American Cutaneous Leishmaniasis, Lepromatous Leprosy, and Pulmonary Tuberculosis Coinfection with Downregulation of the T-helper 1 Cell Response

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Cutaneous leishmaniasis, leprosy, and tuberculosis are caused by intracellular pathogens whose development depends on impaired cell-mediated immunity. We report an exceptional triple association of American cutaneous leishmaniasis, lepromatous leprosy, and pulmonary tuberculosis in a man with no recognized immunodeficiency. Normal immunological assessment of the interferon-γ pathway does not support the hypothesis of a genetic defect in any of the genes involved in the T helper (Th)–1 cytokine cascade in this patient. Unresponsiveness to interleukin (IL)–12 of his T cells after stimulation with Leishmania guyanensis, Mycobacterium bovis bacille Calmette-Guérin, and Mycobacterium leprae antigens suggested the inability to mount an appropriate Th cell response to upregulate the IL-12 receptor expression.

Cutaneous leishmaniasis, leprosy, and tuberculosis are all endemic in tropical South America. Leprosy and cutaneous leishmaniasis both encompass a broad spectrum of clinical and immunological manifestations that depend on the strength of the cell-mediated immune response [1, 2]. The pauci-bacillary/parasitic type of disease (characterized by indeterminate, polar tuberculoid, and borderline tuberculoid leprosy/localized cutaneous leishmaniasis) occurs in patients with effective specific T cell response. In contrast, the multibacillary/parasitic type of disease (characterized by mild-borderline, borderline lepromatous, or polar lepromatous leprosy/mucocutaneous and by disseminated cutaneous and/or diffuse anergic cutaneous leishmaniasis) occurs in patients with depressed specific T cell response. Cell-mediated immunity also plays a crucial role in the containment of tuberculosis [3].

Host defense against intracellular pathogens depends on effective cell-mediated immunity. The description of 2 functionally distinct CD4+ T cell subsets, Th1 cells, which produce IL-2 and IFN-γ, and Th2 cells, which produce IL-4, IL-5, IL-10, and IL-13, made it possible to characterize the CD4+ T cell subpopulations involved in various pathological processes [4, 5]. Th1 cell response is generally associated with resistance to intracellular pathogens, whereas Th2 cell response is associated with progressive disease due to these same pathogens. The activation of infected macrophages by Th1 cytokines—in particular, by IFN-γ released by Th1 cells—is a major effector mechanism of cell-mediated immunity. IFN-γ is produced by Th1 cells and natural killer (NK) cells and binds to IFN-γ receptor (IFN-γ R1/R2 complexes) at the macrophage surface. The production of IFN-γ by Th1 cells and NK cells is mainly regulated by IL-12, which is produced by antigen-presenting cells, such as macrophages and dendritic cells, and which binds to IL-12 receptor (IL-12 Rβ1/β2 com-
plexes) at the surface of Th1 and NK cells [4, 5]. The responsiveness of T cells to IL-12 depends on the expression of a functional IL-12 receptor made of 2 subunits, the β1 and β2 chains [6]. In humans, the presence or absence of expression of the IL-12Rβ2 chain can be a marker for Th1 and Th2 cells, respectively [7]. IL-12 receptor expression can be regulated by the variable Th1 and Th2 cytokine production in the local environment [8].

We report an exceptional triple association of American cutaneous leishmaniasis, lepromatous leprosy, and pulmonary tuberculosis in a man with no recognized immunodeficiency. Genetic factors of susceptibility to intracellular pathogens that may have been responsible for this unusual triple coinfection are discussed.

CASE REPORT

A 44-year-old man, a migrant from Maranhão, Brazil, who had been in French Guiana for 8 months, was admitted to Cayenne Hospital in March 2002 with a 6-month history of disseminated skin lesions. At admission, dermatological examination revealed symmetrically distributed disseminated erythematous papules and nodules that had smooth surfaces and ill-defined margins. Lesions were particularly marked on the face (figure 1A), earlobes (figure 1B), trunk, and limbs. A lateral loss of eyebrows was also noted. These lesions were suggestive of lepromatous leprosy. The recent appearance of a few erythema nodosum leprosum–like lesions on the back and the limbs was also noted. In addition, a scalpy erythematous infiltration with a small ulceration on the left ear (figure 1B) and 3 small ulcerated lesions on the forehead (figure 1A), back, and right thigh were suggestive of cutaneous leishmaniasis. Physical examination was also remarkable for a mild thickening of ulnar nerves.

Results of blood studies were as follows: hemoglobin level, 105 g/L; WBC count, 7,7 × 10⁹ cells/L; total lymphocyte count, 1.2 × 10⁹ cells/L; CD4⁺ T lymphocyte count, 0.437 × 10⁹ cells/L (35% of the total lymphocyte count); CD8⁺ T lymphocyte count, 0.449 × 10⁹ cells/L; platelet count, 404 × 10⁹ platelets/L; and C-reactive protein level, 142 mg/L. Serological tests were negative for HIV, human T cell lymphotropic virus types 1 and 2, and viral hepatitis B and C. A Venereal Disease Research Laboratory test was nonreactive, but the result of a Treponema pallidum hemagglutination assay was positive (titer 1:320), which was consistent with a history of treponematosis. Intra-dermal reaction to tuberculin was positive (size of reaction, 14 mm in diameter). Radiograph and CT scans of the chest revealed a miliary pattern and a bilateral apical fibrosis suggestive of pulmonary tuberculosis (figure 2).

Smears of gastric aspirate samples were positive for acid-fast bacilli, and culture of gastric aspirate samples was positive for Mycobacterium tuberculosis. Polar lepromatous leprosy was confirmed by results of smears of skin from both earlobes, the analysis of which revealed many acid-fast bacilli, and by results of histological examination of a skin biopsy specimen from the right earlobe, which showed a free subepidermal grenz zone (band of Unna), a granulomatous infiltrate of foamy macrophages, and numerous acid-fast bacilli with globi and a bacteriological index of 6+ on Fite staining (figure 3A). Cutaneous leishmaniasis was confirmed by results of smears of skin from the left earlobe that revealed numerous leishmania amastigotes (figure 3B).

Thalidomide therapy (200 mg daily) was initiated on day 1 because of probable erythema nodosum leprosum lesions (type 2 reaction). Within a few days, erythema nodosum leprosum lesions dramatically improved, and the C-reactive protein level decreased to 18 mg/L on day 5 after initiation of treatment. Thalidomide therapy was decreased to 100 mg daily on day 10. Multibacillary leprosy treatment with rifampin (600 mg monthly), clofazimine (100 mg daily), and dapsone (100 mg daily) was initiated on day 5. On day 10,
because of evidence of tuberculosis, dapsone therapy was stopped, and treatment was switched to rifampin (600 mg daily), isoniazid (250 mg daily), pyrazinamide (1.5 g daily), and ethambutol (1 g daily) therapy, in addition to clofazimine. On day 14, he received an intramuscularly administered single dose (375 mg) of pentamidine isethionate (7 mg/kg of pentamidine isethionate, which consisted of 4 mg/kg of pentamidine base), which successfully cured his cutaneous leishmaniasis. Evolution of disease appeared favorable at 6-month follow-up; the antimycobacterial therapy seemed effective.

**METHODS**

PBMCs were isolated over a Ficoll-Hypaque gradient after venipuncture and were resuspended in RPMI medium supplemented with L-glutamine (2 mmol/L), penicillin (100 U/mL), streptomycin (100 µg/mL; all purchased from Sigma), and 5% human heat-inactivated AB serum. IFN-γ production was measured in culture supernatants by use of a specific IFN-γ ELISA (Pharmingen) with a sensitivity of 10 pg/mL. IFN-γ production by PBMCs stimulated with phytohemagglutinin (2.5 µg/mL) was measured after 48 h of culturing. To analyze T cell responsiveness to IL-12, we measured IFN-γ production by PBMCs stimulated in the presence of *Leishmania guyanensis*, *Mycobacterium bovis* bacille Calmette-Guérin (BCG), or *Mycobacterium leprae* antigens (5 µg/mL of each antigen), prepared as described elsewhere [9], and in the presence or absence of IL-12 (50 ng/mL; Tebu) during 72 h of culturing.

Cytokine expression was analyzed by semiquantitative RT-PCR in cutaneous biopsy specimens of the left earlobe and the right thigh of the patient. Total RNA was isolated as described elsewhere [10]. First-strand cDNA synthesis was performed on RNA by use of a first-strand cDNA synthesis kit (Amersham-Pharmacia Biotech). Semiquantitative RT-PCR analysis was performed by use of the competitor plasmids pQA-1 and pQB-3 (provided by David Shire, Sanofi Recherche, Labège, France), with the β-actin gene as housekeeping gene [11]. A constant amount of cDNA was amplified in the presence of 5-fold competitor dilutions. After separation of the PCR products for PFGE in agarose gel containing ethidium bromide, we calculated the ratio of the concentration of the cytokine gene (pg/µL) to the relative concentration of the β-actin gene (pg/µL).

**Figure 2.** Chest radiograph revealing bilateral miliary shadowing and apical fibrosis suggestive of pulmonary tuberculosis.

**Figure 3.** Light microscopy of cutaneous biopsy specimens. A, Granulomatous infiltrate and acid-fast bacilli with globi (arrows) in a cutaneous biopsy specimen obtained from the right earlobe (Fite stain; original magnification, ×1000). B, Leishmania amastigotes (arrows) in smear of skin samples obtained from the lesion of the left earlobe (May-Grünwald-Giemsa stain; original magnification, ×1000).
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RESULTS

In our patient, there was no deficiency in IFN-\(\gamma\) production by PBMCs stimulated with phytohemagglutinin (IFN-\(\gamma\) level was 806 pg/mL in our patient and 789 pg/mL in a healthy control subject). The responsiveness to IL-12 of nonstimulated PBMCs was normal when analyzing IFN-\(\gamma\) production (figure 4A). However, we found unresponsiveness to IL-12 of PBMCs stimulated with either L. guyanensis (figure 4B), M. bovis BCG (figure 4C), or M. leprae (figure 4D) antigens when analyzing IFN-\(\gamma\) production. The expression of IL-4, IL-13, IL-10, IFN-\(\gamma\), TNF-\(\alpha\), and IL-12 (p40) mRNAs analyzed by semiquantitative RT-PCR showed a mixture of Th2 (IL-4 and IL-13) and Th1 (IFN-\(\gamma\)) cytokines in the 2 biopsy specimens (IL-4, 633 U; IL-13, 250 U; IL-10, 166 U; IFN-\(\gamma\), 248 U; TNF-\(\alpha\), 425 U; and IL-12, 185 U). TNF-\(\alpha\) was normally expressed in IFN-\(\gamma\)-stimulated PBMCs in our patient (TNF-\(\alpha\) level increased 20-fold with lipopolysaccharide (LPS) antigens alone and 104-fold with addition of IFN-\(\gamma\) to LPS antigens, compared with nonstimulated PBMCs; TNF-\(\alpha\) levels increased 30- to 110-fold in the control subject).

DISCUSSION

To our knowledge, the concomitant occurrence of American cutaneous leishmaniasis, lepromatous leprosy, and pulmonary tuberculosis has never been previously reported in the literature. We cannot exclude the notion that this triple coinfection could be a coincidence in a patient coming from an area where these diseases are endemic. However, these diseases have close pathophysiological mechanisms because all 3 are caused by intracellular pathogens, and development of these infections depends on an impaired cell-mediated immune response. Moreover, in our patient, all 3 infections were more closely associated with the multibacillary/parasitic type of disease rather than with the pauci-bacillary/parasitic type. Indeed, the patient had polar lepromatous leprosy, a cutaneous leishmaniasis with several lesions and numerous parasites detected on skin smears, and a disseminated form of tubercu-
loss revealed by a miliary pattern that was observed on CT scans of the chest. We can, therefore, suppose that the triple coinfection in our patient could be a significant association rather than a coincidence and that an underlying impaired cell-mediated immunity might have been responsible for particular susceptibility to these diseases.

However, clinical examination and standard biological investigations did not reveal any recognized primary or secondary immunodeficiencies in our patient. A role for genetic factors in variable susceptibility to infections caused by intracellular pathogens, such as mycobacteria, has been indicated by familial clustering, results of twin studies, complex segregation analyses, and results of human leukocyte antigen association studies [3]. Mutations in 5 genes encoding essential proteins of the Th1 cytokine cascade (IL12B, which encodes the p40 subunit of IL-12; IL12RB1, which encodes the β1 chain of the IL-12 receptor; IFNGR1 and IFNGR2, which encode the 2 chains of the IFN-γ receptor; and STAT1, which encodes the IFN-γ-associated signal transducer and activator of transcription) have been recognized and are associated with heightened susceptibility to infections caused by intracellular pathogens because of an impaired IFN-γ-mediated immunity [3, 12]. Mutations in the human natural resistance–associated macrophage protein 1 (NRAMP1) gene (which encodes a membrane protein that may regulate the intraphagosomal replication of pathogens) also appear to play a major role in the innate susceptibility to tuberculosis, leprosy, and leishmaniasis [3, 13].

In our patient, when assessing the IFN-γ pathway, we found no deficiency in responsiveness to IL-12 of nonstimulated PBMCs, which was consistent with the presence of functional IL-12 receptors; IFN-γ production by PBMCs stimulated with a mitogen (phytohemagglutinin) was normal; and there was no deficiency in TNF-α production in response to IFN-γ, which was consistent with the presence of functional IFN-γ receptors. These findings do not support the hypothesis of a genetic defect in any of the genes involved in the Th1 cytokine cascade in our patient.

We found unresponsiveness to IL-12 of PBMCs stimulated with either L. guyanensis, M. bovis BCG, and M. leprae antigens when analyzing IFN-γ production. Because we showed that our patient probably did not have a genetic defect in the structure or function of the IL-12 receptor, these findings suggest, instead, that he was unable to mount an appropriate Th response to upregulate the IL-12 receptor expression. Each infection might synergistically have enhanced susceptibility to the other 2 pathogens by inducing downregulation of the Th1 pathway due to Th2 cytokine production in active lesions, which led the immune response toward a Th2 pathway responsible for the persistence of infections. Unresponsiveness to IL-12 has previously been described in patients with either leishmaniasis, tuberculosis, or lepromatous leprosy and can be explained by reduced IL-12Rβ1 and/or IL-12Rβ2 expression in antigen-specific CD4+ T cells, depending on variability in Th1 and Th2 cell differentiation [9, 14, 15].

In patients with tuberculosis, an increased production of transforming growth factor–β has been shown to reduce IL-12Rβ1 and IL-12Rβ2 expression [14]. Similarly, in patients with leishmaniasis, Th2 cytokines, such as IL-13, have been shown to play the main role for rendering specific CD4+ T cells unresponsive to IL-12 by inhibiting the expression of the IL-12Rβ2 chain [9]. Of interest, unresponsiveness of specific CD4+ T cells to IL-12 has been found only in patients with active cutaneous leishmaniasis—that is, in patients with detectable parasites (such as untreated patients) and in patients who have not responded to treatment but not in cured patients. These results suggest that a state of unresponsiveness to IL-12 of Leishmania-specific T cells is responsible for the persistence of infection [16]. In our patient, analysis of the cytokine profile in tissue specimens obtained from the lesion sites showed a mixed Th2 and Th1 cytokine expression pattern; interpretation was difficult because of the presence of both leishmaniasis and leprosy. Similar to patients with cured leishmaniasis, it is likely that responsiveness to IL-12 of our patient’s T cells would have normalized with successful treatment. Additional studies are needed to better understand the mechanisms underlying the depressed Th1 cell response responsible for susceptibility to infections caused by intracellular pathogens, such as mycobacteria and Leishmania species.

References


