Abstract

Patients with American cutaneous leishmaniasis were studied before therapy (active lesion) and at the end of therapy (cured patients). Assays of lymphocyte proliferative responses of peripheral blood mononuclear cells induced in vitro by Leishmania braziliensis promastigote antigens (Lb) were performed. Antigen-stimulated cells were harvested for CD4 and CD8 phenotype analysis and the levels of gamma interferon (IFN-γ) and interleukin 4 (IL-4) produced were also determined in the culture supernatants. Two different patterns of Lb-induced T cell responses were observed: a) predominance of responding CD4+ cells and mixed type 1 and type 2 cytokine production (IFN-γ and IL-4) during the active disease, and b) similar proportions of responding CD4+ and CD8+ cells, and type 1 cytokine production (presence of IFN-γ and very low IL-4) at the end of therapy (healed lesions). This last pattern is probably associated with a beneficial T cell response.

Leishmania are important intracellular protozoan parasites that produce either tegumentary (cutaneous, mucocutaneous, diffuse) or visceral diseases in man in many areas throughout the world. The outcome of infection in each type of leishmaniasis depends on the complex and intriguing interaction of virulence factors and host immunologic responses. It is agreed that the resolution of human infection is dependent primarily on events within the T cell compartment (1-7).

In the mouse model, there is evidence that the mechanisms for cure or resistance to Leishmania major infection are associated with macrophage activation, leading to the destruction of intracellular parasites. Type 1 cytokines such as IFN-γ and TNF-α and -β when produced by the Th1 subset of CD4+ lymphocytes play a pivotal role in this process of macrophage activation and parasite destruction (8-11). On the other hand, the mechanisms for aggravation of the disease in mice are related to the effects of type 2 cytokines such as IL-4, IL-10 and TGF-β, which are primarily produced by the Th2 subset of CD4+ lymphocytes as well as other cell types. These cytokines are able to inhibit the differentiation of Th1 CD4+ lymphocytes and the production of IFN-γ and TNF (8,11, 12).

CD8+ T lymphocytes also appear to be important in the immunologic mechanisms
responsible for cure of murine leishmaniasis caused by L. major, L. amazonensis, and L. donovani (13-15). Activated antigen-specific CD8+ T cells have been shown to produce IFN-γ and can have a cytolytic effect on parasitized macrophages (16,17).

In humans the most common clinical form of American tegumentary leishmaniasis (ATL) is the localized cutaneous leishmaniasis (LCL) where a single or a few skin ulcers occur with a tendency toward self-healing or susceptibility to classical antimicrobial therapy. The scarceness of parasites in the lesions and the presence of hypersensitivity to leishmanial antigens are the main immunoparasitological features of LCL.

Studies from our laboratory (5-7) on LCL patients examined before antimicrobial therapy (active disease) and at the end of therapy (cure) have shown that the lymphoproliferative responses of peripheral blood mononuclear cells (PBMC) after stimulation in vitro with total L. braziliensis promastigote antigens (Lb), as measured by [3H]-thymidine incorporation, were not significantly different before and at the end of therapy, although they tended to decline.

The phenotypes of leishmanial antigen-reactive T cells stimulated in vitro were also investigated in PBMC cultures. After five days in culture, responding cells were separated by centrifugation over a discontinuous Percoll (Sigma Chemical Co., St. Louis, MO) gradient incubated in the presence of monoclonal antibodies for CD3+ (T3-RD1, Coulter Immunology, Hialeah, FL), CD4+ (T4-FITC, Coulter Immunology) and CD8+ (T8-RD1, Coulter Immunology) and finally analyzed by flow cytometry. The supernatant of each culture was also collected and stored at -70°C for later determination of cytokine concentrations.

Comparing the proportions of CD4+ and CD8+ L. braziliensis-reactive T cells before therapy (BT) and at the end of therapy (ET) we observed an increase in the percentage of CD8+ cells (BT = 23.9 ± 11.7%; ET = 42.6 ± 21.7%; P<0.05), a decline in the proportion of CD4+ cells (BT = 61.2 ± 18.3%; ET = 40.9 ± 21.7%; P<0.05, Mann-Whitney) and a consequent reduction in the CD4+/CD8+ ratio (BT = 2.5; ET = 0.9).

These results suggest that CD8+ T cells may be implicated in the mechanisms of cure of LCL. However, it was not clear whether the process of cure was associated only with the increased percentages of CD8+ Leishmania-reactive T cells or whether it also depended on the balance between CD4+ and CD8+ cells.

The levels of IFN-γ and IL-4 production by Leishmania-reactive T cells were also determined by testing the supernatant of antigen-stimulated PBMC cultures. IFN-γ was measured by a solid-phase ELISA (Intertest, Genzyme Corporation, Cambridge, MA) and IL-4 was also measured by ELISA (Intertest 4, Genzyme Corporation). The mean levels of IFN-γ in supernatants from Leishmania-stimulated cell cultures were 2255 ± 563 pg/ml before therapy (active disease) and 3005 ± 900 pg/ml at the end of therapy (cure). The mean levels of IL-4 on the same occasions were BT = 415.8 ± 633.1 pg/ml and ET = not detectable. Hence, in association with the CD4+-CD8+ switch in cured patients we also observed a slight, but not significant increase in IFN-γ production at the end of therapy, as well as a highly significant decrease in IL-4 production in cured patients.

In this respect, two different patterns of Lb-induced T cell responses were detected (6,7): a) the predominance of a CD4+ T cell response with production of a mixed type 1 (IFN-γ) and type 2 (IL-4) cytokine profile during active LCL, and b) similar proportions of responding CD4+ and CD8+ T cells, with production of IFN-γ and absence of IL-4 in cured patients (after therapy). This last pattern characterizes an apparently beneficial T cell response.

The lymphokine profiles determined in the skin lesions of active cases of ATL (18) have also shown a mixture of type 1 and type
2 lymphokines with relative predominance of mRNA for type 1 lymphokines.

At least three hypotheses can be advanced to explain the immunological changes observed after therapy: a) the observed decrease in the proportion of Lb-reactive CD4+ cells, associated with production of IFN-γ but not IL-4 in cured patients, may point to a better modulation of the T cell-mediated immune responses after destruction of parasites by antimony therapy and the decrease in parasite load. We may speculate that differences in parasite antigens found in active vs healing lesions would be relevant in the mechanisms for differential T cell activation; in addition, a reduction of parasite load by antimonial therapy may alter the T cell responses to Lb antigens. Moreover, results from our laboratory (19) on vaccination of human volunteers with a crude promastigote Leishmania vaccine have shown that the majority of the Leishmania-responding T cells in assays of the lymphoproliferative response to the parasite antigen presented a CD8+ phenotype and IFN-γ production. In this case the CD8+ T cell response seems to be involved in the mechanisms of protective immunity since there is evidence that the vaccine is able to induce protection in approximately 50% of cases (20). b) CD8+ T cells may play an important role in the cure of leishmaniasis by producing type 1 lymphokines, leading to the activation of macrophages for parasite destruction. c) There is also evidence that CD8+ T cells have a cytotoxic effect on parasitized macrophages, with a beneficial effect on the course of the disease (17).

Finally, this observed immunologic pattern associated with cure and/or protection in LCL can be helpful in determining the prognosis of active disease and as an important parameter for the selection of an antigen candidate for a future vaccine.

References


