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REDUCTION IN THE NUMBER OF UCHL-1$^+$ CELLS AND IL-2 PRODUCTION IN THE PERIPHERAL BLOOD OF PATIENTS WITH VISCERAL LEISHMANIASIS[1]

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PBMC from patients with visceral leishmaniasis (VL), before and after successful antimony therapy, were analyzed for their phenotypes and for their ability to produce IL-2 and IFN-γ and to proliferate against PHA and leishmanial Ag. In agreement with results of earlier studies, PBMC from active VL patients showed a markedly reduced proliferative response and IL-2 and IFN-γ production, compared with those of healthy controls. The levels of CD4$^+$ and CD8$^+$ T cells were within the normal range, but there was a significant decrease in UCHL-1$^+$ cells (helper-inducer), compared with healthy individuals. The inhibited cellular responses, and lymphokine secretion and decreased level of UCHL-1$^+$ cells in the PBMC of the VL patients returned to the normal range after successful chemotherapy. PBMC from active VL patients were fractionated into adherent cells and nonadherent cells, and the nonadherent cells were further fractionated into UCHL-1$^+$ and UCHL-1$^-$ subpopulations. Results from cell depletion and reconstitution experiments suggest that the IL-2 production by nonadherent cells stimulated with PHA was inhibited by adherent cells, but the IL-2 production by nonadherent cells in response to specific Ag was not. In contrast, UCHL-1$^-$ cells seem to mediate the inhibition of Ag-driven IL-2 production by nonadherent cells but not mitogen-stimulated IL-2 secretion by nonadherent cells. Ag-specific IL-2 production principally involves UCHL-1$^+$ cells.

Leishmania donovani are protozoan parasites that infect mononuclear phagocytes and are causative agents of VL.[3] The disease has been found to be associated with impairment of T cell responsiveness against parasites and mitogens during the active stage of the disease. Thus, VL patients failed to respond to *L. donovani* Ag in terms of delayed-type hypersensitivity (1, 2), lymphocyte proliferation (3, 4), and IL-2 and IFN-γ production in vitro (5). These immune responses were restored after a successful course of antimony therapy (4–7). The mechanism of immune suppression is complex. We have previously demonstrated, using cryopreserved PBMC, that patients with active VL possess an adherent cell population capable of suppressing IL-2 production by autologous PBMC after recovery (7).

In order to investigate further whether the immune impairment is due to a cellular defect, we have studied the T cell subsets during *L. donovani* infection and the effect of selective depletion and reconstitution of some of these subsets on IL-2 production. In particular, we examined the populations of PBMC expressing the 180-kDa and 200–220-kDa components of leukocyte common Ag (CD45).

The mAb UCHL-1 (8) and 4B4 (9) recognize the 180-kDa component of the CD45 family, whereas Leu-18 (10, 11) and 2H4 (12) recognize determinants expressed only on the higher m.w. forms (200–220 kDa) that are termed CD45R. Thus, it is possible to divide PBMC, and particularly CD4$^+$ T cells, into UCHL-1$^+$ (CD45$^+$, 4B4$^+$, Leu-18$^-$, 2H4$^-$) and UCHL-1$^-$ (CD45R$^+$, 4B4$^-$, Leu-18$^+$, 2H4$^+$) populations. The UCHL-1$^+$, CD4$^+$ T cells (helper-inducer) have been shown to be good producers of helper signals for PWM-induced Ig synthesis, early producers of IL-2, and the main producers of IFN-γ (8, 10). The UCHL-1$^-$, CD4$^+$ T cells (supressor-inducer) provide only minimal help to B cells for PWM-induced Ig synthesis, function as inducers of CD8$^+$ Ts cells, and are late producers of IL-2 and very poor producers of IFN-γ (10, 12). These surface markers are also known to be cellular activation determinants distinguishing 'virgin' (UCHL-1$^-$) and 'memory' (UCHL-1$^+$) cells. Significant changes in the levels of these two subsets of T cells, compared with healthy controls, have been reported for several clinical diseases, such as leprosy (13), tuberculosis (14), rheumatoid arthritis (15, 16), diabetes (17), SLE (18), and multiple sclerosis (19). It is, therefore, of interest to investigate the levels of UCHL-1 T cell subsets in clinical VL, a disease in which immunologic impairment in the response to mitogen as well as to specific Ag is particularly apparent. The results reported here indicate that there is a significant reduction in the percentage of UCHL-1$^+$ cells in the PBMC population of active VL patients, compared with healthy controls. The level of UCHL-1$^+$ cells is restored to the normal range following successful chemotherapy. Furthermore, whereas the plastic-adherent cells are involved in the inhibition of mitogen-induced IL-2 production by non-

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[3] Abbreviation used in this paper: VL, visceral leishmaniasis.

adherent cells, the suppression of Ag-specific IL-2 secretion is mediated by the UCHL-1$^-$ population of cells.

MATERIALS AND METHODS

Subjects. Five Sicillian patients, one woman and four men, ranging in age from 25 to 55 years, were studied. Patient evaluation included history and physical examinations. They all had characteristic signs and symptoms of active VL, including irregular fever, hepatosplenomegaly, anemia, leukopenia, and hyperglobulinemia. The diagnosis was confirmed by the indirect hemagglutination assay, counterimmunoelectrophoresis, indirect immunofluorescence using a local clinical isolate of *L. donovani* (20), and demonstration of leishmania parasites in bone marrow aspirates by using Giemsa staining or culturing. Fifteen healthy laboratory workers served as normal controls. Patients were studied at the time of positive diagnosis and after antimony therapy and disease cure. Antimony therapy was carried out with meglumine antimoniate (Glucantime; 85 mg of Sb^{5+} per ml). The drug was administered i.m. four times per day, equaling a daily dose of 100 mg/kg, for 15 days. Treatment was repeated after 2 weeks at least once, according to the clinical conditions of the patients.

Tissue culture medium. Tissue culture medium consisted of RPMI 1640 (Flow Laboratories, Herts., UK) supplemented with glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μg/ml), 2-ME (50 μM), and 5, 10, or 20% FCS (Flow).

Leishmania Ag. L. donovani 25 was used as the source of promastigotes and soluble Ag in all studies. This strain was characterized by isoenzyme analysis (7). Leishmania Ag were prepared by six cycles of freezing (−70°C) and thawing (37°C) of a suspension of 2×10^8 parasites/ml in PBS. The extract was then centrifuged at 20,000 × *g* for 10 min. The supernatant was collected and kept in aliquots at −20°C.

Isolation of PBMC. Human PBMC were isolated as described previously (21). Briefly, heparinized venous blood was diluted 1/2 with RPMI 1640 buffered with HEPES, layered on Ficoll-Hypaque (Sigma Chemical Co., St. Louis, MO), and centrifuged at 400 × *g* for 30 min at room temperature. Cells recovered from the interface were washed twice in RPMI 1640 containing 10% FCS. These cells contained between 75 and 85% of the original leukocytes, with over 95% viability as assessed by trypan blue exclusion.

Enumeration of T cell subsets by mAb. Cell surface phenotype was determined by indirect immunofluorescence using mouse mAb. Cells expressing CD3, CD4, CD8, CD22, and CD11c Ag were enumerated by using Leu mAb (Becton Dickinson, Mountain View, CA), according to the manufacturer's instructions, and 180-kDa determinants of the leukocyte common Ag ($CD45R^-$), by UCHL-1 mAb (8) (a generous gift of Dr. P. C. L. Beverley, University College, London). In all cases, the second-stage antibody was fluorescein-labeled goat anti-mouse IgG (Meloy Laboratories, Springfield, VA) (22).

Cell fractionation. PBMC (1×10^7) were incubated (in 5 ml of medium plus 20% FCS) for 4 h in 100-mm plastic petri dishes (Falcon, Oxnard, CA), at 37°C and 5% CO_2. The dishes were precoated with FCS (2 h at 37°C). Nonadherent cells were removed by vigorous shaking with three changes of warm medium. The adherent cells were harvested by incubation of the dishes with cold PBS, containing 0.2% EDTA and 5% FCS, for 40 min at 4°C, followed by vigorous pipetting. The adherent cell population was more than 80% $CD11c^+$. In some experiments, subpopulations of human nonadherent cells defined by binding of UCHL-1 mAb were separated into mutually exclusive $UCHL\text{-}1^+$ and $UCHL\text{-}1^-$ subpopulations, using a panning technique (23). Briefly, PBMC nonadherent cells (2×10^6/ml) were incubated in PBS, pH 7.4, containing 1% FCS (PBS-FCS), with an appropriate dilution of UCHL-1 mAb for 1 h at 4°C. The cells were then washed four times in ice-cold PBS-FCS and were plated onto plastic dishes precoated with rabbit anti-mouse Ig (Wellcome Research Laboratories, Beckenham, UK). The dishes (10 cm in diameter; Falcon) had been coated with $F(ab')_2$ rabbit anti-mouse IgG (100 μg in 15 ml of 0.05 M Tris, pH 9.4) overnight at 4°C and washed twice with PBS and three times with PBS-FCS before use. After incubation for 1 h at 4°C, the nonattached cells were gently aspirated, washed twice, and resuspended in RPMI containing 5% FCS. Such cells were >80% $UCHL\text{-}1^-$, 20% $UCHL\text{-}1^+$, 1% $CD11c^+$, and 5% $CD22^+$. The adherent fraction, >85% $UCHL\text{-}1^+$, was removed with a rubber policeman and vigorous flushing.

In vitro stimulation of IL-2 and IFN-γ production. PBMC and the fractionated cells were adjusted to 5×10^6 cells/ml in medium and incubated for 24 h, with or without 5 μg/ml PHA (Wellcome Research Laboratories) or 1×10^7 organisms equivalent/ml leishmanial Ag. This concentration of PHA is not cytotoxic, as tested on cloned CTL (7). Supernatants were collected, filtered through 0.22-μm Millex filters (Millipore S.A., Molshiem, France), and stored at −70°C until tested.

Assay for IL-2 activity. IL-2 activity was determined by its capacity to support the growth of a murine IL-2-dependent CTL cell line (24). CTL were resuspended at 1×10^5 cells/ml, and 100 μl were cultured with 50 μl of test sample (at a final concentration of 5% FCS) for 24 h at 37°C and 5% CO_2. Six hours before harvesting, the cultured cells were pulsed with 1 μCi of [^{3}H]thymidine (24 Ci/mmol) (Radiochemical Centre, Amersham, UK). Cells were harvested using an automatic cell harvester (Titertex), and radioactivity was measured with a β-counter (Beckman Instruments Inc., Milano, Italy). The results from quadruplicate cultures were expressed as units per ml (mean ± SD). IL-2 from Genzyme Corporation (Boston, MA) was used to generate a standard curve for determination of units of IL-2 activity.

Assay of IFN-γ activity. Biologically active IFN in culture supernatants was assayed by an IFN immunoradiometric commercial kit (Centocor γ-IFN RIA; Centocor Inc., Malvern, PA). The IFN-γ present in the supernatants was bound to polystyrene beads coated with mouse mAb specific for human IFN-γ, and the immune complex was bound to an anti-IFN-γ mAb labeled with ^{125}I. Unbound labeled antibody was removed by aspiration and the bound radioactivity was determined by counting of the beads in a Beckman γ 5500 counting system. The bound radioactivity was proportional to the concentration of IFN-γ in the sample, within the working range (1–1024 U/ml) of the assay. A standard curve was obtained by plotting the IFN-γ concentration of the standard bound radioactivity.

Lymphocyte proliferation assay. PBMC from patients and controls were resuspended at 5×10^5 cells/ml in culture medium, and 100 μl of these suspensions were incubated with PHA (1 μg/ml) (Wellcome Research Laboratories) or 1×10^7 organisms equivalent/ml leishmanial Ag for 72 h at 37°C and 5% CO_2. The cultures were pulsed with 1 μCi of [^{3}H]thymidine for 6 h and harvested, and the uptake of radioactivity was measured with a β-counter (Beckman), as described above for the IL-2 assay. The results of quadruplicate cultures were expressed as [^{3}H]thymidine incorporation, in cpm (mean ± SD).

Statistical analysis. SD and SEM were calculated, and statistical significance was analyzed by Student's *t*-test; $p < 0.05$ was considered statistically significant.

RESULTS

Proliferative response and IL-2 and IFN-γ production. PBMC from VL patients at the time of diagnosis and after successful antimony treatment, together with those from healthy individuals, were tested for their proliferative response and IL-2 and IFN-γ production when stimulated with PHA and leishmanial Ag *in vitro*. The optimal concentrations of PHA and leishmanial Ag were determined in preliminary experiments and had been used in earlier studies (7). As expected from results of these earlier studies, PBMC from patients with active VL produced significantly lower levels of proliferative response to PHA, compared with healthy controls. They also secreted markedly lower amounts of IL-2 and IFN-γ when stimulated with PHA *in vitro* (Table I). These responses were restored to the levels of normal controls following successful chemotherapy. Proliferation and IL-2 and IFN-γ production by the PBMC in response to leishmanial Ag were also significantly higher in patients after successful chemotherapy, compared with those before treatment (Table I).

$UCHL\text{-}1^+$ cells in PBMC of VL patients. To seek a possible explanation for the reduction in proliferative response and lymphokine production in the PBMC of VL patients, the cells were analyzed for $UCHL\text{-}1^+$ subpopulation. This subset of T cells are known to be the major IL-2 and IFN-γ producers (8, 10). Table II shows that, although there was a significant reduction in the total lymphocyte count in the PBMC of patients with active VL, compared with controls, the percentages of $CD4^+$ and $CD8^+$ T cells remained comparable in all three groups. In contrast, these patients had markedly reduced levels of

TABLE I

Proliferative response and IL-2 and IFN-γ production by PBMC of VL patients before and after successful chemotherapy

Subjects	*In Vitro* Stimulation[a]	Proliferation (cpm × 10^{-3})	Production (U/ml)	
			IL-2	IFN-γ
Patients before chemotherapy	L.Ag	2.8 ± 1.0[b]	26 ± 13[b]	14 ± 5[b]
	PHA	109.0 ± 16.5[c]	322 ± 101[c]	139 ± 30[c]
Patients after chemotherapy	L.Ag	12.9 ± 2.9	162 ± 58	134 ± 26
	PHA	174.5 ± 7.6	539 ± 56	179 ± 37
Healthy Controls	L.Ag	0.1 ± 0.1	14 ± 6	17 ± 8
	PHA	148.4 ± 9.1	691 ± 138	223 ± 39

[a] L.Ag, leishmanial Ag; for details, see *Materials and Methods*.
[b] Figures are significantly different from those of patients after chemotherapy (line 3); mean ± 1 SEM, $n = 5$.
[c] Figures are significantly different from those of healthy controls (line 6); mean ± 1 SD, $n = 5$.

UCHL-1$^+$ cells, compared with healthy controls. UCHL-1$^+$ cells returned to normal levels following successful chemotherapy (Table II).

Effects of depletion of adherent and UCHL-1$^+$ cells. PBMC from patients with active VL or from healthy individuals were depleted of adherent cells or UCHL-1$^+$ cells, and the residual populations were tested for their ability to produce IL-2 after stimulation with leishmanial Ag or PHA in vitro. Table III shows that depletion of adherent cells enhanced IL-2 production following PHA stimulation but had no significant effect on Ag-specific activation of IL-2 production. In contrast, depletion of UCHL-1$^+$ cells markedly reduced Ag-specific stimulation of IL-2 secretion but had little or no effect on PHA-induced IL-2 production. The cell depletion procedure also had little effect on IL-2 production by PBMC from healthy individuals (Table III). Thus, these results suggest that, in VL patients, mitogen-induced IL-2 production is inhibitable by adherent cells, whereas Ag-specific IL-2 secretion involves UCHL-1$^+$ cells.

Effect of adherent cells and UCHL-1$^-$ cells on IL-2 production. PBMC from active VL patients were fractionated into plastic-adherent, nonadherent, UCHL-1$^+$, and UCHL-1$^-$ subpopulations. Reconstitution experiments were carried out to investigate the influence of these populations on IL-2 production by the nonadherent cells. Table IV shows that the nonadherent cells produced significantly higher levels of IL-2 than unfractionated cells when stimulated with PHA. This elevated IL-2 secretion by nonadherent cells in response to PHA was reduced to the level of the unfractionated cells by reconstitution with adherent cells but not with UCHL-1$^+$ or UCHL-1$^-$ cells. In contrast, Ag-specific IL-2 production was not affected by the depletion of adherent cells, and the IL-2 produced by nonadherent cells in response to leishmanial Ag was also not influenced by the addition of adherent cells. It was, however, significantly enhanced by reconstitution with UCHL-1$^+$ cells and markedly reduced to background levels by the addition of UCHL-1$^-$ cells to the culture. The reduction of IL-2 production by UCHL-1$^-$ cells in culture is unlikely to be attributable to a dilution of the nonadherent cells by the non-IL-2-producing UCHL-1$^-$ cells, because cell titration experiments have shown that nonadherent cells produced significant levels of IL-2 even at a concentration of 1×10^6 cells/ml (data not shown).

DISCUSSION

In the murine leishmanial model, it is generally believed that Th1 subsets of CD4$^+$ T cells secreting IL-2 and IFN-γ are host protective, whereas the Th2 cells producing IL-4 and IL-5 are disease enhancing (25–27). Thus, Th1 cells predominate in the spleen and lymph nodes of resistant strains of mice recovered from *Leishmania major* infection, whereas Th2 cells are the major cell type in these lymphoid organs in the susceptible strain of mice with progressive *L. major* infection. Whether such a dichotomy of CD4$^+$ T cell responses also occurs during VL infection is at present unclear. The classification of Th1 and Th2 subsets in humans is also controversial. However, it seems clear that certain cell surface markers such as those of the T200 series are capable of separating human CD4$^+$ T cells into two distinct categories, the helper-inducer (UCHL-1$^+$) and the suppressor-inducer (UCHL-1$^-$). Data reported here suggest that, compared with the PBMC of healthy controls, the PBMC from active VL patients have reduced levels of UCHL-1$^+$ cells, which are responsible for IL-2 and IFN-γ production. It is unclear at present whether the UCHL-1$^+$ cells reported here are analogous to the Th1 cells in murine cutaneous leishmaniasis. However, the reduction in the number of UCHL-1$^+$ cells (Table II) plus the inhibition of IL-2 secretion by the UCHL-1$^-$ cells (Table IV) may account for the immunologic impairment consistently found in VL patients (1–5).

In an earlier report (7), we demonstrated that plastic-adherent cells from VL patients were able to inhibit the PHA-induced IL-2 secretion by PBMC from patients successfully treated with antimony. We now show that these adherent cells were not able to affect Ag-specific IL-2 secretion by the nonadherent cells. Instead, this Ag-stimulated IL-2 production was markedly inhibited by the UCHL-1$^-$ population. UCHL-1$^-$ cells, however, were not able to influence PHA-induced IL-2 secretion. Thus, the mitogen-induced and the Ag-stimulated types of IL-2 production were regulated by two separate populations of cells, whose actions appeared to be mutually exclusive. This finding may explain the earlier observation (28) that

TABLE II

T cell subset analysis of PBMC from VL patients before and after successful chemotherapy

Subjects	Total Lymphocytes (× 10^3/ml)	Subsets [% (number/ml)]		
		CD4$^+$	CD8$^+$	UCHL-1$^+$
Patients before chemotherapy	1461 ± 306[a]	47 ± 7 (675 ± 160)	40 ± 4 (539 ± 263)	25 ± 7 (367 ± 140)[a]
Patients after chemotherapy	1740 ± 638	43 ± 4 (706 ± 246)	30 ± 6 (604 ± 159)	36 ± 6 (654 ± 315)
Healthy Controls	2425 ± 176	40 ± 7 (958 ± 285)	29 ± 2 (692 ± 151)	40 ± 9 (949 ± 278)

[a] Figures are significantly different from healthy controls; mean ± 1 SD, $n = 5$.

TABLE III

Effects of the depletion of adherent or UCHL-1$^+$ cells on IL-2 production by PBMC of VL patients[a]

Subjects	*In Vitro* Stimulation	IL-2 Production [U/ml (% increase)][b]		
		Unfractionated cells	Adherent-depleted cells	UCHL-1$^+$-depleted cells
Patients	L.Ag[c]	26 ± 2	33 ± 4 (16 ± 17)	9 ± 1 (−64 ± 7)[d]
	PHA	328 ± 30	564 ± 38 (75 ± 18)[d]	440 ± 45 (33 ± 4)
Healthy Controls	L.Ag	7 ± 4	9 ± 4	8 ± 3
	PHA	755 ± 31	785 ± 98 (3.9)	700 ± 52 (−7.2)

[a] PBMC were obtained from active VL patients before chemotherapy. The depletion of adherent cells and UCHL-1$^+$ cells was described in *Materials and Methods*.

[b] Percentage of increase was calculated as [(U/ml fractionated − U/ml unfractionated)/(U/ml unfractionated)] × 100. Negative values represent decreases in IL-2 production.

[c] L.Ag, leishmanial Ag.

[d] Figures are significantly different from those of the unfractionated cells stimulated with leishmanial Ag or PHA, respectively (column 3).

TABLE IV

Effects of adherent cells (A), UCHL-1$^+$ cells, or UCHL-1$^-$ cells on IL-2 production by nonadherent cells (NA) of PBMC from VL patients[a]

Subjects	*In Vitro* Stimulation	IL-2 Production (U/ml)				
		Unfractionated cells	NA	NA + A	NA + UCHL-1$^+$	NA + UCHL-1$^-$
Patients	L.Ag[b]	23 ± 1	27 ± 2	23 ± 2	42 ± 2[c]	9 ± 5[c]
	PHA	150 ± 70	280 ± 5[c]	133 ± 76	265 ± 25[c]	257 ± 7[c]
Healthy Controls	L.Ag	5 ± 2	4 ± 1	4 ± 1	4 ± 2	6 ± 2
	PHA	245 ± 40	290 ± 90	280 ± 45	210 ± 60	230 ± 45

[a] PBMC were obtained from active VL patients before chemotherapy. Cell fractionated was described in detail in *Materials and Methods*. All cultures contained cell numbers equivalent to that expected in the original unfractionated cell population (5×10^6 cells/ml).

[b] L.Ag, leishmanial Ag.

[c] Figures are significantly different from those of the unfractionated cells stimulated with leishmanial Ag or pHA, respectively (column 3).

the Ag-specific unresponsiveness in Indian Kala-azar patients was not reversible by the depletion of CD8$^+$ T cells, because it is conceivable that the inhibition of immune responses in these patients may be mediated by UCHL-1$^-$, CD4$^+$ T cells, as described here.

The 180-kDa (UCHL-1) determinants are expressed on monocyte/macrophages and polymorphonuclear leukocytes as well as T cells (CD4 and CD8) (29). On peripheral T cells, the presence of UCHL-1 Ag seems to be related to immunologic memory function (30). The majority of the UCHL-1$^+$ and UCHL-1$^-$ cells studied in the present report were CD3$^+$ T cells (data not shown), because the reconstitution experiments (Table IV) were carried out with these populations of cells separated from nonadherent cells purified by Ficoll-Hypaque gradients. The expression of UCHL-1 on T cells during their life span may be biphasic (30). Immature thymocytes are >95% UCHL-1$^+$, and they lose their marker with concomitant gain of CD45R (200-kDa) determinants on maturation to virgin mature T cells (UCHL-1$^-$). These T cells then leave the thymus and do not reexpress UCHL-1 until antigenic stimulation has occured. At this time, CD45R determinants are lost and UCHL-1 determinants reappear. The extrathymus stage is thought to be unidirectional, i.e., UCHL-1$^-$ → UCHL-1$^+$ only. It is, therefore, intriguing that the PBMC of VL patients should show fewer UCHL-1$^+$ cells in the presence of continuous parasitic Ag stimulation. Furthermore, restoration of the level of UCHL-1$^+$ cells to the normal range was achieved after successful chemotherapy, a process that greatly reduces the parasite load in the patients. It may be that the UCHL-1$^+$ cells are more susceptible to Ag-induced deletion or, alternatively, this population is abrogated by the UCHL-1$^-$ cells in a manner analogous to that in the murine system, whereby Th2 cells can secrete a factor (cytokine synthesis-inhibiting factor, IL-10) that prevents the Th1 cells from secreting IL-2 and IFN-γ (31). Another possibility is the preferential entrapment of the memory UCHL-1$^+$ T cells in the sites of infection, in such a way that the level of circulating memory T cells is significantly reduced. The accumulation of helper-inducer (CD4$^+$, 4B4$^+$) T cells in the lesions of tuberculoid leprosy has been reported (13). However, in this case, 4B4$^+$ T cells also outnumbered 2H4$^+$ T cells in the peripheral blood of these patients.

In this report, we have so far concentrated on IL-2 production. The profiles of secretion of other lymphokines and their modulation by UCHL-1$^+$ and UCHL-1$^-$ cells in leishmaniasis is currently under investigation. Finally, the pattern of immunologic unresponsiveness among the VL patients of different geographical regions appears to vary. Thus, unlike Sicillian and Kenyan patients, Indian (4, 28) and Brazillian (5) VL patients showed only leishmanial-specific hyporesponsiveness and no diminution of the PHA-induced response. It would be of interest to investigate whether UCHL-1$^-$ cells also play a role in the immunologic impairment in these patients.

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