

CONCISE COMMUNICATIONS

Down-Regulation of Th1 Type of Response in Early Human American Cutaneous Leishmaniasis

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This study examined the T cell responses in the early phase of *Leishmania braziliensis* infection. Cytokine profiles, lymphoproliferative responses, and skin test results in 25 patients with early cutaneous leishmaniasis (ECL; illness duration <60 days) were compared with those in persons with late cutaneous leishmaniasis (LCL; illness duration >2 months). Absent or low lymphoproliferative responses were observed in 8 (32%) of 25 patients and an absence of interferon (IFN)- γ production in 9 (41%) of 22 patients prior to therapy. IFN- γ production in ECL (mean \pm SD) was lower than in LCL (293 ± 346 vs. 747 ± 377 pg/mL, respectively; $P < .01$). In contrast, interleukin (IL)-10 production in ECL (mean \pm SD) was higher than in LCL (246 ± 56 vs. 50 ± 41 pg/mL, respectively; $P < .01$). Restoration of lymphoproliferative responses and IFN- γ production was achieved when monoclonal antibody to IL-10 or IL-12 was added to the cultures. These results show that T cell responses during early-phase infection are down-regulated by IL-10 and may facilitate parasite multiplication.

The major host immune defense mechanism against leishmania is activation of macrophages by interferon (IFN)- γ derived from T cells [1]. Absence of IFN- γ production is responsible for the development of visceral leishmaniasis and diffuse cutaneous leishmaniasis [2, 3]. In contrast, in Central and South American cutaneous leishmaniasis, lymphocytes produce IFN- γ in response to leishmania antigens, and IFN- γ -activated macrophages can kill leishmania in vitro [4]. It is possible that the development of disease may depend on a transient dysregulation of T cell responses during the initial phase of infection. The purpose of this study was to characterize the early cellular immune response in cutaneous leishmaniasis to better understand the pathogenesis of this disease.

Subjects and Methods

Patients. Twenty-five patients with cutaneous ulcers of <2 months' duration were recruited from the endemic area of Corte de Pedra, Bahia, Brazil. Diagnosis of cutaneous leishmaniasis was confirmed by parasite isolation or by a positive skin test. All isolated parasites were identified as *Leishmania braziliensis* by monoclonal antibodies (MAbs). Serodeme analysis was done by an indirect immunofluorescence assay using panels of anti-leishmania MAbs specific for *L. braziliensis*, *L. mexicana*, and *L. donovani*. Immunologic studies were done before therapy in all patients and in 10 patients after antimony therapy. As controls, 20 patients with cutaneous ulcers of >60 days' duration, paired by age and sex, were identified and evaluated prior to therapy. All patients were treated with glucantime (Rhodia, São Paulo, Brazil; 20 mg/kg of weight for 20 days).

Leishmania antigen and intradermal skin test. The leishmanial lysate used for lymphocyte stimulation and for intradermal skin test was prepared from an *L. amazonensis* strain (MHOM-BR-86 BA-125). The immune responses to *L. braziliensis* and *L. amazonensis* antigens are very similar; however, because *L. amazonensis* is easier to grow in cultures, we used this strain to make the antigen.

Lymphocyte blastogenesis assay and cytokine production were performed with peripheral blood mononuclear cells (PBMC) isolated from a ficoll-hypaque gradient of heparinized venous blood. PBMC were cultured in triplicate in 96-well flat-bottom plates in RPMI 1640 medium (Gibco, Grand Island, NY) containing penicillin, streptomycin, and 10% heat-inactivated human AB serum. Cells were stimulated with 10 μ g/mL leishmania antigen or pokeweed mitogen (PWM) [4]. In subgroups of cultures, interleukin (IL)-12 (Genetics Institute, Cambridge, MA) at 500 U/mL or MAbs against human IL-4 or human IL-10 at 250 μ g/mL (DNAX

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Informed consent was obtained from the patients or their parents, and human experiment guidelines of the Hospital University Professor Edgard Santos were followed in the conduct of the research.

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Research Institute, Palo Alto, CA) were added in the presence or absence of leishmania antigen. After 5 days, cells were pulsed with 1 μ Ci of 3 [H]thymidine (6.7 Ci/mM; New England Nuclear Life Sciences Products, Boston) during the last 6 h of culture and collected with a Skatron harvester (Flow Laboratories, Rockville, MD). Data were represented as stimulation index (SI), calculated by dividing the counts per minute (cpm) of the stimulated cultures by the cpm of the unstimulated cultures.

IFN- γ and IL-10 in supernatants of lymphocyte cultures were measured using reagents provided by Immunex (Seattle). In brief, 3×10^6 PBMC/mL were stimulated with *L. amazonensis* lysate at 20 μ g/mL or with phytohemagglutinin. Anti-IL-10 or -IL-4 MAbs (250 μ g/mL) were added to subgroups of cultures. Supernatants were collected after 72 h, and levels of IFN- γ and IL-10 were determined by sandwich ELISA, as described elsewhere [5]. The data represent the mean of duplicates. Standard curves with recombinant cytokines were used to express the results in picograms per milliliter.

Statistical analysis. We used the Wilcoxon rank sum test to compare the SIs and levels of IFN- γ and IL-10. The paired Wilcoxon rank sum test was used to compare IFN- γ levels before and after therapy.

Results

The duration of illness in patients with early cutaneous leishmaniasis (ECL) ranged from 15 to 60 days. Patients with a short duration of illness (<2 weeks) had acneiform lesions or small bleeding ulcers that clearly differed from the classical cutaneous leishmaniasis ulcer observed in patients with late cutaneous leishmaniasis (LCL; >60 days of illness).

Figure 1 shows the lymphocyte proliferation after stimulation with leishmania antigen and PWM and the levels of IFN- γ and IL-10 in lymphocyte supernatants of patients with ECL and LCL. The SI (mean \pm SD) of PBMC cultures from patients with ECL after stimulation with leishmania antigen was significantly lower than in patients with LCL (79 ± 116 vs. 154 ± 107 , respectively; $P < .01$). The response to PWM in ECL did not differ from that observed in LCL (139 ± 107 vs. 155 ± 122 , respectively; $P > .05$).

The mean IFN- γ level (\pm SD) in supernatants of PBMC from patients with ECL after stimulation with leishmania antigen (239 ± 346 pg/mL) was significantly lower than that observed with LCL (747 ± 377 pg/mL; $P < .05$; figure 1B). In 9 patients with ECL, IFN- γ production was absent. In contrast to the poor IFN- γ production, IL-10 levels (246 ± 56 pg/mL) in leishmania antigen-stimulated PBMC from patients with ECL were higher than those observed in patients with LCL (50 ± 41 pg/mL; $P < .05$). There were no significant differences in the PWM-stimulated responses when the levels of IFN- γ and IL-10 in ECL were compared with those in LCL (1666 ± 484 and 516 ± 65 pg/mL vs. 1827 ± 512 and 527 ± 193 pg/mL, respectively; $P > .05$).

Whereas the skin test was positive and a marked lymphocyte

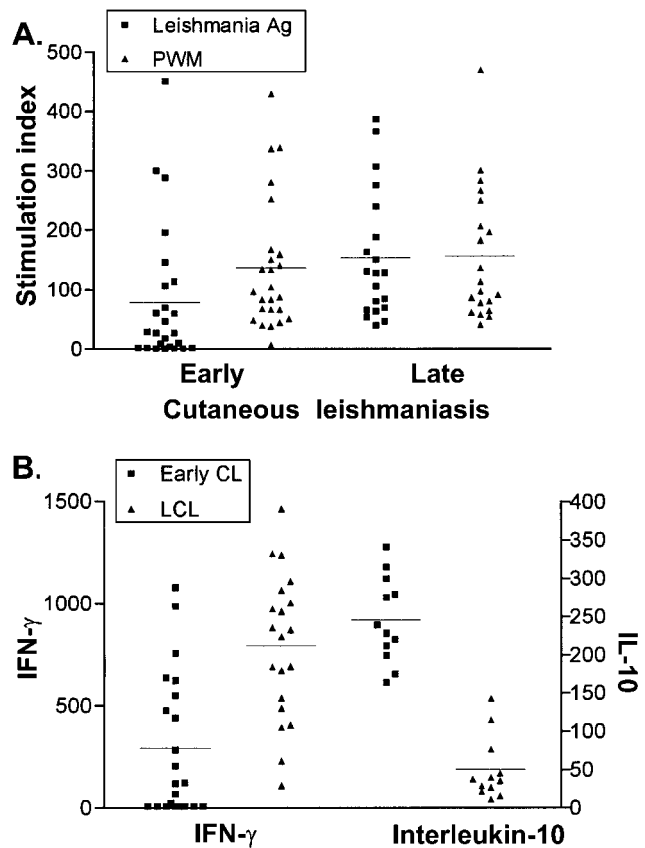


Figure 1. T cell responses in persons with early cutaneous leishmaniasis (ECL) and late cutaneous leishmaniasis (LCL). *A*, Lymphocyte proliferative response to leishmania antigen (Ag; ■) and pokeweed mitogen (PWM; ▲) in patients with ECL and LCL. *B*, Interferon (IFN)- γ and interleukin (IL)-10 levels in lymphocyte supernatants from persons with early (■) and late (▲) cutaneous lesions. Lines represent means.

proliferative response and IFN- γ production upon leishmania antigen stimulation were observed in all 20 patients with LCL, these functions were markedly altered in the majority of patients with ECL. In the 25 ECL patients, 18 (70%) had a negative response in ≥ 1 of these 3 tests.

Enhancement of lymphocyte proliferation and IFN- γ production was observed after addition of MAb to IL-10 but not after addition of MAb to IL-4 (figure 2A). In 4 patients, the mean (\pm SD) level of IFN- γ was 32 ± 25 pg/mL in PBMC cultures stimulated with leishmania antigen and 61 ± 85 pg/mL in cultures containing antigen and anti-IL-4. In contrast, IFN- γ production was 436 ± 290 pg/mL ($P < .05$) in cultures stimulated with leishmania antigen and anti-IL-10. The control isotypes did not modify responses of either unstimulated or antigen-stimulated cultures. Addition of IL-12 restored lymphocyte proliferative responses in antigen-stimulated cultures of 4 patients with ECL (figure 2B). The mean (\pm SD) SI in

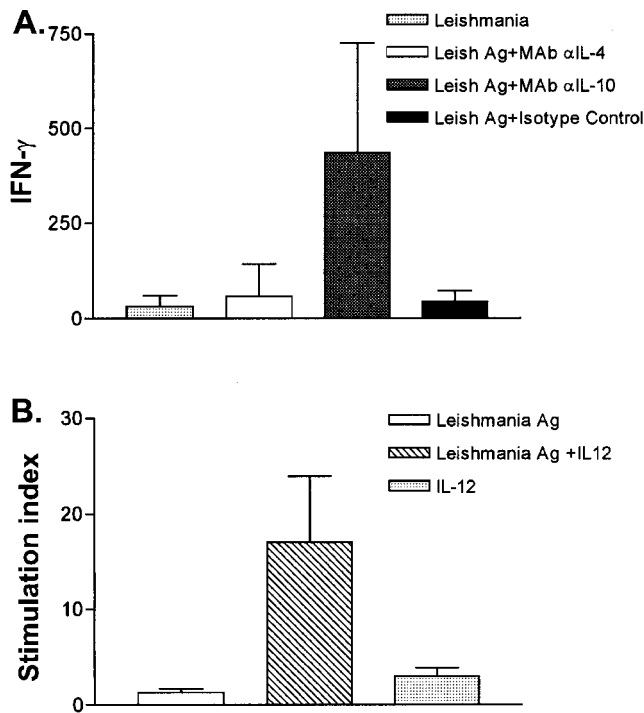


Figure 2. Interleukin (IL)-10 human monoclonal antibody (MAb) and IL-12 restore T cell function in early cutaneous leishmaniasis. *A*, Mean \pm SD of interferon (IFN)- γ levels in supernatant of lymphocyte cultures of 4 patients with early cutaneous leishmaniasis stimulated with leishmania antigen (Leish Ag) alone; Leish Ag and α -IL-4 or α -IL-10; or isotype control at 250 μ g/mL. *B*, Mean \pm SD of stimulation indexes of cultures with addition of Leish Ag, Leish Ag + IL-12 (500 U/mL), or IL-12 alone.

culture stimulated with leishmania antigen and IL-12 was 17 ± 19 , and in cultures stimulated with antigen alone it was 1.4 ± 1.1 ($P < .05$). IL-12 did not induce significant lymphocyte proliferation in cultures without leishmania antigen.

Restoration of lymphocyte proliferation to leishmania antigen and IFN- γ production was documented in all subjects evaluated after therapy. In 7 patients who had low IFN- γ levels before therapy (10 ± 6 pg/mL), these levels increased after therapy to 324 ± 169 pg/mL ($P < .05$).

Discussion

The immunologic response in American cutaneous leishmaniasis is characterized by the ability of lymphocytes to proliferate and produce IFN- γ when stimulated by leishmania antigen [4, 6]. Although mRNA for both Th1 and Th2 cytokines is expressed in cells from patient's lesions [7, 8], production of Th1-type cytokines appears to be predominant [6, 9]. In cutaneous leishmaniasis, the absence of a cellular immune response has been associated with diffuse cutaneous leishmaniasis

[3]. In the present study, persons with a short duration of illness had a transitory depression of Th1-type response characterized by absence of delayed type hypersensitivity, IFN- γ production, and lymphocyte proliferative responses.

Although IL-4 has been considered to be the most important cytokine involved in the pathogenesis of cutaneous leishmaniasis in experimental models [10], more recently its role has been reconsidered [11, 12]. In the present study, IFN- γ production in leishmania antigen-stimulated PBMC of patients with ECL could not be restored by addition of MAb to IL-4. The demonstration that neutralization of IL-10 or the addition of IL-12 enhanced IFN- γ production and lymphocyte proliferation in PBMC cultures of patients with ECL indicates that IL-10 may play a major role in this phenomenon. IL-10 has a powerful suppressive effect in cutaneous inflammatory responses [13], and it may act through suppression of IL-12 and IFN- γ activities. In fact, expression of mRNA for IL-10 has been observed at high levels in cutaneous leishmaniasis [8, 14], and IL-12 production is absent soon after introduction of promastigotes into mammalian cells [15].

Our results show that a modulation of a Th1-type response occurs during the early phases of *L. braziliensis* infection. This phenomenon may allow the parasite to survive and multiply, leading to the development of disease.

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