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# *Trypanosoma cruzi* induces suppression of DNA synthesis and inhibits expression of interleukin-2 receptors by stimulated human B lymphocytes

F. KIERSZENBAUM, E. MORETTI* & M. B. SZTEIN† *Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan and † Center for Vaccine Development, Department of Pediatrics, University of Maryland at Baltimore, Baltimore, Maryland, U.S.A.

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## SUMMARY

*Trypanosoma cruzi*, the causative agent of Chagas' disease, suppresses immune responses during the acute phase and has been shown to induce multiple cellular alterations in activated human T lymphocytes. However, no information is available regarding the effects of this parasite on human B cells. Using an *in vitro* culture system, in which purified *T. cruzi* are co-cultured with either peripheral blood mononuclear cells (PBMC) or B-cell-enriched preparations (BCE), we studied whether the organism can induce alterations in DNA synthesis after stimulation with Pansorbin (PS). This response was markedly reduced by the parasite at both suboptimal and optimal PS concentrations, and the extent of the inhibition was augmented as the parasite concentration was increased. Maximal reduction in DNA synthesis was observed when the trypanosomes were incorporated into the cultures at 0 time (i.e. together with PS); the effect was of a much lesser magnitude and undetectable when the parasites were added at 24 and 48 hr, respectively. These results imply that *T. cruzi* affects a relatively early event during B-cell stimulation. This inference was confirmed by the finding that the proportion of PS-stimulated B cells expressing interleukin-2 (IL-2) receptors was significantly reduced when the parasite was present in the culture. Addition of recombinant human IL-2 did not restore B-cell responsiveness to normal levels. Suppressed B-cell responses were also observed when *T. cruzi* was separated from the PBMC or the BCE by a cell-impermeable filter, indicating that a soluble factor(s) released by the organism mediated the effect. Accordingly, supernatants of *T. cruzi* suspensions were found to be suppressive. These results demonstrate for the first time that *T. cruzi* can affect human B-cell responses and that the mechanism involves inhibition of IL-2 receptor expression.

## INTRODUCTION

Infection by *Trypanosoma cruzi*, the etiological agent of Chagas' disease, is widely distributed in South and Central America, where millions of people are affected. Cases have also been occasionally reported in North America, some of them resulting from the transfusion of infected blood.[1-4] Multiple manifestations of immunosuppression have been documented during the acute phase of Chagas' disease in both laboratory animals and patients.[5 13] This immunosuppression probably facilitates the dissemination of the trypanosomes in mammalian hosts, as immunosuppression of chronically infected hosts exacerbates the disease.[14] Recently, using an *in vitro* model system in which purified *T. cruzi* are co-cultured with human PBMC, we have been able to explore the mechanisms of T lymphocyte immunosuppression induced by this organism, which include inhibition of the expression of the receptors for interleukin (IL-2)[15] and transferrin.[16] We have also documented down-regulation by *T. cruzi* of stimulated T-cell expression of CD3, CD4 and CD8 molecules, which play important roles in the activation process.[17 22] In mice, deficient lympho-proliferative responses to stimulation with a bacterial endotoxin[14] and suppressed antibody formation during acute *T. cruzi* infection[23] have been reported, indicating that the parasite effects B-cell function as well. However, no information is available about the direct effects of *T. cruzi* on human B-cell function, and the mechanisms by which this trypanosome induces B-cell suppression have not

Abbreviations: BCE, B-cell-enriched PBMC; [$^3$H]TdR, tritiated thymidine; MFCh, mean channel number of the logarithm of fluorescence intensity; PS, Pansorbin (killed *Staphylococcus aureus* Cowan I); PBMC, peripheral blood mononuclear cells; serum-free or complete medium, RPMI-1640 medium with penicillin and streptomycin without or supplemented with 5% heat-inactivated foetal bovine serum, respectively; TIF, preparation which contains trypanosomal immunosuppressive factor(s).

Correspondence: Dr. F. Kierszenbaum, Dept. of Microbiology and Public Health, Michigan State University, East Lansing, MI 48824, U.S.A.

* Present address: Servicio Nacional de Chagas, Córdoba, Argentina.

been defined. In this work, our *in vitro* model system was used to study whether *T. cruzi* interaction with human PBMC or B-cell-enriched preparations (BCE) results in down-regulation of B-cell responses.

## MATERIALS AND METHODS

*Biological reagents and monoclonal antibodies*

Bovine serum albumin (BSA) (fraction V, 98–99% albumin) was purchased from Sigma Chemical Co. (St Louis, MO). Pansorbin (PS, killed *Staphylococcus aureus* Cowan I) was obtained from Calbiochem (La Jolla, CA). Recombinant human interleukin-2 (rhIL-2) was a generous gift from Dr Peter Sorter (Hoffman-LaRoche, Nutley, NJ). The concentration of this cytokine that maximally supported the growth of the IL-2-dependent CTLL-2 cell line was 100 U/ml.[24] Magnetic beads coupled with anti-CD2 (Pan T), anti-CD4 (specific for $T_{helper/inducer}$ cells) and anti-CD8 (specific for $T_{cytotoxic/suppressor}$ cells) monoclonal antibodies were acquired from Advanced Magnetics Inc. (Cambridge, MA). Phycoerythrin (PE)-conjugated monoclonal anti-CD20 (B1; Pan B) was purchased from Coulter Immunodiagnostics (Hialeah, FL). Monoclonal anti-CD19 (Leu-12; Pan B), monoclonal FITC-labelled anti-CD25 (anti-IL-2 receptor, p55 chain-specific) and normal mouse IgG labelled with either PE or FITC, were obtained from Becton-Dickinson (Mountain View, CA).

*Peripheral blood mononuclear cells (PBMC)*

Blood was drawn from healthy volunteers by venipuncture. The PBMC were purified by centrifugation (400 *g*, 20°, 30 min) through a mixture of Ficoll–Hypaque of density 1·077. After two washings with serum-free medium, the cells were resuspended at the desired concentration in complete medium. The cell viability of these suspensions, determined by trypan blue exclusion, was consistently >99%.

*Parasites*

Bloodstream (trypomastigote) forms of *T. cruzi* (Tulahuén isolate) were derived from Crl-CD1(ICR) Swiss mice (Charles River Laboratories, Portage, MI) infected subcutaneously 10 days previously with $5 \times 10^5$ organisms. The flagellates were purified by centrifugation (400 *g*, 20°, 45 min) through Ficoll–Hypaque (density 1·077)[25] followed by diethylaminoethyl-cellulose chromatography.[26] After two washings with RPMI-1640 medium supplemented with 5% heat-inactivated (56°, 30 min) foetal bovine serum (referred to in the text as complete medium), the parasites were resuspended at the desired concentration in the same medium. All parasite suspensions consisted of 100% trypomastigotes (>99% viable), were devoid of leucocytes and very occasionally contained <0·1% erythrocytes.

*Preparation of* T. cruzi *supernatants (TIF)*

Suspensions of *T. cruzi* at $1 \times 10^7$ parasites/ml in complete medium were incubated at 37° and 5% $CO_2$ for 24 hr and filtered through a 0·45-μm pore size sterile filter. This material, which was dialysed (10,000 molecular weight cut off membrane) against serum-free medium, aliquoted and stored at −20° until used, is referred to in the text as TIF, for containing a trypanosomal immunosuppressive factor(s).[27]

*B-cell enrichment*

Four millilitres of PBMC suspension at $5 \times 10^7$ cells/ml in complete medium were mixed with a suspension consisting of 4 ml anti-CD2-, 1 ml anti-CD4- and 1 ml anti-CD8-coated magnetic beads (all previously washed five times with sterile complete medium). The mixture was incubated at 0° for 20 min and the beads were then removed by using a magnet. The cells were recovered by centrifugation, resuspended in 4 ml of complete medium and subjected to a second incubation with anti-CD2-, anti-CD4- and anti-CD8-coated beads under the same conditions. After removal of the beads, the cells were washed twice with complete medium, counted microscopically using a haemacytometer and resuspended at $1 \times 10^7$ cells/ml in complete medium. This type of cell preparation is referred to in the text as BCE. Aliquots were stained with PE-labelled anti-CD19 or PE-labelled anti-CD20 to determine B-cell contents by flow cytometric analysis. The purification procedure yielded preparations containing 25–40% $CD19^+$ or $CD20^+$ cells.

*Assays for [$^3$H]TdR incorporation*

Cultures set up in 96-well flat-bottomed well plates, at 0·1 ml per well, consisted of either PBMC or BCE alone or co-cultured with purified *T. cruzi* added at 0 time, i.e. together PS. The concentrations of PBMC, BCE, *T. cruzi* and PS, as well as the lengths of [$^3$H]TdR pulses (with 1 μCi[$^3$H]TdR; specific activity 2 Ci/mmole; Amersham, Arlington Heights, IL) used in each experiment, are described in the Results section. The cultures were terminated by automated harvesting and incorporated radioactivity was determined by using a scintillation counter. In some experiments, *T. cruzi* suspension or fresh complete medium (control) was added at different times after PS stimulation, all other conditions remaining unchanged. We also set up cultures in which TIF which had been dialysed against fresh culture medium was substituted for 90% of the culture medium. All conditions were tested in quadruplicate. The significance of differences between means was established by Student's *t*-test. The percentage of suppression was calculated by using the equation:

$$\% \text{ suppression} = \frac{[(\text{control} - \text{background}) - (\text{experimental} - \text{background})]}{(\text{control} - \text{background})} \times 100.$$

*Cell–parasite contact requirement studies*

To establish whether physical PBMC- or BCE–parasite contact was required for the production of suppressive effects, we separated the human cells from *T. cruzi* with 0·45-μm pore size filters (Millicell HA, Millipore, Bedford, MA). PBMC or BCE ($1 \times 10^6$ cells in 0·5 ml of complete medium) were placed in wells of 24-well culture plates, and a sterile Millicell-HA filter insert, into which 0·4 ml of complete medium containing or lacking $1 \times 10^7$ *T. cruzi* trypomastigotes was added, was introduced. Where appropriate, PS was added to the wells so that the final concentration would be 200 μg/ml. When *T. cruzi* was allowed to be in direct contact with PBMC or BCE, both the organisms and the human cells were placed in the culture well and an insert containing 0·4 ml of complete medium was introduced, all other conditions remaining unchanged. For determination of [$^3$]TdR incorporation, 100-μl aliquots of each culture were transferred after 48 hr of culture to 96-well plates and were mixed with 25 μl of [$^3$H]TdR solution in complete medium (1 μCi). The cultures

were terminated 12 hr later by automated harvesting and processed for scintillation counting.

*Cultures of BCE with* T. cruzi *and immunofluorescence staining*
BCE preparations were incubated in complete medium (at 37°, 5% $CO_2$; 24-well plates) for 72 hr with or without *T. cruzi* in the presence or absence of PS. The concentrations of human cells, *T. cruzi* and PS used in our experiments are described in the Results section. At the end of the cultures, single cell suspensions were obtained by vigorous pipetting. The cells were centrifuged and washed with phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA) and 0·1% sodium azide (PBS + BSA + azide) and were directly stained for single- or two-colour flow cytometric analysis according to standard procedures.[24,28] For single-colour immunofluorescence staining, the cells were incubated with PE-labelled anti-CD19 or anti-CD20 for 30 min at 0°, washed with cold PBS + BSA + azide, fixed with 1% formaldehyde in PBS + BSA + azide, and kept at 4° until analysed by flow cytometry. For two-colour staining, cells were incubated with a mixture of FITC-labelled anti-IL-2R and either PE-labelled anti-CD19 or PE-labelled-anti-CD20, washed and fixed. Cells stained with PE-labelled normal mouse IgG or both PE labelled normal mouse IgG plus FITC-labelled normal mouse IgG were used as controls for background fluorescence for single and two-colour fluorescence, respectively.

*Flow cytometric analyses*
Single-colour immunofluorescence was determined using a FACScan flow cytometer (Becton-Dickinson). The excitation wavelength was 488 nm at 15 mW. Ten thousand cells, gated to exclude parasites, erythrocytes, platelets and non-viable cells were accumulated for each histogram. The logarithms of fluorescence intensities were distributed over four decades. The percentage of positive cells was estimated against a background of cells stained with PE-labelled normal mouse IgG. The mean channel number of the logarithm of the fluorescence intensity (MFCh) was the parameter used to compare the relative surface density of markers in the studied cell populations.

For dual-parameter correlated flow cytometry, FITC and PE fluorescences were collected through four-decade logarithmic amplifiers. Erythrocytes, parasites, platelets and non-viable cells were excluded from analysis by setting an appropriate gate on forward versus side light scatter parameters. Thirty-thousand cells were collected for each sample. Two-parameter data, displayed as contour maps, were used to set gates in the $CD19^+$ or $CD20^+$ cell regions. The percentages of $CD19^+$ or $CD20^+$ cells that concomitantly expressed IL-2R were calculated from IL-2R fluorescence intensity (single-colour histograms) after substraction of the fluorescence intensities of cell populations stained with FITC-normal IgG. This type of graphic representation facilitated comparisons between BCE populations incubated in the presence or absence of *T. cruzi*.

## RESULTS

### Suppression by *T. cruzi* of PS-induced DNA synthesis by human B-cell lymphocytes

Addition of purified trypanosomes to human PBMC cultures stimulated with PS resulted in markedly reduced [$^3$H]TdR incorporation (Fig. 1). This suppressive phenomenon was observed with both optimal and suboptimal PS concentrations. Parasite titrations using an optimal concentration of PS (200 μg/ml) showed that the magnitude of the suppressive effect was dependent upon the number of organisms present in the culture, being first detectable at $5 \times 10^6$ *T. cruzi*/ml (Fig. 2).

To define the time during which *T. cruzi* induces maximal suppression, parasites were added to cultures at different times after PS stimulation. As shown in Fig. 3, maximal suppression of DNA synthesis occurred when the organisms were present from initiation, i.e. added together with PS. The effect was less pronounced when the parasites were added at 24 hr and undetectable if added at 48 hr.

### *T. cruzi*-induced suppression of IL-2R expression by PS-stimulated cells

That *T. cruzi*-induced suppression of human B-cell proliferation was greatest when the cells were exposed to the parasites from 0

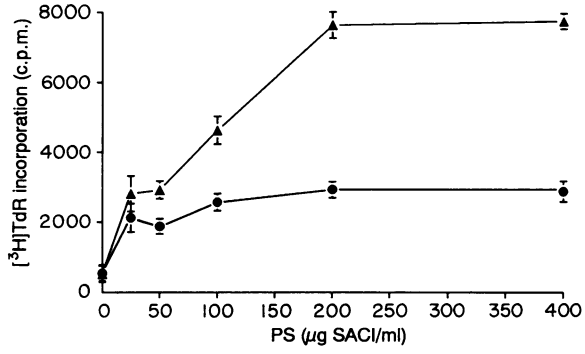


**Figure 1.** *T. cruzi*-induced suppression of human B-lymphocyte proliferation over a wide range of PS [(killed *Staphylococcus aureus* Cowan I (SACI)] concentrations. Suspensions of PBMC ($2 \times 10^6$ per ml) were stimulated with the indicated concentration of PS and were incubated with (●) or without (▲) *T. cruzi* ($5 \times 10^6$ per ml); [$^3$H]TdR pulse = 48–60 hr. For PS concentrations greater than 50 μg/ml, the suppressive effect of *T. cruzi* was statistically significant ($P < 0.01$). This set of data is representative of two separate repeat experiments performed with cells from different donors.

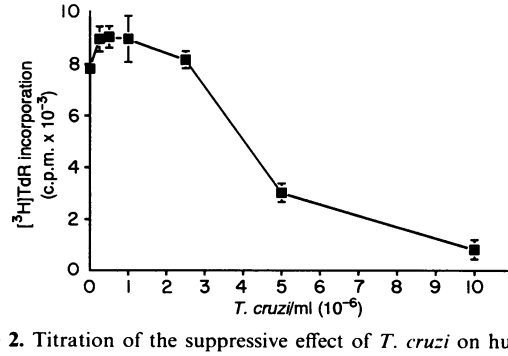


**Figure 2.** Titration of the suppressive effect of *T. cruzi* on human B-lymphocyte proliferation. Suspensions of PBMC ($2 \times 10^6$ per ml) were incubated alone or with PS (200 μg/ml), with or without increasing concentrations of *T. cruzi*; [$^3$H]TdR pulse = 48–60 hr. For parasite concentrations greater than $2{\cdot}5 \times 10^6$ per ml, the suppressive effect was statistically significant ($P < 0.01$). This set of data is representative of two separate repeat experiments performed with cells from different donors.

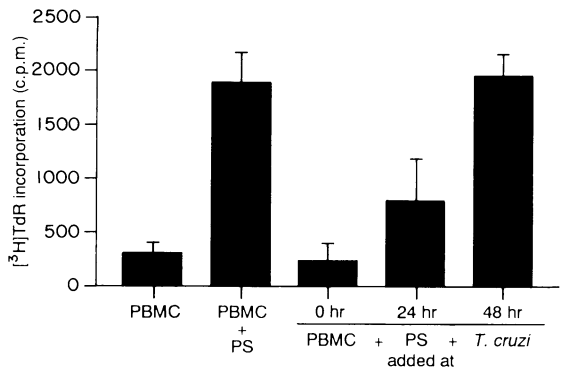


**Figure 3.** Kinetics of the suppressive effect of *T. cruzi*. Suspensions of PBMC ($2 \times 10^6$ per ml) were incubated alone or with PS (200 $\mu$g ml); *T. cruzi* was added at the indicated times to attain a final concentration of $1 \times 10^7$ organisms/ml [$^3$H]TdR pulse = 48–60 hr. Statistically significant ($P < 0.01$) effects were observed when the parasite was added at 0 and 24 hr. This set of data is representative of two separate repeat experiments performed with cells from different donors.

**Table 1.** Demonstration of *T. cruzi*-induced suppression using either PBMC or BCE

| Culture condition | Counts per minute | % suppression |
|---|---|---|
| PBMC + medium | 258 ± 79 | |
| PBMC + PS | 1977 ± 39 | |
| PBMC + PS + *T. cruzi* | 546 ± 39* | 83 |
| BCE + medium | 259 ± 16 | |
| BCE + PS | 2628 ± 206 | |
| BCE + PS + *T. cruzi* | 539 ± 18* | 79 |

Suspensions of PBMC or BCE ($1 \times 10^6$ cells ml) were cultured without or with $1 \times 10^7$ parasites ml for 96 hr. [$^3$H]TdR pulse = 72–96 hr. PS was used at 200 $\mu$g ml. This set of data is representative of four separate repeat experiments using cells from different donors.

* The difference between this value and the corresponding positive control (cells + PS) was statistically significant ($P < 0.001$).

time suggested that a relatively early event(s) during B-lymphocyte activation was being targeted. In previous work, we found that human T-cell suppression by *T. cruzi* involved IL-2R expression and occurred despite the presence of adequate levels of IL-2.[15] Since IL-2R expression is known to be an early requirement for activated B cells to be able to proceed through their cell cycle,[29] we studied whether *T. cruzi* also inhibited IL-2R expression by activated B cells. However, in our experience, the proportion of B cells (i.e. CD20$^+$ or CD19$^+$ cells) present in normal PBMC preparations ranges from 4% to 15%. Demonstrating a statistically significant decrease in the percentage of IL-2R$^+$ B lymphocytes from these low values would have been difficult. For this reason, we decided to use BCE. To validate this approach we set up lympho-proliferation experiments in which PBMC and BCE were tested side by side for sensitivity to *T. cruzi*-induced suppression. The results indicated that both cell preparations were affected by the parasites (Table 1).

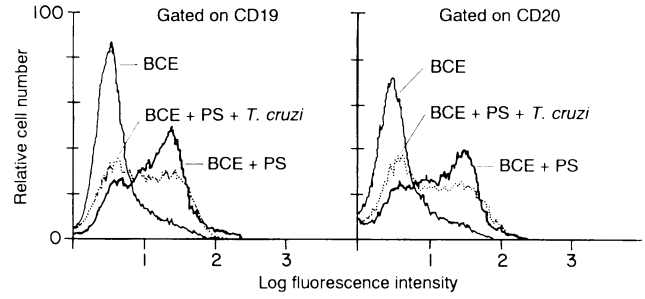


**Figure 4.** Effects of *T. cruzi* on IL-2R expression by PS-stimulated human B lymphocytes. Cultures of BCE ($1 \times 10^6$ per ml) were incubated in medium alone or containing PS (400 $\mu$g ml), without or with *T. cruzi* ($1 \times 10^7$ organisms ml) for 72 hr and then stained with FITC anti-IL-2R and PE anti-CD19 or PE-anti-CD20. Two-colour fluorescence analysis was performed as described in the Materials and Methods. The results are displayed as the logarithm of IL-2R fluorescence intensity. Represented in the figure are the population distributions of CD19$^+$ and CD20$^+$ incubated with or without PS in the presence or absence of *T. cruzi*. The percentages and MFCh values (given in parentheses) were: (a): unstimulated CD19$^+$ cells, 10·6% (31); CD19$^+$ + PS, 53·4% (33); CD19$^+$ + PS + *T. cruzi*, 41·6% (35); (b) unstimulated CD20$^+$ cells, 10·4% (30); CD20$^+$ cells + PS, 50·0% (40); CD20$^+$ + PS + *T. cruzi*, 40·7% (40). This set of data is representative of three separate repeat experiments with cells from different donors.

To study the effect of *T. cruzi* on IL-2R expression by BCE stimulated with PS, the cells were stained with FITC anti-IL-2R and either PE-anti-CD19 or PE-anti-CD20 monoclonal antibodies, and were then analysed by flow cytometry. IL-2R expression was examined by gating on the CD19$^+$ or CD20$^+$ cell populations; the mean fluorescence intensity and the percentage of IL-2R$^+$ cells were calculated from single-colour histograms (Fig. 4). The results demonstrated significant reductions in the percentage of IL-2R$^+$ cells in either the PS-stimulated CD19$^+$ or CD20$^+$ population co-cultured with *T. cruzi* (Fig. 4). It should be noted that no meaningful difference in the MFCh value corresponding to IL-2R expression was observed between PS-stimulated BCE incubated with or without *T. cruzi* (Fig. 4).

Because IL-2 is known to up-regulate IL-2R expression by B cells and IL-2R expression is required for B-cell proliferation,[29] we tested whether the addition of rHIL-2 would restore responsiveness to PS, but this was not the case (data not shown).

### Reduced B-cell DNA synthesis is mediated by a soluble parasite product(s)

The finding of suppressed B-cell responses in co-cultures with *T. cruzi* did not clarify whether the effect resulted from direct cell-parasite contact or was mediated by a soluble parasite product(s). To answer this question, we used a culture system in which both cell types were either on the same or separate compartments created by the presence of Millicell-HA insert in the well. As shown in Table 2, significant suppression, in terms of reduced [$^3$H]TdR incorporation during the last 12 hr of 60-hr cultures, occurred whether or not physical contact between parasites and PBMC or BCE had been allowed. It is noteworthy that the level of *T. cruzi*-induced suppression varied among repeat experiments, even when cells from the same donor were tested, but the suppressive effect was always demonstrable and statistically significant ($P < 0.05$; data not shown).

**Table 2.** Suppression of the PS-elicited response of PBMC or BCE by a soluble *T. cruzi* product(s)

| Present in the Well | Insert | Counts per minute | % Suppression |
|---|---|---|---|
| PBMC + medium | Medium | 525 ± 90 | |
| PBMC + PS | Medium | 7156 ± 453 | |
| PBMC + PS | *T. cruzi* | 2421 ± 211* | 71 |
| PBMC + PS + *T. cruzi* | Medium | 815 ± 95* | 96 |
| BCE + medium | Medium | 275 ± 32 | |
| BCE + PS | Medium | 13,536 ± 1367 | |
| BCE + PS | *T. cruzi* | 5731 ± 135* | 59 |
| BCE + PS + *T. cruzi* | Medium | 8299 ± 375* | 40 |

PBMC or BCE were cultured in wells of 24-well plates with or without PS, in the absence or presence of parasites. For PBMC, BCE and *T. cruzi* concentrations, see the Materials and Methods. A Millicel HA insert (filter pore size = 0·45 nm) was used to separate the organisms from the human cells, or parasites were placed in the well together with the human cells (in these cases, an insert containing medium alone was placed in the well). The cultures were pulsed with 1 $\mu$Ci[$^3$H]TdR during the last 12 hr of a 60-hr culture period. The experiments with PBMC and BCE were performed separately and each set of data is typically representative of two separate repeat experiments performed with cells from different donors.

* The difference between this value and the corresponding positive control (cells + PS in the well and medium alone in the insert) was statistically significant ($P < 0{\cdot}01$).

The suppressive effect of *T. cruzi* on B cells, being demonstrable after parasite separation with a cell-impermeable filter, suggested that a soluble parasite product mediated the phenomenon. If so, the suppressive activity should be found in supernatants of *T. cruzi* suspensions. This was confirmed by the results of experiments in which such supernatants were added to PBMC cultures. Thus, in a typically representative experiment, the c.p.m. values obtained with unstimulated PBMC and with PS-stimulated PBMC were 874 ± 174 and 5337 ± 703, respectively, whereas the value obtained with PS-stimulated cells in cultures in which 90% of the medium had been replaced with TIF which had been dialysed against fresh culture medium was 1406 ± 395 c.p.m. (for an 88% reduction in responsiveness; $P < 0{\cdot}001$).

## DISCUSSION

These results show for the first time that *T. cruzi* can suppress DNA synthesis by stimulated human B lymphocytes. The suppressive mechanism was found (i) to involve at least inhibition of IL-2R expression, (ii) to be mediated by a parasite-secretion product(s), and (iii) not to be corrected by the addition of rhIL-2.

Maximal parasite-induced suppression was observed when *T. cruzi* was added to the cultures at initiation, suggesting that the parasite targeted a relatively early event(s) during B-lymphocyte activation (Fig. 3). While suppressed IL-2R expression might be the main targeted event, the present results do not disclose the even earlier molecular processes that result in this effect.

Whereas the presence of *T. cruzi* resulted in readily demonstrable decreases in the percentage of IL-2R$^+$ B cells, no meaningful change in the MFCh corresponding to IL-2R expression was observed. This indicated that, in the cells which remained IL-2R$^+$ positive, the IL-2R surface density was comparable to that of stimulated cells cultured in the absence of *T. cruzi* (Fig. 4). At first glance, this observation would seem to contrast with human T-cell suppression by the parasite, which results in marked decreases in both the percentage of IL-2R$^+$ cells and surface IL-2R density.[15] However, reductions in MFCh values might have been difficult to measure in the activated B cells because these cells express significantly lower numbers of IL-2R molecules than activated T cells,[30] and any reduction in fluorescence intensity might have made the cells appear as if they were IL-2R$^-$. For this reason alone, the lack of decline in fluorescence intensity might represent more a technical limitation than a real phenomenon.

In earlier work, we showed that *T. cruzi* viability is required for the parasite to exert suppression.[15] However, we have demonstrated that, at suppressive concentrations, the parasite does not consume significant levels of essential medium nutrients[31] nor does it absorb, consume or inactivate IL-2.[15] In the present study, we found that exogenous rhIL-2 did not restore B-cell responsiveness. Therefore, the noted suppression of B-cell DNA synthesis and IL-2R expression is unlikely to result from medium depletion or removal of IL-2 by the parasite. Instead, suppression might be due to a large extent to curtailed IL-2 utilization as a consequence of decreased IL-2R expression.

B-cell proliferative responses are considerably more effective when macrophages are present, probably because the latter cells contribute IL-1.[29] However, the suppressive effect of this parasite on B cells is unlikely to result from inhibited IL-1 production by infected macrophages because *T. cruzi*, in fact, stimulates IL-1 production by adherent human PBMC and does not affect IL-1 production induced with a bacterial lipopolysaccharide.[15] In this context, it is noteworthy that *T. cruzi* also suppresses human T lymphocytes activated via an alternative pathway (i.e. with anti-CD2 monoclonal antibodies), which is known to be macrophage independent.[27,32]

Two lines of evidence demonstrated that *T. cruzi*-induced suppression of B lymphocytes could be mediated by a soluble secretion product(s). The effect occurred when the parasites were separated from either the PBMC or BCE by means of a cell-impermeable filter (Table 2), and supernatants from parasite suspensions (TIF) were also suppressive. While these results do not uncover the biochemical nature or the physico-chemical characteristics of the B-cell-suppressive molecule(s) present in TIF, it is noteworthy that the molecule(s) present in TIF which suppress human T-lymphocyte responses was established to be of protein nature, with a molecular weight between 30,000 and 100,000.[27] Whether the same molecule(s) affects both T and B cells remains to be studied. It should be noted that the B-cell suppressant present in TIF preparations, not being dialysable, must have a molecular weight > 10,000.

While the suppression which accompanies acute Chagas' disease may have an explanation in the present and past findings from our laboratories,[15,16,27] the molecular mechanisms by which *T. cruzi* brings about reduced IL-2R expression remain to be

explored. They could involve excessive or accelerated shedding of IL-2R or alterations in transcription of the genes coding for receptor proteins, messenger RNA stability or translation, or receptor protein transport to the lymphocyte membrane. Preliminary studies have shown that the levels of soluble IL-2R in the conditioned medium of parasite-BCE co-cultures are lower than those present in cultures lacking *T. cruzi* (data not shown). In this light, increased receptor shedding would not appear to be a likely explanation. The remaining possibilities, as well as the identification of the parasite factor(s) mediating the suppressive effect, are exciting subjects for further research, which may also help improve our understanding of key events governing lymphocyte activation.

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