INFECTION AND IMMUNITY, Apr. 1990, p. 1114–1116
0019-9567/90/041114-03$02.00/0
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Vol. 58, No. 4

NOTES

Trypanosoma cruzi-Induced Suppression of Human Peripheral Blood Lymphocytes Activated via the Alternative (CD2) Pathway

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Received 31 October 1989/Accepted 2 January 1990

Coculture of blood forms of *Trypanosoma cruzi* with human peripheral blood mononuclear cells stimulated with anti-$CD2_2$ and anti-$CD2_3$ monoclonal antibodies, i.e., via an antigen-independent pathway of T-lymphocyte activation, resulted in marked immunosuppression compared with that in parallel cultures in which parasites were absent. This effect was evidenced by a decreased lymphoproliferative capacity, a significant reduction in the proportion of cells expressing interleukin-2 receptors, and a significant diminution in the cell surface density of this receptor.

Chagas' disease, which is caused by the protozoan *Trypanosoma cruzi*, affects millions of people in South and Central America, and cases have been occasionally reported in North America (7, 13, 17, 21). The acute phase of both the experimental and human forms of the disease is accompanied by immunosuppression (5, 6, 8, 9, 14–16, 18, 19; J. C. Voltarelli, E. A. Donadi, and R. P. Falcao, Letter, Trans. R. Soc. Trop. Med. Hyg. **81**:169–170, 1987), which is thought to reduce resistance to the establishment and dissemination of the organism in the host. We have previously shown that the presence of *T. cruzi* inhibits lymphoproliferation and the expression of interleukin-2 (IL-2) receptors (IL-2R) induced by either phytohemagglutinin (PHA) or anti-CD3 monoclonal antibodies (2, 10, 11). Meuer and co-workers (12) uncovered a new, antigen-independent pathway of human T lymphocyte activation triggered by anti-$CD2_2$ plus anti-$CD2_3$ monoclonal antibodies (formerly designated anti-$T11_2$ and anti-$T11_3$, respectively) which, in contrast to PHA or anti-CD3, activate human lymphocytes in a macrophage-independent manner (3). To study whether *T. cruzi* could also affect lymphoproliferation initiated by this antigen- and macrophage-independent pathway, we studied the effects of the parasite on proliferation and IL-2R expression by human lymphocytes stimulated with the anti-CD2 antibodies.

T. cruzi trypomastigotes (Tulahuen isolate) were purified from the blood of Crl-CD1(ICR) Swiss mice (Charles River Laboratories, Portage, Mich.) that were infected subcutaneously 9 to 11 days previously with 5×10^5 organisms. The parasites were purified by centrifugation over Ficoll-Hypaque ($400 \times g$, 20°C, 45 min), followed by diethylaminoethylcellulose chromatography (4, 20). After the parasites were washed with serum-free RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) containing 100 IU of penicillin and 100 μg of streptomycin per ml, they were suspended in the same medium supplemented with 5% heat-inactivated (56°C, 20 min) fetal bovine serum (referred to as complete medium). The suspension consisted of 100% trypomastigotes (>99% viable).

Normal human peripheral blood mononuclear cells (PBMCs) were purified by centrifugation over a mixture of Ficoll-Hypaque. After three washings with serum-free medium, the cells were suspended at the desired concentration in complete medium. Cell viability was >99%.

PHA was purchased from Sigma Chemical Co. (St. Louis, Mo.). Anti-CD3 (OKT3) and fluorescein-labeled anti-IL-2R (anti-CD25) monoclonal antibodies were purchased from Ortho Diagnostics (Raritan, N.J.) and Becton Dickinson and Co. (Mountain View, Calif.), respectively.

To measure lymphoproliferation by PBMCs, these cells (1.25×10^6 cells per ml) were incubated at 37°C in 5% CO_2 in complete medium alone or in complete medium containing PHA (5 μg/ml), anti-CD3 (12.5 ng/ml), or a mixture of monoclonal antibodies anti-$CD2_2$ and anti-$CD2_3$, which were a generous gift from Stuart F. Schlossman (Harvard University, Boston, Mass.), at final concentrations representing a 1/100 dilution of the original preparations. The cultures were set up in 96-well plates (100 μl per well) and were incubated for 96 h together with or without *T. cruzi* at 5×10^6 organisms per ml. Each culture received 1 μCi of $[^3H]$thymidine (specific activity, 2 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) per well 24 h before termination by automated harvesting. All conditions were tested in triplicate, and the results were expressed as the mean counts per minute ± 1 standard deviation.

For flow cytometric determinations, PBMC cultures (1.25×10^6 cells per ml) were incubated in complete medium (37°C; 5% CO_2; 24-well plates; final culture volume, 1 ml) for 48 h with or without *T. cruzi* (5×10^6 organisms per ml) in the presence or absence of PHA (5 μg/ml), anti-CD3 (25 ng/ml), or a mixture of anti-$CD2_2$ and anti-$CD2_3$ diluted as described above (this mixture is referred to as anti-CD2). The cells were then washed three times, incubated with fluorescein-labeled anti-CD25 for 30 min at 4°C, washed again, fixed in 1% formaldehyde, and stored at 4°C in the dark until they were analyzed with a flow cytometer (FACS

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TABLE 1. Suppression of PBMC proliferative responses to PHA, anti-CD3, and anti-$CD2_2$ plus anti-$CD2_3$ by *T. cruzi*[a]

Mitogenic stimulus	cpm (10^3) obtained in the presence of the following no. (10^6) of *T. cruzi* organisms/ml:				
	0	2.5	5	7.5	10
None	0.6 ± 0.1	1.3 ± 0.3	1.9 ± 0.9	2.0 ± 0.6	3.0 ± 0.1
PHA	61.7 ± 3.0	20.9 ± 1.1	6.3 ± 0.1	3.1 ± 0.5	1.8 ± 1.3
Anti-CD3	37.2 ± 2.6	22.1 ± 1.4	12.0 ± 0.5	5.6 ± 0.3	2.3 ± 0.4
Anti-CD2	14.2 ± 0.5	6.5 ± 0.2	2.4 ± 0.1	0.9 ± 0.3	0.4 ± 0.1

[a] Cocultures of PBMCs with and without the indicated *T. cruzi* concentrations were stimulated with 5 μg of PHA per ml or 12.5 ng of anti-CD3 per ml or with a 1/100 dilution of the original preparations of anti-$CD2_2$ plus anti-$CD2_3$ and were incubated at 37°C for 96 h. Results are means ± standard deviations of triplicate cultures. All of the results obtained by using a mitogen and in the presence of *T. cruzi* represent statistical ($P < 0.05$, Student's *t* test) reductions with respect to the corresponding control value obtained without trypanosomes.

IV; Becton Dickinson). A minimum of 10,000 cells, which were gated to exclude erythrocytes, platelets, nonviable cells, and *T. cruzi*, were accumulated for each histogram. The percentage of positive cells was estimated against a background of cells stained with normal mouse immunoglobulin G. Mean channel numbers of the logarithm of fluorescence intensities of the positive cell populations (MFCh) were used to compare the relative surface densities of the relevant lymphocyte marker in the presence or absence of *T. cruzi*. Parallel cultures in 96-well plates were set up to verify, in terms of reduced lymphoproliferation, that immunosuppression had taken place.

A comparison of the effects of the presence of *T. cruzi* in the cultures on the capacity of PBMCs to respond to stimulation with PHA, anti-CD3, or anti-CD2 revealed significant suppression in all instances (Table 1). Under all of these stimulatory conditions, the extent of the recorded suppression was greater as the parasite concentration was increased.

We also measured the expression of IL-2R by anti-CD2-stimulated PBMCs to establish whether this critical event in lymphocyte activation was also modulated by *T. cruzi*, as is the case with PHA-stimulated cells (2). Parallel experiments performed with PHA and anti-CD2 indicated that, in both cases, the percentage of cells expressing IL-2R was significantly reduced in the presence of *T. cruzi* (Fig. 1). Furthermore, as evidenced by the pronounced shift of the histograms to the left, there was also a marked reduction in the surface density of the receptor per cell of the IL-$2R^+$ cell populations, denoted by a drop in MFCh values.

These results showed that exposure of PBMCs to *T. cruzi* suppresses their capacity to proliferate and express IL-2R upon stimulation with anti-CD2 monoclonal antibodies. These effects provided evidence that the lymphocyte alterations induced by this parasite do not differ qualitatively from those seen when PHA or anti-CD3 is used as the mitogen. Because CD2 is not a component of the T-cell receptor–CD3 complex, activation by anti-CD2 antibodies must involve an initial pathway that is distinct from that which is involved when the mitogenic signal is conveyed by specific antigen binding. Therefore, the parasite can tamper with T-cell activation, proliferation triggered by mitogenic agents which stimulate T cells by different pathways, or both. Furthermore, because the CD2 pathway is known to be independent of the presence of macrophages (12), it appears that there is no requirement for this accessory cell type in the production of *T. cruzi*-induced suppression. This conclusion is in line with previous results from our laboratory showing that *T. cruzi* suppresses PBMC responses to PHA and concanavalin A, whether or not the adherent cell subpopulation is depleted (1); that the parasite does not inhibit but actually stimulates interleukin-1 production by human adherent PBMCs (2); and that the flagellate does not alter enhanced interleukin-1 production by human macrophages treated with a bacterial endotoxin (2). Since *T. cruzi* does not absorb, consume, or inactivate IL-2, suppressed mitogenic responses are unlikely to result from removal of this essential cytokine by the parasite (1). We have also ruled out increased PBMC death, mitogen absorption, and consumption of essential medium nutrients as possible causes of the suppressive effects of *T. cruzi* (1, 2).

Further work is under way in our laboratories to unravel the mechanisms by which the parasite downregulates key lymphocyte functions. The ability of *T. cruzi* to suppress human PBMCs, which was demonstrated in our in vitro studies, may be involved in the production of the immunosuppression that has been shown to occur during the acute phase of Chagas' disease (19; Voltarelli et al., letter).

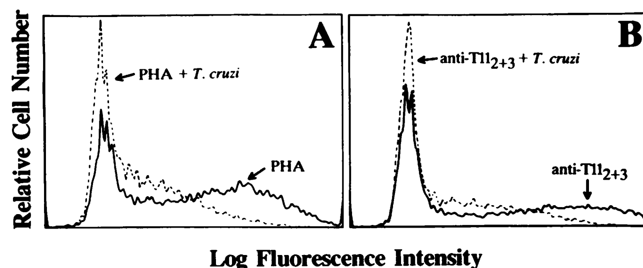

FIG. 1. Reduction by *T. cruzi* of the expression of IL-2R by PBMCs stimulated with PHA or anti-$CD2_2$ plus anti-$CD2_3$. (A) Cells incubated with or without PHA in the absence or presence of *T. cruzi*. (B) Cells incubated with or without anti-CD2 in the absence or presence of *T. cruzi*. The PBMCs were collected after 48 h of culture and were stained with fluorescein-labeled anti-IL-2R for flow cytometry. For panel A, the percentages of IL-$2R^+$ cells and the corresponding MFCh values for PBMCs + PHA were 55.7 and 160, respectively; for PBMC + PHA + *T. cruzi*, the values were 35.9 and 112, respectively. For panel B, the percentages of IL-$2R^+$ cells and the corresponding MFCh values for PBMC + anti-CD2 were 48.9 and 167, respectively; for PBMC + anti-CD2 + *T. cruzi*, the values were 41.0 and 124, respectively. This set of results is representative of three separate repeat experiments.

This study was supported by in part by Public Health Service grants AI-26542 and CA-42219 from the National Institutes of Health.

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