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Immunization with *Trypanosoma cruzi* Epimastigote Antigens Incorporated into Iscoms Protects against Lethal Challenge in Mice

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An immunoglobulin G3 monoclonal antibody obtained by immunizing mice with a cell membrane preparation of epimastigotes of *Trypanosoma cruzi* was shown to agglutinate live epimastigotes, lyse blood-form trypanosomes, and partially protect mice by passive transfer. The main antigens recognized by the monoclonal antibody were located in the flagella of epimastigotes and blood-form trypanosomes. Antigens of epimastigotes, purified by affinity chromatography with the monoclonal antibody, were shown to be highly glycosylated and revealed a wide band with an M_r between 45,000 and 68,000 in sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting. Immunization of mice with a small concentration of the affinity purified antigens incorporated into an antigen delivery system prepared with Quil A (Isotec AB, Lulea, Sweden), a saponin derivative, induced strong antibody and cell-mediated immune responses and protected 100% of the immunized animals against death due to challenge with 100 100% lethal doses of blood form trypanosomes. Protection was due to the use of the antigen delivery system, since mice immunized with equal concentrations of antigens alone or in combination with saponin had 100% mortality. The results suggest that small concentrations of epimastigote antigens obtained by biochemical methods and incorporated into the proper antigen delivery system may serve as a vaccine against Chagas' disease.

Trypanosoma cruzi, the causative agent of Chagas' disease, is endemic from the south of Mexico to Argentina and Chile (36, 37) and occurs sporadically in the United States (32). It is estimated that 30 million individuals are either infected with or exposed to the parasite. Effective and safe therapeutic agents for both individual and mass treatments are still not available (25). Prophylaxis with insecticides to control the insect vector is being used (26) but is a continuous source of concern because of the impact on the environment. New concepts for the development of vaccines against parasitic diseases (13, 20, 28) and new information on the antigenic structure of *T. cruzi* and on the pathogenesis and immunopathology of the infection it causes (14, 34, 35) should renew research activity on vaccines against Chagas' disease. Early publications and more recent reports have indicated that partial resistance to *T. cruzi* in mice may be developed by immunization with killed (8, 33), attenuated (27), or chemically inactivated (1) trypomastigotes as well as with a glycoprotein purified from the epimastigote stage of the parasite (39). The immune mechanisms responsible for resistance are still unclear (11). A strong antibody response appears to be essential (21, 22), and recent reports have suggested an important role for cell-mediated immune mechanisms (4, 17).

In this study, antigens of epimastigotes of *T. cruzi* were purified by affinity chromatography, incorporated into a recently developed antigen delivery system, and examined for the induction of resistance against challenge with virulent blood form trypanosomes.

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MATERIALS AND METHODS

T. cruzi. Cloned (16) organisms of the strain Tulahuen (15) were used. Epimastigotes were obtained from acellular cultures as previously described (5), and trypanosomes were obtained from peripheral blood of Swiss-Webster male mice acutely infected with the parasite.

MAbs. A cell membrane-enriched fraction of epimastigotes was used to immunize BALB/c female mice for the preparation of monoclonal antibodies (MAbs) as previously described (7). The MAbs were assayed for reactivity against antigens of *T. cruzi* by using direct agglutination with live epimastigotes, an indirect imunofluorescent antibody test, and an enzyme-linked immunosorbent assay (ELISA) (5). In addition, each MAb was examined for its ability to lyse blood trypanosomes in a complement-mediated lysis assay (23) and for induction of partial protection by passive transfer. For the passive transfer assay, 0.25 ml of undiluted MAb was injected intravenously into mice 2 days before their intraperitoneal infection with 10^4 blood form trypanosomes. Three more injections were administered intraperitoneally 2, 5, and 7 days after infection. Thereafter, the mice were monitored for time to death and total mortality. Control mice were similarly injected with supernatants from SP2/0 myeloma cells.

Affinity purification of antigens and preparation of immunostimulating complex (iscom). The MAb (87-13-1E3) chosen for purification of epimastigote antigens was coupled to Affi-Gel 10 (Bio-Rad Laboratories, Richmond, Calif.) as previously described (2). The affinity gel was packed into a chromatographic column and washed extensively with 0.1 M phosphate-buffered saline (pH 7.2) (PBS) containing 0.5% Triton X-100, 0.001 M phenylmethylsulfonyl fluoride and 0.001 M tosyl lysine chloromethyl ketone (TLCK) (Sigma

Chemical Co., St. Louis, Mo.) (sample buffer) and with 3.0 M sodium thiocyanate in PBS (elution buffer).

Epimastigotes, obtained from cultures in exponential growth (100% epimastigotes) (5), were washed three times by centrifugation with PBS containing 1% bovine serum albumin and sonicated with three pulses of 30 s each (60 cycles) in an ice bath. The sonic extract was then centrifuged at 10,000 × *g* for 10 min, the sediment was discarded, and the supernatant was centrifuged again at 32,000 × *g* for 2 h at 4°C. The supernatant of this last centrifugation was discarded, and the sediment was solubilized in sample buffer and loaded into the affinity column. The column was incubated for 1 h at room temperature and overnight at 4°C. Thereafter, unbound material was washed with sample buffer, and bound antigens were eluted with the elution buffer. Eluted antigens were dialyzed at 4°C against 10 volumes of distilled water, concentrated by using an Amicon cell with a 1,000-M_r cutoff membrane (Amicon, Danvers, Mass.), and lyophilized. The ability of the eluted antigens to bind antibody was monitored by using the ELISA with MAbs and pooled sera from humans or mice chronically infected with *T. cruzi*. Protein and carbohydrate determinations were performed by using the Bio-Rad protein assay kit with bovine serum albumin as a standard and the anthrone test with glucose as a standard, respectively. The eluted antigens were shown to be heavily glycosylated, containing approximately 60% carbohydrate and 30% protein. In sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels (13% polyacrylamide) stained either with Coomassie blue or with periodic acid-Schiff reagent (41), a wide band with an M_r between 45,000 and 68,000 was observed (data not shown).

Eluted antigens were then used to prepare the iscoms as previously described (24, 30). Briefly, lyophilized antigens were solubilized in a 2% solution of MEGA-10 detergent and mixed with the saponin derivative Quil-A (Iscotec AB, Lulea, Sweden) to a final concentration of 0.1%. The mixture was dialyzed extensively against PBS. Free Quil-A was removed by pelleting the iscoms by centrifugation at 100,000 × *g* for 16 h at 4°C through a cushion of 5 and 10% sucrose. The pelleted iscoms were resuspended in PBS, examined for proper morphology by electron microscopy, filtered through a 0.45-μm-pore-size filter, and stored at 4°C until used. In the electron microscope, iscoms show a cagelike structure approximately 40 nm in diameter and composed of circular subunits approximately 12 nm in diameter in which the antigen appears to be enclosed (Fig. 1).

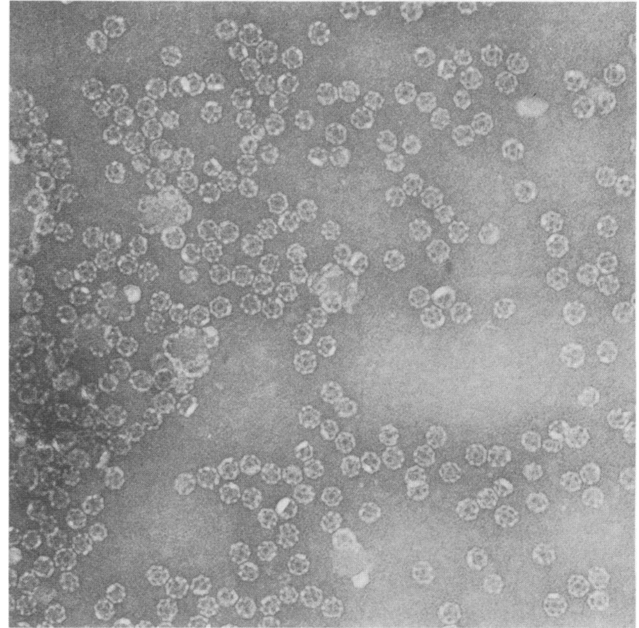

FIG. 1. Electron micrograph of iscoms containing antigens of *T. cruzi*. Magnification, ×7,500.

Immunization of mice. Outbred 20-g Swiss-Webster mice (Simonsen Labs, Gilroy, Calif.) were used. Mice immunized with iscom containing affinity purified antigens received 5 μg of the preparation injected subcutaneously. Two more immunizations with 2 μg of iscom were performed 15 and 30 days after the first injection. Control mice were injected with the same concentration of antigens used alone or antigens in combination with 30 μg of saponin per mouse. Additional control mice were injected similarly with 30 μg of either Quil A, the matrix of the iscoms, or saponin alone per mouse. Seven days after the last immunization episode, blood was collected from the orbital sinuses of three mice of each group and tested individually in the ELISA to determine the antibody response (5). In addition, serum samples from these mice were pooled and used in immunoblots (3) to determine the antigens recognized by antibodies formed as a result of the immunization procedures.

The cell-mediated immune response was assayed by using a delayed-type hypersensitivity assay in three mice of each group, 7 days after the last immunization episode. Briefly, epimastigotes in exponential growth were killed by incubation in 1% buffered Formalin for 30 min at room temperature and then washed five times by centrifugation with PBS. Then 10^7 organisms in 20 μl of PBS were injected into the right footpad of each mouse, and PBS was injected into the contralateral footpad. Swellings of both footpads were measured at 24 and 48 h with a caliper. The results are reported as the difference between the swelling of the footpad injected with antigen and the swelling of the footpad injected with the diluent.

One week after the last immunization, each mouse was challenged with an intraperitoneal inoculation of 10^5 blood trypanosomes of *T. cruzi* Tulahuen. Parasitemias were determined by using five samples of 5 μl each of tail blood as previously described (2). Mortality was recorded daily.

Statistical analysis of the data was performed by using the Student *t* test, the Mann-Whitney *U* test (18), and analysis of variance.

RESULTS

Of the MAbs examined, MAb 87-13-1E3, an immunoglobulin G3, agglutinated live epimastigotes into large clumps, lysed 50 to 75% of the blood form trypanosomes in the complement-mediated lysis assay, induced partial protection when passively transferred to acutely infected mice (two survivors among 5 infected mice), tested positive in the immunofluorescent antibody test, and resulted in an absorbance of 0.085 in the ELISA with the epimastigote lysate (the ELISA control value was 0.006). In addition, results of the immunofluorescent antibody test revealed that 87-13-1E3 bound to antigens located in the flagellum of epimastigotes and blood form trypanosomes and in organelles located near the nuclei of epimastigotes (Fig. 2). Because of these properties and because protective antigens have been suspected to occur in the flagella of epimastigotes (40), MAb 87-13-1E3

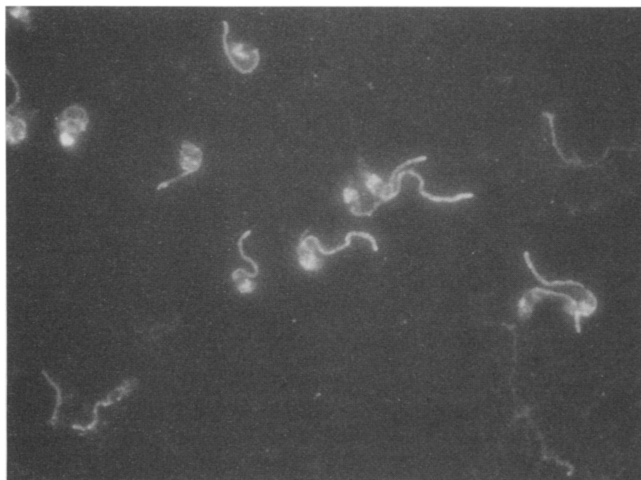

FIG. 2. IFA results of Formalin-killed epimastigotes reacting with MAb 87-13-1E3. Staining is mostly on the flagella and in organelles near the nuclei of the organisms.

was chosen for affinity isolation of epimastigote antigens to examine them for the ability to induce resistance against *T. cruzi* in mice.

Antibody and cell-mediated immune responses. Of all immunized mice, those injected with iscom containing affinity purified antigens had the strongest antibody response (Table 1). Immunization with antigens in combination with saponin also induced the formation of antibodies to *T. cruzi*, but the absorbance readings were significantly lower than the readings noted with sera from iscom-immunized mice. The antibody responses of mice immunized with antigen alone approached negative results, and sera from mice injected with Quil A or saponin alone had absorbance readings similar to those of the negative control sera (Table 1). Immunoblots performed with pooled sera collected 7 days after the last immunization episode revealed that antibodies formed by iscom-immunized mice strongly recognized antigens distributed into a wide band between M_rs 66,000 and 40,000 (Fig. 3, lane 1). In addition, other epitopes in antigens with approximate M_rs of 30,000 and 15,000 were faintly recognized by antibodies in the sera of these mice. Antibodies formed by mice immunized with antigen alone faintly recognized antigens with the same M_r except the 15,000-M_r antigen (lane 2). Antibodies formed by mice injected with antigen combined with saponin faintly recognized the 40,000- to 66,000-M_r antigens and strongly recognized the 30,000-M_r antigen (lane 4). The results obtained with sera of mice injected with saponin alone and with sera of mice chronically infected with *T. cruzi* are shown in lanes 3 and 5, respectively.

TABLE 1. ELISA results in mice immunized with affinity purified antigens and controls

Immunization	Mouse no.	Mean absorbance ± SD
Iscom	1	0.108 ± 0.008
	2	0.192 ± 0.006
	3	0.143 ± 0.019
Saponin-antigen	1	0.036 ± 0.006
	2	0.024 ± 0.004
	3	0.080 ± 0.007
Antigen alone	1	0.024 ± 0.001
	2	0.017 ± 0.004
	3	0.011 ± 0.001
Saponin alone	1	0.015 ± 0.004
	2	0.013 ± 0.001
	3	0.014 ± 0.001
Positive control[a]		0.329 ± 0.009
Negative control[b]		0.014 ± 0.003

[a] Pooled sera from mice chronically infected with *T. cruzi*.
[b] Pooled sera from normal mice.

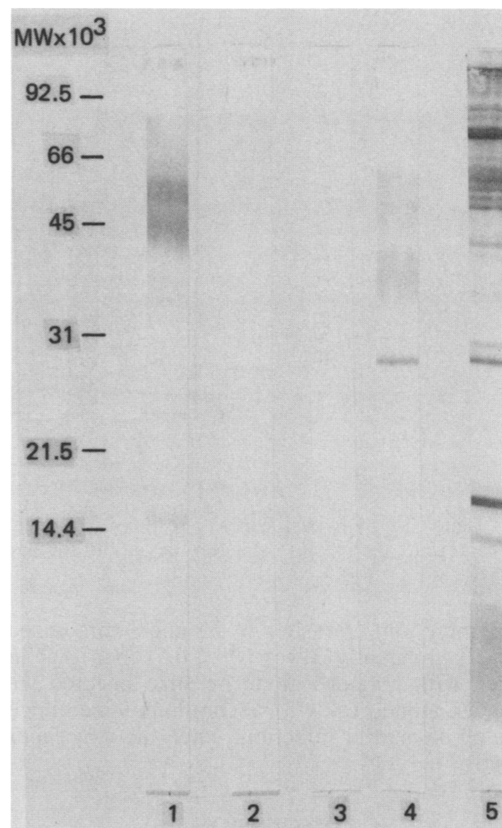

FIG. 3. Antigen recognition by antibodies in sera of immunized mice. Pooled sera from three mice were used. Lanes: 1, mice immunized with iscom; 2, mice immunized with antigen alone; 3, mice injected with saponin only; 4, mice immunized with saponin and antigen; 5, epimastigote antigens recognized by antibodies in sera of mice chronically infected with *T. cruzi*.

The strongest delayed-type hypersensitivity response was noted in iscom-immunized mice. The response in these animals was significantly higher ($P < 0.05$) than the response noted in mice that had been immunized with antigens in combination with saponin, antigens alone, or saponin alone (Fig. 4).

The challenge of each mouse with a lethal inoculum of *T. cruzi* revealed that the iscom-immunized mice developed remarkable resistance to the parasite; none of the animals had died up to 35 days after infection (Table 2). In contrast, 100% of mice immunized with antigens alone, antigens in combination with saponin, and controls injected with either saponin or Quil A died between days 13 and 14 of the

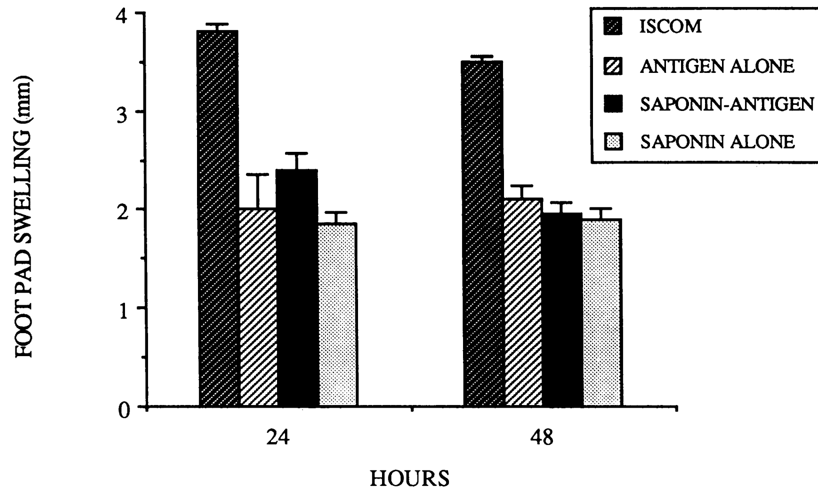

FIG. 4. Delayed-type hypersensitivity responses in mice immunized with iscom, antigen alone, saponin and antigen, or saponin alone. Each bar represents the mean (+standard deviation) footpad swelling of three mice.

challenge infection. Parasitemia in mice immunized with iscom was significantly lower ($P < 0.5$) than that in mice immunized with antigens alone or mice injected with the iscom matrix alone (Table 3). Iscom-immunized mice were still alive 50 days after infection, when the experiment was terminated.

DISCUSSION

Our results indicate that concentrations as low as 5 μg of affinity purified antigens of the epimastigote stage of *T. cruzi* are sufficient to induce remarkable protection against challenge with blood form trypanosomes in mice, provided that the antigens are incorporated into the proper antigen delivery system. Although numerous reports have demonstrated the immunogenic capacity of whole epimastigotes or of their antigens to induce partial protection against *T. cruzi* (33, 38), most of the recent work on the search for protective antigens of *T. cruzi* has been focused on the metacyclic and blood form trypanosome stages of the organism (43). Recent publications, however, have suggested that partial prevention of the natural infection of animals under field conditions may be achieved by immunization with killed epimastigotes (8). The fact that epimastigotes are potent activators of the alternate pathway of complement and readily lysed by normal serum (11) and their apparent inability to penetrate cells suggest that they are a poor source of protective antigens. It should be noted, however, that all stages of the life cycle of *T. cruzi*, epimastigotes, blood form and metacyclic trypomastigotes, and intracellular amastigotes, share a large number of antigens (6). Moreover, although antigenic preparations from each one of the stages of *T. cruzi* can be used for diagnosis of the infection by serological methods (5), epimastigotes are by far the stage most frequently used for this purpose (12). The advantage of using epimastigotes as a source of antigens for diagnosis of Chagas' disease and as potential providers of antigens for immunization studies resides in the fact that these organisms can be grown readily and in large numbers in several defined acellular culture media. Large quantities of organisms can be easily obtained, and antigens may be isolated from them by simple biochemical and immunochemical procedures.

Of interest for the development of vaccines against parasites, particularly against *T. cruzi*, was the observation that a small concentration of antigen incorporated into iscom was sufficient to induce strong antibody and cell-mediated immune responses and to generate significant protection. The use of small concentrations of immunogen may be of crucial importance in a vaccine against *T. cruzi*, because it may

TABLE 2. Percent mortality in mice immunized with purified antigen of *T. cruzi*

Immunization	*n*	% Cumulative mortality on day:						
		10	11	12	13	14	15	35
Iscom	6	0	0	0	0	0	0	0
Saponin-antigen	6	33.3	83.3	83.3	100			
Antigen alone	10	20	80	90	90	100		
Saponin	5	20	60	60	100			
Quil A	5	20	20	80	100			

TABLE 3. Parasitemia[a] in mice immunized with iscom, antigen alone, or iscom matrix (saponin) and challenged with *T. cruzi* blood trypanosomes

Group[b]	Mouse no.	Mean no. ± SE of parasites per 5 μl of blood on day:	
		7	10
Iscom	1	72 ± 33	128 ± 28
	2	32 ± 27	72 ± 32
	3	32 ± 27	192 ± 132
Saponin	1	644 ± 217	4,725 ± 243
	2	804 ± 196	4,324 ± 147
	3	885 ± 121	3,455 ± 191
Antigen alone	1	481 ± 98	3,948 ± 382
	2	676 ± 127	4,735 ± 351
	3	865 ± 121	4,472 ± 877

[a] Parasitemias were determined in three mice of each group. Five samples of 5 μl each of tail blood were used.

[b] Differences between iscom and saponin and iscom and antigen alone were statistically significant ($P < 0.05$) on both days 7 and 10 as determined by one-way analysis of variance with the Scheffe *S* test.

prevent or minimize the possibility of autoimmune responses. Although the autoimmune phenomenon induced by antigens of *T. cruzi* is still a subject of controversy (19, 20, 31), the possibility of its existence has had some negative impact in efforts to develop a vaccine against the parasite. At present, there is no firm evidence that any of the autoimmune reactions linked to Chagas' disease is unmistakably elicited by antigens of parasite origin. It is clear that further experiments to definitely prove or disprove the autoimmunity phenomenon in Chagas' disease are needed. However, as suggested previously (19), the possibility that autoimmune processes are nonexistent in Chagas' disease should be considered, and programs oriented at developing vaccines against the disease should be stimulated. The high immunogenicity of the small antigen concentration was due to the use of the iscom technology, since use of equal amounts of antigen alone or in combination with saponin, which is one of the best adjuvants to generate significant antibody and cell-mediated immune responses (9), was not effective. The iscom matrix Quil A is a triterpene glycoside purified from saponin. Although saponin has had some use in veterinary medicine, its toxicity prevents its use in vaccines for humans (10). In iscoms, Quil A is used in concentrations that are 5 to 10% of the concentration of the antigen, although the adjuvant effect is potentiated at least 10 times. The antigen concentration can be reduced to 0.1 times the concentration used in other systems and still evoke high antibody and cell-mediated immune responses, as has been reported with several viruses and with antigens of *Toxoplasma gondii* (42). Iscoms are stable and can be produced in large scale and lyophilized. Their efficiency in presenting antigens to the immune system and low toxicity for animals, including monkeys, suggest a potential use in vaccines for humans. Vaccines for veterinary use employing iscom technology are already being employed, and studies to evaluate the toxicity of iscoms in humans are in progress (29).

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REFERENCES

1. **Andrews, N. W., M. J. M. Alves, R. I. Schumacher, and W. Colli.** 1985. *Trypanosoma cruzi*: protection in mice immunized with 8-methoxypsoralen-inactivated trypomastigotes. Exp. Parasitol. **60**:255–262.
2. **Araujo, F. G.** 1984. Resistance to *Trypanosoma cruzi* induced by immunization with antigens isolated by affinity chromatography. Braz. J. Med. Biol. Res. **17**:157–164.
3. **Araujo, F. G.** 1986. Analysis of *Trypanosoma cruzi* antigens bound by specific antibodies and by antibodies to related trypanosomatids. Infect. Immun. **53**:179–185.
4. **Araujo, F. G.** 1989. Development of resistance to *Trypanosoma cruzi* in mice depends on a viable population of L3T4$^+$ (CD4$^+$) T lymphocytes. Infect. Immun. **57**:2246–2248.
5. **Araujo, F. G., and D. Guptill.** 1984. Use of antigen preparations of the amastigote stage of *Trypanosoma cruzi* in the serology of Chagas' disease. Am. J. Trop. Med. Hyg. **33**:362–371.
6. **Araujo, F. G., and J. S. Remington.** 1981. Characterization of stages and strains of *Trypanosoma cruzi* by analysis of cell membrane components. J. Immunol. **127**:855–859.
7. **Araujo, F. G., S. D. Sharma, V. Tsai, P. Cox and J. S. Remington.** 1982. Monoclonal antibodies to stages of *Trypanosoma cruzi*: characterization and use for antigen detection. Infect. Immun. **37**:344–349.
8. **Basombrio, M. A.** 1990. *Trypanosoma cruzi*: partial prevention of the natural infection of guinea pigs with a killed parasite vaccine. Exp. Parasitol. **71**:1–8.
9. **Bomford, R.** 1980. The comparative selectivity of adjuvants for humoral and cell-mediated immunity. I. Effect on the antibody response to bovine serum albumin and sheep red blood cells of Freund's incomplete and complete adjuvants, alhydrogel, *Corynebacterium parvum*, *Bordetella pertussis*, muramyl dipetide and saponin. Clin. Exp. Immunol. **39**:426–434.
10. **Bomford, R.** 1989. Adjuvants for anti-parasite vaccines. Parasitol. Today **5**:41–46.
11. **Brener, Z., and A. U. Krettli.** 1990. Immunology of Chagas' disease, p. 247–261. *In* D. J. Wyler (ed.), Modern parasite biology. Cellular, immunological, and molecular aspects. W. H. Freeman and Co., New York.
12. **Camargo, M. E., and G. K. F. Takeda.** 1979. Diagnostico de laboratorio, p. 175–198. *In* Z. Brener and Z. Andrade (ed.), *Trypanosoma cruzi* e doença de Chagas. Guanabara-Koogan, Rio de Janeiro, Brazil.
13. **Capron, A., and J. P. Dessaint.** 1988. Vaccination against parasitic infections: some alternative concepts for the definition of protective antigens. Ann. Inst. Pasteur. **139**:109–117.
14. **Choromanski, L., and R. E. Kuhn.** 1987. Use of parasite antigens and interleukin-2 to enhance suppressed immune responses during *Trypanosoma cruzi* infection in mice. Infect. Immun. **55**:403–408.
15. **de Titto, E. H., and F. G. Araujo.** 1987. Mechanism of cell invasion by *Trypanosoma cruzi*: importance of sialidase activity. Acta Trop. **44**:273–282.
16. **de Titto, E. H., D. M. Israelski, and F. G. Araujo.** 1987. Circulating *Trypanosoma cruzi* from the same cloned population show differences in the ability to infect cells and cause lethal infection in mice. Experientia **43**:1227–1229.
17. **Gazzinelli, R. T., V. M. C. Leme, J. R. Cançado, G. Gazzinelli, and J. Scharfstein.** 1990. Identification and partial characterization of *Trypanosoma cruzi* antigens recognized by T cells and immune sera from patients with Chagas' disease. Infect. Immun. **58**:1437–1444.
18. **Glantz, S. A.** 1987. Primer of biostatistics. McGraw-Hill Book Co., San Francisco.
19. **Kierszenbaum, F.** 1986. Autoimmunity in Chagas' disease. J. Parasitol. **72**:201–203.
20. **Kierszenbaum, F.** 1989. Chagas' disease, p. 173–195. *In* F. Y. Liew (ed.), Vaccination strategies of tropical diseases. CRC Press, Inc., Boca Raton, Fla.
21. **Kierszenbaum, F., and J. G. Howard.** 1976. Mechanism 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**:1208–1212.
22. **Krettli, A. U., and Z. Brener.** 1982. Resistance against *Trypanosoma cruzi* associated to anti-living trypomastigote antibodies. J. Immunol. **128**:2009–2012.
23. **Krettli, A. U., P. Weizs-Carrington, and R. S. 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.
24. **Lovgren, K., J. Lindmark, R. Pipkorn, and B. Morein.** 1987. Antigenic presentation of small molecules and peptides conjugated to a preformed iscom as carrier. J. Immunol. Methods **98**:137–143.
25. **Marr, J. J., and R. Docampo.** 1986. Chemotherapy for Chagas' disease: a perspective of current therapy and considerations for future research. Rev. Infect. Dis. **8**:884–903.
26. **Marsden, P.** 1988. South American trypanosomiasis and leishmaniasis: endemic diseases of continental dimensions affecting poor, neglected, and underfunded people, p. 77–92. *In* A. Sher and P. T. Englund (ed.), The biology of parasitism. Alan R. Liss, Inc., New York.
27. **Menezes, H.** 1976. The protective effect of the epimastigote forms of the PF strain of *Trypanosoma cruzi* against a virulent homologous infection. Tropenmed. Parasitol. **27**:418–421.
28. **Mitchell, G. F.** 1988. The way ahead for vaccines and vaccina-

tion: symposium summary. Vaccine **6**:200–205.
29. **Morein, B.** 1988. The iscom antigen-presenting system. Nature (London) **332**:287–288.
30. **Morein, B., B. Sundquist, S. Hoglund, K. Dalsgaard, and A. Osterhaus.** 1985. Iscom, a novel structure for antigenic presentation of membrane proteins from enveloped viruses. Nature (London) **308**:457–460.
31. **Mortatti, R. C., L. C. S. Maia, A. V. Oliveira, and M. E. Munk.** 1990. Immunopathology of experimental Chagas' disease: binding of T cells to *Trypanosoma cruzi*-infected heart tissue. Infect. Immun. **58**:3588–3593.
32. **Navin, T. R., R. R. Roberto, D. D. Juranek, K. Limpakarnjanarat, E. W. Mortenson, J. R. Clover, R. E. Yescott, C. Taclindo, F. Steurer, and D. Allain.** 1985. Human and sylvatic *Trypanosoma cruzi* infection in California. Am. J. Public Health **75**:366–369.
33. **Neal, R. A., and P. Johnson.** 1977. Immunization against *Trypanosoma cruzi* using killed antigens and with saponin as adjuvant. Acta Trop. **34**:87–96.
34. **Peterson, D. S., R. A. Wrightman, and J. E. Manning.** 1986. Cloning of a major surface antigen gene of *Trypanosoma cruzi* and identification of a nonapeptide repeat. Nature (London) **322**:566–568.
35. **Reed, S. G.** 1988. In vivo administration of recombinant IFN-γ induces macrophage activation, and prevents acute disease, immune suppression, and death in experimental *Trypanosoma cruzi* infections. J. Immunol. **140**:4342–4347.
36. **Schettino, P. M. S., I. Haro Arteaga, and T. U. Berrueta.** 1988. Chagas disease in Mexico. Parasitol. Today **4**:348–352.
37. **Schofield, C. J.** 1985. Control of Chagas' disease vectors. Br. Med. J. **41**:187–194.
38. **Scott, M. T., R. A. Neal, and N. C. Woods.** 1989. Immunization of marmosets with *Trypanosoma cruzi* cell surface glycoprotein (GP90). Trans. R. Soc. Trop. Med. Hyg. **79**:451–454.
39. **Scott, M. T., and D. Snary.** 1979. Protective immunisation of mice using cell surface glycoprotein from *Trypanosoma cruzi*. Nature (London) **282**:73–74.
40. **Segura, E. L., C. Vazquez, A. Bronzina, J. M. Campos, J. A. Cerisola, and S. M. Gonzalez-Cappa.** 1977. Antigens of the subcellular fractions of *Trypanosoma cruzi*. II. Flagellar and membrane fractions. J. Protozool. **24**:540–543.
41. **Takacs, B.** 1979. Electrophoresis of proteins in polyacrylamide slab gels, p. 81–105. In I. Lefkovits and B. Pernis (ed.), Immunological methods. Academic Press, Inc., New York.
42. **Uggla, A., F. G. Araujo, A. Lunden, K. Lovgren, J. S. Remington, and B. Morein.** 1988. Immunizing effects in mice of two *Toxoplasma gondii* iscom preparations. J. Vet. Med. **B35**:311–314.
43. **Yoshida, N.** 1983. Surface antigens of metacyclic trypomastigotes of *Trypanosoma cruzi*. Infect. Immun. **40**:836–839.