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Suppression by *Trypanosoma brucei rhodesiense* of the Capacities of Human T Lymphocytes To Express Interleukin-2 Receptors and Proliferate after Mitogenic Stimulation

FELIPE KIERSZENBAUM,[1]* SUBRAMANIAN MUTHUKKUMAR,[1] LISA A. BELTZ,[1]†
AND MARCELO B. SZTEIN[2]

Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan 48824,[1] and Center for Vaccine Development, Department of Pediatrics, University of Maryland School of Medicine, Baltimore, Maryland 21201[2]

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We studied the suppressive effects induced in phytohemagglutinin (PHA)-stimulated human peripheral blood mononuclear cells (PBMC) by purified blood forms of *Trypanosoma brucei rhodesiense*. The parasite was found to markedly impair lymphocyte proliferation (measured in terms of [^{3}H]thymidine incorporation). The extent of this effect increased with parasite concentration and was not due to mitogen absorption, depletion of medium nutrients, or PBMC killing by the parasite. Significant reductions in interleukin-2 receptor (IL-2R) expression, determined by flow cytometric analysis, were also observed in PHA-stimulated PBMC cultured in the presence of *T. b. rhodesiense* as evidenced by marked decreases in the surface density of the receptor. Concomitant decreases in the percentage of IL-2R$^+$ cells were recorded in approximately half of the experiments. A discrete, dimly stained subpopulation of IL-2R$^+$ cells was consistently demonstrable whether or not a reduction in the percentage of IL-2R$^+$ cells occurred. Living, but not glutaraldehyde-fixed, parasites suppressed IL-2R expression. In kinetic studies, a low but reproducible level of suppression of IL-2R was demonstrable as early as 6 h after PHA stimulation; the extent of this effect became considerably more pronounced as additional culture time elapsed. Levels of IL-2 biological activity in cocultures of *T. b. rhodesiense* with PHA-stimulated PBMC were comparable with or higher than those present in control cultures lacking the parasite. Therefore, insufficient levels of this cytokine would be an unlikely explanation for the noted suppression of IL-2R expression and lymphoproliferation. These effects of *T. b. rhodesiense* could represent an important component of the mechanism by which immunosuppression develops in African sleeping sickness.

Infections caused by African trypanosomes of the *brucei* group are accompanied by multiple manifestations of immunosuppression which have been extensively documented in terms of alterations in T- and B-cell responses to antigens and mitogens (1, 2, 7, 9, 10, 16, 18–20). Recent reports have also identified diminished interleukin-2 receptor (IL-2R) expression by lymphocytes from mice infected with *Trypanosoma brucei brucei* (18, 19). Whereas all of these immunological abnormalities observed in infected mammalian hosts attest to the wide-ranging suppressive effects of African trypanosomes, very little, if anything, is known about the mechanism(s) that lead to their occurrence. In our initial approach to this subject we looked into whether African trypanosomes can affect the functions of normal lymphocytes and made an attempt to define the underlying mechanisms. To this end, we used an in vitro system recently used with success to examine aspects of the immunosuppression induced by *Trypanosoma cruzi* (3, 4, 11, 12). In this work, we studied the ability of *Trypanosoma brucei rhodesiense* to affect the capacity of human lymphocytes to proliferate, express IL-2R, and produce IL-2 after mitogenic stimulation. We will show herein that the parasite markedly curtails IL-2R expression and lymphoproliferation by human phytohemagglutinin (PHA)-activated peripheral blood mononuclear cells (PBMC) despite the presence of sufficient levels of IL-2 activity in the system.

* Corresponding author.
† Present address: Oncology Center, The Johns Hopkins University, Baltimore, MD 21205.

MATERIALS AND METHODS

Parasites. The KETRI-2285 isolate of *T. b. rhodesiense* used in this work, originally from the Kenya Trypanosome Research Institute, was kindly provided by Allen B. Clarkson, New York University. The organisms were initially stored under liquid nitrogen and then maintained by serial intraperitoneal passages in Crl-CD1(ICR) Swiss mice (Charles River Laboratories, Portage, Mich.). The flagellates were purified from the blood of mice infected intraperitoneally 2 to 3 days previously with 2×10^5 to 5×10^5 organisms by chromatography through diethylaminoethyl-cellulose (13) using a buffer containing 103 mM Tris (pH 7.4), 1.5% glucose, and 58 mM NaCl for column equilibration and elution. The recovered flagellates were washed twice by centrifugation with RPMI 1640 medium (GIBCO, Grand Island, N.Y.) containing 10% heat-inactivated (56°C, 20 min) fetal bovine serum (Sigma Chemical Co., St. Louis, Mo.), 100 IU of penicillin per ml, and 100 μg of streptomycin per ml. This medium will be referred to in the text as complete medium. Parasite concentrations were determined microscopically, using a hemacytometer, and adjusted to the desired level in the same medium. The suspensions consisted of 100% trypomastigotes, >99.9% motile.

Killed parasites. *T. b. rhodesiense* parasites were killed by suspending them at 2×10^7 organisms per ml in 0.025% glutaraldehyde in phosphate-buffered saline (PBS) and incubation at 4°C for 15 min. After being washed with cold PBS, the fixed trypanosomes were resuspended in 0.1 M lysine (4°C, 5 min). The parasites were then washed twice with and resuspended at 5×10^7 organisms per ml in complete

medium. Glutaraldehyde-treated *T. b. rhodesiense* was stored at 4°C until use.

Preparation of PBMC. Blood was obtained from healthy volunteers. The PBMC were purified by centrifugation through a mixture of Ficoll-Hypaque of density 1.077. After two washings with complete medium, the PBMC were resuspended in the same medium, counted, and adjusted to the desired concentration. Cell viability, determined by trypan blue dye exclusion, was invariably >99%.

Lymphoproliferation assays. Cultures of PBMC in 96-well plates (100 μl per well; 1.25×10^6 cells per ml) were incubated at 37°C and 5% CO_2 in the presence or absence of PHA (Sigma), with or without *T. b. rhodesiense*, for various periods of time, and pulsed with 1 μCi of [^{3}H]thymidine ([^{3}H]TdR; specific activity = 2 Ci/mmol; Amersham, Arlington Heights, Ill.) per well. The concentrations of parasites and the lengths of the [^{3}H]TdR pulses are described in the Results section. Unless otherwise noted, PHA was used at 5 μg/ml. All cultures were terminated by automated harvesting and processed for liquid scintillation counting. Each condition was tested in triplicate or quadruplicate. The statistical significance of differences in levels of [^{3}H]TdR incorporation was determined by Student's *t* test.

Flow cytometric determinations. Cultures of PBMC in 24-well plates (1 ml per well at 1.25×10^6 cells per ml) were incubated at 37°C and 5% CO_2 in the presence or absence of PHA (5 μg/ml) with or without *T. b. rhodesiense*. The cells were washed with PBS containing 1% bovine serum albumin and 0.1% sodium azide (PBS + BSA + azide) and were incubated with fluorescein (FITC)-labeled anti-IL-2R monoclonal antibody (anti-CD25, immunoglobulin G1 (IgG1) specific for an epitope of the p55 chain; Becton Dickinson, San Jose, Calif.) for 30 min at 4°C, followed by two washings with PBS + BSA + azide. Cells stained with FITC-labeled normal mouse IgG1 were used as controls for background fluorescence. The stained cells were fixed in 1% formaldehyde and stored at 4°C in the dark until analyzed by using a FACScan flow cytometer (Becton Dickinson). A minimum of 10,000 cells, gated on forward versus 90° light scatter to exclude erythrocytes, platelets, nonviable cells, and *T. b. rhodesiense*, were accumulated for each histogram. There was no detectable binding of FITC-anti-IL-2R monoclonal antibody to *T. b. rhodesiense*. The percentage of positive cells was estimated against a background of cells stained with FITC-normal mouse IgG1. Mean channel numbers of the logarithm of fluorescence intensities of the positive cell populations (MFCh) were used to compare the relative density of the relevant lymphocyte marker in the presence or absence of *T. b. rhodesiense*. The logarithm of fluorescence intensities was distributed over four decades.

Determination of IL-2. The IL-2-dependent CTLL-2 cell line was used to determine the biological activity of IL-2. The conditions for growing these cells and performing the assay have been described in detail (4, 14). Results were expressed as units of IL-2 per milliliter with reference to a laboratory IL-2 standard arbitrarily assigned a value of 1,000 U of IL-2/ml. This standard was a concanavalin A-stimulated rat splenocyte culture supernatant. Whether *T. b. rhodesiense* was capable of absorbing or consuming IL-2 was established by measuring residual levels of this cytokine in filtrates (0.45-μm pore size) of solutions of recombinant human IL-2 (rhIL-2; specific activity = 1.6×10^7 IU/mg; a kind gift from Peter Sorter, Hoffmann-LaRoche, Nutley, N.J.) incubated at 37°C for 13 h with 1×10^7 or 2×10^7 parasites per ml. For this purpose, we used a commercially available kit (Intertest 2; Genzyme, Boston, Mass.), which was also used to measure IL-2 antigen in the culture supernatants of some experiments, in terms of manufacturer's defined units.

Absorption of complete medium and PHA solutions with *T. b. rhodesiense*. Solutions of PHA (concentrations described under Results) in complete medium were absorbed at 37°C and 5% CO_2 with 2×10^7 *T. b. rhodesiense* parasites per ml for 13 h or with 1×10^7 *T. b. rhodesiense* parasites per ml for 40 h. The parasites were then removed by filtration through sterile 0.45-μm-pore-size filters. The filtrates were used in lymphoproliferation assays as the source of PHA. For control purposes, parallel mock absorptions and filtrations of complete medium were performed under the same conditions in the absence of trypanosomes.

RESULTS AND DISCUSSION

Suppressive effects of *T. b. rhodesiense* on PHA-stimulated human PBMC. In the presence of *T. b. rhodesiense*, PHA-activated PBMC displayed a markedly decreased capacity to express IL-2R (Fig. 1). This was denoted by significant reductions in both the percentage of IL-2R-positive cells and shifts to the left of the logarithm of fluorescence intensity, representing the density of expression of the marker on the cell surface (a typical example is shown in Fig. 1, left panel). Whereas both parameters were found to be decreased in approximately half of the experiments, only reduced fluorescence intensity was observed in the other half (a typical example is shown in Fig. 1, right panel). It should be noted that whether or not a reduction in the percentage of IL-2R$^+$ occurred, the appearance of a distinct, dimly fluorescent cell subpopulation was readily demonstrable. It has been shown that among PHA-stimulated PBMC, those expressing the lower numbers of IL-2R are less responsive to IL-2 than those whose surface IL-2R density is higher (5). Viewed against this background, our finding suggests that *T. b. rhodesiense* might suppress the immune system, at least in part, by reducing the capacity of a significant proportion of stimulated cells to express IL-2R at surface levels commensurate with optimal responses.

Unstimulated lymphocytes do not express IL-2R, but we did look into whether *T. b. rhodesiense* would induce expression of this receptor by PBMC in the absence of PHA; no evidence for such an effect could be found (data not shown).

Suppression by *T. b. rhodesiense* was also demonstrable in terms of significantly reduced lymphoproliferation ($P < 0.05$, Student's *t* test), monitored by [^{3}H]TdR uptake, and was dependent on the parasite concentration (Fig. 2). This was also the case when the effect was determined in terms of IL-2R expression (Table 1). Because significant suppression was consistently observed with concentrations $\geq 1 \times 10^7$ parasites per ml, this or greater levels (depending on parasite availability) were used in subsequent experiments.

To establish whether suppression resulted from consumption of either essential medium nutrients or mitogen by *T. b. rhodesiense* we incubated solutions of PHA in complete medium with suppressive concentrations of the parasite. The results depicted in Fig. 3 showed that [^{3}H]TdR incorporation measured from 48 to 72 h in cultures set up with absorbed medium or PHA solutions was not significantly different from that recorded with cultures in which mock-absorbed reagents had been used instead. Although the data presented in Fig. 3 were derived from an experiment in which 13-h absorptions were performed, similar results were obtained with supernatants from 40-h absorptions (data not shown).

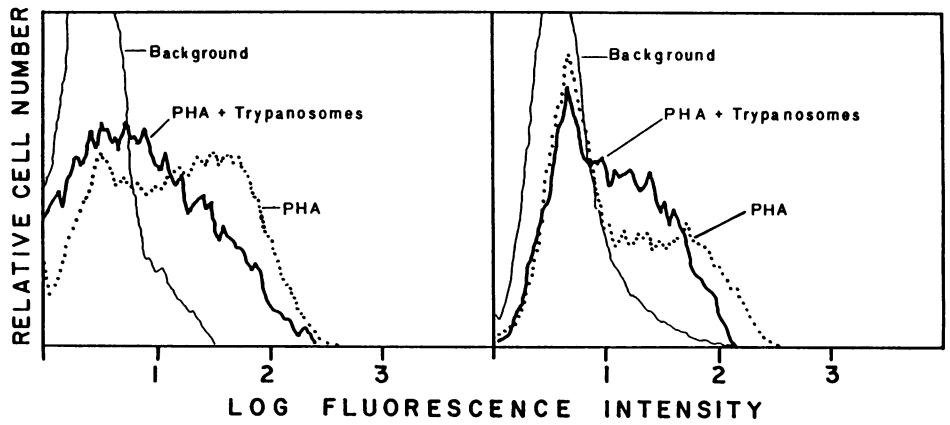

FIG. 1. Suppression by *T. b. rhodesiense* of the expression of IL-2R by PHA-stimulated PBMC. Where added, parasites were present at a final concentration of 10^7 organisms per ml. The cells were stained for flow cytometric analysis of IL-2R expression. These sets of results were obtained after 13 h of culture. Left panel: experiment in which both the percentage of IL-$2R^+$ cells and the mean fluorescence intensity (MFCh) of the positive cell population were markedly reduced by the presence of parasites; the percentages of IL-$2R^+$ cells (and MFCh values) were 49.3% (56) and 39.4% (40) in the absence and presence of trypanosomes, respectively. Right panel: experiment in which *T. b. rhodesiense* caused a marked reduction in MFCh without a significant change in the percentage of IL-$2R^+$ cells; the percentages of IL-$2R^+$ cells (and MFCh values) were 44.1% (62) and 46.3% (48) in the absence and presence of parasites, respectively. These experiments were performed separately with cells from different donors, and each set of results is representative of at least two repeat experiments performed with cells from different donors. The MFCh parameter is a logarithmic function; it represents the mean channel number of the logarithm of the fluorescence intensity of a stained cell population.

Therefore, the noted suppression was not likely the result of nutrient or PHA consumption or absorption.

Any cold thymidine released from *T. b. rhodesiense* disintegrating during the culture period would be expected to compete with hot thymidine for incorporation into newly synthesized DNA, artifactually reducing counts per minute. However, IL-2R expression, which represented an independent parameter of suppression and could not have been affected by cold thymidine, was also decreased. Moreover, we have recently found that the level of reduction of [^{3}H]TdR uptake subsides significantly within 48 to 72 h after all of the organisms added to the culture disintegrate (22). This reversibility could not have taken place if excessive amounts of cold thymidine had been present. It is noteworthy that supernatants of *T. b. rhodesiense* suspensions, free of organisms and dialyzed versus fresh medium, also suppressed IL-2R expression by PHA-stimulated human PBMC (22), indicating further that the effect did not result from parasite consumption of essential nutrients.

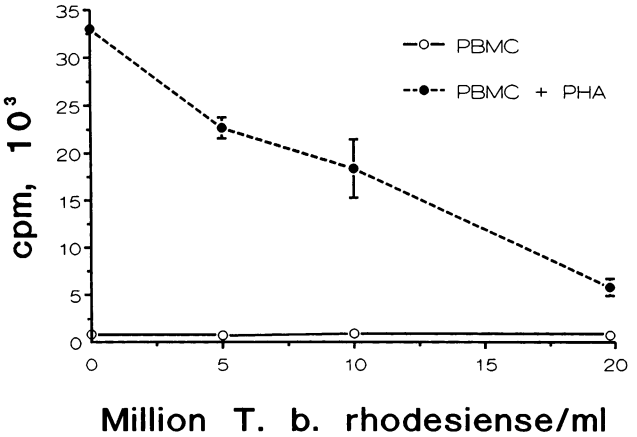

FIG. 2. Levels of suppression of PHA-stimulated PBMC by increasing concentrations of *T. b. rhodesiense*. Cultures of PBMC containing the indicated concentration of parasites were incubated at 37°C and 5% CO_2 for 72 h, and pulsed with 1 μCi of [^{3}H]TdR during the last 24 h. Points and vertical bars represent the means of quadruplicate values and their standard deviation. Standard deviations for the PBMC alone were too small to exceed the size of the points. This set of results is typically representative of three separate repeat experiments performed with cells from different donors.

Several additional types of controls and tests were set up to find out if parasite-induced suppression was an artifact. Binding of fluorescein-labeled anti-IL-2R monoclonal antibody to *T. b. rhodesiense* was not demonstrable by flow cytometry (data not shown). We also tested the possibility that *T. b. rhodesiense* might have suppressed PBMC responses to PHA via absorption or consumption of endogenously produced IL-2. However, levels of IL-2 in solutions which had been incubated with or without 1×10^7 to 2×10^7 parasites per ml were found to be comparable (data not shown).

Effect of *T. b. rhodesiense* on IL-2 secretion by PHA-

TABLE 1. Effect of *T. b. rhodesiense* on IL-2R expression by PHA-stimulated PBMC[a]

Experimental condition	% IL-$2R^+$ cells	MFCh[b]
PBMC alone	10.9	34
PBMC + PHA	57.1	63
PBMC + PHA + 5×10^6 parasites/ml	56.9	57
PBMC + PHA + 1×10^7 parasites/ml	45.2	38

[a] PBMC cultures with or without 5 μg of PHA per ml, in the absence or presence of the indicated *T. b. rhodesiense* concentration, were incubated at 37°C for 16 h, at which time the cells were harvested and stained for flow cytometric analysis of IL-2R. This set of data is representative of three separate experiments performed with cells from different donors.

[b] MFCh is a logarithmic function representing the mean channel number of the logarithm of the fluorescence intensity of the stained cell population.

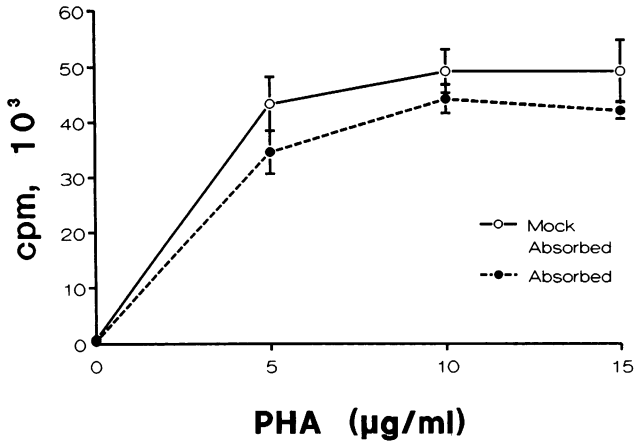

FIG. 3. Mitogenic capacity of PHA solutions before and after absorption with *T. b. rhodesiense*. Complete medium alone or containing the indicated PHA concentrations was incubated without or with 2×10^7 parasites per ml at 37°C for 13 h, filtered (0.45-μm pore size), and used as the culture medium in 96-h lymphocyte proliferation assays. The cultures were pulsed with 1 μCi of [^{3}H]TdR during the last 24 h. Points and vertical bars represent the means of quadruplicate values and their standard deviations. This set of results is representative of two separate experiments with identical design, using cells from different donors.

stimulated PBMC. Because IL-2 upregulates IL-2R expression (8, 15, 17, 21), we tested the possibility that suppression might have resulted from reduced IL-2 production by the mitogen-stimulated PBMC. Culture supernatants were collected at various times after activation and assayed for IL-2 antigen or biological activity. Determination of IL-2 biological activity in culture filtrates collected at 24, 48, and 72 h after PHA stimulation revealed normal or higher-than-normal levels of IL-2 in parasite-containing cultures (Table 2). No IL-2-like activity was detectable in the filtrates of suspensions of *T. b. rhodesiense* alone in the culture medium. Moreover, in four separate experiments in which IL-2 antigen was determined by using an enzyme-linked immunoassay we found increased rather than decreased levels of this cytokine in the cultures which contained parasites (data not shown). Thus, it appears unlikely that insufficient IL-2 could have caused the noted suppressive effects. In this context, it is noteworthy that we have also observed that *T. b. rhodesiense* inhibits the ability of PHA-stimulated PBMC to traverse through the cell cycle and that this inhibitory effect could not be reversed by the addition of exogenous IL-2 (22). At the present time, it is unclear whether the observed increase in IL-2 activity is due to enhanced cytokine production, to IL-2 accumulation due to reduced utilization because of decreased IL-2R expression, or to a combination of both. Whether IL-2 present in the culture medium influences mononuclear cells other than T cells which, in turn, could downregulate PHA-stimulated T cells is a possibility deserving attention.

TABLE 2. Effects of *T. b. rhodesiense* on IL-2 production by PHA-stimulated PBMC[a]

Experimental condition	Time (h)	IL-2 (U/ml)	
		Expt 1	Expt 2
PBMC	24	5	8
PBMC + PHA	24	843	341
PBMC + PHA + *T. b. rhodesiense*	24	1,863	306[b]
PBMC	48	12	10
PBMC + PHA	48	320	42
PBMC + PHA + *T. b. rhodesiense*	48	1,779	151
PBMC	72	15	16
PBMC + PHA	72	6	13
PBMC + PHA + *T. b. rhodesiense*	72	126	38

[a] Supernatants from PHA-stimulated PBMC cultures lacking or containing 2×10^7 parasites per ml were collected at the indicated periods of culture and assayed for IL-2 biological activity. Experiments 1 and 2 were performed separately, using cells from different donors.

[b] This IL-2 reduction was the only one ever seen in a parasite-containing culture, represents a decrease of less than 10% with respect to the positive control value (341 U/ml), and is not significant.

Lymph node cells from mice infected with *T. b. brucei* have been reported to display not only a reduced IL-2R expression capacity but also a diminished ability to produce IL-2 after concanavalin A stimulation (19). This reduced IL-2 production would appear to conflict with the results obtained in our studies with human PBMC exposed to *T. b. rhodesiense* in vitro. Whereas the use of different parasites, lymphoid cells, mitogens, and assay conditions might account for the apparent discrepancy, it is worth noting that another trypanosome, *Trypanosoma cruzi*, has been shown to reduce IL-2 production by mouse (4, 6) but not human (4) lymphocytes.

We also measured viable PBMC concentrations in all cultures at various times and found them to be similar whether the parasite was present or not. For example, in a typically representative experiment originally set up to establish the kinetics of suppressed IL-2R expression the concentrations of viable (trypan blue-excluding) PBMC at 6, 12, and 20 h fluctuated between 1.25×10^6 and 1.3×10^6 PBMC per ml in complete medium, between 1.1×10^6 and 1.3×10^6 PBMC per ml in the presence of PHA, and between 1.0×10^6 and 1.4×10^6 PBMC per ml in the presence of both PHA and *T. b. rhodesiense*. These values compared closely with the initial PBMC concentration (1.25×10^6 viable PBMC per ml). The concentration of live parasites was also determined and found to decrease with time, from 2×10^7 organisms per ml at zero time to 0.5×10^6, 2.3×10^5, and 2×10^4 at 6, 12, and 20 h, respectively. Consequently, greater numbers of dead PBMC in the presence of *T. b. rhodesiense* could not explain decreased [^{3}H]TdR uptake.

PBMC were cocultured with live or glutaraldehyde-fixed *T. b. rhodesiense* to establish whether parasite viability was a requirement for immunosuppression to occur. Living but not dead trypanosomes were able to reduce IL-2R expression by PHA-activated PBMC (data not shown). Thus, parasite viability appears to be an absolute requirement for its immunosuppressive activity.

Kinetics of *T. b. rhodesiense*-induced immunosuppression. Significant suppression of IL-2R expression by *T. b. rhodesiense* was detectable as early as 6 h after PHA stimulation and remained demonstrable during the remainder of the 20-h observation period (Table 3). In the experiment described in Table 3, decreases in both the percentage of IL-2R-positive cells and the density of IL-2R expression were recorded.

Because in our experiments the concentration of viable *T. b. rhodesiense* decreased gradually to very low levels within 15 to 20 h, the suppressive effect was probably induced within the very early hours after lymphocyte activation. Support for this notion was provided by the results of kinetic studies demonstrating suppression as early as 6 h after mitogen stimulation (Table 3). Thus, whereas until

TABLE 3. Kinetics of *T. b. rhodesiense*-induced suppression of PHA-stimulated PBMC[a]

Experimental condition	Time (h)	% IL-2R$^+$ cells	MFCh
PBMC + PHA	6	18.2	41
PBMC + PHA + *T. b. rhodesiense*	6	6.1	39
PBMC + PHA	12	29.9	85
PBMC + PHA + *T. b. rhodesiense*	12	16.9	69
PBMC + PHA	16	36.9	126
PBMC + PHA + *T. b. rhodesiense*	16	21.4	98
PBMC + PHA	20	39.1	143
PBMC + PHA + *T. b. rhodesiense*	20	30.4	102

[a] PHA-stimulated PBMC were cultured at 37°C without or with 10^7 *T. b. rhodesiense* parasites per ml for the indicated amounts of time and stained for flow cytometric analysis of IL-2R expression.

recently most studies on the mechanisms of *T. b. rhodesiense*-induced immunosuppression centered on the immunological alterations occurring in mammalian hosts, our results provide a foundation for focusing on parasite-targeted molecular events taking place shortly after lymphocyte activation. These events may include critical changes in calcium fluxes, phosphatidyl inositol turnover, cell membrane potential, IL-2R gene transcription, IL-2R messenger RNA stability or translation, receptor protein transport to the lymphocyte membrane, and/or increased IL-2R shedding. Some of these possibilities are receiving attention in our laboratories.

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REFERENCES

1. **Bakhiet, M., T. Olsson, P. Van der Meide, and K. Kristensson.** 1990. Depletion of CD88+ T cells suppress growth of *Trypanosoma brucei brucei* and interferon gamma production in infected rats. Clin. Exp. Immunol. **81**:195–199.
2. **Bancroft, G. J., C. J. Sutton, A. G. Morris, and B. A. Askonas.** 1983. Production of interferons during African trypanosomiasis. Clin. Exp. Immunol. **52**:135–143.
3. **Beltz, L. A., F. Kierszenbaum, and M. B. Sztein.** 1989. Selective suppressive effects of *Trypanosoma cruzi* on activated human lymphocytes. Infect. Immun. **57**:2301–2305.
4. **Beltz, L. A., M. B. Sztein, and F. Kierszenbaum.** 1988. Novel mechanism for *Trypanosoma cruzi*-induced suppression of human lymphocytes. Inhibition of IL-2 receptor expression. J. Immunol. **141**:289–294.
5. **Cantrell, D. A., and K. A. Smith.** 1983. Transient expression of interleukin 2 receptors. Consequences for T cell growth. J. Exp. Med. **158**:1895–1911.
6. **Choromanski, L., and R. E. Kuhn.** 1985. Interleukin 2 enhances specific and nonspecific immune responses in experimental Chagas' disease. Infect. Immun. **50**:354–357.
7. **Corsini, A. C., C. E. Clayton, B. A. Askonas, and B. M. Ogilvie.** 1977. Suppressor cells and loss of B cell potential in mice infected with *Trypanosoma brucei*. Clin. Exp. Immunol. **29**: 122–131.
8. **Depper, J. M., W. J. Leonard, C. Drogula, M. Kronke, T. A. Waldmann, and W. C. Greene.** 1985. Interleukin 2 (IL-2) augments transcription of the IL-2 receptor gene. Proc. Natl. Acad. Sci. USA **82**:4230–4234.
9. **Hudson, K. M., C. Byner, J. Freeman, and R. J. Terry.** 1976. Immunodepression, high IgM levels and evasion of the immune response in murine trypanosomiasis. Nature (London) **264**:256–258.
10. **Jayawardena, A. N., and B. H. Waksman.** 1977. Suppressor cells in experimental trypanosomiasis. Nature (London) **265**: 539–541.
11. **Kierszenbaum, F., W. R. Cuna, L. A. Beltz, and M. B. Sztein.** 1989. *Trypanosoma cruzi* reduces the number of high-affinity IL-2 receptors on activated human lymphocytes by suppressing the expression of the p55 and p70 receptor components. J. Immunol. **143**:275–279.
12. **Kierszenbaum, F., M. B. Sztein, and L. A. Beltz.** 1989. Decreased human IL-2 receptor expression due to a protozoan pathogen. Immunol. Today **10**:129–131.
13. **Lanham, S. M., and D. G. Godfrey.** 1970. Isolation of salivarian trypanosomes from man and other mammals using DEAE-cellulose. Exp. Parasitol. **28**:521–534.
14. **Leichtling, K. D., S. A. Serrate, and M. B. Sztein.** 1990. Thymosin alpha 1 modulates the expression of high affinity interleukin-2 receptors on normal human lymphocytes. Int. J. Immunopharmacol. **12**:19–29.
15. **Malek, T. R., and J. D. Ashwell.** 1985. Interleukin 2 upregulates expression of its receptor on a T cell clone. J. Exp. Med. **161**:1575–1580.
16. **Mitchell, L. A., T. W. Pearson, and J. Gauldie.** 1986. Interleukin-1 and interleukin-2 production in resistant and susceptible inbred mice infected with *Trypanosoma congolense*. Immunology **57**:291–296.
17. **Reem, G. H., N. H. Yeh, D. L. Urdal, P. L. Kilian, and J. J. Farrar.** 1985. Induction and upregulation by interleukin 2 of high-affinity interleukin 2 receptors on thymocytes and T cells. Proc. Natl. Acad. Sci. USA **82**:8663–8666.
18. **Sileghem, M., A. Darji, L. Remels, R. Hamers, and P. De Baetselier.** 1989. Different mechanisms account for the suppression of interleukin 2 production and the suppression of interleukin 2 receptor expression in *Trypanosoma brucei*-infected mice. Eur. J. Immunol. **19**:119–124.
19. **Sileghem, M., R. Hamers, and P. De Baetselier.** 1987. Experimental *Trypanosoma brucei* infections selectively suppress both interleukin 2 production and interleukin 2 receptor expression. Eur. J. Immunol. **17**:1417–1421.
20. **Sizemore, R., and J. M. Mansfield.** 1984. Lymphocyte function in experimental African trypanosomiasis. Cell. Immunol. **87**: 684–691.
21. **Smith, K. A., and D. A. Cantrell.** 1985. Interleukin 2 regulates its own receptors. Proc. Natl. Acad. Sci. USA **82**:864–868.
22. **Sztein, M. B., and F. Kierszenbaum.** 1991. A soluble factor from *Trypanosoma brucei rhodesiense* which prevents progression of activated human T lymphocytes through the cell cycle. Immunology **73**:180–185.