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Mucosal leishmaniasis: in situ characterization of the host inflammatory response, before and after treatment

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Abstract

Mucosal leishmaniasis (ML) generally shows progressive tissue destruction, not yet fully elucidated, associated with an intense inflammatory response. To contribute to the understanding of this process and of how treatment interferes with it, we studied several anatomopathological parameters, including those analyzed by immunohistochemistry, such as *Leishmania* antigens, cells participating in the immune response and cytokine expression. Biopsies were taken from 20 patients with ML before and after treatment. A mixed Th1 and Th2 pattern response occurred inside ML before treatment, persist after treatment. Nevertheless, this mixed response was smaller than in active lesions, with reduced but present numbers of cells expressing TNF-α, IFN-γ and IL-4 and sustained numbers of cells expressing IL-10. We may conclude that specific treatment causes a reduction of inflammatory lesions and disappearance of amastigote forms of *Leishmania* although the factors related to the pathogenesis of the lesion, such as T CD4+ and T CD8+ lymphocytes and *Leishmania* antigens, persist in treated lesions. The maintenance of these inflammatory patterns may be due to a specific host-parasite relationship response, strongly indicating the need for continuous surveillance of LM patients at risk of reactivation, despite effective cicatrization after therapy.

Keywords: Mucosal leishmaniasis (ML); Mucocutaneous leishmaniasis; Immunohistochemistry; Treatment

1. Introduction

In Brazil, American tegumentary leishmaniasis (ATL) is caused mainly by *L. (V) braziliensis* (Grimaldi et al., 1989). Exclusive mucosal involvement, named mucosal leishmaniasis (ML), occurs in a minority of patients as a result of the

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metastatic spread of cutaneous lesions. Destructive lesions without spontaneous healing characterize ML and are generally localized in the nose and mouth, but may also affect the larynx and trachea, causing severe facial deformation and respiratory disturbances (Marsden, 1986).

The amastigote forms of *Leishmania* reside inside macrophages, inducing a complex interaction with the host immune response (Hall and Joiner, 1991). The host response patterns of helper lymphocytes, Th1 and Th2, may interact, leading either to control of the infection or to disease. These patterns show different profiles of preferential production of cytokines and of other cell products involved in the final activation of effector cells and in tissue lesion (Mossmann, et al., 1986; Jankovic et al., 2001).

The pathogenesis of ML has been associated with hyperactivity of the specific T cell immune response, with an exuberant, usually progressive and not completely elucidated inflammatory response involving extensive tissue destruction (Carvalho et al., 1985).

A more in-depth study of the immunopathological mechanisms involved in ML has always met the obstacle of the lack of an experimental model reproducing the disease as it occurs in man, especially the mucosal involvement (Barral et al., 1993).

Studies on the pathogenic mechanism of ML in humans are based mainly on phenotypic analysis and on the quantification of cytokines, or their synthesis, in peripheral blood using mononuclear cells stimulated in vitro with *Leishmania* antigens or using the polymerase chain reaction to extract mRNA from lesion material (Pirmez et al., 1993; Toledo et al., 2001; Da-Cruz et al., 2002).

Over the last few years, immunohistochemistry has become an important tool for the understanding of the immunopathology of a wide variety of infectious diseases, including leishmaniasis, since it permits the detection of the infectious agent and the identification of the types of cells and cytokines involved in the inflammatory in situ tissue response. However, to our knowledge, no previous studies have evaluated the pattern of in situ cytokine production associated with the identification of inflammatory cells before and after ML treatment.

The aim of the present study was to apply this method to study the anatomopathological aspects of ML, including the identification of the phenotypic profile of the cell population and tissue cytokines before and after treatment.

2. Material and methods

2.1. Patients, inclusion criteria and post-treatment biopsy

The study was conducted on patients seen at the Department of Infectious and Parasitic Diseases, University Hospital of the School of Medicine, University of São Paulo (FM-USP), seeking treatment for mucosal involvement suspected of Leishmaniasis. The inclusion criteria were based on clinical and endoscopic rhino-laryngologic findings, positive Montenegro delayed skin reaction, positive anti-*Leishmania* antibodies in serum, and histopathological examination of the biopsy, which showed amastigote forms of *Leishmania* or *Leishmania* antigens demonstrated by immunohistochemical reaction. When a patient did not present amastigote forms or *Leishmania* antigens in the lesion, inclusion in the study was based on the remaining criteria mentioned above, as well as on the presence of histopathological findings compatible with leishmaniasis and on mucosal lesion healing after specific treatment. The Ziehl–Nielsen and Grocott reactions were applied in all cases to rule out the possibility of mycobacterial or fungal infection. Patients treated for leishmaniasis or receiving immunosuppressive drugs during the last year before the study were excluded.

All patients were fully informed and 20 of them consented to participate in the study, which was conducted after approval by the Ethics Board of the School of Medicine (Protocol no. 742/98).

Clinical and otorhinolaryngologic examinations were performed in the patients included in the study monthly after the treatment with specific anti-leishmaniasis medication. Additionally, two biopsies were obtained from these same patients:

the first before treatment and the second at least 6 months after the detection of mucosal lesion healing. The criterion of mucosal lesion healing was defined as macroscopic absence of hyperemia, edema, ulceration, and granulomatous or vegetative lesion determined by clinical and endoscopic rhino-laryngological examination.

2.2. Histopathology

The biopsies were embedded in paraffin and sections were stained with hematoxylin-eosin (HE) at the Laboratory of the Discipline of Transmissible Diseases, FM-USP. The presence or absence before and after treatment (qualitative histological events) of the following parameters was determined: amastigote forms of *Leishmania*, *Leishmania* antigens detected by immunohistochemistry and granuloma in the lamina propria, as well as ulcers/erosion and crusts in the epithelium. Additionally, histopathologic alterations were scored (semiquantitative histological events) as 0, absent; 1, mild; 2, moderate; and 3, intense. The alterations scored were pseudoepitheliomatous hyperplasia, acanthosis, spongiosis, neutrophil exudation, and lymphocyte and macrophage infiltration for the epithelium; presence and characteristics of necrosis, collagen hyalinization, infiltration of macrophage, lymphocytes, plasma cells, and presence of granulomas for the lamina propria; and swelling, necrosis, hyalinization, vasculitis, thrombosis, and concentric thickening for blood vessels.

2.3. Immunohistochemistry

Silanized slides containing sections of fragments from biopsies obtained before treatment of leishmaniasis and after lesion healing were submitted to immunohistochemistry for the detection of the following cell subpopulations: T CD4+ lymphocytes, T CD8+ lymphocytes, CD68+ macrophages, CD1a+ Langerhans cells, CD20+ lymphocytes B, and CD57+ natural killer cells. Expression of the following cytokines was determined: IFN-γ, TNF-α, IL-4, and IL-10 (Fig. 1). The technical specifications concerning the antibodies used in the immunohistochemical reactions and the controls of the reactions are presented in Table 1.

Immunohistochemistry was carried out as follows: deparaffinization of histological sections (4 μm) with xylene, rehydration in a decreasing ethanol series, blockade of endogenous tissue peroxidase in 3% hydrogen peroxide, antigen recovery (Table 1) when necessary, incubation with a primary antibody, incubation with the polyvalent biotinylated secondary antibody (DAKO, A/S Denmark-K0492), and incubation with the streptavidin-biotin-peroxidase complex (SABC) (DAKO, A/S Denmark-K0492). The catalyzed signal amplification method (DAKO A/S Denmark-K1500) was used for the detection of T CD4+ and T CD8+ lymphocytes and Langerhans CD1a+ cells; after incubation with SABC (CSA/DAKO kit, CA-USA), an amplification reagent (biotinyl tyramide) was applied, followed by the streptavidin-peroxidase-conjugate. For the cytokines, before application of the primary and secondary antibodies, the slides was placed in a 0.1% saponin solution (Sigma Chemical Co, MO/USA-S7900) and a second peroxidase blockade were performed before SABC application.

All reactions were developed in the same way using a diaminobenzidine chromogen solution (Sigma Chemical Co., MO, USA-D5637) and counterstaining was performed with Harris hematoxylin. Next, the slides were dehydrated in a growing ethanol series and mounted with Permount resin (Fisher Chemicals, NJ, USA).

2.3.1. Quantitative analysis of cell subpopulations and of cytokine-expressing cells

CD4+ and CD8+ T lymphocytes, B lymphocytes, macrophages, natural killer cells and Langerhans cells were counted separately in the epithelium and lamina propria using a 1 cm^2 grid divided into one hundred squares of 1 mm^2, which we considered a field for counting, adapted to the ocular of a light microscope at 40 times magnification (ocular: ten times magnification). Cells expressing IFN-γ, TNF-α, IL-4 and IL-10 were counted by the same method in the lamina propria. An independent observer who was unaware of the origin of the material, randomly

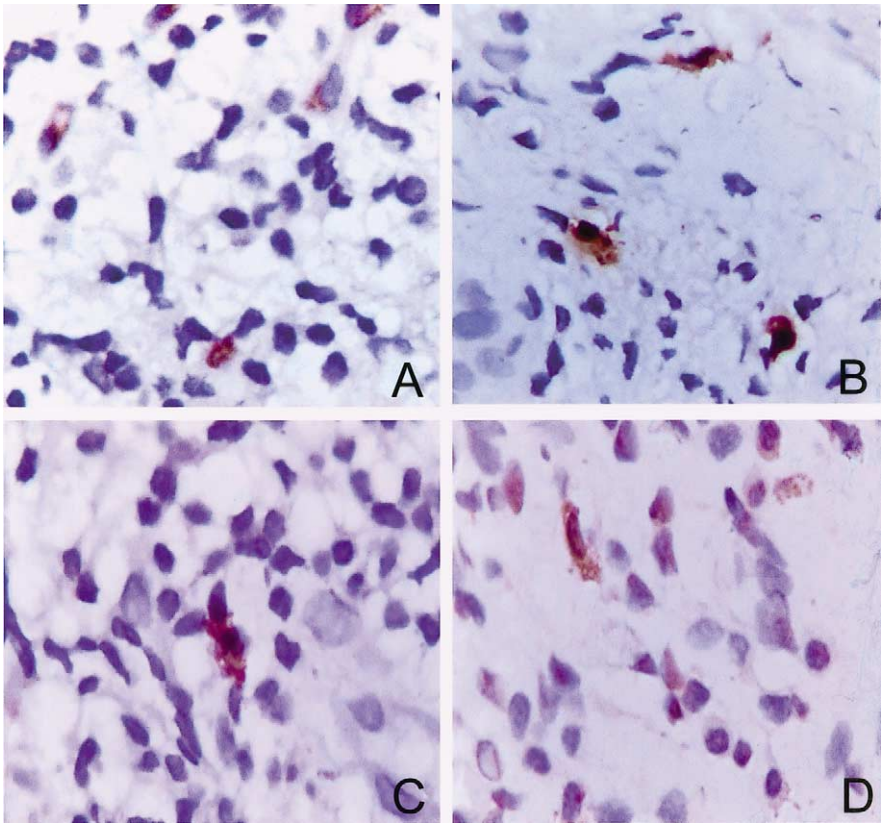

Fig. 1. Cytokine-expressing cells, magnification × 400 (A: IFN-γ, B: TNF-α, C: IL-4, D: IL-10).

counted ten chosen fields from the histological area analyzed.

2.4. Statistical analysis

Statistical analysis was performed using the Statistica for WINDOWS software version 5.1 (StatSoft Inc., 1998, Tulsa, USA). The Mann–Whitney test and Spearman correlation were used to compare the time needed for healing and the clinical characteristics. Quantitative and semi-quantitative data were analyzed by the Wilcoxon test, and frequency data by the sign test method for comparison of the results obtained before and after treatment. The level of significance was set at $P < 0.05$. All tests were two-tailed, with a test power of 90%.

3. Results

3.1. Demographic and therapeutic events of the patients

Sixteen males and four females aged 19–78 years (mean age ± S.D.: 54.4 ± 15) were included in the study. Fourteen (70%) patients only showed lesions in the nasal fossa and six (30%) only on the palate. The duration of symptoms ranged from 5 to 360 months, with a mean of 64 ± 86. Four patients had other diseases associated with ML, three were hypertensive, and one was diabetic. Nine patients received *N*-methyl glucamine antimoniate (Glucantime®, Aventis Pharma Ltda) at the daily dose of 20 mg per Sb^V per kg, with a mean dose of 38.911 ± 11.116 mg per Sb^V (range: 24.000–59.200 mg per Sb^V). Electrocardiographic abnormalities were detected in ten patients before

Table 1
Technical specification of the antibodies used in the immunohistochemical reactions of the paraffin-embedded material

Antibody description	Dilution	Brand/code	Antigen recovery	Positive control used	Negative control used
Polyclonal mouse anti-*Leishmania* ab[a]	1:1000	Produced at LDPTD[b], Faculty of Medicine, University of São Paulo	Trypsin	Skin of a mouse infected with *L. amazonensis* in the footpad	Knee skin obtained from an orthopedic surgery
Anti-human CD1a+ cell mouse monoclonal ab	Pure	Immunotech Company, Marseille/France-1590	CSA[c]	Tuberculosis lymph node	–
Anti-human CD4+ T cell mouse monoclonal ab	1:1000	DAKO Corporation, CA/USA-M834	CSA	Tuberculosis lymph node	–
Anti-human TCD8+ cell mouse monoclonal ab.	1:50	DAKO Corporation, CA/USA-M7103	CSA	Tuberculosis lymph node	–
Anti-human CD57+ cell mouse monoclonal ab.	1:100	DAKO Corporation CA/USA-M1014		Tuberculosis lymph node	
Anti-human CD68+ cell mouse monoclonal ab	1:50	DAKO Corporation, CA/USA-M876	Trypsin	Tuberculosis lymph node	–
Anti-human IL-4 goat monoclonal ab	1:40	R&D Systems Inc., MN/USA-AB204-NA	Trypsin	Atopic dermatitis (skin)	–
Anti-human IL-10 monoclonal ab	1:10	R&D Systems Inc., MN/USA-MAB 217	Trypsin	Atopic dermatitis (skin)	–
Anti-human TNF-α rabbit polyclonal ab	1:400	Genzyme Diagnostics, MA/USA-IP300	Trypsin	Adenocarcinoma of the prostate	–
Anti-human IFN-γ goat polyclonal ab	1:300	Genzyme Diagnostics, MA/USA-IP500	Trypsin	Tuberculoid leprosy (skin)	–

[a] Antibody.
[b] Laboratory of the Discipline of Pathology of Transmissible Disease, University of São Paulo-Brazil (Amato et al., 1998).
[c] Catalyzed Signal Amplification (DAKO A/S Denmark-K1500).

treatment, and because of the well-known cardiotoxicity of antimonial compounds (Berman, 1997), pentamidine isothionate (Pentacarinat®, Aventis Pharma Ltd) was administrated to these patients, with the mean dose required for lesion healing being 2.542±689 mg (range: 2.000–4.200 mg). One patient, in addition to electrocardiographic alterations detected before treatment, also had diabetes mellitus, a disease that contraindicates the use of pentamidine (Sands et al., 1985), and, therefore, was treated with amphotericin B (Fungizon®, Bristol–Myers Squib Brazil S/A) at a total dose of 1860 mg.

3.2. Lesion healing

Healing of the mucosal lesion occurred within a mean time of 4.3 months after the end of treatment (range: 3–6 months; median: 4 months), with no significant correlation being observed between this period of time and patient clinical characteristics such as age, duration of symptoms, extension and

Table 2
Frequency of significant qualitative histological events before and after treatment

Event	Before treatment	After treatment	Sign test P
Amastigotes in the lamina propria	50% (10/20)	0% (0/20)	<0.05
Antigen in the lamina propria	65% (13/20)	40% (8/20)	<0.05
Ulcers erosion	85% (17/20)	40% (8/20)	<0.05
Crusts	90% (18/20)	40% (8/20)	<0.05
Granulomas in the lamina propria	50% (10/20)	5% (1/20)	<0.05

location of the lesion, or disease condition associated.

3.3. Histological events

The frequency of significant qualitative histological events is shown in Table 2. The same pattern of a decreased but not abolished response was observed upon detailed analysis of semiquantitative events (Table 3). For most events, the pattern of decrease was significant, but some events persisted in one or more patients after therapy despite apparent macroscopic cure.

3.3.1. Cell surface markers

Quantitative analysis of the density of cell subtypes is shown in Table 4. A decrease in the frequency of CD4+ cells was observed in the lamina propria but not in the epithelium after treatment, without significant alterations in the frequency of CD8+ cells. CD68+ cells decreased at both sites after treatment, whereas the fre-

Table 3
Semiquantitative data of histological events, presented as mean, S.D. and range

Semiquantitative event	Site	Before diagnosis mean (S.D.) range[a]	After treatment mean (S.D.) range	Wilcoxon test P
Pseudoepitheliomatous hyperplasia	Epithelium	1.4 (0.8) 0–2	0.8 (0.7) 0–2	<0.05
Acanthosis	Epithelium	1.7 (0.5) 0–2	1.2 (0.5) 0–2	<0.05
Spongiosis	Epithelium	1.8 (0.8) 0–3	1.1 (0.8) 0–2	<0.05
Neutrophils	Epithelium	2.1 (0.8) 0–3	1.1 (0.7) 0–2	<0.05
	L. propria	2.1 (0.8) 0–3	1.4 (0.8) 0–2	<0.05
Lymphocytes	Epithelium	1.1 (0.4) 0–2	0.7 (0.4) 0–1	<0.05
	L. propria	2.2 (0.5) 1–3	1.5 (0.8) 0–3	<0.05
Macrophages	Epithelium	0.7 (0.7) 0–2	0.3 (0.4) 0–1	<0.05
	L. propria	1.9 (0.6) 1–3	1.1 (0.6) 0–2	<0.05
Plasma cells	L. propria	2.4 (0.5) 1–3	1.7 (1.0) 0–3	<0.05
Fibrinoid necrosis	L. propria	0.2 (0.5) 0–2	0 (0) 0–0	N.s.[b]
Necrosis with debris	L. propria	1.4 (0.8) 0–3	0.7 (0.8) 0–2	<0.05
Fibrinoid alterations	L. propria	0.2 (0.5) 0–2	0.1 (0.3) 0–1	N.s.
Fragmentation	L. propria	1.5 (0.7) 0–2	1.15 (0.7) 0–2	N.s.
Hyalinization	L. propria	0.15 (0.3) 0–1	0.2 (0.6) 0–3	N.s.
Endothelial swelling	Vessel	1.5 (0.7) 0–3	1.1 (0.5) 0–2	<0.05
Fibrinoid necrosis	Vessel	0.35 (0.7) 0–2	0 (0) 0–0	N.s.
Hyalinization	Vessel	0.2 (0.2) 0–1	0.3 (0.3) 0–1	N.s.
Vasculitis	Vessel	1.15 (0.8) 0–2	0.4 (0.5) 0–2	<0.05
Thrombosis	Vessel	0.1 (0.3) 0–1	0 (0) 0–0	N.s.
Concentric thickening	Vessel	0.25 (0.7) 0–3	0.2 (0.5) 0–2	N.s.

[a] Standard deviation.
[b] Without statistical significance.

Table 4
Quantitative cell subsets before and after treatment

Event (cells per mm^2)	Site	Before treatment mean ± S.D.[a]	After treatment mean ± S.D.	Wilcoxon test P
CD4+	Epithelium	30.8 ± 44.8	22.0 ± 43.6	N.s.[b]
	L. propria	270.9 ± 305.1	135.8 ± 129.1	< 0.05
CD8+	Epithelium	70.1 ± 68.6	51.8 ± 63.2	N.s.
	L. propria	247.6 ± 189.1	212.1 ± 192.9	N.s.
CD68+	Epithelium	20.6 ± 32.9	7.8 ± 8.9	< 0.05
	L. propria	230.5 ± 156.3	53.1 ± 74.2	< 0.05
CD20+	Epithelium	6.8 ± 16.6	5.2 ± 3.3	N.s.
	L. propria	91.0 ± 101.0	54.0 ± 81.9	N.s.
CD1A+	Epithelium	14.2 ± 17.1	8.4 ± 11.2	N.s.
	L. propria	3.5 ± 2.7	3.0 ± 3.5	N.s.
CD57+	Epithelium	3.3 ± 11.3	3.0 ± 4.3	N.s.
	L. propria	33.2 ± 28.6	21.6 ± 21.7	N.s.

[a] Standard deviation.

quency of CD1A+, CD20+ and CD57+ cells remained unchanged at both sites.

3.3.2. *Quantitative analysis of cells expressing cytokines*

Quantitative analysis of cells expressing cytokines was shown in Table 5. The frequency of cells expressing IFN-γ, TNF-α and IL-4 was lower after treatment, while the frequency of IL-10-producing cells was similar, independent of the time when a biopsy was taken.

3.3.3. *Analysis of groups with persistence or not of Leishmania antigens in tissue*

There was no significant difference concerning the qualitative and semiquantitative histological events or quantitative cells subsets, or even the analysis of cells expressing cytokines, when the biopsies were sorted according to persistence or not of *Leishmania* antigens in the mucosa after treatment. However, the small numbers of patients in each group may obscure some fine relationships between these events.

4. Discussion

In the present study, we analyzed the histological course of ML before and after specific therapy, including the determination of tissue cell subpopulations and cellular cytokine production during the regression process, and the detection of *Leishmania* antigens. Previous studies have used immunohistochemistry to quantify the cell phenotype profile only before treatment, especially in cutaneous leishmaniasis (CL; Pirmez et al., 1990; Barral et al., 1987; Esterre et al., 1992).

Table 5
Quantitative analysis of cells expressing cytokines before and after treatment

Event (cells per mm^2)	Site	Before treatment mean ± S.D.[a]	After treatment mean ± S.D.	Wilcoxon test P
IFN-γ+	L. propria	23.5 ± 10.4	14.7 ± 8.6	< 0.05
TNF-α+	L. propria	29.7 ± 17.7	15.3 ± 10.0	< 0.05
IL-10+	L. propria	8.9 ± 12.1	5.6 ± 7.8	N.s[b]
IL-4+	L. propria	7.2 ± 6.4	3.3 ± 2.2	< 0.05

[a] Standard deviation.
[b] Without statistical significance.

Usually, the criterion used to determine lesion healing in ATL is the clinical one, in combination with otorhinolaryngologic evaluation in the case of ML, and has been employed in several studies (Nonata et al., 1997; Falcoff et al., 1994; Singer et al., 1975; Amato et al., 2000). Thus, these criteria were used for the definition of lesion healing in our series. Additionally, serologic tests are of questionable usefulness for follow-up after treatment (Oliveira et al., 1995).

In 40% of the patients with healed lesions and septal perforation we detected crusts and small ulcers/erosions only upon histopathological examination. These erosions/ulcers were very small and could not be visualized by clinical or otorhinolaryngologic examination. The presence of a crust after the healing of nasal lesions is known to be due to the air whirling caused by septal perforation and also to the retention of particulate matter in the atrophic mucosa that has lost its hair cells. On this basis, crust manipulation by the patients themselves may result in superficial erosions or ulcerations that are not detected by otorhinolaryngologic examination. We may assume that these microscopic erosions/ulcerations do not indicate that the lesions did not heal.

In the present study, despite evident clinical otorhinolaryngologic regression of the tissue inflammatory lesions after treatment, the data clearly demonstrated the absence of complete resolution of the lesions, as indicated by presence of parasite antigens and maintenance of microscopic inflammatory events, findings that might explain the frequent recurrence of ML (Franke et al., 1990). This concept of controlled but not eliminated inflammation and infection is supported by our semiquantitative results showing the reduction/elimination of more frequent events such as the presence of ulcers, necrosis and identifiable amastigote forms, while the fine mechanism of inflammation such as the inflammatory cell infiltrate persisted. The same finding has been described for CL in which clinical cure does not always coincide with histopathological cure, i.e. the inflammatory infiltrate may persist after treatment despite healing of the lesions, (Botelho et al., 1998).

There was no significant difference in the parameters comparatively analyzed before and after treatment regarding the presence of *Leishmania* antigens after treatment determined by immunohistochemistry. Our suggested explanation for this was that the main immune-inflammatory host mechanisms that induced LM might persist after treatment due to persistence of the parasite or of its antigens in a clinically healed lesion, as shown by the present data and by previous studies, which inclusively used other techniques for searching *Leishmania* (Schubach et al., 1998a,b).

In this study we observed a reduction of endothelial cell swelling after treatment, and also of cells expressing TNF-α. The morphological finding of venules with a thick endothelium is the expression of endothelial activation (Silva et al., 1998). Several cytokines, and IL-1 and TNF-α in particular, induce endothelial cells to produce and express cytoadhesion molecules that permit cell migration (Beutler and Cerami, 1989). TNF-α seems to play an important role in host defense in an experimental model of leishmaniasis (Titus et al., 1989). Higher serum TNF-α levels have been observed in human ML than in CL, and these levels decrease after treatment in ML, a finding that may be one of the factors related to the generally chronic and destructive course of ML, in contrast to CL (Da-Cruz et al., 1996). Additionally, another study reported the absence of cellular expression of TNF-α in patients with diffuse-cutaneous leishmaniasis, an anergic form of ATL associated with high intracellular parasitism and a low presence of inflammatory cells, indicating that this cytokine plays a protective role in human ATL (Silva et al., 1998). The reduction of TNF-α in ML observed in our study after treatment possibly represents one of the factors that contributed to lesion healing, reflecting reduced endothelial activation and consequently reduced cell migration to the site of lesion and tissue injury.

Some aspects of the present results are noteworthy, such as the presence of CD4+ T and CD8+ T lymphocytes in the healed tissue lesion. This finding, taken together with the persistence of *Leishmania* antigens in the lesions after clinical cure, may be related to the maintenance of a local inflammatory response and to the continued

activation of these cells. In addition, the persistence of CD4+ and CD8+ T lymphocytes in the lesions may act as a defense mechanism protecting against reinfection in regions endemic for leishmaniasis (Schubach et al., 1998b; Da-Cruz et al., 2002; Muller et al., 1994).

IL-4 seems to play an important role in the pathogenesis of ML A study using mRNA extracted from material obtained from the lesions of patients with CL and ML demonstrated larger amounts of this cytokine in ML than in CL (Pirmez et al., 1993). Based on our results, we suggest that the local decrease in this cytokine due to a reduction in antigen load after treatment may alter the response of CD4+ T lymphocytes, with the preferential expression of Th1 type cytokines, which in turn may lead to a much more effective macrophage action in killing the parasite based on the antagonistic effect of IL-4 over IFN-γ.

The decrease in IFN-γ expression after treatment might be related to treatment itself, which results in a reduction of parasite burden and, consequently, in a reduced macrophage stimulation. The production of IFN-γ by CD4+ T lymphocytes is known to depend on the complex and continuous interaction between these cells and the release of IL-12 and IL-18 by macrophages (Okamura et al., 1998). A role of CD8+ T lymphocytes as additional IFN-γ producers in this process cannot be excluded.

Contrary to expectation, quantitative analysis did not show a significant difference in tissue IL-10 expression before and after tissue healing, as reported by others for cutaneous leishmaniasis and diffuse cutaneous leishmaniasis (Toledo, et al. 2001; Barral-Netto et al., 1998). This macrophage-deactivating cytokine is known to play a role by leading to a reduced antigen presentation by these cells and by inhibiting cytokine production by Th1 cells (De Waal et al., 1991; Cassatella et al., 1993). However, IL-10 production is not limited to cells inducing a Th2 response, but may also occur in macrophages, B cells, and mast cells (Barral-Netto et al., 1998). To our knowledge, there are no reports about the comparative tissue expression of this cytokine before and after treatment of LM. Perhaps the persistence of IL-10 in the lesions after treatment was one of the factors that contributed to the lack of elimination of the parasite or its antigens in LM patients, in view of the inhibitory action of this cytokine on macrophages. Thus, further studies are needed for a better clarification of this occurrence.

The results of the present study demonstrate that the marked reduction/disappearance of inflammatory lesions and the disappearance of the amastigote forms were mainly attributable to treatment, although CD4+ and CD8+ T lymphocyte infiltration or *Leishmania* antigens significantly persisted in the treated lesions. We were able to characterize an in situ mixed Th1/Th2 host response in the active lesions. One of the aspects to be emphasized is the modification of the tissue response after treatment, especially the significant reduction of IL-4 expression. This finding may possible indicates a more effective host response permitting lesion healing.

Considering the chronic nature of LM, the present data support the application of new therapeutic measures such as the use of TNF-α and/or IL-4 inhibitors. They could also be of help in studies on the prevention of the disease and contribute to the evaluation of post-treatment control.

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