 1961. Immunologic studies of *Trypanosoma cruzi*. III. Duration of acquired immunity in mice initially infected with a North American strain of *T. cruzi*. J. Infect. Dis. 108:1213–1217.
9. **Kierszenbaum, F., and J. G. Howard.** 1976. Mechamisms 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.
10. **Kierszenbaum, F., and M. N. Pienkowski.** 1979. Thymus-dependent control of host defense mechanisms against *Trypanosoma cruzi* infection. Infect. Immun. 24:117–120.
11. **Krettli, A. U., and Z. Brener.** 1976. Protective effects of specific antibodies in *Trypanosoma cruzi* infections. J. Immunol. 116:755–760.
12. **Krettli, A. U., P. Weiss-Carrington, and R. Nussenzweig.** 1979. Membrane bound antibodies to bloodstream *Trypanosoma cruzi* in mice: strain differences in susceptibility to complement-mediated lysis. Clin. Exp. Immunol. 37:416–423.
13. **Lachmann, P. J., and M. J. Hobart.** 1978. Complement technology, p. 5A.1–5A.23. *In* D. M. Weir (ed.), Handbook of experimental immunology. 3rd ed. Blackwell Scientific Publications, Oxford.
14. **Madeira, E. D., A. F. B deAndrade, M. M. Bunn-Moreno, and M. Barcinski.** 1979. Antibody-dependent cellular cytotoxicity of *Trypanosoma cruzi*: characterization of the effector cell from normal human blood. Infect. Immun. 25:34–38.
15. **Manning, D. D.** 1975. Heavy chain isotype suppression: a review of the immunosuppressive effects of heterologous anti-Ig heavy chain antisera. J. Reticuloendothel. Soc. 18:63–86.
16. **McHardy, N.** 1977. Passive immunization of mice against *Trypanosoma cruzi* using convalescent mouse serum. Tropenmed. Parasitol. 28:195–201.
17. **McHardy,** 1978. Effect of sex of mice in relation to their response to immunization with vaccines prepared from *Trypanosoma cruzi*. Trans. R. Soc. Trop. Med. Hyg. 72:201–202.
18. **Pizzi, T., and R. Prager.** 1952. Estabilizacion de la virulencia de una cepa de *Trypanosoma cruzi* por pasaje seriado en ratones de constitucion genética uniforme: analisis cuantitativo del curso de la infeccion. Biologica 16/17:3–12.
19. **Roberson, E. L., W. L. Chapman, Jr., and W. L. Hanson.** 1973. The effects of total-body x-irradiation on *Trypanosoma cruzi* infection (Chagas' disease) in mice and rats. Z. Parasitenkd. 41:83–94.
20. **Sanderson, C. J., I. A. Clark, and G. A. Taylor.** 1975. Different effector cell-types in antibody-dependent cell-mediated cytotoxicity. Nature (London) 253:376–377.

21. **Santoro, F., J. Bernal, and A. Capron.** 1979. Complement activation by parasites. A review. Acta Trop. **36**: 5–17.
22. **Santos-Buch, CH.** 1979. American trypanosomiasis: Chagas' disease. Int. Rev. Exp. Pathol. **19**:63–100.
23. **Schmunis, G. A., S. M. Gonzales-Cappa, O. C. Traversa, and J. F. Yanovsky.** 1971. The effect of immunodepression due to neonatal thymectomy on infection with *Trypanosoma cruzi* in mice. Trans. R. Soc. Trop. Med. Hyg. **65**:89–91.
24. **Teixeira, A. R.** 1979. Chagas' disease: Trends in immunological research and prospects for immunoprophylaxis. Bull. W.H.O. **57**:697–710.
25. **Trischmann, T. M.** 1980. *Trypanosoma cruzi*. Ability of T-cell-enriched and depleted lymphocyte populations to passively protect mice. Exp. Parasitol. **49**:225–232.