Antigen-specific Immunosuppression in Visceral Leishmaniasis Is Cell Mediated

Edgar M. Carvalho, Olivia Bacellar, Aldina Barral, Roberto Badaro, and Warren D. Johnson, Jr.
Division of Immunology, Department of Medicine, University of Bahia, Salvador, Brazil; and Division of International Medicine, Department of Medicine, Cornell University Medical College, New York 10021

Abstract

Visceral leishmaniasis is associated with an antigen-specific immunosuppression during the acute disease. Patients become responsive to *Leishmania* antigen in both in vivo and in vitro assays after successful antimony therapy. The cell type involved in the suppression of lymphocyte reactivity to *Leishmania* antigen was studied by selective depletion of mononuclear cell (MNC) populations and in co-cultivation experiments. Adherent cells were depleted on plastic and by passage on nylon wool columns. High-avidity Fc^+ cells were depleted by adherence to BSA–anti-BSA complexes and $OKT4^+$ and $OKT8^+$ cells were depleted by treatment with monoclonal antibody (anti-$OKT4^+$ and $OKT8^+$) and complement. Depletion of MNC preparations of adherent cells, high-avidity Fc^+ cells, OKT_4^+ cells and OKT_8^+ cells failed to restore the lymphocyte reactivity to *Leishmania* antigen. Antimony therapy was associated with restoration of the proliferative responses of unseparated MNC (before treatment 460 ± 76 cpm and after treatment $4,293\pm1,442$ cpm). Co-culture of frozen cells obtained before chemotherapy with autologous MNC obtained after treatment reduced the response of posttreatment cells to *Leishmania* antigen by 80%. We conclude that the antigenic specific suppression of lymphocyte proliferation in visceral leishmaniasis is cell mediated.

Introduction

Visceral leishmaniasis is characterized by a depression of the cellular immune response to *Leishmania* antigen. Patients have negative intradermal skin tests (1, 2), absent lymphocyte blastogenesis (3–6), and decreased production of interleukin 2 and γ-interferon (7). They respond to mitogens in these assays and most patients also respond to recall antigens during the acute disease. After successful treatment leishmaniasis patients rapidly become responsive to *Leishmania* antigen (3).

The mechanism of the immunosuppression is unknown. It has been suggested that either serum factors (4, 8), decreased numbers of T cells in peripheral blood (7, 9, 10), absence of antigen-reactive cells in peripheral blood (11), or the presence of suppressor cells might account for the diminished responsiveness. In the present study we were not able to restore the immune response to *Leishmania* antigen in a blastogenesis assay by depleting the mononuclear cell population of either macrophages, B cells, Fc receptor high-avidity cells, or $OKT8^+$ and $OKT4^+$ cells. However, we have determined that cells from unresponsive patients before therapy will abolish the ability of those same patients' cells to respond after therapy.

Methods

Patients. Patients with active visceral leishmaniasis admitted at the Federal University Hospital, in Salvador, Bahia, Brazil were studied. The patients had classic visceral leishmaniasis with fever, weight loss, hepatosplenomegaly, anemia, leukopenia, and hyperglobulinemia. The diagnosis was established by the demonstration of amastigote forms of *Leishmania* in Giemsa-stained bone marrow aspirates. Immunofluorescent antibody titers to *Leishmania* donovani (12) were higher than 1:512 in all patients. Patients with visceral leishmaniasis were treated with *N*-methylglucamine (Glucantime, [Rhodia SA, Sao Paulo, Brazil] SbV; 28 mg/kg per day) for a minimum of 10–14 d. If the patient remained febrile and symptomatic, therapy with Glucantime was continued. None of the patients required > 30 d of treatment. Healthy hospital employees served as controls.

Mononuclear cell suspension. Mononuclear cells were obtained from heparinized peripheral blood by density gradient centrifugation using lymphocyte separating medium (Bionetics Laboratory Products, Kensington, MD) (13). The cells were washed twice, incubated at 37°C to elute surface proteins and washed again in RPMI 1640 (Gibco, Grand Island, NY). This cell suspension will be referred to as mononuclear cells.

Nonadherent lymphocyte preparation. To deplete the suspension of monocytes, 10×10^6 mononuclear cells were plated in 100×15-mm plastic Petri dishes (Falcon Labware, Oxnard, CA) at 37°C for 45 min in medium containing 10% FCS. The adherent cells were harvested and the procedure was repeated. This population will be referred to as nonadherent cells. It contained $2.0\pm0.7\%$ esterase-positive cells. The initial mononuclear cell population had $28\pm3\%$ esterase-positive cells.

In some experiments cells were passaged in nylon wool columns after the initial plating in Petri dishes (14). A 12-ml plastic syringe was packed with 0.6 g of nylon fibers (E. I. Dupont de Nemours & Co., Inc., Wilmington, DE). After the column was rinsed with medium containing 5% FCS and warmed to 37°C, cells in medium containing FCS were applied to the column. The syringe was further incubated at 37°C for 45 min. Nonadherent cells were eluted with RPMI. The percentage of T cells in this population was 87%; 1% were B cells and 1% were monocytes. There were 66% T cells and 9% B cells before the depletion.

High-avidity Fc^- lymphocyte population. To remove the cells with high-avidity Fc receptors for IgG, the mononuclear cells were allowed to attach to Petri dishes with immobilized BSA–anti-BSA immune complexes (15). To prepare these complexes, 2 ml of BSA (5 mg/ml) in PBS was added to 60×15-mm Petri dishes for 45 min at 37°C. After extensive washing with PBS, 2 ml of IgG anti-BSA (1:20 dilution) (Cappel Laboratories, Cochranville, PA) was added to the dishes and incubated for 45 min at room temperature. The plates were washed five times with PBS and 8×10^6 cells in 2 ml of medium were added. The plates were incubated for 1 h at room temperature and the nonadherent cells were recovered. This population will be referred as

high-avidity Fc⁻ lymphocytes. It also contained 1% Fc⁺ lymphocytes and 5% monocytes.

Depletion of OKT₄⁺ or OKT₈⁺ cells. To deplete T cells with the OKT8 or OKT4 marker, nonadherent cells were obtained by passage on Petri dishes and were adjusted to a concentration of 5 × 10⁶/ml. The cells were mixed with anti-OKT4 or anti-OKT8 monoclonal antibody (Ortho Diagnostic Systems Inc., Raritan, NJ) for 30 min on ice. After washing three times the cells were incubated with rabbit serum at a 1:20 dilution.

Separation of T cells, freezing, and co-cultivation procedures. These studies were designed to determine if the normal blastogenic response of post-leishmaniasis treatment cells to *Leishmania* antigen would be diminished by cultivation with the unresponsive mononuclear cells obtained before treatment. T cells were prepared from pretreatment mononuclear cells by isolating sheep erythrocyte rosette-forming cells by the procedure of West et al. (16). The only modification was that the sheep erythrocytes were not treated with neuraminidase. Briefly, 10 × 10⁶ lymphocytes in 1 ml were mixed with 10 × 10⁸ sheep erythrocytes in 4 ml of RPMI and 2 ml of FCS. After incubation for 1 h at 4°C, T cells were separated by density gradient centrifugation and sheep erythrocytes were lysed with a hypotonic solution. Viability of the lymphocytes was over 95% after this procedure.

The freezing procedure was performed using the method of Birkeland (17). The lymphocyte pellet was resuspended in freezing medium containing 50% RPMI, 40% human AB serum, and 10% DMSO. The cells (2 × 10⁶ cells/ml) were aliquoted in 1-ml volumes into Nunc vials (Nunc, Intermed Co., Copenhagen, Denmark). The freezing procedure utilized a cryobiological freezing apparatus (biological freezer type BF-5, Union Carbide Corp., Indianapolis, IN). The freezing apparatus was started on the "E," and gradually lowered to "A," and maintained there overnight. The cells were then stored in liquid nitrogen at −195°C.

After periods of 1–3 mo the cell suspension was rapidly thawed by placing it in a 37°C water bath for 2–3 min. The tubes were removed from the bath before the last ice on the tube melted. The suspension was immediately transferred to a centrifuge tube and 10 ml of RPMI medium with 20% AB serum was added slowly. The cells were washed twice and then centrifuged for 10 min at 1500 rpm at room temperature before use. Trypan blue staining showed > 90% viable cells. The co-culture experiments were performed by culturing different numbers of these pretreatment cells (5 × 10³ to 5 × 10⁴) with 1 × 10⁵ posttreatment mononuclear cells for 5 d in the presence of *Leishmania* antigen. In three healthy control subjects T cells were isolated from peripheral blood and frozen in the manner described above. The subjects were bled 1 wk later and the previously frozen T cells were added to the fresh mononuclear cells. In these experiments the cells were stimulated with *Candida albicans* antigen (final dilution of 1:40) and pokeweed mitogen (final dilution 1:100).

Lymphocyte blastogenesis assay. 2 × 10⁵ cells in 0.2 ml of RPMI 1640, supplemented with antibiotics and 15% heat-inactivated human AB serum, were cultured in triplicate in flat bottom microtiter plates (Linbro Chemical Co., New Haven, CT) for blastogenesis assays. Cell cultures were incubated (37°C, 5% CO₂–95% air) for 5 d with either *Leishmania* antigen in a concentration of 5 μg of protein per ml of culture, purified protein derivative (PPD)[1] 10 μg/ml of culture (Connaught Laboratories, Willow Dale, Ontario, Canada), *C. albicans* in a final dilution of 1:40, or PWM (Gibco) at a final concentration of 1:100. In three experiments, indomethacin in a final concentration of 1 μg/ml was added with the *Leishmania* antigen. Blastogenesis was quantitated by liquid scintillation spectometry, after a 4.5-h pulse of cell cultures with 1 μCi of [³H]thymidine (6.7 Ci/mM; New England Nuclear, Boston, MA). The results are expressed as the mean responses of [³H]thymidine incorporation in triplicate cultures.

Identification of mononuclear cells. The following procedures were used to determine the percentage of lymphocyte subpopulations and monocytes: T cells were characterized by their ability to bind sheep red

1. *Abbreviations used in this paper:* PPD, purified protein derivative.

blood cells (18); B cells were identified by detecting surface immunoglobulin with fluoresceinated goat anti–human globulin (Hyland Laboratories, Deerfield, IL) (19); non-T, non-B high-avidity Fc receptor cells were identified by indirect immunofluorescence after exposure of the cells to normal human serum (19); OKT4⁺ and OKT8⁺ cells were quantitated after exposure of the cells to anti-OKT4 or anti-OKT8 monoclonal antibody followed by fluoresceinated anti–mouse IgG (20); and monocytes were identified by nonspecific esterase staining (21).

Delayed hypersensitivity skin testing. PPD (Connaught Laboratories, Swiftwater, PA) and *C. albicans* antigen (Hollister-Steir Laboratories, Spokane, WA) were utilized in 0.1-ml volumes containing 5 TU and 1:1,000 dilution, respectively. Responses were considered to be positive if there was > 10-mm induration at 48 h.

Results

The age, sex, and lymphocyte reactivity to *Leishmania donovani chagasi*, PPD, and *C. albicans* antigens, and PWM mitogen in 11 patients with visceral leishmaniasis are shown in Table I. In vitro responses to *Leishmania* antigen were either absent or minimal in all 11 patients. The responsiveness of patient's cells was not enhanced by stimulation with different concentrations of *Leishmania* antigen (1.5, 5, and 25 μg of *Leishmania* antigen). Mononuclear cells cultured with antigen for periods of 3, 5, and 7 d were equally unresponsive (Table I footnote). Eight of the 11 patients responded (≥ 2,000 cpm) to either PPD, candida, or both antigens. All patients responded to PWM.

In an attempt to restore the lymphocyte blastogenesis to *Leishmania* antigen, mononuclear cells obtained from three patients with acute visceral leishmaniasis were depleted of monocytes and selected lymphocyte populations and [³H]-thymidine uptake was quantitated before and after these manipulations (Table II). To determine the role of adherent cells in suppressing the lymphocyte response to *Leishmania* anti-

Table I. Lymphocyte Blastogenesis to Leishmania, *PPD, and* C. albicans *Antigens and to PWM in Patients with Visceral* Leishmaniasis

Patients' age/sex	Leishmania (5 μm/ml)*	PPD (10 μg/ml)	C. albicans (1:100)	PWM (1:100)
yr			cpm	
1/M	855±70	530±64	2,023±299	24,978±806
6/M	422±93	315±72	944±230	10,430±701
9/M	544±29	9,765±146	612±71	10,306±908
14/M	153±16	10,889±570	425±166	13,869±1,230
15/F	138±29	254±24	244±31	28,715±9,725
15/F	1,425±250	1,553±514	2,041±441	33,158±1,184
17/M	453±82	719±65	2,326±310	33,158±2,212
19/M	561±96	17,321±887	30,016±4,847	17,046±1,136
22/M	1,583±11	3,016±368	4,511±1,021	17,186±124
24/M	2,567±477	1,290±110	1,631±195	5,829±100
67/M	302±34	11,072±996	4,416±767	8,989±529

Data represent mean±SE of [³H]thymidine incorporation. Background responses (cells without stimulation) were 467±107 cpm.
* In four experiments dose-response curves using 1.5, 5, and 25 μg of *Leishmania* antigen yielded 438±140, 442±101, and 285±56 cpm × 10⁻³ after 5 d of in vitro cultivation. The mean response of cells from three patients with acute disease cultured for 3, 5, and 7 d with *Leishmania* antigen (1 μg/ml) was 472±134, 571±192, and 324±63 cpm × 10⁻³, respectively.

Table II. Procedures Used to Attempt Restoration of Lymphocyte Reactivity to Leishmania Antigen in Patients with Acute Visceral Leishmaniasis

Treatment	Uptake	Percentage of target cells before and after depletion
	cpm	
None	490±244*	28 (M)
Plastic adherence	427±223	2 (M)
None	541±229	25 (M, B cells)
Nylon wool	406±68	1 (M, B cells)
None	378±104	12 (non-T, non-B, Fc+ cells)
BSA–anti-BSA complex	803±310	1 (non-T, non-B, Fc+ cells)
None	390±89	23 OKT8+ cells
Anti-OKT8	608±407	<1 OKT8+ cells
None	1,271±769	42 OKT4+ cells
Anti-OKT4	109±28	3 OKT4+ cells

Data for each depletion method represents the mean±SEM for three patients.
* Data represents [3H]thymidine uptake of cultures stimulated with Leishmania antigen.

gen, these cells were removed from the mononuclear cell preparations by either plating the cells in Petri dishes or passing the cells through nylon wool columns. The percentage of macrophages, as determined by nonspecific esterase staining, was 28±3% before plating in Petri dishes and was 2±1% after depletion. Passage of the cells in nylon wool columns similarly reduced the percentage of macrophages from 25% to 1%. Depletion of adherent cells by either method did not restore the response of the remaining mononuclear cells to Leishmania antigen. Before passage in Petri dishes the [3H]thymidine uptake of lymphocytes cultured with Leishmania antigen was 490±244 cpm, and after depletion it was 427±223 cpm. [3H]-Thymidine uptake before the depletion by nylon wool adherence was 541±229 cpm, and after depletion was 406±68 cpm. The reduced number of macrophages in the culture was not the explanation for the absence of response since [3H]-thymidine uptake after either C. albicans or PWM stimulation was preserved after depletion of adherent cells by both procedures. For example, in one experiment the in vitro response after 5 d cultivation with C. albicans antigen was 8,115±23 cpm after monocyte depletion and was 10,645±807 cpm before depletion. To extend the studies of the potential role of macrophages in suppressing lymphocyte proliferation, indomethacin was added to cultures of mononuclear cells stimulated with Leishmania antigen. Indomethacin did not restore the response to Leishmania antigen: [3H]thymidine uptake was 372±21 cpm (data not shown).

To determine the role of non-T, non-B, Fc receptor-positive cells in the suppression of lymphocyte reactivity, mononuclear cells from three patients were plated on a monolayer of BSA–anti-BSA immune complexes. This procedure depletes the high-avidity Fc receptor-positive cells (14). We reduced the number of Fc+ cells from 12% to 1% by this method (Table II). After the passage in plates containing the BSA–anti-BSA complexes, the mononuclear cell preparations contained 76% T cells, 13% B cells, and 5% monocytes. Depletion of Fc+ receptor cells did not restore the response to Leishmania antigen. [3H]Thymidine uptake of cultures stimulated with Leish-

mania antigen before passage on BSA–anti-BSA was 378±104 cpm and after depletion of Fc+ cells was 803±310 cpm.

To determine if depletion of OKT4+ or OKT8+ cells could revert lymphocyte blastogenesis to Leishmania antigen, mononuclear cells were treated with either anti-OKT8 or anti-OKT4 monoclonal antibody and complement. Treatment with anti-OKT8 plus complement reduced the percentage of OKT8+ cells from 23±3% to < 1% (Table II). Depletion of OKT8+ cells did not restore lymphocyte reactivity to Leishmania antigen. [3H]Thymidine uptake before OKT8+ cell lysis was 390±89 cpm, and after lysis it was 608±407 cpm. Depletion of OKT4+ cells also failed to restore lymphocyte blastogenesis to Leishmania antigen. Before OKT4+ cell depletion the [3H]thymidine uptake was 1,271±769 and after depletion it was 109±28.

Since lymphocytes from visceral leishmaniasis patients do not produce interleukin 2 (IL-2) when stimulated by Leishmania antigen, purified IL-2 (Genzyme Corporation, Boston, MA) in concentrations ranging from 10 to 1,000 U/ml was added to mononuclear cells stimulated with Leishmania antigen. [3H]Thymidine uptake of cultures containing only media was 149±51 cpm, in cultures containing only IL-2 it was 2,619±1,869, and in cultures containing antigen and IL-2 it was 1,995±1,318. Healthy subjects' mononuclear cells cultivated with media alone or in media containing IL-2 yielded 200±58 and 4,566±3,175 cpm, respectively.

T cell subpopulations in visceral leishmaniasis. The numbers of OKT4+ and OKT8+ cells per cubic millimeter of peripheral blood was determined in 10 visceral leishmaniasis patients (Table III). Decreased numbers of helper cells were found in four patients. There was a marked lymphopenia (< 700 mm³) in two patients. There was also a reduction in the number of OKT8+ cells in all but one of the patients with decreased OKT4 cell numbers. The OKT4+/OKT8+ ratio of the whole group was 1.6±0.7, which is not different (P > 0.05) from the ratio determined in healthy subjects in Brazil (2.2±0.7). All 10 patients were unresponsive to Leishmania antigens irrespective of whether they had normal numbers of OKT4+ and OKT8+ cells.

The role of cells in the immunosuppression to antigen. Restoration of the lymphocyte reactivity to Leishmania antigen is associated with successful treatment of visceral leishmaniasis

Table III. Number of Lymphocytes and OKT4+ and OKT8+ Cells in Peripheral Blood of Visceral Leishmaniasis Patients*

Age/sex	Lymphocytes	OKT4+	OKT8+	OKT4/OKT8 ratio
yr	No./ml‡			
2/F	2,015	544	604	0.9
2/M	2,400	744	504	1.5
3/F	3,796	1,746	1,100	1.6
6/M	1,584	997	427	2.3
17/M	2,200	888	289	3.0
17/M	414	128	87	1.5
19/M	1,760	458	492	0.9
21/M	2,800	1,372	672	2.0
21/M	832	258	242	1.0
38/M	620	130	92	1.4

* The expected absolute number of OKT4+ cells ranges from 468 to 1,143 and expected number of OKT8+ cells ranges from 192 to 726.
‡ Value given as no./ml of peripheral blood.

Table IV. Immunosuppression in Visceral Leishmaniasis: Evidence for the Role of T Cells

Cell source	[³H]Thymidine uptake*	
	Leishmania antigen	PWM
	cpm × 10³	
Mononuclear cells before antimony therapy	460±76	11,346±8
Mononuclear cells 1–3 mo after antimony therapy	4,293±1,442	8,072±5
Co-cultivation of pre- and posttherapy cells‡	530±149	5,917±5

* Results shown are the mean±SEM of studies of four patients.
‡ Mononuclear cell obtained after treatment (1 × 10⁵) were co-cultured with 5 × 10⁴ previously frozen pretreatment T cells from the same patient for 5 d in the presence of antigen or mitogen.

(6). To further evaluate the role of cells in suppressing lymphocyte reactivity, co-culture experiments were performed using mononuclear cells from cured patients and cells from the same patients obtained before treatment. Pre- and posttreatment cells from four patients were studied in this manner. Table IV shows that cells obtained during the acute disease were able to suppress blastogenesis of autologous lymphocytes obtained after successful treatment. In these experiments the addition of 5 × 10⁴ cells obtained during active disease decreased the response of the posttreatment mononuclear cells to *Leishmania* antigen from 4,293±1,442 cpm to 509±149 cpm, an 88% reduction. Co-cultivation of pre- and posttreatment cells did not significantly reduced the PWM response of the posttreatment cells (8,072±592 cpm to 5,917±571 cpm) (*P* > 0.05).

Fig. 1 shows that the cell-mediated suppression was dose dependent. In a representative experiment the [³H]thymidine uptake of mononuclear cells obtained after treatment was 7,618±1,027 cpm after *Leishmania* antigen exposure. When 5 × 10³ pretreatment cells were cultured with 1 × 10⁵ mononuclear cells the ³H incorporation was decreased to 2,360±70 cpm (69% reduction). When 5 × 10⁴ pretreatment cells were added the uptake decreased to 672±350 cpm (91% reduction). Surface markers in the erythrocyte rosette-forming cell population after the freezing procedure showed that 64% reacted with anti-OKT11 and that 7% of these cells were esterase positive.

To determine if a nonspecific effect of the freezing technique could account for the depression of lymphocyte reactivity to antigens, three healthy subjects with good lymphocyte reactivity to *C. albicans* were studied. These subjects had their cells frozen and after 1 wk they were co-cultured with fresh cells and stimulated by *C. albicans* antigen. The [³H]-thymidine uptake of the fresh cells was 4,560±1,321 cpm. Co-culture of 1 × 10⁵ fresh mononuclear cells with previously (5 × 10⁴) frozen T cells did not suppress the lymphocyte responsiveness (4,609±1,848 cpm). Autologous responsive posttreatment cells were also frozen and then co-cultured with fresh autologous responsive mononuclear cells. In this experiment, [³H]thymidine uptake of fresh cells cultured with *Leishmania* antigen was 17,971±845 cpm. Co-culture of 1 × 10⁵ fresh autologous cells with previously frozen autologous cells (5 × 10⁴) yielded 16,174±1,336 cpm after 5 d of in vitro
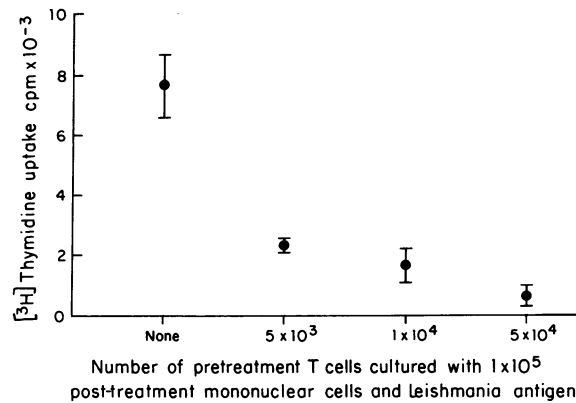

Figure 1. Leishmania antigen-specific cell-mediated immunosuppression.

cultivation. Control cultures with mononuclear cells cultured with autologous previously frozen cells in the absence of *Leishmania* antigen yielded 1,098±70 cpm.

Discussion

The present study documents that immunosuppression in visceral leishmaniasis is antigen specific and is cell mediated. Mononuclear cells from acute visceral leishmaniasis patients had a specific inability to respond to *Leishmania* antigen but most were able to proliferate when stimulated with either PPD or *C. albicans*. Studies testing delayed-type hypersensitivity reactions in leishmaniasis patients also have shown a lack of response to *Leishmania* antigen (1, 22).

The precise mechanisms of immunosuppression in visceral leishmaniasis are unknown. A decrease in the number of peripheral blood T cells is observed in one-fourth of patients (3). A decrease in OKT4⁺ is also seen in some patients. Antigen-reactive cells trapped in lymphoid tissues may account for these observations and contribute to the lack of response to *Leishmania* antigen by peripheral blood cells. However, the lack of lymphocytes in T-dependent areas of lymph nodes and the spleen does not favor this view point (23, 24). Furthermore, although there is an absence of IL-2 production by peripheral blood cells from patients with acute leishmaniasis (7), the addition of this lymphokine to the lymphocytes does not restore their responsiveness (11).

In co-culture experiments we were able to show that mononuclear cells obtained prior to the patients' antimony treatment suppressed the lymphocyte blastogenesis of cells obtained from the same patient after successful therapy. Mononuclear cells from healthy subjects did not suppress the lymphocyte response to PWM and *C. albicans* after the same freezing procedure used for the visceral leishmaniasis patients' cells. Furthermore, pretreatment cells from visceral leishmaniasis patients with active disease did not suppress the response of their posttreatment cells to either PWM or to *C. albicans*.

Attempts to restore responsiveness to *Leishmania* antigen in mononuclear cell preparations from patients with acute leishmaniasis were uniformly unsuccessful. Decreasing macrophage numbers by passage in Petri dishes or on nylon wool column did not restore the lymphocyte response. The addition of indomethacin also had no effect on [³H]thymidine uptake after stimulation with *Leishmania* antigen. Similarly, lymphocyte responsiveness was not restored by attempts to deplete the mononuclear cell preparations of either Fc⁺ cells, OKT4⁺,

or OKT8$^+$ cells. Sacks et al also were unable to reverse the specific unresponsiveness of mononuclear cells from patients with acute leishmaniasis by depletion of Leu-2$^+$ (OKT8$^+$) T cells (11). Each of the different cell types has been shown to have suppressor effects in different patient populations and systems. Macrophages have been reported to mediate suppression of lymphocyte blastogenesis in diffuse cutaneous leishmaniasis (25, 26) and in tuberculosis (27). Cell lines with the T8 phenotype isolated from borderline leprosy patients are capable of suppressing blastogenesis to leprosy antigen (28). Fc$^+$ cells are able to suppress lymphocyte blastogenesis in the presence of immune complexes (29).

The inability to restore *Leishmania* antigen responsiveness to cells obtained during acute disease by depletion of lymphocyte subpopulations is in apparent conflict with the co-cultivation experiments indicating that a suppressor cell is present during acute disease. There are several potential explanations for these findings: (*a*) The targeted subpopulations were markedly decreased but not totally depleted—the few cells remaining may have been sufficient to exert a suppressive effect, (*b*) The suppressive effect on the potential responder cells may not be reversed by removal of the suppressor cell, and (*c*) The suppressor cell may have a phenotype other than those targeted for in the depletion experiments.

Acknowledgments

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