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NOTES

Th1/Th2 Cytokine Profile in Patients Coinfected with HIV and *Leishmania* in Brazil▽†

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To evaluate the effects of HIV on immune responses in cutaneous leishmaniasis (CL), we quantified cytokine levels from plasma and stimulated peripheral blood mononuclear cells (PBMCs) from individuals infected with HIV and/or CL. Gamma interferon (IFN-γ) and interleukin 13 (IL-13) levels and the ratio of IFN-γ to IL-10 produced in response to stimulation with soluble *Leishmania* antigens were significantly lower in HIV-*Leishmania*-coinfected patients than in CL-monoinfected patients.

The clinical outcome of *Leishmania* infection depends on the balance between Th1 and Th2 cytokines (4, 9, 31). HIV infection alters the Th1/Th2 balance in patients with visceral leishmaniasis, resulting in increased Th2 cytokine responses and decreased levels of interleukin 12 (IL-12) and IL-18 (31). Here, we evaluate the effects of HIV on cytokine responses in cutaneous leishmaniasis (CL).

Eight HIV-1-infected patients with CL, 9 HIV-1-seronegative patients with CL, and 21 HIV-infected patients were studied. Institutional review board approval was obtained from Comissão Nacional de Ética em Pesquisa (CONEP), FIOCRUZ, and UCSD's Human Research Protection Program.

Peripheral blood mononuclear cells (PBMCs) were separated by Ficoll-Hypaque centrifugation (Amersham Biosciences) and resuspended in RPMI 1640 (Sigma-Aldrich) supplemented with 2 mM L-glutamine, penicillin (100 U/ml), streptomycin (100 μg/ml) (GIBCO), and 5% heat-inactivated human AB serum. Cells were stimulated with soluble *Leishmania* antigen (SLA) at 10 μg/ml (11), HIV p24 antigen at 2 μg/ml (Protein Sciences, Meriden, CT), or medium alone and incubated for 48 h at 37°C with 5% CO_2. Supernatants were collected, stored at −20°C, and analyzed for IL-2, IL-4, IL-10, IL-13, tumor necrosis factor alpha (TNF-α), and gamma interferon (IFN-γ) using the Luminex 200 system (Austin, TX) (8). The dynamic range of this multiplex assay is from 0.2 pg/ml to 32,000 pg/ml. Only 6 HIV-1-infected subjects with CL, 9 HIV-1 subjects, and 5 subjects with CL had supernatant analyzed for IL-13. Plasma samples were analyzed using the cytometric bead array human Th1/Th2 cytokine kit II (BD, San Jose, CA) (6). The limit of detection for this assay ranged from 2.6 pg/ml for IL-4, 2.8 pg/ml for IL-10 and TNF-α, and 7.1 pg/ml for IFN-γ. IFN-γ/IL-10, IFN-γ/IL-13, and TNF-α/IL-10 ratios were calculated using measured values. Patients who had undetectable levels of cytokines were excluded from the cytokine ratio analysis. Flow cytometry was performed using FACSort (Becton Dickinson, Mountain View, CA).

Statistical analysis was performed using Prism (GraphPad, San Diego, CA) software. Supernatant cytokine concentrations were normalized by subtracting the amount of cytokine produced in culture with medium alone. The Mann-Whitney test was used for median comparisons.

Epidemiological and clinical characteristics of the patients with CL are shown in Table 1. The median $CD4^+$ T cell count in the HIV group was higher than that in the HIV-*Leishmania*-coinfected group (415 cells/mm^3 [range, 108 to 1,005 cells/mm^3] versus 203 cells/mm^3 [range, 78 to 615 cells/mm^3]; $P = 0.05$). The median plasma viral load was similar between groups ($\log_{10}$ 3.3 [1.9 to 5.8] copies/ml versus $\log_{10}$ 4.8 copies/ml; $P = 0.3$).

PBMCs from HIV-1-infected individuals with CL produced less IFN-γ and IL-13 in response to SLA than those from HIV-1-seronegative patients with CL (Fig. 1; see also Table S1 in the supplemental material). A significant difference in the median IFN-γ/IL-10 ratio between coinfected individuals (0.2; range, 0.1 to 0.4) and those with CL alone (4.7; range, 0.3 to 152.5) ($P = 0.004$) was also noted (Fig. 1). Moreover, decreased levels of IFN-γ and the median IFN-γ/IL-10 ratio were also observed in the plasma of coinfected patients compared to those with CL alone (see Table S1 in the supplemental material).

PBMCs from patients with HIV-*Leishmania* coinfection produced significantly more IFN-γ (median, 49 versus 5.6 pg/ml; $P = 0.02$) and IL-10 (median, 227 versus 0.0 pg/ml;

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TABLE 1. Clinical and epidemiological characteristics of the HIV-*Leishmania*-coinfected and *Leishmania*-infected patients[a]

Patient ID	Age (yr)	Gender	Clinical status	No. of T $CD4^+$ lymphocytes/mm^3	Viral load (log_{10} copies/ml)
HIV/Leish3	42	F	Cutaneous mucosal active	93	5.4
HIV/Leish5	22	F	Cutaneous active	291	1.9
HIV/Leish6	36	M	Cutaneous treated	165	5.3
HIV/Leish8	62	F	Cutaneous treated	457	2.7
HIV/Leish9	59	M	Cutaneous active	78	5.0
HIV/Leish12	36	M	Cutaneous active	615	1.9
HIV/Leish13	36	M	Cutaneous active	104	5.0
HIV/Leish14	25	M	Cutaneous mucosal active	241	4.6
Median (range)	36 (22–62)			203[b] (78–615)	4.8 (1.9–5.4)
Leish24	69	M	Cutaneous mucosa active		
Leish28	37	M	Cutaneous active		
Leish31	20	M	Cutaneous active		
Leish32	22	F	Cutaneous active		
Leish33	66	M	Cutaneous active		
Leish35	63	M	Cutaneous active		
Leish36	27	M	Cutaneous mucosal active		
Leish37	76	M	Cutaneous active		
Leish38	38	F	Cutaneous mucosal active		
Median (range)	38 (20–76)				

[a] HIV-infected group ($n = 22$): median number of $CD4^+$ T cells, 415 cells/mm^3 (range, 108 to 1,005 cells/mm^3); median viral load, log_{10} 3.3 copies/ml (range, 1.9 to 5.8 copies/ml); median age, 39 years (range, 24 to 72 years); 62% male. M, male; F, female.
[b] The median CD4 count in the HIV-infected group was significantly different from the coinfected group (Mann-Whitney U test).

$P = 0.03$) than those of patients with HIV-1 infection alone in response to HIV-1 p24 Ag (Fig. 2). Except for one outlier, the IFN-γ/IL-10 ratios were similar in the two groups (Fig. 2).

HIV-1 potentiates *Leishmania* infection in humans and vice versa (30). Most reports of HIV infection unleashing leishmaniasis as an opportunistic infection have been associated with viscerotropic *Leishmania* strains (1, 13, 23, 24). However, scattered studies have reported more aggressive manifestations of CL, such as relapsing and diffuse cutaneous disease, in HIV-infected patients (15, 19, 22, 25, 26). Mucocutaneous disease may also be more severe in the setting of HIV coinfection (2, 20).

The balance between inflammatory and regulatory cytokines creates a controlled immune response that promotes parasite killing but not tissue destruction during CL infection (5, 7). In subjects uninfected with HIV, the levels of IFN-γ and IL-10 produced by immune cells during asymptomatic *Leishmania* infections and those with limited cutaneous pathology are balanced. In contrast, in mucocutaneous leishmaniasis, high levels of IFN-γ and TNF-α and low levels of IL-10 are associated with destruction of the mucous membranes in the nose, mouth, and throat cavities and surrounding tissues (14).

In contrast to both visceral leishmaniasis and HIV infection where IFN-γ responses are diminished (10, 12, 17), during CL infection, levels of IFN-γ measured at the lesion and from circulating PBMCs remain close to normal (21). This study demonstrates that PBMCs from HIV-1-infected CL subjects produce less IFN-γ in response to SLA than HIV-1-seronegative CL subjects. While many cytokine responses are blunted in HIV-1 infection, in this study the IL-10 response was not decreased in the coinfected subjects, resulting in a decreased IFN-γ/IL-10 ratio of cytokine production. Although production of the Th2 cytokine IL-13 was significantly lower in the coinfected group, there was a trend for the IFN-γ/IL-13 ratio to also be decreased, mirroring the IFN-γ/IL-10 ratio (data not shown). Alterations in the Th1/Th2 cytokine ratios may be a mechanism by which more severe cutaneous disease develops in HIV-1–*Leishmania* coinfection.

The impairment in IFN-γ production appears to be specific to *Leishmania* antigens, since despite lower $CD4^+$ T cell counts and higher median HIV-1 viral loads in the HIV-*Leishmania*-coinfected subjects, PBMCs from coinfected patients produced more IFN-γ in response to HIV-1 p24 antigen than PBMCs from subjects infected only with HIV-1. Infection with cutaneous strains of *Leishmania* results in potent IFN-γ responses (27), and this may explain the discrepancy. Other potential explanations include cross priming of the HIV-specific T cells by *Leishmania* infection or differences in T cell functionality depending upon the phase of immune recovery in antiretroviral-treated patients (16, 18, 28–30). Due to the cross-sectional nature of our study, we are unable to elucidate the mechanism involved here.

In HIV-seronegative patients, PBMCs from subjects with mucocutaneous leishmaniasis produce higher levels of TNF-α than those of subjects with cutaneous disease (5). HIV-infected individuals have higher levels of circulating TNF-α than uninfected subjects, and this may contribute to the more severe mucosal disease seen in coinfection (3). Although we observed a slight trend toward higher levels of TNF-α in response to SLA in the HIV-*Leishmania*-coinfected group than in the subjects with CL alone, the TNF-α/IL-10 ratios were similar in both groups. Although we speculate that an altered TNF-α/IL-10 ratio in coinfected individuals may contribute to the increased dermatopathology seen in these individuals, our cohort did not have enough power to detect this effect.

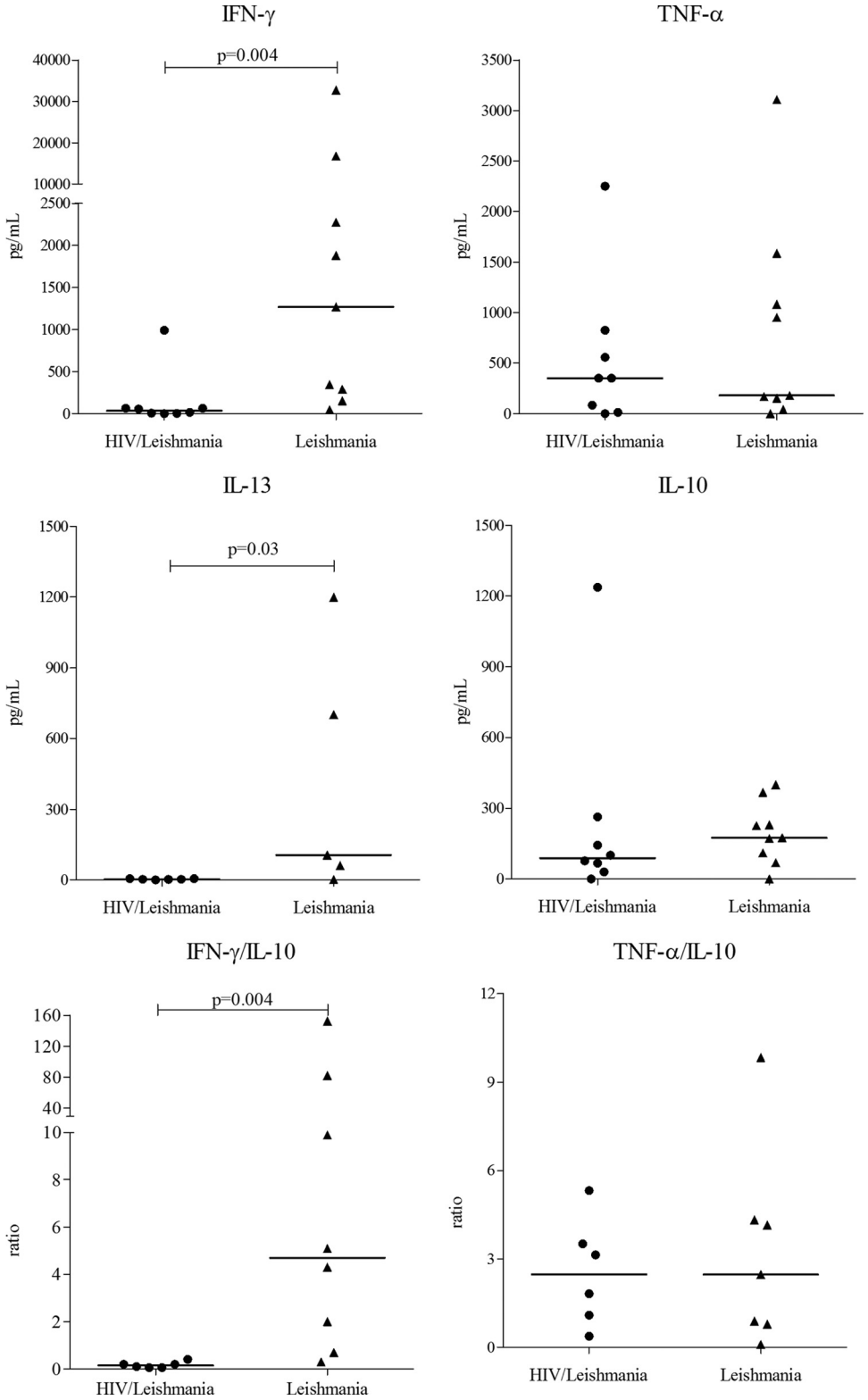

FIG. 1. Graphs demonstrating IFN-γ, TNF-α, IL-4, and IL-10 production by PBMCs from HIV-*Leishmania*-coinfected ($n = 8$) and *Leishmania*-monoinfected ($n = 9$) patients and IL-13 production by PBMCs from HIV-*Leishmania*-coinfected ($n = 6$) and *Leishmania*-monoinfected ($n = 5$) patients. PBMCs were stimulated *in vitro* with soluble *Leishmania* antigen (SLA), and supernatants were collected after 48 h. In the bottom panels, the ratios of IFN-γ/IL-10 and TNF-α/IL-10 cytokine production between the HIV-*Leishmania*-coinfected ($n = 6$) and *Leishmania*-monoinfected ($n = 8$ and 7, respectively) patients are demonstrated. Patients who had undetectable levels of cytokines (HIV/Leish3 and HIV/Leish9) were excluded from the cytokine ratio analysis. Median values are indicated by horizontal bars. A Mann-Whitney test was used to compare groups. For the individual cytokines, significant differences were found between groups for IFN-γ and IL-13 ($P = 0.004$ and $P = 0.03$, respectively). A significant difference was also found for the IFN-γ/IL-10 ratio ($P = 0.004$) but not the TNF-α/IL-10 ratio.

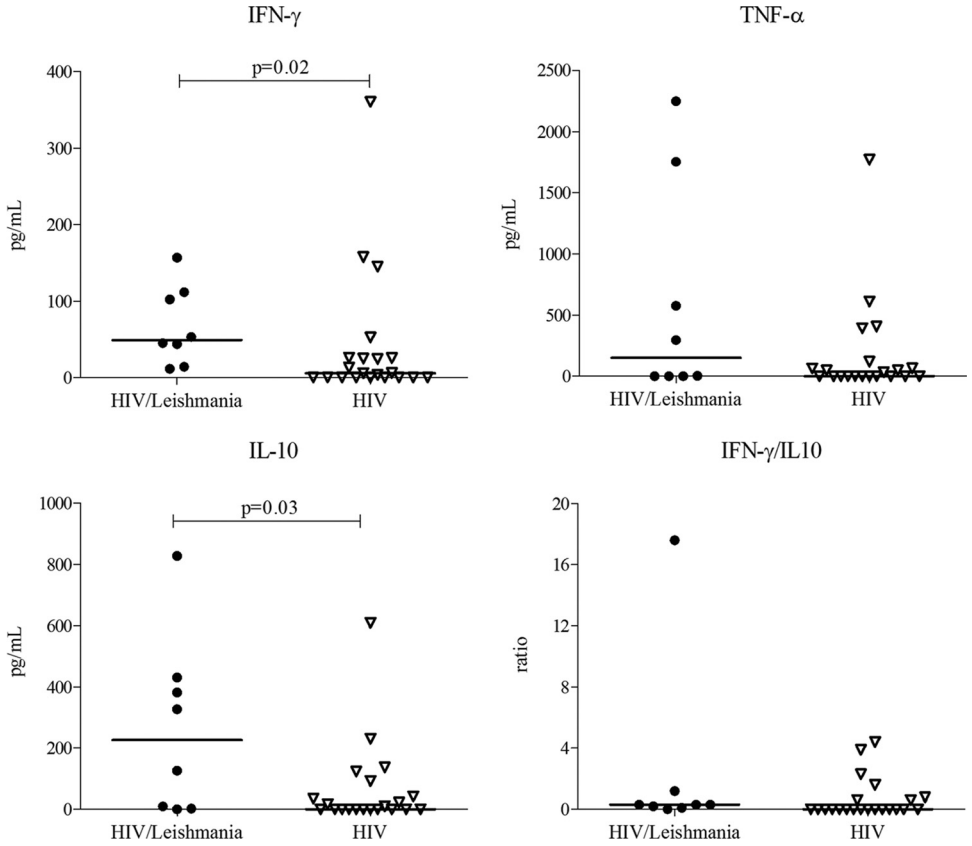

FIG. 2. Graphs demonstrating IFN-γ, TNF-α, IL-4, and IL-10 production by PBMCs from HIV-infected ($n = 21$) and HIV-*Leishmania*-coinfected ($n = 8$) patients. PBMCs were stimulated *in vitro* with HIV p24 protein, and supernatants were collected after 48 h. In the last panel, the ratio of IFN-γ/IL-10 cytokine production between the HIV-*Leishmania*-coinfected ($n = 7$) and HIV-monoinfected ($n = 7$) patients is demonstrated. Medians are indicated by horizontal bars. A Mann-Whitney test was used to compare groups. For the individual cytokines, significant differences between groups were found for IFN-γ and IL-10 ($P = 0.02$ and $P = 0.03$, respectively). A significant difference was not found between the groups for the IFN-γ/IL-10 ratio ($P = 0.07$).

In summary, low IFN-γ production and the altered ratio of inflammatory and regulatory cytokines creates a milieu that supports replication of the *Leishmania* parasites. These alterations may explain how disseminated cutaneous infection and visceralization of species typically causing only cutaneous disease may occur in the setting of HIV and *Leishmania* coinfection.

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