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Use of Parasite Antigens and Interleukin-2 to Enhance Suppressed Immune Responses during *Trypanosoma cruzi* Infection in Mice

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Mice infected with *Trypanosoma cruzi* exhibit an early and profound suppression of parasite-specific and nonspecific immune responses. Earlier studies have shown that this suppression is due, at least in part, to suppressor macrophages, deficiency in production of interleukin-2 (IL-2), and reduced T helper (T_h)-cell activity. In the present study, the effect of exogenously supplied IL-2 on enhancement of parasite-specific T_h-cell activity, anti-parasite immunoglobulin G (IgG) and IgM antibody levels, parasitemia, and longevity was examined in infected mice. The results showed that administration of IL-2 with and without antigenic stimulation with trinitrophenylated *T. cruzi* significantly enhanced parasite-specific IgM and IgG levels. Injection of IL-2 and trinitrophenylated *T. cruzi* together significantly enhanced parasite-specific T_h-cell activity and was more effective in enhancement of parasite-specific antibody levels. In addition, it was found that IL-2 alone had a rapid and lasting effect in reducing parasitemia. These results suggest that deficiency in IL-2 may play a major role in host susceptibility to *T. cruzi*.

During the course of experimental Chagas' disease (American trypanosomiasis) a profound nonspecific immunosuppression develops (3–5, 17–19), and recent evidence suggests that parasite-specific immunosuppression also develops (23). This immunosuppression is due, at least in part, to suppressor macrophages (4, 9), deficient T helper (T_h)-cell activity (15, 16), and reduced production of interleukin-2 (IL-2; 7, 23). The addition of IL-2 to cultures of splenocytes of infected mice or the injection of IL-2 into infected mice partially restores responses to heterologous antigens (2, 15, 16, 23) and enhances immunity to *Trypanosoma cruzi*, as reflected in decreased parasitemia and increased longevity (2).

It is likely that exogenously supplied IL-2 overcomes immunosuppression and enhances antiparasite immunity in experimental Chagas' disease by increasing the reactivity of T cells. In previous studies (1, 24), we have examined anti-*T. cruzi* T_h-cell activity by inducing immune responses to Formalin-fixed trypanosomes to which trinitrophenyl (TNP) groups have been attached. Measurement of B-cell responses in producing anti-TNP plaque-forming cells indirectly indicates the stimulation of anti-*T. cruzi* T_h cells, and therefore, one can obtain a relative quantification of the activity of antigen-specific T_h cells. Use of the carrier effect provides a means to examine the effect of various experimental manipulations on parasite-specific responses.

In the present study, we examined the effect of injecting IL-2 alone, TNP-*T. cruzi* alone, and TNP-*T. cruzi* plus IL-2 on parasite-specific T_h-cell activity and parasite-specific immunoglobulin G (IgG) and IgM responses. We also examined the effect of in vivo administration of IL-2 on parasitemia and longevity of *T. cruzi*-infected mice.

MATERIALS AND METHODS

Mice. Female, 10-week-old C3HeB/FeJ mice (Jackson Laboratory, Bar Harbor, Maine) were used in all experiments. Mice 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.

Infection. Mice were infected intraperitoneally with 10^3 blood-form trypomastigotes of the Brazil strain of *T. cruzi*. Maintenance and characteristics of the parasite have been reported previously (10).

Preparation of haptenated *T. cruzi*. Epimastigotes of *T. cruzi* were harvested from liver infusion tryptose medium as previously described (14). For antigen preparations, epimastigotes were washed three times by centrifugation in phosphate-buffered saline (PBS) and fixed in 1% Formalin in PBS for 30 min. After fixation, parasites were washed five times with PBS and labeled with trinitrobenzene sulfonic acid (Eastman Kodak Co., Rochester, N.Y.) as previously described (1). Next, TNP-*T. cruzi* were washed five times in PBS and stored at 4°C in 1% Formalin. Before being used for immunizations, TNP-*T. cruzi* were washed three times in PBS and adjusted to the desired concentration.

Immunizations. Dose-response and specificity of anti-TNP direct plaque-forming cell (DPFC) response were determined after normal mice were challenged with graded doses of TNP-*T. cruzi*. The in vivo anti-TNP response was determined after intraperitoneal injection of the stated number (see below) of TNP-*T. cruzi* in 0.2 ml of PBS for 5 days before the assays for DPFC.

IL-2. Recombinant human IL-2 was purchased from Genzyme, Boston, Mass. The vial of lyophilized IL-2 was reconstituted with 0.5% deoxycholate (deoxycholic acid; Sigma Chemical Co., St. Louis, Mo.) in PBS and then allowed to stand for 5 min without shaking and diluted with PBS to obtain a 0.5% deoxycholate concentration and 5,000 U of IL-2 per ml. Activity of IL-2 was determined by using the methods described by Gillis et al. (6).

Administration of IL-2 and TNP-*T. cruzi* in vivo. Recombinant IL-2 was injected intraperitoneally into C3H mice infected with *T. cruzi* or into uninfected controls. A dose of 1,500 U of IL-2 was chosen based on results obtained previously (2). The number of TNP-*T. cruzi* injected with or without IL-2 was 5×10^7 (see Results). Protocols for the various experiments are described in the Results section.

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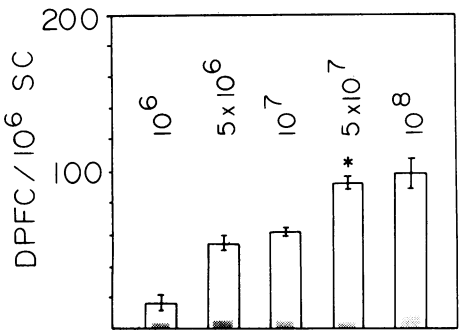

FIG. 1. Dose-response for stimulation of TNP-SRBC DPFC with TNP-*T. cruzi* in normal mice (± standard deviation). □, Anti-TNP-SRBC DPFC results significantly different ($P < 0.05$) from results with 5×10^7 TNP-*T. cruzi* (*); ■, anti-SRBC DPFC results (not significantly different).

DPFC assay. Spleen cells from infected or uninfected mice were removed and teased apart in 2.5 ml of Hanks balanced salt solution containing 0.1% gelatin (pH 7.6; Difco Laboratories, Detroit, Mich.). The resulting cell suspension was drawn through a 23-gauge needle and expelled through a 26-gauge needle to obtain a single-cell suspension. Erythrocytes were removed from the cell suspension by hypotonic shock, and the remaining nucleated cells were used.

The slide modification of the plaque assay of Jerne et al. (8) was used to enumerate DPFC to trinitrophenylated sheep erythrocytes (TNP-SRBC) as indicator cells. Guinea pig serum (GIBCO Laboratories, Grand Island, N.Y.) at 1:30 dilution in Dulbecco PBS was used as a source of complement. DPFC assays were performed 5 days after the last injection of IL-2 or TNP-*T. cruzi* or both.

RIA. The level of *T. cruzi*-specific antibody in the sera of mice was determined by using biotinylated goat anti-mouse IgM or IgG (Tago Inc., Burlingame, Calif.) and [^{3}H]avidin in a solid-phase radioimmunoassay (RIA) as previously described (22). Avidin D (Vector Laboratories, Burlingame, Calif.) was labeled with tritium by using *N*-succinimidyl-[2,3-^{3}H]propionate (New England Nuclear Corp., Boston, Mass.). The 96-well flexible U-bottom polyvinyl chloride plates (Falcon 3911; Becton Dickinson Labware, Oxnard, Calif.) were coated with 5×10^5 epimastigotes or 1×10^5 fibroblast-derived trypomastigotes and fixed with 0.125% glutaraldehyde (22). All serum samples were assayed at the same time, and the level of parasite-specific antibody in serum was expressed as counts per minute.

Preparation of parasites for coating plates. Anti-*T. cruzi* antibody levels were determined by using both epimastigotes and trypomastigotes to assure that antibody levels would reflect all possible antigens exposed to the infected hosts. Culture-form epimastigotes were prepared from liver infusion tryptose medium as previously described (14). Fibroblast-derived trypomastigotes were obtained from SVB6KH fibroblasts (25) infected with bloodstream forms of the Brazil strain of *T. cruzi*. The cultures were maintained in T-75 Corning flasks (Corning Glass Works, Corning, N.Y.) with 5 ml of RPMI 1640 with 10% fetal bovine serum as previously described (10). Fibroblast-derived trypomastigotes were harvested and separated from debris on Histopaque 1077 (Sigma).

Effect of IL-2 on anti-*T. cruzi* immunity in vivo. Infected mice received four injections of 1,500 U of IL-2 3 days apart, starting at 7 days postinfection (d.p.i.). Infected mice not treated with IL-2 served as controls. Mice in these groups were marked, and parasitemia of individual animals and longevity were monitored. Parasitemia was determined also in mice treated with a single dose of IL-2; these mice were used later for determination of DPFC and thus were excluded from evaluation of survival time. Parasitemia was determined as described previously (11) by counting the number of parasites in 4 μl of blood beneath an 18-mm cover slip.

Statistics. DPFC data were normalized by a $\log_2$ transformation before analysis and comparison of means by the Student-Newman-Keuls test (26). Differences were considered significant at the $P < 0.05$ level. Values for parasitemia, survival time, and IgM and IgG antibody levels were analyzed by the Student-Newman-Keuls test and were considered significant at $P < 0.05$.

RESULTS

Antibody responses to TNP-*T. cruzi* in vivo. To determine the amount of antigen (TNP-*T. cruzi*) necessary to obtain optimal anti-TNP-*T. cruzi* DPFC responses, normal uninfected C3HeB/FeJ mice were challenged with graded doses of TNP-*T. cruzi*. The results shown in Fig. 1 indicate that 5×10^7 TNP-*T. cruzi* per mouse elicited specific anti-TNP DPFC responses to a level significantly higher ($P < 0.05$) than 10^7 TNP-*T. cruzi* but not significantly different when compared with a dose of 10^8 TNP-*T. cruzi*. For subsequent studies, therefore, a dose of 5×10^7 TNP-*T. cruzi* was used.

Effect of IL-2 and TNP-*T. cruzi* on anti-TNP-*T. cruzi* responses during infection. Untreated, infected mice developed relatively low levels of anti-TNP and anti-SRBC DPFC, which remained somewhat constant during the course of infection and likely reflected a low level of cross-reactivity or a low level of polyclonal activation of B cells (Fig. 2 and Table 1). Because splenomegaly occurred in infected mice, DPFC data are presented as both DPFC per spleen and per 10^6 spleen cells for completeness. Injection of TNP-*T. cruzi* and IL-2 simultaneously at various times during the course of infection significantly enhanced anti-TNP DPFC responses. When responses were examined on the basis of DPFC per 10^6 spleen cells (Fig. 2), the highest responsiveness was noted when both TNP-*T. cruzi* and IL-2 were provided on day 13 of infection and the DPFC data

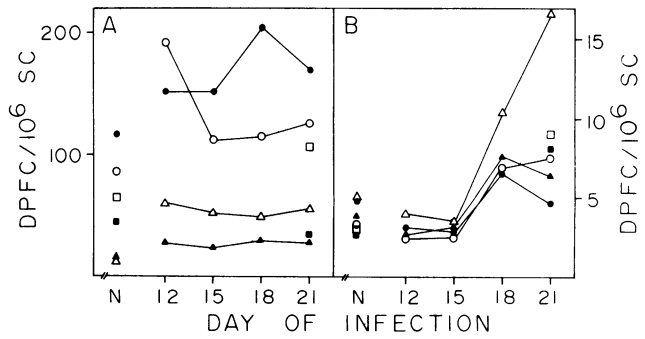

FIG. 2. Anti-TNP-SRBC (A) and anti-SRBC (B) DPFC responses of spleen cells from normal (N) and *T. cruzi*-infected mice on indicated d.p.i. ●, Single doses of IL-2 and TNP-*T. cruzi* given at 7, 10, 13, or 16 d.p.i. and DPFC assay performed 5 days later; ▲, only IL-2 given as indicated above; ○, only TNP-*T. cruzi* given as indicated above; □, doses of IL-2 given at 7, 10, 13, and 16 d.p.i. and TNP-*T. cruzi* single immunization given at 16 d.p.i. with DPFC assay performed on 21 d.p.i.; ■, only doses of IL-2 given as indicated above; △, nontreated normal or infected mice.

TABLE 1. Anti-TNP-SRBC and anti-SRBC DPFC responses per spleen at indicated days and with various treatments after *T. cruzi* infection

IL-2/TNP-*T. cruzi* treatment (days)	10^3 DPFC ± SD per spleen at d.p.i.:				
	Control	12	15	18	21
vs TNP-SRBC					
No treatment	1.1 ± 0.2	9.3 ± 1.9	8.3 ± 1.2	9.7 ± 1.2	11.1 ± 1.2
7/7	7.8 ± 1.6	20.6 ± 5.8			
10/10			27.8 ± 4.4		
13/13				34.1 ± 5.8	
16/16					36.2 ± 2.1
7/–	1.1 ± 0.1	3.8 ± 1.8			
10/–			3.7 ± 1.7		
13/–				5.9 ± 1.6	
16/–					6.1 ± 0.9
–/7	8.5 ± 2.4	29.3 ± 7.1			
–/10			19.3 ± 4.6		
–/13				22.4 ± 4.5	
–/16					29.0 ± 4.6
7,10,13,16/16	6.5 ± 0.9				19.6 ± 2.1
7,10,13,16/–	3.9 ± 0.9				5.8 ± 0.6
vs SRBC					
No treatment	0.4 ± 0.2	0.6 ± 0.5	0.5 ± 0.2	2.0 ± 0.3	3.3 ± 0.4
7/7	0.3 ± 0.1	0.4 ± 0.2			
10/10			0.5 ± 0.2		
13/13				1.1 ± 0.4	
16/16					1.0 ± 0.5
7/–	0.3 ± 0.0	0.4 ± 0.2			
10/–			0.5 ± 0.2		
13/–				1.5 ± 0.3	
16/–					1.3 ± 0.6
–/7	0.3 ± 0.1	0.4 ± 0.1			
–/10			0.4 ± 0.1		
–/13				1.3 ± 0.6	
–/16					1.8 ± 0.4
7,10,13,16/16	0.3 ± 0.1				1.7 ± 1.1
7,10,13,16/–	0.3 ± 0.0				1.6 ± 0.6

were determined 5 days later (day 18). If the number of DPFC per spleen rather than per 10^6 cells is examined, the number of anti-TNP DPFC continued to increase throughout the infection (Table 1).

Injection of TNP-*T. cruzi* alone was also effective in stimulating anti-TNP DPFC in infected mice. Responses of mice injected with TNP-*T. cruzi* on day 7 of infection (and the anti-TNP DPFC responses determined 5 days later) were much greater than responses of mice injected with TNP-*T. cruzi* at later times in infection, suggesting a diminution of anti-*T. cruzi* T_h-cell activity later in infection (Fig. 2 and Table 1). Responses of infected mice to TNP-*T. cruzi* alone were much lower at days 15, 18, and 21 compared with responses of mice receiving both TNP-*T. cruzi* and IL-2. Also, multiple injections of IL-2 on days 7, 10, 13, and 16, with injection of TNP-*T. cruzi* on day 16, were not as effective in enhancing anti-TNP responses as was a single injection of TNP-*T. cruzi* and IL-2 on day 16.

Anti-SRBC DPFC responses of treated and untreated, normal and infected mice are included in Fig. 2 and Table 1 as controls and to indicate the insignificantly low level of polyclonal activation.

Enhancement of parasite-specific antibody levels in vivo. The relative levels of anti-*T. cruzi* IgM and IgG in infected and normal mice were determined by using a [^{3}H]avidin RIA with epimastigotes (Table 2) or trypomastigotes (Table 3) as the antigen. It was found that both IgM and IgG responses increased in unmanipulated, infected mice through day 18, at which time they reached a plateau.

Injection of IL-2 and TNP-*T. cruzi* at various times during the course of infection greatly enhanced anti-*T. cruzi* IgM and IgG antibody responses (Tables 2 and 3). Likewise, injection of TNP-*T. cruzi* alone had a similar effect on the enhancement of parasite-specific antibody levels. Injection of IL-2 alone into infected mice resulted in a slight enhancement of antiparasite antibody responses on days 12 and 15 and even less enhancement on days 18 and 21 of infection. Thus, the administration of IL-2 alone resulted in far less enhancement of antibody levels than did the administration of IL-2 and TNP-*T. cruzi* or TNP-*T. cruzi* alone. It was also found that multiple injections of IL-2 on days 7, 10, 13, and 16 with or without TNP-*T. cruzi* in the final injection were not more effective than single injections in enhancing parasite-specific antibody responses on day 21 of infection.

Effect of IL-2 on parasitemia and longevity in infected mice. In confirmation of an earlier report (2), it was found that four injections of 1,500 U of IL-2 on days 7, 10, 13, and 16 of infection resulted in decreased parasitemia and increased longevity. Untreated, infected mice died on day 26 ± 0.0 days (standard deviation) and had parasitemia values (per ml) of 2.2×10^6 and 4.5×10^6 on days 23 and 26, respectively. Infected mice receiving four injections of IL-2 survived 32.3 ± 3.8 days, with parasitemia values of 0.9×10^6 and 2.4×10^6 on days 23 and 26, respectively. Parasitemia levels of these two groups of *T. cruzi*-infected mice were significantly different ($P < 0.05$) on days 14, 17, 20, 23, and 26.

In this series of experiments, it was also determined that a single injection of IL-2 during the course of infection would result in substantially reduced parasitemia levels very

TABLE 2. Detection of *T. cruzi*-specific IgM and IgG antibodies in the sera of C3HeB/FeJ mice during the course of infection versus epimastigotes

IL-2/TNP-*T. cruzi* treatment (days)	Immunoglobulin levels at d.p.i. (cpm, 10^3):									
	Control		12		15		18		21	
	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
No treatment	9.32 ± 0.34	5.28 ± 0.36	12.53 ± 0.46[a]	5.98 ± 0.44	14.14 ± 0.78[a]	6.12 ± 0.64	17.36 ± 1.33	7.11 ± 0.61[b]	16.25 ± 1.87	7.99 ± 0.96[b]
7/7	18.65 ± 1.72	7.32 ± 0.42	25.09 ± 2.42	8.44 ± 0.03						
10/10					24.77 ± 0.73	8.43 ± 0.13				
13/13							22.89 ± 1.01	8.76 ± 0.30		
16/16									22.17 ± 1.35	9.49 ± 0.45
7/–	10.02 ± 2.14	6.16 ± 0.72	16.47 ± 1.53[a]	7.09 ± 0.36						
10/–					17.97 ± 2.26[a]	6.56 ± 10.83				
13/–							18.50 ± 2.52	8.45 ± 0.11[b]		
16/–									17.34 ± 2.24	9.27 ± 0.11[b]
–/7	15.75 ± 0.41	7.32 ± 0.35	24.78 ± 0.34	7.63 ± 0.22						
–/10					23.12 ± 0.99	8.19 ± 0.06				
–/13							23.89 ± 0.70	8.02 ± 0.25		
–/16									22.15 ± 1.37	8.95 ± 0.09
7,10,13,16/16	18.07 ± 0.72	7.13 ± 0.64							18.65 ± 2.03	9.39 ± 0.21
7,10,13,16/–	10.68 ± 1.80	5.60 ± 0.31							17.01 ± 2.38	8.87 ± 0.78

[a] Significant difference ($P < 0.05$) between IgM levels of untreated and IL-2-treated mice.
[b] Significant difference ($P < 0.05$) between IgG levels of untreated and IL-2-treated mice.

TABLE 3. Detection of *T. cruzi*-specific IgM and IgG antibodies in the sera of C3HeB/FeJ mice during the course of infection versus trypomastigotes

IL-2/TNP-*T. cruzi* treatment (days)	Immunoglobulin levels at d.p.i. (cpm, 10^3):									
	Control		12		15		18		21	
	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
No treatment	4.67 ± 0.09	2.28 ± 0.08	7.24 ± 0.32[a]	3.78 ± 0.13	9.50 ± 0.28[a]	4.00 ± 0.12	12.50 ± 0.50	4.95 ± 0.45	12.12 ± 1.14	4.95 ± 0.41
7/7	12.83 ± 0.64	3.68 ± 0.40	18.93 ± 3.82	4.47 ± 0.33						
10/10					18.22 ± 0.19[b]	5.04 ± 0.21				
13/13							17.11 ± 1.94	5.18 ± 0.23		
16/16									16.16 ± 1.68	5.45 ± 0.07
7/–	6.46 ± 0.07	2.73 ± 0.40	10.10 ± 0.92[a]	4.13 ± 0.17						
10/–					13.72 ± 2.22[a]	4.19 ± 0.86				
13/–							13.24 ± 1.05	5.03 ± 0.27		
16/–									12.48 ± 2.23	5.18 ± 0.12
–/7	10.48 ± 1.17	3.39 ± 0.32	16.97 ± 2.51	4.41 ± 0.14						
–/10					16.41 ± 1.06[b]	4.26 ± 0.41				
–/13							17.54 ± 0.54	4.92 ± 0.15		
–/16									16.64 ± 2.10	5.54 ± 0.16
7,10,13,16/16	12.26 ± 0.67	4.02 ± 0.49							13.78 ± 1.17	5.71 ± 0.42
7,10,13,16/–	6.33 ± 0.76	2.75 ± 0.28							12.26 ± 2.26	5.57 ± 1.46

[a] Significant difference ($P < 0.05$) between IgM levels of untreated and IL-2-treated mice.
[b] Significant difference ($P < 0.05$) between IgM levels of mice treated with TNP-*T. cruzi* and those treated with IL-2 and TNP-*T. cruzi*.

shortly after the administration of the lymphokine. For instance, infected mice given IL-2 on day 16 were found to have significantly reduced levels of parasitemia on days 17 and 20 compared with the levels in untreated, infected mice or infected mice receiving TNP-*T. cruzi* alone. Parasitemia values for mice receiving IL-2 on day 16 were as follows (per ml): day 14, 1.9×10^5; day 17, 2.7×10^5; and day 20, 5.2×10^5. Control values were 1.7×10^5, 4.6×10^5, and 7.3×10^5 for days 14, 17, and 20, respectively. Similar reductions in parasitemia were observed in mice receiving injections of TNP-*T. cruzi* and IL-2.

DISCUSSION

In earlier studies, we and others (2, 7, 15, 16, 23) demonstrated that IL-2 could overcome immunosuppression and restore immune responsiveness against nominal antigens and could effect enhanced immune responses against parasite antigens in mice infected with *T. cruzi*. IL-2 was effective immunotherapeutically in infected mice, as evidenced by increased longevity and reduced parasitemia levels. It was not determined in these earlier studies, however, how exogenously supplied IL-2 acts to stimulate improved immune responses in infected mice, although it was thought that the lymphokine acts to stimulate T_h-cell activity.

In the present study, we examined the effect of the administration of IL-2 with or without the administration of TNP-*T. cruzi*. This approach allowed the estimation of the level of anti-*T. cruzi* T_h-cell activity by determining the number of anti-TNP DPFC responses, an exploitation of the carrier effect (12, 13). It was also possible by this approach to determine the effect of IL-2 in the presence or absence of antigenic stimulation with culture forms of *T. cruzi*, because this stimulation affected the levels of anti-*T. cruzi* IgG and IgM.

It was shown earlier (1) and confirmed here that infected mice can generate anti-TNP DPFC when this hapten is conjugated to fixed trypanosomes. TNP attached to other carriers, i.e., SRBC or keyhole limpet hemocyanin, does not elicit anti-TNP DPFC in *T. cruzi*-infected mice (1) if the TNP is attached to a carrier to which the mice have not had previous exposure. These observations suggest that the mechanisms of immunosuppression were not as effective in the presence of sensitized antigen-specific T_h cells. In this regard, the results of the present study showed that infected mice were more responsive to TNP-*T. cruzi* when challenged early (day 7) versus late (day 10, 13, or 16) in infection. This would suggest that immunosuppressive mechanisms were induced and regulated control of T-cell responses by day 10 of infection. Injection of IL-2 and TNP-*T. cruzi* at various times during infection, however, resulted in sustained, substantial increases in reactivity through day 16 of infection.

It was previously reported that multiple injections of IL-2 during the course of infection can significantly increase longevity and decrease parasitemia in mice infected with *T. cruzi* (2). In the present study, we examined the effect of repeated injections of 1,500 U of IL-2 with or without TNP-*T. cruzi* in the final injection to determine whether continued administration of IL-2 enhanced T_h-cell activity to a level greater than a single injection did. It was found, however, that multiple injections of IL-2 on 7, 10, 13, and 16 d.p.i. with TNP-*T. cruzi* injected on day 16 resulted in far lower anti-TNP responses than did injection of TNP-*T. cruzi* alone or TNP-*T. cruzi* and IL-2 on day 16. These results indicate that multiple injections of IL-2 not only did not result in enhanced responses but were much less efficacious than a single treatment was in enhancing T_h-cell activity.

The present study also demonstrates, however, the positive effect of multiple injections of IL-2 in increasing longevity and reducing parasitemia in *T. cruzi*-infected mice. In addition, it was noted that a single injection of IL-2 was rapidly effective in reducing parasitemia and this effect lasted for several days of observation. The present study confirms and extends a previous report (2) by demonstrating that the intraperitoneal injection of IL-2 augmented the mouse parasite-specific antibody response. It is not clear why multiple injections of IL-2 were effective in providing a high degree of protection for infected mice but simultaneously did not stimulate high levels of anti-TNP-*T. cruzi* responses as measured by anti-TNP DPFC.

In an attempt to analyze the specific antibody response of normal or *T. cruzi*-infected mice exposed to different treatments (Tables 2 and 3), the RIA was used to detect parasite-specific antibody levels. In our RIA experiments, we used epimastigotes and trypomastigotes of *T. cruzi* because, even though there are some unique antigens, they express and share a majority of the same cell surface antigens (20, 21). By using this approach, it was found that the levels of parasite-specific IgM and IgG responses during the course of infection generally peaked within 18 and 21 d.p.i., respectively; however the sera from infected mice which were given TNP-*T. cruzi* and IL-2 showed a peak level of specific IgM at 12 d.p.i. It was also shown that the administration of IL-2 with TNP-*T. cruzi* into normal mice resulted in enhancement of anti-*T. cruzi* IgM levels compared with the levels in animals treated with TNP-*T. cruzi* but not receiving IL-2. It is clear, therefore, that the administration of IL-2 stimulated antibody production in normal and *T. cruzi*-infected mice.

Although the results of the experiments show that the addition of IL-2 to immunodeficient mice stimulated enhanced parasite-specific T_h-cell activity, greater levels of parasite-specific antibody, and increased resistance to *T. cruzi*, the mechanism which regulates the production of IL-2 by T cells to essentially undetectable levels in infected mice is not known. Previous studies have shown that nonspecific immunosuppression is mediated by macrophages, and it is likely that these cells regulate IL-2 production in infected mice.

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