Differential Interferon-γ Production Characterizes the Cytokine Responses to *Leishmania* and *Mycobacterium leprae* Antigens in Concomitant Mucocutaneous Leishmaniasis and Lepromatous Leprosy

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Background. Tegumentary leishmaniasis and leprosy display similar spectra of disease phenotypes, which are dependent on cell-mediated immunity to specific antigens. Diffuse cutaneous leishmaniasis and lepromatous leprosy represent the anergic end of the spectrum, whereas mucocutaneous leishmaniasis and tuberculoid leprosy are associated with marked antigen-specific cellular immune response.

Methods. We characterized and compared the cell-mediated response to *Leishmania* and *Mycobacterium leprae* antigens in a patient with an intriguing association of mucocutaneous leishmaniasis with lepromatous leprosy, which are at opposite ends of the immunopathological spectra of these diseases. This was done by performance of skin tests and by assessment of the cell proliferation and cytokine production of peripheral blood mononuclear cells (PBMCs).

Results. Strong skin-test reactions and PBMC proliferation were observed in response to *Leishmania* antigens but not to *M. leprae* antigens. The stimulation of PBMCs with *Leishmania* and *M. leprae* antigens induced comparable levels of tumor necrosis factor–α, interleukin-5, and interleukin-10. However, the interferon-γ response to *Leishmania* antigens was remarkably high, and that to *M. leprae* antigens was almost nil.

Conclusions. We found that concomitant leprosy and tegumentary leishmaniasis can produce opposite polar forms associated, respectively, with absent or exaggerated cell-mediated immune responses to each pathogen. This suggests that independent mechanisms influence the clinical outcome of each infection. Moreover, interferon-γ appears to play a major role in the clinical expression of these intracellular infections.

Leprosy and American tegumentary leishmaniasis are both caused by intracellular organisms. These diseases are characterized by a spectrum of clinical manifestations that are dependent on T cell–mediated immunity [1, 2]. The hyperergic end of the spectrum is characterized by the scarcity of organisms and potent T cell–mediated responses to *Mycobacterium leprae* or *Leishmania* antigens, demonstrated by high levels of specific antigen-induced in vitro T cell proliferation and by strong skin-test reactions to lepromin or leishmanin. These are features of tuberculoid leprosy and mucosal leishmaniasis [1, 3]. On the other hand, the anergic end of the spectrum, represented by lepromatous leprosy and diffuse cutaneous leishmaniasis, is characterized by the absence of pathogen-specific cell-mediated immunity and by massive numbers of intracellular bacilli or parasites [1, 2].

CD4^{+} T cells can be separated into 2 subsets, Th1 cells and Th2 cells, on the basis of the repertoire of lymphokines that they produce after stimulation with antigens [4]. Th1 cells, which produce IFN-γ and IL-2, are associated with protection against intracellular pathogens, whereas Th2 cells, which produce IL-4, IL-5, and IL-10, are involved in the aggravation and path-

ologic manifestations of intracellular infections [5, 6]. IFN-γ, a characteristic product of the Th1 subset of CD4$^+$ cells, activates macrophages to kill intracellular microbes, such as *Leishmania* and *M. leprae* [7, 8]. Patients who have hyperergic clinical forms of disease, such as tuberculoid leprosy and mucosal leishmaniasis, have higher levels of IFN-γ production than do patients with other forms in the clinical spectra of these diseases [9, 10]. TNF-α has been shown to synergize with IFN-γ in the elimination of *Leishmania major* amastigotes by activated macrophages and to participate in the killing of mycobacteria by macrophages [11, 12]. Although a protective role has been proposed for TNF in experimental murine leishmaniasis [13], patients with severe forms of leishmaniasis, either multiparasitic forms (i.e., visceral or diffuse cutaneous leishmaniases) or pauciparasitic forms (i.e., mucocutaneous leishmaniasis), have significantly higher serum levels of TNF-α than do patients with benign forms (i.e., localized cutaneous leishmaniasis and subclinical visceral leishmaniasis) [14, 15]. In leprosy, the highest levels of serum TNF-α have been found in patients with the multibacillary form, lepromatous leprosy [14]. Although IL-5 has been associated with a nonhealer phenotype in murine leishmaniasis [16], its role is less clear in humans, with regard to both leishmaniasis and leprosy [17, 18]. On the other hand, an aggravating role has been suggested for IL-10 in human leishmaniasis, as well as in lepromatous leprosy [19, 20].

We characterized and compared the responsiveness to *Leishmania* and *M. leprae* antigens in a patient with an unusual case of associated mucocutaneous leishmaniasis and lepromatous leprosy, opposite polar forms in the immunopathological spectra of these intracellular infections, by means of skin tests and the assessment of the proliferation of PBMCs and the production of relevant cytokines.

CASE REPORT

A 46-year-old man, who was born in Bahia State, Brazil, and had been living in Rio de Janeiro State since he was 7 years old, examined in December 1997 at a primary care unit, presented with erythema nodosum, skin infiltration, and madarosis (figure 1). The patient was an agricultural worker and an alcohol abuser with congestive heart failure. Lepromatous leprosy was diagnosed. The multidrug therapy regimen recommended by the World Health Organization (WHO), consisting of supervised, monthly administration of 600 mg rifampin, 300 mg clofazimine, and 100 mg dapsone and daily self-administration of 100 mg dapsone and 50 mg clofazimine was initiated but was irregularly used by the patient. On May 2002, a nasal lesion was noted. This lesion did not improve with multidrug therapy for leprosy, so the patient was referred to Evandro Chagas Hospital (Rio de Janeiro, Brazil) in July 2003 for an

Figure 1. Photograph of a patient with concomitant mucocutaneous leishmaniasis and lepromatous leprosy showing loss of the external third of the eyebrow *(arrow),* a feature of polar lepromatous leprosy.

investigation of the etiology of his illness. At admission, a dermatological examination revealed bilateral madarosis, xerosis, plaque and edema of the left hand, infiltration of the nostrils, and ulceration with partial destruction of the nasal septum (figure 2). The patient also had an atrophic scar measuring 3 cm in diameter on the posterior face of the right ear, with loss of a small part of the ear lobule, which had resulted from a chronic cutaneous ulcer that had developed in 1996, when the patient was in Roraima State in the Amazon region. A physical examination revealed a blood pressure of 110×70 mm Hg, cardiomegaly, a low-intensity mitral systolic murmur, hepatomegaly, and no limb edema.

The laboratory blood values were as follows: hemoglobin, 121 g/L; WBC count, 5.5×10^9 cells/L; total lymphocyte count, 1.87×10^9 cells/L; eosinophil count, 1.76×10^9 cells/L; platelet count, 267×10^9 platelets/L; blood urea nitrogen, 35 mg/dL; creatinine, 0.8 mg/dL; and glucose, 86 mg/dL. The results of liver function tests were normal, with an amylase level of 57 mg/dL and a lipase level of 127 mg/dL. Serological tests were negative for HIV. A Venereal Disease Research Laboratory test and a *Treponema pallidum* hemagglutination assay both had negative results. An anti-*Leishmania* indirect immunofluorescence test yielded a positive titer of 40. Parasitological examination of stool samples revealed the presence of *Strongyloides stercoralis* larvae. Urinalysis showed proteinuria (grade 2+ by dipstick analysis).

Figure 2. Photographs of the patient that show a mucocutaneous leishmaniasis lesion that has partially destroyed the nasal septum

Chest radiography revealed an enlarged cardiac image. Electrocardiography and echocardiography revealed signs of severe dilated cardiomyopathy. Examination of the upper airways with a flexible fiberoptic endoscope revealed intense inflammation in the right nasal septum; edema of the septum, vocal cords, and uvula; hyperemia of the nasal mucous membranes; and lepromatous nodules in the soft palate (figure 3).

Polar lepromatous leprosy was confirmed on the basis of positive results from smears of skin samples that were obtained bilaterally from the earlobes, elbows, and knees, with an average bacterial index of 4+ with Ziehl-Nielsen staining. Mucocutaneous leishmaniasis was diagnosed by isolation of *Leishmania* from a biopsy specimen from the nasal lesion. The parasites that were grown in Novy-McNeal-Nicolle medium were identified as *Leishmania braziliensis* by use of isoenzyme analysis. [21, 22].

Meglumine antimoniate (Glucantime; Aventis) was administered intravenously for the treatment of leishmaniasis with a low-dose schedule of 5 mg/kg per day of pentavalent antimony for 30 days [23]. This scheme was chosen for its relatively low toxicity, because of concern about the patient's alcoholic cardiomyopathy. The patient was examined every other day by means of electrocardiography for QTc interval calculation, and blood tests to evaluate renal and hepatic functions were performed weekly. Multidrug therapy, as described above, was given to treat the leprosy. The leishmaniasis lesion healed 4 months after the end of antimonial therapy, leaving partial mutilation of the nostrils. At the time this report was written, the patient was still receiving regular multidrug therapy and exhibited no disease activity, and follow-up skin smears showed a bacterial index of 0.25+.

METHODS

Skin tests. The lepromin test was performed as follows: 0.1 mL of a suspension of autoclaved armadillo-derived bacilli (1.6×10^8 bacteria/mL)—which was produced by the Oswaldo Cruz Foundation (Rio de Janeiro, Brazil) in accordance with the WHO guidelines [24]—was injected intradermally on the inner side of the forearm. Fernandez and Mitsuda reactions were defined by the presence of a skin induration of ⩾5 mm, 48 h and 4 weeks after antigen inoculation, respectively [25]. The leishmanin skin test was performed as follows: 0.1 mL of leishmanin produced from *Leishmania amazonensis* (WHO reference strain IFLA/BR/67/PH8) promastigotes, containing 40 μg total nitrogen per milliliter of PBS with 0.4% phenol (Bio-Manguinhos), was injected intradermally on the inner side of the forearm. An induration with a diameter of ⩾5 mm 48 h after antigen inoculation was considered to be a positive Montenegro reaction.

Laboratory assays. *Leishmania* and *M. leprae* crude antigen extracts were used in the in vitro assays. *L. braziliensis* (WHO reference strain MHOM/BR/75/2903) and *L. amazonensis* (WHO reference strain IFLA/BR/67/PH8) were separately cultured in Novy-McNeal-Nicolle medium [21], supplemented with RPMI 1640 medium (Sigma) and 10% heat-inactivated fetal calf serum (Sigma). The parasites were washed 3 times by centrifugation at 900 *g* for 10 min at 4°C in PBS and were disrupted by 10 repeated cycles of freezing and thawing, followed by ultrasonication (Ultra-tip Labsonic System; Lab-Line) at 40 watts for 15 min in an ice bath. An irradiated, armadillo-derived whole *M. leprae* antigen preparation (2×10^9 bacteria/mg; kindly provided by Drs. E. P.
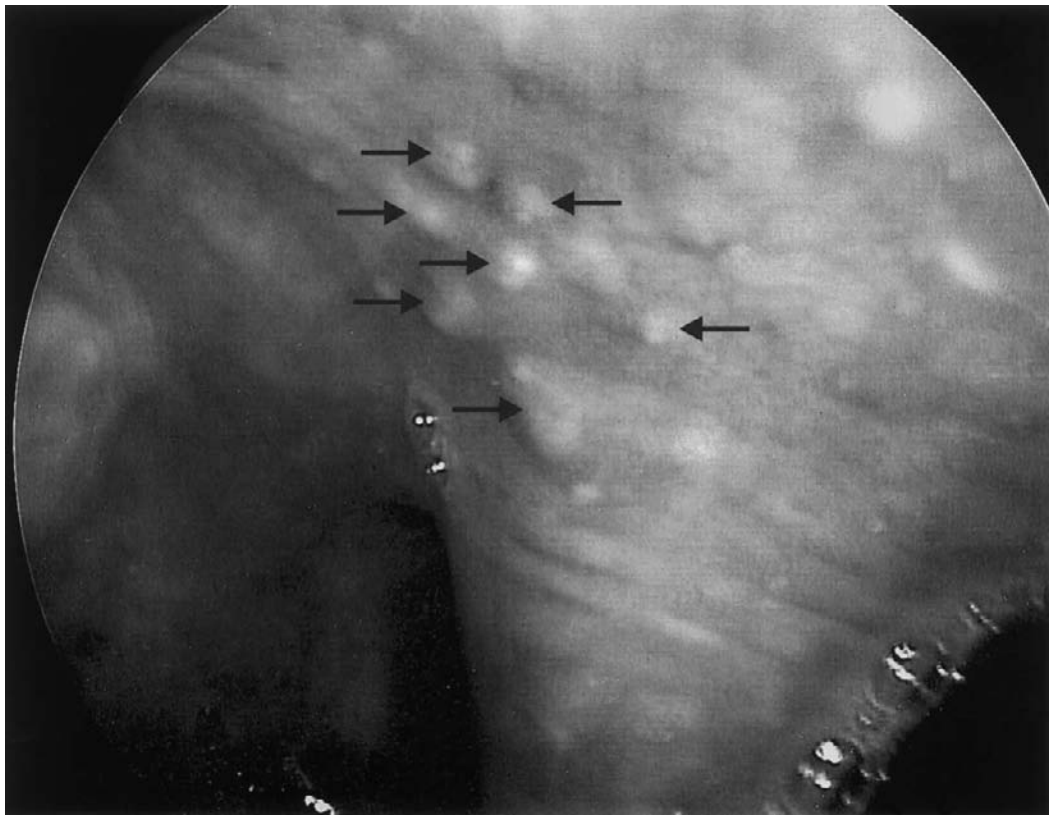
Figure 3. Fiberoptic endoscopic image showing multiple lepromas *(arrows)* in the soft palate

Sampaio and M. C. Pessolani, Leprosy Laboratory, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil), was sonicated according to the same protocol used for the *Leishmania* antigen extracts. The antigen concentration in each preparation was adjusted to 1 mg/mL protein nitrogen. All samples were kept at $-20°C$ until use.

PBMCs were obtained from venous blood samples by centrifugation through a Ficoll-Hypaque gradient (Sigma) and were resuspended in RPMI 1640 medium (Sigma) supplemented with 10% heat-inactivated AB Rh+ serum, 10 mmol/L HEPES, 1.5 mmol/L L-glutamine, 0.04 mmol/L 2-mercaptoethanol, and antibiotics (200 IU/mL penicillin and 200 mg/mL streptomycin) (all from Sigma) and adjusted to 10^6 cells/mL.

The proliferative response of PBMCs was evaluated in triplicate culture wells in 96-well flat-bottomed microtiter plates (Nunc). The plates, containing the cell suspension in a final volume of 200 μL/well in the presence of concanavalin A (ConA; 20 μg/mL) or antigen extracts of *L. braziliensis, L. amazonensis,* or *M. leprae* (50 μg/mL) or without stimuli, were incubated for 5 days at 37°C in a humidified 5% CO_2 atmosphere. Cell proliferation was estimated by [₃H]-thymidine incorporation, as described elsewhere [26]. Results were expressed as stimulation indices, which are defined as the ratio between the mean cell counts for stimulated and nonstimulated triplicate cultures.

The cytokine levels in supernatants from both PBMC cultures that were stimulated with *Leishmania* or *M. leprae* antigen extracts or with ConA and PBMC cultures that were not stimulated were measured by ELISA. PBMCs were cultured in 24-well, flat-bottomed plates (Nunc) in a final volume of 1 mL/well and were stimulated in vitro as described above. The mean cell counts for triplicate cultures was compared with standard curves obtained with recombinant IL-5, IL-10, TNF-α and IFN-γ (Pharmingen). Following a protocol previously established in our laboratory (data not shown), supernatants were collected on day 1 for determination of TNF-α levels, on day 2 for IL-5 levels, on day 3 for IL-10 levels, and on day 5 for IFN-γ levels, and all of the supernatants were stored at $-70°C$ until use.

The enzyme-linked immunospot assay (ELISPOT) was used to compare the frequencies of IFN-γ–producing cells after stimulation with *Leishmania* or *M. leprae* antigens. The 96-well, polyvinylidene fluoride plates (Millipore) were coated with 1.5 μg mouse anti-human IFN-γ monoclonal antibody (Pharmingen) per well. After overnight incubation at 4°C, the wells were washed with PBS and occupied sites were blocked with RPMI
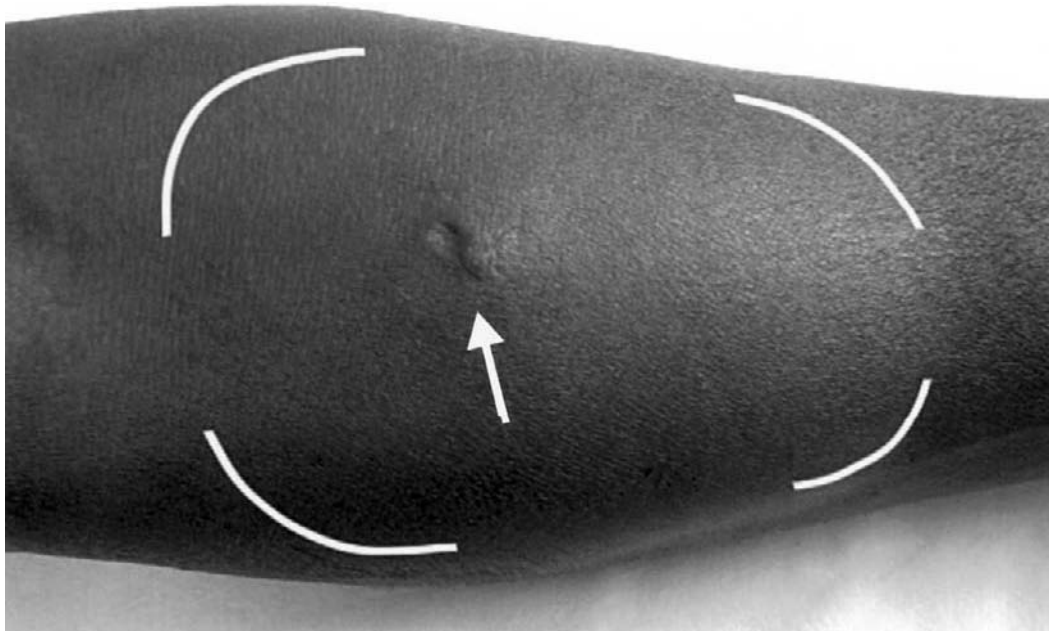
Figure 4. Photograph showing a strong, delayed-type hypersensitivity reaction to the leishmanin skin test, presenting as a large area of skin induration *(white lines)* and vesicular lesions *(arrow)* at the site of antigen inoculation.

1640 medium that was supplemented with 10% fetal bovine serum, 1 mmol/L ʟ-glutamine, and 1% penicillin/streptomycin (Sigma). PBMCs (5×10^4 cells/well) were preincubated in the presence or absence of the antigen extracts (50 μg/mL) or ConA (20 μg/mL), in a final volume of 200 μL/well, for 2 h at 37°C in a 5% CO_2 atmosphere. The cells were then transferred into the precoated polyvinylidene fluoride plates. After 40 h incubation at 37°C in a 5% CO_2 atmosphere, the cells were removed, and biotinylated anti-IFN-γ monoclonal antibody (Pharmingen) was added (0.1 μg/100 μL). Four hours later, the plate was washed, and 100 μL of streptavidin-alkaline phosphatase (BioRad) diluted at a ratio of 1:500 was added to each well. After 1 h at 37°C, the plate was washed again and incubated with 100 μL Tris buffer substrate (5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium) (Sigma), for 5–20 min at 25° C. Dark violet spots on plate membranes were counted by stereoscopic microscopy.

RESULTS

Skin tests. A positive reaction to the Montenegro (i.e., leishmanin) test was observed, with a mean diameter of 106 mm of skin induration (figure 4). The lepromin skin test yielded no Fernandez or Mitsuda reactions.

Laboratory assays. The laboratory results given here are representative of several experiments, 3 in the case of proliferative response and ELISA, and 2 in the case of ELISPOT. Proliferative responses induced by *L. braziliensis* antigens (stimulation index, 10.3) and by ConA (stimulation index, 19.0) were strong, in contrast to the proliferative response induced by *M. leprae* antigens, which was insignificant (stimulation index, 1.7). The response to *L. amazonensis* antigens was similar (stimulation index, 7.1) to that induced by *L. braziliensis* antigens. The levels of TNF-α, IL-5, and IL-10 measured in supernatants of PBMC cultures stimulated with *L. braziliensis* and *M. leprae* antigens were similar. However, the IFN-γ response to *L. braziliensis* antigens was >10 times stronger than the INF-γ response to *M. leprae* antigens and was also stronger than that induced by ConA (figure 5). The cytokine levels in the cultures that were stimulated with *L. amazonensis* antigens (not shown) were equivalent to those seen in the cultures that were stimulated with *L. braziliensis* antigens. An ELISPOT was performed to further compare the IFN-γ responses of PBMC to *Leishmania* and *M. leprae* antigens. The frequency of cells that produced IFN-γ was $868/10^5$ cells after stimulation with *L. braziliensis* antigens, which was >2-fold greater than the frequency seen in the presence of the polyclonal T cell activator ConA ($375/10^5$ cells). The frequency seen after stimulation with *L. amazonensis* antigen was even higher, at $1457/10^5$ cells. In contrast to the high frequencies seen after stimulation with *Leishmania* antigens, no cells producing IFN-γ were detected after stimulation with *M. leprae* antigen.

DISCUSSION

In spite of the overlapping geographical distribution of leishmaniasis and leprosy, reports of concomitant leishmaniasis and leprosy have been relatively scarce, and most of the reports
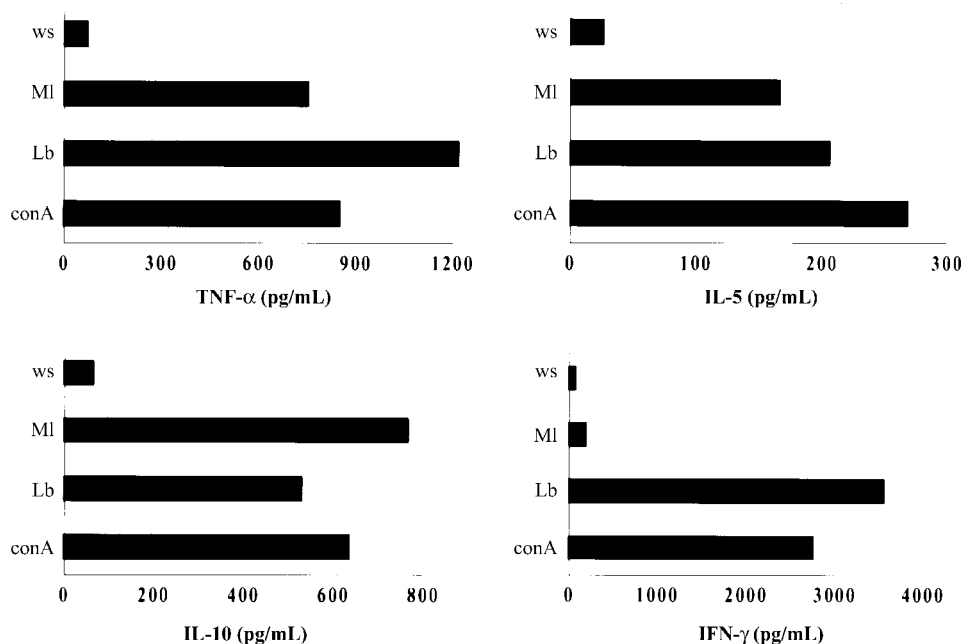
Figure 5. Cytokine levels in PBMCs cultured in the presence of *Mycobacterium leprae* (Ml) or *Leishmania braziliensis* (Lb) whole antigen extracts or concanavalin A (ConA), or without stimuli (ws), as assessed by ELISA.

have involved clinical and diagnostic aspects [27]. These diseases display a spectrum of clinical forms. Paucibacillary/parasitic forms, such as tuberculoid leprosy and localized cutaneous leishmaniasis, represent the expression of the relative resistance of the host that is due to preserved T cell responses to *M. leprae* or *Leishmania* antigens, and these forms are associated with a better prognosis, good response to therapy, and even a tendency toward self-healing [1, 2].

On the other hand, the multibacillary/parasitic forms, lepromatous leprosy and diffuse cutaneous leishmaniasis, are characterized by pathogen-specific suppression of T cell–mediated responses, uncontrolled progression of infection, and poor response to therapy [1, 2]. The *Leishmania*-specific immune responses in patients with mucosal leishmaniasis are strongly biased toward the Th1 pole and are significantly stronger than those observed in patients with localized cutaneous leishmaniasis [2, 3, 10]. These up-regulated Th1-type responses are associated with chronic and severe tissue damage, as well as with a scarcity of parasites in the mucosal lesions [28].

The similarities of leprosy and cutaneous leishmaniasis, in terms of their clinical, immunological, and pathological aspects, have justified the search for a common immunogenetic basis for susceptibility to these intracellular infections [29]. On the basis of this view, the occurrence of concomitant mycobacterial and leishmanial infections have been interpreted as being the result of impaired cell-mediated immunity leading to particular

susceptibility to these diseases [30]. However, accumulating evidence indicates that the immunodeficiency associated with lepromatous leprosy is specific to *M. leprae* infection [31].

To our knowledge, this is the first report of association of anergic multibacillary leprosy with hyperergic pauciparasitic leishmaniasis accompanied by the characterization of cytokine responses to each pathogen. We found that concomitant leprosy and tegumentary leishmaniasis can result in opposite polar forms associated, respectively, with absent or exaggerated cell-mediated immune responses to the specific pathogen. This suggests that independent mechanisms influence the clinical outcome of each infection. Furthermore, among the various cytokines assayed, the only clear difference seen was in the levels of IFN-γ produced in response to *Leishmania* and *M. leprae* antigens, indicating a key role for this cytokine in the clinical expression of these diseases. As revealed by the parameters that were evaluated (namely, skin-test reactivity, PBMC proliferation, and IFN-γ production), the cell-mediated responses induced by *Leishmania* antigens were very strong, as is commonly observed in mucocutaneous leishmaniasis [3, 10]. On the other hand, the corresponding responses that were induced by *M. leprae* antigen were almost nil, as would be expected for a patient with polar lepromatous leprosy [1, 9]. However, cells from patients with paucibacillary forms of leprosy were fully capable of proliferating and producing IFN-γ in response to the preparation of *M. leprae* antigen that was used (data not shown). To explain the specific T cell anergy found in patients

with lepromatous leprosy, an immunosuppressive role for CD8^{+} T cells, IL-4, and IL-10 has been suggested [20, 32]. In our investigation, the absence of cell proliferation and IFN-γ production in the cultures of PBMCs from this patient that were stimulated with *M. leprae* antigen could not be ascribed to IL-10–mediated inhibition, because the levels of this cytokine in such cultures were similar to those found in the PBMC cultures stimulated with *Leishmania* antigens (figure 5). Moreover, the presence of neutralizing anti-human IL-10 antibody did not increase IFN-γ levels or cell proliferation in cultures stimulated with *M. leprae* antigen (data not shown).

Further studies are needed to understand the pathogenesis of American tegumentary leishmaniasis and leprosy and, in particular, to determine the similarities and differences in the immunological mechanisms involved in their clinical expression.

Acknowledgments

References

1. de Vries RR. Genetic control of immunopathology induced by *Mycobacterium leprae*. Am J Trop Med Hyg **1991**; 44:12–6.
2. Convit J, Ulrich M, Fernandez CT, Tapia FJ, Caceres-Dittmar G, Castes M, Rondon AJ. The clinical and immunological spectrum of American cutaneous leishmaniasis. Trans R Soc Trop Med Hyg **1993**; 87:444–8.
3. Castes M, Agnelli A, Verde O, Rondon AJ. Characterization of the cellular immune response in American cutaneous leishmaniasis. Clin Immunol Immunopathol **1983**; 27:176–86.
4. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol **1986**; 136:2348–57.
5. Modlin RL. Th1-Th2 paradigm: insights from leprosy. J Invest Dermatol **1994**; 102:828–32.
6. Reiner SL, Locksley RM. The regulation of immunity to *Leishmania major*. Annu Rev Immunol **1995**; 13:151–77.
7. Murray HW, Rubin BY, Rothermel CD. Killing of intracellular *Leishmania donovani* by lymphokine-stimulated human mononuclear phagocytes: evidence that interferon-gamma is the activating lymphokine. J Clin Invest **1983**; 72:1506–10.
8. Ramasesh N, Adams LB, Franzblau SG, Krahenbuhl JL. Effects of activated macrophages on *Mycobacterium leprae*. Infect Immun **1991**; 59:2864–9.
9. Arnoldi J, Gerdes J, Flad HD. Immunohistologic assessment of cytokine production of infiltrating cells in various forms of leprosy. Am J Pathol **1990**; 137:749–53.
10. Bacellar O, Lessa H, Schriefer A, et al. Up-regulation of Th1-type responses in mucosal leishmaniasis patients. Infect Immun **2002**; 70:6734–40.
11. Green SJ, Crawford RM, Hockmeyer JT, Meltzer MS, Nacy CA. *Leishmania major* amastigotes initiate the ʟ-arginine–dependent killing mechanism in IFN-γ–stimulated macrophages by induction of tumor necrosis factor–α. J Immunol **1990**; 145:4290–7.
12. Bekker LG, Freeman S, Murray PJ, Ryffel B, Kaplan G. TNF-alpha controls intracellular mycobacterial growth by both inducible nitric oxide synthase–dependent and inducible nitric oxide synthase–independent pathways. J Immunol **2001**; 166:6728–34.
13. Titus RG, Sherry B, Cerami A. Tumor necrosis factor plays a protective role in experimental murine cutaneous leishmaniasis. J Exp Med **1989**; 170:2097–104.
14. Pisa P, Gennene M, Soder O, Ottenhoff T, Hansson M, Kiessling R. Serum tumor necrosis factor levels and disease dissemination in leprosy and leishmaniasis. J Infect Dis **1990**; 161:988–91.
15. Castes M, Trujillo D, Rojas ME, et al. Serum levels of tumor necrosis factor in patients with American cutaneous leishmaniasis. Biol Res **1993**; 26:233–8.
16. Nashed BF, Maekawa Y, Takashima M, et al. Different cytokines are required for induction and maintenance of the Th2 type response in DBA/2 mice resistant to infection with *Leishmania major*. Microbes Infect **2000**; 2:1435–43.
17. Shabaana AK, Venkatasubramani R, Narayan NS, Hoessli DC, Dharmalingam K. Cytokine profiles in paraffin-embedded biopsy samples of lepromatous leprosy patients: semi-quantitative measure of cytokine mRNA using RT-PCR. Int J Lepr Other Mycobact Dis **2001**; 69:204–14.
18. Pompeu MM, Brodskyn C, Teixeira MJ, et al. Differences in gamma interferon production in vitro predict the pace of the in vivo response to *Leishmania amazonensis* in healthy volunteers. Infect Immun **2001**; 69:7453–60.
19. Bourreau E, Prevot G, Gardon J, Pradinaud R, Launois P. High intra-lesional interleukin-10 messenger RNA expression in localized cutaneous leishmaniasis is associated with unresponsiveness to treatment. J Infect Dis **2001**; 184:1628–30.
20. Sieling PA, Abrams JS, Yamamura M, Salgame P, Bloom BR, Rea TH, Modlin RL. Immunosuppressive roles for IL-10 and IL-4 in human infection. In vitro modulation of T cell responses in leprosy. J Immunol **1993**; 150:5501–10.
21. Nicolle C. Culture des corps de Leishman isolés de la rate dans trois cas d'ánémie splenique infantile. Bull Soc Pathol Exot **1908**; 1:121–6.
22. Cupolillo E, Grimaldi G Jr, Momen H. A general classification of new world *Leishmania* using numerical zymotaxonomy. Am J Trop Med Hyg **1994**; 50:296–311.
23. Oliveira-Neto MP, Schubach A, Mattos M, Gonçalves-Costa SC, Pirmez C. A low-dose antimony treatment in 159 patients with American cutaneous leishmaniasis: extensive follow-up studies (up to 10 years). Am J Trop Med Hyg **1997**; 57:651–5.
24. World Health Organization (WHO). Recommended safety requirements for the preparation of lepromin: a WHO Memorandum. Bull World Health Organ **1979**; 57:921–3.
25. Convit J, Aranzazu N, Pinardi M, Ulrich M. Immunological changes observed in indeterminate and lepromatous leprosy patients and Mitsuda-negative contacts after the inoculation of a mixture of *Mycobacterium leprae* and BCG. Clin Exp Immunol **1979**; 36:214–20.
26. De Luca PM, Mayrink W, Pinto JA, et al. A randomized double-blind placebo-controlled trial to evaluate the immunogenicity of a candidate vaccine against American tegumentary leishmaniasis. Acta Trop **2001**; 80:251–60.
27. Goulart IM, Patrocinio LG, Nishioka S de A, Patrocinio JA, Ferreira MS, Fleury RN. Concurrent leprosy and leishmaniasis with mucosal involvement. Lepr Rev **2002**; 73:283–4.
28. Lessa HA, Machado P, Lima F, et al. Successful treatment of refractory mucosal leishmaniasis with pentoxifylline plus antimony. Am J Trop Med Hyg **2001**; 65:87–9.
29. Blackwell JM, Black GF, Peacock CS, et al. Immunogenetics of leishmanial and mycobacterial infections: the Belem Family Study. Philos Trans R Soc Lond B Biol Sci **1997**; 352:1331–45.

30. Delobel P, Launois P, Djossou F, Sainte-Marie D, Pradinaud R. American cutaneous leishmaniasis, lepromatous leprosy, and pulmonary tuberculosis coinfection with downregulation of the T-helper 1 cell response. Clin Infect Dis **2003**; 37:628–33.

31. Bach MA, Launois P. Mechanisms of *Mycobacterium leprae*–specific T-cell deficiency in lepromatous leprosy. Biochimie **1988**; 70:1013–8.

32. Salgame P, Modlin R, Bloom BR. On the mechanism of human T cell suppression. Int Immunol **1989**; 1:121–9.