

Human _ Leishmaniasis@cytokines.bahia.br

M. Barral-Netto^{1,2},
C. Brodskyn², E.M. Carvalho²
and A. Barral^{1,2}

¹Centro de Pesquisas Gonçalo Moniz, FIOCRUZ, Salvador, BA, Brasil
²Serviço de Imunologia, Hospital Universitário Prof. Edgard Santos,
Universidade Federal da Bahia, Salvador, BA, Brasil

Abstract

The cell-mediated immune response is critical in the resistance to and recovery from leishmaniasis. Cytokines are central elements in mounting an immune response and have received a great deal of attention in both human and experimental leishmaniasis. IFN- γ is responsible for macrophage activation leading to leishmanicidal mechanisms. Understanding the balance of cytokines that lead to enhanced production of or synergize with IFN- γ , and those cytokines that counterbalance its effects is fundamental for developing rational immunotherapeutic or immunoprophylactic approaches to leishmaniasis. Here we focus on the cytokine balance in human leishmaniasis, particularly IL-10 as an IFN- γ opposing cytokine, and IL-12 as an IFN- γ inducer. The effects of these cytokines were evaluated in terms of several parameters of the human immune response. IL-10 reduced lymphocyte proliferation, IFN- γ production and cytotoxic activity of responsive human peripheral blood mononuclear cells. Neutralization of IL-10 led to partial restoration of lymphoproliferation, IFN- γ production and cytotoxic activity in unresponsive visceral leishmaniasis patients. IL-12 also restored the responses of peripheral blood mononuclear cells from visceral leishmaniasis patients. The responses obtained with IL-12 are higher than those obtained with anti-IL-10, even when anti-IL-10 is combined with anti-IL-4.

Key words

- Cytokines
- Human leishmaniasis
- Immunoregulation
- IL-12
- IL-10

Correspondence

M. Barral-Netto
Centro de Pesquisas Gonçalo Moniz
FIOCRUZ
Rua Waldemar Falcão, 121, Brotas
40295-001 Salvador, BA
Brasil
Fax: 55 (071) 356-2255
E-mail: barral@ufba.br

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Introduction

Leishmaniasis is endemic in many parts of the world, with a prevalence of 12 million individuals (1). The clinical presentations of leishmaniasis are critically influenced by the host immune response. Cure or disease progression has been related to the predominance of a Th1 type of immune response (IFN- γ being the prototypic cytokine) or to a Th2 type of response (with an important production of IL-10), respectively. IFN- γ is responsible for macrophage activation leading to leishmanicidal mechanisms, whereas IL-10 leads to macrophage deactivation (2). Effort is focused on the understanding of the early events that may influence the develop-

ment of a Th1 or a Th2 type of response, and the interplay of several cytokines in order to determine their relative role in mounting or impeding an effective host protective response.

We will divide the present review into three areas: 1) pathogenesis, and clinical and immunological aspects of human leishmaniasis; 2) IL-12 and host protection, and 3) IL-10 and disease progression.

Pathogenesis, and clinical and immunological aspects of human leishmaniasis

Since most studies in the cytokine literature deal with experimental Leishmania in-

fections, we think it is appropriate to briefly review some aspects of the human disease in order to better appreciate the findings in human leishmaniasis.

Following inoculation into the skin, the flagellated promastigote penetrates into the macrophage, transforms into amastigotes and multiplies. Leishmanicidal activity is probably due to the increased capacity of the macrophages to produce toxic oxygen and nitrogen radicals in response to IFN- γ . Nitric oxide production and *Leishmania* killing by human macrophages through the ligation of Fc ϵ RII/CD23 have been shown (3). A summary of the role of IFN- γ in human leishmaniasis is presented in Table 1.

The course of leishmaniasis has been associated with different cytokine patterns,

which parallel cure or non-cure of disease in mice. The polarized responses do not change easily in vivo after 2 to 3 weeks of *Leishmania* infection in mice. A change from a Th1 to a Th2 population in *Leishmania*-specific cells has been obtained by the use of IL-4 in vitro (4) or in vivo (5). The combination of anti-IL-4 or IL-12 with antimony (6,7) promoted a change from a Th2 to a Th1 type of response in *L. major*-infected animals.

Th1 x Th2 dichotomy is probably influenced by cytokine patterns present during the very early stages of *Leishmania* survival inside the macrophage. Upon its entry into the human macrophage *Leishmania* induces contrasting signals. It induces the production of TNF- α , which leads to macrophage activation, or to the production of TGF- β or IL-10, linked to macrophage deactivation and inhibition of IFN- γ (8,9). IFN- γ produced by NK cells, which may depend on the production of IL-12, is also implicated in initial Th1 development (10). Initial survival of *Leishmania* inside the macrophage probably depends on which of these or similar cytokines predominate in the microenvironment of infection. A recent observation suggests the participation of different isolates of *Leishmania aethiopica* in inducing the production of IFN- γ or IL-10 by peripheral blood mononuclear cells (PBMC) from patients (11). *Leishmania* penetration also induces the production of several other products by macrophages, which may influence parasite survival (12-15).

The clinical manifestations of tegumentary leishmaniasis include the self-healing cutaneous disease (CL) and the destructive and hyper-responsive mucosal form (ML). More rarely the anergic form of diffuse cutaneous leishmaniasis (DCL) is observed. The most frequent aspect observed in CL cases is an ulcer with elevated borders and a sharp crater. CL patients exhibit anti-*Leishmania* cell-mediated immunity (CMI). Mucosal involvement, with disfiguring destruction of palate, uvula, pharynx, gums and upper lip,

Table 1 - Interferon- γ in human leishmaniasis.

VL, Visceral leishmaniasis; CL, cutaneous leishmaniasis; DCL, diffuse cutaneous leishmaniasis; ML, mucosal leishmaniasis.

Observation	Reference
Increases macrophage leishmanicidal activity	46,47
Increases Sb ^v uptake by macrophages	48
Not produced by lymphocytes from patients with active VL or DCL	25,26,37,49
IFN- γ restored in cured VL patients	50
IFN- γ mRNA present in lesions from CL patients	19,20
Higher production by PBMC from ML than from CL patients	51
Effective in the treatment of VL or CL patients	52,53

Table 2 - IL-12 in human leishmaniasis.

Observation	Reference
In visceral leishmaniasis patients	
Increases lymphocyte proliferation	37,38
Increases IFN- γ production and anti-tumor target cell cytotoxic activity	38
In mucosal leishmaniasis patients	
IL-12 increases (and anti-IL-12 decreases) parasite-driven anti-autologous macrophage cytotoxicity	56
In cutaneous leishmaniasis patients	
IL-12 mRNA present in most lesions from active CL patients	21

occurs in approximately 3% of patients infected by *L. braziliensis*. ML is considered to be the hyper-responsive pole of the disease due to the potent anti-*Leishmania* CMI responses observed in these patients. CL or ML patients may not exhibit a positive anti-leishmanial response (delayed-type hypersensitivity (DTH) and lymphocyte in vitro proliferation) before two to three months of infection. With disease progression, ML patients tend to develop larger intradermal skin test reactions, and their lymphocytes exhibit higher proliferative responses and production of IFN- γ than cells from CL patients. The initial lesion of DCL resembles those of CL without ulceration. The lesions present predominantly as erythematous papules or nodules. DCL occurs in the absence of anti-parasite CMI. Patients with DCL have a complete anergy to leishmanial antigen. DTH is negative and their lymphocytes do not respond to leishmanial antigen either by proliferation or by lymphokine production. DCL patients present an antigen-specific immunosuppression, mounting normal responses to other antigens. The characteristics of the different forms of human tegumentary leishmaniasis have been recently reviewed (1,16).

The actual role of the cytokine network in human leishmaniasis needs to be better defined. A preferential participation of Th1 or Th2 cell types within human cutaneous lesions is not completely clear as evaluated by cytokine gene expression in CL or DCL (17-23), or in visceral leishmaniasis (VL) patients (24-26).

IL-12 and host protection

IL-12 has received a great deal of attention in leishmaniasis (Table 2). IL-12 is produced following in vivo infection of mice by *Leishmania* (27,28), and is important to control Th2 expansion and promote the predominance of a Th1 type of response (28-30). In vitro, however, the parasite is able to inhibit IL-12 production by the infected macrophages (31,32).

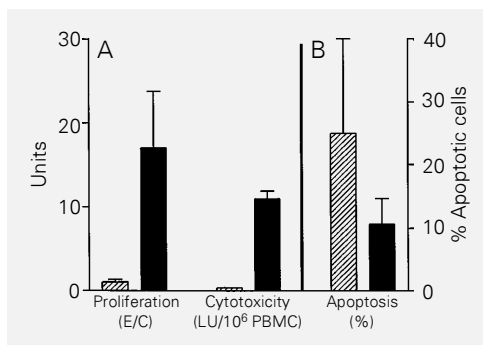


Figure 1 - Effect of IL-12 on the responsiveness of PBMC from visceral leishmaniasis (VL) patients. Panel A shows that the addition of IL-12 + *Leishmania* antigen (black bars) reversed both lymphocyte proliferation (proliferation reported as stimulation index = E/C) and cytotoxic responses against K562 tumor target cells (cytotoxicity reported as lytic units (LU) per million PBMC), as compared to cells receiving *Leishmania* antigen alone (hatched bars). In Panel B the addition of IL-12 + *Leishmania* antigen (black bars) partially reversed the rate of antigen-induced (hatched bars) PBMC apoptosis in VL patients (results are reported as percentage of apoptotic cells as evidenced by propidium iodide staining and FACS analyses). Bars represent the mean \pm SD of at least six determinations.

Treatment of susceptible animals with IL-12 renders them resistant (30,33-35), and has also been used as an effective adjuvant for a killed vaccine for *L. major* (36).

In human leishmaniasis, expression of IL-12 mRNA has been shown in active CL cases (21). In VL patients, IL-12 enhances Th1 responses (37), restoring lymphocyte proliferative responses, IFN- γ production and cytotoxic responses, as shown in Figure 1 (38). IL-12 also decreases spontaneous or Ag-induced PBMC apoptosis in VL patients (Figure 1).

IL-12 used in combination with *Leishmania* antigen restores proliferation of PBMC from VL patients more strongly than the use of anti-IL-4 or anti-IL-10 monoclonal antibodies, or even of both monoclonals combined (Figure 2).

IL-10 and disease progression

The prominent role of IL-4 as the leading Th2 cytokine in murine leishmaniasis was

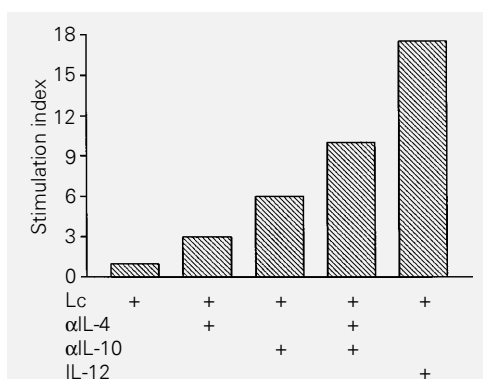


Figure 2 - IL-12 restored lymphocyte proliferation more effectively than did neutralization of IL-10 or IL-4. All PBMC cultures from visceral leishmaniasis patients were stimulated with *L. chagasi* antigen (Lc) and received (+) or not received (-) IL-12, anti-IL-4 or anti-IL-10 monoclonal antibodies. Results are reported as stimulation index (E/C) in relation to unstimulated cultures.

Figure 3 - Production of IFN- γ or IL-10 in different clinical forms of human leishmaniasis. PBMC from cutaneous (CL), mucosal (ML) or visceral leishmaniasis (VL) patients were stimulated with *Leishmania* antigen, and supernatants were evaluated for the production of IFN- γ or IL-10 by ELISA.

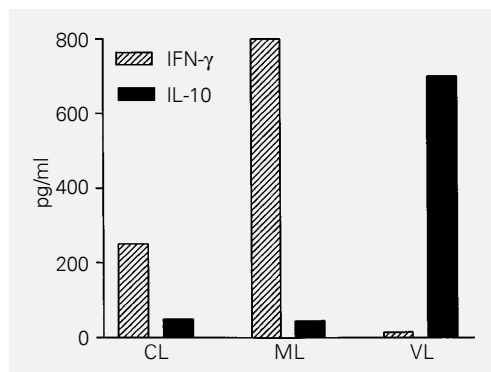
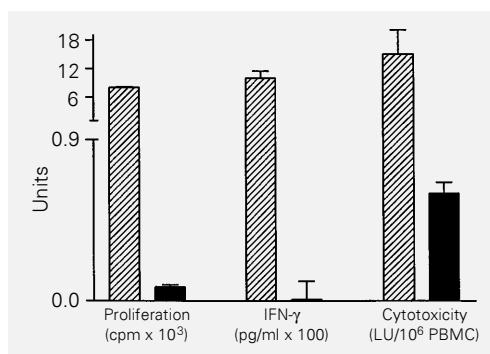


Figure 4 - Effect of IL-10 on the responsiveness of PBMC from tegumentary (CL or ML) leishmaniasis patients. The addition of IL-10 + *Leishmania* antigen (black bars) diminished lymphocyte proliferation (proliferation reported as cpm in thousands), IFN- γ production (pg/ml in hundreds) and cytotoxic responses against K562 tumor target cells (cytotoxicity reported as lytic units (LU) per million PBMC), as compared to cells receiving *Leishmania* antigen alone (hatched bars). Bars represent the mean \pm SD of at least six determinations.



not consistently shown in human leishmaniasis. IL-10 seems to represent the main macrophage-“deactivating” cytokine in opposition to IFN- γ (Figure 3), being present in many different clinical presentations of human leishmaniasis (Table 3). IL-10, however, is not a strict Th2 cytokine, since it can be produced by macrophages, B cells and mast cells, besides Th2 cells.

IL-10 blunts several immunological responses mediated by lymphocytes from tegumentary leishmaniasis patients (Figure 4). IL-10 is responsible for decreased antigen presentation by macrophages and inhibition of cytokine production by Th1 or other cells, including NK cells (39-45), and these effects may be responsible for its inhibitory action in leishmaniasis.

A predominance of Th1 cytokines in CL, with small amounts of IL-10 in DCL, has been shown in Brazilian patients (19). A comparison of cytokine mRNA expression in intralesional cells obtained from acute (less than 2 months) or chronic CL lesions was recently performed, with variable amounts of IL-10 detected in all cases. Chronic lesions exhibited higher expression of IL-10 mRNA than recent ones (22). In order to address the relationship between clinical status and cytokine pattern, we have performed a study analyzing 6 patients with DCL who exhibited transient clinical improvement following therapy. There was, as expected, a preferential differentiation of a Th2 type of response during the active phase of DCL, characterized by a high expression of IL-4 and IL-10 in most patients, with no expression of IFN- γ . The comparison of the DCL cytokine pattern with those of CL patients shows a clear difference in IFN- γ expression, which is expressed in CL but not in DCL (23). After specific therapy, the cytokine pattern observed in DCL patients changed dramatically. An important expression of IFN- γ mRNA and reduced amounts of IL-10 mRNA were observed (23).

Since lymphadenopathy is the earliest

Table 3 - IL-10 in human leishmaniasis.

Observation	Reference
In visceral leishmaniasis patients	
IL-10 blunts IL-12-driven IFN- γ production by PBMC	26,37
Anti-IL-10 monoclonal antibody partially restores lymphocyte proliferation and IFN- γ production.	26
High concentrations in serum but low production by Ag-stimulated PBMC	25
In diffuse cutaneous leishmaniasis patients	
IL-10 mRNA is elevated during active disease and decreases during clinical remission	23
In cutaneous leishmaniasis patients	
IL-10 decreases anti-tumor target (K562) cytotoxicity	54
IL-10 mRNA present in most active lesions	21
IL-10 mRNA weakly present in lesions	20
In mucosal leishmaniasis patients	
IL-10 decreases parasite-driven anti-autologous macrophage cytotoxicity	56
IL-10 mRNA abundantly expressed in lesions	20
IL-10 mRNA present but not predominant in relation to IL-4 mRNA	55

clinical stage of human leishmaniasis available for investigation, we have determined the cytokine profile for IL-2, IL-4, IL-10, IL-12, TGF- β and IFN- γ in lymph node cells from 10 patients with early cutaneous leishmanial infection by RT-PCR. At the time of diagnosis, none of the patients had a skin lesion but all had *Leishmania* cultivated from the lymph node. mRNA for IL-4 and IFN- γ were observed in several cases, but none showed a signal for IL-10. On the other hand, IL-10 mRNA has been demonstrated in lymph nodes from VL patients (26).

Conclusions

Studies in human leishmaniasis confirm the relevant roles of IFN- γ and IL-12 as the major cytokines involved in host protection, whereas IL-10 takes the place as the leading cytokine responsible for parasite survival and disease progression. Other less explored cytokines may also prove important in immunoregulation in human leishmaniasis. Future strategies for vaccination or immunotherapy must take into account such findings, which do not always parallel mouse studies.

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