Immunology 1987 **60** 309–315

Suppression of human lymphocyte responses by *Trypanosoma cruzi*

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Accepted for publication 17 October 1986

SUMMARY

Virtually nothing is known about the basis for the immunosuppression associated with human *T. cruzi* infection. We have used an *in vitro* system to explore this effect. Incubation of human peripheral blood mononuclear cells (PBMC) with blood forms of *T. cruzi* abrogated their responses to suboptimal, optimal and supraoptimal doses of Con A, PHA or PWM, whether or not monocytes were depleted. Killed parasites were not suppressive. Maximal suppression (74%) occurred when the parasites were present during the entire culture period (96 hr), although significant suppression (33%) was seen when the organisms were added 24, 48 or 72 hr after initiation, suggesting that the early stages of lymphocyte activation had been impaired and that a second generation of cells was also affected. The 4-day supernatant medium of a *T. cruzi* suspension supported PBMC responses to Con A as well as medium incubated alone, indicating that suppression did not result from parasite removal of essential nutrients. Furthermore, 96 hr after mitogenic stimulation, the proportions of viable PBMC in cultures containing or lacking the parasites were comparable. Although *T. cruzi* binds Con A and PHA, this absorption was not the cause of reduced responsiveness since optimal concentrations of Con A and PHA remained in solution under our conditions. Levels of IL-2 in PHA-stimulated PBMC cultures were markedly reduced in the presence of *T. cruzi*. However, exogenous IL-2 failed to restore lymphocyte responsiveness. *T. cruzi* neither absorbed nor inactivated IL-2. Thus, the noted suppression appeared to involve at least deficient production and utilization of IL-2.

INTRODUCTION

Experimental and human infection by *Trypanosoma cruzi* (the causative agent of Chagas' disease) are accompanied (particularly during the acute period) by severe alterations of the humoral and cellular arms of the immune system (Brener, 1980; Kuhn, 1981; Clinton *et al.*, 1975; Teixeira *et al.*, 1978; Maleckar & Kierszenbaum, 1983; Ramos, Schädtler-Siwon & Ortiz-Ortiz, 1979). This condition has been regarded as a means by which the parasite eludes immunological defences while it establishes itself in the host (Brener, 1980; Kuhn, 1981). Studies with murine model systems of Chagas' disease have produced evidence suggesting several mechanisms of immunosuppression, including alteration of accessory cell function (Cunningham & Kuhn, 1980; Kierszenbaum, 1982), reduced levels of T cells in the spleen (Hayes & Kierszenbaum, 1981), and altered lymphokine-producing ability (Harel-Bellan *et al.*, 1983; Reed, Inverso & Roters, 1984a, b; Tarleton & Kuhn, 1983). In contrast, our knowledge of human lymphocyte alterations in human *T. cruzi* infection is negligible and, given the differences between murine and human Chagas' disease, extrapolations would be unwarranted. In this work, an *in vitro* system was used to study the effects of *T. cruzi* on human lymphoproliferative responses induced by mitogens. It will be shown in this paper that co-culture with the parasite suppresses human lymphocytes at a relatively early stage of the activation process, and that altered lymphocyte functions include a markedly reduced capacity to both produce and utilize interleukin-2 (IL-2).

Abbreviations: Con A, concanavalin A; c.p.m., counts per minute; FBS, heat-inactivated (56°, 20 min) fetal bovine serum; IL-2, interleukin-2; IL-2_{ch}, crude human IL-2; IL-2_{ph}, purified human IL-2; IL-2_{rat}, crude rat IL-2; PBMC, human peripheral blood mononuclear cells; PHA, phytohaemagglutinin; PWM, pokeweed mitogen.

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MATERIALS AND METHODS

Animals

The 4-week-old Crl-CD1(ICR)BR Swiss mice used to maintain and produce blood forms of *T. cruzi*, and the female Lewis rats used as a source of spleen cells to produce IL-2 (IL-2_{rat}) were purchased from Charles River Laboratory (Portage, MI).

Parasites

The Tulahuén strain of *T. cruzi* was used in this work. Trypomastigotes were purified from the blood of mice (infected intraperitoneally 2 weeks previously with 2×10^5 organisms) by density gradient centrifugation over a mixture of Ficoll–Hypaque of density 1·077 (Budzko & Kierszenbaum, 1974) followed by chromatography through a diethylaminoethyl-

cellulose column (Villalta & Leon, 1979). The parasites were washed twice by centrifugation (800 *g*, 20 min, 4°) in RPMI-1640 medium containing L-glutamine (Gibco, Grand Island, NY), penicillin (100 units/ml) and streptomycin (100 μg/ml). Parasite suspensions were prepared at the desired concentrations (see Results) in the same medium supplemented with 5% heat-inactivated (56°, 20 min) fetal bovine serum (FBS, Gibco) (RPMI + 5% FBS). In some experiments, trypomastigotes grown in cultures of rat heart myoblasts (Lima & Kierszenbaum, 1982) or epimastigotes grown in Warren's medium (Warren, 1960) were used. When killed blood trypomastigotes were needed, the organisms were incubated with 0·025% glutaraldehyde in phosphate-buffered saline (20°, 2 min), washed by centrifugation, quenched with 0·1 M lysine in phosphate-buffered saline and washed twice with RPMI + 5% FBS.

Mitogens

Concanavalin A (Con A), phytohaemagglutinin (PHA) and pokeweed mitogen (PWM) were purchased from Sigma Chemical Co. (St Louis, MO).

IL-2 preparations

The IL-2-containing supernatant used to maintain HT-2 cells (see below) was prepared by stimulating rat spleen cells (1×10^6 cells/ml) with 2 μg Con A/ml in the presence of 5×10^{-5} M 2-mercaptoethanol, using the medium described above. The supernatants were collected after incubating these cultures at 37° and 5% CO_2 for 48 hr in an atmosphere saturated with water vapour, and stored at −20° until used. This material will be referred to in the text as IL-2_{rat}. Purified human IL-2 (IL-2_{ph}) was purchased from Collaborative Research (Lexington, MA). Crude preparations of human IL-2 (IL-2_{ch}) consisted of the 48-hr supernatants of peripheral blood mononuclear cell (PBMC) (5×10^6 PBMC/ml) cultures stimulated with 25 μg PHA/ml (Tilden & Balch, 1982). In some cases, production of IL-2_{ch} in the presence or absence of *T. cruzi* was compared; the concentration of parasites, when present, was 5×10^6 organisms/ml, and all other conditions remained the same.

Cells

The PBMC used in this work were from healthy volunteers. Their purification was by centrifugation over Lymphoprep (Nyegaard, Oslo) at 340 *g* and 20° for 45 min, and they were washed three times with serum-free RPMI-1640 medium prior to use; cell viability, determined by trypan blue dye exclusion, was always >99%. The final suspensions of these cells were prepared in RPMI + 5% FBS. The IL-2-dependent HT-2 cell line (kindly provided by Dr Philippa Marrack from the University of Colorado Health Sciences Center, Denver, CO) was used to measure IL-2 activity in biological fluids. These cells were maintained in RPMI + 10% FBS at 37° and 5% CO_2 by mixing equal volumes of cell culture and the IL-2_{rat} preparation (see above).

Depletion of non-specific esterase-positive cells

Suspensions of PBMC (3·5 ml at 5×10^6 cells/ml) were incubated at 37° (5% CO_2 incubator) for 1 hr in a 60-mm diameter sterile plastic petri-dish. The non-adherent cells were removed and subjected to the same procedure once more, and then centrifuged (280 *g*, 10 min, 4°). The adherent, non-specific esterase-positive cells were further depleted by chromatography over a Sephadex G-10 (Pharmacia, Piscataway, NJ) column (Mishell, Mishell & Shigii, 1980). The non-specific esterase test has been described elsewhere (Yam, Li & Crosby, 1971).

Blastogenesis assay

Cell cultures were set up in triplicate in 96-well microculture plates. Each culture contained $1{\cdot}25 \times 10^5$ PBMC and the appropriate mitogen concentration (see Results) in a total volume of 0·1 ml. When parasites or other reagents were to be present, they were contained in 0·025 ml and substituted for the equivalent volume of RPMI + 5% FBS. All cultures were incubated at 37° and 5% CO_2 for 96 hr (unless otherwise stated) and pulsed with 1 μCi [^{3}H] thymidine (specific activity 2 mCi/mmol, Amersham, Arlington Heights, IL) during the last 24 hr. Cultures were interrupted by harvesting (MASH II, M. A. Bioproducts, Walkersville, MD) and radioactivity was measured in a liquid scintillation spectrometer.

Absorption of Con A solutions with T. cruzi

Solutions of Con A (concentrations described under Results) were incubated with 5×10^6 blood forms of *T. cruzi* per millilitre at 37° (CO_2 incubator) for 24 hr. The parasites were then removed by filtration through sterile 0·22-μm Millipore filters (Bedford, MA). The filtrate was used as the culture medium in blastogenesis assays to test PBMC responses to the residual amount of mitogen.

Incubation of RPMI + 5% FBS with T. cruzi

After incubating RPMI + 5% FBS medium with or without 5×10^6 blood forms of *T. cruzi* per millilitre at 37° (CO_2 incubator) for 4 days and filtration (0·22 μm pore size), the filtrates were used in blastogenesis assays to test PBMC responses to various concentrations of Con A.

Measurement of IL-2 activity

Cultures of HT-2 cells were set up in triplicate in microculture wells, each containing 4×10^3 cells. The final volume of these cultures was 0·2 ml, including 0·1 ml of two-fold dilutions of the biological material to be tested. The cultures were incubated at 37° for 48 hr (5% CO_2) and pulsed with 1 μCi [^{3}H] thymidine during the last 24 hr. Cell harvesting and measurement of radioactivity incorporated into synthesized DNA was as described above.

T. cruzi *incubation with IL-*2_{ch}

Solutions of IL-2_{ch} were incubated with purified blood trypomastigotes at final concentrations varying from $1{\cdot}25 \times 10^6$ to 2×10^7 organisms/ml at 37° (5% CO_2) for 48 hr. After removing the parasites by filtration through sterile 0·22-μm pore-size filters, the filtrates were tested for IL-2 activity as described above. For control purposes, aliquots of IL-2_{ch} were subjected to the same conditions except that the parasites were absent.

Presentation of results and statistical analysis

Each set of results presented in the tables is typically representative of two to four experiments with a similar protocol. The results represent the mean of triplicate determinations ± one SEM. Differences between means were considered to be statistically significant if $P < 0{\cdot}05$ by Student's *t*-test.

Table 1. Suppression of Con A-induced PBMC responses by blood forms of *T. cruzi*

Parasite concentration (organisms/ml)	C.p.m. ($\times 10^{-3}$) obtained with Con A at the following concentrations (μg/ml)			
	0	4	8	16
0	7·0±0·1	44·4±1·0	35·2±0·8	23·8±0·3
$2·5 \times 10^6$	6·7±0·2	33·0±0·9*	35·0±0·4	10·1±0·1*
$5·0 \times 10^6$	4·6±0·2	15·4±0·2*	10·1±0·1*	0·5±0·2*
$7·5 \times 10^6$	2·7±0·2	8·7±1·1*	15·1±0·2*	0·7±0·1*

The cultures were incubated for 96 hr and were pulsed with 1 μCi [^{3}H] thymidine during the last 24 hr; the parasites were added at zero time, i.e. immediately after the mitogen.

*$P \leq 0·05$, with respect to the control value (no parasites present).

RESULTS

Suppression of PBMC responses to mitogens by *T. cruzi*

When present in the cultures, purified blood forms of *T. cruzi* suppressed PBMC responses to Con A (Table 1). The concentration of Con A producing optimal responses varied among repeat experiments (data not shown), probably due to the use of PBMC from different donors and different batches of the mitogen. However, significant suppression by *T. cruzi* was observed in all experiments. Although in some experiments a significant reduction of PBMC responses to the tested mitogens was produced with $2·5 \times 10^6$ blood forms/ml, the minimal concentration of parasites causing such effect in most experiments was 5×10^6 organisms/ml and this was used in all subsequent experiments. Of interest is the fact that tissue culture-derived trypomastigotes and epimastigotes grown in an axenic medium also suppressed Con A-induced lymphoproliferative PBMC responses (data not shown).

The suppressive effect of blood trypomastigotes was also seen when either PHA or PWM were used to stimulate the PBMC, and occurred over a wide range of mitogen concentrations, including suboptimal, optimal and supraoptimal doses (Table 2).

Since *T. cruzi* can bind Con A and PHA (Pereira *et al.*, 1980), we considered the possibility that the parasite might have reduced the concentration of these mitogens to suboptimal levels. To test this possibility, PBMC were stimulated with solutions of Con A or PHA that had been either absorbed with 5×10^6 organisms/ml for 24 hr or mock-absorbed without parasites. Absorption of Con A solutions with *T. cruzi* shifted peak responses towards the higher levels (Table 3), corroborating the ability of the parasite to bind this mitogen. However, enough mitogen remained in the solutions, which initially contained 6 or 8 μg Con A/ml to induce optimal PBMC responses. Significant PBMC stimulation was also produced by solutions of PHA after absorption with *T. cruzi*.

Also considered were the possibilities that (i) *T. cruzi* consumed nutrients required for optimal lymphocyte proliferation, and (ii) reduced levels of [^{3}H] thymidine incorporation resulted from a greater loss of PBMC viability due to the presence of *T. cruzi*. A conditioned medium that had been incubated with a suppressive concentration of *T. cruzi* for 96 hr was as effective in supporting [^{3}H] thymidine incorporation by PBMC as mock-absorbed medium (Table 4). When the proportions of trypan-blue-excluding PBMC were determined in Con A-stimulated cultures at the end of the 96-hr incubation period, the values obtained in the absence of *T. cruzi* in repeat experiments were 77–83%, whereas in the presence of the organisms the corresponding values were 72–74%.

We also investigated whether monocytes/macrophages, the accessory cell function of which may have been altered upon their infection by *T. cruzi*, were a requirement for parasite-

Table 2. Suppression of PBMC responses induced with suboptimal, optimal and supraoptimal concentrations of Con A, PHA or PWM by blood forms of *T. cruzi*

Mitogen	Mitogen concentration (μg/ml)	[^{3}H]Thymidine incorporation (c.p.m. $\times 10^{-3}$)	
		Parasites absent	Parasites present
Con A	0	1·4±0·2	1·6±0·4
	0·4	14·4±1·0	1·4±0·1*
	4	45·2±2·9	2·0±0·1*
	8	40·5±1·0	2·9±0·0*
	16	1·9±0·6	1·1±0·1
PHA	0	0·7±0·1	2·2±0·2
	6·3	27·6±1·0	1·3±0·1*
	12·5	18·7±0·2	0·3±0·1*
	25	42·9±1·6	5·7±0·2*
	50	26·8±0·3	2·3±0·3*
PWM	0	0·2±0·0	0·1±0·0
	2·5	3·3±0·4	0·4±0·0†
	5	2·9±0·1	0·9±0·1†
	10	2·8±0·2	1·3±0·1†

Ninety-six-hr cultures were pulsed with 1 μCi [^{3}H] thymidine during the last 24 hr; the parasites were added at zero time. The experiments with Con A, PHA and PWM were conducted separately.

*$P \leq 0·001$ and †$P \leq 0·05$ for the reductions in c.p.m. with respect to the corresponding control value (parasites absent).

Table 3. Mitogenic capacity of Con A solutions before and after absorption with a suppressive concentration of *T. cruzi*

Mitogen	[^{3}H] Thymidine incorporation (c.p.m. $\times 10^{-3}$) after: Mock absorption	*T. cruzi* absorption
None	7·6±0·4	
Con A 4 μg/ml	21·1±2·3	5·1±1·4
Con A 6 μg/ml	6·3±0·3	19·5±1·0
Con A 8 μg/ml	6·5±0·3	17·0±1·0
None	7·7±0·5	
PHA 5 μg/ml	55·5±0·2	50·6±3·3
PHA 7·5 μg/ml	55·9±1·2	45·7±3·6
PHA 10 μg/ml	55·4±1·5	42·4±3·1

The solutions of Con A and PHA were mock-absorbed (same physical treatments, no parasites) or absorbed with 5×10^6 parasites/ml for 24 hr, filtered through 0·22-μm pore-size filters, and then used to stimulate PBMC in 96-hr cultures in the absence of parasites. The cultures were pulsed with 1 μCi [^{3}H] thymidine during the last 24 hr.

Table 4. Ability of RPMI+5%FBS medium to support Con A-induced responses after incubation with *T. cruzi*

Con A (μg/ml)	[^{3}H] Thymidine incorporation (c.p.m. $\times 10^{-3}$) in: Untreated medium	Medium preincubated with parasites
0	3·5±0·1	3·3±0·1
4	24·9±0·6	21·9±0·3
6	31·5±0·9	23·1±0·4
8	31·1±3·9	19·0±0·6

The 96-hr PBMC cultures were performed in the absence of *T. cruzi*. The culture media consisted of filtered (0·22-μm filter) RPMI+5% FBS that had been incubated in the absence ('untreated') or presence of 5×10^6 parasites/ml. Con A was added at zero time. The cultures were pulsed with 1 μCi [^{3}H] thymidine during the last 24 hr.

induced suppression to occur. When PBMC populations, the monocyte/macrophage contents of which had been reduced from 6–9·7% to <0·7%, were stimulated with Con A or PHA in the presence of *T. cruzi*, their responses were still significantly suppressed. Thus, the lymphocyte responses in the presence of medium alone, 8 μg Con A/ml and 25μg PHA/ml were 4002±1618, 29,768±900 and 52,743±1443 c.p.m., respectively, whereas in the presence of 5×10^6 parasites/ml the responses amounted to 3832±1133, 17,248±496 and 15,367±348 c.p.m., respectively.

Table 5. Effects of addition of *T. cruzi* at different times after PBMC stimulation with Con A

Time of addition of *T. cruzi* (hr)	[^{3}H] Thymidine incorporation (c.p.m. $\times 10^{-3}$) PBMC alone	PBMC+*T. cruzi*	(% R)*
0	43·4±1·2	11·2±0·9	(74·2)
24	45·0±2·2	30·0±0·7	(33·3)
48	47·4±1·3	31·1±0·7	(34·4)
72	41·5±0·4	27·7±1·7	(33·3)

Ninety-six-hr cultures, stimulated with 6 μg Con A/ml and pulsed with 1 μCi [^{3}H] thymidne during the last 24 hr.

*% R, percentage reduction in c.p.m. due to the presence of parasites. All % R values represent statistically significant reductions in c.p.m. ($P \leq 0{\cdot}05$).

No suppression was seen when glutaraldehyde-killed blood trypomastigotes were substituted for living organisms in the PBMC cultures (data not shown).

Experiments were then designed to establish the period of time during which the suppressive effect of the parasite was exerted. In these PBMC cultures, trypomastigotes were added at various times after mitogenic stimulation. Maximal suppression was produced when the organisms were present in the cultures from the beginning (zero time), although significant suppression occurred when the parasite was incorporated into the PBMC cultures 24, 48 or 72 hr later (Table 5).

Effect of *T. cruzi* on IL-2 production and utilization

Since IL-2 is produced by stimulated T cells and plays a key role in lymphocyte proliferation, we set out to establish whether *T. cruzi* suppressed PBMC responses by affecting IL-2 production. Levels of IL-2 were measured after 48 hr of PBMC incubation with an optimal dose of PHA in the presence or absence of 5×10^6 blood trypomastigotes per ml. As shown in Table 6, the levels of IL-2 activity found in the filtrates of PHA-stimulated PBMC cultures were significantly smaller when the parasites were present. If *T. cruzi* suppressed mitogen-induced responses by PBMC merely by impairing IL-2 production, exogenous IL-2 should correct the deficiency, as was seen by investigators who studied antibody production to *T. cruzi*-unrelated antigens by lymphocytes from infected mice (Reed *et al.*, 1984a, b; Tarleton & Kuhn, 1983). However, exogenous IL-2_{ph} failed to restore PBMC responsiveness to Con A in experiments in which the concentration of the lymphokine was either 10 units/ml or 114 units/ml (Table 7). These results led us to examine the possibility that the parasites were absorbing IL-2, making it unavailable to the PBMC. Experiments were conducted in which IL-2_{ch} (produced under the same conditions as used to elicit PBMC responses with PHA) was incubated with suppressive concentrations of *T. cruzi*. The results showed that 5×10^6 organisms/ml did not remove significant amounts of IL-2 activity (Table 8). Similar results were obtained when the parasite concentration was increased up to four-fold (data not shown).

DISCUSSION

Our work produced results showing that living but not killed *T.*

Table 6. Reduced production of IL-2 by PBMC incubated with *T. cruzi*

Material tested	[^{3}H] Thymidine incorporation (c.p.m $\times 10^{-3}$) by HT-2 cells grown in the presence of the material tested diluted:				
	Undiluted	1:2	1:4	1:8	1:16
PHA	1·3±0·3	2·6±0·0	1·5±0·2	2·3±0·6	2·3±0·4
PBMC+PHA	28·1±1·0	17·6±2·0	7·3±1·0	3·7±0·5	2·2±0·2
PBMC+PHA+*T. cruzi*	2·9±0·7*	2·4±0·8†	1·3±0·3†	2·1±0·4	0·9±0·0†

The tested materials consisted of 48-hr supernatants of PBMC cultures (5×10^6 PBMC/ml) stimulated with 25μg PHA/ml in the absence (PBMC+PHA) or presence (PBMC+PHA+*T. cruzi*) of 5×10^6 parasites/ml. The HT-2 cell cultures were incubated with these solutions for 48 hr. The cultures were pulsed with 1 μCi [^{3}H] thymidine during the last 24 hr. 'PHA' was a solution of the mitogen incubated without cells for 48 hr.

*$P \leq 0{\cdot}001$ and †$P \leq 0{\cdot}05$: difference with respect to the value obtained with PBMC+PHA.

Table 7. Failure of exogenous IL-2_{ph} to restore Con A responsiveness of PBMC

Exp. no.	Con A (μg/ml)	IL-2 (units/ml)	[^{3}H] Thymidine incorporation (c.p.m. $\times 10^{-3}$) by PBMC in the presence of:			
			Con A	Con A+IL-2_{ph}‡	Con A+*T. cruzi*	Con A+*T. cruzi*+IL-2_{ph}
1	0	10	1·4±0·2	0·7±0·1	1·6±0·4	2·1±0·3
	4	10	45·2±2·9	39·5±5·3	2·0±0·1†	1·8±0·9†
	8	10	40·5±1·0	25·5±4·8	2·9±0·1†	2·4±0·8†
2	0	114	0·5±0·3	5·2±0·1	0·5±0·2	1·1±0·2
	4	114	22·4±2·1	26·2±7·7	0·7±0·1*	1·1±0·2*
	8	114	37·9±5·1	NA§	1·6±0·5*	0·7±0·1*

*$P \leq 0{\cdot}001$ and †$P \leq 0{\cdot}05$: difference with respect to the value obtained with Con A alone or Con A+IL-2_{ph}.

‡IL-2_{ph} was present during the 96-hr culture period.

§NA, datum not available.

Table 8. *T. cruzi* does not absorb or consume IL-2

IL-2_{ch} dilution	[^{3}H] Thymidine incorporation (c.p.m. $\times 10^{-3}$) by HT-2 grown in the presence of:			
	IL-2 before absorption	IL-2 after absorption with *T. cruzi* at:		
		$1{\cdot}25 \times 10^6$ (organisms/ml)	$2{\cdot}5 \times 10^6$ (organisms/ml)	5×10^6 (organisms/ml)
1/16	44·9±2·5	42·8±0·7	44·1±0·6	40·1±2·4
1/32	27·3±2·8	23·2±2·3	25·9±0·2	22·5±1·9
1/64	12·0±2·0	10·1+0·3	11·9±0·7	10·8±0·9

Aliquots of the IL-2_{ch} preparation were incubated with *T. cruzi* for 48 hr and filtered through 0·22-μm pore-size filters before use. The IL-2 assay was as described for Table 6.

cruzi can suppress human lymphocyte responses to a variety of mitogens, and established experimental conditions to explore the possible mechanisms(s).

Although *T. cruzi* is known to absorb Con A and PHA (Pereira *et al.*, 1980), two independent findings indicated that the parasite did not reduce PBMC responses by making less mitogen available to them. First, the suppressive effect of *T. cruzi* was seen over a wide range of mitogen concentrations, including supraoptimal levels (Table 2), minimizing the possibility that mitogen absorption solely accounted for the reduced responses. Second, optimal stimulatory levels of Con A and PHA remained in solutions of these mitogens after absorption with 5×10^6 parasites/ml (Table 3). We could also rule out the possibility that *T. cruzi* competed with the PBMC for essential nutrients because culture medium incubated with a suppressive parasite concentration supported blastogenesis as well as mock-treated medium (Table 4).

Since parasite-induced suppression was observed with PBMC preparations before and after depletion of non-specific esterase-positive cells, which include monocytes and macrophages, it would appear that the parasite could directly affect lymphoid cells. However, the possibility that diminished accessory cell activity resulting from monocyte/macrophage infection contributes to the noted suppression cannot be ruled out.

The extent of suppression of Con A-induced PBMC responses was greater when the parasites were incorporated into the cultures at zero time (i.e. together with the mitogen) than when added after 24, 48 or 72 hr (Table 5). These observations suggested that *T. cruzi* interfered with the early stages of lymphocyte activation, and that cells that had undergone activation in the absence of the parasites were less or no longer susceptible to the suppressive effect. However, the reduced PBMC responses seen when these cells were mixed with the trypomastigotes 24, 48 or 72 hr after initiation of the cultures were statistically significant, and probably reflected a suppressive effect of the parasites on lymphocytes activated by the mitogen at times later than zero time, including a second generation of cells responding to the mitogen.

We found lower levels of IL-2 activity in the supernatants of PHA-stimulated PBMC when *T. cruzi* was present (Table 6). Reduced IL-2 activity has also been reported by investigators studying suppressed *in vitro* antibody production by lymphocytes from *T. cruzi*-infected mice (Reed *et al.*, 1984a, b; Tarleton & Kuhn, 1983). However, we were unable to restore PBMC responses to Con A when exogenous $IL\text{-}2_{ph}$ was added, even at a relatively high concentration (114 units/ml) (Table 7). In this respect, our results with human PBMC would seem to differ from those obtained by others with spleen cells from *T. cruzi*-infected mice (Reed *et al.*, 1984a, b; Tarleton & Kuhn, 1983), with which a certain degree of restoration was afforded by adding IL-2. This apparent disagreement might be rooted in differences between species, or might be due to the use of different assay systems: the induction of antibody-forming cells by antigens administered to infected hosts was measured in the work with the mouse cells (Reed *et al.*, 1984a, b; Tarleton & Kuhn, 1983) whereas our assay measured proliferative responses of normal human lymphocytes to mitogens. It should be noted, however, that Harel-Bellan *et al.* (1983), who used *T. cruzi*-infected mouse spleen cells to measure Con A-induced lymphoproliferative responses, could not restore the responsiveness of these cells with exogenous IL-2. Whether IL-2 can correct some but not all types of lymphocyte alterations caused by *T. cruzi* infection or by the addition of this parasite to cell cultures is an interestng question deserving further attention. The failure of exogenous IL-2 to restore the lymphoproliferative capacity of the PBMC might have been due to (i) absorption or consumption of IL-2 by *T. cruzi*, (ii) an irreversible PBMC alteration, (iii) a reduced capacity of PBMC to bind or respond to IL-2, and/or (iv) a need for additional cytokines, the production of which might have also been altered. Since absorption with up to 2×10^7 parasites/ml did not remove significant amounts of IL-2 activity, removal of this lymphokine by *T. cruzi* seemed unlikely. On this basis, reduced levels of IL-2 activity in PHA-stimulated PBMC cultures containing the parasite would probably be due to reduced production. The other three possibilities remain open subjects for further studies.

ACKNOWLEDGMENT

This work was supported by grant AI-14848 from the United States Public Health Service.

REFERENCES

Brener Z. (1980) Immunity to *Trypanosoma cruzi*. *Adv. Parasitol.* **18,** 247.

Budzko D.B. & Kierszenbaum F. (1974) Isolation of *Trypanosoma cruzi* from blood. *J. Parasitol.* **60,** 1037.

Clinton B.A., Ortiz-Ortiz L., Garcia W., Martinez T. & Capin R. (1975) *Trypanosoma cruzi*: early immune response in infected mice. *Exp. Parasitol.* **37,** 417.

Cunningham D.S. & Kuhn R.E. (1980) *Trypanosoma cruzi*-induced suppression of the primary immune response in murine cell cultures to T cell-dependent and -independent antigens. *J. Parasitol.* **66,** 16.

Harel-Bellan A., Joskowicz M., Fradelizi D. & Eisen H. (1983) Modification of T cell proliferation and interleukin 2 production in mice infected with *Trypanosoma cruzi*. *Proc. natl. Acad. Sci. U.S.A.* **80,** 3466.

Hayes M.M. & Kierszenbaum F. (1981) Experimental Chagas' disease: kinetics of lymphocyte responses and immunological control of the transition from acute to chronic *Trypanosoma cruzi* infection. *Infect. Immun.* **31,** 1117.

Lima M.F. & Kierszenbaum F. (1982) Biochemical requirements for intracellular invasion by *Trypanosoma cruzi*: protein synthesis. *J. Protozool.* **29,** 566.

Kierszenbaum F. (1982) Immunologic deficiency during experimental Chagas' disease (*Trypanosoma cruzi* infection): role of adherent, nonspecific esterase-positive splenic cells. *J. Immunol.* **129,** 2202.

Kuhn R.E. (1981) Immunology of *Trypanosoma cruzi* infections. In: *Parasitic Diseases* (ed. J. M. Mansfield), Vol. 1, p. 137. Marcel Dekker, New York.

Maleckar J.R. & Kierszenbaum F. (1983) Inhibition of mitogen-induced proliferation of mouse T and B lymphocytes by bloodstream forms of *Trypanosoma cruzi*. *J. Immunol.* **130,** 908.

Mishell B.B., Mishell R.I. & Shigii J.M. (1980) Adherence. In: *Selected Methods in Cellular Immunology* (eds B. B. Mishell and S. M. Shiigi), p. 175. Freeman, San Francisco.

Pereira M.E.A., Loures M.A., Villalta F. & Andrade A.F.B. (1980) Lectin receptors as markers for *Trypanosoma cruzi*. Developmental stages and a study of the interaction of wheat germ agglutinin with sialic acid residues on epimastigote cells. *J. exp. Med.* **152,** 1375.

Ramos C., Schädtler-Siwon I. & Ortiz-Ortiz L. (1979) Suppressor cells present in the spleens of *Trypanosoma cruzi*-infected mice. *J. Immunol.* **122,** 1243.

Reed S.G., Inverso J.A. & Roters S.B. (1984a) Heterologous antibody responses in mice with chronic *T. cruzi* infection: depressed T helper

function restored with supernatants containing interleukin 2. *J. Immunol.* **133,** 1558.

REED S.G., INVERSO J.A. & ROTERS S.B. (1984b) Suppressed antibody responses to sheep erythrocytes in mice with chronic *Trypanosoma cruzi* infections are restored with interleukin 2. *J. Immunol.* **133,** 3333.

TARLETON R.L. & KUHN R.E. (1983) Restoration of *in vitro* immune responses of spleen cells from mice infected with *Trypanosoma cruzi* by supernatants containing interleukin 2. *J. Immunol.* **133,** 1570.

TEIXEIRA A.R.L., TEIXEIRA G., MACEDO V. & PRATA A. (1978) Acquired cell-mediated immunodepression in acute Chagas' disease. *J. clin. Invest.* **62,** 1132.

TILDEN A.B. & BALCH C.M. (1982) A comparison of PGE_2 effects on human suppressor cell function and on interleukin 2 function. *J. Immunol.* **129,** 2469.

VILLALTA F. & LEON W. (1979) Effect of purification by DEAE-cellulose column on infectivity of *Trypanosoma cruzi* blood forms. *J. Parasitol.* **65,** 88.

WARREN L.G. (1960) Metabolism of *Schizotrypanum cruzi* Chagas. I. Effects of culture age and substrate concentration on respiratory rate. *J. Parasitol.* **46,** 529.

YAM L.T., LI C.Y. & CROSBY W.H. (1971) Cytochemical identification of monocytes and granulocytes. *Am. J. clin. Pathol.* **55,** 283.